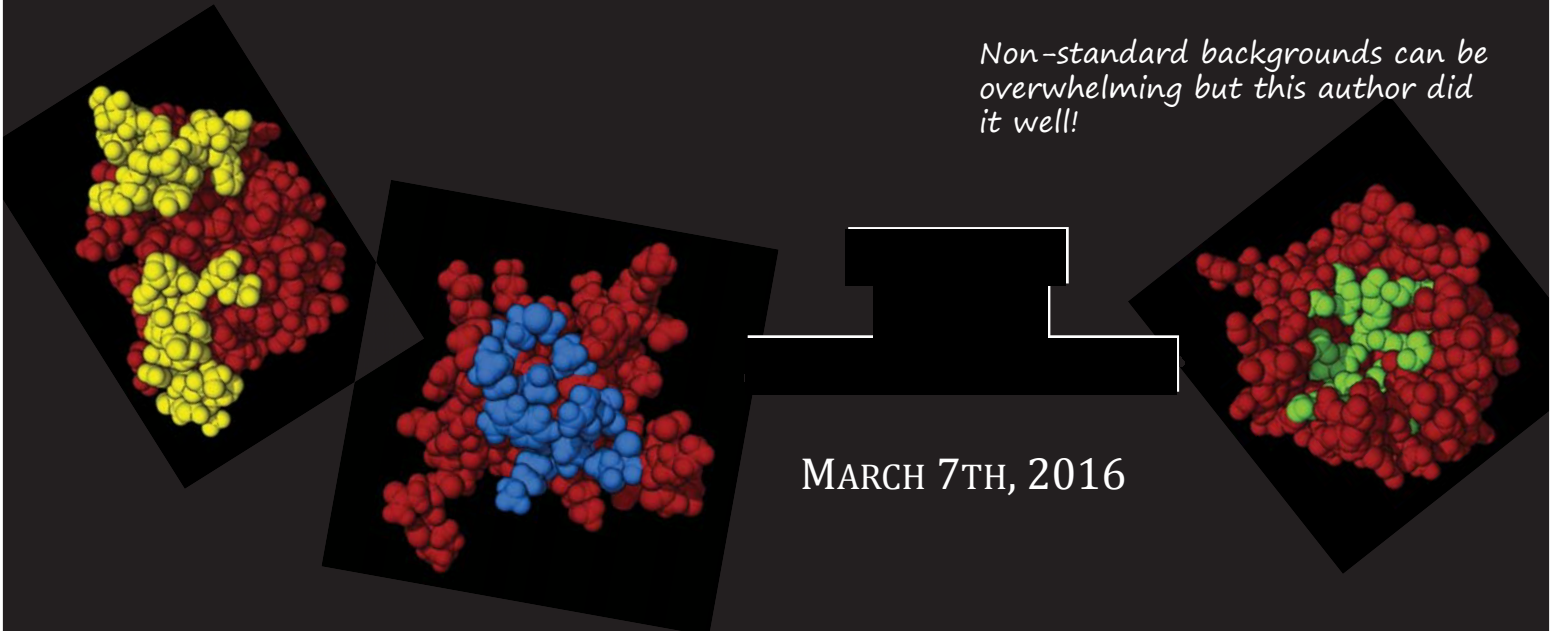


DEVELOPMENT OF THERMOSTABLE AFFINITY AGENTS FOR LOW-COST POINT-OF-CARE DIAGNOSTICS

Non-standard backgrounds can be overwhelming but this author did it well!

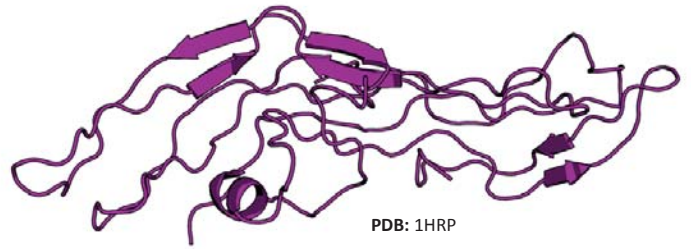
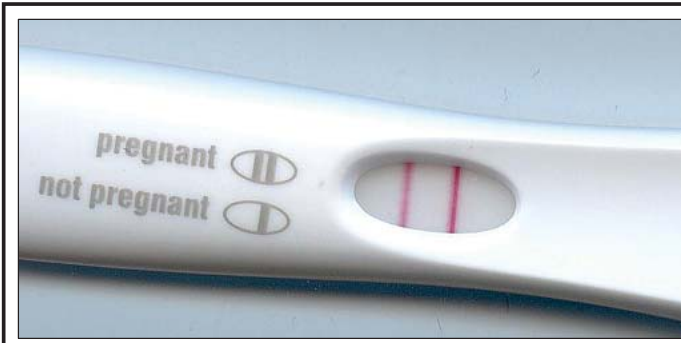


NEED FOR POINT-OF-CARE DIAGNOSTICS

Access to Care by Infrastructure Category ¹			
Region	Access to no infrastructure	Access to minimal infrastructure	Access to moderate/advanced infrastructure
Africa	25%	47%	28%
Asia	13%	29%	58%

- Tuberculosis: **9.6 million new cases, 1.5 million deaths** in 2014²
- **37% of new cases** (3.6 million) went undiagnosed
- Assay with **85% sensitivity, 97% specificity**, and **no infrastructure requirements** could save **400,000 lives** annually³

RAPID DIAGNOSTIC TESTS (RDTs)



PDB: 1HRP

Human chorionic gonadotropin (hCG)

- Detect disease biomarkers in patient fluids
- Require no intensive training or medical infrastructure
- Use monoclonal or polyclonal antibodies (IgG or IgM) for capture and detection of patient antigens

We might have recommended removing the bullets to reduce visual noise!

