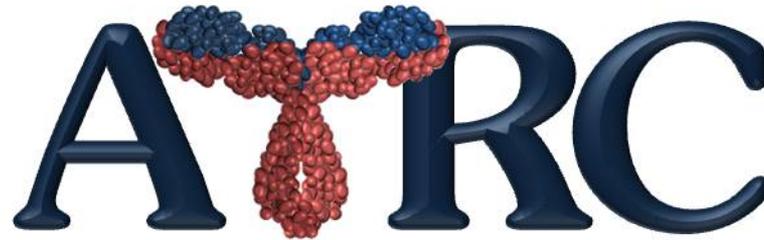


ATRC @ UCSF OVERVIEW



*Antibody technology research center

Goals of the ATRC

Robust approaches for rAbs to the proteome

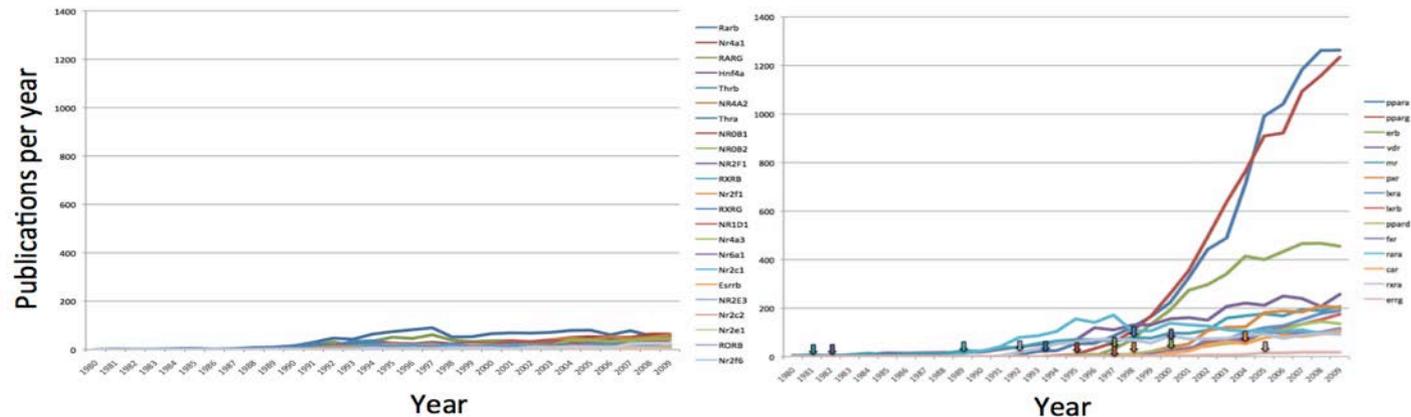
- Initially focus (largely) on secreted proteins
 - Single and multipass membrane proteins/proteases
 - PTMs (phospho-specific rAbs)

