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(54) Title: COMPOSITIONS FOR PURIFYING AND CRYSTALLIZING MOLECULES OF INTEREST

(57) Abstract: A composition of matter is provided. The composition includes at least one ligand capable of binding a target molecule or cell of interest, the at least one ligand being attached to at least one coordinating moiety selected capable of directing the composition of matter to form a non-covalent complex when co-incubated with a coordinator ion or molecule. Also provided are methods of using such compositions for target purification, crystallization and immunization.

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COMPOSITIONS FOR PURIFYING AND CRYSTALLIZING MOLECULES OF INTEREST

FIELD AND BACKGROUND OF THE INVENTION

The present invention relates to compositions, which can be used for purifying and crystallizing molecules of interest.

Proteins and other macromolecules are increasingly used in research, diagnostics and therapeutics. Proteins are typically produced by recombinant techniques on a large scale with purification constituting the major cost (up to 60 % of the total cost) of the production processes. Thus, large-scale use of recombinant protein products is hindered because of the high cost associated with purification.

Current protein purification methods are dependent on the use of a combination of various chromatography techniques. These techniques separate mixtures of proteins on the basis of their charge, degree of hydrophobicity or size among other characteristics. Several different chromatography resins are available for use with each of these techniques, allowing accurate tailoring of the purification scheme to the particular protein targeted for isolation. The essence of each of these separation methods is that proteins can be caused either to move at different rates down a long column, achieving a physical separation that increases as they pass further down the column, or to adhere selectively to the separation medium, enabling differential elution by different solvents. In some cases, the column is designed such that impurities bind thereto while the desired protein is found in the "flow-through."

Affinity precipitation (AP) is the most effective and advanced approach for protein precipitation [Mattiasson (1998); Hilbrig and Freitag (2003) J Chromatogr B Analyt Technol Biomed Life Sci. 790(1-2):79-90]. Current state of the art AP employs ligand coupled “smart polymers”. “Smart polymers” [or stimuli-responsive "intelligent" polymers or Affinity Macro Ligands (AML)] are polymers that respond with large property changes to small physical or chemical stimuli, such as changes in pH, temperature, radiation and the like. These polymers can take many forms; they may be dissolved in an aqueous solution, adsorbed or grafted on aqueous-solid interfaces, or cross-linked to form hydrogels [Hoffman J Controlled Release (1987) 6:297-305; Hoffman Intelligent polymers. In: Park K, ed. Controlled drug delivery. Washington: ACS Publications, (1997) 485–98; Hoffman Intelligent polymers in medicine and biotechnology. Artif Organs (1995) 19:458-467]. Typically, when the polymer's critical response is stimulated, the smart polymer in solution will show a sudden onset of turbidity as it phase-separates; the surface-adsorbed or grafted smart polymer will collapse, converting the interface from hydrophilic to hydrophobic; and the smart polymer (cross-linked in the form of a hydrogel) will exhibit a sharp collapse and release much of its swelling solution. These phenomena are reversed when the stimulus is reversed, although the rate of reversion often is slower when the polymer has to redissolve or the gel has to re-swell in aqueous medium.

"Smart" polymers may be physically mixed with, or chemically conjugated to, biomolecules to yield a large family of polymer-biomolecule systems that can respond to biological as well as to physical and chemical stimuli. Biomolecules that may be polymer-conjugated include proteins and oligopeptides, sugars and polysaccharides, single- and double-stranded oligonucleotides and DNA plasmids, simple lipids and phospholipids, and a wide spectrum of recognition ligands and synthetic drug molecules.

A number of structural parameters control the ability of smart polymers to specifically precipitate proteins of interest; smart polymers should contain reactive groups for ligand coupling; not interact strongly with the impurities; make the ligand available for interaction with the target protein; give complete phase separation of the polymer upon a change of medium property; form compact precipitates; exclude trapping of impurities into the gel structure and be easily solubilized after the precipitate is formed.

Although many different natural as well as synthetic polymers have been utilized in AP [Mattiasson (1998) J. Mol. Recognit. 11:211] the ideal smart polymers remain elusive, as affinity precipitations performed with currently available smart polymers, fail to meet one or several of the above-described requirements [Hlibrig and Freitag (2003), supra].

The availability of efficient and simple protein purification techniques may also be useful in protein crystallization, in which protein purity extensively affects crystal growth. The conformational structure of proteins is a key to understanding their biological functions and to ultimately designing new drug therapies. The conformational structures of proteins are conventionally determined by x-ray diffraction from their crystals. Unfortunately, growing protein crystals of sufficient high quality is very difficult in most cases, and such difficulty is the main limiting factor in the scientific determination and identification of the structures of protein samples. Prior art methods for growing protein crystals from super-saturated solutions are tedious and time-consuming, and less than two percent of the over 100,000 different proteins have been grown as crystals suitable for x-ray diffraction studies.

Membrane proteins present the most challenging group of proteins for crystallization. The number of 3D structures available for membrane proteins is still around 20 while the number of membrane proteins is expected to constitute a third of the proteome. Numerous obstacles need to be traversed when wishing to crystallize a membrane protein. These include, low abundance of proteins from natural sources, the need to solubilize hydrophobic membrane proteins from their native environment (i.e., the lipid bilayer) and their tendency to denaturate, aggregate and/or degrade in the detergent solution. The choice of the solubilizing detergent presents another problem as some detergents may interfere with binding of a stabilizing partner to the target protein.

Two approaches have been attempted in the crystallization of membrane proteins.

Until very recently, the majority of X-ray crystal structures of membrane proteins have been determined using crystals grown directly from solutions of protein-detergent complexes. Crystal growth of protein-detergent complexes can be considered equivalent to that of soluble proteins only the solute being crystallized is a complex of protein and detergent, rather than solely protein. The actual lattice contacts are formed by protein-protein interactions, although crystal packing brings the detergent moieties into close apposition as well. In order to increase the surface area available to make these protein-protein contacts studies suggested adding an antibody fragment which will increase the chances of producing crystals [Hunte and Michel (2002) Curr. Opin. Struct. Biol. 12:503-508]. However, applying this technology to various membrane proteins is difficult as it requires the generation of monoclonal antibodies, which are specific to each membrane protein.

Furthermore, it is argued that no detergent micelle can fully and accurately reproduce the lipid bilayer environment of the protein.

Thus, efforts to crystallize membrane proteins must be directed towards producing crystals within a bilayer environment. A number of attempts have been made to generate crystals of membrane proteins using this approach. These include the generation of crystals of bacteriorhodopsin grown in the presence of a lipidic cubic phase, which forms gel-like substance containing continuous bilayer structures [Landau and Rosenbuch (1996) Proc. Natl. Acad. Sci. USA 93:14532-14535] and crystallization in cubo which was proven successful in the crystallization of archaeal seven-transmembrane helix proteins [Gordeliy (2002) Nature 419:484-487; Luecke (2001) Science 293:1499-1503; Kolbe (2000) Science 288:1390-1396; Royant (2001) Proc. Natl. Acad. Sci. USA 98:10131-10136]. However, crystals of other membrane proteins using the in cubo approach were not of as high a quality as crystals grown directly from protein-detergent complex solutions [Chiu (2000) Acta. Crystallogr. D. 56:781-784].

There is thus a widely recognized need for, and it would be highly advantageous to have, compositions and methods using same for the purification and crystallization of molecules which are devoid of the above limitations.

SUMMARY OF THE INVENTION

According to one aspect of the present invention there is provided a composition of matter comprising at least one ligand capable of binding a target molecule or cell of interest, the at least one ligand being attached to at least one coordinating moiety selected capable of directing the composition of matter to form a non-covalent complex when co-incubated with a coordinator ion or molecule.

According to another aspect of the present invention there is provided a method of purifying a target molecule or cell of interest, the method comprising: (a) contacting a sample including the target molecule or cell of interest with a composition including: (i) at least one ligand capable of binding the target molecule or cell of interest, the at least one ligand being attached to at least one coordinating moiety; and (ii) a coordinator capable of non-covalently binding the at least one coordinating moiety, the at least one coordinating moiety and the coordinator being capable of forming a complex when co-incubated; and (b) collecting a precipitate including the complex bound to the target molecule or cell of interest, thereby purifying the target molecule or cell of interest.

According to further features in preferred embodiments of the invention described below, the method further comprising recovering the molecule of interest from the precipitate.

According to yet another aspect of the present invention there is provided a method of detecting predisposition to, or presence of a disease associated with a molecule of interest in a subject, the method comprising contacting a biological sample obtained from the subject with a composition including: (i) at least one ligand capable of binding the molecule of interest, the at least one ligand being attached to at least one coordinating moiety; and (ii) a coordinator capable of non-covalently binding the at least one coordinating moiety, the at least one coordinating moiety and the coordinator being capable of forming a complex when co-incubated, wherein formation of the complex including the molecule of interest is indicative of predisposition to, or presence of the disease associated with the molecule of interest in the subject.

According to still another aspect of the present invention there is provided a composition for crystallizing a molecule of interest, the composition comprising: (i) at least one ligand capable of binding the molecule of interest, the at least one ligand being attached to at least one coordinating moiety; and (ii) a coordinator capable of non-covalently binding the at least one coordinating moiety, wherein the at least one coordinating moiety and the coordinator are capable of forming a complex when co-incubated and whereas the composition is selected so as to define the relative spatial positioning and orientation of the molecule of interest when bound thereto, thereby facilitating formation of a crystal therefrom under inducing crystallization conditions.

According to an additional aspect of the present invention there is provided a method of crystallizing a molecule of interest, the method comprising contacting a sample including the molecule of interest with a crystallizing composition including: (i) at least one ligand capable of binding the molecule of interest, the at least one ligand being attached to at least one coordinating moiety; and (ii) a coordinator capable of non-covalently binding the at least one coordinating moiety, wherein the at least one coordinating moiety and the coordinator are capable of forming a complex when co-incubated and whereas the crystallizing composition is selected so as to define the relative spatial positioning and orientation of the molecule of interest when bound thereto, thereby facilitating formation of a crystal therefrom under inducing crystallization conditions.

According to yet an additional aspect of the present invention there is provided a composition-of-matter comprising a molecule having a first region capable of binding a molecule of interest and a second region capable of binding a coordinator ion or molecule, the second region being designed such that the molecule forms a polymer when exposed to the coordinator ion or molecule.

According to still an additional aspect of the present invention there is provided a method of depleting a target molecule or cell of interest from a sample, the method comprising: (a) contacting the sample including the target molecule or cell of interest with a composition including: (i) at least one ligand capable of binding the molecule of interest, the at least one ligand being attached to at least one coordinating moiety; and (ii) a coordinator capable of non-covalently binding the at least one coordinating moiety, the at least one coordinating moiety and the coordinator being capable of forming a complex when co-incubated; and (b) removing a precipitate including the complex bound to the target molecule or cell of interest to thereby deplete the target molecule or cell of interest from the sample.

According to a further aspect of the present invention there is provided a method of enhancing immunogenicity of a target molecule of interest, the method comprising contacting the target molecule of interest with a composition including: (i) at least one ligand capable of binding the target molecule of interest, the at least one ligand being attached to at least one coordinating moiety; and (ii) a coordinator capable of non-covalently binding the at least one coordinating moiety, wherein contacting is effected such that the at least one coordinating moiety and the coordinator forms a complex including the target molecule of interest, thereby enhancing immunogenicity of the target molecule of interest.

According to still further features in the described preferred embodiments the molecule of interest is selected from the group consisting of a protein, a nucleic acid sequence, a small molecule chemical and an ion.

According to still further features in the described preferred embodiments the at least one ligand is selected from the group consisting of a growth factor, a hormone, a nucleic acid sequence, an antibody, an epitope tag, an avidin, a biotin, a enzymatic substrate and an enzyme.

According to still further features in the described preferred embodiments the at least one ligand is attached to the at least one coordinating moiety via a linker.

According to still further features in the described preferred embodiments the coordinating moiety is selected from the group consisting of a chelator, a biotin, a nucleic acid sequence, an epitope tag, an electron poor molecule and an electron-rich molecule.

According to still further features in the described preferred embodiments the coordinator ion or molecule is selected from the group consisting of a metal ion, an avidin, a nucleic acid sequence, an electron poor molecule and an electron-rich molecule.

The present invention successfully addresses the shortcomings of the presently known configurations by providing compositions and methods for the purification of molecules.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, suitable methods and materials are described below. In case of conflict, the patent specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

BRIEF DESCRIPTION OF THE DRAWINGS

The invention is herein described, by way of example only, with reference to the accompanying drawings. With specific reference now to the drawings in detail, it is stressed that the particulars shown are by way of example and for purposes of illustrative discussion of the preferred embodiments of the present invention only, and are presented in the cause of providing what is believed to be the most useful and readily understood description of the principles and conceptual aspects of the invention. In this regard, no attempt is made to show structural details of the invention in more detail than is necessary for a fundamental understanding of the invention, the description taken with the drawings making apparent to those skilled in the art how the several forms of the invention may be embodied in practice.

In the drawings:

FIGs. 1a-f schematically illustrate several configurations of the compositions of the present invention. Figures 1a-c show ligands bound to two coordinating moieties. Figures 1d-f show ligands bound to multiple coordinating moieties. Z denotes the coordinating moiety.

FIGs. 2a-b schematically illustrate precipitation of a target molecule using the compositions of the present invention. A ligand covalently attached to a bis-chelator is incubated in the presence of a target molecule (Figure 2a). Addition of a metal (M^+, M^{2+}, M^{3+}, M^{4+}) binds the chelator and forms a matrix including the target molecule non-covalently bound to the metal ion (Figure 2b).

FIGs. 3a-e schematically illustrate stepwise recovery of the target molecule from the precipitate. Figure 3a shows the addition of a free chelator, which competes with the binding of the ligand-bound chelator to the metal. Figure 3b shows gravity-based separation of the ligand-bound target molecule from the free competing chelator and the complexed metal (Figure 3c). Figure 3d shows loading of the ligand-bound target molecule on an immobilized metal column to allow binding of the complex. Under proper elution conditions the target molecule is eluted while the ligand-coordinating moiety molecule is not. A desalting stage may be added for further purification of the target molecule. Regeneration of the ligand-chelator molecule is achieved by addition of a competing chelator to the column, followed by dialysis or ultrafiltration (Figure 3e).

FIG. 4 schematically illustrates direct elution of the target molecule from the precipitate, wherein the chelator-metal complex is maintained, while binding between the target molecule and the ligand decreases.

FIG. 5 schematically illustrates regeneration of the precipitating unit (i.e., ligand-coordinating moiety) following elution of the target molecule. In this case, recovery is achieved by the addition of a competing chelator and application of an appropriate separation procedure, such as, dialysis and ultrafiltration.

FIGs. 6a-c schematically illustrate precipitation of a target molecule using nucleic acid sequences as the coordinating moiety. A ligand with a covalently bound bis-nucleotide sequence (coordinating moiety) is incubated in the presence of a target molecule (Figure 6a). Addition of a complementary sequence results in the formation of matrix including ligand-coordinating moiety:target molecule:the complementary sequence (coordinator molecule, Figure 6b). Non-symmetrical coordinating sequences are shown as well (Figure 6c).

FIGs. 7a-b schematically illustrate precipitation of a target molecule using biotin as the coordinating moiety. A ligand with a covalently bound bis-biotin or biotin derivative such as: DSB-X Biotin is incubated in the presence of a target molecule (Figure 7a). Introduction of avidin (or its derivatives) creates a network comprising ligand-coordinating moiety (biotin): target molecule: avidin (Figure 7b).

FIGs. 8a-c schematically illustrate precipitation of a target molecule using electron rich molecules as the coordinating moiety. A ligand with a covalently bound bis-electron rich entity is incubated in the presence of a target molecule (Figure 8a). Addition of a bis (also tris, tetra) electron poor derivative with the propensity to form a complex results in a non-covalent network comprising ligand-coordinating moiety (electron poor molecule): target molecule: bis-electron poor moiety (Figure 8b). The picric acid and indole system can also be used according to the present invention (Figure 8c).

FIG. 9 schematically illustrates precipitation of a target antibody with protein A (ProA) bound used as a ligand. Addition of an appropriate coordinator results in a network of: Protein A-coordinating moiety : coordinator : target molecule.

FIGs. 10a-b schematically illustrate the use of the complexes of the present invention for crystallization of membrane proteins. The general formation of 2D (or 3D) structures in the presence of crystallizing composition is presented, where the coordinators are not interconnected between themselves (Figure 10a). A more detailed example utilizing a specific ligand modified with two antigens, and a monoclonal antibody (mAb) directed at the specific antigen, serving as the coordinator, is illustrated in Figure 10b.

FIGs. 11a-b schematically illustrate the use of metallo complexes (Figure 11a) and nucleo-complexes (Figure 11b) for the formation of crystals of membrane proteins.

FIG. 11c schematically illustrates a three-dimensional membrane complex using the compositions of the present invention. The hydrophobic domain of the protein is surrounded by detergent micelles. Z denotes a multi valent coordinator (i.e., at least bi-valent coordinator).

FIG. 12 schematically illustrates the formation of a non-covalent composition consisting of three ligands bound to a single metal coordinator, through suitable chelators which are bound to the ligands through covalent linkers.

FIGs. 13a-b schematically illustrate the modification of three ligands of interest to include the hydroxamate derivatives (Figure 13a), such that a tri-non-covalent ligand complex is formed in the presence of Fe^{3+} ions (Figure 13b).

FIG. 14 schematically illustrates a two-step synthesis procedure for the generation of ligand-chelator molecules.

FIGs. 15a-b schematically illustrate the formation of di (Figure 15a) and tri (Figure 15b) non-covalent ligands, by utilizing the same ligand-linker-chelator molecule, while changing only, the cation present in the medium.

FIGs. 16a-c schematically illustrate the compositions of the present invention coordinated by electron poor / rich relations. By modifying a ligand with an electron poor moiety (Figure 16a) and synthesizing a tri covalent electron rich moiety (Figure 16b), a complex of the structure seen in Figure 16c is formed.

FIG. 17 schematically illustrates a two step synthesis process for the preparation of ligand-electron rich or ligand-electron poor derivatives.

FIG. 18 schematically illustrates the use of peptides for the formation of ligand complexes utilizing electron rich and electron poor moieties.

FIG. 19 schematically illustrates the formation of ligand complexes which utilize a chelator-metal as well as electron rich and poor relationships.

FIG. 20 schematically illustrates a single step synthesis procedure for the preparation of a chelator-electron poor derivative.

FIGs. 21a-b schematically illustrate formation of di and tri non-covalent electron poor moieties by utilizing the same chelator-electron poor (catechol-TNB) derivative and changing only the cation in the medium.

FIGs. 22a-b schematically illustrate the addition of a peptide containing an electron rich moiety to form a dimer and a trimer.

FIGs. 23a-b schematically illustrate the formation of a polymer complex by the addition of a composition including ligand attached to two chelators which are coordinated through electron rich/poor relations.

FIG. 24 schematically illustrates one possibility of limiting the freedom of motion of non-covalent protein dimers. After non-covalent dimmers are formed via a ligand-linker-chelator with the addition of an appropriate metal, the addition of a covalent electron poor moiety (e.g., trinitrobenzene-trinitrobenzene = TNB-TNB) leads to the simultaneous binding of two accessible electron rich residues (e.g., Trp) on two adjacent proteins thereby imposing motion constraints and allowing formation of a crystal structure.

