



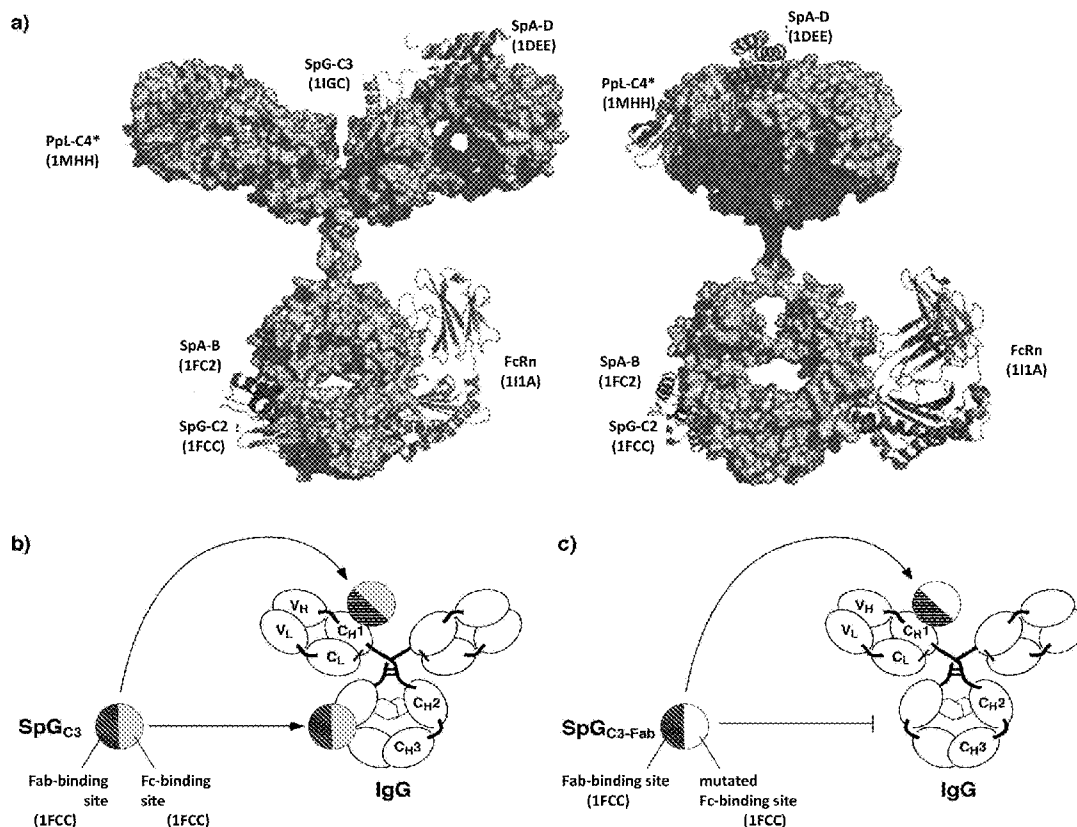
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(19) **United States**(12) **Patent Application Publication**
Kontermann et al.(10) **Pub. No.: US 2014/0220017 A1**(43) **Pub. Date: Aug. 7, 2014**(54) **SERUM HALF-LIFE EXTENSION USING
IGBD**(30) **Foreign Application Priority Data**

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(71) Applicant: **UNIVERSITÄT STUTTGART,**
Stuttgart (DE)**Publication Classification**(72) Inventors: **Roland Kontermann**, Nürtingen (DE);
Felix Unverdorben, Stuttgart (DE);
Meike Hutt, Schomdorf (DE)(51) **Int. Cl.**
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CPC **A61K 47/48715** (2013.01); **A61K 47/48676**
(2013.01)(21) Appl. No.: **14/345,845**USPC .. **424/134.1**; 530/391.9; 530/391.5; 435/188;
530/387.3; 536/23.4; 435/320.1; 435/252.3;
435/254.11; 435/419; 435/328; 514/44 R(86) PCT No.: **PCT/EP2012/068802**§ 371 (c)(1),
(2), (4) Date: **Mar. 19, 2014****Related U.S. Application Data**(60) Provisional application No. 61/538,310, filed on Sep.
23, 2011.(57) **ABSTRACT**

The present invention relates to complexes comprising (i) an immunoglobulin (Ig) binding moiety and (ii) a pharmaceutically active moiety, wherein the Ig binding moiety specifically binds to the constant domain 1 of the heavy chain (C_{H1}) of an Ig molecule and their use for therapy and prophylaxis.



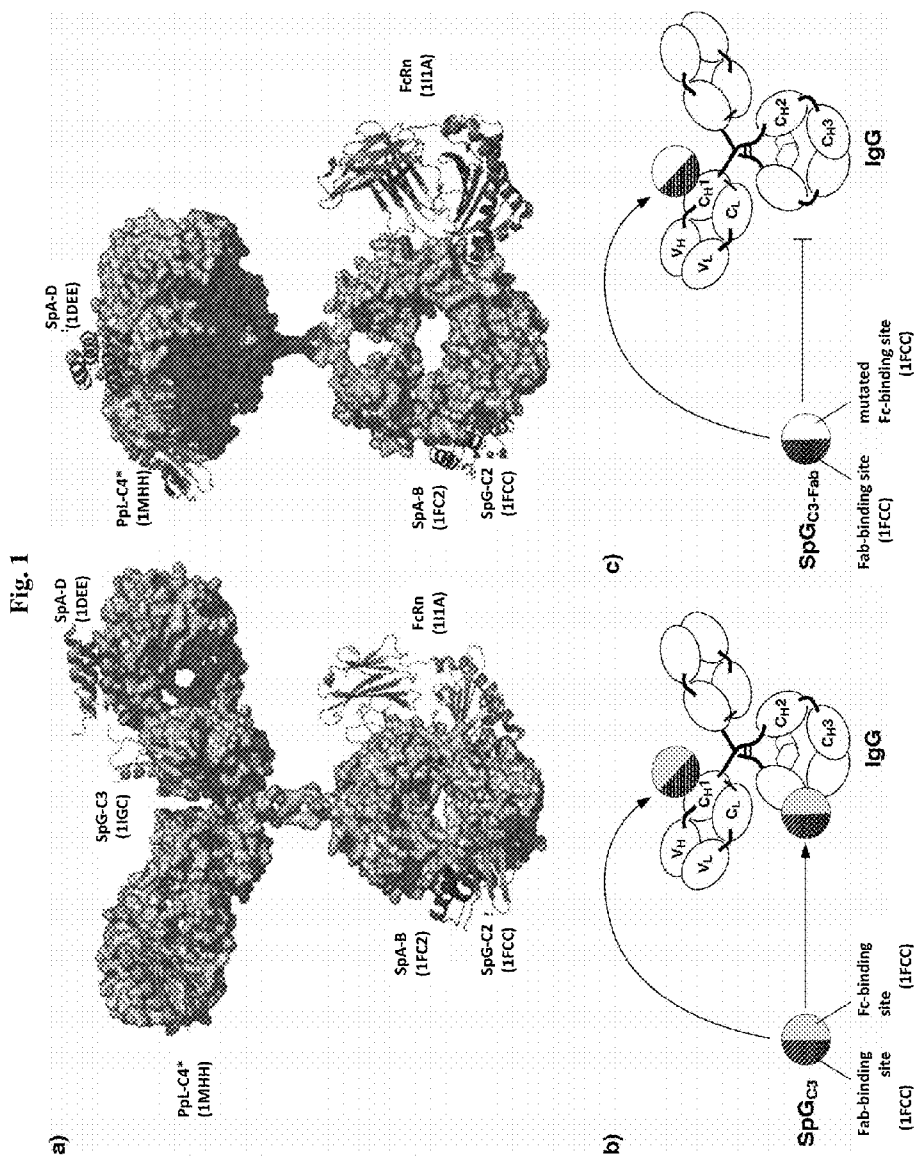


Fig. 2

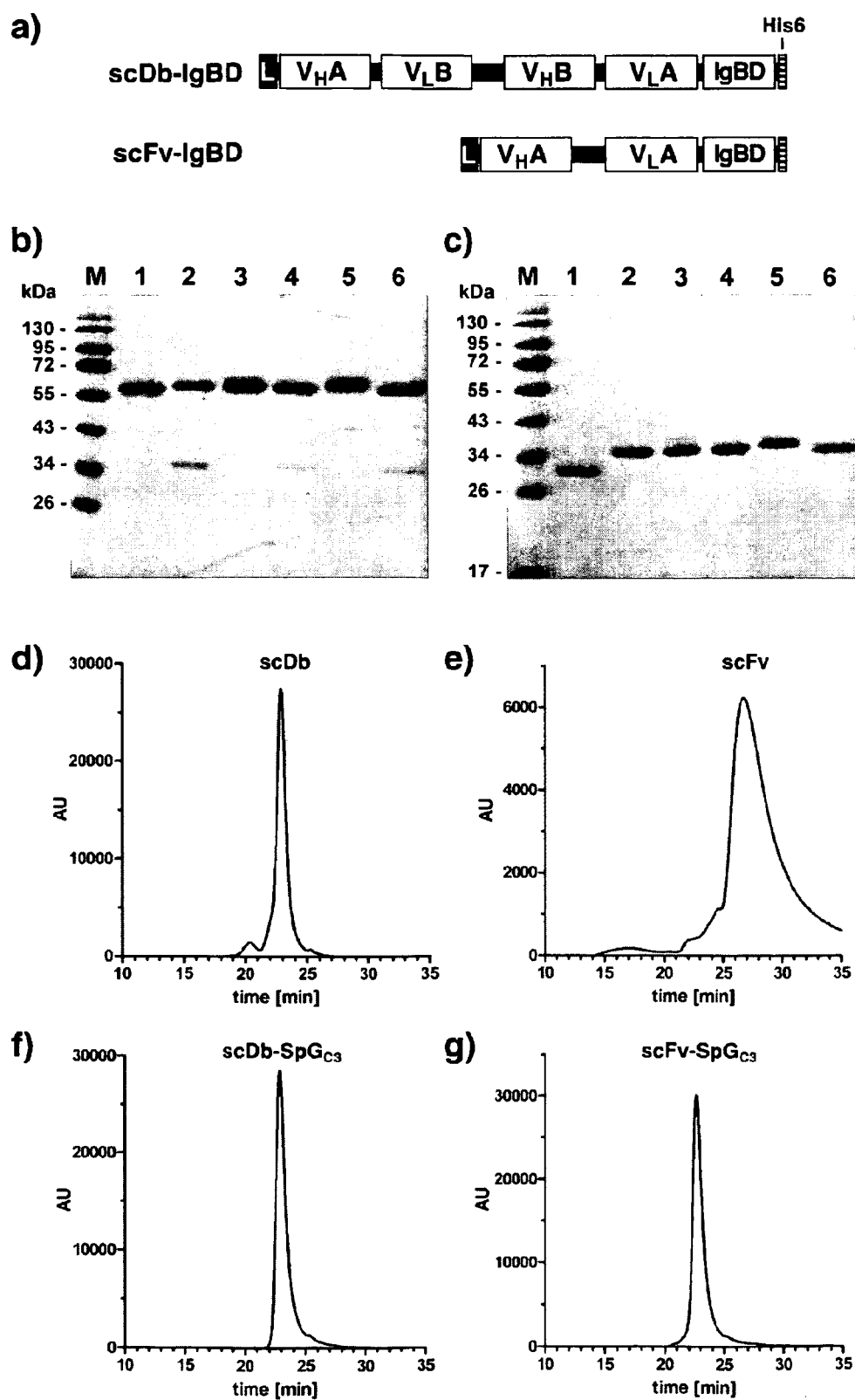


Fig. 3

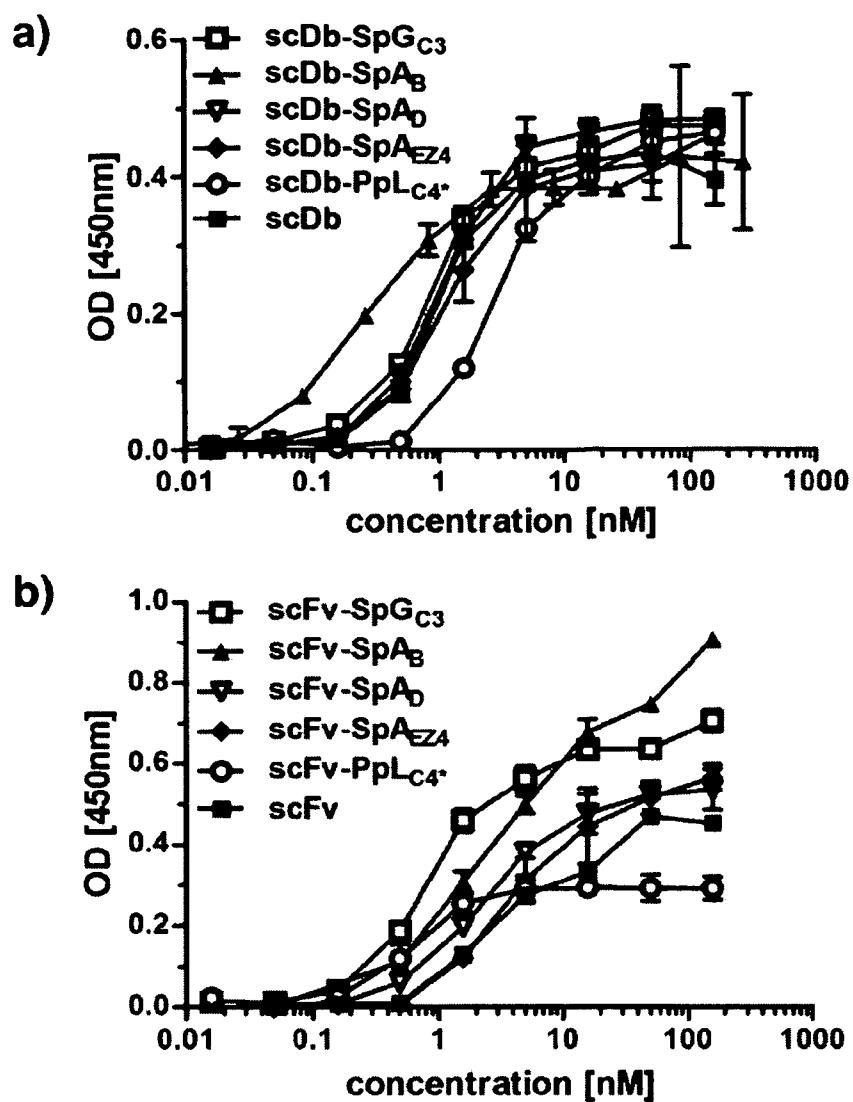


Fig. 4 a)

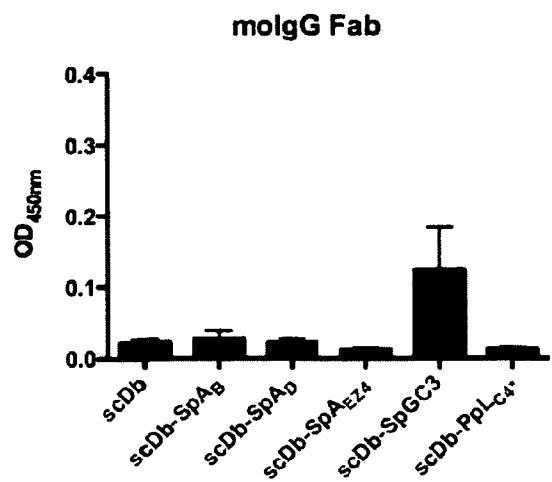
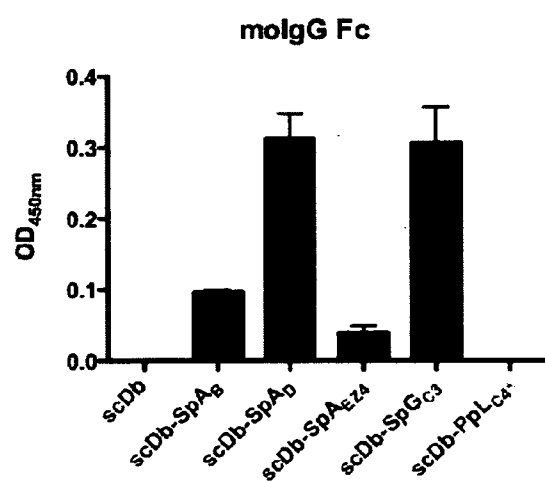
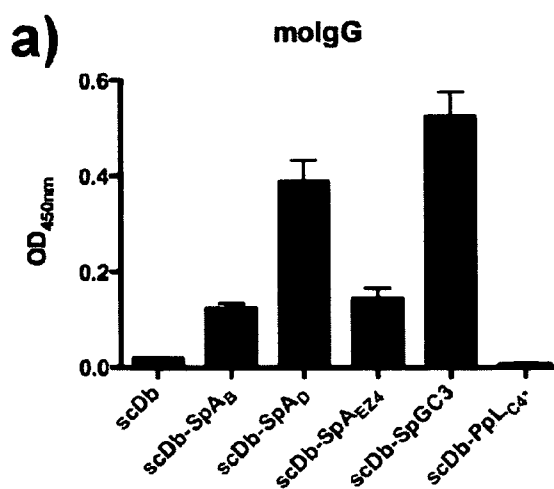


Fig. 4 b)

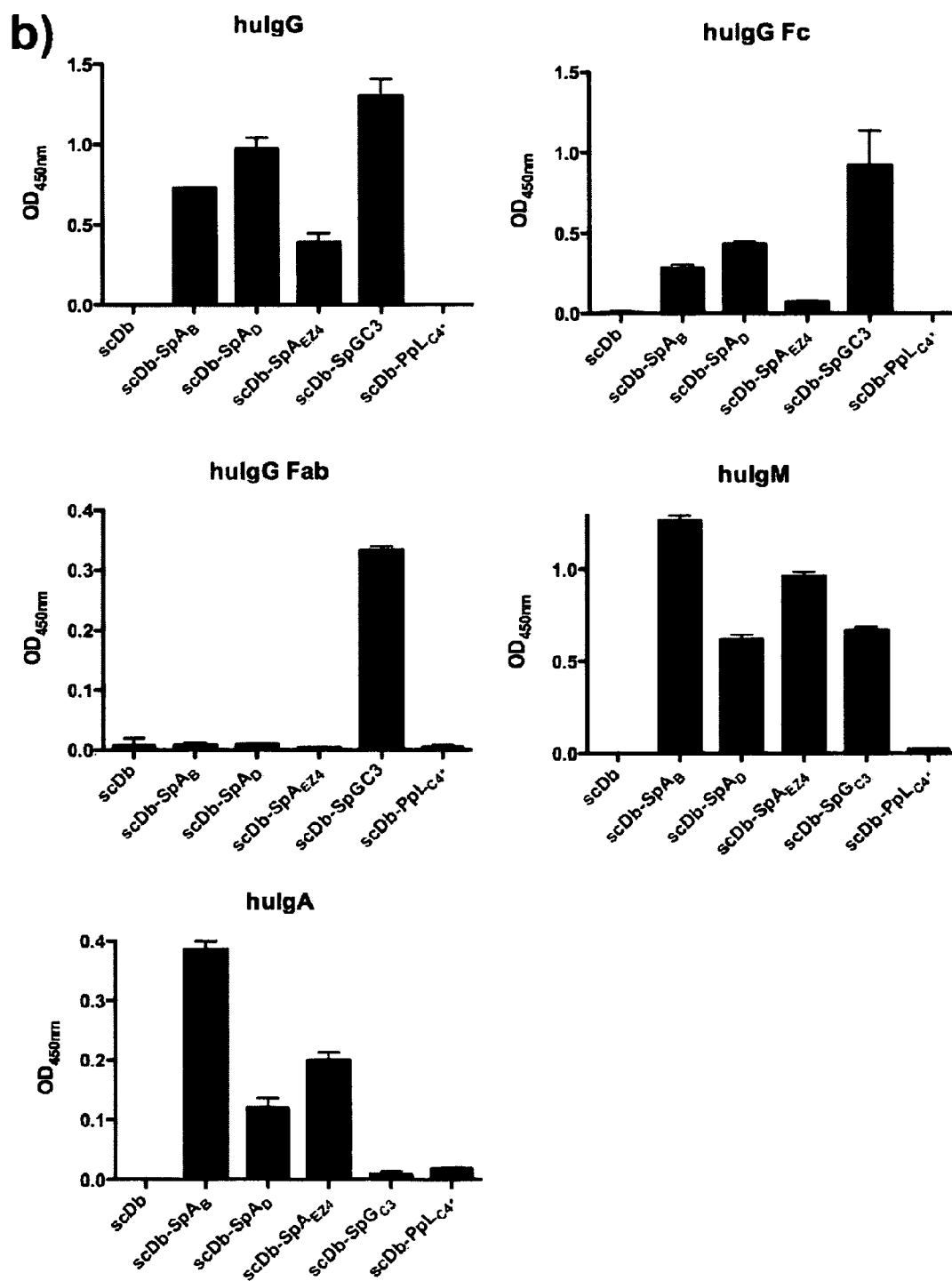


Fig. 5, part 1

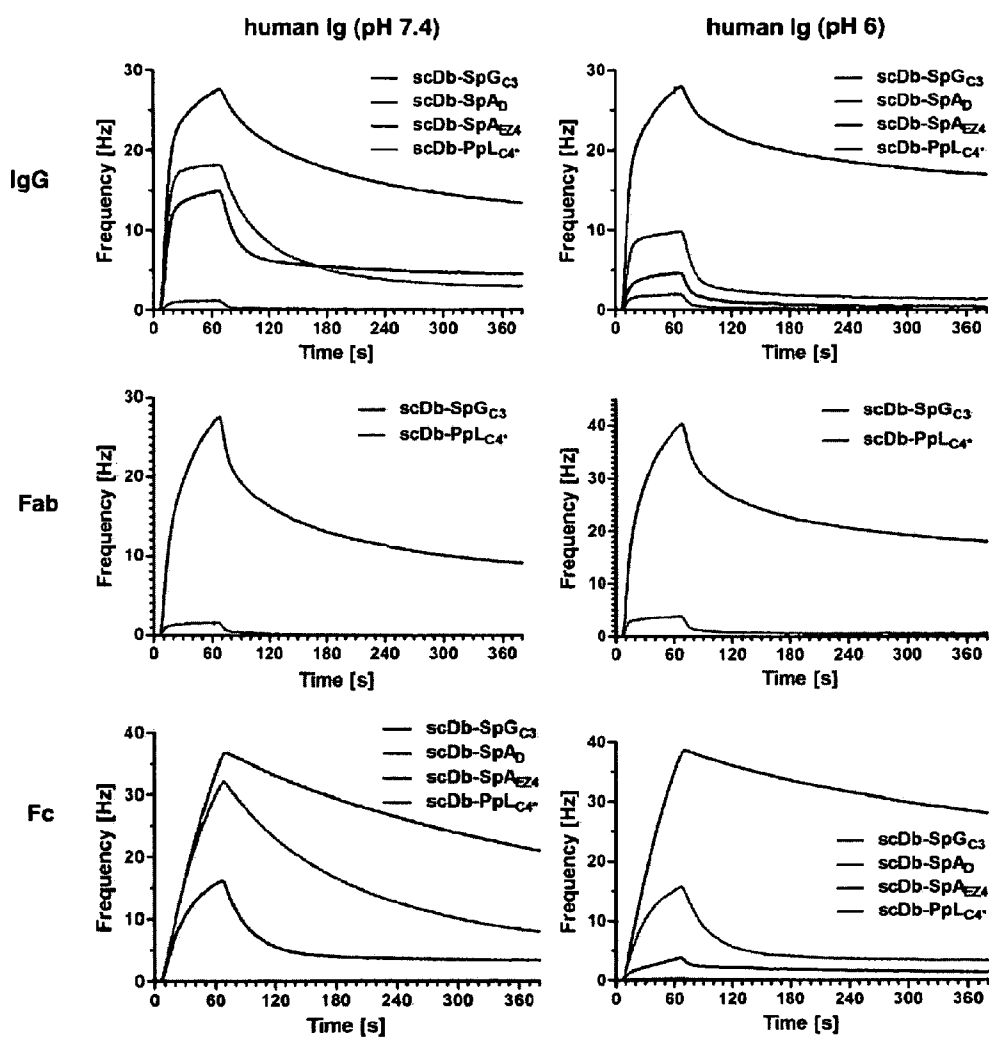


Fig. 5, part 2

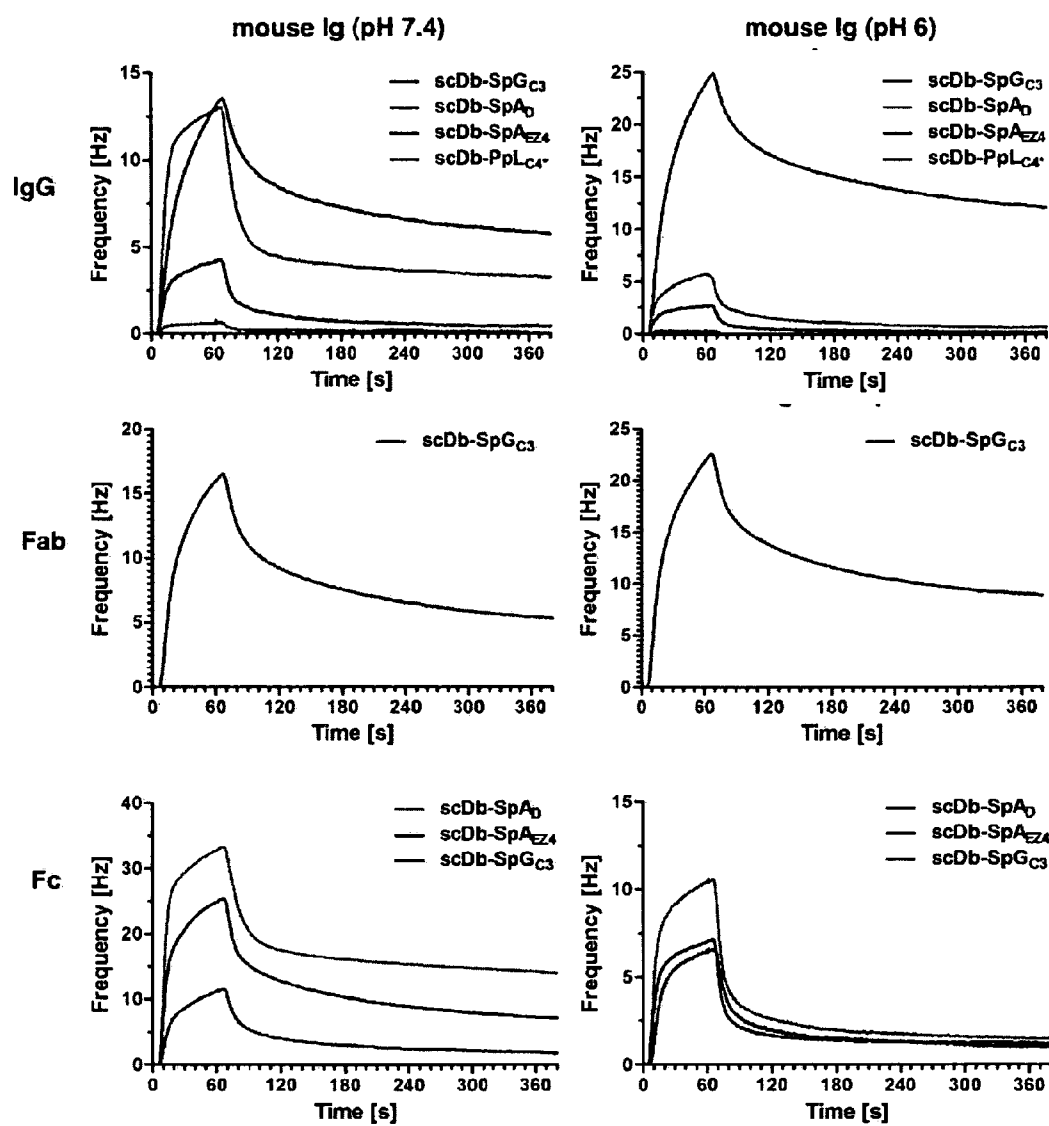


Fig. 6

Affinity measurements

construct	protein	K _D (nM) pH 7.4	K _D (nM) pH 6
SpA _B ¹	human IgG	9	350
	mouse IgG	380	15000
SpA _D	human IgG	22	148
	human IgG Fab	-	n.d.
	human IgG Fc	14	30
	mouse IgG	56	773
	mouse IgG Fab	-	n.d.
	mouse IgG Fc	54	980
SpA _{EZ4}	human IgG	37	65
	human IgG Fab	-	n.d.
	human IgG Fc	24	87
	mouse IgG	78	2480
	mouse IgG Fab	-	n.d.
	mouse IgG Fc	120	1000
SpG _{C3}	human IgG	6	5
	human IgG Fab	190	150
	human IgG Fc	10	3.6
	mouse IgG	58	46
	mouse IgG Fab	190	190
	mouse IgG Fc	24	n.d.
PpL _{C4} *	human IgG	1510	1130
	human IgG Fab	3100	n.d.
	human IgG Fc	-	n.d.
	mouse IgG	-	n.d.
	mouse IgG Fab	-	n.d.
	mouse IgG Fc	-	n.d.

n.d., not determined.

