Fig. 1A

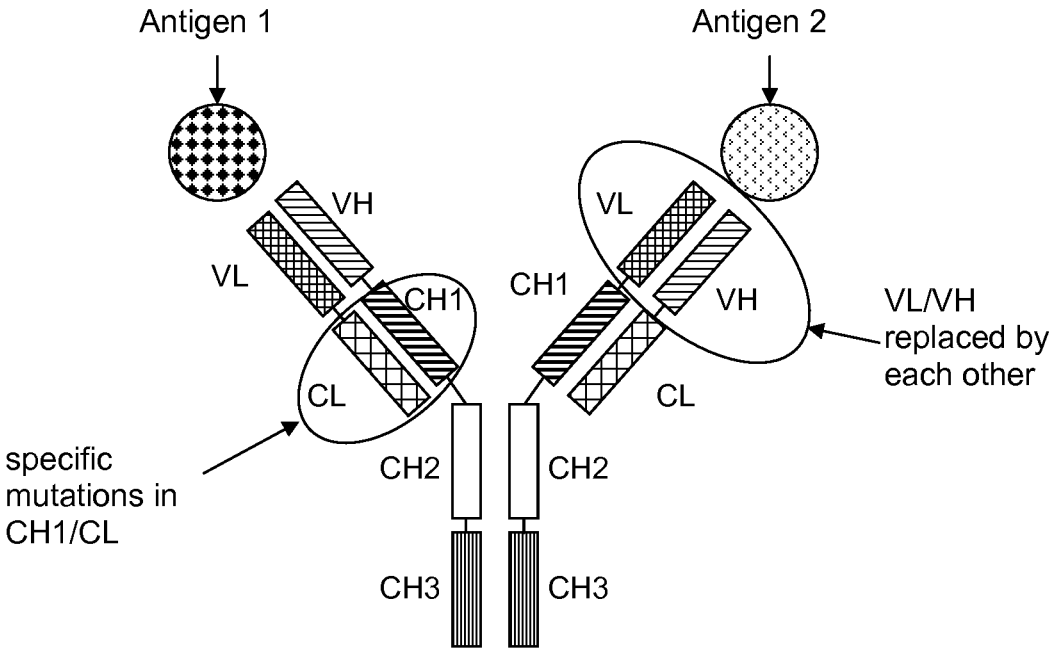

Antigen 1
Antigen 2
VH
VL
CH1
VL
CH1
VH
VL/VH
replaced by
each other
CL
CL
specific
mutations in
CH1/CL
CH2
CH2
CH3
CH3

Fig. 1B

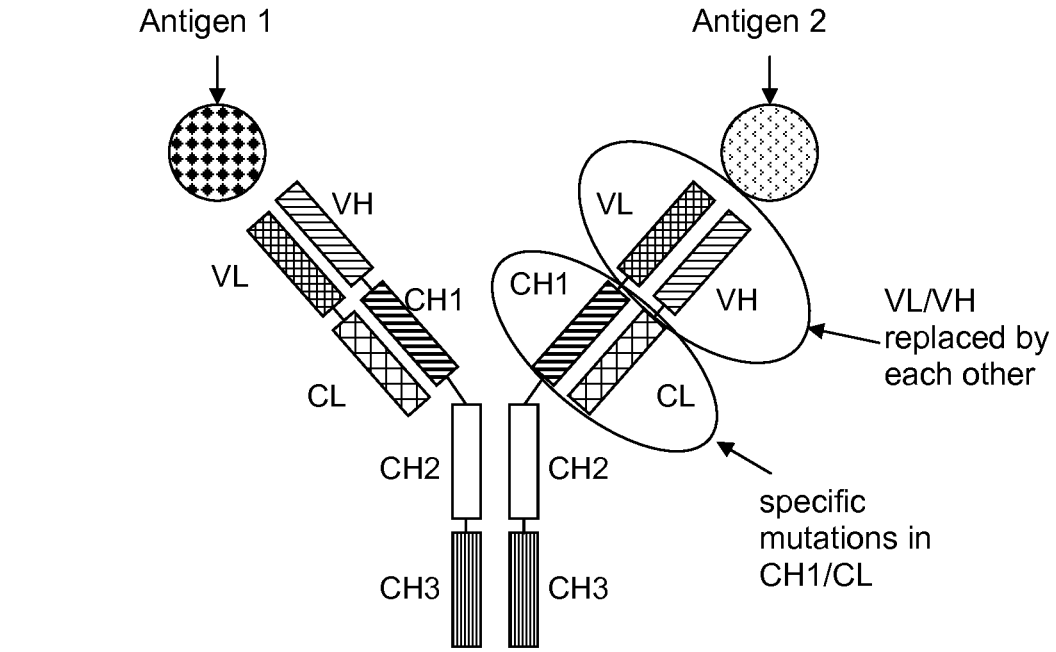

Antigen 1
Antigen 2
VH
VL
VL
CH1
CH1
VH
VL/VH
replaced by
each other
CL
CL
CH2
CH2
specific
mutations in
CH1/CL
CH3
CH3

