

Yeast Surface Display FAQs

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Format

- **Methods tips**, as opposed to research results (which will be **Tuesday @ 3 PM**).
- **FAQ & A**

Why use yeast display?

- **Affinity**, **expression**, & **stability** maturation
 - Fast
 - Robust
 - Large improvements
- Quantitative screening
- Eucaryotic secretory processing
 - Glycosylation
 - Disulfides

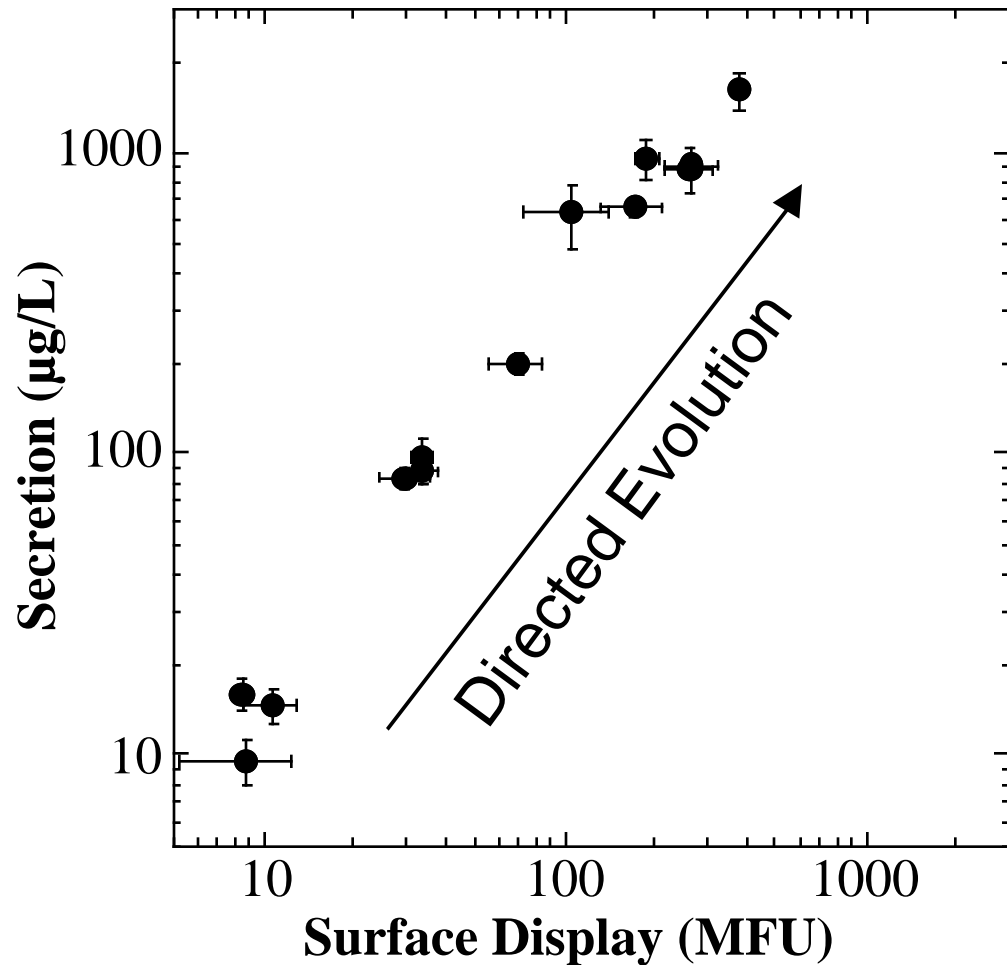
Yeast Display **Affinity** Maturation

Binder	Target	# rounds	Start K_d	Final K_d	Improve- ment	Ref
KJ16	TCR	1	500 nM	150 nM	3 x	<i>Prot. Eng.</i> 10:1303 '97
4-4-20	Fluorescein	4	0.3 nM	270 fM	1100x	<i>PNAS</i> 97:10701 '00
scTCR	pMHC	1	1.5 μM	9 nM	170 x	<i>PNAS</i> 97:5387, '00
scTCR	SEC	2	3 μM	7 nM	430x	<i>J. Mol Biol.</i> , 307:1305, '01
MFE-23 scFv	CEA	2	17 nM	12 pM	1400x	<i>Unpub.</i>

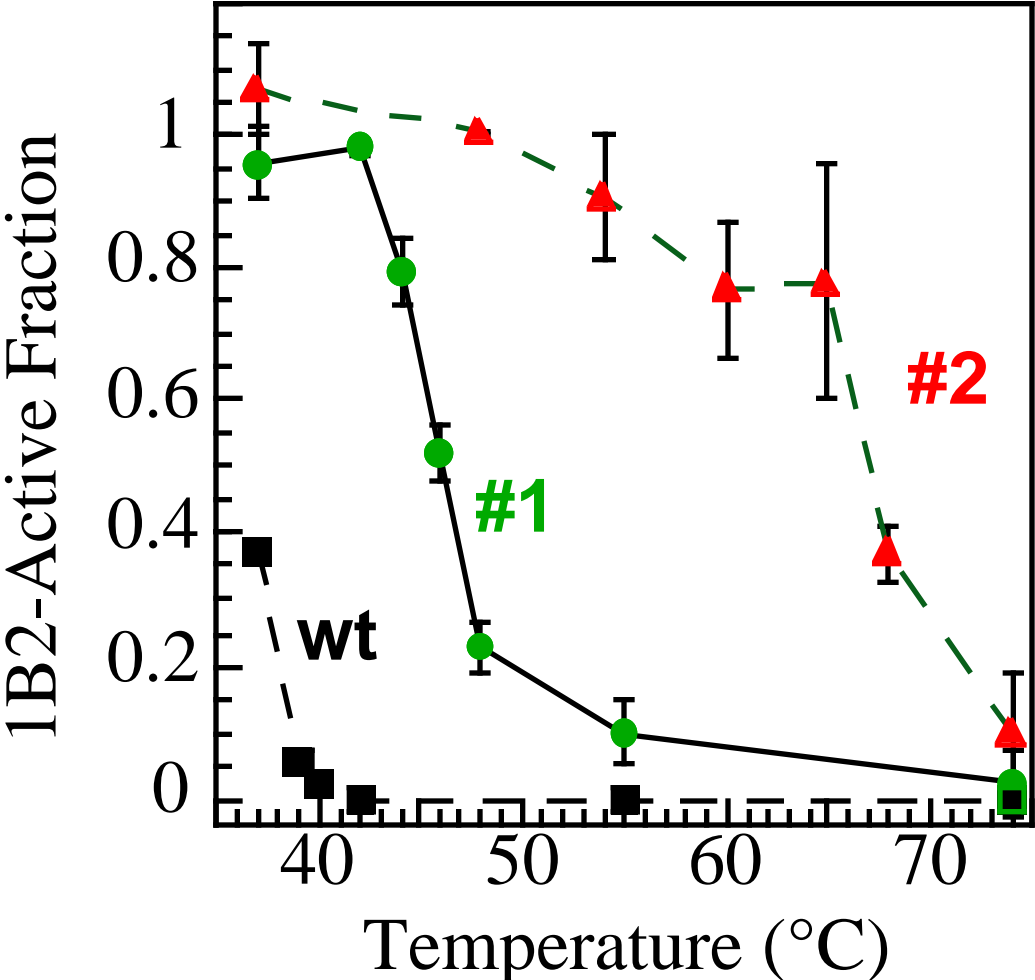
“Displayability” Correlates with Soluble Expression Level

