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DIRECCIÓN DE NUEVAS CREACIONES
PRESENTACIÓN DE OPOSICIÓN

1. Oposición a solicitud de:

- ☒ Patente de invención
☐ Registro de Diseño Industrial
☐ Patente de Modelo de Utilidad
☐ Esquema de Trazado de Circuitos Integrados

Número de expediente: 12-081-792

Solicitante: ABBOTT LABORATORIES.

Apoderado: ALICIA LLOREDA RICAURTE.

Título de la Nueva Creación: INMUNOGLOBULINAS CON DOMINIO VARIABLE DUAL Y USOS DE LAS MISMAS

Gaceta: 655 de 31 de octubre de 2012, No. de publicación 1686

2. Datos del Opositor

☐ Persona Natural

☒ Persona Jurídica

TECNOQUIMICAS S.A.

Nombre o denominación /Razón social completa

Documento de identificación: C.C. ☐ C.E. ☐ NIT ☒ Otro ☐ Cuál _____

Número 890 300 466-5

Dirección Calle 23 No. 7-39 Ciudad Cali

Teléfono (092) 882.55.55 Fax: (092) 883.88.59 E-mail: _____



Autorizo expresamente a la Superintendencia de Industria y Comercio para que todas las comunicaciones sean enviadas a través de la dirección de correo electrónico

3. Datos del representante o apoderado:

REYES VILLAMIZAR JOSE LUIS

Apellidos y Nombre

79'152.473

Documento de identificación

44655

T.P. No.

Dirección del representante

Cra 17 No. 88-23 Of.205

Dirección electrónica

info@reyes-abogados.com

No. Fax

621.25.42

Número telefónico

621.26.31

En caso de haber presentado poder general para asuntos que se adelanten ante la Delegatura de Propiedad Industrial, sírvase indicar el número de su radicación

2

6. Fundamentos de la oposición

Causal: Incumplimiento de requisitos de patentabilidad (Título II, Capítulo I, Decisión 486 de 2000)

Justificación: VER ANEXO

Prórroga:

SI

☒

NO

☐

7. Anexos

☒

Comprobante de pago de la tasa para la presentación de la solicitud No. _____

Fecha

30-01-13

☒

Poder, si fuera el caso con el que se acredita la representación.

☒

Pruebas de los fundamentos de la oposición

☒

Copias para traslado

8. Firma

JOSÉ LUIS REYES VILLAMIZAR

Firma

SI

Nombre y apellidos

79.152.473

C.C.

44655

T.P.

Señor

SUPERINTENDENTE DE INDUSTRIA Y COMERCIO

E. S. D.

REF: EXPEDIENTE 12-081-792

TÍTULO SOLICITADO: "INMUNOGLOBULINAS CON DOMINIO VARIABLE DUAL Y USOS DE LAS MISMAS"

PUBLICACIÓN: Gaceta 655 de 31 de octubre de 2012, N° de publicación 1686.

PRIORIDAD: US 61/255.686 28/10/2009

SOLICITANTE: ABBOTT LABORATORIES.

APODERADO: ALICIA LLOREDA RICAURTE

TRÁMITE: PRESENTACION DE OPOSICIÓN

JOSÉ LUIS REYES VILLAMIZAR, mayor de edad, identificado como aparece al pie de mi firma, abogado en ejercicio, titular de la tarjeta profesional número 44655 del Consejo Superior de la Judicatura, obrando en calidad de apoderado de la sociedad **TECNOQUÍMICAS S.A.**, en ejercicio de lo dispuesto por el artículo 42 de la Decisión 486 de 2000, muy respetuosamente me permito formular la siguiente

I. PETICIÓN

Sírvase negar el beneficio patentario a la solicitud contenida en el expediente **12-081-792**, por no cumplir con lo dispuesto en la Decisión 486 de 2000, con base en los fundamentos que se expondrán a continuación.

II. LEGÍTIMO INTERÉS

Asiste a **TECNOQUÍMICAS S.A.**, legítimo interés para presentar esta oposición, pues la eventual concesión de la solicitud restringiría injustificadamente el empleo de ciertas sustancias, derivados y procesos que a nuestro juicio carecen de nivel inventivo, en detrimento de sus legítimos intereses comerciales. De igual manera mis clientes están interesados, por su misma condición de industrias farmacéuticas, en evitar la concesión de privilegios de patente que incumplan los requisitos materiales de patentabilidad establecidos en las normas vigentes, y que supongan un riesgo para la libre competencia.

III. FUNDAMENTOS DE LA OPOSICIÓN

La solicitud colombiana en trámite 12-079-711, referida a proteínas de unión multivalentes y multiespecíficas, procedimientos para prepararlas, incumple los requisitos materiales para el otorgamiento de patente de invención, a la luz de los argumentos y pruebas que se plantearán a continuación:

La reivindicación principal, en este caso, obedece a las siguientes reclamaciones:

*1. Una proteína de unión, CARACTERIZADA PORQUE comprende una cadena de polipéptidos, en donde dicha cadena de polipéptidos comprende VD1-(X1)_n-VD2-C-(X2)_n, en donde;
VD1 es un primer dominio variable de la cadena pesada;
VD2 es un segundo dominio variable de la cadena pesada;
C es un dominio constante de la cadena pesada;
X1 es un conector con la condición que no sea CH1;
X2 es una región Fc; y
n es 0 o 1;
en donde la proteína de unión tiene la capacidad de unirse a un par de antígenos seleccionados del grupo que consiste de TNF (seq.2) y NGF; TNF (seq. 3) y NGF; TNF (seq. 4) y NGF; TNF (seq. 5) y NGF; TNF (seq. 6) y NGF; TNF (seq. 2) y SOST; TNF (seq. 3) y SOST; TNF (seq. 4) y SOST; TNF (seq. 5) y SOST; TNF (seq. 6) y SOST; TNF (seq. 2) y PGE2; TNF (seq. 3) y PGE2; TNF (seq. 4) y PGE2; TNF (seq. 5) y PGE2; TNF (seq. 6) y PGE2; TNF (seq. 1) y LPA; TNF (seq. 2) y LPA; TNF (seq. 3) y LPA; TNF (SEQ: 4) y LPA; TNF (seq. 5) y LPA; y TNF (seq. 6) y LPA.*

En primer término, debe anotarse que la solicitud carece de precisión y claridad, en tanto los anticuerpos en ella incorporados no han sido definidos según las técnicas aceptadas internacionalmente para esta clase de solicitudes, esto es, por

su secuencia o por el número del certificado de depósito, sino mediante una definición de fórmula general conocida de anticuerpos duales que describen dominios variables de cadena pesada, ligados a un dominio constante de cadena pesada, un conector y una región Fc, definición ésta que resulta a todas luces insuficiente para delimitar el alcance de la materia pretendida, y dificulta en forma injustificada el debido entendimiento de la misma.

No existe, en efecto, caracterización técnica de los anticuerpos referidos, pues los mismos se han definido, o bien en función de un “resultado esperado”, o bien de las moléculas a las que dichos anticuerpos han de unirse, pero no de acuerdo con sus propias secuencias o características. Aceptar esta redacción permitiría que cualquier molécula de unión que cumpla las condiciones de orden descriptivo antes mencionadas, se entienda amparada dentro de la reivindicación, y llevaría a amparar un número indeterminado de compuestos. Como ya se ha dicho, se considera que la simple sustitución de sitios de unión, no definidos por sus características, sino por sus antígenos o proteínas de unión, no constituye una identificación aceptable de la proteína reclamada.

Nótese cómo, en efecto, la estructura en cuestión puede ser tan amplia que entre los anticuerpos reclamados se incluyen compuestos de secuencias presentes en los anticuerpos *Adalimumab* e *Infliximab*, entre otros, todos conocidos ampliamente en el estado de la técnica, e incluso con patentes que se encuentran próximas a expirar.

Pero aún si se definieran los anticuerpos correctamente y se presentaran debidamente sus secuencias, se estima que la solicitud presenta faltas graves en materia de unidad de invención, dadas por la existencia de proteínas de funciones diferentes y de estructuras en secuencias de aminoácidos igualmente distintas.

Es por ello, que al existir diferentes parejas de moléculas a las cuales se unen las proteínas de unión reclamadas, cada una de ellas debería emplearse para definir un grupo inventivo en función de su propia secuencia.

Por otra parte, la solicitud en estudio incluye materia exceptuada de patentabilidad como claramente se observa en las reivindicaciones 59 - 61:

59. Un método para tratar a un sujeto para una enfermedad o un trastorno CARACTERIZADO PORQUE comprende la administración al sujeto de la proteína de unión de una cualquiera de las reivindicaciones 1-36 y 55 de modo tal que se logra dicho tratamiento.

El método de tratamiento referido en dichas reclamaciones viola flagrantemente lo dispuesto por la D. 486 y dada la notoria falta de claridad y novedad, tal como se verá a continuación, no puede ser objeto de patente.

Es necesario mencionar además que si bien algunas modalidades particulares y opcionales de las reivindicaciones señalan secuencias que eventualmente cumplirían con la resolución del problema técnico, las demás reivindicaciones no dependen de dichas características, por lo que esto únicamente se reclama en el capítulo reivindicatorio como un ejemplo aislado dentro del amplio espectro de posibles proteínas duales reclamadas de forma imprecisa.

En adición a lo expuesto, y aun cuando se estima que la solicitud no cumple siquiera con los requisitos formales para ser valorado de fondo, es necesario observar que existen documentos del estado de la técnica que ya han reclamado la misma materia bajo la fórmula empleada:

- En primer término, la publicación americana US 2009/0215992 “*Dual variable domain immunoglobulin and uses thereof*” publicado por Wu et al., del 27 de agosto de 2009.

Este documento describe una proteína de unión multiespecíficas y multivalentes de la misma forma que la solicitud en estudio la reclama, específicamente describe los métodos para realizarlas, y sus usos en prevención y/o tratamiento de enfermedades inflamatorias crónicas y agudas (ver abstract) y en el párrafo 13 de la descripción de la anterioridad:

[0013] In one embodiment the invention provides a binding protein comprising a polypeptide chain, wherein said polypeptide chain comprises $VD1-(X1)_n-VD2-C-(X2)_n$, wherein VD1 is a first variable domain, VD2 is a second variable domain, C is a constant domain, X1 represents an amino acid or polypeptide, X2 represents an Fc region and n is 0 or 1. In a preferred embodiment the VD1 and VD2 in the binding protein are heavy chain variable domains. More preferably the heavy chain variable domain is selected from the group consisting of a murine heavy chain variable domain, a human heavy chain variable domain, a CDR grafted heavy chain variable domain, and a humanized heavy chain variable domain. In a preferred embodiment VD1 and VD2 are capable of binding the same antigen. In another embodiment VD1 and VD2 are capable of binding different antigens. Preferably C is a heavy chain constant domain. More preferably X1 is a linker with the proviso that X1 is not CH1. Most preferably X1 is a linker selected from the group consisting of AKTTPKLEEGEFSEAR; AKTTPKLEEGEFSEARV; AKTTPKLGG; SAKTTPKLGG; AKTTPKLEEGEFSEARV; SAKTTP; SAKTTPKLGG; RADAAP; RADAAPTVS; RADAAAAGGPGS; RADAAA(G₄S)₄; SAKTTP; SAKTTPKLGG; SAKTTPKLEEGEFSEARV; ADAAP; ADAAPTVSIFPP; TVAAP; TVAAPSVFIFPP; QPKAAP; QPKAAPSVTLFPP; AKTTPP; AKTTPPSVTPLAP; AKTTAP; AKTTAPSVYPLAP; ASTKGP; ASTKGPSVFPLAP, GGGGSGGGGSGGGGS; GENKVEYAPALMALS; GPAKELTPLKEAKVS; and GHEAAAVMQVQYPAS. Preferably X2 is an Fc region. More preferably X2 is a variant Fc region.

Como se observa, la anterioridad hace la divulgación expresa mencionando que la proteína comprende una cadena de polipéptido (párrafo 0017) donde dicha cadena de polipéptidos comprende $VD1-(X1)_n-VD2-C-(X2)_n$, donde VD1 es un dominio variable de cadena pesada, VD2 es un segundo dominio variable de cadena pesada, C es un dominio pesado de cadena constante, X1 es un enlazante con la condición que no es CH1 y X2 es una región fc (página 3, párrafo 0017) y n es 0 o 1.

De acuerdo a lo anterior, es claro observar que la fórmula reclamada en la solicitud en trámite ya era conocida y había sido reclamada por otra patente anterior, lo que implica que la creación ahora reivindicada deba negarse de plano por carecer de Novedad.

En adición a lo expuesto, la solicitud se refiere a moléculas de unión que se ligan a diferentes receptores y antígenos, los cuales, se encuentran descritos en la anterioridad ya que el mismo se refiere específicamente a moléculas que se unen tanto a TNF como NGF (ver párrafo 0041, página 8). Por otra parte, de una selección dada de parejas como la que se observa en las reclamaciones de la presente solicitud, no es posible identificar un efecto técnico que supere lo descrito

por la anterioridad dado que no se observa un efecto sorprendente de los anticuerpos duales reclamados sobre la simple selección arbitraria de parejas de antígenos.

Aun así, y aunque no se defina que el par al que se une la proteína erróneamente definida, consiste en la pareja TNF y NGF, (párrafo 0013, 0249), dichas moléculas blanco si son expresamente mencionadas como alternativas para la obtención del diseño de las moléculas de anticuerpo duales por lo que la selección no puede ser una característica decisiva, en la medida en que no existe ningún efecto sorprendente de esta pareja (y no otra) sobre los anticuerpos de unión duales del estado de la técnica:

[0041] In another embodiment the binding proteins of the invention are capable of binding one or more targets selected from the group consisting of: ...

NGFB (NGF); NGFR; NgR-Lingo; NgR-Nogo66 (Nogo); NgR-p75; NgR-Troy; NME1 (NM23A); NOX5; NPPB; NR0B1; NR0B2; NR1D1; NR1D2; NR1H2; NR1H3; NR1H4; NR1I2; NR1I3; NR2C1; NR2C2; NR2E1; NR2E3; NR2F1; NR2F2; NR2F6; NR3C1; NR3C2; NR4A1; NR4A2; NR4A3; NR5A1; NR5A2; NR6A1; NRP1; NRP2; NT5E; NTN4; ODZ1; OPRD1; P2RX7; PAP; PART1; PATE; PAWR; PCA3; PCNA; PDGFA; PDGFB; PECAM1; PF4 (CXCL4); PGF; PGR; phosphacan; PIAS2; PIK3CG; PLAU (uPA); PLG; PLXDC1; PPBP (CXCL7); PPID; PR1; PRKCQ; PRKD1; PRL; PROC; PROK2; PSAP; PSCA; PTAFR; PTEN; PTGS2 (COX-2); PTN; RAC2 (p21Rac2); RARB; RGS1; RGS13; RGS3; RNF110 (ZNF144); ROBO2; S100A2; SCGB1D2 (lipophilin B); SCGB2A1 (mammaglobin 2); SCGB2A2 (mammaglobin 1); SCYE1 (endothelial Monocyte-activating cytokine); SDF2; SERPINA1; SERPINA3; SERPINB5 (maspin); SERPINE1 (PAI-1); SERPINF1; SHBG; SLA2; SLC2A2; SLC33A1; SLC43A1; SLIT2; SPP1; SPRR1B (Spr1); ST6GAL1; STAB 1; STAT6; STEAP; STEAP2; TB4R2; TBX21; TCP10; TDGF1; TEK; TGFA; TGFB1; TGFB1I1; TGFB2; TGFB3; TGFB1; TGFB1R1; TGFB1R2; TGFB1R3; TH1L; THBS1 (thrombospondin-1); THBS2; THBS4; THPO; TIE (Tie-1); TIMP3; tissue factor; TLR10; TLR2; TLR3; TLR4; TLR5; TLR6; TLR7; TLR8; TLR9; TNF; TNF-a; TNFAIP2 (B94);
(...)

- Publicación americana US 2007/0071675 "*Dual variable domain immunoglobulin and uses thereof*" publicado por Wu et al., el 29 de marzo de 2007.

Este documento enseña una proteína de unión que comprende una cadena de polipéptido que comprende dominios de cadena pesada (párrafo 0012), donde el polipéptido comprende dominios de unión que se ligan de igual forma de que la solicitud tanto TNF como NGF (párrafo 0040), con lo cual era obvio para una persona versada en la materia caracterizar secuencias que se uniera a TNF y NGF e incluirlas en la fórmula ya conocida de anticuerpos duales enseñada por US 2009/0215992.

De esta forma, la obtención de anticuerpos que incluyeran los dominios de unión tanto a TNF y NGF que corresponden a la primera pareja de anticuerpos definidos por las reivindicaciones habían sido descritos en su totalidad bajo las respectivas formulas, y moléculas de unión.

Por lo tanto, con los documentos citados en el presente escrito, un técnico medio en la materia habría fácilmente derivado la materia de la solicitud en trámite bajo una selección de las características técnicas sugeridas ya que dicha anterioridad presentan los conocimientos y datos necesarios para que el experto medio en la materia llegara a el objeto reclamado.

IV. FUNDAMENTOS DE DERECHO

Son fundamentos de derecho el Capítulo I, (Requisitos de Patentabilidad) y el artículo 42 (Oposiciones) de la Decisión 486 de 2000.

