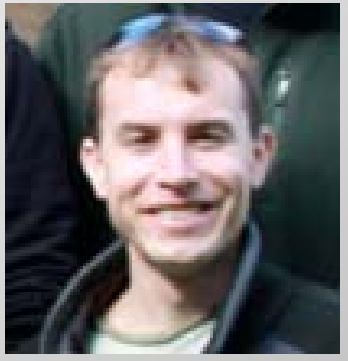
Nature-inspired design of motif-specific antibody scaffolds*

Nature Biotechnology, October 2013, Vol. 31, #10

* Scientific information in the following slides is from this article, if not stated otherwise



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Journal Club 2, 26.11.2013

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Outline

1. Background (traditional and recombinant AB production)

2. Motivation for the study, aims

3. Study design, Models, Methods

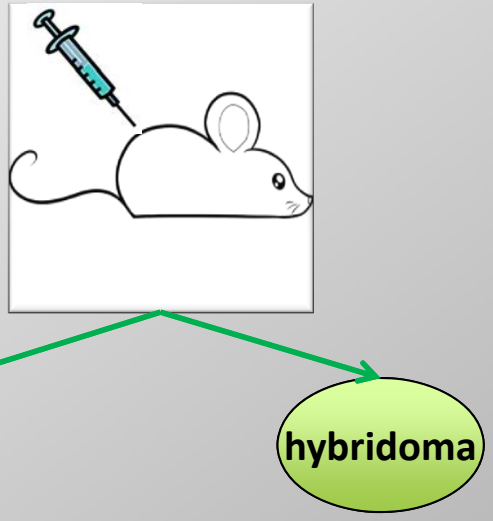
4. Results

5. Discussion: Significance and relevance of the results

Background

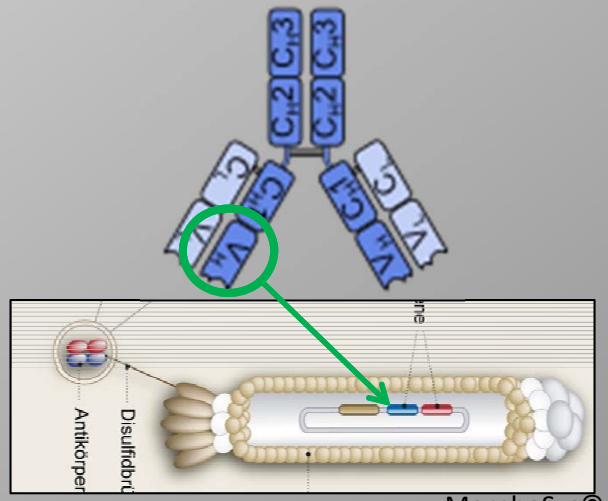
Production of AB

Immunization



Display

Genetic information, Expression and Display



MorphoSys©

Diversity $10^{7\text{-}13}$

Feature	Polyclonal sera	Hybridoma mAB	*In vitro*-selected mAB
Therapeutic use	Not for chronic diseases	Requires humanization	Yes
Toxic antigen	Difficult	Difficult	Possible
Minimal time for selection	Months	Months	Weeks
Predetermination of epitope structure, conformation specificity, avoid cross-reactivity	Not possible	Not possible	Adjustment of selection conditions, introducing competitors
Costs	Animal facility	Animal facility	In vitro automated liquid handling

H. Hoogenboom Nature Biotechnology, 2005 S. Dübel, Trends in Biotechnology, 2010

Background

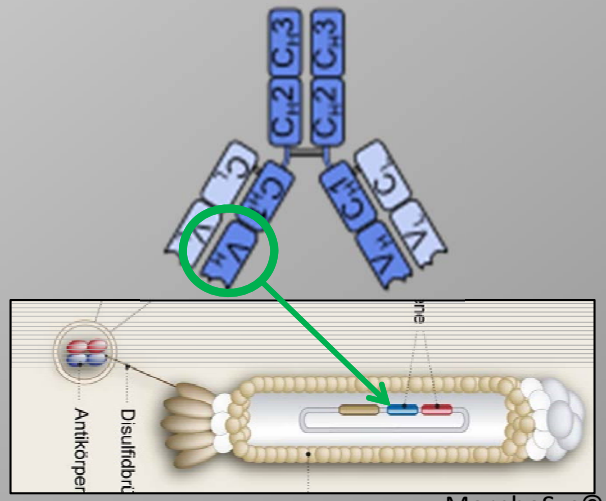
Production of AB

Immunization

hybridoma

Display

Genetic information, Expression and Display



MorphoSys©

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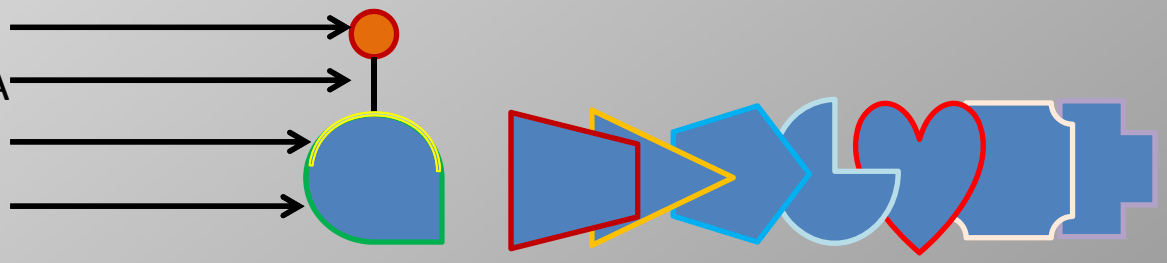
H. Hoogenboom Nature Biotechnology, 2005 S. Dübel, Trends in Biotechnology, 2010

Background

Production of AB specific to post translational modifications (PTMs)

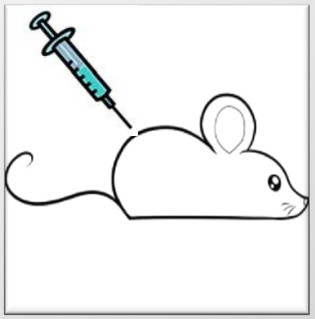
Ideal antibody against PTM

1. Exclusive PTM
2. Exclusive targeted AA
3. High affinity
4. Renewability
5. Cheap and quick
6. All types of applications