(*JMB* 292:949, '99; *Nat. Biotech.* 18:754, '00)

- 13 mutant scTCRs
- Linear across 2 orders of magnitude
- **Expression ~ 10 mg/L in flasks**



Thermal stability from 37°C ⇒ 65 °C



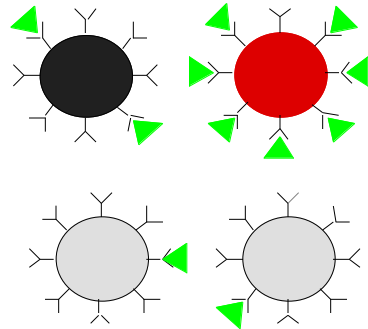
Does quantitative screening matter?

- Optimal labeling strategies
- Normalization for expression differences

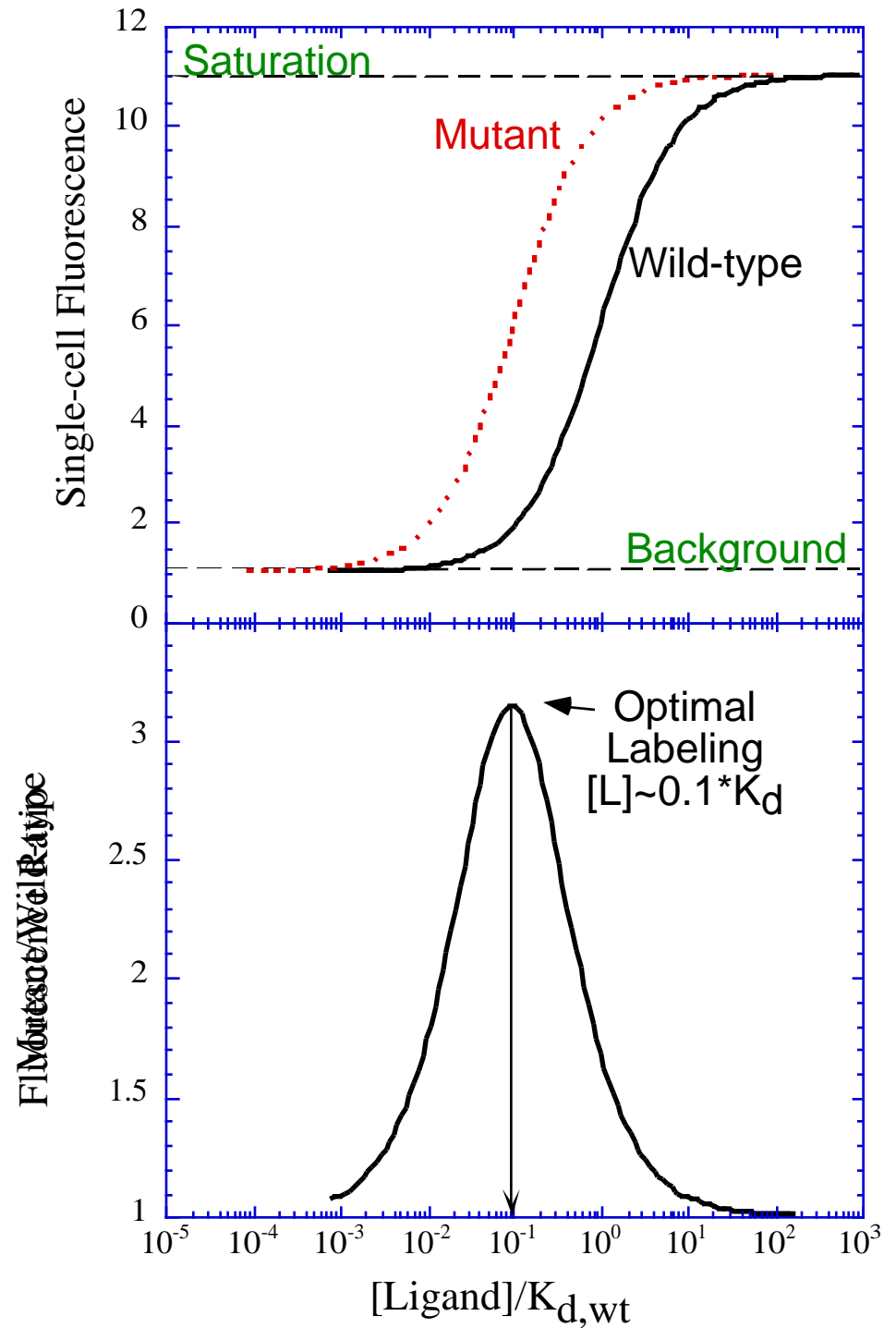
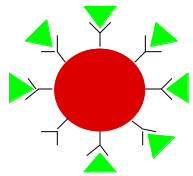
Optimal equilibrium screening