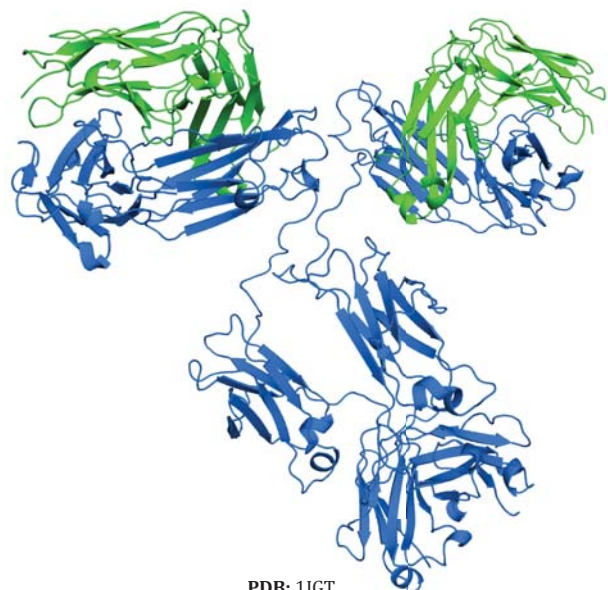
3

NON-IDEAL ANTIBODY CHARACTERISTICS

- Thermal denaturation

This slide had animations (not shown) to transition between many thoughts

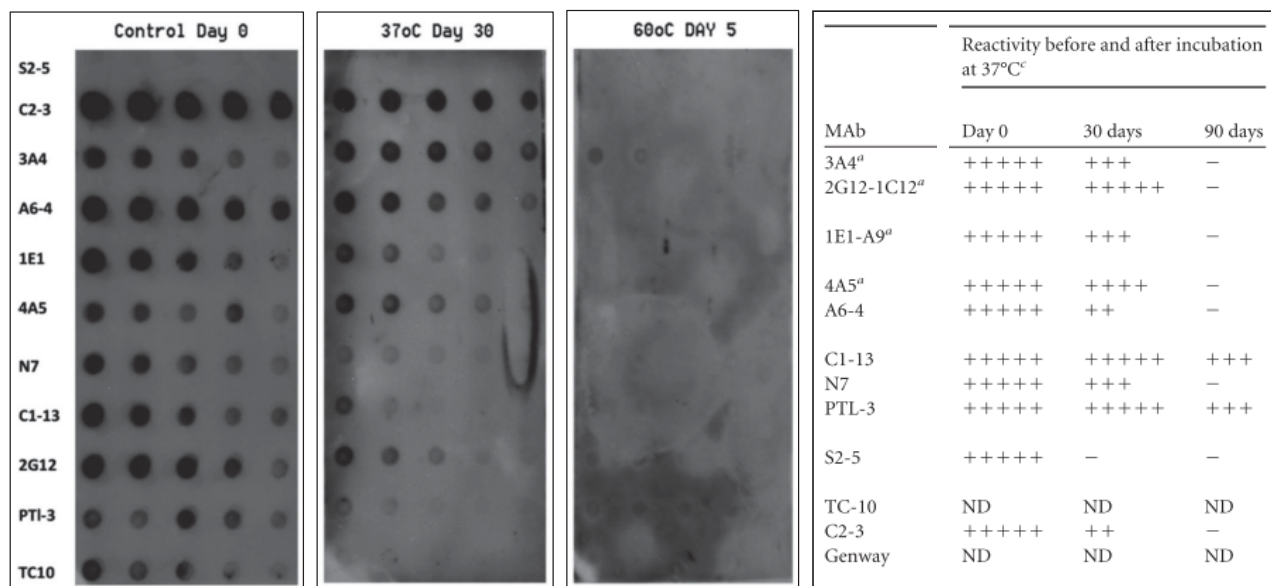
It sets the viewer up to come back to the slide to see where they've been and where they're going—great idea!



PDB: 1IGT

4

INSTABILITY OF DIAGNOSTIC ANTIBODIES



The visuals all match, great!

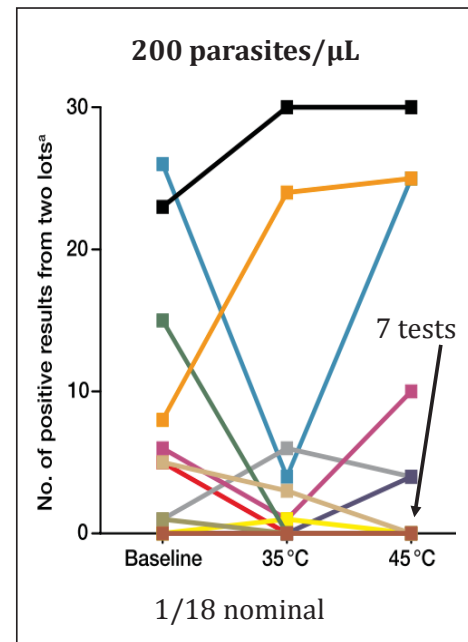
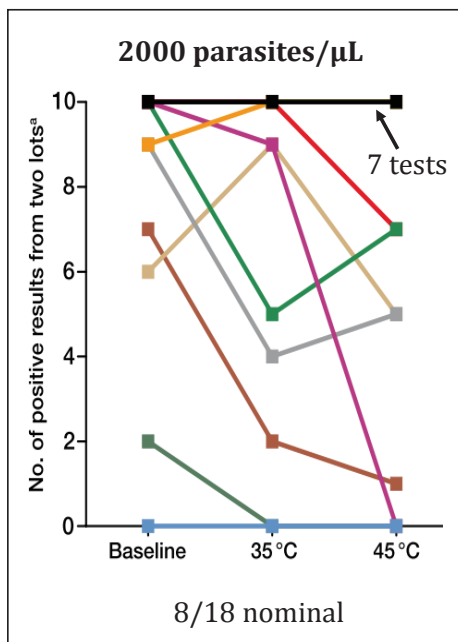
5

1. Lee et al. J. Clin Micro. (2012)

TEST PERFORMANCE DIMINISHED BY HEAT

- Malaria rapid diagnostic tests incubated for 60 days, 75% humidity¹
- Shipments of RDTs in Cambodia spent >3,400 hours above 30°C²
- At some sites in Senegal and Ethiopia, RDTs stored at 30°C for >80% of time, 40°C for 18% of time³

The take-away message on this complicated slide is present in the title!



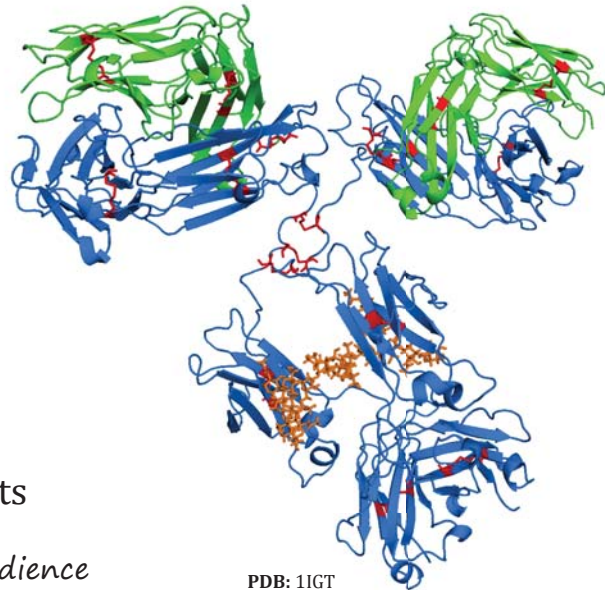
6

1. WHO. Malaria RDT Survey, Round 4. (2012)
 2. Jorgensen et al. Am J. Trp. Med. Hyg. (2006)
 3. Albertini et al. Malar. J. (2012)

NON-IDEAL ANTIBODY CHARACTERISTICS

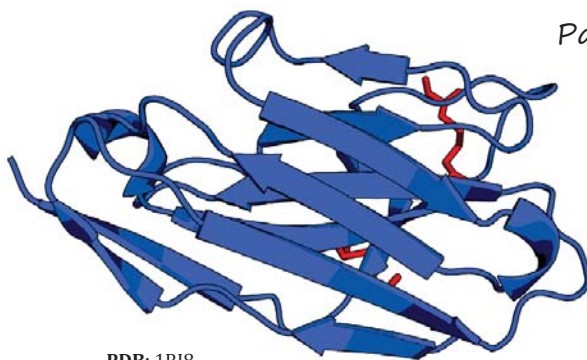
- Thermal denaturation
- Complex recombinant production due to **glycosylation** and disulfide bonds
- Generation times of 3-12 months
- Lot-to-lot variation in polyclonal blends
- Non-specific binding to immune elements

Coming back to a slide we've seen helps the audience understand!



7

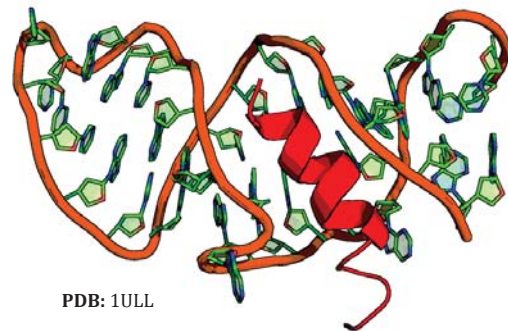
CANDIDATE BINDING SCAFFOLDS FOR ANTIBODY REPLACEMENT



Single-domain camelid antibodies

- Low expression yields
- Poor solubility
- Native disulfide bonds

Parallel structure helps us draw comparisons

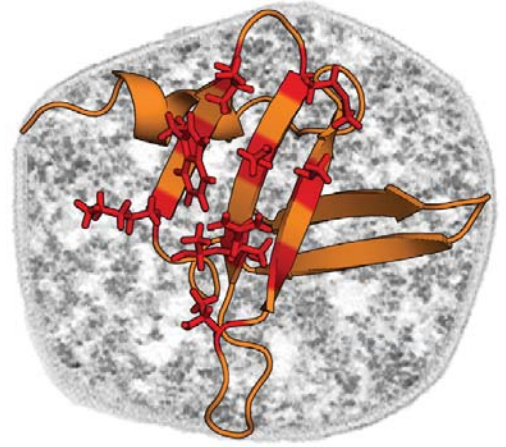


DNA aptamers

- Black box development process
- Frequent false positives
- Low affinities

8

NATURE-GUIDED DESIGN: Sso7D



- **Native host:** *Sulfolobus solfataricus*
- **Protein:** Sso7d
- **Function:** Histone analog

Pretty slide!