Fig. 7

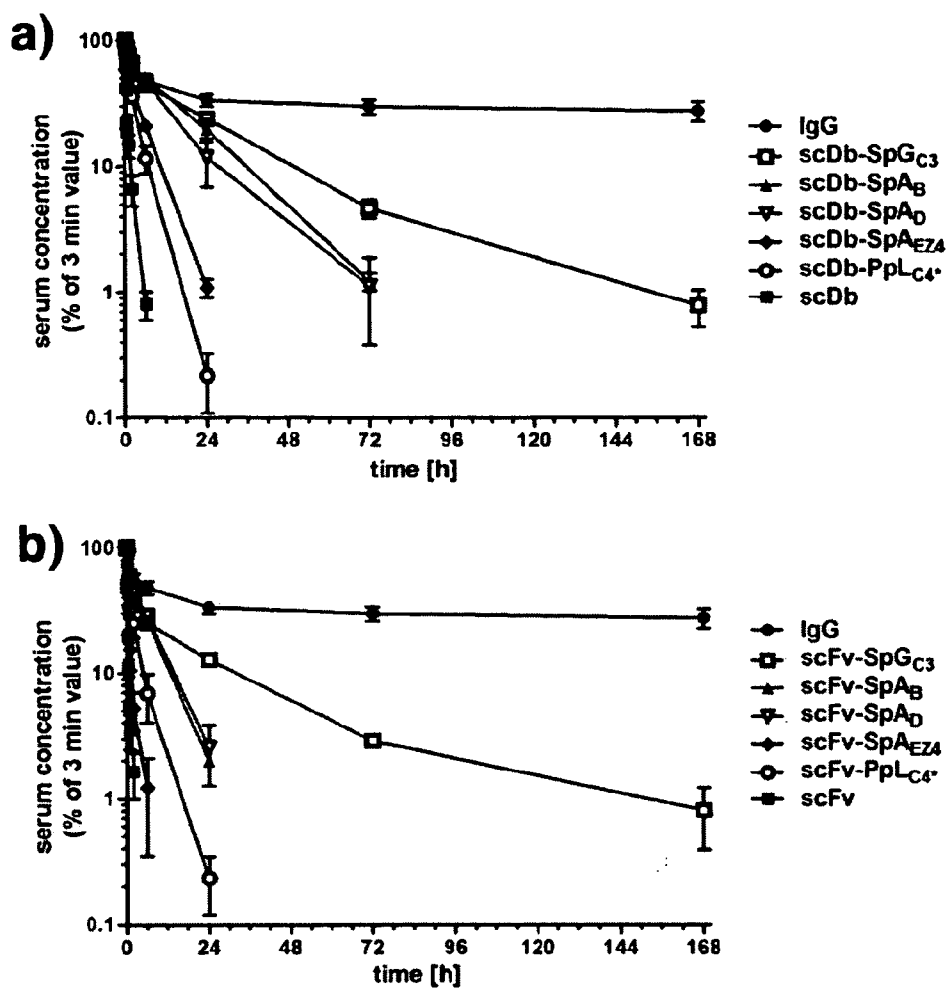
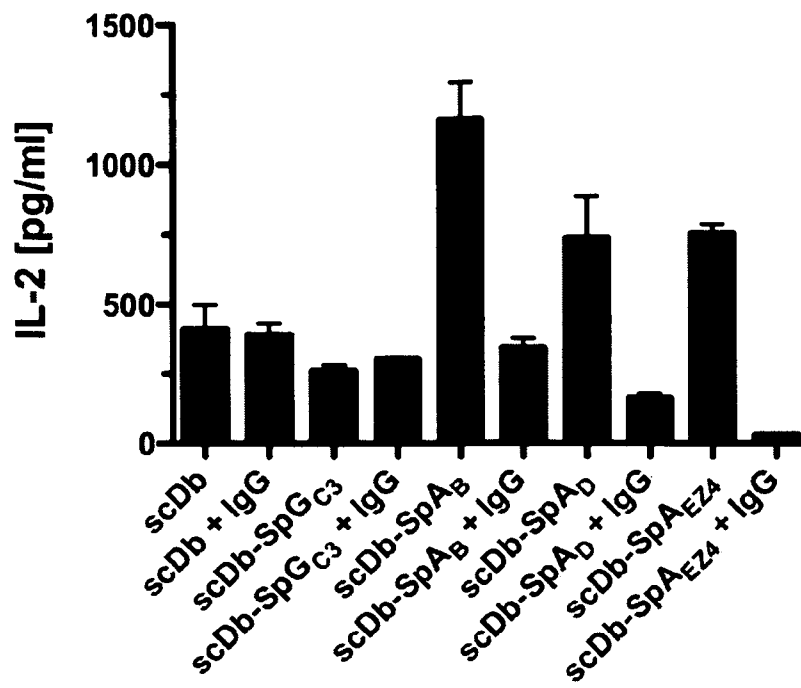


Fig. 8

Pharmacokinetic properties

construct	M _r (kDa)	S _r (nm)	t _{1/2} α (h)	t _{1/2} β (h)	AUC (%h)
scDb	54.5	2.6	0.2 ± 0.1	1.3 ± 0.3	52 ± 17
scDb-SpA _B	59.9	2.6	2.2 ± 0.5	11.8 ± 1.6	1407 ± 352
scDb-SpA _D	60.1	2.2	2.0 ± 0.8	9.0 ± 4.6	1042 ± 403
scDb-SpA _{EZ4}	59.7	2.4	1.0 ± 0.3	4.2 ± 0.6	435 ± 79
scDb-SpG _{C3}	59.6	2.7	2.8 ± 1.2	23.3 ± 5.9	1879 ± 279
scDb-PpL _{C4} *	60.3	2.3	0.8 ± 0.1	2.4 ± 0.6	233 ± 136
scFv	26.7	1.2	0.1 ± 0.01	0.6 ± 0.2	16 ± 4
scFv-SpA _B	33.6	2.7	1.0 ± 0.1	4.3 ± 0.7	376 ± 200
scFv-SpA _D	33.8	2.3	1.1 ± 0.3	4.9 ± 1.0	434 ± 222
scFv-SpA _{EZ4}	33.5	2.4	0.4 ± 0.2	1.1 ± 0.4	63 ± 29
scFv-SpG _{C3}	33.4	2.7	0.8 ± 0.1	20.8 ± 11.0	1040 ± 589
scFv-PpL _{C4} *	34.0	2.5	0.6 ± 0.2	2.7 ± 1.0	207 ± 128

Fig. 9



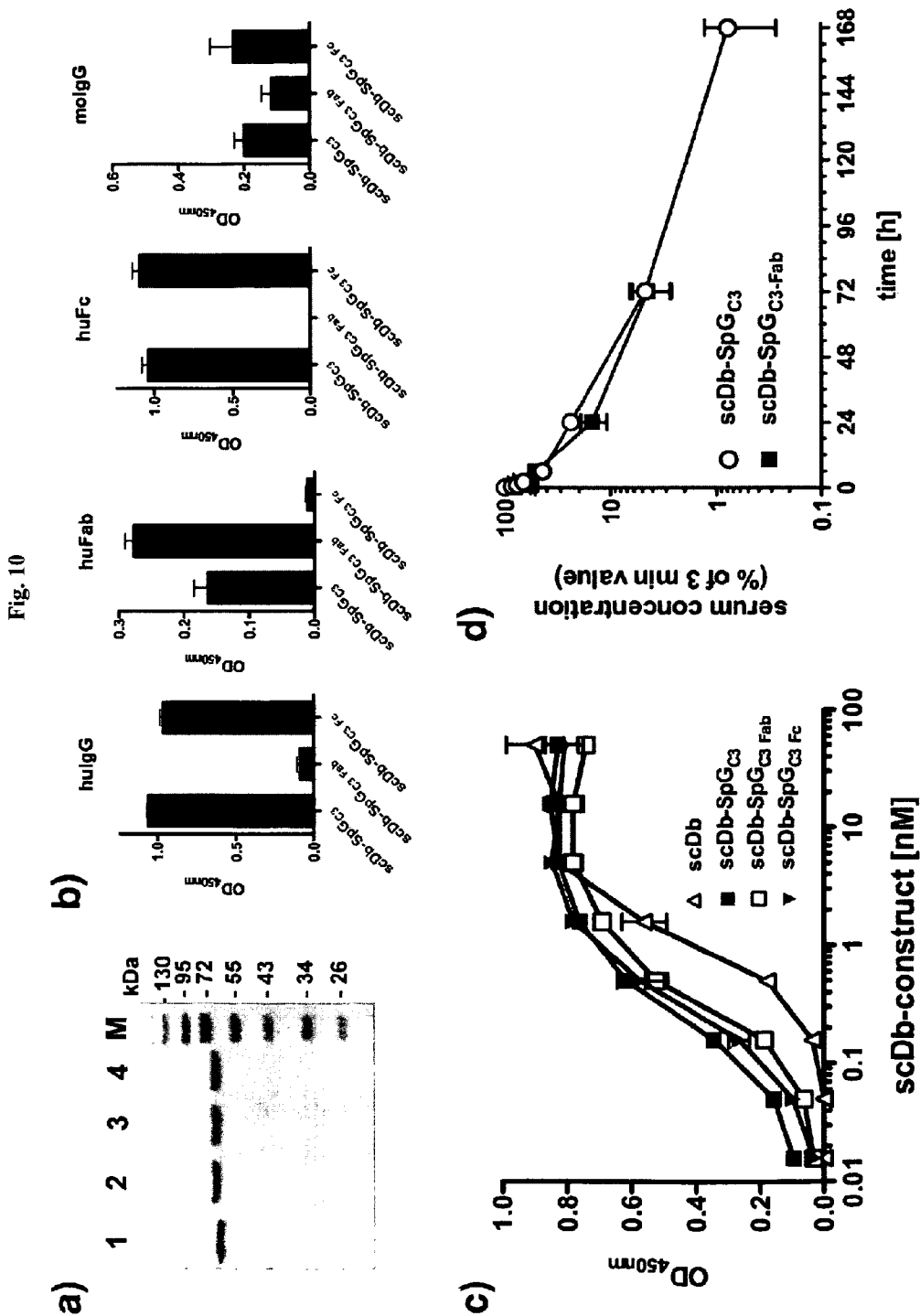


Fig. 11

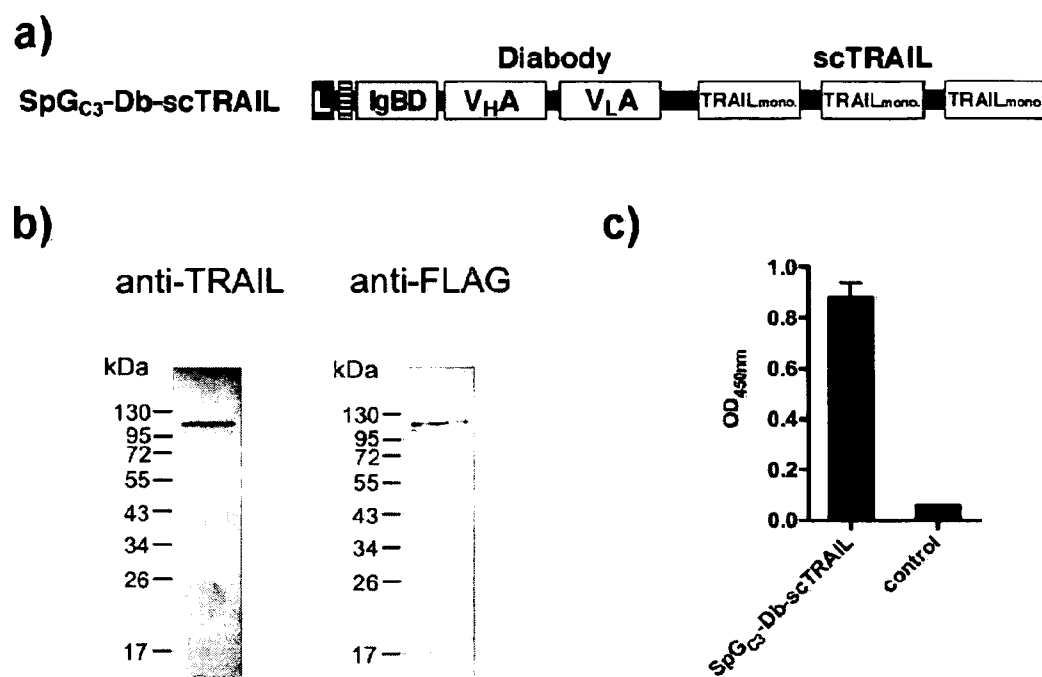


Fig. 12

IgG CH1 domain sequences

EU index	122	123	124	125	126	127	...	207	208	209	210	211	?	212	213	214
human γ 1	Gly	Pro	Ser	Val	Phe	Pro	...	Ser	Asn	Thr	Lys	Val	-	Asp	Lys	Lys
human γ 2	Gly	Pro	Ser	Val	Phe	Pro	...	Ser	Asn	Thr	Lys	Val	-	Asp	Lys	Thr
human γ 3	Gly	Pro	Ser	Val	Phe	Pro	...	Ser	Asn	Thr	Lys	Val	-	Asp	Lys	Arg
human γ 4	Gly	Pro	Ser	Val	Phe	Pro	...	Ser	Asn	Thr	Lys	Val	-	Asp	Lys	Arg
human μ	Ala	Pro	Thr	Leu	Phe	Pro	...	Asn	Gly	Asn	Lys	-	-	Glu	Lys	Asn
human ϵ	Ser	Pro	Ser	Val	Phe	Pro	...	Ser	Ser	Thr	Asp	Trp	Val	Asp	Asn	Lys
human δ	Ala	Pro	Asp	Val	Phe	Pro	...	Ser	Lys	Ser	Lys	-	-	Lys	Glu	Ile
human α 1	Ser	Pro	Lys	Val	Phe	Pro	...	Asn	Pro	Ser	Gln	-	-	Asp	Val	Thr
human α 2	Ser	Pro	Lys	Val	Phe	Pro	...	Asn	Pro	Ser	Gln	-	-	Asp	Val	Thr
mouse γ 1	Pro	Pro	Ser	Val	Tyr	Pro	...	Ser	Ser	Thr	Lys	Val	-	Asp	Lys	Lys
mouse γ 2a	Ala	Pro	Ser	Val	Tyr	Pro	...	Ser	Ser	Thr	Lys	Val	-	Asp	Lys	Lys
mouse γ 2b	Ala	Pro	Ser	Val	Tyr	Pro	...	Ser	Ser	Thr	Thr	Val	-	Asp	Lys	Lys
mouse γ 3	Ala	Pro	Ser	Val	Tyr	Pro	...	Ser	Lys	Thr	Glu	Leu	-	Ile	Lys	Arg
rat g1	Ala	Pro	Ser	Val	Tyr	Pro	...	Ser	Ser	Thr	Lys	Val	-	Asp	Lys	Lys

Differences to human γ 1 are shown in bold letters

[illegible]

Fig. 14, part 1
scDb-SpG-C3 (anti-CEA x anti-CD3)

ATG GAG ACA GAC ACA CTC CTG CTA TGG GTA CTG CTG TGG GTT CCA GGT TCC ACT GGT	< 60
M E T D T L L L W V L L L W V P G S T G	
GAC GCG GCC CAG CCG ATG GCC CAG GTG AAA CTG CAG CAG TCT GGG GCA GAA CTT GTG	< 120
D A A Q P A M A Q V K L L Q Q S G A E L V	
AGG TCA GGG ACC TCA AAG TTG TCC TGC ACA GGT TCT GGC TTC AAC ATT AAA GAC TCC	< 180
R S G T S V K L S C T A S G F N I K D S	
TAT ATG CAC TGG TTG AGG CAG GGG CCT GAA CAG GGC CTG GAG TGG ATT GGA TGG ATT GAT	< 240
Y M H W L R Q G P E Q G L E W I G W I D	
CCT GAG AAT GGT GAT ACT GAA TAT GCC CCG AAG TTC CAG GGC AAG GCC ACT TTT ACT ACA	< 300
P E N G D T E Y A P K F Q G K A T F T T	
GAC ACA TCC TCC AAC ACA GCC TAC CTG CAG CTC AGC AGC CTG ACA TCT GAG GAC ACT GCC	< 360
D T S S N T A Y L Q L S S L T S E D T A	
GTC TAT TAT TGT AAT GAG GGG ACT CCG ACT GGG CCG TAC TAC TTT GAC TAC TGG GGC CAA	< 420
V Y Y C N E G T P T G P Y Y F D Y W G Q	
GGG ACC ACG CTC ACC GTC TCC TCA GGC GGT GGC GGA TCG GAT ATC CAG ATG ACC CAG TCC	< 480
G T T V T V S S G G G G S D I Q M T Q S	
CGG AGT TCC CTG TCC GCC TCT GTG GGC GAT AGA GTC ACC ATC ACC TGT CGT GCC AGT CAG	< 540
P S S L S A S V G D R V T I T C R A S Q	
GAC ATC CGT AAT TAT CTG AAC TGG TAT CAA CAG AAA CCA GGA AAA GCT CCG AAA CTA CTG	< 600
D I R N Y L N W Y Q Q K P G K A P K L L	
ATT TAC TAT ACC TCC CGC CTG GAG TCT GGA GTC CCT TCT CGC TTC TCT GGT TCT TCT	< 660
I Y Y T S R L E S G V P S R F S G S S	
GGG ACG GAT TAC ACT CTC ACC ATC AGC AGT CTG CAA CCG GAG GAC TTC GCA ACC TAT TAC	< 720
G T D Y T L T I S S L Q P E D F A T Y Y	
TGT CAG CAA GGT AAT ACT CTG CCG TGG ACG TTC GGA CAG GGC ACC AAG GTG GAG ATC AAA	< 780
C Q Q G G N T L P W T F G Q G T K V E I K	
CGT GGA GGC GGT AGC GGT GGC CGC GGC GGC GGC TCA GGT TCA GAT GTT CAG CTG	< 840
R G G G G S G G R A S G G G G S E V Q L	
GTG GAG TCT GGC GGT GTC GTG CAG CCA GGG GGC TCA CTC CGT TTG TCC TGT GCA GGT	< 900
V E S C G C L V Q P G C S L R L S C A A	

Fig. 14, part 2

TCT GGC TAC TCC TTT ACC GGC TAC ACT ATG AAC TGG GTG CGT CAG GCC CCA GGT ARG GGC < 960
S G Y S F T G Y T M N W V R Q A P G K G

CTG GAA TGG GTT GCA CTG ATT AAT CCT TAT AAA GGT GTT TCC ACC TAT AAC CAG AAA TTC < 1020
L E W V A L I N P Y K G V S T Y N Q K F

AAG GAT CGT TTC ACG ATA TCC GTA GAT AAA TCC AAA AAC ACA GCC TAC CTG CAA ATG AAC < 1080
K D R F T I S V D K S K N T A Y L Q M N

AGC CTG CGT GCT GAG GAC ACT GCA GTC TAT TAT TGT GCT AGA AGC GGA TAC TAC GGC GAT < 1140
S L R A E D T A V Y Y C A R S G Y Y G D

AGC GAC TGG TAT TTT GAC GTC TGG GGT CAA GGA ACC CTA GTC ACC GTC TCC TCG GGA GGC < 1200
S D W Y F D V W G Q G T L V T V S S G G

GGG GGT TCG GAC ATC GAG CTC ACC CAG TCT CCA GCC ATC ATG TCT GCA TCT CCA GGG GAG < 1260
G G S D I E L T Q S P A I M S A S P G E

AAG GTC ACC ATA ACC TGC AGT GCC AGC TCA AGT GTA AGT TAC ATG CAC TGG TTC CAG CAG < 1320
K V T I T C S A S S V S Y M H W F Q Q

AAG CCA GGC ACT TCT CCC AAA CTC TGG ATT TAT AGC ACA TCC AAC CTG GCT TCT GGA GTC < 1380
K P G T S P K L W I Y S T S N L A S G V

CCT GCT CGC TTC AGT GGC AGT GGA TCT GGG ACC TCT TAC TCT CTC ACA ATC AGC CGA ATG < 1440
P A R F S G S G S G T S Y S L T I S R M

GAG GCT GAA GAT GCT GCC ACT TAT TAC TGC CAG CAA AGG AGT AGT TAC CCA CTC ACG TTC < 1500
E A E D A A T Y Y C Q R S S Y P L T F

GGT GCT GGC ACC AAG CTG GAG CTG AAA CGG GCG GCC GCA GGC GGA TCT GGC GGC ACC ACC < 1560
G A G T K L E L K R A A A G G S G G T T

TAC AAG CTG GTG ATC AAC GGC AAC ACC CTG AAG GGC GAG ACA ACC ACC AAG GCC GTG GAC < 1620
Y K L V I N G K T L K G E T T T K A V D

GCC GAG ACA GCC GAG AAG GCC TTC AAG CAG TAC GCC AAC GAC AAC GGC GTG GAC GGC GTG < 1680
A E T A E K A F K Q Y A N D N G V D G V

TGG ACC TAC GAC GAC GCC ACC AAG ACC TTC ACC GTG ACC GAG GGC GGA TCC CAC CAC CAC < 1740
W T Y D D A T K T F T V T E G G S H H H

CAT CAC CAC TGA TGA < 1755
H H H * *

Fig. 15, part 1

SpG-C3-Db-scTRAIL (anti-human EGFR)

ATG GAC TGG ACC TGG CGC GTG TTT TGC CTG CTC GCC GTG GCT CCT GGG GCC CAC AGC CTC	< 60
M D W T W R V F C L L A V A P G A H S L	
GAG GCC AGC GAC TAC AAA GAC GAT GAC GAT AAA GGA GCC ACC ACC TAC AAG CTG GTG ATC	< 120
E A S D Y K D D D K G A T T Y K L V I	
AAC GGC AAG ACC CTG AAG GGC GAG ACA ACC ACC AAG GCC GTG GAC GCC GAG ACA GCC GAG	< 180
N G K T L K G E T T T K A V D A E T A E	
AAG GCC TTC AAG CAG TAC GCC AAC GAC AAC GGC GTG GAC GGC GTG TGG ACC TAC GAC GAC	< 240
K A F K Q Y A N D N G V D G V W T Y D D	
GCC ACC AAG ACC TTC ACC GTG ACC GAG GGC GGA TCC GGA GGT GGT TCA GGA GGT GAG	< 300
A T K T F T V T E G G S G G G G S G G E	
GTG CAG CTG GTC GAG TCT GGC GGC GGA CTG GTG CAG CCT GGC GGC TCC CTG AGA CTG TCT	< 360
V Q L V E S G G G L V Q P G G S L R L S	
TGC GCT GCC TCC GGC TTC TCC CTG ACC AAC TAC GGC GTG CAC TGG GTC CGG CAG GCT CCC	< 420
C A A S G F S L T N Y G V H W V R Q A P	
GGC AAG GGA CTG GAA TGG CTG GGC GTG ATT TGG TCC GGC GGC AAC ACC GAC TAC AAC ACC	< 480
G K G L E W L G V I W S G G N T D Y N T	
CCT TTC ACC TCC CGG TTC ACC ATC TCC CGG GAC AAC TCC AAG AAC ACC CTG TAC CTG CAG	< 540
P F T S R F T I S R D N S K N T L Y L Q	
ATG AAC TCC CTG CGG GCC GAG GAC ACC GCC GTG TAC TAC TGC GCC AGG GCT CTG ACC TAC	< 600
M N S L R A E D T A V Y Y C A R A L T Y	
TAC GAC TAC GAG TTC GCC TAC TGG GGC CAG GGC ACC ACA GTG ACC GTG TCT AGT GGC GGT	< 660
Y D Y E F A Y W G Q G T T V T V S S G G	
GGC GGC TCT GAT ATT CAG CTG ACC CAG TCC CCC TCC TTC CTG TCC GCC TCC GTG GGC GAC	< 720
G G S D I Q L T Q S P S F L S A S V G D	
AGA GTG ACC ATC ACC TGC CGG GCC TCC CAG TCC ATC GGC ACC AAC ATC CAC TGG TAT CAG	< 780
R V T I T C R A S Q S I G T N I H W Y Q	
CAG AAG CCT GGC AAG GCC CCT AAG CTG CTG ATC AAG TAC GCC TCC GAG TCT ATC TCC GGC	< 840
Q K P G K A P K L L I K Y A S E S I S G	
GTG CCT TCC CGG TTC TCC GGC TCC GGC TCT GGA ACC GAG TTC ACC CTG ACC ATC TCC AGC	< 900
V P S R F S G S G S G T E P T L T I S S	
CTG CAG CCT GAG GAC TTC GCC ACC TAC TAC TGC CAG CAG AAC AAC AAC TGG CCT ACC ACC	< 960
L Q P E D F A T Y Y C Q Q N N N W P T T	
TTC GGC GCT GGC ACC AAG CTG GAA ATC AAG AGA GCG GCC GCA GGC AAC GGC ACC AGC AAC	< 1020
F G A G T K L E I K R A A A G N G T S N	
GGG ACA TCC GAA TTC ACG CGT GGC ACC AGC GAG GAA ACC ATT AGC ACC GTC CAG GAA AAG	< 1080
G T S E F T R G T S E E T I S T V Q E K	
CAG CAG AAC ATC AGC CCC CTG GTC CGG GAG AGA GGC CCC CAG AGA GTC GCC GCC CAC ATC	< 1140
Q Q N I S P L V R E R G P Q R V A A H I	
ACC GGC ACC CGG GGC AGA AGC AAC ACC CTG AGC AGC CCC AAC AGC AAG AAC GAG AAG GCC	< 1200
T G T R G R S N T L S S P N S K N E K A	
CTG GGC CGG AAG ATC AAC AGC TGG GAG AGC AGC AGA AGC GGC CAC AGC TTT CTG AGC AAC	< 1260
L G R K I N S W E S S R S G H S F L S N	
CTG CAC CTG CGG AAC GGC GAG CTG GTC ATC CAC GAG AAG GGC TTC TAC TAC ATC TAC AGC	< 1320
L H L R N G E L V I H E K G F Y Y I Y S	
CAG ACC TAC TTC AGA TTC CAA GAA GAG ATC AAA GAG AAC ACC AAG AAC GAC AAG CAG ATG	< 1380
Q T Y F R F Q E E I K E N T K N D K Q M	

Fig. 15, part 2

GTG CAG TAC ATC TAC AAG TAC ACC AGC TAC CCC CAC CCC ATC CTG CTG ATG AAG TCC GCC < 1440
V Q Y I Y K Y T S Y P D P I L L M K S A

CGG AAC AGC TGC TGG TCC AAG GAC GCC GAG TAC GGC CTG TAC AGC ATC TAC CAG GGC GGC < 1500
R N S C W S K D A E Y G L Y S I Y Q G G

ATC TTC GAG CTG AAA GAG AAC GAC CGG ATC TTC GTG AGC GTG ACC AAC GAG CAC CTG ATC < 1560
I P E L K E N D R I F V S V T N E H L I

GAC ATG GAC CAC GAG GCC AGC TTT TTC GGC GCA TTC CTG GTC GGC GGA GGG GGA TCC GGC < 1620
D M D H E A S F F G A F L V G G G G S G

GGA GGA AGC ACC TCC GAA GAG ACT ATC TCT ACA GTC CAG GAA AAA CAG CAG AAT ATC TCC < 1680
G G S T S E E T I S T V Q E K Q Q N I S

CCT CTC GTG CGG GAG CGG GGA CCT CAG CGG GTG GCC GCC CAT ATT ACA GGC ACA AGA GGC < 1740
P L V R E R G P Q R V A A H I T G T R G

CGG TCC AAC ACC CTG TCC TCC CCC AAC TCT AAG AAT GAA AAG GCC CTC GGG ACA AAG ATC < 1800
R S N T L S S P N S K N E K A L G R K I

AAC TCC TGG GAG TCC AGC CGC TCC GGC CAC TCC TTT CTG TCC AAT CTG CAC CTG AGA AAT < 1860
N S W E S S R S G H S F L S N L H L R N

