DUAL SPECIFIC BINDING PROTEINS DIRECTED AGAINST TNFα

[001] This application claims priority to U.S. Provisional Application Serial No. 61/794,964, filed March 15, 2013, which is hereby incorporated by reference in its entirety.

[002] Multivalent and multispecific binding proteins are disclosed that bind TNFα, IL-13, PGE2, and/or NGF, as well as methods of making and using the binding proteins in the diagnosis, prevention, and/or treatment of acute and chronic inflammatory diseases, cancer, and other diseases.

[003] Engineered proteins, such as multispecific binding proteins capable of binding two or more antigens, are known in the art. Such multispecific binding proteins can be generated using cell fusion, chemical conjugation, or recombinant DNA techniques. There are a variety of multispecific binding protein structures known in the art and many structures and methods have distinct disadvantages.

[004] Bispecific antibodies have been produced using quadroma technology. However, the presence of mis-paired by-products and significantly reduced production yields with this technology means that sophisticated purification procedures are required. Bispecific antibodies can also be produced by chemical conjugation of two different mAbs. However, this approach does not yield homogeneous preparations.

[005] Other approaches used previously include coupling of two parental antibodies with a hetero-bifunctional crosslinker, production of tandem single-chain Fv molecules, diabodies, bispecific diabodies, single-chain diabodies, and di-diabodies. However, each of these approaches have disadvantages. In addition, a multivalent antibody construct comprising two Fab repeats in the heavy chain of an IgG and capable of binding four antigen molecules has been described (see PCT Publication No. WO 0177342 and Miller et al. (2003) J. Immunol. 170(9): 4854-61).

[006] US Patent No. 7,612,181 (incorporated herein by reference in its entirety) provides a novel family of binding proteins capable of binding two or more antigens with high affinity, which are called dual variable domain binding proteins (DVD binding protein) or dual variable domain immunoglobulins (DVD-Ig™). DVD-Ig molecules are proteins that may be used to bind two distinct epitopes on the same molecule or two different molecules simultaneously. DVDs are unique binding proteins comprised of two variable domains fused to N-terminal constant regions. The variable

domains may be directly fused to one another or connected via synthetic peptide linkers of assorted length and amino acid composition. DVD-Ig proteins may be engineered with intact and functional Fc domains, allowing then to mediate appropriate effector functions. DVD-Ig format, due to its flexibility of choice of variable domain pair, orientation of two antigen-binding domains, and the length of the linker that joins them, may provide for novel therapeutic modalities.

[007] While a variety of structures are provided in the art, some with advantages and disadvantages, specific constructs are required for preparing multivalent binding proteins with specific properties and which bind to specific targets. Additionally, new variable domain sequences can further improve the properties of binding proteins. For example, there remains a need for constructs exhibiting better targeting and/or desired efficiency of binding to TNFα and a second target chosen from IL-13, PGE2, or NGF, e.g., to prevent, diagnose, and/or treat autoimmune, inflammatory, or neurological disorders. There is thus a need in the art for improved multivalent binding proteins capable of binding TNFα, IL-13, PGE2, and/or NGF.

[008] Accordingly, disclosed herein are dual variable domain immunoglobulins using the binding protein framework disclosed in US Patent No. 7,612,181 (incorporated herein by reference in its entirety) and containing particular first and second polypeptide chains, each comprising first and second variable domains comprising sequences (e.g., sequences selected from those listed in Table 1) that form functional binding sites for binding targets such as TNF-α, IL-13, PGE2, and/or NGF. In some embodiments, the first and second polypeptide chains of the binding protein each independently comprise VD1-(X1)n-VD2-C-X2, wherein: VD1 is a first variable domain; VD2 is a second variable domain; C is a constant domain; X1 is a linker; X2 is an Fc region that is either present or absent; n is 0 or 1, and wherein the VD1 domains on the first and second polypeptide chains form a first functional target binding site for TNF-α, IL-13, PGE2, or NGF, and the VD2 domains on the first and second polypeptide chains form a second functional target binding site for TNF-α, IL-13, PGE2, or NGF. In some embodiments, an Fc domain is present on one polypeptide chain and absent on the other, or absent on both polypeptide chains. In some embodiments, the sequences of the first and second variable domains on each polypeptide chain (i.e., VD1 and VD2) are selected from the sequences in Table 1 to form functional binding sites. In some embodiments, the sequences of the first and second variable domains each contain the three CDRs (i.e., CDRs 1-3) from the selected sequences listed in Table 1 and are arranged in the same order as shown in

Table 1, thereby forming functional binding sites (i.e., the binding domains are capable of binding to their target antigen TNF-α, IL-13, PGE2, or NGF). In some embodiments, the paired variable domain sequences on the first and second polypeptide chains (i.e., the VD1 sequence on the first chain paired with the VD1 sequence on the second chain and the VD2 sequence on the first chain paired with the VD2 sequence on the second chain) form functional binding sites for binding targets TNF-α, IL-13, PGE2, and/or NGF. In some embodiments, the binding proteins are capable of binding to TNF-α, IL-13, PGE2, and/or NGF with improved binding affinity and/or neutralization potency.

Brief Description of the Drawings

[009] Figure 1 is a schematic representation of Dual Variable Domain (DVD) binding protein construct according to certain embodiments of the present disclosure.

Detailed Description

[010] TNF-α plays a role in the pathology associated with a variety of diseases involving immune and inflammatory elements, such as autoimmune diseases, particularly those assocated with inflammation, including Crohn's disease, psoriasis (including plaque psoriasis), arthritis (including rheumatoid arthritis, psoratic arthritis, osteoarthritis, or juvenile idiopathic arthritis), multiple sclerosis, systemic lupus erythematosus, and ankylosing spondylitis.

[011] Interleukin 13 (IL-13) is a 17-kDa glycoprotein produced by activated T cells of the Th2 lineage. The function of IL-13 includes immunoglobulin isotype switching to IgE in human B cells and suppressing inflammatory cytokine production. IL-13 is associated primarily with the induction of airway inflammation such as asthma. It has also been linked to other allergic diseases, fibrotic conditions, cancer and infectious diseases

[012] Disclosed herein are improved binding proteins against TNFα, IL-13, PGE2, and/or NGF

Binding Proteins

[013] In some embodiments, a binding protein is disclosed comprising first and second polypeptide chains, each independently comprising VD1-(X1)n-VD2-C-X2, wherein: VD1 is a first variable domain; VD2 is a second variable domain; C is a

constant domain; X1 is a linker; X2 is an Fc region that is either present or absent; n is independently 0 or 1 on the first and second chains, and wherein the VD1 domains on the first and second polypeptide chains form a first functional target binding site and the VD2 domains on the first and second polypeptide chains form a second functional target binding site. In some embodiments, the binding protein is capable of binding one or more of TNFα, IL-13, PGE2, and NGF. In some embodiments, the binding protein comprises VD1 sequences on the first and second polypeptide chains (i.e., a VD1 sequence on the first chain paired with a VD1 sequence on the second chain) that together form a binding domain capable of binding TNFα and IL-13, TNFα and PGE2, or TNFα and NGF. In an embodiment, binding proteins capable of binding TNFα and IL-13, TNFα and PGE2, or TNFα and NGF with high affinity are provided. In some embodiments, the binding protein is capable of binding TNFα at the VD1 position and a second target (IL-13, PGE2, or NGF) at the VD2 position. In some embodiments, the binding protein is capable of binding a second target (IL-13, PGE2, or NGF) at the VD1 position and TNFα at the VD2 position.

[014] The binding proteins disclosed herein comprise VD1 and VD2 binding domains that are capable of binding to first and second target antigens. As used herein, a VD1 domain or a VD2 domain, or a VD1 position or VD2 position, may refer to either the variable domain sequence on one polypeptide chain (e.g., a VD1 heavy chain sequence) or to the variable domain sequences on both the first and second polypeptide chain (e.g., a VD1 heavy chain sequence and a VD1 light chain sequence) that together form the functional binding site.

[015] In some embodiments, the VD1 sequences that form the VD1 binding site are selected from the paired sequences in Table 1 (for example, the paired sequences of SEQ ID NO: 32 and 33 in Table 1 that together form a binding site for IL-13). In some embodiments, the VD2 sequences that form the VD2 binding site are selected from the paired sequences in Table 1 (for example, the paired sequences of SEQ ID NO: 32 and 33 in Table 1 that together form a binding site for IL-13). In some embodiments, the VD1 and/or VD2 sequences comprise CDRs 1-3 of the sequences selected from Table 1 but have different variable domain framework sequences (e.g., variable domains that are CDR grafted, affinity matured, humanized, humanized and backmutated, or other functional variants of the sequences disclosed in Table 1).

[016] When the binding protein comprises the CDRs from a sequence selected from Table 1, the CDRs are arranged in the order specified by the sequence in Table 1 and separated by suitable framework sequences to form a functional

binding site. The paired sequences selected from Table 1 that form a functional binding site for a target (i.e., a binding site for TNFα, IL-13, PGE2, or NGF), or the CDRs from those sequences, may be placed in either the VD1 or VD2 positions on the first and second polypeptide chains to form a binding site at either the VD1 or VD2 domain. For instance, matching heavy and light chain variable domain sequences from Table 1 that form a binding site for IL-13 (e.g., SEQ ID NO: 34 and 35) can be placed in the VD1 positions on the first and second polypeptide chains to form a VD1 binding site for IL-13. In another example, the matching heavy and light chain sequences from Table 1 that form a binding site for IL-13 (e.g., SEQ ID NO: 34 and 35) can be placed in the VD2 positions on the first and second polypeptide chains to form a VD2 binding site for IL-13. The same or different sequences may occupy both the VD1 and VD2 positions. For example, SEQ ID NO: 34 and 35 may be used to form a binding domain at the VD1 position and at the VD2 position, or SEQ ID NO: 34 and 35 may form the binding domain at one of the VD1 and VD2 positions, while a different sequence pair can be selected to form the binding domain at the other position. Similarly, any of the other sequence pairs in Table 1 may be selected for use in either or both of the VD1 and VD2 positions on the first and second polypeptide chains.

[017] In some embodiments, the variable domain sequences on the first and second polypeptide chains that form a functional target binding site for IL-13 in a binding protein (i.e., at the VD1 and/or VD2 positions) can comprise the paired variable domain sequences selected from those in Table 1 or CDRs 1-3 from those sequences. For instance, the variable domains that form a functional target binding site for IL-13 can comprise SEQ ID NO: 32 and SEQ ID NO: 33, SEQ ID NO: 34 and SEQ ID NO: 35, SEQ ID NO: 36 and SEQ ID NO: 37, or CDRs 1-3 from those paired variable domain sequences. For example, the variable domains that form a functional target binding site for IL-13 can comprise CDRs 1-3 from SEQ ID NO: 32 on one polypeptide chain paired with CDRs 1-3 from SEQ ID NO: 33 on the other chain.

[018] In some embodiments, the variable domain sequences on the first and second polypeptide chains that form a functional target binding site for TNF in a binding protein (i.e., at the VD1 and/or VD2 positions) can comprise the paired variable domain sequences selected from those in Table 1 or CDRs 1-3 from those sequences. For instance, the variable domains that form a functional target binding site for TNF can comprise SEQ ID NO: 38 and SEQ ID NO: 39, SEQ ID NO: 40 and SEQ ID NO: 41, SEQ ID NO: 42 and SEQ ID NO: 43, SEQ ID NO: 48 and SEQ ID

NO: 49, or CDRs 1-3 from those paired variable domain sequences. For example, the variable domains that form a functional target binding site for TNF can comprise CDRs 1-3 from SEQ ID NO: 38 on one polypeptide chain paired with CDRs 1-3 from SEQ ID NO: 39 on the other chain.

[019] In some embodiments, the variable domain sequences on the first and second polypeptide chains that form a functional target binding site for PGE2 in a binding protein (i.e., at the VD1 and/or VD2 positions) can comprise the paired variable domain sequences selected from those in Table 1 or CDRs 1-3 from those sequences. For instance, the variable domains that form a functional target binding site for PGE2 can comprise SEQ ID NO: 50 and SEQ ID NO: 51, SEQ ID NO: 52 and SEQ ID NO: 53, SEQ ID NO: 54 and SEQ ID NO: 55, or CDRs 1-3 from those paired variable domain sequences. For example, the variable domains that form a functional target binding site for PGE2 can comprise CDRs 1-3 from SEQ ID NO: 50 on one polypeptide chain paired with CDRs 1-3 from SEQ ID NO: 51 on the other chain.

[020] In some embodiments, the variable domain sequences on the first and second polypeptide chains that form a functional target binding site for NGF in a binding protein (i.e., at the VD1 and/or VD2 positions) can comprise the paired variable domain sequences selected from those in Table 1 or CDRs 1-3 from those sequences. For instance, the variable domains that form a functional target binding site for NGF can comprise SEQ ID NO: 56 and SEQ ID NO: 57, or CDRs 1-3 from those paired variable domain sequences. For example, the variable domains that form a functional target binding site for NGF can comprise CDRs 1-3 from SEQ ID NO: 56 on one polypeptide chain paired with CDRs 1-3 from SEQ ID NO: 57 on the other chain.

[021] In an embodiment, a binding protein comprises a functional target binding site for TNF (i.e., a TNF binding site at either the VD1 or VD2 position) and a functional target binding site for IL-13 (i.e., an IL-13 binding site at either the VD2 or VD1 position). In an embodiment, the TNF binding site comprises CDRs 1-3 from SEQ ID NO: 38 and CDRs 1-3 from SEQ ID NO: 39, CDRs 1-3 from SEQ ID NO: 40 and CDRs 1-3 from SEQ ID NO: 41, CDRs 1-3 from SEQ ID NO: 42 and CDRs 1-3 from SEQ ID NO: 43, or CDRs 1-3 from SEQ ID NO: 48 and CDRs 1-3 from SEQ ID NO: 49. In an embodiment, the TNF binding site comprises SEQ ID NO: 38 and SEQ ID NO: 39, SEQ ID NO: 40 and SEQ ID NO: 41, SEQ ID NO: 42 and SEQ ID NO: 43, or SEQ ID NO: 48 and SEQ ID NO: 49. In an embodiment, the IL-13 binding site comprises CDRs 1-3 from SEQ ID NO: 32 and CDRs 1-3 from SEQ ID NO: 33, CDRs

1-3 from SEQ ID NO: 34 and CDRs 1-3 from SEQ ID NO: 35, or CDRs 1-3 from SEQ ID NO: 36 and CDRs 1-3 from SEQ ID NO: 37. In an embodiment, the IL-13 binding site comprises SEQ ID NO: 32 and SEQ ID NO: 33, SEQ ID NO: 34 and SEQ ID NO: 35, or SEQ ID NO: 36 and SEQ ID NO: 37. In an embodiment, the X1 linker on the first and/or second polypeptide chain comprises any one of SEQ ID NOs: 1-31. In an embodiment, the binding protein comprises first and second polypeptide chains comprising any of the paired heavy and light chain SEQ ID NOs listed in Table 2. In an embodiment, the binding protein is capable of binding TNF with a K_D of at most about 5.8 $x10^{-11}$ M, as measured by surface plasmon resonance, and/or capable of neutralizing TNF with an IC50 of at most about 0.731 nM, as measured in a TNF neutralization assay, and/or the binding protein is capable of binding IL-13 with a K_D of at most about 1.2 $x10^{-9}$ M, as measured by surface plasmon resonance, and/or capable of neutralizing IL-13 with an IC50 of at most about 1.379 nM, as measured in an IL-13 neutralization assay.

[022] In an embodiment, a binding protein comprises a functional target binding site for TNF (i.e., a TNF binding site at either the VD1 or VD2 position) and a functional target binding site for PGE2 (i.e., a PGE2 binding site at either the VD2 or VD1 position). In an embodiment, the TNF binding site comprises CDRs 1-3 from SEQ ID NO: 38 and CDRs 1-3 from SEQ ID NO: 39, CDRs 1-3 from SEQ ID NO: 40 and CDRs 1-3 from SEQ ID NO: 41, CDRs 1-3 from SEQ ID NO: 42 and CDRs 1-3 from SEQ ID NO: 43, or CDRs 1-3 from SEQ ID NO: 48 and CDRs 1-3 from SEQ ID NO: 49. In an embodiment, the TNF binding site comprises SEQ ID NO: 38 and SEQ ID NO: 39, SEQ ID NO: 40 and SEQ ID NO: 41, SEQ ID NO: 42 and SEQ ID NO: 43, or SEQ ID NO: 48 and SEQ ID NO: 49. In an embodiment, the PGE2 binding site comprises CDRs 1-3 from SEQ ID NO: 50 and CDRs 1-3 from SEQ ID NO: 51, CDRs 1-3 from SEQ ID NO: 52 and CDRs 1-3 from SEQ ID NO: 53, or CDRs 1-3 from SEQ ID NO: 54 and CDRs 1-3 from SEQ ID NO: 55. In an embodiment, the PGE2 binding site comprises SEQ ID NO: 50 and SEQ ID NO: 51, SEQ ID NO: 52 and SEQ ID NO: 53, or SEQ ID NO: 54 and SEQ ID NO: 55. In an embodiment, the X1 linker on the first and/or second polypeptide chain comprises any one of SEQ ID NOs: 1-31. In an embodiment, the binding protein comprises first and second polypeptide chains comprising any of the paired heavy and light chain SEQ ID NOs listed in Table 3. In an embodiment, the binding protein is capable of neutralizing TNF with an IC50 of at most about 3.076, or about 2.876 nM, as measured in a TNF neutralization assay, and/or the binding protein is capable of neutralizing PGE2 with an IC50 of at most

about 124.8 nM, 34.78 nM, 12.05 nM, or 1.136 nM, as measured in a PGE2 neutralization assay.

[023] In an embodiment, a binding protein comprises a functional target binding site for TNF (i.e., a TNF binding site at either the VD1 or VD2 position) and a functional target binding site for NGF (i.e., an NGF binding site at either the VD2 or VD1 position). In an embodiment, the TNF binding site comprises CDRs 1-3 from SEQ ID NO: 38 and CDRs 1-3 from SEQ ID NO: 39, CDRs 1-3 from SEQ ID NO: 40 and CDRs 1-3 from SEQ ID NO: 41, CDRs 1-3 from SEQ ID NO: 42 and CDRs 1-3 from SEQ ID NO: 43, or CDRs 1-3 from SEQ ID NO: 48 and CDRs 1-3 from SEQ ID NO: 49. In an embodiment, the TNF binding site comprises SEQ ID NO: 38 and SEQ ID NO: 39, SEQ ID NO: 40 and SEQ ID NO: 41, SEQ ID NO: 42 and SEQ ID NO: 43, or SEQ ID NO: 48 and SEQ ID NO: 49. In an embodiment, the NGF binding site comprises CDRs 1-3 from SEQ ID NO: 56 and CDRs 1-3 from SEQ ID NO: 57. In an embodiment, the NGF binding site comprises SEQ ID NO: 56 and SEQ ID NO: 57. In an embodiment, the X1 linker on the first and/or second polypeptide chain comprises any one of SEQ ID NOs: 1-31. In an embodiment, the binding protein comprises first and second polypeptide chains comprising any of the paired heavy and light chain SEQ ID NOs listed in Table 4. In an embodiment, the binding protein is capable of neutralizing TNF with an IC50 of at most about 0.673 nM, or about 0.279 nM, as measured in a TNF neutralization assay, and/or the binding protein is capable of inhibiting NGF with an IC50 of at most about 7.455 nM, or about 2.895 nM, as measured in an NGF inhibition assay.

[024] In some embodiments, a binding protein as described above comprises an X1 linker on each of the first and second polypeptide chain and an X2 Fc region on one of the two chains. The X1 linkers are independently present or absent on each chain (i.e., n is independently chosen from 0 or 1 on each chain). The X1 linkers on the first and second polypeptide chains, if present, can have the same or different sequences. In one embodiment, the X1 on the first and second polypeptide chains are short ("S") (e.g., 6 amino acid or shorter) linkers. In another embodiment, the X1 on the first and second polypeptide chains are long ("L") (e.g., greater than 6 amino acid) linkers. In another embodiment, the X1 on the first chain is a short linker and the X1 on the second chain is a long linker. In another embodiment, the X1 on the first chain is a long linker and the X1 on the second chain is a short linker. In some embodiments, the X1 linkers on the first and/or second polypeptide chains are independently selected from any one of SEQ ID NO: 1-31. In

some embodiments, X1 on the first and/or second polypeptide chain of a binding protein is not a complete CH1 or CL domain, but may comprise portions of those domains. In some embodiments, X1 on the first chain is not CH1, and X1 on the second chain is not CL, or X1 on the first chain is not CL and X1 on the second chain is not CH1. In some embodiments, the choice of X1 linker on the first and/or second polypeptide chain can affect the binding kinetics of the binding protein (e.g., selecting a GS-based linker may significantly improve binding affinity and/or potency).

[025] In some embodiments, X2 (the Fc region) is present on the first polypeptide chain and absent on the second polypeptide chain, while in other embodiments X2 is present on the second chain and absent on the first chain, or X2 is absent on both the first and second chains. In some embodiments, X2 is a variant sequence Fc region. In certain embodiments, the Fc region is an Fc region from an IgG1, IgG2, IgG3, IgG4, IgA, IgM, IgE, or IgD. In some embodiments, the binding protein is a crystallized binding protein.

[026] In some embodiments, the first polypeptide chain of a binding protein, as described above, is a heavy chain, and the second polypeptide chain is a light chain. In certain embodiments, where the first polypeptide chain is a heavy chain and the second polypeptide chain is a light chain, X1 is independently present or absent on each chain (i.e., n is independently chosen from 0 or 1 on each chain), and X2 is present on the heavy chain and absent on the light chain. In some embodiments, the binding protein comprises an X1 linker on the heavy and/or light polypeptide chain that is independently selected from any one of SEQ ID NO: 1-31.

[027] In some embodiments, any of the binding proteins described above can comprise two first polypeptide chains and two second polypeptide chains and four functional binding sites. For instance, a first and second polypeptide chain may be paired on one arm of a binding protein to form two functional binding sites (at the VD1 and VD2 positions), while a second set of first and second polypeptide chains may be paired on the other arm of the binding protein to form two additional functional binding sites (at the VD1 and VD2 positions). An example of a four chain structure having two arms, each arm comprising a first and second polypeptide chain and two functional binding sites, is shown in Figure 1. In some embodiments, the binding domains at the VD1 and VD2 positions on the first and second arms are identical. In other embodiments, the first and second arms contain different domains at the VD1 and VD2 positions. In some embodiments, the VD1 and VD2 binding domains comprise

variable domain sequences selected from Table 1, or comprise the CDRs from the selected sequences.

[028] In various embodiments, the binding proteins described above can comprise constant region amino acid sequences selected from wild type and mutated sequences. In some embodiments, a wild type human kappa light chain constant region sequence is used. In some embodiments, a wild type human lamda light chain constant region sequence is used. In some embodiments, wild type or mutant human IgG heavy chain constant region sequences are used. In some embodiments, wild type or mutant human IgG1 heavy chain constant region sequences are used. In certain embodiments, the mutated sequence is the one shown in Table 4a. In some embodiments, the binding proteins disclosed herein comprise a wild type human kappa light chain constant region sequence and also comprise a wild type human heavy chain IgG1 constant region sequence.

[029] In one embodiment, binding proteins comprising a polypeptide chain that binds TNFα and IL-13, TNFα and PGE2, or TNFα and NGF, wherein the polypeptide chain comprises VD1-(X1)n-VD2-C-X2, wherein VD1 is a first variable domain, VD2 is a second variable domain, C is a constant domain, X1 represents an amino acid or polypeptide, X2 represents an Fc region that is either present or absent, and n is 0 or 1, are provided. In an embodiment, the VD1 and/or VD2 in the binding protein are heavy chain variable domains. In an embodiment, the VD1 and/or VD2 in the binding protein are light chain variable domains. In another embodiment, VD1 and VD2 are capable of binding the same antigen. In another embodiment, VD1 and VD2 are capable of binding different antigens. In still another embodiment, C is a heavy chain constant domain. For example, X1 is a linker with the proviso that X1 is not CH1.

[030] In an embodiment, the binding protein disclosed herein comprises a polypeptide chain that binds TNFα and IL-13, TNFα and PGE2, or TNFα and NGF, wherein the polypeptide chain comprises VD1-(X1)n-VD2-C-X2, wherein VD1 is a first heavy chain variable domain, VD2 is a second heavy chain variable domain, C is a heavy chain constant domain, X1 is a linker, and X2 is an Fc region that is either present or absent. In an embodiment, X1 is a linker with the proviso that it is not CH1.

[031] In an embodiment, the binding protein disclosed herein comprises a polypeptide chain that binds TNFα and IL-13, TNFα and PGE2, or TNFα and NGF, wherein the polypeptide chain comprises VD1-(X1)n-VD2-C, wherein VD1 is a first

light chain variable domain, VD2 is a second light chain variable domain, C is a light chain constant domain, X1 is a linker, and X2 is absent. In an embodiment, X1 is a linker with the proviso that it is not CL.

[032] In another embodiment, a binding protein that binds TNFα and IL-13, TNFα and PGE2, or TNFα and NGF comprising two polypeptide chains, wherein the first polypeptide chain comprises VD1-(X1)n-VD2-C-X2, wherein VD1 is a first heavy chain variable domain, VD2 is a second heavy chain variable domain, C is a heavy chain constant domain, X1 is a first linker, and X2 is an Fc region; and the second polypeptide chain comprises VD1-(X1)n-VD2-C, wherein VD1 is a first light chain variable domain, VD2 is a second light chain variable domain, C is a light chain constant domain, X1 is a second linker, and X2 d is absent (i.e., there is no Fc on the second polypeptide chain) In some embodiments, the X1 on the first and second polypeptide chains are the same. In other embodiments, the X1 on the first and second polypeptide chains are different. In some embodiments the first X1 is not a CH1 domain and/or the second X1 is not a CL domain. In one embodiment, the first X1 and the second X1 are short (e.g., 6 amino acid) linkers. In another embodiment, the first X1 and the second X1 are long (e.g., greater than 6 amino acid) linkers. In another embodiment, the first X1 is a short linker and the second X1 is a long linker. In another embodiment, the first X1 is a long linker and the second X1 is a short linker.

[033] In an embodiment, the disclosure provides Dual Variable Domain (DVD) binding proteins comprising four polypeptide chains, wherein each of the first two polypeptide chains comprises VD1-(X1)n-VD2-C-X2, wherein VD1 is a first heavy chain variable domain, VD2 is a second heavy chain variable domain, C is a heavy chain constant domain, X1 is a first linker, and X2 is an Fc region; and each of the second two polypeptide chain comprises VD1-(X1)n-VD2-C-X2, wherein VD1 is a first light chain variable domain, VD2 is a second light chain variable domain, C is a light chain constant domain, X1 is a second linker, and X2 is absent (i.e., there is no Fc on the second two polypeptide chains). Such a DVD binding protein has four antigen binding sites. In some embodiments, the first two polypeptide chains are identical, and the second two polypeptide chains are identical, with one of the first polypeptide chains paired with one of the second polypeptide chains, forming two target binding sites, on each arm of the DVD binding protein. In some embodiments, the X1 on the first and second polypeptide chains are the same. In other embodiments, the X1 on the first and second polypeptide chains are different. In some embodiments, the first

X1 is not a complete CH1 domain and/or the second X1 is not a complete CL domain. In another embodiment, the binding proteins disclosed herein are capable of binding TNFα and IL-13, TNFα and PGE2, or TNFα and NGF. Accordingly, in some embodiments, the binding proteins comprise at least two variable domain sequences (e.g., VD1 and VD2) capable of binding TNFα and IL-13, TNFα and PGE2, or TNFα and NGF, in any orientation (i.e., capable of binding TNFα, IL-13, PGE2, or NGF at the VD1 position, and the same at the VD2 position). In some embodiments, VD1 and VD2 are independently chosen. Therefore, in some embodiments, VD1 and VD2 can comprise the same SEQ ID NO and, in other embodiments, VD1 and VD2 can comprise different SEQ ID NOS.

[034] In an embodiment, the disclosure provides a binding protein comprising first and second polypeptide chains, each independently comprising VD1-(X1)n-VD2-C-X2, wherein VD1 is a first variable domain; VD2 is a second variable domain; C is a constant domain; X1 is a linker with the proviso that it is not CH1; X2 is an Fc region that is present on one polypeptide chain and absent on the other chain; and n is 0 or 1, wherein the VD1 domains on the first and second polypeptide chains form a first functional target binding site and the VD2 domains on the first and second polypeptide chains form a second functional target binding site, and wherein (a) the binding protein is capable of binding TNFα and IL-13, wherein (i) the variable domains that form a functional target binding site for TNFα comprise a sequence selected from the group consisting of SEQ ID NOs: 38-49 and/or the binding protein is capable of binding TNFα with a K_D of at most about $5.8x10^{-11}$ M, as measured by surface plasmon resonance, and/or (ii) the variable domains that form a functional target binding site for IL-13 comprise a sequence selected from the group consisting of SEQ ID NO: 32-37, and/or the binding protein is capable of binding IL-13 with a K_D of at most about $1.2x10^{-9}$ M, as measured by surface plasmon resonance; (b) the binding protein is capable of binding TNFα and PGE2, wherein (i) the variable domains that form a functional target binding site for TNFα comprise a sequence selected from the group consisting of SEQ ID NOs: 38-49 and/or the binding protein is capable of inhibiting TNFα with an IC50 of at most about 3.076 nM, as measured by a TNFα neutralization assay, as measured by surface plasmon resonance, and/or (ii) the variable domains that form a functional target binding site for PGE2 comprise a sequence selected from the group consisting of SEQ ID NO: 50-55, and/or the binding protein is capable of inhibiting PGE2 with an IC50 of at most about 124.8 nM, as measured by a PGE2 neutralization assay; or (c) the binding protein is capable of

binding TNFα and NGF, wherein (i) the variable domains that form a functional target binding site for TNFα comprise a sequence selected from the group consisting of SEQ ID NOs: 38-49 and/or the binding protein is capable of inhibiting TNFα with an IC50 of at most about 0.673 nM, as measured by a TNFα neutralization assay, and/or (ii) the variable domains that form a functional target binding site for NGF comprise a sequence selected from the group consisting of SEQ ID NO: 56-57, and/or the binding protein is capable of inhibiting NGF with an IC50 of at most about 7.455 nM, as measured by a TF-1 cell proliferation bioassay.

[035] In another embodiment, a binding is provided protein comprising first and second polypeptide chains, each independently comprising VD1-(X1)n-VD2-C-X2, wherein VD1 is a first variable domain; VD2 is a second variable domain; C is a constant domain; X1 is a linker with the proviso that it is not CH1; X2 is an Fc region that is present on one polypeptide chain and absent on the other chain; and n is 0 or 1, wherein the VD1 domains on the first and second polypeptide chains form a first functional target binding site and the VD2 domains on the first and second polypeptide chains form a second functional target binding site, and wherein (a) the binding protein is capable of binding TNFα and IL-13, wherein (i) the variable domains that form a functional target binding site for TNFα comprise: CDRs 1-3 from SEQ ID NO: 38 and CDRs 1-3 from SEQ ID NO: 39, CDRs 1-3 from SEQ ID NO: 40 and CDRs 1-3 from SEQ ID NO: 41, CDRs 1-3 from SEQ ID NO: 42 and CDRs 1-3 from SEQ ID NO: 43, CDRs 1-3 from SEQ ID NO: 44 and CDRs 1-3 from SEQ ID NO: 45, or CDRs 1-3 from SEQ ID NO: 46 and CDRs 1-3 from SEQ ID NO: 47; CDRs 1-3 from SEQ ID NO: 48 and CDRs 1-3 from SEQ ID NO: 49; and/or the binding protein is capable of binding TNFα with a K_D of at most about $5.8x10^{-11}$ M, as measured by surface plasmon resonance, and/or (ii) the variable domains that form a functional target binding site for IL-13 comprise CDRs 1-3 from SEQ ID NO: 32 and CDRs 1-3 from SEQ ID NO: 33; CDRs 1-3 from SEQ ID NO: 34 and CDRs 1-3 from SEQ ID NO: 35; or CDRs 1-3 from SEQ ID NO: 36 and CDRs 1-3 from SEQ ID NO: 37; and/or the binding protein is capable of binding IL-13 with a K_D of at most about $1.2x10^{-9}$ M, as measured by surface plasmon resonance; (b) the binding protein is capable of binding TNFα and PGE2, wherein (i) the variable domains that form a functional target binding site for TNFα comprise: CDRs 1-3 from SEQ ID NO: 38 and CDRs 1-3 from SEQ ID NO: 39, CDRs 1-3 from SEQ ID NO: 40 and CDRs 1-3 from SEQ ID NO: 41, CDRs 1-3 from SEQ ID NO: 42 and CDRs 1-3 from SEQ ID NO: 43, CDRs 1-3 from SEQ ID NO: 44 and CDRs 1-3 from SEQ ID NO: 45, or CDRs 1-3

from SEQ ID NO: 46 and CDRs 1-3 from SEQ ID NO: 47; CDRs 1-3 from SEQ ID NO: 48 and CDRs 1-3 from SEQ ID NO: 49; and/or the binding protein is capable of inhibiting TNFα with an IC50 of at most about 3.076 nM, as measured by a TNFα neutralization assay, and/or (ii) the variable domains that form a functional target binding site for PGE2 comprise CDRs 1-3 from SEQ ID NO: 50 and CDRs 1-3 from SEQ ID NO: 51; CDRs 1-3 from SEQ ID NO: 52 and CDRs 1-3 from SEQ ID NO: 53; or CDRs 1-3 from SEQ ID NO: 54 and CDRs 1-3 from SEQ ID NO: 55; and/or the binding protein is capable of inhibiting PGE2 with an IC50 of at most about 124.8 nM, as measured by a PGE2 neutralization assay; or (c) the binding protein is capable of binding TNFα and NGF, wherein (i) the variable domains that form a functional target binding site for TNFα comprise: CDRs 1-3 from SEQ ID NO: 38 and CDRs 1-3 from SEQ ID NO: 39, CDRs 1-3 from SEQ ID NO: 40 and CDRs 1-3 from SEQ ID NO: 41, CDRs 1-3 from SEQ ID NO: 42 and CDRs 1-3 from SEQ ID NO: 43, CDRs 1-3 from SEQ ID NO: 44 and CDRs 1-3 from SEQ ID NO: 45, or CDRs 1-3 from SEQ ID NO: 46 and CDRs 1-3 from SEQ ID NO: 47; CDRs 1-3 from SEQ ID NO: 48 and CDRs 1-3 from SEQ ID NO: 49; and/or the binding protein is capable of inhibiting TNFα with an IC50 of at most about 0.673 nM, as measured by a TNFα neutralization assay, and/or (ii) the variable domains that form a functional target binding site for NGF comprise CDRs 1-3 from SEQ ID NO: 56 and CDRs 1-3 from SEQ ID NO: 57; and/or the binding protein is capable of inhibiting NGF with an IC50 of at most about 7.455 nM, as measured by a TF-1 cell proliferation bioassay.

[036] In an embodiment, a binding protein comprises a first polypeptide chain comprising a first VD1-(X1)n-VD2-C-X2, wherein VD1 is a first heavy chain variable domain; VD2 is a second heavy chain variable domain; C is a heavy chain constant domain; X1 is a linker with the proviso that it is not CH1; X2 is an Fc region; n is 0 or 1, and wherein the second polypeptide chain comprises a second VD1-(X1)n-VD2-C, wherein VD1 is a first light chain variable domain; VD2 is a second light chain variable domain; C is a light chain constant domain; X1 is a linker with the proviso that it is not CH1; n is 0 or 1; and the chain does not comprise an Fc region; and n is 0 or 1, wherein the VD1 domains on the first and second polypeptide chains form a first functional target binding site and the VD2 domains on the first and second polypeptide chains form a second functional target binding site.

[037] In another embodiment, (a) the binding protein is capable of binding TNFα and IL-13, wherein (i) the variable domains that form a functional target binding site for TNFα comprise: (1) SEQ ID NO: 38 and SEQ ID NO: 39, (2) SEQ ID

NO: 40 and SEQ ID NO: 41, (3) SEQ ID NO: 42 and SEQ ID NO: 43, (4) SEQ ID NO: 44 and SEQ ID NO: 45, (5) SEQ ID NO: 46 and SEQ ID NO: 47; (6) SEQ ID NO: 48 and SEQ ID NO: 49; and/or (ii) the variable domains that form a functional target binding site for IL-13 comprise: (1) SEQ ID NO: 32 and SEQ ID NO: 33, (2) SEQ ID NO: 34 and SEQ ID NO: 35, or (3) SEQ ID NO: 36 and SEQ ID NO: 37.

[038] In another embodiment, the binding protein comprises two first polypeptide chains and two second polypeptide chains, wherein the binding protein comprises four functional target binding sites. In another embodiment, X1 is any one of SEQ ID NO: 1-31. In another embodiment, X1 is not CL In another embodiment, the Fc region is an Fc region from an IgG1, IgG2, IgG3, IgG4, IgA, IgM, IgE, or IgD.

[039] In another embodiment, the disclosure provides a binding protein capable of bindingTNFα and IL-13, comprising any DVD-Ig VH and VL from Table 2.

[040] In another embodiment, the disclosure provides a binding protein capable of bindingTNFα and PGE2, comprising any DVD-Ig VH and VL from Table 3.

[041] In another embodiment, the disclosure provides a binding protein capable of binding TNFα and NGF, comprising any DVD-Ig VH and VL from Table 4.

[042] In another embodiment, the binding protein comprises a paired heavy chain and a light chain sequence as shown in Table 1 herein, forming a functional binding site from the paired heavy and light chains.

[043] In an embodiment, any of the heavy chain, light chain, two chain, or four chain embodiments includes at least one X1 linker comprising AKTTPKLEEGEFSEAR (SEQ ID NO: 1); AKTTPKLEEGEFSEARV (SEQ ID NO: 2); AKTTPKLGG (SEQ ID NO: 3); SAKTTPKLGG (SEQ ID NO: 4); SAKTTP (SEQ ID NO: 5); RADAAP (SEQ ID NO: 6); RADAAPTVS (SEQ ID NO: 7); RADAAAAGGPGS (SEQ ID NO: 8); RADAAAA$(G_4S)_4$ (SEQ ID NO: 9); SAKTTPKLEEGEFSEARV (SEQ ID NO: 10); ADAAP (SEQ ID NO: 11); ADAAPTVSIFPP (SEQ ID NO: 12); TVAAP (SEQ ID NO: 13); TVAAPSVFIFPP (SEQ ID NO: 14); QPKAAP (SEQ ID NO: 15); QPKAAPSVTLFPP (SEQ ID NO: 16); AKTTPP (SEQ ID NO: 17); AKTTPPSVTPLAP (SEQ ID NO: 18); AKTTAP (SEQ ID NO: 19); AKTTAPSVYPLAP (SEQ ID NO: 20); ASTKGP (SEQ ID NO: 21); ASTKGPSVFPLAP (SEQ ID NO: 22), GGGGSGGGGSGGGGS (SEQ ID NO: 23); GENKVEYAPALMALS (SEQ ID NO: 24); GPAKELTPLKEAKVS (SEQ ID NO: 25); or GHEAAAVMQVQYPAS (SEQ ID NO: 26); TVAAPSVFIFPPTVAAPSVFIFPP (SEQ ID NO: 27); ASTKGPSVFPLAPASTKGPSVFPLAP (SEQ ID NO: 28); GGGGSGGGGS (SEQ ID

NO: 29); GGSGGGGSG (SEQ ID NO: 30); or G/S based sequences (e.g., G4S and G4S repeats; SEQ ID NO: 31). In an embodiment, X1 is not a constant region, is not a CH region, and/or is not a CL region. In an embodiment, X2 is an Fc region. In another embodiment, X2 is a variant Fc region. In an embodiment, the linker is GGGGSGGGGS (SEQ ID NO: 29) on the first chain and/or GGSGGGGSG (SEQ ID NO: 30) on the second chain. In an embodiment, the linker is GGSGGGGSG (SEQ ID NO: 30) on the first chain and/or GGGGSGGGGS (SEQ ID NO: 29) on the second chain.

[044] In an embodiment, X2 is an Fc region. In another embodiment, the Fc region is a variant Fc region. In still another embodiment, the Fc region, if present, is a native sequence Fc region or a variant sequence Fc region. In yet another embodiment, the Fc region is an Fc region from an IgG1, an Fc region from an IgG2, an Fc region from an IgG3, an Fc region from an IgG4, an Fc region from an IgA, an Fc region from an IgM, an Fc region from an IgE, or an Fc region from an IgD.

Binding Protein Properties

[045] The development and production of a binding protein for use as a human therapeutic agent, e.g., as an anti-inflammatory agent or neurological agent, may require more than the identification of a binding protein capable of binding to a desired target or targets. The binding proteins disclosed herein exhibit favorable properties in one or more of the following categories (a) the binding kinetics (on-rate, off-rate and affinity) for both the inner and outer antigen-binding domains, (b) potencies in various biochemical and cellular bioassays, (c) in vivo efficacies in relevant tumor models, (d) pharmacokinetic and pharmacodynamics properties, (e) manufacturability, including protein expression level in selected cell lines, scalability, post-translational modification, physicochemical properties such as monomer percentage, solubility, and stability (intrinsic, freeze/thaw, storage stability, etc.), (f) formulation properties, (g) potential immunogenicity risk, (h) toxicological properties, and (i) binding mode and valency. Binding mode and valency may affect binding properties and cellular potencies of a molecule.

[046] The binding proteins disclosed herein exhibit favorable properties in some or each of the categories listed above, including surprisingly high binding affinity at both the VD1 and VD2 position, as compared to other binding proteins for the same targets but comprising different variable domains and/or linker sequences. It has also been found, unexpectedly, that the binding proteins disclosed herein may, in some

embodiments, exhibit a superior combination of one or more properties, such as one or more of: effective binding kinetics, improved neutralization ability, enhanced in vivo efficacy, superior formulatability, a desirable glycosylation pattern, a favorable pharmacokinetic profile, and efficient expression in host cells, as compared to other binding proteins for the same targets but comprising different variable domains and/or linker sequences.