9

CRITICAL ATTRIBUTES FOR DIAGNOSTIC BINDING PROTEINS

Chemical/Physical Properties

Bio manufacturing

Activity

Stability

Setting the audience up for another set of slides where lots of complicated information is connected together

Colors allows us to differentiate which topic we're on

10

CHEMICAL/PHYSICAL PROPERTIES OF Sso7D

Chemical/Physical Properties

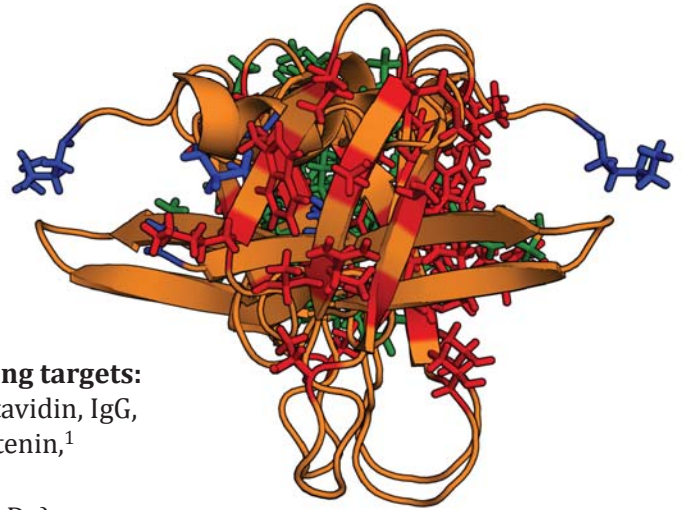
- Structurally-isolated binding face
- Compatibility with many antigens
- Small molecular footprint
- No native cysteine residues
- Terminal modifications won't interfere with binding
- Compatible with cytometry-based screening

Biomanufacturing

Activity

Stability

- **Demonstrated binding targets:**
 - Lysozyme, streptavidin, IgG, fluorescein, β -catenin,¹ RCNM virus²
- **MW:** 7 kDa (Ab: 150 kDa)
- **Radius of gyration:** 1.31 nm (Ab: 5.39 nm)



11

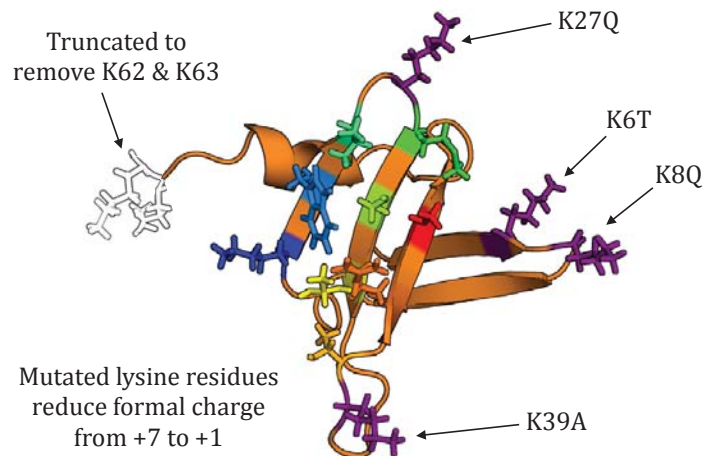
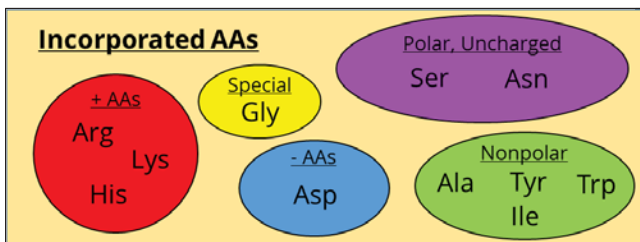
1. Gera et al. J. Mol. Bio. (2011)
2. Hussain et al. Biotech Prog. (2012)

WITTRUP LAB Sso7D LIBRARY

1 10 20 30 40 50 60

ATVKFTYQGEKQVDISKIKKVVWRVGMISFTYDEGGGATGRGAVSEKDAPKELLQMLEKQ

- Only residues within binding face randomized (saturation mutagenesis)
- Special method used to ensure no premature stop codons
- Limited suite of 11 amino acids



Theoretical Library Diversity: 1.4×10^9

12

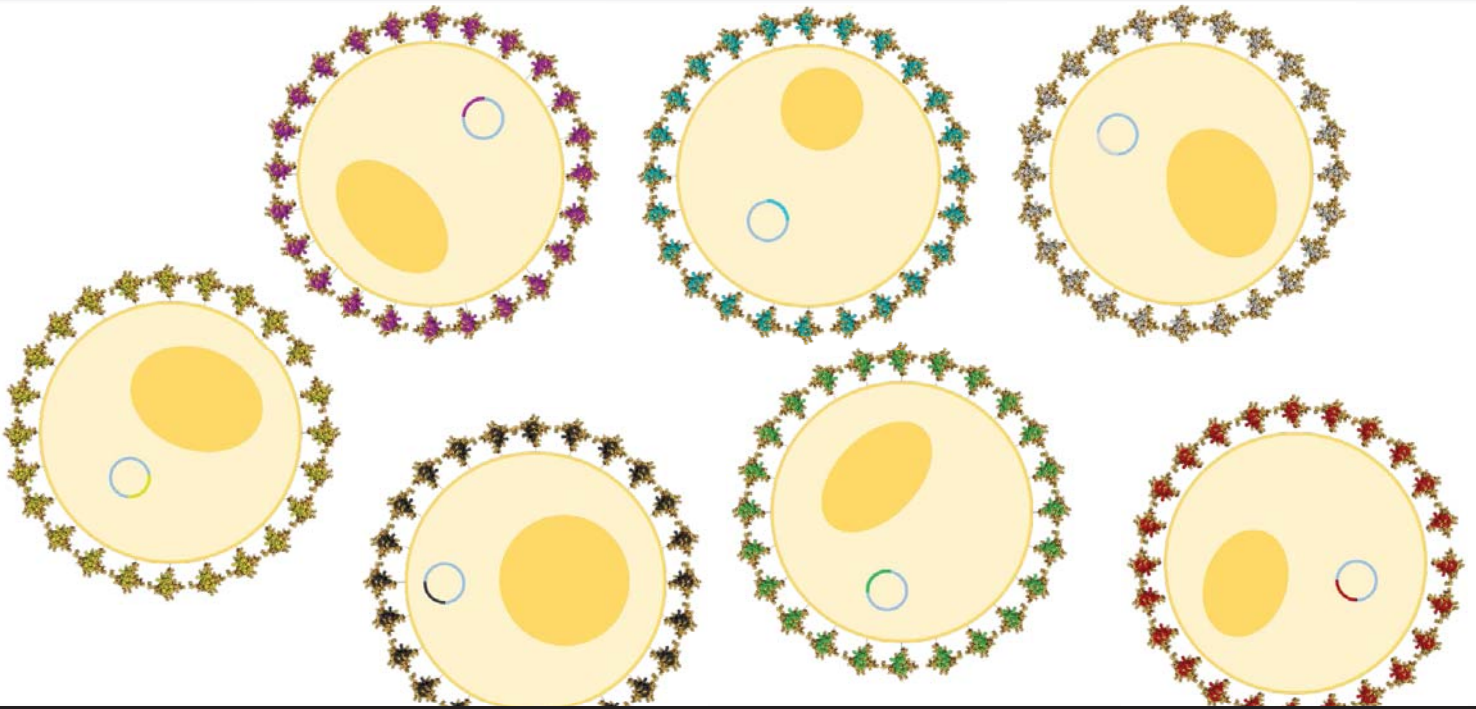
Color in the cartoon in the bottom left connects us back to the image in the top right

