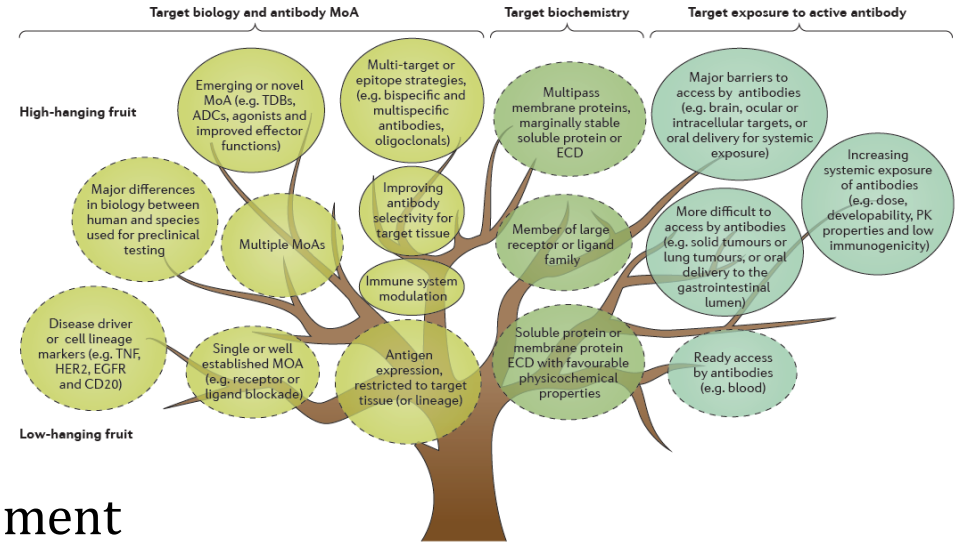
Human Antibody Identification Services: Exiris Library

Monoclonal Antibodies: the next challenges



- Mature field
- > 70 mAbs approved
- > 600 mAbs in development

Nat Rev Drug Discov. 2018 Mar;17(3):197-223.

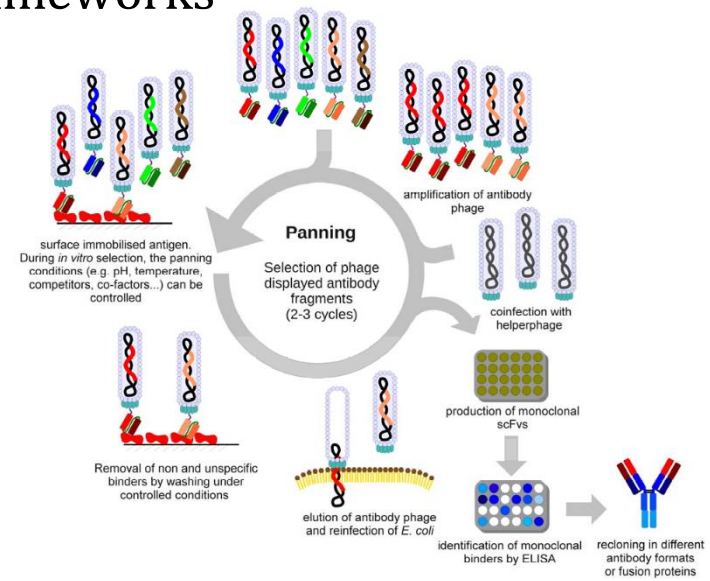
- Challenges:
  - Novel MOAs: agonist mAbs, mAbs inhibiting enzyme functions
  - Multispecific mAbs

Exiris

A high complexity, synthetic, phage-displayed antibody library

Features:

- High complexity: 1.34×10^{10}
- scFV format, fully human
- Highly validated VH3/VK1 frameworks



Advantages:

- Maximize probability of identifying high affinity binders
- Maximize identification of mAbs with good developability
- Facile identification of low abundance clones with novel biologic properties
- Straightforward development of bispecific binders

Patent filed EP3293293

Complexity of 1.34×10^{10}

Complexity	Probability to identify a binder with Kd <1 nM
10^7	1 %
10^8	9.6 %
10^9	63.2 %
10^{10}	99.9%

The probability **P** of identifying a ligand within a library that has an affinity lower than the threshold value **p** depends on the number of different ligands **N**, present in the library according to the formula $\mathbf{P = 1 - e^{-Np}}$

Complexity> 10^9 is needed to have high probability of identifying high affinity binders

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Library design principles

- **Library diversity only in HC-CDR3**
  - **Major determinant of antibody diversity**
- **Focus on HC-CDR3 loop lengths frequently present in therapeutic mAbs**
  - **Increase probability of success during development**
- **Reduce combinatorial bias (oversampling of short HC-CDR3, undersampling of long HC-CDR3)**
  - **Adjust percent fraction of loop lengths and reduce aa diversity going from shorter to longer HCDR3**

