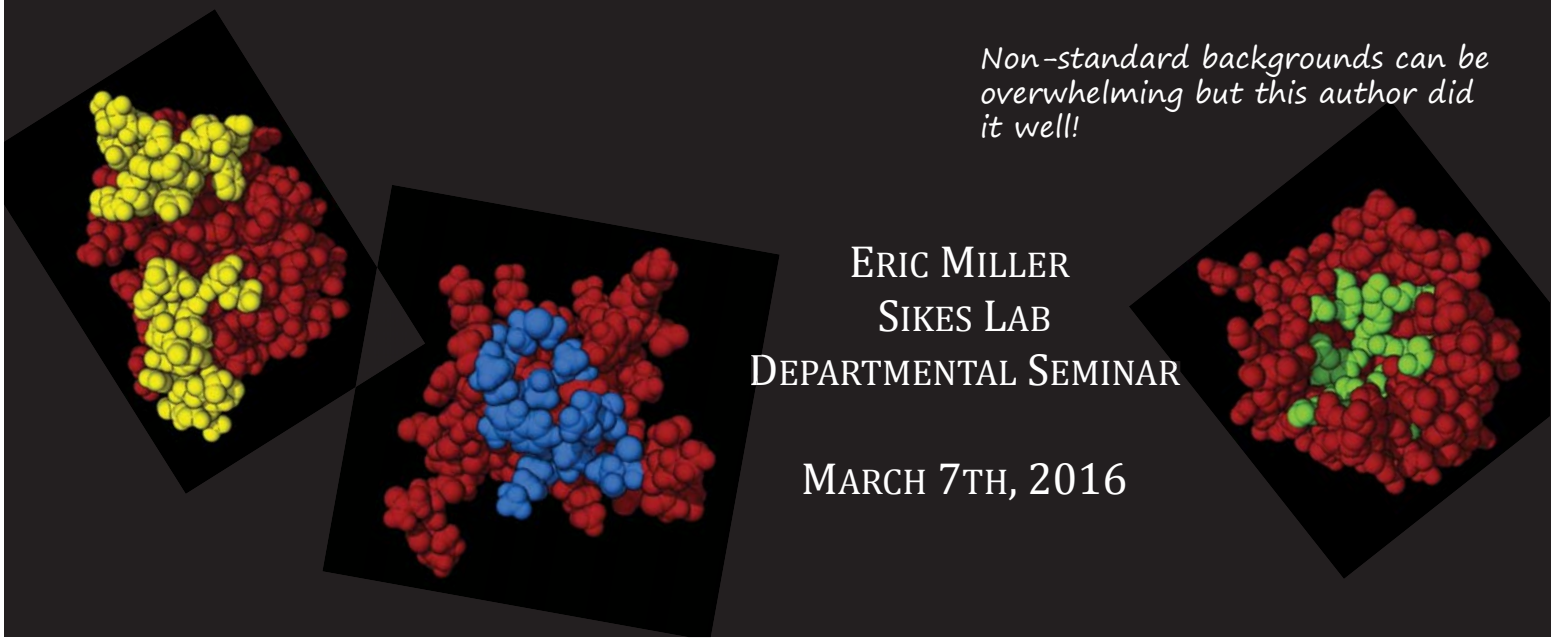
# Development of Thermostable Affinity Agents For Low-Cost Point-of-Care Diagnostics



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

Eric Miller
Sikes Lab
Departmental Seminar

March 7th, 2016

# 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% |

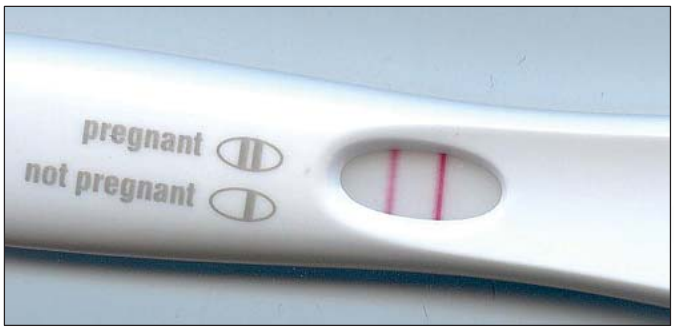
- Tuberculosis: 9.6 million new cases, 1.5 million deaths in 2014[2]
- 37% of new cases (3.6 million) went undiagnosed
- Assay with 85% sensitivity, 97% specificity, and no infrastructure requirements could save 400,000 lives annually[3]

