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**KIUCHI et al.**

(54) **OBSERVATION METHOD USING BINDING AND DISSOCIATION PROBE**

(71) Applicant: **KYOTO UNIVERSITY**, Kyoto-shi (JP)

(72) Inventors: **Tai KIUCHI**, Kyoto (JP); **Naoki WATANABE**, Kyoto (JP); **Takushi MIYOSHI**, Kyoto (JP); **Ryo SASAKI**, Kyoto (JP)

(73) Assignee: **KYOTO UNIVERSITY**, Kyoto-shi (JP)

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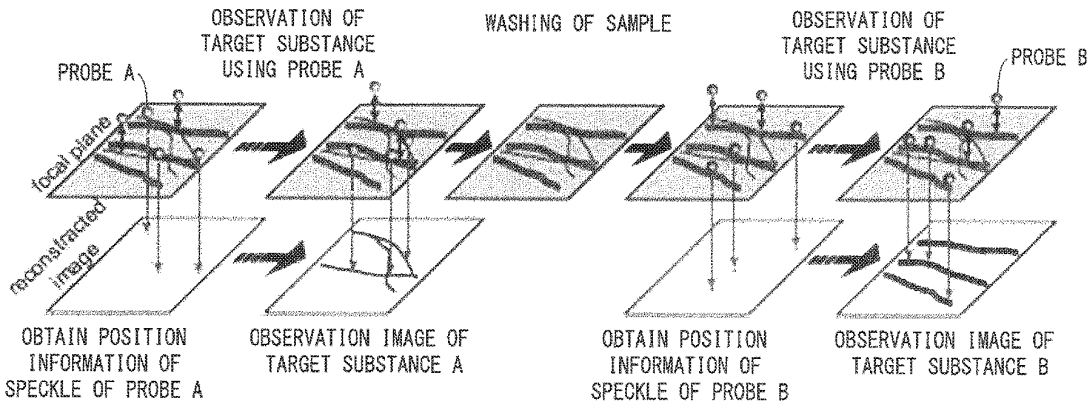
(57) **ABSTRACT**

An observation method of a sample containing a target substance, the observation method including an imaging step in which a step of obtaining a speckle image including, as a speckle, light emitted from a luminescent substance in which a medium is brought into contact with the sample is performed a plurality of times so as to obtain a plurality of speckle images, the medium containing a probe that contains the luminescent substance emitting light and that repeatedly binds to and dissociates from the target substance directly and specifically, and an observation image generation step of generating an observation image of the target substance in the sample from the plurality of speckle images, wherein a half-life of a probe-target complex formed by binding between the probe and the target substance is equal to or more than 10 milliseconds and equal to or less than 3 seconds.



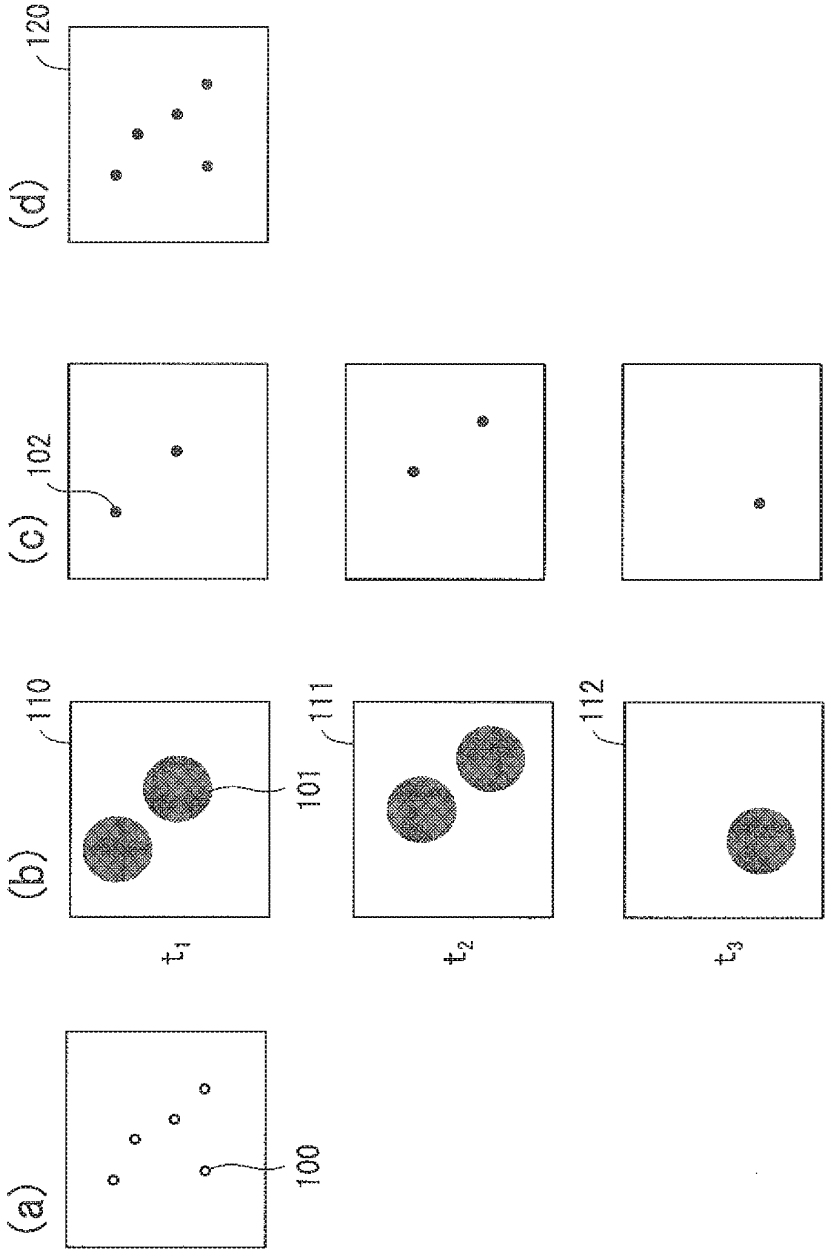



FIG. 1

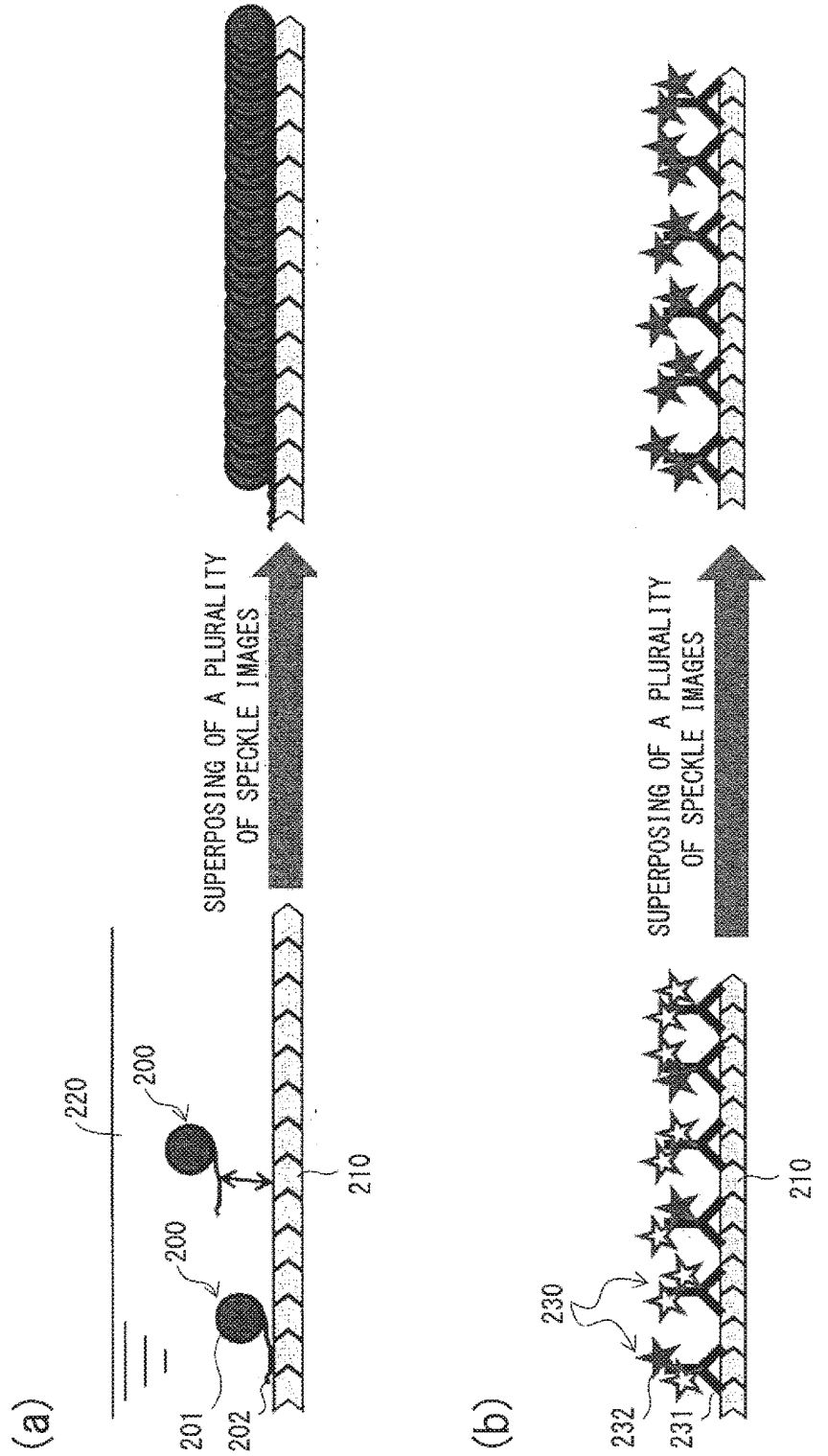

(a)
220
200
200
201
202
210
SUPERPOSING OF A PLURALITY OF SPECKLE IMAGES
(b)
230
232
231
210
SUPERPOSING OF A PLURALITY OF SPECKLE IMAGES

FIG. 2

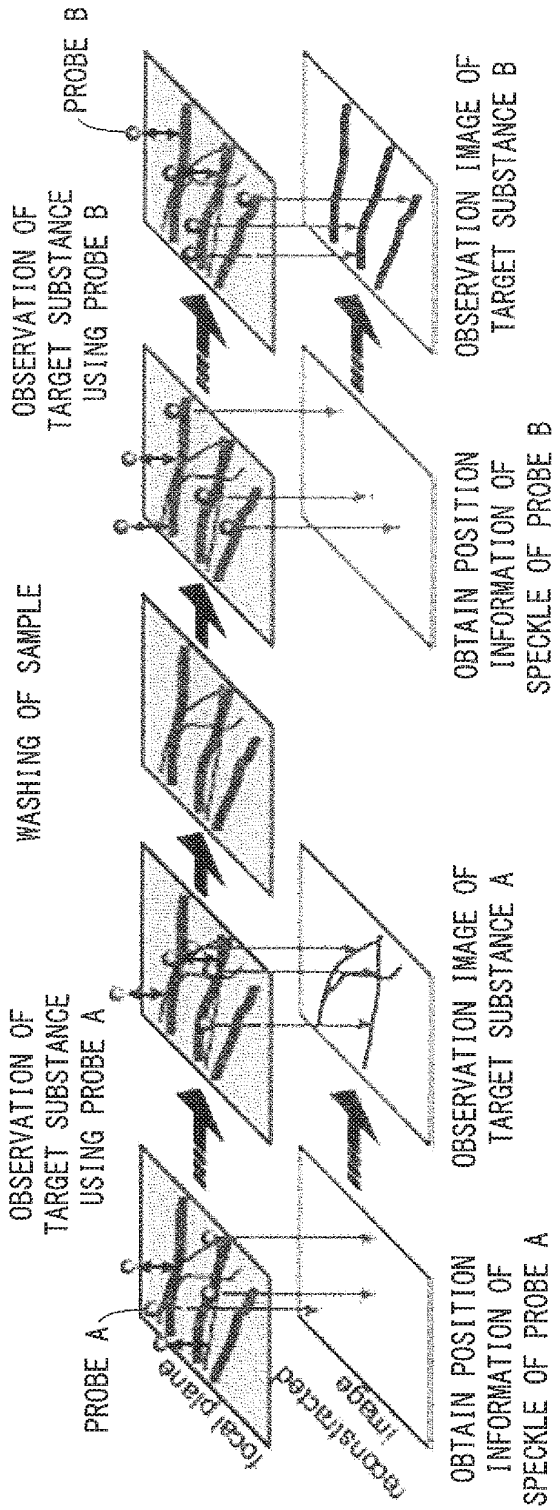

PROBE A
OBSERVATION OF TARGET SUBSTANCE USING PROBE A
WASHING OF SAMPLE
OBSERVATION OF TARGET SUBSTANCE USING PROBE B
PROBE B
(bce) plane
reconstructed image
OBTAIN POSITION INFORMATION OF SPECKLE OF PROBE A
OBSERVATION IMAGE OF TARGET SUBSTANCE A
OBTAIN POSITION INFORMATION OF SPECKLE OF PROBE B
OBSERVATION IMAGE OF TARGET SUBSTANCE B

FIG. 3

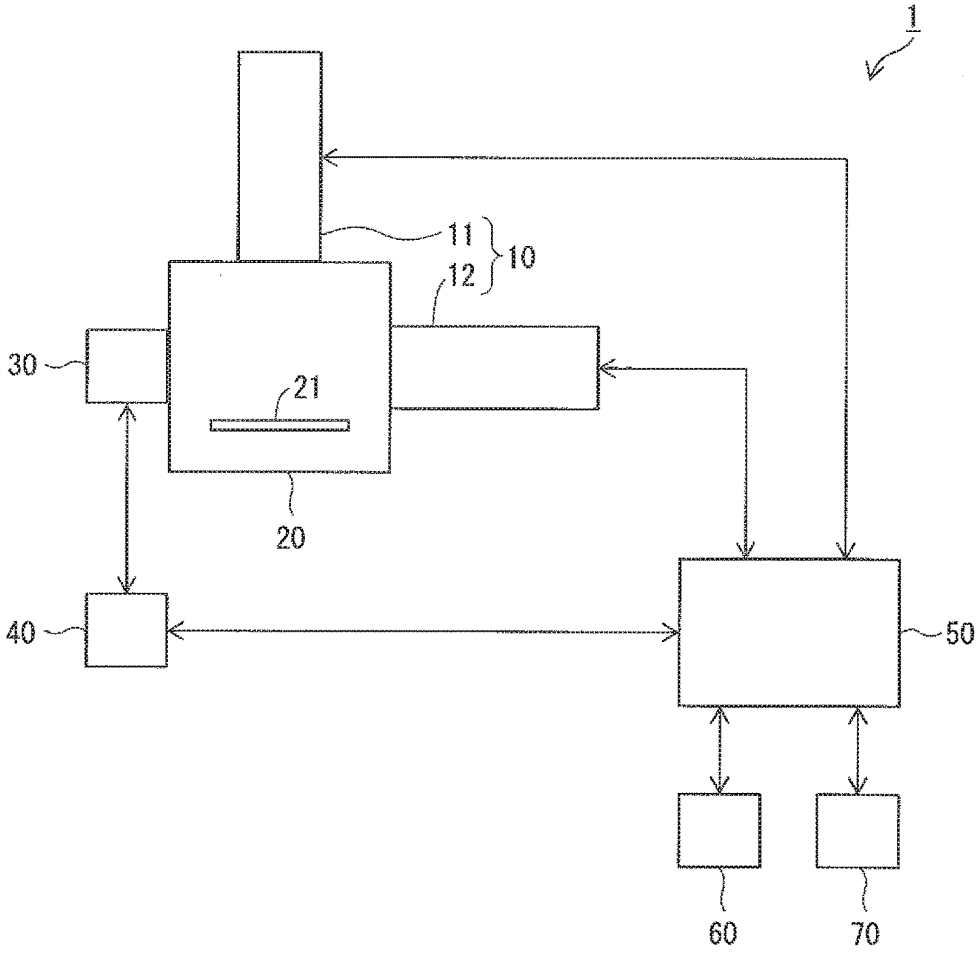



FIG. 4

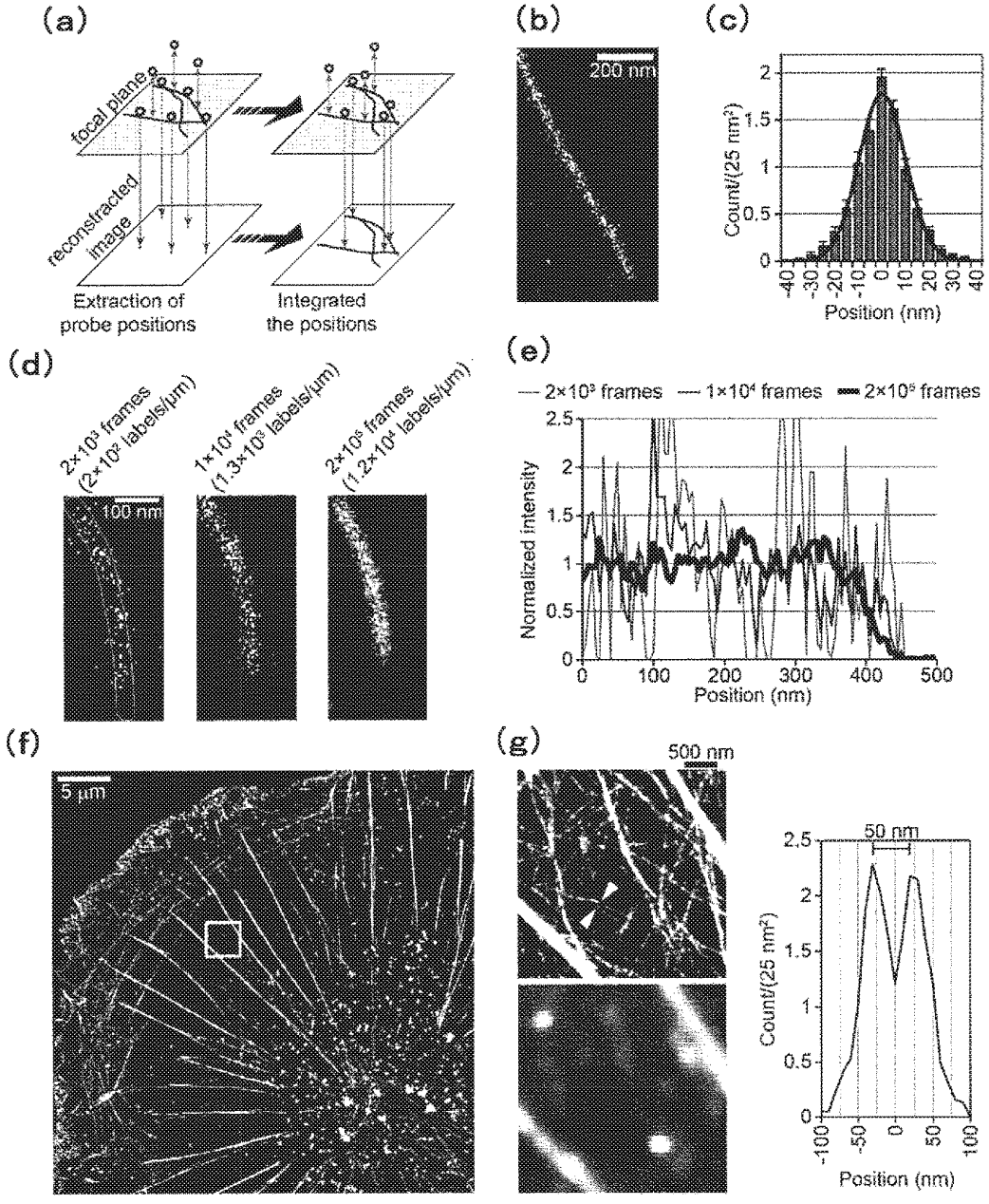

(a)
focal plane
reconstracted image
Extraction of probe positions
Integrated the positions
(b)
200 nm
(c)
Count/(25 nm²)
Position (nm)
(d)
2×10³ frames (2×10² labels/μm)
1×10⁴ frames (1.3×10³ labels/μm)
2×10⁵ frames (1.2×10⁴ labels/μm)
100 nm
(e)
2×10³ frames
1×10⁴ frames
2×10⁵ frames
Normalized intensity
Position (nm)
(f)
5 μm
(g)
500 nm
50 nm
Count/(25 nm²)
Position (nm)

FIG. 5

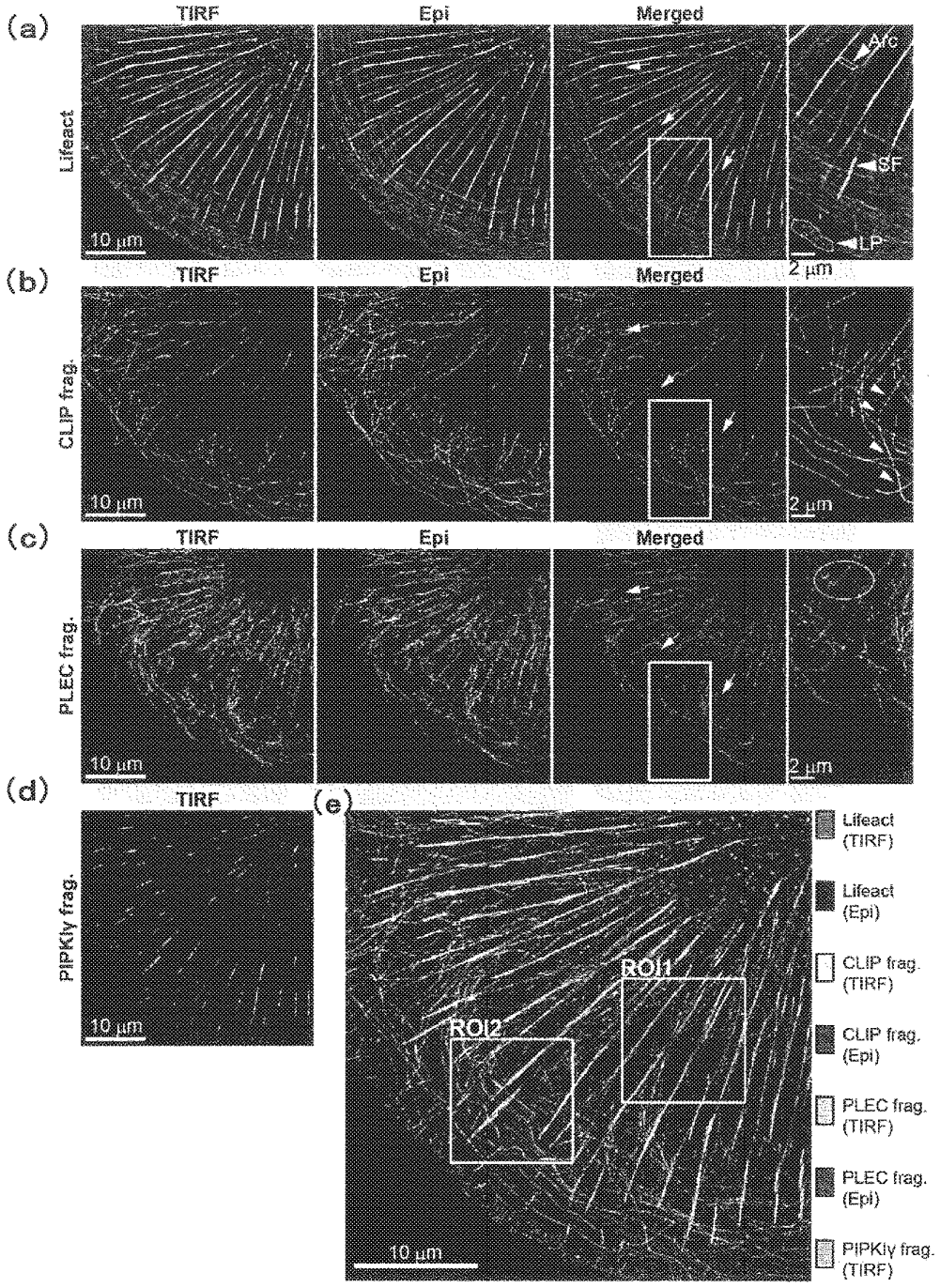

(a)
TIRF
Epi
Merged
Lifeact
Arc
SF
LP
10 μm
2 μm
(b)
TIRF
Epi
Merged
CLIP frag.
10 μm
2 μm
(c)
TIRF
Epi
Merged
PLEC frag.
10 μm
2 μm
(d)
TIRF
PIPKIγ frag.
10 μm
(e)
ROI1
ROI2
10 μm
Lifeact (TIRF)
Lifeact (Epi)
CLIP frag. (TIRF)
CLIP frag. (Epi)
PLEC frag. (TIRF)
PLEC frag. (Epi)
PIPKIγ frag. (TIRF)

FIG. 6

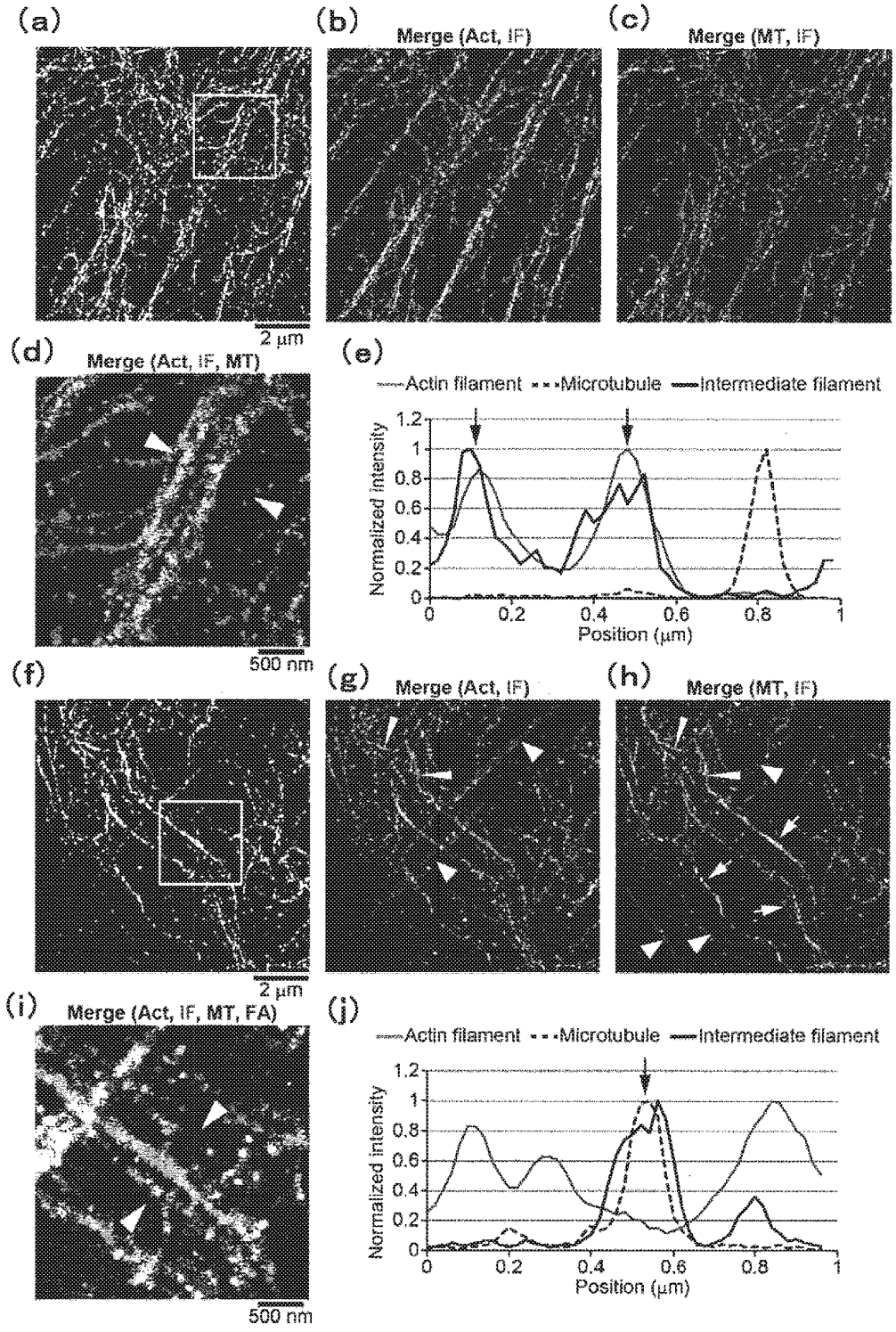

(a)
(b)
Merge (Act, IF)
(c)
Merge (MT, IF)
2 μm
(d)
Merge (Act, IF, MT)
500 nm
(e)
Actin filament
Microtubule
Intermediate filament
Normalized intensity
Position (μm)
(f)
2 μm
(g)
Merge (Act, IF)
(h)
Merge (MT, IF)
(i)
Merge (Act, IF, MT, FA)
500 nm
(j)
Actin filament
Microtubule
Intermediate filament
Normalized intensity
Position (μm)

FIG. 7

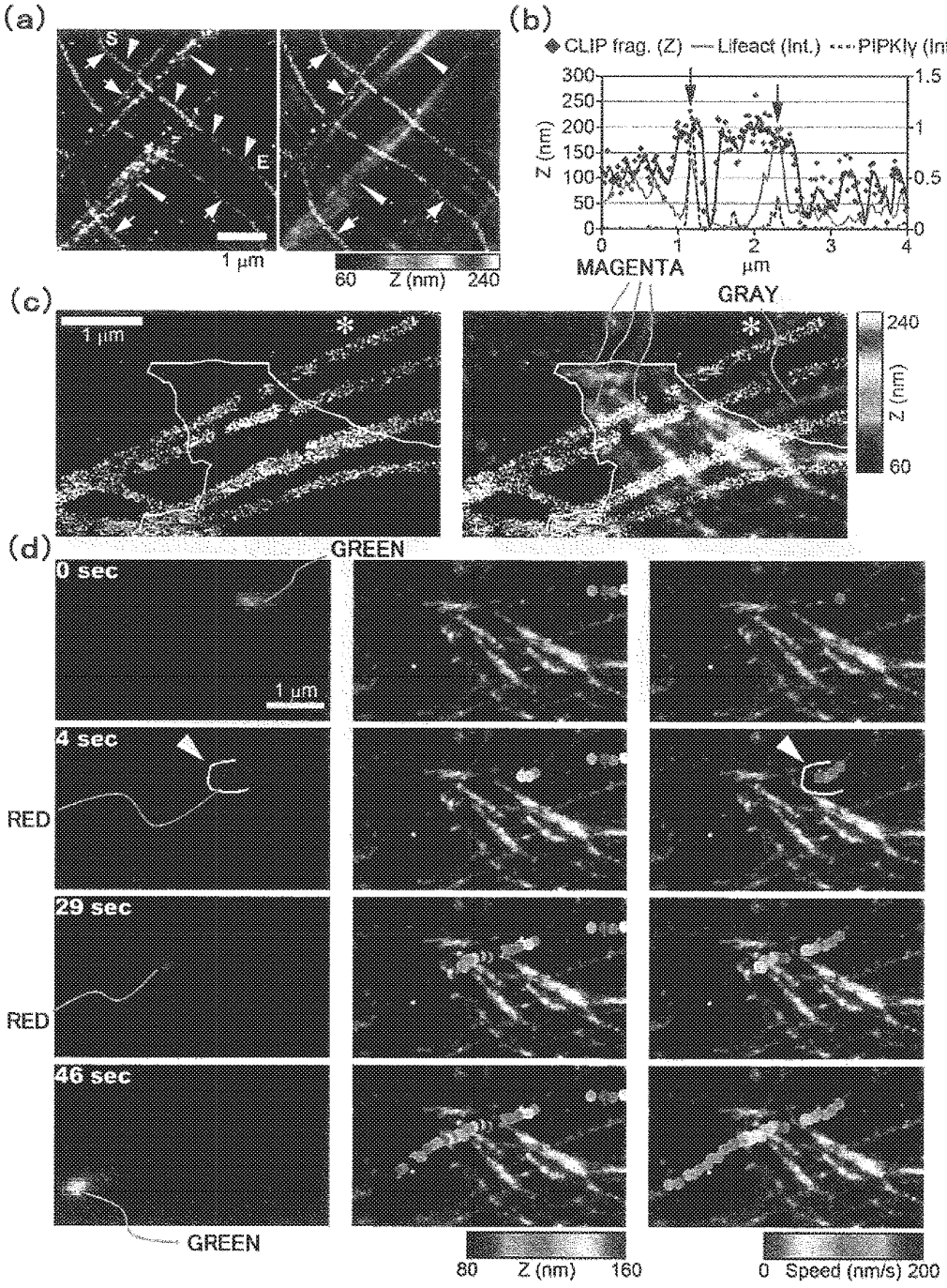

(a)
S
E
1 μm
60 Z (nm) 240
(b)
CLIP frag. (Z)
Lifeact (Int.)
PIPKIγ (Int.)
Z (nm)
Normalized intensity
MAGENTA
GRAY
μm
(c)
1 μm
240
Z (nm)
60
(d)
GREEN
0 sec
1 μm
4 sec
RED
29 sec
RED
46 sec
GREEN
80 Z (nm) 160
0 Speed (nm/s) 200

FIG. 8

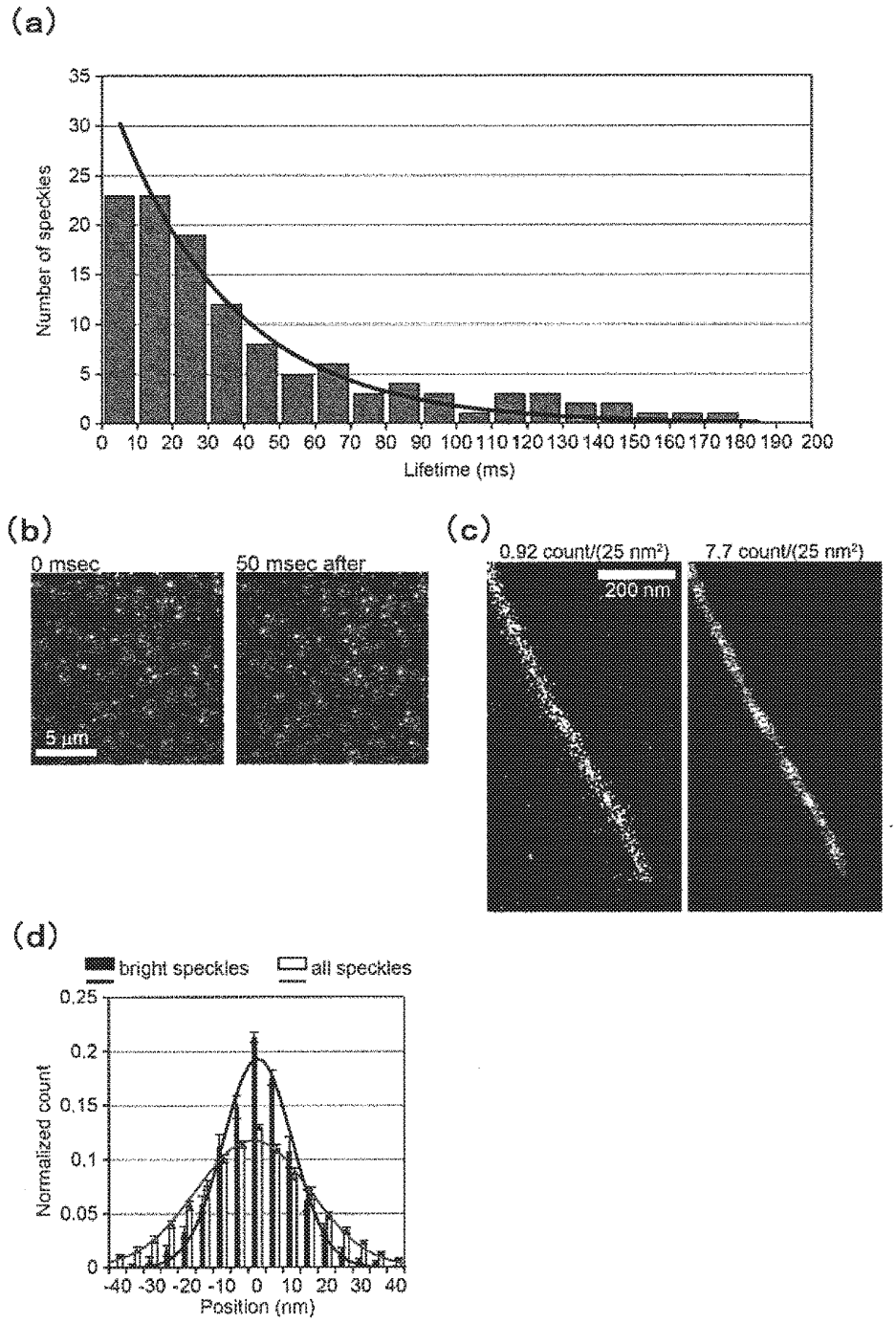

(a)
Number of speckles
Lifetime (ms)
(b)
0 msec
50 msec after
5 μm
(c)
0.92 count/(25 nm²)
7.7 count/(25 nm²)
200 nm
(d)
bright speckles
all speckles
Normalized count
Position (nm)

FIG. 9

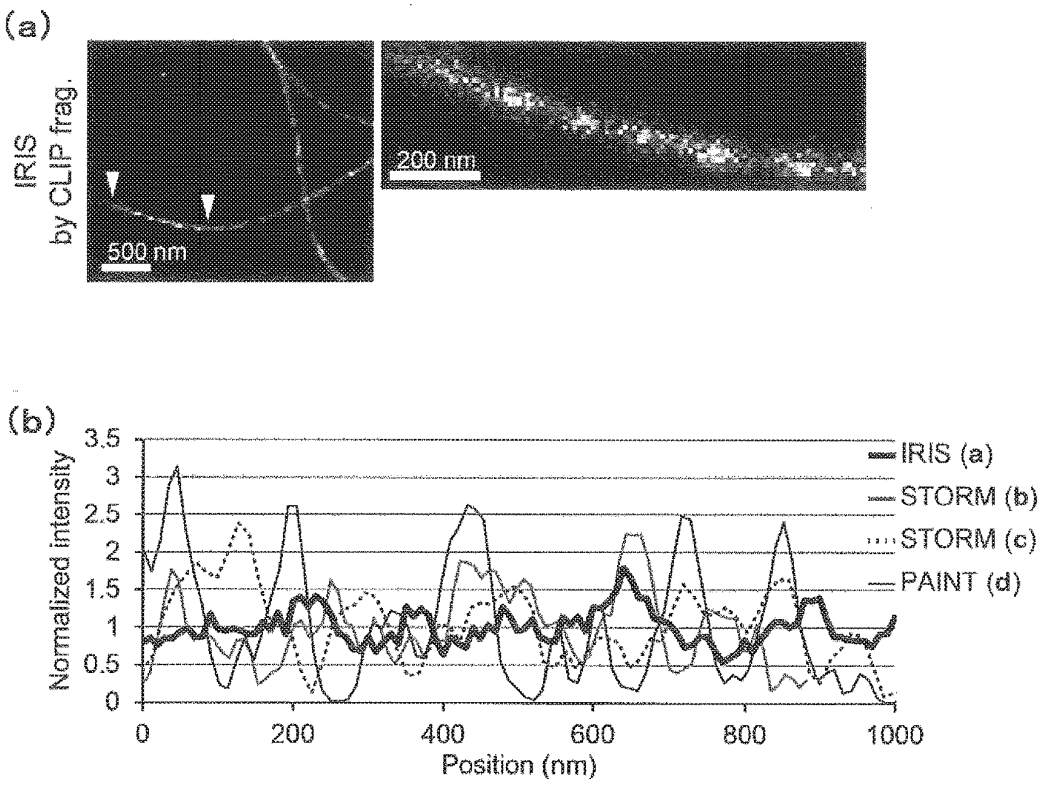
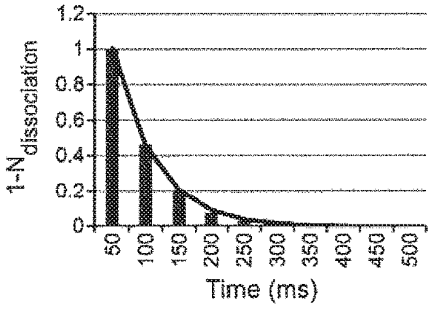
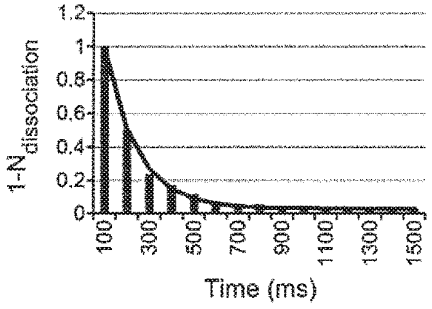

(a)
IRIS
by CLIP frag.
500 nm
200 nm
(b)
Normalized intensity
3.5
3
2.5
2
1.5
1
0.5
0
0
200
400
600
800
1000
Position (nm)
IRIS (a)
STORM (b)
STORM (c)
PAINT (d)

FIG. 10

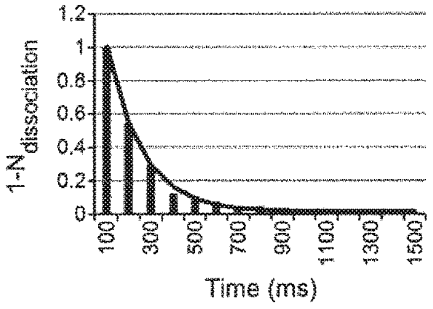
CLIP-170 frag. (3-309), half-life 44 ms



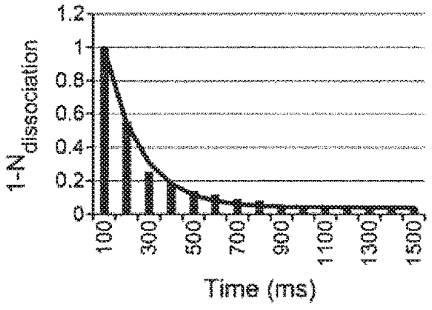

APC frag. (2536-2843), half-life 100 ms



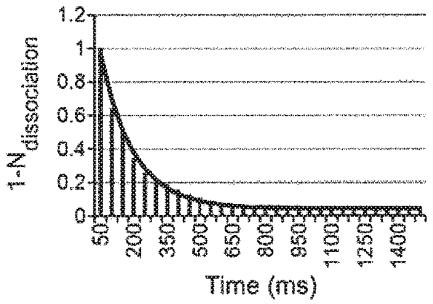

MAP4 frag. (1-908), half-life 109 ms



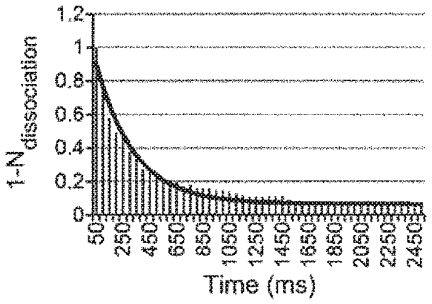

Tau isoform3, half-life 110 ms



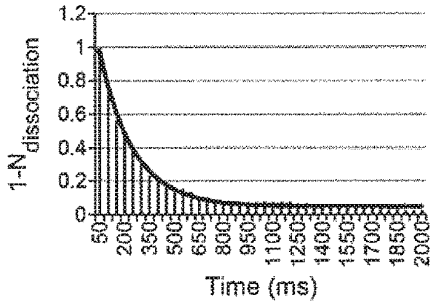

PLEC frag. (4022-4364), half-life 103 ms




Paxillin full length, half-life 196 ms




Src frag. (3-251), half-life 141 ms




PIPKIγ frag. (641-668), half-life 496 ms

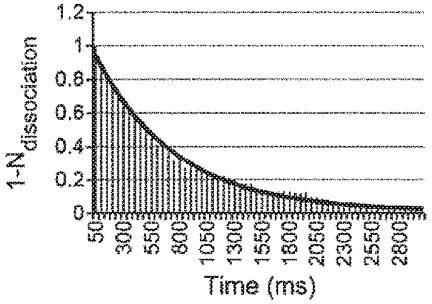



FIG. 11

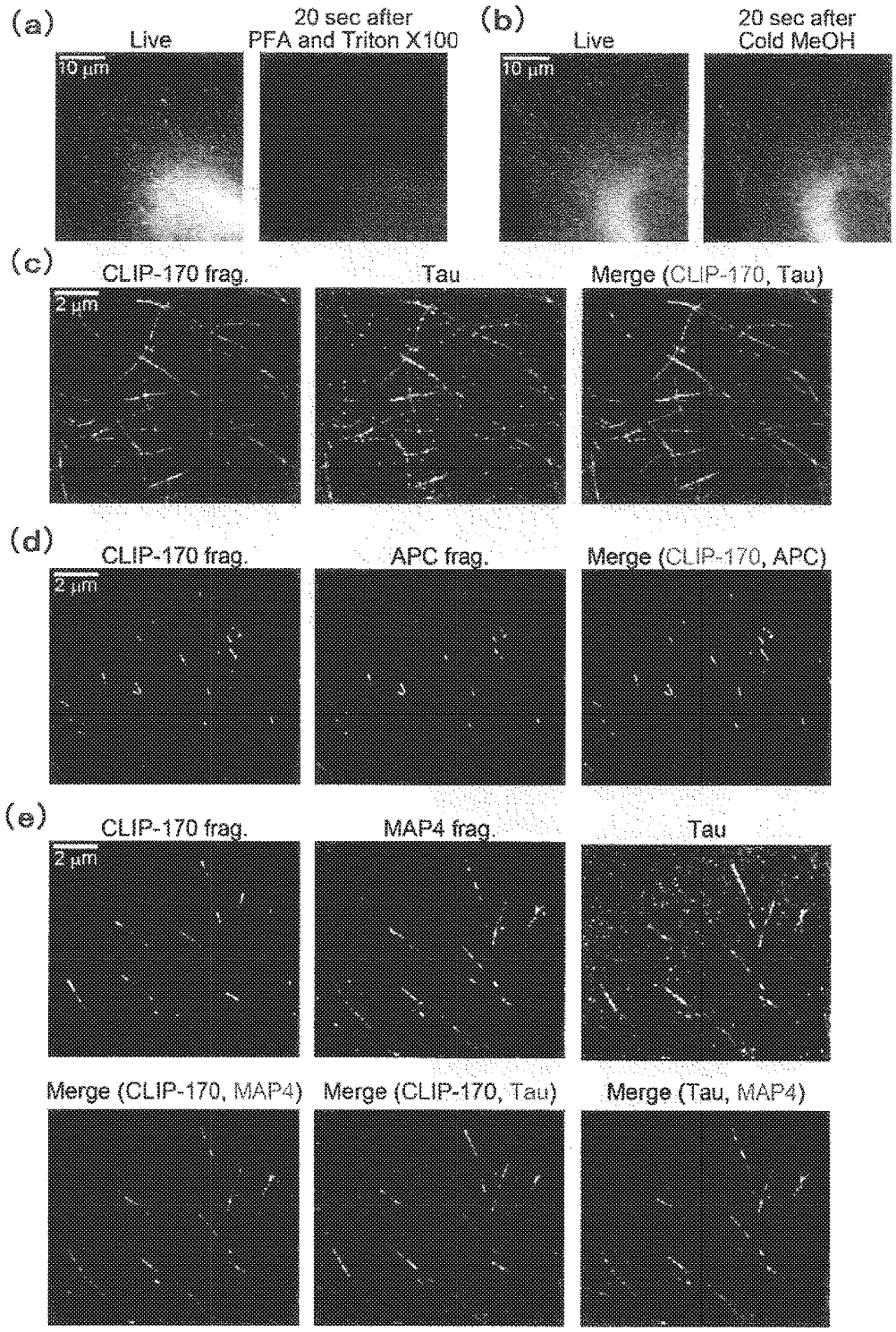

(a)
Live
20 sec after PFA and Triton X100
10 μm
(b)
Live
20 sec after Cold MeOH
10 μm
(c)
CLIP-170 frag.
Tau
Merge (CLIP-170, Tau)
2 μm
(d)
CLIP-170 frag.
APC frag.
Merge (CLIP-170, APC)
2 μm
(e)
CLIP-170 frag.
MAP4 frag.
Tau
2 μm
Merge (CLIP-170, MAP4)
Merge (CLIP-170, Tau)
Merge (Tau, MAP4)

