ATRC @ UCSF
OVERVIEW

*Antibody technology research center

Goals of the ATRC

Robust approaches for rAbs to the proteome

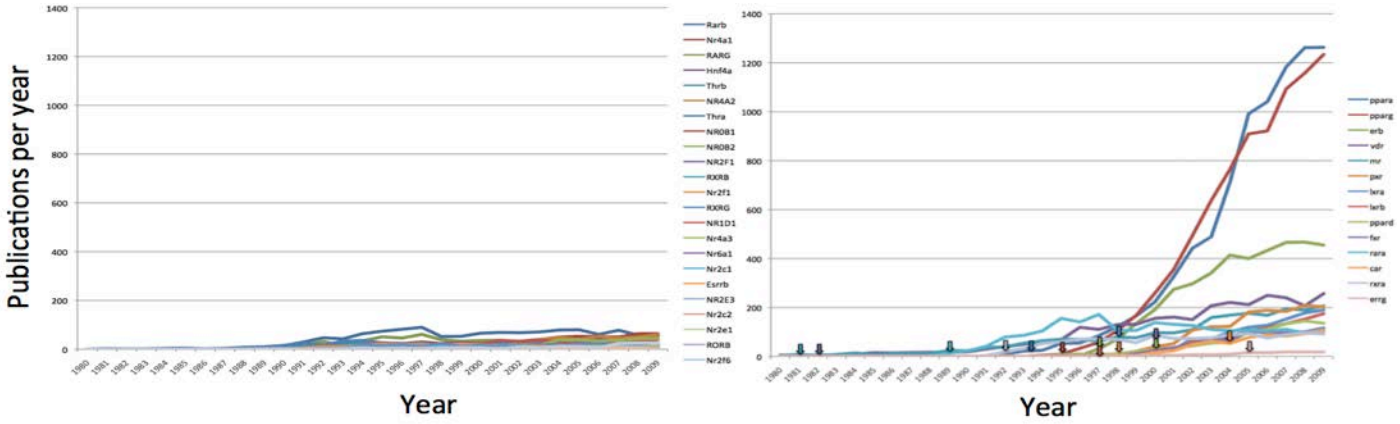
- Initially focus (largely) on secreted proteins
  - Single and multipass membrane proteins/proteases
  - PTMs (phospho-specific rAbs)

Technologies

- Phage Ab
- Automation and high throughput screening
- In vitro antigen and Ab expression
- Automatable cell selection

Existing antibodies

Functional Abs not available for >90% of proteome



Where available

- Half or more are not specific
- Lot-to-lot and vendor-to-vendor variability
- Relatively expensive
- Not renewable (not cloned)
- Typically IgG only

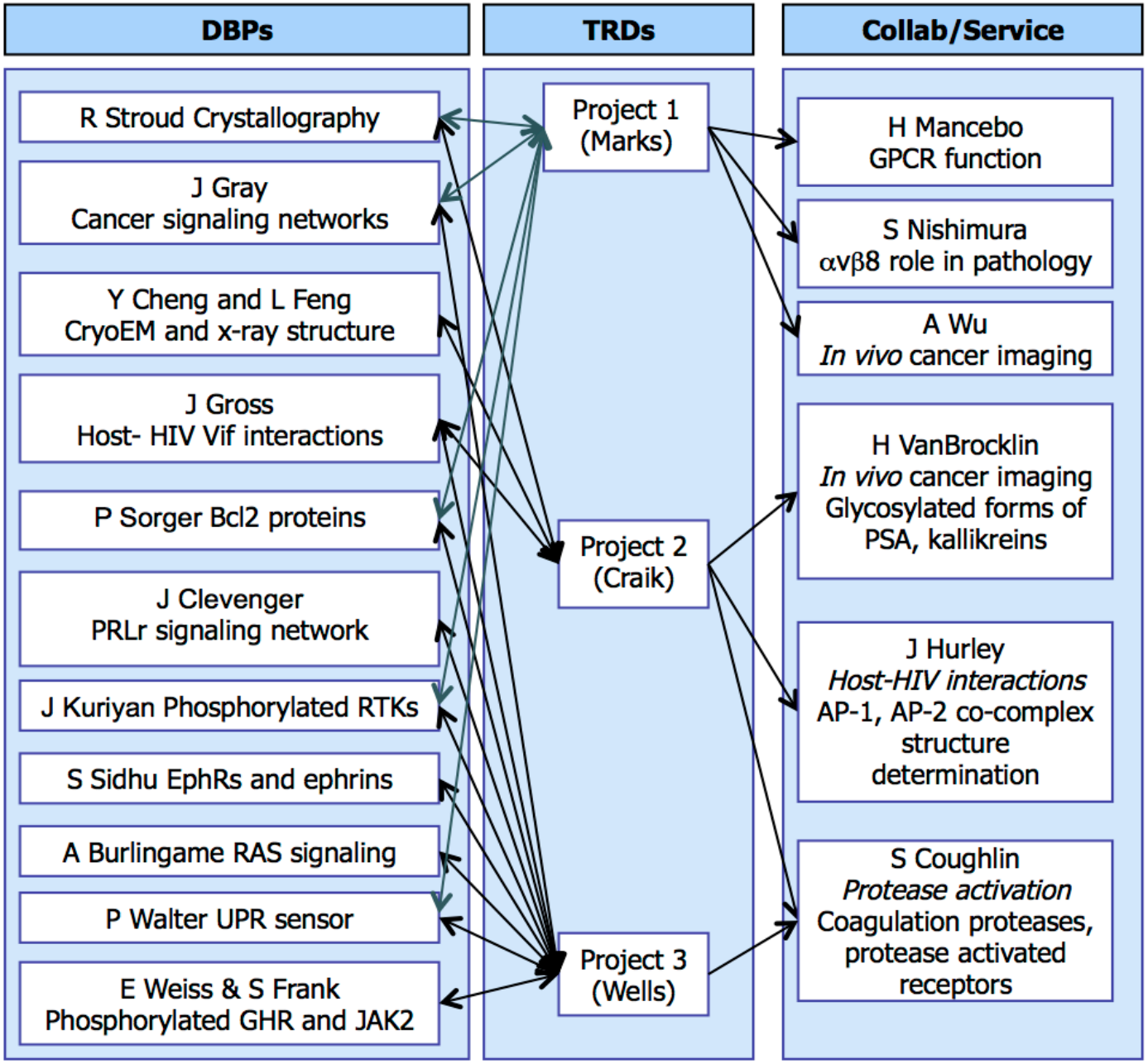
ATRC Solution

Phage Ab based rAb generation

- rAbs defined by their sequence
- Forever renewable
- Can be made as fragment or with any Fc (species/isotype)

A technology-driven extension and expansion of what we are doing at a cottage industry level

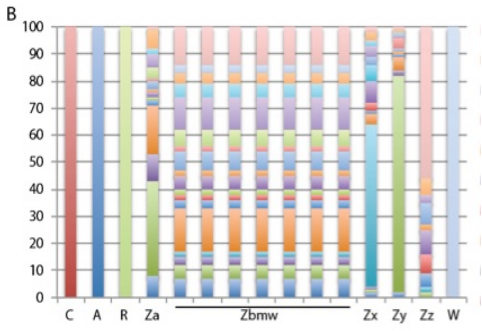
- Generating robust phage Ab libraries
- Automation of rAb generation
- Generating rAbs to challenging antigens
- Providing technology training