[047] For instance, it has unexpectedly been found that many of the binding proteins disclosed herein bind their targets with an affinity roughly comparable (i.e., within the same order of magnitude) to that of both their individual parent antibodies. See, for example, the comparison of parental antibodies and binding proteins in Tables 5-8 and 10. This is surprising as loss in binding affinity may have been anticipated a priori from the use of a dual variable domain binding structure. In some embodiments, the binding proteins disclosed herein exhibit surprisingly favorable physicochemical characteristics, including solubility, viscosity, stability on freeze thaw, and/or lack of other significant changes during thermal stress, as compared to other binding proteins for the same targets but comprising different variable domains and/or linker sequences. In some embodiments, the binding proteins disclosed herein exhibit reduced immunogenicity in vivo, as compared to dual administration of separate antibodies for the same targets, and/or as compared to other binding proteins for the same targets but comprising different variable domains and/or linker sequences.

[048] In various embodiments, the binding proteins disclosed herein exhibit improved properties, e.g., improved safety, increased stability, greater potency, a reduced inflammatory or immune response, or other beneficial in vivo human therapeutic properties, as compared to other treatments for inflammatory, autoimmune, or neurological conditions. Treatments suitable for comparison can include, e.g., administration of a small molecule anti-inflammatory or neurological agent, dual administration of separate antibodies for the same targets bound by the antibodies disclosed herein, or administration of other binding proteins for the same targets but comprising different variable domains and/or linker sequences. In some embodiments, the binding proteins disclosed herein exhibit improved properties over a current standard of care treatment for an autoimmune, inflammatory, or neurological condition. For instance, the binding protein can exhibit improved binding kinetics, superior in vivo therapeutic efficacy, enhanced formulatability (including reduced aggregation and improved storage stability), improved pharmacokinetics, a reduced inflammatory or immune response, and/or enhanced host cell expression levels.

Preparation of Binding Proteins

[049] In another aspect, the disclosure provides a method of making a binding protein that binds TNFα and/or IL-13 is provided. In an embodiment, the method of making a binding protein that binds TNFα and/or IL-13 comprises the steps of a) obtaining a first parent antibody, or antigen binding portion thereof, that binds TNFα; b) obtaining a second parent antibody, or antigen binding portion thereof, that binds IL-13; c) determining the sequences of the variable domains of the parent antibodies or antigen binding portions thereof; d) preparing construct(s) encoding any of the binding proteins described herein using those variable domain sequences; and e) expressing the polypeptide chains, such that a binding protein that binds TNFα and IL-13, TNF and PGE2, or TNF and NGF is generated.

[050] A method of making a binding protein that binds TNFα and/or PGE2 is provided. In an embodiment, the method of making a binding protein that binds TNFα and/or PGE2 comprises the steps of a) obtaining a first parent antibody, or antigen binding portion thereof, that binds TNFα; b) obtaining a second parent antibody, or antigen binding portion thereof, that binds PGE2; c) preparing construct(s) encoding any of the binding proteins described herein; and d) expressing the polypeptide chains, such that a binding protein that binds the first and the second antigen is generated.

[051] A method of making a binding protein that binds TNFα and/or NGF is provided. In an embodiment, the method of making a binding protein that binds TNFα and/or NGF comprises the steps of a) obtaining a first parent antibody, or antigen binding portion thereof, that binds TNFα; b) obtaining a second parent antibody, or antigen binding portion thereof, that binds NGF; c) preparing construct(s) encoding any of the binding proteins described herein; and d) expressing the polypeptide chains, such that a binding protein that binds the first and the second antigen is generated.

[052] In any of the embodiments herein, the VD1 heavy chain variable domain, if present, and light chain variable domain, if present, can be from a first parent antibody or antigen binding portion thereof; the VD2 heavy chain variable domain, if present, and light chain variable domain, if present, can be from a second parent antibody or antigen binding portion thereof. The first and second parent antibodies can be the same or different.

[053] In one embodiment, the first parent antibody or antigen binding portion thereof, binds a first antigen, and the second parent antibody or antigen binding portion thereof, binds a second antigen. In an embodiment, the first and second antigens are the same antigen. In another embodiment, the parent antibodies bind different epitopes on the same antigen. In another embodiment, the first and second antigens are different antigens. In another embodiment, the first parent antibody or antigen binding portion thereof, binds the first antigen with a potency different from the potency with which the second parent antibody or antigen binding portion thereof, binds the second antigen. In yet another embodiment, the first parent antibody or antigen binding portion thereof, binds the first antigen with an affinity different from the affinity with which the second parent antibody or antigen binding portion thereof, binds the second antigen.

[054] In another embodiment, the first parent antibody or antigen binding portion thereof, and the second parent antibody or antigen binding portion thereof, are a human antibody, CDR grafted antibody, humanized antibody, and/or affinity matured antibody.

[055] In another embodiment, the binding protein possesses at least one desired property exhibited by the first parent antibody or antigen binding portion thereof, or the second parent antibody or antigen binding portion thereof. Alternatively, the first parent antibody or antigen binding portion thereof and the second parent antibody or antigen binding portion thereof possess at least one desired property exhibited by the binding protein. In an embodiment, the desired property is one or more antibody parameters. In another embodiment, the antibody parameters are antigen specificity, affinity to antigen, potency, biological function, epitope recognition, stability, solubility, production efficiency, immunogenicity, pharmacokinetics, bioavailability, tissue cross reactivity, or orthologous antigen binding. In an embodiment, the binding protein is multivalent. In another embodiment, the binding protein is multispecific. The multivalent and or multispecific binding proteins described herein have desirable properties particularly from a therapeutic standpoint. For instance, the multivalent and or multispecific binding protein may (1) be internalized (and/or catabolized) faster than a bivalent antibody by a cell expressing an antigen to which the antibodies bind; (2) be an agonist binding protein; and/or (3) induce cell death and/or apoptosis of a cell expressing an antigen to which the multivalent binding protein is capable of binding. The “parent antibody”, which provides at least one antigen binding specificity of the multivalent and or multispecific

binding protein, may be one that is internalized (and/or catabolized) by a cell expressing an antigen to which the antibody binds; and/or may be an agonist, cell death-inducing, and/or apoptosis-inducing antibody, and the multivalent and or multispecific binding protein as described herein may display improvement(s) in one or more of these properties. Moreover, the parent antibody may lack any one or more of these properties, but may acquire one or more of them when constructed as a multivalent binding protein as described herein.

[056] In another embodiment, the binding protein has an on rate constant (K_{on}) to one or more targets of at least about $10^2M^{-1}s^{-1}$; at least about $10^3M^{-1}s^{-1}$; at least about $10^4M^{-1}s^{-1}$; at least about $10^5M^{-1}s^{-1}$; or at least about $10^6M^{-1}s^{-1}$, as measured by surface plasmon resonance. In an embodiment, the binding protein has an on rate constant (K_{on}) to one or more targets from about $10^2M^{-1}s^{-1}$ to about $10^3M^{-1}s^{-1}$; from about $10^3M^{-1}s^{-1}$ to about $10^4M^{-1}s^{-1}$; from about $10^4M^{-1}s^{-1}$ to about $10^5M^{-1}s^{-1}$; or from about $10^5M^{-1}s^{-1}$ to about $10^6M^{-1}s^{-1}$, as measured by surface plasmon resonance.

[057] In another embodiment, the binding protein has an off rate constant (K_{off}) for one or more targets of at most about $10^{-3}s^{-1}$; at most about $10^{-4}s^{-1}$; at most about $10^{-5}s^{-1}$; or at most about $10^{-6}s^{-1}$, as measured by surface plasmon resonance. In an embodiment, the binding protein has an off rate constant (K_{off}) to one or more targets of about $10^{-3}s^{-1}$ to about $10^{-4}s^{-1}$; of about $10^{-4}s^{-1}$ to about $10^{-5}s^{-1}$; or of about $10^{-5}s^{-1}$ to about $10^{-6}s^{-1}$, as measured by surface plasmon resonance.

[058] In another embodiment, the binding protein has a dissociation constant (K_d) to one or more targets of at most about 10^{-7}M; at most about 10^{-8}M; at most about 10^{-9}M; at most about 10^{-10}M; at most about 10^{-11}M; at most about 10^{-12}M; or at most 10^{-13}M. In an embodiment, the binding protein has a dissociation constant (K_d) to its targets of about 10^{-7}M to about 10^{-8}M; of about 10^{-8}M to about 10^{-9}M; of about 10^{-9}M to about 10^{-10}M; of about 10^{-10}M to about 10^{-11}M; of about 10^{-11}M to about 10^{-12}M; or of about 10^{-12} to M about 10^{-13}M.

[059] In another embodiment, the binding protein is a conjugate further comprising an agent. In an embodiment, the agent is an immunoadhesion molecule, an imaging agent, a therapeutic agent, or a cytotoxic agent. In an embodiment, the imaging agent is a radiolabel, an enzyme, a fluorescent label, a luminescent label, a bioluminescent label, a magnetic label, or biotin. In another embodiment, the radiolabel is ^{3}H, ^{14}C, ^{35}S, ^{90}Y, ^{99}Tc, ^{111}In, ^{125}I, ^{131}I, ^{177}Lu, ^{166}Ho, or ^{153}Sm. In yet another embodiment, the therapeutic or cytotoxic agent is an anti-metabolite, an alkylating

agent, an antibiotic, a growth factor, a cytokine, an anti-angiogenic agent, an anti-mitotic agent, an anthracycline, toxin, or an apoptotic agent.

[060] In another embodiment, the binding protein is a crystallized binding protein and exists as a crystal. In an embodiment, the crystal is a carrier-free pharmaceutical controlled release crystal. In another embodiment, the crystallized binding protein has a greater half life in vivo than the soluble counterpart of the binding protein. In yet another embodiment, the crystallized binding protein retains biological activity.

[061] In another embodiment, the binding protein described herein is glycosylated. For example, the glycosylation pattern is a human glycosylation pattern.

[062] An isolated nucleic acid encoding any one of the binding proteins disclosed herein is also provided. A further embodiment provides a vector comprising the isolated nucleic acid disclosed herein wherein the vector is pcDNA; pTT (Durocher et al. (2002) Nucleic Acids Res. 30(2); pTT3 (pTT with additional multiple cloning site; pEFBOS (Mizushima and Nagata (1990) Nucleic Acids Res. 18(17); pBV; pJV; pcDNA3.1 TOPO; pEF6 TOPO; pBOS; pHybE; or pBJ. In an embodiment, the vector is a vector disclosed in US Patent Publication No. 20090239259.

[063] In another aspect, a host cell is transformed with the vector disclosed herein. In an embodiment, the host cell is a prokaryotic cell, for example, E.coli. In another embodiment, the host cell is a eukaryotic cell, for example, a protist cell, an animal cell, a plant cell, or a fungal cell. In an embodiment, the host cell is a mammalian cell including, but not limited to, CHO, COS, NS0, SP2, PER.C6, or a fungal cell, such as *Saccharomyces cerevisiae*, or an insect cell, such as Sf9. In an embodiment, two or more binding proteins, e.g., with different specificities, are produced in a single recombinant host cell. For example, the expression of a mixture of antibodies has been called Oligoclonics™ (Merus B.V., The Netherlands) US Patent Nos. 7,262,028 and 7,429,486.

[064] A method of producing a binding protein disclosed herein comprising culturing any one of the host cells disclosed herein in a culture medium under conditions sufficient to produce the binding protein is provided. In an embodiment, 50%-75% of the binding protein produced by this method is a dual specific tetravalent binding protein. In another embodiment, 75%-90% of the binding protein produced by this method is a dual specific tetravalent binding protein. In another embodiment, 90%-95% of the binding protein produced is a dual specific tetravalent binding protein.

[065] One embodiment provides a composition for the release of a binding protein wherein the composition comprises a crystallized binding protein, an ingredient, and at least one polymeric carrier. In an embodiment, the polymeric carrier is poly (acrylic acid), a poly (cyanoacrylate), a poly (amino acid), a poly (anhydride), a poly (depsipeptide), a poly (ester), poly (lactic acid), poly (lactic-co-glycolic acid) or PLGA, poly (b-hydroxybutryate), poly (caprolactone), poly (dioxanone), poly (ethylene glycol), poly ((hydroxypropyl) methacrylamide, poly [(organo)phosphazene], a poly (ortho ester), poly (vinyl alcohol), poly (vinylpyrrolidone), a maleic anhydride- alkyl vinyl ether copolymer, a pluronic polyol, albumin, alginate, cellulose, a cellulose derivative, collagen, fibrin, gelatin, hyaluronic acid, an oligosaccharide, a glycaminoglycan, a sulfated polysaccharide, or blends and copolymers thereof. In an embodiment, the ingredient is albumin, sucrose, trehalose, lactitol, gelatin, hydroxypropyl-β- cyclodextrin, methoxypolyethylene glycol, or polyethylene glycol.

[066] Another embodiment provides a method for treating a mammal comprising the step of administering to the mammal an effective amount of a composition disclosed herein.

[067] A pharmaceutical composition comprising a binding protein disclosed herein and a pharmaceutically acceptable carrier is provided. In a further embodiment, the pharmaceutical composition comprises at least one additional therapeutic agent for treating a disorder. For example, the additional agent may be a therapeutic agent, an imaging agent, a cytotoxic agent, an angiogenesis inhibitor (including but not limited to an anti-VEGF antibody or a VEGF-trap), a kinase inhibitor (including but not limited to a KDR and a TIE-2 inhibitor), a co-stimulation molecule blocker (including but not limited to anti-B7.1, anti-B7.2, CTLA4-Ig, anti-CD20), an adhesion molecule blocker (including but not limited to an anti-LFA-1 antibody, an anti-E/L selectin antibody, a small molecule inhibitor), an anti-cytokine antibody or functional fragment thereof (including but not limited to an anti-IL-18, an anti-TNF, and an anti-IL-6/cytokine receptor antibody), methotrexate, cyclosporin, rapamycin, FK506, a detectable label or reporter, a TNF antagonist, an antirheumatic, a muscle relaxant, a narcotic, a non-steroid anti-inflammatory drug (NSAID), an analgesic, an anesthetic, a sedative, a local anesthetic, a neuromuscular blocker, an antimicrobial, an antipsoriatic, a corticosteriod, an anabolic steroid, an erythropoietin, an immunization, an immunoglobulin, an immunosuppressive, a growth hormone, a hormone replacement drug, a radiopharmaceutical, an antidepressant, an antipsychotic, a

stimulant, an asthma medication, a beta agonist, an inhaled steroid, an epinephrine or analog, a cytokine, or a cytokine antagonist.

Therapeutic and Diagnostic Uses

[068] A method for treating a human subject suffering from a disorder in which the target, or targets, capable of being bound by the binding protein disclosed herein is detrimental, comprising administering to the human subject a binding protein disclosed herein such that the activity of the target, or targets, in the human subject is inhibited and one or more symptoms is alleviated or treatment is achieved is provided. The binding proteins provided herein can be used to treat humans suffering from autoimmune diseases such as, for example, those associated with inflammation. In an embodiment, the binding proteins provided herein or antigen-binding portions thereof, are used to treat asthma, allergies, allergic lung disease, allergic rhinitis, atopic dermatitis, chronic obstructive pulmonary disease (COPD), fibrosis, cystic fibrosis (CF), fibrotic lung disease, idiopathic pulmonary fibrosis, liver fibrosis, lupus, hepatitis B-related liver diseases and fibrosis, sepsis, systemic lupus erythematosus (SLE), glomerulonephritis, inflammatory skin diseases, psoriasis, diabetes, insulin dependent diabetes mellitus, infectious diseases caused by HIV, inflammatory bowel disease (IBD), ulcerative colitis (UC), Crohn's disease (CD), rheumatoid arthritis (RA), osteoarthritis (OA), multiple sclerosis (MS), graft-versus-host disease (GVHD), transplant rejection, ischemic heart disease (IHD), celiac disease, contact hypersensitivity, alcoholic liver disease, Behcet's disease, atherosclerotic vascular disease, occular surface inflammatory diseases, or Lyme disease.

[069] In another embodiment, the disorder or condition to be treated comprises the symptoms caused by viral infection in a human which is caused by, for example, HIV, the human rhinovirus, an enterovirus, a coronavirus, a herpes virus, an influenza virus, a parainfluenza virus, a respiratory syncytial virus or an adenovirus.

[070] The binding proteins provided herein can be used to treat neurological disorders. In an embodiment, the binding proteins provided herein, or antigen-binding portions thereof, are used to treat neurodegenerative diseases and conditions involving neuronal regeneration and spinal cord injury.

[071] In an embodiment, diseases that can be treated or diagnosed with the compositions and methods disclosed herein include, but are not limited to, primary and metastatic cancers, including carcinomas of breast, colon, rectum, lung, oropharynx, hypopharynx, esophagus, stomach, pancreas, liver, gallbladder and bile

ducts, small intestine, urinary tract (including kidney, bladder and urothelium), female genital tract (including cervix, uterus, and ovaries as well as choriocarcinoma and gestational trophoblastic disease), male genital tract (including prostate, seminal vesicles, testes and germ cell tumors), endocrine glands (including the thyroid, adrenal, and pituitary glands), and skin, as well as hemangiomas, melanomas, sarcomas (including those arising from bone and soft tissues as well as Kaposi's sarcoma), tumors of the brain, nerves, eyes, and meninges (including astrocytomas, gliomas, glioblastomas, retinoblastomas, neuromas, neuroblastomas, Schwannomas, and meningiomas), solid tumors arising from hematopoietic malignancies such as leukemias, and lymphomas (both Hodgkin's and non-Hodgkin's lymphomas).

[072] Another embodiment provides for the use of the binding protein in the treatment of a disease or disorder, wherein the disease or disorder is rheumatoid arthritis, osteoarthritis, juvenile chronic arthritis, septic arthritis, Lyme arthritis, psoriatic arthritis, reactive arthritis, spondyloarthropathy, systemic lupus erythematosus, Crohn's disease, ulcerative colitis, inflammatory bowel disease, insulin dependent diabetes mellitus, thyroiditis, asthma, allergic diseases, psoriasis, dermatitis scleroderma, graft versus host disease, organ transplant rejection, acute or chronic immune disease associated with organ transplantation, sarcoidosis, atherosclerosis, disseminated intravascular coagulation, Kawasaki's disease, Grave's disease, nephrotic syndrome, chronic fatigue syndrome, Wegener's granulomatosis, Henoch-Schoenlein purpurea, microscopic vasculitis of the kidneys, chronic active hepatitis, uveitis, septic shock, toxic shock syndrome, sepsis syndrome, cachexia, infectious diseases, parasitic diseases, acquired immunodeficiency syndrome, acute transverse myelitis, Huntington's chorea, Parkinson's disease, Alzheimer's disease, stroke, primary biliary cirrhosis, hemolytic anemia, malignancies, heart failure, Addison's disease, sporadic, polyglandular deficiency type I and polyglandular deficiency type II, Schmidt's syndrome, adult (acute) respiratory distress syndrome, alopecia, alopecia areata, arthropathy, Reiter's disease, psoriatic arthropathy, ulcerative colitic arthropathy, enteropathic synovitis, chlamydia, yersinia and salmonella associated arthropathy, atheromatous disease/arteriosclerosis, atopic allergy, autoimmune bullous disease, pemphigus vulgaris, pemphigus foliaceus, pemphigoid, linear IgA disease, autoimmune haemolytic anaemia, Coombs positive haemolytic anaemia, acquired pernicious anaemia, juvenile pernicious anaemia, myalgic encephalitis/Royal Free Disease, chronic mucocutaneous candidiasis, giant cell arteritis, primary sclerosing hepatitis, cryptogenic autoimmune hepatitis, acquired

immunodeficiency related diseases, hepatitis B, hepatitis C, common varied immunodeficiency (common variable hypogammaglobulinaemia), dilated cardiomyopathy, female infertility, ovarian failure, premature ovarian failure, fibrotic lung disease, cryptogenic fibrosing alveolitis, post-inflammatory interstitial lung disease, interstitial pneumonitis, connective tissue disease associated interstitial lung disease, mixed connective tissue disease associated lung disease, systemic sclerosis associated interstitial lung disease, rheumatoid arthritis associated interstitial lung disease, systemic lupus erythematosus associated lung disease, dermatomyositis/polymyositis associated lung disease, Sjögren's disease associated lung disease, ankylosing spondylitis associated lung disease, vasculitic diffuse lung disease, haemosiderosis associated lung disease, drug-induced interstitial lung disease, fibrosis, radiation fibrosis, bronchiolitis obliterans, chronic eosinophilic pneumonia, lymphocytic infiltrative lung disease, postinfectious interstitial lung disease, gouty arthritis, autoimmune hepatitis, type-1 autoimmune hepatitis (classical autoimmune or lupoid hepatitis), type-2 autoimmune hepatitis (anti-LKM antibody hepatitis), autoimmune mediated hypoglycaemia, type B insulin resistance with acanthosis nigricans, hypoparathyroidism, acute immune disease associated with organ transplantation, chronic immune disease associated with organ transplantation, osteoarthrosis, primary sclerosing cholangitis, psoriasis type 1, psoriasis type 2, idiopathic leucopaenia, autoimmune neutropaenia, renal disease NOS, glomerulonephritides, microscopic vasulitis of the kidneys, lyme disease, discoid lupus erythematosus, male infertility idiopathic or NOS, sperm autoimmunity, multiple sclerosis (all subtypes), sympathetic ophthalmia, pulmonary hypertension secondary to connective tissue disease, Goodpasture's syndrome, pulmonary manifestation of polyarteritis nodosa, acute rheumatic fever, rheumatoid spondylitis, Still's disease, systemic sclerosis, Sjörgren's syndrome, Takayasu's disease/arteritis, autoimmune thrombocytopaenia, idiopathic thrombocytopaenia, autoimmune thyroid disease, hyperthyroidism, goitrous autoimmune hypothyroidism (Hashimoto's disease), atrophic autoimmune hypothyroidism, primary myxoedema, phacogenic uveitis, primary vasculitis, vitiligo acute liver disease, chronic liver diseases, alcoholic cirrhosis, alcohol-induced liver injury, choleosatatis, idiosyncratic liver disease, drug-induced hepatitis, non-alcoholic steatohepatitis, allergy and asthma, group B streptococci (GBS) infection, mental disorders, depression, schizophrenia, Th2 Type and Th1 Type mediated diseases, acute and chronic pain, different forms of pain, cancers, lung cancer, breast cancer, stomach cancer, bladder cancer, colon cancer, pancreatic cancer, ovarian cancer, prostate cancer, rectal cancer, hematopoietic

malignancies, leukemia, lymphoma, Abetalipoprotemia, acrocyanosis, acute and chronic parasitic or infectious processes, acute leukemia, acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML), acute or chronic bacterial infection, acute pancreatitis, acute renal failure, adenocarcinomas, aerial ectopic beats, AIDS dementia complex, alcohol-induced hepatitis, allergic conjunctivitis, allergic contact dermatitis, allergic rhinitis, allograft rejection, alpha-l-antitrypsin deficiency, amyotrophic lateral sclerosis, anemia, angina pectoris, anterior horn cell degeneration, anti cd3 therapy, antiphospholipid syndrome, anti-receptor hypersensitivity reactions, aortic and peripheral aneuryisms, aortic dissection, arterial hypertension, arteriosclerosis, arteriovenous fistula, ataxia, atrial fibrillation (sustained or paroxysmal), atrial flutter, atrioventricular block, B cell lymphoma, bone graft rejection, bone marrow transplant (BMT) rejection, bundle branch block, Burkitt's lymphoma, burns, cardiac arrhythmias, cardiac stun syndrome, cardiac tumors, cardiomyopathy, cardiopulmonary bypass inflammation response, cartilage transplant rejection, cerebellar cortical degenerations, cerebellar disorders, chaotic or multifocal atrial tachycardia, chemotherapy associated disorders, chronic myelocytic leukemia (CML), chronic alcoholism, chronic inflammatory pathologies, chronic lymphocytic leukemia (CLL), chronic obstructive pulmonary disease (COPD), chronic salicylate intoxication, colorectal carcinoma, congestive heart failure, conjunctivitis, contact dermatitis, cor pulmonale, coronary artery disease, Creutzfeldt-Jakob disease, culture negative sepsis, cystic fibrosis, cytokine therapy associated disorders, dementia pugilistica, demyelinating diseases, dengue hemorrhagic fever, dermatitis, dermatologic conditions, diabetes, diabetes mellitus, diabetic ateriosclerotic disease, diffuse Lewy body disease, dilated congestive cardiomyopathy, disorders of the basal ganglia, Down's syndrome in middle age, drug-induced movement disorders induced by drugs which block CNS dopamine receptors, drug sensitivity, eczema, encephalomyelitis, endocarditis, endocrinopathy, epiglottitis, epstein-barr virus infection, erythromelalgia, extrapyramidal and cerebellar disorders, familial hematophagocytic lymphohistiocytosis, fetal thymus implant rejection, Friedreich's ataxia, functional peripheral arterial disorders, fungal sepsis, gas gangrene, gastric ulcer, glomerular nephritis, graft rejection of any organ or tissue, gram negative sepsis, gram positive sepsis, granulomas due to intracellular organisms, hairy cell leukemia, Hallervorden-Spatz disease, Hashimoto's thyroiditis, hay fever, heart transplant rejection, hemachromatosis, hemodialysis, hemolytic uremic syndrome/thrombolytic thrombocytopenic purpura, hemorrhage, hepatitis A, His bundle arrythmias, HIV infection/HIV neuropathy, Hodgkin's disease, hyperkinetic

movement disorders, hypersensitity reactions, hypersensitivity pneumonitis, hypertension, hypokinetic movement disorders, hypothalamic-pituitary-adrenal axis evaluation, idiopathic Addison's disease, idiopathic pulmonary fibrosis, antibody mediated cytotoxicity, Asthenia, infantile spinal muscular atrophy, inflammation of the aorta, influenza a, ionizing radiation exposure, iridocyclitis/uveitis/optic neuritis, ischemia- reperfusion injury, ischemic stroke, juvenile rheumatoid arthritis, juvenile spinal muscular atrophy, Kaposi's sarcoma, kidney transplant rejection, legionella, leishmaniasis, leprosy, lesions of the corticospinal system, lipedema, liver transplant rejection, lymphederma, malaria, malignamt lymphoma, malignant histiocytosis, malignant melanoma, meningitis, meningococcemia, metabolic/idiopathic, migraine headache, mitochondrial multi-system disorder, mixed connective tissue disease, monoclonal gammopathy, multiple myeloma, multiple systems degenerations (Mencel Dejerine-Thomas Shi-Drager and Machado-Joseph), mycobacterium avium intracellulare, mycobacterium tuberculosis, myelodyplastic syndrome, myocardial infarction, myocardial ischemic disorders, nasopharyngeal carcinoma, neonatal chronic lung disease, nephritis, nephrosis, neurodegenerative diseases, neurogenic muscular atrophies, neutropenic fever, non-hodgkins lymphoma, occlusion of the abdominal aorta and its branches, occulsive arterial disorders, okt3 therapy, orchitis/epidydimitis, orchitis/vasectomy reversal procedures, organomegaly, osteoporosis, pancreas transplant rejection, pancreatic carcinoma, paraneoplastic syndrome/hypercalcemia of malignancy, parathyroid transplant rejection, pelvic inflammatory disease, perennial rhinitis, pericardial disease, peripheral atherlosclerotic disease, peripheral vascular disorders, peritonitis, pernicious anemia, pneumocystis carinii pneumonia, pneumonia, POEMS syndrome (polyneuropathy, organomegaly, endocrinopathy, monoclonal gammopathy, and skin changes syndrome), post perfusion syndrome, post pump syndrome, post-MI cardiotomy syndrome, preeclampsia, progressive supranucleo palsy, primary pulmonary hypertension, radiation therapy, Raynaud's phenomenon and disease, Raynoud's disease, Refsum's disease, regular narrow QRS tachycardia, renovascular hypertension, reperfusion injury, restrictive cardiomyopathy, sarcomas, scleroderma, senile chorea, senile dementia of Lewy body type, seronegative arthropathies, shock, sickle cell anemia, skin allograft rejection, skin changes syndrome, small bowel transplant rejection, solid tumors, specific arrythmias, spinal ataxia, spinocerebellar degenerations, streptococcal myositis, structural lesions of the cerebellum, subacute sclerosing panencephalitis, syncope, syphilis of the cardiovascular system, systemic anaphalaxis, systemic inflammatory response syndrome, systemic onset juvenile

rheumatoid arthritis, T-cell or FAB ALL telangiectasia, thromboangitis obliterans, thrombocytopenia, toxicity, transplants, trauma/hemorrhage, type III hypersensitivity reactions, type IV hypersensitivity, unstable angina, uremia, urosepsis, valvular heart diseases, varicose veins, vasculitis, venous diseases, venous thrombosis, ventricular fibrillation, viral and fungal infections, vital encephalitis/aseptic meningitis, vital-associated hemaphagocytic syndrome, Wernicke-Korsakoff syndrome, Wilson's disease, xenograft rejection of any organ or tissue, acute coronary syndromes, acute idiopathic polyneuritis, acute inflammatory demyelinating polyradiculoneuropathy, acute ischemia, adult Still's disease, anaphylaxis, anti-phospholipid antibody syndrome, aplastic anemia, atopic eczema, atopic dermatitis, autoimmune dermatitis, autoimmune disorder associated with streptococcus infection, autoimmune enteropathy, autoimmune hearing loss, autoimmune lymphoproliferative syndrome (ALPS), autoimmune myocarditis, autoimmune premature ovarian failure, blepharitis, bronchiectasis, bullous pemphigoid, cardiovascular disease, catastrophic antiphospholipid syndrome, celiac disease, cervical spondylosis, chronic ischemia, cicatricial pemphigoid, clinically isolated syndrome (cis) with risk for multiple sclerosis, childhood onset psychiatric disorder, dacryocystitis, dermatomyositis, diabetic retinopathy, disk herniation, disk prolaps, drug induced immune hemolytic anemia, endometriosis, endophthalmitis, episcleritis, erythema multiforme, erythema multiforme major, gestational pemphigoid, Guillain-Barré syndrome (GBS), Hughes syndrome, idiopathic Parkinson's disease, idiopathic interstitial pneumonia, IgE-mediated allergy, immune hemolytic anemia, inclusion body myositis, infectious ocular inflammatory disease, inflammatory demyelinating disease, inflammatory heart disease, inflammatory kidney disease, IPF/UIP, iritis, keratitis, keratojuntivitis sicca, Kussmaul disease or Kussmaul-Meier disease, Landry's paralysis, Langerhan's cell histiocytosis, livedo reticularis, macular degeneration, microscopic polyangiitis, morbus bechterev, motor neuron disorders, mucous membrane pemphigoid, multiple organ failure, myasthenia gravis, myelodysplastic syndrome, myocarditis, nerve root disorders, neuropathy, non-A non-B hepatitis, optic neuritis, osteolysis, pauciarticular JRA, peripheral artery occlusive disease (PAOD), peripheral vascular disease (PVD), peripheral artery, disease (PAD), phlebitis, polyarteritis nodosa (or periarteritis nodosa), polychondritis, poliosis, polyarticular JRA, polyendocrine deficiency syndrome, polymyositis, polymyalgia rheumatica (PMR), primary Parkinsonism, prostatitis, pure red cell aplasia, primary adrenal insufficiency, recurrent neuromyelitis optica, restenosis, rheumatic heart disease, sapho (synovitis, acne, pustulosis, hyperostosis, and osteitis), secondary amyloidosis, shock lung, scleritis, sciatica,

secondary adrenal insufficiency, silicone associated connective tissue disease, sneddon-wilkinson dermatosis, spondilitis ankylosans, Stevens-Johnson syndrome (SJS), temporal arteritis, toxoplasmic retinitis, toxic epidermal necrolysis, transverse myelitis, TRAPS (tumor necrosis factor receptor, type 1 allergic reaction, type II diabetes, urticaria, usual interstitial pneumonia (UIP), vasculitis, vernal conjunctivitis, viral retinitis, Vogt-Koyanagi-Harada syndrome (VKH syndrome), wet macular degeneration, or wound healing.

[073] In some embodiments, any one of the binding proteins disclosed herein can be used to treat a disorder listed above. In certain embodiments, the binding protein used to treat any of the disorders discussed herein is one or more of the binding proteins listed in Tables 2-4.

[074] In an embodiment, a binding protein disclosed herein is used to treat arthritis, rheumatoid arthritis, psoriatic arthritis, ankylosing spondylitis, ANCA vasculitis, polymyalgia rheumatica, or dry eye. In an embodiment, the binding protein is used to treat arthritis. In an embodiment, the binding protein is used to treat rheumatoid arthritis. In an embodiment, the binding protein is used to treat psoriatic arthritis. In an embodiment, the binding protein is used to treat ankylosing spondylitis. In some embodiments, the binding protein is any one of the binding proteins disclosed herein. In certain embodiments, the binding protein is one or more of the binding proteins listed in Tables 2-4.

[075] In some embodiments, a binding protein disclosed herein may be used to treat one of the conditions above (e.g., rheumatoid arthritis) and exhibit improved results over TNF monotherapy. For instance, a binding protein may persist in circulation longer than an anti-TNF antibody, thereby providing for a longer-duration treatment effect and enabling the potential for reduced administration frequency, which in turn may reduce the risk of the administered agent inducing an immune response.

[076] In some embodiments, a binding protein may produce a greater reduction in inflammation associated with rheumatoid arthritis than can be achieved by administering an anti-TNF antibody, or a greater reduction than is achieved by the sum of inhibition after dual administration of separate antibodies to TNFα and IL-13, TNF and PGE2, or TNF and NGF. Inflammation may be evaluated, e.g., by measuring the level of IL-6, CXCL-1, PGE-2, CXCL-5, G-CSF, or MMP3 expression. In some embodiments, a binding protein may be used to reduce inflammation,

cartilage loss, and/or bone destruction by an amount greater than can be achieved using aTNF antibody or using TNFα and IL-13, TNF and PGE2, or TNF and NGF antibodies administered as a combination of monotherapies. Therefore, targeting a combination of inflammatory mediators using a binding protein disclosed herein may more fully control a patient's symptoms than could be achieved by individual monotherapies.

[077] In an embodiment, the binding proteins, or antigen-binding portions thereof, are used to treat cancer or in the prevention or inhibition of metastases from the tumors described herein either when used alone or in combination with radiotherapy and/or chemotherapeutic agents.

[078] In another aspect, methods of treating a patient suffering from a disorder comprising the step of administering any one of the binding proteins disclosed herein before, concurrently, or after the administration of a second agent, are provided. In an embodiment, the second agent is budenoside, epidermal growth factor, a corticosteroid, cyclosporin, sulfasalazine, an aminosalicylate, 6-mercaptopurine, azathioprine, metronidazole, a lipoxygenase inhibitor, mesalamine, olsalazine, balsalazide, an antioxidant, a thromboxane inhibitor, an IL-1 receptor antagonist, an anti-IL-1β mAbs, an anti-IL-6 or IL-6 receptor mAb, a growth factor, an elastase inhibitor, a pyridinyl-imidazole compound, an antibody or agonist of TNF, LT, IL-1, IL-2, IL-6, IL-7, IL-8, IL-12, IL-13, IL-15, IL-16, IL-18, IL-23, EMAP-II, GM-CSF, FGF, or PDGF, an antibody to CD2, CD3, CD4, CD8, CD-19, CD25, CD28, CD30, CD40, CD45, CD69, CD90 or a ligand thereof, methotrexate, cyclosporin, FK506, rapamycin, mycophenolate mofetil, leflunomide, an NSAID, ibuprofen, prednisolone, a phosphodiesterase inhibitor, an adenosine agonist, an antithrombotic agent, a complement inhibitor, an adrenergic agent, IRAK, NIK, IKK, p38, a MAP kinase inhibitor, an IL-1β converting enzyme inhibitor, a TNFα-converting enzyme inhibitor, a T-cell signalling inhibitor, a metalloproteinase inhibitor, sulfasalazine, azathioprine, a 6-mercaptopurine, an angiotensin converting enzyme inhibitor, a soluble cytokine receptor, a soluble p55 TNF receptor, a soluble p75 TNF receptor, sIL-1RI, sIL-1RII, sIL-6R, an antiinflammatory cytokine, IL-4, IL-10, IL-11, IL-13, or TGFβ. In a particular embodiment, the pharmaceutical compositions disclosed herein are administered to a patient by parenteral, subcutaneous, intramuscular, intravenous, intrarticular, intrabronchial, intraabdominal, intracapsular, intracartilaginous, intracavitary, intracelial, intracerebellar, intracerebroventricular, intracolic, intracervical, intragastric, intrahepatic, intramyocardial, intraosteal, intrapelvic, intrapericardiac, intraperitoneal,

intrapleural, intraprostatic, intrapulmonary, intrarectal, intrarenal, intraretinal, intraspinal, intrasynovial, intrathoracic, intrauterine, intravesical, bolus, vaginal, rectal, buccal, sublingual, intranasal, or transdermal administration.

[079] Anti-idiotype antibodies to the binding proteins disclosed herein are also provided. An anti-idiotype antibody includes any protein or peptide-containing molecule that comprises at least a portion of an immunoglobulin molecule such as, but not limited to, at least one complementarily determining region (CDR) of a heavy or light chain or a ligand binding portion thereof, a heavy chain or light chain variable region, a heavy chain or light chain constant region, a framework region, or any portion thereof, that can be incorporated into a binding protein provided herein.

[080] A method of determining the presence, amount or concentration of TNF(and IL-13, TNF(and PGE2, or TNF(and NGF, or fragment thereof, in a test sample is provided. The method comprises assaying the test sample for the antigen, or fragment thereof, by an immunoassay. The immunoassay (i) employs at least one binding protein and at least one detectable label and (ii) comprises comparing a signal generated by the detectable label as a direct or indirect indication of the presence, amount or concentration of the antigen, or fragment thereof, in the test sample to a signal generated as a direct or indirect indication of the presence, amount or concentration of the antigen, or fragment thereof, in a control or a calibrator. The calibrator is optionally part of a series of calibrators in which each of the calibrators differs from the other calibrators in the series by the concentration of the antigen, or fragment thereof. The method can comprise (i) contacting the test sample with at least one capture agent, which binds to an epitope on the antigen, or fragment thereof, so as to form a capture agent/antigen, or fragment thereof, complex, (ii) contacting the capture agent/antigen, or fragment thereof, complex with at least one detection agent, which comprises a detectable label and binds to an epitope on the antigen, or fragment thereof, that is not bound by the capture agent, to form a capture agent/antigen, or fragment thereof/detection agent complex, and (iii) determining the presence, amount or concentration of the antigen, or fragment thereof, in the test sample based on the signal generated by the detectable label in the capture agent/antigen, or fragment thereof/detection agent complex formed in (ii), wherein at least one capture agent and/or at least one detection agent is the at least one binding protein.

[081] Alternatively, the method can comprise (i) contacting the test sample with at least one capture agent, which binds to an epitope on the antigen, or fragment thereof, so as to form a capture agent/antigen, or fragment thereof, complex, and simultaneously or sequentially, in either order, contacting the test sample with detectably labeled antigen, or fragment thereof, which can compete with any antigen, or fragment thereof, in the test sample for binding to the at least one capture agent, wherein any antigen, or fragment thereof, present in the test sample and the detectably labeled antigen compete with each other to form a capture agent/antigen, or fragment thereof, complex and a capture agent/detectably labeled antigen, or fragment thereof, complex, respectively, and (ii) determining the presence, amount or concentration of the antigen, or fragment thereof, in the test sample based on the signal generated by the detectable label in the capture agent/detectably labeled antigen, or fragment thereof, complex formed in (ii), wherein at least one capture agent is the at least one binding protein and wherein the signal generated by the detectable label in the capture agent/detectably labeled antigen, or fragment thereof, complex is inversely proportional to the amount or concentration of antigen, or fragment thereof, in the test sample.

The test sample can be from a patient, in which case the method can further comprise diagnosing, prognosticating, or assessing the efficacy of therapeutic/prophylactic treatment of the patient. If the method further comprises assessing the efficacy of therapeutic/prophylactic treatment of the patient, the method optionally further comprises modifying the therapeutic/prophylactic treatment of the patient as needed to improve efficacy. The method can be adapted for use in an automated system or a semi-automated system. Accordingly, the methods described herein also can be used to determine whether or not a subject has or is at risk of developing a given disease, disorder or condition. Specifically, such a method can comprise the steps of: (a) determining the concentration or amount in a test sample from a subject of analyte, or fragment thereof, (e.g., using the methods described herein, or methods known in the art); and (b) comparing the concentration or amount of analyte, or fragment thereof, determined in step (a) with a predetermined level, wherein, if the concentration or amount of analyte determined in step (a) is favorable with respect to a predetermined level, then the subject is determined not to have or be at risk for a given disease, disorder or condition. However, if the concentration or amount of analyte determined in step (a) is unfavorable with respect to the predetermined level, then the subject is determined to have or be at risk for a given disease, disorder or condition.

[082] Additionally, provided herein is method of monitoring the progression of disease in a subject. Optimally the method comprising the steps of: (a) determining the concentration or amount in a test sample from a subject of analyte; (b) determining the concentration or amount in a later test sample from the subject of analyte; and (c) comparing the concentration or amount of analyte as determined in step (b) with the concentration or amount of analyte determined in step (a), wherein if the concentration or amount determined in step (b) is unchanged or is unfavorable when compared to the concentration or amount of analyte determined in step (a), then the disease in the subject is determined to have continued, progressed or worsened. By comparison, if the concentration or amount of analyte as determined in step (b) is favorable when compared to the concentration or amount of analyte as determined in step (a), then the disease in the subject is determined to have discontinued, regressed or improved.

[083] Optionally, the method further comprises comparing the concentration or amount of analyte as determined in step (b), for example, with a predetermined level. Further, optionally the method comprises treating the subject with one or more pharmaceutical compositions for a period of time if the comparison shows that the concentration or amount of analyte as determined in step (b), for example, is unfavorably altered with respect to the predetermined level.

[084] Also provided is a kit for assaying a test sample for TNFα, IL-13, PGE2, and/or NGF, or fragments thereof. The kit comprises at least one component for assaying the test sample for an antigen, or fragment thereof, and instructions for assaying the test sample for an antigen, or fragment thereof, wherein the at least one component includes at least one composition comprising the binding protein disclosed herein, wherein the binding protein is optionally detectably labeled.

[085] Unless otherwise defined herein, scientific and technical terms used herein have the meanings that are commonly understood by those of ordinary skill in the art. In the event of any latent ambiguity, definitions provided herein take precedent over any dictionary or extrinsic definition. Unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular. The use of "or" means "and/or" unless stated otherwise. The use of the term "including", as well as other forms, such as "includes" and "included", is not limiting. All ranges given in the application encompass the endpoints unless stated otherwise.