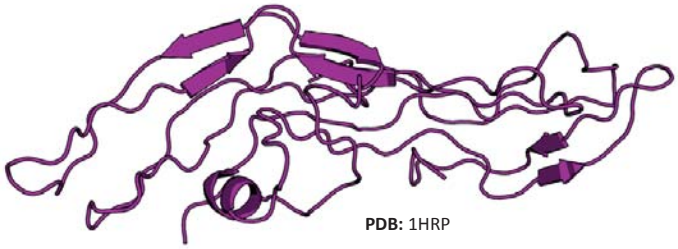


*Pop of red color is used to highlight important information*

1. Girosi et al. Nature. (2006)
2. WHO. TB Report. (2015)
3. Urdea et al. Nature. (2006)

# Rapid Diagnostic Tests (RDTs)




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!*

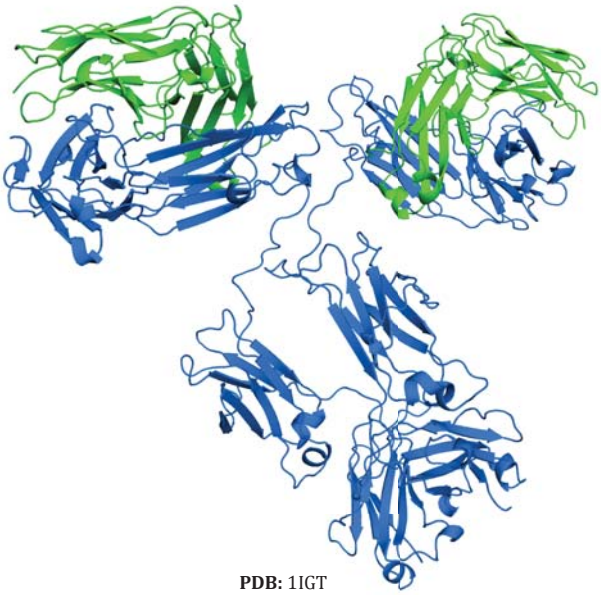


# Non-Ideal Antibody Characteristics

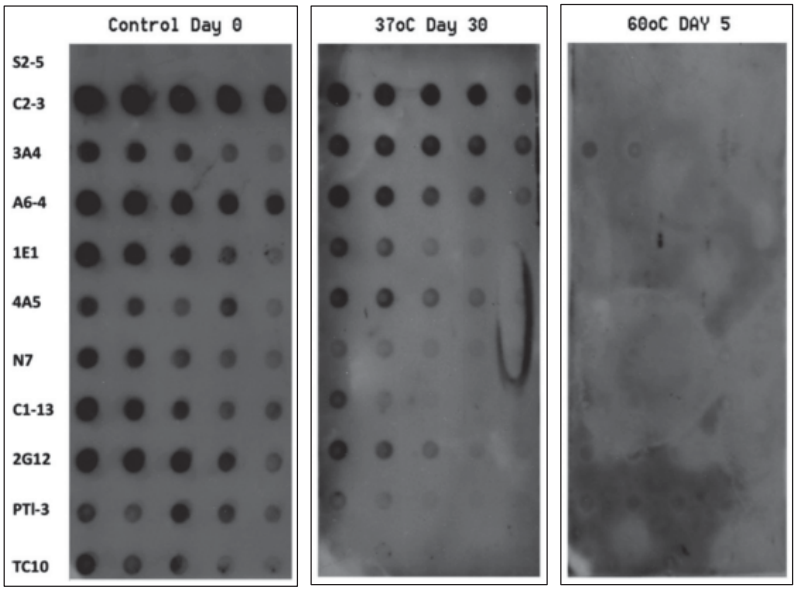
- Thermal denaturation

*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!*



# Instability of Diagnostic Antibodies




| MAb | Reactivity before and after incubation at 37°C[c] Day 0 | 30 days | 90 days |
|---|---|---|---|
| 3A4[a] | +++++ | +++ | – |
| 2G12-1C12[a] | +++++ | +++++ | – |
| 1E1-A9[a] | +++++ | +++ | – |
| 4A5[a] | +++++ | ++++ | – |
| A6-4 | +++++ | ++ | – |
| C1-13 | +++++ | +++++ | +++ |
| N7 | +++++ | +++ | – |
| PTL-3 | +++++ | +++++ | +++ |
| S2-5 | +++++ | – | – |
| TC-10 | ND | ND | ND |
| C2-3 | +++++ | ++ | – |
| Genway | ND | ND | ND |

*The visuals all match, great!*

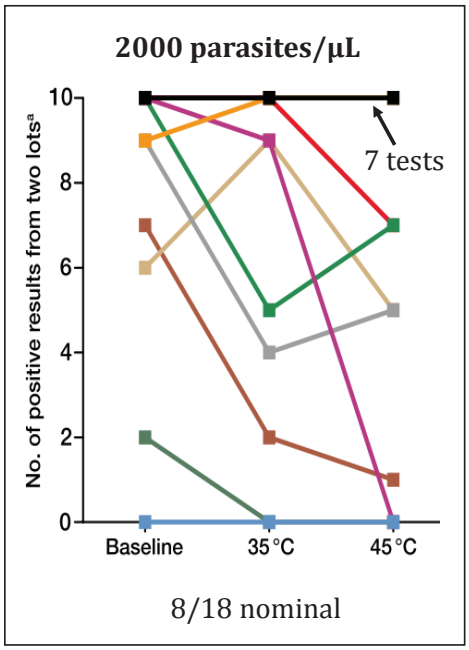


1. Lee et al. J. Clin Micro. (2012)

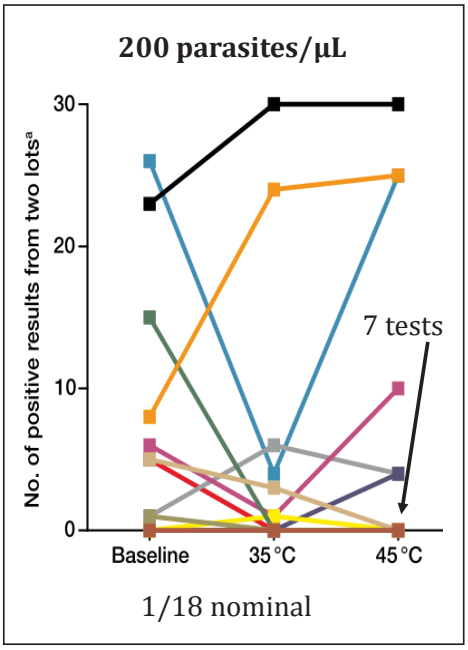
# Test Performance Diminished By Heat

- Malaria rapid diagnostic tests incubated for 60 days, 75% humidity[1]
- Shipments of RDTs in Cambodia spent >3,400 hours above 30°C[2]
- At some sites in Senegal and Ethiopia, RDTs stored at 30°C for >80% of time, 40°C for 18% of time[3]

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




8/18 nominal




1/18 nominal

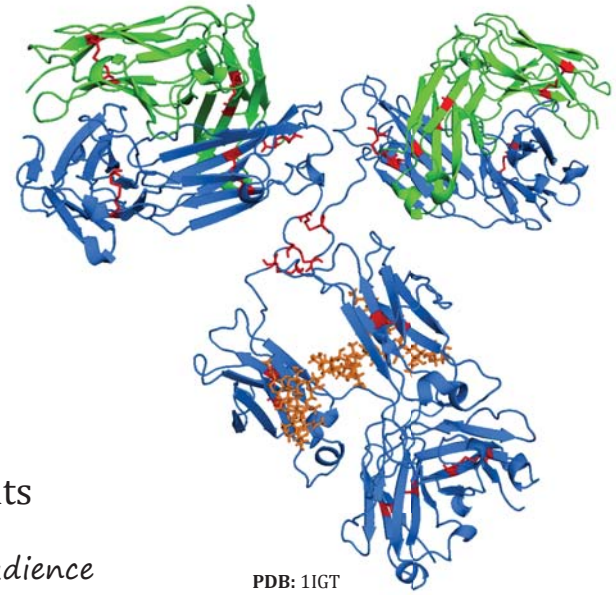


1. WHO. Malaria RDT Survey, Round 4. (2012)
2. Jorgensen et al. Am J. Trp. Med. Hyg. (2006)
3. Albertini et al. Malar. J. (2012)

