Base Pair BIOTECHNOLOGIES The Aptamer Discovery Company

Rapid DNA Aptamer Binding Characterization and ELASA Development Using Biolayer Interferometry (BLI)

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Abstract

Nucleic acid aptamers are high affinity, high selectivity ligands produced in vitro by a process commonly known as "SELEX". Primarily due to the expiration of major intellectual property restricting their use, the aptamer class of affinity reagents is poised for considerable growth in a variety of diagnostic application areas [1,2]. Specifically, the ability to inexpensively synthesize a well-defined chemical reagent represents a major advantage of aptamers over antibodies.

While the selection of DNA and RNA aptamers has been described for some time, the SELEX process was traditionally performed against a single target at a time requiring weeks to months for successful execution. We have developed a proprietary process for multiplexing SELEX to discover aptamers against multiple targets simultaneously, thereby greatly increasing the throughput of aptamer discovery. The increased productivity, however, requires us to address aptamer validation (i.e. Kd determination) in a high throughput fashion as well. In contrast to surface plasmon resonance, biolayer interferometry (BLI) has no flow cell or microfluidic channels susceptible to clogging. BLI experiments on ForteBio's Octet Red 96™ are performed in a standard 96 well plate. Additionally, sensors come with facile chemistries for target immobilization requiring little or no experiment-to-experiment optimization.

Herein we describe the use of BLI instrumentation for rapid validation of putative aptamer sequences. Such aptamers are generally selected to bind protein or peptide targets of high diagnostic potential. Finally, taking these validations a step further, we describe the use of BLI for further screening of multiple aptamer clones for complementarity in an ELISA-like pairs of "sandwich" assays. Others have termed such aptamer-based ELISAs as "Enzyme Linked Aptamer Sorbent Assay" or "ELASA". Because kinetics are inherently followed, the optimization of such assays by BLI allows a highly educated subsequent translation of the assay to conventional instrumentation such as fluorescent plate readers.

Introduction

Bio-Layer Interferometry (BLI) is a label-free technology for measuring biomolecular interactions. The binding between a ligand immobilized on the biosensor tip surface and an analyte in solution produces an increase in optical thickness at the biosensor tip. These changes in turn generate a shift in the white light interference pattern that can be measured (Δλ) in real time.

Sensor referencing. As in surface plasmon resonance (SPR), specific binding events are measured relative to some reference sensor/surface to account for non-specific adsorption and any other optical effects not related to true molecular interaction. We frequently employ several methods of sensor referencing in BLI studies for aptamer Kd determination. These methods (see ***Figure 2*** at right) can be viewed as increasing in stringency for true aptamer:protein interaction. For instance, in ***Figure 2, Panel C***, the reference sensor surface comprises an immobilized "sham" or randomized DNA of equal length of the aptamer under test. Thus, the reference sensor exactly mimics the local charge presented for binding on the aptamer sensor.

"ELASA" development on BLI. In addition to routine Kd determination for validating our discovered aptamers, we are presently developing optimal conditions for an ELISA-like approach, enzyme linked *aptamer* sorbent assay or "ELASA" in the BLI instrument. BLI inherently allows the following of binding and reaction kinetics which is more informative than end-point readings in a fluorescent or colorimetric platereader, for example.

Methods

Aptamer selection. Multiple protein targets were immobilized on a solid phase surface (similar to a protein microarray). A randomized DNA library was then exposed to the array for multiple rounds of multiplexed, competitive selection and enrichment. In the final round, proprietary methods are employed to enable mapping DNA sequences to particular protein targets on the array. Following DNA sequencing, all subsequent binding studies were performed on clonal DNA materials.

Methods continued

BLI binding studies. Biomolecular interaction studies were performed using ForteBio's Octet Red 96™ instrument (ForteBio Inc., Menlo Park, CA). Both streptavidin sensors as well as amine reactive "AR2G" sensors.

Sensor preparation and kinetic analysis. For Kd determination, aptamers are typically synthesized with a 3'-biotin and immobilized directly to streptavidin functionalized sensors. Sensors are then "blocked" with a biotin-PEG (PEG molecular weight 350 Da, Nanocs, Boston, MA) prior to addition of protein (or peptide) analyte. Raw data are processed by ForteBio's resident instrument software and fit by a number of available binding models.