V. PRUEBAS

Ruego que se tenga como tal, sin perjuicio de las que puedan presentarse al vencimiento de la prórroga solicitada, y de aquellas que el Despacho considere oficiosamente, las siguientes:

- ❖ La publicación internacional WO 2009/0215992 "*Dual variable domain immunoglobulin and uses thereof*" publicado por Wu et al., del 28 de febrero de 2008. Portada, página 1,2, 8 y reivindicaciones.
- ❖ La publicación internacional WO 2007/071675 del 29 de marzo de 2007. Portada, páginas 2, 8 y reivindicaciones.

VI. ANEXOS

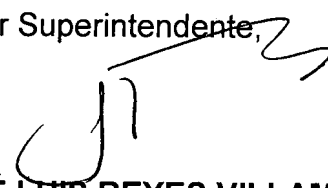
Para efectos del trámite correspondiente, me permito anexar al presente escrito los siguientes anexos:

1. Certificado de existencia y representación legal de Tecnoquímicas S.A.
2. Poder para actuar.
3. Constancia de pago de las tasas de tramitación de oposición.
4. Constancia de pago para sustentar la solicitud de prórroga a que se refiere el artículo 42, inciso 2º de la Decisión 486.
5. Copia para correr traslado al solicitante.
6. Las mencionadas en el acápite de pruebas

VII. NOTIFICACIONES

El suscrito recibirá notificaciones en la secretaría de su Despacho o en mi oficina localizada en la Carrera 17 No. 88-23, Oficina 205, Fax (1) 621.25.42 de esta ciudad. Tecnoquímicas S.A. las recibirá en sus oficinas localizadas en la Calle 23 N° 7-39 de Cali.

Señor Superintendente,


JOSÉ LUIS REYES VILLAMIZAR

C.C. 79.152.473 de Usaquén
T.P.A. 44.655 de C.S.J.



US 20090215992A1

(19) **United States**

(12) **Patent Application Publication**
Wu et al.

(10) **Pub. No.: US 2009/0215992 A1**

(43) **Pub. Date: Aug. 27, 2009**

(54) **DUAL VARIABLE DOMAIN
IMMUNOGLOBULIN AND USES THEREOF**

Related U.S. Application Data

(63) Continuation-in-part of application No. 11/507,050,
filed on Aug. 18, 2006.

(60) Provisional application No. 60/709,911, filed on Aug.
19, 2005, provisional application No. 60/732,892,
filed on Nov. 2, 2005.

Publication Classification

(51) **Int. Cl.**
C07K 16/00 (2006.01)
C07K 17/00 (2006.01)

(52) **U.S. Cl. 530/387.3; 530/387.1; 530/391.1**

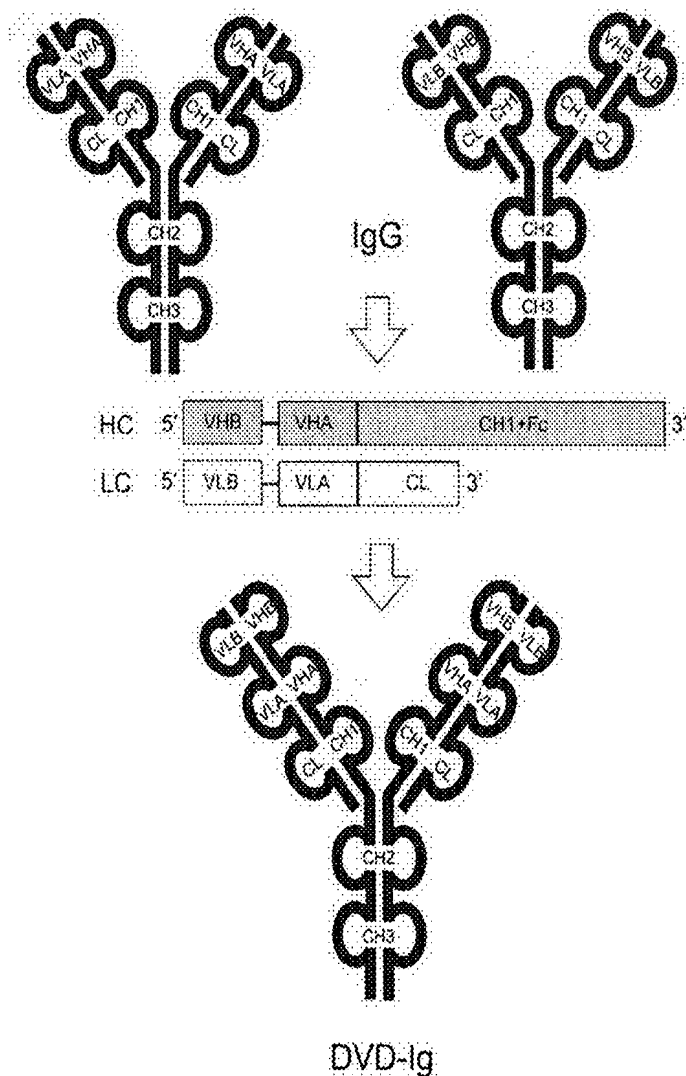
(57) **ABSTRACT**

The present invention relates to engineered multivalent and multispecific binding proteins, methods of making, and specifically to their uses in the prevention and/or treatment of acute and chronic inflammatory and other diseases.

Correspondence Address:
YANKWICH & ASSOCIATES, P.C.
(AND ABBOTT BIORESEARCH CENTER)
201 BROADWAY
CAMBRIDGE, MA 02139 (US)

(21) **Appl. No.: 11/890,215**

(22) **Filed: Aug. 3, 2007**



DUAL VARIABLE DOMAIN IMMUNOGLOBULIN AND USES THEREOF

CROSS-REFERENCE TO RELATED APPLICATION

[0001] This application is a continuation in part of U.S. patent application Ser. No. 11/507,050 filed Aug. 18, 2006, which claims the benefit of priority to U.S. Provisional Application Ser. No. 60/709,911 filed Aug. 19, 2005, and to U.S. Provisional Application No. 60/732,892 filed Nov. 2, 2005.

FIELD OF THE INVENTION

[0002] The present invention relates to multivalent and multispecific binding proteins, methods of making, and specifically to their uses in the prevention and/or treatment of acute and chronic inflammatory diseases, cancer, and other diseases.

BACKGROUND OF THE INVENTION

[0003] Engineered proteins, such as multispecific antibodies capable of binding two or more antigens are known in the art. Such multispecific binding proteins can be generated using cell fusion, chemical conjugation, or recombinant DNA techniques.

[0004] Bispecific antibodies have been produced using the quadroma technology (see Milstein, C. and A. C. Cuello, *Nature*, 1983. 305 (5934): p. 537-40) based on the somatic fusion of two different hybridoma cell lines expressing murine monoclonal antibodies with the desired specificities of the bispecific antibody. Because of the random pairing of two different Ig heavy and light chains within the resulting hybrid-hybridoma (or quadroma) cell line, up to ten different immunoglobulin species are generated of which only one is the functional bispecific antibody. The presence of mispaired by-products, and significantly reduced production yields, means sophisticated purification procedures are required.

[0005] Bispecific antibodies can be produced by chemical conjugation of two different mAbs (see Staerz, U. D., et al., *Nature*, 1985. 314 (6012): p. 628-31). This approach does not yield homogeneous preparation. Other approaches have used chemical conjugation of two different monoclonal antibodies or smaller antibody fragments (see Brennan, M., et al., *Science*, 1985. 229 (4708): p. 81-3).

[0006] Another method is the coupling of two parental antibodies with a hetero-bifunctional crosslinker, but the resulting preparations of bispecific antibodies suffer from significant molecular heterogeneity because reaction of the crosslinker with the parental antibodies is not site-directed. To obtain more homogeneous preparations of bispecific antibodies two different Fab fragments have been chemically crosslinked at their hinge cysteine residues in a site-directed manner (see Glennie, M. J., et al., *J Immunol*, 1987. 139 (7): p. 2367-75). But this method results in Fab'2 fragments, not full IgG molecule.

[0007] A wide variety of other recombinant bispecific antibody formats have been developed in the recent past (see Kriangkum, J., et al., *Biomol Eng*, 2001. 18 (2): p. 3140). Amongst them tandem single-chain Fv molecules and diabodies, and various derivatives thereof, are the most widely used formats for the construction of recombinant bispecific antibodies. Routinely, construction of these molecules starts from two single-chain Fv (scFv) fragments that recognize different antigens (see Economides, A. N., et al., *Nat Med*,

2003. 9 (1): p. 47-52). Tandem scFv molecules (taFv) represent a straightforward format simply connecting the two scFv molecules with an additional peptide linker. The two scFv fragments present in these tandem scFv molecules form separate folding entities. Various linkers can be used to connect the two scFv fragments and linkers with a length of up to 63 residues (see Nakanishi, K., et al., *Annu Rev Immunol*, 2001. 19: p. 423-74). Although the parental scFv fragments can normally be expressed in soluble form in bacteria, it is, however, often observed that tandem scFv molecules form insoluble aggregates in bacteria. Hence, refolding protocols or the use of mammalian expression systems are routinely applied to produce soluble tandem scFv molecules. In a recent study, in vivo expression by transgenic rabbits and cattle of a tandem scFv directed against CD28 and a melanoma-associated proteoglycan was reported (see Gracie, J. A., et al., *J Clin Invest*, 1999. 104 (10): p. 1393-401). In this construct, the two scFv molecules were connected by a CH1 linker and serum concentrations of up to 100 mg/L of the bispecific antibody were found. Various strategies including variations of the domain order or using middle linkers with varying length or flexibility were employed to allow soluble expression in bacteria. A few studies have now reported expression of soluble tandem scFv molecules in bacteria (see Leung, B. P., et al., *J Immunol*, 2000. 164 (12): p. 6495-502; Ito, A., et al., *J Immunol*, 2003. 170 (9): p. 4802-9; Kami, A., et al., *J Neuroimmunol*, 2002. 125 (1-2): p. 134-40) using either a very short Ala3 linker or long glycine/serine-rich linkers. In a recent study, phage display of a tandem scFv repertoire containing randomized middle linkers with a length of 3 or 6 residues was employed to enrich for those molecules that are produced in soluble and active form in bacteria. This approach resulted in the isolation of a preferred tandem scFv molecule with a 6 amino acid residue linker (see Arndt, M. and J. Krauss, *Methods Mol Biol*, 2003. 207: p. 305-21). It is unclear whether this linker sequence represents a general solution to the soluble expression of tandem scFv molecules. Nevertheless, this study demonstrated that phage display of tandem scFv molecules in combination with directed mutagenesis is a powerful tool to enrich for these molecules, which can be expressed in bacteria in an active form.

[0008] Bispecific diabodies (Db) utilize the diabody format for expression. Diabodies are produced from scFv fragments by reducing the length of the linker connecting the VH and VL domain to approximately 5 residues (see Peipp, M. and T. Valerius, *Biochem Soc Trans*, 2002. 30 (4): p. 507-11). This reduction of linker size facilitates dimerization of two polypeptide chains by crossover pairing of the VH and VL domains. Bispecific diabodies are produced by expressing two polypeptide chains with, either the structure VHA-VLB and VHB-VLA (VH-VL configuration), or VLA-VHB and VLB-VHA (VL-VH configuration) within the same cell. A large variety of different bispecific diabodies have been produced in the past and most of them can be expressed in soluble form in bacteria. However, a recent comparative study demonstrates that the orientation of the variable domains can influence expression and formation of active binding sites (see Mack, M., G. Riethmuller, and P. Kufer, *Proc Natl Acad Sci USA*, 1995. 92 (15): p. 7021-5). Nevertheless, soluble expression in bacteria represents an important advantage over tandem scFv molecules. However, since two different polypeptide chains are expressed within a single cell inactive homodimers can be produced together with active het-

erodimers. This necessitates the implementation of additional purification steps in order to obtain homogenous preparations of bispecific diabodies. One approach to force the generation of bispecific diabodies is the production of knob-into-hole diabodies (see Holliger, P., T. Prospero, and G. Winter, *Proc Natl Acad Sci USA*, 1993. 90 (14): p. 6444-8.18). This was demonstrated for a bispecific diabody directed against HER2 and CD3. A large knob was introduced in the VH domain by exchanging Val37 with Phe and Leu45 with Trp and a complementary hole was produced in the VL domain by mutating Phe98 to Met and Tyr87 to Ala, either in the anti-HER2 or the anti-CD3 variable domains. By using this approach the production of bispecific diabodies could be increased from 72% by the parental diabody to over 90% by the knob-into-hole diabody. Importantly, production yields did only slightly decrease as a result of these mutations. However, a reduction in antigen-binding activity was observed for several analyzed constructs. Thus, this rather elaborate approach requires the analysis of various constructs in order to identify those mutations that produce heterodimeric molecule with unaltered binding activity. In addition, such approach requires mutational modification of the immunoglobulin sequence at the constant region, thus creating non-native and non-natural form of the antibody sequence, which may result in increased immunogenicity, poor in vivo stability, as well as undesirable pharmacokinetics.

[0009] Single-chain diabodies (scDb) represent an alternative strategy to improve the formation of bispecific diabody-like molecules (see Holliger, P. and G. Winter, *Cancer Immunol Immunother*, 1997. 45 (34): p. 128-30; Wu, A. M., et al., *Immunotechnology*, 1996. 2 (1): p. 21-36). Bispecific single-chain diabodies are produced by connecting the two diabody-forming polypeptide chains with an additional middle linker with a length of approximately 15 amino acid residues. Consequently, all molecules with a molecular weight corresponding to monomeric single-chain diabodies (50-60 kDa) are bispecific. Several studies have demonstrated that bispecific single chain diabodies are expressed in bacteria in soluble and active form with the majority of purified molecules present as monomers (see Holliger, P. and G. Winter, *Cancer Immunol Immunother*, 1997. 45 (3-4): p. 128-30; Wu, A. M., et al., *Immunotechnology*, 1996. 2 (1): p. 21-36; Pluckthun, A. and P. Pack, *Immunotechnology*, 1997. 3 (2): p. 83-105; Ridgway, J. B., et al., *Protein Eng*, 1996. 9 (7): p. 617-21). Thus, single-chain diabodies combine the advantages of tandem scFvs (all monomers are bispecific) and diabodies (soluble expression in bacteria).

[0010] More recently diabody have been fused to Fc to generate more Ig-like molecules, named di-diabody (see Lu, D., et al., *J Biol Chem*, 2004. 279 (4): p. 2856-65). In addition, multivalent antibody construct comprising two Fab repeats in the heavy chain of an IgG and capable of binding four antigen molecules has been described (see WO 0177342A1, and Miller, K., et al., *J Immunol*, 2003. 170 (9): p. 4854-61).

[0011] There is a need in the art for improved multivalent binding proteins capable of binding two or more antigens. The present invention provides a novel family of binding proteins capable of binding two or more antigens with high affinity.

SUMMARY OF THE INVENTION

[0012] This invention pertains to multivalent binding proteins capable of binding two or more antigens. The present

invention provides a novel family of binding proteins capable of binding two or more antigens with high affinity.

[0013] In one embodiment the invention provides a binding protein comprising a polypeptide chain, wherein said polypeptide chain comprises VD1-(X1)n-VD2-C-(X2)n, wherein VD1 is a first variable domain, VD2 is a second variable domain, C is a constant domain, X1 represents an amino acid or polypeptide, X2 represents an Fc region and n is 0 or 1. In a preferred embodiment the VD1 and VD2 in the binding protein are heavy chain variable domains. More preferably the heavy chain variable domain is selected from the group consisting of a murine heavy chain variable domain, a human heavy chain variable domain, a CDR grafted heavy chain variable domain, and a humanized heavy chain variable domain. In a preferred embodiment VD1 and VD2 are capable of binding the same antigen. In another embodiment VD1 and VD2 are capable of binding different antigens. Preferably C is a heavy chain constant domain. More preferably X1 is a linker with the proviso that X1 is not CH1. Most preferably X1 is a linker selected from the group consisting of AKTTPKLEEGEFSEAR; AKTTPKLEEGEFSEARV; AKTTPKLGG; SAKTTPKLGG; AKTTPKLEEGEFSEARV; SAKTTP; SAKTTPKLGG; RADAAP; RADAAPTVS; RADAAAAGGPGS; RADAAA(G₄S)₄; SAKTTP; SAKTTPKLGG; SAKTTPKLEEGEFSEARV; ADAAP; ADAAPTVSIFPP; TVAAP; TVAAPSVFIFPP; QPKAAP; QPKAAPSVTLFPP; AKTTP; AKTTPPSVTPLAP; AKTIAP; AKTIAPSVYPLAP; ASTKGP; ASTKGPSVFPLAP; GGGGSGGGGSGGGGS; GENKVEYAPALMALS; GPAKELTPLKEAKVS; and GHEAAVMQVQYPAS. Preferably X2 is an Fc region. More preferably X2 is a variant Fc region.

[0014] In a preferred embodiment the binding protein disclosed above comprises a polypeptide chain, wherein said polypeptide chain comprises VD1-(X1)n-VD2-C-(X2)n, wherein VD1 is a first heavy chain variable domain, VD2 is a second heavy chain variable domain, C is a heavy chain constant domain, X1 is a linker with the proviso that it is not CH1, and X2 is an Fc region.