YEAST SURFACE DISPLAY PRIMER

Another great series of animations that along with the author's speech takes the audience through a step-by-step understanding

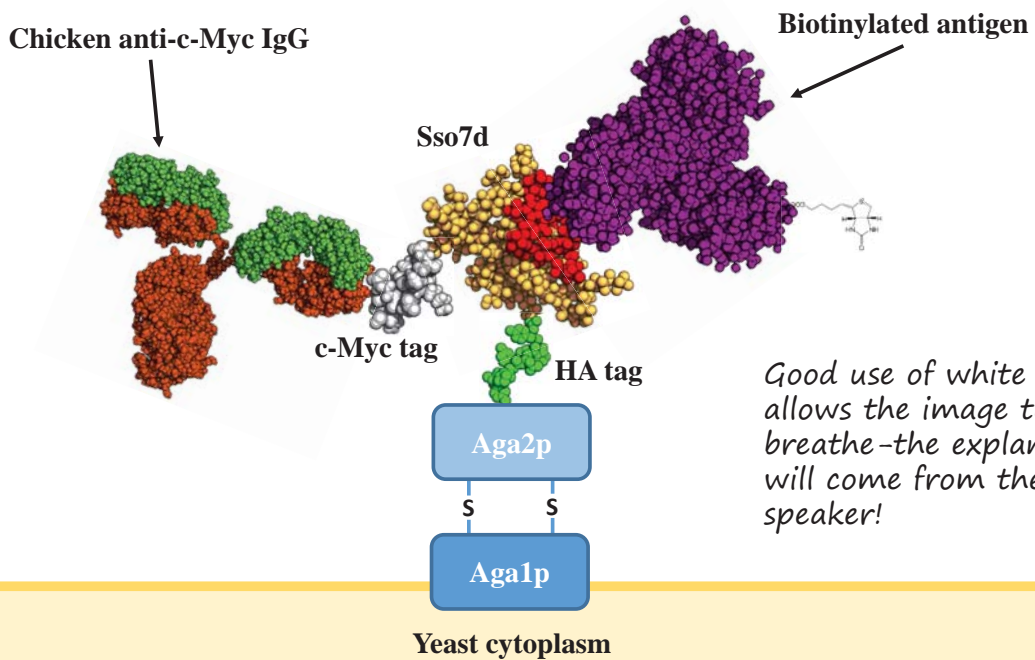
YEAST SURFACE DISPLAY PRIMER

YEAST SURFACE DISPLAY PRIMER



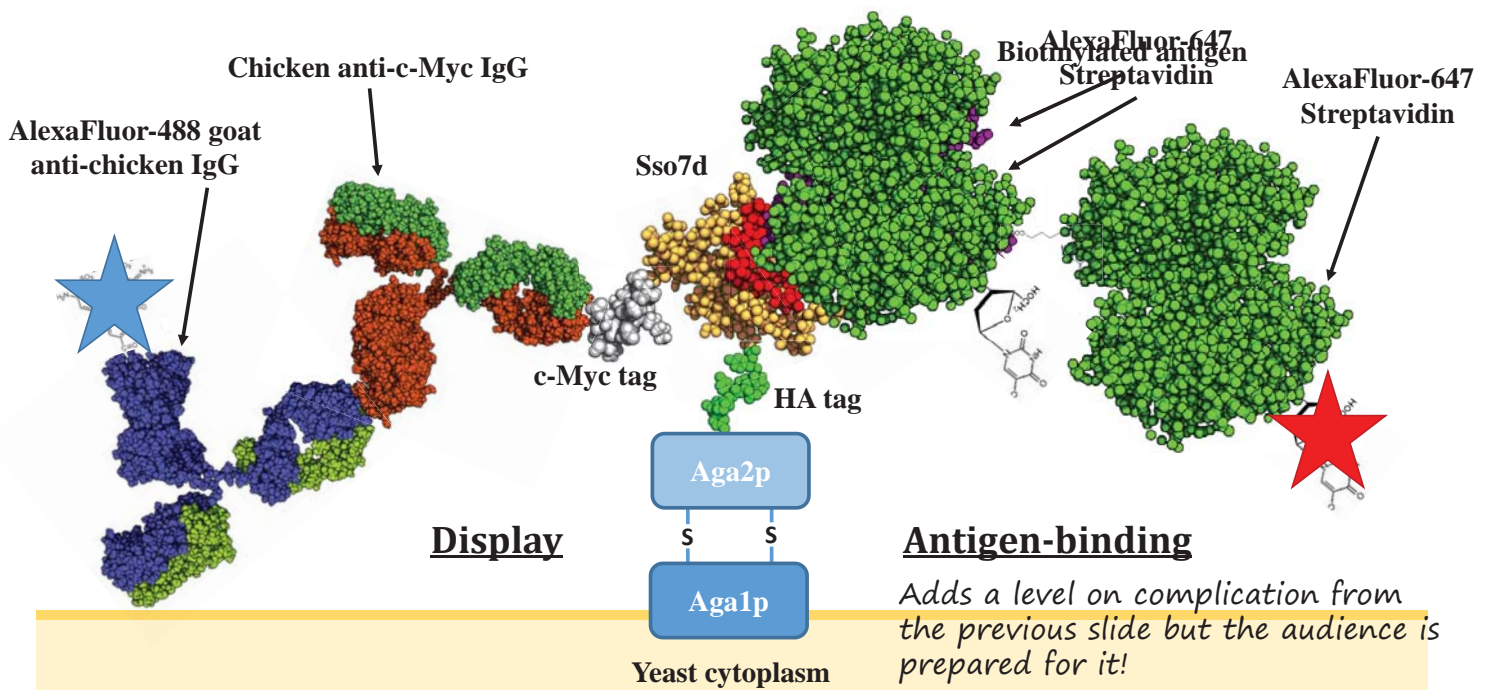
15

YEAST SURFACE DISPLAY PRIMER



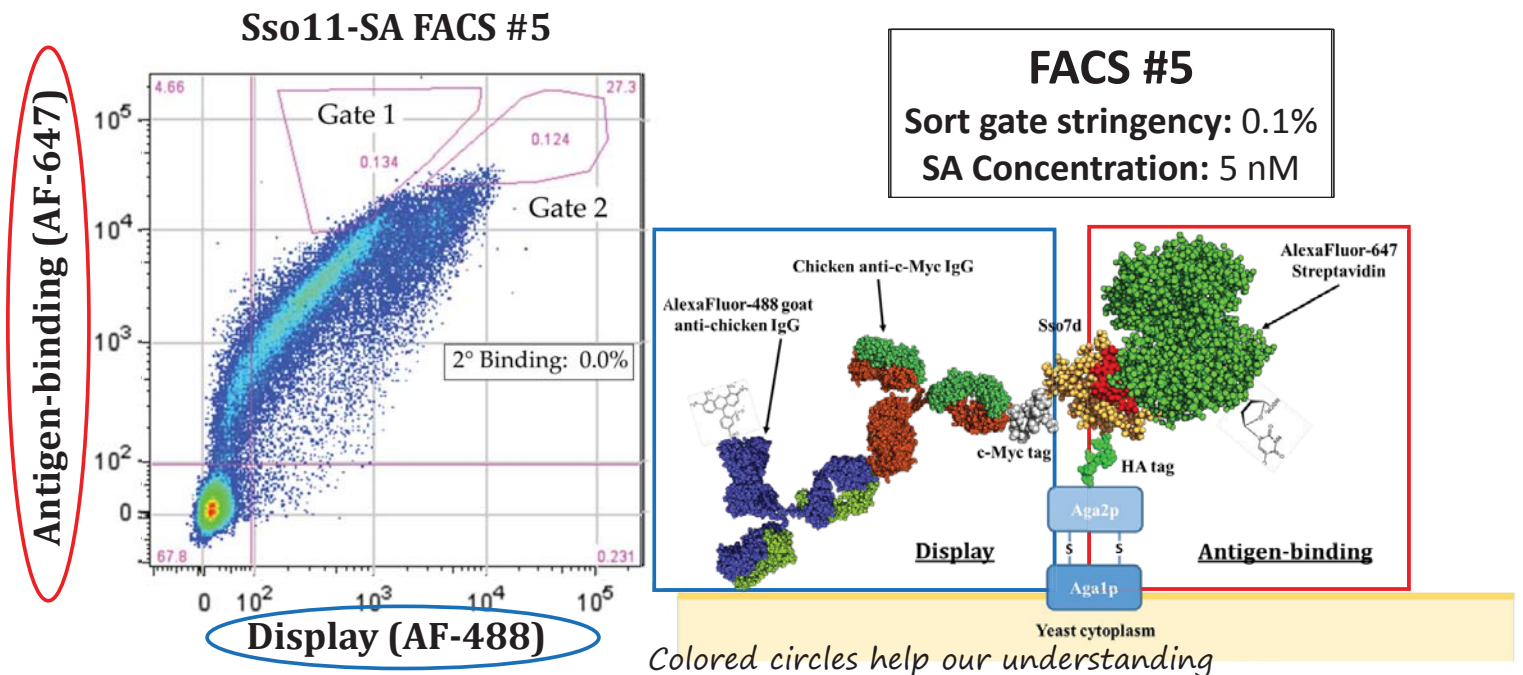
16

YEAST SURFACE DISPLAY PRIMER



17

MODEL STREPTAVIDIN BINDER DEVELOPMENT



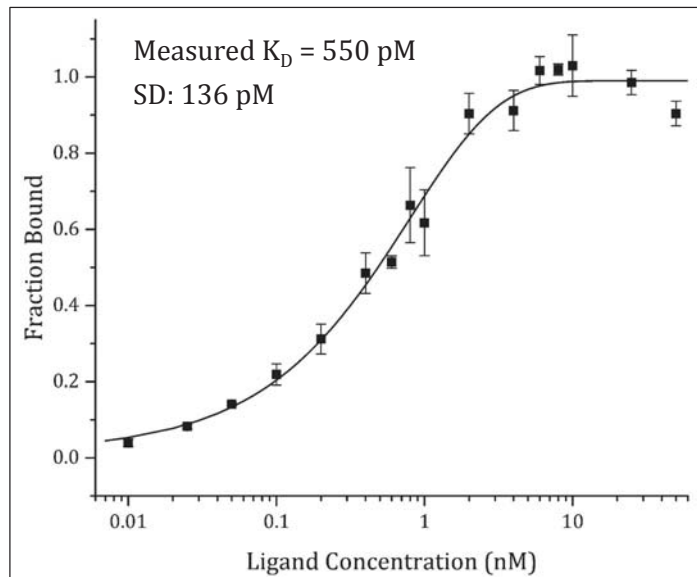
18

Sso11-SA FEATURES PICOMOLAR AFFINITY

Sso11-SA:

ATVKFTYQGEEKQVDISKIKIVARDGQYIDFKYDEGGGAYGYGWVSEKDAPKELLQMLEKQ

Red color is used to highlight important pieces of the information



19

BIOMANUFACTURING OF Sso7D