# 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!*

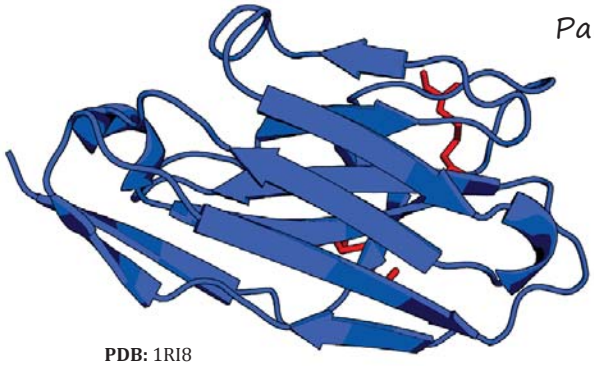


**PDB:** 1IGT



# Candidate Binding Scaffolds for Antibody Replacement

*Parallel structure helps us draw comparisons*



**PDB:** 1RI8

**Single-domain camelid antibodies**

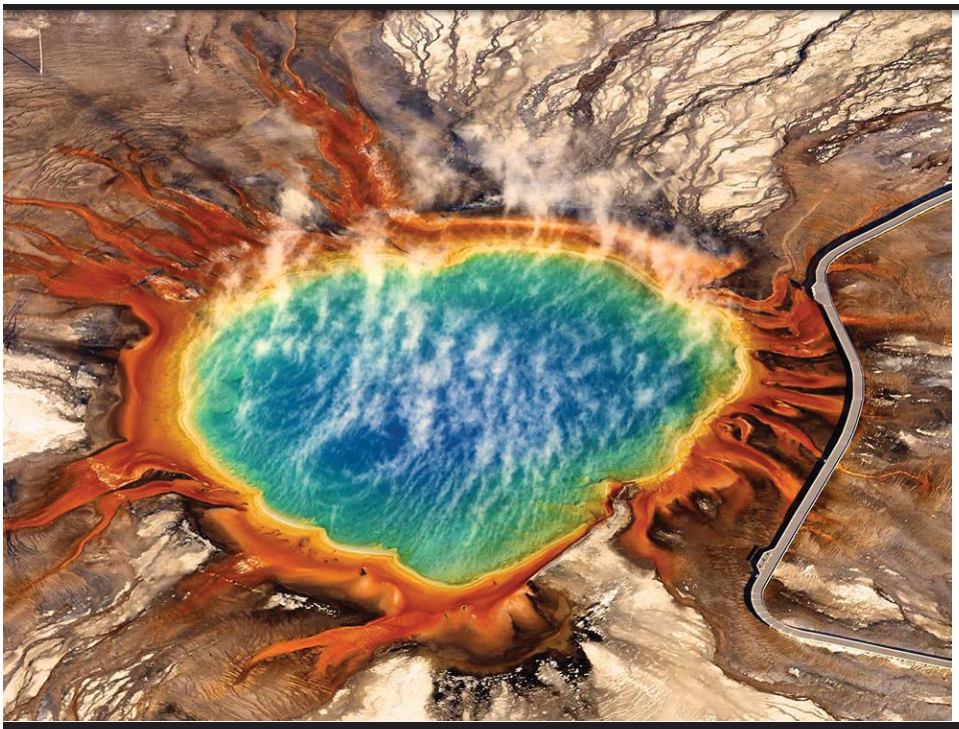
- Low expression yields
- Poor solubility
- Native disulfide bonds

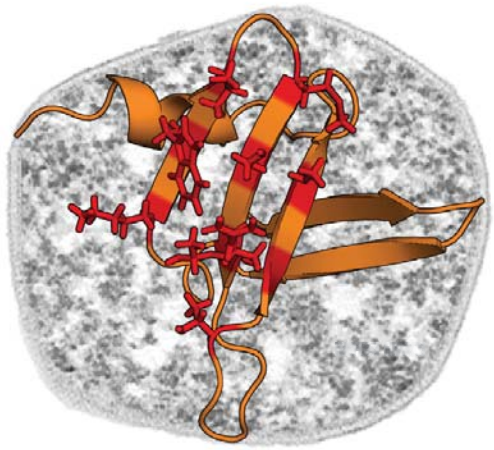
**PDB:** 1ULL

**DNA aptamers**

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

# Nature-Guided Design: Sso7d





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

*Pretty slide!*



# Critical Attributes for Diagnostic Binding Proteins

**Chemical/Physical Properties**

**Biomanufacturing**

**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*

# Chemical/Physical Properties of Sso7d

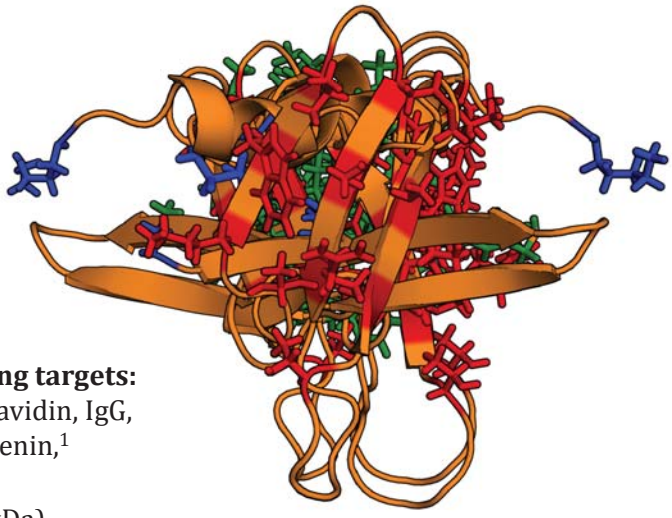
**Chemical/Physical Properties**

- 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 virus[2]
- **MW:** 7 kDa (Ab: 150 kDa)
- **Radius of gyration:** 1.31 nm (Ab: 5.39 nm)



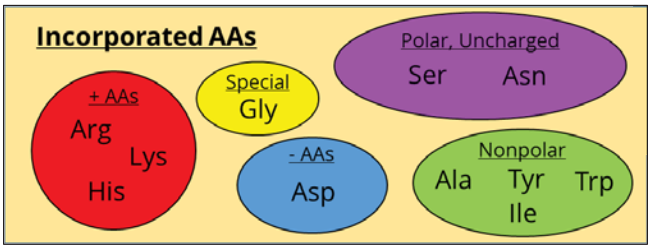
1. Gera et al. J. Mol. Bio. (2011)
2. Hussain et al. Biotech Prog. (2012)

