Figure 1A

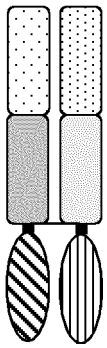
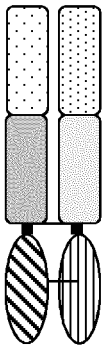


Figure 1B



First Variable region of light chain VL1

First Variable region of heavy chain VH1

Constant regions cKappa and CH1

Second variable region of light chain VL2

Second variable region of heavy chain VH2

Disulphide bond

Figure 2

(a) Heavy chain variable domain of anti-albumin antibody (no ds) (SEQ ID NO:1)
EVQLLESGGGLVQPGGSLRLSCAVSGIDLSNYAINWVRQAPGKGLEWIGIIWASGTTFYATWAKGRFTI
SRDNSKNTVYLQMNSLRAEDTAVYYCARTVPGYSTAPYFDLWGQGTLVTVSS

(b) Heavy chain variable domain of anti-albumin antibody (ds) (SEQ ID NO:2)
EVQLLESGGGLVQPGGSLRLSCAVSGIDLSNYAINWVRQAPGKCLEWIGIIWASGTTFYATWAKGRFTI
SRDNSKNTVYLQMNSLRAEDTAVYYCARTVPGYSTAPYFDLWGQGTLVTVSS

(c) Light chain variable domain of anti-albumin antibody (no ds) (SEQ ID NO:3)
DIQMTQSPSSVSASVGDRVTITCQSSPSVWSNFLSWYQQKPGKAPKLLIYEASKLTSGVPSRFSGSGSG
TDFTLTISSLQPEDFATYYCGGGYSSISDTTFGGGTKVEIKRT

(d) Light chain variable domain of anti-albumin antibody (ds) (SEQ ID NO:4)
DIQMTQSPSSVSASVGDRVTITCQSSPSVWSNFLSWYQQKPGKAPKLLIYEASKLTSGVPSRFSGSGSG
TDFTLTISSLQPEDFATYYCGGGYSSISDTTFGCGTKVEIKRT

(e) Linker 1 (SEQ ID NO:5)
SGGGGSGGGGTGGGGS

(f) Linker 2 (SEQ ID NO:6)
GGGGSGGGGSGGGGS

(g) A26 Fab Heavy-(G4S,G4T,G4S)-645dsFv(gH5) (SEQ ID NO:7)
EVQLVESGGGLVQPGGSLRLSCAASGFTFTNYGIHWIRQAPGKGLEWVASISPSGGLTYYRDSVKGRFT
ISRDDAKNSPYLQMNSLRAEDTAVYYCATGGEGIFDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGG
TAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPS
NTKVDKKVEPKSCSGGGGSGGGGTGGGGSEVQLLESGGGLVQPGGSLRLSCAVSGIDLSNYAINWVRQA
PGKCLEWIGIIWASGTTFYATWAKGRFTISRDNSKNTVYLQMNSLRAEDTAVYYCARTVPGYSTAPYFD
LWGQGTLVTVSS

(h) A26 Fab Light-(3xG4S)-645dsFv(gL4) (SEQ ID NO:8)
DIQMTQSPSSLSASVGDRVTITCRATQSIYNALAWYQQKPGKAPKLLIYNANTLHTGVPSRFSASGSGT
DSTLTISSLQPEDFATYYCQQYYDYPLTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNN
FYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTK
SFNRGECGGGGSGGGGSGGGGSDIQMTQSPSSVSASVGDRVTITCQSSPSVWSNFLSWYQQKPGKAPKL
LIYEASKLTSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCGGGYSSISDTTFGCGTKVEIKRT

FIGURE 3

645gH1 heavy chain variable domain (SEQ ID NO: 9)
EVQLLESGGGLVQPGGSLRLSCAVSGIDLSNYAINWVRQAPGKCLEWIGIIWASGTTFYATWAKGRFTI
SRDSTTVYLQMNSLRAEDTAVYYCARTVPGYSTAPYFDLWGQGTLVTVSS

