

CREATIVE SINGLE DOMAIN ANTIBODY DISCOVERY BIOLABS AND ENGINEERING

www.creative-biolabs.com/sdab





Over 10 Years Extensive Experience In Antibody Discovery & Development

CONTENT

EXCELSIOR FLEXIBLE OPEN

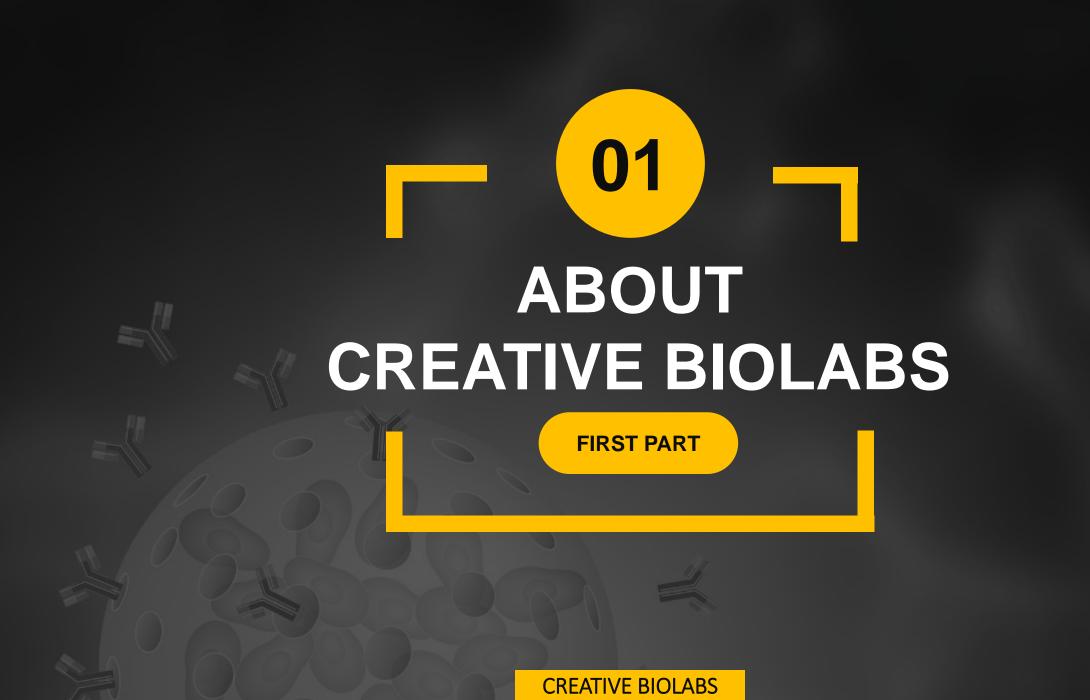
High-Quality Service Provider For Researchers All Over The World!

sdAb Discovery

- Immune sdAb Library
 - Construction and Screening
- Premade sdAb Library Screening
- Intrabody Discovery
- Anti-Membrane Protein sdAb Discovery
- Anti-Idiotype sdAb Discovery
- Anti-BBB sdAb Discovery
- Anti-Albumins sdAb Discovery
- Custom sdAb Production

sdAb Development

- De Novo sdAb
 - Sequencing
- □ sdAb Affinity Maturation
- □ sdAb Humanization
- Bispecific sdAb
 - Engineering
- □ sdAb Conjugation
- sdAb Stability
- Improvement
- Antibody Camelization



INTRODUCTION

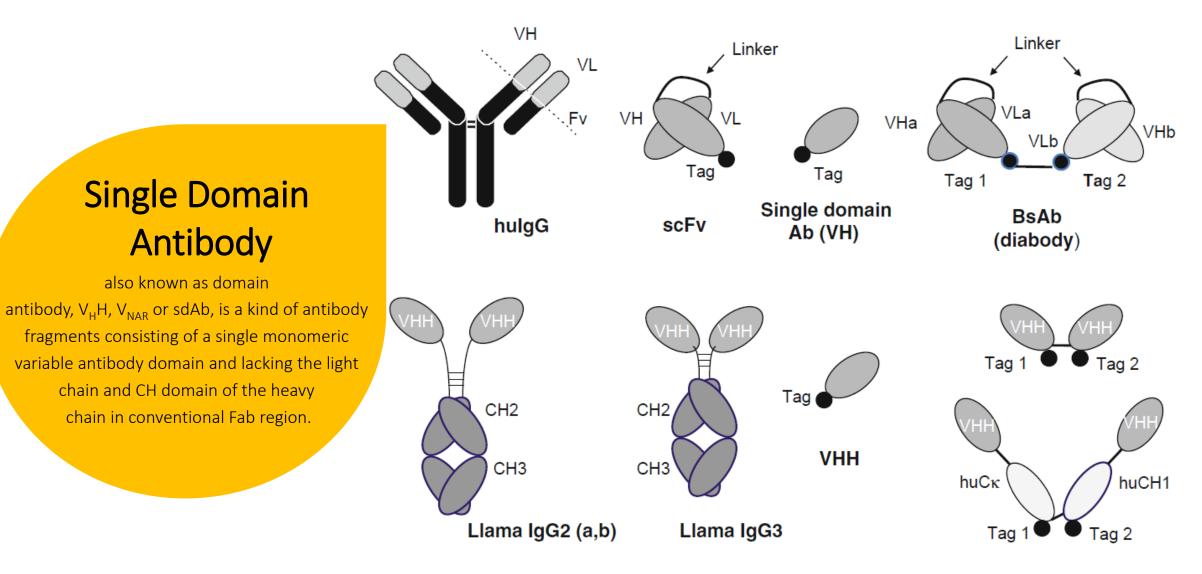


Experts in Custom Antibody Service

- ✓ Monoclonal Antibody Generation In All Species
- ✓ Affinity Maturation
- ✓ Bispecific Antibody Engineering
- ✓ Native[™] Antibody Discovery
- ✓ De novo Antibody Sequencing
- ✓ Antibody Humanization
- ✓ Antibody Murinization, Caninization & Camelization

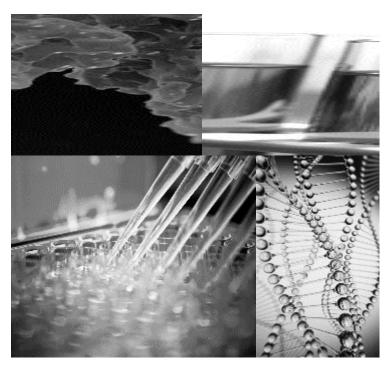
- ✓ Antibody Development Against Membrane Proteins
- ✓ Human Antibody Production Using Transgenic Mice
- ✓ Antibody-Drug Conjugate
- ✓ Chimeric Antigen Receptor (CAR) Services
- ✓ Immunogenicity Assessment
- ✓ Stable Cell Line Construction

INTRODUCTION



Llama BsAb

INTRODUCTION



ADVANTAGES OF sdAb

Smallest antibody fragment with only ~15 kDa

Recognize novel/hidden

antibodies cannot

epitopes that conventional

High stability to function and

and intracellular environment

01

03

- blood-brain barrier
- 05
- Short plasma half-life and better clearance as diagnostic tool
- exist within extreme conditions



