inhibits cellular responsiveness. B cell activation, B cell proliferation and antibody secretion is thus aborted.

TABLE 1.	Receptors for the Fc Regions of Immunoglobulin Isotypes		
Receptor	**Binding**	**Cell Type**	**Effect of Ligation**
FcγRI (CD64)	IgG1 10^8 M^{-1}	Macrophages Neutrophils Eosinophils Dendritic cells	Uptake Stimulation Activation of respiratory burst Induction of killing
FcγRII-A (CD32)	IgG1 2×10^6 M^{-1}	Macrophages Neutrophils Eosinophils Dendritic cells Platelets Langerhan cells	Uptake Granule Release
FcγRII-B2 (CD32)	*IgG1* 2×10^6 M^{-1}	Macrophages Neutrophils Eosinophils	Uptake Inhibition of Stimulation
FcγRII-B1 (CD32)	IgG1 2×10^6 M^{-1}	B cells Mast cells	No Uptake Inhibition of Stimulation
FcγRIII (CD16)	IgG1 5×10^5 M^{-1}	NK cells Eosinophil Macrophages Neutrophils Mast Cells	Induction of Killing
FcεRI	IgE 10^{10} M^{-1}	Mast cells Eosinophil Basophils	Secretion of granules
FcαRI (CD89)	IgA1, IgA2 10^7 M^{-1}	Macrophages Neutrophils Eosinophils	Uptake Induction of Killing

3. SUMMARY ~~OF THE INVENTION~~

[0014] The present ~~invention~~disclosure relates to covalent diabodies and/or covalent diabody molecules and to their use in the treatment of a variety of diseases and disorders including cancer, autoimmune disorders, allergy disorders and infectious diseases caused by bacteria, fungi or viruses. Preferably, the diabody of the present ~~invention~~disclosure can bind to two different epitopes on two different cells wherein the first epitope is expressed on a different cell type than the second epitope, such that the diabody can bring the two cells together.

[0015] In one embodiment, the present ~~invention~~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 (VL1) 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 (VH1), 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 (VL1)(VH1) 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 embodiment, the present ~~invention~~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 (VL1) 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 (VH1), 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 (VL1)(VH1) 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.

[0016a] In one aspect, there is provided a diabody molecule comprising a first polypeptide chain and a second polypeptide chain, wherein said chains comprise a heterodimer-promoting domain and are covalently bonded to one another;

which 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 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 domain and second domain are covalently linked such that the first domain and second domain 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 (VH1), and (iii) a sixth domain comprising an Fc domain, 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 the first domain and the fifth domain associate to form a first binding site (VL1)(VH1) 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

wherein said heterodimer-promoting domain links:

(1) said second and said third domains; or

(2) *said fifth and said sixth domains;*

and is selected from the group consisting of SEQ ID NOs: 278, 280, 281, 283, 284, 286, 287, 288, 289, 290, 291, 293, 295 and 297.

[0016b] In another aspect, there is provided a diabody molecule comprising a first polypeptide chain and a second polypeptide chain, wherein said chains comprise a heterodimer-promoting domain and are covalently bonded to one another;

which 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 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 a hinge domain and an Fc

domain or portion thereof, which first domain and second domain are covalently linked such that the first domain and second domain 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 (VH1), and (iii) a sixth domain comprising SEQ ID NO:23 (FNRGEC) 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 the first domain and the fifth domain associate to form a first binding site (VL1)(VH1) 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

wherein said heterodimer-promoting domain links:

(1) said second and said third domains; or

(2) said fifth and said sixth domains;

and is selected from the group consisting of SEQ ID NOs: 278, 280, 281, 283, 284, 286, 287, 288, 289, 290, 291, 293, 295 and 297.

[0016c] In another aspect, there is provided a diabody molecule comprising a first polypeptide chain and a second polypeptide chain, wherein said chains comprise a heterodimer-promoting domain and are covalently bonded to one another;

which 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 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 a hinge domain, which first domain and second domain are covalently linked such that the first domain and second domain 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 (VH1), and (iii) a sixth domain comprising SEQ ID NO:23 (FNRGEC), 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 the first domain and the fifth domain associate to form a first binding site (VL1)(VH1) 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

wherein said heterodimer-promoting domain links:

(1) said second and said third domains; or

(2) said fifth and said sixth domains;

and is selected from the group consisting of SEQ ID NOs: 278, 280, 281, 283, 284, 286, 287, 288, 289, 290, 291, 293, 295 and 297.