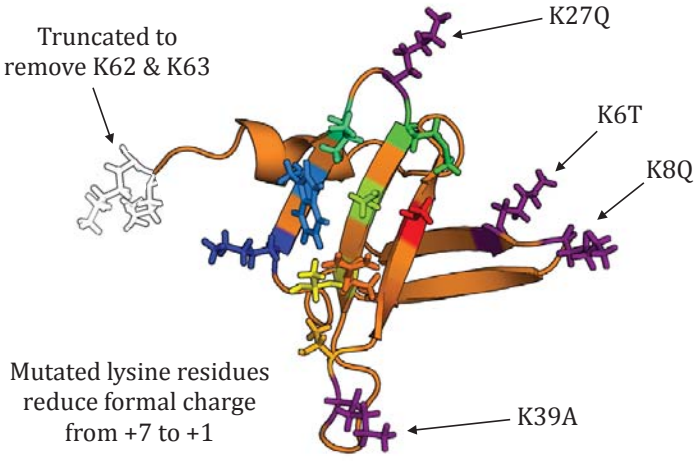
# Wittrup Lab Sso7d Library

1 10 20 30 40 50 60

ATVKFTYQGEEKQVDISKIKKVWRVGQMISFTYDEGGGATGRGAVSEKDAPKELLQMLEKQ

- 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: $1.4 \times 10^9$



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

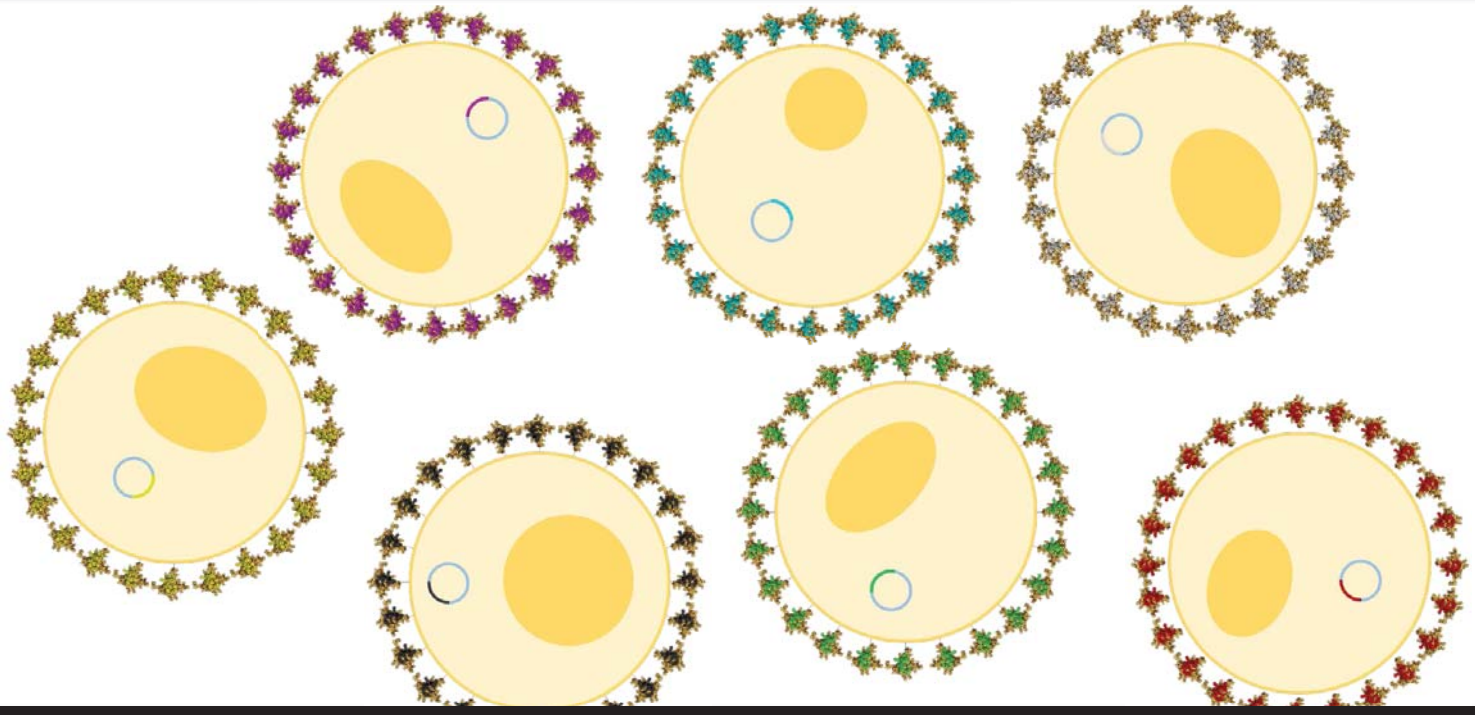
# Yeast Surface Display Primer

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



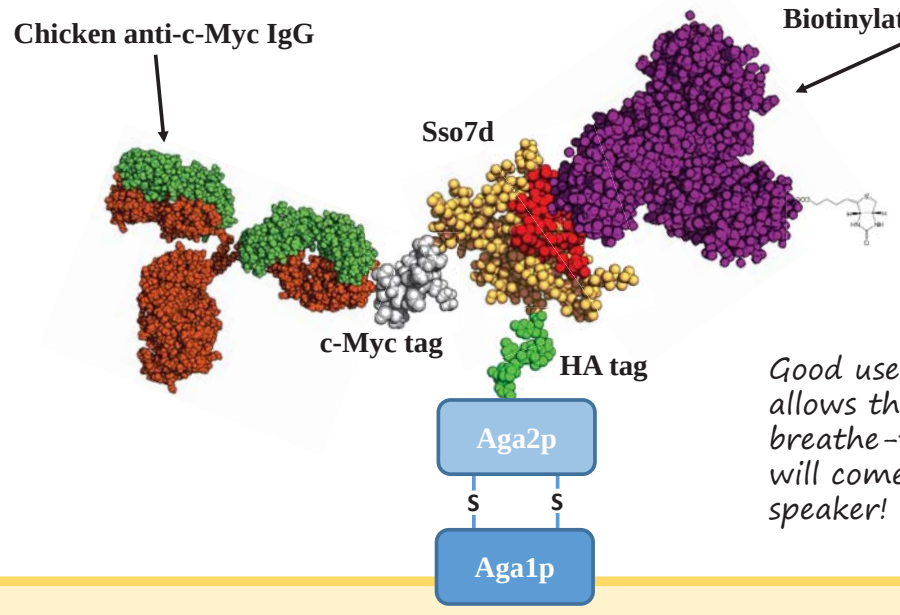
# Yeast Surface Display Primer

# Yeast Surface Display Primer



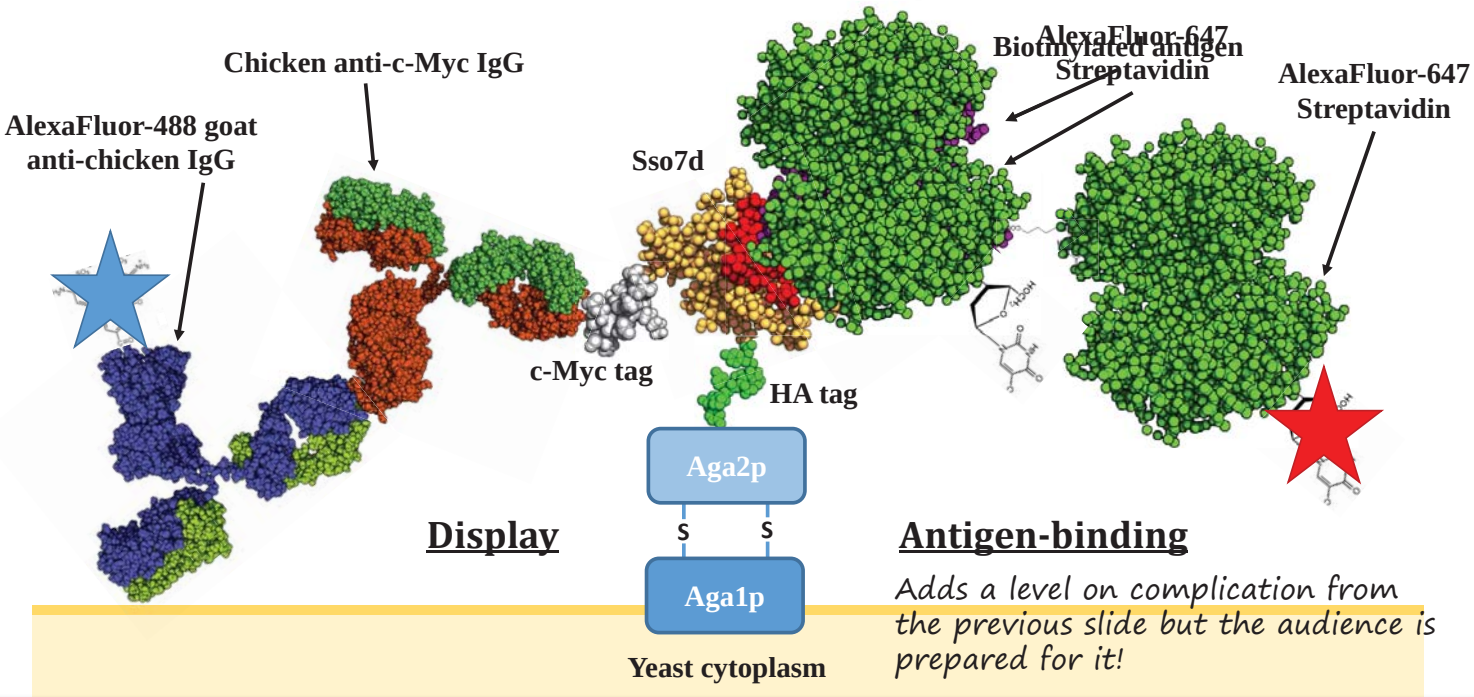


# Yeast Surface Display Primer




*Good use of white space allows the image to breathe–the explanation will come from the speaker!*