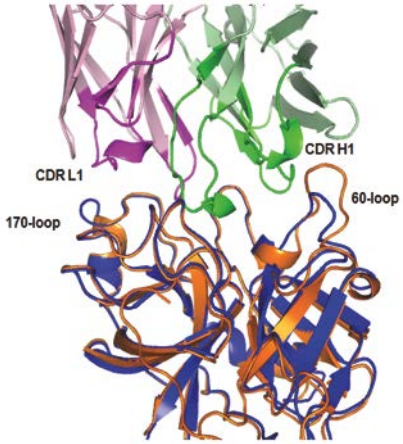
ATRC is funded by a P41 mechanism, that requires TRD, DBP, CSP

TR&Ds

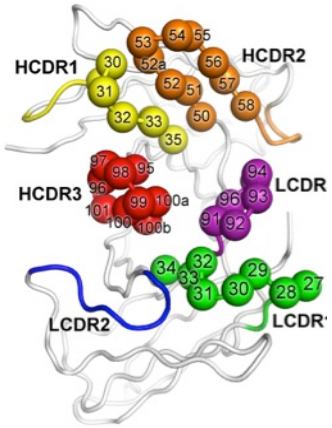
- TR&D 1 (Marks):
  - Next generation phage Ab libraries
  - Robust rAb generation to secreted and single and multipass membrane proteins



- TR&D 2 (Craik):
  - Generate secreted and type 2 TM serine proteases, receptors and intramembrane proteases
  - rAbs to protease targets including inhibitory rAbs

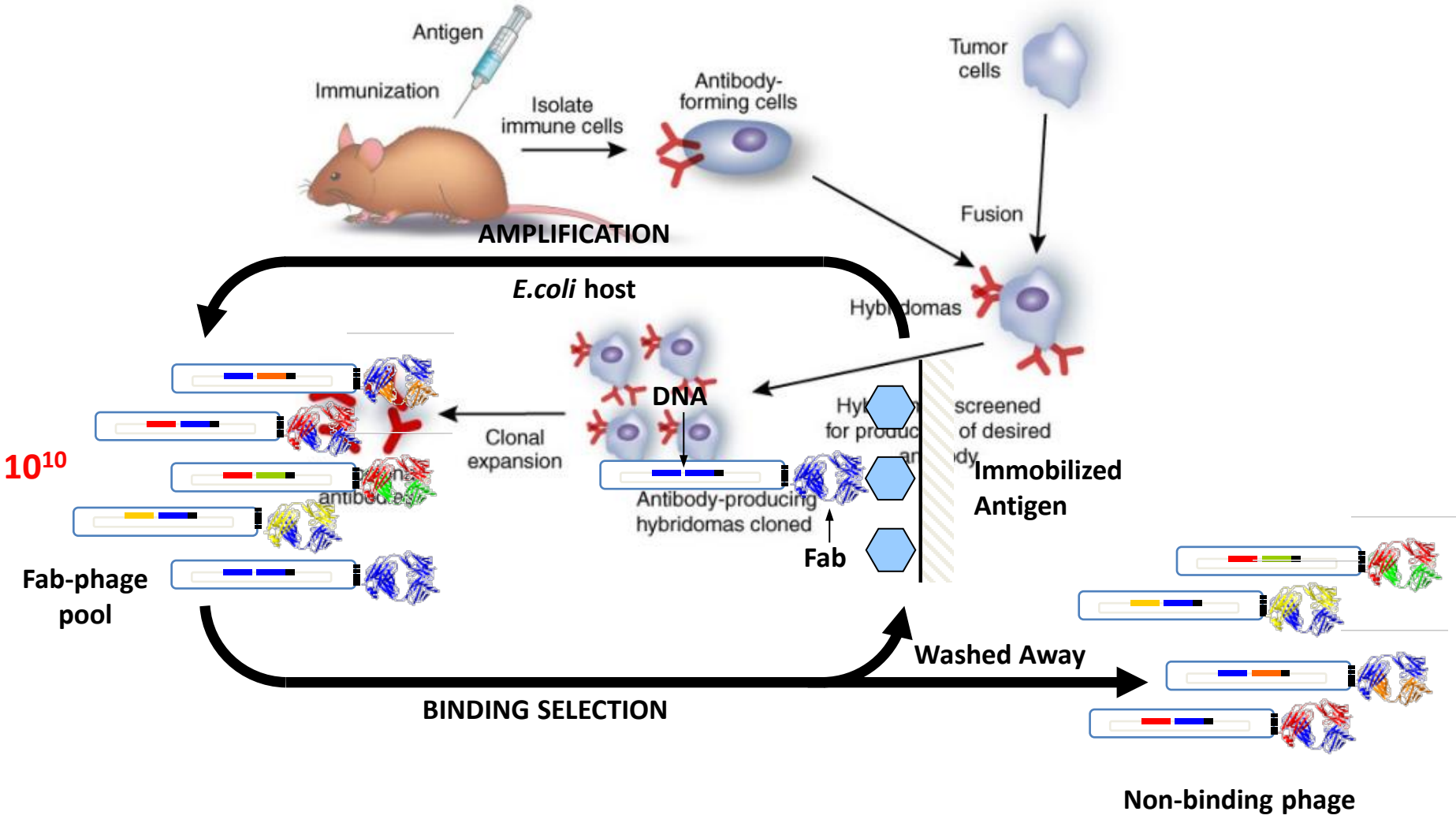


- TR&D 3 (Wells):
  - Phage libraries/technologies to generate rAbs to phosphorylated antigens, neo- and conformational-epitopes
  - Automation of selections

