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(54) Title: METHODS OF INDENTIFYING ANTIBODIES THAT BIND OLIGOMERIC FORMS OF CHEMOKINES AND USES THEREOF

(57) Abstract: The present invention is directed to methods of identifying an antibody or an antigen-binding portion thereof that specifically recognizes and binds an oligomeric form of a chemokine.

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METHODS OF IDENTIFYING ANTIBODIES THAT BIND OLIGOMERIC FORMS OF CHEMOKINES AND USES THEREOF

Related Applications

[0001] This application claims the benefit of priority of U.S. provisional patent application No. 60/837,003, filed on August 11, 2006, the entire contents of which are incorporated by reference herein in their entirety.

Background of the Invention

[0002] Chemokines are small structurally related chemo-attractant cytokines that signal cells to migrate. Almost 40 chemokines have been identified to date which, based on the position of the conserved N-terminal cysteine residues, fall into four families. Two of these families have been well characterized, the CC and the CXC families. The CC family includes regulated on activation, normal T-cell expressed, and secreted, monocyte chemoattractant protein-1, and MIP-1 (macrophage inflammatory peptides-1). The CXC family includes interleukin-8 (Rossi *et al.*, (2000) *Annu. Rev. Immunol* 18: 217-242; Bazan *et al.*, (1997) *Nature* 385: 640-644; Kelner *et al.*, (1994) *Science* 266: 1395-1399; and Rollins (1997) *Blood* 90: 909-928). The C chemokine (lymphotactin) and the CX_3C chemokine (fractalkine) families have been identified more recently. The chemokine proteins signal through G-protein-coupled seven transmembrane domain receptors and are involved in many biological processes including immunosurveillance, activation, and recruitment of specific cell populations during disease states, where increased levels of chemokines are usually found (Wells *et al.*, (1998) *Trends Pharmacol. Sci.*, 19: 376-380; Rossi *et al.* above; Luster *et al.*, (1998) *N. Engl. J. Med.* 7: 436-445; Gale *et al.*, (1999) *BioEssays* 21: 17-28)

[0003] Most, if not all, chemokines bind to heparin sulfate, a glycosaminoglycan (GAG) found ubiquitously at the cell surface and in the extracellular matrix (Witt *et al.*, (1994)

Curr. Biol., 4: 394-400; Kuschert *et al.*, (1999) *Biochem.* 38: 12959-12968; David *et al.*, (1998) *Matrix Biol.* 17: 461-63; Zimmermann *et al.*, (1999) *FASEB J.* 13: 91-100). This binding is thought to be functionally important and current models indicate that heparin sulfate (HS) either enhances the local concentration of chemokines in the vicinity of the G-protein-coupled receptor or provides a haptotactic gradient of the protein along cell surfaces. For example, leukocyte migration along the endothelium surface, and migration into the tissues at the site of inflammation, is believed to depend on the local presentation of chemokines by such cell surface-expressed GAGs (Tanaka *et al.*, (1996) *J. Exp. Med.* 184: 1987-1997; Tanaka *et al.*, (1998) *Arthritis & Rheum* 41: 1365-1377; Weber *et al.*, (1999) *Eur. J. Immunol.* 29: 700-712).

[0004] Many chemokines have been shown to oligomerize and it is believed that such oligomerization plays a fundamental role in chemokine function, *e.g.*, activation and recruitment of lymphocytes and monocytes in a disease state. Accordingly, it would be beneficial to identify agents, *e.g.*, antibodies, that selectively recognize and bind the oligomeric versus the monomeric form of a chemokine.

Summary of the Invention

[0005] The present invention provides methods of identifying compounds, *e.g.*, antibodies or antigen-binding portions thereof, or small molecules, which selectively bind an oligomeric, *e.g.*, homodimeric or heterodimeric, form of a chemokine. Such compounds are useful for modulating the function of an oligomeric form of a chemokine and/or for detecting an oligomeric form of a chemokine, for example, in various disease states associated with increased chemokine levels.

[0006] In one aspect, the present invention provides a method of identifying a compound, *e.g.*, an antibody or an antigen-binding portion thereof, which selectively

binds to an oligomeric form and not to a monomeric form of a chemokine. The method includes: (a) contacting a compound, *e.g.*, an antibody, or an antigen-binding portion thereof, with a peptide including a glycosaminoglycan binding site of a chemokine; or (b) contacting an compound, *e.g.*, an antibody, or an antigen-binding portion thereof, with a chemokine in the presence of a cation, wherein the cation is present at a concentration sufficient to promote and/or stabilize chemokine oligomerization; (c) determining whether the compound, *e.g.*, the antibody, or antigen-binding portion thereof, binds the peptide in (a) or the chemokine in (b); and (d) identifying a compound, *e.g.*, an antibody, or an antigen-binding portion thereof, which selectively binds to an oligomeric form and not to a monomeric form of the chemokine, based on the ability of the compound, *e.g.*, antibody, or antigen-binding portion thereof, to bind the peptide in (a) or the chemokine in (b).

[0007] In another aspect, the present invention provides a method of identifying a compound, *e.g.*, an antibody, or an antigen-binding portion thereof, which selectively binds to an oligomeric form, and not to a monomeric form, of a chemokine by: (a) contacting a compound, *e.g.*, an antibody, or antigen-binding portion thereof, with a peptide including a glycosaminoglycan binding site of a chemokine; (b) determining whether the compound, *e.g.*, antibody, or an antigen-binding portion, thereof binds the peptide; and (c) identifying a compound, *e.g.*, antibody, or antigen-binding portion, which selectively binds to an oligomeric form, and not to a monomeric form, of the chemokine based on the ability of the compound, *e.g.*, antibody, or an antigen-binding portion thereof, to bind the peptide.

[0008] In yet another aspect, the present invention provides a method of identifying a compound, *e.g.*, an antibody, or an antigen-binding portion thereof, which selectively

binds to an oligomeric form, and not to a monomeric form, of a chemokine by: (a) contacting a compound, *e.g.*, antibody, or an antigen-binding portion thereof, with a chemokine in the presence of a cation, wherein the cation is present at a concentration sufficient to promote and/or stabilize chemokine oligomerization; (b) determining whether the compound, *e.g.*, antibody, or an antigen-binding portion thereof, binds the chemokine in the presence of the cation; and (c) identifying a compound, *e.g.*, antibody, or antigen-binding portion thereof, which selectively binds to an oligomeric form, and not to a monomeric form, of a chemokine based on the ability of the compound, *e.g.*, antibody, or an antigen-binding portion thereof, to bind the chemokine in the presence of the cation.

[0009] In a further aspect, the present invention provides a method for identifying a compound which selectively binds to an oligomeric form, and not to a monomeric form, of a chemokine by: (a) contacting a compound, *e.g.*, antibody or antigen-binding portion thereof, or a small molecule, with a monomeric form of a chemokine; (b) contacting the compound, e.g., antibody or antigen-binding portion thereof, or a small molecule, with an oligomeric form of a chemokine; (c) determining whether the compound binds to the monomeric form of the chemokine or the oligomeric form of the chemokine, and (d) identifying a compound which selectively binds to an oligomeric form of the chemokine based on the ability of the compound to bind to the oligomeric form and not to the monomeric form, of the chemokine.

[0010] In some embodiments of the present invention, the chemokine may be IL-8 (interleuk GROα (growth regulated oncogene-alpha), GROβ (growth regulated oncogene-beta), GROγ (growth regulated oncogene-gamma), ENA-78 (epithelial cell-derived neutrophi activating peptide), LDGF-PBP (leukocyte derived growth factor-platelet basic protein), GCF

(granulocyte chemotactic protein-2), PF4 (platelet factor-4), Mig (monokine induced by int gamma), IP-10 (interferon-inducible protein 10), SDF-1α (stromal cell-derived factor-1 alp SDF-1β (stromal cell-derived factor-1 beta), BUNZO/STRC33 (stereocilin33), I-TAC (inte inducible T cell alpha chemoattractant), BLC/BCA-1 (B cell-attracting chemokine 1), MIP- (macrophage inflammatory protein 1-alpha), MIP-1β (macrophage inflammatory protein 1- MDC (macrophage derived chemokine), TECK (thymus expressed chemokine), TARC (Th specific chemokine thymus and activation-regulated chemokine), RANTES (regulated upor activation, normally T expressed, and presumably secreted), HCC-1 (human CC chemokine CK1 (dendritic cell derived chemokine-1), MIP-3α (macrophage inflammatory protein 3-al MIP-3β (macrophage inflammatory protein 3-beta), MCP-1 (monocyte chemoattractant pro MCP-2 (monocyte chemoattractant protein-2), MCP-3 (monocyte chemoattractant protein-3 MCP-4 (monocyte chemoattractant protein-4), Eotaxin, Eotaxin-2/MPIF-2 (myeloid progen inhibitory factor 2), I-309 (small inducible cytokine A1); MIP-5/HCC-2 (macrophage inflammatory protein 5/human CC chemokine-2), MPIF-1 (myeloid progenitor inhibitory fa 6Ckine/SLC (secondary lymphoid-tissue chemokine), CTACK (cutaneous T cell-attracting chemokine), MEC (mucosae-associated epithelial chemokine), Lymphotactin or Fractalkine subtypes thereof.

[0011] In a particular embodiment, the chemokine is SDF-1α and the peptide used in the me of the invention includes amino acid residues 20-33, 20-31 or 24-27 of SDF-1α.

[0012] In one embodiment of the invention, the glycosaminoglycan may be heparin, heparin sulfate, chondroitin sulfate, dermatan sulfate, keratin sulfate, hyaluronase or derivatives or combinations thereof.

[0013] In another embodiment, an oligomeric form of a chemokine is a dimeric form, *e.g.*, a homodimeric form or a heterodimeric form.

[0014] In yet other embodiments of the methods of the invention, a cation is a multivalent cation, such as, for example, a trivalent cation, *e.g.*, Fe^{3+}or Al^{3+}, or a divalent cation, *e.g.*, Ca^{2+}, Mg^{2+}, Sr^{2+}, Ba^{2+}, Mn^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+} and Cd^{2+}. In a particular embodiment, a cation is Ca^{2+}. In some embodiments, the cation may be present at a concentration of at least 1 mM, 1.1 mM, 1.2 mM, 1.3 mM, 1.4 mM, 1.5mM, 1.6 mM, 1.7 mM, 1.8 mM, 1.9 mM, or 2 mM.

[0015] In some embodiments of the present invention, the concentration of a cation is decreased by the addition of a chelating agent, *e.g.*, EDTA (ethylenediamine tetraacetate), porphine, DTPA (Diethylenetriaminepentaacetic acid), Dimercaprol (2,3-dimercapto-1-propanol) and NTA (N,N-bis(carboxymethyl)glycine; Triglycollamic acid), DETA (diethylenetriamine) and AEEA (aminoethylethaloamine). In a particular embodiment, a chelating agent used in the methods of the invention is EDTA (ethylenediamine tetraacetate).

[0016] In some embodiments of the invention, the compound is an antibody, *e.g.*, a polyclonal antibody, a monoclonal antibody, a humanized antibody, chimeric antibody or a human antibody. In a particular embodiment, an antibody is a human antibody, such as 1H2 or 1D3 or 2E5. An antigen-binding portion of an antibody may be Fab, Fab', F(ab')2, Fabc, ScFv or Fv. In other embodiments, a compound is a small molecule.

[0017] In another aspect, the present invention provides compounds, *e.g.*, antibodies, or antigen-binding portions thereof, identified using the method of the invention, as well as pharmaceutical compositions including a therapeutically effective amount of a compound, *e.g.*, an antibody or an antigen-binding portion thereof, or a small molecule, identified using the methods of the invention, and a pharmaceutically effective carrier.

[0018] In yet another aspect, the present invention provides methods for inhibiting the binding of an oligomeric chemokine to a chemokine receptor. The methods include contacting, *e.g.*, *in vitro* or *in vivo*, the chemokine with an effective amount of a compound, *e.g.*, an antibody, or an antigen-binding portion thereof, or a small molecule, identified using the methods of the invention, thereby inhibiting the binding of the oligomeric chemokine to the chemokine receptor.

[0019] In yet a further aspect, the present invention provides methods for inhibiting the binding of an oligomeric form of a chemokine to a glycosaminoglycan. The methods include contacting, *e.g.*, *in vitro* or *in vivo*, the chemokine with an effective amount of a compound, *e.g.*, an antibody, or an antigen-binding portion thereof, or a small molecule, identified using the methods of the invention, thereby inhibiting the binding of the oligomeric chemokine to the chemokine receptor.

[0020] In one embodiment, a compound, *e.g.*, an antibody used for inhibiting the binding of an oligomeric chemokine to a receptor and/or to a glycosaminoglycan is 1H2 or 1D3 or 2E5.

[0021] In another aspect, the present invention provides methods for treating a chemokine-mediated disorder in a subject, *e.g.*, a human, in need thereof. The methods include administering to the subject an effective amount of a compound, *e.g.*, antibody or an antigen-binding thereof, or a small molecule, identified using the methods of the invention, thereby treating a chemokine-mediated disorder. A chemokine-mediated disorder may be an inflammatory disorder, *e.g.*, chronic inflammation, chronic obstructive pulmonary disease (COPD), acute respiratory distress syndrome (ARDS), inflammatory bowel disease, leprosy, encephalomyelitis, artherosclerosis, uveoretinitis, osteoarthritis, asthma, dermatitis, or eczema; an immune disorder, *e.g.*, multiple

sclerosis, HIV infection, psoriasis, allograft rejection, tumor-specific immunity, immune complex glomerulonephritis and lupus; or an angiogenic disorder, *e.g.*, cancer.

[0022] In one embodiment, a compound, *e.g.*, an antibody or antigen-binding portion thereof, or a small molecule, identified using the methods of the invention is administered locally to a site of inflammation in a subject.

[0023] In another aspect, the present invention provides methods for diagnosing or prognosing a chemokine-mediated disorder in a subject, *e.g.*, a human in need thereof. The methods include (a) contacting a biological sample and a control sample with a compound identified using the methods of the invention; and (b) detecting an oligomeric form of a chemokine in the biological sample and the control sample, where the subject is diagnosed or prognosed with a chemokine-mediated disorder based on a difference in the amount of an oligomeric form of a chemokine present in the biological sample as compared to the control sample.

[0024] In another embodiment, an antibody which may be used in the therapeutic, prognostic or diagnostic methods of the invention is 1H2 or 1D3 or 2E5.

[0025] In a yet further aspect, the present invention provides a method of identifying a compound, *e.g.*, an antibody or an antigen-binding portion thereof, that selectively binds to an oligomeric form, and not to a monomeric form, of SDF-1α. The method includes: (a) contacting a compound, *e.g.*, antibody, or antigen-binding portion thereof, with a peptide comprising a glycosaminoglycan binding site of SDF-1α; or (b) contacting a compound, *e.g.*, antibody, or antigen binding portion thereof, with SDF-1α in the presence of a cation, where the cation is present at a concentration sufficient to promote and/or stabilize SDF-1α oligomerization; (c) determining whether the compound, *e.g.*, antibody, or antigen-binding portion thereof, binds the peptide in (a) or the SDF-1α in

(b); and (d) identifying a compound, *e.g.*, antibody, or antigen-binding portion, which selectively binds to an oligomeric form, and not to a monomeric form, of SDF-1α, based on the ability of the compound, *e.g.*, antibody, or an antigen-binding portion thereof, to bind the peptide in (a) or to bind the SDF-1α in (b).

[0026] Other features and advantages of the invention will be apparent from the following detailed description, figures and claims.

Brief Description of the Drawings

[0027] Figures 1A-1F are graphs depicting the results from an experiment measuring the binding of B-type (*i.e.*, 1D3, 1H2 and 2E5) and Z-type (*i.e.*, 1C6, 2A5 and 1A7) Fabs to the monomeric form of SDF-1α immobilized on a chip. The Y-axis represents the signal obtained from binding of the Fabs, which was directly proportional to the molecular weight of the analyte or ligand bound, and the X-axis represents the time over which the signal was measured.

[0028] Figures 2A-2C are graphs depicting the results from an experiment measuring the binding of B-type Fabs 1D3, 1H2 and 2E5 to SDF-1α, where the Fabs were immobilized onto an anti-CH1 chip and the antigen, SDF-1α, was flowed across. The graphs depict the signal measured over time.

[0029] Figures 3A-3B are graphs depicting the results from an experiment comparing the binding of a B-type Fab, 1H2, immobilized onto an anti-CH1 chip to SDF-1α relative to the binding of a Z-type Fab, 1C6, immobilized onto an anti-CH1 chip to SDF-1α.

[0030] Figure 4 depicts the results from a time course experiment where a signal resulting from the binding of a B-type Fab 1D3 or a Z-type Fab 1C6, immobilized onto an anti-CH1 chip to SDF-1α was measured after dilution of SDF-1α.

[0031] Figure 5 depicts the stoichiometry of the binding of a B-type Fab molecule (1D3) to SDF-1α, demonstrating that about 1.75 molecules of SDF-1α bound per Fab.

[0032] Figures 6A-6C depict the results from an experiment designed to investigate the effect of a chelating agent, EDTA, on the binding of various B-type IgGs to SDF-1α, which was indicative of whether SDF-1α was found to be present predominantly in a monomeric form in the presence of EDTA or an oligomeric form.

[0033] Figures 7A-7C depict the results from an experiment designed to investigate the effect of heparin on the binding of 1D3 and 1C6 IgGs to SDF-1α. As depicted in Figure 7A, SDF-1α bound to biotinylated heparin immobilized onto a streptavidin chip.

[0034] Figures 8A-8C depict the results of a time course experiment designed to measure the effect of a divalent cation, Ca^{2+}, on SDF-1α oligomerization and in turn on binding of B-type IgGs to SDF-1α by fluorescence polarization.

[0035] Figures 9A-9B depict the effect of Ca^{2+} on SDF-1α oligomerization, measured using fluorescence resonance energy transfer (FRET).

[0036] Figure 10 depicts the results of an experiment designed to investigate the effect of Ca^{2+} on binding of SDF-1α to immobilized B-type IgGs in the presence of heparin.

[0037] Figures 11A-11B depict the results of an experiment designed to investigate the effect of pH on binding of SDF-1α to B-type and Z-type IgGs immobilized onto a chip.

[0038] Figure 12 depicts the results of an experiment investigating the effect of Ca^{2+} on loss of binding of B-type antibodies to SDF-1α observed at a low (*i.e.*, acidic) pH.

[0039] Figures 13A-13B depict the results of an experiment designed to investigate the effect of a protein denaturant on the folding of various B-type and Z-type antibodies.

[0040] Figures 14A-14B depict a mass spectrometric analysis approach on molecular epitope sequence identification of SDF1-α with different peptide overlaps generated by various enzyme cleavages.

[0041] Figure 15 depicts a schematic of the amino acid sequence of SDF-1α (SEQ ID NO:9) including the various domains that have been identified in the chemokine summarized from mass spectrometric data and literature citation.

[0042] Figure 16A is a schematic representation of the tertiary epitope regions identified on the structure of SDF-1α, using mass spectrometry and PDB code 1A15 as a template. Figure 16B is a schematic representation of the tertiary structure of the various synthetic peptides (SEQ ID NOs: 10-19) shown on the three dimensional structure of SDF-1α, generated using the structure PDB code 1A15 as the template.

[0043] Figures 17A-17B are bar graphs depicting the results from an experiment designed to identify the region(s) of SDF-1α which bind the various Z-type antibodies that bind a monomeric form of SDF-1α.

[0044] Figures 18A-18B are bar graphs depicting the results from an experiment designed to identify the region(s) of SDF-1α which bind the various B-type antibodies that bind an oligomeric form of SDF-1α.

[0045] Figure 19 depicts the results of an experiment demonstrating that heparin binding to SDF-1α blocks the binding of antibodies to SDF-1α.

[0046] Figure 20A shows the nucleotide sequence (SEQ ID NO:1) and amino acid sequence (SEQ ID NO:2) of the heavy chain variable region of the 1D3 human monoclonal antibody. The complementarity determining regions (CDRs) are delineated and the V, D and J germline derivations are indicated. Figure 20B shows the nucleotide sequence (SEQ ID NO:3) and the amino acid sequence (SEQ ID NO:4) of the light chain variable region of the 1D3 human monoclonal antibody. The CDRs are delineated and the V and J germline derivations are indicated.

[0047] Figure 21A shows the nucleotide sequence (SEQ ID NO:5) and amino acid sequence (SEQ ID NO:6) of the heavy chain variable region of the 1H2 human monoclonal antibody. The complementarity determining regions (CDRs) are delineated and the V, D and J germline derivations are indicated. Figure 21B shows the nucleotide sequence (SEQ ID NO:7) and the amino acid sequence (SEQ ID NO:8) of the light chain variable region of the 1H2 human monoclonal antibody. The CDRs are delineated and the V and J germline derivations are indicated.

Detailed Description of the Invention

[0048] Many chemokines have been shown to oligomerize and it is believed that such oligomerization plays a fundamental role in chemokine function, *e.g.*, activation and recruitment of lymphocytes and monocytes, particularly in various disease states associated with increased levels of chemokines.

[0049] The dimerization motifs in various chemokines tend to be structurally conserved and tend to fall primarily into two categories, the CC chemokines and the CXC chemokines. The chemokines in the CC family are believed to dimerize by the formation of a two-stranded anti-parallel β-sheet involving residues near the N-termini, while the CXC family members are believed to dimerize through the first strand of the

β-sheet which is preformed in the monomeric structure (Fernandez *et al.*, (2002) *Annu Rev. Pharmacol. Toxicol.* 42: 469-99; Fernando *et al.* (2004) *J. Biol. Chem.* 279: 36175-78). There is increasing evidence that oligomerization (*e.g.*, dimerization) of chemokines is related to their glycosaminoglycan (GAG) binding properties. For example, it has been reported that in the presence of heparin, the apparent molecular weights of various chemokines are significantly elevated consistent with the formation of higher order structures (*e.g.*, oligomers). Additionally, chemokines have been shown to oligomerize on immobilized heparin and the GAG binding site in most chemokines is thought to lie at or near the dimer-interface (Fernandez *et al.* (2004) *J. Biol. Chem.* 279: 36175-78; .Koopmann *et al.*, (1997) *J. Biol. Chem*, 272: 10103-09; McCornack *et al.*, (2004) *Biochem.*. 43: 10090-101).

[0050] It has been reported previously that antibodies that bind at the dimer-interface (which includes or overlaps with the heparin-binding site) bind a monomeric form of a chemokine (Deforge *et al.*, (2000) *Cytokine* 11: 1620-1629). Therefore, this invention is based, at least in part, on the surprising and unexpected finding that an antibody that binds the heparin-binding site of a chemokine (*e.g.*, SDF-1α) selectively binds the oligomeric (*e.g.*, dimeric) form versus the monomeric form of the chemokine.

[0051] It has also been reported that chemokine dimerization occurs in the presence of anions (Veldkamp *et al.*, (2005) *Protein Science* 14:1071-1081). Accordingly, this invention is also based, at least in part, on the surprising and unexpected finding that in the presence of cations (*e.g.*, Ca^{2+}) chemokines form dimers or higher order oligomers.

[0052] The present invention features, at least in part, methods of identifying compounds (*e.g.*, antibodies or antigen-binding portions thereof, and small molecules) that selectively bind oligomeric, *e.g.*, heterodimeric or homodimeric, forms of

chemokines. Increased levels of chemokines have been reported in various disease states and oligomerization of chemokines is favored at increased chemokine levels. Accordingly, the compounds identified using the methods of the invention are useful for treating, preventing, diagnosing or prognosing diseases or disorders associated with increased chemokine levels (*e.g.*, chemokine-mediated disorders). Without wishing to be bound by theory, it is believed that the compounds, *e.g.*, antibodies, antigen-binding portions thereof and small molecules, identified using the methods of the invention modulate chemokine activity, for example, by inhibiting binding of an oligomeric form of a chemokine to a chemokine receptor and/or by inhibiting the binding of an oligomeric form of a chemokine to a glycosaminoglycan.

[0053] In one aspect, the present invention is directed to a method of identifying a compound, *e.g.*, antibody or an antigen-binding portion thereof, which selectively binds to an oligomeric form, and not to a monomeric form, of a chemokine. The method includes: (a) contacting a compound, *e.g.*, antibody, or an antigen-binding portion thereof, with a peptide including a glycosaminoglycan binding site of a chemokine; or (b) contacting a compound, *e.g.*, antibody, or an antigen-binding portion thereof, with a chemokine in the presence of a cation, wherein the cation is present at a concentration sufficient to promote and/or stabilize chemokine oligomerization; (c) determining whether the compound, *e.g.*, antibody, or antigen-binding portion thereof, binds the peptide in (a) or the chemokine in the presence of a cation in (b); and (d) identifying a compound, *e.g.*, antibody, or an antigen-binding portion thereof, which selectively binds to an oligomeric form, and not to a monomeric form, of the chemokine, based on the ability of the compound, *e.g.*, antibody, or antigen-binding portion thereof, to bind the peptide in (a) or chemokine in (b).

[0054] In order that the present invention may be more readily understood, certain terms are first defined. Additional definitions are set forth throughout the detailed description.

I. Definitions

[0055] The terms "chemokine" and "chemokine-like molecules," as used herein, refer to any of many structurally related glycoproteins with one or more biological activities, including for example, leukocyte activation and/or chemotactic activity. Chemokines are typically about 70 to 90 amino acids in length, although, they may be larger. In some embodiments, "chemokines" and "chemokine-like molecules" are characterized by virtue of their interaction with seven transmembrane spanning G protein-linked receptor on target cells. In other embodiments, "chemokines" and "chemokine-like molecules" are characterized by virtue of their interaction with glycosaminoglycans (GAGs) on surface of target cells. In yet other embodiments, "chemokines" and "chemokine-like molecules," may have both of these characteristics. Structurally, "chemokines" and "chemokine-like molecules" may be characterized based on the spacing of two conserved amino-terminal cysteines which are involved in disulfide bond formation. For example, in certain "chemokines" and "chemokine-like molecules," the two cysteines are separated by another amino acid residues (CXC) and in other "chemokines," and "chemokine-like molecules," the two cysteines are adjacent to each other (CC). The terms "chemokine" and "chemokine-like molecule" also refer to any polypeptide or protein having an activity of a chemokine. Such proteins and polypeptides include both those which may be produced by the cells of a host (*e.g.*, a human) and those which may be produced by an infectious agent which infects a host (*e.g.*, a bacteria or a virus). Exemplary chemokines include, but are not limited to the

ones shown below in Table I. Table I also includes the known target cell(s) for each of the exemplary chemokines and receptor, where known.