[086] Generally, nomenclatures used in connection with cell and tissue culture, molecular biology, immunology, microbiology, genetics and protein and nucleic acid chemistry and hybridization described herein are those well known and commonly used in the art. The methods and techniques provided herein are generally performed according to conventional methods well known in the art and as described in various general and more specific references that are cited and discussed throughout the present specification unless otherwise indicated. Enzymatic reactions and purification techniques are performed according to manufacturer's specifications, as commonly accomplished in the art or as described herein. The nomenclatures used in connection with, and the laboratory procedures and techniques of, analytical chemistry, synthetic organic chemistry, and medicinal and pharmaceutical chemistry described herein are those well known and commonly used in the art. Standard techniques are used for chemical syntheses, chemical analyses, pharmaceutical preparation, formulation, and delivery, and treatment of patients.

[087] That the disclosure may be more readily understood, select terms are defined below.

[088] The term "antibody" refers to an immunoglobulin (Ig) molecule, which is generally comprised of four polypeptide chains, two heavy (H) chains and two light (L) chains, or a functional fragment, mutant, variant, or derivative thereof, that retains the epitope binding features of an Ig molecule. Such fragment, mutant, variant, or derivative antibody formats are known in the art. In an embodiment of a full-length antibody, each heavy chain is comprised of a heavy chain variable region (VH) and a heavy chain constant region (CH). The CH is comprised of three domains, CH1, CH2 and CH3. Each light chain is comprised of a light chain variable region (VL) and a light chain constant region (CL). The CL is comprised of a single CL domain. The VH and VL can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDRs), interspersed with regions that are more conserved, termed framework regions (FRs). Generally, each VH and VL is composed of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, and FR4. Immunoglobulin molecules can be of any type (e.g., IgG, IgE, IgM, IgD, IgA and IgY), class (e.g., IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2), or subclass.

[089] The term "bispecific antibody" refers to an antibody that binds one antigen (or epitope) on one of its two binding arms (one pair of HC/LC), and binds a different antigen (or epitope) on its second binding arm (a different pair of HC/LC). A

bispecific antibody has two distinct antigen binding arms (in both specificity and CDR sequences), and is monovalent for each antigen to which it binds. Bispecific antibodies include those generated by quadroma technology (Milstein and Cuello (1983) Nature 305(5934): 537-40), by chemical conjugation of two different monoclonal antibodies (Staerz et al. (1985) Nature 314(6012): 628-31), or by knob-into-hole or similar approaches which introduces mutations in the Fc region (Holliger et al. (1993) Proc. Natl. Acad. Sci. USA 90(14): 6444-6448).

[090] An "affinity matured" antibody or binding protein refers to an antibody or binding protein with one or more alterations in one or more CDR or framework (FR) regions thereof, which result an improvement in the affinity for an antigen, compared to a parent antibody or binding protein which does not possess those alteration(s). Exemplary affinity matured antibodies or binding protein will have nanomolar or even picomolar affinities for the target antigen. Affinity matured antibodies or binding protein may be produced by procedures known in the art, e.g., Marks et al. (1992) BioTechnology 10:779-783 describes affinity maturation by VH and VL domain shuffling. Random mutagenesis of CDR and/or framework residues is described by Barbas et al. (1994) Proc. Nat. Acad. Sci. USA 91:3809-3813; Schier et al. (1995) Gene 169:147-155; Yelton et al. (1995) J. Immunol. 155:1994-2004; Jackson et al. (1995) J. Immunol. 154(7):3310-9; Hawkins et al. (1992) J. Mol. Biol. 226:889-896 and mutation at selective mutagenesis positions, contact or hypermutation positions with an activity enhancing amino acid residue as described in US Patent No. 6,914,128.

[091] The term "CDR-grafted" antibody or binding protein refers to an antibody or binding protein that comprises heavy and light chain variable region sequences in which the sequences of one or more of the CDR regions of VH and/or VL are replaced with CDR sequences of another antibody or binding protein. For example, the two antibodies or binding protein can be from different species, such as antibodies or binding protein having murine heavy and light chain variable regions in which one or more of the murine CDRs has been replaced with human CDR sequences.

[092] The term "humanized" antibody or binding protein refers to an antibody or binding protein from a non-human species that has been altered to be more "human-like", i.e., more similar to human germline sequences. One type of humanized antibody or binding protein is a CDR-grafted antibody or binding protein, in which non-human CDR sequences are introduced into human VH and VL sequences to replace the corresponding human CDR sequences. A humanized antibody or

binding protein also encompasses a variant, derivative, analog or fragment of an antibody or or binding protein that comprises framework region (FR) sequences having substantially (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identity to) the amino acid sequence of a human antibody and at least one CDR having substantially the amino acid sequence of a non-human antibody. A humanized antibody or binding protein may comprise substantially all of at least one variable domain (Fab, Fab', F(ab') 2, FabC, Fv) in which the sequence of all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin (i.e., donor antibody) and the sequence of all or substantially all of the FR regions are those of a human immunoglobulin. The humanized antibody or binding protein also may include the CH1, hinge, CH2, CH3, and CH4 regions of the heavy chain. In an embodiment, a humanized antibody or binding protein may also comprise at least a portion of a human immunoglobulin Fc region. In some embodiments, a humanized antibody or binding protein only contains a humanized light chain. In some embodiments, a humanized antibody or binding protein only contains a humanized heavy chain. In some embodiments, a humanized antibody or binding protein only contains a humanized variable domain of a light chain and/or humanized variable domain of a heavy chain. In some embodiments, a humanized antibody or binding protein contains a humanized light chain as well as at least a variable domain of a heavy chain. In some embodiments, a humanized antibody or binding protein contains a humanized heavy chain as well as at least a variable domain of a light chain.

[093] The terms "dual variable domain binding protein" and "dual variable domain immunoglobulin" refer to a binding protein that has two variable domains in each of its two binding arms (e.g., a pair of HC/LC) (see PCT Publication No. WO 02/02773), each of which is able to bind to an antigen. In an embodiment, each variable domain binds different antigens or epitopes. In another embodiment, each variable domain binds the same antigen or epitope. In another embodiment, a dual variable domain binding protein has two identical antigen binding arms, with identical specificity and identical CDR sequences, and is bivalent for each antigen to which it binds. In an embodiment, the DVD binding proteins may be monospecific, i.e., capable of binding one antigen or multispecific, i.e., capable of binding two or more antigens. DVD binding proteins comprising two heavy chain DVD polypeptides and two light chain DVD polypeptides are referred to as a DVD-Ig™. In an embodiment, each half of a four chain DVD binding protein comprises a heavy chain DVD polypeptide, and a light chain DVD polypeptide, and two antigen binding sites. In an

embodiment, each binding site comprises a heavy chain variable domain and a light chain variable domain with a total of 6 CDRs involved in antigen binding per antigen binding site.

[094] The term "antiidiotypic antibody" refers to an antibody raised against the amino acid sequence of the antigen combining site of another antibody. Antiidiotypic antibodies may be administered to enhance an immune response against an antigen.

[095] The term "biological activity" refers to any one or more biological properties of a molecule (whether present naturally as found in vivo, or provided or enabled by recombinant means). Biological properties include, but are not limited to, binding a receptor, inducing cell proliferation, inhibiting cell growth, inducing other cytokines, inducing apoptosis, and enzymatic activity.

[096] The term "neutralizing" refers to counteracting the biological activity of an antigen when a binding protein specifically binds to the antigen. In an embodiment, the neutralizing binding protein binds to an antigen (e.g., a cytokine) and reduces its biologically activity by at least about 20%, 40%, 60%, 80%, 85% or more.

[097] "Specificity" refers to the ability of a binding protein to selectively bind an antigen.

[098] "Affinity" is the strength of the interaction between a binding protein and an antigen, and is determined by the sequence of the CDRs of the binding protein as well as by the nature of the antigen, such as its size, shape, and/or charge. Binding proteins may be selected for affinities that provide desired therapeutic end-points while minimizing negative side-effects. Affinity may be measured using methods known to one skilled in the art (US 20090311253).

[099] The term "potency" refers to the ability of a binding protein to achieve a desired effect, and is a measurement of its therapeutic efficacy. Potency may be assessed using methods known to one skilled in the art (US 20090311253).

[0100] The term "cross-reactivity" refers to the ability of a binding protein to bind a target other than that against which it was raised. Generally, a binding protein will bind its target tissue(s)/antigen(s) with an appropriately high affinity, but will display an appropriately low affinity for non-target normal tissues. Individual binding proteins are generally selected to meet two criteria. (1) Tissue staining appropriate for the known expression of the antibody target. (2) Similar staining pattern between

human and tox species (mouse and cynomolgus monkey) tissues from the same organ. These and other methods of assessing cross-reactivity are known to one skilled in the art (US 20090311253).

[0101] The term "biological function" refers the specific *in vitro* or *in vivo* actions of a binding protein. Binding proteins may target several classes of antigens and achieve desired therapeutic outcomes through multiple mechanisms of action. Binding proteins may target soluble proteins, cell surface antigens, as well as extracellular protein deposits. Binding proteins may agonize, antagonize, or neutralize the activity of their targets. Binding proteins may assist in the clearance of the targets to which they bind, or may result in cytotoxicity when bound to cells. Portions of two or more antibodies may be incorporated into a multivalent format to achieve distinct functions in a single binding protein molecule. The *in vitro* assays and *in vivo* models used to assess biological function are known to one skilled in the art (US 20090311253).

[0102] A "stable" binding protein is one in which the binding protein essentially retains its physical stability, chemical stability and/or biological activity upon storage. A multivalent binding protein that is stable *in vitro* at various temperatures for an extended period of time is desirable. Methods of stabilizing binding proteins and assessing their stability at various temperatures are known to one skilled in the art (US 20090311253).

[0103] The term "solubility" refers to the ability of a protein to remain dispersed within an aqueous solution. The solubility of a protein in an aqueous formulation depends upon the proper distribution of hydrophobic and hydrophilic amino acid residues, and therefore, solubility can correlate with the production of correctly folded proteins. A person skilled in the art will be able to detect an increase or decrease in solubility of a binding protein using routine HPLC techniques and methods known to one skilled in the art (US 20090311253).

[0104] Binding proteins may be produced using a variety of host cells or may be produced in vitro, and the relative yield per effort determines the "production efficiency." Factors influencing production efficiency include, but are not limited to, host cell type (prokaryotic or eukaryotic), choice of expression vector, choice of nucleotide sequence, and methods employed. The materials and methods used in binding protein production, as well as the measurement of production efficiency, are known to one skilled in the art (US 20090311253).

[0105] The term "immunogenicity" means the ability of a substance to induce an immune response. Administration of a therapeutic binding protein may result in a certain incidence of an immune response. Potential elements that might induce immunogenicity in a multivalent format may be analyzed during selection of the parental antibodies, and steps to reduce such risk can be taken to optimize the parental antibodies prior to incorporating their sequences into a multivalent binding protein format. Methods of reducing the immunogenicity of antibodies and binding proteins are known to one skilled in the art (US 20090311253).

[0106] The terms "label" and "detectable label" mean a moiety attached to a member of a specific binding pair, such as an antibody/binding protein or its analyte to render a reaction (e.g., binding) between the members of the specific binding pair, detectable. The labeled member of the specific binding pair is referred to as "detectably labeled." Thus, the term "labeled binding protein" refers to a protein with a label incorporated that provides for the identification of the binding protein. In an embodiment, the label is a detectable marker that can produce a signal that is detectable by visual or instrumental means, e.g., incorporation of a radiolabeled amino acid or attachment to a polypeptide of biotinyl moieties that can be detected by marked avidin (e.g., streptavidin containing a fluorescent marker or enzymatic activity that can be detected by optical or colorimetric methods). Examples of labels for polypeptides include, but are not limited to, the following: radioisotopes or radionuclides (e.g., ^{3}H, ^{14}C, ^{35}S, ^{90}Y, ^{99}Tc, ^{111}In, ^{125}I, ^{131}I, ^{177}Lu, ^{166}Ho, or ^{153}Sm); chromogens, fluorescent labels (e.g., FITC, rhodamine, lanthanide phosphors), enzymatic labels (e.g., horseradish peroxidase, luciferase, alkaline phosphatase); chemiluminescent markers; biotinyl groups; predetermined polypeptide epitopes recognized by a secondary reporter (e.g., leucine zipper pair sequences, binding sites for secondary antibodies, metal binding domains, epitope tags); and magnetic agents, such as gadolinium chelates. Representative examples of labels commonly employed for immunoassays include moieties that produce light, e.g., acridinium compounds, and moieties that produce fluorescence, e.g., fluorescein. In this regard, the moiety itself may not be detectably labeled but may become detectable upon reaction with yet another moiety.

[0107] The term "conjugate" refers to a binding protein that is chemically linked to a second chemical moiety, such as a therapeutic or cytotoxic agent. The term "agent" includes a chemical compound, a mixture of chemical compounds, a biological macromolecule, or an extract made from biological materials. In an

embodiment, the therapeutic or cytotoxic agents include, but are not limited to, pertussis toxin, taxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicin, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, and puromycin and analogs or homologs thereof. When employed in the context of an immunoassay, the conjugate antibody may be a detectably labeled antibody used as the detection antibody.

[0108] The terms "crystal" and "crystallized" refer to a binding protein (e.g., an antibody), or antigen binding portion thereof, that exists in the form of a crystal. Crystals are one form of the solid state of matter, which is distinct from other forms such as the amorphous solid state or the liquid crystalline state. Crystals are composed of regular, repeating, three-dimensional arrays of atoms, ions, molecules (e.g., proteins such as antibodies), or molecular assemblies (e.g., antigen/antibody complexes). These three-dimensional arrays are arranged according to specific mathematical relationships that are well-understood in the field. The fundamental unit, or building block, that is repeated in a crystal is called the asymmetric unit. Repetition of the asymmetric unit in an arrangement that conforms to a given, well-defined crystallographic symmetry provides the "unit cell" of the crystal. Repetition of the unit cell by regular translations in all three dimensions provides the crystal. See Giege, R. and Ducruix, A. Barrett, CRYSTALLIZATION OF NUCLEIC ACIDS AND PROTEINS, A PRACTICAL APPROACH, 2nd ea., pp. 20 1-16, Oxford University Press, New York, New York, (1999).

[0109] The term "vector" refers to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of vector is a "plasmid", which refers to a circular double stranded DNA loop into which additional DNA segments may be ligated. Another type of vector is a viral vector, wherein additional DNA segments may be ligated into the viral genome. Other vectors include RNA vectors. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (e.g., bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (e.g., non-episomal mammalian vectors) can be integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Certain vectors are capable of directing the expression of genes to which they are operatively linked. Such vectors are referred to herein as "recombinant expression

vectors" (or simply, "expression vectors"). In general, expression vectors of utility in recombinant DNA techniques are often in the form of plasmids. In the present specification, "plasmid" and "vector" may be used interchangeably as the plasmid is the most commonly used form of vector. However, other forms of expression vectors are also included, such as viral vectors (e.g., replication defective retroviruses, adenoviruses and adeno-associated viruses), which serve equivalent functions. A group of pHybE vectors (US Patent Application Serial No. 61/021,282) may be used for parental antibody and DVD-binding protein cloning. V1, derived from pJP183; pHybE-hCg1,z,non-a V2, may be used for cloning of antibody and DVD heavy chains with a wildtype constant region. V2, derived from pJP191; pHybE-hCk V3, may be used for cloning of antibody and DVD light chains with a kappa constant region. V3, derived from pJP192; pHybE-hCl V2, may be used for cloning of antibody and DVDs light chains with a lambda constant region. V4, built with a lambda signal peptide and a kappa constant region, may be used for cloning of DVD light chains with a lambda-kappa hybrid V domain. V5, built with a kappa signal peptide and a lambda constant region, may be used for cloning of DVD light chains with a kappa-lambda hybrid V domain. V7, derived from pJP183; pHybE-hCg1,z,non-a V2, may be used for cloning of antibody and DVD heavy chains with a (234,235 AA) mutant constant region.

[0110] The terms "recombinant host cell" or "host cell" refer to a cell into which exogenous DNA has been introduced. Such terms refer not only to the particular subject cell, but to the progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term "host cell" as used herein. In an embodiment, host cells include prokaryotic and eukaryotic cells. In an embodiment, eukaryotic cells include protist, fungal, plant and animal cells. In another embodiment, host cells include but are not limited to the prokaryotic cell line *E.Coli*; mammalian cell lines CHO, HEK 293, COS, NS0, SP2 and PER.C6; the insect cell line Sf9; and the fungal cell *Saccharomyces cerevisiae*.

[0111] The term "transfection" encompasses a variety of techniques commonly used for the introduction of exogenous nucleic acid (e.g., DNA) into a host cell, e.g., electroporation, calcium-phosphate precipitation, DEAE-dextran transfection and the like.

[0112] The term "cytokine" refers to a protein released by one cell population that acts on another cell population as an intercellular mediator. The term "cytokine"

includes proteins from natural sources or from recombinant cell culture and biologically active equivalents of the native sequence cytokines.

[0113] The term "biological sample" means a quantity of a substance from a living thing or formerly living thing. Such substances include, but are not limited to, blood, (e.g., whole blood), plasma, serum, urine, amniotic fluid, synovial fluid, endothelial cells, leukocytes, monocytes, other cells, organs, tissues, bone marrow, lymph nodes and spleen.

[0114] The term "component" refers to an element of a composition. In relation to a diagnostic kit, for example, a component may be a capture antibody, a detection or conjugate antibody, a control, a calibrator, a series of calibrators, a sensitivity panel, a container, a buffer, a diluent, a salt, an enzyme, a co-factor for an enzyme, a detection reagent, a pretreatment reagent/solution, a substrate (e.g., as a solution), a stop solution, and the like that can be included in a kit for assay of a test sample. Thus, a "component" can include a polypeptide or other analyte as above, that is immobilized on a solid support, such as by binding to an anti-analyte (e.g., anti-polypeptide) antibody. Some components can be in solution or lyophilized for reconstitution for use in an assay.

[0115] "Control" refers to a composition known to not analyte ("negative control") or to contain analyte ("positive control"). A positive control can comprise a known concentration of analyte. "Control," "positive control," and "calibrator" may be used interchangeably herein to refer to a composition comprising a known concentration of analyte. A "positive control" can be used to establish assay performance characteristics and is a useful indicator of the integrity of reagents (e.g., analytes).

[0116] "Predetermined cutoff" and "predetermined level" refer generally to an assay cutoff value that is used to assess diagnostic/prognostic/therapeutic efficacy results by comparing the assay results against the predetermined cutoff/level, where the predetermined cutoff/level already has been linked or associated with various clinical parameters (e.g., severity of disease, progression/nonprogression/improvement, etc.). While the present disclosure may provide exemplary predetermined levels, it is well-known that cutoff values may vary depending on the nature of the immunoassay (e.g., antibodies employed, etc.). It further is well within the ordinary skill of one in the art to adapt the disclosure herein for other immunoassays to obtain immunoassay-specific cutoff values for those other

immunoassays based on this disclosure. Whereas the precise value of the predetermined cutoff/level may vary between assays, correlations as described herein (if any) may be generally applicable.

[0117] "Pretreatment reagent," e.g., lysis, precipitation and/or solubilization reagent, as used in a diagnostic assay as described herein is one that lyses any cells and/or solubilizes any analyte that is/are present in a test sample. Pretreatment is not necessary for all samples, as described further herein. Among other things, solubilizing the analyte (e.g., polypeptide of interest) may entail release of the analyte from any endogenous binding proteins present in the sample. A pretreatment reagent may be homogeneous (not requiring a separation step) or heterogeneous (requiring a separation step). With use of a heterogeneous pretreatment reagent there is removal of any precipitated analyte binding proteins from the test sample prior to proceeding to the next step of the assay.

[0118] "Quality control reagents" in the context of immunoassays and kits described herein, include, but are not limited to, calibrators, controls, and sensitivity panels. A "calibrator" or "standard" typically is used (e.g., one or more, such as a plurality) in order to establish calibration (standard) curves for interpolation of the concentration of an analyte, such as an antibody or an analyte. Alternatively, a single calibrator, which is near a predetermined positive/negative cutoff, can be used. Multiple calibrators (i.e., more than one calibrator or a varying amount of calibrator(s)) can be used in conjunction so as to comprise a "sensitivity panel."

[0119] The term "specific binding partner" is a member of a specific binding pair. A specific binding pair comprises two different molecules that specifically bind to each other through chemical or physical means. Therefore, in addition to antigen and antibody specific binding, other specific binding pairs can include biotin and avidin (or streptavidin), carbohydrates and lectins, complementary nucleotide sequences, effector and receptor molecules, cofactors and enzymes, enzyme inhibitors and enzymes, and the like. Furthermore, specific binding pairs can include members that are analogs of the original specific binding members, for example, an analyte-analog. Immunoreactive specific binding members include antigens, antigen fragments, and antibodies, including monoclonal and polyclonal antibodies as well as complexes, fragments, and variants (including fragments of variants) thereof, whether isolated or recombinantly produced.

[0120] The term "Fc region" defines the C-terminal region of an immunoglobulin heavy chain, which may be generated by papain digestion of an intact antibody or binding protein. The Fc region may be a native sequence Fc region or a variant Fc region. The Fc region of an immunoglobulin generally comprises two constant domains, a CH2 domain and a CH3 domain, and optionally comprises a CH4 domain. Replacements of amino acid residues in the Fc portion to alter effector function are known in the art (e.g., US Patent Nos. 5,648,260 and 5,624,821). The Fc region mediates several important effector functions, e.g., cytokine induction, antibody dependent cell mediated cytotoxicity (ADCC), phagocytosis, complement dependent cytotoxicity (CDC), and half-life/ clearance rate of antibody or binding protein and antigen-antibody or antigen-binding protein complexes. In some cases these effector functions are desirable for a therapeutic immunoglobulin but in other cases might be unnecessary or even deleterious, depending on the therapeutic objectives.

[0121] The term "antigen-binding portion" of a binding protein means one or more fragments of a binding protein that retain the ability to specifically bind to an antigen. The antigen-binding portion of a binding protein can be performed by fragments of a full-length binding protein, including bispecific, dual specific, or multi-specific formats; specifically binding to two or more different antigens. Examples of binding fragments encompassed within the term "antigen-binding portion" of an binding protein include (i) an Fab fragment, a monovalent fragment consisting of the VL, VH, CL and CH1 domains; (ii) an $F(ab')_2$ fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; (iii) an Fd fragment consisting of the VH and CH1 domains; (iv) an Fv fragment consisting of the VL and VH domains of a single arm of an antibody or binding protein, (v) a dAb fragment, which comprises a single variable domain; and (vi) an isolated complementarity determining region (CDR). Furthermore, although the two domains of the Fv fragment, VL and VH, encoded 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). Such single chain antibodies or binding proteins are also intended to be encompassed within the term "antigen-binding portion" of an antibody or binding protein. Other forms of single chain antibodies, such as diabodies are also encompassed. In addition, single chain antibodies or binding protein also include "linear" antibodies or binding protein comprising a pair of tandem Fv segments

(VH-CH1-VH-CH1) which, together with complementary light chain polypeptides, form a pair of antigen binding regions.

[0122] The term "multivalent binding protein" means a binding protein comprising two or more antigen binding sites. In an embodiment, the multivalent binding protein is engineered to have three or more antigen binding sites, and may not be a naturally occurring antibody. The term "multispecific binding protein" refers to a binding protein capable of binding two or more related or unrelated targets. In an embodiment, the dual variable domain (DVD) binding proteins provided herein comprise two or more antigen binding sites and are tetravalent or multivalent binding proteins.

[0123] The term "linker" means an amino acid residue or a polypeptide comprising two or more amino acid residues joined by peptide bonds that are used to link two polypeptides (e.g., two VH or two VL domains). Such linker polypeptides are well known in the art (see, e.g., Holliger et al. (1993) Proc. Natl. Acad. Sci. USA 90:6444-6448; Poljak et al. (1994) Structure 2:1121-1123).

[0124] The terms "Kabat numbering", "Kabat definitions" and "Kabat labeling" are used interchangeably herein. These terms, which are recognized in the art, refer to a system of numbering amino acid residues which are more variable (i.e., hypervariable) than other amino acid residues in the heavy and light chain variable regions of an antibody or binding protein, or an antigen binding portion thereof (Kabat et al. (1971) Ann. NY Acad. Sci. 190:382-391 and, 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). For the heavy chain variable region, the hypervariable region ranges from amino acid positions 31 to 35 for CDR1, amino acid positions 50 to 65 for CDR2, and amino acid positions 95 to 102 for CDR3. For the light chain variable region, the hypervariable region ranges from amino acid positions 24 to 34 for CDR1, amino acid positions 50 to 56 for CDR2, and amino acid positions 89 to 97 for CDR3.

[0125] The term "CDR" means a complementarity determining region within an immunoglobulin variable region sequence. There are three CDRs in each of the variable regions of the heavy chain and the light chain, which are designated CDR1, CDR2 and CDR3, for each of the heavy and light chain variable regions. The term "CDR set" refers to a group of three CDRs that occur in a single variable region capable of binding the antigen. The exact boundaries of these CDRs have been

defined differently according to different systems. The system described by Kabat (Kabat et al. (1987) and (1991)) not only provides an unambiguous residue numbering system applicable to any variable region of an antibody or binding protein, but also provides precise residue boundaries defining the three CDRs in each heavy or light chain sequence. These CDRs may be referred to as Kabat CDRs. Chothia and coworkers (Chothia and Lesk (1987) J. Mol. Biol. 196:901-917; Chothia et al. (1989) Nature 342:877-883) found that certain sub- portions within Kabat CDRs adopt nearly identical peptide backbone conformations, despite having great diversity at the level of amino acid sequence. These sub-portions were designated as L1, L2 and L3 or H1, H2 and H3 where the "L" and the "H" designates the light chain and the heavy chain regions, respectively. These regions may be referred to as Chothia CDRs, which have boundaries that overlap with Kabat CDRs. Other boundaries defining CDRs overlapping with the Kabat CDRs have been described by Padlan (1995) FASEB J. 9:133-139 and MacCallum (1996) J. Mol. Biol. 262(5):732-45). Still other CDR boundary definitions may not strictly follow one of the herein systems, but will nonetheless overlap with the Kabat CDRs, although they may be shortened or lengthened in light of prediction or experimental findings that particular residues or groups of residues or even entire CDRs do not significantly impact antigen binding. The methods used herein may utilize CDRs defined according to any of these systems, although certain embodiments use Kabat or Chothia defined CDRs.

[0126] The term "epitope" means a region of an antigen that is bound by a binding protein, e.g., a polypeptide and/or other determinant capable of specific binding to an immunoglobulin or T-cell receptor. In certain embodiments, epitope determinants include chemically active surface groupings of molecules such as amino acids, sugar side chains, phosphoryl, or sulfonyl, and, in certain embodiments, may have specific three dimensional structural characteristics, and/or specific charge characteristics. In an embodiment, an epitope comprises the amino acid residues of a region of an antigen (or fragment thereof) known to bind to the complementary site on the specific binding partner. An antigenic fragment can contain more than one epitope. In certain embodiments, a binding protein specifically binds an antigen when it recognizes its target antigen in a complex mixture of proteins and/or macromolecules. Binding proteins "bind to the same epitope" if the antibodies or binding proteins cross-compete (one prevents the binding or modulating effect of the other). In addition, structural definitions of epitopes (overlapping, similar, identical) are informative; and functional definitions encompass structural (binding) and functional

(modulation, competition) parameters. Different regions of proteins may perform different functions. For example specific regions of a cytokine interact with its cytokine receptor to bring about receptor activation whereas other regions of the protein may be required for stabilizing the cytokine. To abrogate the negative effects of cytokine signaling, the cytokine may be targeted with a binding protein that binds specifically to the receptor interacting region(s), thereby preventing the binding of its receptor. Alternatively, a binding protein may target the regions responsible for cytokine stabilization, thereby designating the protein for degradation. The methods of visualizing and modeling epitope recognition are known to one skilled in the art (US 20090311253).

[0127] "Pharmacokinetics" refers to the process by which a drug is absorbed, distributed, metabolized, and excreted by an organism. To generate a multivalent binding protein molecule with a desired pharmacokinetic profile, parent monoclonal antibodies with similarly desired pharmacokinetic profiles are selected. The PK profiles of the selected parental monoclonal antibodies can be easily determined in rodents using methods known to one skilled in the art (US 20090311253).

[0128] "Bioavailability" refers to the amount of active drug that reaches its target following administration. Bioavailability is function of several of the previously described properties, including stability, solubility, immunogenicity and pharmacokinetics, and can be assessed using methods known to one skilled in the art (US 20090311253).

[0129] The term "surface plasmon resonance" means an optical phenomenon that allows for the analysis of real-time biospecific interactions by detection of alterations in protein concentrations within a biosensor matrix, for example using the BIAcore® system (BIAcore International AB, a GE Healthcare company, Uppsala, Sweden and Piscataway, NJ). For further descriptions, see Jönsson et al. (1993) Ann. Biol. Clin. 51:19-26. The term "K_{on}" means the on rate constant for association of a binding protein (e.g., an antibody or DVD-Ig) to the antigen to form the, e.g., DVD-Ig/antigen complex. The term "K_{on}" also means "association rate constant", or "ka", as is used interchangeably herein. This value indicating the binding rate of a binding protein to its target antigen or the rate of complex formation between a binding protein, e.g., an antibody, and antigen also is shown by the equation below:

Antibody ("Ab") + Antigen ("Ag")→Ab-Ag

[0130] The term "K_{off}" means the off rate constant for dissociation, or "dissociation rate constant", of a binding protein (e.g., an antibody or DVD-Ig) from the, e.g., DVD-Ig/antigen complex as is known in the art. This value indicates the dissociation rate of a binding protein, e.g., an antibody, from its target antigen or separation of Ab-Ag complex over time into free antibody and antigen as shown by the equation below:

Ab + Ag←Ab-Ag

[0131] The terms "K_d" and "equilibrium dissociation constant" means the value obtained in a titration measurement at equilibrium, or by dividing the dissociation rate constant (K_{off}) by the association rate constant (K_{on}). The association rate constant, the dissociation rate constant and the equilibrium dissociation constant, are used to represent the binding affinity of a binding protein (e.g., an antibody or DVD-Ig) to an antigen. Methods for determining association and dissociation rate constants are well known in the art. Using fluorescence–based techniques offers high sensitivity and the ability to examine samples in physiological buffers at equilibrium. Other experimental approaches and instruments such as a BIAcore® (biomolecular interaction analysis) assay, can be used (e.g., instrument available from BIAcore International AB, a GE Healthcare company, Uppsala, Sweden). Additionally, a KinExA® (Kinetic Exclusion Assay) assay, available from Sapidyne Instruments (Boise, Idaho), can also be used.

[0132] The term "variant" means a polypeptide that differs from a given polypeptide in amino acid sequence by the addition (e.g., insertion), deletion, or conservative substitution of amino acids, but that retains the biological activity of the given polypeptide (e.g., a variant TNF(antibody can compete with anti- TNF(antibody for binding to TNF(). A conservative substitution of an amino acid, i.e., replacing an amino acid with a different amino acid of similar properties (e.g., hydrophilicity and degree and distribution of charged regions) is recognized in the art as typically involving a minor change. These minor changes can be identified, in part, by considering the hydropathic index of amino acids, as understood in the art (see, e.g., Kyte et al. (1982) J. Mol. Biol. 157: 105-132). The hydropathic index of an amino acid is based on a consideration of its hydrophobicity and charge. It is known in the art that amino acids of similar hydropathic indexes in a protein can be substituted and the protein still retains protein function. In one aspect, amino acids having hydropathic

indexes of ± 2 are substituted. The hydrophilicity of amino acids also can be used to reveal substitutions that would result in proteins retaining biological function. A consideration of the hydrophilicity of amino acids in the context of a peptide permits calculation of the greatest local average hydrophilicity of that peptide, a useful measure that has been reported to correlate well with antigenicity and immunogenicity (see, e.g., US Patent No. 4,554,101). Substitution of amino acids having similar hydrophilicity values can result in peptides retaining biological activity, for example immunogenicity, as is understood in the art. In one aspect, substitutions are performed with amino acids having hydrophilicity values within ± 2 of each other. Both the hydrophobicity index and the hydrophilicity value of amino acids are influenced by the particular side chain of that amino acid. Consistent with that observation, amino acid substitutions that are compatible with biological function are understood to depend on the relative similarity of the amino acids, and particularly the side chains of those amino acids, as revealed by the hydrophobicity, hydrophilicity, charge, size, and other properties. The term "variant" also includes polypeptide or fragment thereof that has been differentially processed, such as by proteolysis, phosphorylation, or other post-translational modification, yet retains its biological activity or antigen reactivity, e.g., the ability to bind to TNF(. The term "variant" encompasses fragments of a variant unless otherwise defined. A variant may be 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, 90%, 89%, 88%, 87%, 86%,85%, 84%, 83%, 82%, 81%, 80%, 79%, 78%, 77%, 76%, or 75% identical to the wildtype sequence.

Generation of binding proteins

[0133] The binding proteins disclosed herein can be generated using various techniques. Expression vectors, host cell and methods of generating the binding protein are provided and are well known in the art. For instance, the variable domains of the DVD binding protein can be obtained from parent antibodies, including polyclonal Abs and mAbs capable of binding antigens of interest. These antibodies may be naturally occurring or may be generated by recombinant technology. The person of ordinary skill in the art is well familiar with many methods for producing antibodies, including, but not limited to using hybridoma techniques, selected lymphocyte antibody method (SLAM), use of a phage, yeast, or RNA-protein fusion display or other library, immunizing a non-human animal comprising at least some of the human immunoglobulin locus, and preparation of chimeric, CDR-grafted, and humanized antibodies. See, e.g., US Patent Publication No. 20090311253 A1. Variable domains may also be prepared using affinity maturation techniques.

A. Criteria for selecting parent monoclonal antibodies

[0134] An embodiment is provided comprising selecting parent antibodies with at least one or more properties desired in the DVD binding protein molecule. In an embodiment, the desired property is one or more antibody parameters, such as, for example, antigen specificity, affinity to antigen, potency, biological function, epitope recognition, stability, solubility, production efficiency, immunogenicity, pharmacokinetics, bioavailability, tissue cross reactivity, or orthologous antigen binding. See, e.g., US Patent Publication No. 20090311253.

B. Construction of binding protein molecules

[0135] The binding protein may be designed such that two different light chain variable domains (VL) from the two different parent monoclonal antibodies are linked in tandem directly or via a linker by recombinant DNA techniques, followed by the light chain constant domain CL. Similarly, the heavy chain comprises two different heavy chain variable domains (VH) linked in tandem, directly or via a linker, followed by the constant domain CH1 and Fc region (Figure 1).

[0136] The variable domains can be obtained using recombinant DNA techniques from parent antibodies generated by any one of the methods described herein. In an embodiment, the variable domain is a murine heavy or light chain variable domain. In another embodiment, the variable domain is a CDR grafted or a humanized variable heavy or light chain domain. In an embodiment, the variable domain is a human heavy or light chain variable domain.

[0137] The linker sequence may be a single amino acid or a polypeptide sequence. In an embodiment, the choice of linker sequences is based on crystal structure analysis of several Fab molecules. There is a natural flexible linkage between the variable domain and the CH1/CL constant domain in Fab or antibody molecular structure. This natural linkage comprises approximately 10-12 amino acid residues, contributed by 4-6 residues from the C-terminus of a V domain and 4-6 residues from the N-terminus of a CL/CH1 domain. DVD-Ig binding proteins were generated using N-terminal 5-6 amino acid residues, or 11-12 amino acid residues, of CL or CH1 as a linker in the light chain and heavy chains, respectively. The N-terminal residues of CL or CH1 domains, particularly the first 5-6 amino acid residues, can adopt a loop conformation without strong secondary structures, and therefore can act as flexible linkers between the two variable domains. The N-terminal residues of CL or CH1 domains are natural extension of the variable domains, as they are part of the Ig

sequences, and therefore their use minimizes to a large extent any immunogenicity potentially arising from the linkers and junctions.

[0138] In a further embodiment, of any of the heavy chain, light chain, two chain, or four chain embodiments, includes at least one linker comprising AKTTPKLEEGEFSEAR (SEQ ID NO: 1); AKTTPKLEEGEFSEARV (SEQ ID NO: 2); AKTTPKLGG (SEQ ID NO: 3); SAKTTPKLGG (SEQ ID NO: 4); SAKTTP (SEQ ID NO: 5); RADAAP (SEQ ID NO: 6); RADAAPTVS (SEQ ID NO: 7); RADAAAAGGPGS (SEQ ID NO: 8); RADAAAA(G_4S)$_4$ (SEQ ID NO: 9); SAKTTPKLEEGEFSEARV (SEQ ID NO: 10); ADAAP (SEQ ID NO: 11); ADAAPTVSIFPP (SEQ ID NO: 12); TVAAP (SEQ ID NO: 13); TVAAPSVFIFPP (SEQ ID NO: 14); QPKAAP (SEQ ID NO: 15); QPKAAPSVTLFPP (SEQ ID NO: 16); AKTTPP (SEQ ID NO: 17); AKTTPPSVTPLAP (SEQ ID NO: 18); AKTTAP (SEQ ID NO: 19); AKTTAPSVYPLAP (SEQ ID NO: 20); ASTKGP (SEQ ID NO: 21); ASTKGPSVFPLAP (SEQ ID NO: 22), GGGGSGGGGSGGGGS (SEQ ID NO: 23); GENKVEYAPALMALS (SEQ ID NO: 24); GPAKELTPLKEAKVS (SEQ ID NO: 25); or GHEAAAVMQVQYPAS (SEQ ID NO: 26); TVAAPSVFIFPPTVAAPSVFIFPP (SEQ ID NO: 27); ASTKGPSVFPLAPASTKGPSVFPLAP (SEQ ID NO: 28); GGGGSGGGGS (SEQ ID NO: 29); GGSGGGGSG (SEQ ID NO: 30); or G/S based sequences (e.g., G4S and G4S repeats; SEQ ID NO: 31). In an embodiment, X2 is an Fc region. In another embodiment, X2 is a variant Fc region.

[0139] Other linker sequences may include any sequence of any length of a CL/CH1 domain but not all residues of a CL/CH1 domain; for example the first 5-12 amino acid residues of a CL/CH1 domain; the light chain linkers can be from Cκ or Cλ; and the heavy chain linkers can be derived from CH1 of any isotype, including Cγ1, Cγ2, Cγ3, Cγ4, Cα1, Cα2, Cδ, Cε, and Cμ. Linker sequences may also be derived from other proteins such as Ig-like proteins (e.g., TCR, FcR, KIR); G/S based sequences (e.g., G4S repeats; SEQ ID NO: 31); hinge region-derived sequences; and other natural sequences from other proteins.

[0140] In an embodiment, a constant domain is linked to the two linked variable domains using recombinant DNA techniques. In an embodiment, a sequence comprising linked heavy chain variable domains is linked to a heavy chain constant domain and a sequence comprising linked light chain variable domains is linked to a light chain constant domain. In an embodiment, the constant domains are human heavy chain constant domains and human light chain constant domains respectively. In an embodiment, the DVD heavy chain is further linked to an Fc region. The Fc

region may be a native sequence Fc region or a variant Fc region. In another embodiment, the Fc region is a human Fc region. In another embodiment, the Fc region includes Fc region from IgG1, IgG2, IgG3, IgG4, IgA, IgM, IgE, or IgD.

[0141] In an embodiment, an antibody or functional antigen binding fragment thereof is disclosed, comprising an antibody having a functional binding site capable of binding TNFα, IL-13, PGE2, or NGF, and having a variable region comprising paired VH and VL sequences selected from the pairs listed in Table 1, or comprising the CDR regions of those VH and VL regions. For instance, the antibody or functional antigen binding fragment thereof may be capable of binding IL-13 and have a variable region comprising SEQ ID NOs: 32 and 33. Likewise, an antibody or functional antigen binding fragment thereof can be capable of binding TNF, PGE2, or NGF and have a variable region comprising paired sequences selected from those in Table 1. In an embodiment, a functional antigen binding fragment of an antibody described above is disclosed, wherein the antigen binding fragment retains variable sequences sufficient to form a binding site capable of binding the target antigen. For example, the antigen binding fragment may comprise the CDR regions taken from the paired VH and VL sequences in Table 1, or the full VH and VL sequences with or without an Fc domain. A functional antigen binding fragment may include, among other examples, a humanized, fully human, camelized, single-chain, chimeric, synthetic, recombinant, hybrid, mutated, back-mutated, or CDR-grafted antibody, or a Fab, F(ab')2, Fv, scFv, Fd, dAb fragment, a VHH (also referred to as a nanobody), or any other antibody fragment that retains antigen-binding function, including bi-specific or multi-specific antibodies.

[0142] In an embodiment, a binding protein (e.g., a dual variable domain binding protein) is disclosed comprising variable domains selected from those in Table 1. In some embodiments, the binding protein comprises first and second polypeptide chains, each of which comprises VD1-(X1)n-VD2-C-X2, and wherein the first and second chains of the binding protein together form two functional binding sites, wherein those binding sites are capable of binding TNFα and IL-13, TNF and PGE2, or TNF and NGF. In some embodimdnts, the VD1 and VD2 sequences are independently chosen (i.e., the choice of VH and VL sequences for the VD1 position does not impact the choice of sequences for the VD2 position, and vice versa). In an embodiment, each functional binding site comprises paired VH and VL sequences selected from the pairs listed in Table 1 (e.g., the paired VH and VL sequences of SEQ ID NO: 32 and 33, forming a binding site for IL-13), or comprising the CDR

regions of those VH and VL sequences. In some embodiments, the first chain comprises a first VH sequence at position VD1 and a second VH sequence at position VD2, both of which are selected from Table 1, or the VD1 and VD2 domains contain the CDR sequences from those selected VH sequences, while the second chain comprises a first VL sequence at position VD1 and a second VL sequence at position VD2, both of which are selected from Table 1, or the VD1 and VD2 domains contain the CDR sequences from those selected VL sequences. In other embodiments, the VH-VL arrangement of the first or second binding site is flipped across the two polypeptide chains, such that each chain comprises a VH sequence joined to a VL sequence, while the two chains together still form two functional binding sites. For instance, the first polypeptide chain may comprise a VH sequence at the VD1 position and a VL sequence at the VD2 position, while the second chain would comprise the paired VL sequence at the VD1 position (forming a first functional binding site) and the paired VH sequence at the VD2 position (forming a second binding site).