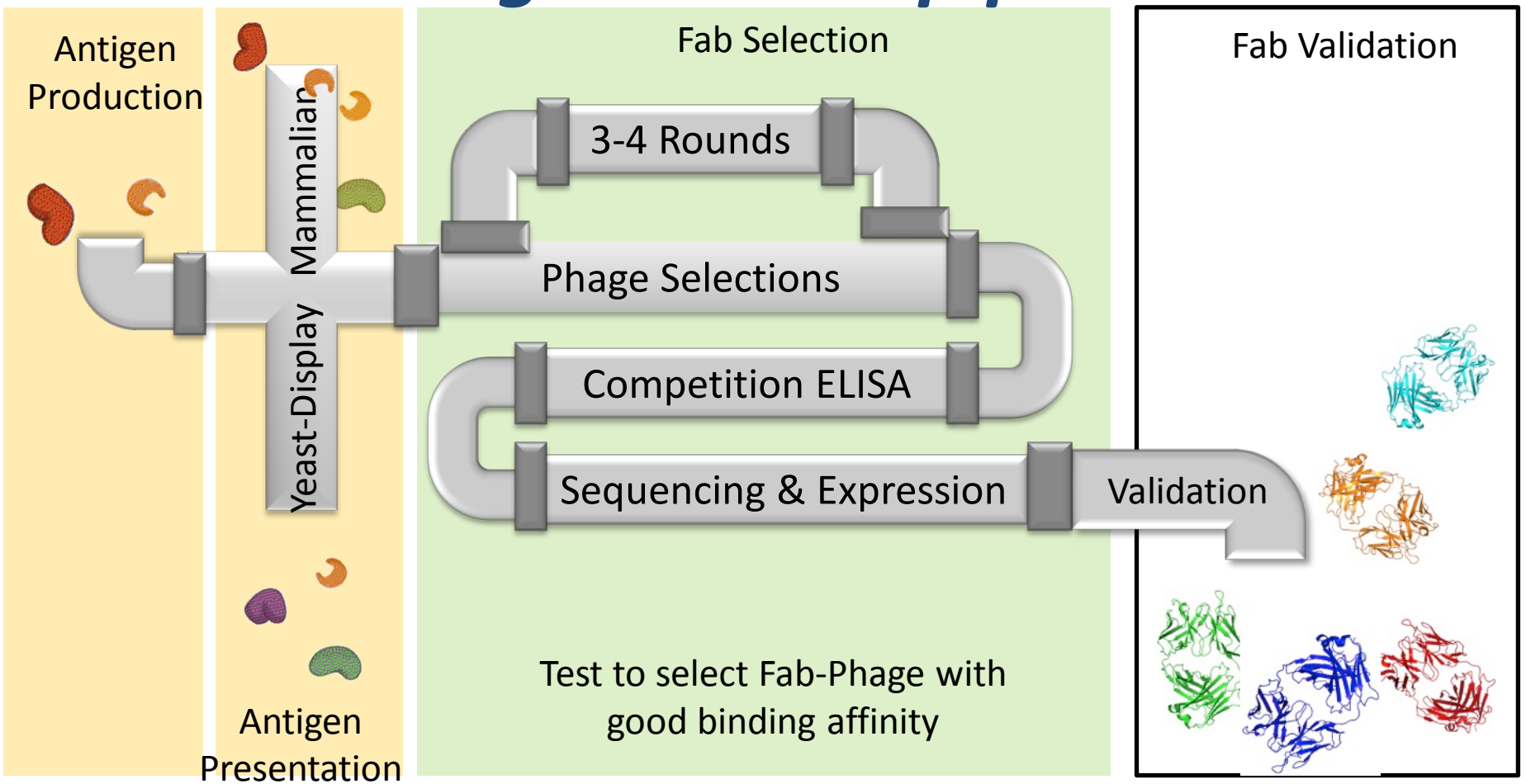


Technologies: *in vivo* vs *in vitro* Abs

Adapted from www.medscape.com



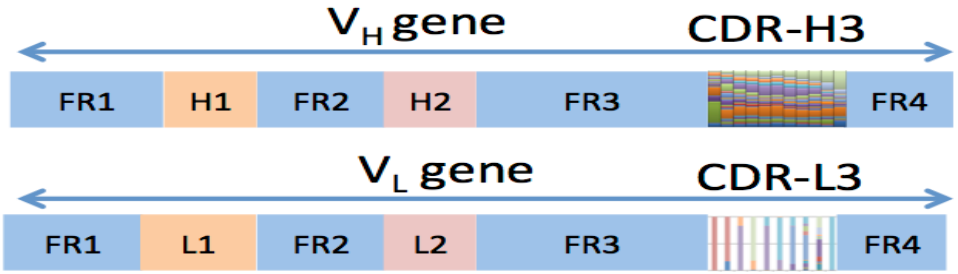
Ab generation pipeline



Antigen QC: SDS-PAGE; SEC

rAb QC: Sequence, affinity, specificity

TRD 1: Nature-inspired synthetic antibody libraries

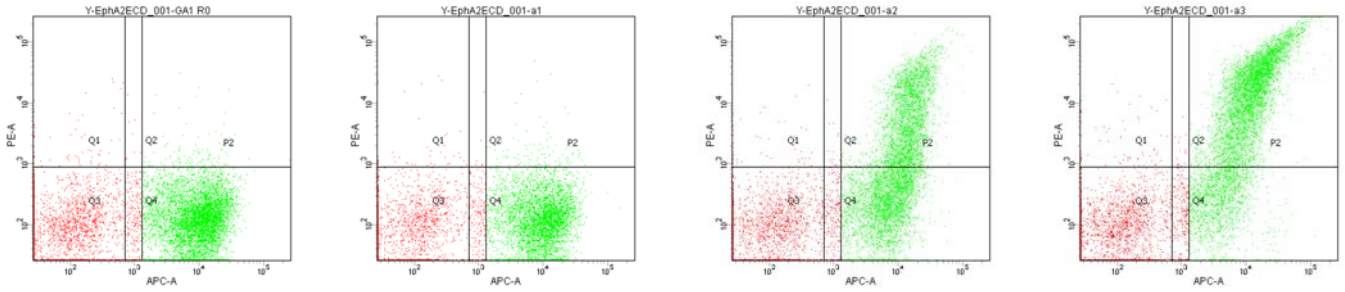


6 VH scaffolds

5 VL scaffolds

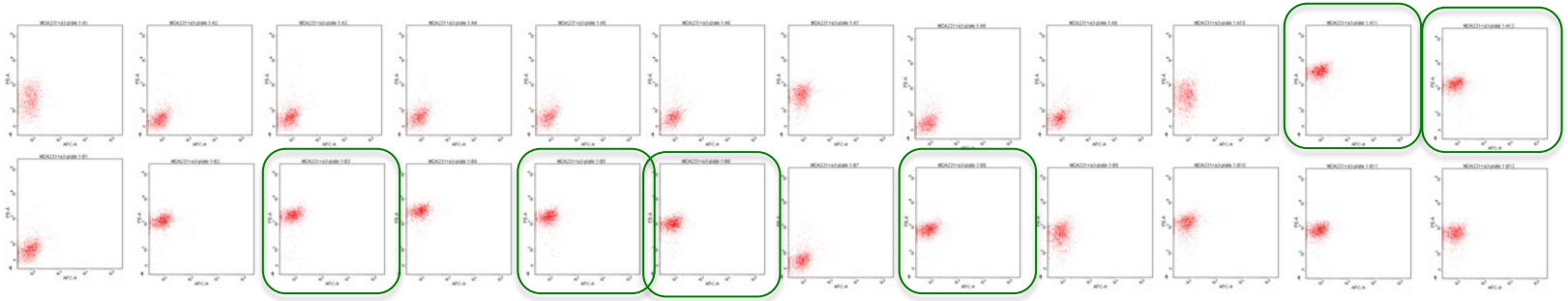
R0 R1 R2 R3

Y-EphA2ECD



> 30 unique antibodies

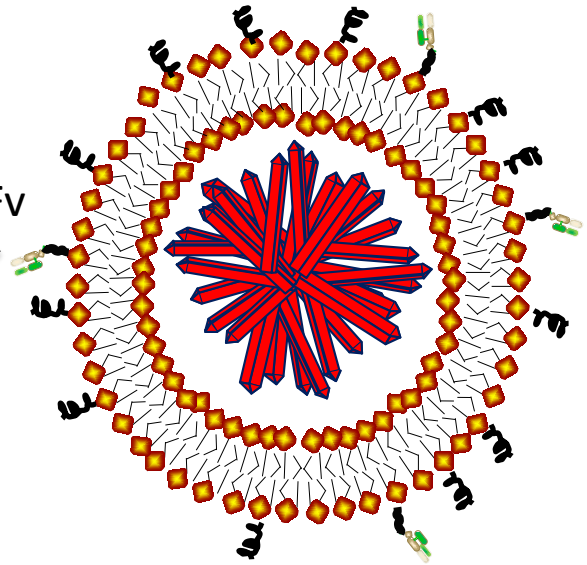
MDA-MB-231 (EphA2 +) cells



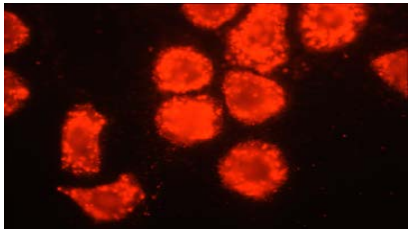
Antibody fragments for nanoliposome targeting

Anti-HER2 liposomal doxorubicin
MM-302
Registration Phase 2
HER2+, progress on T DM-1