"Direct" ELASA demonstration on BLI. For demonstration of "direct" ELASA (Figure 2) amine reactive second generation sensors (AR2G from ForteBio) were loaded with protein target in various concentrations. EDC (1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide) coupling amended with NHS (*N*-hydroxysuccinimide) was performed according to the standard AR2G recommended sensor protocol. Reactive sensors were then "quenched" using 1M Tris buffer, pH 8. After quenching and a buffer wash, the protein-loaded sensor was exposed to 1 µM biotinylated aptamer. Following aptamer binding, sensors were dipped in a 1:2000 dilution of stock streptavidin-HRP conjugate. Finally, sensors were transferred to wells containing the common HRP substrate, TMB.

Sandwich ELASA on BLI". In contrast to *Figure 1*, we are presently developing "sandwich" ELASAs in which a primary aptamer is immobilized through a 3'-amine functionality. Aptamers are synthesized (IDT, Coralville, IA) with 3'-amine and immobilized using identical EDC/NHS coupling as described above. Following addition of varying concentrations of protein, a secondary biotinylated aptamer and HRP-based reporting are also performed identically to the "direct" ELASA described above.

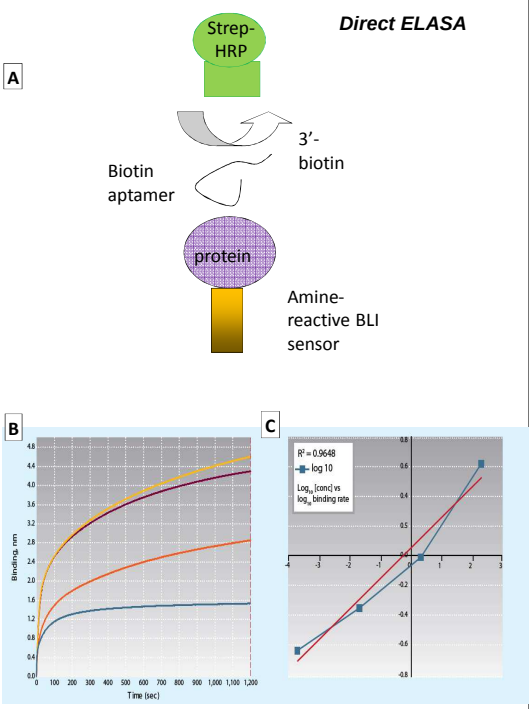

Figure 1. Example of protein quantitation using biotinylated aptamer and secondary streptavidin-HRP binding. (A) Direct (non-sandwich) ELASA format. **(B)** BLI sensor response with varied protein concentrations bound to sensor. **(C)** Standard curve of $\log_{10}$ protein concentration vs. $\log_{10}$ strep-HRP binding rate.

Results

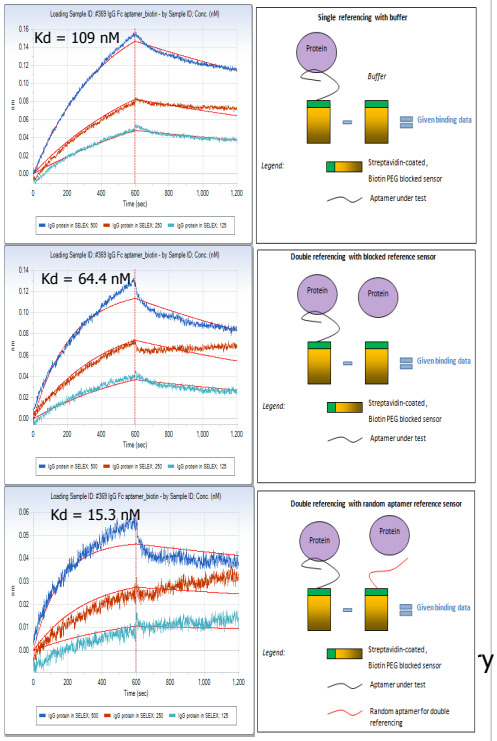

Figure 2 **Examples of various referencing schemes in biolayer interferometry (BLI) of aptamer binding.** Association and dissociation graph of 1:1 fitting model of IgG Fc aptamer (biotinylated) to IgG protein concentrations 500, 250 and 125 nM; **(A)** Single reference method, **(B)** Double reference method with blocked reference sensor, **(C)** Double reference method with random aptamer reference sensor.

Summary

The data presented here represent preliminary adaptations of standard antibody-based methods for protein detection and quantitation to aptamer-based recognition. While not fully optimized in terms of sensitivity, these data also demonstrate the utility of BLI in rapid methods development and optimization over end-point measurements

References

1. Jackson GW, Strych U: Report #BIO071A - **Nucleic Acid Aptamers for Diagnostics and Therapeutics: Global Markets.** BCC Research; 2010.
2. Bunka DHJ, Stockley PG: **Aptamers come of age – at last.** Nature Reviews Microbiology 2006, 4:588–596.

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