[0143] In an embodiment, two first chain polypeptides and two second chain polypeptides are combined to form a DVD-Ig binding protein having two arms and four binding sites. An example of a DVD-Ig binding protein structure having two arms and four binding sites is shown in Figure 1. In an embodiment, a DVD-Ig binding protein comprises at the VD1 and VD2 positions on each arm at least one, or at least two, at least three, or at least four, of the VH and VL sequence pairs listed in Table 1, in any orientation. In some embodiments, sequence pairs forming the binding sites are independently chosen (e.g., the choice of VH and VL sequences for the VD1 position on one arm does not impact the choice of sequences for the VD1 position on the other arm, nor does it affect the choice of sequences for the VD2 positions on either arm). The VH and VL sequences provided in Table 1 below comprise complementarity determining regions (CDRs) and framework sequences. In some embodiments, one or more of the framework sequences are replaced, without loss of function, by other framework sequences, e.g., from binding proteins that are known in the art to bind to the same antigen.

[0144] In another embodiment, two heavy chain DVD-Ig polypeptides and two light chain DVD-Ig polypeptides are combined to form a DVD-Ig binding protein. Tables 1A-1C list amino acid sequences of VH and VL regions of exemplary antibodies useful for treating disease. In an embodiment, a DVD-Ig comprising at least two of the VH and/or VL regions listed in Table 1, in any orientation, is provided. In some embodiments, VD1 and VD2 are independently chosen. Therefore, in some

embodiments, VD1 and VD2 comprise the same SEQ ID NO and, in other embodiments, VD1 and VD2 comprise different SEQ ID NOS. The VH and VL domain sequences provided below comprise complementarity determining regions (CDRs) and framework sequences that are either known in the art or readily discernible using methods known in the art. In some embodiments, one or more of these CDRs and/or framework sequences are replaced, without loss of function, by other CDRs and/or framework sequences from binding proteins that are known in the art to bind to the same antigen.

Table 1: List of Amino Acid Sequences of VH and VL Regions of Antibodies for Generating Binding Proteins, Including Multivalent Binding Proteins

SEQ ID No.	ABT Unique ID	Protein region	Sequence 1234567890123456789012345678 90
32	AB397VH	VH-IL13 (seq 1)	EVTLRESGPGLVKPTQTLTLTCTLYGFSLS TSLMGVDWIRQPPGKGLEWLAHIWWDDVKR YNPALKSRLTISKDTSKNQVVLKLTSVDPV DTATYYCARTVSSGYIYYAMDYWGQGTLVT VSS
33	AB397VL	VL-IL13 (seq 1)	DIQMTQSPSSLSASVGDRVTISCRASQDIR NYLNWYQQKPGKAPKLLIFYTSKLHSGVPS RFSGSGSGTDYTLTISSLQPEDIATYYCQQ GLTPPLTFGGGTKVEIKR
34	AB398VH	VH-IL13 (seq 2)	EVQLVQSGAEVKKPGASVKVSCKASGYTFT TYGVSWVRQAPGQGLEWMGEIYPGNYNTYY NEKFRGRVTMTTDTSTSTAYMELRSLRSDD TAVYYCARWRTSYFSDYGYFDYWGQGTTVT VSS
35	AB398VL	VL-IL13 (seq 2)	DVVMTQSPLSLPVTLGQPASISCRSSQSLV HSHGNTYLHWYQQRPGQSPRLLIYTVSNRF SGVPDRFSGSGSGTDFTLKISRVEAEDVGV YYCSQSTHVPYTFGGGTKVEIKR
36	AB399VH	VH-IL13 (seq 3)	EVQLVQSGAEVKKPGASVKVSCKASGYTFT SYWMHWVRQAPGQGLEWIGNINPKGGSNIY NEKFQGRVTMTRDTSISTAYMELSRLRSDD TAVYYCARLDYFGDSFDLWGQGTTVTVSS
37	AB399VL	VL-IL13 (seq 3)	DIQMTQSPSSLSASVGDRVTITCRASQGIR NYLNWYQQKPGKAPKLLIYYASNLEVGVPS RFSGSGSGTDYTLTISSLQPEDFATYYCQQ DNRFPYTFGGGTKVEIKR
38	AB436VH	VH - TNF (seq 1)	EVQLVESGGGLVQPGGSLRLSCAASGFTFS NYGVTWVRQAPGKGLEWVSMIWADGSTEYA SSVKGRFTISRDNSKNTLYLQMNSLRAEDT AVYYCAREWQHGPVAYWGQGTLVTVSS
39	AB436VL	VL - TNF (seq 1)	DIQMTQSPSSLSASVGDRVTITCRASQLVS SAVAWYQQKPGKAPKLLIYWASARHTGVPS RFSGSGSGTDFTLTISSLQPEDFATYYCQQ HYKTPFTFGQGTKLEIKR

SEQ ID No.	ABT Unique ID	Protein region	Sequence 1234567890123456789012345678 90
40	AB437VH	VH - TNF (seq 2)	EVQLVESGGGLVQPGGSLRLSCAASGFTFS NYGVEWVRQAPGKGLEWVSGIWADGSTHYA DTVKSRFTISFDNSKNTLYLQMNSLRAEDT AVYYCAREWQHGPVAYWGQGTLVTVSS
41	AB437VL	VL - TNF (seq 2)	DIQMTQSPSSLSASVGDRVTITCKASQLVS SAVAWYQQKPGKAPKLLIYWASTLHTGVPS RFSGSGSGTDFTLTISSLQPEDFATYYCQQ HYRTPFTFGQGTKLEIKR
42	AB441VH	VH - TNF (seq 3)	EVQLVQSGAEVKKPGASVKVSCKASGYTFA NYGIIWVRQAPGQGLEWMGWINTYTGKPTY AQKFQGRVTMTTDTSTSTAYMELSSLRSED TAVYYCARKLFTTMDVTDNAMDYWGQGTTV TVSS
43	AB441VL	VL - TNF (seq 3)	DIQMTQSPSSLSASVGDRVTITCKASQDIS QYLNWYQQKPGKAPKLLIYYTSRLQSGVPS RFSGSGSGTDFTLTISSLQPEDFATYFCQQ GNTWPPTFGQGTKLEIKR
48	AB444VH	VH - TNF (seq 3)	EVQLVQSGAEVKKPGASVKVSCKASGYTFN NYGIIWVRQAPGQGLEWMGWINTYTGKPTY AQKFQGRVTMTTDTSTSTAYMELSSLRSED TAVYYCARKLFNTVAVTDNAMDYWGQGTTV TVSS
49	AB444VL	VL - TNF (seq 3)	DIQMTQSPSSLSASVGDRVTITCRASQDIE NYLNWYQQKPGKAPKLLIYYTSPLQSGVPS RFSGSGSGTDFTLTISSLQPEDFATYFCQQ GNTQPPTFGQGTKLEIKR
50	AB046VH	VH - PGE2 (seq 1)	EVQLVQSGAEVKKPGASVKVSCKASGYTFT KYWLGWVRQAPGQGLEWMGDIYPGYDYTHY NEKFKDRVTLTTDTSTSTAYMELRSLRSDD TAVYYCARSDGSSTYWGQGTLVTVSS
51	AB046VL	VL - PGE2 (seq 1)	DVLMTQTPLSLPVTPGEPASISCTSSQNIV HSNGNTYLEWYLQKPGQSPQLLIYKVSNRF SGVPDRFSGSGSGTDFTLKISRVEAEDVGV YYCFQVSHVPYTFGGGTKVEIKR
52	AB131VH	VH - PGE2 (AB016) (seq 2)	EVQLVESGGGLVQPGGSLRLSCAASGFSFS KYWLGWVRQAPGKGLEWVSDIYPGYDYTHY NEKFKDRFTISADTSKNTAYLQMNSLRAED TAVYYCARSDGSSTYWGQGTLVTVSS
53	AB131VL	VL - PGE2 (AB016) (seq 2)	DIQMTQSPSSLSASVGDRVTITCTSSQNIV HSNGNTYLEWYQQKPGKAPKLLIYKVSNRF SGVPSRFSGSGSGTDFTLTISSLQPEDFAT YYCFQVSHVPYTFGQGTKVEIKR
54	AB135VH	VH - PGE2 (AB022) (seq 3)	EVQLQQSGPELVTPGASVKISCKASGYTFT KYWLGWVKQSHGKSLEWIGDIYPGYDYTHY NEKFKDTATLTVDKSSSIAYMEIRGLTSED SAVYYCARSDGSSTYWGQGTLVTVSA

SEQ ID No.	ABT Unique ID	Protein region	Sequence 1234567890123456789012345678 90
55	AB135VL	VL - PGE2 (AB022)(seq 3)	DVQMIQSPSSLSASLGDIVTMTCTSSQNIV RSNGNTYLEWFQQKPGKAPKLLIYKVSNRF SGVPSRFSGSGSRYGTDFTLTISSLEDEDLAT YFCFQVSHVPYTFGGGTKLEIKR
56	AB267VH	VH - NGF	EVQLVESGGGLVQPGGSLRLSCAASGFSLT NNNVNWVRQAPGKGLEWVGGVWAGGATDYN SALKSRFTISRDNSKNTAYLQMNSLRAEDT AVYYCARDGGYSSSTLYAMDAWGQGTLVTV SS
57	AB267VL	VL - NGF	DIQMTQSPSSLSASVGDRVTITCRASEDIY NALAWYQQKPGKAPKLLIYNTDTLHTGVPS RFSGSGSGTDYTLTISSLQPEDFATYFCQH YFSYPRTFGQGTKVEIKR

[0145] CDRs 1-3 of each VH and VL sequence listed in table 1 are underlined. For instance, CDRs 1-3 are underlined for SEQ ID NO: 32 at amino acid positions 31-37 (CDR1), 52-67 (CDR2), and 100-112 (CDR3). Detailed description of specific DVD binding proteins capable of binding specific targets, and methods of making the same, is provided in the Examples section below.

C. Production of binding proteins

[0146] The binding proteins provided herein may be produced by any of a number of techniques known in the art. For example, expression from host cells, wherein expression vector(s) encoding the DVD-Ig heavy and DVD-Ig light chains is (are) transfected into a host cell by standard techniques. Although it is possible to express the DVD-Ig binding proteins provided herein in either prokaryotic or eukaryotic host cells, DVD-Ig binding proteins are preferably expressed in eukaryotic cells, for example, mammalian host cells, because such eukaryotic cells (and in particular mammalian cells) are more likely than prokaryotic cells to assemble and secrete a properly folded and immunologically active DVD-Ig binding protein.

[0147] In an exemplary system for recombinant expression of DVD-Ig proteins, a recombinant expression vector encoding both the DVD-Ig heavy chain and the DVD-Ig light chain is introduced into dhfr- CHO cells by calcium phosphate-mediated transfection. Within the recombinant expression vector, the DVD-Ig heavy and light chain genes are each operatively linked to CMV enhancer/AdMLP promoter regulatory elements to drive high levels of transcription of the genes. The recombinant expression vector also carries a DHFR gene, which allows for selection of CHO cells that have been transfected with the vector using methotrexate selection/amplification.

The selected transformant host cells are cultured to allow for expression of the DVD-Ig heavy and light chains and intact DVD-Ig protein is recovered from the culture medium. Standard molecular biology techniques are used to prepare the recombinant expression vector, transfect the host cells, select for transformants, culture the host cells and recover the DVD-Ig protein from the culture medium. A method of synthesizing a DVD-Ig protein provided herein by culturing a host cell provided herein in a suitable culture medium until a DVD-Ig protein is synthesized is also provided. The method can further comprise isolating the DVD-Ig protein from the culture medium.

[0148] An important feature of a DVD-Ig binding protein is that it can be produced and purified in a similar way to a conventional antibody. The production of DVD-Ig binding protein results in a homogeneous, single major product with desired dual-specific activity, without the need for sequence modification of the constant region or chemical modifications. Other previously described methods to generate "bi-specific", "multi-specific", and "multi-specific multivalent" full length binding proteins can lead to the intracellular or secreted production of a mixture of assembled inactive, mono-specific, multi-specific, multivalent, full length binding proteins, and multivalent full length binding proteins with a combination of different binding sites.

[0149] Surprisingly, the design of the "dual-specific multivalent full length binding proteins" provided herein leads to a dual variable domain light chain and a dual variable domain heavy chain that assemble primarily to the desired "dual-specific multivalent full length binding proteins".

[0150] In some embodiments, at least 50%, at least 75% and at least 90% of the assembled, and expressed dual variable domain immunoglobulin molecules are the desired dual-specific tetravalent protein, and therefore possess enhanced commercial utility. Thus, in various embodiments, a method to express a dual variable domain light chain and a dual variable domain heavy chain in a single cell leading to a single primary product of a "dual-specific tetravalent full length binding protein" is provided.

[0151] Methods of expressing a dual variable domain light chain and a dual variable domain heavy chain in a single cell leading to a "primary product" of a "dual-specific tetravalent full length binding protein", where the "primary product" is more than 50%, such as more than 75% and more than 90%, of all assembled protein,

comprising a dual variable domain light chain and a dual variable domain heavy chain are provided.

Uses of binding proteins

[0152] Given their ability to bind to two or more antigens, the binding proteins provided herein can be used to detect the antigens (e.g., in a biological sample, such as serum or plasma), using a conventional immunoassay, such as an enzyme linked immunosorbent assays (ELISA), a radioimmunoassay (RIA), or tissue immunohistochemistry. The binding protein is directly or indirectly labeled with a detectable substance to facilitate detection of the bound or unbound antibody. 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 is luminol and examples of suitable radioactive materials include ^{3}H, ^{14}C, ^{35}S, ^{90}Y, ^{99}Tc, ^{111}In, ^{125}I, ^{131}I, ^{177}Lu, ^{166}Ho, and ^{153}Sm.

[0153] In an embodiment, the binding proteins provided herein are capable of neutralizing the activity of their antigen targets both *in vitro* and *in vivo*. Accordingly, such binding proteins can be used to inhibit antigen activity, e.g., in a cell culture containing the antigens, in human subjects or in other mammalian subjects having the antigens with which a binding protein provided herein cross-reacts. In another embodiment, a method for reducing antigen activity in a subject suffering from a disease or disorder in which the antigen activity is detrimental is provided. A binding protein provided herein can be administered to a human subject for therapeutic purposes.

[0154] The term "a disorder in which antigen activity is detrimental" is intended to include diseases and other disorders in which the presence of the antigen in a subject suffering from the disorder has been shown to be or is suspected of being either responsible for the pathophysiology of the disorder or a factor that contributes to a worsening of the disorder. Accordingly, a disorder in which antigen activity is detrimental is a disorder in which reduction of antigen activity is expected to alleviate the symptoms and/or progression of the disorder. Such disorders may be evidenced,

for example, by an increase in the concentration of the antigen in a biological fluid of a subject suffering from the disorder (e.g., an increase in the concentration of antigen in serum, plasma, synovial fluid, etc., of the subject). Non-limiting examples of disorders that can be treated with the binding proteins provided herein include those disorders discussed below and in the section pertaining to pharmaceutical compositions comprising the binding proteins.

[0155] DVD binding proteins are useful as therapeutic agents to simultaneously block two different targets to enhance efficacy/safety and/or increase patient coverage.

[0156] Additionally, DVD binding proteins provided herein can be employed for tissue-specific delivery (target a tissue marker and a disease mediator for enhanced local PK thus higher efficacy and/or lower toxicity), including intracellular delivery (targeting an internalizing receptor and an intracellular molecule), delivering to inside brain (targeting transferrin receptor and a CNS disease mediator for crossing the blood-brain barrier). DVD binding protein can also serve as a carrier protein to deliver an antigen to a specific location via binding to a non-neutralizing epitope of that antigen and also to increase the half-life of the antigen. Furthermore, DVD binding protein can be designed to either be physically linked to medical devices implanted into patients or target these medical devices (see Burke et al. (2006)Advanced Drug Deliv. Rev. 58(3): 437-446; Hildebrand et al. (2006) Surface and Coatings Technol. 200(22-23): 6318-6324; Drug/ device combinations for local drug therapies and infection prophylaxis, Wu (2006) Biomaterials 27(11):2450-2467; Mediation of the cytokine network in the implantation of orthopedic devices, Marques (2005) Biodegradable Systems in Tissue Engineer. Regen. Med. 377-397). Briefly, directing appropriate types of cell to the site of medical implant may promote healing and restoring normal tissue function. Alternatively, inhibition of mediators (including but not limited to cytokines), released upon device implantation by a DVD coupled to or target to a device is also provided.

[0157] Binding protein molecules provided herein are useful as therapeutic molecules to treat various diseases, e.g., wherein the targets that are recognized by the binding proteins are detrimental. Such binding proteins may bind one or more targets involved in a specific disease. In an embodiment, the DVD-Ig proteins of the invention are used to treat or diagnose human autoimmune or inflammatory disorders, asthma, rheumatoid arthritis, osteoarthritis, sepsis, systemic lupus erythematosis, multiple sclerosis, neurological disorders, or oncological disorders.

Pharmaceutical Compositions

[0158] In various embodiments, pharmaceutical compositions comprising one or more binding proteins, either alone or in combination with prophylactic agents, therapeutic agents, and/or pharmaceutically acceptable carriers are provided. The pharmaceutical compositions comprising binding proteins provided herein are for use in, but not limited to, diagnosing, detecting, or monitoring a disorder, in preventing, treating, managing, or ameliorating a disorder or one or more symptoms thereof, and/or in research. The formulation of pharmaceutical compositions, either alone or in combination with prophylactic agents, therapeutic agents, and/or pharmaceutically acceptable carriers, are known to one skilled in the art (US Patent Publication No. 20090311253 A1).

[0159] Methods of administering a pharmaceutical composition or a prophylactic or therapeutic agent provided herein include, but are not limited to, parenteral administration (e.g., intradermal, intramuscular, intraperitoneal, intravenous and subcutaneous), epidural administration, intratumoral administration, mucosal administration (e.g., intranasal and oral routes) and pulmonary administration (e.g., aerosolized compounds administered with an inhaler or nebulizer). The formulation of pharmaceutical compositions for specific routes of administration, and the materials and techniques necessary for the various methods of administration are available and known to one skilled in the art (e.g., US Patent Publication No. 20090311253 A1).

[0160] Dosage regimens may be adjusted to provide the optimum desired response (e.g., a therapeutic or prophylactic response). For example, a single bolus may be administered, several divided doses may be administered over time or the dose may be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation. It is especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. The term "dosage unit form" refers to physically discrete units suited as unitary dosages for the mammalian subjects to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms provided herein are dictated by and directly dependent on (a) the unique characteristics of the active compound and the particular therapeutic or prophylactic effect to be achieved, and (b) the limitations inherent in the art of compounding such an active compound for the treatment of sensitivity in individuals.

An exemplary, non-limiting range for a therapeutically or prophylactically effective amount of a binding protein provided herein is 0.1-20 mg/kg, for example, 1-10 mg/kg. It is to be noted that dosage values may vary with the type and severity of the condition to be alleviated. It is to be further understood that for any particular subject, specific dosage regimens may be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of the compositions, and that dosage ranges set forth herein are exemplary only and are not intended to limit the scope or practice of the claimed composition.

Combination Therapy

[0161] A binding protein provided herein also can also be administered with one or more additional therapeutic agents useful in the treatment of various diseases, the additional agent being selected by the skilled artisan for its intended purpose. For example, the additional agent can be a therapeutic agent art-recognized as being useful to treat the disease or condition being treated by the antibody provided herein. The combination can also include more than one additional agent, e.g., two or three additional agents.

[0162] Combination therapy agents include, but are not limited to, antineoplastic agents, radiotherapy, chemotherapy such as DNA alkylating agents, cisplatin, carboplatin, anti-tubulin agents, paclitaxel, docetaxel, taxol, doxorubicin, gemcitabine, gemzar, anthracyclines, adriamycin, topoisomerase I inhibitors, topoisomerase II inhibitors, 5-fluorouracil (5-FU), leucovorin, irinotecan, receptor tyrosine kinase inhibitors (e.g., erlotinib, gefitinib), COX-2 inhibitors (e.g., celecoxib), kinase inhibitors, and siRNAs.

[0163] Combinations to treat autoimmune and inflammatory diseases may include the addition of non-steroidal anti-inflammatory drug(s), also referred to as NSAIDS, which include drugs like ibuprofen. Other combinations are corticosteroids including prednisolone; the well known side-effects of steroid use can be reduced or even eliminated by tapering the steroid dose required when treating patients in combination with the binding proteins provided herein. Non-limiting examples of therapeutic agents for rheumatoid arthritis with which a binding protein provided herein, or a binding portion thereof, can be combined include the following: cytokine suppressive anti-inflammatory drug(s) (CSAIDs); antibodies to or antagonists of other human cytokines or growth factors, for example, TNF, LT, IL-1, IL-2, IL-3, IL-4, IL-5,

IL-6, IL-7, IL-8, IL-15, IL-16, IL-18, IL-21, IL-23, interferons, EMAP-II, GM-CSF, FGF, and PDGF. Binding proteins provided herein, or antigen binding portions thereof, can be combined with antibodies to cell surface molecules such as CD2, CD3, CD4, CD8, CD25, CD28, CD30, CD40, CD45, CD69, CD80 (B7.1), CD86 (B7.2), CD90, CTLA or their ligands including CD154 (gp39 or CD40L).

[0164] Combinations of therapeutic agents may interfere at different points in the autoimmune and subsequent inflammatory cascade; one or more of the following may therefore be administered in combination with a binding protein disclosed herein. Examples include a binding protein disclosed herein and a TNF antagonist like a chimeric, humanized or human TNF antibody, Adalimumab, (PCT Publication No. WO 97/29131), CA2 (Remicade™), CDP 571, a soluble p55 or p75 TNF receptor, or derivative thereof (p75TNFR1gG (Enbrel™) or p55TNFR1gG (Lenercept)), a TNFα converting enzyme (TACE) inhibitor; or an IL-1 inhibitor (an Interleukin-1-converting enzyme inhibitor, IL-1RA, etc.). Other combinations include a binding protein disclosed herein and Interleukin 11. Yet another combination include key players of the autoimmune response which may act parallel to, dependent on or in concert with IL-12 function; especially relevant are IL-18 antagonists including an IL-18 antibody, a soluble IL-18 receptor, or an IL-18 binding protein. It has been shown that IL-12 and IL-18 have overlapping but distinct functions and a combination of antagonists to both may be most effective. Yet another combination is a binding protein disclosed herein and a non-depleting anti-CD4 inhibitor. Yet other combinations include a binding protein disclosed herein and an antagonist of the co-stimulatory pathway CD80 (B7.1) or CD86 (B7.2) including an antibody, a soluble receptor, or an antagonistic ligand.

[0165] The binding proteins provided herein may also be combined with an agent, such as methotrexate, 6-MP, azathioprine sulphasalazine, mesalazine, olsalazine chloroquinine/hydroxychloroquine, pencillamine, aurothiomalate (intramuscular and oral), azathioprine, cochicine, a corticosteroid (oral, inhaled and local injection), a beta-2 adrenoreceptor agonist (salbutamol, terbutaline, salmeteral), a xanthine (theophylline, aminophylline), cromoglycate, nedocromil, ketotifen, ipratropium, oxitropium, cyclosporin, FK506, rapamycin, mycophenolate mofetil, leflunomide, an NSAID, for example, ibuprofen, a corticosteroid such as prednisolone, a phosphodiesterase inhibitor, an adensosine agonist, an antithrombotic agent, a complement inhibitor, an adrenergic agent, an agent which interferes with signalling by proinflammatory cytokines such as TNF-α or IL-1 (e.g., IRAK, NIK, IKK , p38 or a MAP kinase inhibitor), an IL-1β converting enzyme inhibitor, a TNFα converting

enzyme (TACE) inhibitor, a T-cell signalling inhibitor such as a kinase inhibitor, a metalloproteinase inhibitor, sulfasalazine, azathioprine, a 6-mercaptopurine, an angiotensin converting enzyme inhibitor, a soluble cytokine receptor or derivative thereof (e.g., a soluble p55 or p75 TNF receptor or the derivative p75TNFRIgG (Enbrel™) or p55TNFRIgG (Lenercept), sIL-1RI, sIL-1RII, sIL-6R), an antiinflammatory cytokine (e.g., IL-4, IL-10, IL-11, IL-13 and TGFβ), celecoxib, folic acid, hydroxychloroquine sulfate, rofecoxib, etanercept, infliximab, naproxen, valdecoxib, sulfasalazine, methylprednisolone, meloxicam, methylprednisolone acetate, gold sodium thiomalate, aspirin, triamcinolone acetonide, propoxyphene napsylate/apap, folate, nabumetone, diclofenac, piroxicam, etodolac, diclofenac sodium, oxaprozin, oxycodone hcl, hydrocodone bitartrate/apap, diclofenac sodium/misoprostol, fentanyl, anakinra, human recombinant, tramadol hcl, salsalate, sulindac, cyanocobalamin/fa/pyridoxine, acetaminophen, alendronate sodium, prednisolone, morphine sulfate, lidocaine hydrochloride, indomethacin, glucosamine sulf/chondroitin, amitriptyline hcl, sulfadiazine, oxycodone hcl/acetaminophen, olopatadine hcl, misoprostol, naproxen sodium, omeprazole, cyclophosphamide, rituximab, IL-1 TRAP, MRA, CTLA4-IG, IL-18 BP, anti-IL-18, Anti-IL15, BIRB-796, SCIO-469, VX-702, AMG-548, VX-740, Roflumilast, IC-485, CDC-801, or Mesopram. Combinations include methotrexate or leflunomide and in moderate or severe rheumatoid arthritis cases, cyclosporine.

[0166] In one embodiment, the binding protein or antigen-binding portion thereof, is administered in combination with one of the following agents for the treatment of rheumatoid arthritis: a small molecule inhibitor of KDR, a small molecule inhibitor of Tie-2; methotrexate; prednisone; celecoxib; folic acid; hydroxychloroquine sulfate; rofecoxib; etanercept; infliximab; leflunomide; naproxen; valdecoxib; sulfasalazine; methylprednisolone; ibuprofen; meloxicam; methylprednisolone acetate; gold sodium thiomalate; aspirin; azathioprine; triamcinolone acetonide; propxyphene napsylate/apap; folate; nabumetone; diclofenac; piroxicam; etodolac; diclofenac sodium; oxaprozin; oxycodone hcl; hydrocodone bitartrate/apap; diclofenac sodium/misoprostol; fentanyl; anakinra, human recombinant; tramadol hcl; salsalate; sulindac; cyanocobalamin/fa/pyridoxine; acetaminophen; alendronate sodium; prednisolone; morphine sulfate; lidocaine hydrochloride; indomethacin; glucosamine sulfate/chondroitin; cyclosporine; amitriptyline hcl; sulfadiazine; oxycodone hcl/acetaminophen; olopatadine hcl; misoprostol; naproxen sodium; omeprazole; mycophenolate mofetil; cyclophosphamide; rituximab; IL-1 TRAP; MRA; CTLA4-IG;

IL-18 BP; IL-12/23; anti-IL 18; anti-IL 15; BIRB-796; SCIO-469; VX-702; AMG-548; VX-740; Roflumilast; IC-485; CDC-801; or mesopram.

[0167] Non-limiting examples of therapeutic agents for inflammatory bowel disease with which a binding protein provided herein can be combined include the following: budenoside; epidermal growth factor; a corticosteroid; cyclosporin, sulfasalazine; aminosalicylates; 6-mercaptopurine; azathioprine; metronidazole; a lipoxygenase inhibitor; mesalamine; olsalazine; balsalazide; an antioxidant; a thromboxane inhibitor; an IL-1 receptor antagonist; an anti-IL-1β mAb; an anti-IL-6 mAb; a growth factor; an elastase inhibitor; a pyridinyl-imidazole compound; an antibody to or antagonist of other human cytokines or growth factors, for example, TNF, LT, IL-1, IL-2, IL-6, IL-7, IL-8, IL-15, IL-16, IL-17, IL-18, EMAP-II, GM-CSF, FGF, or PDGF. Antibodies provided herein, or antigen binding portions thereof, can be combined with an antibody to a cell surface molecule such as CD2, CD3, CD4, CD8, CD25, CD28, CD30, CD40, CD45, CD69, CD90 or their ligands. The antibodies provided herein, or antigen binding portions thereof, may also be combined with an agent, such as methotrexate, cyclosporin, FK506, rapamycin, mycophenolate mofetil, leflunomide, an NSAID, for example, ibuprofen, a corticosteroid such as prednisolone, a phosphodiesterase inhibitor, an adenosine agonist, an antithrombotic agent, a complement inhibitor, an adrenergic agent, an agent which interferes with signalling by proinflammatory cytokines such as TNFα or IL-1 (e.g., an IRAK, NIK, IKK, p38 or MAP kinase inhibitor), an IL-1β converting enzyme inhibitor, a TNFα converting enzyme inhibitor, a T-cell signalling inhibitor such as a kinase inhibitor, a metalloproteinase inhibitor, sulfasalazine, azathioprine, a 6-mercaptopurine, an angiotensin converting enzyme inhibitor, a soluble cytokine receptor or derivative thereof (e.g., a soluble p55 or p75 TNF receptor, sIL-1RI, sIL-1RII, sIL-6R) or an antiinflammatory cytokine (e.g., IL-4, IL-10, IL-11, IL-13 or TGFβ) or a bcl-2 inhibitor.

[0168] Examples of therapeutic agents for Crohn's disease in which a binding protein can be combined include the following: a TNF antagonist, for example, an anti-TNF antibody, Adalimumab (PCT Publication No. WO 97/29131; HUMIRA), CA2 (REMICADE), CDP 571, a TNFR-Ig construct, (p75TNFRIgG (ENBREL) or a p55TNFRIgG (LENERCEPT)) inhibitor or a PDE4 inhibitor. Antibodies provided herein, or antigen binding portions thereof, can be combined with a corticosteroid, for example, budenoside and dexamethasone. Binding proteins provided herein or antigen binding portions thereof, may also be combined with an agent such as sulfasalazine, 5-aminosalicylic acid and olsalazine, or an agent that interferes with the

synthesis or action of a proinflammatory cytokine such as IL-1, for example, an IL-1β converting enzyme inhibitor or IL-1ra. Antibodies provided herein or antigen binding portion thereof may also be used with a T cell signaling inhibitor, for example, a tyrosine kinase inhibitor or an 6-mercaptopurine. Binding proteins provided herein, or antigen binding portions thereof, can be combined with IL-11. Binding proteins provided herein, or antigen binding portions thereof, can be combined with mesalamine, prednisone, azathioprine, mercaptopurine, infliximab, methylprednisolone sodium succinate, diphenoxylate/atrop sulfate, loperamide hydrochloride, methotrexate, omeprazole, folate, ciprofloxacin/dextrose-water, hydrocodone bitartrate/apap, tetracycline hydrochloride, fluocinonide, metronidazole, thimerosal/boric acid, cholestyramine/sucrose, ciprofloxacin hydrochloride, hyoscyamine sulfate, meperidine hydrochloride, midazolam hydrochloride, oxycodone hcl/acetaminophen, promethazine hydrochloride, sodium phosphate, sulfamethoxazole/trimethoprim, celecoxib, polycarbophil, propoxyphene napsylate, hydrocortisone, multivitamins, balsalazide disodium, codeine phosphate/apap, colesevelam hcl, cyanocobalamin, folic acid, levofloxacin, methylprednisolone, natalizumab or interferon-gamma

[0169] Non-limiting examples of therapeutic agents for multiple sclerosis with which binding proteins provided herein can be combined include the following: a corticosteroid; prednisolone; methylprednisolone; azathioprine; cyclophosphamide; cyclosporine; methotrexate; 4-aminopyridine; tizanidine; interferon-β1a (AVONEX; Biogen); interferon-β1b (BETASERON; Chiron/Berlex); interferon α-n3) (Interferon Sciences/Fujimoto), interferon-α (Alfa Wassermann/J&J), interferon β1A-IF (Serono/Inhale Therapeutics), Peginterferon α 2b (Enzon/Schering-Plough), Copolymer 1 (Cop-1; COPAXONE; Teva Pharmaceutical Industries, Inc.); hyperbaric oxygen; intravenous immunoglobulin; clabribine; an antibody to or antagonist of other human cytokines or growth factors and their receptors, for example, TNF, LT, IL-1, IL-2, IL-6, IL-7, IL-8, IL-23, IL-15, IL-16, IL-18, EMAP-II, GM-CSF, FGF, or PDGF. Binding proteins provided herein can be combined with an antibody to a cell surface molecule such as CD2, CD3, CD4, CD8, CD19, CD20, CD25, CD28, CD30, CD40, CD45, CD69, CD80, CD86, CD90 or their ligands. Binding proteins provided herein, may also be combined with an agent, such as methotrexate, cyclosporine, FK506, rapamycin, mycophenolate mofetil, leflunomide, an NSAID, for example, ibuprofen, a corticosteroid such as prednisolone, a phosphodiesterase inhibitor,an adensosine agonist,an antithrombotic agent, a complement inhibitor, an adrenergic agent, an

agent which interferes with signalling by a proinflammatory cytokine such as TNFα or IL-1 (e.g.,IRAK, NIK, IKK, p38 or a MAP kinase inhibitor), an IL-1β converting enzyme inhibitor, a TACE inhibitor, a T-cell signaling inhibitor such as a kinase inhibitor, a metalloproteinase inhibitor, sulfasalazine, azathioprine, a 6-mercaptopurine, an angiotensin converting enzyme inhibitor, a soluble cytokine receptor or derivatives thereof (e.g., a soluble p55 or p75 TNF receptor, sIL-1RI, sIL-1RII, sIL-6R), an antiinflammatory cytokine (e.g., IL-4, IL-10, IL-13 or TGFβ) or a bcl-2 inhibitor.

[0170] Examples of therapeutic agents for multiple sclerosis with which binding proteins provided herein can be combined include interferon-β, for example, IFNβ1a and IFNβ1b; copaxone, corticosteroids, caspase inhibitors, for example inhibitors of caspase-1, IL-1 inhibitors, TNF inhibitors, and antibodies to CD40 ligand and CD80.

[0171] Non-limiting examples of therapeutic agents for asthma with which binding proteins provided herein can be combined include the following: albuterol, salmeterol/fluticasone, montelukast sodium, fluticasone propionate, budesonide, prednisone, salmeterol xinafoate, levalbuterol hcl, albuterol sulfate/ipratropium, prednisolone sodium phosphate, triamcinolone acetonide, beclomethasone dipropionate, ipratropium bromide, azithromycin, pirbuterol acetate, prednisolone, theophylline anhydrous, methylprednisolone sodium succinate, clarithromycin, zafirlukast, formoterol fumarate, influenza virus vaccine, methylprednisolone, amoxicillin trihydrate, flunisolide, allergy injection, cromolyn sodium, fexofenadine hydrochloride, flunisolide/menthol, amoxicillin/clavulanate, levofloxacin, inhaler assist device, guaifenesin, dexamethasone sodium phosphate, moxifloxacin hcl, doxycycline hyclate, guaifenesin/d-methorphan, p-ephedrine/cod/chlorphenir, gatifloxacin, cetirizine hydrochloride, mometasone furoate, salmeterol xinafoate, benzonatate, cephalexin, pe/hydrocodone/chlorphenir, cetirizine hcl/pseudoephed, phenylephrine/cod/promethazine, codeine/promethazine, cefprozil, dexamethasone, guaifenesin/pseudoephedrine, chlorpheniramine/hydrocodone, nedocromil sodium, terbutaline sulfate, epinephrine, methylprednisolone, metaproterenol sulfate.

[0172] Non-limiting examples of therapeutic agents for COPD with which binding proteins provided herein can be combined include the following: albuterol sulfate/ipratropium, ipratropium bromide, salmeterol/fluticasone, albuterol, salmeterol xinafoate, fluticasone propionate, prednisone, theophylline anhydrous, methylprednisolone sodium succinate, montelukast sodium, budesonide, formoterol fumarate, triamcinolone acetonide, levofloxacin, guaifenesin, azithromycin,

beclomethasone dipropionate, levalbuterol hcl, flunisolide, ceftriaxone sodium, amoxicillin trihydrate, gatifloxacin, zafirlukast, amoxicillin/clavulanate, flunisolide/menthol, chlorpheniramine/hydrocodone, metaproterenol sulfate, methylprednisolone, mometasone furoate, p-ephedrine/cod/chlorphenir, pirbuterol acetate, p-ephedrine/loratadine, terbutaline sulfate, tiotropium bromide, (R,R)-formoterol, TgAAT, Cilomilast, Roflumilast.

[0173] Non-limiting examples of therapeutic agents for psoriasis with which binding proteins provided herein can be combined include the following: small molecule inhibitor of KDR, small molecule inhibitor of Tie-2, calcipotriene, clobetasol propionate, triamcinolone acetonide, halobetasol propionate, tazarotene, methotrexate, fluocinonide, betamethasone diprop augmented, fluocinolone acetonide, acitretin, tar shampoo, betamethasone valerate, mometasone furoate, ketoconazole, pramoxine/fluocinolone, hydrocortisone valerate, flurandrenolide, urea, betamethasone, clobetasol propionate/emoll, fluticasone propionate, azithromycin, hydrocortisone, moisturizing formula, folic acid, desonide, pimecrolimus, coal tar, diflorasone diacetate, etanercept folate, lactic acid, methoxsalen, hc/bismuth subgal/znox/resor, methylprednisolone acetate, prednisone, sunscreen, halcinonide, salicylic acid, anthralin, clocortolone pivalate, coal extract, coal tar/salicylic acid, coal tar/salicylic acid/sulfur, desoximetasone, diazepam, emollient, fluocinonide/emollient, mineral oil/castor oil/na lact, mineral oil/peanut oil, petroleum/isopropyl myristate, psoralen, salicylic acid, soap/tribromsalan, thimerosal/boric acid, celecoxib, infliximab, cyclosporine, alefacept, efalizumab, tacrolimus, pimecrolimus, PUVA, UVB, sulfasalazine.

[0174] Examples of therapeutic agents for SLE (Lupus) with which binding proteins provided herein can be combined include the following: NSAIDS, for example, diclofenac, naproxen, ibuprofen, piroxicam, indomethacin; COX2 inhibitors, for example, Celecoxib, rofecoxib, valdecoxib; anti-malarials, for example, hydroxychloroquine; Steroids, for example, prednisone, prednisolone, budenoside, dexamethasone; Cytotoxics, for example, azathioprine, cyclophosphamide, mycophenolate mofetil, methotrexate; inhibitors of PDE4 or purine synthesis inhibitor, for example Cellcept. Binding proteins provided herein may also be combined with agents such as sulfasalazine, 5-aminosalicylic acid, olsalazine, Imuran and agents which interfere with synthesis, production or action of proinflammatory cytokines such as IL-1, for example, caspase inhibitors like IL-1β converting enzyme inhibitors and IL-1ra. Binding proteins provided herein may also be used with T cell signaling inhibitors,

for example, tyrosine kinase inhibitors; or molecules that target T cell activation molecules, for example, CTLA-4-IgG or anti-B7 family antibodies, anti-PD-1 family antibodies. Binding proteins provided herein, can be combined with IL-11 or anti-cytokine antibodies, for example, fonotolizumab (anti-IFNg antibody), or anti-receptor receptor antibodies, for example, anti-IL-6 receptor antibody and antibodies to B-cell surface molecules. Antibodies provided herein or antigen binding portion thereof may also be used with LJP 394 (abetimus), agents that deplete or inactivate B-cells, for example, Rituximab (anti-CD20 antibody), lymphostat-B (anti-BlyS antibody), TNF antagonists, for example, anti-TNF antibodies, Adalimumab (PCT Publication No. WO 97/29131; HUMIRA), CA2 (REMICADE), CDP 571, TNFR-Ig constructs, (p75TNFRIgG (ENBREL) and p55TNFRIgG (LENERCEPT)) and bcl-2 inhibitors, because bcl-2 overexpression in transgenic mice has been demonstrated to cause a lupus like phenotype (see MarquinaThe pharmaceutical compositions provided herein may include a "therapeutically effective amount" or a "prophylactically effective amount" of a binding protein provided herein. A "therapeutically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired therapeutic result. A therapeutically effective amount of the binding protein may be determined by a person skilled in the art and may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the binding protein to elicit a desired response in the individual. A therapeutically effective amount is also one in which any toxic or detrimental effects of the antibody, or antibody binding portion, are outweighed by the therapeutically beneficial effects. A "prophylactically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired prophylactic result. Typically, since a prophylactic dose is used in subjects prior to or at an earlier stage of disease, the prophylactically effective amount will be less than the therapeutically effective amount.

Diagnostics

[0175] The disclosure herein also provides diagnostic applications including, but not limited to, diagnostic assay methods, diagnostic kits containing one or more binding proteins, and adaptation of the methods and kits for use in automated and/or semi-automated systems. The methods, kits, and adaptations provided may be employed in the detection, monitoring, and/or treatment of a disease or disorder in an individual. This is further elucidated below.

D. Method of assay

[0176] The present disclosure also provides a method for determining the presence, amount or concentration of an analyte, or fragment thereof, in a test sample using at least one binding protein as described herein. Any suitable assay as is known in the art can be used in the method. Examples include, but are not limited to, immunoassays and/or methods employing mass spectrometry.

[0177] Immunoassays provided by the present disclosure may include sandwich immunoassays, radioimmunoassay (RIA), enzyme immunoassay (EIA), enzyme-linked immunosorbent assay (ELISA), competitive-inhibition immunoassays, fluorescence polarization immunoassay (FPIA), enzyme multiplied immunoassay technique (EMIT), bioluminescence resonance energy transfer (BRET), and homogenous chemiluminescent assays, among others.

[0178] A chemiluminescent microparticle immunoassay, in particular one employing the ARCHITECT® automated analyzer (Abbott Laboratories, Abbott Park, IL), is an example of an immunoassay.

[0179] Methods employing mass spectrometry are provided by the present disclosure and include, but are not limited to MALDI (matrix-assisted laser desorption/ionization) or by SELDI (surface-enhanced laser desorption/ionization).

[0180] Methods for collecting, handling, processing, and analyzing biological test samples using immunoassays and mass spectrometry would be well-known to one skilled in the art, are provided for in the practice of the present disclosure (US 2009-0311253 A1).

E. Kit

[0181] A kit for assaying a test sample for the presence, amount or concentration of an analyte, or fragment thereof, in a test sample is also provided. The kit comprises at least one component for assaying the test sample for the analyte, or fragment thereof, and instructions for assaying the test sample for the analyte, or fragment thereof. The at least one component for assaying the test sample for the analyte, or fragment thereof, can include a composition comprising a binding protein, as disclosed herein, and/or an anti-analyte binding protein (or a fragment, a variant, or a fragment of a variant thereof), which is optionally immobilized on a solid phase.

