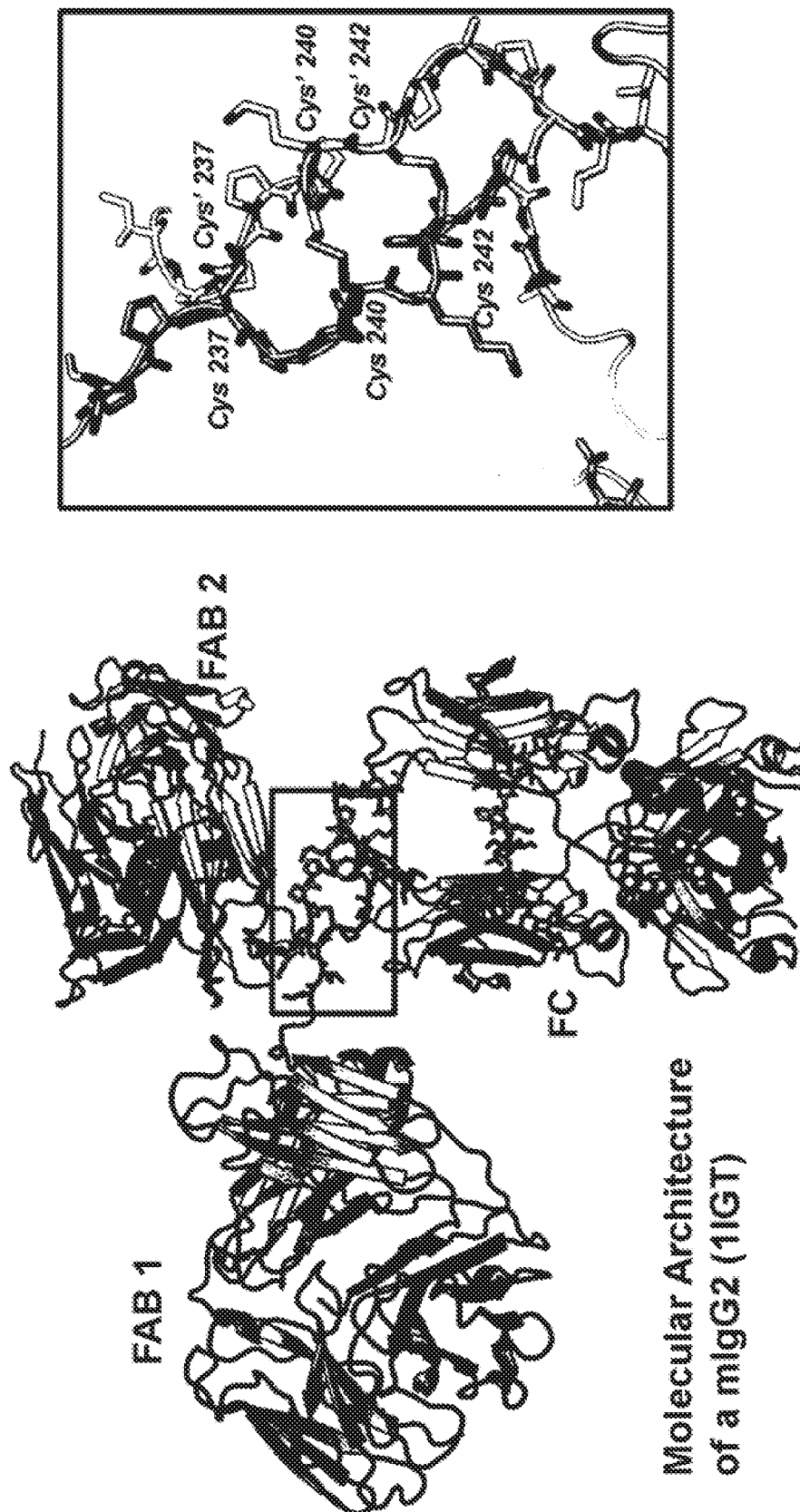


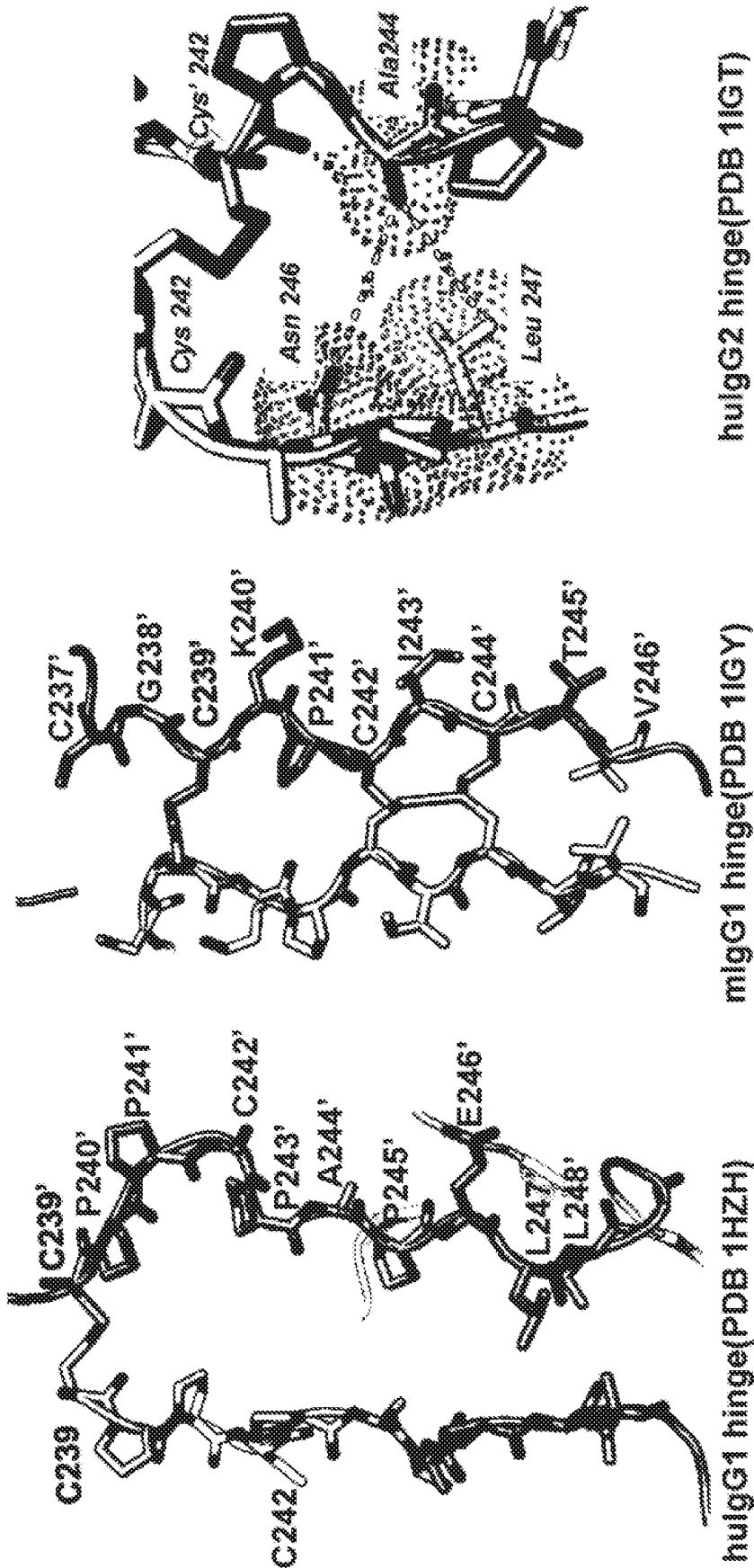
FIG. 1

TARGETING THE HINGE REGION TO FORM HETERODIMERS



