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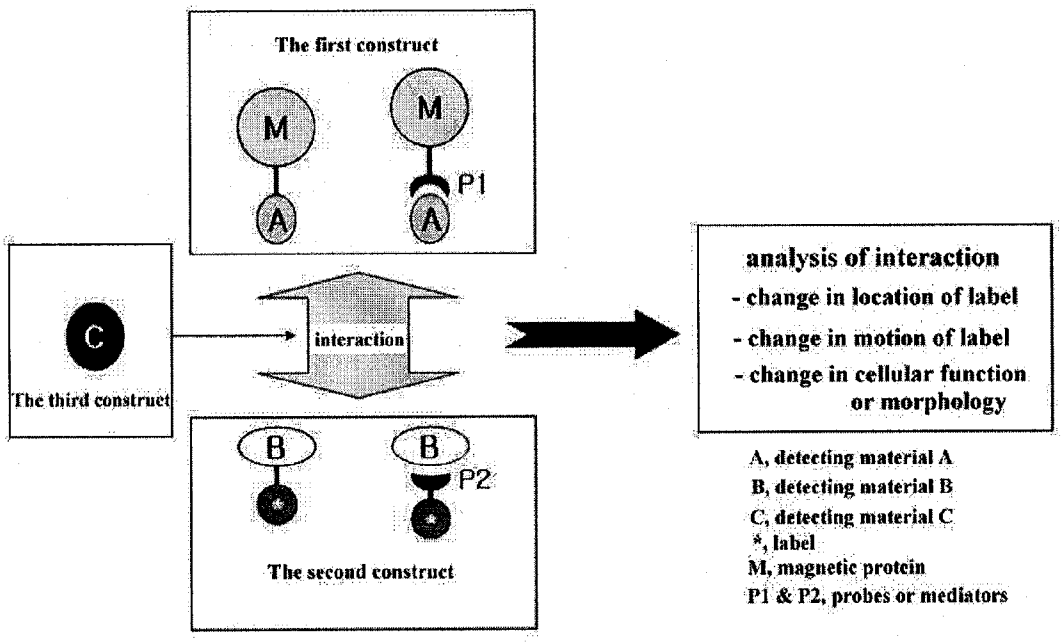
(54) **Title:** SYSTEM FOR DETECTING MOLECULAR INTERACTIONS



(57) **Abstract:** The present invention relates to a method for detecting interactions between various interest molecules, such as bioactive molecules, the method being used for analyzing in vitro and in vivo. According to the present invention, "first detecting material" having a magnetic protein bonded thereto, which is translocated by an externally applied magnetic force, and "second detecting material" having a label bonded thereto are provided. Accordingly, a complex of the "first detecting material" and the "second detecting material", which is formed by interactions in vitro and in vivo, can be detected by varying the strength of the externally applied magnetic force. As a result, a target molecule can be easily detected and screened.

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SYSTEM FOR DETECTING MOLECULAR INTERACTIONS

Technical field

The present invention relates to a method for detecting interactions, *in vitro and/or in vivo*, between interest molecules, such as bioactive molecules. Further, the present invention relates to a method for detecting interactions, *in vitro and/or in vivo*, between interest molecules, such as bioactive molecules, and for screening target molecules.

Background Art

In general, a bioactive chemical compound refers to a chemical compound, which binds to biomolecules such as proteins, nucleic acids, mono-/oligo-/poly-saccharides or lipids and it is configured to control the function or biological activity thereof, *in vivo*. Such a bioactive chemical compound is extracted from an organism or prepared by chemical synthesis. Various antibiotics, for example, "CYCLOSPORINE A" (Novartis AG) and "FK506" (Fujisawa), which are used for reducing immune rejection following an organ transplantation, were isolated from a microorganism, a plant or a marine organism. Such a natural or synthetic bioactive chemical compound is developed as a new drug through pharmacological activity tests and clinical tests thereof, while employing an animal model and a human model.

A protein performs its biological functions *in vivo* by binding to other proteins. In general, the complement proteins interact with each other, and a bioactive chemical compound binds specifically to a specific portion of the 3-dimensional protein structure. The interaction between two proteins strongly implies that they are functionally related. Further, the bioactive chemical compound binding to a specific portion of a protein especially relevant to diseases has potential as a therapeutics which

diagnoses, prevents, treats or alleviates a disease by controlling the activity of the protein. Accordingly, various methods for screening bioactive molecules as drug candidates and detecting novel target proteins as therapeutic targets have been studied, wherein the methods detecting the interaction between a "bait" whose function and feature are known and a "prey" which is an interaction partner of the bait. As mentioned above, identification and isolation of a novel target protein are considered as primary study for obtaining useful information of activity, effectiveness and adverse effects of bioactive chemical compounds. Additionally, the target protein helps to understand the biological pathway and signal transduction system, and provides information on fundamental cellular regulation, physiology and/or mechanism of disease. Accordingly, such information is a powerful tool for, e.g., new drug developments, modification of existing drugs and discovery of novel pharmaceutical usage via detecting and screening of bioactive molecules binding to the target protein(s).

Since the identification of human genome, various studies related to functions, of which the bioactive chemical compound is used to perform during many metabolisms and signal transduction processes, have been conducted rapidly. As new study fields such as chemical genomics, chemical biology and chemical proteomics have been created, a number of technologies such as analysis design, functional genomics, proteomics, automatic engineering and bioinformatics have been able to follow the development.

The methods for detecting interactions between molecules *in vitro* are as follows: conventional biochemical method of, for example, cross-linking, radio-labeled ligand binding, X-ray crystallography and affinity chromatography, affinity blotting, immunoprecipitation and mass spectrometry (E. M. Phizicky and S. Fields, Microbiol. Rev., 59: 94-123, 1995; A. R. Mendelsohn and R. Brent, Science, 284: 1948-1950, 1999). However, the above-mentioned conventional methods require protein

production and isolation processes therefor, which in turn demand much effort. In addition, since these methods are to be carried out *in vitro*, it cannot provide the exact information on *in vivo* interactions.

In order to overcome the above problems of the conventional methods, functional cloning methods, such as yeast two-hybrid (Y2H), yeast three-hybrid (Y3H) (Licitra, E.J., and Liu, J.O., 1996, A three-hybrid system for detecting small ligand-protein receptor interactions. Proc. Natl. Acad. Sci. USA 93, 12817-12821), drug-western (Tanaka, H., Ohima, N., and Hidaka, H. 1999, Isolation of cDNAs encoding cellular drug-binding proteins using a novel expression cloning procedure: drug-western. Mol. Pharmacol. 55, 356-363), phage display cloning (Sche, P.P., McKenzie, K.M., White, J.D., and Austin, D.J. 1999, Display cloning: functional identification of natural product receptors using cDNA-phage display. Chem. Biol. 6, 707-716), fluorescence resonance energy transfer (FRET; Moshinsky DJ, Ruslim L., Blake RA, Tang F., A widely applicable, high-throughput TR-FRET assay for the measurement of kinase autophosphorylation: VEGFR-2 as a prototype. J. Biomol. Screen. 2003 Aug; 8(4):447-52) and mRNA display cloning (McPherson, M., Yang, Y., Hammond, P.W., and Kreider, B.L., 2002, Drug receptor identification from multiple tissues using cellular-derived RNA display libraries. Chem. Biol. 9, 691-698), etc. were developed.

Among the methods, the yeast two-hybrid system is characterized in that a gene expression is performed through a reconstruction of a transcriptional factor obtained by interactions of two expressed proteins. The expressed proteins expected to interact with each other have been cloned into a DNA binding domain (DBD) and a transcriptional activation domain (TAD), respectively, through recombinant DNA technology, and then the proteins are allowed to be expressed within a yeast cell (Fields and Song, Nature 340: 245-246. 1989). This system can be conducted *in vivo* and offers a relatively easy test for conducting. Further, since interactions between two recombinant proteins can be

monitored through activity of a reporter gene, sensitivity control is possible depending on the selections of kind and nature of the reporter gene. These are the merits of the above system. In addition, since gene manipulation technology can be used to entice mutations, the system can be used to detect domains or amino acids that are critical to interactions and a measurement of binding affinity is possible as well. However, since this system is unreliable for genomes that are big and complex and its interpretation may become difficult, implementing the same to humans may also be difficult. Also, the system should be selected based on the original location of the protein in a cell, and a transcription activity can be affected depending on the cell metabolism and growth thereof. Further, it is difficult to obtain a high quality library, and the hybrid proteins can be toxic.

Alternatively, the Y3H system is characterized in that detections on interactions between bioactive chemical compounds and proteins are conducted in living cells. In particular, the Y3H system includes three hybrid molecules, i.e. a DNA-binding protein (DBD) fused with a ligand binding domain (LBD) of a bioactive chemical compound, a hybrid protein containing a transcriptional activation domain (TAD) fused with a second LBD, and a bivalent hybrid molecule. However, the Y3H, like the Y2H, is limited in that it should be conducted in a simple cell such as a yeast cell. Also, it is not suitable for analyzing a full-length membrane protein and difficult to apply for interactions between bioactive chemical compounds and proteins requiring a posttranslational modification or an accessory protein in a yeast cell. Further, the yeast cell has a drawback in that it has genetically a lower permeability for bioactive chemical compound when compared to mammalian cells.