GGG GAG CTG GTC ATT CAC GAA AAG GGG TTT TAC TAT ATC TAC TCT CAG ACA TAC TTT AGG < 1920
G E L V I H E K G F Y Y I Y S Q T Y F R

TTT CAG GAA GAA ATT AAA GAA AAT ACA AAG AAT GAT AAA CAG ATG GTC CAG TAT ATC TAT < 1980
F Q E E I K E N T K N D K Q M V Q Y I Y

AAA TAC ACT TCC TAC CCT GAT CCT ATT CTG CTG ATG AAA AGC GCC AGA AAC AGC TGT TGG < 2040
K Y T S Y P D P I L L M K S A R N S C W

AGC AAG GAT GCC GAA TAT GGG CTC TAC TCT ATC TAC CAG GGG GGG ATT TTT GAA CTT AAG < 2100
S K D A E Y G L Y S I Y Q G G I F E L K

GAG AAT GAC AGA ATC TTT GTG TCT GTG ACA AAT GAG CAT CTG ATT GAT ATG GAT CAC GAA < 2160
E N D R I F V S V T N E H L I D M D H E

GCC TCA TTC TTT GGA GCC TTT CTT GTG GGA GGG GGC GGA TCT GGT GGC GGA TCC ACC TCT < 2220
A S F F G A F L V G G G G S G G G S T S

GAG GAA ACA ATA TCC ACC GTC CAG GAG AAG CAA CAA AAC ATT TCC CCC CTC GTG CGC GAA < 2280
E E T I S T V Q E K Q Q N I S P L V R E

CGG GGC CCA CAG AGG GTC GCC GCT CAC ATT ACA GCG ACC AGG GGC CGC AGC AAT ACC CTG < 2340
R G P Q R V A A H I T G T R G R S N T L

TCC AGC CCG AAC TCC AAA AAT GAG AAA GCG CTG GGG CGG AAG ATT AAT TCC TGG GAA AGC < 2400
S S P N S K N E K A L G R K I N S W E S

TCC AGA AGC GGG CAC TCC TTC CTC AGC AAT CTG CAT CTG CGC AAC GGG GAA CTC GTG ATT < 2460
S R S G H S F L S N L H L R N G E L V I

CAT GAG AAG GGA TTC TAT TAT ATC TAT TCC CAG ACA TAC TTC CGC TTC CAA GAG GAA ATT < 2520
H E K G F Y Y I Y S Q T Y F R F Q E E I

AAA GAG AAC ACT AAA AAC GAT AAA CAA ATG GTT CAA TAC ATC TAC AAA TAT ACC TCT TAC < 2580
K E N T K N D K Q M V Q Y I Y K Y T S Y

CCA GAT CCC ATC CTC CTC ATG AAG AGT GCC AGA AAC TCC TGC TGG TCT AAG GAT GCG GAA < 2640
P D P I L L M K S A R N S C W S K D A E

TAC GGA TTG TAC TCC ATC TAT CAA GGG GGA ATC TTT GAG TTG AAA GAA AAT GAT CGC ATT < 2700
Y G L Y S I Y Q G G I F E L K E N D R I

TTC GTG TCC GTC ACG AAT GAG CAC CTC ATA GAC ATG GAT CAT GAA GCG AGT TTC TTC GGG < 2760
F V S V T N E H L I D M D H E A S F F G

GCT TTC CTC GTG GGT TGA C < 2779
A F L V G *

Fig. 16

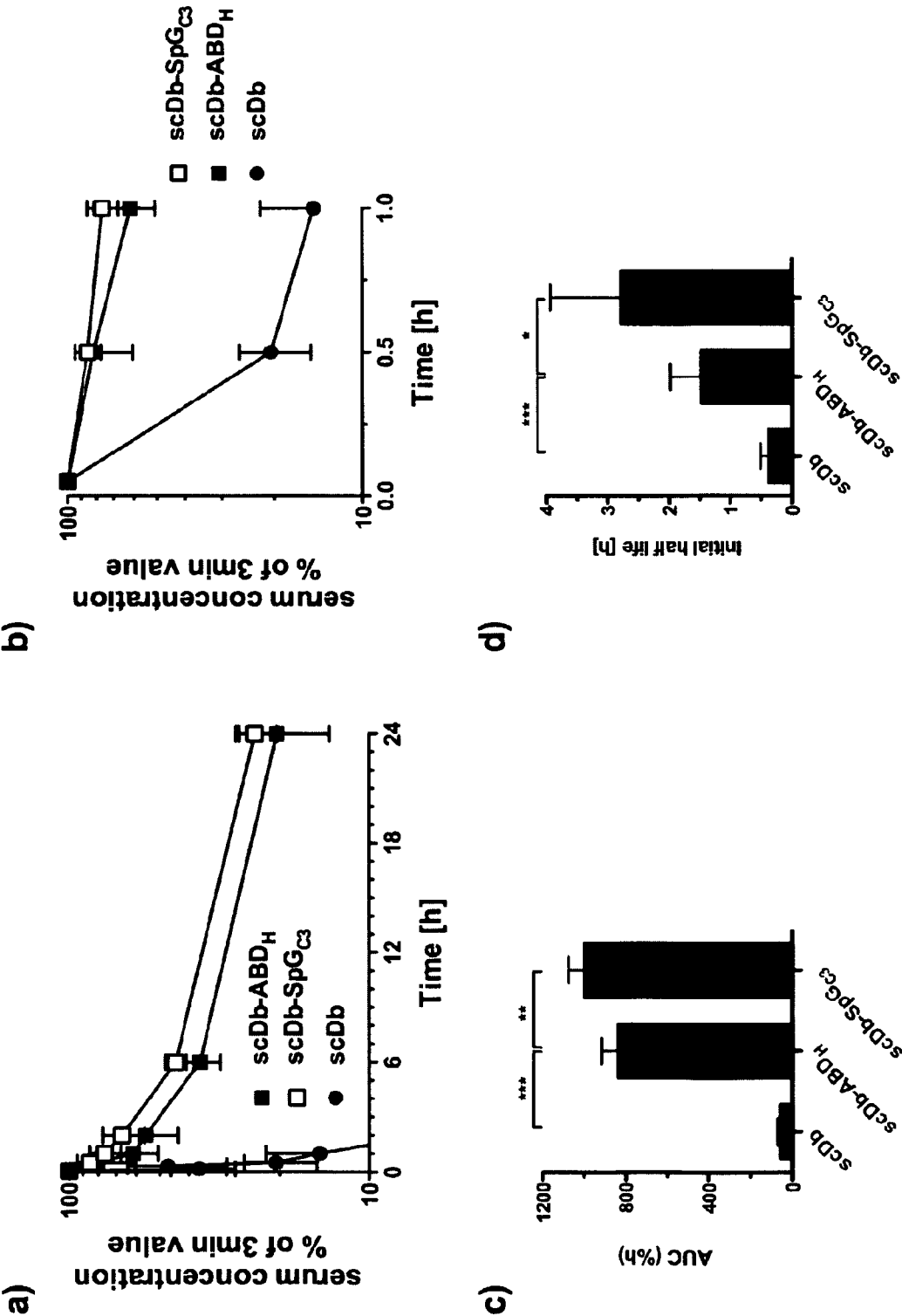
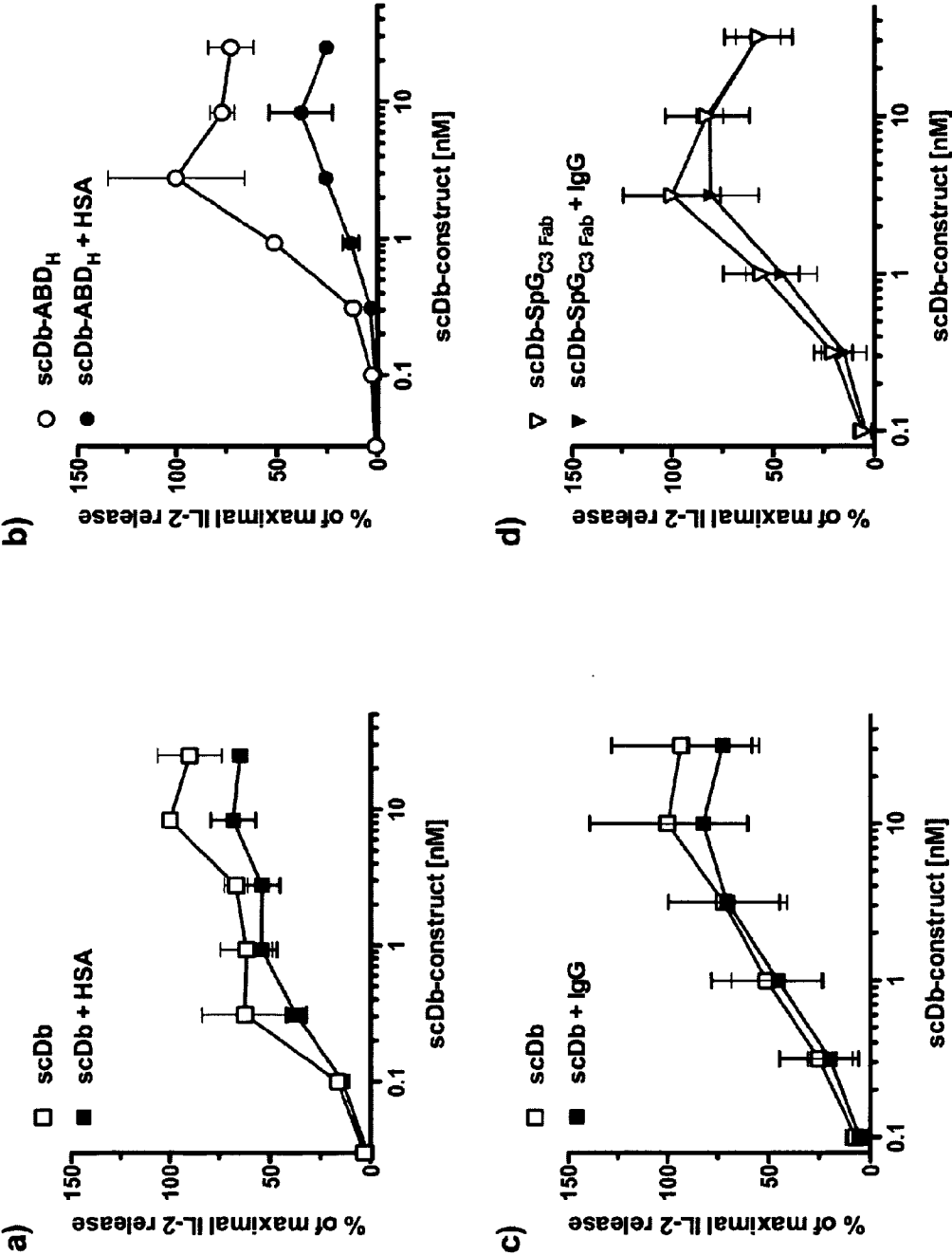


Fig. 17



SERUM HALF-LIFE EXTENSION USING IGBD

[0001] The present invention relates to complexes comprising (i) an immunoglobulin (Ig) binding moiety and (ii) a pharmaceutically active moiety, wherein the Ig binding moiety specifically binds to the constant domain 1 of the heavy chain (C_H1) of an Ig molecule and their use for therapy and prophylaxis.

BACKGROUND

[0002] Most of the therapeutic applications of pharmaceuticals benefit from maintaining a therapeutic effective concentration over a prolonged period of time, often requiring a frequent administration or infusions, or a loco-regional application or subcutaneously of the drug utilizing a slow adsorption into the blood stream in order to maintain an effective concentration over a prolonged period of time. When a drug is administered by rapid intravenous injection into the vascular system, its removal from the blood almost always occurs in a biphasic fashion (see Greenblatt (1985) *Ann. Rev. Med.* 36:421-427). This can be mathematically described by a two-compartment model, which resolves the body into a central compartment and a peripheral compartment (see Dhillon and Gill: *Basic Pharmacokinetics*). These compartments have no distinct physiological or anatomical delimitation, however, the central compartment is considered to comprise tissues that are highly perfused (e.g. heart, lungs, kidneys, liver and brain) whilst the peripheral compartment comprises less well-perfused tissues (e.g. muscle, fat and skin). A two-compartment model assumes that upon drug administration into the central compartment, e.g. into the blood stream, the drug distributes between the central compartment and the peripheral compartment. However, the drug does not achieve instantaneous distribution, i.e. equilibration, between the two compartments. The drug concentration-time profile shows a curve, with the log drug concentration-time plot showing a biphasic response which can be used to distinguish whether a drug shows a one- or two-compartment model (see Dhillon and Gill: *Basic Pharmacokinetics*). Immediately after the dose is given, there is a phase of rapid drug disappearance from the blood, usually lasting from a few minutes to an hour or two, which may lead to a very substantial decrement in drug concentrations in blood. This initial phase (described by the initial or distribution plasma half-life; $t_{1/2\alpha}$) of rapid drug disappearance is determined mainly by reversible distribution of drug out of the "central" compartment, of which the vascular system is a component, into storage sites in peripheral tissues; very little of this initial rapid decline is determined by elimination or clearance. After distribution is complete, the blood concentration curve enters a less rapid phase of drug disappearance, termed the elimination phase (described by the terminal or elimination plasma half-life; $t_{1/2\beta}$), during which drug disappearance is determined mainly by irreversible clearance. The pattern of drug decline during this elimination phase is used to calculate the elimination plasma half-life which is generally determined only after drug distribution equilibrium has been attained (see Greenblatt (1985) *Ann. Rev. Med.* 36:421-427). Both, the initial plasma half-life and the terminal plasma half-life of a substance, e.g. a pharmaceutical, can be influenced in order to extend the bioavailability of such substance in the body by preventing its rapid clearance from the blood.

[0003] Small molecule pharmaceuticals, in particular most small protein therapeutics, including many of the alternative

recombinant antibody formats (Kontermann (2010) *Curr. Opin. Mol. Ther.* 12:176-183) but also the emerging class of alternative scaffold proteins (Nuttall & Walsh (2008) *Curr. Opin. Pharmacol.* 8:609-615; Gebauer & Skerra (2009) *Curr. Opin. Chem. Biol.* 13:245-255), suffer from a short serum half-life mainly due to their rapid clearance from circulation (Batra et al. (2002) *Curr. Opin. Biotechnol.* 13:603-608). These limitations of small size drugs has led to the development and implementation of half-life extension strategies to prolong circulation of these recombinant antibodies in the blood and thus to improve administration and pharmacokinetic as well as pharmacodynamic properties.

[0004] Extension of the half-life can help to reduce the number of applications and to lower doses, thus are beneficial for therapeutic but also economic reasons. Strategies to extend the plasma half-life of pharmaceuticals and therapeutic proteins have, therefore, attracted increasing interest (Pisal et al., (2010) *J. Pharmaceut. Sci.* 99:2557-2575; Kontermann (2009) *BioDrugs* 23:93-109; Kontermann (2011) *Curr. Opin. Biotechnol.* in press).

[0005] Several mechanisms are involved in clearance of drugs from circulation including peripheral blood-mediated elimination by proteolysis, renal and hepatic elimination, and elimination by receptor-mediated endocytosis (Tang et al. (2004) *J. Pharmaceut. Sci.* 93:2184-2204). Molecules possessing a small size, i.e. a low molecular mass with a threshold in the range of 40-50 kDa, are rapidly cleared by renal filtration and degradation. Responsible for renal clearance is the glomerular filtration barrier (GBM) formed by the fenestrated endothelium, the glomerular basement membrane and the slit diaphragm located between the podocyte foot processes (Tryggvason & Wartiovaara (2005) *Physiology* 20:96-101). While the fenestrae between the glomerular endothelial cells are rather large (50-100 nm) allowing free diffusion of molecules, the slit diaphragm represents the ultimate macromolecular barrier, forming an isoporous, zipper-like filter structure with numerous small, 4-5 nm diameter pores and a lower number of 8-10 nm diameter pores (Haraldsson & Sorensson (2004) *New Physiol. Sci.* 19:7-10; Wartiovaara et al. (2004) *J. Clin. Invest.* 114:1475-1483). Molecules with a hydrodynamic radius smaller than approximately 4-5 nm are therefore rapidly cleared from the blood. In addition, the charge of a protein contributes to renal filtration. Proteoglycans of the endothelial cells and the GBM form an anionic barrier, which partially prevents the traversal of negatively charged plasma macromolecules (Tryggvason & Wartiovaara (2005) *Physiology* 20:96-101). Consequently, the size of a protein therapeutic, i.e. its hydrodynamic radius, but also its physicochemical properties represent starting points in order to improve half-life. Furthermore, some plasma proteins such as serum albumin and IgG molecules possess an extraordinary long half-life in the range of 2-4 weeks in humans, which clearly discriminates these molecules from all the other plasma proteins (Kontermann (2009) *BioDrugs* 23:93-109). Responsible is a recycling through the neonatal Fc receptor (FcRn, Brambell receptor) (Roopenian & Akilesh (2007) *Nat. Rev. Immunol.* 7:715-725). Albumin and IgGs taken up by cells, e.g. endothelial cells, through macropinocytosis bind to the FcRn in a pH-dependent manner in the acidic environment of the early endosome. This binding diverges albumin and IgG from degradation in the lysosomal compartment and redirects them to the plasma membrane, where they are released back into the blood plasma due to the neutral pH. This offers additional opportunities to extend or modulate the

half-life of proteins, e.g. through fusion to albumin or the Fc-region of IgG (Kontermann (2009) *BioDrugs* 23, 93-109). Finally, protein drugs that bind to a cellular surface receptor will be internalized by receptor-mediated endocytosis and subjected to lysosomal degradation if the protein drug stays bound to the receptor (Tang et al. (2004) *J. Pharmaceut. Sci.* 93:2184-2204; Lao & Kamei (2008) *Biotechnol. Prog.* 24:2-7). Hence, engineering of the interaction of the therapeutic protein with its receptor(s) at acidic pH can therefore also prolong half-life of the protein by allowing recycling of the unbound molecules into the blood stream as shown for engineered G-CSF and an anti-IL6 receptor antibody (Sarkar et al. (2002) *Nat. Biotechnol.* 20:908-913; Igawa et al. (2010) *Nat. Biotechnol.* 28:1203-1208).

[0006] Several half-life extension strategies have been developed in recent years (Kontermann (2009) *BioDrugs* 23:93-109; Kontermann (2011) *Curr. Opin. Biotechnol.* in press), including strategies such as PEGylation and hyperglycosylation with the aim to increase the hydrodynamic volume of the protein to reduce renal clearance, as well as strategies utilizing recycling processes executed by the neonatal Fc receptor (FcRn), which is responsible for the extraordinary long half-lives of serum IgGs and of serum albumin (Kim et al. (2006) *Clin. Immunol.* 122:146-155). For example, albumin has been employed for half-life extension through the generation of albumin fusion proteins. Several albumin fusion proteins, e.g. albuterol- α 2b and a coagulation factor IX-HSA fusion protein, have already entered clinical trials (Nelson et al. (2010) *Gastroenterology* 139:1267-1276; Metzner et al. (2009) *Thromb. Haemost.* 102:634-644). In addition, various molecules exhibiting albumin-binding activity have been used for half-life extension. For this approach, the albumin-binding moiety is coupled or fused to the therapeutic protein leading to reversible binding to serum albumin after administration. Such albumin-binding molecules include fatty acids, organic molecules, peptides, single-chain Fv, domain antibodies, nanobodies but also domains from naturally occurring proteins capable of binding albumin (for review see: Kontermann (2009) *BioDrugs* 23:93-109). For example, an albumin-binding domain (ABD) from streptococcal protein G was used to prolong the plasma half-life of recombinant antibodies and Affibody molecules (Stork et al. (2007) *Protein Eng. Des. Sel.* 20:569-576; Andersen et al. (2010) *J. Biol. Chem.* 286:5234-5241). Fusion of the ABD resulted in similar half-lives as seen for an albumin fusion protein and an improved tumor accumulation as shown for a bispecific single-chain diabody (Stork et al. (2007) *Protein Eng. Des. Sel.* 20:569-576; Stork et al. (2009) *J. Biol. Chem.* 284:25612-25619). These studies, however, also revealed that albumin and ABD fusion proteins do not reach the long half-life of IgG molecules. Attempts to further prolong half-life by applying an ABD with increased affinity for albumin resulted only in a marginal improvement (Hopp et al., 2010, *Protein Eng. Des. Sel.* 23:827-834). Non-covalent interaction with serum IgG also represents a feasible alternative to binding to serum albumin. This approach was already tested with a bispecific diabody with affinity for mouse Fc γ 1, which prolonged the terminal plasma half-life of the diabody from 1.7 h to 10 h in mice (Holliger et al. (1997) *Nat. Biotechnol.* 15:632-636).

[0007] However, many disadvantages are associated with above strategies of extending the plasma half-life of pharmaceuticals. The usage of PEG, polysialic acid and HES requires their chemical conjugation to the pharmaceutical, which con-

sequently complicates the production and analysis of the final product. PEG is not biologically degradable and may accumulate in the body of a patient which may lead to further complications. Moreover, it has been shown that these modifications were only able to prolong the serum half-life of pharmaceuticals to a limited extent. Similarly, also the serum half-life extension via the conjugation, fusion or binding of the pharmaceutical to serum albumin or via Fc-fusion proteins remains significantly below the serum half-life of IgG. There is thus, a clear need for the development of new strategies allowing for the extension of the serum half-life of pharmaceuticals, especially of therapeutic proteins, which overcome these disadvantages.

[0008] Present inventors surprisingly found that the fusion of pharmaceuticals to an immunoglobulin-binding domain (IgBD) solves this problem. IgBDs are known from various bacterial proteins, e.g. staphylococcal protein A (SpA), streptococcal protein G (SpG) and protein L of *Peptostreptococcus* (PpL) (Tashiro & Montelione (1995) *Curr. Biol.* 5:471-481; Sidorin & Solov'eva (2011) *Biochemistry (Mosc.)* 76:363-378). These IgBDs have a length of 50 to 60 amino acid residues and form either a 3- α -helix bundle or a compact structure composed of a 4-stranded β -sheet and one α -helix (Tashiro & Montelione (1995) *Curr. Biol.* 5:471-481). IgBDs are, thus, particularly stable which benefits the production and storage properties of fusion proteins comprising them.

[0009] IgBDs show a high affinity to serum immunoglobulins, with most of them binding to the same location on the Fc domain of an immunoglobulin as the neonatal Fc receptor, e.g. in IgG the primary binding site is located at the C μ 2-C μ 3 interface of one heavy chain (Deisenhofer (1981) *Biochemistry* 20:2361-2370). They are thus, competing with the FcRn binding and may negatively influence the recycling of the immunoglobulin molecule via the FcRn. For these reasons, IgBDs have so far not been considered for the extension of the serum half-life of pharmaceuticals. However, some bacterial IgBDs are also capable of binding to different regions of the Fab fragment (Tashiro & Montelione (1995) *Curr. Biol.* 5:471-481). Present inventors were able to show that the fusion of pharmaceuticals to an IgBD significantly prolongs the serum half-life of such pharmaceutical, probably due to the fact that these IgBDs do not compete with the Fc receptor binding.

[0010] The fusion or conjugation of a pharmaceutical to such IgBD thus, represents an advantageous possibility of extending their serum half-life as the binding of such fusion protein to an immunoglobulin molecule has a twofold effect; firstly, the clearance by renal filtration and degradation is limited or prevented, and secondly, the recycling of the fusion protein via the FcRn is allowed for.

[0011] The complexes of the present invention provide inter alia the following advantageous properties increase of the solubility of the respective pharmaceutically active moiety in vivo, increase of the in vitro stability of the respective pharmaceutically active moiety, which results in an extended shelf-life of such fusion protein. In cases wherein the pharmaceutical active moiety is a protein or peptide, a further advantage of fusing such moiety to an IgBD of the present invention is the increased expression of such fusion proteins, e.g. in mammalian expression systems. In addition, the complexing of the pharmaceutical moiety to an immunoglobulin binding moiety, in particular if the pharmaceutical moiety is a protein or peptide allows an easier and/or faster purification of such pharmaceutical.

SUMMARY OF THE INVENTION

[0012] In a first aspect, the present invention relates to a complex comprising (i) an immunoglobulin (Ig) binding moiety and (ii) a pharmaceutically active moiety, wherein the Ig binding moiety specifically binds to the constant domain 1 of the heavy chain (C_{H1}) of an Ig molecule.

[0013] In a second aspect, the present invention provides a nucleic acid molecule comprising a sequence encoding the complex of the first aspect.

[0014] In a third aspect, the present invention provides a vector comprising the nucleic acid of the second aspect.

[0015] In a fourth aspect, the present invention provides an isolated cell containing the complex of the first aspect and/or the nucleic acid molecule of the second aspect and/or the vector of the third aspect.

[0016] In a fifth aspect, the present invention provides a pharmaceutical composition comprising the complex of the first aspect, the nucleic acid of the second aspect, the vector of the third aspect and/or the cell of the fourth aspect and a pharmaceutical acceptable carrier and/or excipient.

[0017] In a sixth aspect, the present invention provides the complex of the first aspects, the nucleic acid of the second aspect, the vector of third aspect, the cell of the fourth aspect, the pharmaceutical composition of the fifth aspect for use in extending the serum half-life.

[0018] In a seventh aspect, the present invention provides the complex of the first aspects, the nucleic acid of the second aspect, the vector of third aspect, the cell of the fourth aspect, the pharmaceutical composition of the fifth aspect for use as a medicament.

FIGURES

[0019] FIG. 1: Summary of IgBD bound to human IgG1. IgBDs from protein A (SpA_B , SpA_D), protein G (SpG_{C2} , SpG_{C3}) and protein L (PpL_{C4*}) in complex with IgG were visualized on a human IgG1 model (Clark (1997) Chem. Immunol. 65:88-110). In addition, the extracellular region of human FcRn bound to the Fc region was included (Burmeister et al. (1994) Nature 372:379-383). PDB entries are indicated for each IgBD and the FcRn. The structures were visualized with the PyMOL Molecular Graphics System (Version 1.3, Schrödinger, LLC). b) Schematic illustration of binding of SpG_{C3} , possessing one binding site for CH1 and one binding site for the Fc part, to IgG. c) Schematic illustration of binding of SpG_{C3-Fab} , possessing one binding site for CH1 and a mutated inactive Fc binding site, to the CH1 domain of IgG only.

[0020] FIG. 2: Construction of scDb-IgBDs and scFv-IgBDs. a) Composition of the scDb-IgBD and scFv-IgBD fusion protein. IgBDs are fused to the C-terminus of a bispecific scDb or a scFv. b) SDS-PAGE analysis of purified scDb-CEACD3 (1), scDb- SpA_B (2), scDb- SpA_D (3), scDb- SpA_{E24} (4), scDb- SpG_{C3} (5), and scDb- PpL_{C4*} (6) under reducing conditions. c) SDS-PAGE analysis of purified anti-CEA scFv (1), scFv- SpA_B (2), scFv- SpA_D (3), scFv- SpA_{E24} (4), scFv- SpG_{C3} (5), and scFv- PpL_{C4*} (6) under reducing conditions. Two micrograms of the proteins were analyzed per lane and the gel was stained with Coomassie brilliant blue G-250 (M, molecular weight standards). d-g) Purified scDb, scFv as well as the scDb- SpG_{C3} and scFv- SpG_{C3} fusion proteins were analyzed by SEC.

[0021] FIG. 3. Binding of scDb-IgBD and scFv-IgBD fusion proteins to CEA in ELISA. Increasing concentrations

of the scDb-IgBD (a) or scFv-IgBD (b) fusion proteins were analyzed for binding to immobilized CEA.

[0022] FIG. 4: Binding of scDb-IgBD to IgG, Fab and Fc analyzed by ELISA. scDb, scDb- SpA_B , scDb- SpA_D , scDb- SpA_{E24} , scDb- SpG_{C3} , and scDb- PpL_{C4*} were analyzed for binding to immobilized mouse (a) and human (b) serum IgG as well as Fab and Fc fragments thereof. Furthermore, human IgM and human IgA were analyzed for binding of these fusion proteins.

[0023] FIG. 5. Binding of scDb-IgBD to IgG, Fab and Fc analyzed by quartz crystal microbalance measurements. Human and mouse IgG as well as Fab and Fc fragments thereof were immobilized on a QCM chip and binding of the scDb-IgBD fusion proteins was determined at 1.5 μ M (Fab fragments), 500 nM (IgGs), 40 nM (human IgG) or x nM (IgG-Fc fragments), and 1.28 nM (mouse IgG-Fc fragments).