Background

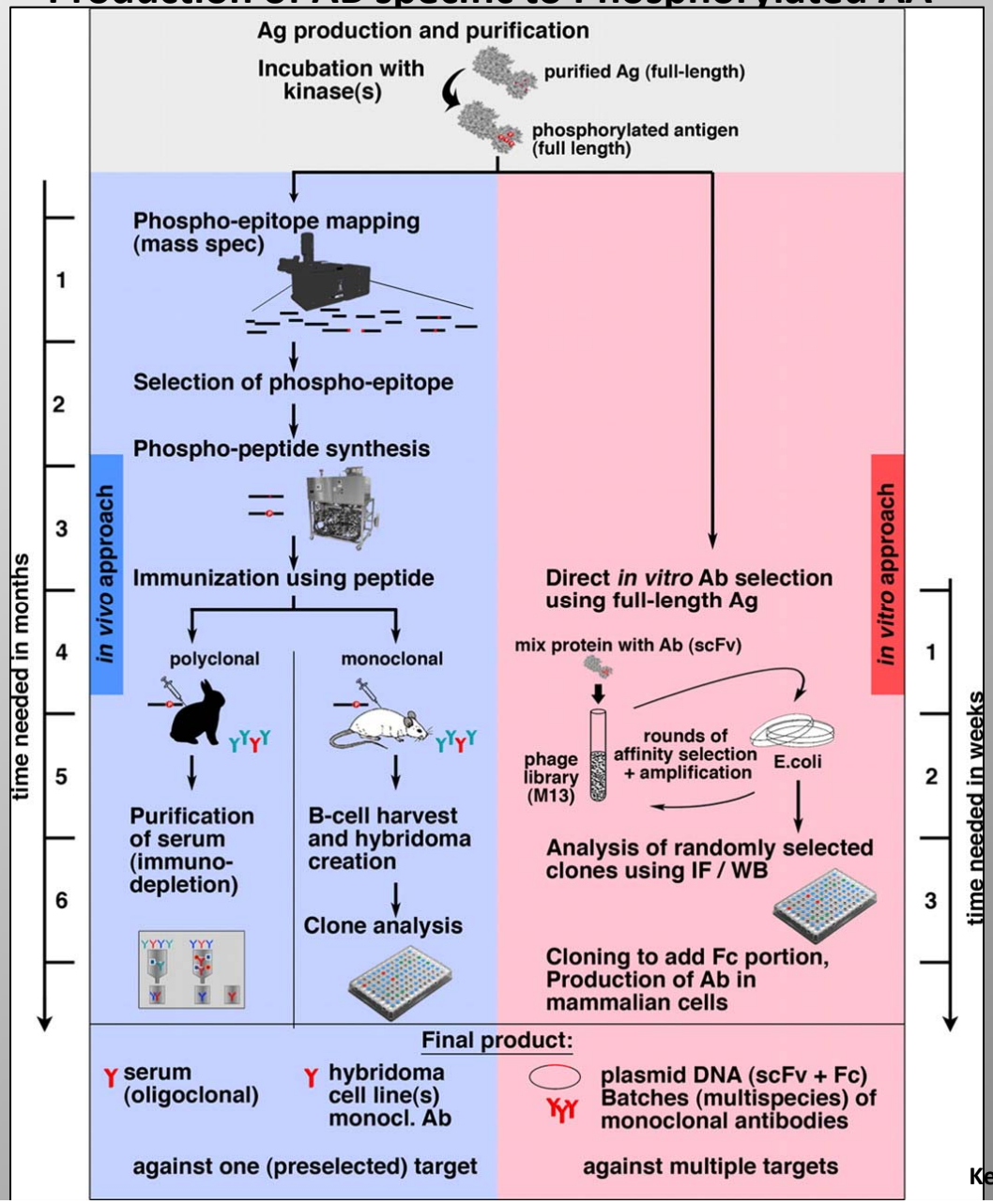
Production of AB specific to Phosphorylated AA

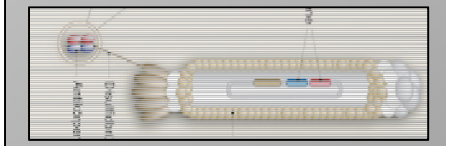


1. Exclusive PTM +
2. Targeted AA -/+
3. High affinity +
4. Renewability -
5. Cheap and quick -
6. All types of applications -/+

Threat:
Antigen processing

O Vielemeyer et a.,
The Journal of Biological Chemistry,
July 2009





1. Exclusive PTM +
2. Targeted AA +
3. High affinity +
4. Renewability -
5. Cheap and quick -/+
6. All types of applications -/+

Threat:
Far more rear AB, selection complexity (20 times more clones for selection)

Kehoe JW, Mol Cell Proteomics. 2006

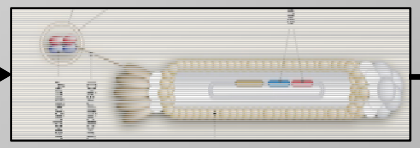
Background

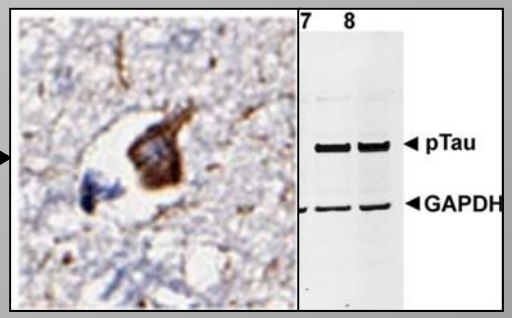
Production of AB specific to Phosphorylated AA