[0015] In another embodiment VD1 and VD2 in the binding protein are light chain variable domains. Preferably the light chain variable domain is selected from the group consisting of a murine light chain variable domain, a human light chain variable domain, a CDR grafted light chain variable domain, and a humanized light chain variable domain. In one embodiment VD1 and VD2 are capable of binding the same antigen. In another embodiment VD1 and VD2 are capable of binding different antigens. Preferably C is a light chain constant domain. More preferably X1 is a linker with the proviso that X1 is not CL1. Preferably X1 is a linker selected from the group consisting of AKTTPKLEEGEFSEAR; AKTTPKLEEGEFSEARV; AKTTPKLGG; SAKTTPKLGG; AKTTPKLEEGEFSEARV; SAKTTP; SAKTTPKLGG; RADAAP; RADAAPTVS; RADAAAAGGPGS; RADAAA(G₄S)₄; SAKTTP; SAKTTPKLGG; SAKTTPKLEEGEFSEARV; ADAAP; ADAAPTVSIFPP; TVAAP; TVAAPSVFIFPP; QPKAAP; QPKAAPSVTLFPP; AKTTP; AKTTPPSVTPLAP; AKTIAP; AKTIAPSVYPLAP; ASTKGP; and ASTKGPSVFPLAP. Preferably the binding protein does not comprise X2.

[0016] In a preferred embodiment the binding protein disclosed above comprises a polypeptide chain, wherein said polypeptide chain comprises VD1-(X1)n-VD2-C-(X2)n, wherein VD1 is a first light chain variable domain, VD2 is a

cytic syndrome, Wernicke-Korsakoff syndrome, Wilson's disease, xenograft rejection of any organ or tissue.

[0038] In another aspect the invention provides a method of treating a patient suffering from a disorder comprising the step of administering any one of the binding proteins disclosed above before, concurrent, or after the administration of a second agent, as discussed above. In a preferred embodiment the second agent is selected from the group consisting of budenoside, epidermal growth factor, corticosteroids, cyclosporin, sulfasalazine, aminosalicylates, 6-mercaptopurine, azathioprine, metronidazole, lipoxigenase inhibitors, mesalamine, olsalazine, balsalazide, antioxidants, thromboxane inhibitors, IL-1 receptor antagonists, anti-IL-1 β monoclonal antibodies, anti-L-6 or IL-6 receptor monoclonal antibodies, growth factors, elastase inhibitors, pyridinylimidazole compounds, antibodies or agonists of TNF, LT, IL-1, IL-2, IL-6, IL-7, IL-8, IL-12, IL-13, IL-15, IL-16, IL-18, IL-23, EMAP-II, GM-CSF, FGF, and PDGF, antibodies of CD2, CD3, CD4, CD8, CD-19, CD25, CD28, CD30, CD40, CD45, CD69, CD90 or their ligands, methotrexate, cyclosporin, FK506, rapamycin, mycophenolate mofetil, leflunomide, NSAIDs, ibuprofen, corticosteroids, prednisolone, phosphodiesterase inhibitors, adenosine agonists, anti-thrombotic agents, complement inhibitors, adrenergic agents, IRAK, NIK, IKK, p38, MAP kinase inhibitors, IL-1 β converting enzyme inhibitors, TNF α converting enzyme inhibitors, T-cell signalling inhibitors, metalloproteinase inhibitors, sulfasalazine, azathioprine, 6-mercaptopurines, angiotensin converting enzyme inhibitors, soluble cytokine receptors, soluble p55 TNF receptor, soluble p75 TNF receptor, sIL-1RI, sIL-1RII, sIL-6R, antiinflammatory cytokines, IL-4, IL-10, IL-11, IL-13 and TGF β .

[0039] In a preferred embodiment the pharmaceutical compositions disclosed above are administered to the subject by at least one mode selected from parenteral, subcutaneous, intramuscular, intravenous, intrarticular, intrabronchial, intraabdominal, intracapsular, intracartilaginous, intracavitary, intracelial, intracerebellar, intracerebroventricular, intracolic, intracervical, intragastric, intrahepatic, intramyocardial, intraosteal, intrapelvic, intrapericardiac, intraperitoneal, intrapleural, intraprostatic, intrapulmonary, intrarectal, intrarenal, intraretinal, intraspinal, intrasynovial, intrathoracic, intrauterine, intravesical, bolus, vaginal, rectal, buccal, sublingual, intranasal, and transdermal.

[0040] One aspect of the invention provides at least one anti-idiotypic antibody to at least one binding protein of the present invention. The anti-idiotypic antibody includes any protein or peptide containing molecule that comprises at least a portion of an immunoglobulin molecule such as, but not limited to, at least one complementarily determining region (CDR) of a heavy or light chain or a ligand binding portion thereof, a heavy chain or light chain variable region, a heavy chain or light chain constant region, a framework region, or any portion thereof, that can be incorporated into a binding protein of the present invention.

[0041] In another embodiment the binding proteins of the invention are capable of binding one or more targets selected from the group consisting of ABCF1; ACVR1; ACVR1B; ACVR2; ACVR2B; ACVRL1; ADORA2A; AggreCAN; AGR2; AICDA; AIF1; AIG1; AKAP1; AKAP2; AMH; AMHR2; ANGPT1; ANGPT2; ANGPTL3; ANGPTL4; ANPEP; APC; APOC1; AR; AZGP1 (zinc- α -glycoprotein); B7.1; B7.2; BAD; BAFF; BAG1; BAI1; BCL2; BCL6; BDNF; BLNK; BLR1 (MDR15); BlyS; BMP1; BMP2;

BMP3B (GDF10); BMP4; BMP6; BMP8; BMPR1A; BMPR1B; BMPR2; BPAG1 (plectin); BRCA1; C19orf10 (IL27w); C3; C4A; C5; C5R1; CANT1; CASP1; CASP4; CAV1; CCBP2 (D6/JAB61); CCL1 (1-309); CCL11 (eotaxin); CCL13 (MCP-4); CCL15 (MIP-1d); CCL16 (HCC4); CCL17 (TARC); CCL18 (PARC); CCL19 (MIP-3b); CCL2 (MCP-1); MCAF; CCL20 (MIP-3a); CCL21 (MIP-2); SLC; exodus-2; CCL22 (MDC/STC-1); CCL23 (MPIF-1); CCL24 (MPIF-2/eotaxin-2); CCL25 (TECK); CCL26 (eotaxin-3); CCL27 (CTACK/ILC); CCL28; CCL3 (MIP-1a); CCL4 (MIP-1b); CCL5 (RANTES); CCL7 (MCP-3); CCL8 (mcp-2); CCNA1; CCNA2; CCND1; CCNE1; CCNE2; CCR1 (CKR1/HM145); CCR2 (mcp-1RB/RA); CCR3 (CKR3/CMKBR3); CCR4; CCR5 (CMKBR5/ChemR13); CCR6 (CMKBR6/CKR-L3/STRL22/DRY6); CCR7 (CKR7/EBI1); CCR8 (CMKBR8/TER1/CKR-L1); CCR9 (GPR-9-6); CCRL1 (VSHK1); CCRL2 (L-CCR); CD164; CD19; CD1C; CD20; CD200; CD-22; CD24; CD28; CD3; CD37; CD38; CD3E; CD3G; CD3Z; CD4; CD40; CD40L; CD44; CD45RB; CD52; CD69; CD72; CD74; CD79A; CD79B; CD8; CD80; CD81; CD83; CD86; CDH1 (E-cadherin); CDH10; CDH12; CDH13; CDH18; CDH19; CDH20; CDH5; CDH7; CDH8; CDH9; CDK2; CDK3; CDK4; CDK5; CDK6; CDK7; CDK9; CDKN1A (p21Wap1/Cip1); CDKN1B (p27Kip1); CDKN1C; CDKN2A (p16INK4a); CDKN2B; CDKN2C; CDKN3; CEBPB; CER1; CHGA; CHGB; Chitinase; CHST10; CKLFSF2; CKLFSF3; CKLFSF4; CKLFSF5; CKLFSF6; CKLFSF7; CKLFSF8; CLDN3; CLDN7 (claudin-7); CLN3; CLU (clusterin); CMKLR1; CMKOR1 (RDC1); CNR1; COL18A1; COL1A1; COL4A3; COL6A1; CR2; CRP; CSF1 (M-CSF); CSF2 (GM-CSF); CSF3 (G-CSF); CTLA4; CTNBN1 (b-catenin); CTSB (cathepsin B); CX3CL1 (SCYD1); CX3CR1 (V28); CXCL1 (GRO1); CXCL10 (IP-10); CXCL11 (I-TAC/IP-9); CXCL12 (SDF1); CXCL13; CXCL14; CXCL16; CXCL2 (GRO2); CXCL3 (GRO3); CXCL5 (ENA-78/LIX); CXCL6 (GCP-2); CXCL9 (MIG); CXCR3 (GPR9/CKRL2); CXCR4; CXCR6 (TYMSTR/STRL33/Bonzo); CYB5; CYC1; CYSLTR1; DAB2IP; DES; DKFZp451J0118; DNCL1; DPP4; E2F1; ECGF1; EDG1; EFNA1; EFNA3; EFN2; EGF; EGFR; ELAC2; ENG; ENO1; ENO2; ENO3; EPHB4; EPO; ERBB2 (Her-2); EREG; ERK8; ESR1; ESR2; F3 (TF); FADD; FasL; FASN; FCER1A; FCER2; FCGR3A; FGF; FGF1 (aFGF); FGF10; FGF11; FGF12; FGF12B; FGF13; FGF14; FGF16; FGF17; FGF18; FGF19; FGF2 (bFGF); FGF20; FGF21; FGF22; FGF23; FGF3 (int-2); FGF4 (HST); FGF5; FGF6 (HST-2); FGF7 (KGF); FGF8; FGF9; FGFR3; FIGF (VEGFD); FIL1 (EPSILON); FIL1 (ZETA); FLJ12584; FLJ25530; FLRT1 (fibronectin); FLT1; FOS; FOSL1 (FRA-1); FY (DARC); GABRP (GABAa); GAGEB1; GAGEC1; GALNAC4S-6ST; GATA3; GDF5; GF11; GGT1; GM-CSF; GNAS1; GNRH1; GPR2 (CCR10); GPR31; GPR44; GPR81 (FKSG80); GRCC10 (C10); GRP; GSN (Gelsolin); GSTP1; HAVCR2; HDAC4; HDAC5; HDAC7A; HDAC9; HGF; HIF1A; HIP1; histamine and histamine receptors; HLA-A; HLA-DRA; HM74; HMOX1; HUMCYT2A; ICEBERG; ICOSL; ID2; IFN- α ; IFNA1; IFNA2; IFNA4; IFNA5; IFNA6; IFNA7; IFNB1; IFN-gamma; IFNW1; IGBP1; IGF1; IGF1R; IGF2; IGFBP2; IGFBP3; IGFBP6; IL-1; IL10; IL10RA; IL10RB; IL11; IL11RA; IL-12; IL12A; IL12B; IL12RB1; IL12RB2; IL13; IL13RA1; IL13RA2; IL14; IL15; IL15RA; IL16; IL17; IL17B; IL17C; IL17R; IL18; IL18BP; IL18R1; IL18RAP; IL19; IL1A; IL1B; IL1F10; IL1F5; IL1F6; IL1F7; IL1F8;

IL1F9; IL1HY1; IL1R1; IL1R2; IL1RAP; IL1RAPL1; IL1RAPL2; IL1RL1; IL1RL2 IL1RN; IL2; IL20; IL20RA; IL21R; IL22; IL22R; IL22RA2; IL23; IL24; IL25; IL26; IL27; IL28A; IL28B; IL29; IL2RA; IL2RB; IL2RG; IL3; IL30; IL3RA; IL4; IL4R; IL5; IL5RA; IL6; IL6R; IL6ST (glycoprotein 130); IL7; IL7R; IL8; IL8RA; IL8RB; IL8RB; IL9; IL9R; ILK; INHA; INHBA; INSL3; INSL4; IRAK1; IRAK2; ITGA1; ITGA2; ITGA3; ITGA6 (α6 integrin); ITGAV; ITGB3; ITGB4 (β4 integrin); JAG1; JAK1; JAK3; JUN; K6HF; KAI1; KDR; KITLG; KLF5 (GC Box BP); KLF6; KLK10; KLK12; KLK13; KLK14; KLK15; KLK3; KLK4; KLK5; KLK6; KLK9; KRT1; KRT19 (Keratin 19); KRT2A; KRTHB6 (hair-specific type II keratin); LAMA5; LEP (leptin); Lingo-p75; Lingo-Troy; LPS; LTA (TNF-β); LTβ; LTβ4R (GPR16); LTβ4R2; LTβR; MACMARCKS; MAG or Omgp; MAP2K7 (c-Jun); MDK; MIB1; midkine; MIF; MIP-2; MKI67 (Ki-67); MMP2; MMP9; MS4A1; MSMB; MT3 (metallothionein-III); MTSS1; MUC1 (mucin); MYC; MYD88; NCK2; neurocan; NFKB1; NFKB2; NGFB (NGF); NGFR; NgR-Lingo; NgR-Nogo66 (Nogo); NgR-p75; NgR-Troy; NME1 (NM23A); NOX5; NPPB; NR0B1; NR0B2; NR1D1; NR1D2; NR1H2; NR1H3; NR1H4; NR1I2; NR1I3; NR2C1; NR2C2; NR2E1; NR2E3; NR2F1; NR2F2; NR2F6; NR3C1; NR3C2; NR4A1; NR4A2; NR4A3; NR5A1; NR5A2; NR6A1; NRP1; NRP2; NT5E; NTN4; ODZ1; OPRD1; P2RX7; PAP; PART1; PATE; PAWR; PCA3; PCNA; PDGFA; PDGFB; PECAM1; PF4 (CXCL4); PGF; PGR; phosphacan; PIAS2; PIK3CG; PLAU (uPA); PLG; PLXDC1; PPBP (CXCL7); PPID; PR1; PRKCQ; PRKD1; PRL; PROC; PROK2; PSAP; PSCA; PTAFR; PTEN; PTGS2 (COX-2); PTN; RAC2 (p21Rac2); RARB; RGS1; RGS13; RGS3; RNF110 (ZNF144); ROBO2; S100A2; SCGB1D2 (lipophilin B); SCGB2A1 (mammaglobin 2); SCGB2A2 (mammaglobin 1); SCYE1 (endothelial Monocyte-activating cytokine); SDF2; SERPINA1; SERPINA3; SERPINB5 (maspin); SERPINE1 (PAI-1); SERPINF1; SHBG; SLA2; SLC2A2; SLC33A1; SLC43A1; SLIT2; SPP1; SPRR1B (Spr1); ST6GAL1; STAB 1; STAT6; STEAP; STEAP2; TB4R2; TBX21; TCP10; TDGF1; TEK; TGFA; TGFB1; TGFB1I1; TGFB2; TGFB3; TGFB1; TGFB1R1; TGFB1R2; TGFB1R3; TH1L; THBS1 (thrombospondin-1); THBS2; THBS4; THPO; TIE (Tie-1); TIMP3; tissue factor; TLR10; TLR2; TLR3; TLR4; TLR5; TLR6; TLR7; TLR8; TLR9; TNF; TNF-α; TNFAIP2 (B94); TNFAIP3; TNFRSF11A; TNFRSF1A; TNFRSF1B; TNFRSF21; TNFRSF5; TNFRSF6 (Fas); TNFRSF7; TNFRSF8; TNFRSF9; TNFSF10 (TRAIL); TNFSF11 (TRANCE); TNFSF12 (APO3L); TNFSF13 (April); TNFSF13B; TNFSF14 (HVEM-L); TNFSF15 (VEG1); TNFSF18; TNFSF4 (OX40 ligand); TNFSF5 (CD40 ligand); TNFSF6 (FasL); TNFSF7 (CD27 ligand); TNFSF8 (CD30 ligand); TNFSF9 (4-1BB ligand); TOLLIP; Toll-like receptors; TOP2A (topoisomerase Iia); TP53; TPM1; TPM2; TRADD; TRAF1; TRAF2; TRAF3; TRAF4; TRAF5; TRAF6; TREM1; TREM2; TRPC6; TSLP; TWEAK; VEGF; VEGFB; VEGFC; versican; VHL C5; VLA-4; XCL1 (lymphotactin); XCL2 (SCM-1b); XCR1 (GPR5/CCXCR1); YY1; and ZFPM2.

[0042] In another embodiment the invention provides a binding protein comprising a polypeptide chain, wherein said polypeptide chain comprises VD1-(X1)n-VD2-C-(X2)n, wherein;

VD1 is a first heavy chain variable domain obtained from a first parent antibody or antigen binding portion thereof; VD2

is a second heavy chain variable domain obtained from a second parent antibody or antigen binding portion thereof; C is a heavy chain constant domain; (X1)n is a linker with the proviso that it is not CH1, wherein said (X1)n is either present or absent; and (X2)n is an Fc region, wherein said (X2)n is either present or absent. Preferably, the Fc region is absent from the binding protein.

[0043] In another embodiment, the invention provides a binding protein comprising a polypeptide chain, wherein said polypeptide chain comprises VD1-(X1)n-VD2-C-(X2)n, wherein, VD1 is a first light chain variable domain obtained from a first parent antibody or antigen binding portion thereof; VD2 is a second light chain variable domain obtained from a second parent antibody or antigen binding portion thereof; C is a light chain constant domain; (X1)n is a linker with the proviso that it is not CH1, wherein said (X1)n is either present or absent; and (X2)n does not comprise an Fc region, wherein said (X2)n is either present or absent. Preferably (X2)n is absent from the binding protein.

