



Development of a Synthetic, Closed-Cycle Sensing System for an Implantable Glucose Sensor

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Overview

- Developed a closed-cycle continuous glucose sensing system that can produce a consistent, measurable response to physiologically relevant levels of glucose while functioning under biologically relevant conditions.
- Synthesized a library of structurally and chemically diverse sensing system component materials that have tunable affinity and reversible binding capabilities with glucose in aqueous media and fractionated serum matrix.
- Constructed a binding affinity model and interaction database to assist our efforts in identifying component materials that showed competitive interaction with glucose with optimized sensitivity.
- Utilized a unique binding assay technique to demonstrate the competitive response between glucose and the sensing system components.

Results

We have designed a chemical system in which the components of the self-contained closed-cycle system will competitively interact with glucose (Figure 1) to respond proportionately to physiologically relevant blood glucose levels. Demonstration of the sensing system required the interaction of two components: 1) the competitive agent/signaling component, which is a dendrimer-boronic acid construct (DBA) and 2) the glucose competitive DBA binding environment, which is an immobilized monosaccharide mimic (iDIOL) (Figure 1). Libraries of DBA and iDIOL components were strategically designed to incorporate an assortment of functional groups with different reactive properties that, in turn, generated reproducible glucose competition curves with tunable sensitivity responses (Figures 2 and 4).¹

Coordinated identification of DBA:iDIOL pairs that competitively interacted with glucose with increased sensitivity was based on our evaluation of the binding affinity (K_{eq}) of a DBA for an iDIOL versus the DBA for glucose. A K_{eq} model and relational database based on a three component (DBA:iDIOL:glucose) competitive assay was used to generate a scatter plot of interaction data (Figures 3 and 5).² Based on the location of a representative data point on the interaction graph, the response of glucose and/or each iDIOL for each DBA was evaluated and scored for inclusion into the screening

