High Throughput Kinetic Assay Using FortéBio Octet RED96 System to Support Discovery and Fine-tuning of Aptamer Based Diagnostic Agents

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Research & Development
AM Biotechnologies, LLC
Outline

• Background
• Bead-based X-Aptamer selection process
• Aptamer optimization process
• Octet applications at AM Biotech
• Summary
Aptamers

Diagnostics

Environmental analytics

Therapeutics

Imaging tools

Delivery tools (Targeted therapy)

Affinity chromatography

Target validation

Biomarker discovery

Biosensors
# Aptamers vs. Antibodies in Diagnostic Applications

<table>
<thead>
<tr>
<th>Attribute</th>
<th>Aptamers</th>
<th>Antibodies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Affinity</td>
<td>very strong</td>
<td>very strong</td>
</tr>
<tr>
<td>Specificity</td>
<td>can be customized</td>
<td>can be an issue</td>
</tr>
<tr>
<td>Synthesis</td>
<td>chemically / in vitro</td>
<td>biologically / in vivo</td>
</tr>
<tr>
<td>Chemical modification</td>
<td>easy</td>
<td>complex</td>
</tr>
<tr>
<td>Speed of discovery</td>
<td>weeks</td>
<td>months</td>
</tr>
<tr>
<td>Consistency of performance</td>
<td>lot-to-lot variation is not an issues</td>
<td>lot-to-lot variation issues</td>
</tr>
<tr>
<td>Immunogenicity</td>
<td>low</td>
<td>high</td>
</tr>
<tr>
<td>Storage</td>
<td>ambient temp</td>
<td>refrigerated</td>
</tr>
<tr>
<td>Shipping</td>
<td>ambient temp</td>
<td>dry ice / overnight</td>
</tr>
<tr>
<td>Production cost</td>
<td>low</td>
<td>high</td>
</tr>
<tr>
<td>Target</td>
<td>miscellaneous</td>
<td>can be limited</td>
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</tbody>
</table>
AM Biotech®

- Founded in 2006
- Core technologies
  - Bead-based X-Aptamer selection process
  - Conventional aptamer optimization
- Utilize phosphorodithioate (PS2) backbone modifications and base modifications
- Superior binding and nuclease resistance
Bead-Based X-Aptamer Selection Technology (patented)

Enables the use of modified sequences that cannot be enzymatically amplified (e.g. PS2 and combinations of base modifications)
Bead-Based X-Aptamer Selection Technology (patented)

protein binds beads with high affinity sequences

first stage magnetic selection of library beads

anti-tag magnetic particles bind protein

recovered beads: true & false positives

cleave oligos from beads into solution

second-stage solution pull-down with target protein + magnetic particles

next gen sequencing to identify true positives

resynthesize high-affinity aptamers for characterization

customer can easily perform these steps
Conventional Aptamer Optimization Strategy

1\textsuperscript{st} generation screening (modification ‘walk’)

- Synthesize aptamers containing a single modification (e.g. PS2)
- Octet screening
- 5 to 10 times enhanced binding

2\textsuperscript{nd} generation screening

- Synthesize aptamers containing multiple modifications
- Strategically incorporate into multiple positions
- Octet screening
- 500 to 1000 times enhanced binding

3\textsuperscript{rd} generation screening (optional)

- Optimizations from the 2\textsuperscript{nd} generation
- Octet screening
- Enhanced binding with optimum number of modifications

If the bead-based selection was NOT used, AM Biotech\textsuperscript{®} is able to fine-tune conventional aptamers using the ‘walking’ strategy.
Octet Technology: Bio-Layer Interferometry (BLI)

- A layer of molecules immobilized to the tip of the sensor creates an interference pattern at the detector.
- A change in the number of molecules bound causes a measured shift in the pattern.
Main Octet Functions at AM Biotech

• Affinity ranking for candidate X-Aptamers
  • Kinetic analysis for $K_D$ determination
  • Off-rate screening for $K_D$ determination
• HTS of crude X-Aptamers
• Binding investigations in serum
• Counter-screen assays
• Solution competition assays
• Two-site binding assays for diagnostics
Aptamer Optimization: The 1st Generation Screening

1st generation screening (PS2 ‘walk’)
- Synthesize aptamers containing a single PS2 (or other modification)
- Octet screening
- 5 to 10 times enhanced binding

2nd generation screening
- Synthesize aptamers containing multiple modifications
- Strategically incorporate into multiple positions
- Octet screening
- 500 to 1000 times enhanced binding

3rd generation screening (optional)
- Optimizations from the 2nd generation
- Octet screening
- Enhanced binding with optimum number of modifications

Unmodified aptamer
- $K_D$ in the nM range

Modified aptamer/s
- $K_D$ in the pM range
The First Generation Screening of Aptamer Variants (PS2 ‘walk’)

1. Synthesize aptamer variants containing only a single PS2
2. Octet screening against the target protein at a suitable concentration range
3. Obtain $K_D$ values by kinetic analysis
4. Identify positions with a 5-10 times enhancement in the binding
5. Repeat screen of the high impact variants for reproducibility

Shift (nm) vs Time (s)

1. Baseline
2. Association
3. Dissociation
The PS2 ‘Walk’

K_D (nM)

- 11.3
- 10.7
- 11.1
- 8.38
- 7.99
- 7.94
- 8.39
- 8.18
- 7.74
- 8.9
- 6.8
- 7.4
- 6.8
- 7.3
- 7.32
- 7.99
- 7.3
- 7.94
- 8.39
- 5.25
- 5.54
- 3.52
- 2.56
- 5.65
- 3.69
- 4.74
- 5.94
- 6.5
- 6.56
- 7.34
- 7.48
- 8.03
- 6.74
- 8.9
- 6.1
- 7.48
- 7.39
- 8.03
- 6.74