The phage display libraries have been developed as a method for screening an antibody binding specifically to an antigen by using antibody libraries. Recently, a method which identifies an interaction map via the two-hybrid system has been

published. Here, the map is created by screening candidate proteins with bioinformatics technology after finding via peptide libraries a consensus sequence that binds to a specific protein (Allen JB, Walberg MW, Edwards MC, Elledge SJ., Finding prospective partners in the library: the two-hybrid system and phage display to find a match, Trends Biochem Sci. 1995 Dec; 20(12): 511-6). However, since its fundamental process is performed *in vitro*, it has a drawback of having a high false positive rate.

Alternatively, a bioinformatics method has been developed, which predicts interactions between proteins. This method predicts interactions between proteins through molecular phylogenies or multiple sequence alignment, based on the database of genome sequence information. Also, there are relatively newly developed virtual screening methods such as structural genomics, molecular fingerprint, and various cluster analyzing methods.

As mentioned above, even though various methods for detecting interactions of proteins have been proposed so far, the need for finding more effective interaction detection method has been continuous.

Further, various methods for screening a bioactive chemical compound by detecting interactions of the compound and a target protein have been developed. For example, Ding, S. et. al. identified a chemical compound, which induces a Pl9 stem cell to differentiate to a nerve cell, by screening purine molecule library using affinity chromatography (Ding, S., T.Y.H., Brinker, A., Peters, E.C., Hur, W., Gray, N.S., and Schultz, P. G. 2003, Synthetic small molecules that control stem cell fate. Proc. Natl. Acad. Sci. USA 100, 7632-7637), as a biochemical method. However, this method can be used only when the affinity is high, and in order for effective detection, numerous target proteins should be provided. Since most of the bioactive chemical compounds are hydrophobic, the compounds bind competitively to the target molecules in hydrophilic environment. Therefore, a protein would bind to a non-specific binding partner as well

as a specific binding partner if an extract to be detected is applied to an agarose or other supports where a bioactive chemical compound is attached. Thus, in this method, washing process should be carried out in order to remove a binding molecule with non-specific binding affinity.

In order to overcome the shortcomings of the biochemical method, a genomic method was introduced. Zheng et al. used yeast-three hybrid system to detect heterozygote and enhanced drug sensitivity in a host cell. The potent relationship between phenotype and genotype is the biggest advantage of this method. That is, one hit identified using this method provides directly a gene clone to a target protein (Jaeger, S., Eriani, G., and Martin, F. 2004, Results and prospects of the yeast three-hybrid system. FEBS Lett. 566, 7-12). In this system, a novel target protein candidate can be used in the fused form as a part of a multivalent protein complex. This system, however, is allowed to be used only in yeast.

The chemical genomics, which uses a bioactive chemical compound in order to determine the effect of gene mutation on a cell, identifies an active chemical compound from a group of chemical compounds or from a combinatorial library (Schreiber, S.L. 2003, The small molecule approach to biology. Chem. & Eng. 1199 News 81, 51-61; Crews, CM., and Splittgerber, U. 1999, chemical genetics: exploring and controlling cellular processes with chemical probes. Trends Biochem. Sci. 24, 317-320). In this method, hundreds or thousands of chemical compounds are arrayed with gene manipulated cells in wells of a microtiter plate in order to detect interest phenotype (Clemons, P.A., Koehler, A.N., Wager, B.K., Springs, T.G., Spring, D.R., King, R.W., Schreiber, S.L., and Foley, M. A. 2001, A one-bead, one-stock solution approach to chemical genetics: part 2. Chem. Biol. 6, 71-83). For example, a report construct (reporter) having a promoter inducing the expression of a GFP or a commercial marker will be included in a cell, in order to confirm the expression of a certain gene or a group

of genes. However, this method has also two significant problems: first, the chemical genomics is not suitable to detect a bioactive chemical compound which functions at nanomolar concentration; secondly, only a couple of active molecules can be detected from hundreds of thousands of materials, using this method.

As mentioned above, most of the conventional technologies for detecting the interaction between proteins or for detecting the interaction between a protein and a bioactive chemical compound can be carried out only in limited kinds of cells, such as, yeast or bacteria cell. And most of the conventional technologies should be carried out *in vitro*. Since the most of the conventional technologies require gene manipulation and expression process, it is difficult to implement the above technologies as they are limited by the size of DNA. Further, the higher false positive rate due to other parameters lowers reliability of the result.

The inventors of the present invention have developed a new method for detecting molecular interactions of bioactive molecules. Such method is completed by the following order: after introducing into a cell a bioactive chemical compound attached to a localizer showing magnetism to be shifted under magnetic field and other bioactive chemical compounds attached to a label material, a magnetic field is applied to the above cell and the location of the label shifted under magnetic field is detected, followed by detection and verification of the molecular interaction of bioactive molecules. An example of the methods has been published as International Patent Application No. PCT/KR2005/002352. Despite this, a constant improvement is still possible like any other outstanding technologies. As disclosed in the above international patent application method, a bioactive chemical compound is required to be bonded to a localizer such as a magnetic particle. However, because of the different nature, binding between the metal-based magnetic particle and the bioactive chemical compound could be difficult to achieve. Further, in case the bioactive chemical

compound is a protein or a molecule that binds to proteins, it could be preferable to express simultaneously the localizer and the bioactive chemical compound *in vivo* by genetic manipulation when screening and valuating a target protein. Accordingly, in the above case of the bioactive chemical compound being a protein or a molecule that binds to proteins, it is necessary to provide a localizer, which can be easily bonded to the bioactive chemical compound and can be simultaneously expressed with the above bioactive chemical compound *in vivo* by genetic manipulation. Further, a complex of the localizer and the bioactive chemical compound is also necessary.

Disclosure of Invention

Technical Problem

The object of the present invention, in general, is to provide a real-time detection method for detecting interactions between interest molecules, such as biomolecules, *in vivo* (i.e., in cell, in tissue or in body) and *in vitro*, and for screening target molecules. In particular, this invention is for providing a method for directly detecting interactions, *in vivo* and *in vitro*, between "the first detecting material" of a certain biomolecule and "the second detecting material" of a biomolecule to be detected, valuated and/or screened.

Another object of the present invention is to provide a method for directly detecting interactions, *in vivo* and *in vitro*, between "the first detecting material" and "the second detecting material", and for screening a target molecule.

Still another object of the present invention is to provide a kit or a chip for directly detecting interactions of "the first detecting material" and "the second detecting material", *in vivo* and *in vitro*.

Still another object of the present invention is to provide a method for detecting a target molecule which blocks, inhibits, activates or induces interactions between "the

first detecting material" and "the second detecting material", and for screening the target molecule.

Still another object of the present invention is to provide a method for indirectly detecting interactions, *in vivo* and *in vitro*, between "the first detecting material" and "the second detecting material" by monitoring the change in cellular morphology or function.

Technical Solution

In order to achieve the above-mentioned purposes of the present invention, the inventors of the present invention designed two constructs. More specifically, the first construct comprises a magnetic protein, which is translocated by a magnetic force, and the first detecting material, such as a biomolecule to be analyzed, wherein the first detecting material is attached to the magnetic protein directly or indirectly by the use of, for example, a linker. In the first construct, the magnetic protein and the first detecting material of biomolecule can be provided as a fused form. Further, the first construct can be provided as a structure, which are formed by an indirect binding of at least one of the first detecting materials of biomolecules and magnetic proteins to which probes are fused to help to recognize the biomolecules. The second construct includes one or more second detecting materials, such as biomolecules, to which at least one label is attached for detection. In the second construct, the label and the biomolecule to be analyzed can be provided in a directly fused form. Further, the second construct can be provided as a structure, which are formed by an indirect binding of at least one of the second detecting materials, e.g. biomolecules, and labels to which the probes are fused to help recognize the biomolecules. A detection of the complex formed by the interaction of the first detecting material and the second detecting material is performed by approaching them near enough to interact with each other in the same field or system,

followed by applying externally the magnetic force and detecting the label which has changed in location or motion by employing a suitable apparatus. Further, it is possible to indirectly detect interactions by monitoring the change of the cellular morphology or function after applying an external magnetic force according to the present invention. In this invention, if the first detecting material is working as "bait", then the second detecting material is working as "prey", and vice versa. In the present invention, the change of the location or motion can be monitored by the use of, for example, an optical method employing, such as microscope, and can be monitored by a scanner, radioactive label detecting device, fluorescence polarization reader (FP reader), spectrophotometer, MRI (magnetic resonance imaging), SQUID, fluorescence detector and luminescence detector, etc.

In particular, the method for detecting molecular interactions of the present invention comprises the steps of: i) providing a first construct of a binding complex of a first detecting material and a magnetic protein which is translocated by an externally applied magnetic force; ii) providing a second construct comprising a second detecting material to which a label is attached for detection; iii) approaching the first construct and the second construct, in the same field or system, near enough to interact with each other; and iv) applying an external driving force followed by detecting the label which has changed in location or motion.

