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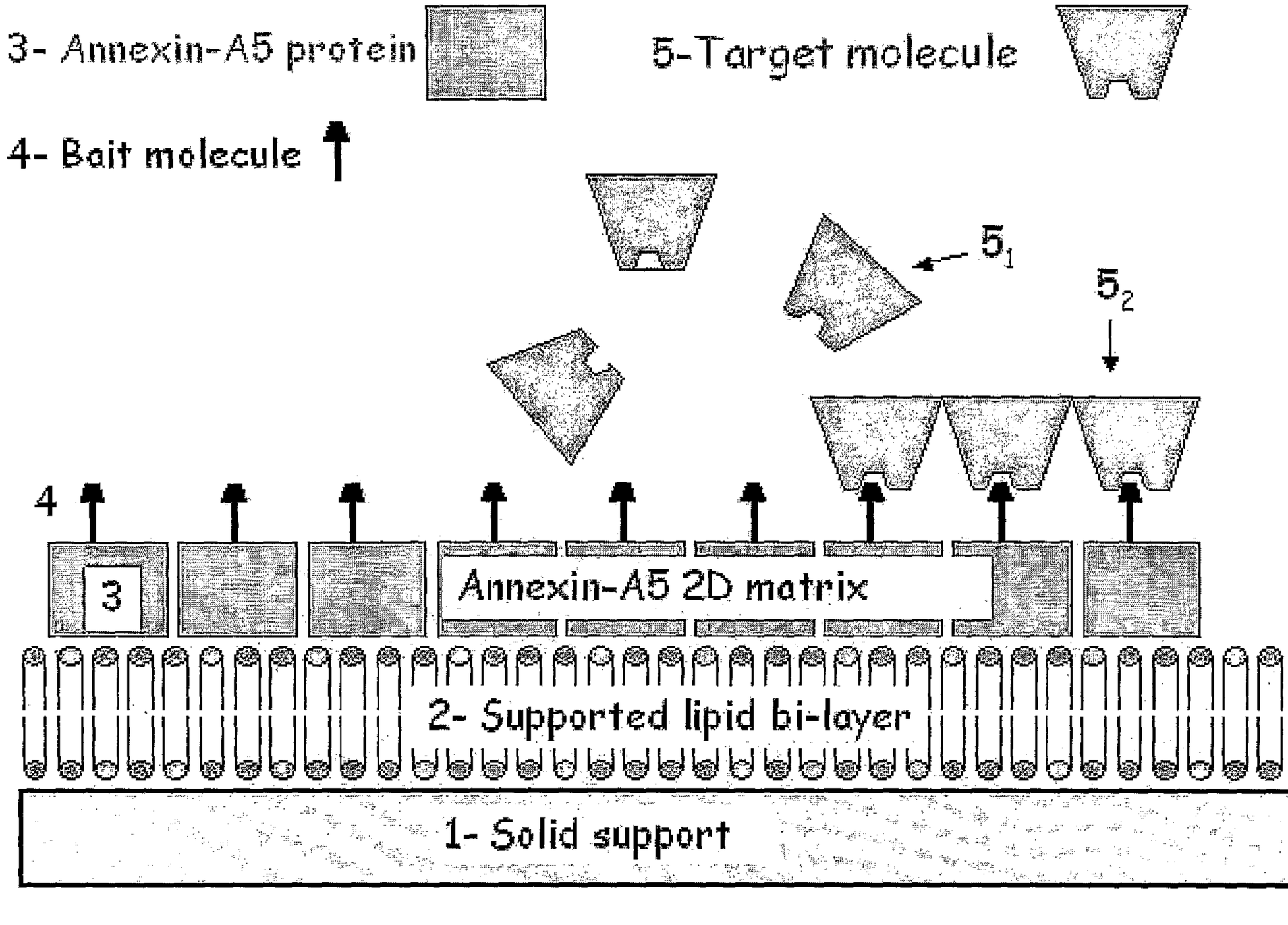

(57) Abrégé/Abstract:
The present invention pertains to a device for binding a target entity onto a bait entity that is immobilized on said device, comprising : a) a lipid layer having a negative net charge in an aqueous solution at a neutral pH; b) a two-dimensional matrix of anchoring

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(57) Abrégé(suite)/Abstract(continued):
complexes that are bound to said lipid layer, wherein each of said anchoring complexes comprises : (i) a fusion complex comprising an Annexin protein fused to a partner rmolecule; and (ii) a bait entity. It also concerns various uses of said device, including for detection and pharmaceutical purposes.

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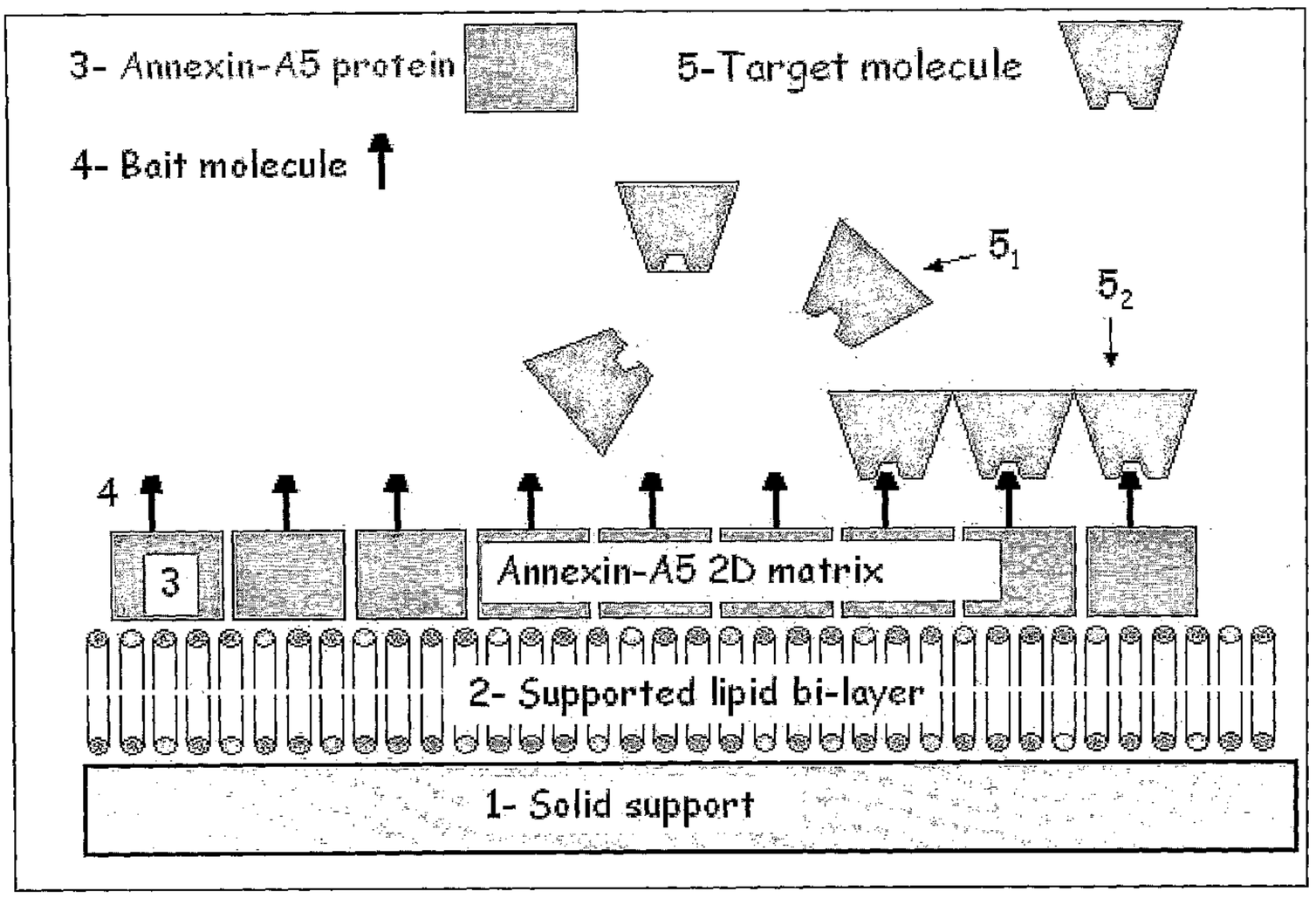

(57) Abstract: The present invention pertains to a device for binding a target entity onto a bait entity that is immobilized on said device, comprising : a) a lipid layer having a negative net charge in an aqueous solution at a neutral pH; b) a two-dimensional matrix of anchoring complexes that are bound to said lipid layer, wherein each of said anchoring complexes comprises : (i) a fusion complex comprising an Annexin protein fused to a partner rmolecule; and (ii) a bait entity. It also concerns various uses of said device, including for detection and pharmaceutical purposes.

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A DEVICE FOR BINDING A TARGET ENTITY TO A BAIT ENTITY AND DETECTION METHODS USING THE SAME

FIELD OF THE INVENTION

The present invention relates generally to methods and devices for binding a target entity to a bait entity, as well as to the corresponding detection methods.

The methods and the devices of the invention are used in the industrial field for various purposes, such as i) in biomedical analysis, proteomics, genomics, biosensor and microarray technologies for assaying for the presence of a target entity (e.g. antigens, antibodies, cell proteins, cell membranes, ligand molecules, peptides, drugs, nucleic acids, sugar residues, lipids etc.), or ii) in pharmaceutical analysis for screening for target entities of interest, such as pharmaceutical candidate target molecules of interest that bind to a cell receptor of therapeutical interest, or iii) in biomedical and therapeutical applications for delivering therapeutical molecules of interest to target receptors, target cells or target organs of interest, in patients in need thereof.

BACKGROUND OF THE INVENTION

There is a need in the art for novel devices and novel methods that may allow improved detection of the binding of target entities, notably target molecules, onto bait entities, notably bait molecules, of interest, particularly bait proteins of interest, such as bait antibodies and various other bait receptor proteins or bait peptides, notably those of biologically relevance, including those of diagnostic and pharmacological relevance.

Notably, there is a need in the art to easy, low cost and high throughput methods for screening target candidate drug molecules that bind to, or are active against, therapeutically relevant bait molecules, notably bait proteins.

Current assays for the presence of an analyte in a solution, such as those commonly used for diagnostics, for instance, involve the use of a bait receptor molecule, notably an antibody, which has been raised against the target molecule, notably an antigen. Multianalyte assays known in the art involve the use of multiple bait receptor molecules, notably antibodies, and are directed towards assaying for multiple target analytes.

Automation and/or miniaturisation of binding assays are required if large numbers of target molecules are to be assayed simultaneously. Materials, surface coatings, and detection methods used for biomolecule assays are thus highly needed in the art.

There is also a need in the art for novel methods that may allow delivering therapeutically useful ingredients, notably drugs and pharmaceutically active molecules,of interest, notably those of pharmacological and therapeutical relevance, to target entities, notably target cells and receptor molecules,of interest. Current assays for delivering a drug of interest to target receptors of interest or target cells of interest involve the use of vectors, principally viral vectors and non-viral vectors, notably synthetic liposomes and polymers.

Delivering the therapeutical molecules of interest to target cells and receptors is problematic and requires controlled immobilization of specific bait entities to the targeting vectors, so that the immobilized bait entities are enabled to bind to target entities of interest.

Also, a recurrent technical problem that is encountered while manufacturing miniaturised biomolecule assay devices, notably protein assay devices, relates to a suitable binding of the “bait-receptor” molecule, notably a "bait-receptor" protein, so as to ensure a maximum availability of said “bait-receptor” molecule to the corresponding target molecules(s) of interest.

Available methods for linking the bait receptor molecule to the assay substrate make use of either 1) direct physical adsorption of the

bait molecule or of molecules acting as receptors for the bait molecule to said substrate surface (e.g. Enzyme Linked ImmunoSorbent Assays, ELISA), or alternatively 2) chemical modification of the surface material of the substrate with linker molecules to which the bait molecule is bound, either covalently or non-covalently.

However, the above linking methods possess various technical drawbacks. Adsorbing the bait proteins directly onto the surface area of the substrate often causes an alteration of the structural conformation of said bait protein, which bait protein no more remains optimally available to its corresponding target molecules. In addition, direct adsorption of the bait protein or of molecules acting as receptors for the bait protein result in a random, uncontrolled, orientation of the bait protein, as well as in an uncontrolled surface density of the bait protein.

In addition, the use of covalent linkers between the surface area of the substrate and the bait receptor protein or molecules acting as receptors for the bait protein is complex and costly and results in an uncontrolled density of properly-oriented bait molecules for subsequent reactions. These drawbacks lead to a low ratio of correct binding of the bait proteins onto the selected substrate, in view of the initial amount of the bait protein material which is used.

There is thus a need in the art for improved biomolecule assay methods, notably protein assay methods, as well as corresponding devices.

Further, there is a need in the art for novel devices that are useful for the delivery of pharmaceutical molecules of interest to a target receptor, a target cell, a target tissue or a target organ in patients in need thereof.

SUMMARY OF THE INVENTION

A first object of the invention consists of a device for binding a target entity onto a bait entity that is immobilized on said device, said device comprising:

a) a lipid layer which comprises one or more lipids, said lipid layer having a negative net charge in an aqueous solution at a neutral pH;

b) a two-dimensional matrix of anchoring complexes that are bound to said lipid layer, wherein each of said anchoring complexes comprises :

(i) a fusion complex comprising an Annexin protein fused to a partner molecule, wherein :

- said Annexin protein is bound to said lipid layer, and
- said partner molecule consists of an organic or a mineral compound;

(ii) a bait molecule selected from the group consisting of :

- said partner molecule that is fused to said Annexin protein;;
- a molecule that is covalently or non-covalently bound to said partner molecule;
- a molecule that is indirectly bound to said partner molecule through one or more intermediate molecules that are covalently or non-covalently bound to said partner molecule.

In a device according to the present invention, said lipid layer may be selected from the group consisting of :

ai) a lipid bi-layer, including a lipid bi-layer coating a solid substrate;

aii) a lipid mono-layer, including a lipid mono-layer formed at the interface between air and an aqueous solution;

aiii) a liposome in an aqueous solution, including a liposome consisting of a vesicle with one or more lipid bi-layers enclosing an aqueous core.

By "fusion complex", it is intended herein a hybrid molecule that comprises, or consists of, an Annexin protein moiety that is covalently linked to a partner molecule, notably a protein, a peptide, or a nucleic acid. When said second molecule is a protein or a peptide, covalent linking with said Annexin moiety is performed either through a normal peptide bond via recombinant DNA technology methods or through a chemical bond, said chemical bond being either a normal peptide bond or any other chemical bond, via protein chemistry methods. Further, in said fusion complex, the Annexin protein may be either directly linked to said second molecule or may be separated from said second molecule by a spacer chain, notably an amino acid spacer chain having an amino acid length that may vary from 1 to 20 amino acid residues, most preferably hydrophilic amino acid residues.

In the device according to the invention, said two-dimensional matrix of anchoring complexes consists of a two-dimensional (2D) protein matrix that contains a fusion complex between an Annexin protein and a partner molecule, said partner molecule being preferably selected from the group consisting of a protein , a peptide, or a nucleic acid. Said 2D protein matrix results from the assembly of said Annexin moiety of said fusion complex on said lipid layer by specific and non-covalent binding. Said fusion complex is oriented and stably bound to said lipid layer.

In a device according to the invention, said bait molecule, is part of said anchoring complex, said anchoring complex consisting of :

i) either the fusion complex as defined above, wherein the bait molecule is the partner molecule that is fused to said Annexin moiety of said fusion complex,

ii) or a complex between the fusion complex defined above and said bait molecule, wherein said bait molecule is bound, covalently or non-covalently, to the partner molecule of said fusion complex,

iii) or a complex between the fusion complex defined above, the bait molecule and intermediate molecules, said intermediate molecules

binding both to said bait molecule and to the partner molecule of said fusion complex, when said bait molecule is linked to the partner molecule of said fusion complex via intermediate molecules.

When said lipid layer is:

ai) a lipid bi-layer coating a solid substrate;

aii) a lipid mono-layer formed at the interface between air and an aqueous solution,

another object of the invention consists of a system for detecting the binding of a target entity molecule onto a bait entity, wherein said system comprises a plurality of detection devices as defined above. This invention also relates to a method for detecting the binding of a target entity molecule onto a bait entity molecule, wherein said method comprises the steps of:

a) providing a sample to be tested;

b) bringing into contact the sample to be tested with a detection device or with a detection system as defined above; and

c) detecting the complexes eventually formed between (i) the bait entity (ies) contained in said detection device or in said detection system and (ii) the target entities eventually present within said tested sample.

When said lipid layer is:

ai) a lipid bi-layer coating a solid substrate;

aii) a lipid mono-layer formed at the interface between air and an aqueous solution,

this invention also pertains to a method for assaying for the presence of a target entity in a sample comprising the steps of :

a) providing an anchoring complex between an Annexin protein and a bait entity, which binds to said target entity

b) mixing the sample with said anchoring complex, whereby complexes between the bait moiety of said anchoring complex and the target molecule are allowed to be formed;

c) immobilizing the anchoring complexes obtained at step b), eventually under the form of complexes with said target entity, at the surface of a lipid layer, said lipid layer comprising a combination of (i) one or more lipids with (ii) one or more phospholipids, said one or more phospholipids having a negative net charge in an aqueous solution at a neutral pH;

d) detecting the complexes that are formed between the bait moiety of said anchoring complex and the target entity when said target entity is present in said sample.

When said lipid layer is a liposome in an aqueous solution, another object of the invention consists of a device for delivering entities notably drug and molecules, of pharmacological and therapeutical interest, to target entities, notably target cells and receptor molecules, of interest. Said device comprising:

a) liposomes comprising one or more lipid layers and an inner core containing one or more pharmaceutically active ingredients dissolved or suspended in an aqueous solution, wherein said lipid layer(s) comprise(s) one or more lipids and has a negative net charge in an aqueous solution at a neutral pH;

b) a two-dimensional matrix of anchoring complexes that are bound to the outer lipid layer, wherein each of said anchoring complexes comprises :

(i) a fusion complex comprising an Annexin protein fused to a partner molecule, wherein :

- said Annexin protein is bound to said lipid layer, and
- said partner molecule consists of an organic or a mineral compound;

(ii) a bait entity selected from the group consisting of :

- said partner molecule that is fused to said Annexin protein;;
- a molecule that is covalently or non-covalently bound to said partner molecule;
- a molecule that is indirectly bound to said partner molecule through one or more intermediate molecules that are covalently or non-covalently bound to said partner molecule.

Throughout the present description, it is disclosed the binding of a target entity, notably a target molecule, to a bait entity, notably a bait molecule, said bait entity being part of an anchoring complex containing an Annexin moiety, said anchoring complex being assembled as a 2D protein matrix stably bound, in an oriented manner, to a lipid layer surface via the specific interaction between the Annexin protein and negatively charged molecules, e.g. negatively charged phospholipids, contained in said lipid layer. The present invention is based on the intrinsic property of Annexin proteins, notably Annexin-A5, to form 2D protein matrices of high and well-defined density, including 2D crystalline assemblies (Mosser et al., 1991; Voges et al. 1994; Brisson et al., 1999; Oling et al., 2001; Reviakine et al., 1998), on lipid surfaces containing negatively charged phospholipids in the presence of calcium ions, said 2D protein matrices being stable, almost irreversibly bound, upon rinsing in calcium-containing buffer solutions (Govorukhina et al., 2002; Oling et al., 2001; Richter et al., submitted).

BRIEF DESCRIPTION OF THE FIGURES

FIGURE 1 illustrates a scheme of 2D assemblies of bait-anchoring complexes on lipid layers

In the specific embodiment shown in Figure 1, the anchoring complex consists of the bait molecule linked directly to an Annexin moiety, and said Annexin moiety consists of the Annexin-A5 protein.

Three types of lipid layers are represented, respectively :

- 1: a lipid bi-layer coating a solid substrate;
- 2: a lipid mono-layer at the air-water interface;
- 3: a liposome in aqueous solution

FIGURE 2 illustrates a scheme of a specific embodiment of an assay device, also herein termed detection device, according to the invention, wherein the lipid layer consists of a lipid bi-layer coating a solid substrate, and wherein the Annexin protein consists of the Annexin-A5 protein.

At the bottom of figure 1, there is represented the solid substrate (1) which is coated with a lipid bi-layer (2).

The Annexin-A5 moiety of the fusion complex (3) is bound to the external surface of the lipid bi-layer, forming a 2D self-assembled protein matrix. The bait molecule (4) is directly linked to said Annexin moiety of said fusion complex . There is also represented molecules of the target entity (5) which is tested, either as free target molecules in the liquid solvent (5_1), or as target molecules which are bound to bait molecules (5_2).

FIGURE 3 illustrates a scheme of various modes of binding of a target molecule to a bait molecule that is part of an anchoring complex containing an Annexin-A5 protein moiety bound to a lipid layer.

Mode 1. The fusion complex consists of Annexin-A5 fused to the bait molecule.

Mode 2. The anchoring complex comprises :

- a fusion complex consisting of an Annexin A5 protein fused to a partner molecule, and
- the bait molecule which is bound to the partner molecule of said fusion complex.

Mode 3. The anchoring complex comprises :

- a fusion complex consisting of an Annexin A5 protein fused to a partner molecule,

- an intermediate molecule (i) that is bound by one side to the partner molecule of said fusion complex, and (ii) that is bound by another side to the bait molecule; and
- the bait molecule that is bound to the intermediate molecule.

In Mode 2A and Mode 3A, an example of a preferred embodiment is shown, in which the fusion complex comprises the Annexin-A5 protein fused to the ZZ domain of protein A from *Staphylococcus aureus* (Loewenadler et al., 1987; Nilsson et al., 1987), by recombinant DNA technology :

- In Mode 2A, the ZZ domain of protein A binds an IgG molecule, said IgG molecule being the bait molecule;
- In Mode 3A, the ZZ domain of protein A binds an IgG molecule that is directed against the bait molecule. In this embodiment, the IgG molecule is an intermediate molecule between the fusion complex and the bait molecule.

FIGURE 4 illustrates a sheme of a specific embodiment of the detection of target molecules bound to bait molecules contained in a 2D matrix of anchoring complexes containing an Annexin-A5 moiety

I- Scheme representing the use of a labelled target-binding molecule to detect target molecules bound to bait molecules that are part of an 2D matrix of Annexin-A5-containing anchoring complexes on a lipid layer, said lipid layer being: i) either a lipid bi-layer coating a solid substrate; or ii) a lipid mono-layer at the air-water interface.Annexin.

II- In a preferred embodiment, the target-binding molecule consists of a complex between an Annexin-A5—ZZ fusion protein and an anti-target antibody, said complex being labelled, notably fluorescently labelled, preferably at the level of the Annexin-A5—ZZ fusion protein moiety.

FIGURE 5illustrates the two-dimensional projected structure of the 2-D self-assemblies formed by Annexin-A5 on phospholipid surfaces.

1) Trimer of Annexin-A5 formed upon binding to a lipid surface containing negatively charged phospholipids, in the presence of calcium ions. An Annexin-A5 monomer is coloured in red. The four Annexin domains are numbered I to IV. Trimers of Annexin-A5 are found in 2D crystals with p6 symmetry (Fig. 5-2,3A), in 2D crystals with p3 symmetry (not shown) and in high-density close-packed arrangements (Fig. 5-3B) (Mosser et al., 1991; Voges et al., 1994; Oling et al., 2001; Reviakine et al., 1998; Govorukhina et al., 2002; Richter and Brisson, 2003; Richter and Brisson, submitted).

2) On lipid mono-layers (here composed of the mixture DOPC:DOPS, 4:1, w:w) at the air-water interface, Annexin-A5 trimers form 2D crystals with p6 symmetry. The figure presents a 2D projection map of a p6 2D crystal of Annexin-A5, obtained by analysis of electron microscopy images (Oling et al., 2001). The blue circle surrounds six Annexin-A5 trimers located at the vertices of a hexagon, plus a central Annexin-A5 trimer.

3A) On a (DOPC:DOPS, 4:1, w:w) lipid bi-layer formed on a mica support, 2D crystalline assemblies with p6 symmetry are also observed by Atomic Force Microscopy (Reviakine et al., 1998).

3B) On a (DOPC:DOPS, 4:1, w:w) phospholipid bi-layer formed on a silica support, Annexin-A5 trimers form 2D high-density close-packed arrangements, as shown by Atomic Force Microscopy (Richter and Brisson, 2003).

Scale bars: 10 nm.

FIGURE 6- Model of a fusion complex between Annexin-A5 and a second protein, named Protein X, obtained by recombinant DNA technology methods

In the example shown here, the fusion complex is formed of Annexin-A5 fused at its C-terminal end to the N-terminal end of protein X, with a short linker sequence separating the 2 proteins (dashed segment).

FIGURE 7- Expression and purification of the fusion complex Annexin-A5—ZZ , by SDS-PAGE analysis

A- Lane 1: Cell Soluble Extract (2 µL). The Annexin-A5—ZZ fusion protein, indicated by the arrow, migrates at an apparent MW of 42 kDa (theoretical MW: 50,074 Da). Lane 2: molecular weight markers.

B- Purification of Annexin-A5—ZZ fusion protein by gel filtration with a Superdex 75 column (Amersham BioSciences). The Annexin-A5—ZZ fusion protein (arrow) elutes in a sharp peak.

C- Purification of Annexin-A5—ZZ fusion protein by Mono-Q anion exchange chromatography. Pure fractions of the Annexin-A5—ZZ fusion protein (arrow) elute at ~ 270 mM NaCl.

FIGURE 8- Model of a fusion complex between Annexin-A5 and Protein X, by chemical cross-linking.

In the example shown here, the fusion protein is formed by covalent cross-linking between a mutated form of Annexin-A5 (T163C; C314S), which presents a single sulfhydryl group located at amino-acid position 163, and protein X, said protein X being previously activated by the hetero-bi-functional cross-linker N-succinimidyl 3-(2-pyridyldithio)propionate (SPDP) (Pierce).

The double mutant (T163C;C314S) Annexin-A5 presents all known lipid-binding properties characteristic of Annexin-A5 and in consequence the double-mutant (T163C; C314S) Annexin-A5 is called hereafter Annexin-A5.

As it is known, the N-succinimidyl group reacts with primary amines at alkaline pH (Wong, 1991). Upon mixing Annexin-A5-SH with 2-pyridyl-disulfide-activated protein X, a disulfide-bonded complex consisting of [Annexin-A5-S-S-Protein X] is formed.

Due to the non-specific nature of the reaction between SPDP and any protein, various types of [Annexin-A5-S-S-Protein X] complexes can be formed.

Other hetero-bi-functional cross-linkers, reacting with both primary amines and sulfhydryl groups, may be used instead of SPDP, such as SMTP, SULFO-LC-SMTP, LC-SPDP, SMCC, SULFO-SMCC, MBS, SULFO-MBS, SMPB, SULFO-SMPB (Pierce Biotechnology, USA).

FIGURES 9A and **9B** illustrate the production and the purification of a chemically cross-linked fusion complex between Annexin-A5 and protein G from *Streptococcus sp.*

Figure 9A illustrates the production of fusion complexes between the double mutant Annexin A5 (T163C;C314S) and SPDP-modified protein G, by chemical cross-linking.

I : Annexin-$A5_B$; II : Protein G; III : ProteinG-SPDP; IV : Annexin-$A5_B$ + ProteinG-SPDP; V : Annexin-$A5_B$ + ProteinG-SPDP + β-mercapto-ethanol;

1 : Annexin-$A5_B$; 2 : Protein G; 3 : Annexin-A_5B dimer; 4 : Fusion complex Annexin-$A5_B$-ProteinG; 5 : Multimer Annexin-$A5_B$- Protein G.

It is to be noticed that Annexin-$A5_B$ means the double mutant of Annexin A5 (T163C;C314S).