Phosphorylated Tau protein

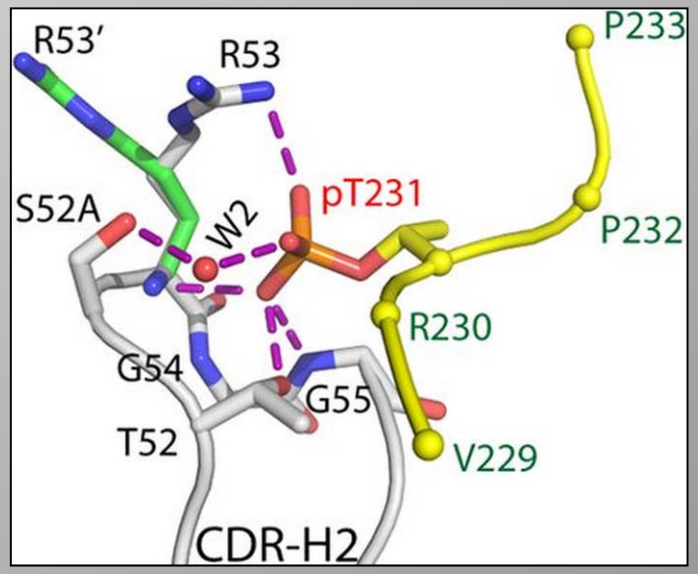
Kd 100-1nM

Library 3.8×10^8





Phosphopeptide mixture



H.H. Shih et al.,
The Journal of Biological Chemistry,
November 2012

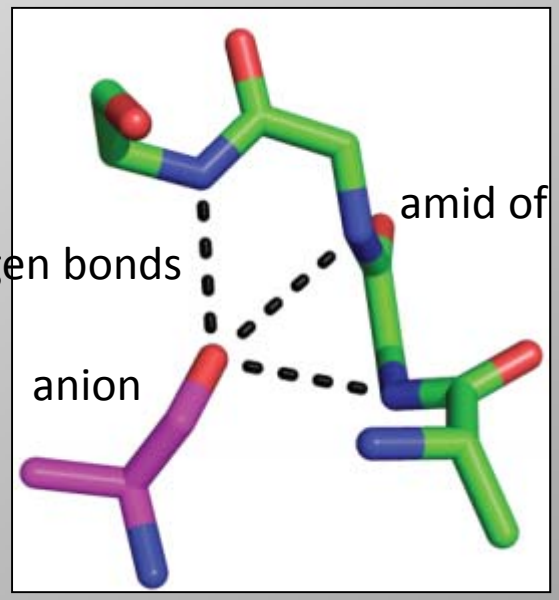
Motivation for the study and aim

Reduction of the selection steps in display technology and fixation of specificity to the PTM with targeted AA

1. Exclusive PTM
2. Exclusive targeted AA
3. High affinity
4. Renewability
5. Cheap and quick
6. All types of applications

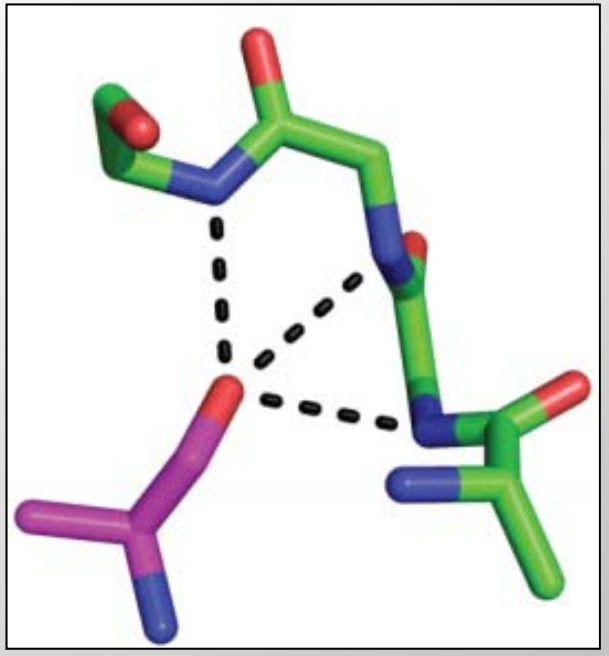
Study design, <u>models</u>, methods

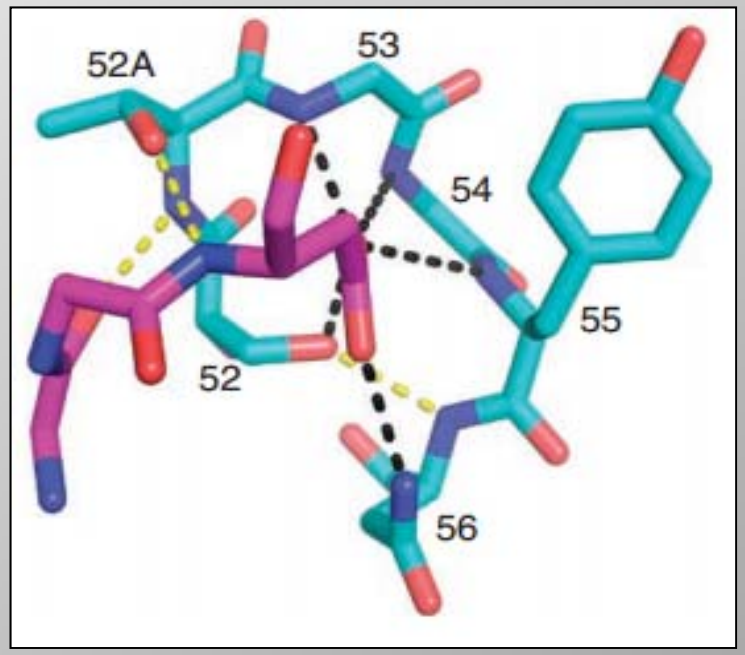
Common structure of anion (phosphate group) binding motif in protein super-families: kinases and ATPases



Study design, models, methods

Screen existing mAB scaffolds library for the desired anion recognition region

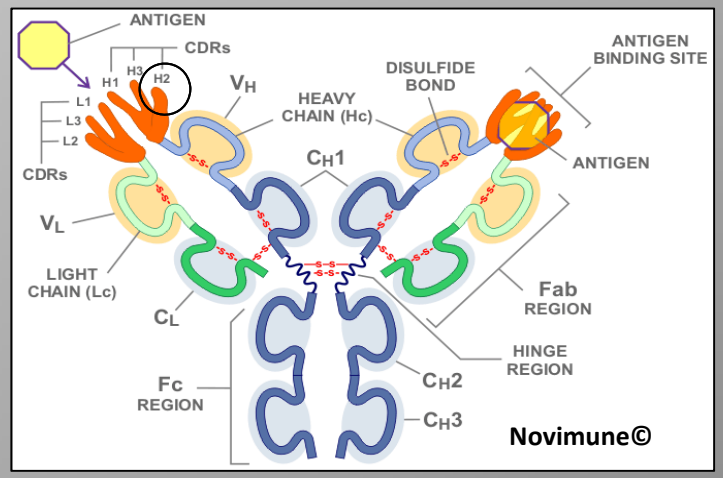


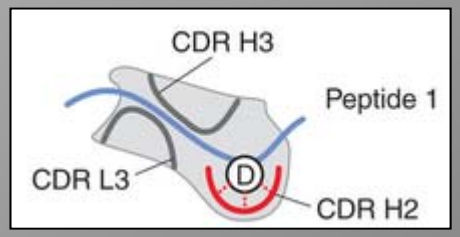


Asp of antigen peptide

KGNYVVTDH

reader region





anion recognition

STGGYN

O. Stoevesandt,Nature Biotechnology, October 2013

Study design, models, methods

Antigen peptide + CDR H2 region +

KGNYVVTDH **STGGYN**

Generation:

1. Antigen phosphopeptide
   - Phosphorylated AA
   - Sequence of rest of the peptide
2. Phage libraries

Selection:

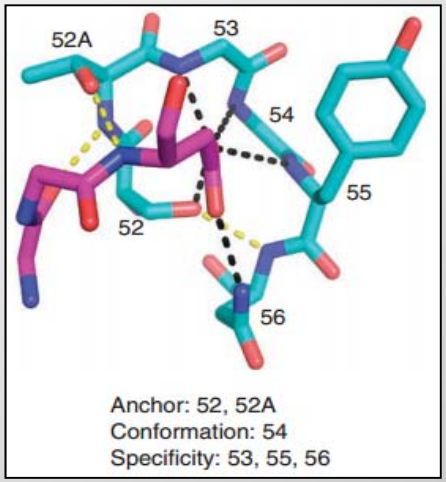
Phage display selection
ELISA
WB

Structure analysis:

X-ray crystallography
Modeling

Results

Does parent H2 scaffold **STGGYN** (anion recognition) binds phosphopeptides?



KGNYVVTDH

↓

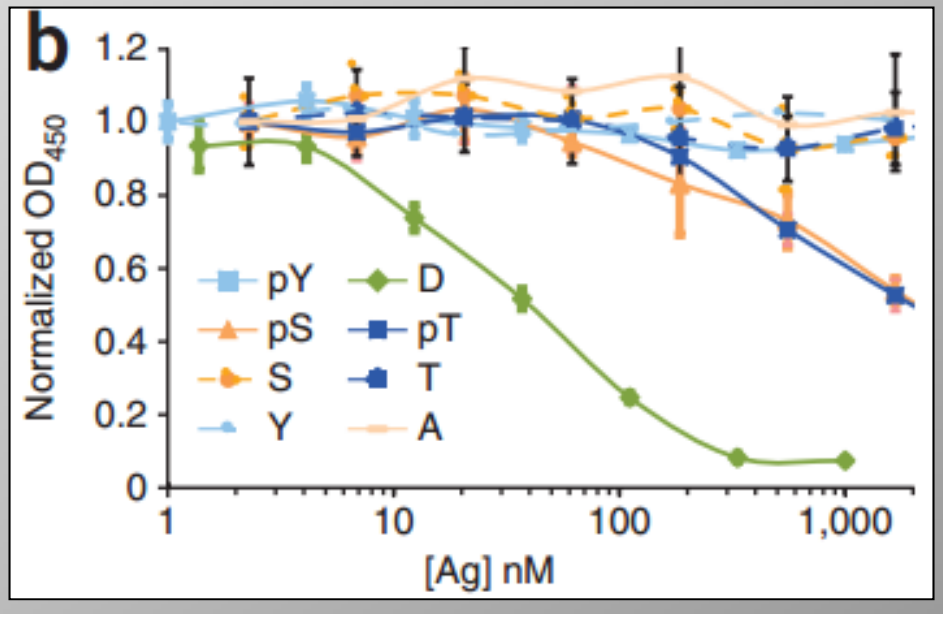
KGNYVVTYH **KGNYVVTpYH**

KGNYVVTSH **KGNYVVTpSH**

KGNYVVTTH **KGNYVVTpTH**

KGNYVVTAH

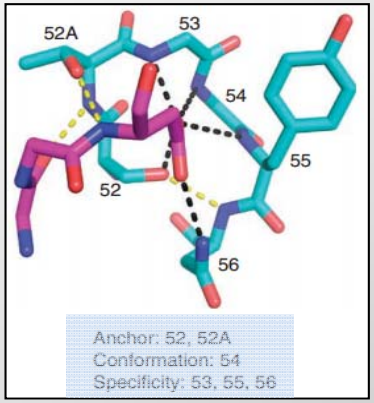
Competition Fab-phage binding to the immobilized wild-type peptide