[0182] Optionally, the kit may comprise a calibrator or control, which may comprise isolated or purified analyte. The kit can comprise at least one component for assaying the test sample for an analyte by immunoassay and/or mass spectrometry. The kit components, including the analyte, binding protein, and/or anti-analyte binding protein, or fragments thereof, may be optionally labeled using any art-known detectable label. The materials and methods for the creation provided for in the practice of the present disclosure would be known to one skilled in the art (US 2009-0311253 A1).

F. Adaptation of kit and method

[0183] The kit (or components thereof), as well as the method of determining the presence, amount or concentration of an analyte in a test sample by an assay, such as an immunoassay as described herein, can be adapted for use in a variety of automated and semi-automated systems (including those wherein the solid phase comprises a microparticle), as described, for example, in US Patent Nos. 5,089,424 and 5,006,309, and as commercially marketed, for example, by Abbott Laboratories (Abbott Park, IL) as ARCHITECT®.

[0184] Other platforms available from Abbott Laboratories include, but are not limited to, AxSYM®, IMx® (see, for example, US Patent No. 5,294,404, PRISM®, EIA (bead), and Quantum™ II, as well as other platforms. Additionally, the assays, kits and kit components can be employed in other formats, for example, on electrochemical or other hand-held or point-of-care assay systems. The present disclosure is, for example, applicable to the commercial Abbott Point of Care (i-STAT®, Abbott Laboratories) electrochemical immunoassay system that performs sandwich immunoassays. Immunosensors and their methods of manufacture and operation in single-use test devices are described, for example in, US Patent No. 5,063,081, 7,419,821, and 7,682,833; and US Publication Nos. 20040018577, 20060160164 and US 20090311253.

[0185] It will be readily apparent to those skilled in the art that other suitable modifications and adaptations of the methods described herein are obvious and may be made using suitable equivalents without departing from the scope of the embodiments disclosed herein. Having now described certain embodiments in detail, the same will be more clearly understood by reference to the following examples, which are included for purposes of illustration only and are not intended to be limiting.

EXAMPLES

Example 1: Generation and Characterization of Dual Variable Domain (DVD) Binding Proteins

[0186] Two and four-chain dual variable domain (DVD) binding proteins using variable domains from parent antibodies were generated by synthesizing polynucleotide fragments encoding DVD binding protein variable heavy and DVD binding protein variable light chain sequences and cloning the fragments into a pHybC-D2 vector according to art known methods. The DVD binding protein constructs were cloned into and expressed in 293 cells and purified according to art known methods. DVD VH and VL chains for the DVD binding proteins are provided below. The SEQ ID NOs listed in the leftmost column of Tables 2-4 refer to the sequences for the full variable domains of the DVD binding proteins identified in each row of the Table. Each row in the rightmost column of Tables 2-4 provides three SEQ ID NOs. The first number refers to the SEQ ID NO of the outer variable domain sequence, the second number refers to the SEQ ID NO of the linker, and the third number refers to the SEQ ID NO of the inner variable domain sequence, which together are found within the full DVD variable domain sequence (i.e., the full DVD variable domain comprising VD1-X1-VD2).

Table 2: DVD Binding Proteins That Bind TNF and IL-13

SEQ ID NO	DVD-Ig Variable Domain Name	Outer Variable Domain Name (VD1)	Linker	Inner Variable Domain Name (VD2)	SEQ ID NO VD1 – X1 – VD2 Formula
120	DVD2683H	AB436VH	GS-H10	AB397VH	38-29-32
121	DVD2683L	AB436VL	GS-L10	AB397VL	39-30-33
122	DVD2684H	AB436VH	HG-short	AB397VH	38-21-32
123	DVD2684L	AB436VL	LK-short	AB397VL	39-13-33
124	DVD2686H	AB436VH	HG-short	AB397VH	38-21-32
125	DVD2686L	AB436VL	LK-long	AB397VL	39-14-33
126	DVD2687H	AB436VH	HG-long	AB397VH	38-22-32
127	DVD2687L	AB436VL	LK-short	AB397VL	39-13-33
128	DVD2688H	AB397VH	GS-H10	AB436VH	32-29-38
129	DVD2688L	AB397VL	GS-L10	AB436VL	33-30-39
130	DVD2689H	AB397VH	HG-short	AB436VH	32-21-38
131	DVD2689L	AB397VL	LK-short	AB436VL	33-13-39
132	DVD2691H	AB397VH	HG-short	AB436VH	32-21-38
133	DVD2691L	AB397VL	LK-long	AB436VL	33-14-39
134	DVD2692H	AB397VH	HG-long	AB436VH	32-22-38

135	DVD2692L	AB397VL	LK-short	AB436VL	33-13-39
136	DVD2733H	AB437VH	GS-H10	AB397VH	40-29-32
137	DVD2733L	AB437VL	GS-L10	AB397VL	41-30-33
138	DVD2734H	AB437VH	HG-short	AB397VH	40-21-32
139	DVD2734L	AB437VL	LK-short	AB397VL	41-13-33
140	DVD2736H	AB437VH	HG-short	AB397VH	40-21-32
141	DVD2736L	AB437VL	LK-long	AB397VL	41-14-33
142	DVD2737H	AB437VH	HG-long	AB397VH	40-22-32
143	DVD2737L	AB437VL	LK-short	AB397VL	41-13-33
144	DVD2738H	AB397VH	GS-H10	AB437VH	32-29-40
145	DVD2738L	AB397VL	GS-L10	AB437VL	33-30-41
146	DVD2739H	AB397VH	HG-short	AB437VH	32-21-40
147	DVD2739L	AB397VL	LK-short	AB437VL	33-13-41
148	DVD2741H	AB397VH	HG-short	AB437VH	32-21-40
149	DVD2741L	AB397VL	LK-long	AB437VL	33-14-41
150	DVD2742H	AB397VH	HG-long	AB437VH	32-22-40
151	DVD2742L	AB397VL	LK-short	AB437VL	33-13-41
152	DVD2783H	AB441VH	GS-H10	AB397VH	42-29-32
153	DVD2783L	AB441VL	GS-L10	AB397VL	43-30-33
154	DVD2784H	AB441VH	HG-short	AB397VH	42-21-32
155	DVD2784L	AB441VL	LK-short	AB397VL	43-13-33
156	DVD2786H	AB441VH	HG-short	AB397VH	42-21-32
157	DVD2786L	AB441VL	LK-long	AB397VL	43-14-33
158	DVD2787H	AB441VH	HG-long	AB397VH	42-22-32
159	DVD2787L	AB441VL	LK-short	AB397VL	43-13-33
160	DVD2788H	AB397VH	GS-H10	AB441VH	32-29-42
161	DVD2788L	AB397VL	GS-L10	AB441VL	33-30-43
162	DVD2789H	AB397VH	HG-short	AB441VH	32-21-42
163	DVD2789L	AB397VL	LK-short	AB441VL	33-13-43
164	DVD2791H	AB397VH	HG-short	AB441VH	32-21-42
165	DVD2791L	AB397VL	LK-long	AB441VL	33-14-43
166	DVD2792H	AB397VH	HG-long	AB441VH	32-22-42
167	DVD2792L	AB397VL	LK-short	AB441VL	33-13-43
168	DVD3008H	AB444VH	GS-H10	AB397VH	48-29-32
169	DVD3008L	AB444VL	GS-L10	AB397VL	49-30-33
170	DVD3009H	AB444VH	HG-short	AB397VH	48-21-32
171	DVD3009L	AB444VL	LK-short	AB397VL	49-13-33
172	DVD3011H	AB444VH	HG-short	AB397VH	48-21-32
173	DVD3011L	AB444VL	LK-long	AB397VL	49-14-33
174	DVD3012H	AB444VH	HG-long	AB397VH	48-22-32
175	DVD3012L	AB444VL	LK-short	AB397VL	49-13-33
176	DVD3013H	AB397VH	GS-H10	AB444VH	32-29-48
177	DVD3013L	AB397VL	GS-L10	AB444VL	33-30-49

178	DVD3014H	AB397VH	HG-short	AB444VH	32-21-48
179	DVD3014L	AB397VL	LK-short	AB444VL	33-13-49
180	DVD3016H	AB397VH	HG-short	AB444VH	32-21-48
181	DVD3016L	AB397VL	LK-long	AB444VL	33-14-49
182	DVD3017H	AB397VH	HG-long	AB444VH	32-22-48
183	DVD3017L	AB397VL	LK-short	AB444VL	33-13-49
184	DVD3083H	AB436VH	GS-H10	AB398VH	38-29-34
185	DVD3083L	AB436VL	GS-L10	AB398VL	39-30-35
186	DVD3084H	AB436VH	HG-short	AB398VH	38-21-34
187	DVD3084L	AB436VL	LK-short	AB398VL	39-13-35
188	DVD3086H	AB436VH	HG-short	AB398VH	38-21-34
189	DVD3086L	AB436VL	LK-long	AB398VL	39-14-35
190	DVD3087H	AB436VH	HG-long	AB398VH	38-22-34
191	DVD3087L	AB436VL	LK-short	AB398VL	39-13-35
192	DVD3088H	AB398VH	GS-H10	AB436VH	34-29-38
193	DVD3088L	AB398VL	GS-L10	AB436VL	35-30-39
194	DVD3089H	AB398VH	HG-short	AB436VH	34-21-38
195	DVD3089L	AB398VL	LK-short	AB436VL	35-13-39
196	DVD3091H	AB398VH	HG-short	AB436VH	34-21-38
197	DVD3091L	AB398VL	LK-long	AB436VL	35-14-39
198	DVD3092H	AB398VH	HG-long	AB436VH	34-22-38
199	DVD3092L	AB398VL	LK-short	AB436VL	35-13-39
200	DVD3093H	AB437VH	GS-H10	AB398VH	40-29-38
201	DVD3093L	AB437VL	GS-L10	AB398VL	41-30-39
202	DVD3094H	AB437VH	HG-short	AB398VH	40-21-38
203	DVD3094L	AB437VL	LK-short	AB398VL	41-13-39
204	DVD3096H	AB437VH	HG-short	AB398VH	40-21-38
205	DVD3096L	AB437VL	LK-long	AB398VL	41-14-39
206	DVD3097H	AB437VH	HG-long	AB398VH	40-22-38
207	DVD3097L	AB437VL	LK-short	AB398VL	41-13-39
208	DVD3098H	AB398VH	GS-H10	AB437VH	34-29-40
209	DVD3098L	AB398VL	GS-L10	AB437VL	35-30-41
210	DVD3099H	AB398VH	HG-short	AB437VH	34-21-40
211	DVD3099L	AB398VL	LK-short	AB437VL	35-13-41
212	DVD3101H	AB398VH	HG-short	AB437VH	34-21-40
213	DVD3101L	AB398VL	LK-long	AB437VL	35-14-41
214	DVD3102H	AB398VH	HG-long	AB437VH	34-22-40
215	DVD3102L	AB398VL	LK-short	AB437VL	35-13-41
216	DVD3103H	AB441VH	GS-H10	AB398VH	42-29-38
217	DVD3103L	AB441VL	GS-L10	AB398VL	43-30-39
218	DVD3104H	AB441VH	HG-short	AB398VH	42-21-38
219	DVD3104L	AB441VL	LK-short	AB398VL	43-13-39
220	DVD3106H	AB441VH	HG-short	AB398VH	42-21-38

221	DVD3106L	AB441VL	LK-long	AB398VL	43-14-39
222	DVD3107H	AB441VH	HG-long	AB398VH	42-22-38
223	DVD3107L	AB441VL	LK-short	AB398VL	43-13-39
224	DVD3108H	AB398VH	GS-H10	AB441VH	34-29-42
225	DVD3108L	AB398VL	GS-L10	AB441VL	35-30-43
226	DVD3109H	AB398VH	HG-short	AB441VH	34-21-42
227	DVD3109L	AB398VL	LK-short	AB441VL	35-13-43
228	DVD3111H	AB398VH	HG-short	AB441VH	34-21-42
229	DVD3111L	AB398VL	LK-long	AB441VL	35-14-43
230	DVD3112H	AB398VH	HG-long	AB441VH	34-22-42
231	DVD3112L	AB398VL	LK-short	AB441VL	35-13-43
232	DVD3113H	AB444VH	GS-H10	AB398VH	48-29-38
233	DVD3113L	AB444VL	GS-L10	AB398VL	49-30-39
234	DVD3114H	AB444VH	HG-short	AB398VH	48-21-38
235	DVD3114L	AB444VL	LK-short	AB398VL	49-13-39
236	DVD3116H	AB444VH	HG-short	AB398VH	48-21-38
237	DVD3116L	AB444VL	LK-long	AB398VL	49-14-39
238	DVD3117H	AB444VH	HG-long	AB398VH	48-22-38
239	DVD3117L	AB444VL	LK-short	AB398VL	49-13-39
240	DVD3118H	AB398VH	GS-H10	AB444VH	34-29-48
241	DVD3118L	AB398VL	GS-L10	AB444VL	35-30-49
242	DVD3119H	AB398VH	HG-short	AB444VH	34-21-48
243	DVD3119L	AB398VL	LK-short	AB444VL	35-13-49
244	DVD3121H	AB398VH	HG-short	AB444VH	34-21-48
245	DVD3121L	AB398VL	LK-long	AB444VL	35-14-49
246	DVD3122H	AB398VH	HG-long	AB444VH	34-22-48
247	DVD3122L	AB398VL	LK-short	AB444VL	35-13-49
248	DVD3143H	AB436VH	GS-H10	AB399VH	38-29-36
249	DVD3143L	AB436VL	GS-L10	AB399VL	39-30-37
250	DVD3144H	AB436VH	HG-short	AB399VH	38-21-36
251	DVD3144L	AB436VL	LK-short	AB399VL	39-13-37
252	DVD3146H	AB436VH	HG-short	AB399VH	38-21-36
253	DVD3146L	AB436VL	LK-long	AB399VL	39-14-37
254	DVD3147H	AB436VH	HG-long	AB399VH	38-22-36
255	DVD3147L	AB436VL	LK-short	AB399VL	39-13-37
256	DVD3153H	AB437VH	GS-H10	AB399VH	40-29-36
257	DVD3153L	AB437VL	GS-L10	AB399VL	41-30-37
258	DVD3154H	AB437VH	HG-short	AB399VH	40-21-36
259	DVD3154L	AB437VL	LK-short	AB399VL	41-13-37
260	DVD3156H	AB437VH	HG-short	AB399VH	40-21-36
261	DVD3156L	AB437VL	LK-long	AB399VL	41-14-37
262	DVD3157H	AB437VH	HG-long	AB399VH	40-22-36
263	DVD3157L	AB437VL	LK-short	AB399VL	41-13-37

264	DVD3158H	AB399VH	GS-H10	AB437VH	36-29-40
265	DVD3158L	AB399VL	GS-L10	AB437VL	37-30-41
266	DVD3159H	AB399VH	HG-short	AB437VH	36-21-40
267	DVD3159L	AB399VL	LK-short	AB437VL	37-13-41
268	DVD3163H	AB441VH	GS-H10	AB399VH	42-29-36
269	DVD3163L	AB441VL	GS-L10	AB399VL	43-30-37
270	DVD3164H	AB441VH	HG-short	AB399VH	42-21-36
271	DVD3164L	AB441VL	LK-short	AB399VL	43-13-37
272	DVD3166H	AB441VH	HG-short	AB399VH	42-21-36
273	DVD3166L	AB441VL	LK-long	AB399VL	43-14-37
274	DVD3167H	AB441VH	HG-long	AB399VH	42-22-36
275	DVD3167L	AB441VL	LK-short	AB399VL	43-13-37
276	DVD3168H	AB399VH	GS-H10	AB441VH	36-29-42
277	DVD3168L	AB399VL	GS-L10	AB441VL	37-30-43
278	DVD3169H	AB399VH	HG-short	AB441VH	36-21-42
279	DVD3169L	AB399VL	LK-short	AB441VL	37-13-43
280	DVD3173H	AB444VH	GS-H10	AB399VH	48-29-63
281	DVD3173L	AB444VL	GS-L10	AB399VL	49-30-37
282	DVD3174H	AB444VH	HG-short	AB399VH	48-21-36
283	DVD3174L	AB444VL	LK-short	AB399VL	49-13-37
284	DVD3176H	AB444VH	HG-short	AB399VH	48-21-36
285	DVD3176L	AB444VL	LK-long	AB399VL	49-14-37
286	DVD3177H	AB444VH	HG-long	AB399VH	48-22-36
287	DVD3177L	AB444VL	LK-short	AB399VL	49-13-37

Table 3: DVD Binding Proteins That Bind TNF and PGE2

SEQ ID NO	DVD-Ig Variable Domain Name	Outer Variable Domain Name (VD1)	Linker	Inner Variable Domain Name (VD2)	SEQ ID NO
288	DVD2693H	AB436VH	GS-H10	AB048VH	38-29-50
289	DVD2693L	AB436VL	GS-L10	AB048VL	39-30-51
290	DVD2694H	AB436VH	HG-short	AB048VH	38-21-50
291	DVD2694L	AB436VL	LK-short	AB048VL	39-13-51
292	DVD2696H	AB436VH	HG-short	AB048VH	38-21-50
293	DVD2696L	AB436VL	LK-long	AB048VL	39-14-51
294	DVD2697H	AB436VH	HG-long	AB048VH	38-22-50
295	DVD2697L	AB436VL	LK-short	AB048VL	39-13-51
296	DVD2698H	AB048VH	GS-H10	AB436VH	50-29-38
297	DVD2698L	AB048VL	GS-L10	AB436VL	51-30-39
298	DVD2699H	AB048VH	HG-short	AB436VH	50-21-38
299	DVD2699L	AB048VL	LK-short	AB436VL	51-13-39
300	DVD2701H	AB048VH	HG-short	AB436VH	50-21-38
301	DVD2701L	AB048VL	LK-long	AB436VL	51-14-39
302	DVD2702H	AB048VH	HG-long	AB436VH	50-22-38
303	DVD2702L	AB048VL	LK-short	AB436VL	51-13-39

304	DVD2743H	AB437VH	GS-H10	AB048VH	40-29-50
305	DVD2743L	AB437VL	GS-L10	AB048VL	41-30-51
306	DVD2744H	AB437VH	HG-short	AB048VH	40-21-50
307	DVD2744L	AB437VL	LK-short	AB048VL	41-13-51
308	DVD2746H	AB437VH	HG-short	AB048VH	40-21-50
309	DVD2746L	AB437VL	LK-long	AB048VL	41-14-51
310	DVD2747H	AB437VH	HG-long	AB048VH	40-22-50
311	DVD2732L	AB437VL	LK-short	AB048VL	41-13-51
312	DVD2748H	AB048VH	GS-H10	AB437VH	50-29-40
313	DVD2748L	AB048VL	GS-L10	AB437VL	51-30-41
314	DVD2749H	AB048VH	HG-short	AB437VH	50-21-40
315	DVD2749L	AB048VL	LK-short	AB437VL	51-13-41
316	DVD2751L	AB048VH	HG-short	AB437VH	50-21-40
317	DVD2751L	AB048VL	LK-long	AB437VL	51-14-41
318	DVD2752H	AB048VH	HG-long	AB437VH	50-22-40
319	DVD2752L	AB048VL	LK-short	AB437VL	51-13-41
320	DVD2793H	AB441VH	GS-H10	AB048VH	42-29-50
321	DVD2793L	AB441VL	GS-L10	AB048VL	43-30-51
322	DVD2794H	AB441VH	HG-short	AB048VH	42-21-50
323	DVD2794L	AB441VL	LK-short	AB048VL	43-13-51
324	DVD2796H	AB441VH	HG-short	AB048VH	42-21-50
325	DVD2796L	AB441VL	LK-long	AB048VL	43-14-51
326	DVD2797H	AB441VH	HG-long	AB048VH	42-22-50
327	DVD2797L	AB441VL	LK-short	AB048VL	43-13-51
328	DVD2798H	AB048VH	GS-H10	AB441VH	50-29-42
329	DVD2798L	AB048VL	GS-L10	AB441VL	51-30-43
330	DVD2799H	AB048VH	HG-short	AB441VH	50-21-42
331	DVD2799L	AB048VL	LK-short	AB441VL	51-13-43
332	DVD2801H	AB048VH	HG-short	AB441VH	50-21-42
333	DVD2801L	AB048VL	LK-long	AB441VL	51-14-43
334	DVD2802H	AB048VH	HG-long	AB441VH	50-22-42
335	DVD2727L	AB048VL	LK-short	AB441VL	51-13-43
336	DVD3018H	AB444VH	GS-H10	AB048VH	48-29-50
337	DVD3018L	AB444VL	GS-L10	AB048VL	49-30-51
338	DVD3019H	AB444VH	HG-short	AB048VH	48-21-50
339	DVD3019L	AB444VL	LK-short	AB048VL	49-13-51
340	DVD3021H	AB444VH	HG-short	AB048VH	48-21-50
341	DVD3021L	AB444VL	LK-long	AB048VL	49-14-51
342	DVD3022H	AB444VH	HG-long	AB048VH	48-22-50
343	DVD3022L	AB444VL	LK-short	AB048VL	49-13-51
344	DVD3023H	AB048VH	GS-H10	AB444VH	50-29-48
345	DVD3023L	AB048VL	GS-L10	AB444VL	51-30-49
346	DVD3024H	AB048VH	HG-short	AB444VH	50-21-48
347	DVD3024L	AB048VL	LK-short	AB444VL	51-13-49
348	DVD3026L	AB048VH	HG-short	AB444VH	50-21-48
349	DVD3026L	AB048VL	LK-long	AB444VL	51-14-49
350	DVD3027H	AB048VH	HG-long	AB444VH	50-22-48
351	DVD3027L	AB048VL	LK-short	AB444VL	51-13-49

352	DVD3203H	AB436VH	GS-H10	AB131VH	38-29-52
353	DVD3203L	AB436VL	GS-L10	AB131VL	39-30-53
354	DVD3204H	AB436VH	HG-short	AB131VH	38-21-52
355	DVD3204L	AB436VL	LK-short	AB131VL	39-13-53
356	DVD3206H	AB436VH	HG-short	AB131VH	38-21-52
357	DVD3206L	AB436VL	LK-long	AB131VL	39-14-53
358	DVD3207H	AB436VH	HG-long	AB131VH	38-22-52
359	DVD3207L	AB436VL	LK-short	AB131VL	39-13-53
360	DVD3208H	AB131VH	GS-H10	AB436VH	52-29-38
361	DVD3208L	AB131VL	GS-L10	AB436VL	53-30-39
362	DVD3209H	AB131VH	HG-short	AB436VH	52-21-38
363	DVD3209L	AB131VL	LK-short	AB436VL	53-13-39
364	DVD3211H	AB131VH	HG-short	AB436VH	52-21-38
365	DVD3211L	AB131VL	LK-long	AB436VL	53-14-39
366	DVD3212H	AB131VH	HG-long	AB436VH	52-22-38
367	DVD3212L	AB131VL	LK-short	AB436VL	53-13-39
368	DVD3213H	AB437VH	GS-H10	AB131VH	40-29-52
369	DVD3213L	AB437VL	GS-L10	AB131VL	41-30-53
370	DVD3214H	AB437VH	HG-short	AB131VH	40-21-52
371	DVD3214L	AB437VL	LK-short	AB131VL	41-13-53
372	DVD3216H	AB437VH	HG-short	AB131VH	40-21-52
373	DVD3216L	AB437VL	LK-long	AB131VL	41-14-53
374	DVD3217H	AB437VH	HG-long	AB131VH	40-22-52
375	DVD3217L	AB437VL	LK-short	AB131VL	41-13-53
376	DVD3218H	AB131VH	GS-H10	AB437VH	52--29-40
377	DVD3218L	AB131VL	GS-L10	AB437VL	53-30-41
378	DVD3219H	AB131VH	HG-short	AB437VH	52-21-40
379	DVD3219L	AB131VL	LK-short	AB437VL	53-13-41
380	DVD3221H	AB131VH	HG-short	AB437VH	52-21-40
381	DVD3221L	AB131VL	LK-long	AB437VL	53-14-41
382	DVD3222H	AB131VH	HG-long	AB437VH	52-22-40
383	DVD3222L	AB131VL	LK-short	AB437VL	53-13-41
384	DVD3223H	AB441VH	GS-H10	AB131VH	42-29-52
385	DVD3223L	AB441VL	GS-L10	AB131VL	43-30-53
386	DVD3224H	AB441VH	HG-short	AB131VH	42-21-52
387	DVD3224L	AB441VL	LK-short	AB131VL	43-13-53
388	DVD3226H	AB441VH	HG-short	AB131VH	42-21-52
389	DVD3226L	AB441VL	LK-long	AB131VL	43-14-53
390	DVD3227H	AB441VH	HG-long	AB131VH	42-22-52
391	DVD3227L	AB441VL	LK-short	AB131VL	43-13-53
392	DVD3228H	AB131VH	GS-H10	AB441VH	52-29-42
393	DVD3228L	AB131VL	GS-L10	AB441VL	53-30-43
394	DVD3229H	AB131VH	HG-short	AB441VH	52-21-42
395	DVD3229L	AB131VL	LK-short	AB441VL	53-13-43
396	DVD3231L	AB131VH	HG-short	AB441VH	52-21-42
397	DVD3231L	AB131VL	LK-long	AB441VL	53-14-43
398	DVD3232H	AB131VH	HG-long	AB441VH	52-22-42
399	DVD3232L	AB131VL	LK-short	AB441VL	53-13-43

400	DVD3233H	AB444VH	GS-H10	AB131VH	48-29-52
401	DVD3233L	AB444VL	GS-L10	AB131VL	49-30-53
402	DVD3234H	AB444VH	HG-short	AB131VH	48-21-52
403	DVD3234L	AB444VL	LK-short	AB131VL	49-13-53
404	DVD3236H	AB444VH	HG-short	AB131VH	48-21-52
405	DVD3236L	AB444VL	LK-long	AB131VL	49-14-53
406	DVD3237H	AB444VH	HG-long	AB131VH	48-22-52
407	DVD3237L	AB444VL	LK-short	AB131VL	49-13-53
408	DVD3238H	AB131VH	GS-H10	AB444VH	52-29-48
409	DVD3238L	AB131VL	GS-L10	AB444VL	53-30-49
410	DVD3239H	AB131VH	HG-short	AB444VH	52-21-48
411	DVD3239L	AB131VL	LK-short	AB444VL	53-13-49
412	DVD3241H	AB131VH	HG-short	AB444VH	52-21-48
413	DVD3241L	AB131VL	LK-long	AB444VL	53-14-49
414	DVD3242H	AB131VH	HG-long	AB444VH	52-22-48
415	DVD3242L	AB131VL	LK-short	AB444VL	53-13-49
416	DVD3263H	AB436VH	GS-H10	AB135VH	38-29-54
417	DVD3263L	AB436VL	GS-L10	AB135VL	39-30-55
418	DVD3264H	AB436VH	HG-short	AB135VH	38-21-54
419	DVD3264L	AB436VL	LK-short	AB135VL	39-13-55
420	DVD3267H	AB131VH	HG-long	AB135VH	52-22-54
421	DVD3267L	AB131VL	LK-short	AB135VL	53-13-55
422	DVD3273H	AB131VH	GS-H10	AB443VH	52-29-46
423	DVD3273L	AB131VL	GS-L10	AB443VL	53-30-47
424	DVD3274H	AB131VH	HG-short	AB443VH	52-21-46
425	DVD3274L	AB131VL	LK-short	AB443VL	53-13-47
426	DVD3276H	AB131VH	HG-short	AB443VH	52-21-46
427	DVD3276L	AB131VL	LK-long	AB443VL	53-14-47
428	DVD3277H	AB131VH	HG-long	AB443VH	52-22-46
429	DVD3277L	AB131VL	LK-short	AB443VL	53-13-47
430	DVD3279H	AB135VH	HG-short	AB437VH	54-21-40
431	DVD3279L	AB135VL	LK-short	AB437VL	55-13-41
432	DVD3283H	AB441VH	GS-H10	AB135VH	42-29-54
433	DVD3283L	AB441VL	GS-L10	AB135VL	43-30-55
434	DVD3284H	AB441VH	HG-short	AB135VH	42-21-54
435	DVD3284L	AB441VL	LK-short	AB135VL	43-13-55
436	DVD3286H	AB441VH	HG-short	AB135VH	42-21-54
437	DVD3286L	AB441VL	LK-long	AB135VL	43-14-55
438	DVD2697H	AB441VH	HG-long	AB135VH	42-22-54
439	DVD2697L	AB441VL	LK-short	AB135VL	43-13-55

Table 4: DVD Binding Proteins That Bind TNF and NGF

SEQ ID NO	DVD-Ig Variable Domain Name	Outer Variable Domain Name (VD1)	Linker	Inner Variable Domain Name (VD2)	SEQ ID NO
440	DVD2713H	AB436VH	GS-H10	AB267VH	38-29-56
441	DVD2713L	AB436VL	GS-L10	AB267VL	39-30-57

442	DVD2714H	AB436VH	HG-short	AB267VH	38-21-56
443	DVD2714L	AB436VL	LK-short	AB267VL	39-13-57
444	DVD2716H	AB436VH	HG-short	AB267VH	38-21-56
445	DVD2716L	AB436VL	LK-long	AB267VL	39-14-57
446	DVD2717H	AB436VH	HG-long	AB267VH	38-22-56
447	DVD2717L	AB436VL	LK-short	AB267VL	39-13-57
448	DVD2718H	AB267VH	GS-H10	AB436VH	56-29-38
449	DVD2718L	AB267VL	GS-L10	AB436VL	57-30-39
450	DVD2719H	AB267VH	HG-short	AB436VH	56-21-38
451	DVD2719L	AB267VL	LK-short	AB436VL	57-13-39
452	DVD2721H	AB267VH	HG-short	AB436VH	56-21-38
453	DVD2721L	AB267VL	LK-long	AB436VL	57-14-39
454	DVD2722H	AB267VH	HG-long	AB436VH	56-22-38
455	DVD2722L	AB267VL	LK-short	AB436VL	57-13-39
456	DVD2763H	AB437VH	GS-H10	AB267VH	40-29-56
457	DVD2763L	AB437VL	GS-L10	AB267VL	41-30-57
458	DVD2764H	AB437VH	HG-short	AB267VH	40-21-56
459	DVD2764L	AB437VL	LK-short	AB267VL	41-13-57
460	DVD2766H	AB437VH	HG-short	AB267VH	40-21-56
461	DVD2766L	AB437VL	LK-long	AB267VL	41-14-57
462	DVD2767H	AB437VH	HG-long	AB267VH	40-22-56
463	DVD2767L	AB437VL	LK-short	AB267VL	41-13-57
464	DVD2768H	AB267VH	GS-H10	AB437VH	56-29-40
465	DVD2768L	AB267VL	GS-L10	AB437VL	57-30-41
466	DVD2769H	AB267VH	HG-short	AB437VH	56-21-40
467	DVD2769L	AB267VL	LK-short	AB437VL	57-13-41
468	DVD2771H	AB267VH	HG-short	AB437VH	56-21-40
469	DVD2771L	AB267VL	LK-long	AB437VL	57-14-41
470	DVD2772H	AB267VH	HG-long	AB437VH	56-22-40
471	DVD2772L	AB267VL	LK-short	AB437VL	57-13-41
472	DVD2813H	AB441VH	GS-H10	AB267VH	42-29-56
473	DVD2813L	AB441VL	GS-L10	AB267VL	43-30-57
474	DVD2814H	AB441VH	HG-short	AB267VH	42-21-56
475	DVD2814L	AB441VL	LK-short	AB267VL	43-13-57
476	DVD2816H	AB441VH	HG-short	AB267VH	42-21-56
477	DVD2816L	AB441VL	LK-long	AB267VL	43-14-57
478	DVD2817H	AB441VH	HG-long	AB267VH	42-22-56
479	DVD2817L	AB441VL	LK-short	AB267VL	43-13-57
480	DVD2818H	AB267VH	GS-H10	AB441VH	56-29-42
481	DVD2818L	AB267VL	GS-L10	AB441VL	57-30-43
482	DVD2819H	AB267VH	HG-short	AB441VH	56-21-42
483	DVD2819L	AB267VL	LK-short	AB441VL	57-13-43
484	DVD2821H	AB267VH	HG-short	AB441VH	56-21-42
485	DVD2821L	AB267VL	LK-long	AB441VL	57-14-43
486	DVD2822H	AB267VH	HG-long	AB441VH	56-22-42
487	DVD2822L	AB267VL	LK-short	AB441VL	57-13-43
488	DVD3038H	AB444VH	GS-H10	AB267VH	48-29-56

489	DVD3038L	AB444VL	GS-L10	AB267VL	49-30-57
490	DVD3039H	AB444VH	HG-short	AB267VH	48-21-56
491	DVD3039L	AB444VL	LK-short	AB267VL	49-13-57
492	DVD3041H	AB444VH	HG-short	AB267VH	48-21-56
493	DVD3041L	AB444VL	LK-long	AB267VL	49-14-57
494	DVD3042H	AB444VH	HG-long	AB267VH	48-22-56
495	DVD3042L	AB444VL	LK-short	AB267VL	49-13-57
496	DVD3043H	AB267VH	GS-H10	AB444VH	56-29-48
497	DVD3043L	AB267VL	GS-L10	AB444VL	57-30-49
498	DVD3044H	AB267VH	HG-short	AB444VH	56-21-48
499	DVD3044L	AB267VL	LK-short	AB444VL	57-13-49
500	DVD3046H	AB267VH	HG-short	AB444VH	56-21-48
501	DVD3046L	AB267VL	LK-long	AB444VL	57-14-49
502	DVD3047H	AB267VH	HG-long	AB444VH	56-22-48
503	DVD3047L	AB267VL	LK-short	AB444VL	57-13-49

All DVD binding proteins listed above in Tables 2-4 may further comprise a human light chain Kappa constant region and a wild-type human heavy chain IgG1 constant region. The constant domain sequences are shown below in Table 4a.

Table 4a: Human IgG Heavy and Light Chain Constant Domains

Protein	SEQ ID NO	Sequence
		1234567890123456789012345678901234567890123456789012345678901234567890123
Wild type hIgG1 constant region		ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTF PAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKT HTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN WYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP APIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWE SNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNH YTQKSLSLSPGK
Mutant hIgG1 constant region (IgG1, z, non-a mut (234,235))		ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTF PAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKT HTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN WYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP APIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWE SNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNH YTQKSLSLSPGK
Ig kappa constant region		TVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQE SVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
Ig Lambda constant region		QPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVE TTTPSKQSNNKYAASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS

Example 2: Assays Used To Determine the Functional Activity Of Parent Antibodies And DVD-Ig Proteins

Example 2.1: IL-13 Bioassay and Neutralization Assay

[0187] A549 cells were plated at 1.5-2 x 10^5 cells per well in a 100 µL volume and incubated overnight at 37°C, 5% CO_2. Following a 16-20 hour overnight incubation, the original 100 µl media seeding volume was removed and 100 µL of 400 ng/mL (2x concentrated) rhTNF-α was added to all wells. The plates were placed at 37°C, 5% CO_2 until the addition of IL-13 and antibody or DVD-Ig protein. A 20 µg/mL working stock of antibody or DVD-Ig protein (4x concentrated) was prepared in complete F12 medium. An eight point serial dilution was performed (5 µg/mL-0.0003 µg/mL) in complete F12 in Marsh dilution plates. Sixty uL /well of each antibody or DVD-Ig protein dilution was added in quadruplicate to a 96 well v-bottom (Costar# 3894) plate and 60 µL of a 4x concentrated (20 ng/mL) solution of IL-13 was added to all wells except the cell only control. Following a 1 hour incubation, 100 µL of the above IL-13/Antibody or DVD-Ig protein complex was added to the A549 cells. All well volumes were equal to 200 µL. The final concentration of recombinant IL-13 was 5 ng/mL and rhTNF-(was 200 ng/mL. All plate reagents were then 1x concentrated. After a 16-20 hour incubation, the well contents (200 µL) were transferred into a 96-well round bottom plate (Costar# 3799) and placed in a –20°C freezer. The supernatants were tested for hTARC levels by ELISA in the Assay Lab. Neutralization potency was determined by calculating percent inhibition relative to the 5 ng/mL IL-13 alone control value. Reported IC_{50} values (sigmoidal curve dose responses) were calculated using GraphPad Prism. See Table 5.

Table 5: IL-13 Neutralization Assay With IL-13 Parent Antibody and DVD-Ig Protein

Parent Antibody or DVD-Ig ID	N-terminal Variable Domain (VD)	C-terminal Variable Domain (VD)	N-Terminal VD IL13 Neutralization Assay IC50 nM	C-Terminal VD IL13 Neutralization AssayIC50 nM
AB397	IL-13 (seq 1)		0.066	
AB398	IL-13 (seq 2)		0.068	
AB399	IL-13 (seq 3)		0.075	
DVD2683	TNF (seq 1)	IL-13 (seq 1)		0.133
DVD2684	TNF (seq 1)	IL-13 (seq 1)		1.379
DVD2686	TNF (seq 1)	IL-13 (seq 1)		0.094

DVD2687	TNF (seq 1)	IL-13 (seq 1)		0.022
DVD2688	IL-13 (seq 1)	TNF (seq 1)	0.094	
DVD2689	IL-13 (seq 1)	TNF (seq 1)	0.147	
DVD2691	IL-13 (seq 1)	TNF (seq 1)	0.105	
DVD2692	IL-13 (seq 1)	TNF (seq 1)	0.131	
DVD2733	TNF (seq 2)	IL-13 (seq 1)		0.119
DVD2734	TNF (seq 2)	IL-13 (seq 1)		0.955
DVD2736	TNF (seq 2)	IL-13 (seq 1)		0.167
DVD2737	TNF (seq 2)	IL-13 (seq 1)		0.061
DVD2738	IL-13 (seq 1)	TNF (seq 2)	0.149	
DVD2739	IL-13 (seq 1)	TNF (seq 2)	0.132	
DVD2741	IL-13 (seq 1)	TNF (seq 2)	0.118	
DVD2742	IL-13 (seq 1)	TNF (seq 2)	0.064	
DVD2783	TNF (seq 3)	IL-13 (seq 1)		0.897
DVD2784	TNF (seq 3)	IL-13 (seq 1)		0.214
DVD2786	TNF (seq 3)	IL-13 (seq 1)		0.189
DVD2787	TNF (seq 3)	IL-13 (seq 1)		0.054
DVD2788	IL-13 (seq 1)	TNF (seq 3)	0.072	
DVD2789	IL-13 (seq 1)	TNF (seq 3)	0.078	
DVD2791	IL-13 (seq 1)	TNF (seq 3)	0.245	
DVD2792	IL-13 (seq 1)	TNF (seq 3)		0.361
DVD3008	TNF (seq 4)	IL-13 (seq 1)		0.278
DVD3009	TNF (seq 4)	IL-13 (seq 1)		0.563
DVD3011	TNF (seq 4)	IL-13 (seq 1)		0.242
DVD3012	TNF (seq 4)	IL-13 (seq 1)		0.207
DVD3013	IL-13 (seq 1)	TNF (seq 4)	0.077	
DVD3014	IL-13 (seq 1)	TNF (seq 4)	0.132	
DVD3016	IL-13 (seq 1)	TNF (seq 4)	0.081	
DVD3017	IL-13 (seq 1)	TNF (seq 4)	0.111	
DVD3083	TNF (seq 1)	IL-13 (seq 2)		0.098
DVD3084	TNF (seq 1)	IL-13 (seq 2)		0.587
DVD3086	TNF (seq 1)	IL-13 (seq 2)		0.12
DVD3087	TNF (seq 1)	IL-13 (seq 2)		0.082
DVD3088	IL-13 (seq 2)	TNF (seq 1)	0.103	
DVD3089	IL-13 (seq 2)	TNF (seq 1)	0.074	
DVD3091	IL-13 (seq 2)	TNF (seq 1)	0.056	
DVD3092	IL-13 (seq 2)	TNF (seq 1)	0.06	
DVD3093	TNF (seq 2)	IL-13 (seq 2)		0.067
DVD3094	TNF (seq 2)	IL-13 (seq 2)		0.444
DVD3096	TNF (seq 2)	IL-13 (seq 2)		0.064
DVD3097	TNF (seq 2)	IL-13 (seq 2)		0.072
DVD3098	IL-13 (seq 2)	TNF (seq 2)	0.059	
DVD3099	IL-13 (seq 2)	TNF (seq 2)	0.014	
DVD3101	IL-13 (seq 2)	TNF (seq 2)	0.037	
DVD3102	IL-13 (seq 2)	TNF (seq 2)	0.025	
DVD3103	TNF (seq 3)	IL-13 (seq 2)		0.042
DVD3104	TNF (seq 3)	IL-13 (seq 2)		1.052
DVD3106	TNF (seq 3)	IL-13 (seq 2)		0.074
DVD3107	TNF (seq 3)	IL-13 (seq 2)		0.029

DVD3108	IL-13 (seq 2)	TNF (seq 3)		
DVD3109	IL-13 (seq 2)	TNF (seq 3)	0.044	
DVD3111	IL-13 (seq 2)	TNF (seq 3)	0.018	
DVD3112	IL-13 (seq 2)	TNF (seq 3)	0.017	
DVD3113	TNF (seq 4)	IL-13 (seq 2)		0.065
DVD3114	TNF (seq 4)	IL-13 (seq 2)		0.781
DVD3116	TNF (seq 4)	IL-13 (seq 2)		0.081
DVD3117	TNF (seq 4)	IL-13 (seq 2)		0.015
DVD3118	IL-13 (seq 2)	TNF (seq 4)	0.013	
DVD3119	IL-13 (seq 2)	TNF (seq 4)	0.051	
DVD3121	IL-13 (seq 2)	TNF (seq 4)	0.013	
DVD3122	IL-13 (seq 2)	TNF (seq 4)	0.011	
DVD3143	TNF (seq 1)	IL-13 (seq 3)		0.057
DVD3144	TNF (seq 1)	IL-13 (seq 3)		0.209
DVD3146	TNF (seq 1)	IL-13 (seq 3)		0.051
DVD3147	TNF (seq 1)	IL-13 (seq 3)		0.745
DVD3153	TNF (seq 2)	IL-13 (seq 3)		0.064
DVD3154	TNF (seq 2)	IL-13 (seq 3)		0.175
DVD3156	TNF (seq 2)	IL-13 (seq 3)		0.062
DVD3157	TNF (seq 2)	IL-13 (seq 3)		0.035
DVD3158	IL-13 (seq 3)	TNF (seq 2)	0.137	
DVD3159	IL-13 (seq 3)	TNF (seq 2)	0.087	
DVD3163	TNF (seq 3)	IL-13 (seq 3)		0.076
DVD3164	TNF (seq 3)	IL-13 (seq 3)		0.335
DVD3166	TNF (seq 3)	IL-13 (seq 3)		0.072
DVD3167	TNF (seq 3)	IL-13 (seq 3)		0.046
DVD3168	IL-13 (seq 3)	TNF (seq 3)	0.081	
DVD3169	IL-13 (seq 3)	TNF (seq 3)	0.089	
DVD3173	TNF (seq 4)	IL-13 (seq 3)		0.079
DVD3174	TNF (seq 4)	IL-13 (seq 3)		0.527
DVD3176	TNF (seq 4)	IL-13 (seq 3)		0.152
DVD3177	TNF (seq 4)	IL-13 (seq 3)		0.078

All DVD-Ig proteins containing VDs from AB397, AB398, or AB399 in either the N-terminal or C-terminal position showed neutralization in the A549 IL-13 neutralization assay.

