FORTÉBIO

The next generation of SPR-based interaction analysis

Pioneer and Pioneer FE systems

Pioneer SPR systems from ForteBio employ a novel gradient injection technology that combines the accuracy and sensitivity of traditional SPR analysis with the benefits of simpler assay set-up, shorter run times, reduced sample consumption, and higher throughput. Next Generation SPR is based on the well-established concept of Taylor dispersion. Pioneer instruments precisely deliver analyte sample to the SPR flow cell in a continuous concentration gradient using an innovative sample dispersion and delivery system. A wide range of concentrations is covered in a single injection, eliminating the need to run multiple dilutions of sample to obtain accurate kinetic and affinity constants. OneStep® and NeXtStep™ gradient injections are two examples of how Next Generation SPR can improve data quality and reduce the time to both develop and run assays.

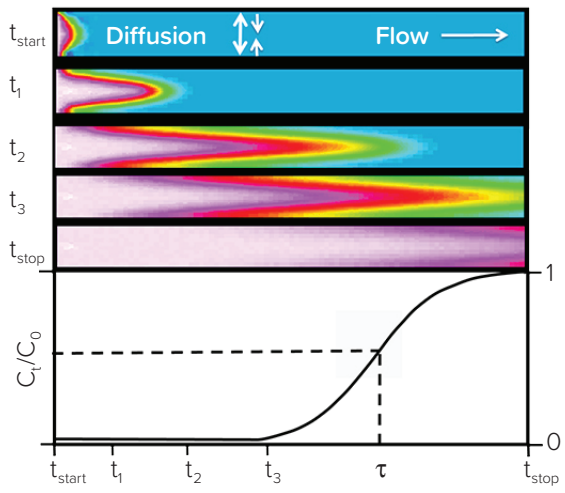

Figure 1: OneStep gradient formation in the Pioneer injection line (top) and analyte concentration measured at the SPR flow cell (bottom). Blue indicates buffer and pink indicates sample. The gradient formation and its relationship to analyte concentration at the flow cell is illustrated using five simulated snapshots (t_{start} – t_{stop}) of the injection line at different times.

OneStep Injections

A OneStep gradient injection disperses analyte in the sample through an injection line filled with buffer in the Pioneer fluidics *en route* to the SPR flow cell. This method, based on Taylor dispersion, produces a sigmoidal concentration gradient of analyte in the injection line. As the sample gradient flows over the sensor, binding data is collected in real time, incorporating the full range of analyte concentrations presented to the surface, from low to high. Figure 1 shows the injection line and the analyte concentration at the SPR flow cell:

IMPROVE KINETIC CHARACTERIZATION

Traditional SPR kinetic characterization relies on analyzing the time-resolved binding of an interaction over multiple concentrations of analyte. The OneStep gradient produces different analyte concentrations over time, in a single injection — eliminating the need for a dilution series of analyte and requiring only one sample and one injection to characterize the kinetics of an affinity interaction. This:

- Saves time and sample material
- Reduces the variance introduced by pipetting multiple samples and target immobilization among different channels
- Improves analysis of unstable targets which need to be tested quickly before all activity is lost

A OneStep Injection's high-resolution concentration gradient also enables more accurate analyses as compared to the traditional multi-concentration injections needed with traditional SPR. OneStep gradients can test analyte concentrations across 3–4 orders of magnitude, encompassing thousands of concentration data points. This heightened resolution is especially important for complex or heterogeneous interactions and is helpful in identifying non-specific or promiscuous interactions.

Analyte diffusion coefficients can also be determined from a OneStep analysis. Diffusion can offer additional insight into the binding analysis, as the solution behavior of the analyte and formation of aggregates or higher order species can have an impact. This information is not accessible with traditional SPR measurements.

REDUCE ASSAY DEVELOPMENT TIME

Traditional SPR assay development is cumbersome and begins with finding a suitable method to immobilize the target molecule and then finding optimal conditions to observe binding of the analyte. An analyte binding test is often performed to determine activity of the target and to catch a glimpse of the binding kinetics between analyte and target.

OneStep Injections simplify assay development by performing a full kinetic characterization at the time of the binding test. When a mid to high concentration of analyte analyzed with a OneStep Injection, a concentration gradient is produced that allows for the identification of optimal conditions as well as characterization of the interaction under the present conditions. Even if the maximum concentration used on the Pioneer system is high enough to saturate the binding interaction, the OneStep gradient typically introduces low enough concentrations to accurately determine binding affinities.