Fluorescent ligand: 

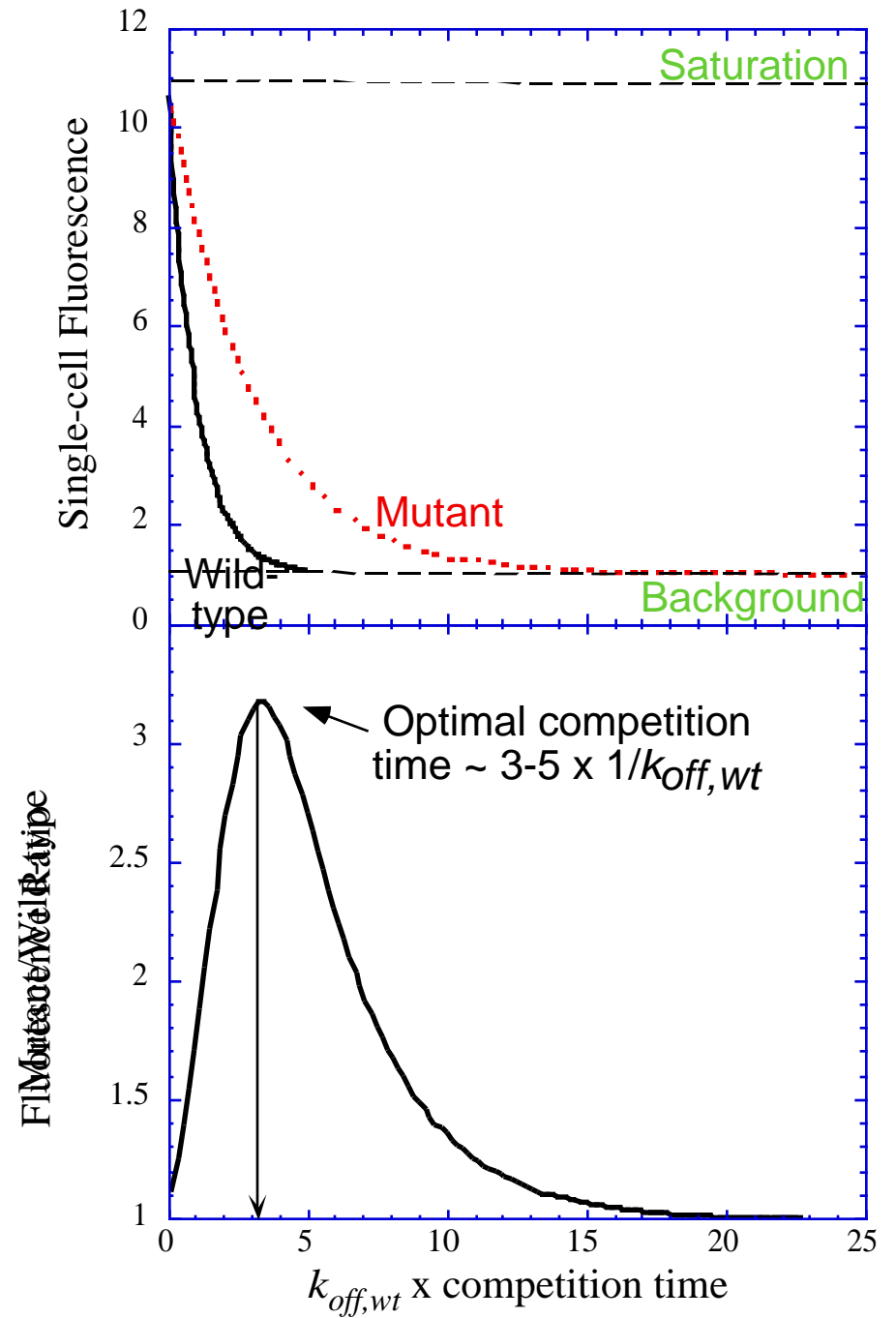
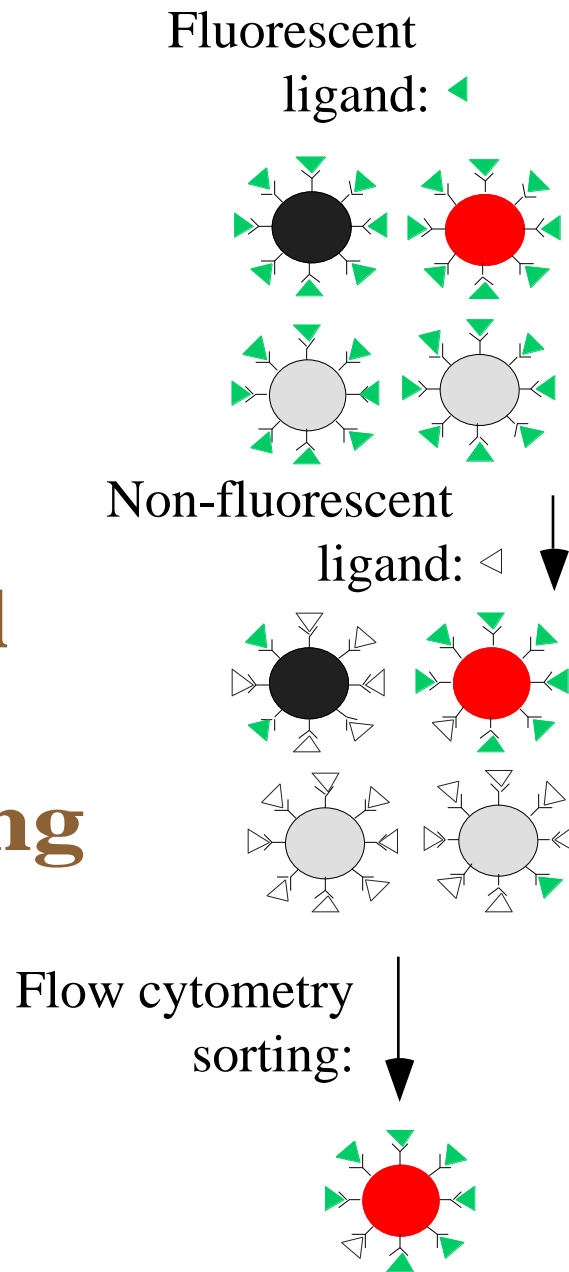
“Library”