Table I

Chemokine (Systemic Name)	Chromosome	Target cell	Receptor
IL-8 CXCL8	4	Neutrophil, basophil, T cell	CXCR1, 2
GROα CXCL1	4	Neutrophil	CXCR2 >> 1
GROβ CXCL2	4	Neutrophil	CXCR2
GROγ CXCL3	4	Neutrophil	CXCR2
ENA-78 CXCL5	4	Neutrophil	CXCR2
LDGF-PBP CXCL7	4	Fibroblast, neutrophil	CXCR2
GCP-2 CXCL6	4	Neutrophil	CXCR2
PF4 CXCL4	4	Fibroblast	Unknown
Mig CXCL9	4	Activated T cell	CXCR3
IP-10 CXCL10	4	Activated T cell ($T_H1 > T_H2$)	CXCR3
SDF-1α/β CXCL12	10	$CD34^+$ bone marrow cell, T cell, dendritic cell, B cell, naive B cell, activated CD4 T cell	CXCR4
BUNZO/STRC33 CXCL16	17	T cell, NK T cell	CXCR6
I-TAC CXCL11	4	Activated T cell	CXCR3
BLC/BCA-1 CXCL13	4	Naive B cells, activated CD4 T cells	CXCR5
MIP-1α CCL3	17	Monocyte/macrophage, T cell ($T_H1 > T_H2$), NK cell, basophil, immature dendritic cell, bone marrow cell	CCR1, 5
MIP-1β CCL4	17	Monocyte/macrophage, T cell ($T_H1 > T_H2$), NK cell, basophil, immature dendritic cell, bone marrow cell	CCR1, 5
MDC	16	Immature dendritic cell, IA	CCR4

CCL22		NK cell, T cell ($T_H2 > T_H1$), thymocyte	
TECK CCL25	19	Macrophage, thymocytes, dendritic cell	CCR9
TARC CCL17	16	T-cell ($T_H2 > T_H1$), immature dendritic cell, IA NK cell, T cell ($T_H2 > T_H1$), thymocyte	CCR4
RANTES CCL5	17	Monocyte/macrophage, T cell (memory T cell > T cell; $T_H1 > T_H2$), NK cell, basophil, eosinophil, dendritic cell	CCR1, 3, 5
HCC-1 CCL14	17	Monocyte	CCR1
HCC-4 CCL16	17	Monocyte	CCR1
DC-CK1 CCL18	17	Naive T cell > T cell	Unknown
MIP-3α CCL20	2	T cell (memory T cell > T cell), peripheral blood mononuclear cell, bone marrow cell-dendritic cell	CCR6
MIP-3β CCL19	9	Naive T cell, mature dendritic cell, B cell	CCR7
MCP-1 CCL2	17	T cell, monocyte, basophil	CCR2
MCP-2 CCL8	17	T cell, monocyte, eosinophil, basophil	CCR2
MCP-3 CCL7	17	T cell, monocyte, eosinophil, basophil, dendritic cell	CCR2
MCP-4 CCL13	17	T cell, monocyte, eosinophil, basophil, dendritic cell	CCR2, 3
Eotaxin CCL11	17	Eosinophil	CCR3
Eotaxin-2/MPIF-2 CCL24	?	T cell (?), eosinophil, basophil	CCR3
I-309 CCL1	17	Neutrophil (TCA-3 only), T cell	CCR8
MIP-5/HCC-2 CCL15	17	T cell, monocyte, neutrophil (?), dendritic cell	CCR1, 3
MPIF-1 CCL23	?	Monocyte, T cell (resting), neutrophil (?)	Unknown
6Ckine CCL21	9	Naive T cell, B cell, mesangial cells (?)	CCR7
CTACK CCL27	9	T cell	CCR10
MEC CCL28	5	T cell, eosinophil	CCR10, 3
Lymphotactin XCL1	1	T cell, NK cell	XCR1

Fractalkine CXCL1	16	T cell, monocyte, neutrophil (?)	CX3CR1

[0056] The term "chemokine-mediated disorder," as used herein, includes disease states and/or symptoms associated with a disease state, where increased levels of one or more chemokines are found. It is understood that oligomerization of chemokines occurs when increased levels of chemokines are found. Accordingly, the term "chemokine-mediated disorder," also refers to disease states and/or symptoms associated with disease states where chemokine oligomerization occurs. In general, the term "chemokine-mediated disorder" refers to any disorder, the onset, progression or the persistence of the symptoms of which requires the participation of a chemokine molecule, in particular, an oligomeric form of a chemokine. Exemplary chemokine-mediated disorders include, but are not limited to, for example, inflammatory disorders, immune disorders and angiogenic disorders such as, for example, chronic inflammation, chronic obstructive pulmonary disease (COPD), acute respiratory distress syndrome (ARDS), inflammatory bowel disease, tuberculoid leprosy, encephalomyelitis, artherosclerosis, uveoretinitis, osteoarthritis, asthma, dermatitis, eczema, multiple sclerosis, HIV infection, psoriasis, allograft rejection, tumor-specific immunity, immune complex glomerulonephritis, lupus and angiogenesis. Additional chemokine-mediated disorders are set forth in Table II, which reflects the disease association of some of the chemokines.

Table II

Chemokines Associated with Disease States

Disease States	**Site**	**Chemokine**
Cystic Fibrosis	Lavage Fluid	IL-8, ENA-78, MCP-1
Acute Pulmonary Diseases	Tissue	IL-8, ENA-78, MCP-1, Rantes
Asthmatic Reactions	Lavage Fluid	MCP-1, MIP-1A, Rantes
Endotoxemia and Sepsis	Plasma	IL-8, MIP-1A, MCP-1, Rantes

Rheumatoid Arthritis	Synovial Fluid	IL-8, ENA-78, MCP-1, MIP1 alpha
Osteoarthritis	Synovial Fluid	MIP-1B
Psoriatic Scale	Tissue Extract	IL-8, GROa,B,G, MCP-1, IP-10, ENA-78
Gastrointestinal Inflammation	Tissue	IL-8, MCP-1, MIP1A/B, Rantes, IP-10
Arteriosclerosis	Tissue	MCP-1, MIP1A/B, Rantes, IL-8, GROB
Immune Complex Glomerulonephritis	Tissue	IL-8, MCP-1
Uveoretinitis	Tissue	IL-8, IP-10, MCP-1, Rantes, MIP-1A/B
Tuberculoid Leprosy	Tissue	IP-10
Post-Major Surgery	Plasma	IL-8
Wound Healing Site	Tissue	MCP-1 and IP-10
Cytomegalovirus Encephalomyelitis	Cerebrospinal Fluid	MCP-1
Atopic and Contact Dermatitis	Tissue	Rantes, Eotaxin, IL-8, MCP-1, IP-10

[0057] The terms "chemokine activity" and "activity of an oligomeric form of a chemokine," as used herein, refer to any biological (*e.g.*, molecular or cellular or physiological) activity of a chemokine (or an oligomeric form of a chemokine). For example, in some embodiments, a chemokine activity is the binding of an oligomeric form of a chemokine to a chemokine receptor. In other embodiments, a chemokine activity is the binding of an oligomeric form of a chemokine to a glycosaminoglycan. In some embodiments, a chemokine activity may include both of these activities. Chemokine activity may be measured using one or more of the assays discussed herein and those that are well known in the art.

[0058] The term "oligomeric form of a chemokine," as used herein, includes a form other than the monomeric form of a chemokine. Oligomeric forms encompassed by the present invention include, but are not limited to, dimeric, tetrameric and higher order forms of a chemokine. Dimeric forms include both homodimeric and heterodimeric forms of chemokines. Oligomeric forms of a chemokine may be readily detected using one or more methods described herein and those known in the art including, but not limited to, for example, X-ray crystallography, nuclear magnetic resonance, mass spectrometry, and fluorescence spectroscopy, ,analytical ultracentrifugation, surface plasmon resonance (Biacore technology).

[0059] The term "oligomerization," as used herein, relates to the formation of a higher order chemokine structure which is not a monomer. In other words, the term "oligomerization" relates to the association of monomeric forms of a chemokine to form higher order structures, including, but not limited to dimeric forms, tetrameric forms and other oligomeric forms, including both homo-oligomeric forms and hetero-oligomeric forms of chemokines.

[0060] As used herein, the term "hetero-oligomeric" refers to the stable association of two or more different chemokine proteins or polypeptide chains either through covalent or non-covalent interaction, for example, disulphide bonding.

[0061] The term "a peptide comprising a glycosaminoglycan binding site," as used herein, includes a peptide derived from a chemokine or designed based on a chemokine, which includes a glycosaminoglycan (GAG) binding site. The peptide may include one or more GAG binding sites (*e.g.*, at least one, or at least two, or at least three, or at least four GAG binding sites). The peptide can be of any length so long as it includes a binding site for a GAG (*e.g.*, a heparin or a heparin sulfate binding site). In some

embodiments of the methods of the invention, the length of such a peptide may be less than the length of the full amino acid sequence of a chemokine monomer, by at least one amino acid. For example, if the length of the amino acid sequence for a chemokine monomer is n, a peptide useful in the methods of the invention can be of any length equal to or less than (n-1), so long as it includes a GAG binding site. The term "a peptide comprising a glycosaminoglycan binding site," also includes peptides which include the GAG binding site of a chemokine (*e.g.*, amino acid residues 24-27 of SDF-1α) flanked by amino acid residues not derived from the chemokine. For example, such a peptide includes the GAG binding site of a chemokine flanked by heterologous sequences at one or both ends. In some embodiments, a peptide comprising a GAG binding site is at least 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100 or more amino acid residues in length. A peptide comprising a GAG binding site may either be a natural peptide, a recombinant peptide or a synthetic peptide. In some embodiments, a peptide comprising a GAG binding site is derived from, or is designed based on, SDF-1α, and includes amino acid residues 20-31, 20-33 or 24-27 of SDF-1α.

[0062] The terms "immunoglobulin" and "antibody" (used interchangeably herein) include a protein having a basic four-polypeptide chain structure consisting of two heavy and two light chains, said chains being stabilized, for example, by interchain disulfide bonds, which has the ability to specifically bind an antigen. The term "single-chain immunoglobulin" or "single-chain antibody" (used interchangeably herein) refers to a protein having a two-polypeptide chain structure consisting of a heavy and a light chain, said chains being stabilized, for example, by interchain peptide linkers, which has the ability to specifically bind an antigen. The term "domain" refers to a globular region of a heavy or light chain polypeptide comprising peptide loops (*e.g.*, comprising 3 to 4

peptide loops) stabilized, for example, by β-pleated sheet and/or intrachain disulfide bond. Domains are further referred to herein as "constant" or "variable," based on the relative lack of sequence variation within the domains of various class members in the case of a "constant" domain, or the significant variation within the domains of various class members in the case of a "variable" domain. Antibody or polypeptide "domains" are often referred to interchangeably in the art as antibody or polypeptide "regions." The "constant" domains of an antibody light chain are referred to interchangeably as "light chain constant regions," "light chain constant domains," "CL" regions or "CL" domains. The "constant" domains of an antibody heavy chain are referred to interchangeably as "heavy chain constant regions," "heavy chain constant domains," "CH" regions or "CH" domains). The "variable" domains of an antibody light chain are referred to interchangeably as "light chain variable regions," "light chain variable domains," "VL" regions or "VL" domains). The "variable" domains of an antibody heavy chain are referred to interchangeably as "heavy chain constant regions," "heavy chain constant domains," "VH" regions or "VH" domains).

[0063] Immunoglobulins or antibodies can exist in monomeric or polymeric form, for example, IgM antibodies which exist in pentameric form and/or IgA antibodies which exist in monomeric, dimeric or multimeric form. Other than "bispecific" or "bifunctional" immunoglobulins or antibodies, an immunoglobulin or antibody is understood to have each of its binding sites identical. A "bispecific" or "bifunctional antibody" is an artificial hybrid antibody having two different heavy/light chain pairs and two different binding sites. Bispecific antibodies can be produced by a variety of methods including fusion of hybridomas or linking of Fab' fragments. See, *e.g.*, Songsivilai & Lachmann, (1990) *Clin. Exp. Immunol.* 79:315-321; Kostelny *et al.*, (1992) *J. Immunol.* 148:1547-1553.

[0064] Exemplary antibodies identified using the methods of the invention include the antibodies described herein, *e.g.*, 1D3, 1H2, or 2E5, which selectively bind the oligomeric form of SDF-1α. These antibodies and their uses are described in co-pending U.S. provisional patent application no. 60/837,004, filed on August 11, 2006, the entire contents of which are expressly incorporated by reference herein.

[0065] The term "antigen-binding portion" of an antibody (or "antibody portion") includes fragments of an antibody that retain the ability to specifically bind to an antigen (*e.g.*, a chemokine or a chemokine-like molecule). It has been shown that the antigen-binding function of an antibody can be performed by fragments of a full-length antibody. Examples of binding fragments encompassed within the term "antigen-binding portion" of an antibody include (i) a Fab fragment, a monovalent fragment consisting of the VL, VH, CL and CH1 domains; (ii) a $F(ab')_2$ fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; (iii) a Fd fragment consisting of the VH and CH1 domains; (iv) a Fv fragment consisting of the VL and VH domains of a single arm of an antibody, (v) a dAb fragment (Ward *et al.*, (1989) *Nature* 341:544-546), which consists of a VH domain; and (vi) an isolated complementarity determining region (CDR). Furthermore, although the two domains of the Fv fragment, VL and VH, are coded for by separate genes, they can be joined, using recombinant methods, by a synthetic linker that enables them to be made as a single protein chain in which the VL and VH regions pair to form monovalent molecules (known as single chain Fv (scFv); see *e.g.*, Bird *et al.*, (1988) *Science* 242:423-426; and Huston *et al.*, (1988) *Proc. Natl. Acad. Sci. USA* 85:5879-5883). Such single chain antibodies are also intended to be encompassed within the term "antigen-binding portion" of an antibody. Other forms of single chain antibodies, such as diabodies are also encompassed. Diabodies are bivalent, bispecific antibodies in which VH and VL domains are

expressed on a single polypeptide chain, but using a linker that is too short to allow for pairing between the two domains on the same chain, thereby forcing the domains to pair with complementary domains of another chain and creating two antigen binding sites (see *e.g.*, Holliger, P. *et al.*, (1993) *Proc. Natl. Acad. Sci. USA* 90:6444-6448; Poljak, R. J. *et al.*, (1994) *Structure* 2:1121-1123). Still further, an antibody or antigen-binding portion thereof may be part of a larger immunoadhesion molecules, formed by covalent or non-covalent association of the antibody or antibody portion with one or more other proteins or peptides. Examples of such immunoadhesion molecules include use of the streptavidin core region to make a tetrameric scFv molecule (Kipriyanov, S. M. *et al.*, (1995) *Human Antibodies and Hybridomas* 6:93-101) and use of a cysteine residue, a marker peptide and a C-terminal polyhistidine tag to make bivalent and biotinylated scFv molecules (Kipriyanov, S. M. *et al.*, (1994) *Mol. Immunol.*, 31:1047-1058). Antibody portions, such as Fab and $F(ab')_2$ fragments, can be prepared from whole antibodies using conventional techniques, such as papain or pepsin digestion, respectively, of whole antibodies. Moreover, antibodies, antibody portions and immunoadhesion molecules can be obtained using standard recombinant DNA techniques, as described herein. Preferred antigen binding portions are complete domains or pairs of complete domains.

[0066] "Specific binding," "specifically binds," "selective binding," and "selectively binds," as used herein, mean that the compound, *e.g.*, antibody or antigen-binding portion thereof, exhibits appreciable affinity for a particular antigen or epitope and, generally, does not exhibit significant cross-reactivity with other antigens and epitopes. "Appreciable" or preferred binding includes binding with an affinity of at least 10^6, 10^7, 10^8, 10^9 M^{-1}, or 10^{10} M^{-1}. Affinities greater than 10^7 M^{-1}, preferably greater than 10^8 M^{-1} are more preferred. Values intermediate of those set forth herein are also intended to be

within the scope of the present invention and a preferred binding affinity can be indicated as a range of affinities, for example, 10^6 to 10^{10} M^{-1}, preferably 10^7 to 10^{10} M^{-1}, more preferably 10^8 to 10^{10} M^{-1}. An antibody that "does not exhibit significant cross-reactivity" is one that will not appreciably bind to an undesirable entity (*e.g.*, an undesirable proteinaceous entity). For example, in one embodiment, a compound, *e.g.*, antibody or antigen-binding portion thereof, or a small molecule, that specifically binds to a chemokine will appreciably bind that chemokine but will not significantly react with other chemokines and non-chemokine proteins or peptides. Using the methods of the invention, a compound, *e.g.*, antibody or antigen-binding portion thereof, or a small molecule, is identified which appreciably binds an oligomeric form (*e.g.*, a dimeric form or a tetrameric form or a homodimeric form or a heterodimeric form) of a chemokine and does not specifically react with the monomeric form. An antibody specific for a particular epitope will, for example, not significantly cross-react with remote epitopes on the same or other chemokines or non-chemokine antigens. Specific or selective binding can be determined according to any art-recognized means for determining such binding, including, for example, according to Scatchard analysis and/or competitive binding assays.

[0067] The term "humanized immunoglobulin" or "humanized antibody" refers to an immunoglobulin or antibody that includes at least one humanized immunoglobulin or antibody chain (*i.e.*, at least one humanized light or heavy chain). The term "humanized immunoglobulin chain" or "humanized antibody chain" (*i.e.*, a "humanized immunoglobulin light chain" or "humanized immunoglobulin heavy chain") refers to an immunoglobulin or antibody chain (*i.e.*, a light or heavy chain, respectively) having a variable region that includes a variable framework region substantially from a human immunoglobulin or antibody and complementarity determining regions (CDRs) (*e.g.*, at

least one CDR, preferably two CDRs, more preferably three CDRs) substantially from a non-human immunoglobulin or antibody, and further includes constant regions (*e.g.*, at least one constant region or portion thereof, in the case of a light chain, and preferably three constant regions in the case of a heavy chain). The term "humanized variable region" (*e.g.*, "humanized light chain variable region" or "humanized heavy chain variable region") refers to a variable region that includes a variable framework region substantially from a human immunoglobulin or antibody and complementarity determining regions (CDRs) substantially from a non-human immunoglobulin or antibody.

[0068] The term "human antibody" includes antibodies having variable and constant regions corresponding to human germline immunoglobulin sequences as described by Kabat et al. (See Kabat, *et al.*, (1991) *Sequences of proteins of Immunological Interest, Fifth Edition*, U.S. Department of Health and Human Services, NIH Publication No. 91-3242). The human antibodies of the invention may include amino acid residues not encoded by human germline immunoglobulin sequences (*e.g.*, mutations introduced by random or site-specific mutagenesis in vitro or by somatic mutation in vivo), for example in the CDRs and in particular CDR3. The human antibody can have at least one position replaced with an amino acid residue, *e.g.*, an activity enhancing amino acid residue which is not encoded by the human germline immunoglobulin sequence. The human antibody can have up to twenty positions replaced with amino acid residues which are not part of the human germline immunoglobulin sequence. In other embodiments, up to ten, up to five, up to three or up to two positions are replaced. In a preferred embodiment, these replacements are within the CDR regions as described in detail below.

[0069] The term "recombinant human antibody" includes human antibodies that are prepared, expressed, created or isolated by recombinant means, such as antibodies expressed using a recombinant expression vector transfected into a host cell, antibodies isolated from a recombinant, combinatorial human antibody library, antibodies isolated from an animal (*e.g.*, a mouse) that is transgenic for human immunoglobulin genes (see *e.g.*, Taylor, L. D. *et al.*, (1992) *Nucl. Acids Res.* 20:6287-6295) or antibodies prepared, expressed, created or isolated by any other means that involves splicing of human immunoglobulin gene sequences to other DNA sequences. Such recombinant human antibodies have variable and constant regions derived from human germline immunoglobulin sequences (See Kabat E. A., *et al.*, (1991) *Sequences of Proteins of Immunological Interest, Fifth Edition*, U.S. Department of Health and Human Services, NIH Publication No. 91-3242). In certain embodiments, however, such recombinant human antibodies are subjected to in vitro mutagenesis (or, when an animal transgenic for human Ig sequences is used, in vivo somatic mutagenesis) and thus the amino acid sequences of the VH and VL regions of the recombinant antibodies are sequences that, while derived from and related to human germline VH and VL sequences, may not naturally exist within the human antibody germline repertoire in vivo. In certain embodiments, however, such recombinant antibodies are the result of selective mutagenesis approach or backmutation or both.

[0070] An "isolated antibody" includes an antibody that is substantially free of other antibodies having different antigenic specificities (*e.g.*, an isolated antibody that specifically binds a chemokine and is substantially free of antibodies or antigen-binding portions thereof that specifically bind other antigens, including other chemokines). An isolated antibody that specifically binds a chemokine may bind the same chemokine

and/or chemokine-like molecules from other species. Moreover, an isolated antibody may be substantially free of other cellular material and/or chemicals.

[0071] The term "chimeric immunoglobulin" or antibody refers to an immunoglobulin or antibody whose variable regions derive from a first species and whose constant regions derive from a second species. Chimeric immunoglobulins or antibodies can be constructed, for example by genetic engineering, from immunoglobulin gene segments belonging to different species.

[0072] An "antigen" is an entity (*e.g.*, a proteinaceous entity or peptide) to which an antibody or antigen-binding portion thereof specifically or selectively binds. In various embodiments of the present invention, an antigen is a chemokine or a chemokine-like molecule. In a particular embodiment according to the invention, an antigen is SDF-1α. The complete amino acid sequence of an exemplary human SDF-1α is set forth in Genbank® Accession number NM_00069.

[0073] The term " k_{off}," as used herein, is intended to refer to the off rate constant for dissociation of an antibody from the antibody/chemokine complex. The chemokine may either be in a monomeric state or in an oligomeric state.

[0074] The term " K_D," as used herein, is intended to refer to the dissociation constant of a particular antibody-chemokine interaction, where the chemokine may be either in monomeric form or an oligomeric form.

[0075] The term "modifying," as used herein, is intended to refer to changing a compound identified using the methods of the invention, *e.g.*, changing one or more amino acids in the antibodies or antigen-binding portions thereof. The change can be produced by adding, substituting or deleting an amino acid at one or more positions. The

change can be produced using known techniques, such as PCR mutagenesis. For example, in some embodiments, an antibody or an antigen-binding portion thereof identified using the methods of the invention can be modified, to thereby increase the binding of the antibody or antigen-binding portion thereof to an oligomeric form of a chemokine. In other embodiments, an antibody or an antigen-binding portion thereof, is modified subsequent to its identification, to reduce its immunogenicity for use in humans.

[0076] The term "inhibiting binding to a chemokine receptor," as used herein, refers to the ability of a compound, *e.g.*, antibody or an antigen-binding portion thereof, or a small molecule, identified using the methods of the present invention, to decrease or prevent the binding of a chemokine (*e.g.*, an oligomeric form of a chemokine) to its cognate receptor. In some embodiments, such a compound, *e.g.*, antibody or antigen-binding portion thereof, or a small molecule, inhibits binding of an oligomeric form of a chemokine to its cognate receptor by binding to the chemokine in a manner which prevents it from binding to its receptor, *e.g.*, in a case where the receptor binding site on the oligomeric form of the chemokine overlaps with the site to which the compound, *e.g.*, antibody or antigen-binding portion thereof, or small molecule binds. Alternatively, the binding of a compound, *e.g.*, antibody or antigen-binding portion thereof, or a small molecule, to an oligomeric form of a chemokine may alter or distort its conformation such that it is unable to bind its cognate receptor. In another embodiment, a compound, *e.g.*, antibody or antigen-binding portion thereof, or a small molecule, identified using the methods of the invention, inhibits the binding of an oligomeric form of a chemokine to its cognate receptor by sequestering the oligomeric form away from the receptor. In yet other embodiments, a compound, *e.g.*, antibody or antigen-binding portion thereof, or a small molecule, identified using the methods of the invention, inhibits binding of an

oligomeric form of a chemokine to its cognate receptor by increasing the affinity of the oligomeric form of a chemokine for a GAG, *e.g.*, in a case where both a chemokine receptor and a GAG are present on a target cell, thereby decreasing the concentration of available oligomeric form of the chemokine to bind to a receptor. Inhibition of the binding of a chemokine to a receptor can be readily measured using one or more *in vitro* and/or *in vivo* assays well known in the art and those described herein (Coulin *et al.*, (1997) *Eur. J. Biochem.*, 248: 507-515; Campanella *et al.*,(2003) *J. Biol. Chem.*, 278: 17066-17074). It is understood that inhibition of binding to a chemokine receptor can be measured by assaying for inhibition of one or more activities of a receptor, *e.g.*, binding to a receptor and/or a signaling activity of the receptor.

[0077] The term "inhibiting binding to a glycosaminoglycan," as used herein, refers to the ability of a compound, *e.g.*, antibody or antigen-binding portion thereof, or a small molecule, identified using the methods of the invention, to inhibit or decrease the binding of a chemokine (*e.g.*, an oligomeric form of a chemokine) to a glycosaminoglycan (GAG). In some embodiments, such a compound, *e.g.*, antibody or antigen-binding portion thereof, or a small molecule, inhibits the binding of an oligomeric form of a chemokine to a GAG by binding to the chemokine in a manner which prevents it from binding to a GAG, *e.g.*, on the surface of a target cell. In another embodiment, a compound, *e.g.*, antibody or antigen-binding portion thereof, or a small molecule, identified using the methods of the invention, inhibits the binding of an oligomeric form of a chemokine to a GAG by sequestering the oligomeric form away from the GAG. In yet another embodiment, a compound, *e.g.*, antibody or antigen-binding portion thereof, or a small molecule, inhibits the binding of an oligomeric form of a chemokine to GAG by increasing the affinity of the oligomeric form of the chemokine for its receptor, *e.g.*, in a case where both receptor and GAG are present on a

target cell, thereby decreasing the concentration of available oligomeric form of chemokine to bind to a GAG. Inhibition of the binding of a chemokine to a GAG using a compound, *e.g.*, antibody or an antigen-binding portion thereof, or a small molecule, identified using the methods of the invention, can be readily measured using one or more assays well known in the art and those described herein. For example, the assays disclosed in Kuschert *et al.*, (1999) *Biochemistry* 38: 12959-68; McCornack *et al.*, (2004) *Biochemistry* 43: 10090-10101; and Bryan *et al.*, (2003) *The Embo J.* 22, 833–846, may be used.

II. Methods for Identifying Compounds that Bind an Oligomeric Form of a Chemokine

[0078] Methods of the invention can be used for screening for and/or identifying compounds, *e.g.*, antibodies, or antigen-binding portions thereof, or small molecules, that selectively bind oligomeric forms and not monomeric forms of chemokines. These methods are described in more detail below.

[0079] In one aspect, a compound identified using the methods of the invention is, for example, an antibody or an antigen-binding portion thereof.

[0080] Antibodies encompassed by the present invention include both monoclonal and polyclonal antibodies. Monoclonal antibodies to chemokines can be produced by generation of antibody-producing hybridomas in accordance with known methods (*see, e.g., Goding,* (1983) *Monoclonal antibodies: principles and practice.* Academic Press Inc., New York; *Yokoyama,* (1992) "Production of Monoclonal Antibodies" in *Current Protocols in Immunology*. Greene Publishing Assoc. and John Wiley & Sons). Polyclonal antibodies to chemokines can be produced by inoculation of a mammalian subject with chemokines or fragments thereof in accordance with known methods. Chimeric antibodies may also be produced in accordance with known methods.

[0081] Portions of antibodies can be obtained *via* chemical or enzymatic treatment of an intact or complete antibody or antibody chain. Portions can also be obtained by recombinant means.

[0082] In another aspect of the present invention, a compound identified using the methods of the invention is, for example, a small molecule. Exemplary small molecules include, but are not limited to, peptides, peptidomimetics, amino acids, amino acid analogs, polynucleotides, polynucleotide analogs, nucleotides, nucleotide analogs, organic or inorganic compounds (*i.e*,. including heteroorganic and organometallic compounds) having a molecular weight less than about 10,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 5,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 1,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 500 grams per mole, and salts, esters, and other pharmaceutically acceptable forms of such compounds.

[0083] A small molecule can be obtained using any of the numerous approaches in combinatorial library methods known in the art, including: biological libraries; spatially addressable parallel solid phase or solution phase libraries; synthetic library methods requiring deconvolution; the "one-bead one-compound" library method; and synthetic library methods using affinity chromatography selection. The biological library approach is limited to peptide libraries, while the other four approaches are applicable to peptide, non-peptide oligomer or libraries of small molecule compounds (Lam, K.S. (1997) *Anticancer Drug Des.* 12:145). Examples of methods for the synthesis of molecular libraries can be found in the art, for example in: DeWitt *et al.* (1993) *Proc. Natl. Acad. Sci. U.S.A.* 90:6909; Erb *et al.* (1994) *Proc. Natl. Acad. Sci. USA* 91:11422;

Zuckermann *et al.* (1994). *J. Med. Chem.* 37:2678; Cho *et al.* (1993) *Science* 261:1303; Carrell *et al.* (1994) *Angew. Chem. Int. Ed. Engl.* 33:2059; Carell *et al.* (1994) *Angew. Chem. Int. Ed. Engl.* 33:2061; and in Gallop *et al.* (1994) *J. Med. Chem.* 37:1233.

[0084] Libraries of compounds may be presented in solution (*e.g.*, Houghten (1992) *Biotechniques* 13:412-421), or on beads (Lam (1991) *Nature* 354:82-84), chips (Fodor (1993) *Nature* 364:555-556), bacteria (Ladner USP 5,223,409), spores (Ladner USP '409), plasmids (Cull *et al.* (1992) *Proc Natl Acad Sci USA* 89:1865-1869) or on phage (Scott and Smith (1990) *Science* 249:386-390); (Devlin (1990) *Science* 249:404-406); (Cwirla *et al.* (1990) *Proc. Natl. Acad. Sci.* 87:6378-6382); (Felici (1991) *J. Mol. Biol.* 222:301-310); (Ladner *supra.*).