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FIG. 2

HUMAN IGG1 CRYSTAL STRUCTURE



SEQUENCE ALIGNMENT FOR IGG1, IGG2 AND IGG4

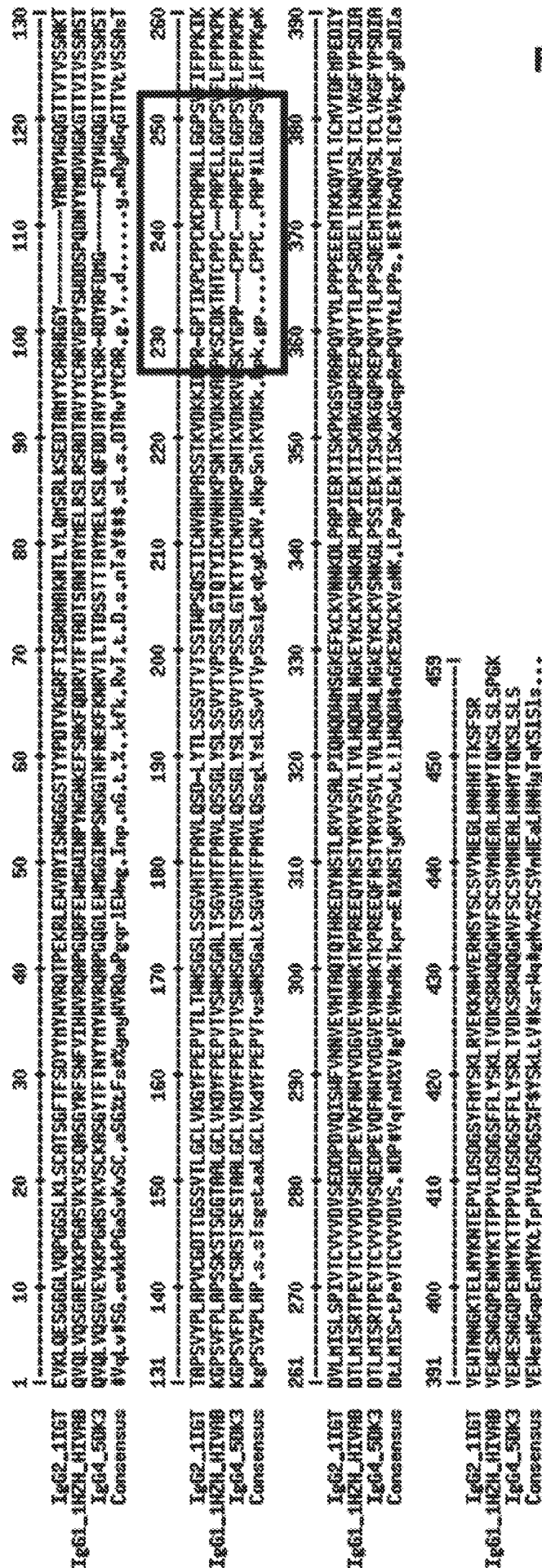


FIG. 3

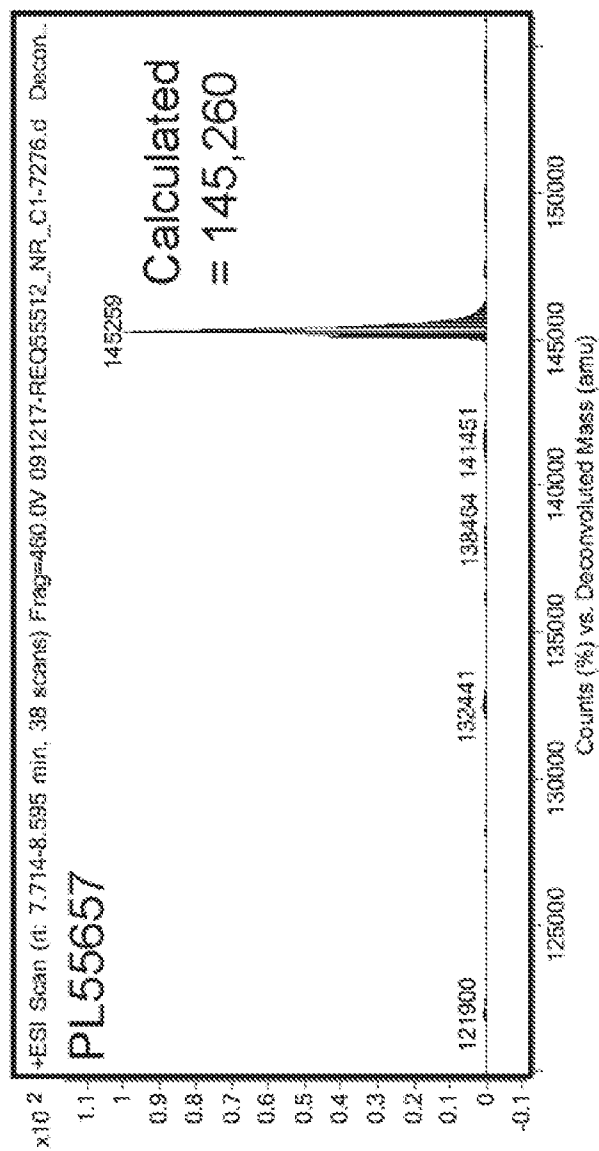
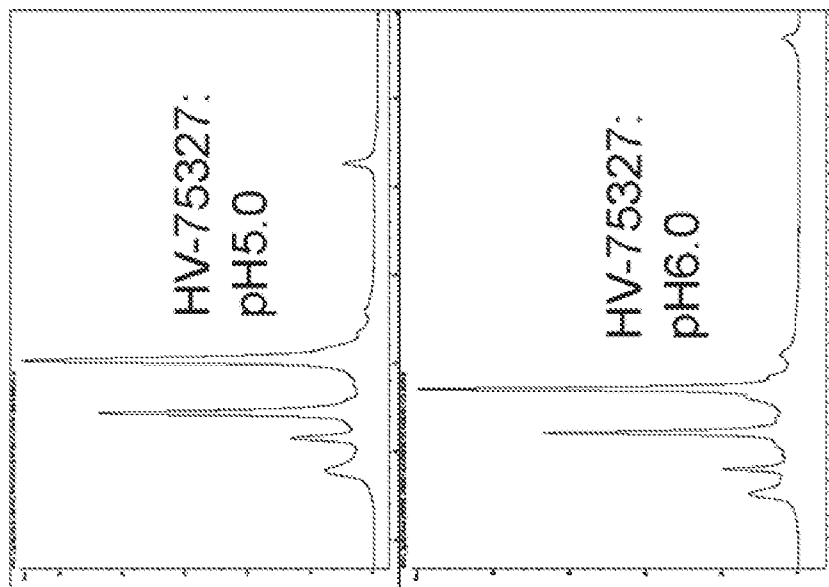
FIG. 4

