Faster interaction measurements using MP-SPR KineticTitration

KineticTitration significantly reduces time required to run an assay with different concentrations. It is also useful for interactions that are difficult to regenerate or when regeneration damages the ligand on the surface. In the measurement, analyte samples are flown over the surface in a series from low to high concentration, without dissociation and regeneration between the samples as required in other methods. The dissociation rate is measured after the last analyte sample.

Here, KineticTitration was utilized to measure binding of small molecular weight drug (tolcapone, 273 Da) to an immobilized protein (human serum albumin, HSA). The results revealed two different binding sites, first with $K_D = 9.7 \times 10^{-7}$ M, $k_a = 1.99 \times 10^3$ $(M*s)^{-1}$ and $k_d = 1.93 \times 10^{-3}$ s^{-1} and second with $K_D2 = 1.9 \times 10^{-6}$ M, $k_a2 = 1.41 \times 10^2$ $(M*s)^{-1}$, $k_d2 = 2.67 \times 10^{-4}$ s^{-1}. The dissociation of Tolcapone from HSA is slow, so the typical multi-step measurement cycle would have taken 3 times more time than using KineticTitration.

Introduction

Surface Plasmon Resonance (SPR) is a popular real-time label-free method to assess biomolecular interactions especially in the fields of drug discovery and biosensor development.

Multi-Parametric Surface Plasmon Resonance (MP-SPR) scans a broad angular range and records whole SPR curve. Hence from the same measurement, MP-SPR provides more information about changes at the surface than traditional SPR. Additionally, MP-SPR records also bulk (solvent) effect simultaneously with the binding signal in every channel. Thanks to the PureKinetics™ feature, bulk effect can be separated from molecular binding events in real-time, providing more reliable results and enabling measurements, which are not possible using traditional SPR instruments. PureKinetics™ enables measurement of kinetics in altering dissociation buffers (Application Note #147) or when big refractive index changes occur between running buffer and the samples (even 5% DMSO can be measured).

To quantify affinity and kinetic parameters of the biomolecular interaction, binding at not less than two concentrations of the analyte needs to be measured. Concentrations need to be in the range close to the dissociation constant (K_D), and usually a few more concentrations are required, as affinity is typically unknown. Conventionally, the dissociation rate is measured after each analyte concentration. Additionally, if dissociation is slow, the remaining part of the bound analyte is removed using a run of regeneration solution between the sample injections. Ideal regeneration breaks the bonds between the ligand and the analyte without deactivating the molecules on the surface. However, such regeneration is sometimes difficult to achieve and also the ligand is damaged.

The KineticTitration option allows the injection of samples sequentially and the dissociation is measured only at the end of the whole run. This shortens the time required for the whole measurement cycle (Figure 1).

KineticTitration is especially useful when:

- results are needed quickly
- there is less time available for assay optimization (such as for finding a suitable regeneration solution)
- surface is difficult or impossible to regenerate without losing the activity of the ligand

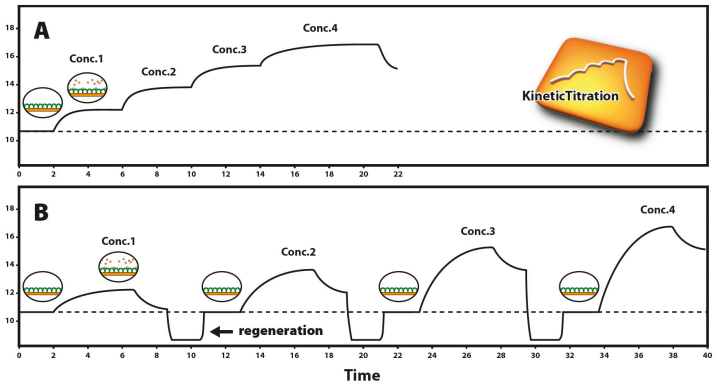

Figure 1. Analyte binding on a ligand surface measured using (A) KineticTitration and (B) conventional multi-cycle measurement. KineticTitration measurements do not require dissociation and regeneration steps between sample injections, thus reducing time required for the whole measurement by half (depending on assay and number of concentrations).