Improved bioavailability for therapeutic applications

Outstanding penetrability

which is able to cross the

- Expressible in both 07 eukaryotic and prokaryotic systems
- Excellent chaperone for the 08 crystallization of challenging targets

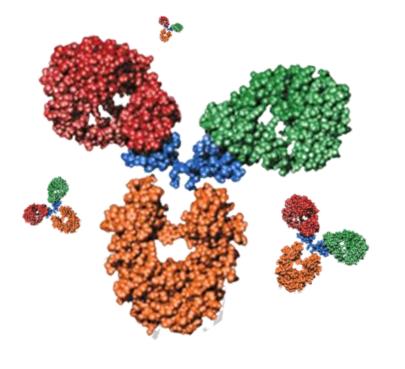


Great potential in downstream engineering (e.g. fusion protein and humanization)

02 SINGLE DOMAIN ANTIBODY DISCOVERY

SECOND PART

CREATIVE BIOLABS



1 Immune Phage Display Library Construction and Screening



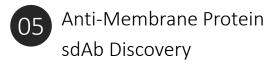
Premade sdAb Library Screening



Intrabody Discovery



Anti-Albumins sdAb Discovery





Anti-Idiotype sdAb Discovery

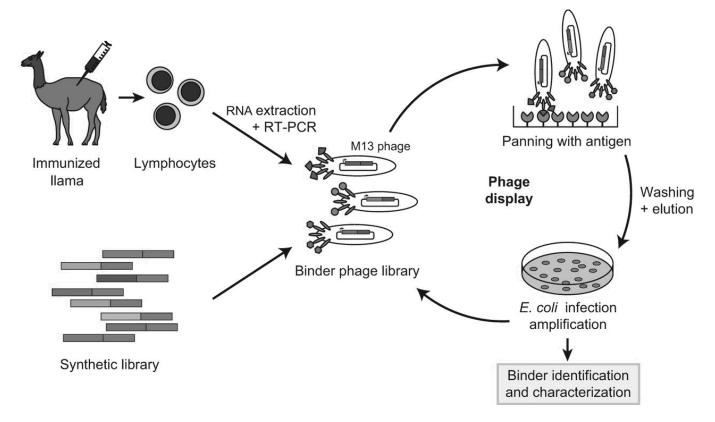


Anti-BBB sdAb Discovery



Custom sdAb Production

2.1 Immune sdAb Library Construction and Screening

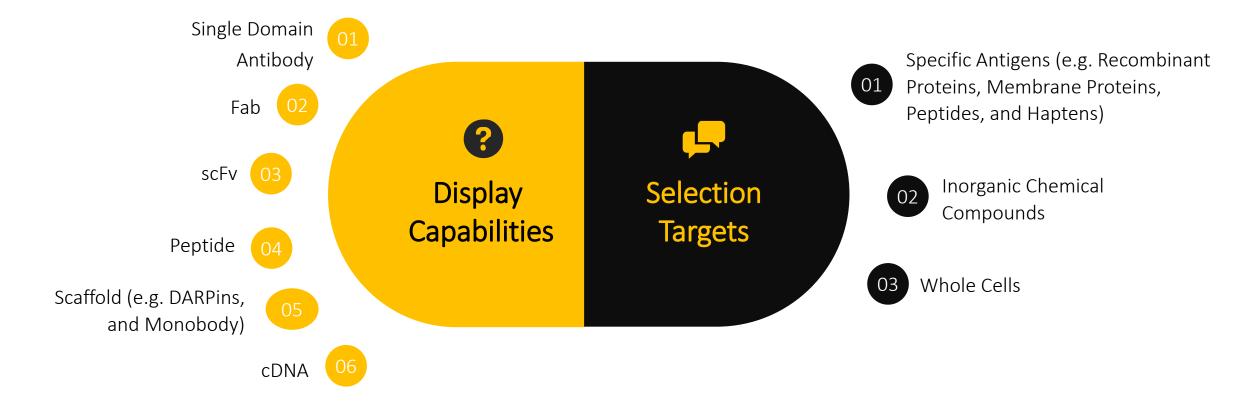


Phage display is a long-lasting laboratory platform for the large-scale study of molecules interaction, such as proteinprotein, protein-peptide and protein-DNA interactions and molecule selection.

Relying on engineering bacteriophages to display interested molecules on their surface, phage display can result in a linkage between genotype and phenotype.

10

2.1 Immune Phage Display Library Technology



2.1 Immune sdAb Library Construction and Screening

Raising sdAb Against Challenging Targets



We raised excellent phospho-specific single domain antibodies against two phosphorylation sites.

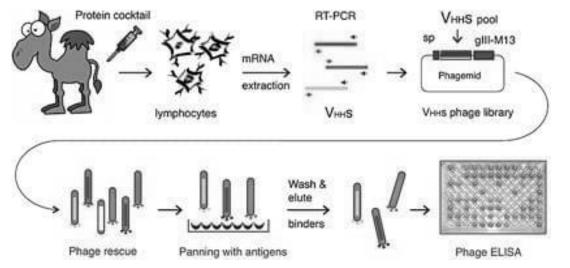
Prof. Stefan Schulz Jena University Hospital, Institute of Pharmakology and Toxikology Drackendorfer Str. 1, D-07747 Jena Email: stefan.schulz@mti.uni-jena.de



We raised high-affinity monoclonal single domain antibodies against nucleotides.

Dr. Erich Koller F. Hoffmann-La Roche Ltd. Pharmaceutical Sciences, pRED Building 69, Rm 155, CH-4070 Basel, Switzerland Tel: +41 61 687 21 41 Email: erich.koller@roche.com

2.1 Immune sdAb Library Construction and Screening



Standard Protocol

- Animal Immunization
- RNA Isolation
- ➢ RT-PCR
- Amplification of V_HH
 Expression Cassette
- Ligation and Transformation
- Prepare M13KO7 Helper Phage
- Prepare Antibody Library

Phages

- Antibody Library Biopanning
 - Solid-Phase
 - Solution-Sorting with Plate
 - Solution-Sorting with Beads
- Polyclonal Phage ELISA
- Monoclonal Phage ELISA
- DNA Sequencing
- Soluble sdAb ELISA
- > Bioinformatics Analysis
- Recombinant sdAb Production

2.1 Immune sdAb Library Construction and Screening



In terms of the advanced phage display technology, **Creative Biolabs** has unparalleled capabilities for the construction of $V_H H$ or V_{NAR} based single domain antibody libraries through immunized camel, llama, alpaca or shark.

Our scientists have vast experience constructing and screening immunized phage display sdAb libraries. We usually obtain immunized sdAb libraries with an overwhelming capacity of 10-100 million that can derivate antibodies with excellent affinity/specificity.

2.1 Immune sdAb Library Construction and Screening



We commonly cooperate with a USDA registered research facility that has NIH/OLAW assurances. This is an approved blood collection facility under EC 1069/2009 for export of animal blood products to EU countries. The NIH and USDA assurances as well as EC 1069/2009 assurance are available upon request.

2.1 Immune sdAb Library Construction and Screening

Standard Immunization Procedure (3-week interval)

We are open to perform custom immunization procedure to meet your specific requirement

High-quality RNA will be extracted at the same day after the production bleed to ensure the best starting material for library construction.