(a) **Selection of Compounds Using a Peptide Including a GAG-binding Site**

[0085] In one aspect, the present invention provides a method of identifying a compound, *e.g.*, antibody, or an antigen-binding portion thereof, or a small molecule, which selectively binds to an oligomeric form, and not to a monomeric form, of a chemokine by: (a) contacting a compound, *e.g.*, antibody, or antigen-binding portion thereof, or a small molecule, with a peptide including a glycosaminoglycan binding site of a chemokine; (b) determining whether the compound, *e.g.*, antibody, or an antigen-binding portion thereof, or a small molecule, binds the peptide; and (c) identifying a compound, *e.g.*, antibody, or antigen-binding portion, or a small molecule, which selectively binds to an oligomeric form, and not to a monomeric form, of the chemokine based on the ability of the compound, *e.g.*, antibody, or an antigen-binding portion thereof, or a small molecule to bind the peptide.

[0086] A peptide containing a GAG binding site may either be derived from a naturally occurring chemokine (*e.g.*, by enzymatic cleavage of the chemokine) or may be

designed based on a glycosaminoglycan binding site of a chemokine and synthetically produced. Synthetic peptides may be prepared by standard techniques known in the art such as those described in Bodansky, M. *Principles of Peptide Synthesis*, Springer Verlag, Berlin (1993) and Grant, G.A (ed.). *Synthetic Peptides: A User's Guide*, W.H. Freeman and Company, New York (1992). Automated peptide synthesizers are commercially available (*e.g.*, Advanced ChemTech Model 396; Milligen/ Biosearch 9600).

[0087] The GAG binding site on a chemokine can be readily identified using one or more methods described herein and those known in the art. For example, it is known that GAGs (*e.g.*, heparin), which are negatively charged, interact with the most positively charged residues on a chemokine, which are typically found in the first β-strand of most chemokines and constitute a typical BBXB heparin-binding motif, where B is a basic amino acid residue (Sadir *et al.*, (2001) *J. Biol. Chem.* 276: 8288-8296, the entire contents of which are incorporated by reference herein). Therefore, a GAG-binding site of a chemokine can be predicted based on the sequence analysis of the chemokine. Further, validation of such binding sites can be carried out by, for example, mutagenesis techniques which are well known in the art.

[0088] GAG-binding sites on chemokines may also be identified, for example, on the basis of sequence alignments between the polypeptide sequence of a known chemokine, whose GAG-binding site has been identified, and that of a putative chemokine, using alignment programs such as the programs GAP or BESTFIT and default gap weights. Peptides comprising an amino acid sequence substantially identical to a known GAG binding site may also be used. The term "substantial identity" means that two polypeptide sequences, when optimally aligned, such as by the programs GAP or

BESTFIT using default gap weights, share at least 80-90% sequence identity, preferably at least 90-95% sequence identity, and more preferably at least 95% sequence identity or more (*e.g.*, 99% sequence identity or more).

[0089] Optimal alignment of sequences for comparison can be conducted, *e.g.*, by the local homology algorithm of Smith & Waterman, (1981) *Adv. Appl. Math.* 2:482, by the homology alignment algorithm of Needleman & Wunsch, (1970) *J. Mol. Biol.* 48:443, by the search for similarity method of Pearson & Lipman, (1988) *Proc. Nat'l. Acad. Sci. USA* 85:2444, by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, WI), or by visual inspection (*see generally* Ausubel *et al., Current Protocols in Molecular Biology*). One example of algorithm that is suitable for determining percent sequence identity and sequence similarity is the BLAST algorithm, which is described in Altschul *et al.*, (1990) *J. Mol. Biol.* 215:403. Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information (publicly accessible through the National Institutes of Health NCBI internet server). Typically, default program parameters can be used to perform the sequence comparison, although customized parameters can also be used. For amino acid sequences, the BLASTP program uses as defaults a wordlength (W) of 3, an expectation (E) of 10, and the BLOSUM62 scoring matrix (*see Henikoff & Henikoff,* (1989) *Proc. Natl. Acad. Sci. USA* 89:10915).

[0090] A GAG-binding site of a chemokine may also be identified on the basis of a structural analysis of the chemokine using techniques that are well known in the art and those described herein. For example, NMR studies of a GAG-chemokine complex would be expected to provide an indication of the GAG binding site on the chemokine,

as the residues whose resonances shift in the presence of the GAG's are likely involved in GAG binding. Such NMR techniques are described in, for example, McCormack *et al.* (2003) *J. Biol. Chem* 278: 1946-56.

[0091] Additionally, a GAG binding site may be identified by epitope mapping experiments, such as those described in the Examples below. For example, various peptidic fragments of a chemokine can be obtained, *e.g.*, by enzymatic cleavage, and the peptidic fragments can be tested for interaction with a GAG using one or more assays described herein and well known in the art. For example, commercially available affinity columns including a GAG, for example, heparin or heparin sulfate, may be used to determine whether a particular peptide, either derived from a chemokine by, for example, enzymatic cleavage, or a synthetic peptide designed based on a chemokine sequence binds the GAG on the column. It is understood that any assay well known in the art and those described herein, suitable for investigating an interaction between a GAG and a peptide may be used in the methods of the invention. Exemplary assays that may be used are described in, *e.g.*, McCornack *et al.*, (2003) *J. Biol. Chem.* 278: 1946-1956; and Kuscher *et al.*, (1999) *Biochem.* 38: 12959-12968.

[0092] In some embodiments, a peptide comprising a GAG binding site is derived from the chemokine SDF-1α, and may include amino acid residues 20-31, 20-33 or 24-27 of SDF-1α. A peptide of any length may be used in the methods of the invention, however, typically such a peptide is at least one, two, three, four, five, six, seven, eight, nine, or ten or more amino acids shorter than the full-length sequence of a chemokine monomer.

[0093] Once a peptide containing a GAG-binding site is generated, it may be used in the methods of the invention to identify a compound, *e.g.*, antibody, or an antigen-binding

portion thereof, or a small molecule which selectively binds to an oligomeric form, but not to a monomeric form, of a chemokine.

[0094] The peptide containing a GAG-binding site may be immobilized onto an affinity column or a chip and a compound, *e.g.*, antibodies and/or antigen-binding portions thereof, or small molecules, may be flowed across the column or chip. Alternatively, a compound, *e.g.*, antibody or an antigen-binding portion thereof, may be immobilized onto a chip (*e.g.*, an anti-CH1 chip) or an affinity column, and a peptide containing a GAG-binding site may be flowed across the column or chip. Suitable solid surfaces, *e.g.*, columns or chips, for use in the methods of the invention include, for example, glasses, ceramics, plastics, metals, alloys, carbon, papers, agarose, silica, quartz, cellulose, polyacrylamide, polyamide, and gelatin, as well as other polymer supports, other solid-material supports, or flexible membrane supports. Polymers that may be used as substrates include, but are not limited to: polystyrene; poly(tetra)fluoroethylene (PTFE); polyvinylidenedifluoride; polycarbonate; polymethylmethacrylate; polyvinylethylene; polyethyleneimine; polyoxymethylene (POM); polyvinylphenol; polylactides; polymethacrylimide (PMI); polyalkenesulfone (PAS); polypropylene; polyethylene; polyhydroxyethylmethacrylate (HEMA); polydimethylsiloxane; polyacrylamide; polyimide; and various block co-polymers. The substrate can also comprise a combination of materials, whether water-permeable or not, in multi-layer configurations.

[0095] A variety of methods are known in the art for attaching molecules to solid supports. See, generally, Affinity Techniques, Enzyme Purification: Part B, Meth. Enz. 34 (ed. W. B. Jakoby and M. Wilchek, Acad. Press, N.Y. 1974) and Immobilized Biochemicals and Affinity Chromatography, *Adv. Exp. Med. Biol.* 42 (ed. R. Dunlap, Plenum Press, N.Y. 1974). Arenkov *et al.*, for example, have described a way to

immobilize proteins while preserving their function by using microfabricated polyacrylamide gel pads to capture proteins, and then accelerating diffusion through the matrix by microelectrophoresis (Arenkov *et al.* (2000), *Anal Biochem* 278(2):123-31). The patent literature also describes a number of different methods for attaching molecules to solid supports. For example, U.S. Patent No. 4,282,287 describes a method for modifying a polymer surface through the successive application of multiple layers of biotin, avidin, and extenders. U.S. Patent No. 4,562,157 describes a technique for attaching biochemical ligands to surfaces by attachment to a photochemically reactive arylazide. U.S. Patent No. 4,681,870 describes a method for introducing free amino or carboxyl groups onto a silica matrix, in which the groups may subsequently be covalently linked to a protein in the presence of a carbodiimide. In addition, U.S. Patent No. 4,762,881 describes a method for attaching a polypeptide chain to a solid substrate by incorporating a light-sensitive unnatural amino acid group into the polypeptide chain and exposing the product to low-energy ultraviolet light.

[0096] The compound, *e.g.*, an antibody or antigen-binding portion thereof, may be coated onto the solid surface at a coating density of about 10,000-50,000 RU, preferably 10,000-15,000 RU or 10,000-20,000 RU. The peptide containing a GAG binding site may be coated onto the solid surface at a coating density of about ,800-1,000 RU.

[0097] Compounds, *e.g.*, antibodies or antigen-binding portions thereof can be tested for binding to a peptide including a glycosaminoglycan binding site by, for example, standard assays known in the art, such as ELISA, FACS analysis and/or Biacore analysis. In a typical ELISA assay, microtiter plates are coated with a peptide containing a GAG binding site in PBS, and then blocked with 5% bovine serum albumin in PBS. Dilutions of antibody or antigen-binding portions thereof are added to each well and incubated for

1-2 hours at 37°C. The plates are washed with PBS/Tween and then incubated with a secondary reagent (*e.g.*, anti-species secondary antibody) conjugated to alkaline phosphatase for 1 hour at 37°C. After washing, the plates are developed with pNPP substrate (1 mg/ml), and analyzed at OD of 405-650 nm.

[0098] Determining the ability of a compound, *e.g.*, antibody or antigen-binding portion thereof, to bind to a peptide including a glycosaminoglycan binding site can also be accomplished using a technology such as real-time Biomolecular Interaction Analysis (BIA). Sjolander, S. and Urbaniczky, C. (1991) *Anal. Chem.* 63:2338-2345 and Szabo *et al.* (1995) *Curr. Opin. Struct. Biol.* 5:699-705. As used herein, "BIA" is a technology for studying biospecific interactions in real time, without labeling any of the interactants (*e.g.*, BIAcore™). Changes in the optical phenomenon of surface plasmon resonance (SPR) can be used as an indication of real-time reactions between biological molecules.

[0099] Compounds, *e.g.*, antibodies or antigen-binding portions thereof, can be further tested for reactivity with a peptide including a glycosaminoglycan binding site by, for example, Western blotting. For example, a peptide including a GAG binding site or a putative GAG binding site can be subjected to sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis. After electrophoresis, the separated peptides are transferred to nitrocellulose membranes, blocked with 20% mouse serum, and probed with the antibodies or antigen-binding portions to be tested. Antibody binding can be detected using anti-species specific secondary antibody linked to alkaline phosphatase and developed with BCIP/NBT substrate tablets (Sigma Chem. Co., St. Louis, MO). Other techniques for evaluating the ability of antibodies to bind a peptide including a GAG binding site are well known in the art.

[00100] Compounds, *e.g.*, antibodies or antigen-binding portions thereof, or small molecules, identified as binding to a peptide containing a GAG binding site and, thereby as binding to an oligomeric form of a chemokine, can be further tested using functional assays, *e.g.*, to investigate the effect of the antibody on chemokine activity (*e.g.*, an activity mediated by an oligomeric form of a chemokine). For example, chemokine activity can be determined by detecting induction of a cellular second messenger of a chemokine receptor (*e.g.*, intracellular Ca^{2+}, diacylglycerol, IP_3 and the like). Such suitable assays are described in, for example (Koopmann *et al.*, (1997) *J. Biol. Chem.*, 272:10103-09).

[00101] In some embodiments, antibodies or antigen-binding portions identified using the methods of the present invention exhibit one or more of the following characteristics: they bind to a chemokine (*e.g.*, SDF-1α) with a K_D of 1 X 10^{-7} M or less; they bind to a chemokine (*e.g.*, SDF-1α) in an immunoprecipitation assay; they block binding of a chemokine (*e.g.*, SDF-1α) to cells (*e.g.*, CEM cells); they block chemokine induced calcium flux; they block chemokine induced migration of cells (*e.g.*, CEM cells); and/or they block capillary tube formation in HuVEC cells.

(b) Selection of Compounds Using an Agent which favors Chemokine Oligomerization

[00102] In yet another aspect, the present invention provides a method of identifying an compound, *e.g.*, antibody, or an antigen-binding portion thereof, or a small molecule, which selectively binds to an oligomeric form, and not to a monomeric form, of a chemokine by: (a) contacting a compound, *e.g.*, antibody, or an antigen-binding portion thereof, or a small molecule, with a chemokine in the presence of a cation, where the cation is present at a concentration sufficient to promote and/or stabilize chemokine olgomerization; (b) determining whether the compound, *e.g.*,

antibody, or an antigen-binding portion thereof, or a small molecule, binds the chemokine; and (c) identifying a compound, *e.g.*, antibody, or antigen-binding portion thereof, or a small molecule, which selectively binds to an oligomeric form, and not to a monomeric form, of a chemokine based on the ability of the compound, *e.g.*, antibody, or an antigen-binding portion thereof, or a small molecule, to bind the chemokine.

[00103] The phrase "cation is present at a concentration sufficient to promote and/or stabilize chemokine oligomerization," as used herein, includes contacting compound, *e.g.*, antibody or antigen-binding portion thereof, or a small molecule, with a chemokine in the presence of a positively charged ion, where the positively charged ion is present at a concentration which favors chemokine oligomerization. A concentration of a cation sufficient to promote and/or stabilize chemokine oligomerization is any amount of a cation, in the presence of which chemokine oligomerization is promoted and/or an amount of a cation that prevents or slows down a shift towards a monomeric form of a chemokine, thereby stabilizing an oligomeric form of the chemokine. Any cation may be used in the methods of the invention, so long as it promotes oligomerization of a chemokine and/or prevents or slows down over time, a shift towards a monomeric form of the chemokine, thereby stabilizing an oligomeric form of a chemokine. Such a cation may be for example, a multivalent cation such as, for example, a trivalent cation or a divalent cation. In an exemplary embodiment, a cation useful in the methods of the invention is a divalent cation, for example, Ca^{2+}, Mg^{2+}, Sr^{2+}, Ba^{2+}, Mn^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+} and Cd^{2+}. Alternatively, a cation may be a monovalent cation, *e.g.*, K^{+}. A concentration of a cation sufficient to stabilize an oligomeric form of a chemokine can be at least 1 mM, 1.1 mM, 1.2 mM, 1.3 mM, 1.4 mM, 1.5 mM. 1.6 mM. 1.7 mM, 1.8 mM, 1.9 mM or 2 mM. In a particular embodiment,

a concentration of a cation sufficient to stabilize an oligomeric form of a cation is at least 1 mM.

[00104] Identification of a cation which promotes and/or stabilizes chemokine oligomerization can be made, for example, by assaying for chemokine oligomerization in the presence and in the absence of the cation. Similarly, determination of a concentration of a cation which promotes and/or stabilizes chemokine oligomerization can be made, for example, by assaying for chemokine oligomerization at varying concentrations of a cation.

[00105] In some embodiments, a decrease and/or absence of a cation is achieved by adding a chelating agent. Accordingly, the term "decrease in concentration of a cation," as used herein, includes a complete absence of a cation or the presence of a cation at a concentration which is not sufficient to stabilize an oligomeric form of a chemokine. A decrease in the concentration of a cation may be achieved, for example, by specifically excluding a cation from the conditions under which a compound, *e.g.*, antibody, or antibody-portion thereof, or a small molecule, is contacted with a chemokine. Alternatively, such a decrease can be achieved by adding a chelating agent or chelator (*e.g.*, EDTA).

[00106] The terms "chelating agent" and "chelator," as used interchangeably herein, are well known in the art and refer to any substance which can form one or more bonds to a single metal ion. Exemplary chelating agents include, but are not limited to, EDTA (ethylenediamine tetraacetate), porphine, DTPA (Diethylenetriaminepentaacetic acid), Dimercaprol (2,3-dimercapto-1-propanol) and NTA (N,N-bis(carboxymethyl)glycine; Triglycollamic acid), DETA (diethylenetriamine) and AEEA (aminoethylethaloamine).

[00107] Chemokine oligomerization in the presence of a compound which facilitates oligomerization such as, for example, a cation, can be measured using one or more assays known in the art and/or described herein. For example, oligomerization of a chemokine can be detected based on any one of the following: a determination of a molar mass of a putative oligomer (see., *e.g.*, Williams *et al.*, (2005) *Shock*, 23: 371-376), by chromatography (see., *e.g.*, Hoogewerf et al., (1997) Biochemistry, 36: 13570-13578); or by assaying for a chemokine monomer-dimer equilibrium (see, *e.g.*, Veldkamp *et al.*, (2005) *Protein Science* 14: 1071-1081).

[00108] Binding of a compound, *e.g.*, antibody or antigen-binding portion thereof, or a small molecule, to a chemokine in the presence of a cation at a concentration sufficient to promote and/or stabilize oligomerization of a chemokine, can be measured as described in section II(a) above.

[00109] In a further aspect, the present invention provides a method for identifying a compound which selectively binds to an oligomeric form, and not to a monomeric form, of a chemokine by: (a) contacting a compound, *e.g.*, antibody or antigen-binding portion thereof, or a small molecule, with a monomeric form of a chemokine; (b) contacting the compound, *e.g.*, antibody or antigen-binding portion thereof, or a small molecule, with an oligomeric form of a chemokine; (c) determining whether the compound binds to the monomeric form of the chemokine or the oligomeric form of the chemokine; and (d) identifying a compound, *e.g.*, antibody or antigen-binding portion thereof, or a small molecule, which selectively binds to an oligomeric form based on the ability of the compound to bind to the oligomeric form and not to the monomeric form, of the chemokine.

[00110] Any agent and/or condition which promotes chemokine oligomerization may be used for obtaining an oligomeric form of a chemokine. Exemplary agents include those described herein, for example, a cation, and those known in the art as promoting chemokine oligomerization, *e.g.*, non-acidic pH, phosphate, sulfate and heparin (see, *e.g.*, Veldkamp *et al.* (2005) *Protein Science* 14: 1071-81). Alternatively, amino acid residues that favor oligomerization may be introduced in a chemokine molecule, thereby promoting the formation of an oligomeric form of a chemokine.

[00111] In another embodiment, a monomeric form of a chemokine may be obtained by mutating one or more amino acid residues at or near, for example, the dimer interface of a chemokine, to thereby prevent the chemokine from oligomerizing.

[00112] Methods for the identification of a monomeric form of a chemokine and an oligomeric form of a chemokine are well known in the art and include those that are described herein.

III. Methods of Using Compounds Identified Using the Methods of the Invention

[00113] The present invention also provides methods of using compounds, *e.g.*, antibodies and antigen-binding portions thereof, or small molecules, identified using the methods of the invention.

[00114] In light of their diverse biological activities, it is not surprising that chemokines have been implicated in a number of physiological and disease conditions, including lymphocyte trafficking, wound healing, hematopoietic regulation and immunological disorders such as allergy, asthma, multiple sclerosis, inflammatory bowel disease and arthritis. Oligomeric forms of chemokines are believed to play an important role in many disease states. Accordingly, once a compound, *e.g.*, antibody or antigen-binding portion thereof, that selectively binds an oligomeric form, and not to a

monomeric form, of a chemokine is identified, such a compound, *e.g*, antibody or antigen-binding portion can be used for treating, diagnosing or prognosing a chemokine-mediated disorder.

[00115] The term "chemokine-mediated disorder," as used herein, includes disease states and/or symptoms associated with a disease state, where increased levels of one or more chemokines are found. It is understood that oligomerization of chemokines occurs when increased levels of chemokines are found. Accordingly, the term "chemokine-mediated disorder," also refers to disease states and/or symptoms associated with disease states where chemokine oligomerization occurs. In general, the term "chemokine-mediated disorder" refers to any disorder, the onset, progression or the persistence of the symptoms of which requires the participation of a chemokine molecule, in particular, an oligomeric form of a chemokine. Exemplary chemokine-mediated disorders include, but are not limited to, for example, inflammatory disorders, immune disorders and angiogenic disorders such as, for example, chronic inflammation, chronic obstructive pulmonary disease (COPD), acute respiratory distress syndrome (ARDS), inflammatory bowel disease, tuberculoid leprosy, encephalomyelitis, artherosclerosis, uveoretinitis, osteoarthritis, asthma, dermatitis, eczema, multiple sclerosis, HIV infection, psoriasis, allograft rejection, tumor-specific immunity, immune complex glomerulonephritis, lupus and angiogenesis. Additional chemokine-mediated disorders are those included in Table II.

[00116] The term "inflammatory disorder" includes any disease or a symptom associated with a disease, which is characterized by, caused by, results from or becomes affected by inflammation and, in particular, inflammation resulting from increased levels of one or more chemokines. The inflammation may be local or systemic.

[00117] The term "immune disorder" includes any disease or a symptom associated with a disease, which affects or is mediated by one or more cell types of the immune system and is associated with increased levels of one or more chemokines.

[00118] The term "angiogenic disorder," includes a disease, disorder or condition characterized or caused by aberrant or unwanted, *e.g.*, stimulated or suppressed, formation of blood vessels (angiogenesis). Exemplary angiogenic disorders includes neoplasia and cancer.

[00119] The term "treatment" or "treat" or "treating" refers to effective inhibition, suppression or cessation of a chemokine activity so as to prevent or delay the onset, retard the progression or ameliorate the symptoms of a chemokine-mediated disorder.

[00120] As used herein, the term "subject" includes warm-blooded animals, preferably mammals, including humans.

[00121] As used herein, the term "administering" to a subject includes dispensing, delivering or applying a compound, *e.g.*, antibody or antigen-binding portion thereof, or a small molecule, identified using the methods of the invention, to a subject by any suitable route of delivery. In some embodiment, an effective amount of a compound, *e.g.*, antibody or antigen-binding portion thereof, identified using the methods of the invention is administered locally, *e.g.*, to a site of inflammation in a subject.

[00122] The term "effective amount," as used herein, refers to that amount of a compound, *e.g.*, antibody or an antigen-binding portion thereof, or a small molecule, identified using the methods of the invention which is sufficient to effect treatment, prognosis or diagnosis of a chemokine-mediated disorder, as described herein, when administered to a subject. A therapeutically effective amount will vary depending upon

the subject and disease condition being treated, the weight and age of the subject, the severity of the disease condition, the manner of administration and the like, which can readily be determined by one of ordinary skill in the art. The dosages for administration can range from, for example, about 1 ng to about 10,000 mg, about 5 ng to about 9,500 mg, about 10 ng to about 9,000 mg, about 20 ng to about 8,500 mg, about 30 ng to about 7,500 mg, about 40 ng to about 7,000 mg, about 50 ng to about 6,500 mg, about 100 ng to about 6,000 mg, about 200 ng to about 5,500 mg, about 300 ng to about 5,000 mg, about 400 ng to about 4,500 mg, about 500 ng to about 4,000 mg, about 1 μg to about 3,500 mg, about 5 μg to about 3,000 mg, about 10 μg to about 2,600 mg, about 20 μg to about 2,575 mg, about 30 μg to about 2,550 mg, about 40 μg to about 2,500 mg, about 50 μg to about 2,475 mg, about 100 μg to about 2,450 mg, about 200 μg to about 2,425 mg, about 300 μg to about 2,000, about 400 μg to about 1,175 mg, about 500 μg to about 1,150 mg, about 0.5 mg to about 1,125 mg, about 1 mg to about 1,100 mg, about 1.25 mg to about 1,075 mg, about 1.5 mg to about 1,050 mg, about 2.0 mg to about 1,025 mg, about 2.5 mg to about 1,000 mg, about 3.0 mg to about 975 mg, about 3.5 mg to about 950 mg, about 4.0 mg to about 925 mg, about 4.5 mg to about 900 mg, about 5 mg to about 875 mg, about 10 mg to about 850 mg, about 20 mg to about 825 mg, about 30 mg to about 800 mg, about 40 mg to about 775 mg, about 50 mg to about 750 mg, about 100 mg to about 725 mg, about 200 mg to about 700 mg, about 300 mg to about 675 mg, about 400 mg to about 650 mg, about 500 mg, or about 525 mg to about 625 mg, of an antibody or antigen-binding portion thereof, identified using the methods of the invention. Dosage regiments may be adjusted to provide the optimum therapeutic response. An effective amount is also one in which any toxic or detrimental effects (*i.e.*, side effects) of a compound, *e.g.*, an antibody or antigen-binding portion thereof, or a small molecule, are minimized and/or outweighed by the beneficial effects.

[00123] As used herein "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like that are physiologically compatible. In one embodiment, the carrier is suitable for parenteral administration. Alternatively, the carrier can be suitable for intravenous, intraperitoneal or intramuscular administration. In another embodiment, the carrier is suitable for oral administration. Pharmaceutically acceptable carriers include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. Sterile injectable solutions can be prepared by incorporating a compound, *e.g.*, antibody or an antigen-binding portion thereof, identified using the methods of the invention in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization.

[00124] The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the antibody or antigen-binding portion thereof, or a small molecule, identified using the methods of the invention, use thereof in the pharmaceutical compositions of the invention is contemplated.

[00125] Supplementary compounds can also be incorporated into the compositions, for example, compounds which act additively or synergistically with compounds, *e.g.*, antibodies or antigen-binding portions thereof, or small molecules, identified using the methods of the invention, to modulate activity of an oligomeric form of a chemokine.

[00126] One factor that may be considered when determining a therapeutically or prophylactically effective amount of a compound, *e.g.*, antibody or an antigen-binding

portion thereof, or a small molecule, is the concentration of a chemokine, *e.g.*, at the site of an inflammatory response, in a subject. Due to a finding of chemokine oligomerization in the presence of divalent cations, *e.g.*, Ca^{2+}, discussed in more details in the Examples *infra*, in some embodiments, an amount of Ca^{2+} is measured, *e.g.*, at a site of an inflammation, to predict the amount of an oligomeric form of a chemokine that may be present at the site. Also, therapeutically effective amount of a compound, *e.g.*, antibody or an antigen-binding portion thereof, or a small molecule, may be determined based the stoichiometry of the interaction between a compound, *e.g.*, antibody or antigen-binding portion thereof, or a small molecule, and a chemokine. For example, as discussed in the Examples *infra*, based on stoichiometry analysis, it was determined that about 1 molecule of an antibody or antigen-binding portion thereof, binds about 1.75 molecules of a chemokine (*e.g.*, SDF-1α). Accordingly, a therapeutically effective amount of an antibody or antigen-binding portion thereof, which binds an oligomeric form of the chemokine SDF-1α, can be determined from the stoichiometry analysis. A non-limiting range for a therapeutically or prophylactically effective amounts of an antibody or antigen-binding portion thereof, identified using the methods of the invention is, for example, 0.01 nM-10 μM or 0.01 mg/kg to about 100 mg/kg body weight per treatment.

[00127] In some embodiments, compounds, *e.g.*, antibodies or antigen-binding portions thereof, or small molecules, identified using methods of the invention, are used for detecting oligomeric forms of a chemokine, *e.g.*, at sites of inflammation and tumor growth. Accordingly, the present invention also provides a method of diagnosing a chemokine-mediated disorder in a subject in need thereof by: contacting a biological sample with a compound identified using the methods of the invention; and detecting an oligomeric form of a chemokine in the biological sample, wherein the subject is

diagnosed with a chemokine mediated disorder based on the amount of an oligomeric form of a chemokine present in the biological sample. A subject may be diagnosed with a chemokine-mediated disorder if an amount of an oligomeric chemokine present in the biological sample is at least 2-fold, or 5-fold, or 10-fold, or 15-fold, or 20-fold, or 25-fold, or 30-fold or more higher than the amount present in a control biological sample.