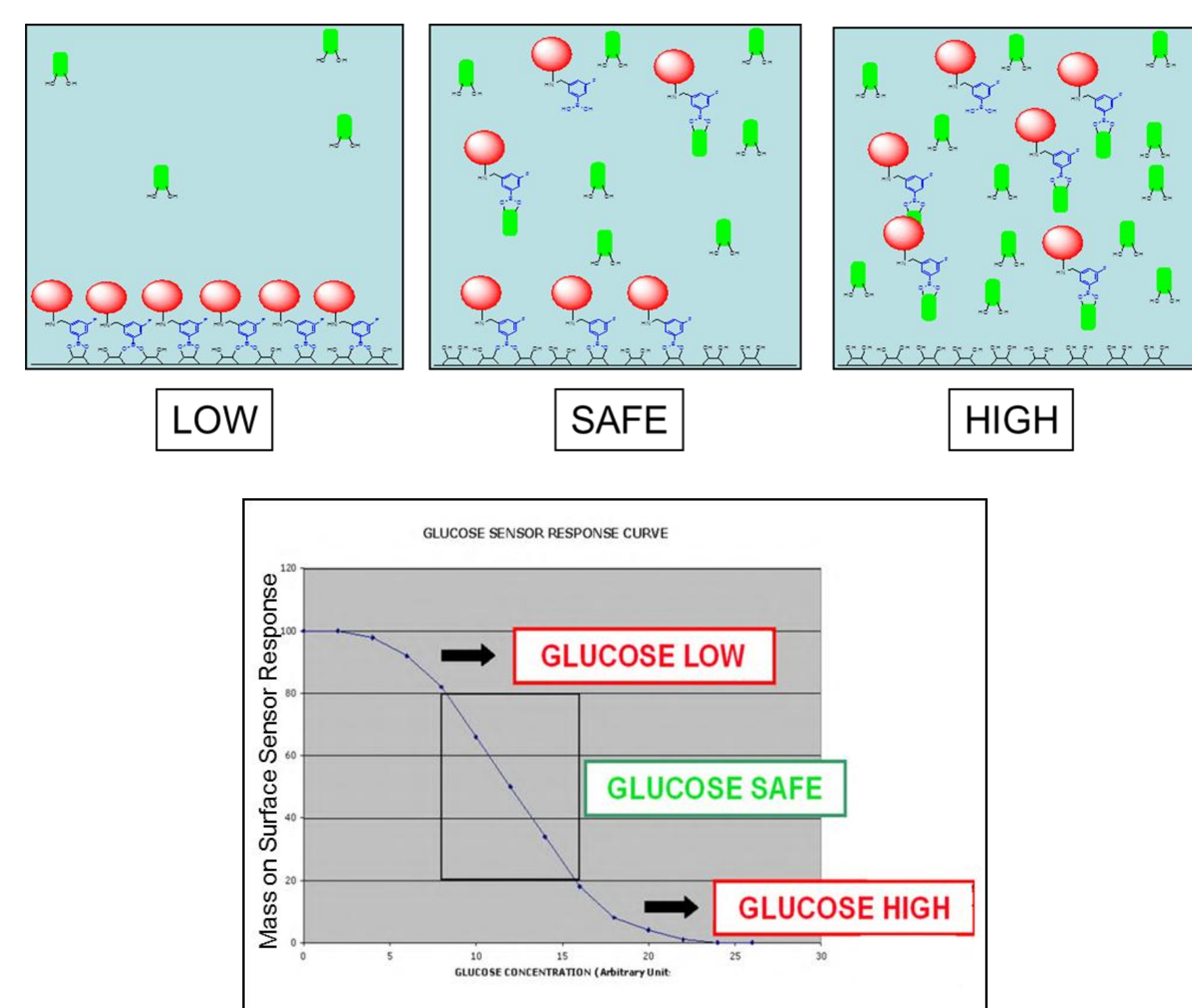


Figure 1. Schematic representing the sensing system's competitive interaction with glucose. The competitive interaction of glucose with the DBA and the iDIOL produced an inverse, proportional signal that is responsive to fluctuating levels of glucose. Due to the higher optimized affinity of the DBA competitor for glucose over that of the immobilized iDIOL, glucose will preferentially displace the DBA from the iDIOL resulting in a mass change on surface and signal.