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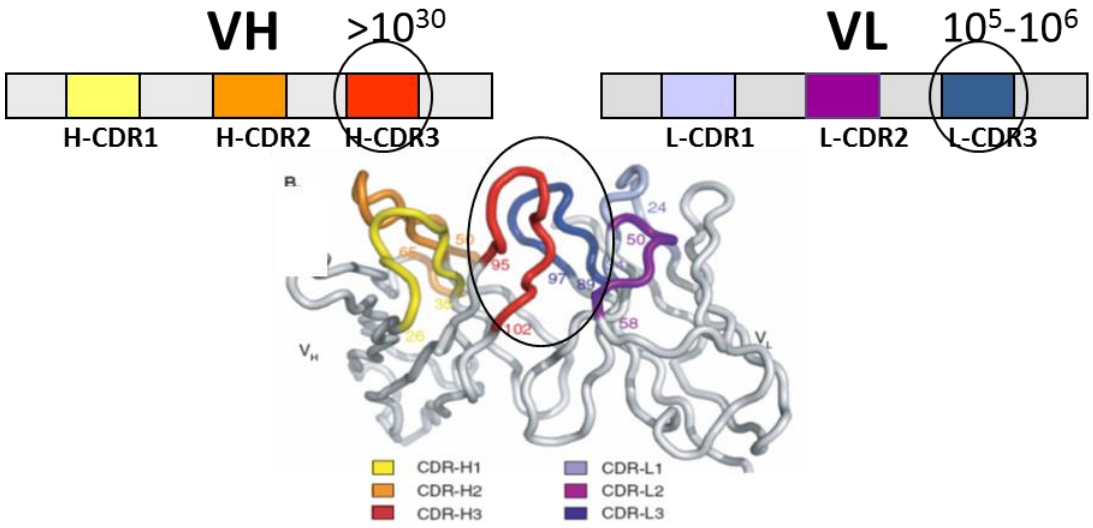
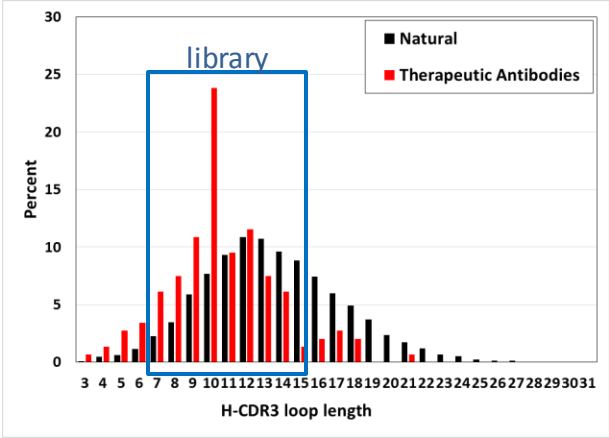
Diversity only in HC-CDR3



HC-CDR3 is the major contributor to antibody diversity

Specific loop length distribution

HC-CDR3 loop length distribution
Natural (black), Therapeutic mAbs (red)



Germline HC-CDR3 length distribution is broad. Therapeutic antibodies show a pronounced maximum at HC-CDR3 loop length 7-14 possibly reflecting good physicochemical properties (solubility, no aggregation, etc) and good expression properties

Exiris library loop length distribution based on natural distribution but especially focused on the main portion of therapeutic mAbs loop lengths (7-15 aa)

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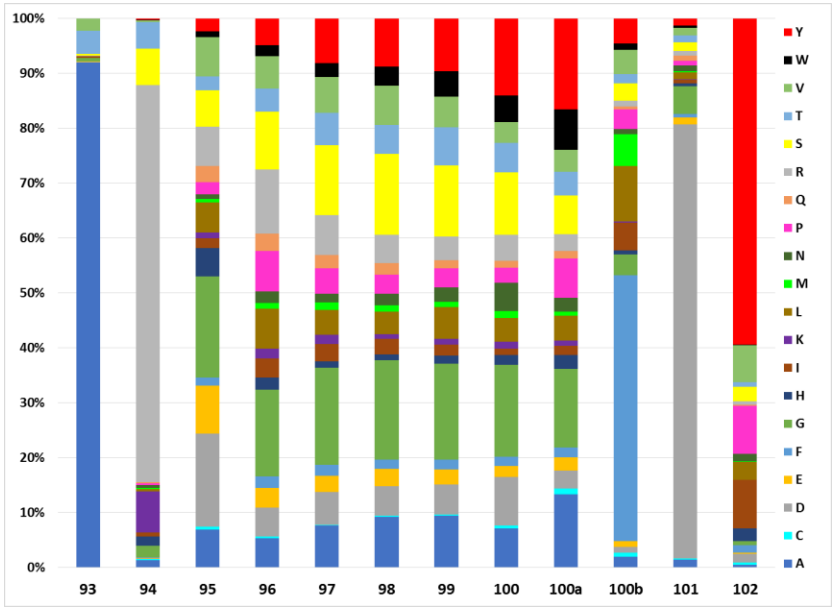
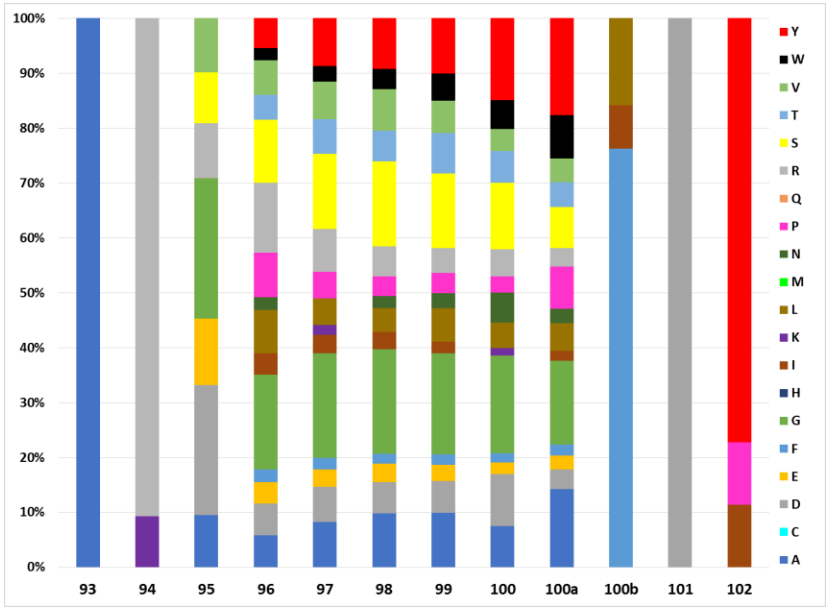
Non-random amino acid distribution