Further, the method for screening a target molecule of the present invention comprises the steps of: i) providing a first construct of a binding complex of a first detecting material and a magnetic protein which is translocated by externally applied magnetic force; ii) providing a second construct comprising a second detecting material to which a label is attached for detection; iii) approaching the first construct and the second construct, in the same field or system, near enough to interact with each other; iv) applying external magnetic force followed by detecting the label changed in location

or motion; and v) isolating and identifying the molecule from the second construct. The screening of the target molecule can be further carried out using a conventional method, for example, RT-PCR, genomic DNA PCR or using Mass Spectroscopy.

Further, in accordance with the present invention, three constructs are provided in order to detect interactions among the molecules and to screen a target molecule. Specifically, the method of the present invention comprises the steps of: i) providing a first construct of a binding complex of a first detecting material and a magnetic protein which is translocated by an externally applied magnetic force; ii) providing a second construct comprising a second detecting material to which a label is attached for detection; iii) providing a third construct, which mediates interactions between the first and the second constructs and includes a target molecule; iv) approaching the three constructs, in the same field or system, near enough to interact with each other; v) applying an external magnetic force followed by detecting the label having changed in location or motion; and vi) isolating and identifying the molecule from the third construct. The screening of the target molecules can be further performed by a conventional method, for example, RT-PCR, genomic DNA PCR or a Mass Spectroscopy.

In the present invention, the molecule to be detected in the first, the second or the third construct can be provided as a library.

In one example, the first construct of the present invention is prepared by binding a specific molecule such as a biomolecule to a metal storage protein (referred to as 'magnetic protein') having magnetism influenced by the externally applied magnetic force. The above magnetic protein can be provided by being bonded to two or more specific molecules. Further, the second construct is prepared by attaching a label configured to detect partner molecules, i.e. other biomolecules, which interact with biomolecules among the first construct. The interest target molecule can be detected in

real-time after the first construct and the second construct are introduced into the same cell followed by applying a magnetic field as a driving force and by determining the translocated label. Further, in the present invention, the detection can be performed by changing the strength of the magnetic field. And the detection by comparison is performed by a negative control that does not show any change in location or motion, or using a positive control that shows change in location or motion, while varying the strength of the external magnetic field.

In another embodiment, the present invention comprises the steps of: i) providing a specific probe by binding it to a magnetic protein; ii) introducing the magnetic protein having the probe bonded thereto into a cell containing a bioactive molecule having a label attached thereto; iii) introducing other bioactive molecule, which is to be bonded to the probe, into the cell; iv) moving the magnetic protein by applying a magnetic field to the cell; v) monitoring location of the label; and vi) isolating and identifying the bioactive molecule from the cell in which the label has changed in location.

Also, a system for detecting interactions of molecules in accordance with the present invention includes: a reactor; a first construct comprising a magnetic protein, which is translocated by an externally applied magnetic force, and a first detecting material to which the magnetic protein is bonded, or comprising a base sequence for encoding the magnetic protein and the first detecting material; and a second construct comprising a label for detection and a second detecting material to which the label is bonded, or comprising a base sequence for encoding the label and the second detecting material. The above system is characterized in that, when introducing the first and the second constructs into the reactor for an interaction therebetween and applying an externally applied magnetic force thereto, the label is varied in location or motion according to an interaction between the first and the second detecting materials.

When performing this invention *in vivo*, it can be done: for example, in prokaryote or eukaryote; in an organism, a tissue or a cell of mammals; and in an organism, a tissue or a cell of plants. Particularly, the method of this invention can be carried out in an organism, a tissue or a cell of zebra fish, *C-elegans*, yeast, fly or frog. The first construct, the second construct and the third construct are introduced into a cell by the use of, e.g., lipid (or liposome) or the binding complex thereof; or by electroporation or magnetofection. In particular, when carrying out this invention in a living cell, this invention can be carried out in a culture plate/dish. Further, microarrayed cells can be employed for this invention.

The externally applied driving force in the present invention depends on features, such as the physical, chemical, biological and electrostatic feature, of the magnetic protein of the first construct.

In the present invention, the "bioactive molecule" is understood as including all the materials, which represent biological activities *in vivo*, such as nucleic acid, mono-/oligo-/poly-nucleotide, protein, mono-/oligo-/poly-peptide, amino acid, mono-/oligo-/poly-saccharide, lipid, vitamin, chemical compound and the materials constructing thereof.

In the present invention, the "bait" refers to a bioactive molecule used for detecting interaction with other bioactive molecule.

In the present invention, the "prey" refers to a bioactive molecule to be detected or screened, which is an interaction partner of the "bait".

In the present invention, a "target molecule" refers to the bait or the prey, which interacts with the prey or the bait, respectively. Also, the target molecule means all the materials to be identified, which activate or induce the interactions between the bait and the prey or which block or inhibit the interactions between the bait and the prey.

In the present invention, the “magnetic protein” refers to a metal storage protein

having magnetism influenced by a magnetic force externally applied thereto. For example, such a metal storage protein includes ferritin, bacterioferritin, ferritin-like protein, magnetosome, metal binding protein, and so on.

In the present invention, the "detecting material" refers to a material to be assayed and the first detecting material refers to one or more detecting materials that are directly or indirectly bonded to the first construct. Further, the second detecting material refers to one or more detecting materials that are directly or indirectly bonded to the second construct. The third detecting material is to indicate one or more detecting materials that are directly or indirectly bonded to the third construct.

In the present invention, the externally applied driving force means all types of forces which result in a translocation or motion of the magnetic protein defined above. The external driving force includes, for example, electro-magnetic force, magnetic force and so on.

In the present invention, the label of the second construct which is used for detection, comprises a fluorescent material, which emits fluorescence by itself or develops fluorescence by the interaction with the first construct or other materials, for example, fluorescent dye, such as, FITC and rhodamine, etc; fluorescent protein, such as, GFP, YFP, CFP and RFP, etc.; tetracystein motif; and fluorescent nanoparticle. In the present invention, the label of the second construct comprises a luminescent material, which emits luminescence by itself or develops luminescence by the interaction with the first construct or other materials, for example, luciferase. Further, the label of the second construct comprises radio-active label, such as ^{32}P, ^{35}S, ^{3}H and ^{14}C, etc.

In the present invention, a binding between a bioactive molecule and a magnetic protein, or a binding between a bioactive molecule and a label includes, for example, a physical, chemical, electrostatic or biological direct or indirect binding. The bioactive molecule can be attached to the magnetic protein or the label by the above

binding.

In the present invention, the probe of the first construct or the second construct, which is used for detection, comprises, for example, an antibody, protein, protein domain, protein motif and peptide, etc. The probe, which binds to the bioactive molecule by chemical, physical, biological or electrostatic binding, can combine the bioactive molecule and the magnetic protein or combine the bioactive molecule and the label, directly or indirectly.

Advantageous Effects

As mentioned above, in accordance with the present invention, the detection of a complex formed by a molecular interaction can be performed by reacting, in the same field or system, a first construct comprising a bioactive molecule and a magnetic protein of which location is changed according to magnetism externally applied thereto; and a second construct comprising another bioactive molecule to which a label is attached for detection, and detecting the change of location or motion of the label under magnetic field. Also, translocation of the complex can be detected by changing the strength of the magnetic field. Therefore, the present invention can be used for discovering a novel pharmaceutical use from existing drugs or improving the pharmaceutical activities of the existing drugs as well as in detecting and screening a target protein or a new drug candidate, without damaging the interest cell and without any limitation in size of the target molecule for detection, *in vitro and in vivo*.

Brief Description of Drawings

The above and other objects and features of the present invention will become apparent from the following description of embodiments given in conjunction with the accompanying drawings, in which:

Fig. 1 is a schematic drawing of the present invention.

Fig. 2 is a schematic drawing showing a construction of the present invention, which is for detecting an interaction between a first and a second construct.

Fig. 3 is a schematic drawing showing a method for detecting the interaction between the first and the second construct, which is mediated by a third construct.

Fig. 4 is a schematic drawing showing a method for detecting an interaction between a first and a second detecting material by using the third construct, the third construct being a hybridized ligand having a probe-binding factor and a detecting material bonded therewith.

Fig. 5 is a confocal microscope image showing a magnetic protein translocated by an externally applied magnetic force.

Fig. 6 is a confocal microscope image showing a magnetic protein translocated by an externally applied magnetic force, where a non-FAC-treated cell and a cell where only a green fluorescent protein is expressed are used for a negative control.

Fig. 7 is a confocal microscope image confirming a translocation of an EYFP-RelA protein caused by the binding of an IκBα protein and a RelA protein. Further, Fig. 7 is a confocal microscope image confirming a translocation of an mRFP-βTrCP protein caused by the binding of an IκBα protein and a βTrCP protein, the binding being resulted from a TNFα treatment.

Fig. 8 is a confocal microscope image confirming a translocation of an EGFP-FRB protein caused by the binding of a FKBP12 protein and a FRB protein, the binding being induced by the rapamycin treatment.