Anti-HER2 scFv

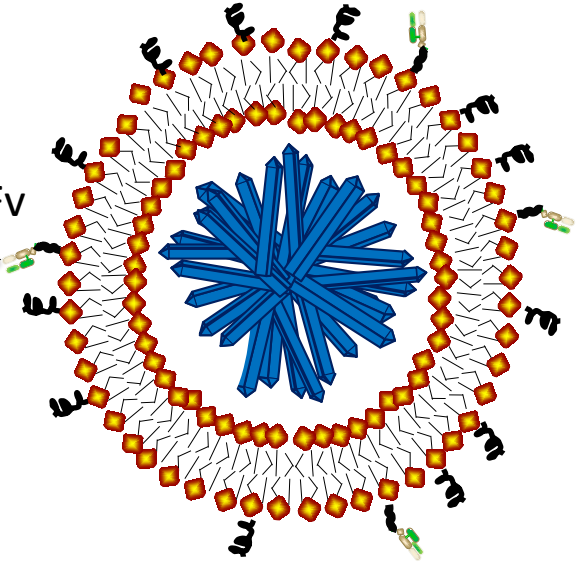


scFv F5

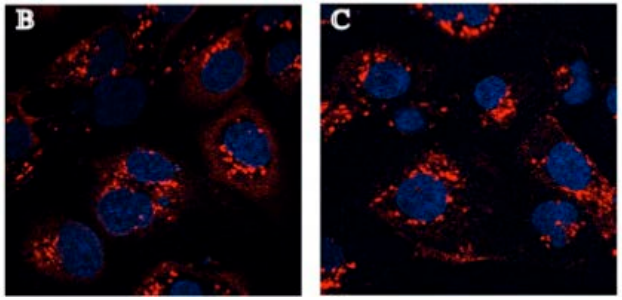


Anti-EphA2 liposomal docetaxel
MM-310
Completed manufacturing
Phase 1 Q2 2016

Anti-EphA2 scFv



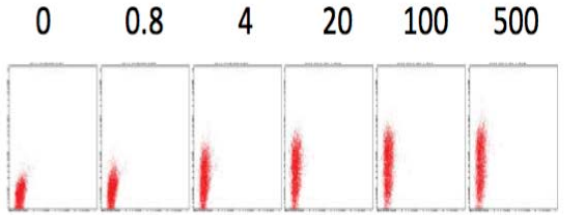
scFv
D2-1A7



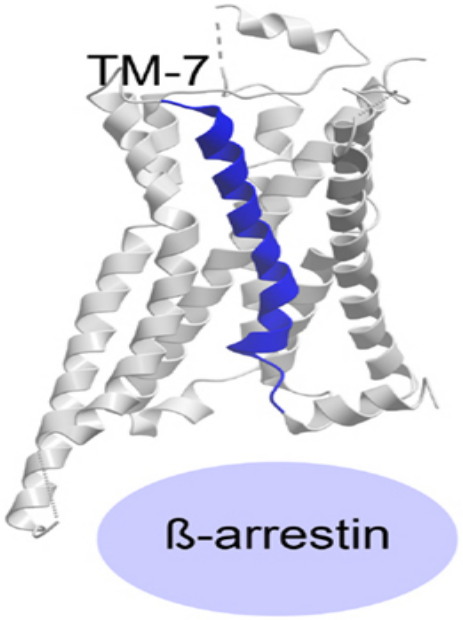
rAbs to CXCR1 with functional activity

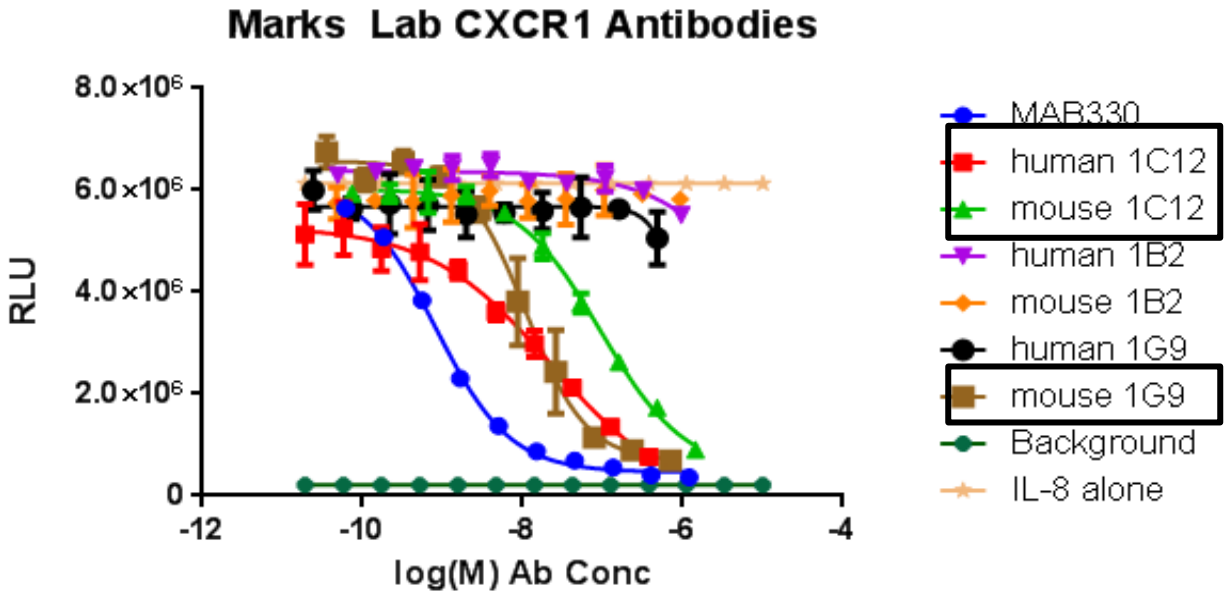
Immune phage library
- GPCR DNA immunization

Select on transfected CHO cells

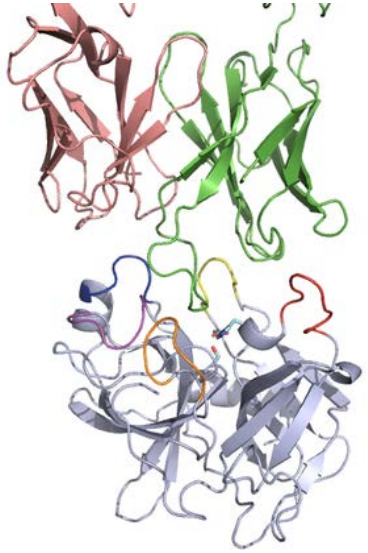


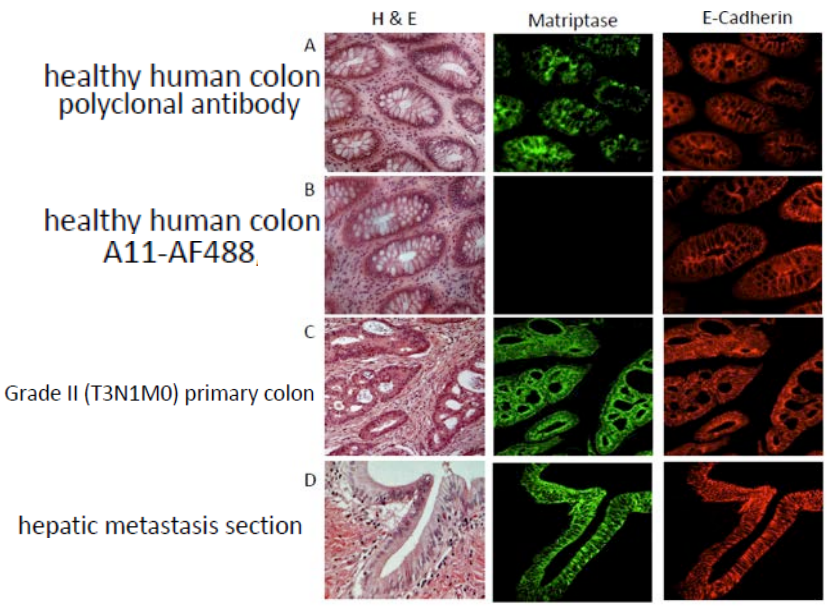
B-arrestin assay for mAb biologic activity: inhibit IL-6 CXCR1 activation