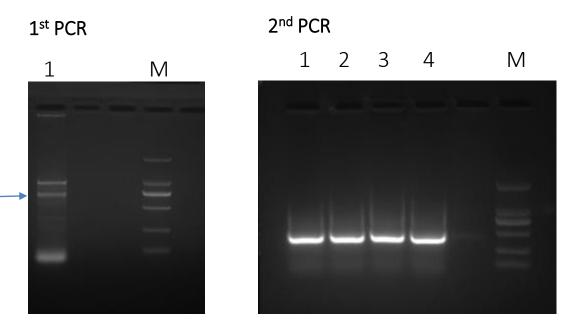
Day	Procedure
0	Primer Injection & Pre-bleed. 20 mL serum per animal. Inject antigen mixed 1:1 with CFA.
21	2 nd Injection. Inject antigen mixed 1:1 with IFA.
42	3 rd Injection. Inject antigen mixed 1:1 with IFA.
49	1 st Test bleed.
63	4 th Injection. Inject antigen mixed 1:1 with IFA.
70	2 nd Test bleed.
84	1 st Boost (Option). Inject antigen mixed 1:1 with IFA.
91	3 rd Test bleed.
105	2 nd Boost (Option). Inject antigen mixed 1:1 with IFA.
112	Production bleed/whole blood with heparin.

2.1 Immune sdAb Library Construction and Screening

Phage Display Antibody Library Construction

Degenerate primers have been used to amplify the $V_H H$ fragments and generated $V_H H$ libraries for the corresponding species (llama, alpaca or camel).

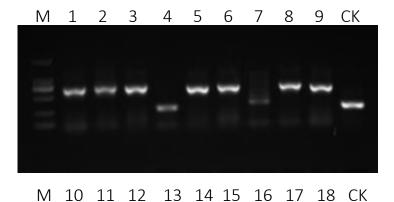
Creative Biolabs has designed and validated degenerate primers for amplifying V_H H-Fc gene and V_H H gene. We have used these primers to generate V_H H libraries that can be expressed on the surface of bacteriophage with our pCDisplay vector.

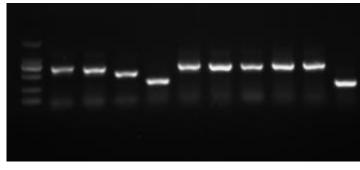


2.1 Immune sdAb Library Construction and Screening

Library QC (Sample Report)

- According to the example, 18 random clones from the end library was subjected to QC colony PCR. 14 out of 18 clones carry sense V_HH genes.
- Random clones from the end library was subjected to DNA sequencing.





Lane1-18: PCR products for random clones from the end library.

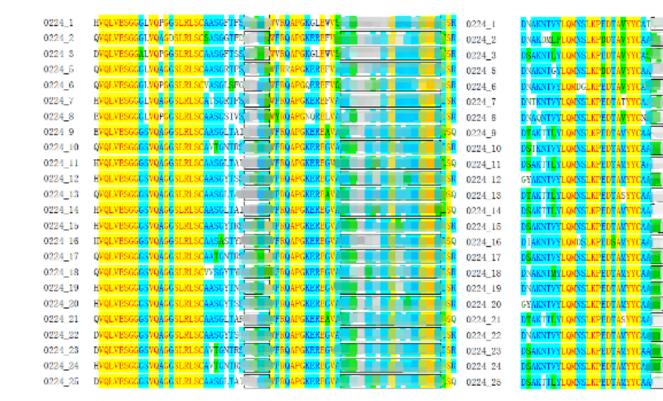
Lane CK: PCR products with the empty phagemid as the template, negative control.

Lane M: DL2000 DNA Marker (2000,1000, 750, 500, 250, 100 bp)

2.1 Immune sdAb Library Construction and Screening

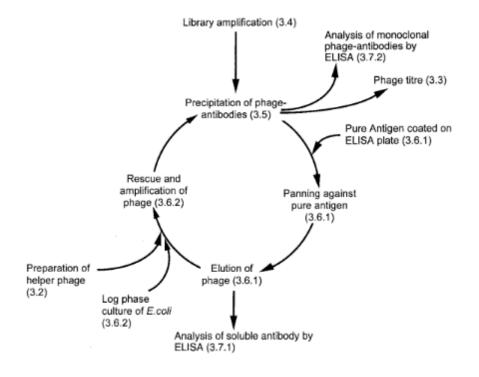
Library QC (Sample Report)

- The V_H H sequences were aligned together.
- All sequences are unique, indicating good diversity of the end library.

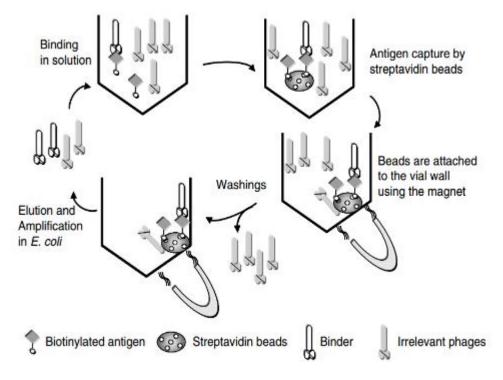


2.1 Immune sdAb Library Construction and Screening

Library Screening Strategies



Option 1. Solid-Phase Screening



Option 2. Solution-Sorting Screening (plate or beads)

2.1 Immune sdAb Library Construction and Screening

Example of Screening Report

Round	Conditions	Input	Output	Enriching factor
1 st	Target protein: <mark>50</mark> μg/mL AG3 Washing: <mark>0.1</mark> % Tween20 PBST, 9 times Elution: <mark>Trypsin digestion</mark> Pre counter select: 2% M-PBS	2.40×10 ¹¹	2.72×10 ⁴	8.81×10 ⁶
	Target protein: <mark>30</mark> µg/mL AG3 Washing: <mark>0.1</mark> % Tween20 PBST, 9 times Elution: <mark>Trypsin digestion</mark> Pre counter select: 2% M-PBS	2.80×10 ¹¹	1.44×10 ⁵	<mark>1.94×10⁶</mark>
2 nd -N	Target protein: <mark>no coating</mark> Washing: <mark>0.1</mark> % Tween20 PBST, 9 times Elution: <mark>Trypsin digestion</mark> Pre counter select: 2% M-PBS	3.50×10 ¹⁰	1.12×10 ³	3.13×10 ⁷
	Target protein: <mark>30</mark> μg/mL AG3 Washing: <mark>0.1</mark> % Tween20 PBST, 9 times Elution: <mark>Trypsin digestion</mark> Pre counter select: 2% M-PBS	3.32×10 ¹¹	8.96×10 ⁶	3.71×10 ⁴
	Target protein: <mark>no coating</mark> Washing: <mark>0.1</mark> % Tween20 PBST, 9 times Elution: <mark>Trypsin digestion</mark> Pre counter select: 2% M-PBS	1.84×10 ¹⁰	1.02×10 ³	1.81×10 ⁷

2.1 Immune sdAb Library Construction and Screening

Binder Selection Strategies



Phage amplification of binder phage clones obtained from enriched phage library. Verify the specific binders, including:

- Phage amplification
- Phage ELISA against the target
- Phage DNA extraction
- Phage DNA sequencing