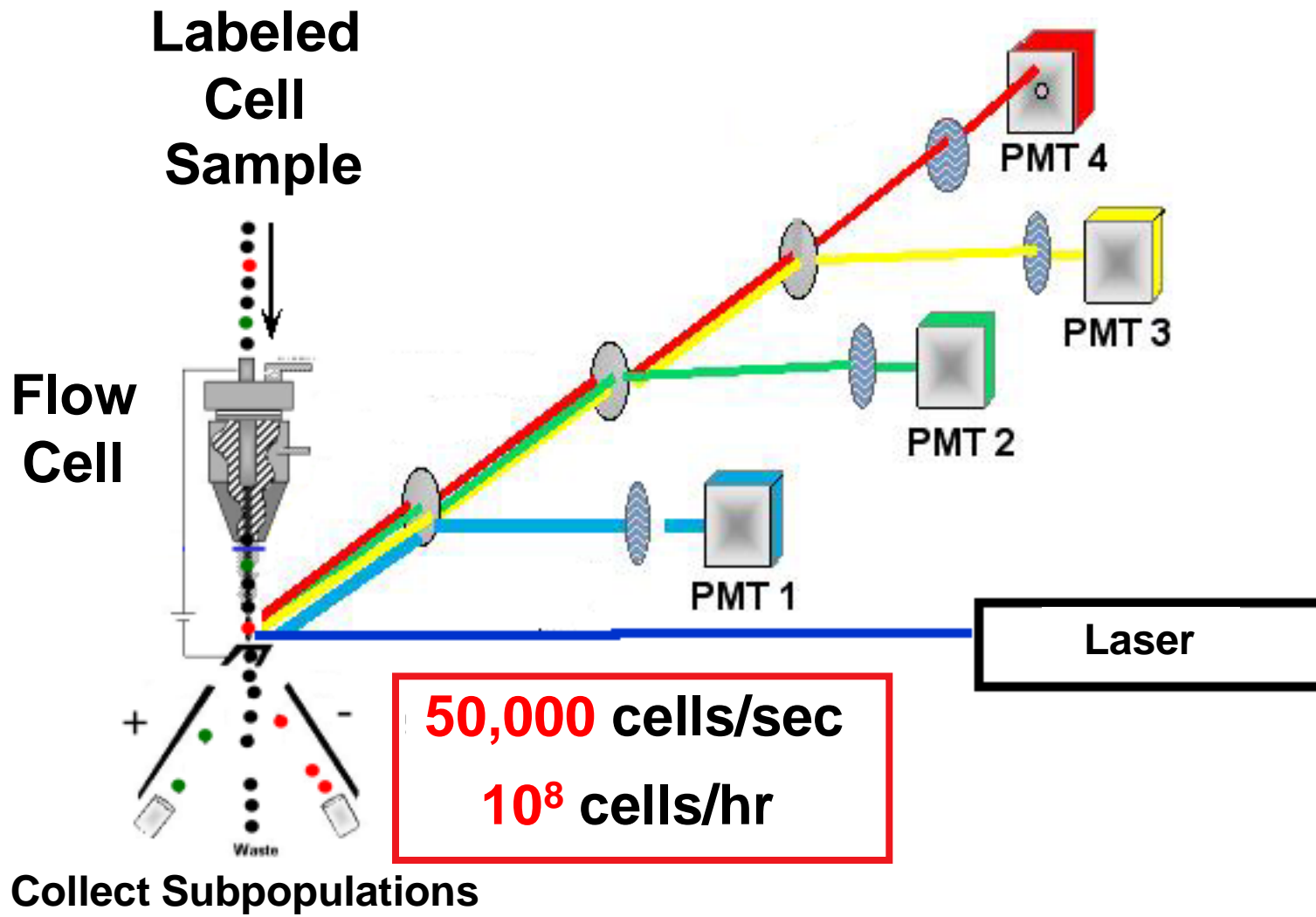
Flow cytometry sorting:



Optimal kinetic screening

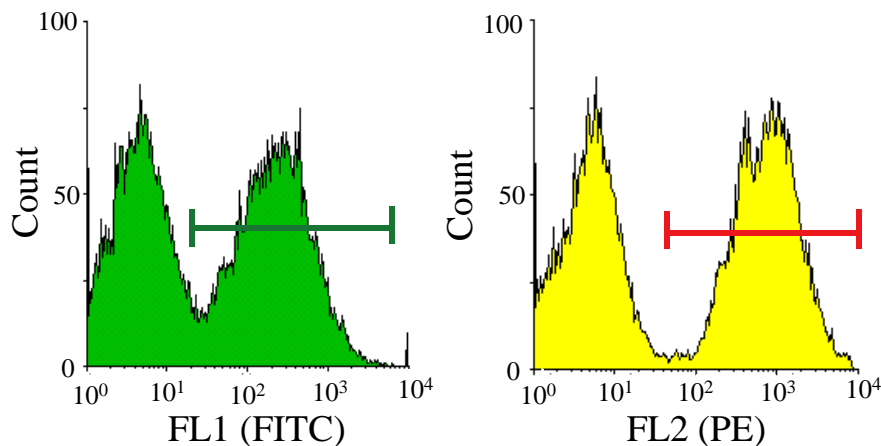


Flow Cytometry

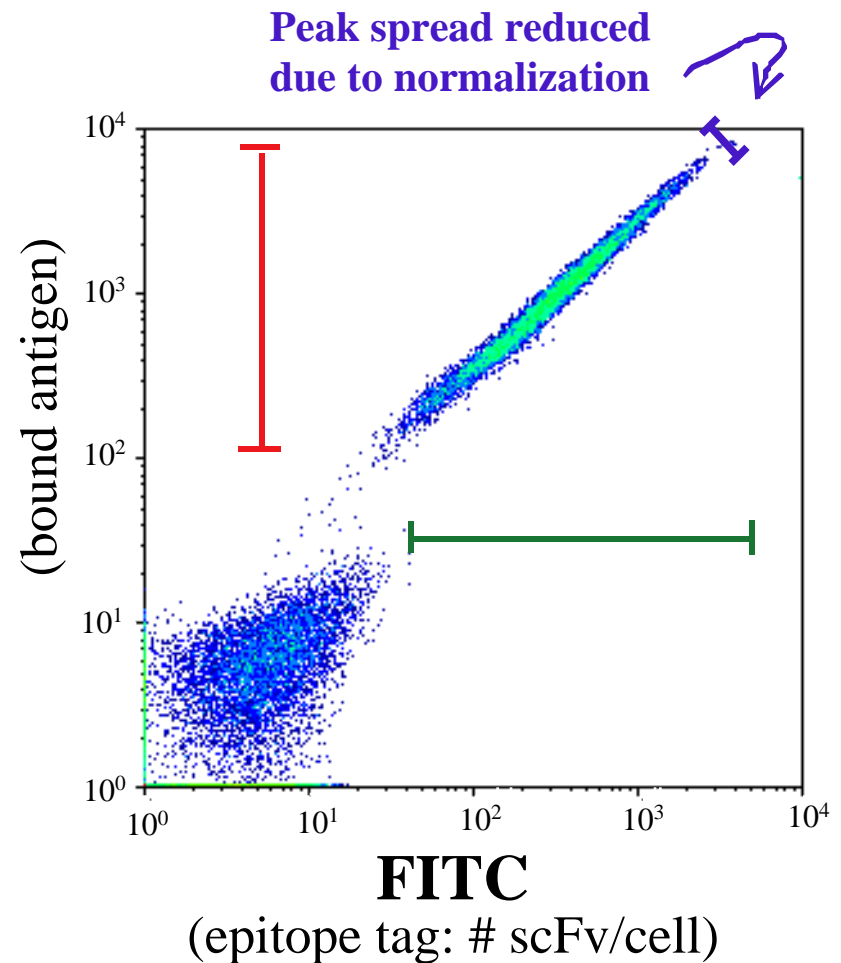


Normalization by expression level

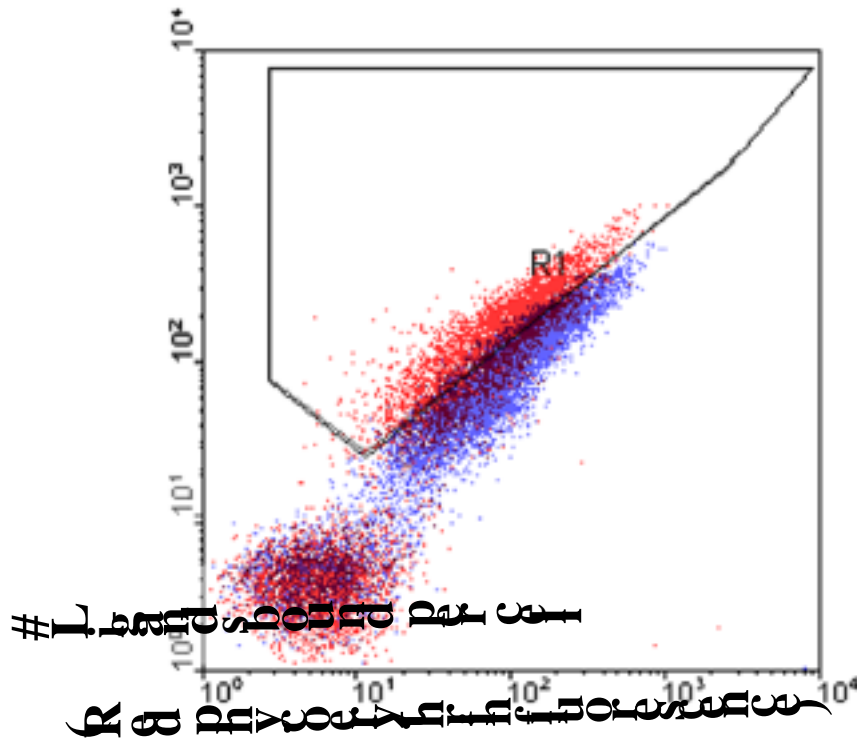
- Broad distribution of population (~ 100 - fold)
- Correct for single-cell expression variation with epitope tag signal



PE



Case study in the value of normalization



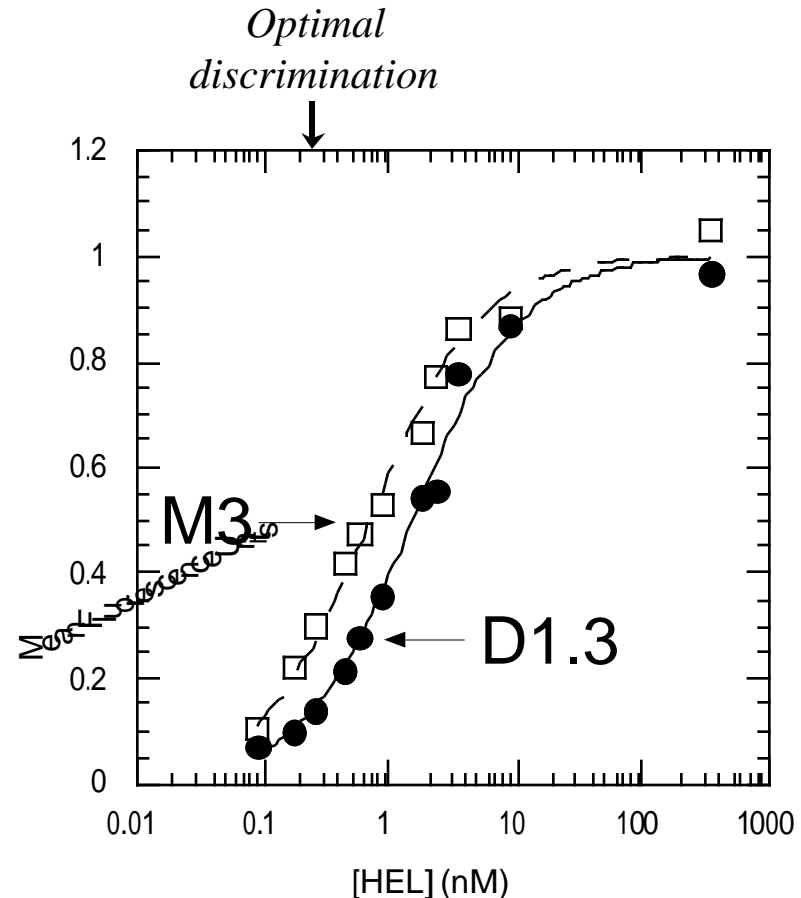
Protein fusions displayed per cell
(Green fluorescein fluorescence)

