I. said first polypeptide chain comprises, in the N-terminal to C-terminal direction: (i) a first domain comprising a binding region of a light chain variable domain of a first immunoglobulin (VL1) specific for an epitope (1); (ii) a second domain comprising a binding region of a heavy chain variable domain of a second immunoglobulin (VH2) specific for an epitope (2); and (iii) a positively or negatively charged polypeptide domain that spontaneously assumes a helical conformation, wherein said negatively charged polypeptide domain is an E-coil separator (SEQ ID NO: 299) and said positively charged polypeptide domain is a K-coil separator (SEQ ID NO: 300);

which first domain and second domain are covalently linked such that the first domain and second domain do not associate to form said epitope (1) binding site or said epitope (2) binding site;

II. said second polypeptide chain comprises, in the N-terminal to C-terminal direction: (i) a fourth domain comprising a binding region of a light chain variable domain of the second immunoglobulin (VL2) specific for said epitope (2); (ii) a fifth domain comprising a binding region of a heavy chain variable domain of the first immunoglobulin (VH1) specific for said epitope (1); (iii) a positively or negatively charged polypeptide domain that spontaneously assumes a helical conformation linked to said fifth domain, wherein said negatively charged polypeptide domain is an E-coil separator (SEQ ID NO: 299) and said positively charged polypeptide domain is a K-coil separator (SEQ ID NO: 300), wherein when said first polypeptide chain comprises said E-coil separator, said second polypeptide chain comprises said K-coil separator, and when said first polypeptide chain comprises said K-coil separator, said second polypeptide chain comprises said E-coil separator;

which fourth domain and fifth domain are covalently linked such that the fourth domain and fifth domain do not associate to form said epitope (1) binding site or said epitope (2) binding site;

wherein the first domain and the fifth domain associate to form a binding site (VL1)(VH1) that binds said epitope (1);

wherein the second domain and the fourth domain associate to form a binding site (VL2)(VH2) that binds said epitope (2)

[0015] In one example, the present disclosure is directed to a covalent bispecific diabody, which diabody comprises a first and a second polypeptide chain, which first polypeptide chain comprises (i) a first domain comprising a binding region of a light chain variable domain of a first immunoglobulin (VLl) specific for a first epitope, (ii) a second domain comprising a binding region of a heavy chain variable domain of a second immunoglobulin (VH2) specific for a second epitope, and, optionally, (iii) a third domain

comprising at least one cysteine residue, which first and second domains are covalently linked such that the first and second domains do not associate to form an epitope binding site; which second polypeptide chain comprises (i) a fourth domain comprising a binding region of a light chain variable domain of the second immunoglobulin (VL2), (ii) a fifth domain comprising a binding region of a heavy chain variable domain of the first immunoglobulin (VHl), and, optionally, (iii) a sixth domain comprising at least one cysteine residue, which fourth and fifth domains are covalently linked such that the fourth and fifth domains do not associate to form an epitope binding site; and wherein the first polypeptide chain and the second polypeptide chain are covalently linked, with the proviso that the covalent link is not a peptide bond; wherein the first domain and the fifth domain associate to form a first binding site (VLl)(VHl) that binds the first epitope; wherein the second domain and the fourth domain associate to form a second binding site (VL2)(VH2) that binds the second epitope.

[0016] In another example, the present disclosure is directed to a covalent bispecific diabody, which diabody comprises a first and a second polypeptide chain, which first polypeptide chain comprises (i) a first domain comprising a binding region of a light chain variable domain of a first immunoglobulin (VLl) specific for a first epitope, (ii) a second domain comprising a binding region of a heavy chain variable domain of a second immunoglobulin (VH2) specific for a second epitope and (iii) a third domain comprising an Fc domain or portion thereof, which first and second domains are covalently linked such that the first and second domains do not associate to form an epitope binding site; which second polypeptide chain comprises (i) a fourth domain comprising a binding region of a light chain variable domain of the second immunoglobulin (VL2), (ii) a fifth domain comprising a binding region of a heavy chain variable domain of the first immunoglobulin (VHl), which fourth and fifth domains are covalently linked such that the third and fourth domains do not associate to form an epitope binding site; and wherein the first polypeptide chain and the second polypeptide chain are covalently linked, with the proviso that the covalent link is not a peptide bond; wherein the first domain and the fifth domain associate to form a first binding site (VLl)(VHl) that binds the first epitope; wherein the second domain and the fourth domain associate to form a second binding site (VL2)(VH2) that binds the second epitope.

[0017] In certain examples, the present disclosure is directed to diabody molecule, which molecule comprises a first and a second polypeptide chain, which first polypeptide

chain comprises (i) a first domain comprising a binding region of a light chain variable domain of a first immunoglobulin (VLl) specific for a first epitope, (ii) a second domain comprising a binding region of a heavy chain variable domain of a second immunoglobulin (VH2) specific for a second epitope and (iii) a third domain comprising an Fc domain or portion thereof, which first and second domains are covalently linked such that the first and second domains do not associate to form an epitope binding site; which second polypeptide chain comprises (i) a fourth domain comprising a binding region of a light chain variable domain of the second immunoglobulin (VL2), (ii) a fifth domain comprising a binding region of a heavy chain variable domain of the first immunoglobulin (VHl), and (iii) a sixth domain comprising at least one cysteine residue, which fourth and fifth domains are covalently linked such that the fourth and fifth domains do not associate to form an epitope binding site; and wherein the first polypeptide chain and the second polypeptide chain are covalently linked, with the proviso that the covalent link is not a peptide bond; wherein the first domain and the fifth domain associate to form a first binding site (VLl)(VHl) that binds the first epitope; wherein the second domain and the fourth domain associate to form a second binding site (VL2)(VH2) that binds the second epitope.

[0018] In certain examples, the present disclosure is directed to a covalent bispecific diabody, which diabody is a dimer of diabody molecules, each diabody molecule comprising a first and a second polypeptide chain, which first polypeptide chain comprises (i) a first domain comprising a binding region of a light chain variable domain of a first immunoglobulin (VLl) specific for a first epitope, (ii) a second domain comprising a binding region of a heavy chain variable domain of a second immunoglobulin (VH2) specific for a second epitope and (iii) a third domain comprising an Fc domain or portion thereof, which first and second domains are covalently linked such that the first and second domains do not associate to form an epitope binding site; and which second polypeptide chain comprises (i) a fourth domain comprising a binding region of a light chain variable domain of the second immunoglobulin (VL2), (ii) a fifth domain comprising a binding region of a heavy chain variable domain of the first immunoglobulin (VHl), and (iii) a sixth domain comprising at least one cysteine residue, which fourth and fifth domains are covalently linked such that the fourth and fifth domains do not associate to form an epitope binding site; and wherein the first polypeptide chain and the second polypeptide chain of each diabody molecule are covalently linked, with the

proviso that the covalent link is not a peptide bond; wherein the first domain and the fifth domain of each diabody molecule associate to form a first binding site (VLl)(VHl) that binds the first epitope; wherein the second domain and the fourth domain of each diabody molecule associate to form a second binding site (VL2)(VH2) that binds the second epitope.