2.1 Immune sdAb Library Construction and Screening

Example of Phage ELISA

Sample	OD ₄₉₀		
Campie	Coating: AG3	no coating	
1-A	0.241	0.072	
2-A	0.476	0.074	
M13K07	0.173	0.083	
1% M-PBS	0.137	0.072	
Positive control [serum]	1.573		

1-A: amplified phages of the 1st eluate 2-A: amplified phages of the 2nd eluate Primary antibody: rabbit anti-M13 pAb Secondary antibody: HRP-goat anti rabbit pAb

Clones	OD ₄₉₀		
Ciones –	Coating: AG3	No coating	
1	0.631	0.091	
2	0.691	0.092	
3	0.321	0.083	
4	0.635	0.081	
5	0.702	0.090	
6	0.716	0.098	
7	0.268	0.097	
8	0.519	0.090	
9	0.649	0.091	
10	0.697	0.093	
11	0.777	0.085	
12	0.632	0.090	
13	0.725	0.087	
14	0.726	0.108	
15	0.665	0.095	
16	0.671	0.093	
17	0.666	0.095	
18	0.682	0.095	
19	0.707	0.091	
20	0.751	0.091	

	0.704	0.007	
21	0.721	0.087	
22	0.718	0.093	
23	0.713	0.096	
24	0.690	0.083	
25	0.651	0.085	
26	0.690	0.079	
27	0.682	0.076	
28	0.611	0.079	
29	0.656	0.076	
30	0.710	0.079	
31	0.691	0.085	
32	0.622	0.079	
33	0.644	0.101	
34	0.682	0.096	
35	0.654	0.088	
36	0.648	0.097	
37	0.677	0.083	
38	0.669	0.098	
39	0.653	0.101	
40	0.620	0.091	
M13K07	0.166	0.081	
1%M-PBS	0.127	0.072	
Positive control [serum]	1.588		

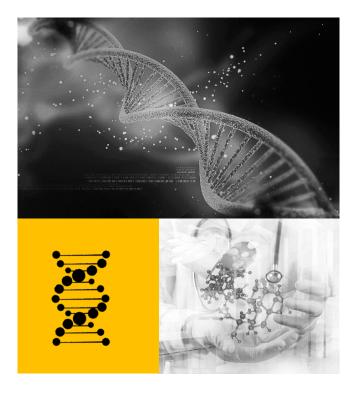
Magic[™] Therapeutic Antibody Discovery Platform



As a pioneer and undisputed global leader in antibody discovery and manufacture, **Creative Biolabs** has established the exclusive Magic[™] Therapeutic Antibody Discovery Platform (MTADP) to obtain all possible promising antibodies in phage display library.

For diagnostic and therapeutic antibody production, the only way to get good antibodies with all required properties is to have a large number of antibody candidates first. It is very common that a suitable antibody (pair) can only be discovered from 100-300 regular binders raised using different methods in different animals, thus have very diverse properties and sequences.

Magic[™] Therapeutic Antibody Discovery Platform



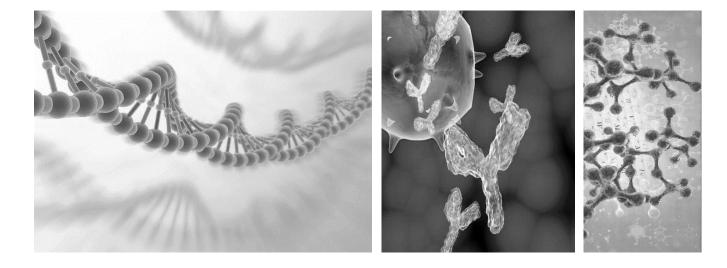
Identification of all the potential candidates in antibody library is a critical step in single domain antibody development. An enriched library derived from phage display could consists of millions of clones which is not possible to be covered by the conventional validation methods.

As a solution to these problems, our unique Magic[™] platform is all about time to results. The more antibodies you can get from the screening, the more and more reliable-functional antibodies you can send for downstream evaluation.

Through this powerful platform, a large number of antibody candidates specific for the target can be isolated at one time. Once antibodies targeting different epitopes with different affinity and specificity are obtained, we are able to fast and precisely identify the clones with the highest affinity and specificity.



Magic[™] Therapeutic Antibody Discovery Platform





High Success Rates

We have a proven record of successfully selecting the antibodies with high affinity and specificity using Magic[™] Platform.



Rapid Turn Around

Receive results as soon as 4-6 weeks.



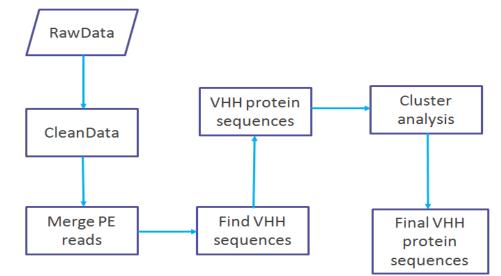
Cost-effective

Our high-quality data and high success rates ensures the identification of all possible candidates in the library, avoids costly repeat of conventional selection, which saves your money and time in ultimate.

Over Ten Times New Binders Discovered From Magic[™] Platform (Sample Report)

Project Description

- A phage display V_H H antibody library was constructed with a diversity of ~10⁸.
- After the biopanning and ELISA validation, 5 V_H H was discovered from the enriched library.
- In order to cover all the diversity of the enriched sub-library, Magic[™] Platform was performed to investigate the V_HH pool.



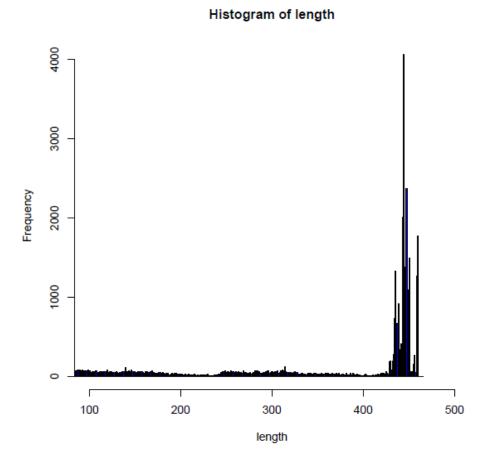
Expectations				
NucFreq	Nucleotide sequence and abundance			
ProFreq	Protein sequence and abundance			

Over Ten Times New Binders Discovered From Magic[™] Platform (Sample Report)

Most sequences centered around 450 bp

Sequence pattern (* stand for target sequence):

GTTATTACTCGCAGCAAGCGGCGCGCGCATGCC***** GC TAGCGAACAAAACTCATCTCAGAAGAGGATCT



Over Ten Times New Binders Discovered From Magic[™] Platform (Sample Report)

V_HH Protein Sequences (Raw)