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



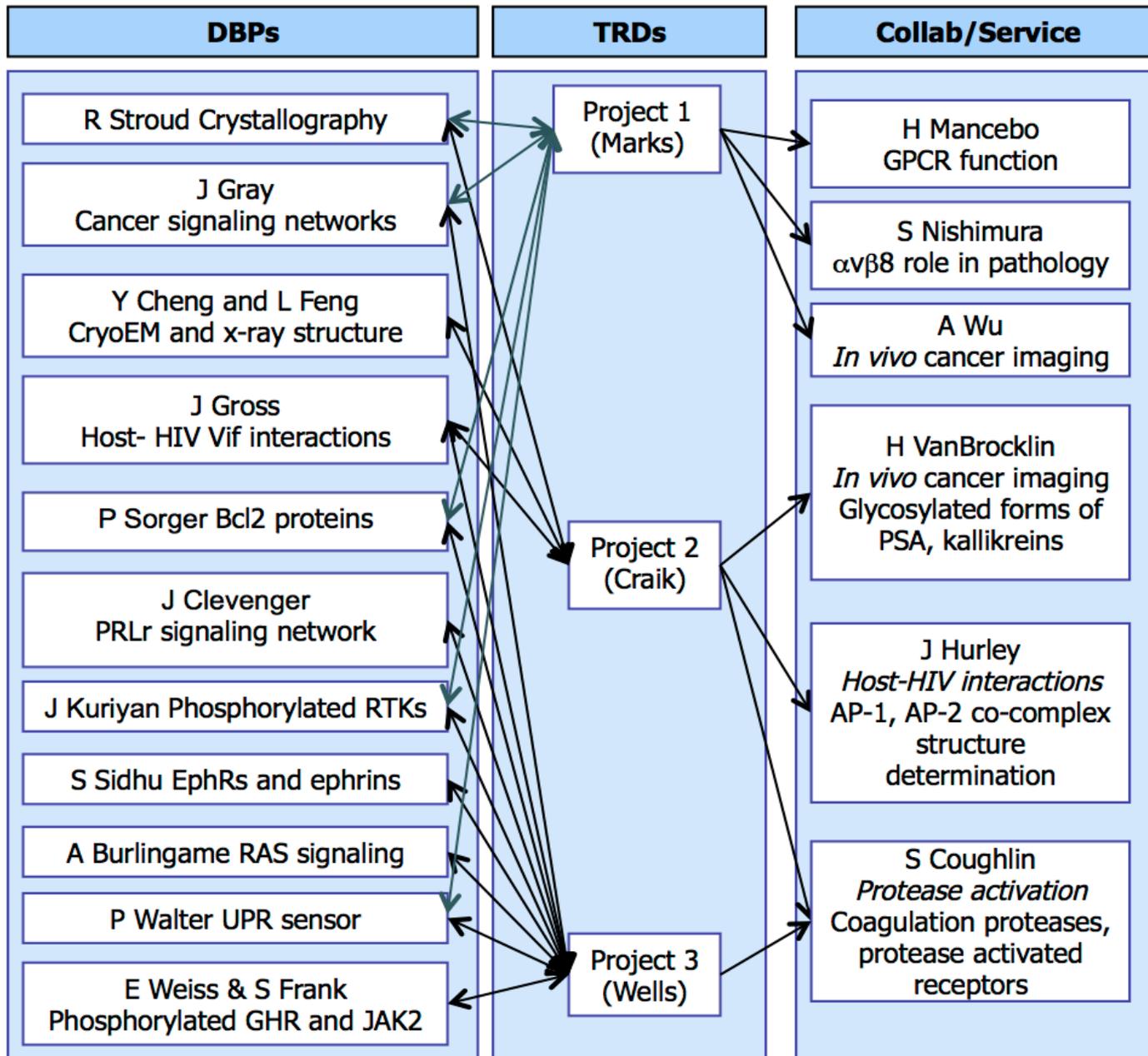
ATRC Solution

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

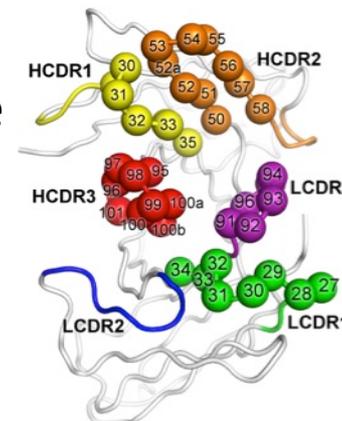
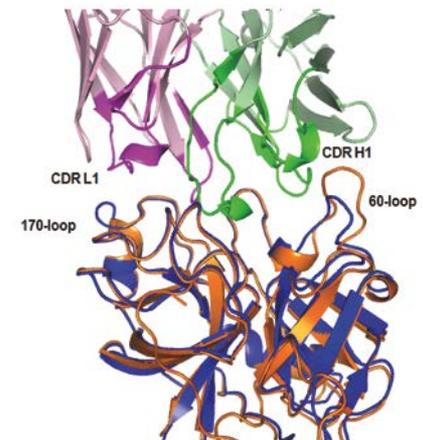
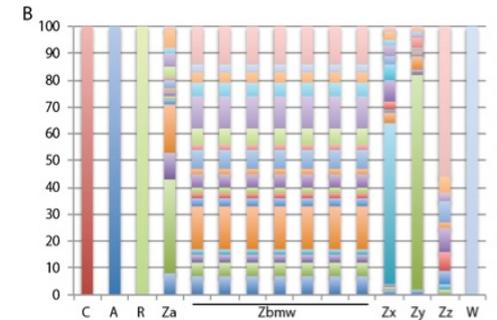
- Generating robust phage Ab libraries
- Automation of rAb generation
- Generating rAbs to challenging antigens
- Providing technology training