FIG. 12

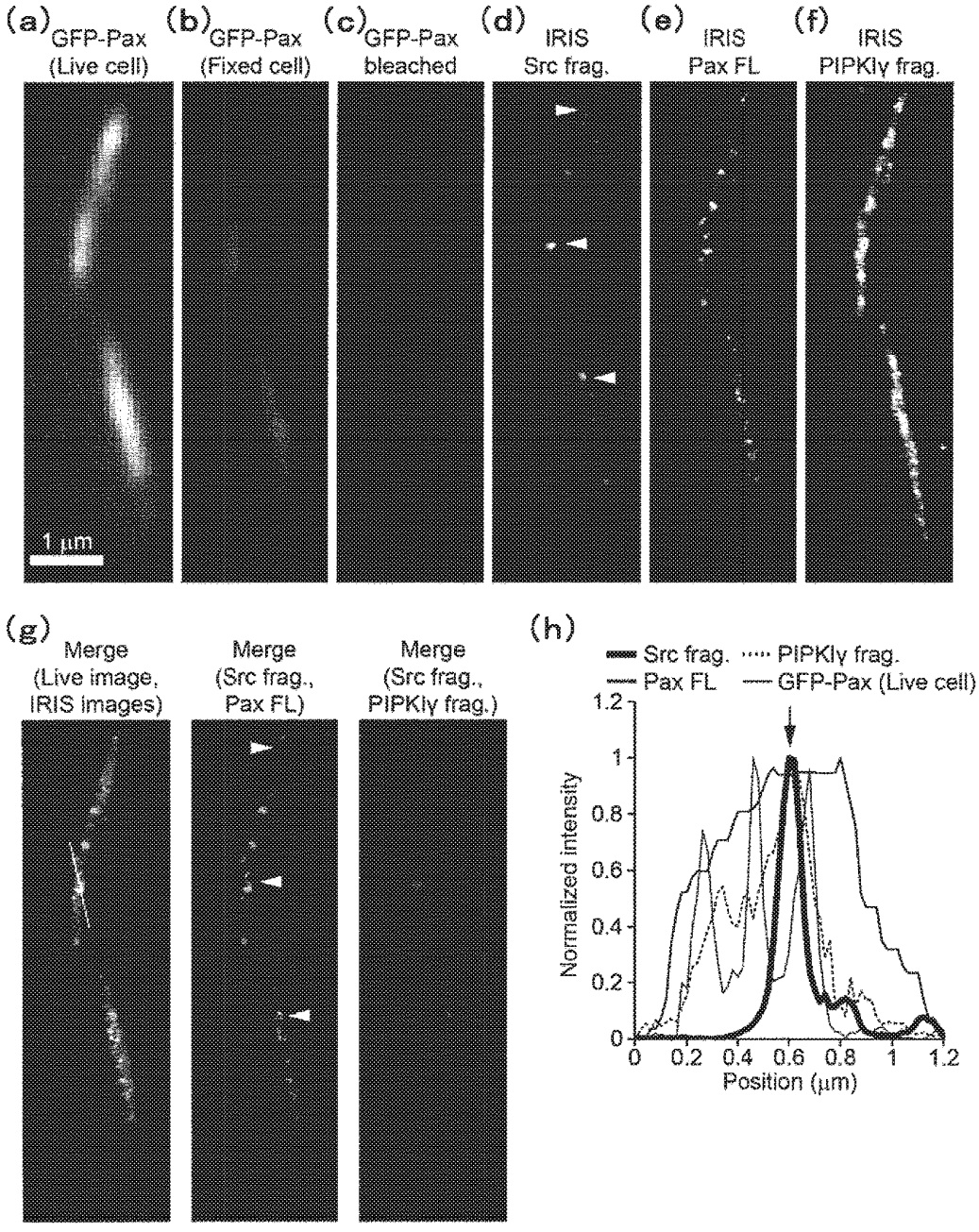
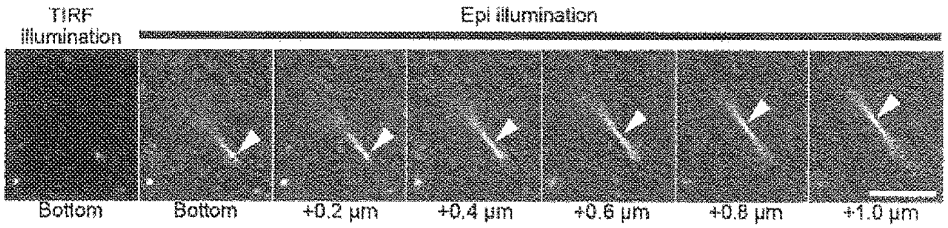

(a) GFP-Pax (Live cell)
(b) GFP-Pax (Fixed cell)
(c) GFP-Pax bleached
(d) IRIS Src frag.
(e) IRIS Pax FL
(f) IRIS PIPKIγ frag.
1 μm
(g) Merge (Live image, IRIS images)
Merge (Src frag., Pax FL)
Merge (Src frag., PIPKIγ frag.)
(h)
Src frag.
PIPKIγ frag.
Pax FL
GFP-Pax (Live cell)
Normalized intensity
Position (μm)
0
0.2
0.4
0.6
0.8
1
1.2

FIG. 13

(a)


TIRF illumination
Epi illumination
Bottom
Bottom
+0.2 μm
+0.4 μm
+0.6 μm
+0.8 μm
+1.0 μm

(b)

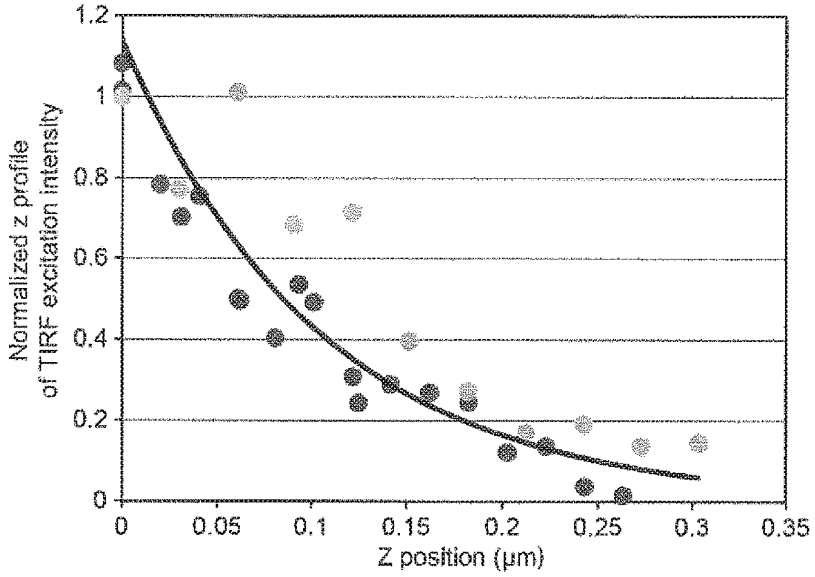

Normalized z profile of TIRF excitation intensity
1.2
1
0.8
0.6
0.4
0.2
0
0
0.05
0.1
0.15
0.2
0.25
0.3
0.35
Z position (μm)

FIG. 14

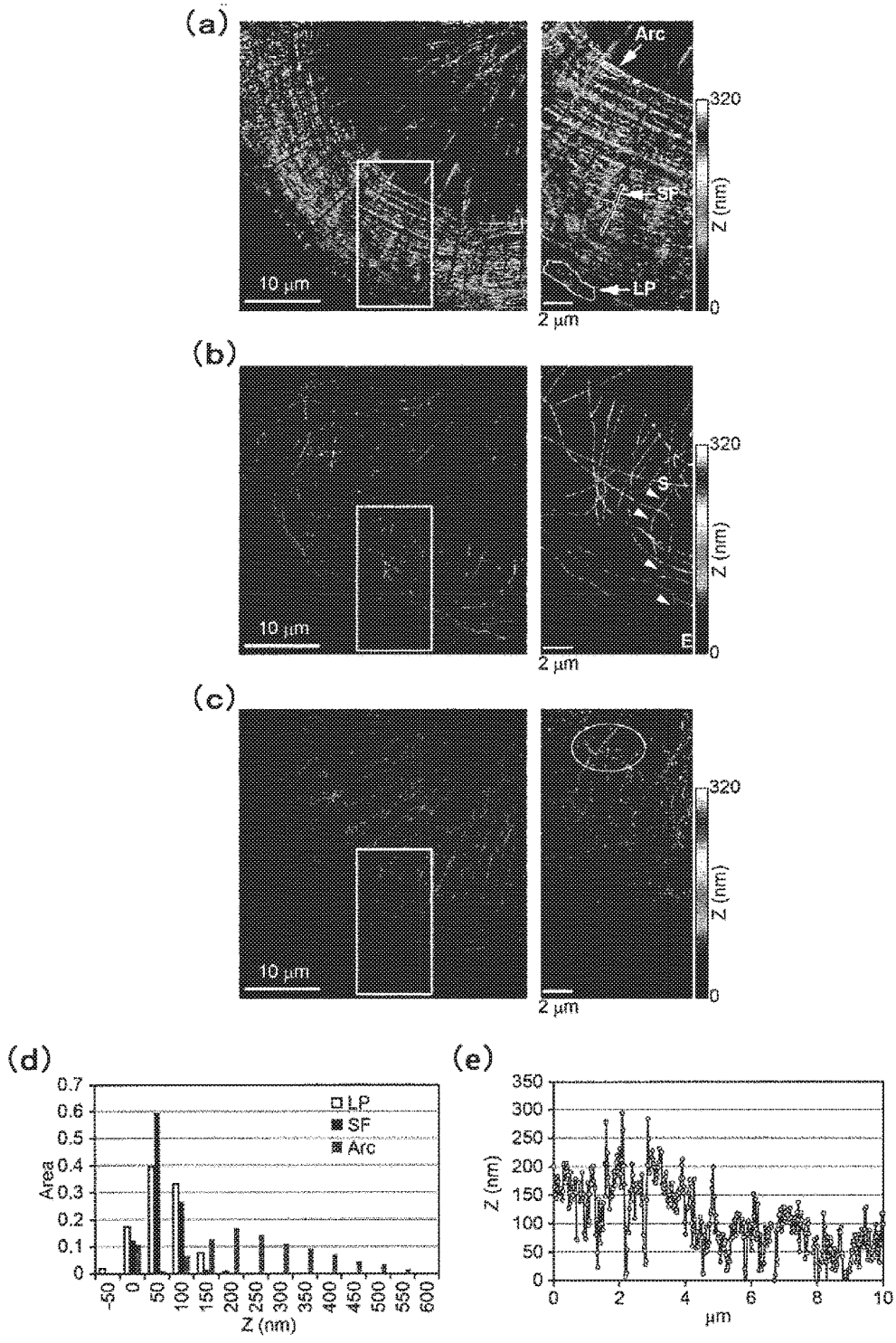

(a)
Arc
SP
LP
320
Z (nm)
0
10 μm
2 μm
(b)
S
320
Z (nm)
0
10 μm
2 μm
(c)
320
Z (nm)
0
10 μm
2 μm
(d)
LP
SF
Arc
Area
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
-50
0
50
100
150
200
250
300
350
400
450
500
550
600
Z (nm)
(e)
350
300
250
200
150
100
50
0
Z (nm)
0
2
4
6
8
10
μm

FIG. 15

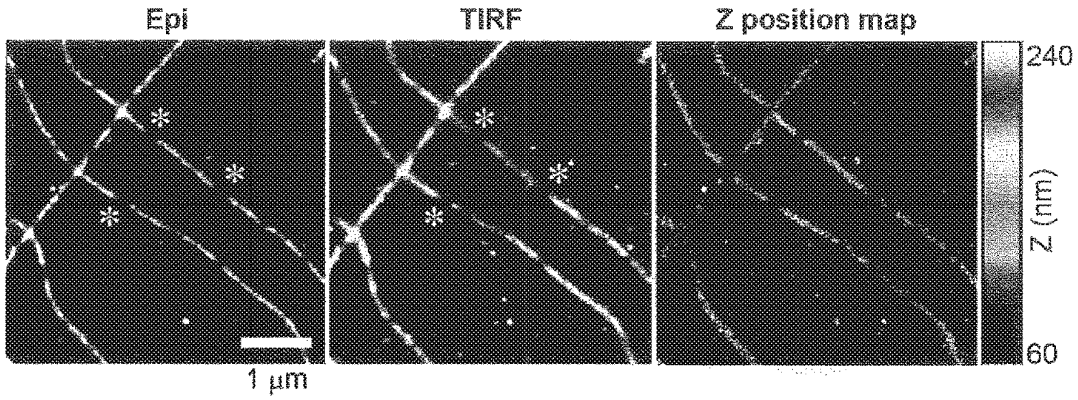



FIG. 16

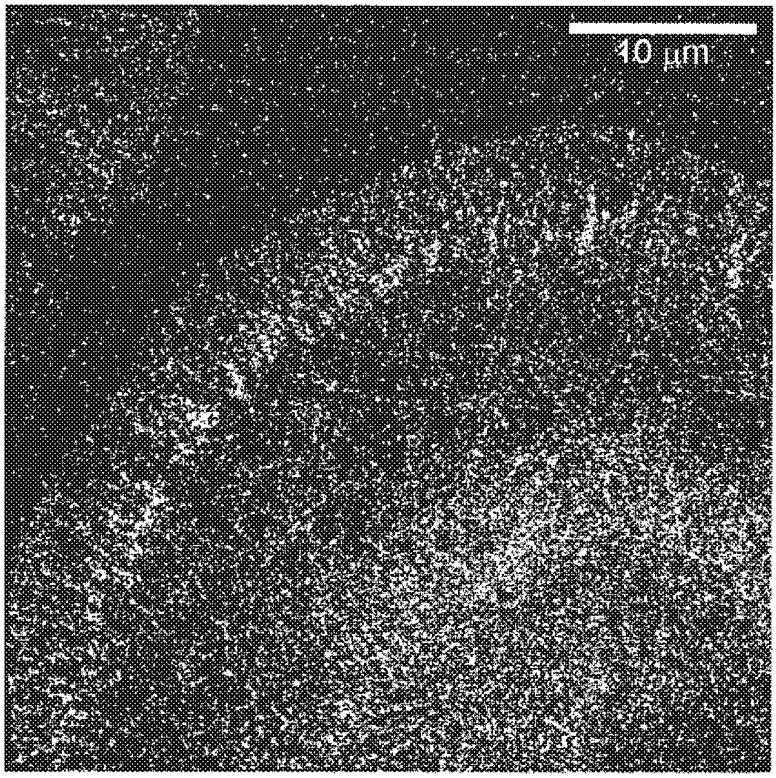



FIG. 17

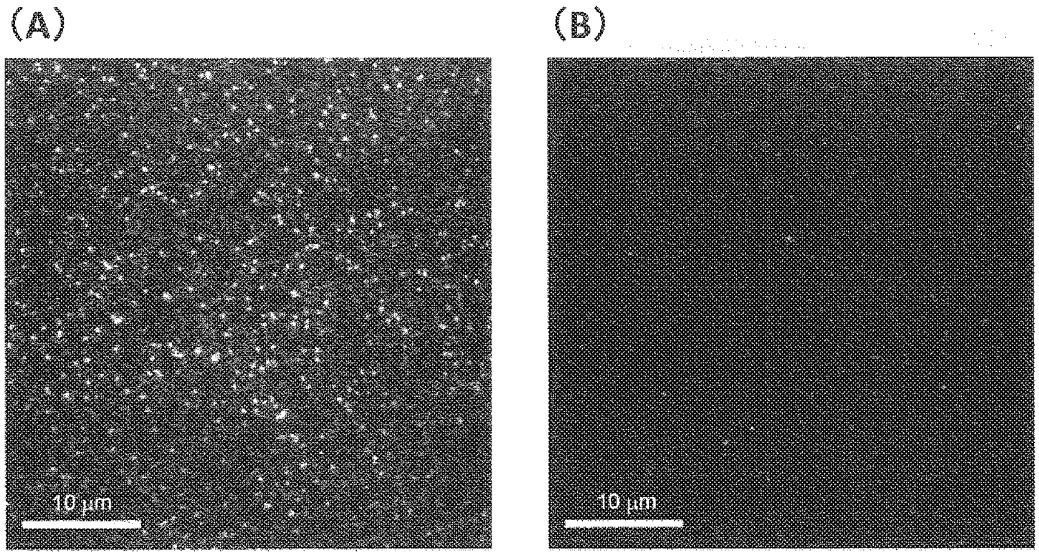



FIG. 18

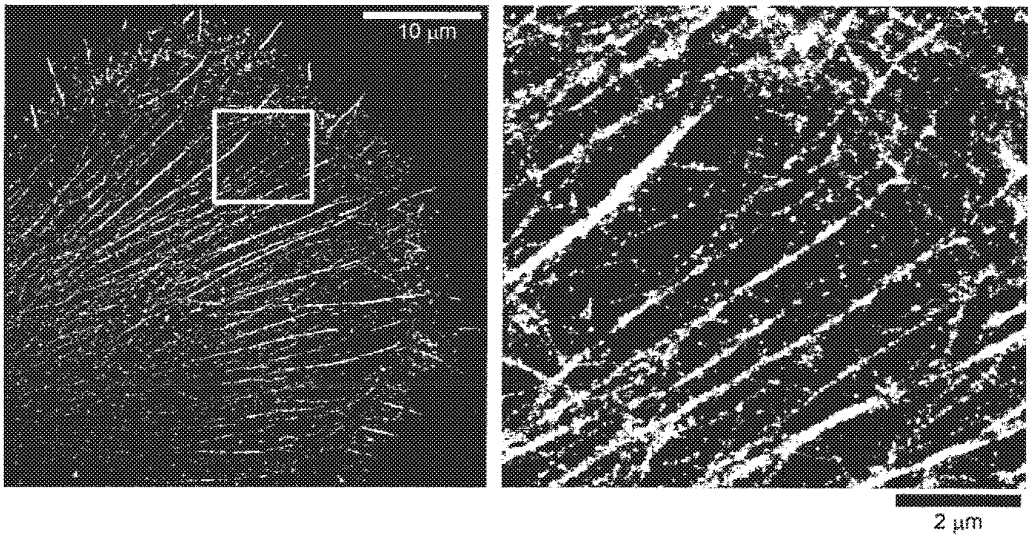



FIG. 19

# OBSERVATION METHOD USING BINDING AND DISSOCIATION PROBE

## CROSS REFERENCE TO RELATED APPLICATIONS

**[0001]** This application is based upon and claims the benefit of priority from prior Japanese Patent Application No. 2015-048692, filed Mar. 11, 2015, the entire contents of which are incorporated herein by this reference. This is a Continuation Application of PCT Application No. PCT/JP2016/057817, filed Mar. 11, 2016, which was not published under PCT Article 21 (2) in English.

## BACKGROUND OF THE INVENTION

### Field of the Invention

**[0002]** The present invention is related to an observation method using a binding and dissociation probe by which a super-resolution image of a sample can be obtained.

### Description of the Related Art

**[0003]** In recent years, super-resolution microscopy (STED, SIM, PALM/STORM; resolution capability:10 nm through 100 nm) with a resolution capability exceeding that of optical microscopes (>200 nm) has been developed (Nat. Rev. Mol. Cell Biol., 9, 929-943, 2008), and major microscope manufacturing companies (Nikon, Carl Zeiss, Leica) are selling their super-resolution microscope apparatuses. In the field of biological science particularly, important discoveries are expected from applications of PALM (photo-activated localization microscopy, Japanese National Publication of International Patent Application No. 2008-542826, Science, 313, 1642-1645, 2006)/STORM (stochastic optical reconstruction microscopy, Nature Methods, 3, 793-795 and United States Patent Application No. US2008/0032414). In each super-resolution microscopy, fluorescent dye is made to bind to a target so as to label the target, and the spatial distribution of the fluorescent dye is observed with a high resolution capability in Stimulated Emission Depletion (STED), structured illumination microscopy (SIM), and localization microscopy (PALM/STORM).

**[0004]** The outline of localization microscopy is as follows. First, a target substance to be observed is labeled with a luminescent substance such as a fluorescent substance. Then, the luminescent substance with which the target substance is labeled is made to emit light at a low density so that speckle images are obtained in which the speckles of emissions of light are separated individually, i.e., in which luminescent substances of single molecules are separated. The central positions of the individual speckles can be obtained in the respective speckle images that have been obtained. The above step of obtaining speckle images including separated speckles so as to obtain information of the positions of speckles is repeated a plurality of times, e.g., hundreds of times through hundreds of thousands of times, and the central positions of roughly all luminescent substances on the target substance are obtained, and thereby an observation image of the target substance is constructed. Explanations will be schematically given for the localization microscopy by referring to FIG. **1**. For example, it is assumed as shown in FIG. **1** (*a*) that five molecules **100** of target substances to be observed are distributed. The five molecules **100** of the target substance are respectively labeled with luminescent substances and are made to emit light separately in such a manner that the speckles of light emission do not overlap each other. For example, as shown in FIG. **1** (*b*), three speckle images **110**, **111** and **112** having separate speckles **101** are obtained at different times t**1**, t**2** and t**3**. A gauss function is fit to the image of each speckle **101** in each of the speckle images **110**, **111** and **112** so as to obtain the central positions of the speckles, and position information **102** which is information of the central positions is recorded (FIG. **1** (*c*)). Pieces of position information **102** of individual recorded speckles are synthesized so as to draw an observation image **120** as shown in FIG. **1** (*d*). The above method can be implemented by using for example DAOSTORM, a computer program (Nature methods, 8 279-280, 2011). In localization microscopy, as schematically shown in FIG. (**1***b*), it is necessary that images of the molecules **100**, which are labeled target substances, be picked up over time while the molecules **100** are sequentially made to emit light at a relatively low density so as to obtain a plurality of speckle images so that the speckles of luminescent substances of single molecules can be separated.

**[0005]** PALM/STORM use a fluorescent substance that can perform activation or switching through irradiation with light for making a fluorescent substance emit light at a low density so as to adjust a light irradiation condition in order to stochastically perform activation or switching on the state of the fluorescent substance, and thereby obtain speckle images in which speckles are separated individually.

**[0006]** Proceedings of the national Academy of Sciences of the United States of America 103, 18911-18916 (2006) reports a method referred to as PAINT (point accumulation for imaging in nanoscale topography). The document discloses that super-resolution observation of a form of a lipid bilayer was conducted through PAINT by using the fluorescent dye Nile-red that swiftly goes back and forth between an aqueous solution and the lipid bilayer.

**[0007]** Recently, as a multicolor super-resolution microscopy to which PAINT above is applied, a method has been reported in which a target substance is labeled with an antibody that was made to fuse with a DNA oligomer and a fluorescent DNA oligomer which is a complementary base sequence that temporarily binds to and dissociates from the DNA oligomer is used (Exchange-PAINT, Nature methods, 11, 313-318, 2014).

## SUMMARY OF THE INVENTION

**[0008]** The present inventors have found that a luminescent probe can be used for labeling different positions on a target substance in respective speckle images so that the labeling density with respect to the target substance can be substantially increased by increasing the number of the speckle images that are picked up and a high resolution observation image exceeding a diffraction limit can be generated by the localization microscopy from respective speckle images when a plurality of images of light emission speckles (speckle images) are picked up at different times and while keeping a contact state between the luminescent probe and a sample, by using the luminescent probe, as a binding and dissociation luminescent probe, that is a luminescent probe which repeatedly binds to and dissociates from a target substance directly and specifically and for which the half-life of a probe-target complex formed by binding between the luminescent probe and the target sub-

stance is in a prescribed scope, and the present inventors have completed the present invention. The present invention incorporates the following inventions.

(1) An observation method of a sample containing a target substance, the observation method comprising:

**[0009]** an imaging step in which a step of obtaining a speckle image including, as a speckle, light emitted from a luminescent substance under a prescribed condition in a state in which a medium is brought into contact with the sample is performed a plurality of times at different times respectively so as to obtain a plurality of speckle images, the medium containing a probe that contains the luminescent substance emitting light under the prescribed condition and that repeatedly binds to and dissociates from the target substance directly and specifically; and

**[0010]** an observation image generation step of generating an observation image of the target substance in the sample from the plurality of speckle images, wherein

**[0011]** a half-life of a probe-target complex formed by binding between the probe and the target substance is equal to or more than 10 milliseconds and equal to or less than 3 seconds.

(2) The method according to (1), wherein

**[0012]** the observation image generation step is a step in which information of a position of a speckle included in each of the plurality of speckle images is obtained for each of the plurality of speckle images and the observation image is generated on the basis of the information from the plurality of speckle images.

(3) The method according to (1), wherein

**[0013]** the sample includes two or more target substances, the imaging step is sequentially performed on the sample by using the probe that is specific to each of the target substances, and

**[0014]** the observation image generation step is a step in which observation images of the respective target substances in the sample are respectively generated from the plurality of speckle images obtained from the respective imaging steps.

(4) The method according to (3), further comprising

**[0015]** a multiple-observation image generation step in which observation images of the respective target substances in the sample generated in the observation image generation step are superposed so as to generate a multiple-observation image, which is an observation image of the two or more target substances in the sample.

(5) The method according to (1), wherein

**[0016]** the luminescent substance is a fluorescent substance, and the prescribed condition is irradiation with excitation light.

(6) The method according to (1), wherein

**[0017]** a combination between the probe and the target substance is selected from a group of:

**[0018]** a combination wherein the probe is (a1) a polypeptide, linked to the luminescent substance, which consists of an amino acid sequence of sequence number 19, (a2) a polypeptide, linked to the luminescent substance, which consists of the amino acid sequence described in (a1) where one or a plurality of amino acids have been substituted, deleted, inserted or added and for which a half-life of a probe-target complex formed by binding between the probe and the target substance is equal to or more than 10 milliseconds and equal to or less than 3 seconds, or (a3) a polypeptide, linked to the luminescent substance, which consists of an amino acid sequence having at least a 70% identity with the amino acid sequence described in (a1) and for which a half-life of a probe-target complex formed by binding between the probe and the target substance is equal to or more than 10 milliseconds and equal to or less than 3 seconds, and the target substance is an actin polymer;

**[0019]** a combination wherein the probe is (b1) a polypeptide, linked to the luminescent substance, which consists of an amino acid sequence that is a partial amino acid sequence of an amino acid sequence of sequence number 12, that at least partially contains an amino acid sequence of 3-309 and that has 407 or fewer amino acids, an amino acid sequence that is a partial amino acid sequence of an amino acid sequence of sequence number 14, that at least partially contains an amino acid sequence of 2536-2843 and that has 408 or fewer amino acids, an amino acid sequence that is a partial amino acid sequence of an amino acid sequence of sequence number 14, that at least partially contains an amino acid sequence of 2781-2819 and that has 138 or fewer amino acids, an amino acid sequence that is a partial amino acid sequence of an amino acid sequence of sequence number 4, that at least partially contains an amino acid sequence of 1-908 and that has 1008 or fewer amino acids, an amino acid sequence that is a partial amino acid sequence of an amino acid sequence of sequence number 4, that at least partially contains an amino acid sequence of 659-908 and that has 394 or fewer amino acids, an amino acid sequence of sequence number 5, or an amino acid sequence of sequence number 6, (b2) a polypeptide, linked to the luminescent substance, which consists of the amino acid sequence described in (b1) where one or a plurality of amino acids have been substituted, deleted, inserted or added and for which a half-life of a probe-target complex formed by binding between the probe and the target substance is equal to or more than 10 milliseconds and equal to or less than 3 seconds, or (b3) a polypeptide, linked to the luminescent substance, which consists of an amino acid sequence having at least a 70% identity with the amino acid sequence described in (b1) and for which a half-life of a probe-target complex formed by binding between the probe and the target substance is equal to or more than 10 milliseconds and equal to or less than 3 seconds, and the target substance is a microtubule;

**[0020]** a combination wherein the probe is (c1) a polypeptide, linked to the luminescent substance, which consists of an amino acid sequence that is a partial amino acid sequence of an amino acid sequence of sequence number 8, that at least partially contains an amino acid sequence of 3777-4684 and that has 1008 or fewer amino acids, an amino acid sequence that is a partial amino acid sequence of an amino acid sequence of sequence number 8, that at least partially contains an amino acid sequence of

3777-4364 and that has 688 or fewer amino acids, an amino acid sequence that is a partial amino acid sequence of an amino acid sequence of sequence number 8, that at least partially contains an amino acid sequence of 3777-4313 and that has 637 or fewer amino acids, or an amino acid sequence that is a partial amino acid sequence of an amino acid sequence of sequence number 8, that at least partially contains an amino acid sequence of 4022-4364 and that has 443 or fewer amino acids, (c2) a polypeptide, linked to the luminescent substance, which consists of the amino acid sequence described in (c1) where one or a plurality of amino acids have been substituted, deleted, inserted or added and for which a half-life of a probe-target complex formed by binding between the probe and the target substance is equal to or more than 10 milliseconds and equal to or less than 3 seconds, or (c3) a polypeptide, linked to the luminescent substance, which consists of an amino acid sequence having at least a 70% identity with the amino acid sequence described in (c1) and for which a half-life of a probe-target complex formed by binding between the probe and the target substance is equal to or more than 10 milliseconds and equal to or less than 3 seconds, and the target substance is an intermediate filament; and

**[0021]** a combination wherein the probe is (d1) a polypeptide, linked to the luminescent substance, which consists of an amino acid sequence of sequence number 15, an amino acid sequence that is a partial amino acid sequence of an amino acid sequence of sequence number 15, that at least partially contains an amino acid sequence of 54-557 and that has 556 or fewer amino acids, an amino acid sequence that is a partial amino acid sequence of an amino acid sequence of sequence number 15, that at least partially contains an amino acid sequence of 54-498 and that has 545 or fewer amino acids, an amino acid sequence that is a partial amino acid sequence of an amino acid sequence of sequence number 15, that at least partially contains an amino acid sequence of 167-557 and that has 491 or fewer amino acids, an amino acid sequence that is a partial amino acid sequence of an amino acid sequence of sequence number 16, that at least partially contains an amino acid sequence of 1-251 and that has 351 or fewer amino acids, an amino acid sequence that is a partial amino acid sequence of an amino acid sequence of sequence number 16, that at least partially contains an amino acid sequence of 3-251 and that has 349 or fewer amino acids or an amino acid sequence of sequence number 18, (d2) a polypeptide, linked to the luminescent substance, which consists of the amino acid sequence described in (d1) where one or a plurality of amino acids have been substituted, deleted, inserted or added and for which a half-life of a probe-target complex formed by binding between the probe and the target substance is equal to or more than 10 milliseconds and equal to or less than 3 seconds, or (d3) a polypeptide, linked to the luminescent substance, which consists of an amino acid sequence having at least a 70% identity with the amino acid sequence described in (d1) and for which a half-life of a probe-target complex formed by binding between the probe and the target substance is equal to or more than 10 milliseconds and equal to or less than 3 seconds, and the target substance is a focal adhesion.

(7) The method according to (1), wherein

**[0022]** the probe contains an antibody or a fragment of an antibody, the antibody or the fragment being to the target substance and the antibody or the fragment being linked to the luminescent substance.

(8) The method according to (7), wherein the fragment of the antibody is a Fab fragment.

(9) A probe used for labeling a target substance, wherein

**[0023]** the probe contains a luminescent substance that emits light under a prescribed condition,

**[0024]** the probe can repeatedly bind to and dissociate from the target substance directly and specifically, and

**[0025]** a half-life of a probe-target complex formed by binding to the target substance is equal to or more than 10 milliseconds and equal to or less than 3 seconds.

(10) The probe according to (9), wherein

**[0026]** the target substance is an actin polymer and the probe is (a1) a polypeptide, linked to the luminescent substance, which consists of an amino acid sequence of sequence number 19, (a2) a polypeptide, linked to the luminescent substance, which consists of the amino acid sequence described in (a1) where one or a plurality of amino acids have been substituted, deleted, inserted or added and for which a half-life of a probe-target complex formed by binding between the probe and the target substance is equal to or more than 10 milliseconds and equal to or less than 3 seconds, or (a3) a polypeptide, linked to the luminescent substance, which consists of an amino acid sequence having at least a 70% identity with the amino acid sequence described in (a1) and for which a half-life of a probe-target complex formed by binding between the probe and the target substance is equal to or more than 10 milliseconds and equal to or less than 3 seconds,

**[0027]** the target substance is a microtubule and the probe is (b1) a polypeptide, linked to the luminescent substance, which consists of an amino acid sequence that is a partial amino acid sequence of an amino acid sequence of sequence number 12, that at least partially contains an amino acid sequence of 3-309 and that has 407 or fewer amino acids, an amino acid sequence that is a partial amino acid sequence of an amino acid sequence of sequence number 14, that at least partially contains an amino acid sequence of 2536-2843 and that has 408 or fewer amino acids, an amino acid sequence that is a partial amino acid sequence of an amino acid sequence of sequence number 14, that at least partially contains an amino acid sequence of 2781-2819 and that has 138 or fewer amino acids, an amino acid sequence that is a partial amino acid sequence of an amino acid sequence of sequence number 4, that at least partially contains an amino acid sequence of 1-908 and that has 1008 or fewer amino acids, an amino acid sequence that is a partial amino acid sequence of an amino acid sequence of sequence number 4, that at least partially contains an amino acid sequence of 659-908 and that has 394 or fewer amino acids, an amino acid sequence of sequence number 5 or an amino acid sequence of sequence number 6, (b2) a polypeptide, linked to the luminescent substance, which consists of the amino

acid sequence described in (b1) where one or a plurality of amino acids have been substituted, deleted, inserted or added and for which a half-life of a probe-target complex formed by binding between the probe and the target substance is equal to or more than 10 milliseconds and equal to or less than 3 seconds, or (b3) a polypeptide, linked to the luminescent substance, which consists of an amino acid sequence having at least a 70% identity with the amino acid sequence described in (b1) and for which a half-life of a probe-target complex formed by binding between the probe and the target substance is equal to or more than 10 milliseconds and equal to or less than 3 seconds,

**[0028]** the target substance is an intermediate filament and the probe is (c1) a polypeptide, linked to the luminescent substance, which consists of an amino acid sequence that is a partial amino acid sequence of an amino acid sequence of sequence number 8, that at least partially contains an amino acid sequence of 3777-4684 and that has 1008 or fewer amino acids, an amino acid sequence that is a partial amino acid sequence of an amino acid sequence of sequence number 8, that at least partially contains an amino acid sequence of 3777-4364 and that has 688 or fewer amino acids, an amino acid sequence that is a partial amino acid sequence of an amino acid sequence of sequence number 8, that at least partially contains an amino acid sequence of 3777-4313 and that has 637 or fewer amino acids or an amino acid sequence that is a partial amino acid sequence of an amino acid sequence of sequence number 8, that at least partially contains an amino acid sequence of 4022-4364 and that has 443 or fewer amino acids, (c2) a polypeptide, linked to the luminescent substance, which consists of the amino acid sequence described in (c1) where one or a plurality of amino acids have been substituted, deleted, inserted or added and for which a half-life of a probe-target complex formed by binding between the probe and the target substance is equal to or more than 10 milliseconds and equal to or less than 3 seconds, or (c3) a polypeptide, linked to the luminescent substance, which consists of an amino acid sequence having at least a 70% identity with the amino acid sequence described in (c1) and for which a half-life of a probe-target complex formed by binding between the probe and the target substance is equal to or more than 10 milliseconds and equal to or less than 3 seconds, or

**[0029]** the target substance is a focal adhesion and the probe is (d1) a polypeptide, linked to the luminescent substance, which consists of an amino acid sequence of sequence number 15, an amino acid sequence that is a partial amino acid sequence of an amino acid sequence of sequence number 15, that at least partially contains an amino acid sequence of 54-557 and that has 556 or fewer amino acids, an amino acid sequence that is a partial amino acid sequence of an amino acid sequence of sequence number 15, that at least partially contains an amino acid sequence of 54-498 and that has 545 or fewer amino acids, an amino acid sequence that is a partial amino acid sequence of an amino acid sequence of sequence number 15, that at least partially contains an amino acid sequence of 167-557 and that has 491 or fewer amino acids, an amino acid sequence that is a partial amino acid sequence of an amino acid sequence of sequence number 16, that at least partially contains an amino acid sequence of 1-251 and that has 351 or fewer amino acids, an amino acid sequence that is a partial amino acid sequence of an amino acid sequence of sequence number 16, that at least partially contains an amino acid sequence of 3-251 and that has 349 or fewer amino acids, or an amino acid sequence of sequence number 18, (d2) a polypeptide, linked to the luminescent substance, which consists of the amino acid sequence described in (d1) where one or a plurality of amino acids have been substituted, deleted, inserted or added and for which a half-life of a probe-target complex formed by binding between the probe and the target substance is equal to or more than 10 milliseconds and equal to or less than 3 seconds or (d3) a polypeptide, linked to the luminescent substance, which consists of an amino acid sequence having at least a 70% identity with the amino acid sequence described in (d1) and for which a half-life of a probe-target complex formed by binding between the probe and the target substance is equal to or more than 10 milliseconds and equal to or less than 3 seconds.

(11) The probe according to (9), wherein

**[0030]** the probe contains an antibody or a fragment of an antibody, the antibody or the fragment being to the target substance and the antibody or the fragment being linked to the luminescent substance.

(12) The probe according to (11), wherein

**[0031]** the fragment of the antibody is a Fab fragment.

(13) A reagent kit for labeling a target substance, wherein

**[0032]** the reagent kit at least includes the probe according to (9).

(14) A screening method of a site in which identifies a target substance in the probe according to (9), the screening method comprising:

**[0033]** an immobilization step in which a candidate substance of the site or a substance partially containing the candidate substance is fixed to a solid support;

**[0034]** an observation step in which a target substance linked to a luminescent substance and a solid support obtained in the immobilization step are observed in a medium while the target substance linked to a luminescent substance and the solid support obtained in the immobilization step are kept in contact, in a condition that allows observation, in units of 1 molecule, of light emission from the luminescent substance in a probe-target complex formed by binding between the target substance and the candidate substance, and

**[0035]** a screening step in which the candidate substance resulting in a half-life of the probe-target complex that is equal to or more than 10 milliseconds and equal to or less than 3 seconds is selected as the site on the basis of observation in the observation step.

(15) The method according to (14) wherein

**[0036]** the candidate substance is an antibody or a fragment of an antibody from a library of hybridoma that produces an antibody to the target substance, and

**[0037]** the antibody is fixed to a solid support in the immobilization step.

**[0038]** The present document incorporates the contents of the disclosure of Japanese patent application No. 2015-048692, on the basis of which the priority is claimed for the present application.

**[0039]** According to the present invention, operations and effects that are remarkably more advantageous than those of existing super-resolution microscopy can be provided because (i) the problem of a labeling density, which has been the cause of reduced reliability of existing super-resolution microscopy, can be resolved and (ii) it is easy to visualize a plurality of target substances by protein-based exchangeable probes and there are no limitations on the number of target substances.

## BRIEF DESCRIPTION OF THE DRAWINGS

**[0040]** The present invention will be more apparent from the following detailed description when the accompanying drawings are referenced.

**[0041]** FIG. **1** explains the outline of the localization microscopy. FIG. **1** (*a*) shows the distribution of the actual probe molecules. FIG. **1** (*b*) shows speckle images containing fluorescent speckles picked up at different times. FIG. **1** (*c*) shows position information of speckles in the respective speckle images. FIG. **1** (*d*) shows an observation image reconstructed on the basis of the position information.

**[0042]** FIG. **2** (*a*) is a schematic view for explaining the principle by which the labeling density can be increased by using the method of the present invention. FIG. **2** (*b*) is a schematic view for explaining the principle of STORM.

**[0043]** FIG. **3** is a schematic view for explaining procedures for observing a plurality of target substances in one sample by using the method of the present invention.

**[0044]** FIG. **4** is a functional block diagram of a microscope apparatus used for implementing the method of the present invention.

**[0045]** FIG. **5** are related to super-resolution microscopy according to the present invention. Super-resolution microscopy according to the present invention will be referred to as IRIS (image reconstruction by integrating exchangeable single-molecule localization). FIG. **5** (*a*) shows the outline of IRIS. Transient associations of single-molecule fluorescent probes with their targets are visualized, and central positions of the probes are identified with nanometer accuracy. Integrating the position information from many frames produces a super-resolution image of the target substance. FIG. **5** (*b*) shows a super-resolution image of a single actin filament in vitro by TIRF (total internal reflection fluorescence) microscopy using Atto 488-Lifeact. An observation image was reconstructed by using high-brightness speckles in order to improve the localization accuracy (see FIG. **9***c*). FIG. **5** (*c*) shows the cross-sectional profile of single actin filaments (n=10 filaments, gray bars). The black curve shows the Gaussian fit to the mean profile, with a FWHM of 23 nm. Error bars show S.E.M. FIG. **5** (*d*) shows dependency of image quality on the labeling density in vitro. The labeling density of Atto 488-Lifeact per unit length is indicated at the top of each view. FIG. **5** (*e*) shows line profiles of the labeling density of Atto 488-Lifeact along the frame in FIG. **5** (*d*). The labeling density is shown after being normalized by the mean labeling density of an entire actin filament. FIG. **5** (*f*) shows an IRIS image of actin filaments in a cell using Atto 488-Lifeact and total internal reflection illumination. The image was reconstructed from $5\times10^5$ frames. FIG. **5** (*g*) shows a comparison between the IRIS super-resolution image (upper left) and the added SiMS image (lower left) of the region in the frame in FIG. **5** (*f*). The right graph shows cross-sectional profiles of two adjacent filaments between the two arrowheads in the upper left image. Atto 488-Lifeact was excited by the simultaneous irradiation with 473-nm and 488-nm laser beams in the TIRF mode to obtain a strong signal from each speckle. The stage drift of the microscope was corrected with a bright-field image of nonfluorescent beads in observation of an actin filament in vitro and was corrected with a bright-field image of the cell itself in observation of an actin filament in the cell (see "Methods" in the examples).

**[0046]** FIG. **6** (*a*) shows super-resolution images of actin filaments using Lifeact. FIG. **6** (*b*) shows super-resolution images of microtubules using a CLIP-170 fragment (amino acid residues 3-309 of sequence number 12). FIG. **6** (*c*) shows super-resolution images of intermediate filaments using a Plecin-1 (PLEC) fragment (amino acid residue 4022-4364 of sequence number 8). FIG. **6** (*d*) shows a super-resolution image of a focal adhesion using a phosphatidylinositol-(4)-phosphate 5-kinase type Iγ-90 (PIPKIγ) fragment (641-668) (sequence number 18). Except for a case when a PIPKIγ fragment was used as a probe, SiMS images (speckle images) of individual probes were obtained by alternately conducting total internal reflection illumination and epi-illumination. Specifically, imaging (exposure time was 50 ms/frame, 20 Hz) for 250 consecutive frames of speckle images based on the total internal reflection fluorescence in accordance with the condition explained in "procedures for imaging of multicolor super-resolution by IRIS" in "methods" of examples and imaging (exposure time was 50 ms/frame, 20 Hz) for 250 consecutive frames of speckle images based on the epi-fluorescence were repeated for observation of actin filament, microtubule and intermediate filament. For imaging of focal adhesions, speckle images based on epi-illumination were not obtained and imaging (exposure time was 50 ms/frame, 20 Hz) of 500 consecutive frames based on total internal reflection illumination was repeated. Then, the super-resolution images in the cell bottom (TIRF=total internal reflection fluorescence) and the entire cell peripheral regions (Epi=epi-fluorescence) were merged so as to reconstruct a super-resolution image. In FIG. **6** (*a*) through FIG. **6** (*c*), images obtained by merging TIRF images and epi-fluorescence images are shown, and the enlarged images of the regions enclosed by the frames are shown as the rightmost images. FIG. **6** (*e*) shows an image resulting from merging seven super-resolution images. The images were respectively reconstructed from $2\times10^5$ frames (Lifeact (TIRF), Lifeact (Epi)), $4\times10^4$ frames (CLIP frag. (TIRF), CLIP frag. (Epi)), $1.2\times10^5$ frames (PLEC frag. (TIRF), PLEC frag. (Epi)) and $4\times10^4$ frames (PIPKIγ frag. (TIRF)). The number of frames of speckle images used for constructing the TIRF image of FIG. **6** (*a*) is $2\times10^5$, the number of frames of speckle images used for constructing the Epi image of FIG. **6** (*a*) is $2\times10^5$, the number of frames of speckle images used for constructing the TIRF image of FIG. **6** (*b*) is $4\times10^4$, the number of frames of speckle images used for constructing the Epi of FIG. **6** (*b*) is $4\times10^4$, the number of frames of speckle images used for constructing the TIRF image of FIG. **6** (*c*) is $1.2\times10^5$, the number of frames of speckle images used for constructing the Epi of FIG. **6** (*c*) is $1.2\times10^5$, and the number of frames of speckle images used for constructing the TIRF image of FIG. **6** (*d*) is $4\times10^4$. Also, the probe concentration in the imaging solution for speckle imaging was 2.4 nM for the imaging in FIG. **6** (*a*), 8 nM for the imaging in FIG. **6** (*b*), 9.2 nM for the imaging in FIG. **6** (*c*) and 84 nM for the imaging in FIG. **6** (*d*). Note that the above probe concen-

trations are the actual probe concentration in the case when Atto488-Lifeact was used as the probe and are conversion values based on the fluorescence intensity of labeling fluorescent proteins in the case when other probes were used.

**[0047]** FIG. **7** show region-specific proximity between cytoskeletons and focal adhesions. FIG. **7**(*a*) shows an epi-fluorescence super-resolution image of intermediate filaments in the lamella region in ROI**1** in FIG. **6** (*e*). FIG. **7** (*b*) shows a merged super-resolution image of the intermediate filaments (IF) and actin filaments (Act). FIG. **7** (*c*) shows a merged super-resolution image of intermediate filaments (IF) and microtubules (MT). FIG. **7** (*d*) shows a merged super-resolution of intermediate filaments (IF), actin filaments (Act) andmicrotubules (MT) in super-resolution image (a). FIG. **7** (*e*) shows cross-sectional profiles of three types of cytoskeletons between the arrowheads in FIG. **7** (*d*). Intermediate filaments are tangled with actin stress fibers (at the positions of arrows) in the lamellar region but are not tangled with microtubules. FIG. **7** (*f*) shows a TIRF super-resolution image of intermediate filaments in the peripheral region of ROI**2** in FIG. **6** (*e*). FIG. **7** (*g*) shows a merged TIRF super-resolution image of intermediate filaments (IF) and actin filaments (Act). FIG. **7** (*h*) shows a merged TIRF super-resolution image of intermediate filaments (IF) and microtubules (MT). FIG. **7** (*i*) shows a merged TIRF super-resolution image of intermediate filaments (IF), actin filaments (Act), microtubules (MT) and focal adhesions (FA) in the region in the frame in super-resolution image (f). In FIG. **7** (*g*), which shows a TIRF super-resolution image, the intermediate filaments (IF) are denoted by narrow arrowheads and the actin filaments (Act) are denoted by thick arrowheads. In FIG. **7** (*h*), which shows a TIRF super-resolution image, the intermediate filaments (IF) are denoted by narrow arrowheads and the microtubules (MT) are denoted by thick arrowheads. FIG. **7** (*j*) shows cross-sectional profiles of three types of cytoskeletons between the arrowheads in FIG. **7** (*i*). In this peripheral region, the intermediate filaments overlap a microtubule at the position of the arrow but do not overlap actin filaments.

**[0048]** FIG. **8** show movements of microtubule tips in the vicinity of actin stress fibers and focal adhesions. The left image of FIG. **8** (*a*) is an image resulting from merging a z position map (FIG. **16**) of microtubules (MT) and a TIRF super-resolution image of a focal adhesion (FA) in the central region in FIG. **7** (*i*). The right image of FIG. **8** (*a*) is an image resulting from merging a z position map (FIG. **16**) of microtubules (MT) and a TIRF super-resolution image of actin filaments (Act) in the central region in FIG. **7** (*i*). The z positions of microtubules were calculated from the signal intensity ratio between an epi-fluorescence super-resolution image and a TIRF super-resolution image (see "Methods" in the examples). In the left image of FIG. **8** (*a*), the microtubules (MT) are denoted by arrows and the focal adhesions (FA) are denoted by narrow arrowheads. In the right image of FIG. **8** (*a*), the microtubules (MT) are denoted by arrows and the actin filaments (Act) are denoted by narrow arrowheads. FIG. **8** (*b*) shows the line profiles (CLIP frag) of the z position of a microtubule, the intensity (Lifeact) of an actin filament and the intensity (PIPKIγ) of a focal adhesion along the interval between S and E of the microtubules denoted by the thick arrowheads in FIG. **8** (*a*). The curve of the thick solid line shows a movement average of four data points for the z position of the microtubule. The left image of FIG. **8** (*c*) is a map of the z positions of microtubules, and the right image of FIG. **8** (*c*) is an image resulting from merging the TIRF super-resolution images of actin filaments (grey) and focal adhesions (magenta). The focal adhesion was visualized by combining a super-resolution image obtained by using the Src fragment (residues 3-251 of sequence number 15) as a probe and a super-resolution image obtained by using the Paxillin full length as a probe. In FIG. **8** (*d*), the left panels are live-cell epi-fluorescence observation images (red) and TIRF observation images (green) of EB1-EGFP obtained before IRIS imaging. The middle panels are results of causing overlap between the z position of the tip of the EB1-labeled microtubule, the actin filament and focal adhesion shown in the right image of FIG. **8** (*c*), and the right panels are results of causing overlap between the speed of the portion of the tip of the EB1-labeled microtubule, the actin filament and focal adhesion shown in the right image of FIG. **8** (*c*). The microtubule analyzed with the trajectory of EB1 is indicated by an asterisk in FIG. **8** (*c*).