1	EVELOPERAGE FUTGOE: TERCATORE: FELVARANCE PROVIDER FOR TAVADAVES AFTE: INDIAAR TVY, ORTHER PROTATY ICAARNYA VYTYL, VAPODE A DEGOG TOVTY IS	125	6529 1.	290501
2	DVGLUEDOKLEVANOOKLELISCAP DOR TOTIKLEND APROAPOKEREPYASISTIK SVECISIAYTED SYECKETTI SEDVIEKTYTLORENLEPHITAVITYCAAR SOAR TORPOTTITTUKETVOOD IQVIV	131	5726 0.	,212147
3	DVQLQBS0CBL#QPCCSLBLSCVASOCTFAIYTRCNFEQAPCKEREFYAAITETOCTTYTCOSVECRFILSEORAKETTFLQRSGLEFEDTAVTTCARAGESCVSTFTPSRSENED/W0QCTQVTVSS	128	1173 0.	. 052655
4	DVCLOB SOCILITONOS SLELACAN SOCIFISTYA TO VEGATE KEREPYANI SE SAGE TABAO SVEGEFTISKORAKSTVPLORIS SLEPEDTA VTPCANFEVANTTYTIS SOARE V NYKOOTOVTY SS	127	1109 0.	.049782
5	INCLOSED AND DESCRIPTION AND AND AND AND AND AND AND AND AND AN	127	976 0.	043812
6	DWGLOPISCOCLYGAGOSLELSCOR SGETVERYLMANFEGASIGREREPYACITETCYETCHADIVECRESEBORARISERVLGRSSLEPEDTAYTYCAAVLFOLRTV STEPSEPTOPYCGGTGVTVYSS	126	788.0.	035873
T	DWGLOBSCOCLEGTCCSLTLSCAT SCREPTILY ARAN FROM PORTREPTANT SERVICE TO TOTAL STREPTIC ACCOUNTS AND TOTAL STREPTIC AND TOTAL STREPT	127	724	0.0825
. 8	DWQLQB9000LFQT005LTLSCAT90E5F3LYAMANFEQAPOKEKEFYAGVSEBORTAYADAVECRF71SRDNAENTVTLRMRSLEF8D7AVTFCAARQLANTSAYLYE90D7AVV5007QVTVSS	126	506 0.	.022714
. 9	DWQLQBSOGGLEQTGOSLTLSCAT SQRSFIL, VANANFEQAPOKEREPTACITE TOTADITYEGEF SEIRONARSINVLQRSILEFEDTATTVCAAVLPGLETS STEP SDFGTVVGQGTQVTVSS	126	401 0.	010001
10	DVCLOPSIGNUEVGASSCIERS OF AN INTERVIEW AND A PERSON AND A SERVICE AND A	127	268.1	.01(51)
11	DVGLOBSOCCLEGTGGELTLSCATSOREPSLYAMANFEGAPOREREPYACVERSORTAYADAVECRETTISRONAMITYTLGRTELEPEDTATYTCARENVAVLSTVVVTPORTASVOCTVVTVSS	126	263 0.	016295
12	DWGLQESCOCLEQUOCSLELSCOV DORTF SCYTRCHFEQUPCKIRTF LAAIBT DOCCTKY ACGYRCRFTLEREYERYT1SLQRSCLEPEDTAATYCYARPCAL LEP1ESCYTR.#000CTCYTYSS	124	206 0.	.013734
13	DWQLQ8 SOCKLEQ TO CSLEL SCAT SOR SLTLY AND VER DAPOREER PROATER SORTATED STECRET I SEDWAARTYTLORTSLEP SOTATTTCAARSYAVL STVYTTPORTA SPOOTOFTYSS	126	262 0.	.011761
14	DWEEQROOOD VOMOUTH BE ACAA DOTTING THE TAG VERONANDER PARA FEEDMOTT IN ACCOVERE FEEDMONT IN ACCOVER FEEDMONT IN ACCOVER AND AND THE AVAILABLE AVAILABL	127	257.0	.011537
38	INVOLUESDOOL PARKER/LIELSCAR DORTFORY AND REPARCHEREPYAATSERGEDTYYECOVECREPTING MARSTYYL GRINDLEPEDTRYFCAASCAVITER FLITE ODDY ATROUTING ST	127	244.0	. 01 (963)
.16	DPOLOBBOOCLF97005LTLBCATSORSF5LYAMANFEOAPOKEREFYACVEROUTAFADAVECRFTISRONTEKTYTLOMESLEPEDTAVTTCAAESCAESCAESCAFSCHP0TTTTT00DTVOOCT077455	127	229	0.01028
17	DWGLQB SOCCLEQPGCSLBL SCTT SRT DTYNFLDWTEQAPONQED, VAS IT SVDSTNYADSVECRFT I SEDWAERTVTLQERSLESSOTA FTYCHARREFTGGCRED/WGQCTQFTYSS	128	196 0.	.008798

Compared with the five $V_H H$ binders rediscovered using the conventional method

09082014_AG1_1	DVQLQESGGGLVQAGGLALSCAPSORIGIL FRQAPGKEREFVASST	SRDNTKKTYLOMENIKEEDIAYYYCAA	MAG-AG1-2
09082014_AG1_3	DVQLQESGGGLVRSGDSLRLSCTTSGRLTT VFRQAPGKEREFVAAS	SRDNT KKTVY LOMENLK PEDT AV TY CAAP	MAG-AG1-5
09082014_AG1_6	DVQLQEYGGGLVQGGEFLRLSCAAS <mark>GRIFS</mark>	SRDNARNTY LOWNS LKEEDT AV TECAAF	MAG-AG1-15
09082014_AG1_9	DVQLQESGGGLVQTGGSLTLSCATSGREFT FRQAPGKEREFVAGY	SRDNAANTYLLOMTSLKEEDTATYSCAL WGQGTQVTVSS	MAG-AG1-1
09082014_AG1_13	DVQLQESGGGLVQAQSELRLACAASGGTFSVFRQAPGKGREFVAAIS	JRDNAKSTVELCMNSLKEEDTAVTECAAL WGQGTQVTVSS	MAG-AG1-14

Over Ten Times New Binders Discovered From Magic[™] Platform (Sample Report)

V_HH Protein Sequences (Clustered)

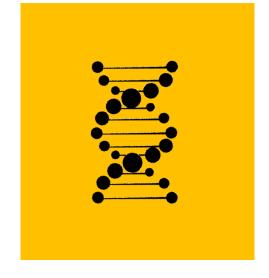
- 61 V_{H} H binders have been identified.
- These new sequences have been separated into 22 groups according to the characteristics of CDR domains and labeled with different colors.

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NAMESSO ANT COLD, TARKET STARE VERALASSEEPANT RESERVANT RESERVANT DESKARTSTEMTSLAPESTATISK PERALTSTEMAKET NOUTUVIS	1.25	161 0.90
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NUMBERGORDSTONDUTUSCHTERGENE EINE PEREFERETENT REDITIONERFEITERGENERFENTERENTEN ANTONISCHTERETERENTIGENERFEITER	127	26 0.00
PULOE SOUR POTOSOLTI, SOURT SOURCE SAMA PERGAPREMENTANT DESITA VALANMENT DESIMANTY TLANTSLAFSSTAVYTCA SPRAAELETPEDED VOOTSVYT DI	124	24 0.00
NUMERICAL AND CONTRACTOR AND	1.28	34 0.00
NU AR SOLID, NAMES & STORY CORPORATION TO A A RECOMPTION OF THE AND THE AND THE AREA OF TH	124	306 0.01
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RULOH SORDEL NOARSSELE, ACAA SORTY SEL TAS FEBUARHEURBEPRAALSE SATTELYADDIESH PEBUGARUTVPLOBELER PELABOLER PELA	127	257 0.05
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Over Ten Times New Binders Discovered From Magic[™] Platform (Sample Report)

Result Summary

- From the conventional strategy, 5 different sequences were discovered, and they are rediscovered by the Magic[™] Platform. The most frequent sequences found in the conventional method are corresponding to the most abundant cluster in the Magic[™] result.
- Through the Magic[™] Platform, we found 56 NEW V_HH binders. These new discovered sequences have been clustered into 22 groups according to the characteristics of CDR domains.