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

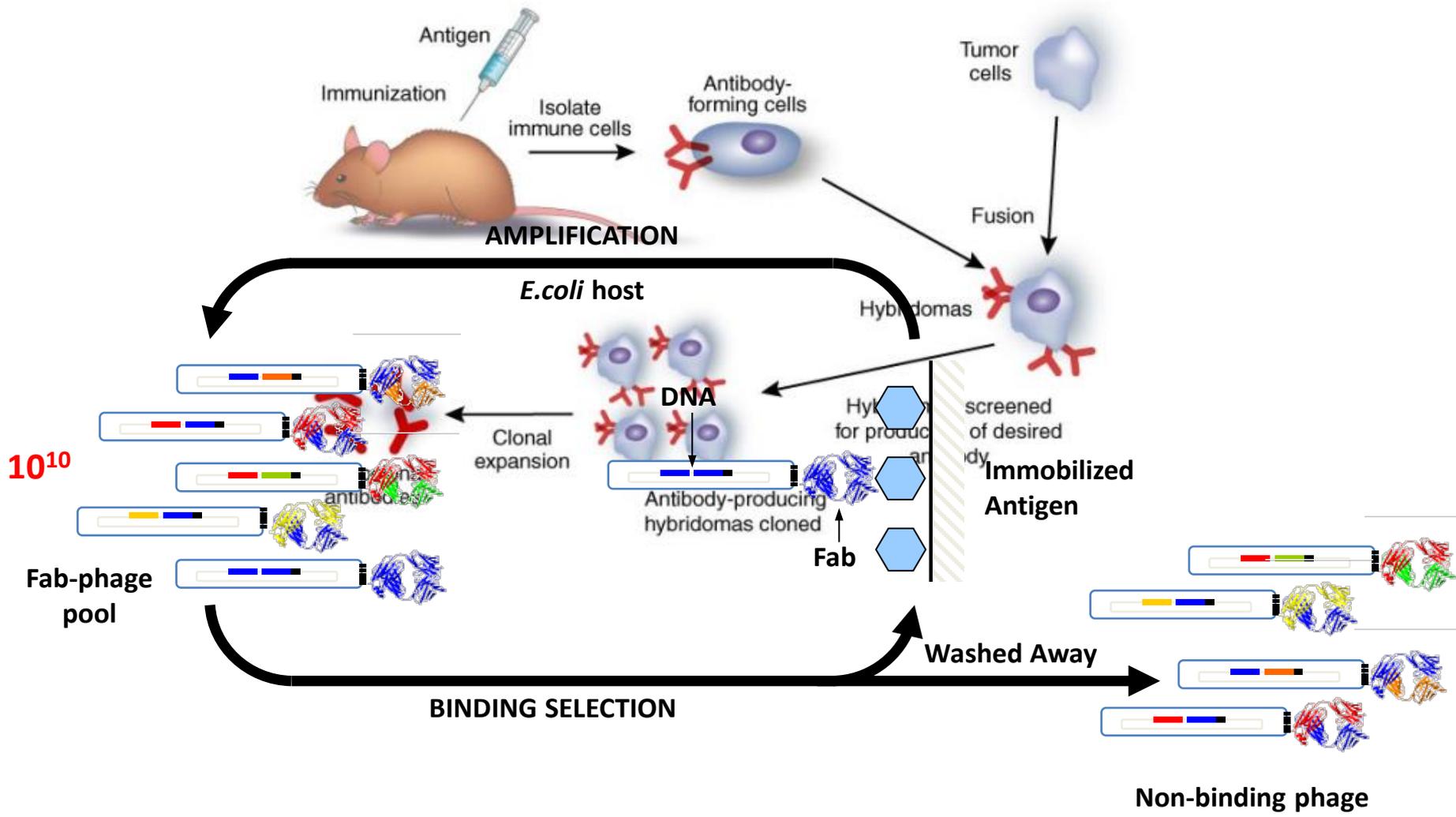
TR&Ds

- 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

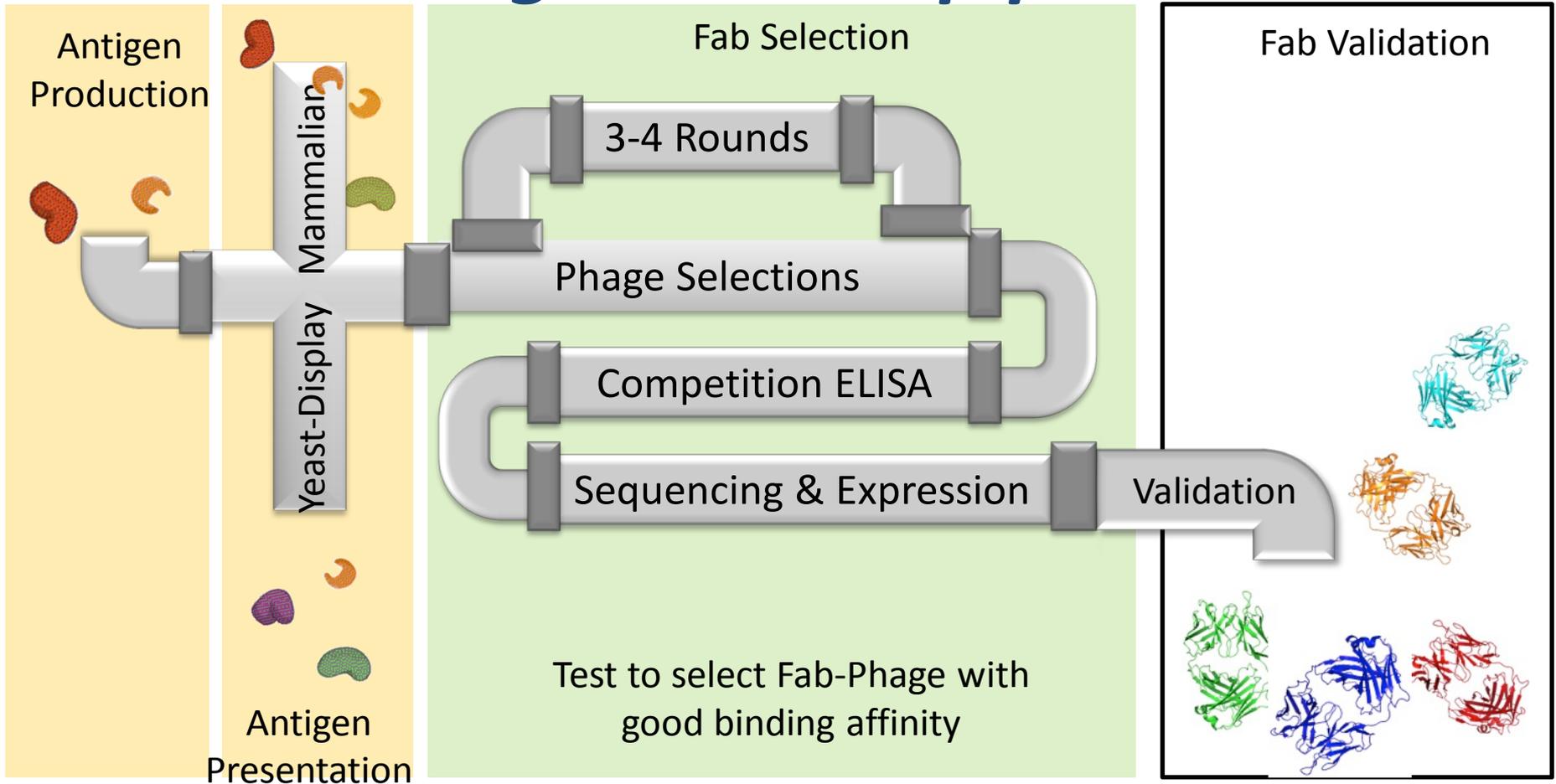


Technologies: in vivo vs in vitro Abs

Adapted from www.medscape.com



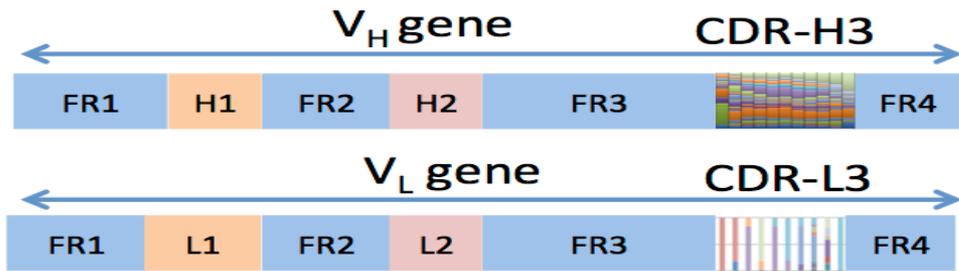
Ab generation pipeline



Antigen QC: SDS-PAGE; SEC

rAb QC: Sequence, affinity, specificity

TRD 1: Nature-inspired synthetic antibody libraries



6 V_H scaffolds

5 V_L scaffolds

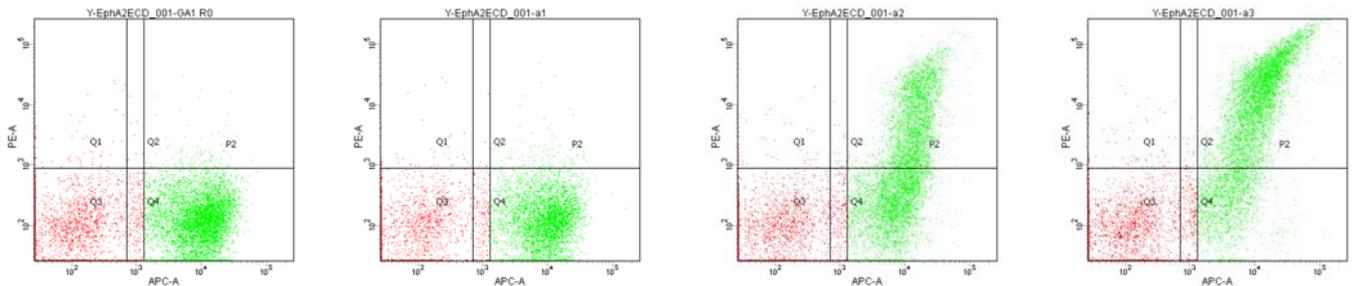
R0

R1

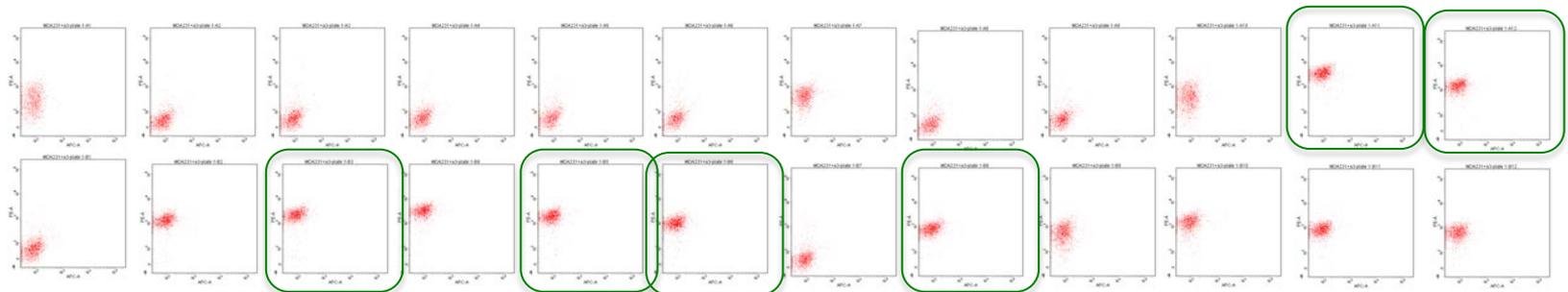
R2

R3

Y-EphA2ECD



> 30 unique antibodies
MDA-MB-231 (EphA2 +) cells



Antibody fragments for nanoliposome targeting