[00128] The term "biological sample" is intended to include tissues, cells and biological fluids isolated from a subject, as well as tissues, cells and fluids present within a subject. That is, the detection method of the invention can be used to detect an oligomeric form of a chemokine in a biological sample *in vitro* as well as *in vivo*. In some embodiments, an antibody or antigen binding portion thereof, identified using the methods of the invention is introduced into a subject. For example, the antibody can be labeled with a radioactive marker whose presence and location in a subject can be detected by standard imaging techniques.

[00129] Compounds, *e.g.*, antibodies or antigen-binding portions, or small molecules, identified using the methods of the invention may be labeled with a detectable substance using well known techniques. Suitable detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials and radioactive materials. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, β-galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; and examples of suitable radioactive material include ^{14}C, ^{123}I, ^{124}I, ^{125}I, ^{131}I, ^{99m}Tc, ^{35}S or ^{3}H.

[00130] In some embodiments, a compound identified using the methods of the invention, binds to the same site on a chemokine as a GAG and, consequently, blocks the binding of a GAG to the chemokine. In another embodiment, a compound identified using the methods of the invention binds to a site on a chemokine which is opposite from the site where the GAG binds and consequently, does not block the binding of a GAG to the chemokine. For example, such a compound may recognize the dimer/oligomer interface of the chemokine from the direction where the C-terminal helices of both monomers stack against each other and where GAG does not bind.

[00131] Accordingly, in some embodiments, a method of the invention includes inhibiting the binding of an oligomeric form of a chemokine to its cognate receptor by a compound, *e.g.*, antibody or antigen-binding portion thereof, or a small molecule, identified using the methods of the invention. The method includes contacting a chemokine with an effective amount of a compound, *e.g.*, antibody or antigen-binding portion thereof, or a small molecule, identified using the methods of the invention, thereby inhibiting the binding of an oligomeric form of the chemokine to its cognate receptor.

[00132] Without wishing to be bound by theory, it is believed that compounds, *e.g.*, antibodies and antigen-binding portions thereof, or small molecules, identified using the methods of the invention, which inhibit binding of a chemokine oligomeric form to a receptor may have at least one or more of the following properties: modulating (*e.g.*, increasing or decreasing) of at least one activity or function characteristic of a chemokine receptor such as, for example, a binding activity or a signaling activity (*e.g.*, activation of a mammalian G protein, induction of rapid and transient increase in the concentration of cytosolic free calcium); and modulation of a cellular response function

(*e.g.*, stimulation of chemotaxis, exocytosis or inflammatory mediator release by leukocytes). Modulation of a chemokine receptor activity, *e.g.*, a binding activity, can be detected and/or measured using one or more methods known in the art and those described herein such as, for example, using membrane fractions containing receptor or cells expressing receptors. Modulation of a signaling activity can be detected using one or more methods known in the art and those described herein such as, for example, enzymatic assays for G protein activity responsive to receptor binding (*e.g.*, exchange of GTP for GDP on the G protein-α subunit, using membrane fractions) (see, *e.g.*, Coulin *et al.*, (1997) *Eur. J. Biochem.*, 248: 507-515; Campanella *et al.*,(2003) *J. Biol. Chem.*, 278: 17066-17074).

[00133] Additional methods of using a compound, *e.g.*, antibody or antigen-binding portions, or small molecules, identified using the methods of the invention include methods of inhibiting binding of an oligomeric form of a chemokine to a GAG, *e.g.*, on the surface of a target cell. In some embodiments, such methods include contacting a chemokine with an effective amount of a compound, *e.g.*, antibody or antigen-binding portion thereof, or a small molecule, identified using the methods of the invention, thereby inhibiting binding of the oligomeric form of a chemokine to a GAG. Inhibition of binding to a GAG can be measured by any suitable assays such as those described herein and those known in the art, *e.g.*, *in vitro* heparin binding assays and binding assays using cells expressing GAGs (Bryan *et al.*, (2003) *The Embo J.* 22, 833–846; Proudfeet *et al.*, (2003) *Proc. Natl. Acad. Sci* 100: 1885–1890; and Lau *et al.* (2004) *J. Biol. Chem.* 279:22294-305).

[00134] Also within the scope of the invention are kits comprising one or more compounds, *e.g.*, antibody or antigen-binding portion thereof, and/or a small molecule,

identified using the methods of the invention and instructions for use. Kits typically include a label indicating the intended use of the contents of the kit. The term label includes any writing, marketing materials or recorded material supplied on or with the kit, or which otherwise accompanies the kit.

[00135] This invention is further illustrated by the following examples which should not be construed as limiting. The contents of all references, patents and published patent applications cited throughout this application, as well as the Figures, are incorporated herein by reference.

Examples

[00136] In the examples that follow, certain antibodies are referred to as B-type antibodies, *e.g.*, 1D3, 1H2 and 2E5, and other antibodies are referred to as Z-type antibodies, *e.g.*, 1C6, 2A5 and 1A7.

Example 1: Characterization of binding of B-type and Z-type Fabs to SDF-1α

[00137] Binding of B-type and Z-type Fabs to a chip containing immobilized chemokine SDF-1α was investigated. In an exemplary experiment, about 10 μg/ml of SDF-1α at a coating density of 984 RUwas immobilized onto a chip. SDF-1α (Peptrotech, Catalog Id: 300-28A) was immobilized to a matrix of carboxyl methylated dextran chip, CM5, obtained from Biacore. SDF-1α was covalently linked to the CM5 chip using amine coupling chemistry. Generally, the carboxyl groups on the matrix are activated using succinimide (NHS) and cabodiimide (EDC) to form active esters, which react with amine groups on SDF-1α, thereby immobilizing it. After coupling, ehthanolamine is added to deactivate the remaining active esters. About 1000 RUs of signal corresponds to about 1ng/mm^2 of coupled protein, which, in turn, corresponds to about 6 mg/mL of bulk concentration. The surface concentration of SDF-1α is very

close to its dimerization constant, *i.e.*, about 150 μM (or about 1mg/ml). Dimerization of SDF-1α, which is concentration dependent, is stabilized through non-covalent interactions. The high pH of the deactivation buffer, ethanolamine, disrupts the non-covalent interactions between surface dimers/oligomers of SDF-1α and only the molecules that are covalently linked to the matrix remain, while the remaining molecules are washed away. This is confirmed from the drop in signal after deactivation. Some SDF-1α molecules that are covalently bound to the matrix may result in oligomers if they are close enough.

[00138] B-type or Z-type Fabs were flowed across this SDF-1α coated chip at a rate of 20μL/minute. While, the Z-type Fabs showed binding to the chemokine SDF-1α immobilized on the chip, the B-type Fabs showed relatively weak binding. The results from one such experiment are depicted in Figure 1A-1F. As demonstrated in Figures 1A-1F, the monomeric form of SDF-1α depicted relatively weak binding to the B-type Fabs compared to the Z-type Fabs.

[00139] The binding of various B-type and Z-type Fabs used is summarized below in Table III.

Table III

Sample	Kd (nM)	k_{off} (s^{-1})	k_{on} (1/Ms)	Rank
1C6	3.6	4.0×10^{-4}	1.1×10^{5}	1
2A5	4.6	4.5×10^{-4}	9.6×10^{4}	2
1A7	6.4	4.2×10^{-4}	6.6×10^{4}	3
1D3	151	1.7×10^{-3}	1.1×10^{4}	1
1H2	176	1.6×10^{-3}	9.1×10^{3}	2
2E5	201	1.6×10^{-3}	8.2×10^{3}	3

[00140] In another exemplary experiment, either B-type or Z-type Fabs were immobilized onto an anti-CH1 chip (chip type CM5) and the chemokine SDF-1α was flowed across the chip. About 10 μg/ml of each Fab was used for coating and the coating density was about 15,000 RU. A 2.5μM solution of SDF-1α was flowed across the chip. Interesting, while the SDF-1α bound to the Fabs if used immediately after the requisite dilution, no binding was observed with B-type Fabs if the chemokine was allowed to reach an equilibrium after dilution. The results of an exemplary experiment are depicted in Figures 2A-2C and 3A-3B and are summarized below in Tables IV and V. As depicted in Figure 2A-2B, while SDF-1α bound to the B-type Fabs when used immediately after the requisite dilution, no binding was observed after SDF-1α was allowed to reach an equilibrium.

[00141] As depicted in Figure 3A, which represents the binding of the Z-type Fab (1C6) to SDF-1α, the binding was comparable to that observed when the SDF-1α was immobilized onto a chip. Figure 3B, represents the binding of the B-type Fab (1H2) to SDF-1α, demonstrating that the B-type Fab showed no binding after SDF-1α was allowed to reach equilibrium.

Table IV

Sample	Kd (nM)	Koff (1/s)	Kon (1/Ms)
1D3	5.8	8.3×10^{-4}	1.4×10^{5}
1H2	6.3	7.3×10^{-4}	1.2×10^{5}
2E5	4.2	5.1×10^{-4}	1.2×10^{5}

[00142] Of all the B-type Fabs tested, the 1H2 antibody appeared to have the weakest binding to SDF-1α.

Table V

Sample	Kd (nM)	Koff (1/s)	Kon (1/Ms)
1C6	1.4	3.9×10^{-4}	2.7×10^{5}
2A5	1.1	4.8×10^{-4}	5.2×10^{5}
1A7	1.9	5.2×10^{-4}	2.7×10^{5}

[00143] The Z-type Fabs demonstrated binding which was comparable to the binding when the SDF-1α was immobilized onto the chip. Of all the Z-type Fabs tested, the 1A7 Fab demonstrated the weakest binding.

[00144] Because SDF-1α is expected to be in monomeric form when bound at low density to the chip, the results of the binding experiments suggest that B-type Fabs bind to the dimeric form of SDF-1α, whereas the Z-type Fabs have no preference.

[00145] In another experiment, a time-course analysis after the requisite dilution was performed for binding of the B-type and Z-type Fabs to SDF-1α. Binding of a fixed concentration (*i.e*,. 100 nM) of SDF-1α to a chip containing either B-type or Z-type Fabs immobilized on the chip was measured over a duration of time. As depicted in Figure 4, while the binding of Z-type Fabs to SDF-1α was substantially unchanged over a duration of time, the binding of B-type Fabs SDF-1 was substantially reduced. Because the equilibrium of SDF-1α is expected to shift from a dimeric state to a monomeric state over time, these results also demonstrate that the B-type Fabs bind the dimeric state of the SDF-1α antigen, while the Z-type Fabs have no preference and bind both the monomeric and the dimeric state of the antigen.

[00146] Stoichiometry of the binding of the Fab molecules to SDF-1α was calculated based on the following equation:

(RUSDF-1α/RUFab) * (M. WtFab/M. WtSDF-1α)

where RU refers to the signal which is directly proportional to the molecular weight (M. Wt) of the analyte or ligand bound to the chip. In an exemplary experiment, B-type Fabs were bound onto an anti-CH1 chip and saturating amounts of SDF-1α were used. As depicted in Figure 5, RUSDF-1α was about 350, RUFab was about 1250; M. WtFab was about 50,000 and M. Wt.SDF-1α was about 8000. Accordingly, based on the above equation, it was determined that about 1.75 molecules of SDF-1α bound per Fab molecule.

Example 2: Characterization of binding of B-type and Z-type IgGs to SDF-1α

[00147] In another experiment, the binding of B-type and Z-type IgGs to SDF-1α was investigated. The B-type or Z-type IgGs were immobilized onto a chip and SDF-1α was flowed across the chip. The results from one such experiment are summarized in Table VI below.

Table VI

MAb	K_D (nM)	k on (s-1)	K off (1/Ms)
1D3	0.63	0.8×10^6	5.0×10^{-4}
1H2	1.32	0.6×10^6	8.4×10^{-4}
2A5	0.37	2.9×10^6	11×10^{-4}
1C6	0.46	1.4×10^6	6.4×10^{-4}

[00148] As shown in Table VI, the B-type IgGs specifically bound to SDF-1α dimers, however Z-type IgGs had no preference.

Example 3: Effect of various factors on the binding of IgGs to SDF-1α

[00149] In another experiment, the effect of various factors on binding of B-type or Z-type IgGs to SDF-1α was investigated.

1. **Effect of EDTA**

[00150] In an exemplary experiment, the effect of EDTA on binding was measured at defined time intervals after dilution of the chemokine to monitor the effects of loss of SDF-1α dimers on binding to B-type IgGs.

[00151] The effect of three separate buffers: HBS-EP; HBS-P and HBS-N on the binding of a B-type IgG (1H2) to SDF-1α was measured. The three buffers were substantially identical except that the buffer HBS-P lacked EDTA and the buffer HBS-N lacked the surfactant, P20. The results from one such experiment are depicted in Figures 6A-6C.

[00152] The binding of the B-type IgGs to SDF-1α was altered in the presence of EDTA (*i.e.*, buffer HBS-EP), as depicted in Figure 6A and 6B. However, in the absence of EDTA (*i.e.*, buffer HBS-N), the binding of the B-type IgG (1H2) was substantially similar to the binding of Z-type IgGs (1C6 and 2A5).

[00153] These results suggest that EDTA alters the monomer-dimer equilibrium of SDF-1α, thereby effecting the binding of the IgGs (in particulat the B-type IgGs) to SDF-1α.

2. **Effect of Heparin**

[00154] In another experiment, the effect of heparin on the binding of IgGs to SDF-1α was investigated. SDF-1α was shown to bind biotinylated heparin immobilized on a streptavidin chip. As shown in Figure 7A, the Kd of such binding was about 81 nM, the on-rate was about 6.7×10^5 and the off-rate was about 5.4×10^{-2} s^{-1}. Additionally, it was demonstrated that the presence of heparin in solution increased the amount of SDF-1α dimer present in solution. As depicted in Figure 7B-7C, the presence of heparin in solution appeared to alter the binding of the B-type IgGs to SDF-1α, whereas, the binding of the Z-type IgGs did not appear to be affected. Therefore, the presence of heparin in solution appeared to increase the amount of SDF-1α dimers, which improved the binding of a B-type IgG to SDF-1α over time (Figure 7B), whereas the binding of a Z-type IgG was not effected (Figure 7C).

3. **Effect of Cations**

[00155] In another experiment, the effect of a divalent cation, for example, Ca^{2+}, on the binding of B-type Fabs to SDF-1α was investigated by Fluorescence Resonance Energy Transfer (FRET). About 50 mM SDF-1α was used in this experiment. The results of one such experiment are depicted in Figure 8A-8C and Figure 9A-9B.

[00156] As depicted in Figure 8A-8C, while FITC labeling of SDF-1α disrupted the binding of the B-type IgGs to SDF-1α over time, the presence of either Ca^{2+} or heparin resulted in rescuing the binding. As shown in Figure 9A, while SDF-1α dimerization of SDF-Dansyl and SDF-FITC decreased over time, such dimerization was not decreased in the presence of Ca^{2+}, as depicted in Figure 9B.

[00157] Figure 10 depicts the results of an experiment designed to investigate the effect of Ca^{2+} on binding of SDF-1α to immobilized B-type IgGs in the presence of heparin. As depicted in Figure 10, when B-type IgGs were immobilized onto a chip and SDF-1α and heparin were flowed across the chip, blocking of binding to B-type IgGs was observed. However, no blocking was observed in the presence of $CaCl_2$.

4. **Effect of pH**

[00158] In another experiment, the effect of pH on the binding of SDF-1α to B-type IgGs was investigated. Either B-type or Z-type IgGs were immobilized (5000 RU) onto a chip and 25 nM SDF-1α was flowed across the chip for approximately 1 minute. Results from an exemplary experiment are depicted in Figure 11A-11B. As depicted in Figure 11A, acidic pH disrupted the binding of B-type IgGs to SDF-1α; however, acidic pH did not appear to have a substantial effect on the binding of Z-type IgGs, as demonstrated by Figure 11B.

[00159] Interesting, the loss of binding at a low pH could be rescued by Ca^{2+}, as shown in Figure 12. Binding of B-type IgGs to SDF-1α was restored upon the addition of about 1 mM $CaCl_2$ at about pH 5.5. These results suggest that the dimeric form of SDF-1α is predominantly present in the presence of sufficient Ca^{2+} ions.

Example 4: The B-type and Z-type IgGs have comparable physical stability

[00160] In order to ascertain that the differences in properties of the B-type and Z-type antibodies were not associated with their respective physical properties, differential scanning calorimetry was used.

[00161] Calorimetric measurements of melting temperatures (T_m) were carried out on a VP-Capillary DSC differential scanning microcalorimeter platform that is combined with an autosampler (MicroCal LLC, Northampton, MA, USA). Sample cell volume was 0.144 mL. Denaturation data of the antibodies was obtained by heating the samples, at a concentration of 2.0μM, from 30 to 95 °C at a rate of 1 °C/min. The protein samples were present in phosphate-buffered saline (PBS) at pH 7.4. The same buffer was used in the reference cell to obtain the molar heat capacity by comparison. The observed thermograms were baseline corrected and normalized data was analyzed based on a 2-step model, using the software Origin v7.0.

[00162] The results of one such experiment are summarized below in Table VII.

Table VII

mAb	T_m1 (°C)	T_m2 (°C)
2A5	68.6	75
1C6	59.6	66.8
1H2	59.9	65.7
1D3	57.8	65.8

[00163] As summarized in Table VII, while 2A5 appeared to have the best physical stability, the other antibodies were comparable.

[00164] In another experiment, the effect of a protein denaturant, guanidine hydrochloride (GdHCl) on the folding of B-type and Z-type antibodies was

measured. The results of one such experiment are summarized below in Table VIII and depicted in Figure 13A-13B.

Table VIII

mAb	Unfolding Midpoint (M)
2A5	2.63
1C6	1.96
1D3	1.94
1H2	1.94

Again, as demonstrated by the folding experiment, 2A5 appeared to be more stable than the other antibodies.

Example 5: Identification of molecular epitopes on SDF-1α that bind to the B-type and Z-type antibodies

[00165] Based on Examples 1-4 discussed above, it was reasonable to conclude that the B-type antibodies specifically bind to the dimeric form of SDF-1α, while the Z-type antibodies did not appear to have a preference. In the next set of experiments, mass spectrometry was used to identify molecular epitopes on SDF-1α that bind to the Z-type antibody.

[00166] Two approaches were taken in determining the epitope sequence information: epitope extraction and epitope excision (Figure 14A). In epitope extraction, SDF-1α was subjected to enzymatic digestion, and then its peptide fragments were mixed with antibody-bound POROS resin to check for binders; whereas in epitope excision, the digestion was performed *in situ* while SDF-1α was bound to the antibody. After washing away the non-binders, the epitope containing peptide(s) that was still bound onto the antibody resin was taken together directly for MALDI-Q-TOF, matrix-assisted laser desorption ionization quadrupole/time-of-flight, mass spectrometry

sequence analysis. Different enzymes were used for cleaving the amino acid sequence of SDF-1α, such that the entire length of the sequence was covered. The various peptides generated by the enzyme cleavage are depicted in Figure 14B. With different overlaps, the SDF-1a peptides in both reduced and native forms were examined for epitope binding.

[00167] Figure 15 depicts the various domains in SDF-1α, which have been previously characterized. For example, Dealwis *et al.*, *Proc. Natl. Acad. Sci USA* 95: 126941-12646 (1998) (PDB: 1A15) previously identified the receptor binding site on SDF-1α and Sadir *et al.*, *J. Biol. Chem.* 276: 8288-8296 (2001) identified the heparin recognition site on SDF-1α, both of which are shown in Figure 16. As shown in this Figure, the heparin-binding site spans amino acid residues 24-27. The data from both the extraction and the excision experiments with Z-type antibodies are also summarized in Figure 15. Z-IgGs were immobilized onto POROS aldehyde resin with an average density of 13 μg Ab per one μl resin. SDF-α intact protein or peptide digests were generated by 0.5 to 2 %(wt) from various endoproteinases. Incubation with antibody resin was done with 1:1 Ag:Ab molar ratio at room temperature. The resin was then washed three times to remove non-binding peptides. All digestion, incubation, and wash steps were performed in 20mM ammonium acetate. The peptide(s) that remain bound on the antibody resin was mixed 1:2 with α-cyano-4-hydroxycinnamic acid in 1% formic acid and 35% acetonitrile directly on the sample plate. Experiments with use of single enzyme were mapped in parallel against both 2A5 and 1C6 IgGs; with use of multiple enzymes only 2A5 IgG was carried out for further characterization and described here in details.

[00168] . At least three molecular epitopes may be found on SDF-1α obtained by mass spectrometric data. From the epitope extraction of reduced SDF-1α experiment, only one peptide, amino acid residues 1-24, was isolated from the endoproteinase Lys-C digestion. This N-terminal peptide, while bound to the antibody resin, can be digested further by the secondary enzyme to cut closer to the epitope region. When digested with endoproteinase Glu-C, two shorter fragments were found to be bound to the antibody, *i.e.*, fragments including amino acid residues 1-15 and 7-15. However, when digested with endoproteinase trypsin, all peptides including the ones with amino acid residues 1-8 and 12-20 lost binding. This observation reveals the first epitope having the tightest affinity as being near the N-terminus of SDF-α, spanning amino acid residues 7-15. It also demonstrates the presence of both arginines, Arg8 and Arg12, as being crucial for the binding affinity of the 2A5 IgG. Two other epitope regions were identified by the excision experiment. Results from the native SDF1-α with endoproteinase Lys-C revealed two additional peptides, amino acid residues 28-40 and 45-54. This also coincides with the finding of weaker binders from the extraction experiment with endoproteinases trypsin and Arg-C.

[00169] These epitopes are referred to as epitopes A, B and C (see Figure 16A). Epitope A spans amino acid residues 7-15; epitope B spans amino acid residues 28-40 and epitope C spans amino acid residues 45-54. Epitope A overlaps with two receptor binding sites, amino acid residues 1-8 and residues 12-17. The heparin site spans amino acid residues 24-27, which lies within epitope B. The epitope of an IgG (*i.e.*, 2A5) on SDF-1α monomer identified by mass spectrometry was mapped on the 3-dimensional structure of .SDF-1α dimer molecule (PDB: 1A15), using Insight-II molecule modeling program (Accelrys, Inc., San Diego). Drawing inferences from the biophysical experiments involving B-type antibodies and molecular epitope data of the 2A5 antibody

from the mass spectrometric experiments, the residues that could possibly be part of epitope of B-type antibodies were identified on the 3-dimensional dimer structure of SDF-1α (PDB:1A15) (see Figure 16A).

[00170] In a subsequent experiment, synthetic peptides were generated. Region 1 peptides spanned the N-terminal region, and the receptor binding region amino acid residues 7-18; Region 2 peptides spanned the dimer interface region, amino acid residues 20-33; and Region 3 region peptides spanned the receptor binding region, amino acid residues 37-50. The sequences of the peptides are shown in Figure 16B.

[00171] About 10,000 RU of Z-IgGs, specifically 2A5 and 1C6, were immobilized onto a surface and the various synthetic peptides were flowed across the surface. IgG4 isotype antibody was used as a control. The binding experiments were performed in 20 mM ammonium acetate buffer. The results from an exemplary experiment are shown in Figures 17A-17B. The data from IgG4 control was subtracted from the data obtained with the Z-type antibodies.

[00172] In another experiment, about 5000 RU of B-type IgGs (1H2 and 1D3) were immobilized onto a surface and the various peptides were flowed across the surface. The binding experiments were performed in 20 mM ammonium acetate buffer. IgG4 was used as a control and was subtracted from the data. The results are depicted in Figure 18A-18B.

[00173] The results from the peptide binding studies demonstrate that Z-type antibodies do not recognize the dimer interface or the heparin binding site on SDF-1α, whereas, the B-type antibodies bind near or at the dimer interface and the heparin binding site.

[00174] Additionally, based on the peptide binding data, it appears that residues at amino acid positions 8, 12, 24, 27, 41, 43, 47 and 48 of SDF-1α are crucial for recognition by B-type and Z-type antibodies.

Example 6: Heparin binding to SDF-1α blocks the binding of B-type antibodies to SDF-1α

[00175] The blocking of SDF-1 binding to heparin sulphate was determined by capturing biotinylated heparin sulphate (Sigma) on a Streptavidin chip (Biacore, Uppsala, Sweden) then measuring the SDF-1 binding with and without an excess of monoclonal antibodies Experiments were carried out in PBS. The results of one such experiment are shown in Figure 19.

[00176] The specification is most thoroughly understood in light of the teachings of the references cited within the specification which are hereby incorporated by reference. The embodiments within the specification provide an illustration of embodiments in this invention and not be construed to limit its scope. The skilled artisan readily recognizes that many other embodiments are encompassed by this invention. All publications and invention are incorporated by reference in their entirety. To the extent that the material incorporated by reference contradicts or is inconsistent with the present specification, the present specification will supercede any such material. The citation of any references herein is not an admission that such references are prior art to the present invention.

[00177] Unless otherwise indicated, all numbers expressing quantities of ingredients, cell culture, treatment conditions, and so forth used in the specification, including claims, are to be understood as being modified in all instances by the term "about." Accordingly, unless otherwise indicated to the contrary, the numerical

parameters are approximations and may vary depending upon the desired properties sought to be obtained by the present invention. Unless otherwise indicated, the term "at least" preceding a series of elements is to be understood to refer to every element in the series. Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

Claims

What is claimed is:

1. A method of identifying a compound, that selectively binds to an oligomeric form, and not to a monomeric form, of a chemokine, the method comprising:

(a) contacting a compound, with a peptide comprising a glycosaminoglycan binding site of a chemokine; or

(b) contacting a compound in the presence of a cation, wherein the cation is present at a concentration sufficient to promote and/or stabilize oligomerization of a chemokine;

(c) determining whether the compound, binds the peptide in (a) or the chemokine in (b); and

(d) identifying a compound, which selectively binds to an oligomeric form, and not to a monomeric form, of the chemokine based on the ability of the compound, to bind the peptide in (a) or to bind the chemokine in (b).

2. The method of claim 1, wherein the compound is an antibody or an antigen-binding portion thereof.

3. The method of claim 1, wherein the compound is a small molecule.

4. A method of identifying an antibody or an antigen-binding portion thereof, that selectively binds to an oligomeric form, and not to a monomeric form, of a chemokine, the method comprising:

(a) contacting an antibody, or antigen-binding portion thereof, with a peptide comprising a glycosaminoglycan binding site of a chemokine; or

(b) contacting an antibody with a chemokine in the presence of a cation, wherein the cation is present at a concentration sufficient to promote and/or stabilize oligomerization of a chemokine;

(c) determining whether the antibody, or an antigen-binding portion thereof, binds the peptide in (a) or the chemokine in (b); and

(d) identifying an antibody, or antigen-binding portion, which selectively binds to an oligomeric form, and not to a monomeric form, of the chemokine based on the ability of the antibody, or an antigen-binding portion thereof, to bind the peptide in (a) or to bind the chemokine in (b).

5. The method of claim 1 or claim 4, wherein the chemokine is selected from the group consisting of SDF-1α, SDF-1β, IP-10, IL-8, MIP-1α, MIP-1β, RANTES, MCP-1, MCP-2, MCP-3, MCP-4, GROα, GROβ, GROγ, ENA-78, LDGF-PBP, GCP-2, PF4, Mig, BUNZO/STRC33, I-TAC, BLC/BCA-1, MDC, TECK, TARC, HCC-1, HCC-4, DC-CK1, MIP-3α, MIP-3β, Eotaxin, Eotaxin-2, I-309, HCC-2, MPIF-1, 6Ckine, CTACK, MEC, Lymphotactin and Fractalkine.

6. The method of claim 1 or claim 4, wherein the chemokine is SDF-1α.

7. The method of claim 1 or claim 4, wherein the peptide comprises amino acid residues 20-33 of SDF-1α.

8. The method of claim 1 or claim 4, wherein the peptide comprises amino acid residues 20-31 of SDF-1α.

9. The method of claim 1 or claim 4, wherein the peptide comprises amino acid residues 24-27 of SDF-1α.

10. The method of claim 1 or claim 4, wherein the glycosaminoglycan is selected from the group consisting of heparin, heparin sulfate, chondroitin sulfate, dermatan sulfate, keratin sulfate, hyaluronase and derivatives and combinations thereof.