Phospho T and S bind, but with low affinity

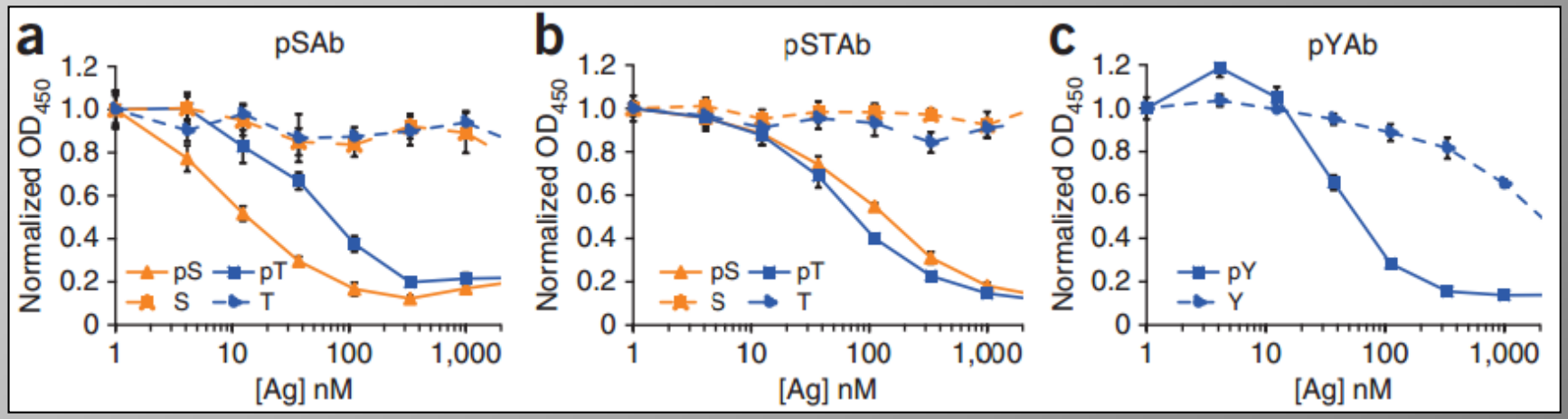
Results

Adjustment of the parent H2 scaffold for binding of **phospho**peptides



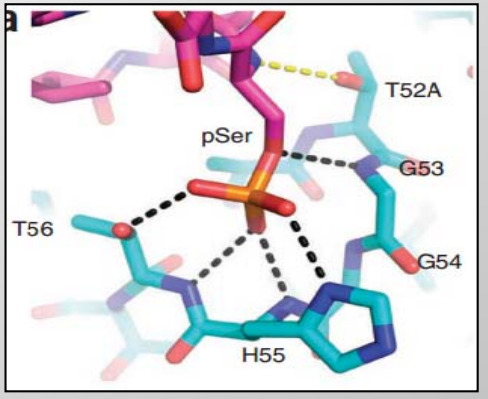
S T G G Y N
52 52A 53 54 55 56

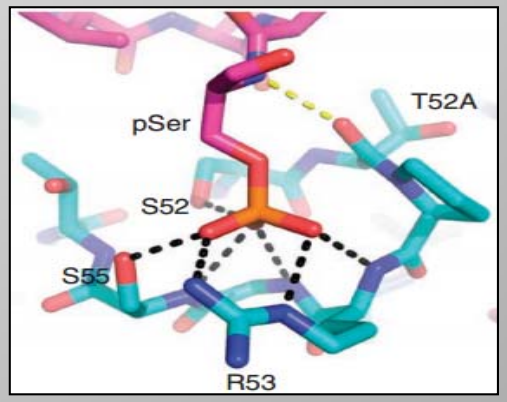
Phage library
Phage display selection

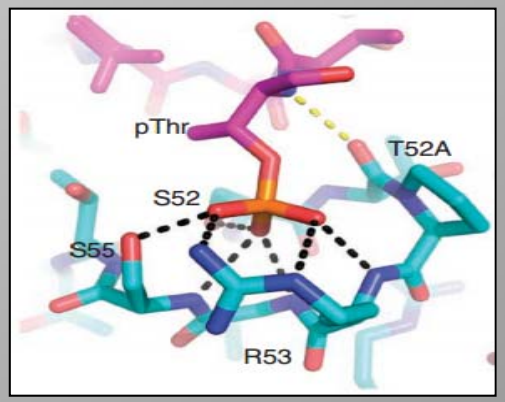


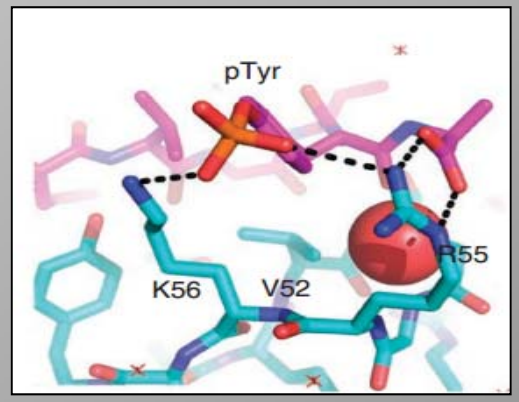
Results

What do the AA in the H2 scaffold actually do?







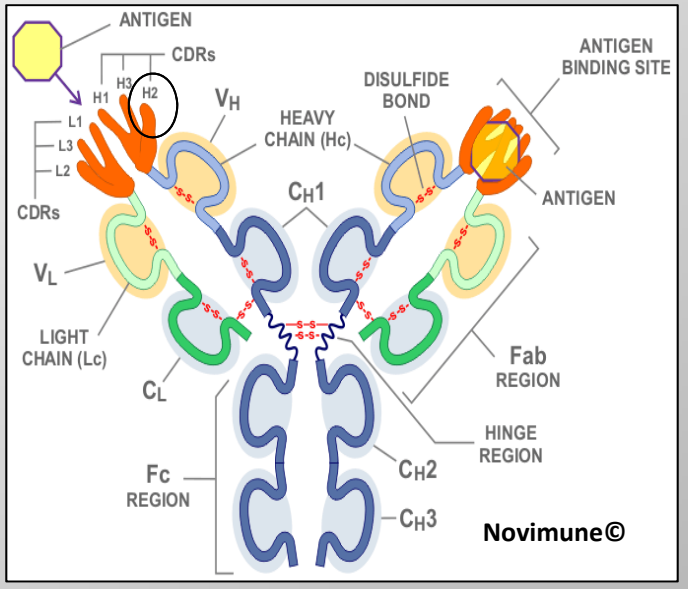


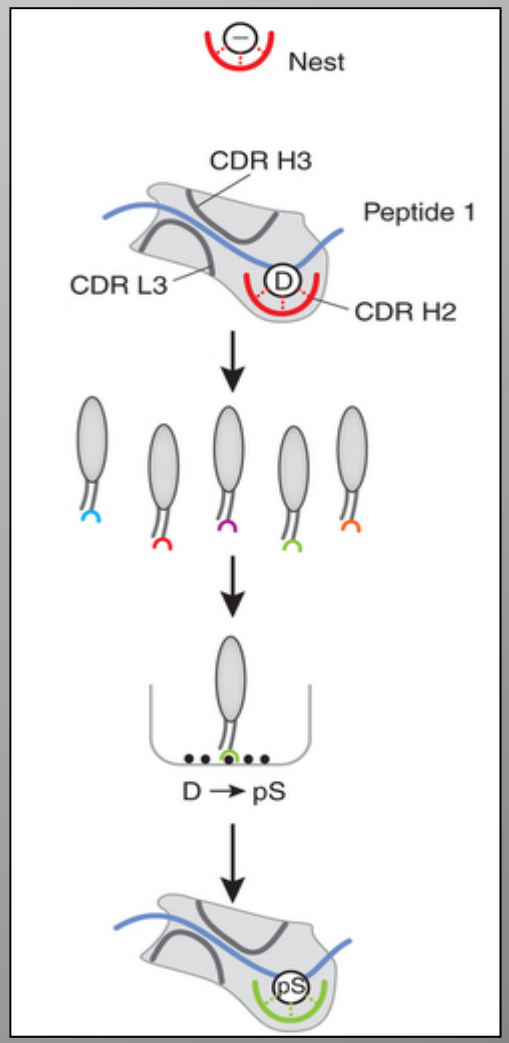
Anchoring (52), Conformation (54), Specificity (53, 55, 56)