Chemical/Physical Properties

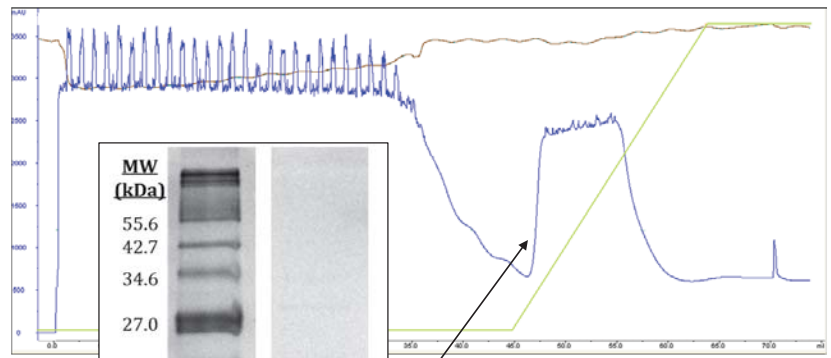
Bio manufacturing

- Bacterial expression (no disulfide bonds)
- Facile, low-cost purification
- High yields of soluble protein

Activity

Stability

Audience returns to their familiar roadmap—great way to remind them where they were and where they're going!



Sso11-SA

- **Time:** 24 hours
- **Yield:** 42.2 mg/L_{culture}
- **Molar Yield:** 4.5 μmol/L_{culture}
- **Mass/Test:** 0.25 μg
- **# of Tests:** ~170,000
- **Cost/Test:** \$0.00027

pAb-SA

- **Time:** 720 hours¹
- **Yield:** 3.2 mg/L_{egg yolk}
- **Molar Yield:** 0.02 μmol/L_{egg yolk}
- **Mass/Test:** 2 μg
- **# of Tests:** ~1,600
- **Cost/Test:** \$0.0276 (\$69/5 mg)

