Synthetic Proteins: New Tools for New Biology

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Donnelly Centre for Cellular and Biomolecular Research
University of Toronto

The Central Dogma

DNA

(Easy): Sequencing

RNA

(Pretty Easy): RNAi

Protein

(Hard): ?

Why Study Proteins?

- We work in the genomics era…
- But we live in a protein world
- Proteins drive biology and disease

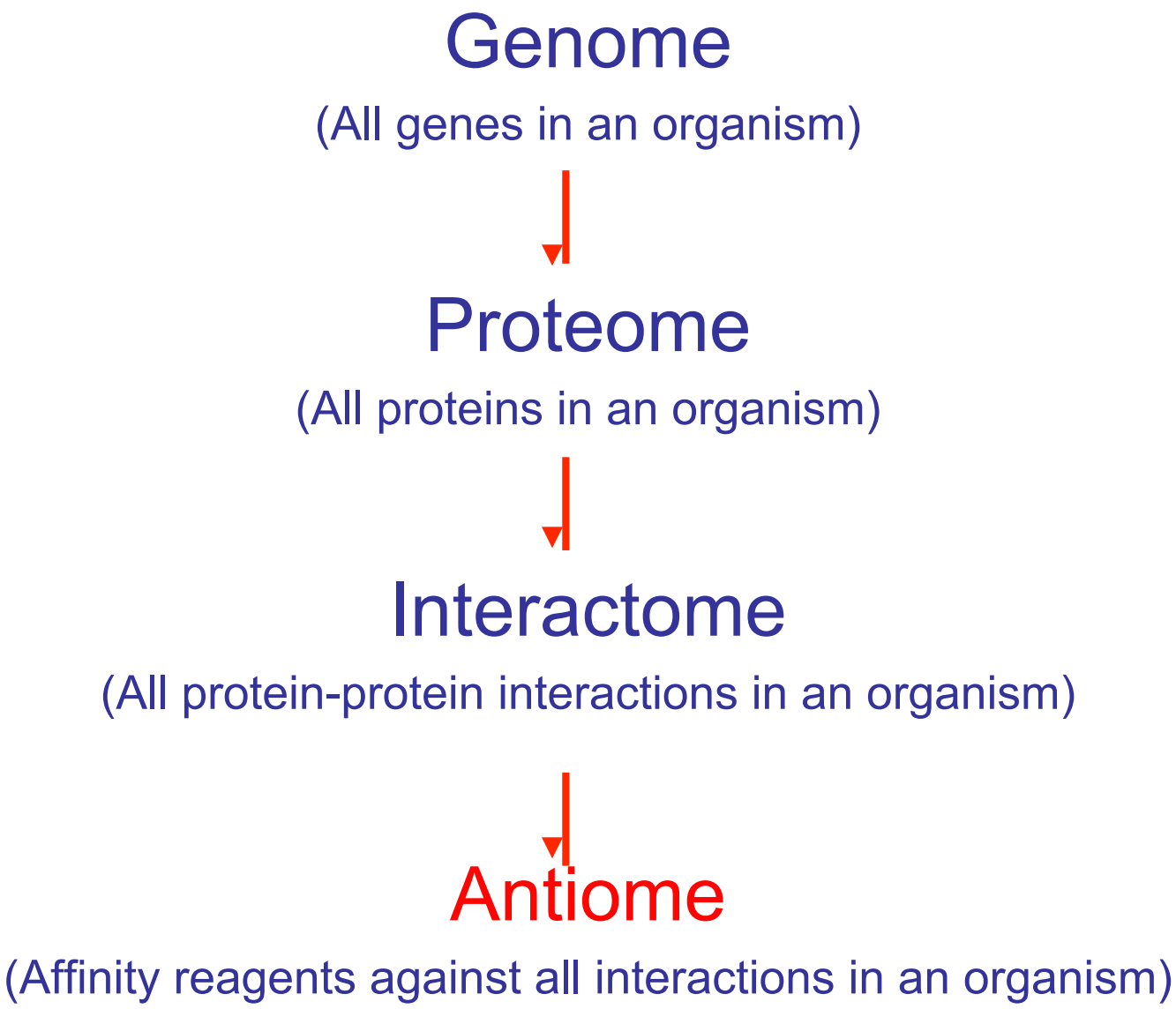
Central Problem:

Proteins are important…

But proteins are hard.

What can I do?

GO BIG → Invent an ‘ome


Genome
(All genes in an organism)
Proteome
(All proteins in an organism)
Interactome
(All protein-protein interactions in an organism)
Antiome
(Affinity reagents against all interactions in an organism)

Mission Statement

Develop technology that enables protein-level targeting of all protein-protein interactions

Problem

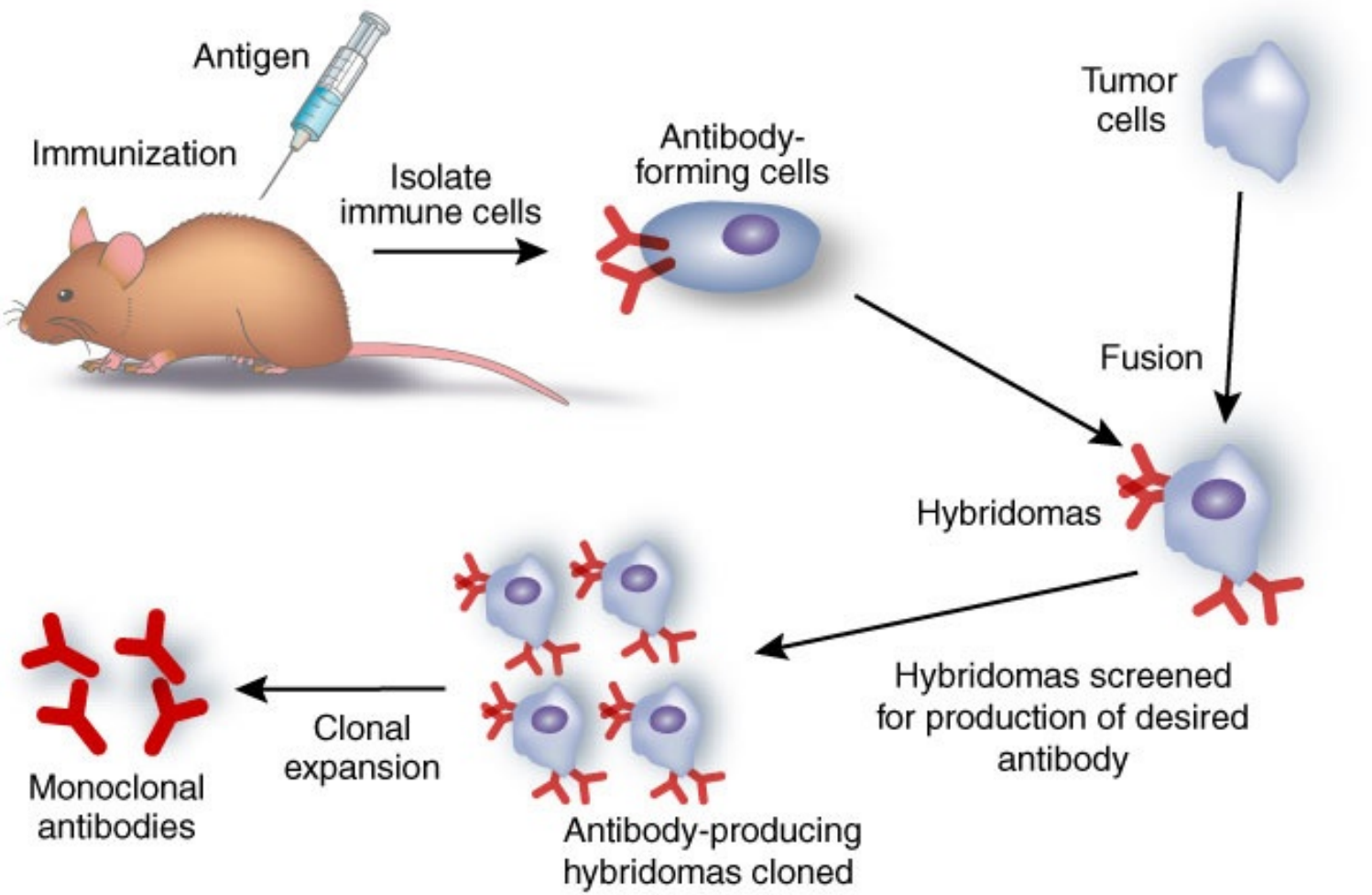
DNA and RNA are easy
Proteins are hard

Mission Statement

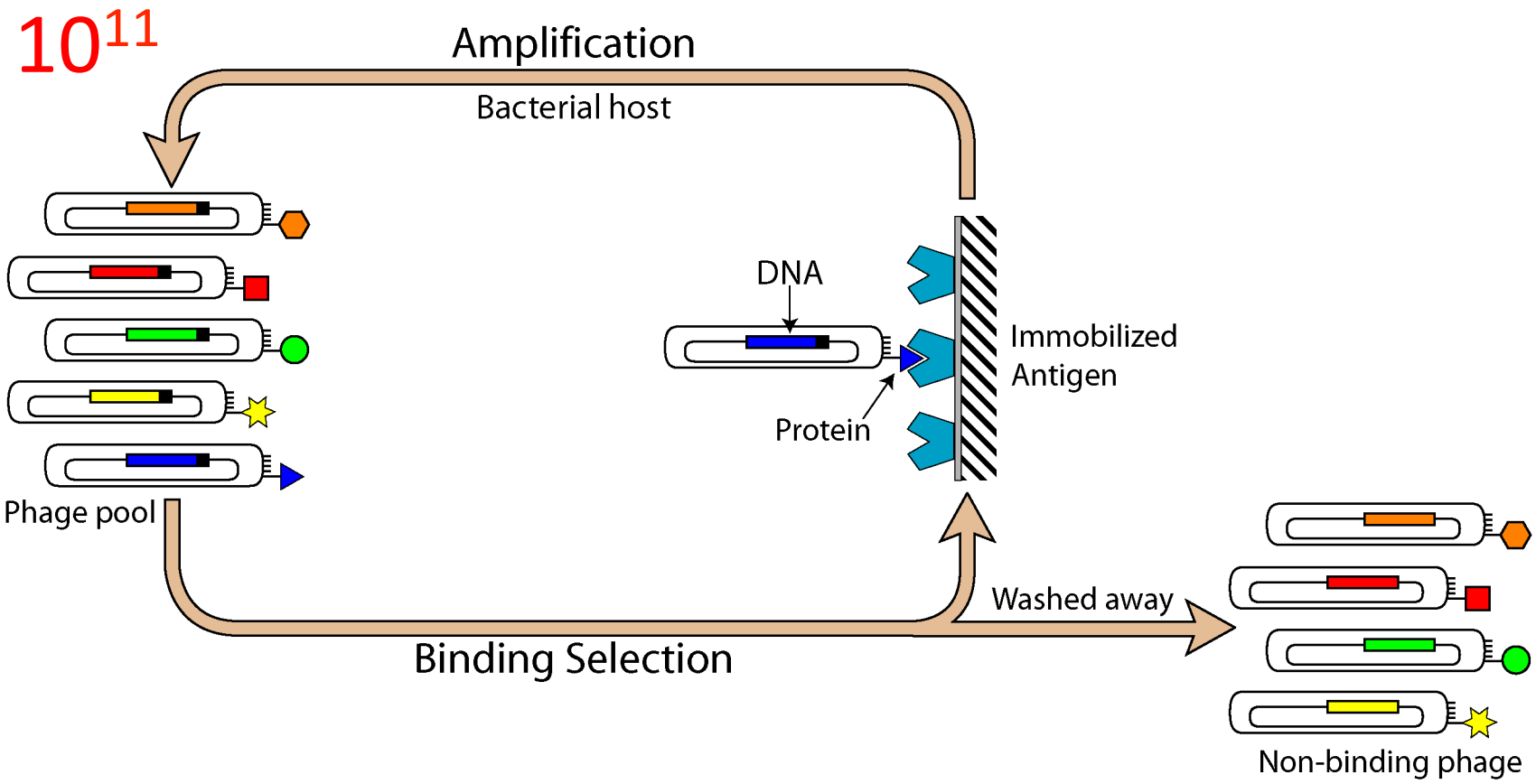
You got to use what you got to get just what you want-ah

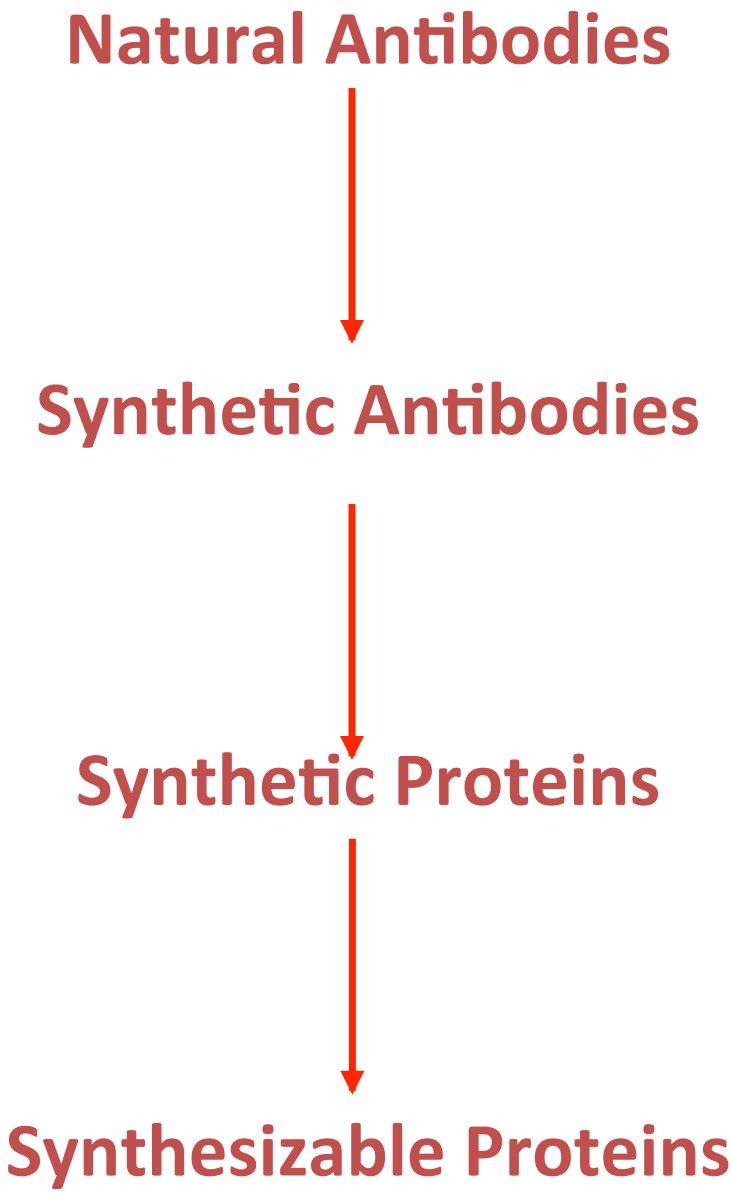
James Brown (1971) *Hot Pants*

What we got?
We got mice



What we got?
We got phage display

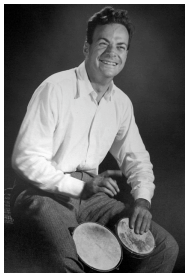



Natural Antibodies
Synthetic Antibodies
Synthetic Proteins
Synthesizable Proteins

Why Synthetic Proteins?
D.I.Y. is FUN

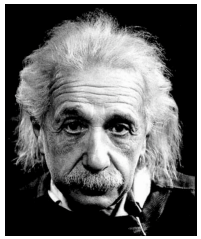
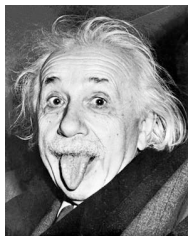
If I can't create it, I don't understand it.

(*Richard Feynman*)

Everything should be made as simple as possible, but no simpler.

(*Albert Einstein*)

Give it away, give it away, give it away now.

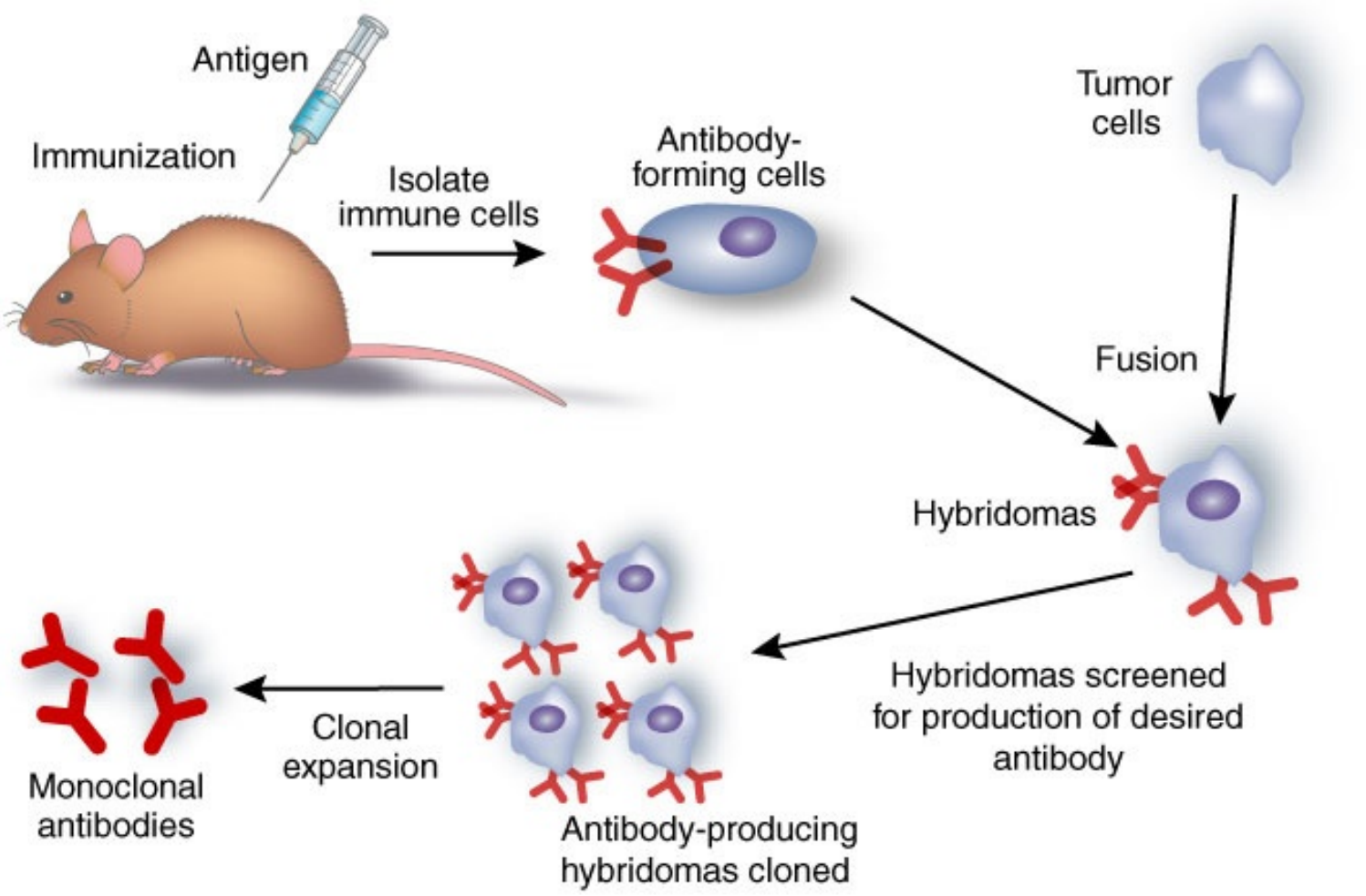
(*Red Hot Chili Peppers*)