FIG. 25 schematically illustrates chelators and metals, which can be used as the coordinating moiety and coordinator ion, respectively, in the compositions of the present invention.

FIG. 26 schematically illustrates electron rich and electron poor moieties which can be used as the coordinating moiety in the compositions of the present invention.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present invention is of compositions, which can be used for purifying and crystallizing molecules of interest.

The principles and operation of the present invention may be better understood with reference to the drawings and accompanying descriptions.

Before explaining at least one embodiment of the invention in detail, it is to be understood that the invention is not limited in its application to the details set forth in the following description or exemplified by the Examples. The invention is capable of other embodiments or of being practiced or carried out in various ways. Also, it is to be understood that the phraseology and terminology employed herein is for the purpose of description and should not be regarded as limiting.

Cost effective commercial-scale production of proteins, such as therapeutic proteins, depends largely on the development of fast and efficient methods of purification since it is the purification step which typically contributes most of the cost involved in large scale production of proteins.

There is thus, a need for simple, cost effective processes, which can be used to purify proteins and other commercially important molecules.

The state of the art approach in protein purification is Affinity Precipitation (AP) which is based on the use of "smart" polymers coupled to a recognition unit, which binds the protein of interest. These smart polymers respond to small changes in environmental stimuli with large, sometimes discontinuous changes in their physical state or properties, resulting in phase separation from aqueous solution or order-ofmagnitude changes in hydrogel size and precipitation of the molecule of interest. However, at present, the promise of smart polymers has not been realized due to several drawbacks including, entrapment of impurities during the precipitation process, adsorption of impurities to the polymeric matrix, decreased affinity of the protein recognition unit and working conditions which may lead to a purified protein with reduced activity.

While reducing the present invention to practice, the present inventors designed novel compositions, which can be used for cost-effective and efficient purification of proteins as well as other molecules and cells of interest.

As is illustrated hereinbelow and in the Examples section which follows, the compositions of the present invention specifically bind target molecules to form non-covalent complexes which can be precipitated and collected under mild conditions. Furthermore, contrary to prior art purifying compositions, the compositions of the present invention are not immobilized (such as to a smart polymer) which reduces affinity of the ligand towards the target molecule, limits the amount of ligand used, necessitates the use of sophisticated laboratory equipment (HPLC) requiring high maintenance, leads to column fouling and limits column usage to a single covalently bound ligand.

Thus, according to one aspect of the present invention there is provided a composition-of-matter, which is suitable for purification of a target molecule or cell of interest.

The target molecule can be a macromolecule such as a protein, a carbohydrate, a glycoprotein or a nucleic acid sequence (e.g., DNA such as plasmids, RNA) or a small molecule such as a chemical. Although most of the examples provided herein describe proteinacious target molecules, it will be appreciated that the present invention is not limited to such targets.

The target cell can be a eukaryotic cell, a prokaryotic cell or a viral cell.

The composition-of-matter of the present invention includes at least one ligand capable of binding the molecule or cell of interest and at least one coordinating moiety which is selected capable of directing the composition of matter to form a non-covalent complex when co-incubated with a coordinator ion or molecule.

As used herein the term “ligand” refers to a synthetic or a naturally occurring molecule preferably exhibiting high affinity (e.g., $K_D < 10^{-5}$) binding to the target molecule of interest and as such the two are capable of specifically interacting. When the target of interest is a cell, the ligand is selected capable of binding a protein, a carbohydrate or chemical, which is expressed on the surface of the cell (e.g., cellular marker). Preferably, ligand binding to the molecule or cell of interest is a non-covalent binding. The ligand according to this aspect of the present invention may be mono, bi (antibody, growth factor) or multi-valent ligand and may exhibit affinity to one or more molecules or cells of interest (e.g., bi-specific antibodies). Examples of ligands which may be used in accordance with the present invention include, but are not limited to, antibodies, mimetics (e.g., Affibodies® see: U.S. Pat. Nos. 5,831,012, 6,534,628 and 6,740,734) or fragments thereof, epitope tags, antigens, biotin and derivatives thereof, avidin and derivatives thereof, metal ions, receptors and fragments thereof (e.g., EGF binding domain), enzymes (e.g., proteases) and mutants thereof (e.g., catalytic inactive), substrates (e.g., heparin), lectins (e.g., concanavalin A), carbohydrates (e.g., heparin), nucleic acid sequences [e.g., aptamers and Spiegelmers [Wlotzka® (2002) Proc. Natl. Acad. Sci. USA 99:8898-02], dyes which often interact with the catalytic site of an enzyme mimicking the structure of a natural substrate or co-factor and consisting of a chromophore (e.g., azo dyes, anthraquinone, or phathalocyanine), linked to a reactive group (e.g., a mono- or dichlorotriazine ring, see, Denzili (2001) J Biochem Biophys Methods. 49(1-3):391-416), small molecule chemicals, receptor ligands (e.g., growth factors and hormones), mimetics having the same binding function but distinct chemical structure, or fragments thereof (e.g., EGF domain), ion ligands (e.g., calmodulin), protein A, protein G and protein L or mimetics thereof (e.g., PAM, see Fassina (1996) J. Mol. Recognit. 9:564-9], chemicals (e.g., cibacron Blue which bind enzymes and serum albumin; amino acids e.g., lysine and arginine which bind serine proteases) and magnetic molecules such as high spin organic molecules and polymers (see http://www.chem.unl.edu/rajca/highspin.html).

As used herein the phrase "coordinating moiety" refers to any molecule having sufficient affinity (e.g., $K_D < 10^{-5}$) to a coordinator ion or molecule. The coordinating moiety can direct the composition of matter of this aspect of the present invention to form a non-covalent complex when co-incubated with a coordinator ion or molecule. Examples of coordinating moieties which can be used in accordance with the present invention include but are not limited to, epitopes (antigenic determinants antigens to which the paratope of an antibody binds), antibodies, chelators (e.g., His-tag, see other example in Example 1 of the Examples section which follows, Figures 1, 25 and 26), biotin (see Figure 7), nucleic acid sequences (see Figure 6), protein A or G (Figure 9), electron poor molecules and electron rich molecules (see Example 2 of the Examples section which follows and Figure 8) and other molecules described hereinabove (see examples for ligands).

It will be appreciated that a number of coordinating moieties can be bound to the ligand described above (see Figures 1a-f).

It will be further appreciated that different coordinating moieties can be attached to the ligand such as a chelator and an electron rich/poor molecule to form a complex such as is shown in Figure 19. Such a combination of binding moieties may mediate the formation of polymers or ordered sheets (i.e., networks) containing the molecule of interest as is illustrated in Figures 23a-b and 24, respectively.

To avoid competition and/or further problems in the recovery of the molecule of interest from the complex, the coordinating moiety is selected so as to negate the possibility of coordinating moiety-ligand interaction or coordinating moiety-target molecule interaction. For example, if the ligand is an antigen having an affinity towards an immunoglobulin of interest than the coordinating moiety is preferably not an epitope tag or an antibody capable of binding the antigen.

As used herein the phrase "coordinator ion or molecule" refers to a soluble entity (i.e., molecule or ion), which exhibits sufficient affinity (i.e., $K_D < 10^{-5}$) to the coordinating moiety and as such is capable of directing the composition of matter of this aspect of the present invention to form a non-covalent complex. Examples of coordinator molecules which can be used in accordance with the present invention include but are not limited to, avidin and derivatives thereof, antibodies, electron rich molecules, electron poor molecules and the like. Examples of coordinator ions which can be used in accordance with the present invention include but are not limited to, mono, bis or tri valent metals. Figure 25 illustrates examples of chelators and metals which can be used as a coordinator ion by the present invention. Figure 26 lists examples of electron rich molecules and electron poor molecules which can be used by the present invention. Methods of generating antibodies and antibody fragments as well as single chain antibodies are described in Harlow and Lane, Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory, New York, 1988, incorporated herein by reference; Goldenberg, U.S. Pat. Nos. 4,036,945 and 4,331,647, and references contained therein; See also Porter, R. R. [Biochem. J. 73: 119-126 (1959); Whitlow and Filpula, Methods 2: 97-105 (1991); Bird *et al.*, Science 242:423-426 (1988); Pack *et al.*, Bio/Technology 11:1271-77 (1993); and U.S. Pat. No. 4,946,778].

Preferably, the composition of this aspect of the present invention includes the coordinator ion or molecule.

The ligand of this aspect of the present invention may be bound directly to the coordinating moiety, depending on the chemistry of the two. Measures are taken, though, to maintain recognition (e.g., affinity) of the ligand to the molecule of interest. When needed (e.g., steric hindrance), the ligand may be bound to the coordinating moiety via a linker. A general synthetic pathway for modification of representative chelators with a general ligand is shown in Figure 14. Margherita et al. (1993) J. Biochem. Biophys. Methods 38:17-28 provides synthetic procedures which may be used to attach the ligand to the coordinating moiety of the present invention.

When the ligand and coordinating moiety bound thereto are both proteins (e.g., growth factor and epitope tag, respectively), synthesis of a fusion protein can be effected by molecular biology methods (e.g., PCR) or biochemical methods (solid phase peptide synthesis).

Complexes of the present invention be of various complexity levels, such as, monomers (see Figures 12 and 13a-b depicting a three ligand complex), dimers, polymers (see Figure 23a-b depicting formation of a polymer via a combined linker as described in Example 3 of the Examples section), sheets (see Figure 24 in which sheets are formed when a single surface exposed Trp residue of a target molecule forms electron rich/poor relations with a TNB---TNB entity) and lattices which may form three dimensional (3D) structures (such as when more than one surface exposed Trp residues form electron rich/poor relations). It is well established that the higher complexity of the complex the more rigid is the structure enabling use thereof in crystallization procedures as further described hereinbelow. Furthermore, large complexes will phase separate more rapidly, negating the use of further centrifugation steps.

The compositions of the present invention can be packed in a purification kit which may include additional buffers and additives, as described hereinbelow. It will be appreciated that such kits may include a number of ligands for purifying a number of molecules from a single sample. However, to simplify precipitation (e.g., using the same reaction buffer, temperature conditions, pH and the like) and further purification steps, the coordinating moieties and coordinator ions or molecules are selected the same.

As mentioned hereinabove, the compositions of the present invention may be used to purify a molecule or cell of interest from a sample.

Thus, according to another aspect of the present invention there is provided a method of purifying a molecule of interest.

As used herein the term "purifying" refers to at least separating the molecule of interest from the sample by changing its solubility upon binding to the composition of the present invention and precipitation thereof (i.e., phase separation).

The method of this aspect of the present invention is effected by contacting a sample including the molecule of interest with a composition of the present invention and collecting a precipitate which includes a complex formed from the composition-of-matter of the present invention and the molecule of interest, thereby purifying the molecule of interest.

As used herein the term "sample" refers to a solution including the molecule of interest and possibly one or more contaminants (i.e., substances that are different from the desired molecule of interest). For example when the molecule of interest is a secreted recombinant polypeptide, the sample can be the conditioned medium, which may include in addition to the recombinant polypeptide, serum proteins as well as metabolites and other polypeptides, which are secreted from the cells. When the sample includes no contaminants, purifying refers to concentrating.

In order to initiate purification, the composition-of-matter of the present invention is first contacted with the sample. This is preferably effected by adding the ligand attached to the coordinating moiety to the sample allowing binding of the molecule of interest to the ligand and then adding the coordinator ion or molecule to allow complex formation and precipitation of the molecule of interest. In order to avoid rapid formation of complexes (which may result in the entrapment of contaminants) slow addition of the coordinator to the sample while stirring is preferred. Controllable rate of precipitation can also be achieved by adding free coordinating entity (i.e., not bound to the ligand), which may also lead to the formation of smaller complexes which may be beneficial in a variety of applications such as for the formation of immunogens, further described hereinbelow.

Once the complex described above is formed (seconds to hours), precipitation of the complex may be facilitated by centrifugation (e.g., ultra-centrifugation), although in some cases (for example, in the case of large complexes) centrifugation is not necessary.

Depending on the intended use the molecule of interest, the precipitate may be subjected to further purification steps in order to recover the molecule of interest from the complex. This may be effected by using a number of biochemical methods which are well known in the art. Examples include, but are not limited to, fractionation on a hydrophobic interaction chromatography (e.g. on phenyl sepharose), ethanol precipitation, isoelectric focusing, reverse phase HPLC, chromatography on silica, chromatography on heparin sepharose, anion exchange chromatography, cation exchange chromatography, chromatofocusing, SDS-PAGE, ammonium sulfate precipitation, hydroxylapatite chromatography, gel electrophoresis, dialysis, and affinity chromatography (e.g. using protein A, protein G, an antibody, a specific substrate, ligand or antigen as the capture reagent).

It will be appreciated that simple addition of clean reaction solution (e.g., buffer) may be added to the precipitate to elute low affinity bound impurities which were precipitated during complex formation.

It will be further appreciated that any of the above-described purification procedures may be repetitively applied on the sample (i.e., precipitate) to increase the yield and or purity of the target molecule.

Preferably, the composition of matter and coordinator ion or molecule are selected so as to enable rapid and easy isolation of the target molecule from the complex formed. For example, the molecule of interest may be eluted directly from the complex, provided that the elution conditions employed do not disturb binding of the coordinating moiety to the coordinator (see Figures 4-5). For example, when the coordinating moiety used in the complex is a chelator, high ionic strength may be applied to elute the molecule of interest, since it is well established that it does not effect metal-chelator interactions. Alternatievly, elution with chaotropic salt may be used, since it has been shown that metal-chelator interactions are resistant to high salt conditions enabling elution of the target molecule at such conditions [Porath (1983) Biochemistry 22:1621-1630].

The complex can be re-solubilized by the addition of free (unmodified) chelator (i.e., coordinating moiety), which competes with the coordinator metal (Figure 3). Ultrafiltration or dialysis may be used, thereafter, to remove most of the chelated metal and the competing chelator. The solubilized complex (i.e., molecule of interest:ligand-coordinating moiety) can then be loaded on an immobilized metal affinity column [e.g., iminodiacetic acid (IDA) and nitrilotriacetic acid (NTA)]. It will be appreciated that when high affinity chelators are used (e.g., catechol), measures are taken to use immobilized metal affinity ion column modified with the same or with other chelator having similar binding affinities toward the immobilized metal, to avoid elution of the ligand:chelator agent from the column instead of binding to it.

Application of suitable elution conditions will result in the elution of the target molecule keeping the ligand-coordinating moiety bound to the column. A final desalting procedure may be applied to obtain the final product.

Regeneration of the ligand-coordinating moiety is of high economical value, since synthesis of such a fusion molecule may contribute most of the cost and labor involved in the methodology described herein. Thus, for example, regeneration of the ligand-coordinating moiety can be achieved by loading the above-described column with a competing chelator or changing column pH followed by ultrafiltration that may separate between the free chelator and the desired ligand-coordinating moiety.

The above-described purification methodology can be applied for the isolation of various recombinant and natural substances which are of high research or clinical value such as recombinant growth factors and blood protein products *(e.g.,* von Willebrand Factor and Factor VIII which are therapeutic proteins effective in replacement therapy for von Willebrand's disease and Hemophilia A, respectively).

As mentioned hereinabove, the compositions of the present invention may be used to isolate particular populations of cells as well.

It is well established that due to shortage in human organs, in-vitro organogenesis is emerging as an optimal substitute. To this end, stem cells which are capable of differentiating to any desired cell lineage must be isolated. Thus, for example, to isolate hematopoietic stem/progenitor cells a number of ligands may be employed which bind to surface markers which are unique to this cell population, such as CD34 and CD105 [see Pierelli (2001) Leuk. Lymphoma 42(6):1195-206].

Another example is the isolation of erythrocytes using lectin ligands, such as concanavalin A [Sharon (1972) Science 177:949; Goldstein (1965) Biochemistry 4:876].

Viral cell isolation may be effected using various ligands which are specific for viral cells of interest [see http://www.bdbiosciences.com/clontech/archive/JAN04UPD/Adeno-X.shtml].

Specifically, retroviruses may be isolated by the compositions of the present invention which are designed to include a heparin ligand [Kohleisen (1996) J Virol Methods 60(1):89-101].

Cell isolation using the above-described methodology may be effected with preceding steps of sample de-bulking which is effected to isolate cells based on cell density or size (e.g., centrifugation) and further steps of selective cell-enrichment (e.g., FACS).

On top of their purifying capabilities, the compositions of the present invention may also be used to deplete a sample from undesired molecules or cells.

This is effected by contacting the sample including the undesired target molecule or cell of interest with the composition of the present invention such that a complex is formed (described above) and removing the precipitate. The clarified sample is the supernatant.

This method have various uses such as in depleting tumor cells from bone marrow samples, depleting B cells and monocytes for the isolation and enrichment of T cells and $CD8^+$ cells or CD 4^+ cells from peripheral blood, spleen, thymus, lymph or bone marrow samples, depleting pathogens and unwanted substances (e.g., prions, toxins) from biological samples, protein purification (e.g., depleting high molecular weight proteins such as BSA) and the like.

As mentioned hereinabove multiple ligands may be employed for the depletion of a number of targets from a given sample such as for the removal of highly abundant proteins from biological fluids (e.g., albumin, IgG, anti-trypsin, IgA, transferrin and haptoglobin, see http://www.chem.agilent.com/cag/prod/ca/51882709small.pdf).

The unique properties of the novel compositions of the present invention provide numerous advantages over prior art precipitation compositions (e.g., smart polymers), some of these these advantages are summerized infra.

(i) Low cost purification; the present methodology does not rely upon sophisticated laboratory equipment such as HPLC, thereby circumventing machine maintenance and operating costs.

(ii) Easy up scaling; the present methodology is not restricted by limited capacity of affinity columns having diffusion limitations. Essentially, the amount of added precipitating complex is unlimited.

(iii) Mild precipitation process; averts limitations resulting from substantial changes in pH, ionic strength or temperature.

(iv) Control over the precipitation process; precipitation may be governed by, slow addition of an appropriate coordinator ion or molecule to the precipitation mixture; use of mono and/or multi-valent coordinators; use of coordinator ions or molecules with different affinities towards the coordinating moiety; addition of the non-immobilized free coordinating moieties to avoid non-specific binding and entrapment of impurities prior to, during or following formation of a non-covalent polymer, sheet or lattice [Mattiasson et al., (1998) J. Mol. Recognit. 11:211-216; Hilbrig and Freitag (2003) J. Chromatogr. B 790:79-90]; as well as by varrying temperature conditions. It is well established that various molecules exhibit lower solubility as the temperature decreases, therefore, controlling temperature conditions may regulate the rate and degree of precipitation. It will be appreciated, though, that low temperature conditions may lead to entrapment of impurities due to a fast precipitation process, while high temperature conditions may lead to low yields of the target molecule (e.g., denaturing temperatures). Thus measures are taken to achieve optimal temperature conditions, while considering the above parameters.

(v) Reduced contamination background; contaminants cannot bind the coordinator entity and as such they cannot bind tightly to the non-covalent matrix, allowing their removal prior to the elution step. Furthermore, contaminations deriving from the ligand biological background (molecules which co-purified with the ligand) may become modified as well as the ligand itself [provided that the ligand and the contaminants share the same chemistry (e.g., both being proteins)], and might become part of the precipitating complex. Under suitable elution conditions, the target molecule will be recovered, while the modified contaminations will not.