Example 2.2: PGE2 Bioassay and Neutralization Assay

[0188] The ability of anti-PGE2 antibodies and anti-PGE2 containing DVD-Ig molecules to inhibit the cellular response of PGE2 was determined in a Ca++ flux assay in HEK293Gα16 cells stably transfected with human EP4 receptor. Cells were plated in black/clear poly-D-lysine plates, (Corning #3667, Corning, N.Y.) and incubated with Ca++sensitive dye (Molecular Devices) for 90 minutes. Stock PGE2 (in 200 proof ethanol) was diluted with FLIPR buffer (containing 1xHBSS (Invitrogen, Carlsbad, California), 20 mM HEPES (Invitrogen, Carlsbad, California), 0.1% BSA

(Sigma, St. Louis, Mo.) and 2.5 mM Probenecid (Sigma, St. Louis, Mo.)). Anti-PGE2 antibodies, DVD-Ig molecules or isotype matched control antibodies were also pre-diluted in FLIPR buffer. 25 µl of PGE2 or pre-incubated PGE2/antibody mixture or pre-incubated PGE2/DVD-Ig molecule mixture was added to the wells pre-plated with cells. A dose response of PGE2 was done by a serial titration of PGE2 and was determined FLIPR1 or Tetra (Molecular Devices). EC50 was determined using GraphPad Prism 5 (GraftPad Software, La Jolla, California). For testing antibodies and DVD-Ig molecules, PGE2 at EC50 concentration was incubated with varying concentrations of test articles or isotype matched antibody (negative control) for 20 minutes, added to dye-loaded human EP4 in HEK293Gα16 cells. Ca++ flux was monitored using FLIPR1 and data was analyzed using GraphPad Prism 5. PGE2 inhibition results are shown in Table 6 for the DVD-Ig constructs that contain the different TNF sequences.

Table 6: PGE2 Neutralization Assay With PGE2 Parent Antibody and DVD-Ig Protein

Parent Antibody or DVD-Ig ID	N-terminal Variable Domain (VD)	C-terminal Variable Domain (VD)	N-Terminal VD PGE2 Neutralization Assay IC50 nM	C-Terminal VD PGE2 Neutralization Assay IC50 nM
AB048	PGE2 (seq 1)		0.401	
AB131	PGE2 (seq 2)		29.66	
AB135	PGE2 (seq 3)		N/A	
DVD2693	TNF (seq 1)	PGE2 seq 1		0.719
DVD2694	TNF (seq 1)	PGE2 seq 1		0.886
DVD2696	TNF (seq 1)	PGE2 seq 1		0.7
DVD2697	TNF (seq 1)	PGE2 seq 1		0.571
DVD2698	PGE2 seq 1	TNF (seq 1)	0.856	
DVD2699	PGE2 seq 1	TNF (seq 1)	0.869	
DVD2701	PGE2 seq 1	TNF (seq 1)	0.869	
DVD2702	PGE2 seq 1	TNF (seq 1)	0.801	
DVD2743	TNF (seq 2)	PGE2 seq 1		0.647
DVD2744	TNF (seq 2)	PGE2 seq 1		0.507
DVD2746	TNF (seq 2)	PGE2 seq 1		1.136
DVD2747	TNF (seq 2)	PGE2 seq 1		0.722
DVD2748	PGE2 seq 1	TNF (seq 2)	0.994	
DVD2749	PGE2 seq 1	TNF (seq 2)	0.566	
DVD2751	PGE2 seq 1	TNF (seq 2)	0.552	
DVD2752	PGE2 seq 1	TNF (seq 2)	0.949	
DVD2793	TNF (seq 3)	PGE2 seq 1		0.366
DVD2794	TNF (seq 3)	PGE2 seq 1		0.493
DVD2796	TNF (seq 3)	PGE2 seq 1		0.589
DVD2797	TNF (seq 3)	PGE2 seq 1		0.646

DVD2798	PGE2 seq 1	TNF (seq 3)	0.523	
DVD2799	PGE2 seq 1	TNF (seq 3)	0.597	
DVD2801	PGE2 seq 1	TNF (seq 3)	0.904	
DVD2802	PGE2 seq 1	TNF (seq 3)	0.883	
DVD3018	TNF (seq 4)	PGE2 seq 1		0.461
DVD3019	TNF (seq 4)	PGE2 seq 1		0.460
DVD3021	TNF (seq 4)	PGE2 seq 1		0.612
DVD3022	TNF (seq 4)	PGE2 seq 1		0.344
DVD3023	PGE2 seq 1	TNF (seq 4)	0.876	
DVD3024	PGE2 seq 1	TNF (seq 4)	0.663	
DVD3026	PGE2 seq 1	TNF (seq 4)	0.639	
DVD3027	PGE2 seq 1	TNF (seq 4)	0.396	
DVD3203	TNF (seq 1)	PGE2 (AB016) seq 2		34.78
DVD3204	TNF (seq 1)	PGE2 (AB016) seq 2		34.5
DVD3206	TNF (seq 1)	PGE2 (AB016) seq 2		10.01
DVD3207	TNF (seq 1)	PGE2 (AB016) seq 2		12.05
DVD3208	PGE2 (AB016) seq 2	TNF (seq 1)	20.49	
DVD3209	PGE2 (AB016) seq 2	TNF (seq 1)	109.7	
DVD3211	PGE2 (AB016) seq 2	TNF (seq 1)	31.27	
DVD3212	PGE2 (AB016) seq 2	TNF (seq 1)	20.86	
DVD3213	TNF (seq 2)	PGE2 (AB016) seq 2		29.58
DVD3214	TNF (seq 2)	PGE2 (AB016) seq 2		23.35
DVD3216	TNF (seq 2)	PGE2 (AB016) seq 2		68735
DVD3217	TNF (seq 2)	PGE2 (AB016) seq 2		>100000
DVD3218	PGE2 (AB016) seq 2	TNF (seq 2)	60.14	
DVD3219	PGE2 (AB016) seq 2	TNF (seq 2)	>100000	
DVD3221	PGE2 (AB016) seq 2	TNF (seq 2)	>100000	
DVD3222	PGE2 (AB016) seq 2	TNF (seq 2)	>100000	
DVD3223	TNF (seq 3)	PGE2 (AB016) seq 2		>100000
DVD3224	TNF (seq 3)	PGE2 (AB016) seq 2		>100000
DVD3226	TNF (seq 3)	PGE2 (AB016) seq 2		>100000
DVD3227	TNF (seq 3)	PGE2 (AB016) seq 2		>100000
DVD3228	PGE2 (AB016) seq 2	TNF (seq 3)	34.96	
DVD3229	PGE2 (AB016)	TNF (seq 3)	124.8	

	seq 2			
DVD3231	PGE2 (AB016) seq 2	TNF (seq 3)	>100000	
DVD3232	PGE2 (AB016) seq 2	TNF (seq 3)	11.25	
DVD3233	TNF (seq 4)	PGE2 (AB016) seq 2		27.26
DVD3234	TNF (seq 4)	PGE2 (AB016) seq 2		111.3
DVD3236	TNF (seq 4)	PGE2 (AB016) seq 2		>100000
DVD3237	TNF (seq 4)	PGE2 (AB016) seq 2		>100000
DVD3238	PGE2 (AB016) seq 2	TNF (seq 4)	1186	
DVD3239	PGE2 (AB016) seq 2	TNF (seq 4)	62.05	
DVD3241	PGE2 (AB016) seq 2	TNF (seq 4)	>100000	
DVD3242	PGE2 (AB016) seq 2	TNF (seq 4)	>100000	
DVD3263	TNF (seq 1)	PGE2 (AB022) seq 3		9.203
DVD3264	TNF (seq 1)	PGE2 (AB022) seq 3		30.21
DVD3267	TNF (seq 1)	PGE2 (AB022) seq 3		6.832
DVD3273	TNF (seq 2)	PGE2 (AB022) seq 3		11.91
DVD3274	TNF (seq 2)	PGE2 (AB022) seq 3		34.84
DVD3276	TNF (seq 2)	PGE2 (AB022) seq 3		27048
DVD3277	TNF (seq 2)	PGE2 (AB022) seq 3		32.08
DVD3279	PGE2 (AB022) seq 3	TNF (seq 2)	30.71	
DVD3283	TNF (seq 3)	PGE2 (AB022) seq 3		21.94
DVD3284	TNF (seq 3)	PGE2 (AB022) seq 3		47.6
DVD3286	TNF (seq 3)	PGE2 (AB022) seq 3		65.22
DVD3287	TNF (seq 3)	PGE2 (AB022) seq 3		257

All DVD-Ig proteins containing VDs from AB048, AB131, or AB135 in either the N-terminal or C-terminal position showed neutralization in the EP4 PGE2 neutralization assay.

Example 2.3: HuTNFα bioassay and Neutralization Assay

[0189] L929 cells were grown to a semi-confluent density and harvested using 0.05% tryspin (Gibco#25300). The cells were washed with PBS, counted and resuspended at 1E6 cells/mL in assay media containing 4 μg/mL actinomycin D. The

cells were seeded in a 96-well plate (Costar#3599) at a volume of 50 µl and 5E4 cells/well. The DVD-Ig™ and control IgG were diluted to a 4x concentration in assay media and serial 1:3 dilutions were prepared. The huTNFα was diluted to 400 pg/ml in assay media. An antibody sample (200 µl) was added to the huTNFα (200 µL) in a 1:2 dilution scheme and allowed to incubate for 0.5 hour at room temperature.

[0190] The DVD-Ig™ / huTNFα solution was added to the plated cells at 100 µl for a final concentration of 100 pg/mL huTNFα and 25 nM – 0.00014 nM DVD-Ig™. The plates were incubated for 20 hours at 37°C, 5 % CO_2. To quantitate viability, 100 µL was removed from the wells and 10 µL of WST-1 reagent (Roche cat# 11644807001) was added. Plates were incubated under assay conditions for 3.5 hours, centrifuged at 500 xg and 75 µL supernatant transferred to an ELISA plate (Costar cat#3369). The plates were read at OD 420-600 nm on a Spectromax 190 ELISA plate reader. An average EC50 from several assays is included in Table 7 for the DVD-Ig constructs containing the various TNF sequences.

Table 7: huTNFα Neutralization Assay With TNFα Parent Antibody and DVD-Ig Protein

Parent Antibody or DVD-Ig ID	N-terminal Variable Domain (VD)	C-terminal Variable Domain (VD)	N-Terminal VD huTNFα Neutralization Assay IC50 nM	C-Terminal VD huTNFα Neutralization Assay IC50 nM
AB436	TNF (seq 1)		0.006	
AB437	TNF (seq 2)		0.012	
AB441	TNF (seq 3)		0.037	
AB444	TNF (seq 4)		0.034	
DVD2683	TNF (seq 1)	IL-13 (seq 1)	0.006	
DVD2684	TNF (seq 1)	IL-13 (seq 1)	0.007	
DVD2686	TNF (seq 1)	IL-13 (seq 1)	0.103	
DVD2687	TNF (seq 1)	IL-13 (seq 1)	0.048	
DVD2688	IL-13 (seq 1)	TNF (seq 1)		0.093
DVD2689	IL-13 (seq 1)	TNF (seq 1)		0.076
DVD2691	IL-13 (seq 1)	TNF (seq 1)		0.041
DVD2692	IL-13 (seq 1)	TNF (seq 1)		0.04
DVD2733	TNF (seq 2)	IL-13 (seq 1)	0.009	
DVD2734	TNF (seq 2)	IL-13 (seq 1)	0.017	
DVD2736	TNF (seq 2)	IL-13 (seq 1)	0.009	
DVD2737	TNF (seq 2)	IL-13 (seq 1)	0.012	
DVD2738	IL-13 (seq 1)	TNF (seq 2)		0.452
DVD2739	IL-13 (seq 1)	TNF (seq 2)		0.517
DVD2741	IL-13 (seq 1)	TNF (seq 2)		0.202
DVD2742	IL-13 (seq 1)	TNF (seq 2)		0.129

DVD2783	TNF (seq 3)	IL-13 (seq 1)	0.008	
DVD2784	TNF (seq 3)	IL-13 (seq 1)	0.006	
DVD2786	TNF (seq 3)	IL-13 (seq 1)	0.008	
DVD2787	TNF (seq 3)	IL-13 (seq 1)	0.005	
DVD2788	IL-13 (seq 1)	TNF (seq 3)		0.23
DVD2789	IL-13 (seq 1)	TNF (seq 3)		0.186
DVD2791	IL-13 (seq 1)	TNF (seq 3)		0.064
DVD2792	IL-13 (seq 1)	TNF (seq 3)		0.136
DVD3008	TNF (seq 4)	IL-13 (seq 1)	0.029	
DVD3009	TNF (seq 4)	IL-13 (seq 1)	0.031	
DVD3011	TNF (seq 4)	IL-13 (seq 1)	0.024	
DVD3012	TNF (seq 4)	IL-13 (seq 1)	0.034	
DVD3013	IL-13 (seq 1)	TNF (seq 4)		0.229
DVD3014	IL-13 (seq 1)	TNF (seq 4)		0.49
DVD3016	IL-13 (seq 1)	TNF (seq 4)		0.148
DVD3017	IL-13 (seq 1)	TNF (seq 4)		0.234
DVD3083	TNF (seq 1)	IL-13 (seq 2)	0.011	
DVD3084	TNF (seq 1)	IL-13 (seq 2)	0.014	
DVD3086	TNF (seq 1)	IL-13 (seq 2)	0.023	
DVD3087	TNF (seq 1)	IL-13 (seq 2)	0.015	
DVD3088	IL-13 (seq 2)	TNF (seq 1)		0.472
DVD3089	IL-13 (seq 2)	TNF (seq 1)		0.52
DVD3091	IL-13 (seq 2)	TNF (seq 1)		0.192
DVD3092	IL-13 (seq 2)	TNF (seq 1)		0.096
DVD3093	TNF (seq 2)	IL-13 (seq 2)	0.012	
DVD3094	TNF (seq 2)	IL-13 (seq 2)	0.021	
DVD3096	TNF (seq 2)	IL-13 (seq 2)	0.019	
DVD3097	TNF (seq 2)	IL-13 (seq 2)	0.019	
DVD3098	IL-13 (seq 2)	TNF (seq 2)	0.012	
DVD3099	IL-13 (seq 2)	TNF (seq 2)	0.003	
DVD3101	IL-13 (seq 2)	TNF (seq 2)		0.308
DVD3102	IL-13 (seq 2)	TNF (seq 2)		0.294
DVD3103	TNF (seq 3)	IL-13 (seq 2)	0.016	
DVD3104	TNF (seq 3)	IL-13 (seq 2)	0.033	
DVD3106	TNF (seq 3)	IL-13 (seq 2)	0.018	
DVD3107	TNF (seq 3)	IL-13 (seq 2)	0.015	
DVD3108	IL-13 (seq 2)	TNF (seq 3)		0.183
DVD3109	IL-13 (seq 2)	TNF (seq 3)		0.731
DVD3111	IL-13 (seq 2)	TNF (seq 3)		0.146
DVD3112	IL-13 (seq 2)	TNF (seq 3)		0.387
DVD3113	TNF (seq 4)	IL-13 (seq 2)	0.01	
DVD3114	TNF (seq 4)	IL-13 (seq 2)	0.016	
DVD3116	TNF (seq 4)	IL-13 (seq 2)	0.025	
DVD3117	TNF (seq 4)	IL-13 (seq 2)	1.96	
DVD3118	IL-13 (seq 2)	TNF (seq 4)		0.065
DVD3119	IL-13 (seq 2)	TNF (seq 4)		0.163
DVD3121	IL-13 (seq 2)	TNF (seq 4)		0.036
DVD3122	IL-13 (seq 2)	TNF (seq 4)		0.018
DVD3143	TNF (seq 1)	IL-13 (seq 3)	0.008	

DVD3144	TNF (seq 1)	IL-13 (seq 3)	0.007	
DVD3146	TNF (seq 1)	IL-13 (seq 3)	0.006	
DVD3147	TNF (seq 1)	IL-13 (seq 3)	0.007	
DVD3153	TNF (seq 2)	IL-13 (seq 3)	0.021	
DVD3154	TNF (seq 2)	IL-13 (seq 3)	0.013	
DVD3156	TNF (seq 2)	IL-13 (seq 3)	0.022	
DVD3157	TNF (seq 2)	IL-13 (seq 3)	0.012	
DVD3158	IL-13 (seq 3)	TNF (seq 2)		0.62
DVD3159	IL-13 (seq 3)	TNF (seq 2)		0.414
DVD3163	TNF (seq 3)	IL-13 (seq 3)	0.015	
DVD3164	TNF (seq 3)	IL-13 (seq 3)	0.021	
DVD3166	TNF (seq 3)	IL-13 (seq 3)	0.016	
DVD3167	TNF (seq 3)	IL-13 (seq 3)	0.018	
DVD3168	IL-13 (seq 3)	TNF (seq 3)		0.146
DVD3169	IL-13 (seq 3)	TNF (seq 3)		0.169
DVD3173	TNF (seq 4)	IL-13 (seq 3)	0.009	
DVD3174	TNF (seq 4)	IL-13 (seq 3)	0.014	
DVD3176	TNF (seq 4)	IL-13 (seq 3)	0.015	
DVD3177	TNF (seq 4)	IL-13 (seq 3)	0.022	
DVD2693	TNF (seq 1)	PGE2 seq 1	0.001	
DVD2694	TNF (seq 1)	PGE2 seq 1	0.001	
DVD2696	TNF (seq 1)	PGE2 seq 1	NA	
DVD2697	TNF (seq 1)	PGE2 seq 1	0.000	
DVD2698	PGE2 seq 1	TNF (seq 1)		0.085
DVD2699	PGE2 seq 1	TNF (seq 1)		0.064
DVD2701	PGE2 seq 1	TNF (seq 1)		0.036
DVD2702	PGE2 seq 1	TNF (seq 1)		0.106
DVD2743	TNF (seq 2)	PGE2 seq 1	0.006	
DVD2744	TNF (seq 2)	PGE2 seq 1	0.003	
DVD2746	TNF (seq 2)	PGE2 seq 1	0.006	
DVD2747	TNF (seq 2)	PGE2 seq 1	0.003	
DVD2748	PGE2 seq 1	TNF (seq 2)		2.876
DVD2749	PGE2 seq 1	TNF (seq 2)		NA
DVD2751	PGE2 seq 1	TNF (seq 2)		NA
DVD2752	PGE2 seq 1	TNF (seq 2)		0.384
DVD2793	TNF (seq 3)	PGE2 seq 1	0.006	
DVD2794	TNF (seq 3)	PGE2 seq 1	0.003	
DVD2796	TNF (seq 3)	PGE2 seq 1	0.010	
DVD2797	TNF (seq 3)	PGE2 seq 1	0.002	
DVD2798	PGE2 seq 1	TNF (seq 3)		0.381
DVD2799	PGE2 seq 1	TNF (seq 3)		3.076
DVD2801	PGE2 seq 1	TNF (seq 3)		0.116
DVD2802	PGE2 seq 1	TNF (seq 3)		0.195
DVD3018	TNF (seq 4)	PGE2 seq 1	0.008	
DVD3019	TNF (seq 4)	PGE2 seq 1	0.009	
DVD3021	TNF (seq 4)	PGE2 seq 1	0.007	
DVD3022	TNF (seq 4)	PGE2 seq 1	0.016	
DVD3023	PGE2 seq 1	TNF (seq 4)		0.203
DVD3024	PGE2 seq 1	TNF (seq 4)		0.934

DVD3026	PGE2 seq 1	TNF (seq 4)		0.038
DVD3027	PGE2 seq 1	TNF (seq 4)		0.076
DVD3203	TNF (seq 1)	PGE2 (AB016) seq 2	0.071	
DVD3204	TNF (seq 1)	PGE2 (AB016) seq 2	0.001	
DVD3206	TNF (seq 1)	PGE2 (AB016) seq 2	0.001	
DVD3207	TNF (seq 1)	PGE2 (AB016) seq 2	0.005	
DVD3208	PGE2 (AB016) seq 2	TNF (seq 1)		0.172
DVD3209	PGE2 (AB016) seq 2	TNF (seq 1)		0.536
DVD3211	PGE2 (AB016) seq 2	TNF (seq 1)		0.014
DVD3212	PGE2 (AB016) seq 2	TNF (seq 1)		0.039
DVD3213	TNF (seq 2)	PGE2 (AB016) seq 2	0.010	
DVD3214	TNF (seq 2)	PGE2 (AB016) seq 2	0.020	
DVD3216	TNF (seq 2)	PGE2 (AB016) seq 2	0.022	
DVD3217	TNF (seq 2)	PGE2 (AB016) seq 2	0.008	
DVD3218	PGE2 (AB016) seq 2	TNF (seq 2)		0.413
DVD3219	PGE2 (AB016) seq 2	TNF (seq 2)		2.102
DVD3221	PGE2 (AB016) seq 2	TNF (seq 2)		0.479
DVD3222	PGE2 (AB016) seq 2	TNF (seq 2)		0.243
DVD3223	TNF (seq 3)	PGE2 (AB016) seq 2	0.002	
DVD3224	TNF (seq 3)	PGE2 (AB016) seq 2	0.004	
DVD3226	TNF (seq 3)	PGE2 (AB016) seq 2	0.011	
DVD3227	TNF (seq 3)	PGE2 (AB016) seq 2	0.002	
DVD3228	PGE2 (AB016) seq 2	TNF (seq 3)		0.052
DVD3229	PGE2 (AB016) seq 2	TNF (seq 3)		0.086
DVD3231	PGE2 (AB016) seq 2	TNF (seq 3)		0.112
DVD3232	PGE2 (AB016) seq 2	TNF (seq 3)		0.254
DVD3233	TNF (seq 4)	PGE2 (AB016) seq 2	0.005	
DVD3234	TNF (seq 4)	PGE2 (AB016) seq 2	0.009	
DVD3236	TNF (seq 4)	PGE2 (AB016) seq 2	0.006	

DVD3237	TNF (seq 4)	PGE2 (AB016) seq 2	0.010	
DVD3238	PGE2 (AB016) seq 2	TNF (seq 4)		0.168
DVD3239	PGE2 (AB016) seq 2	TNF (seq 4)		1.616
DVD3241	PGE2 (AB016) seq 2	TNF (seq 4)		0.112
DVD3242	PGE2 (AB016) seq 2	TNF (seq 4)		0.130
DVD3263	TNF (seq 1)	PGE2 (AB022) seq 3	0.008	
DVD3264	TNF (seq 1)	PGE2 (AB022) seq 3	0.006	
DVD3267	TNF (seq 1)	PGE2 (AB022) seq 3	0.002	
DVD3273	TNF (seq 2)	PGE2 (AB022) seq 3	0.020	
DVD3274	TNF (seq 2)	PGE2 (AB022) seq 3	0.008	
DVD3276	TNF (seq 2)	PGE2 (AB022) seq 3	0.083	
DVD3277	TNF (seq 2)	PGE2 (AB022) seq 3	0.051	
DVD3279	PGE2 (AB022) seq 3	TNF (seq 2)		
DVD3283	TNF (seq 3)	PGE2 (AB022) seq 3	0.002	
DVD3284	TNF (seq 3)	PGE2 (AB022) seq 3	0.012	
DVD3286	TNF (seq 3)	PGE2 (AB022) seq 3	0.002	
DVD3287	TNF (seq 3)	PGE2 (AB022) seq 3	0.018	
DVD2713	TNF (seq 1)	NGF	0.006	
DVD2714	TNF (seq 1)	NGF	0.008	
DVD2716	TNF (seq 1)	NGF	0.014	
DVD2717	TNF (seq 1)	NGF	0.278	
DVD2718	NGF	TNF (seq 1)		0.249
DVD2719	NGF	TNF (seq 1)		0.126
DVD2721	NGF	TNF (seq 1)		0.029
DVD2722	NGF	TNF (seq 1)		0.190
DVD2763	TNF (seq 2)	NGF	0.004	
DVD2764	TNF (seq 2)	NGF	0.012	
DVD2766	TNF (seq 2)	NGF	0.010	
DVD2767	TNF (seq 2)	NGF	0.010	
DVD2768	NGF	TNF (seq 2)		0.189
DVD2769	NGF	TNF (seq 2)		0.222
DVD2771	NGF	TNF (seq 2)		0.060
DVD2772	NGF	TNF (seq 2)		0.128
DVD2813	TNF (seq 3)	NGF	0.016	
DVD2814	TNF (seq 3)	NGF	0.012	
DVD2816	TNF (seq 3)	NGF	0.009	
DVD2817	TNF (seq 3)	NGF	0.013	

DVD2818	NGF	TNF (seq 3)		0.072
DVD2819	NGF	TNF (seq 3)		0.279
DVD2821	NGF	TNF (seq 3)		0.090
DVD2822	NGF	TNF (seq 3)		0.107
DVD3038	TNF (seq 4)	NGF	0.002	
DVD3039	TNF (seq 4)	NGF	0.012	
DVD3041	TNF (seq 4)	NGF	0.006	
DVD3042	TNF (seq 4)	NGF	0.006	
DVD3043	NGF	TNF (seq 4)		0.128
DVD3044	NGF	TNF (seq 4)		0.673
DVD3046	NGF	TNF (seq 4)		0.050
DVD3047	NGF	TNF (seq 4)		-

All DVD-Ig proteins containing VDs from AB436, AB437, AB441, or AB444 in either the N-terminal or C-terminal position showed neutralization in the L929 huTNFα neutralization assay.

Example 2.4: Inhibition of NGF in TF-1 Cell Proliferation bioassay

[0191] TF-1. are cultured in RPMI 1640 (Invitrogen) +10% Fetal Bovine Serum (Hyclone) +L-glutamine (Invitrogen) +rhu GM-CSF (R&D Systems,) TF-1 cells are serum starved 24 hours in RPMI 1640 + L-glutamine at 1 x 10^5 cells per mL and incubated overnight at 37°C, 5% CO_2. The day of the experiment TF- 1 cells are plated in opaque walled 96-well plates at 2.5 x 10^4 cells per well in a 100 μL volume + assay media (RPMI-1640 +L-glutamine + 4% FBS) Stimulate the cells by adding NGF/DVD-Ig protein or antibody to the cells. The DVD-Ig™ protein and control IgG were diluted to a 4x concentration in assay media and serial 1:5 dilutions were performed. The huNGF was diluted to 8 ng/mL in assay media. The DVD-Ig™ protein (50 ul) and huNGF (50 uL) solutions were added to the plated for a final concentration of 2 ng/mL huNGF and 25 nM – 0.000003 nM DVD-Ig™ protein. The plates were incubated for 72 hour at 37°C, 5 % CO_2. To quantitate viability, the Cell Titer Glo kit (Promega cat# TB288) was used (100 ul of solution added to each well following manufacturer's instructions). The plates were read using luminescence on a Spectromax 190 ELISA plate reader. See Table 8.

Table 8: NGF Inhibition Assay With NGF Parent Antibodies and DVD-Ig Proteins

Parent Antibody or DVD-Ig ID	N-terminal Variable Domain (VD)	C-terminal Variable Domain (VD)	N-Terminal VD NGF Neutralization Assay IC50 nM	C-Terminal VD NGF Neutralization Assay IC50 nM
AB267	NGF		0.007	
DVD2713	TNF (seq 1)	NGF		2.895

DVD2714	TNF (seq 1)	NGF		6.852
DVD2716	TNF (seq 1)	NGF		0.6242
DVD2717	TNF (seq 1)	NGF		0.936
DVD2718	NGF	TNF (seq 1)	0.008	
DVD2719	NGF	TNF (seq 1)	0.019	
DVD2721	NGF	TNF (seq 1)	0.041	
DVD2722	NGF	TNF (seq 1)	0.053	
DVD2763	TNF (seq 2)	NGF		0.871
DVD2764	TNF (seq 2)	NGF		4.097
DVD2766	TNF (seq 2)	NGF		
DVD2767	TNF (seq 2)	NGF		0.646
DVD2768	NGF	TNF (seq 2)	0.003	
DVD2769	NGF	TNF (seq 2)	0.001	
DVD2771	NGF	TNF (seq 2)	0.037	
DVD2772	NGF	TNF (seq 2)	0.04	
DVD2813	TNF (seq 3)	NGF		7.455
DVD2814	TNF (seq 3)	NGF		0.019
DVD2816	TNF (seq 3)	NGF		2.89
DVD2817	TNF (seq 3)	NGF		1.275
DVD2818	NGF	TNF (seq 3)	0.003	
DVD2819	NGF	TNF (seq 3)	0.006	
DVD2821	NGF	TNF (seq 3)	0.011	
DVD2822	NGF	TNF (seq 3)	0.003	
DVD3038	TNF (seq 4)	NGF		0.093
DVD3039	TNF (seq 4)	NGF		0.088
DVD3041	TNF (seq 4)	NGF		>10
DVD3042	TNF (seq 4)	NGF		>10
DVD3043	NGF	TNF (seq 4)	NA	
DVD3044	NGF	TNF (seq 4)	NA	
DVD3046	NGF	TNF (seq 4)	0.145	
DVD3047	NGF	TNF (seq 4)	>10	

All DVD-Ig proteins containing VDs from AB267 in either the N-terminal or C-terminal position showed neutralization in the TF-1 NGF neutralization assay.

Example 2.5: Affinity Determination Using BIACORE Technology

Table 9: Reagents Used in Biacore Analyses

Antigen	Vendor Designation	Vendor	Catalog #
TNFα	Recombinant Human TNF-α / TNFSF1A	R&D systems	210-TA
IL-13	Recombinant Human IL-13	R&D systems	213-IL
NGF	Recombinant Human β-NGF	R&D systems	256-GF

BIACORE Methods:

[0192] The BIACORE assay (GE, Healthcare Piscataway, NJ) determined the affinity of antibodies or DVD-Ig protein with kinetic measurements of on-rate and off-rate constants. Binding of antibodies or DVD-Ig proteins to a target antigen (for example, a purified recombinant target antigen) was determined by surface plasmon resonance-based measurements with a Biacore T200 using running HBS-EP + *buffer from GE Healthcare* at 25°C. All chemicals were obtained from GE Healthcare or otherwise from a different source as described in the text. For example, approximately 5000 RU of goat anti-mouse IgG, (Fcγ), fragment specific polyclonal antibody (Pierce Biotechnology Inc, Rockford, IL) diluted in 10 mM sodium acetate (pH 4.5) was directly immobilized across a CM5 research grade biosensor chip using a standard amine coupling kit according to manufacturer's instructions. Unreacted moieties on the biosensor surface were blocked with ethanolamine. Modified carboxymethyl dextran surface in flowcell 1 was used as a reference surface. Rate constants were derived by making kinetic binding measurements at different antigen concentrations ranging from 0.8-100 nM. Binding was recorded as a function of time and kinetic rate constants were calculated. In this assay, association rate was evaluated for 5 min and dissociation was monitored for 10 min. For kinetic screening analysis, rate equations derived from the 1:1 binding model were fitted simultaneously to association and dissociation phases of all injections (using global fit analysis with Rmax fit locally to account for capture variations) with the use of Biaevaluation software. Purified antibodies or DVD-Ig proteins were diluted in HEPES-buffered saline for capture across goat anti-mouse IgG specific reaction surfaces. Antibodies or DVD-Ig proteins to be captured as a ligand were injected over reaction matrices at a flow rate of 5 µl/minute. The association and dissociation rate constants, k_{on} ($M^{-1}s^{-1}$) and k_{off} (s^{-1}) were determined under a continuous flow rate of 50µl/minute. Rate constants were derived by making kinetic binding measurements at different antigen concentrations ranging from 0.8-100 nM. Binding was recorded as a function of time and kinetic rate constants were calculated. In this assay, association rate was evaluated for 5 min and dissociation was monitored for 10 min. Results are shown in Table 10.

Table 10: BIACORE Analysis of Parental Antibodies and DVD-Ig Proteins

Parent Antibody or DVD-Ig ID	N-terminal Variable Domain (VD)	C-terminal Variable Domain (VD)	k_{on} (M-1s-1)	k_{off} (s-1)	k_D (M)
AB436	TNF (seq 1)		1.10E+07	7.90E-05	7.10E-12
AB437	TNF (seq 2)		1.20E+07	1.00E-04	8.60E-12
AB441	TNF (seq 3)		7.50E+06	1.80E-05	2.40E-12
AB444	TNF (seq 4)		1.10E+07	5.50E-05	4.80E-12
AB397	IL-13 (seq 1)		9.20E+05	1.20E-04	1.30E-10
AB398	IL-13 (seq 2)		6.30E+05	2.80E-05	4.50E-11
AB399	IL-13 (seq 3)		3.40E+06	6.40E-05	1.90E-11
DVD2683	TNF (seq 1)		1.50E+07	9.00E-05	5.80E-12
DVD2683		IL-13 (seq 1)	6.80E+04	5.90E-05	8.70E-10
DVD2684	TNF (seq 1)		1.40E+07	9.10E-05	6.60E-12
DVD2684		IL-13 (seq 1)	4.80E+04	5.90E-05	1.20E-09
DVD2686	TNF (seq 1)		1.50E+07	4.80E-05	3.10E-12
DVD2686		IL-13 (seq 1)	1.40E+05	4.40E-05	3.20E-10
DVD2687	TNF (seq 1)		1.80E+07	7.10E-05	4.00E-12
DVD2687		IL-13 (seq 1)	1.10E+05	5.00E-05	4.70E-10
DVD2691	IL-13 (seq 1)		9.50E+05	1.30E-04	1.30E-10
DVD2691		TNF (seq 1)	2.00E+06	5.00E-05	2.50E-11
DVD2733	TNF (seq 2)		1.60E+07	9.00E-05	5.60E-12
DVD2733		IL-13 (seq 1)	7.90E+04	8.60E-05	1.10E-09
DVD2734	TNF (seq 2)		1.50E+07	1.40E-04	9.60E-12
DVD2734		IL-13 (seq 1)	5.80E+04	4.80E-05	8.20E-10
DVD2736	TNF (seq 2)		1.80E+07	6.40E-05	3.70E-12
DVD2736		IL-13 (seq 1)	1.50E+05	4.60E-05	3.00E-10
DVD2737	TNF (seq 2)		1.70E+07	5.80E-05	3.40E-12
DVD2737		IL-13 (seq 1)	1.20E+05	6.20E-05	5.00E-10
DVD3008	TNF (seq 4)		1.60E+07	1.20E-04	7.10E-12
DVD3008		IL-13 (seq 1)	1.10E+05	1.50E-05	1.40E-10
DVD3009	TNF (seq 4)		1.50E+07	5.70E-05	3.90E-12
DVD3009		IL-13 (seq 1)	8.70E+04	3.20E-05	3.60E-10
DVD3083	TNF (seq 1)		1.70E+07	6.80E-05	3.90E-12
DVD3083		IL-13 (seq 2)	5.20E+04	2.00E-06	3.80E-11
DVD3084	TNF (seq 1)		1.50E+07	1.30E-04	8.60E-12
DVD3084		IL-13 (seq 2)	8.70E+04	1.10E-06	1.20E-11

DVD3086	TNF (seq 1)		1.70E+07	8.50E-05	5.00E-12
DVD3086		IL-13 (seq 2)	6.50E+04	<1E-06	<1.5E-11
DVD3087	TNF (seq 1)		1.70E+07	6.20E-05	3.70E-12
DVD3087		IL-13 (seq 2)	5.10E+04	<1E-06	<2.0E-11
DVD3092	IL-13 (seq 2)		5.80E+05	2.50E-05	4.40E-11
DVD3092		TNF (seq 1)	1.80E+06	1.10E-04	5.80E-11
DVD3093	TNF (seq 2)		1.90E+07	9.70E-05	5.20E-12
DVD3093		IL-13 (seq 2)	5.10E+04	<1E-06	<2.0E-11
DVD3094	TNF (seq 2)		7.40E+06	1.20E-04	1.60E-11
DVD3094		IL-13 (seq 2)	8.90E+04	5.90E-06	6.70E-11
DVD3096	TNF (seq 2)		1.90E+07	1.40E-04	7.20E-12
DVD3096		IL-13 (seq 2)	7.00E+04	5.30E-08	7.60E-13
DVD3097	TNF (seq 2)		2.00E+07	8.60E-05	4.30E-12
DVD3097		IL-13 (seq 2)	4.80E+04	<1E-06	<2.1E-11
DVD3106	TNF (seq 3)		3.90E+06	8.50E-05	2.20E-11
DVD3106		IL-13 (seq 2)	5.70E+05	6.40E-05	1.10E-10
DVD3107	TNF (seq 3)		2.80E+06	1.50E-04	5.30E-11
DVD3107		IL-13 (seq 2)	8.10E+05	7.00E-05	8.70E-11
DVD3113	TNF (seq 4)		1.80E+07	2.70E-05	1.50E-12
DVD3113		IL-13 (seq 2)	7.50E+04	1.20E-06	1.50E-11
DVD3114	TNF (seq 4)		1.50E+07	6.90E-05	4.70E-12
DVD3114		IL-13 (seq 2)	1.70E+05	1.20E-06	6.90E-12
DVD3116	TNF (seq 4)		1.40E+07	5.00E-05	3.60E-12
DVD3116		IL-13 (seq 2)	5.80E+04	<1E-06	<1.7E-11
DVD3143	TNF (seq 1)		1.50E+07	5.40E-05	3.50E-12
DVD3143		IL-13 (seq 3)	1.70E+05	6.20E-05	3.70E-10
DVD3144	TNF (seq 1)		1.30E+07	8.60E-05	6.50E-12
DVD3144		IL-13 (seq 3)	6.20E+04	7.30E-06	1.20E-10
DVD3146	TNF (seq 1)		1.40E+07	8.20E-05	6.00E-12
DVD3146		IL-13 (seq 3)	1.90E+05	4.60E-05	2.40E-10
DVD3147	TNF (seq 1)		1.30E+07	9.50E-05	7.40E-12
DVD3147		IL-13 (seq 3)	1.30E+05	<1E-06	<7.7E-12
DVD3153	TNF (seq 2)		1.50E+07	8.30E-05	5.40E-12
DVD3153		IL-13 (seq 3)	1.80E+05	8.20E-05	4.50E-10
DVD3154	TNF (seq 2)		1.40E+07	1.30E-04	9.40E-12
DVD3154		IL-13 (seq 3)	5.30E+04	3.00E-05	5.80E-10
DVD3156	TNF (seq 2)		1.70E+07	5.20E-05	3.10E-12
DVD3156		IL-13 (seq 3)	1.60E+05	5.40E-05	3.40E-10
DVD3157	TNF (seq 2)		1.10E+07	5.00E-05	4.70E-12
DVD3157		IL-13 (seq 3)	1.10E+05	1.10E-06	9.50E-12

DVD3158	IL-13 (seq 3)		1.20E+06	6.70E-05	5.70E-11
DVD3158		TNF (seq 2)	1.30E+06	6.30E-05	4.70E-11
DVD3159	IL-13 (seq 3)		1.40E+06	5.40E-05	3.90E-11
DVD3159		TNF (seq 2)	1.20E+06	2.80E-05	2.30E-11
DVD3161	IL-13 (seq 3)	TNF (seq 2)			
DVD3163	TNF (seq 3)		1.30E+07	5.00E-05	3.90E-12
DVD3163		IL-13 (seq 3)	1.40E+05	8.40E-05	6.10E-10
DVD3164	TNF (seq 3)		4.00E+06	6.50E-05	1.60E-11
DVD3164		IL-13 (seq 3)	5.50E+04	4.80E-05	8.70E-10
DVD3166	TNF (seq 3)		4.70E+06	1.30E-04	2.70E-11
DVD3166		IL-13 (seq 3)	2.80E+05	5.10E-05	1.80E-10
DVD3168	IL-13 (seq 3)		1.10E+06	7.40E-05	6.40E-11
DVD3168		TNF (seq 3)	8.00E+05	3.60E-05	4.50E-11
DVD3169	IL-13 (seq 3)		1.90E+06	2.00E-05	1.00E-11
DVD3169		TNF (seq 3)	6.50E+05	<1E-06	<1.5E-12
DVD3173	TNF (seq 4)		7.00E+06	2.20E-05	3.20E-12
DVD3173		IL-13 (seq 3)	2.40E+05	8.80E-05	3.70E-10
DVD3174	TNF (seq 4)		4.40E+06	1.40E-05	3.30E-12
DVD3174		IL-13 (seq 3)	8.50E+04	7.70E-05	9.00E-10
DVD3176	TNF (seq 4)		5.30E+06	1.30E-06	2.50E-13
DVD3176		IL-13 (seq 3)	5.50E+05	4.80E-05	8.70E-11
DVD2693	TNF (seq 1)	PGE2 seq 1	4.4E+06	4.9E-05	1.1E-11
DVD2694	TNF (seq 1)	PGE2 seq 1	3.9E+06	6.2E-05	1.6E-11
DVD2696	TNF (seq 1)	PGE2 seq 1	4.1E+06	5.4E-05	1.3E-11
DVD2697	TNF (seq 1)	PGE2 seq 1	4.4E+06	4.7E-05	1.1E-11
DVD2698	PGE2 seq 1	TNF (seq 1)	2.1E+05	2.3E-05	1.1E-10
DVD2699	PGE2 seq 1	TNF (seq 1)	1.4E+05	5.8E-05	4.0E-10
DVD2701	PGE2 seq 1	TNF (seq 1)	3.6E+05	5.3E-05	1.5E-10
DVD2702	PGE2 seq 1	TNF (seq 1)	4.7E+05	5.2E-05	1.1E-10
DVD2743	TNF (seq 2)	PGE2 seq 1	4.6E+06	4.9E-05	1.1E-11
DVD2744	TNF (seq 2)	PGE2 seq 1	3.9E+06	7.5E-05	1.9E-11
DVD2746	TNF (seq 2)	PGE2 seq 1	4.7E+06	3.0E-05	6.4E-12
DVD2747	TNF (seq 2)	PGE2 seq 1	4.5E+06	5.1E-05	1.1E-11
DVD2748	PGE2 seq 1	TNF (seq 2)	1.3E+05	3.4E-05	2.7E-10
DVD2749	PGE2 seq 1	TNF (seq 2)	1.2E+05	3.6E-05	3.1E-10
DVD2751	PGE2 seq 1	TNF (seq 2)	2.7E+05	4.2E-05	1.5E-10
DVD2752	PGE2 seq 1	TNF (seq 2)	3.4E+05	7.0E-05	2.0E-10
DVD2797	TNF (seq 3)	PGE2 seq 1	3.4E+06	1.9E-05	5.5E-12
DVD2798	PGE2 seq 1	TNF (seq 3)	1.8E+05	3.2E-05	1.7E-10
DVD2799	PGE2 seq 1	TNF (seq 3)	6.5E+04	4.8E-05	7.3E-10