Natural Antibodies

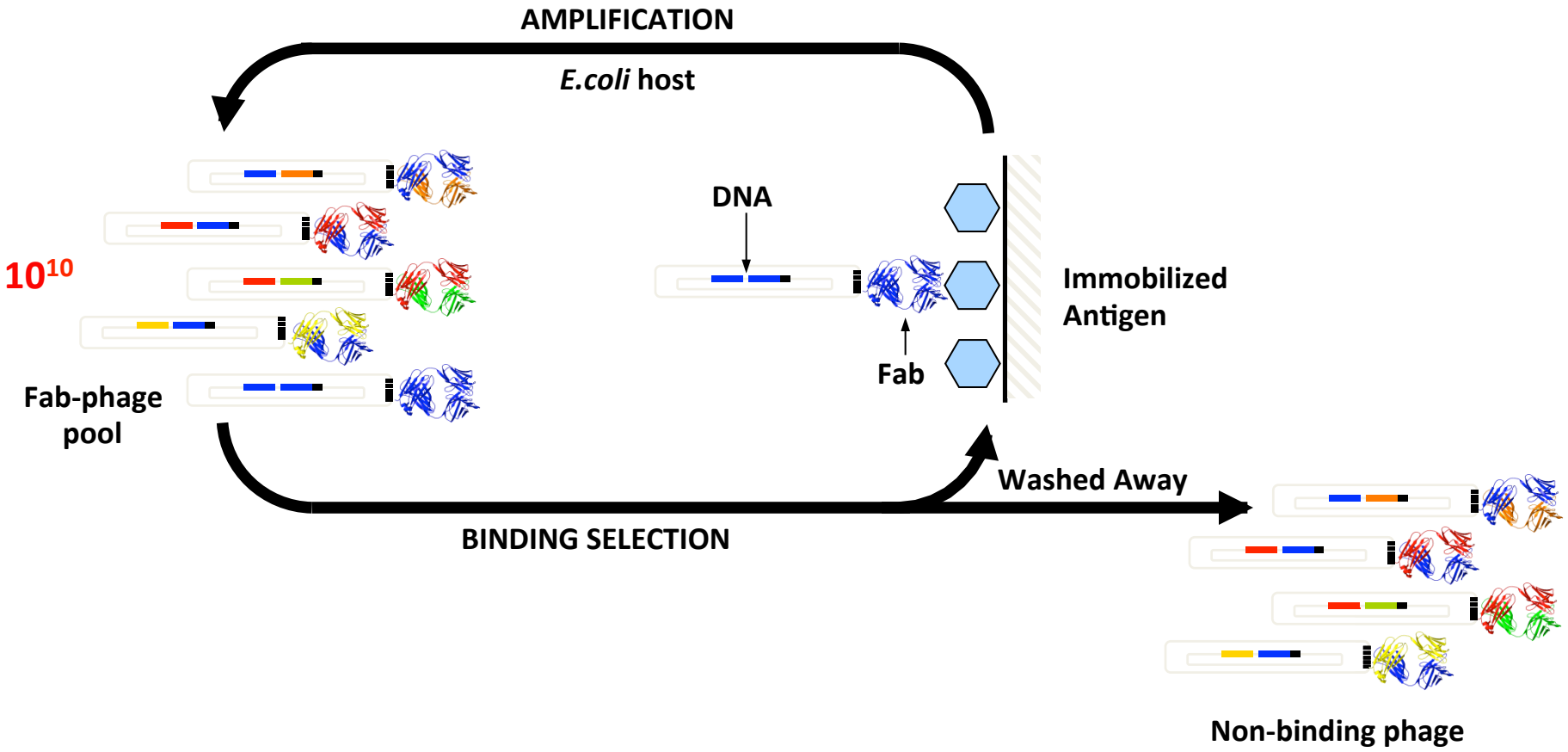
Synthetic Antibodies

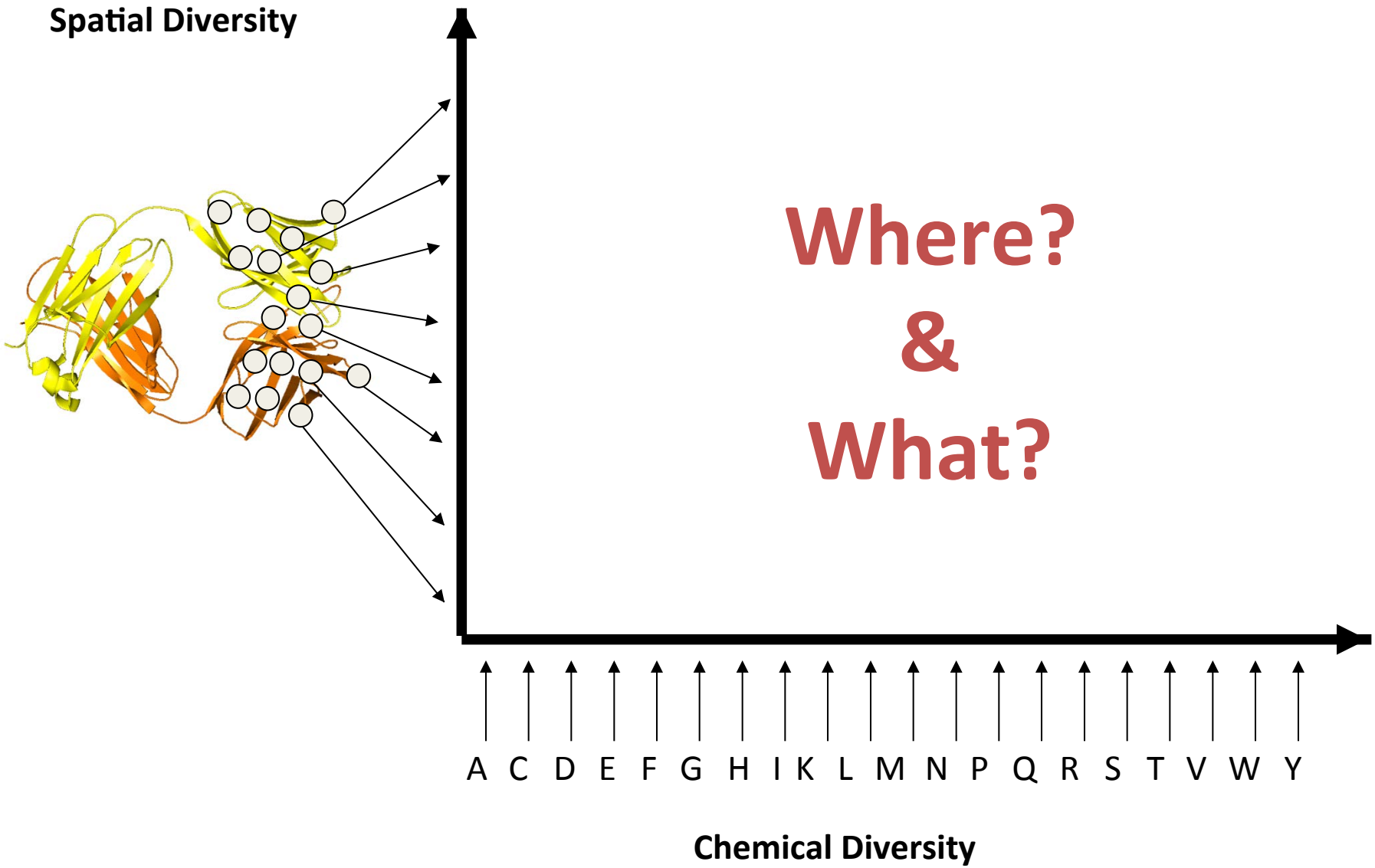
Hybridoma Monoclonal Antibodies

Molecular Lottery



Antibody Phage Display
Molecular Poker

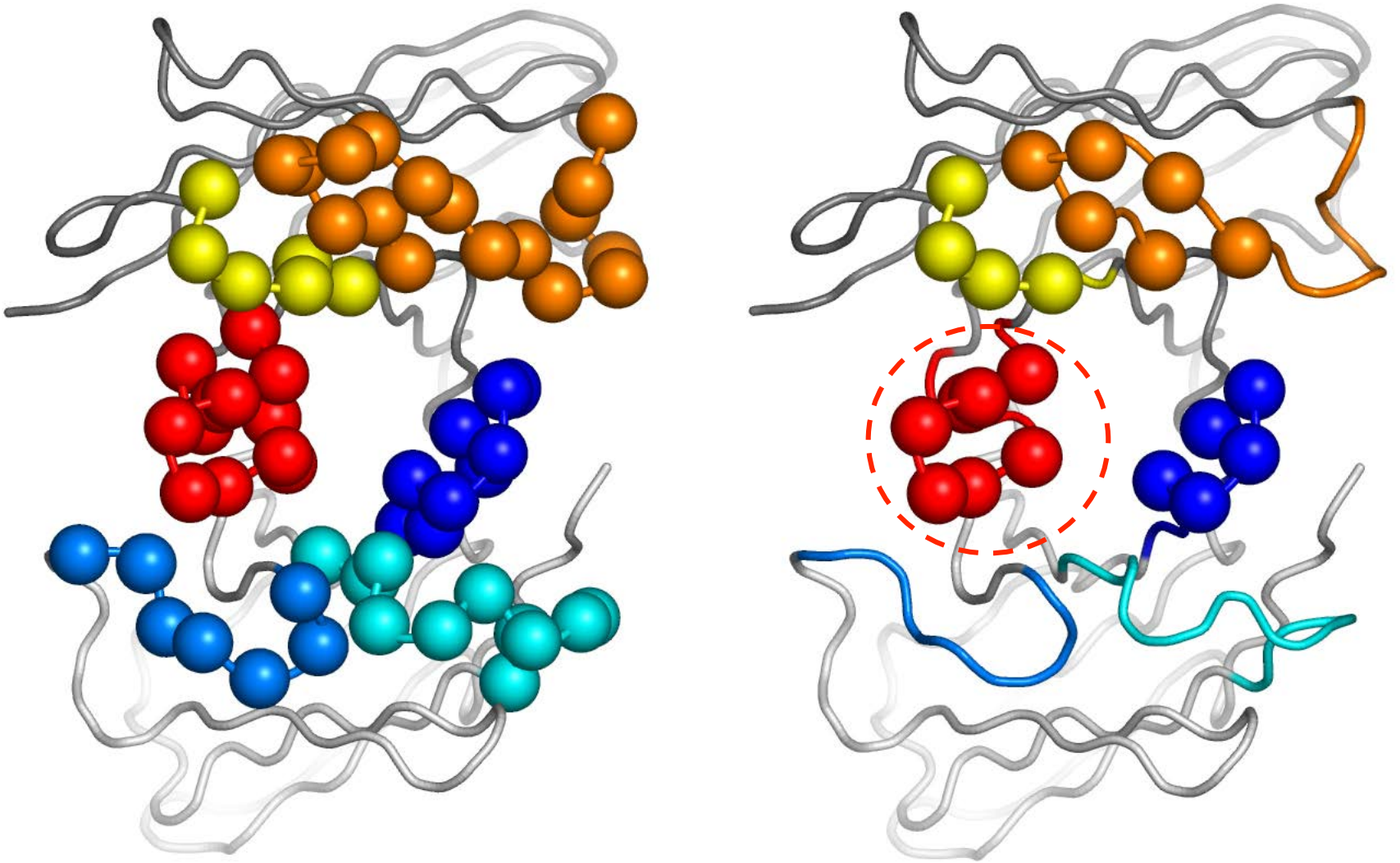


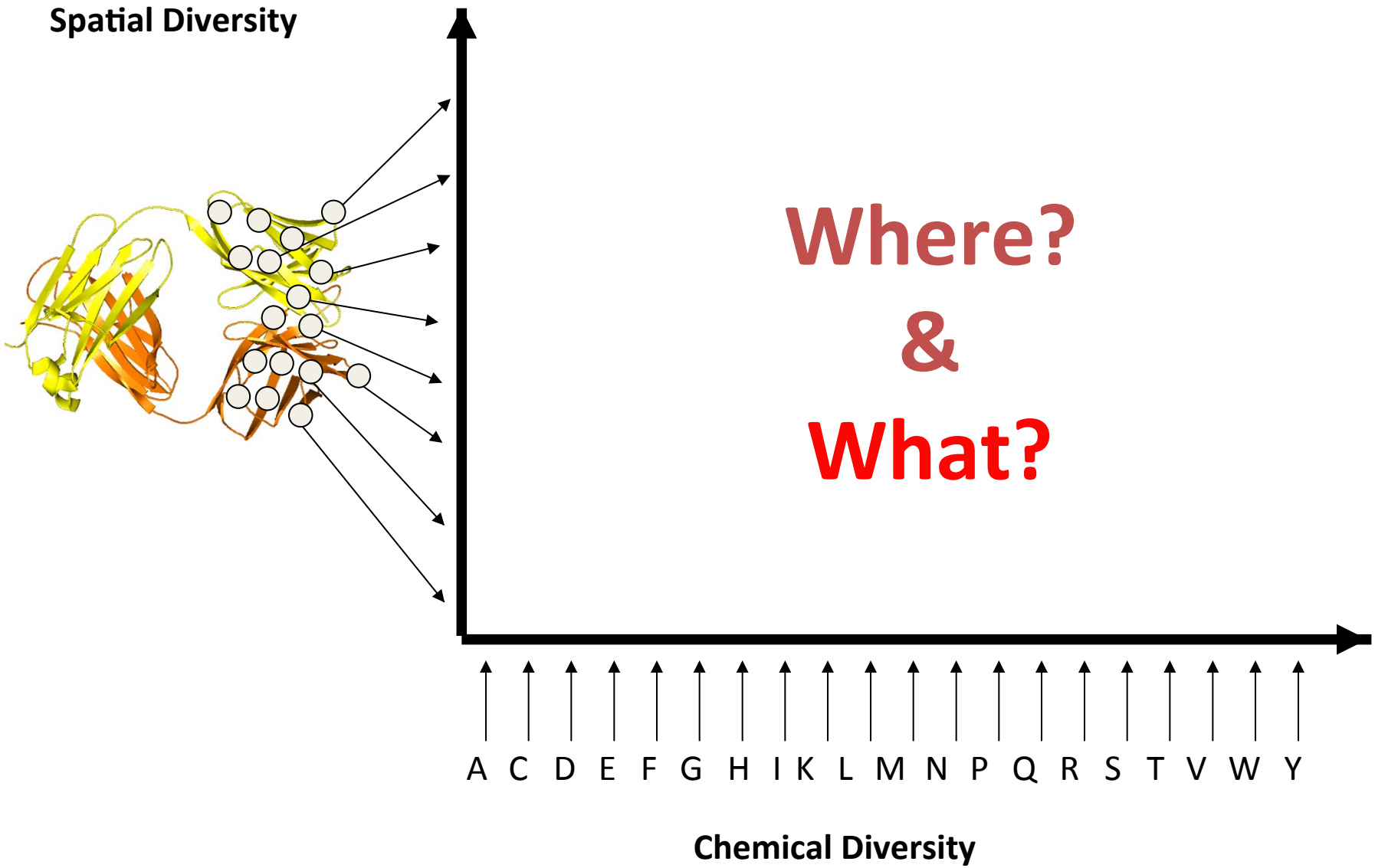

Spatial Diversity
Where?
&
What?
A C D E F G H I K L M N P Q R S T V W Y
Chemical Diversity

Focusing Spatial Diversity

Natural Repertoire

Synthetic Repertoire




Spatial Diversity
Where?
&
What?
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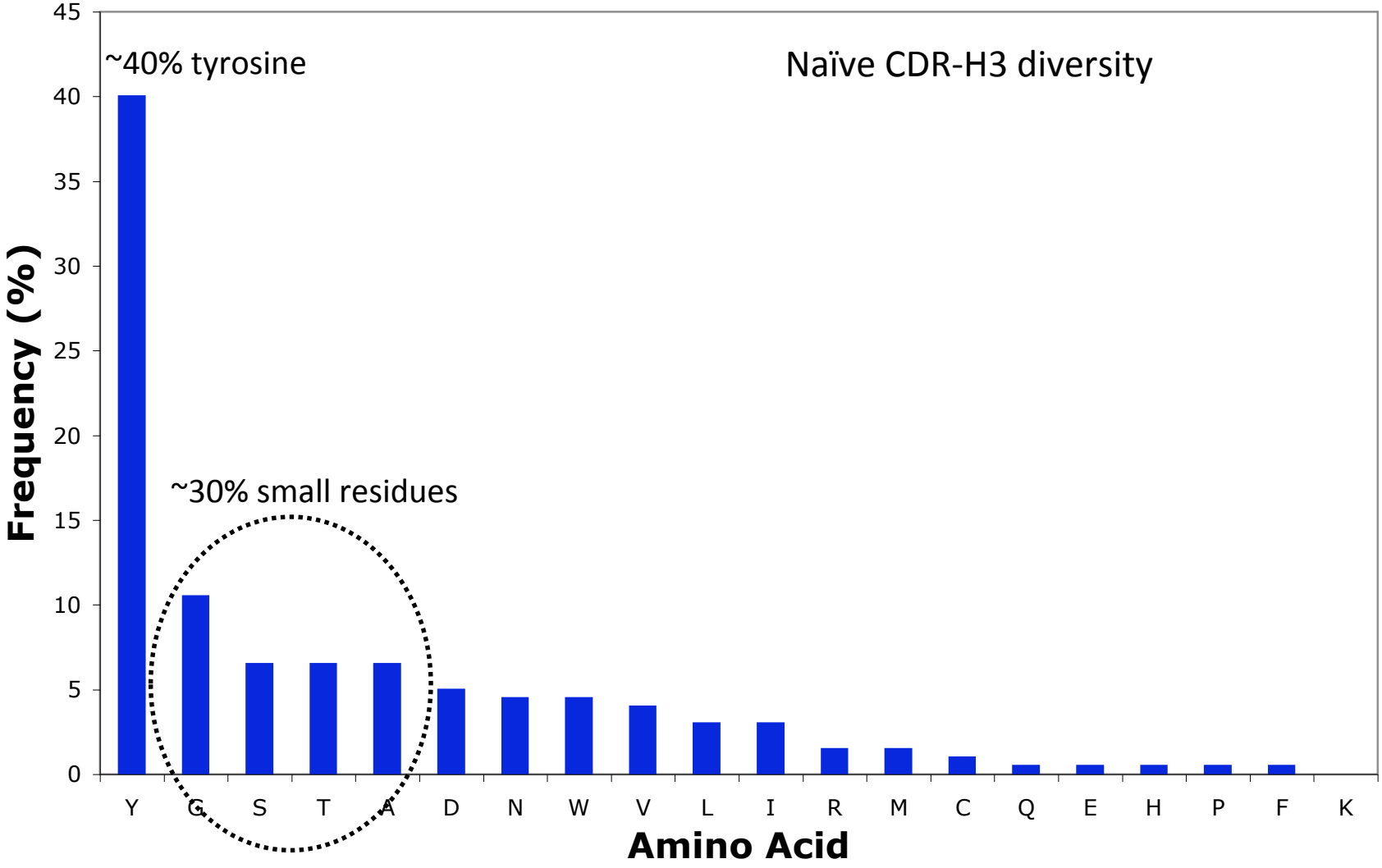
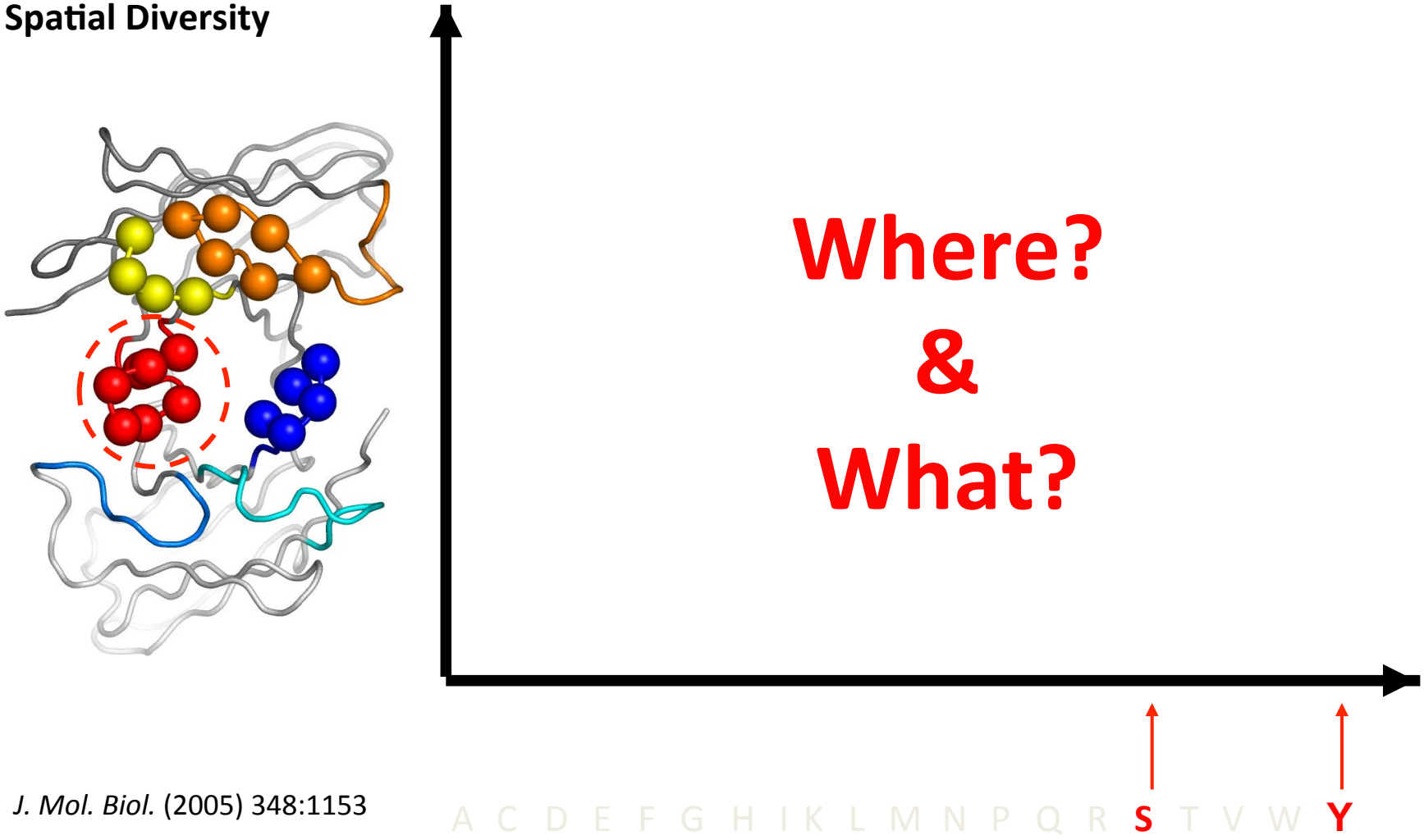
Chemical Diversity