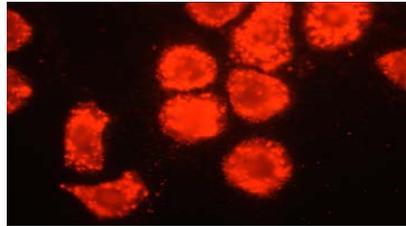
Anti-HER2 liposomal doxorubicin

MM-302

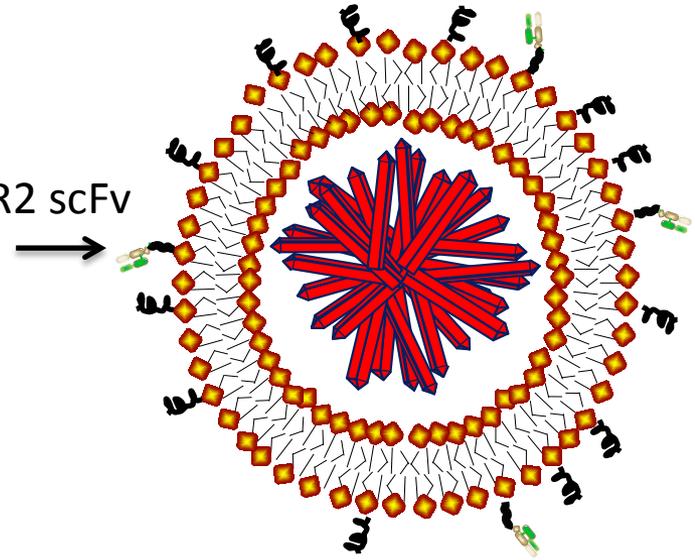
Registration Phase 2

HER2+, progress on T DM-1

scFv F5



Anti-HER2 scFv



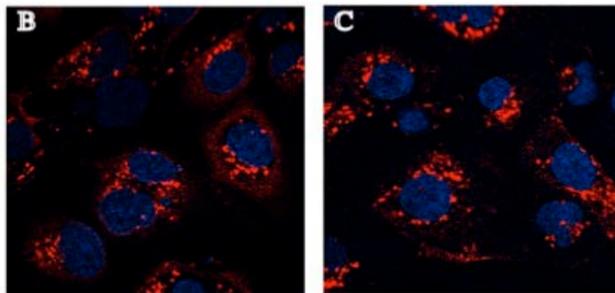
Anti-EphA2 liposomal docetaxel

MM-310

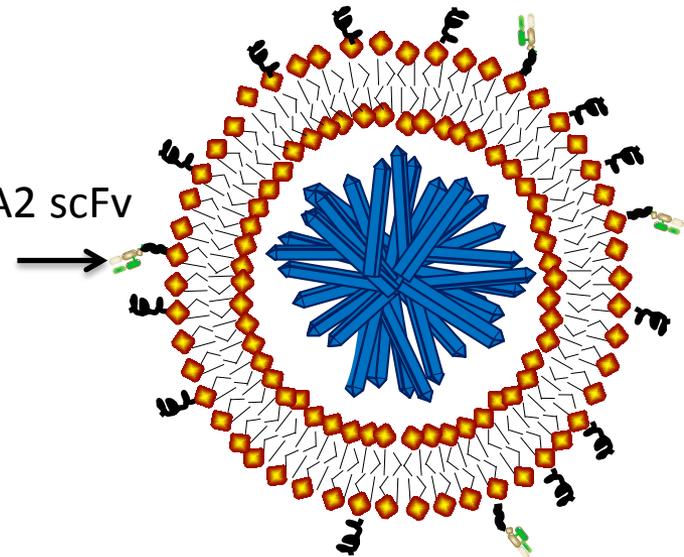
Completed manufacturing

Phase 1 Q2 2016

scFv
D2-1A7



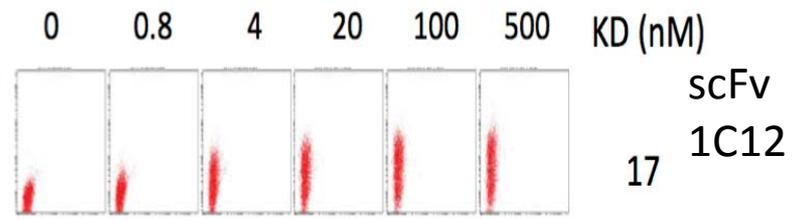
Anti-EphA2 scFv



rAbs to CXCR1 with functional activity

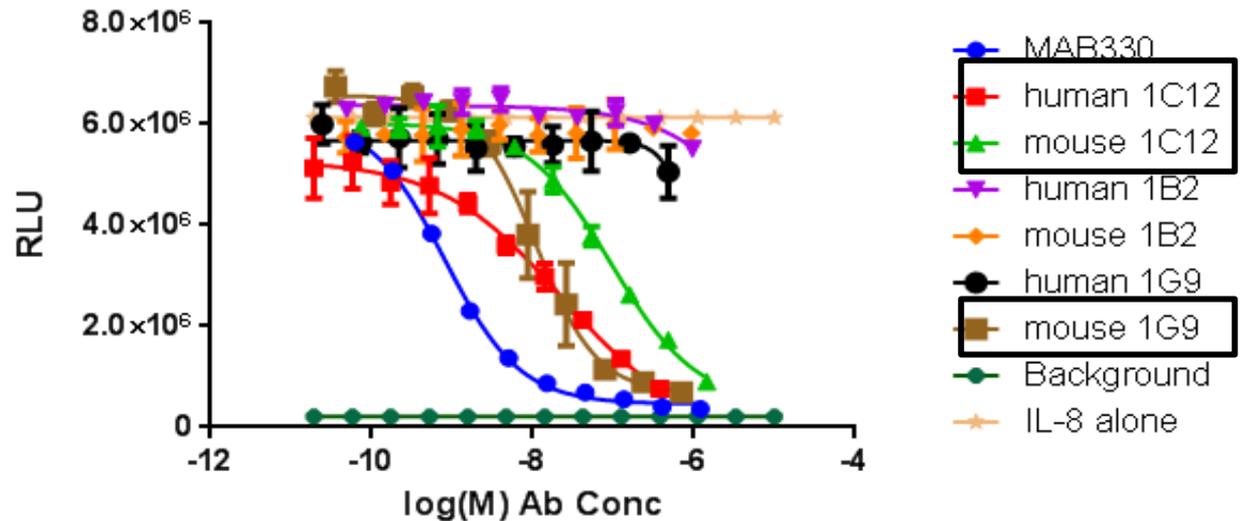
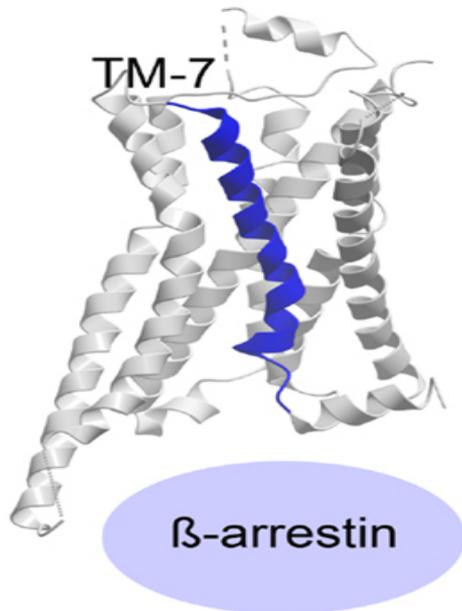
Immune phage library
- GPCR DNA immunization

Select on
transfected
CHO cells

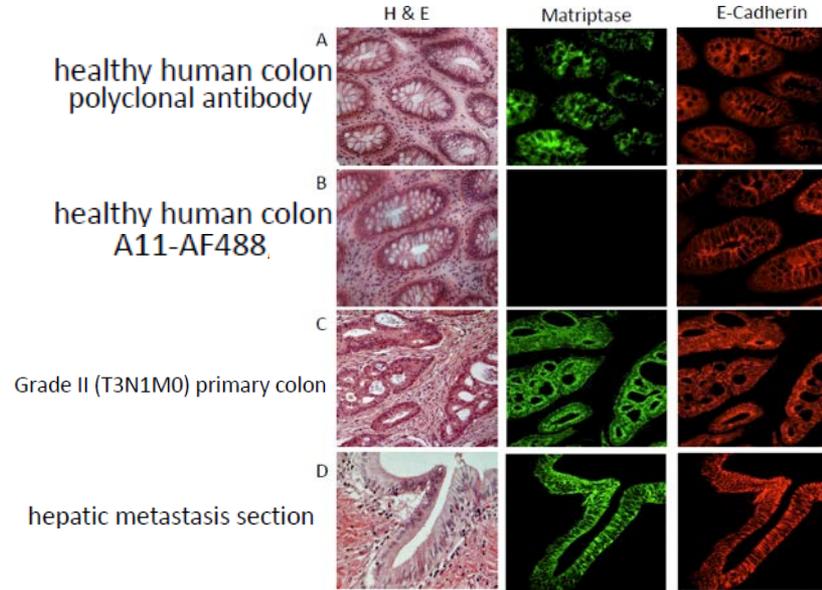
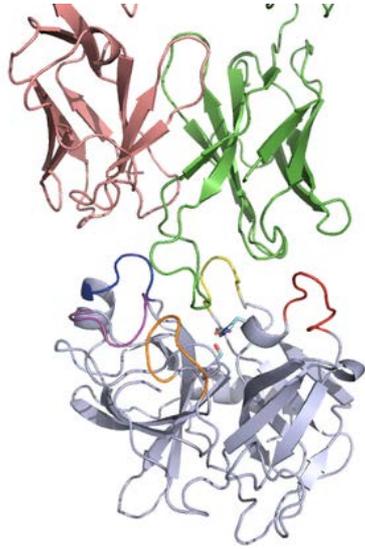