SUMMARY TABLE: HINGE DESIGNS

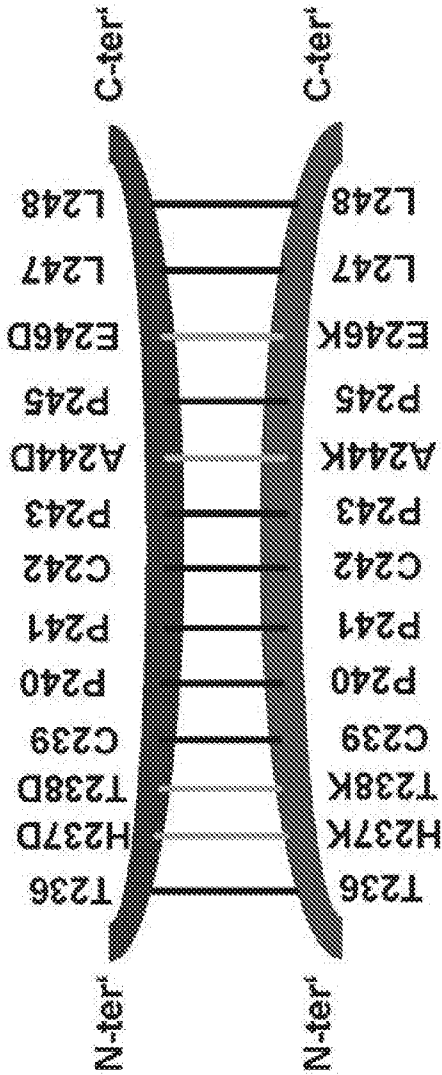
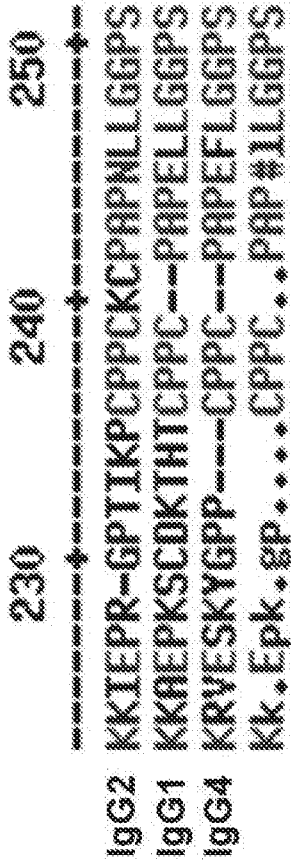
Protein Lots	Protein Sequence Identity	BoReg ID	Harvest ID	Titer mg/L	Lot Amount (mg)	Design ID	Design	MSQC	Comments	Measured Mass (Da)	Expected Mass (Da)	Error (Da)	P.I.	CIEX
PL-S5655	IPS:105287	5278	HV- 75325	15 mg/L	1	CHZ00	HC ~ THTCPPCPAPPELL HC' ~ THTCPPCPAPPELL	pass	Control	145079	145081	-2	8.71	mono Peak
PL-S5657	IPS:572023	5279	HV- 75327	8 mg/L	0.45	CHZ01	HC ~ THTCPPCDODDC HC' ~ THTCPPCKKKKC	pass		145259	145260	-1	8.98	multiple peaks
PL-S5658	IPS:572024	5280	HV- 75328	6 mg/L	0.45	CHZ02	HC ~ THTCPPCPDODDC HC' ~ THTCPPCFKKKC	fail	2 + HC**	145255	145211	44	8.98	
PL-S5659	IPS:572025	5281	HV- 75329	9 mg/L	0.57	CHZ03	HC ~ THTCPPCFDPEHC HC' ~ THTCPPCPKPKKC	fail	2 + HC	145197	145162	35	8.98	
PL-S5660	IPS:572027	5282	HV- 75330	9 mg/L	0.58	CHZ04	HC ~ THTCPPCFDPEHC HC' ~ THTCPPCFKPKKC	fail	2 + HC	145167	145145	22	8.99	
PL-S5661	IPS:572029	5283	HV- 75331	11 mg/L	0.87	CHZ05	HC ~ THTCPPCFDPEHLL HC' ~ THTCPPCPKPKLL	fail	2 + HC	145191	145167	24	8.99	
PL-S5662	IPS:572031	5284	HV- 75332	13 mg/L	1.18	CHZ06	HC ~ THTCPPCFDPEHLL HC' ~ THTCPPCPKPKLL	fail	2 + HC	145196	145181	14	8.84	
PL-S5663	IPS:572033	5285	HV- 75333	9 mg/L	0.55	CHZ07	HC ~ THDCPPCPDPEHLL HC' ~ THKPPCFKPKLL	fail	2 + HC	145240	145208	32	8.98	
PL-S5664	IPS:572035	5286	HV- 75334	12 mg/L	0.9	CHZ08	HC ~ THTCPPCFDPEHLL HC' ~ THKPPCPKPKLL	fail	2 + HC	145168	145138	30	8.96	
PL-S5665	IPS:572037	5287	HV- 75335	9 mg/L	0.63	CHZ09	HC ~ TDDCPPPCFDPEHLL HC' ~ THKCFDPEKPKLL	pass		145175	145177	-2	8.98	multiple peaks
PL-S5666	IPS:572038	5288	HV- 75336	13 mg/L	1.04	CHZ10	HC ~ THTCPPCPAPPELL HC' ~ THTCPPCPKPKLL	fail	2 + HC	145190	145125	64	8.84	
PL-S5667	IPS:572039	5289	HV- 75337	16 mg/L	1.12	CHZ11	HC ~ THTCPPCFAPPELL HC' ~ THTCPPCFKPKLL	pass		145133	145135	-2	8.76	multiple peaks
PL-S5668	IPS:572040	5290	HV- 75338	12 mg/L	1	CHZ12	HC ~ LKCPKCPDPEHLL HC' ~ LKCPKCFKPKLL	fail	2 + HC	145191	145166	25	9.09	
PL-S5669	IPS:572041	5291	HV- 75339	12 mg/L	0.73	CHZ13	HC ~ ----CFKCPDPEHLL HC' ~ ----CFKCPKPKLL	fail	2 + HC	144512	144489	23	8.99	

FIG. 6

ANALYTICAL CEX AND MASS SPEC - CZH01



THE CHARGED ZIPPER HINGE—DESIGN CZH09



Sequence numbering from 1HZH IgG1 crystal structure

CZH09	
HC Hinge 1	HC Hinge 2
T236	T236
H237K	H237D
T238K	T238D
C239	C239
P240	P240
P241	P241
C242	C242
P243	P243
A244K	A244D
P245	P245
E246K	E246D
L247	L247
L248	L248