Focusing Chemical Diversity



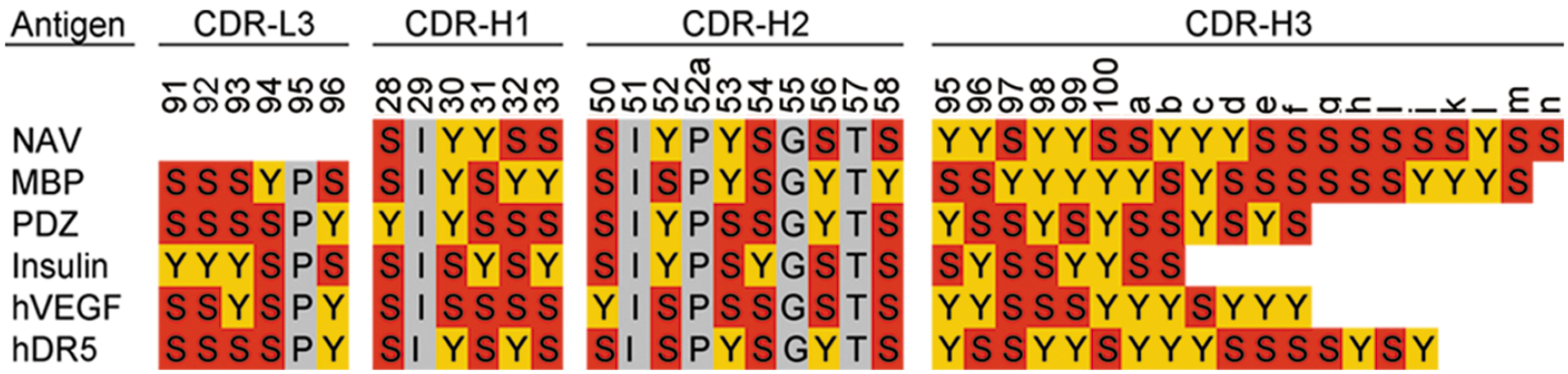
Adapted from Zemlin *et al*. JMB (2003)

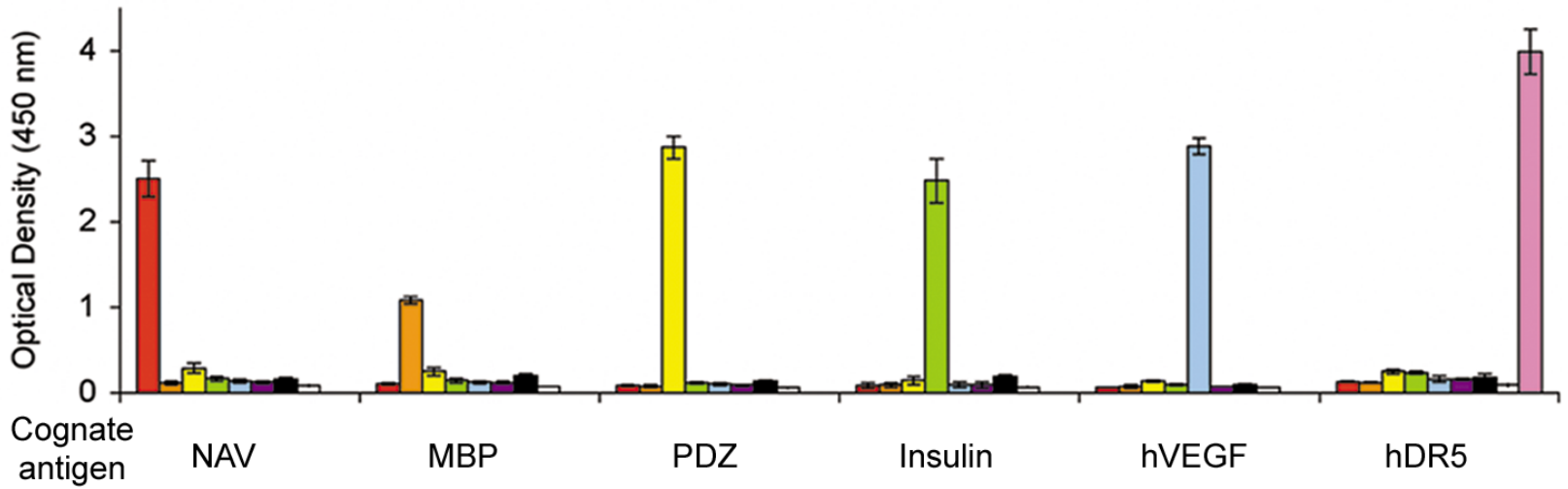
Focused Space and Chemistry



J. Mol. Biol. (2005) 348:1153

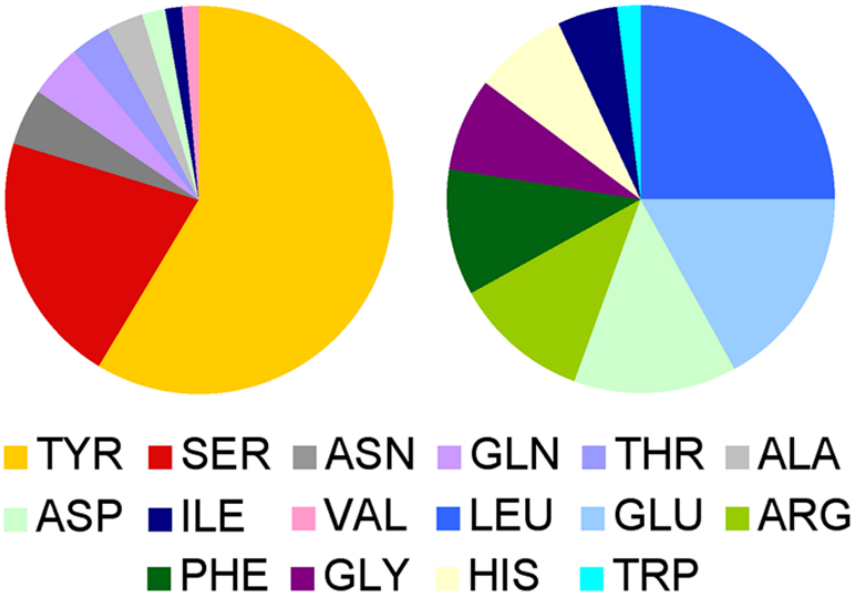
Binary Binders



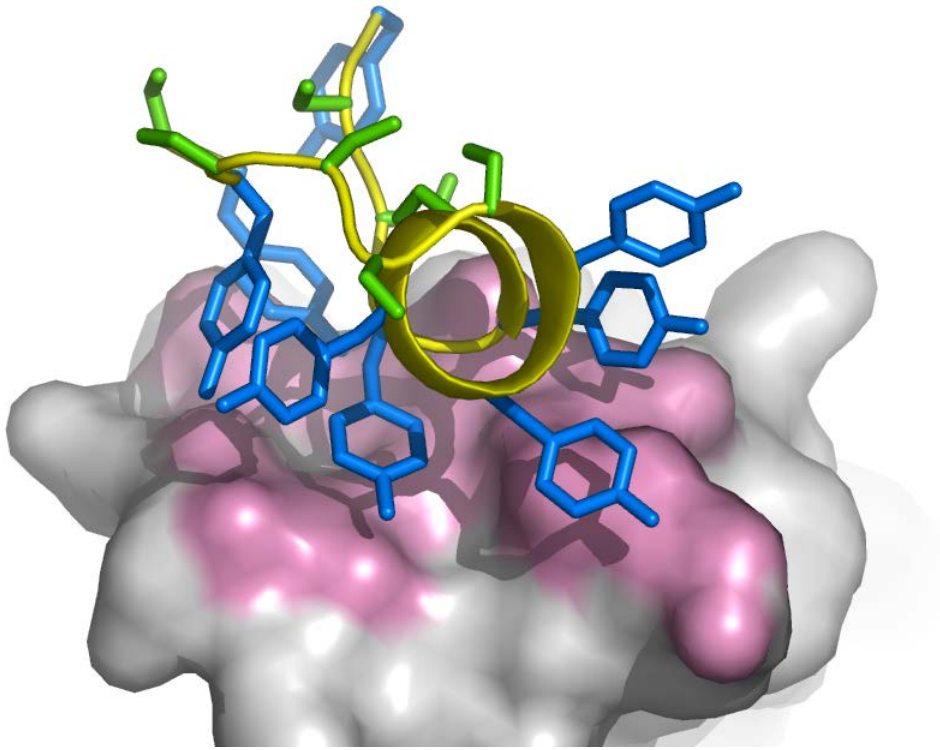


Interface composition

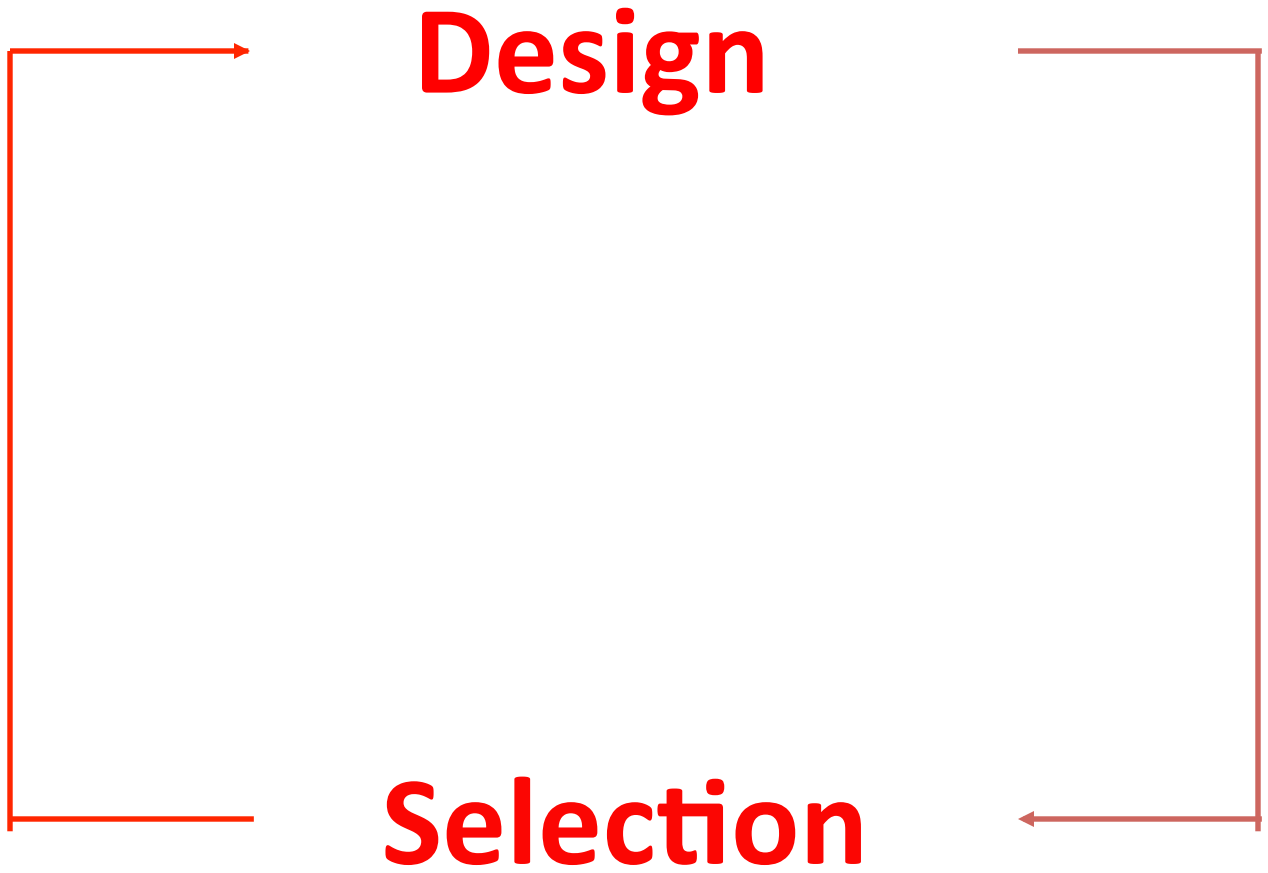
Antibody Antigen



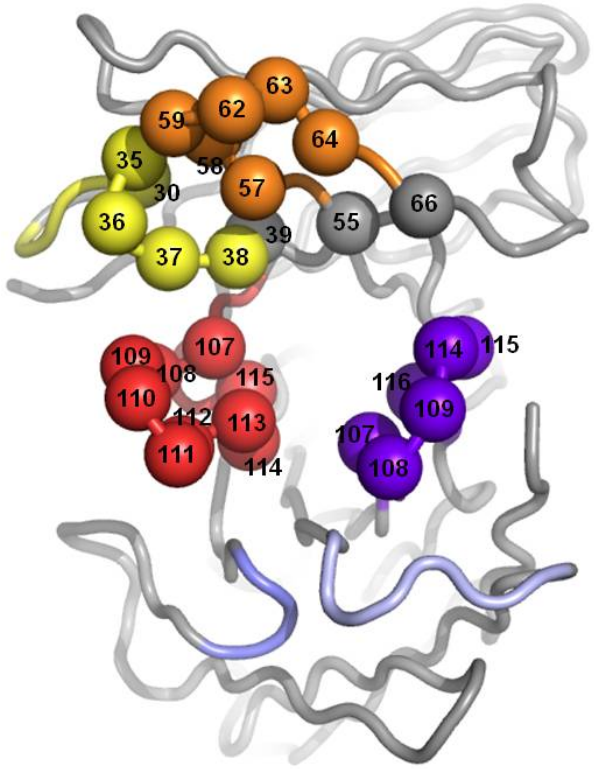
CDRH3:Antigen interface



Fred Fellouse


Design
Selection

The Toronto Synthetic Antibody Library



CDR-L3

105	106	107	108	109	114	115	116	117
Q	Q	X	X	X	X	PL	FI	T

CDR-H1

27	28	29	30	35	36	37	38	39
G	F	N	IL	YS	YS	YS	YS	IM

CDR-H2

55	56	57	58	59	62	63	64	65	66
YS	I	YS	PS	YS	YS	GS	YS	T	YS

CDR-H3

105	106	107	108	109	110	111	112	113	114	115	116	117
A	R	X	X	X	X	X	X	X	AG	FILM	D	Y

Theoretical Diversity					Actual
CDR-L3	CDR-H1	CDR-H2	CDR-H3	Total	Diversity
896	128	256	5×10^{22}	1×10^{30}	3×10^{10}

Persson et al. (2012) J. Mol. Biol.