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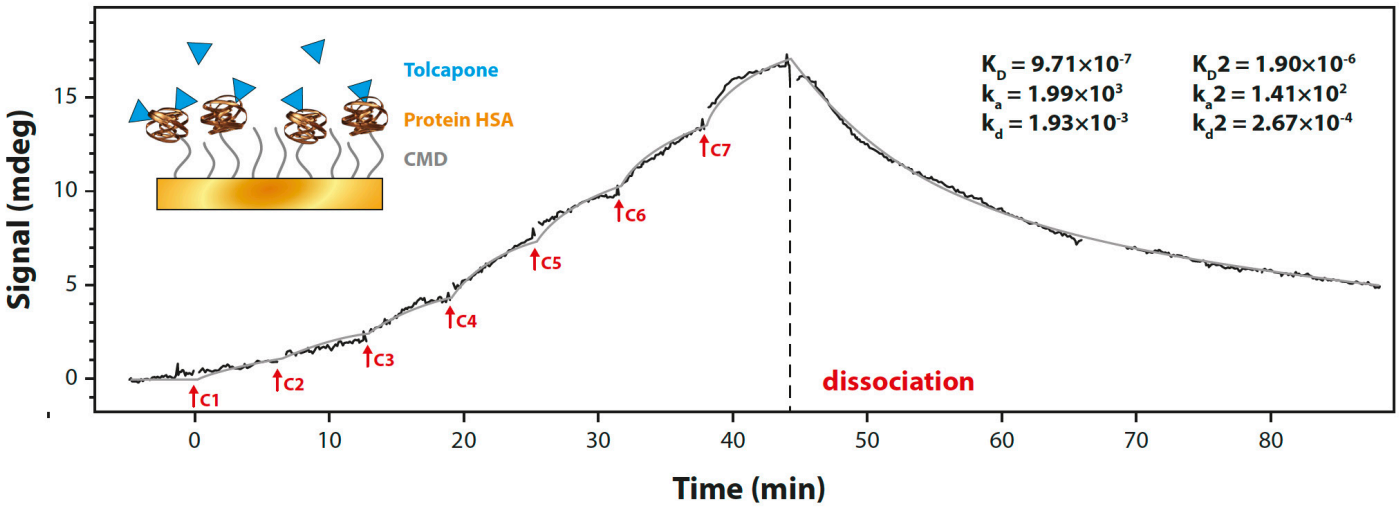

Figure 2. Small molecular weight drug tolcapone (7 concentrations) binding to human serum albumin (HSA) measured using KineticTitration. Measured data was fitted using TraceDrawer™ software to determine affinity and kinetic constants. Dissociation rate was measured after the last sample. The biomolecular interaction is a two state binding event.

Materials and methods

Human Serum Albumin (HSA) was immobilized on a Carboxymethyldextran (CMD) sensor surface using amino coupling chemistry – a mixture of NHS and EDC (NHS = N-hydroxysuccinimide, EDC = N-[3-dimethylaminopropyl]-N-ethyl-carbodiimide hydrochloride). After protein injection, the surface was deactivated with 1 M ethanolamine. Seven samples of tolcapone (273 Da) were injected from lowest to highest concentration in the range of 0.25 – 25 µM. Each sample injection lasted 6 minutes before the next sample was introduced. The dissociation rate was measured after the last (highest) concentration. Data was analyzed using TraceDrawer™ for MP-SPR Navi™ to determine affinity and kinetic constants of the interaction.

Results and discussion

Tolcapone binding to HSA was determined using the fully automated 4-channel MP-SPR Navi™ 420A ILVES instrument in KineticTitration mode. Interaction measurement revealed two binding sites for tolcapone in the HSA protein (Figure 2).

Conclusions

KineticTitration feature of MP-SPR Navi™ 420A ILVES allows faster measurements of molecular interactions when compared to other SPR instruments. In KineticTitration measurement, samples are introduced without requiring any dissociation and regeneration steps between the different concentrations of one analyte.

MP-SPR with KineticTitration is a time-saving solution for biomolecular interaction measurements. It is also a solution for assays with regeneration-sensitive-ligand or when regeneration is not known. KineticTitration can also be combined with PureKinetics™ making it an exceptionally powerful combo for drug discovery and biosensor development.

See also how MP-SPR was utilized to measure drug delivery nanoparticles interactions (Application Note #152) and small molecules interactions with living cell monolayer (Application Note

Recommended instrumentation for reference assay experiments

MP-SPR Navi™ 420A ILVES

Sensor surface: Carboxymethyl dextran (CMD)

Software: MP-SPR Navi™ Controller, DataViewer and TraceDrawer™ for MP-SPR Navi™

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