(vi) Binding in homogenous solutions; it is well established that binding in homogeneous solution is more rapid and more effective than in heterogeneous phases such as in affinity chromatography [AC, Schneider et al., (1981) Ann. NY Acad. Sci. 369, 257-263; Lowe (2001) J. Biochem. Biophys. Methods 49, 561-574]. For example, high molecular mass polymers (used in AP) are known to form highly coiled and viscous structures in solutions that hinder the access of incoming macromolecules such as the target molecules as in many affinity separation strategies. [Vaida et al., (1999) Biotechnol. Bioeng. 64:418].

(vii) No immobilization of the ligand – further described hereinabove.

(viii) Easy resolubilzation of the complex; the complex is generated by non-covalent interactions.

(ix) Sanitizing under harsh conditions; the composition is <u>not</u> covalently bound to a matrix and as such can be removed from any device, allowing application of sanitizing conditions to clean the device (column) from non-specifically bound impurities.

The ability of the compositions of the present invention to arrange molecules of interest in ordered complexes such as in dimers, trimers, polymers, sheets or lattices also enables use thereof in facilitating crystallization of macromolecules such as proteins, in particular membraneous proteins. As is well known in the art, a crystal structure represents ordered arrangement of a molecule in a three dimensional space. Such ordered arrangement can be egenerated by reducing the number of free molecules in a given space (see Figures 10a-b and 11a-c).

Thus, according to yet another aspect of the present invention there is provided a composition for crystallizing a molecule of interest.

As used herein the term "crystallizing" refers to the solidification of the molecule of interest so as to form a regularly repeating internal arrangement of its atoms and often external plane faces.

The composition of this aspect of the present invention includes at least one ligand capable of binding the molecule of interest, wherein the ligand is attached to at least one coordinating moiety; and a coordinator capable of non-covalently binding the at least one coordinating moiety, wherein the at least one coordinating moiety and the coordinator are capable of forming a complex when co-incubated and whereas the composition is selected so as to define the relative spatial positioning and orientation of the molecule of interest when bound thereto, thereby facilitating formation of a crystal therefrom under inducing crystallization conditions.

It will be appreciated that the use of covalent multi ligand complexes has been previously attempted in the crystallization of soluble proteins [Dessen (1995) Biochemistry 34:4933-4942; Moothoo (1998) Acta. Cryst. D54 1023-1025; Bhattacharyya (1987) J. Biol. Chem. 262:1288-1293]. However, synthesis of multi-ligand complexes which have more than two ligands per molecule is technically difficult and expensive; Furthermore, the three-dimensional structure of the target protein should be known in advance to synthesize multi ligand complexes which have the optimal distance between the ligands to bind enough target molecules to occupy all target binding sites in the multi-ligand complex, as such, these ligands were never used for the crystallization of membrane proteins.

The present invention circumvents these, by synthesizing only the basic unit in the non-covalent multi-ligand, (having the general structure of: Ligand—coordinating moiety) which is far easier to achieve, faster and cheaper. This basic unit, would form non-covalent tri-ligand only by adding the multi valent coordinator ion or molecule. Thus, a single synthesis step is used to form di, tri, tetra or higher multi ligands that may be used for crystallization experiments.

In order to produce crystals of a molecule of interest (preferably of membrane proteins) the compositions of the preset invention are contacted with a sample, which includes the molecule of interest preferably provided at a predetermined purity and concentration.

Typically, the crystallization sample is a liquid sample. For example, when the molecule of interest is a membrane protein, the crystallization sample, according to this aspect of the present invention, is a membrane preparation. Methods of generating membrane preparations are described in Strategies for Protein Purification and Characterization - A Laboratory Course Manual" CSHL Press (1996).

Once the molecule of interest is bound to the composition of the present invention, such that its relative spatial positioning and orientation are well defined, the sample is subjected to suitable crystallization conditions. Several crystalization approaches which are known in the art can be applied to the sample in order to facilitate crystalization of the molecule of interest. Examples of crystallization approaches include, but are not limited to, the free interface diffusion method [Salemme, F. R. (1972) Arch. Biochem. Biophys. 151:533-539], vapor diffusion in the hanging or sitting drop method (McPherson, A. (1982) Preparation and Analysis of Protein Crystals, John Wiley and Son, New York, pp 82-127), and liquid dialysis (Bailey, K. (1940) Nature 145:934-935).

Presently, the hanging drop method is the most commonly used method for growing macromolecular crystals from solution; this approach is especially suitable for generating protein crystals. Typically, a droplet containing a protein solution is spotted on a cover slip and suspended in a sealed chamber that contains a reservoir with a higher concentration of precipitating agent. Over time, the solution in the droplet equilibrates with the reservoir by diffusing water vapor from the droplet, thereby slowly increasing the concentration of the protein and precipitating agent within the droplet, which in turn results in precipitation or crystallization of the protein.

Crystals obtained using the above-described methodology, have a resolution of preferably less than 3 Å, more preferably less than 2.5 Å, even more preferably less than 2 Å.

Compositions of the present invention may have evident utility in assaying analytes from complex mixtures such as serum samples, which may have obvious diagnostic advantages.

Thus, the present invention envisages a method of detecting predisposition to, or presence of a disease associated with a molecule of interest in a subject.

An example of a disease which is associated with a molecule of interest is prostate cancer which may be detected by the presence of prostate specific antigen [PSA, e.g., >0.4 ng/ml, Boccon-Gibod Int J Clin Pract. (2004) 58(4):382-90].

The compositions of the present invention are contacted with a biological sample obtained from the subject whereby the level of complex formation including the molecule of interest is indicative of predisposition to, or presence of the disease associated with the molecule of interest in the subject.

As used herein the phrase "biological sample" refers to a sample of tissue or fluid isolated from a subject, including but not limited to, for example, plasma, serum, spinal fluid, lymph fluid, the external sections of the skin, respiratory, intestinal, and genitourinary tracts, tears, saliva, milk, blood cells, tumors, neuronal tissue, organs, and also samples of in vivo cell culture constituents.

To facilitate detection and quantification of the molecule of interest in the complexes, the biological sample or the composition is preferably labeled (e.g., fluorescent, radioactive labeling).

Compositions of the present invention may also be utilized to qualify and quantify substances present in a liquid or gaseous samples which may be of great importance in clinical, environmental, health and safety, remote sensing, military, food/beverage and chemical processing applications.

Abnormal protein interaction governs the development of many pathogenic disorders. For example, abnormal interactions and misfolding of synaptic proteins in the nervous system are important pathogenic events resulting in neurodegeneration in various neurological disorders. These include Alzheimer's disease (AD), Parkinson's disease (PD), and dementia with Lewy bodies (DLB). In AD, misfolded amyloid beta peptide 1-42 (Abeta), a proteolytic product of amyloid precursor protein metabolism, accumulates in the neuronal endoplasmic reticulum and extracellularly as aggregates (i.e., plaques). The compositions of the present invention can be used to disturb such macromolecular complexes to thereby treat such disorders.

Methods of administration and generation of pharmaceutical compositions are described by, for example, Fingl, et al., (1975) "The Pharmacological Basis of Therapeutics", Ch. 1 p.1.

The compositions of the present invention can be included in a diagnostic or therapeutic kits. For example, compositions of a specific disease can be packaged in a one or more containers with appropriate buffers and preservatives and used for diagnosis or for directing therapeutic treatment.

Thus, the ligand and coordinating moiety can be placed in one container and the coordinator molecule or ion can be placed in a second container. Preferably, the containers include a label. Suitable containers include, for example, bottles, vials, syringes, and test tubes. The containers may be formed from a variety of materials such as glass or plastic.

In addition, other additives such as stabilizers, buffers, blockers and the like may also be added.

A number of methods are known in the art for enhancing the immunogenic potential of antigens. For example, hapten carrier conjugation which involves cross-linking of the antigenic molecule (e.g., peptides) to larger carriers such as KLH, BSA thyroglobulin and ovalbumin is used to elevate the molecular size of the molecule, a parameter known to govern immunogenicity [see Harlow and Lane (1998) A laboratory manual Infra]. However, covalent cross-linking of the antigenic molecule leads to structural alterations therein, thereby limiting antigenic presentation. Non-covalent immobilization of the antigenic molecule to various substrates have been attempted to circumvent this problem [Sheibani Frazier (1998) BioTechniques 25:28]. Accordingly, compositions of the present invention may be used to mediate the same.

Thus, the present invention also envisages a method of enhancing immunogenicity of a molecule of interest using the compositions of the present invention. As used herein the term "immunogenicity" refers to the ability of a molecule to evoke an immune response (e.g., antibody response) within an organism.

The method is effected by contacting the molecule of interest with the composition of the present invention whereby the complex thus formed serves as an immunogen. Such a complex can be injected to an animal host to generate an immune response.

Thus, for example, to generate an antibody response, the above-described immunogenic composition is subcutaneously injected into the animal host (e.g., rabbit or mouse). Following 1-4 injections (i.e., boosts), serum is collected (about 14 weeks of first injection) and antibody titer is determined such as by using the above-described methods of analyte detection in samples, where the ligand is protein A for example. Alternatively or additionally, affinity chromatography or ELISA is effected.

It will be appreciated that the compositions of the present invention may have numerous other utilities, which are not distinctly described herein such as those utilities, which are attributed to affinity chromatography [see e.g., Wen-Chien and Kelvin (2004) Analytical Biochemistry 324:1-10].

Additional objects, advantages, and novel features of the present invention will become apparent to one ordinarily skilled in the art upon examination of the following examples, which are not intended to be limiting. Additionally, each of the various embodiments and aspects of the present invention as delineated hereinabove and as claimed in the claims section below finds experimental support in the following examples.

EXAMPLES

Reference is now made to the following examples, which together with the above descriptions, illustrate the invention in a non limiting fashion.

Generally, the nomenclature used herein and the laboratory procedures utilized in the present invention include molecular, biochemical, microbiological and recombinant DNA techniques. Such techniques are thoroughly explained in the literature. See, for example, "Molecular Cloning: A laboratory Manual" Sambrook et al., (1989); "Current Protocols in Molecular Biology" Volumes I-III Ausubel, R. M., ed. (1994); Ausubel et al., "Current Protocols in Molecular Biology", John Wiley and Sons, Baltimore, Maryland (1989); Perbal, "A Practical Guide to Molecular Cloning", John Wiley & Sons, New York (1988); Watson et al., "Recombinant DNA", Scientific American Books, New York; Birren et al. (eds) "Genome Analysis: A Laboratory Manual Series", Vols. 1-4, Cold Spring Harbor Laboratory Press, New York (1998); methodologies as set forth in U.S. Pat. Nos. 4,666,828; 4,683,202; 4,801,531; 5,192,659 and 5,272,057; "Cell Biology: A Laboratory Handbook", Volumes I-III Cellis, J. E., ed. (1994); "Current Protocols in Immunology" Volumes I-III Coligan J. E., ed. (1994); Stites et al. (eds), "Basic and Clinical Immunology" (8th Edition), Appleton & Lange, Norwalk, CT (1994); Mishell and Shiigi (eds), "Selected Methods in Cellular Immunology", W. H. Freeman and Co., New York (1980); available immunoassays are extensively described in the patent and scientific literature, see, for example, U.S. Pat. Nos. 3,791,932; 3,839,153; 3,850,752; 3,850,578; 3,853,987; 3,867,517; 3,879,262; 3,901,654; 3,935,074; 3,984,533; 3,996,345; 4,034,074; 4,098,876; 4,879,219; 5,011,771 and 5,281,521; "Oligonucleotide Synthesis" Gait, M. J., ed. (1984); "Nucleic Acid Hybridization" Hames, B. D., and Higgins S. J., eds. (1985); "Transcription and Translation" Hames, B. D., and Higgins S. J., Eds. (1984); "Animal Cell Culture" Freshney, R. I., ed. (1986); "Immobilized Cells and Enzymes" IRL Press, (1986); "A Practical Guide to Molecular Cloning" Perbal, B., (1984) and "Methods in Enzymology" Vol. 1-317, Academic Press; "PCR Protocols: A Guide To Methods And Applications", Academic Press, San Diego, CA (1990); Marshak et al., "Strategies for Protein Purification and Characterization - A Laboratory Course

Manual" CSHL Press (1996); all of which are incorporated by reference as if fully set forth herein. Other general references are provided throughout this document. The procedures therein are believed to be well known in the art and are provided for the convenience of the reader. All the information contained therein is incorporated herein by reference.

EXAMPLE 1

Synthesis of non covalent multi ligand complexes utilizing chelator-metal complexes

The ability of chelators to bind metals, with different specificities and affinities is well described in the literature. To generate the non-covalent multi ligand complex of the present invention, a linker, (of a desired length) is modified to bind a specific ligand, and a chelator to generate the following general structure of: ligand----linker----chelator.

Then, by the addition of an appropriate metal, a non-covalent multi-ligand complex should be formed. (Figure 12)

For example, a hydroxamate (which is a known Fe^{3+} chelator) derivative is synthesized (Figure 13a) such that in the presence of Fe^{3+} ions, a non-covalent multi-ligand complex is formed (Figure 13b). A general synthetic pathway for modification of representative chelators with a general ligand is shown in Figure 14. Such a synthesis can be similar to the one presented by Margherita et al., 1999 supra.

The utilization of chelators for the preparation of a non-covalent multi-ligand complex, may have an additional advantage which arises from the ability of some chelators to bind different metals with different stochiometries, as in the case of $[1,10\text{-phenanthroline}]_2\text{-}Cu^{2+}$, or $[1,10\text{-phenanthroline}]_3\text{-}Ru^{3+}$ [Onfelt et al., (2000) Proc. Natl. Acad. Sci. USA 97:5708-5713].

This phenomenon can be utilized for formation of di (Figure 15a) and tri (Figure 15b) non-covalent multi-ligand complexes, utilizing the same: ligand----linker----chelator derivative.

EXAMPLE 2

Synthesis of non-covalent multi ligand complexes utilizing electron rich-poor complexes

Electron acceptors form molecular complexes readily with the "π excessive" heterocyclic indole ring system. Indole picric acid was the first complex of this type to be described nearly 130 years ago [Baeyer, and Caro, (1877) Ber. 10:1262] and the same electron acceptor was used a few years later to isolate indole from jasmine flower oil. Picric acid had since been used frequently for isolating and identifying indoles as complexes from reaction mixtures. Later, 1,3,5-trinitro benzene was introduced as a complexing agent and often used for the same purpose [Merchant, and Salagar, (1963) Current Sci. 32:18]. Other solid complexes of indoles have been prepared with electron acceptors such as: styphnic acid [Marion, L., and Oldfield, C. W., (1947) Cdn. J. Res. 25B 1], picryl halides [Triebs, W., (1961) Chem. Ber. 94:2142], 2,4,5,7-tetranitro-9-fluorenone [Hutzinger, O., and Jamieson, W. D., Anal. Biochem. (1970) **35**, 351-358], and with 1-fluoro-2,4-dinitorbenzene and 1-chloro-2,4-dinitorbenzene [Elguero et al., (1967) Anals Real Soc. Espan. Fis. Quim. (Madrid) ser. B 63, 905 (1967); Wilshire, J. F. K., Australian J. Chem. **19**, 1935 (1966)].

Figure 16a, illustrates one example of a ligand----linker----electron poor (E. poor) derivative, and Figure 16b, presents an example of an electron rich covalent trimer that could be used. It is expected, that by mixing together the trinitrobenzene (Figure 16a) and the indole (Figure 16b) derivatives, a multi-ligand complex will be formed (Figure 16c). It will be appreciated that the reverse complex could be synthesized as well, i.e., a ligand derivative with an electron rich moiety, and an electron poor covalent trimer.]

A possible synthetic pathway for the preparation of the above ligand derivatives is shown in Figure 17.

Synthetic peptides (or any peptide) containing Trp residues (or any other electron rich or poor moieties) may also be of use for the preparation of non-covalent multi ligand complexes. Figure 18 shows an example of a synthetic peptide with four Trp residues (four electron rich moieties) that can be formed, a tetra-non-covalent-ligand in the presence of a ligand derivative modified with an electron poor moiety (trinitrobenzene).

EXAMPLE 3

Synthesis of non-covalent multi ligand complexes utilizing a combination of electron rich-poor and chelator-metal relationships

One can combine the two complexing abilities as described in Examples 1 and 2 above, so as to form non-covalent multi ligand complexes. An example of the general structure of such a non-covalent multi ligand complex is shown in Figure 19.

To this end, a chelator that is covalently bound to an electron poor moiety is desired. A synthetic pathway for generating such a combination is presented in Figure 20.

For example, a chelator (e.g., catechol) that is capable to bind both to M^{2+}, and M^{3+} metals, is capable in the presence of M^{2+} and M^{3+} metals, to form a non-covalent-di-ligand, (Figure 21a), or a non-covalent-tri-ligand (Figure 21b).

The presence of a peptide (or polypeptide) with a Trp residue (or any other electron rich residue) might lead to the formation of the structures shown in Figures 22a-b.

The combination of the two above binding relationships (chelator-metal together with electron rich-poor) may introduce additional advantages. For example, the ability to form non-covalent-multi-ligand-polymeric complexes. This may be achieved by synthesizing two chelators and an electron rich moiety between them (Figure 23a). In the presence of a ligand----E. poor derivative the complex which is drawn in Figure 23b is expected to form, which represents a Non-Covalent Polymer of ligands.

Once a dimer, trimer, tetramer etc. is formed, (by a ligand----chelator derivative for example) it may be desired to limit the freedom of motion of the above, in order to achieve more order. If the protein of interest has an electron rich moiety (such as Trp) that is accessible to a covalent di-electron-poor moiety (such as di-trinitrobnezene, TNB---TNB for example) then a complex might be formed between two non-covalent dimers. (Figure 24). This may lead to the formation of ordered sheets of proteins and multi-ligands.

It is appreciated that certain features of the invention, which are, for clarity, described in the context of separate embodiments, may also be provided in combination in a single embodiment. Conversely, various features of the invention, which are, for brevity, described in the context of a single embodiment, may also be provided separately or in any suitable subcombination.

Although the invention has been described in conjunction with specific embodiments thereof, it is evident that many alternatives, modifications and variations will be apparent to those skilled in the art. Accordingly, it is intended to embrace all such alternatives, modifications and variations that fall within the spirit and broad scope of the appended claims. All publications, patents and patent applications mentioned in this specification are herein incorporated in their entirety by reference into the specification, to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated herein by reference. In addition, citation or identification of any reference in this application shall not be construed as an admission that such reference is available as prior art to the present invention.

WHAT IS CLAIMED IS:

1. A composition of matter comprising at least one ligand capable of binding a target molecule or cell of interest, said at least one ligand being attached to at least one coordinating moiety selected capable of directing the composition of matter to form a non-covalent complex when co-incubated with a coordinator ion or molecule.

2. The composition of claim 1, wherein said complex is a polymeric complex.

3. The composition of claim 1, further comprising said coordinator ion or molecule.

4. The composition of claim 1, wherein said target molecule of interest is selected from the group consisting of a protein, a nucleic acid sequence, a small molecule chemical and an ion.

5. The composition of claim 1, wherein said target cell of interest is selected from the group consisting of a eukaryotic cell, a prokaryotic cell and a viral cell.

6. The composition of claim 1, wherein said at least one ligand is selected from the group consisting of a growth factor, a hormone, a nucleic acid sequence, an antibody, an epitope tag, an avidin, a biotin, a enzymatic substrate and an enzyme.

7. The composition of claim 1, wherein said at least one ligand is attached to said at least one coordinating moiety via a linker.

