Streamlining methods for screening antibody performance

Pall ForteBio User Meeting
Atlanta, GA
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The Edgewood Chemical Biological Center's (ECBC) science and technology expertise has protected the United States from the threat of chemical weapons since 1917.

Since that time, ECBC has expanded its mission to include biological materials and emerges today as the nation's premier authority on chemical and biological defense.

Today, ECBC has partnerships with nearly every federal agency is a national resource for chemical and biological defense. TECHNOLOGY DRIVEN. WARFIGHTER FOCUSED.
ECBC BioTechnology
Innovative Solutions Developing Quality Products for the Soldier

Molecular Biology
- Sequencing & Cloning
- Protein Engineering
- PCR probes
- Primer Design
- Microbial and Viral Genetics

Cell Culture
- Hybridoma Development
- Optimization
- Cell-based assays
- Toxicology
- Cell Banking

Manufacturing
- Scale-up Process Engineering
- Process optimization
- 5-1500 Liter Production
- Purification
- Milling/drying
- BSL-2

Analytical Services
- Purity
- Functional Activity
- Physical Properties
- Solubility
- Stability
- Molecular Interactions

Capabilities: Detection, Diagnostics Test & Evaluation, Toxicology and Decontamination
Biomaterials: Antibodies, Antigens, Simulants, Recombinant Proteins and Enzymes

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ECBC Biotechnology

Antibody production, characterization and storage
ECBC BioTechnology

- DOD sponsored antibody repository
- Award winning standardized testing and database design
- A2LA accreditation for verifying antibody concentrations, purity, and behavior in solution (DLS)
- Database includes $T_m$ and affinity characteristics

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Antibody Technology Program

- ECBC provides antibody to performers
- Performers required to improve thermostability to retain 80% activity at 70°C
- Antibody affinity should improve 100-fold
- Performers provide enhanced product to ECBC Biotechnology for testing:
  - Purity tested via Experion
  - Concentration tested via NanoDrop
  - Antibody solubility tested with DLS
  - $T_m$ estimated using DSC
  - Thermostability and affinity assessed using SPR
  - Thermostability assessed by ELISA

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Surface Plasmon Resonance (SPR)

SPR detects a change in the angle of reflected light based on mass changes at the surface of the chip.

Biacore T200 allows for highly sensitive detection of mass changes, allowing for binding characterization of low molecular weight proteins and low affinity interactions.
The Many Uses of SPR

• Early test platform for hybridoma supernatant quantitative characterization and ranking

• To study ligand specificity

• Early assay development pairs analysis

• Assess kinetics of purified ligands

• Assess activity of denatured protein

• Assess low affinity binding interactions

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BLI detects the interaction of reflected light waves at the tip of the glass-fiber biosensor.

**Octet RED**₃₈⁴

Octet RED₃₈⁴ creates flow without microfluidics by placing biosensors directly into samples, which allows for rapid, real-time monitoring of molecular binding events.
The Many Uses of BLI

- Use BLI to assess the kinetics of antibodies in complex matrices
- Eliminate refractive index problems
- Eliminate microfluidics
- Cost efficient sensors
- Time for testing is dramatically reduced; while producing similar results to SPR

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Generating Fab Profile

BotFab5 binding to HA33A

Using SPR

\[ k_a = 1.26 \pm 0.003 \text{e}^{6 \text{M}^{-1} \text{s}^{-1}} \]
\[ k_d = 5.26 \pm 0.007 \text{e}^{-3 \text{s}^{-1}} \]
\[ K_D = 4.19 \text{nM} \]

Five serial dilutions from: 75nM-930pM

Using BLI

\[ k_a = 1.01 \pm 0.002 \text{e}^{6 \text{M}^{-1} \text{s}^{-1}} \]
\[ k_d = 4.06 \pm 0.005 \text{e}^{-3 \text{s}^{-1}} \]
\[ K_D = 4.03 \text{nM} \]

Five serial dilutions from: 3nM-187pM

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Generating Mouse IgG Profile

Bot IgG binding to HA33A

Using SPR

\[ k_a = 2.45 \pm 0.003 \times 10^6 \text{M}^{-1} \text{s}^{-1} \]
\[ k_d = 5.20 \pm 0.007 \times 10^{-3} \text{s}^{-1} \]
\[ K_D = 2.12 \text{nM} \]