[0019] In yet other examples, the present disclosure is directed to a covalent tetrapecific diabody, which diabody is a dimer of diabody molecules, the first diabody molecule comprising a first and a second polypeptide chain, which first polypeptide chain comprises (i) a first domain comprising a binding region of a light chain variable domain of a first immunoglobulin (VLl) specific for a first epitope, (ii) a second domain comprising a binding region of a heavy chain variable domain of a second immunoglobulin (VH2) specific for a second epitope and (iii) a third domain comprising an Fc domain or portion thereof, which first and second domains are covalently linked such that the first and second domains do not associate to form an epitope binding site; and which second polypeptide chain comprises (i) a fourth domain comprising a binding region of a light chain variable domain of the second immunoglobulin (VL2), (ii) a fifth domain comprising a binding region of a heavy chain variable domain of the first immunoglobulin (VHl), and (iii) a sixth domain comprising at least one cysteine residue, which fourth and fifth domains are covalently linked such that the fourth and fifth domains do not associate to form an epitope binding site; and wherein the first polypeptide chain and the second polypeptide chain are covalently linked, with the proviso that the covalent link is not a peptide bond; wherein the first domain and the fifth domain associate to form a first binding site (VLl)(VHl) that binds the first epitope; wherein the second domain and the fourth domain associate to form a second binding site (VL2)(VH2) that binds the second epitope; and the second diabody molecule comprising a first and a second polypeptide chain, which first polypeptide chain comprises (i) a first domain comprising a binding region of a light chain variable domain of a third immunoglobulin (VL3) specific for a third epitope, (ii) a second domain comprising a binding region of a heavy chain variable domain of a fourth immunoglobulin (VH4) specific for a fourth epitope and (iii) a third domain comprising an Fc domain or portion thereof, which first and second domains are covalently linked such that the first and second domains do not associate to form an epitope binding site; and which second polypeptide chain comprises (i) a fourth domain comprising a binding region of a light chain variable domain of the fourth

immunoglobulin (VL4), (ii) a fifth domain comprising a binding region of a heavy chain variable domain of the third immunoglobulin (VH3), and (iii) a sixth domain comprising at least one cysteine residue, which fourth and fifth domains are covalently linked such that the fourth and fifth domains do not associate to form an epitope binding site; and wherein the first polypeptide chain and the second polypeptide chain are covalently linked, with the proviso that the covalent link is not a peptide bond; wherein the first domain and the fifth domain associate to form a first binding site (VL3)(VH3) that binds the third epitope; wherein the second domain and the fourth domain associate to form a second binding site (VL4)(VH4) that binds the fourth epitope.

[0020] In certain examples of the disclosure the first epitope, second epitope, and where applicable, third epitope and fourth epitope can be the same. In other examples, the first epitope, second epitope, and where applicable, third epitope and fourth epitope can each different from the other. In certain examples of the disclosure comprising a third epitope binding domain, the first epitope and third epitope can be the same. In certain examples of the disclosure comprising a fourth epitope binding domain, the first epitope and fourth epitope can be the same. In certain examples of the disclosure comprising a third epitope binding domain, the second epitope and third epitope can be the same. In certain examples of the disclosure comprising a fourth epitope binding domain, the second epitope and fourth epitope can be the same. In preferred examples of the disclosure, the first eptitope and second epitope are different. In yet other examples of the disclosure comprising a third epitope binding domain and a fourth epitope binding domain, the third epitope and fourth epitope can be different. It is to be understood that any combination of the foregoing is encompassed in the present disclosure.

[0021] In particular examples of the disclosure, the first domain and the fifth domain of the diabody or diabody molecule can be derived from the same immunoglobulin. In another example, the second domain and the fourth domain of the diabody or diabody molecule can be derived from the same immunoglobulin. In yet another example, the first domain and the fifth domain of the diabody or diabody molecule can be derived from a different immunoglobulin. In yet another example, the second domain and the fourth domain of the diabody or diabody molecule can be derived from a different immunoglobulin. It is to be understood that any combination of the foregoing is encompassed in the present disclosure.

[0022] In certain examples of the disclosure, the covalent linkage between the first polypeptide chain and second polypeptide chain of the diabody or diabody molecule can be via a disulfide bond between at least one cysteine residue on the first polypeptide chain and at least one cysteine residue on the second polypeptide chain. The cysteine residues

on the first or second polypeptide chains that are responsible for disulfide bonding can be found anywhere on the polypeptide chain including within the first, second, third, fourth, fifth and sixth domains. In a specific example the cysteine residue on the first polypeptide chain is found in the first domain and the cysteine residue on the second polypeptide chain is found in the fifth domain. The first, second, fourth and fifth domains correspond to the variable regions responsible for binding. In preferred examples, the cysteine residues responsible for the disulfide bonding between the first and second polypeptide chains are located within the third and sixth domains, respectively. In a particular example, the third domain of the first polypeptide chain comprises the C-terminal 6 amino acids of the human kappa light chain, FNRGEC (SEQ ID NO: 23), which can be encoded by the amino acid sequence (SEQ ID NO: 17). In another example, the sixth domain of the second polypeptide chain comprises the C-terminal 6 amino acids of the human kappa light chain, FNRGEC (SEQ ID NO: 23), which can be encoded by the amino acid sequence (SEQ ID NO: 17). In still another example, the third domain of the first polypeptide chain comprises the amino acid sequence VEPKSC (SEQ ID NO: 77), derived from the hinge domain of a human IgG, and which can be encoded by the nucleotide sequence (SEQ ID NO: 78). In another example, the sixth domain of the second

polypeptide chain comprises the amino acid sequence VEPKSC (SEQ ID NO: 77),

derived from the hinge domain of a human IgG, and which can be encoded by the nucleotide sequence (SEQ ID NO: 78). In certain examples, the third domain of the first polypeptide chain comprises the C-terminal 6 amino acids of the human kappa light chain, FNRGEC (SEQ ID NO: 23); and the sixth domain of the second polypeptide chain comprises the amino acid sequence VEPKSC (SEQ ID NO: 77). In other examples, the sixth domain of the second polypeptide chain comprises the C-terminal 6 amino acids of the human kappa light chain, FNRGEC (SEQ ID NO: 23); and the third domain of the first polypeptide chain comprises the amino acid sequence VEPKSC (SEQ ID NO: 77). In yet other examples, the third domain of the first polypeptide chain comprises the C-terminal 6 amino acids of the

human kappa light chain, FNRGEC (SEQ ID NO: 23); and the sixth domain of the second polypeptide chain comprises a hinge domain. In other examples, the sixth domain of the second polypeptide chain comprises the C-terminal 6 amino acids of the human kappa light chain, FNRGEC (SEQ ID NO: 23); and the third domain of the

first polypeptide chain comprises the hinge domain. In yet other examples, the third domain of the first polypeptide chain comprises the C-terminal 6 amino acids of the human kappa light chain, FNRGEC (SEQ ID NO: 23); and the sixth domain of the first polypeptide chain comprises an Fc domain, or portion thereof. In still other examples, the sixth domain of the second polypeptide chain comprises the C-terminal 6 amino acids of the human kappa light chain, FNRGEC (SEQ ID NO: 23); and the third domain of the first polypeptide chain comprises an Fc domain, or portion thereof.

[0023] In other examples, the cysteine residues on the first or second polypeptide that are responsible for the disulfide bonding can be located outside of the first, second or third domains on the first polypeptide chain and outside of the fourth, fifth and sixth domain on the second polypeptide chain. In particular, the cysteine residue on the first polypeptide chain can be N-terminal to the first domain or can be C-terminal to the first domain. The cysteine residue on the first polypeptide chain can be N-terminal to the second domain or can be C-terminal to the second domain. The cysteine residue on

the first polypeptide chain can be N-terminal to the third domain or can be C-terminal to the third domain. Further, the cysteine residue on the second polypeptide chain can be N-terminal to the fourth domain or can be C-terminal to the fourth domain. The cysteine residue on the second polypeptide chain can be N-terminal to the fifth domain or can be C-terminal to the fifth domain. Accordingly, the cysteine residue on the second polypeptide chain can be C-terminal to the sixth domain or can be N-terminal to the sixth domain. In a particular example, disulfide bond can between at least two cysteine residues on the first polypeptide chain and at least two cysteine residues on the second polypeptide chain. In a particular example, wherein the third domain and sixth domain do not comprise an Fc domain, or portion thereof, the cysteine residue can be at the C-terminus of the first polypeptide chain and at the C-terminus of the second polypeptide chain. It is to be understood that any combination of the foregoing is encompassed in the present disclosure.

[0024] In specific examples of the disclosure described supra, the covalent diabody of the disclosure encompasses dimers of diabody molecules, wherein each diabody

molecule comprises a first and second polypeptide chain. In certain examples the diabody molecules can be covalently linked to form the dimer, with the proviso that the covalent linkage is not a peptide bond. In preferred examples, the covalent linkage is a disulfide bond between at least one cysteine residue

on the first polypeptide chain of each of the diabody molecules of the dimer. In yet more preferred examples of this disclosure, the covalent linkage is a disulfide bond between at least

one cysteine residue on the first polypeptide chain of each of the diabody molecules forming the dimer, wherein said at least one cysteine residue is located in the third domain of each first polypeptide chain.