**[0049]** FIG. **9** show characteristic evaluation of Atto 488-Lifeact. FIG. **9** (*a*) shows speckle lifetime distribution of Atto488-Lifeact in fixed XTC cells. The SiMS images (speckle images) were picked up consecutively with an exposure time of 10 ms/frame and at a frame rate of 100 Hz by using a 488 nm laser (with the main body output of 50 mW but reaching the sample after being attenuated by AOTF etc.). The measurement of the speckle lifetime (having obtained 100 pixels×100 pixels) of a narrow scope of the sample was performed using the Speckle TrackerJ plug-in so that 100 Hz is achieved, i.e. so that 1 frame was able to be obtained per 10 ms. The black line shows a half-life of 23 ms fit to the single exponential curve of the lifetime distribution of speckles between 20 ms and 110 ms. The photobleaching rate of Atto488-Lifeact was negligible in the term of 200 ms. FIG. **9***b* shows analysis of Atto488-Lifeact speckles using DAOSTORM (circles in the image). The SiMS images of Atto488-Lifeact in a cell were obtained with an exposure time of 50 ms. The central position of each speckle was determined with nanometer accuracy. The distribution of speckles changed greatly in the frame next to a frame in which an image was picked up 50 milliseconds later. FIG. **9** (*c*) shows super-resolved images of a single actin filament in vitro by TIRF microscopy using Atto488-Lifeact. The left image is an image obtained by the reconstruction from only high-brightness speckles (top 12% of all measured speckles) and is the same as that shown in FIG. **5** (*b*). The right image was reconstructed from all speckles. The labeling density on the actin filament is indicated at the top of each image. FIG. **9** (*d*) shows the cross-sectional profiles of single actin filaments in vitro (n=10 filaments) in the image reconstructed from high-brightness speckles (black) or in the image reconstructed from all speckles (white). Normalization was conducted so that all counts became **1**. The black curve shows a Gaussian fit curve with a FWHM of 23 nm (high-brightness speckles) and the white curve shows a Gaussian fit curve with a FWHM of 38 nm (all speckles). The error bar represents ±S.E.M.

**[0050]** FIG. **10** show a comparison of labeling patterns along the longitudinal direction of microtubules between IRIS and other super-resolution techniques. FIG. **10** (*a*) shows super-resolution images of microtubules by IRIS using CLIP-170 as probes. These images are part of the epi-fluorescence IRIS observation image of FIG. **6** (*b*). FIG. **10** (*b*) shows line profiles of labeling intensity along microtubules in the right image of FIG. **10** (*a*) (assumed to be IRIS

(a)), the super-resolution of a microtubule by STORM using the anti-β tubulin described in document 21 (assumed to be STORM (b)), the super-resolution image of a microtubule by STORM using the anti-β tubulin described in document 22 (assumed to be STORM (c)), and the super-resolution image of a microtubule by Exchange-PAINT using the anti-**3** tubulin described in document 23 (assumed to be PAINT (d)). The labeling density is shown after being normalized by the mean labeling density of an entire actin filament.

**[0051]** FIG. **11** shows the lifetime distribution data of the selected IRIS probes on the targets. 100 to 112 lifetimes were plotted in accordance with a complementary cumulative distribution function (1-Ndissociation) for each probe. Ndissociation is the cumulative relative frequency of dissociated probes. The half-life of probes on the targets was determined by fitting the life time distribution data by a single exponential function. Part of the measurement results shown on table 1 is shown here.

**[0052]** FIG. **12** show labeling patterns of microtubules by a CLIP-170 fragment (amino acid residues 3-309 of sequence number 12), an APC fragment (amino acid residues 2536-2843 of sequence number 14), a MAP4 fragment (amino acid residues 1-908 of sequence number 4) and Tau isoform 3 (full length of sequence number 5). The patterns are different depending upon procedures for fixing cells. FIG. **12** (*a*) and FIG. **12** (*b*) show live-cell imaging during the fixation operation. In FIG. **12** (*a*), the accumulation of EB1-EGFP at the tips of microtubules disappeared in a process by 3.7% PFA and 0.5% Triton-X 100. In FIG. **12** (*b*), EB1-EGFP was fixed in a state in which it was accumulated at the tips of the microtubules in a process by cold methanol. FIG. **12** (*c*) shows TIRF IRIS images (observation images) of microtubules using CLIP-170 fragment and Tau isoform 3 in cells processed by PFA and Triton-X 100. With these probes, IRIS images of the entire microtubules were obtained. The image resulting from merging these IRIS images indicates that the entire microtubules visualized by these probes correspond to each other. FIG. **12** (*d*) shows the TIRF IRIS images of microtubules using a CLIP-170 fragment and an APC fragment in cells preprocessed with cold methanol for 5 seconds and next with PFA and Triton X-100 as a preprocess. These probes resulted in IRIS images of microtubules where the microtubule tips were visualized with much stronger signals than were the entire microtubules. The image resulting from merging these images shows a coincidence of the microtubule tips visualized by these probes. FIG. **12** (*e*) shows TIRF IRIS images of microtubules using a CLIP-170 fragment, a MAP4 fragment and Tau isoform 3 in cold methanol-preprocessed cells. The microtubule tips were labeled strongly with a CLIP-170 fragment while they were weakly labeled with a MAP4 fragment and Tau isoform 3 (see merged images in the lower tier).

**[0053]** FIG. **13** show labeling patterns of focal adhesions by Paxillin full length (FL) (full length of sequence number 15), the Src fragment (amino acid residues 3-251 of sequence number 16) and a PIPKIγ fragment (residues 641-668) (sequence number 18). FIG. **13** (*a*) shows live-cell images in which GFP-Paxillin was used for visualizing focal adhesions for comparison. FIG. **13** (*b*) shows images of cells obtained by fixing and providing a permeabilization process to the living cells after the imaging of FIG. **13** (*a*). FIG. **13** (*c*) shows a state in which a remaining fluorescence of GFP-Paxillin was completely bleached by irradiation with a strong excitation laser. FIG. **13** (*d*) through FIG. **13** (*f*) are IRIS images in the order of the Src fragment, Paxillin FL, and a PIPKIγ fragment after the photobleaching of FIG. **13** (*c*). In an IRIS image view of FIG. **13** (*d*), generated by using the Src fragment, some dot structures appeared (arrowheads in FIG. **13** (*d*)) in the focal adhesion. These dot structures were not reconstructed by probes that did not dissolve, but were reconstructed by probes that repeatedly bound. In Paxillin FL, a focal adhesion was labeled partially. The left image of FIG. **13** (*g*) is a result of merging FIGS. **13** (*a*) (*d*) (*e*) and (*f*), the center image is a result of merging FIGS. **13** (*d*) and (*e*), and the right image is a result of merging FIGS. **13** (*d*) and (*f*). In the center image, the dot structures visualized by Src fragments (portions denoted by the arrowheads) have many portions that do not correspond to the focal adhesions visualized by Paxillin FL. FIG. **13** (*h*) shows the cross-sectional profiles of the focal adhesions in three IRIS images and the living-cell image of GFP-Paxillin. The measured scope is denoted by the line in the left image of FIG. **13** (*g*). The dot structures visualized by Src fragments are not completely visualized by Paxillin FL (arrow in FIG. **13** (*h*)). These proteins have different binding partners in focal adhesions (documents 17, 38 and 39). These differences between IRIS images may indicate a distribution of partners that respective proteins can associate with in focal adhesions.

**[0054]** FIG. **14** show the z profile of the TIRF excitation intensity. FIG. **14** (*a*) shows fluorescence images of HyLight488-labeled microtubules obtained by total internal reflection fluorescence (TIRF) and epi fluorescence. The epi fluorescence images were obtained as z-stack images (0.2 μm step size). The z-stack epi-fluorescence images were used to determine the z-directional distance of each point along the tilted microtubule (see "Methods"). The bar represents 5 μm. FIG. **14** (*b*) shows the Z profile of the TIRF excitation intensity.

**[0055]** FIG. **15** (*a*) shows z-position mapping of actin bundles, FIG. **15** (*b*) shows z-position mapping of microtubules, and FIG. **15** (*c*) shows z-position mapping of intermediate filaments. The respective images of FIGS. **15** (*a*), (*b*) and (*c*) were calculated and generated from the total internal reflection fluorescence (TIRF) IRIS image and the epi-fluorescence IRIS image shown in FIGS. **6** (*a*), (*b*) and (*c*) (see "Methods"). The right image is an enlarged image of the region in the frame shown in the left image. FIG. **15** (*d*) shows a distribution of z positions of actin bundles in lamellipodia (LP), stress fibers (SF) and actin arcs (Arc). In these layered actin structures, a calculated z position represents the height position of the center of gravity in the z axial direction of an actin filament. FIG. **15** (*e*) shows the z-position profile along the longitudinal direction of a microtubule. The microtubule submerges toward the periphery of a cell (arrowheads in FIG. **15** (*b*)). S and E in FIG. **15** (*b*) represent the starting and ending points of the line profile.

**[0056]** FIG. **16** shows an epi-fluorescence super-resolution image of microtubules (left), a TIRF image (center) and the z-position mapping shown in FIG. **8** (*a*) (right). In CLIP-170 fragment-visualized microtubules, signals were partially lost (asterisks in the left and center images).

**[0057]** FIG. **17** shows a result of IRIS super-resolution imaging using a probe containing a Fab fragment (Fab probe). It is a result of imaging a distribution of p40, which is a subunit of an Arp2/3 complex, by using a Fab probe that is produced from anti-p40 polyclonal antibody.

**[0058]** FIG. **18** show binding of FLAG-EGFP to a solid-phased antibody. An antibody contained in hybridoma culture supernatant was fixed to a glass surface and a FLAG-EGFP solution was added so as to observe it with a TIRF microscope. Each speckle is FLAG-EGFP that has bound to a solid-phased antibody. FIG. **18** (A) shows a reaction positive example. Speckles representing binding between FLAG-EGFP and antibodies are observed at a high density. An observation result of a great number of speckles indicates that solid-phased antibodies are anti-FLAG antibodies having a binding capacity to FLAG-EGFP. FIG. **18** (B) shows a reaction negative example. Few speckles are observed. This is a similar level to a case where FLAG-EGFP was added to a glass surface that was not solid phased.

**[0059]** FIG. **19** shows super-resolution images of FLAG fused actins obtained by using a Fab probe derived from an anti-FLAG monoclonal antibody selected from a hybridoma library (203 milliseconds as the half-life of a probe-target complex). This cell has forcibly expressed a FLAG tag fused actin. The right image is an enlarged image of the region in the white frame in the left image.

## DESCRIPTION OF THE EMBODIMENTS

**[0060]** In super-resolution microscopy, an increased resolution capability has brought the new problems of a low labeling density and unevenness thereof. According to a sampling theorem for example, it has been shown that one labeling substance has to be included at 10 nm in a target in order to obtain a spatial resolution capability of 20 nm (J. Cell Sci., 126, 3505-3513, 2013). In conventional super-resolution imaging of proteins in cells, target proteins are labeled by using the expression of target proteins with which fluorescent proteins have been made to fuse or by using a fluorescent antibody. This imposes limitations on a labeling density, depending upon the expression amount ratio to endogenous target proteins and upon the size (about 10 nm) of an antibody itself. Also, because fluorescent dyes that have bound to a target substance are used, the maximum number of types of target substances that can be visualized in one sample is two or three. The limitations on the number of proteins that can be stained have been a long-standing issue in the study of a cell consisting of various types of proteins.

**[0061]** In Exchange-PAINT above as well, target substances have to be labeled with antibodies that were made to fuse with DNA oligomers, and thus it is expected that a plurality of antibodies will interfere with each other spatially in a region at or below the diffraction limit. In principle, the greater the number of types of antibodies there are, the more difficult it is to perform labeling evenly.

**[0062]** Uneven labeling leads to reconstruction of false super-resolution, which is problematic.

**[0063]** Proceedings of the national Academy of Sciences of the United States of America 103, 18911-18916 (2006), which discloses PAINT, does not at all describe increasing of the labeling density of target substances by labeling substances.

**[0064]** Thus, it is an object of the present invention to provide a super-resolution microscope observation method that can obtain position information of luminescent substances at a high density, the luminescent substances being used for labeling.

**[0065]** According to the method of the present invention, it is possible to label target substances at a high density in a sample by using luminescent probes and to generate a highly accurate observation image of a target substance.

### 1. Principle of IRIS

**[0066]** The present inventors have named the observation method of the present invention IRIS (image reconstruction by integrating exchangeable single-molecule localization).

**[0067]** FIG. **2** (*a*) explains functions of a luminescent probe in the observation method of the present invention. A probe **200** contains a luminescent substance **201** that emits light under a prescribed condition (for example a fluorescent substance that emits fluorescence when irradiated with excitation light), and usually further contains a binding substance **202** that is involved in binding to and dissociation from a target substance that is linked to the luminescent substance **201**. The probe **200** can repeatedly bind to and dissociate from a target substance **210** directly and specifically. Characteristics of binding of the probe **200** are as below. When the probe **200** is in a state in which it has bound to the target substance **210**, the light emitted by the luminescent substance **201** can be picked up as a speckle in a speckle image, and when the probe **200** has dissociated from the target substance **210**, the light emitted by the luminescent substance **201** is not picked up as a speckle in an image because the probe is in a disordered thermal motion in a medium **220**. In an imaging step of the present invention, a step is performed a plurality of times (e.g. hundreds of times through hundreds of thousands of times respectively at different times) in which a speckle image including, as a speckle, light emitted from the luminescent substance **201** under a prescribed condition is obtained in a state in which the medium **220** containing the probe **200** is brought into contact with a sample containing the target substance **210**. As is shown when superposing the obtained plurality speckle images, an imaging step of the present invention can attain the same effect as that attained by labeling the target substances **210** with probes **200** at a high density and picking up images of them. Theoretically, there is no upper limit on the density of the labeling in the above. Also, when the concentration of the probes **200** in the medium **220** is adjusted appropriately, it is possible to identify a plurality of individual speckles in a separate manner in one speckle image.

**[0068]** Meanwhile, in PALM, STORM and Exchange-PAINT, which have conventionally been known as localization microscopy, an antibody to which a luminescent substance is linked is used for labeling a target substance. In an example of STORM for example, as shown in FIG. **2** (*b*), luminescent antibodies **230** resulting from linking luminescent substances **232** and antibodies **231** for the target substance **210** are made to bind to the target substance **210** and images are picked up a plurality of times (e.g. hundreds of times through hundreds of thousands of times) at different times while making the luminescent substances **232** emit light discretely at a density that is low enough to prevent the speckles from overlapping. By superposing the obtained plurality of speckle images, an image with all the luminescent substances **232** emitting light can be obtained. However, the labeling density at which the target substance is labeled with the luminescent antibodies **230** is limited by the size (about 10 nm) of the antibodies themselves. In order to achieve a resolution capability of Xnm, it is necessary to label a target substance at intervals of X/2 nm (Nyquist Sampling Theorem). The luminescent antibody **230** has a

size of at least about 10 nm, so making labeling at a high density is impossible. Also, in Exchange-PAINT, a plurality of target substances in one sample are labeled with a plurality of antibodies that are specific to the respective target substances, which causes interference between the plurality of antibodies, making it further difficult to perform labeling at a high density. The observation method according to the present invention can solve the above problem of labeling density in conventional localization microscopy.

2. Observation of a Plurality of Target Substances

**[0069]** The observation method according to the present invention can preferably be implemented even when a plurality of target substances exist in one sample.

**[0070]** When a plurality of target substances exist in one sample, the observation method according to the present invention are implemented through the following procedures. A medium containing a probe that repeatedly binds to and dissociates from one type from among a plurality of target substances directly and specifically is brought into contact with a sample so that an imaging step of the present invention is conducted and thereby a plurality of speckle images including speckles of light emitted from the probes that have bound to that one type of the target substance are obtained. Next, probes are removed by washing the sample and a medium containing a probe that repeatedly binds to and dissociates from one different type from among a plurality of target substances directly and specifically is brought into contact with the sample so that an imaging step of the present invention is conducted and thereby a plurality of speckle images including speckles of light emitted from the probes that have bound to that one different type of the target substance are obtained. These procedures are performed until a plurality of speckle images are obtained for each of all the target substances that are to be observed. The wash of the sample between the respective imaging steps can be implemented by for example performing at least one time an operation in which an appropriate washing medium such as the above medium etc. not containing the probe is brought into contact with the sample the sample is washed. Observation images of respective target substances can be generated from a plurality of speckle images for respective target substances obtained by using respective probes. Also, by superposing observation images of respective target substances in the same sample, a multiple-observation image including observation images of a plurality of target substances in the sample can also be generated.

**[0071]** A probe used in the present invention is a probe that can repeatedly bind to and dissociate from a target substance directly and specifically. "Bind to a target substance directly" used herein refers to binding between a probe and a target substance that is not through another binding substance such as an antibody etc. When a probe and a target substance are binding through at least one type of binding selected from a group consisting of for example hydrogen binding, hydrophilic and/or hydrophobic binding, electrostatic binding and van der Waals binding, the probe and the target substance can be treated as binding directly.

**[0072]** In the present invention, because a probe and a target substance bind to and dissociate from each other directly, the original state of the sample can be recovered when the sample is washed so as to remove the probe after performing an imaging step in which the sample is processed with the probe so as to make the sample emit light. Even in a case when an operation is repeatedly performed sequentially for each target substance, in which a medium containing a probe is brought into contact with a sample, an imaging step is performed and washing is performed in order to obtain observation images of a plurality of target substances included in one sample, and each imaging step can be performed in a state where only a probe for one type of a target substance that is to be observed exists and probes for other target substances do not exist. This prevents interference from occurring between probes and makes it possible to observe each target substance in a sample that is in a more natural state.

**[0073]** FIG. **3** schematically shows procedures for obtaining each observation image by using one sample containing target substances A and target substances B. First, an imaging step is performed in a state where a medium containing probes A that are specific to target substances A is brought into contact with a sample so as to obtain a plurality of speckle images. Next, the sample is washed so as to remove the probes A. Thereafter, an imaging step is performed in a state where a medium containing probes B that are specific to target substances B is brought into contact with the sample so as to obtain a plurality of speckle images. An observation image is generated for each of target substance A and target substance B from a plurality of speckle images for the respective target substances. It is also possible to generate a multiple-observation image by superposing an observation image of target substance A and an observation image of target substance B.

3. Explanations for Sample and Probe

**[0074]** A sample used for observation is typically a biological sample such as a cell, a tissue etc. consisting of a plurality of cells. When a sample is a biological sample, it is preferable to use a sample in a fixed state, and it is particularly preferable to use a sample that has further received a permeabilization process as necessary.

**[0075]** A sample contains at least one target substance. "Target substance" used herein refers to an object, as an observation target, that is contained in a sample. Examples of a target substance may include a structure body that constitutes a cytoskeleton preferably such as an actin polymer, a microtubule, an intermediate filament, a focal adhesion, etc. A target substance is preferably a structure that is, like the above structure, formed as a result of many constituents assembling, the constituents having the same structure or having a structure having common characteristics. Origin organisms for a structure body that constitutes a biological sample such as a cell, a tissue, etc. that can be an observation sample and a cytoskeleton that can be a target substance are not particularly limited. For example, a structure originating from vertebrates such as mammals (humans, rabbits, rodents, etc.), amphibians (frogs etc. *xenopus* for example), fish including bony fish and cartilaginous fish, reptiles, birds, etc., invertebrates such as mollusks, protochordates, echinoderm, cnidarians, arthropods, etc., and unicellular organisms such as eukaryotic unicellular organisms (yeast etc.) can be used. Among them, structure bodies constituting cytoskeletons are held by a wide variety of species, and a method of observing a structure body constituting a cytoskeleton whose effectiveness has been confirmed in the examples can be applied regardless of the type of origin organism. It is preferable that a target substance be an object having a shape that does not substantially change

during an imaging step of picking up a plurality of speckle images, and a target substance may receive a fixation process as necessary.

**[0076]** A target substance is preferably a target substance containing a protein, and is more preferably a protein target substance that exits together with one or more different proteins, which are not the target substance, and that are in an observation target sample. In the present embodiment of the present invention, a probe specifically binds to a protein that is a target substance from among a plurality of types of proteins, making it possible to selectively visualize a target substance.

**[0077]** A probe has a property of repeatedly binding to and dissociating from one specific type of target substances in a sample directly and specifically. The meaning of "binding to a target substance directly" is as mentioned previously.

**[0078]** While a probe may be a luminescent substance that by itself has a property of binding to and dissociating from a target substance, it usually contains a luminescent substance and a binding substance that is linked to that luminescent substance and that has a property of repeatedly binding to and dissociating from a target substance directly and specifically.

**[0079]** A probe and a target substance have the following binding characteristics. Specifically, the half-life of a probe-target complex formed by binding between a probe and a target substance is preferably equal to or more than 10 milliseconds and equal to or less than 3 seconds, more preferably equal to or more than 10 milliseconds and equal to or less than 2 seconds, more preferably equal to or more than 10 milliseconds and equal to or less than 1 second, more preferably equal to or more than 10 milliseconds and equal to or less than 900 milliseconds, more preferably equal to or more than 10 milliseconds and equal to or less than 800 milliseconds, more preferably equal to or more than 10 milliseconds and equal to or less than 700 milliseconds, more preferably equal to or more than 10 milliseconds and equal to or less than 600 milliseconds, more preferably equal to or more than 10 milliseconds and equal to or less than 500 milliseconds, more preferably equal to or more than 20 milliseconds, and more preferably equal to or less than 300 milliseconds, particularly preferably equal to or less than 250 milliseconds. The above half-life herein is preferably a half-life of a probe-target complex formed by binding between a probe and a target substance in a case when a medium containing a probe and a sample containing a target substance that is to be observed are brought into contact under a condition for performing an imaging step. The half-life is defined by a period of time before the number of probes that have bound to target substances at a given moment is reduced to half through dissociation. Measurement procedures for the half-life are as below. A medium containing a probe is brought into contact with a sample containing a target substance that is to be observed and a period of time between when light emission speckle based on the probe appears and when it disappears is measured for each light emission speckle while performing observation under a condition that is used for observation. Then, periods of time between the appearance and disappearance of the speckles are plotted in accordance with a complementary cumulative relative frequency function (1-Ndissociation). Ndissociation is a cumulative relative frequency of a probe that dissociated. A cumulative relative frequency is a ratio of the speckles that disappeared within a given period of time to the total number of measured speckles (i.e. probes) (i.e. probes that dissociated within a given period of time), and represents the total number as 1. Then, by fitting the above complementary cumulative relative frequency function with an exponent function, that half-life is calculated.

**[0080]** The period of time between the appearance and disappearance of a light emission speckle based on a probe can be measured by the following procedures. Specifically, while keeping a medium containing a probe in contact with a target substance and providing a prescribed condition necessary for emitting light (for example irradiation with excitation light), speckle images including speckles based on the light emission of luminescent substances of probes are consecutively picked up with an exposure time of X seconds (for example 0.050 seconds, 0.100 seconds, etc.) and at a frame rate of 1/XHz. Then, the period of time is measured between when a probe that has bound to a target appears in a speckle image and when it disappears through dissociation. In the above, when observation is performed for Y consecutive frames, and for a speckle not observed in frames before and after them, the period of time between the appearance and disappearance of that speckle can be treated as YX seconds.

**[0081]** A probe with the above half-life equal to or more than 10 milliseconds provides a period of time between binding to and dissociation from a target substance that is sufficiently long to allow an ordinary highly sensitive imaging device such as an EM-CCD camera etc. to pick up an image of a speckle based on the luminescent substance of the probe that has been bound. When by contrast the half-life is too long, a region including a probe having a long binding time to a target substance represents a signal that is particularly strong in a reconstructed observation image, which can cause uneven labeling. This makes it difficult to obtain an accurate distribution of target substances. From the results obtained thus far, it is possible to obtain a speckle image and an observation image that are relatively even when a probe whose half-life is equal to or less than 3 seconds is used and the shorter the half-life of a probe that is used is, the easier it is to obtain a speckle image and an observation image that are even. This point of view has made obvious that a half-life that is equal to or less than 500 milliseconds is particularly preferable. Also, as a general rule, the longer a half-life is, the longer an exposure time for one frame and imaging intervals have to be, which sometimes requires a long period of time for obtaining speckle images that are needed for generating a reconstruction image. From this point of view, it is preferable that the half-life be equal to or less than 3 seconds or that the half-life be further shorter.

**[0082]** In the present invention, a step of obtaining each speckle image will be referred to as a "frame imaging step". In each frame imaging step, a speckle image including a speckle of light emitted from a luminescent substance under a prescribed condition is picked up by using an imaging device. A term in which one speckle image is picked up will be referred to as a "frame", a term between the starting time of a frame and the starting time of the next frame will be referred to as an "interval", and a term between the ending time of a frame and the starting time of the next frame will be referred to as an "inter-frame term". A period of time for one frame (exposure time) can be determined in accordance with the binding half-life between a probe and a target substance in an appropriate manner. It is preferable that a period of time for one frame (exposure time) be longer than

the binding half-life between a probe and a target substance, but the scope of the present embodiment is not limited to this.

**[0083]** Target substances and probes that meet the above requirements can be selected appropriately while a combination between a target substance and a binding substance of a probe is preferably selected from a group of:

**[0084]** a combination wherein the probe is (a1) a polypeptide, linked to the luminescent substance, which consists of an amino acid sequence of sequence number 19, (a2) a polypeptide, linked to the luminescent substance, which consists of the amino acid sequence described in (a1) where one or a plurality of amino acids have been substituted, deleted, inserted or added, and for which a half-life of a probe-target complex formed by binding between the probe and the target substance is equal to or more than 10 milliseconds and equal to or less than 3 seconds, or (a3) a polypeptide, linked to the luminescent substance, which consists of an amino acid sequence having at least a 70% identity with the amino acid sequence described in (a1) and for which a half-life of a probe-target complex formed by binding between the probe and the target substance is equal to or more than 10 milliseconds and equal to or less than 3 seconds, and the target substance is an actin polymer;

**[0085]** a combination wherein the probe is (b1) a polypeptide, linked to the luminescent substance, which consists of an amino acid sequence which is a partial amino acid sequence of an amino acid sequence of sequence number 12, which at least partially contains an amino acid sequence of 3-309 and which has 407 or fewer, preferably 357 or fewer, more preferably 327 or fewer and most preferably 307 amino acids, an amino acid sequence which is a partial amino acid sequence of an amino acid sequence of sequence number 14, which at least partially contains an amino acid sequence of 2536-2843 and which has 408 or fewer, preferably 358 or fewer, more preferably 328 or fewer and most preferably 308 amino acids, an amino acid sequence which is a partial amino acid sequence of an amino acid sequence of sequence number 14, which at least partially contains an amino acid sequence of 2781-2819 and which has 138 or fewer, preferably 88 or fewer, more preferably 58 or fewer and most preferably 38 amino acids, an amino acid sequence which is a partial amino acid sequence of an amino acid sequence of sequence number 4, which at least partially contains an amino acid sequence of 1-908 and which has 1008 or fewer, preferably 958 or fewer, more preferably 928 or fewer and most preferably 908 amino acids, an amino acid sequence which is a partial amino acid sequence of an amino acid sequence of sequence number 4, which at least partially contains an amino acid sequence of 659-908 and which has 394 or fewer, preferably 344 or fewer, more preferably 314 or fewer and most preferably 294 amino acids, an amino acid sequence of sequence number 5 or an amino acid sequence of sequence number 6, (b2) a polypeptide, linked to the luminescent substance, which consists of the amino acid sequence described in (b1) where one or a plurality of amino acids have been substituted, deleted, inserted or added and for which a half-life of a probe-target complex formed by binding between the probe and the target substance is equal to or more than 10 milliseconds and equal to or less than 3 seconds or (b3) a polypeptide, linked to the luminescent substance, which consists of an amino acid sequence having at least a 70% identity with the amino acid sequence described in (b1) and for which a half-life of a probe-target complex formed by binding between the probe and the target substance is equal to or more than 10 milliseconds and equal to or less than 3 seconds, and the target substance is a microtubule;

**[0086]** a combination wherein the probe is (c1) a polypeptide, linked to the luminescent substance, which consists of an amino acid sequence which is a partial amino acid sequence of an amino acid sequence of sequence number 8, which at least partially contains an amino acid sequence of 3777-4684 and which has 1008 or fewer, preferably 958 or fewer, more preferably 928 or fewer and most preferably 908 amino acids, an amino acid sequence which is a partial amino acid sequence of an amino acid sequence of sequence number 8, which at least partially contains an amino acid sequence of 3777-4364 and which has 688 or fewer, preferably 638 or fewer, more preferably 608 or fewer and most preferably 588 amino acids, an amino acid sequence which is a partial amino acid sequence of an amino acid sequence of sequence number 8, which at least partially contains an amino acid sequence of 3777-4313 and which has 637 or fewer, preferably 587 or fewer, more preferably 557 or fewer and most preferably 537 amino acids, or an amino acid sequence which is a partial amino acid sequence of an amino acid sequence of sequence number 8, which at least partially contains an amino acid sequence of 4022-4364 and which has 443 or fewer, preferably 393 or fewer, more preferably 363 or fewer and most preferably 343 amino acids, (c2) a polypeptide, linked to the luminescent substance, which consists of the amino acid sequence described in (c1) where one or a plurality of amino acids have been substituted, deleted, inserted or added and for which a half-life of a probe-target complex formed by binding between the probe and the target substance is equal to or more than 10 milliseconds and equal to or less than 3 seconds, or (c3) a polypeptide, linked to the luminescent substance, which consists of an amino acid sequence having at least a 70% identity with the amino acid sequence described in (c1) and for which a half-life of a probe-target complex formed by binding between the probe and the target substance is equal to or more than 10 milliseconds and equal to or less than 3 seconds, and the target substance is an intermediate filament; and (d1) a polypeptide, linked to the luminescent substance, which consists of an amino acid sequence of sequence number 15, an amino acid sequence which is a partial amino acid sequence of an amino acid sequence of sequence number 15, which at least partially contains an amino acid sequence of 54-557 and which has 556 or fewer, more preferably 524 or fewer and most preferably 504 amino acids, an amino acid sequence which is a partial amino acid sequence of an amino acid sequence of sequence number 15, which at least partially contains an amino acid sequence of 54-498 and which has 545 or fewer, preferably 495 or fewer, more preferably 465 or fewer and most preferably 445 amino acids, an amino acid

sequence which is a partial amino acid sequence of an amino acid sequence of sequence number 15, which at least partially contains an amino acid sequence of 167-557 and which has 491 or fewer, preferably 441 or fewer, more preferably 411 or fewer and most preferably 391 amino acids, an amino acid sequence which is a partial amino acid sequence of an amino acid sequence of sequence number 16, which at least partially contains an amino acid sequence of 1-251 and which has 351 or fewer, preferably 301 or fewer, more preferably 271 or fewer and most preferably 251 amino acids, an amino acid sequence which is a partial amino acid sequence of an amino acid sequence of sequence number 16, which at least partially contains an amino acid sequence of 3-251 and which has 349 or fewer, preferably 299 or fewer, more preferably 269 or fewer and most preferably 249 amino acids, or an amino acid sequence of sequence number 18, (d2) a polypeptide, linked to the luminescent substance, which consists of the amino acid sequence described in (d1) where one or a plurality of amino acids have been substituted, deleted, inserted or added and for which a half-life of a probe-target complex formed by binding between the probe and the target substance is equal to or more than 10 milliseconds and equal to or less than 3 seconds or (d3) a polypeptide, linked to the luminescent substance, which consists of an amino acid sequence having at least a 70% identity with the amino acid sequence described in (d1) and for which a half-life of a probe-target complex formed by binding between the probe and the target substance is equal to or more than 10 milliseconds and equal to or less than 3 seconds, and the target substance is a focal adhesion. It is also preferable that a more preferable scope of half-lives of probe-target complexes in the above respective combinations be in accordance with the above and particularly be a period of time that is equal to or more than 10 milliseconds and equal to or less than 500 milliseconds or shorter. In the above combinations, more preferable scopes for a half-life of a probe-target complex are as described above and it is particularly preferable that a half-life of a probe-target complex be equal to or more than 10 milliseconds and equal to or less than 500 milliseconds or that a half-life of a probe-target complex be further shorter.

**[0087]** Origin organisms for an actin polymer, a microtubule, an intermediate filament and/or focal adhesion in the above combinations are not particularly limited, however a substance originating from vertebrates such as mammals (humans, rabbits, rodents, etc.), amphibians (frogs etc. *xenopus* for example), fish including bony fishes and cartilaginous fishes, reptiles, birds, etc., invertebrates such as mollusks, protochordates, echinoderm, cnidarians, arthropods, etc., a unicellular organism such as eukaryotic unicellular organisms (yeast etc.), and a substance resulting from artificially introducing variation to them can be used.

**[0088]** "Actin polymer" used herein refers to a structure body formed through polymerization of an actin molecule, and typically to an actin filament.

**[0089]** In this document, a "microtubule" refers to for example a structure in which 13 protofilaments where heterodimers of atubulin and (tubulin are connected in a fibrous manner are collected to form a tubular structure body having a diameter of 25 nm.

**[0090]** In this document, intermediate filaments of type I, type II, type III and type IV are known as an "intermediate filament", and all of them may be observation targets of the present invention. Plectin can bind to all types of intermediate filaments.

**[0091]** In this document, "focal adhesion" refers to a structure made by for example a plurality of proteins (integrin, paxillin, vinculin, talin, etc.) being provided at adhesion points between a cell and an extracellular matrix.

**[0092]** "One or a plurality" for substitution, deletion, insertion or addition of an amino acid in the above (a2), (b2), (c2) and (d2) refers to for example 1 to 50, preferably 1 to 25, more preferably 1 to 20, more preferably 1 to 15, more preferably 1 to 10, more preferably 1 to 7, more preferably 1 to 5, more preferably 1 to 4, more preferably 1 to 3, and most preferably 1 or 2. Further preferably, the above expression of "one or a plurality" refers to preferably equal to or less than 20%, more preferably equal to or less than 10% and most preferably equal to or less than 5% of the number of amino acids of the polypeptides in the above (a1), (b1), (c1) and (d1).

**[0093]** Substitution of an amino acid is preferably conservative amino acid substitution. "conservative amino acid substitution" refers to substitution between amino acids having similar physicochemical functions such as an electric charge, a side chain, a polarity, an aromatic property, etc. Amino acids having similar physicochemical functions can be categorized into for example a basic amino acid (arginine, lysine, histidine), an acidic amino acid (aspartic acid, glutamic acid), a non-charged polar amino acid (asparagine, glutamine, serine, threonine, cysteine, tyrosine), a non-polar amino acid (glycine, leucine, isoleucine, alanine, valine, proline, phenylalanine, tryptophan, methionine), a branched-chain amino acid (leucine, valine, isoleucine), an aromatic amino acid (phenylalanine, tyrosine, tryptophan, histidine), etc. Addition of one or a plurality of amino acids in each amino acid sequence is preferably addition of a total of one or a plurality of amino acids to at least one of the N-terminus and the C-terminus of that amino acid sequence. Also, deletion of one or a plurality of amino acids in each amino acid sequence is preferably deletion of a total of one or a plurality of amino acids from at least one of the N-terminus and the C-terminus of that amino acid sequence.

**[0094]** Identity with the amino acid sequences respectively described in the above (a1), (b1), (c1) and (d1) in the above (a3), (b3), (c3), and (d3) is preferably equal to or more than 80%, more preferably equal to or more than 85%, more preferably equal to or more than 90%, more preferably equal to or more than 95%, more preferably equal to or more than 97%, more preferably equal to or more than 98%, and most preferably equal to or more than 99%. In the present invention, the value of identity of amino acid sequences is calculated in a default setting by using software that computes identity between a plurality of amino acid sequences (for example FASTA, DANASYS and BLAST). The value of identity of amino acid sequences is calculated by calculating the number of amino acid residues that match when a pair of amino acid sequences is aligned in such a manner that the matching degree becomes the maximum and is calculated as a ratio of the number of the matching amino acid residues to the total number of the amino acid residues of the amino acid sequences that were compared. In this example, when there are gaps, the above total number of the amino acid residues is the number of amino acid residues obtained

by counting one gap as one amino acid residue. When thus calculated, the total numbers of all the amino acid residues are different between the amino acid sequences that are compared, and the identity is calculated on the basis of the greater total number of the amino acid residues. For a detailed method of determining identity, reference is to be made to for example Altschul et al, Nuc. Acids. Res. 25, 3389-3402, 1977 and Altschul et al, J. Mol. Biol. 215, 403-410, 1990.

**[0095]** Also, more preferably, polypeptides in the above (a3), (b3), (c3) and (d3) are polypeptides having preferably equal to or more than 75%, more preferably equal to or more than 80%, more preferably equal to or more than 85%, more preferably equal to or more than 90%, more preferably equal to or more than 95%, more preferably equal to or more than 97%, more preferably equal to or more than 98%, and most preferably equal to or more than 99%, of similarly to the amino acid sequences respectively described in the above (a1), (b1), (c1) and (d1). The value of similarity of amino acid sequences is calculated by calculating the total of the number of amino acid residues that match when a pair of amino acid sequences is aligned in such a manner that the matching degree becomes the maximum and the amino acid residues have similar physicochemical functions, and is calculated as a ratio of the total number to the total number of the amino acid residues of the amino acid sequences that were compared. In this example, similarity of amino acid sequences can be calculated by a computer by using software similar to that described for the identity of amino acid sequences. A method of calculating the total number of amino acid residues is as described above for amino acid identity. The meaning of amino acid residues having similar physicochemical functions is as described above.

**[0096]** In a preferable embodiment of the present invention, a binding substance contained in a probe (a site for identification of a target substance) is an antibody or a fragment of an antibody, to a target substance, and particularly preferably a fragment of an antibody.

**[0097]** A technique for producing an antibody to an arbitrary target substance has already been established. Thus, using an antibody or a fragment of an antibody produced in accordance with a target substance as a binding substance of a probe makes it possible to use the technique of the present invention for observation of a target substance ranging in a wide scope.

**[0098]** It is sufficient if a target substance in this embodiment is a target substance presenting antigenicity, and it is typically a protein.

**[0099]** An antibody that an antibody or a fragment of an antibody contained in a probe is from is typically immunoglobulin G (IgG), however a different isotype is possible, and it may be for example immunoglobulin M (IgM), immunoglobulin D (IgD), immunoglobulinA (IgA), immunoglobulin E (IgE), etc. When these types of immunoglobulin contain a plurality of subclasses, one belonging to an arbitrary subclass is possible. Examples of another type of antibody may include a single domain antibody. These antibodies may be a variation or may be in a form in which it is made to fuse with a different polypeptide.

**[0100]** Although origins of an antibody are not particularly limited, for example, an antibody originating from for example nonhuman animals such as mice, rats, llamas, camels, etc. and from humans etc. can be used. Also, an antibody may be a chimeric antibody formed by causing a fusion of domains of antibodies of a plurality of origins.

**[0101]** An antibody may be a polyclonal antibody or may be a monoclonal antibody.

**[0102]** "Fragment" of an antibody in the present embodiment refers to a "functional fragment" having avidity to a target substance (antigen). A fragment of an antibody may be a variant or may be in a form in which it is made to fuse with a different polypeptide.

**[0103]** Examples of a fragment of an antibody may include a Fab fragment, a Fab' fragment, a F $(ab')_2$ fragment, an scFv (single-strand Fv) fragment, a VHH fragment of a single domain antibody (for example a commercial name of a nanobody), etc. A Fab fragment can be obtained by cutting an antibody with papain, a protein breakdown enzyme. A F $(ab')_2$ fragment can be obtained by cutting an antibody with pepsin. A Fab' fragment can be obtained by further processing a F $(ab')_2$ fragment under a reduction condition. An scFv fragment is a result of linking a heavy-chain variable region and a light-chain variable region of an antibody with a linker of a polypeptide so as to make them single-stranded and can be produced by a gene engineering method that utilizes a polynucleotide having a base sequence that encodes the heavy-chain variable region, the linker and a light-chain variable region.

**[0104]** In the present embodiment, the half-life of a probe-target complex formed by binding of a probe containing an antibody or a fragment of an antibody to a target substance is equal to or more than 10 milliseconds and equal to or less than 3 seconds. Generally, because an antibody has a bivalent binding capacity to an antigen (i.e., an antibody of 1 molecule has two antigen binding sites), the binding tendency to an antigen is strong and the half-life of a probe-target complex between an antibody and an antigen far exceeds the upper limit of the above scope. Thus, it is preferable that a fragment of an antibody that can repeatedly bind to and dissociate from a target substance be used and that a fragment of an antibody have a univalent binding capacity to a target substance, which is an antigen (i.e., 1 molecule has 1 antigen binding site). Examples of an antibody fragment having a univalent binding capacity to a target substance may include a Fab fragment, a Fab' fragment, an scFv fragment, a VHH fragment of a single domain antibody (for example a commercial name of nanobody), etc.

**[0105]** Methods of producing the above antibody and fragment of an antibody are not particularly limited. A preferable embodiment for a method of screening for an antibody presenting the above prescribed half-life from candidates for antibodies adjusted by an arbitrary method such as a hybridoma method etc. will be explained later.

**[0106]** Luminescent substances contained in probes are not particularly limited as long as they are substances emitting light that makes observation possible under a prescribed condition. As a luminescent substance, a fluorescent substance that can emit fluorescence when irradiated with excitation light is particularly preferable. Examples of fluorescent substances may include a fluorescent protein such as a green fluorescent protein (GFP), an enhanced green fluorescent protein (EGFP), a red fluorescent protein (RFP), TagRFP, etc.; fluorescent dye such as Atto (trademark) 488, Atto (trademark) 550, Dylight (trademark) 488, Dylight (trademark) 550, CF (trademark) dye (CF680R, CF488A, CF543, etc.), etc.; and a quantum dot.

**[0107]** In an imaging step of the present invention, a mixture of a medium, a probe and an observation target sample is preferably exposed at intervals that are sufficiently shorter than a period of time between when the luminescent substance starts to be exposed to the prescribed condition and when it photobleaches the light (photobleaching period of time) or may be exposed consecutively, in the prescribed condition (irradiation with excitation light for example) for making a luminescent substance included in the probe emit light. For example, in an embodiment in which a luminescent substance is a fluorescent substance, a mixture of a medium, a probe and an observation target sample may be irradiated with excitation light at intervals that are sufficiently shorter than a photobleaching period of time or may be irradiated consecutively.

**[0108]** A luminescent substance in a probe and a binding substance can be linked through chemical binding via an appropriate linker component as necessary. Examples of chemical binding may include covalent binding, coordinate binding, etc., and covalent binding is preferable in view of stability. When a luminescent substance and a binding substance are both polypeptides, a probe can be formed as a fused polypeptide resulting from the luminescent substance and the binding substance linking to each other through normal peptide binding.

**[0109]** An imaging step of the present invention is performed in a state in which a medium containing a probe and a sample are in contact with each other. Media are not particularly limited as long as they allow a probe and a target substance to maintain the above binding characteristics, however they are usually liquid, and the liquid is preferably an aqueous solution, is more preferably an aqueous buffer solution adjusted to an appropriate pH, is more preferably an aqueous buffer solution adjusted to a pH of 6.1 to pH 7.5, and more preferably contains active oxygen remover. As an active oxygen remover, at least one type or two or more types selected from glucose oxidase, catalase, 2-mercaptoethanol, glucose, etc. can be used. An example of a proper amount of glucose oxidase may be 200 μg/ml, an example of a proper amount of catalase may be 35 μg/ml, an example of a proper amount of 2-mercaptoethanol may be 0.5%, and an example of a proper amount of glucose may be 4.5 mg/ml. As a buffer component for preparing an aqueous buffer solution, HEPES, Tris, etc. can be used.

**[0110]** It is preferable that the concentration of probes in a medium have appropriately been adjusted to a concentration that allows identification of emission of light of a luminescent substance as a separate speckle for each molecule in one speckle image.

**[0111]** Temperature conditions in a case when a medium containing a probe is brought into contact with a sample are not particularly limited, and for example 20 degrees Celsius through 30 degrees, and more preferably an ambient temperature, specifically 25 degrees Celsius, can be adopted.

## 4. Embodiment of Observation Method of Present Invention that Uses Microscope

**[0112]** Devices used for an observation method of the present invention are not particularly limited.

**[0113]** FIG. **4** shows an example of a device that can be used for an embodiment of the present invention in which a luminescent substance contained in a probe is a fluorescent substance that emits fluorescence when irradiated with excitation light.

**[0114]** A microscope apparatus **1** shown in FIG. **4** includes excitation illumination devices **10** (including a first excitation illumination device **11** and a second excitation illumination device **12**), a microscope body **20**, a camera **30**, a camera controller **40**, a control unit **50**, a display device **60** and a storage unit **70**.

**[0115]** The excitation illumination devices **10** (including the first excitation illumination device **11** and the second excitation illumination device **12**) are devices that provide the microscope body **20** with excitation light for making a fluorescent substance emit light. Any device can be used as the excitation illumination devices **10** (including the first excitation illumination device **11** and the second excitation illumination device **12**) as long as it can provide excitation light of a wavelength in accordance with the fluorescent substance. Although they are not shown, each of the first excitation illumination device **11** and the second excitation illumination device **12** may be a system combining a plurality of devices and usually includes a laser beam source, a shutter, and a total reflection mirror. The laser beam source is a light source that emits excitation light. The shutter is a device that switches between supply and suspend of excitation light to the microscope body **20**. The total reflection mirror is a mechanism for totally reflecting excitation light emitted from the laser beam source toward a stage **21** of the microscope body **20**.

**[0116]** The microscope body **20** may be an inverted microscope for example. The microscope body **20** includes the stage **21** for mounting thereon a sample that is to be observed. To the microscope body **20**, the camera **30** for picking up a fluorescence image of the sample mounted on the stage **21** is connected. As the camera **30**, for example a CCD camera having a plurality of pixels can be used.

**[0117]** The microscope body **20** is provided with a an objective lens for issuing excitation light toward the stage **21**, an imaging lens for condensing, on the light receiving plane of the camera **30**, fluorescence radiated from a fluorescent substance in the sample, etc. The above objective lens and the imaging lens constitute an imaging optical system.

**[0118]** The microscope body **20** including the stage **21** and the imaging optical system is configured to be able to provide total internal reflection illumination by which excitation light is totally reflected on the interface between the glass in contact with the sample and the sample. The total internal reflection illumination makes it possible to illuminate the sample with evanescent light that leaks to the sample side from the glass when the excitation light is totally reflected. In the shown embodiment, the second excitation illumination device **12** is equivalent to a device for providing excitation light through total internal reflection illumination.

**[0119]** The microscope body **20** is further configured to be able to switch between the above total internal reflection illumination and epi-illumination to use them. In the shown embodiment, the first excitation illumination device **11** is equivalent to a device for providing excitation light based on epi-illumination. Using a speckle image of fluorescence based on the total internal reflection illumination of excitation light and a speckle image of fluorescence based on epi-illumination makes it possible to calculate the position in the depth direction (z direction) in an observation image. Alternatively, it is also possible to make a probe emit intensive fluorescence by issuing excitation light based on

epi-illumination and excitation light based on total internal reflection illumination simultaneously.

**[0120]** The excitation illumination devices **10** do not always have to include the first excitation illumination device **11** and the second excitation illumination device **12** and may have only one of the first and second excitation illumination devices **11** and **12**.

**[0121]** The control unit **50** is a computer that totally controls the microscope apparatus **1**, and is connected to the excitation illumination devices **10** (first excitation illumination device **11** and second excitation illumination device **12**), the display unit **60**, the storage unit **70**, and the camera controller **40**. The control unit **50** has at least a control signal generation function of generating a control signal for controlling these devices, a speckle image obtainment function of obtaining a speckle image via the camera controller **40**, an image analysis function of analyzing an obtained speckle image, a light source control function of controlling driving of a light source included in the excitation illumination devices **10**, and an image forming function of generating an observation image from a plurality of speckle images. The control unit **50** constitutes an image generation unit that generates an observation image as the overall function.

**[0122]** The camera controller **40** performs driving control of the camera **30**. The camera controller **40** operates the camera **30** on the basis of a control signal input from the control unit **50** so as to obtain a speckle image of fluorescence, and outputs the obtained speckle image to the control unit **50**.

**[0123]** The display unit **60** is a display (display device), a printer (printing device), etc., and provides a function of displaying and printing an image based on image data output from the control unit **50** (data of a speckle image, data of an observation image, etc.).

**[0124]** The storage unit **70** can be configured by a storage device such as a semiconductor memory, a hard disk etc. A program used in the control unit **50** and data provided from the control unit **50** (such as a speckle image etc.) are stored in a state in which they can be read by the control unit **50**.

**[0125]** Hereinafter, explanations will be given for the observation method of the present invention, by the microscope apparatus **1**, that uses a fluorescent substance emitting fluorescence when irradiated with excitation light as a luminescent substance contained in a probe.

**[0126]** An imaging step using the microscope apparatus **1** is a step in which a step is performed a plurality of times at different times respectively, where a sample that has been brought into contact with a medium containing a probe on the stage **21** is irradiated with excitation light by using the excitation light illumination devices **10** (one or both of the first excitation illumination device **11** and the second excitation illumination device **12**) and a speckle image including, as a speckle of fluorescence, fluorescence emitted from a substance contained in a probe that has bound to a target substance in the sample is obtained by the camera **30**, and thereby a plurality of speckle images are obtained. A step of obtaining each speckle image will be referred to as a "frame imaging step". In each frame imaging step, the camera **30** picks up a speckle image of fluorescence emitted from a fluorescent substance each time excitation light is issued. A speckle image has a different pattern for each frame.