8. The composition of claim 1, wherein said coordinating moiety is selected from the group consisting of a chelator, a biotin, a nucleic acid sequence, an epitope tag, an electron poor molecule and an electron-rich molecule.

9. The composition of claim 1, wherein said coordinator ion or molecule is selected from the group consisting of a metal ion, an avidin, a nucleic acid sequence, an electron poor molecule and an electron-rich molecule.

10. A method of purifying a target molecule or cell of interest, the method comprising:

(a) contacting a sample including the target molecule or cell of interest with a composition including:
    (i) at least one ligand capable of binding the target molecule or cell of interest, said at least one ligand being attached to at least one coordinating moiety; and
    (ii) a coordinator capable of non-covalently binding said at least one coordinating moiety, said at least one coordinating moiety and said coordinator being capable of forming a complex when co-incubated; and
(b) collecting a precipitate including said complex bound to the target molecule or cell of interest, thereby purifying the target molecule or cell of interest.

11. The method of claim 10, wherein the molecule of interest is selected from the group consisting of a protein, a nucleic acid sequence, a small molecule chemical and an ion.

12. The method of claim 10, wherein the target cell of interest is selected from the group consisting of a eukaryotic cell, a prokaryotic cell and a viral cell.

13. The method of claim 10, wherein said at least one ligand is selected from the group consisting of a growth factor, a hormone, a nucleic acid sequence, an antibody, an epitope tag, an avidin, a biotin, a enzymatic substrate and an enzyme.

14. The method of claim 10, wherein said at least one ligand is attached to said at least one coordinating moiety via a linker.

15. The method of claim 10, wherein said coordinating moiety is selected from the group consisting of a chelator, a biotin, a nucleic acid sequence, an epitope tag, an electron poor molecule and an electron-rich molecule.

16. The method of claim 10, wherein said coordinator ion or molecule is selected from the group consisting of a metal ion, an avidin, a nucleic acid sequence, an electron poor molecule and an electron-rich molecule.

17. The method of claim 10, further comprising recovering the molecule of interest from said precipitate.

18. A method of detecting predisposition to, or presence of a disease associated with a molecule of interest in a subject, the method comprising contacting a biological sample obtained from the subject with a composition including:

(i) at least one ligand capable of binding the molecule of interest, said at least one ligand being attached to at least one coordinating moiety; and

(ii) a coordinator capable of non-covalently binding said at least one coordinating moiety, said at least one coordinating moiety and said coordinator being capable of forming a complex when co-incubated,

wherein formation of said complex including the molecule of interest is indicative of predisposition to, or presence of the disease associated with the molecule of interest in the subject.

19. The method of claim 18, wherein the molecule of interest is selected from the group consisting of a protein, a nucleic acid sequence, a small molecule chemical and an ion.

20. The method of claim 18, wherein said at least one ligand is selected from the group consisting of a growth factor, a hormone, a nucleic acid sequence, an antibody, an epitope tag, an avidin, a biotin, a enzymatic substrate and an enzyme.

21. The method of claim 18, wherein said at least one ligand is attached to said at least one coordinating moiety via a linker.

22. The method of claim 18, wherein said coordinating moiety is selected from the group consisting of a chelator, a biotin, a nucleic acid sequence, an epitope tag, an electron poor molecule and an electron-rich molecule.

23. The method of claim 18, wherein said coordinator ion or molecule is selected from the group consisting of a metal ion, an avidin, a nucleic acid sequence, an electron poor molecule and an electron-rich molecule.

24. A composition for crystallizing a molecule of interest, the composition comprising:

(i) at least one ligand capable of binding the molecule of interest, said at least one ligand being attached to at least one coordinating moiety; and

(ii) a coordinator capable of non-covalently binding said at least one coordinating moiety, wherein said at least one coordinating moiety and said coordinator are capable of forming a complex when co-incubated and whereas the composition is selected so as to define the relative spatial positioning and orientation of the molecule of interest when bound thereto, thereby facilitating formation of a crystal therefrom under inducing crystallization conditions.

25. The composition of claim 24, wherein the molecule of interest is selected from the group consisting of a protein, a nucleic acid sequence, a small molecule chemical and an ion.

26. The composition of claim 24, wherein said at least one ligand is selected from the group consisting of a growth factor, a hormone, a nucleic acid sequence, an antibody, an epitope tag, an avidin, a biotin, a enzymatic substrate and an enzyme.

27. The composition of claim 24, wherein said at least one ligand is attached to said at least one coordinating moiety via a linker.

28. The composition of claim 24, wherein said coordinating moiety is selected from the group consisting of a chelator, a biotin, a nucleic acid sequence, an epitope tag, an electron poor molecule and an electron-rich molecule.

29. The composition of claim 24, wherein said coordinator ion or molecule is selected from the group consisting of a metal ion, an avidin, a nucleic acid sequence, an electron poor molecule and an electron-rich molecule.

30. A method of crystallizing a molecule of interest, the method comprising contacting a sample including the molecule of interest with a crystallizing composition including:

(i) at least one ligand capable of binding the molecule of interest, said at least one ligand being attached to at least one coordinating moiety; and

(ii) a coordinator capable of non-covalently binding said at least one coordinating moiety, wherein said at least one coordinating moiety and said coordinator are capable of forming a complex when co-incubated and whereas said crystallyzing composition is selected so as to define the relative spatial positioning and orientation of the molecule of interest when bound thereto, thereby facilitating formation of a crystal therefrom under inducing crystallization conditions.

31. The method of claim 30, wherein the molecule of interest is selected from the group consisting of a protein, a nucleic acid sequence and a small molecule chemical.

32. The method of claim 30, wherein said at least one ligand is selected from the group consisting of a growth factor, a hormone, a nucleic acid sequence, an antibody, an epitope tag, an avidin, a biotin, a enzymatic substrate and an enzyme.

33. The method of claim 30, wherein said at least one ligand is attached to said at least one coordinating moiety via a linker.

34. The method of claim 30, wherein said coordinating moiety is selected from the group consisting of a chelator, a biotin, a nucleic acid sequence, an epitope tag, an electron poor molecule and an electron-rich molecule.

35. The method of claim 30, wherein said coordinator ion or molecule is selected from the group consisting of a metal, an avidin, a nucleic acid sequence, an electron poor molecule and an electron-rich molecule.

36. A composition-of-matter comprising a molecule having a first region capable of binding a molecule of interest and a second region capable of binding a coordinator ion or molecule, said second region being designed such that said molecule forms a polymer when exposed to said coordinator ion or molecule.

37. The composition of claim 36, wherein said second region is capable of binding more than two coordinator ions or molecules.

38. The composition of claim 36, wherein binding of said coordinator ion or molecule is non covalent binding.

39. A method of depleting a target molecule or cell of interest from a sample, the method comprising:

(a) contacting the sample including the target molecule or cell of interest with a composition including:
    (i) at least one ligand capable of binding the molecule of interest, said at least one ligand being attached to at least one coordinating moiety; and
    (ii) a coordinator capable of non-covalently binding said at least one coordinating moiety, said at least one coordinating moiety and said coordinator being capable of forming a complex when co-incubated; and
(b) removing a precipitate including said complex bound to the target molecule or cell of interest to thereby deplete the target molecule or cell of interest from the sample

40. The method of claim 39, wherein the molecule of interest is selected from the group consisting of a protein, a nucleic acid sequence, a small molecule chemical and an ion.

41. The method of claim 39, wherein the target cell of interest is selected from the group consisting of a eukaryotic cell, a prokaryotic cell and a viral cell.

42. The method of claim 39, wherein said at least one ligand is selected from the group consisting of a growth factor, a hormone, a nucleic acid sequence, an antibody, an epitope tag, an avidin, a biotin, a enzymatic substrate and an enzyme.

43. The method of claim 39, wherein said at least one ligand is attached to said at least one coordinating moiety via a linker.

44. The method of claim 39, wherein said coordinating moiety is selected from the group consisting of a chelator, a biotin, a nucleic acid sequence, an epitope tag, an electron poor molecule and an electron-rich molecule.

45. The method of claim 39, wherein said coordinator ion or molecule is selected from the group consisting of a metal ion, an avidin, a nucleic acid sequence, an electron poor molecule and an electron-rich molecule.

46. A method of enhancing immunogenicity of a target molecule of interest, the method comprising contacting the target molecule of interest with a composition including:

(i) at least one ligand capable of binding the target molecule of interest, said at least one ligand being attached to at least one coordinating moiety; and

(ii) a coordinator capable of non-covalently binding said at least one coordinating moiety,

wherein contacting is effected such that said at least one coordinating moiety and said coordinator forms a complex including the target molecule of interest, thereby enhancing immunogenicity of the target molecule of interest.

47. The method of claim 46, wherein the molecule of interest is selected from the group consisting of a protein, a nucleic acid sequence, a small molecule chemical and an ion.

48. The method of claim 46, wherein said at least one ligand is selected from the group consisting of a growth factor, a hormone, a nucleic acid sequence, an antibody, an epitope tag, an avidin, a biotin, a enzymatic substrate and an enzyme.

49. The method of claim 46, wherein said at least one ligand is attached to said at least one coordinating moiety via a linker.

50. The method of claim 46, wherein said coordinating moiety is selected from the group consisting of a chelator, a biotin, a nucleic acid sequence, an epitope tag, an electron poor molecule and an electron-rich molecule.

51. The method of claim 46, wherein said coordinator ion or molecule is selected from the group consisting of a metal ion, an avidin, a nucleic acid sequence, an electron poor molecule and an electron-rich molecule.

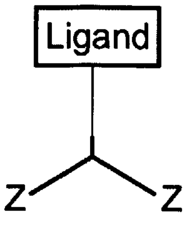


Fig. 1a

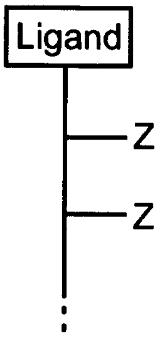


Fig. 1b

Fig. 1c

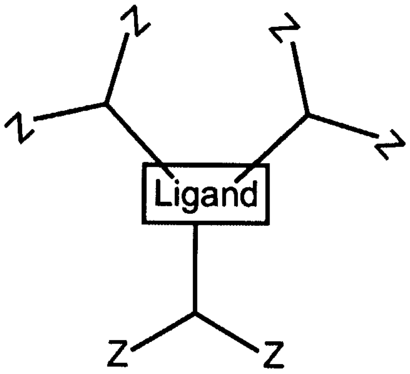


Fig. 1d

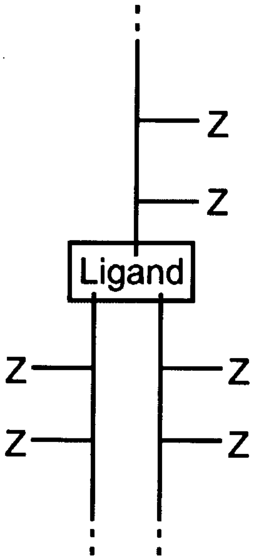


Fig. 1e

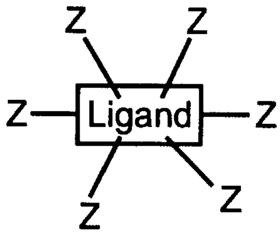


Fig. 1f

Z = chelator and/nucleotide sequence and/biotin, (and its derivatives) and/an electron rich or poor entity, etc...

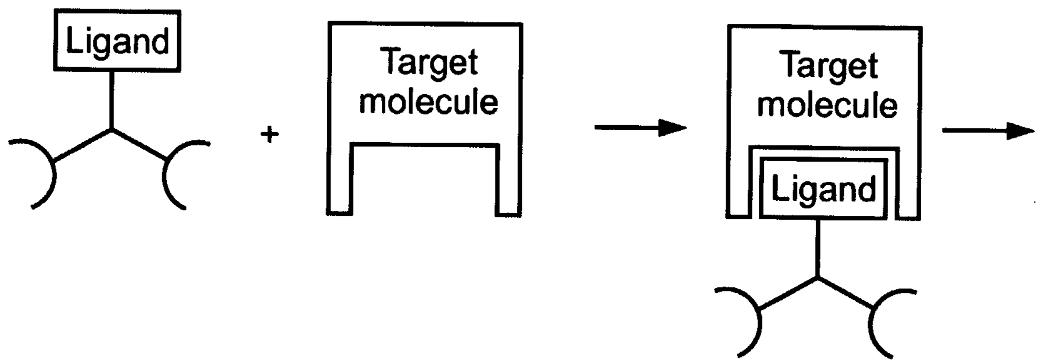

Ligand
+
Target molecule
Target molecule
Ligand

Fig. 2a

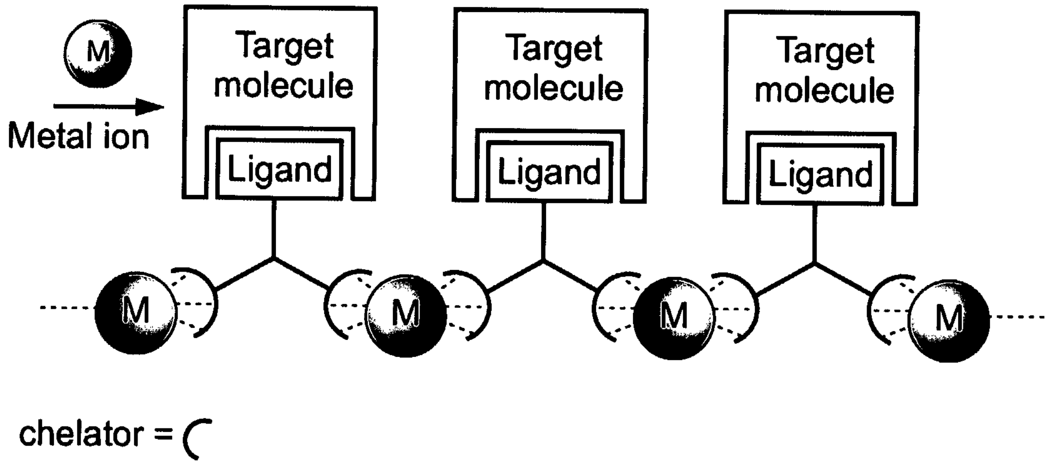

M
Metal ion
Target molecule
Ligand
Target molecule
Ligand
Target molecule
Ligand
M
M
M
M
chelator =

Fig. 2b

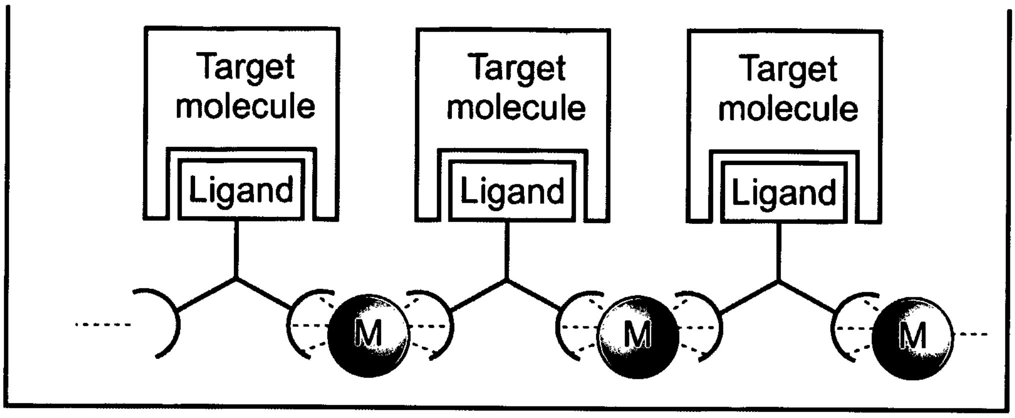


Fig. 3a

addition of a competing free chelator

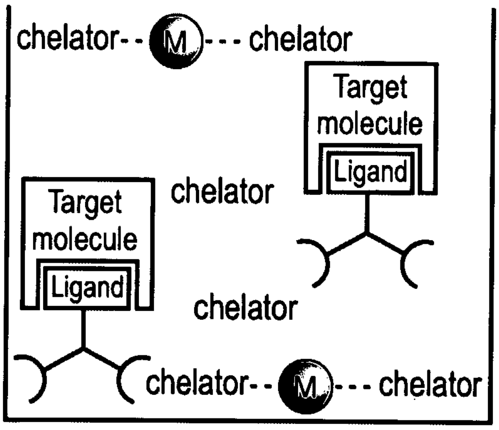


Fig. 3b

Ultrafiltration will remove free chelator and complexed metal

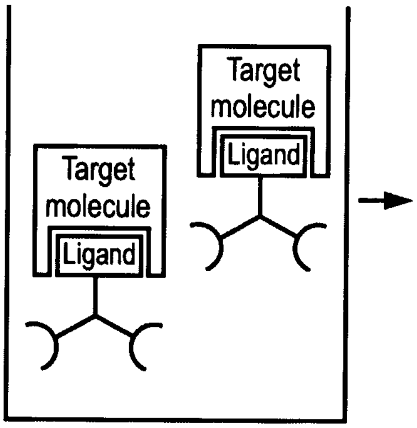


Fig. 3c

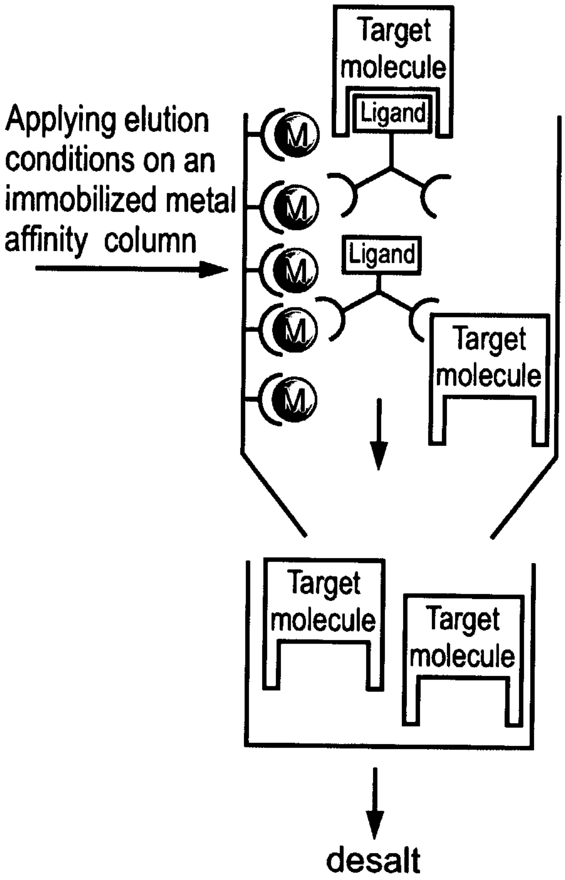

Applying elution conditions on an immobilized metal affinity column
Target molecule
Ligand
M
Ligand
Target molecule
Target molecule
Target molecule
desalt