Natural HCDR3 loop length 10



Library HCDR3 loop length 10

In each HC-CDR3 loop position we incorporated residues preferentially found in nature, but rare variants were excluded

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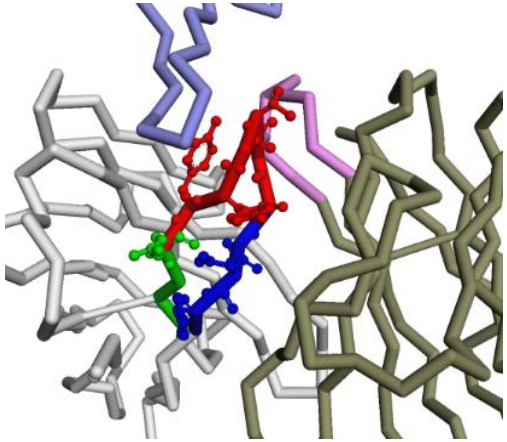
Non-random amino acid distribution



The initial (**dark blue**) and final (**green**) segments of the HC-CDR3 loop do not in general directly contribute to antigen recognition. Direct contacts with the antigen (**light blue**) are mostly mediated by the central segment of the HC-CDR3 (**red**).

In each HC-CDR3 loop position the initial and final parts were kept relatively constant; in longer loops less variability was introduced in order to cover a larger fraction of possible theoretical variants

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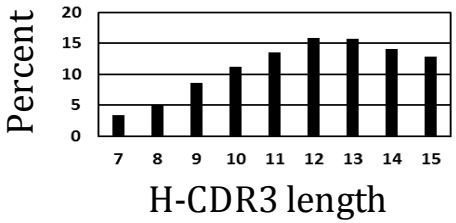
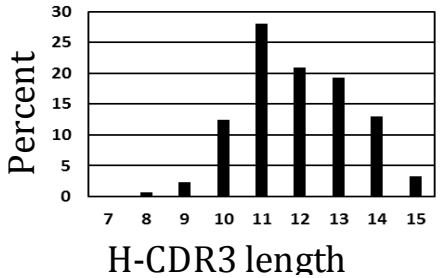
Non-random amino acid distribution



Adjust fractions of H-CDR3 loop length



CDR3H length	% fraction	Pos 94	Pos 95	hypervariable positions	Pos 101-1	Pos 101	Pos 102	# of possible unique variants	# of total variants present	# unique variants present	Fraction of possible unique variants covered	Average multiplicity of unique variants
7	3.3	1	5	16	8	1	4	6.55E+05	4.5E+08	6.55E+05	1.00	679.5
8	5.1	1	5	16	8	1	4	1.05E+07	6.8E+08	1.05E+07	1.00	64.9
9	8.6	1	5	16	8	1	4	1.68E+08	1.2E+09	1.68E+08	1.00	6.9
10	11.2	1	5	16	8	1	4	2.68E+09	1.5E+09	1.15E+09	0.43	1.3
11	13.6	1	5	16	8	1	4	4.29E+10	1.8E+09	1.78E+09	0.04	1.0
12	15.8	1	5	16	8	1	4	6.87E+11	2.1E+09	2.12E+09	3.1E-03	1.0
13	15.6	1	5	16	8	1	4	1.10E+13	2.1E+09	2.09E+09	1.9E-04	1.0
14	14.0	1	5	16	8	1	4	1.76E+14	1.9E+09	1.87E+09	1.1E-05	1.0
15	12.8	1	5	16	8	1	4	2.81E+15	1.7E+09	1.72E+09	6.1E-07	1.0