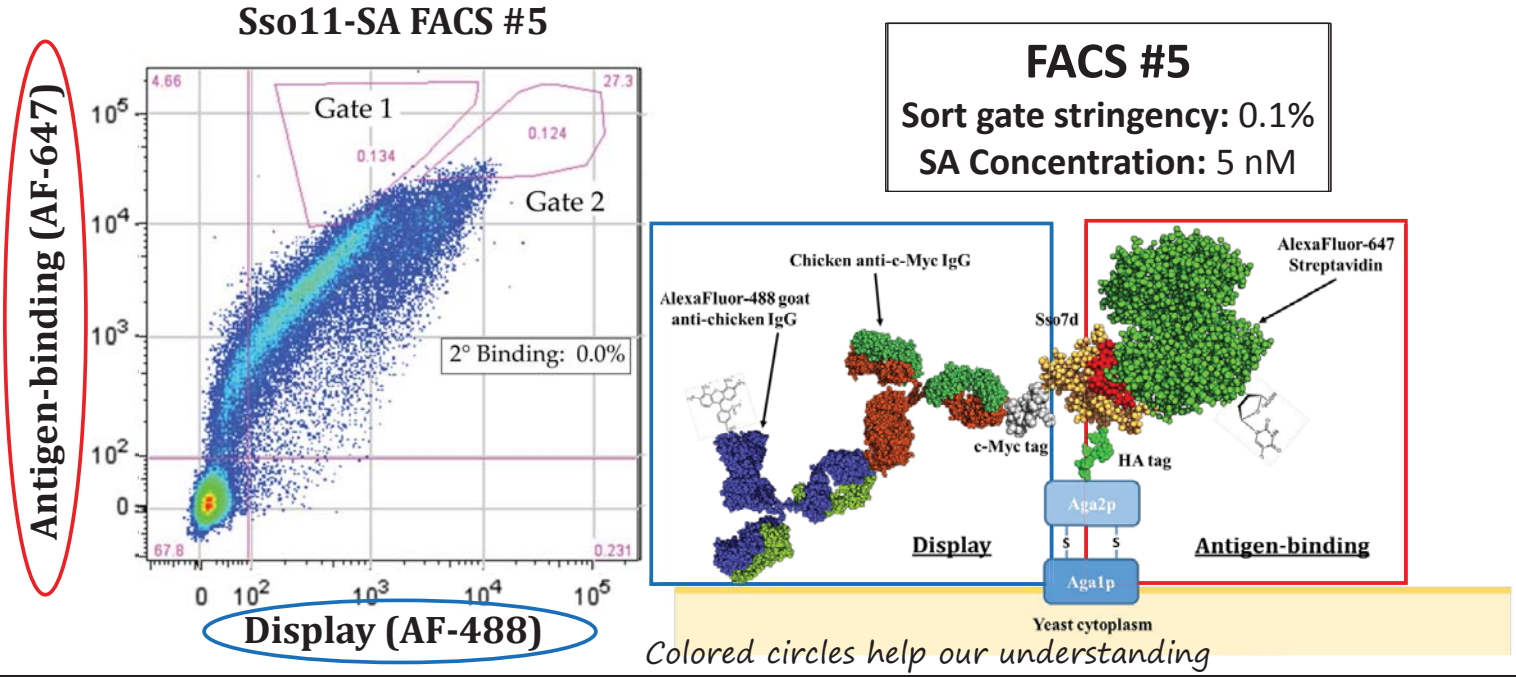
# Yeast Surface Display Primer




Adds a level on complication from the previous slide but the audience is prepared for it!



# Model Streptavidin Binder Development

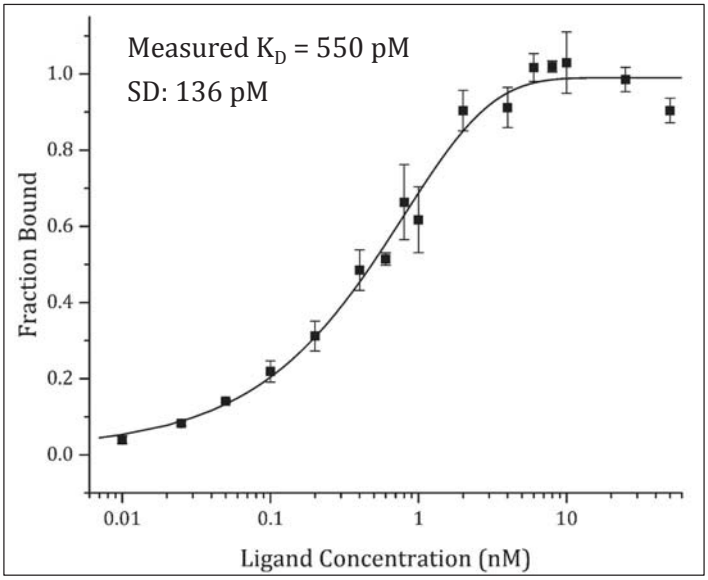



Colored circles help our understanding

# Sso11-SA Features Picomolar Affinity

**Sso11-SA:**
ATVKFTYQGEEKQVDISKIKIVARDGQYIDFKYDEGGGAYGYGWVSEKDAPKELLQMLEKQ

*Red color is used to highlight important pieces of the information*






# Biomanufacturing of Sso7d

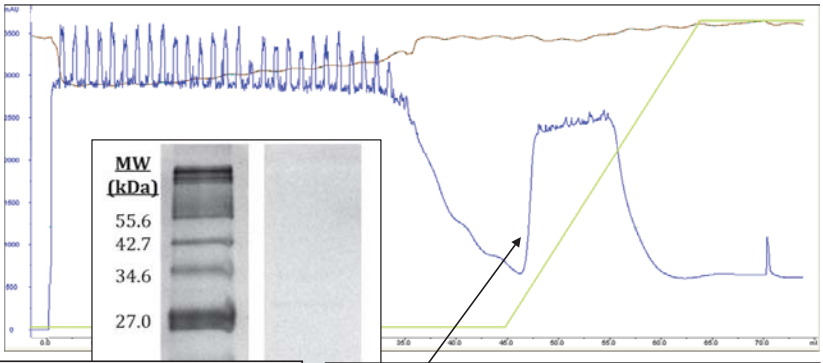
**Chemical/Physical Properties**

**Biomanufacturing**
- 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 hours[1]
- **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)