Results

Adjust anion recognition region (H2)

1. Exclusive PTM ☺
2. Exclusive targeted AA ☺





KGNYVVTpYH

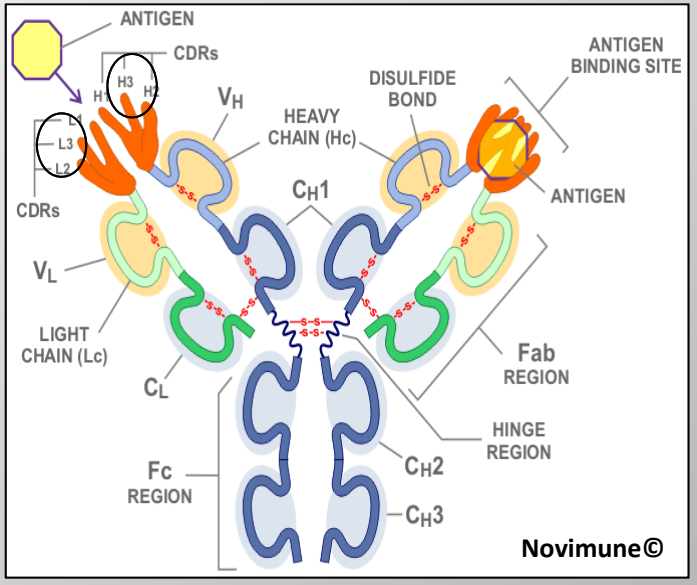
KGNYVVTDH ⟶ KGNYVVTpSH

KGNYVVTpTH

Results

Adjust reader region (H3, L3)

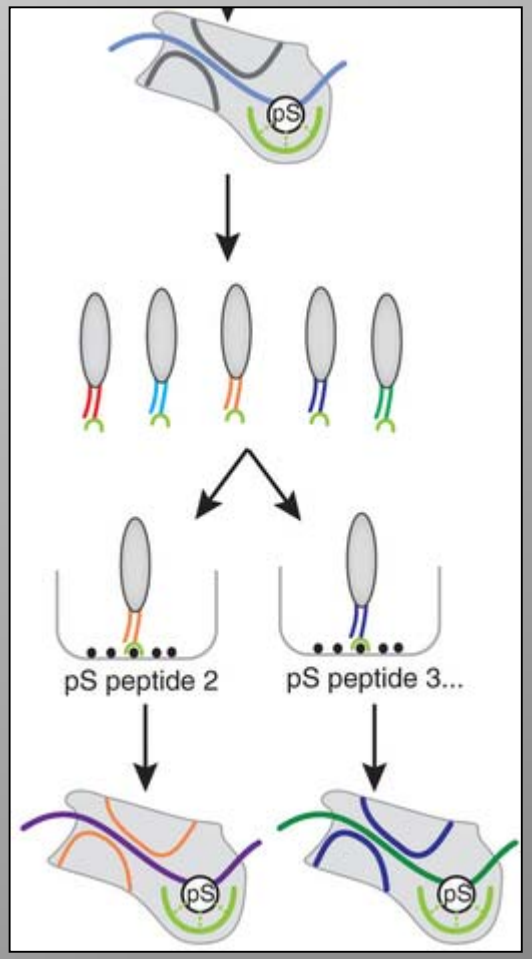
3. High affinity !
4. Renewability !



KGNYVVTpYH **XXXXXXXpYX**

KGNYVVTpSH → **XXXXXXXpSX**

KGNYVVTpTH **XXXXXXXpTX**



Results

Library for reader region
Display to the number of targets

Target

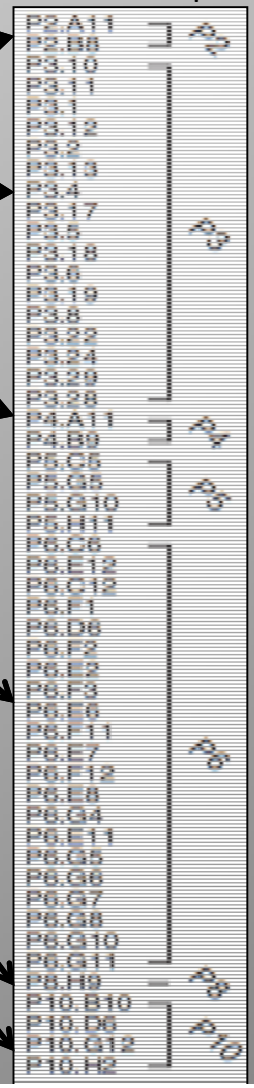
Peptide	Sequence
P1: Caspase 3 (S12)	NTENSVDSK**pS**IKNLEPKII
P2: RIPK3 (S227)	REVELPTEP**pS**LVYEAV
P3: RIPK3 (S199)	LFVNVNRKA**pS**TASDVYSF
P4: Smad2 (T8)	MSSILPF**pT**PPVVKRLL
P5: CREB (S133)	RREILSRRP**pS**YRKILNDL
P6: HtrA2 (S212)	RRRVRVRLL**pS**GDTYEAVV
P7: Akt1 (T308)	KEGIKDGATMK**pT**F
P8: Akt1 (S473)	ERRPHFPQF**pS**YSASGTA
P9: PKC Θ (S695)	DQNMFRNF**pS**FMNPGMER
P10: Sgk1 (S422)	EAAEAFLGF**pS**YAPPTDSF