Adjust level of of aa diversity

CDR3H length	% fraction	Pos 94	Pos 95	hypervariable positions	Pos 101-1	Pos 101	Pos 102	# of possible unique variants	# unique variants present	Fraction of possible unique variants covered	Average multiplicity of unique variants
7	0.1	3	10	16	16	1	4	7.86E+06	6.68E+06	0.8	2.2
8	1.0	3	9	16	16	1	4	1.13E+08	7.86E+07	0.7	1.7
9	2.5	3	8	15	4	1	4	2.92E+08	1.99E+08	0.7	1.7
10	11.5	2	7	15	3	1	3	1.44E+09	9.47E+08	0.7	1.6
11	28.3	2	6	14	3	1	2	7.59E+09	2.98E+09	0.4	1.3
12	19.8	1	5	13	2	1	1	8.16E+09	2.26E+09	0.3	1.2
13	17.7	1	4	11	1	1	1	9.43E+09	2.10E+09	0.2	1.1
14	13.1	1	3	9	1	1	1	1.05E+10	1.62E+09	0.2	1.1
15	6.0	1	2	8	1	1	1	1.72E+10	7.85E+08	0.05	1.0

Both percentage of each loop length and aa composition in each loop position were adjusted in order to minimize redundancy of shorter loops and maximize coverage of longer loops

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Final design

- **H-CDR3, complexity 1.34 x 10^{10}**
- **Exclude Cys, His, Met, Gln**
- **Focus on loop distribution found in therapeutic mAbs (Loop lengths: 7-15)**
- **Naturally found amino acid frequency, exclude rare variants**
- **Modulate aa diversity for each loop to maximize coverage**

H-CDR3 length	fraction of total loops in library (%)	number of hyper-variable positions	number of different amino acids at hyper-variable positions	Pos 93	Pos 94	Pos 95	Pos 96	Pos 97	Pos 98	Pos 99	Pos 100	Pos 100a	Pos 100b	Pos 100c	Pos 100d	Pos 100e	Pos 100f	Pos 100g	Pos 101	Pos 102	Number of theoretically possible variants	Actual number of clones present	Fraction of theoretically possible variants actually present (Poisson estimate)	Actual number of variants present (Poisson estimate)	Redundancy (number of times each variant is present on average)
7	0.1	3	16	1	3	10	16	16	16	16									1	4	7.86E+06	1.49E+07	0.85	6.68E+06	2.2
8	1.0	4	16	1	3	9	16	16	16	16	16								1	4	1.13E+08	1.34E+08	0.69	7.86E+07	1.7
9	2.5	5	15	1	3	8	15	15	15	15	15	4							1	4	2.92E+08	3.35E+08	0.68	1.99E+08	1.7
10	11.5	6	15	1	2	7	15	15	15	15	15	15	3						1	3	1.44E+09	1.55E+09	0.66	9.47E+08	1.6
11	28.3	7	14	1	2	6	14	14	14	14	14	14	14	3					1	2	7.59E+09	3.79E+09	0.39	2.98E+09	1.3
12	19.8	8	13	1	1	5	13	13	13	13	13	13	13	13	2				1	1	8.16E+09	2.65E+09	0.28	2.26E+09	1.2
13	17.7	9	11	1	1	4	11	11	11	11	11	11	11	11	11	1			1	1	9.43E+09	2.37E+09	0.22	2.10E+09	1.1
14	13.1	10	9	1	1	3	9	9	9	9	9	9	9	9	9	9	1		1	1	1.05E+10	1.76E+09	0.15	1.62E+09	1.1
15	6.0	11	8	1	1	2	8	8	8	8	8	8	8	8	8	8	8	1	1	1	1.72E+10	8.04E+08	0.05	7.85E+08	1.0

Outcome: no or very low redundancy, excellent representation of intermediate and long loops

Greatly improved diversity

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Library validation: (1) Quality control

NGS of 2773807 sequences:

- ✓ Correct amino acid composition
- ✓ Relative frequency of amino acids as designed
- ✓ 94% of clones with inserts
- ✓ 6-7% of clones with insertions or deletions
- ✓ 88% correct sequences
- ✓ very few duplications, in line with design

