The aptamer discovery company™

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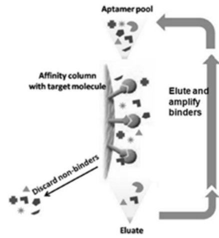
Aptamer Background

"*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].

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.

Base Pair Bio advantages

Patented *multiplex* selection of aptamers:

- ***Currently we can select aptamers to ~25 targets <u>in multiplex</u>***
- ***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: *3-4 weeks*
- Aptamer Screening
- Deep, "Next-Gen" Sequencing
- Proprietary Sequence Analysis
- Provision of Clonal Test Materials, typ. 1-10 clones

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

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

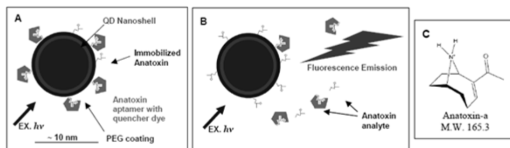


Figure 2. Schematic of anatoxin-a (ATX) sensing chemistry (approximate scale). In a competitive-binding fluorescence resonance energy transfer (FRET) assay, quantum dots (QDs) are conjugated to ATX or ATX analogs. Aptamers which bind ATX with high specificity are identified by in vitro selection or "SELEX". 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

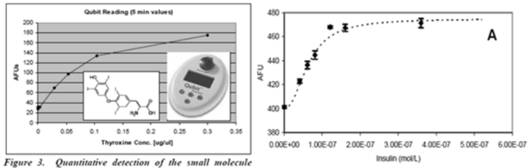
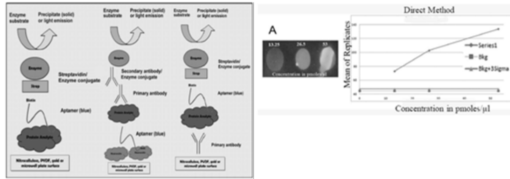


Figure 3. Quantitative detection of the small molecule thyroxine (T4) via FRET. Insets show structure of T4 and portable (4.5 x 6.5 x 1.8 inch) Qubit™ fluorometer (Invitrogen) used to acquire data.

Insulin LOD ~ 20 nM

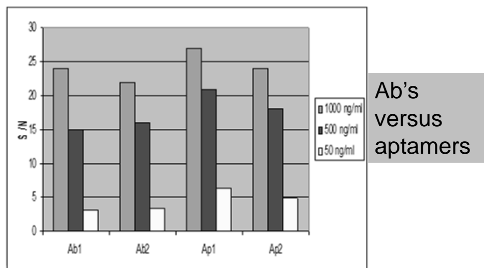
T4: LOD ~10 ppm

Aptamer "dot-blots"



Assay Type	Limit of Detection (nM)	Total amount of material detected
Direct Method	259.7	259.7 femtomoles
Indirect (Sandwich) Method 1	5.3	1.6 picomoles
Indirect (Sandwich) Method 2	0.0265	7.95 femtomoles

Novel "Luminex-like" assay for IgG



Assay Details

Strip contains a Target Protein and Non Target Protein, Strip developed with Aptamer specific for Target Protein and does not bind to Non Target Protein

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

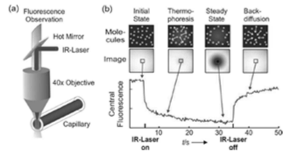
Calorimetry (A) and Fluorescence Polarization (B)

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.