11. The method of claim 1 or claim 4, wherein the oligomeric form is a dimeric form of the chemokine.

12. The method of claim 11, wherein the dimeric form is a homodimeric form of the chemokine.

13. The method of claim 11, wherein the dimeric form is a heterodimeric form of the chemokine.

14. The method of claim 1 or claim 4, wherein the cation is a multivalent cation.

15. The method of claim 1 or claim 4, wherein the cation is a trivalent cation.

16. The method of claim 1 or claim 4, wherein the cation is a divalent cation.

17. The method of claim 16, wherein the divalent cation is selected from the group consisting of Ca^{2+}, Mg^{2+}, Sr^{2+}, Ba^{2+}, Mn^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+} and Cd^{2+} 18.

18. The method of claim 17, wherein the divalent cation is Ca^{2+}.

19. The method of claim 1 or claim 4, wherein the cation is present at a concentration of at least 1 mM.

20. The method of claim 1 or claim 4, wherein the concentration of the cation is decreased by the addition of a chelating agent.

21. The claim of claim 20, wherein the chelating agent is selected from the group consisting of EDTA (ethylenediamine tetraacetate), porphine, DTPA (Diethylenetriaminepentaacetic acid), Dimercaprol (2,3-dimercapto-1-propanol) and NTA (N,N-bis(carboxymethyl)glycine; Triglycollamic acid), DETA (diethylenetriamine) and AEEA (aminoethylethaloamine).

22. The method of claim 4, wherein the antigen-binding portion of the antibody is selected from the group consisting of Fab, Fab', F(ab')2, Fabc, ScFv and Fv.

23. The method of claim 4, wherein the antibody is selected from the group consisting of a polyclonal antibody, monoclonal antibody, a humanized antibody, chimeric antibody and a human antibody.

24. The method of claim 23, wherein the antibody is a human antibody.

25. The method of claim 24, wherein the human antibody selectively binds an oligomeric form of SDF-1α.

26. The method of claim 25, wherein the human antibody is selected from the group consisting of 1D3, 2H5 and 1H2.

27. The method of claim 4, wherein the antibody blocks the binding of an oligomeric form of a chemokine to its receptor.

28. The method of claim 4, wherein the antibody blocks the binding of the oligomeric form of a chemokine to a glycosaminoglycan.

29. A method of identifying an antibody or an antigen-binding portion thereof, which selectively binds to an oligomeric form, and not a monomeric form, of a chemokine, the method comprising:

(a) contacting an antibody, or antigen-binding portion thereof, with a peptide comprising a glycosaminoglycan binding site of a chemokine;

(b) determining whether the antibody, or an antigen-binding portion thereof, binds the peptide; and

(c) identifying an antibody, or antigen-binding portion thereof, which selectively binds to an oligomeric form, and not to a monomeric form, of the chemokine based on the ability of the antibody, or an antigen-binding portion thereof, to bind the peptide.

30. A method of identifying an antibody or an antigen-binding portion thereof, which selectively binds to an oligomeric form, and not a monomeric form, of a chemokine, the method comprising:

(a) contacting an antibody with a chemokine in the presence of a cation, wherein the cation is present at a concentration sufficient to promote and/or stabilize oligomerization of a chemokine;

(b) determining whether the antibody, or an antigen-binding portion thereof, binds the chemokine; and

(c) identifying an antibody, or antigen-binding portion, which selectively binds to an oligomeric form, and not to a monomeric form, of the chemokine, based on the ability of the antibody, or an antigen-binding portion thereof, to bind the chemokine in the presence of the cation.

31. An antibody or an antigen-binding portion thereof, identified using the method of claim 4.

32. The antibody of claim 31, wherein the antibody is selected from the group consisting of 1H2, 1D3 and 2H5.

33. A pharmaceutical composition comprising a therapeutically effective amount of an antibody, or an antigen-binding portion thereof, identified using the method of claim 4, and a pharmaceutically acceptable carrier.

34. A method for inhibiting the binding of an oligomeric chemokine to a chemokine receptor comprising contacting said chemokine with an effective amount of an antibody, or an antigen-binding portion thereof, identified using the method of claim 4, thereby inhibiting the binding of an oligomeric chemokine to a chemokine receptor.

35. A method of inhibiting the binding of an oligomeric chemokine to a glycosaminoglycan comprising contacting said chemokine with an effective amount of an antibody, or an antigen-binding portion thereof, identified using the method of claim 4, thereby inhibiting the binding of an oligomeric chemokine to a glycosaminoglycan.

36. The method of any one of claims 34-35, wherein the antibody is selected from the group consisting of 1H2, 1D3 and 2H5.

37. The method of any one of claims 34-35, wherein the chemokine is contacted with the antibody *in vitro*.

38 A method of treating a chemokine-mediated disorder in a subject in need thereof , comprising administering to the subject an effective amount of compound, identified using the method of claim 1, thereby treating a chemokine-mediated disorder.

39. A method of diagnosing or prognosing a chemokine-mediated disorder in a subject in need thereof, comprising:

(a) contacting a biological sample and a control sample with a compound identified using the method of claim 1; and

(b) detecting an oligomeric form of a chemokine in the biological sample and the control sample,

wherein the subject is diagnosed or prognosed with a chemokine mediated disorder based on a difference in the amount of an oligomeric form of a chemokine present in the biological sample as compared to the control sample.

40. The method of claim 39, wherein the compound is contacted with a biological sample isolated from a subject.

41. The method of claim 39, wherein the compound is contacted with a biological sample in a subject.

42. The method of any one of claims 38-39, wherein the chemokine-mediated disorder is an inflammatory disorder.

43. The method of claim 42, wherein the inflammatory disorder is selected from the group consisting of chronic inflammation, chronic obstructive pulmonary disease (COPD), acute respiratory distress syndrome (ARDS), inflammatory bowel disease, tuberculoid leprosy, encephalomyelitis, artherosclerosis, uveoretinitis, osteoarthritis, asthma, dermatitis, and eczema.

44. The method of any one of claims 38-39, wherein the chemokine-mediated disorder is an immune disorder.

45. The method of claim 44, wherein the immune disorder is selected from the group consisting of multiple sclerosis, HIV infection, psoriasis, allograft rejection, tumor-specific immunity, immune complex glomerulonephritis and lupus.

46. The method of any one of claims 38-39, wherein the chemokine-mediated disorder is an angiogenic disorder.

47. The method of claim 46, wherein the angiogenic disorder is cancer.

48. The method of any one of claims 38-39, wherein the subject is a human.

49. The method of claim 40, wherein the antibody is selected from the group consisting of 1H2, 1D3 and 2H5.

50. The method of claim 40, wherein the antibody or antigen-binding portion thereof, that selectively binds to an oligomeric form and not to a monomeric form of a chemokine, is administered locally to a site of inflammation in a subject.

51. A method of identifying an antibody or an antigen-binding portion thereof, that selectively binds to an oligomeric form, and not to a monomeric form, of SDF-1α, the method comprising:

(a) contacting an antibody, or antigen-binding portion thereof, with a peptide comprising a glycosaminoglycan binding site of SDF-1α; or

(b) contacting an antibody, or antigen binding portion thereof, with SDF-1α in the presence of a cation, wherein the cation is present at a concentration sufficient to promote/ stabilize the oligomerization of SDF-1α;

(c) determining whether the antibody, or antigen-binding portion thereof, binds the peptide in (a) or the SDF-1α in (b); and

(d) identifying an antibody, or antigen-binding portion, which selectively binds to an oligomeric form, and not to a monomeric form, of SDF-1α based on the ability of the antibody, or an antigen-binding portion thereof, to bind the peptide in (a) or to bind the SDF-1α in (b).

52. The method of claim 51, wherein the peptide comprising a glycosaminoglycan binding site of SDF-1α is selected from the group consisting of a peptide comprising amino acid residues 20-33 of SDF-1α, a peptide comprising amino acid residues 20-31 of SDF-1α and a peptide comprising amino acid residues 24-27 of SDF-1α.

53. The method of claim 51, wherein the cation is Ca^{2+}.

54. The method of claim 53, wherein the concentration of Ca^{2+} is at least 1 mM.

55. An antibody or antigen-binding portion thereof, identified using the method of claim 51.

56. A method of identifying a compound that selectively binds to an oligomeric form of a chemokine and not to a monomeric form of the chemokine, the method comprising:

(a) contacting a monomeric form of a chemokine with a compound;

(b) contacting an oligomeric form of a chemokine with the compound;

(c) determining whether the compound binds to the monomeric form of the chemokine or the oligomeric form of the chemokine, and

(d) identifying a compound which selectively binds to an oligomeric form, and not to a monomeric form, of the chemokine based on the ability of the compound to bind the oligomeric form and not the monomer jc form of the chemokine.

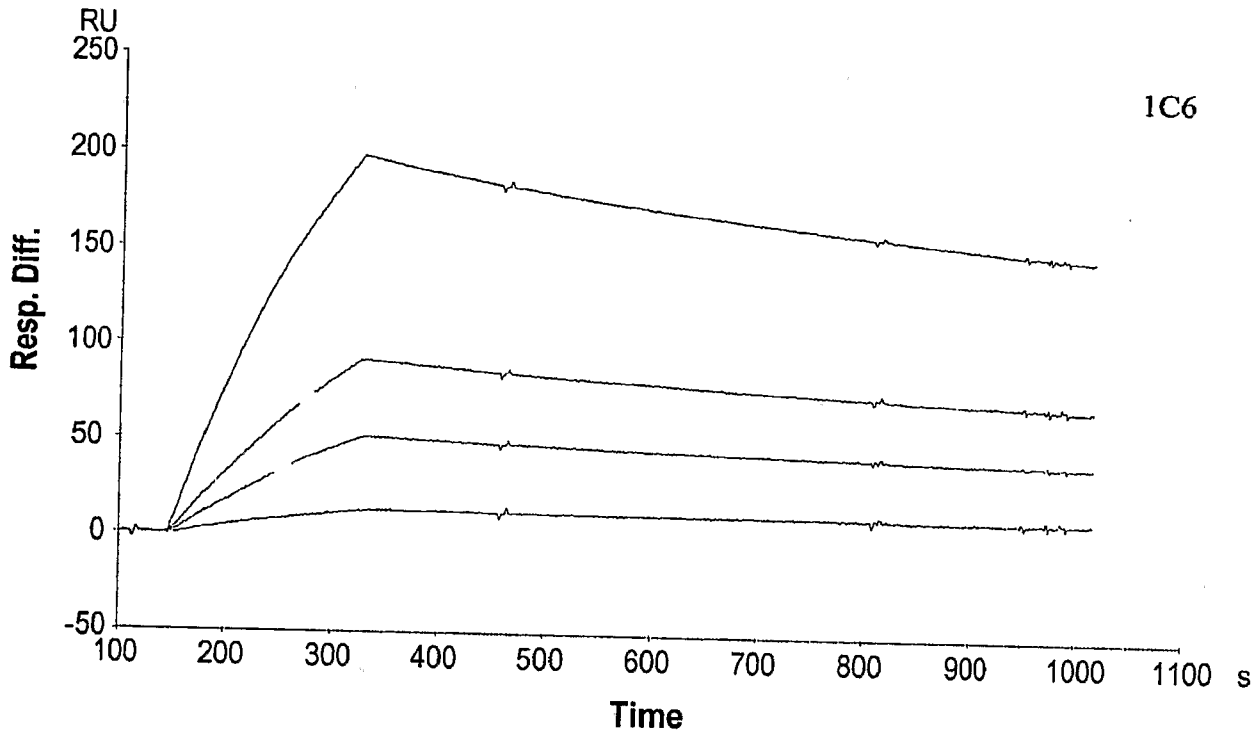

RU
250
200
150
100
50
0
-50
Resp. Diff.
1C6
100
200
300
400
500
600
700
800
900
1000
1100
s
Time

Fig. 1A

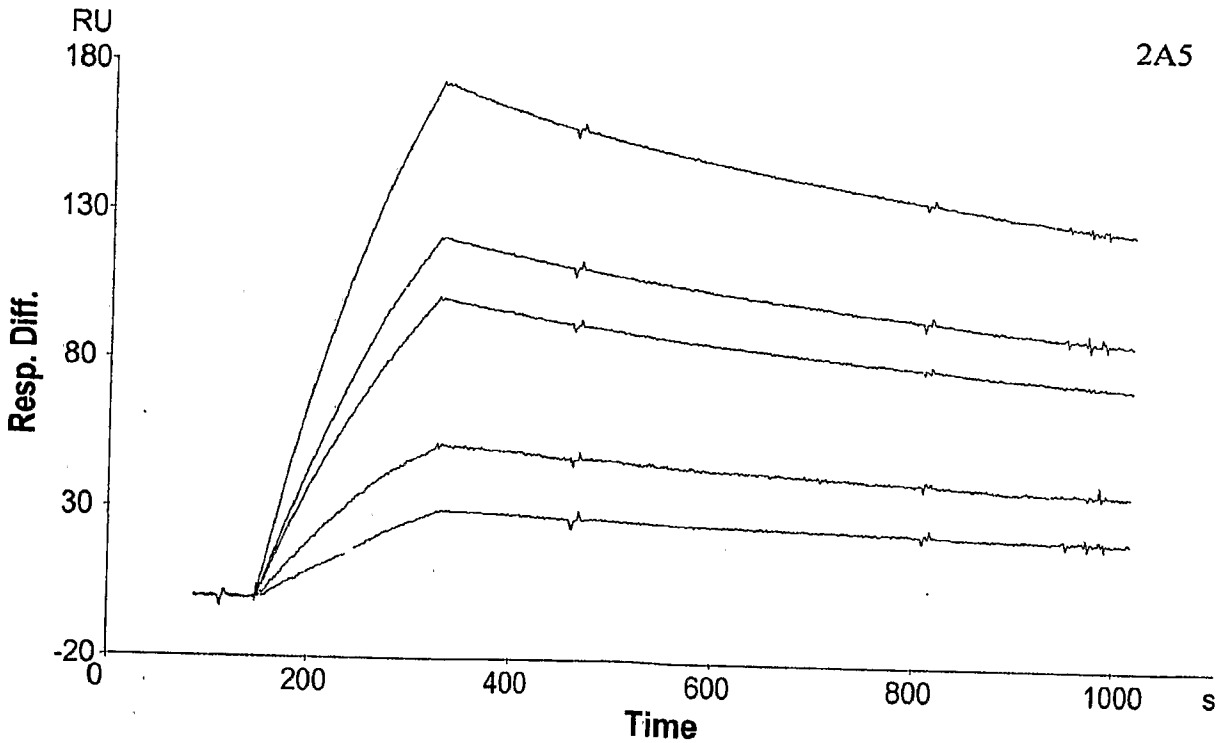

RU
180
130
80
30
-20
Resp. Diff.
2A5
0
200
400
600
800
1000
s
Time

Fig. 1B

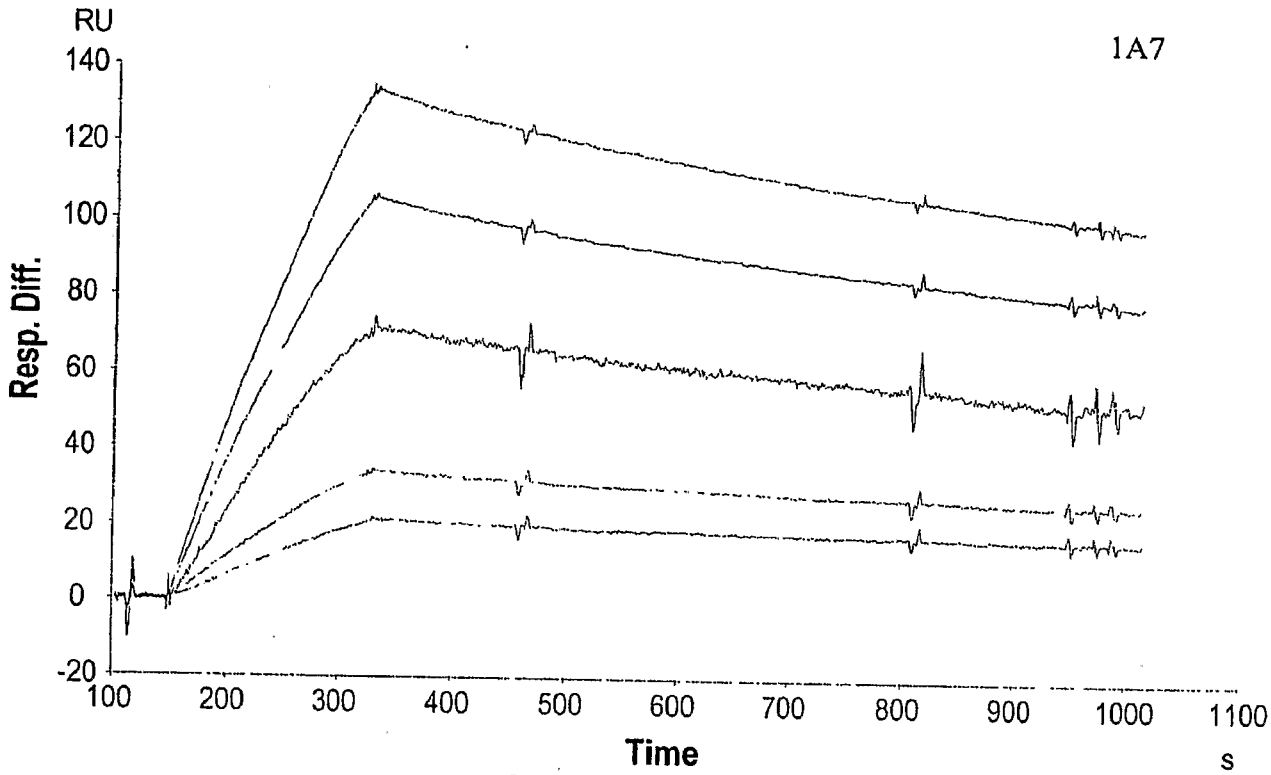

RU
1A7
140
120
100
80
60
40
20
0
-20
Resp. Diff.
100
200
300
400
500
600
700
800
900
1000
1100
Time
s

Fig. 1C

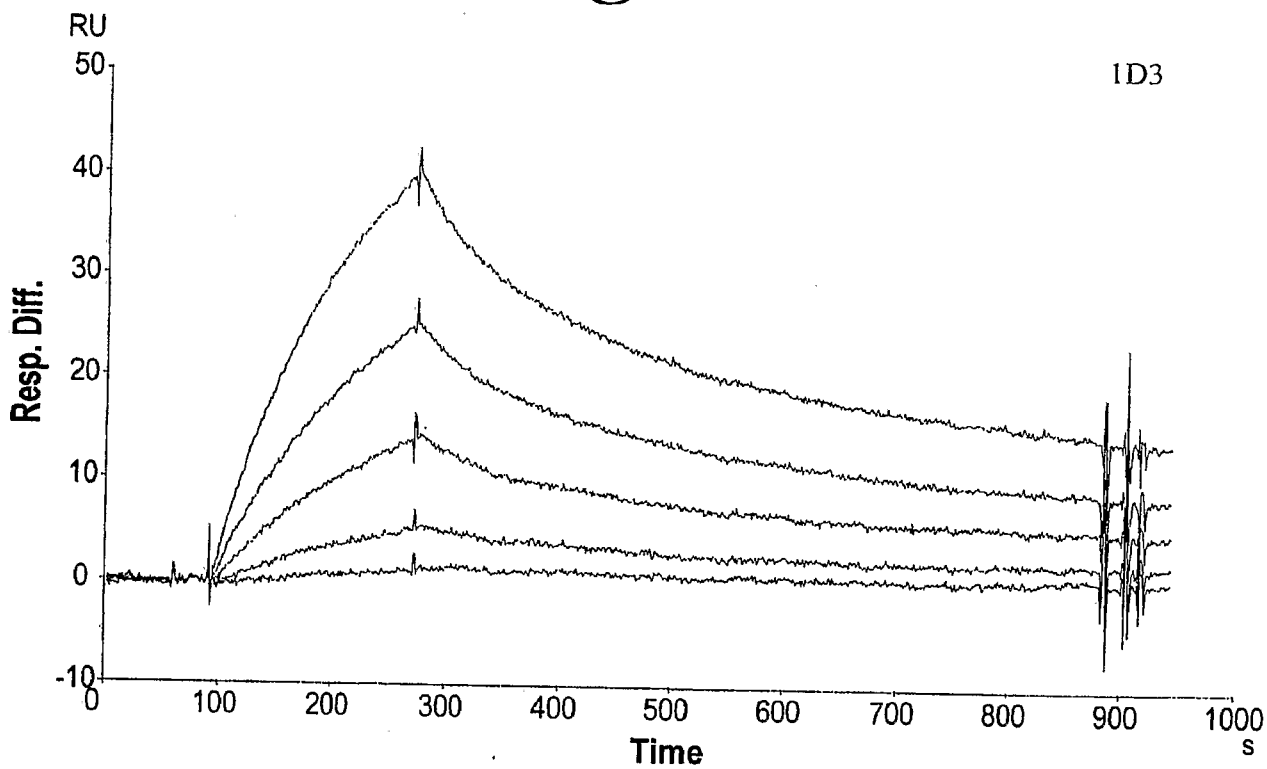

RU
1D3
50
40
30
20
10
0
-10
Resp. Diff.
0
100
200
300
400
500
600
700
800
900
1000
Time
s

Fig. 1D

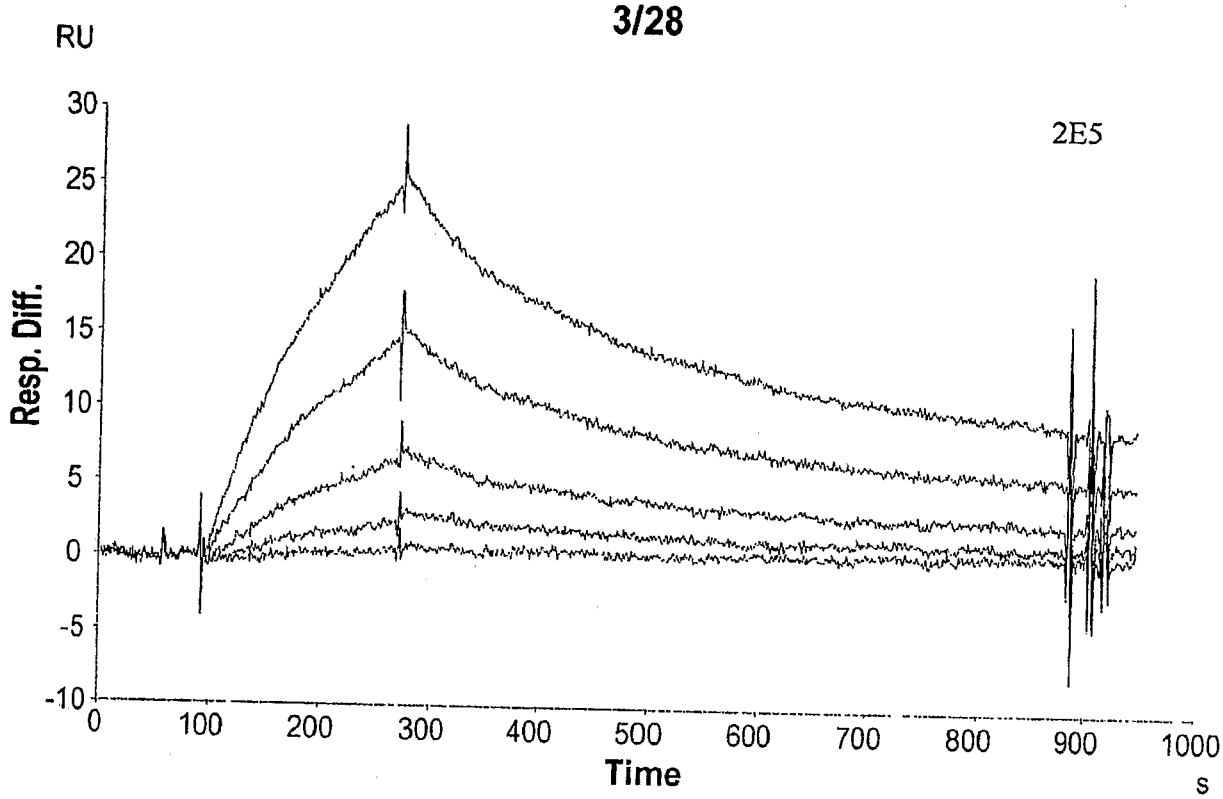

RU
2E5
Resp. Diff.
30
25
20
15
10
5
0
-5
-10
0
100
200
300
400
500
600
700
800
900
1000
Time
s

Fig. 1E

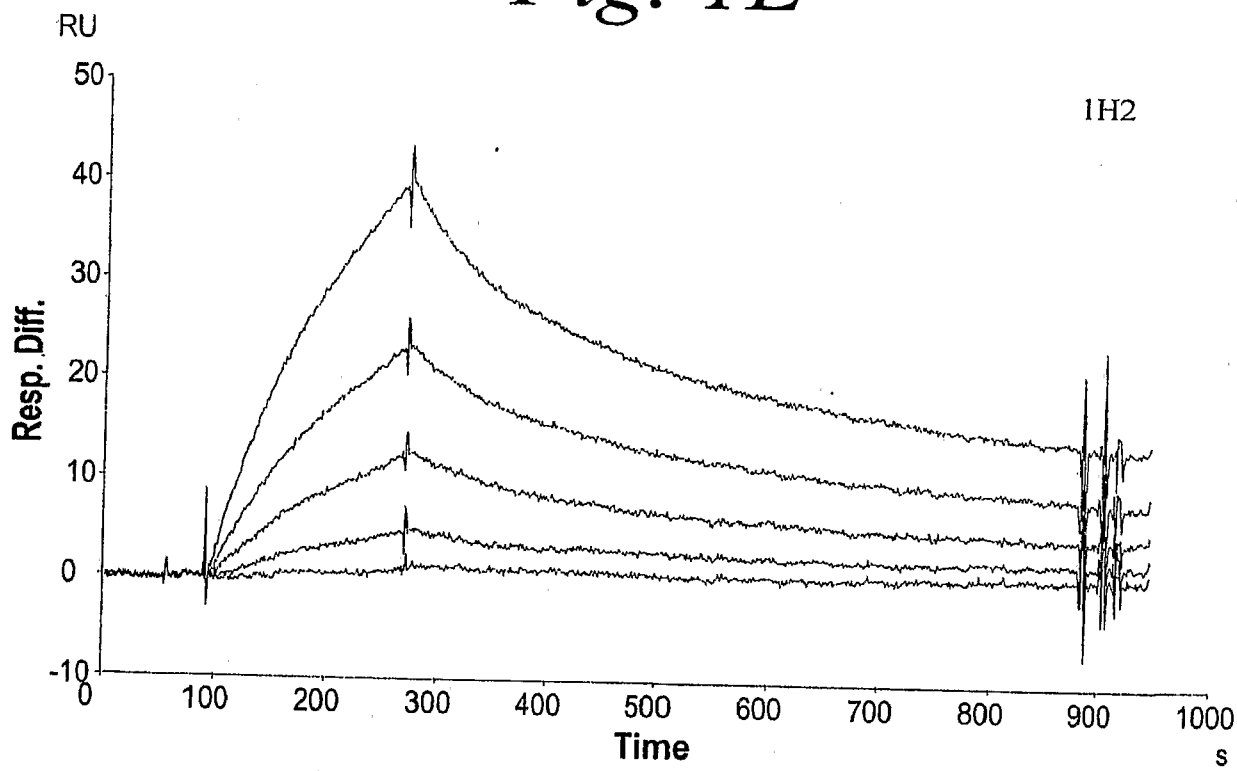

RU
1H2
Resp. Diff.
50
40
30
20
10
0
-10
0
100
200
300
400
500
600
700
800
900
1000
Time
s