Fig. 9 is a confocal microscope image showing the result of an mRFP-IκBα protein translocated after an IKKβ protein activated by a TNFα treatment is combined with an IκBα protein which is a substrate of the IKKβ protein

Mode for the Invention

Preferred embodiments of this invention are described in the following examples. Other embodiments within the scope of the claims herein will be apparent to those skilled in the art from consideration of the specification or practice of the invention as disclosed herein. It is intended that the specification, together with the examples, be considered exemplary only, with the scope and the spirit of the invention being indicated by the claims which follow the examples. The examples herein are meant to exemplify the various aspects of carrying out the invention and not intended to limit the scope of the invention in any way. The examples do not include detailed descriptions of conventional methods employed. Such methods are well-known to those skilled in the art and are described in numerous publications. In addition, all publications referred herein are integrated hereto as a reference purpose.

(First Embodiment)

Confirming change in location within a cell by employing a magnetic protein fused with fluorescent protein

Ferritin originated from human or bacterioferritin originated from bacteria has been conventionally used as a magnetic protein. The human ferritin is a spherical-shaped protein complex composed of two kinds of twenty-four subunits. It has an outside diameter of about 12 nm and an inner diameter of about 9 nm, and includes more than 2500 iron atoms (Chasteen, N.D. and Harrison, P.M. 1999, Mineralization in ferritin: an efficient means of iron storage. J. Struc. Biol. 126, 182-194). Human ferritin genes, FTH1 (GenBank Acc. No. BC013724) and FTL (GenBank Acc. No. BC016346), are purchased from Open BioSystems Inc. of U.S. pBluescript II SK(+) (purchased from Stratagene) was used as a cloning vector. The IRES sequence used for producing a double-expression vector employs pIRES2-EGFP (purchased at

Clontech). pcDNA3.1/Zeo(+) (purchased from Invitrogen) was used as an expression vector for a mammalian cell. As a fluorescent label protein, EGFP (pEGFP-N1; purchased from Clontech) and mRFP (Cambell, R.E., Tour, O., Palmer, A.E., Steinbach, P.A., Baird, G.S., Zacharias, D.A. and Tsien, R.Y. 2002, Amonomeric red fluorescent protein. Proc. Nat'l. Acad. Sci. USA. 99, 7877-7882) are employed. By using a primer having an Xho I linker, 5'-ATA TAC TCG AGC CAC CAT GAC GAC CGC GTC CAC CTC G-3' (SEQ ID No. 1) and another primer having an Xba I/Apa I linker, 5'-GAT CCT CTA GAG GGC CCT TAG CTT TCA TTA TCA CTG TCT CC-3' (SEQ ID No. 2), the CDS (coding sequence) of a FTH1 gene is amplified by PCR. Further, a primer having an Xho I linker, 5'-ATA TAC TCG AGC CAC CAT GAG CTC CCA GAT TCG TCA GA-3' (SEQ ID No. 3) and another primer having an Xba I/Apa I linker, 5'-GAT CCT CTA GAG GGC CCT TAG TCG TGC TTG AGA GTG AGC-3' (SEQ ID No. 4) are used to amplify by PCR the CDS of a FTL gene. By processing the PCR-amplified FTH1 and FTL products with Xho I and Xba I restriction enzymes, followed by cloning the products into the Xho I and Xba I restriction enzyme positions of the pcDNA3.1/Zeo(+) vector, a pcDNA-FTH1 vector and a pcDNA-FTL vector are produced. After amplifying the CDS of an EGFP gene by PCR by using a primer having an EcoR I linker, 5'-AAC CGG AAT TCC CAC CAT GGT GAG CAA GGG CGA GGA G-3' (SEQ ID No. 5) and another primer having a Sal I linker, 5'-TGC GTG CGT CGA CCC TTG TAC AGC TCG TCC ATG CCG-3' (SEQ ID No. 6), followed by cloning the genes into the EcoR I and Xho I restriction enzyme positions of the pcDNA-FTH1 vector and the pcDNA-FTL vector, a pBioMAGIC-GFP-FTH1 vector and a pBioMAGIC-GFP-FTL vector are produced.

Further, after processing the PCR-amplified FTH1 and FTL genes with Xho I and Apa I restriction enzymes, followed by cloning the genes into Xho I and Apa I restriction enzyme positions of a pBluescript II SK(+) vector, a pBSII-FTH1 vector and

a pBSII-FTL vector are produced. After amplifying the CDS of a mRFP gene via PCR by employing a primer having an Xba I linker, 5'-CCT AGT CTA GAC CAC CAT GGC CTC CTC CGA GGA CGT C-3' (SEQ ID No. 7) and another primer having a Sal I linker, 5'-TGC GTG CGT CGA CCC AGG GCG CCG GTG GAG TGG C-3' (SEQ ID No. 8), followed by cloning the gene into Xba I and Xho I restriction enzyme positions of the pBSII-FTH1 and the pBSII-FTL vectors, a pBSII-mRFP-FTH1 vector and a pBSII-mRFP-FTL vector are produced. After extracting a mRFP-FTH1 gene and a mRFP-FTL gene by processing the pBSII-mRFP-FTH1 vector and the pBSII-mRFP-FTL vector with Xba I and Apa I restriction enzymes, followed by cloning the genes into the Xba I and the Apa I restriction enzyme positions of a pcDNA3.1/Zeo(+) vector, a pBioMAGIC-mRFP-FTH1 vector and a pBioMAGIC-mRFP-FTL vector are produced.

On the other hand, after extracting a FTH1 gene and a FTL gene by processing a pcDNA-FTH1 vector and a pcDNA-FTL vector with Xho I and Xba I restriction enzymes, followed by cloning the genes into a pBioMAGIC-mRFP-FTL vector and a pBioMAGIC-mRFP-FTH1 vector, respectively, a pBioMAGIC-FTH1-mRFP-FTL vector and a pBioMAGIC-FTL-mRFP-FTH1 vector are produced. Further, by extracting a GFP-FTH1 gene and a GFP-FTL gene by processing a pBioMAGIC-GFP-FTH1 vector and a pBioMAGIC-GFP-FTL vector with EcoR I and Xba I restriction enzymes, followed by cloning the genes into the pBioMAGIC-mRFP-FTL vector and the pBioMAGIC-mRFP-FTH1 vector, respectively, a pBioMAGIC-GFP-FTH1-mRFP-FTL vector and a pBioMAGIC-GFP-FTL-mRFP-FTH1 vector are produced. After amplifying by PCR an IRES sequence by employing a primer having an Xba I linker, 5'-GCT AGT CTA GAG CCC CTC TCC CTC CCC CCC C-3' (SEQ ID No. 9) and another primer having an Xba I linker, 5'-GAT CCT CTA GAT GTG GCC ATA TTA TCA TCG TGT T-3' (SEQ ID No. 10) and processing the sequence with an Xba I

restriction enzyme, followed by cloning the sequence into the Xba I restriction enzyme position of the pBioMAGIC-FTH1-mRFP-FTL, the pBioMAGIC-FTL-mRFP-FTH1, the pBioMAGIC-GFP-FTH1-mRFP-FTL and the pBioMAGIC-GFP-FTL-mRFP-FTH1 vectors, a pBioMAGIC-1 (pBioMAGIC-FTH1-IRES-mRFP-FTL) vector, a pBioMAGIC-2 (pBioMAGIC-FTL-IRES-mRFP-FTH1) vector, a pBioMAGIC-CON1 (pBioMAGIC-GFP-FTH1-IRES-mRFP-FTL) vector and a pBioMAGIC-CON2 (pBioMAGIC-GFP-FTL-IRES-mRFP-FTH1) vector are produced.

A cell line of human cervical cancer, a HeLa cell (purchased from ATCC) is incubated with DMEM (purchased from Invitrogen) containing 10% Fetal Bovine Serum (FBS, purchased from Invitrogen). After subculturing a HeLa cell at a rate of 4000 cells/well in a 16-well chambered slideglass (purchased from Nunc), the cell is incubated at 37℃ for 24 hrs in a CO_2 incubator. After mixing the pBioMAGIC-GFP-FTH1 vector and the pBioMAGIC-mRFP-FTH1 vector, the resulting mixture is introduced into the HeLa cell by using Lipofectamine 2000 (a product name: purchased from Invitrogen). Then again, each of the pBioMAGIC-GFP-FTH1 vector and the pBioMAGIC-mRFP-FTL vector is introduced into the HeLa cell by using the Lipofectamine 2000. Further, a pEGFP-N1 vector is introduced into the HeLa cell as a negative control. After incubating the DNA-introduced HeLa cell at 37 °C in the CO_2 incubator for 24 hours and adding DMEM containing 20~500 μM ferric ammonium citrate (FAC; purchased from Sigma), the cell is incubated again at 37 °C in the CO_2 incubator for 24 hours. Upon placing the slideglass having the incubated cell to the top of the focal plate of a confocal laser microscope (Carl Zeiss), permanent magnet or electromagnet force is applied to its bottom surface. By adjusting the focal plate of the confocal laser microscope to the bottom of cell, the ferritin protein complex moved to the bottom of the cell is observed. (Fig. 5) The ferritin protein complex is observed to be motionless in the non-FAC treated cell, whereas, in the FAC-treated cell, the location

of the ferritin protein complex is moved to the bottom of the cell and observed that green fluorescent or red fluorescent light is observed. (Fig. 6)