20

ACTIVITY OF Sso11-SA

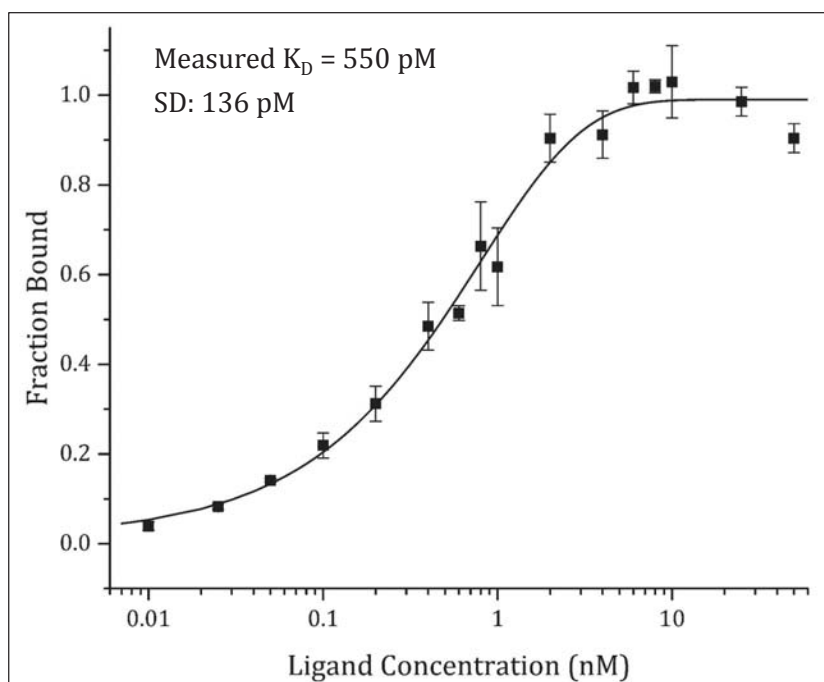
Chemical/Physical Properties

Biomanufacturing

Activity

- High-affinity binding interactions
- Surface-immobilized activity

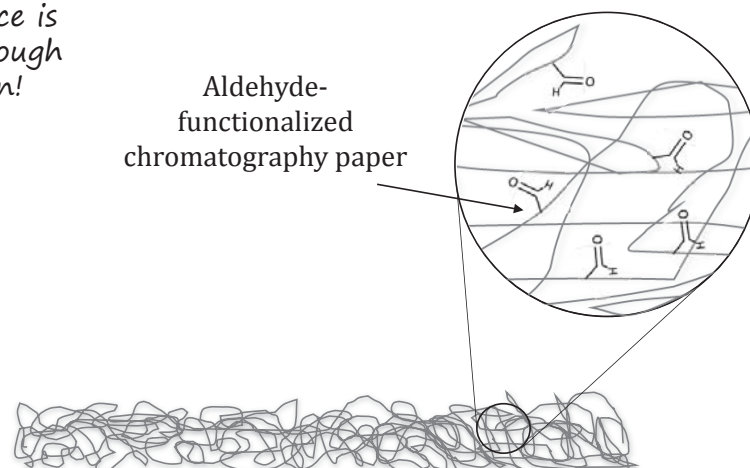
Stability



21

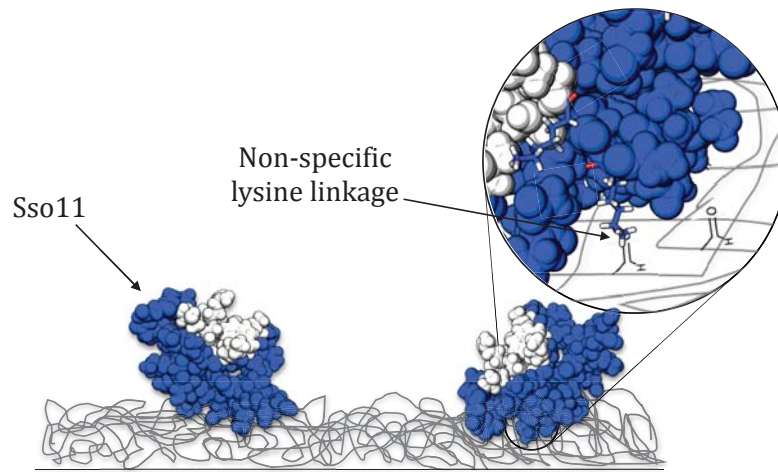
PAPER ASSAY SCHEMATIC

Another series of animated slides where the audience is walked sequentially through complicated information!



22

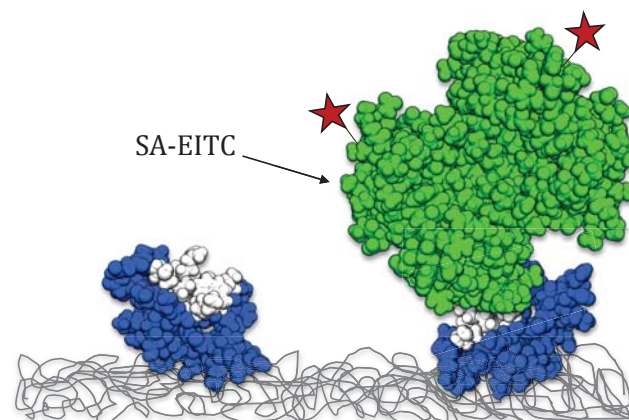
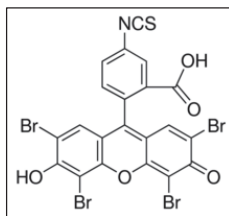
PAPER ASSAY SCHEMATIC



23

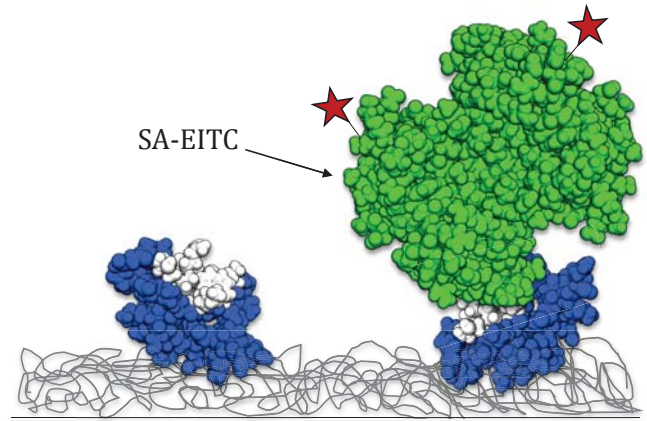
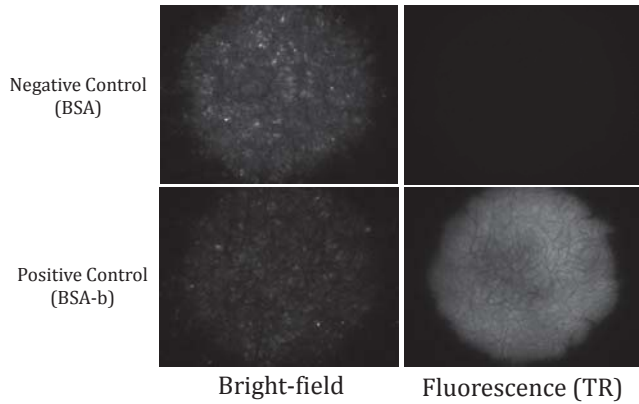
PAPER ASSAY SCHEMATIC

Eosin isothiocyanate (EITC)



24

PAPER ASSAY SCHEMATIC



25

LOW-NANOMOLAR SURFACE-IMMOBILIZED BINDING SIGNAL

Chemical/Physical Properties

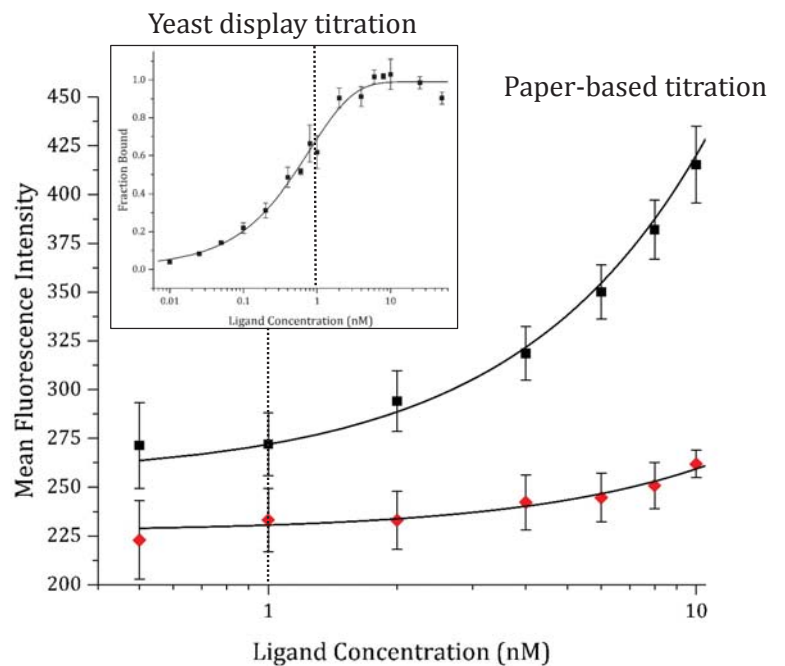
Biomanufacturing

Activity

- High-affinity binding interactions
- Surface-immobilized activity
- Limited non-specific binding

Stability

Back to the roadmap! You may worry it is redundant but it really helps an audience retain information!