B-arrestin assay for mAb biologic activity: inhibit IL-6 CXCR1 activation

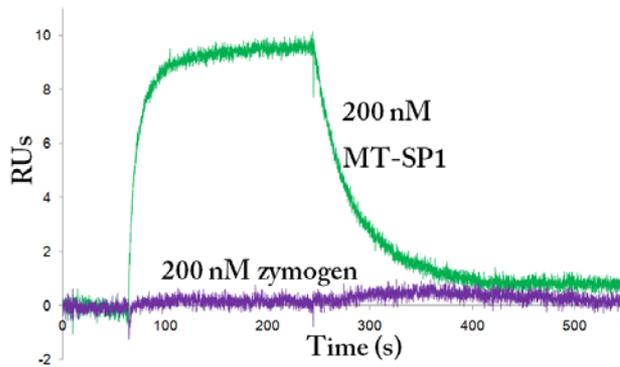
Marks Lab CXCR1 Antibodies



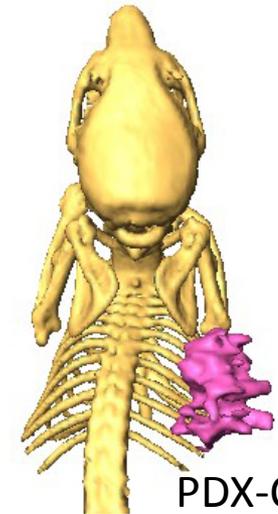
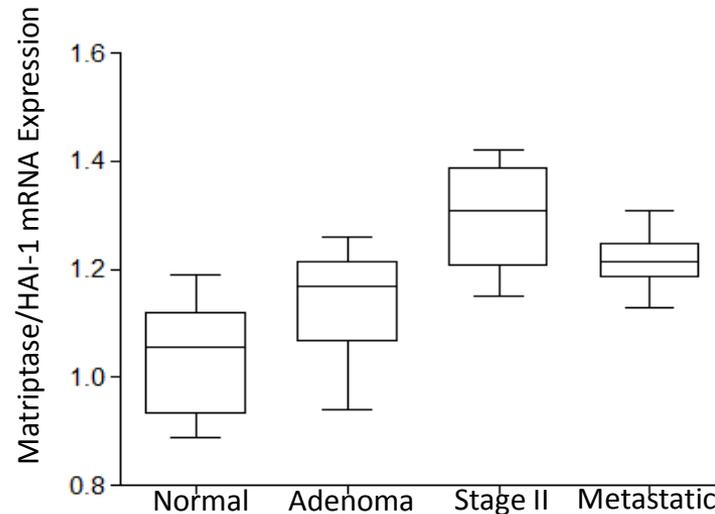
Ab Activity Based Probe to Matriptase Provides Diagnostic Information in Colon Cancer



HT29-Colon

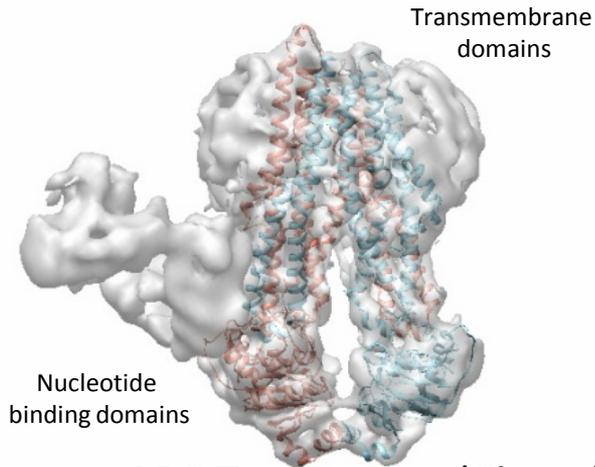


Schneider E, et al. *J. Mol Bio* **412** (2012)
 LeBeau AM, et al. *PNAS* **110** (2013)



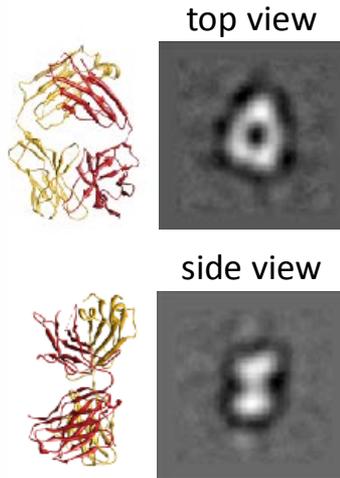
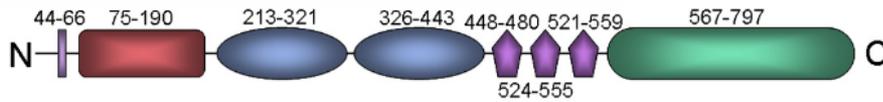
PDX-Colon

Conformationally Selective Fabs to Challenging Targets

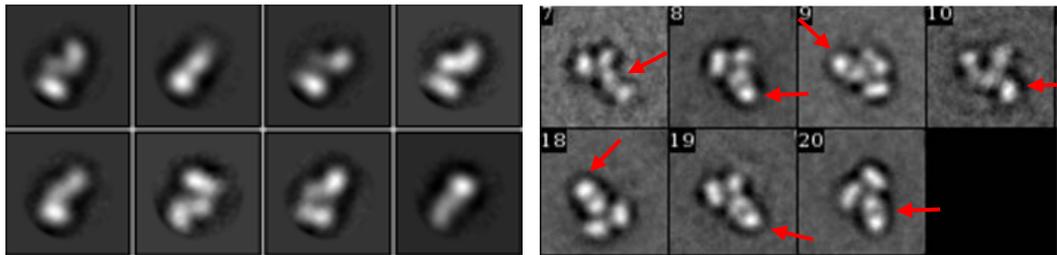
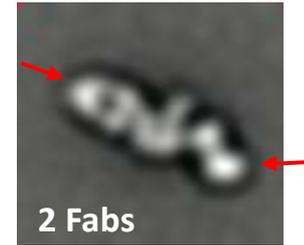
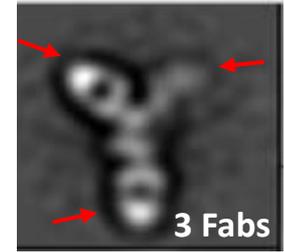
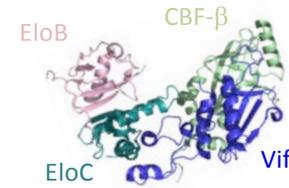


ABC Transporter (Chang)

Kim J et al. Nature (2015)