**[0127]** In each frame imaging step, the control unit **50** operates the camera **30** via the camera controller **40** so as to pick up an image of fluorescence emitted from a fluorescent substance, and thereby obtains a speckle image. An obtained speckle image is output to the control unit **50** from the camera controller **40**. The control unit **50** also stores a thus-obtained speckle image in the storage unit **70**.

**[0128]** In an imaging step, the control unit **50** repeatedly performs the above frame imaging step at appropriate intervals. Examples of the number of times of the frame imaging step may include hundreds of times through hundreds of thousands of times including for example 1,000 to 999,000 times.

**[0129]** An observation image generation step in the present invention is a step in which an observation image of a target substance that binds to the probe in the sample is generated from a plurality of speckle images obtained in the above imaging step. Specifically, the control unit **50** executes an appropriate computer program so as to obtain, for each of the plurality of speckle images recorded in the storage unit **70**, information of position of a speckle included in a speckle image so as to generate an observation image by integrating the pieces of information from the plurality of speckle images. In this example, information of the position of each speckle is typically information of the central position (position of the center of gravity) of each speckle, and can be obtained by using for example DAOSTORM, a computer program (Nature methods, 8 279-280, 2011). In the above, in order to increase the accuracy, only a speckle of luminance that is equal to or higher than a prescribed threshold may be used for generating an observation image in each of the speckle images. When generating an observation image, an observation image can be generated by drawing, at the central position of each speckle of a blank image, a point having an appropriate size. Specifically, by turning the size of pixels of a blank image into an appropriate size (for example a square pixel with the length of each side being 5 nm through 20 nm) and by plotting the central position of each speckle to each pixel, an observation image can be generated. The control unit **50** records the data of the generated observation image in the storage unit **70** and also displays it in the display unit **60**.

**[0130]** When a plurality of target substances exist in one sample, it is sufficient to perform the above imaging step sequentially by using probes that are specific to the respective target substances. "Probes that are specific to respective target substances" refer to probes that repeatedly bind to and dissociate from the respective target substances directly and specifically. It is preferable that the sample be washed sufficiently between the respective imaging steps. Then, the control unit **50** can generate an observation image of each target substance in the sample from a plurality of speckle images, stored in the storage unit **70**, obtained in the respective imaging steps. The control unit **50** can also synthesize pieces of data of observation images of a plurality of target substances in the same sample so as to output the result to the display unit **60** and display a multiple-observation image resulting from superposing observation images of a plurality of target substances.

**[0131]** The above is an embodiment of the observation method of the present invention that uses a probe containing a fluorescent substance as a luminescent substance. When a luminescent substance is a luminescent substance that is not a fluorescent substance, the method of the present invention can be implemented through the same procedures as that described above if means for providing a condition in a sample mounted on the stage **21** for that luminescent sub-

stance to radiate light instead of the excitation illumination devices **10** in the microscope apparatus **1** are arranged and the camera **30** that can pick up an image of light emitted by the luminescent substance in the probe that has bound to a target substance in the sample as a speckle is used.

5. Probe and Kit

**[0132]** The present invention also provides the above probe itself and a kit including at least the above probe.

**[0133]** The above probe may be provided solely in solid form such as powder, may be provided in liquid form that has dispersed or has been dissolved in an appropriate liquid medium or may be provided in solid form such as powder together with an appropriate solid component (such as a diluting agent etc.). In other words, a probe of the present invention may be provided solely by itself or may be provided as a probe-containing composition that includes at least a probe and may include a different complementary component.

**[0134]** Any kit may be used as the above kit as long as it includes at least a probe of the above various forms and it may further include a different element used for observing a target substance. Examples of a different element for the above kit may include a medium such as a liquid medium for dissolving or dispersing the probe etc., and a reagent to be used for a process of observing a sample etc. The above medium may be a medium that includes a probe and that is used for bringing it into contact with a sample for performing observation. The probe and a different element in the above kit are usually wrapped separately so that they will not be mixed physically.

6. Screening Method

**[0135]** The present invention also provides a screening method of a site (also referred to as a target substance identification site or a binding substance) in which a target substance is identified in the probe accordingly, the screening method including:

**[0136]** an immobilization step in which a candidate substance of the site or a substance partially containing the candidate substance is fixed to a solid support;

**[0137]** an observation step in which a target substance linked to a luminescent substance and a solid support obtained in the immobilization step are observed in a medium while the target substance linked to a luminescent substance and the solid support obtained in the immobilization step are kept in contact, in a condition that allows observation, in units of 1 molecule, of light emission from the luminescent substance in a probe-target complex formed by binding between the target substance and the candidate substance, and

**[0138]** a screening step in which the candidate substance resulting in a half-life of the probe-target complex that is equal to or more than 10 milliseconds and equal to or less than 3 seconds is selected as the site on the basis of observation in the observation step.

**[0139]** According to this embodiment of the present invention, it is possible to efficiently perform screening for a substance that presents a desired binding tendency to a target substance from a library of candidate substances.

**[0140]** Examples of a solid support used for an immobilization step may include an inner wall surface of each well of for example a multiwell plate.

**[0141]** Means for fixing a candidate substance to a solid support in an immobilization step are not particularly limited, and when for example a candidate substance is an antibody or an antibody fragment, it is preferable that a Fab domain of an antibody or an antibody fragment be immobilized to the solid support in a state in which it can bind to the target substance. For example, by fixing to a solid support a protein having a binding capability to an Fc domain of an antibody (for example, Protein G) and then immobilizing the antibody to the solid support to which the protein has been immobilized, it is possible to immobilize the antibody to the solid support in a state in which the Fab domain can bind to the target substance. Methods of immobilizing the protein to a solid support are not particularly limited, however it is possible to immobilize the protein via a functional group introduced to a surface of a solid support.

**[0142]** As a medium used in an observation step, the same medium as that used in an imaging step of the present invention can be used.

**[0143]** Types of luminescent substances are not particularly limited in a target substance linked to the luminescent substance used in an observation step, however a fluorescent substance that can emit fluorescence when irradiated with excitation light is particularly preferable. Specific examples of a fluorescent substance are the same as those of the fluorescent substance described above for a probe.

**[0144]** In an observation step, in a medium, a target substance linked to a luminescent substance and a solid support to which a candidate substance is fixed are observed in a state in which they are in contact with each other, in a condition that allows observation, in units of 1 molecule, of emission of light by the luminescent substance in a probe-target complex of the target substance and the candidate substance. In this step, the fact that light emission of a target substance that has not bound cannot be detected because it causes thermal motions randomly in the medium, whereas emission of light from the target substance that has bound to the candidate substance can be detected and is utilized. In order to observe emission of light by the luminescent substance in units of 1 molecule, it is effective to reduce the concentration of the target substance in the medium. Even when a candidate substance is a bivalent antibody or antibody fragment having two antigen binding sites, reducing the concentration of the target substance in the medium makes it possible to observe binding of the target substance individually in each antigen binding site.

**[0145]** Observation means in an observation step may appropriately be selected in accordance with the luminescent substance. When the luminescent substance is a fluorescent substance, observation is possible by using a fluorescence microscope (for example, a TIRF fluorescence microscope).

**[0146]** In a screening step, on the basis of observation in the observation step, the candidate substance leading to the half-life of the probe-target complex that is equal to or more than 10 milliseconds and equal to or less than 3 seconds is selected as a target substance identification site. The half-life is more preferably equal to or more than 10 milliseconds and equal to or less than 2 seconds, more preferably equal to or more than 10 milliseconds and equal to or less than 1 second, more preferably equal to or more than 10 milliseconds and equal to or less than 900 milliseconds, more preferably equal to or more than 10 milliseconds and equal to or less than 800 milliseconds, more preferably equal to or more than 10 milliseconds and equal to or less than 700 milliseconds,

more preferably equal to or more than 10 milliseconds and equal to or less than 600 milliseconds, more preferably equal to or more than 10 milliseconds and equal to or less than 500 milliseconds, more preferably equal to or more than 20 milliseconds and equal to or less than 300 milliseconds, and particularly preferably equal to or less than 250 milliseconds. The half-life is defined by a period of time before the number of target substances that have bound to candidate substances on solid supports at a given moment is reduced to half through dissociation. Measurement procedures for the half-life are as below. A medium containing a target substance labeled with a luminescent substance is brought into contact with a solid support to which the candidate substance has been fixed, and a period of time between when a light emission speckle appears and when it disappears is measured, for each light emission speckle, while performing observation under a condition used for observation. Then, periods of time between the appearance and disappearance of the speckles are plotted in accordance with a complementary cumulative relative frequency function (1-Ndissociation). Then, by fitting the above complementary cumulative relative frequency with an exponent function, that half-life is calculated.

**[0147]** A period of time between appearance and disappearance of a light emission speckle based on a labeled target substance is measured by the following procedures. Specifically, while keeping a medium containing a labeled target substance in contact with a solid support to which the candidate substance has been immobilized and providing a prescribed condition necessary for emitting light (for example irradiation with excitation light), speckle images including speckles based on the light emission of the labeled target substance are consecutively picked up with an exposure time of X seconds (for example 0.050 seconds, 0.100 seconds, etc.) and at a frame rate of 1/XHz. Then, a period of time is measured between when a target substance that has bound to a candidate substance appears in a speckle image and when it disappears through dissociation. In the above, observation is performed for Y consecutive frames, and for a speckle not observed in frames before and after them, the period of time between the appearance and disappearance of that speckle can be treated as YX seconds.

**[0148]** In a screening method of the present invention, the candidate substance is more preferably an antibody or a fragment of an antibody that binds to a prescribed target substance and fixes the antibody to a solid support in the immobilization step. When screening is performed for antibodies binding to prescribed target substances, ELISA is generally used in which target substances are immobilized to solid supports and candidate antibodies are brought into contact with the solid supports. In a screening method according to the present embodiment, contrary to ELISA, an antibody (the candidate substance) is solid phased. Then, in the medium, an antigen (target substance) linked to a luminescent substance and the solid support are observed in a state in which they are in contact with each other, in a condition that allows observation, in units of 1 molecule, of emission of light by the luminescent substance in a probe-target complex formed by binding of the target substance and the candidate substance. This method has advantages as described below.

**[0149]** 1) It is possible to measure the half-life of binding of an antibody and an antigen. Because binding/dissociation between an antibody and an antigen is visualized through the appearance and disappearance of a fluorescent single molecule, the half-life of that binding can be measured directly for each single molecule of an antigen.

**[0150]** 2) It is possible to measure univalent binding/dissociation of an antigen and an antibody. Epitope that allows identification of a monoclonal antibody is at one location in an antigen. Accordingly, an antigen that has bound to 1 antigen binding site of a solid-phased antibody cannot bind to a different antigen binding site existing nearby, and an antigen inevitably binds with the antibody molecule in a univalent state. When an antigen has been solid phased, an antibody can bind to one or two antigens, making it impossible to measure univalent binding/dissociation of an antigen and an antibody. A method in which this antibody is solid phased makes it possible to estimate the half-life of binding of an antibody fragment such as a Fab fragment that binds in a univalent manner without producing it.

**[0151]** 3) It is possible to perform screening for an antibody fragment from a library of hybridoma in an inexpensive and simple manner. In one embodiment of this screening method, main structure factors are a solid support resulting from immobilizing an antibody contained in each supernatant of a hybridoma library and a labeling body of antigen epitope. The half-life of binding of an antibody fragment and an antigen can be estimated even without producing a light-emission-labeled antibody fragment from an antibody of each supernatant of a library, making it possible to perform screening on candidate substances at a very low cost. Also, visualization is performed in units of molecules, measurement is possible even when the amount of antibodies that have been solid phased is small. Thereby, immense amounts of labor and time taken for cultivation of a hybridoma library can be saved.

**[0152]** The present screening method has solved a major problem in evaluation of the affinity of an antibody by ELISA. 1) In ELISA, an antigen is solid phased, and accordingly an antibody may bind to an antigen regardless of whether the binding is univalent or bivalent. Further, by a secondary antibody used for measuring a binding amount of primary antibodies, a primary antibody is crosslinked. This prevents measurement of the half-life of univalent binding of an antibody and an antigen. 2) The binding amount of antibodies obtained in ELISA is influenced not only by the affinity of antibodies but also by the amount of antibodies produced by hybridoma. This makes it difficult to evaluate the property of binding of one antibody in ELISA. 3) In addition, because washing is conducted a plurality of times in a reaction step in ELISA, there is a risk that an antibody that has bound with a short half-life that is equal to or less than 3 seconds, which is needed in the present invention, may be lost before being visualized. The method of the present embodiment has solved these problems by solid phasing an antibody instead of an antigen and visualizing binding/dissociation of an antigen and an antibody in actual time. The method of the present embodiment is more suitable for selecting an antibody having a short binding half-life, which is the aim of the present invention, than ELISA in principle in that it can evaluate univalent binding/dissociation, it is not influenced by the production amount of antibodies, and it can also measure a short binding half-life.

**[0153]** According to a screening method of the present invention, a library of polypeptides and a library of phage display can be solid phased as candidate substances for a

target substance identification site of a probe in addition to a antibody or an antibody fragment. A screening method of the present invention can also be applied to solid-phasing of these for performing screening for a polypeptide that binds to and dissociates from a target substance.

**[0154]** The present invention will be explained in more detail by the following examples, however they are just exemplary and do not limit the present invention. All the experiments below were conducted at an ambient temperature, i.e., 25 degrees Celsius, unless limitations are particularly given.

## 1. Experiment 1

### Methods

### Plasmid and Reagent

**[0155]** Expression plasmid (pFLAG-EGFP-C1) encoding EGFP (sequence number 1) having an N terminus tagged with FLAG (sequence number 2) and an expression plasmid (p3×FLAG-EGFP-N3) encoding EGFP having a C terminus tagged with 3×FLAG (sequence number 3) were constructed respectively by using a pEGFP-C1 vector and a pEGFP-N3 vector (Clontech Laboratories, Inc).

**[0156]** EST clones encoding mouse MAP4, human Tau isoforms 3 and 4, mouse KIF1A, human plectin-1 and *Xenopus laevis* talin-1 were purchased from OpenBiosystems.

**[0157]** cDNA encoding human FAK was purchased from the DNASU Plasmid Repository.

**[0158]** The GenBank/EMBL/DDBJ accession numbers for each sequence are as follows:

**[0159]** BC055332 (MAP4), BC114948 (Tau isoform 3), BC101936 (Tau isoform 4), BC062891 (KIF1A), BM559026 (Plectin-1), CF282569 (Talin1) and BC035404 (FAK).

**[0160]** cDNAs encoding human EB1, rat CLIP-170, human CLASP2γ and human APC were provided by Y. Mimori-Kiyosue (RIKEN).

**[0161]** As plasmids encoding human paxillin, chicken Src and human vinculin, those described in documents 31 to 33 were used.

**[0162]** Each cDNA was inserted into a pFLAG-EGFP-C1 vector or a p3×FLAG-EGFP-N3 vector by using a PCR. For a probe having an N terminus at the fusion position of GFP in table 1 below, a pFLAG-EGFP-C1 vector was used. For a probe having a C terminus at the fusion position of GFP in table 1 below, a p3×FLAG-EGFP-N3 vector was used.

**[0163]** An expression plasmid encoding PIPKIγ fragment (amino acid residues 641-668) having an N terminus tagged with FLAG-EGFP was constructed by inserting a synthetic cDNA encoding PTDERSWVYSPLHYSAQAPPASDGESDT (sequence number 18) into a pFLAG-EGFP-C1 vector.

**[0164]** Lifeact peptide (MGVADLIKKFESISKEE (sequence number 19)) with an Atto 488 fluorescent body linked to an N terminus was purchased from Sigma-Aldrich.

**[0165]** The amino acid sequence of EGFP is denoted by sequence number 1.

**[0166]** The amino acid sequence of FLAG is denoted by sequence number 2.

**[0167]** The amino acid sequence of 3×FLAG is denoted by sequence number 3.

**[0168]** The amino acid sequence of mouse MAP4 is denoted by sequence number 4.

**[0169]** The amino acid sequence of human Tau isoform 3 is denoted by sequence number 5.

**[0170]** The amino acid sequence of human Tau isoform 4 is denoted by sequence number 6.

**[0171]** The amino acid sequence of mouse KIF1A is denoted by sequence number 7.

**[0172]** The amino acid sequence of human Plectin-1 is denoted by sequence number 8.

**[0173]** The amino acid sequence of *Xenopus laevis* Talin1 (amino acid residues 1-2353) is denoted by sequence number 9.

**[0174]** The amino acid sequence of human FAK is denoted by sequence number 10.

**[0175]** The amino acid sequence of human EB1 is denoted by sequence number 11.

**[0176]** The amino acid sequence of rat CLIP-170 is denoted by sequence number 12.

**[0177]** The amino acid sequence of human CLASP2γ is denoted by sequence number 13.

**[0178]** The amino acid sequence of human APC is denoted by sequence number 14.

**[0179]** The amino acid sequence of human Paxillin is denoted by sequence number 15.

**[0180]** The amino acid sequence of chicken Src is denoted by sequence number 16.

**[0181]** The amino acid sequence of human Vinculin is denoted by sequence number 17.

**[0182]** The amino acid sequence of human PIPKIγ-90 fragment (amino acid residues 641-668) is denoted by sequence number 18.

**[0183]** The amino acid sequence of Lifeact peptide is denoted by sequence number 19.

### Production and Screening of Exchangeable Protein Probes

**[0184]** In order to find exchangeable probes for super-resolution images of microtubules, intermediate filaments and focal adhesions, a test was conducted by using, as candidate molecules for probes, a protein (polypeptide) and a protein fragment that are known to be able to localize in each target structure. "Exchangeable" used herein refers to being able to repeatedly bind to and dissociate from a target structure.

**[0185]** Table 1 shows the tested probe candidates. The test was conducted a plurality of times by using, as a probe candidate, a protein or a fragment of a protein mentioned in the section of "plasmid and reagent".

**[0186]** An expression plasmid for expression as a molecule made to fuse with EGFP was constructed for each probe candidate in accordance with the documents described in table 1.

**[0187]** HEK (Human embryonic kidney)-293F cells were transfected with a plasmid encoding a probe candidate protein tagged with FLAG-EGFP or 3×FLAG-EGFP. 3 to 4 days later, the cells were dissolved in a cell lysis buffer (10 mM HEPES, pH 7.2, 90 mM KCl, 3 mM MgCl2, 0.2% Triton X-100, 100 μM DTT) containing a protease inhibitor cocktail (Nacalai Tesque). Centrifugal separation was performed on the lysate and the supernatant liquid was collected. In order to perform screening for a probe for IRIS, the binding capacity of a probe candidate in the supernatant liquid with respect to the structure of an XCT cell that was fixed with paraformaldehyde (PFA) and that received a

permeabilization process was tested. The appearance and disappearance of single molecule speckles (Single-molecule speckles, SiMS) in the structure was tested. Screening was performed for a probe for IRIS in accordance with the following criteria:

(1) It is possible to confirm a distribution of target substances in the structure in an image resulting from adding SiMS images;

(2) It is possible to wash and remove a probe after SiMS imaging (imaging step);

(3) A probe that has bound can dissociate from the target substance swiftly (half-life is equal to or less than 500 ms); and

(4) It is possible to reconstruct an image of a target substance by integrating the central positions of the respective speckles.

**[0188]** Table 1 describes that "localization" is positive (P) when above criterion (1) is met, that "washability" is positive (P) when above criterion (2) is met, and that "IRIS image" is positive (P) when above criterion (3) is met.

**[0189]** Probes used for an IRIS experiment were purified in the following procedures. Specifically, each probe was overexpressed in HEK-293F cells and collected with anti-DYKDDDDK (Flag) antibody beads (Wako). The beads were washed four times with an excess amount of HEPES-buffered solution (10 mM Hepes pH 7.2, 90 mM KCl, 3 mM MgCl2, 100 μM DTT). Proteins that had bound to beads after the washing were processed with the HEPES-buffered solution containing 0.5 mg/ml DYKDDDDK (Flag) peptide (Wako) or 3×FLAG peptide (Sigma-Aldrich) for 30 minutes and were eluted.

**[0190]** The localization test of the above (1), whose result is shown in table 1, was performed in the following procedures. First, the supernatant liquid of lysate of a cell that expressed each probe candidate was brought into contact with an XCT cell that was fixed on a coverglass and that received a permeabilization process. A coverglass on which the sample was mounted was arranged in the observation chamber of a fluorescence microscope apparatus, which will be described in detail in the section of "procedures for imaging of multicolor super-resolution by IRIS" below, and speckle images of 10000 frames were obtained with an exposure time of 50 ms or 100 ms for one frame and at a frame rate of 20 Hz (20 frames per second) or with an exposure time of 100 ms and at a frame rate of 10 Hz (10 frames per second) while irradiating the sample with a 488 nm-laser beam line (with a main body output of 50 mW but reaching the sample after being attenuated by AOTF etc.) for total internal reflection fluorescence observation. The obtained speckle images of 10000 frames were integrated so as to confirm whether or not a distribution of target substances in the cell (microtubule, intermediate filament, focal adhesion or actin filament) was able to be confirmed. When a distribution of a target substances was able to be confirmed, the result of the localization test was treated as P (positive).

**[0191]** The localization test of the above (2), whose result is shown in table 1, was performed in the following procedures. After the picking up of speckle images explained in the previous paragraph, 1 ml of the supernatant liquid containing respective probe candidates in the observation chamber was aspirated by using an aspirator and 1 ml of an imaging solution (however, it was not supplemented with active oxygen-scavenging mix) that does not contain a probe and that will be described below was added. This switching of imaging solution was conducted slowly so that the observation position would not shift. Next, an imaging solution not containing a probe and not containing the active oxygen-scavenging mix was switched 10 to 20 times. After the switching, for confirmation, for an XCT cell sample in the observation chamber, speckle images of 10 frames were picked up under a similar condition to that of the localization test described in the previous paragraph, and when the number of speckles confirmed in the speckle images (i.e., the number of probes that had bound) became sufficiently smaller (equal to or less than about 10%) than the number of the speckles in the speckle images before the washing operation, washing was determined to be possible and the result was treated as P (positive).

**[0192]** The measurement of binding half-life, whose result is shown in table 1, was performed in the following procedures. Speckle images of 10000 frames picked up in the localization test were used in order to measure a period of time between the appearance of a probe that has bound to a target in a speckle image and disappearance through dissociation in a semi-manual mode by using Speckle TrackerJ, an ImageJ plug-in. In case of for example picking up of images with an exposure time of 50 ms and at a frame rate of 20 Hz, it is assumed that a period of time between the appearance and disappearance of a speckle that is observed in only one of consecutive frames is 50 ms and a period of time between the appearance and disappearance of a speckle observed in only two consecutive frames is 100 ms. Then, the number of binding probes with respect to periods of time between the appearance and disappearance was plotted in accordance with a complementary cumulative relative frequency function (1-Ndissociation). Ndissociation is a cumulative relative frequency of probes that dissociated. Then, by fitting the complementary cumulative relative frequency function with an exponent function, the half-life was calculated. However, the binding half-life with respect to an actin filament of Atto488-Lifeact was measured by using a method that is described in detail in the section for FIG. **9** in the Brief Description of the Drawings.

**[0193]** The IRIS image test of the above (3) whose result is described in table 1 was performed in the following procedures. By using all the speckle images of 10000 frames picked up in the localization test, the central point of a probe that has bound to a target in each frame of the speckle images was determined with nanometer accuracy by using DAOSTORM. By adding a large number of pieces of central point information in the fluorescence images of 10000 frames, a reconstruction image (observation image) was generated. Whether or not a distribution of a target molecule was able to be observed in that reconstruction image was determined, and when a distribution of a target molecule was observed, the result was treated as P (positive).

Procedures for Imaging of Multicolor Super-Resolution by IRIS

**[0194]** *Xenopus laevis* XTC cells were cultured in 70% Leibovitz's L15 medium supplemented with 10% fetal bovine serum. A multicolor super-resolution image was produced from a large number of fluorescence single molecule speckle (SiMS) images that were sequentially obtained from a fixed and XTC cell (20,000 to 500,000 frames per probe) with various exchangeable probes. The cells were allowed to spread on a 0.1 mg/ml poly (L-lysine) and 10

g/ml fibronectin-coated coverglass in 70% Leibovitz's L15 medium without serum and distinct stress fibers and focal adhesions were formed (document 33). 2 hours later, the cells were fixed and received a permeabilization process with a cytoskeleton buffer containing 3.7% PFA and 0.5% Triton X-100 in (10 mM Mes pH6.1, 90 mM KCl, 3 mM MgCl2, 2 mM glycol ether diamine tetraacetic acid (EGTA)). After performing blocking with 4% bovine serum albumin for 30 minutes, the purified IRIS probes were brought into contact with the cells in an imaging solution including the HEPES-buffered solution (10 mM Hepes pH 7.2, 90 mM KCl, 3 mM MgCl2, 100 μM DTT) with an oxygen-scavenging mix (200 μg/ml glucose oxidase, 35 μg/ml catalase, 4.5 mg/ml glucose, 0.5% 2-mercaptoethanol) (document 34). The concentration of the probe was 1 nM through 100 nM. When the oxygen-scavenging mix was not used, laser-induced photodamage was apparent after obtaining SiMS images several tens of thousands of times.

**[0195]** For the imaging of actin filaments in vitro, monomeric actin was prepared from rabbit skeletal muscle in a method described in documents 33, 35 and 36. Phalloidin-stabilized F-actin was observed on a 1 mg/ml poly (L-lysine)-coated coverglass in the imaging solution.

**[0196]** SiMS images were obtained by using an inverted microscope (Olympus IX83-ZDC) equipped with an Olympus PlanApo 1.45×100 through a numerical aperture (NA) objective lens, a 2×intermediate lens and an EM-CCD camera (Evolve 512, Roper), and controlled by MetaMorph software (Molecular Device). The focus was automatically maintained at the bottom of the cell by a z drift compensation system during the long-term imaging. The IRIS probe was alternately excited with a 473-nm laser beam (50 mW) for epi-illumination microscopy and a 488-nm laser beam (50 mW) for epi-fluorescence observation in the following procedures. In the epi-illumination mode, the incidence angle of the 473-nm laser beam was tilted so as to reduce background fluorescence from out-of-focus probes that have not bound. The epi-fluorescence image and TIRF image were used to estimate the z position of the target object (see below). Specifically, images were picked up by repeating the following procedures:

(a) imaging in bright field;

(b) imaging of a SiMS image (speckle image) with epi-fluorescence (exposure time for one frame: 50 ms, frame rate: 20 Hz (20 frames per second), number of frames picked up consecutively: 250 frames); and

(c) imaging of SiMS image (speckle image) with TIRF (exposure time for one frame: 50 ms, frame rate: 20 Hz (20 frames per second), number of frames picked up consecutively: 250 frames).

**[0197]** An image obtained in a bright field was used to correct a drift of the microscope stage in a lateral direction (see below) In case of observation of focal adhesions, epi-fluorescence observation was not performed and TIRF observation was performed (frame rate: 20 Hz, 500 frames). Each one of the procedures required 27 seconds and was repeated 160 to 240 times (probe/target substance=CLIP-170 fragment) (amino acid residues 3-309 of sequence number 12)/microtubule, 40 times (probe/target substance=PIPKIγ fragment (641-668) (sequence number 18), Paxillin (overall length of sequence number 15) and the Src fragment (amino acid residues 3-251 of sequence number 16)/focal adhesion), 800 times (probe/target substance=Lifeact (sequence number 19)/actin filament), and 400 to 480 times (probe/target substance=Plectin-1 fragment (amino acid residues 4022-4364 of sequence number 8/intermediate filament). In order to maintain the oxygen-scavenging capacity in the imaging solution, an imaging solution containing the probe was replaced with a fresh imaging solution every 40 sets when the CLIP-170 fragment, the PIPKIγ fragment, paxillin and the Src fragment were used and every 80 sets when Lifeact and the plectin-1 fragment were used. For multicolor imaging of three types of cytoskeletons and focal adhesions, a pick up of SiMS images was conducted in the order of the CLIP-170 fragment, the PIPKIγ fragment (or the Src fragment and paxillin), Lifeact and the plectin-1 fragment. After obtaining SiMS images on the basis of each probe, washing was conducted 10 times by using the HEPES-buffered solution. The remaining fluorescence of a probe was completely photobleached in the HEPES-buffered solution supplemented with an oxygen-scavenging mix, and the next probe was made to react.

**[0198]** In the present example, imaging steps of obtaining observation images were conducted under the above conditions unless otherwise described.

Procedures for Image Reconstruction in IRIS

**[0199]** A super-resolution image was reconstructed by plotting the central points of each fluorescent speckle on a blank image with subpixel accuracy. The number of plotted points was typically $10^6$ to $10^8$. A central point was estimated with subpixel accuracy by fitting of a point-spread function (PSF) of this microscope using a computer program known as DAOSTORM (document 14). In order to correct a stage drift of the microscope, the drift distance was calculated by an autocorrelation function, i.e., $A_N(x_{drift}, y_{drift})$ of the bright-field images obtained at each set of imaging procedures.

[Numerical expression 1]

$$A_N(x_{drift}, y_{drift}) = \sum_{y=y_0}^{y_m} \sum_{x=x_0}^{x_m} [I_0(x, y) \times I_N(x + x_{drift}, y + y_{drift})]$$

where $x_{drift}$ and $y_{drift}$ are drift distances in the directions of the x axis and y axis, respectively. $I_0(x, y)$ and $IN(x+x_{drift}, y+y_{drift})$ are intensities at the pixel positions (x, y) and $(x+x_{drift}, y+y_{drift})$ in the bright field images obtained in the 1st and Nth sets, respectively.

**[0200]** The product of $I_0(x, y)$ and $IN(x+x_{drift}, y+y_{drift})$ is integrated within a prescribed region in the bright-field image. $A_N(x_{drift}, y_{drift})$, which is a function of variables $x_{drift}$ and $y_{drift}$, becomes the maximum when the two bright-field images coincide. The $x_{drift}$ and $y_{drift}$ values leading to a maximum value as $A_N(x_{drift}, y_{drift})$ were calculated by using a customized plug-in in ImageJ software (http://rsb.info.nih.gov/ij/). First, the drift of the bright-field image in the Nth set was corrected using the $x_{drift}$ and $y_{drift}$ values with pixel accuracy. To further determine the drift distance with subpixel accuracy, the bright-field image and the corrected image in the first and Nth sets were enlarged by using a bicubic method. Using the $A_N(x_{drift}, y_{drift})$ of the enlarged images, the drift distances were determined with subpixel accuracy. The central positions of speckles in the SiMS images in the Nth set were corrected with the drift distances.

By plotting the corrected central positions, a super-resolution image was produced. The positions of speckles consecutively observed in 10 or more frames or in 20 or more frames were not used when generating a reconstruction image (observation image) based on Lifeact or generating a reconstruction image (observation image) based on the plectin-1 fragment. These two probes in some cases bound to a target substance when strong excitation laser output was employed.

Image Process for Mapping z Position of Observation Target Object

**[0201]** Because a TIRF excitation light intensity exponentially decreases with increasing distance from the coverglass, the height of the observation target object was estimated from the ratio of a TIRF image and an epi-fluorescence image. The z position from the coverglass surface was measured by the method, described in document 18, using fluorescent microtubules that were tilted with respect to the coverglass in a low-melting-point agarose gel. HyLight 488-labeled tubulin was purchased from Cytoskeleton. The labeled microtubules were prepared according to the method described in document 18. Images of the tilted microtubules each having one end touching the coverglass were picked up by TIRF and epi-fluorescence. Epi-fluorescence images were obtained as z stack images (0.2-μm step size) (FIG. **14** (*a*)). In the intensity line profile along the microtubules of epi-fluorescence images, the x-y position of the highest intensity was used to determine the intersection of the tilted microtubule and the focal plane (FIG. **14** (*a*) arrows). By connecting the intersections among the z-stacked images, the z-directional distance of each point along the tilted microtubule was obtained. The z profile of the excitation light intensity based on a TIRF method was determined by associating the z-directional distance of each point with the ratio of the intensity of the microtubule in a TIRF image and the intensity of the microtubule in an epi-fluorescence image (FIG. **14** (*b*)). This z profile was fitted with a single exponential decay function (FIG. **14** (*b*)). The scale of the z positions were again adjusted by a factor of 0.82, taking into consideration the difference in the refractive index between the immersion oil and the imaging solution (document 18). The inverse function of the exponential function was used to determine the z position of the observation target object by the following numerical expression.

$$z = -\alpha_z \ln\left(\beta \frac{F^{TIRF}}{F^{Epi}}\right) \qquad \text{[Numerical expression 2]}$$

(where $\alpha_z$ is the z position at which the intensity of TIRF illumination is 1/e, β is a parameter for calibrating the difference in the laser output between TIRF observation and epi-fluorescence observation, and $F^{TIRF}$ and $F^{Epi}$ are the fluorescence intensities of the target object in a TIRF image and an epi-fluorescence image, respectively)

**[0202]** The z-position maps of three types of cytoskeletons were converted from the image of a ratio of the IRIS image (observation image) based on TIRF to the IRIS image (observation image) based on an epi-fluorescence image. For this purpose, the peak intensity of the fluorescent speckle was also fitted by using DAOSTORM. The IRIS images were reconstructed by plotting the peak intensity at the central position of the speckle. In the obtained z-position map, the fluorescence intensity in an image resulting from adding the IRIS image based on TIRF and the IRIS image based on epi-fluorescence was masked by a threshold and noise in a region having no cytoskeletons was removed. In a layered structure such as an actin stress fiber, etc., the calculated z position represents the height of the center of gravity of the structure in the z axial direction.

**[0203]** The z position of the microtubule plus end traced by live-cell imaging of EB1-EGFP was obtained by converting a ratio of an average intensity in a 0.4-μm-diameter region of the microtubule plus end in the TIRF image to that in a corresponding region in the epi-fluorescence image.

Live-Cell Imaging of the Movement of Microtubule Plus End

**[0204]** XTC cells were transfected with an expression plasmid of EGFP fused EB1. 3 to 4 days later, live-cell imaging of EB1-EGFP was conducted on the cells at one-second intervals. In each interval term, an imaging with an exposure time of 100 ms of fluorescence by excitation light for epi-fluorescence observation and imaging with an exposure time of 100 ms of fluorescence by excitation light for total reflection fluorescence observation were conducted. In the above, each excitation light was emitted under the above conditions for IRIS super-resolution except that the excitation light was emitted after having its laser power reduced to about 20% of that used for an IRIS super-resolution imaging in order to avoid damaging live cells. At each imaging time point, two fluorescence images were obtained by alternately using total internal reflection illumination and epi-illumination with an exposure time of 100 milliseconds. An EB1-labeled microtubule plus end was traced by using Speckle TrackerJ, an ImageJ plug-in (documents 33 and 37). The z position of the traced microtubule plus end was calculated by the above method. The speed of the site of the microtubule plus end was calculated with a linear approximation of its x-y positions at five consecutive imaging time points.

Results

**[0205]** The results of screening tests on probe candidates were as below.

TABLE 1

| TARGET SUBSTANCE | PROBE | SEQUENCE NUMBER | AMINO ACID NUMBER | EGFP FUSION POSITION | LOCALIZATION | WASHABILITY | HALF-LIFE | IRIS IMAGE | DOCUMENT |
|---|---|---|---|---|---|---|---|---|---|
| MICROTUBULE | CLIP-170 | 12 | 3-309 | N TERMINUS | P | P | 44 ms | P | 41 |
| | APC | 14 | 2536-2843 | C TERMINUS | P | P | 100 ms | P | 43.44 |
| | APC | 14 | 2781-2819 | N TERMINUS | P | P | 27 ms | P | 43.44 |
| | MAP4 | 4 | 1-908 | N TERMINUS | P | P | 109 ms | P | 47 |

TABLE 1-continued

| TARGET SUBSTANCE | PROBE | SEQUENCE NUMBER | AMINO ACID NUMBER | EGFP FUSION POSITION | LOCALI-ZATION | WASH-ABILITY | HALF-LIFE | IRIS IMAGE | DOCUMENT |
|---|---|---|---|---|---|---|---|---|---|
| | MAP4 | 4 | 659-908 | N TERMINUS | P | P | 106 ms | P | 47 |
| | TAU ISOFORM 3 | 5 | 1-383 | N TERMINUS | P | P | 110 ms | P | 48.49 |
| | TAU ISOFORM 4 | 6 | 1-352 | N TERMINUS | P | P | 60 ms | P | 48.49 |
| INTERMEDIATE | Plectin-1 | 8 | 3777-4684 | N TERMINUS | P | P | 457 ms | P | 16 |
| FILAMENT | Plectin-1 | 8 | 3777-4364 | N TERMINUS | P | P | 59 ms | P | 16 |
| | Plectin-1 | 8 | 3777-4313 | N TERMINUS | P | P | 52 ms | P | 16 |
| | Plectin-1 | 8 | 4022-4364 | N TERMINUS | P | P | 103 ms | P | 16 |
| | Plectin-1 | 8 | 4066-4364 | N TERMINUS | P | P | 7.7 s | NA | 16 |
| FOCAL | Paxillin | 15 | 1-557 | N TERMINUS | P | P | 196 ms | P | 50 |
| ADHESION | Paxillin | 15 | 54-557 | N TERMINUS | P | P | 246 ms | P | 50 |
| | Paxillin | 15 | 54-498 | N TERMINUS | P | P | 91 ms | P | 50 |
| | Paxillin | 15 | 167-557 | N TERMINUS | P | P | 138 ms | P | 50 |
| | Src | 16 | 1-251 | C TERMINUS | P | P | 161 ms | P | 59 |
| | Src | 16 | 3-251 | C TERMINUS | P | P | 141 ms | P | 59 |
| | PIPKIγ90 | 18 | 641-668 | N TERMINUS | P | P | 496 ms | P | 17 |
| ACTIN FILAMENT | Atto488-Lifeact | 19 | — | — | — | — | 23 ms | — | — |

**[0206]** In the table, P represents positive while NA indicates that measurement was not conducted (not accessed).

**[0207]** In the sequence table, sequence number 18 represents an amino acid sequence of fragments 641-668 of PIPKIγ90.

**[0208]** A probe candidate that had not passed either a localization test or a washing test received neither a test of an IRIS image nor half-life measurement, and thus is not described in the above table 1. A probe candidate that did not pass a test is estimated to have a very long or very short binding half-life to a target substance or to not bind to a target substance.

**[0209]** Because the test described in detail in FIG. **9** and the explanations for the figure have independently confirmed that Atto488-Lifeact is effective for an actin filament and that the half-life thereof is 23 nm, these facts are described in the above table as reference results.

Super-Resolution of Actin Filament in High Labeling Density by IRIS

**[0210]** The present inventors confirmed whether or not a super-resolution image can be generated through the IRIS method by using Lifeact, which is a widely used actin marker. Lifeact is a short peptide that stains an actin filament in a live cell or a fixed cell (document 10). Lifeact has a property of being exchanged within 0.4 seconds on an actin filament (document 10). The high-speed exchangeability of Lifeact is confirmed through single-molecule speckle (SiMS) microscopy (documents 11 to 13). The dwell time of Atto488-labeled Lifeact showed a single exponential decay of a half-life of 23 milliseconds (FIG. **9** (*a*)) The present inventors selected a concentration of 2.4 nM as the Atto488-labeled Lifeact concentration in the imaging solution and obtained $2\times10^5$ SiMS images of Lifeact on an actin filament in vitro through consecutive imaging using a total internal reflection illumination of 488 nm with an exposure time of 50 ms/frame and at a frame rate of 20 Hz. The central position of each fluorescent speckle was determined by using a computer program named DAOSTORM (document 14) (FIG. **9** (*b*)). Pieces of position information from a great number of Lifeact speckles in the above speckle images of $2\times10^5$ frames were integrated so as to reconstruct an image of actin filaments (FIG. **5** (*b*) and FIG. **9** (*c*)). The average width of single actin filaments was 23 nm as the full-width at half-maximum (FWHM) in an image reconstructed by using only high-brightness speckles (highest 12% approximately) in order to guarantee a high accuracy of localization (FIG. **5** (*c*) and FIG. **9** (*d*)).

**[0211]** A major problem in conventional super-resolution microscopy is that using an antibody and a photoactivatable fluorescent protein makes it difficult to label an observation target structure in a sufficient density. The actin subunit and the antibody have the widths of 6 nm and 12 nm, respectively, and accordingly a single actin filament having 360 subunits per 1 μm can only be used for labeling up to a density of at most 180 subunits per 1 μm in the labeling of antibodies. This labeling density is equivalent to the labeling density in an observation image reconstructed from speckle images of $2\times10^3$ frames in the IRIS method of the present inventors. As shown in the left view of FIG. **5** (*d*) and in FIG. **5** (*e*), actin filaments show a pattern that is not continuous in the longitudinal direction in an observation image reconstructed from speckle images of $2\times10^3$ in the IRIS method. Even a labeling density that was 6.5 times its original was not sufficient for continuous staining of actin filaments (FIG. **5** (*d*)), however it was possible to achieve a labeling density of $1.2\times10^4$ for an observation image reconstructed from speckle images of $2\times10^5$ frames and to obtain consecutive super-resolution images of actin filaments (FIG. **5** (*d*) and FIG. **5** (*e*)). As described above, it was made obvious that the IRIS method of the present invention can eliminate the problem of a labeling density that had conventionally been an obstacle to dissolving two or three types of target substances coexisting close to each other.

**[0212]** For a cell that was fixed and had received a permeabilization process, in an observation image generated by using the IRIS method that utilizes a Lifeact probe of an actin filament, it was possible to dissolve two parallel actin bundles that were apart by 50 nm (FIG. **5** (*d*) through FIG. **5** (*f*)). By contrast, in an image resulting from adding SiMS images (speckle images) (which is equivalent to an image that can be obtained by a conventional immunofluorescence method), it was not possible to dissolve two such actin bundles (lower left view of FIG. **5** (*g*)). According to the IRIS method, it is possible to obtain consecutive observation

images of an actin filament or a microtubule (below). This is remarkable progress from the conventional super-resolution microscopy (FIG. **10**).

Establishment of Screening Method of IRIS Probe

**[0213]** The present inventors established an effective method for swiftly determining an IRIS probe for a different cell structure while taking into consideration a necessary molecular characteristic determined by data using a Lifeact probe. A probe candidate was generated by making a protein and EGFP fuse with each other, the protein and the EGFP being known to bind to a target substance. Live-cell fluorescence single molecule speckle (SiMS) microscopy (documents 11 and 12) is also effective for testing a probe candidate. However, the present inventors discovered that the binding specificity and dissociation dynamics of a probe candidate can easily be clarified by bringing crude lysate of a cell that expresses EGFP fused probe candidate into contact with a fixed cell. The inventors selected an IRIS probe in the following four steps:

(i) SiMS images (speckle images) of 10,000 frames were picked up at intervals of 50 ms or 100 ms, and probe candidates that were not able to localize in targets in an integrated SiMS image were excluded;

(ii) probe candidates that were not able to be easily removed through washing were excluded;

(iii) probe candidates having high dissociation speeds (with a half-life of 10 ms through 500 ms, see FIG. **11**) were selected; and

(iv) it was confirmed, in a reconstruction IRIS image (observation image), that probe candidates were able to localize in targets.

**[0214]** As shown in table 1, 18 types were selected by the above screening method from among the probe candidates. By a plurality of probes for microtubule and focal adhesion, different sites of their structure bodies were able to be visualized (FIG. **12** and FIG. **13**). Also, independently from the above, it has been confirmed that Atto488-Lifeact is effective as a probe for an actin filament and is effective for the visualization of an actin filament (FIG. **9** etc.). This suggests that IRIS is effective for mapping a distribution of probe identification sites in one structure body.

Super-Resolution Image of a Plurality of Target Substances by IRIS

**[0215]** It was discovered that Lifeact, a CLIP-170 fragment (residues 3-309), a Plecin-1 fragment (residues 4022-4364) and a phosphatidyl inositol-(4)-phosphate 5-kinase type Iγ-90 (PIPKIγ) fragment (residues 641-668) are preferable respectively for observation of actin, microtubules, intermediate filaments and focal adhesion. By utilizing the exchangeability of IRIS probes, images in which the IRIS probes bound to 4 different types of cytoskeleton structures were obtained sequentially.

**[0216]** Further, the present inventors investigated a three-dimensional (3D) network of an actin filament, a microtubule and an intermediate filament. An image based on each IRIS probe was obtained by alternately using total internal reflection illumination and epi-illumination, and super-resolution images at the bottom and in the entire peripheral region of the cell were reconstructed respectively by using the obtained images. These images show that actin arcs running parallel to the cell contour gradually rise as if they were climbing on the radial actin bundles localizing at the bottom (arrows in FIG. **6** (*a*)) with decreasing distance to the center. It was confirmed that microtubules and intermediate filaments behind the lamellipodium (lobopodium) base were eliminated from the cell bottom at several locations (arrows in FIG. **6** (*b*) and FIG. **6** (*c*)).

**[0217]** The z position of each observation target object was estimated by using a signal ratio between an IRIS image based on TIRF and an IRIS image based on epi-fluorescence on the basis of measurements that used images of tilted fluorescent microtubules described in an above method (document 18). The 3D images as shown in FIG. **15** clarified the structure of the target substance. Lamellipodia (LP), stress fibers (SF) and actin arcs (Arc) were distributed with height positions of their centers of gravity (average ±S.D.) of 42±43 nm, 34±30 nm and 217±132 nm, respectively (arrows in FIG. **15** (*a*) and FIG. **15** (*d*)). The z positions of the microtubules became lower from 150 nm through 200 nm to 50 nm through 100 nm in the vicinity of the cell perimeters (arrowheads in FIG. **15** (*b*) and FIG. **15** (*e*)). Intermediate filaments formed mesh-shaped structures throughout the cell body, and some of the filaments were located at a height of approximately 200 nm behind the lamellipodium (ellipse in FIG. **15** (*c*)).

**[0218]** An IRIS image makes it possible to observe a spatial relationship between a plurality of cytoskeletal structures in a single cell at a resolution capability exceeding the diffraction limit. In a lamellar region, intermediate filaments were tangled with actin stress fibers in many cases, but they are not tangled with microtubules (FIG. **7** (*a*) through FIG. **7** (*c*)). The tangled actins and intermediate filaments appear to be linked to each other. Their cross-sectional profiles show that actin stress fibers overlapped intermediate filaments (arrows in FIG. **7** (*e*)). In peripheral regions, intermediate filaments did not tangle with actin stress fibers (FIG. **7** (*g*) and FIG. **7** (*i*)), whereas some intermediate filaments ran along microtubules (arrows in FIG. **7** (*h*)). The cross-sectional profiles show that intermediate filaments overlapped microtubules but did not overlap actin filaments (arrows in FIG. **3** (*i*), and FIG. **3** (*j*)). Thus, IRIS can reveal a region-specific proximity between 4 cytoskeletal components.

Behavior of Plus End of Microtubule in Live Cell and Comparison with Super-Resolution of Cytoskeleton Network

**[0219]** The present inventors discovered that the heights of microtubules locally change in the vicinity of focal adhesions and stress fibers. When microtubules crossed focal adhesions and stress fibers, they were lifted to positions at 200 nm from positions at 100 nm from the glass surface (arrowheads in FIG. **8** (*a*), FIG. **8** (*b*) and FIG. **16**). Components of focal adhesions are located at a height of 30 nm through 80 nm from the glass surface (document 19). It appears that the lifted microtubules climbed on actin stress fibers without touching focal adhesions.

**[0220]** The present inventors further investigated a relationship between a microtubule plus end and a cytoskeleton network in that portion by observing the behavior of EB1-EGFP in a live cell and then reconstructing a super-resolution image of a 3D cytoskeleton network after fixation. The imaging system of the present inventors makes it possible to perform live-cell 3D imaging with acousto-optic tunable filters for swiftly switching between total internal reflection illumination and epi-illumination. The trajectory of the tip of

the EB1-labeled microtubule was compared with focal adhesions and stress fibers in a super-resolution image (asterisk in FIG. **8** (*c*)). When the tip of the microtubule glowing was brought into contact with a stress fiber, the tip moved upward so as to move away from a focal adhesion existing below (FIG. **8** (*d*)). When the tip of the EB1-labeled microtubule was brought into contact with a stress fiber, the growth slowed down and the moving direction changed (arrow in FIG. **8** (*d*)). These pieces of data suggest that the speed and the direction of growth of a microtubule are greatly influenced by the collision and subsequent interaction with an actin stress fiber. As described above, a combination between IRIS and live-cell imaging makes it possible to clarify formation processes of a plurality of cytoskeletal structures that dynamically interact with each other.

Consideration

**[0221]** Consideration 1: Comparison with PAINT

**[0222]** Document 20 (Proceedings of the national Academy of Sciences of the United States of America 103, 18911-18916 (2006)) has reported a method called PAINT (point accumulation for imaging in nanoscale topography). This document has reported that super-resolution observation based on PAINT was conducted on lipid bilayer morphology by using Nile-red, which is a fluorescent dye that rapidly shuttles between an aqueous solution and the lipid bilayer. However, Nile-red is not a probe that binds to and dissociates from a specific bilayer molecule type. This prevents highly accurate determination of the position of a specific lipid molecule type that constitutes a lipid layer. By contrast, a probe of IRIS can bind to and dissociate from a specific target protein from among various types of proteins existing in a cell so as to make it possible to determine the position of the molecule thereof highly accurately. In addition, the concept of PAINT does not include a concept of obtaining a distribution of a plurality of target substances through probe exchange of IRIS. High resolution-capability imaging of various target substances by IRIS cannot be realized by the concept of PAINT. Also, this document has does not disclose improving of resolution by increasing the number of times of observation in PAINT. The reason appears to be that even an increased number of times of observation does not fix a lipid layer, which is a target substance, and thus the resolution does not improve through a change in the shape. In addition, the reason also seems to be that the problem of labeling density first became widely recognized a few years later than the document as an unsolved problem among those skilled in the art (documents 4 through 7).