[0025] In certain examples of the disclosure, the first domain on the first polypeptide chain can be N-terminal to the second domain or can be C-terminal to the second domain. The first domain on the first polypeptide chain can be N-terminal to the third domain or can be C-terminal to the third domain. The second domain on the first polypeptide chain can be N-terminal to the first domain or can be C-terminal to the first domain. Further, the second domain on the first polypeptide chain can be N-terminal to the third domain or can be C-terminal to the third domain. Accordingly, the third domain on the first polypeptide chain can be N-terminal to the first domain or can be C-terminal to the first domain. The third domain on the first polypeptide chain can be N-terminal to the second domain or can be C-terminal to the second domain. With respect to the second polypeptide chain, the fourth domain can be N-terminal to the fifth domain or can be C-terminal to the fifth domain. The fourth domain can be N-terminal to the sixth domain or can be C-terminal to the sixth domain. The fifth domain on the second polypeptide chain can be N-terminal to the fourth domain or can be C-terminal to the fourth domain. The fifth domain on the second polypeptide chain can be N-terminal to the sixth domain or can be C-terminal to the sixth domain. Accordingly the sixth domain on the second polypeptide chain can be

N-terminal to the fourth domain or can be C-terminal to the fourth domain. The sixth domain on the second polypeptide chain can be N-terminal to the fifth domain or can be C-terminal to the fifth domain. It is to be understood that any combination of the

foregoing is encompassed in the present disclosure.

[0026] In certain examples, first domain and second domain can be located C-terminal to the third domain on the first polypeptide chain; or the first domain and second domain can be located N-terminal to the third domain on the first polypeptide chain. With respect to the second polypeptide chain, the fourth domain and fifth domain can be located

C-terminal to the sixth domain, or the fourth domain and fifth domain can be located N-terminal to the sixth domain. In certain examples, the present disclosure

is directed to a covalent bispecific diabody, which diabody is a dimer of diabody molecules, each diabody molecule comprising a first and a second polypeptide chain, which first polypeptide chain comprises (i) a first domain comprising a binding region of a light chain variable domain of a first immunoglobulin (VLl) specific for a first epitope,

(ii) a second domain comprising a binding region of a heavy chain variable domain of a second immunoglobulin (VH2) specific for a second epitope and (iii) a third domain comprising an Fc domain or portion thereof, which first and second domains are covalently linked such that the first and second domains do not associate to form an epitope binding site and wherein the third domain is located N-terminal to both the first domain and second domain; and which second polypeptide chain comprises (i) a fourth domain comprising a binding region of a light chain variable domain of the second immunoglobulin (VL2), (ii) a fifth domain comprising a binding region of a heavy chain variable domain of the first immunoglobulin (VHl), and (iii) a sixth domain comprising at least one cysteine residue, which fourth and fifth domains are covalently linked such that the fourth and fifth domains do not associate to form an epitope binding site; and wherein the first polypeptide chain and the second polypeptide chain of each diabody molecule are covalently linked, with the proviso that the covalent link is not a peptide bond; wherein the first domain and the fifth domain of each diabody molecule associate to form a first binding site (VLl)(VHl) that binds the first epitope; wherein the second domain and the fourth domain of each diabody molecule associate to form a second binding site (VL2)(VH2) that binds the second epitope.

[0027] In yet another example, the present disclosure is directed to a covalent tetrapecific diabody, which diabody is a dimer of diabody molecules, the first diabody molecule comprising a first and a second polypeptide chain, which first polypeptide chain comprises (i) a first domain comprising a binding region of a light chain variable domain of a first immunoglobulin (VLl) specific for a first epitope, (ii) a second domain comprising a binding region of a heavy chain variable domain of a second immunoglobulin (VH2) specific for a second epitope and (iii) a third domain comprising an Fc domain or portion thereof, which first and second domains are covalently linked such that the first and second domains do not associate to form an epitope binding site and wherein the third domain is located N-terminal to both the first domain and second

domain; and which second polypeptide chain comprises (i) a fourth domain comprising a binding region of a light chain variable domain of the second immunoglobulin (VL2), (ii) a fifth domain comprising a binding region of a heavy chain variable domain of the first immunoglobulin (VHl), and (iii) a sixth domain comprising at least one cysteine residue, which fourth and fifth domains are covalently linked such that the fourth and fifth domains do not associate to form an epitope binding site; and wherein the first polypeptide chain and the second polypeptide chain are covalently linked, with the proviso that the covalent link is not a peptide bond; wherein the first domain and the fifth domain associate to form a first binding site (VLl)(VHl) that binds the first epitope; wherein the second domain and the fourth domain associate to form a second binding site (VL2)(VH2) that binds the second epitope; and the second diabody molecule comprises a first and a second polypeptide chain, which first polypeptide chain comprises (i) a first domain comprising a binding region of a light chain variable domain of a third immunoglobulin (VL3) specific for a third epitope, (ii) a second domain comprising a binding region of a heavy chain variable domain of a fourth immunoglobulin (VH4) specific for a fourth epitope and (iii) a third domain comprising an Fc domain or portion thereof, which first and second domains are covalently linked such that the first and second domains do not associate to form an epitope binding site and wherein the third domain is located N-terminal to both the first domain and second domain; and which second polypeptide chain comprises (i) a fourth domain comprising a binding region of a light chain variable domain of the fourth immunoglobulin (VL4), (ii) a fifth domain comprising a binding region of a heavy chain variable domain of the third immunoglobulin (VH3), and (iii) a sixth domain comprising at least one cysteine residue, which fourth and fifth domains are covalently linked such that the fourth and fifth domains do not associate to form an epitope binding site; and wherein the first polypeptide chain and the second polypeptide chain are covalently linked, with the proviso that the covalent link is not a peptide bond; wherein the first domain and the fifth domain associate to form a first binding site (VL3)(VH3) that binds the third epitope; wherein the second domain and the fourth domain associate to form a second binding site (VL4)(VH4) that binds the fourth epitope.

[0028] As discussed above, the domains on the individual polypeptide chains are covalently linked. In specific examples, the covalent link between the first and second domain, first and third domain, second and third domain, fourth and fifth domain, fourth and sixth domain, and/or fifth and sixth domain can be a peptide bond. In particular, the

first and second domains, and the fourth and fifth domains can be separated by the third domain and sixth domain, respectively, or by additional amino acid residues, so long as the first and second, and fourth and fifth domains do not associate to form a binding site. The number of amino acid residues can be 0, 1, 2, 3, 4, 5, 6, 7, 8 or 9 amino acid residues. In one preferred example, the number of amino acid residues between the domains is 8.

[0029] In certain examples of the disclosure, the domains of the first and second polypeptid chain comprising an Fc domain, i.e., optionally, the third and sixth domains, respectively, can further comprise a hinge domain such that the domain comprises a hinge- Fc region. In alternative examples, the first polypeptide chain or the second polypeptide chain can comprise a hinge domain without also comprising an Fc domain. The heavy chains, light chains, hinge regions, Fc domains, and/or hinge-Fc domains for use in the disclosure can be derived from any immunoglobulin type including IgA, IgD, IgE, IgG or IgM. In a preferred example, the immunoglobulin type is IgG, or any subtype thereof, i.e., IgG_1, IgG_2, IgG_3 or IgG_4. In other examples, the immunoglobulin from which the light and heavy chains are derived is humanized or chimerized.

[0030] Further, the first epitope and second epitopes, and, where applicable, third epitope and fourth epitope, to which the diabody or diabody molecule binds can be different epitopes from the same antigen or can be different epitopes from different antigens. The antigens can be any molecule to which an antibody can be generated. For example, proteins, nucleic acids, bacterial toxins, cell surface markers, autoimmune markers, viral proteins, drugs, etc. In particular examples, at least one epitope binding site of the diabody is specific for an antigen on a particular cell, such as a B-cell, a T-cell, a phagocytic cell, a natural killer (NK) cell or a dendritic cell.