Five serial dilutions from: 25nM-310pM

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Using BLI

\[ k_a = 1.17 \pm 0.014 \times 10^6 \text{M}^{-1} \text{s}^{-1} \]
\[ k_d = 1.81 \pm 0.019 \times 10^{-3} \text{s}^{-1} \]
\[ K_D = 1.55 \text{nM} \]

Five serial dilutions from: 3nM-187pM

TED RECOM
TECHNOLOGY DRIVEN. WARFIGHTER FOCUSED.
Generating scFv Profile

Bot scFv binding to HA33A

Using SPR

Using BLI

Five serial dilutions from: 25nM-310pM

\[ k_a = 1.92 \pm 0.003 \text{e}^6 \text{M}^{-1} \text{s}^{-1} \]

\[ k_d = 2.59 \pm 0.005 \text{e}^{-3} \text{s}^{-1} \]

\[ K_D = 135 \text{nM} \]

Five serial dilutions from: 3nM-187pM

\[ k_a = 1.74 \pm 0.005 \text{e}^6 \text{M}^{-1} \text{s}^{-1} \]

\[ K_d = 3.57 \pm 0.005 \text{e}^{-3} \text{s}^{-1} \]

\[ K_D = 205 \text{nM} \]

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Generating Human IgG

Bot Human IgG binding to HA33A

Using SPR

$\textit{k}_a = 1.83 \pm 0.005 \text{e}^6 \text{M}^{-1} \text{s}^{-1}$

$K_d = 3.21 \pm 0.008 \text{e}^{-3} \text{s}^{-1}$

$K_D = 17.5 \text{pM}$

Five serial dilutions from: 25nM-310pM

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Using BLI

$\textit{k}_a = 8.50 \pm 0.007 \text{e}^6 \text{M}^{-1} \text{s}^{-1}$

$K_d = 6.42 \pm 0.002 \text{e}^{-3} \text{s}^{-1}$

$K_D = 7.55 \text{pM}$

Five serial dilutions from: 3nM-187pM

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TECHNOLOGY DRIVEN. WARFIGHTER FOCUSED.
Active Concentration

SPR

Bot ScFv at 75°C

Percent Activity

Time at 75°C (minutes)

BLI

Bot ScFv at 75°C

Percent Activity

Incubation Time (minutes)

Human IgG at 75°C

Percent Activity

Time at 75°C (minutes)

Human IgG at 75°C

Percent Activity

Incubation Time (minutes)

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## Comparative Results

<table>
<thead>
<tr>
<th>Instrument</th>
<th>Antibody</th>
<th>KD</th>
<th>Time (50% active at 75°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biacore T200</td>
<td>Fab</td>
<td>4.19nM</td>
<td>45 mins</td>
</tr>
<tr>
<td>ForteBio Octet</td>
<td>Fab</td>
<td>4.03nM</td>
<td>60 mins</td>
</tr>
<tr>
<td>Biacore T200</td>
<td>Mouse IgG</td>
<td>2.12nM</td>
<td>7 mins</td>
</tr>
<tr>
<td>ForteBio Octet</td>
<td>Mouse IgG</td>
<td>1.55nM</td>
<td>12 mins</td>
</tr>
<tr>
<td>Biacore T200</td>
<td>scFv</td>
<td>135nM</td>
<td>30 mins</td>
</tr>
<tr>
<td>ForteBio Octet</td>
<td>scFv</td>
<td>2.05nM</td>
<td>&gt;60 mins</td>
</tr>
<tr>
<td>Biacore T200</td>
<td>Human IgG</td>
<td>17.5pM</td>
<td>&gt;60 mins</td>
</tr>
<tr>
<td>ForteBio Octet</td>
<td>Human IgG</td>
<td>7.55pM</td>
<td>&gt;60 mins</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Biacore T200</th>
<th>ForteBio Octet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Run Time</td>
<td>7hrs</td>
<td>2hrs</td>
</tr>
<tr>
<td>5mins</td>
<td></td>
<td>12mins</td>
</tr>
<tr>
<td>% Reduction in Run Time</td>
<td></td>
<td><strong>72%</strong></td>
</tr>
</tbody>
</table>

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Working for the Soldier

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## What Interferents Could Affect an Assay’s Sensitivity?