[0044] In a preferred embodiment the binding protein of the invention comprises first and second polypeptide chains, wherein said first polypeptide chain comprises a first VD1-(X1)n-VD2-C-(X2)n, wherein VD1 is a first heavy chain variable domain obtained from a first parent antibody or antigen binding portion thereof; VD2 is a second heavy chain variable domain obtained from a second parent antibody or antigen binding portion thereof; C is a heavy chain constant domain; (X1)n is a linker with the proviso that it is not CH1, wherein said (X1)n is either present or absent; and (X2)n is an Fc region, wherein said (X2)n is either present or absent; and wherein said second polypeptide chain comprises a second VD1-(X1)n-VD2-C-(X2)n, wherein VD1 is a first light chain variable domain obtained from a first parent antibody or antigen binding portion thereof; VD2 is a second light chain variable domain obtained from a second parent antibody or antigen binding portion thereof; C is a light chain constant domain; (X1)n is a linker with the proviso that it is not CH1, wherein said (X1)n is either present or absent; and (X2)n does not comprise an Fc region, wherein said (X2)n is either present or absent. More preferably the binding protein comprises two first polypeptide chains and two second polypeptide chains. Most preferably (X2)n is absent from the second polypeptide. Preferably the Fc region, if present in the first polypeptide is selected from the group consisting of native sequence Fc region and a variant sequence Fc region. More preferably the Fc region is selected from the group consisting of an Fc region from an IgG1, IgG2, IgG3, IgG4, IgA, IgM, IgE, and IgD.

[0045] In a preferred embodiment the binding protein of the invention is a DVD-Ig capable of binding two antigens comprising four polypeptide chains, wherein, first and third polypeptide chains comprise VD1-(X1)n-VD2-C-(X2)n, wherein, VD1 is a first heavy chain variable domain obtained from a first parent antibody or antigen binding portion thereof; VD2 is a second heavy chain variable domain obtained from a second parent antibody or antigen binding portion thereof; C is a heavy chain constant domain; (X1)n is a linker with the proviso that it is not CH1, wherein said (X1)n is either present or absent; and (X2)n is an Fc region, wherein said (X2)n is either present or absent; and wherein second and fourth polypeptide chains comprise VD1-(X1)n-VD2-C-(X2)n, wherein VD1 is a first light chain variable domain obtained from a first parent antibody or antigen binding portion thereof; VD2 is a second light chain variable domain

We claim:

1. A binding protein comprising a polypeptide chain, wherein said polypeptide chain comprises VD1-(X1)_n-VD2-C-(X2)_n, wherein;

VD1 is a first heavy chain variable domain obtained from a first parent antibody or antigen binding portion thereof;

VD2 is a second heavy chain variable domain obtained from a second parent antibody or antigen binding portion thereof;

C is a heavy chain constant domain;

(X1)_n is a linker with the proviso that it is not CH1, wherein said (X1)_n is either present or absent; and

(X2)_n is an Fc region, wherein said (X2)_n is either present or absent.

2. A binding protein according to claim 1, wherein (X2)_n is absent.

3. A binding protein comprising a polypeptide chain, wherein said polypeptide chain comprises VD1-(X1)_n-VD2-C-(X2)_n, wherein;

VD1 is a first light chain variable domain obtained from a first parent antibody or antigen binding portion thereof;

VD2 is a second light chain variable domain obtained from a second parent antibody or antigen binding portion thereof;

C is a light chain constant domain;

(X1)_n is a linker with the proviso that it is not CH1, wherein said (X1)_n is either present or absent; and

(X2)_n does not comprise an Fc region, wherein said (X2)_n is either present or absent.

4. A binding protein according to claim 3, wherein (X2)_n is absent.

5. A binding protein comprising first and second polypeptide chains, wherein, said first polypeptide chain comprises a first VD1-(X1)_n-VD2-C-(X2)_n, wherein

VD1 is a first heavy chain variable domain obtained from a first parent antibody or antigen binding portion thereof;

VD2 is a second heavy chain variable domain obtained from a second parent antibody or antigen binding portion thereof;

C is a heavy chain constant domain;

(X1)_n is a linker with the proviso that it is not CH1, wherein said (X1)_n is either present or absent; and

(X2)_n is an Fc region, wherein said (X2)_n is either present or absent; and

wherein said second polypeptide chain comprises a second VD1-(X1)_n-VD2-C-(X2)_n, wherein

VD1 is a first light chain variable domain obtained from a first parent antibody or antigen binding portion thereof;

VD2 is a second light chain variable domain obtained from a second parent antibody or antigen binding portion thereof;

C is a light chain constant domain;

(X1)_n is a linker with the proviso that it is not CH1, wherein said (X1)_n is either present or absent; and

(X2)_n does not comprise an Fc region, wherein said (X2)_n is either present or absent.

6. The binding protein of claim 5, wherein the binding protein comprises two first polypeptide chains and two second polypeptide chains.

7. The binding protein of claim 5, wherein the Fc region is selected from the group consisting of native sequence Fc region and a variant sequence Fc region.

8. The binding protein of claim 5, wherein the Fc region is selected from the group consisting of an Fc region from an IgG1, IgG2, IgG3, IgG4, IgA, IgM, IgE, and IgD.

9. The binding protein of claim 5, wherein said VD1 of the first polypeptide chain and said VD1 of the second polypeptide chain are obtained from the same parent antibody or antigen binding portion thereof.

10. The binding protein of claim 5, wherein said VD1 of the first polypeptide chain and said VD1 of the second polypeptide chain are obtained from different parent antibody or antigen binding portion thereof.

11. The binding protein of claim 5, wherein said VD2 of the first polypeptide chain and said VD2 of the second polypeptide chain are obtained from the same parent antibody or antigen binding portion thereof.

12. The binding protein of claim 5, wherein said VD2 of the first polypeptide chain and said VD2 of the second polypeptide chain are obtained from different parent antibody or antigen binding portion thereof.

13. The binding protein of claim 5, wherein said first parent antibody or antigen binding portion thereof, and said second parent antibody or antigen binding portion thereof, are the same antibody.

14. The binding protein of claim 5, wherein said first parent antibody or antigen binding portion thereof, and said second parent antibody or antigen binding portion thereof, are different antibodies.

15. The binding protein of claim 5, wherein said first parent antibody or antigen binding portion thereof, binds a first antigen and said second parent antibody or antigen binding portion thereof, bind a second antigen.

16. The binding protein of claim 15, wherein said first antigen and said second antigen are the same antigen.

17. The binding protein of claim 15, wherein said first antigen and said second antigen are different antigens.

18. The binding protein of claim 16, wherein said first and said second parent antibodies bind different epitopes on said antigen.

19. The binding protein of claim 15, wherein said first parent antibody or antigen binding portion thereof, binds said first antigen with a potency different from the potency with which said second parent antibody or antigen binding portion thereof, binds said second antigen.

20. The binding protein of claim 15, wherein said first parent antibody or antigen binding portion thereof, binds said first antigen with an affinity different from the affinity with which said second parent antibody or antigen binding portion thereof, binds said second antigen.

21. The binding protein of claim 5, wherein said first parent antibody or antigen binding portion thereof, and said second parent antibody or antigen binding portion thereof, are selected from the group consisting of, human antibody, CDR grafted antibody, and humanized antibody.

22. The binding protein of claim 5, wherein said first parent antibody or antigen binding portion thereof, and said second parent antibody or antigen binding portion thereof, are selected from the group consisting of a Fab fragment, a F(ab')₂ fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; a Fd fragment consisting of the VH and CH1 domains; a Fv fragment consisting of the VL and VH domains of a single arm of an antibody, a dAb fragment, an isolated complementarity determining region (CDR), a single chain antibody, and diabodies.

23. The binding protein of claim 5, wherein said binding protein possesses at least one desired property exhibited by said first parent antibody or antigen binding portion thereof, or said second parent antibody or antigen binding portion thereof.

24. The binding protein of claim 23, wherein said desired property is selected from one or more antibody parameters.

25. The binding protein of claim 24, wherein said antibody parameters are selected from the group consisting of antigen specificity, affinity to antigen, potency, biological function, epitope recognition, stability, solubility, production efficiency, immunogenicity, pharmacokinetics, bioavailability, tissue cross reactivity, and orthologous antigen binding.

26. A DVD-Ig capable of binding two antigens comprising four polypeptide chains, wherein first and third polypeptide chains comprise VD1-(X1)_n-VD2-C-(X2)_n, wherein

VD1 is a first heavy chain variable domain obtained from a first parent antibody or antigen binding portion thereof; VD2 is a second heavy chain variable domain obtained from a second parent antibody or antigen binding portion thereof;

C is a heavy chain constant domain;

(X1)_n is a linker with the proviso that it is not CH1, wherein said (X1)_n is either present or absent; and

(X2)_n is an Fc region, wherein said (X2)_n is either present or absent; and

wherein second and fourth polypeptide chains comprise VD1-(X1)_n-VD2-C-(X2)_n, wherein

VD1 is a first light chain variable domain obtained from a first parent antibody or antigen binding portion thereof; VD2 is a second light chain variable domain obtained from a second parent antibody or antigen binding portion thereof;

C is a light chain constant domain;

(X1)_n is a linker with the proviso that it is not CH1, wherein said (X1)_n is either present or absent; and

(X2)_n does not comprise an Fc region, wherein said (X2)_n is either present or absent.

27. A method for generating a Dual Variable Domain Immunoglobulin capable of binding two antigens comprising the steps of

a) obtaining a first parent antibody or antigen binding portion thereof, capable of binding a first antigen;

b) obtaining a second parent antibody or antigen binding portion thereof, capable of binding a second antigen;

c) constructing first and third polypeptide chains comprising VD1-(X1)_n-VD2-C-(X2)_n, wherein

VD1 is a first heavy chain variable domain obtained from said first parent antibody or antigen binding portion thereof;

VD2 is a second heavy chain variable domain obtained from said second parent antibody or antigen binding portion thereof;

C is a heavy chain constant domain;

(X1)_n is a linker with the proviso that it is not CH1, wherein said (X1)_n is either present or absent; and

(X2)_n is an Fc region, wherein said (X2)_n is either present or absent;

d) constructing second and fourth polypeptide chains comprising VD1-(X1)_n-VD2-C-(X2)_n, wherein

VD1 is a first light chain variable domain obtained from said first parent antibody or antigen binding portion thereof;

VD2 is a second light chain variable domain obtained from said second parent antibody or antigen binding portion thereof; C is a light chain constant domain;

(X1)_n is a linker with the proviso that it is not CH1, wherein said (X1)_n is either present or absent; and

(X2)_n does not comprise an Fc region, wherein said (X2)_n is either present or absent;

e) expressing said first, second, third and fourth polypeptide chains;

such that a Dual Variable Domain Immunoglobulin capable of binding said first and said second antigen is generated.

28. The method of claim 27, wherein said first parent antibody or antigen binding portion thereof, and said second parent antibody or antigen binding portion thereof, are selected from the group consisting of, human antibody, CDR grafted antibody, and humanized antibody.

29. The method of claim 27, wherein said first parent antibody or antigen binding portion thereof, and said second parent antibody or antigen binding portion thereof, are selected from the group consisting of a Fab fragment, a F(ab')₂ fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; a Fd fragment consisting of the VH and CH1 domains; a Fv fragment consisting of the VL and VH domains of a single arm of an antibody, a dAb fragment, an isolated complementarity determining region (CDR), a single chain antibody, and diabodies.

30. The method of claim 27 wherein said first and said second antigen are the same antigen.

31. The method of claim 27 wherein said first and said second antigen are different antigens.

32. The method of claim 31 wherein said first and said second antigen are different epitopes on said antigen.

33. The method of claim 27, wherein said first parent antibody or antigen binding portion thereof possesses at least one desired property exhibited by the Dual Variable Domain Immunoglobulin.

34. The method of claim 27, wherein said second parent antibody or antigen binding portion thereof possesses at least one desired property exhibited by the Dual Variable Domain Immunoglobulin.

35. The method of claim 27, wherein the Fc region is selected from the group consisting of a native sequence Fc region and a variant sequence Fc region.

36. The method of claim 27, wherein the Fc region is selected from the group consisting of an Fc region from an IgG1, IgG2, IgG3, IgG4, IgA, IgM, IgE, and IgD.

37. The method of claim 33, wherein said desired property is selected from one or more antibody parameters.

38. The method of claim 34, wherein said desired property is selected from one or more antibody parameters.

39. The method of claim 37 wherein said antibody parameters are selected from the group consisting of antigen specificity, affinity to antigen, potency, biological function, epitope recognition, stability, solubility, production efficiency, immunogenicity, pharmacokinetics, bioavailability, tissue cross reactivity, and orthologous antigen binding.

40. The method of claim 38 wherein said antibody parameters are selected from the group consisting of antigen specificity, affinity to antigen, potency, biological function, epitope recognition, stability, solubility, production efficiency, immunogenicity, pharmacokinetics, bioavailability, tissue cross reactivity, and orthologous antigen binding.

41. The method of claim 27 wherein said first parent antibody or antigen binding portion thereof, binds said first antigen with a different affinity than the affinity with which said second parent antibody or antigen binding portion thereof, binds said second antigen.

42. The method of claim 27 wherein said first parent antibody or antigen binding portion thereof, binds said first antigen with a different potency than the potency with which said second parent antibody or antigen binding portion thereof, binds said second antigen.

43. A method for generating a Dual Variable Domain Immunoglobulin capable of binding two antigens with desired properties comprising the steps of

- a) obtaining a first parent antibody or antigen binding portion thereof, capable of binding a first antigen and possessing at least one desired property exhibited by the Dual Variable Domain Immunoglobulin;
- b) obtaining a second parent antibody or antigen binding portion thereof, capable of binding a second antigen and possessing at least one desired property exhibited by the Dual Variable Domain Immunoglobulin;
- c) constructing first and third polypeptide chains comprising VD1-(X1)n-VD2-C-(X2)n, wherein;
VD1 is a first heavy chain variable domain obtained from said first parent antibody or antigen binding portion thereof;

VD2 is a second heavy chain variable domain obtained from said second parent antibody or antigen binding portion thereof;

C is a heavy chain constant domain;

(X1)n is a linker with the proviso that it is not CH1, wherein said (X1)n is either present or absent; and

(X2)n is an Fc region, wherein said (X2)n is either present or absent;

d) constructing second and fourth polypeptide chains comprising VD1-(X1)n-VD2-C-(X2)n, wherein;
VD1 is a first light chain variable domain obtained from said first parent antibody or antigen binding portion thereof;

VD2 is a second light chain variable domain obtained from said second parent antibody or antigen binding portion thereof;

C is a light chain constant domain;

(X1)n is a linker with the proviso that it is not CH1, wherein said (X1)n is either present or absent; and

(X2)n does not comprise an Fc region, wherein said (X2)n is either present or absent;

e) expressing said first, second, third and fourth polypeptide chains;

such that a Dual Variable Domain Immunoglobulin capable of binding said first and said second antigen with desired properties is generated.

* * * * *



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(54) **DUAL VARIABLE DOMAIN
IMMUNOGLOBULIN AND USES THEREOF**

(76) Inventors: **Chengbin Wu**, Shrewsbury, MA (US);
Tariq Ghayur, Holliston, MA (US);
Richard W. Dixon, Jefferson, MA
(US); **Jochen G. Salfeld**, North
Grafton, MA (US)

Correspondence Address:
ABBOTT BIORESEARCH
100 RESEARCH DRIVE
WORCESTER, MA 01605-4314 (US)

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(57) **ABSTRACT**

Related U.S. Application Data

(60) Provisional application No. 60/709,911, filed on Aug.
19, 2005. Provisional application No. 60/732,892,
filed on Nov. 2, 2005.

The present invention relates to engineered multivalent and
multispecific binding proteins, methods of making, and
specifically to their uses in the prevention and/or treatment
of acute and chronic inflammatory and other diseases.

DUAL VARIABLE DOMAIN IMMUNOGLOBULIN AND USES THEREOF

CROSS-REFERENCE TO RELATED APPLICATION

[0001] This application claims the benefit of priority to U.S. provisional application No. 60/709,911 filed Aug. 19, 2005, and to U.S. provisional application No. 60/732,892 filed Nov. 2, 2005.

FIELD OF THE INVENTION

[0002] The present invention relates to multivalent and multispecific binding proteins, methods of making, and specifically to their uses in the prevention and/or treatment of acute and chronic inflammatory, cancer, and other diseases.

BACKGROUND OF THE INVENTION

[0003] Engineered proteins, such as multispecific antibodies capable of binding two or more antigens are known in the art. Such multispecific binding proteins can be generated using cell fusion, chemical conjugation, or recombinant DNA techniques.

[0004] Bispecific antibodies have been produced using the quadroma technology (see Milstein, C. and A. C. Cuellar, *Nature*, 1983. 305(5934): p. 537-40) based on the somatic fusion of two different hybridoma cell lines expressing murine monoclonal antibodies with the desired specificities of the bispecific antibody. Because of the random pairing of two different Ig heavy and light chains within the resulting hybrid-hybridoma (or quadroma) cell line, up to ten different immunoglobulin species are generated of which only one is the functional bispecific antibody. The presence of mispaired by-products, and significantly reduced production yields, means sophisticated purification procedures are required.

