Base Pair
BIOTECHNOLOGIES

Application Note

BOROTIN™ Dual Function Boronate-Biotin Probe for Quantitative Measurement of Protein Glycation/Glycosylation

Introduction: Glycosylation is one of the most prevalent post translational modifications of proteins and has been shown to play an important role in both protein structure and interaction. There has been increasing evidence that atypical modifications are implicated in many disease types, thus the detection and quantitative understanding of oligosaccarides has become increasingly important in many research areas.

BOROTIN™ Principle: The BOROTIN™ dual function patent pending probe developed by Base Pair Biotechnologies, Inc. is a convenient molecule for any application in which a cis-diol (i.e. saccharide) group is desired to be quantified. The probe shown in Figure 1 below can be used alone to quantify total cis-diol levels or used in conjunction with any capture affinity reagent (aptamer or antibody) for greater assay specificity. Base Pair Biotechnologies has developed example protocols for use with the probe which leverage the reversible, covalent binding of boronic acid for cis-diols at elevated pH.

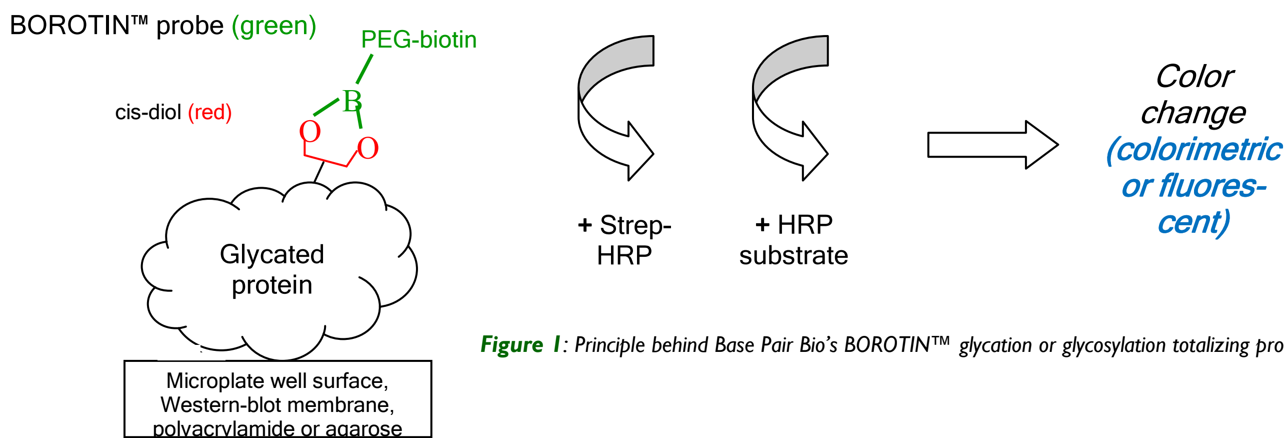

Figure 1: Principle behind Base Pair Bio's BOROTIN™ glycation or glycosylation totalizing probe.

Boronates have been used since the early 1980's in affinity chromatography to separate glycosylated proteins, enzymes and RNA [1, 2]. All of these separations rely on the highly specific, reversible-covalent interaction between boronic acid and cis-diols of sugars.

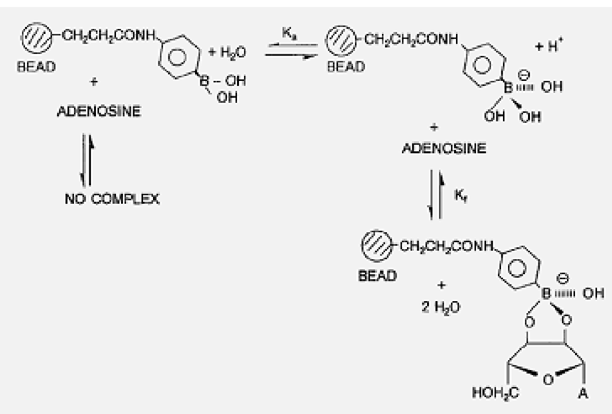

Figure 2: Ionization and diester formation of adenosine, a representative cis-diol, with immobilized boronic acid. The "BEAD" represents any insoluble matrix to which the aryl boronic acid is attached . In aqueous solution, only the ionized tetrahedral boronate forms appreciable amounts of stable complex.

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Because this reversible interaction is pH dependent, our BOROTIN™ probe can be used in a highly selective, "tunable" fashion for recognition of cis-diols (i.e. glycation and glycosylation groups) on captured proteins. In this example we used ForteBio's proprietary Biolayer Interferometry (BLI) systems for protein immobilization and analysis. These systems enable real-time analysis of biomolecular interactions in micro volume sample sizes in just a few minutes.