<table>
<thead>
<tr>
<th>Interferent</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>Violet Signal Smoke</td>
</tr>
<tr>
<td>Burning Vegetation</td>
<td>BSA, Fraction V</td>
</tr>
<tr>
<td>Burning Diesel</td>
<td>Yellow Signal Smoke</td>
</tr>
<tr>
<td>Nutrient Broth</td>
<td>BHI Broth</td>
</tr>
<tr>
<td>Tryptic Soy Broth</td>
<td>Loamy Soil</td>
</tr>
<tr>
<td>Tween 80, 1% in PBS</td>
<td>Sandy Soil</td>
</tr>
<tr>
<td>Burning Rags</td>
<td>VERO Cell Supernatant</td>
</tr>
<tr>
<td>Brucella Broth</td>
<td>HC Smoke</td>
</tr>
<tr>
<td>PBS</td>
<td>Burning Rubber</td>
</tr>
<tr>
<td>G Media w/trace minerals</td>
<td>Burning Fog Oil</td>
</tr>
<tr>
<td>Green Signal Smoke</td>
<td>Sage Pollen</td>
</tr>
<tr>
<td>Red Signal Smoke</td>
<td>Clay Soil</td>
</tr>
<tr>
<td><em>Aspergillus niger</em></td>
<td>Malathion</td>
</tr>
</tbody>
</table>
Interferent Affinity Screen

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Loamy soil completely inhibits scFv function

Diesel fuel inhibits murine IgG and scFv activity, but does not significantly affect binding kinetics

Loamy soil impacts kinetics, but not activity, of Fab

All antibodies appear unaffected by Green Signal Smoke
A Different Kind of Dirty

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• We want our service members to know if they’ve been exposed to something contaminated with *Salmonella*

• We will use our antibodies to develop an antibody-based assay that can be used with a smart phone
Characteristics of *Salmonella*

- Live bacteria
- Gram negative rod
- About 2μm in length
- Motile
How Can we Analyze Antibody Behavior with Live Bacteria?

- We have antibodies that bind to live culture Salmonella
- We want to see the kinetic behavior of these antibodies, but we don’t want to put live bacteria in our microfluidic systems

**Solution:** Use BLI
Can BLI detect something that big?

YES!

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Antibody Pairs For Salmonella Detection

1. Antibody 1 Capture
2. Salmonella Capture
3. Antibody 2 Association
4. Antibody 2 Dissociation

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Antibody 2 Kinetics
Fit and Residuals

Fitting View

Residual View

\[ k_a : 2 \times 10^4 \text{M}^{-1}\text{s}^{-1} \]
\[ k_d : 1.8 \times 10^{-5} \text{s}^{-1} \]
\[ K_D : 1.37 \text{nM} \]
Conclusions from Salmonella Experiments

- Using BLI, we are able to analyze whole bacterial cells in culture.
- We are not only able to view the qualitative interaction between the cell and the antibody, we can use antibody pairs to quantitate the interaction.
- This antibody pair should work well in our intended assays.
Goals of the program:
- To reimagine how proteins are constructed
- To develop novel medicines and diagnostics as countermeasures to chemical and biological threats

Specifically:
- To develop biologically active non-natural polymers that are structurally similar to naturally occurring proteins without their limitations such as:
  - Sensitivity to heat denaturation
  - Sensitivity to chemical degradation

Performers provide enhanced product to ECBC Biotechnology for testing

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Acknowledgements

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Katherine Rhea (Excet, Inc)
Edward Emm (Leidos)
Heather Welsh (US Army)
Melody Zacharko (Excet, Inc)

Funding Agencies:

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Questions?

E-mail me: patricia.e.buckley4.civ@mail.mil
Supplemental Slides
Scout Analyte Concentration in 1XKB

Association of Ricin A Chain

Dissociation of Ricin A Chain

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mAb with Ricin A Chain in Sage Pollen

1:20-1:1280 Sage Pollen with antigen

Negative Controls: 1:20 Sage pollen and 1xKB

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mAb with Ricin A Chain in Sandy Soil

1:20-1:1280 Sandy Soil with antigen

Negative Controls: 1:20 Sandy Soil and 1xKB
mAb with Ricin A Chain in Burning Diesel Fuel

1:10-1:320 Burning Diesel Fuel with antigen

Negative Control: 1xKB

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mAb with Ricin A Chain in Burning Diesel Fuel

1:20-1:1280 Burning Diesel Fuel with antigen

Negative Control: 1:20 Fuel or 1xKB

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