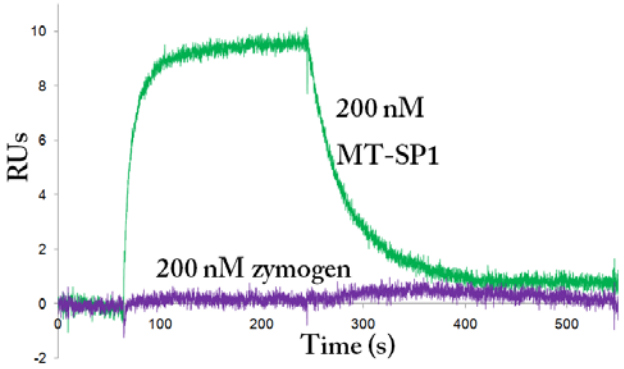


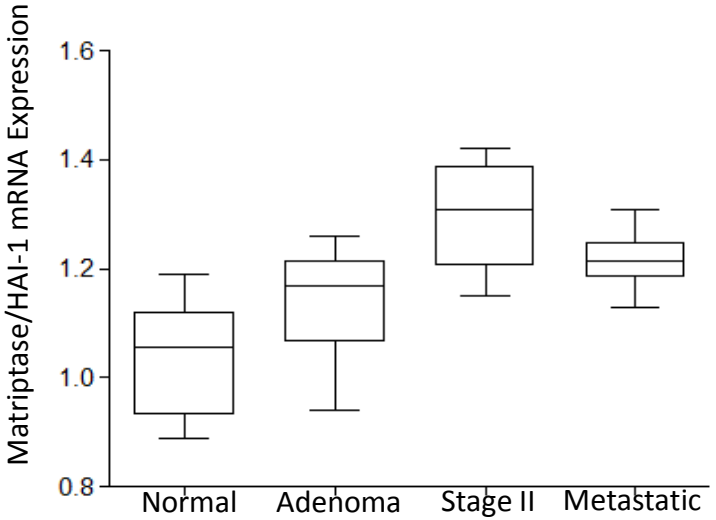


Ab Activity Based Probe to Matriptase Provides Diagnostic Information in Colon Cancer



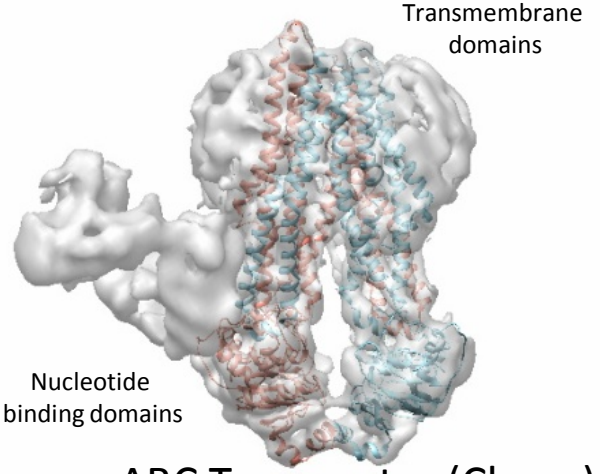






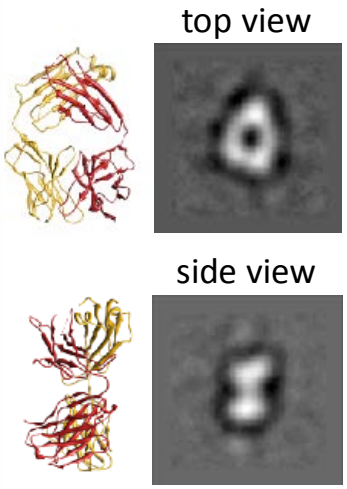
Schneider E, et al. *J. Mol Bio* **412** (2012)
LeBeau AM, et al. *PNAS* **110** (2013)

Conformationally Selective Fabs to Challenging Targets

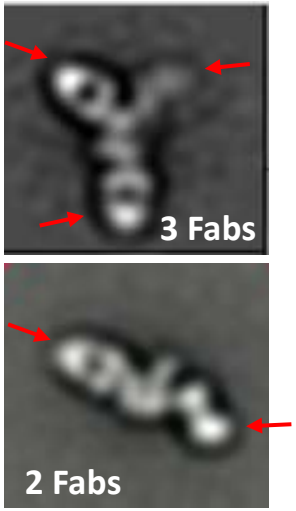


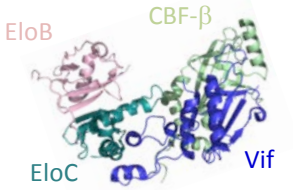
ABC Transporter (Chang)

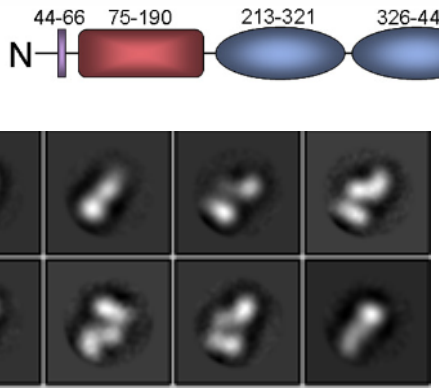
Kim J et al. Nature (2015)



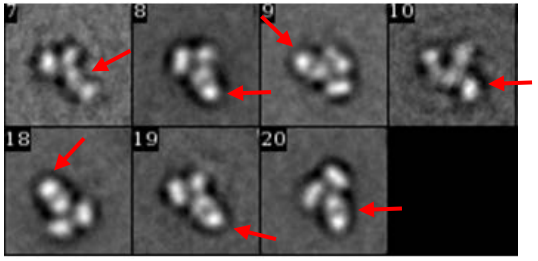
HIV-host complexes: VCBC (Gross)





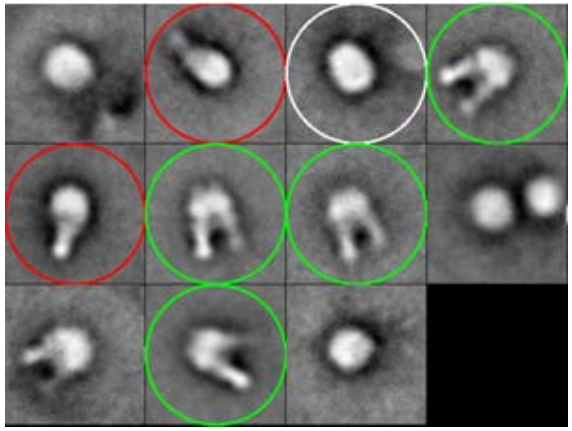


TMPRSS6



TMPRSS6 + Fab

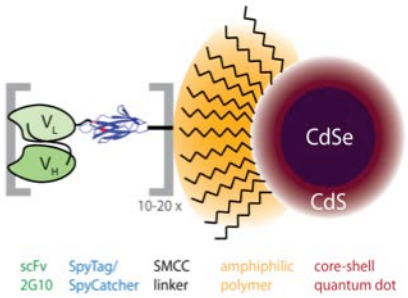
TMPRSS6 membrane anchored serine protease (Bayer)

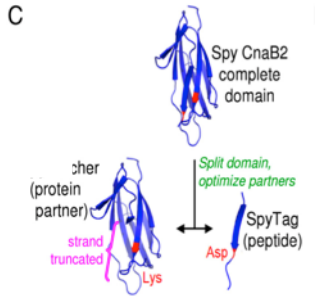


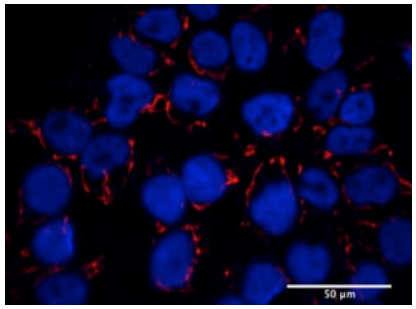
Dimeric Chloride Channel (Jan)