**[0223]** The problem of labeling density was proposed in 2008 (document 6), whereas it had long been an unsolved problem in super-resolution microscope observation (documents 4, 5 and 7). As a method of increasing a target density, the review of 2013 (document 5) introduced an attempt to make fluorescent dye, which is an organic compound that is smaller than an antibody and a fluorescent protein, stably bind to a target substance and to make a single domain antibody such as Nanobody etc., which stably binds, stably bind to a target substance. This means that PAINT had not been recognized as a method of increasing a target density. Also, even the method introduced in the above review did not achieve a labeling density equivalent to that realized by the present invention, and the problem of a labeling density had remained unsolved. From these points, it can be said that there had not been an idea of using a binding and dissociation probe for solving the problem of a labeling density. In FIG. **10**, an observation image of a microtubule based on IRIS and those based on STORM and Exchange-PAINT, which are conventionally known super-resolution microscopy, are compared (documents 21 to 23). According to the line profiles of label intensities along the longitudinal direction of microtubules, the observation image based on IRIS shows a more continuous pattern than those of observation images based on STORM and Exchange-PAINT (FIG. **10** (*b*)). Also, it is easy for the labeling density of actin filaments based on IRIS to become 60 times the maximum density of antibodies that bind to actin filaments. From these results, it is obvious that IRIS according to the present invention can solve the problem of labeling density that has caused a deterioration in reliability of the conventional super-resolution microscopy.

**[0224]** In addition to the ability to overcome the problem of labeling density, according to IRIS of the present invention, it is easy to visualize a plurality of target substances by protein-based exchangeable probes and there are no limitations on the number of target substances. In super-resolution microscopy that has conventionally been known and available, only up to 2 target substances can be observed (documents 21, 22, 24 and 25). Recently, Exchange-PAINT, which makes it possible to conduct super-resolution observation of a plurality of target substances, was announced (document 23 (Nature methods, 11, 313-318, 2014)). Exchange-PAINT uses characteristic labeling means of hybridizing short and fluorescence-labeled DNA with complementary DNA conjugated to antibody molecules. Exchange-PAINT is similar to IRIS in that it sequentially visualizes a plurality of target substances one by one by sequentially hybridizing pairs of various types of oligonucleotides. However, in Exchange-PAINT, uneven labeling and/or interference between antibodies sometimes lead to inaccurate analysis of the distribution of labeling substance (FIG. **10** (*b*)). By contrast, an IRIS probe is washed and removed and thereafter the next IRIS probe is brought into contact with the sample, resulting in no interference between a plurality of probes that are respectively for a plurality of target substances. The data of the present inventors shows the region-specific proximity of three types of cytoskeleton structures and focal adhesions with an accuracy exceeding the diffraction limit. In principle, the number of target substances that can be observed by IRIS is not limited even when they coalesce in a narrow region. This effect is remarkably advantageous in comparison with existing super-resolution microscopy.

Consideration 2: Probe

**[0225]** As shown in the above table, the present inventors have confirmed that probes of Document 19 are actually effective for the IRIS method. The above probes have been probes that use a binding partner known to associate with a target substance material, however the scope of the invention is not limited to this example. Using a phage display or an assay that confirms interactions between proteins of yeast-two hybrid system etc. makes it possible to perform screening for useful IRIS probes.

**[0226]** A plurality of IRIS probes for a microtubule and a focal adhesion visualized different sites of their structure bodies. A MAP4 fragment and a tau protein weakly visualized a microtubule plus end when EB1 existed (FIG. **12** (*e*)).

A CLIP-170 fragment strongly labeled a microtubule tip when EB1 existed and continuously labeled the entire microtubule when EB1 did not exist (FIG. **12** (*c*) and FIG. **12** (*e*)). The result corresponds to in-vitro data (document 26). Further, Paxillin and the Src fragment visualized different portions of focal adhesion (FIG. **13**). Thus, IRIS can also be applied to mapping analysis of a site in which a plurality of protein fragments bind. Development of an IRIS probe that binds to a molecule in a specific state also makes it is possible to perform super-resolution mapping on the molecule in the specific state.

2. Experiment 2

**[0227]** 2.1. Method of Producing a Fab Probe from a Polyclonal Antibody

**[0228]** An anti-p40 antibody including antiserum was produced from a rabbit by using an antigen *Xenopus laevis* derived p40 produced in the present inventors' laboratory. Rabbit antiserum was put into an affinity column filled with antigens and polyclonal antibodies were made to adsorb on the column. pH in the column was reduced in a stepwise manner (pH5 to pH2) and the antibodies made to adsorb were eluted. 2 ml of fraction eluted at a high pH (pH2 through pH3.5) was added to 1.4 ml of a 50% slurry solution of Protein A Protein beads (Protein A Sepharose CL-4B, GE) and the antibodies were made to adsorb on the beads over 1 night at 4 degrees Celsius. After using PBS to wash and remove antibodies that had not been made to adsorb, the beads were suspended in 1 ml of PBS. To this suspension of beads, 50 μl of 1 μg/μl DyLight 488 NHS Ester (Thermo-Scientific) in which DMSO was dissolved was added, and antibodies were fluorescence-labeled at an ambient temperature over one hour. DyLight 488 NHS Ester that had made no reactions was washed and removed by using PBS and only beads were collected through centrifugation. In order to produce a Fab fragment from an antibody that was made to adsorb on beads, 1 ml of 7 μg/ml papain dissolved in a Digestion Buffer (50 mM Tris-HCl pH8.0, 10 mM Cysteine-HCl, 2 mM EDTA) was added and reactions were caused for 1 hour at 37 degrees Celsius. After the centrifugation, supernatant containing a Fab fragment was collected and 1 μl of 1 mg/ml leupeptin was added in order to inhibit the activity of papain. The obtained Fab fragment was put into an affinity column filled with antigens, and the Fab fragment was made to adsorb. pH in the column was reduced in a stepwise manner and a Fab fragment of a fraction eluted at a high pH (pH 5 to pH 4) was obtained. As described above, in the production of a Fab fragment from a polyclonal antibody, column purification was conducted twice. By using the affinity column of the first time, an antibody having a weak antigen-binding force was purified. From that antibody, a Fab fragment having a further weak antigen-binding force was produced. By using the affinity column of the second time, a Fab fragment in which an antigen-binding force still remained was purified. A Fab fragment prepared in this method was derived from IgG.

2.2. Iris Super-Resolution Imaging of Actin

**[0229]** Preparation of Arp2/3 complex observation cell sample By following the procedures described in detail in "procedures for imaging of multicolor super-resolution by IRIS" of experiment 1, a *Xenopus laevis* XTC cell was fixed and received a permeabilization process. After a blocking step with 4% bovine serum albumin for 30 minutes, the Fab probes were brought into contact with the cells in an imaging solution comprising the HEPES-buffered solution (10 mM Hepes pH 7.2, 90 mM KCl, 3 mM MgCl2, 100 μM DTT, 0.1% Triton X-100) supplemented with an oxygen-scavenging mix (200 μg/ml glucose oxidase, 35 μg/ml catalase, 4.5 mg/ml glucose, 0.5% 2-mercaptoethanol). The Fab probe concentration was 100 nM in the imaging solution.

Imaging and Image Reconstruction

**[0230]** Similarly to "procedures for imaging of multicolor super-resolution by IRIS" of experiment 1, SiMS images (speckle images) were obtained by using an inverted microscope (Olympus IX83-ZDC) equipped with an Olympus PlanApo 100×/1.45-numerical aperture (NA) objective lens, a 2×intermediate lens and an EM-CCD camera (Evolve 512, Roper) and controlled by MetaMorph software (Molecular Device). The Fab probe fluorescence-labeled by being irradiated with a 488 nm laser beam (50 mW) was excited for TIRF observation. SiMS images (speckle images) of a total of 33,750 frames were picked up by conducting consecutive imaging of 250 frames for each under a condition that the exposure time was 100 milliseconds for 1 frame and the frame rate (imaging speed) was 10 Hz (10 frames per second).

**[0231]** The procedures for image reconstruction from the above SiMS images of 33,750 frames are as described in detail in "procedures for image reconstruction in IRIS" of experiment 1.

**[0232]** The half-life of a probe-target complex formed between a purified Fab probe and a target antigen was obtained in a similar method to that in Experiment 1. In other words, by using the SiMS images picked up in the above, a period of time between when the Fab probe that had bound to the target appeared in a speckle image and it disappeared through dissociation was measured in a semi-manual mode by using Speckle TrackerJ, a plug-in of ImageJ. Then, the number of binding probes with respect to the period of time between the appearance and disappearance was plotted in accordance with a complementary cumulative relative frequency function (1-Ndissociation). Then, by fitting the complementary cumulative relative frequency function with an exponent function, the half-life was calculated. The result showed that the half-life of a probe-target complex formed between the Fab probe and a target antigen was 216 milliseconds.

2.3. Results

**[0233]** FIG. **17** shows a reconstruction image based on the Fab probe generated from a polyclonal antibody derived from a rabbit with respect to a p40 subunit of an Arp2/3 complex, which is an actin polymerization promotion factor. It is known that much p40 is distributed in a peripheral region of a cell and a distribution of Fab probes was visualized as such.

3. Experiment 3

**[0234]** Screening of Fab Probe

**[0235]** A library (1200 samples) of hybridoma was generated by using FLAG peptide (sequence number 2) as an antigen. After immunizing a mouse with an antigen, lymphocytes were collected from an iliac lymph node and were made to fuse with a mouse myeloma cell, and hybridoma

was generated. The library of hybridoma was made through dilution culture. Culture supernatant was collected for each sample from the hybridoma library and was made to react with a 96 well glass bottom plate in which Protein G was solid phased and thereby an antibody in the culture supernatant was fixed to the surface of the bottom glass.

**[0236]** The 96 well glass bottom plate in which Protein G was solid phased and immobilization of the antibody were conducted in the following procedures. (3-aminopropyl) triethoxysilane was dissolved in a mixture of methanol and acetic acid (mixing ratio 100:5) and a 3% solution was produced. This solution was put in a 96 well multi-well plate having each of its wells made of glass, the solution was incubated for 30 minutes at an ambient temperature, and the glass surfaces of the well bottoms were coated. (3-aminopropyl) triethoxysilane that had made no reactions was washed a plurality of times with methanol so as to remove it and thereafter the plate was air dried so that (3-aminopropyl) triethoxysilane was made to remain on the glass surfaces. Next, a 0.1% glutaraldehyde solution was added to each well so as to cause reactions for 30 minutes at an ambient temperature and glutaraldehyde was made to bind to the glass surfaces. A 50 ng/μl Protein G (Thermo Scientific) solution was added to each well and was kept in contact with the glass surfaces to which glutaraldehyde bound, for 1 night at 4 degrees Celsius. Protein G causes covalent bonding with (3-aminopropyl) triethoxysilane via glutaraldehyde and is solid phased on the glass surfaces. Blocking was conducted on the glass surfaces on which Protein G was solid phased, with a 3% BSA solution for 1 night at 4 degrees Celsius. Thereafter, a hybridoma culture supernatant containing an antibody was added to each well and was left to stand for 1 night at 4 degrees Celsius. An antibody contained in the culture supernatant binds, specifically and strongly, to Protein G via the Fc domain thereof and is fixed to the glass surface. After removing the hybridoma culture supernatant, the glass surfaces were washed with a cell lysis buffer (10 mM Hepes pH 7.2, 3 mM MgCl2, 0.2% Triton X-100, 100 μM DTT), and received an observation step, which will be described later. In order to obtain a fused protein of FLAG peptide and FLAG peptide that becomes an antigen, an HEK-293F cell was transfected with a plasmid that encoded FLAG-EGFP. After culturing the transfected cell for 3 to 4 days, it was dissolved in a cell lysis buffer (10 mM Hepes pH 7.2, 3 mM MgCl2, 0.2% Triton X-100, 100 μM DTT) containing a protease inhibitor cocktail (Nacalai Tesque). After centrifugally separating the lysate, the supernatant liquid was collected. The FLAG-EGFP concentration contained in the supernatant liquid was estimated from the light emission intensity of EGFP, and a 50 nM FLAG-EGFP solution was prepared by diluting it with a cell lysis buffer.

**[0237]** A 50 nM FLAG-EGFP solution was added to each well to which an antibody was fixed. By using a TIRF microscope, SiMS images (speckle images) of 500 frames were picked up with an exposure time of 50 ms for 1 frame and at a frame rate of 20 Hz (20 frames per second). As a result of observing 1200 wells, 10 to 300 speckles (0.006 to 0.178 speckles/μm2) were observed in the field of view of the microscope (41 μm×41 μm). Each speckle corresponds to EGFP of a single molecule. As examples of speckle images, FIG. **18**A shows an image in which about 250 speckles were observed and FIG. **18**B shows an image in which about 20 speckles were observed. In the case where 50 nM FLAG-EGFP solutions were added to wells in which antibodies had not been solid phased, the numbers of the speckles in the field of view were 10 to 20 (0.006 to 0.012/μm2). Then, the half-lives of binding between antibodies and FLAG-EGFP were measured by treating, as positive examples, a 26 well in which 40 (0.023/μm2) or more speckles had been observed in the field of view. Each of the wells had different half-lives. The half-life in the well shown in FIG. **18**A was 55 ms.

**[0238]** From the 26 well in which 40 or more speckles had been observed in the field of view of the microscope as the positive examples, antibodies having half-lives of the probe-target complex that were equal to or more than 10 milliseconds and equal to or less than 3 seconds were selected. Hybridoma producing these antibodies was converted into monoclone and 2 ml to 10 ml of the culture supernatant was collected. 40 μl of a 50% slurry solution of Protein A beads (Protein A Sepharose CL-4B, GE) was added to the culture superresolution, and the antibodies were made to adsorb on the beads for 1 night at 4 degrees Celsius. After using PBS to wash and remove antibodies that had not adsorbed, the beads were suspended in 100 μl of PBS. To this suspension of beads, 16 μl of 0.5 μg/μl DyLight 488 NHS Ester (ThermoScientific) dissolved in DMSO was added, and the antibodies were fluorescence-labeled for 1 hour at an ambient temperature. DyLight 488 NHS Ester that had made no reactions was washed and removed with PBS and only beads were collected after centrifugation. In order to produce a Fab fragment from an antibody that bound to the beads, 20 μl of 0.01 mg/ml papain dissolved in Digestion Buffer (50 mM Tris-HCl pH8.0, 10 mM Cysteine-HCl, 2 mM EDTA) was added and reactions were caused for 1 hour at 37 degrees Celsius. After the centrifugation, supernatant containing a Fab fragment was collected and 2 μl of 0.01 mg/ml leupeptin was added in order to inhibit the activity of papain.

2.2. IRIS Super-Resolution Imaging of Actin

**[0239]** Preparation of a FLAG Tag Fused Actin Expression XTC Cell Sample

**[0240]** FLAG tag fused actin encoded by the base sequence described by sequence number 20 was expressed with an XTC cell.

**[0241]** Expression vector-(CLONETECH) encoding pEGFP-actin was cut with restriction enzyme Age-I, Bgl-II (NEB) to remove EGFP from the vector. Synthetic cDNA encoding FLAG peptide (DYKDDDDK) was inserted into the vector and an expression plasmid encoding an actin for which FLAG was tagged to an N terminus was constructed.

**[0242]** A *Xenopus laevis* XTC cell was transfected with the above expression plasmid in the following procedures. 3 μl of an expression plasmid of 1 μg/μ of FLAG fused actin was dissolved in 200 μl of a serum-free medium (70% dilution Leibovitz's L-15 medium) and 8μ of Polyethylenimine, linear, M.W. 25,000 (Polysciences) of 1 mg/ml was added and it was vortexed. After leaving it to stand for 30 minutes at an ambient temperature, a medium with serum of 1 ml (70% dilution Leibovitz's L-15 medium, 10% FCS supplement) was added. The solution containing this plasmid and the media of XTC cells spread in 6 wells were switched, and the cells were made to take in plasmids for 1 night.

**[0243]** 3 to 4 days later than the transfecting, the cells were fixed and received the permeabilization process in the procedures described in detail in "procedures for imaging of multicolor super-resolution by IRIS" of experiment 1. After

a blocking step with 4% bovine serum albumin for 30 minutes, the Fab probes were each brought into contact with the cells in an imaging solution comprising the HEPES-buffered solution (10 mM Hepes pH 7.2, 3 mM MgCl2, 100 μM DTT, 1 μg/ml leupeptin) supplemented with an oxygen-scavenging mix (200 μg/ml glucose oxidase, 35 μg/ml catalase, 4.5 mg/ml glucose, 0.5% 2-mercaptoethanol). The Fab probe concentration was 3 nM in the imaging solution.

Imaging and Image Reconstruction

**[0244]** Similarly to "procedures for imaging of multicolor super-resolution by IRIS" of experiment 1, SiMS images (speckle images) were obtained by using an inverted microscope (Olympus IX83-ZDC) equipped with an Olympus PlanApo 100×/1.45-numerical aperture (NA) objective lens, a 2×intermediate lens and an electron-multiplying EM-CCD camera (Evolve 512, Roper) and controlled by MetaMorph software (Molecular Device). The Fab probe fluorescence-labeled by being irradiated with a 488 nm laser beam (50 mW) was excited for TIRF observation. SiMS images (speckle images) of a total of 120,000 frames were picked up by conducting consecutive imaging of 500 frames for each under a condition such that the exposure time was 50 milliseconds for 1 frame and the frame rate (imaging speed) was 20 Hz (20 frames per second).

**[0245]** The procedures for image reconstruction from the above SiMS images of 120,000 frames are as described in detail in "procedures for image reconstruction in IRIS" of experiment 1.

2.4. Results

**[0246]** FIG. **19** shows IRIS super-resolution images of FLAG fused actin by a Fab probe derived from an anti-FLAG monoclonal antibody selected by the above screening method. The half-life of a probe-target complex of a Fab probe and an FLAG fused actin used for the generation of these IRIS super-resolution images was 203 milliseconds.

**[0247]** The present screening method made it possible to select a Fab probe appropriate for IRIS super-resolution imaging.

4. Documents

**[0248]** The documents referred to herein are described below.

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**[0272]** 24. Shroff, H. et al. Dual-color superresolution imaging of genetically expressed probes within individual adhesion complexes. Proc. Natl. Acad. Sci. USA 104, 20308-20313, doi:10.1073/pnas.0710517105 (2007).

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**[0306]** The present invention can provide a super-resolution microscope observation method that makes it possible to obtain, at a high density, position information of luminescent substances used for labeling and to generate a high resolution observation image exceeding the diffraction limit, and also it is made possible to clarify the formation process of a plurality of cytoskeleton structures that interact dynamically, by a combination between the present invention and a living-cell image technique, and thus the present invention has a high industrial applicability.

**[0307]** All publications, patents and patent applications referred to herein are incorporated herein in their entirety by reference.

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Pro Gly Thr Pro Gly Ser Arg Ser Arg Thr Pro Ser Leu Pro Thr Pro
145                 150                 155                 160

Pro Thr Arg Glu Pro Lys Lys Val Ala Val Val Arg Thr Pro Pro Lys
                165                 170                 175

Ser Pro Ser Ser Ala Lys Ser Arg Leu Gln Thr Ala Pro Val Pro Met
            180                 185                 190
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Pro Asp Leu Lys Asn Val Lys Ser Lys Ile Gly Ser Thr Glu Asn Leu
        195                 200                 205

Lys His Gln Pro Gly Gly Gly Lys Val Gln Ile Val Tyr Lys Pro Val
    210                 215                 220

Asp Leu Ser Lys Val Thr Ser Lys Cys Gly Ser Leu Gly Asn Ile His
225                 230                 235                 240

His Lys Pro Gly Gly Gly Gln Val Glu Val Lys Ser Glu Lys Leu Asp
                245                 250                 255

Phe Lys Asp Arg Val Gln Ser Lys Ile Gly Ser Leu Asp Asn Ile Thr
            260                 265                 270

His Val Pro Gly Gly Gly Asn Lys Lys Ile Glu Thr His Lys Leu Thr
        275                 280                 285

Phe Arg Glu Asn Ala Lys Ala Lys Thr Asp His Gly Ala Glu Ile Val
    290                 295                 300

Tyr Lys Ser Pro Val Val Ser Gly Asp Thr Ser Pro Arg His Leu Ser
305                 310                 315                 320

Asn Val Ser Ser Thr Gly Ser Ile Asp Met Val Asp Ser Pro Gln Leu
                325                 330                 335

Ala Thr Leu Ala Asp Glu Val Ser Ala Ser Leu Ala Lys Gln Gly Leu
            340                 345                 350


<210> SEQ ID NO 7
<211> LENGTH: 1689
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 7

Met Ala Gly Ala Ser Val Lys Val Ala Val Arg Val Arg Pro Phe Asn
1               5                   10                  15

Ser Arg Glu Met Ser Arg Asp Ser Lys Cys Ile Ile Gln Met Ser Gly
            20                  25                  30

Ser Thr Thr Thr Ile Val Asn Pro Lys Gln Pro Lys Glu Thr Pro Lys
        35                  40                  45

Ser Phe Ser Phe Asp Tyr Ser Tyr Trp Ser His Thr Ser Pro Glu Asp
    50                  55                  60

Ile Asn Tyr Ala Ser Gln Lys Gln Val Tyr Arg Asp Ile Gly Glu Glu
65                  70                  75                  80

Met Leu Gln His Ala Phe Glu Gly Tyr Asn Val Cys Ile Phe Ala Tyr
                85                  90                  95

Gly Gln Thr Gly Ala Gly Lys Ser Tyr Thr Met Met Gly Lys Gln Glu
            100                 105                 110

Lys Asp Gln Gln Gly Ile Ile Pro Gln Leu Cys Glu Asp Leu Phe Ser
        115                 120                 125

Arg Ile Asn Asp Thr Thr Asn Asp Asn Met Ser Tyr Ser Val Glu Val
    130                 135                 140

Ser Tyr Met Glu Ile Tyr Cys Glu Arg Val Arg Asp Leu Leu Asn Pro
145                 150                 155                 160

Lys Asn Lys Gly Asn Leu Arg Val Arg Glu His Pro Leu Leu Gly Pro
                165                 170                 175

Tyr Val Glu Asp Leu Ser Lys Leu Ala Val Thr Ser Tyr Asn Asp Ile
            180                 185                 190

Gln Asp Leu Met Asp Ser Gly Asn Lys Ala Arg Thr Val Ala Ala Thr
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        195                 200                 205

Asn Met Asn Glu Thr Ser Ser Arg Ser His Ala Val Phe Asn Ile Ile
    210                 215                 220

Phe Thr Gln Lys Arg His Asp Ala Glu Thr Asn Ile Thr Thr Glu Lys
225                 230                 235                 240

Val Ser Lys Ile Ser Leu Val Asp Leu Ala Gly Ser Glu Arg Ala Asp
                245                 250                 255

Ser Thr Gly Ala Lys Gly Thr Arg Leu Lys Glu Gly Ala Asn Ile Asn
            260                 265                 270

Lys Ser Leu Thr Thr Leu Gly Lys Val Ile Ser Ala Leu Ala Glu Met
        275                 280                 285

Asp Ser Gly Pro Asn Lys Asn Lys Lys Lys Lys Lys Thr Asp Phe Ile
    290                 295                 300

Pro Tyr Arg Asp Ser Val Leu Thr Trp Leu Leu Arg Glu Asn Leu Gly
305                 310                 315                 320

Gly Asn Ser Arg Thr Ala Met Val Ala Ala Leu Ser Pro Ala Asp Ile
                325                 330                 335

Asn Tyr Asp Glu Thr Leu Ser Thr Leu Arg Tyr Ala Asp Arg Ala Lys
            340                 345                 350

Gln Ile Arg Cys Asn Ala Ile Ile Asn Glu Asp Pro Asn Asn Lys Leu
        355                 360                 365

Ile Arg Glu Leu Lys Asp Glu Val Thr Arg Leu Arg Asp Leu Leu Tyr
    370                 375                 380

Ala Gln Gly Leu Gly Asp Ile Thr Asp Met Thr Asn Ala Leu Val Gly
385                 390                 395                 400

Met Ser Pro Ser Ser Ser Leu Ser Ala Leu Ser Ser Arg Ala Ala Ser
                405                 410                 415

Val Ser Ser Leu His Glu Arg Ile Leu Phe Ala Pro Gly Ser Glu Glu
            420                 425                 430

Ala Ile Glu Arg Leu Lys Glu Thr Glu Lys Ile Ile Ala Glu Leu Asn
        435                 440                 445

Glu Thr Trp Glu Glu Lys Leu Arg Arg Thr Glu Ala Ile Arg Met Glu
    450                 455                 460

Arg Glu Ala Leu Leu Ala Glu Met Gly Val Ala Met Arg Glu Asp Gly
465                 470                 475                 480

Gly Thr Leu Gly Val Phe Ser Pro Lys Lys Thr Pro His Leu Val Asn
                485                 490                 495

Leu Asn Glu Asp Pro Leu Met Ser Glu Cys Leu Leu Tyr Tyr Ile Lys
            500                 505                 510

Asp Gly Val Thr Arg Val Gly Arg Glu Asp Ala Glu Arg Arg Gln Asp
        515                 520                 525

Ile Val Leu Ser Gly His Phe Ile Lys Glu Glu His Cys Ile Phe Arg
    530                 535                 540

Ser Asp Ser Arg Gly Gly Gly Glu Ala Val Val Thr Leu Glu Pro Cys
545                 550                 555                 560

Glu Gly Ala Asp Thr Tyr Val Asn Gly Lys Lys Val Thr Glu Pro Ser
                565                 570                 575

Ile Leu Arg Ser Gly Asn Arg Ile Ile Met Gly Lys Ser His Val Phe
            580                 585                 590

Arg Phe Asn His Pro Glu Gln Ala Arg Gln Glu Arg Glu Arg Thr Pro
        595                 600                 605
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Cys Ala Glu Thr Pro Ala Glu Pro Val Asp Trp Ala Phe Ala Gln Arg
    610                 615                 620

Glu Leu Leu Glu Lys Gln Gly Ile Asp Met Lys Gln Glu Met Glu Gln
625                 630                 635                 640

Arg Leu Gln Glu Leu Glu Asp Gln Tyr Arg Arg Glu Arg Glu Glu Ala
                645                 650                 655

Thr Tyr Leu Leu Glu Gln Gln Arg Leu Asp Tyr Glu Ser Lys Leu Glu
            660                 665                 670

Ala Leu Gln Lys Gln Met Asp Ser Arg Tyr Tyr Pro Glu Val Asn Glu
        675                 680                 685

Glu Glu Glu Glu Pro Glu Asp Glu Val Gln Trp Thr Glu Arg Glu Cys
    690                 695                 700

Glu Leu Ala Leu Trp Ala Phe Arg Lys Trp Lys Trp Tyr Gln Phe Thr
705                 710                 715                 720

Ser Leu Arg Asp Leu Leu Trp Gly Asn Ala Ile Phe Leu Lys Glu Ala
                725                 730                 735

Asn Ala Ile Ser Val Glu Leu Lys Lys Lys Val Gln Phe Gln Phe Val
            740                 745                 750

Leu Leu Thr Asp Thr Leu Tyr Ser Pro Leu Pro Pro Asp Leu Leu Pro
        755                 760                 765

Pro Glu Ala Ala Lys Asp Arg Glu Thr Arg Pro Phe Pro Arg Thr Ile
    770                 775                 780

Val Ala Val Glu Val Gln Asp Gln Lys Asn Gly Ala Thr His Tyr Trp
785                 790                 795                 800

Thr Leu Glu Lys Leu Arg Gln Arg Leu Asp Leu Met Arg Glu Met Tyr
                805                 810                 815

Asp Arg Ala Ala Glu Val Pro Ser Ser Val Val Glu Asp Cys Asp Asn
            820                 825                 830

Val Val Thr Gly Gly Asp Pro Phe Tyr Asp Arg Phe Pro Trp Phe Arg
        835                 840                 845

Leu Val Gly Arg Ala Phe Val Tyr Leu Ser Asn Leu Leu Tyr Pro Val
    850                 855                 860

Pro Leu Val His Arg Val Ala Ile Val Ser Glu Lys Gly Glu Val Lys
865                 870                 875                 880

Gly Phe Leu Arg Val Ala Val Gln Ala Ile Ser Ala Asp Glu Glu Ala
                885                 890                 895

Pro Asp Tyr Gly Ser Gly Val Arg Gln Ser Gly Thr Ala Lys Ile Ser
            900                 905                 910

Phe Asp Asp Gln His Phe Glu Lys Phe Gln Ser Glu Ser Cys Pro Val
        915                 920                 925

Val Gly Met Ser Arg Ser Gly Thr Ser Gln Glu Glu Leu Arg Ile Val
    930                 935                 940

Glu Gly Gln Gly Gln Gly Ala Asp Ala Gly Pro Ser Ala Asp Glu Val
945                 950                 955                 960

Asn Asn Asn Thr Cys Ser Ala Val Pro Pro Glu Gly Leu Met Asp Ser
                965                 970                 975

Pro Glu Lys Ala Ala Leu Asp Gly Pro Leu Asp Thr Ala Leu Asp His
            980                 985                 990

Leu Arg Leu Gly Ser Thr Phe Thr  Phe Arg Val Thr Val  Leu Gln Ala
        995                 1000                1005
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Ser Ser  Ile Ser Ala Glu Tyr  Ala Asp Ile Phe Cys  Gln Phe Asn
    1010                1015                1020

Phe Ile  His Arg His Asp Glu  Ala Phe Ser Thr Glu  Pro Leu Lys
    1025                1030                1035

Asn Thr  Gly Arg Gly Pro Pro  Leu Gly Phe Tyr His  Val Gln Asn
    1040                1045                1050

Ile Ala  Val Glu Val Thr Lys  Ser Phe Ile Glu Tyr  Ile Lys Ser
    1055                1060                1065

Gln Pro  Ile Val Phe Glu Val  Phe Gly His Tyr Gln  Gln His Pro
    1070                1075                1080

Phe Pro  Pro Leu Cys Lys Asp  Val Leu Ser Pro Leu  Arg Pro Ser
    1085                1090                1095

Arg Arg  His Phe Pro Arg Val  Met Pro Leu Ser Lys  Pro Val Pro
    1100                1105                1110

Ala Thr  Lys Leu Ser Thr Met  Thr Arg Pro Ser Pro  Gly Pro Cys
    1115                1120                1125

His Cys  Lys Tyr Asp Leu Leu  Val Tyr Phe Glu Ile  Cys Glu Leu
    1130                1135                1140

Glu Ala  Asn Gly Asp Tyr Ile  Pro Ala Val Val Asp  His Arg Gly
    1145                1150                1155

Gly Met  Pro Cys Met Gly Thr  Phe Leu Leu His Gln  Gly Ile Gln
    1160                1165                1170

Arg Arg  Ile Thr Val Thr Leu  Leu His Glu Thr Gly  Ser His Ile
    1175                1180                1185

Arg Trp  Lys Glu Val Arg Glu  Leu Val Val Gly Arg  Ile Arg Asn
    1190                1195                1200

Thr Pro  Glu Thr Asp Glu Ala  Leu Ile Asp Pro Asn  Ile Leu Ser
    1205                1210                1215

Leu Asn  Ile Leu Ser Ser Gly  Tyr Val His Pro Ala  Gln Asp Asp
    1220                1225                1230

Arg Thr  Phe Tyr Gln Phe Glu  Ala Ala Trp Asp Ser  Ser Met His
    1235                1240                1245

Asn Ser  Leu Leu Leu Asn Arg  Val Thr Pro Tyr Arg  Glu Lys Ile
    1250                1255                1260

Tyr Met  Thr Leu Ser Ala Tyr  Ile Glu Met Glu Asn  Cys Thr Gln
    1265                1270                1275

Pro Ala  Val Ile Thr Lys Asp  Phe Cys Met Val Phe  Tyr Ser Arg
    1280                1285                1290

Asp Ala  Lys Leu Pro Ala Ser  Arg Ser Ile Arg Asn  Leu Phe Gly
    1295                1300                1305

Ser Gly  Ser Leu Arg Ala Thr  Glu Gly Asn Arg Val  Thr Gly Val
    1310                1315                1320

Tyr Glu  Leu Ser Leu Cys His  Val Ala Asp Ala Gly  Ser Pro Gly
    1325                1330                1335

Met Gln  Arg Arg Arg Arg Arg  Val Leu Asp Thr Ser  Val Ala Tyr
    1340                1345                1350

Val Arg  Gly Glu Glu Asn Leu  Ala Gly Trp Arg Pro  Arg Ser Asp
    1355                1360                1365

Ser Leu  Ile Leu Asp His Gln  Trp Glu Leu Glu Lys  Leu Ser Leu
    1370                1375                1380

Leu Gln  Glu Val Glu Lys Thr  Arg His Tyr Leu Leu  Leu Arg Glu
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    1385                1390                1395

Lys Leu  Glu Thr Thr Gln Arg  Pro Gly Pro Glu Val  Leu Ser Pro
    1400                1405                1410

Ala Ser  Ser Glu Asp Ser Glu  Ser Arg Ser Ser Ser  Gly Ala Ser
    1415                1420                1425

Ser Pro  Leu Ser Ala Glu Gly  Gln Pro Ser Pro Leu  Glu Ala Pro
    1430                1435                1440

Asn Glu  Arg Gln Arg Glu Leu  Ala Val Lys Cys Leu  Arg Leu Leu
    1445                1450                1455

Met His  Thr Phe Asn Arg Glu  Tyr Thr His Ser His  Val Cys Ile
    1460                1465                1470

Ser Ala  Ser Glu Ser Lys Leu  Ser Glu Met Ser Val  Thr Leu Met
    1475                1480                1485

Arg Asp  Pro Ser Met Ser Pro  Leu Gly Ala Ala Thr  Leu Thr Pro
    1490                1495                1500

Ser Ser  Thr Cys Pro Ser Leu  Ile Glu Gly Arg Tyr  Gly Ala Thr
    1505                1510                1515

Asp Val  Arg Thr Pro Gln Pro  Cys Ser Arg Pro Ala  Ser Pro Glu
    1520                1525                1530

Pro Glu  Leu Leu Pro Glu Leu  Asp Ser Lys Lys Thr  Pro Ser Pro
    1535                1540                1545

Val Arg  Ala Thr Glu Thr Glu  Lys Glu Pro Gln Arg  Leu Leu Val
    1550                1555                1560

Pro Asp  Ile Gln Glu Ile Arg  Val Ser Pro Ile Val  Ser Lys Lys
    1565                1570                1575

Gly Tyr  Leu His Phe Leu Glu  Pro His Thr Ala Gly  Trp Ala Lys
    1580                1585                1590

Arg Phe  Val Val Val Arg Arg  Pro Tyr Ala Tyr Met  Tyr Asn Ser
    1595                1600                1605

Asp Lys  Asp Thr Val Glu Arg  Phe Val Leu Asn Leu  Ser Thr Ala
    1610                1615                1620

Gln Val  Glu Tyr Ser Glu Asp  Gln Gln Ala Met Leu  Lys Thr Pro
    1625                1630                1635

Asn Thr  Phe Ala Val Cys Thr  Glu His Arg Gly Ile  Leu Leu Gln
    1640                1645                1650

Ala Asn  Ser Asp Lys Asp Met  His Asp Trp Leu Tyr  Ala Phe Asn
    1655                1660                1665

Pro Leu  Leu Ala Gly Thr Ile  Arg Ser Lys Leu Ser  Arg Arg Arg
    1670                1675                1680

Ser Ala  Gln Met Arg Val
    1685


<210> SEQ ID NO 8
<211> LENGTH: 4684
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 8

Met Val Ala Gly Met Leu Met Pro Arg Asp Gln Leu Arg Ala Ile Tyr
1               5                  10                  15

Glu Val Leu Phe Arg Glu Gly Val Met Val Ala Lys Lys Asp Arg Arg
            20                  25                  30
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Pro Arg Ser Leu His Pro His Val Pro Gly Val Thr Asn Leu Gln Val
        35                  40                  45

Met Arg Ala Met Ala Ser Leu Arg Ala Arg Gly Leu Val Arg Glu Thr
    50                  55                  60

Phe Ala Trp Cys His Phe Tyr Trp Tyr Leu Thr Asn Glu Gly Ile Ala
65                  70                  75                  80

His Leu Arg Gln Tyr Leu His Leu Pro Pro Glu Ile Val Pro Ala Ser
                85                  90                  95

Leu Gln Arg Val Arg Arg Pro Val Ala Met Val Met Pro Ala Arg Arg
            100                 105                 110

Thr Pro His Val Gln Ala Val Gln Gly Pro Leu Gly Ser Pro Pro Lys
        115                 120                 125

Arg Gly Pro Leu Pro Thr Glu Glu Gln Arg Val Tyr Arg Arg Lys Glu
    130                 135                 140

Leu Glu Glu Val Ser Pro Glu Thr Pro Val Val Pro Ala Thr Thr Gln
145                 150                 155                 160

Arg Thr Leu Ala Arg Pro Gly Pro Glu Pro Ala Pro Ala Thr Asp Glu
                165                 170                 175

Arg Asp Arg Val Gln Lys Lys Thr Phe Thr Lys Trp Val Asn Lys His
            180                 185                 190

Leu Ile Lys Ala Gln Arg His Ile Ser Asp Leu Tyr Glu Asp Leu Arg
        195                 200                 205

Asp Gly His Asn Leu Ile Ser Leu Leu Glu Val Leu Ser Gly Asp Ser
    210                 215                 220

Leu Pro Arg Glu Lys Gly Arg Met Arg Phe His Lys Leu Gln Asn Val
225                 230                 235                 240

Gln Ile Ala Leu Asp Tyr Leu Arg His Arg Gln Val Lys Leu Val Asn
                245                 250                 255

Ile Arg Asn Asp Asp Ile Ala Asp Gly Asn Pro Lys Leu Thr Leu Gly
            260                 265                 270

Leu Ile Trp Thr Ile Ile Leu His Phe Gln Ile Ser Asp Ile Gln Val
        275                 280                 285

Ser Gly Gln Ser Glu Asp Met Thr Ala Lys Glu Lys Leu Leu Leu Trp
    290                 295                 300

Ser Gln Arg Met Val Glu Gly Tyr Gln Gly Leu Arg Cys Asp Asn Phe
305                 310                 315                 320

Thr Ser Ser Trp Arg Asp Gly Arg Leu Phe Asn Ala Ile Ile His Arg
                325                 330                 335

His Lys Pro Leu Leu Ile Asp Met Asn Lys Val Tyr Arg Gln Thr Asn
            340                 345                 350

Leu Glu Asn Leu Asp Gln Ala Phe Ser Val Ala Glu Arg Asp Leu Gly
        355                 360                 365

Val Thr Arg Leu Leu Asp Pro Glu Asp Val Asp Val Pro Gln Pro Asp
    370                 375                 380

Glu Lys Ser Ile Ile Thr Tyr Val Ser Ser Leu Tyr Asp Ala Met Pro
385                 390                 395                 400

Arg Val Pro Asp Val Gln Asp Gly Val Arg Ala Asn Glu Leu Gln Leu
                405                 410                 415

Arg Trp Gln Glu Tyr Arg Glu Leu Val Leu Leu Leu Leu Gln Trp Met
            420                 425                 430

Arg His His Thr Ala Ala Phe Glu Glu Arg Arg Phe Pro Ser Ser Phe
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        435                 440                 445

Glu Glu Ile Glu Ile Leu Trp Ser Gln Phe Leu Lys Phe Lys Glu Met
    450                 455                 460

Glu Leu Pro Ala Lys Glu Ala Asp Lys Asn Arg Ser Lys Gly Ile Tyr
465                 470                 475                 480

Gln Ser Leu Glu Gly Ala Val Gln Ala Gly Gln Leu Lys Val Pro Pro
                485                 490                 495

Gly Tyr His Pro Leu Asp Val Glu Lys Glu Trp Gly Lys Leu His Val
            500                 505                 510

Ala Ile Leu Glu Arg Glu Lys Gln Leu Arg Ser Glu Phe Glu Arg Leu
        515                 520                 525

Glu Cys Leu Gln Arg Ile Val Thr Lys Leu Gln Met Glu Ala Gly Leu
    530                 535                 540

Cys Glu Glu Gln Leu Asn Gln Ala Asp Ala Leu Leu Gln Ser Asp Val
545                 550                 555                 560

Arg Leu Leu Ala Ala Gly Lys Val Pro Gln Arg Ala Gly Glu Val Glu
                565                 570                 575

Arg Asp Leu Asp Lys Ala Asp Ser Met Ile Arg Leu Leu Phe Asn Asp
            580                 585                 590

Val Gln Thr Leu Lys Asp Gly Arg His Pro Gln Gly Glu Gln Met Tyr
        595                 600                 605

Arg Arg Val Tyr Arg Leu His Glu Arg Leu Val Ala Ile Arg Thr Glu
    610                 615                 620

Tyr Asn Leu Arg Leu Lys Ala Gly Val Ala Ala Pro Ala Thr Gln Val
625                 630                 635                 640

Ala Gln Val Thr Leu Gln Ser Val Gln Arg Arg Pro Glu Leu Glu Asp
                645                 650                 655

Ser Thr Leu Arg Tyr Leu Gln Asp Leu Leu Ala Trp Val Glu Glu Asn
            660                 665                 670

Gln His Arg Val Asp Gly Ala Glu Trp Gly Val Asp Leu Pro Ser Val
        675                 680                 685

Glu Ala Gln Leu Gly Ser His Arg Gly Leu His Gln Ser Ile Glu Glu
    690                 695                 700

Phe Arg Ala Lys Ile Glu Arg Ala Arg Ser Asp Glu Gly Gln Leu Ser
705                 710                 715                 720

Pro Ala Thr Arg Gly Ala Tyr Arg Asp Cys Leu Gly Arg Leu Asp Leu
                725                 730                 735

Gln Tyr Ala Lys Leu Leu Asn Ser Ser Lys Ala Arg Leu Arg Ser Leu
            740                 745                 750

Glu Ser Leu His Ser Phe Val Ala Ala Ala Thr Lys Glu Leu Met Trp
        755                 760                 765

Leu Asn Glu Lys Glu Glu Glu Glu Val Gly Phe Asp Trp Ser Asp Arg
    770                 775                 780

Asn Thr Asn Met Thr Ala Lys Lys Glu Ser Tyr Ser Ala Leu Met Arg
785                 790                 795                 800

Glu Leu Glu Leu Lys Glu Lys Lys Ile Lys Glu Leu Gln Asn Ala Gly
                805                 810                 815

Asp Arg Leu Leu Arg Glu Asp His Pro Ala Arg Pro Thr Val Glu Ser
            820                 825                 830

Phe Gln Ala Ala Leu Gln Thr Gln Trp Ser Trp Met Leu Gln Leu Cys
        835                 840                 845
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Cys Cys Ile Glu Ala His Leu Lys Glu Asn Ala Ala Tyr Phe Gln Phe
    850                 855                 860

Phe Ser Asp Val Arg Glu Ala Glu Gly Gln Leu Gln Lys Leu Gln Glu
865                 870                 875                 880

Ala Leu Arg Arg Lys Tyr Ser Cys Asp Arg Ser Ala Thr Val Thr Arg
                885                 890                 895

Leu Glu Asp Leu Leu Gln Asp Ala Gln Asp Glu Lys Glu Gln Leu Asn
            900                 905                 910

Glu Tyr Lys Gly His Leu Ser Gly Leu Ala Lys Arg Ala Lys Ala Val
        915                 920                 925

Val Gln Leu Lys Pro Arg His Pro Ala His Pro Met Arg Gly Arg Leu
    930                 935                 940

Pro Leu Leu Ala Val Cys Asp Tyr Lys Gln Val Glu Val Thr Val His
945                 950                 955                 960

Lys Gly Asp Glu Cys Gln Leu Val Gly Pro Ala Gln Pro Ser His Trp
                965                 970                 975

Lys Val Leu Ser Ser Ser Gly Ser Glu Ala Ala Val Pro Ser Val Cys
            980                 985                 990

Phe Leu Val Pro Pro Pro Asn Gln  Glu Ala Gln Glu Ala  Val Thr Arg
        995                 1000                1005

Leu Glu  Ala Gln His Gln Ala  Leu Val Thr Leu Trp  His Gln Leu
    1010                1015                1020

His Val  Asp Met Lys Ser Leu  Leu Ala Trp Gln Ser  Leu Arg Arg
    1025                1030                1035

Asp Val  Gln Leu Ile Arg Ser  Trp Ser Leu Ala Thr  Phe Arg Thr
    1040                1045                1050

Leu Lys  Pro Glu Glu Gln Arg  Gln Ala Leu His Ser  Leu Glu Leu
    1055                1060                1065

His Tyr  Gln Ala Phe Leu Arg  Asp Ser Gln Asp Ala  Gly Gly Phe
    1070                1075                1080

Gly Pro  Glu Asp Arg Leu Met  Ala Glu Arg Glu Tyr  Gly Ser Cys
    1085                1090                1095

Ser His  His Tyr Gln Gln Leu  Leu Gln Ser Leu Glu  Gln Gly Ala
    1100                1105                1110

Gln Glu  Glu Ser Arg Cys Gln  Arg Cys Ile Ser Glu  Leu Lys Asp
    1115                1120                1125

Ile Arg  Leu Gln Leu Glu Ala  Cys Glu Thr Arg Thr  Val His Arg
    1130                1135                1140

Leu Arg  Leu Pro Leu Asp Lys  Glu Pro Ala Arg Glu  Cys Ala Gln
    1145                1150                1155

Arg Ile  Ala Glu Gln Gln Lys  Ala Gln Ala Glu Val  Glu Gly Leu
    1160                1165                1170

Gly Lys  Gly Val Ala Arg Leu  Ser Ala Glu Ala Glu  Lys Val Leu
    1175                1180                1185

Ala Leu  Pro Glu Pro Ser Pro  Ala Ala Pro Thr Leu  Arg Ser Glu
    1190                1195                1200

Leu Glu  Leu Thr Leu Gly Lys  Leu Glu Gln Val Arg  Ser Leu Ser
    1205                1210                1215

Ala Ile  Tyr Leu Glu Lys Leu  Lys Thr Ile Ser Leu  Val Ile Arg
    1220                1225                1230
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Gly Thr  Gln Gly Ala Glu Glu  Val Leu Arg Ala His  Glu Glu Gln
    1235                1240                1245

Leu Lys  Glu Ala Gln Ala Val  Pro Ala Thr Leu Pro  Glu Leu Glu
    1250                1255                1260

Ala Thr  Lys Ala Ser Leu Lys  Lys Leu Arg Ala Gln  Ala Glu Ala
    1265                1270                1275

Gln Gln  Pro Thr Phe Asp Ala  Leu Arg Asp Glu Leu  Arg Gly Ala
    1280                1285                1290

Gln Glu  Val Gly Glu Arg Leu  Gln Gln Arg His Gly  Glu Arg Asp
    1295                1300                1305

Val Glu  Val Glu Arg Trp Arg  Glu Arg Val Ala Gln  Leu Leu Glu
    1310                1315                1320

Arg Trp  Gln Ala Val Leu Ala  Gln Thr Asp Val Arg  Gln Arg Glu
    1325                1330                1335

Leu Glu  Gln Leu Gly Arg Gln  Leu Arg Tyr Tyr Arg  Glu Ser Ala
    1340                1345                1350

Asp Pro  Leu Gly Ala Trp Leu  Gln Asp Ala Arg Arg  Arg Gln Glu
    1355                1360                1365

Gln Ile  Gln Ala Met Pro Leu  Ala Asp Ser Gln Ala  Val Arg Glu
    1370                1375                1380

Gln Leu  Arg Gln Glu Gln Ala  Leu Leu Glu Glu Ile  Glu Arg His
    1385                1390                1395

Gly Glu  Lys Val Glu Glu Cys  Gln Arg Phe Ala Lys  Gln Tyr Ile
    1400                1405                1410

Asn Ala  Ile Lys Asp Tyr Glu  Leu Gln Leu Val Thr  Tyr Lys Ala
    1415                1420                1425

Gln Leu  Glu Pro Val Ala Ser  Pro Ala Lys Lys Pro  Lys Val Gln
    1430                1435                1440

Ser Gly  Ser Glu Ser Val Ile  Gln Glu Tyr Val Asp  Leu Arg Thr
    1445                1450                1455

His Tyr  Ser Glu Leu Thr Thr  Leu Thr Ser Gln Tyr  Ile Lys Phe
    1460                1465                1470

Ile Ser  Glu Thr Leu Arg Arg  Met Glu Glu Glu Glu  Arg Leu Ala
    1475                1480                1485

Glu Gln  Gln Arg Ala Glu Glu  Arg Glu Arg Leu Ala  Glu Val Glu
    1490                1495                1500

Ala Ala  Leu Glu Lys Gln Arg  Gln Leu Ala Glu Ala  His Ala Gln
    1505                1510                1515

