



## Determining kinetics and affinities of protein interactions using a parallel real-time label-free biosensor, the Octet

Yasmina Abdiche\*, Dan Malashock, Alanna Pinkerton, Jaume Pons

Rinat Laboratories, Pfizer Inc., South San Francisco, CA 94080, USA

### ARTICLE INFO

#### Article history:

Received 10 January 2008

Available online 25 March 2008

#### Keywords:

Biacore

Octet

ProteOn

Protein/Protein interaction

Affinity

### ABSTRACT

ForteBio's Octet optical biosensor harnesses biolayer interferometry to detect and quantify molecular interactions using disposable fiber-optic biosensors that address samples from an open shaking microplate without any microfluidics. We recruited a monoclonal antibody against a panel of peptides to compare the Octet directly with Biacore's well-established 3000 platform and Bio-Rad's recently launched ProteOn XPR36 array system, which use surface plasmon resonance (SPR) to detect the binding of one analyte over four surfaces and six analytes over six surfaces, respectively. A sink method was used to prevent analyte from rebinding the ligand-coated Octet tips and enabled us to extract accurate kinetic rate constants, as judged by their close agreement with those determined by SPR. Although the Octet is not sensitive enough to detect the binding of small molecules directly, it can access their affinities indirectly via solution competition experiments. We conducted similar experiments on the SPR instruments to validate these measurements. The Octet is emerging as a versatile complement to other more sophisticated biosensors, and the ProteOn provides high-quality data near the sensitivity of Biacore but in a more multiplexed format. Our results provide a benchmark for assessing the performance of the above-mentioned sensors.

© 2008 Elsevier Inc. All rights reserved.

Cutting-edge biosensors are revolutionizing our understanding of biomolecular interactions and are established as routine biophysical tools in research laboratories worldwide. Until recently, Biacore AB (now part of GE Healthcare) had been the only significant vendor of commercial biosensors due in part to its patent surrounding the use of a hydrogel matrix for monitoring interaction analysis by exploiting surface plasmon resonance (SPR)<sup>1</sup> and its expanding repertoire of customized platforms. Recently, however, other companies have emerged with complementary technologies that differ in detection, sample delivery, and/or throughput [1]. One such platform is the Octet, commercialized by ForteBio (Menlo Park, CA, USA). This simple dip-and-read assay harnesses biolayer interferometry incorporated onto inexpensive disposable optical fiber biosensors to measure a parallel set of eight interactions from an open shaking plate (Fig. 1). Thus, the sensors move to the samples, rendering unnecessary the microfluidics that traditionally deliver samples to a stationary Biacore sensor chip. By handling samples on single-use tips without microfluidics, the Octet offers a unique

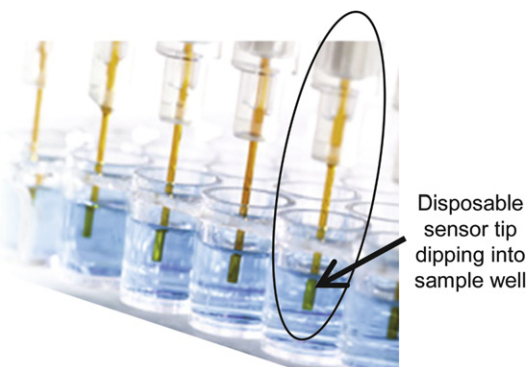
platform with significant advantages over other biosensors that pertain to the way in which the assay can be configured. For example, ligands can be coupled onto tips offline in large batches and do not need to be regenerated. If samples are sufficiently stable under experimental conditions, they can be reused within the assay or recovered for use in other assays, and this is particularly appealing when they are precious. Furthermore, clogging is not an issue on the Octet, and virtually no maintenance is required on the unit.