Fig. 1C

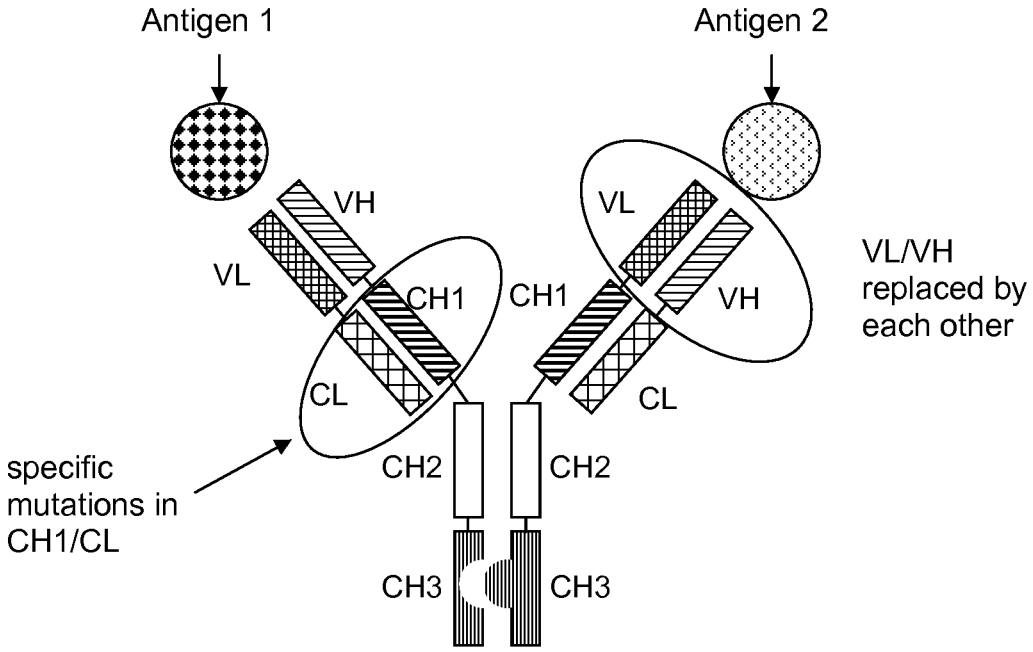
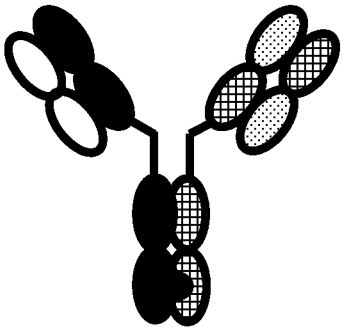
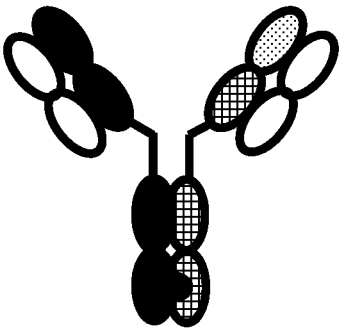

Antigen 1
Antigen 2
VH
VL
CH1
CH1
VL
VH
VL/VH
replaced by
each other
CL
CL
specific
mutations in
CH1/CL
CH2
CH2
CH3
CH3

Fig. 2A



desired bispecific antibody with VH-VL exchange/replacement in one binding arm



Main side product: result of Bence-Jones interaction of crossed HC with uncrossed LC

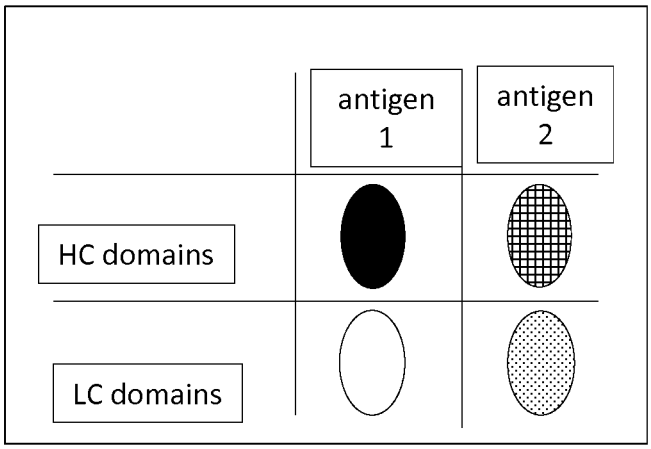
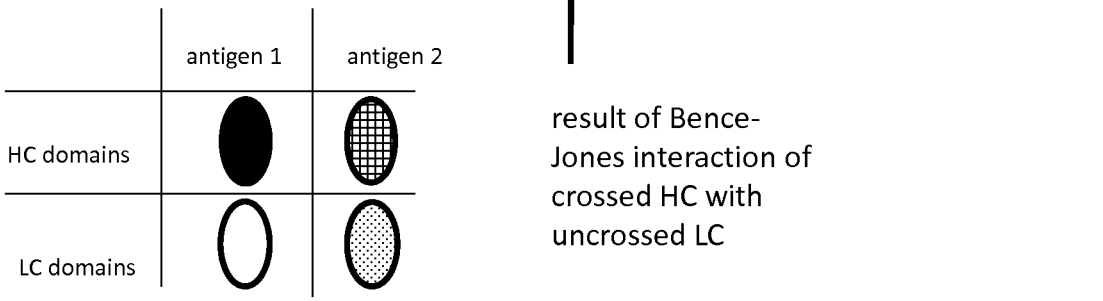


