

Case study XI

CASE STUDY_AFFINITY MATURATION OF HUMAN MONOCLONAL ANTIBODY

- Aim: To affinity mature a human monoclonal antibody MAB by NNK saturation randomization of CDR regions of heavy and light antibody variable domains

- Affinity improvement from low nM to mid-low pM

Antigen	MAB		
	Ka (1/Ms)	Kd (1/s)	KD (M)
Human target A-His	4.8E+06	6.4E-03	1.3E-09
Human target A-Fc	3.7E+06	9.1E-04	2.4E-10
Cynomolgus Monkey target A-Fc	3.3E+06	8.4E-04	2.6E-10

SPR binding assay data of parental antibody.

Library design and construction:

- Identification of VH and VL CDR1,2,3 according to kabat numbering
- PCR assembly of synthetic genes using primers that introduce mutations (NNK) in CDR regions
- Cloning in phagemid (VH and VL sub-libraries):
 - 1- VH_CDR1 Fab affinity maturation library
 - 2- VH_CDR2 Fab affinity maturation library
 - 3- VH_CDR3 Fab affinity maturation library
 - 4- VL_CDR1 Fab affinity maturation library
 - 5- VL_CDR2 Fab affinity maturation library
 - 6- VL_CDR3 Fab affinity maturation library

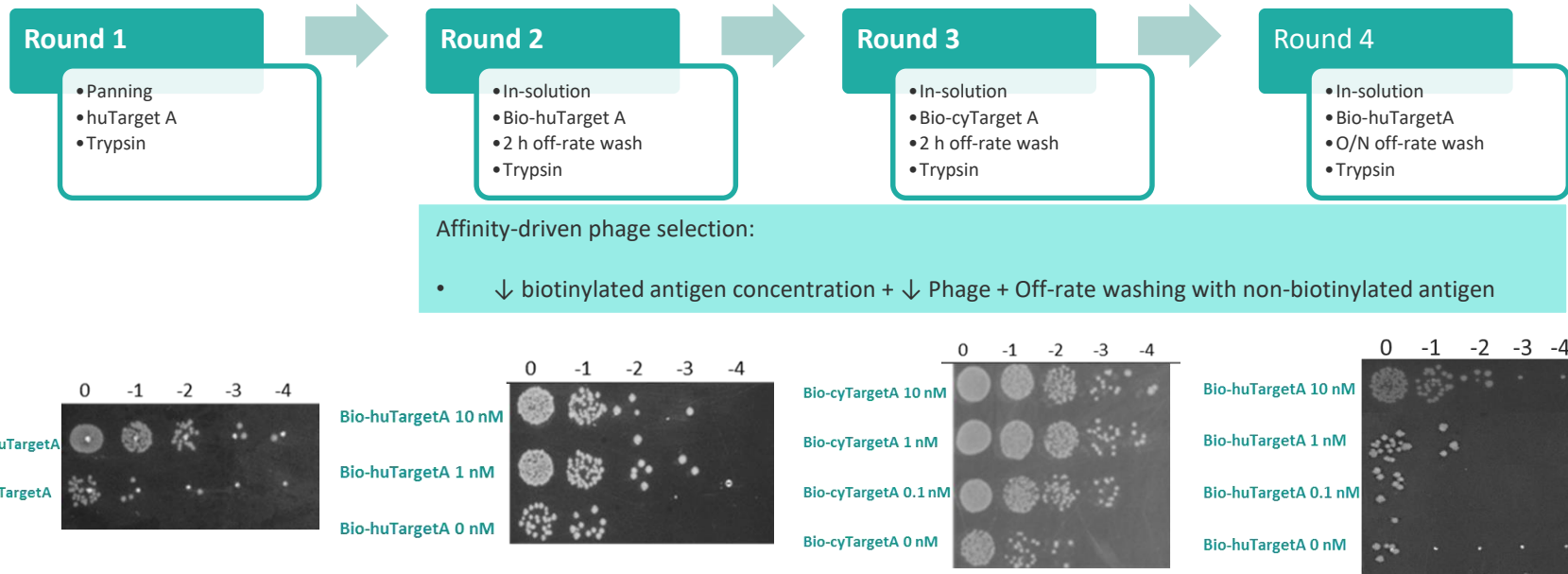
Library ID	Heavy Chain			Light Chain			Nr. of CDR residues varied	Theoretical diversity	Library size (CFU)	Fab insert %
	HCDR1	HCDR2	HCDR3	LCDR1	LCDR2	LCDR3				
VH_CDR1	Mut	WT	WT	WT	WT	WT	5	3.2E+06	1,2E+08	100
VH_CDR2	WT	Mut	WT	WT	WT	WT	4	1.6E+05	8,0E+07	100
VH_CDR3	WT	WT	Mut	WT	WT	WT	5	3.2E+06	5,0E+7	100
VL_CDR1	WT	WT	WT	Mut	WT	WT	5	3.2E+06	5,0E+7	100
VL_CDR2	WT	WT	WT	WT	Mut	WT	5	3.2E+06	5,0E+7	100
VL_CDR3	WT	WT	WT	WT	WT	Mut	5	3.2E+06	8,0E+7	100

Heavy and light chain affinity library construction design. Mut- mutated WT- wild-type.