Fig. 3d

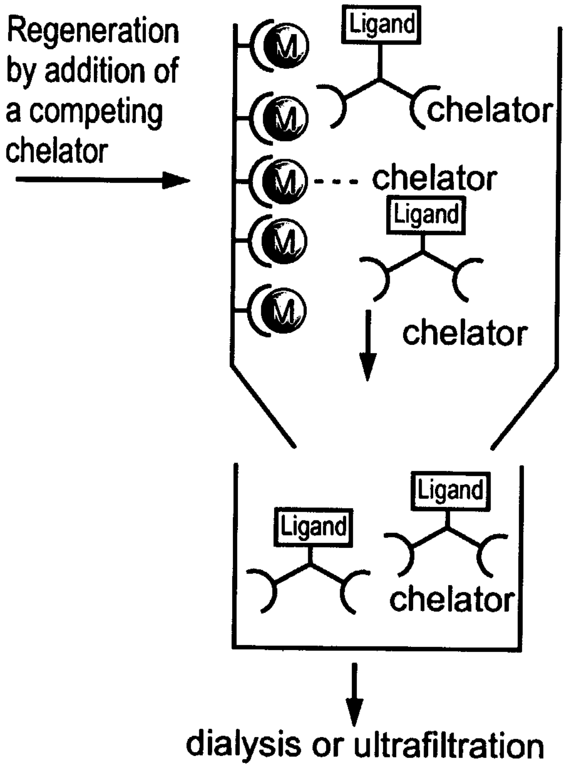

Regeneration by addition of a competing chelator
Ligand
chelator
M
chelator
Ligand
chelator
Ligand
Ligand
chelator
dialysis or ultrafiltration

Fig. 3e

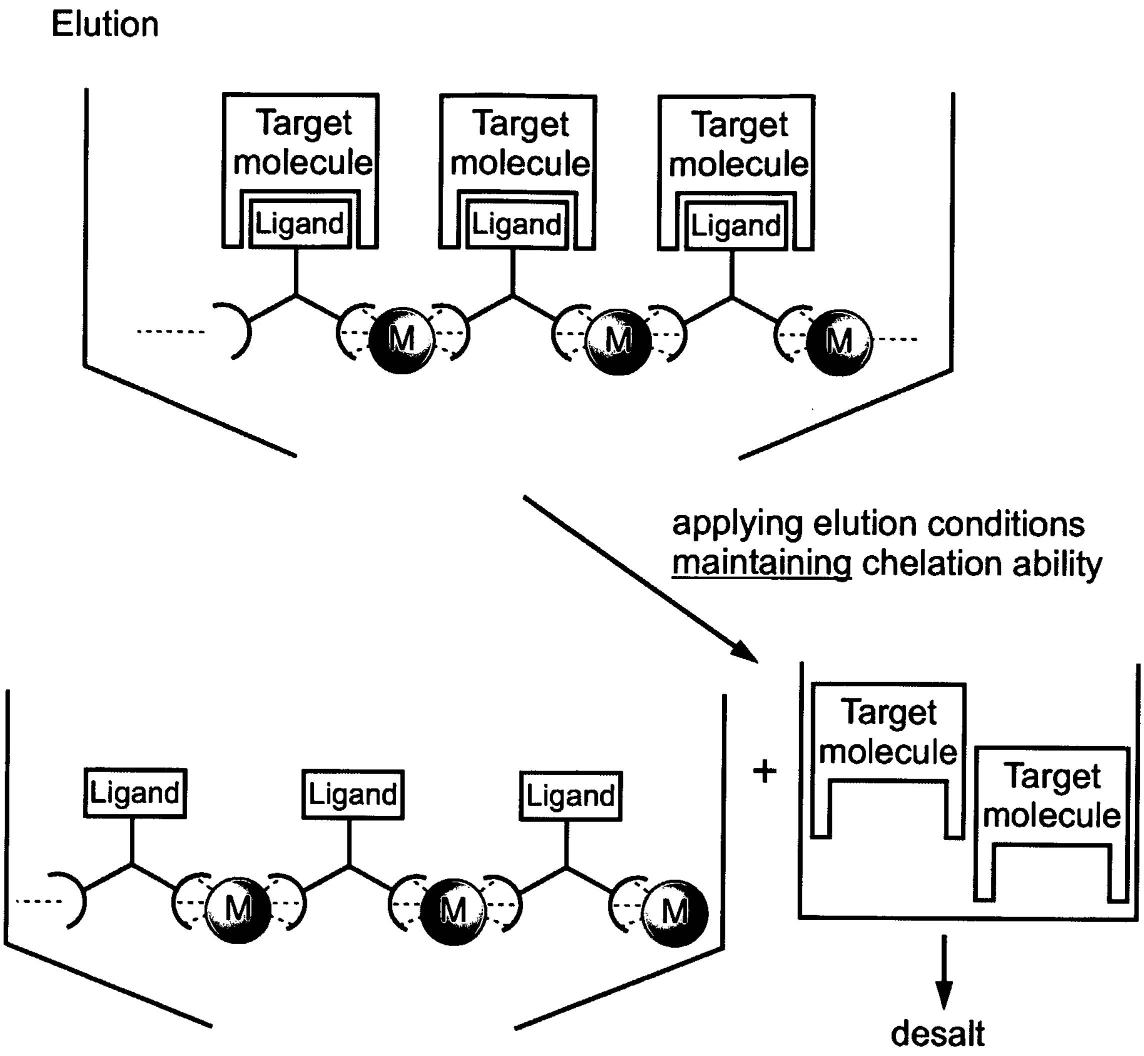

Elution
Target molecule
Ligand
M
applying elution conditions
maintaining chelation ability
+
desalt

Fig. 4

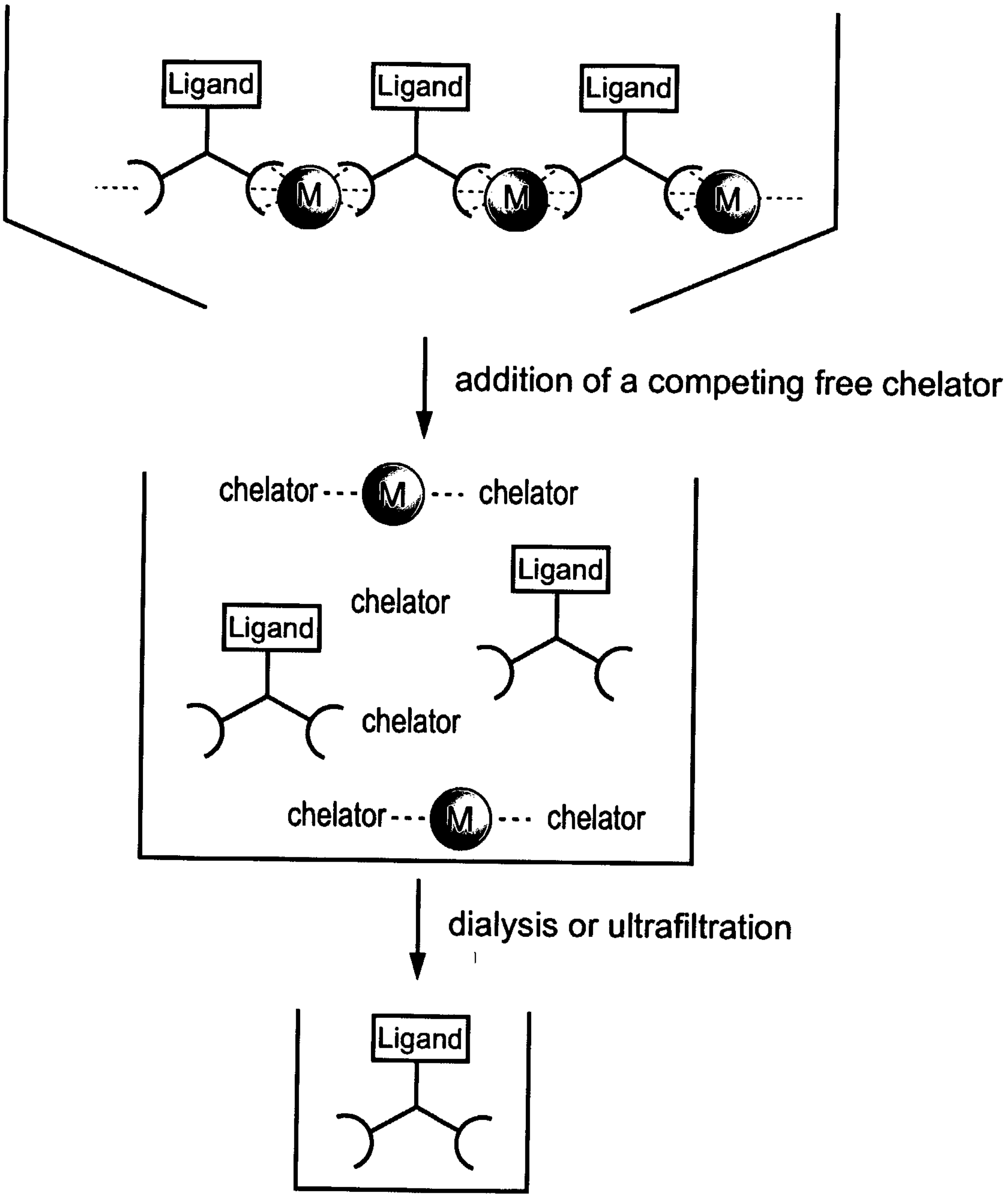

Ligand
Ligand
Ligand
M
M
M
addition of a competing free chelator
chelator
M
chelator
Ligand
chelator
Ligand
chelator
chelator
M
chelator
dialysis or ultrafiltration
Ligand

Fig. 5

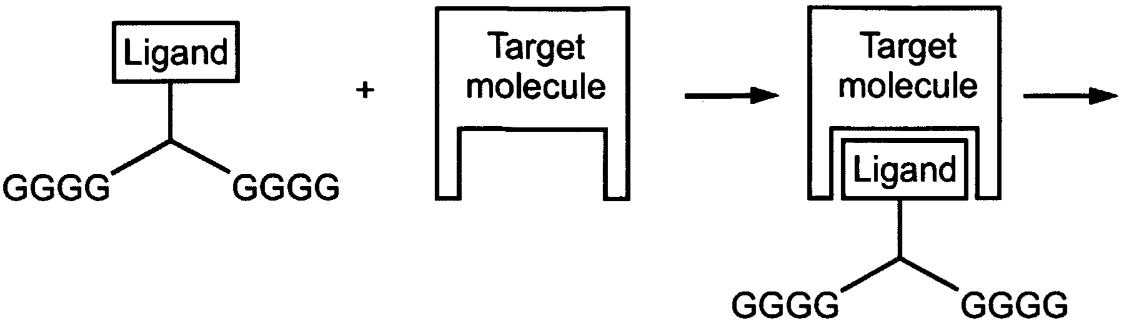


Fig. 6a

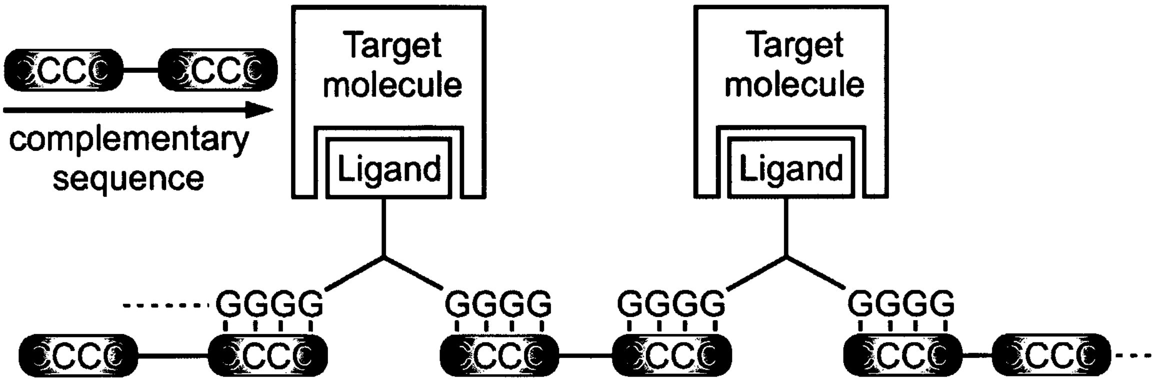


Fig. 6b

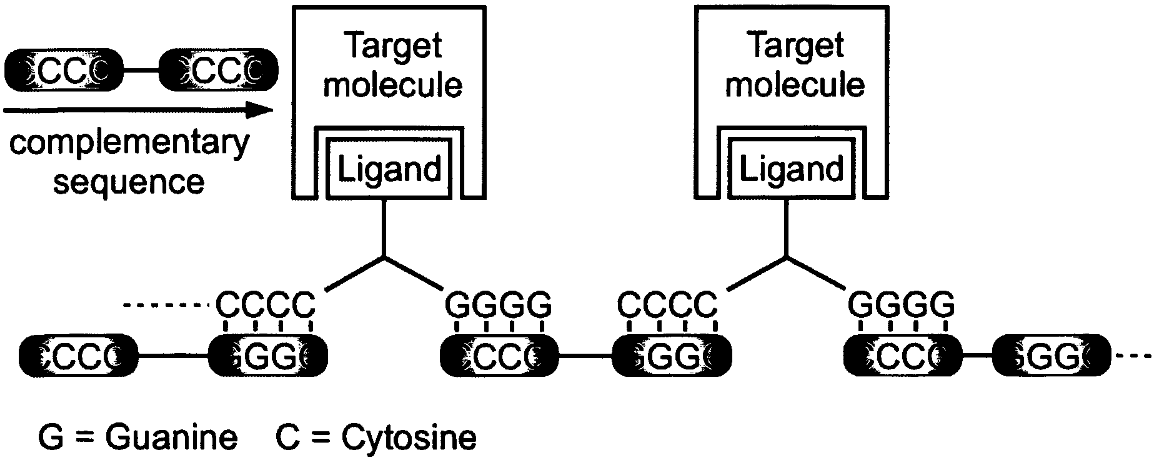


Fig. 6c

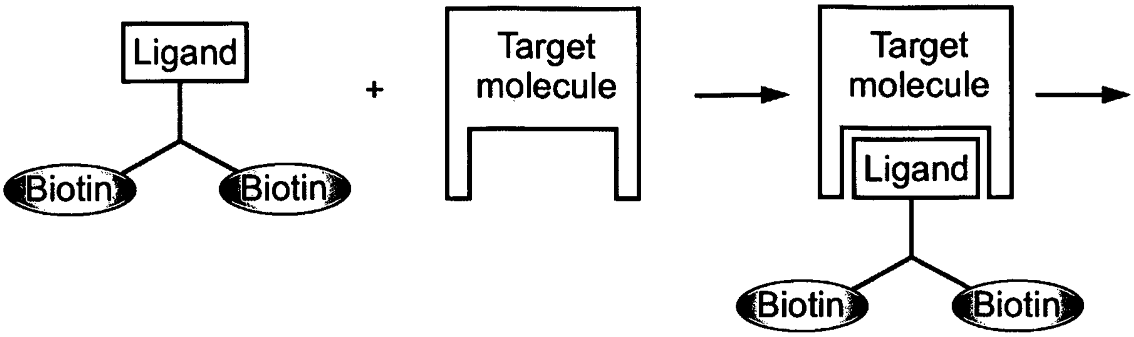
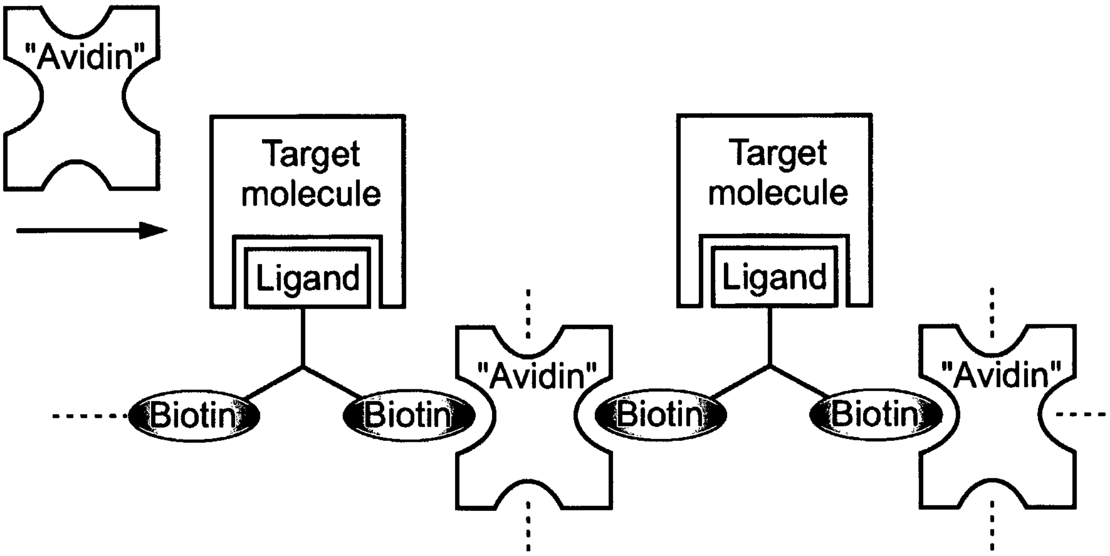


Fig. 7a



"Avidin" = avidin, nitroavidin, iodoavidin, or any other avidin derivative.

Biotin = Biotin, DSB-X Biotin, or any other biotin derivative.