ANALYTICAL CEX AND MASS SPEC -- CZH09

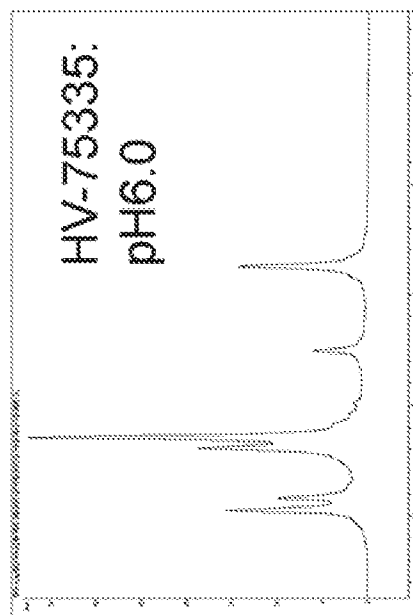
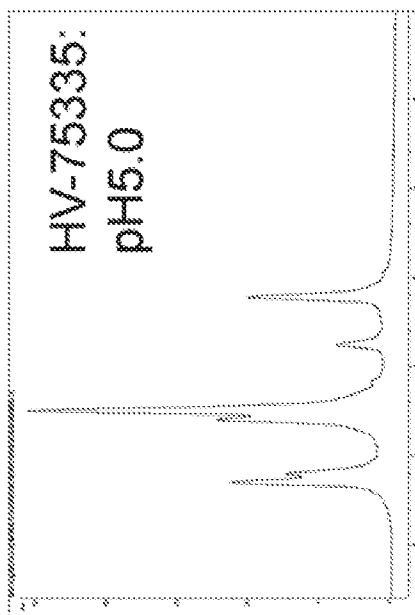
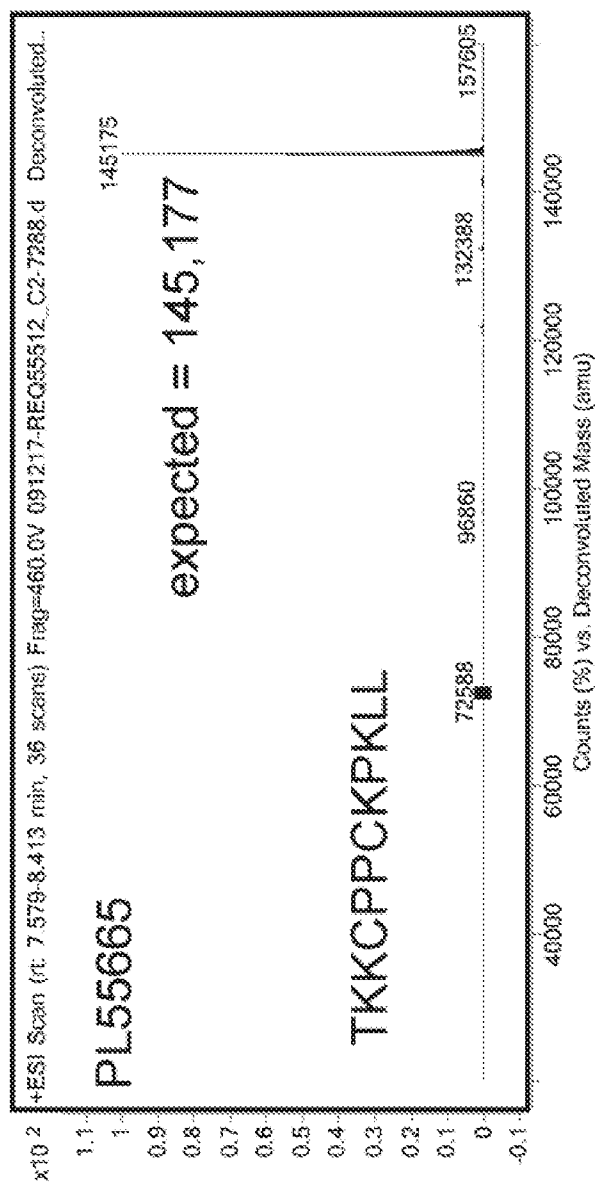