Beyond Natural Antibodies

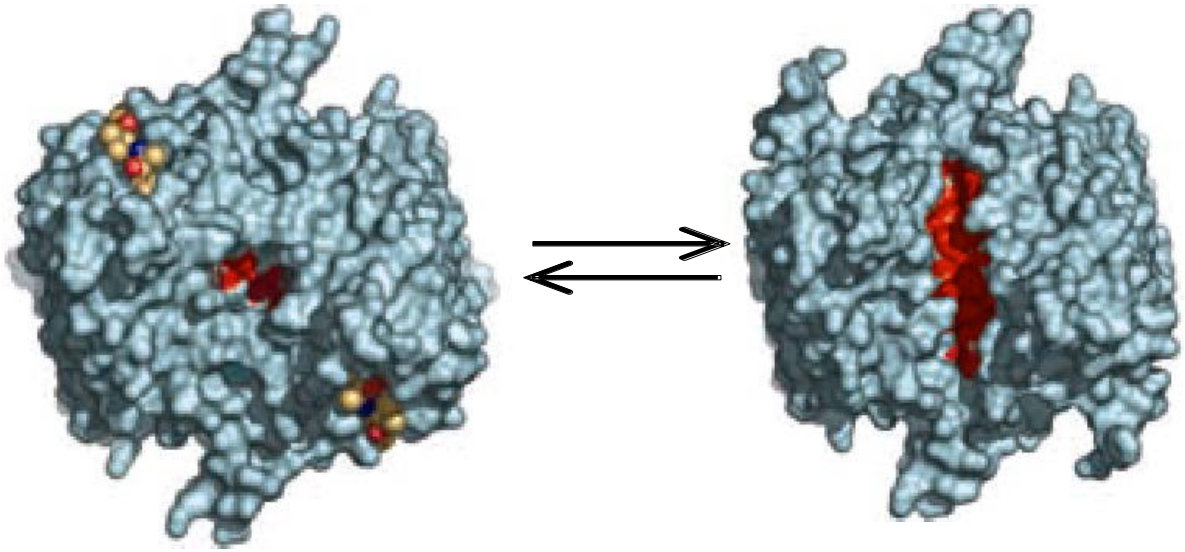
Conformation-specific antibodies

Antibodies against structured RNA

Detecting post-translational modifications

Targeting integral membrane proteins

Conformation Specific Anti-Caspase-1



On-state Off-state

UCSF
Jim Wells
Junjun Gao

Proc. Natl. Acad. Sci. USA (2009) 106:3071

	On form	Off form
On selective	**12 nM**	> uM
Off selective	150 nM	**3 nM**

Beyond Natural Antibodies

Conformation-specific antibodies

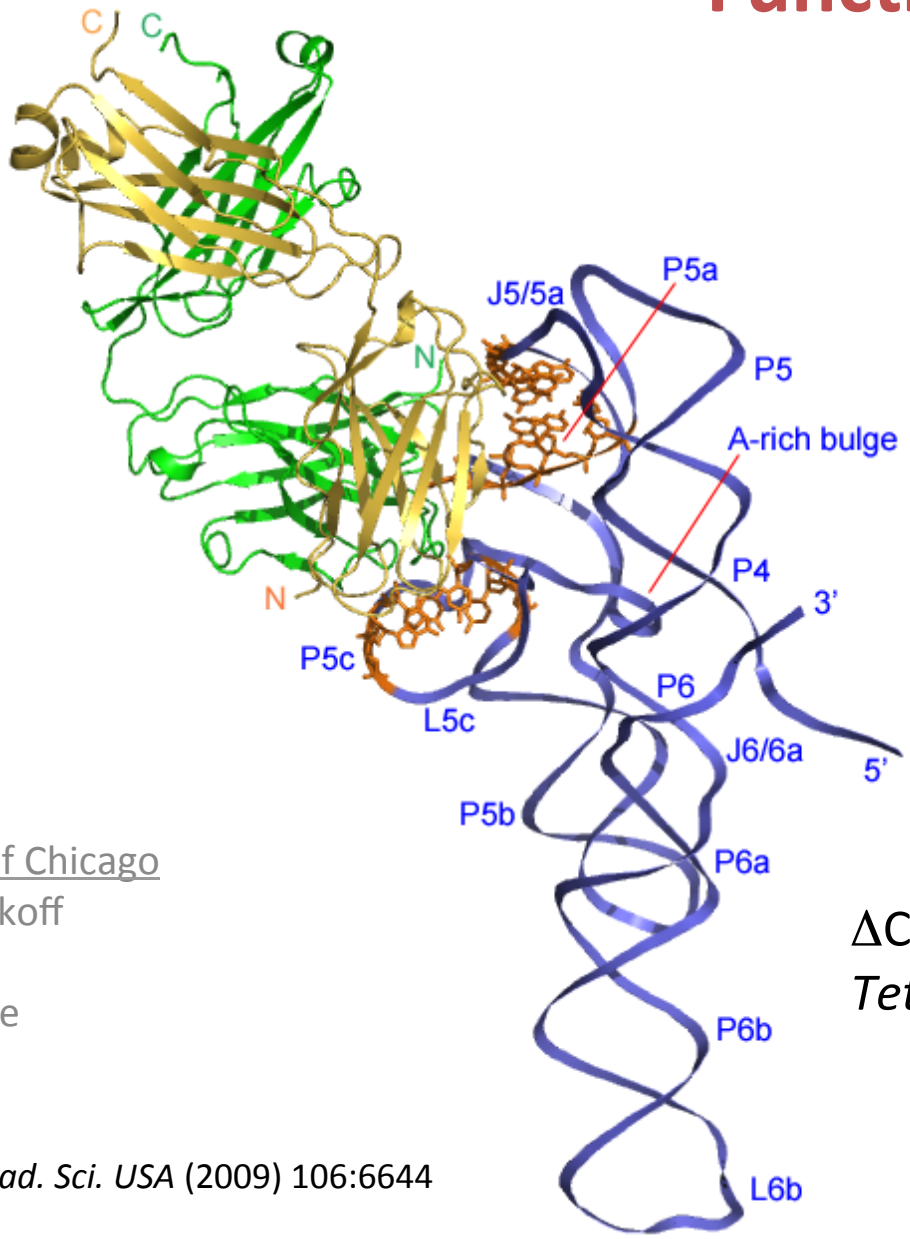
Antibodies against structured RNA

Detecting post-translational modifications

Targeting integral membrane proteins

Functional RNAs

Fab



University of Chicago
Tony Kossiakoff
Joe Piccirilli
Shohei Koide
Jin Dong Li

ΔC209 P4P6 domain of
Tetrahymena Group I intron

Proc. Natl. Acad. Sci. USA (2009) 106:6644

Beyond Natural Antibodies

Conformation-specific antibodies

Antibodies against structured RNA

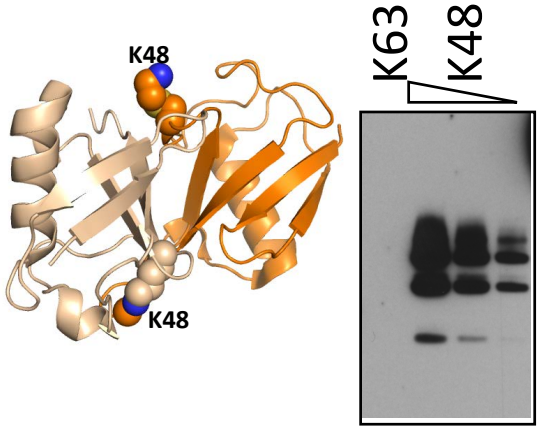
Detecting post-translational modifications

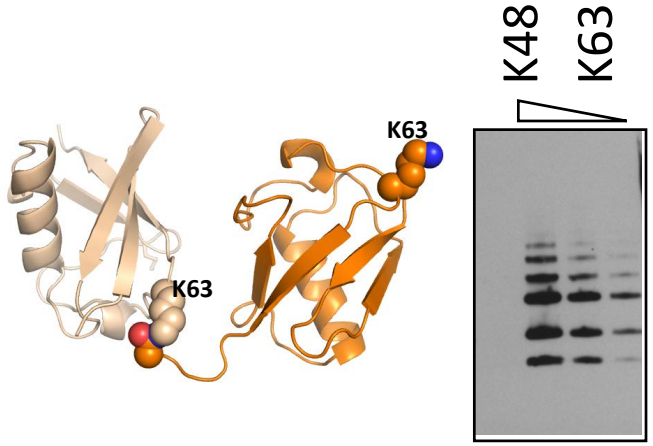
Targeting integral membrane proteins

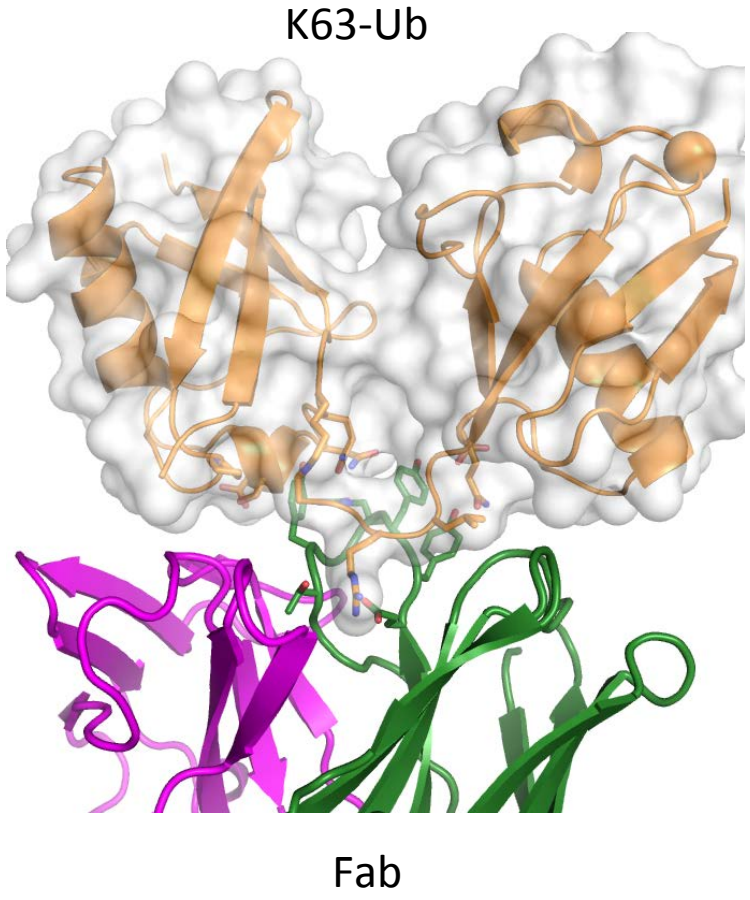
Cross-Link Specific Anti-Ubiquitins

Genentech
Vishva Dixit
Bob Kelley
Sarah Hymowitz
Ingrid Wertz
Kim Newton
Nat Gordon

Cell (2008) 134:668







Beyond Natural Antibodies

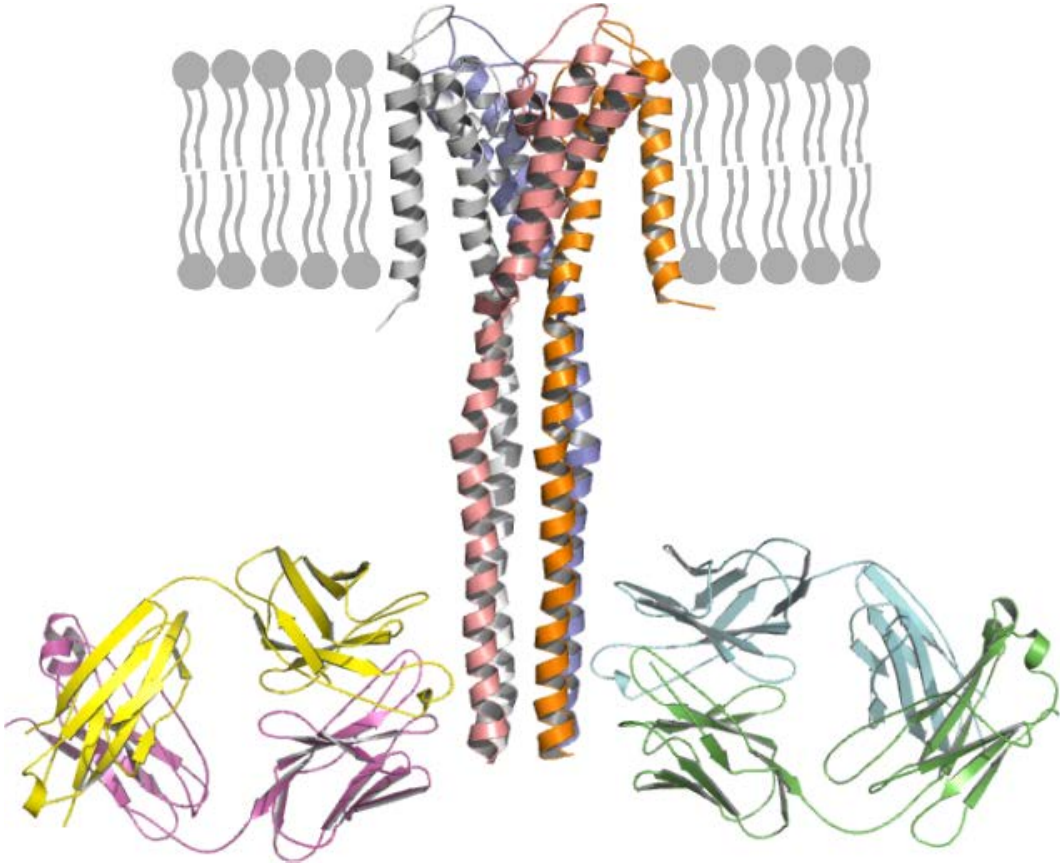
Conformation-specific antibodies

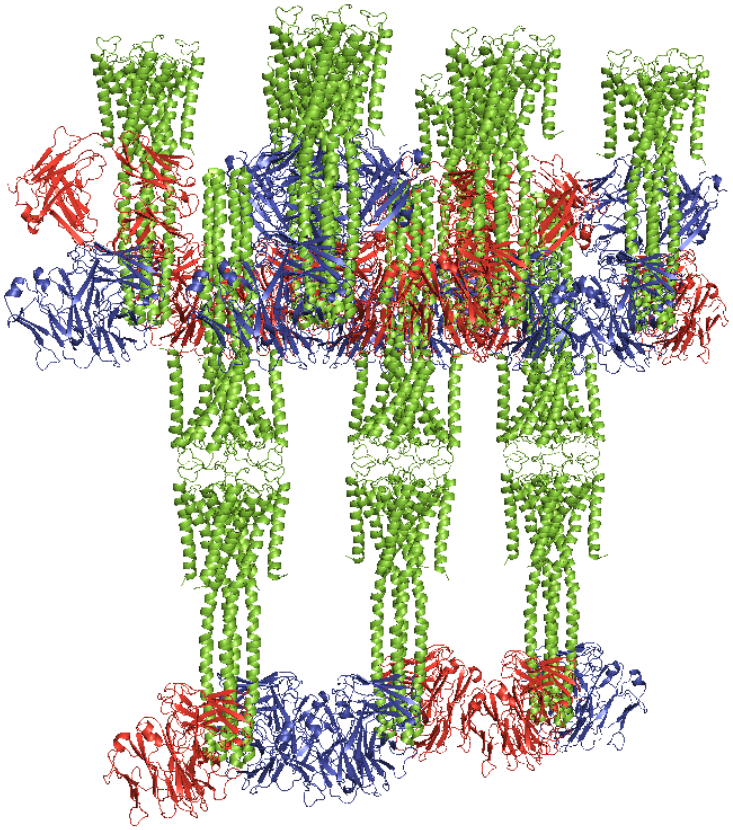
Antibodies against structured RNA

Detecting post-translational modifications

Targeting integral membrane proteins

Crystal Structure of the Full-Length KcsA

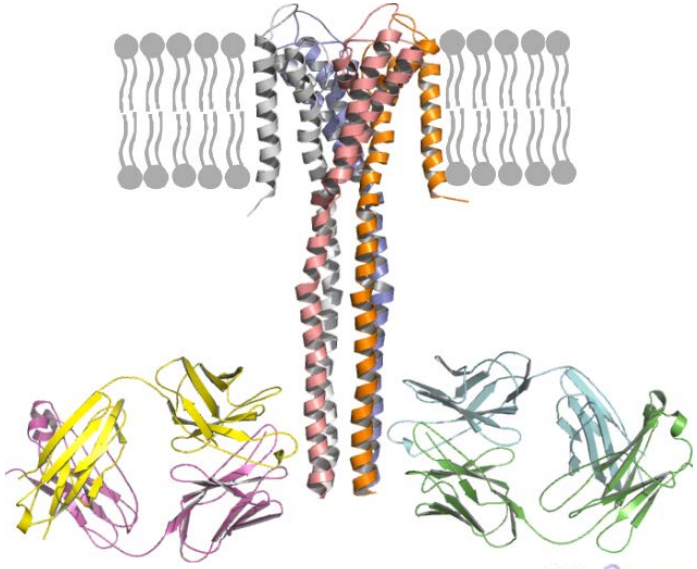


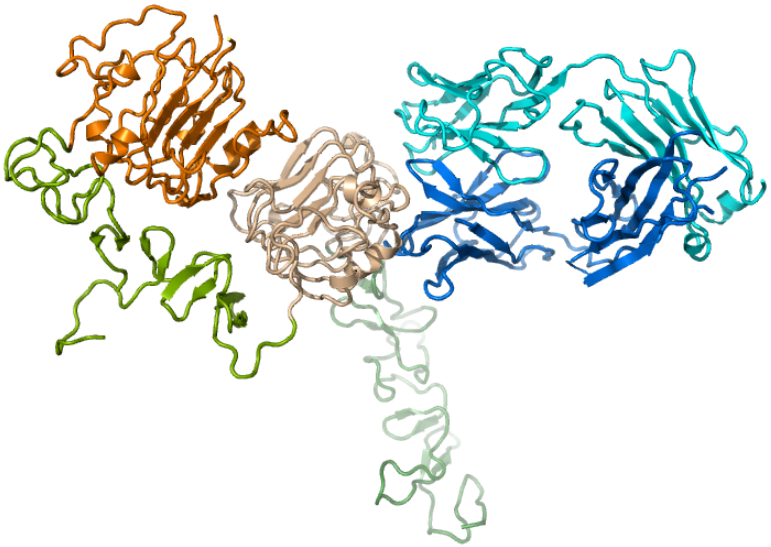


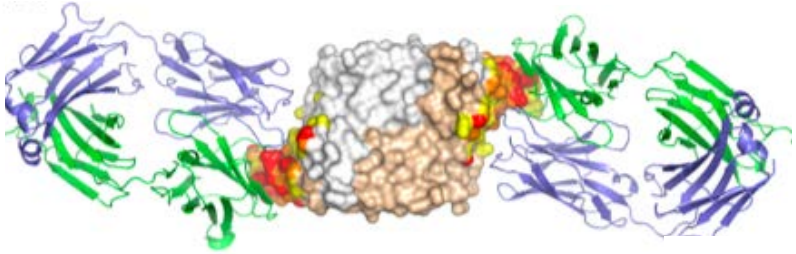
University of Chicago
Tony Kossiakoff
Eduardo Perozo
Shohei Koide
Serdar Uysal
Valeria Vasquez

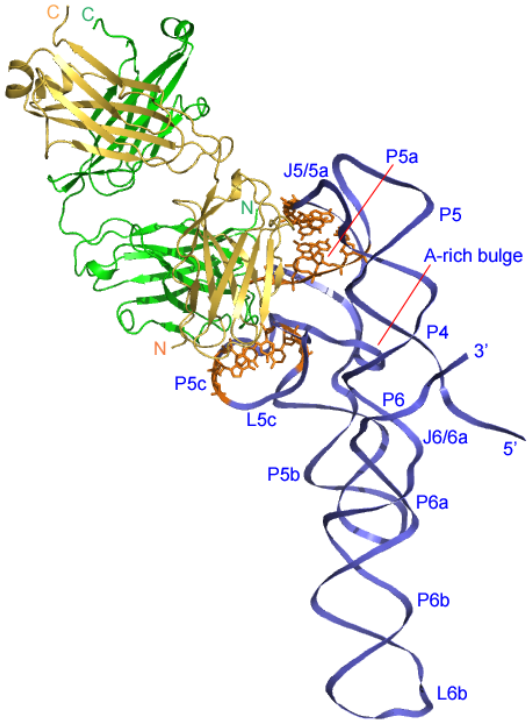
Fabs do not affect ion conductance
Fabs mediate crystal packing

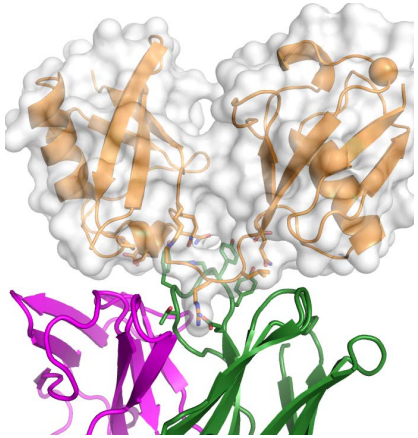
Proc. Natl. Acad. Sci. USA (2008) 105:82

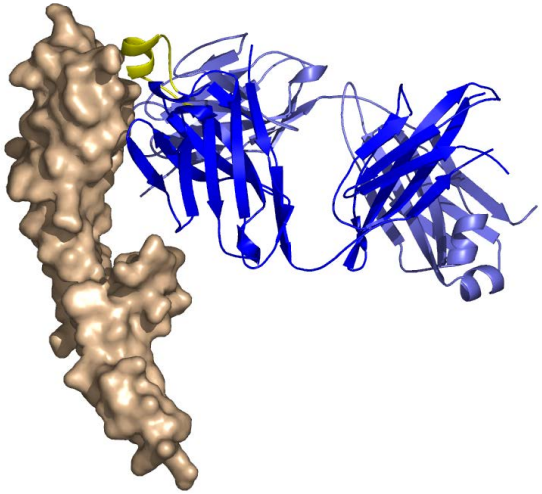







C
C
N
N
J5/5a
P5a
P5
A-rich bulge
P4
3'
P5c
L5c
P6
J6/6a
5'
P5b
P6a
P6b
L6b

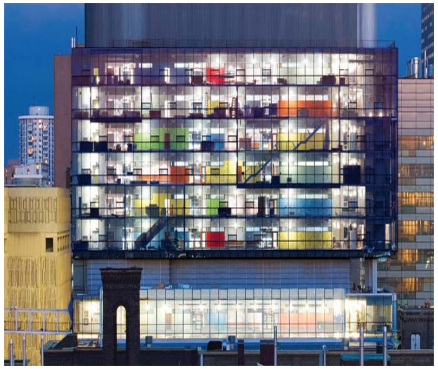




TORONTO RECOMBINANT ANTIBODY CENTRE

Donnelly Centre for Cellular + Biomolecular Research UNIVERSITY OF TORONTO | UNIVERSITY OF TORONTO