Fig. 2B



	antigen 1	antigen 2
HC domains	●	◍
LC domains	○	◔

result of Bence-Jones interaction of crossed HC with uncrossed LC

Fig. 3A

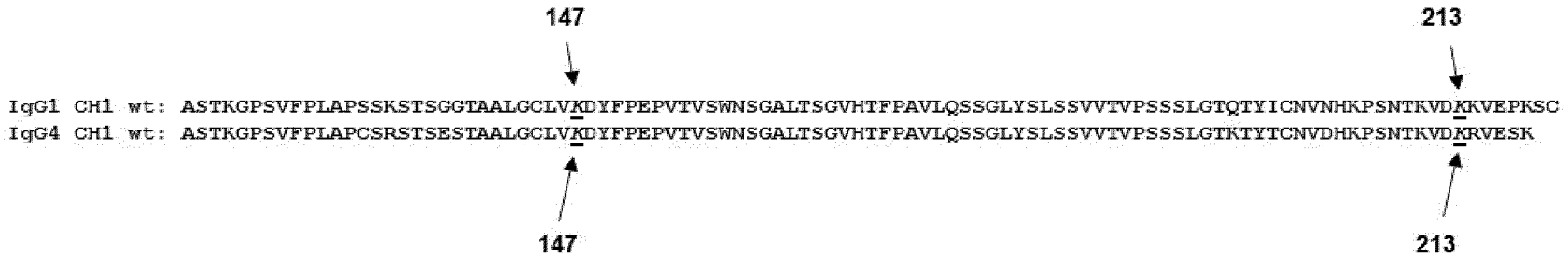


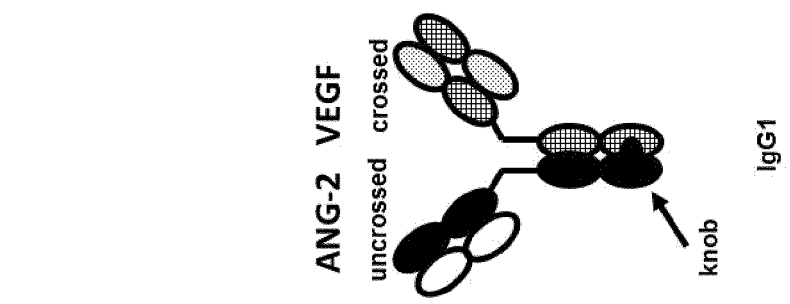
Fig. 3B



Fig. 3C

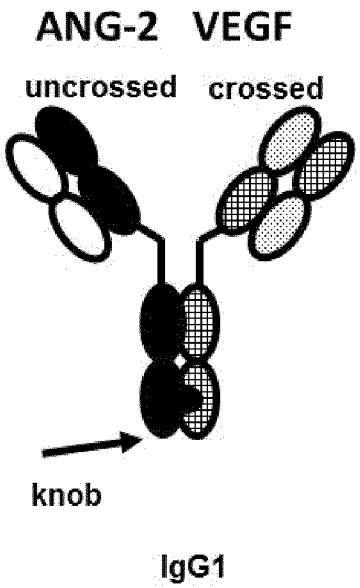


Fig. 4A



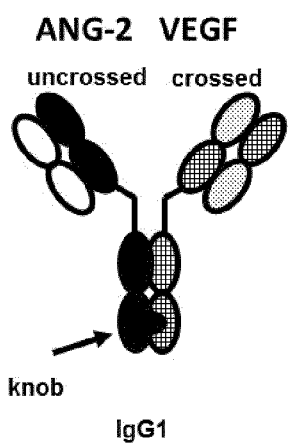
	No VH/VL exchange – uncrossed					VH/VL exchange – crossed			Bence-Jones mispairing (VL-CH1/VL-CL-Pairing) (amount antibody with 2 Ang2-LC's) % by MS
	CL isotype	CL ANG-2 (position 124)	CL ANG-2 (position 123)	CH1 ANG-2 (position 147)	CH1 ANG-2 (position 213)	CL isotype	CH1 VEGF	CL VEGF	
Ang2VEGF -0273	kappa	wt: Q124	wt: E123	wt: K147	wt: K213	kappa	wt	wt	~20
Ang2VEGF -0396	kappa	**Q124K**	wt	**K147E**	wt	kappa	wt	wt	~3
Ang2VEGF -0397	kappa	**Q124K**	wt	wt	**K213E**	kappa	wt	wt	~3
Ang2VEGF -0394	kappa	wt	**E123K**	**K147E**	wt	kappa	wt	wt	~15
Ang2VEGF -0395	kappa	wt	**E123K**	wt	**K213E**	kappa	wt	wt	~15