[0031] In certain examples, at least one epitope binding site

of the diabody or diabody molecule is specific for a Fc receptor, which Fc receptor can be an activating Fc receptor or an inhibitory Fc receptor. In particular examples, the Fc receptor is a Fcγ receptor, and the Fcγ receptor is a FcγRI, FcγRII or FcγRIII receptor. In more preferred examples, the FcγRIII receptor is the FcγRIIIA (CD 16A) receptor or the FcγRIIIB (CD 16B) receptor, and, more preferably, the FcγRIII receptor is the FcγRIIIA (CD 16A) receptor. In another preferred example, the FcγRII receptor is the FcγRIIA (CD32A) receptor or the FcγRIIB (CD32B) receptor, and more preferably the FcγRIIB (CD32B) receptor. In a particularly preferred example, one binding site of the diabody is specific for CD32B and the other binding site is specific for CD16A. In a specific

example of the disclosure, at least one epitope binding site of the diabody or diabody molecule is specific for an activating Fc receptor and at least one other site is specific for an inhibitory Fc receptor. In certain examples the activating Fc receptor is CD32A and the inhibitory Fc receptor is CD32B. In other examples the activating Fc receptor is BCR and the inhibitory Fc receptor is CD32B. In still other examples, the activating Fc receptor is IgERI and the inhibitory Fc receptor is CD32B.

[0031a] In another aspect, there is provided a diabody molecule comprising a first polypeptide chain and a second polypeptide chain, wherein said first polypeptide chain and said second polypeptide chain are covalently linked to one another, with the proviso that the covalent link is not a peptide bond, wherein:

said first polypeptide chain comprises: (i) a first domain comprising a binding region of a light chain variable domain of a first immunoglobulin (VL1) specific for CD32B, (ii) a second domain comprising a binding region of a heavy chain variable domain of a second immunoglobulin (VH2) specific for CD79b, and (iii) a third domain comprising at least one cysteine residue, and an E-coil separator (SEQ ID NO:299) or a K-coil separator (SEQ ID NO:300), which first domain and second domain are covalently linked such that the first domain and second domain do not associate to form a first epitope binding site;

said second polypeptide chain comprises: (i) a fourth domain comprising a binding region of a light chain variable domain of the second immunoglobulin (VL2) specific for CD79b, (ii) a fifth domain comprising a binding region of a heavy chain variable domain of the first immunoglobulin (VH1) specific for CD32B, and (iii) a sixth domain comprising at least one cysteine residue, and an E-coil separator (SEQ ID NO:299) or a K-coil separator (SEQ ID NO:300), which fourth domain and fifth domain are covalently linked such that the fourth domain and fifth domain do not associate to form an epitope binding site;

wherein said cysteine residue of said third and sixth domains form a disulfide bond between said first and second polypeptide chains;

wherein when said first polypeptide chain comprises said E-coil separator, said second polypeptide chain comprises said K-coil separator; and when said first polypeptide chain comprises said K-coil separator, said second polypeptide chain comprises said E-coil separator;

wherein the first domain and the fifth domain associate to form a first binding site (VL1)(VH1) that binds CD32B;

wherein the second domain and the fourth domain associate to form a second binding site (VL2)(VH2) that binds CD79b.

[0031b] In another aspect, there is provided a diabody that is a dimer of two diabody molecules, wherein:

(A) the first of said two diabody molecules comprises a first polypeptide chain and a second polypeptide chain, wherein said first polypeptide chain and said second polypeptide chain of said first diabody molecule are covalently linked to one another, with the proviso that the covalent link is not a peptide bond, and wherein:

said first polypeptide chain of said first diabody molecule comprises: (i) a first domain comprising a binding region of a light chain variable domain of a first immunoglobulin (VL1) specific for CD32B, (ii) a second domain comprising a binding region of a heavy chain variable domain of a second immunoglobulin (VH2) specific for CD79b, and (iii) a third domain comprising at least one cysteine residue, an E-coil separator (SEQ ID NO:299) or a K-coil separator (SEQ ID NO:300), and a CH2 domain and a CH3 domain;

which first domain and second domain are covalently linked such that the first domain and second domain do not associate to form a first epitope binding site;
said second polypeptide chain of said first diabody molecule comprises: (i) a fourth domain comprising a binding region of a light chain variable domain of the second immunoglobulin (VL2) specific for CD79b, (ii) a fifth domain comprising a binding region of a heavy chain variable domain of the first immunoglobulin (VH1) specific for CD32B, and (iii) a sixth domain comprising at least one cysteine residue, and an E-coil separator (SEQ ID NO:299) or a K-coil separator (SEQ ID NO:300), which fourth domain and fifth domain are covalently linked such that the fourth domain and fifth domain do not associate to form an epitope binding site;
wherein when said first polypeptide chain of said first diabody molecule comprises said E-oil separator, said second polypeptide chain of said first diabody molecule comprises said K-coil separator; and when said first polypeptide chain of said first diabody molecule comprises said K-coil separator, said second polypeptide chain of said first diabody molecule comprises said E-coil separator;
wherein said cysteine residue of said third and sixth domains of said first diabody molecule form a disulfide bond between said first and second polypeptide chains of said first diabody molecule;
wherein the first domain and the fifth domain of said first diabody molecule associate to form a first binding site (VL1)(VH1) that binds CD32B;
wherein the second domain and the fourth domain of said first diabody molecule associate to form a second binding site (VL2)(VH2) that binds CD79b; and
(B) the second of said two diabody molecules comprises a first polypeptide chain and a second polypeptide chain, wherein said first polypeptide chain and said second polypeptide chain of said second diabody molecule are covalently linked to one another, with the proviso that the covalent link is not a peptide bond, and wherein:
said first polypeptide chain of said second diabody molecule comprises: (i) a first domain comprising a binding region of a light chain variable domain of a first immunoglobulin (VL1) specific for CD32B, (ii) a second domain comprising a binding region of a heavy chain variable domain of a second immunoglobulin (VH2) specific for CD79b, and (iii) a third domain comprising at least one cysteine residue, an E-coil separator (SEQ ID NO:299) or a K-coil separator (SEQ ID NO:300), and a CH2 domain and a CH3 domain; which first domain and second domain are covalently linked such that the first domain and second domain do not associate to form a first epitope binding site;
said second polypeptide chain of said second diabody molecule comprises (i) a fourth domain comprising a binding region of a light chain variable domain of the second immunoglobulin (VL2) specific for CD79b, (ii) a fifth domain comprising a binding region of a heavy chain variable domain of the first immunoglobulin (VH1) specific for CD32B, and (iii) a sixth domain comprising at least one cysteine residue, and an E-coil separator (SEQ ID NO:299) or a K-coil separator (SEQ ID NO:300), which fourth domain and fifth domain are covalently linked such that the fourth domain and fifth domain do not associate to form an epitope binding site;
wherein when said first polypeptide chain of said second diabody molecule comprises said E-coil separator, said second polypeptide chain of said second diabody molecule comprises said K-coil separator; and when said first polypeptide chain of said second diabody molecule comprises said K-coil separator, said second polypeptide chain of said second diabody molecule comprises said E-coil separator;
wherein said cysteine residue of said third and sixth domains of said second diabody molecule form a disulfide bond between said first and second polypeptide chains of said second diabody molecule;

wherein the first domain and the fifth domain of said second diabody molecule associate to form a first binding site (VL1)(VH1) that binds CD32B;
wherein the second domain and the fourth domain of said second diabody molecule associate to form a second binding site (VL2)(VH2) that binds CD79b;
wherein said CH2 domain and CH3 domain of said first polypeptide chain of said first diabody molecule associates with said CH2 domain and CH3 domain of said first polypeptide chain of said second diabody molecule to form an Fc region.