645gL1 light chain variable domain (SEQ ID NO: 10)
DIVMTQSPSSVSASVGDRVTITCQSSPSVWSNFLSWYQQKPGKAPKLLIYEASKLTSGVPSRFKGSGSG
TDFTLTISSLQPEDFATYYCGGGYSSISDTTFGCGTKVEIK

A26 Fab Heavy-(3xG4S)-645dsFv(gH1) (SEQ ID NO:11)
EVQLVESGGGLVQPGGSLRLSCAASGFTFTNYGIHWIRQAPGKGLEWVASISPSGGLTYYRDSVKGRFT
ISRDDAKNSPYLQMNSLRAEDTAVYYCATGGEGIFDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGG
TAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPS
NTKVDKKVEPKSCSGGGGSGGGGSGGGGSEVQLLESGGGLVQPGGSLRLSCAVSGIDLSNYAINWVRQA
PGKCLEWIGIIWASGTTFYATWAKGRFTISRDSTTVYLQMNSLRAEDTAVYYCARTVPGYSTAPYFDLW
GQGTLVTVSS

A26 Fab Light-(3xG4S)-645dsFv(gL1) (SEQ ID NO:12)
DIQMTQSPSSLSASVGDRVTITCRATQSIYNALAWYQQKPGKAPKLLIYNANTLHTGVPSRFSASGSGT
DSTLTISSLQPEDFATYYCQQYYDYPLTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNN
FYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTK
SFNRGECSGGGGSGGGGSGGGGSDIVMTQSPSSVSASVGDRVTITCQSSPSVWSNFLSWYQQKPGKAPK
LLIYEASKLTSGVPSRFKGSGSGTDFTLTISSLQPEDFATYYCGGGYSSISDTTFGCGTKVEIK

FIGURE 4

645 gH5gL4 (SEQ ID NO: 13)

GAGGTTCAGCTGCTGGAGTCTGGAGGCGGGCTTGTCCAGCCTGGAGGGAGCCTGCGTCTCTCTTGTGCA
GTAAGCGGCATCGACCTGTCCAACTACGCGATTAACTGGGTACGTCAGGCACCGGGTAAAGGTCTGGAA
TGGATCGGCATCATCTGGGCCTCTGGTACGACCTTCTACGCTACTTGGGCCAAAGGTCGTTTCACCATC
TCCCGTGACAACTCTAAAAACACCGTGTACCTGCAGATGAACTCTCTGCGTGCGGAAGACACTGCGGTT
TACTATTGCGCGCGTACCGTTCCGGGCTATTCTACTGCACCGTACTTCGACCTGTGGGGTCAGGGTACT
CTGGTTACCGTCTCGAGTGGAGGTGGCGGTTCTGGCGGTGGCGGTTCCGGTGGCGGTGGATCGGGAGGT
GGCGGTTCTGATATCCAGATGACCCAGAGTCCAAGCAGTGTTTCCGCCAGCGTAGGCGATCGTGTGACT
ATTACCTGTCAGTCCTCTCCGAGCGTTTGGTCCAACTTCCTGAGCTGGTACCAGCAGAAACCGGGTAAA
GCCCCGAAACTGCTGATCTACGAGGCGTCTAAACTGACCTCTGGTGTACCGTCCCGTTTCTCTGGCTCT
GGCTCTGGTACGGACTTCACTCTGACCATCTCCTCTCTGCAGCCGGAAGACTTTGCAACGTACTACTGC
GGTGGTGGTTACTCTTCCATCTCTGACACCACGTTCGGTGGAGGCACCAAAGTTGAAATCAAACGTACG
CATCACCATCACCATCACCATCACCATCAC

645 gH5gL4 (SEQ ID NO: 14)

EVQLLESGGGLVQPGGSLRLSCAVSGIDLSNYAINWVRQAPGKGLEWIGIIWASGTTFYATWAKGRFTI
SRDNSKNTVYLQMNSLRAEDTAVYYCARTVPGYSTAPYFDLWGQGTLVTVSSGGGGSGGGGSGGGGSGG
GGSDIQMTQSPSSVSASVGDRVTITCQSSPSVWSNFLSWYQQKPGKAPKLLIYEASKLTSGVPSRFSGS
GSGTDFTLTISSLQPEDFATYYCGGGYSSISDTTFGGGTKVEIKRTHHHHHHHHHH