Figure 9B illustrates the purification of the [Annexin-$A5_B$-Protein G] fusion complexes, by gel filtration.

I : Fractions from 1st peak; II : fractions from 2nd peak; III : fractions from 3rd peak; IV : fractions from 4th peak.

1 : Annexin-$A5_B$; 2 : Protein G; 3 : (Annexin-$A5_B$)$_2$ = disulfide bonded dimer; 4 : fusion complexes [Annexin-$A5_B$-Protein G]; 5 : Multimer [Annexin-$A5_B$-Protein G].

FIGURES 10,11,12,13,14 illustrate detection assays using a detection device according to the invention. The detection method used here is the quartz crystal microbalance with dissipation monitoring (QCM-D) (Rodhal et al., 1995).

Figure 10 illustrates the detection of the binding of IgG to a pre-formed 2D self-assembled matrix of Annexin A5-ZZ on [PC:PS (4:1)] SLB, by QCM-D.

Figure 11 illustrates the detection of the binding of IgG to a 2D self-assembled matrix of [Annexin A5/Annexin A-ZZ) on a [PC:PS (4:1)] SLB, by QCMD.

Figure 12 illustrates the detection of the binding of complexes of [Annexin A5-ZZ/IgG), preformed in solution, on a [PC:PS (4:1)] SLB, by QCM-D.

Figure 13 illustrates the detection of the binding of IgGs to a 2D matrix of [Annexin $A5_B$-Protein G] cross-linked complexes on a [PC:PS (4:1)] SLB, by QCM-D.

Figure 14 illustrates the use of an anchoring complex [Annexin-A5-S-S-Annexin A5] for anchoring liposomes.

DETAILED DESCRIPTION OF THE INVENTION

The inventors have now designed new devices comprising a bait molecule that efficiently bind specifically to a target molecule.

More precisely, the inventors have designed new devices wherein target molecules specifically bind to bait molecules that are immobilized on said devices and wherein said bait molecules are comprised in anchoring complexes that are stably bound to a lipid layer.

Thus, the new devices of the invention may be used in various methods wherein the specific binding of a bait molecule to a target molecule is sought.

In certain embodiments, the new devices of the invention are used in methods wherein the detection of a specific binding event between a bait molecule and a target molecule is sought. Such embodiments include the use of a device according to the invention in methods for the screening of candidate target molecules that specifically bind to the bait molecules included in said devices.

In certain other embodiments, the new devices of the invention are used in methods wherein the specific binding of a bait molecule included in said device to a target molecule that is expressed at the cell membrane of a target cell is sought. Such embodiments include the use of a device according to the invention for performing the binding of said device on the cell membrane of a target cell in view of delivering one or more therapeutically useful molecules to said target cell.

An object of the invention consists of a device for binding a target entity onto a bait entity that is immobilized on said device, comprising:

a) a lipid layer which comprises one or more lipids , said lipid layer having a negative net charge in an aqueous solution at a neutral pH;

b) a two-dimensional matrix of anchoring complexes that are bound to said lipid layer, wherein each of said anchoring complexes comprises :

(i) a fusion complex comprising an Annexin protein fused to a partner molecule, wherein :

- said Annexin protein is bound to said phopholipid layer, and
- said partner molecule consists of an organic or a mineral compound;

(ii) a bait entity selected from the group consisting of :

- said partner molecule that is fused to said Annexin protein;;
- a molecule that is covalently or non-covalently bound to said partner molecule;
- a molecule that is indirectly bound to said partner molecule through one or more intermediate molecules that are covalently or non-covalently bound to said partner molecule.

Thus, the inventors have now found that it can be performed improved binding and detection methods of target entities, notably target molecules, which make use of a device consisting of:

a) a lipid layer which comprises one or more lipids, said lipid layer having a negative net charge in an aqueous saline solution at a neutral pH;

said lipid layer, being:

ai) a lipid bi-layer, e.g. a lipid bi-layer coating a solid substrate;

aii) a lipid mono-layer, e;g. a lipid mono-layer formed at the interface between air and an aqueous solution;

aiii) a liposome in an aqueous solution, e.g. a liposome consisting of a vesicle with one or more lipid bi-layers enclosing an aqueous core;

b) a two-dimensional (2D) protein matrix that contains a fusion complex between an Annexin protein and a partner molecule, notably a protein, a peptide, or an nucleic acid.

Said 2D protein matrix resulting from the assembly of said Annexin moiety of said fusion complex on said lipid layer by specific and non-covalent binding.

Said fusion complex being oriented and stably bound to said lipid layer.

c) a bait molecule, is part of said anchoring complex, said anchoring complex consisting of :

i) either the fusion complex as defined above, wherein the bait molecule is the partner molecule that is fused to said Annexin moiety of said fusion complex,

ii) or a complex between the fusion complex defined above and said bait molecule, wherein said bait molecule is bound, covalently or non-covalently, to the partner molecule of said fusion complex,

iii) or a complex between the fusion complex defined above, the bait molecule and intermediate molecules that bind both to said bait molecule and to the partner molecule of said fusion complex, when said bait molecule is linked to the partner molecule of said fusion complex via intermediate molecules.

In certain embodiments, said anchoring complexes are non-covalently bound to said lipid layer.

In certain other embodiments, said anchoring complexes may be covalently bound to said lipid layer. Illustratively, covalent bounds may be generated between said anchoring complexes and lipid molecules comprised in said lipid layer subsequently to the formation of said two-dimensional matrix of anchoring complexes on said lipid layer. The one skilled in the art may use well-known techniques for generating covalent bonds between a protein and a lipid molecule. Illustratively, covalent bonds may be generated between alpha- or epsilon- groups of one or more lysine residues contained in the anchoring complexes, more specifically of the Annexin moiety of the anchoring complexes, and carboxyl groups comprised in the lipid molecules of said lipid layer.

The term "entity" is intended herein to encompass small molecules, biological molecules as well as supra-molecular assemblies, including notably cells, membrane vesicles and fragments derived from cells, natural or artificial liposomes, and inorganic particles.

According to the invention, generally, a bait entity consists of a bait protein, a peptide or an nucleic acid.

According to the invention, said Annexin protein comprised in said fusion complex preferably consists of an Annexin-A5 protein or a modified form of Annexin-A5.

The inventors have found that, using a fusion complex between (i) an Annexin protein and (ii) a partner molecule, the Annexin fusion complex presents the known properties of the Annexin-A5 protein *per se* to self-assemble into a stable, high-density 2D matrix, on a lipid layer.

Generally, in a device according to the invention, said partner molecule consists of a partner protein. Throughout the present specification, said partner protein may also be termed "second protein".

More specifically, the inventors have found that, using a fusion complex between (i) an Annexin-A5 protein and (ii) a second protein, the

Annexin fusion complex presents the known properties of the Annexin-A5 protein *per se* to self-assemble into a stable, high-density 2D matrix, on alipid layer.

In said fusion complexes, said partner molecule generally consists of an organic compound, and advantageously a polypeptide, i.e. a peptide or a protein.

In other embodiments of a fusion complex, said partner molecule may consist of a mineral molecule.

In certain embodiments, said lipid layer consists of a lipid bi-layer. These embodiments encompass a lipid bi-layer coating a solid substrate.

In certain other embodiments, said lipid layer consists of a lipid mono-layer. These embodiments encompass a lipid monolayer formed at the interface between air and an aqueous solution.

In still certain other embodiments, said lipid layer consists of the external lipid layer of a liposome in an aqueous solution. These embodiments encompass a lipid layer consisting of a liposome, said liposome consisting of a vesicle comprising one or more lipid bi-layers enclosing an aqueous core.

Further, the inventors have found that the fusion complexes made of either the Annexin-A5 protein fused to the ZZ domain of protein A from *Staphylococcus aureus* (Loewenadler et al., 1987; Nilsson et al., 1987) by recombinant DNA technology or the Annexin-A5 protein fused to a peptide containing the Arg-Gly-Asp (RGD) cell-adhesion sequence (Ruoslahti and Pierschbacher (1987), form 2D crystals on lipid mono-layers at the air-water interface and on solid-supported lipid bi-layers, said 2D crystals exhibiting p6 symmetry and geometrical characteristics similar to the 2D crystals formed by Annexin-A5 and described in Oling et al. (2001), Reviakine et al. (1998). In consequence, the self-assembly properties of Annexin-A5 which are responsible for the formation of trimers and of 2D crystals of trimers are conserved in the Annexin-A5—ZZ fusion complex.

Still further, the inventors have shown that the surface density of fusion complexes on the lipid layer can be adjusted by using mixtures of (i) fusion complexes and (ii) Annexin- molecules in a defined ratio of fusion complex molecules to Annexin molecules. Notably, the surface density of fusion complex molecules on the lipid layer may be adjusted by using mixtures of (i) fusion complex molecules and (ii) Annexin A5 molecules in a defined ratio of fusion complex molecules to Annexin A5 molecules.

The molecular ratio of fusion complex molecules to Annexin-A5 molecules in a lipid-bound 2D assembly of anchoring complexes is the same as the molecular ratio between these molecules in the aqueous mixture that serves as the starting material that is added to a pre-formed lipid layer. Consequently, the surface density of fusion complexes on said lipid layer is predetermined and controllable. The maximal surface density of Annexin-A5 is ~3,3 x 10^{12} molecules /cm^2 or ~ 200 ng/ cm^2.

In a device according to the invention, the molecular ratio of (i) fusion complexes to (ii) non-fused Annexin molecules in the lipid-bound 2D assemblies of anchoring complexes can range from 1:0 to 0:1. However, preferably, said ratio ranges from 0.5:0.5 to 0.01:0.99, more preferably from 0.4:0.6 to 0.1: 0.99 and most preferably from 0.3:0.7 to 0.1:0.9

Still further, the inventors have shown that the property of an Annexin protein, including an Annexin-A5 protein, to bind to lipid layers in the presence of calcium ions in a quasi-irreversible manner upon extensive rinsing with calcium-containing solutions is preserved for both fusion complexes and anchoring complexes.

Still further, the inventors have found that when an anchoring complex is included in said 2D protein matrix bound on the lipid layer, the bait molecule of said anchoring complex exhibits a high exposure to the solvent phase, and thus to any molecule contained in the solvent phase, that has the property to bind to said bait molecule.

Still further, the inventors have found that the 2D matrices of anchoring complexes consisting of Annexin-A5 linked to an RGD-containing peptide on a lipid bi-layer facilitate cell adhesion.

Still further, the inventors have found that the 2D matrices of anchoring complexes comprising disulfide-linked Annexin-A5 (T163C;C314S) dimers on lipid layers are able to anchor liposomes containing negatively charged phospholipids in the presence of calcium ions.

Thus, according to the invention, it has been manufactured a novel binding and detection device comprising a bait protein, said detection device taking benefit from the technical advantages of the Annexin-A5-based anchoring complex forming a 2D matrix on a lipid layer that are described above.

More precisely, the invention discloses a device for detecting the binding of a target molecule onto a bait molecule, wherein said bait molecule is immobilized on said device as part of an anchoring complex which comprises said fusion complex between an Annexin-A5 protein and a partner molecule, said fusion complex being bound on the lipid layer, said lipid layer consisting either i) of a lipid bi-layer coating a solid substrate; or ii) of a lipid mono-layer formed at the air-water interface.

As already mentioned, the inventors have found that there was a high availability of the partner molecule of the fusion complex as well as of the bait molecule of the anchoring complex to their corresponding ligand(s), due to the oriented binding of the fusion complex which is used, because the Annexin moiety is bound to the lipid layer through its convex side surface, whereas the partner molecule and the bait molecule are exposed to the solvent on the side of the Annexin moiety which is opposite to the side in contact with the lipid layer.

Further, a high surface density of the fusion complexes is allowed by the 2D self-assembly properties of the Annexin protein, when said protein is bound to the lipid layer.

Another object of the invention consists of a system for detecting the binding of a target entity molecule onto a bait entity, wherein said system comprises a plurality of detection devices as defined above.

Most preferably, according to the invention, the Annexin protein consists of an Annexin-A5 protein or a modified form of Annexin-A5.

In a first preferred embodiment, the lipid layer is a lipid bi-layer coating a solid substrate, said solid substrate of the invention device may be of various types, provided it suitably allows the formation of a homogeneous lipid bi-layer coating. In said first embodiment of a device according to the invention, said solid substrate is part of the device.

Preferably, said solid substrate is selected from the group of substrates consisting of mica, silica, silicon, mineral glass and gold.

As a mica substrate, muscovite mica is preferred, such as that marketed by Company JBG-Metafix (Montdidier, France) under the reference) muscovite rubis mica, quality Clear-Scratch-Less CLSS

As a silica substrate, silica-coated quartz crystals such as those marketed by Company Q-Sense (Gothenburg, Sweden) are preferred.

As a silicon substrate, a silicon wafer such as that marketed by the CEA (Grenoble, France)is preferred.

As mineral glass, microscope cover glass such as that marketed by the Company Fisher scientific (Pittsburgh, PA, USA) is preferred.

A "lipid layer" according to the invention consists of a layer comprising lipid molecules and wherein said layer has a negative net charge in an aqueous solution at a neutral pH. Thus, said lipid layer comprises one or more kinds of compounds that impart to said lipid layer a negative net charge in an aqueous solution at a neutral pH. Compound(s) that impart a negative net charge in an aqueous solution at a neutral pH may consist of any compound having a negative net charge in an aqueous solution at a neutral pH. In certain embodiments of the lipid layer, the compounds that impart a negative net charge in an aqueous solution at a neutral pH consist of phospholipids having a

negative net charge in an aqueous solution at a neutral pH. In certain embodiments, the compounds that impart a negative net charge in an aqueous solution at a neutral pH consist of polymers having a negative net charge in an aqueous solution at a neutral pH, such as polyphosphate-containing molecules, polysulfate-containing molecules, like heparin molecules or any polysulfate-containing derivatative of heparin.

By a "lipid mono-layer" according to the invention, it is intended a single lipid layer as defined above. In certain embodiments, said lipid monolayer is located at the interface between a liquid medium and air, said liquid medium preferably consisting of an aqueous or an hydrophilic liquid medium, such as water or any other aqueous solution included a saline water solution or any buffer aqueous solution.

By a "lipid bi-layer" according to the invention, it is intended a bi-layer comprising two lipid layers as defined above, said two lipid layers being associated, one with the other, through non-covalent interaction.

In certain embodiments, said lipid bi-layer comprises two lipid layers that consist respectively of:

(i) a first molecular layer consisting of a lipid layer comprising a combination of (i) one or more amphiphilic molecules, most preferably one or more lipids, with (ii) one or more phospholipids, said one or more phospholipids having a negative net charge in an aqueous solution at a neutral pH, and wherein said lipid layer is bound, non-covalently, on one side to the solid substrate of the invention's device and on the opposite side to the second molecular layer of the bi-layer; and

(ii) a second molecular layer consisting of a lipid layer comprising a combination of (i) one or more lipids with (ii) one or more phospholipids, said one or more phospholipids having a negative net charge in an aqueous solution at a neutral pH, and wherein said lipid layer is non-covalently bound on one side to the first molecular layer

and wherein the opposite side of said lipid layer is exposed to the solvent and is bound by affinity to the Annexin moiety of the fusion complexes.

In a first preferred embodiment of a lipid layer, said lipid layer comprises, or even consists of, a lipid layer comprising a combination of (i) one or more lipids with (ii) one or more phospholipids, said one or more phospholipids having a negative net charge in an aqueous solution at a neutral pH. Preferred phospholipids having a negative net charge in an aqueous solution at a neutral pH are 1,2-dioleoyl-sn-glycero-3-phosphoserine (DOPS), 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoserine (POPS), 1,2-dioleoyl-sn-glycero-3-phosphatidic acid (DOPA), 1,2-dioleoyl-sn-glycero-3-phosphoglycerol (DOPG), cardiolipins.

In this first preferred embodiment of a lipid layer, said lipid layer comprises, or even consists of amphiphilic molecules with C_{14} or longer alkyl or alkenyl chains, such as C_{16}, C_{18}, C_{20}. Preferred amphiphilic molecules are lipid molecules such as 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC), 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC), 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC), egg lecithin, 1,2-dioleoyl-sn-glycero-3-phosphoserine (DOPS), 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoserine (POPS), 1,2-dioleoyl-sn-glycero-3-phosphatidic acid (DOPA), 1,2-dioleoyl-sn-glycero-3-phosphoglycerol (DOPG), cardiolipins, N-[1-(2,3-Dioleoxyloxy]propyl]-N,N,N-trimethylammonium methyl sulfate (DOTAP).

When a lipid bi-layer comprising two molecular layers as defined previously is used, then said first molecular layer is oriented with the hydrophilic part of the lipids interacting with the solid substrate and the extremity of the hydrophobic chains of the lipids being associated with the extremity of the hydrophobic chains of the second molecular layer. Said first embodiment of the lipid bi-layer is particularly suitable when the

solid substrate which is used consists of mica, silica, silicon or mineral glass.

By a "phospholipid having a negative net charge", it is intended natural or synthetic phospholipids, such as 1,2-dioleoyl-sn-glycero-3-phosphoserine (DOPS), 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoserine (POPS), 1,2-dioleoyl-sn-glycero-3-phosphatidic acid (DOPA), 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphatidic acid (POPA), 1,2-dioleoyl-sn-glycero-3-phosphoglycerol (DOPG), 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoglycerol (POPG), cardiolipins or a mixture of them such as brain lipid extracts.

Alternatively, negatively charged lipids like sulfatides may be used instead of phospholipids having a negative net charge.

The one or more phospholipids having a negative net charge in an aqueous solution at a neutral pH impart to the lipid layer the property of binding the Annexin moiety of the fusion complex. Notably, Annexin, particularly Annexin-A5, binds with a high affinity to such lipids that contain a phosphoserine group in the presence of calcium ions.

Most preferably, the phospholipids having a negative net charge in an aqueous solution at a neutral pH are selected from the group consisting of 1,2-di-myristoyl-sn-glycero-3-phosphoserine (DMPS), 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoserine (POPS), 1,2-dioleoyl-sn-glycero-3-phosphoserine (DOPS), 1,2-dioleoyl-sn-glycero-3-phosphoglycerol (DOPG) and cardiolipin. Most preferably, DOPS is used.

In the lipid layer, the content of said one or more phospholipids having a negative net charge in an aqueous solution at a neutral pH varies from 2% to 100% by weight, advantageously from 20% to 40% by weight, based on the total weight of said lipid layer.

The one skilled in the art may adapt the content of the lipid layer in said negatively charged phospholipids, depending on which solid substrate is used. For instance, the content in said negatively charged phospholipids varies preferably from 20% to 100% by weight, based on

the total weight of the lipid layer, when mica is used as the solid substrate. In another illustrative example, the content in said negatively charged lipids varies preferably from 20% to 30% by weight, based on the total weight of the lipid layer, when silica is used as the solid substrate.

When the content in negatively charged phospholipids within the lipid layer is smaller than the lower limit range above, a 2D matrix of the fusion complexes of lower density is obtained, which may prohibit the manufacture of a detection device allowing a high detection sensitivity.

The other lipids that are comprised in the lipid layer may be phospholipids of various suitable types. These other phospholipids are preferably selected from the group consisting of lecithins including 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC), 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC), 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC), egg lecithin.

In a most preferred embodiment, particularly when mica, silica, silicon or mineral glass is used as the solid substrate, the lipid layer consists of a mixture of 1,2-dioleoyl-sn-glycero-3-phosphoserine (DOPS) and 1,2-dioleoylsn-glycero-3-phosphocholine (DOPC).

According to said first preferred embodiment, a lipid bi-layer is formed by depositing lipid vesicles formed with lipids described above over a solid substrate, said lipid vesicles being prepared notably by sonication, as described under Example 5-1. Generally, the one skilled in the art will find suitable technical protocols for forming the lipid bi-layer from pre-formed phospholipid vesicles in the articles of Keller and Kasemo (1998); Richter et al. (2003), Richter and Brisson (2003) and Richter and Brisson (2005).

As already specified above, in certain embodiments of a device according to the invention, said lipid layer consists of a layer of phospholipid vesicles either adsorbed on the solid substrate, or bound to

a 2D matrix of fusion complex made of a disulfide-linked complex of Annexin-A5 (T163C; C314S) bound to a phospholipid bi-layer.

In an alternative embodiment, the lipid bi-layer is replaced by negatively-charged polymers, polyphosphate-containing molecules, poly-sulfate-containing molecules, such as heparin molecules.

In yet another alternative embodiment, the lipid bi-layer is separated from the solid substrate by a cushion or a layer of polymer molecules (Sackmann and Tanaka, 2000).

As previously specified, certain embodiments of a lipid layer consist of a lipid mono-layer at the interface between air and an aqueous solution,

In a specific embodiment of lipid mono-layer according to the invention, said lipid mono-layer consists of a mono-molecular layer consisting of a lipid layer comprising a combination of (i) one or more amphiphilic molecules, most preferably one or more lipids, with (ii) one or more phospholipids, said one or more phospholipids having a negative net charge in an aqueous solution at a neutral pH, and wherein said lipid mono-layer is formed at the interface between air and an aqueous solution and is exposed to the solvent and is bound to the Annexin moiety of the fusion complexes.

In a first preferred embodiment of the lipid mono-layer, said mono-layer comprises, or even consists of a combination of (i) one or more lipids with (ii) one or more phospholipids, said one or more phospholipids having a negative net charge in an aqueous solution at a neutral pH. Preferred phospholipids having a negative net charge in an aqueous solution at a neutral pH are 1,2-dioleoyl-sn-glycero-3-phosphoserine (DOPS), 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoserine (POPS), 1,2-dioleoyl-sn-glycero-3-phosphatidic acid (DOPA), 1,2-dioleoyl-sn-glycero-3-phosphoglycerol (DOPG), cardiolipins.

In this first preferred embodiment of the lipid mono-layer, said mono-layer comprises, or even consists of amphiphilic molecules with

C_{14} or longer alkyl or alkenyl chains, such as C_{16}, C_{18}, C_{20}. Preferred amphiphilic molecules are lipid molecules such as 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC), 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC), 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC), egg lecithin, 1,2-dioleoyl-sn-glycero-3-phosphoserine (DOPS), 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoserine (POPS), 1,2-dioleoyl-sn-glycero-3-phosphatidic acid (DOPA), 1,2-dioleoyl-sn-glycero-3-phosphoglycerol (DOPG), cardiolipins, N-[1-(2,3-Dioleoxyloxy]propyl]-N,N,N-trimethylammonium methyl sulfate (DOTAP).

According to said first preferred embodiment, said lipid mono-layer is oriented with the hydrophilic part of the lipids exposed to the aqueous solution with the hydrophobic chains of the lipids being exposed to air.

By a “phospholipid having a negative net charge”, it is intended natural or synthetic phospholipids, such as 1,2-dioleoyl-sn-glycero-3-phosphoserine (DOPS), 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoserine (POPS), 1,2-dioleoyl-sn-glycero-3-phosphatidic acid (DOPA), 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphatidic acid (POPA), 1,2-dioleoyl-sn-glycero-3-phosphoglycerol (DOPG), 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoglycerol (POPG), cardiolipins or a mixture of them such as brain lipid extracts.

Alternatively, negatively charged lipids like sulfatides may be used instead of phospholipids having a negative net charge.

The one or more phospholipids having a negative net charge in an aqueous solution at a neutral pH impart to the lipid mono-layer the property of binding the Annexin moiety of the fusion complex. Notably, Annexin, particularly Annexin-A5, binds with a high affinity to such lipids that contain a phosphoserine group in the presence of calcium ions.

Most preferably, the phospholipids having a negative net charge in an aqueous solution at a neutral pH are selected from the group consisting of 1,2-di-myristoyl-sn-glycero-3-phosphoserine (DMPS), 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoserine (POPS), 1,2-dioleoyl-sn-

glycero-3-phosphoserine (DOPS), 1,2-dioleoyl-sn-glycero-3-phosphoglycerol (DOPG) and cardiolipin. Most preferably, DOPS is used.

In the lipid mono-layer, the content of said one or more phospholipids having a negative net charge in an aqueous solution at a neutral pH varies from 2% to 100% by weight, advantageously from 20% to 40% by weight, based on the total weight of said lipid mono-layer.

The one skilled in the art may adapt the content of the lipid mono-layer in said negatively charged phospholipids. For instance, the content in said negatively charged phospholipids varies preferably from 20% to 100% by weight, based on the total weight of the lipid mono-layer.

When the content in negatively charged phospholipids within the lipid mono-layer is smaller than the lower limit range above, a 2D matrix of the fusion complexes of lower density is obtained, which may prohibit the manufacture of a detection device allowing a high detection sensitivity.

The other lipids that are comprised in the lipid mono-layer may be phospholipids of various suitable types. These other phospholipids are preferably selected from the group consisting of lecithins including 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC), 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC), 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC), egg lecithin.

In a most preferred embodiment, the lipid mono-layer consists of a mixture of 1,2-dioleoyl-sn-glycero-3-phosphoserine (DOPS) and 1,2-dioleoylsn-glycero-3-phosphocholine (DOPC).

According to the invention, the main advantageous properties of the Annexin moiety of the fusion complex are the capacity of forming high-density 2D protein matrices on phospholipid surfaces and the fact that said 2D protein matrices are stably bound to said phospholipid surfaces. In addition, the property of self-assembling into trimers and 2D crystalline assemblies based on trimers, as exhibited by Annexin-A5 and several other Annexins, including Annexin-A4 and Annexin-A12, provides

additional advantages, like providing knowledge and control of the surface density of anchoring complexes. Annexin-A5 is preferred.

As it is widely known in the art, Annexin-A5 comprises four Annexin repetitive homology domains (Huber et al., 1990; Concha et al., 1993). It is also known that Annexins bind with high affinity to lipid surfaces containing negatively charged phospholipids in the presence of calcium ions (Tait et al., 1989; Blackwood and Ernst, 1990; Pigault et al., 1994; Meers, 1996). Annexin-A5 self-assembles spontaneously into Annexin-A5 trimers and into 2D crystalline arrangements, made of Annexin-A5 trimers, at the level of lipid mono-layers at the air-water interface (Mosser et al., 1991; Voges et al. 1994; Brisson et al., 1999), as well as at the level of solid-supported phospholipid bi-layers formed on mica (Reviakine et al., 1998; Richter and Brisson, 2005) (Figure 5-2, 5-3A). It is also known that Annexin-A5 forms close-packed 2D self-assemblies of trimers on solid-supported phospholipid bi-layers formed on silica-coated silicon substrates (Richter and Brisson, 2003; Richter et al., 2005) (Figure 5-3B).

By an “Annexin” protein it is intended herein a protein selected from the group consisting of Annexin-A1, Annexin-A2, Annexin-A3, Annexin-A4, Annexin-A5, Annexin-A6, Annexin-A7, Annexin-A8, Annexin-A9, Annexin-A12, Annexin-A, Annexin-B, Annexin-C and Annexin-D, as well as anyone of their “functionally active “protein derivatives.

By an “Annexin-A5” protein, it is intended herein a protein of the Annexin-A5 family, including Annexin-A5 from a species selected from the group consisting of *Rattus, Homo sapiens, Mus, Gallus* and *Bos*, as well as any one of their "functionally active" protein derivatives.

A “functionally active“ derivative of an Annexin protein according to the invention encompasses any protein which is derived from any one the naturally occurring Annexin proteins, and which allows the formation of high-density 2D close packed assemblies of Annexin moieties which

are stably bound to a phospholipid layer, especielly to a phospholipid bi-layer.

The functionally active derivative of an Annexin protein also encompasses proteins having one or more amino-acid residue differences with regard to the aminoacid sequence of the naturally occurring corresponding Annexin which is taken as the reference protein. A functionally active derivative of an Annexin protein also encompasses proteins that comprise one or more addition(s), substitution(s) or deletion(s) of one amino acid residue, compared to the reference Annexin-A5 protein, and wherein the modification(s) does not alter the formation of a 2D close packed arrangement of Annexin moieties which are stably bound to a phospholipid bi-layer.