We report on how the Octet can be used to quantify the kinetics and affinities of protein interactions and validate these measurements by comparing them directly with those collected on SPR-based biosensors, namely the recently released parallel ProteOn XPR36 array and the widely used serial flow Biacore 3000 platform, which we consider our standard. We address three main themes. First, we establish that the direct binding of small molecules such as peptides to ligands on the tip is beyond the sensitivity of the Octet, limiting its use to the study of large analytes (generally > 50 kDa in molecular mass depending on the specifics of the interaction). Second, we demonstrate that the Octet can return accurate kinetic rate constants for large analytes through the use of a sink method that abolishes any rebinding of the analyte to ligand-coated tips, a strategy that has been commonly employed for years in other well-based systems such as the IAsys technology [2]. Third, we highlight how solution competition experiments on all three systems confirm the affinities measured by direct binding.

\* Corresponding author. Fax: +650 615 7350.

E-mail address: [yasmina.abdiche@pfizer.com](mailto:yasmina.abdiche@pfizer.com) (Y. Abdiche).

<sup>1</sup> Abbreviations used: SPR, surface plasmon resonance; CGRP, calcitonin gene-related peptide; rCGRP $\alpha$ , rat CGRP- $\alpha$ ; hCGRP $\alpha$ , human CGRP- $\alpha$ ; Mes, 2-(N-morpholino)ethanesulfonic acid; EDTA, ethylenediaminetetraacetic acid; HBS-EP, Hepes-buffered saline EDTA polysorbate P20; EDC, N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide; NHS, N-hydroxysuccinimide; BSA, bovine serum albumin.



**Fig. 1.** The Octet's dip-and-read well-based format. An isolated view of an individual sensor is circled. Samples are handled without any microfluidics because a row of up to eight sensors moves to the samples held in an open shaking microplate. The actual plate used in the instrument is black. Reproduced with permission from ForteBio Inc., Menlo Park, CA, USA.

For the purpose of illustrating these applications, we adopt a model system, namely the interactions of a murine monoclonal antibody called "4901" with its antigen, the calcitonin gene-related peptide (CGRP). This interaction was chosen because the reagents are commercially available, they regenerate well, and their affinities fall within a measurable range. Furthermore, CGRP is implicated in migraine and other types of pain, and interfering with its biological activity is of therapeutic interest, rendering this model system relevant from a drug discovery perspective [3]. Raised against a C-terminal epitope of rat CGRP- $\alpha$  (rCGRP $\alpha$ ), antibody 4901 also binds other isoforms [4], and we include full-length and truncated forms of human CGRP- $\alpha$  (hCGRP $\alpha$ ) in this study. The four peptides studied here varied in molecular mass from 609 to 3806 Da and bound 4901 with a 1000-fold range in affinity. Our results demonstrate that the Octet and ProteOn can return kinetic rate constants and binding affinities as accurately as the well-established Biacore 3000. The main limitation of the Octet is its sensitivity, meaning that only certain assay orientations are possible. The results of our study can be used as a benchmark to test the performance of any or all of the above-mentioned instruments.

## Materials and methods

### Materials

Octet QK equipped with amine reactive (AR) and streptavidin (SBC or FA) biosensor tips and coupling buffer (100 mM 2-(*N*-morpholino)ethanesulfonic acid (MES), pH 5.0) were purchased from ForteBio. Biacore 3000 equipped with CM5 and streptavidin sensor chips, HEPES-buffered saline ethylenediaminetetraacetic acid (EDTA) polysorbate P20 (HBS-EP) buffer (10 mM HEPES (pH 7.4), 150 mM NaCl, 3.4 mM EDTA, and 0.005% (v/v) polysorbate P20), and coupling reagents (10 mM sodium acetate (pH 5.0), *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide (EDC), *N*-hydroxysuccinimide (NHS), and 1 M ethanolamine-HCl (pH 8.5)) were purchased from Biacore (Uppsala, Sweden, now part of GE Healthcare, Piscataway, NJ, USA). ProteOn XPR36, GLM and neutravidin (NLC) sensor chips, and coupling reagents (sulfo-NHS and EDC) were purchased from Bio-Rad (Hercules, CA, USA). Monoclonal anti-CGRP antibody 4901 (cat. no. C7113) produced in mice and polyclonal anti-rat CGRP (cat. no. C8198) produced in rabbit were purchased from Sigma Chemical (St. Louis, MO, USA). rCGRP $\alpha$  (1–37 and 19–37) and hCGRP $\alpha$  (1–37, 1–19, 26–37, and 32–37) were custom-ordered from Global Peptide (Fort Collins, CO, USA) amidated at the C terminal. Full-length (1–37) peptides were also purchased modified at their N terminal with LC-biotin. The following immuno-

pure-grade reagents were purchased from Pierce (Rockford, IL, USA): streptavidin (cat. no. 21122), Gentle IgG Elution Buffer (cat. no. 1851520), and Fab Preparation Kit (cat. no. 44885). The Fab kit was used as directed to digest full-length 4901 antibody into Fab fragments using papain resin, and the product was confirmed to be more than 90% pure by standard SDS-PAGE under reducing conditions.