[0005] Bispecific antibodies can be produced by chemical conjugation of two different mAbs (see Staerz, U. D., et al., *Nature*, 1985. 314(6012): p. 628-31). This approach does not yield homogeneous preparation. Other approaches have used chemical conjugation of two different monoclonal antibodies or smaller antibody fragments (see Brennan, M., et al., *Science*, 1985. 229(4708): p. 81-3). Another method is the coupling of two parental antibodies with a hetero-bifunctional crosslinker, but the resulting preparations of bispecific antibodies suffer from significant molecular heterogeneity because reaction of the crosslinker with the parental antibodies is not site-directed. To obtain more homogeneous preparations of bispecific antibodies two different Fab fragments have been chemically crosslinked at their hinge cysteine residues in a site-directed manner (see Glennie, M. J., et al., *J Immunol*, 1987. 139(7): p. 2367-75). But this method results in Fab'2 fragments, not full IgG molecule.

[0006] A wide variety of other recombinant bispecific antibody formats have been developed in the recent past (see Kriangkum, J., et al., *Biomol Eng*, 2001. 18(2): p. 3140). Amongst them tandem single-chain Fv molecules and diabodies, and various derivatives thereof, are the most widely used formats for the construction of recombinant bispecific antibodies. Routinely, construction of these molecules starts from two single-chain Fv (scFv) fragments that recognize

different antigens (see Economides, A. N., et al., *Nat Med*, 2003. 9(1): p. 47-52). Tandem scFv molecules (taFv) represent a straightforward format simply connecting the two scFv molecules with an additional peptide linker. The two scFv fragments present in these tandem scFv molecules form separate folding entities. Various linkers can be used to connect the two scFv fragments and linkers with a length of up to 63 residues (see Nakanishi, K., et al., *Annu Rev Immunol*, 2001. 19: p. 423-74). Although the parental scFv fragments can normally be expressed in soluble form in bacteria, it is, however, often observed that tandem scFv molecules form insoluble aggregates in bacteria. Hence, refolding protocols or the use of mammalian expression systems are routinely applied to produce soluble tandem scFv molecules. In a recent study, in vivo expression by transgenic rabbits and cattle of a tandem scFv directed against CD28 and a melanoma-associated proteoglycan was reported (see Gracie, J. A., et al., *J Clin Invest*, 1999. 104(10): p. 1393-401). In this construct, the two scFv molecules were connected by a CH1 linker and serum concentrations of up to 100 mg/L of the bispecific antibody were found. Various strategies including variations of the domain order or using middle linkers with varying length or flexibility were employed to allow soluble expression in bacteria. A few studies have now reported expression of soluble tandem scFv molecules in bacteria (see Leung, B. P., et al., *J Immunol*, 2000. 164(12): p. 6495-502; Ito, A., et al., *J Immunol*, 2003. 170(9): p. 4802-9; Karni, A., et al., *J Neuroimmunol*, 2002. 125(1-2): p. 134-40) using either a very short Ala3 linker or long glycine/serine-rich linkers. In a recent study, phage display of a tandem scFv repertoire containing randomized middle linkers with a length of 3 or 6 residues was employed to enrich for those molecules that are produced in soluble and active form in bacteria. This approach resulted in the isolation of a preferred tandem scFv molecule with a 6 amino acid residue linker (see Arndt, M. and J. Krauss, *Methods Mol Biol*, 2003. 207: p. 305-21). It is unclear whether this linker sequence represents a general solution to the soluble expression of tandem scFv molecules. Nevertheless, this study demonstrated that phage display of tandem scFv molecules in combination with directed mutagenesis is a powerful tool to enrich for these molecules, which can be expressed in bacteria in an active form.

[0007] Bispecific diabodies (Db) utilize the diabody format for expression. Diabodies are produced from scFv fragments by reducing the length of the linker connecting the VH and VL domain to approximately 5 residues (see Peipp, M. and T. Valerius, *Biochem Soc Trans*, 2002. 30(4): p. 507-11). This reduction of linker size facilitates dimerization of two polypeptide chains by crossover pairing of the VH and VL domains. Bispecific diabodies are produced by expressing two polypeptide chains with either the structure VHA-VLB and VHB-VLA (VH-VL configuration), or VLA-VHB and VLB-VHA (VL-VH configuration) within the same cell. A large variety of different bispecific diabodies have been produced in the past and most of them can be expressed in soluble form in bacteria. However, a recent comparative study demonstrates that the orientation of the variable domains can influence expression and formation of active binding sites (see Mack, M., G. Riethmüller, and P. Kufer, *Proc Natl Acad Sci USA*, 1995. 92(15): p. 7021-5). Nevertheless, soluble expression in bacteria represents an important advantage over tandem scFv molecules. However, since two different polypeptide chains are expressed within

a single cell inactive homodimers can be produced together with active heterodimers. This necessitates the implementation of additional purification steps in order to obtain homogenous preparations of bispecific diabodies. One approach to force the generation of bispecific diabodies is the production of knob-into-hole diabodies (see Holliger, P., T. Prospero, and G. Winter, *Proc Natl Acad Sci USA*, 1993. 90(14): p. 6444-8.18). This was demonstrated for a bispecific diabody directed against HER2 and CD3. A large knob was introduced in the VH domain by exchanging Val37 with Phe and Leu45 with Trp and a complementary hole was produced in the VL domain by mutating Phe98 to Met and Tyr87 to Ala, either in the anti-HER2 or the anti-CD3 variable domains. By using this approach the production of bispecific diabodies could be increased from 72% by the parental diabody to over 90% by the knob-into-hole diabody. Importantly, production yields did only slightly decrease as a result of these mutations. However, a reduction in antigen-binding activity was observed for several analyzed constructs. Thus, this rather elaborate approach requires the analysis of various constructs in order to identify those mutations that produce heterodimeric molecule with unaltered binding activity. In addition, such approach requires mutational modification of the immunoglobulin sequence at the constant region, thus creating non-native and non-natural form of the antibody sequence, which may result in increased immunogenicity, poor in vivo stability, as well as undesirable pharmacokinetics.

[0008] Single-chain diabodies (scDb) represent an alternative strategy to improve the formation of bispecific diabody-like molecules (see Holliger, P. and G. Winter, *Cancer Immunol Immunother*, 1997. 45(3-4): p. 128-30; Wu, A. M., et al., *Immunotechnology*, 1996. 2(1): p. 21-36). Bispecific single-chain diabodies are produced by connecting the two diabody-forming polypeptide chains with an additional middle linker with a length of approximately 15 amino acid residues. Consequently, all molecules with a molecular weight corresponding to monomeric single-chain diabodies (50-60 kDa) are bispecific. Several studies have demonstrated that bispecific single chain diabodies are expressed in bacteria in soluble and active form with the majority of purified molecules present as monomers (see Holliger, P. and G. Winter, *Cancer Immunol Immunother*, 1997. 45(34): p. 128-30; Wu, A. M., et al., *Immunotechnology*, 1996. 2(1): p. 21-36; Pluckthun, A. and P. Pack, *Immunotechnology*, 1997. 3(2): p. 83-105; Ridgway, J. B., et al., *Protein Eng*, 1996. 9(7): p. 617-21). Thus, single-chain diabodies combine the advantages of tandem scFvs (all monomers are bispecific) and diabodies (soluble expression in bacteria).

[0009] More recently diabody have been fused to Fc to generate more Ig-like molecules, named di-diabody (see Lu, D., et al., *J Biol Chem*, 2004. 279(4): p. 2856-65). In addition, multivalent antibody construct comprising two Fab repeats in the heavy chain of an IgG and capable of binding four antigen molecules has been described (see WO 0177342A1, and Miller, K., et al., *J Immunol*, 2003. 170(9): p. 4854-61).

[0010] There is a need in the art for improved multivalent binding proteins capable of binding two or more antigens. The present invention provides a novel family of binding proteins capable of binding two or more antigens with high affinity.

SUMMARY OF THE INVENTION

[0011] This invention pertains to multivalent binding proteins capable of binding two or more antigens. The present invention provides a novel family of binding proteins capable of binding two or more antigens with high affinity.

[0012] In one embodiment the invention provides a binding protein comprising a polypeptide chain, wherein said polypeptide chain comprises VD1-(X1)_n-VD2-C(X2)_n, wherein VD1 is a first variable domain, VD2 is a second variable domain, C is a constant domain, X1 represents an amino acid or polypeptide, X2 represents an Fc region and n is 0 or 1. In a preferred embodiment the VD1 and VD2 in the binding protein are heavy chain variable domains. More preferably the heavy chain variable domain is selected from the group consisting of a murine heavy chain variable domain, a human heavy chain variable domain, a CDR grafted heavy chain variable domain, and a humanized heavy chain variable domain. In a preferred embodiment VD1 and VD2 are capable of binding the same antigen. In another embodiment VD1 and VD2 are capable of binding different antigens. Preferably C is a heavy chain constant domain. More preferably X1 is a linker with the proviso that X1 is not CH1. Most preferably X1 is a linker selected from the group consisting of AKTTPKLEEGEFSEAR; AKTTPKLEEGEFSEARV; AKTTPKLGG; SAKTTPKLGG; AKTTPKLEEGEFSEARV; SAKTTP; SAKTTPKLGG; RADAAP; RADAAPTVS; RADAAAAGGPGS; RADAAA(G₄S)₄; SAKTTP; SAKTTPKLGG; SAKTTPKLEEGEFSEARV; ADAAP; ADAAPTVSIFPP; TVAAP; TVAAPSVFIFPP; QPKAAP; QPKAAPSVTLFPP; AKTTP; AKTTPPSVTPLAP; AKTTAP; AKTTAPSVYPLAP; ASTKGP; ASTKGPSVFPLAP; GGGGSGGGGSGGGGS; GENKVEYAPALMALS; GPAKELTPLKEAKVS; and GHEAAAVMQVQYPAS. Preferably X2 is an Fc region. More preferably X2 is a variant Fc region.

[0013] In a preferred embodiment the binding protein disclosed above comprises a polypeptide chain, wherein said polypeptide chain comprises VD1-(X1)_n-VD2-C(X2)_n, wherein VD1 is a first heavy chain variable domain, VD2 is a second heavy chain variable domain, C is a heavy chain constant domain, X1 is a linker with the proviso that it is not CH1, and X2 is an Fc region.

[0014] In another embodiment VD1 and VD2 in the binding protein are light chain variable domains. Preferably the light chain variable domain is selected from the group consisting of a murine light chain variable domain, a human light chain variable domain, a CDR grafted light chain variable domain, and a humanized light chain variable domain. In one embodiment VD1 and VD2 are capable of binding the same antigen. In another embodiment VD1 and VD2 are capable of binding different antigens. Preferably C is a light chain constant domain. More preferably X1 is a linker with the proviso that X1 is not CL1. Preferably X1 is a linker selected from the group consisting of AKTTPKLEEGEFSEAR; AKTTPKLEEGEFSEARV; AKTTPKLGG; SAKTTPKLGG; AKTTPKLEEGEFSEARV; SAKTTP; SAKTTPKLGG; RADAAP; RADAAPTVS; RADAAAAGGPGS; RADAAA(G₄S)₄; SAKTTP; SAKTTPKLGG; SAKTTPKLEEGEFSEARV; ADAAP; ADAAPTVSIFPP; TVAAP; TVAAPSVFIFPP; QPKAAP; QPKAAPSVTLFPP; AKTTP; AKTTPPSVTPLAP; AKTTAP; AKTTAPSVYPLAP; ASTKGP; and ASTKGPSVFPLAP. Preferably the binding protein does not comprise X2.

ovascular hypertension, reperfusion injury, restrictive cardiomyopathy, sarcomas, scleroderma, senile chorea, Senile Dementia of Lewy body type, seronegative arthropathies, shock, sickle cell anemia, skin allograft rejection, skin changes syndrome, small bowel transplant rejection, solid tumors, specific arrhythmias, spinal ataxia, spinocerebellar degenerations, streptococcal myositis, structural lesions of the cerebellum, Subacute sclerosing panencephalitis, Syncope, syphilis of the cardiovascular system, systemic anaphylaxis, systemic inflammatory response syndrome, systemic onset juvenile rheumatoid arthritis, T-cell or FAB ALL, Telangiectasia, thromboangitis obliterans, thrombocytopenia, toxicity, transplants, trauma/hemorrhage, type III hypersensitivity reactions, type IV hypersensitivity, unstable angina, uremia, urosepsis, urticaria, valvular heart diseases, varicose veins, vasculitis, venous diseases, venous thrombosis, ventricular fibrillation, viral and fungal infections, vital encephalitis/aseptic meningitis, vital-associated hemaphagocytic syndrome, Wernicke-Korsakoff syndrome, Wilson's disease, xenograft rejection of any organ or tissue.

[0037] In another aspect the invention provides a method of treating a patient suffering from a disorder comprising the step of administering any one of the binding proteins disclosed above before, concurrent, or after the administration of a second agent, as discussed above. In a preferred embodiment the second agent is selected from the group consisting of budenoside, epidermal growth factor, corticosteroids, cyclosporin, sulfasalazine, aminosalicylates, 6-mercaptopurine, azathioprine, metronidazole, lipoxygenase inhibitors, mesalamine, olsalazine, balsalazide, antioxidants, thromboxane inhibitors, IL-1 receptor antagonists, anti-IL-1 β monoclonal antibodies, anti-IL-6 monoclonal antibodies, growth factors, elastase inhibitors, pyridinyl-imidazole compounds, antibodies or agonists of TNF, LT, IL-1, IL-2, IL-6, IL-7, IL-8, IL-12, IL-13, IL-15, IL-16, IL-18, IL-23, EMAP-II, GM-CSF, FGF, and PDGF, antibodies of CD2, CD3, CD4, CD8, CD-19, CD25, CD28, CD30, CD40, CD45, CD69, CD90 or their ligands, methotrexate, cyclosporin, FK506, rapamycin, mycophenolate mofetil, leflunomide, NSAIDs, ibuprofen, corticosteroids, prednisolone, phosphodiesterase inhibitors, adenosine agonists, antithrombotic agents, complement inhibitors, adrenergic agents, IRAK, NIK, IKK, p38, MAP kinase inhibitors, IL-1 β converting enzyme inhibitors, TNF α converting enzyme inhibitors, T-cell signalling inhibitors, metalloproteinase inhibitors, sulfasalazine, azathioprine, 6-mercaptopurines, angiotensin converting enzyme inhibitors, soluble cytokine receptors, soluble p55 TNF receptor, soluble p75 TNF receptor, sIL-1R1, sIL-1R11, sIL-6R, antiinflammatory cytokines, IL-4, IL-10, IL-11, IL-13 and TGF β .

[0038] In a preferred embodiment the pharmaceutical compositions disclosed above are administered to the subject by at least one mode selected from parenteral, subcutaneous, intramuscular, intravenous, intrarticular, intrabronchial, intraabdominal, intracapsular, intracartilaginous, intracavitary, intracelial, intracerebellar, intracerebroventricular, intracolic, intracervical, intragastric, intrahepatic, intramyocardial, intraosteal, intrapelvic, intrapericardiac, intraperitoneal, intrapleural, intraprostatic, intrapulmonary, intrarectal, intrarenal, intraretinal, intraspinal, intrasynovial, intrathoracic, intrauterine, intravesical, bolus, vaginal, rectal, buccal, sublingual, intranasal, and transdermal.

[0039] One aspect of the invention provides at least one anti-idiotypic antibody to at least one binding protein of the present invention. The anti-idiotypic antibody includes any protein or peptide containing molecule that comprises at least a portion of an immunoglobulin molecule such as, but not limited to, at least one complementarily determining region (CDR) of a heavy or light chain or a ligand binding portion thereof, a heavy chain or light chain variable region, a heavy chain or light chain constant region, a framework region, or; any portion thereof, that can be incorporated into a binding protein of the present invention.