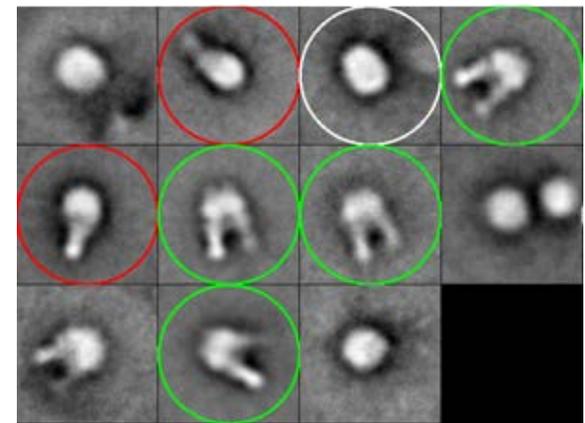
HIV-host complexes:
VCBC (Gross)



TMPRSS6

TMPRSS6 + Fab

TMPRSS6 membrane anchored serine protease (Bayer)

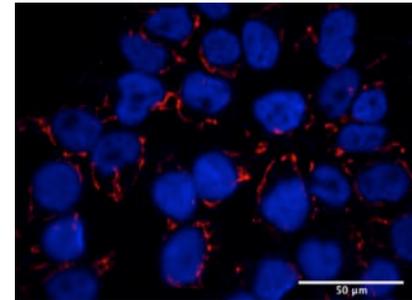
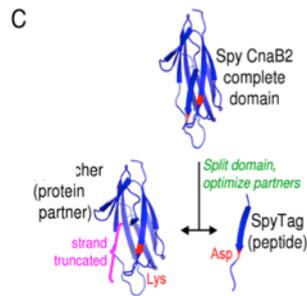
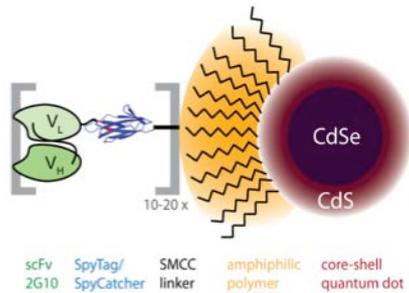


Dimeric Chloride Channel (Jan)

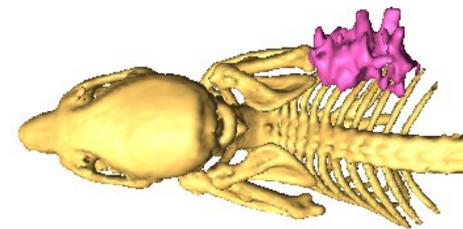
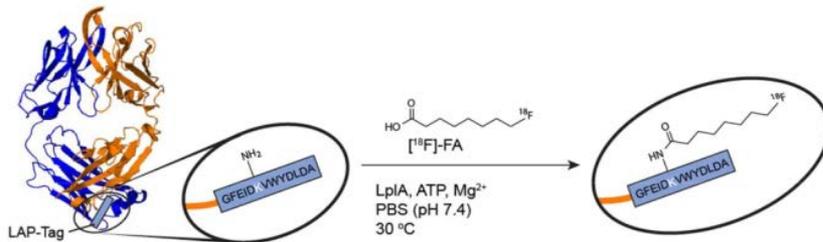
Engineered Antibody Projects



Antibody-Qdot conjugates using the SpyTag/SpyCatcher system



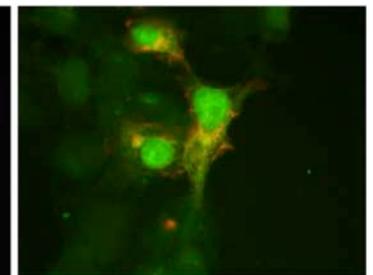
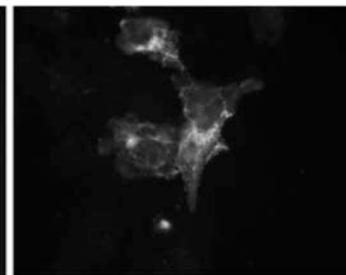
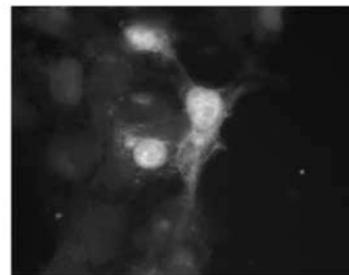
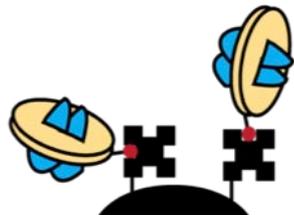
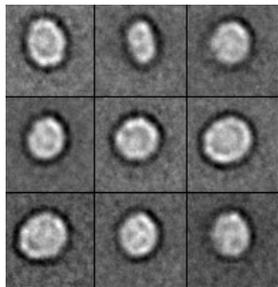
Labeling using LplA acceptor peptide (LAP) peptide or aldehyde peptide (CxPxR)



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



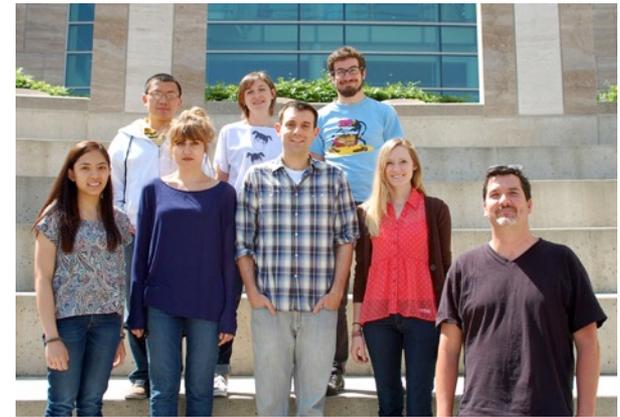
Robert Stroud



Cheng Lab



Gross Lab

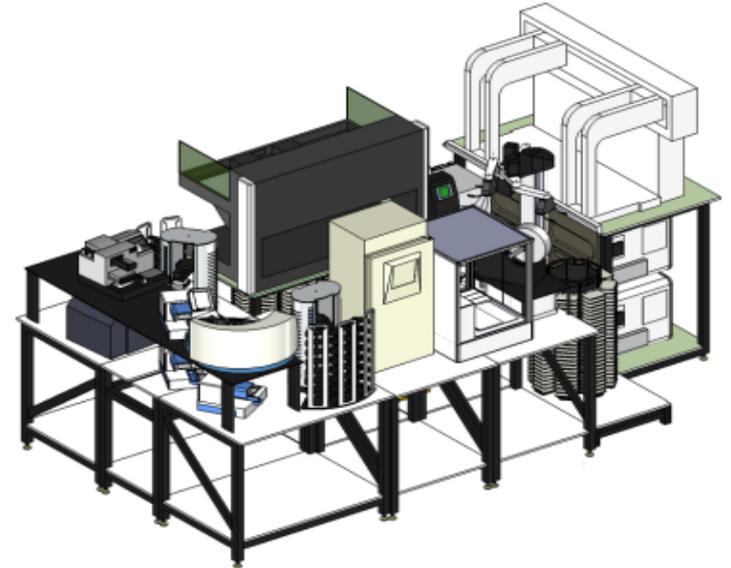
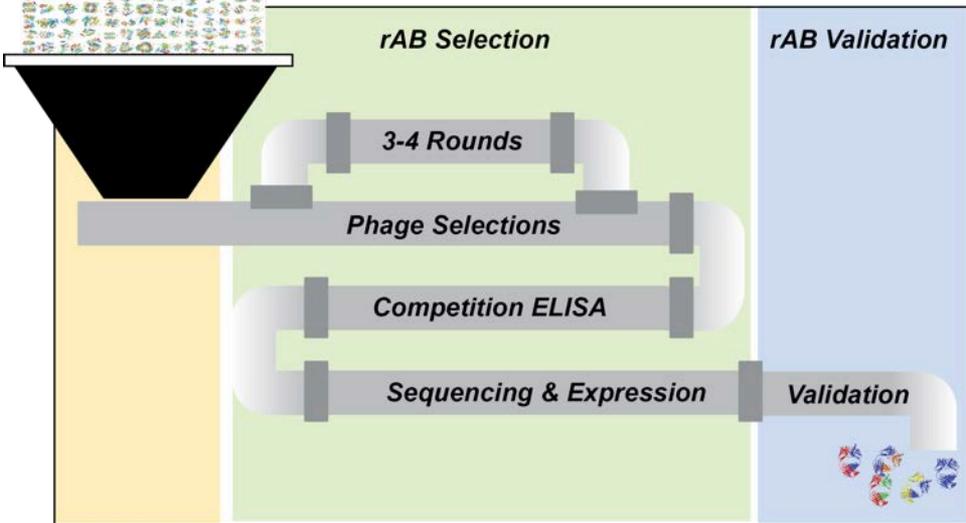