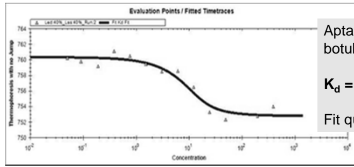
Microscale Thermophoresis



www.nanotemper.de

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 +/- 0.3 nM

Fit quality = 0.93

Backscattering interferometry and aptamers to small molecules

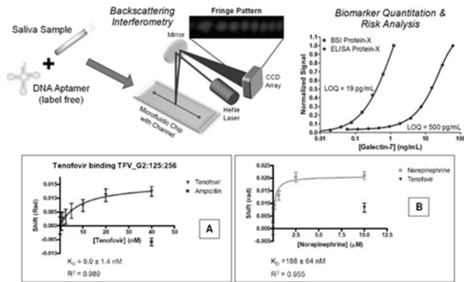
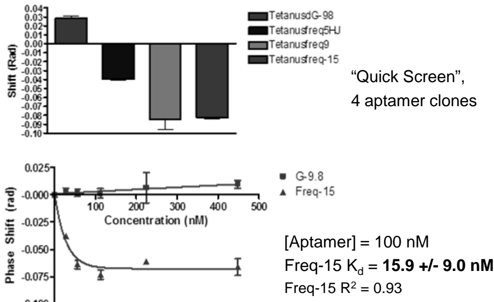


Figure 1. Examples of aptamer:small-molecule binding studies using backscattering interferometry (BSI). (A) A tenofovir specific aptamer is shown to have 9 nM binding and does not bind the small molecule ampicillin **(B)** An aptamer selected to norepinephrine has a Kd of 188 nM and shows some degree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

BSI measurements, cont'd



"Quick Screen",
4 aptamer clones

[Aptamer] = 100 nM

Freq-15 K_d = **15.9 +/- 9.0 nM**

Freq-15 R^2 = 0.93

Publications (selected)

http://www.basepairbio.com/research-and-publications/publications/

Peer-reviewed publications:

1. Zhang, X, Potty, AS, Jackson, GW, Stepanov, V, Tang, A, Liu, Y, Kourentzi, K, Strych, U, Fox, GE, Willson, RC: **Engineered 5S ribosomal RNAs displaying aptamers recognizing vascular endothelial growth factor and malachite green.** J Mol Recognit 2009, 22(2):154-161. PMID: 19195013.
2. Potty, ASR, Kourentzi, K, Jackson, GW, Legge, G, & Willson, RC. **Biophysical Characterization of DNA Aptamer Interactions with Vascular Endothelial Growth Factor**. Biopolymers (2008) 91(2): 145-56. PMID: 19025993.

Presentation and poster abstracts:

1. Ilavarasi Gandhi, Mithil Soni, Hardik Jani, Kaushik Narendran, Mark Morris, George W Jackson: **"BorotinTM": A dual function boronate-biotin molecular probe for measuring glycation/glycosylation of proteins.** Southwest Regional Meeting of the American Chemical Society, Nov. 4-7, 2012. Baton Rouge, LA.
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.
3. Gandhi I, Narendran K, Jackson GW: **Rapid DNA aptamer binding characterization and ELASA development using biolayer interferometry.** 44th Annual AACC Oak Ridge Conference: Emerging Technologies for 21st Century Clinical Diagnostics. April 19-20, 2012.
4. Gandhi I, Soni M, Pillai A, Narendran K, Drabek R, Ballerstadt R, Jackson GW: **Selection and characterization of DNA aptamers specific to glycated proteins.** In Southwest Regional Meeting of the American Chemical Society, Nov. 9-12, 2011. Austin, TX
5. Narendran K, Gandhi I, Soni M, Pillai A, Drabek R, Ballerstadt R, Jackson GW: **Demonstration and optimization of multiple aptamer-ELISA ("ELASA") assays.** In Southwest Regional Meeting of the American Chemical Society, Nov. 9-12, 2011. Austin, TX
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
8. Sonny O. Ang, Cassie Hartline, Tiejuan Mi, Sourindra N. Maiti, George W. Jackson, Hiroki Torikai, Helen Huls, Elizabeth Shpall, Dean A. Lee, Richard E. Champlin, Laurence J.N. Cooper. **Generating a chimeric antigen receptor to redirect T-cell specificity after infusion.** 14th Annual Meeting of the American Society of Gene & Cell Therapy (ASGCT), May 18-21, 2011, Seattle, WA.
9. George W. Jackson, Ulrich Strych, Ed Frank, Richard C. Willson, Ralph Ballerstadt, & Roger J. McNichols. **Portable FRET Sensing of Proteins, Hormones, and Toxins Using DNA Aptamers and Quantum Dots.** Technical Proceedings of the 2009 Nanotechnology Conference and Trade Show, Nanotech 2009, Houston, Texas, May 3-7, 2009.

Thank you from Houston!

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