A specific embodiment of a functionally active derivative of an Annexin-A5 protein is illustrated in the examples, said functionally active derivative being the mutated [T163C; C314S] Annexin-A5 protein, that derives from the naturally occurring Annexin-A5 from *Rattus norvegicus*.

The least homologous Annexin-A5 proteins among the group of those from *Rattus, Homo sapiens, Mus, Gallus* and *Bos* share 77% amino acid identity.

Preferably, an Annexin protein moiety which is part of one fusion complex according to the invention has at least 50% aminoacid identity as regards the corresponding naturally occurring Annexin protein. More preferably, said Annexin protein moiety has at least 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% aminoacid identity as regards the corresponding naturally occurring Annexin protein. As an illustrative example, generally, an Annexin functionally active derivative wherein the sole modifications consist of the deletion of the N-terminal sequence preceding the first repetitive region and of the C-terminal sequence following the fourth repetitive region has about 90% amino acid

sequence identity as regards to the corresponding naturally occurring Annexin protein.

To determine the percentage of identity between two amino acid sequences, the sequence are aligned for optimal comparison purposes. For example, gaps can be introduced in one or both of a first and a second amino acid sequence for optimal alignment and non-homologous sequences can be disregarded for comparison purposes.

For optimal comparison purposes, the percent of identity of two amino acid sequences can be determined with CLUSTAL W (version 1.82) with the following parameters : (1) CPU MODE = ClustalW mp ; (2) ALIGNMENT = « full » ; (3) OUTPUT FORMAT = « aln w/numbers » ; (4) OUTPUT ORDER = « aligned » ; (5) COLOR ALIGNMENT = « no » ; (6) KTUP (word size) = « default » ; (7) WINDOW LENGTH = « default » ; (8) SCORE TYPE = « percent » ; (9) TOPDIAG = « default » ; (10) PAIRGAP = « default » ; (11) PHYLOGENETIC TREE/TREE TYPE = « none » ; (12) MATRIX = « default » ; (13) GAP OPEN = « default » ; (14) END GAPS = « default » ; (15) GAP EXTENSION = « default » ; (16) GAP DISTANCES = « default » ; (17) TREE TYPE = « cladogram » et (18) TREE GRAP DISTANCES = « hide ».

Particularly preferred Annexin-A5 moieties that may be comprised in a fusion complex of the invention consist of the Annexin-A5 proteins selected from the group consisting of the aminoacid sequences of SEQ ID N°1 to SEQ ID N°5 as well as their functionally active derivatives.

For illustratively defining the family of proteins which is encompassed by the functionally active derivatives of the Annexin-A5 proteins of SEQ ID N°1 to SEQ ID N° 5, it is to be noted the following characteristics of the sequences below.

For the rat Annexin-A5 protein of SEQ ID N°1, the four repetitive regions are located, respectively, in the following defined regions: (i) 20 – 80, (ii) 92-152, (iii) 176-236 and (iv) 251-311.

For mouse the Annexin-A5 protein of SEQ ID N°2, the four repetitive regions are located, respectively, in the following defined regions (i) 21-81, (ii) 93-153, (iii) 177-237 and (iv) 252-312.

For the human Annexin-A5 protein of SEQ ID N°3, the four repetitive regions are located, respectively, in the following defined regions (i) 22-82, (ii) 94-154, (iii) 178-238 and (iv) 253-313.

For the bovine Annexin-A5 protein of SEQ ID N°4, the four repetitive regions are located, respectively, in the following defined regions (i) 22-82, (ii) 94-154, (iii) 178-238 and (iv) 253-313.

For the chiken Annexin-A5 protein of SEQ ID N°5, the four repetitive regions are located, respectively, in the following defined regions (i)23-83, (ii) 95-155, (iii) 179-239 and (iv) 254-314.

An illustrative embodiment of an Annexin-A5 moiety as defined above consists of the rat-derived mutated [T163C; C314S] Annexin-A5 protein which is comprised in one fusion complex according to the invention, namely the fusion complex of SEQ ID N°6 herein, consisting of rat Annexin-A5 fused to the ZZ domain of protein A from *Staphylococcus aureus*).

As already mentioned in the present specification, an Annexin protein which is included in a fusion complex of the invention encompasses other Annexins than Annexin-A5 and may be selected among the group consisting of Annexin-1 (SEQ ID N°7), Annexin-2 (SEQ ID N°8), Annexin-3 (SEQ ID N°16), Annexin-4 (SEQ ID N°10), Annexin-6 (SEQ ID N°9), Annexin-7 (SEQ ID N°12), Annexin-8 (SEQ ID N°11), Annexin-9 (SEQ ID N°18), Annexin-A (SEQ ID N°17), Annexin-B (SEQ ID N°15), Annexin C (SEQ ID N°13), Annexin-D (SEQ ID N°14) as well as any one of their functionally active derivatives.

By “fusion complex”, it is intended herein a hybrid molecule that comprises, or consists of, an Annexin protein moiety that is covalently linked to a second moiety, that is herein also termed “partner molecule”, notably a protein, a peptide, or an oligonucleotide, When said partner

molecule is a protein or a peptide, covalent linking with said Annexin moiety is effected either through a normal peptide bond via recombinant DNA technology methods or through a chemical bond, said chemical bond being either a normal peptide bond or any other chemical bond, via protein chemistry methods, as illustrated in Figures 6 and 7. Further, in said fusion complex, the Annexin protein may be either directly linked to said second molecule or may be separated from said second molecule by a spacer chain, notably an amino acid spacer chain having an amino acid length that may vary from 1 to 20 amino acid residues, most preferably hydrophilic amino acid residues.

In a fusion protein formed by recombinant DNA technology methods as defined in the present specification, the Annexin moiety is located either at the N-terminal end or in contrast at the C-terminal end of its amino acid sequence.

In one preferred embodiment, the Annexin moiety is located at the N-terminal end of said fusion protein, in which case the second protein is located at the C-terminal end of said fusion protein.

An illustrative example of a fusion complex produced by protein chemistry between the Annexin protein and the second protein is obtained through the cross-linking agent SPDP (N-succinimidyl 3-(2-pyridylthio)propionate).

As already mentioned in the present specification, in a device according to the invention, the bait molecule that is used has affinity for one or more target molecules, the presence of which is sought in the sample to be tested.

As already mentioned, said bait molecule is immobilized on the device as part of an anchoring complex comprising, or consisting of, the fusion protein between an Annexin protein and a partner molecule, notably a second protein.

In one embodiment, said anchoring complex consists exclusively of said fusion complex, in which embodiment the partner molecule which

is fused with the Annexin-A5 protein consists of the bait molecule itself, in which case said bait molecule consists of a bait protein (Figure 2-Mode 1).

In another embodiment, said anchoring complex comprises said fusion complex, wherein the partner molecule, e.g. the partner protein, contained in said fusion complex is, directly or indirectly, non-covalently bound to the said bait molecule. The partner molecule contained in the fusion complex is directly non-covalently bound to the said bait molecule when said bait molecule is simply non-covalently bound with said second protein (Figure 2-Mode 2).

In still another embodiment, the partner molecule, e;g. the partner protein, contained in the fusion complex is indirectly non-covalently bound to the said bait molecule when said bait molecule is bound to an intermediate protein, said intermediate protein being itself directly or indirectly non-covalently bound to the second protein comprised in said fusion complex (Figure 2-Mode 3).

In the embodiments above also, the bait molecule may consist of a bait protein.

In a preferred embodiment of the device according to the invention, in the fusion complex between the Annexin protein and a second protein, said second protein consists of a protein having affinity for the Fc moiety of an antibody.

Preferably, said protein having affinity for the Fc moiety of an antibody consists of the ZZ domain analogous to part of the protein A from *Staphylococcus aureus*, such as that described by Uhlen et al. (1990). Still preferably, said protein having affinity with the Fc moiety of an antibody consists of the G protein from *Streptococcus sp.*, or of part of the G protein from *Streptococcus sp* , such as that described by Elliasson et al. (1988).

According to this preferred embodiment, the protein having affinity for the Fc moiety of an antibody may be the bait molecule, in which case

the detection device allows the detection of the presence of antibodies in a sample to be tested.

Still according to this preferred embodiment, the second protein, consisting of a protein having affinity for the Fc moiety of an antibody, does not consist of the bait molecule, in which case the bait molecule may be either (i) an antibody which is directly bound on said second protein (Figure 3-Mode 2A), or (ii) a bait molecule of interest which is bound to a bait-binding intermediate molecule (Figure 3-Mode 3), notably a bait-specific antibody (Figure 3-Mode3A), said bait-binding intermediate molecule directly binds to said second protein (Figure 3-Mode 3A).

In another preferred embodiment of the device according to the invention, in the fusion complex between the Annexin protein and a second protein, said second protein consists of an antibody fragment comprising the Complementary Determining Regions (CDRs) of an antibody.

According to this preferred embodiment, said antibody fragment may consist of the bait molecule itself, in which case the detection device allows detecting target molecules that have affinity with said antibody fragment.

Still according to this preferred embodiment, said antibody fragment consists of a bait-specific antibody fragment, to which the bait molecule is directly non-covalently bound.

In a preferred aspect of this preferred embodiment, said antibody fragment is selected from the group consisting of an Fab or a single-chain variable fragment (scFv) derived from a parent antibody.

In still another preferred embodiment of the detection device according to the invention, in the fusion complex between the Annexin protein and a second protein, said second protein consists of said bait molecule, which thus consists of a bait protein.

In another preferred embodiment of the detection device according to the invention, said anchoring complex consists of :

(i) a fusion complex between an Annexin-A5 protein and an antibody fragment comprising the CDR domain of a bait-specific antibody; and
(ii) the bait molecule which is non covalently bound to the bait-specific antibody fragment of said fusion complex. Said bait molecule may consist of a bait protein.

In still a further embodiment of the detection device according to the invention, said anchoring complex consists of :

(i) a fusion complex between an Annexin protein and a protein having affinity for the Fc portion of an antibody; and
(ii) an antibody which is non-covalently bound by its Fc domain onto said fusion complex, said antibody being said bait molecule.

In yet a further embodiment of the detection device according to the invention, said anchoring complex consists of :

(i) a fusion complex between an Annexin protein and a protein having affinity for the Fc portion of an antibody;
(ii) a bait-specific antibody which is non-covalently bound by its Fc domain onto said fusion complex; and
(iii) the bait molecule which is non-covalently bound onto said bait-specific antibody. Said bait molecule may consist of a bait protein.

In yet a further embodiment of the detection device according to the invention, said anchoring complex consists of :

(i) a fusion complex between an Annexin protein and a protein which is the bait protein

In yet a further embodiment of the detection device according to the invention, said anchoring complex consists of :

(i) a fusion complex between an Annexin protein and a second protein having affinity for the bait molecule
(ii) the bait molecule which is bound to said second protein. Said bait molecule may consist of a bait protein

Preferably, in a detection device according to the invention, said bait molecule is selected from the group consisting of biological

molecules, their derivatives, and their assemblies as well as from the group of organic molecules as well as from the group of polymers as well as from the group of inorganic molecules and their aggregates.

Most preferably, in a detection device according to the invention, said bait molecule is selected from the group consisting of antigen-specific antibodies, pathogen-specific antibodies, tumor cell specific antibodies, growth factor receptors, hormone receptors, lipid-binding proteins, neurotransmitter receptors, catecholamine receptors, amino acid derivative receptors, cytokine receptors, extracellular matrix receptors, lectins, cytokines, serpins, proteases, kinases, phosphatases, ras-like GTPases, hydrolases, steroid hormone receptors, transcription factors, heat-shock transcription factors, DNA-binding proteins, zinc-finger proteins, leucine-zipper proteins, homeodomain proteins, intracellular signal transduction modulators and effectors, apoptosis-related factors, DNA synthesis factors, DNA repair factors, DNA recombination factors, cell-surface antigens, hepatitis C virus (HCV) proteases or HIV proteases and antibodies, growth factors, hormones, drugs, oligonucleotides, nucleic acids, sugar residues, lipids, small molecules, polymers, inorganic molecules and their aggregates

The one skilled in the art may manufacture a fusion complex according to the invention quite easily, notably through the well-known recombinant DNA technology methods for manufacturing expression vectors that encode fusion proteins.

For general methods disclosing recombinant genetic engineering production of proteins, the one skilled in the art may notably refer to the book of Sambrook et al. (1989). The one skilled in the art may also refer to the book of Ausubel et al. (1989).

An illustrative embodiment of the production of a fusion complex which can be used for manufacturing a device according to the invention is given in the examples herein, i.e. the production of the fusion protein

comprising the Annexin-A5 protein of SEQ ID N°1 with the ZZ domain of protein A from the *Staphylococcus aureus.*

In the specific embodiment of a fusion complex that is illustrated in Example 1, the Annexin-A5 moiety of SEQ ID N°1 with the double mutation [T 163C; C 314S] and the second protein moiety (the ZZ domain) are covalently linked together through a peptide bond in the final recombinant fusion protein.

Thus, in one preferred embodiment of the invention device, in said fusion complex, the Annexin moiety and the partner molecule, e;g. the second protein moiety, are covalently linked together through a normal peptide bond.

In one specific embodiment of said fusion complex, this fusion complex consists of a recombinant protein.

In another embodiment of the invention device, said fusion complex consists of an Annexin protein that is chemically covalently bound to a partner molecule, e.g. a second protein moiety, through a covalent bond other than a peptide bond, as illustrated in Example 2.

This further embodiment above is notably illustrated by the chemical cross-linking between the Annexin-A5 [T163C; C314S] moiety and the second protein through a cysteine residue incorporated in the Annexin-A5 moiety and a cysteine-reactive group grafted to the second protein. In such a specific embodiment, it may be used a functionally active derivative of the Annexin-A5 protein of SEQ ID N°1, wherein the cysteine residue located in the aminoacid position 314 is replaced by a serine residue and wherein the threonine residue located in the aminoacid position 163 is replaced by a cysteine residue. Grafting of a cysteine-reactive group to any protein is performed according to well known methods for the one skilled in the art by means of hetero-bi-functional cross-linking reagents. For general methods disclosing chemical modification of proteins by protein chemistry, the one skilled in the art may notably refer to the book of Wong (1991).

Thus in a specific embodiment of the detection device according to the invention, the Annexin-A5 protein and the second protein are covalently linked together through a chemical bond between a cysteine residue of the Annexin-A5 protein and a cysteine-reactive group grafted on the second protein.

In certain preferred embodiments of a device according to the invention, said device also comprises a substrate and said lipid layer is coated on said substrate.

According to these preferred embodiments, said substrate may consists of a solid substrate.

A solid substrate may be prefereably selected from the group consisting of mica, silicon, mineral glass and gold.

In other preferred embodiments, said substrate consists of the air-liquid interface of a liquid medium.

As it is readily understood, following the above general description of the device according to the invention, said device allows a high level of miniaturization of target molecules screening means and is preferably used as a biochip for screening simultaneously for the presence either of multiple target molecules in an assay sample or of one target molecule using multiple bait molecules.

Therefore, the present invention also concerns a system that comprises more than one type of bait molecules included in anchoring complexes, namely a collection of bait molecules included in anchoring complexes.

Thus, the present invention also pertains to a system for detecting the binding of a target molecule onto a bait molecule, wherein said system comprises a plurality of detection devices as defined above.

Preferably, in said detection system, each detection device of said plurality of detection devices comprises a unique bait molecule, notably a unique bait protein. It must be noticed than one given bait molecule may

specifically bind to more than one target molecule that is present in an assay sample to be tested.

Preferably, in said detection system, two distinct detection devices that are included therein comprise distinct bait molecules, notably distinct bait proteins. The detection devices that are the subject-matter of the present invention, and particularly those that are included in a detection system as defined hereabove, may comprise the solid substrate under the form of particles that are made of this solid substrate or alternatively under the form of particles that are coated with this solid substrate.

In another embodiment of a device according to the invention, the solid substrate is under the form of a collection of solid particles. In this specific embodiment of the invention's device, said particles, preferably silica particles, silica-coated particles or glass beads, are coated with a lipid bi-layer of the kind already defined above (Mornet et al., 2005), onto which are bound anchoring complexes of the invention.

Preferably, according to this specific embodiment, each particle or bead which is part of the device contains, bound thereto, a large number of molecules of one given type of bait protein.

An illustrative example of a detection system according to the invention that is manufactured under the form of a biochip, consists of a system comprising a serial of anchoring complexes comprising a serial of bait molecules, which serial of bait molecules consists of a serial of distinct target-specific antibodies, each target-specific antibody being directed against a distinct specific antigen, and/or against distinct epitopes of a given antigen, so as to screen an assay sample for the presence of one or more target molecules consisting of one or more antigens recognized by the corresponding target-specific antibody.

The device or the detection system according to the invention is used for performing methods for assaying the binding of a target molecule contained in an assay sample, including any screening method

of target molecules of biological interest, including target molecules of therapeutical interest.

Thus, a further object of the invention consists of a method for detecting the binding of a target molecule onto a bait molecule, wherein said method comprises the steps of:

a) providing a sample to be tested;

b) bringing into contact the sample to be tested with a detection device or with a detection system as defined above; and

c) detecting the complexes eventually formed between (i) the bait molecule(s) contained in said detection device or in said detection system and (ii) the target molecule(s) eventually present within said tested sample.

This invention also relates to a method for assaying for the presence of a target molecule in a sample comprising the steps of :

a) providing a fusion complex between an Annexin protein and a bait molecule which binds to said target molecule;

b) mixing the sample with said fusion complex, whereby complexes between the bait moiety of said fusion complex and the target molecule are allowed to be formed;

c) immobilizing the fusion complexess obtained at step b), eventually under the form of complexes with said target molecule, at the surface of a phospholipid bi-layer coating a solid substrate, said phospholipid bi-layer comprising a combination of (i) one or more lipids with (ii) one or more phospholipids, said one or more phospholipids having a negative net charge in an aqueous solution at a neutral pH;

c) detecting the complexes that are formed between the bait moiety of said fusion complex and the target molecule when said target molecule is present in said sample.

This invention also relates to a method for assaying for the presence of a target molecule in a sample comprising the steps of :

a) providing a bait molecule which binds to said target molecule; said bait molecule may be i) part of a fusion complex with an Annexin protein; ii) a molecule that binds to a second molecule that is part of an Annexin-A5 fusion complex; iii) a molecule that binds to an intermediate molecule that itself binds to a second molecule that is part of an Annexin-A5 fusion complex;

b) mixing the sample with said bait molecule, whereby complexes between the bait molecule and the target molecule are allowed to be formed;

c) immobilizing the complexes obtained at step b), eventually under the form of complexes with said target molecule, at the surface of a lipid layer, said lipid layer being i) either a lipid bi-layer coating a solid substrate; ii) or a lipid mono-layer formed at the interface between air and an aqueous solution;, said lipid layer comprising a combination of (i) one or more lipids with (ii) one or more phospholipids, said one or more phospholipids having a negative net charge in an aqueous solution at a neutral pH;

d) detecting the complexes that are formed between the bait moiety of said anchoring complex and the target molecule when said target molecule is present in said sample.

In a preferred embodiment of the method above, the fusion complex comprises a protein having affinity for the Fc moiety of an antibody, said antibody being either the bait protein, or a bait-specific antibody, or directed against a molecule that binds to the bait molecule.

In a further preferred embodiment of the method above, the fusion complex comprises a protein consisting of the ZZ domain of protein A from *Staphylococcus aureus*.

In a further preferred embodiment of the method above, the fusion complex comprises a protein consisting of the G protein from *Streptococcus sp*.

In a further preferred embodiment of the method above, the fusion complex comprises a bait protein consisting of an antibody fragment comprising the CDR domains of a target-specific antibody.

In a further preferred embodiment of the method above, the fusion complex comprises an antibody fragment comprising the CDR domains of a bait-specific antibody.

In a further preferred embodiment of the method above, the fusion complex comprises a bait protein selected from the group consisting of growth factor receptors, hormone receptors, neurotransmitter receptors, catecholamine receptors, amino acid derivative receptors, cytokine receptors, extracellular matrix receptors, lectins, cytokines, serpins, proteases, kinases, phosphatases, ras-like GTPases, hydrolases, steroid hormone receptors, transcription factors, heat-shock transcription factors, DNA-binding proteins, zinc-finger proteins, leucine-zipper proteins, homeodomain proteins, intracellular signal transduction modulators and effectors, apoptosis-related factors, DNA synthesis factors, DNA repair factors, DNA recombination factors, cell-surface antigens, hepatitis C virus (HCV) proteases or HIV proteases and antibodies, oligonucleotides, nucleic acids, sugar residues, lipids, small molecules, polymers, inorganic molecules and their aggregates.

- In an illustrative embodiment, the detection, the quantification or the characterization of the target molecules is performed by mass-spectrometry or alternatively by one of known surface-sensitive methods, like fluorescence, ellipsometry, QCM-D, surface plasmon resonance.
- In another illustrate embodiment, the detection, the quantification or the characterization of the target molecules is performed by using target-specific antibodies, said target-specific antibodies being labeled prior to performing the assay method.

In such a specific embodiment, the detection, quantification or characterization of the complexes formed between the bait molecule (s) and the target molecule (s) of the invention's device is carried out

through the detection of the detectable molecule that will be finally bound to said device.

The detectable molecule may be a radioactive isotope such as ^{3}[H], ^{14}[C], ^{125}[I] or ^{32}[P]. The detectable molecule may also be a fluorescent molecule such as a small fluorescent dye molecule or a protein of the green fluorescent protein (GFP) family. The detectable molecule may be an enzyme, such as the well-known β-galactosidase, luciferase, peroxydase, alkaline phosphatase, acetylcholinesterase, or catalase. Any other conventional detectable molecule widely used in the art is herein encompassed by a detectable molecule usable according to the assay method of the invention.

As another embodiment of the detection step, the detection, quantification or characterization of the complexes formed between the bait molecule(s) and the target molecule(s) can be carried out using antibodies directed against a specific target molecule of interest, the presence of which in the assay sample is sought.

In a further embodiment of the detection step, the detection, the quantification or the characterization, of the complexes formed between the bait molecule(s) and the target molecule(s) is carried out using the method of the Quartz Crystal Microbalance with Dissipation Monitoring (QCM-D) as illustrated in Example 5 and in Figures 10 to 14.

As it appears clearly from the specification above, the assay device and methods of the invention gather numerous technical advantages that cannot be found in such an advantageous combination in the prior art devices and methods.

Firstly, the assay device of the invention, once conceived by the inventors, can be easily and reproducibly manufactured. As an illustration, the manufacture of either the lipid bi-layer on the solid substrate or of the mono-layer at an air-water interface, and the further formation of a high-density matrix of the fusion complexes thereto may be performed in less than a one-hour period of time.

Secondly, as it has been already mentioned in the above description, the controlled orientation of the Annexin-A5 moieties on the lipid layer allows an appropriate exposure of the bait proteins of interest to the solvent, ensuring maximal efficiency of capture of the target molecules that are eventually contained in the assay sample to be analyzed.

Thirdly, the lipid-layer covers the entire surface of either the air-water interface or the solid substrate, and the lipid layer is covered by a continuous 2D assembly of Annexin-A5 or Annexin-A5 derivatives, both of them preventing non-specific adsorption of molecules.

Fourthly, the quasi-irreversible immobilization of the bait proteins at the surface of the lipid layer is obtained through the properties of the Annexin-A5 protein moieties, including the functionally active derivatives of the Annexin-A5 proteins.

Fifthly, the complete coverage of the lipid layer by the 2D matrix of anchoring complexes is obtained through the 2D self-assembly properties of the Annexin-A5 moiety.

Further, in a device according to the invention, the bait proteins, as well as the complexes between the bait proteins and their corresponding target molecules, are separated from the solid substrate by the phospholipid bi-layer, which avoids the problems of the bait protein denaturation which are generally encountered with prior art methods and devices.

This strategy may be extended to other molecules, notably proteins, that present the basic property of forming 2D molecular matrices of high-density, and stable, on lipid layers. The strategy may be extended notably to proteins that present the property of forming 2D crystals on lipid layers, as this is the case for Annexins, notably Annexin-A5 on lipid mono-layers (Mosser et al., 1991; Voges et al. 1994; Brisson et al., 1999a; Brisson et al., 1999b) and phospholipid bi-layers (Reviakine et al., 1998).. Examples of proteins that form 2D crystals by specific

interaction with lipids incorporated into lipid mono-layers at the air-water interface can be found in Brisson et al., 1999a and in Ellis and Hebert (2001). An example of a protein that presents the basic property of forming stable high-density 2D arrangements on lipid surfaces is streptavidin, which forms 2D crystals on lipid mono-layers containing biotinylated lipids (Darst et al., 1991; Brisson et al., 1999b; Farah et al., 2001; Ratanabanangkoon and Gast, 2003), as well as on lipid bi-layers containing biotinylated lipids coating solid-supports (Reviakine and Brisson, 2001; Richter and Brisson, 2003). The binding and the detection of target molecules could be realized by use of bait molecules that will be linked either directly or indirectly to the 2D matrix of anchoring complexes containing streptavidin. Linking between the bait molecule and streptavidin may involve: 1) a fusion complex obtained by recombinant DNA technology, ii) the chemical cross-linking with a biotinylated bi-functional reagent of either the bait molecule, the second molecule of the fusion complex, or an intermediate molecule of the anchoring complex located between the bait molecule and the second molecule. Another example of a protein that forms stable high-density 2D matrix on lipid surfaces is the B_5 moiety of cholera toxin from *Vibrio cholerae* which binds to G_{M1} ganglioside-containing lipid surfaces (Ludwig et al., 1986; Mosser et al., 1992; Brisson et al., 1999b). Other examples are proteins engineered with a polyhistidine extension which form high-density 2D matriceson lipid surfaces containing lipid molecules covalently modified with a N",N"-bis[carboxymethyl]-L-lysine-(nitriloacetic acid)-nickel-chelating group (Kubalek et al., 1994; Brisson et al., 1999a). Examples of such proteins are the protein HupR from *Rhodobacter capsulatus* (Venien-Bryan et al., 1997) and the extracellular fragment of the vascular endothelium cadherin VE-EC_{1-4} (Al-Kurdi et al., 2004). Yet another example of proteins that self-assemble as 2D protein matrices on various types of lipid surfaces consists of the S-layer family of proteins (Schuster and Sleytr, 2000; Moll et al., 2002).

As previously specified, certain embodiments of a device according to the invention provide useful vectors for targeting the delivery therapeutical molecules of interest towards specific cell types, specific tissue types or specific organs, in patients in need thereof.

According to these embodiments, a device of the invention is under the form of lipid vesicles, i.e. liposomes, wherein at least the outer lipid layer which is exposed to the solvent has a negative net charge in an aqueous solution at a neutral pH and is coated with the two-dimensional matrix of anchoring complexes comprising bait molecules that may specifically bind to target molecules that are present in a biological fluid, at the membrane surface or in the intracellular content of the targeted cell(s), of the targeted tissue(s) or of the targeted organ(s). According to these embodiments, the inner part of said lipid vesicles comprises a liquid medium in which one or more therapeutically useful compounds are dissolved or suspended. The inner part of the lipid vesicles or liposomes may herein also be termed “core” or “inner core” and consists of the inner central part of said lipid vesicles or liposomes that contains a liquid medium, preferably an aqueous liquid medium wherein one or more pharmaceutically active ingredients are dissolved or suspended.

According to these embodiments, devices according to the invention may be administered to a patient, either by a systemic or a local route. Then, said devices that comprise bait molecules specifically bind to cells, tissues or organs that express the corresponding target molecules at their surface. Then, the devices that are bound to the target cells, tissues or organs through specific binding of the bait molecules onto the target molecules are internalized within the cells and deliver the therapeutically useful molecules that were initially contained in the inner part of the lipid vesicles of the devices according to the invention.