[0031c] In another aspect, there is provided a diabody that is a dimer of two diabody molecules, wherein:

(A) the first of said two diabody molecules comprises a first polypeptide chain and a second polypeptide chain, wherein said first polypeptide chain and said second polypeptide chain of said first diabody molecule are covalently linked to one another, with the proviso that the covalent link is not a peptide bond, and wherein:
said first polypeptide chain of said first diabody molecule comprises: (i) a first domain comprising a binding region of a light chain variable domain of a first immunoglobulin (VL1) specific for CD79b, (ii) a second domain comprising a binding region of a heavy chain variable domain of a second immunoglobulin (VH2) specific for CD32B, and (iii) a third domain comprising at least one cysteine residue, an E-coil separator (SEQ ID NO:299) or a K-coil separator (SEQ ID NO:300), and a CH2 domain and a CH3 domain; which first domain and second domain are covalently linked such that the first domain and second domain do not associate to form a first epitope binding site;
said second polypeptide chain of said first diabody molecule comprises: (i) a fourth domain comprising a binding region of a light chain variable domain of the second immunoglobulin (VL2) specific for CD32B, (ii) a fifth domain comprising a binding region of a heavy chain variable domain of the first immunoglobulin (VH1) specific for CD79b, and (iii) a sixth domain comprising at least one cysteine residue, and an E-coil separator (SEQ ID NO:299) or a K-coil separator (SEQ ID NO:300), which fourth domain and fifth domain are covalently linked such that the fourth domain and fifth domain do not associate to form an epitope binding site;
wherein when said first polypeptide chain of said first diabody molecule comprises said E-coil separator, said second polypeptide chain of said first diabody molecule comprises said K-coil separator; and when said first polypeptide chain of said first diabody molecule comprises said K-coil separator, said second polypeptide chain of said first diabody molecule comprises said E-coil separator;
wherein said cysteine residue of said third and sixth domains of said first diabody molecule form a disulfide bond between said first and second polypeptide chains of said first diabody molecule;
wherein the first domain and the fifth domain of said first diabody molecule associate to form a first binding site (VL1)(VH1) that binds CD79b;
wherein the second domain and the fourth domain of said first diabody molecule associate to form a second binding site (VL2)(VH2) that binds CD32B; and

(B) the second of said two diabody molecules comprises a first polypeptide chain and a second polypeptide chain, wherein said first polypeptide chain and said second polypeptide chain of said second diabody molecule are covalently linked to one another, with the proviso that the covalent link is not a peptide bond, and wherein:
said first polypeptide chain of said second diabody molecule comprises: (i) a first domain comprising a binding region of a light chain variable domain of a first immunoglobulin (VL1) specific for CD79b, (ii) a second domain comprising a binding region of a heavy chain variable domain of a second immunoglobulin (VH2) specific for

CD32B, and (iii) a third domain comprising at least one cysteine residue, an E-coil separator (SEQ ID NO:299) or a K-coil separator (SEQ ID NO:300), and a CH2 domain and a CH3 domain; which first domain and second domain are covalently linked such that the first domain and second domain do not associate to form a first epitope binding site;

said second polypeptide chain of said second diabody molecule comprises: (i) a fourth domain comprising a binding region of a light chain variable domain of the second immunoglobulin (VL2) specific for CD32B, (ii) a fifth domain comprising a binding region of a heavy chain variable domain of the first immunoglobulin (VH1) specific for CD79b, and (iii) a sixth domain comprising at least one cysteine residue, and an E-coil separator (SEQ ID NO:299) or a K-coil separator (SEQ ID NO:300), which fourth domain and fifth domain are covalently linked such that the fourth domain and fifth domain do not associate to form an epitope binding site;

wherein when said first polypeptide chain of said second diabody molecule comprises said E-coil separator, said second polypeptide chain of said second diabody molecule comprises said K-coil separator; and when said first polypeptide chain of said second diabody molecule comprises said K-coil separator, said second polypeptide chain of said second diabody molecule comprises said E-coil separator;

wherein said cysteine residue of said third and sixth domains of said second diabody molecule form a disulfide bond between said first and second polypeptide chains of said second diabody molecule;

wherein the first domain and the fifth domain of said second diabody molecule associate to form a first binding site (VL1)(VH1) that binds CD79b;

wherein the second domain and the fourth domain of said second diabody molecule associate to form a second binding site (VL2)(VH2) that binds CD32B;

wherein said CH2 domain and CH3 domain of said first polypeptide chain of said first diabody molecule associates with said CH2 domain and CH3 domain of said first polypeptide chain of said second diabody molecule to form an Fc region.

[0032] In cases where one epitope binding site is specific for CD16A, the VL and VH domains can be the same as or similar to the VL and VH domains of the mouse antibody 3G8, the sequence of which has been cloned and is set forth herein. In other cases where one epitope binding site is specific for CD32A, the VL and VH domains can be the same as or similar to the VL and VH domains of the mouse antibody IV.3. In yet other cases where one epitope binding site is specific for CD32B, the VL and VH domains can be the same as or similar to the VL and VH domains of the mouse antibody 2B6, the sequence of which has been cloned and is set forth herein. It is to be understood that any of the VL or VH domains of the 3G8, 2B6 and IV.3 antibodies can be used in any combination. The present disclosure is also directed to a bispecific diabody or diabody molecule wherein the first epitope is specific for CD32B, and the second epitope is specific for CD16A.

[0033] In other examples, an epitope binding site can be specific for a pathogenic antigen. As used herein, a pathogenic antigen is an antigen involved in a specific pathogenic disease, including cancer, infection and autoimmune disease. Thus, the pathogenic antigen can be a tumor antigen, a bacterial antigen, a viral antigen, or an autoimmune antigen. Exemplary pathogenic antigens include, but are not limited to lipopolysaccharide, viral

antigens selected from the group consisting of viral antigens from human immunodeficiency virus, Adenovirus, Respiratory Syncitial Virus, West Nile Virus (e.g., E16 and/or E53 antigens) and hepatitis virus, nucleic acids (DNA and RNA) and collagen. Preferably, the pathogenic antigen is a neutralizing antigen. In a preferred example, where one epitope binding site is specific for CD16A or CD32A, the other epitope binding site is specific for a pathogenic antigen excluding autoimmune antigens. In yet another preferred example, where one epitope binding site is specific for CD32B, the other epitope binding site is specific for any pathogenic antigen. In specific examples, the diabody molecule of the disclosure binds two different antigens on the same cell, for

example, one antigen binding site is specific for an activating Fc receptor while the other is specific for an inhibitory Fc receptor. In other examples, the diabody molecule binds two distinct viral neutralizing epitopes, for example, but not limited to, E16 and E53 of West Nile Virus.

[0034] In yet another example of the present disclosure, the diabodies of the disclosure can be used to treat a variety of diseases and disorders. In another aspect, there is provided a pharmaceutical composition comprising the diabody molecule as described herein and a pharmaceutically acceptable carrier. In another aspect, there is provided use of the diabody molecule described herein or the pharmaceutical composition described herein for the manufacture of a medicament for inhibiting B-cell proliferation. In another aspect, there is provided use of the diabody molecule described herein or the pharmaceutical composition described herein for the manufacture of a medicament for inhibiting B-cell activation. In another aspect, there is provided use of the diabody molecule described herein or the pharmaceutical composition described herein for the manufacture of a medicament for the treatment of an autoimmune disorder. Accordingly, the present disclosure is directed to a method for treating a disease or disorder comprising administering to a patient in need thereof an effective amount of a covalent diabody or diabody molecule of the disclosure in which at least one binding site is specific for a pathogenic antigen, such as an antigen expressed on the surface of a cancer cell or on the surface of a bacterium or virion and at least one other binding site is specific for a Fc receptor, e.g., CD16A.

[0035] In yet another example, the disclosure is directed to a method for treating a disease or disorder comprising administering to a patient in need thereof an effective amount of a diabody or diabody molecule of the disclosure, in which at least one binding site is specific for CD32B and at least one other binding site is specific for CD16A.

[0036] In yet another example, the disclosure is directed to a method for inducing immune tolerance to a pathogenic antigen comprising administering to a patient in need there an effective amount of a covalent diabody or dovalent diabody molecule of the disclosure, in which at least one binding site is specific for CD32B and at least one other binding site is specific for said pathogenic antigen. In this example, the pathogenic antigen can be an allergen or another molecule to which immune tolerance is desired, such as a protein expressed on transplanted tissue.

[0037] In yet another example, the present disclosure is directed to a method for detoxification comprising administering to a patient in need thereof an effective amount of a covalent diabody or diabody molecule of the disclosure, in which at least one binding site is specific for a cell surface marker and at least one other binding site is specific for a toxin. In particular examples, the diabody of the disclosure administered is one where one binding site is specific for a cell surface marker such as an Fc and the other binding site is specific for a bacterial toxin or for a drug. In one example, the cell surface marker is not found on red blood cells.