[0016d] In another aspect, there is provided a diabody molecule comprising a first polypeptide chain and a second polypeptide chain, wherein said first polypeptide chain comprises a heterodimer-promoting domain and said first polypeptide chain and said second polypeptide chain are covalently bonded to one another;

which first polypeptide chain comprises: (i) a third domain comprising an Fc domain or portion thereof, (ii) said heterodimer-promoting domain, (iii) a first domain comprising a binding region of a light chain variable domain of a first immunoglobulin (VL1) specific for a first epitope, and (iv) a second domain comprising a binding region of a heavy chain variable domain of a second immunoglobulin (VH2) specific for a second epitope, which first domain and second domain are covalently linked such that the first domain and second domain 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), and (ii) a fifth domain comprising a binding region of a heavy chain variable domain of the first immunoglobulin (VH1), which fourth domain and fifth domain are covalently linked such that the fourth and fifth domains do not associate to form an epitope binding site;

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

wherein the third domain is linked to said heterodimer-promoting domain and said third domain and said heterodimer-promoting domain are N-terminal to both the first domain and the second domain; and

wherein said heterodimer-promoting domain links said second and said third domains to one another and is selected from the group consisting of SEQ ID NOs: 278, 280, 281, 283, 284, 286, 287, 288, 289, 290, 291, 293, 295 and 297.

[0016e] In another aspect, there is provided a diabody molecule comprising a first polypeptide chain and a second polypeptide chain, wherein said chains comprise a heterodimer-promoting domain and are covalently bonded to one another, wherein:

(A) said first polypeptide chain comprises:
  (i) a domain (A) comprising a binding region of a light chain variable domain of a first immunoglobulin (VL1) specific for an epitope (1);
  (ii) a domain (B) comprising a binding region of a heavy chain variable domain of a second immunoglobulin (VH2) specific for an epitope (2); and
  (iii) a domain (C) comprising a light chain constant region (CL) domain or portion thereof;

(B) said second polypeptide chain comprises:
  (i) a domain (D) comprising a binding region of a light chain variable domain of the second immunoglobulin (VL2) specific for said epitope (2);
  (ii) a domain (E) comprising a binding region of a heavy chain variable domain of the first immunoglobulin (VH1) specific for said epitope (1);
  (iii) a domain (F) comprising a heavy chain constant region 1 (CH1) domain or portion thereof, and
  (iv) a hinge region a heavy chain constant region 2 (CH2) and a heavy chain constant region 3 (CH3);

wherein:
said domains (A) and (B) do not associate with one another to form an epitope binding site;
said domains (D) and (E) do not associate with one another to form an epitope binding site;
said domains (A) and (E) associate to form a binding site that binds said epitope (1); said domains (B) and (D) associate to form a binding site that binds said epitope (2);
said domains (C) and (F) are associated together via a disulfide bond to form a CH1 domain or a portion thereof;
and wherein said heterodimer-promoting domain links:
(1) said domains (B) and (C); or
(2) said domains (E) and (F);
to one another and is selected from the group consisting of SEQ ID NOs.:278, 280, 281, 283, 284, 286, 287, 288, 289, 290, 291, 293, 295 and 297.

[0017] In certain ~~aspects~~embodiments, the present ~~invention~~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 (VL1) 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 (VH1), 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 (VL1)(VH1) 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 embodiments, the present ~~invention~~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 (VL1) 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 (VH1), 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 (VL1)(VH1) 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 embodiments, the present ~~invention~~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 (VL1) 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 (VH1), 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 (VL1)(VH1) 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 ~~aspects~~embodiments of the ~~invention~~disclosure the first epitope, second epitope, and where applicable, third epitope and fourth epitope can be the same. In other ~~aspects~~embodiments, the first epitope, second epitope, and where applicable, third epitope and fourth epitope can each different from the other. In certain ~~aspects~~embodiments of the ~~invention~~disclosure comprising a third epitope binding domain, the first epitope and third epitope can be the same. In certain ~~aspects~~embodiments of the ~~invention~~disclosure comprising a fourth epitope binding domain, the first epitope and fourth epitope can be the same. In certain ~~aspects~~embodiments of the ~~invention~~disclosure comprising a third epitope binding domain, the second epitope and third epitope can be the same. In certain ~~aspects~~embodiments of the ~~invention~~disclosure comprising a fourth epitope binding domain, the second epitope and fourth epitope can be the same. In preferred ~~aspects~~embodiments of the ~~invention~~disclosure, the first eptitope and second epitope are different. In yet other ~~aspects~~embodiments of the ~~invention~~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 ~~invention~~disclosure.