Engineered Antibody Projects

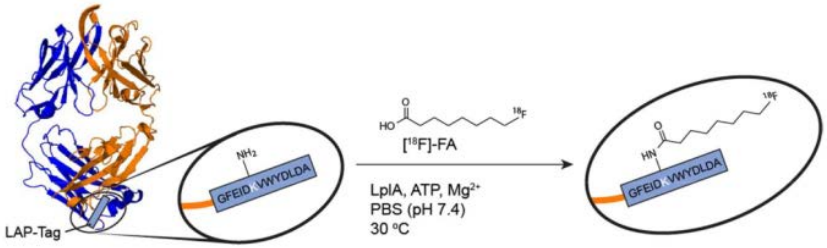
Antibody-Qdot conjugates using the SpyTag/SpyCatcher system

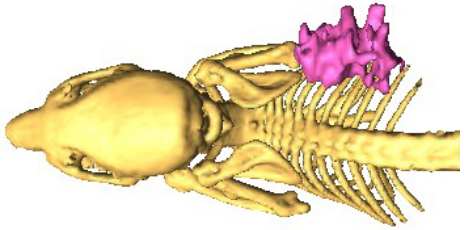






Labeling using LplA acceptor peptide (LAP) peptide or aldehyde peptide (CxPxR)

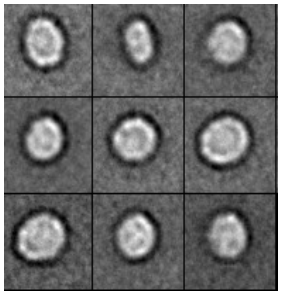


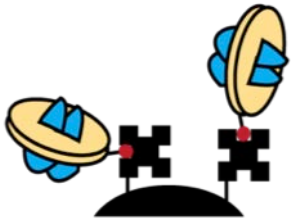


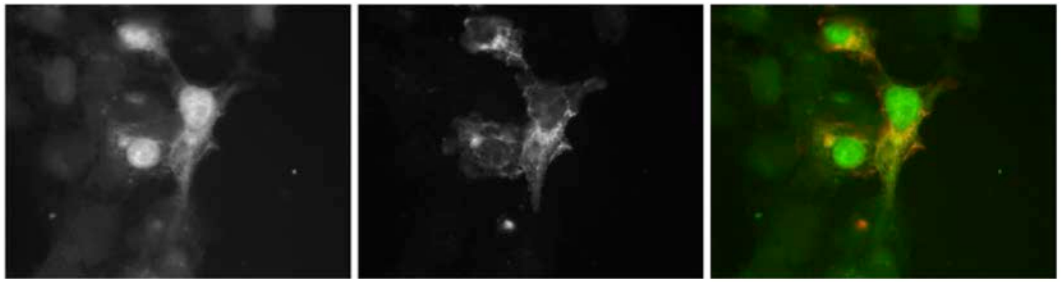
PDX-Colon

Drake CR et al. ACS Chem Biol. 2016 Mar 31.

Nanobodies selected against VGLUT2 reconstituted in nanodiscs





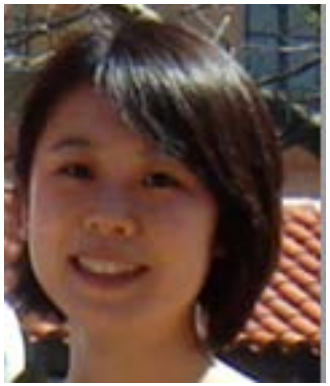


Many Thanks

Natalia Sevillano

Kathrin Zuberbuhler

Melody Lee



Jim Marks

Jim Wells

Robert Stroud

Cheng Lab

Gross Lab

UCSF University of California San Francisco

CTSI at UCSF

THE UNIVERSITY OF CALIFORNIA 1868

qb3 ucb·ucsc·ucsf

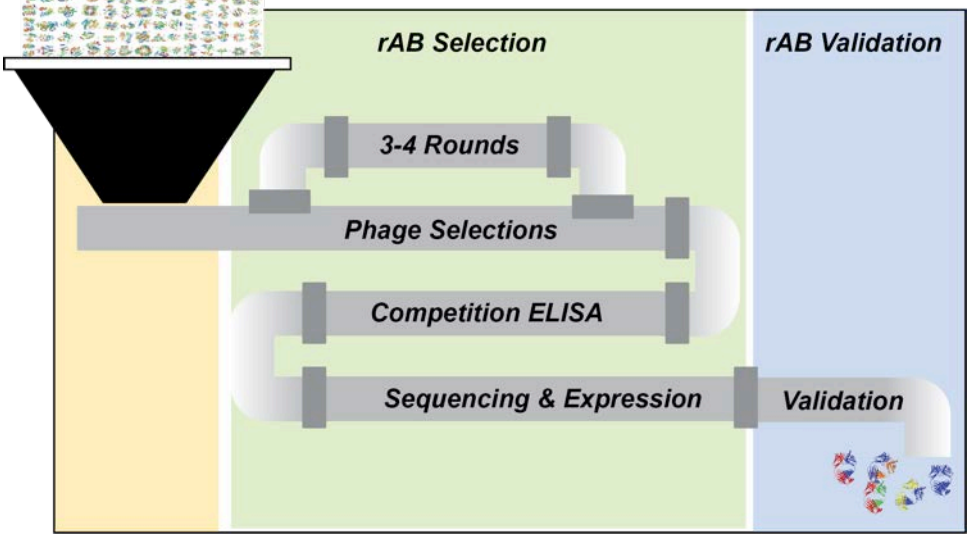
NATIONAL INSTITUTES OF HEALTH

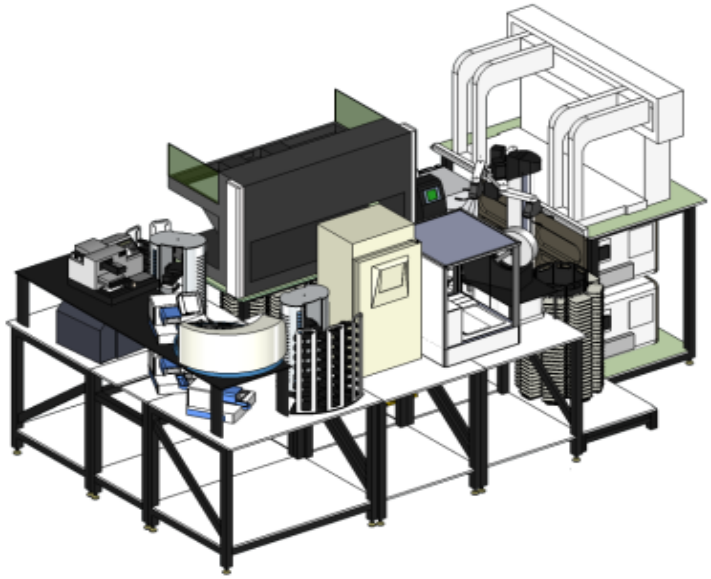
TRD3 capabilities

- Industrialized Selections
- Mutant and PTM specific selections
- How is the Surfaceome changed by RAS transformation?
- Multiplexed antibody detection for proteomics
  - BaNGS (Bar-coded antibodies NGS)
  - PhaNGS (Phage-antibodies NGS)

Industrialized Antibody Pipeline

Recombinant Antibody Network





~3000 Fab
346 TFs
211 Epigenetic Factors
Ave affinity: 10-20nM
Expression: 1-10 mg/mL