WHAT IS CLAIMED IS

Claim 1. A diabody molecule comprising a first polypeptide chain and a second polypeptide chain, said polypeptide chains being covalently bonded to one another, wherein:

I. said first polypeptide chain comprises, in the N-terminal to C-terminal direction: (i) a first domain comprising a binding region of a light chain variable domain of a first immunoglobulin (VL1) specific for an epitope (1); (ii) a second domain comprising a binding region of a heavy chain variable domain of a second immunoglobulin (VH2) specific for an epitope (2); and (iii) a positively or negatively charged polypeptide domain that spontaneously assumes a helical conformation, wherein said negatively charged polypeptide domain is an E-coil separator (SEQ ID NO: 299) and said positively charged polypeptide domain is a K-coil separator (SEQ ID NO: 300);

which first domain and second domain are covalently linked such that the first domain and second domain do not associate to form said epitope (1) binding site or said epitope (2) binding site;

II. said second polypeptide chain comprises, in the N-terminal to C-terminal direction: (i) a fourth domain comprising a binding region of a light chain variable domain of the second immunoglobulin (VL2) specific for said epitope (2); (ii) a fifth domain comprising a binding region of a heavy chain variable domain of the first immunoglobulin (VH1) specific for said epitope (1); (iii) a positively or negatively charged polypeptide domain that spontaneously assumes a helical conformation linked to said fifth domain, wherein said negatively charged polypeptide domain is an E-coil separator (SEQ ID NO: 299) and said positively charged polypeptide domain is a K-coil separator (SEQ ID NO: 300), wherein when said first polypeptide chain comprises said E-coil separator, said second polypeptide chain comprises said K-coil separator, and when said first polypeptide chain comprises said K-coil separator, said second polypeptide chain comprises said E-coil separator;

which fourth domain and fifth domain are covalently linked such that the fourth domain and fifth domain do not associate to form said epitope (1) binding site or said epitope (2) binding site;

wherein the first domain and the fifth domain associate to form a binding site (VL1)(VH1) that binds said epitope (1);

wherein the second domain and the fourth domain associate to form a binding site (VL2)(VH2) that binds said epitope (2).

Claim 2. The diabody molecule of claim 1, wherein said first polypeptide chain additionally comprises a third domain, said third domain being separated from said second domain by said charged polypeptide domain, wherein said third domain is an Fc domain, a heavy chain constant region 2 (CH2) and/or a heavy chain constant region 3 (CH3).

Claim 3. The diabody molecule of any one of claims 1-2, wherein said second polypeptide chain additionally comprises a sixth domain, said sixth domain being separated from said fifth domain by said charged polypeptide domain, wherein said sixth domain is an Fc domain, a heavy chain constant region 2 (CH2) and/or a heavy chain constant region 3 (CH3).

Claim 4. The diabody molecule of any one of claims 1-2, wherein said diabody additionally comprises a third polypeptide chain and a fourth polypeptide chain, said third and fourth polypeptide chains being covalently bonded to one another, wherein:

III. said third polypeptide chain comprises, in the N-terminal to C-terminal direction: (i) a seventh domain comprising a binding region of a light chain variable domain of a third immunoglobulin (VL3) specific for an epitope (3), (ii) an eighth domain comprising a binding region of a heavy chain variable domain of a fourth immunoglobulin (VH4) specific for an epitope (4), (iii) a positively or negatively charged polypeptide domain that spontaneously assumes a helical conformation, wherein said negatively charged polypeptide domain is an E-coil separator (SEQ ID

NO: 299) and said positively charged polypeptide domain is a K-coil separator (SEQ ID NO: 300), and (iv) a ninth domain, wherein said ninth domain is an Fc domain, a heavy chain constant region 2 (CH2) and/or a heavy chain constant region 3 (CH3) domain;

which seventh domain and eighth domain are covalently linked such that the seventh domain and eighth domain do not associate together to form said epitope (3) binding site or said epitope (4) binding site;

IV. said fourth polypeptide chain comprises, in the N-terminal to C-terminal direction: (i) a tenth domain comprising a binding region of a light chain variable domain of the fourth immunoglobulin (VL4) specific for said epitope (4), (ii) an eleventh domain comprising a binding region of a heavy chain variable domain of the third immunoglobulin (VH3) specific for said epitope (3), (iii) a positively or negatively charged polypeptide domain that spontaneously assumes a helical conformation linked to said eleventh domain, wherein said negatively charged polypeptide domain is an E-coil separator (SEQ ID NO: 299) and said positively charged polypeptide domain is a K-coil separator (SEQ ID NO: 300), wherein when said third polypeptide chain comprises said E-coil separator, said fourth polypeptide chain comprises said K-coil separator, and when said third polypeptide chain comprises said K-coil separator, said fourth polypeptide chain comprises said E-coil separator;

which tenth domain and eleventh domain are covalently linked such that the tenth domain and eleventh domain do not associate together to form said epitope (3) binding site or said epitope (4) binding site;

wherein the seventh domain and the eleventh domain associate together to form a binding site (VL3)(VH3) that binds said epitope (3);

wherein the eighth domain and the tenth domain associate together to form a binding site (VL4)(VH4) that binds said epitope (4).

Claim 5. The diabody molecule of claim 1, wherein said first polypeptide chain additionally comprises a polypeptide portion of a protein that binds to a serum protein, said polypeptide portion being capable of binding to said serum protein.

Claim 6. The diabody molecule of claim 5, wherein said second polypeptide chain additionally comprises a polypeptide portion of a protein that binds to a serum protein, said polypeptide portion being capable of binding to said serum protein.

Claim 7. The diabody molecule of claim 5 or 6, wherein said protein that binds to a serum protein is an albumin binding protein.

Claim 8. The diabody molecule of claim 7, wherein said albumin binding protein is streptococcal protein G and said polypeptide portion is an albumin-binding domain (ABD) of said streptococcal protein G.

Claim 9. The diabody molecule of claim 8, wherein said albumin-binding domain (ABD) of said streptococcal protein G is albumin-binding domain 3 (ABD3) of protein G of Streptococcus strain G148.

Claim 10. The diabody molecule of any one of claims 5-9, wherein said diabody molecule exhibits an in vivo serum half-life of greater than 2 hours.

Claim 11. The diabody molecule of claim 10, wherein said diabody molecule exhibits an in vivo serum half-life of greater than 10 hours.

Claim 12. The diabody molecule of claim 11, wherein said diabody exhibits an in vivo serum half-life greater than 20 hours.

Claim 13. The diabody molecule of any one of claims 1-12, wherein said diabody molecule binds an epitope of CD32B and an epitope of CD16.

Claim 14. The diabody molecule of any of claims 1-12, wherein said diabody molecule binds to the Natural Killer Group 2D (NKG2D) receptor.

Claim 15. The diabody molecule of any one of claims 1-12, wherein said diabody molecule binds to the T-cell receptor (TCR).

Claim 16. The diabody molecule of any one of claims 1-15, wherein said diabody molecule binds to a tumor-associated antigen.

Claim 17. The diabody molecule of claim 16, wherein said tumor-associated antigen is a breast cancer antigen, an ovarian cancer antigen, a prostate cancer antigen, a cervical cancer antigen, a pancreatic carcinoma antigen, a lung cancer antigen, a bladder cancer antigen, a colon cancer antigen, a testicular cancer antigen, a glioblastoma cancer antigen, an antigen associated with a B cell malignancy, an antigen associated with multiple myeloma, an antigen associated with non-Hodgkin's lymphoma, or an antigen associated with chronic lymphocytic leukemia.