- Established in 2010
- Fully equipped for developing humanized antibodies for therapeutic and diagnostic applications
- 25-plus core scientists (40% PhDs)



The Donnelly Centre

- Integrated discovery platform
- Scientific leadership
- Disease focus: cancer, infection, inflammation…
- you bring it we swing it, but you pay to play

- Graduate students and postdoctoral fellows welcome
- Tenure track positions at the Donnelly Centre

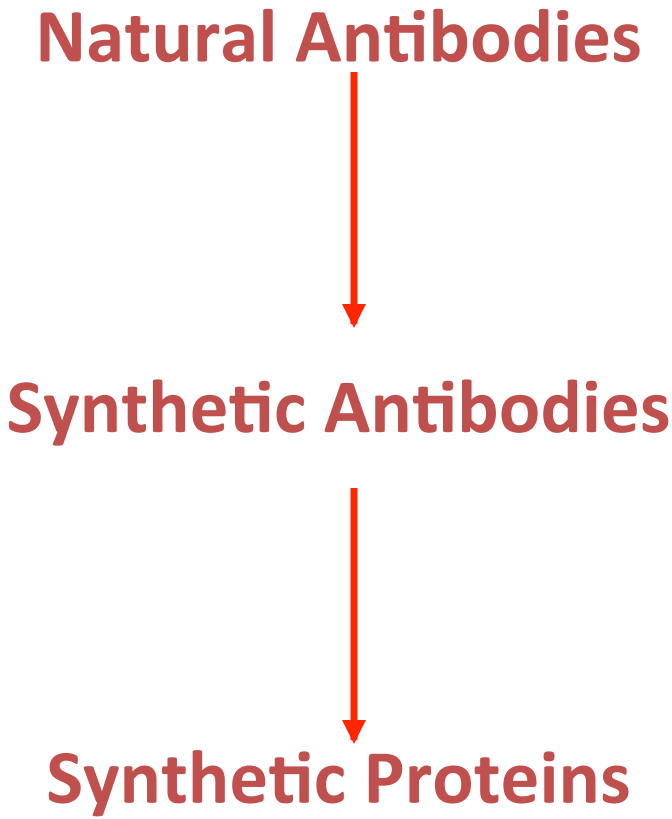
UCSF
University of California
San Francisco

UNIVERSITY OF TORONTO

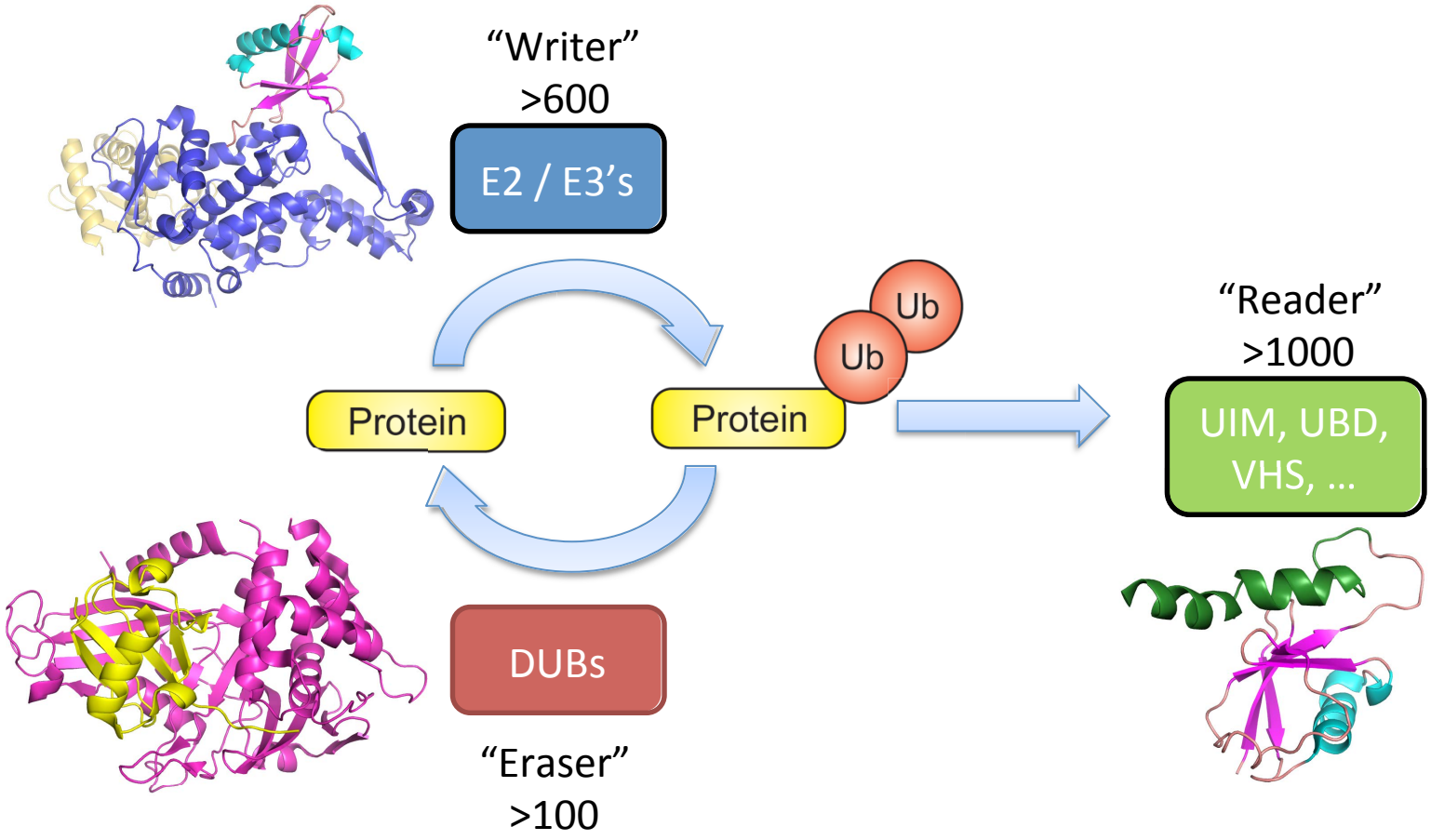
Recombinant Antibody Network

THE UNIVERSITY OF CHICAGO

Three integrated automation centers creating renewable, open-source, high quality binding reagents to the proteome.

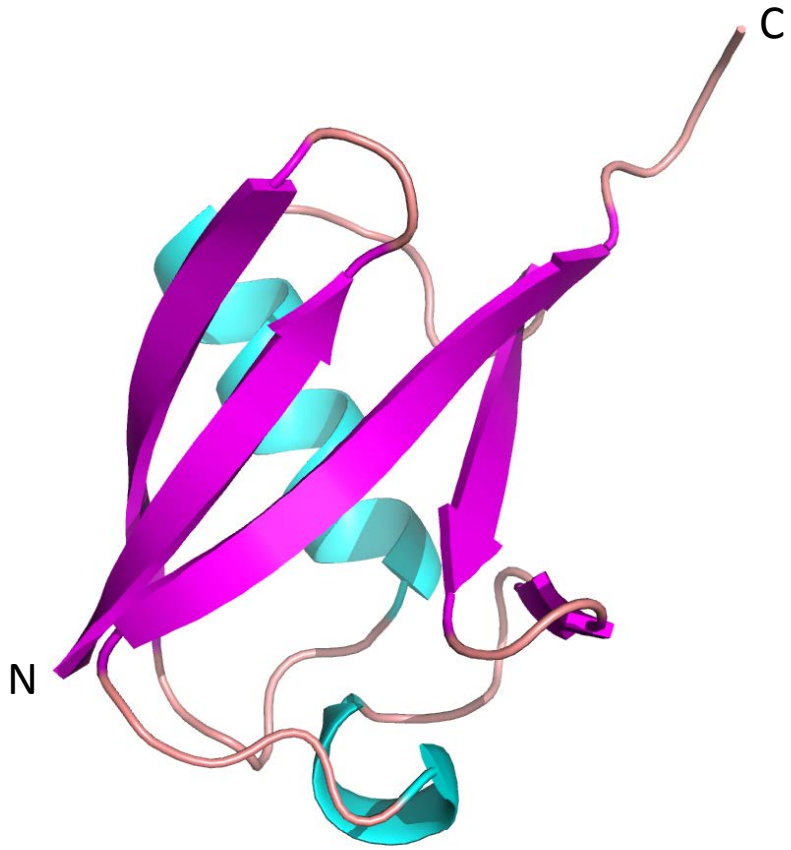

Natural Antibodies
Synthetic Antibodies
Synthetic Proteins

Modulation of the Ubiquitin System

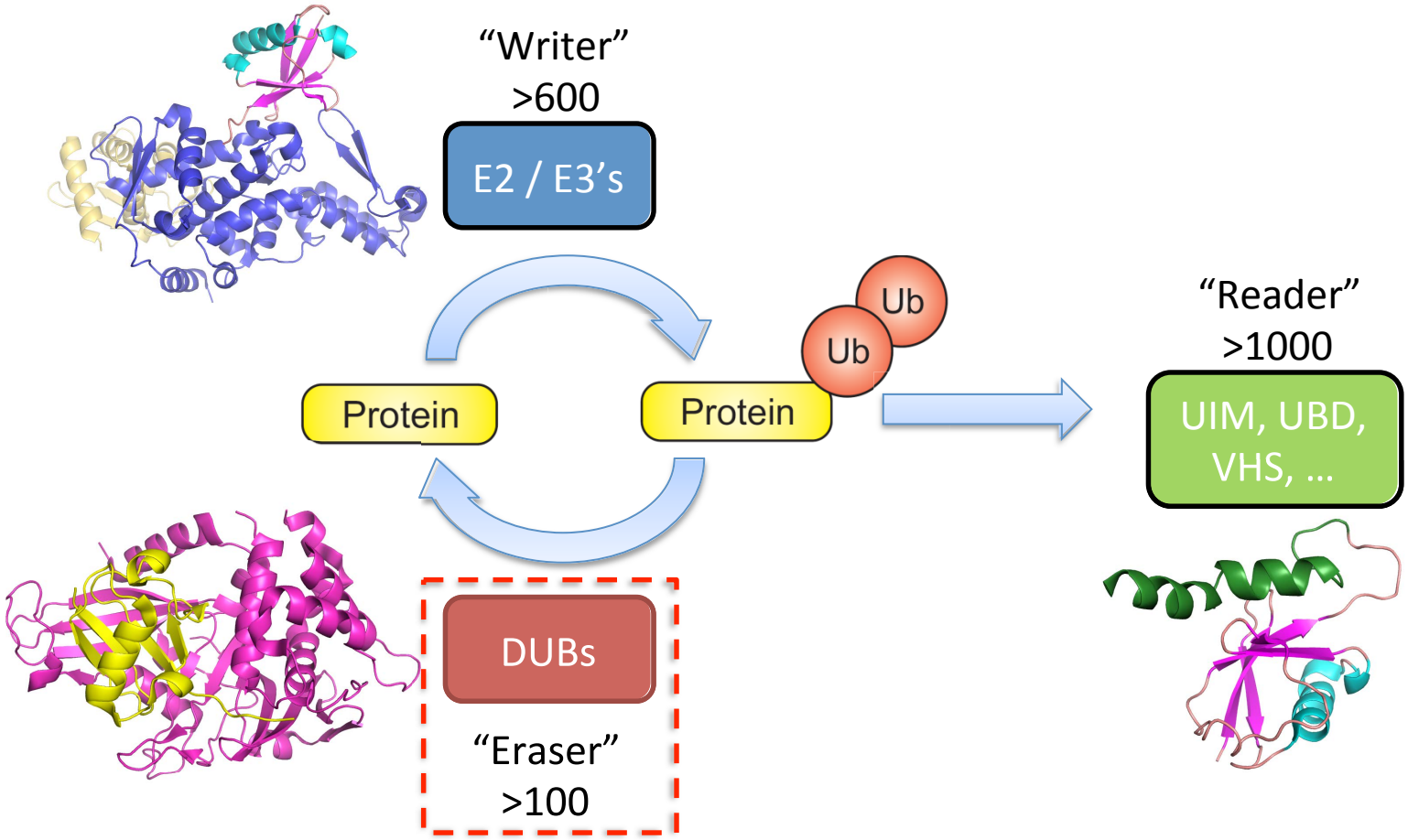


Use Ubiquitin Against Itself

- 76 amino acids
- Stable (T_m = 64°C)
- Large binding surface
- Weak interactions

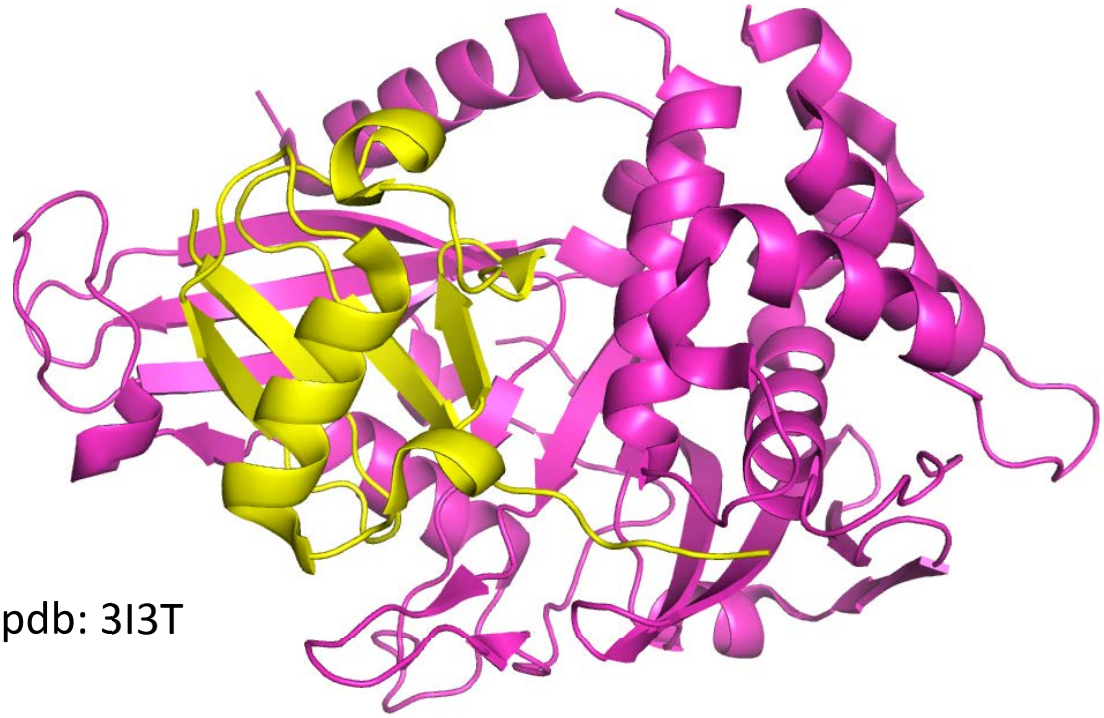


Targeting the "Erasers"



Ernst et al. (2013) Science

Several co-complexes show that ubiquitin specific proteases (USPs) recognize ubiquitin using a similar binding mode.

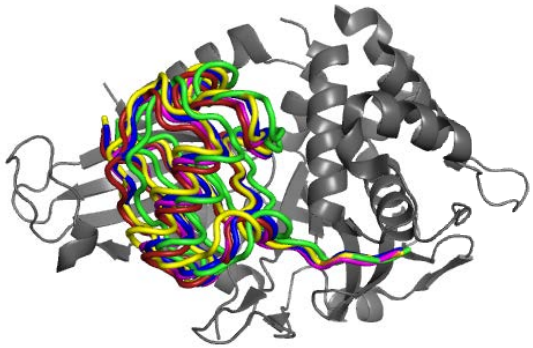


ubiquitin

USP21

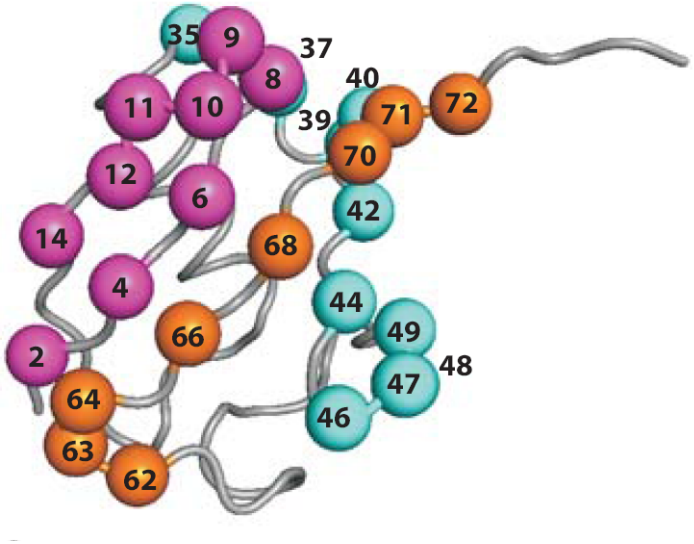
pdb: 3I3T

Buried surface:
~2000 Å^2



Sirano Dhe-Paganon, SGC, Toronto

A phage displayed library of Ubiquitin variants



27 contact residues

Region 1

2	3	4	5	6	7	8	9	10	11	12	13	14
Q	I	F	V	K	T	L	T	G	K	T	I	T

Region 2

35	36	37	38	39	40	41	42	43	44	45	46	47	48	49
G	I	P	P	D	Q	Q	R	L	I	F	A	G	K	Q

Region 3

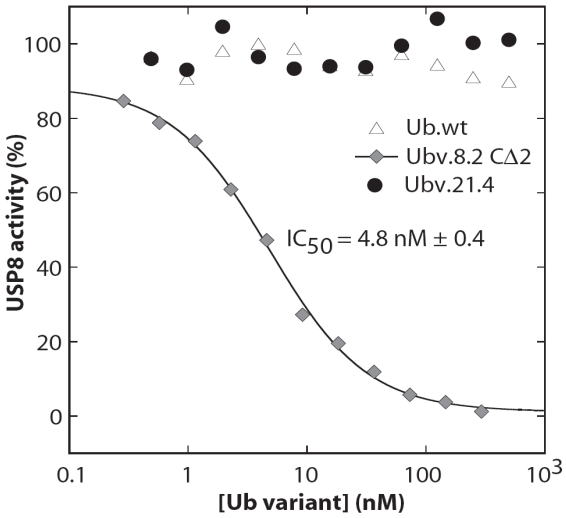
62	63	64	65	66	67	68	69	70	71	72
Q	K	E	S	T	L	H	L	V	L	R

- Mutation rate adjusted to 5 – 8 mutations per variant, on average

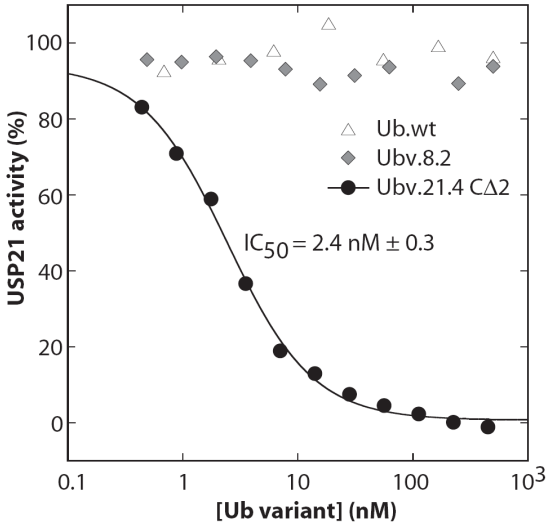
- Practical library size: >**10^{10}** ubiquitin variants

