Determination of the Kinetic Rate Constants and K_D of a Small Molecule-Protein Interaction Using Simulject™ Methodology on the BiOptix 404pi

Introduction

Determining the kinetic rate constants (k_a and k_d) and the equilibrium dissociation constants (K_D) for small molecule-protein interactions is an important application for label-free instrumentation in the drug discovery and diagnostic industries. Often, small molecule-protein interactions can be difficult to regenerate on biosensor surfaces. In those cases where no appropriate regeneration condition can be found, alternate biosensor methods of kinetic analysis are required. In this application note, we demonstrate a technique referred to as Simulject that requires no surface regeneration. In a Simulject experiment, two concentrations of analyte are injected simultaneously and independently across two flow cells in a BiOptix 404pi biosensor. Previously, it has been shown that two appropriately chosen concentrations of analyte are all that is required to determine the binding parameters for an interaction (1).

Experimental

Carbonic anhydrase II from bovine erythrocytes (Sigma) was amine-coupled to an average surface capacity of 4100 RU (±1.5%) on two flow cells of a BiOptix CMD200 sensor chip using standard EDC/NHS coupling chemistry with blocking of the remaining active ester groups by ethanolamine. Two reference flow cells were activated and blocked with no immobilized protein. The running buffer was PBS, 0.05% polysorbate 20, pH 7.4. Two concentrations of 4-(aminomethyl)benzenesulfonamide (ABS) (Fluka) were injected in duplicate at 100 µL/min at 20˚C as indicated in Table 1. Five buffer blanks were injected for "warming up" the surface and double-referencing before the ABS injections. Figure 1 illustrates schematically the Simulject experiment.

Table 1

Ligand	Analyte	Analyte Concentrations (µM)	Association (sec)	Dissociation (sec)
CA II	ABS	3.7 (flow cell 3), 100 (flow cell 4)	60	60

BiOptix 404pi biosensor run in 2x2 mode

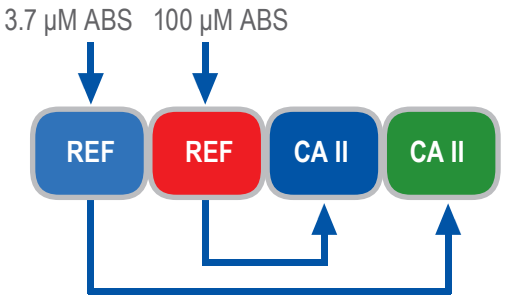

Figure 1 Simulject experimental scheme. ABS was injected simultaneously at 3.7 µM and 100 µM over two independent flow cells at the same surface capacity of CA II.

Results

Data were collected on the BiOptix 404pi instrument and were processed using Scrubber 2.0c (Biologic Software). The data were globally fit from two different flow cells with nearly identical surface capacities of CA II to a 1:1 interaction model as shown in Figure 2. Table 2 shows the fitted k_a, k_d and K_D values by the Simuljet™ method versus a traditional biosensor experiment with five different concentrations of ABS injected.

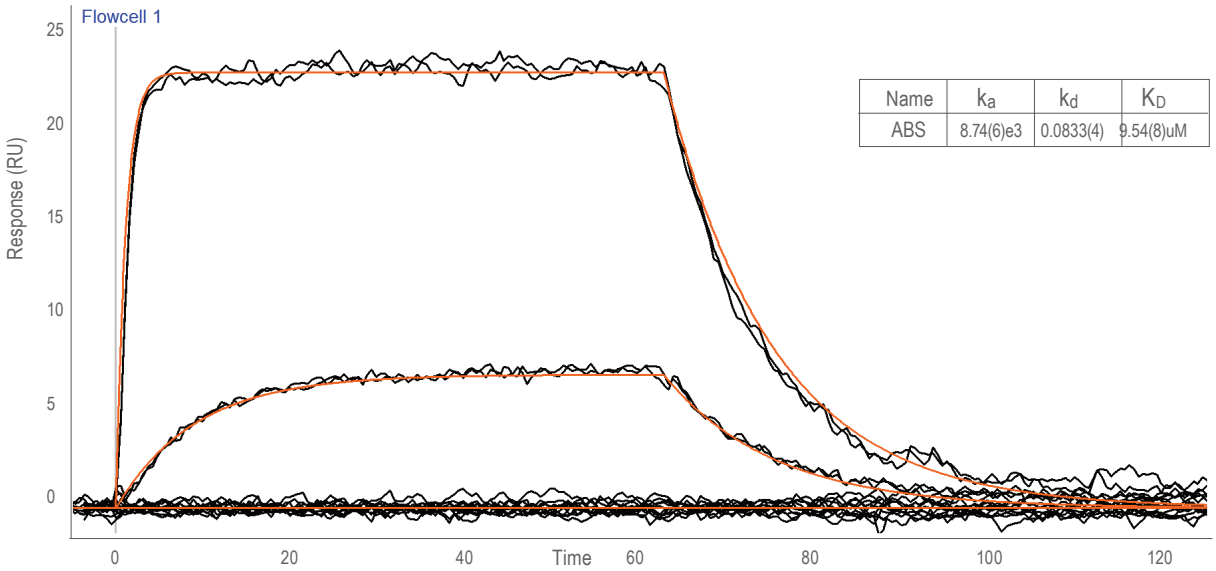

Figure 2 Simulject™ data of ABS binding to CA II. ABS was injected simultaneously at 3.7 µM and 100 µM over two independent flow cells at the same surface capacity of CA II. The data were fit globally (red line) to a simple 1:1 kinetic interaction model over two independent flow cells.

Table 2

Method	Association (k_a)	Dissociation (k_d)	Affinity (K_D)
Simulject - Two ABS concentrations	8.7×10^4 M^{-1} s^{-1}	0.083 s^{-1}	9.5 µM
Conventional - Five ABS concentrations (see reference 2)	9.8×10^4 M^{-1} s^{-1}	0.093 s^{-1}	9.6 µM

Conclusions

Kinetic and K_D data generated using the Simulject method on the BiOptix 404pi biosensor are identical within the 95% confidence intervals reported previously for the ABS/CA II interaction performed with a multiple concentration series using conventional biosensor methodology (2). The Simulject technique is enabled by the unique flow cell architecture present in the BiOptix 404pi biosensor, which allows for simultaneous injections of two different concentrations of analyte over independent flow paths. Simulject offers an accurate and precise alternative for measuring the binding kinetics and K_D of molecular interactions in which no regeneration condition can be found.

References

(1) Onell, A. and Andersson, K. (2005) J Mol. Recognit. 18: 307-317.
(2) BiOptix Application Note, “Label-Free Detection of Small Molecule-Protein Interactions.”