- Kinetic competition, CEA binding
- **Mutant $1/5 \times k_{off}$**
- Ligand histograms overlap extensively
- Mutant isolated in 3 sorting rounds with window shown

Trial Sort: Very Fine Discrimination

Van Antwerp & Wittrup, Biotech. Prog. 16:31, '00

- D1.3 scFv against hen egg lysozyme (HEL)
- Previous phage affinity maturation
D1.3 ($K_d = 1.4 \pm 0.1$ nM) vs.
M3 ($K_d = 0.6 \pm 0.2$ nM)
(*Hawkins et al., JMB 234:958, '93*)
- Mix M3:D1.3 at 1:1,000
- Sort top 0.1%
- **125-fold mean single pass enrichment**

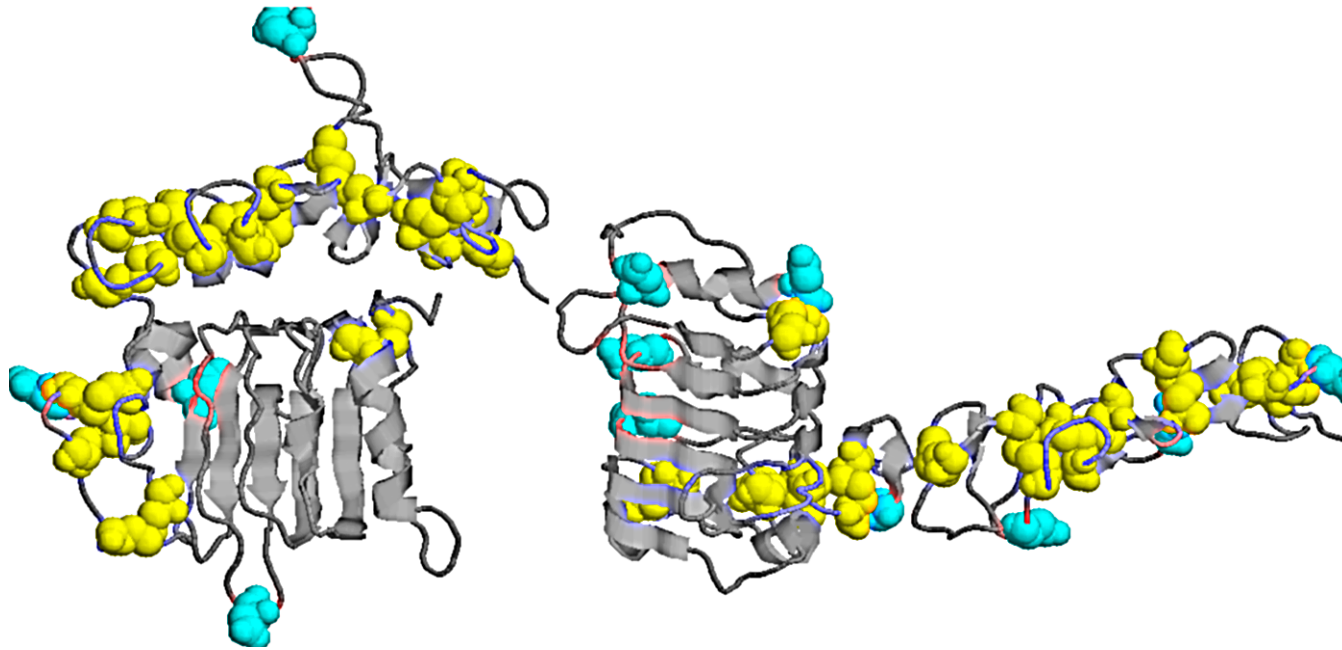


What proteins have been displayed on yeast?

- **scFvs (~ 30 to date),
scTCRs**
- MHC Class I & II
- GFP
- Cytokines & growth factors
- Constrained peptide loops
- Selectins, EGFR ECD

EGFR ectodomain is displayed and binds EGF

- Overexpressed in ~ 1/3 epithelial cancers
- What epitopes are neutralizing?

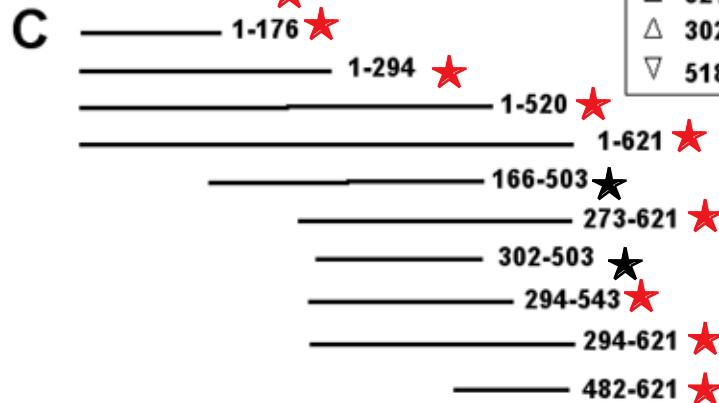
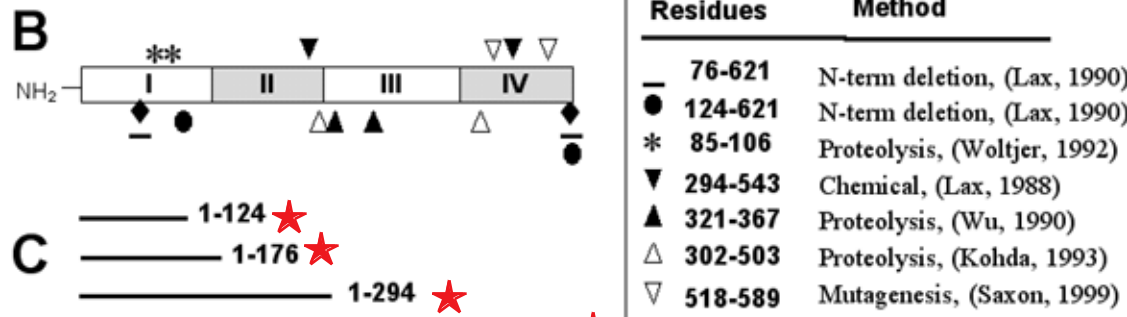