[0040] In another embodiment the binding proteins of the invention are capable of binding one or more targets selected from the group consisting of ABCF1; ACVR1; ACVR1B; ACVR2; ACVR2B; ACVRL1; ADORA2A; Aggrecan; AGR2; AICDA; AIF1; AIG1; AKAP1; AKAP2; AMH; AMHR2; ANGPT1; ANGPT2; ANGPTL3; ANGPTL4; ANPEP; APC; APOC1; AR; AZGP1 (zinc-a-glycoprotein); B7.1; B7.2; BAD; BAFF; BAG1; BAI1; BCL2; BCL6; BDNF; BLNK; BLR1 (MDR15); BlyS; BMP1; BMP2; BMP3B (GDF10); BMP4; BMP6; BMP8; BMPR1A; BMPR1B; BMPR2; BPAG1 (plectin); BRCA1; C19orf10 (IL27w); C3; C4A; C5; C5R1; CANT1; CASP1; CASP4; CAV1; CCBP2 (D6/JAB61); CCL1 (1-309); CCL11 (eotaxin); CCL13 (MCP-4); CCL15 (MIP-1d); CCL16 (HCC-4); CCL17 (TARC); CCL18 (PARC); CCL19 (MIP-3b); CCL2 (MCP-1); MCAF; CCL20 (MIP-3a); CCL21 (MIP-2); SLC; exodus-2; CCL22 (MDC/STC-1); CCL23 (MPIF-1); CCL24 (MPIF-2/eotaxin-2); CCL25 (TECK); CCL26 (eotaxin-3); CCL27 (CTACK/ILC); CCL28; CCL3 (MIP-1a); CCL4 (MIP-1b); CCL5 (RANTES); CCL7 (MCP-3); CCL8 (mcp-2); CCNA1; CCNA2; CCND1; CCNE1; CCNE2; CCR1 (CKR1/HM145); CCR2 (mcp-1RB/RA); CCR3 (CKR3/CMKBR3); CCR4; CCR5 (CMKBR5/ChemR13); CCR6 (CMKBR6/CKR-L3/STRL22/DRY6); CCR7 (CKR7/EBI1); CCR8 (CMKBR8/TER1/CKR-L1); CCR9 (GPR-9-6); CCRL1 (VSHK1); CCRL2 (L-CCR); CD164; CD19; CD1C; CD20; CD200; CD-22; CD24; CD28; CD3; CD37; CD38; CD3E; CD3G; CD3Z; CD4; CD40; CD40L; CD44; CD45RB; CD52; CD69; CD72; CD74; CD79A; CD79B; CD8; CD80; CD81; CD83; CD86; CDH1(E-cadherin); CDH10; CDH12; CDH13; CDH18; CDH19; CDH20; CDH5; CDH7; CDH8; CDH9; CDK2; CDK3; CDK4; CDK5; CDK6; CDK7; CDK9; CDKN1A (p21 Wap1/Cip1); CDKN1B (p27Kip1); CDKN1C; CDKN2A (p16INK4a); CDKN2B; CDKN2C; CDKN3; CEBPB; CER1; CHGA; CHGB; Chitinase; CHST10; CKLFSF2; CKLFSF3; CKLFSF4; CKLFSF5; CKLFSF6; CKLFSF7; CKLFSF8; CLDN3; CLDN7 (claudin-7); CLN3; CLU (clusterin); CMKLR1; CMKOR1 (RDC1); CNR1; COL18A1; COL1A1; COL4A3; COL6A1; CR2; CRP; CSF1(M-CSF); CSF2 (GM-CSF); CSF3 (G-CSF); CTLA4; CTNNB1 (b-catenin); CTSB (cathepsin B); CX3CL1 (SCYD1); CX3CR1 (V28); CXCL1 (GRO1); CXCL10 (IP-10); CXCL11(I-TAC/IP-9); CXCL12 (SDF1); CXCL13; CXCL14; CXCL16; CXCL2 (GRO2); CXCL3 (GRO3); CXCL5 (ENA-78/LIX); CXCL6 (GCP-2); CXCL9 (MIG); CXCR3 (GPR9/CKR-L2); CXCR4; CXCR6 (TYMSTR/STRL33/Bonzo); CYB5; CYC1; CYS-LTR1; DAB21P; DES; DKFZp451J0118; DNCL1; DPP4; E2F1; ECGF1; EDG1; EFNA1; EFNA3; EFN2; EGF; EGFR; ELAC2; ENG; ENO1; ENO2; ENO3; EPHB4; EPO; ERBB2 (Her-2); EREG; ERK8; ESR1; ESR2; F3 (TF); FADD; FasL; FASN; FCER1A; FCER2; FCGR3A; FGF;

TLR5; TLR6; TLR7; TLR8; TLR9; TNF; TNF- α ; TNFAIP2 (B94); TNFAIP3; TNFRSF11A; TNFRSF1A; TNFRSF1B; TNFRSF21; TNFRSF5; TNFRSF6 (Fas); TNFRSF7; TNFRSF8; TNFRSF9; TNFSF10 (TRAIL); TNFSF11 (TRANCE); TNFSF12 (APO3L); TNFSF13 (April); TNFSF13B; TNFSF14 (HVEM-L); TNFSF15 (VEGI); TNFSF18; TNFSF4 (OX40 ligand); TNFSF5 (CD40 ligand); TNFSF6 (FasL); TNFSF7 (CD27 ligand); TNFSF8 (CD30 ligand); TNFSF9 (4-1BB ligand); TOLLIP, Toll-like receptors; TOP2A (topoisomerase α); TP53; TPM1; TPM2; TRADD; TRAF1; TRAF2; TRAF3; TRAF4; TRAF5; TRAF6; TREM1; TREM2; TRPC6; TSLP; TWEAK; VEGF; VEGFB; VEGFC; versican; VHL C5; VLA-4; XCL1 (lymphotactin); XCL2 (SCM-1b); XCR1 (GPR5/CCXCR1); YY1; and ZFPM2.

[0041] FIG. 1A is a schematic representation of Dual Variable Domain (DVD)-Ig constructs and shows the strategy for generation of a DVD-Ig from two parent antibodies; FIG. 1B, is a schematic representation of constructs DVD1-Ig, DVD2-Ig, and two chimeric mono-specific antibodies from hybridoma clones 2D13.E3 (anti-IL-1 α) and 13F5.G5 (anti-IL-1 β).

[0042] This invention pertains to multivalent and/or multispecific binding proteins capable of binding two or more antigens. Specifically, the invention relates to dual variable domain immunoglobulins (DVD-Ig), and pharmaceutical compositions thereof, as well as nucleic acids, recombinant expression vectors and host cells for making such DVD-Igs. Methods of using the DVD-Igs of the invention to detect specific antigens, either in vitro or in vivo are also encompassed by the invention.

[0044] Generally, nomenclatures used in connection with, and techniques of, cell and tissue culture, molecular biology, immunology, microbiology, genetics and protein and nucleic acid chemistry and hybridization described herein are those well known and commonly used in the art. The methods and techniques of the present invention are generally performed according to conventional methods well known in the art and as described in various general and more specific references that are cited and discussed throughout the present specification unless otherwise indicated. Enzymatic

-continued

Asn Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Phe
145 150 155 160
Ile Tyr Thr Ala Ser Thr Arg Ala Thr Asp Ile Pro Ala Arg Phe Ser
165 170 175
Gly Ser Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln
180 185 190
Ser Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Tyr Asn Asn Trp Pro
195 200 205
Ser Ile Thr Phe Gly Gln Gly Thr Arg Leu Glu Ile Lys Arg
210 215 220

<210> SEQ ID NO 117
<211> LENGTH: 108
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: PEPTIDE
<222> LOCATION: (1)..(108)
<223> OTHER INFORMATION: Sequence of 1D4.1 VL region

<400> SEQUENCE: 117

Asp Ile Val Met Thr Gln Ser Pro Asp Ser Leu Ala Val Ser Leu Gly
1 5 10 15
Glu Arg Ala Thr Ile Asn Cys Lys Ala Ser Gln Ser Val Ser Asn Asp
20 25 30
Val Ala Trp Tyr Gln Gln Lys Pro Gly Gln Pro Pro Lys Leu Leu Ile
35 40 45
Tyr Tyr Ala Ser Asn Arg Tyr Thr Gly Val Pro Asp Arg Phe Ser Gly
50 55 60
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Ala
65 70 75 80
Glu Asp Val Ala Val Tyr Tyr Cys Gln Gln Asp Tyr Asn Ser Pro Trp
85 90 95
Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys Arg
100 105

We claim:

1. A binding protein comprising a polypeptide chain, wherein said polypeptide chain comprises VD1-(X1)n-VD2-C-(X2)n, wherein VD1 is a first variable domain, VD2 is a second variable domain, C is a constant domain, X1 represents an amino acid or polypeptide, X2 represents an Fc region and n is 0 or 1.

2. The binding protein according to claim 1, wherein said VD1 and VD2 are heavy chain variable domains.

3. The binding protein according to claim 2, wherein said heavy chain variable domain is selected from the group consisting of a murine heavy chain variable domain, a human heavy chain variable domain, a CDR grafted heavy chain variable domain, and a humanized heavy chain variable domain.

4. The binding protein according to claim 2, wherein VD1 and VD2 are capable of binding the same antigen.

5. The binding protein according to claim 2, wherein VD1 and VD2 are capable of binding different antigens.

6. The binding protein according to claim 2, wherein C is a heavy chain constant domain.

7. The binding protein according to claim 6, wherein X1 is a linker with the proviso that X1 is not CH1.

8. The binding protein according to claim 7, wherein the linker is selected from the group consisting of AKTTP-KLEEGEFSEAR; AKTTPKLEEGEFSEARV; AKTTP-KLGG; SAKTTPKLGG; AKTTPKLEEGEFSEARV; SAKTTP; SAKTTPKLGG; RADAAP; RADAAPTVS; RADAAAAGGPGS; RADAAA(G₄S)₄; SAKTTP; SAKTTPKLGG; SAKTTPKLEEGEFSEARV; ADAAP; ADAAPTVSIFPP; TVAAP; TVAAPSVFIFPP; QPKAAP; QPKAAPSVTLFPP; AKTTPP; AKTTPPSVTPLAP; AKT-TAP; AKTTAPSVYPLAP; ASTKGP; ASTKGPSVFPLAP; GGGGSGGGGSGGGGS; GENKVEYAPALMALS; GPA-KELTPLKEAKVS; and GHEAAAVMQVYPAS.

9. The binding protein according to claim 7, wherein X2 is an Fc region.

10. The binding protein according to claim 9, wherein said Fc region is a variant Fc region.

11. A binding protein comprising a polypeptide chain, wherein said polypeptide chain comprises VD1-(X1)n-VD2-C-(X2)n, wherein VD1 is a first heavy chain variable

domain, VD2 is a second heavy chain variable domain, C is a heavy chain constant domain, X1 is a linker with the proviso that it is not CH1, and X2 is an Fc region.

12. The binding protein according to claim 1, wherein said VD1 and VD2 are light chain variable domains.

13. The binding protein according to claim 12, wherein said light chain variable domain is selected from the group consisting of a murine light chain variable domain, a human light chain variable domain, a CDR grafted light chain variable domain, and a humanized light chain variable domain.

14. The binding protein according to claim 12, wherein VD1 and VD2 are capable of binding the same antigen.

15. The binding protein according to claim 12, wherein VD1 and VD2 are capable of binding different antigens.

16. The binding protein according to claim 12, wherein C is a light chain constant domain.

17. The binding protein according to claim 16, wherein X1 is a linker with the proviso that X1 is not CL1.

18. The binding protein according to claim 17, wherein the linker is selected from the group consisting of AKTTPKLEEGEFSEAR; AKTTPKLEEGEFSEARV; AKTTPKLG; SAKTTPKLGG; AKTTPKLEEGEFSEARV; SAKTTP; SAKTTPKLGG; RADAAP; RADAAPTVS; RADAAGGPGS; RADAAGGPGS₄; SAKTTP; SAKTTPKLGG; SAKTTPKLEEGEFSEARV; ADAAP; ADAAPTVSIFPP; TVAAP; TVAAPSVFIFPP; QPKAAP; QPKAAPSVTLFPP; AKTTPP; AKTTPPSVTPLAP; AKTAP; AKTAPSVYPLAP; ASTKGP; ASTKGPSVFPLAP; GGGGSGGGGSGGGGS; GENKVEYAPALMALS; GPAKELTPLKEAKVS; and GHEAAAVMQVQYPAS.

19. The binding protein according to claim 17, wherein the binding protein does not comprise X2.

20. A binding protein comprising a polypeptide chain, wherein said polypeptide chain comprises VD1-(X1)_n-VD2-C-(X2)_n, wherein VD1 is a first light chain variable domain, VD2 is a second light chain variable domain, C is a light chain constant domain, X1 is a linker with the proviso that it is not CH1, and X2 does not comprise an Fc region.

21. A binding protein comprising first and second polypeptide chains, wherein said first polypeptide chain comprises VD1-(X1)_n-VD2-C-(X2)_n, wherein VD1 is a first heavy chain variable domain, VD2 is a second heavy chain variable domain, C is a heavy chain constant domain, X1 is a linker with the proviso that it is not CH1, and X2 is an Fc region; and said second polypeptide chain comprises VD1-(X1)_n-VD2-C-(X2)_n, wherein VD1 is a first light chain variable domain, VD2 is a second light chain variable domain, C is a light chain constant domain, X1 is a linker with the proviso that it is not CH1, and X2 does not comprise an Fc region.

22. A binding protein comprising four polypeptide chains, wherein two polypeptide chains comprise VD1-(X1)_n-VD2-C-(X2)_n, wherein VD1 is a first heavy chain variable domain, VD2 is a second heavy chain variable domain, C is a heavy chain constant domain, X1 is a linker with the proviso that it is not CH1, and X2 is an Fc region; and two polypeptide chains comprises VD1-(X1)_n-VD2-C-(X2)_n, wherein VD1 is a first light chain variable domain, VD2 is a second light chain variable domain, C is a light chain constant domain, X1 is a linker with the proviso that it is not CH1, and X2 does not comprise an Fc region.

23. The binding protein according to claim 1, wherein said binding protein is capable of binding one or more targets.

24. The binding protein according to claim 23, wherein the target is selected from the group consisting of ABCF1; ACVR1; ACVR1B; ACVR2; ACVR2B; ACVRL1; ADORA2A; Aggrecan; AGR2; AICDA; AIF1; AIG1; AKAP1; AKAP2; AMH; AMHR2; ANGPT1; ANGPT2; ANGPTL3; ANGPTL4; ANPEP; APC; APOC1; AR; AZGP1 (zinc-α-glycoprotein); B7.1; B7.2; BAD; BAFF; BAG1; BAI1; BCL2; BCL6; BDNF; BLNK; BLR1 (MDR15); BlyS; BMP1; BMP2; BMP3B (GDF10); BMP4; BMP6; BMP8; BMPR1A; BMPR1B; BMPR2; BPAG1 (plectin); BRCA1; C19orf10 (IL27w); C3; C4A; C5; C5R1; CANT1; CASP1; CASP4; CAV1; CCBP2 (D6/JAB61); CCL1 (I-309); CCL11 (eotaxin); CCL13 (MCP-4); CCL15 (MIP-1d); CCL16 (HCC-4); CCL17 (IARC); CCL18 (PARC); CCL19 (MIP-3b); CCL2 (MCP-1); MCAF; CCL20 (MIP-3a); CCL21 (MIP-2); SLC; exodus-2; CCL22 (MDC/STC-1); CCL23 (MPIF-1); CCL24 (MPIF-2/eotaxin-2); CCL25 (TECK); CCL26 (eotaxin-3); CCL27 (CTACK/ILC); CCL28; CCL3 (MIP-1a); CCL4 (MIP-1b); CCL5 (RANTES); CCL7 (MCP-3); CCL8 (mcp-2); CCNA1; CCNA2; CCND1; CCNE1; CCNE2; CCR1 (CKR1/HM145); CCR2 (mcp-1RB/RA); CCR3 (CKR3/CMKBR3); CCR4; CCR5 (CMKBR5/ChemR13); CCR6 (CMKBR6/CKR-L3/STRL22/DRY6); CCR7 (CKR7/EBI1); CCR8 (CMKBR8/TER1/CKR-L1); CCR9 (GPR-9-6); CCRL1 (VSHK1); CCRL2 (L-CCR); CD164; CD19; CD1C; CD20; CD200; CD-22; CD24; CD28; CD3; CD37; CD38; CD3E; CD3G; CD3Z; CD4; CD40; CD40L; CD44; CD45RB; CD52; CD69; CD72; CD74; CD79A; CD79B; CD8; CD80; CD81; CD83; CD86; CDH1 (E-cadherin); CDH10; CDH12; CDH13; CDH18; CDH19; CDH20; CDH5; CDH7; CDH8; CDH9; CDK2; CDK3; CDK4; CDK5; CDK6; CDK7; CDK9; CDKN1A (p21Wap1/Cip1); CDKN1B (p27Kip1); CDKN1C; CDKN2A (p16INK4a); CDKN2B; CDKN2C; CDKN3; CEBPB; CER1; CHGA; CHGB; Chitinase; CHST10; CKLFSF2; CKLFSF3; CKLFSF4; CKLFSF5; CKLFSF6; CKLFSF7; CKLFSF8; CLDN3; CLDN7 (claudin-7); CLN3; CLU (clusterin); CMKLR1; CMKOR1 (RDC1); CNR1; COL18A1; COL1A1; COL4A3; COL6A1; CR2; CRP; CSF1 (M-CSF); CSF2 (GM-CSF); CSF3 (G-CSF); CTLA4; CTNNB1 (b-catenin); CTSB (cathepsin B); CX3CL1 (SCYD1); CX3CR1 (V28); CXCL1 (GRO1); CXCL10 (IP-10); CXCL11 (I-TAC/IP-9); CXCL12 (SDF1); CXCL13; CXCL14; CXCL16; CXCL2 (GRO2); CXCL3 (GRO3); CXCL5 (ENA-78/LIX); CXCL6 (GCP-2); CXCL9 (MIG); CXCR3 (GPR9/CKR-L2); CXCR4; CXCR6 (TYMSTR/STRL33/Bonzo); CYB5; CYC1; CYSLTR1; DAB21P; DES; DKFZp451J0118; DNCL1; DPP4; E2F1; ECGF1; EDG1; EFNA1; EFNA3; EFN2; EGF; EGFR; ELAC2; ENG; ENO1; ENO2; ENO3; EPHB4; EPO; ERBB2 (Her-2); EREG; ERK8; ESR1; ESR2; F3 (TF); FADD; FasL; FASN; FCER1A; FCER2; FCGR3A; FGF; FGF1 (aFGF); FGF10; FGF11; FGF12; FGF12B; FGF13; FGF14; FGF16; FGF17; FGF18; FGF19; FGF2 (bFGF); FGF20; FGF21; FGF22; FGF23; FGF3 (int-2); FGF4 (HST); FGF5; FGF6 (HST-2); FGF7 (KGF); FGF8; FGF9; FGFR3; FIGF (VEGFD); FIL1 (EPSILON); FIL1 (ZETA); FLJ12584; FLJ25530; FLRT1 (fibronectin); FLT1; FOS; FOSL1 (FRA-1); FY (DARC); GABRP (GABAa); GAGEB1; GAGEC1; GALNAC4S-6ST; GATA3; GDF5; GFI1; GGT1; GM-CSF; GNAS1; GNRH1; GPR2 (CCR10); GPR31; GPR44; GPR81 (FKSG80); GRCC10 (C10); GRP; GSN (Gelsolin); GSTP1; HAVCR2; HDAC4; HDAC5; HDAC7A; HDAC9;