TRD3 capabilities

- 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



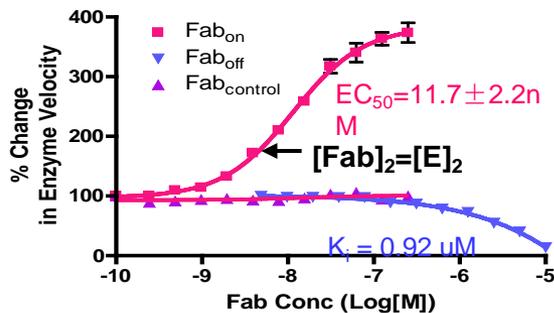
~3000 Fab
346 TFs
211 Epigenetic Factors
Ave affinity: 10-20nM
Expression: 1-10 mg/mL

Hornsby et al 2015

Selection for high resolution antibodies

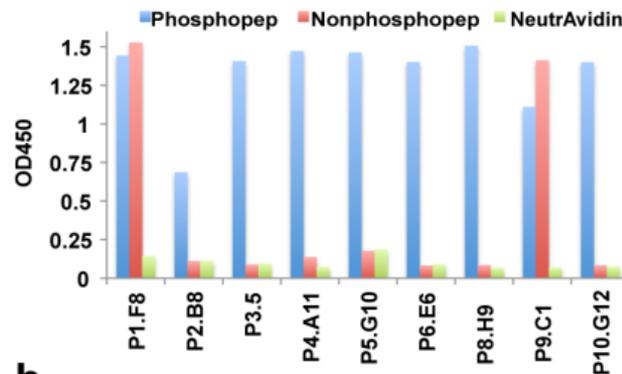
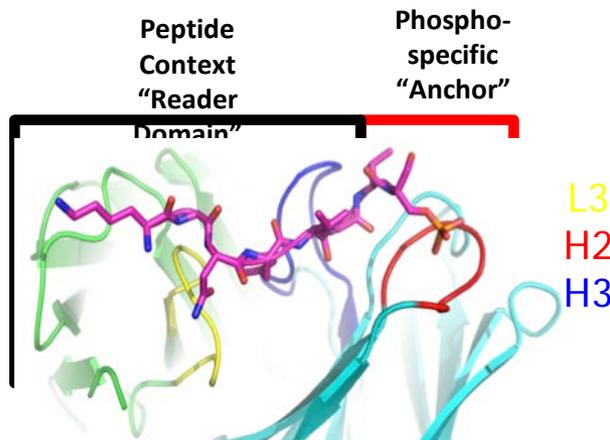
Conformations: On/Off state

	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

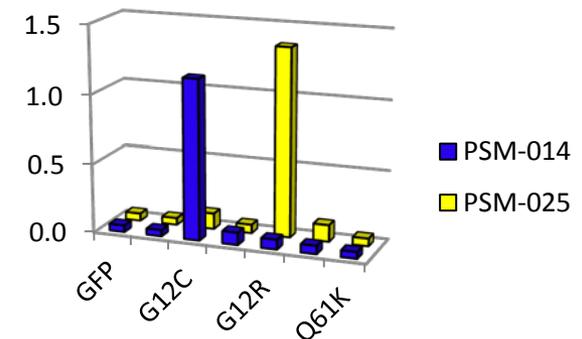
PTMs: Phosphorylation



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

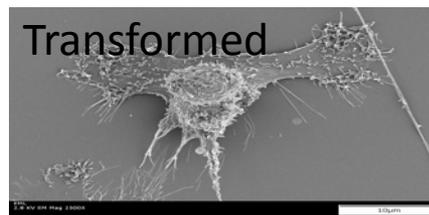
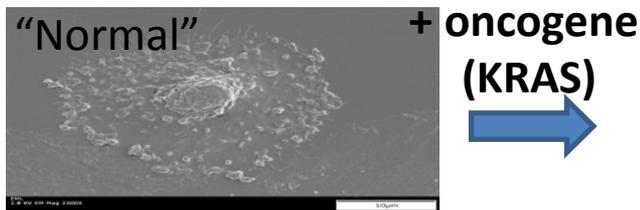
Mutations: KRAS