Fig. 1F

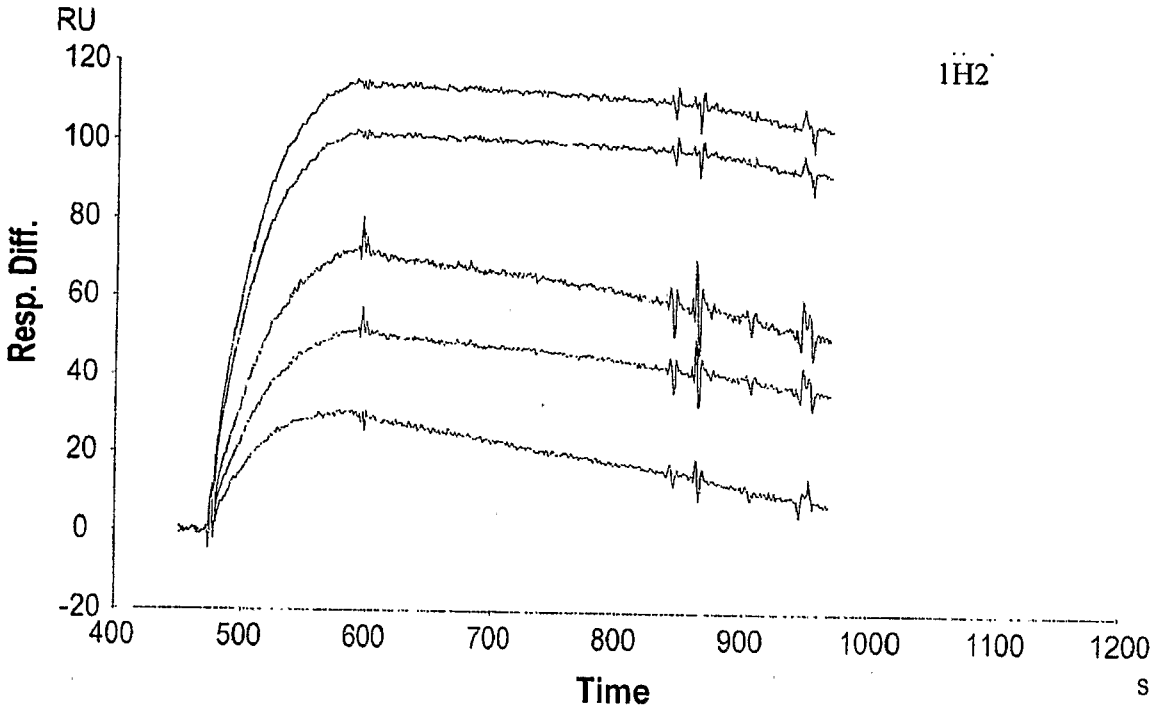

RU
120
100
80
60
40
20
0
-20
Resp. Diff.
1H2
400
500
600
700
800
900
1000
1100
1200
Time
s

Fig. 2A

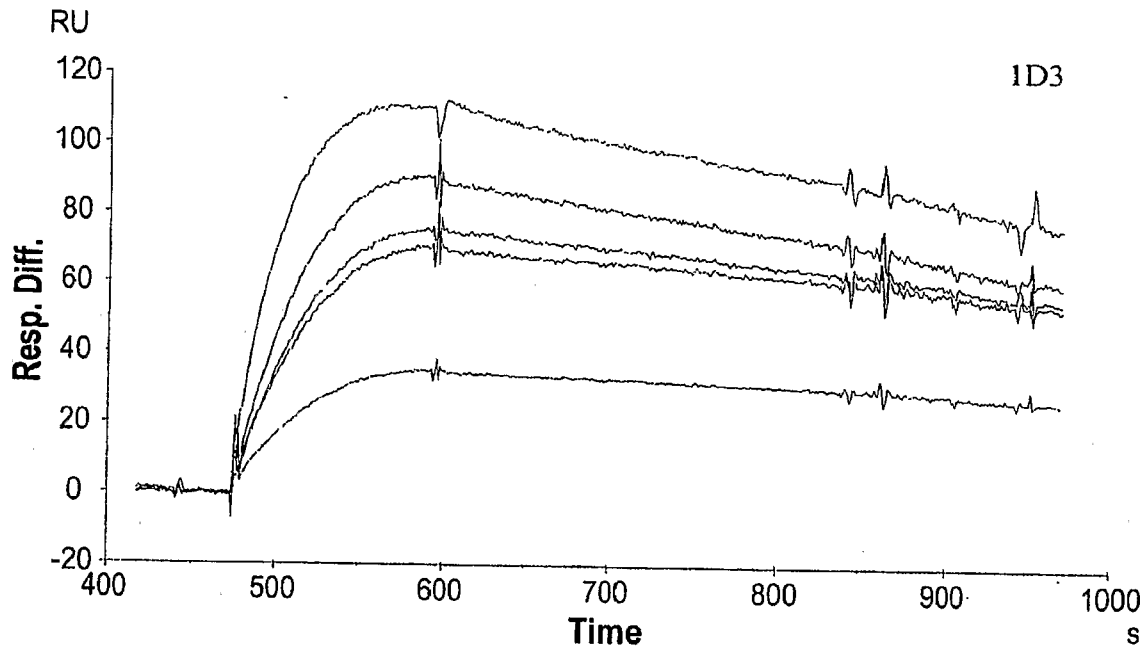

RU
120
100
80
60
40
20
0
-20
Resp. Diff.
1D3
400
500
600
700
800
900
1000
Time
s

Fig. 2B

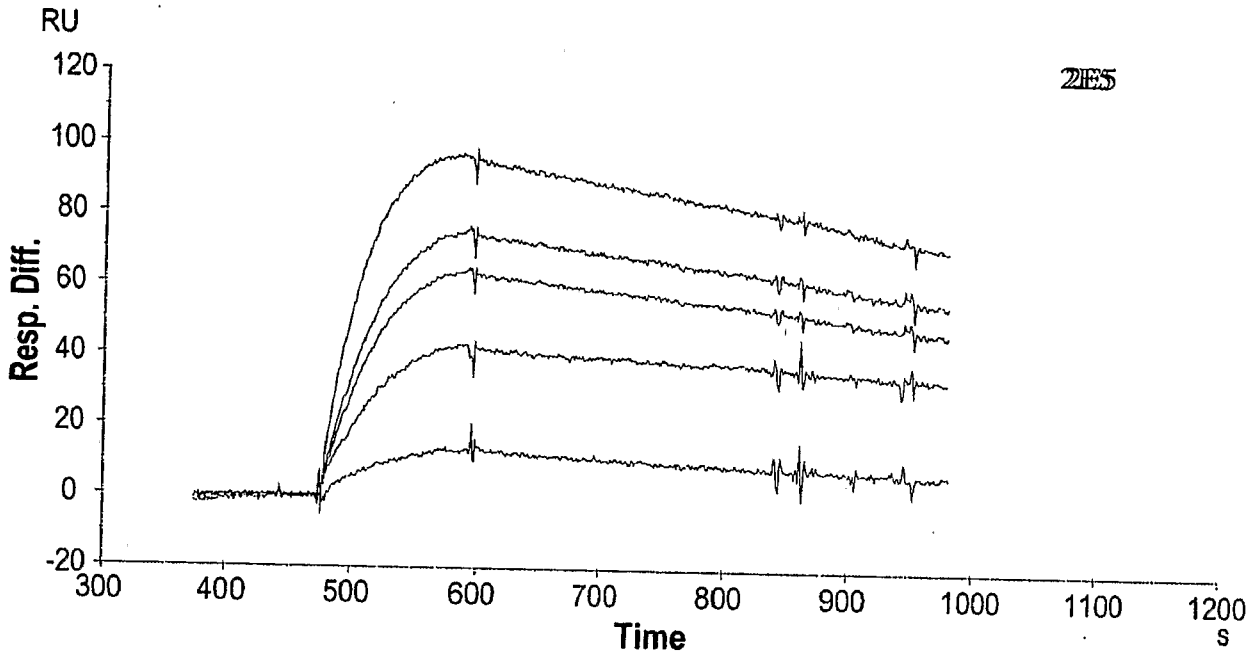

RU
120
100
80
60
40
20
0
-20
Resp. Diff.
2E5
300
400
500
600
700
800
900
1000
1100
1200
Time
s

Fig. 2C

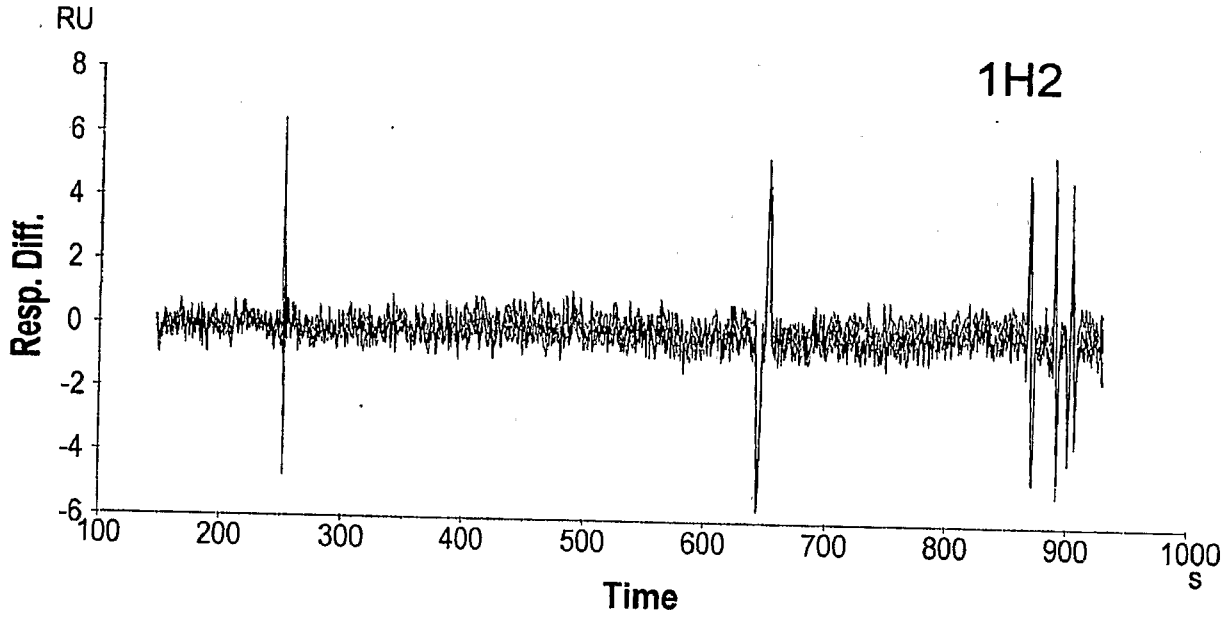

RU
8
6
4
2
0
-2
-4
-6
Resp. Diff.
1H2
100
200
300
400
500
600
700
800
900
1000
s
Time

Fig. 3A

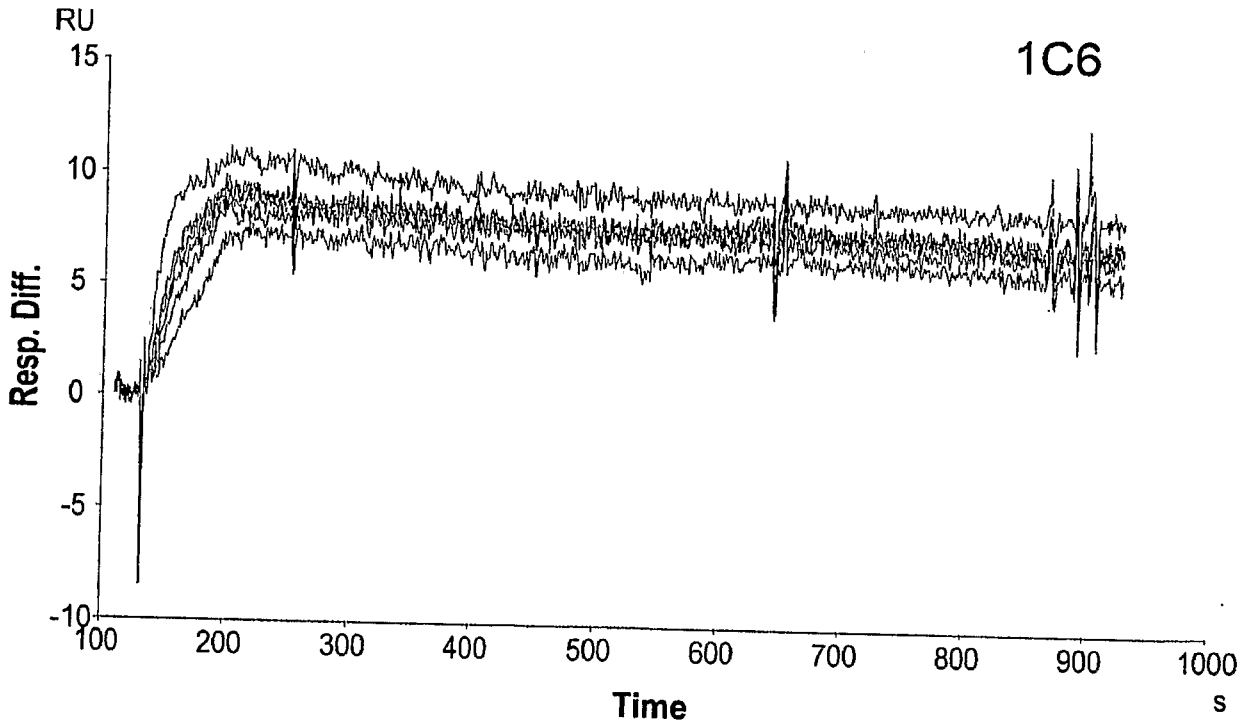

RU
15
10
5
0
-5
-10
Resp. Diff.
1C6
100
200
300
400
500
600
700
800
900
1000
s
Time

Fig. 3B

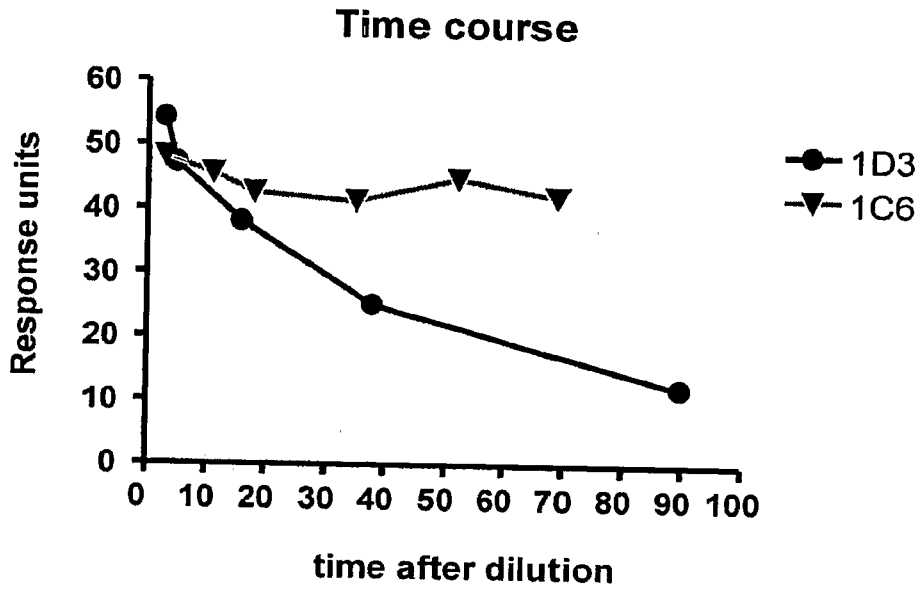

Time course
Response units
60
50
40
30
20
10
0
0 10 20 30 40 50 60 70 80 90 100
time after dilution
1D3
1C6

Fig. 4

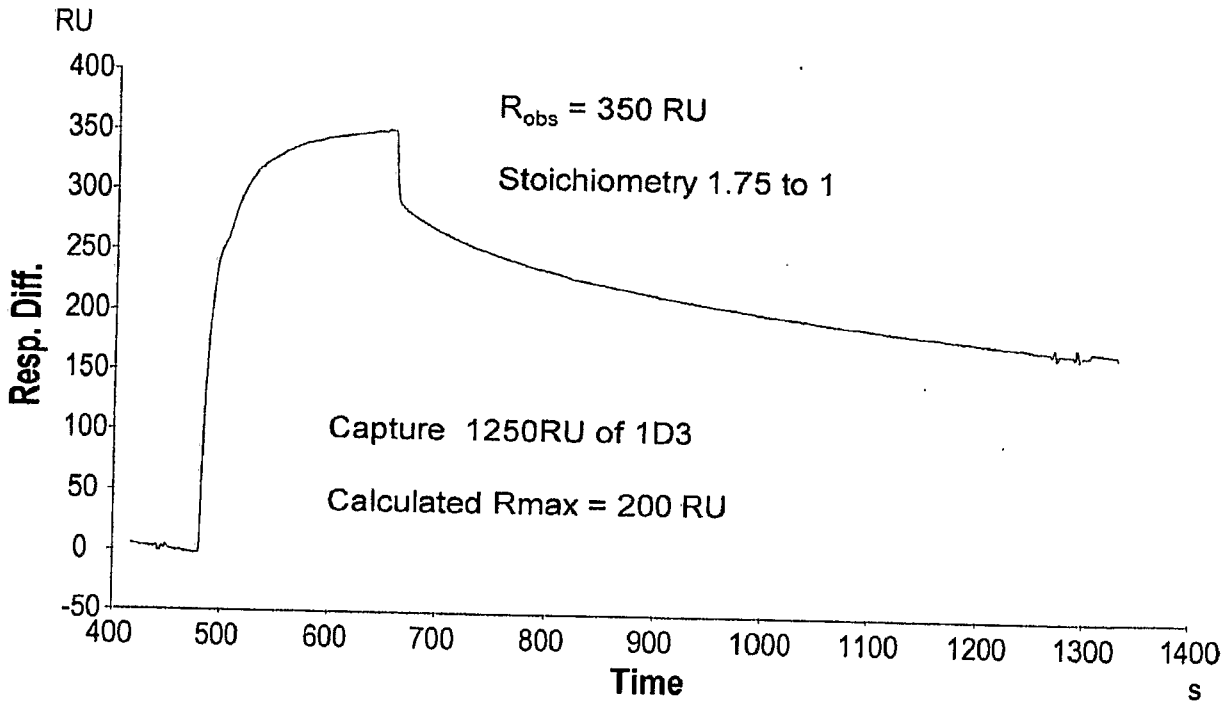

RU
Resp. Diff.
400
350
300
250
200
150
100
50
0
-50
400 500 600 700 800 900 1000 1100 1200 1300 1400
Time
s
R_{obs} = 350 RU
Stoichiometry 1.75 to 1
Capture 1250RU of 1D3
Calculated Rmax = 200 RU

Fig. 5

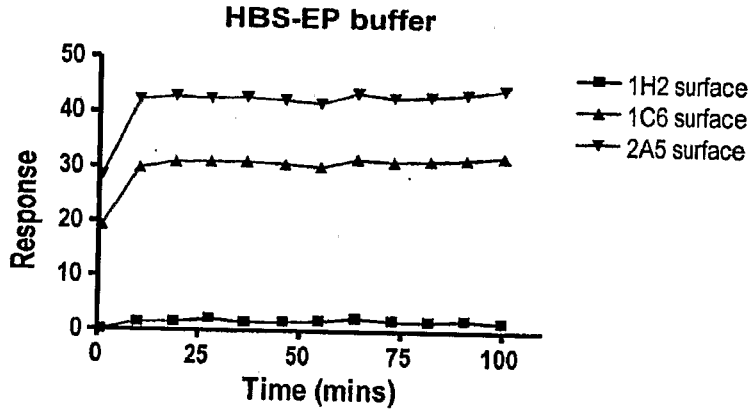


Fig. 6A

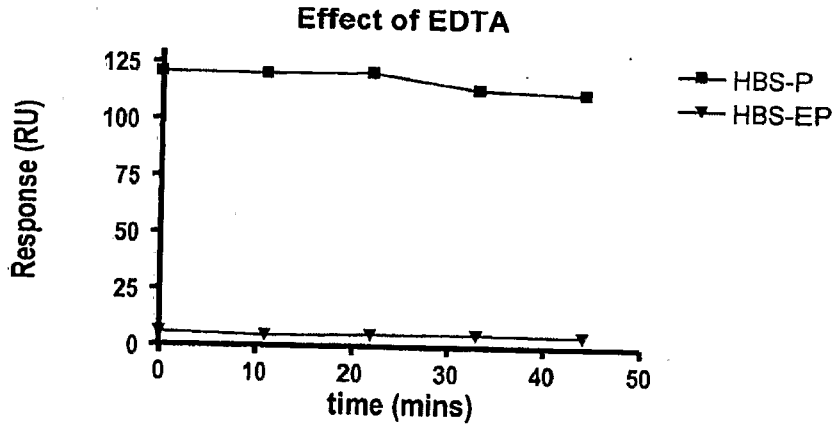


Fig. 6B

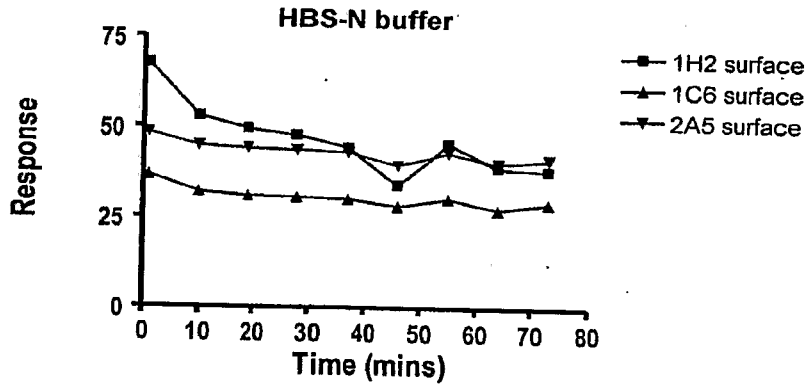


Fig. 6C

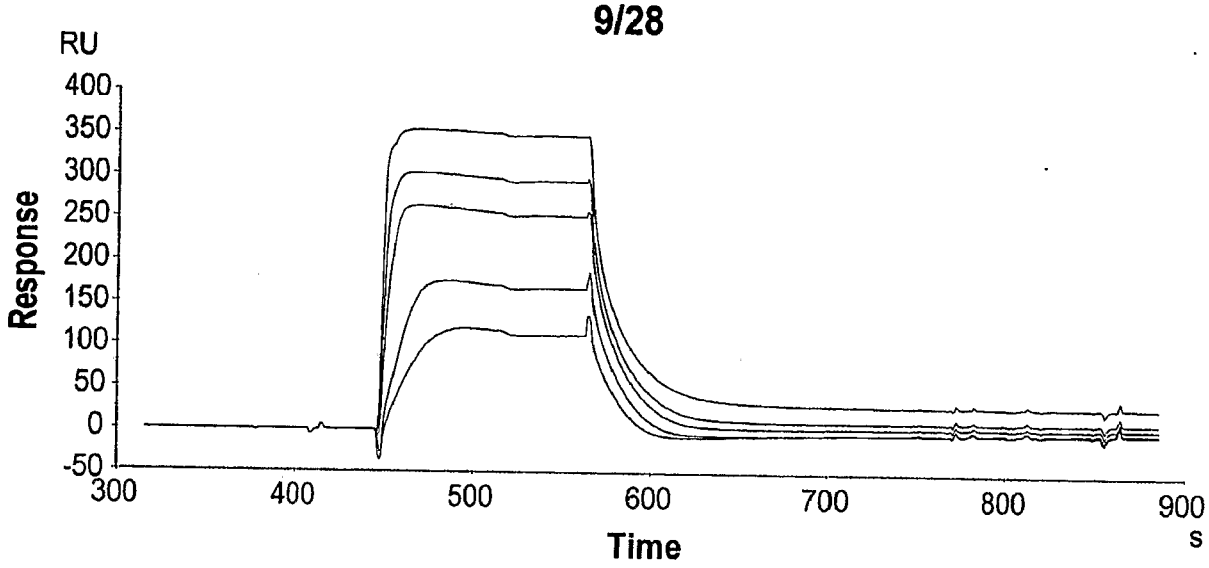


Fig. 7A

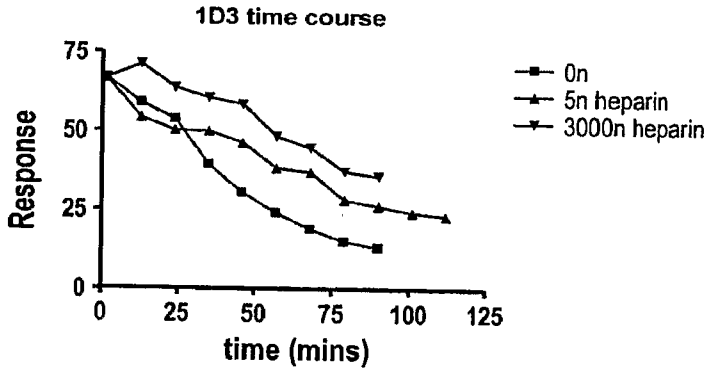


Fig. 7B

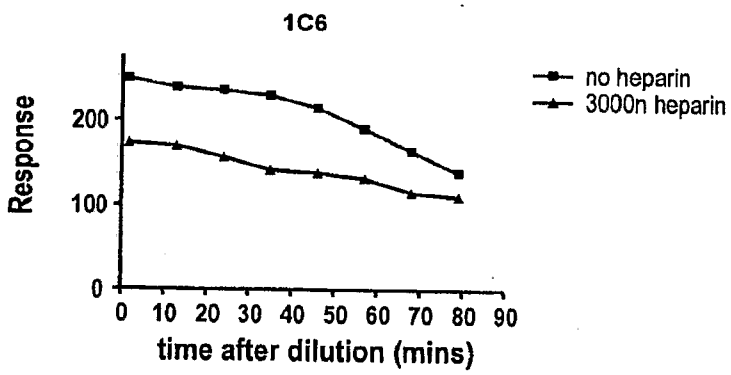


Fig. 7C

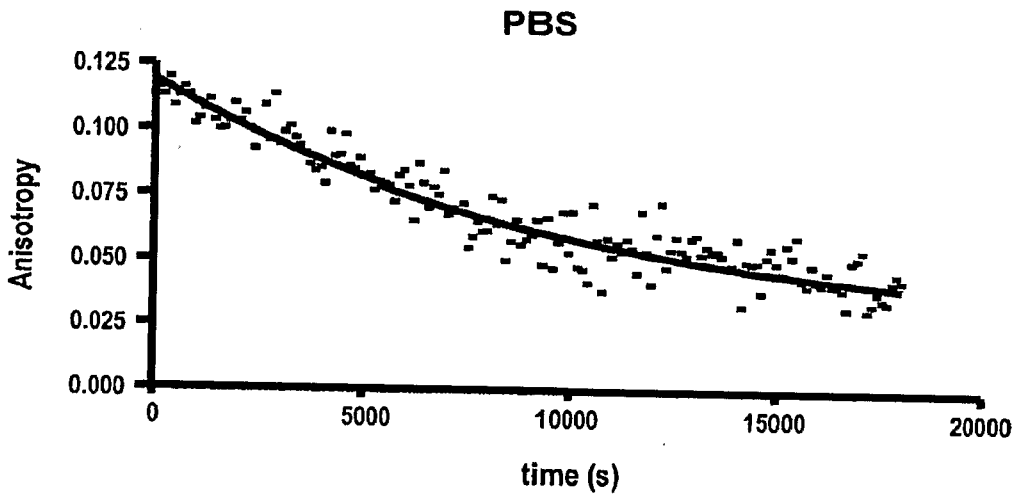

PBS
Anisotropy
0.125
0.100
0.075
0.050
0.025
0.000
0
5000
10000
15000
20000
time (s)