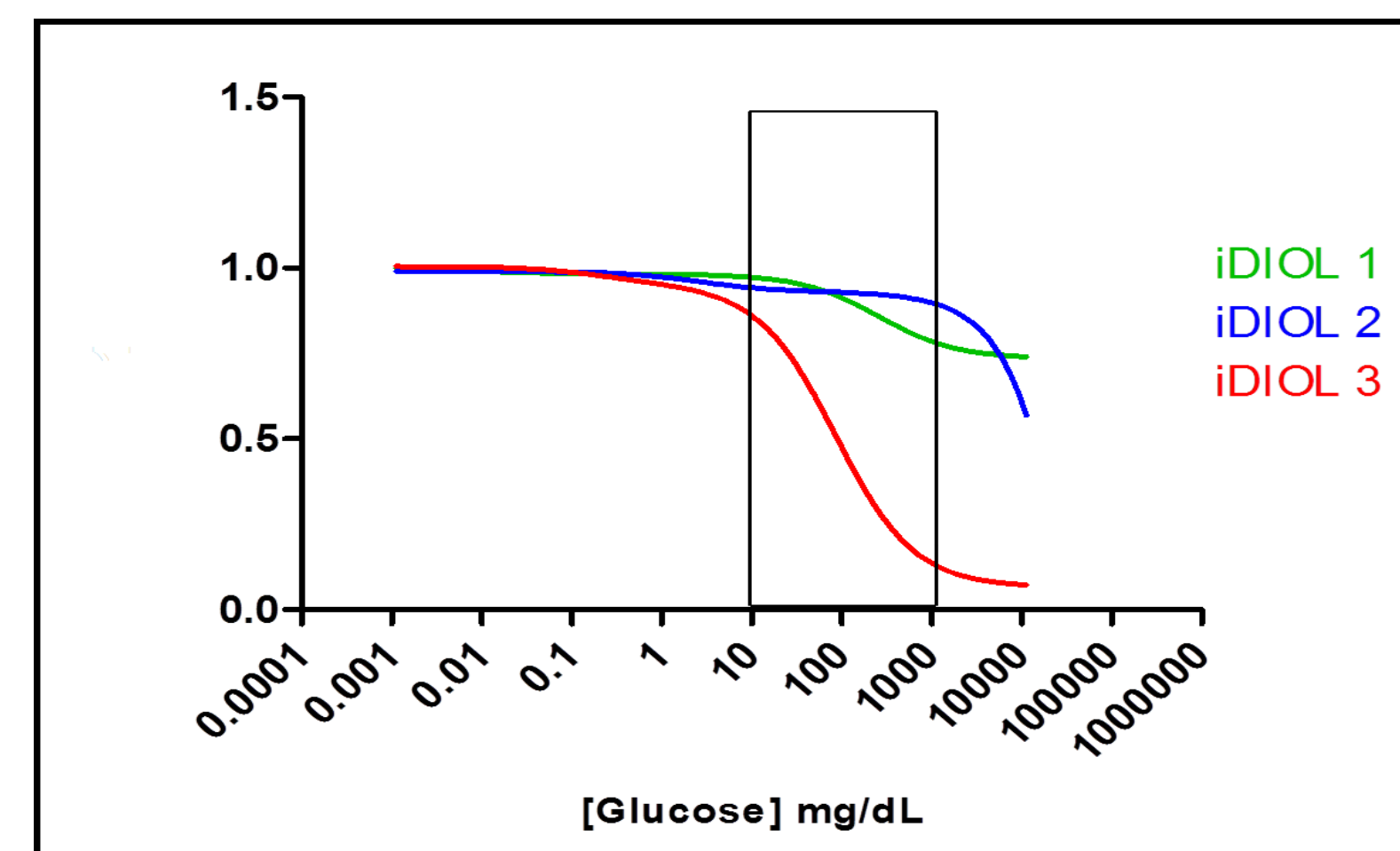
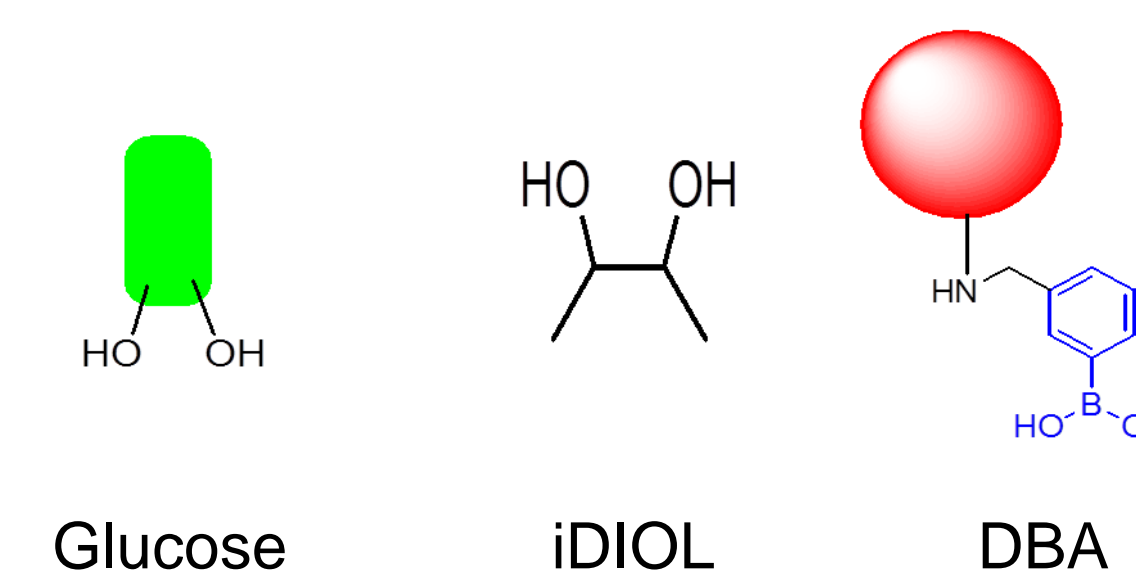


Figure 2. Glucose competition curves comparing the glucose concentration profiles of one DBA on various iDIOL immobilized surfaces.

I_{50} Values: DBA:iDIOL 1 > 100 mg/dL; DBA:iDIOL 2 ~ 10,000 mg/dL; DBA:iDIOL 3 ~ 100mg/dL