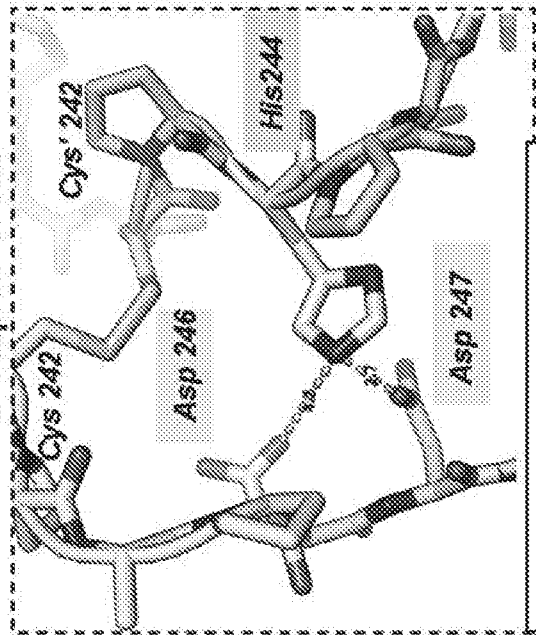
FIG. 8



STRUCTURAL GUIDANCE FOR THE CHARGED ZIPPER HINGE

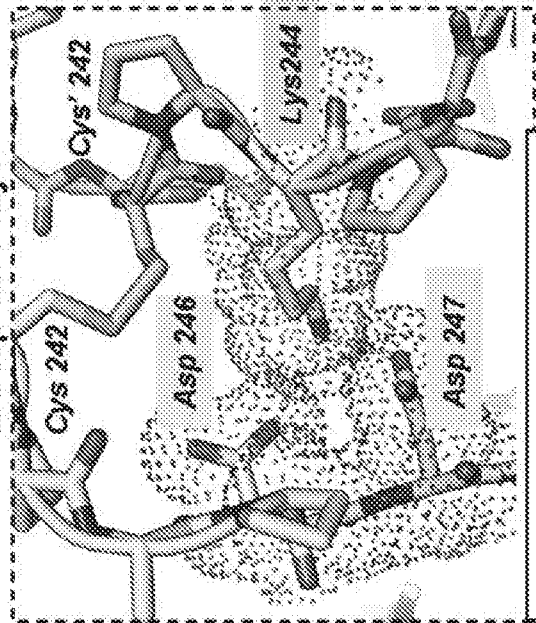
Correct pairing

2xAsp vs 1xHis

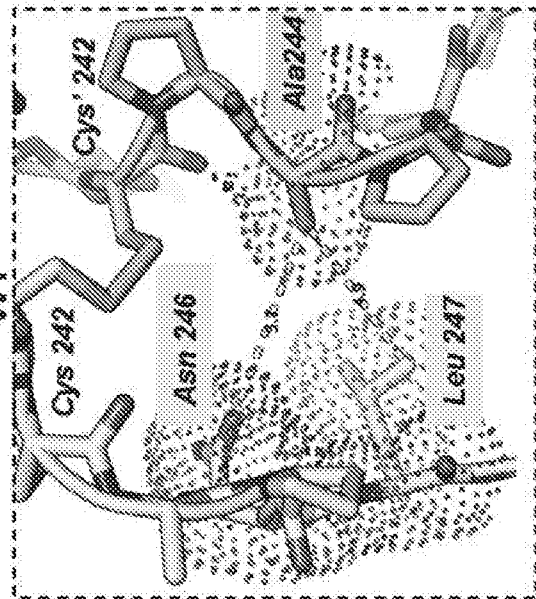


Incorrect pairing

2xAsp vs 1xLys



WT



230 240 250
IgG2 KKIEPR-GPTIKPCPPCKCPAPNLLGGPS
KKAEPKSCDKTHICPPC---PAPPELLGGPS
IgG4 KRVESKYGP-----CPPC---PAPPELLGGPS
Kk.Epk.ep....CPPC..PAP#1LGGPS

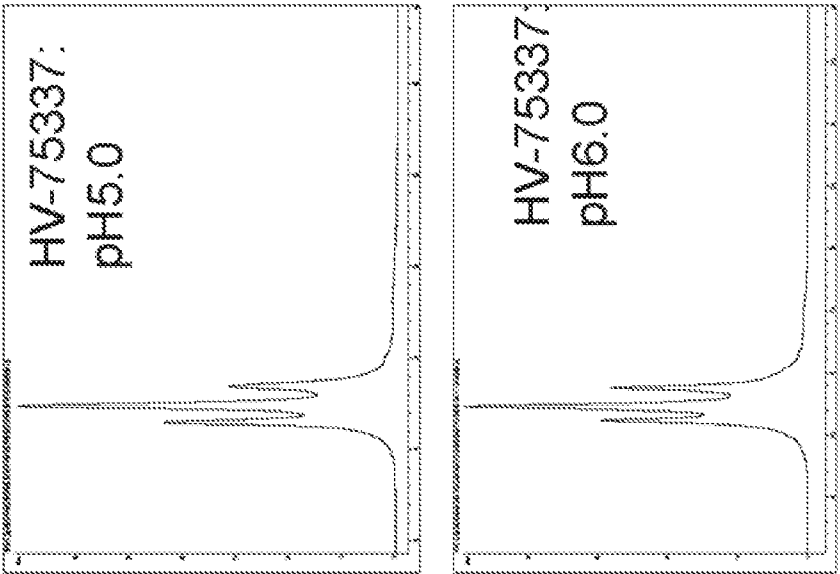
Sequence numbering from 1IGT IgG2
crystal structure

FIG. 9

CZH11	
HC Hinge 1	HC Hinge 2
C242	C242
P243	P243
A244H	A244
P245	P245
E246	E246D
L247	L247D

CZH10	
HC Hinge 1	HC Hinge 2
C242	C242
P243	P243
A244K	A244
P245	P245
E246	E246D
L247	L247D