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- Technology: Phage display libraries of Fab variants combined with Affinity-driven phage selections



Example of titration of *E. coli* infected with phage from Fab affinity maturation libraries selected after several selection rounds on Target A without or with off-rate wash.

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P.E. ELISA

Sequence diversity

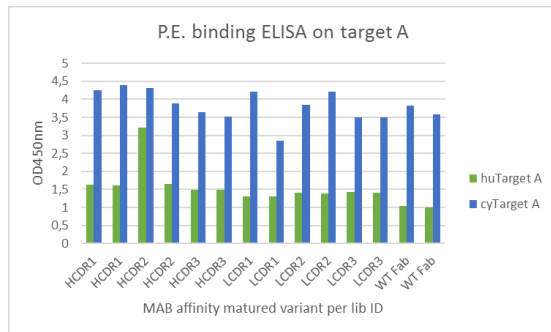
Off-rate analysis

ELISA binding data and off-rate of selected Fab affinity maturation variants. Data for WT Fab is shown for comparison.

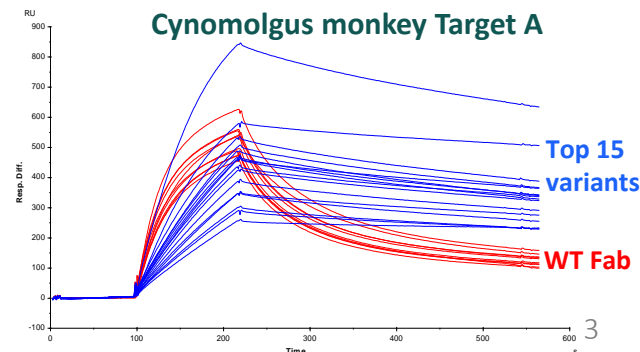
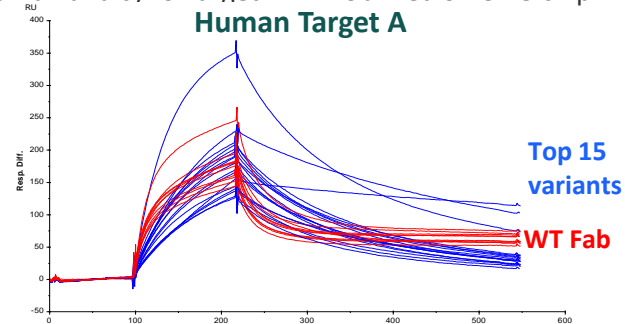
Fab library ID	Human target A P.E. ELISA Hit-rate*	Cyno target A P.E. ELISA Hit-rate*
VH_CDR1	94 % (n=124)	99 % (n=124)
VH_CDR2	92 % (n=122)	94 % (n=122)
VH_CDR3	54 % (n=122)	54 % (n=122)
VL_CDR1	88 % (n=124)	97 % (n=124)
VL_CDR2	84 % (n=122)	88 % (n=122)
VL_CDR3	76 % (n=122)	78 % (n=122)

Fab library ID	Nr. of different MAB variants
VH_CDR1	107 (n=111)
VH_CDR2	89 (n=92)
VH_CDR3	6 (n=56)
VL_CDR1	55 (n=107)
VL_CDR2	59 (n=95)
VL_CDR3	72 (n=95)

*OD450nm on target A > 5 fold ELISA background (PBS)



Human and cyno Target A immobilized on CM5 chip



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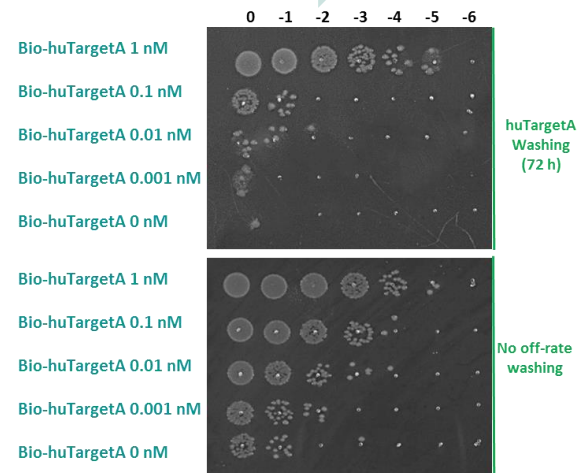
Identification of
beneficial mutations