Ubiquitin variants are potent inhibitors of their cognate USPs

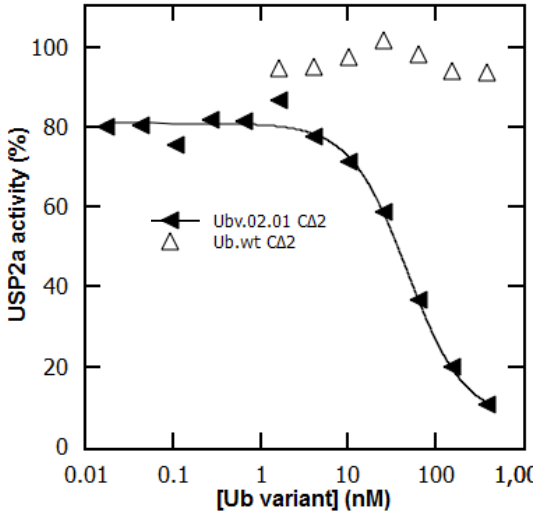
USP8



USP21



USP2



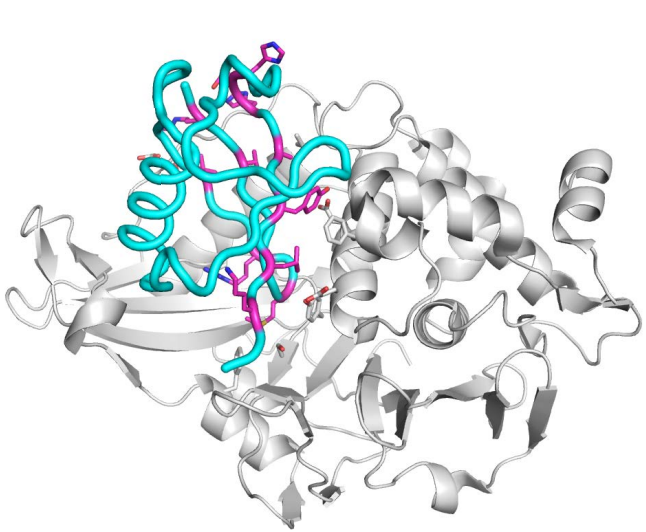
IC_{50} = 4.8 nM

IC_{50} = 2.4 nM

IC_{50} = 38 nM

Ernst et al. (2013) *Science*

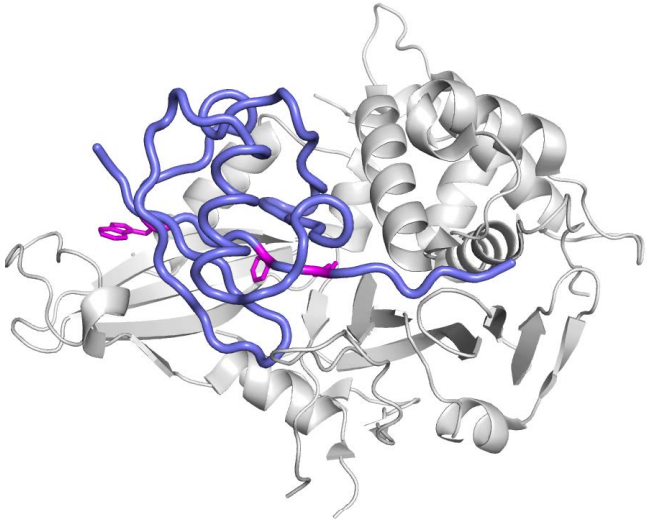
Co-crystal structures shows that Ub variants mimic Ub



Ubv.8.2

USP8

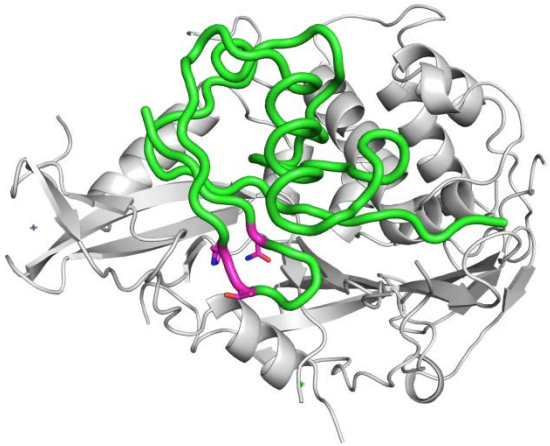
PDB: 3N3K
Resolution 2.6Å



Ubv.21.4

USP21

PDB: 3MTN
Resolution 2.7Å



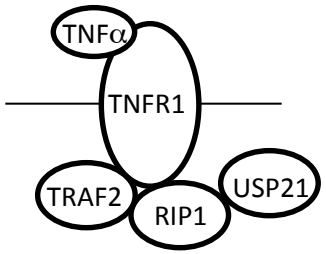
Ubv.2.1

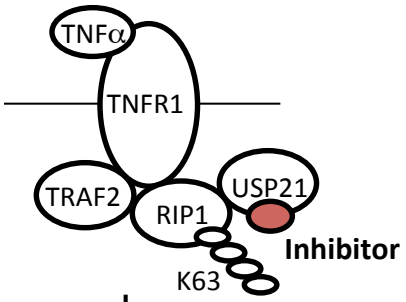
USP2a

Resolution 1.7Å

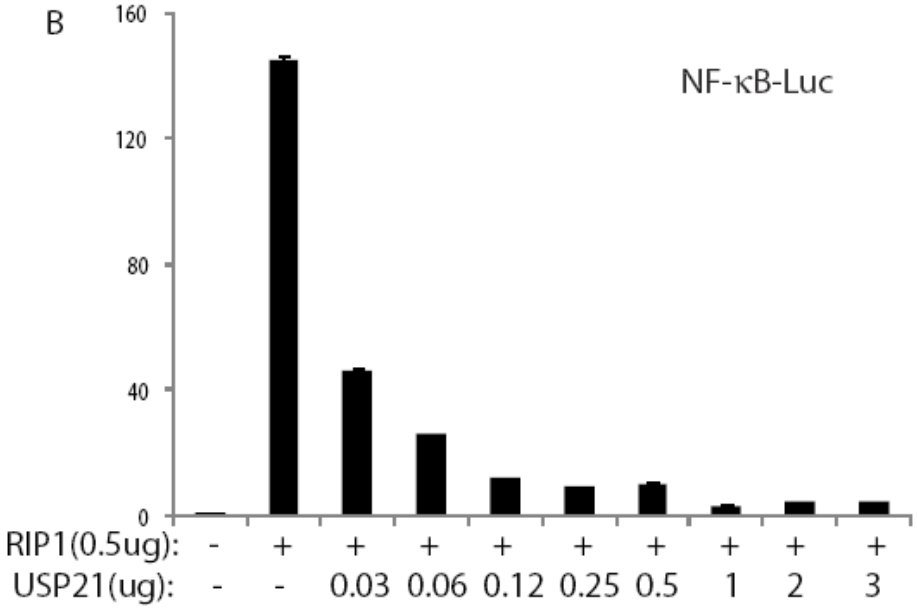
Sirano Dhe-Paganon, Mirela Neculai, SGC, Toronto

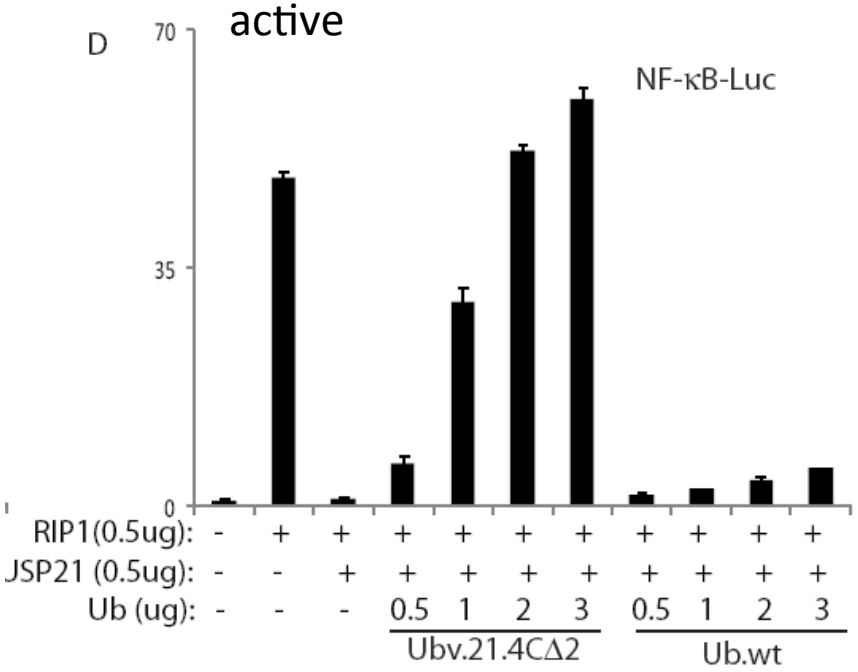
Inhibition of USP21 by Ubv.21.4 restores nf-kB signaling





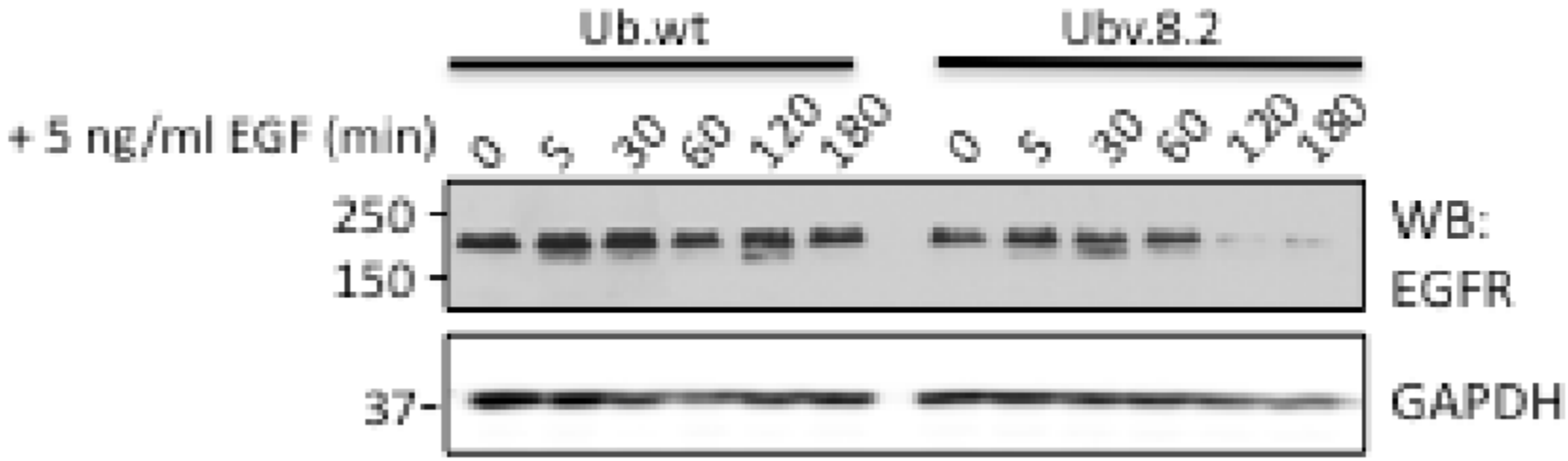
nf-κB
active





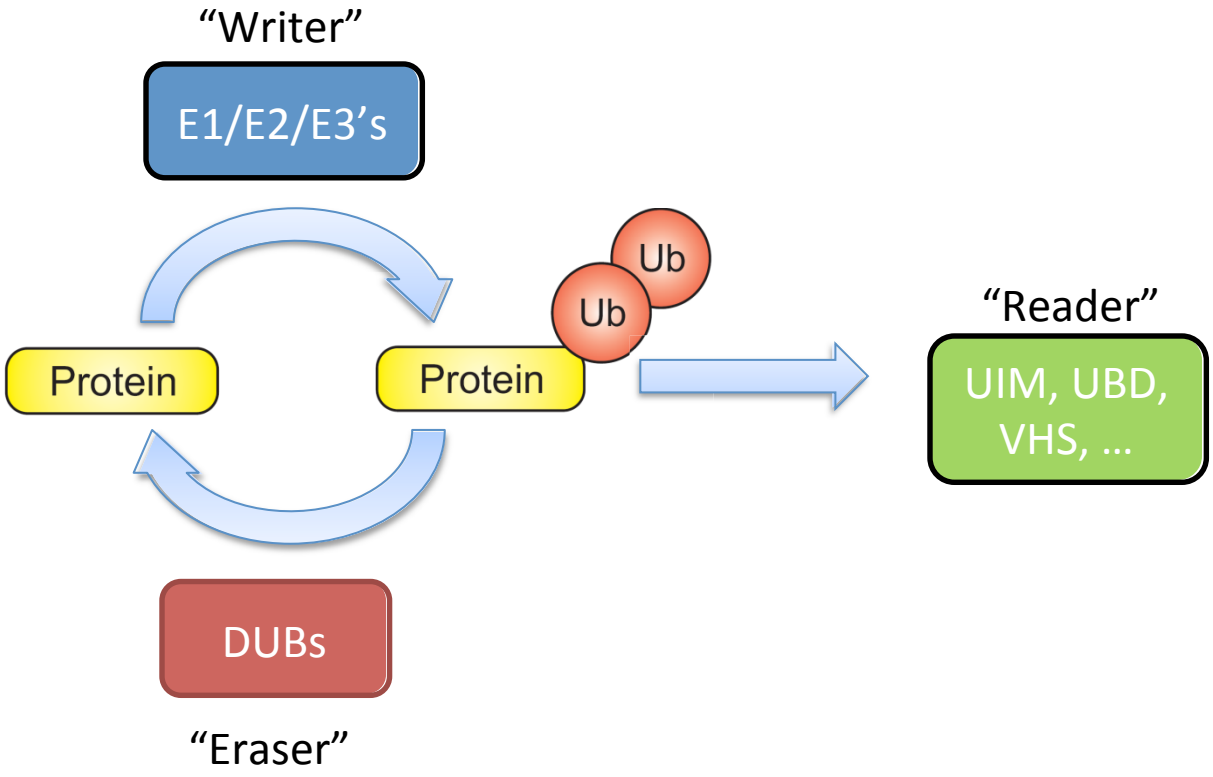
Jianhua Yang, BCM, Houston

Inhibition of USP8 by Ubv.8.2 down-regulates EGFR



Mike Moran, University of Toronto

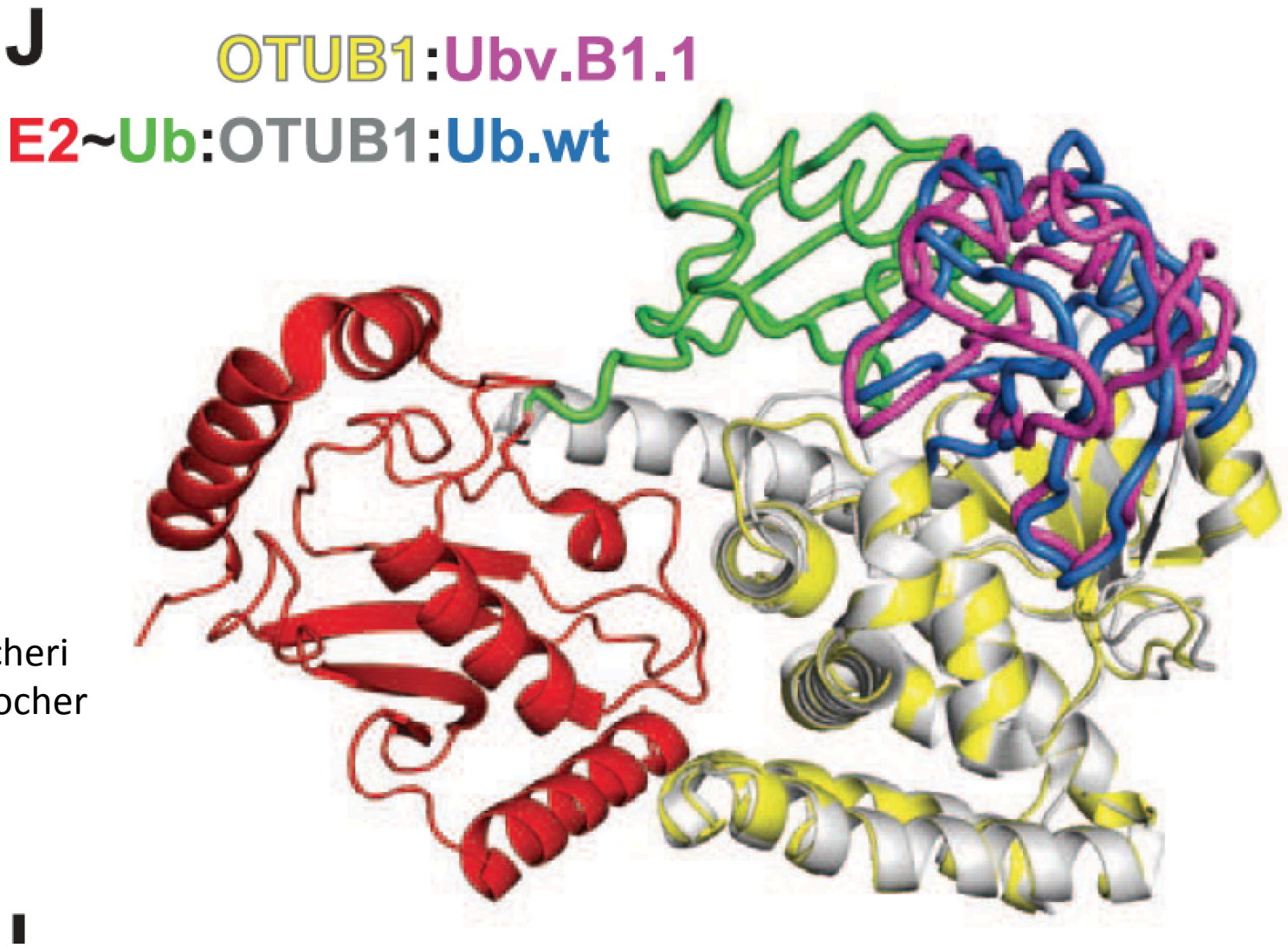
Going Beyond USPs…What Can We Target?