2.2 Premade Phage Display V_HH Library Screening



Libraries	Display Technology	Library Format	Species	Library Size
CaV _H HL-1	Phage Display	Naïve V _H H	Camel	1.5×10^{9}
CaV _H HL-2	Phage Display	Naïve V _H H	Camel	2.0×10^{9}
LlaV _H HL-1	Phage Display	Naïve V _H H	Llama	2.0×10^{9}
HuSdL-1	Phage Display	Camelized synthetic V _H H	Human	1.5×10^{9}
HuSdL-2	Phage Display	Camelized synthetic V _H H	Human	2.5 × 10 ¹⁰

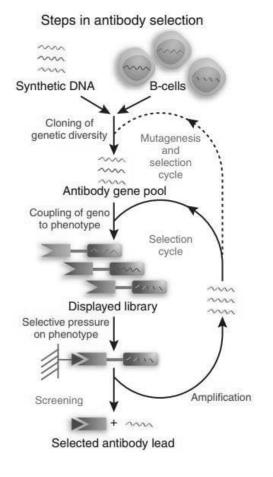
2.2 Premade Phage Display V_HH Library Screening

Creative Biolabs has built up a HuSdL[™] Human Single Domain Antibody Library that allows rapid discovery of large numbers of high-potency camelized human single domain antibodies against any therapeutic targets.



- Most robust and straightforward
- Low immunogenicity: our library produces camelized human antibodies that have a human origin, thus the lowest immunogenic potential in humans, especially for long-term and multiple-dose administration.
- Adequate developability: the library was preselected based on the thermostability and expressibility (in *E. coli*) of the displayed antibodies. In particular, in the library, the antibody repertoire was heat-treated to remove clones that could not withstand heat-induced aggregation.

2.2 Premade Phage Display V_HH Library Screening





Our premade single domain antibody library was constructed based on either camelized human VH3 in FR2 or naïve camelid V_HH repertoire.

Accepted Targets:

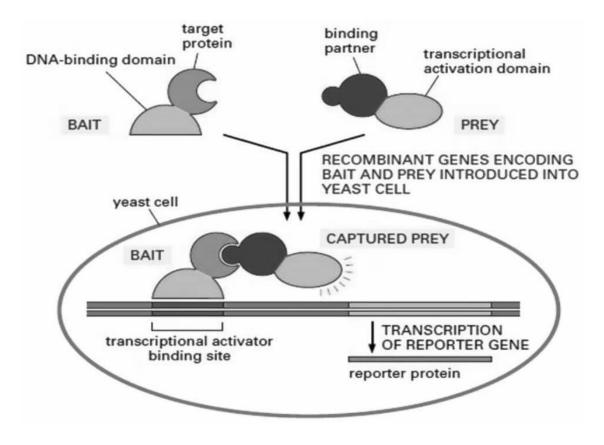
- ✓ Whole Cells/Tissues
- ✓ Peptides/Haptens
- ✓ Recombinant Proteins
- ✓ Membrane Proteins
- ✓ Other *in vivo* Targets

Screening Strategies:

- ✓ Solid-Phase Screening
- ✓ Solution-Sorting Screening
- ✓ Cell-based Screening
- ✓ In vivo Screening
- ✓ Ex vivo Screening
- ✓ More...

Creative Biolabs can also license these premade sdAb libraries to our clients who prefer to screen the novel sdAbs themselves!

2.3 Intrabody Discovery



Creative Biolabs has built up A novel intrabody discovery platform to screen and validate novel single domain antibodies that can bind to intracellular targets specifically within various cellular compartments.

Platform

- Two-Hybrid Detection System
- Advanced Phage Display Platform

Typical Features

- In vivo antigen expression.
- A solution to identify binders to proteins or domains, which are unstable *ex vivo*, difficult to purify, or expensive to purchase.
- Can validate specific antibody in either high- or lowthroughout manner

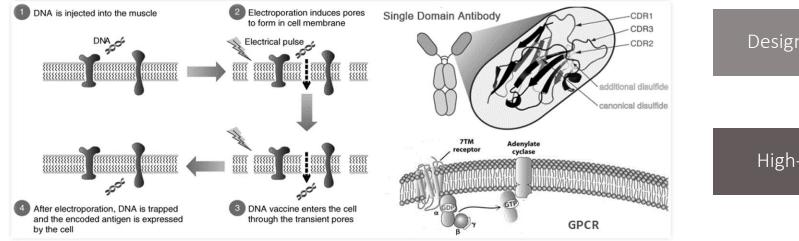
2.4 Anti-Membrane Protein sdAb Discovery



Creative Biolabs has developed a robust and highly effective strategy for generating sdAbs targeting membrane proteins using DNA immunization, whole cell immunization and many other strategies.

Followed by high-throughput screening on cell lines expressing target membrane proteins in native conformation and subsequent analysis by FACS, we have successfully selected a great many potent single domain antibodies at nanomolar or sub-nanomolar level, with desired functionalities.

2.4 Anti-Membrane Protein sdAb Discovery via DNA Immunization



Electroporation

Other unique methods

Improved immunization strategies

Optimized boost schedule

Whole cell immunization

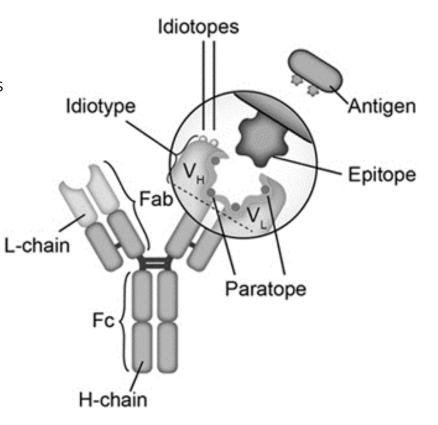
Design the best fit immunization procedure High-quality Immune Library Construction High-throughput screening on cell lines Specific Binders Validation

2.5 Anti-Idiotype sdAb Discovery

Creative Biolabs offers anti-idiotypic sdAb production service through immunizing camel/llama/alpaca/shark with target antibodies in the forms of whole IgG, scFv, Fab, Fab' or F(ab')₂.

Applications

- Antibody drug pharmacokinetic/pharmacodynamics (PK/PD) studies
- Antibody drug immunogenicity (immune response, IR) studies
- Preclinical research of therapeutic antibodies
- Anti-drug antibodies (ADA) for clinical development
- Controls in ligand binding neutralizing assays
- Controls in antibody blocking assays



2.6 Anti-BBB sdAb Discovery



Creative Biolabs offers blood-brain barrier specific antibodies with implications for the development of biologics-based treatment of brain disorders.