1. Gassmann et al. The FASEB Journal. (1990)

# Activity of Sso11-SA

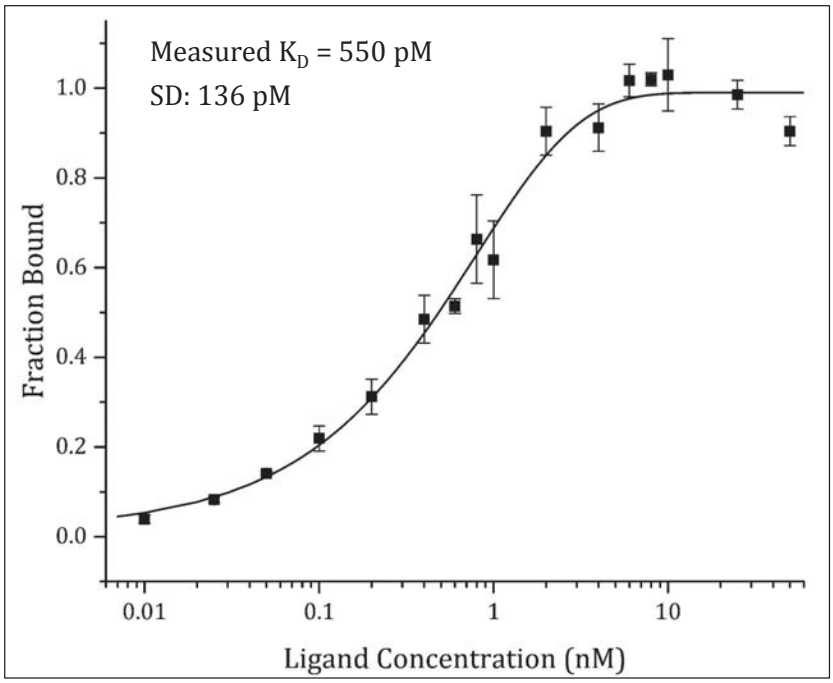
**Chemical/Physical Properties**

**Biomanufacturing**

**Activity**

- High-affinity binding interactions
- Surface-immobilized activity

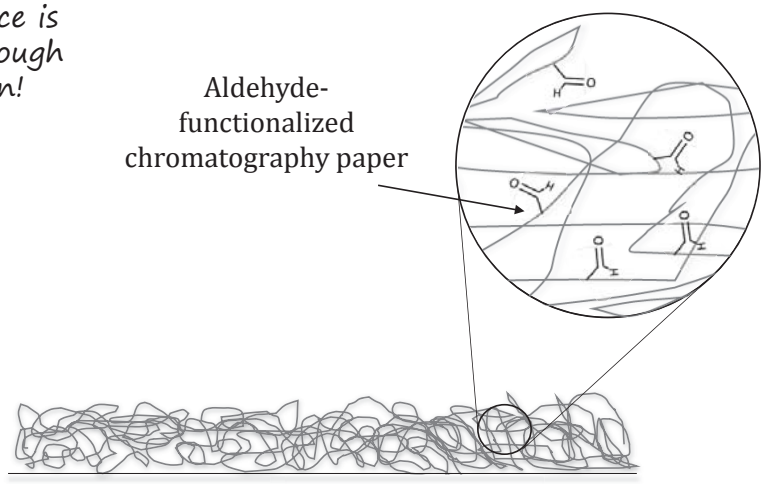
**Stability**



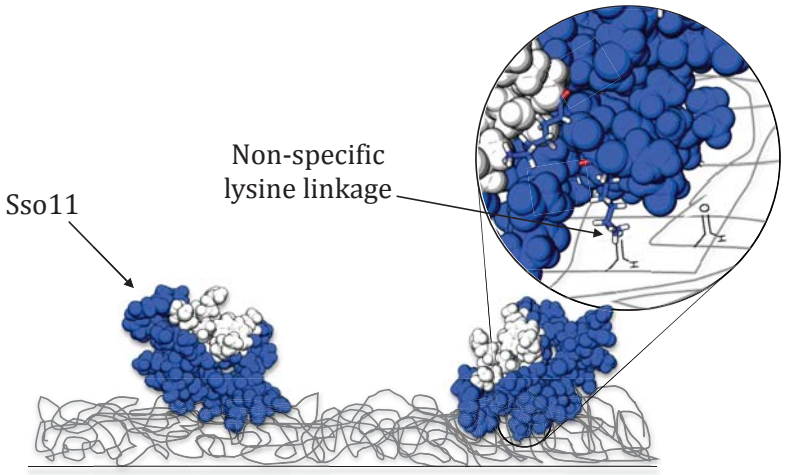



# Paper Assay Schematic

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



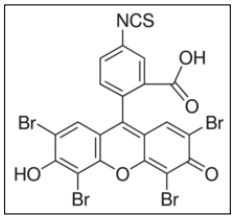
# Paper Assay Schematic

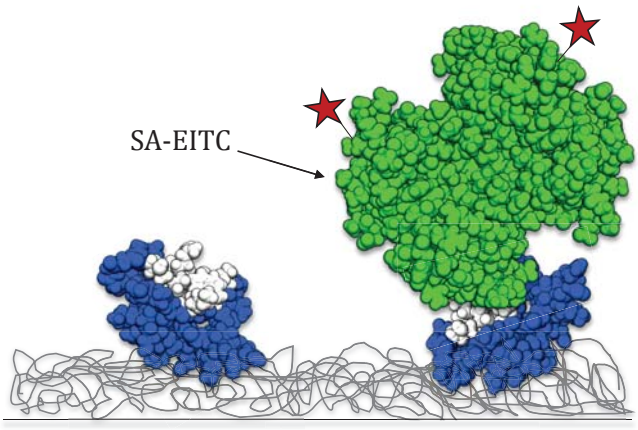




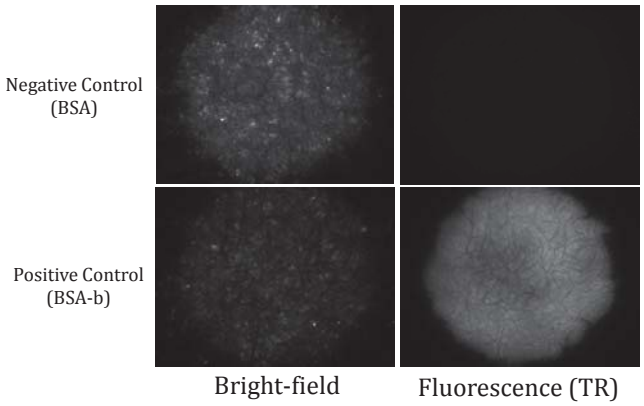

# Paper Assay Schematic

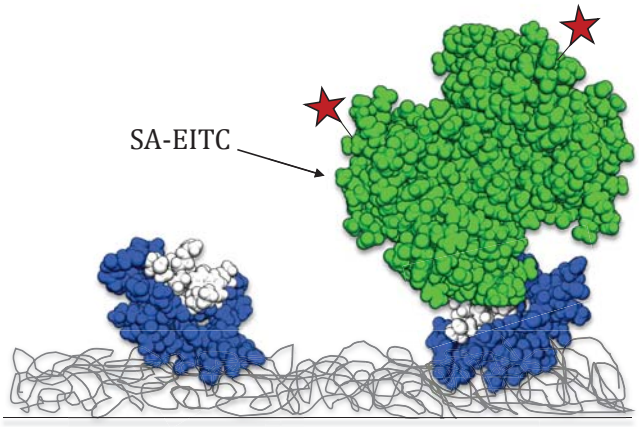