- 5.65
- 3.69
- 7.74
- 8.9
- 6.1
- 7.48
- 7.39
- 8.03
- 6.74

affinity enhanced by a factor of 2 to 5 times

no observed binding within the range of 0.5 – 50 nM protein concentration
Aptamer Optimization: The 2\textsuperscript{nd} Generation Screening

1\textsuperscript{st} generation screening (PS2 ‘walk’)

- Synthesize aptamers containing a single PS2 (or other modification)
- Octet screening
- 5 to 10 times enhanced binding

2\textsuperscript{nd} generation screening

- Synthesize aptamers containing multiple PS2
- Strategically incorporate into multiple positions
- Octet screening
- 500 to 1000 times enhanced binding

3\textsuperscript{rd} generation screening (optional)

- Optimizations from the 2\textsuperscript{nd} generation
- Octet screening
- Enhanced binding with optimum number of modifications

<table>
<thead>
<tr>
<th>Unmodified aptamer</th>
</tr>
</thead>
<tbody>
<tr>
<td>( K_D ) in the nM range</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Modified aptamer/s</th>
</tr>
</thead>
<tbody>
<tr>
<td>( K_D ) in the pM range</td>
</tr>
</tbody>
</table>
The Identification of a Fine-Tuned Aptamer

Design and synthesis of aptamers with multiple PS2 modifications

Aptamers contain strategically incorporated PS2 at multiple positions

Octet screening

Identification of aptamers with 500 – 1000 times enhanced binding

"Parent" aptamer (unmodified)

Dithioate (PS2) modified aptamer

$K_D, \text{ unmodified} = 2.1 \pm 0.3 \text{ nM}$

$R^2 = 0.99 \quad \chi^2 = 0.05$

$K_D, \text{ PS2 modified} = 0.015 \pm 0.0055 \text{ nM}$

$R^2 = 0.99 \quad \chi^2 = 0.08$
<table>
<thead>
<tr>
<th>Therapeutic Target</th>
<th>$K_D$, unmodified</th>
<th>Modification</th>
<th>$K_D$, modified</th>
<th>Binding Enhancement</th>
</tr>
</thead>
<tbody>
<tr>
<td>VEGF$_{165}$</td>
<td>2.1 nM</td>
<td>PS2</td>
<td>15 pM</td>
<td>140x</td>
</tr>
<tr>
<td></td>
<td></td>
<td>FS2</td>
<td>2.3 pM</td>
<td>913x</td>
</tr>
<tr>
<td>α-Thrombin</td>
<td>1.1 nM</td>
<td>PS2</td>
<td>1.2 pM</td>
<td>916x</td>
</tr>
<tr>
<td>α-Thrombin</td>
<td>2.4 nM</td>
<td>T-indole</td>
<td>1.4 pM</td>
<td>1700x</td>
</tr>
<tr>
<td>IgE</td>
<td>11.3 nM</td>
<td>PS2</td>
<td>10.4 pM</td>
<td>1000x</td>
</tr>
</tbody>
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Main Octet Functions at AM Biotech

- Affinity ranking for candidate X-aptamers
  - Kinetic analysis for $K_D$ determination
  - Off-rate screening for $K_D$ determination
- HTS of crude X-aptamers
- Binding investigations in serum
- Counter-screen assays
- Solution competition assays
- Two-site binding assays for diagnostics
Binding Investigations in Serum

**Typical Binding Assay in Buffer**

- **Baseline (buffer)**
- **Association**
- **Dissociation (buffer)**

**Same Binding Assay in Serum**

- **Buffer**
- **Aptamer Loading**
- **Blocking (serum + 1% BSA)**
- **Wash (buffer + 1% BSA)**
- **Association (Protein + serum + 1% BSA)**
- **Dissociation (serum + 1% BSA)**

Reference (0 nM protein)

\[ K_{D, \text{buffer}} = 4.4 \pm 0.9 \text{ pM} \]

\[ K_{D, \text{serum}} = 74.9 \pm 1.2 \text{ pM} \]
Counter-screen Assays for Undesired Affinities

- Target Protein (10 nM)
- BSA (500 nM)
- IgG (100 nM)
- MAPK1 (100 nM)
- Thrombin (100 nM)
Solution Competition Assays

$K_D, \text{ aptamer 2} > K_D, \text{ aptamer 1}$

Loading sample: Aptamer 2

Buffer

Buffer + aptamer 1

Association

Dissociation

Time (sec)

Loading sample: Aptamer 2

Buffer

Buffer + aptamer 2

Buffer + aptamer 1

Loading sample: Aptamer 1

Buffer

Buffer + aptamer 2

Buffer + aptamer 1
Two-site Binding X-Aptamers for Diagnostic Assay Platform
Detection of Two-site Binding in a Diagnostic Assay

Thrombin binding (Association 1)

2'-F-PS2-RNA aptamer binding (Association 2)

Dissociation

2'-F-PS2-RNA aptamer binding to T-indole-TBA-Thrombin complex
Summary

• The Octet technology has rapidly become an integral part of the core technologies employed at AM Biotech for aptamer discovery and optimization
  • Research & Development — aptamer optimization, HTP titer assays, $K_D$ determination, diagnostic assays, binding in serum
  • Product development — rapid library screen, $K_D$ determination, off-rate screening, counter-screening

• BLI protocols enable rapid, parallel optimization and screening of modified aptamer variants in both simple and complex matrices

• The Octet technology saves a significant amount of time and effort over other screening technologies while delivering excellent data
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FortéBio

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