[0024] FIG. 6: Affinities. Affinities of the scDb-IgBD fusion proteins for human and mouse IgG and Fab and Fc fragments thereof determined at neutral pH (7.4) and acidic pH (6.0) by quartz crystal microbalance measurements using an Attana A100 and sensorchips with chemically conjugated immunoglobulins.

[0025] FIG. 7. Plasma half-life of scDb-IgBD and scFv-IgBD fusion proteins in comparison to unmodified proteins (scDb, scFv) and IgG. ScDb-IgBD (a) and scFv-IgBD (b) fusion proteins were i.v. injected into CD1 mice (25 μ g/animal) and serum concentrations of the antibody molecules were determined at different time points by ELISA. Data were normalized considering maximal concentration at the first time point (3 min).

[0026] FIG. 8: Biochemical and pharmacokinetic properties of scDb-IgBD and scFv-IgBD fusion proteins in the mouse. ScDb-IgBD and scFv-IgBD fusion proteins were i.v. injected into CD1 mice (25 μ g/animal) and serum concentrations of the antibody molecules were determined at different time points by ELISA. Data were normalized considering maximal concentration at the first time point (3 min). $t_{1/2\alpha}$ indicates the initial plasma half-life; $t_{1/2\beta}$ indicates the terminal plasma half-life; AUC indicates the bioavailability of the tested scDb-IgBD and scFv-IgBD fusion proteins. The molecular masses were calculated from the amino acid sequences. Stokes radii (Sr) were determined by size exclusion chromatography.

[0027] FIG. 9. Immunostimulatory activity of scDb-IgBD fusion proteins. The bispecific anti-CEA/xanti-CD3 scDb-IgBD fusion proteins were analyzed in vitro for triggering of IL-2 release from human PBMCs in a target cell-dependent manner in the absence or presence of human IgG (100 μ g/ml). CEA-positive target cells (LS1S74T) were grown in microtiter plates and subsequently PBMCs and fusion proteins were added and incubated for 24 h. Subsequently, IL-2 released from activated T cells was determined by ELISA. Unmodified scDb was included as control.

[0028] FIG. 10: SpG_{C3} Mutants. a) SDS-PAGE analysis of scDb (1), scDb- SpG_{C3} (2), scDb- SpG_{C3-Fab} (3), scDb- SpG_{C3-Fc} (4). Gel was stained with Coomassie brilliant blue G-250. b) Binding of scDb- SpG_{C3} , scDb- SpG_{C3-Fab} , scDb- SpG_{C3-Fc} to human IgG, Fab and Fc analyzed by ELISA. scDb- SpG_{C3} , scDb- SpG_{C3-Fab} , scDb- SpG_{C3-Fc} were analyzed for binding to immobilized human serum IgG as well as Fab and Fc fragments thereof. c) Binding of scDb, scDb- SpG_{C3} , scDb- SpG_{C3-Fab} , scDb- SpG_{C3-Fc} to CEA analyzed by ELISA. d) Plasma half-life of scDb- SpG_{C3-Fab} ; scDb- SpG_{C3-Fab} was i.v. injected into CD1 mice (25 μ g/animal) and

serum concentrations of the antibody molecules were determined at different time points by ELISA. Data were normalized considering maximal concentration at the first time point (3 min).

[0029] FIG. 11: Production and IgG-binding of an SpG-C3-Diabody-scTRAIL fusion protein. a) Composition of the fusion protein composed of an N-terminal SpG_{C3} domain, an anti-EGFR diabody and a single-chain derivative of TRAIL (scTRAIL). b) Detection of purified fusion protein by Western blot with anti-TRAIL or anti-FLAG-tag antibodies. A band corresponding to the expected size of approximately 100 kDa is detected. c) Binding of the fusion protein (10 µg/ml) to immobilized human serum IgG in ELISA. Bound fusion protein was detected with an anti-FLAG-tag antibody and an HRP-conjugated anti-mouse antibody. The fusion protein was omitted in the control.

[0030] FIG. 12: Amino acid sequences of the SpG-C3 binding epitope on the CH1 domains of human, mouse, and rat IgG according to EU index as in Kabat.

[0031] FIG. 13: Nucleic acid and amino acid sequence of scFv-SpG-C3 fusion protein (anti-CEA). SpG_{C3} sequence is marked with a grey box, the leader sequence is underlined.

[0032] FIG. 14: Nucleic acid and amino acid sequence of scDb-SpG-C3 fusion protein (anti-CEA×anti-CD3). SpG_{C3} sequence is marked with a grey box, the leader sequence is underlined.

[0033] FIG. 15: Nucleic acid and amino acid sequence of SpG-C3-Db-scTRAIL (anti-human EGFR) fusion protein. SpG_{C3} sequence is marked with a grey box, the leader sequence is underlined.

[0034] FIG. 16: Pharmacokinetic properties of scDb-SpG_{C3} and scDb-ABD_H. a) ScDb, scDb-SpG_{C3} and scDb-ABD_H fusion proteins were i.v. injected into CD1 mice (25 µg/animal) and serum concentrations at different time points were determined by ELISA. The 3 min value was set to 100% for normalization. b) Plasma concentrations shown for the first 1 h. c) AUC determined over the first 24 h. d) Initial plasma half-lives determined for the first 3 time points (up to 1 h). Plasma half-lives and AUC_{0-24h} of scDb, scDb-SpG_{C3} and scDb-ABD_H were calculated from the serum concentrations by Excel and statistics were performed using a T-test with GraphPad Prism.

[0035] FIG. 17: Comparison of IL-2 release using scDb-SpG_{C3-Fab} and scDb-ABD_H fusion proteins. LS174T cells were incubated with varying concentrations of scDb (a and c), scDb-ABD_H (b) or scDb-SpG_{C3-Fab} (d) before adding human PBMCs. The scDb fusion proteins were either preincubated without (white symbols) or with 1 mg/ml HSA (a, b) or 100 µg/ml human IgG (c, d) corresponding approximately to 1/50 of the normal plasma concentrations (black symbols). After 24 h, IL-2 release into the supernatant was determined by ELISA.

DETAILED DESCRIPTION

[0036] Before the present invention is described in detail below, it is to be understood that this invention is not limited to the particular methodology, protocols and reagents described herein as these may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to limit the scope of the present invention which will be limited only by the appended claims. Unless defined otherwise, all technical and scientific terms used herein have the same meanings as commonly understood by one of ordinary skill in the art.

[0037] Preferably, the terms used herein are defined as described in "A multilingual glossary of biotechnological terms: (IUPAC Recommendations)", Leuenberger, H. G. W., Nagel, B. and Kölbl, H. eds. (1995), Helvetica Chimica Acta, CH-4010 Basel, Switzerland).

[0038] Several documents are cited throughout the text of this specification. Each of the documents cited herein (including all patents, patent applications, scientific publications, manufacturer's specifications, instructions, GenBank Accession Number sequence submissions etc.), whether supra or infra, is hereby incorporated by reference in its entirety. Nothing herein is to be construed as an admission that the invention is not entitled to antedate such disclosure by virtue of prior invention.

DEFINITIONS

[0039] Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", and variations such as "comprises" and "comprising", will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps.

[0040] The term "complex" as used herein, refers to a whole that comprehends a number of individual components, parts or moieties which are in close proximity to each other and fulfil a common or interrelated function. The individual parts of such complex may fulfil differing functions in order to achieve the common function of the complex, i.e. one part of the complex may mediate one function (e.g. the binding of the complex) whilst the other part of the complex may mediate a different function (e.g. the activity of the complex) in order to fulfil the common function (e.g. of a site specific activity). The individual moieties of a complex may be of the same or of differing nature, i.e. they may be composed of the same, a similar or of differing chemical entities such as but not limited to nucleotides, amino acids, nucleic acids, peptides, polypeptides, proteins, carbohydrates, and/or lipids. Exemplified, a complex may comprise a number of associated proteins, or a mixture of one or more proteins and one or more nucleic acids or a mixture of one or more proteins and one or more lipids and/or carbohydrates. It is understood that any other combination of identical, similar or differing chemical entities is also encompassed. The individual moieties of a complex may or may not be interconnected. Typically, the individual parts of a complex are connected via covalent or non-covalent bonds.

[0041] The terms "polynucleotide" and "nucleic acid" are used interchangeably herein and are understood as a polymeric or oligomeric macromolecule made from nucleotide monomers. Nucleotide monomers are composed of a nucleobase, a five-carbon sugar (such as but not limited to ribose or 2'-deoxyribose), and one to three phosphate groups. Typically, a polynucleotide is formed through phosphodiester bonds between the individual nucleotide monomers. In the context of the present invention referred to nucleic acid molecules include but are not limited to ribonucleic acid (RNA), deoxyribonucleic acid (DNA), and mixtures thereof such as e.g. RNA-DNA hybrids. The nucleic acids, can e.g. be synthesized chemically, e.g. in accordance with the phosphotriester method (see, for example, Uhlmann & Peyman (1990) Chemical Reviews 90:543-584). "Aptamers" are nucleic acids which bind with high affinity to a polypeptide. Aptamers can be isolated by selection methods such as SELEmir146-a (see e.g. Jayasena (1999) Clin. Chem.

45:1628-50; Klug and Famulok (1994) M. Mol. Biol. Rep. 20:97-107; U.S. Pat. No. 5,582,981) from a large pool of different single-stranded RNA molecules. Aptamers can also be synthesized and selected in their mirror-image form, for example as the L-ribonucleotide (Nolte et al. (1996) Nat. Biotechnol. 14:1116-1119; Klussmann et al. (1996) Nat. Biotechnol. 14:1112-1115). Forms which have been isolated in this way enjoy the advantage that they are not degraded by naturally occurring ribonucleases and, therefore, possess greater stability.

[0042] The terms “protein” and “polypeptide” are used interchangeably herein and refer to any peptide-bond-linked chain of amino acids, regardless of length or post-translational modification. Proteins usable in the present invention (including protein derivatives, protein variants, protein fragments, protein segments, protein epitops and protein domains) can be further modified by chemical modification. This means such a chemically modified polypeptide comprises other chemical groups than the 20 naturally occurring amino acids. Examples of such other chemical groups include without limitation glycosylated amino acids and phosphorylated amino acids. Chemical modifications of a polypeptide may provide advantageous properties as compared to the parent polypeptide, e.g. one or more of enhanced stability, increased biological half-life, or increased water solubility.

[0043] As used herein, the term “variant” is to be understood as a polynucleotide or protein which differs in comparison to the polynucleotide or protein from which it is derived by one or more changes in its length or sequence. The polypeptide or polynucleotide from which a protein or nucleic acid variant is derived is also known as the parent or parental polypeptide or polynucleotide. The term “variant” comprises “fragments” or “derivatives” of the parent molecule. Typically, “fragments” are smaller in length or size than the parent molecule, whilst “derivatives” exhibit one or more differences in their sequence in comparison to the parent molecule. Also encompassed are modified molecules such as but not limited to post-translationally modified proteins (e.g. glycosylated, biotinylated, phosphorylated, ubiquitinated, palmitoylated, or proteolytically cleaved proteins) and modified nucleic acids such as methylated DNA. Also mixtures of different molecules such as but not limited to RNA-DNA hybrids, are encompassed by the term “variant”. Typically, a variant is constructed artificially, preferably by gene-technological means whilst the parent polypeptide or polynucleotide is a wild-type protein or polynucleotide. However, also naturally occurring variants are to be understood to be encompassed by the term “variant” as used herein. Further, the variants usable in the present invention may also be derived from homologs, orthologs, or paralogs of the parent molecule or from artificially constructed variant, provided that the variant exhibits at least one biological activity of the parent molecule, i.e. is functionally active.

[0044] The changes in the nucleotide or amino acid sequence may be nucleotide or amino acid exchanges, insertions, deletions, 5'- or 3' truncations, N- or C-terminal truncations, or any combination of these changes, which may occur at one or several sites. In preferred embodiments, a variant usable in the present invention exhibits a total number of up to 200 (up to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, or 200) changes in the nucleotide or amino acid sequence (i.e. exchanges, insertions, deletions, and/or truncations). Amino acid exchanges may be

conservative and/or non-conservative. Alternatively or additionally, a “variant” as used herein, can be characterized by a certain degree of sequence identity to the parent polypeptide or parent polynucleotide from which it is derived. More precisely, a protein variant in the context of the present invention exhibits at least 70% sequence identity to its parent polypeptide. A polynucleotide variant in the context of the present invention exhibits at least 70% sequence identity to its parent polynucleotide. Preferably, the sequence identity of protein variants is over a continuous stretch of 20, 30, 40, 45, 50, 60, 70, 80, 90, 100 or more amino acids. Preferably, the sequence identity of polynucleotide variants is over a continuous stretch of 60, 90, 120, 135, 150, 180, 210, 240, 270, 300 or more nucleotides.

[0045] The term “at least 70% sequence identity” is used throughout the specification with regard to polypeptide and polynucleotide sequence comparisons. This expression preferably refers to a sequence identity of at least 70%, at least 71%, at least 72%, at least 73%, at least 74%, at least 75%, at least 76%, at least 77%, at least 78%, at least 79%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% to the respective reference polypeptide or to the respective reference polynucleotide.

[0046] In case where two sequences are compared and the reference sequence is not specified in comparison to which the sequence identity percentage is to be calculated, the sequence identity is to be calculated with reference to the longer of the two sequences to be compared, if not specifically indicated otherwise. If the reference sequence is indicated, the sequence identity is determined on the basis of the full length of the reference sequence indicated by SEQ ID, if not specifically indicated otherwise. For example, a peptide sequence consisting of 358 amino acids compared to the amino acid sequence of an IgG molecule may exhibit a maximum sequence identity percentage of 80.09% (358/447) while a sequence with a length of 224 amino acids may exhibit a maximum sequence identity percentage of 50.11% (224/447). The similarity of nucleotide and amino acid sequences, i.e. the percentage of sequence identity, can be determined via sequence alignments. Such alignments can be carried out with several art-known algorithms, preferably with the mathematical algorithm of Karlin and Altschul (Karlin & Altschul (1993) Proc. Natl. Acad. Sci. USA 90:5873-5877), with hmalign (HMMER package, <http://hmmer.wustl.edu/>) or with the CLUSTAL algorithm (Thompson et al. (1994) Nucleic Acids Res. 22:4673-4680) available e.g. on <http://www.ebi.ac.uk/Tools/clustalw/> or on <http://www.ebi.ac.uk/Tools/clustalw2/index.html> or on http://npsa-pbil.ibcp.fr/cgi-bin/npsa_automat.pl?page=/NPSA/npsa_clustalw.html. Preferred parameters used are the default parameters as they are set on <http://www.ebi.ac.uk/Tools/clustalw/> or <http://www.ebi.ac.uk/Tools/clustalw2/index.html>. The grade of sequence identity (sequence matching) may be calculated using e.g. BLAST, BLAT or BlastZ (or BlastX). A similar algorithm is incorporated into the BLASTN and BLASTP programs of Altschul et al. (1990) J. Mol. Biol. 215:403-410. BLAST polynucleotide searches are performed with the BLASTN program, score=100, word length=12. BLAST protein searches are performed with the BLASTP program, score=50, word length=3. To obtain gapped alignments for comparative purposes, Gapped BLAST is utilized as

described in Altschul et al. (1997) *Nucleic Acids Res.* 25:3389-3402. When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs are used. Sequence matching analysis may be supplemented by established homology mapping techniques like Shuffle-LAGAN (Brudno M. (2003b) *Bioinformatics* 19 Suppl 1:154-162) or Markov random fields. When percentages of sequence identity are referred to in the present application, these percentages are calculated in relation to the full length of the longer sequence, if not specifically indicated otherwise. "Hybridization" can also be used as a measure of sequence identity or homology between two nucleic acid sequences. A nucleic acid sequence encoding F, N, or M2-1, or a portion of any of these can be used as a hybridization probe according to standard hybridization techniques. Hybridization conditions are known to those skilled in the art and can be found, for example, in *Current Protocols in Molecular Biology*, John Wiley & Sons, N. Y., 6.3.1-6.3.6, 1991. "Moderate hybridization conditions" are defined as equivalent to hybridization in 2× sodium chloride/sodium citrate (SSC) at 30° C., followed by a wash in 1×SSC, 0.1% SDS at 50° C. "Highly stringent conditions" are defined as equivalent to hybridization in 6× sodium chloride/sodium citrate (SSC) at 45° C., followed by a wash in 0.2×SSC, 0.1% SDS at 65° C.

[0047] The term "immunoglobulin (Ig)" as used herein refers to immunity conferring glycoproteins of the immunoglobulin superfamily. "Surface immunoglobulins" are attached to the membrane of effector cells by their transmembrane region and encompass molecules such as but not limited to B-cell receptors, T-cell receptors, class I and II major histocompatibility complex (MHC) proteins, beta-2 microglobulin (β 2M), CD3, CD4 and CD8. Typically, the term "antibody" as used herein refers to secreted immunoglobulins which lack the transmembrane region and can thus, be released into the bloodstream and body cavities. Human antibodies are grouped into different isotypes based on the heavy chain they possess. There are five types of human Ig heavy chains denoted by the Greek letters: α , δ , γ , and μ . The type of heavy chain present defines the class of antibody, i.e. these chains are found in IgA, IgD, IgE, IgG, and IgM antibodies, respectively, each performing different roles, and directing the appropriate immune response against different types of antigens. Distinct heavy chains differ in size and composition; α and γ comprise approximately 450 amino acids, while μ and ϵ have approximately 550 amino acids (Janeway et al. (2001) *Immunobiology*, Garland Science). IgA is found in mucosal areas, such as the gut, respiratory tract and urogenital tract, as well as in saliva, tears, and breast milk and prevents colonization by pathogens (Underdown & Schiff (1986) *Annu. Rev. Immunol.* 4:389-417). IgD mainly functions as an antigen receptor on B cells that have not been exposed to antigens and is involved in activating basophils and mast cells to produce antimicrobial factors (Geisberger et al. (2006) *Immunology* 118:429-437; Chen et al. (2009) *Nat. Immunol.* 10:889-898). IgE is involved in allergic reactions via its binding to allergens triggering the release of histamine from mast cells and basophils. IgE is also involved in protecting against parasitic worms (Pier et al. (2004) *Immunology, Infection, and Immunity*, ASM Press). IgG provides the majority of antibody-based immunity against invading pathogens and is the only antibody isotype capable of crossing the placenta to give passive immunity to fetus (Pier et al. (2004) *Immunology, Infection, and Immunity*, ASM Press). In humans there are four different IgG subclasses (IgG1, 2, 3,

and 4), named in order of their abundance in serum with IgG1 being the most abundant (~66%), followed by IgG2 (~23%), IgG3 (~7%) and IgG (~4%). The biological profile of the different IgG classes is determined by the structure of the respective hinge region. IgM is expressed on the surface of B cells in a monomeric form and in a secreted pentameric form with very high avidity. IgM is involved in eliminating pathogens in the early stages of B cell mediated (humoral) immunity before sufficient IgG is produced (Geisberger et al. (2006) *Immunology* 118:429-437).

[0048] Antibodies are not only found as monomers but are also known to form dimers of two Ig units (e.g. IgA), tetramers of four Ig units (e.g. IgM of teleost fish), or pentamers of five Ig units (e.g. mammalian IgM). Antibodies are typically made of four polypeptide chains comprising two identical heavy chains and identical two light chains which are connected via disulfide bonds and resemble a "Y"-shaped macromolecule. Each of the chains comprises a number of immunoglobulin domains out of which some are constant domains and others are variable domains. Immunoglobulin domains consist of a 2-layer sandwich of between 7 and 9 antiparallel β -strands arranged in two β -sheets. Typically, the heavy chain of an antibody comprises four Ig domains with three of them being constant (C_H domains: C_{H1} , C_{H2} , C_{H3}) domains and one of the being a variable domain (V_H). The light chain typically comprises one constant Ig domain (C_L) and one variable Ig domain (V_L). Exemplified, the human IgG heavy chain is composed of four Ig domains linked from N- to C-terminus in the order V_H - C_{H1} - C_{H2} - C_{H3} (also referred to as V_H - $C\gamma 1$ - $C\gamma 2$ - $C\gamma 3$), whereas the human IgG light chain is composed of two immunoglobulin domains linked from N- to C-terminus in the order V_L - C_L , being either of the kappa or lambda type ($V\kappa$ - $C\kappa$ or $V\lambda$ - $C\lambda$).

[0049] Exemplified, the constant chain of human IgG comprises 447 amino acids. Throughout the present specification and claims, the numbering of the amino acid positions in an immunoglobulin are that of the "EU index" as in Kabat, E. A., Wu, T. T., Perry, H. M., Gottesman, K. S., and Foeller, C., (1991) *Sequences of proteins of immunological interest*, 5th ed. U.S. Department of Health and Human Service, National Institutes of Health, Bethesda, Md. The "EU index as in Kabat" refers to the residue numbering of the human IgG1EU antibody. Accordingly, C_H domains in the context of IgG are as follows: " C_{H1} " refers to amino acid positions 118-220 according to the EU index as in Kabat; " C_{H2} " refers to amino acid positions 237-340 according to the EU index as in Kabat; and " C_{H3} " refers to amino acid positions 341-447 according to the EU index as in Kabat.

[0050] Papain digestion of antibodies produces two identical antigen binding fragments, called "Fab fragments" (also referred to as "Fab portion" or "Fab region") each with a single antigen binding site, and a residual "Fc fragment" (also referred to as "Fc portion" or "Fc region") whose name reflects its ability to crystallize readily. The crystal structure of the human IgG Fc region has been determined (Deisenhofer (1981) *Biochemistry* 20:2361-2370). In IgG, IgA and IgD isotypes, the Fc region is composed of two identical protein fragments, derived from the C_{H2} and C_{H3} domains of the antibody's two heavy chains; in IgM and IgE isotypes, the Fc regions contain three heavy chain constant domains (C_{H2} -4) in each polypeptide chain. In addition, smaller immunoglobulin molecules exist naturally or have been constructed artificially. The term "Fab' fragment" refers to a Fab fragment additionally comprise the hinge region of an Ig molecule

whilst “F(ab')₂ fragments” are understood to comprise two Fab' fragments being either chemically linked or connected via a disulfide bond. Whilst “single domain antibodies (sdAb)” (Desmyter et al. (1996) Nat. Structure Biol. 3:803-811) and “Nanobodies” only comprise a single V_H domain, “single chain Fv (scFv)” fragments comprise the heavy chain variable domain joined via a short linker peptide to the light chain variable domain (Huston et al. (1988) Proc. Natl. Acad. Sci. USA 85, 5879-5883). Divalent single-chain variable fragments (di-scFvs) can be engineered by linking two scFvs (scFvA-scFvB). This can be done by producing a single peptide chain with two V_H and two V_L regions, yielding “tandem scFvs” (V_HA-V_LA-V_HB-V_LB). Another possibility is the creation of scFvs with linkers that are too short for the two variable regions to fold together, forcing scFvs to dimerize. Usually linkers with a length of 5 residues are used to generate these dimers. This type is known as “diabodies”. Still shorter linkers (one or two amino acids) between a V_H and V_L domain lead to the formation of monospecific trimers, so-called “tribodies” or “tribodies”. Bispecific diabodies are formed by expressing to chains with the arrangement V_HA-V_LB and V_HB-V_LA or V_LA-V_HB and V_LB-V_HA, respectively. Single-chain diabodies (scDb) comprise a V_HA-V_LB and a V_HB-V_LA fragment which are linked by a linker peptide (P) of 12-20 amino acids, preferably 14 amino acids, (V_HA-V_LB-P-V_HB-V_LA). “Bi-specific T-cell engagers (BiTEs)” are fusion proteins consisting of two scFvs of different antibodies wherein one of the scFvs binds to T cells via the CD3 receptor, and the other to a tumor cell via a tumor specific molecule (Kufer et al. (2004) Trends Biotechnol. 22:238-244). Dual affinity retargeting molecules (“DART” molecules) are diabodies additionally stabilized through a C-terminal disulfide bridge.

[0051] The term “immunoglobulin (Ig) binding moiety” as used herein refers to a moiety or part of a complex which interacts with an immunoglobulin. Typically an Ig binding moiety comprises a polypeptide or a protein which binds to the heavy and/or to the light chain of an Ig, preferably an antibody. An Ig binding moiety may comprise an “Ig binding domain (IgBD)” as well as further domains fulfilling additional functions such as but not limited to stabilizing the Ig binding moiety, or promoting the Ig binding potential of the IgBD.

[0052] The term “Ig binding domain (IgBD)” as used herein refers to domains which mediate the actual binding of the Ig binding moiety to the Ig molecule. An IgBD may bind to any of the domains of an Ig molecule, i.e. to the variable domains V_H or V_L and/or to the constant domains C_H1, C_H2, C_H3 and/or C_L of an Ig molecule. Typically an IgBD has an affinity to bind to an Ig molecule at neutral pH (i.e. pH 7), however also binding at lower or higher pH values, e.g. at pH values 5, 6, or 8, may occur. The affinity of an IgBD to bind to Ig molecules may lie below 10⁻⁶ M, often below 10⁻⁷ M, or even below 10⁻⁸ M. Typically, IgBDs are derived from Ig binding proteins of gram-positive bacteria. These include but are not limited to Protein A from *Staphylococcus aureus*, streptococcal Protein G, and Protein L from *Peptostreptococcus magnus* (now: *Finegoldia magna*).

[0053] “Protein A (SpA)” is a 40-60 kDa surface protein originally found in the cell wall of *Staphylococcus aureus*. It binds immunoglobulins, most notably IgGs, from many mammalian species through an interaction of two α-helices of its IgBDs (A, B, C, D, E) with the C_H2 and C_H3 domains in the Fc fragment of an Ig molecule. Protein A binds with high

affinity to human IgG1 and IgG2 as well as mouse IgG2a and IgG2b but only with moderate affinity to human IgM, IgA and IgE as well as to mouse IgG3 and IgG1.

[0054] “Protein G (SpG)” is an immunoglobulin-binding protein expressed in group C and G streptococcal strains which is similar to Protein A but exhibits different specificities. It is a cell surface protein of about 65-kDa that binds to the Fc region of IgG molecules (in particular to IgG1, IgG2 or IgG4) as well as to serum albumin. The amino acid sequences of the individual IgBDs are identical from streptococcal strains G148, GX7805, and GX7809 (Guss et al. (1986) EMBO J. 5:1567-1575). Protein G consists of repetitively arranged domains with the C-terminal domains (C1, C2, C3, also referred to as domains B1-B3) being responsible for IgG binding and the domains in the N-terminal half of the protein (domains A1, A2, A3) binding to serum albumin. The single IgBDs of Protein G show a common secondary structure consisting of a central α-helix packed against a four-stranded, antiparallel-parallel-antiparallel β-sheet. The amino acid sequences of domains C1 and C2 are 90% and 93%, respectively, identical to the sequence of domain C3. The amino acid sequences of Ig-binding domains C1, C2, and C3 of streptococcal Protein G are as followed:

SpG-C1: (SEQ ID NO: 16)
 TYKLILNGKTLKGETTTEAVDAATAEKVFKQYANDNGVDGEWYDDATKT

FTVTE

SpG-C2: (SEQ ID NO: 17)
 TYKLVLINGKTLKGETTTEAVDAATAEKVFKQYANDNGVDGEWYDDATKT

FTVTE

SpG-C3: (SEQ ID NO: 1)
 TYKLVLINGKTLKGETTTKAVDAETA EKAFKQYANDNGVDGVWYDDATKT

FTVTE

[0055] Whilst in most streptococcal strains Protein G comprises all three Ig-binding domains (C1-C3), some strains contain a Protein G only comprising two Ig-binding domains. Exemplified, streptococcal strain GX7809 contains a Protein G with only two Ig-binding domains, wherein the first domain is identical to C1 of G148 and GX7805 and the last domain identical to C3 of G148 and GX7805.

[0056] The interaction between the IgBD of Protein G, in particular the C1 and C2 domains of SpG, and the Fc fragment of an immunoglobulin is mediated by the α-helix and the third β-strand within the IgBD (Gronenborn & Clore (1993) J. Mol. Biol. 233:331-335). The C3 domain interacts with the Fab fragment of an Ig molecule by binding to the surface exposed region of the C_H1 domain of the Ig molecule. This interaction of the C3 domain with the Fab fragment is mediated through an antiparallel alignment of the second β-strand from domain C3 with the seventh β-strand from the C_H1 domain of the Ig molecule which affects the extension of the four stranded β-sheet of domain C3 into the C_H1 domain (Derrick & Wigley (1994) J. Mol. Biol. 243:906-918). More specifically, the C3 domain interacts with the amino acid positions 122-127 and/or 207-214 of the C_H1 domain according to the EU index as in Kabat (see FIG. 1).