Time consuming DMSO corrections are also alleviated. Traditional SPR requires at least six DMSO standards to produce a calibration curve. The refractive index correction to account for varying concentrations of DMSO in samples can be performed with OneStep Injections on Pioneer systems with just two standards. It's also more amenable to repeat calibrations to correct for changing sensor compositions (decaying protein surface, build-up of non-specific analytes, etc.).

WORK WITH DIFFICULT SAMPLES

Unstable or unregenerable targets. The immobilization of biomolecules on SPR biosensors is a common pain point, as it can lead to destabilized molecules or the requirement to regenerate the immobilized molecule after analyte binding. A OneStep Injection's more rapid measurement can accommodate rapidly decaying immobilized molecule activity, and unregenerable molecules benefit since it does not require multiple analyte injections and regeneration of the immobilized molecule.

Complex interactions. Heterogeneity either on the part of the analyte or the immobilized target can be difficult to resolve in SPR assays. The heterogeneity in binding results in response curves that display the simultaneous binding of more than one event — *e.g.* two different forms of analyte to one target, one analyte to two different forms of target, etc. When assay development does not eliminate the complexity, deconvoluting these multiple events with traditional SPR requires testing up to 10 analyte concentrations to accurately determine more than one set of kinetic parameters (k_a, k_d, R_{max}). A single OneStep injection has the analytical resolution to deconvolute and analyze multiple kinetic parameter sets because the analyte concentration is a function of time, providing thousands of analyte concentrations in one gradient injection.

ANALYZE A WIDER RANGE OF MOLECULES

The molecular weight of analytes is not limited with the OneStep method. It has been successfully used to analyze interactions of small molecule fragments (≥70 Da), small molecule compounds (100–400 Da), DNA/RNA oligonucleotides, lipids, peptides, proteins, protein oligomers, and aggregate species.

AFFINITY AND KINETICS DATA FROM PRIMARY SCREENS

In screening fragments, OneStep Injections have been demonstrated to obtain reliable affinity (K_D) and kinetics (k_a, k_d) data directly from the primary screen, combining the first three steps (initial screen, primary yes/no screen, affinity K_D screen) in the traditional SPR workflow into one step. The Pioneer FE system can process a complete screen from new library to characterized hits in less than 1 week, compared to 2.5 weeks for a traditional 4-channel SPR system. Identification of promiscuous binders is also easier with OneStep Injections, as the gradient resolves linear and super-stoichiometric binding events.

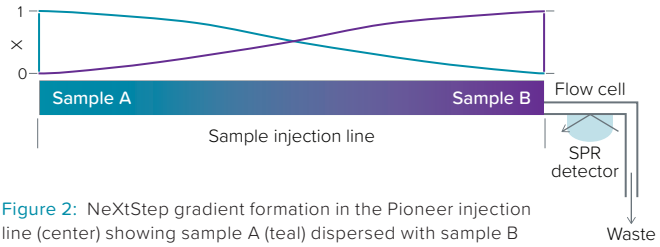

Figure 2: NeXtStep gradient formation in the Pioneer injection line (center) showing sample A (teal) dispersed with sample B (purple) and the gradient approaching the flow cell and detector.

NeXtStep Injections

NeXtStep Injections are another type of gradient injection where two samples are dispersed with one another, producing a crossed (as sample B concentration decreases, sample A concentration increases respectively) sigmoidal concentration profile (Figure 2). This is distinct from OneStep Injections, which disperse one sample with buffer. The two different samples injected by NeXtStep can then be used for competition and inhibition gradient assays.

INCREASE SPEED FOR COMPETITION/INHIBITION ASSAYS

When screening for competition and inhibition, NeXtStep Injections provide increased speed and decreased sample consumption compared to traditional SPR assays. NeXtStep Injections require only one sample per competition/inhibition analyte (compared to eight samples with traditional SPR) and is nearly five-fold faster *per analyte* than a comparable SPR assay. NeXtStep methods employ multiple assay formats to determine competition mechanisms or inhibition concentrations (IC50) in a single injection. There is no need for a sample dilution series as the NeXtStep gradient measures a wide concentration range encompassing all concentrations necessary for analysis.

RELIABLE DATA

Taylor Dispersion is a well-established effect in fluid mechanics that has been accepted for over half a century. The OneStep method and application was first published in 2012[1], and since then, users of the technology have published their results in numerous peer-reviewed journals.

References

1. Modeling Taylor Dispersion Injections: Determination of Kinetic/Affinity Interaction Constants and Diffusion Coefficients in Label-Free Biosensing, Quinn JG, *Anal. Biochem.*, 421(2) 391–410, 2012.

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