I. Method: In order to demonstrate the ability to quantify the amount of a glycated protein we used Biolayer Interferometry (BLI) in an ELISA-like approach. Using ForteBio's "Dip and Read" amine reactive AR2G sensors along with the "Octet RED 96" BLI instrument we quantified the amount of BOROTIN™ probe interacting with cis-diols on the protein. A streptaviin-horseradish peroxidase (step-HRP) conjugate was bound to the BOROTIN™ probe, exposed to the immobilized protein, then followed by a standard HRP substrate. The conversion of the HRP substrate resulted in precipitation of material on the BLI sensor which resulted in a large signal change. The conversion rate of the substrate was then used to quantify the original (glycated) protein amount. To perform this experiment, gly HSA, was covalently immobilized to second generation biosensors (AR2G) using standard EDC/sulfo-NHS coupling. Three different pH's were used initially to determine the optimal pH for protein immobilization. Following optimization three different concentrations of gly HSA and several controls were immobilized. The BOROTIN™ probe was then applied to each sensor at the selected pH for covalent boronate-sugar interaction (***Figure 2)***. The sensors were then briefly washed in buffer and subsequently dipped in an equal concentration of streptavidin-HRP conjugate and finally DAB substrate.

Tips For Optimal Performance

♦ 200 µl/well of reagent is required for a 96 well plate, for optimal performance keep the shake speed of well to be 1000rpm.

♦ Equilibrate reagents and samples to room temperature prior to preparation. For frozen samples, thaw and mix thoroughly prior to use.

♦ Use EDC and s-NHS within 1 hour of mixing them together. Use or refreeze EDC and s-NHS aliquots within 10 hours of thawing.

♦ Hydrating the biosensors is required prior to using them in an assay. Hydrate the sensors in nuclease-free water.

♦ Ensure that the Octet instrument is turned on and the lamp is warmed up to room temperature for at least 40 minutes prior to starting the assay.

♦ ForteBio recommends assaying at 30°C. Assaying at other temperatures may require different assay times than discussed in this protocol.

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II. Quantitation assay for different concentration of gly HSA immobilized at pH 4.0 on AR2G sensors, bound to BOROTIN™ probe with controls:

Protocol	AR2G biosensor tray (ForteBio part no. 18-5092)
Protein (dilutions) loading to biosensor	Equilibrate biosensors for 120 sec. in water, load 20 mM EDC and 10 mM NHS for 300 sec., load dilution series of gly HSA protein (25, 2.5, 0.25 ug/ml) in 10 mM acetate buffer pH 4.0 for 3600 sec. Since the amount of protein loading is low, we need to have increased loading time.
Quench after protein binding	Quench the remaining unreacted surface with 1 M Tris, pH 8.5 for 300 sec., change to running buffer, 1 X PBS pH 9.0 for 600 sec.
Associate BOROTIN™ probe to the immobilized protein	Load 100 X dilution of BOROTIN™ probe stock (~370 uM) for 900 sec.
Wash after Association	With 1 X PBS pH 9.0 for 600sec.
Streptravidin-HRP	1:3000 Streptavidin-HRP enzyme conjugate for 600 sec.
Wash after detection conjugate	With 1 X PBS pH 9.0 for 600sec.
Substrate binding	200ul of a 1:10 dilution of DAB substrate stock for 900 sec.