DVD2801	PGE2 seq 1	TNF (seq 3)	3.8E+05	6.6E-06	1.8E-11
DVD2802	PGE2 seq 1	TNF (seq 3)	2.6E+05	4.1E-05	1.6E-10
DVD3023	PGE2 seq 1	TNF (seq 4)	3.5E+05	4.8E-05	1.4E-10
DVD3024	PGE2 seq 1	TNF (seq 4)	1.2E+05	2.9E-06	2.4E-11
DVD3026	PGE2 seq 1	TNF (seq 4)	6.8E+05	3.5E-05	5.1E-11
DVD3027	PGE2 seq 1	TNF (seq 4)	6.1E+05	5.3E-05	8.6E-11
DVD3203	TNF (seq 1)	PGE2 (AB016) seq 2	4.4E+06	3.1E-05	7.1E-12
DVD3204	TNF (seq 1)	PGE2 (AB016) seq 2	3.6E+06	6.6E-05	1.8E-11
DVD3206	TNF (seq 1)	PGE2 (AB016) seq 2	4.1E+06	4.9E-05	1.2E-11
DVD3207	TNF (seq 1)	PGE2 (AB016) seq 2	4.7E+06	4.3E-05	9.3E-12
DVD3208	PGE2 (AB016) seq 2	TNF (seq 1)	3.0E+05	7.0E-05	2.3E-10
DVD3209	PGE2 (AB016) seq 2	TNF (seq 1)	1.8E+05	8.3E-05	4.6E-10
DVD3211	PGE2 (AB016) seq 2	TNF (seq 1)	5.0E+05	3.2E-05	6.3E-11
DVD3212	PGE2 (AB016) seq 2	TNF (seq 1)	3.1E+05	3.7E-05	1.2E-10
DVD3213	TNF (seq 2)	PGE2 (AB016) seq 2	4.8E+06	4.1E-05	8.6E-12
DVD3214	TNF (seq 2)	PGE2 (AB016) seq 2	3.9E+06	6.4E-05	1.6E-11
DVD3216	TNF (seq 2)	PGE2 (AB016) seq 2	4.4E+06	5.1E-05	1.2E-11
DVD3217	TNF (seq 2)	PGE2 (AB016) seq 2	5.0E+06	3.4E-05	6.8E-12
DVD3218	PGE2 (AB016) seq 2	TNF (seq 2)	2.2E+05	5.8E-05	2.6E-10
DVD3219	PGE2 (AB016) seq 2	TNF (seq 2)	1.3E+05	2.7E-05	2.1E-10
DVD3221	PGE2 (AB016) seq 2	TNF (seq 2)	3.3E+05	1.4E-05	4.4E-11
DVD3222	PGE2 (AB016) seq 2	TNF (seq 2)	3.6E+05	5.5E-05	1.5E-10
DVD3224	TNF (seq 3)	PGE2 (AB016) seq 2	2.8E+06	4.0E-05	1.4E-11
DVD3226	TNF (seq 3)	PGE2 (AB016) seq 2	3.5E+06	2.5E-05	7.0E-12
DVD3228	PGE2 (AB016) seq 2	TNF (seq 3)	2.4E+05	3.4E-05	1.5E-10
DVD3229	PGE2 (AB016) seq 2	TNF (seq 3)	1.0E+05	2.7E-06	2.6E-11
DVD3231	PGE2 (AB016) seq 2	TNF (seq 3)	3.8E+05	1.3E-06	3.6E-12
DVD3232	PGE2 (AB016) seq 2	TNF (seq 3)	3.4E+05	3.5E-05	1.0E-10
DVD3238	PGE2 (AB016) seq 2	TNF (seq 4)	4.3E+05	3.1E-05	7.3E-11
DVD3239	PGE2 (AB016) seq 2	TNF (seq 4)	1.6E+05	4.9E-06	3.1E-11

DVD3241	PGE2 (AB016) seq 2	TNF (seq 4)	6.1E+05	2.6E-05	4.3E-11
DVD3242	PGE2 (AB016) seq 2	TNF (seq 4)	6.4E+05	3.6E-05	5.6E-11
DVD3263	TNF (seq 1)	PGE2 (AB022) seq 3	4.5E+06	4.1E-05	9.1E-12
DVD3273	TNF (seq 2)	PGE2 (AB022) seq 3	4.3E+06	5.8E-05	1.4E-11
DVD3274	TNF (seq 2)	PGE2 (AB022) seq 3	3.7E+06	6.7E-05	1.8E-11
DVD3277	TNF (seq 2)	PGE2 (AB022) seq 3	4.6E+06	2.4E-05	5.2E-12
DVD3283	TNF (seq 3)	PGE2 (AB022) seq 3	3.2E+06	2.6E-05	8.4E-12
DVD3284	TNF (seq 3)	PGE2 (AB022) seq 3	2.7E+06	3.9E-05	1.4E-11
DVD3286	TNF (seq 3)	PGE2 (AB022) seq 3	3.0E+06	1.3E-05	4.5E-12
DVD3287	TNF (seq 3)	PGE2 (AB022) seq 3	3.1E+06	3.3E-05	1.1E-11
DVD2713	TNF (seq 1)	NGF	4.40E+06	6.90E-05	1.60E-11
DVD2713	TNF (seq 1)	NGF	6.90E+04	<1E-06	<1.4E-11
DVD2714	TNF (seq 1)	NGF	4.20E+06	7.40E-05	1.80E-11
DVD2714	TNF (seq 1)	NGF	2.90E+04	<1E-06	<3.4E-11
DVD2716	TNF (seq 1)	NGF	4.20E+06	7.00E-05	1.70E-11
DVD2716	TNF (seq 1)	NGF	4.40E+05	2.50E-06	5.70E-12
DVD2717	TNF (seq 1)	NGF	4.00E+06	6.50E-05	1.60E-11
DVD2717	TNF (seq 1)	NGF	9.90E+04	1.30E-06	1.30E-11
DVD2763	TNF (seq 2)	NGF	4.80E+06	7.20E-05	1.50E-11
DVD2763	TNF (seq 2)	NGF	6.90E+04	<1E-06	<1.4E-11
DVD2764	TNF (seq 2)	NGF	3.90E+06	8.70E-05	2.20E-11
DVD2764	TNF (seq 2)	NGF	3.20E+04	<1E-06	<3.1E-11
DVD2766	TNF (seq 2)	NGF	4.60E+06	5.70E-05	1.20E-11
DVD2766	TNF (seq 2)	NGF	3.70E+05	<1E-06	<2.7E-12
DVD2767	TNF (seq 2)	NGF	5.00E+06	6.80E-05	1.40E-11
DVD2767	TNF (seq 2)	NGF	8.70E+04	2.70E-06	3.20E-11
DVD2813	TNF (seq 3)	NGF	3.20E+06	6.10E-05	1.90E-11
DVD2813	TNF (seq 3)	NGF	3.30E+04	<1E-06	<3.0E-11
DVD2816	TNF (seq 3)	NGF	3.90E+06	3.10E-05	8.00E-12
DVD2816	TNF (seq 3)	NGF	6.00E+04	4.80E-06	8.00E-11
DVD3038	TNF (seq 4)	NGF	5.40E+06	2.40E-05	4.40E-12
DVD3038	TNF (seq 4)	NGF	1.10E+05	<1E-06	<9.1E-12

All DVD-Ig proteins characterized by Biacore technology exhibited binding. All variable domains bound with similar high affinity as the parent antibodies.

Example 3: Characterization Of Antibodies and DVD-Ig Proteins

[0193] The ability of purified DVD-Ig protein to inhibit a functional activity was determined, e.g., using the cytokine bioassay as described in Example 1. The binding affinities of the DVD-Ig protein to recombinant human antigen were determined using surface plasmon resonance (Biacore®) measurement as described in Example 2. The IC_{50} values from the bioassays and the affinity of the antibodies and DVD-Ig proteins were ranked. The DVD-Ig protein that fully maintain the activity of the parent mAbs were selected as candidates for future development. The top 2-3 most favorable DVD-Ig proteins were further characterized.

Example 3.1: Pharmacokinetic Analysis Of Humanized Antibodies or DVD-Ig Protein

[0194] Pharmacokinetic studies are carried out in Sprague-Dawley rats and cynomolgus monkeys. Male and female rats and cynomolgus monkeys are dosed intravenously or subcutaneously with a single dose of 4mg/kg mAb or DVD-Ig protein and samples are analyzed using antigen capture ELISA, and pharmacokinetic parameters are determined by noncompartmental analysis. Briefly, ELISA plates are coated with goat anti-biotin antibody (5 mg/ml, 4°C, overnight), blocked with Superblock (Pierce), and incubated with biotinylated human antigen at 50 ng/ml in 10% Superblock TTBS at room temperature for 2 hours. Serum samples are serially diluted (0.5% serum, 10% Superblock in TTBS) and incubated on the plate for 30 minutes at room temperature. Detection is carried out with HRP-labeled goat anti human antibody and concentrations are determined with the help of standard curves using the four parameter logistic fit. Values for the pharmacokinetic parameters are determined by non-compartmental model using WinNonlin software (Pharsight Corporation, Mountain View, CA). Humanized mAbs with good pharmacokinetics profile (T1/2 is 8-13 days or better, with low clearance and excellent bioavailability 50-100%) are selected.

Example 3.2: Physicochemical And In Vitro Stability Analysis Of Humanized Monoclonal Antibodies and DVD-Ig Proteins

Size Exclusion Chromatography

[0195] Antibodies or DVD-Ig proteins were diluted to 2.5 mg/mL with water and 20 mL was analyzed on a Shimadzu HPLC system using a TSK gel G3000 SWXL column (Tosoh Bioscience, cat# k5539-05k). Samples were eluted from the column with 211 mM sodium sulfate, 92 mM sodium phosphate, pH 7.0, at a flow rate of 0.3 mL/minutes. The HPLC system operating conditions were as follows:

[0196] Mobile phase: 211 mM Na_2SO_4, 92 mM $Na_2HPO_4*7H_2O$, pH 7.0

[0197] Gradient: Isocratic

[0198] Flow rate: 0.3 mL/minute

[0199] Detector wavelength: 280 nm

[0200] Autosampler cooler temp: 4°C

[0201] Column oven temperature: Ambient

[0202] Run time: 50 minutes

[0203] Table 11 contains purity data of parent antibodies and DVD-Ig proteins expressed as percent monomer (unaggregated protein of the expected molecular weight) as determined by the above protocol.

Table 11: Purity of Parent Antibodies and DVD-Ig Proteins as Determined by Size Exclusion Chromatography

Parent Antibody or DVD-Ig ID	N-terminal Variable Domain (VD)	C-terminal Variable Domain (VD)	% Monomer (purity)
AB436	TNF (seq 1)		85.7
AB437	TNF (seq 2)		86.9
AB441	TNF (seq 3)		82.7
AB444	TNF (seq 4)		75.3
AB397	IL-13 (seq 1)		93
AB398	IL-13 (seq 2)		92.8
AB399	IL-13 (seq 3)		98.8
DVD2683	TNF (seq 1)	IL-13 (seq 1)	89.4
DVD2684	TNF (seq 1)	IL-13 (seq 1)	91.6
DVD2686	TNF (seq 1)	IL-13 (seq 1)	96.2
DVD2687	TNF (seq 1)	IL-13 (seq 1)	88.9
DVD2688	IL-13 (seq 1)	TNF (seq 1)	76
DVD2689	IL-13 (seq 1)	TNF (seq 1)	77.7
DVD2691	IL-13 (seq 1)	TNF (seq 1)	80.2
DVD2692	IL-13 (seq 1)	TNF (seq 1)	72.7
DVD2733	TNF (seq 2)	IL-13 (seq 1)	97.3
DVD2734	TNF (seq 2)	IL-13 (seq 1)	86.4
DVD2736	TNF (seq 2)	IL-13 (seq 1)	91.1
DVD2737	TNF (seq 2)	IL-13 (seq 1)	85
DVD2738	IL-13 (seq 1)	TNF (seq 2)	53.7
DVD2739	IL-13 (seq 1)	TNF (seq 2)	55.2
DVD2741	IL-13 (seq 1)	TNF (seq 2)	54.8
DVD2742	IL-13 (seq 1)	TNF (seq 2)	48.9
DVD2783	TNF (seq 3)	IL-13 (seq 1)	79.2

DVD2784	TNF (seq 3)	IL-13 (seq 1)	76.6
DVD2786	TNF (seq 3)	IL-13 (seq 1)	77.9
DVD2787	TNF (seq 3)	IL-13 (seq 1)	74.8
DVD2788	IL-13 (seq 1)	TNF (seq 3)	56.5
DVD2789	IL-13 (seq 1)	TNF (seq 3)	59.6
DVD2791	IL-13 (seq 1)	TNF (seq 3)	61
DVD2792	IL-13 (seq 1)	TNF (seq 3)	61
DVD3008	TNF (seq 4)	IL-13 (seq 1)	84.9
DVD3009	TNF (seq 4)	IL-13 (seq 1)	79.9
DVD3011	TNF (seq 4)	IL-13 (seq 1)	77.2
DVD3012	TNF (seq 4)	IL-13 (seq 1)	75.2
DVD3013	IL-13 (seq 1)	TNF (seq 4)	49.1
DVD3014	IL-13 (seq 1)	TNF (seq 4)	68.9
DVD3016	IL-13 (seq 1)	TNF (seq 4)	69.4
DVD3017	IL-13 (seq 1)	TNF (seq 4)	72.1
DVD3083	TNF (seq 1)	IL-13 (seq 2)	91.7
DVD3084	TNF (seq 1)	IL-13 (seq 2)	97.8
DVD3086	TNF (seq 1)	IL-13 (seq 2)	95.8
DVD3087	TNF (seq 1)	IL-13 (seq 2)	94.3
DVD3088	IL-13 (seq 2)	TNF (seq 1)	67
DVD3089	IL-13 (seq 2)	TNF (seq 1)	79.5
DVD3091	IL-13 (seq 2)	TNF (seq 1)	76.3
DVD3092	IL-13 (seq 2)	TNF (seq 1)	86.6
DVD3093	TNF (seq 2)	IL-13 (seq 2)	93.4
DVD3094	TNF (seq 2)	IL-13 (seq 2)	93.5
DVD3096	TNF (seq 2)	IL-13 (seq 2)	97.5
DVD3097	TNF (seq 2)	IL-13 (seq 2)	93
DVD3098	IL-13 (seq 2)	TNF (seq 2)	76.4
DVD3099	IL-13 (seq 2)	TNF (seq 2)	59.8
DVD3101	IL-13 (seq 2)	TNF (seq 2)	57.4
DVD3102	IL-13 (seq 2)	TNF (seq 2)	59.6
DVD3103	TNF (seq 3)	IL-13 (seq 2)	75
DVD3104	TNF (seq 3)	IL-13 (seq 2)	78.3
DVD3106	TNF (seq 3)	IL-13 (seq 2)	81.9
DVD3107	TNF (seq 3)	IL-13 (seq 2)	80.1
DVD3108	IL-13 (seq 2)	TNF (seq 3)	53.7
DVD3109	IL-13 (seq 2)	TNF (seq 3)	56.3
DVD3111	IL-13 (seq 2)	TNF (seq 3)	76.6
DVD3112	IL-13 (seq 2)	TNF (seq 3)	58.1
DVD3113	TNF (seq 4)	IL-13 (seq 2)	84.8
DVD3114	TNF (seq 4)	IL-13 (seq 2)	90.9
DVD3116	TNF (seq 4)	IL-13 (seq 2)	91.3
DVD3118	IL-13 (seq 2)	TNF (seq 4)	66.4
DVD3119	IL-13 (seq 2)	TNF (seq 4)	65.1
DVD3121	IL-13 (seq 2)	TNF (seq 4)	73.5
DVD3122	IL-13 (seq 2)	TNF (seq 4)	68.9
DVD3143	TNF (seq 1)	IL-13 (seq 3)	96.3
DVD3144	TNF (seq 1)	IL-13 (seq 3)	97.3

DVD3146	TNF (seq 1)	IL-13 (seq 3)	99.4
DVD3147	TNF (seq 1)	IL-13 (seq 3)	87.5
DVD3153	TNF (seq 2)	IL-13 (seq 3)	97.90
DVD3154	TNF (seq 2)	IL-13 (seq 3)	98.00
DVD3156	TNF (seq 2)	IL-13 (seq 3)	98.40
DVD3157	TNF (seq 2)	IL-13 (seq 3)	90.50
DVD3158	IL-13 (seq 3)	TNF (seq 2)	100
DVD3163	TNF (seq 3)	IL-13 (seq 3)	93.7
DVD3164	TNF (seq 3)	IL-13 (seq 3)	94.6
DVD3166	TNF (seq 3)	IL-13 (seq 3)	88.7
DVD3167	TNF (seq 3)	IL-13 (seq 3)	76
DVD3168	IL-13 (seq 3)	TNF (seq 3)	100
DVD3169	IL-13 (seq 3)	TNF (seq 3)	70
DVD3173	TNF (seq 4)	IL-13 (seq 3)	86.9
DVD3174	TNF (seq 4)	IL-13 (seq 3)	88
DVD3176	TNF (seq 4)	IL-13 (seq 3)	88.2
DVD3177	TNF (seq 4)	IL-13 (seq 3)	65.9
AB048	PGE2 (seq 1)		
AB131	PGE2 (AB016) seq 2		100
AB135	PGE2 (AB022) seq 3		100
DVD2693	TNF (seq 1)	PGE2 (seq 1)	92.2
DVD2694	TNF (seq 1)	PGE2 (seq 1)	94.3
DVD2696	TNF (seq 1)	PGE2 (seq 1)	98.2
DVD2697	TNF (seq 1)	PGE2 (seq 1)	96.4
DVD2698	PGE2 (seq 1)	TNF (seq 1)	97.3
DVD2699	PGE2 (seq 1)	TNF (seq 1)	96.4
DVD2701	PGE2 (seq 1)	TNF (seq 1)	96.3
DVD2702	PGE2 (seq 1)	TNF (seq 1)	94.1
DVD2743	TNF (seq 2)	PGE2 (seq 1)	96.7
DVD2744	TNF (seq 2)	PGE2 (seq 1)	98
DVD2746	TNF (seq 2)	PGE2 (seq 1)	100
DVD2747	TNF (seq 2)	PGE2 (seq 1)	98.6
DVD2748	PGE2 (seq 1)	TNF (seq 2)	98
DVD2749	PGE2 (seq 1)	TNF (seq 2)	97.3
DVD2751	PGE2 (seq 1)	TNF (seq 2)	97.6
DVD2752	PGE2 (seq 1)	TNF (seq 2)	98.5
DVD2793	TNF (seq 3)	PGE2 (seq 1)	75.4
DVD2794	TNF (seq 3)	PGE2 (seq 1)	64.5
DVD2796	TNF (seq 3)	PGE2 (seq 1)	75.5
DVD2797	TNF (seq 3)	PGE2 (seq 1)	98.6
DVD2798	PGE2 (seq 1)	TNF (seq 3)	98
DVD2799	PGE2 (seq 1)	TNF (seq 3)	97.3
DVD2801	PGE2 (seq 1)	TNF (seq 3)	97.6
DVD2802	PGE2 (seq 1)	TNF (seq 3)	98.5
DVD3018	TNF (seq 4)	PGE2	75.4
DVD3019	TNF (seq 4)	PGE2	64.5

DVD3021	TNF (seq 4)	PGE2	75.5
DVD3022	TNF (seq 4)	PGE2	76.7
DVD3023	PGE2	TNF (seq 4)	98.1
DVD3024	PGE2	TNF (seq 4)	97
DVD3026	PGE2	TNF (seq 4)	98.6
DVD3027	PGE2	TNF (seq 4)	98.9
DVD3203	TNF (seq 1)	PGE2 (AB016) seq 2	86.1
DVD3204	TNF (seq 1)	PGE2 (AB016) seq 2	95.2
DVD3206	TNF (seq 1)	PGE2 (AB016) seq 2	84.9
DVD3207	TNF (seq 1)	PGE2 (AB016) seq 2	86.2
DVD3208	PGE2 (AB016) seq 2	TNF (seq 1)	95.6
DVD3209	PGE2 (AB016) seq 2	TNF (seq 1)	95.4
DVD3211	PGE2 (AB016) seq 2	TNF (seq 1)	87.3
DVD3212	PGE2 (AB016) seq 2	TNF (seq 1)	90.8
DVD3213	TNF (seq 2)	PGE2 (AB016) seq 2	95.3
DVD3214	TNF (seq 2)	PGE2 (AB016) seq 2	97.6
DVD3216	TNF (seq 2)	PGE2 (AB016) seq 2	91.4
DVD3217	TNF (seq 2)	PGE2 (AB016) seq 2	93.9
DVD3218	PGE2 (AB016) seq 2	TNF (seq 2)	96.9
DVD3219	PGE2 (AB016) seq 2	TNF (seq 2)	97.3
DVD3221	PGE2 (AB016) seq 2	TNF (seq 2)	91.4
DVD3222	PGE2 (AB016) seq 2	TNF (seq 2)	93.9
DVD3223	TNF (seq 3)	PGE2 (AB016) seq 2	75.8
DVD3224	TNF (seq 3)	PGE2 (AB016) seq 2	88.7
DVD3226	TNF (seq 3)	PGE2 (AB016) seq 2	80.9
DVD3227	TNF (seq 3)	PGE2 (AB016) seq 2	78.8
DVD3228	PGE2 (AB016) seq 2	TNF (seq 3)	93.4
DVD3229	PGE2 (AB016) seq 2	TNF (seq 3)	93.5
DVD3231	PGE2 (AB016) seq 2	TNF (seq 3)	83.8
DVD3232	PGE2 (AB016) seq 2	TNF (seq 3)	93.6
DVD3233	TNF (seq 4)	PGE2 (AB016) seq 2	73.1
DVD3234	TNF (seq 4)	PGE2 (AB016) seq 2	74.6
DVD3236	TNF (seq 4)	PGE2 (AB016) seq 2	67.5
DVD3237	TNF (seq 4)	PGE2 (AB016) seq 2	70.8
DVD3238	PGE2 (AB016) seq 2	TNF (seq 4)	92.3
DVD3239	PGE2 (AB016) seq 2	TNF (seq 4)	95.5

DVD3241	PGE2 (AB016) seq 2	TNF (seq 4)	91.7
DVD3242	PGE2 (AB016) seq 2	TNF (seq 4)	96.6
DVD3263	TNF (seq 1)	PGE2 (AB022) seq 3	82.8
DVD3264	TNF (seq 1)	PGE2 (AB022) seq 3	
DVD3267	TNF (seq 1)	PGE2 (AB022) seq 3	
DVD3273	TNF (seq 2)	PGE2 (AB022) seq 3	100
DVD3274	TNF (seq 2)	PGE2 (AB022) seq 3	100
DVD3276	TNF (seq 2)	PGE2 (AB022) seq 3	100
DVD3277	TNF (seq 2)	PGE2 (AB022) seq 3	
DVD3279	PGE2 (AB022) seq 3	TNF (seq 2)	
DVD3283	TNF (seq 3)	PGE2 (AB022) seq 3	83.4
DVD3284	TNF (seq 3)	PGE2 (AB022) seq 3	91.4
DVD3286	TNF (seq 3)	PGE2 (AB022) seq 3	
DVD3287	TNF (seq 3)	PGE2 (AB022) seq 3	
DVD2713	TNF (seq 1)	NGF	96.7
DVD2714	TNF (seq 1)	NGF	92.4
DVD2716	TNF (seq 1)	NGF	93.1
DVD2717	TNF (seq 1)	NGF	84
DVD2718	NGF	TNF (seq 1)	70.5
DVD2719	NGF	TNF (seq 1)	68.2
DVD2721	NGF	TNF (seq 1)	72.6
DVD2722	NGF	TNF (seq 1)	72
DVD2763	TNF (seq 2)	NGF	88.6
DVD2764	TNF (seq 2)	NGF	85.9
DVD2766	TNF (seq 2)	NGF	87.9
DVD2767	TNF (seq 2)	NGF	85.50
DVD2768	NGF	TNF (seq 2)	75.40
DVD2769	NGF	TNF (seq 2)	73.60
DVD2771	NGF	TNF (seq 2)	76.20
DVD2772	NGF	TNF (seq 2)	72.60
DVD2813	TNF (seq 3)	NGF	80.30
DVD2814	TNF (seq 3)	NGF	76.20
DVD2816	TNF (seq 3)	NGF	82.90
DVD2817	TNF (seq 3)	NGF	78.3
DVD2818	NGF	TNF (seq 3)	75
DVD2819	NGF	TNF (seq 3)	74.9
DVD2821	NGF	TNF (seq 3)	76.1
DVD2822	NGF	TNF (seq 3)	78.7
DVD3038	TNF (seq 4)	NGF	81.6

DVD3039	TNF (seq 4)	NGF	74.2
DVD3041	TNF (seq 4)	NGF	69.4
DVD3042	TNF (seq 4)	NGF	66.2
DVD3043	NGF	TNF (seq 4)	73.8
DVD3044	NGF	TNF (seq 4)	69.9
DVD3046	NGF	TNF (seq 4)	72.5
DVD3047	NGF	TNF (seq 4)	72.6

DVD-Ig proteins showed an excellent SEC profile with most DVD-Ig proteins showing >90% monomer. This DVD-Ig protein profile was similar to that observed for parent antibodies.

SDS-PAGE

[0204] Antibodies and DVD-Ig proteins are analyzed by sodium dodecyl sulfate - polyacrylamide gel electrophoresis (SDS-PAGE) under both reducing and non-reducing conditions. Adalimumab lot AFP04C is used as a control. For reducing conditions, the samples are mixed 1:1 with 2X tris glycine SDS-PAGE sample buffer (Invitrogen, cat# LC2676, lot# 1323208) with 100 mM DTT, and heated at 60°C for 30 minutes. For non-reducing conditions, the samples are mixed 1:1 with sample buffer and heated at 100°C for 5 minutes. The reduced samples (10 mg per lane) are loaded on a 12% pre-cast tris-glycine gel (Invitrogen, cat# EC6005box, lot# 6111021), and the non-reduced samples (10 mg per lane) are loaded on an 8%-16% pre-cast tris-glycine gel (Invitrogen, cat# EC6045box, lot# 6111021). SeeBlue Plus 2 (Invitrogen, cat#LC5925, lot# 1351542) is used as a molecular weight marker. The gels are run in a XCell SureLock mini cell gel box (Invitrogen, cat# EI0001) and the proteins are separated by first applying a voltage of 75 to stack the samples in the gel, followed by a constant voltage of 125 until the dye front reached the bottom of the gel. The running buffer used is 1X tris glycine SDS buffer, prepared from a 10X tris glycine SDS buffer (ABC, MPS-79-080106)). The gels are stained overnight with colloidal blue stain (Invitrogen cat# 46-7015, 46-7016) and destained with Milli-Q water until the background is clear. The stained gels are then scanned using an Epson Expression scanner (model 1680, S/N DASX003641).

Sedimentation Velocity Analysis

[0205] Antibodies or DVD-Ig proteins are loaded into the sample chamber of each of three standard two-sector carbon epon centerpieces. These centerpieces have a 1.2 cm optical path length and are built with sapphire windows. PBS is used for a reference buffer and each chamber contained 140 µL. All samples are

examined simultaneously using a 4-hole (AN-60Ti) rotor in a Beckman ProteomeLab XL-I analytical ultracentrifuge (serial # PL106C01).

[0206] Run conditions are programmed and centrifuge control is performed using ProteomeLab (v5.6). The samples and rotor are allowed to thermally equilibrate for one hour prior to analysis (20.0 ± 0.1 °C). Confirmation of proper cell loading is performed at 3000 rpm and a single scan is recorded for each cell. The sedimentation velocity conditions are the following:

[0207] Sample Cell Volume: 420 mL

[0208] Reference Cell Volume: 420 mL

[0209] Temperature: 20°C

[0210] Rotor Speed: 35,000 rpm

[0211] Time: 8:00 hours

[0212] UV Wavelength: 280 nm

[0213] Radial Step Size: 0.003 cm

[0214] Data Collection: One data point per step without signal averaging.

[0215] Total Number of Scans: 100

LC-MS molecular weight measurement of intact antibodies

[0216] Molecular weight of intact antibodies and DVD-Ig proteins are analyzed by LC-MS. Each antibody or DVD-Ig protein is diluted to approximately 1 mg/mL with water. An 1100 HPLC (Agilent) system with a protein microtrap (Michrom Bioresources, Inc, cat# 004/25109/03) is used to desalt and introduce 5 mg of the sample into an API Qstar pulsar i mass spectrometer (Applied Biosystems). A short gradient is used to elute the samples. The gradient is run with mobile phase A (0.08% FA, 0.02% TFA in HPLC water) and mobile phase B (0.08% FA and 0.02% TFA in acetonitrile) at a flow rate of 50 mL/minute. The mass spectrometer is operated at 4.5 kvolts spray voltage with a scan range from 2000 to 3500 mass to charge ratio.

LC-MS Molecular Weight Measurement of Antibody and DVD-Ig Protein Light and Heavy Chains

[0217] Molecular weight measurement of antibody and DVD-Ig protein light chain (LC), heavy chain (HC) and deglycosylated HC are analyzed by LC-MS. Antibodies and DVD-Ig proteins are diluted to 1 mg/mL with water and the sample is

reduced to LC and HC with a final concentration of 10 mM DTT for 30 minutes at 37°C. To deglycosylate the antibodies and DVD-Ig proteins, 100 mg of the antibody or DVD-Ig protein is incubated with 2 mL of PNGase F, 5 mL of 10% N-octylglucoside in a total volume of 100 mL overnight at 37 °C. After deglycosylation the sample is reduced with a final concentration of 10 mM DTT for 30 minutes at 37°C. An Agilent 1100 HPLC system with a C4 column (Vydac, cat# 214TP5115, S/N 060206537204069) is used to desalt and introduce the sample (5 mg) into an API Qstar pulsar i mass spectrometer (Applied Biosystems). A short gradient is used to elute the sample. The gradient is run with mobile phase A (0.08% FA, 0.02% TFA in HPLC water) and mobile phase B (0.08% FA and 0.02% TFA in acetonitrile) at a flow rate of 50 mL/minute. The mass spectrometer is operated at 4.5 kvolts spray voltage with a scan range from 800 to 3500 mass to charge ratio.

Peptide Mapping

[0218] The antibody or DVD-Ig protein is denatured for 15 minutes at room temperature with a final concentration of 6 M guanidine hydrochloride in 75 mM ammonium bicarbonate. The denatured samples are reduced with a final concentration of 10 mM DTT at 37°C for 60 minutes, followed by alkylation with 50 mM iodoacetic acid (IAA) in the dark at 37°C for 30 minutes. Following alkylation, the sample is dialyzed overnight against four liters of 10 mM ammonium bicarbonate at 4°C. The dialyzed sample is diluted to 1 mg/mL with 10 mM ammonium bicarbonate, pH 7.8 and 100 mg of antibody or DVD-Ig protein is either digested with trypsin (Promega, cat# V5111) or Lys-C (Roche, cat# 11 047 825 001) at a 1:20 (w/w) trypsin/Lys-C:antibody or DVD-Ig protein ratio at 37°C for 4 hours. Digests are quenched with 1 mL of 1 N HCl. For peptide mapping with mass spectrometer detection, 40 mL of the digests are separated by reverse phase high performance liquid chromatography (RPHPLC) on a C18 column (Vydac, cat# 218TP51, S/N NE9606 10.3.5) with an Agilent 1100 HPLC system. The peptide separation is run with a gradient using mobile phase A (0.02% TFA and 0.08% FA in HPLC grade water) and mobile phase B (0.02% TFA and 0.08% FA in acetonitrile) at a flow rate of 50 mL/minutes. The API QSTAR Pulsar i mass spectromer is operated in positive mode at 4.5 kvolts spray voltage and a scan range from 800 to 2500 mass to charge ratio.

Disulfide Bond Mapping

[0219] To denature the antibody, 100 mL of the antibody or DVD-Ig protein is mixed with 300 mL of 8 M guanidine HCl in 100 mM ammonium bicarbonate. The pH

is checked to ensure that it is between 7 and 8 and the samples are denatured for 15 minutes at room temperature in a final concentration of 6 M guanidine HCl. A portion of the denatured sample (100 mL) is diluted to 600 mL with Milli-Q water to give a final guanidine-HCl concentration of 1 M. The sample (220 mg) is digested with either trypsin (Promega, cat # V5111, lot# 22265901) or Lys-C (Roche, cat# 11047825001, lot# 12808000) at a 1:50 trypsin or 1:50 Lys-C: antibody or DVD-Ig protein (w/w) ratios (4.4 mg enzyme: 220 mg sample) at 37°C for approximately 16 hours. An additional 5 mg of trypsin or Lys-C is added to the samples and digestion is allowed to proceed for an additional 2 hours at 37°C. Digestions are stopped by adding 1 mL of TFA to each sample. Digested samples are separated by RPHPLC using a C18 column (Vydac, cat# 218TP51 S/N NE020630-4-1A) on an Agilent HPLC system. The separation is run with the same gradient used for peptide mapping using mobile phase A (0.02% TFA and 0.08% FA in HPLC grade water) and mobile phase B (0.02% TFA and 0.08% FA in acetonitrile) at a flow rate of 50 mL/minute. The HPLC operating conditions are the same as those used for peptide mapping. The API QSTAR Pulsar i mass spectromer is operated in positive mode at 4.5 kvolts spray voltage and a scan range from 800 to 2500 mass-to-charge ratio. Disulfide bonds are assigned by matching the observed MWs of peptides with the predicted MWs of tryptic or Lys-C peptides linked by disulfide bonds.

Free Sulfhydryl Determination

[0220] The method used to quantify free cysteines in an antibody or DVD-Ig protein is based on the reaction of Ellman's reagent, 5,5¢- dithio-bis (2-nitrobenzoic acid) (DTNB), with sulfhydryl groups (SH) which gives rise to a characteristic chromophoric product, 5-thio-(2-nitrobenzoic acid) (TNB). The reaction is illustrated in the formula:

[0221] DTNB + RSH ® RS-TNB + TNB- + H+

[0222] The absorbance of the TNB- is measured at 412 nm using a Cary 50 spectrophotometer. An absorbance curve is plotted using dilutions of 2 mercaptoethanol (b-ME) as the free SH standard and the concentrations of the free sulfhydryl groups in the protein are determined from absorbance at 412 nm of the sample.

[0223] The b-ME standard stock is prepared by a serial dilution of 14.2 M b-ME with HPLC grade water to a final concentration of 0.142 mM. Then standards in triplicate for each concentration are prepared. Antibody or DVD-Ig protein is

concentrated to 10 mg/mL using an amicon ultra 10,000 MWCO centrifugal filter (Millipore, cat# UFC801096, lot# L3KN5251) and the buffer is changed to the formulation buffer used for adalimumab (5.57 mM sodium phosphate monobasic, 8.69 mM sodium phosphate dibasic, 106.69 mM NaCl, 1.07 mM sodium citrate, 6.45 mM citric acid, 66.68 mM mannitol, pH 5.2, 0.1% (w/v) Tween). The samples are mixed on a shaker at room temperature for 20 minutes. Then 180 mL of 100 mM Tris buffer, pH 8.1 is added to each sample and standard followed by the addition of 300 mL of 2 mM DTNB in 10 mM phosphate buffer, pH 8.1. After thorough mixing, the samples and standards are measured for absorption at 412 nm on a Cary 50 spectrophotometer. The standard curve is obtained by plotting the amount of free SH and OD_{412} nm of the b-ME standards. Free SH content of samples are calculated based on this curve after subtraction of the blank.

Weak Cation Exchange Chromatography

[0224] Antibody or DVD-Ig protein is diluted to 1 mg/mL with 10 mM sodium phosphate, pH 6.0. Charge heterogeneity is analyzed using a Shimadzu HPLC system with a WCX-10 ProPac analytical column (Dionex, cat# 054993, S/N 02722). The samples are loaded on the column in 80% mobile phase A (10 mM sodium phosphate, pH 6.0) and 20% mobile phase B (10 mM sodium phosphate, 500 mM NaCl, pH 6.0) and eluted at a flow rate of 1.0 mL/minute.

Oligosaccharide Profiling

[0225] Oligosaccharides released after PNGase F treatment of antibody or DVD-Ig protein are derivatized with 2-aminobenzamide (2-AB) labeling reagent. The fluorescent-labeled oligosaccharides are separated by normal phase high performance liquid chromatography (NPHPLC) and the different forms of oligosaccharides are characterized based on retention time comparison with known standards.

[0226] The antibody or DVD-Ig protein is first digested with PNGaseF to cleave N-linked oligosaccharides from the Fc portion of the heavy chain. The antibody or DVD-Ig protein (200 mg) is placed in a 500 mL Eppendorf tube along with 2 mL PNGase F and 3 mL of 10% N-octylglucoside. Phosphate buffered saline is added to bring the final volume to 60 mL. The sample is incubated overnight at 37°C in an Eppendorf thermomixer set at 700 RPM. Adalimumab lot AFP04C is also digested with PNGase F as a control.

[0227] After PNGase F treatment, the samples are incubated at 95°C for 5 minutes in an Eppendorf thermomixer set at 750 RPM to precipitate out the proteins, then the samples are placed in an Eppendorf centrifuge for 2 minutes at 10,000 RPM to spin down the precipitated proteins. The supernatent containing the oligosaccharides are transferred to a 500 mL Eppendorf tube and dried in a speed-vac at 65°C.

[0228] The oligosaccharides are labeled with 2AB using a 2AB labeling kit purchased from Prozyme (cat# GKK-404, lot# 132026). The labeling reagent is prepared according to the manufacturer's instructions. Acetic acid (150 mL, provided in kit) is added to the DMSO vial (provided in kit) and mixed by pipeting the solution up and down several times. The acetic acid/DMSO mixture (100 mL) is transferred to a vial of 2-AB dye (just prior to use) and mixed until the dye is fully dissolved. The dye solution is then added to a vial of reductant (provided in kit) and mixed well (labeling reagent). The labeling reagent (5 mL) is added to each dried oligosaccharide sample vial, and mixed thoroughly. The reaction vials are placed in an Eppendorf thermomixer set at 65°C and 700-800 RPM for 2 hours of reaction.

[0229] After the labeling reaction, the excess fluorescent dye is removed using GlycoClean S Cartridges from Prozyme (cat# GKI-4726). Prior to adding the samples, the cartridges are washed with 1 mL of milli-Q water followed with 5 ishes of 1 mL 30% acetic acid solution. Just prior to adding the samples, 1 mL of acetonitrile (Burdick and Jackson, cat# AH015-4) is added to the cartridges.

[0230] After all of the acetonitrile passes through the cartridge, the sample is spotted onto the center of the freshly washed disc and allowed to adsorb onto the disc for 10 minutes. The disc is washed with 1 mL of acetonitrile followed by five ishes of 1 mL of 96% acetonitrile. The cartridges are placed over a 1.5 mL Eppendorf tube and the 2-AB labeled oligosaccharides are eluted with 3 ishes (400 mL each ish) of milli Q water.

[0231] The oligosaccharides are separated using a Glycosep N HPLC (cat# GKI-4728) column connected to a Shimadzu HPLC system. The Shimadzu HPLC system consisted of a system controller, degasser, binary pumps, autosampler with a sample cooler, and a fluorescent detector.

Stability at Elevated Temperatures

[0232] The buffer of antibody or DVD-Ig protein is either 5.57 mM sodium phosphate monobasic, 8.69 mM sodium phosphate dibasic, 106.69 mM NaCl, 1.07

mM sodium citrate, 6.45 mM citric acid, 66.68 mM mannitol, 0.1% (w/v) Tween, pH 5.2; or 10 mM histidine, 10 mM methionine, 4% mannitol, pH 5.9 using Amicon ultra centrifugal filters. The final concentration of the antibodies or DVD-Ig proteins is adjusted to 2 mg/mL with the appropriate buffers. The antibody or DVD-Ig protein solutions are then filter sterized and 0.25 mL aliquots are prepared under sterile conditions. The aliquots are left at either -80°C, 5°C, 25°C, or 40°C for 1, 2 or 3 weeks. At the end of the incubation period, the samples are analyzed by size exclusion chromatography and SDS-PAGE.

[0233] The stability samples are analyzed by SDS-PAGE under both reducing and non-reducing conditions. The procedure used is the same as described herein. The gels are stained overnight with colloidal blue stain (Invitrogen cat# 46-7015, 46-7016) and destained with Milli-Q water until the background is clear. The stained gels are then scanned using an Epson Expression scanner (model 1680, S/N DASX003641). To obtain more sensitivity, the same gels are silver stained using silver staining kit (Owl Scientific) and the recommended procedures given by the manufacturer is used.

Dynamic Scanning Fluorimetry

[0234] The DVD-Ig proteins were dialysed in 10mM citrate 10mM phosphate buffer, pH 6.0 to get a final concentration of 1 mg/ml. Triplicates were run for each DVD-Ig protein. For each sample, 27 µl of the DVD-Ig protein was added in a well of a 96 well plate and mixed with 3 µl of 4X diluted SYPRO Orange dye (Invitrogen). The dye is supplied in DMSO at a concentration of 5000X and was diluted to the working concentration of 4X in water. The plate was centrifuged for 30 seconds to ensure that both the dye and the protein settle to the bottom of the wells and complete mixing was ensured by gentle aspiration by a pipette tip. The plate was then sealed with an adhesive film.