Claim 18. The diabody molecule of claim 16, wherein said tumor-associated antigen is A33; ADAM-9; ALCAM; B1; BAGE; beta-catenin; CA125; Carboxypeptidase M; CD5; CD19; CD20; CD22; CD23; CD25; CD27; CD28; CD32B; CD36; CD40; CD45; CD46; CD56; CD79a; CD79b; CD103; CD154; CDK4; CEA; CTLA4; Cytokeratin 8; EGF-R; an Ephrin receptor; ErbB1; ErbB3; ErbB4; GAGE-1; GAGE-2; GD2; GD3; GM2; gp100; HER-2/neu; human papillomavirus-E6; human papillomavirus-E7; Integrin Alpha-V-Beta-6; JAM-3; KID3; KID31; KSA; LUCA-2; MAGE-1; MAGE-3; MART; MUC-1; MUM-1; N-acetylglucosaminyltransferase; Oncostatin M; p15; PIPA; PSA; PSMA; RAAG10; ROR1; SART; sTn; TES7; the TNF-α receptor; the TNF-ß receptor; the TNF-γ receptor; the Transferrin Receptor or the VEGF receptor.

Claim 19. The diabody molecule of claim 18, wherein said tumor-associated antigen is HER-2/neu.

Claim 20. A diabody molecule comprising a first polypeptide chain and a second polypeptide chain, wherein said first polypeptide chain and said second polypeptide chain are covalently linked to one another, with the proviso that the covalent link is not a peptide bond, wherein:

said first polypeptide chain comprises: (i) a first domain comprising a binding region of a light chain variable domain of a first immunoglobulin (VL1) specific for CD32B, (ii) a second domain comprising a binding region of a heavy chain variable domain of a second immunoglobulin (VH2) specific for CD79b, and (iii) a third domain

comprising at least one cysteine residue, and an E-coil separator (SEQ ID NO:299) or a K-coil separator (SEQ ID NO:300), which first domain and second domain are covalently linked such that the first domain and second domain do not associate to form a first epitope binding site;

said second polypeptide chain comprises: (i) a fourth domain comprising a binding region of a light chain variable domain of the second immunoglobulin (VL2) specific for CD79b, (ii) a fifth domain comprising a binding region of a heavy chain variable domain of the first immunoglobulin (VH1) specific for CD32B, and (iii) a sixth domain comprising at least one cysteine residue, and an E-coil separator (SEQ ID NO:299) or a K-coil separator (SEQ ID NO:300), which fourth domain and fifth domain are covalently linked such that the fourth domain and fifth domain do not associate to form an epitope binding site;

wherein said cysteine residue of said third and sixth domains form a disulfide bond between said first and second polypeptide chains;

wherein when said first polypeptide chain comprises said E-coil separator, said second polypeptide chain comprises said K-coil separator; and when said first polypeptide chain comprises said K-coil separator, said second polypeptide chain comprises said E-coil separator;

wherein the first domain and the fifth domain associate to form a first binding site (VL1)(VH1) that binds CD32B;

wherein the second domain and the fourth domain associate to form a second binding site (VL2)(VH2) that binds CD79b.

Claim 21. The diabody molecule of claim 20, wherein said first polypeptide chain or said second polypeptide chain further comprises an Fc domain, a heavy chain constant region 2 (CH2) and/or a heavy chain constant region 3 (CH3).

Claim 22. The diabody molecule of claim 21, wherein said Fc domain, said CH2 and/or said CH3 is linked to said E-coil separator or said K-coil separator.

Claim 23. The diabody molecule of claim 21, wherein said Fc domain, said CH2 and/or said CH3 is linked to the N-terminus of said first domain or said fourth domain.

Claim 24. A diabody that is a dimer of two diabody molecules, wherein:

(A) the first of said two diabody molecules comprises a first polypeptide chain and a second polypeptide chain, wherein said first polypeptide chain and said second polypeptide chain of said first diabody molecule are covalently linked to one another, with the proviso that the covalent link is not a peptide bond, and wherein:

said first polypeptide chain of said first diabody molecule comprises: (i) a first domain comprising a binding region of a light chain variable domain of a first immunoglobulin (VL1) specific for CD32B, (ii) a second domain comprising a binding region of a heavy chain variable domain of a second immunoglobulin (VH2) specific for CD79b, and (iii) a third domain comprising at least one cysteine residue, an E-coil separator (SEQ ID NO:299) or a K-coil separator (SEQ ID NO:300), and a CH2 domain and a CH3 domain; which first domain and second domain are covalently linked such that the first domain and second domain do not associate to form a first epitope binding site;

said second polypeptide chain of said first diabody molecule comprises: (i) a fourth domain comprising a binding region of a light chain variable domain of the second immunoglobulin (VL2) specific for CD79b, (ii) a fifth domain comprising a binding region of a heavy chain variable domain of the first immunoglobulin (VH1) specific for CD32B, and (iii) a sixth domain comprising at least one cysteine residue, and an E-coil separator (SEQ ID NO:299) or a K-coil separator (SEQ ID NO:300), which fourth domain and fifth domain are covalently linked such that the fourth domain and fifth domain do not associate to form an epitope binding site;

wherein when said first polypeptide chain of said first diabody molecule comprises said E-coil separator, said second polypeptide chain of said first diabody molecule comprises said K-coil separator; and when said first polypeptide chain of said first diabody molecule comprises said K-coil separator, said second polypeptide chain of said first diabody molecule comprises said E-coil separator;

wherein said cysteine residue of said third and sixth domains of said first diabody molecule form a disulfide bond between said first and second polypeptide chains of said first diabody molecule;

wherein the first domain and the fifth domain of said first diabody molecule associate to form a first binding site (VL1)(VH1) that binds CD32B;

wherein the second domain and the fourth domain of said first diabody molecule associate to form a second binding site (VL2)(VH2) that binds CD79b; and

(B) the second of said two diabody molecules comprises a first polypeptide chain and a second polypeptide chain, wherein said first polypeptide chain and said second polypeptide chain of said second diabody molecule are covalently linked to one another, with the proviso that the covalent link is not a peptide bond, and wherein:

said first polypeptide chain of said second diabody molecule comprises: (i) a first domain comprising a binding region of a light chain variable domain of a first immunoglobulin (VL1) specific for CD32B, (ii) a second domain comprising a binding region of a heavy chain variable domain of a second immunoglobulin (VH2) specific for CD79b, and (iii) a third domain comprising at least one cysteine residue, an E-coil separator (SEQ ID NO:299) or a K-coil separator (SEQ ID NO:300), and a CH2 domain and a CH3 domain; which first domain and second domain are covalently linked such that the first domain and second domain do not associate to form a first epitope binding site;

said second polypeptide chain of said second diabody molecule comprises (i) a fourth domain comprising a binding region of a light chain variable domain of the second immunoglobulin (VL2) specific for CD79b, (ii) a fifth domain comprising a binding region of a heavy chain variable domain of the first immunoglobulin (VH1) specific for CD32B, and (iii) a sixth domain comprising at least one cysteine residue, and an E-coil separator (SEQ ID NO:299) or a K-coil separator (SEQ ID NO:300), which fourth domain and fifth domain are covalently linked such that the fourth domain and fifth domain do not associate to form an epitope binding site;

wherein when said first polypeptide chain of said second diabody molecule comprises said E-coil separator, said second polypeptide chain of said second diabody molecule comprises said K-coil separator; and when said first polypeptide chain of said second diabody molecule comprises said K-coil separator, said second polypeptide chain of said second diabody molecule comprises said E-coil separator;

wherein said cysteine residue of said third and sixth domains of said second diabody molecule form a disulfide bond between said first and second polypeptide chains of said second diabody molecule;

wherein the first domain and the fifth domain of said second diabody molecule associate to form a first binding site (VL1)(VH1) that binds CD32B;

wherein the second domain and the fourth domain of said second diabody molecule associate to form a second binding site (VL2)(VH2) that binds CD79b;

wherein said CH2 domain and CH3 domain of said first polypeptide chain of said first diabody molecule associates with said CH2 domain and CH3 domain of said first polypeptide chain of said second diabody molecule to form an Fc region.