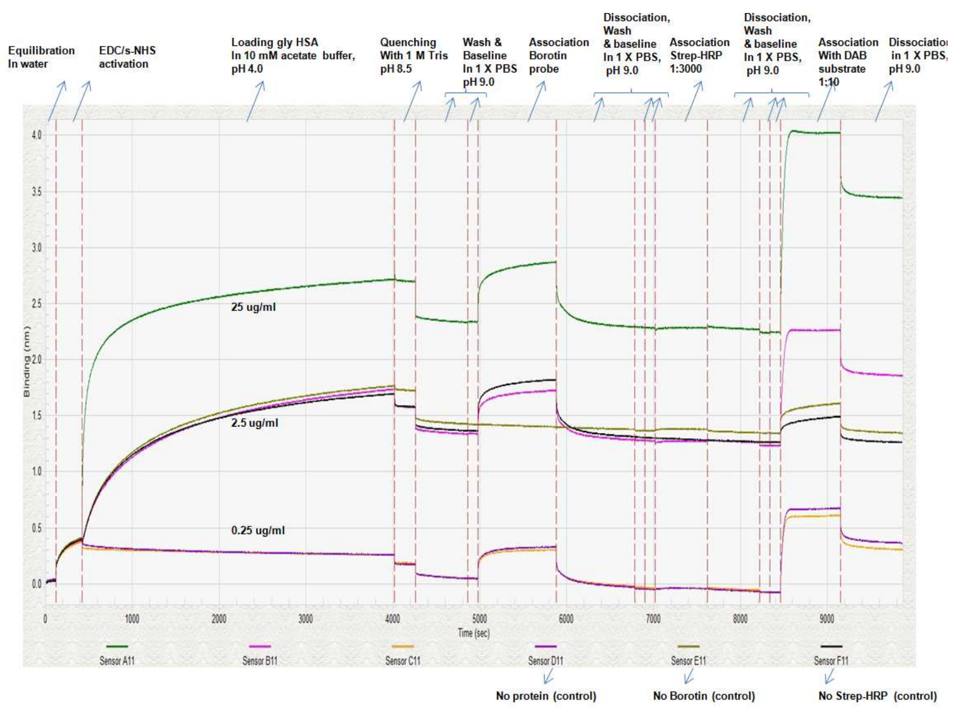

Figure 3: Kinetic data showing all steps for quantitation of glycated HSA protein bound to BOROTIN™ using BLI

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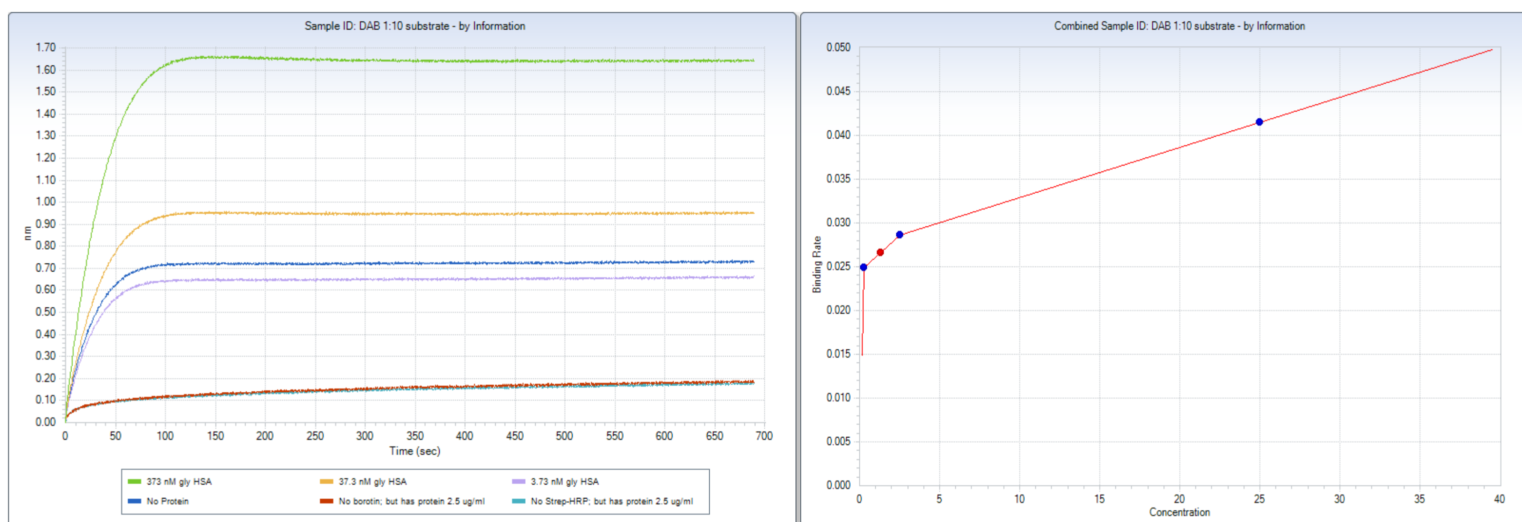
III. Quantitating the DAB substrate step of previous kinetics assay

Figure 4*: Data extracted from HRP substrate step (****Figure 3****). Data are referenced-sensor-corrected and then substrate conversion rates (slopes) are used to quantify the amount of glycated protein in the sample as reported by BOROTIN™ probe*



Conclusion: The data presented here demonstrates the feasibility of using our novel BOROTIN™ probe for quantifying the amount of glycated or glycosylated protein in a sample and eventually discriminating between non glycated and glycated protein. The BLI instrumentation conveniently allows us to monitor both kinetics and endpoint signals, however, based on the data it should be quite feasible to make endpoint measurements alone in more standard instrumentation such as colormetric or fluorescent plate readers using commonly available HRP substrates

FOR ADDITIONAL INFORMATION ON BOROTIN™ PROBES CONTACT US AT *INFO@BASEPAIRBIO.COM*

REFERENCES:

1. Hageman JH, Kuehn GD: **Boronic Acid Matrices for the Affinity Purification of Glycoproteins and Enzymes**. In *Practical Protein Chromatography*. New Jersey: Humana Press; , **11**:45-72.
2. 2. Burroughs S, Wang B: **Boronic acid-based lectin mimics (boronolectins) that can recognize cancer biomarker, the Thomsen-Friedenrich antigen**. *Chembiochem* 2010, **11**:2245-2246.

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