645 gH5gL4ds (SEQ ID NO: 15)

GAGGTTCAGCTGCTGGAGTCTGGAGGCGGGCTTGTCCAGCCTGGAGGGAGCCTGCGTCTCTCTTGTGCA
GTAAGCGGCATCGACCTGTCCAACTACGCGATTAACTGGGTACGTCAGGCACCGGGTAAATGCCTGGAA
TGGATCGGCATCATCTGGGCCTCTGGTACGACCTTCTACGCTACTTGGGCCAAAGGTCGTTTCACCATC
TCCCGTGACAACTCTAAAAACACCGTGTACCTGCAGATGAACTCTCTGCGTGCGGAAGACACTGCGGTT
TACTATTGCGCGCGTACCGTTCCGGGCTATTCTACTGCACCGTACTTCGACCTGTGGGGTCAGGGTACT
CTGGTTACCGTCTCGAGTGGAGGTGGCGGTTCTGGCGGTGGCGGTTCCGGTGGCGGTGGATCGGGAGGT
GGCGGTTCTGATATCCAGATGACCCAGAGTCCAAGCAGTGTTTCCGCCAGCGTAGGCGATCGTGTGACT
ATTACCTGTCAGTCCTCTCCGAGCGTTTGGTCCAACTTCCTGAGCTGGTACCAGCAGAAACCGGGTAAA
GCCCCGAAACTGCTGATCTACGAGGCGTCTAAACTGACCTCTGGTGTACCGTCCCGTTTCTCTGGCTCT
GGCTCTGGTACGGACTTCACTCTGACCATCTCCTCTCTGCAGCCGGAAGACTTTGCAACGTACTACTGC
GGTGGTGGTTACTCTTCCATCTCTGACACCACGTTCGGTTGTGGCACCAAAGTTGAAATCAAACGTACG
CATCACCATCACCATCACCATCACCATCAC

645 gH5gL4ds (SEQ ID NO: 16)

EVQLLESGGGLVQPGGSLRLSCAVSGIDLSNYAINWVRQAPGKCLEWIGIIWASGTTFYATWAKGRFTI
SRDNSKNTVYLQMNSLRAEDTAVYYCARTVPGYSTAPYFDLWGQGTLVTVSSGGGGSGGGGSGGGGSGG
GGSDIQMTQSPSSVSASVGDRVTITCQSSPSVWSNFLSWYQQKPGKAPKLLIYEASKLTSGVPSRFSGS
GSGTDFTLTISSLQPEDFATYYCGGGYSSISDTTFGCGTKVEIKRTHHHHHHHHHH

FIGURE 5

LINKER (SEQ ID NO: 17)

GGGGSGGGGSGGGGSGGGGS

LINKER (SEQ ID NO. 18)

SGGGGSGGGGSGGGGS

cKappa (SEQ ID NO. 19)

VAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSST
LTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

CH1 (SEQ ID NO. 20)

ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVV
TVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSC

FIGURE 6

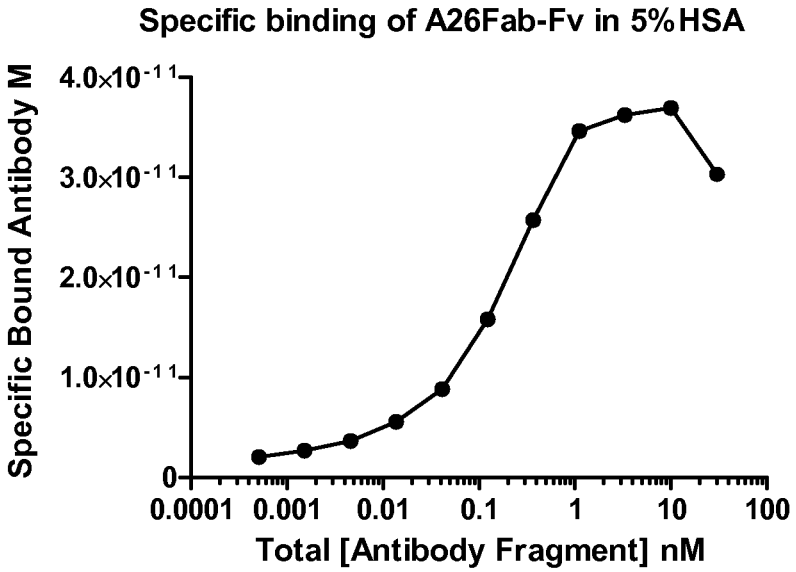

Specific binding of A26Fab-Fv in 5%HSA
Specific Bound Antibody M
4.0x10-11
3.0x10-11
2.0x10-11
1.0x10-11
0
0.0001
0.001
0.01
0.1
1
10
100
Total [Antibody Fragment] nM

FIGURE 7

A26-645gH5gL4 Transient expression of FabA-dsscFv in HEK293 cell line

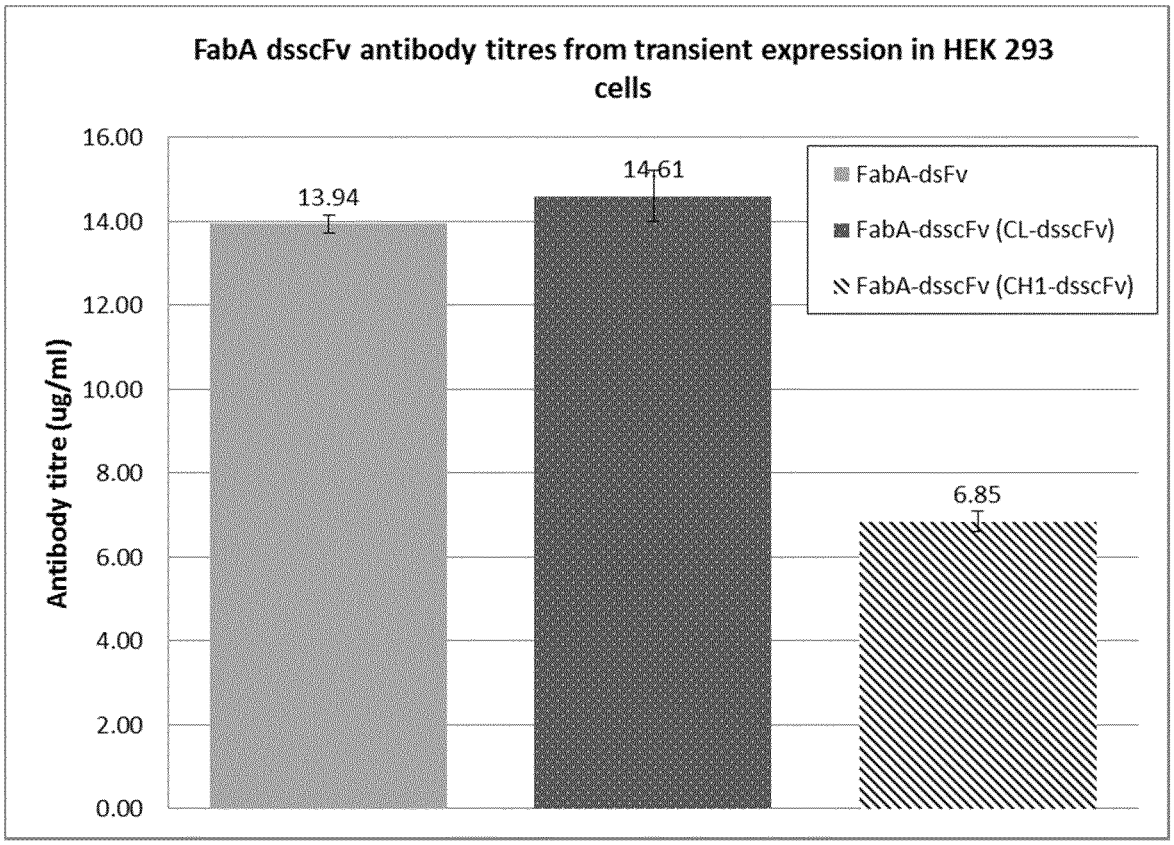
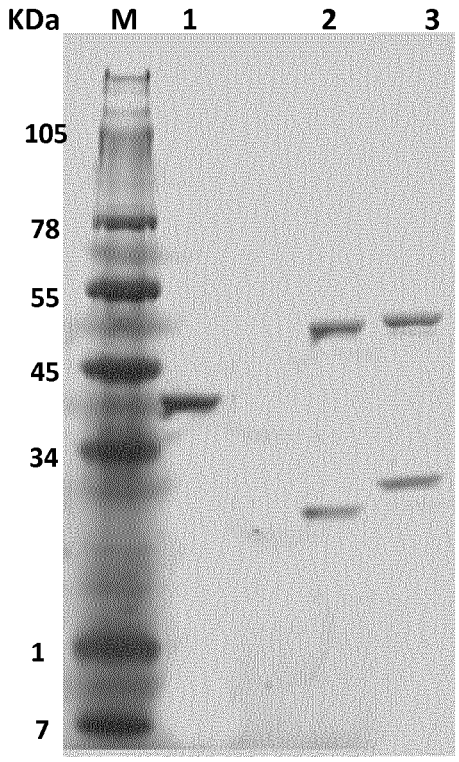