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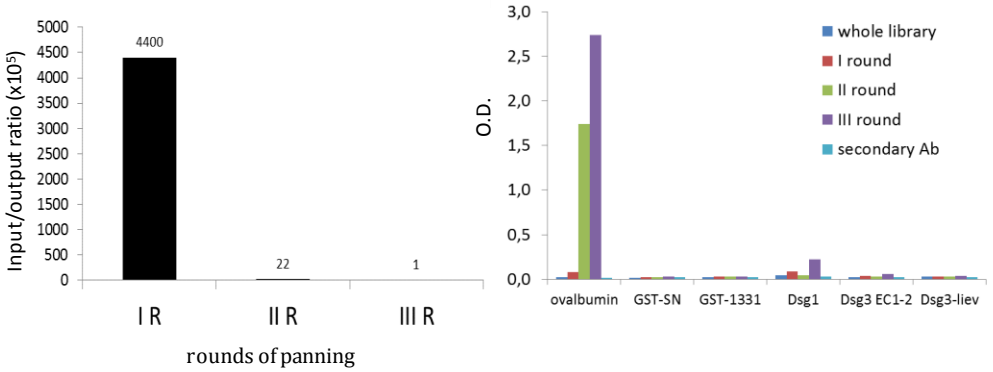
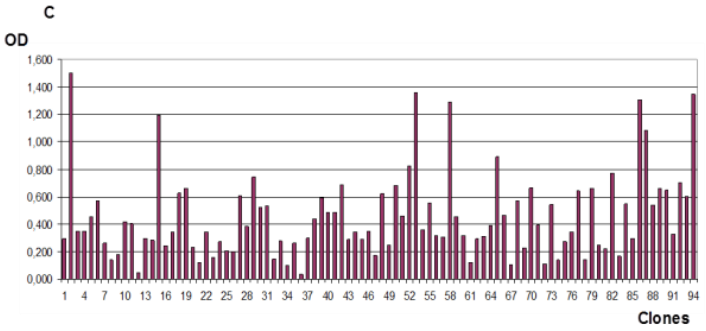
Library validation: (2) Panning against test antigen (ovalbumin)

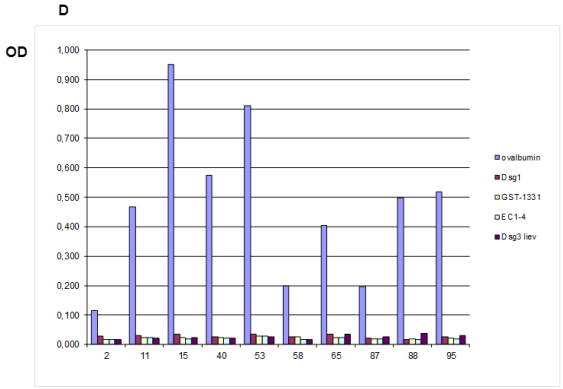
1. panning



2. Individual clones



3. Selected clones



4. Sequencing

CLONE	V_H (DP47)
2	...CAKV..VTGVLWGF..DYW...
11	...CAKD..YAGDYGYF..DYW...
58	...CAKD..YASGWGYF..DYW...
15	...CAKD..FGRGYGYF..DYW...
65	...CAKD..FRSGYGYF..DYW...
40	...CAQD..VRRGCGYF..DYW...
53	...CAKD..VARGYGYF..DYW...
95	...CAKD..VWRGYGYF..DYW...
88	...CAKD..VGRGVGYF..DYW...
87	...CAKV..VGGVLYAF..DYW...

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Library validation: (2) Panning against panel of test antigens – summary results

	Number of rounds	# analyzed clones	# positive clones	% anti-target positive clones
BSA	3	47	89	53%
OVA	3	71	94	76%
GST	3	20	90	22%
DSG1	3	81	94	86%
FGFR4	3	73	77	95%
target 1	3	49	49	100%
target 2	3	135	135	100%

	Specificity (vs other targets)	Sequenced clones	# of unique sequences
BSA	12/12	5	1
OVA	10/10	10	10
GST	15/15	10	8
DSG1	10/10	10	10
FGFR4	16/20	10	4
target 1	16/16	10	8
target 2	75/135	75	31

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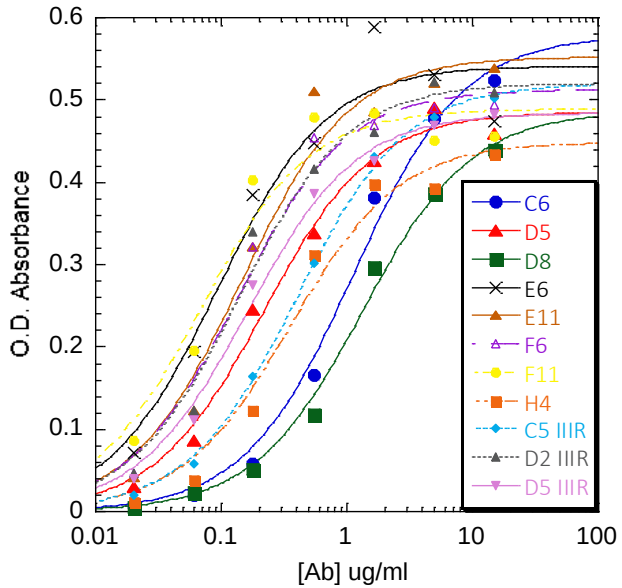
Project 1: selection of high affinity binders (IgG1) against recombinant cell surface receptor

Kd (nM) of identified IgGs (ELISA)