**Eosin isothiocyanate (EITC)**



# Paper Assay Schematic






# Low-Nanomolar Surface-Immobilized Binding Signal

**Chemical/Physical Properties**

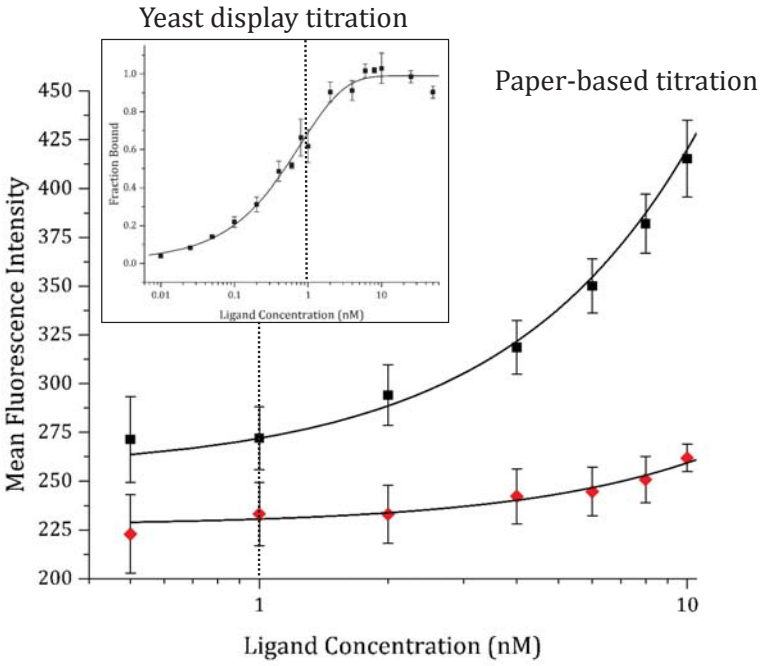
**Biomanufacturing**

**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!*



# Stability of Sso7d

**Chemical/Physical Properties**

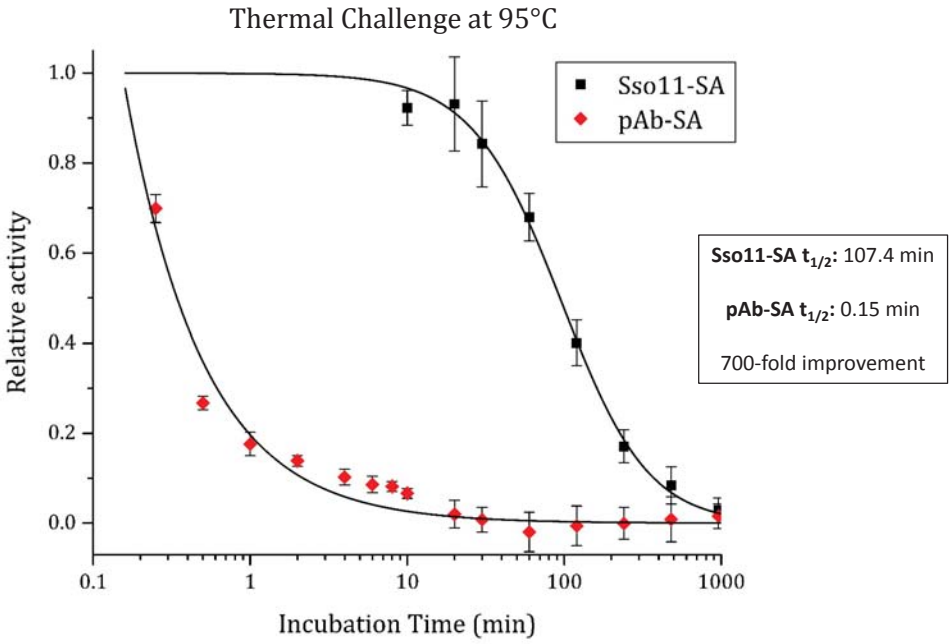
**Biomanufacturing**

**Activity**

**Stability**

- Chemical stability
- Thermal stability
- Dehydration tolerance

- **pH Range:** 0.33-12.5[1]
- **$[Gdn\text{-}HCl]_{1/2}$:** 3.5 ± 0.78 M
- **Wild-type $T_m$:** 98°C






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**

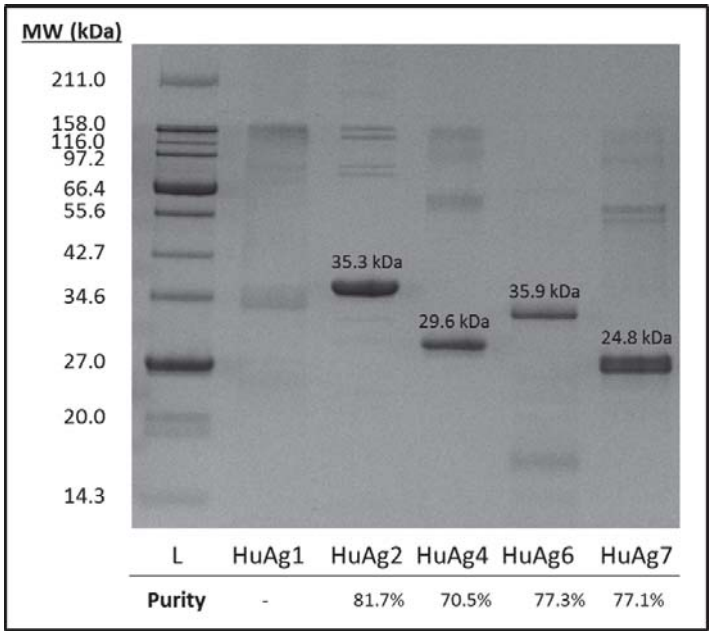
- Sub-nanomolar affinity
- Demonstrated surface activity to low nanomolar concentrations