FIGURE 8

SDS-PAGE of purified FabA-dsscFv (HEK293)



M Standard Marker
1 FabA-dsFv
FabA-dsscFv
2 FabA-dsscFv (CL-dsscFv)
3 FabA-dsscFv (CH1-dsscFv)

Predicted Mr	kDa
FabA-dsFv	
CL-vL	36.3
CH1-vH	37.3
FabA-dsscFv (CL-dsscFv)	
CL-dssFv	50.5
HC	23.1
FabA-dsscFv (CH1-dsscFv)	
CHI-dsscFv	50.2
LC	25.6

FIGURE 9

Affinity to HSA and AntigenA binding (HEK293)

	Analyte		Concentrations used	ka (1/Ms)	kd (1/s)	KD (M)	KD (nM)
FabA-dsscFv (CL-dsscFv)	HSA	Titration	50nM, 25nM, 12.5nM, 6.25nM	1.83E+05	2.40E-04	1.31E-09	1.31
		Single concentration	50nM	1.65E+05	2.11E-04	1.28E-09	1.28
FabA-dsscFv (CH1-dsscFv)	HSA	Titration	50nM, 25nM, 12.5nM, 6.25nM	1.72E+05	2.22E-04	1.29E-09	1.29
		Single concentration	50nM	1.60E+05	1.99E-04	1.25E-09	1.25
FabA-dsFv	HSA	Titration	50nM, 25nM, 12.5nM, 6.25nM	7.51E+04	1.51E-04	2.01E-09	2.01
		Single concentration	50nM	6.06E+04	1.19E-04	1.96E-09	1.96

FIGURE 10

	Analyte		Conc.	ka (1/Ms)	kd (1/s)	KD (M)	KD (pM)
FabA-dsscFv (CH1-dsscFv)	AntigenA	Single conc.	25nM	1.66E+05	2.34E-05	1.41E-10	141
FabA-dsscFv (CL-dsscFv)	AntigenA	Single conc.	25nM	1.78E+05	1.82E-05	1.02E-10	102
FabA-dsFv	AntigenA	Single conc.	25nM	1.70E+05	1.53E-05	9.00E-11	90

Figure 11

FabA-dsscFv (CHO)