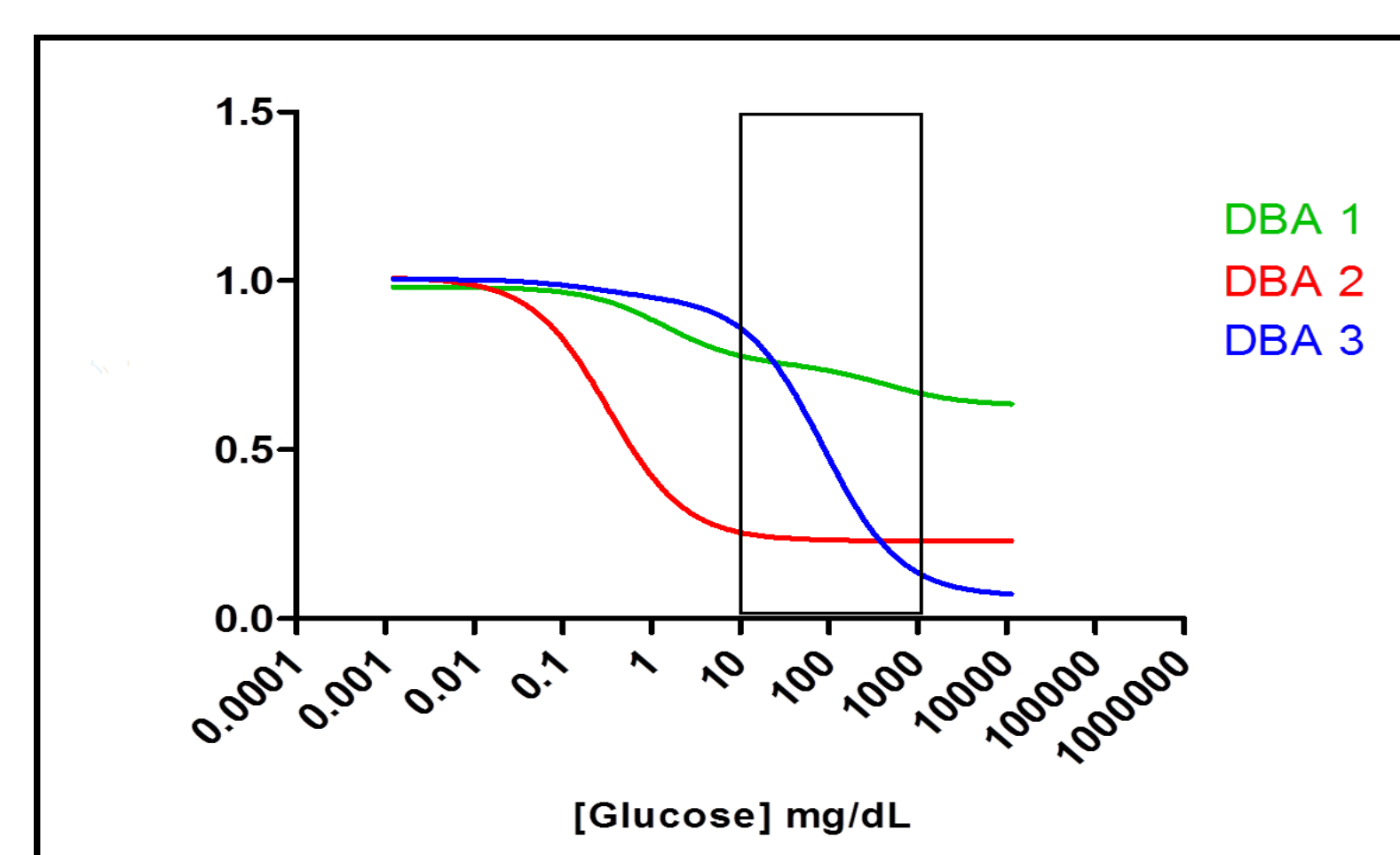


Figure 4. Glucose competition curves comparing the glucose concentration profiles of various DBAs on a iDIOL immobilized surface.

I_{50} Values: DBA 1:iDIOL > 10,000 mg/dL; DBA 2:iDIOL 0.5 mg/dL; DBA 3:iDIOL ~ 100 mg/dL