Illustratively, the bait molecules that are part of the anchoring complexes comprised in such embodiments of a device according to the invention may consist of bait molecules that specifically bind to antigens

that are specifically expressed or present at the cell surface of cancer cells. According to these embodiments, the therapeutically useful molecules that are contained in the inner part of the lipid vesicles of said devices are selected from the group consisting of the pharmaceutical ingredients that are active against cancer, such as well known cytotoxic ingredients that are currently used for treating cancer patients.

Thus, the present invention further relates to a vector for targeting therapeutical molecules comprising a device according to claim 1, wherein said device consists of lipid vesicles having :

a) an outer lipid layer comprising one or more lipids, said lipid layer having a negative net charge in an aqueous solution at a neutral pH;

b) a two-dimensional matrix of anchoring complexes that are bound to said lipid layer, wherein each of said anchoring complexes comprises :

(i) a fusion complex comprising an Annexin protein fused to a partner molecule, wherein :

- said Annexin protein is bound to said phopholipid layer, and
- said partner molecule consists of an organic or a mineral compound;

(ii) a bait entity selected from the group consisting of :

- said partner molecule that is fused to said Annexin protein;;
- a molecule that is covalently or non-covalently bound to said partner molecule;
- a molecule that is indirectly bound to said partner molecule through one or more intermediate molecules that are covalently or non-covalently bound to said partner molecule; and

c) in the inner part of said lipid vesicles, a liquid medium comprising one or more pharmaceutically active molecules that are dissolved or that are suspended therein.

As mentioned above, in a vector for targeting therapeutical molecules according to the invention, the bait molecule specifically binds

to a target molecule that is expressed at the surface of the targeted cell, of the targeted tissue or of the targeted organ of interest.

Preferably, in a vector above, the anchoring complexes consist of an Annexin protein fused to the bait molecule, thus a fusion complex between said Annexin molecule and said bait molecule.

The bait molecule may be of any kind of a bait molecule disclosed in the present specification.

The present invention is further illustrated, without in any way being limited to, the examples below.

EXAMPLES

Example 1- Production of a fusion complex between Annexin-A5 [T163C ; C314S] and a second protein by recombinant DNA technology methods , wherein said second protein consists of the ZZ domain of protein A from *Staphylococcus aureus*

EXAMPLE 1-1: Construction of Annexin-A5 [T163C; C314S]—ZZ expression vector

The expression vector containing the coding sequence of rat Annexin-A5 linked to the coding sequence of the ZZ domain analogous to protein A from *Staphylococcus aureus* was constructed by standard methods of molecular biology.

The rat Annexin-A5 coding sequence was excised by NcoI digestion from a pKK233-2-Annexin-A5 expression vector (Pepinsky et al., 1988) and cloned into the expression vector pGELAF+ (Schanstra et al., 1993) between two NcoI restriction sites, resulting in the pGEF-A5 expression vector.

Site-directed mutagenesis was performed by standard procedures (Kunkel, 1985) to insert the double mutation [T163C; C314S], resulting in the pGEF-A5B expression vector. All the assays available to us were

performed to verify that the (T163C; C314S) double-mutant Annexin-A5protein presents all the known properties of wild type Annexin-A5, particularly in what concerns its binding to lipid membranes, and the formation of 2D crystalline arrays of trimers on lipid mono-layers (Oling et al., 2001; Govorukhina et al., 2002), and on mica-supported lipid bi-layers (Reviakine et al., 1998). For this reason, the term Annexin-A5 used here refers to the double-mutant (T163C; C314S) Annexin-A5.

In order to fuse the ZZ coding sequence at the 3'end of the Annexin-A5 gene, the stop codon of Annexin-A5 in pGEF-A5B was removed and replaced by TAA. The PCR fragment produced with the primers: 5'-GAAGAGCTCAGGGCCATAAAACAAG –3' [SEQ ID N° 19] (SacI site underlined) and 5'-CATGCTAGCTAAGTCATCCTCGCCTCCACAGA-3' [SEQ ID N° 20] (NheI site underlined) was digested with SacI and NheI and ligated into SacI/NheI digested pGEF-A5B, resulting in pGEF-A5B'.

A ZZ fragment lacking the signal sequence and containing a NheI and a BamHI restriction sites was produced by PCR from the pEZZ18 vector (Amersham BioSciences). The primers used were 5'-TGTGCTAGCCAAGCCGTAAACAAATTCAAC-3' [SEQ ID N° 21] (NheI site underlined) and

5'-GCAGGATCCCTATACCGAGCTCGAATTCGCGTCTAC-3'

[SEQ ID N° 22] (BamHI site underlined). The PCR product was digested with NheI and BamHI and introduced by ligation into NheI/BamHI digested pGEF-A5B', resulting in pGEF-A5BZZ. The sequence coding for the Annexin-A5—ZZ protein was excised by Xba1/BamH1 and cloned into the pET-11b expression vector (Novagen), resulting into the pET-A5BZZ expression vector.

The nucleotidic sequence corresponding to the Annexin-A5—ZZ fusion protein was verified by standard methods and assessed to code for the amino acid sequence of SEQ ID N°6.

EXAMPLE 1-2: Expression of the Annexin-A5—ZZ fusion protein

Escherichia coli BL21 (DE3) cells were transformed by heat shock with plasmid pET-A5BZZ. Cells were plated on LB plates containing ampicillin (100 µg/mL), and incubated overnight at 37°C.

One clone was collected and incubated in 25 ml LB-ampicillin medium at 37°C for ~ 20 hr, ending up with an OD_{600} ~3. The necessary volume was taken and diluted to an OD of 0,1 in 400 mL LB-ampicillin medium.

The culture was then incubated at 30°C until OD reaches 0,6-0,7, after which induction was started with 0,4 mM IPTG and incubation was carried out for another 16 hr at 30°C. The final OD_f was measured.

The cells were harvested by centrifugation (10 min, 6,700g) and the pellet was resuspended in a volume of buffer containing 10 mM Tris, 1 mM EDTA, 0.01% NaN_3, 10% glycerol, pH 7.5, equal to 6,7 x OD_f.

The cell suspension was sonicated at 4°C with a Branson sonicator operated in a pulse mode with five steps of sonication at 13 W for 1 min with 15 sec intervals. Membrane fragments and large debris were separated by centrifugation at 48,000 g for 1 hour at 4°C. The supernatant, referred to as cell soluble extract (CSE) was collected and stored until use at 4°C.

The Annexin-A5—ZZ fusion protein is expressed at high levels, as shown in Figure 7A.

EXAMPLE 1-3: Purification of Annexin-A5-ZZ fusion protein

The CSE was filtered over 0,22 µm filters and applied in 5 mL fractions to a Superdex 75 exclusion column (Amersham BioSciences) pre-equilibrated with a buffer containing 20 mM Tris, pH8, 0.02% NaN_3 (buffer A). Elution of the proteins was performed with buffer A. The Annexin-A5—ZZ fusion protein elutes at ~ 55 mL, as shown by SDS-PAGE (Figure 7B).

The fractions containing Annexin-A5—ZZ were pooled and applied to a MonoQ HR5/5 anion-exchange column (Amersham BioSciences) pre-equilibrated with buffer A. Elution was performed with a 0 to 0.5 M NaCl gradient in buffer A. The Annexin-A5—ZZ fusion protein elutes as a pure protein at ~ 270 mM NaCl (SDS-PAGE analysis shown in Figure 7C).

The mass of the purified protein was estimated by MALDI-TOF mass spectrometry analysis at 50077 Da, the theoretical mass without the initial methionine being 50074 Da.

An amount of ~ 40 mg pure Annexin-A5—ZZ protein is produced starting from 175 mg protein total protein in CSE from 400 mL cell culture (yield ~ 23%). The proteinis stable for a period longer than one year when stored at 4°C.

The ability of Annexin-A5-ZZ complexes to form stable 2D self-assemblies and to bind IgGs is demonstrated by QCM-D (Figures 10,11,12).

Example 2- Production of the fusion complex between Annexin-A5 [T163C ; C314S] and a second protein by covalent chemical linkage, wherein said second protein consists of protein G from *Streptococcus sp.*

The formation of fusion complexes between recombinant protein G (Pierce Biotechnology, USA) from *Streptococcus sp.* and Annexin-A5 (T163C;C314S) is performed using the hetero-bi-functional reagent *N*-

Succinimidyl 3-[2-pyridyldithio]-propionate (SPDP) (Pierce Biotechnology) in two steps: i) protein G is coupled to SPDP; ii) covalent complexes are formed between Annexin-A5 (T163C; C314S) which presents a single accessible sulfydryl group and (2-pyridyl-disulfide-activated protein G).

In order to obtain protein G derivatized with a small number of SPDP molecules per protein G molecule, protein G and SPDP are mixed in equimolar ratio. SPDP, dissolved in DMSO at 1 mg/mL, is mixed with an appropriate amount of protein G at 1 mg/mL in 160 mM borate buffer, pH 7,9. The reaction was performed by incubation for 2 hr at ambiant temperature.

As Annexin-A5 (T163C; C314S) has a natural tendency to oxidize into disulfide-linked dimers, freshly reduced Annexin-A5 is prepared by reducing the disulfide-linked dimers of Annexin-A5 with DTT just before coupling with protein G-SPDP. 1 ml of a solution containing 1 mg Annexin-A5 dimer in 20 mM Tris, 300 mM NaCl, 0,01% NaN_3, pH 8 is mixed with 100 µl of 100 mM DTT in 10 mM HEPES, pH 6,4. After 30 min incubation at ambient temperature, the excess DTT is eliminated by gel filtration on a HiTrap desalting column (5 mL) eluted with 10 mM HEPES, 150 mM NaCl, pH 7,4.

Cross-linking between Annexin-A5 and (protein G-SPDP) is performed at a molar ratio (Annexin-A5 / proteinG) equal to 2/1, as follows: the appropriate volumes of Annexin-A5 (~ 0,4 mg/mL) in 10 mM HEPES, 150 mM NaCl, pH 7,4 and of (protein G-SPDP) (~ 0,2 mg/mL) in 160 mM borate buffer, pH 7,9 were mixed and incubated for more than 16 hr at ambient temperature.

The characterization of the cross-linked products is performed by SDS-PAGE. Proteins migrating at positions expected for (Annexin-A5-proteinG) hetero-dimers and $(Annexin\text{-}A5)_2$-proteinG hetero-trimers are observed (Figure 9 A).

The covalent cross-linked complexes between Annexin-A5 and protein G are purified by gel filtration on Superdex 75 (Amersham BioSciences) eluted in buffer A, followed by anion exchange chromatography on Mono-Q eluted with a 0-0.5 M NaCl gradient in buffer A. The fractions are analyzed by SDS-PAGE. Peaks corresponding to the AnnexinA5-proteinG hetero-dimers and $(Annexin\text{-}A5)_2$-proteinG hetero-trimers are separated (Figure 9B).

50 µg of proteinG—Annexin-A5 complexes are obtained, starting from 1 mg Annexin-A5 dimer and 0,4 mg protein G, corresponding to ~ 6% yield for protein G.

The ability of proteinG—Annexin-A5 complexes to form stable 2D self-assemblies and to bind rat IgGs is demonstrated by QCM-D (Figure 13).

Example 3- Production of the anchoring complex made of a dimer of Annexin-A5 [T163C ; C314S]

The formation of anchoring complexes between two Annexin-A5 (T163C;C314S) molecules associated via a disulfide bond is performed by means of the reagent 4,4'-dithiodipyridine (DTDP).

The reduced form of Annexin-A5 (T163C;C314S) is obtained as described in Example 2.

The following protocol is recommended to oxydize reduced Annexin-A5 (T163C;C314S) with DTDP: reduced Annexin-A5 is mixed with DTDP at a molar ratio [Annexin-A5/DTDP] of 2.5, 50 mM sodium phosphate, pH 7.4, for 30 min. at ~ 20°C. The yield in disulfide-linked Annexin-A5 dimer is close to 75%. The disulfide-linked Annexin-A5 dimer is purified by anion exchange chromatography with a MonoQ HR5/5 column (Amersham BioSciences) pre-equilibrated with buffer A. The Annexin-A5 dimer elutes at ~ 280 mM NaCl. The mass of the purified protein is verified by MALDI-TOF.

Alternatively, the formation of (Annexin-A5-S-S-Annexin-A5) is obtained by spontaneous oxydation of the reduced Annexin-A5 (T163C;C314S) molecules.

Example 4- Production of an anchoring complex between Annexin-A5 [T163C ; C314S] and a RGD-containing peptide by covalent chemical linkage and application for anchoring target cells

Example 4-1 Production of an anchoring complex between Annexin-A5 [T163C ; C314S] and a RGD-containing peptide by covalent chemical linkage

The formation of anchoring complexes between Annexin-A5 (T163C;C314S) and a peptide containing the Arg–Gly–Asp (RGD) amino-acide sequence is performed by means of the reagent 4,4'-dithiodipyridine (DTDP).

The peptide sequence is GCRGYGRGDSPG (VandeVondele et al., 2003), which contains both the cell-adhesion motif RGD and a cysteine residue that is used to form a disulfide bond with (Annexin-A5 (T163C; C314S).

The reduced form of Annexin-A5 (T163C;C314S) is obtained as described in Example 2.

The following protocol is recommended: reduced Annexin-A5 is mixed with the GCRGYGRGDSPG and with DTDP at a molar ratio [Annexin-A5/RGD-peptide/DTDP] equal to 1/4/1 in 50 mM sodium phosphate, pH 7.4, for 30 min. at ~ 20°C. The yield in anchoring complex Annexin-A5—RGD peptide is close to 60%.

Alternatively, the formation of Annexin-A5—RGD peptide is obtained by spontaneous oxydation between the reduced Annexin-A5 (T163C;C314S) and the RGD-peptide.

The mass of the Annexin-A5—RGD peptide is verified by MALDI-TOF.

Example 4-2 Application of 2D matrices of Annexin-A5—RGD peptide on lipid bi-layers coating a solid substrate for anchoring target cells

The adhesion of human vascular endothelial cells to 2D matrices of Annexin-A5—RGD peptide on lipid bi-layers coating a glass substrate has been achieved. The adhesion of cells on the 2D matrices of Annexin-A5—RGD peptide is equivalent to the standard protocoles used in the art for cell adhesion. As a control experiment, no adhesion is observed when 2D matrices of Annexin-A5 (lacking the RGD peptide) on lipid bi-layers coating a glass substrate are used.

Example 5: Manufacture of a detection device according to the invention

EXAMPLE 5-1 : Formation of a stable 2D self-assembled layer of Annexin-A5-ZZ fusion complex on a supported phospholipid bi-layer and subsequent binding of a mouse monoclonal antibody, by QCM-D

The results are shown in figure 10.

This figure represents a typical example of an experiment in which a target molecule –here a mouse monoclonal antibody (IgG)- is detected by specific binding to a 2D self-assembled matrix of the fusion complex Annexin-A5—ZZ formed on a solid-supported phospholipid bi-layer (SLB).

I- Methods:

I-1) QCM-D

QCM-D measurements (Rodahl et al., 1995) were performed with the Q-SENSE D300 system equipped with an Axial Flow Chamber (QAFC 302) (Q-SENSE AB, Gothenburg, Sweden). as described in

Richter et al. (2003). Briefly, upon adsorption of analytes to the surface of a silica-coated quartz crystal, changes in the resonance frequency, ΔF, related to attached mass, and in the dissipation, ΔD, related to frictional losses in the adlayer, are measured in real time.

In Figures 10-13, the abscissa represents the time, expressed in minutes. The left ordinate (ΔF, blue) is expressed in Hz units and represents the variation of the normalized resonance frequency of the crystal at the 15 MHz harmonics. According to the Sauerbrey equation, the mass of adsorbed material is linearly related to ΔF, ($m = -C \times \Delta F$, with $C = 17.7\ ng \cdot cm^{-2} \cdot Hz^{-1}$) (Sauerbrey, 1959). The right ordinate (ΔD, orange) represents the variation in Dissipation.

I-2) Preparation of lipid vesicles

1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC) and 1,2-dioleoyl-sn-glycero-3-phospatidylserine (DOPS) (Avanti Polar Lipids (Alabama, USA) are dissolved in chloroform, mixed in desired amounts, dried first under a stream of nitrogen and subsequently in a vacuum desiccator overnight. Lipids are resuspended at ~ 1 mg/mL final concentration in a buffer containing 150 mM NaCl, 2 mM NaN_3 and 10 mM HEPES, pH 7.4 (buffer B), prepared in ultrapure water. Lipid suspensions are homogenized by 5 cycles of freeze-thawing and vortexing. Small unilamellar vesicles (SUVs) are obtained by sonication with a tip-sonicator (Misonix, NY, USA) operated in a pulsed mode at 30% duty cycle for 30 min with refrigeration, followed by centrifugation at 16,000 g for 10 min to remove titanium particles. SUV suspensions are stored at 4°C under nitrogen until use.

II- Experiment:

At position 1 in Figure 10, a solution of 100 µg/mL DOPC:DOPS (4:1; w:w) SUVs in buffer C (buffer B supplemented with 2 mM $CaCl_2$) was flushed on top of a silica-coated quartz crystal through the QCM-D

chamber. The changes of ΔF and ΔD, exhibiting a characteritic two-phase behavior, together with the ΔF and ΔD values obtained at the plateau, namely ΔF ~ - 25 HZ and ΔD ~ 0, are characteristic of the formation of a homogeneous, defect-free, SLB on the silica-coated quartz crystal. For general methods disclosing the formation of lipid bilayers by deposition of lipid vesicles on solid substrates, the one skilled in the art may notably refer to the following articles (Keller and Kasemo, 1991; Richter et al., 2003; Richter and Brisson, 2003; Richter et al., 2005).

In 2, the QCM-D chamber is flushed with buffer C to remove the excess of SUVs.

In 3, a solution of 20 µg/mL Annexin-A5—ZZ fusion protein in buffer C is injected. The specific binding of the fusion complex to the SLB results in a decrease of the frequency, ΔF ~ - 31 Hz, and a slight increase in dissipation, $\Delta D \sim 1{,}5x10^{-6}$. As reference, a 2D matrix of Annexin-A5 covering entirely a SLB gives rise to values of ΔF ~ - 17 Hz and $\Delta D \sim 0{,}2x10^{-6}$. In 4, several rinses with buffer C are performed (blue arrows), which does not induce any release of bound material. These results indicate that the Annexin-A5—ZZ fusion complex forms a stably bound, 2D self-assembled matrix over the SLB.

In 5, a solution of 20 µg/mL of IgG in buffer C is added, resulting in a rapid decrease of the frequency, ΔF stabilizing at ~ - 53 Hz. The change in dissipation, $\Delta D_{final} \sim 3x10^{-6}$, is characteristic of the presence of a flexible layer of adsorbed molecules on the sensor crystal. The IgG molecules are immobilized on the Annexin-A5—ZZ layer, as extensive rinsing with buffer C (R in Figure 9) does not induce any release of bound material.

Control experiments were performed, which indicated that the IgG molecules do not bind non specifically to the SLB, nor to an Annexin-A5 matrix.

In 6, a solution of buffer C supplemented with 2 mM EDTA, a calcium-chelator, is flushed through the QCM-D chamber, resulting in the instantaneous displacement of the Annexin-A5—ZZ / IgG complexes bound to the SLB.

EXAMPLE 5-2 : Formation of a stable 2D self-assembled matrix of (Annexin-A5 / Annexin-A5-ZZ) on a supported phospholipid bilayer and subsequent binding of a mouse monoclonal antibody, by QCM-D

The results are shown in Figure 11.

The principle of this experiment is similar to the one presented in Figure 9, except that a mixture of (Annexin-A5 / Annexin-A5—ZZ) (4:1; w:w) is used to form the 2D self-assembled matrix onto which the IgG molecules are subsequently immobilized.

In 1, injection of 100 µg/mL DOPC:DOPS (4:1; w:w) SUVs in buffer C The QCM-D chamber is then flushed with buffer C to remove the excess of SUVs (R in Figure 11).

In 2, a solution containing a mixture of (Annexin-A5 / Annexin-A5—ZZ) (4:1; w:w) at a total concentration of 20 µg/mL, in buffer C, is injected. At the plateau, binding of the (Annexin-A5 / Annexin-A5—ZZ) mixture gives rise to $\Delta F \sim -20$ Hz. Taking into account that maximal ΔF values of –17 Hz and –31 Hz are obtained for pure Annexin-A5 (not shown) and pure Annexin-A5—ZZ (Figure 9), respectively, and that the content of Annexin-A5—ZZ in the investigated mixture (Annexin-A5 / Annexin-A5—ZZ) is 20%, the observed ΔF value found here, namely –20 Hz, is in agreement with what is expected from an ideal mixing of Annexin-A5 and Annexin-A5—ZZ.

In 3, the injection of 20 µg/mL of IgG in buffer C results in the rapid decrease of the frequency, ΔF stabilizing at ~ -15 Hz. This value, obtained with 20% Annexin-A5—ZZ, is in agreement with the value of –53 Hz obtained with a 2D matrix of pure (100%) Annexin-A5—ZZ (as

described above in Example 5-1), as it is likely that in the latter case the IgGs will not saturate all Annexin-A5—ZZ binding sites due to steric hindrance effect, the size of an IgG molecule being significantly larger (~ 150 kDa) than the size of an Annexin-A5—ZZ fusion complex (~ 50 kDa).

EXAMPLE 5-3: Detection of the binding of complexes of (Annexin-A5—ZZ / IgG) pre-formed in solution on a [PC:PS (4:1)] SLB, by QCM-D

The results are shown in Figure 12.

In this experiment, non-covalent complexes between Annexin-A5—ZZ molecules and mouse monoclonal IgG molecules were formed in solution, by mixing in 1,5 mL buffer C,, for 15 minutes at ambiant temperature, 40 µg Annexin-A5—ZZ and 120 µg IgG, , which corresponds to an equimolar ratio of both species.

In 2, this mixture was injected over a DOPC:DOPS (4:1; w:w) SLB, formed in (1). Binding of the (Annexin-A5—ZZ / IgG) complexes results in a decrease of ΔF of -85 Hz. This value is almost identical to that obtained when the IgGs are added to a pre-formed Annexin-A5—ZZ layer (described in Example 5-1 and Figure 10). This experiment indicates that complexes can be formed first in solution before being immobilized, via the specific interaction of the Annexin-A5 moiety on PS-containing lipid surfaces.. The bound material is stable upon rinsing (R in Figure 12).

EXAMPLE 5-4: Detection of the binding of a rat monoclonal antibody (IgG) to a 2D self-assembled matrix of a chemically cross-linked fusion complex between Annexin-A5 (T163C; C314S) and protein G from *Streptococcus sp.* on a [PC:PS (4:1)] SLB, by QCM-D

The results are shown in Figure 13.

In this experiment, a chemically cross-linked complex made of Annexin-A5 and protein G was prepared, as described in Example 2 and Figures 9A,B), and was used for immobilizing rat monoclonal IgG molecules. Rat monoclonal antibodies react with protein G, but do not react with protein A.

In 1, injection of 100 µg/mL DOPC:DOPS (4:1; w:w) SUVs in buffer C. The QCM-D chamber is then flushed with buffer C to remove the excess of SUVs (R in Figure 13).

In 2, a solution containing chemically cross-linked complex of (Annexin-A5—protein G) at a total concentration of 10 µg/mL in buffer C is injected. At the plateau, binding of the complex corresponds to $\Delta F \sim -35$ Hz. This value is expected for a 2D matrix of (Annexin-A5—protein G) covering entirely the SLB surface.

In 3, the injection of 20 µg/mL of rat monoclonal antibodies in buffer C results in the rapid decrease of the frequency, ΔF stabilizing at ~ -25 Hz.

EXAMPLE 6: Application of 2D matrices of the (Annexin-A5-S-S-Annexin-A5) fusion complex bound to lipid bi-layers coating a solid substrate for anchoring target liposomes

The property of 2D matrices of the (Annexin-A5-S-S-Annexin-A5) fusion complex bound to lipid bi-layers coating a solid substrate to anchor target liposomes is described in Example 6 and illustrated in Figure 14.

In 1, injection of 100 µg/mL DOPC:DOPS (4:1; w:w) SUVs in buffer C, which results in the formation of a supported lipid bi-layer..

In 2, a solution containing 40 µg/mL (Annexin-A5-S-S-Annexin-A5) dimer in buffer C is injected. At the plateau, binding of the (Annexin-A5-S-S-Annexin-A5) gives rise to $\Delta F \sim -38$ Hz. This value indicates that the (Annexin-A5-S-S-Annexin-A5) dimer is bound to the lipid bi-layer via one

Annexin-A5 moiety, while the second moiety is exposed to the aqueous solution, as illustrated in Figure 14.

In 3, injection of 100 µg/mL DOPC:DOPS (4:1; w:w) SUVs in buffer C, which results in the rapid binding of liposomes. Control experiments with pure DOPC liposomes and with DOPC:DOPS (4:1; w:w) SUVs in the absence of calcium ions, in which no adsorption of liposomes is observed, indicate that the binding of SUVs observed in (3) is specific.

This experiment demonstrates that 2D matrices of the (Annexin-A5-S-S-Annexin-A5) fusion complex bound to lipid bi-layers coating a solid substrate are able to anchor target liposomes, and by extension cell membranes containing negatively charged phospholipids.

Example 7: Formation of a 2D crystalline matrix of the fusion complex Annexin-A5-ZZ on a lipid mono-layer at the air-water interface

In this experiment, a lipid mono-layer is formed at the air-water interface by applying 0.6 µl of a lipid mixture containing 150 µM DOPS - 450 µM DOPC dissolved in chloroform:hexane (1:1, v:v) over a 17 µl droplet of 0.1 mg/ml Annexin-A5-ZZ in a buffer containing 2 mM $CaCl_2$, 150 mM NaCl, 10 mM HEPES, 3 mM NaN_3, pH 7.4, following standard procedures described in Brisson et al. (1999a,b). After incubation times ranging from 30 minutes to 16 hr, the interfacial film is transferred to an electron microscopy (EM) coated with a perforated carbon film and negatively stained with 1% uranyl acetate.

EM observations are performed with a Philips CM120 electron microscope operating at 120 kV, equipped with a 2k x 2k Gatan-794 slow-scan CCD camera. Electron micrographs are recorded under low-dose conditions at a magnification of 45,000x and underfocus values close to 300 nm. The images are analyzed by Fourier transformation.

Annexin-A5—ZZ form 2D crystals with p6 symmetry structurally homologous to Annexin-A5.

TABLE 1

SEQUENCES OF THE INVENTION

SEQ ID N°	Type	Description
1	Protein	Rat Annexin-A5
2	Protein	Mouse Annexin A5
3	Protein	Human Annexin A5
4	Protein	Bovine Annexin A5
5	Protein	Chicken Annexin A5
6	Protein	Rat Annexin A5[T 163C; C 314S] -ZZ fusion protein
7	Protein	Human Annexin-1
8	Protein	Human Annexin-2
9	Protein	Human Annexin-6
10	Protein	Human Annexin-4
11	Protein	Human Annexin-8
12	Protein	Human Annexin-7
13	Protein	Hydat Annexin-C
14	Protein	Human Annexin-D
15	Protein	Human Annexin-B
16	Protein	Human Annexin-3
17	Protein	Human Annexin-A
18	Protein	Human Annexin-9
19-22	DNA	Primers

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SEQUENCE LISTING

<110> Centre National de la Recherche Scientifique

<110> Université de Bordeaux 1

<120> A DEVICE FOR BINDING A TARGET ENTITY TO A BAIT ENTITY AND DETECTION METHODS USING THE SAME

<130> R296US-CNRS

<140>

<141>

<160> 22

<170> PatentIn Ver. 2.1

<210> 1
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<212> PRT
<213> Rattus sp.