Fig. 7b

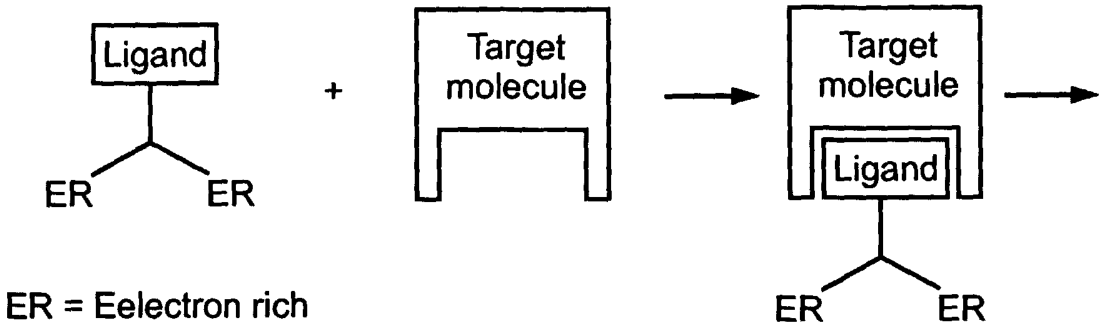


Fig. 8a

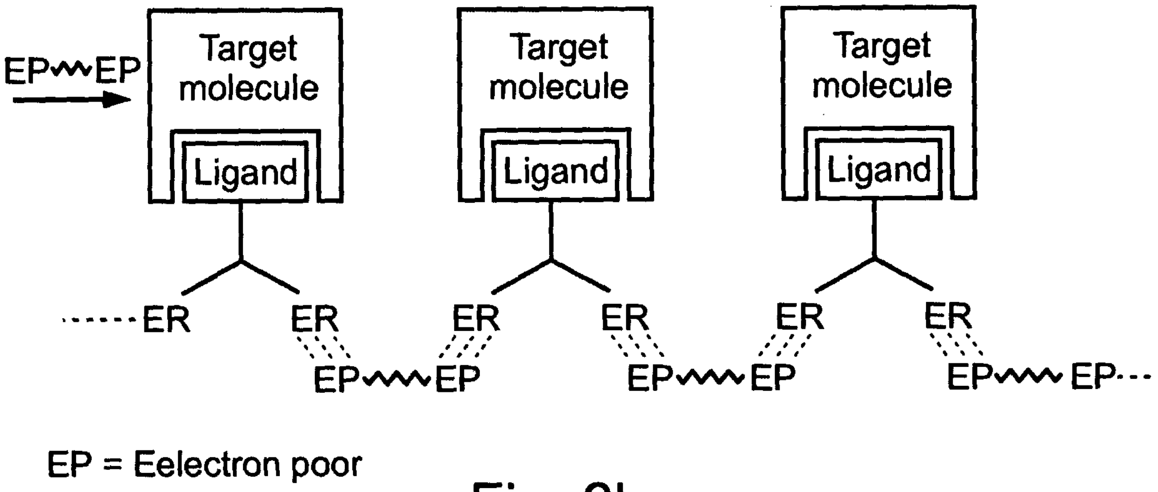


Fig. 8b

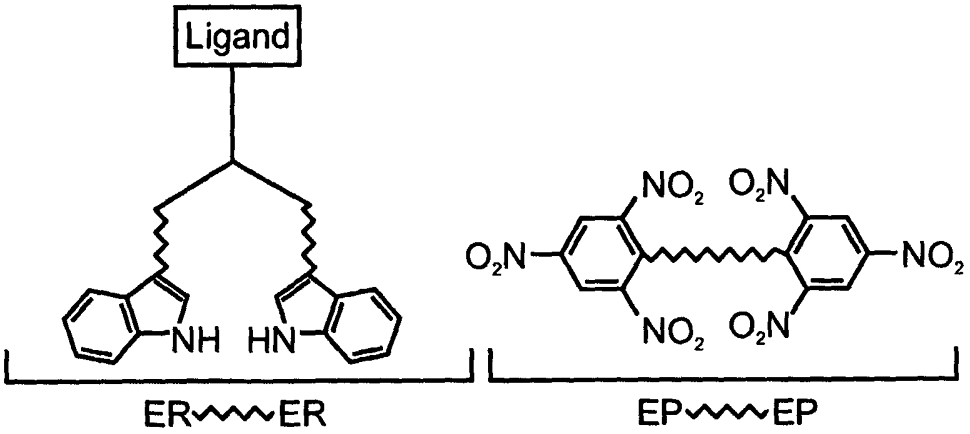
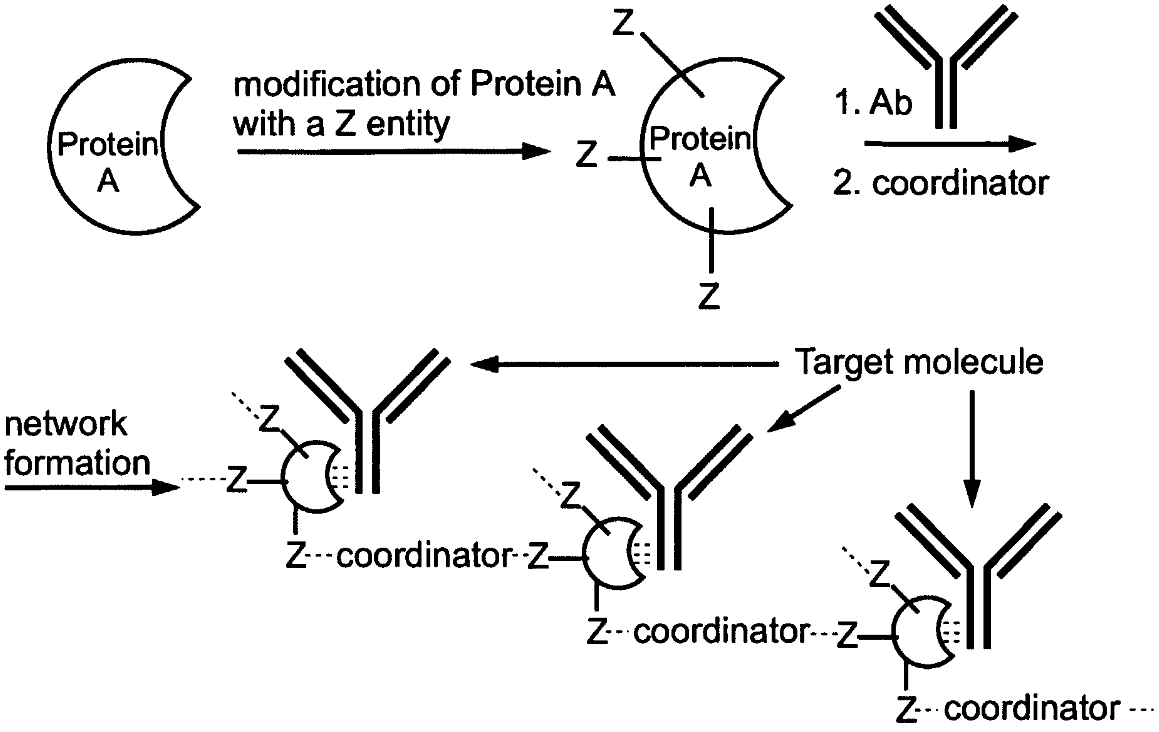


Fig. 8c



Z = chelator, biotin, nucleotide sequence, rich/poor entity etc.

coordinator = metal, avidin, complementary nucleotide sequence, electron rich/poor entity etc...

Fig. 9

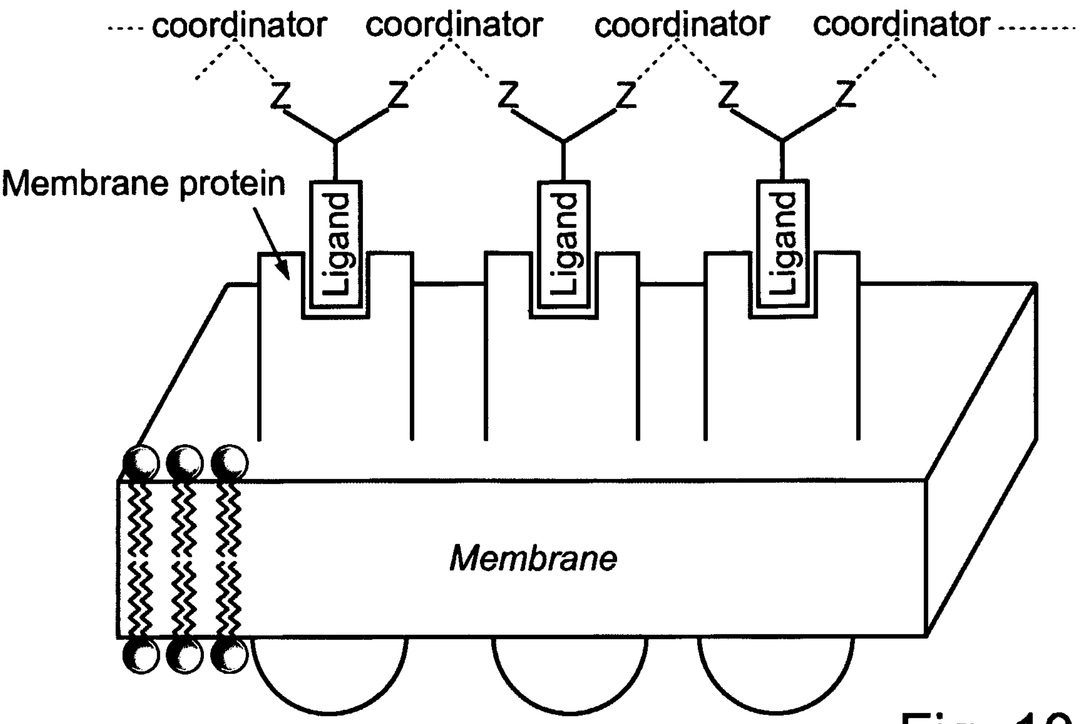


Fig. 10a

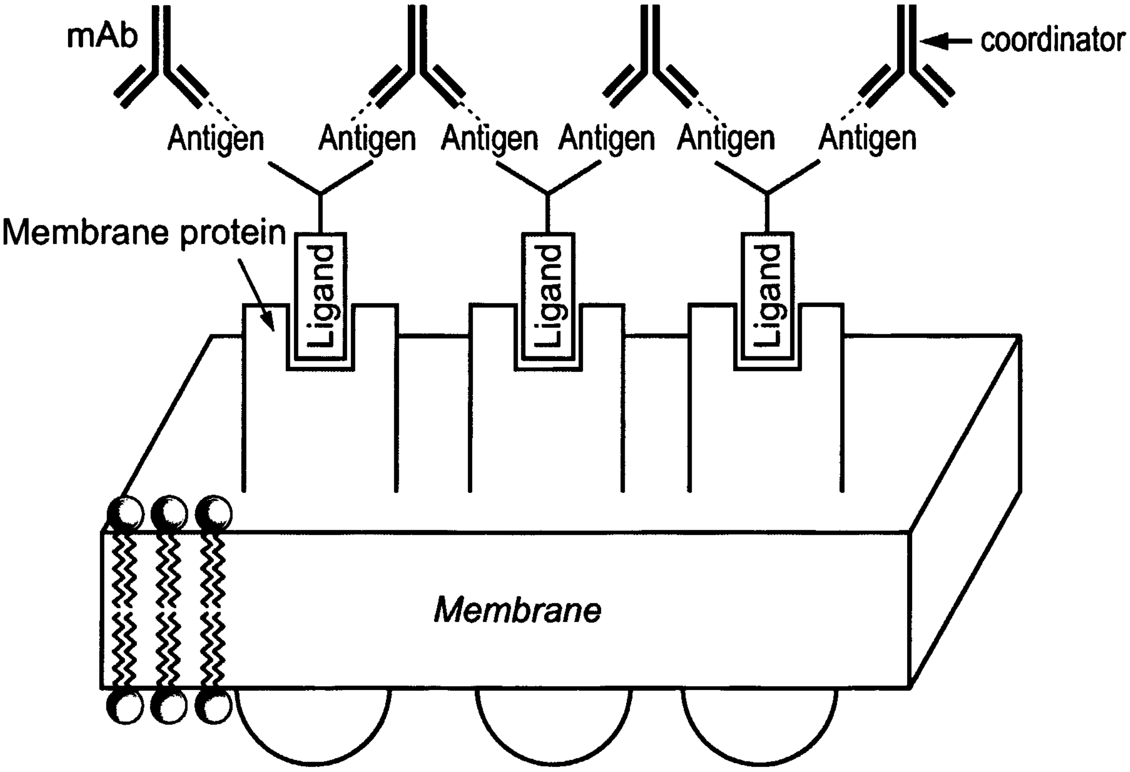


Fig. 10b

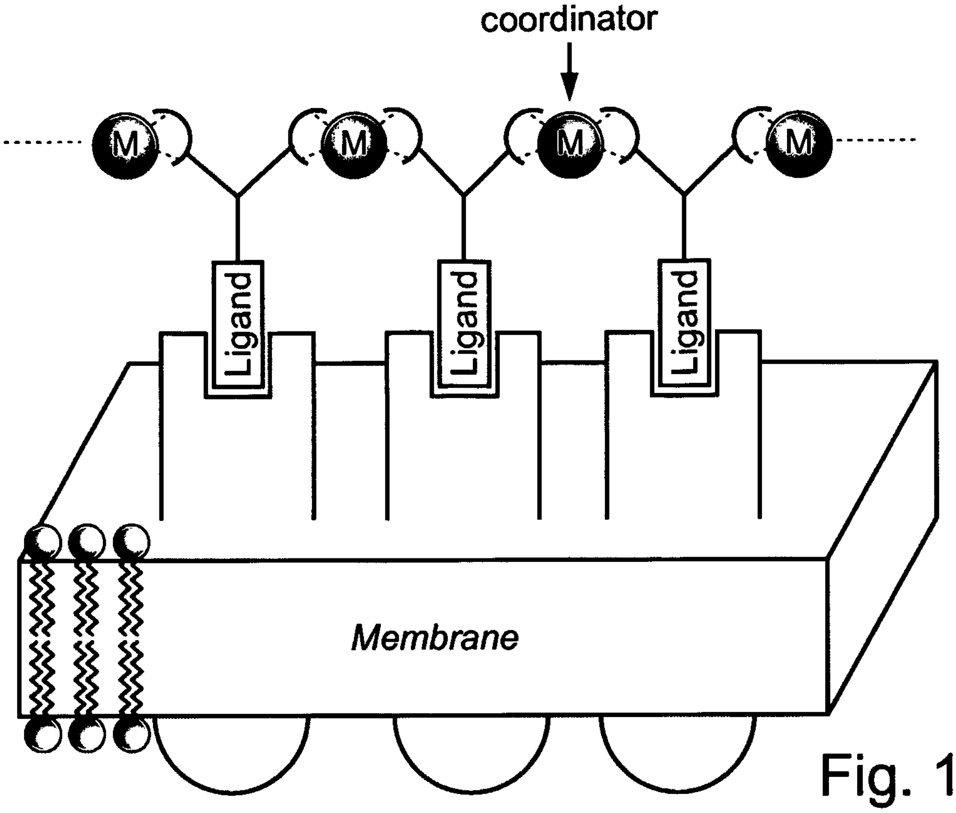


Fig. 11a

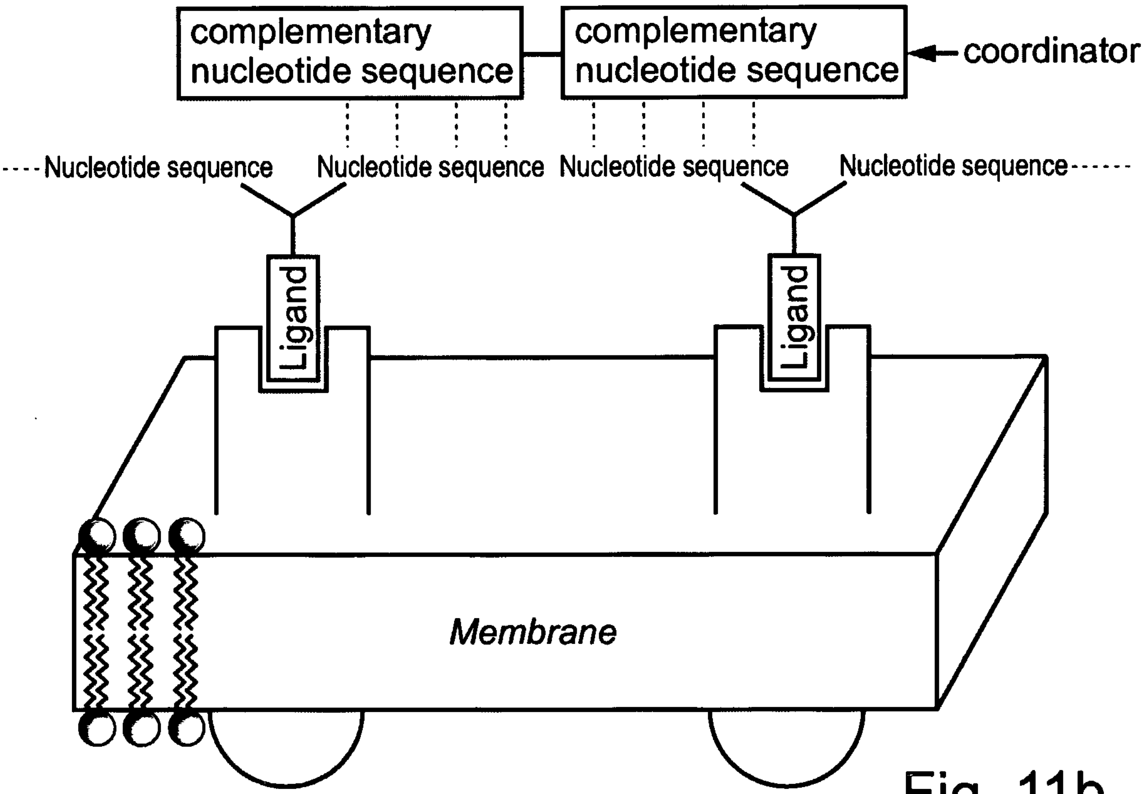


Fig. 11b

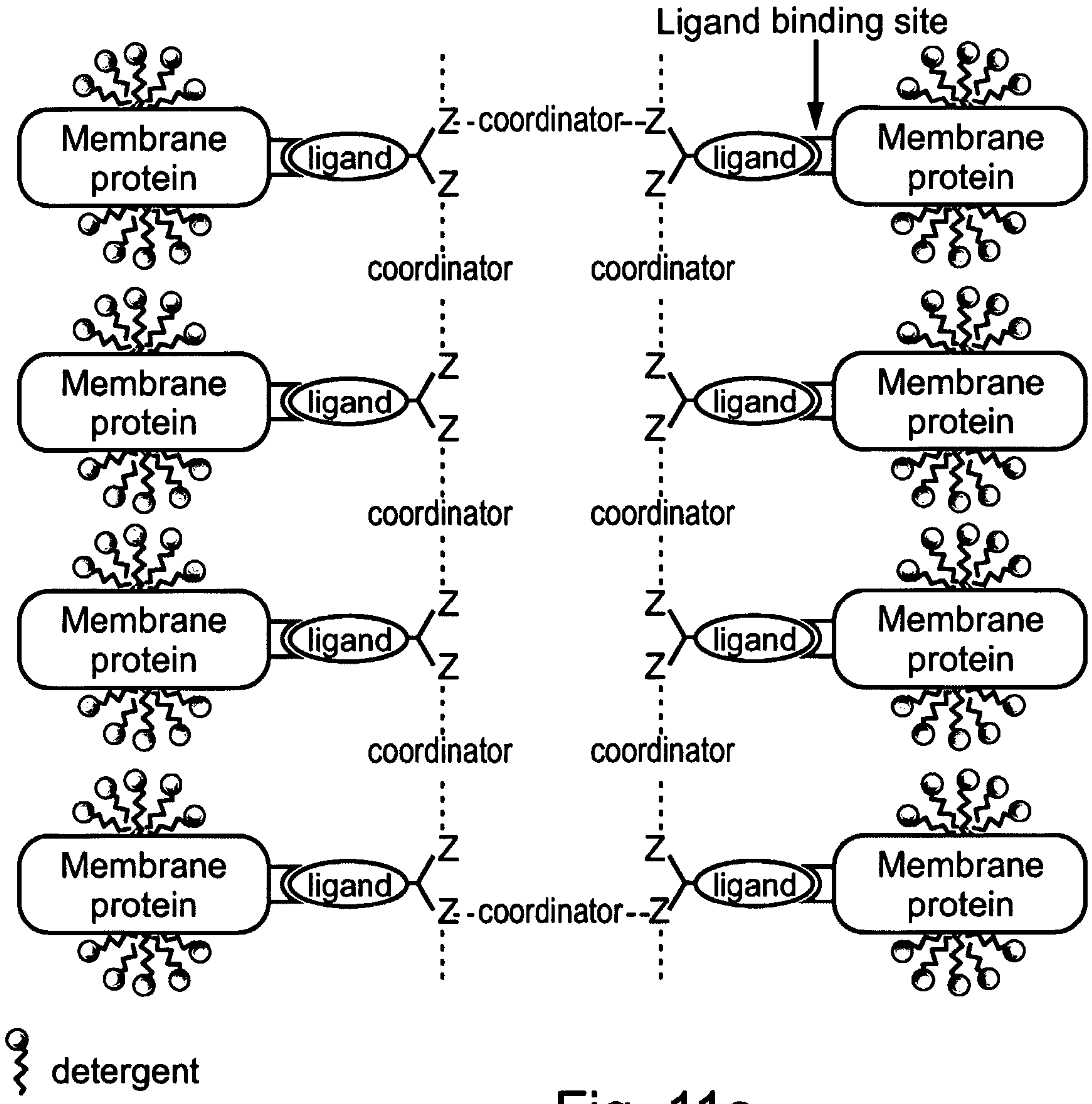

Ligand binding site
Membrane protein
ligand
Z
Z-coordinator--Z
coordinator
detergent