Second Embodiment

Detection of the interactions of various biomolecules *in vivo*

1) Detection of the interaction *in vivo* between proteins: a pcDNA-IκBα-FTH1 vector is produced by cloning the CDS of a PCR-amplified IκBα gene (Genbank Acc. No. NM_020529) into a pcDNA-FTH1 vector. By amplifying a gene of RelA protein (Genbank Acc. No. NM_021975) via PCR, the protein being a binding partner of a IκBα protein in cell, followed by cloning the gene into a pEYFP-N1 vector (purchased from Clonetech), a pEYFP-RelA vector is produced. After mixing a pcDNA-IκBα-FTH1 vector, a pBioMAGIC-mRFP-FTL vector and a pEYFP-RelA vector, the resulting mixture is introduced into a HeLa cell, as discussed in the first embodiment. By observing the FAC-treated cell exposed to a magnetic field and in a non-magnetic-field-applied condition with a confocal laser microscope, a change in yellow fluorescent light caused by an interaction between the IκBα protein and the RelA protein is confirmed. (Fig. 7)

2) Detection of a protein complex forming *in vivo* by bioactive low molecular weight chemical compounds: A FRB protein and a FKBP12 protein are interacted by having rapamycin as a mediator (Sabers, C.J., Martin, M.M., Brunn, G.J., Williams, J.M., Dumont, F.J., Wiederrecht, G. and Abraham, R.T. 1995, Isolation of a protein target of FKBP12-rapamycin complex in mammalian cells. J. Biol. Chem. 270, 815-822). A pcDNA-FKBP12-FHT1 vector is produced by cloning a PCR-amplified FKBP12 gene (Genbank Acc. No. NM_125831) into the pcDNA-FTH1 vector. A pEGFP-FRB vector is produced by cloning the CDS of a PCR-amplified FRB gene (Genbank Acc. No. NM_004958) into the pEGFP-N1 vector. A mixture of the

pcDNA-FKBP12-FHT1, the pBioMAGIC-mRFP-FTL and the pEGFP-FRB vectors is, according to the manner identical to the one discussed in the first embodiment, introduced into a HeLa cell. After treating the DNA-introduced HeLa cell with FAC for 24 hours and then with rapamycin (purchased from Calbiochem) for 10 minutes, respectively, the resulting cell is observed with a confocal laser microscope while being exposed to a magnetic field and in a non-magnetic-field-applied condition. From the observation, a change in green fluorescent light caused by an interaction between the FKBP12-rapamycin complex and the FRB protein is detected (Fig. 8).

(Third Embodiment)

Detection of change in protein complex by signal transduction

To detect a change of protein complex caused by signal transduction *in vivo*, a system of TNF-α/IκBα signal transduction is selected. An IκBα controls a variety of signals by forming a complex consisting of NF-κB proteins such as RelA/p65, p50, C-Rel and the like. Upon treating a cell with TNF- α , the 32nd serine and the 36th serine of an IκBα are phosphorylated by the increase of IκBα kinase (IKK) activity, and thereby causing the IκBα protein and the βTrCP protein to combine [Brown, et al., Science 267: 1485, (1995)].

1) Detecting a protein complex forming by signal transduction: By inserting the PCR-amplified IκBα gene and the CDS of a ECFP gene into a pcDNA-FTH1 vector, a pcDNA-ECFP-IκBα-FTH1 vector is produced. Further, a pmRFP-βTrCP vector, of which the PCR-amplified βTrCP gene is fused with a mRFP gene, is produced. After introducing, simultaneously, the pcDNA-ECFP-IκBα-FTH1 vector, the pcDNA-FTL vector, the pEYFP-RelA vector and the pmRFP-βTrCP vector into the HeLa cell and treating the cell with FAC for 24 hours, followed by treating of a 10 ng/ml TNF-α for 5 minutes, a confocal image of a living cell is taken with a confocal laser microscope

while being exposed to a magnetic field and in a non-magnetic-field-applied condition. The EYFP-RelA fusion protein forming a complex with the IκBα is observed to be moved by externally applied magnetic force regardless of TNF-α treating, whereas the mRFP-βTrCP fusion protein forming a complex with the phosphorylated IκBα is observed to be moved only when treated with the TNF-α. (Fig. 7)

2) Detecting an interaction between a protein kinase and a substrate caused by a signal transduction: A pBioMAGIC-IKKβ-FTH vector is produced by cloning the CDS of a PCR-amplified IKKβ gene into a FTH1 expression vector and a pmRFP-IκBα vector is produced by cloning the CDS of a PCR-amplified IκBα gene into a mRFP expression vector. After introducing, simultaneously, the pBioMAGIC-IKKβ-FTH vector and the pmRFP-IκBα vector into the HeLa cell and treating the cell with FAC for 24 hours, followed by treating of a 10 ng/ml TNF-α for 5 minutes, a confocal image of a living cell is taken with a confocal laser microscope while being exposed to a magnetic field. As the IKKβ protein is activated by the signal transduction of the TNF-α, thereby being combined with an IκBα protein, which is a substrate of the IKKβ protein, a green fluorescent light is observed to be in motion. (Fig. 9)

While the invention has been shown and described with respect to the preferred embodiments, it will be understood by those skilled in the art that various changes and modifications may be made without departing from the scope of the invention as defined in the following claims. The present invention also includes such changes and modifications.

What is claimed is:

1. A method for detecting molecular interactions, the method comprising the steps of:

i) providing a first construct including a magnetic protein, which is translocated by an externally applied magnetic force, and a first detecting material to which the magnetic protein is bonded, or a base sequence for encoding the magnetic protein and the first detecting material;

ii) providing a second construct including a label for detection and a second detecting material to which the label is bonded, or a base sequence for encoding the label and the second detecting material;

iii) approaching the first and the second construct, in the same field or system, to a location where an interaction between the first and the second construct is possible; and

iv) detecting an interaction between the first and the second detecting material by detecting the label, which is varied in location or motion, when applying the external magnetic force.

2. The method of claim 1, in case the interaction between the first and the second construct is performed in a cell, further comprising the step of detecting the change by the interaction in the cellular morphology or function.

3. The method of claim 1 or 2, wherein the first and the second detecting materials are bioactive molecules.

4. The method of claim 3, wherein the bioactive molecules are one or more

molecules selected from a group consisting of nucleic acid, nucleotide, protein, peptide, amino acid, saccharide, lipid, vitamin, chemical compounds and the materials constructing thereof.

5. The method of claim 1 or 2, wherein the externally applied magnetic force is a magnetic field.

6. The method of claim 5, wherein the magnetic field is an electro-magnetic or a permanent magnetic field.

7. The method of claim 5 or 6, wherein the detection step is carried out while the strength of the magnetic field is changed.

8. The method of any one of claims 1 to 7, wherein the magnetic protein is a metal storage protein.

9. The method of any one of claims 1 to 7, wherein the magnetic protein is ferritin, bacterioferritin, ferritin-like protein, magnetosome, or metal-binding protein.

10. The method of any one of claims 1 to 9, wherein the label is a radio-active, fluorescent or luminescent material.

11. The method of claim 10, wherein the fluorescent material or the luminescent material emits fluorescence or luminescence by itself, or develops fluorescence or luminescence when it interacts with the molecule of the first construct or another molecule.

12. The method of claim 10, wherein the fluorescent material is fluorescent dye, tetracystein motif, fluorescent protein, such as GFP, YFP, CFP and RFP, or fluorescent nano-particle.

13. The method of any one of claims 1 to 12, wherein the magnetic protein of the first construct binds to two or more of the first detecting materials directly or indirectly.

14. The method of any one of claims 1 to 13, wherein the magnetic protein of the first construct binds to the first detecting material by an electrostatic, physical, chemical or biological binding.

15. The method of any one of claims 1 to 14, wherein the label of the second construct binds to the second detecting material directly or indirectly.

16. The method of any one of claims 1 to 15, wherein the label of the second construct binds to the second detecting material by an electrostatic, physical, chemical or biological binding.

17. The method of any one of claims 1 to 16, wherein one or more probes, selected from a group consisting of an antibody, protein, protein domain, protein motif and peptide, are used for binding the magnetic protein and the first detecting material in the first construct, and for binding the label and the second detecting material in the second construct.

18. The method of any one of claims 1 to 17, wherein the interaction between the

first construct and the second construct is carried out in a cell.

19. The method of claim 18, wherein the cell is a prokaryotic or eukaryotic cell.

20. The method of claim 18 or 19, wherein the first construct and the second construct are introduced into a cell by transducible peptide or fusogenic peptide, lipid or liposome, or the binding complex thereof, or by electroporation or magnetofection.

21. The method of any one of claims 18 to 20, the interaction between the first construct and the second construct is performed in cells within an culture plate or dish, or in microarrayed cells.

22. The method of any one of claims 1 to 17, wherein the interaction between the first construct and the second construct is performed *in vitro*.

23. The method of any one of claims 5 to 22, wherein the material, translocated or non-translocated when changing the strength of a magnetic field applied thereto, is compared as a positive control or a negative control, respectively.

24. The method of any one of claims 1 to 23, wherein the detecting material of the first construct or the second construct is provided as a library.

25. A method for screening a target molecule, the method comprising the steps of isolating and identifying the second detecting material included in the second construct, after detecting the interactions between molecules according to any one method of claims 1 to 24.