Hornsby et al 2015

UCSF University of California San Francisco

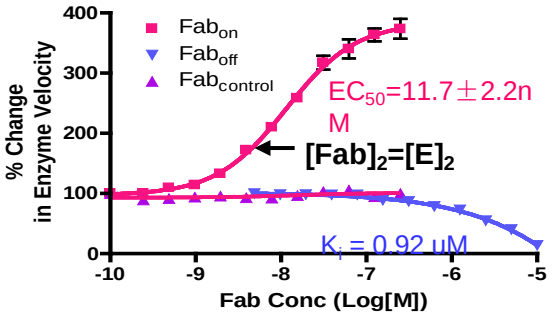
THE UNIVERSITY OF CHICAGO

UNIVERSITY OF TORONTO

Selection for high resolution antibodies

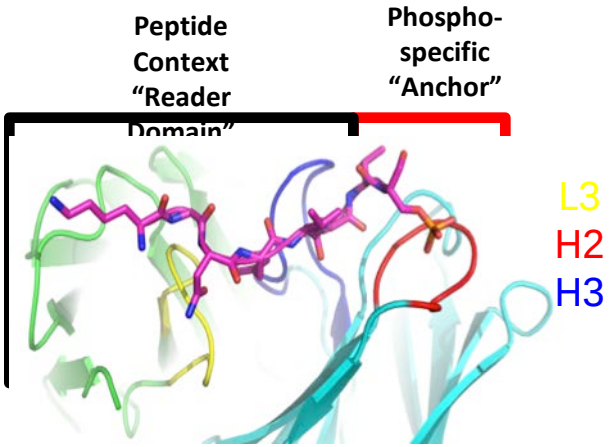
Conformations: On/Off state

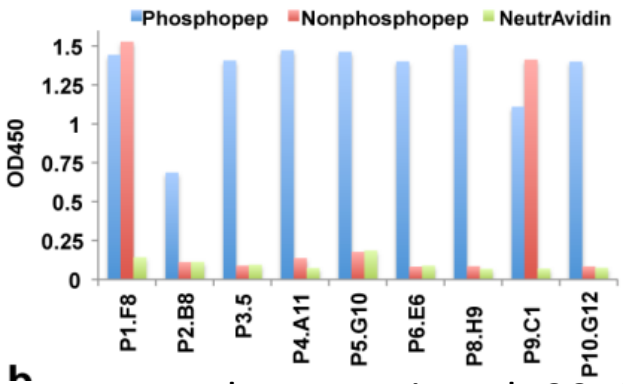
	Fab_{on}			Fab_{off}		
	"on" form	"off" form	apo	"on" form	"off" form	apo
$K_D(10^{-9}M)$	2.5	>1000	330	99	4.7	17
$k_{on}(10^4 M^{-1}s^{-1})$	66	N.D.	0.8	1.6	135	55
$k_{off}(10^{-3}s^{-1})$	1.7	N.D.	2.6	1.6	6.4	9.5