HGF; HIF1A; HIP1; histamine and histamine receptors; HLA-A; HLA-DRA; HM74; HMOX1; HUMCYT2A; ICEBERG; ICOSL; ID2; IFN- α ; IFNA1; IFNA2; IFNA4; IFNA5; IFNA6; IFNA7; IFNB1; IFN γ ; IFNW1; IGBP1; IGF1; IGF1R; IGF2; IGFBP2; IGFBP3; IGFBP6; IL-1; IL10; IL10RA; IL10RB; IL11; IL11RA; IL-12; IL12A; IL12B; IL12RB1; IL12RB2; IL13; IL13RA1; IL13RA2; IL14; IL15; IL15RA; IL16; IL17; IL17B; IL17C; IL17R; IL18; IL18BP; IL18R1; IL18RAP; IL19; IL1A; IL1B; IL1F10; IL1F5; IL1F6; IL1F7; IL1F8; IL1F9; IL1HY1; IL1R1; IL1R2; IL1RAP; IL1RAPL1; IL1RAPL2; IL1RL1; IL1RL2; IL1RN; IL2; IL20; IL20RA; IL21R; IL22; IL22R; IL22RA2; IL23; IL24; IL25; IL26; IL27; IL28A; IL28B; IL29; IL2RA; IL2RB; IL2RG; IL3; IL30; IL3RA; IL4; IL4R; IL5; IL5RA; IL6; IL6R; IL6ST (glycoprotein 130); IL7; IL7R; IL8; IL8RA; IL8RB; IL9; IL9R; ILK; INHA; INHBA; INSL3; INSL4; IRAK1; IRAK2; ITGA1; ITGA2; ITGA3; ITGA6 (a6 integrin); ITGAV; ITGB3; ITGB4 (b 4 integrin); JAG1; JAK1; JAK3; JUN; K6HF; KAI1; KDR; KITLG; KLF5 (GC Box BP); KLF6; KLK10; KLK12; KLK13; KLK14; KLK15; KLK3; KLK4; KLK5; KLK6; KLK9; KRT1; KRT19 (Keratin 19); KRT2A; KRTHB6 (hair-specific type II keratin); LAMA5; LEP (leptin); Lingo-p75; Lingo-Troy; LPS; LTA (TNF-b); LTB; LTB4R (GPR16); LTB4R2; LTBR; MACMARCKS; MAG or Omgp; MAP2K7 (c-Jun); MDK; MIB1; midkine; MIF; MIP-2; MKI67 (Ki-67); MMP2; MMP9; MS4A1; MSMB; MT3 (metallothionein-III); MTSS1; MUC1 (mucin); MYC; MYD88; NCK2; neurocan; NFKB1; NFKB2; NGFB (NGF); NGFR; NgR-Lingo; NgR-Nogo66 (Nogo); NgR-p75; NgR-Troy; NME1 (NM23A); NOX5; NPPB; NR0B1; NR0B2; NR1D1; NR1D2; NR1H2; NR1H3; NR1H4; NR1J2; NR1J3; NR2C1; NR2C2; NR2E1; NR2E3; NR2F1; NR2F2; NR2F6; NR3C1; NR3C2; NR4A1; NR4A2; NR4A3; NR5A1; NR5A2; NR6A1; NRP1; NRP2; NT5E; NTN4; ODZ1; OPRD1; P2RX7; PAP; PART1; PATE; PAWR; PCA3; PCNA; PDGFA; PDGFB; PECAM1; PF4 (CXCL4); PGF; PGR; phosphacan; PIAS2; PIK3CG; PLAUI (uPA); PLG; PLXDC1; PPBP (CXCL7); PPID; PR1; PRKCQ; PRKD1; PRL; PROC; PROK2; PSAP; PSCA; PTAFR; PTEN; PTGS2 (COX-2); PTN; RAC2 (p21Rac2); RARB; RGS1; RGS13; RGS3; RNF110 (ZNF144); ROBO2; S100A2; SCGB1D2 (lipophilin B); SCGB2A1 (mammaglobin 2); SCGB2A2 (mammaglobin 1); SCYE1 (endothelial Monocyte-activating cytokine); SDF2; SERPINA1; SERPINA3; SERPINB5 (maspin); SERPINE1 (PAI-1); SERPINF1; SHBG; SLA2; SLC2A2; SLC33A1; SLC43A1; SLIT2; SPPI; SPRR1B (Spr1); ST6GAL1; STAB1; STAT6; STEAP; STEAP2; TB4R2; TBX21; TCP10; TDGF1; TEK; TGFA; TGFB11; TGFB11I; TGFB2; TGFB3; TGFB1; TGFB1R1; TGFB2R2; TGFB3R3; TH1L; THBS1 (thrombospondin-1); THBS2; THBS4; THPO; TIE (Tie-1); TIMP3; tissue factor; TLR10; TLR2; TLR3; TLR4; TLR5; TLR6; TLR7; TLR8; TLR9; TNF; TNF- α ; TNFAIP2 (B94); TNFAIP3; TNFRSF1A; TNFRSF1A; TNFRSF1B; TNFRSF21; TNFRSF5; TNFRSF6 (Fas); TNFRSF7; TNFRSF8; TNFRSF9; TNFSF10 (TRAIL); TNFSF11 (TRANCE); TNFSF12 (APO3L); TNFSF13 (April); TNFSF13B; TNFSF14 (HVEM-L); TNFSF15 (VEGI); TNFSF18; TNFSF4 (OX40 ligand); TNFSF5 (CD40 ligand); TNFSF6 (FasL); TNFSF7 (CD27 ligand); TNFSF8 (CD30 ligand); TNFSF9 (4-1BB ligand); TOLLIP; Toll-like receptors; TOP2A (topoisomerase IIA); TP53; TPM1; TPM2; TRADD; TRAF1; TRAF2; TRAF3; TRAF4; TRAF5;

TRAF6; TREM1; TREM2; TRPC6; TSLP; TWEAK; VEGF; VEGFB; VEGFC; versican; VHL C5; VLA-4; XCL1 (lymphotactin); XCL2 (SCM-1b); XCR1 (GPR5/CCXCR1); YY1; and ZFPM2.

25. The binding protein according to claim 1, wherein said binding protein is capable of binding a two targets, wherein the two targets are selected from the group consisting of CD138 and CD20; CD138 and CD40; CD20 and CD3; CD38 & CD138; CD38 and CD20; CD38 and CD40; CD40 and CD20; CD19 and CD20; CD-8 and IL-6; PDL-1 and CTLA-4; CTLA-4 and BTNO2; CSPGs and RGM A; IGF1 and IGF2; IGF1/2 and Erb2B; IL-12 and IL-18; IL-12 and TWEAK; IL-13 and ADAM8; IL-13 and CL25; IL-13 and IL-1beta; IL-13 and IL-25; IL-13 and IL-4; IL-13 and IL-5; IL-13 and IL-9; IL-13 and LHR agonist; IL-13 and MDC; IL-13 and MIF; IL-13 and PED2; IL-13 and SPRR2a; IL-13 and SPRR2b; IL-13 and TARC; IL-13 and TGF- β ; IL-1 α and IL-1 β ; MAG and RGM A; NgR and RGM A; NogoA and RGM A; OMGP and RGM A; RGM A and RGM B; Te38 and TNF α ; TNF α and IL-12; TNF α and IL-12p40; TNF α and IL-13; TNF α and IL-15; TNF α and IL-17; TNF α and IL-18; TNF α and IL-1beta; TNF α and IL-23; TNF α and MIF; TNF α and PEG2; TNF α and PGE4; TNF α and VEGF; and VEGFR and EGFR; TNF α and RANK ligand; TNF α and Blys; TNF α and GP130; TNF α and CD-22; and TNF α and CTLA-4.

26. The binding protein according to claim 23, wherein the binding protein is capable of modulating a biological function of one or more targets.

27. The binding protein according to claim 23, wherein the binding protein is capable of neutralizing one or more targets.

28. The binding protein according to any one of claims 23-25, wherein the target is selected from the group consisting of cytokine, chemokine, cell surface protein, enzyme and receptor.

29. The binding protein according to claim 28 wherein the cytokine is selected from the group consisting of lymphokines, monokines, and polypeptide hormones.

30. The binding protein according to claim 29, wherein said cytokines are IL-1 α and IL-1 β .

31. The binding protein according to claim 30, wherein the binding protein comprises a DVD heavy chain amino acid sequence selected from the group consisting of SEQ ID NO. 33, SEQ ID NO. 37, SEQ ID NO. 41, SEQ ID NO. 45, SEQ ID NO. 47, SEQ ID NO. 51, SEQ ID NO. 53, SEQ ID NO. 55, SEQ ID NO. 57, and SEQ ID NO. 59; and a DVD light chain amino acid sequence selected from the group consisting of SEQ ID NO. 35, SEQ ID NO. 39, SEQ ID NO. 43, SEQ ID NO. 46, SEQ ID NO. 49, SEQ ID NO. 52, SEQ ID NO. 54, SEQ ID NO. 56, SEQ ID NO. 58, and SEQ ID NO. 60.

32. The binding protein according to claim 29, wherein said cytokines are TNF- α and IL-13.

33. The binding protein according to claim 29, wherein said cytokines are IL-12 and IL-18.

34. The binding protein according to claim 33, wherein the binding protein comprises a DVD heavy chain amino acid sequence selected from the group consisting of SEQ ID NO. 83, SEQ ID NO. 90, SEQ ID NO. 93, SEQ ID NO. 95, and SEQ ID NO. 114; and a DVD light chain amino acid sequence selected from the group consisting of SEQ ID NO. 86, SEQ ID NO. 91, SEQ ID NO. 94, SEQ ID NO. 46, SEQ ID NO. 96, and SEQ ID NO. 116.

35. The binding protein according to claim 28 wherein the chemokine is selected from the group consisting of CCR2, CCR5 and CXCL-13.

36. The binding protein according to claim 28 wherein the cell surface protein is an integrin.

37. The binding protein according to claim 28 wherein the cell surface proteins are CD-20 and CD3.

38. The binding protein according to claim 37, wherein the binding protein comprises a DVD heavy chain amino acid sequence is SEQ ID NO. 97, and a DVD light chain SEQ ID NO. 101.

39. The binding protein according to claim 28 wherein the enzyme is selected from the group consisting of kinases and proteases.

40. The binding protein according to claim 28 wherein the receptor is selected from the group consisting of lymphokine receptor, monokine receptor, and polypeptide hormone receptor.

41. The binding protein according to claim 28, wherein said binding protein has an on rate constant (K_{on}) to said one or more targets selected from the group consisting of: at least about $10^2 M^{-1} s^{-1}$; at least about $10^3 M^{-1} s^{-1}$; at least about $10^4 M^{-1} s^{-1}$; at least about $10^5 M^{-1} s^{-1}$; and at least about $10^6 M^{-1} s^{-1}$, as measured by surface plasmon resonance.

42. The binding protein according to claim 28, wherein said binding protein has an off rate constant (K_{off}) to said one or more targets selected from the group consisting of: at most about $10^{-3} s^{-1}$; at most about $10^{-4} s^{-1}$; at most about $10^{-5} s^{-1}$; and at most about $10^{-6} s^{-1}$, as measured by surface plasmon resonance.

43. The binding protein according to claim 28, wherein said binding protein has a dissociation constant (K_D) to said one or more targets selected from the group consisting of: at most about $10^{-7} M$; at most about $10^{-8} M$; at most about $10^{-9} M$; at most about $10^{-10} M$; at most about $10^{-11} M$; at most about $10^{-12} M$; and at most $10^{-13} M$.

44. A binding protein conjugate comprising a binding protein described in any one of claims 1-43, said binding protein conjugate further comprising an agent selected from the group consisting of; an immunoadhesion molecule, an imaging agent, a therapeutic agent, and a cytotoxic agent.

45. The binding protein conjugate according to claim 44, wherein said agent is an imaging agent selected from the group consisting of a radiolabel, an enzyme, a fluorescent label, a luminescent label, a bioluminescent label, a magnetic label, and biotin.

46. The binding protein conjugate according to claim 45, wherein said imaging agent is a radiolabel selected from the group consisting of: 3H , ^{14}C , ^{35}S , ^{90}Y , ^{99}Tc , ^{111}In , ^{125}I , ^{131}I , ^{177}Lu , ^{166}Ho , and ^{153}Sm .

47. The binding protein conjugate according to claim 45, wherein said agent is a therapeutic or cytotoxic agent selected from the group consisting of; an anti-metabolite, an alkylating agent, an antibiotic, a growth factor, a cytokine, an anti-angiogenic agent, an anti-mitotic agent, an anthracycline, toxin, and an apoptotic agent.

48. The binding protein according to claim 1, wherein said binding protein is a crystallized binding protein.

49. The crystallized binding protein according to claim 48, wherein said crystal is a carrier-free pharmaceutical controlled release crystal.

50. The crystallized binding protein according to claim 48, wherein said binding protein has a greater half life in vivo than the soluble counterpart of said binding protein.

51. The crystallized binding protein according to claim 48, wherein said binding protein retains biological activity.

52. An isolated nucleic acid encoding a binding protein amino acid sequence of any one of claims 1-22.

53. A vector comprising an isolated nucleic acid according to claim 52.

54. The vector of claim 53 wherein said vector is selected from the group consisting of pcDNA, pTT, pTT3, pEFBOS, pBV, pJV, pcDNA3.1 TOPO, pEF6 TOPO, and pBJ.

55. A host cell comprising a vector according to claim 53.

56. The host cell according to claim 55, wherein said host cell is a prokaryotic cell.

57. The host cell according to claim 56, wherein said host cell is *E. Coli*.

58. The host cell according to claim 55, wherein said host cell is a eukaryotic cell.

59. The host cell according to claim 58, wherein said eukaryotic cell is selected from the group consisting of protist cell, animal cell, plant cell and fungal cell.

60. The host cell according to claim 58, wherein said eukaryotic cell is an animal cell selected from the group consisting of; a mammalian cell, an avian cell, and an insect cell.

61. The host cell according to claim 58, wherein said host cell is a CHO cell.

62. The host cell according to claim 58, wherein said host cell is COS.

63. The host cell according to claim 58, wherein said host cell is a yeast cell.

64. The host cell according to claim 63, wherein said yeast cell is *Saccharomyces cerevisiae*.

65. The host cell according to claim 58, wherein said host cell is an insect Sf9 cell.

66. A method of producing a binding protein, comprising culturing a host cell described in any one of claims 55-65 in culture medium under conditions sufficient to produce the binding protein.

67. The method of claim 66, wherein 50%-75% of the binding protein produced is a dual specific tetravalent binding protein.

68. The method of claim 66, wherein 75%-90% of the binding protein produced is a dual specific tetravalent binding protein.

69. The method of claim 66, wherein 90%-95% of the binding protein produced is a dual specific tetravalent binding protein.

70. A protein produced according to the method of claim 66.

71. A pharmaceutical composition comprising the binding protein of any one of claims 1-51 and 70, and a pharmaceutically acceptable carrier.

72. The pharmaceutical composition of claim 71 further comprising at least one additional therapeutic agent.

73. The pharmaceutical composition of claim 72, wherein said additional agent is selected from the group consisting of: Therapeutic agent, imaging agent, cytotoxic agent, angiogenesis inhibitors; kinase inhibitors; co-stimulation molecule blockers; adhesion molecule blockers; anti-cytokine antibody or functional fragment thereof; methotrexate; cyclosporin; rapamycin; FK506; detectable label or reporter; a TNF antagonist; an antirheumatic; a muscle relaxant, a

narcotic, a non-steroid anti-inflammatory drug (NSAID), an analgesic, an anesthetic, a sedative, a local anesthetic, a neuromuscular blocker, an antimicrobial, an antipsoriatic, a corticosteroid, an anabolic steroid, an erythropoietin, an immunization, an immunoglobulin, an immunosuppressive, a growth hormone, a hormone replacement drug, a radiopharmaceutical, an antidepressant, an antipsychotic, a stimulant, an asthma medication, a beta agonist, an inhaled steroid, an epinephrine or analog, a cytokine, and a cytokine antagonist.

74. A method for treating a subject for a disease or a disorder by administering to the subject the binding protein of any one of claims 1-51 and 70 such that treatment is achieved.