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



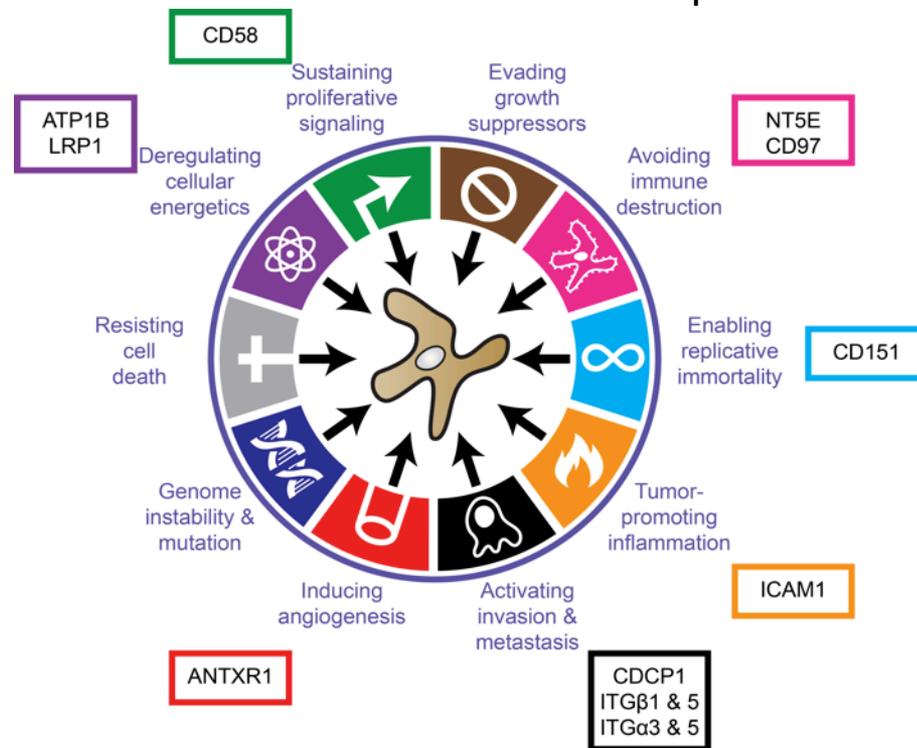
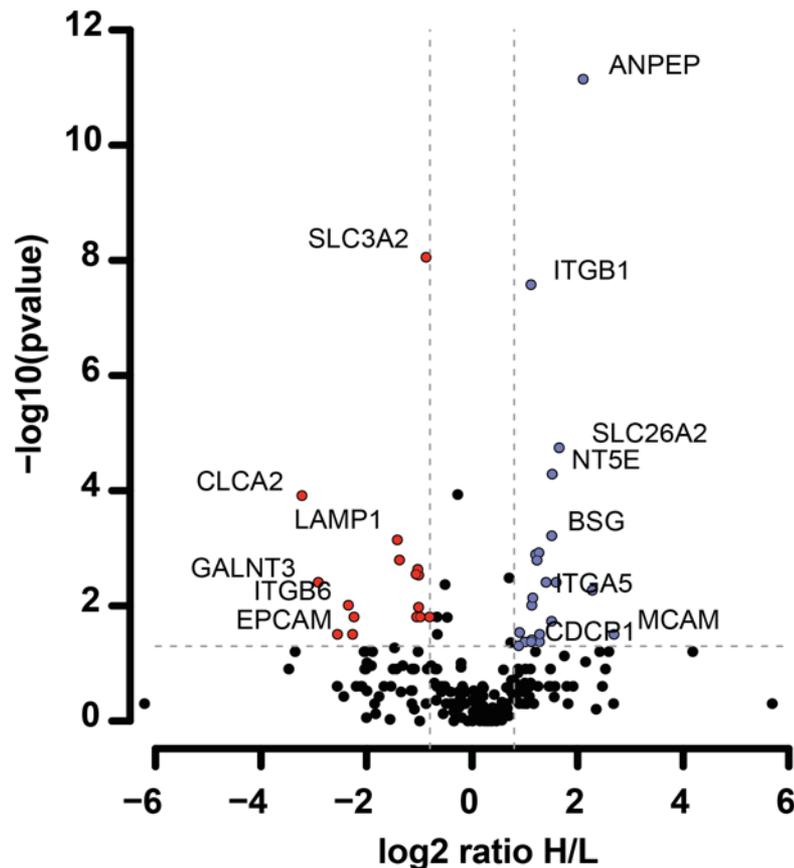
P. Marinec, Unpublished,

How oncogenes remodel cell surfaces



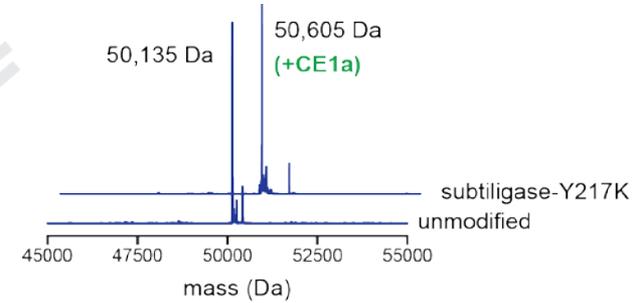
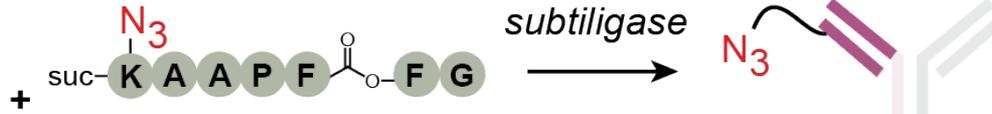
A Martinko
J Blonder
G Whiteley
S. Bandopadhyay
unpublished

MCF10A KRAS
Hydrazide SILAC Comparison

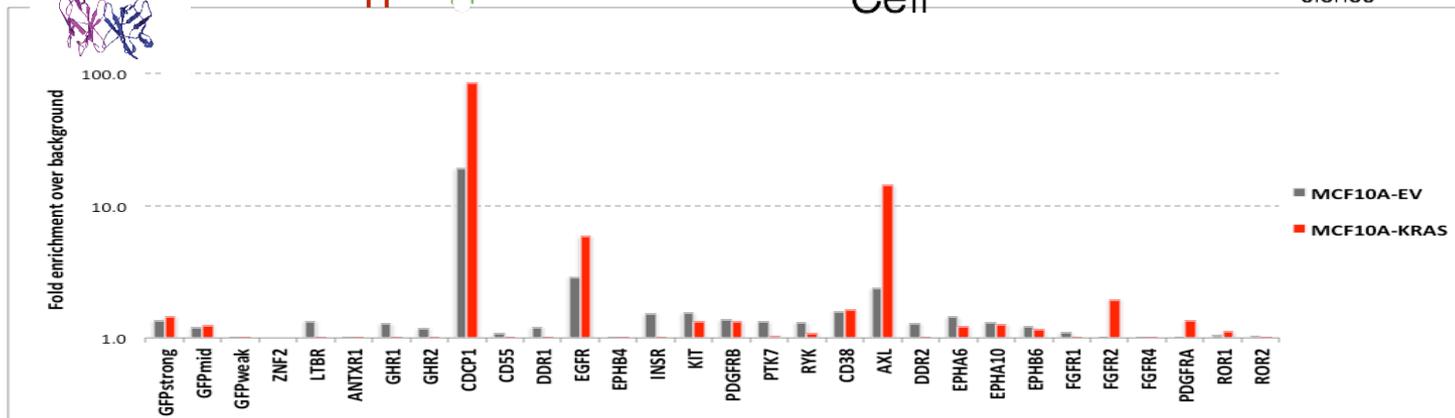
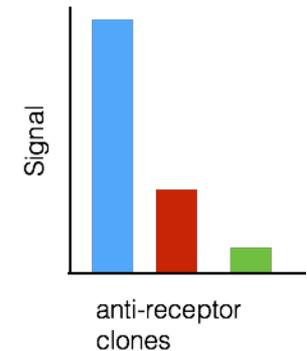
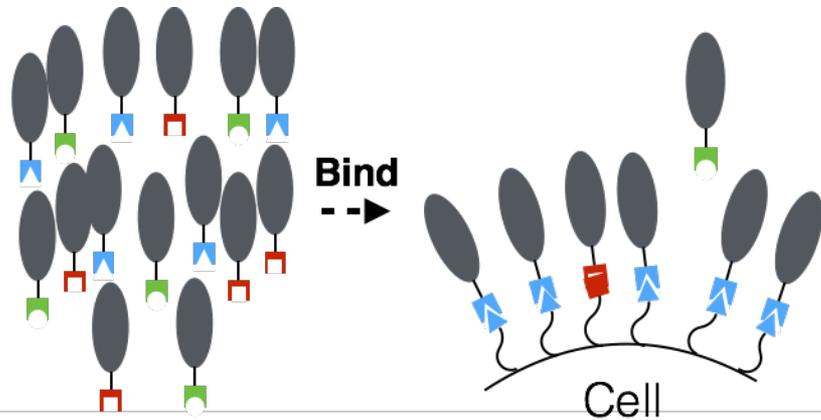


DNA-barcoding for multiplex detection

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