26. A method for screening a target molecule, the method comprising the steps of isolating and identifying the first detecting material included in the first construct, after detecting the interactions between molecules according to any one method of claims 1 to 24.

27. The method of claim 25 or 26, wherein the detecting material of the first construct or the second construct is provided as a library.

28. A method for detecting molecular interactions, the method comprising the steps of:

i) providing a first construct including a magnetic protein, which is translocated by an externally applied magnetic force, and a probe to which the magnetic protein is bonded, or a base sequence for encoding the magnetic protein and the probe;

ii) providing a second construct including a label and a second detecting material to which the label is bonded, or a base sequence for encoding the label and the second detecting material;

iii) providing a third construct, which mediates an interaction between the first construct and the second construct, including a probe–binding factor which binds to the probe and a third detecting material, or a base sequence for encoding the probe-binding factor and the third detecting material;

iv) approaching the first, the second and the third construct, in the same field or system, to a location where an interaction between the constructs is possible; and

v) detecting an interaction between the second and the third detecting material by detecting the label, which is varied in location or motion, when applying the external magnetic force.

29. The method of claim 28, in case the interaction is performed in a cell, further comprising the step of detecting the change by the interaction in the cellular morphology or function.

30. The method of claim 28 or 29, wherein the probe or the detecting material of the first, the second, or the third construct is provided as a library.

31. A method for screening a target molecule, the method comprising the steps of:

i) providing a first construct including a magnetic protein, which is translocated by an externally applied magnetic force, and a probe to which the magnetic protein is bonded, or a base sequence for encoding the magnetic protein and the probe;

ii) providing a second construct including a label and a second detecting material to which the label is bonded, or a base sequence for encoding the label and the second detecting material;

iii) providing a third construct, which mediates the interaction between the first and the second construct, including a probe–binding factor which binds to the probe and a third detecting material, or a base sequence for encoding the probe-binding factor and the third detecting material;

iv) approaching the first, the second and the third construct, in the same field or system, to a location where an interaction between the constructs are possible;

v) detecting an interaction between the second and the third detecting material by detecting the label, which is varied in location or motion, when applying the external magnetic force; and

vi) isolating and identifying target molecules included in the second or the third construct.

32. The method of claim 31, in case the interaction is performed in a cell, further comprising the step of detecting the change by the interaction in the cellular morphology or function.

33. A system for detecting interactions of molecules, the system comprising:

a reactor;

a first construct including a magnetic protein, which is translocated by an externally applied magnetic force, and a first detecting material to which the magnetic protein is bonded, or a base sequence for encoding the magnetic protein and the first detecting material;

a second construct including a label for detection and a second detecting material to which the label is bonded, or a base sequence for encoding the label and the second detecting material; and

wherein the system is characterized in that, when introducing the first and the second constructs into the reactor for an interaction therebetween and applying an externally applied magnetic force thereto, the label is varied in location or motion according to an interaction between the first and the second detecting materials.

34. A system for detecting interactions of molecules, the system comprising:

a reactor;

a first construct including a magnetic protein, which is translocated by an externally applied magnetic force, and a probe to which the magnetic protein is bonded, or a base sequence for encoding the magnetic protein and the probe;

a second construct including a label and a second detecting material to which the label is bonded, or a base sequence for encoding the label and the second detecting

material;

a third construct, which mediates the interaction between the first and the second construct, including a probe–binding factor which binds to the probe and a third detecting material, or a base sequence for encoding the probe-binding factor and the third detecting material; and

wherein the system is characterized in that, when introducing the first, the second and the third constructs into the reactor for an interaction therebetween and applying an externally applied magnetic force thereto, the label is varied in location or motion according to an interaction between the second and the third detecting materials.

35. The system of claim 33 or 34, wherein the reactor is a cell and at least one of the first, the second and the third construct is provided as a vector.

36. The system of claim 35, further comprising the means for detecting the change by the interactions of molecules in the cellular morphology or function.

37. The system of claim 33 or 34, wherein the detecting materials are bioactive molecules.

38. The system of claim 37, wherein the bioactive molecules are one or more molecules selected from a group consisting of nucleic acid, nucleotide, protein, peptide, amino acid, saccharide, lipid, vitamin, chemical compounds and the materials constructing thereof.

39. The system of claim 33 or 34, wherein the magnetic protein is a metal storage protein.

40. The system of claim 33 or 34, wherein the magnetic protein is ferritin, bacterioferritin, ferritin-like protein, magnetosom, or metal-binding protein.

41. The system of any one of claim 33 or 34, wherein the label is a radio-active, fluorescent or luminescent material.

42. The system of claim 41, wherein the fluorescent material or the luminescent material emits fluorescence or luminescence by itself, or develops fluorescence or luminescence when it binds to the molecule of the first construct or another molecule.

43. The system of claim 42, wherein the fluorescent material is fluorescent dye, tetracystein motif, fluorescent protein, such as GFP, YFP, CFP and RFP, or fluorescent nano-particle.

44. The system of claim 33 or 34, wherein the system is provided as a kit or a chip.

Fig. 1

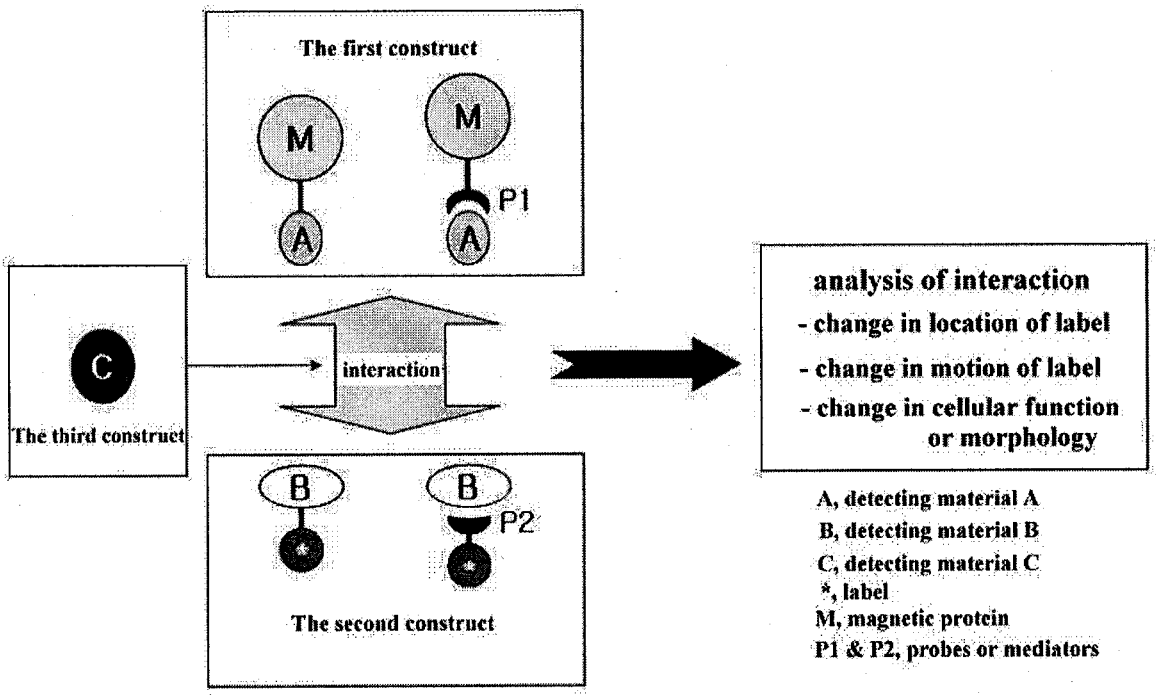

The first construct
M
M
P1
A
A
C
The third construct
interaction
B
B
P2
The second construct
analysis of interaction
- change in location of label
- change in motion of label
- change in cellular function or morphology
A, detecting material A
B, detecting material B
C, detecting material C
*, label
M, magnetic protein
P1 & P2, probes or mediators