[0021] In particular ~~aspects~~embodiments of the ~~invention~~disclosure, the first domain and the fifth domain of the diabody or diabody molecule can be derived from the same immunoglobulin. In another ~~aspect~~embodiment, the second domain and the fourth domain of the diabody or diabody molecule can be derived from the same immunoglobulin. In yet another ~~aspect~~embodiment, the first domain and the fifth domain of the diabody or diabody molecule can be derived from a different immunoglobulin. In yet another ~~aspect~~embodiment, 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 ~~invention~~disclosure.

[0022] In certain ~~aspects~~embodiments of the ~~invention~~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 embodiment 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 embodiments, 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 ~~aspect of this~~ embodiment, 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 ~~aspect of this~~ embodiment, 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 ~~aspect of this~~ embodiment, 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 ~~aspect of this~~ embodiment, 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 ~~aspects of this~~ embodiments, 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 ~~aspects of this~~ embodiments, 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 ~~aspects of this~~ embodiments, 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 ~~aspects of this~~ embodiments, 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 ~~aspects of this~~ embodiments, 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 ~~aspects of this~~ embodiments, 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 embodiments, 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 ~~aspect~~embodiment, 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 ~~aspect~~embodiment, 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 ~~invention~~disclosure.

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

molecule comprises a first and second polypeptide chain. In certain ~~aspects of this~~ embodiments 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 ~~aspects of this~~ embodiments, 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 ~~aspects~~embodiments of this ~~invention~~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 ~~aspects~~embodiments of the ~~invention~~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 ~~invention~~disclosure.

[0026] In certain embodiments, 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 ~~aspects of this~~ embodiments, the present ~~invention~~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 (VL1) 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 (VH1), 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 (VL1)(VH1) 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 embodiment, the present ~~invention~~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 (VL1) 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 (VH1), 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 (VL1)(VH1) 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 ~~aspects~~embodiments, 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 ~~aspect~~embodiment, the number of amino acid residues between the domains is 8.

[0029] In certain ~~aspects~~embodiments of the ~~invention~~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 embodiments, 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 ~~invention~~disclosure can be derived from any immunoglobulin type including IgA, IgD, IgE, IgG or IgM. In a preferred ~~aspect~~embodiment, the immunoglobulin type is IgG, or any subtype thereof, *i.e.*, IgG_1, IgG_2, IgG_3 or IgG_4. In other ~~aspects~~embodiments, 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 ~~aspects~~embodiments, 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 ~~aspects of the present~~ embodiments, 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 ~~aspects~~embodiments, the Fc receptor is a Fcγ receptor, and the Fcγ receptor is a FcγRI, FcγRII or FcγRIII receptor. In more preferred ~~aspects~~embodiments, 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 ~~aspect~~embodiment, the FcγRII receptor is the FcγRIIA (CD32A) receptor or the FcγRIIB (CD32B) receptor, and more preferably the FcγRIIB (CD32B) receptor. In a particularly preferred ~~aspect~~embodiment, one binding site of the diabody is specific for CD32B and the other binding site is specific for CD16A. In a specific

embodiment of the ~~invention~~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 ~~aspects of this~~ embodiments the activating Fc receptor is CD32A and the inhibitory Fc receptor is CD32B. In other ~~aspects of this~~ embodiments the activating Fc receptor is BCR and the inhibitory Fc receptor is CD32B. In still other ~~aspects of this~~ embodiments, the activating Fc receptor is IgERI and the inhibitory Fc receptor is CD32B.

[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 ~~invention~~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 ~~aspects~~embodiments, 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 ~~aspect~~embodiment, 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 ~~aspect~~embodiment, where one epitope binding site is specific for CD32B, the other epitope binding site is specific for any pathogenic antigen. In specific embodiments, the diabody molecule of the ~~invention~~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 embodiments, 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 embodiment of the present ~~invention~~disclosure, the diabodies of the ~~invention~~disclosure can be used to treat a variety of diseases and disorders. Accordingly, the present ~~invention~~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 ~~invention~~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 embodiment, the ~~invention~~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 ~~invention~~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 embodiment, the ~~invention~~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 ~~invention~~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 ~~aspects of~~ this embodiment, 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 embodiment, the present ~~invention~~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 ~~invention~~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 ~~aspects~~embodiments, the diabody of the ~~invention~~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 ~~aspect~~embodiment, the cell surface marker is not found on red blood cells.