Ala Lys  Ala Gln Ala Glu Arg  Glu Ala Lys Glu Leu  Gln Gln Arg
    1520                1525                1530

Met Gln  Glu Glu Val Val Arg  Arg Glu Glu Ala Ala  Val Asp Ala
    1535                1540                1545

Gln Gln  Gln Lys Arg Ser Ile  Gln Glu Glu Leu Gln  Gln Leu Arg
    1550                1555                1560

Gln Ser  Ser Glu Ala Glu Ile  Gln Ala Lys Ala Arg  Gln Ala Glu
    1565                1570                1575

Ala Ala  Glu Arg Ser Arg Leu  Arg Ile Glu Glu Glu  Ile Arg Val
    1580                1585                1590

Val Arg  Leu Gln Leu Glu Ala  Thr Glu Arg Gln Arg  Gly Gly Ala
    1595                1600                1605

Glu Gly  Glu Leu Gln Ala Leu  Arg Ala Arg Ala Glu  Glu Ala Glu
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    1610                1615                1620

Ala Gln  Lys Arg Gln Ala Gln  Glu Glu Ala Glu Arg  Leu Arg Arg
    1625                1630                1635

Gln Val  Gln Asp Glu Ser Gln  Arg Lys Arg Gln Ala  Glu Val Glu
    1640                1645                1650

Leu Ala  Ser Arg Val Lys Ala  Glu Ala Glu Ala Ala  Arg Glu Lys
    1655                1660                1665

Gln Arg  Ala Leu Gln Ala Leu  Glu Glu Leu Arg Leu  Gln Ala Glu
    1670                1675                1680

Glu Ala  Glu Arg Arg Leu Arg  Gln Ala Glu Val Glu  Arg Ala Arg
    1685                1690                1695

Gln Val  Gln Val Ala Leu Glu  Thr Ala Gln Arg Ser  Ala Glu Ala
    1700                1705                1710

Glu Leu  Gln Ser Lys Arg Ala  Ser Phe Ala Glu Lys  Thr Ala Gln
    1715                1720                1725

Leu Glu  Arg Ser Leu Gln Glu  Glu His Val Ala Val  Ala Gln Leu
    1730                1735                1740

Arg Glu  Glu Ala Glu Arg Arg  Ala Gln Gln Gln Ala  Glu Ala Glu
    1745                1750                1755

Arg Ala  Arg Glu Glu Ala Glu  Arg Glu Leu Glu Arg  Trp Gln Leu
    1760                1765                1770

Lys Ala  Asn Glu Ala Leu Arg  Leu Arg Leu Gln Ala  Glu Glu Val
    1775                1780                1785

Ala Gln  Gln Lys Ser Leu Ala  Gln Ala Glu Ala Glu  Lys Gln Lys
    1790                1795                1800

Glu Glu  Ala Glu Arg Glu Ala  Arg Arg Arg Gly Lys  Ala Glu Glu
    1805                1810                1815

Gln Ala  Val Arg Gln Arg Glu  Leu Ala Glu Gln Glu  Leu Glu Lys
    1820                1825                1830

Gln Arg  Gln Leu Ala Glu Gly  Thr Ala Gln Gln Arg  Leu Ala Ala
    1835                1840                1845

Glu Gln  Glu Leu Ile Arg Leu  Arg Ala Glu Thr Glu  Gln Gly Glu
    1850                1855                1860

Gln Gln  Arg Gln Leu Leu Glu  Glu Glu Leu Ala Arg  Leu Gln Arg
    1865                1870                1875

Glu Ala  Ala Ala Ala Thr Gln  Lys Arg Gln Glu Leu  Glu Ala Glu
    1880                1885                1890

Leu Ala  Lys Val Arg Ala Glu  Met Glu Val Leu Leu  Ala Ser Lys
    1895                1900                1905

Ala Arg  Ala Glu Glu Glu Ser  Arg Ser Thr Ser Glu  Lys Ser Lys
    1910                1915                1920

Gln Arg  Leu Glu Ala Glu Ala  Gly Arg Phe Arg Glu  Leu Ala Glu
    1925                1930                1935

Glu Ala  Ala Arg Leu Arg Ala  Leu Ala Glu Glu Ala  Lys Arg Gln
    1940                1945                1950

Arg Gln  Leu Ala Glu Glu Asp  Ala Ala Arg Gln Arg  Ala Glu Ala
    1955                1960                1965

Glu Arg  Val Leu Ala Glu Lys  Leu Ala Ala Ile Gly  Glu Ala Thr
    1970                1975                1980

Arg Leu  Lys Thr Glu Ala Glu  Ile Ala Leu Lys Glu  Lys Glu Ala
    1985                1990                1995
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Glu Asn  Glu Arg Leu Arg Arg  Leu Ala Glu Asp Glu  Ala Phe Gln
    2000                 2005                 2010

Arg Arg  Arg Leu Glu Glu Gln  Ala Ala Gln His Lys  Ala Asp Ile
    2015                 2020                 2025

Glu Glu  Arg Leu Ala Gln Leu  Arg Lys Ala Ser Asp  Ser Glu Leu
    2030                 2035                 2040

Glu Arg  Gln Lys Gly Leu Val  Glu Asp Thr Leu Arg  Gln Arg Arg
    2045                 2050                 2055

Gln Val  Glu Glu Glu Ile Leu  Ala Leu Lys Ala Ser  Phe Glu Lys
    2060                 2065                 2070

Ala Ala  Ala Gly Lys Ala Glu  Leu Glu Leu Glu Leu  Gly Arg Ile
    2075                 2080                 2085

Arg Ser  Asn Ala Glu Asp Thr  Leu Arg Ser Lys Glu  Gln Ala Glu
    2090                 2095                 2100

Leu Glu  Ala Ala Arg Gln Arg  Gln Leu Ala Ala Glu  Glu Glu Arg
    2105                 2110                 2115

Arg Arg  Arg Glu Ala Glu Glu  Arg Val Gln Lys Ser  Leu Ala Ala
    2120                 2125                 2130

Glu Glu  Glu Ala Ala Arg Gln  Arg Lys Ala Ala Leu  Glu Glu Val
    2135                 2140                 2145

Glu Arg  Leu Lys Ala Lys Val  Glu Glu Ala Arg Arg  Leu Arg Glu
    2150                 2155                 2160

Arg Ala  Glu Gln Glu Ser Ala  Arg Gln Leu Gln Leu  Ala Gln Glu
    2165                 2170                 2175

Ala Ala  Gln Lys Arg Leu Gln  Ala Glu Glu Lys Ala  His Ala Phe
    2180                 2185                 2190

Ala Val  Gln Gln Lys Glu Gln  Glu Leu Gln Gln Thr  Leu Gln Gln
    2195                 2200                 2205

Glu Gln  Ser Val Leu Asp Gln  Leu Arg Gly Glu Ala  Glu Ala Ala
    2210                 2215                 2220

Arg Arg  Ala Ala Glu Glu Ala  Glu Glu Ala Arg Val  Gln Ala Glu
    2225                 2230                 2235

Arg Glu  Ala Ala Gln Ser Arg  Arg Gln Val Glu Glu  Ala Glu Arg
    2240                 2245                 2250

Leu Lys  Gln Ser Ala Glu Glu  Gln Ala Gln Ala Arg  Ala Gln Ala
    2255                 2260                 2265

Gln Ala  Ala Ala Glu Lys Leu  Arg Lys Glu Ala Glu  Gln Glu Ala
    2270                 2275                 2280

Ala Arg  Arg Ala Gln Ala Glu  Gln Ala Ala Leu Arg  Gln Lys Gln
    2285                 2290                 2295

Ala Ala  Asp Ala Glu Met Glu  Lys His Lys Lys Phe  Ala Glu Gln
    2300                 2305                 2310

Thr Leu  Arg Gln Lys Ala Gln  Val Glu Gln Glu Leu  Thr Thr Leu
    2315                 2320                 2325

Arg Leu  Gln Leu Glu Glu Thr  Asp His Gln Lys Asn  Leu Leu Asp
    2330                 2335                 2340

Glu Glu  Leu Gln Arg Leu Lys  Ala Glu Ala Thr Glu  Ala Ala Arg
    2345                 2350                 2355

Gln Arg  Ser Gln Val Glu Glu  Glu Leu Phe Ser Val  Arg Val Gln
    2360                 2365                 2370
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Met Glu  Glu Leu Ser Lys Leu  Lys Ala Arg Ile Glu  Ala Glu Asn
    2375                2380                2385

Arg Ala  Leu Ile Leu Arg Asp  Lys Asp Asn Thr Gln  Arg Phe Leu
    2390                2395                2400

Gln Glu  Glu Ala Glu Lys Met  Lys Gln Val Ala Glu  Glu Ala Ala
    2405                2410                2415

Arg Leu  Ser Val Ala Ala Gln  Glu Ala Ala Arg Leu  Arg Gln Leu
    2420                2425                2430

Ala Glu  Glu Asp Leu Ala Gln  Gln Arg Ala Leu Ala  Glu Lys Met
    2435                2440                2445

Leu Lys  Glu Lys Met Gln Ala  Val Gln Glu Ala Thr  Arg Leu Lys
    2450                2455                2460

Ala Glu  Ala Glu Leu Leu Gln  Gln Gln Lys Glu Leu  Ala Gln Glu
    2465                2470                2475

Gln Ala  Arg Arg Leu Gln Glu  Asp Lys Glu Gln Met  Ala Gln Gln
    2480                2485                2490

Leu Ala  Glu Glu Thr Gln Gly  Phe Gln Arg Thr Leu  Glu Ala Glu
    2495                2500                2505

Arg Gln  Arg Gln Leu Glu Met  Ser Ala Glu Ala Glu  Arg Leu Lys
    2510                2515                2520

Leu Arg  Val Ala Glu Met Ser  Arg Ala Gln Ala Arg  Ala Glu Glu
    2525                2530                2535

Asp Ala  Gln Arg Phe Arg Lys  Gln Ala Glu Glu Ile  Gly Glu Lys
    2540                2545                2550

Leu His  Arg Thr Glu Leu Ala  Thr Gln Glu Lys Val  Thr Leu Val
    2555                2560                2565

Gln Thr  Leu Glu Ile Gln Arg  Gln Gln Ser Asp His  Asp Ala Glu
    2570                2575                2580

Arg Leu  Arg Glu Ala Ile Ala  Glu Leu Glu Arg Glu  Lys Glu Lys
    2585                2590                2595

Leu Gln  Gln Glu Ala Lys Leu  Leu Gln Leu Lys Ser  Glu Glu Met
    2600                2605                2610

Gln Thr  Val Gln Gln Glu Gln  Leu Leu Gln Glu Thr  Gln Ala Leu
    2615                2620                2625

Gln Gln  Ser Phe Leu Ser Glu  Lys Asp Ser Leu Leu  Gln Arg Glu
    2630                2635                2640

Arg Phe  Ile Glu Gln Glu Lys  Ala Lys Leu Glu Gln  Leu Phe Gln
    2645                2650                2655

Asp Glu  Val Ala Lys Ala Gln  Gln Leu Arg Glu Glu  Gln Gln Arg
    2660                2665                2670

Gln Gln  Gln Gln Met Glu Gln  Glu Arg Gln Arg Leu  Val Ala Ser
    2675                2680                2685

Met Glu  Glu Ala Arg Arg Arg  Gln His Glu Ala Glu  Glu Gly Val
    2690                2695                2700

Arg Arg  Lys Gln Glu Glu Leu  Gln Gln Leu Glu Gln  Gln Arg Arg
    2705                2710                2715

Gln Gln  Glu Glu Leu Leu Ala  Glu Glu Asn Gln Arg  Leu Arg Glu
    2720                2725                2730

Gln Leu  Gln Leu Leu Glu Glu  Gln His Arg Ala Ala  Leu Ala His
    2735                2740                2745

Ser Glu  Glu Val Thr Ala Ser  Gln Val Ala Ala Thr  Lys Thr Leu
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    2750                2755                2760

Pro Asn  Gly Arg Asp Ala Leu  Asp Gly Pro Ala Ala  Glu Ala Glu
    2765                2770                2775

Pro Glu  His Ser Phe Asp Gly  Leu Arg Arg Lys Val  Ser Ala Gln
    2780                2785                2790

Arg Leu  Gln Glu Ala Gly Ile  Leu Ser Ala Glu Glu  Leu Gln Arg
    2795                2800                2805

Leu Ala  Gln Gly His Thr Thr  Val Asp Glu Leu Ala  Arg Arg Glu
    2810                2815                2820

Asp Val  Arg His Tyr Leu Gln  Gly Arg Ser Ser Ile  Ala Gly Leu
    2825                2830                2835

Leu Leu  Lys Ala Thr Asn Glu  Lys Leu Ser Val Tyr  Ala Ala Leu
    2840                2845                2850

Gln Arg  Gln Leu Leu Ser Pro  Gly Thr Ala Leu Ile  Leu Leu Glu
    2855                2860                2865

Ala Gln  Ala Ala Ser Gly Phe  Leu Leu Asp Pro Val  Arg Asn Arg
    2870                2875                2880

Arg Leu  Thr Val Asn Glu Ala  Val Lys Glu Gly Val  Val Gly Pro
    2885                2890                2895

Glu Leu  His His Lys Leu Leu  Ser Ala Glu Arg Ala  Val Thr Gly
    2900                2905                2910

Tyr Lys  Asp Pro Tyr Thr Gly  Gln Gln Ile Ser Leu  Phe Gln Ala
    2915                2920                2925

Met Gln  Lys Gly Leu Ile Val  Arg Glu His Gly Ile  Arg Leu Leu
    2930                2935                2940

Glu Ala  Gln Ile Ala Thr Gly  Gly Val Ile Asp Pro  Val His Ser
    2945                2950                2955

His Arg  Val Pro Val Asp Val  Ala Tyr Arg Arg Gly  Tyr Phe Asp
    2960                2965                2970

Glu Glu  Met Asn Arg Val Leu  Ala Asp Pro Ser Asp  Asp Thr Lys
    2975                2980                2985

Gly Phe  Phe Asp Pro Asn Thr  His Glu Asn Leu Thr  Tyr Leu Gln
    2990                2995                3000

Leu Leu  Glu Arg Cys Val Glu  Asp Pro Glu Thr Gly  Leu Cys Leu
    3005                3010                3015

Leu Pro  Leu Thr Asp Lys Ala  Ala Lys Gly Gly Glu  Leu Val Tyr
    3020                3025                3030

Thr Asp  Ser Glu Ala Arg Asp  Val Phe Glu Lys Ala  Thr Val Ser
    3035                3040                3045

Ala Pro  Phe Gly Lys Phe Gln  Gly Lys Thr Val Thr  Ile Trp Glu
    3050                3055                3060

Ile Ile  Asn Ser Glu Tyr Phe  Thr Ala Glu Gln Arg  Arg Asp Leu
    3065                3070                3075

Leu Arg  Gln Phe Arg Thr Gly  Arg Ile Thr Val Glu  Lys Ile Ile
    3080                3085                3090

Lys Ile  Ile Ile Thr Val Val  Glu Glu Gln Glu Gln  Lys Gly Arg
    3095                3100                3105

Leu Cys  Phe Glu Gly Leu Arg  Ser Leu Val Pro Ala  Ala Glu Leu
    3110                3115                3120

Leu Glu  Ser Arg Val Ile Asp  Arg Glu Leu Tyr Gln  Gln Leu Gln
    3125                3130                3135
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Arg Gly  Glu Arg Ser Val Arg  Asp Val Ala Glu Val  Asp Thr Val
    3140                 3145                 3150

Arg Arg  Ala Leu Arg Gly Ala  Asn Val Ile Ala Gly  Val Trp Leu
    3155                 3160                 3165

Glu Glu  Ala Gly Gln Lys Leu  Ser Ile Tyr Asn Ala  Leu Lys Lys
    3170                 3175                 3180

Asp Leu  Leu Pro Ser Asp Met  Ala Val Ala Leu Leu  Glu Ala Gln
    3185                 3190                 3195

Ala Gly  Thr Gly His Ile Ile  Asp Pro Ala Thr Ser  Ala Arg Leu
    3200                 3205                 3210

Thr Val  Asp Glu Ala Val Arg  Ala Gly Leu Val Gly  Pro Glu Phe
    3215                 3220                 3225

His Glu  Lys Leu Leu Ser Ala  Glu Lys Ala Val Thr  Gly Tyr Arg
    3230                 3235                 3240

Asp Pro  Tyr Thr Gly Gln Ser  Val Ser Leu Phe Gln  Ala Leu Lys
    3245                 3250                 3255

Lys Gly  Leu Ile Pro Arg Glu  Gln Gly Leu Arg Leu  Leu Asp Ala
    3260                 3265                 3270

Gln Leu  Ser Thr Gly Gly Ile  Val Asp Pro Ser Lys  Ser His Arg
    3275                 3280                 3285

Val Pro  Leu Asp Val Ala Cys  Ala Arg Gly Cys Leu  Asp Glu Glu
    3290                 3295                 3300

Thr Ser  Arg Ala Leu Ser Ala  Pro Arg Ala Asp Ala  Lys Ala Tyr
    3305                 3310                 3315

Ser Asp  Pro Ser Thr Gly Glu  Pro Ala Thr Tyr Gly  Glu Leu Gln
    3320                 3325                 3330

Gln Arg  Cys Arg Pro Asp Gln  Leu Thr Gly Leu Ser  Leu Leu Pro
    3335                 3340                 3345

Leu Ser  Glu Lys Ala Ala Arg  Ala Arg Gln Glu Glu  Leu Tyr Ser
    3350                 3355                 3360

Glu Leu  Gln Ala Arg Glu Thr  Phe Glu Lys Thr Pro  Val Glu Val
    3365                 3370                 3375

Pro Val  Gly Gly Phe Lys Gly  Arg Thr Val Thr Val  Trp Glu Leu
    3380                 3385                 3390

Ile Ser  Ser Glu Tyr Phe Thr  Ala Glu Gln Arg Gln  Glu Leu Leu
    3395                 3400                 3405

Arg Gln  Phe Arg Thr Gly Lys  Val Thr Val Glu Lys  Val Ile Lys
    3410                 3415                 3420

Ile Leu  Ile Thr Ile Val Glu  Glu Val Glu Thr Leu  Arg Gln Glu
    3425                 3430                 3435

Arg Leu  Ser Phe Ser Gly Leu  Arg Ala Pro Val Pro  Ala Ser Glu
    3440                 3445                 3450

Leu Leu  Ala Ser Gly Val Leu  Ser Arg Ala Gln Phe  Glu Gln Leu
    3455                 3460                 3465

Lys Asp  Gly Lys Thr Thr Val  Lys Asp Leu Ser Glu  Leu Gly Ser
    3470                 3475                 3480

Val Arg  Thr Leu Leu Gln Gly  Ser Gly Cys Leu Ala  Gly Ile Tyr
    3485                 3490                 3495

Leu Glu  Asp Thr Lys Glu Lys  Val Ser Ile Tyr Glu  Ala Met Arg
    3500                 3505                 3510
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Arg Gly  Leu Leu Arg Ala Thr  Thr Ala Ala Leu Leu  Leu Glu Ala
    3515                 3520                 3525

Gln Ala  Ala Thr Gly Phe Leu  Val Asp Pro Val Arg  Asn Gln Arg
    3530                 3535                 3540

Leu Tyr  Val His Glu Ala Val  Lys Ala Gly Val Val  Gly Pro Glu
    3545                 3550                 3555

Leu His  Glu Gln Leu Leu Ser  Ala Glu Lys Ala Val  Thr Gly Tyr
    3560                 3565                 3570

Arg Asp  Pro Tyr Ser Gly Ser  Thr Ile Ser Leu Phe  Gln Ala Met
    3575                 3580                 3585

Gln Lys  Gly Leu Val Leu Arg  Gln His Gly Ile Arg  Leu Leu Glu
    3590                 3595                 3600

Ala Gln  Ile Ala Thr Gly Gly  Ile Ile Asp Pro Val  His Ser His
    3605                 3610                 3615

Arg Val  Pro Val Asp Val Ala  Tyr Gln Arg Gly Tyr  Phe Ser Glu
    3620                 3625                 3630

Glu Met  Asn Arg Val Leu Ala  Asp Pro Ser Asp Asp  Thr Lys Gly
    3635                 3640                 3645

Phe Phe  Asp Pro Asn Thr His  Glu Asn Leu Thr Tyr  Arg Gln Leu
    3650                 3655                 3660

Leu Glu  Arg Cys Val Glu Asp  Pro Glu Thr Gly Leu  Arg Leu Leu
    3665                 3670                 3675

Pro Leu  Lys Gly Ala Glu Lys  Ala Glu Val Val Glu  Thr Thr Gln
    3680                 3685                 3690

Val Tyr  Thr Glu Glu Glu Thr  Arg Arg Ala Phe Glu  Glu Thr Gln
    3695                 3700                 3705

Ile Asp  Ile Pro Gly Gly Gly  Ser His Gly Gly Ser  Thr Met Ser
    3710                 3715                 3720

Leu Trp  Glu Val Met Gln Ser  Asp Leu Ile Pro Glu  Glu Gln Arg
    3725                 3730                 3735

Ala Gln  Leu Met Ala Asp Phe  Gln Ala Gly Arg Val  Thr Lys Glu
    3740                 3745                 3750

Arg Met  Ile Ile Ile Ile Ile  Glu Ile Ile Glu Lys  Thr Glu Ile
    3755                 3760                 3765

Ile Arg  Gln Gln Gly Leu Ala  Ser Tyr Asp Tyr Val  Arg Arg Arg
    3770                 3775                 3780

Leu Thr  Ala Glu Asp Leu Phe  Glu Ala Arg Ile Ile  Ser Leu Glu
    3785                 3790                 3795

Thr Tyr  Asn Leu Leu Arg Glu  Gly Thr Arg Ser Leu  Arg Glu Ala
    3800                 3805                 3810

Leu Glu  Ala Glu Ser Ala Trp  Cys Tyr Leu Tyr Gly  Thr Gly Ser
    3815                 3820                 3825

Val Ala  Gly Val Tyr Leu Pro  Gly Ser Arg Gln Thr  Leu Ser Ile
    3830                 3835                 3840

Tyr Gln  Ala Leu Lys Lys Gly  Leu Leu Ser Ala Glu  Val Ala Arg
    3845                 3850                 3855

Leu Leu  Leu Glu Ala Gln Ala  Ala Thr Gly Phe Leu  Leu Asp Pro
    3860                 3865                 3870

Val Lys  Gly Glu Arg Leu Thr  Val Asp Glu Ala Val  Arg Lys Gly
    3875                 3880                 3885

Leu Val  Gly Pro Glu Leu His  Asp Arg Leu Leu Ser  Ala Glu Arg
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    3890                3895                3900

Ala Val  Thr Gly Tyr Arg Asp  Pro Tyr Thr Glu Gln  Thr Ile Ser
    3905                3910                3915

Leu Phe  Gln Ala Met Lys Lys  Glu Leu Ile Pro Thr  Glu Glu Ala
    3920                3925                3930

Leu Arg  Leu Leu Asp Ala Gln  Leu Ala Thr Gly Gly  Ile Val Asp
    3935                3940                3945

Pro Arg  Leu Gly Phe His Leu  Pro Leu Glu Val Ala  Tyr Gln Arg
    3950                3955                3960

Gly Tyr  Leu Asn Lys Asp Thr  His Asp Gln Leu Ser  Glu Pro Ser
    3965                3970                3975

Glu Val  Arg Ser Tyr Val Asp  Pro Ser Thr Asp Glu  Arg Leu Ser
    3980                3985                3990

Tyr Thr  Gln Leu Leu Arg Arg  Cys Arg Arg Asp Asp  Gly Thr Gly
    3995                4000                4005

Gln Leu  Leu Leu Pro Leu Ser  Asp Ala Arg Lys Leu  Thr Phe Arg
    4010                4015                4020

Gly Leu  Arg Lys Gln Ile Thr  Met Glu Glu Leu Val  Arg Ser Gln
    4025                4030                4035

Val Met  Asp Glu Ala Thr Ala  Leu Gln Leu Arg Glu  Gly Leu Thr
    4040                4045                4050

Ser Ile  Glu Glu Val Thr Lys  Asn Leu Gln Lys Phe  Leu Glu Gly
    4055                4060                4065

Thr Ser  Cys Ile Ala Gly Val  Phe Val Asp Ala Thr  Lys Glu Arg
    4070                4075                4080

Leu Ser  Val Tyr Gln Ala Met  Lys Lys Gly Ile Ile  Arg Pro Gly
    4085                4090                4095

Thr Ala  Phe Glu Leu Leu Glu  Ala Gln Ala Ala Thr  Gly Tyr Val
    4100                4105                4110

Ile Asp  Pro Ile Lys Gly Leu  Lys Leu Thr Val Glu  Glu Ala Val
    4115                4120                4125

Arg Met  Gly Ile Val Gly Pro  Glu Phe Lys Asp Lys  Leu Leu Ser
    4130                4135                4140

Ala Glu  Arg Ala Val Thr Gly  Tyr Lys Asp Pro Tyr  Ser Gly Lys
    4145                4150                4155

Leu Ile  Ser Leu Phe Gln Ala  Met Lys Lys Gly Leu  Ile Leu Lys
    4160                4165                4170

Asp His  Gly Ile Arg Leu Leu  Glu Ala Gln Ile Ala  Thr Gly Gly
    4175                4180                4185

Ile Ile  Asp Pro Glu Glu Ser  His Arg Leu Pro Val  Glu Val Ala
    4190                4195                4200

Tyr Lys  Arg Gly Leu Phe Asp  Glu Glu Met Asn Glu  Ile Leu Thr
    4205                4210                4215

Asp Pro  Ser Asp Asp Thr Lys  Gly Phe Phe Asp Pro  Asn Thr Glu
    4220                4225                4230

Glu Asn  Leu Thr Tyr Leu Gln  Leu Met Glu Arg Cys  Ile Thr Asp
    4235                4240                4245

Pro Gln  Thr Gly Leu Cys Leu  Leu Pro Leu Lys Glu  Lys Lys Arg
    4250                4255                4260

Glu Arg  Lys Thr Ser Ser Lys  Ser Ser Val Arg Lys  Arg Arg Val
    4265                4270                4275
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Val Ile  Val Asp Pro Glu Thr  Gly Lys Glu Met Ser  Val Tyr Glu
    4280                 4285                 4290

Ala Tyr  Arg Lys Gly Leu Ile  Asp His Gln Thr Tyr  Leu Glu Leu
    4295                 4300                 4305

Ser Glu  Gln Glu Cys Glu Trp  Glu Glu Ile Thr Ile  Ser Ser Ser
    4310                 4315                 4320

Asp Gly  Val Val Lys Ser Met  Ile Ile Asp Arg Arg  Ser Gly Arg
    4325                 4330                 4335

Gln Tyr  Asp Ile Asp Asp Ala  Ile Ala Lys Asn Leu  Ile Asp Arg
    4340                 4345                 4350

Ser Ala  Leu Asp Gln Tyr Arg  Ala Gly Thr Leu Ser  Ile Thr Glu
    4355                 4360                 4365

Phe Ala  Asp Met Leu Ser Gly  Asn Ala Gly Gly Phe  Arg Ser Arg
    4370                 4375                 4380

Ser Ser  Ser Val Gly Ser Ser  Ser Ser Tyr Pro Ile  Ser Pro Ala
    4385                 4390                 4395

Val Ser  Arg Thr Gln Leu Ala  Ser Trp Ser Asp Pro  Thr Glu Glu
    4400                 4405                 4410

Thr Gly  Pro Val Ala Gly Ile  Leu Asp Thr Glu Thr  Leu Glu Lys
    4415                 4420                 4425

Val Ser  Ile Thr Glu Ala Met  His Arg Asn Leu Val  Asp Asn Ile
    4430                 4435                 4440

Thr Gly  Gln Arg Leu Leu Glu  Ala Gln Ala Cys Thr  Gly Gly Ile
    4445                 4450                 4455

Ile Asp  Pro Ser Thr Gly Glu  Arg Phe Pro Val Thr  Asp Ala Val
    4460                 4465                 4470

Asn Lys  Gly Leu Val Asp Lys  Ile Met Val Asp Arg  Ile Asn Leu
    4475                 4480                 4485

Ala Gln  Lys Ala Phe Cys Gly  Phe Glu Asp Pro Arg  Thr Lys Thr
    4490                 4495                 4500

Lys Met  Ser Ala Ala Gln Ala  Leu Lys Lys Gly Trp  Leu Tyr Tyr
    4505                 4510                 4515

Glu Ala  Gly Gln Arg Phe Leu  Glu Val Gln Tyr Leu  Thr Gly Gly
    4520                 4525                 4530

Leu Ile  Glu Pro Asp Thr Pro  Gly Arg Val Pro Leu  Asp Glu Ala
    4535                 4540                 4545

Leu Gln  Arg Gly Thr Val Asp  Ala Arg Thr Ala Gln  Lys Leu Arg
    4550                 4555                 4560

Asp Val  Gly Ala Tyr Ser Lys  Tyr Leu Thr Cys Pro  Lys Thr Lys
    4565                 4570                 4575

Leu Lys  Ile Ser Tyr Lys Asp  Ala Leu Asp Arg Ser  Met Val Glu
    4580                 4585                 4590

Glu Gly  Thr Gly Leu Arg Leu  Leu Glu Ala Ala Ala  Gln Ser Thr
    4595                 4600                 4605

Lys Gly  Tyr Tyr Ser Pro Tyr  Ser Val Ser Gly Ser  Gly Ser Thr
    4610                 4615                 4620

Ala Gly  Ser Arg Thr Gly Ser  Arg Thr Gly Ser Arg  Ala Gly Ser
    4625                 4630                 4635

Arg Arg  Gly Ser Phe Asp Ala  Thr Gly Ser Gly Phe  Ser Met Thr
    4640                 4645                 4650
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Phe Ser  Ser Ser Ser Tyr Ser  Ser Ser Gly Tyr Gly  Arg Arg Tyr
    4655                 4660                 4665

Ala Ser  Gly Ser Ser Ala Ser  Leu Gly Gly Pro Glu  Ser Ala Val
    4670                 4675                 4680

Ala


<210> SEQ ID NO 9
<211> LENGTH: 2353
<212> TYPE: PRT
<213> ORGANISM: Xenopus laevis

<400> SEQUENCE: 9

Met Val Ala Leu Ser Leu Lys Ile Ser Ile Gly Asn Val Val Lys Thr
1               5                   10                  15

Met Gln Phe Glu Pro Ser Thr Met Ile Tyr Asp Ala Cys Arg Ile Ile
            20                  25                  30

Arg Glu Lys Val Pro Glu Ala Gln Ile Gly Gln Pro Asn Asp Phe Gly
        35                  40                  45

Leu Phe Leu Ser Asp Glu Asp Pro Lys Lys Gly Ile Trp Leu Glu Ala
    50                  55                  60

Gly Lys Ala Leu Asp Tyr Tyr Met Leu Arg Asn Gly Asp Thr Leu Glu
65                  70                  75                  80

Tyr Arg Lys Lys Gln Arg Pro Leu Glu Ile Arg Met Leu Asp Gly Thr
                85                  90                  95

Val Lys Thr Val Met Val Asp Asp Ser Asn Thr Met Ser Asp Leu Leu
            100                 105                 110

Met Thr Ile Cys Ala Arg Ile Gly Ile Thr Asn Tyr Asp Glu Tyr Ser
        115                 120                 125

Leu Val Arg Glu Ile Met Glu Glu Lys Lys Glu Glu Val Thr Gly Thr
    130                 135                 140

Leu Lys Arg Asp Lys Thr Leu Leu Arg Asp Asp Lys Lys Met Glu Lys
145                 150                 155                 160

Leu Lys Gln Lys Leu His Thr Asp Asp Glu Leu Asn Trp Leu Asp Pro
                165                 170                 175

Gly Arg Thr Leu Arg Glu Gln Gly Val Asp Glu Asn Glu Thr Leu Leu
            180                 185                 190

Leu Arg Arg Lys Phe Phe Tyr Ser Asp Gln Asn Val Asp Ser Arg Asp
        195                 200                 205

Pro Val Gln Leu Asn Leu Leu Tyr Val Gln Ala Arg Asp Asp Ile Leu
    210                 215                 220

Asn Gly Ser His Pro Val Ser Phe Asp Lys Ala Cys Glu Phe Ala Gly
225                 230                 235                 240

Tyr Gln Cys Gln Val Gln Phe Gly Pro His Asn Glu Val Lys His Lys
                245                 250                 255

Pro Gly Phe Leu Glu Leu Lys Asp Phe Leu Pro Lys Glu Tyr Ile Lys
            260                 265                 270

Gln Lys Gly Glu Arg Lys Ile Phe Leu Ala His Lys Gln Cys Gly Asn
        275                 280                 285

Met Ser Glu Ile Glu Ala Lys Ala Arg Tyr Val Lys Leu Ala Arg Ser
    290                 295                 300

Leu Lys Thr Tyr Gly Val Ser Phe Phe Leu Val Lys Glu Lys Met Lys
305                 310                 315                 320
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```
Gly Lys Asn Lys Leu Val Pro Arg Leu Leu Gly Ile Thr Lys Glu Cys
                325                 330                 335

Val Met Arg Val Asp Glu Lys Thr Lys Glu Val Ile Gln Glu Trp Asn
            340                 345                 350

Leu Thr Asn Ile Lys Arg Trp Ala Ala Ser Pro Lys Ser Phe Thr Leu
        355                 360                 365

Asp Phe Gly Asp Tyr Gln Asp Gly Tyr Tyr Ser Val Gln Thr Thr Glu
    370                 375                 380

Gly Glu Gln Ile Ala Gln Leu Ile Ala Gly Tyr Ile Asp Ile Ile Leu
385                 390                 395                 400

Lys Lys Lys Lys Ser Lys Asp His Phe Gly Leu Glu Gly Asp Glu Glu
                405                 410                 415

Ser Thr Met Leu Glu Asp Ser Val Ser Pro Lys Lys Ser Thr Leu Leu
            420                 425                 430

Gln Gln Gln Phe Asn Gln Val Gly Lys Val Glu His Gly Ser Val Ala
        435                 440                 445

Leu Pro Ala Ile Met Arg Ser Gly Ala Ser Gly Pro Glu Asn Phe Gln
    450                 455                 460

Val Gly Ser Met Pro Gln Ala Gln Gln His Ile Thr Ser Gly Gln Met
465                 470                 475                 480

His Leu Gly His Met Pro Pro Leu Thr Ser Ala Gln Gln Ala Leu Thr
                485                 490                 495

Gly Thr Ile Asn Ser Ser Met Gln Ala Ile Asn Ala Ala Gln Ala Thr
            500                 505                 510

Leu Asp Asp Phe Asp Ser Leu Pro Pro Leu Gly Asp Asp Ala Ala Ser
        515                 520                 525

Lys Ala Trp Arg Lys Asn Lys Met Asp Asn Ser Lys His Glu Ile His
    530                 535                 540

Ser Gln Val Asp Ala Ile Thr Ala Gly Thr Ala Ser Val Val Asn Leu
545                 550                 555                 560

Thr Ala Gly Asn Pro Ala Asp Thr Asp Tyr Thr Ala Val Gly Cys Ala
                565                 570                 575

Val Thr Thr Ile Ser Ser Asn Leu Thr Glu Met Ser Arg Gly Val Lys
            580                 585                 590

Leu Leu Ala Ala Leu Met Glu Asp Glu Gly Gly Asn Gly Arg Gln Leu
        595                 600                 605

Leu His Ala Ala Lys Asn Leu Ala Gly Ala Val Ser Asp Leu Leu Lys
    610                 615                 620

Thr Ala Gln Pro Ala Ser Thr Glu Pro Arg Gln Val Leu Met Gln Ala
625                 630                 635                 640

Ala Gly Asn Val Gly Met Thr Ser Gly Glu Leu Leu Lys Gln Ile Gly
                645                 650                 655

Glu Cys Asp Thr Asp Ser Gln Phe Gln Asp Met Leu Val Gln Leu Ala
            660                 665                 670

Lys Ala Val Ala Ser Ala Ala Ala Ala Leu Val Leu Lys Ala Lys Asn
        675                 680                 685

Val Ala Glu Arg Thr Asp Asp Gly Ala Gln Gln Thr Gln Val Ile Ala
    690                 695                 700

Ala Ala Thr Gln Cys Ala Leu Ser Thr Ser Gln Leu Val Ala Cys Thr
705                 710                 715                 720

Lys Val Val Ala Pro Thr Ile Ser Ser Pro Val Cys Gln Glu Gln Leu
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               725                 730                 735

Leu Glu Ala Gly Lys Gln Val Ala Lys Ser Val Glu Gly Cys Val Glu
            740                 745                 750

Ala Ser Glu Ala Ala Val Glu Asp Pro Glu Leu Leu Lys Ser Val Gly
        755                 760                 765

Val Ala Ala Ser Gly Val Thr Gln Ala Leu Asn Asn Leu Leu Gln His
    770                 775                 780

Ile Lys Lys His Ala Ser Gly Gly Pro Ser Thr Gly Arg Tyr Asp Gln
785                 790                 795                 800

Ala Thr Asp Thr Ile Leu Asn Val Thr Glu Asn Ile Phe Ser Ser Met
                805                 810                 815

Gly Asp Ala Gly Glu Met Val Arg Gln Ala Arg Ile Leu Ala Gln Ala
            820                 825                 830

Thr Ser Asp Leu Val Gly Ala Ile Lys Ala Asp Ala Glu Arg Glu Ser
        835                 840                 845

Asp Leu Glu Asn Ser Arg Lys Leu Leu Cys Ala Ala Lys Leu Leu Ala
    850                 855                 860

Asp Ala Thr Ala Arg Met Val Glu Ala Ala Lys Gly Ala Ala Ala His
865                 870                 875                 880

Pro Asp Ser Glu Glu Gln Gln Gln Lys Leu Arg Glu Ala Ala Glu Gly
                885                 890                 895

Leu Arg Met Ala Thr Asn Ala Ala Ala Gln Asn Ala Ile Lys Lys Lys
            900                 905                 910

Leu Val His Lys Leu Glu Gln Ala Ala Lys Gln Ala Ala Ala Ser Ala
        915                 920                 925

Thr Gln Thr Ile Ala Ala Ala Gln Asn Ala Ala Ser Ser Asn Lys Asn
    930                 935                 940

Pro Ala Ala Gln Gln Gln Leu Val Gln Ser Cys Lys Val Val Ala Glu
945                 950                 955                 960

Gln Ile Pro Met Leu Val Gln Gly Val Arg Gly Ser Gln Ser Gln Pro
                965                 970                 975

Asp Ser Pro Ser Ala Gln Leu Ser Leu Ile Ser Ala Ser Gln Asn Phe
            980                 985                 990

Leu Gln Pro Gly Ala Lys Leu Val  Thr Ala Gly Lys Ser  Ala Val Pro
        995                 1000                1005

Thr Val  Ser Asp Pro Ala Ser  Ala Met Gln Leu Gly  Gln Cys Thr
    1010                1015                1020

Lys Asn  Leu Ala Ser Ala Leu  Ala Glu Leu Arg Thr  Ala Ala Gln
    1025                1030                1035

Lys Ala  His Glu Ala Cys Gly  Pro Leu Glu Ile Asp  Ser Ala Leu
    1040                1045                1050

Asn Val  Val Arg Ser Leu Glu  Gln Asp Leu Gln Glu  Ala Arg Ala
    1055                1060                1065

Ala Ala  Arg Glu Gly Lys Leu  Gln Pro Leu Pro Gly  Glu Thr Met
    1070                1075                1080

Glu Lys  Cys Ala Gln Asp Leu  Gly Ser Ser Thr Lys  Ala Val Ser
    1085                1090                1095

Ser Ser  Ile Ala Gln Leu Leu  Gly Glu Ile Val His  Gly Asn Glu
    1100                1105                1110

Asn Tyr  Thr Gly Arg Ala Ala  Arg Asp Val Ala Gln  Ala Leu Arg
    1115                1120                1125
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Ser Leu  Ala Gln Ala Ser Arg  Gly Val Ala Ala Asn  Ser Thr Asp
    1130                1135                1140

Pro Ala  Val Gln Asn Ala Met  Leu Glu Cys Ala Glu  Asp Val Met
    1145                1150                1155

Asp Lys  Ala Gly Asn Leu Ile  Glu Glu Ala Lys Arg  Ala Val Gly
    1160                1165                1170

Lys Pro  Thr Asp Pro Glu Gly  Gln Gln Arg Leu Val  Gln Val Ala
    1175                1180                1185

Lys Ala  Val Ser Gln Ala Leu  Ser Arg Cys Val Asn  Cys Leu Pro
    1190                1195                1200

Gly Gln  Arg Asp Val Asp Ala  Ala Ile Lys Ser Ile  Gly Glu Ala
    1205                1210                1215

Ser Lys  Ile Leu Leu Ala Ser  Ser Phe Pro Ser Gly  Thr Lys Asn
    1220                1225                1230

Phe Gln  Glu Ala Gln Ser Gln  Leu Asn Gln Ala Ala  Ala Gly Leu
    1235                1240                1245

Asn Gln  Ser Ala Asn Glu Leu  Val Gln Ala Ser Arg  Thr Thr Pro
    1250                1255                1260

Gln Glu  Leu Ala Lys Ala Ser  Gly Lys Tyr Ser Gln  Asp Phe Asn
    1265                1270                1275

Glu Phe  Leu Gln Ala Gly Val  Glu Met Ala Gly Gln  Ser Gln Asn
    1280                1285                1290

Lys Glu  Asp Gln Ala Gln Val  Val Ser Asn Leu Lys  Ser Ile Ser
    1295                1300                1305

Leu Ser  Ser Ser Lys Leu Leu  Leu Ala Ala Lys Ala  Leu Ser Ala
    1310                1315                1320

Asp Pro  Ala Ala Pro Asn Leu  Lys Asn Gln Leu Ala  Ala Ala Ala
    1325                1330                1335

Arg Ala  Val Thr Asp Ser Ile  Asn Gln Leu Ile Thr  Val Cys Thr
    1340                1345                1350

Gln Gln  Ala Pro Gly Gln Lys  Glu Cys Asp Asn Ala  Leu Arg Glu
    1355                1360                1365

Leu Glu  Thr Val Arg Glu Leu  Leu Gln Asn Pro Thr  Gln Pro Val
    1370                1375                1380

Asn Asp  Gln Ser Tyr Phe His  Cys Leu Asp Ser Val  Met Glu Asn
    1385                1390                1395

Ser Lys  Val Leu Gly Glu Ser  Met Ala Gly Ile Ser  Gln Asn Ala
    1400                1405                1410

Lys Thr  Ser Asn Leu Pro Glu  Phe Gly Glu Ser Val  Gly Ala Ala
    1415                1420                1425

Ser Lys  Ala Leu Cys Gly Leu  Thr Glu Ala Ala Ala  Gln Ala Ala
    1430                1435                1440

Tyr Leu  Val Gly Val Ser Asp  Ala Asn Ser His Ala  Gly Met Gln
    1445                1450                1455

Gly Leu  Val Asp Pro Thr Gln  Phe Ala Arg Ala Asn  Gln Ala Ile
    1460                1465                1470

Gln Met  Ala Cys Gln Asn Leu  Gly Asp Pro Ala Cys  Thr Gln Ser
    1475                1480                1485

Gln Val  Leu Ser Ala Ala Thr  Ile Val Ala Lys His  Thr Ser Ala
    1490                1495                1500
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Leu Cys  Asn Ala Cys Arg Val  Ala Ser Thr His Thr  Ser Asn Pro
    1505                1510                1515

Val Ala  Lys Arg Gln Phe Val  Gln Ser Ala Lys Glu  Val Ala Asn
    1520                1525                1530

Ser Thr  Ala Asn Leu Val Lys  Thr Ile Lys Ala Leu  Asp Gly Thr
    1535                1540                1545

Phe Asn  Asp Glu Asn Arg Val  Lys Cys Arg Asn Ala  Thr Val Pro
    1550                1555                1560

Leu Ile  Gln Ala Val Glu Asn  Leu Thr Ala Phe Ala  Ser Asn Pro
    1565                1570                1575

Glu Phe  Ala Ser Val Pro Ala  Gln Ile Ser Pro Glu  Gly Leu Arg
    1580                1585                1590

Ala Met  Glu Pro Ile Val Thr  Ala Ala Lys Leu Met  Leu Glu Ser
    1595                1600                1605

Ser Ser  Gly Leu Ile Gln Thr  Ala Arg Ser Leu Ala  Ala Asn Pro
    1610                1615                1620

Lys Asp  Pro Pro Gln Trp Ser  Val Leu Ala Gly His  Ser Arg Asn
    1625                1630                1635

Val Ser  Asp Ser Ile Lys Lys  Leu Ile Thr Asn Met  Arg Asp Lys
    1640                1645                1650

Ala Pro  Gly Gln Arg Glu Cys  Asp Gln Ala Ile Glu  Leu Leu Asn
    1655                1660                1665

Gln Ala  Val Arg Asp Leu Asp  Gln Ala Ser Leu Glu  Ala Ile Ser
    1670                1675                1680

Gln Gln  Leu Ala Pro Arg Glu  Gly Ile Ser Gln Glu  Ala Leu His
    1685                1690                1695

Asn Gln  Met Gln Thr Ser Val  Gln Glu Ile Ser Asn  Leu Ile Glu
    1700                1705                1710

Pro Met  Ala Ala Ala Ala Arg  Ala Asp Ser Ser Gln  Leu Gly His
    1715                1720                1725

Lys Val  Ser Gln Met Ala Gln  Tyr Phe Glu Pro Leu  Thr His Ala
    1730                1735                1740

Ser Ile  Gly Thr Ala Ser Lys  Thr Ile Asn His Gln  Gln Gln Met
    1745                1750                1755

Asn Leu  Leu Asp Gln Thr Lys  Thr Leu Ala Glu Ser  Ala Leu Gln
    1760                1765                1770

Met Leu  Tyr Thr Ala Lys Glu  Ala Gly Gly Asn Pro  Lys Val Ala
    1775                1780                1785

Ala Gln  Thr Gln Glu Ala Leu  Asp Glu Ala Ala Gln  Met Met His
    1790                1795                1800

Glu Ala  Val Gly Asp Leu Thr  Val Thr Leu Asn Glu  Ala Ala Ser
    1805                1810                1815

Ala Ala  Gly Ala Val Gly Gly  Met Val Asp Ser Ile  Thr Gln Ala
    1820                1825                1830

Ile Asn  Lys Leu Asp Glu Glu  Pro Thr Gly Glu Pro  Glu Gly Ser
    1835                1840                1845

Phe Val  Asp Tyr Gln Thr Thr  Met Val Lys Thr Ala  Lys Ala Ile
    1850                1855                1860

Ala Val  Thr Val Gln Glu Met  Val Thr Lys Ser Thr  Thr Asn Pro
    1865                1870                1875

Asp Glu  Leu Gly Thr Leu Ala  Asn Gln Leu Thr Asn  Glu Tyr Ser
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    1880                1885                1890

Gln Leu  Ala His Glu Ala Lys  Pro Ala Ala Met Thr  Ala Glu Asn
    1895                1900                1905

Glu Glu  Ile Gly Ser His Ile  Lys Gln Arg Val Gln  Glu Leu Gly
    1910                1915                1920

His Asn  Cys Ser Leu Leu Val  Thr Lys Ala Gly Ala  Leu Gln Cys
    1925                1930                1935

Ser Pro  Asn Asp Ser Tyr Thr  Lys Lys Glu Leu Ile  Glu Ser Ala
    1940                1945                1950

Arg Arg  Val Ser Glu Lys Val  Ser His Val Leu Ala  Ala Leu Gln
    1955                1960                1965

Ala Gly  Asn Arg Gly Thr Gln  Ala Cys Ile Thr Ala  Ala Ser Ala
    1970                1975                1980

Val Ser  Gly Ile Ile Ala Asp  Leu Asp Thr Thr Ile  Met Phe Ala
    1985                1990                1995

Thr Ala  Gly Thr Leu Asn Arg  Glu Asn Ala Glu Thr  Phe Ala Asp
    2000                2005                2010

His Arg  Glu Gly Ile Leu Lys  Thr Ala Lys Ala Leu  Val Glu Asp
    2015                2020                2025

Thr Lys  Val Leu Val Gln Asn  Ala Thr Ser Ser Gln  Glu Lys Leu
    2030                2035                2040

Ala Gln  Ala Ala Gln Ser Ser  Val Thr Thr Ile Thr  Arg Leu Ala
    2045                2050                2055

Glu Thr  Val Lys Leu Gly Ala  Ala Ser Leu Gly Ala  Glu Asp Pro
    2060                2065                2070

Glu Thr  Gln Val Val Leu Ile  Asn Ala Val Lys Asp  Val Ala Lys
    2075                2080                2085

Ala Leu  Gly Asp Leu Ile Ser  Ala Thr Lys Ser Ala  Ala Gly Lys
    2090                2095                2100

Ser Ser  Asp Asp Pro Ser Val  Tyr Gln Leu Lys Asn  Ser Ala Lys
    2105                2110                2115

Val Met  Val Thr Asn Val Thr  Ser Leu Leu Lys Thr  Val Lys Ala
    2120                2125                2130