Generation of combinatorial Fab affinity
maturation library

Affinity-driven phage display
selections

Library ID	Nr. selected variants
VH_CDR1	10
VH_CDR2	14
VH_CDR3	7
VL_CDR1	10
VL_CDR2	10
VL_CDR3	10

Library ID	Heavy Chain			Light Chain			Library size (CFU)	Fab insert %
	CDR1	CDR2	CDR3	CDR1	CDR2	CDR3		
VH_CDR1+2+3	Mut	Mut	Mut	WT	WT	WT	1,0E+08	96
VL_CDR1+2+3	WT	WT	WT	Mut	Mut	Mut	2,9E+07	100
VH_VL_CDR1+2+3	Mut	Mut	Mut	Mut	Mut	Mut	8,8E+07	96



Example of titration of *E. coli* infected with phage from combinatorial Fab affinity maturation library selected after five selection rounds on biotinylated Target A with or without 72 h off-rate wash.

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P.E. ELISA

Sequence diversity

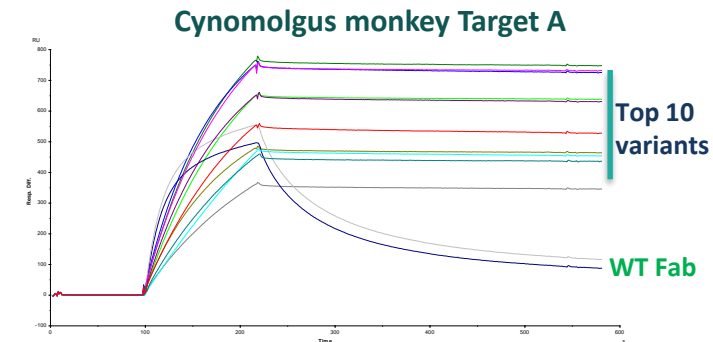
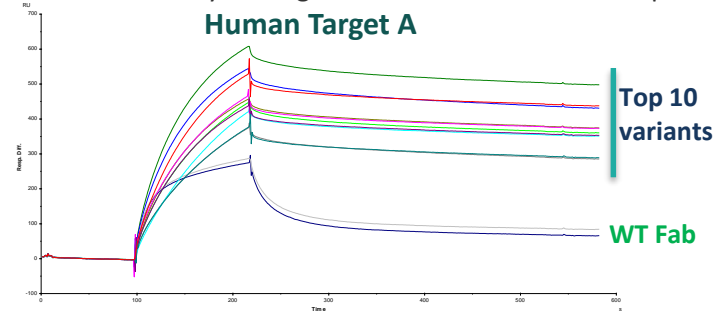
Off-rate analysis

Fab library ID	Human target A P.E. ELISA Hit-rate*	Cyno target A P.E. ELISA Hit-rate*
VH_VL_CDR1+2+3	88 % (n=184)	90 % (n=184)

*OD450nm on target A > 3 fold ELISA background (PBS)

122 unique Fab
variants identified
(n=157)

Human and cyno Target A immobilized on CM5 chip



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- Combination of CDRs randomization for Fab affinity maturation library generation with monovalent phage display affinity-based selection allowed the isolation of a very diverse panel of Fabs variants with improved off-rates compared to parental MAB

	Human Target A			Cynomolgus monkey Target A		
Clone ID	kd (1/s)	SE (kd; s-1)	R0	kd (1/s)	SE (kd; s-1)	R0
MAB WT Fab	8,20E-04	4,14E-05	160	2,91E-03	3,35E-05	339
Variant 1	3,53E-04	2,87E-06	386	5,19E-05	1,67E-06	640
Variant 2	3,56E-04	2,72E-06	481	1,00E-04	2,32E-06	542
Variant 3	3,63E-04	2,67E-06	382	7,85E-05	2,13E-06	463
Variant 4	3,89E-04	3,21E-06	412	3,92E-05	1,70E-06	738
Variant 5	4,02E-04	3,08E-06	415	6,01E-05	2,58E-06	471
Variant 6	4,35E-04	3,49E-06	400	3,98E-05	2,05E-06	644
Variant 7	9,12E-04	7,31E-06	162	4,65E-05	4,06E-06	260
Variant 8	5,79E-04	3,57E-06	303	5,09E-05	2,16E-06	466
Variant 9	4,55E-04	4,93E-06	561	5,32E-05	3,42E-06	758
Variant 10	4,79E-04	3,26E-06	326	5,43E-05	2,27E-06	442

Off-rate values of selected Fab affinity maturation variants. Data for WT Fab is shown for comparison.

- Conversion to human IgG and affinity assessment of MAB variants