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<221> VARIANT
<222> (163)
<223> T replaced by C

<220>
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<222> (314)
<223> C replaced by S

<400> 1

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Ala Asp Ala Glu Val Leu Arg Lys Ala Met Lys Gly Leu Gly Thr Asp
             20                  25                  30

Glu Asp Ser Ile Leu Asn Leu Leu Thr Ala Arg Ser Asn Ala Gln Arg
         35                  40                  45

Gln Gln Ile Ala Glu Glu Phe Lys Thr Leu Phe Gly Arg Asp Leu Val
     50                  55                  60

Asn Asp Met Lys Ser Glu Leu Thr Gly Lys Phe Glu Lys Leu Ile Val
 65                  70                  75                  80

Ala Leu Met Lys Pro Ser Arg Leu Tyr Asp Ala Tyr Glu Leu Lys His
                 85                  90                  95

Ala Leu Lys Gly Ala Gly Thr Asp Glu Lys Val Leu Thr Glu Ile Ile
            100                 105                 110

Ala Ser Arg Thr Pro Glu Glu Leu Arg Ala Ile Lys Gln Ala Tyr Glu
        115                 120                 125

Glu Glu Tyr Gly Ser Asn Leu Glu Asp Asp Val Val Gly Asp Thr Ser
    130                 135                 140

Gly Tyr Tyr Gln Arg Met Leu Val Val Leu Gln Ala Asn Arg Asp
145                 150                 155                 160
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Pro Asp Thr Ala Ile Asp Asp Ala Gln Val Glu Leu Asp Ala Gln Ala
165 170 175

Leu Phe Gln Ala Gly Glu Leu Lys Trp Gly Thr Asp Glu Glu Lys Phe
180 185 190

Ile Thr Ile Leu Gly Thr Arg Ser Val Ser His Leu Arg Arg Val Phe
195 200 205

Asp Lys Tyr Met Thr Ile Ser Gly Phe Gln Ile Glu Glu Thr Ile Asp
210 215 220

Arg Glu Thr Ser Gly Asn Leu Glu Asn Leu Leu Leu Ala Val Val Lys
225 230 235 240

Ser Ile Arg Ser Ile Pro Ala Tyr Leu Ala Glu Thr Leu Tyr Tyr Ala
245 250 255

Met Lys Gly Ala Gly Thr Asp Asp His Thr Leu Ile Arg Val Ile Val
260 265 270

Ser Arg Ser Glu Ile Asp Leu Phe Asn Ile Arg Lys Glu Phe Arg Lys
275 280 285

Asn Phe Ala Thr Ser Leu Tyr Ser Met Ile Lys Gly Asp Thr Ser Gly
290 295 300

Asp Tyr Lys Lys Ala Leu Leu Leu Leu Cys Gly Gly Glu Asp Asp
305 310 315

<210> 2
<211> 319
<212> PRT
<213> Mus sp.

<400> 2
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Ala Asp Ala Glu Val Leu Arg Lys Ala Met Lys Gly Leu Gly Thr Asp
20 25 30

Glu Asp Ser Ile Leu Asn Leu Leu Thr Ser Arg Ser Asn Ala Gln Arg
35 40 45

Gln Glu Ile Ala Gln Glu Phe Lys Thr Leu Phe Gly Arg Asp Leu Val
50 55 60

Asp Asp Leu Lys Ser Glu Leu Thr Gly Lys Phe Glu Lys Leu Ile Val
65 70 75 80

Ala Met Met Lys Pro Ser Arg Leu Tyr Asp Ala Tyr Glu Leu Lys His
85 90 95

Ala Leu Lys Gly Ala Gly Thr Asp Glu Lys Val Leu Thr Glu Ile Ile
100 105 110

Ala Ser Arg Thr Pro Glu Glu Leu Ser Ala Ile Lys Gln Val Tyr Glu
115 120 125

Glu Glu Tyr Gly Ser Asn Leu Glu Asp Asp Val Val Gly Asp Thr Ser
130 135 140

Gly Tyr Tyr Gln Arg Met Leu Val Val Leu Leu Gln Ala Asn Arg Asp
145 150 155 160

Pro Asp Thr Ala Ile Asp Asp Ala Gln Val Glu Leu Asp Ala Gln Ala
165 170 175

Leu Phe Gln Ala Gly Glu Leu Lys Trp Gly Thr Asp Glu Glu Lys Phe
180 185 190

Ile Thr Ile Phe Gly Thr Arg Ser Val Ser His Leu Arg Arg Val Phe
195 200 205

Asp Lys Tyr Met Thr Ile Ser Gly Phe Gln Ile Glu Glu Thr Ile Asp
210 215 220

Arg Glu Thr Ser Gly Asn Leu Glu Gln Leu Leu Leu Ala Val Val Lys
225 230 235 240

Ser Ile Arg Ser Ile Pro Ala Tyr Leu Ala Glu Thr Leu Tyr Tyr Ala
245 250 255

Met Lys Gly Ala Gly Thr Asp Asp His Thr Leu Ile Arg Val Val Val
260 265 270

Ser Arg Ser Glu Ile Asp Leu Phe Asn Ile Arg Lys Glu Phe Arg Lys
275 280 285

Asn Phe Ala Thr Ser Leu Tyr Ser Met Ile Lys Gly Asp Thr Ser Gly
290 295 300

Asp Tyr Lys Lys Ala Leu Leu Leu Leu Cys Gly Gly Glu Asp Asp
305 310 315

<210> 3
<211> 320
<212> PRT
<213> Homo sapiens

<400> 3
Met Ala Gln Val Leu Arg Gly Thr Val Thr Asp Phe Pro Gly Phe Asp
1 5 10 15

Glu Arg Ala Asp Ala Glu Thr Leu Arg Lys Ala Met Lys Gly Leu Gly
20 25 30

Thr Asp Glu Glu Ser Ile Leu Thr Leu Leu Thr Ser Arg Ser Asn Ala
35 40 45

Gln Arg Gln Glu Ile Ser Ala Ala Phe Lys Thr Leu Phe Gly Arg Asp
50 55 60

Leu Leu Asp Asp Leu Lys Ser Glu Leu Thr Gly Lys Phe Glu Lys Leu
65 70 75 80

Ile Val Ala Leu Met Lys Pro Ser Arg Leu Tyr Asp Ala Tyr Glu Leu
85 90 95

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Lys His Ala Leu Lys Gly Ala Gly Thr Asn Glu Lys Val Leu Thr Glu
            100                 105                 110

Ile Ile Ala Ser Arg Thr Pro Glu Glu Leu Arg Ala Ile Lys Gln Val
        115                 120                 125

Tyr Glu Glu Glu Tyr Gly Ser Ser Leu Glu Asp Asp Val Val Gly Asp
    130                 135                 140

Thr Ser Gly Tyr Tyr Gln Arg Met Leu Val Val Leu Leu Gln Ala Asn
145                 150                 155                 160

Arg Asp Pro Asp Ala Gly Ile Asp Glu Ala Gln Val Glu Gln Asp Ala
                165                 170                 175

Gln Ala Leu Phe Gln Ala Gly Glu Leu Lys Trp Gly Thr Asp Glu Glu
            180                 185                 190

Lys Phe Ile Thr Ile Phe Gly Thr Arg Ser Val Ser His Leu Arg Lys
        195                 200                 205

Val Phe Asp Lys Tyr Met Thr Ile Ser Gly Phe Gln Ile Glu Glu Thr
    210                 215                 220

Ile Asp Arg Glu Thr Ser Gly Asn Leu Glu Gln Leu Leu Leu Ala Val
225                 230                 235                 240

Val Lys Ser Ile Arg Ser Ile Pro Ala Tyr Leu Ala Glu Thr Leu Tyr
                245                 250                 255

Tyr Ala Met Lys Gly Ala Gly Thr Asp Asp His Thr Leu Ile Arg Val
            260                 265                 270

Met Val Ser Arg Ser Glu Ile Asp Leu Phe Asn Ile Arg Lys Glu Phe
        275                 280                 285

Arg Lys Asn Phe Ala Thr Ser Leu Tyr Ser Met Ile Lys Gly Asp Thr
    290                 295                 300

Ser Gly Asp Tyr Lys Lys Ala Leu Leu Leu Leu Cys Gly Glu Asp Asp
305                 310                 315                 320
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<210> 4
<211> 321
<212> PRT
<213> Bos sp.

<400> 4

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Met Ala Gln Val Leu Arg Gly Thr Val Ala Asp Phe Pro Gly Phe Asp
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Glu Arg Ala Asp Ala Glu Thr Leu Arg Lys Ala Met Lys Gly Leu Gly
            20                  25                  30

Thr Asp Glu Glu Ser Ile Leu Thr Leu Leu Thr Ser Arg Ser Asn Ala
        35                  40                  45
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Gln Arg Gln Glu Ile Ala Val Ala Phe Lys Thr Leu Phe Gly Arg Asp
50 55 60

Leu Leu Asp Asp Leu Lys Ser Glu Leu Thr Gly Lys Phe Glu Lys Leu
65 70 75 80

Ile Val Ala Leu Met Lys Pro Ser Arg Leu Tyr Asp Ala Tyr Glu Leu
85 90 95

Lys His Ala Leu Lys Gly Ala Gly Thr Asp Glu Lys Val Leu Thr Glu
100 105 110

Ile Ile Ala Ser Arg Thr Pro Glu Glu Leu Arg Ala Ile Lys Gln Val
115 120 125

Tyr Glu Glu Glu Tyr Gly Ser Ser Leu Glu Asp Asp Val Val Gly Asp
130 135 140

Thr Ser Gly Tyr Tyr Gln Arg Met Leu Val Val Leu Leu Gln Ala Asn
145 150 155 160

Arg Asp Pro Asp Ala Arg Ile Asp Glu Ala Gln Val Glu Gln Asp Ala
165 170 175

Gln Ala Leu Phe Gln Ala Gly Glu Leu Lys Trp Gly Thr Asp Glu Glu
180 185 190

Lys Phe Ile Thr Ile Phe Gly Thr Arg Ser Val Ser His Leu Arg Arg
195 200 205

Val Phe Asp Lys Tyr Met Thr Ile Ser Gly Phe Gln Ile Glu Glu Thr
210 215 220

Ile Asp Arg Glu Thr Ser Gly Asn Leu Glu Gln Leu Leu Leu Ala Val
225 230 235 240

Val Lys Ser Ile Arg Ser Ile Pro Ala Tyr Leu Ala Glu Thr Leu Tyr
245 250 255

Tyr Ala Met Lys Gly Ala Gly Thr Asp Asp His Thr Leu Ile Arg Val
260 265 270

Val Val Ser Arg Ser Glu Ile Asp Leu Tyr Asn Ile Arg Lys Glu Phe
275 280 285

Arg Lys Asn Phe Gly Thr Ser Leu Tyr Ser Met Ile Lys Gly Asp Thr
290 295 300

Ser Gly Asp Tyr Lys Lys Ala Leu Leu Leu Leu Cys Gly Gly Glu Asp
305 310 315 320

Asp

<210> 5
<211> 321
<212> PRT
<213> Gallus sp.

<400> 5
Met Ala Lys Tyr Thr Arg Gly Thr Val Thr Ala Phe Ser Pro Phe Asp

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  1               5                  10                  15

Ala Arg Ala Asp Ala Glu Ala Leu Arg Lys Ala Met Lys Gly Met Gly
            20                  25                  30

Thr Asp Glu Glu Thr Ile Leu Lys Ile Leu Thr Ser Arg Asn Asn Ala
        35                  40                  45

Gln Arg Gln Glu Ile Ala Ser Ala Phe Lys Thr Leu Phe Gly Arg Asp
    50                  55                  60

Leu Val Asp Asp Leu Lys Ser Glu Leu Thr Gly Lys Phe Glu Thr Leu
65                  70                  75                  80

Met Val Ser Leu Met Arg Pro Ala Arg Ile Phe Asp Ala His Ala Leu
                85                  90                  95

Lys His Ala Ile Lys Gly Ala Gly Thr Asn Glu Lys Val Leu Thr Glu
            100                 105                 110

Ile Leu Ala Ser Arg Thr Pro Ala Glu Val Gln Asn Ile Lys Gln Val
        115                 120                 125

Tyr Met Gln Glu Tyr Glu Ala Asn Leu Glu Asp Lys Ile Thr Gly Glu
    130                 135                 140

Thr Ser Gly His Phe Gln Arg Leu Leu Val Val Leu Leu Gln Ala Asn
145                 150                 155                 160

Arg Asp Pro Asp Gly Arg Val Asp Glu Ala Leu Val Glu Lys Asp Ala
                165                 170                 175

Gln Val Leu Phe Arg Ala Gly Glu Leu Lys Trp Gly Thr Asp Glu Glu
            180                 185                 190

Thr Phe Ile Thr Ile Leu Gly Thr Arg Ser Val Ser His Leu Arg Arg
        195                 200                 205

Val Phe Asp Lys Tyr Met Thr Ile Ser Gly Phe Gln Ile Glu Glu Thr
    210                 215                 220

Ile Asp Arg Glu Thr Ser Gly Asp Leu Glu Lys Leu Leu Leu Ala Val
225                 230                 235                 240

Val Lys Cys Ile Arg Ser Val Pro Ala Tyr Phe Ala Glu Thr Leu Tyr
                245                 250                 255

Tyr Ser Met Lys Gly Ala Gly Thr Asp Asp Asp Thr Leu Ile Arg Val
            260                 265                 270

Met Val Ser Arg Ser Glu Ile Asp Leu Leu Asp Ile Arg His Glu Phe
        275                 280                 285

Arg Lys Asn Phe Ala Lys Ser Leu Tyr Gln Met Ile Gln Lys Asp Thr
    290                 295                 300

Ser Gly Asp Tyr Arg Lys Ala Leu Leu Leu Leu Cys Gly Gly Asp Asp
305                 310                 315                 320

Glu
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R296USP1.APP.txt

<210> 6
<211> 448
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:fusion protein

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Met Ala Leu Arg Gly Thr Val Thr Asp Phe Ser Gly Phe Asp Gly Arg
1 5 10 15

Ala Asp Ala Glu Val Leu Arg Lys Ala Met Lys Gly Leu Gly Thr Asp
20 25 30

Glu Asp Ser Ile Leu Asn Leu Leu Thr Ala Arg Ser Asn Ala Gln Arg
35 40 45

Gln Gln Ile Ala Glu Glu Phe Lys Thr Leu Phe Gly Arg Asp Leu Val
50 55 60

Asn Asp Met Lys Ser Glu Leu Thr Gly Lys Phe Glu Lys Leu Ile Val
65 70 75 80

Ala Leu Met Lys Pro Ser Arg Leu Tyr Asp Ala Tyr Glu Leu Lys His
85 90 95

Ala Leu Lys Gly Ala Gly Thr Asp Glu Lys Val Leu Thr Glu Ile Ile
100 105 110

Ala Ser Arg Thr Pro Glu Glu Leu Arg Ala Ile Lys Gln Ala Tyr Glu
115 120 125

Glu Glu Tyr Gly Ser Asn Leu Glu Asp Asp Val Val Gly Asp Thr Ser
130 135 140

Gly Tyr Tyr Gln Arg Met Leu Val Val Leu Leu Gln Ala Asn Arg Asp
145 150 155 160

Pro Asp Cys Ala Ile Asp Asp Ala Gln Val Glu Leu Asp Ala Gln Ala
165 170 175

Leu Phe Gln Ala Gly Glu Leu Lys Trp Gly Thr Asp Glu Glu Lys Phe
180 185 190

Ile Thr Ile Leu Gly Thr Arg Ser Val Ser His Leu Arg Arg Val Phe
195 200 205

Asp Lys Tyr Met Thr Ile Ser Gly Phe Gln Ile Glu Glu Thr Ile Asp
210 215 220

Arg Glu Thr Ser Gly Asn Leu Glu Asn Leu Leu Leu Ala Val Val Lys
225 230 235 240

Ser Ile Arg Ser Ile Pro Ala Tyr Leu Ala Glu Thr Leu Tyr Tyr Ala
245 250 255

Met Lys Gly Ala Gly Thr Asp Asp His Thr Leu Ile Arg Val Ile Val
260 265 270

Ser Arg Ser Glu Ile Asp Leu Phe Asn Ile Arg Lys Glu Phe Arg Lys
275 280 285

Asn Phe Ala Thr Ser Leu Tyr Ser Met Ile Lys Gly Asp Thr Ser Gly
290 295 300

Asp Tyr Lys Lys Ala Leu Leu Leu Leu Ser Gly Gly Glu Asp Asp Leu
305 310 315 320

Ala Ser Gln Ala Val Asp Asn Lys Phe Asn Lys Glu Gln Gln Asn Ala
325 330 335

Phe Tyr Glu Ile Leu His Leu Pro Asn Leu Asn Glu Glu Gln Arg Asn
340 345 350

Ala Phe Ile Gln Ser Leu Lys Asp Asp Pro Ser Gln Ser Ala Asn Leu
355 360 365

Leu Ala Glu Ala Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys Val Asp
370 375 380

Asn Lys Phe Asn Lys Glu Gln Gln Asn Ala Phe Tyr Glu Ile Leu His
385 390 395 400

Leu Pro Asn Leu Asn Glu Glu Gln Arg Asn Ala Phe Ile Gln Ser Leu
405 410 415

Lys Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala Lys Lys
420 425 430

Leu Asn Asp Ala Gln Ala Pro Lys Val Asp Ala Asn Ser Ser Ser Val
435 440 445

<210> 7
<211> 346
<212> PRT
<213> Homo sapiens

<400> 7
Met Ala Met Val Ser Glu Phe Leu Lys Gln Ala Trp Phe Ile Glu Asn
1 5 10 15

Glu Glu Gln Glu Tyr Val Gln Thr Val Lys Ser Ser Lys Gly Gly Pro
20 25 30

Gly Ser Ala Val Ser Pro Tyr Pro Thr Phe Asn Pro Ser Ser Asp Val
35 40 45

Ala Ala Leu His Lys Ala Ile Met Val Lys Gly Val Asp Glu Ala Thr
50 55 60

Ile Ile Asp Ile Leu Thr Lys Arg Asn Asn Ala Gln Arg Gln Gln Ile
65 70 75 80

Lys Ala Ala Tyr Leu Gln Glu Thr Gly Lys Pro Leu Asp Glu Thr Leu
85 90 95

Lys Lys Ala Leu Thr Gly His Leu Glu Glu Val Val Leu Ala Leu Leu
100 105 110

Lys Thr Pro Ala Gln Phe Asp Ala Asp Glu Leu Arg Ala Ala Met Lys
115 120 125

Gly Leu Gly Thr Asp Glu Asp Thr Leu Ile Glu Ile Leu Ala Ser Arg
130 135 140

Thr Asn Lys Glu Ile Arg Asp Ile Asn Arg Val Tyr Arg Glu Glu Leu
145 150 155 160

Lys Arg Asp Leu Ala Lys Asp Ile Thr Ser Asp Thr Ser Gly Asp Phe
165 170 175

Arg Asn Ala Leu Leu Ser Leu Ala Lys Gly Asp Arg Ser Glu Asp Phe
180 185 190

Gly Val Asn Glu Asp Leu Ala Asp Ser Asp Ala Arg Ala Leu Tyr Glu
195 200 205

Ala Gly Glu Arg Arg Lys Gly Thr Asp Val Asn Val Phe Asn Thr Ile
210 215 220

Leu Thr Thr Arg Ser Tyr Pro Gln Leu Arg Arg Val Phe Gln Lys Tyr
225 230 235 240

Thr Lys Tyr Ser Lys His Asp Met Asn Lys Val Leu Asp Leu Glu Leu
245 250 255

Lys Gly Asp Ile Glu Lys Cys Leu Thr Ala Ile Val Lys Cys Ala Thr
260 265 270

Ser Lys Pro Ala Phe Phe Ala Glu Lys Leu His Gln Ala Met Lys Gly
275 280 285

Val Gly Thr Arg His Lys Ala Leu Ile Arg Ile Met Val Ser Arg Ser
290 295 300

Glu Ile Asp Met Asn Asp Ile Lys Ala Phe Tyr Gln Lys Met Tyr Gly
305 310 315 320

Ile Ser Leu Cys Gln Ala Ile Leu Asp Glu Thr Lys Gly Asp Tyr Glu
325 330 335

Lys Ile Leu Val Ala Leu Cys Gly Gly Asn
340 345

<210> 8
<211> 339
<212> PRT
<213> Homo sapiens

<400> 8

Met Ser Thr Val His Glu Ile Leu Cys Lys Leu Ser Leu Glu Gly Asp
1 5 10 15

His Ser Thr Pro Pro Ser Ala Tyr Gly Ser Val Lys Ala Tyr Thr Asn
20 25 30

Phe Asp Ala Glu Arg Asp Ala Leu Asn Ile Glu Thr Ala Ile Lys Thr
35 40 45

Lys Gly Val Asp Glu Val Thr Ile Val Asn Ile Leu Thr Asn Arg Ser

50 55 60

Asn Ala Gln Arg Gln Asp Ile Ala Phe Ala Tyr Gln Arg Arg Thr Lys
65 70 75 80

Lys Glu Leu Ala Ser Ala Leu Lys Ser Ala Leu Ser Gly His Leu Glu
85 90 95

Thr Val Ile Leu Gly Leu Leu Lys Thr Pro Ala Gln Tyr Asp Ala Ser
100 105 110

Glu Leu Lys Ala Ser Met Lys Gly Leu Gly Thr Asp Glu Asp Ser Leu
115 120 125

Ile Glu Ile Ile Cys Ser Arg Thr Asn Gln Glu Leu Gln Glu Ile Asn
130 135 140

Arg Val Tyr Lys Glu Met Tyr Lys Thr Asp Leu Glu Lys Asp Ile Ile
145 150 155 160

Ser Asp Thr Ser Gly Asp Phe Arg Lys Leu Met Val Ala Leu Ala Lys
165 170 175

Gly Arg Arg Ala Glu Asp Gly Ser Val Ile Asp Tyr Glu Leu Ile Asp
180 185 190

Gln Asp Ala Arg Asp Leu Tyr Asp Ala Gly Val Lys Arg Lys Gly Thr
195 200 205

Asp Val Pro Lys Trp Ile Ser Ile Met Thr Glu Arg Ser Val Pro His
210 215 220

Leu Gln Lys Val Phe Asp Arg Tyr Lys Ser Tyr Ser Pro Tyr Asp Met
225 230 235 240

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

Leu Asn Leu Val Gln Cys Ile Gln Asn Lys Pro Leu Tyr Phe Ala Asp
260 265 270

Arg Leu Tyr Asp Ser Met Lys Gly Lys Gly Thr Arg Asp Lys Val Leu
275 280 285

Ile Arg Ile Met Val Ser Arg Ser Glu Val Asp Met Leu Lys Ile Arg
290 295 300

Ser Glu Phe Lys Arg Lys Tyr Gly Lys Ser Leu Tyr Tyr Tyr Ile Gln
305 310 315 320

Gln Asp Thr Lys Gly Asp Tyr Gln Lys Ala Leu Leu Tyr Leu Cys Gly
325 330 335

Gly Asp Asp

<210> 9
<211> 673
<212> PRT
<213> Homo sapiens

<400> 9

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Met Ala Lys Pro Ala Gln Gly Ala Lys Tyr Arg Gly Ser Ile His Asp
  1               5                  10                  15

Phe Pro Gly Phe Asp Pro Asn Gln Asp Ala Glu Ala Leu Tyr Thr Ala
            20                  25                  30

Met Lys Gly Phe Gly Ser Asp Lys Glu Ala Ile Leu Asp Ile Ile Thr
        35                  40                  45

Ser Arg Ser Asn Arg Gln Arg Gln Glu Val Cys Gln Ser Tyr Lys Ser
    50                  55                  60

Leu Tyr Gly Lys Asp Leu Ile Ala Asp Leu Lys Tyr Glu Leu Thr Gly
65                  70                  75                  80

Lys Phe Glu Arg Leu Ile Val Gly Leu Met Arg Pro Pro Ala Tyr Cys
                85                  90                  95

Asp Ala Lys Glu Ile Lys Asp Ala Ile Ser Gly Ile Gly Thr Asp Glu
            100                 105                 110

Lys Cys Leu Ile Glu Ile Leu Ala Ser Arg Thr Asn Glu Gln Met His
        115                 120                 125

Gln Leu Val Ala Ala Tyr Lys Asp Ala Tyr Glu Arg Asp Leu Glu Ala
    130                 135                 140

Asp Ile Ile Gly Asp Thr Ser Gly His Phe Gln Lys Met Leu Val Val
145                 150                 155                 160

Leu Leu Gln Gly Thr Arg Glu Glu Asp Asp Val Val Ser Glu Asp Leu
                165                 170                 175

Val Gln Gln Asp Val Gln Asp Leu Tyr Glu Ala Gly Glu Leu Lys Trp
            180                 185                 190

Gly Thr Asp Glu Ala Gln Phe Ile Tyr Ile Leu Gly Asn Arg Ser Lys
        195                 200                 205

Gln His Leu Arg Leu Val Phe Asp Glu Tyr Leu Lys Thr Thr Gly Lys
    210                 215                 220

Pro Ile Glu Ala Ser Ile Arg Gly Glu Leu Ser Gly Asp Phe Glu Lys
225                 230                 235                 240

Leu Met Leu Ala Val Val Lys Cys Ile Arg Ser Thr Pro Glu Tyr Phe
                245                 250                 255

Ala Glu Arg Leu Phe Lys Ala Met Lys Gly Leu Gly Thr Arg Asp Asn
            260                 265                 270

Thr Leu Ile Arg Ile Met Val Ser Arg Ser Glu Leu Asp Met Leu Asp
        275                 280                 285

Ile Arg Glu Ile Phe Arg Thr Lys Tyr Glu Lys Ser Leu Tyr Ser Met
    290                 295                 300

Ile Lys Asn Asp Thr Ser Gly Glu Tyr Lys Lys Thr Leu Leu Lys Leu
305                 310                 315                 320

Ser Gly Gly Asp Asp Asp Ala Ala Gly Gln Phe Phe Pro Glu Ala Ala
                325                 330                 335
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Gln Val Ala Tyr Gln Met Trp Glu Leu Ser Ala Val Ala Arg Val Glu
            340                 345                 350

Leu Lys Gly Thr Val Arg Pro Ala Asn Asp Phe Asn Pro Asp Ala Asp
        355                 360                 365

Ala Lys Ala Leu Arg Lys Ala Met Lys Gly Leu Gly Thr Asp Glu Asp
    370                 375                 380

Thr Ile Ile Asp Ile Ile Thr His Arg Ser Asn Val Gln Arg Gln Gln
385                 390                 395                 400

Ile Arg Gln Thr Phe Lys Ser His Phe Gly Arg Asp Leu Met Thr Asp
                405                 410                 415

Leu Lys Ser Glu Ile Ser Gly Asp Leu Ala Arg Leu Ile Leu Gly Leu
            420                 425                 430

Met Met Pro Pro Ala His Tyr Asp Ala Lys Gln Leu Lys Lys Ala Met
        435                 440                 445

Glu Gly Ala Gly Thr Asp Glu Lys Ala Leu Ile Glu Ile Leu Ala Thr
    450                 455                 460

Arg Thr Asn Ala Glu Ile Arg Ala Ile Asn Glu Ala Tyr Lys Glu Asp
465                 470                 475                 480

Tyr His Lys Ser Leu Glu Asp Ala Leu Ser Ser Asp Thr Ser Gly His
                485                 490                 495

Phe Arg Arg Ile Leu Ile Ser Leu Ala Thr Gly His Arg Glu Glu Gly
            500                 505                 510

Gly Glu Asn Leu Asp Gln Ala Arg Glu Asp Ala Gln Val Ala Ala Glu
        515                 520                 525