KGNYVVTpYH **XXXXXXXpYX**

KGNYVVTpSH → **XXXXXXXpSX**

KGNYVVTpTH **XXXXXXXpTX**

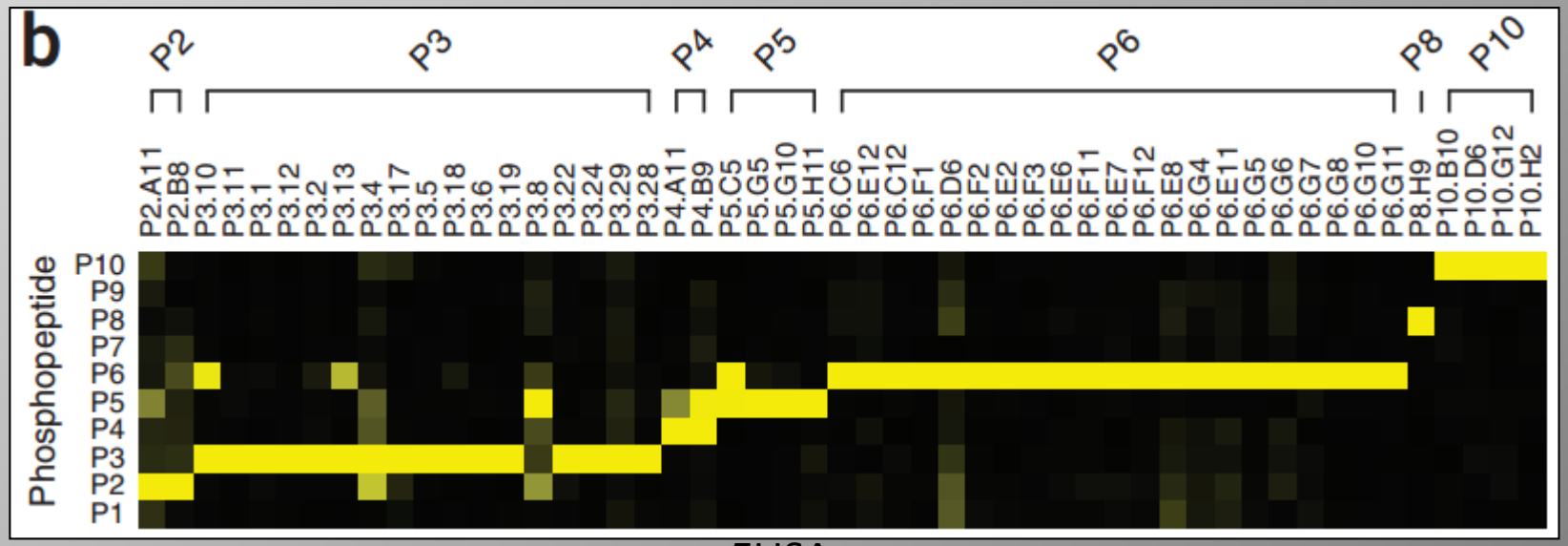
Selected scFvs with fixed H2 phosphate recognition region and selective to the specific target



Results

Specificity of the selected scFvs validated by ELISA

Peptide	Sequence	Number of unique scFvs	Number of phospho-specific scFvs[a]	K_D (nM)[b]
P1: Caspase 3 (S12)	NTENSVDSK**pS**IKNLEPKII	5	0	n.d.
P2: RIPK3 (S227)	REVELPTEP**pS**LVYEAV	6	2	102 ± 15 (P2.A11)
P3: RIPK3 (S199)	LFVNVNRKA**pS**TASDVYSF	23	17	250 ± 13 (P3.28)
P4: Smad2 (T8)	MSSILPF**pT**PPVVKRLL	3	2	78 ± 14 (P4.B9)
P5: CREB (S133)	RREILSRRP**pS**YRKILNDL	4	4	151 ± 8 (P5.G10)
P6: HtrA2 (S212)	RRRVRVRLL**pS**GDTYEAVV	21	21	2430 ± 150 (P6.C12)
P7: Akt1 (T308)	KEGIKDGATMK**pT**F	0	0	n.d.
P8: Akt1 (S473)	ERRPHFPQF**pS**YSASGTA	1	1	>5000[c] (P8.H9)
P9: PKC Θ (S695)	DQNMFRNF**pS**FMNPGMER	1	0	n.d.
P10: Sgk1 (S422)	EAAEAFLGF**pS**YAPPTDSF	4	4	42.2 ± 2.8 (P10.D6)