Val Glu  Asp Glu Ala Thr Lys  Gly Thr Arg Ala Leu  Glu Ala Thr
    2135                2140                2145

Ile Glu  His Ile Arg Gln Glu  Leu Ala Val Phe Ser  Ser Pro Glu
    2150                2155                2160

Pro Pro  Pro His Thr Ser Thr  Pro Glu Asp Phe Ile  Arg Met Thr
    2165                2170                2175

Lys Gly  Ile Thr Met Ala Thr  Ala Lys Ala Val Ala  Ala Gly Asn
    2180                2185                2190

Ser Cys  Arg Gln Glu Asp Val  Ile Ala Thr Ala Asn  Leu Ser Arg
    2195                2200                2205

Arg Ala  Ile Ala Asp Met Leu  Arg Ser Cys Lys Glu  Ala Val Tyr
    2210                2215                2220

His Pro  Glu Val His Ala Asp  Val Arg Met Arg Ala  Thr Arg Phe
    2225                2230                2235

Gly Lys  Glu Cys Ala Ile Gly  Tyr Leu Gln Leu Leu  Glu His Val
    2240                2245                2250

Leu Leu  Ile Leu Gln Lys Pro  Ser Pro Glu Leu Lys  Gln Gln Leu
    2255                2260                2265
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Ala Ala  Tyr Ser Lys Gln Val  Ala Gly Ser Val Thr  Glu Leu Ile
    2270                2275                2280

Gln Ala  Ala Glu Ala Met Lys  Gly Thr Glu Trp Val  Asp Pro Glu
    2285                2290                2295

Asp Pro  Thr Val Ile Ala Glu  Asn Glu Leu Leu Gly  Ala Ala Ala
    2300                2305                2310

Ala Ile  Glu Ala Ala Ala Lys  Lys Leu Glu Gln Leu  Lys Pro Arg
    2315                2320                2325

Ala Lys  Pro Lys Gln Ala Asp  Glu Ser Leu Asn Phe  Glu Glu Gln
    2330                2335                2340

Ile Leu  Glu Ala Ala Lys Ser  Ile Ala Ala
    2345                2350


<210> SEQ ID NO 10
<211> LENGTH: 1006
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 10

Met Ala Ala Ala Tyr Leu Asp Pro Asn Leu Asn His Thr Pro Asn Ser
1               5                   10                  15

Ser Thr Lys Thr His Leu Gly Thr Gly Met Glu Arg Ser Pro Gly Ala
            20                  25                  30

Met Glu Arg Val Leu Lys Val Phe His Tyr Phe Glu Ser Asn Ser Glu
        35                  40                  45

Pro Thr Thr Trp Ala Ser Ile Ile Arg His Gly Asp Ala Thr Asp Val
    50                  55                  60

Arg Gly Ile Ile Gln Lys Ile Val Asp Ser His Lys Val Lys His Val
65                  70                  75                  80

Ala Cys Tyr Gly Phe Arg Leu Ser His Leu Arg Ser Glu Glu Val His
                85                  90                  95

Trp Leu His Val Asp Met Gly Val Ser Ser Val Arg Glu Lys Tyr Glu
            100                 105                 110

Leu Ala His Pro Pro Glu Glu Trp Lys Tyr Glu Leu Arg Ile Arg Tyr
        115                 120                 125

Leu Pro Lys Gly Phe Leu Asn Gln Phe Thr Glu Asp Lys Pro Thr Leu
    130                 135                 140

Asn Phe Phe Tyr Gln Gln Val Lys Ser Asp Tyr Met Leu Glu Ile Ala
145                 150                 155                 160

Asp Gln Val Asp Gln Glu Ile Ala Leu Lys Leu Gly Cys Leu Glu Ile
                165                 170                 175

Arg Arg Ser Tyr Trp Glu Met Arg Gly Asn Ala Leu Glu Lys Lys Ser
            180                 185                 190

Asn Tyr Glu Val Leu Glu Lys Asp Val Gly Leu Lys Arg Phe Phe Pro
        195                 200                 205

Lys Ser Leu Leu Asp Ser Val Lys Ala Lys Thr Leu Arg Lys Leu Ile
    210                 215                 220

Gln Gln Thr Phe Arg Gln Phe Ala Asn Leu Asn Arg Glu Glu Ser Ile
225                 230                 235                 240

Leu Lys Phe Phe Glu Ile Leu Ser Pro Val Tyr Arg Phe Asp Lys Glu
                245                 250                 255

Cys Phe Lys Cys Ala Leu Gly Ser Ser Trp Ile Ile Ser Val Glu Leu
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           260                 265                 270

Ala Ile Gly Pro Glu Glu Gly Ile Ser Tyr Leu Thr Asp Lys Gly Cys
        275                 280                 285

Asn Pro Thr His Leu Ala Asp Phe Thr Gln Val Gln Thr Ile Gln Tyr
    290                 295                 300

Ser Asn Ser Glu Asp Lys Asp Arg Lys Gly Met Leu Gln Leu Lys Ile
305                 310                 315                 320

Ala Gly Ala Pro Glu Pro Leu Thr Val Thr Ala Pro Ser Leu Thr Ile
                325                 330                 335

Ala Glu Asn Met Ala Asp Leu Ile Asp Gly Tyr Cys Arg Leu Val Asn
            340                 345                 350

Gly Thr Ser Gln Ser Phe Ile Ile Arg Pro Gln Lys Glu Gly Glu Arg
        355                 360                 365

Ala Leu Pro Ser Ile Pro Lys Leu Ala Asn Ser Glu Lys Gln Gly Met
    370                 375                 380

Arg Thr His Ala Val Ser Val Ser Glu Thr Asp Asp Tyr Ala Glu Ile
385                 390                 395                 400

Ile Asp Glu Glu Asp Thr Tyr Thr Met Pro Ser Thr Arg Asp Tyr Glu
                405                 410                 415

Ile Gln Arg Glu Arg Ile Glu Leu Gly Arg Cys Ile Gly Glu Gly Gln
            420                 425                 430

Phe Gly Asp Val His Gln Gly Ile Tyr Met Ser Pro Glu Asn Pro Ala
        435                 440                 445

Leu Ala Val Ala Ile Lys Thr Cys Lys Asn Cys Thr Ser Asp Ser Val
    450                 455                 460

Arg Glu Lys Phe Leu Gln Glu Ala Leu Thr Met Arg Gln Phe Asp His
465                 470                 475                 480

Pro His Ile Val Lys Leu Ile Gly Val Ile Thr Glu Asn Pro Val Trp
                485                 490                 495

Ile Ile Met Glu Leu Cys Thr Leu Gly Glu Leu Arg Ser Phe Leu Gln
            500                 505                 510

Val Arg Lys Tyr Ser Leu Asp Leu Ala Ser Leu Ile Leu Tyr Ala Tyr
        515                 520                 525

Gln Leu Ser Thr Ala Leu Ala Tyr Leu Glu Ser Lys Arg Phe Val His
    530                 535                 540

Arg Asp Ile Ala Ala Arg Asn Val Leu Val Ser Ser Asn Asp Cys Val
545                 550                 555                 560

Lys Leu Gly Asp Phe Gly Leu Ser Arg Tyr Met Glu Asp Ser Thr Tyr
                565                 570                 575

Tyr Lys Ala Ser Lys Gly Lys Leu Pro Ile Lys Trp Met Ala Pro Glu
            580                 585                 590

Ser Ile Asn Phe Arg Arg Phe Thr Ser Ala Ser Asp Val Trp Met Phe
        595                 600                 605

Gly Val Cys Met Trp Glu Ile Leu Met His Gly Val Lys Pro Phe Gln
    610                 615                 620

Gly Val Lys Asn Asn Asp Val Ile Gly Arg Ile Glu Asn Gly Glu Arg
625                 630                 635                 640

Leu Pro Met Pro Pro Asn Cys Pro Pro Thr Leu Tyr Ser Leu Met Thr
                645                 650                 655

Lys Cys Trp Ala Tyr Asp Pro Ser Arg Arg Pro Arg Phe Thr Glu Leu
            660                 665                 670
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Lys Ala Gln Leu Ser Thr Ile Leu Glu Glu Glu Lys Ala Gln Gln Glu
        675                 680                 685

Glu Arg Met Arg Met Glu Ser Arg Arg Gln Ala Thr Val Ser Trp Asp
    690                 695                 700

Ser Gly Gly Ser Asp Glu Ala Pro Pro Lys Pro Ser Arg Pro Gly Tyr
705                 710                 715                 720

Pro Ser Pro Arg Ser Ser Glu Gly Phe Tyr Pro Ser Pro Gln His Met
                725                 730                 735

Val Gln Thr Asn His Tyr Gln Asp Ser Thr Val Leu Asp Leu Arg Gly
            740                 745                 750

Ile Gly Gln Val Leu Pro Thr His Leu Met Glu Glu Arg Leu Ile Arg
        755                 760                 765

Gln Gln Gln Glu Met Glu Glu Asp Gln Arg Trp Leu Glu Lys Glu Glu
    770                 775                 780

Arg Phe Leu Lys Pro Asp Val Arg Leu Ser Arg Gly Ser Ile Asp Arg
785                 790                 795                 800

Glu Asp Gly Ser Leu Gln Gly Pro Ile Gly Asn Gln His Ile Tyr Gln
                805                 810                 815

Pro Val Gly Lys Pro Asp Pro Ala Ala Pro Pro Lys Lys Pro Pro Arg
            820                 825                 830

Pro Gly Ala Pro Gly His Leu Gly Ser Leu Ala Ser Leu Ser Ser Pro
        835                 840                 845

Ala Asp Ser Tyr Asn Glu Gly Val Lys Leu Gln Pro Gln Glu Ile Ser
    850                 855                 860

Pro Pro Pro Thr Ala Asn Leu Asp Arg Ser Asn Asp Lys Val Tyr Glu
865                 870                 875                 880

Asn Val Thr Gly Leu Val Lys Ala Val Ile Glu Met Ser Ser Lys Ile
                885                 890                 895

Gln Pro Ala Pro Pro Glu Glu Tyr Val Pro Met Val Lys Glu Val Gly
            900                 905                 910

Leu Ala Leu Arg Thr Leu Leu Ala Thr Val Asp Glu Thr Ile Pro Leu
        915                 920                 925

Leu Pro Ala Ser Thr His Arg Glu Ile Glu Met Ala Gln Lys Leu Leu
    930                 935                 940

Asn Ser Asp Leu Gly Glu Leu Ile Asn Lys Met Lys Leu Ala Gln Gln
945                 950                 955                 960

Tyr Val Met Thr Ser Leu Gln Gln Glu Tyr Lys Lys Gln Met Leu Thr
                965                 970                 975

Ala Ala His Ala Leu Ala Val Asp Ala Lys Asn Leu Leu Asp Val Ile
            980                 985                 990

Asp Gln Ala Arg Leu Lys Met Leu  Gly Gln Thr Arg Pro  His
        995                 1000                1005


<210> SEQ ID NO 11
<211> LENGTH: 268
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 11

Met Ala Val Asn Val Tyr Ser Thr Ser Val Thr Ser Asp Asn Leu Ser
1               5                   10                  15

Arg His Asp Met Leu Ala Trp Ile Asn Glu Ser Leu Gln Leu Asn Leu
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           20                  25                  30

Thr Lys Ile Glu Gln Leu Cys Ser Gly Ala Ala Tyr Cys Gln Phe Met
        35                  40                  45

Asp Met Leu Phe Pro Gly Ser Ile Ala Leu Lys Lys Val Lys Phe Gln
    50                  55                  60

Ala Lys Leu Glu His Glu Tyr Ile Gln Asn Phe Lys Ile Leu Gln Ala
65                  70                  75                  80

Gly Phe Lys Arg Met Gly Val Asp Lys Ile Ile Pro Val Asp Lys Leu
                85                  90                  95

Val Lys Gly Lys Phe Gln Asp Asn Phe Glu Phe Val Gln Trp Phe Lys
            100                 105                 110

Lys Phe Phe Asp Ala Asn Tyr Asp Gly Lys Asp Tyr Asp Pro Val Ala
        115                 120                 125

Ala Arg Gln Gly Gln Glu Thr Ala Val Ala Pro Ser Leu Val Ala Pro
    130                 135                 140

Ala Leu Asn Lys Pro Lys Lys Pro Leu Thr Ser Ser Ser Ala Ala Pro
145                 150                 155                 160

Gln Arg Pro Ile Ser Thr Gln Arg Thr Ala Ala Ala Pro Lys Ala Gly
                165                 170                 175

Pro Gly Val Val Arg Lys Asn Pro Gly Val Gly Asn Gly Asp Asp Glu
            180                 185                 190

Ala Ala Glu Leu Met Gln Gln Val Asn Val Leu Lys Leu Thr Val Glu
        195                 200                 205

Asp Leu Glu Lys Glu Arg Asp Phe Tyr Phe Gly Lys Leu Arg Asn Ile
    210                 215                 220

Glu Leu Ile Cys Gln Glu Asn Glu Gly Glu Asn Asp Pro Val Leu Gln
225                 230                 235                 240

Arg Ile Val Asp Ile Leu Tyr Ala Thr Asp Glu Gly Phe Val Ile Pro
                245                 250                 255

Asp Glu Gly Gly Pro Gln Glu Glu Gln Glu Glu Tyr
            260                 265


<210> SEQ ID NO 12
<211> LENGTH: 1320
<212> TYPE: PRT
<213> ORGANISM: Rattus norvegicus

<400> SEQUENCE: 12

Met Ser Met Leu Lys Pro Ser Gly Leu Lys Ala Pro Thr Lys Ile Leu
1               5                   10                  15

Lys Pro Gly Ser Thr Ala Leu Lys Thr Pro Ala Ala Ala Ala Ala Pro
            20                  25                  30

Leu Glu Lys Thr Val Pro Ser Glu Lys Ala Ser Gly Pro Pro Ser Ser
        35                  40                  45

Glu Thr Gln Glu Glu Phe Val Asp Asp Phe Arg Val Gly Glu Arg Val
    50                  55                  60

Trp Val Asn Gly Asn Lys Pro Gly Phe Ile Gln Phe Leu Gly Glu Thr
65                  70                  75                  80

Gln Phe Ala Pro Gly Gln Trp Ala Gly Ile Val Leu Asp Glu Pro Ile
                85                  90                  95

Gly Lys Asn Asp Gly Ser Val Ala Gly Val Arg Tyr Phe Gln Cys Glu
            100                 105                 110
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Pro Leu Lys Gly Ile Phe Thr Arg Pro Ser Lys Leu Thr Arg Lys Val
        115                 120                 125

Gln Ala Glu Asp Glu Ala Asn Gly Leu Gln Thr Ala His Ala Arg Ala
    130                 135                 140

Ala Ser Pro Leu Ser Thr Ala Ala Ala Thr Met Val Ser Ser Ser Pro
145                 150                 155                 160

Ala Thr Pro Ser Asn Ile Pro Gln Lys Pro Ser Gln Pro Val Ala Lys
                165                 170                 175

Glu Thr Ser Ala Thr Pro Gln Ile Ser Asn Leu Thr Lys Thr Ala Ser
            180                 185                 190

Glu Ser Ile Ser Asn Leu Ser Glu Ala Gly Ser Val Lys Lys Gly Glu
        195                 200                 205

Arg Glu Leu Lys Ile Gly Asp Arg Val Leu Val Gly Gly Thr Lys Ala
    210                 215                 220

Gly Val Val Arg Phe Leu Gly Glu Thr Asp Phe Ala Lys Gly Glu Trp
225                 230                 235                 240

Cys Gly Val Glu Leu Asp Glu Pro Leu Gly Lys Asn Asp Gly Ala Val
                245                 250                 255

Ala Gly Thr Arg Tyr Phe Gln Cys Gln Pro Lys Tyr Gly Leu Phe Ala
            260                 265                 270

Pro Val His Lys Val Thr Lys Ile Gly Phe Pro Ser Thr Thr Pro Ala
        275                 280                 285

Lys Ala Lys Ala Ala Ala Val Arg Arg Val Met Ala Thr Thr Pro Ala
    290                 295                 300

Ser Leu Lys Arg Ser Pro Ser Ala Ser Ser Leu Ser Ser Met Ser Ser
305                 310                 315                 320

Val Ala Ser Ser Val Ser Ser Lys Pro Ser Arg Thr Gly Leu Leu Thr
                325                 330                 335

Glu Thr Ser Ser Arg Tyr Ala Arg Lys Ile Ser Gly Thr Thr Ala Leu
            340                 345                 350

Gln Glu Ala Leu Lys Glu Lys Gln Gln His Ile Glu Gln Leu Leu Ala
        355                 360                 365

Glu Arg Asp Leu Glu Arg Ala Glu Val Ala Lys Ala Thr Ser His Val
    370                 375                 380

Gly Glu Ile Glu Gln Glu Leu Ala Leu Ala Arg Asp Gly His Asp Gln
385                 390                 395                 400

His Val Leu Glu Leu Glu Ala Lys Met Asp Gln Leu Arg Thr Met Val
                405                 410                 415

Glu Ala Ala Asp Arg Glu Lys Val Glu Leu Leu Asn Gln Leu Glu Glu
            420                 425                 430

Glu Lys Arg Lys Val Glu Asp Leu Gln Phe Arg Val Glu Glu Glu Ser
        435                 440                 445

Ile Thr Lys Gly Asp Leu Glu Thr Gln Thr Lys Leu Glu His Ala Arg
    450                 455                 460

Ile Lys Glu Leu Glu Gln Ser Leu Leu Phe Glu Lys Thr Lys Ala Asp
465                 470                 475                 480

Lys Leu Gln Arg Glu Leu Glu Asp Thr Arg Val Ala Thr Val Ser Glu
                485                 490                 495

Lys Ser Arg Ile Met Glu Leu Glu Lys Asp Leu Ala Leu Arg Val Gln
            500                 505                 510

Glu Val Ala Glu Leu Arg Arg Arg Leu Glu Ser Ser Lys Pro Pro Gly
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        515                 520                 525

Asp Val Asp Met Ser Leu Ser Leu Leu Gln Glu Ile Ser Ala Leu Gln
    530                 535                 540

Glu Lys Leu Glu Val Thr His Thr Asp His Gln Asn Glu Val Thr Ser
545                 550                 555                 560

Leu Lys Asp His Phe Gly Thr Arg Glu Glu Met Phe Gln Lys Glu Ile
                565                 570                 575

Lys Ala Leu His Ala Ala Thr Glu Lys Leu Ser Lys Glu Asn Glu Ser
            580                 585                 590

Leu Arg Ser Lys Leu Asp His Ala Asn Lys Glu Asn Ser Asp Val Ile
        595                 600                 605

Ala Leu Trp Lys Ser Lys Leu Glu Thr Ala Ile Ala Ser His Gln Gln
    610                 615                 620

Ala Met Glu Glu Leu Lys Val Ser Phe Ser Lys Gly Ile Gly Thr Asp
625                 630                 635                 640

Ser Ala Glu Phe Ala Glu Leu Lys Thr Gln Ile Glu Arg Leu Arg Leu
                645                 650                 655

Asp Tyr Gln His Glu Ile Glu Ser Leu Gln Ser Lys Gln Asp Ser Glu
            660                 665                 670

Arg Ser Ala His Ala Lys Glu Met Glu Ser Met Lys Ala Lys Leu Met
        675                 680                 685

Lys Ile Ile Lys Glu Lys Glu Asp Ser Leu Glu Ala Val Lys Ala Arg
    690                 695                 700

Leu Asp Thr Ala Glu Asp Gln His Leu Val Glu Met Glu Glu Met Leu
705                 710                 715                 720

Ser Lys Leu Gln Glu Ala Glu Ile Lys Lys Glu Lys Phe Ala Ser Ala
                725                 730                 735

Ser Glu Glu Ala Val Ser Thr Gln Thr Ser Met Gln Asp Thr Val Asn
            740                 745                 750

Lys Leu His Gln Lys Glu Glu Gln Phe Asn Met Leu Ser Ser Glu Leu
        755                 760                 765

Glu Lys Leu Arg Glu Asn Leu Thr Asp Met Glu Ala Lys Phe Lys Glu
    770                 775                 780

Lys Asp Glu Arg Glu Asp Gln Leu Val Lys Ala Lys Glu Lys Leu Glu
785                 790                 795                 800

Asn Asp Ile Ala Glu Ile Met Lys Met Ser Gly Asp Asn Ser Ser Gln
                805                 810                 815

Leu Thr Lys Met Asn Asp Glu Leu Arg Leu Lys Glu Arg Ser Val Glu
            820                 825                 830

Glu Leu Gln Leu Lys Leu Thr Lys Ala Asn Glu Asn Ala Ser Leu Leu
        835                 840                 845

Gln Lys Ser Ile Gly Glu Val Thr Leu Lys Ala Glu Gln Ser Gln Gln
    850                 855                 860

Glu Ala Ala Lys Lys His Glu Glu Glu Lys Lys Glu Leu Glu Asn Lys
865                 870                 875                 880

Leu Leu Glu Leu Glu Lys Lys Met Glu Thr Ser His Tyr Gln Cys Gln
                885                 890                 895

Asp Leu Lys Ala Lys Tyr Glu Lys Ala Ser Ser Glu Thr Lys Ile Lys
            900                 905                 910

His Glu Glu Ile Leu Gln Asn Phe Gln Lys Met Leu Val Asp Thr Glu
        915                 920                 925
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Asp Lys Leu Lys Ala Ala Gln Glu Ala Asn Arg Asp Leu Met Gln Asp
    930                 935                 940

Met Glu Glu Leu Lys Ser Gln Ala Asp Lys Ala Lys Ala Ala Gln Thr
945                 950                 955                 960

Ala Glu Asp Ala Met Gln Ile Met Glu Gln Met Thr Lys Glu Lys Thr
               965                 970                 975

Glu Thr Leu Ala Ser Leu Glu Asp Thr Lys Gln Thr Asn Ala Lys Leu
           980                 985                 990

Gln Ser Glu Leu Asp Thr Leu Lys  Glu Asn Asn Leu Lys  Thr Val Glu
       995                 1000                1005

Glu Leu  Asn Lys Ser Lys Glu  Leu Leu Asn Glu Glu  Asn Gln Lys
    1010                1015                1020

Met Glu  Glu Phe Lys Lys Glu  Ile Glu Thr Leu Lys  Gln Ala Ala
    1025                1030                1035

Ala Gln  Lys Ser Gln Gln Leu  Ser Ala Leu Gln Glu  Glu Asn Val
    1040                1045                1050

Lys Leu  Ala Glu Glu Leu Gly  Arg Thr Arg Asp Glu  Val Thr Ser
    1055                1060                1065

His Gln  Lys Leu Glu Glu Glu  Arg Ser Val Leu Asn  Asn Gln Leu
    1070                1075                1080

Leu Glu  Met Lys Lys Ser Leu  Pro Ser Asn Thr Leu  Arg Glu Ser
    1085                1090                1095

Glu Tyr  Arg Lys Asp Ala Asp  Glu Glu Lys Ala Ser  Leu Gln Lys
    1100                1105                1110

Ser Ile  Ser Leu Thr Ser Ala  Leu Leu Thr Glu Lys  Asp Ala Glu
    1115                1120                1125

Leu Glu  Lys Leu Arg Asn Glu  Val Thr Val Leu Arg  Gly Glu Asn
    1130                1135                1140

Ala Ser  Ala Lys Ser Leu His  Ser Val Val Gln Thr  Leu Glu Ser
    1145                1150                1155

Asp Lys  Val Lys Leu Glu Leu  Lys Val Lys Asn Leu  Glu Leu Gln
    1160                1165                1170

Leu Lys  Glu Asn Lys Arg Gln  Leu Ser Ser Ser Ser  Gly Asn Thr
    1175                1180                1185

Asp Val  Gln Thr Glu Glu Asp  Glu Arg Ala Gln Glu  Ser Gln Gln
    1190                1195                1200

Met Ile  Asp Phe Leu Asn Ser  Val Ile Val Asp Leu  Gln Arg Lys
    1205                1210                1215

Asn Gln  Asp Leu Lys Met Lys  Val Glu Met Met Ser  Glu Gly Ala
    1220                1225                1230

Leu Asn  Gly Asn Gly Glu Asp  Pro Asn Ser Tyr Asp  Ser Asp Asp
    1235                1240                1245

Gln Glu  Lys Gln Ser Lys Lys  Lys Pro Arg Leu Phe  Cys Asp Ile
    1250                1255                1260

Cys Asp  Cys Phe Asp Leu His  Asp Thr Glu Asp Cys  Pro Thr Gln
    1265                1270                1275

Ala Gln  Met Ser Glu Asp Pro  Pro His Ser Thr His  His Gly Ser
    1280                1285                1290

Arg Ser  Glu Glu Arg Pro Tyr  Cys Glu Ile Cys Glu  Met Phe Gly
    1295                1300                1305
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His Trp  Ala Thr Asn Cys Asn  Asp Asp Glu Thr Phe
    1310                1315                1320


<210> SEQ ID NO 13
<211> LENGTH: 1294
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 13

Met Ala Met Gly Asp Asp Lys Ser Phe Asp Asp Glu Glu Ser Val Asp
1               5                  10                  15

Gly Asn Arg Pro Ser Ser Ala Ala Ser Ala Phe Lys Val Pro Ala Pro
            20                  25                  30

Lys Thr Ser Gly Asn Pro Ala Asn Ser Ala Arg Lys Pro Gly Ser Ala
        35                  40                  45

Gly Gly Pro Lys Val Gly Gly Ala Ser Lys Glu Gly Gly Ala Gly Ala
    50                  55                  60

Val Asp Glu Asp Asp Phe Ile Lys Ala Phe Thr Asp Val Pro Ser Ile
65                  70                  75                  80

Gln Ile Tyr Ser Ser Arg Glu Leu Glu Glu Thr Leu Asn Lys Ile Arg
                85                  90                  95

Glu Ile Leu Ser Asp Asp Lys His Asp Trp Asp Gln Arg Ala Asn Ala
            100                 105                 110

Leu Lys Lys Ile Arg Ser Leu Leu Val Ala Gly Ala Ala Gln Tyr Asp
        115                 120                 125

Cys Phe Phe Gln His Leu Arg Leu Leu Asp Gly Ala Leu Lys Leu Ser
    130                 135                 140

Ala Lys Asp Leu Arg Ser Gln Val Val Arg Glu Ala Cys Ile Thr Val
145                 150                 155                 160

Ala His Leu Ser Thr Val Leu Gly Asn Lys Phe Asp His Gly Ala Glu
                165                 170                 175

Ala Ile Val Pro Thr Leu Phe Asn Leu Val Pro Asn Ser Ala Lys Val
            180                 185                 190

Met Ala Thr Ser Gly Cys Ala Ala Ile Arg Phe Ile Ile Arg His Thr
        195                 200                 205

His Val Pro Arg Leu Ile Pro Leu Ile Thr Ser Asn Cys Thr Ser Lys
    210                 215                 220

Ser Val Pro Val Arg Arg Arg Ser Phe Glu Phe Leu Asp Leu Leu Leu
225                 230                 235                 240

Gln Glu Trp Gln Thr His Ser Leu Glu Arg His Ala Ala Val Leu Val
                245                 250                 255

Glu Thr Ile Lys Lys Gly Ile His Asp Ala Asp Ala Glu Ala Arg Val
            260                 265                 270

Glu Ala Arg Lys Thr Tyr Met Gly Leu Arg Asn His Phe Pro Gly Glu
        275                 280                 285

Ala Glu Thr Leu Tyr Asn Ser Leu Glu Pro Ser Tyr Gln Lys Ser Leu
    290                 295                 300

Gln Thr Tyr Leu Lys Ser Ser Gly Ser Val Ala Ser Leu Pro Gln Ser
305                 310                 315                 320

Asp Arg Ser Ser Ser Ser Ser Gln Glu Ser Leu Asn Arg Pro Phe Ser
                325                 330                 335

Ser Lys Trp Ser Thr Ala Asn Pro Ser Thr Val Ala Gly Arg Val Ser
            340                 345                 350
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Ala Gly Ser Ser Lys Ala Ser Ser Leu Pro Gly Ser Leu Gln Arg Ser
        355                 360                 365

Arg Ser Asp Ile Asp Val Asn Ala Ala Ala Gly Ala Lys Ala His His
    370                 375                 380

Ala Ala Gly Gln Ser Val Arg Ser Gly Arg Leu Gly Ala Gly Ala Leu
385                 390                 395                 400

Asn Ala Gly Ser Tyr Ala Ser Leu Glu Asp Thr Ser Asp Lys Leu Asp
                405                 410                 415

Gly Thr Ala Ser Glu Asp Gly Arg Val Arg Ala Lys Leu Ser Ala Pro
            420                 425                 430

Leu Ala Gly Met Gly Asn Ala Lys Ala Asp Ser Arg Gly Arg Ser Arg
        435                 440                 445

Thr Lys Met Val Ser Gln Ser Gln Pro Gly Ser Arg Ser Gly Ser Pro
    450                 455                 460

Gly Arg Val Leu Thr Thr Thr Ala Leu Ser Thr Val Ser Ser Gly Val
465                 470                 475                 480

Gln Arg Val Leu Val Asn Ser Ala Ser Ala Gln Lys Arg Ser Lys Ile
                485                 490                 495

Pro Arg Ser Gln Gly Cys Ser Arg Glu Ala Ser Pro Ser Arg Leu Ser
            500                 505                 510

Val Ala Arg Ser Ser Arg Ile Pro Arg Pro Ser Val Ser Gln Gly Cys
        515                 520                 525

Ser Arg Glu Ala Ser Arg Glu Ser Ser Arg Asp Thr Ser Pro Val Arg
    530                 535                 540

Ser Phe Gln Pro Leu Ala Ser Arg His His Ser Arg Ser Thr Gly Ala
545                 550                 555                 560

Leu Tyr Ala Pro Glu Val Tyr Gly Ala Ser Gly Pro Gly Tyr Gly Ile
                565                 570                 575

Ser Gln Ser Ser Arg Leu Ser Ser Ser Val Ser Ala Met Arg Val Leu
            580                 585                 590

Asn Thr Gly Ser Asp Val Glu Glu Ala Val Ala Asp Ala Leu Lys Lys
        595                 600                 605

Pro Ala Arg Arg Arg Tyr Glu Ser Tyr Gly Met His Ser Asp Asp Asp
    610                 615                 620

Ala Asn Ser Asp Ala Ser Ser Ala Cys Ser Glu Arg Ser Tyr Ser Ser
625                 630                 635                 640

Arg Asn Gly Ser Ile Pro Thr Tyr Met Arg Gln Thr Glu Asp Val Ala
                645                 650                 655

Glu Val Leu Asn Arg Cys Ala Ser Ser Asn Trp Ser Glu Arg Lys Glu
            660                 665                 670

Gly Leu Leu Gly Leu Gln Asn Leu Leu Lys Asn Gln Arg Thr Leu Ser
        675                 680                 685

Arg Val Glu Leu Lys Arg Leu Cys Glu Ile Phe Thr Arg Met Phe Ala
    690                 695                 700

Asp Pro His Gly Lys Arg Val Phe Ser Met Phe Leu Glu Thr Leu Val
705                 710                 715                 720

Asp Phe Ile Gln Val His Lys Asp Asp Leu Gln Asp Trp Leu Phe Val
                725                 730                 735

Leu Leu Thr Gln Leu Leu Lys Lys Met Gly Ala Asp Leu Leu Gly Ser
            740                 745                 750
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Val Gln Ala Lys Val Gln Lys Ala Leu Asp Val Thr Arg Glu Ser Phe
        755                 760                 765

Pro Asn Asp Leu Gln Phe Asn Ile Leu Met Arg Phe Thr Val Asp Gln
    770                 775                 780

Thr Gln Thr Pro Ser Leu Lys Val Lys Val Ala Ile Leu Lys Tyr Ile
785                 790                 795                 800

Glu Thr Leu Ala Lys Gln Met Asp Pro Gly Asp Phe Ile Asn Ser Ser
                805                 810                 815

Glu Thr Arg Leu Ala Val Ser Arg Val Ile Thr Trp Thr Thr Glu Pro
            820                 825                 830

Lys Ser Ser Asp Val Arg Lys Ala Ala Gln Ser Val Leu Ile Ser Leu
        835                 840                 845

Phe Glu Leu Asn Thr Pro Glu Phe Thr Met Leu Leu Gly Ala Leu Pro
    850                 855                 860

Lys Thr Phe Gln Asp Gly Ala Thr Lys Leu Leu His Asn His Leu Arg
865                 870                 875                 880

Asn Thr Gly Asn Gly Thr Gln Ser Ser Met Gly Ser Pro Leu Thr Arg
                885                 890                 895

Pro Thr Pro Arg Ser Pro Ala Asn Trp Ser Ser Pro Leu Thr Ser Pro
            900                 905                 910

Thr Asn Thr Ser Gln Asn Thr Leu Ser Pro Ser Ala Phe Asp Tyr Asp
        915                 920                 925

Thr Glu Asn Met Asn Ser Glu Asp Ile Tyr Ser Ser Leu Arg Gly Val
    930                 935                 940

Thr Glu Ala Ile Gln Asn Phe Ser Phe Arg Ser Gln Glu Asp Met Asn
945                 950                 955                 960

Glu Pro Leu Lys Arg Asp Ser Lys Lys Asp Asp Gly Asp Ser Met Cys
                965                 970                 975

Gly Gly Pro Gly Met Ser Asp Pro Arg Ala Gly Gly Asp Ala Thr Asp
            980                 985                 990

Ser Ser Gln Thr Ala Leu Asp Asn  Lys Ala Ser Leu Leu  His Ser Met
        995                 1000                1005

Pro Thr  His Ser Ser Pro Arg  Ser Arg Asp Tyr Asn  Pro Tyr Asn
    1010                1015                1020

Tyr Ser  Asp Ser Ile Ser Pro  Phe Asn Lys Ser Ala  Leu Lys Glu
    1025                1030                1035

Ala Met  Phe Asp Asp Asp Ala  Asp Gln Phe Pro Asp  Asp Leu Ser
    1040                1045                1050

Leu Asp  His Ser Asp Leu Val  Ala Glu Leu Leu Lys  Glu Leu Ser
    1055                1060                1065

Asn His  Asn Glu Arg Val Glu  Glu Arg Lys Ile Ala  Leu Tyr Glu
    1070                1075                1080

Leu Met  Lys Leu Thr Gln Glu  Glu Ser Phe Ser Val  Trp Asp Glu
    1085                1090                1095

His Phe  Lys Thr Ile Leu Leu  Leu Leu Leu Glu Thr  Leu Gly Asp
    1100                1105                1110

Lys Glu  Pro Thr Ile Arg Ala  Leu Ala Leu Lys Val  Leu Arg Glu
    1115                1120                1125

Ile Leu  Arg His Gln Pro Ala  Arg Phe Lys Asn Tyr  Ala Glu Leu
    1130                1135                1140

Thr Val  Met Lys Thr Leu Glu  Ala His Lys Asp Pro  His Lys Glu
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    1145                1150                1155

Val Val  Arg Ser Ala Glu Glu  Ala Ala Ser Val Leu  Ala Thr Ser
    1160                1165                1170

Ile Ser  Pro Glu Gln Cys Ile  Lys Val Leu Cys Pro  Ile Ile Gln
    1175                1180                1185

Thr Ala  Asp Tyr Pro Ile Asn  Leu Ala Ala Ile Lys  Met Gln Thr
    1190                1195                1200

Lys Val  Ile Glu Arg Val Ser  Lys Glu Thr Leu Asn  Leu Leu Leu
    1205                1210                1215

Pro Glu  Ile Met Pro Gly Leu  Ile Gln Gly Tyr Asp  Asn Ser Glu
    1220                1225                1230

Ser Ser  Val Arg Lys Ala Cys  Val Phe Cys Leu Val  Ala Val His
    1235                1240                1245

Ala Val  Ile Gly Asp Glu Leu  Lys Pro His Leu Ser  Gln Leu Thr
    1250                1255                1260

Gly Ser  Lys Met Lys Leu Leu  Asn Leu Tyr Ile Lys  Arg Ala Gln
    1265                1270                1275

Thr Gly  Ser Gly Gly Ala Asp  Pro Thr Thr Asp Val  Ser Gly Gln
    1280                1285                1290

Ser


<210> SEQ ID NO 14
<211> LENGTH: 2843
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 14

Met Ala Ala Ala Ser Tyr Asp Gln Leu Leu Lys Gln Val Glu Ala Leu
1               5                   10                  15

Lys Met Glu Asn Ser Asn Leu Arg Gln Glu Leu Glu Asp Asn Ser Asn
            20                  25                  30

His Leu Thr Lys Leu Glu Thr Glu Ala Ser Asn Met Lys Glu Val Leu
        35                  40                  45

Lys Gln Leu Gln Gly Ser Ile Glu Asp Glu Ala Met Ala Ser Ser Gly
    50                  55                  60

Gln Ile Asp Leu Leu Glu Arg Leu Lys Glu Leu Asn Leu Asp Ser Ser
65                  70                  75                  80

Asn Phe Pro Gly Val Lys Leu Arg Ser Lys Met Ser Leu Arg Ser Tyr
                85                  90                  95

Gly Ser Arg Glu Gly Ser Val Ser Ser Arg Ser Gly Glu Cys Ser Pro
            100                 105                 110

Val Pro Met Gly Ser Phe Pro Arg Arg Gly Phe Val Asn Gly Ser Arg
        115                 120                 125

Glu Ser Thr Gly Tyr Leu Glu Glu Leu Glu Lys Glu Arg Ser Leu Leu
    130                 135                 140

Leu Ala Asp Leu Asp Lys Glu Glu Lys Glu Lys Asp Trp Tyr Tyr Ala
145                 150                 155                 160

Gln Leu Gln Asn Leu Thr Lys Arg Ile Asp Ser Leu Pro Leu Thr Glu
                165                 170                 175

Asn Phe Ser Leu Gln Thr Asp Met Thr Arg Arg Gln Leu Glu Tyr Glu
            180                 185                 190

Ala Arg Gln Ile Arg Val Ala Met Glu Glu Gln Leu Gly Thr Cys Gln
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        195                 200                 205

Asp Met Glu Lys Arg Ala Gln Arg Arg Ile Ala Arg Ile Gln Gln Ile
    210                 215                 220

Glu Lys Asp Ile Leu Arg Ile Arg Gln Leu Leu Gln Ser Gln Ala Thr
225                 230                 235                 240

Glu Ala Glu Arg Ser Ser Gln Asn Lys His Glu Thr Gly Ser His Asp
                245                 250                 255

Ala Glu Arg Gln Asn Glu Gly Gln Gly Val Gly Glu Ile Asn Met Ala
            260                 265                 270

Thr Ser Gly Asn Gly Gln Gly Ser Thr Thr Arg Met Asp His Glu Thr
        275                 280                 285

Ala Ser Val Leu Ser Ser Ser Ser Thr His Ser Ala Pro Arg Arg Leu
    290                 295                 300

Thr Ser His Leu Gly Thr Lys Val Glu Met Val Tyr Ser Leu Leu Ser
305                 310                 315                 320

Met Leu Gly Thr His Asp Lys Asp Asp Met Ser Arg Thr Leu Leu Ala
                325                 330                 335

Met Ser Ser Ser Gln Asp Ser Cys Ile Ser Met Arg Gln Ser Gly Cys
            340                 345                 350

Leu Pro Leu Leu Ile Gln Leu Leu His Gly Asn Asp Lys Asp Ser Val
        355                 360                 365

Leu Leu Gly Asn Ser Arg Gly Ser Lys Glu Ala Arg Ala Arg Ala Ser
    370                 375                 380

Ala Ala Leu His Asn Ile Ile His Ser Gln Pro Asp Asp Lys Arg Gly
385                 390                 395                 400

Arg Arg Glu Ile Arg Val Leu His Leu Leu Glu Gln Ile Arg Ala Tyr
                405                 410                 415

Cys Glu Thr Cys Trp Glu Trp Gln Glu Ala His Glu Pro Gly Met Asp
            420                 425                 430

Gln Asp Lys Asn Pro Met Pro Ala Pro Val Glu His Gln Ile Cys Pro
        435                 440                 445

Ala Val Cys Val Leu Met Lys Leu Ser Phe Asp Glu Glu His Arg His
    450                 455                 460

Ala Met Asn Glu Leu Gly Gly Leu Gln Ala Ile Ala Glu Leu Leu Gln
465                 470                 475                 480

Val Asp Cys Glu Met Tyr Gly Leu Thr Asn Asp His Tyr Ser Ile Thr
                485                 490                 495

Leu Arg Arg Tyr Ala Gly Met Ala Leu Thr Asn Leu Thr Phe Gly Asp
            500                 505                 510

Val Ala Asn Lys Ala Thr Leu Cys Ser Met Lys Gly Cys Met Arg Ala
        515                 520                 525

Leu Val Ala Gln Leu Lys Ser Glu Ser Glu Asp Leu Gln Gln Val Ile
    530                 535                 540

Ala Ser Val Leu Arg Asn Leu Ser Trp Arg Ala Asp Val Asn Ser Lys
545                 550                 555                 560

Lys Thr Leu Arg Glu Val Gly Ser Val Lys Ala Leu Met Glu Cys Ala
                565                 570                 575

Leu Glu Val Lys Lys Glu Ser Thr Leu Lys Ser Val Leu Ser Ala Leu
            580                 585                 590

Trp Asn Leu Ser Ala His Cys Thr Glu Asn Lys Ala Asp Ile Cys Ala
        595                 600                 605
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Val Asp Gly Ala Leu Ala Phe Leu Val Gly Thr Leu Thr Tyr Arg Ser
    610                 615                 620

Gln Thr Asn Thr Leu Ala Ile Ile Glu Ser Gly Gly Gly Ile Leu Arg
625                 630                 635                 640

Asn Val Ser Ser Leu Ile Ala Thr Asn Glu Asp His Arg Gln Ile Leu
                645                 650                 655

Arg Glu Asn Asn Cys Leu Gln Thr Leu Leu Gln His Leu Lys Ser His
            660                 665                 670

Ser Leu Thr Ile Val Ser Asn Ala Cys Gly Thr Leu Trp Asn Leu Ser
        675                 680                 685

Ala Arg Asn Pro Lys Asp Gln Glu Ala Leu Trp Asp Met Gly Ala Val
    690                 695                 700

Ser Met Leu Lys Asn Leu Ile His Ser Lys His Lys Met Ile Ala Met
705                 710                 715                 720

Gly Ser Ala Ala Ala Leu Arg Asn Leu Met Ala Asn Arg Pro Ala Lys
                725                 730                 735

Tyr Lys Asp Ala Asn Ile Met Ser Pro Gly Ser Ser Leu Pro Ser Leu
            740                 745                 750

His Val Arg Lys Gln Lys Ala Leu Glu Ala Glu Leu Asp Ala Gln His
        755                 760                 765

Leu Ser Glu Thr Phe Asp Asn Ile Asp Asn Leu Ser Pro Lys Ala Ser
    770                 775                 780

His Arg Ser Lys Gln Arg His Lys Gln Ser Leu Tyr Gly Asp Tyr Val
785                 790                 795                 800

Phe Asp Thr Asn Arg His Asp Asp Asn Arg Ser Asp Asn Phe Asn Thr
                805                 810                 815

Gly Asn Met Thr Val Leu Ser Pro Tyr Leu Asn Thr Thr Val Leu Pro
            820                 825                 830

Ser Ser Ser Ser Ser Arg Gly Ser Leu Asp Ser Ser Arg Ser Glu Lys
        835                 840                 845

Asp Arg Ser Leu Glu Arg Glu Arg Gly Ile Gly Leu Gly Asn Tyr His
    850                 855                 860

Pro Ala Thr Glu Asn Pro Gly Thr Ser Ser Lys Arg Gly Leu Gln Ile
865                 870                 875                 880

Ser Thr Thr Ala Ala Gln Ile Ala Lys Val Met Glu Glu Val Ser Ala
                885                 890                 895

Ile His Thr Ser Gln Glu Asp Arg Ser Ser Gly Ser Thr Thr Glu Leu
            900                 905                 910

His Cys Val Thr Asp Glu Arg Asn Ala Leu Arg Arg Ser Ser Ala Ala
        915                 920                 925

His Thr His Ser Asn Thr Tyr Asn Phe Thr Lys Ser Glu Asn Ser Asn
    930                 935                 940

Arg Thr Cys Ser Met Pro Tyr Ala Lys Leu Glu Tyr Lys Arg Ser Ser
945                 950                 955                 960

Asn Asp Ser Leu Asn Ser Val Ser Ser Ser Asp Gly Tyr Gly Lys Arg
                965                 970                 975

Gly Gln Met Lys Pro Ser Ile Glu Ser Tyr Ser Glu Asp Asp Glu Ser
            980                 985                 990

Lys Phe Cys Ser Tyr Gly Gln Tyr  Pro Ala Asp Leu Ala  His Lys Ile
        995                 1000                1005
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```
His Ser  Ala Asn His Met Asp  Asp Asn Asp Gly Glu  Leu Asp Thr
    1010                1015                1020

Pro Ile  Asn Tyr Ser Leu Lys  Tyr Ser Asp Glu Gln  Leu Asn Ser
    1025                1030                1035

Gly Arg  Gln Ser Pro Ser Gln  Asn Glu Arg Trp Ala  Arg Pro Lys
    1040                1045                1050

His Ile  Ile Glu Asp Glu Ile  Lys Gln Ser Glu Gln  Arg Gln Ser
    1055                1060                1065

Arg Asn  Gln Ser Thr Thr Tyr  Pro Val Tyr Thr Glu  Ser Thr Asp
    1070                1075                1080

Asp Lys  His Leu Lys Phe Gln  Pro His Phe Gly Gln  Gln Glu Cys
    1085                1090                1095

Val Ser  Pro Tyr Arg Ser Arg  Gly Ala Asn Gly Ser  Glu Thr Asn
    1100                1105                1110

Arg Val  Gly Ser Asn His Gly  Ile Asn Gln Asn Val  Ser Gln Ser
    1115                1120                1125

Leu Cys  Gln Glu Asp Asp Tyr  Glu Asp Asp Lys Pro  Thr Asn Tyr
    1130                1135                1140

Ser Glu  Arg Tyr Ser Glu Glu  Glu Gln His Glu Glu  Glu Glu Arg
    1145                1150                1155

Pro Thr  Asn Tyr Ser Ile Lys  Tyr Asn Glu Glu Lys  Arg His Val
    1160                1165                1170

Asp Gln  Pro Ile Asp Tyr Ser  Leu Lys Tyr Ala Thr  Asp Ile Pro
    1175                1180                1185

Ser Ser  Gln Lys Gln Ser Phe  Ser Phe Ser Lys Ser  Ser Ser Gly
    1190                1195                1200

Gln Ser  Ser Lys Thr Glu His  Met Ser Ser Ser Ser  Glu Asn Thr
    1205                1210                1215

Ser Thr  Pro Ser Ser Asn Ala  Lys Arg Gln Asn Gln  Leu His Pro
    1220                1225                1230

Ser Ser  Ala Gln Ser Arg Ser  Gly Gln Pro Gln Lys  Ala Ala Thr
    1235                1240                1245

Cys Lys  Val Ser Ser Ile Asn  Gln Glu Thr Ile Gln  Thr Tyr Cys
    1250                1255                1260

Val Glu  Asp Thr Pro Ile Cys  Phe Ser Arg Cys Ser  Ser Leu Ser
    1265                1270                1275

Ser Leu  Ser Ser Ala Glu Asp  Glu Ile Gly Cys Asn  Gln Thr Thr
    1280                1285                1290

Gln Glu  Ala Asp Ser Ala Asn  Thr Leu Gln Ile Ala  Glu Ile Lys
    1295                1300                1305

Glu Lys  Ile Gly Thr Arg Ser  Ala Glu Asp Pro Val  Ser Glu Val
    1310                1315                1320

Pro Ala  Val Ser Gln His Pro  Arg Thr Lys Ser Ser  Arg Leu Gln
    1325                1330                1335

Gly Ser  Ser Leu Ser Ser Glu  Ser Ala Arg His Lys  Ala Val Glu
    1340                1345                1350

Phe Ser  Ser Gly Ala Lys Ser  Pro Ser Lys Ser Gly  Ala Gln Thr
    1355                1360                1365

Pro Lys  Ser Pro Pro Glu His  Tyr Val Gln Glu Thr  Pro Leu Met
    1370                1375                1380

Phe Ser  Arg Cys Thr Ser Val  Ser Ser Leu Asp Ser  Phe Glu Ser
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    1385                1390                1395

Arg Ser  Ile Ala Ser Ser Val  Gln Ser Glu Pro Cys  Ser Gly Met
    1400                1405                1410

Val Ser  Gly Ile Ile Ser Pro  Ser Asp Leu Pro Asp  Ser Pro Gly
    1415                1420                1425

Gln Thr  Met Pro Pro Ser Arg  Ser Lys Thr Pro Pro  Pro Pro Pro
    1430                1435                1440

Gln Thr  Ala Gln Thr Lys Arg  Glu Val Pro Lys Asn  Lys Ala Pro
    1445                1450                1455

Thr Ala  Glu Lys Arg Glu Ser  Gly Pro Lys Gln Ala  Ala Val Asn
    1460                1465                1470

Ala Ala  Val Gln Arg Val Gln  Val Leu Pro Asp Ala  Asp Thr Leu
    1475                1480                1485