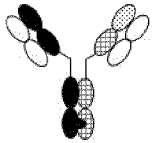
Fig. 4B



	No VH/VL exchange – uncrossed		VH/VL exchange - crossed	
	LC ANG-2	HC ANG-2	HC VEGF	LC VEGF
Ang2VEGF-0273	SEQ ID NO: 1	SEQ ID NO: 2	SEQ ID NO: 3	SEQ ID NO: 4
Ang2VEGF-0396	SEQ ID NO: 5	SEQ ID NO: 6	SEQ ID NO: 3	SEQ ID NO: 4
Ang2VEGF-0397	SEQ ID NO: 5	SEQ ID NO: 7	SEQ ID NO: 3	SEQ ID NO: 4
Ang2VEGF-0394	SEQ ID NO: 8	SEQ ID NO: 6	SEQ ID NO: 3	SEQ ID NO: 4
Ang2VEGF-0395	SEQ ID NO: 8	SEQ ID NO: 7	SEQ ID NO: 3	SEQ ID NO: 4

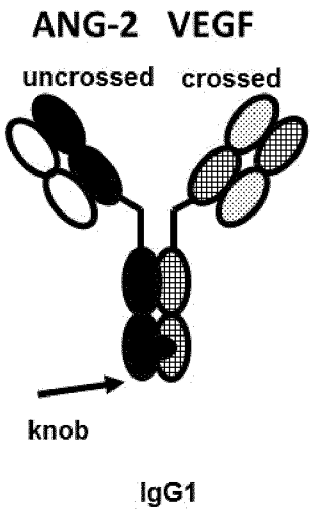
Fig. 5A





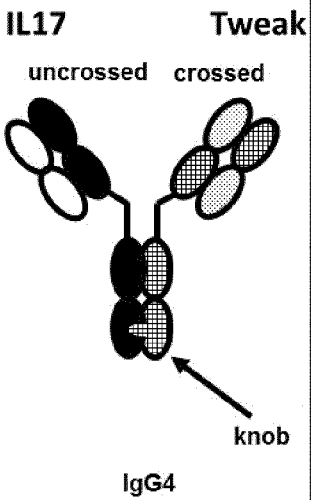
	No VH/VL exchange – uncrossed					VH/VL exchange – crossed			
	CL isotype	CL ANG-2	CL ANG-2	CH1 ANG-2	CH1 ANG-2	CL isotype	CH1 VEGF	CL VEGF	Bence-Jones mispairing (VL-CH1/VL-CL-Pairing) (amount antibody with 2 Ang2-LC's) % by MS
Ang2VEGF-0273	kappa	wt: Q124	wt: E123	wt: K147	wt: K213	kappa	wt	wt: Q124	**~20%**
Ang2VEGF-0274	kappa	**Q124K**	**E123K**	**K147E**	**K213E**	kappa	wt	wt	**0**
Ang2VEGF-0282	kappa	**Q124R**	**E123K**	**K147E**	**K213E**	kappa	wt	**Q124E**	**0**
Ang2VEGF-0283	lambda (wt lambda: E124/ E123)	**E124K**	**E123K**	**K147E**	**K213E**	kappa	wt	wt:	**0**
Ang2VEGF-0284	kappa	**Q124R**	**E123K**	**K147E**	**K213D**	kappa	wt	wt	**0**
Ang2VEGF-0285	kappa	**Q124R**	**E123K**	**K147E**	**K213D**	kappa	wt	**Q124E**	**0**
Ang2VEGF-0286	kappa	**Q124K**	**E123K**	**K147E**	**K213E**	kappa	wt	**Q124E**	**0**