Fig. 11c

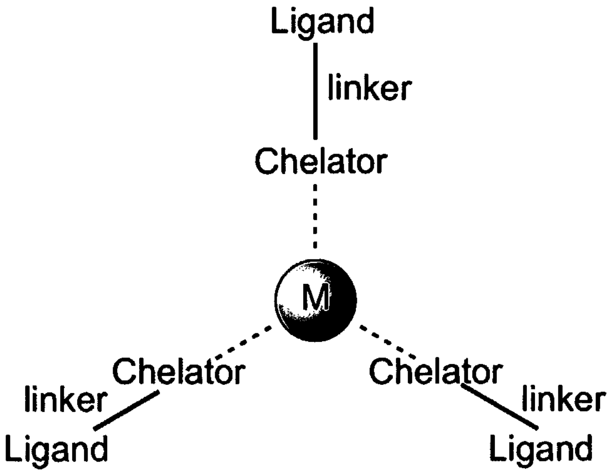
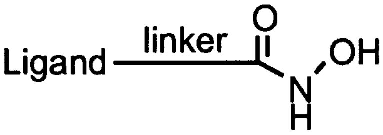


Fig. 12

Fig. 13a



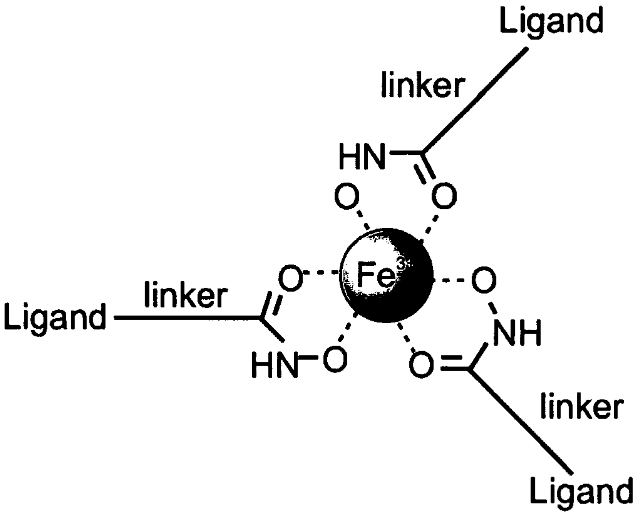
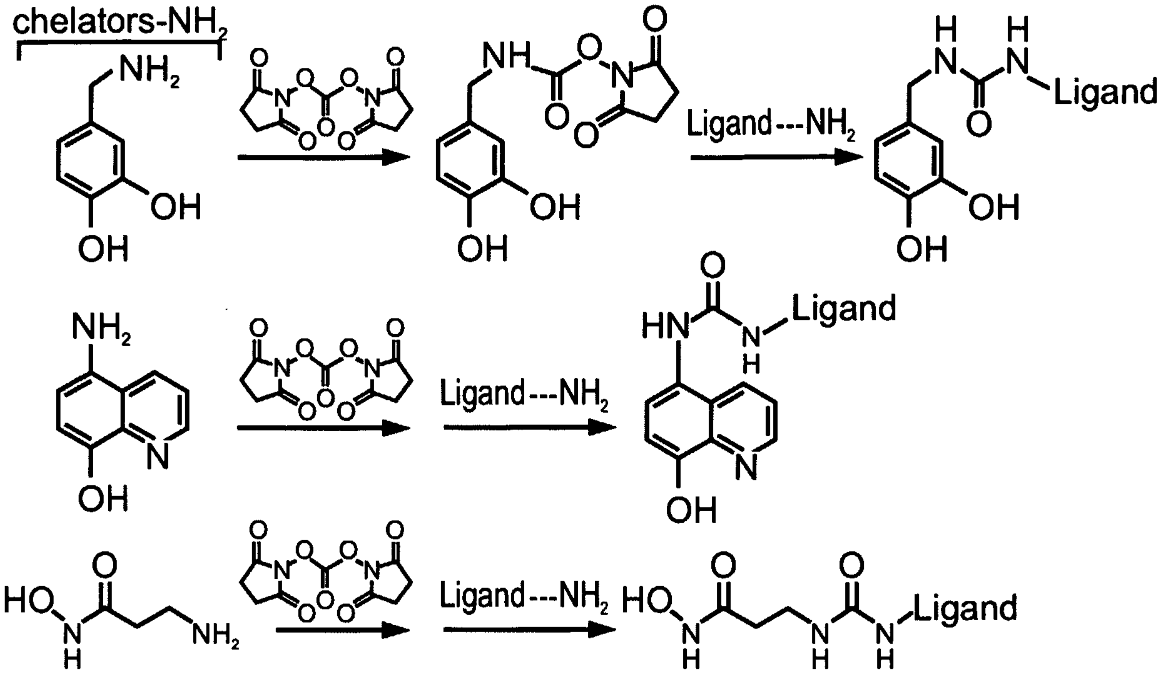


Fig. 13b

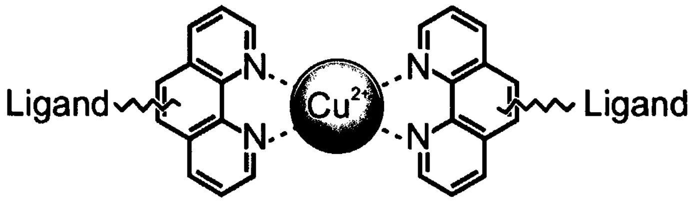


The same could apply for: Ligand --- OH Ligand --- SH Ligand --- COOH

Fig. 14

Formation of **di-ligand** in the presence of **Cu**$^{2+}$ ions:

Fig. 15a



Formation of **tri-ligand** in the presence of **Ru**$^{3+}$ ions:

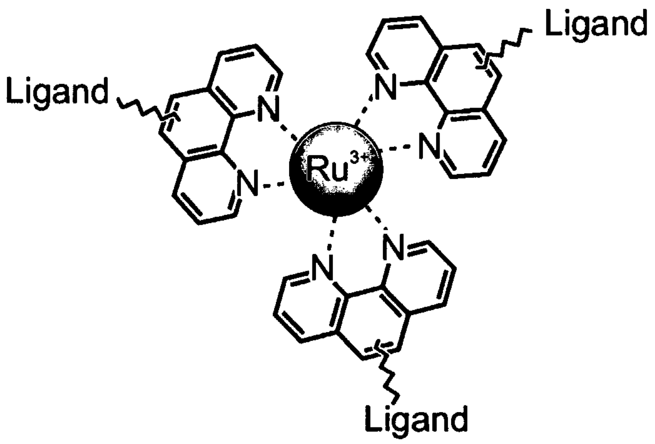


Fig. 15b

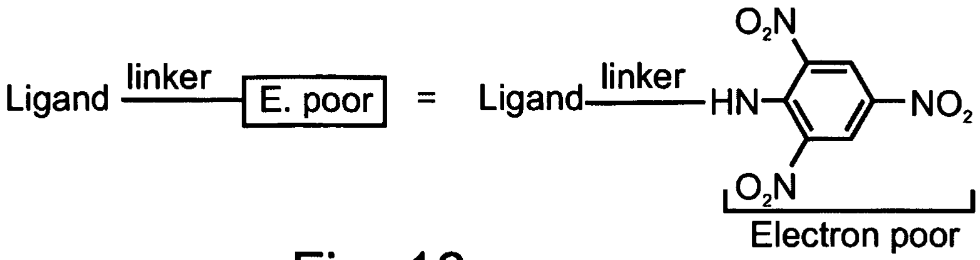


Fig. 16a

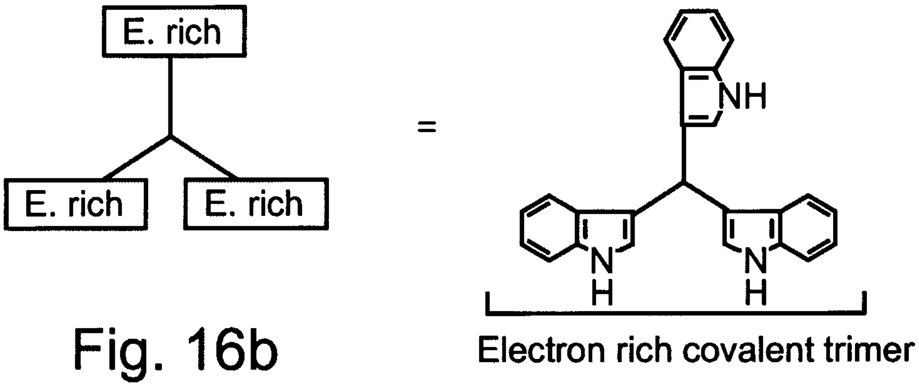


Fig. 16b

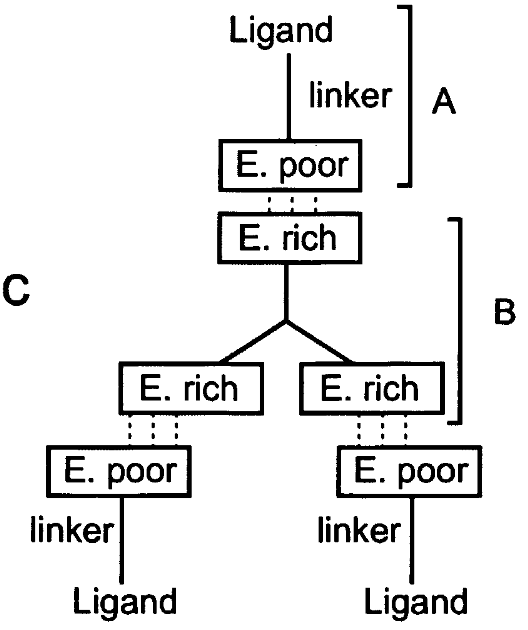
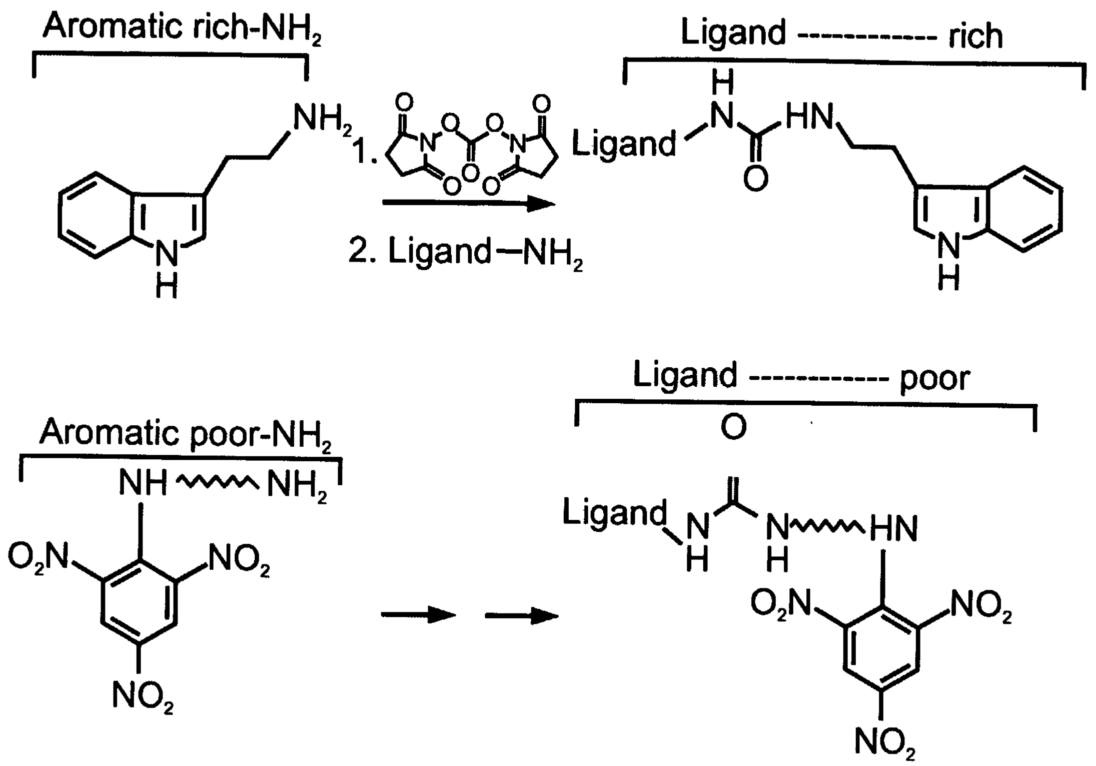


Fig. 16c



The same could apply for: Ligand --- OH Ligand --- SH Ligand --- COOH

Fig. 17

A synthetic peptide with four Trp residues

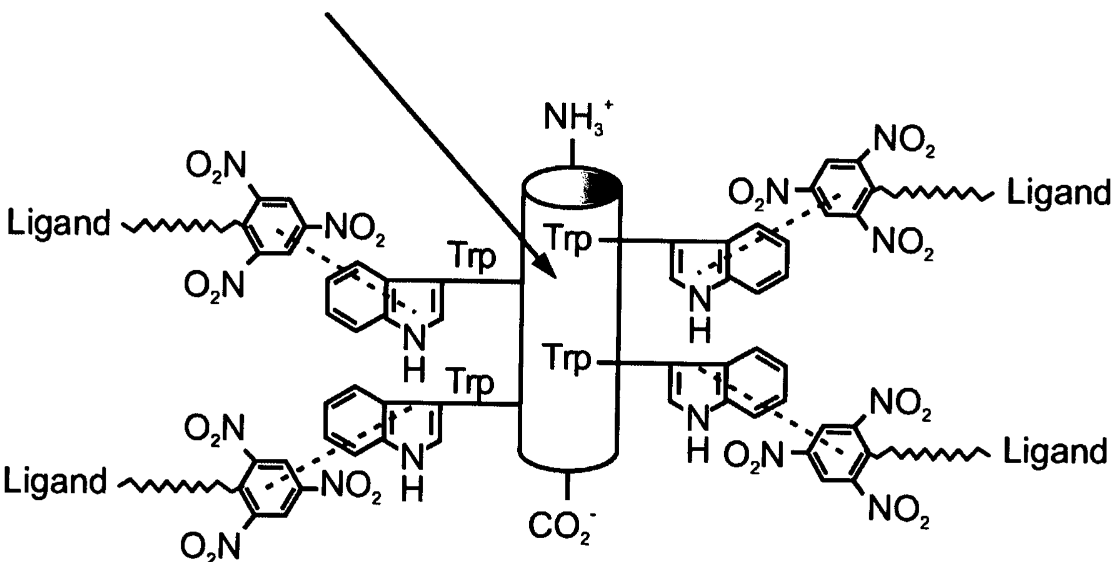


Fig. 18

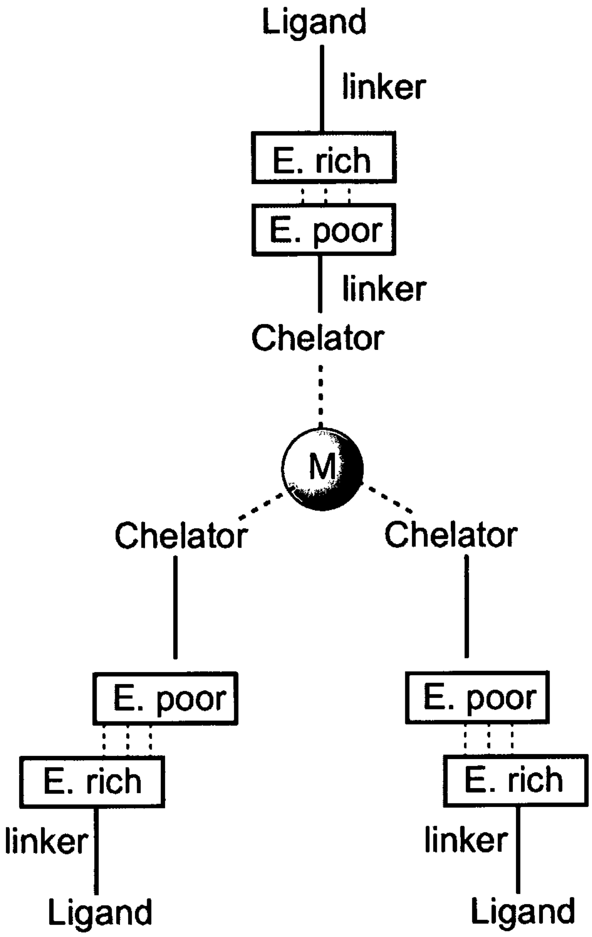

Ligand
linker
E. rich
E. poor
linker
Chelator
M
Chelator
Chelator
E. poor
E. rich
linker
Ligand
E. poor
E. rich
linker
Ligand

Fig. 19

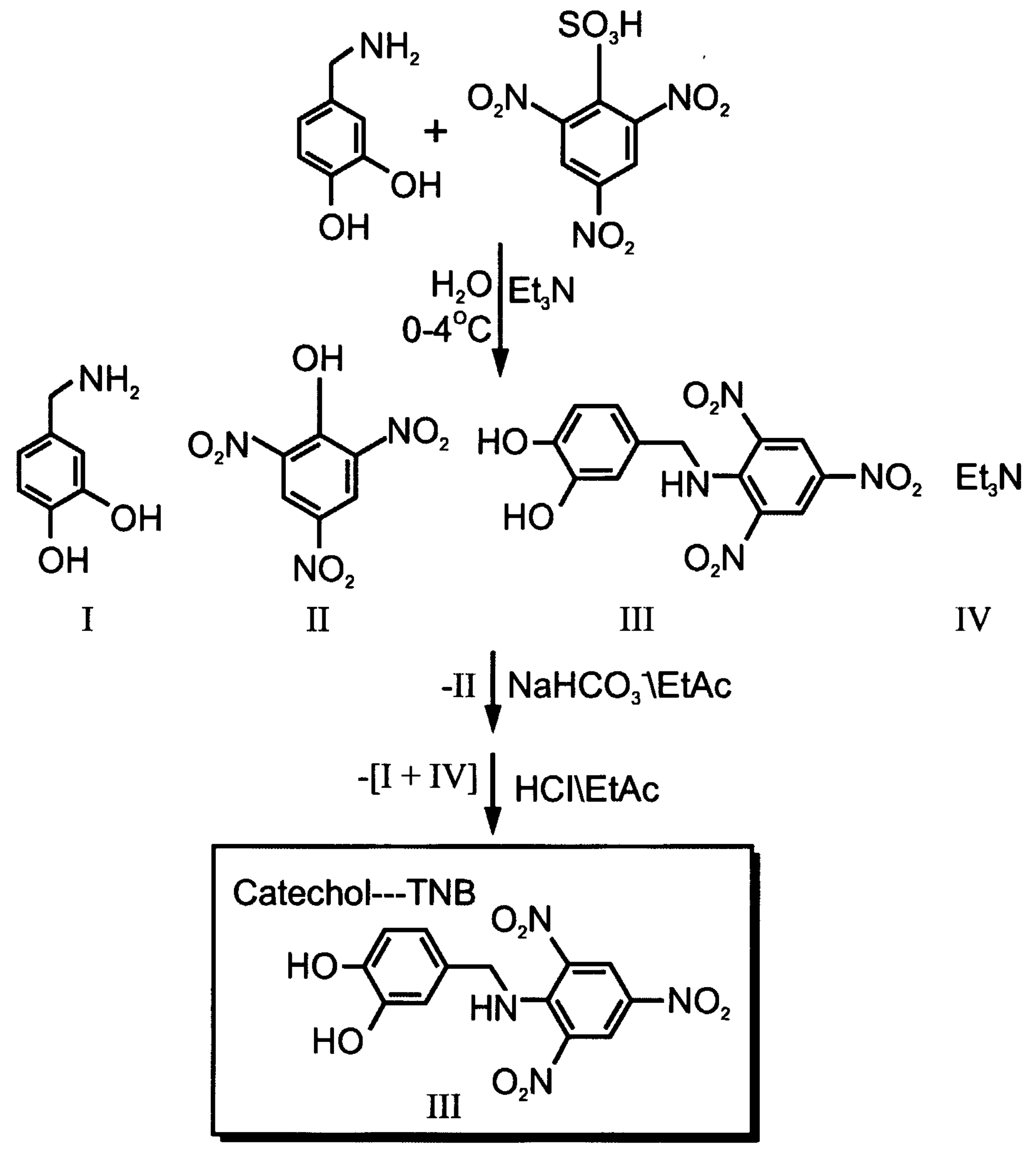

NH2
OH
OH
+
SO3H
O2N
NO2
NO2
H2O
0-4°C
Et3N
I
II
III
IV
OH
HO
HN
Et3N
-II
NaHCO3\EtAc
-[I + IV]
HCl\EtAc
Catechol---TNB
III