Antibody	Kd (nM)
C6	7,5
D5	1,4
D8	8,8
E6	0,6
E11	0,9
F6	0,9
F11	0,4
H4	2,3
C5 IIIR	2,6
D2 IIIR	0,9
D5 IIIR	1,1

80% productive and functional IgG
100% with Kd < 10 nM

Best affinity: **400 pM**
Worst affinity: **8.8 nM**

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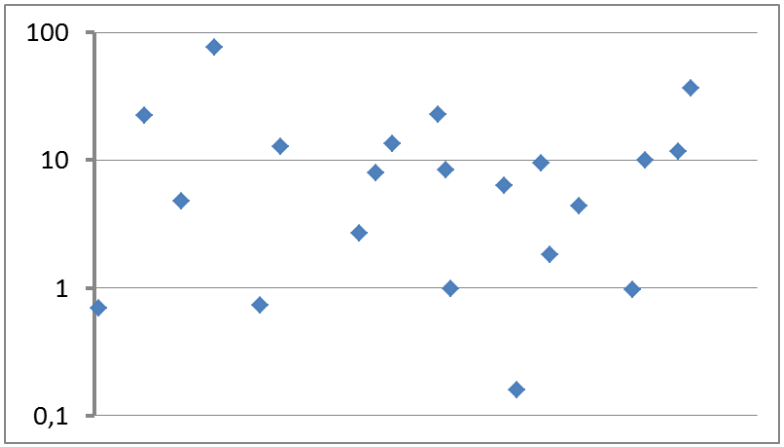
Project 2: selection of high affinity binders (IgG1) against "difficult" target

Kd (nM) of selected IgGs (ELISA)



Kd < 1 nM: 24%
Kd < 5 nM: 43%
Kd < 10 nM: 67%
Kd< 25 nM: 90%

Best affinity: 160 pM
Worst affinity: 76 nM

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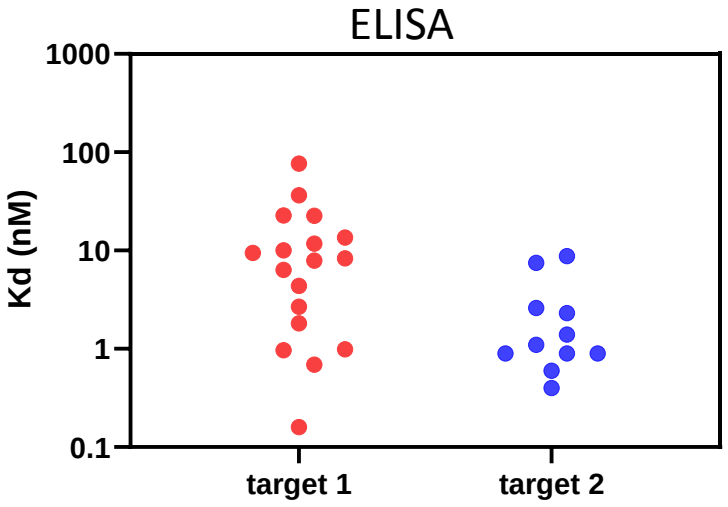
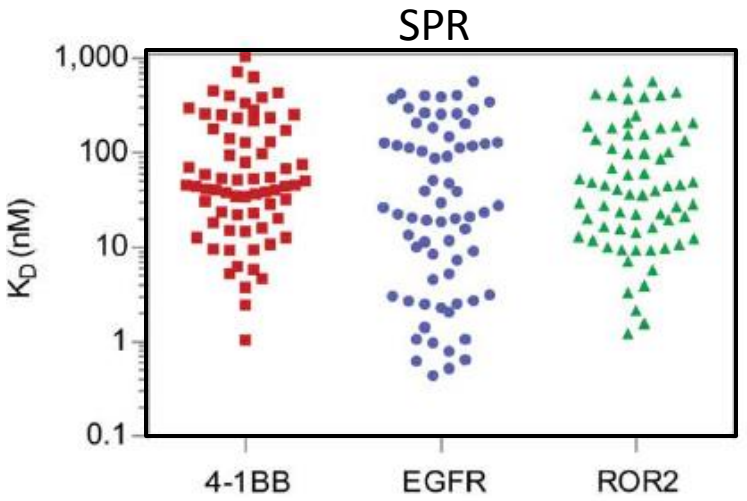
Comparison between Exiris library and competition