[0057] Unlike Protein A and Protein G, which bind to the heavy chain of immunoglobulins, “Protein L (PpL)” from

Peptostreptococcus magnus binds through light chain interactions to those Ig molecules that contain kappa light chains. In the process PpL does not interfere with the antigen-binding site of the Ig molecule. Protein L binds to representatives of all antibody classes, including IgG, IgM, IgA, IgE and IgD as well as to scFv and Fab fragments.

[0058] The availability of a substance (for example a metabolite, drug, signaling molecule, radioactive nuclide, or other substance) in the body is dependent on several factors, such as its concentration in the blood plasma and the speed of its clearance from the body. The overall persistence of a substance in the body, i.e. the length of time a substance spends in the body, is expressed as the “mean resistance time (MRT)”. The MRT depends on various factors such as the individual’s body size, the rate at which the substance moves through and react within the body, and if applicable the amount of a substance, e.g. a pharmaceutical, administered. The MRT is also dependent on the overall ability of the body to eliminate a certain substance, e.g. a drug, from the plasma. In mammals, plasma clearance is achieved by the main clearing organs: the kidney and the liver. The term “plasma clearance” as used herein thus, refers to the volume of plasma that is cleared of a certain substance in a given time and is measured in units of a volumetric flow rate (volume/time).

[0059] The time it takes for the blood concentration of a substance to fall by one half is referred to as the “plasma half-life” or the “serum half-life” of a substance, irrespective of the factors (e.g. plasma clearance, absorbance by the tissue) which cause the decrease in concentration. The term “plasma” refers to the complete soluble fraction of the blood, whilst the term “serum” refers to plasma devoid of coagulation factors, i.e. obtained after coagulation of blood. Both, the plasma half-life and the serum half-life are measured as concentration in the blood.

[0060] A differentiation may be made between the initial and the terminal plasma or serum half-life of a substance. The terms “initial plasma half-life” (or “initial serum half-life”) and “distribution plasma half-life” (or “distribution serum half-life”) are used interchangeably herein and are abbreviated as $t_{1/2\alpha}$. The initial plasma half-life refers to the phase of rapid drug disappearance from the blood which occurs immediately after the dose is given, and which may lead to a very substantial decrement in drug concentrations in the blood. This initial phase of rapid drug disappearance is determined mainly by reversible distribution of drug out of the “central” compartment, of which the vascular system is a component, into storage sites in peripheral tissues; very little of this initial rapid decline is determined by elimination or clearance. Typically, the initial phase lasts from a few minutes (i.e. 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 min) to a few hours (i.e. 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 hours).

[0061] The terms “terminal plasma half-life” (or “terminal serum half-life”) or “elimination plasma half-life” (or “elimination serum half-life”) are used interchangeably herein and are abbreviated as $t_{1/2\beta}$. The elimination plasma half-life is generally determined only after drug distribution equilibrium has been attained, i.e. after the distribution of the administered substance in the various body tissues is complete. The blood concentration curve enters a less rapid phase of drug disappearance, termed the elimination phase, during which drug disappearance is determined mainly by irreversible clearance. Accordingly, the terminal plasma half-life ($t_{1/2\beta}$) is

determined by clearance (CL) and volume of distribution (V_D) and the relationship is described by the following equation:

$$t_{1/2} = \frac{\ln 2 \cdot V_D}{CL}$$

[0062] Depending on the substance in question, the relationship between the initial plasma half-life and the terminal plasma half-life of such substance may be complex, taking into account factors including its accumulation in the tissues and receptor interactions (Toutain & Bousquet-Melou (2004) J. Vet. Pharmacol. Therap. 27:427-439). Both, the initial plasma half-life (or “initial serum half-life”) and the terminal plasma half-life (or “terminal serum half-life”) of a substance, e.g. a pharmaceutical, can be influenced in order to extend the bioavailability of such substance in the body. The bioavailability of a substance may be determined by measuring the concentration of said substance in the blood (plasma or serum) at certain time intervals after administration and establishing the area under the concentration-time-curve. The value of the “Area under the curve (AUC)” is proportional to the amount of the substance being available in the blood-stream.

[0063] Exemplified, the reduction of plasma clearance, e.g. by increasing the hydrodynamic volume of such substance to reduce renal clearance or by utilizing recycling processes via the FcRn, may lead to a prolonged terminal plasma half-life of the respective substance and thereby to an increased bioavailability in the body.

[0064] In the context of the present application it is preferred that the serum half-life, preferably the terminal serum half-life, of a pharmaceutically active moiety can be prolonged by reducing its plasma clearance and allowing for its recycling via the FcRn by complexing the pharmaceutically active moiety to an IgBD, preferably the C3 IgBD of streptococcal Protein G as described in detail above. The terms “prolonged” and “extended” or “prolongation” and “extension” are used interchangeably herein referring to an increase in the length of time, preferably in the lengths of the serum half-life, in particular the initial and/or terminal serum half-life.

[0065] The term “pharmaceutically active moiety” as used herein, is understood to refer to a part or moiety of a complex which mediates a pharmaceutical effect including but not limited to prophylactic, therapeutic, and/or diagnostic effects.

[0066] As used herein, “prevent”, “preventing”, “prevention”, or “prophylaxis” of a disease or disorder means preventing that such disease or disorder occurs in a patient. Accordingly, a moiety having a prophylactic effect prevents the onset of a disease or disorder in a patient.

[0067] As used herein, “treat”, “treating”, “treatment” or “therapy” of a disease or disorder means accomplishing one or more of the following: (a) reducing the severity of the disorder; (b) limiting or preventing development of symptoms characteristic of the disorder(s) being treated; (c) inhibiting worsening of symptoms characteristic of the disorder(s) being treated; (d) limiting or preventing recurrence of the disorder(s) in an individual that has previously had the disorder(s); and (e) limiting or preventing recurrence of symptoms in individuals that were previously symptomatic for the disorder(s). Accordingly, a moiety having a therapeutic effect

treats the symptoms of a disease or disorder by accomplishing one or more of above named effects (a)-(e).

[0068] The terms “identify”, “identifying”, “identification” or “diagnosis” of a disease or disorder are used herein to refer to the determination of the nature and the cause of a disease or disorder. Accordingly, a moiety having a diagnostic effect allows for the determination of the nature and the cause of a disease or disorder.

[0069] “Symptoms” of a disease or disorder are implication of the disease or disorder noticeable by the tissue, organ or organism having such disease or disorder and include but are not limited to pain, weakness, tenderness, strain, stiffness, and spasm of the tissue, an organ or an individual as well as the presence, absence, increase, decrease, of specific indicators such as biomarkers or molecular markers. The term “disease” and “disorder” as used herein, refer to an abnormal condition, especially an abnormal medical condition such as an illness or injury, wherein a tissue, an organ or an individual is not able to efficiently fulfil its function anymore. Typically, but not necessarily, a disease or disorder is associated with specific symptoms or signs indicating the presence of such disease or disorder.

[0070] A pharmaceutically active moiety typically comprises a biological and/or chemical pharmaceutical. “Chemical pharmaceuticals” are typically understood to refer to chemical compounds synthesized artificially which are effective in the prevention, treatment or diagnosis of disorders or diseases. “Biologicals” are typically understood to refer to medical drugs produced using biotechnological means and are used for prophylactic, therapeutic, and/or in vivo diagnostic purposes. Biologicals include but are not limited to peptides, polypeptides, proteins and nucleic acids (e.g. DNA, RNA, or hybrids thereof). Approved therapeutic biologicals include but are not limited to hormones (e.g. insulin, hGH, FSH, Glucagon-like peptide 1, parathyroid hormone, calcitonin, lutropin, glucagon), growth factors (e.g. erythropoietin, G-CSF/GM-CSF, IGF-1), interferons (e.g. IFN- α , IFN- β , IFN- γ), interleukins (e.g. IL-2, IL-11, IL-1Ra), coagulation factors (e.g. factor VIII, factor IX, factor VIIa, thrombin), thrombolytics and anti-coagulants (e.g. t-PA, hirudin, activated protein C), enzymes (e.g. α -glucosidase, glucocerebrosidase, iduronate-2-sulfatase, galactosidase, urate oxidase, DNase), antigen-binding molecule such as antibodies and antibody fragments (e.g. IgG, Fab), and fusion proteins thereof (e.g. TNFR2-Fc, TMP-Fc, CTLA-4-Fc, IL-1R-Fc, LFA-3-Fc, IL-2-DT).

[0071] A “peptide linker” (or short: “linker”) in the context of the present invention refers to an amino acid sequence which sterically separates two parts or moieties of a complex, e.g. two peptides or proteins. Typically such linker consists of between 1 and 100 amino acids having a minimum length of at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 amino acids, and a maximum length of at least 100, 95, 90, 85, 80, 75, 70, 65, 60, 55, 50, 45, 40, 35, 34, 33, 32, 31, 30, 29, 28, 27, 26, 25, 24, 23, 22, 21, 20, 19, 18, 17, 16, or 15 amino acids or less. The indicated preferred minimum and maximum lengths of the peptide linker according to the present invention may be combined, if such a combination makes mathematically sense, e.g. such linker may consist of 1-15, or 12-40, or 25-75, or 1-100 amino acids. Peptide linkers may also provide flexibility among the two moieties that are linked together. Such flexibility is generally increased if the amino acids are small. Accordingly, flexible peptide linkers comprise an increased

content of small amino acids, in particular of glycines and/or alanines, and/or hydrophilic amino acids such as serines, threonines, asparagines and glutamines. Preferably, more than 20%, 30%, 40%, 50%, 60% or more of the amino acids of the peptide linker are small amino acids.

[0072] The term “cleavage site” as used herein refers to an amino acid sequence or nucleotide sequence wherein this sequence directs the division of a complex or a macromolecule (e.g. a nucleic acid or a protein), e.g. because it is recognized by a cleaving enzyme, and/or can be divided. Typically, a polypeptide chain is cleaved by hydrolysis of one or more peptide bonds that link the amino acids and a polynucleotide chain is cleaved by hydrolysis of one or more of the phosphodiester bond between the nucleotides. Cleavage of peptide- or phosphodiester-bonds may originate from chemical or enzymatic cleavage. Enzymatic cleavage refers to such cleavage being attained by proteolytic enzymes including but not limited to restriction endonuclease (e.g. type I, type II, type III, type IV or artificial restriction enzymes) and endo- or exo-peptidases or -proteases (e.g. serine-proteases, cysteine-proteases, metallo-proteases, threonine proteases, aspartate proteases, glutamic acid proteases). Typically, enzymatic cleavage occurs due to self-cleavage or is affected by an independent proteolytic enzyme. Enzymatic cleavage of a protein or polypeptide can happen either co- or post-translational. Accordingly, the term “endopeptidase cleavage site” used herein, refers to a cleavage site within the amino acid or nucleotide sequence where this sequence is cleaved or is cleavable by an endopeptidase (e.g. trypsin, pepsin, elastase, thrombin, collagenase, furin, thermolysin, endopeptidase V8, cathepsins).

[0073] The term “self-cleavage site” as used herein refers to a cleavage site within the amino acid sequence where this sequence is cleaved or is cleavable without such cleavage involving any additional molecule. It is understood that cleavage sites typically comprise several amino acids. Thus, the cleavage site may also serve the purpose of a peptide linker, i.e. sterically separating two peptides or proteins.

[0074] As used herein, the term “vector” refers to a protein or a polynucleotide or a mixture thereof which is capable of being introduced or of introducing the proteins and/or nucleic acid comprised therein into a cell. In the context of the present invention it is preferred that the genes of interest encoded by the introduced polynucleotide are expressed within the cell upon introduction of the vector or vectors. Examples of suitable vectors include but are not limited to plasmids, cosmids, phages, viruses or artificial chromosomes.

[0075] The terms “pharmaceutical”, “medicament” and “drug” are used interchangeably herein, referring to a substance and/or a combination of substances being used for the identification, prevention or treatment of a disease or disorder.

[0076] The terms “preparation” and “composition” are intended to include the formulation of the active compound with encapsulating material as a carrier providing a capsule in which the active component with or without other carriers, is surrounded by a carrier, which is thus in association with the active compound.

[0077] “Pharmaceutically acceptable” means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans.

[0078] The term “active ingredient” refers to the substance in a pharmaceutical composition or formulation that is biologically active, i.e. that provides pharmaceutical value. A pharmaceutical composition may comprise one or more active ingredients which may act in conjunction with or independently of each other. The active ingredient can be formulated as neutral or salt forms. Pharmaceutically acceptable salts include those formed with free amino groups such as but not limited to those derived from hydrochloric, phosphoric, acetic, oxalic, tartaric acids, etc., and those formed with free carboxyl groups such as but not limited to those derived from sodium, potassium, ammonium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, procaine, and the like.

[0079] The term “carrier”, as used herein, refers to a pharmacologically inactive substance such as but not limited to a diluent, excipient, surfactants, stabilizers, physiological buffer solutions or vehicles with which the therapeutically active ingredient is administered. Such pharmaceutical carriers can be liquid or solid. Liquid carrier include but are not limited to sterile liquids, such as saline solutions in water and oils, including but not limited to those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. A saline solution is a preferred carrier when the pharmaceutical composition is administered intravenously. Examples of suitable pharmaceutical carriers are described in “Remington’s Pharmaceutical Sciences” by E. W. Martin.

[0080] Suitable pharmaceutical “excipients” include starch, glucose, lactose, sucrose, gelatine, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like.

[0081] “Surfactants” include anionic, cationic, and non-ionic surfactants such as but not limited to sodium deoxycholate, sodium dodecylsulfate, Triton X-100, and polysorbates such as polysorbate 20, polysorbate 40, polysorbate 60, polysorbate 65 and polysorbate 80.

[0082] “Stabilizers” include but are not limited to mannitol, sucrose, trehalose, albumin, as well as protease and/or nuclease antagonists.

[0083] “Physiological buffer solution” include but are not limited to sodium chloride solution, demineralized water, as well as suitable organic or inorganic buffer solutions such as but not limited to phosphate buffer, citrate buffer, tris buffer (tris(hydroxymethyl)aminomethane), HEPES buffer ([4 (2 hydroxyethyl)piperazinomethanesulphonic acid) or MOPS buffer (3 morpholino-1 propanesulphonic acid). The choice of the respective buffer in general depends on the desired buffer molarity. Phosphate buffer are suitable, for example, for injection and infusion solutions.

[0084] The term “adjuvant” refers to agents that augment, stimulate, activate, potentiate, or modulate the immune response to the active ingredient of the composition at either the cellular or humoral level, e.g. immunologic adjuvants stimulate the response of the immune system to the actual antigen, but have no immunological effect themselves. Examples of such adjuvants include but are not limited to inorganic adjuvants (e.g. inorganic metal salts such as aluminium phosphate or aluminium hydroxide), organic adjuvants (e.g. saponins or squalene), oil-based adjuvants (e.g. Freund’s complete adjuvant and Freund’s incomplete adju-

vant), cytokines (e.g. IL-1 β , IL-2, IL-7, IL-12, IL-18, GM-CSF, and INF- γ) particulate adjuvants (e.g. immuno-stimulatory complexes (ISCOMS), liposomes, or biodegradable microspheres), virosomes, bacterial adjuvants (e.g. monophosphoryl lipid A, or muramyl peptides), synthetic adjuvants (e.g. non-ionic block copolymers, muramyl peptide analogues, or synthetic lipid A), or synthetic polynucleotides adjuvants (e.g. polyarginine or polylysine).

[0085] An “effective amount” or “therapeutically effective amount” is an amount of a therapeutic agent sufficient to achieve the intended purpose. The effective amount of a given therapeutic agent will vary with factors such as the nature of the agent, the route of administration, the size and species of the animal to receive the therapeutic agent, and the purpose of the administration. The effective amount in each individual case may be determined empirically by a skilled artisan according to established methods in the art.

EMBODIMENTS

[0086] In a first aspect the present invention relates to a complex comprising (i) an immunoglobulin (Ig) binding moiety and (ii) a pharmaceutically active moiety, wherein the Ig binding moiety specifically binds to the constant domain 1 of the heavy chain (C_H1) of an Ig molecule.

[0087] In preferred embodiments, the Ig binding moiety prolongs the serum half-life of the pharmaceutically active moiety, i.e. the pharmaceutically active moiety exhibits a prolonged serum half-life when being part of the complex of the first aspect of the invention. In preferred embodiments the complex of the first aspect is thus, used for extending the serum half-life, preferably the serum half-life of the pharmaceutically active moiety. It is particularly preferred that the initial and/or the terminal serum half-life are extended. It is further preferred that the bioavailability, more preferably the bioavailability of the pharmaceutically active moiety, is increased.

[0088] In the context of the present invention it is preferred that the Ig binding moiety binds to mammalian, avian, fish or reptile Ig, in particular to Igs of laboratory animals including but not limited to mouse, rat and rabbit, and/or domestic animals including but not limited to guinea pig, rabbit, horse, donkey, camel, cow, sheep, goat, pig, chicken, duck, goose, parrot, canary bird, cat, dog, goldfish, trout, pangasius, carp, koi, perch, catfish, salmon, turtle, tortoise, snake, and lizard, and/or primates including but not limited to gibbons, lemurs, chimpanzees, bonobos, gorillas, and human beings. It is particularly preferred that the Ig binding moiety binds to the Ig of human beings. Preferably, the binding of the Ig binding moiety to the Ig molecule occurs in vivo, i.e. within the body of a mammal, bird, fish, or reptile, in particular within the body of a mammal, bird, fish, or reptile as specified above. Accordingly, preferably, the Ig binding moiety binds to an Ig molecule in vivo.

[0089] In further embodiments of the present invention, the Ig binding moiety binds to IgA, IgD, IgE, IgG, and/or IgM, preferably to an IgG of subclasses IgG1, IgG2, IgG3, and/or IgG4, more preferably to IgG1, IgG2, and/or IgG4.

[0090] The Ig binding moiety preferably binds to the Fab fragment and/or the Fc portion of an immunoglobulin molecule. It is particularly preferred that the Ig binding moiety binds to the Fab portion of an Ig molecule, and that it optionally also binds to the Fc fragment. Thus, in preferred embodiments, the Ig binding moiety has a structure allowing for the binding to both, the Fc portion and the Fab portion of an

immunoglobulin molecule (e.g. as illustrated in FIG. 1b). In further preferred embodiments the Ig binding moiety has a structure allowing for the binding to either the Fc portion or the Fab portion of an immunoglobulin molecule (e.g. as illustrated in FIG. 1c for a Fab-binding Ig binding moiety). Preferably, the ability to bind to only one of the Fc portion or the Fab portion of an immunoglobulin molecule is due to the functional inactivation (e.g. the structural deletion of all or parts of the Fab- or Fc-binding site, or the functional inactivation via amino acid deletions, replacements, additions, mutations or exchanges) of the respective binding site of Ig binding moiety via genetic engineering. In preferred embodiments, the Ig binding moiety has an affinity to bind to an Ig molecule at neutral pH (i.e. pH 7), preferably with an affinity of below 10^{-6} to below 10^{-9} M, i.e. with an affinity of below 10^{-6} , below 10^{-7} M, below 10^{-8} M, or below 10^{-9} M. It is particularly preferred that the Ig binding moiety binds to the Fab fragment of an Ig molecule with an affinity of 10^{-7} M to 10^{-6} M (i.e. with an affinity of 1×10^{-7} M, 1.1×10^{-7} M, 1.2×10^{-7} M, 1.3×10^{-7} M, 1.4×10^{-7} M, 1.5×10^{-7} M, 1.6×10^{-7} M, 1.7×10^{-7} M, 1.8×10^{-7} M, 1.9×10^{-7} M, 2×10^{-7} M, 2.1×10^{-7} M, 2.2×10^{-7} M, 2.3×10^{-7} M, 2.4×10^{-7} M, 2.5×10^{-7} M, 2.6×10^{-7} M, 2.7×10^{-7} M, 2.8×10^{-7} M, 2.9×10^{-7} M, 3×10^{-7} M, 3.1×10^{-7} M, 3.2×10^{-7} M, 3.3×10^{-7} M, 3.4×10^{-7} M, 3.5×10^{-7} M, 3.6×10^{-7} M, 3.7×10^{-7} M, 3.8×10^{-7} M, 3.9×10^{-7} M, 4×10^{-7} M, 4.1×10^{-7} M, 4.2×10^{-7} M, 4.3×10^{-7} M, 4.4×10^{-7} M, 4.5×10^{-7} M, 4.6×10^{-7} M, 4.7×10^{-7} M, 4.8×10^{-7} M, 4.9×10^{-7} M, 5×10^{-7} M, 5.1×10^{-7} M, 5.2×10^{-7} M, 5.3×10^{-7} M, 5.4×10^{-7} M, 5.5×10^{-7} M, 5.6×10^{-7} M, 5.7×10^{-7} M, 5.8×10^{-7} M, 5.9×10^{-7} M, 6×10^{-7} M, 6.1×10^{-7} M, 6.2×10^{-7} M, 6.3×10^{-7} M, 6.4×10^{-7} M, 6.5×10^{-7} M, 6.6×10^{-7} M, 6.7×10^{-7} M, 6.8×10^{-7} M, 6.9×10^{-7} M, 7×10^{-7} M, 7.1×10^{-7} M, 7.2×10^{-7} M, 7.3×10^{-7} M, 7.4×10^{-7} M, 7.5×10^{-7} M, 7.6×10^{-7} M, 7.7×10^{-7} M, 7.8×10^{-7} M, 7.9×10^{-7} M, 8×10^{-7} M, 8.1×10^{-7} M, 8.2×10^{-7} M, 8.3×10^{-7} M, 8.4×10^{-7} M, 8.5×10^{-7} M, 8.6×10^{-7} M, 8.7×10^{-7} M, 8.8×10^{-7} M, 8.9×10^{-7} M, 9×10^{-7} M, 9.1×10^{-7} M, 9.2×10^{-7} M, 9.3×10^{-7} M, 9.4×10^{-7} M, 9.5×10^{-7} M, 9.6×10^{-7} M, 9.7×10^{-7} M, 9.8×10^{-7} M, 9.9×10^{-7} M, or 1×10^{-6} M) and/or to the Fc portion of an Ig molecule with an affinity of 10^{-8} M to 10^{-7} M (i.e. with an affinity of 1×10^{-8} M, 1.1×10^{-8} M, 1.2×10^{-8} M, 1.3×10^{-8} M, 1.4×10^{-8} M, 1.5×10^{-8} M, 1.6×10^{-8} M, 1.7×10^{-8} M, 1.8×10^{-8} M, 1.9×10^{-8} M, 2×10^{-8} M, 2.1×10^{-8} M, 2.2×10^{-8} M, 2.3×10^{-8} M, 2.4×10^{-8} M, 2.5×10^{-8} M, 2.6×10^{-8} M, 2.7×10^{-8} M, 2.8×10^{-8} M, 2.9×10^{-8} M, 3×10^{-8} M, 3.1×10^{-8} M, 3.2×10^{-8} M, 3.3×10^{-8} M, 3.4×10^{-8} M, 3.5×10^{-8} M, 3.6×10^{-8} M, 3.7×10^{-8} M, 3.8×10^{-8} M, 3.9×10^{-8} M, 4×10^{-8} M, 4.1×10^{-8} M, 4.2×10^{-8} M, 4.3×10^{-8} M, 4.4×10^{-8} M, 4.5×10^{-8} M, 4.6×10^{-8} M, 4.7×10^{-8} M, 4.8×10^{-8} M, 4.9×10^{-8} M, 5×10^{-8} M, 5.1×10^{-8} M, 5.2×10^{-8} M, 5.3×10^{-8} M, 5.4×10^{-8} M, 5.5×10^{-8} M, 5.6×10^{-8} M, 5.7×10^{-8} M, 5.8×10^{-8} M, 5.9×10^{-8} M, 6×10^{-8} M, 6.1×10^{-8} M, 6.2×10^{-8} M, 6.3×10^{-8} M, 6.4×10^{-8} M, 6.5×10^{-8} M, 6.6×10^{-8} M, 6.7×10^{-8} M, 6.8×10^{-8} M, 6.9×10^{-8} M, 7×10^{-8} M, 7.1×10^{-8} M, 7.2×10^{-8} M, 7.3×10^{-8} M, 7.4×10^{-8} M, 7.5×10^{-8} M, 7.6×10^{-8} M, 7.7×10^{-8} M, 7.8×10^{-8} M, 7.9×10^{-8} M, 8×10^{-8} M, 8.1×10^{-8} M, 8.2×10^{-8} M, 8.3×10^{-8} M, 8.4×10^{-8} M, 8.5×10^{-8} M, 8.6×10^{-8} M, 8.7×10^{-8} M, 8.8×10^{-8} M, 8.9×10^{-8} M, 9×10^{-8} M, 9.1×10^{-8} M, 9.2×10^{-8} M, 9.3×10^{-8} M, 9.4×10^{-8} M, 9.5×10^{-8} M, 9.6×10^{-8} M, 9.7×10^{-8} M, 9.8×10^{-8} M, 9.9×10^{-8} M, or 1×10^{-7} M).

[0091] In preferred embodiments the Ig binding moiety specifically binds to the surface-exposed region of the C_H1 domain of an Ig molecule. The term “surface exposed region of an Ig molecule” preferably refers to those amino acids of an

Ig molecule, which are free to specifically interact with a binding moiety, if the binding moiety and the Ig molecule are in solution, preferably in a physiological solution. Preferably the “surface exposed regions” of an Ig molecule are those, which can elicit an immune response, preferably a B cell specific immune response. Preferred is the surface-exposed region of the C_H1 domain of an IgG molecule. Preferably, the Ig binding moiety interacts with the seventh β -stand of the C_H1 domain of the Ig molecule. It is particularly preferred that the Ig binding moiety specifically binds to an epitope formed by amino acid positions 122-127 and/or 207-214 of an Ig molecule according to EU index as in Kabat (see FIG. 12). Preferably, the Ig binding moiety specifically binds to an epitope formed by amino acid positions 122-127 and/or 207-214 according to EU index as in Kabat of a human Ig $\gamma 1$ according to SEQ ID NO: 4, human Ig $\gamma 2$ according to SEQ ID NO: 5, human Ig $\gamma 3$ according to SEQ ID NO: 6, human Ig $\gamma 4$ according to SEQ ID NO: 7, mouse Ig $\gamma 1$ according to SEQ ID NO: 8, mouse Ig $\gamma 2a$ according to SEQ ID NO: 9, mouse Ig $\gamma 2b$ according to SEQ ID NO: 10, mouse Ig $\gamma 3$ according to SEQ ID NO: 11, and/or rat $\gamma 1$ according to SEQ ID NO: 12, or variants thereof. Preferably, the Ig binding moiety has an affinity to the C_H1 domain of an Ig molecule, more preferably to the surface-exposed region of the C_H1 domain of an IgG molecule of below 10^{-6} to below 10^{-9} M or the preferred affinities set out above in more detail.

[0092] In the context of the present invention, the Ig binding moiety preferably comprises an immunoglobulin binding domain (IgBD). Preferably, the IgBD is derived from an Ig binding protein of gram-positive bacteria, more preferably the IgBD is a *streptococcus-derived IgBD*. In preferred embodiments, the Ig binding moiety comprises a C_H1 binding-IgBD, preferably of a streptococcal strain, more preferably a C_H1 binding-IgBD of streptococcal protein G. It is further preferred that the Ig binding moiety comprises the C3 IgBD of streptococcal protein G (the abbreviations “SpG-C3” or “SpG_{C3}”, are used interchangeably herein), more preferably comprising an amino acid sequence according to SEQ ID NO: 1 or variants thereof. In preferred embodiments variants comprise an amino acid sequence of at least 70% identity to the amino acid sequence of SpG-C3, preferably of SEQ ID NO: 1, i.e. comprise an amino acid sequence of at least 70%, of at least 71%, of at least 72%, of at least 73%, of at least 74%, of at least 75%, at least 76%, at least 77%, at least 78%, at least 79%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, or at least 98% to the amino acid sequence according to SEQ ID NO: 1. In particularly preferred embodiments, variants comprise an amino acid sequence of at least 94% identity to the amino acid sequence of SpG-C3, preferably of SEQ ID NO: 1, i.e. comprise an amino acid sequence of at least 94%, at least 95%, at least 96%, at least 97%, or at least 98% to the amino acid sequence according to SEQ ID NO: 1. Thus, it is preferred that variants of SpG-C3, preferably of SEQ ID NO: 1, have between 1 and 14 amino acids substitutions, deletions and/or insertions, e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13 or 14 substitutions, deletions and/or insertions.