ANALYTICAL CEX AND MASS SPEC – CZH11



PL55667

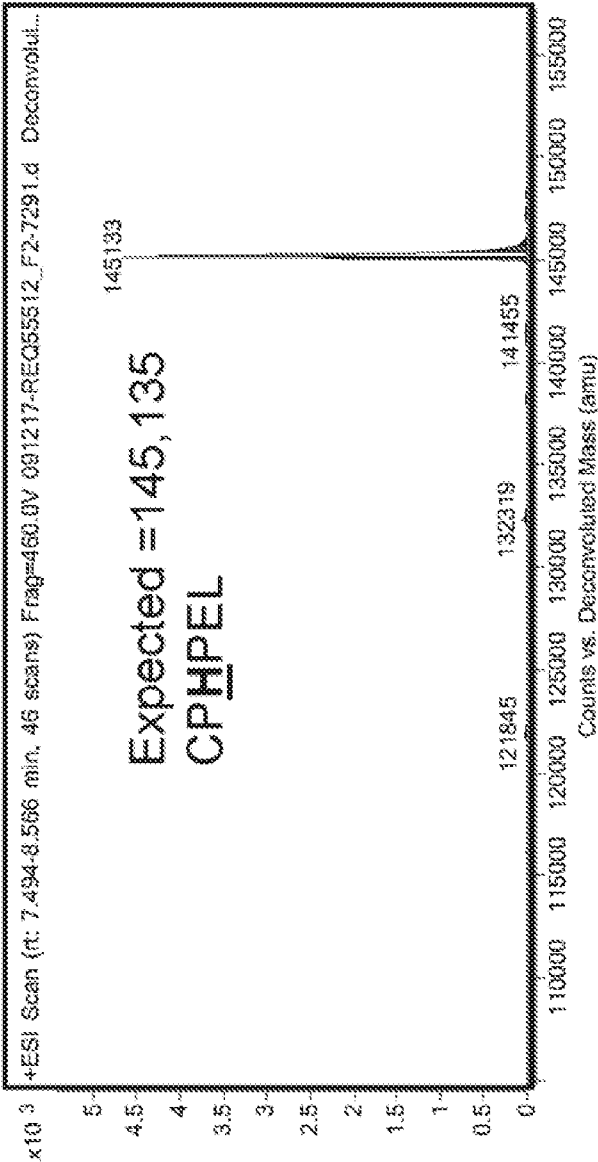


FIG. 10

FIG. 11

TARGETING THE HINGE REGION TO FORM HETERODIMERS +
CH3 CPM V11

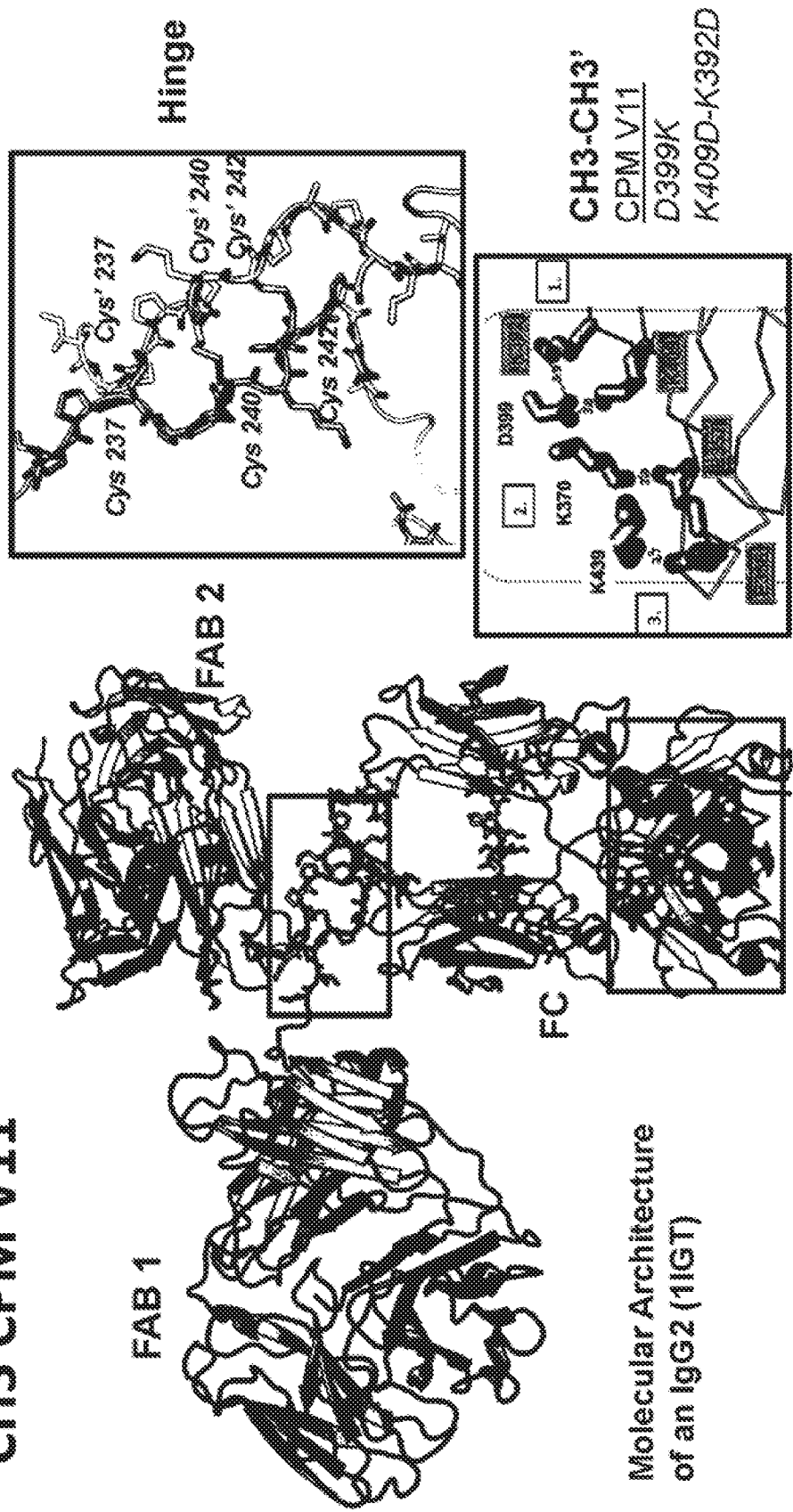


FIG. 12

SUMMARY TABLE : HINGE DESIGNS + CH3-CH3' CPM V11

Protein Lots	Protein Sequence Identity	Seq Reg ID	Harvest ID	Ther mg/L	Lot Amount (mg)	Design	M5QC	Comments	Measured Mass (Da)	Expected Mass (Da)	Error (Da)	P.I.	CEX
PL-55670	IPS-572002	5292	HV- 75340	13 mg/L	1	CH200 + V11 HC - THTCFFCPAPPELL	pass	Control	145067	145068	-1	8.75	Mono Peak
PL-55671	IPS-571998	5293	HV- 75341	11 mg/L	0.76	CH201 + V11 HC - THTCFFCPAPPELL	pass		145247	145247	0	8.88	Mono Peak
PL-55672	IPS-572000	5294	HV- 75342	8 mg/L	0.71	CH202 + V11 HC - THTCFFCPAPPELL	pass		145195	145198	-3	8.66	Mono Peak
PL-55673	IPS-572002	5295	HV- 75343	10 mg/L	0.67	CH203 + V11 HC - THTCFFCPAPPELL	pass		145148	145149	-1	8.88	Mono Peak
PL-55674	IPS-572004	5296	HV- 75344	7 mg/L	0.61	CH204 + V11 HC - THTCFFCPAPPELL	fail	ID unclear	145155	145152	23	8.98	mono Peak
PL-55675	IPS-572006	5297	HV- 75345	11 mg/L	0.78	CH205 + V11 HC - THTCFFCPAPPELL	pass		145152	145154	-2	8.88	mono Peak
PL-55676	IPS-572008	5298	HV- 75346	10 mg/L	0.68	CH206 + V11 HC - THTCFFCPAPPELL	pass		145169	145169	0	8.88	mono Peak
PL-55677	IPS-572010	5299	HV- 75347	8 mg/L	0.65	CH207 + V11 HC - THTCFFCPAPPELL	fail	ID unclear	145216	145195	21	8.99	mono Peak
PL-55679	IPS-572012	5300	HV- 75348	9 mg/L	0.85	CH208 + V11 HC - THTCFFCPAPPELL	pass		145121	145123	-2	8.88	mono Peak