26

STABILITY OF Sso7D

Chemical/Physical Properties

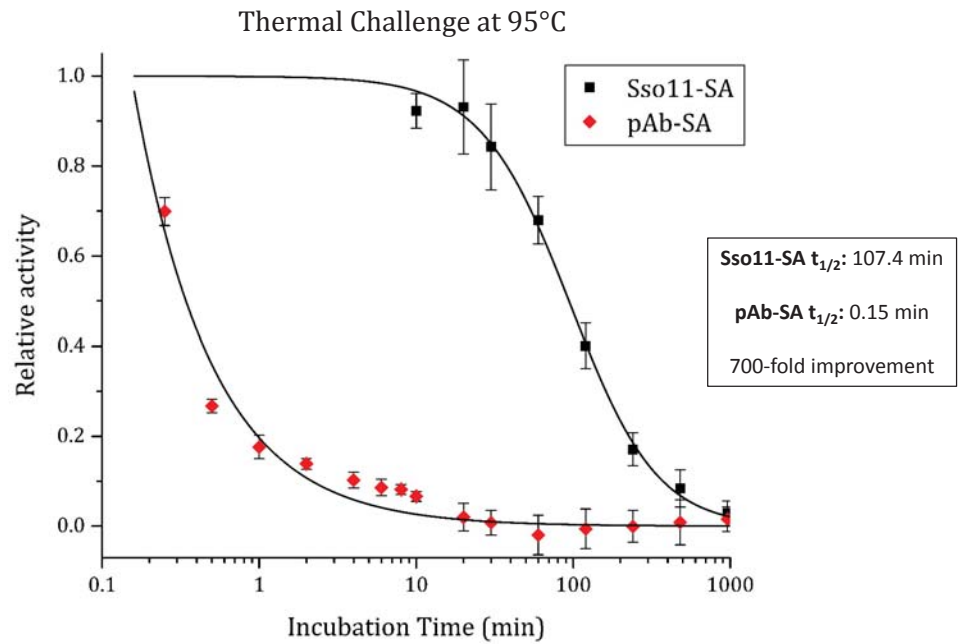
Biomanufacturing

Activity

Stability

- Chemical stability
- Thermal stability
- Dehydration tolerance

- **pH Range:** 0.33-12.5¹
- **[Gdn-HCl]_{1/2}:** 3.5 ± 0.78 M
- **Wild-type T_m:** 98°C



27

1. Gera et al. J. Mol. Bio. (2011)

CONCLUSIONS

Chemical/Physical Properties

- 3-12x improvement in development throughput

Biomanufacturing

- 100-400x improvement in cost
- 10x improvement in molar yield
- 30x improvement in throughput

Activity

- Sub-nanomolar affinity
- Demonstrated surface activity to low nanomolar concentrations

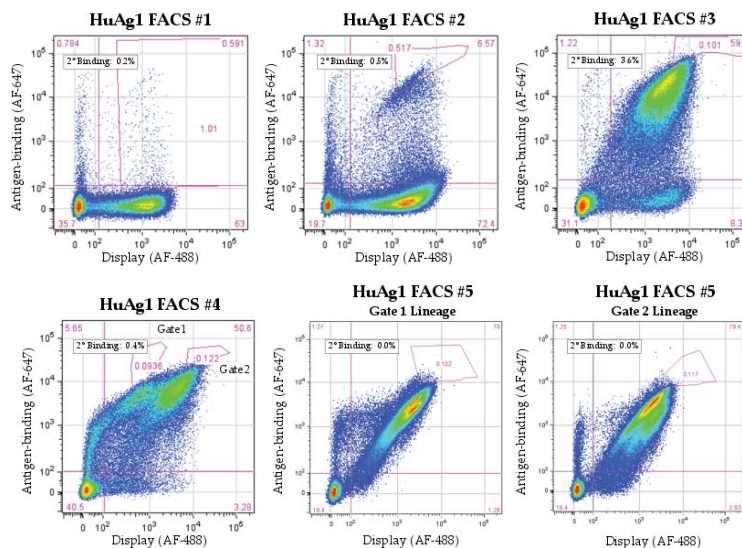
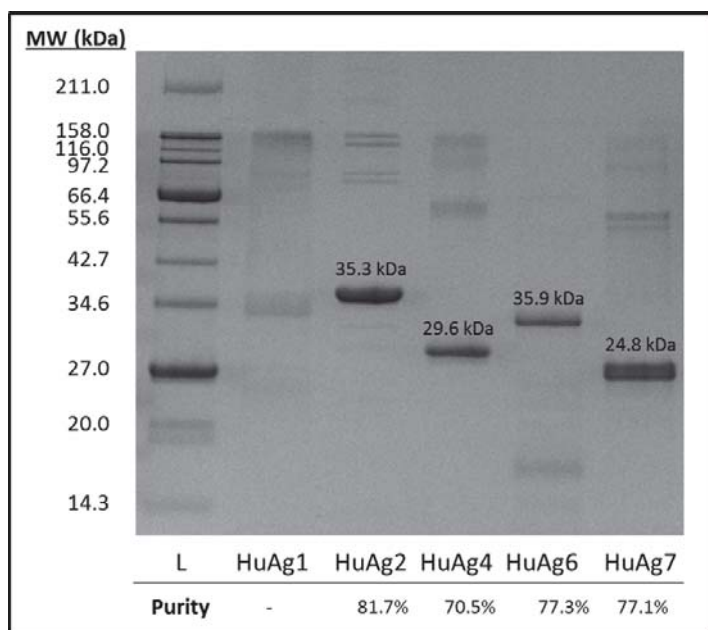
Stability

- 700x improvement in activity retention under thermal challenge

Great job using the parallel structure already set up with the roadmap to drive home the important conclusions

28

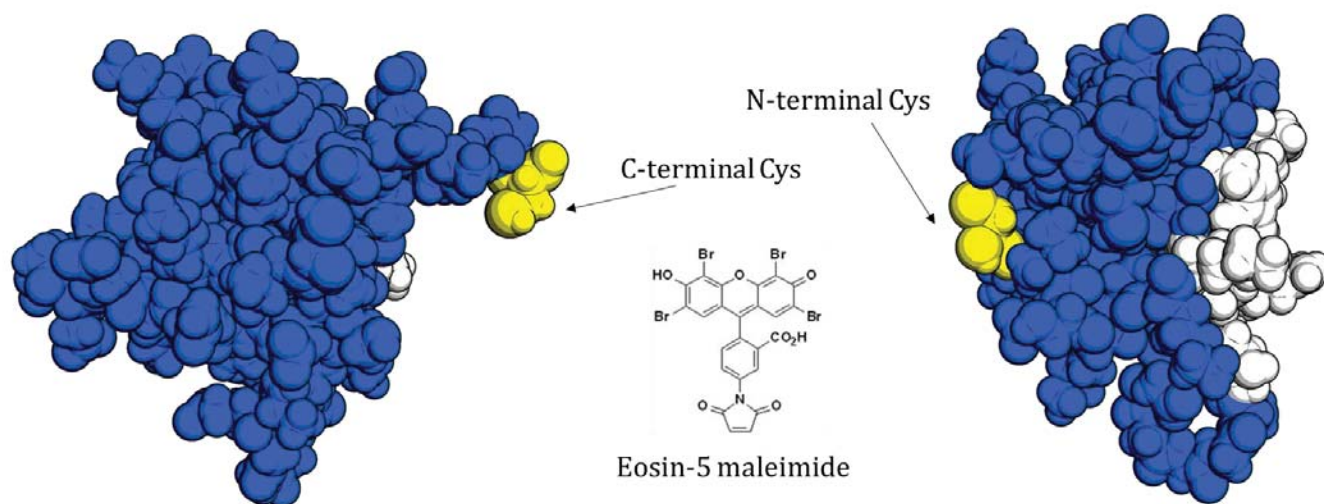
FUTURE DIRECTIONS – TB ANTIGENS



Binders selected: HuAg1 (4), HuAg4 (2), HuAg6 (2)