Ile Leu Glu Ile Ala Asp Thr Pro Ser Gly Asp Lys Thr Ser Leu Glu
    530                 535                 540

Thr Arg Phe Met Thr Ile Leu Cys Thr Arg Ser Tyr Pro His Leu Arg
545                 550                 555                 560

Arg Val Phe Gln Glu Phe Ile Lys Met Thr Asn Tyr Asp Val Glu His
                565                 570                 575

Thr Ile Lys Lys Glu Met Ser Gly Asp Val Arg Asp Ala Phe Val Ala
            580                 585                 590

Ile Val Gln Ser Val Lys Asn Lys Pro Leu Phe Phe Ala Asp Lys Leu
        595                 600                 605

Tyr Lys Ser Met Lys Gly Ala Gly Thr Asp Glu Lys Thr Leu Thr Arg
    610                 615                 620

Ile Met Val Ser Arg Ser Glu Ile Asp Leu Leu Asn Ile Arg Arg Glu
625                 630                 635                 640

Phe Ile Glu Lys Tyr Asp Lys Ser Leu His Gln Ala Ile Glu Gly Asp
                645                 650                 655

Thr Ser Gly Asp Phe Leu Lys Ala Leu Leu Ala Leu Cys Gly Gly Glu
            660                 665                 670
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Asp

<210> 10
<211> 319
<212> PRT
<213> Homo sapiens

<400> 10

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

Met Glu Asp Ala Gln Thr Leu Arg Lys Ala Met Lys Gly Leu Gly Thr
             20                  25                  30

Asp Glu Asp Ala Ile Ile Ser Val Leu Ala Tyr Arg Asn Thr Ala Gln
         35                  40                  45

Arg Gln Glu Ile Arg Thr Ala Tyr Lys Ser Thr Ile Gly Arg Asp Leu
     50                  55                  60

Ile Asp Asp Leu Lys Ser Glu Leu Ser Gly Asn Phe Glu Gln Val Ile
 65                  70                  75                  80

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

Arg Ala Met Lys Gly Ala Gly Thr Asp Glu Gly Cys Leu Ile Glu Ile
            100                 105                 110

Leu Ala Ser Arg Thr Pro Glu Glu Ile Arg Arg Ile Ser Gln Thr Tyr
        115                 120                 125

Gln Gln Gln Tyr Gly Arg Ser Leu Glu Asp Asp Ile Arg Ser Asp Thr
    130                 135                 140

Ser Phe Met Phe Gln Arg Val Leu Val Ser Leu Ser Ala Gly Gly Arg
145                 150                 155                 160

Asp Glu Gly Asn Tyr Leu Asp Asp Ala Leu Val Arg Gln Asp Ala Gln
                165                 170                 175

Asp Leu Tyr Glu Ala Gly Glu Lys Lys Trp Gly Thr Asp Glu Val Lys
            180                 185                 190

Phe Leu Thr Val Leu Cys Ser Arg Asn Arg Asn His Leu Leu His Val
        195                 200                 205

Phe Asp Glu Tyr Lys Arg Ile Ser Gln Lys Asp Ile Glu Gln Ser Ile
    210                 215                 220

Lys Ser Glu Thr Ser Gly Ser Phe Glu Asp Ala Leu Leu Ala Ile Val
225                 230                 235                 240

Lys Cys Met Arg Asn Lys Ser Ala Tyr Phe Ala Glu Lys Leu Tyr Lys
                245                 250                 255

Ser Met Lys Gly Leu Gly Thr Asp Asp Asn Thr Leu Ile Arg Val Met
            260                 265                 270
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Val Ser Arg Ala Glu Ile Asp Met Leu Asp Ile Arg Ala His Phe Lys
275 280 285

Arg Leu Tyr Gly Lys Ser Leu Tyr Ser Phe Ile Lys Gly Asp Thr Ser
290 295 300

Gly Asp Tyr Arg Lys Val Leu Leu Val Leu Cys Gly Gly Asp Asp
305 310 315

<210> 11
<211> 327
<212> PRT
<213> Homo sapiens

<400> 11
Met Ala Trp Trp Lys Ala Trp Ile Glu Gln Glu Gly Val Thr Val Lys
1 5 10 15

Ser Ser Ser His Phe Asn Pro Asp Pro Asp Ala Glu Thr Leu Tyr Lys
20 25 30

Ala Met Lys Gly Ile Gly Thr Asn Glu Gln Ala Ile Ile Asp Val Leu
35 40 45

Thr Lys Arg Ser Asn Thr Gln Arg Gln Gln Ile Ala Lys Ser Phe Lys
50 55 60

Ala Gln Phe Gly Lys Asp Leu Thr Glu Thr Leu Lys Ser Glu Leu Ser
65 70 75 80

Gly Lys Phe Glu Arg Leu Ile Val Ala Leu Met Tyr Pro Pro Tyr Arg
85 90 95

Tyr Glu Ala Lys Glu Leu His Asp Ala Met Lys Gly Leu Gly Thr Lys
100 105 110

Glu Gly Val Ile Ile Glu Ile Leu Ala Ser Arg Thr Lys Asn Gln Leu
115 120 125

Arg Glu Ile Met Lys Ala Tyr Glu Glu Asp Tyr Gly Ser Ser Leu Glu
130 135 140

Glu Asp Ile Gln Ala Asp Thr Ser Gly Tyr Leu Glu Arg Ile Leu Val
145 150 155 160

Cys Leu Leu Gln Gly Ser Arg Asp Asp Val Ser Ser Phe Val Asp Pro
165 170 175

Ala Leu Ala Leu Gln Asp Ala Gln Asp Leu Tyr Ala Ala Gly Glu Lys
180 185 190

Ile Arg Gly Thr Asp Glu Met Lys Phe Ile Thr Ile Leu Cys Thr Arg
195 200 205

Ser Ala Thr His Leu Leu Arg Val Phe Glu Glu Tyr Glu Lys Ile Ala
210 215 220

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

Glu Glu Ala Met Leu Thr Val Val Lys Cys Thr Gln Asn Leu His Ser

245 250 255

Tyr Phe Ala Glu Arg Leu Tyr Tyr Ala Met Lys Gly Ala Gly Thr Arg
260 265 270

Asp Gly Thr Leu Ile Arg Asn Ile Val Ser Arg Ser Glu Ile Asp Leu
275 280 285

Asn Leu Ile Lys Cys His Phe Lys Lys Met Tyr Gly Lys Thr Leu Ser
290 295 300

Ser Met Ile Met Glu Asp Thr Ser Gly Asp Tyr Lys Asn Ala Leu Leu
305 310 315 320

Ser Leu Val Gly Ser Asp Pro
325

<210> 12
<211> 466
<212> PRT
<213> Homo sapiens

<400> 12
Met Ser Tyr Pro Gly Tyr Pro Pro Thr Gly Tyr Pro Pro Phe Pro Gly
1 5 10 15

Tyr Pro Pro Ala Gly Gln Glu Ser Ser Phe Pro Pro Ser Gly Gln Tyr
20 25 30

Pro Tyr Pro Ser Gly Phe Pro Pro Met Gly Gly Gly Ala Tyr Pro Gln
35 40 45

Val Pro Ser Ser Gly Tyr Pro Gly Ala Gly Gly Tyr Pro Ala Pro Gly
50 55 60

Gly Tyr Pro Ala Pro Gly Gly Tyr Pro Gly Ala Pro Gln Pro Gly Gly
65 70 75 80

Ala Pro Ser Tyr Pro Gly Val Pro Pro Gly Gln Gly Phe Gly Val Pro
85 90 95

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25

Claims

1. A device for binding a target entity onto a bait entity that is immobilized on said device, comprising:

a) a lipid layer which comprises one or more lipids, said lipid layer having a negative net charge in an aqueous solution at a neutral pH;

b) a two-dimensional matrix of anchoring complexes that are bound to said lipid layer, wherein each of said anchoring complexes comprises:

(i) a fusion complex comprising an Annexin protein fused to a partner molecule, wherein :

- said Annexin protein is bound to said lipid layer, and
- said partner molecule consists of an organic or a mineral compound;

(ii) a bait entity selected from:

- said partner molecule that is fused to said Annexin protein;
- a molecule that is covalently or non-covalently bound to said partner molecule; or
- a molecule that is indirectly bound to said partner molecule through one or more intermediate molecules that are covalently or non-covalently bound to said partner molecule.

2. The device of claim 1, wherein said lipid layer is selected from a lipid bilayer, a lipid mono-layer or the external lipid layer of a liposome.

3. The device of claim 1, wherein said lipid layer comprises a combination of (i) one or more lipids with (ii) one or more phospholipids, said one or more phospholipids having a negative net charge in an aqueous solution at a neutral pH.

4. The device of claim 1, wherein said lipid layer comprises a combination of (i) one or more lipids with (ii) one or more phospholipids, said one or more phospholipids having a negative net charge in an aqueous solution at a neutral pH and said lipid layer has a content in said one or more phospholipids with a negative net charge from 2% to 100% by weight, based % on the total weight of said phospholipids bi-layer.

5. The device of claim 1, wherein the lipids (i) are selected from lecithine, DMPC, DOPC, POPC, egg-lecithine, DOPS, POPS, DOPA, DOPG, cardiolipin or DOTAP.

6. The device of claim 3, wherein the one or more phospholipids (ii) are selected from fatty acid esters of glycerophosphoserine, glycerophosphocholine, glycerophosphoglycerol or glycerophosphoethanolamine.

7. The device of claim 3, wherein the one or more phospholipids (ii) are selected from DMPS, DOPS, POPS, DOPA, DOPG, cardiolipin or a mixture of them.

8. The device of claim 7, wherein the phospholipids are brain lipid extracts.

9. The device of claim 1, wherein the Annexin protein of the fusion complex is selected from Annexin-A5 proteins consisting of the amino acid sequences SEQ ID N°1-5 and 7-18 and functionally derivatives thereof.

10. The device of claim 1, wherein, in the fusion complex between the Annexin protein and a partner protein, said partner protein consists of a protein having affinity for the Fc moiety of an antibody.

11. The device of claim 1, wherein, in the fusion complex between the Annexin protein and a partner protein, said partner protein consists of the ZZ domain of the A protein from *Staphylococcus aureus*.

12. The device of claim 1, wherein, in the fusion complex between the Annexin protein and a partner protein, said partner protein consists of the G protein from *Streptococcus*.

13. The device of claim 1, wherein, in the fusion complex between the Annexin protein and a partner protein, said partner protein consists of an antibody fragment comprising the CDRs of an antibody.

14. The device of claim 1, wherein, in the fusion complex between the Annexin protein and a partner protein, said partner protein consists of an antibody fragment consisting of Fab or scFv.

15. The device of claim 1, wherein, in the fusion complex between the Annexin protein and a partner protein, said partner protein consists of said bait molecule.

16. The device of claim 1, wherein said anchoring complex consists of:
(i) a fusion complex between an Annexin protein and an antibody fragment comprising the CDR domains of a target-specific antibody.

17. The device of claim 1, wherein said anchoring complex consists of:
(i) a fusion complex between an Annexin protein and an antibody fragment comprising the CDR domains of a bait-specific antibody; and
(ii) the bait molecule which is non covalently bound to the bait-specific antibody fragment of said fusion complex.

18. The device of claim 1, wherein said anchoring complex consists of:
(i) a fusion complex between an Annexin protein and a protein having affinity for the Fc portion of an antibody; and
(ii) an antibody which is non-covalently bound by its Fc portion onto said fusion complex, said antibody being said bait protein.

19. The device of claim 1, wherein said anchoring complex consists of:
(i) a fusion complex between an Annexin protein and a protein having affinity for the Fc portion of an antibody;
(ii) a bait-specific antibody which is non-covalently bound by its Fc portion onto said fusion complex; and
(iii) the bait protein which is non-covalently bound onto said bait-specific antibody.

20. The device of claim 1 , wherein said bait molecule is selected from growth factor receptors, hormone receptors, neurotransmitter receptors, catecholamine receptors,

amino acid derivative receptors, cytokine receptors, extracellular matrix receptors, lectins, cytokines, serpins, proteases, kinases, phosphatases, ras-like GTPases, hydrolases, steroid hormone receptors, transcription factors, heat-shock transcription factors, DNA-binding proteins, zinc-finger proteins, leucine-zipper proteins, homeodomain proteins, intracellular signal transduction modulators and effectors, apoptosis-related factors, DNA synthesis factors, DNA repair factors, DNA recombination factors, cell-surface antigens, hepatitis C virus (HCV) proteases or HIV proteases and antibodies, oligonucleotides, oligosides, small molecules, polymers, inorganic molecules or their aggregates.

21. The device of claim 1, wherein, in said fusion complex, the Annexin protein and the partner protein are covalently linked together through a normal peptide bond.

22. The device of claim 1, wherein said fusion complex consists of a recombinant fusion protein produced by recombinant DNA technology methods.

23. The device of claim 1, wherein, in said fusion complex, the Annexin protein and the partner protein are covalently linked together by a chemical bond other than a peptide bond.

24. The device of claim 1 , wherein, in said fusion complex, the Annexin protein and the partner protein are covalently linked together through a chemical bond between a cysteine residue of the Annexin protein and a cysteine-reactive group of the partner protein.

25. The device of claim 1, wherein said device also comprises a substrate and wherein said lipid layer is coated on said substrate.

26. The device of claim 25, wherein said substrate consists of a solid substrate.

27. The device of claim 26, wherein said solid substrate is selected from mica, silicon, mineral glass or gold.

28. The device of claim 25, wherein said substrate consists of the air-liquid interface of a liquid medium.

29. A system for detecting the binding of a target molecule onto a bait molecule, wherein said system comprises a plurality of detection devices according to any one of claims 1 to 28.

30. The system of claim 29, wherein each detection device of said plurality of detection devices comprises a unique bait protein.

31. The system of claim 29, wherein two distinct detection devices that are included therein comprise distinct bait molecules.

32. A method for detecting the binding of a target molecule onto a bait molecule, wherein said method comprises the steps of:
a) providing a sample to be tested;
b) bringing into contact the sample to be tested with the detection device according to any one of claims 1 to 28 or with the detection system according to any one of claims 29 to 31; and
c) detecting the complexes eventually formed between (i) the bait molecule(s) contained in said detection device or in said detection system and (ii) the target molecule(s) eventually present within said tested sample.

33. A method for assaying for the presence of a target molecule in a sample comprising the steps of :
a) providing a fusion complex between an Annexin protein and a bait molecule which binds to said target molecule;
b) mixing the sample with said fusion complex, whereby complexes between the bait moiety of said fusion complex and the target molecule are allowed to be formed;
c) immobilizing the fusion complexes obtained at step b), eventually under the form of complexes with said target molecule, at the surface of a lipid bi-layer coating a solid

substrate, said lipid bi-layer comprising a combination of (i) one or more lipids with (ii) one or more phospholipids, said one or more phospholipids having a negative net charge in an aqueous solution at a neutral pH; and

d) detecting the complexes that are formed between the bait moiety of said fusion complex and the target molecule when said target molecule is present in said sample.

34. The method of claim 33, wherein the fusion complex comprises a bait molecule consisting of a protein having affinity with the Fc moiety of an antibody.

35. The method of claim 33, wherein the fusion complex comprises a bait molecule consisting of the ZZ domain of the protein A from *Staphylococcus aureus*.

36. The method of claim 33, wherein the fusion complex comprises a bait molecule consisting of a fragment of protein G from *Streptococcus sp.*

37. The method of claim 33, wherein the fusion complex comprises a bait molecule consisting of an antibody fragment comprising the CDR domains of a target-specific antibody.

38. The method of claim 33, wherein the fusion complex comprises a bait molecule selected from growth factor receptors, hormone receptors, neurotransmitter receptors, catecholamine receptors, amino acid derivative receptors, cytokine receptors, extracellular matrix receptors, lectins, cytokines, serpins, proteases, kinases, phosphatases, ras-like GTPases, hydrolases, steroid hormone receptors, transcription factors, heat-shock transcription factors, DNA-binding proteins, zinc-finger proteins, leucine-zipper proteins, homeodomain proteins, intracellular signal transduction modulators and effectors, apoptosis-related factors, DNA synthesis factors, DNA repair factors, DNA recombination factors, cell-surface antigens, hepatitis C virus (HCV) proteases or HIV proteases and antibodies, oligonucleotides, oligosides, small molecules, polymers, inorganic molecules or their aggregates.

39. A vector for targeting therapeutical molecules comprising a device according to claim 1, wherein said device consists of lipid vesicles having:

a) an outer lipid layer comprising one or more lipids, said lipid layer having a negative net charge in an aqueous solution at a neutral pH;

b) a two-dimensional matrix of anchoring complexes that are bound to said lipid layer, wherein each of said anchoring complexes comprises:

(i) a fusion complex comprising an Annexin protein fused to a partner molecule, wherein :

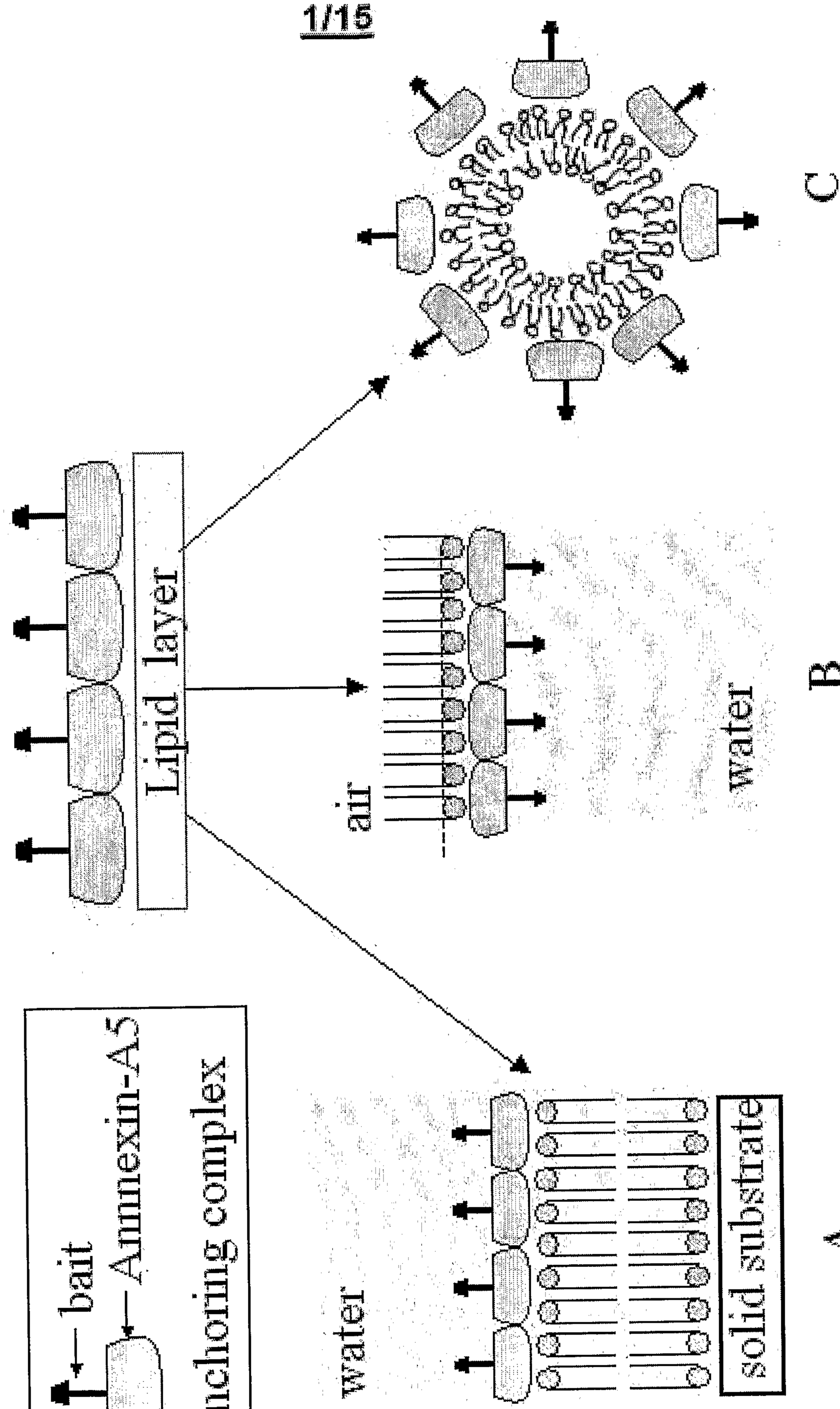
- said Annexin protein is bound to said lipid layer, and
- said partner molecule consists of an organic or a mineral compound;

(ii) a bait entity selected from :

- said partner molecule that is fused to said Annexin protein;
- a molecule that is covalently or non-covalently bound to said partner molecule; or
- a molecule that is indirectly bound to said partner molecule through one or more intermediate molecules that are covalently or non-covalently bound to said partner molecule; and

c) in the inner part of said lipid vesicles, a liquid medium comprising one or more pharmaceutically active molecules that are dissolved or that are suspended therein.

1/15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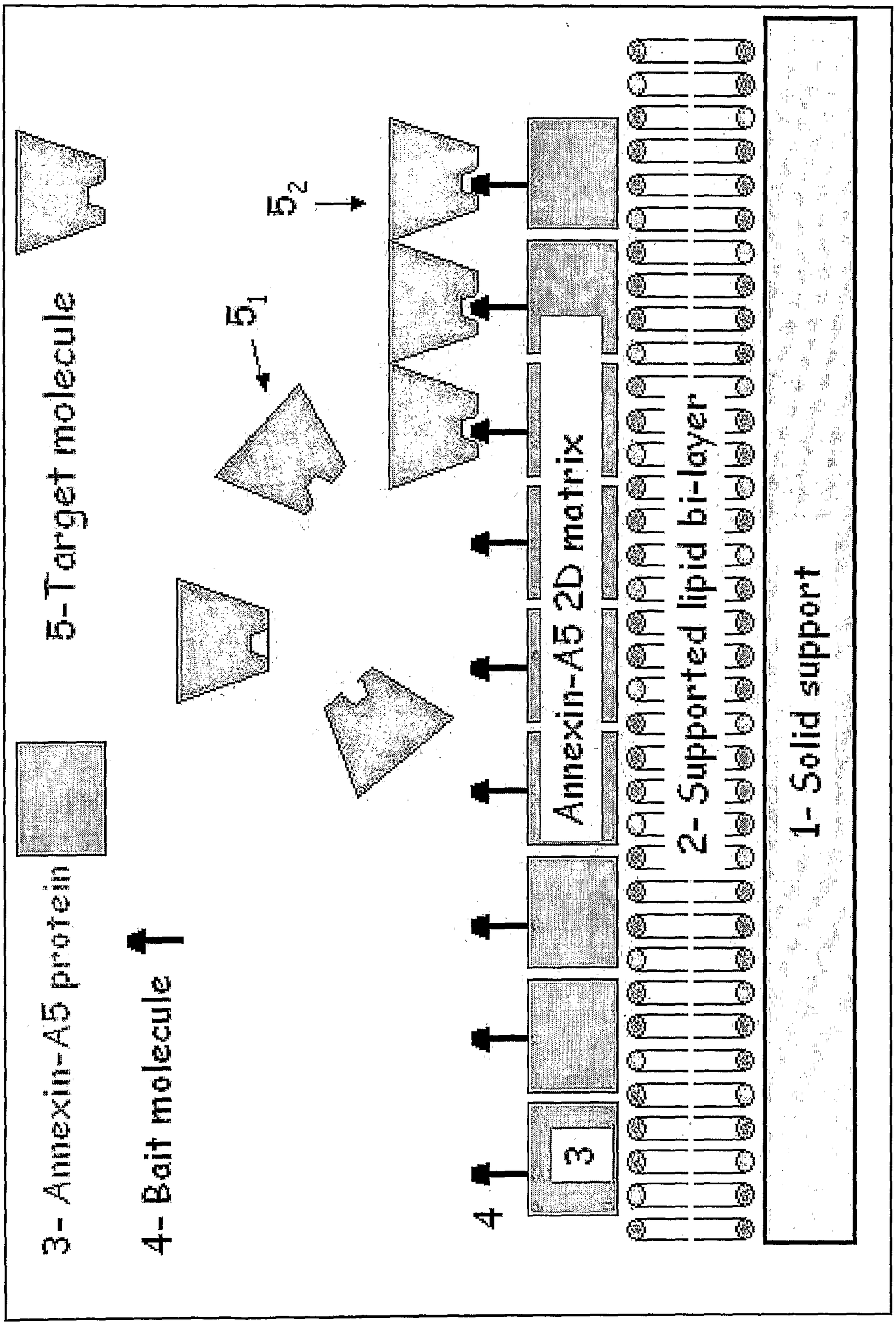
Anchoring complex
A
B
C

Figure 1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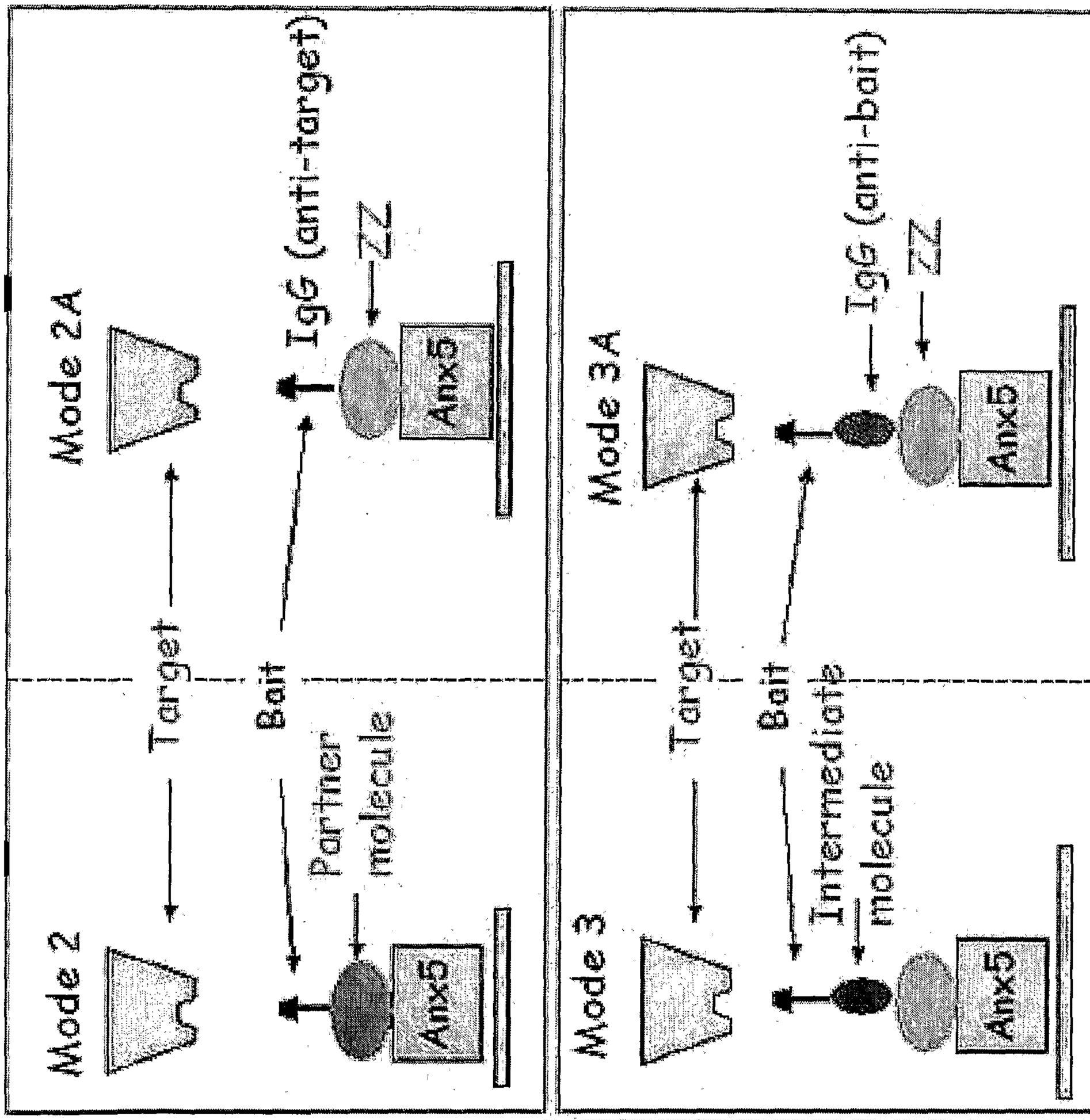
5_2

