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1. Accelerated Molecular Probe Pipeline (AMPP)

2. Biomarkers of viable pathogen cells

3. Point-of-care diagnosis of tuberculosis

4. Epidemiology of non-tuberculous mycobacteria
Accelerated molecular probe pipeline (AMPP)
Re-inventing the monoclonal antibody

**Biomarker Discovery**
- Proteomics
- Transcriptomics
- Metabolomics
- Ab/Ag arrays
- CMVAT/IVIAT
- Systems biology
- Informatics
- Data mining
- Lipidomics
- TraSH/STM
- etc

**Diagnostic detection**
- LFI
- ELISA
- IHC/microscopy
- SPR
- Impedance
- Electrochemical sensors
- FRET
- Enzymatic amplification
- Branched polymers
- Nanotip sensors
- Biosensors
- Antibody arrays
- Flow cytometry
- Microfluidics
- etc

The University of Arizona Biology Project
Accelerated molecular probe pipeline (AMPP)
Re-inventing the monoclonal antibody
Accelerated molecular probe pipeline (AMPP)
Re-inventing the monoclonal antibody

- NIAID Biodefense/EID program
- *Entamoeba histolytica* (intestinal amoebiasis)
- Biomarkers → diagnostic test
- Goal: Affordable replacement for MAbs
Rapid scFv probe selection by yeast display

Yeast-displayed single chain fragment variable (scFv) antibodies
• Diverse libraries (~2 billion) in yeast
• Selectable by magnetic separation and FACS
• 2-3 weeks to antigen-specific, yeast-bound scFv probes
Yeast-displayed single chain fragment variable (scFv) antibodies

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- Easy affinity maturation/enhancement → increased effective diversity

Rapid scFv probe selection by yeast display

Parental scFv

Mutagenize → Sort yeast → Sort yeast → Sort yeast → Analyze clones

mut-ScFv

(2-3 rounds of selection and sorting)
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- Poor activity in solution, an environment for which the scFv were not selected

Rapid scFv probe selection by yeast display
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- Solution: Use the scFv in their selective environments, bound to yeast cell walls
  - “Yeast-scFv” reagents
Yeast-scFv sandwich assays

1. Flow cytometric

- **No antigen** (negative control)
- **EHI_115350** (non-target)
- **EHI_000780** (target)

Lyophilized yeast-scFv reagents

Figure 5. Lyophilization time course assay with clone 23-780

Immunoassay formats for whole yeast-scFv probes

• Antibody sandwich flow cytometry (ASFC)
• Yeast-scFv immunofluorescence microscopy (yeast IFM)
• Yeast-scFv ELISA
• Competitive inhibition flow cytometry (CIFC)
Yeast-scFv sandwich assays

2. Immunofluorescence microscopy

Yeast-scFv sandwich assays
3. Enzymatic (e.g. horseradish peroxidase) assays

GaR-HRP assay

Figure 7. Schematic of the antibody sandwich enzymatic assay (top left). Binding of cognate antigen by yeast probes is visualized by TMB substrate (top right) and can be quantified (bottom left).
Yeast-scFv sandwich assays
4. Competitive inhibition assays

Yeast with displayed scFv

Streptavidin-phycoerythrin

Biotinylated antigen

Antigen

Yeast with displayed scFv

![Graph showing competitive inhibition assays for Yeast-CI detection of Trypanosoma brucei ISG75 protein.](image)

**Yeast-CI detection of *Trypanosoma brucei* ISG75 protein**

Gray SA et al, Biotechnology & Bioengineering, 2010
Pull-down assays: Limits of detection at various sample volumes

Percentage PE positive vs. concentration of 350-PEG Bio antigen for different sample volumes (0.25 mL, 2.5 mL, 25 mL).

LOD (Limits of Detection) is indicated on the graph.
Conclusions:
• scFv antibodies culled from yeast display libraries (like all antibodies) function best in the environments in which they were selected
• Whole cell yeast-scFv affinity reagents function well in diverse assay formats
• Yeast-scFv reagents are stable in lyophilized form
• Advantages:
  • Easy selection
  • Can be produced cheaply in vast quantities
  • Excellent affinity
  • Monoclonal specificity
  • Amenable to optimization

Hale’s Brewpub, Seattle, WA
Accelerated molecular probe pipeline (AMPP)
Re-inventing the monoclonal antibody

Up next:
• Lyophilization/stability studies
• Based on _E. histolytica_ cyst proteome (Ali IK et al, in press), identify and express ~50 candidate _Eh_-specific cyst antigens
• Select yeast-scFv clones for each antigen
• Screen _Eh_-positive and –negative stool samples (ICDDR,B) to identify cyst biomarkers.
• Develop prototype strip test either with scFv or standard IgG probes

**Anticipated outcomes:**
• New _E. histolytica_ POC test
• Validation of yeast-scFv concept

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Five _Entamoeba histolytica_ - positive stool samples from Bangladesh were semi-purified for cysts and analyzed by LC-MS/MS.

**TABLE 1. Unique _E. histolytica_ cyst proteins identified in high abundance by LC-MS/MS**

<table>
<thead>
<tr>
<th>Protein ID</th>
<th>Annotation</th>
<th>Samples Identified In (n=5)</th>
<th>Total MS/MS Hits</th>
<th>E. dispar Homologue % Identity</th>
<th>% Coverage</th>
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<tr>
<td>EHI_101240</td>
<td>nucleic acid binding related</td>
<td>3, 4, 5</td>
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</table>

Ali IK et al, PLoS Neglected Tropical Diseases, in press
AMPP partners

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Funders: NIAID, FIND