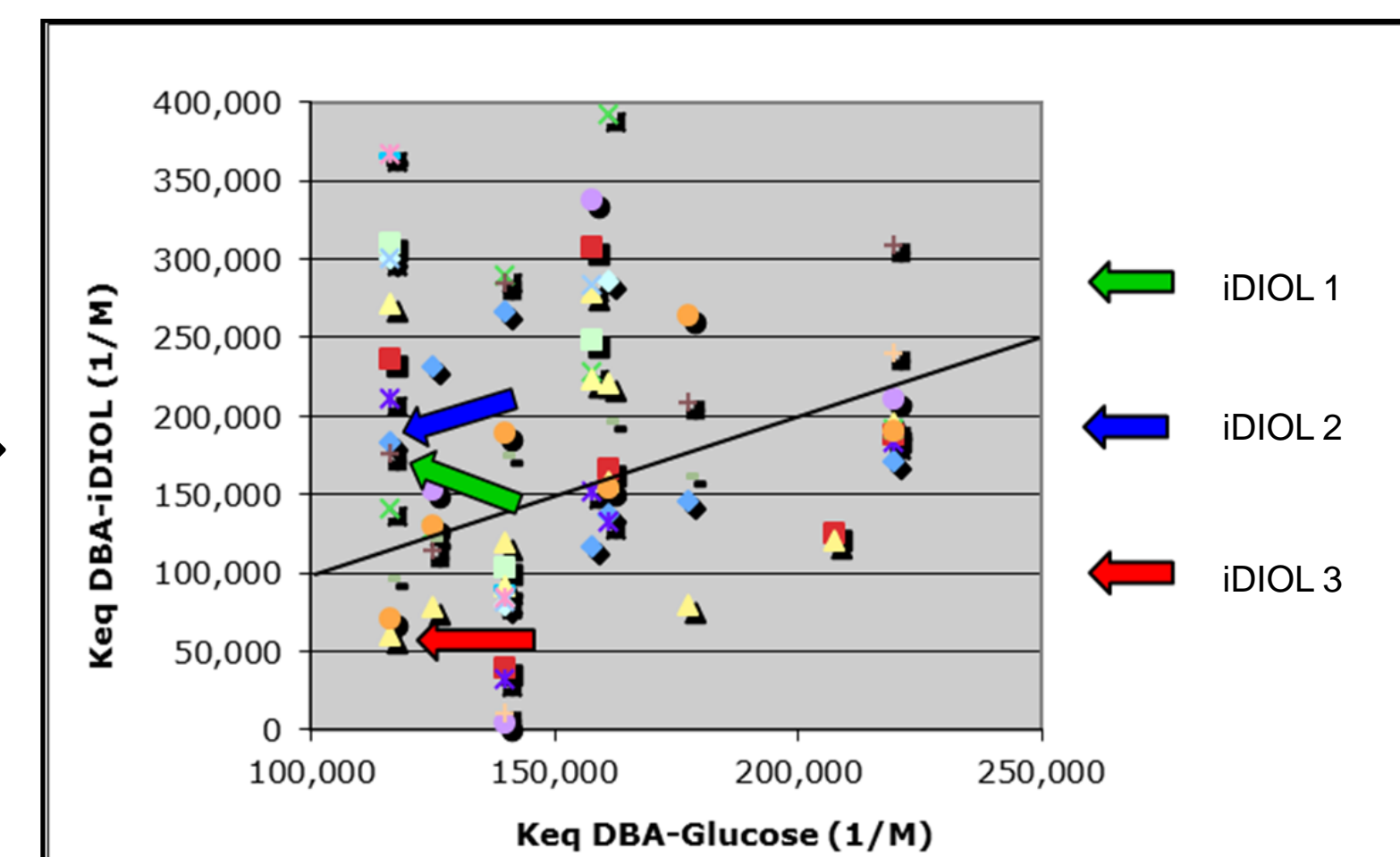


Figure 3. K_{eq} interaction graph comparing DBA-to-Glucose binding affinity (x-axis) to DBA-to-iDIOL binding affinity (y-axis) with respect to the 1:1 K_{eq} interaction line.