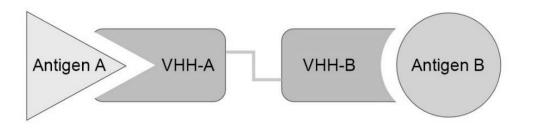
Anti-BBB sdAb can be used as vectors to target drugs or therapeutic peptides into the brain.

Our technology also aims to target endogenous transport systems, including carrier-mediated transporters such as glucose and amino acid carriers, receptor-mediated transcytosis for insulin, transferrin or low-density lipoproteins (LRP1) and the active efflux transporters such as p-glycoprotein.

2.7 Anti-Albumins sdAb Discovery

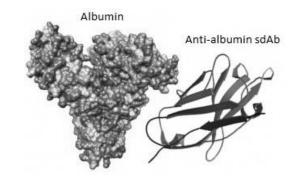


Fusion with an anti-albumin sdAb is a potential option to extend the serum half-life of therapeutic agents. Anti-albumin sdAb will recognize the albumin after properly administration and allow the fused biopharmaceutical agent to be carried around the body, and to take on similar PK parameters with the albumin as well.





- Animal immunization (llama/alpaca/camel/shark)
- Immune library construction/Premade library preparation
- Library screening and validation
- Anti-albumin sdAb production/further development



2.8 Custom sdAb Production

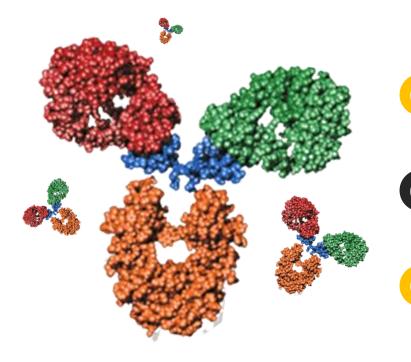
We offer custom service to produce custom single domain antibodies. We have a Non-Exclusive License from the following party that allows us to produce single domain antibodies from camelid (llama, camel, and alpaca). We will pay a royalty to this licensor while our customers have full right to own the resulting single domain antibodies. With this license, we can produce single domain antibodies using any method, not limited to phage display antibody library construction and screening, which is also patent-free.

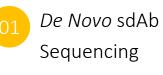


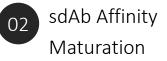
Tech Transfer Department VIB vzw Rijvisschestraat 120 9052 Zwijnaarde Belgium Website: <u>www.vib.be</u>

03 **SINGLE DOMAIN ANTIBODY DEVELOPMENT THIRD PART**

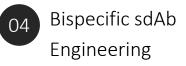
CREATIVE BIOLABS



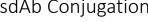




sdAb Humanization









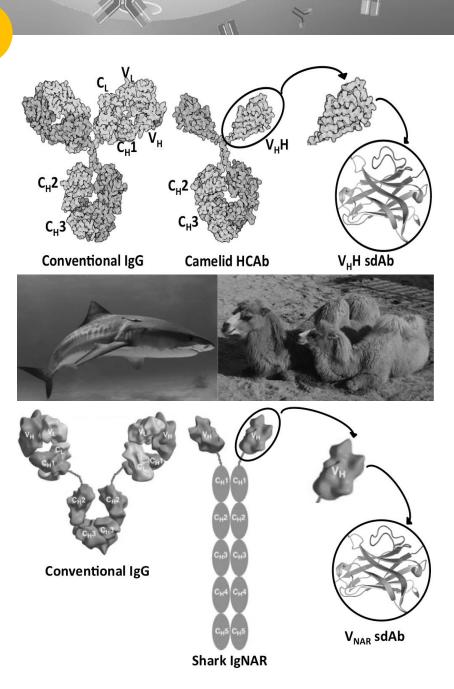
Antibody Camelization

3.1 De Novo sdAb Sequencing

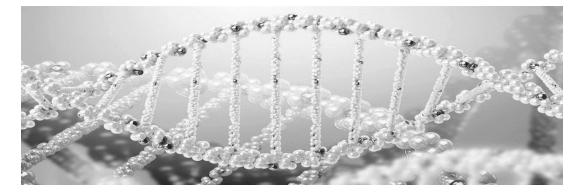
Through the proprietary **Database Assisted Shotgun Sequencing (DASS)** technology, soluble and functional single domain antibody can be *de novo* sequenced by subunit with **100% coverage** and dozens of successful cases proving **100% accuracy**.

Specifications

- Sequencing of the V and J and C segments by DASS
- *De novo* sequencing of the CDR3 region
- Sequencing of isobaric amino acids
 - W can be distinguished from GE, AD and SV
 - R can be distinguished from GV
 - Q can be distinguished from K
 - N can be distinguished from GG
 - Q can be distinguished from GA
 - Leucine will be predicted from the germline sequences and the cutting frequency of chymotrypsin.



3.2 sdAb Affinity Maturation



Creative Biolabs has developed a proprietary DNA mutagenesis technique that is able to create a huge number (e.g. 10¹⁰) variants of the parental antibody with defined positions mutated.

In combination with our first class phage display antibody library construction and screening technologies, 10-100 fold increase in affinity for parental single domain antibodies of low nM affinity is frequently achieved.



A phage display mutant library at a size of 10¹⁰ is created for each parental sdAb.



Frequently use parental sdAb or antigen to wash away weak mutants.



High-throughput biopanning to isolate rare mutants with affinity-increased for 10 or more fold.



Validate a large number, e.g. 80, affinity-matured individual mutants using phage ELISA and antibody ELISA.



Affinity (e.g. KD) determination on top 5 affinity-matured sdAb mutants.



Get sdAbs of an affinity of 0.1 nM over better.

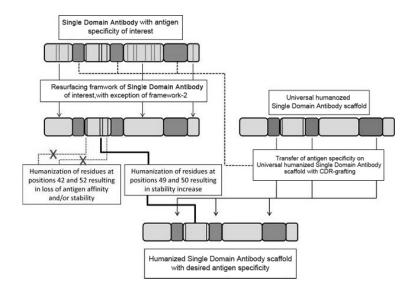
3.3 sdAb Humanization & Human sdAb Discovery



Creative Biolabs has extensive experience in generating human single domain antibodies and humanizing single domain antibodies.

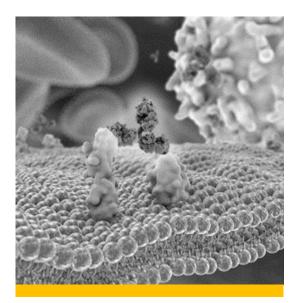
Three approaches to develop human/humanized sdAb

- Screening of the premade synthetic human sdAb library
- Immunizing transgenic mice that harbor human single domain antibody gene repertoires
- sdAb humanization



- Employs soluble, stable, well expressed universal humanized single domain antibody scaffolds
- CDR grafting
- Back-mutation

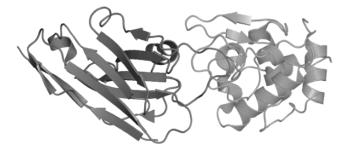
3.4 Bispecific sdAb Engineering



Bispecific antibodies (bsAbs), which are capable of simultaneous binding to two different targets, are considered the most promising solution to increase therapeutic activity by retargeting a large variety of payloads to cancer cells.

> **Strategy:** Link two single domain antibodies *via* a peptidic linker, thereby creating a tandem single domain antibody.