25 disulfides

12 N-linked glycosylation sites

620 amino acids

Various fragments of EGFR expressed functionally on yeast



★ = expressed, binds EGF

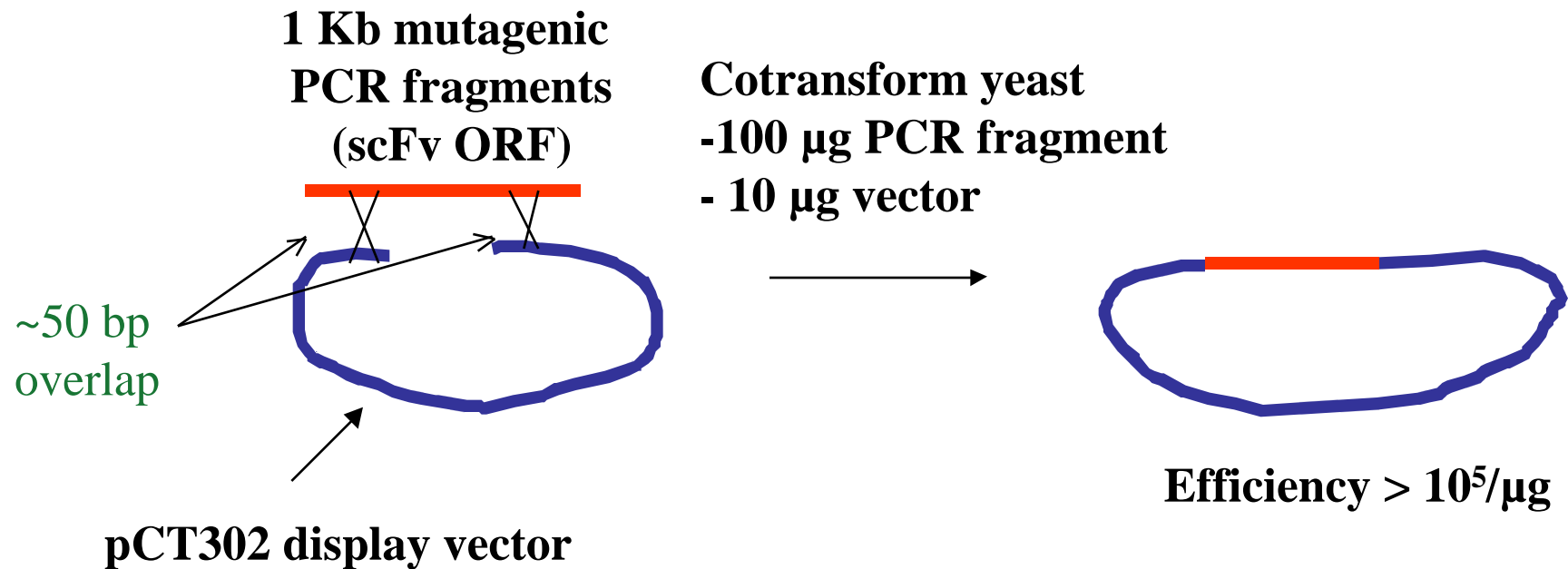
★ = not expressed

Antigens
for scFv
library
screening:

How big are yeast libraries?

- $\sim 10^7$
 - one person, one day's work
 - Default size for affinity & stability maturation
(10^5 has generally been sufficient)
- $\sim 10^9$
 - ~ 50 person-days of effort
 - Nonimmune human scFv library

Gap repair by homologous recombination in yeast

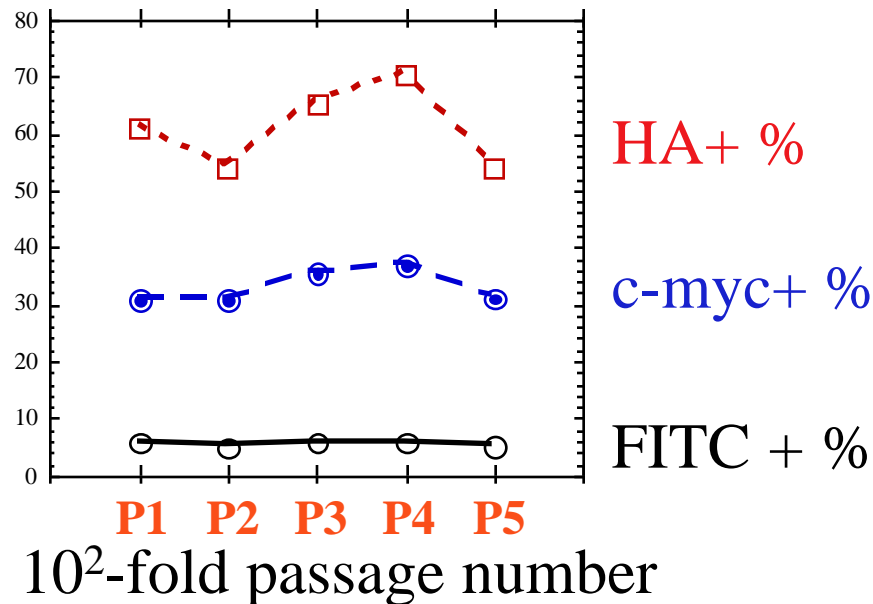
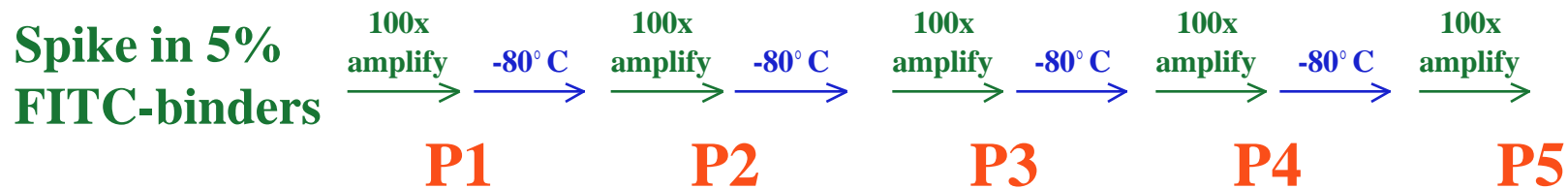