75. The method of claim 74, wherein said disorder is selected from the group comprising rheumatoid arthritis, osteoarthritis, juvenile chronic arthritis, septic arthritis, Lyme arthritis, psoriatic arthritis, reactive arthritis, spondyloarthropathy, systemic lupus erythematosus, Crohn's disease, ulcerative colitis, inflammatory bowel disease, insulin dependent diabetes mellitus, thyroiditis, asthma, allergic diseases, psoriasis, dermatitis scleroderma, graft versus host disease, organ transplant rejection, acute or chronic immune disease associated with organ transplantation, sarcoidosis, atherosclerosis, disseminated intravascular coagulation, Kawasaki's disease, Grave's disease, nephrotic syndrome, chronic fatigue syndrome, Wegener's granulomatosis, Henoch-Schoenlein purpura, microscopic vasculitis of the kidneys, chronic active hepatitis, uveitis, septic shock, toxic shock syndrome, sepsis syndrome, cachexia, infectious diseases, parasitic diseases, acquired immunodeficiency syndrome, acute transverse myelitis, Huntington's chorea, Parkinson's disease, Alzheimer's disease, stroke, primary biliary cirrhosis, hemolytic anemia, malignancies, heart failure, myocardial infarction, Addison's disease, sporadic, polyglandular deficiency type I and polyglandular deficiency type II, Schmidt's syndrome, adult (acute) respiratory distress syndrome, alopecia, alopecia areata, seronegative arthropathy, arthropathy, Reiter's disease, psoriatic arthropathy, ulcerative colitic arthropathy, enteropathic synovitis, chlamydia, *yersinia* and *salmonella* associated arthropathy, spondyloarthropathy, atheromatous disease/arteriosclerosis, atopic allergy, autoimmune bullous disease, pemphigus vulgaris, pemphigus foliaceus, pemphigoid, linear IgA disease, autoimmune haemolytic anaemia, Coombs positive haemolytic anaemia, acquired pernicious anaemia, juvenile pernicious anaemia, myalgic encephalitis/Royal Free Disease, chronic mucocutaneous candidiasis, giant cell arteritis, primary sclerosing hepatitis, cryptogenic autoimmune hepatitis, Acquired Immunodeficiency Disease Syndrome, Acquired Immunodeficiency Related Diseases, Hepatitis B, Hepatitis C, common varied immunodeficiency (common variable hypogammaglobulinaemia), dilated cardiomyopathy, female infertility, ovarian failure, premature ovarian failure, fibrotic lung disease, cryptogenic fibrosing alveolitis, post-inflammatory interstitial lung disease, interstitial pneumonitis, connective tissue disease associated interstitial lung disease, mixed connective tissue disease associated lung disease, systemic sclerosis associated interstitial lung disease, rheumatoid arthritis associated interstitial lung disease, systemic lupus erythematosus associated lung disease, dermatomyositis/polymyositis associated lung disease, Sjögren's disease associated lung disease, ankylosing spondylitis associated lung disease, vasculitic diffuse lung

disease, haemosiderosis associated lung disease, drug-induced interstitial lung disease, fibrosis, radiation fibrosis, bronchiolitis obliterans, chronic eosinophilic pneumonia, lymphocytic infiltrative lung disease, postinfectious interstitial lung disease, gouty arthritis, autoimmune hepatitis, type-1 autoimmune hepatitis (classical autoimmune or lupoid hepatitis), type-2 autoimmune hepatitis (anti-LKM antibody hepatitis), autoimmune mediated hypoglycaemia, type B insulin resistance with acanthosis nigricans, hypoparathyroidism, acute immune disease associated with organ transplantation, chronic immune disease associated with organ transplantation, osteoarthritis, primary sclerosing cholangitis, psoriasis type 1, psoriasis type 2, idiopathic leucopenia, autoimmune neutropenia, renal disease NOS, glomerulonephritides, microscopic vasculitis of the kidneys, Lyme disease, discoid lupus erythematosus, male infertility idiopathic or NOS, sperm autoimmunity, multiple sclerosis (all subtypes), sympathetic ophthalmia, pulmonary hypertension secondary to connective tissue disease, Goodpasture's syndrome, pulmonary manifestation of polyarteritis nodosa, acute rheumatic fever, rheumatoid spondylitis, Still's disease, systemic sclerosis, Sjögren's syndrome, Takayasu's disease/arteritis, autoimmune thrombocytopenia, idiopathic thrombocytopenia, autoimmune thyroid disease, hyperthyroidism, goitrous autoimmune hypothyroidism (Hashimoto's disease), atrophic autoimmune hypothyroidism, primary myxoedema, phacogenic uveitis, primary vasculitis, vitiligo acute liver disease, chronic liver diseases, alcoholic cirrhosis, alcohol-induced liver injury, choleosatis, idiosyncratic liver disease, Drug-Induced hepatitis, Non-alcoholic Steatohepatitis, allergy and asthma, group B streptococci (GBS) infection, mental disorders (e.g., depression and schizophrenia), Th2 Type and Th1 Type mediated diseases, acute and chronic pain (different forms of pain), and cancers such as lung, breast, stomach, bladder, colon, pancreas, ovarian, prostate and rectal cancer and hematopoietic malignancies (leukemia and lymphoma) Abetalipoproteinemia, Acrocyanosis, acute and chronic parasitic or infectious processes, acute leukemia, acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML), acute or chronic bacterial infection, acute pancreatitis, acute renal failure, adenocarcinomas, aerial ectopic beats, AIDS dementia complex, alcohol-induced hepatitis, allergic conjunctivitis, allergic contact dermatitis, allergic rhinitis, allograft rejection, alpha-1-antitrypsin deficiency, amyotrophic lateral sclerosis, anemia, angina pectoris, anterior horn cell degeneration, anti cd3 therapy, antiphospholipid syndrome, anti-receptor hypersensitivity reactions, aortic and peripheral aneurysms, aortic dissection, arterial hypertension, arteriosclerosis, arteriovenous fistula, ataxia, atrial fibrillation (sustained or paroxysmal), atrial flutter, atrioventricular block, B cell lymphoma, bone graft rejection, bone marrow transplant (BMT) rejection, bundle branch block, Burkitt's lymphoma, Burns, cardiac arrhythmias, cardiac stun syndrome, cardiac tumors, cardiomyopathy, cardiopulmonary bypass inflammation response, cartilage transplant rejection, cerebellar cortical degenerations, cerebellar disorders, chaotic or multifocal atrial tachycardia, chemotherapy associated disorders, chronic myelocytic leukemia (CML), chronic alcoholism, chronic inflammatory pathologies, chronic lymphocytic leukemia (CLL), chronic obstructive pulmonary disease (COPD), chronic salicylate intoxication, colorectal carcinoma, congestive heart failure, conjunctivitis, contact dermatitis, cor pulmonale, coronary artery dis-

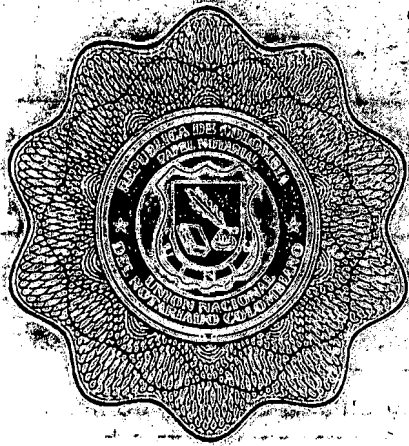
ease, Creutzfeldt-Jakob disease, culture negative sepsis, cystic fibrosis, cytokine therapy associated disorders, Dementia pugilistica, demyelinating diseases, dengue hemorrhagic fever, dermatitis, dermatologic conditions, diabetes, diabetes mellitus, diabetic atherosclerotic disease, Diffuse Lewy body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

orchitis/epididymitis, orchitis/vasectomy reversal procedures, organomegaly, osteoporosis, pancreas transplant rejection, pancreatic carcinoma, paraneoplastic syndrome/hypercalcemia of malignancy, parathyroid transplant rejection, pelvic inflammatory disease, perennial rhinitis, pericardial disease, peripheral atherosclerotic disease, peripheral vascular disorders, peritonitis, pernicious anemia, *pneumocystis carinii* pneumonia, pneumonia, POEMS syndrome (polyneuropathy, organomegaly, endocrinopathy, monoclonal gammopathy, and skin changes syndrome), post perfusion syndrome, post pump syndrome, post-MI cardiomyopathy syndrome, preeclampsia, Progressive supranuclear Palsy, primary pulmonary hypertension, radiation therapy, Raynaud's phenomenon and disease, Raynaud's disease, Refsum's disease, regular narrow QRS tachycardia, renovascular hypertension, reperfusion injury, restrictive cardiomyopathy, sarcomas, scleroderma, senile chorea, Senile Dementia of Lewy body type, seronegative arthropathies, shock, sickle cell anemia, skin allograft rejection, skin changes syndrome, small bowel transplant rejection, solid tumors, specific arrhythmias, spinal ataxia, spinocerebellar degenerations, streptococcal myositis, structural lesions of the cerebellum, Subacute sclerosing panencephalitis, Syphilis, syphilis of the cardiovascular system, systemic anaphylaxis, systemic inflammatory response syndrome, systemic onset juvenile rheumatoid arthritis, T-cell or FAB ALL, Telangiectasia, thromboangitis obliterans, thrombocytopenia, toxicity, transplants, trauma/hemorrhage, type III hypersensitivity reactions, type IV hypersensitivity, unstable angina, uremia, urosepsis, urticaria, valvular heart diseases, varicose veins, vasculitis, venous diseases, venous thrombosis, ventricular fibrillation, viral and fungal infections, viral encephalitis/aseptic meningitis, vital-associated hemaphagocytic syndrome, Wernicke-Korsakoff syndrome, Wilson's disease, xenograft rejection of any organ or tissue.

76. The method according to claim 74, wherein said administering to the subject is by at least one mode selected from parenteral, subcutaneous, intramuscular, intravenous, intrarticular, intrabronchial, intraabdominal, intracapsular, intracartilaginous, intracavitary, intracelal, intracerebellar, intracerebroventricular, intracolic, intracervical, intragastric, intrahepatic, intramyocardial, intraosteal, intrapelvic, intrapericardiac, intraperitoneal, intrapleural, intraprostatic, intrapulmonary, intrarectal, intrarenal, intraretinal, intraspinal, intrasynovial, intrathoracic, intrauterine, intravesical, bolus, vaginal, rectal, buccal, sublingual, intranasal, and transdermal.

* * * * *

AA 3286877 NOTARIA TERCERA



ESCRITURA NUMERO CUATRO MIL QUINIENTOS TREINTA Y NUEVE (No. 4.539)

En la ciudad de Cali, Capital del Departamento del Valle del Cauca, República de Colombia, a los VEINTITRES (23) días del mes de OCTUBRE del año DOS MIL UNO --

(2.001), ante mí, JORGE ENRIQUE CAICEDO ZAMORANO, Notario TERCERO (3o.) del Circulo de Cali, - - - - -

compareció JUAN MANUEL BARBERI OSPINA mayor de edad, vecino de Cali, identificado con la cédula de ciudadanía número 14.949.561 de Cali, y dijo: ----

PRIMERO: Que obra en su carácter de Representante Legal de en nombre y representación de TECNOQUIMICAS S.A. sociedad comercial organizada y existente conforme a las leyes colombianas, con domicilio y sede principal en Cali constituida mediante escritura pública número 2670 de la Notaría Segunda de Cali de fecha 12 de junio de 1957, reformada en distintas oportunidades, según consta en el certificado expedido por la Cámara de Comercio de Cali

SEGUNDO: Que en tal carácter, por este público instrumento, concede Poder Especial pero amplio y suficiente a JOSE LUIS REYES VILLAMIZAR, mayor de edad, vecino de Bogotá, identificado con C.C. # 79.152.473 de Isaquén, abogado en ejercicio, portador de la T.P. 44655 del G.S. de la J., domiciliado en Bogotá, D.C., República de Colombia con dirección carrera 7 No. 88.23 oficina 205, de la misma ciudad, como su representante con poder especial, amplio y suficiente, para obtener la protección de nuestra propiedad industrial y contra la competencia desleal, a cuyo efecto lo autorizo, para que nos represente ante cualesquiera entidad o persona, ejerzan o no jurisdicción, especialmente ante las del orden administrativo, contencioso-administrativo y judicial, en todo lo concerniente a los siguientes asuntos: a) Obtención, formulación de observaciones y oposición a patentes de invención, modelos y dibujos industriales y el registro de licencias de uso de esos derechos; b) Las acciones de oposición, cancelación, nulidad, medidas cautelares, concesión de licencias y en general los procesos civiles, penales, administrativos o contencioso-administrativos relacionados con estos derechos u otros de diferente naturaleza; acciones y procesos que promovamos o se nos promuevan; y c) para

Como Notario Ventitres de este Circulo hago constar que esta fotocopia autentica que he tenido a la vista. 113 AGO 2001

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LUZ MERY CORDELL ELABORACION DE ESCRITURAS Notaria 3 de Cali

que en todos los asuntos arriba determinados, tanto en los términos señalados por los artículos 42 y complementarios de la Decisión 486 de la Comunidad Andina como del artículo 70 del C.P.C pueda sustituir este mandato, transigir, conciliar, admitir los hechos del proceso, desistir, cancelar, recibir, renunciar, y ratificar los actos de agentes oficiosos y ceder a título gratuito y/o oneroso, licenciar, inscribir contratos de cesión y de licencia, inscripción de prendas, levantamiento de prendas, inscribir derechos de retención y en general todos los asuntos que se deban tramitar ante la Superintendencia de Industria y Comercio.

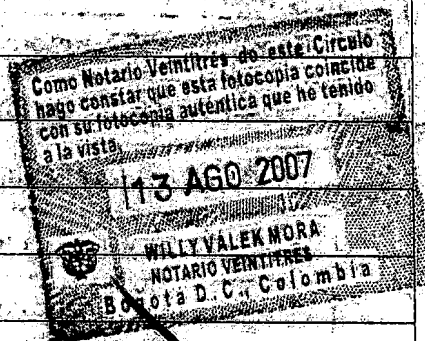
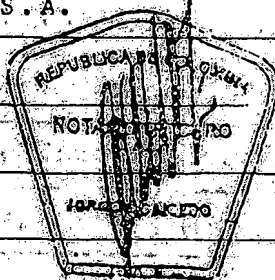
Leído el presente instrumento al otorgante, lo aprobó y en constancia firma conmigo el Notario de todo lo cual doy fe.

Se agregan comprobantes. Derechos \$30.000.00 Decreto 1681 de Septiembre 16 de 1996. Reformado mediante Resolución No. 5839 de Diciembre 27 del año 2.000 y Decreto 1326 de Julio 06 del año 2.001. Se corrió en la hoja número AA 3286877. ENMENDADO "OCTUBRE" VALE.

Juan m. Barberi

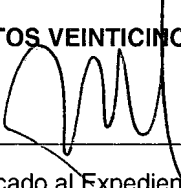
JUAN MANUEL BARBERI OSPINA

En rep. de TECNOQUIMICAS S.A.



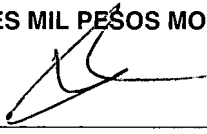
El Notario Tercero de Cali

Dr. JORGE ENRIQUE CALCEDO ZAMORANO

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO NIT : 800.176.089-2 - / -					
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				Bogotá D.C., Enero 30 de 2013 - 15:27:07	
RECIBIDO DE : REYES Y REYES ABOGADOS				NI 900.048.328	
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CONSIGNACION	BANCO DE BOGOTA	062754387	494785966	29/01/2013	290.000.00
*** Conceptos Pagados ***					
CANT. RENTISTICO		CONCEPTO		Vr.UNDITARIO	Vr.CONCEPTO
1 50005-01-04 PRESENTACION DE OBSERVACIONES A SOLICITUDES		12 OPOSICION POR CADA CLASE-MARCAS Y LEMAS COMERCIALES		325.000.00	325.000.00
					\$325.000.00
SON: **TRESCIENTOS VEINTICINCO MIL PESOS MONEDA CORRIENTE***					
Responsable: 					
Recibo de Caja Aplicado al Expediente No. _____					
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Sede Centro: Carrera 13 No. 27 - 00 Pisos 3,4,5 y 10 Bogotá, D.C.- Colombia Web: www.sic.gov.co e-mai: info@sic.gov.co Conmutador: (571) 5870000 Fax: (571) 5870284 Línea: 018000-910165 Call Center: (571) 6513240 Enero 30 de 2013 - 15:27:08					

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*** Conceptos Pagados ***					
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1 50005-01-08	PRORROGA DE TERMINOS	1608 N.CREACION PRORROGA DE TERMINOS O PLAZO ADICIONAL	103.000.00	103.000.00	
				=====	
				\$103.000.00	
SON: **CIENTO TRES MIL PESOS MONEDA CORRIENTE**					
Responsable: 					

Recibo de Caja Aplicado al Expediente No. _____					
SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO  No. 12-081792- -00008-0000 Fecha: 2013-01-30 15:53:56 Dep. 2020 DIR.NUEVASCR Tra. 11 PATENTEIYII Eve: 379 FASENACIONALII Act. 400 OPOSIOBSERVTER Folios: 23					
Sede Centro: Carrera 13 No. 27 - 00 Pisos 3,4,5 y 10 Bogotá, D.C.- Colombia					
Web: www.sic.gov.co e-mai: info@sic.gov.co Conmutador: (571) 5870000 Fax: (571) 5870284 Linea: 018000-910165 Call Center: (571) 6513240					
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