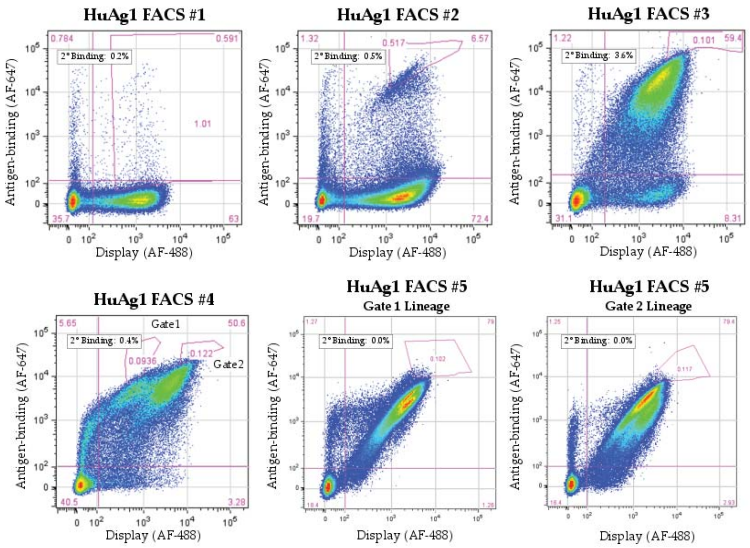
**Stability**

- 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*

# Future Directions – TB Antigens

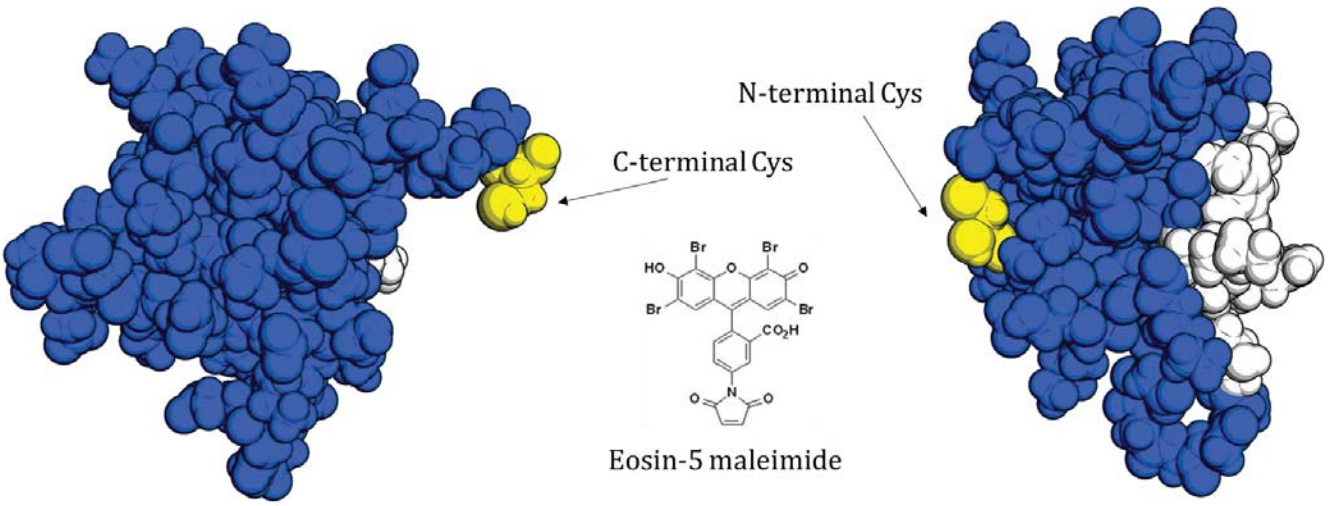



**Binders selected:** HuAg1 (4), HuAg4 (2), HuAg6 (2)



# Future Directions – Site-Specific Chemistry

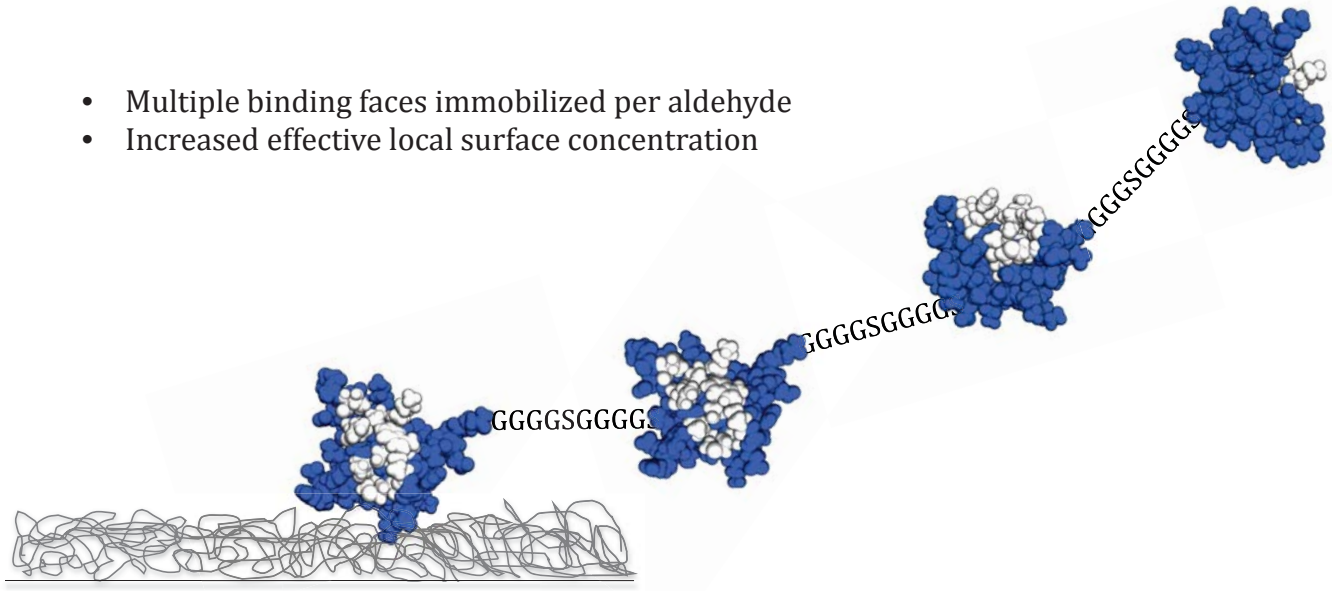



- Site-specific chemical modification
- Oriented surface immobilization

*Arrows help the audience know where they should be looking!*

# Future Directions - Multimerization

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



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