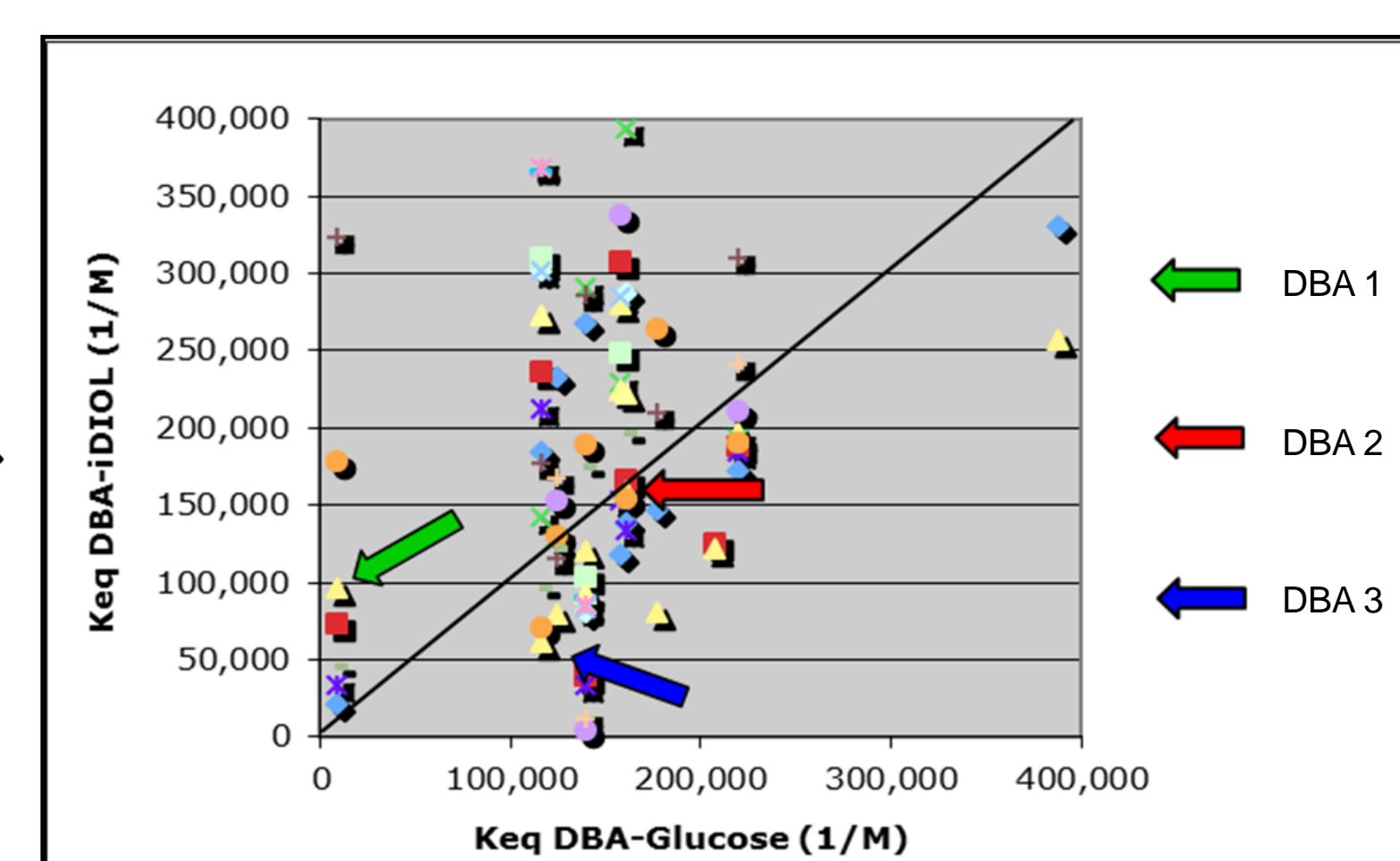


Figure 5. K_{eq} interaction graph comparing DBA-to-Glucose binding affinity (x-axis) to DBA-to-iDIOL binding affinity (y-axis) with respect to the 1:1 K_{eq} interaction line.

Results Continued

process. Glucose competition binding assays were used to screen the performance of each selected DBA:iDIOL pair with glucose. The results show that data extrapolated from the K_{eq} interaction graph streamlined our efforts in identifying which DBA:iDIOL pairs and with what amount of sensitivity they interacted with glucose.

Methods

Glucose Titration Assay and Incubation

A series of mixtures that contained a fixed concentration of the fluorescently labeled DBA with varying amounts of glucose present were incubated in a PBS buffer solution on the iDIOL functionalized surface. The solution was allowed to equilibrate for 5 minutes. Fluorescence spectra were obtained in triplicate.

Conclusions

- A chemical sensing system that can be integrated into an implantable glucose device has been successfully developed
- Proved that K_{eq} constants are capable of predicting what type of competitive response will occur with glucose upon selection of a particular DBA:iDIOL pair
- Demonstrated that the components were not only capable of generating reproducible glucose response curves, but also provided structurally and chemically diverse materials that could be used to optimize the sensitivity of the system response

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References

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