Fig. 8A

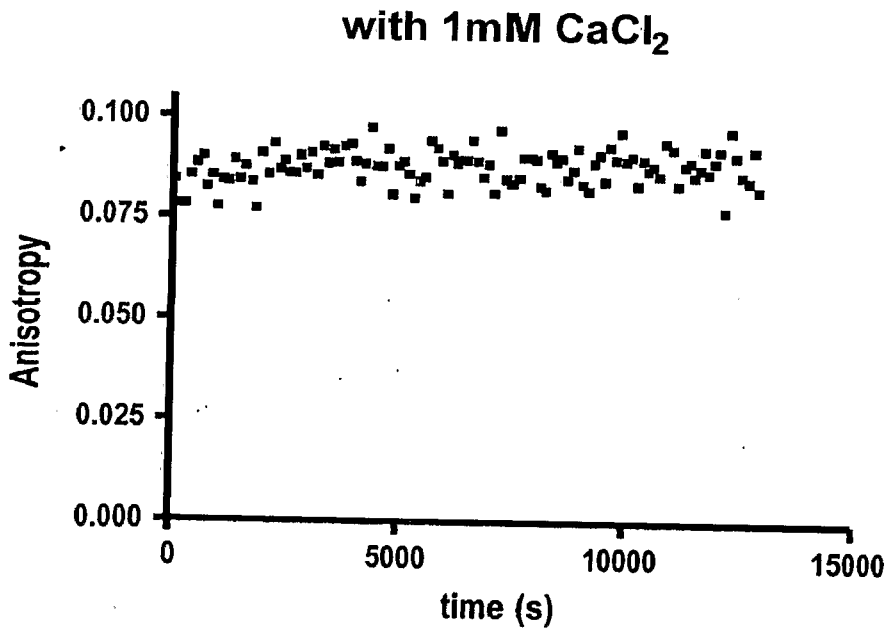

with 1mM $CaCl_2$
Anisotropy
0.100
0.075
0.050
0.025
0.000
0
5000
10000
15000
time (s)

Fig. 8B

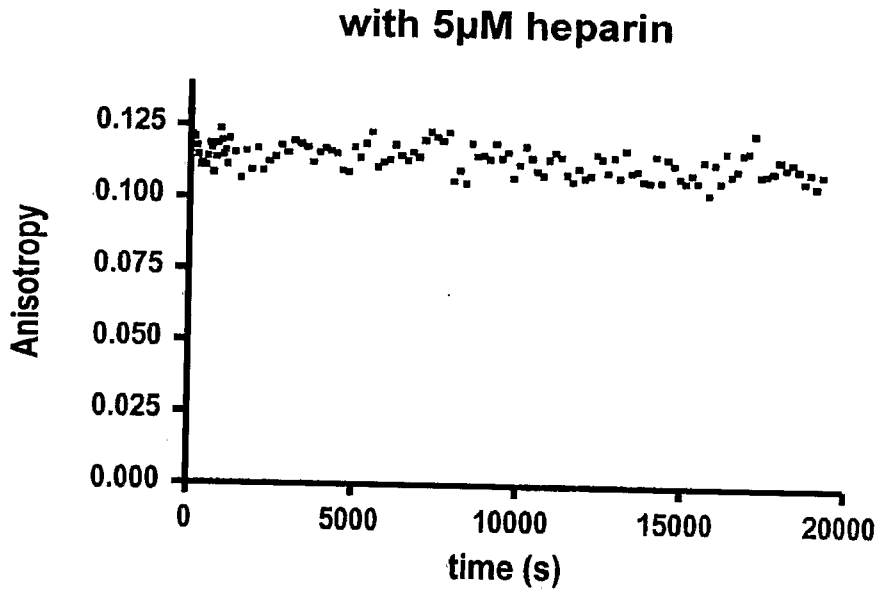

with 5μM heparin
Anisotropy
0.125
0.100
0.075
0.050
0.025
0.000
0
5000
10000
15000
20000
time (s)

Fig. 8C

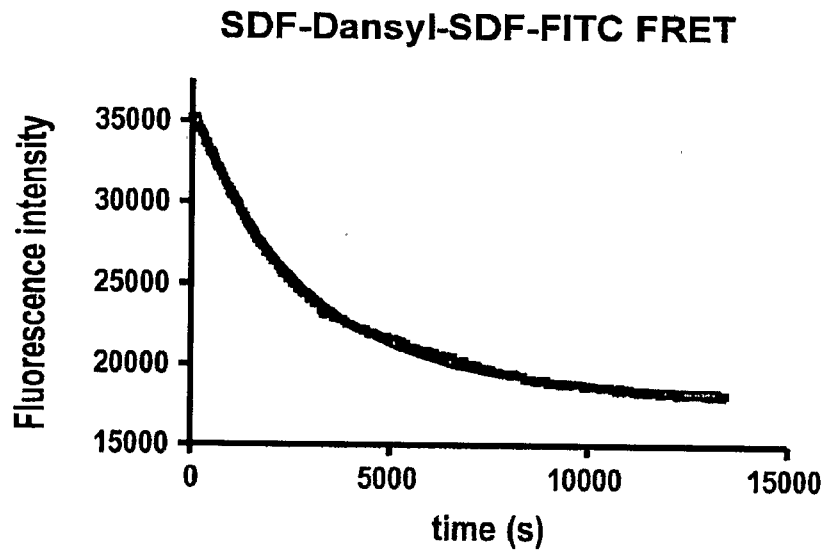

SDF-Dansyl-SDF-FITC FRET
Fluorescence intensity
35000
30000
25000
20000
15000
0
5000
10000
15000
time (s)

Fig. 9A

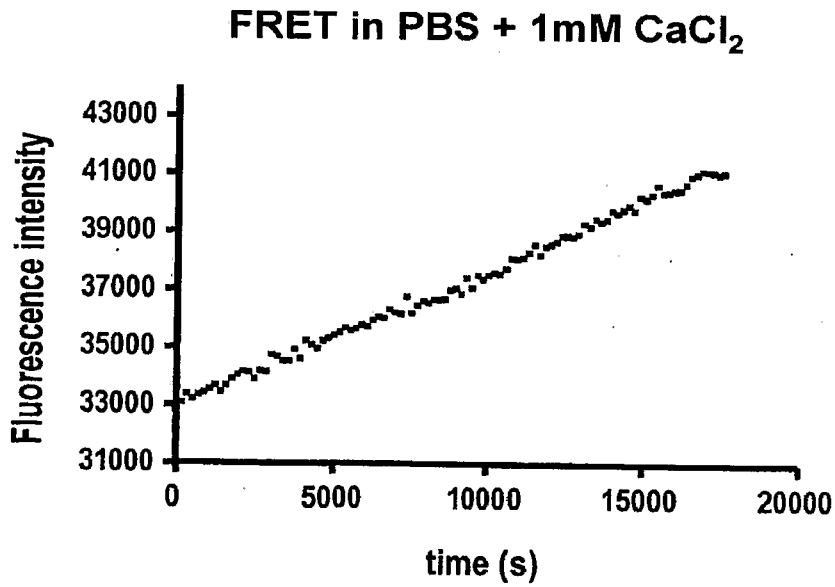

FRET in PBS + 1mM CaCl2
Fluorescence intensity
43000
41000
39000
37000
35000
33000
31000
0
5000
10000
15000
20000
time (s)

Fig. 9B

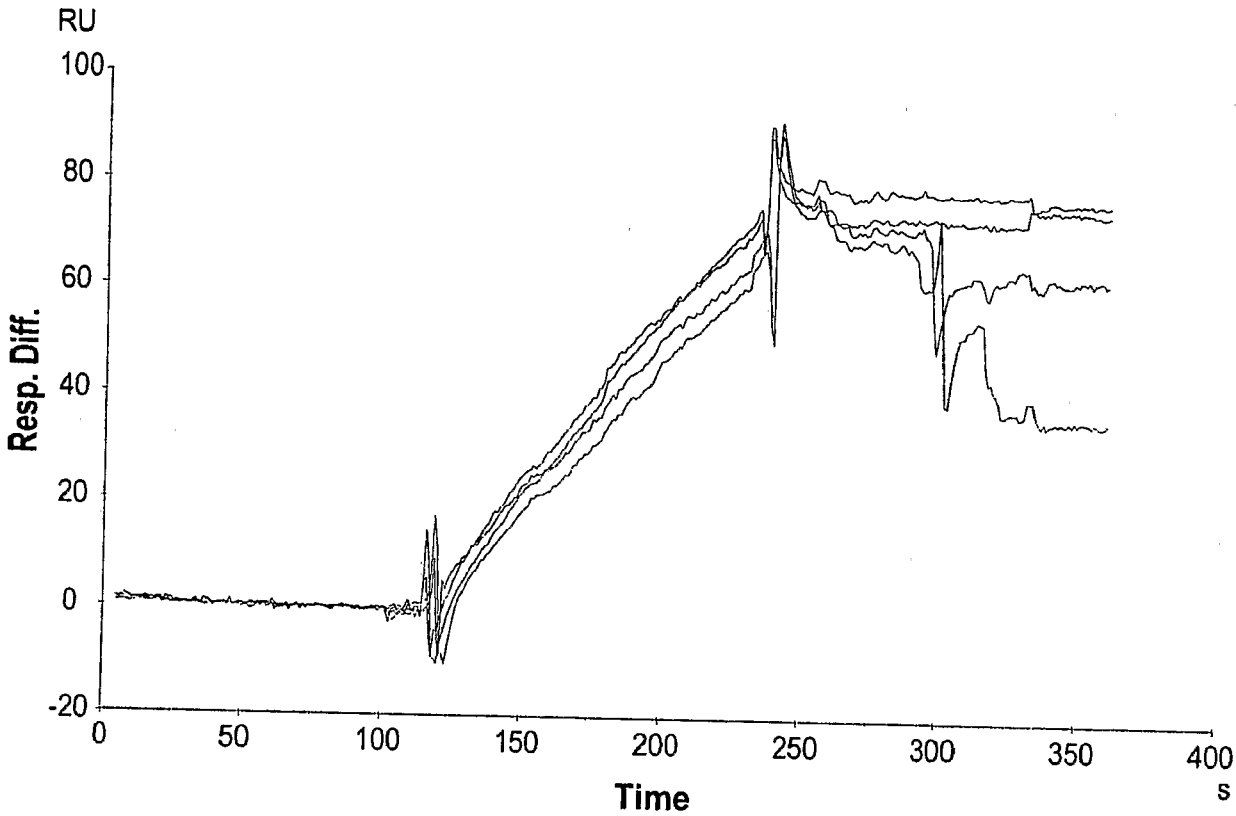

RU
100
80
60
40
20
0
-20
Resp. Diff.
0
50
100
150
200
250
300
350
400
Time
s

Fig. 10

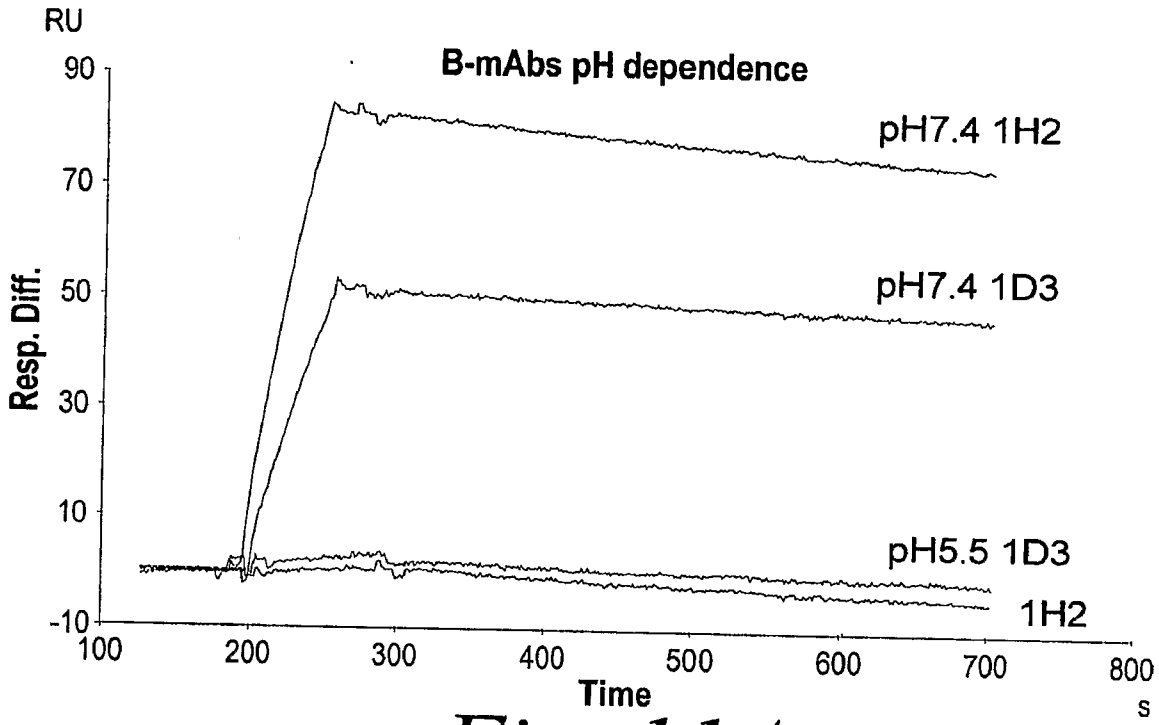


Fig. 11A

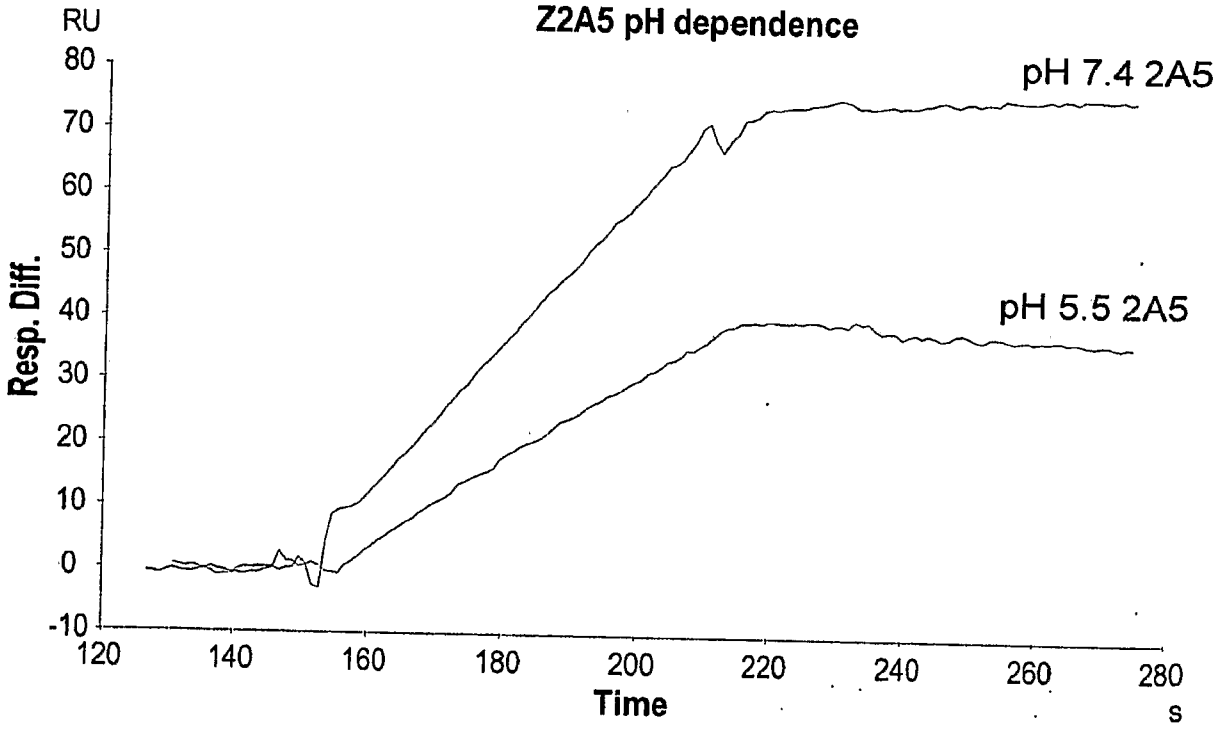


Fig. 11B

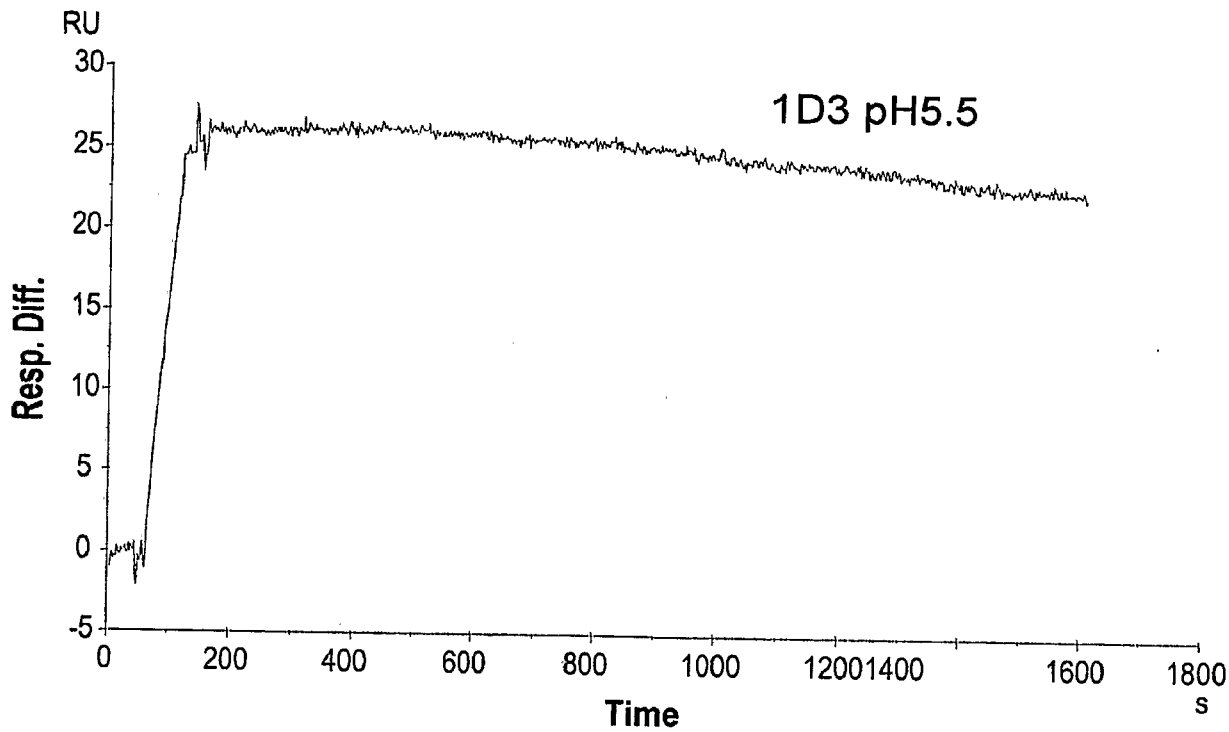

RU
30
25
20
15
10
5
0
-5
Resp. Diff.
1D3 pH5.5
0
200
400
600
800
1000
1200
1400
1600
1800
Time
s

Fig. 12

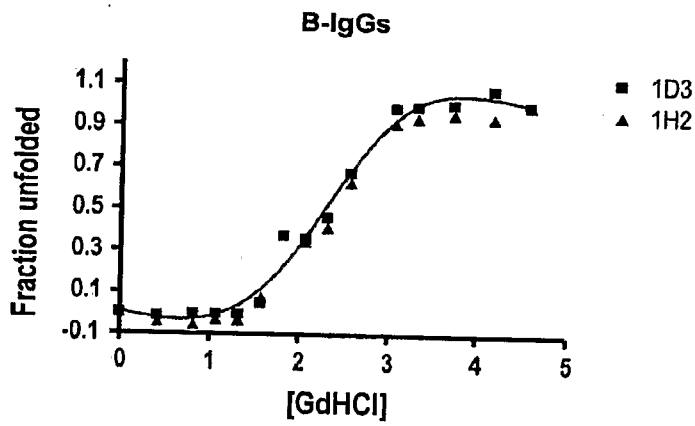


Fig. 13A

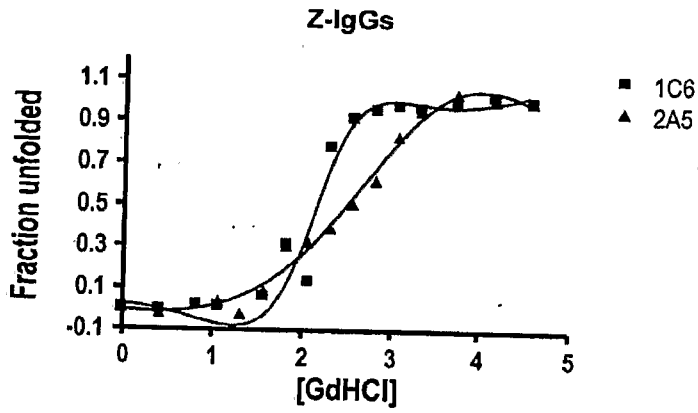


Fig. 13B

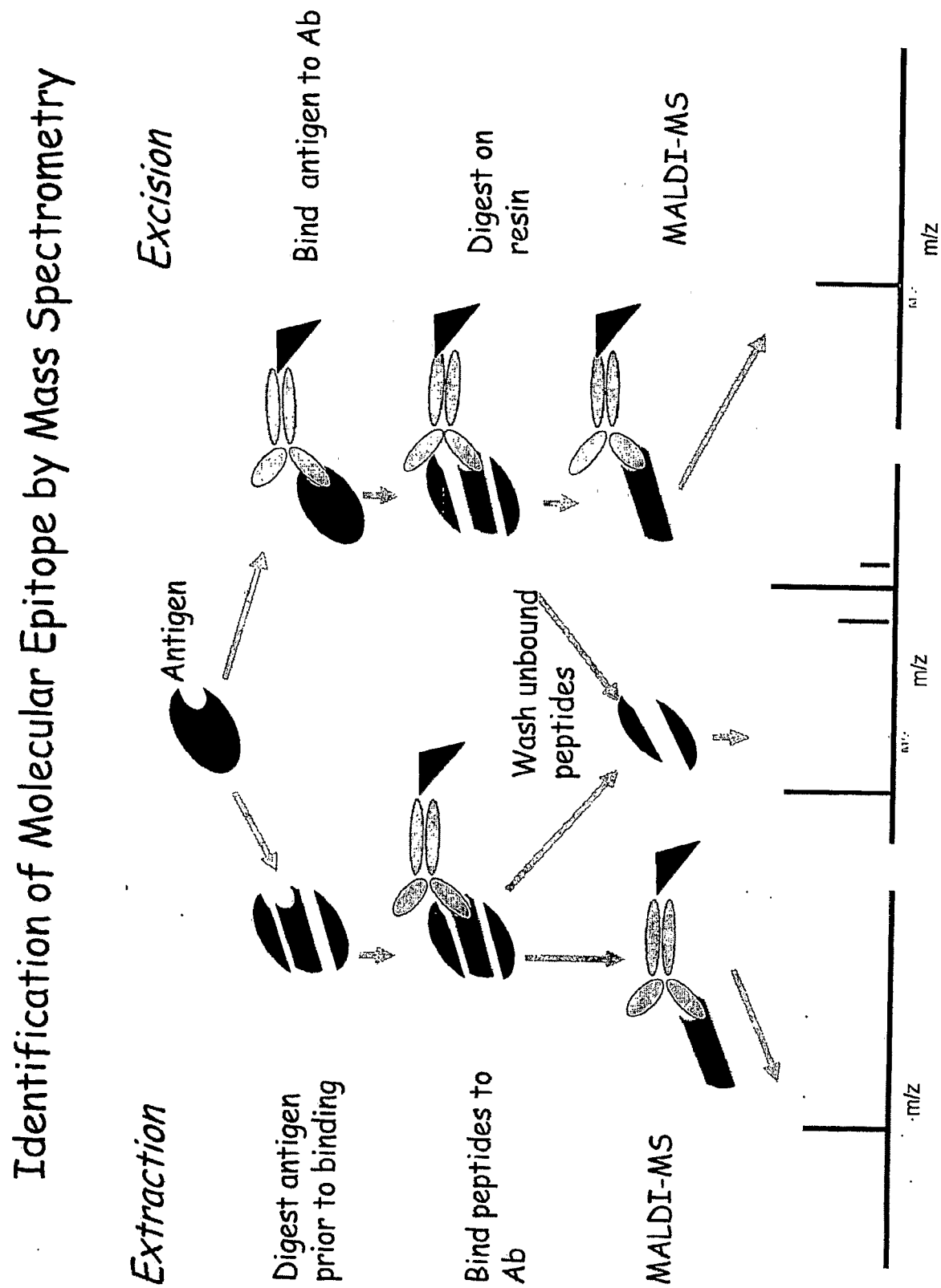
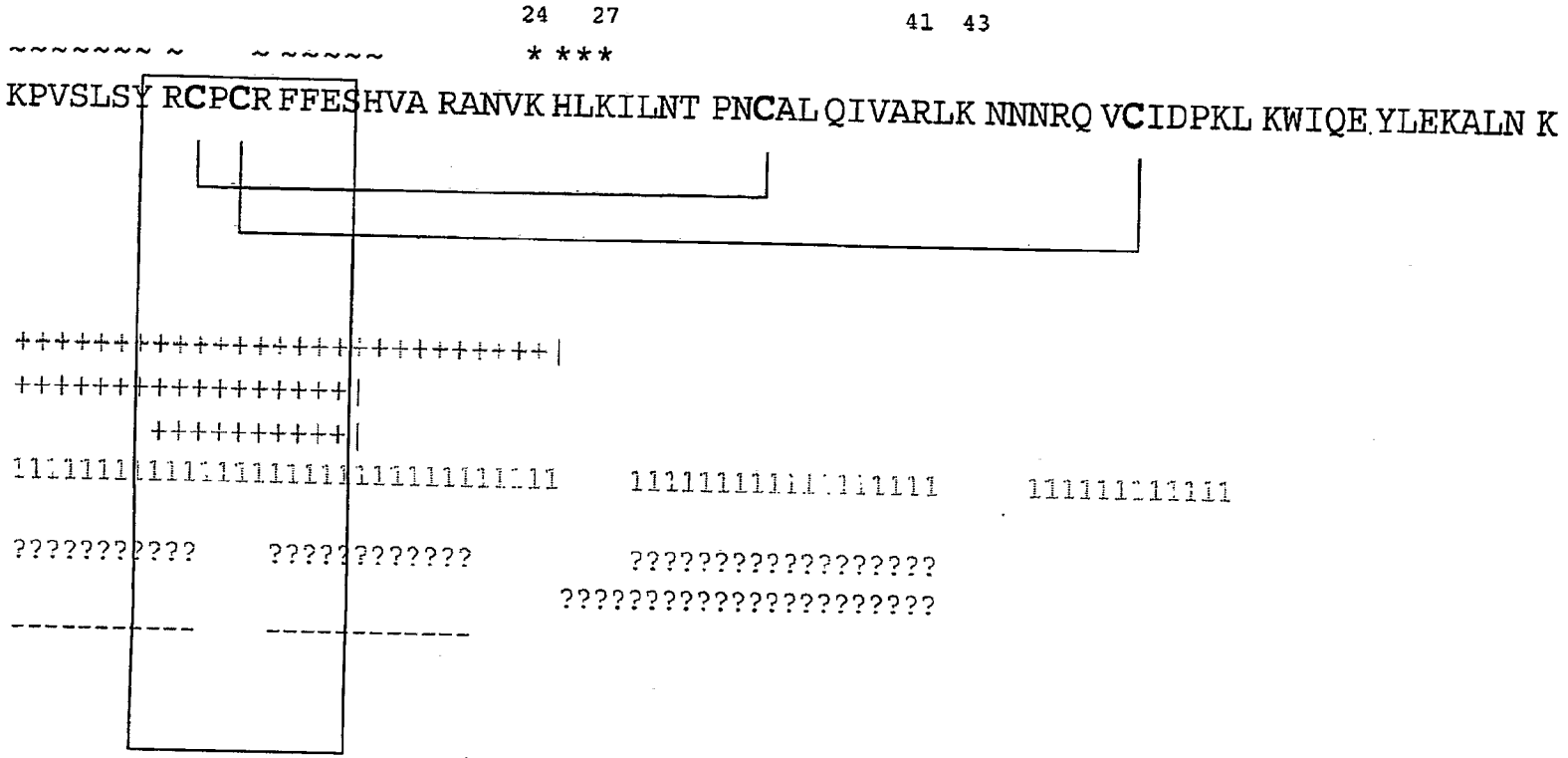