[0093] It is preferred that the plasma half-life, preferably the initial and/or the terminal plasma half-life, or the serum half-life, preferably the initial and/or the terminal serum half-life, of the variant is not altered with respect to the naturally occurring C_H1 -binding IgBD, preferably the Ig binding pro-

tein of a gram-positive bacteria, on which the variant is based. In further preferred embodiments, the plasma half-life, preferably the initial and/or the terminal plasma half-life; or the serum half-life, preferably the initial and/or the terminal serum half-life, of the variant is increased with respect to the naturally occurring C_H1 -binding IgBD on which the variant is based. Preferably, the plasma half-life, more preferably the initial and/or the terminal plasma half-life; or serum half-life, more preferably the initial and/or the terminal serum half-life, is increased by at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, more preferably by at least 80%.

[0094] It is also preferred that the bioavailability of the variant is not altered with respect to the naturally occurring C_H1 -binding IgBD. It is further preferred that the bioavailability of the variant is increased with respect to the naturally occurring C_H1 -binding IgBD. Preferably, the bioavailability of the variant is increased by at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, more preferably by at least 80%.

[0095] In preferred embodiments such variant comprises amino acid exchanges, insertions, deletions, or N- or C-terminal truncations, or any combination of these changes, which may occur at one or several sites. Optionally such variants may alter the binding properties of the IgBD, preferably by increasing the binding affinity of the IgBD to the Ig molecule. An increased binding affinity to an Ig molecule may be achieved by preventing the binding of the IgBD to the Fc- or the Fab-fragment ("C3-Fc" or "C3-Fab", respectively), e.g. by exchanging one or more amino acid positions to contain the amino acid alanine. Preferably, the Fab-binding of the C3 IgBD of streptococcal protein G is prohibited by the amino acid exchanges Thr10Ala, Lys12Ala, and Glu14Ala, as shown in SEQ ID NO: 2 (C3-Fc). The Fc-binding of the C3 IgBD of streptococcal protein G is prohibited by the amino acid exchanges Glu26Ala, Lys27Ala, and Lys30Ala, as shown in SEQ ID NO: 3 (C3-Fab).

[0096] In embodiments of the first aspect of the present invention, the pharmaceutically active moiety comprises a biological and/or chemical pharmaceutical. Preferably, the pharmaceutically active moiety comprises a biological such as but not limited to pharmaceutically, preferably therapeutically, active peptides, polypeptides, or proteins produced via biotechnological means. Suitable biologicals include but are not limited to hormones (e.g. insulin, hGH, FSH, Glucagon-like peptide 1, parathyroid hormone, calcitonin, lutropin, glucagon), blood factors (e.g. factor VIII, factor IX, factor XI), growth factors (e.g. erythropoietin, G-CSF/GM-CSF, IGF-1), interferons (e.g. IFN- α , IFN- β , IFN- γ), interleukins (e.g. IL-2, IL-11, IL-1Ra), coagulation factors (e.g. factor VIII, factor IX, factor VIIa, thrombin), thrombolytics and anticoagulants (e.g. t-PA, hirudin, activated protein C), enzymes (e.g. α -glucosidase, glucocerebrosidase, iduronate-2-sulfatase, galactosidase, urate oxidase, DNase), vaccines (e.g. parasitic, fungal, bacterial, or viral antigens such as e.g. hepatitis B surface antigens), antigen-binding molecule such as antibodies and antibody fragments (e.g. IgG, Fab), and fusion proteins thereof (e.g. TNFR2-Fc, TMP-Fc, CTLA-4-Fc, IL-1R-Fc, LFA-3-Fc, IL-2-DT).

[0097] In preferred embodiments, the pharmaceutically active moiety does not comprise the N-terminal domain of the diphtheria toxin, a cellulose-binding domain (CBD), diagnostic proteins, in particular a firefly luciferase and/or the green

fluorescent protein (GFP) of *Aequorea victoria*. Diagnostic proteins are those, which are capable to fluoresce.

[0098] In the context of the present invention it is particularly preferred that the pharmaceutically active moiety comprises an antigen-binding molecule such as an immunoglobulin molecule. Preferably, the antigen-binding molecule is selected from the group consisting of an antibody fragment, a Fab fragment, a Fab' fragment, a F(ab')₂ fragment, a heavy chain antibody, a single-domain antibody (sdAb), a single-chain variable fragment (scFv), a di-scFv, a bispecific T-cell engager (BITEs), a diabody, a single-chain diabody, a DART molecule, a triple body, an alternative scaffold protein, and a fusion proteins thereof. In particularly preferred embodiments the antigen-binding molecule is a scFv or a diabody or a fusion protein comprising a scFv or a diabody. Preferably, the antigen-binding molecule does not comprise a cellulose-binding domain (CBD).

[0099] Additionally or alternatively, the antigen-binding molecule may further comprise a radioactive moiety, a cytotoxic drug, a chelating moiety, a photosensitizer, or an imaging reagent.

[0100] In preferred embodiments the antigen-binding molecule comprises a radioactive moiety, i.e. a radionuclide. The radioactive moiety may be an isotope of F, Br, Mn, Co, Ga, As, Zr, P, C, S, H, I, In, Lu, Cu, Rh, Bi, At, Y, Re, Ac, Tc, or Hg atom. The radioactive moiety labels the antigen-binding molecule radioactively allowing for its detection, e.g. in the human body, rendering it not only useful for diagnostic approaches (radioimmunoassay: RIA) but also suitable in therapeutic applications (radioimmunotherapy: RIT).

[0101] Photosensitizers are chemical compounds capable of light emission or formation of free radicals and singlet oxygen after being excited by light of a specific wavelength. Photosensitizer are used e.g. for photodynamic therapy. In preferred embodiments photosensitizer include but are not limited to compounds of the porphyrin family, texaphyrin family, the chlorin family and the phthalocyanine family, in particular including HpD, ALA, M-ALA, Vertiporphin, Lutetaphyrin, Temoporfin, Talaporfin, HPPH, Phthalocyanine, and Naphthalocyanine.

[0102] Imaging reagents include bioluminescent, chemiluminescent and fluorescent imaging reagent such as but not limited to luciferase from *Renilla reniformis* and/or *Metridia longa*, peroxalate, polymethines (e.g. cyanine dyes such as Cy3, Cy5, Cy5.5, Cy7) squaraine derivatives, phthalocyanine, porphyrin derivatives, and BODIPY analogous (BODIPY FL, BODIPY R6G, BODIPY TR, BODIPY TMR, BODIPY 581/591, BODIPY 630/650, BODIPY 650/665), as well as fluorescent proteins such as but not limited to CFP, BFP, YFP, DsRED (Chudakov et al. (2010) *Physiol. Rev.* 90: 1103-1163). Preferably, the fluorescent protein is not GFP.

[0103] In preferred embodiments the antigen-binding molecule is a cytotoxic drug which has a toxic effect on cells such as but not limited to antimitotic drugs, drugs prohibiting cell growths and drugs causing cell death. Non-limiting examples of cytotoxic drugs are alkylating agents (e.g. cisplatin, carboplatin, oxaloplatin, mechlorethamine, cyclophosphamide, chlorambucil), anti-metabolites (5-fluorouracil (5-FU), capecitabine (Xeloda®), 6-mercaptopurine (6-MP), methotrexate, gemcitabine), plant alkaloids (e.g. ajmaline, atropine, scopolamine, hyoscyamine, vinca alkaloids, codeine cocaine colchicine morphine, reserpine, tubocurarine, physostigmine, quinidine, quinine, emetine, ergot alkaloids), antitumor antibiotics (e.g. actinomycin-D, bleomycin, and mitomycin-

C, mitoxantrone, and anthracyclines such as daunorubicin, doxorubicin), topoisomerase inhibitors (e.g. topotecan, irinotecanetoposide (VP-16) and teniposide), and mitotic inhibitors (estramustine, taxanes such as paclitaxel and docetaxel, epothilones such as ixabepilone, and vinca alkaloids such as vinblastine, vincristine, vindesine and vinorelbine).

[0104] The antigen-binding molecule may further comprise a chelating moiety capable of binding at least one metal ion, such as but not limited to calcium, magnesium, iron, aluminium, zinc, copper, arsenic, lead, thallium, and mercury ions, by chelation. Such chelating moiety may comprise ethylenediamine tetraacetic acid (EDTA), ethylenediamine tetraacetic acid (calcium disodium versante) (CaNa₂-EDTA), dimercaprol (BAL), dimercaptosuccinic acid (DMSA), dimercapto-propane sulfonate (DMPS), ferritin, deferoxamine and deferasirox, deferiprone (1,2-dimethyl-3-hydroxyl-4-pyridinone), DOTA, DTPA, DADT, DADS, DO3A, N2S2MAMA, Triamidethiol, phosphonates, organic gadolinium complexes, penicillamine, and antibiotic drugs of the tetracycline family. A chelating moiety is of particular interest in chelating therapy, e.g. in the treatment of atherosclerosis, rheumatoid arthritis, and poisoning such as mercury poisoning, copper toxicity, gold toxicity, arsenic poisoning, lead poisoning, acute iron poisoning, and iron overload. Chelating moieties are also important for radiotherapy.

[0105] Preferably, the antigen binding molecule is a fusion protein, which additionally or alternatively further comprises a proapoptotic protein, an immuno-(co)stimulatory protein, immuno-suppressive protein, a cytokine (e.g. interleukins and/or interferons), a chemokine (e.g. an α -, β -, or γ -chemokine), a toxin, a growth factor or an enzyme, preferably a RNase, a prodrug-converting enzyme or a kinase (e.g. AGC kinases, CaM kinases, CK1 kinases, CMGC kinases, STE kinases, TK kinases, and TKL kinases).

[0106] In preferred embodiments proapoptotic protein include but are not limited to Bid, Bik, Puma, and Bim, and proapoptotic cytokines (death ligands) such as but not limited to TNF, TRAIL, and FasL.

[0107] In preferred embodiments immuno-(co)stimulatory protein include but are not limited to B7.1, B7.2, 4-1BBL, LIGHT, ICOSL, GITR, CD40, OX40L, and CD70.

[0108] Immuno-suppressive proteins preferably include but are not limited to IL1-Ra and toxins preferably include but are not limited to *Pseudomonas* exotoxin A and ricin. Preferably, the toxin is not diphtheria toxin.

[0109] In preferred embodiments, cytokines are interleukins and/or interferons. Interleukins (IL) include but are not limited to Interleukin-1, Interleukin-2, Interleukin-3, Interleukin-4, Interleukin-5, Interleukin-6, Interleukin-7, Interleukin-8, Interleukin-9, Interleukin-10, Interleukin-11, Interleukin 12, Interleukin-13, Interleukin-14, Interleukin-15, Interleukin-16, Interleukin-17, Interleukin-18, Interleukin-19, Interleukin-20, Interleukin-21, Interleukin-22, Interleukin-23, Interleukin-24, Interleukin-25, Interleukin-26 Interleukin-27, Interleukin-28, Interleukin-29, Interleukin-30, Interleukin-31, Interleukin-32, Interleukin-33, Interleukin-34 and Interleukin-35. Interferons (IFN) include but are not limited to interferon type I (e.g. IFN- α , IFN- β and IFN- ω), interferon type II (e.g. IFN- γ), and interferon type III. In particular included are interferon A1, interferon A2, interferon A4, interferon A5, interferon A6, interferon A7, interferon A8, interferon A10, interferon A13, interferon A14, interferon A16, interferon A17, interferon A21, interferon B1, TNF, TRAIL, and FasL.

[0110] In preferred embodiments growth factors include but are not limited to Adrenomedullin (AM), Angiopoietin (Ang), Autocrine motility factor, Bone morphogenetic proteins (BMPs), Brain-derived neurotrophic factor (BDNF), Epidermal growth factor (EGF), Erythropoietin (EPO), Fibroblast growth factor (FGF), Glial cell line-derived neurotrophic factor (GDNF), Granulocyte colony-stimulating factor (G-CSF), Granulocyte macrophage colony-stimulating factor (GM-CSF), Growth differentiation factor-9 (GDF9), Hepatocyte growth factor (HGF), Hepatoma-derived growth factor (HDGF), Insulin-like growth factor (IGF), Migration-stimulating factor Myostatin (GDF-8), Nerve growth factor (NGF) and other neurotrophins, Platelet-derived growth factor (PDGF), Thrombopoietin (TPO), Transforming growth factor alpha (TGF- α), Transforming growth factor beta (TGF- β), Vascular endothelial growth factor (VEGF), Wnt Signaling Pathway, and placental growth factor (PlGF).

[0111] RNases include endoribonucleases such as but are not limited to RNase A, RNase H, RNase I, RNase III, RNase L, RNase P, RNase PhyM, RNase T1, RNase T2, RNase U2, RNase V1, and RNase V, and exoribonucleases such as but not limited to Polynucleotide Phosphorylase (PNPase), RNase PH, RNase II, RNase R, RNase D, RNase T, Oligoribonuclease Exoribonuclease I, and Exoribonuclease II.

[0112] Pro-drug-converting enzymes include but are not limited to esterases such as but not limited to acetylcholinesterase, thiolester hydrolases, phosphoric monoester hydrolases, phosphoric diester hydrolases, triphosphoric monoester hydrolases, sulfuric ester hydrolases (sulfatases), diphosphoric monoester hydrolases, and phosphoric triester hydrolases; phosphatases such as but not limited to tyrosine-specific phosphatases, serine/threonine specific phosphatases, dual specificity phosphatases, histidine phosphatase, and lipid phosphatase; and reductases such as but not limited to 5-alpha reductase, dihydrofolate reductase, HMG-CoA reductase, methemoglobin reductase, ribonucleotide reductase, thioredoxin reductase, *E. coli* nitroreductase, methylenetetrahydrofolate reductase, and carboxypeptidase G2, cytosine deaminase, nitroreductase, thymidine kinase.

[0113] Kinases include but are not limited to AGC kinases such as PKA, PKC and PKG, CaM kinases such as calcium/calmodulin-dependent protein kinases and serine/threonine protein kinases (e.g. DAPK2), CK1 such as the casein kinase 1 group, CMGC such as CDK, MAPK, GSK3 and CLK kinases, STE such as homologs of yeast Sterile 7, Sterile 11, and Sterile 20 kinases, tyrosine kinases (TK), the tyrosine-kinase like group of kinases (TKL), receptor-associated tyrosine kinases, MAP kinases, and histidine kinases.

[0114] In particularly preferred embodiments the pharmaceutically active moiety is a peptide-linked or a disulfide-linked single-chain diabody. It is particularly preferred that the pharmaceutically active moiety is a single-chain diabody with a first specificity (A) directed against a target molecule, and a second specificity (B) directed against an effector molecule. Preferably, the single-chain diabody comprises the structure [VH(A)-VL(B)-P-VH(B)-VL(A)] or [VL(B)-VH(A)-P-VL(A)-VH(B)]. In preferred embodiments the first specificity (A) is directed against a tumor-associated antigen or an antigen of a pathogen. Preferably, the tumor-associated antigen is selected from the group consisting of CEA, EGFR, HER2, HER3, HER4, VEGFRs, integrin receptor family, fibroblast activation protein, galectin, EpCAM, CEA, CD44, CD44v, CD2, CD5, CD7, CD19, CD20, CD21, CD22, CD24,

CD25, CD30, CD33, CD38, CD40, CD52, CD56, CD71, CD72, CD73, CD105, CD117, CD123, c-Met, PDGFR, IGF1-R, HMW-MAA, TAG-72, GD2, GD3, GM2, folate receptor, Leg, MUC-1, MUC-2, PSMA, PSCA and uPAR. In further preferred embodiments the second specificity (B) is directed against molecules of cell membranes, cytokines, chemokines, growth factors, proteins of the complement system, proteins of the coagulation system, fibrinolytic proteins, enzymes which are able to convert the inactive precursor of a drug into an active drug on the target structure, peptide hormones, steroid hormones, the constant part of an immunoglobulin, cytotoxic peptide, and pharmaceuticals. Preferably, the second specificity (B) is directed against molecules on the cell membrane of lymphocytes, macrophages, monocytes or granulocytes, more preferably against molecules on the cell membrane of T-cells. It is particularly preferred that the second specificity (B) is directed against CD3, more preferably against the extracellular region of CD3.

[0115] In further embodiments of the present invention the Ig binding moiety and the pharmaceutically active moiety are connected via covalent or non-covalent bond(s). It is particularly preferred that the Ig binding moiety and the pharmaceutically active moiety are connected directly or indirectly via one or more linkers. Preferably, the one or more linkers comprise peptide linkers, more preferably flexible peptide linkers. In preferred embodiments, a peptide linker according to the present invention has a minimum length of at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 amino acids, preferably of at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 amino acids. Preferably, a peptide linker according to the present invention has a maximum length of at least 100, 95, 90, 85, 80, 75, 70, 65, 60, 55, 50, 45, 40, 35, 34, 33, 32, 31, 30, 29, 28, 27, 26, 25, 24, 23, 22, 21, 20, 19, 18, 17, 16, or 15 amino acids or less. Preferably, the linker has a length of 1-40, preferably of 5-20, more preferably of 18-12, most preferably of 10 amino acids. In preferred embodiments of the present invention, the peptide linker has an increased content of small amino acids, in particular of glycines and/or alanines, and/or hydrophilic amino acids such as serines, threonines, asparagines and glutamines. Preferably, more than 20%, 30%, 40%, 50%, 60% or more of the amino acids of the peptide linker are small and/or hydrophilic amino acids. Preferably the amino acids of the linker are selected from glycines and serines. In further preferred embodiments, the peptide linker of the present invention is non-immunogenic; in particularly preferred embodiments, the peptide linker is non-immunogenic to humans. A peptide linker having the sequence GSGGGSGG is particularly preferred.

[0116] In preferred embodiments the Ig binding moiety comprises a streptococcal IgBD, more preferably the C3 IgBD of streptococcal Protein G (SpG-C3), which is connected via a flexible linker to the pharmaceutically active moiety, preferably to an antigen binding molecule selected from the group consisting of an antibody fragment, a Fab fragment, a Fab' fragment, a F(ab')₂ fragment, a heavy chain antibody, a single-domain antibody (sdAb), a single-chain variable fragment (scFv), a di-scFv, a bispecific T-cell engager (BiTEs), a diabody, a single-chain diabody, a DART molecule, a triple body, an alternative scaffold protein, and a fusion protein thereof.

[0117] In particularly preferred embodiments of the first aspect of the present invention, the complex of the present invention comprises an amino acid sequence according to

SEQ ID NO: 13, SEQ ID NO: 14 or SEQ ID NO: 15 or variants thereof. Preferably, such variant has a sequence identity of at least 94%, i.e. of at least 94%, at least, 95%, at least 96%, at least 97%, at least 98%, at least 99% sequence identity, to an amino acid sequence according to SEQ ID NO: 13, SEQ ID NO: 14 or SEQ ID NO: 15.

[0118] In further embodiments the one or more peptide linkers comprise one or more cleavage sites, preferably one or more endopeptidase cleavage sites. It is preferred that the cleavage site allows for the release of the pharmaceutically active moiety once the intended destination is reached. Preferably, an endopeptidase cleavage site relates to cleavage site within the amino acid sequence where this sequence is cleaved or is cleavable by an endopeptidase such as but not limited to trypsin, pepsin, elastase, thrombin, collagenase, furin, thermolysin, endopeptidase V8, metalloproteinases and cathepsins.

[0119] In a second aspect, the present invention provides a nucleic acid molecule comprising a sequence encoding the complex of the first aspect. Preferably such nucleic acid molecule comprises a DNA and/or RNA molecule.

[0120] In a third aspect, the present invention provides a vector comprising the nucleic acid of the second aspect. It is understood that suitable vectors include but are not limited to plasmids, cosmids, phages, viruses and/or artificial chromosomes.

[0121] In a fourth aspect, the present invention provides an isolated cell containing the complex of the first aspect and/or the nucleic acid molecule of the second aspect and/or the vector of the third aspect. It is understood that such cell includes but is not limited to prokaryotic (e.g. a bacterial cell) or eukaryotic cells (e.g. a fungal, plant or animal cell).

[0122] In a fifth aspect, the present invention provides a composition comprising the complex of the first aspect, the nucleic acid of the second aspect, the vector of the third aspect and/or the cell of the fourth aspect and a pharmaceutical acceptable carrier and/or excipient. Preferably, such composition is a pharmaceutical composition. In preferred embodiments the pharmaceutical composition further comprises a pharmaceutically acceptable carrier and/or excipient and optionally one or more additional active substances. Preferably, the composition of the fifth aspect contains a therapeutically effective amount of the compound, preferably in purified form, together with a suitable amount of carrier and/or excipient so as to provide the form for proper administration to the patient. The formulation should suit the mode of administration.

[0123] The pharmaceutical compositions can take the form of solutions, suspensions, emulsion, tablets, pills, capsules, powders, sustained-release formulations and the like. The pharmaceutical composition can be formulated as a suppository, with traditional binders and carriers such as triglycerides.

[0124] For preparing pharmaceutical compositions of the present invention, pharmaceutically acceptable carriers can be either solid or liquid. Solid form compositions include powders, tablets, pills, capsules, lozenges, cachets, suppositories, and dispersible granules. A solid excipient can be one or more substances, which may also act as diluents, flavouring agents, binders, preservatives, tablet disintegrating agents, or an encapsulating material. In powders, the excipient is preferably a finely divided solid, which is in a mixture with the finely divided inhibitor of the present invention. In tablets, the active ingredient is mixed with the carrier having

the necessary binding properties in suitable proportions and compacted in the shape and size desired. Suitable excipients are magnesium carbonate, magnesium stearate, talc, sugar, lactose, pectin, dextrin, starch, gelatin, tragacanth, methylcellulose, sodium carboxymethylcellulose, a low melting wax, cocoa butter, and the like. For preparing suppositories, a low melting wax, such as a mixture of fatty acid glycerides or cocoa butter, is first melted and the active component is dispersed homogeneously therein, as by stirring. The molten homogeneous mixture is then poured into convenient sized moulds, allowed to cool, and thereby to solidify. Tablets, powders, capsules, pills, cachets, and lozenges can be used as solid dosage forms suitable for oral administration.

[0125] Liquid form compositions include solutions, suspensions, and emulsions, for example, water, saline solutions, aqueous dextrose, glycerol solutions or water/propylene glycol solutions. For parenteral injections (e.g. intravenous, intraarterial, intraosseous infusion, intramuscular, subcutaneous, intraperitoneal, intradermal, and intrathecal injections), liquid preparations can be formulated in solution in, e.g. aqueous polyethylene glycol solution. A saline solution is a preferred carrier when the pharmaceutical composition is administered intravenously.

[0126] Preferably, the pharmaceutical composition is in unit dosage form. In such form the composition may be subdivided into unit doses containing appropriate quantities of the active component. The unit dosage form can be a packaged composition, the package containing discrete quantities of the composition, such as packaged tablets, capsules, and powders in vials or ampoules. Also, the unit dosage form can be a capsule, an injection vial, a tablet, a cachet, or a lozenge itself, or it can be the appropriate number of any of these in packaged form.

[0127] The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents.

[0128] Furthermore, such pharmaceutical composition may also comprise other pharmacologically active substance such as but not limited to adjuvants and/or additional active ingredients. Adjuvants in the context of the present invention include but are not limited to inorganic adjuvants, organic adjuvants, oil-based adjuvants, cytokines, particulate adjuvants, virosomes, bacterial adjuvants, synthetic adjuvants, or synthetic polynucleotides adjuvants.

[0129] In a sixth aspect, the present invention provides the complex of the first aspect of the present invention as described in detail above, for the use in extending the serum half-life and/or the plasma half-life. It is preferred that the complex of the first aspect of the present invention is for the use in extending the initial and/or terminal serum half-life. In preferred embodiments the serum half-life, more preferably the initial and/or terminal serum half-life, of the pharmaceutically active moiety is prolonged. Preferably, the serum half-life, more preferably the initial and/or terminal serum half-life, of the pharmaceutically active moiety is prolonged due to its complexing to an immunoglobulin-binding moiety, preferably to an IgBD, more preferably to the C3-IgBD of streptococcal Protein G.

[0130] It is further preferred that the complex of the first aspect of the present invention is for the use in extending the initial and/or terminal plasma half-life. In preferred embodiments the plasma half-life, more preferably the initial and/or terminal plasma half-life, of the pharmaceutically active moiety is prolonged. Preferably, the plasma half-life, more pref-

erably the initial and/or terminal plasma half-life, of the pharmaceutically active moiety is prolonged due to its complexing to an immunoglobulin-binding moiety, preferably to an IgBD, more preferably to the C3-IgBD of streptococcal Protein G.

[0131] In a seventh aspect, the present invention provides the complex of the first aspect of the present invention as described in detail above for use as a medicament. In preferred embodiments the complex is for use in medicine, i.e. for use in the prophylaxis, treatment or diagnosis of a disorder or disease such as but not limited to autoimmune diseases, allergic diseases, cancer type diseases, cutaneous conditions, endocrine diseases, eye diseases and disorders, genetic disorders, infectious diseases, intestinal diseases, neurological disorders, and mental illness. Exemplified, autoimmune diseases include but are not limited to Diabetes mellitus type 1, rheumatoid arthritis, psoriasis, Crohns Disease, autoimmune cardiomyopathy, autoimmune hepatitis, Hashimoto's thyroiditis, and Sjogern's syndrome. Exemplified, allergic diseases include but are not limited to allergic rhinitis, asthma, atopic eczema, anaphylaxis, insect venom allergies, drug allergies, and food allergies. Exemplified, cancer type diseases include but are not limited to Basal cell carcinoma, Bladder cancer, Bone cancer, Brain tumor, Breast cancer, Burkitt lymphoma, Cervical cancer, Colon Cancer, Cutaneous T-cell lymphoma, Esophageal cancer, Retinoblastoma, Gastric (Stomach) cancer, Gastrointestinal stromal tumor, Glioma, Hodgkin lymphoma, Kaposi sarcoma, Leukemias, Lymphomas, Melanoma, Oropharyngeal cancer, Ovarian cancer, Pancreatic cancer, Pleuropulmonary blastoma, Prostate cancer, Throat cancer, Thyroid cancer, and Urethral cancer. Exemplified, cutaneous conditions include but are not limited to Acne, Dermatitis, Eczema, conditions of the skin appendages, conditions of the subcutaneous fat, disturbances of pigmentation, epidermal nevi, epidermal neoplasms, epidermal cysts, erythemas, frostbites, genodermatoses, mucinoses, neurocutaneous conditions (e.g. Wiskott-Aldrich syndrome), and psoriasis. Exemplified, endocrine diseases include but are not limited to Diabetes mellitus type 1 and type 2, Osteoporosis, and Cushing's disease. Exemplified, genetic disorders include but are not limited to color blindness, cystic fibrosis, Down syndrome, Sickle-cell disease, and Turner syndrome. Exemplified, infectious diseases include but are not limited to infections diseases caused by viruses, bacteria, worms, prions or other pathogens or parasites such as African sleeping sickness, AIDS, HIV infection, Anthrax, Borreliosis, Calicivirus infection (Norovirus and Sapovirus), Chickenpox, *Chlamydia* infection, Cholera, *Clostridium* infection, Colorado tick fever (CTF), common cold, Creutzfeldt-Jakob disease, Dengue fever (DEN-1, DEN-2, DEN-3 and DEN-4), Ebola, Enterovirus infection, infections with Human herpesvirus 6 (HHV-6) and Human herpesvirus 7 (HHV-7), Gonorrhea, Streptococcal infections (group A and B), Hand, foot and mouth disease (HFMD), *Helicobacter pylori* infection, Hepatitis (A, B, C, and D), Herpes infection, Papillomavirus infection, Parainfluenza virus infection, Influenza, Lassa fever, Marburg fever, Measles, Meningitis, Mumps, Pasteurellosis, *Pediculus* infection, Plague, Pneumococcal infection, Respiratory syncytial virus infection, Rotavirus infection, Rubella virus infection, *Salmonella* food poisoning and infection, SARS, Scabies infections, Schistosomiasis, Smallpox, Staphylococcal food poisoning and infection, Syphilis, Tetanus, *Trichophyton* infection, Tuberculosis, Typhus, Venezuelan equine

encephalitis, and Yellow fever. Exemplified, intestinal diseases include but are not limited to Gastroenteritis, Ileus, Ileitis, Colitis, Appendicitis, Coeliac disease, Irritable bowel syndrome, Diverticular disease, Diarrhea, Polyp, and Ulcerative colitis. Exemplified, neurological disorders include but are not limited to Amyotrophic Lateral Sclerosis (ALS), Alzheimer's disease, Brain damage, Creutzfeldt-Jakob disease, Cushing's syndrome, Dyslexia, Encephalitis, Epilepsy, Headache, Huntington's disease, Migraine, Multiple sclerosis, Parkinson's disease, Polio, Rabies, Schizophrenia, and Stroke. Exemplified, mental illness include but are not limited to Acute stress disorder, attention-deficit hyperactivity disorder (ADHD), Autistic disorder, Borderline personality disorder, Bulimia *nervosa*, Burn Out, Schizophrenia, Depression, Cognitive disorder, Communication disorder, Eating disorder, Kleptomania, Learning disorders, Male erectile disorder, Melancholia, Obsessive-compulsive disorder (OCD), Paranoia Pathological gambling, Posttraumatic stress disorder (PTSD), Psychotic disorder, Hypersomnia, Insomnia, and Tourette's syndrome.