Figure2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Bait
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ZZ
Anx5
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ZZ
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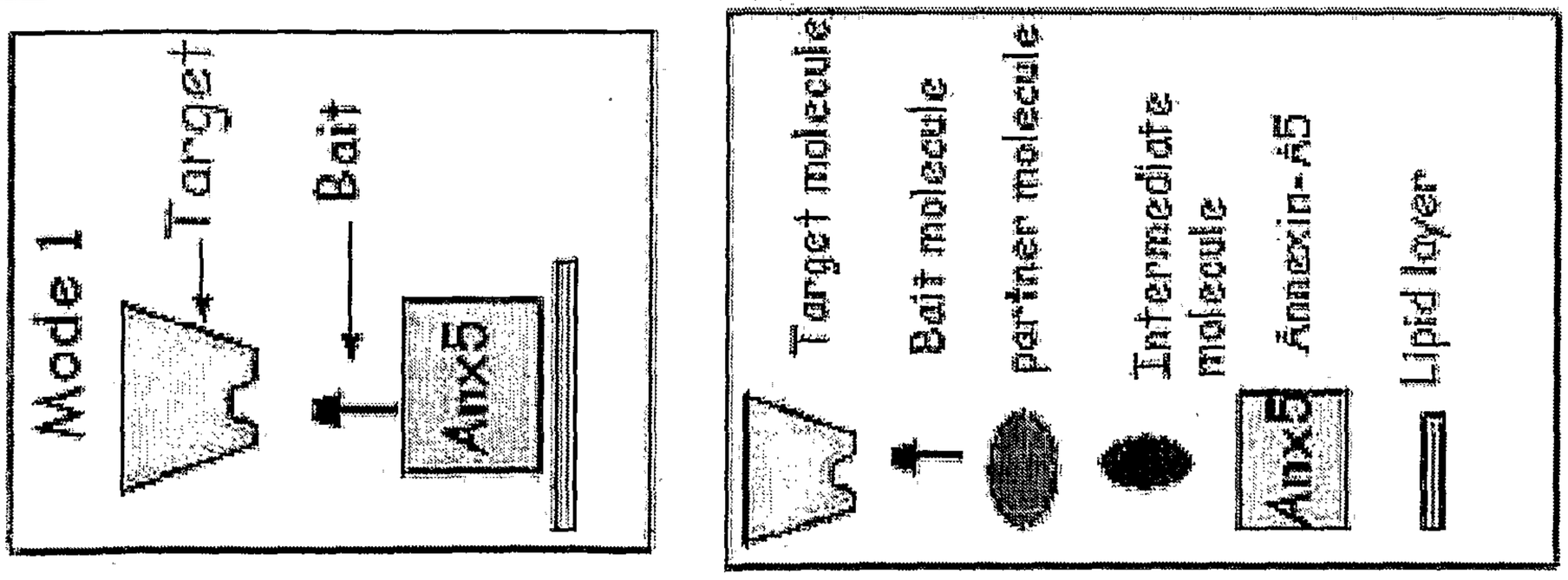
Mode 2
Target
Bait
Partner molecule
Anx5
Mode 3
Target
Bait
Intermediate molecule
Anx5

Figure 3


Mode 1
Target
Bait
Anx5
Target molecule
Bait molecule
partner molecule
Intermediate molecule
Annexin-A5
Lipid layer

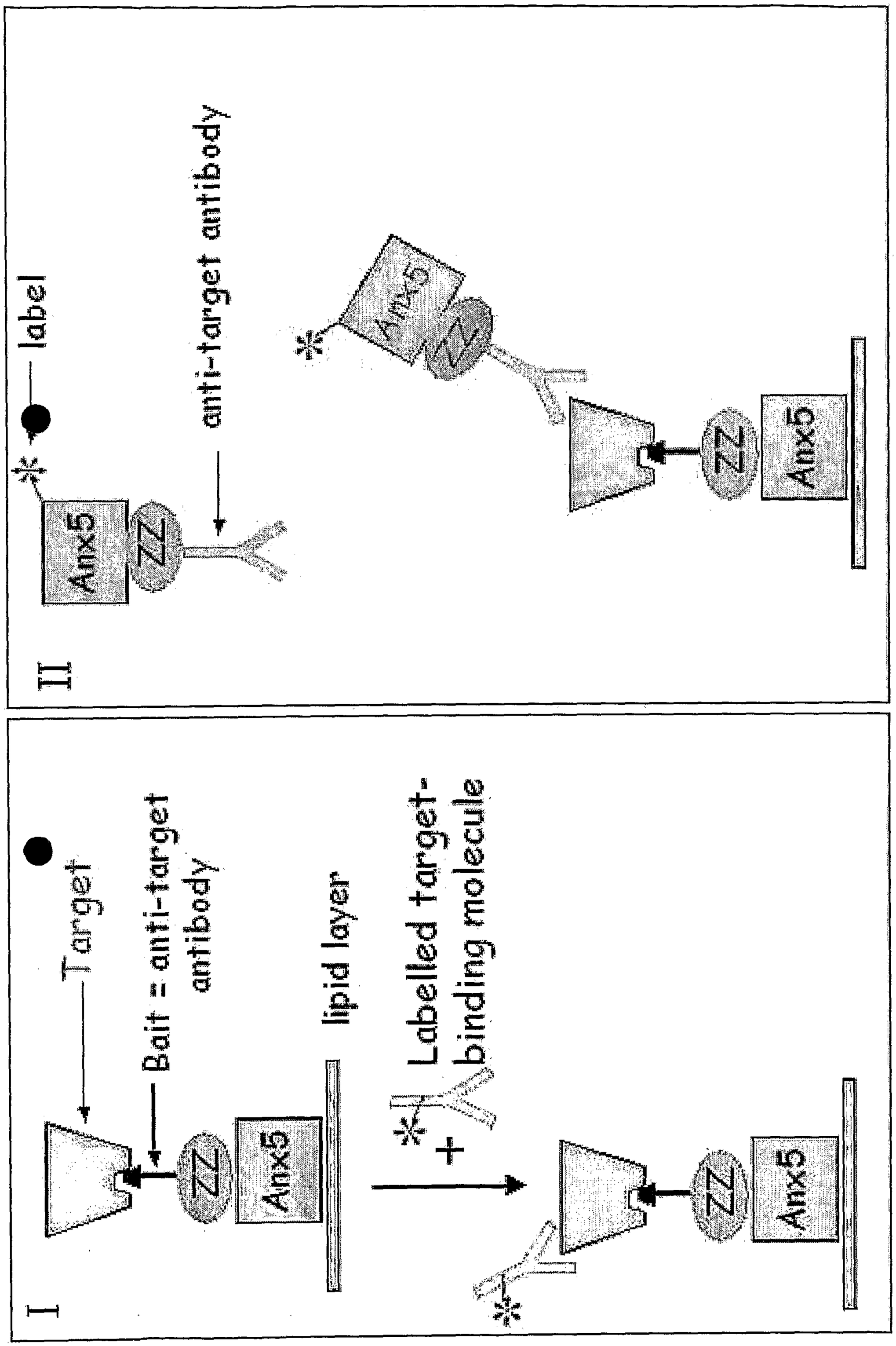

II
Target
Bait = anti-target antibody
lipid layer
Labelled target-binding molecule
label
anti-target antibody
Anx5
ZZ

Figure 4

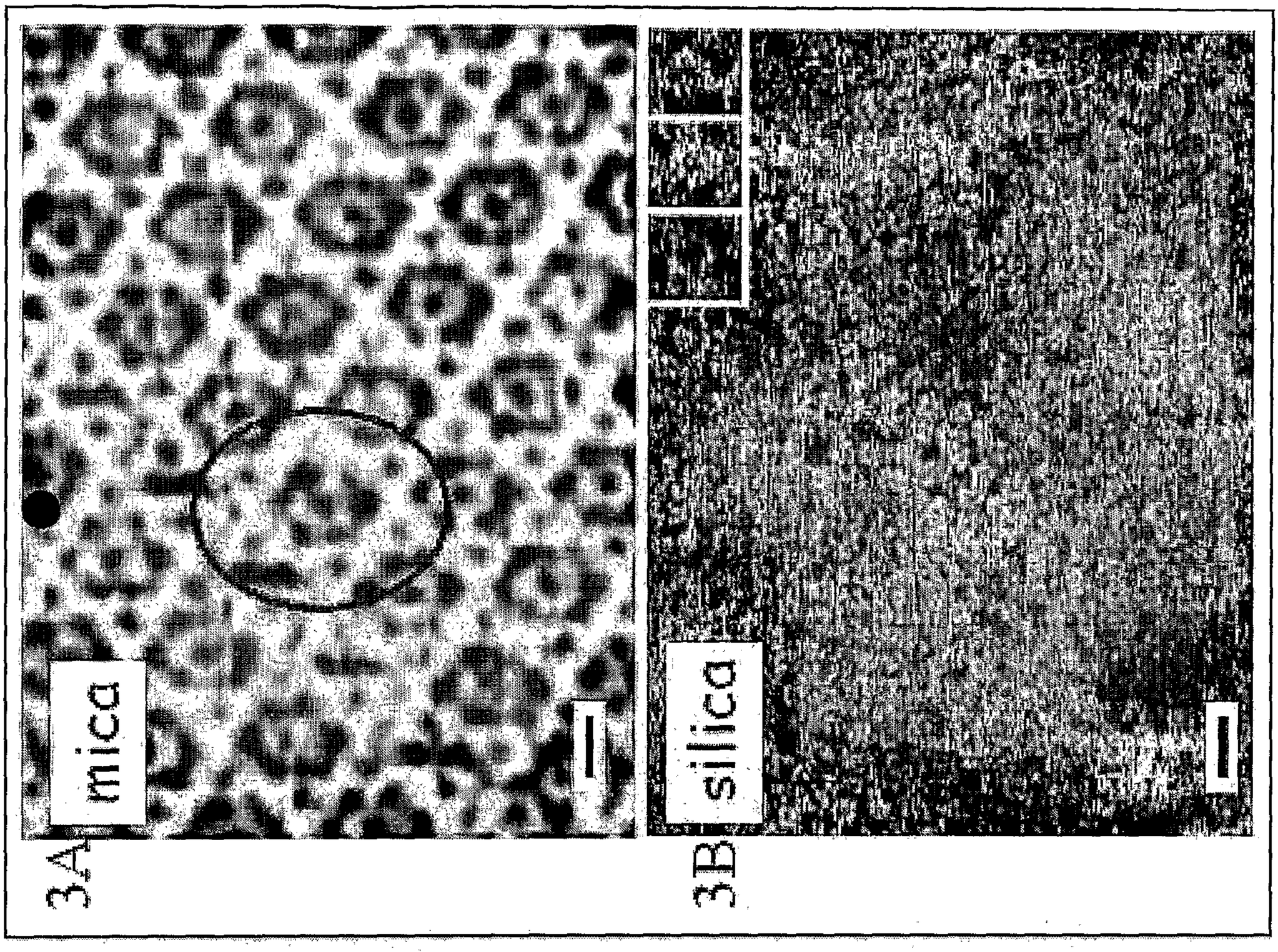
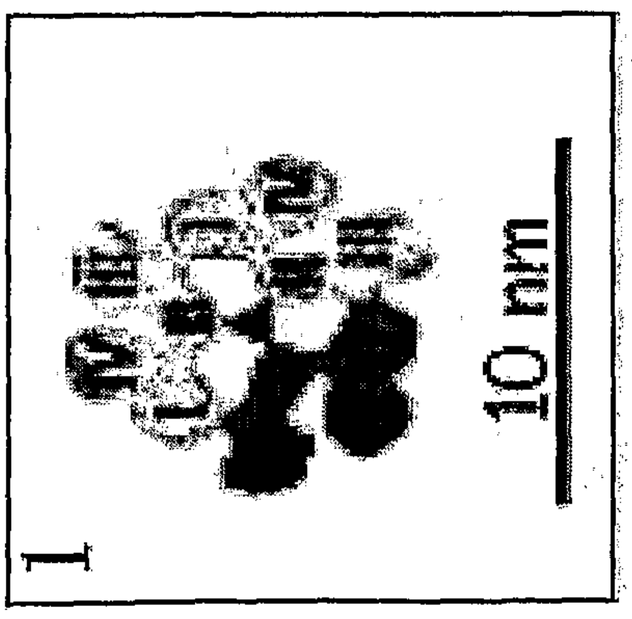
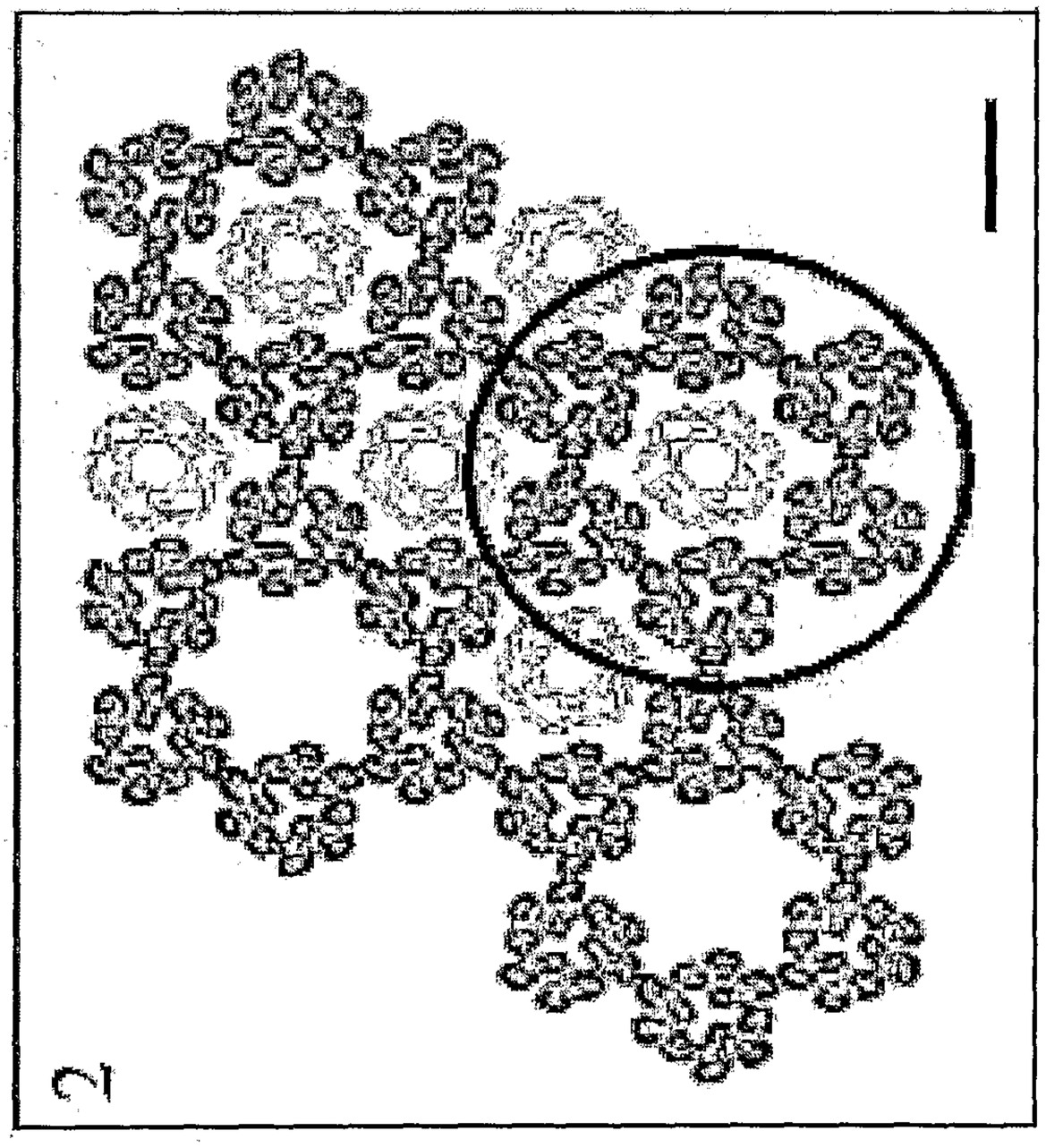


Figure 5





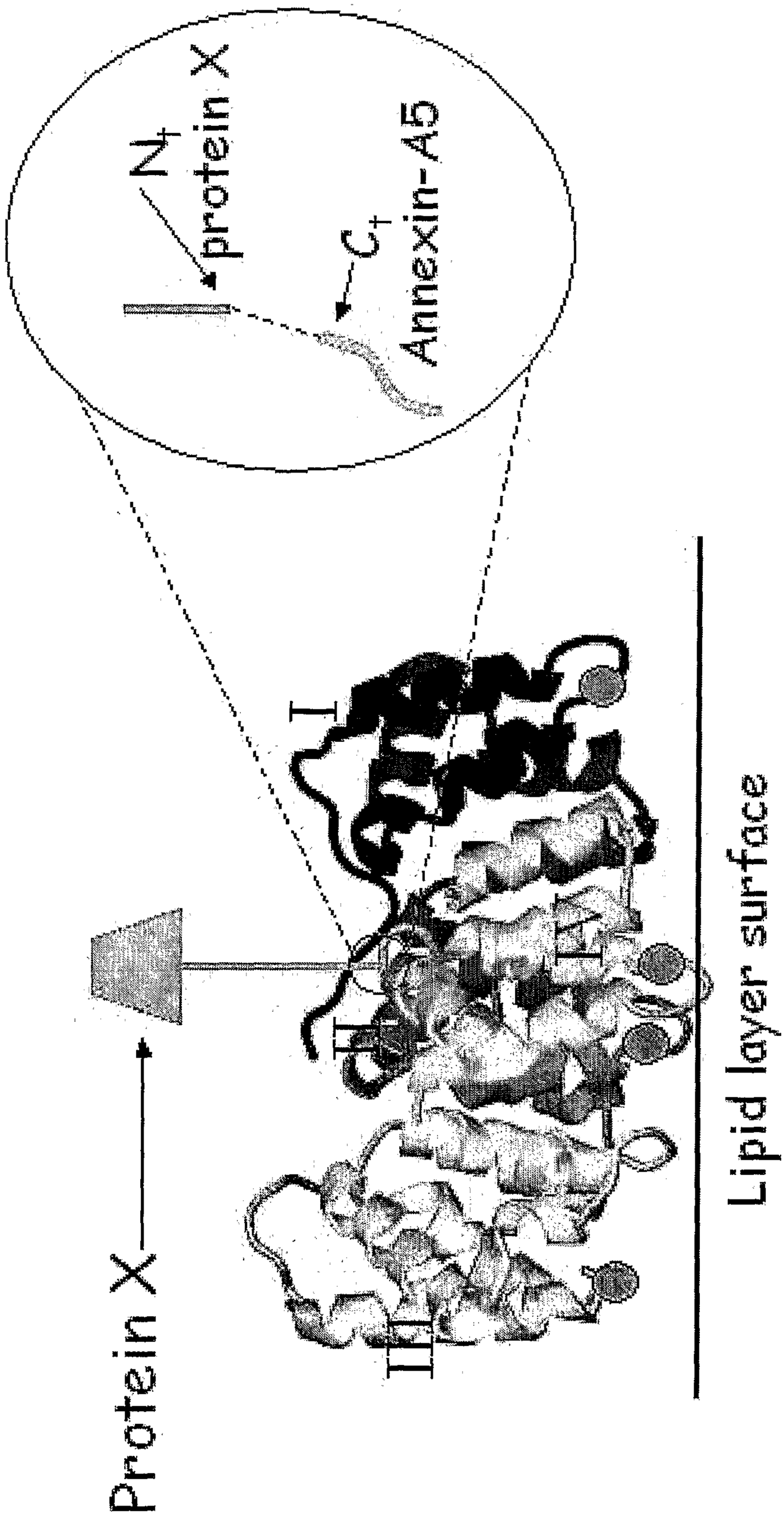


Figure 6

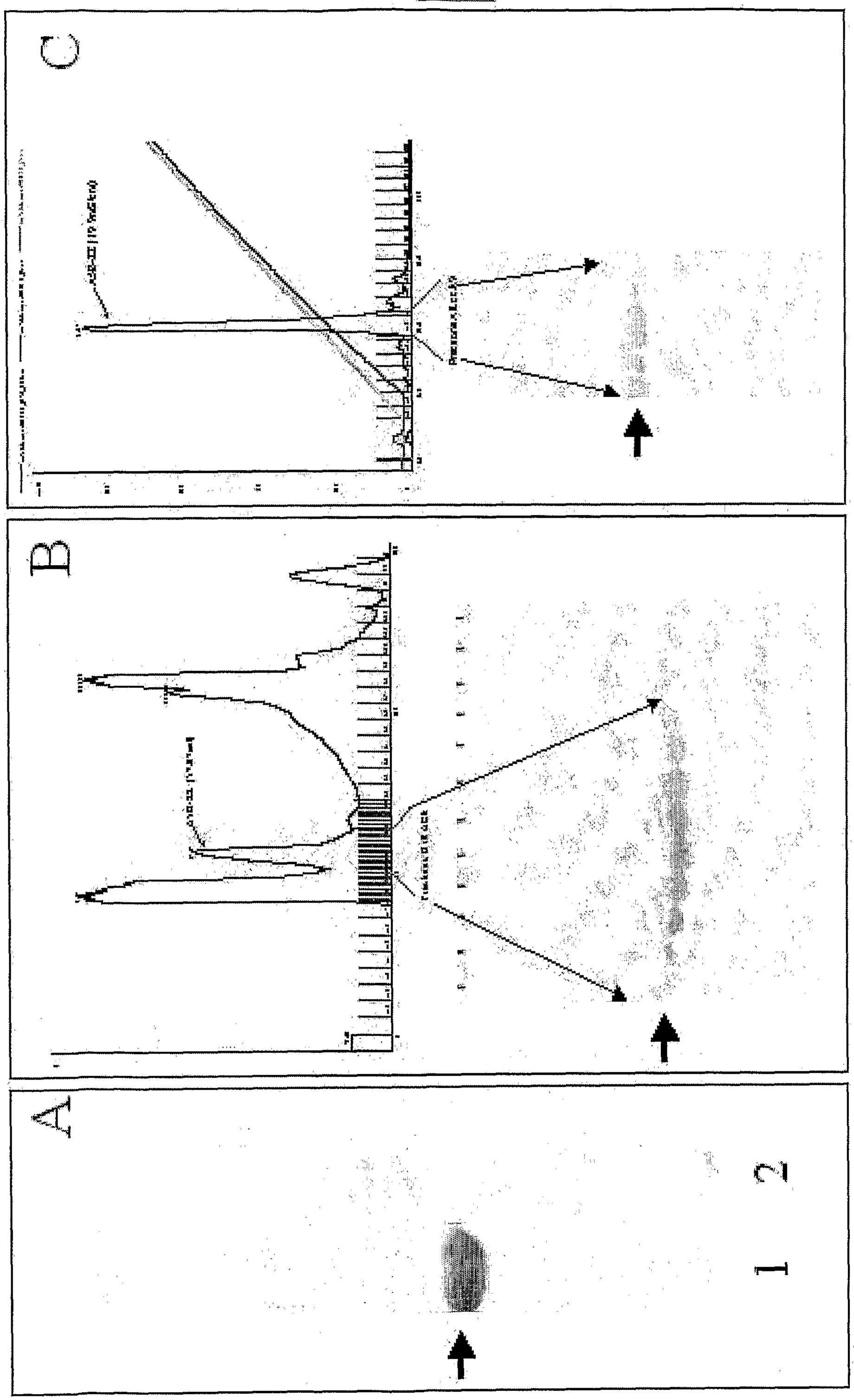


Figure 7

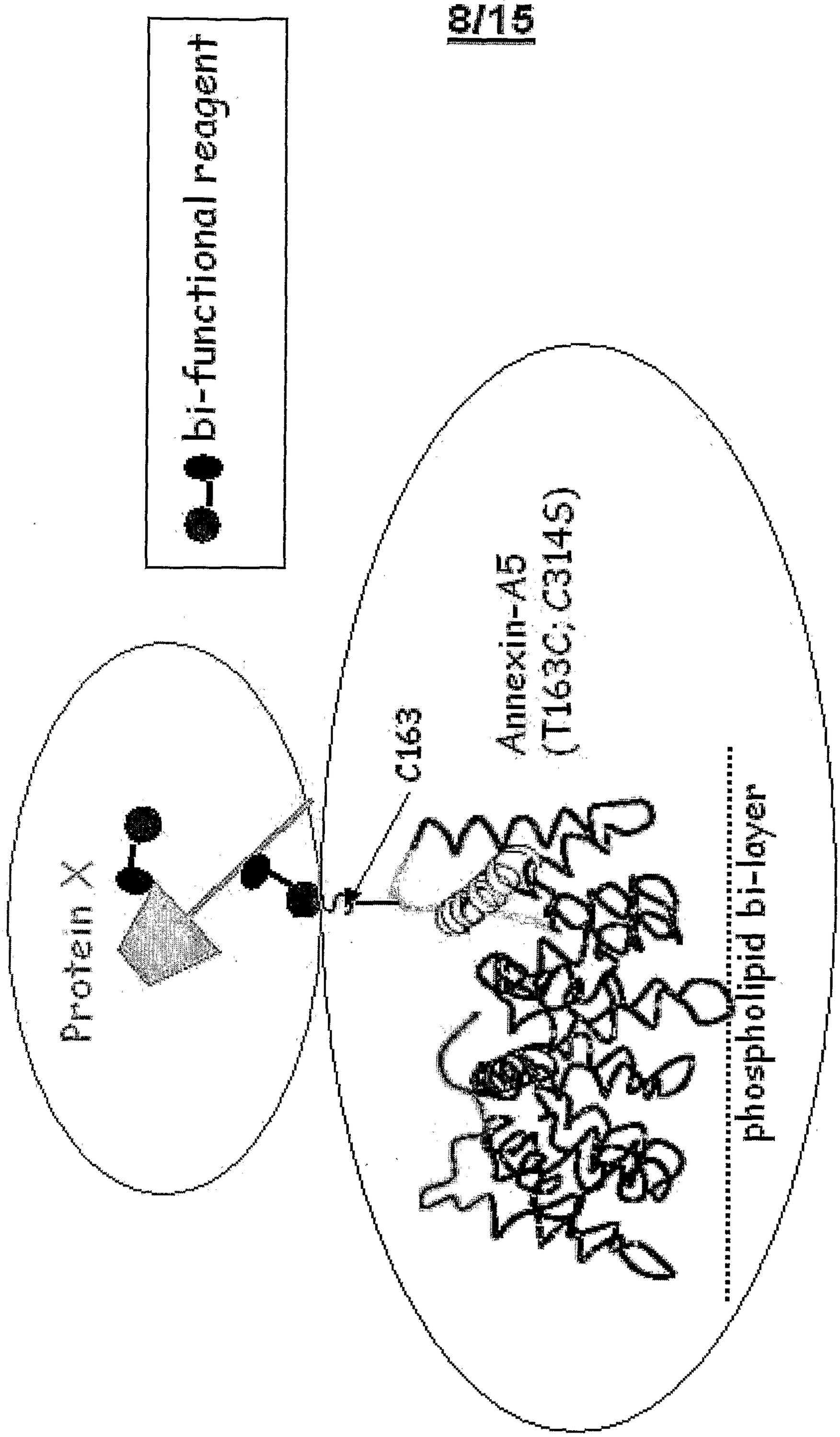

bi-functional reagent
Protein X
C163
Annexin-A5
(T163C; C315)
Phospholipid bi-layer