Exiris library



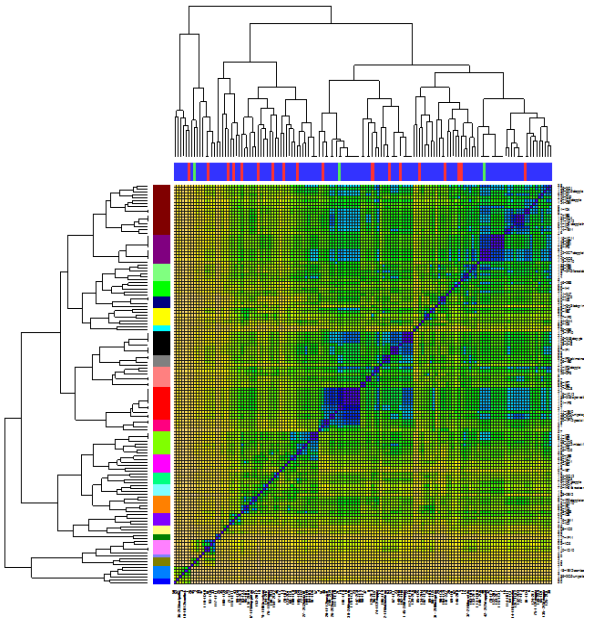
Pfizer library

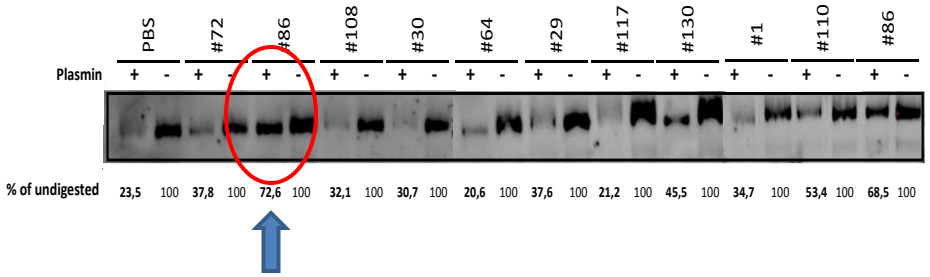
(Van Blarcom *et al.* MABS, 2018)

Superior performance of Exiris library

Project 3: selection of antibodies with specific biological function (inhibitors of proteolytic processing)

Extracellular target. Proteolysis releases cytokine involved in pathologic processes. So far only polyclonal Ab mixtures were reported to inhibit processing. Exiris library screen of 200 binders revealed 19 sequence families. Representative members were cloned, expressed and purified.





mAb 86 binds to target with Kd= 1 nM and strongly inhibits processing

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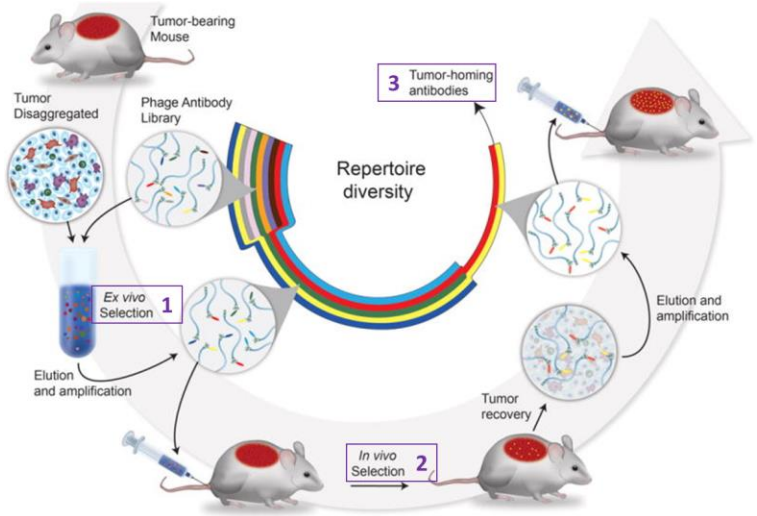
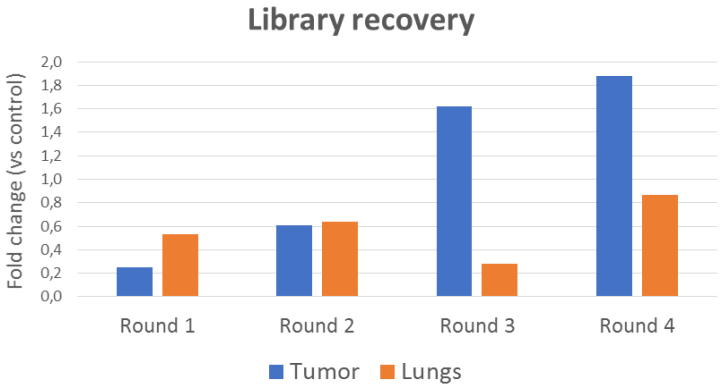
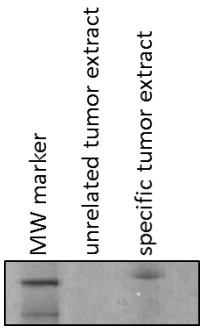
Project 4: In vivo selection



Methods Mol Biol. **2015**, 1324, 205-22
Trends Biotechnol. **2015**, 33, 292-301



The scFv-derived IgG was used for immunoprecipitation from tumor extracts and led to the identification of the target protein by mass spectrometry