29

FUTURE DIRECTIONS – SITE-SPECIFIC CHEMISTRY



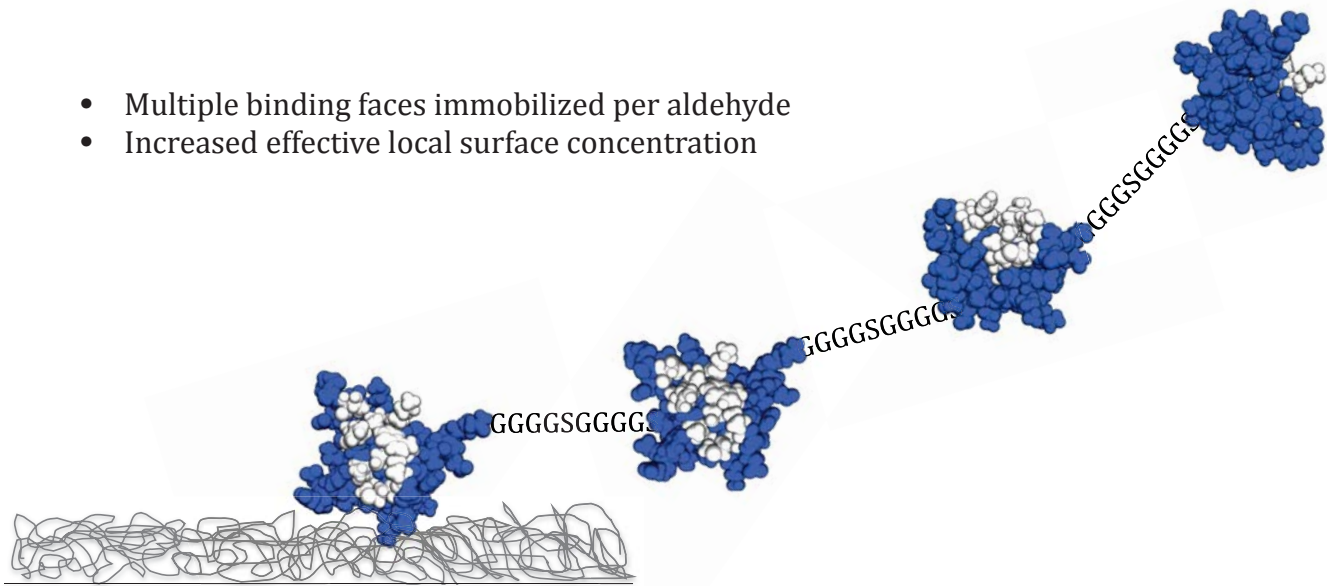
- Site-specific chemical modification
- Oriented surface immobilization

Arrows help the audience know where they should be looking!

30

FUTURE DIRECTIONS - MULTIMERIZATION

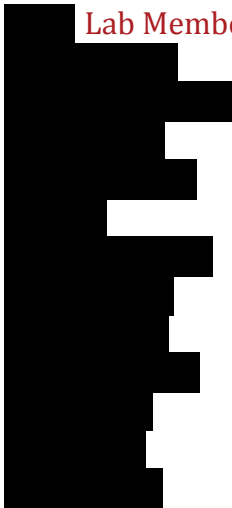
- Multiple binding faces immobilized per aldehyde
- Increased effective local surface concentration



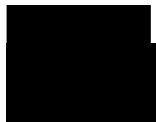
31

ACKNOWLEDGMENTS

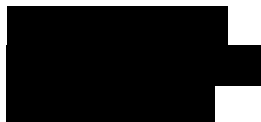
- **Lab Members:**



- **UROP Team**



- **Committee Members**



- **Collaborators**



Lots of acknowledgements—be sure to only thank who you absolutely need to thank in a presentation! Or at least minimize the written portion!

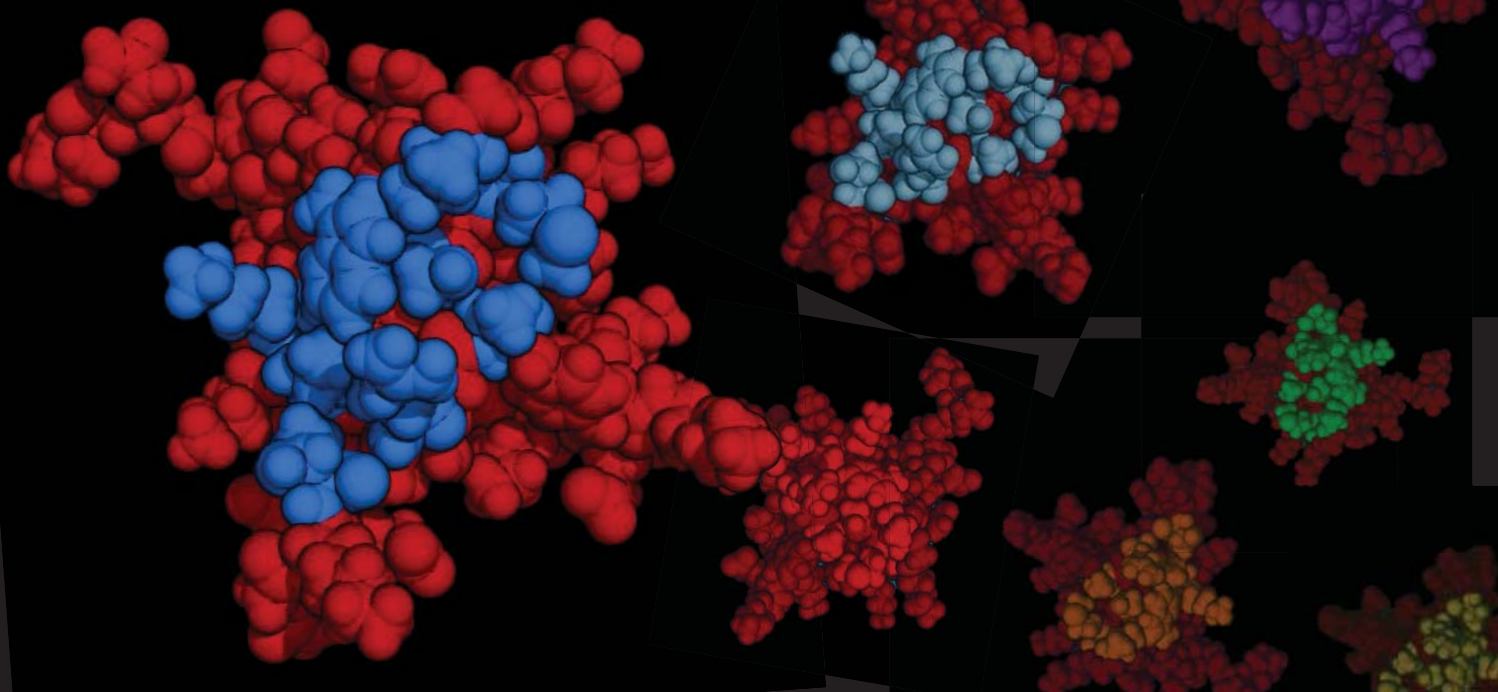
- **Funding Sources**

NIH
Biotechnology Training Program



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QUESTIONS?



APPENDIX