Claim 25. A diabody that is a dimer of two diabody molecules, wherein:

(A) the first of said two diabody molecules comprises a first polypeptide chain and a second polypeptide chain, wherein said first polypeptide chain and said second polypeptide chain of said first diabody molecule are covalently linked to one another, with the proviso that the covalent link is not a peptide bond, and wherein:

said first polypeptide chain of said first diabody molecule comprises: (i) a first domain comprising a binding region of a light chain variable domain of a first immunoglobulin (VL1) specific for CD79b, (ii) a second domain comprising a binding region of a heavy chain variable domain of a second immunoglobulin (VH2) specific for CD32B, and (iii) a third domain comprising at least one cysteine residue, an E-coil separator (SEQ ID NO:299) or a K-coil separator (SEQ ID NO:300), and a CH2 domain and a CH3 domain; which first domain and second domain are covalently linked such that the first domain and second domain do not associate to form a first epitope binding site;

said second polypeptide chain of said first diabody molecule comprises: (i) a fourth domain comprising a binding region of a light chain variable domain of the second immunoglobulin (VL2) specific for CD32B, (ii) a fifth domain comprising a binding region of a heavy chain variable domain of the first immunoglobulin (VH1) specific for CD79b, and (iii) a sixth domain comprising at least one cysteine residue, and an E-coil separator (SEQ ID NO:299) or a K-coil separator (SEQ ID NO:300), which fourth domain and fifth domain are covalently linked such that the fourth domain and fifth domain do not associate to form an epitope binding site;

wherein when said first polypeptide chain of said first diabody molecule comprises said E-coil separator, said second polypeptide chain of said first diabody molecule comprises said K-coil separator; and when said first polypeptide chain of said first diabody molecule comprises said K-coil separator, said second polypeptide chain of said first diabody molecule comprises said E-coil separator;

wherein said cysteine residue of said third and sixth domains of said first diabody molecule form a disulfide bond between said first and second polypeptide chains of said first diabody molecule;

wherein the first domain and the fifth domain of said first diabody molecule associate to form a first binding site (VL1)(VH1) that binds CD79b;

wherein the second domain and the fourth domain of said first diabody molecule associate to form a second binding site (VL2)(VH2) that binds CD32B; and

(B) the second of said two diabody molecules comprises a first polypeptide chain and a second polypeptide chain, wherein said first polypeptide chain and said second polypeptide chain of said second diabody molecule are covalently linked to one another, with the proviso that the covalent link is not a peptide bond, and wherein:

said first polypeptide chain of said second diabody molecule comprises: (i) a first domain comprising a binding region of a light chain variable domain of a first immunoglobulin (VL1) specific for CD79b, (ii) a second domain comprising a binding region of a heavy chain variable domain of a second immunoglobulin

(VH2) specific for CD32B, and (iii) a third domain comprising at least one cysteine residue, an E-coil separator (SEQ ID NO:299) or a K-coil separator (SEQ ID NO:300), and a CH2 domain and a CH3 domain; which first domain and second domain are covalently linked such that the first domain and second domain do not associate to form a first epitope binding site;

said second polypeptide chain of said second diabody molecule comprises: (i) a fourth domain comprising a binding region of a light chain variable domain of the second immunoglobulin (VL2) specific for CD32B, (ii) a fifth domain comprising a binding region of a heavy chain variable domain of the first immunoglobulin (VH1) specific for CD79b, and (iii) a sixth domain comprising at least one cysteine residue, and an E-coil separator (SEQ ID NO:299) or a K-coil separator (SEQ ID NO:300), which fourth domain and fifth domain are covalently linked such that the fourth domain and fifth domain do not associate to form an epitope binding site;

wherein when said first polypeptide chain of said second diabody molecule comprises said E-coil separator, said second polypeptide chain of said second diabody molecule comprises said K-coil separator; and when said first polypeptide chain of said second diabody molecule comprises said K-coil separator, said second polypeptide chain of said second diabody molecule comprises said E-coil separator;

wherein said cysteine residue of said third and sixth domains of said second diabody molecule form a disulfide bond between said first and second polypeptide chains of said second diabody molecule;

wherein the first domain and the fifth domain of said second diabody molecule associate to form a first binding site (VL1)(VH1) that binds CD79b;

wherein the second domain and the fourth domain of said second diabody molecule associate to form a second binding site (VL2)(VH2) that binds CD32B;

wherein said CH2 domain and CH3 domain of said first polypeptide chain of said first diabody molecule associates with said CH2 domain and CH3 domain of said first polypeptide chain of said second diabody molecule to form an Fc region.

Claim 26. The diabody molecule of claim 20, wherein both said E-coil separator and said K-coil separator are linked to an Fc domain, a heavy chain constant region 2 (CH2) and/or a heavy chain constant region 3 (CH3).

Claim 27. The diabody molecule of claim 20, wherein said E-coil separator or said K-coil separator is linked to a polypeptide portion of a serum-binding protein, said polypeptide portion being capable of binding to said serum-binding protein.

Claim 28. The diabody molecule of claim 27, wherein said serum-binding protein is an albumin binding protein.

Claim 29. The diabody molecule of claim 28, wherein said albumin binding protein is streptococcal protein G and said polypeptide portion is an albumin-binding domain (ABD) of said streptococcal protein G.

Claim 30. The diabody molecule of claim 29, wherein said albumin-binding domain (ABD) of said streptococcal protein G is albumin-binding domain 3 (ABD3) of protein G of Streptococcus strain G148.

Claim 31. The diabody molecule of claim 27, wherein said diabody molecule exhibits an in vivo serum half-life greater than 2 hours.

Claim 32. The diabody molecule of claim 31, wherein said diabody molecule exhibits an in vivo serum half-life greater than 10 hours.

Claim 33. The diabody molecule of claim 32, wherein said diabody molecule exhibits an in vivo serum half-life greater than 20 hours.

Claim 34. The diabody molecule of any one of claims 20-24 and 26-33, wherein:

(A) said first polypeptide chain comprises the light chain variable domain, the intervening GGGSGGGG linker (SEQ ID NO:10) and the heavy chain variable domain of SEQ ID NO:252; and

(B) said second polypeptide chain comprises:

(i) the light chain variable domain, the intervening GGGSGGGG linker (SEQ ID NO:10) and the heavy chain variable domain of SEQ ID NO:254; or

(ii) the light chain variable domain, the intervening GGGSGGGG linker (SEQ ID NO:10) and the heavy chain variable domain of SEQ ID NO:256.

Claim 35. The diabody molecule of claim 34, wherein said second polypeptide chain comprises said light chain variable domain, said intervening GGGSGGGG linker (SEQ ID NO:10) and said heavy chain variable domain of SEQ ID NO:254.

Claim 36. The diabody molecule of claim 34, wherein said second polypeptide chain comprises said light chain variable domain, said intervening GGGSGGGG linker (SEQ ID NO:10) and said heavy chain variable domain of SEQ ID NO:256.

Claim 37. The diabody molecule of claim 25, wherein:

(A) said first polypeptide chain comprises:

(i) the light chain variable domain, the intervening GGGSGGGG linker (SEQ ID NO:10) and the heavy chain variable domain of SEQ ID NO:254; or

(ii) the light chain variable domain, the intervening GGGSGGGG linker (SEQ ID NO:10) and the heavy chain variable domain of SEQ ID NO:256; and

(B) said second polypeptide chain comprises the light chain variable domain, the intervening GGGSGGGG linker (SEQ ID NO:10) and the heavy chain variable domain of SEQ ID NO:252.

Claim 38. The diabody molecule of claim 37, wherein said first polypeptide chain comprises said light chain variable domain, said intervening GGGSGGGG linker (SEQ ID NO:10) and said heavy chain variable domain of SEQ ID NO:254.

Claim 39. The diabody molecule of claim 37, wherein said first polypeptide chain comprises said light chain variable domain, said intervening GGGSGGGG linker (SEQ ID NO:10) and said heavy chain variable domain of SEQ ID NO:256.

Claim 40. A pharmaceutical composition comprising the diabody molecule of any one of claims 1-39 and a pharmaceutically acceptable carrier.

Claim 41. Use of the diabody molecule of any one of claims 20-39 or the pharmaceutical composition of claim 40 for the manufacture of a medicament for inhibiting B-cell proliferation.

Claim 42. Use of the diabody molecule of any one of claims 20-39 or the pharmaceutical composition of claim 40 for the manufacture of a medicament for inhibiting B-cell activation.

Claim 43. Use of the diabody molecule of any one of claims 20-39 or the pharmaceutical composition of claim 40 for the manufacture of a medicament for the treatment of an autoimmune disorder.