Fig. 20

Catechol---TNB + M^{2+}

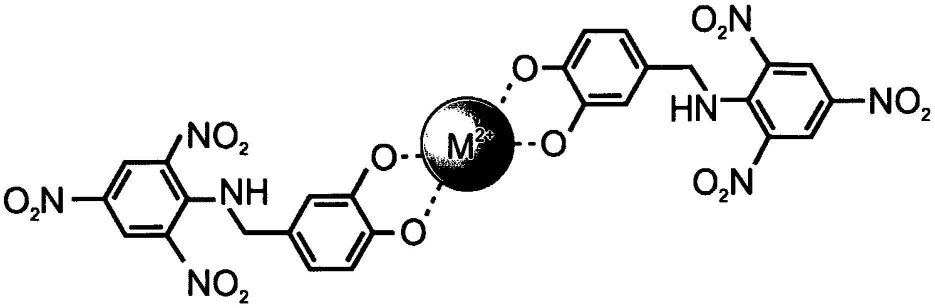


Fig. 21a

Catechol---TNB + M^{3+}

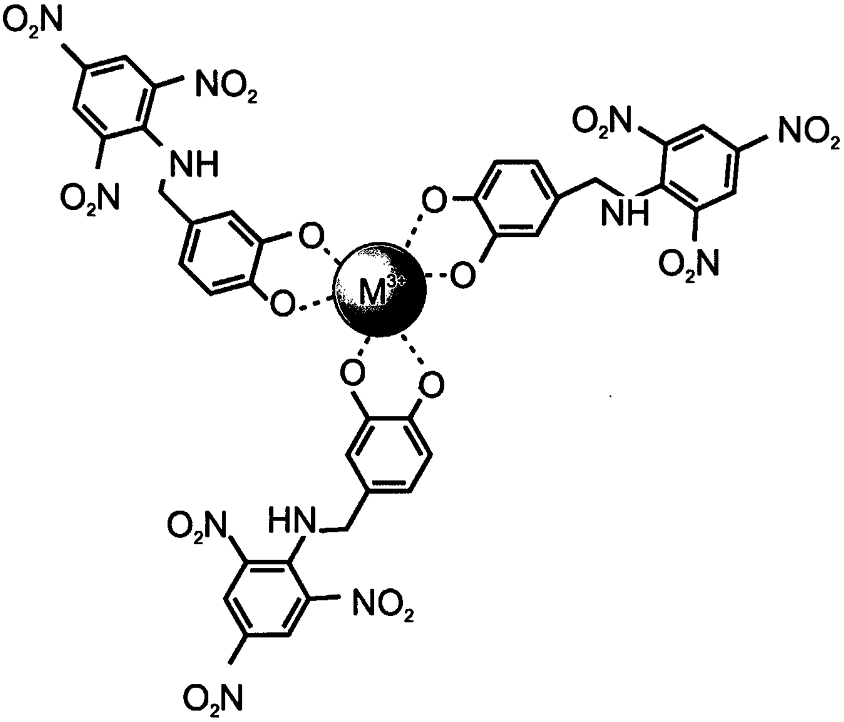


Fig. 21b

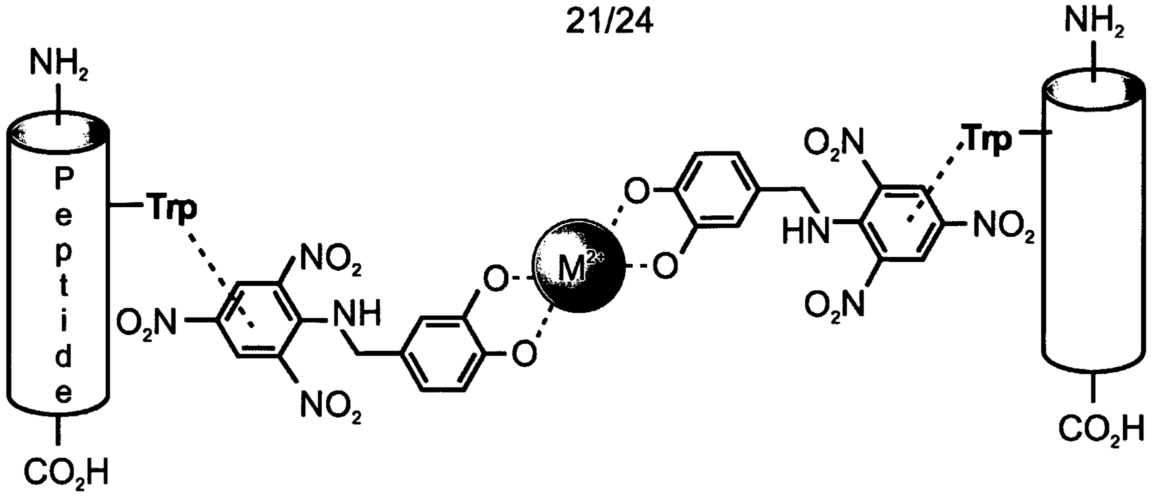


Fig. 22a

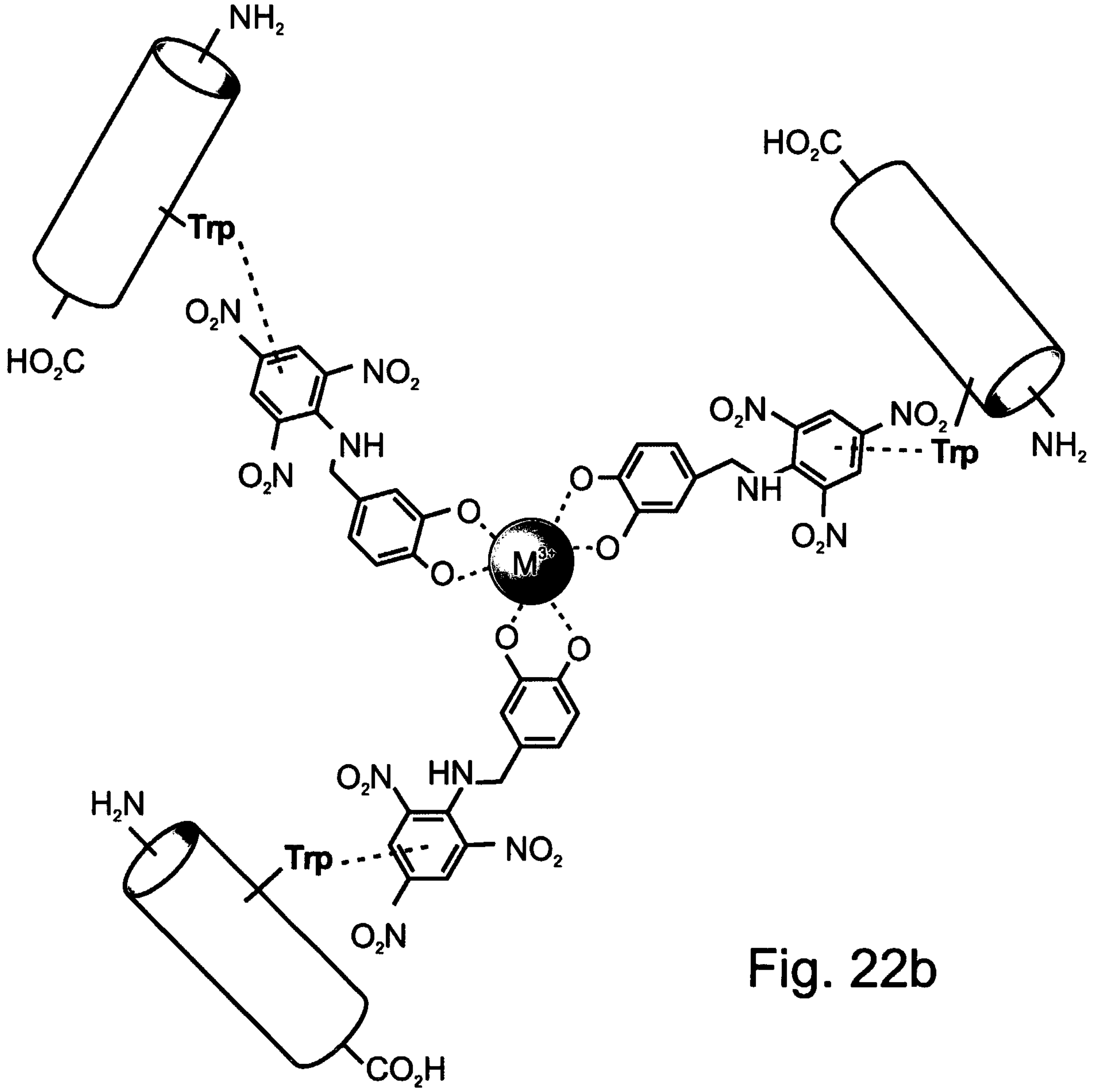


Fig. 22b

Fig. 23a

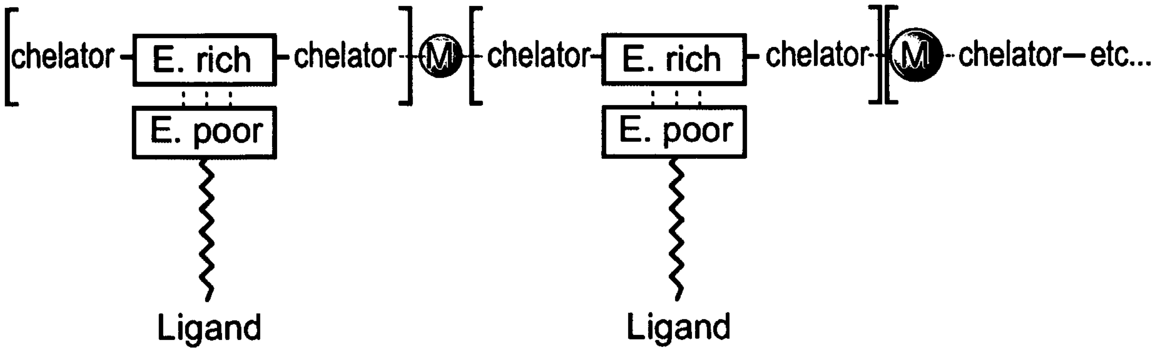


Fig. 23b

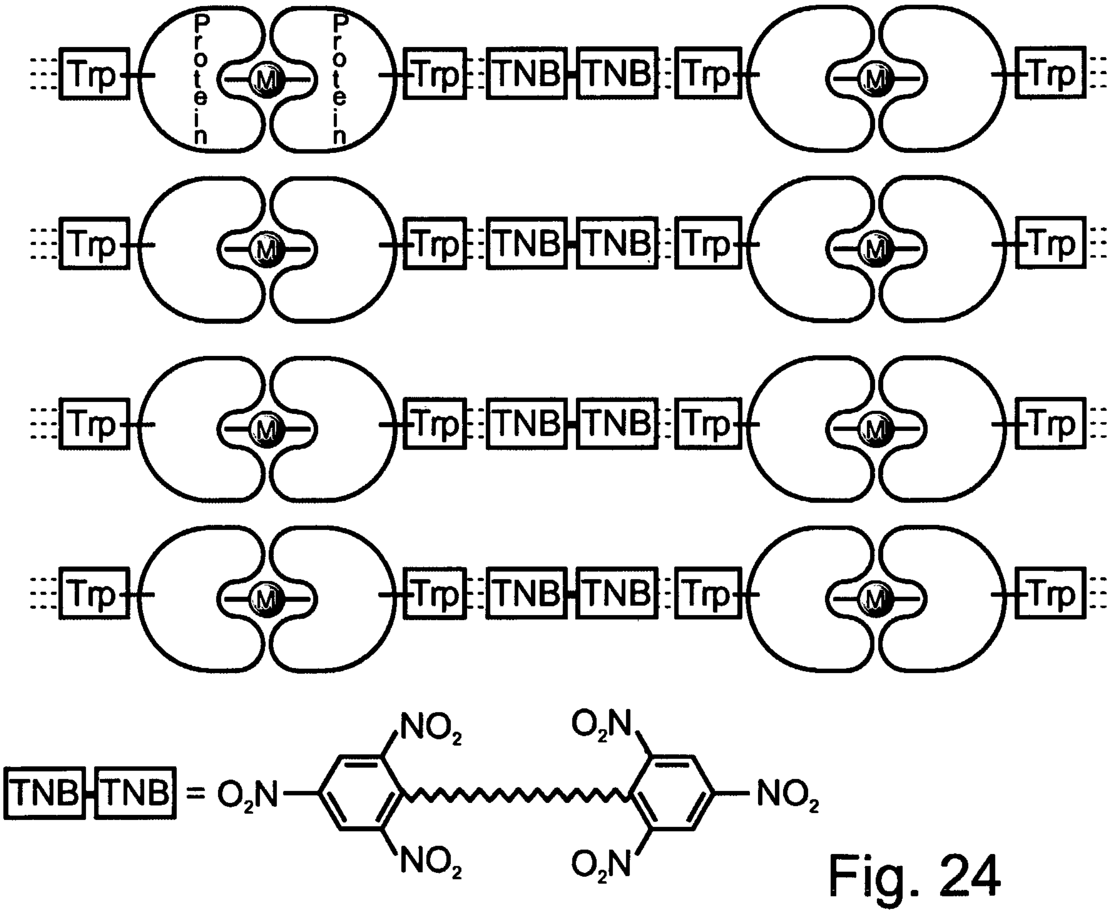
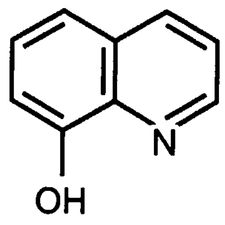
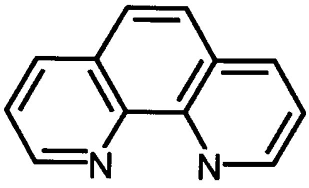


Fig. 24

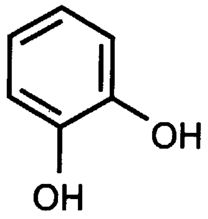
Examples of chelators:



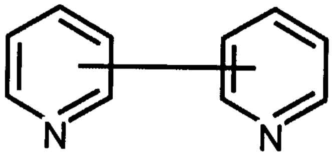
8-Hydroxyquinoline



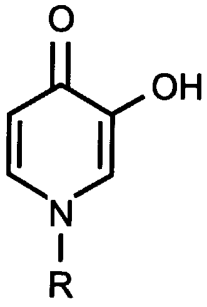
1, 10 Phenanthroline



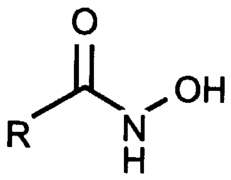
Catechol



bipyridines



Pyridine-4-ones



Hydroxamaate

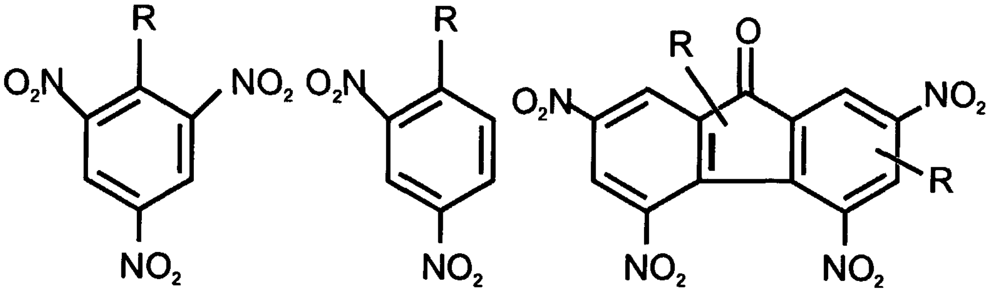
Examples of metals:

any M^{2+}, M^{3+}, or M^{4+}

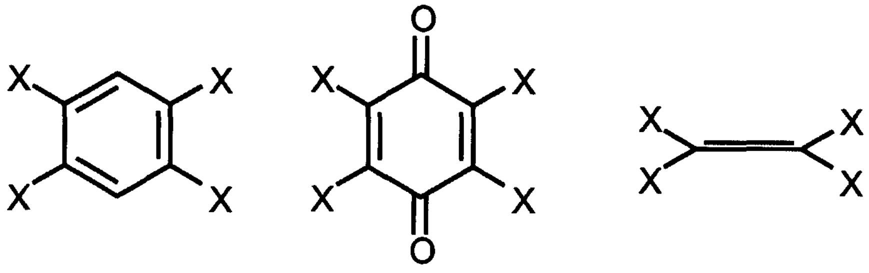
such as: Cu^{2+}, Fe^{3+}, Ru^{3+}, Al^{3+}, Co^{2+}, Cr^{3+}, etc...

Fig. 25

Examples for electron poor molecules:

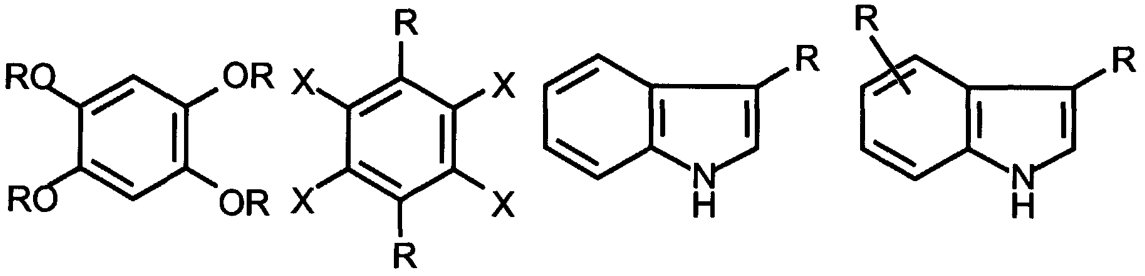


R=NHR, NR$_2$, OR, SR, NHCOR, etc...



X=Cl, Br, I, F, CN, or any withdrawing group

Examples for electron rich molecules:



X=Cl, Br, I, F, CN, or any withdrawing group

R=NHR, NR$_2$, OR, SR, NHCOR, etc...

Fig. 26