PL-55679	IPS-572014	5301	HV- 75349	8 mg/L	0.59	CH209 + V11 HC - THTCFFCPAPPELL	pass		145163	145164	-1	8.75	mono Peak
PL-55680	IPS-572016	5302	HV- 75350	14 mg/L	1.22	CH210 + V11 HC - THTCFFCPAPPELL	fail	ID unclear	145134	145113	21	8.67	mono Peak
PL-55681	IPS-572018	5303	HV- 75351	14 mg/L	1.14	CH211 + V11 HC - THTCFFCPAPPELL	pass		145120	145122	-2	8.99	mono Peak
PL-55682	IPS-572020	5304	HV- 75352	10 mg/L	0.92	CH212 + V11 HC - THTCFFCPAPPELL	fail	ID unclear	145176	145152	24	8.75	2 peaks
PL-55683	IPS-572022	5305	HV- 75353	8 mg/L	0.71	CH213 + V11 HC - THTCFFCPAPPELL	pass		144473	144476	-3	8.66	mono Peak

THERMOSTABILITY ANALYSIS FOR HINGE DESIGNS

FIG. 13

Molecule	Hinge Mutation	+ CH3 CPM V11	BioID	Tm1	Tagg
Standard IgG Antibody	CH200	N	5278-1	73.3	68.9
Standard IgG Antibody	CH201	N	5279-1	73.4	73.8
Standard IgG Antibody	CH209	N	5287-1	73.7	75.5
Standard IgG Antibody	CH211	N	5289-1	73.2	74.4
Standard IgG Antibody	CH200	Y	5292-1	70.7	73.6
Standard IgG Antibody	CH201	Y	5293-1	71.6	74.2
Standard IgG Antibody	CH202	Y	5294-1	71.6	74.3
Standard IgG Antibody	CH203	Y	5295-1	71.9	74.4
Standard IgG Antibody	CH205	Y	5297-1	71.1	71.1
Standard IgG Antibody	CH206	Y	5298-1	70.8	74.2
Standard IgG Antibody	CH208	Y	5300-1	69.9	73.8
Standard IgG Antibody	CH209	Y	5301-1	69.8	74.3
Standard IgG Antibody	CH211	Y	5303-1	69.6	73.6
Standard IgG Antibody	CH213	Y	5305-1	71.1	74.3

← Aggregation is observed before molecule is fully melted.

Observations:

- 1) Hinge mutations do not negatively impact the stability of the Ab.
- 2) CH3 CPM V11 mutations appear to decrease Tm +/- 2 deg