[0132] The following examples are merely illustrative of the present invention and should not be construed to limit the scope of the invention as indicated by the appended claims in any way.

EXAMPLES

Example 1

Construction and Production of scDb-IgBD Fusion Proteins

[0133] DNA encoding the IgBDs (IgBD SpA_B, SpA_D, SpA_{EZ4}, SpG_{C3}, and PpL_{C4*}) including a hexahistidyl-tag at the C-terminus were synthesized by GeneArt (Regensburg, Germany) adding a NotI at the 5' end and an EcoRI and XbaI site at the 3' end. IgBD SpA_B was cloned into mammalian expression vector pSecTagAHis scDb-CEACD3-ABD-L (Hopp et al. (2010) Protein Eng. Des. Sel. 23:827-834) cut with NotI and XbaI. The IgBD SpA_D, SpA_{EZ4}, SpG_{C3}, and PpL_{C4*} were then cloned into scDb-CEACD3-SpA_B as NotI-EcoRI fragments substituting the SpA_B IgBD. Composition of the scDb-IgBD fusion proteins are given in FIG. 2a. HEK293 cells were stably transfected and the fusion proteins scDb-SpA_B, scDb-SpA_D, scDb-SpA_{EZ4}, scDb-SpG_{C3}, and scDb-PpL_{C4*} were purified from cell culture supernatant by IMAC essentially as described previously (Müller et al. (2007) J. Biol. Chem. 282:12650-12660). Yields of 2 to 22 mg/L supernatant were obtained. SDS-PAGE of purified fusion proteins was performed. Two micrograms proteins were analyzed per lane and the gel was stained with Coomassie brilliant blue G-250 (M, molecular weight standards). SDS-PAGE analysis revealed a single band under reducing and non-reducing conditions (FIG. 2b). Compared with unmodified scDb, the molecular mass was increased by approximately 5 kDa under reducing conditions.

Example 2

Construction and Production of scFv-IgBD Fusion Proteins

[0134] DNA encoding the IgBDs (IgBD SpA_B, SpA_D, SpA_{EZ4}, SpG_{C3}, and PpL_{C4*}) including a hexahistidyl-tag at the C-terminus were synthesized by GeneArt (Regensburg, Germany) adding a NotI at the 5' end and an EcoRI and XbaI

site at the 3' end. The DNA was digested with NotI and EcoRI and cloned into vector pSecTagA-scFvCEA-4-1BBL (Müller et al. (2008) J. Immunol. 31:714-722). Composition of the scFv-IgBD fusion proteins are given in FIG. 2a. HEK293 cells were stably transfected and the fusion proteins scFv-SpA_B, scFv-SpA_D, scFv-SpA_{EZ4}, scFv-SpG_{C3}, and scFv-PpL_{C4*} were purified from cell culture supernatant by IMAC essentially as described previously (Müller et al., (2007) J. Biol. Chem. 282:12650-12660). SDS-PAGE of purified fusion proteins was performed. Two micrograms proteins were analyzed per lane and the gel was stained with Coomassie brilliant blue G-250 (M, molecular weight standards). SDS-PAGE analysis revealed a single band under reducing and non-reducing conditions (FIG. 2c). Compared with unmodified scFv, the molecular mass was increased by approximately 5 kDa under reducing conditions.

Example 3

Size Exclusion Chromatography (SEC)

[0135] Purity and Stokes radii of the scDb-IgBD and scFv-IgBD fusion proteins were analyzed by HPLC size exclusion chromatography using a BioSuite 250 (Waters Corporation, Milford, USA) and a flow rate of 0.5 ml/min (FIG. 2d-g). The following standard proteins were used: thyroglobulin, β -amylase, bovine serum albumin, carbonic anhydrase, cytochrome c. All fusion proteins showed a single peak corresponding to monomeric molecules. The measured Stokes radii of the fusion proteins were in the range of 2.3 to 2.7 nm. Interestingly, the Stokes radii of the scFv-IgBD fusion proteins were similar to those of the scDb-IgBD fusion proteins, while the unmodified scFv had a Stokes radius of 1.2 nm (see also FIG. 8).

Example 4

Binding of scDb-IgBD and scFv-IgBD Fusion Proteins to CEA in ELISA

[0136] Increasing concentrations of the scDb-IgBD (a) or scFv-IgBD (b) fusion proteins were analyzed for binding to immobilized CEA by ELISA. Carcinoembryonic antigen (CEA) (300 ng/well) was coated overnight at 4° C. and remaining binding sites were blocked with 2% (w/v) dry milk/PBS. Purified recombinant scDb, scFv, as well as scDb-IgBD and scFv-IgBD fusion proteins were titrated in duplicates and incubated for 1 h at RT. Detection was performed with mouse HRP-conjugated anti-His-tag antibody using TMB substrate (0.1 mg/ml TMB, 100 mM sodium acetate buffer pH 6.0, 0.006% H₂O₂). The reaction was stopped with 50 μ l of 1 M H₂SO₄. Absorbance was measured at 450 nm in an ELISA-reader.

Example 5

Binding of scDb-IgBD Fusion Proteins to Human and Mouse IgG, Human Fab- and Fc-Fragments

[0137] The fusion proteins scDb-SpG_{C3}, scDb-SpA_B, scDb-SpA_D, scDb-SpA_{EZ4}, and scDb-PpL_{C4*} were analyzed for binding to immobilized human serum IgG as well as Fab and Fc fragments thereof by ELISA. Human or mouse IgG, human Fab or human Fc fragments (100 ng/well) was coated overnight at 4° C. and remaining binding sites were blocked with 2% (w/v) dry milk/PBS. Purified recombinant antibodies and serum samples were titrated in duplicates and incu-

bated for 1 h at RT. Detection was performed with mouse HRP-conjugated anti-His-tag antibody using TMB substrate (0.1 mg/ml TMB, 100 mM sodium acetate buffer pH 6.0, 0.006% H_2O_2). The reaction was stopped with 50 μl of 1 M H_2SO_4 . Absorbance was measured at 450 nm in an ELISA-reader. Strongest binding to human serum IgG (hulgG), as well as human Ig Fc (hulgFc) was observed for scDb-SpG_{C3} (FIG. 4b). Also, scDb-SpA_B, scDb-SpA_D, scDb-SpA_{EZ4} were able to bind to hulgG and hulgFc, however their binding was weaker than the binding of scDb-SpG_{C3}. Hardly any binding could be observed for scDb-PpL_{C4*}. Binding to hulgFab could be observed for scDb-SpG_{C3}, whilst all other fusion protein showed hardly any binding to hulgFab. The fusion proteins scDb-SpG_{C3}, scDb-SpA_B, scDb-SpA_D, and scDb-SpA_{EZ4} were also able to bind to hulgM (FIG. 4b) with scDb-SpA_B exhibiting the strongest binding (FIG. 4b). In addition, the fusion proteins scDb-SpA_B, scDb-SpA_D, and scDb-SpA_{EZ4} also showed binding to hulgA.

[0138] Binding of all fusion proteins (except scDb-PpL_{C4*}) was also seen with mouse serum IgG (molgG) as well as mouse Ig Fc (molgFc), although binding was generally weaker than that seen for the human IgGs (FIG. 4a). Binding to and mouse Ig Fab (molgFab) fragments was only observed for scDb-SpG_{C3}.

Example 6

Affinity Measurements

[0139] Affinities of scDb-IgBD fusion proteins for human and mouse serum IgG well as Fab and Fc fragments at neutral or acidic pH were determined by quartz crystal microbalance measurements (Attana A-100 C-Fast system). IgGs as well as Fab and Fc fragments were chemically immobilized on an LNB (low nonspecific binding) carboxyl sensor chip according to the manufacturer's protocol at a density resulting in a signal increase of 65-95 Hz. Binding experiments were performed in PBST (0.1% Tween 20) pH 7.4 or pH 6.0 with a flow rate of 25 $\mu\text{l}/\text{min}$. The chip was regenerated with 25 μl 10 mM glycine-HCl pH 3.0. Before each measurement, a baseline was measured which was subtracted from the binding curve. Data were collected by Attester 3.0 (Version 3.1.1.8, Attana, Stockholm, Sweden) and analyzed by Attache Office Evaluation Software (Version 3.3.4, Attana, Stockholm, Sweden), using a mass transport model for curve fitting (see FIG. the active compound 5, FIG. 5). Strong binding in the low nanomolar range to human and mouse IgG as well as IgG-Fc was observed for the different SpA-IgBD and the SpG_{C3} fusion proteins. Binding to human and mouse Fab fragments was only observed for scDb-SpG_{C3}. The binding of the scDb-SpA-IgBD fusion protein was found to be pH-dependent and was strongly reduced at pH 6. For example, lowering the pH from 7.4 to 6.0 resulted in an approximately 45-fold reduced affinity of scDb-SpA_B for human serum IgG and a 43-fold reduced affinity for mouse serum IgG. A pH-dependent binding may have a direct influence on FcRn-mediated recycling, which requires that the SpA fusion protein stays bound to IgG-FcRn complexes in the acidic environment of the early endosome (pH~6.3 to 6.8) and tubular recycling endosomes (pH~6.5). In contrast, similar or even an increased binding affinity of scDb-SpG_{C3} to human IgFc, human IgFab, mouse IgG and mouse IgFab were observed by lowering the pH value from 7.4 to 6 (FIG. 5).

Example 7

Pharmacokinetics

[0140] CD1 mice were purchased from Elevage Janvier (Le Genest St. Isle, France). Animal care and all experiments

performed were in accordance with federal guidelines and have been approved by university and state authorities. CD1 mice (8-16 weeks, weight between 30-40 g) received an i.v. injection of 25 μg a scDb-IgBD or a scFv-IgBD fusion protein in a total volume of 150 μl . In time intervals of 3 min, 30 min, 1 h, 2 h, 6 h, 1 day, and 3 days blood samples (50 μl) were taken from the tail and incubated on ice. Clotted blood was centrifuged at 13,000 g for 10 min, 4° C. and serum samples stored at -20° C. The active compound Serum concentrations of CEA-binding recombinant antibodies were determined by ELISA. Carcinoembryonic antigen (CEA) (300 ng/well) or IgG (500 ng/well) was coated overnight at 4° C. and remaining binding sites were blocked with 2% (w/v) dry milk/PBS. Purified recombinant antibodies and serum samples were titrated in duplicates and incubated for 1 h at RT. For determination of pH dependence of binding, all incubation and washing steps were performed with PBS adjusted to the indicated pH. Detection was performed with mouse HRP-conjugated anti-His-tag antibody using TMB substrate (0.1 mg/ml TMB, 100 mM sodium acetate buffer pH 6.0, 0.006% H_2O_2). The reaction was stopped with 50 μl of 1 M H_2SO_4 . Absorbance was measured at 450 nm in an ELISA-reader. For comparison, the first value (3 min) was set to 100%. Half-life of scDb-IgBD and scFv-IgBD fusion proteins was analyzed after a single i.v. injection into CD1 mice. The initial plasma half-life ($t_{1/2\alpha}$), the terminal plasma half-life ($t_{1/2\beta}$) and the bioavailability (AUC) were calculated for scDb-IgBD and scFv-IgBD fusion proteins using Excel (FIG. 8). For statistics, Student's t-test was applied. The bioavailability of all fusion proteins was increased in comparison to the non-fused scDb or scFv, respectively. The highest increase in the bioavailability was obtained by scDb-SpG_{C3} and scFv-SpG_{C3} in comparison to the non-fused scDb or scFv, respectively, with scDb-SpG_{C3} exhibiting a 36-fold increase and scFv-SpG_{C3} exhibiting a 65-fold increase in their bioavailability (FIG. 8). Compared to scDb exhibiting a terminal half-life of 1.3 h, the scDb-IgBD fusion proteins showed a strongly prolonged circulation in the blood (FIG. 7a). A terminal half-life of 23.3 h was determined for scDb-SpG_{C3} compared to terminal half-lives of 2.4 h for scDb-PpL_{C4*}, 4.2 h for scDb-SpA_{EZ4}, 9 h for scDb-SpA_D, and 11.8 h for scDb-SpA_B (FIG. 8). Also, scFv-IgBD fusion proteins showed a strongly prolonged circulation in the blood (FIG. 7b). A terminal half-life of 20.8 h was determined for scFv-SpG_{C3} compared to terminal half-lives of 1 to 5 h for scDb-SpA_B, scDb-SpA_D, scDb-SpA_{EZ4}, and scDb-Pp the active compound L_{C4*}.

Example 8

IL-2 Release Assay

[0141] The scDb-fusion proteins were analyzed in vitro for their ability to induce IL-2 release (FIG. 9). Peripheral blood mononuclear cells (PBMC) from healthy donors were isolated from buffy coat as described before (Müller et al. (2007) J. Biol. Chem. 282:12650-12660). 1×10^5 LS174T cells/100 μl /well were seeded in 96-well plates. The next day supernatant was removed and 150 μl of recombinant antibody added. After 1 h preincubation at 37° C., 2×10^5 PBMC/50 μl /well were added. PBMCs had been thawed the day before and seeded on a culture dish. Only cells that remained in suspension were used for the assay. After addition of PBMCs, the 96-well plate was incubated for 24 h at 37° C., 5% CO_2 . Plates were centrifuged and cell-free supernatant collected. Concentration of human IL-2 in the supernatant was determined using the DuoSet IL-2 ELISA kit (R&D Systems) following the manufacturer's protocol. Compared with the unmodified scDb, the scDb-SpA_B, scDb-SpA_D and scDb-SpA_{EZ4} fusion proteins showed a strongly increased IL-2 release in the

absence of human IgG which indicates that the SpA_B domain induces activation of PBMCs (FIG. 9). In contrast, scDb-SpG_{C3} did not induce an IL-2 release exceeding the IL-2 release induced by scDb.

Example 9

SpG_{C3} Mutants

[0142] Variants of scDb-SpG_{C3} lacking the binding site to the Ig Fc fragment (scDb-SpG_{C3-Fab}) or the Ig Fab fragment (scDb-SpG_{C3-Fc}) were produced in stably transfected HEK293 cells, purified by IMAC and analyzed for binding to human IgG, IgG-Fab fragments and IgG-Fc fragments (FIG. 10a). Binding to the human Fc-fragment could be observed for scDb-SpG_{C3} and scDb-SpG_{C3-Fc} whilst scDb-SpG_{C3-Fab} was not able to bind to huIgFc. In contrast, binding to the human Fab-fragment could be observed for scDb-SpG_{C3} and scDb-SpG_{C3-Fab} whilst scDb-SpG_{C3-Fc} was not able to bind to huIgFab (FIG. 10b). scDb-SpG_{C3-Fab} was further analyzed for plasma half-life in CD1 mice as described in Example 7. A terminal half-life of 21.2±5.6 h (n=3) was determined for scDb-SpG_{C3-Fab} (FIG. 10 c and d), demonstrating that binding to the Fab fragment of immunoglobulins is sufficient to retain the long half-life of the wild-type fusion protein (terminal half-life 23.3±5.9 h (n=6)).

Example 10

A SpG_{C3}-Diabody-scTRAIL Fusion Protein

[0143] A SpG_{C3}-diabody-scTRAIL fusion protein was generated by fusing the SpG_{C3} to an anti-EGFR diabody (VH-VL domains of a humanized anti-EGFR antibody huC225 connected by a 5 residue GGGGS linker) fused by an additional linker to a single-chain derivative of human TRAIL (FIGS. 11 and 13). For purification and detection, the protein contains a FLAG-tag at the N-terminus. The fusion protein was produced by stably transfected HEK293 cells and purified by anti-FLAG affinity chromatography from cell culture supernatant. Westernblot with an anti-TRAIL antibody or an anti-FLAG antibody showed a single band of approximately 100 kDa, corresponding to the expected molecular mass. The purified fusion protein showed strong binding to human IgG in ELISA demonstrating that the SpG_{C3} domain is also functional when fused to the N-terminus of a protein.

Example 11

Comparison of the Pharmacokinetic Properties of scDb-SpG_{C3} and scDb-ABD_H

[0144] Similar to IgBDs, an albumin-binding domain (ABD) derived from *Streptococcus* protein G has been shown to strongly improve the half-life of small recombinant proteins by recycling via the FcRn when bound to albumin (Stork et al., 2007, Protein Eng. Des. Sel., 20, 569-576; Andersen et

al., 2010, J. Biol. Chem. 286, 5234-5241). Several mutants of the ABD with altered affinity for mouse and human albumin have been described and tested for their half-life extension properties (Jonsson et al., 2008, Protein Eng. Des. Sel. 21, 515-527; Hopp et al., 2010, Protein Eng. Des. Sel. 23, 827-834). Amongst them, ABD_H (albumin-binding domain with high affinity) has proven to show the best pharmacokinetic properties and seems therefore a suitable fusion protein for comparison. Furthermore, the affinity of the ABD_H towards albumin is similar to the affinity of SpG_{C3} towards IgG (Hopp et al., 2010, Protein Eng. Des. Sel. 23, 827-834). Therefore, we compared the plasma half-lives of scDb-ABD_H and scDb-SpG_{C3} after a single dose i.v. injection (25 µg/animal) into CD1 mice (FIG. 16a and b), as described above in Example 7. Over the first 24 hours, scDb-SpG_{C3} showed a significantly (p<0.01) increased plasma concentration, displayed by an AUC of 997±79% h compared to 836±81% h for the scDb-ABD_H and 56±15% h for unmodified scDb (FIG. 16c). Additionally, initial plasma half-life was calculated (using the first 3 values) and revealed a 1.6-fold increase in t_{1/2α} for scDb-SpG_{C3} (2.4±0.7 h) compared to scDb-ABD_H (1.5±0.5 h), which was significantly different (p<0.05) (FIG. 16d). Further investigating the biphasic profile of the fusion protein pharmacokinetics resulted in a very similar terminal plasma half-life of 20.6±11.5 h for scDb-ABD_H and 21.0±4.8 h for scDb-SpG_{C3}, calculated from 6 h to 24 h. This finding indicates that SpG_{C3} fusion proteins compared to ABD_H fusion proteins, have an improved initial distribution phase resulting in an increased bioavailability as measured by the AUC.

Example 12

Comparison of IL-2 Release by scDb-SpG_{C3-Fab} and scDb-ABD_H in the Absence or Presence of Albumin or IgG

[0145] The potential of the bispecific anti-CEA×anti-CD3 scDb fusion proteins to stimulate T cells was analyzed using an IL-2 release assay. Following the protocol described above in Example 8, scDb-SpG_{C3-Fab} and scDb-ABD_H were used in different protein concentrations ranging from 0.1 nM to 31.6 nM (FIG. 17). While the unmodified scDb as reference molecule showed no or only a marginal reduction in IL-2 release in the presence of IgG or human serum albumin (using 1/50 of the physiological concentrations), scDb-ABD_H showed a strong reduction in signal when preincubated with HSA. In contrast, the SpG_{C3} variant SpG_{C3-Fab}, lacking the binding site for the Ig Fc fragment, showed strong activation even in the presence of IgG similar to the unmodified scDb, demonstrating that SpG_{C3-Fab} is especially suitable for half-life extension of bispecific molecules retargeting effector T cells and that this domain is superior over the established ABD domain.

Sequence Listing—Free Text Information

[0146]

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Amino acid sequence of the C3 domain of SpG:
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SEQ ID NO: 2

Amino acid sequence of C3-Fc:
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Amino acid sequence of C3-Fab:
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Amino acid positions 122-127 and 207-214 of a human
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Amino acid positions 122-127 and 207-214 of a human
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SEQ ID NO: 5

Amino acid positions 122-127 and 207-214 of a human
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SEQ ID NO: 6

Amino acid positions 122-127 and 207-214 of a human
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SEQ ID NO: 7

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SEQ ID NO: 8

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SEQ ID NO: 9

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Ig γ 2b molecule according to EU index as in Kabat:
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SEQ ID NO: 10

Amino acid positions 122-127 and 207-214 of a mouse
Ig γ 3 molecule according to EU index as in Kabat:
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SEQ ID NO: 11

Amino acid positions 122-127 and 207-214 of a rat Ig
g1 molecule according to EU index as in Kabat:
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SEQ ID NO: 12

Amino acid sequence of scFv-SpG-C3 (anti-CEA)
SEQ ID NO: 13

Amino acid sequence of scDb-SpG-C3 (anti-CEA x anti-CD3)
SEQ ID NO: 14

Amino acid sequence of SpG-C3-Db-scTRAIL (anti-human EGFR)
SEQ ID NO: 15

Amino acid sequence of the C1 domain of SpG:
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Amino acid sequence of the C2 domain of SpG:
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<223> OTHER INFORMATION: SpG-C3 binding epitope

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 65 70 75 80
 Tyr Thr Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys
 85 90 95
 Arg Val Glu Leu Lys Thr Pro Leu Gly Asp Thr Thr His Thr Cys Pro
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 Arg Cys Pro Glu Pro Lys Ser Cys Asp Thr Pro Pro Pro Cys Pro Arg
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 Cys Pro Glu Pro Lys Ser Cys Asp Thr Pro Pro Pro Cys Pro Arg Cys
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 Pro Glu Pro Lys Ser Cys Asp Thr Pro Pro Pro Cys Pro Arg Cys Pro
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 Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val
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290    295    300
Leu His Glu Gly Leu His Asn His His Thr Glu Lys Ser Leu Ser His
305    310    315    320

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-continued

Ser Pro Gly Lys

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<210> SEQ ID NO 9
<211> LENGTH: 330
<212> TYPE: PRT
<213> ORGANISM: Mus musculus
<220> FEATURE:
<221> NAME/KEY: BINDING
<222> LOCATION: (5)..(10)
<223> OTHER INFORMATION: SpG-C3 binding epitope
<220> FEATURE:
<221> NAME/KEY: BINDING
<222> LOCATION: (89)..(96)
<223> OTHER INFORMATION: SpG-C3 binding epitope

<400> SEQUENCE: 9
Ala Lys Thr Thr Ala Pro Ser Val Tyr Pro Leu Ala Pro Val Cys Gly
 1             5             10             15
Asp Thr Thr Gly Ser Ser Val Thr Leu Gly Cys Leu Val Lys Gly Tyr
      20             25             30
Phe Pro Glu Pro Val Thr Leu Thr Trp Asn Ser Gly Ser Leu Ser Ser
      35             40             45
Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Asp Leu Tyr Thr Leu
 50             55             60
Ser Ser Ser Val Thr Val Thr Ser Ser Thr Trp Pro Ser Gln Ser Ile
 65             70             75             80
Thr Cys Asn Val Ala His Pro Ala Ser Ser Thr Lys Val Asp Lys Lys
      85             90             95
Ile Glu Pro Arg Gly Pro Thr Ile Lys Pro Cys Pro Pro Cys Lys Cys
     100             105             110
Pro Ala Pro Asn Leu Leu Gly Gly Pro Ser Val Phe Ile Phe Pro Pro
     115             120             125
Lys Ile Lys Asp Val Leu Met Ile Ser Leu Ser Pro Ile Val Thr Cys
     130             135             140
Val Val Val Asp Val Ser Glu Asp Asp Pro Asp Val Gln Ile Ser Trp
 145             150             155             160
Phe Val Asn Asn Val Glu Val His Thr Ala Gln Thr Gln Thr His Arg
     165             170             175
Glu Asp Tyr Asn Ser Thr Leu Arg Val Val Ser Ala Leu Pro Ile Gln
     180             185             190
His Gln Asp Trp Met Ser Gly Lys Glu Phe Lys Cys Lys Val Asn Asn
     195             200             205
Lys Asp Leu Pro Ala Pro Ile Glu Arg Thr Ile Ser Lys Pro Lys Gly
     210             215             220
Ser Val Arg Ala Pro Gln Val Tyr Val Leu Pro Pro Pro Glu Glu Glu
 225             230             235             240
Met Thr Lys Lys Gln Val Thr Leu Thr Cys Met Val Thr Asp Phe Met
     245             250             255
Pro Glu Asp Ile Tyr Val Glu Trp Thr Asn Asn Gly Lys Thr Glu Leu
     260             265             270
Asn Tyr Lys Asn Thr Glu Pro Val Leu Asp Ser Asp Gly Ser Tyr Phe
     275             280             285
Met Tyr Ser Lys Leu Arg Val Glu Lys Lys Asn Trp Val Glu Arg Asn
     290             295             300
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Ser	Tyr	Ser	Cys	Ser	Val	Val	His	Glu	Gly	Leu	His	Asn	His	His	Thr
305					310					315					320

Thr	Lys	Ser	Phe	Ser	Arg	Thr	Pro	Gly	Lys
				325				330	

<210> SEQ ID NO 10
 <211> LENGTH: 335
 <212> TYPE: PRT
 <213> ORGANISM: Mus musculus
 <220> FEATURE:
 <221> NAME/KEY: BINDING
 <222> LOCATION: (4)..(9)
 <223> OTHER INFORMATION: SpG-C3 binding epitope
 <220> FEATURE:
 <221> NAME/KEY: BINDING
 <222> LOCATION: (88)..(95)
 <223> OTHER INFORMATION: SpG-C3 binding epitope

<400> SEQUENCE: 10

Lys	Thr	Thr	Pro	Pro	Ser	Val	Tyr	Pro	Leu	Ala	Pro	Gly	Cys	Gly	Asp
1				5					10					15	
Thr	Thr	Gly	Ser	Ser	Val	Thr	Leu	Gly	Cys	Leu	Val	Lys	Gly	Tyr	Phe
			20					25					30		
Pro	Glu	Ser	Val	Thr	Val	Thr	Trp	Asn	Ser	Gly	Ser	Leu	Ser	Ser	Ser
			35				40					45			
Val	His	Thr	Phe	Pro	Ala	Leu	Leu	Gln	Ser	Gly	Leu	Tyr	Thr	Met	Ser
	50					55					60				
Ser	Ser	Val	Thr	Val	Pro	Ser	Ser	Thr	Trp	Pro	Ser	Gln	Thr	Val	Thr
65					70					75					80
Cys	Ser	Val	Ala	His	Pro	Ala	Ser	Ser	Thr	Thr	Val	Asp	Lys	Lys	Leu
				85					90					95	
Glu	Pro	Ser	Gly	Pro	Ile	Ser	Thr	Ile	Asn	Pro	Cys	Pro	Pro	Cys	Lys
			100					105						110	
Glu	Cys	His	Lys	Cys	Pro	Ala	Pro	Asn	Leu	Glu	Gly	Gly	Pro	Ser	Val
		115					120					125			
Phe	Ile	Phe	Pro	Pro	Asn	Ile	Lys	Asp	Val	Leu	Met	Ile	Ser	Leu	Thr
	130					135					140				
Pro	Lys	Val	Thr	Cys	Val	Val	Val	Asp	Val	Ser	Glu	Asp	Asp	Pro	Asp
145					150					155					160
Val	Gln	Ile	Ser	Trp	Phe	Val	Asn	Asn	Val	Glu	Val	His	Thr	Ala	Gln
				165					170						175
Thr	Gln	Thr	His	Arg	Glu	Asp	Tyr	Asn	Ser	Thr	Ile	Arg	Val	Val	Ser
			180					185					190		
Thr	Leu	Pro	Ile	Gln	His	Gln	Asp	Trp	Met	Ser	Gly	Lys	Glu	Phe	Lys
		195					200					205			
Cys	Lys	Val	Asn	Asn	Lys	Asp	Leu	Pro	Ser	Pro	Ile	Glu	Arg	Thr	Ile
		210				215					220				
Ser	Lys	Ile	Lys	Gly	Leu	Val	Arg	Ala	Pro	Gln	Val	Tyr	Ile	Leu	Pro
225					230					235					240
Pro	Pro	Ala	Glu	Gln	Leu	Ser	Arg	Lys	Asp	Val	Ser	Leu	Thr	Cys	Leu
				245					250					255	
Val	Val	Gly	Phe	Asn	Pro	Gly	Asp	Ile	Ser	Val	Glu	Trp	Thr	Ser	Asn
			260				265						270		
Gly	His	Thr	Glu	Glu	Asn	Tyr	Lys	Asp	Thr	Ala	Pro	Val	Leu	Asp	Ser
		275					280						285		