Fig. 2

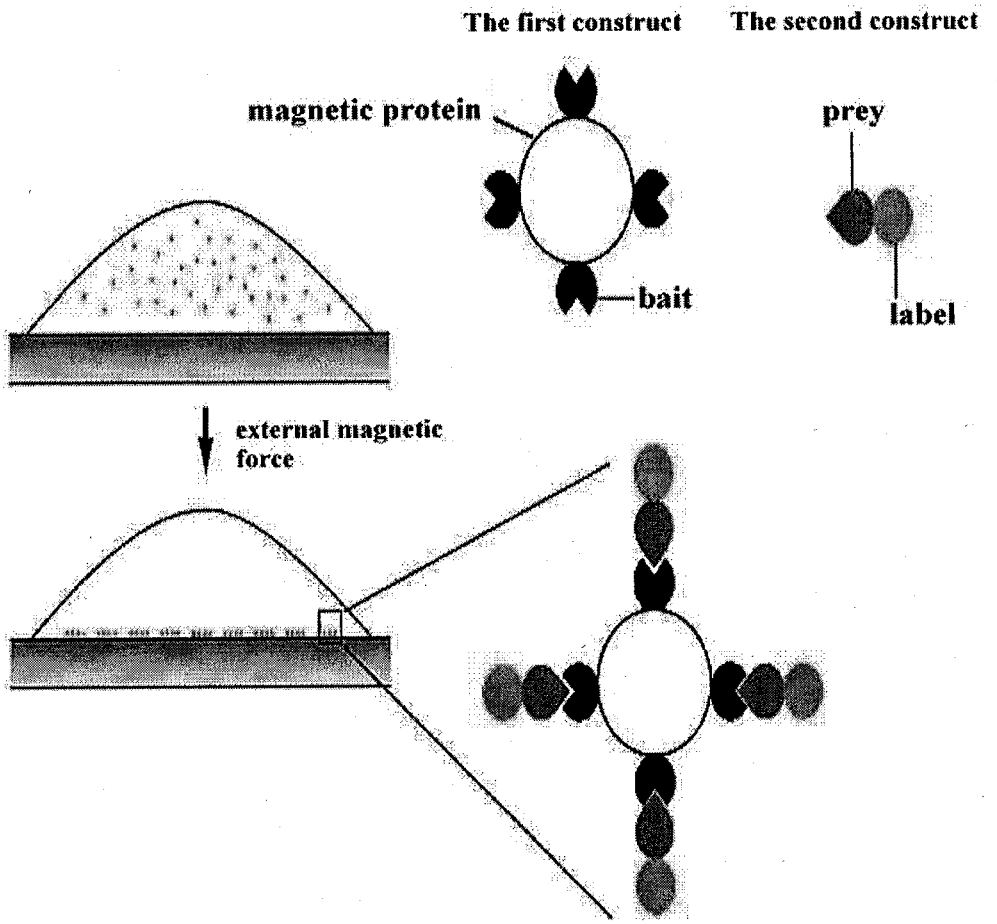

The first construct
The second construct
magnetic protein
prey
bait
label
external magnetic force

Fig. 3

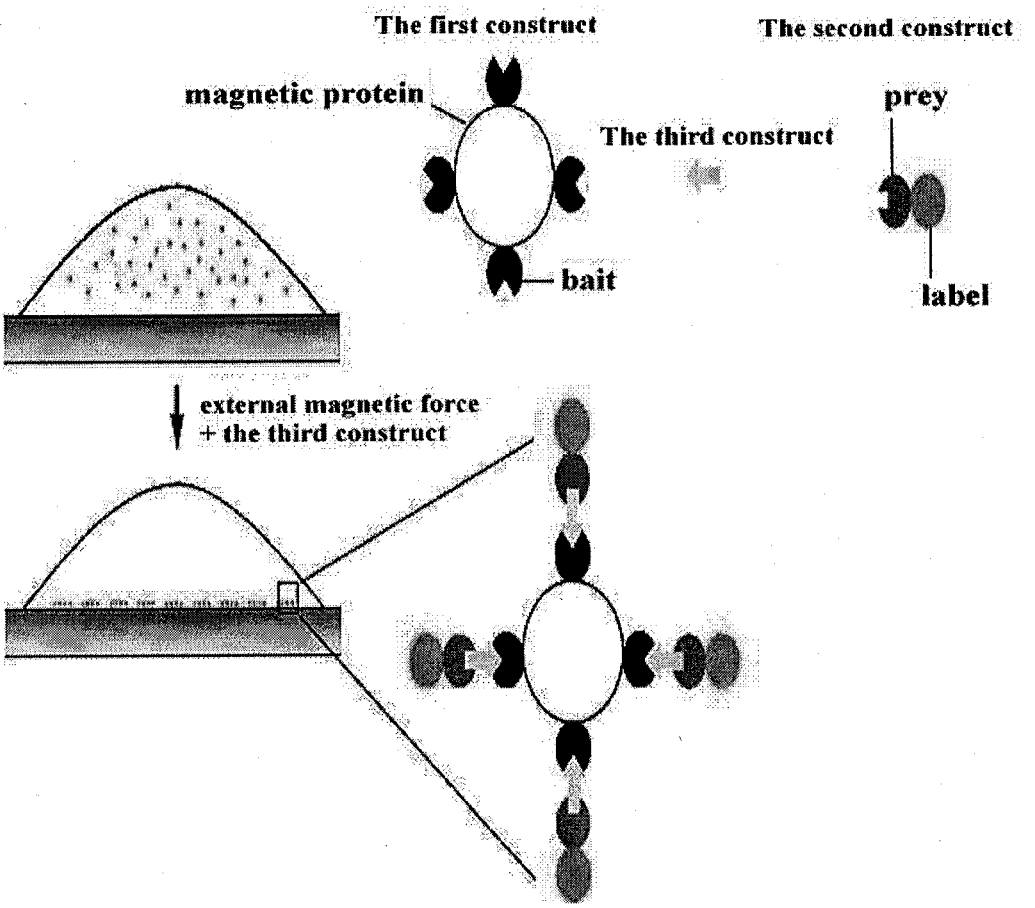

The first construct
The second construct
magnetic protein
prey
The third construct
bait
label
external magnetic force
+ the third construct

Fig. 4

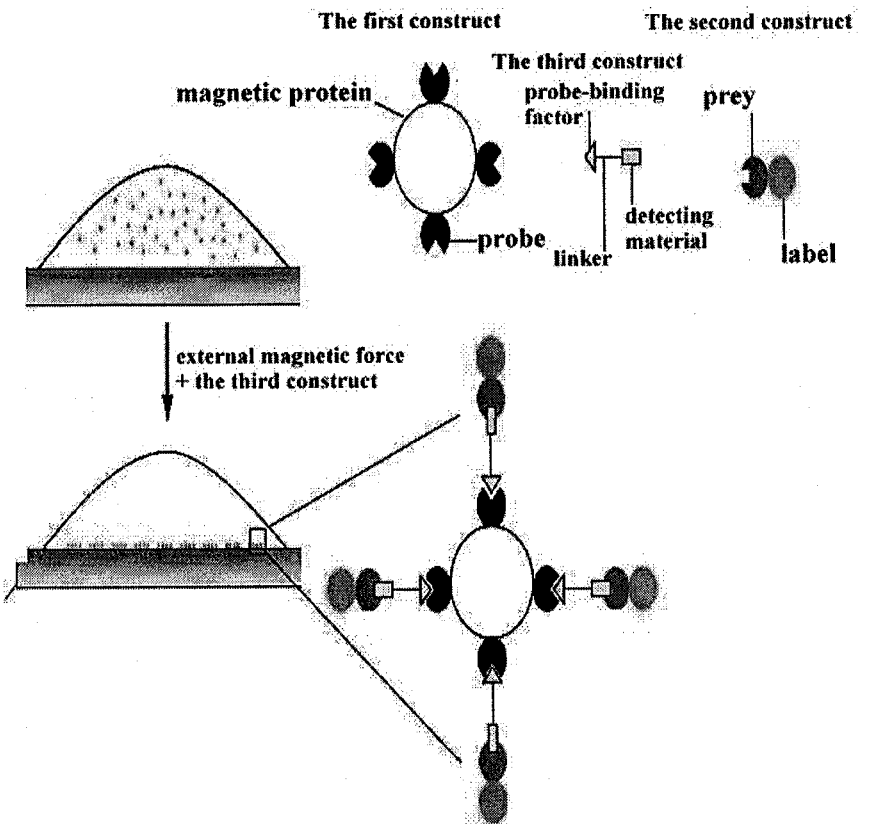

The first construct
The second construct
The third construct
magnetic protein
probe-binding factor
prey
detecting material
probe
linker
label
external magnetic force + the third construct

Fig. 5

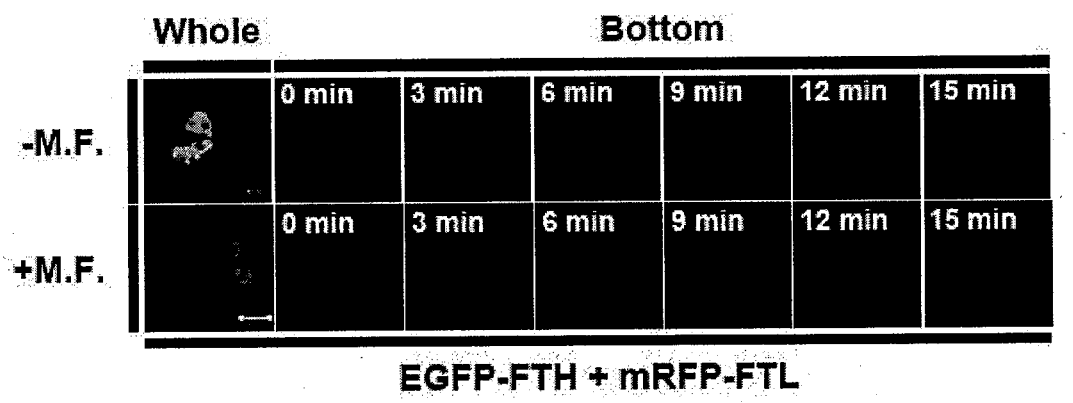

Whole
Bottom
0 min
3 min
6 min
9 min
12 min
15 min
-M.F.
+M.F.
EGFP-FTH + mRFP-FTL