[0235] A real time PCR (Applied Biosciences, 7500 Series) was used for measuring the change in fluorescence intensities with temperature. The plate was heated from 25° C to 95° C at a temperature ramp rate of approximately 0.5°C/minute and emission fluorescence was collected using TAMRA filter. The data was exported to Microsoft Excel and plotted as temperature vs fluorescence for each DVD-Ig protein. Onset of melting was noted as the temperature where the thermogram rises above the baseline fluorescence. SYPRO Orange is a hydrophobic dye and preferentially binds to the exposed hydrophobic residues in an unfolded protein molecule. Hence the onset of unfolding temperature, as measured by an increase in

fluorescence, is an indication of the thermal stability of the DVD-Ig protein. The unfolding temperature for the DVD-Ig proteins can be found in Table 12.

Table 12: Thermal Stability of DVD-Ig Proteins as Determined by Dynamic Scanning Fluorimetry

Parent Antibody or DVD-Ig ID	N-terminal Variable Domain (VD)	C-terminal Variable Domain (VD)	T onset (deg C)
DVD2683	TNF (seq 1)	IL-13 (seq 1)	58
DVD2684	TNF (seq 1)	IL-13 (seq 1)	58.2
DVD2686	TNF (seq 1)	IL-13 (seq 1)	64.3
DVD2687	TNF (seq 1)	IL-13 (seq 1)	57.9
DVD2691	IL-13 (seq 1)	TNF (seq 1)	59.2
DVD2734	TNF (seq 2)	IL-13 (seq 1)	64.31
DVD2736	TNF (seq 2)	IL-13 (seq 1)	65
DVD2737	TNF (seq 2)	IL-13 (seq 1)	65.5
DVD2742	IL-13 (seq 1)	TNF (seq 2)	
DVD2784	TNF (seq 3)	IL-13 (seq 1)	
DVD2786	TNF (seq 3)	IL-13 (seq 1)	
DVD2787	TNF (seq 3)	IL-13 (seq 1)	
DVD2789	IL-13 (seq 1)	TNF (seq 3)	
DVD2791	IL-13 (seq 1)	TNF (seq 3)	
DVD3009	TNF (seq 4)	IL-13 (seq 1)	63.3
DVD3011	TNF (seq 4)	IL-13 (seq 1)	
DVD3083	TNF (seq 1)	IL-13 (seq 2)	66.3
DVD3084	TNF (seq 1)	IL-13 (seq 2)	66.9
DVD3086	TNF (seq 1)	IL-13 (seq 2)	65.9
DVD3087	TNF (seq 1)	IL-13 (seq 2)	65
DVD3093	TNF (seq 2)	IL-13 (seq 2)	65.1
DVD3094	TNF (seq 2)	IL-13 (seq 2)	66
DVD3096	TNF (seq 2)	IL-13 (seq 2)	65.9
DVD3097	TNF (seq 2)	IL-13 (seq 2)	66.2
DVD3103	TNF (seq 3)	IL-13 (seq 2)	
DVD3106	TNF (seq 3)	IL-13 (seq 2)	64.1
DVD3107	TNF (seq 3)	IL-13 (seq 2)	65.1
DVD3113	TNF (seq 4)	IL-13 (seq 2)	65.6
DVD3143	TNF (seq 1)	IL-13 (seq 3)	63
DVD3144	TNF (seq 1)	IL-13 (seq 3)	62.6
DVD3146	TNF (seq 1)	IL-13 (seq 3)	61.8
DVD3147	TNF (seq 1)	IL-13 (seq 3)	61.5
DVD3154	TNF (seq 2)	IL-13 (seq 3)	63.3
DVD3156	TNF (seq 2)	IL-13 (seq 3)	61.4
DVD3157	TNF (seq 2)	IL-13 (seq 3)	62.5
DVD3163	TNF (seq 3)	IL-13 (seq 3)	62.4
DVD3164	TNF (seq 3)	IL-13 (seq 3)	63.4
DVD3166	TNF (seq 3)	IL-13 (seq 3)	60.8
DVD3167	TNF (seq 3)	IL-13 (seq 3)	

DVD3173	TNF (seq 4)	IL-13 (seq 3)	60.8
DVD3174	TNF (seq 4)	IL-13 (seq 3)	61.3
DVD3176	TNF (seq 4)	IL-13 (seq 3)	60.2

[0236] Most DVD-Ig proteins showed an unfolding temperature >50 degrees C. This DVD-Ig protein profile is similar to that observed for parent antibodies.

Solubility Determination

[0237] DVD-Ig protein candidates were dialyzed in 15mM His, pH 6.0. This was followed by concentrating them upto 50 µl in centricons with a 30K cutoff. Solubility was visually confirmed by absence of precipitation after storage at 4°C and quantitatively determined by UV absorbance measurement at 280nm.

Table 13: Solubility of DVD-Ig Proteins

Parent Antibody or DVD-Ig ID	N-terminal Variable Domain (VD)	C-terminal Variable Domain (VD)	Visual observation	Solubility (mg/mL)
DVD2683	TNF (seq 1)	IL-13 (seq 1)	clear	>248
DVD2684	TNF (seq 1)	IL-13 (seq 1)	clear	>154
DVD2686	TNF (seq 1)	IL-13 (seq 1)	clear	>124
DVD2687	TNF (seq 1)	IL-13 (seq 1)	clear	>131
DVD2691	IL-13 (seq 1)	TNF (seq 1)	clear	>110
DVD2734	TNF (seq 2)	IL-13 (seq 1)	clear	>168
DVD2736	TNF (seq 2)	IL-13 (seq 1)	clear	>189
DVD2737	TNF (seq 2)	IL-13 (seq 1)	clear	>165
DVD2742	IL-13 (seq 1)	TNF (seq 2)	clear	>78
DVD2784	TNF (seq 3)	IL-13 (seq 1)	clear	>93
DVD2786	TNF (seq 3)	IL-13 (seq 1)	clear	>75
DVD2787	TNF (seq 3)	IL-13 (seq 1)	clear	>80
DVD2789	IL-13 (seq 1)	TNF (seq 3)	clear	>114
DVD2791	IL-13 (seq 1)	TNF (seq 3)	clear	>83
DVD3009	TNF (seq 4)	IL-13 (seq 1)	clear	>64
DVD3011	TNF (seq 4)	IL-13 (seq 1)	clear	>60
DVD3083	TNF (seq 1)	IL-13 (seq 2)	clear	>128
DVD3084	TNF (seq 1)	IL-13 (seq 2)	clear	>36
DVD3086	TNF (seq 1)	IL-13 (seq 2)	clear	>108
DVD3087	TNF (seq 1)	IL-13 (seq 2)	clear	>174
DVD3093	TNF (seq 2)	IL-13 (seq 2)	clear	>162
DVD3094	TNF (seq 2)	IL-13 (seq 2)	clear	>134
DVD3096	TNF (seq 2)	IL-13 (seq 2)	clear	>118
DVD3097	TNF (seq 2)	IL-13 (seq 2)	clear	>161
DVD3103	TNF (seq 3)	IL-13 (seq 2)	clear	>103
DVD3106	TNF (seq 3)	IL-13 (seq 2)	clear	>171
DVD3107	TNF (seq 3)	IL-13 (seq 2)	clear	>185

DVD3113	TNF (seq 4)	IL-13 (seq 2)	clear	>83
DVD3143	TNF (seq 1)	IL-13 (seq 3)	clear	>160
DVD3144	TNF (seq 1)	IL-13 (seq 3)	clear	>174
DVD3146	TNF (seq 1)	IL-13 (seq 3)	clear	>148
DVD3147	TNF (seq 1)	IL-13 (seq 3)	clear	>83
DVD3154	TNF (seq 2)	IL-13 (seq 3)	clear	>179
DVD3156	TNF (seq 2)	IL-13 (seq 3)	clear	>179
DVD3157	TNF (seq 2)	IL-13 (seq 3)	clear	>142
DVD3163	TNF (seq 3)	IL-13 (seq 3)	clear	>250
DVD3164	TNF (seq 3)	IL-13 (seq 3)	clear	>229
DVD3166	TNF (seq 3)	IL-13 (seq 3)	clear	>160
DVD3167	TNF (seq 3)	IL-13 (seq 3)	clear	>81
DVD3173	TNF (seq 4)	IL-13 (seq 3)	clear	>128
DVD3174	TNF (seq 4)	IL-13 (seq 3)	clear	>90
DVD3176	TNF (seq 4)	IL-13 (seq 3)	clear	>64

[0238] Most DVD-Ig proteins showed clear appearance and could be concentrated to greater than 25 mg/ml. This DVD-Ig protein profile is similar to that observed for parent antibodies.

Incorporation by Reference

[0239] The contents of all cited references (including literature references, patents, patent applications, and websites) that maybe cited throughout this application are hereby expressly incorporated by reference in their entirety for any purpose, as are the references cited therein. The disclosure will employ, unless otherwise indicated, conventional techniques of immunology, molecular biology and cell biology, which are well known in the art.

[0240] The present disclosure also incorporates by reference in their entirety techniques well known in the field of molecular biology and drug delivery. These techniques include, but are not limited to, techniques described in the following publications:

Ausubel et al. (eds.), CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley &Sons, NY (1993);

Ausubel, F.M. et al. eds., SHORT PROTOCOLS IN MOLECULAR BIOLOGY (4th Ed. 1999) John Wiley & Sons, NY. (ISBN 0-471-32938-X);

CONTROLLED DRUG BIOAVAILABILITY, DRUG PRODUCT DESIGN AND PERFORMANCE, Smolen and Ball (eds.), Wiley, New York (1984);

Giege, R. and Ducruix, A. Barrett, CRYSTALLIZATION OF NUCLEIC ACIDS AND PROTEINS, a Practical Approach, 2nd ea., pp. 20 1-16, Oxford University Press, New York, New York, (1999);

Goodson, in MEDICAL APPLICATIONS OF CONTROLLED RELEASE, vol. 2, pp. 115-138 (1984);

Hammerling, et al., in: MONOCLONAL ANTIBODIES AND T-CELL HYBRIDOMAS 563-681 (Elsevier, N.Y., 1981;

Harlow et al. , ANTIBODIES: A LABORATORY MANUAL, (Cold Spring Harbor Laboratory Press, 2nd ed. 1988);

Kabat et al., SEQUENCES OF PROTEINS OF IMMUNOLOGICAL INTEREST (National Institutes of Health, Bethesda, Md. (1987) and (1991);

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;

Kontermann and Dubel eds., ANTIBODY ENGINEERING (2001) Springer-Verlag. New York. 790 pp. (ISBN 3-540-41354-5).

Kriegler, Gene Transfer and Expression, A Laboratory Manual, Stockton Press, NY (1990);

Lu and Weiner eds., CLONING AND EXPRESSION VECTORS FOR GENE FUNCTION ANALYSIS (2001) BioTechniques Press. Westborough, MA. 298 pp. (ISBN 1-881299-21-X).

MEDICAL APPLICATIONS OF CONTROLLED RELEASE, Langer and Wise (eds.), CRC Pres., Boca Raton, Fla. (1974);

Old, R.W. & S.B. Primrose, PRINCIPLES OF GENE MANIPULATION: AN INTRODUCTION TO GENETIC ENGINEERING (3d Ed. 1985) Blackwell Scientific Publications, Boston. Studies in Microbiology; V.2:409 pp. (ISBN 0-632-01318-4).

Sambrook, J. et al. eds., MOLECULAR CLONING: A LABORATORY MANUAL (2d Ed. 1989) Cold Spring Harbor Laboratory Press, NY. Vols. 1-3. (ISBN 0-87969-309-6).

SUSTAINED AND CONTROLLED RELEASE DRUG DELIVERY SYSTEMS, J.R. Robinson, ed., Marcel Dekker, Inc., New York, 1978

Winnacker, E.L. FROM GENES TO CLONES: INTRODUCTION TO GENE TECHNOLOGY (1987) VCH Publishers, NY (translated by Horst Ibelgaufts). 634 pp. (ISBN 0-89573-614-4).

Equivalents

[0241] The disclosure may be embodied in other specific forms without departing from the spirit or essential characteristics thereof. The foregoing embodiments are therefore to be considered in all respects illustrative rather than limiting of the disclosure. Scope of the disclosure is thus indicated by the appended claims rather than by the foregoing description, and all changes that come within the meaning and range of equivalency of the claims are therefore intended to be embraced herein.

We claim:

1. A binding protein comprising first and second polypeptide chains, each independently comprising VD1-(X1)n-VD2-C-X2, wherein

VD1 is a first variable domain;

VD2 is a second variable domain;

C is a constant domain;

X1 is a linker;

X2 is an Fc region that is either present or absent;

n is 0 or 1,

wherein the VD1 domains on the first and second polypeptide chains form a first functional target binding site and the VD2 domains on the first and second polypeptide chains form a second functional target binding site, and wherein the binding protein is capable of binding

(a) TNFα and IL-13, wherein

(i) the variable domains that form a functional target binding site for TNFα comprise a sequence selected from the group consisting of SEQ ID NOs: 38-43 and 48-49, and/or

(ii) the variable domains that form a functional target binding site for IL-13 comprise a sequence selected from the group consisting of SEQ ID NO: 32-37;

(b) TNFα and PGE2, wherein

(i) the variable domains that form a functional target binding site for TNFα comprise a sequence selected from the group consisting of SEQ ID NOs: 38-43 and 48-49, and/or

(ii) the variable domains that form a functional target binding site for PGE2 comprise a sequence selected from the group consisting of SEQ ID NO: 50-55;

or

(c) TNFα and NGF, wherein

(i) the variable domains that form a functional target binding site for TNFα comprise a sequence selected from the group consisting of SEQ ID NOs: 38-43 and 48-49, and/or

(ii) the variable domains that form a functional target binding site for NGF comprise a sequence selected from the group consisting of SEQ ID NO: 56-57.

2. A binding protein comprising first and second polypeptide chains, each independently comprising VD1-(X1)n-VD2-C-X2, wherein

VD1 is a first variable domain;

VD2 is a second variable domain;

C is a constant domain;

X1 is a linker;

X2 is an Fc region that is either present or absent;

n is 0 or 1,

wherein the VD1 domains on the first and second polypeptide chains form a first functional target binding site and the VD2 domains on the first and second polypeptide chains form a second functional target binding site, and wherein the binding protein is capable of binding

(a) TNFα and IL-13, wherein

(i) the variable domains that form a functional target binding site for TNFα comprise:

CDRs 1-3 from SEQ ID NO: 38 and CDRs 1-3 from SEQ ID NO: 39,

CDRs 1-3 from SEQ ID NO: 40 and CDRs 1-3 from SEQ ID NO: 41,

CDRs 1-3 from SEQ ID NO: 42 and CDRs 1-3 from SEQ ID NO: 43, or

CDRs 1-3 from SEQ ID NO: 48 and CDRs 1-3 from SEQ ID NO: 49;

and/or

(ii) the variable domains that form a functional target binding site for IL-13 comprise

CDRs 1-3 from SEQ ID NO: 32 and CDRs 1-3 from SEQ ID NO: 33;

CDRs 1-3 from SEQ ID NO: 34 and CDRs 1-3 from SEQ ID NO: 35; or

CDRs 1-3 from SEQ ID NO: 36 and CDRs 1-3 from SEQ ID NO: 37;

(b) TNFα and PGE2, wherein

(i) the variable domains that form a functional target binding site for TNFα comprise:

CDRs 1-3 from SEQ ID NO: 38 and three CDRs from SEQ ID NO: 39,

CDRs 1-3 from SEQ ID NO: 40 and three CDRs from SEQ ID NO: 41,

CDRs 1-3 from SEQ ID NO: 42 and three CDRs from SEQ ID NO: 43, or

CDRs 1-3 from SEQ ID NO: 48 and CDRs 1-3 from SEQ ID NO: 49;

and/or

(ii) the variable domains that form a functional target binding site for PGE2 comprise

CDRs 1-3 from SEQ ID NO: 50 and CDRs 1-3 from SEQ ID NO: 51;

CDRs 1-3 from SEQ ID NO: 52 and CDRs 1-3 from SEQ ID NO: 53; or

CDRs 1-3 from SEQ ID NO: 54 and CDRs 1-3 from SEQ ID NO: 55;

or

(c) TNFα and NGF, wherein

(i) the variable domains that form a functional target binding site for TNFα comprise:

CDRs 1-3 from SEQ ID NO: 38 and CDRs 1-3 from SEQ ID NO: 39,

CDRs 1-3 from SEQ ID NO: 40 and CDRs 1-3 from SEQ ID NO: 41,

CDRs 1-3 from SEQ ID NO: 42 and CDRs 1-3 from SEQ ID NO: 43, or

CDRs 1-3 from SEQ ID NO: 48 and CDRs 1-3 from SEQ ID NO: 49;

and/or

(ii) the variable domains that form a functional target binding site for NGF comprise

CDRs 1-3 from SEQ ID NO: 56 and CDRs 1-3 from SEQ ID NO: 57.

3. The binding protein of claim 1 or 2, wherein the first polypeptide chain comprises a first VD1-(X1)n-VD2-C-X2, wherein

VD1 is a first heavy chain variable domain;

VD2 is a second heavy chain variable domain;

C is a heavy chain constant domain;

X1 is a linker;

X2 is an Fc region that is either present or absent;

n is 0 or 1, and

wherein the second polypeptide chain comprises a second VD1-(X1)n-VD2-C, wherein

VD1 is a first light chain variable domain;

VD2 is a second light chain variable domain;

C is a light chain constant domain;

X1 is a linker;

n is 0 or 1,

wherein the VD1 domains on the first and second polypeptide chains form a first functional target binding site and the VD2 domains on the first and second polypeptide chains form a second functional target binding site.

4. The binding protein of any one of claims 1-3, wherein the binding protein is capable of binding:

(a) TNFα and IL-13, wherein

(i) the variable domains that form a functional target binding site for TNFα comprise:

SEQ ID NO: 38 and SEQ ID NO: 39,

SEQ ID NO: 40 and SEQ ID NO: 41,

SEQ ID NO: 42 and SEQ ID NO: 43, or

SEQ ID NO: 48 and SEQ ID NO: 49;

and/or

(ii) the variable domains that form a functional target binding site for IL-13 comprise

SEQ ID NO: 32 and SEQ ID NO: 33;

SEQ ID NO: 34 and SEQ ID NO: 35; or

SEQ ID NO: 36 and SEQ ID NO: 37;

(b) TNFα and PGE2, wherein

(i) the variable domains that form a functional target binding site for TNFα comprise:

SEQ ID NO: 38 and SEQ ID NO: 39,

SEQ ID NO: 40 and SEQ ID NO: 41,

SEQ ID NO: 42 and SEQ ID NO: 43, or

SEQ ID NO: 48 and SEQ ID NO: 49;

and/or

(ii) the variable domains that form a functional target binding site for PGE2 comprise

SEQ ID NO: 50 and SEQ ID NO: 51;

SEQ ID NO: 52 and SEQ ID NO: 53; or

SEQ ID NO: 54 and SEQ ID NO: 55;

or

(c) TNFα and NGF, wherein

(i) the variable domains that form a functional target binding site for TNFα comprise:

SEQ ID NO: 38 and SEQ ID NO: 39,

SEQ ID NO: 40 and SEQ ID NO: 41,

SEQ ID NO: 42 and SEQ ID NO: 43, or

SEQ ID NO: 48 and SEQ ID NO: 49;

and/or

(ii) the variable domains that form a functional target binding site for NGF comprise

SEQ ID NO: 56 and SEQ ID NO: 57.

5. The binding protein of any one of claims 1-4, wherein

(a) the binding protein is capable of binding TNFα and IL-13, wherein the binding protein is capable of binding TNFα with a K_D of at most about $5.8x10^{-11}$ M, as measured by surface plasmon resonance, and/or the binding protein is capable of binding IL-13 with a K_D of at most about $1.2x10^{-9}$ M, as measured by surface plasmon resonance;

(b) the binding protein is capable of binding TNFα and PGE2, wherein the binding protein is capable of neutralizing TNFα with an IC50 of at most about

3.076 nM, as measured by a TNFα neutralization assay, and/or the binding protein is capable of neutralizing PGE2 with an IC50 of at most about 124.8 nM, as measured by a PGE2 neutralization assay; or

(c) the binding protein is capable of binding TNFα and NGF, wherein the binding protein is capable of neutralizing TNFα with an IC50 of at most about 0.673 nM, as measured by a TNFα neutralization assay, and/or the binding protein is capable of inhibiting NGF with an IC50 of at most about 7.455 nM, as measured by a TF-1 cell proliferation bioassay.

6. The binding protein of any one of claims 1-5, comprising two first polypeptide chains and two second polypeptide chains and four functional target binding sites.

7. The binding protein of any one of claims 1-6, wherein X1 is any one of SEQ ID NO: 1-31.

8. The binding protein of any one of claims 1-7, wherein X1 is not CH1 or CL.

9. The binding protein of any one of claims 1-8, wherein the Fc region is a variant sequence Fc region, and/or wherein the Fc region is an Fc region from an IgG1, IgG2, IgG3, IgG4, IgA, IgM, IgE, or IgD.

10. The binding protein of any one of claims 1-9, wherein the binding protein comprises

(a) a heavy chain constant region comprising:

(i) a wild type human IgG1 heavy chain sequence, or

(ii) a human IgG1 heavy chain sequence modified by one or more amino acid changes, optionally wherein the changes comprise substitutions at amino acid positions 234 and 235 of the constant region sequence, optionally wherein the changes comprise the substitution of leucines at positions 234 and 235 with alanines;

and/or

(b) a light chain constant region comprising:

(i) a wild type human kappa light chain constant region sequence, or

(ii) a wild type human lamda light chain constant region sequence.

11. The binding protein of any one of claims 1-10, wherein the binding protein is a crystallized binding protein.

12. A binding protein capable of binding
(a) TNFα and IL-13, comprising any DVD-Ig VH and VL sequence pair from Table 2;
(b) TNFα and PGE2, comprising any DVD-Ig VH and VL sequence pair from Table 3; or
(c) TNFα and NGF, comprising any DVD-Ig VH and VL sequence pair from Table 4.

13. A binding protein conjugate comprising the binding protein according to any one of claims 1-12, the binding protein conjugate further comprising an immunoadhesion molecule, an imaging agent, a therapeutic agent, or a cytotoxic agent.

14. The binding protein conjugate of claim 13, wherein the imaging agent is a radiolabel, an enzyme, a fluorescent label, a luminescent label, a bioluminescent label, a magnetic label, or biotin.

15. The binding protein conjugate of claim 14, wherein the radiolabel is ^{3}H, ^{14}C, ^{35}S, ^{90}Y, ^{99}Tc, ^{111}In, ^{125}I, ^{131}I, ^{177}Lu, ^{166}Ho, or ^{153}Sm.

16. The binding protein conjugate of claim 13, wherein the therapeutic or cytotoxic agent is an anti-metabolite, an alkylating agent, an antibiotic, a growth factor, a cytokine, an anti-angiogenic agent, an anti-mitotic agent, an anthracycline, toxin, or an apoptotic agent.

17. An isolated nucleic acid encoding the binding protein amino acid sequence of any one of claims 1-12.

18. A vector comprising the isolated nucleic acid of claim 17.

19. The vector of claim 18, wherein the vector comprises pcDNA, pTT, pTT3, pEFBOS, pBV, pJV, pcDNA3.1 TOPO, pEF6, pHybE, TOPO, or pBJ.

20. A host cell comprising the vector of claim 18 or 19.

21. The host cell of claim 20, wherein the host cell is a prokaryotic cell, *Escherichia coli*, a eukaryotic cell, a protist cell, an animal cell, a plant cell, a fungal cell, a yeast

cell, an Sf9 cell, a mammalian cell, an avian cell, an insect cell, a CHO cell or a COS cell.

22. A method of producing a binding protein, comprising culturing the host cell of claim 20 or 21 in culture medium under conditions sufficient to produce the binding protein.

23. A pharmaceutical composition comprising the binding protein according to any one of claims 1-12, and a pharmaceutically acceptable carrier.

24. The pharmaceutical composition of claim 23, further comprising at least one additional therapeutic agent.

25. The pharmaceutical composition according to claim 24, wherein the additional therapeutic agent is an imaging agent, a cytotoxic agent, an angiogenesis inhibitor, a kinase inhibitor, a co-stimulation molecule blocker, an adhesion molecule blocker, an anti-cytokine antibody or functional fragment thereof, methotrexate, cyclosporin, rapamycin, FK506, a detectable label or reporter, a TNF antagonist, an antirheumatic, a muscle relaxant, a narcotic, a non-steroid anti-inflammatory drug (NSAID), an analgesic, an anesthetic, a sedative, a local anesthetic, a neuromuscular blocker, an antimicrobial, an antipsoriatic, a corticosteriod, an anabolic steroid, an erythropoietin, an immunization, an immunoglobulin, an immunosuppressive, a growth hormone, a hormone replacement drug, a radiopharmaceutical, an antidepressant, an antipsychotic, a stimulant, an asthma medication, a beta agonist, an inhaled steroid, an epinephrine or analog, a cytokine, or a cytokine antagonist.

26. A method of treating a subject for a disease or a disorder by administering the binding protein of any one of claims 1-12 to the subject.

27. The method of claim 26, wherein the disorder is arthritis, osteoarthritis, juvenile chronic arthritis, septic arthritis, Lyme arthritis, psoriatic arthritis, reactive arthritis, spondyloarthropathy, systemic lupus erythematosus, Crohn's disease, ulcerative colitis, inflammatory bowel disease, insulin dependent diabetes mellitus, thyroiditis, asthma, allergic diseases, psoriasis, dermatitis scleroderma, graft versus host disease, organ transplant rejection, acute or chronic immune disease associated with organ transplantation, sarcoidosis, atherosclerosis, disseminated intravascular coagulation, Kawasaki's disease, Grave's disease, nephrotic syndrome, chronic fatigue syndrome, Wegener's granulomatosis, Henoch-Schoenlein purpurea,

microscopic vasculitis of the kidneys, chronic active hepatitis, uveitis, septic shock, toxic shock syndrome, sepsis syndrome, cachexia, infectious diseases, parasitic diseases, acute transverse myelitis, Huntington's chorea, Parkinson's disease, Alzheimer's disease, stroke, primary biliary cirrhosis, hemolytic anemia, malignancies, heart failure, myocardial infarction, Addison's disease, sporadic polyglandular deficiency type I and polyglandular deficiency type II, Schmidt's syndrome, adult (acute) respiratory distress syndrome, alopecia, alopecia areata, seronegative arthopathy, arthropathy, Reiter's disease, psoriatic arthropathy, ulcerative colitic arthropathy, enteropathic synovitis, chlamydia, yersinia and salmonella associated arthropathy, spondyloarthopathy, atheromatous disease/arteriosclerosis, atopic allergy, autoimmune bullous disease, pemphigus vulgaris, pemphigus foliaceus, pemphigoid, linear IgA disease, autoimmune haemolytic anaemia, Coombs positive haemolytic anaemia, acquired pernicious anaemia, juvenile pernicious anaemia, myalgic encephalitis/Royal Free Disease, chronic mucocutaneous candidiasis, giant cell arteritis, primary sclerosing hepatitis, cryptogenic autoimmune hepatitis, Acquired Immunodeficiency Syndrome, Acquired Immunodeficiency Related Diseases, Hepatitis B, Hepatitis C, common varied immunodeficiency (common variable hypogammaglobulinaemia), dilated cardiomyopathy, female infertility, ovarian failure, premature ovarian failure, fibrotic lung disease, cryptogenic fibrosing alveolitis, post-inflammatory interstitial lung disease, interstitial pneumonitis, connective tissue disease associated interstitial lung disease, mixed connective tissue disease associated lung disease, systemic sclerosis associated interstitial lung disease, rheumatoid arthritis associated interstitial lung disease, systemic lupus erythematosus associated lung disease, dermatomyositis/polymyositis associated lung disease, Sjögren's disease associated lung disease, ankylosing spondylitis associated lung disease, vasculitic diffuse lung disease, haemosiderosis associated lung disease, drug-induced interstitial lung disease, fibrosis, radiation fibrosis, bronchiolitis obliterans, chronic eosinophilic pneumonia, lymphocytic infiltrative lung disease, postinfectious interstitial lung disease, gouty arthritis, autoimmune hepatitis, type-1 autoimmune hepatitis (classical autoimmune or lupoid hepatitis), type-2 autoimmune hepatitis (anti-LKM antibody hepatitis), autoimmune mediated hypoglycaemia, type B insulin resistance with acanthosis nigricans, hypoparathyroidism, acute immune disease associated with organ transplantation, chronic immune disease associated with organ transplantation, osteoarthrosis, primary sclerosing cholangitis, psoriasis type 1, psoriasis type 2, idiopathic leucopaenia, autoimmune neutropaenia, renal disease NOS, glomerulonephritides, microscopic vasulitis of the kidneys, lyme

disease, discoid lupus erythematosus, male infertility idiopathic or NOS, sperm autoimmunity, multiple sclerosis (all subtypes), sympathetic ophthalmia, pulmonary hypertension secondary to connective tissue disease, Goodpasture's syndrome, pulmonary manifestation of polyarteritis nodosa, acute rheumatic fever, rheumatoid spondylitis, Still's disease, systemic sclerosis, Sjörgren's syndrome, Takayasu's disease/arteritis, autoimmune thrombocytopaenia, idiopathic thrombocytopaenia, autoimmune thyroid disease, hyperthyroidism, goitrous autoimmune hypothyroidism (Hashimoto's disease), atrophic autoimmune hypothyroidism, primary myxoedema, phacogenic uveitis, primary vasculitis, vitiligo acute liver disease, chronic liver diseases, alcoholic cirrhosis, alcohol-induced liver injury, cholestasis, idiosyncratic liver disease, Drug-Induced hepatitis, Non-alcoholic Steatohepatitis, allergy and asthma, group B streptococci (GBS) infection, mental disorders (*e.g.*, depression and schizophrenia), Th2 Type and Th1 Type mediated diseases, acute and chronic pain (different forms of pain), and cancers such as lung, breast, stomach, bladder, colon, pancreas, ovarian, prostate and rectal cancer and hematopoietic malignancies (leukemia and lymphoma) abetalipoproteinemia, Acrocyanosis, acute and chronic parasitic or infectious processes, acute leukemia, acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML), acute or chronic bacterial infection, acute pancreatitis, acute renal failure, adenocarcinomas, aerial ectopic beats, AIDS dementia complex, alcohol-induced hepatitis, allergic conjunctivitis, allergic contact dermatitis, allergic rhinitis, allograft rejection, alpha-l- antitrypsin deficiency, amyotrophic lateral sclerosis, anemia, angina pectoris, anterior horn cell degeneration, anti cd3 therapy, antiphospholipid syndrome, anti-receptor hypersensitivity reactions, aordic and peripheral aneuryisms, aortic dissection, arterial hypertension, arteriosclerosis, arteriovenous fistula, ataxia, atrial fibrillation (sustained or paroxysmal), atrial flutter, atrioventricular block, B cell lymphoma, bone graft rejection, bone marrow transplant (BMT) rejection, bundle branch block, Burkitt's lymphoma, burns, cardiac arrhythmias, cardiac stun syndrome, cardiac tumors, cardiomyopathy, cardiopulmonary bypass inflammation response, cartilage transplant rejection, cerebellar cortical degenerations, cerebellar disorders, chaotic or multifocal atrial tachycardia, chemotherapy associated disorders, chromic myelocytic leukemia (CML), chronic alcoholism, chronic inflammatory pathologies, chronic lymphocytic leukemia (CLL), chronic obstructive pulmonary disease (COPD), chronic salicylate intoxication, colorectal carcinoma, congestive heart failure, conjunctivitis, contact dermatitis, cor pulmonale, coronary artery disease, Creutzfeldt-Jakob disease, culture negative sepsis, cystic fibrosis, cytokine therapy associated disorders, Dementia pugilistica,

demyelinating diseases, dengue hemorrhagic fever, dermatitis, dermatologic conditions, diabetes, diabetes mellitus, diabetic ateriosclerotic disease, Diffuse Lewy body disease, dilated congestive cardiomyopathy, disorders of the basal ganglia, Down's Syndrome in middle age, drug- induced movement disorders induced by drugs which block CNS dopamine receptors, drug sensitivity, eczema, encephalomyelitis, endocarditis, endocrinopathy, epiglottitis, epstein-barr virus infection, erythromelalgia, extrapyramidal and cerebellar disorders, familial hematophagocytic lymphohistiocytosis, fetal thymus implant rejection, Friedreich's ataxia, functional peripheral arterial disorders, fungal sepsis, gas gangrene, gastric ulcer, graft rejection of any organ or tissue, gram negative sepsis, gram positive sepsis, granulomas due to intracellular organisms, hairy cell leukemia, Hallerrorden-Spatz disease, hashimoto's thyroiditis, hay fever, heart transplant rejection, hemachromatosis, hemodialysis, hemolytic uremic syndrome/thrombolytic thrombocytopenic purpura, hemorrhage, hepatitis A, His bundle arryhthmias, HIV infection/HIV neuropathy, Hodgkin's disease, hyperkinetic movement disorders, hypersensitity reactions, hypersensitivity pneumonitis, hypertension, hypokinetic movement disorders, hypothalamic-pituitary-adrenal axis evaluation, idiopathic Addison's disease, idiopathic pulmonary fibrosis, antibody mediated cytotoxicity, Asthenia, infantile spinal muscular atrophy, inflammation of the aorta, influenza a, ionizing radiation exposure, iridocyclitis/uveitis/optic neuritis, ischemia- reperfusion injury, ischemic stroke, juvenile rheumatoid arthritis, juvenile spinal muscular atrophy, Kaposi's sarcoma, kidney transplant rejection, legionella, leishmaniasis, leprosy, lesions of the corticospinal system, lipedema, liver transplant rejection, lymphederma, malaria, malignamt Lymphoma, malignant histiocytosis, malignant melanoma, meningitis, meningococcemia, metabolic/idiopathic, migraine headache, mitochondrial multi-system disorder, mixed connective tissue disease, monoclonal gammopathy, multiple myeloma, multiple systems degenerations (Mencel Dejerine- Thomas Shy-Drager and Machado-Joseph), myasthenia gravis, mycobacterium avium intracellulare, mycobacterium tuberculosis, myelodyplastic syndrome, myocardial ischemic disorders, nasopharyngeal carcinoma, neonatal chronic lung disease, nephritis, nephrosis, neurodegenerative diseases, neurogenic I muscular atrophies , neutropenic fever, non- hodgkins lymphoma, occlusion of the abdominal aorta and its branches, occulsive arterial disorders, okt3 therapy, orchitis/epidydimitis, orchitis/vasectomy reversal procedures, organomegaly, osteoporosis, pancreas transplant rejection, pancreatic carcinoma, paraneoplastic syndrome/hypercalcemia of malignancy, parathyroid transplant rejection, pelvic inflammatory disease, perennial

rhinitis, pericardial disease, peripheral atherlosclerotic disease, peripheral vascular disorders, peritonitis, pernicious anemia, pneumocystis carinii pneumonia, pneumonia, POEMS syndrome (polyneuropathy, organomegaly, endocrinopathy, monoclonal gammopathy, and skin changes syndrome), post perfusion syndrome, post pump syndrome, post-MI cardiotomy syndrome, preeclampsia, Progressive supranucleo Palsy, primary pulmonary hypertension, radiation therapy, Raynaud's phenomenon and disease, Raynoud's disease, Refsum's disease, regular narrow QRS tachycardia, renovascular hypertension, reperfusion injury, restrictive cardiomyopathy, sarcomas, scleroderma, senile chorea, Senile Dementia of Lewy body type, seronegative arthropathies, shock, sickle cell anemia, skin allograft rejection, skin changes syndrome, small bowel transplant rejection, solid tumors, specific arrythmias, spinal ataxia, spinocerebellar degenerations, streptococcal myositis, structural lesions of the cerebellum, Subacute sclerosing panencephalitis, Syncope, syphilis of the cardiovascular system, systemic anaphalaxis, systemic inflammatory response syndrome, systemic onset juvenile rheumatoid arthritis, T-cell or FAB ALL, Telangiectasia, thromboangitis obliterans, thrombocytopenia, toxicity, transplants, trauma/hemorrhage, type III hypersensitivity reactions, type IV hypersensitivity, unstable angina, uremia, urosepsis, urticaria, valvular heart diseases, varicose veins, ,vasculitis, venous diseases, venous thrombosis, ventricular fibrillation, viral and fungal infections, vital encephalitis/aseptic meningitis, vital-associated hemaphagocytic syndrome, Wernicke- Korsakoff syndrome, Wilson's disease, xenograft rejection of any organ or tissue, acute coronary syndromes, acute idiopathic polyneuritis, acute inflammatory demyelinating polyradiculoneuropathy, acute ischemia, adult Still's disease, anaphylaxis, anti-phospholipid antibody syndrome, aplastic anemia, atopic eczema, atopic dermatitis, autoimmune dermatitis, autoimmune disorder associated with streptococcus infection, autoimmune enteropathy, autoimmune hearing loss, autoimmune lymphoproliferative syndrome (ALPS), autoimmune myocarditis, autoimmune premature ovarian failure, blepharitis, bronchiectasis, bullous pemphigoid, cardiovascular disease, catastrophic antiphospholipid syndrome, celiac disease, cervical spondylosis, chronic ischemia, cicatricial pemphigoid, clinically isolated syndrome (cis) with risk for multiple sclerosis, childhood onset psychiatric disorder, dacryocystitis, dermatomyositis, diabetic retinopathy, disk herniation, disk prolaps, drug induced immune hemolytic anemia, endometriosis, endophthalmitis, episcleritis, erythema multiforme, erythema multiforme major, gestational pemphigoid, Guillain-Barré syndrome (GBS), hay fever, Hughes syndrome, idiopathic Parkinson's disease, idiopathic interstitial pneumonia,

IgE-mediated allergy, immune hemolytic anemia, inclusion body myositis, infectious ocular inflammatory disease, inflammatory demyelinating disease, inflammatory heart disease, inflammatory kidney disease, IPF/UIP, iritis, keratitis, keratoconjunctivitis sicca, Kussmaul disease or Kussmaul-Meier disease, Landry's paralysis, Langerhan's cell histiocytosis, livedo reticularis, macular degeneration, microscopic polyangiitis, morbus bechterev, motor neuron disorders, mucous membrane pemphigoid, multiple organ failure, myelodysplastic syndrome, myocarditis, nerve root disorders, neuropathy, non-A non-B hepatitis, optic neuritis, osteolysis, ovarian cancer, pauciarticular JRA, peripheral artery occlusive disease (PAOD), peripheral vascular disease (PVD), peripheral artery, disease (PAD), phlebitis, polyarteritis nodosa (or periarteritis nodosa), polychondritis, polymyalgia rheumatica, poliosis, polyarticular JRA, polyendocrine deficiency syndrome, polymyositis, post-pump syndrome, primary Parkinsonism, prostate and rectal cancer and hematopoietic malignancies (leukemia and lymphoma), prostatitis, pure red cell aplasia, primary adrenal insufficiency, recurrent neuromyelitis optica, restenosis, rheumatic heart disease, sapho (synovitis, acne, pustulosis, hyperostosis, and osteitis), scleroderma, secondary amyloidosis, shock lung, scleritis, sciatica, secondary adrenal insufficiency, silicone associated connective tissue disease, sneddon-wilkinson dermatosis, spondilitis ankylosans, Stevens-Johnson syndrome (SJS), systemic inflammatory response syndrome, temporal arteritis, toxoplasmic retinitis, toxic epidermal necrolysis, transverse myelitis, TRAPS (tumor necrosis factor receptor, type 1 allergic reaction, type II diabetes, usual interstitial pneumonia (UIP), vernal conjunctivitis, viral retinitis, Vogt-Koyanagi-Harada syndrome (VKH syndrome), wet macular degeneration, or wound healing.

28. The method of claim 26 or 27, wherein the disorder is an autoimmune disorder, asthma, rheumatoid arthritis, osteoarthritis, systemic lupus erythematosus (SLE), multiple sclerosis, sepsis, a neurodegenerative disease, or an oncological disorder.

29. The method of any one of claims 26-28, wherein the binding protein is formulated for parenteral, subcutaneous, intramuscular, intravenous, intrarticular, intrabronchial, intraabdominal, intracapsular, intracartilaginous, intracavitary, intracelial, intracerebellar, intracerebroventricular, intracolic, intracervical, intragastric, intrahepatic, intramyocardial, intraosteal, intrapelvic, intrapericardiac, intraperitoneal, intrapleural, intraprostatic, intrapulmonary, intrarectal, intrarenal, intraretinal, intraspinal, intrasynovial, intrathoracic, intrauterine, intravesical, bolus, vaginal, rectal, buccal, sublingual, intranasal, or transdermal administration.

30. A method of determining the presence, amount, or concentration of at least one target or fragment thereof in a test sample by an immunoassay,

wherein the immunoassay comprises contacting the test sample with at least one binding protein and at least one detectable label, and

wherein the at least one binding protein comprises the binding protein of any one of claims 1-12.

31. The method of claim 30, further comprising:

(i) contacting the test sample with the at least one binding protein, wherein the binding protein binds to an epitope on the target or fragment thereof so as to form a first complex;

(ii) contacting the complex with the at least one detectable label, wherein the detectable label binds to the binding protein or an epitope on the target or fragment thereof that is not bound by the binding protein to form a second complex; and

(iii) detecting the presence, amount, or concentration of the target or fragment thereof in the test sample based on the signal generated by the detectable label in the second complex, wherein the presence, amount, or concentration of the target or fragment thereof is directly correlated with the signal generated by the detectable label.

32. The method of claim 30, further comprising:

(i) contacting the test sample with the at least one binding protein, wherein the binding protein binds to an epitope on the target or fragment thereof so as to form a first complex;

(ii) contacting the complex with the at least one detectable label, wherein the detectable label competes with the target or fragment thereof for binding to the binding protein so as to form a second complex; and

(iii) detecting the presence, amount, or concentration of the target or fragment thereof in the test sample based on the signal generated by the detectable label in the second complex, wherein the presence, amount, or concentration of the target or

fragment thereof is indirectly correlated with the signal generated by the detectable label.

33. A kit for assaying a test sample for the presence, amount, or concentration of a target or fragment thereof in the sample, said kit comprising (a) instructions for assaying the test sample for the target or fragment thereof and (b) at least one binding protein comprising the binding protein of any one of claims 1-12.

34. Use of the binding protein of any one of claims 1-12 in the manufacture of a medicament for treating a disease or disorder.