Successfully example: A few bispecific single domain antibodies with neutralization activity obtained using a specially designed linker based on the hinge region of the llama IgG_{2a} isotype



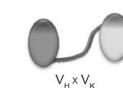




Bispecific tandem single domain antibodies

BsFab





Bispecific trivalent tandem single domain antibodies

3.5 sdAb Conjugation

Benefits

- Performed by professionals in this field
- Considerable conjugation ratio and scale
- Quick turnaround time and economical expenditure
- ♦ High-quality results

Introduction

Bioconjugation is a chemical strategy to form a stable covalent link between two molecules. Conjugated sdAbs have been found significant applications for enhanced stability, sensitive and functionalities in diagnostics and therapeutics.



Applications

- Biological interaction discovery
- Diagnostic applications
- Extend the half-life of single domain antibody

3.5 sdAb Conjugation

sdAb Biotinylation Service

sdAb-Enzyme Conjugation Service

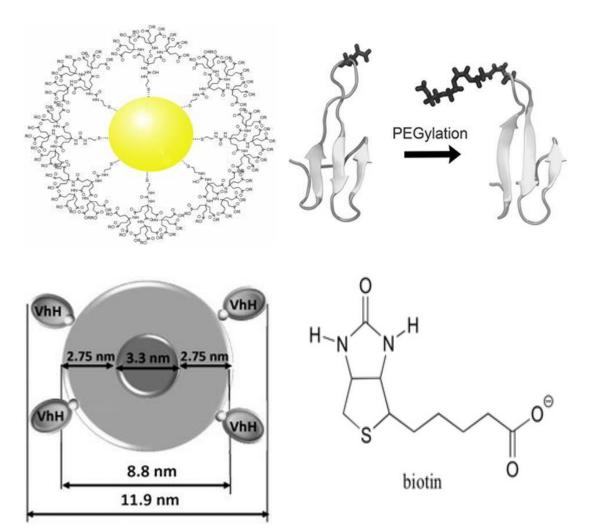
sdAb-Fluorophore Conjugation Service

sdAb-Gold Nanoparticle Labeling Service

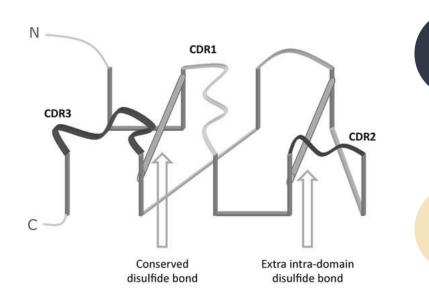
sdAb PEGylation Service

sdAb-Polystyrene Beads Labeling Service

sdAb-Quantum Dots Conjugation Service



3.6 sdAb Stability Improvement



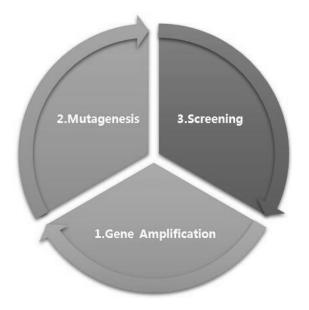


In vitro sdAb stability

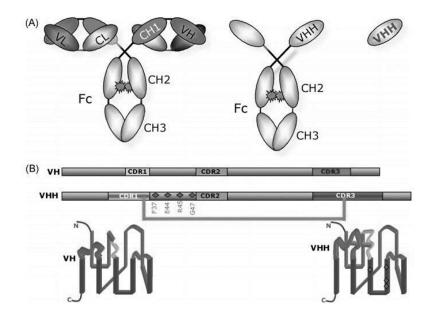
- Physical stability (thermodynamic stability)
- Chemical stability (proteolytic stability)

Approaches

- Two amino acid substitutions within the framework to form an additional intra-domain disulfide bond.
- CDR is grafted onto the stable framework.



3.7 Antibody Camelization



Creative Biolabs provides service of *in silico* design of camlized human antibodies.

- We select the single domain antibody framework with the best homology to human VH backbone, perform CDR grafting *in silico*, and then run computer based antibody modeling to do back mutations.
- Combined with construction and screening of a custom single domain antibody library, camelized (human) antibody sequences of the best affinity are generated.

HuSdL[®] Human Single Domain Antibody Library

Camelized human antibodies

The single service provider in the world!



SELECTED REFERENCES

FOURTH PART

CREATIVE BIOLABS

REFERENCE

We conducted novel sdAb discovery and development projects for following customers with excellent results, who are willing to be our references.

Dr. Emmanuelle VIGNE

Global Biotherapeutics Centre de Recherche de Vitry/Alfortville 13, quai Jules Guesde – BP 14 – 94403 Vitry-sur-Seine Cedex France Tel: + 33 (0) 1.58.93.37.11 Email: Emmanuelle.Vigne@sanofi.com

Dr. John W. Beaber

Associate Director, Advanced Platform Research Molecular Engineering Unit Intrexon Corporation 20358 Seneca Meadows Parkway Germantown, MD 20876 Tel: 650-597-4044 Fax: 301 556 9901 Email: jbeaber@intrexon.com Website: www.dna.com

Dr. Erich Koller

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Prof. Kiyuk Chang

Associate Professor Cardiovascular Center and Cardiology Division Seoul St. Mary's Hospital, College of Medicine The Catholic University of Korea, Seoul, Korea Tel: 82-2-2258-1139 CP: 82-10-9175-2076 Email: kiyuk@catholic.ac.kr

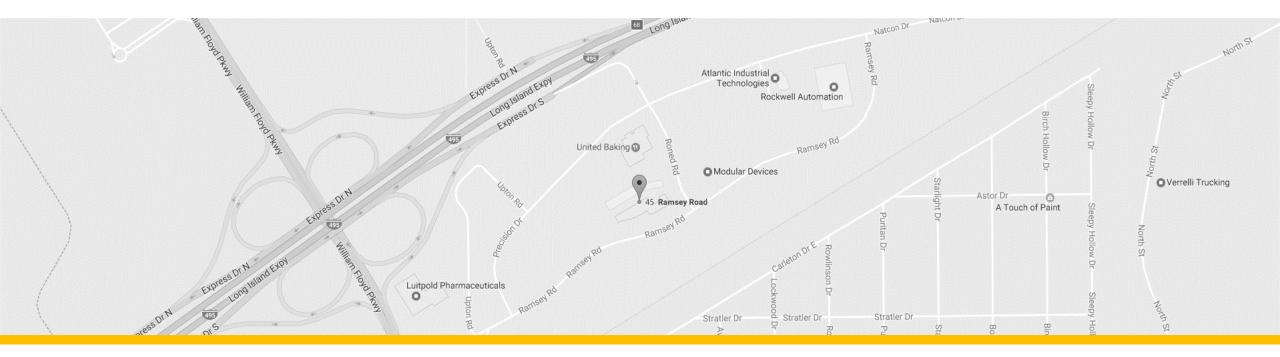
Dr. Eric Marr

Pfizer Inc. 445 Eastern Point Road - Bldg. 98 Groton, CT 06340 USA Email: eric.s.marr@pfizer.com

Prof. Stefan Schulz

Jena University Hospital Institute of Pharmakology and Toxikology Drackendorfer Str. 1 D-07747 Jena Email: stefan.schulz@mti.uni-jena.de

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