Ubiquitin variant binders generated against 21 Ub-binding proteins

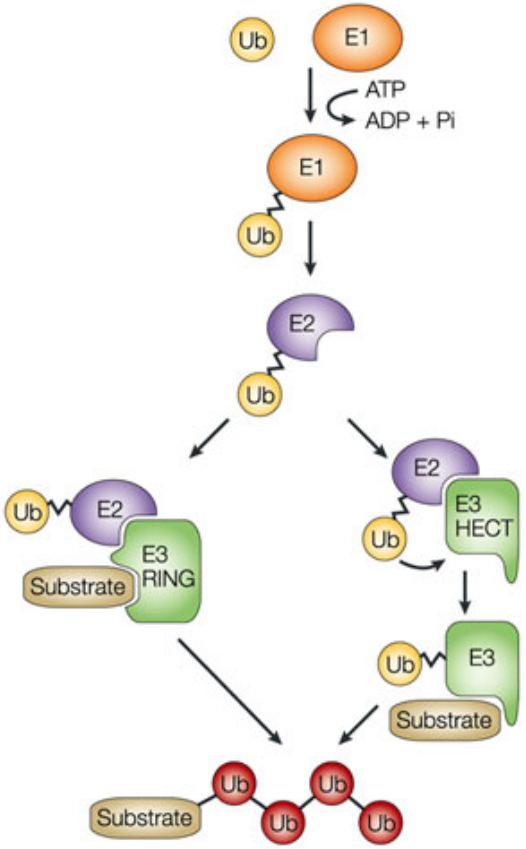
Antigen	Protein family	Unique specific inhibitors	Best affinity / inhibition (nM)	
Ube2G2	E2	29	< 150	E2/E3
Ube2B1	E2	4	< 150	
UbcH5	E2	26	< 150	
UbcH7	E2	6	< 150	
NEDD4	HECT-E3 ligase	33	3.4	
ITCH	HECT-E3 ligase	8	n/a	
β-TrCP-SKP1	F-Box / WD repeat	1	n/a	
USP2a	DUB / USP	61	16	DUBs
USP5	DUB / USP	39	n/a	
USP21	DUB / USP	32	2.4	
USP48	DUB / USP	9	25	
USP8	DUB / USP	11	4.8	
USP10	DUB / USP	1	63	
USP9x	DUB / USP	1	17	
OTUB1	DUB / OTU-family	37	5	
DubA	DUB / OTU-family	6	3	
BRISC	DUB / Metallo protease	1	n/a	
$NEMO_{CoZi}$	Linear Ub-binding domain	22	< 10	UBDs
AIBN-1	Linear Ub-binding domain	8	< 25	
USP37-UIM 1-3	Ub interaction motif	26	< 10	
VPS27p-UIM1	Ub interaction motif	34	113	

OTU-B1 Inhibitor Targets an Exosite



Frank Sicheri
Dan Durocher

Targeting Ligases



Ubiquitin

E1 (2):

Uba1
Uba6

E2 (30):

UBE2A(hHR6A)	UBE2L3(UbcH7)
UBE2B(hHR6B)	UBE2L6(UbcH8) ±
UBE2C(UbcH10)	UBE2N(Ubc13)
UBE2D1(UbcH5A)	UBE2O(E2-230K)
UBE2D2(UbcH5B)	UBE2Q1(NICE-5)
UBE2D3(UbcH5C)	UBE2Q2
UBE2D4(HBUCE1)	UBE2R1(CDC34)
UBE2E1(UbcH6) ±	UBE2R2(CDC34B)
UBE2E2	UBE2S(E2-EPF)
UBE2E3(UbcH9)	UBE2T(HSPC150)
UBE2G1(UBE2G)	UBE2U*
UBE2G2(UBC7)	UBE2V1(UEV-1A)
UBE2H(UBCH)	UBE2V2(MMS2)
UBE2J1(NCUBE1)	UBE2W
UBE2J2(NCUBE2)	UBE2Z(Use1)
UBE2K(HIP2)	BIRC6(apollon)

E3 (>1000):

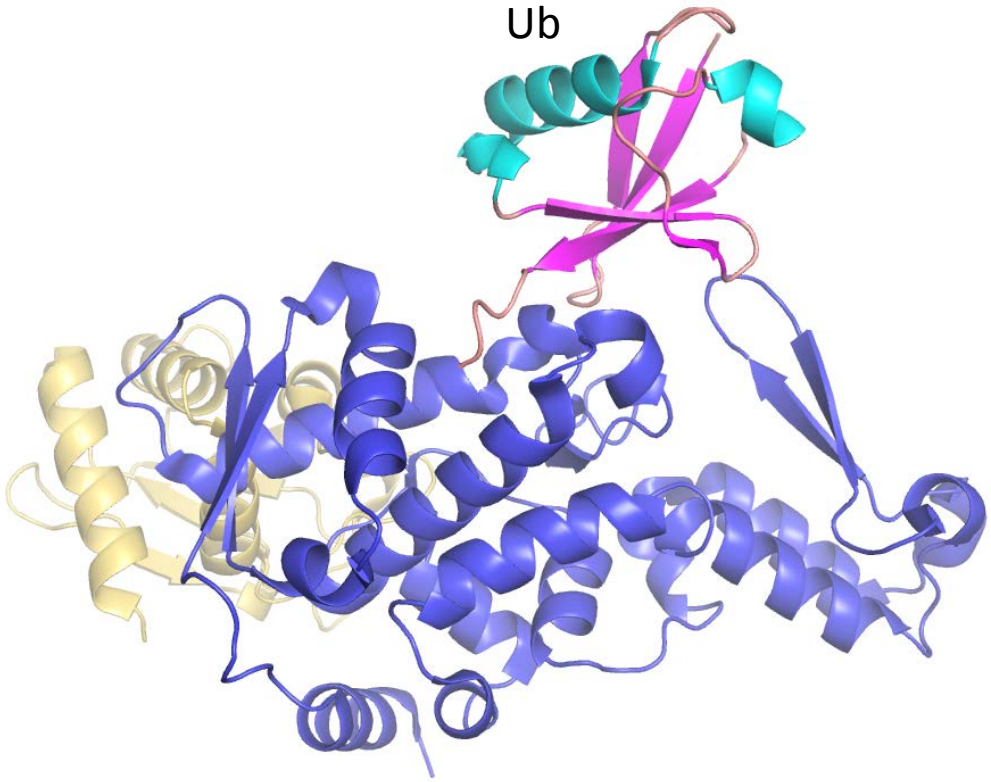
Single/multiple subunit
RING, HECT, U-box, PHD

Targeting E3s

Fiore *et al.,* Nature Reviews, 2003

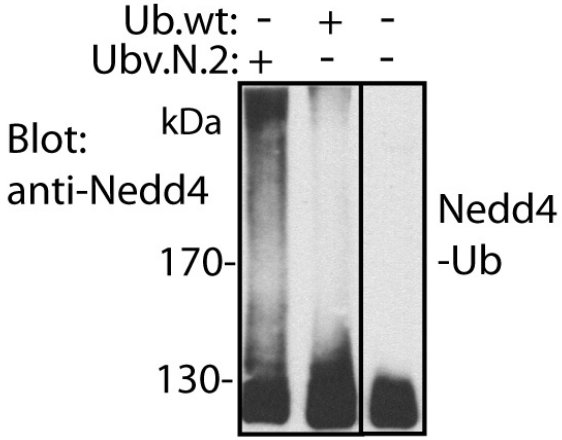
Sjoerd *et al.,* FASEB, 2010

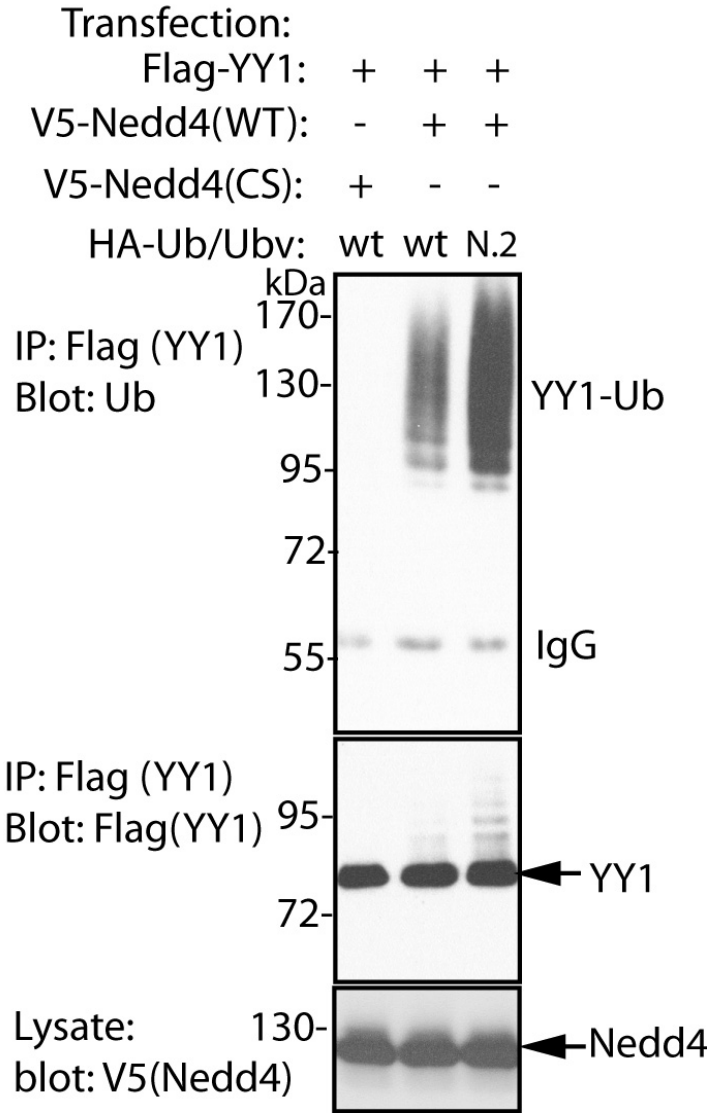
Ubiquitin engages similar surface residues for binding to HECT-domain of NEDD4



Nedd4 and ITCH binders are highly specific

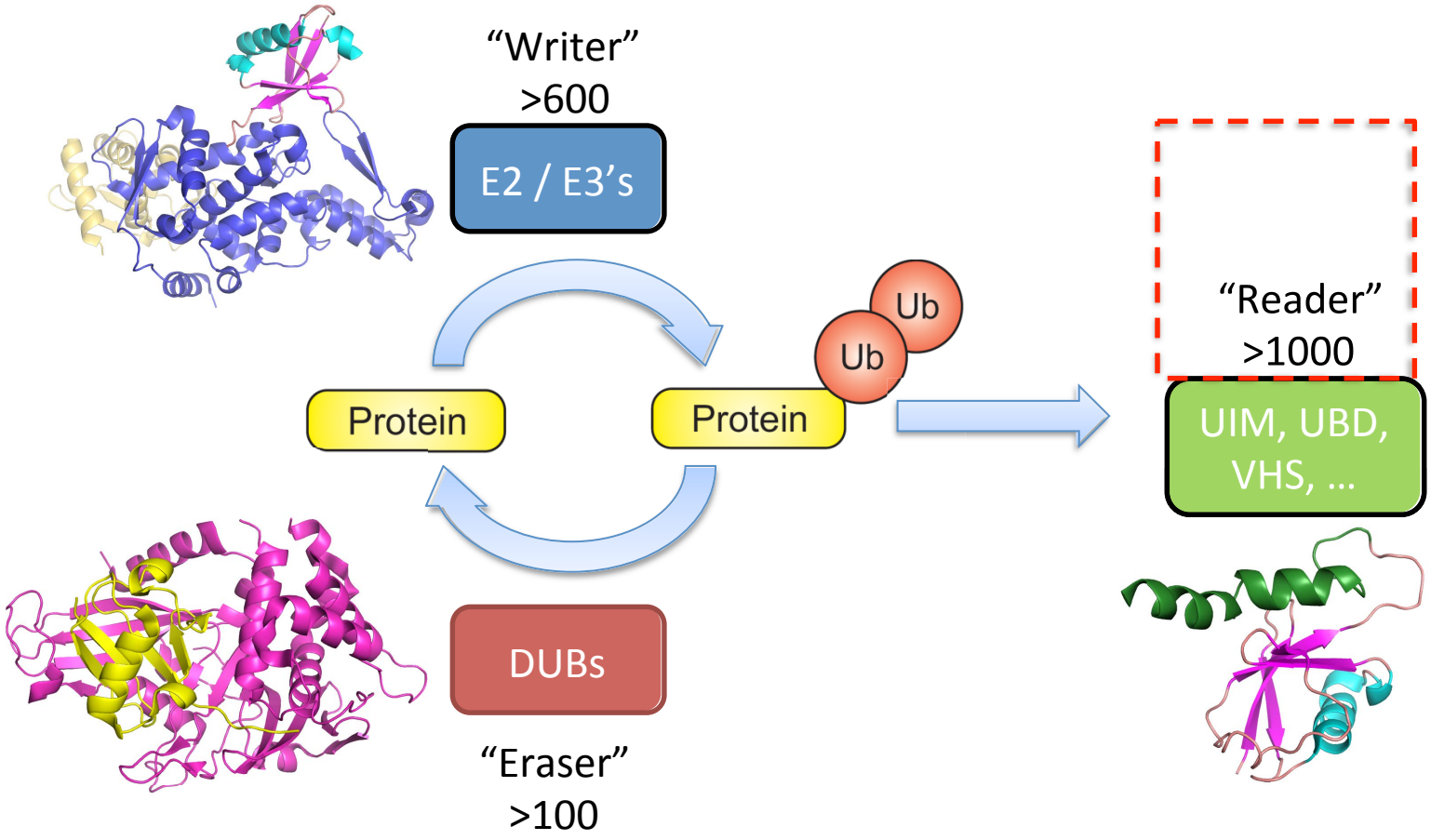
NEDD4 binders are activators



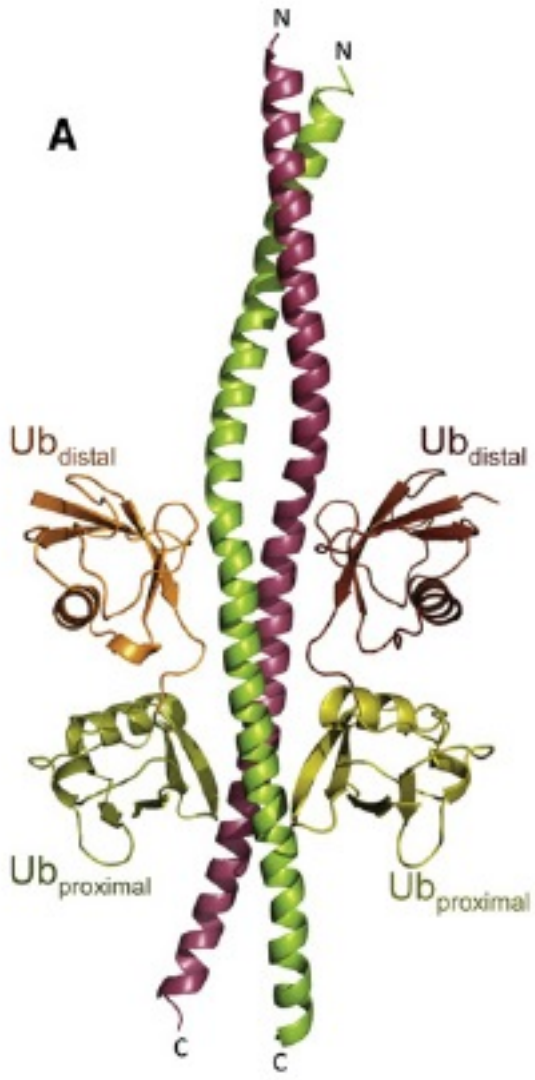


Philipp Alberts and Daniela Rotin, Sick Kids, Toronto

Can we target the “readers”?