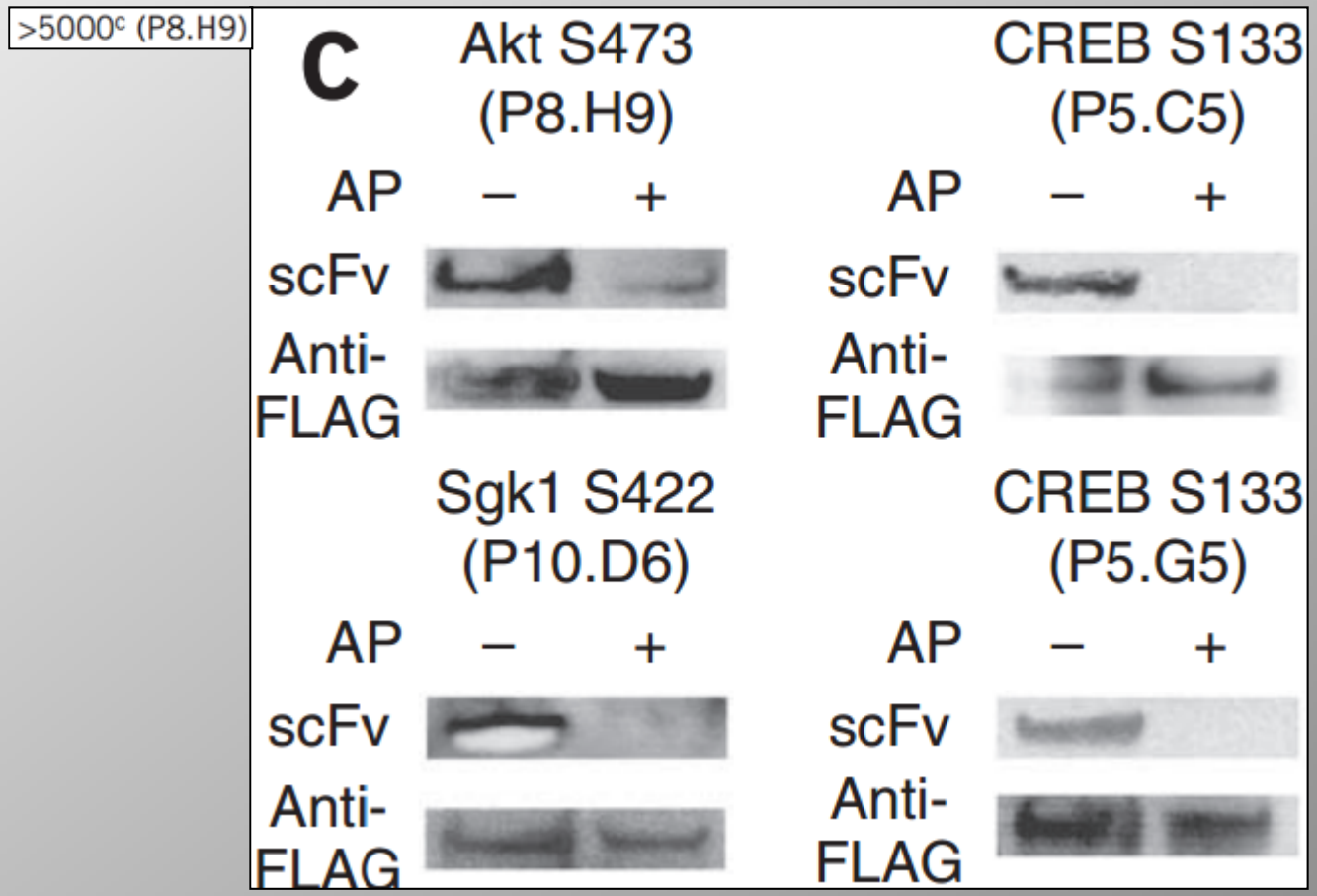


ELISA

Results

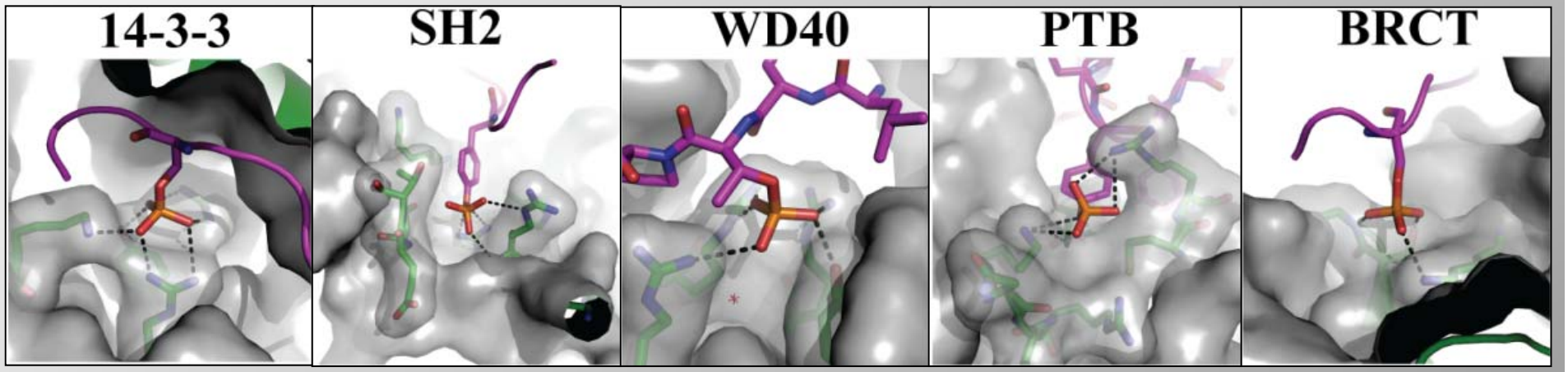
Validation of the selected scFvs with WB on phosphorylated full length proteins

FLAG-tagged target proteins from HEK293T cells +/- treated with alkaline phosphatase (AP)

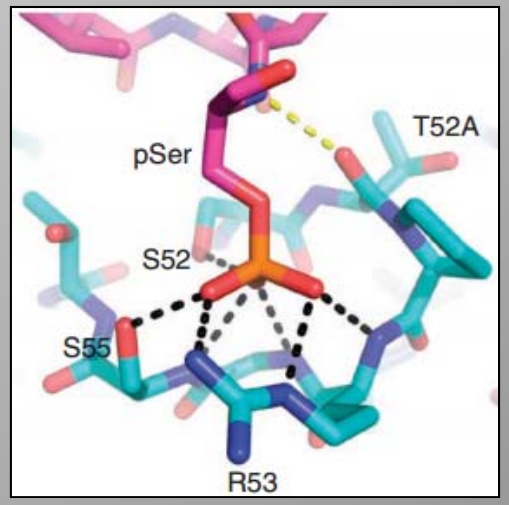


Conclusion

Nature inspired



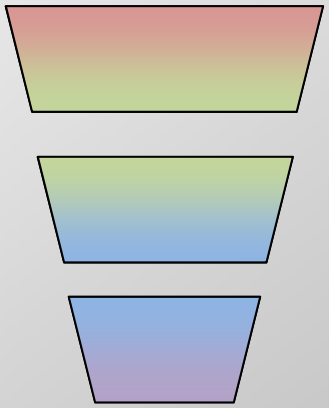
design of motif-specific antibody scaffolds



Discussion

Significance and relevance of the results

- High affinity (42-250 nM Kd)
- Easier/quicker selection in phage display (conserved phosphate binding nest): competition with unphosphorylated peptide



Phage Display Library (random)

Direct Selection of Monoclonal Phosphospecific Antibodies without Prior Phosphoamino Acid Mapping
Ole Vielemeyer, JBC, 2009

Phage Display Library from pre-immunized animal

An Ultra-specific Avian Antibody to Phosphorylated Tau Protein Reveals a Unique Mechanism for Phosphoepitope Recognition
Heather H. Shih, JBC, 2012

Fixed PTM recognizing nest in the library

JT Koerber, Ph.D.
Ph.D. University of California, Berkeley
B.S. University of Illinois
Project: **Engineering Protein Scaffolds to Detect Cellular Apoptosis**
Email: jt.koerber@ucsf.edu

= High throughput biomarker screening

Competing financial interests
J.T.K., W.F.D. and J.A.W. have filed a provisional patent regarding the technology described in this manuscript.