Fig. 6

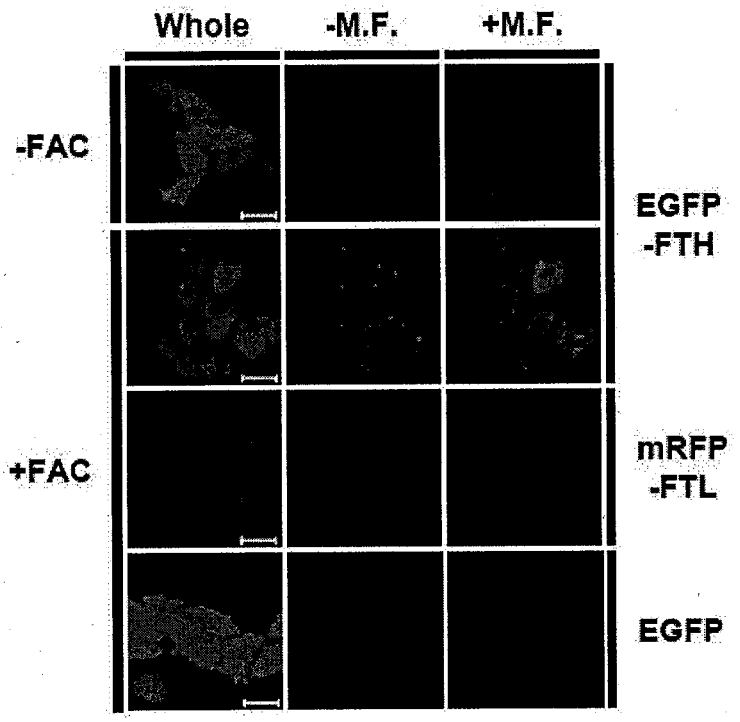

Whole
-M.F.
+M.F.
-FAC
+FAC
EGFP
-FTH
mRFP
-FTL
EGFP

Fig. 7

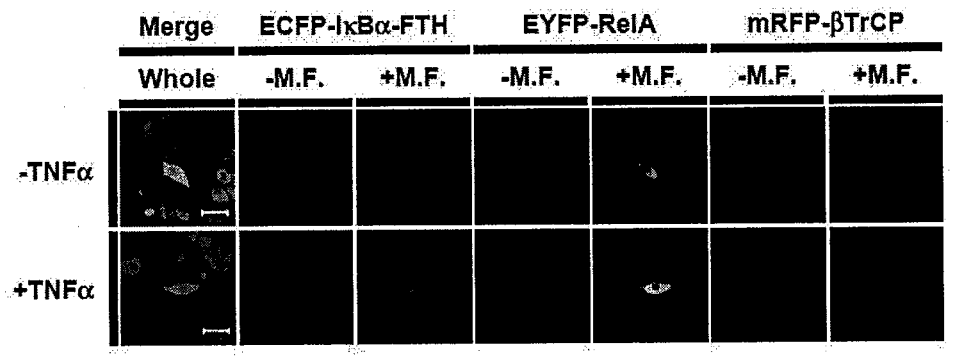

Merge
ECFP-IκBα-FTH
EYFP-RelA
mRFP-βTrCP
Whole
-M.F.
+M.F.
-M.F.
+M.F.
-M.F.
+M.F.
-TNFα
+TNFα

Fig. 8

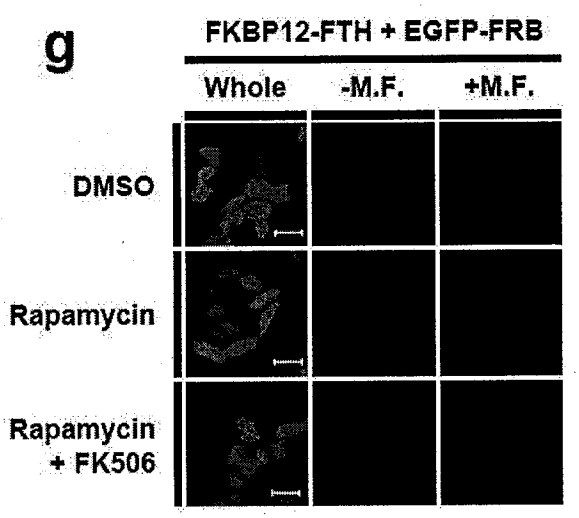
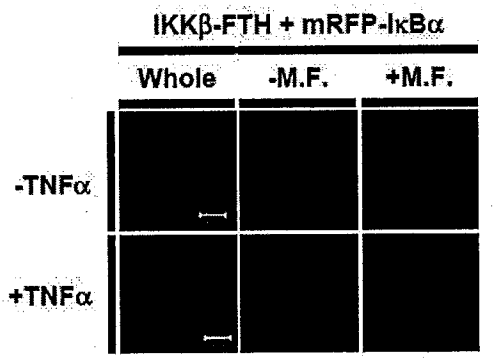

g
FKBP12-FTH + EGFP-FRB
Whole
-M.F.
+M.F.
DMSO
Rapamycin
Rapamycin
+ FK506

Fig. 9


IKKβ-FTH + mRFP-IκBα
Whole
-M.F.
+M.F.
-TNFα
+TNFα

INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR2006/004772

A. CLASSIFICATION OF SUBJECT MATTER

G01N 33/48(2006.01)i

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 8: G01N 33/48, C12Q 1/68, G01N 33/548

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
Korean Patents and Applications for Invention since 1975
Korean Utility models and Application for Utility model since 1975
Japanese Patents and Application for Invention since 1975

Electronic data base consulted during the intertnational search (name of data base and, where practicable, search terms used)
eKIPASS(KIPO internal), Delphion, Pubmed (magnetic protein&molecular interaction&label)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Won, J; Kim, M; Yi, YW; Kim, YH; Jung, N; Kim, TK. A magnetic nanoprobe technology for detecting molecular interactions in live cells. Science. 309 (5731): 121-125; 1 July 2005. See the whole document, especially page 122 (left column).	1-44
A	Roberts, TG; Anker, JN; Kopelman, R. Magnetically modulated optical nanoprobes (MagMOONs) for detection and measurement of biologically important ions against the natural background fluorescence of intracellular environments. Journal of Magnetism and Magnetic Materials. 293(1): 715-724; 2 March 2005. See the whole document, especially abstract and figure 6.	1-44
A	WO0114591A1 (FOX, John, S.) 01 March 2001 See the whole document, especially abstract and page 5.	1-44
A	US06828786B2 (CALIFORNIA INSTITUTE OF TECHNOLOGY) 07 December 2004 See the whole document, especially abstract and claim 1.	1-44
A	JP17091014A (UNIV WASEDA) 07 April 2005 See the whole document, especially abstract.	1-44
A	US05945281A (BECTON, DICKINSON AND COMPANY) 31 August 1999 See the whole document, especially abstract and column 3.	1-44

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of citation or other special reason (as specified)
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"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
07 FEBRUARY 2007 (07.02.2007)	**07 FEBRUARY 2007 (07.02.2007)**

Name and mailing address of the ISA/KR	Authorized officer
Korean Intellectual Property Office 920 Dunsan-dong, Seo-gu, Daejeon 302-701, Republic of Korea Facsimile No. 82-42-472-7140	CHO, Kyung Joo Telephone No. 82-42-481-8287

INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR2006/004772

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item1.b of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, the international search was carried out on the basis of :

   a. type of material
   - [x] a sequence listing
   - [ ] table(s) related to the sequence listing

   b. format of material
   - [ ] on paper
   - [x] in electronic form

   c. time of filing/furnishing
   - [ ] contained in the international application as filed
   - [x] filed together with the international application in electronic form
   - [ ] furnished subsequently to this Authority for the purposes of search

2. [ ] In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.
PCT/KR2006/004772

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO0114591A1	01.03.2001	AU200069200A5	19.03.2001
		CA2381732AA	01.03.2001
		EP1210461A1	05.06.2002
		EP1210461A4	23.11.2005
		WO0200114591A1	01.03.2001
US06828786B2	07.12.2004	US20030158474A1	21.08.2003
JP17091014A	07.04.2005	JP2005091014A2	07.04.2005
US05945281A	31.08.1999	None	

INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR2006/004772

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item1.b of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, the international search was carried out on the basis of :

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3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR2006/004772

A. CLASSIFICATION OF SUBJECT MATTER

G01N 33/48(2006.01)i

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 8: G01N 33/48, C12Q 1/68, G01N 33/548

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
Korean Patents and Applications for Invention since 1975
Korean Utility models and Application for Utility model since 1975
Japanese Patents and Application for Invention since 1975

Electronic data base consulted during the intertnational search (name of data base and, where practicable, search terms used)
eKIPASS(KIPO internal), Delphion, Pubmed (magnetic protein&molecular interaction&label)

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Republic of Korea
Facsimile No. 82-42-472-7140

Authorized officer
CHO, Kyung Joo
Telephone No. 82-42-481-8287

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.
PCT/KR2006/004772

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO0114591A1	01.03.2001	AU200069200A5	19.03.2001
		CA2381732AA	01.03.2001
		EP1210461A1	05.06.2002
		EP1210461A4	23.11.2005
		WO0200114591A1	01.03.2001
US06828786B2	07.12.2004	US20030158474A1	21.08.2003
JP17091014A	07.04.2005	JP2005091014A2	07.04.2005
US05945281A	31.08.1999	None	