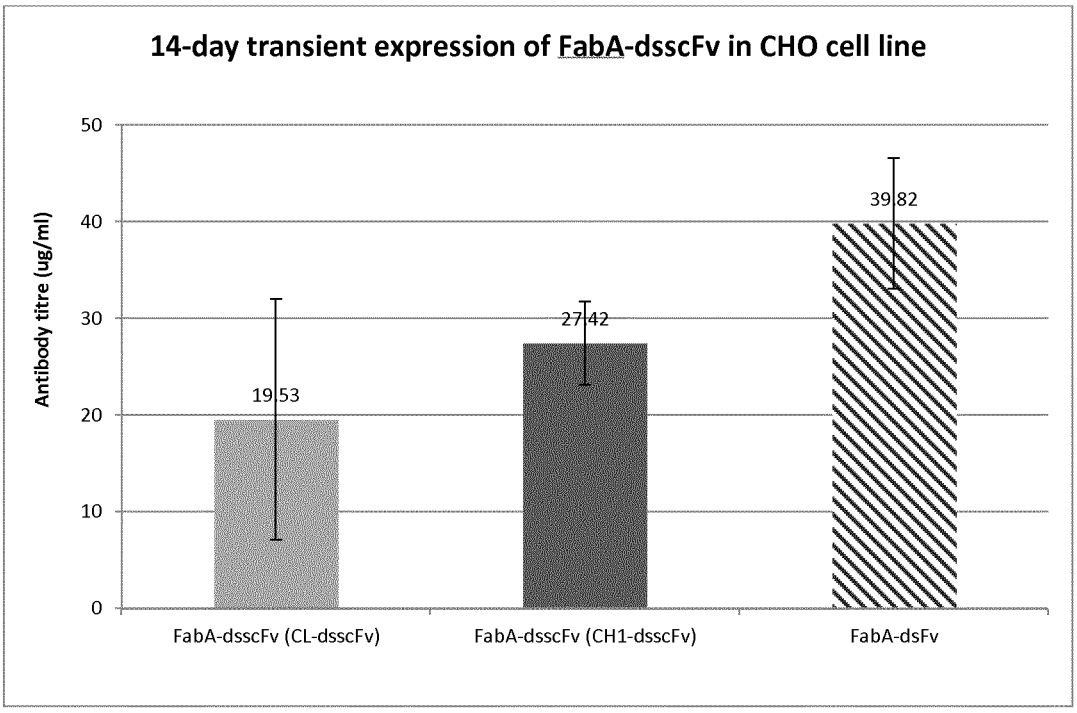
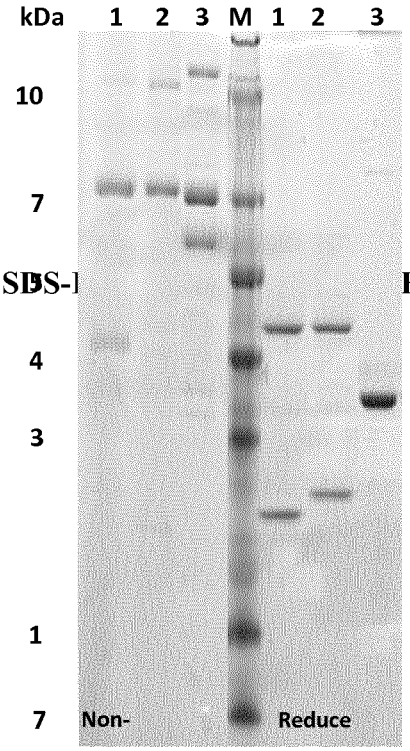


FIGURE 12



SDS-PAGE (CHO)

1 FabA-dsscFv (CL-dsscFv)
2 FabA-dsscFv (CH1-dsscFv)
3 FabA-dsFv

Predicted Mr	kDa
FabA-dsscFv (CL-dsscFv)	
CL-dssFv	50.5
HC	23.1
FabA-dsscFv (CH1-dsscFv)	
CHI-dsscFv	50.2
LC	25.6
FabA-dsFv	
CL-vL	36.3
CH1-vH	37.3

FIGURE 13

Thermostability FabA-dsscFv (CHO)

	Tm 1	Tm 1 std dev	Tm 2	Tm 2 std dev	Tm 3	Tm 3 std dev
FabA-dsscFv (CL-dsscFv)	83.8	0.2	73.6	0.5	52.4	0.7
FabA-dsscFv (CH1-dsscFv)	84.6	0.3	75.5	0.5	57.8	0.6
FabA-dsFv	84	0.2	73.9	0.3	ND	ND