Fig. 5B



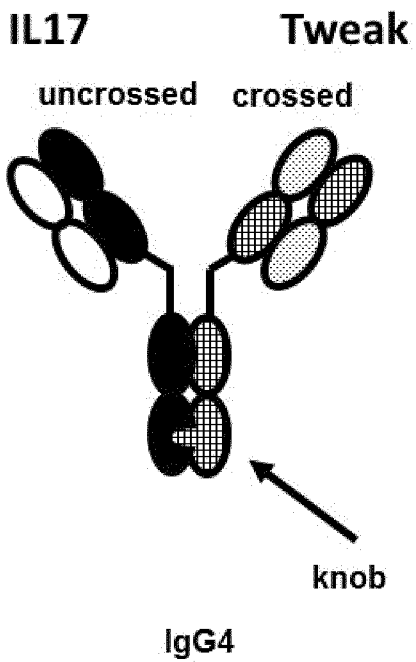
	No VH/VL exchange – uncrossed		VH/VL exchange - crossed	
	LC ANG-2	HC ANG-2	HC VEGF	LC VEGF
Ang2VEGF-0273	SEQ ID NO: 1	SEQ ID NO: 2	SEQ ID NO: 3	SEQ ID NO: 4
Ang2VEGF-0274	SEQ ID NO: 9	SEQ ID NO: 10	SEQ ID NO: 3	SEQ ID NO: 4
Ang2VEGF-0282	SEQ ID NO: 11	SEQ ID NO: 10	SEQ ID NO: 3	SEQ ID NO: 12
Ang2VEGF-0283	SEQ ID NO: 13	SEQ ID NO: 10	SEQ ID NO: 3	SEQ ID NO: 4
Ang2VEGF-0284	SEQ ID NO: 11	SEQ ID NO: 14	SEQ ID NO: 3	SEQ ID NO: 4
Ang2VEGF-0285	SEQ ID NO: 11	SEQ ID NO: 14	SEQ ID NO: 3	SEQ ID NO: 12
Ang2VEGF-0286	SEQ ID NO: 9	SEQ ID NO: 10	SEQ ID NO: 3	SEQ ID NO: 12

Fig. 6A



		No VH/VL exchange – uncrossed				VH/VL exchange – crossed			
	CL isotype	CL IL17 (position 124)	CL IL17 (position 123)	CH1 IL17 (position 147)	CH1 IL17 (position 213)	CL isotype	CH1 TWEAK	CL TWEAK (position 124)	Bence-Jones mispairing (VL-CH1/VL-CL-Pairing) (amount antibody with 2 IL17-LC's) % by MS
TweakIL 17-0096	kappa	wt: Q124	wt: E123	wt: K147	wt: K213	kappa	wt	wt: Q124	**~20%**
TweakIL 17-0097	kappa	**Q124K**	**E123R**	**K147E**	**K213E**	kappa	wt	**Q124E**	**0**
TweakIL 17-0098	kappa	**Q124K**	**E123R**	**K147E**	**K213D**	kappa	wt	**wt**	**0**
TweakIL 17-0099	kappa	**Q124K**	**E123R**	**K147E**	**K213D**	kappa	wt	**Q124E**	**0**
TweakIL 17-0100	kappa	**Q124K**	**E123K**	**K147E**	**K213E**	kappa	wt	**Q124E**	**0**
TweakIL 17-0101	kappa	**Q124K**	**E123K**	**K147E**	**K213E**	kappa	wt	**wt**	**not determ.**

Fig. 6B



	No VH/VL exchange – uncrossed		VH/VL exchange - crossed	
	LC IL17	HC IL17	HC Tweak	LC Tweak
TweakIL17-0096	SEQ ID NO: 15	SEQ ID NO: 16	SEQ ID NO: 17	SEQ ID NO: 18
TweakIL17-0097	SEQ ID NO: 19	SEQ ID NO: 20	SEQ ID NO: 17	SEQ ID NO: 21
TweakIL17-0098	SEQ ID NO: 19	SEQ ID NO: 22	SEQ ID NO: 17	SEQ ID NO: 18
TweakIL17-0099	SEQ ID NO: 19	SEQ ID NO: 22	SEQ ID NO: 17	SEQ ID NO: 21
TweakIL17-0100	SEQ ID NO: 23	SEQ ID NO: 20	SEQ ID NO: 17	SEQ ID NO: 21
TweakIL17-0101	SEQ ID NO: 23	SEQ ID NO: 20	SEQ ID NO: 17	SEQ ID NO: 18