Figure 8

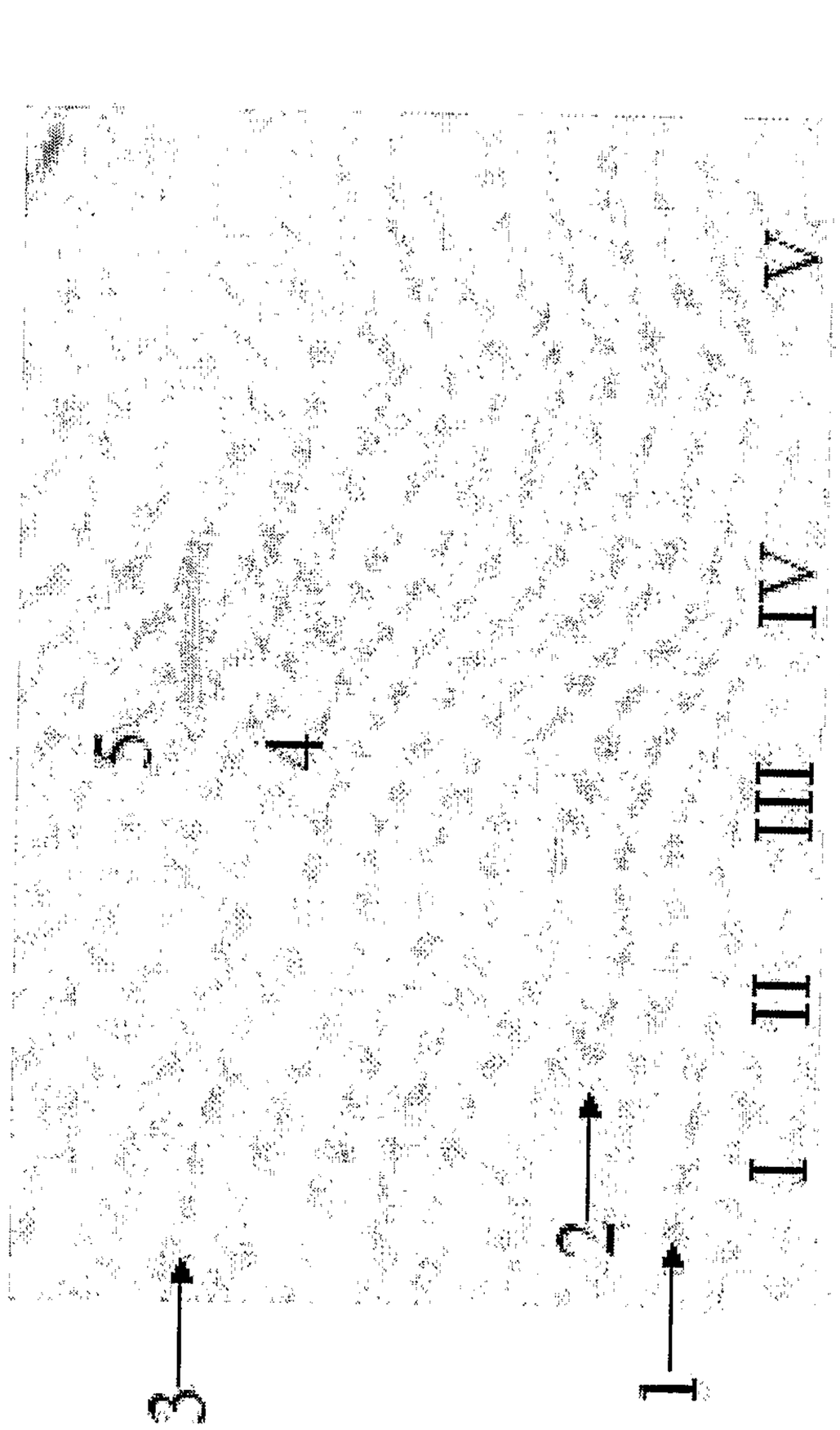


Figure 9A

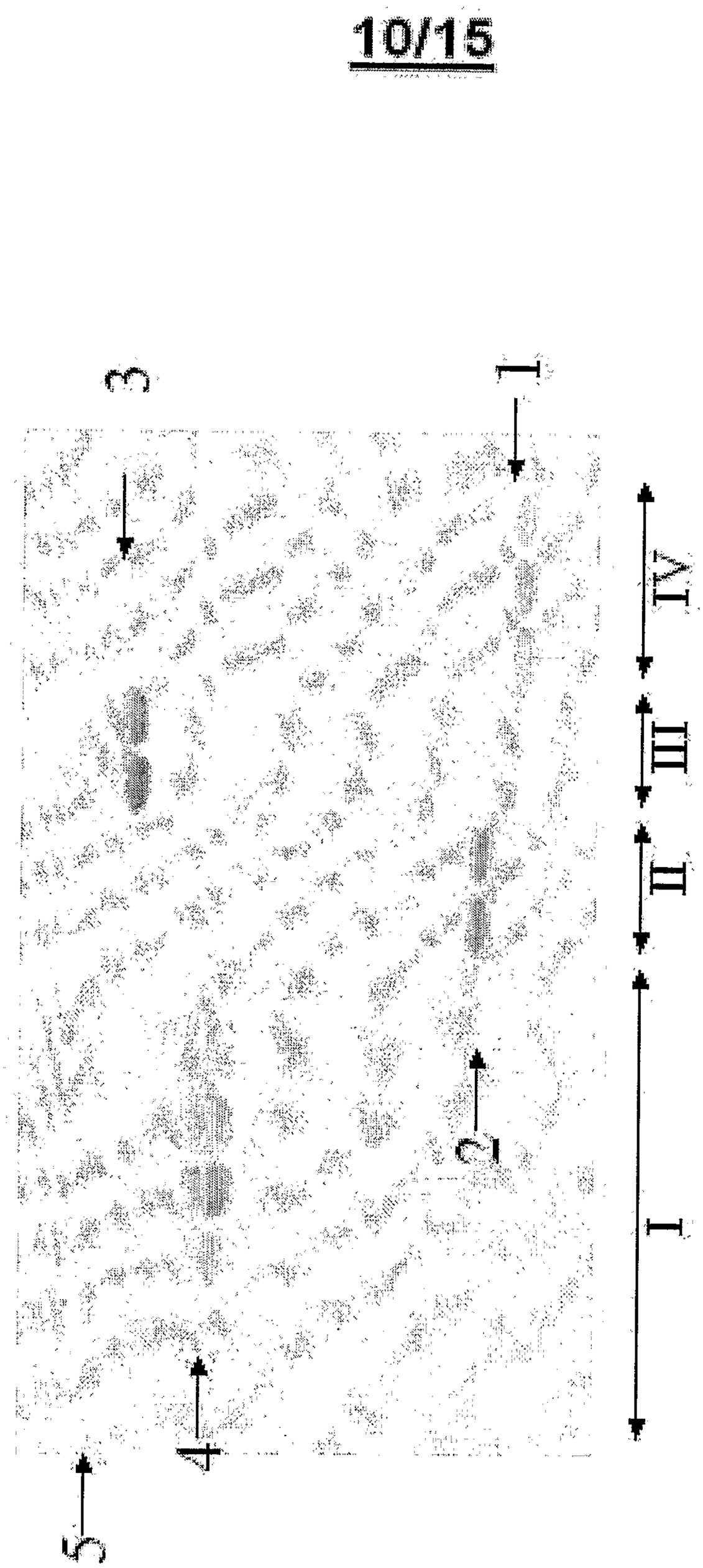


Figure 8B

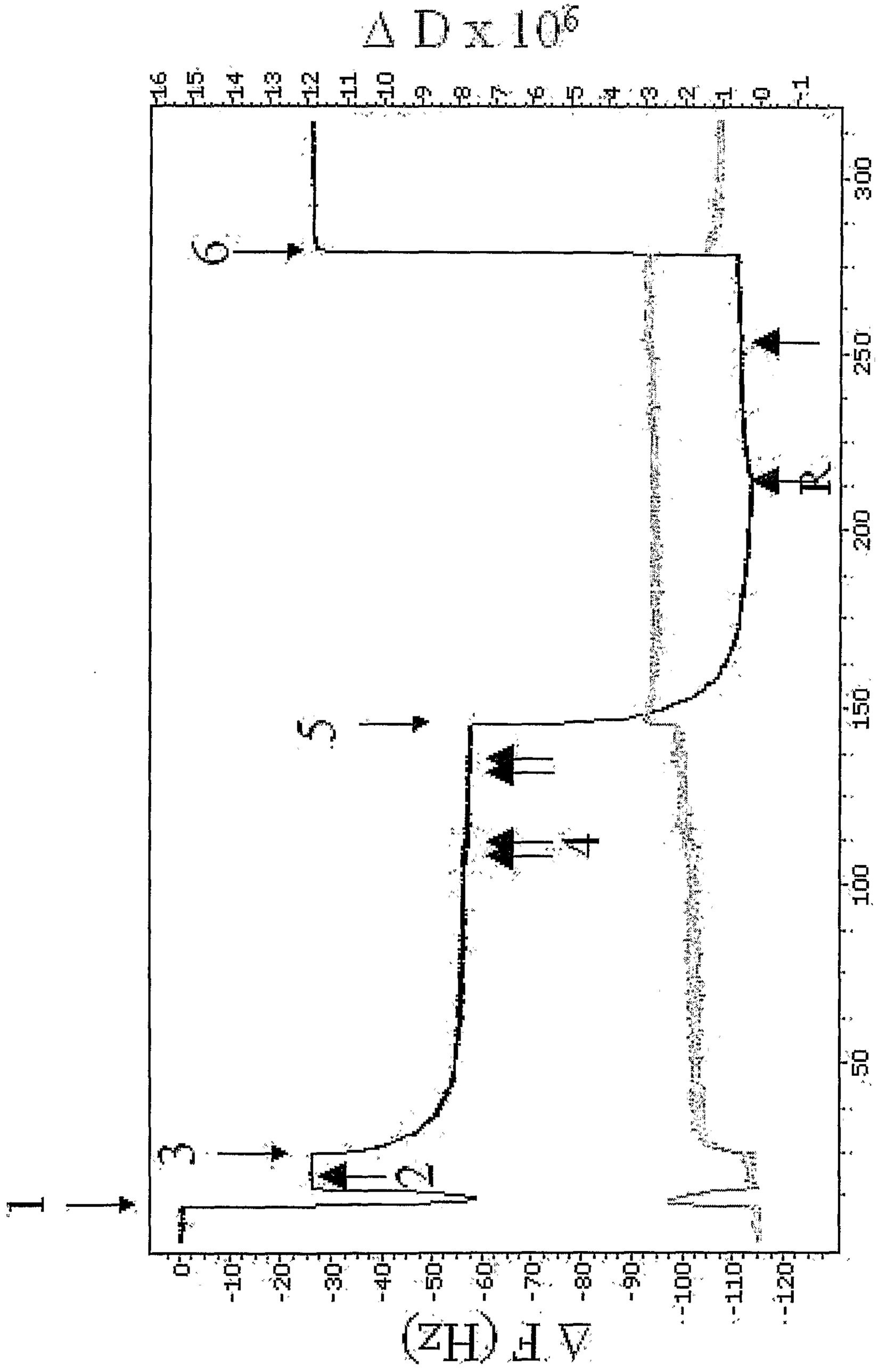

Δ D x 10^6
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
300
250
200
150
100
50
Time (min)
6
R
5
4
3
2
1
0
-10
-20
-30
-40
-50
-60
-70
-80
-90
-100
-110
-120
Δ F (Hz)

Figure 10

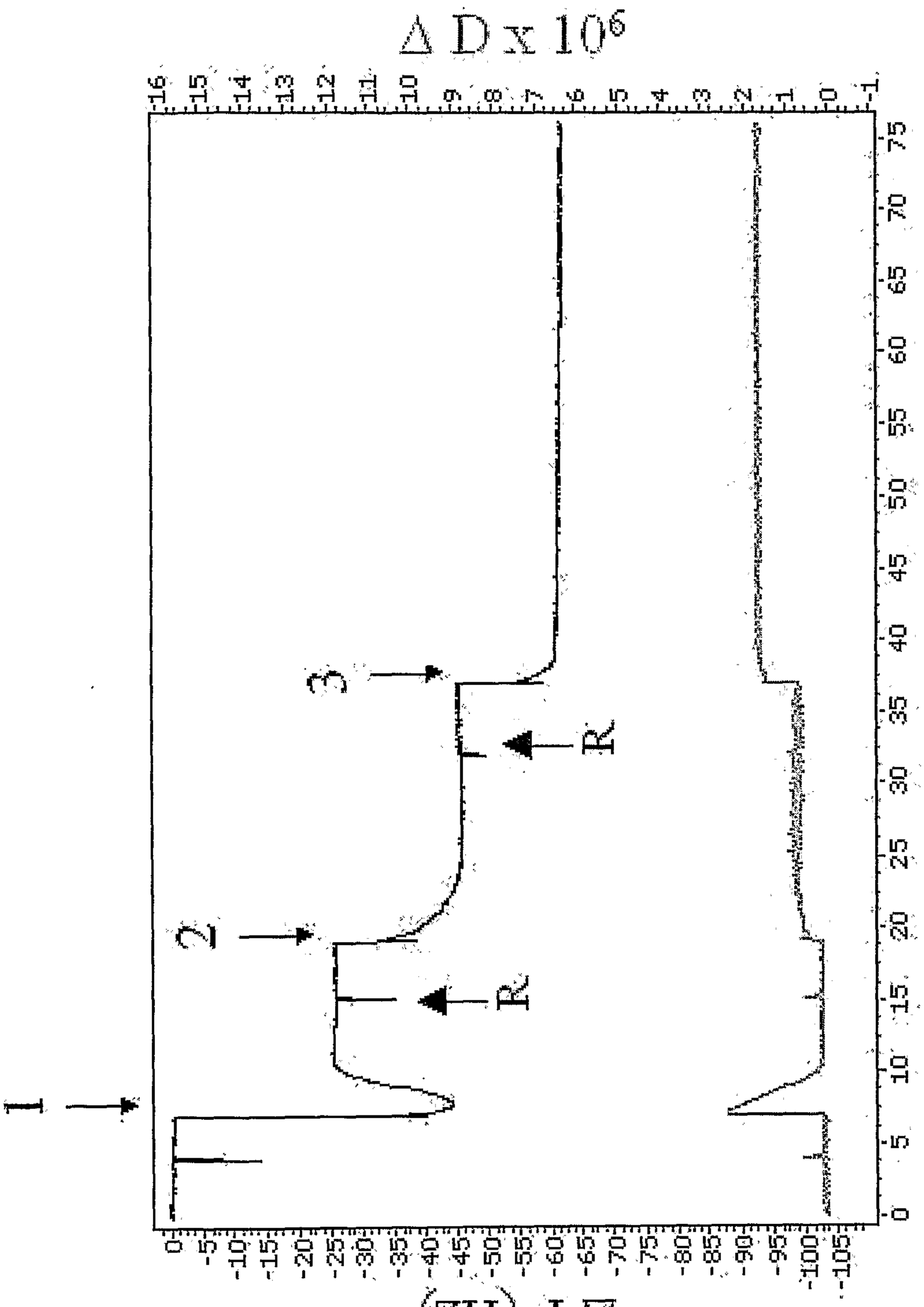


Figure 11

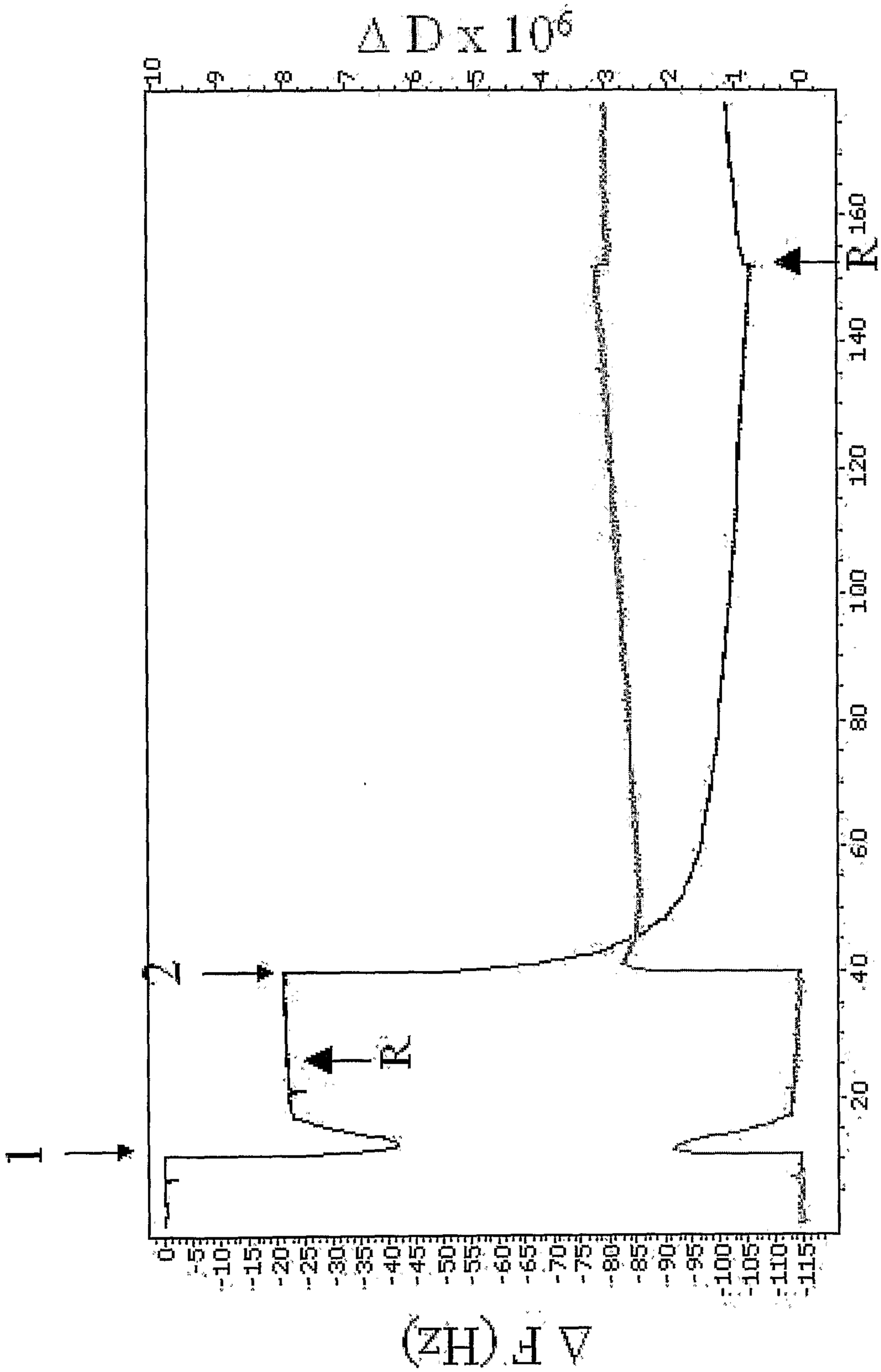

Δ D x 10⁶
Δ F (Hz)
Time (min)
1
2
R
R
R
0
-5
-10
-15
-20
-25
-30
-35
-40
-45
-50
-55
-60
-65
-70
-75
-80
-85
-90
-95
-100
-105
-110
-115
20
40
60
80
100
120
140
160

Figure 12

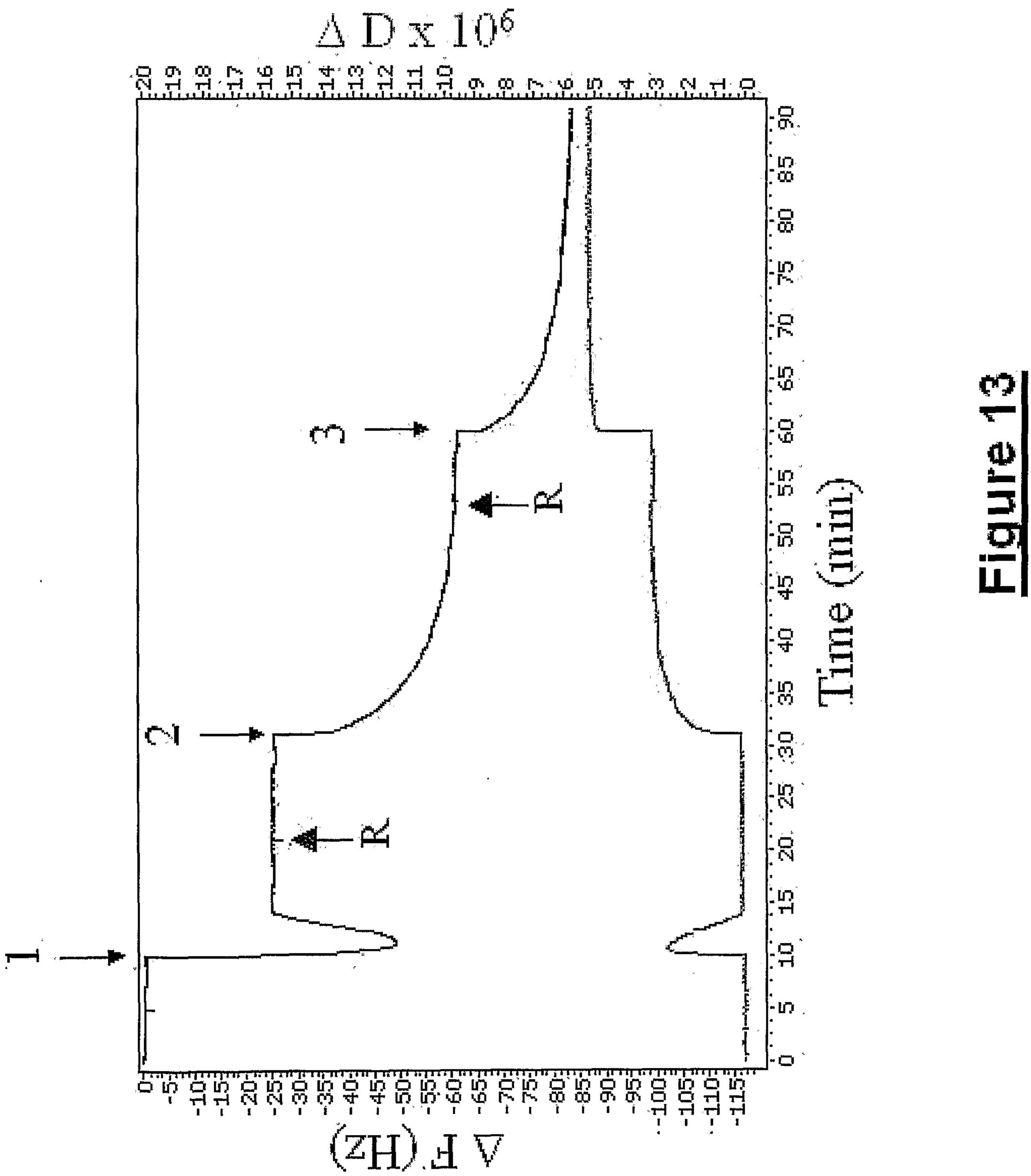

Δ D x 10⁶
20 19 18 17 16 15 14 13 12 11 10 9 8 7 6 5 4 3 2 1 0
90 85 80 75 70 65 60 55 50 45 40 35 30 25 20 15 10 5 0
3
R
2
R
1
0 -5 -10 -15 -20 -25 -30 -35 -40 -45 -50 -55 -60 -65 -70 -75 -80 -85 -90 -95 -100 -105 -110 -115
Δ F (Hz)
Time (min)

Figure 13

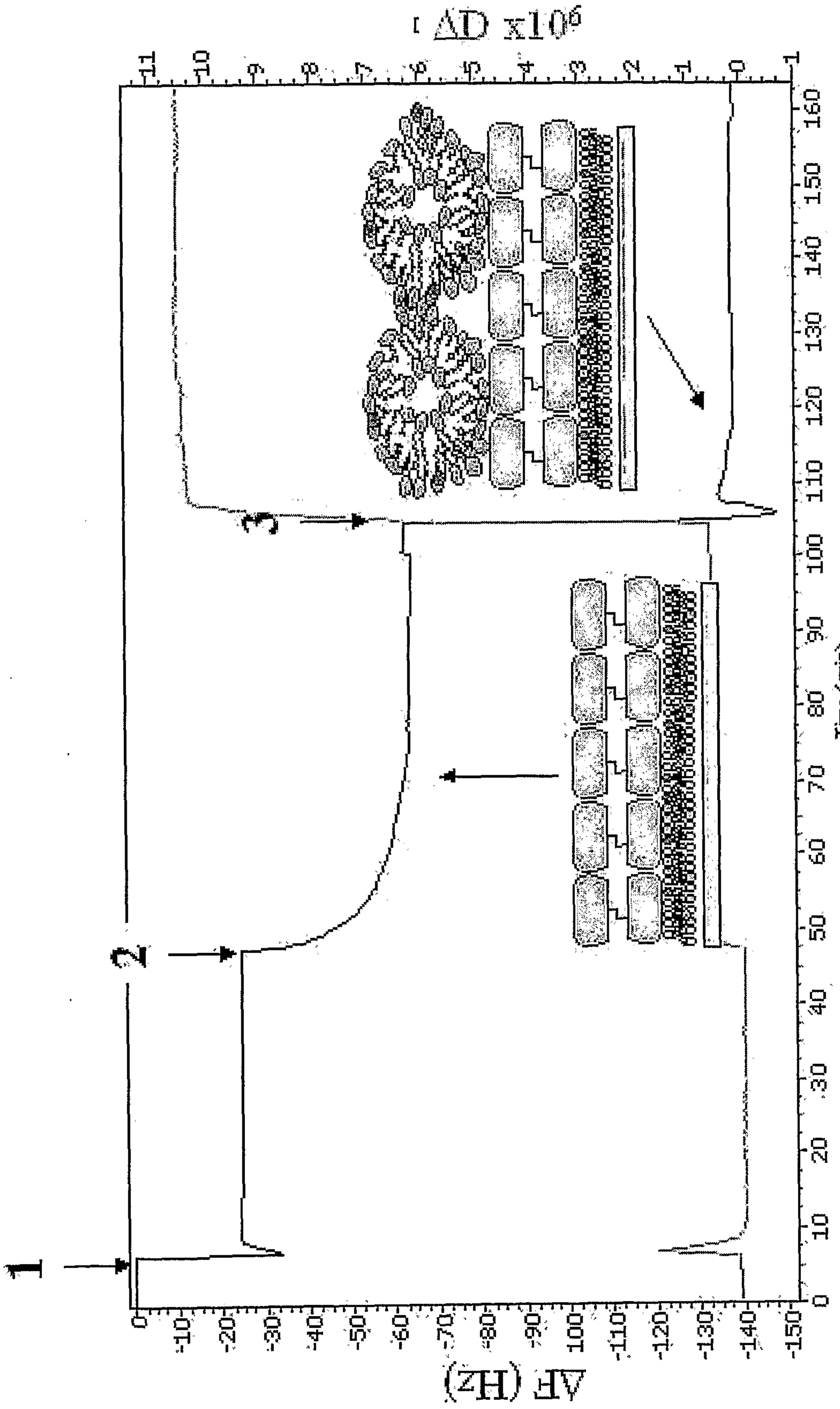


Figure 14

3- Annexin-A5 protein

5-Target molecule

4- Bait molecule