Gao PNAS 2009
Thomson PNAS 2013

PTMs: Phosphorylation

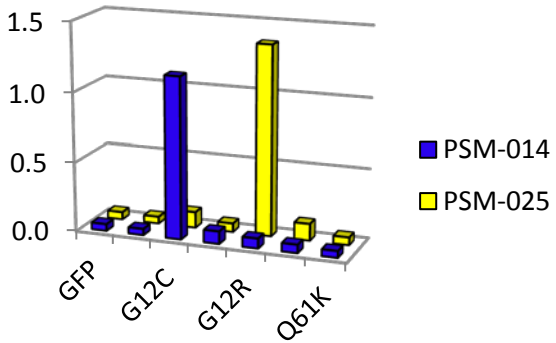




Koerber Nat Biotech 2013
P.Lee, K.Mou, unpublished

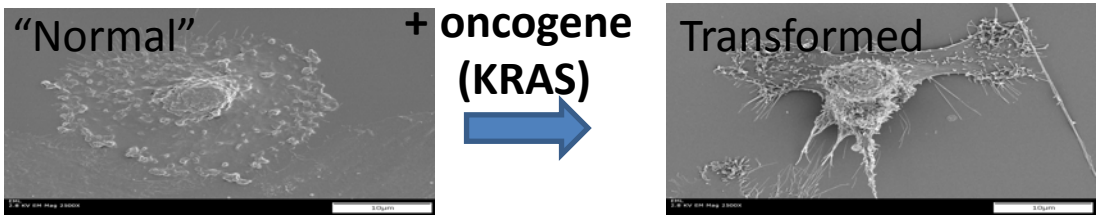
Mutations: KRAS

Gly12	Gly13	Gln61
A	A	E
C	C	H
D	D	K
R	R	L
V	V	P
S	S	R

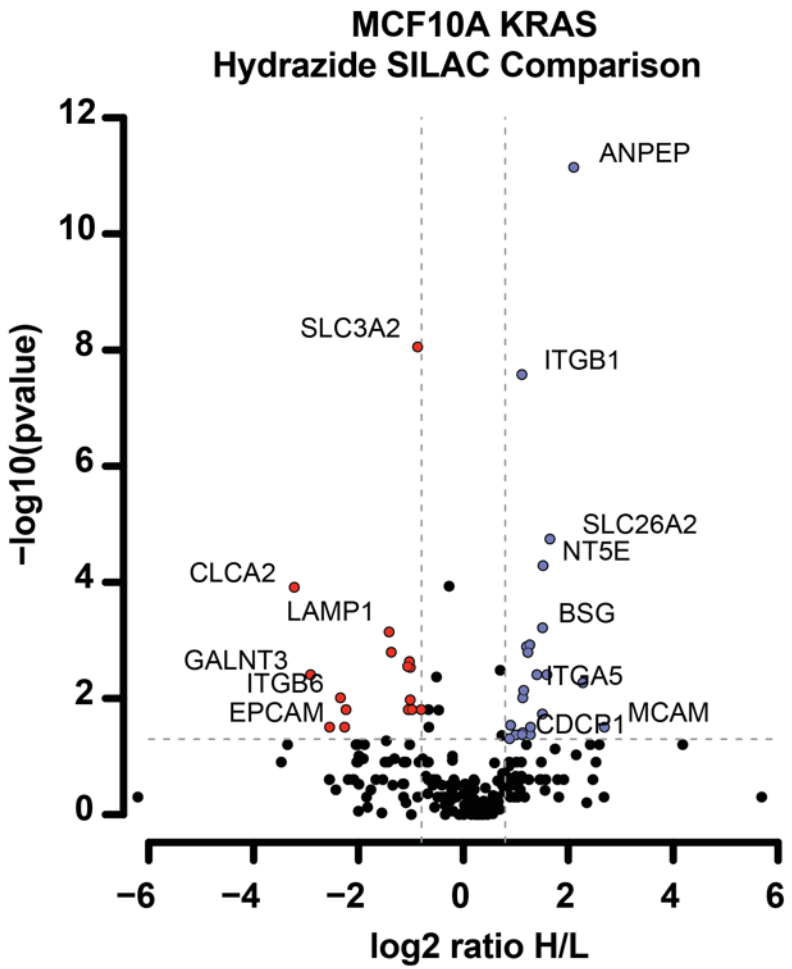


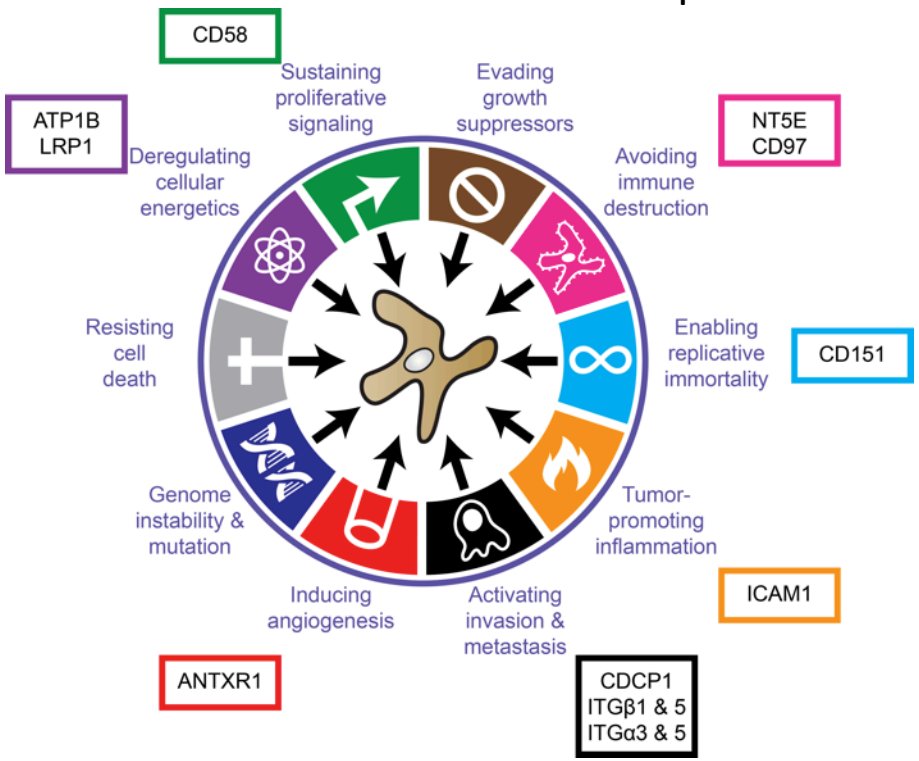
P. Marinec, Unpublished,

How oncogenes remodel cell surfaces



A Martinko
J Blonder
G Whiteley
S. Bandopadhyay
unpublished

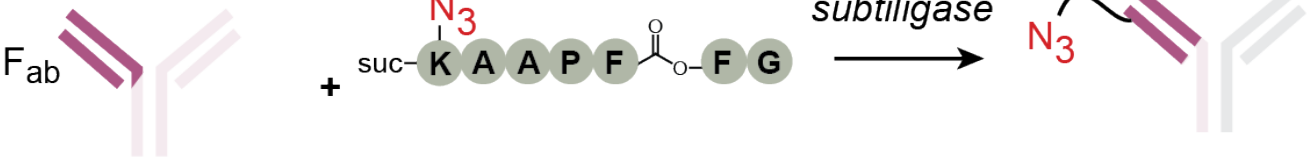


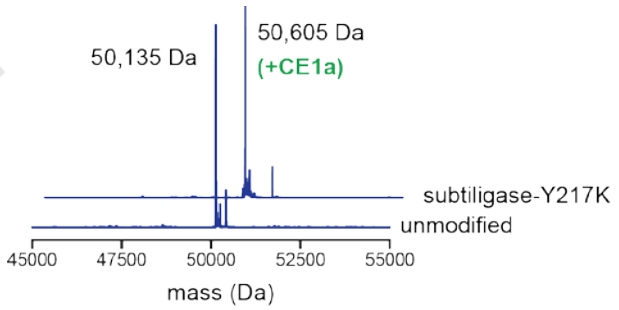


Hanahan, D., & Weinberg, R. A. (2011). *Cell*

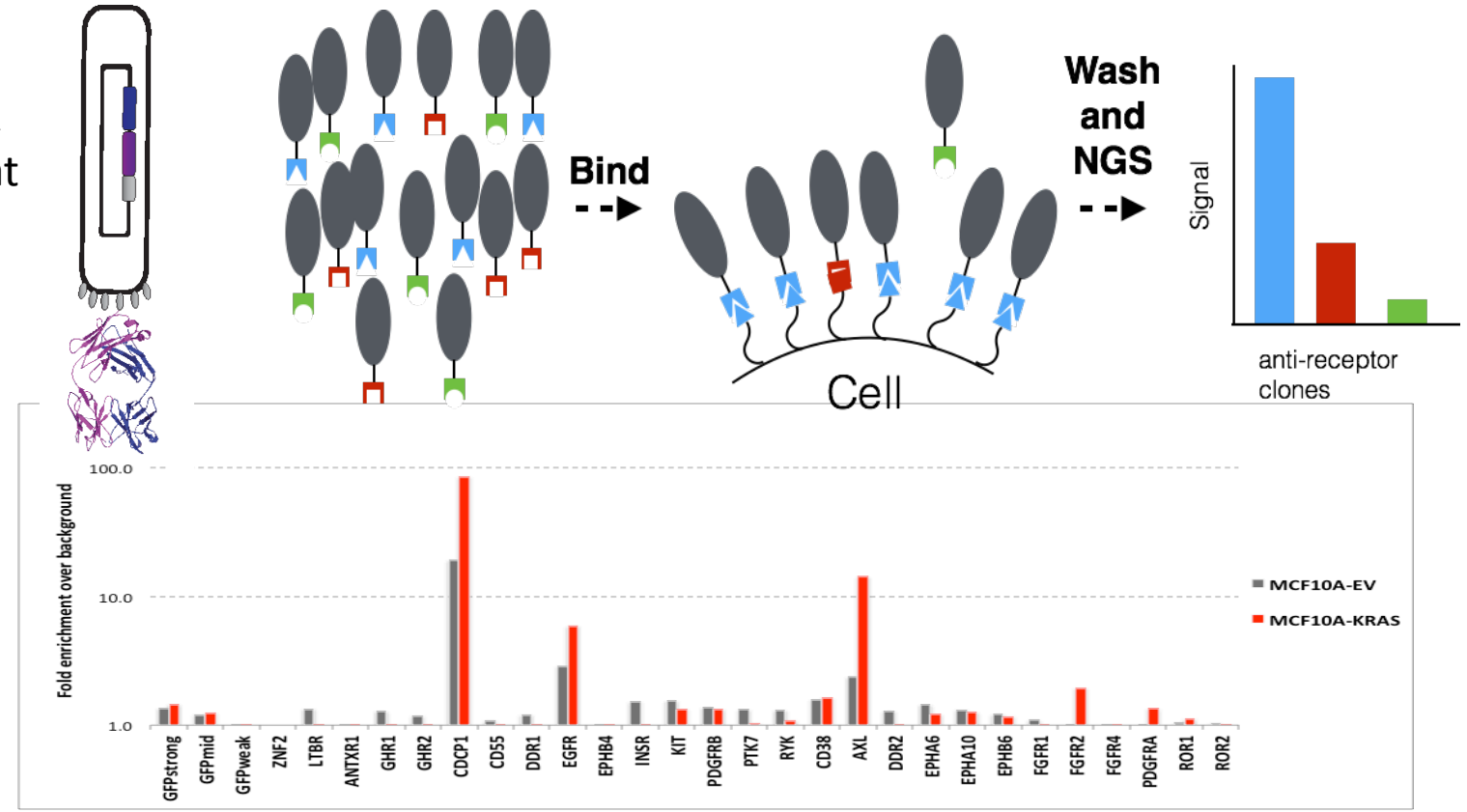
DNA-barcoding for multiplex detection

BaNGS: Amy Weeks





PhaNGS:
Sam Pollock
Jason Moffat
Dev Sidhu



How can FNLCR help?

- Protein Antigens (100/yr)
  - Fc fusions
  - Membrane proteins and complexes
- IgG conversion, expression, distribution (100/yr)
- Access to high priority targets and collaborations