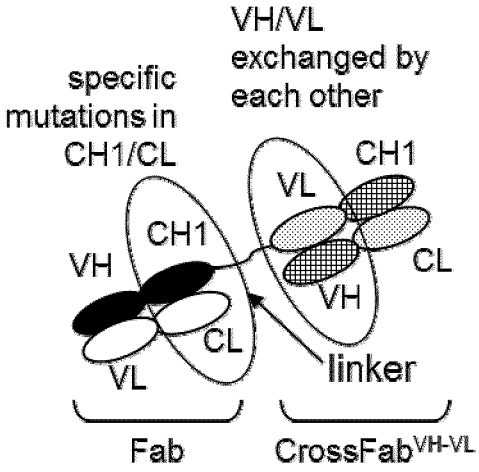


Fig. 7A

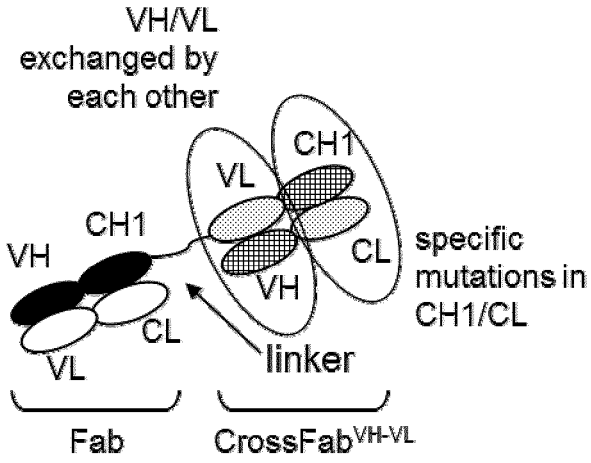


Fig. 7B

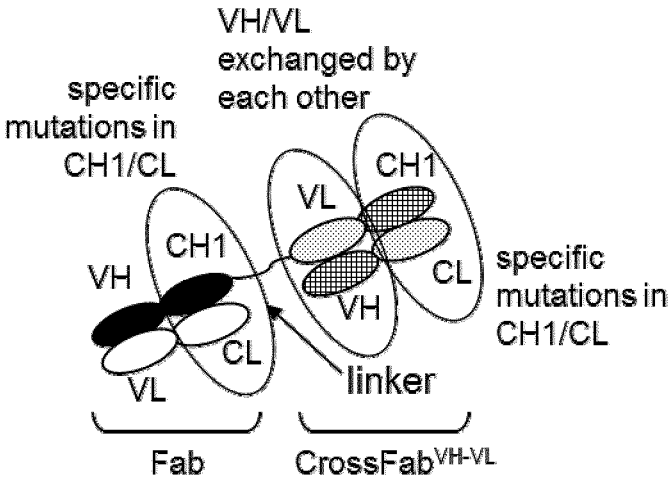


Fig. 7C

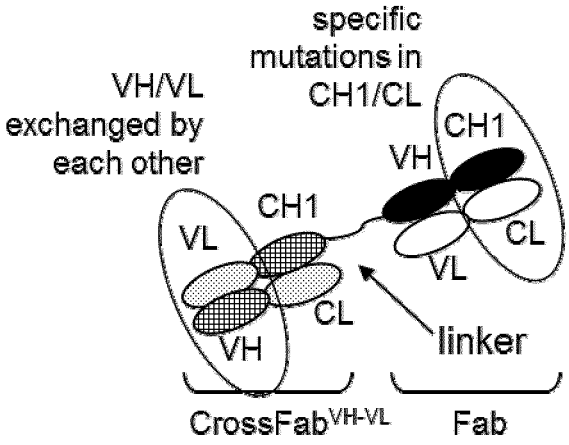

specific
mutations in
CH1/CL
VH/VL
exchanged by
each other
CH1
VH
CL
VL
CH1
VL
CL
VH
linker
CrossFab^VH-VL
Fab

Fig. 7D

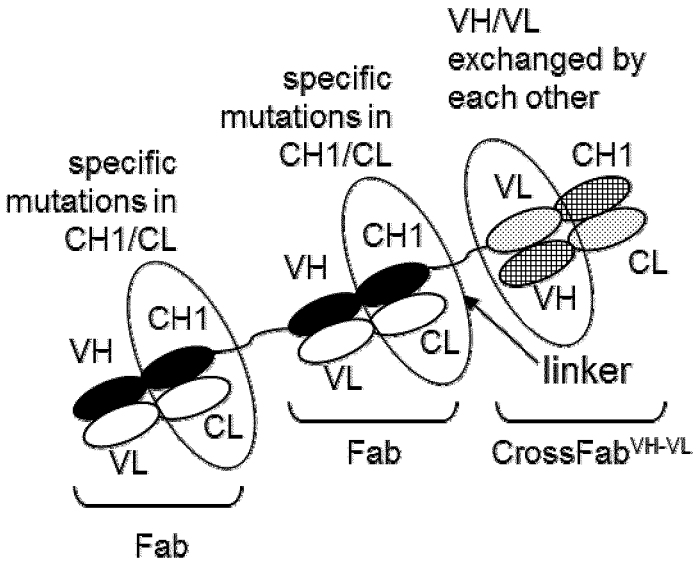

VH/VL
exchanged by
each other
specific
mutations in
CH1/CL
specific
mutations in
CH1/CL
CH1
VL
CH1
VH
CL
VH
CH1
VH
CL
VL
linker
CL
VL
Fab
CrossFab^{VH-VL}
Fab

Fig. 8A

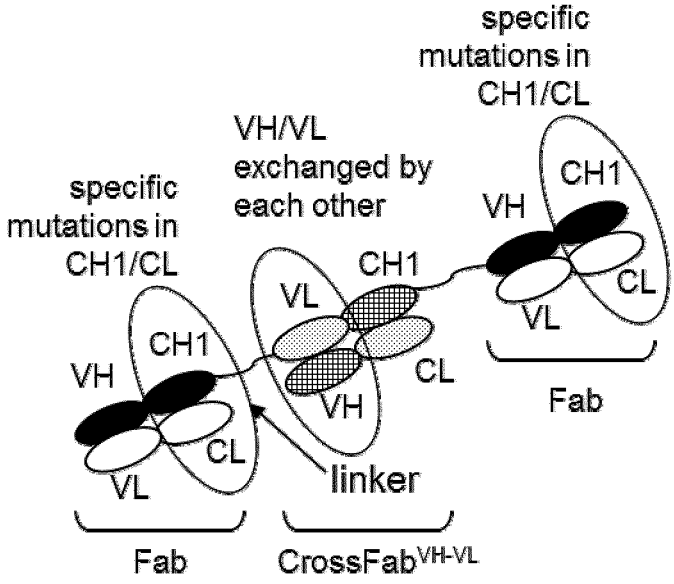

specific
mutations in
CH1/CL
VH/VL
exchanged by
each other
specific
mutations in
CH1/CL
CH1
VH
CL
VL
CH1
VL
CH1
VH
CL
VH
CL
VL
linker
Fab
Fab
CrossFab^{VH-VL}