Leu His  Phe Ala Thr Glu Ser  Thr Pro Asp Gly Phe  Ser Cys Ser
    1490                1495                1500

Ser Ser  Leu Ser Ala Leu Ser  Leu Asp Glu Pro Phe  Ile Gln Lys
    1505                1510                1515

Asp Val  Glu Leu Arg Ile Met  Pro Pro Val Gln Glu  Asn Asp Asn
    1520                1525                1530

Gly Asn  Glu Thr Glu Ser Glu  Gln Pro Lys Glu Ser  Asn Glu Asn
    1535                1540                1545

Gln Glu  Lys Glu Ala Glu Lys  Thr Ile Asp Ser Glu  Lys Asp Leu
    1550                1555                1560

Leu Asp  Asp Ser Asp Asp Asp  Asp Ile Glu Ile Leu  Glu Glu Cys
    1565                1570                1575

Ile Ile  Ser Ala Met Pro Thr  Lys Ser Ser Arg Lys  Ala Lys Lys
    1580                1585                1590

Pro Ala  Gln Thr Ala Ser Lys  Leu Pro Pro Pro Val  Ala Arg Lys
    1595                1600                1605

Pro Ser  Gln Leu Pro Val Tyr  Lys Leu Leu Pro Ser  Gln Asn Arg
    1610                1615                1620

Leu Gln  Pro Gln Lys His Val  Ser Phe Thr Pro Gly  Asp Asp Met
    1625                1630                1635

Pro Arg  Val Tyr Cys Val Glu  Gly Thr Pro Ile Asn  Phe Ser Thr
    1640                1645                1650

Ala Thr  Ser Leu Ser Asp Leu  Thr Ile Glu Ser Pro  Pro Asn Glu
    1655                1660                1665

Leu Ala  Ala Gly Glu Gly Val  Arg Gly Gly Ala Gln  Ser Gly Glu
    1670                1675                1680

Phe Glu  Lys Arg Asp Thr Ile  Pro Thr Glu Gly Arg  Ser Thr Asp
    1685                1690                1695

Glu Ala  Gln Gly Gly Lys Thr  Ser Ser Val Thr Ile  Pro Glu Leu
    1700                1705                1710

Asp Asp  Asn Lys Ala Glu Glu  Gly Asp Ile Leu Ala  Glu Cys Ile
    1715                1720                1725

Asn Ser  Ala Met Pro Lys Gly  Lys Ser His Lys Pro  Phe Arg Val
    1730                1735                1740

Lys Lys  Ile Met Asp Gln Val  Gln Gln Ala Ser Ala  Ser Ser Ser
    1745                1750                1755

Ala Pro  Asn Lys Asn Gln Leu  Asp Gly Lys Lys Lys  Lys Pro Thr
    1760                1765                1770
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Ser Pro  Val Lys Pro Ile Pro  Gln Asn Thr Glu Tyr  Arg Thr Arg
    1775                 1780                 1785

Val Arg  Lys Asn Ala Asp Ser  Lys Asn Asn Leu Asn  Ala Glu Arg
    1790                 1795                 1800

Val Phe  Ser Asp Asn Lys Asp  Ser Lys Lys Gln Asn  Leu Lys Asn
    1805                 1810                 1815

Asn Ser  Lys Val Phe Asn Asp  Lys Leu Pro Asn Asn  Glu Asp Arg
    1820                 1825                 1830

Val Arg  Gly Ser Phe Ala Phe  Asp Ser Pro His His  Tyr Thr Pro
    1835                 1840                 1845

Ile Glu  Gly Thr Pro Tyr Cys  Phe Ser Arg Asn Asp  Ser Leu Ser
    1850                 1855                 1860

Ser Leu  Asp Phe Asp Asp Asp  Asp Val Asp Leu Ser  Arg Glu Lys
    1865                 1870                 1875

Ala Glu  Leu Arg Lys Ala Lys  Glu Asn Lys Glu Ser  Glu Ala Lys
    1880                 1885                 1890

Val Thr  Ser His Thr Glu Leu  Thr Ser Asn Gln Gln  Ser Ala Asn
    1895                 1900                 1905

Lys Thr  Gln Ala Ile Ala Lys  Gln Pro Ile Asn Arg  Gly Gln Pro
    1910                 1915                 1920

Lys Pro  Ile Leu Gln Lys Gln  Ser Thr Phe Pro Gln  Ser Ser Lys
    1925                 1930                 1935

Asp Ile  Pro Asp Arg Gly Ala  Ala Thr Asp Glu Lys  Leu Gln Asn
    1940                 1945                 1950

Phe Ala  Ile Glu Asn Thr Pro  Val Cys Phe Ser His  Asn Ser Ser
    1955                 1960                 1965

Leu Ser  Ser Leu Ser Asp Ile  Asp Gln Glu Asn Asn  Asn Lys Glu
    1970                 1975                 1980

Asn Glu  Pro Ile Lys Glu Thr  Glu Pro Pro Asp Ser  Gln Gly Glu
    1985                 1990                 1995

Pro Ser  Lys Pro Gln Ala Ser  Gly Tyr Ala Pro Lys  Ser Phe His
    2000                 2005                 2010

Val Glu  Asp Thr Pro Val Cys  Phe Ser Arg Asn Ser  Ser Leu Ser
    2015                 2020                 2025

Ser Leu  Ser Ile Asp Ser Glu  Asp Asp Leu Leu Gln  Glu Cys Ile
    2030                 2035                 2040

Ser Ser  Ala Met Pro Lys Lys  Lys Lys Pro Ser Arg  Leu Lys Gly
    2045                 2050                 2055

Asp Asn  Glu Lys His Ser Pro  Arg Asn Met Gly Gly  Ile Leu Gly
    2060                 2065                 2070

Glu Asp  Leu Thr Leu Asp Leu  Lys Asp Ile Gln Arg  Pro Asp Ser
    2075                 2080                 2085

Glu His  Gly Leu Ser Pro Asp  Ser Glu Asn Phe Asp  Trp Lys Ala
    2090                 2095                 2100

Ile Gln  Glu Gly Ala Asn Ser  Ile Val Ser Ser Leu  His Gln Ala
    2105                 2110                 2115

Ala Ala  Ala Ala Cys Leu Ser  Arg Gln Ala Ser Ser  Asp Ser Asp
    2120                 2125                 2130

Ser Ile  Leu Ser Leu Lys Ser  Gly Ile Ser Leu Gly  Ser Pro Phe
    2135                 2140                 2145
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-continued

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His Leu  Thr Pro Asp Gln Glu  Glu Lys Pro Phe Thr  Ser Asn Lys
    2150                 2155                 2160

Gly Pro  Arg Ile Leu Lys Pro  Gly Glu Lys Ser Thr  Leu Glu Thr
    2165                 2170                 2175

Lys Lys  Ile Glu Ser Glu Ser  Lys Gly Ile Lys Gly  Gly Lys Lys
    2180                 2185                 2190

Val Tyr  Lys Ser Leu Ile Thr  Gly Lys Val Arg Ser  Asn Ser Glu
    2195                 2200                 2205

Ile Ser  Gly Gln Met Lys Gln  Pro Leu Gln Ala Asn  Met Pro Ser
    2210                 2215                 2220

Ile Ser  Arg Gly Arg Thr Met  Ile His Ile Pro Gly  Val Arg Asn
    2225                 2230                 2235

Ser Ser  Ser Ser Thr Ser Pro  Val Ser Lys Lys Gly  Pro Pro Leu
    2240                 2245                 2250

Lys Thr  Pro Ala Ser Lys Ser  Pro Ser Glu Gly Gln  Thr Ala Thr
    2255                 2260                 2265

Thr Ser  Pro Arg Gly Ala Lys  Pro Ser Val Lys Ser  Glu Leu Ser
    2270                 2275                 2280

Pro Val  Ala Arg Gln Thr Ser  Gln Ile Gly Gly Ser  Ser Lys Ala
    2285                 2290                 2295

Pro Ser  Arg Ser Gly Ser Arg  Asp Ser Thr Pro Ser  Arg Pro Ala
    2300                 2305                 2310

Gln Gln  Pro Leu Ser Arg Pro  Ile Gln Ser Pro Gly  Arg Asn Ser
    2315                 2320                 2325

Ile Ser  Pro Gly Arg Asn Gly  Ile Ser Pro Pro Asn  Lys Leu Ser
    2330                 2335                 2340

Gln Leu  Pro Arg Thr Ser Ser  Pro Ser Thr Ala Ser  Thr Lys Ser
    2345                 2350                 2355

Ser Gly  Ser Gly Lys Met Ser  Tyr Thr Ser Pro Gly  Arg Gln Met
    2360                 2365                 2370

Ser Gln  Gln Asn Leu Thr Lys  Gln Thr Gly Leu Ser  Lys Asn Ala
    2375                 2380                 2385

Ser Ser  Ile Pro Arg Ser Glu  Ser Ala Ser Lys Gly  Leu Asn Gln
    2390                 2395                 2400

Met Asn  Asn Gly Asn Gly Ala  Asn Lys Lys Val Glu  Leu Ser Arg
    2405                 2410                 2415

Met Ser  Ser Thr Lys Ser Ser  Gly Ser Glu Ser Asp  Arg Ser Glu
    2420                 2425                 2430

Arg Pro  Val Leu Val Arg Gln  Ser Thr Phe Ile Lys  Glu Ala Pro
    2435                 2440                 2445

Ser Pro  Thr Leu Arg Arg Lys  Leu Glu Glu Ser Ala  Ser Phe Glu
    2450                 2455                 2460

Ser Leu  Ser Pro Ser Ser Arg  Pro Ala Ser Pro Thr  Arg Ser Gln
    2465                 2470                 2475

Ala Gln  Thr Pro Val Leu Ser  Pro Ser Leu Pro Asp  Met Ser Leu
    2480                 2485                 2490

Ser Thr  His Ser Ser Val Gln  Ala Gly Gly Trp Arg  Lys Leu Pro
    2495                 2500                 2505

Pro Asn  Leu Ser Pro Thr Ile  Glu Tyr Asn Asp Gly  Arg Pro Ala
    2510                 2515                 2520

Lys Arg  His Asp Ile Ala Arg  Ser His Ser Glu Ser  Pro Ser Arg
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    2525                2530                2535

Leu Pro  Ile Asn Arg Ser Gly  Thr Trp Lys Arg Glu  His Ser Lys
    2540                2545                2550

His Ser  Ser Ser Leu Pro Arg  Val Ser Thr Trp Arg  Arg Thr Gly
    2555                2560                2565

Ser Ser  Ser Ser Ile Leu Ser  Ala Ser Ser Glu Ser  Ser Glu Lys
    2570                2575                2580

Ala Lys  Ser Glu Asp Glu Lys  His Val Asn Ser Ile  Ser Gly Thr
    2585                2590                2595

Lys Gln  Ser Lys Glu Asn Gln  Val Ser Ala Lys Gly  Thr Trp Arg
    2600                2605                2610

Lys Ile  Lys Glu Asn Glu Phe  Ser Pro Thr Asn Ser  Thr Ser Gln
    2615                2620                2625

Thr Val  Ser Ser Gly Ala Thr  Asn Gly Ala Glu Ser  Lys Thr Leu
    2630                2635                2640

Ile Tyr  Gln Met Ala Pro Ala  Val Ser Lys Thr Glu  Asp Val Trp
    2645                2650                2655

Val Arg  Ile Glu Asp Cys Pro  Ile Asn Asn Pro Arg  Ser Gly Arg
    2660                2665                2670

Ser Pro  Thr Gly Asn Thr Pro  Pro Val Ile Asp Ser  Val Ser Glu
    2675                2680                2685

Lys Ala  Asn Pro Asn Ile Lys  Asp Ser Lys Asp Asn  Gln Ala Lys
    2690                2695                2700

Gln Asn  Val Gly Asn Gly Ser  Val Pro Met Arg Thr  Val Gly Leu
    2705                2710                2715

Glu Asn  Arg Leu Asn Ser Phe  Ile Gln Val Asp Ala  Pro Asp Gln
    2720                2725                2730

Lys Gly  Thr Glu Ile Lys Pro  Gly Gln Asn Asn Pro  Val Pro Val
    2735                2740                2745

Ser Glu  Thr Asn Glu Ser Ser  Ile Val Glu Arg Thr  Pro Phe Ser
    2750                2755                2760

Ser Ser  Ser Ser Ser Lys His  Ser Ser Pro Ser Gly  Thr Val Ala
    2765                2770                2775

Ala Arg  Val Thr Pro Phe Asn  Tyr Asn Pro Ser Pro  Arg Lys Ser
    2780                2785                2790

Ser Ala  Asp Ser Thr Ser Ala  Arg Pro Ser Gln Ile  Pro Thr Pro
    2795                2800                2805

Val Asn  Asn Asn Thr Lys Lys  Arg Asp Ser Lys Thr  Asp Ser Thr
    2810                2815                2820

Glu Ser  Ser Gly Thr Gln Ser  Pro Lys Arg His Ser  Gly Ser Tyr
    2825                2830                2835

Leu Val  Thr Ser Val
    2840


<210> SEQ ID NO 15
<211> LENGTH: 557
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 15

Met Asp Asp Leu Asp Ala Leu Leu Ala Asp Leu Glu Ser Thr Thr Ser
1               5                  10                  15
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His Ile Ser Lys Arg Pro Val Phe Leu Ser Glu Glu Thr Pro Tyr Ser
            20                  25                  30

Tyr Pro Thr Gly Asn His Thr Tyr Gln Glu Ile Ala Val Pro Pro Pro
        35                  40                  45

Val Pro Pro Pro Pro Ser Ser Glu Ala Leu Asn Gly Thr Ile Leu Asp
    50                  55                  60

Pro Leu Asp Gln Trp Gln Pro Ser Gly Ser Arg Phe Ile His Gln Gln
65                  70                  75                  80

Pro Gln Ser Ser Ser Pro Val Tyr Gly Ser Ser Ala Lys Thr Ser Ser
                85                  90                  95

Val Ser Asn Pro Gln Asp Ser Val Gly Ser Pro Cys Ser Arg Val Gly
            100                 105                 110

Glu Glu Glu His Val Tyr Ser Phe Pro Asn Lys Gln Lys Ser Ala Glu
        115                 120                 125

Pro Ser Pro Thr Val Met Ser Thr Ser Leu Gly Ser Asn Leu Ser Glu
    130                 135                 140

Leu Asp Arg Leu Leu Leu Glu Leu Asn Ala Val Gln His Asn Pro Pro
145                 150                 155                 160

Gly Phe Pro Ala Asp Glu Ala Asn Ser Ser Pro Pro Leu Pro Gly Ala
                165                 170                 175

Leu Ser Pro Leu Tyr Gly Val Pro Glu Thr Asn Ser Pro Leu Gly Gly
            180                 185                 190

Lys Ala Gly Pro Leu Thr Lys Glu Lys Pro Lys Arg Asn Gly Gly Arg
        195                 200                 205

Gly Leu Glu Asp Val Arg Pro Ser Val Glu Ser Leu Leu Asp Glu Leu
    210                 215                 220

Glu Ser Ser Val Pro Ser Pro Val Pro Ala Ile Thr Val Asn Gln Gly
225                 230                 235                 240

Glu Met Ser Ser Pro Gln Arg Val Thr Ser Thr Gln Gln Gln Thr Arg
                245                 250                 255

Ile Ser Ala Ser Ser Ala Thr Arg Glu Leu Asp Glu Leu Met Ala Ser
            260                 265                 270

Leu Ser Asp Phe Lys Phe Met Ala Gln Gly Lys Thr Gly Ser Ser Ser
        275                 280                 285

Pro Pro Gly Gly Pro Pro Lys Pro Gly Ser Gln Leu Asp Ser Met Leu
    290                 295                 300

Gly Ser Leu Gln Ser Asp Leu Asn Lys Leu Gly Val Ala Thr Val Ala
305                 310                 315                 320

Lys Gly Val Cys Gly Ala Cys Lys Lys Pro Ile Ala Gly Gln Val Val
                325                 330                 335

Thr Ala Met Gly Lys Thr Trp His Pro Glu His Phe Val Cys Thr His
            340                 345                 350

Cys Gln Glu Glu Ile Gly Ser Arg Asn Phe Phe Glu Arg Asp Gly Gln
        355                 360                 365

Pro Tyr Cys Glu Lys Asp Tyr His Asn Leu Phe Ser Pro Arg Cys Tyr
    370                 375                 380

Tyr Cys Asn Gly Pro Ile Leu Asp Lys Val Val Thr Ala Leu Asp Arg
385                 390                 395                 400

Thr Trp His Pro Glu His Phe Phe Cys Ala Gln Cys Gly Ala Phe Phe
                405                 410                 415

Gly Pro Glu Gly Phe His Glu Lys Asp Gly Lys Ala Tyr Cys Arg Lys
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           420                 425                 430

Asp Tyr Phe Asp Met Phe Ala Pro Lys Cys Gly Gly Cys Ala Arg Ala
        435                 440                 445

Ile Leu Glu Asn Tyr Ile Ser Ala Leu Asn Thr Leu Trp His Pro Glu
    450                 455                 460

Cys Phe Val Cys Arg Glu Cys Phe Thr Pro Phe Val Asn Gly Ser Phe
465                 470                 475                 480

Phe Glu His Asp Gly Gln Pro Tyr Cys Glu Val His Tyr His Glu Arg
                485                 490                 495

Arg Gly Ser Leu Cys Ser Gly Cys Gln Lys Pro Ile Thr Gly Arg Cys
            500                 505                 510

Ile Thr Ala Met Ala Lys Lys Phe His Pro Glu His Phe Val Cys Ala
        515                 520                 525

Phe Cys Leu Lys Gln Leu Asn Lys Gly Thr Phe Lys Glu Gln Asn Asp
    530                 535                 540

Lys Pro Tyr Cys Gln Asn Cys Phe Leu Lys Leu Phe Cys
545                 550                 555


<210> SEQ ID NO 16
<211> LENGTH: 533
<212> TYPE: PRT
<213> ORGANISM: Gallus gallus domesticus

<400> SEQUENCE: 16

Met Gly Ser Ser Lys Ser Lys Pro Lys Asp Pro Ser Gln Arg Arg Arg
1               5                   10                  15

Ser Leu Glu Pro Pro Asp Ser Thr His His Gly Gly Phe Pro Ala Ser
            20                  25                  30

Gln Thr Pro Asn Lys Thr Ala Ala Pro Asp Thr His Arg Thr Pro Ser
        35                  40                  45

Arg Ser Phe Gly Thr Val Ala Thr Glu Pro Lys Leu Phe Gly Gly Phe
    50                  55                  60

Asn Thr Ser Asp Thr Val Thr Ser Pro Gln Arg Ala Gly Ala Leu Ala
65                  70                  75                  80

Gly Gly Val Thr Thr Phe Val Ala Leu Tyr Asp Tyr Glu Ser Arg Thr
                85                  90                  95

Glu Thr Asp Leu Ser Phe Lys Lys Gly Glu Arg Leu Gln Ile Val Asn
            100                 105                 110

Asn Thr Glu Gly Asp Trp Trp Leu Ala His Ser Leu Thr Thr Gly Gln
        115                 120                 125

Thr Gly Tyr Ile Pro Ser Asn Tyr Val Ala Pro Ser Asp Ser Ile Gln
    130                 135                 140

Ala Glu Glu Trp Tyr Phe Gly Lys Ile Thr Arg Arg Glu Ser Glu Arg
145                 150                 155                 160

Leu Leu Leu Asn Pro Glu Asn Pro Arg Gly Thr Phe Leu Val Arg Glu
                165                 170                 175

Ser Glu Thr Thr Lys Gly Ala Tyr Cys Leu Ser Val Ser Asp Phe Asp
            180                 185                 190

Asn Ala Lys Gly Leu Asn Val Lys His Tyr Lys Ile Arg Lys Leu Asp
        195                 200                 205

Ser Gly Gly Phe Tyr Ile Thr Ser Arg Thr Gln Phe Ser Ser Leu Gln
    210                 215                 220
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```
Gln Leu Val Ala Tyr Tyr Ser Lys His Ala Asp Gly Leu Cys His Arg
225                 230                 235                 240

Leu Thr Asn Val Cys Pro Thr Ser Lys Pro Gln Thr Gln Gly Leu Ala
                245                 250                 255

Lys Asp Ala Trp Glu Ile Pro Arg Glu Ser Leu Arg Leu Glu Val Lys
            260                 265                 270

Leu Gly Gln Gly Cys Phe Gly Glu Val Trp Met Gly Thr Trp Asn Gly
        275                 280                 285

Thr Thr Arg Val Ala Ile Lys Thr Leu Lys Pro Gly Thr Met Ser Pro
    290                 295                 300

Glu Ala Phe Leu Gln Glu Ala Gln Val Met Lys Lys Leu Arg His Glu
305                 310                 315                 320

Lys Leu Val Gln Leu Tyr Ala Val Val Ser Glu Glu Pro Ile Tyr Ile
                325                 330                 335

Val Thr Glu Tyr Met Ser Lys Gly Ser Leu Leu Asp Phe Leu Lys Gly
            340                 345                 350

Glu Met Gly Lys Tyr Leu Arg Leu Pro Gln Leu Val Asp Met Ala Ala
        355                 360                 365

Gln Ile Ala Ser Gly Met Ala Tyr Val Glu Arg Met Asn Tyr Val His
    370                 375                 380

Arg Asp Leu Arg Ala Ala Asn Ile Leu Val Gly Glu Asn Leu Val Cys
385                 390                 395                 400

Lys Val Ala Asp Phe Gly Leu Ala Arg Leu Ile Glu Asp Asn Glu Tyr
                405                 410                 415

Thr Ala Arg Gln Gly Ala Lys Phe Pro Ile Lys Trp Thr Ala Pro Glu
            420                 425                 430

Ala Ala Leu Tyr Gly Arg Phe Thr Ile Lys Ser Asp Val Trp Ser Phe
        435                 440                 445

Gly Ile Leu Leu Thr Glu Leu Thr Thr Lys Gly Arg Val Pro Tyr Pro
    450                 455                 460

Gly Met Val Asn Arg Glu Val Leu Asp Gln Val Glu Arg Gly Tyr Arg
465                 470                 475                 480

Met Pro Cys Pro Pro Glu Cys Pro Glu Ser Leu His Asp Leu Met Cys
                485                 490                 495

Gln Cys Trp Arg Lys Asp Pro Glu Glu Arg Pro Thr Phe Glu Tyr Leu
            500                 505                 510

Gln Ala Phe Leu Glu Asp Tyr Phe Thr Ser Thr Glu Pro Gln Tyr Gln
        515                 520                 525

Pro Gly Glu Asn Leu
    530


<210> SEQ ID NO 17
<211> LENGTH: 1066
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 17

Met Pro Val Phe His Thr Arg Thr Ile Glu Ser Ile Leu Glu Pro Val
1               5                   10                  15

Ala Gln Gln Ile Ser His Leu Val Ile Met His Glu Glu Gly Glu Val
            20                  25                  30

Asp Gly Lys Ala Ile Pro Asp Leu Thr Ala Pro Val Ala Ala Val Gln
        35                  40                  45
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Ala Ala Val Ser Asn Leu Val Arg Val Gly Lys Glu Thr Val Gln Thr
    50                  55                  60

Thr Glu Asp Gln Ile Leu Lys Arg Asp Met Pro Pro Ala Phe Ile Lys
65                  70                  75                  80

Val Glu Asn Ala Cys Thr Lys Leu Val Gln Ala Ala Gln Met Leu Gln
                85                  90                  95

Ser Asp Pro Tyr Ser Val Pro Ala Arg Asp Tyr Leu Ile Asp Gly Ser
            100                 105                 110

Arg Gly Ile Leu Ser Gly Thr Ser Asp Leu Leu Leu Thr Phe Asp Glu
        115                 120                 125

Ala Glu Val Arg Lys Ile Ile Arg Val Cys Lys Gly Ile Leu Glu Tyr
    130                 135                 140

Leu Thr Val Ala Glu Val Val Glu Thr Met Glu Asp Leu Val Thr Tyr
145                 150                 155                 160

Thr Lys Asn Leu Gly Pro Gly Met Thr Lys Met Ala Lys Met Ile Asp
                165                 170                 175

Glu Arg Gln Gln Glu Leu Thr His Gln Glu His Arg Val Met Leu Val
            180                 185                 190

Asn Ser Met Asn Thr Val Lys Glu Leu Leu Pro Val Leu Ile Ser Ala
        195                 200                 205

Met Lys Ile Phe Val Thr Thr Lys Asn Ser Lys Asn Gln Gly Ile Glu
    210                 215                 220

Glu Ala Leu Lys Asn Arg Asn Phe Thr Leu Glu Lys Met Ser Ala Glu
225                 230                 235                 240

Ile Asn Glu Ile Ile Arg Val Leu Gln Leu Thr Ser Trp Asp Glu Asp
                245                 250                 255

Ala Trp Ala Ser Lys Asp Thr Glu Ala Met Lys Arg Ala Leu Ala Ser
            260                 265                 270

Ile Asp Ser Lys Leu Asn Gln Ala Lys Gly Trp Leu Arg Asp Pro Ser
        275                 280                 285

Ala Ser Pro Gly Asp Ala Gly Glu Gln Ala Ile Arg Gln Ile Leu Asp
    290                 295                 300

Glu Ala Gly Lys Val Gly Glu Leu Cys Ala Gly Lys Glu Arg Arg Glu
305                 310                 315                 320

Ile Leu Gly Thr Cys Lys Met Leu Gly Gln Met Thr Asp Gln Val Ala
                325                 330                 335

Asp Leu Arg Ala Arg Gly Gln Gly Ser Ser Pro Val Ala Met Gln Lys
            340                 345                 350

Ala Gln Gln Val Ser Gln Gly Leu Asp Val Leu Thr Ala Lys Val Glu
        355                 360                 365

Asn Ala Ala Arg Lys Leu Glu Ala Met Thr Asn Ser Lys Gln Ser Ile
    370                 375                 380

Ala Lys Lys Ile Asp Ala Ala Gln Asn Trp Leu Ala Asp Pro Asn Gly
385                 390                 395                 400

Gly Pro Glu Gly Glu Glu Gln Ile Arg Gly Ala Leu Ala Glu Ala Arg
                405                 410                 415

Lys Ile Ala Glu Leu Cys Asp Asp Pro Lys Glu Arg Asp Asp Ile Leu
            420                 425                 430

Arg Ser Leu Gly Glu Ile Ser Ala Leu Thr Ser Lys Leu Ala Asp Leu
        435                 440                 445
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```
Arg Arg Gln Gly Lys Gly Asp Ser Pro Glu Ala Arg Ala Leu Ala Lys
    450                 455                 460

Gln Val Ala Thr Ala Leu Gln Asn Leu Gln Thr Lys Thr Asn Arg Ala
465                 470                 475                 480

Val Ala Asn Ser Arg Pro Ala Lys Ala Ala Val His Leu Glu Gly Lys
                485                 490                 495

Ile Glu Gln Ala Gln Arg Trp Ile Asp Asn Pro Thr Val Asp Asp Arg
            500                 505                 510

Gly Val Gly Gln Ala Ala Ile Arg Gly Leu Val Ala Glu Gly His Arg
        515                 520                 525

Leu Ala Asn Val Met Met Gly Pro Tyr Arg Gln Asp Leu Leu Ala Lys
    530                 535                 540

Cys Asp Arg Val Asp Gln Leu Thr Ala Gln Leu Ala Asp Leu Ala Ala
545                 550                 555                 560

Arg Gly Glu Gly Glu Ser Pro Gln Ala Arg Ala Leu Ala Ser Gln Leu
                565                 570                 575

Gln Asp Ser Leu Lys Asp Leu Lys Ala Arg Met Gln Glu Ala Met Thr
            580                 585                 590

Gln Glu Val Ser Asp Val Phe Ser Asp Thr Thr Thr Pro Ile Lys Leu
        595                 600                 605

Leu Ala Val Ala Ala Thr Ala Pro Pro Asp Ala Pro Asn Arg Glu Glu
    610                 615                 620

Val Phe Asp Glu Arg Ala Ala Asn Phe Glu Asn His Ser Gly Lys Leu
625                 630                 635                 640

Gly Ala Thr Ala Glu Lys Ala Ala Ala Val Gly Thr Ala Asn Lys Ser
                645                 650                 655

Thr Val Glu Gly Ile Gln Ala Ser Val Lys Thr Ala Arg Glu Leu Thr
            660                 665                 670

Pro Gln Val Val Ser Ala Ala Arg Ile Leu Leu Arg Asn Pro Gly Asn
        675                 680                 685

Gln Ala Ala Tyr Glu His Phe Glu Thr Met Lys Asn Gln Trp Ile Asp
    690                 695                 700

Asn Val Glu Lys Met Thr Gly Leu Val Asp Glu Ala Ile Asp Thr Lys
705                 710                 715                 720

Ser Leu Leu Asp Ala Ser Glu Glu Ala Ile Lys Lys Asp Leu Asp Lys
                725                 730                 735

Cys Lys Val Ala Met Ala Asn Ile Gln Pro Gln Met Leu Val Ala Gly
            740                 745                 750

Ala Thr Ser Ile Ala Arg Arg Ala Asn Arg Ile Leu Leu Val Ala Lys
        755                 760                 765

Arg Glu Val Glu Asn Ser Glu Asp Pro Lys Phe Arg Glu Ala Val Lys
    770                 775                 780

Ala Ala Ser Asp Glu Leu Ser Lys Thr Ile Ser Pro Met Val Met Asp
785                 790                 795                 800

Ala Lys Ala Val Ala Gly Asn Ile Ser Asp Pro Gly Leu Gln Lys Ser
                805                 810                 815

Phe Leu Asp Ser Gly Tyr Arg Ile Leu Gly Ala Val Ala Lys Val Arg
            820                 825                 830

Glu Ala Phe Gln Pro Gln Glu Pro Asp Phe Pro Pro Pro Pro Pro Asp
        835                 840                 845

Leu Glu Gln Leu Arg Leu Thr Asp Glu Leu Ala Pro Pro Lys Pro Pro
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    850                 855                 860

Leu Pro Glu Gly Glu Val Pro Pro Pro Arg Pro Pro Pro Pro Glu Glu
865                 870                 875                 880

Lys Asp Glu Glu Phe Pro Glu Gln Lys Ala Gly Glu Val Ile Asn Gln
                885                 890                 895

Pro Met Met Met Ala Ala Arg Gln Leu His Asp Glu Ala Arg Lys Trp
            900                 905                 910

Ser Ser Lys Gly Asn Asp Ile Ile Ala Ala Ala Lys Arg Met Ala Leu
        915                 920                 925

Leu Met Ala Glu Met Ser Arg Leu Val Arg Gly Gly Ser Gly Thr Lys
    930                 935                 940

Arg Ala Leu Ile Gln Cys Ala Lys Asp Ile Ala Lys Ala Ser Asp Glu
945                 950                 955                 960

Val Thr Arg Leu Ala Lys Glu Val Ala Lys Gln Cys Thr Asp Lys Arg
                965                 970                 975

Ile Arg Thr Asn Leu Leu Gln Val Cys Glu Arg Ile Pro Thr Ile Ser
            980                 985                 990

Thr Gln Leu Lys Ile Leu Ser Thr  Val Lys Ala Thr Met  Leu Gly Arg
        995                 1000                1005

Thr Asn  Ile Ser Asp Glu Glu  Ser Glu Gln Ala Thr  Glu Met Leu
    1010                1015                1020

Val His  Asn Ala Gln Asn Leu  Met Gln Ser Val Lys  Glu Thr Val
    1025                1030                1035

Arg Glu  Ala Glu Ala Ala Ser  Ile Lys Ile Arg Thr  Asp Ala Gly
    1040                1045                1050

Phe Thr  Leu Arg Trp Val Arg  Lys Thr Pro Trp Tyr  Gln
    1055                1060                1065


<210> SEQ ID NO 18
<211> LENGTH: 28
<212> TYPE: PRT
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: PIPKI gamma fragment

<400> SEQUENCE: 18

Pro Thr Asp Glu Arg Ser Trp Val Tyr Ser Pro Leu His Tyr Ser Ala
1               5                   10                  15

Gln Ala Pro Pro Ala Ser Asp Gly Glu Ser Asp Thr
            20                  25


<210> SEQ ID NO 19
<211> LENGTH: 17
<212> TYPE: PRT
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Lifeact peptide

<400> SEQUENCE: 19

Met Gly Val Ala Asp Leu Ile Lys Lys Phe Glu Ser Ile Ser Lys Glu
1               5                   10                  15

Glu


<210> SEQ ID NO 20
<211> LENGTH: 1164
<212> TYPE: DNA
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-continued

<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: FLAG-Actin

<400> SEQUENCE: 20

atggactaca aagacgatga cgacaagaga tctcgagcta tggatgatga tatcgccgcg 60

ctcgtcgtcg acaacggctc cggcatgtgc aaggccggct tcgcgggcga cgatgccccc 120

cgggccgtct tcccctccat cgtggggcgc cccaggcacc agggcgtgat ggtgggcatg 180

ggtcagaagg attcctatgt gggcgacgag gcccagagca agagaggcat cctcaccctg 240

aagtacccca tcgagcacgg catcgtcacc aactgggacg acatggagaa aatctggcac 300

cacaccttct acaatgagct gcgtgtggct cccgaggagc accccgtgct gctgaccgag 360

gccccctga accccaaggc caaccgcgag aagatgaccc agatcatgtt tgagaccttc 420

aacaccccag ccatgtacgt tgctatccag gctgtgctat ccctgtacgc ctctggccgt 480

accactggca tcgtgatgga ctccggtgac ggggtcaccc acactgtgcc catctacgag 540

gggtatgccc tcccccatgc catcctgcgt ctggacctgg ctggccggga cctgactgac 600

tacctcatga agatcctcac cgagcgcggc tacagcttca ccaccacggc cgagcgggaa 660

atcgtgcgtg acattaagga gaagctgtgc tacgtcgccc tggacttcga gcaagagatg 720

gccacggctg cttccagctc ctccctggag aagagctacg agctgcctga cggccaggtc 780

atcaccattg gcaatgagcg gttccgctgc cctgaggcac tcttccagcc ttccttcctg 840

ggcatggagt cctgtggcat ccacgaaact accttcaact ccatcatgaa gtgtgacgtg 900

gacatccgca aagacctgta cgccaacaca gtgctgtctg gcggcaccac catgtaccct 960

ggcattgccg acaggatgca gaaggagatc actgccctgg cacccagcac aatgaagatc 1020

aagatcattg ctcctcctga gcgcaagtac tccgtgtgga tcggcggctc catcctggcc 1080

tcgctgtcca ccttccagca gatgtggatc agcaagcagg agtatgacga gtccggcccc 1140

tccatcgtcc accgcaaatg cttc 1164

What is claimed is:

**1**. An observation method of a sample containing a target substance, the observation method comprising:

an imaging step in which a step of obtaining a speckle image including, as a speckle, light emitted from a luminescent substance under a prescribed condition in a state in which a medium is brought into contact with the sample is performed a plurality of times at different times respectively so as to obtain a plurality of speckle images, the medium containing a probe that contains the luminescent substance emitting light under the prescribed condition and that repeatedly binds to and dissociates from the target substance directly and specifically; and

an observation image generation step of generating an observation image of the target substance in the sample from the plurality of speckle images, wherein

a half-life of a probe-target complex formed by binding between the probe and the target substance is equal to or more than 10 milliseconds and equal to or less than 3 seconds.

**2**. The method according to claim **1**, wherein

the observation image generation step is a step in which information of a position of a speckle included in each of the plurality of speckle images is obtained for each of the plurality of speckle images and the observation image is generated on the basis of the information from the plurality of speckle images.

**3**. The method according to claim **1**, wherein

the sample includes two or more target substances,

the imaging step is sequentially performed on the sample by using the probe that is specific to each of the target substances, and

the observation image generation step is a step in which observation images of the respective target substances in the sample are respectively generated from the plurality of speckle images obtained from the respective imaging steps.

**4**. The method according to claim **3**, further comprising

a multiple-observation image generation step in which observation images of the respective target substances in the sample generated in the observation image generation step are superposed so as to generate a multiple-observation image, which is an observation image of the two or more target substances in the sample.

5. The method according to claim 1, wherein

the luminescent substance is a fluorescent substance, and

the prescribed condition is irradiation with excitation light.

6. The method according to claim 1, wherein

a combination between the probe and the target substance is selected from a group of:

a combination wherein the probe is (a1) a polypeptide, linked to the luminescent substance, which consists of an amino acid sequence of sequence number 19, (a2) a polypeptide, linked to the luminescent substance, which consists of the amino acid sequence described in (a1) where one or a plurality of amino acids have been substituted, deleted, inserted or added and for which a half-life of a probe-target complex formed by binding between the probe and the target substance is equal to or more than 10 milliseconds and equal to or less than 3 seconds, or (a3) a polypeptide, linked to the luminescent substance, which consists of an amino acid sequence having at least a 70% identity with the amino acid sequence described in (a1) and for which a half-life of a probe-target complex formed by binding between the probe and the target substance is equal to or more than 10 milliseconds and equal to or less than 3 seconds, and the target substance is an actin polymer;

a combination wherein the probe is (b1) a polypeptide, linked to the luminescent substance, which consists of an amino acid sequence that is a partial amino acid sequence of an amino acid sequence of sequence number 12, that at least partially contains an amino acid sequence of 3-309 and that has 407 or fewer amino acids, an amino acid sequence that is a partial amino acid sequence of an amino acid sequence of sequence number 14, that at least partially contains an amino acid sequence of 2536-2843 and that has 408 or fewer amino acids, an amino acid sequence that is a partial amino acid sequence of an amino acid sequence of sequence number 14, that at least partially contains an amino acid sequence of 2781-2819 and that has 138 or fewer amino acids, an amino acid sequence that is a partial amino acid sequence of an amino acid sequence of sequence number 4, that at least partially contains an amino acid sequence of 1-908 and that has 1008 or fewer amino acids, an amino acid sequence that is a partial amino acid sequence of an amino acid sequence of sequence number 4, that at least partially contains an amino acid sequence of 659-908 and that has 394 or fewer amino acids, an amino acid sequence of sequence number 5, or an amino acid sequence of sequence number 6, (b2) a polypeptide, linked to the luminescent substance, which consists of the amino acid sequence described in (b1) where one or a plurality of amino acids have been substituted, deleted, inserted or added and for which a half-life of a probe-target complex formed by binding between the probe and the target substance is equal to or more than 10 milliseconds and equal to or less than 3 seconds, or (b3) a polypeptide, linked to the luminescent substance, which consists of an amino acid sequence having at least a 70% identity with the amino acid sequence described in (b1) and for which a half-life of a probe-target complex formed by binding between the probe and the target substance is equal to or more than 10 milliseconds and equal to or less than 3 seconds, and the target substance is a microtubule;

a combination wherein the probe is (c1) a polypeptide, linked to the luminescent substance, which consists of an amino acid sequence that is a partial amino acid sequence of an amino acid sequence of sequence number 8, that at least partially contains an amino acid sequence of 3777-4684 and that has 1008 or fewer amino acids, an amino acid sequence that is a partial amino acid sequence of an amino acid sequence of sequence number 8, that at least partially contains an amino acid sequence of 3777-4364 and that has 688 or fewer amino acids, an amino acid sequence that is a partial amino acid sequence of an amino acid sequence of sequence number 8, that at least partially contains an amino acid sequence of 3777-4313 and that has 637 or fewer amino acids, or an amino acid sequence that is a partial amino acid sequence of an amino acid sequence of sequence number 8, that at least partially contains an amino acid sequence of 4022-4364 and that has 443 or fewer amino acids, (c2) a polypeptide, linked to the luminescent substance, which consists of the amino acid sequence described in (c1) where one or a plurality of amino acids have been substituted, deleted, inserted or added and for which a half-life of a probe-target complex formed by binding between the probe and the target substance is equal to or more than 10 milliseconds and equal to or less than 3 seconds, or (c3) a polypeptide, linked to the luminescent substance, which consists of an amino acid sequence having at least a 70% identity with the amino acid sequence described in (c1) and for which a half-life of a probe-target complex formed by binding between the probe and the target substance is equal to or more than 10 milliseconds and equal to or less than 3 seconds, and the target substance is an intermediate filament; and

a combination wherein the probe is (d1) a polypeptide, linked to the luminescent substance, which consists of an amino acid sequence of sequence number 15, an amino acid sequence that is a partial amino acid sequence of an amino acid sequence of sequence number 15, that at least partially contains an amino acid sequence of 54-557 and that has 556 or fewer amino acids, an amino acid sequence that is a partial amino acid sequence of an amino acid sequence of sequence number 15, that at least partially contains an amino acid sequence of 54-498 and that has 545 or fewer amino acids, an amino acid sequence that is a partial amino acid sequence of an amino acid sequence of sequence number 15, that at least partially contains an amino acid sequence of 167-557 and that has 491 or fewer amino acids, an amino acid sequence that is a partial amino acid sequence of an amino acid sequence of sequence number 16, that at least partially contains an amino acid sequence of 1-251 and that has 351 or fewer amino acids, an amino acid sequence that is a partial amino acid sequence of an amino acid sequence of sequence number 16, that at least partially contains an amino

acid sequence of 3-251 and that has 349 or fewer amino acids or an amino acid sequence of sequence number 18, (d2) a polypeptide, linked to the luminescent substance, which consists of the amino acid sequence described in (d1) where one or a plurality of amino acids have been substituted, deleted, inserted or added and for which a half-life of a probe-target complex formed by binding between the probe and the target substance is equal to or more than 10 milliseconds and equal to or less than 3 seconds, or (d3) a polypeptide, linked to the luminescent substance, which consists of an amino acid sequence having at least a 70% identity with the amino acid sequence described in (d1) and for which a half-life of a probe-target complex formed by binding between the probe and the target substance is equal to or more than 10 milliseconds and equal to or less than 3 seconds, and the target substance is a focal adhesion.

**7**. The method according to claim **1**, wherein

the probe contains an antibody or a fragment of an antibody, the antibody or the fragment being to the target substance and the antibody or the fragment being linked to the luminescent substance.

**8**. The method according to claim **7**, wherein

the fragment of the antibody is a Fab fragment.

**9**. A probe used for labeling a target substance, wherein

the probe contains a luminescent substance that emits light under a prescribed condition,

the probe can repeatedly bind to and dissociate from the target substance directly and specifically, and

a half-life of a probe-target complex formed by binding to the target substance is equal to or more than 10 milliseconds and equal to or less than 3 seconds.

**10**. The probe according to claim **9**, wherein

the target substance is an actin polymer and the probe is (a1) a polypeptide, linked to the luminescent substance, which consists of an amino acid sequence of sequence number 19, (a2) a polypeptide, linked to the luminescent substance, which consists of the amino acid sequence described in (a1) where one or a plurality of amino acids have been substituted, deleted, inserted or added and for which a half-life of a probe-target complex formed by binding between the probe and the target substance is equal to or more than 10 milliseconds and equal to or less than 3 seconds, or (a3) a polypeptide, linked to the luminescent substance, which consists of an amino acid sequence having at least a 70% identity with the amino acid sequence described in (a1) and for which a half-life of a probe-target complex formed by binding between the probe and the target substance is equal to or more than 10 milliseconds and equal to or less than 3 seconds,

the target substance is a microtubule and the probe is (b1) a polypeptide, linked to the luminescent substance, which consists of an amino acid sequence that is a partial amino acid sequence of an amino acid sequence of sequence number 12, that at least partially contains an amino acid sequence of 3-309 and that has 407 or fewer amino acids, an amino acid sequence that is a partial amino acid sequence of an amino acid sequence of sequence number 14, that at least partially contains an amino acid sequence of 2536-2843 and that has 408 or fewer amino acids, an amino acid sequence that is a partial amino acid sequence of an amino acid sequence of sequence number 14, that at least partially contains an amino acid sequence of 2781-2819 and that has 138 or fewer amino acids, an amino acid sequence that is a partial amino acid sequence of an amino acid sequence of sequence number 4, that at least partially contains an amino acid sequence of 1-908 and that has 1008 or fewer amino acids, an amino acid sequence that is a partial amino acid sequence of an amino acid sequence of sequence number 4, that at least partially contains an amino acid sequence of 659-908 and that has 394 or fewer amino acids, an amino acid sequence of sequence number 5 or an amino acid sequence of sequence number 6, (b2) a polypeptide, linked to the luminescent substance, which consists of the amino acid sequence described in (b1) where one or a plurality of amino acids have been substituted, deleted, inserted or added and for which a half-life of a probe-target complex formed by binding between the probe and the target substance is equal to or more than 10 milliseconds and equal to or less than 3 seconds, or (b3) a polypeptide, linked to the luminescent substance, which consists of an amino acid sequence having at least a 70% identity with the amino acid sequence described in (b1) and for which a half-life of a probe-target complex formed by binding between the probe and the target substance is equal to or more than 10 milliseconds and equal to or less than 3 seconds,

the target substance is an intermediate filament and the probe is (c1) a polypeptide, linked to the luminescent substance, which consists of an amino acid sequence that is a partial amino acid sequence of an amino acid sequence of sequence number 8, that at least partially contains an amino acid sequence of 3777-4684 and that has 1008 or fewer amino acids, an amino acid sequence that is a partial amino acid sequence of an amino acid sequence of sequence number 8, that at least partially contains an amino acid sequence of 3777-4364 and that has 688 or fewer amino acids, an amino acid sequence that is a partial amino acid sequence of an amino acid sequence of sequence number 8, that at least partially contains an amino acid sequence of 3777-4313 and that has 637 or fewer amino acids or an amino acid sequence that is a partial amino acid sequence of an amino acid sequence of sequence number 8, that at least partially contains an amino acid sequence of 4022-4364 and that has 443 or fewer amino acids, (c2) a polypeptide, linked to the luminescent substance, which consists of the amino acid sequence described in (c1) where one or a plurality of amino acids have been substituted, deleted, inserted or added and for which a half-life of a probe-target complex formed by binding between the probe and the target substance is equal to or more than 10 milliseconds and equal to or less than 3 seconds, or (c3) a polypeptide, linked to the luminescent substance, which consists of an amino acid sequence having at least a 70% identity with the amino acid sequence described in (c1) and for which a half-life of a probe-target complex formed by binding between the probe and the target substance is equal to or more than 10 milliseconds and equal to or less than 3 seconds, or

the target substance is a focal adhesion and the probe is (d1) a polypeptide, linked to the luminescent substance,

which consists of an amino acid sequence of sequence number 15, an amino acid sequence that is a partial amino acid sequence of an amino acid sequence of sequence number 15, that at least partially contains an amino acid sequence of 54-557 and that has 556 or fewer amino acids, an amino acid sequence that is a partial amino acid sequence of an amino acid sequence of sequence number 15, that at least partially contains an amino acid sequence of 54-498 and that has 545 or fewer amino acids, an amino acid sequence that is a partial amino acid sequence of an amino acid sequence of sequence number 15, that at least partially contains an amino acid sequence of 167-557 and that has 491 or fewer amino acids, an amino acid sequence that is a partial amino acid sequence of an amino acid sequence of sequence number 16, that at least partially contains an amino acid sequence of 1-251 and that has 351 or fewer amino acids, an amino acid sequence that is a partial amino acid sequence of an amino acid sequence of sequence number 16, that at least partially contains an amino acid sequence of 3-251 and that has 349 or fewer amino acids, or an amino acid sequence of sequence number 18, (d2) a polypeptide, linked to the luminescent substance, which consists of the amino acid sequence described in (d1) where one or a plurality of amino acids have been substituted, deleted, inserted or added and for which a half-life of a probe-target complex formed by binding between the probe and the target substance is equal to or more than 10 milliseconds and equal to or less than 3 seconds, or (d3) a polypeptide, linked to the luminescent substance, which consists of an amino acid sequence having at least a 70% identity with the amino acid sequence described in (d1) and for which a half-life of a probe-target complex formed by binding between the probe and the target substance is equal to or more than 10 milliseconds and equal to or less than 3 seconds.

**11**. The probe according to claim **9**, wherein

the probe contains an antibody or a fragment of an antibody, the antibody or the fragment being to the target substance and the antibody or the fragment being linked to the luminescent substance.

**12**. The probe according to claim **11**, wherein

the fragment of the antibody is a Fab fragment.

**13**. A reagent kit for labeling a target substance, wherein

the reagent kit at least includes the probe according to claim **9**.

**14**. A screening method of a site which identifies a target substance in the probe according to claim **9**, the screening method comprising:

an immobilization step in which a candidate substance of the site or a substance partially containing the candidate substance is fixed to a solid support;

an observation step in which a target substance linked to a luminescent substance and a solid support obtained in the immobilization step are observed in a medium while the target substance linked to a luminescent substance and the solid support obtained in the immobilization step are kept in contact, in a condition that allows observation, in units of 1 molecule, of light emission from the luminescent substance in a probe-target complex formed by binding between the target substance and the candidate substance, and

a screening step in which the candidate substance resulting in a half-life of the probe-target complex that is equal to or more than 10 milliseconds and equal to or less than 3 seconds is selected as the site on the basis of observation in the observation step.

**15**. The method according to claim **14** wherein

the candidate substance is an antibody or a fragment of an antibody from a library of hybridoma that produces an antibody to the target substance, and

the antibody is fixed to a solid support in the immobilization step.

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