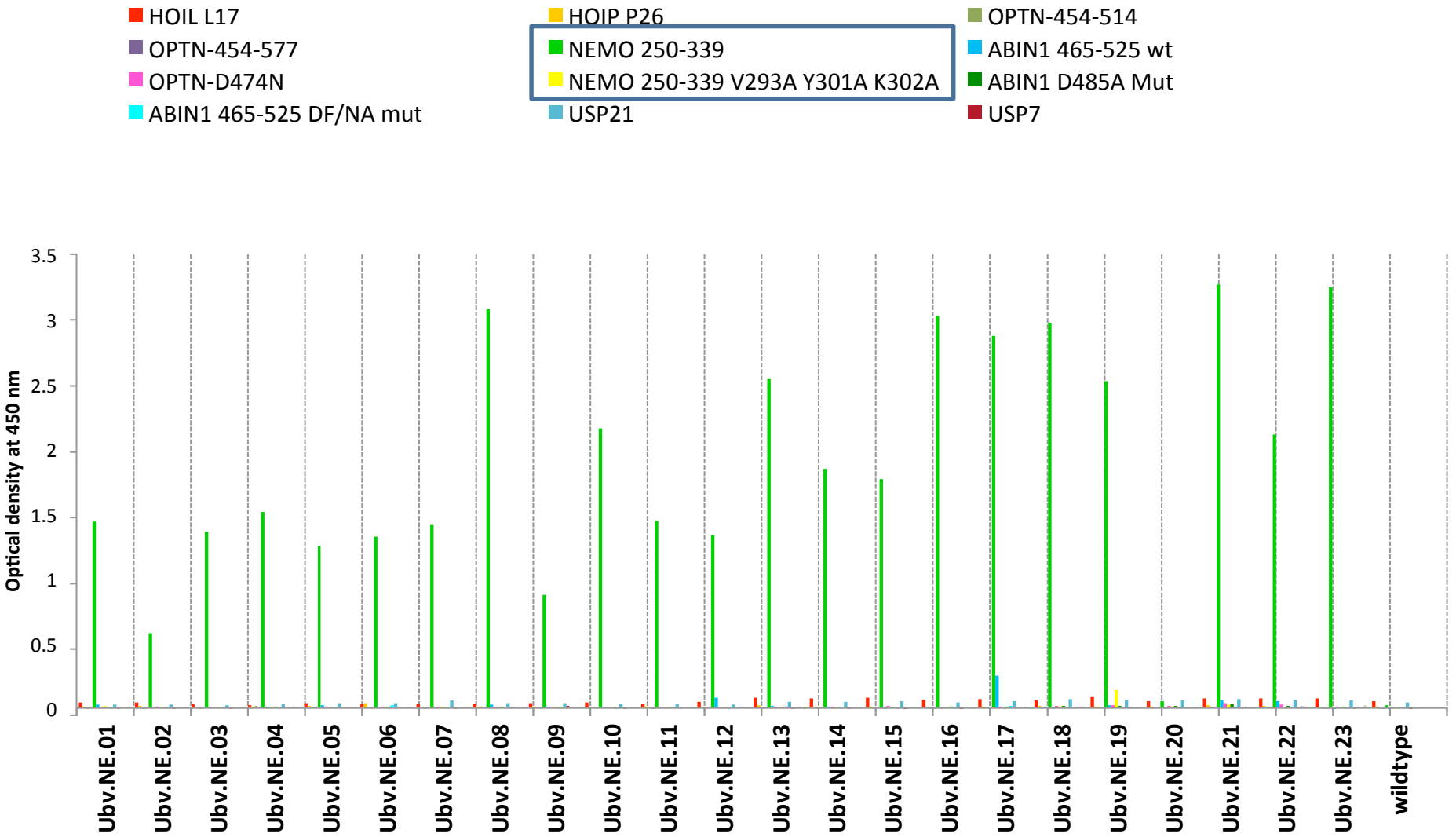


Targeting the NEMO Ubiquitin Interacting Domain



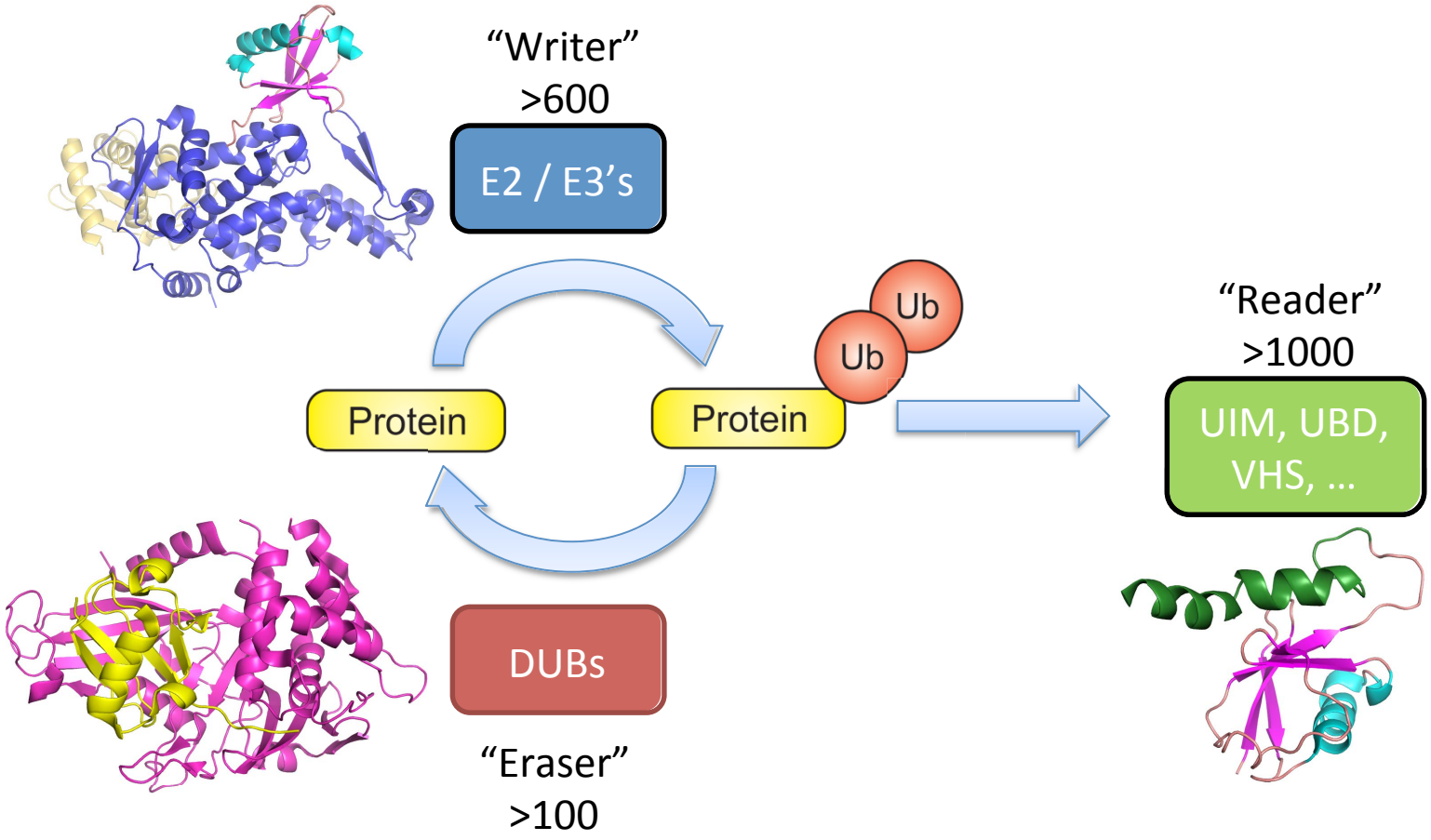
Ivan Dikic, Frankfurt

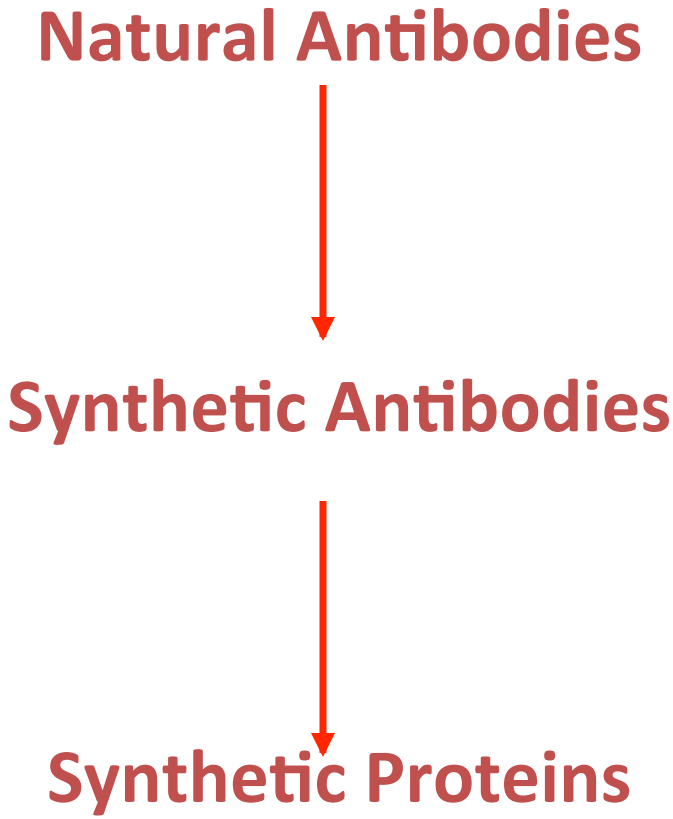
Ubiquitin Variants Are Specific for NEMO-250-339

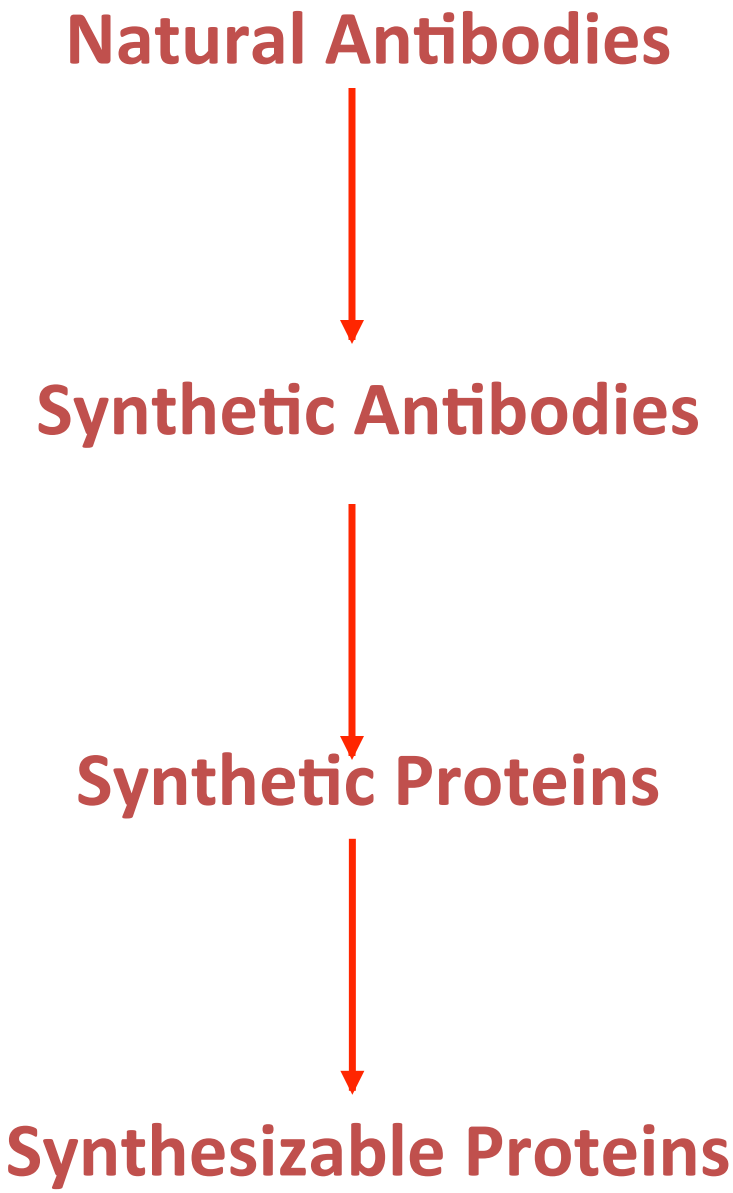


Importantly, the variants do not bind to the point mutant variant of NEMO indicating they bind a similar area as distal Ub in a NEMO linear di-Ub complex .

All components of the Ubiquitin pathway can be targeted




Natural Antibodies
Synthetic Antibodies
Synthetic Proteins


Natural Antibodies
Synthetic Antibodies
Synthetic Proteins
Synthesizable Proteins

REFLEXION PHARMACEUTICALS

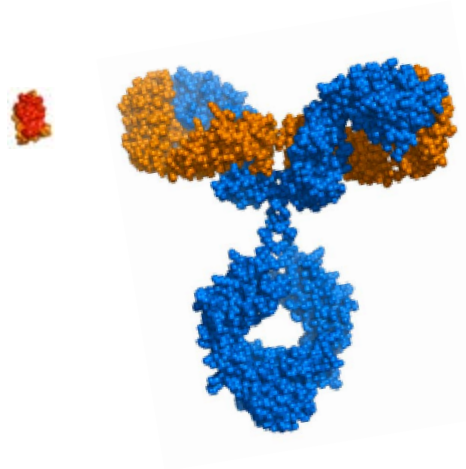
Therapeutic Mirror Image Proteins

Steve Kent
Dana Ault-Riche
Maruti Uppalapati
Kalyan Mandal

D-Protein Therapeutics

Small proteins made entirely of D-amino acids

Designed protein binding surface of 500 to 1,000 Å^2



Advantages:

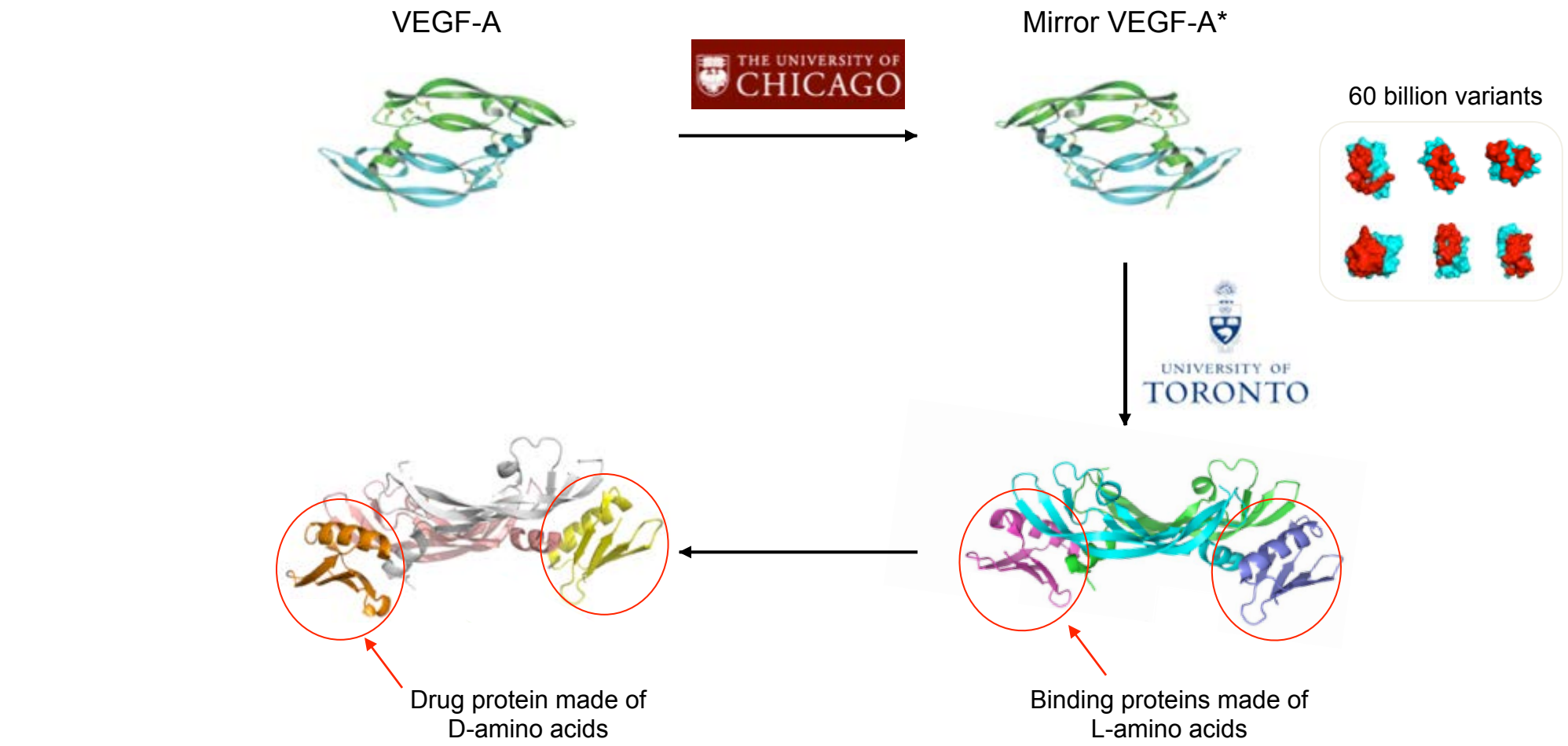
Small, but with very strong affinity and specificity
- Improved safety and effectiveness
- Access to hidden targets

Metabolically stable and non-immunogenic

Chemically manufactured
- Faster regulatory path to approval
- More consistent and homogeneous manufacturing
- Expanded chemistry options

The power of synthetic chemistry applied to proteins

Discovery of VEGF-A antagonist



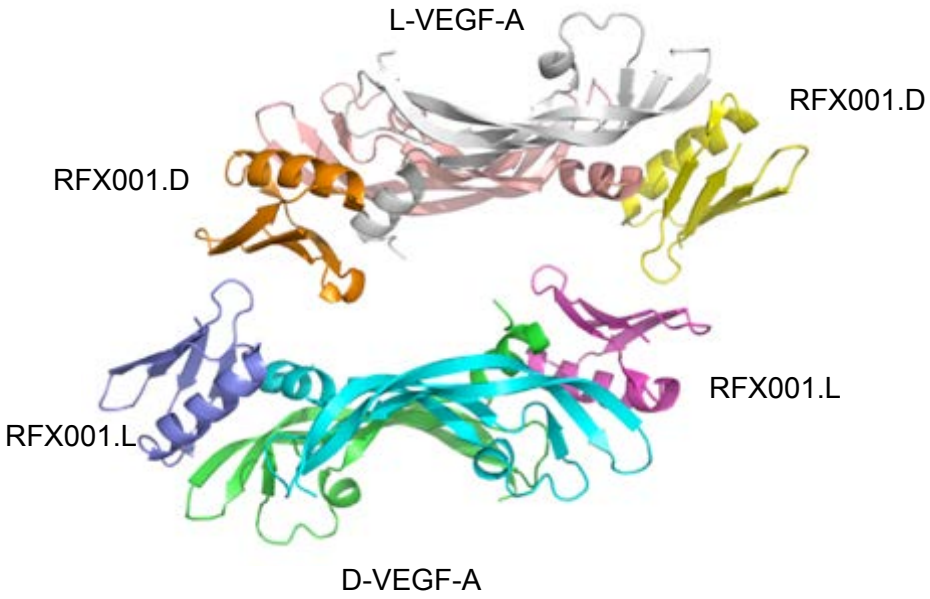
Whitehead Institute

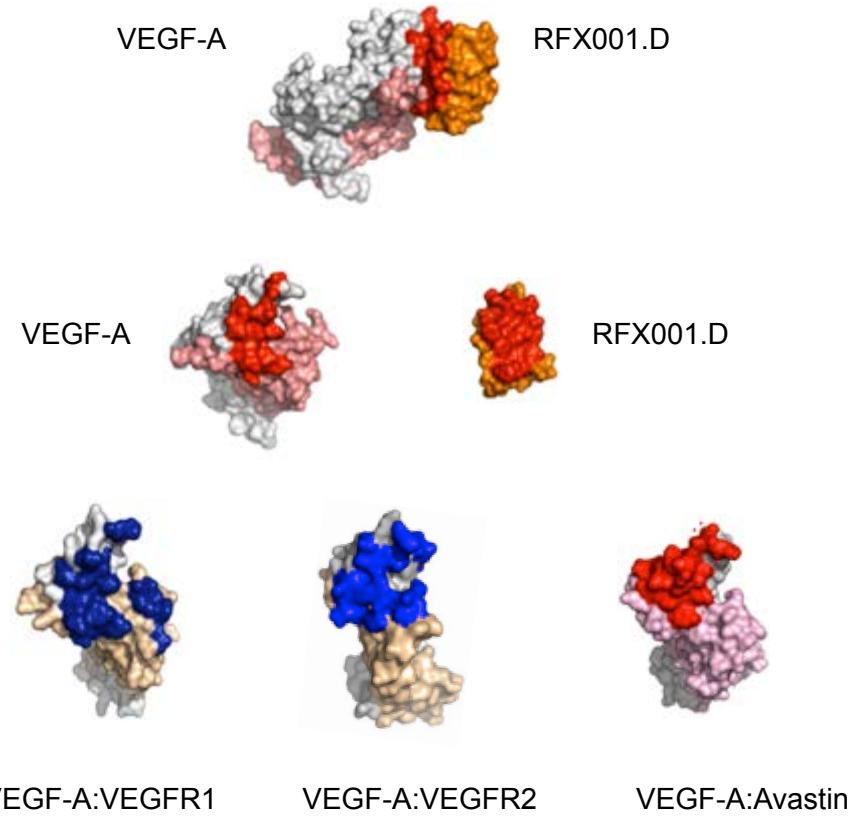
Mirror image phage display:
Schumacher et al, *Science*, 271, 1854-1857 (1996)

*Mandal, *Angew Chem Int Ed* 50, 8029-8033 (2011)

Racemate of a heterochiral protein complex

6 synthetic proteins: 632 amino acids,
Structure weight: 73,201.8 Da
1.6 Å resolution



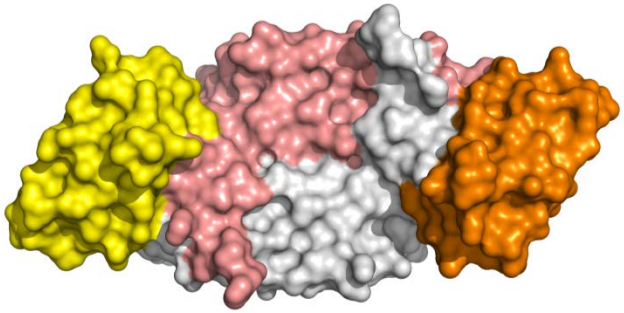


PDB PROTEIN DATA BANK

4GLS/4GLN

Mandal, Uppalapati, et al, *Proc Natl Acad Sci USA* 109, 14779-14784 (2012)

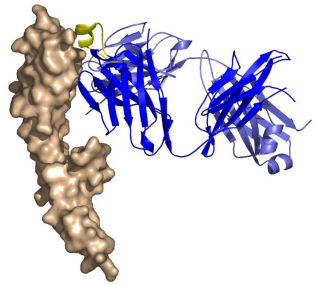
SUMMARY



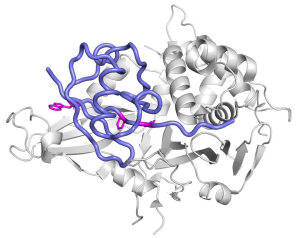
- A new class of chemically manufactured drugs with antibody-like affinity and specificity
- Composed entirely of D-amino acids
- Metabolically inert and non-immunogenic
- High physical stability
- Enables racemic crystallography
- **Expands the potential of therapeutic proteins and research reagents**

Natural Antibodies

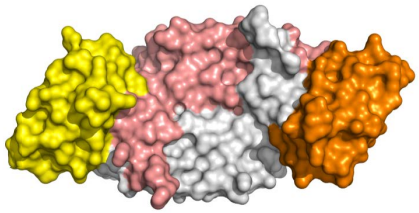
Synthetic Antibodies



Synthetic Proteins



Synthesizable Protein



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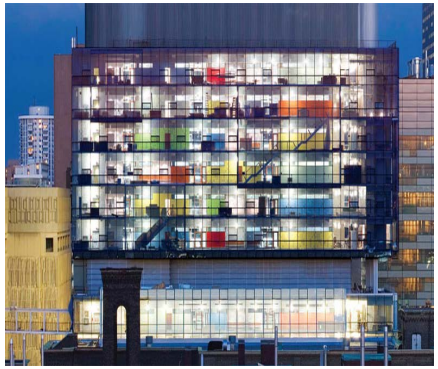
CFI
NIH

The Donnelly Centre

http://tdccbr.med.utoronto.ca/



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