Identification of specific tumor-homing phages

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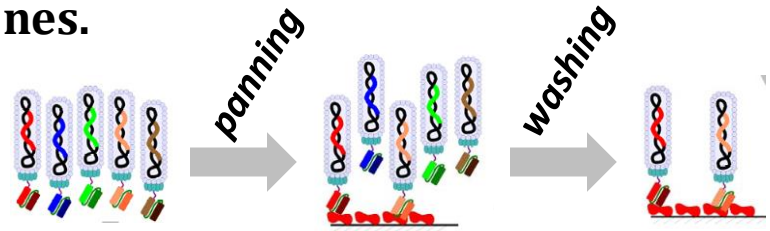
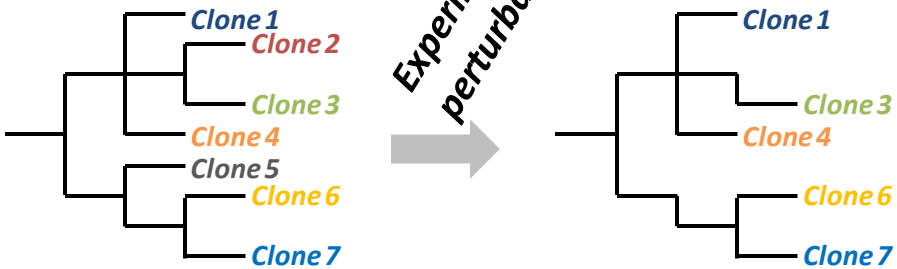
Advantages of the Exiris library: (1) straightforward ID of low abundance clones

During standard library selections high affinity binders are often missed, even by sampling > 5000 clones. Following selection by NGS of a high complexity "H-CDR3-only" –library maximizes the likelihood of identifying low abundance clones.



amplification and deep sequence coverage of HCDR3 by NGS ~ 10^6 sequences

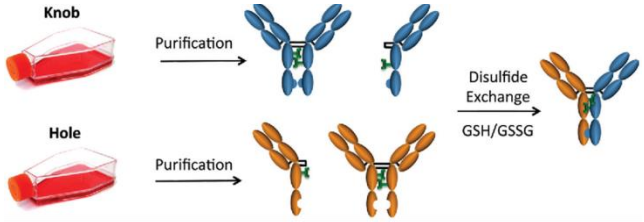
Philogenetic tree of selection

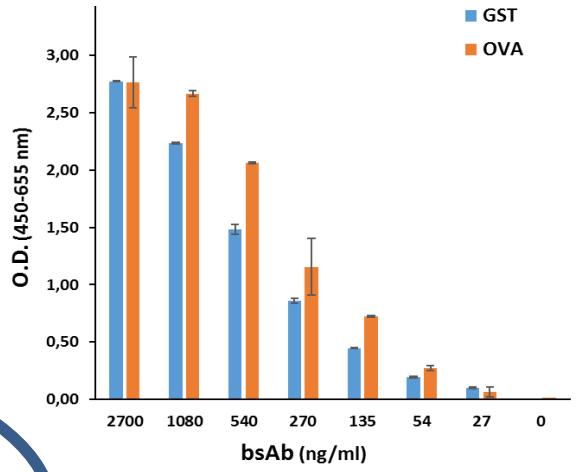


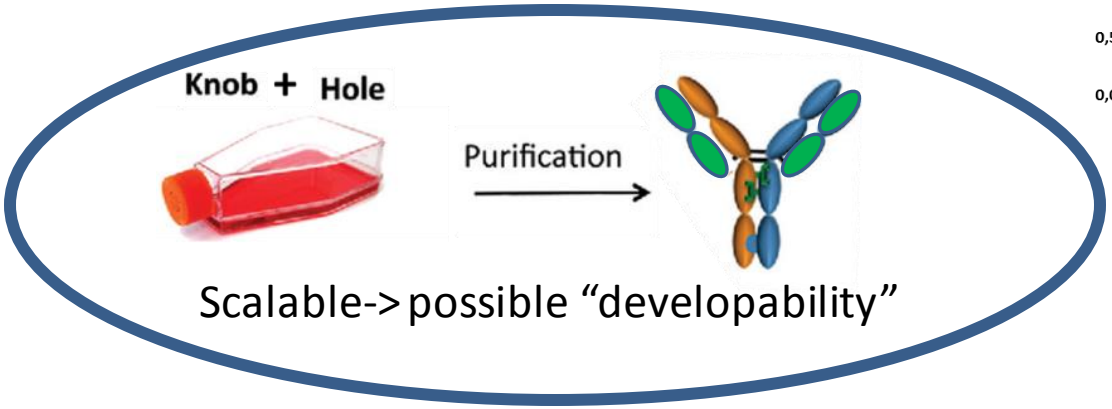
Advantages of the Exiris library: (2) straightforward access to bispecifics

An "H-CDR3-only" –library allows to rapidly generate bispecific mAbs using the "knobs into holes" technique and a single host cell production.

standard method, not scalable







Scalable-> possible "developability"

Successful proof of concept achieved

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