Identification of Molecular Epitope by Mass Spectrometry
Extraction
Excision
Antigen
Digest antigen prior to binding
Bind peptides to Ab
Wash unbound peptides
MALDI-MS
Bind antigen to Ab
Digest on resin
MALDI-MS
m/z
m/z
m/z

Fig. 14A

Different enzyme cleavage to cover entire sequence:

Fig. 14B



Summary of mass spec data on peptide affinity against 2A5 IgG :

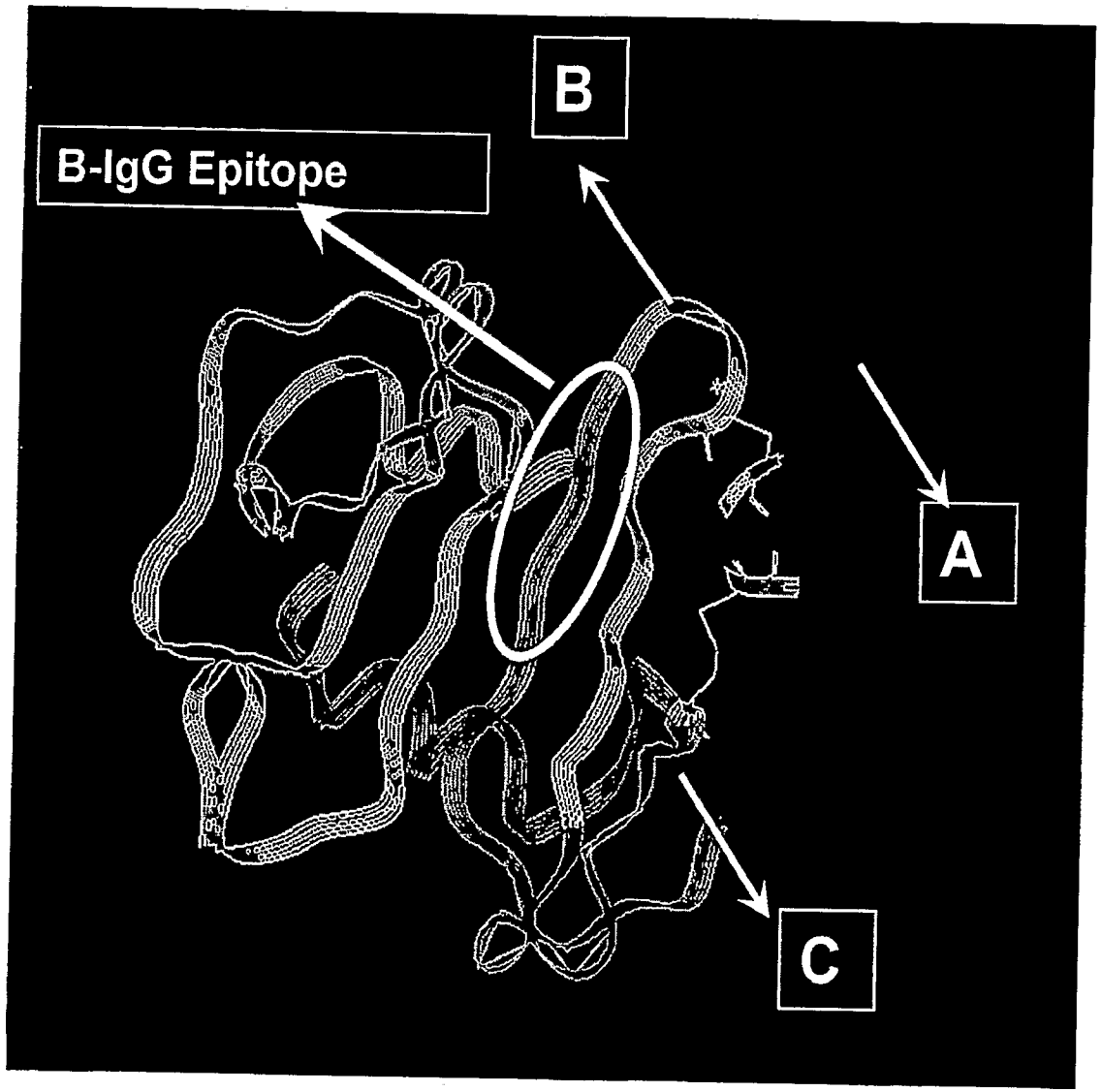
Lys-C
Lys-C, Glu-C *in situ*
Lys-C, Trypsin *in situ*
Trypsin
Arg-C

Binders:	+++++++
Binders linked by disulphides:	11111111
Weak binders:	???????
Non-binders:	-------

~	Receptor binding
*	Heparin recognition
boxed:	epitope peptide 7-15

Fig. 15

Molecular epitope of 2A5



Epitope A: strong signal

B & C: weak signal

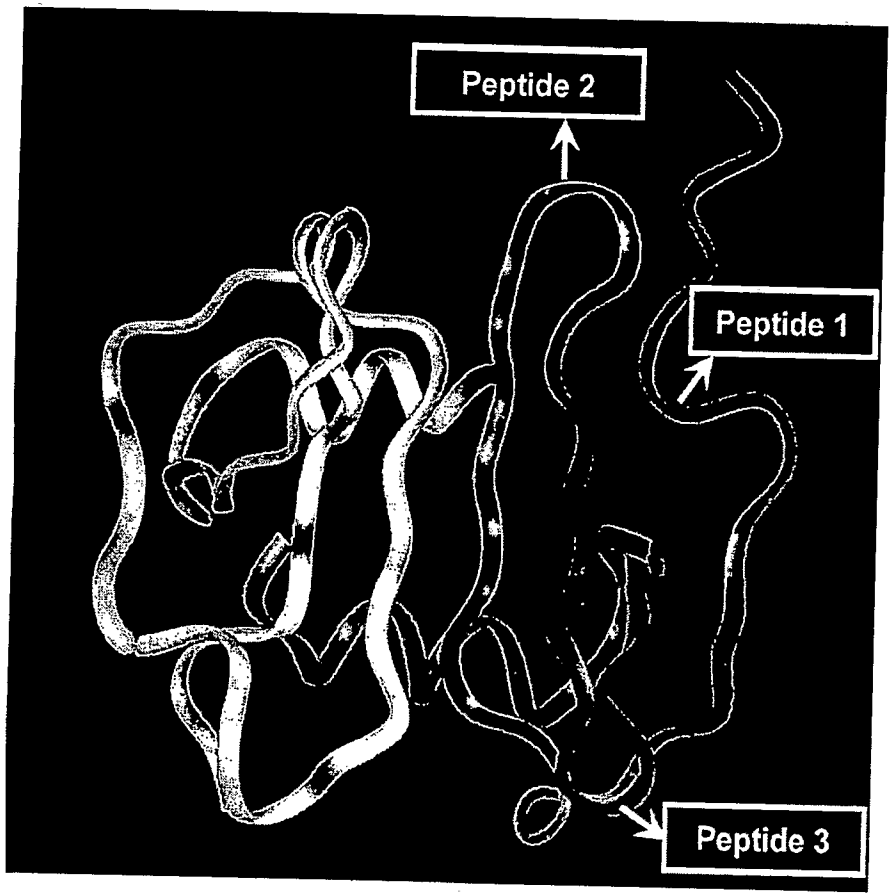
A: 7-15

B: 28-40

C: 45-54

Heparin site: 24-27 (adjacent to epitope B & dimer interface).

Fig. 16A



Peptide 1: N-terminal region, 7-18 (orange)
Peptide 2: Dimer interface and heparin binding region, 20-33 (green)
Peptide 3: Residues 37-50 (pink)

	7 8 9 10 11 12 13 14 15 16 17 18
Peptide 1a:	Y R C P C R F F E S H V
Peptide 1b:	Y R C P C A F F E S H V
Peptide 1c:	Y A C P C R F F E S H V

	20 21 22 23 24 25 26 27 28 29 30 31
Peptide 2a:	R A N V K H L K I L N T
Peptide 2b:	R A N V A H L A I L N T

	27 28 29 30 31 32 33
Peptide 2c:	K I L N T P N

	37 38 39 40 41 42 43 44 45 46
Peptide 3a:	Q I V A R L K N N N

	41 42 43 44 45 46 47 48 49 50
Peptide 3b:	R L K N N N R Q V C

	41 42 43 44 45 46 47 48 49 50
Peptide 3c:	R L K N N N A A V C

	41 42 43 44 45 46 47 48 49 50
Peptide 3d	A L A N N N R Q V C

Fig. 16B

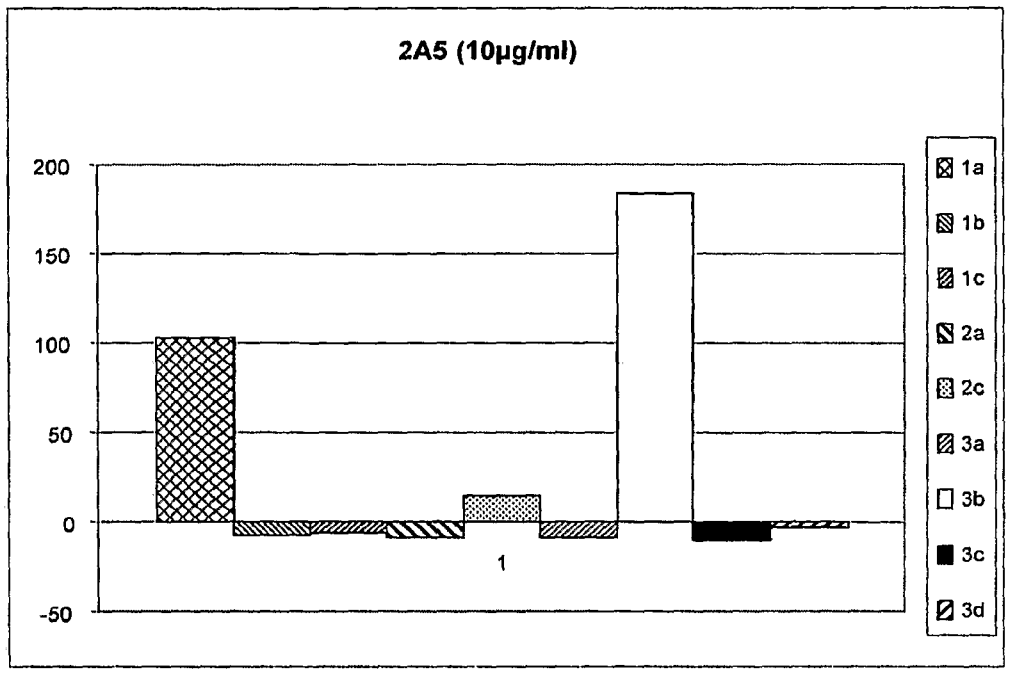


Fig. 17A

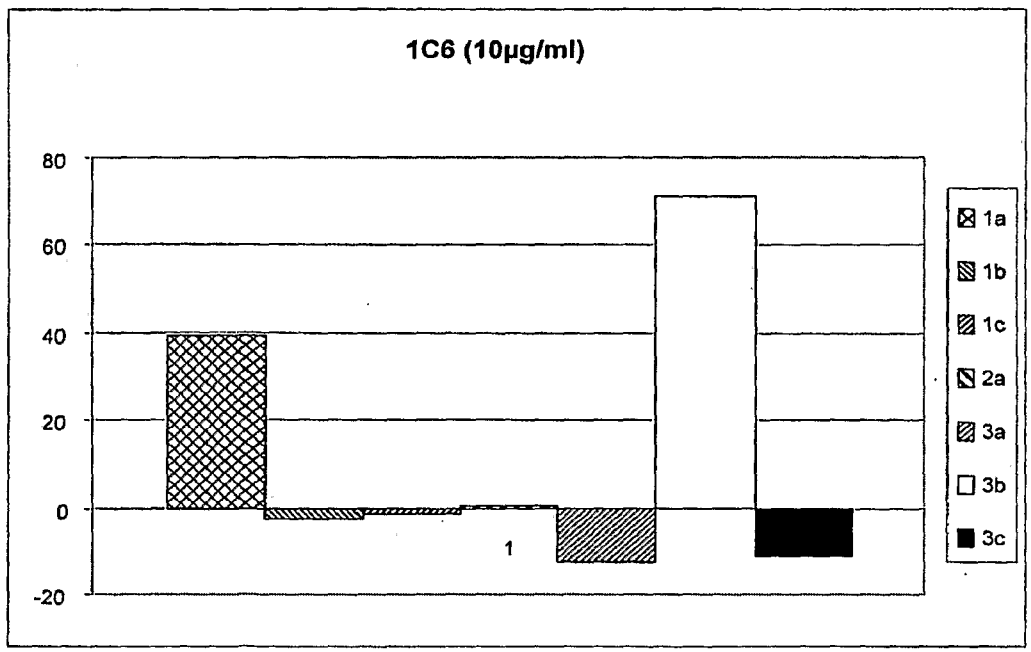


Fig. 17B

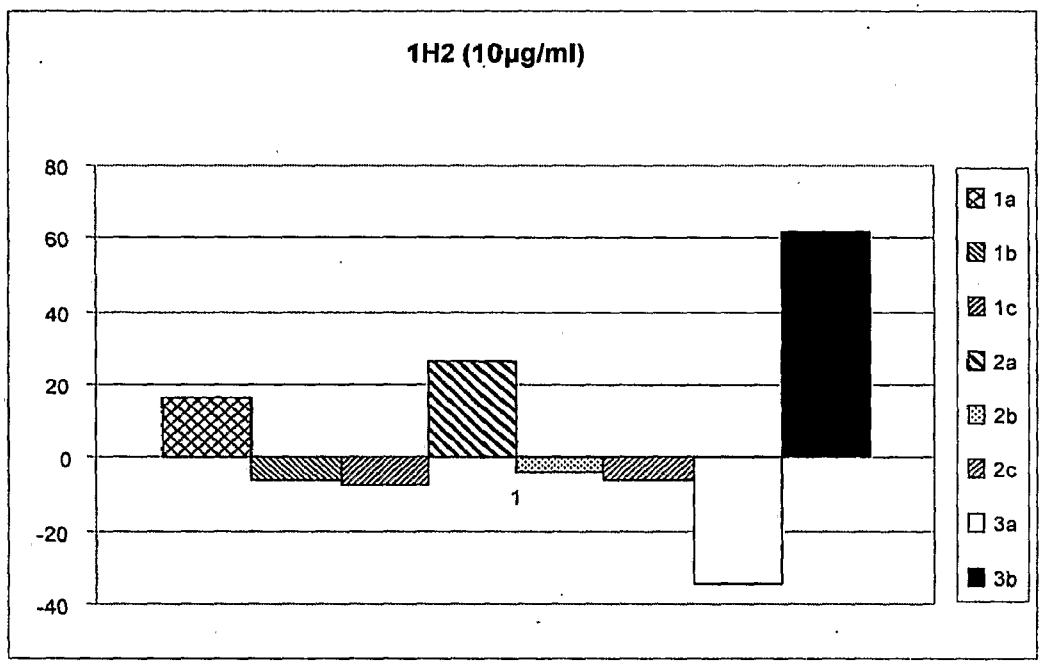


Fig. 18A

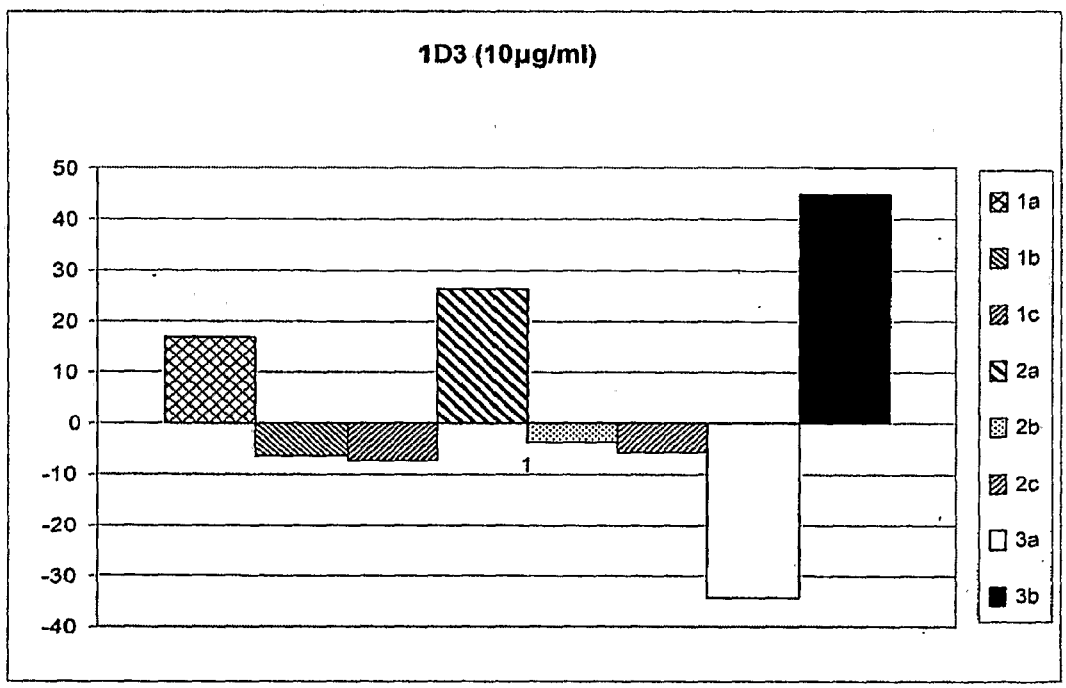


Fig. 18B

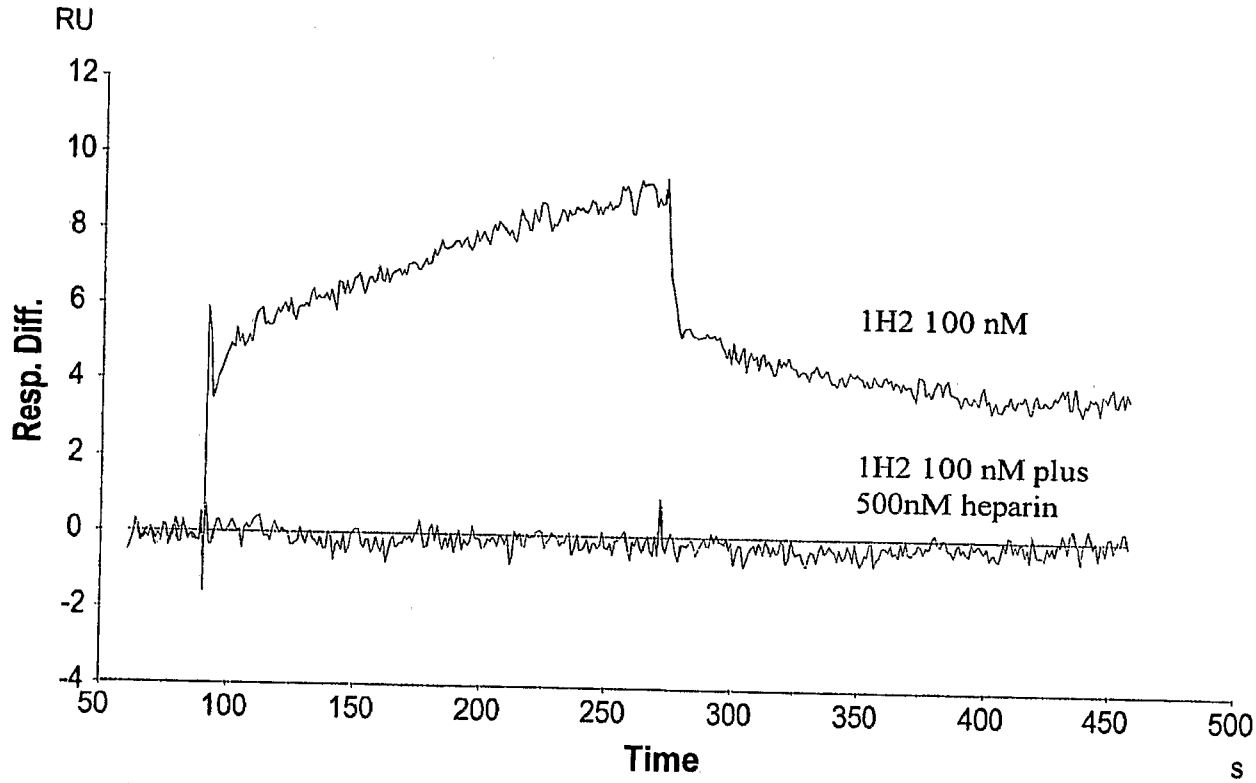

RU
12
10
8
6
4
2
0
-2
-4
Resp. Diff.
1H2 100 nM
1H2 100 nM plus
500nM heparin
50
100
150
200
250
300
350
400
450
500
Time
s

Fig. 19

Anti-SDF-1 1D3 VH

V segment: 1-24
D segment: 6-19
J segment: JH6b
primer encoded

```
                                  E   V   Q   L   V   Q   S   G   A   E   V   K   K   P   G   A   S   V   K
                                  1  GAG GTG CAG CTG GTG CAG TCT GGG GCT GAG GTG AAG AAG CCT GGG GCC TCA GTG AAG
                                                                                  CDR1
                                                                                  ~~~~~~~~~~~~~~~~~~~~
                                  V   S   C   K   V   S   G   Y   T   L   T   K   L   S   V   H   W   V   R
                                 58  GTC TCC TGC AAG GTT TCC GGA TAC ACC CTC ACT AAA TTA TCC GTG CAC TGG GTG CGA
                                                                                  CDR2
                                                                                  ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
                                  Q   A   P   G   K   G   L   E   W   M   G   S   F   D   P   E   D   G   E
                                115  CAG GCT CCT GGA AAA GGG CTT GAG TGG ATG GGA AGT TTT GAT CCT GAA GAT GGT GAA
                                          CDR2
                                          ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
                                  T   I   Y   S   Q   R   F   Q   G   R   V   T   M   T   E   D   T   S   T
                                172  ACA ATC TAC TCA CAG AGG TTC CAG GGC AGA GTC ACC ATG ACC GAG GAC ACA TCT ACA

                                  D   T   A   Y   M   E   L   T   S   L   R   S   E   D   T   A   V   Y   Y
                                229  GAC ACA GCC TAC ATG GAG CTG ACC AGC CTG AGA TCT GAG GAC ACG GCC GTG TAT TAC
                                                                  CDR3
                                                  ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
                                  C   A   T   E   G   Q   W   L   V   A   Y   Y   G   M   D   V   W   G   Q
                                286  TGT GCA ACG GAG GGG CAG TGG CTG GTA GCC TAC TAC GGT ATG GAC GTC TGG GGC CAA

                                  G   T   T   V   T   V   S   S
                                343  GGG ACC ACG GTC ACC GTC TCC TCA
```

Fig. 20A

Anti-SDF-1 1D3 VK

V segment: L18
J segment: JK4
primer encoded

```
   ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
    E   I   V   L   T   Q   S   P   S   S   L   S   A   S   V   G   D   R   V
1   GAA ATT GTG CTC ACC CAG TCT CCA TCC TCC CTG TCT GCA TCT GTA GGA GAC AGA GTC
                                  CDR1
                    ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
    T   I   T   C   R   A   S   Q   G   I   S   S   A   L   A   W   Y   Q   Q
58  ACC ATC ACT TGC CGG GCA AGT CAG GGC ATT AGC AGT GCT TTA GCC TGG TAT CAG CAG
                                                  CDR2
                                    ~~~~~~~~~~~~~~~~~~~~~~~~~~~~
    K   P   G   K   A   P   K   L   L   I   Y   D   A   S   S   L   E   S   G
115 AAA CCA GGG AAA GCT CCT AAG CTC CTG ATC TAT GAT GCC TCC AGT TTG GAA AGT GGG

    V   P   S   R   F   S   G   S   G   S   G   T   D   F   T   L   T   I   S
172 GTC CCA TCA AGG TTC AGC GGC AGT GGA TCT GGG ACA GAT TTC ACT CTC ACC ATC AGC
                                                      CDR3
                                        ~~~~~~~~~~~~~~~~~~~~~~~~~~~~
    S   L   Q   P   E   D   F   A   T   Y   Y   C   Q   Q   F   N   S   Y   P
229 AGC CTG CAG CCT GAA GAT TTT GCA ACT TAT TAC TGT CAA CAG TTT AAT AGT TAC CCG
    CDR3
    ~~~~~~~~
    L   T   F   G   G   G   T   K   V   E   I   K
286 CTC ACT TTC GGC GGA GGG ACC AAG GTG GAG ATC AAA
```

Fig. 20B

Anti-SDF-1 1H2 VH

V segment: 1-24
D segment: 6-19
J segment: JH6b
primer encoded

```
                                                                  CDR1
                                                                  ~~~~~~~~~~~~~~~~~~~~
 Q   V   Q   L   V   Q   S   G   A   E   V   K   K   P   G   A   S   V   K
 1  CAG GTG CAG CTG GTG CAG TCT GGG GCT GAG GTG AAG AAG CCT GGG GCC TCA GTG AAG

                                                                  CDR2
                                                                  ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
 V   S   C   K   V   S   G   Y   T   F   T   K   L   S   V   H   W   V   R
58  GTC TCC TGC AAG GTT TCC GGA TAC ACC TTC ACT AAA TTA TCC GTG CAC TGG GTG CGA

                                                                  CDR2
                                                                  ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
 Q   A   P   G   K   G   L   E   W   M   G   S   F   D   P   E   D   G   E
115  CAG GCT CCT GGA AAA GGG CTT GAG TGG ATG GGA AGT TTT GAT CCT GAA GAT GGT GAA
            CDR2
            ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
 T   I   Y   S   Q   R   F   Q   G   R   V   T   M   T   E   D   T   S   T
172  ACA ATC TAC TCA CAG AGG TTC CAG GGC AGA GTC ACC ATG ACC GAG GAC ACA TCT ACA

 D   T   A   Y   M   E   L   S   S   L   R   S   E   D   T   A   V   Y   Y
229  GAC ACA GCC TAC ATG GAG CTG AGC AGC CTG AGA TCT GAG GAC ACG GCC GTG TAT TAC
                                  CDR3
                                  ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
 C   A   T   E   G   Q   W   L   V   A   Y   Y   G   M   D   V   W   G   Q
286  TGT GCA ACG GAG GGG CAG TGG CTG GTA GCC TAC TAC GGT ATG GAC GTC TGG GGC CAA

 G   T   M   V   T   V   S   S
343  GGG ACC ATG GTC ACC GTC TCC TCA
```

Fig. 21A

Anti-SDF-1 1H2 VK

V segment: L18
J segment: JK4
primer encoded

```
  E   I   V   L   T   Q   S   P   S   S   L   S   A   S   V   G   D   R   V
  1 GAA ATT GTG CTC ACC CAG TCT CCA TCC TCC CTG TCT GCA TCT GTA GGA GAC AGA GTC
                                CDR1
                    ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
  T   I   T   C   R   A   S   Q   G   I   S   S   A   L   A   W   Y   Q   Q
 58 ACC ATC ACT TGC CGG GCA AGT CAG GGC ATT AGC AGT GCT TTA GCC TGG TAT CAG CAG
                                                    CDR2
                                          ~~~~~~~~~~~~~~~~~~~~~~~~~~~~
  K   P   G   K   A   P   K   L   L   I   Y   D   A   S   S   L   E   S   G
115 AAA CCA GGG AAA GCT CCT AAG CTC CTG ATC TAT GAT GCC TCC AGT TTG GAA AGT GGG

  V   P   S   R   F   S   G   S   G   S   G   T   D   F   T   L   T   I   S
172 GTC CCA TCA AGG TTC AGC GGC AGT GGA TCT GGG ACA GAT TTC ACT CTC ACC ATC AGC
                                                    CDR3
                                          ~~~~~~~~~~~~~~~~~~~~~~~~~~~~
  S   L   Q   P   E   D   F   A   T   Y   Y   C   Q   Q   F   N   S   Y   P
229 AGC CTG CAG CCT GAA GAT TTT GCA ACT TAT TAC TGT CAA CAG TTT AAT AGT TAC CCG
  CDR3
  ~~~~~~~
  L   T   F   G   G   G   T   K   V   E   I   K
286 CTC ACT TTC GGC GGA GGG ACC AAG GTG GAG ATC AAA
```

Fig. 21B