### Standard experimental conditions

All interaction analyses were conducted at 25 °C in HBS-EP running buffer unless stated otherwise. Temperature control on the Octet was accomplished by holding the instrument in a 22 °C temperature-controlled room so that it could be heated to 25 °C. Sensor tips were prewet for 5 min in buffer immediately prior to use, and the microplates used in the Octet were filled with 200  $\mu$ l of sample or buffer per well and agitated at 1000 rpm.

### Direct binding of CGRP to amine-coupled 4901 on tip

Amine reactive AR tips were prewet in 0.1 M MES buffer (pH 5.0), which served as the background buffer for the immobilization. This involved establishing a stable baseline (5 min), activating the sensors with a freshly mixed solution of 0.2 M EDC + 0.05 M NHS (5 min), coupling 100  $\mu$ g/ml 4901 (15 min), and then blocking excess reactive esters with ethanolamine (5 min). Final immobilization levels were  $2.5 \pm 0.1$  nm (full-length IgG) and  $2.0 \pm 0.1$  nm (Fab fragment) within a row of eight tips. A new baseline was established in HBS-EP + 1 mg/ml bovine serum albumin (BSA) (5 min) that provided the running buffer for all subsequent binding steps. rCGRP $\alpha$  was prepared as a serial dilution (0, 1.2, 3.7, 11, 33, and 100 nM) and allowed to bind the 4901-saturated tips for 15 min. This was followed by the binding of polyclonal anti-rCGRP $\alpha$  (15 min) at a single concentration that corresponded to a sevenfold dilution of the commercial stock. The full-length 4901 IgG and the 4901 Fab were compared in the same experiment by coupling each onto its own row of tips.

### Optimizing sink conditions on the Octet

Streptavidin-coated FA tips were saturated with 20  $\mu$ g/ml N-biotinylated full-length CGRPs (5 min). Typical capture levels were  $0.42 \pm 0.06$  nm within a row of eight tips, with the standard deviation being within instrument noise. Then 100 or 300 nM 4901 Fab was bound to rCGRP $\alpha$  or hCGRP $\alpha$  for 15 min and allowed to dissociate for 1 h into buffer spiked with a threefold concentration gradient of a competing peptide (0, 1, 3, 9, 27, 81, and 243  $\mu$ M rCGRP $\alpha$  19–37) or an irrelevant peptide (100  $\mu$ M hCGRP $\alpha$  1–19). Dissociation buffer was used only once to ensure its potency. Blank binding cycles containing no Fab were used to correct for baseline drift.

### One-shot kinetics by Octet

FA tips were saturated with 20  $\mu$ g/ml N-biotinylated full-length CGRPs (5 min). The 4901 Fab was prepared as a five-membered threefold serial dilution with a highest concentration of 100 or 300 nM to study the interactions with rCGRP $\alpha$  or hCGRP $\alpha$  on the tip. A duplicate of the middle Fab concentration and two buffer blanks completed a row of eight samples, which were allowed to associate for 15 min. Dissociation was measured for 1 h into buffer spiked with 100  $\mu$ M rCGRP $\alpha$  19–37 to ensure optimal sink conditions as described above. The analysis was sometimes repeated on the same set of tips by reusing them if the binding signal returned to baseline at the end of the dissociation phase (hCGRP $\alpha$  on tip) or regenerating them if it did not (rCGRP $\alpha$  on tip). When

Download English Version:

<https://daneshyari.com/en/article/1176342>

Download Persian Version:

<https://daneshyari.com/article/1176342>

[Daneshyari.com](https://daneshyari.com)