Fig. 8B

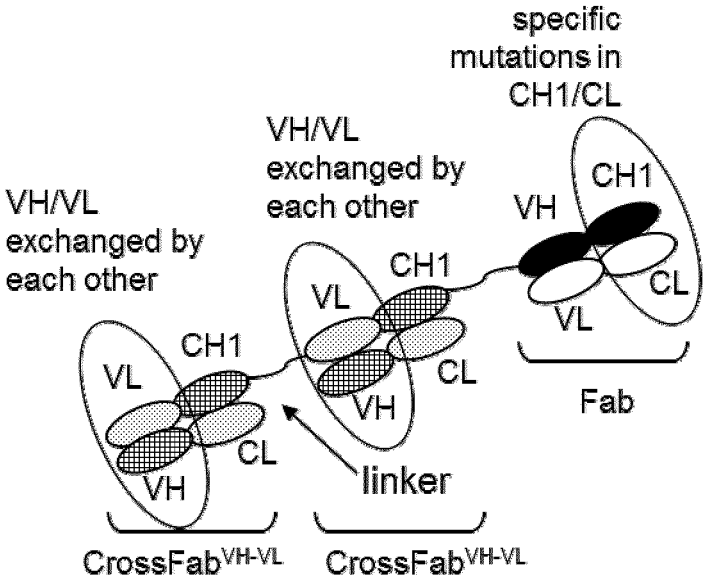

specific
mutations in
CH1/CL
VH/VL
exchanged by
each other
VH/VL
exchanged by
each other
CH1
VH
CL
VL
CH1
VL
CL
VH
CH1
VL
CL
VH
linker
Fab
CrossFab^{VH-VL}
CrossFab^{VH-VL}

Fig. 8C

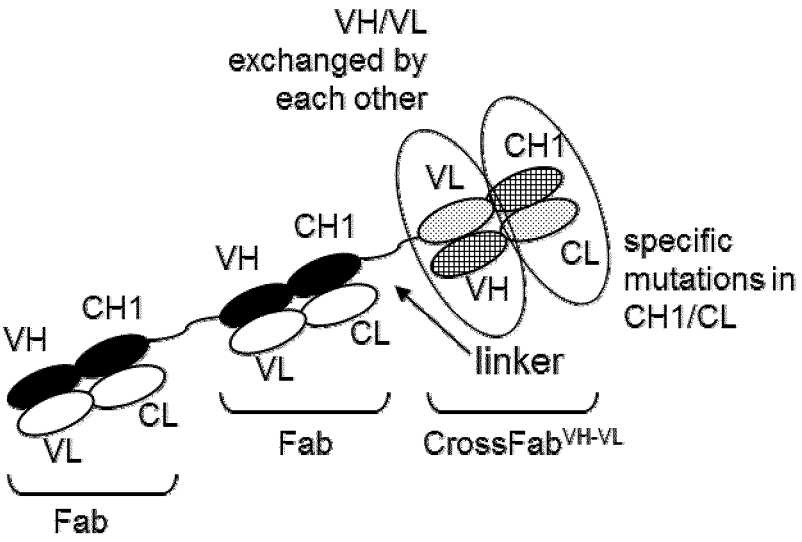

VH/VL
exchanged by
each other
CH1
VL
CL
VH
specific
mutations in
CH1/CL
CH1
VH
CL
VL
linker
VH
CL
VL
Fab
CrossFab^{VH-VL}
Fab

Fig. 8D

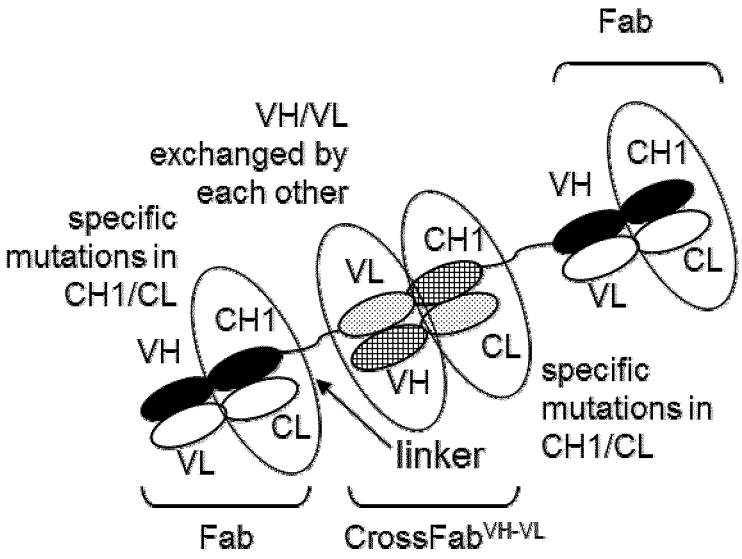

Fab
VH/VL
exchanged by
each other
CH1
VH
CL
VL
specific
mutations in
CH1/CL
CH1
VL
CL
VH
CH1
VH
CL
VL
specific
mutations in
CH1/CL
linker
Fab
CrossFab^{VH-VL}

