The aptamer discovery company™

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"Aptus" = “to fit”. DNA or RNA can be “selected” to bind just about anything.

First drugs now on the market (Macugen approved; several others in trials – www.regadobio.com)

**Advantages of aptamers:**
- Chemically synthesized: cheap and easy to produce
- No organisms involved → GMP easy
- Low/non-immunogenic targets can be addressed
- Can be selected under non-physiologic conditions
- Easily modified for chemical conjugation, labeling, other properties
- Small size: good transport, more moles/gram
- Stable: no cold chain required

**Conventional Aptamer Selection, “SELEX”:**
- Requires weeks to months
- Results in aptamers to just one target
The case for increased aptamer adoption

Historically, would-be adopters could not access aptamers or could only “play academically” (3 “sea changes”)

1. Recently a less ominous intellectual property situation. IP on SELEX expired Dec. 2010

2. Aptamers were expensive and time-consuming to produce
   • Our technology completely disrupts aptamer production costs

3. “Good, selective binding” has not always been replicated outside of academic publications
   • Multiplex-selected aptamers likely to have improved selectivity
   • High-throughput validation tools have greatly improved
   • Enhanced, modified DNA chemistries also recently off-patent

1. Base Pair’s founder has written the only published market research report on aptamers: Jackson GW, Strych U: Report #BIO071B – Nucleic Acid Aptamers for Diagnostics and Therapeutics: Global Markets. BCC Research; Oct 2012.
Patented *multiplex* selection of aptamers:

- *Currently we can select aptamers to ~25 targets in multiplex*
- *3 week* turnaround time for aptamer discovery. 1-2 more weeks for Kd determination
- Kd’s often in *single nanomolar* range

➢ Our competitors require *3-6 months* for aptamer discovery

In addition to high-throughput process, Base Pair has significant know-how and trade secrets for discovering aptamers to highly customized targets (small molecules, peptides, etc).

Prior and Current Customers (partial list)
Phased development workflow

Base Pair’s proprietary
$10^{15}$ DNA pool and
selection expertise

Phase 1, Initial Discovery:
- Aptamer Screening
- Deep, “Next-Gen” Sequencing
- Proprietary Sequence Analysis
- Provision of Clonal Test Materials, typ. 1-10 clones
  3-4 weeks

Phase 2, Extended Analytics:
- High-throughput qualitative screen (1200 clones)
- Kd determination (1-10 clones)
- Flow cytometry and other methods available
  1-2 weeks

Phase 3, (Optional) Assay Development:
Consultation and “hands on” capabilities available for:
- Conjugation chemistries
- Sensor development
- Assay format and instrumentation

Phase 4, Transfer of Ownership:
Model agreements in place for:
- Outright sequence buyout
- Royalty agreements
- Exclusive or non-exclusive ownership
- Specific Fields-of-Use
Aptamer-FRET sensing in free solution

Figure 2. Schematic of anatoxin-a (ATX) sensing chemistry (approximate scale). In a competitive-binding fluorescence resonance energy transfer (FRET) assay, quantum dots (QDs) are conjugated to ATX or ATX analogs. Aptamers which bind ATX with high specificity are identified by in vitro selection or "SELEX". Panel (A): Aptamers synthesized with a terminal fluorophore for quenching of the QD are bound to immobilized ATX. Panel (B) When free environmental ATX is exposed to this reagent mixture, QD-quenching aptamers are released from the QDs resulting in a fluorescence signal proportional to the ATX concentration. (C) Chemical structure of anatoxin-a (protonated form).
Aptamer-FRET sensing in free solution

**Figure 3.** Quantitative detection of the small molecule thyroxine (T4) via FRET. Insets show structure of T4 and portable (4.5 x 6.5 x 1.8 inch) Qubit™ fluorometer (Invitrogen) used to acquire data.

- **T4:** LOD ~10 ppm
- **Insulin LOD:** ~20 nM
Aptamer “dot-blots”

<table>
<thead>
<tr>
<th>Assay Type</th>
<th>Limit of Detection (nM)</th>
<th>Total amount of material detected</th>
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<tbody>
<tr>
<td>Direct Method</td>
<td>259.7</td>
<td>259.7 femtomoles</td>
</tr>
<tr>
<td>Indirect (Sandwich)</td>
<td>5.3</td>
<td>1.6 picomoles</td>
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<tr>
<td>Method 1</td>
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<tr>
<td>Indirect (Sandwich)</td>
<td>0.0265</td>
<td>7.95 femtomoles</td>
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<tr>
<td>Method 2</td>
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Novel “Luminex-like” assay for IgG

Ab’s versus aptamers
Assay Details

A. 1 µL of MRSA protein (0.5 mg/mL), 1 µM Oligo #819, + strep-Au conjugate
B. 2 µL of MRSA protein (0.5 mg/mL), 1 µM Oligo #819, + strep-Au conjugate
C. No protein, No Oligo, + strep-Au conjugate
Biomolecular interaction

Surface plasmon resonance (SPR)

Microscale thermophoresis (MST)

Back-scattering Interferometry

Calorimetry (A) and Fluorescence Polarization (B)

Figure W.7. (from Potty et al., see Appendix B). (Panel A): Calorimetric titration of 2 μM VEGF with 20 μM anti-VEGF DNA aptamer. (Panel B): Aptamer:protein binding isotherms as determined by fluorescence polarization (anisotropy).
Routine Characterization of DNA Aptamer Affinity to Recombinant Protein Targets (ForteBio’s “Interactions”, Spring 2012)

**Figure 1:** Kinetic assay set up for direct immobilization of biotinylated DNA to streptavidin biosensors.

**Figure 2:** Processed kinetic data for 1 μM #387 hCG aptamer-biotin and hCG protein analyte showing overlaid fits with $K_D = 56.6$ nM.
Microscale Thermophoresis

Molecules move in a thermal gradient

Binding induces changes in hydration, net charge, size, or solvation entropy

Aptamer recently made to botulinum toxin Type B

$K_d = 3.1 \pm 0.3 \text{ nM}$

Fit quality = 0.93
Backscattering interferometry and aptamers to small molecules

Figure 1. Examples of aptamer:small-molecule binding studies using backscattering interferometry (BSI). (A) A tenofovir specific aptamer is shown to have 9 nM binding and does not bind the small molecule ampicillin (B) An aptamer selected to norepinephrine has a Kd of 188 nM and shows some degree of cross-reactivity with the small molecule drug, tenofovir.
BSI measurements, cont’d

“Quick Screen”,
4 aptamer clones

[\text{Aptamer}] = 100 \text{ nM}
Freq-15 $K_d$ = 15.9 +/- 9.0 nM
Freq-15 $R^2$ = 0.93
Publications (selected)

Peer-reviewed publications:

Presentation and poster abstracts:
2. Mark Morris, Philipp Baaske, Gernot Längst, George W Jackson: “Binding analytics of DNA aptamers to small molecules determined by microscale thermophoresis (MST)”. Southwest Regional Meeting of the American Chemical Society, Nov. 4-7, 2012. Baton Rouge, LA.
6. Gandhi I, Soni M, Quan CQ, Jackson GW: Towards a competitive, aptamer-mediated DNA amplification assay for ultrasensitive protein quantitation. In Southwest Regional Meeting of the American Chemical Society, Nov. 9-12, 2011. Austin, TX
7. Soni M, Gandhi I, Drabek R, Jackson GW: Simplified, empirical Cot-based sequence diversity measurements for evolving aptamer libraries. In Southwest Regional Meeting of the American Chemical Society, Nov. 9-12, 2011. Austin, TX
Thank you from Houston!

Please visit at Poster #773
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