- No ligation
- No *E. coli* transformation
- < 1% no-insert vector transformants
- < 1% frameshifts

Can a yeast library be amplified?

- Yes, 10^{10} -fold without bias
- \therefore transform once, propagate indefinitely

Yeast library propagated 10^{10} -fold w/o bias (Feldhaus, PNNL)



scFv expression strongly
repressed by propagation
on glucose,
∴ No growth selection bias

Can Magnetic Beads be Used Instead of Flow Cytometry?

(Yeung & Wittrup, in press)

Yeast-
displayed
scFv + Biotinylated
Antigen + Streptavidin-
Dynal
Magnetic Beads
 $\{10^7 \text{ cells/mL}\}/\{OD_{600}\}$

Affinity Maturation

(One 8x improved in 85,000)

Single-pass Enrichment	Loss
600 \pm 200	75 \pm 25%

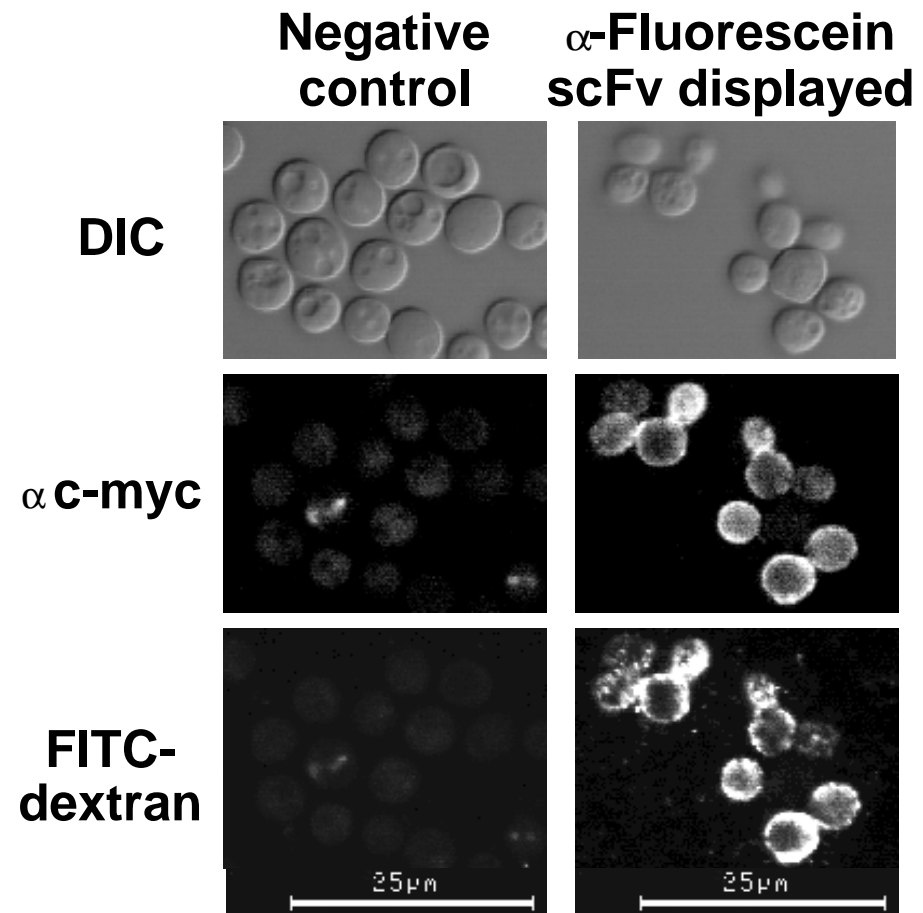
Lead Identification

(One binder in 140,000 nonbinders)

Single-pass Enrichment	Loss
9400 \pm 1800	93 \pm 4%

How accessible are the displayed proteins?

- live, unfixed cells
- 2×10^6 MW dextran
- 1° and 2° IgG + PE
- \therefore
>2.5 Megadaltons of detection reagent accessible to scFv



Isn't multivalent display a problem?

- Although yeast display antibody multivalently, *the soluble antigen is monovalent*; and so avidity artifacts are avoided.

Is it hard to work w/ yeast?

- Standard microbial culture
 - Standard Petri dish, tube, Ehrlenmeyer flask
 - ~ 2 hr doubling time @ 30 °C
- Good general yeast methods references:
 - *Methods In Enzymology* vol. 194, '91
 - Gietz lab transformation page
<http://www.umanitoba.ca/faculties/medicine/units/biochem/gietz/>
- Flow facilities don't mind yeast once they get used to the idea

How can I get started?

- Invitrogen yeast display kit
 - Plasmid pYD1 and yeast EBY100
 - Licensed from UIUC for evaluation purposes
- *Methods in Enzymology* **328**:430, '00
 - Detailed protocols

Summary

- Yeast display
 - **Eucaryotic** secretory processing
 - **Quantitative** precision in screening
- Objective functions
 - **Affinity** (femtomolar K_d , fine discrimination)
 - **Stability & secretion** (e.g. soluble scTCRs)

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