Fig. 8E

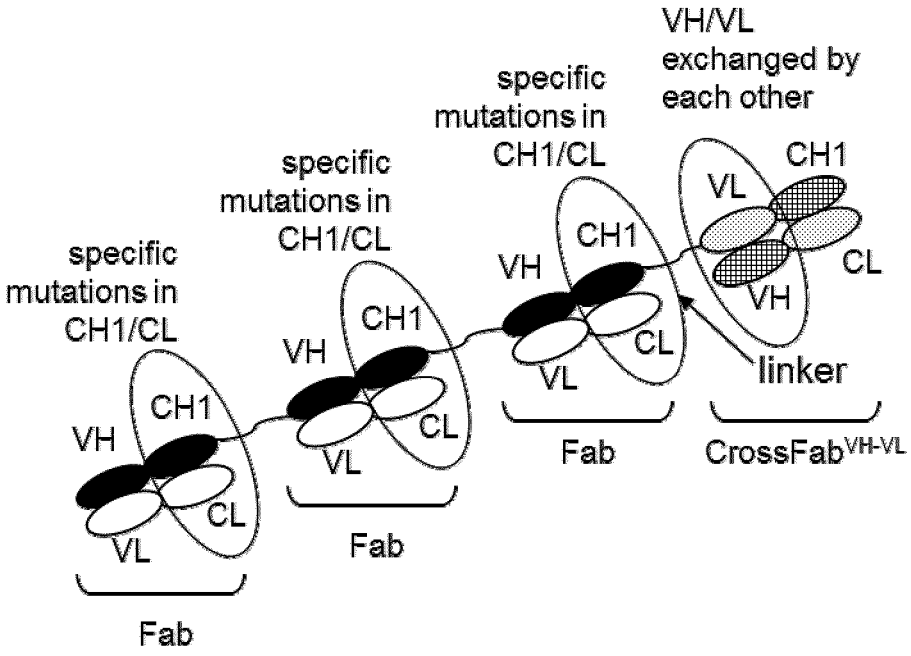

specific
mutations in
CH1/CL
VH
CH1
VL
CL
Fab
specific
mutations in
CH1/CL
VH
CH1
VL
CL
Fab
specific
mutations in
CH1/CL
VH
CH1
VL
CL
Fab
VH/VL
exchanged by
each other
VH
CH1
VL
CL
linker
CrossFab^VH-VL

Fig. 9A

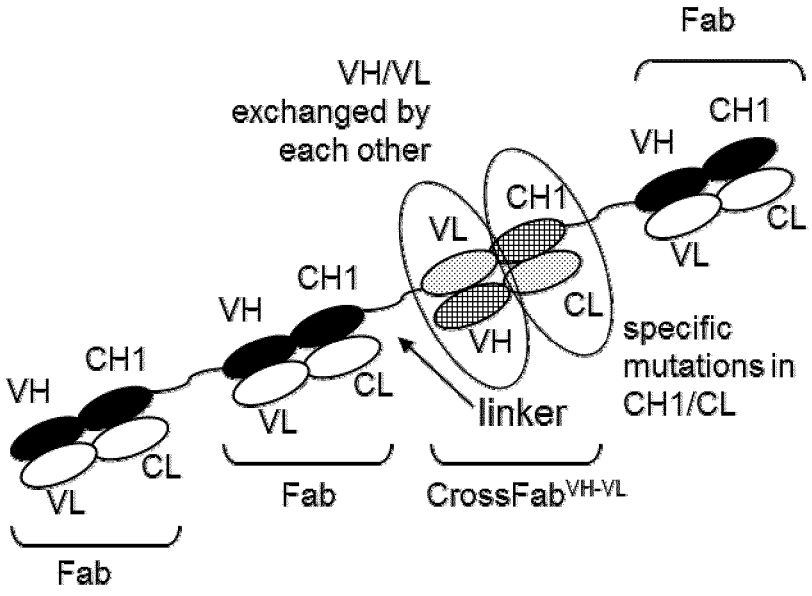

Fab
VH/VL
exchanged by
each other
VH
CH1
VL
CL
CH1
VL
CL
VH
specific
mutations in
CH1/CL
VH
CH1
VL
CL
linker
VH
CH1
VL
CL
Fab
Fab
CrossFab^VH-VL

Fig. 9B