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Asp Gly Ser Tyr Phe Ile Tyr Ser Lys Leu Asn Met Lys Thr Ser Lys
 290 295 300

Trp Glu Lys Thr Asp Ser Phe Ser Cys Asn Val Arg His Glu Gly Leu
 305 310 315 320

Lys Asn Tyr Tyr Leu Lys Lys Thr Ile Ser Arg Ser Pro Gly Lys
 325 330 335

<210> SEQ ID NO 11
 <211> LENGTH: 398
 <212> TYPE: PRT
 <213> ORGANISM: Mus musculus
 <220> FEATURE:
 <221> NAME/KEY: BINDING
 <222> LOCATION: (4)..(9)
 <223> OTHER INFORMATION: SpG-C3 binding epitope
 <220> FEATURE:
 <221> NAME/KEY: BINDING
 <222> LOCATION: (88)..(95)
 <223> OTHER INFORMATION: SpG-C3 binding epitope

<400> SEQUENCE: 11

Thr Thr Thr Ala Pro Ser Val Tyr Pro Leu Val Pro Gly Cys Ser Asp
 1 5 10 15

Thr Ser Gly Ser Ser Val Thr Leu Gly Cys Leu Val Lys Gly Tyr Phe
 20 25 30

Pro Glu Pro Val Thr Val Lys Trp Asn Tyr Gly Ala Leu Ser Ser Gly
 35 40 45

Val Arg Thr Val Ser Ser Val Leu Gln Ser Gly Phe Tyr Ser Leu Ser
 50 55 60

Ser Leu Val Thr Val Pro Ser Ser Thr Trp Pro Ser Gln Thr Val Ile
 65 70 75 80

Cys Asn Val Ala His Pro Ala Ser Lys Thr Glu Leu Ile Lys Arg Ile
 85 90 95

Glu Pro Arg Ile Pro Lys Pro Ser Thr Pro Pro Gly Ser Ser Cys Pro
 100 105 110

Pro Gly Asn Ile Leu Gly Gly Pro Ser Val Phe Ile Phe Pro Pro Lys
 115 120 125

Pro Lys Asp Ala Leu Met Ile Ser Leu Thr Pro Lys Val Thr Cys Val
 130 135 140

Val Val Asp Val Ser Glu Asp Asp Pro Asp Val His Val Ser Trp Phe
 145 150 155 160

Val Asp Asn Lys Glu Val His Thr Ala Trp Thr Gln Pro Arg Glu Ala
 165 170 175

Gln Tyr Asn Ser Thr Phe Arg Val Val Ser Ala Leu Pro Ile Gln His
 180 185 190

Gln Asp Trp Met Arg Gly Lys Glu Phe Lys Cys Lys Val Asn Asn Lys
 195 200 205

Ala Leu Pro Ala Pro Ile Glu Arg Thr Ile Ser Lys Pro Lys Gly Arg
 210 215 220

Ala Gln Thr Pro Gln Val Tyr Thr Ile Pro Pro Pro Arg Glu Gln Met
 225 230 235 240

Ser Lys Lys Lys Val Ser Leu Thr Cys Leu Val Thr Asn Phe Phe Ser
 245 250 255

Glu Ala Ile Ser Val Glu Trp Glu Arg Asn Gly Glu Leu Glu Gln Asp
 260 265 270

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Tyr Lys Asn Thr Pro Pro Ile Leu Asp Ser Asp Gly Thr Tyr Phe Leu
  275                      280                      285

Tyr Ser Lys Leu Thr Val Asp Thr Asp Ser Trp Leu Gln Gly Glu Ile
  290                      295                      300

Phe Thr Cys Ser Val Val His Glu Ala Leu His Asn His His Thr Gln
  305                      310                      315                      320

Lys Asn Leu Ser Arg Ser Pro Glu Leu Glu Leu Asn Glu Thr Cys Ala
                      325                      330                      335

Glu Ala Gln Asp Gly Glu Leu Asp Gly Leu Trp Thr Thr Ile Thr Ile
                      340                      345                      350

Phe Ile Ser Leu Phe Leu Leu Ser Val Cys Tyr Ser Ala Ser Val Thr
                      355                      360                      365

Leu Phe Lys Val Lys Trp Ile Phe Ser Ser Val Val Gln Val Lys Gln
                      370                      375                      380

Thr Ala Ile Pro Asp Tyr Arg Asn Met Ile Gly Gln Gly Ala
  385                      390                      395

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<210> SEQ ID NO 12
<211> LENGTH: 326
<212> TYPE: PRT
<213> ORGANISM: Rattus norvegicus
<220> FEATURE:
<221> NAME/KEY: BINDING
<222> LOCATION: (5)..(10)
<223> OTHER INFORMATION: SpG-C3 binding epitope
<220> FEATURE:
<221> NAME/KEY: BINDING
<222> LOCATION: (89)..(96)
<223> OTHER INFORMATION: SpG-C3 binding epitope

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<400> SEQUENCE: 12

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Ala Glu Thr Thr Ala Pro Ser Val Tyr Pro Leu Ala Pro Gly Thr Ala
  1                      5                      10                      15

Leu Lys Ser Asn Ser Met Val Thr Leu Gly Cys Leu Val Lys Gly Tyr
                      20                      25                      30

Phe Pro Glu Pro Val Thr Val Thr Trp Asn Ser Gly Ala Leu Ser Ser
                      35                      40                      45

Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Gly Leu Tyr Thr Leu
                      50                      55                      60

Thr Ser Ser Val Thr Val Pro Ser Ser Thr Trp Pro Ser Gln Thr Val
  65                      70                      75                      80

Thr Cys Asn Val Ala His Pro Ala Ser Ser Thr Lys Val Asp Lys Lys
                      85                      90                      95

Ile Val Pro Arg Asn Cys Gly Gly Asp Cys Lys Pro Cys Ile Cys Thr
                      100                     105                     110

Gly Ser Glu Val Ser Ser Val Phe Ile Phe Pro Pro Lys Pro Lys Asp
                      115                      120                      125

Val Leu Thr Ile Thr Leu Thr Pro Lys Val Thr Cys Val Val Val Asp
                      130                      135                      140

Ile Ser Gln Asp Asp Pro Glu Val His Phe Ser Trp Phe Val Asp Asp
  145                      150                      155                      160

Val Glu Val His Thr Ala Gln Thr Arg Pro Pro Glu Glu Gln Phe Asn
                      165                      170                      175

Ser Thr Phe Arg Ser Val Ser Glu Leu Pro Ile Leu His Gln Asp Trp
                      180                      185                      190

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Leu Asn Gly Arg Thr Phe Arg Cys Lys Val Thr Ser Ala Ala Phe Pro
 195 200 205
 Ser Pro Ile Glu Lys Thr Ile Ser Lys Pro Glu Gly Arg Thr Gln Val
 210 215 220
 Pro His Val Tyr Thr Met Ser Pro Thr Lys Glu Glu Met Thr Gln Asn
 225 230 235 240
 Glu Val Ser Ile Thr Cys Met Val Lys Gly Phe Tyr Pro Pro Asp Ile
 245 250 255
 Tyr Val Glu Trp Gln Met Asn Gly Gln Pro Gln Glu Asn Tyr Lys Asn
 260 265 270
 Thr Pro Pro Thr Met Asp Thr Asp Gly Ser Tyr Phe Leu Tyr Ser Lys
 275 280 285
 Leu Asn Val Lys Lys Glu Lys Trp Gln Gln Gly Asn Thr Phe Thr Cys
 290 295 300
 Ser Val Leu His Glu Gly Leu His Asn His His Thr Glu Lys Ser Leu
 305 310 315 320
 Ser His Ser Pro Gly Lys
 325

<210> SEQ ID NO 13
 <211> LENGTH: 343
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: ScFv-SpG-C3 (anti-CEA) protein

<400> SEQUENCE: 13

Met Glu Thr Asp Thr Leu Leu Leu Trp Val Leu Leu Leu Trp Val Pro
 1 5 10 15
 Gly Ser Thr Gly Asp Ala Ala Gln Pro Ala Met Ala Gln Val Lys Leu
 20 25 30
 Gln Gln Ser Gly Ala Glu Leu Val Arg Ser Gly Thr Ser Val Lys Leu
 35 40 45
 Ser Cys Thr Ala Ser Gly Phe Asn Ile Lys Asp Ser Tyr Met His Trp
 50 55 60
 Leu Arg Gln Gly Pro Glu Gln Gly Leu Glu Trp Ile Gly Trp Ile Asp
 65 70 75 80
 Pro Glu Asn Gly Asp Thr Glu Tyr Ala Pro Lys Phe Gln Gly Lys Ala
 85 90 95
 Thr Phe Thr Thr Asp Thr Ser Ser Asn Thr Ala Tyr Leu Gln Leu Ser
 100 105 110
 Ser Leu Thr Ser Glu Asp Thr Ala Val Tyr Tyr Cys Asn Glu Gly Thr
 115 120 125
 Pro Thr Gly Pro Tyr Tyr Phe Asp Tyr Trp Gly Gln Gly Thr Thr Val
 130 135 140
 Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly
 145 150 155 160
 Gly Gly Ser Asp Ile Glu Leu Thr Gln Ser Pro Ala Ile Met Ser Ala
 165 170 175
 Ser Pro Gly Glu Lys Val Thr Ile Thr Cys Ser Ala Ser Ser Ser Val
 180 185 190
 Ser Tyr Met His Trp Phe Gln Gln Lys Pro Gly Thr Ser Pro Lys Leu
 195 200 205

-continued

Trp Ile Tyr Ser Thr Ser Asn Leu Ala Ser Gly Val Pro Ala Arg Phe
 210 215 220
 Ser Gly Ser Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile Ser Arg Met
 225 230 235 240
 Glu Ala Glu Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Arg Ser Ser Tyr
 245 250 255
 Pro Leu Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys Arg Ala Ala
 260 265 270
 Ala Gly Gly Ser Gly Gly Thr Thr Tyr Lys Leu Val Ile Asn Gly Lys
 275 280 285
 Thr Leu Lys Gly Glu Thr Thr Thr Lys Ala Val Asp Ala Glu Thr Ala
 290 295 300
 Glu Lys Ala Phe Lys Gln Tyr Ala Asn Asp Asn Gly Val Asp Gly Val
 305 310 315 320
 Trp Thr Tyr Asp Asp Ala Thr Lys Thr Phe Thr Val Thr Glu Gly Gly
 325 330 335
 Ser His His His His His His
 340

<210> SEQ ID NO 14
 <211> LENGTH: 583
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: scDb-SpG-C3 (anti-CEA x anti-CD3) protein

<400> SEQUENCE: 14

Met Glu Thr Asp Thr Leu Leu Leu Trp Val Leu Leu Leu Trp Val Pro
 1 5 10 15
 Gly Ser Thr Gly Asp Ala Ala Gln Pro Ala Met Ala Gln Val Lys Leu
 20 25 30
 Gln Gln Ser Gly Ala Glu Leu Val Arg Ser Gly Thr Ser Val Lys Leu
 35 40 45
 Ser Cys Thr Ala Ser Gly Phe Asn Ile Lys Asp Ser Tyr Met His Trp
 50 55 60
 Leu Arg Gln Gly Pro Glu Gln Gly Leu Glu Trp Ile Gly Trp Ile Asp
 65 70 75 80
 Pro Glu Asn Gly Asp Thr Glu Tyr Ala Pro Lys Phe Gln Gly Lys Ala
 85 90 95
 Thr Phe Thr Thr Asp Thr Ser Ser Asn Thr Ala Tyr Leu Gln Leu Ser
 100 105 110
 Ser Leu Thr Ser Glu Asp Thr Ala Val Tyr Tyr Cys Asn Glu Gly Thr
 115 120 125
 Pro Thr Gly Pro Tyr Tyr Phe Asp Tyr Trp Gly Gln Gly Thr Thr Val
 130 135 140
 Thr Val Ser Ser Gly Gly Gly Gly Ser Asp Ile Gln Met Thr Gln Ser
 145 150 155 160
 Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys
 165 170 175
 Arg Ala Ser Gln Asp Ile Arg Asn Tyr Leu Asn Trp Tyr Gln Gln Lys
 180 185 190
 Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr Tyr Thr Ser Arg Leu Glu
 195 200 205

-continued

Ser	Gly	Val	Pro	Ser	Arg	Phe	Ser	Gly	Ser	Gly	Ser	Gly	Thr	Asp	Tyr
210						215					220				
Thr	Leu	Thr	Ile	Ser	Ser	Leu	Gln	Pro	Glu	Asp	Phe	Ala	Thr	Tyr	Tyr
225					230					235				240	
Cys	Gln	Gln	Gly	Asn	Thr	Leu	Pro	Trp	Thr	Phe	Gly	Gln	Gly	Thr	Lys
				245					250					255	
Val	Glu	Ile	Lys	Arg	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Arg	Ala	Ser	Gly
			260					265					270		
Gly	Gly	Gly	Ser	Glu	Val	Gln	Leu	Val	Glu	Ser	Gly	Gly	Gly	Leu	Val
		275					280					285			
Gln	Pro	Gly	Gly	Ser	Leu	Arg	Leu	Ser	Cys	Ala	Ala	Ser	Gly	Tyr	Ser
	290					295					300				
Phe	Thr	Gly	Tyr	Thr	Met	Asn	Trp	Val	Arg	Gln	Ala	Pro	Gly	Lys	Gly
305					310					315					320
Leu	Glu	Trp	Val	Ala	Leu	Ile	Asn	Pro	Tyr	Lys	Gly	Val	Ser	Thr	Tyr
			325						330					335	
Asn	Gln	Lys	Phe	Lys	Asp	Arg	Phe	Thr	Ile	Ser	Val	Asp	Lys	Ser	Lys
			340					345					350		
Asn	Thr	Ala	Tyr	Leu	Gln	Met	Asn	Ser	Leu	Arg	Ala	Glu	Asp	Thr	Ala
		355					360					365			
Val	Tyr	Tyr	Cys	Ala	Arg	Ser	Gly	Tyr	Tyr	Gly	Asp	Ser	Asp	Trp	Tyr
	370					375					380				
Phe	Asp	Val	Trp	Gly	Gln	Gly	Thr	Leu	Val	Thr	Val	Ser	Ser	Gly	Gly
385					390					395					400
Gly	Gly	Ser	Asp	Ile	Glu	Leu	Thr	Gln	Ser	Pro	Ala	Ile	Met	Ser	Ala
				405					410					415	
Ser	Pro	Gly	Glu	Lys	Val	Thr	Ile	Thr	Cys	Ser	Ala	Ser	Ser	Ser	Val
			420					425					430		
Ser	Tyr	Met	His	Trp	Phe	Gln	Gln	Lys	Pro	Gly	Thr	Ser	Pro	Lys	Leu
		435					440					445			
Trp	Ile	Tyr	Ser	Thr	Ser	Asn	Leu	Ala	Ser	Gly	Val	Pro	Ala	Arg	Phe
	450					455					460				
Ser	Gly	Ser	Gly	Ser	Gly	Thr	Ser	Tyr	Ser	Leu	Thr	Ile	Ser	Arg	Met
465					470					475					480
Glu	Ala	Glu	Asp	Ala	Ala	Thr	Tyr	Tyr	Cys	Gln	Gln	Arg	Ser	Ser	Tyr
				485					490					495	
Pro	Leu	Thr	Phe	Gly	Ala	Gly	Thr	Lys	Leu	Glu	Leu	Lys	Arg	Ala	Ala
			500					505					510		
Ala	Gly	Gly	Ser	Gly	Gly	Thr	Thr	Tyr	Lys	Leu	Val	Ile	Asn	Gly	Lys
		515					520					525			
Thr	Leu	Lys	Gly	Glu	Thr	Thr	Thr	Lys	Ala	Val	Asp	Ala	Glu	Thr	Ala
	530					535					540				
Glu	Lys	Ala	Phe	Lys	Gln	Tyr	Ala	Asn	Asp	Asn	Gly	Val	Asp	Gly	Val
545					550					555					560
Trp	Thr	Tyr	Asp	Asp	Ala	Thr	Lys	Thr	Phe	Thr	Val	Thr	Glu	Gly	Gly
				565					570					575	
Ser	His	His	His	His	His	His									
				580											

<210> SEQ ID NO 15

<211> LENGTH: 925

<212> TYPE: PRT

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<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: SpG-C3-Db-scTRAIL (anti-human EGFR) protein

<400> SEQUENCE: 15

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Met Asp Trp Thr Trp Arg Val Phe Cys Leu Leu Ala Val Ala Pro Gly
 1          5          10          15
Ala His Ser Leu Glu Ala Ser Asp Tyr Lys Asp Asp Asp Asp Lys Gly
      20          25          30
Ala Thr Thr Tyr Lys Leu Val Ile Asn Gly Lys Thr Leu Lys Gly Glu
      35          40          45
Thr Thr Thr Lys Ala Val Asp Ala Glu Thr Ala Glu Lys Ala Phe Lys
      50          55          60
Gln Tyr Ala Asn Asp Asn Gly Val Asp Gly Val Trp Thr Tyr Asp Asp
      65          70          75          80
Ala Thr Lys Thr Phe Thr Val Thr Glu Gly Gly Ser Gly Gly Gly Gly
      85          90          95
Ser Gly Gly Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln
      100          105          110
Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Ser Leu
      115          120          125
Thr Asn Tyr Gly Val His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu
      130          135          140
Glu Trp Leu Gly Val Ile Trp Ser Gly Gly Asn Thr Asp Tyr Asn Thr
      145          150          155          160
Pro Phe Thr Ser Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr
      165          170          175
Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr
      180          185          190
Tyr Cys Ala Arg Ala Leu Thr Tyr Tyr Asp Tyr Glu Phe Ala Tyr Trp
      195          200          205
Gly Gln Gly Thr Thr Val Thr Val Ser Ser Gly Gly Gly Gly Ser Asp
      210          215          220
Ile Gln Leu Thr Gln Ser Pro Ser Phe Leu Ser Ala Ser Val Gly Asp
      225          230          235          240
Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Ser Ile Gly Thr Asn Ile
      245          250          255
His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Lys
      260          265          270
Tyr Ala Ser Glu Ser Ile Ser Gly Val Pro Ser Arg Phe Ser Gly Ser
      275          280          285
Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu
      290          295          300
Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Asn Asn Asn Trp Pro Thr Thr
      305          310          315          320
Phe Gly Ala Gly Thr Lys Leu Glu Ile Lys Arg Ala Ala Ala Gly Asn
      325          330          335
Gly Thr Ser Asn Gly Thr Ser Glu Phe Thr Arg Gly Thr Ser Glu Glu
      340          345          350
Thr Ile Ser Thr Val Gln Glu Lys Gln Gln Asn Ile Ser Pro Leu Val
      355          360          365
Arg Glu Arg Gly Pro Gln Arg Val Ala Ala His Ile Thr Gly Thr Arg

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370	375	380
Gly Arg Ser Asn Thr Leu Ser Ser Pro Asn Ser Lys Asn Glu Lys Ala 385 390 395 400		
Leu Gly Arg Lys Ile Asn Ser Trp Glu Ser Ser Arg Ser Gly His Ser 405 410 415		
Phe Leu Ser Asn Leu His Leu Arg Asn Gly Glu Leu Val Ile His Glu 420 425 430		
Lys Gly Phe Tyr Tyr Ile Tyr Ser Gln Thr Tyr Phe Arg Phe Gln Glu 435 440 445		
Glu Ile Lys Glu Asn Thr Lys Asn Asp Lys Gln Met Val Gln Tyr Ile 450 455 460		
Tyr Lys Tyr Thr Ser Tyr Pro Asp Pro Ile Leu Leu Met Lys Ser Ala 465 470 475 480		
Arg Asn Ser Cys Trp Ser Lys Asp Ala Glu Tyr Gly Leu Tyr Ser Ile 485 490 495		
Tyr Gln Gly Gly Ile Phe Glu Leu Lys Glu Asn Asp Arg Ile Phe Val 500 505 510		
Ser Val Thr Asn Glu His Leu Ile Asp Met Asp His Glu Ala Ser Phe 515 520 525		
Phe Gly Ala Phe Leu Val Gly Gly Gly Gly Ser Gly Gly Gly Ser Thr 530 535 540		
Ser Glu Glu Thr Ile Ser Thr Val Gln Glu Lys Gln Gln Asn Ile Ser 545 550 555 560		
Pro Leu Val Arg Glu Arg Gly Pro Gln Arg Val Ala Ala His Ile Thr 565 570 575		
Gly Thr Arg Gly Arg Ser Asn Thr Leu Ser Ser Pro Asn Ser Lys Asn 580 585 590		
Glu Lys Ala Leu Gly Arg Lys Ile Asn Ser Trp Glu Ser Ser Arg Ser 595 600 605		
Gly His Ser Phe Leu Ser Asn Leu His Leu Arg Asn Gly Glu Leu Val 610 615 620		
Ile His Glu Lys Gly Phe Tyr Tyr Ile Tyr Ser Gln Thr Tyr Phe Arg 625 630 635 640		
Phe Gln Glu Glu Ile Lys Glu Asn Thr Lys Asn Asp Lys Gln Met Val 645 650 655		
Gln Tyr Ile Tyr Lys Tyr Thr Ser Tyr Pro Asp Pro Ile Leu Leu Met 660 665 670		
Lys Ser Ala Arg Asn Ser Cys Trp Ser Lys Asp Ala Glu Tyr Gly Leu 675 680 685		
Tyr Ser Ile Tyr Gln Gly Gly Ile Phe Glu Leu Lys Glu Asn Asp Arg 690 695 700		
Ile Phe Val Ser Val Thr Asn Glu His Leu Ile Asp Met Asp His Glu 705 710 715 720		
Ala Ser Phe Phe Gly Ala Phe Leu Val Gly Gly Gly Gly Ser Gly Gly 725 730 735		
Gly Ser Thr Ser Glu Glu Thr Ile Ser Thr Val Gln Glu Lys Gln Gln 740 745 750		
Asn Ile Ser Pro Leu Val Arg Glu Arg Gly Pro Gln Arg Val Ala Ala 755 760 765		
His Ile Thr Gly Thr Arg Gly Arg Ser Asn Thr Leu Ser Ser Pro Asn 770 775 780		

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Ser Lys Asn Glu Lys Ala Leu Gly Arg Lys Ile Asn Ser Trp Glu Ser
785                790                795                800

Ser Arg Ser Gly His Ser Phe Leu Ser Asn Leu His Leu Arg Asn Gly
                805                810                815

Glu Leu Val Ile His Glu Lys Gly Phe Tyr Tyr Ile Tyr Ser Gln Thr
                820                825                830

Tyr Phe Arg Phe Gln Glu Glu Ile Lys Glu Asn Thr Lys Asn Asp Lys
                835                840                845

Gln Met Val Gln Tyr Ile Tyr Lys Tyr Thr Ser Tyr Pro Asp Pro Ile
                850                855                860

Leu Leu Met Lys Ser Ala Arg Asn Ser Cys Trp Ser Lys Asp Ala Glu
865                870                875                880

Tyr Gly Leu Tyr Ser Ile Tyr Gln Gly Gly Ile Phe Glu Leu Lys Glu
                885                890                895

Asn Asp Arg Ile Phe Val Ser Val Thr Asn Glu His Leu Ile Asp Met
                900                905                910

Asp His Glu Ala Ser Phe Phe Gly Ala Phe Leu Val Gly
                915                920                925

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<210> SEQ ID NO 16
<211> LENGTH: 55
<212> TYPE: PRT
<213> ORGANISM: Peptostreptococcus magnus

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<400> SEQUENCE: 16

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Thr Tyr Lys Leu Ile Leu Asn Gly Lys Thr Leu Lys Gly Glu Thr Thr
1                5                10                15

Thr Glu Ala Val Asp Ala Ala Thr Ala Glu Lys Val Phe Lys Gln Tyr
                20                25                30

Ala Asn Asp Asn Gly Val Asp Gly Glu Trp Thr Tyr Asp Asp Ala Thr
                35                40                45

Lys Thr Phe Thr Val Thr Glu
50                55

```

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<210> SEQ ID NO 17
<211> LENGTH: 55
<212> TYPE: PRT
<213> ORGANISM: Peptostreptococcus magnus

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<400> SEQUENCE: 17

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```

Thr Tyr Lys Leu Val Ile Asn Gly Lys Thr Leu Lys Gly Glu Thr Thr
1                5                10                15

Thr Glu Ala Val Asp Ala Ala Thr Ala Glu Lys Val Phe Lys Gln Tyr
                20                25                30

Ala Asn Asp Asn Gly Val Asp Gly Glu Trp Thr Tyr Asp Asp Ala Thr
                35                40                45

Lys Thr Phe Thr Val Thr Glu
50                55

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1. A complex comprising

- (i) an immunoglobulin (Ig) binding moiety and
- (ii) a pharmaceutically active moiety,

wherein the Ig binding moiety specifically binds to the constant domain 1 of the heavy chain (CH1) of an Ig molecule.

2. The complex of claim 1, wherein the Ig binding moiety specifically binds to the surface-exposed region of the C_H1 domain of an Ig molecule.

3. The complex of claim 1, wherein the Ig binding moiety specifically binds to the Fc portion of the Ig molecule.

4. The complex of claim 1, wherein the Ig molecule is an IgG molecule, preferably an IgG1, IgG2, or IgG4 molecule.

5. The complex of claim 1, wherein the Ig binding moiety specifically binds to an epitope formed by amino acid positions 122-127 and/or 207-214 of an Ig molecule according to EU index.

6. The complex of claim 1, wherein the Ig binding moiety comprises an immunoglobulin binding domain (IgBD), preferably a *streptococcus*-derived IgBD.

7. The complex of claim 1, wherein the Ig binding moiety comprises a C_H1 binding-IgBD of streptococcal protein G.

8. The complex of claim 1, wherein the Ig binding moiety comprises an amino acid sequence according to SEQ ID NO: 1 or variants thereof.

9. The complex of claim 1, wherein the pharmaceutically active moiety comprises a biological and/or chemical pharmaceutical.

10. The complex of claim 9, wherein the biological is a pharmaceutically active polypeptide, preferably an antigen-binding molecule.

11. The complex of claim 10, wherein the antigen-binding molecule is selected from the group consisting of an antibody fragment, a Fab fragment, a Fab' fragment, a F(ab')₂ fragment, a heavy chain antibody, a single-domain antibody (sdAb), a single-chain variable fragment (scFv), a di-scFv, a bispecific T-cell engager (BITEs), a diabody, a single-chain diabody, a DART, a triple body, an alternative scaffold protein, and a fusion protein thereof.

12. The complex of claim 10, wherein said antigen-binding molecule further comprises a radioactive moiety, a cytotoxic drug, a chelating moiety, a photosensitizer, or an imaging reagent.

13. The complex of claim 11, wherein said antigen binding molecule is a fusion protein, which further comprises a proapoptotic protein, an immuno-(co)stimulatory protein, immuno-suppressive protein, a cytokine, a chemokine, a

toxin, a growth factor or an enzyme, preferably an RNase, a prodrug-converting enzyme, or a kinase.

14. The complex of claim 1, wherein the Ig binding moiety and the pharmaceutically active moiety are connected via covalent or non-covalent bond(s).

15. The complex of claim 1, wherein the Ig binding moiety and the pharmaceutically active moiety are connected directly or indirectly via one or more linkers.

16. The complex of claim 14, wherein the one or more linkers comprise peptide linkers, preferably flexible peptide linkers.

17. The complex of claim 16, wherein the one or more peptide linker comprise one or more cleavage sites, preferably one or more endopeptidase cleavage sites.

18. A nucleic acid molecule comprising a sequence encoding the complex of claim 1.

19. A vector comprising the nucleic acid of claim 18.

20. A cell comprising the complex of claim 1, the nucleic acid of claim 18 and/or the vector of claim 19.

21. A pharmaceutical composition comprising the complex of claim 1, the nucleic acid of claim 18 and/or the vector of claim 19.

22. The pharmaceutical composition of claim 21, which further comprises a pharmaceutically acceptable carrier and/or excipient and optionally one or more additional active substances.

23. A method of extending the serum half-life of an agent comprising combining that agent with the complex of claim 1.

24. A method of treating a subject by administration of a medicament comprising the complex of claim 1 to a subject in need thereof.

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