TECHNICAL BRIEF

SensíQ Technologies' OneStep® Gradient Injection Technology Has Changed How Fragment Screening is Performed – So What is the NeXtStep?

Aaron Martin and
Eric L. Reese Ph.D.

Pioneer with NeXtStep Injection technology has the unique ability to determine full kinetics and affinity in the presence of a competitor molecule from a single SPR injection.

Introduction:

The detection and characterization of fragment binding events is dependent upon sensitive biophysical technologies capable of detecting low affinity interactions of low molecular weight compounds. However, standard SPR biosensors are limited in the processing and subsequent data analyses of fragment binding events. The Pioneer FE SPR system provides rapid affinity data (K_D) from primary screening alone thus enabling prompt identification of fragment candidate actives. Using a combination of dynamic injection SPR (diSPR®) and actives selection software, the Pioneer FE is now the "go-to" instrument for real-time, label-free identification and characterization of fragment actives in FBDD screening campaigns.

diSPR® is SensíQ Technologies' unique portfolio of next generation injection techniques available only on Pioneer. At the core of diSPR® is the OneStep® injection methodology. OneStep® is a continuous analyte titration method that provides reliable affinity measurements in a single injection suitable for rapid screening in both direct and competitive binding formats.

Why Use OneStep® for Fragment Screening??

- Make decisions early on. Reliable affinity data (K_D) and kinetic data (k_a, k_d) directly from primary screen.
- Fast time to first result. Fully automated, requiring minimal assay development to arrive at the correct fragment candidate(s) quickly.
- Optimization of workflows. Reliable, automated identification of lead fragment candidates while simultaneously eliminating those candidates that will eventually fail.
- Get the same results each time: An objective approach to the identification of fragment actives.
- More confidence in fragment actives selection due to best-in-class, industry-proven, actives selection software.
- High throughput: Up to 768 samples in 24 hours.

Shortcomings of Fixed Concentration SPR Instrumentation:

SensíQ Technologies' patented gradient injection methods provides K_D's from single injections, allowing SensiQ instruments to eliminate secondary screens such as our competitor's "Affinity Screen". No other SPR providers have gradient injection technology. To compensate for this shortcoming, alternative SPR vendors will suggest that other features may be needed to produce a robust fragment screen, such as increased sensitivity. However, SensiQ Pioneer FE customers have demonstrated that it is the combination of our excellent sensitivity, OneStep® gradient injections and the integrated Active Selection software that provides superior results, not sensitivity alone.

OneStep® gradient injection technology has set a new standard for SPR-based fragment screening. So what is the NeXtStep to further enable the prompt and accurate determination of fragment actives?

The NeXtStep in FBDD, Competition Assays:

Competition assays are very useful in drug discovery, yielding the ability to find active site binders directly by competing fragment hits with a control molecule. SensiQ has developed a new binding competition assay method for rapidly screening fragments, termed NeXtStep. Using a rapid dispersion injection (similar to OneStep®) two sample components are dispersed and injected over a biosensor surface. Figures 1 and 2 show the experimental set of a NeXtStep competition assay. In summary, a two-part experiment design is demonstrated where fragments are NeXtStep injected in the presence and absence of control molecule. Blank NeXtStep injections then consist of either buffer only or control analyte only to subtract the respective background signal. Like OneStep, NeXtStep enables full kinetic analysis in a single injection and site-specific competition is clearly seen as a modulation of binding in the presence of the competitor molecule.

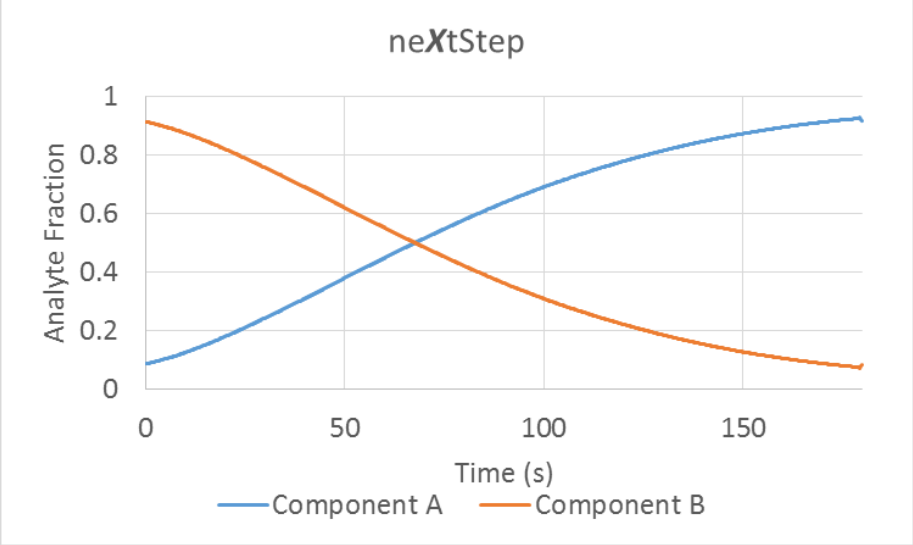

Figure 1: (NeXtStep illustration) NeXtStep rapidly disperses two analyte components forming a sigmoidal concentration gradient.

SensíQ Technologies, Inc.
655 Research Pkwy
Suite 300
Oklahoma City, OK 73104

T+ 1.405.239.8600
F+ 1.405.235.8608

www.sensiqtech.com

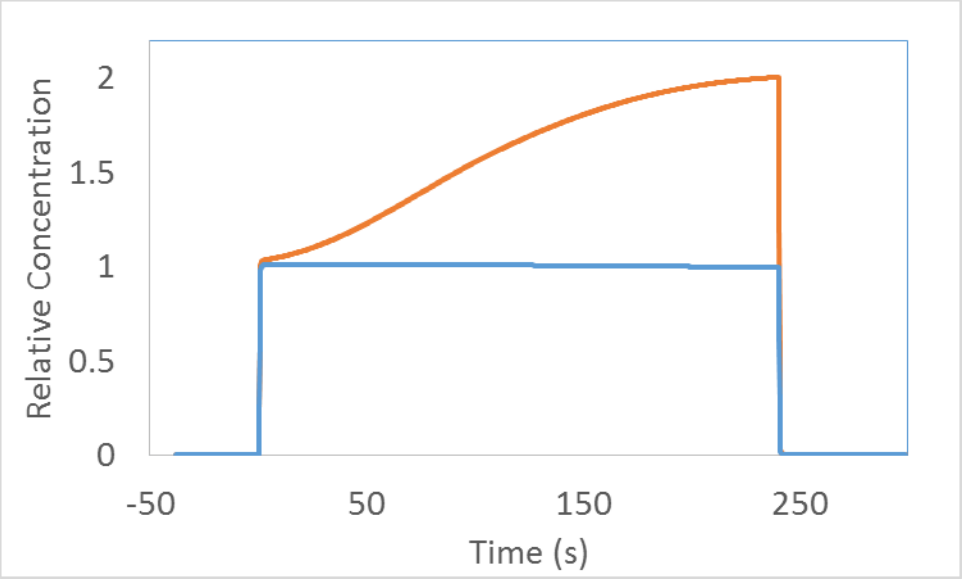

Figure 2A: Shows two NeXtStep injections overlaid: a gradient of ANALYTE (orange curve) in the constant presence of CONTROL and a constant concentration of CONTROL (blue curve) which is the reference (blank) injection.

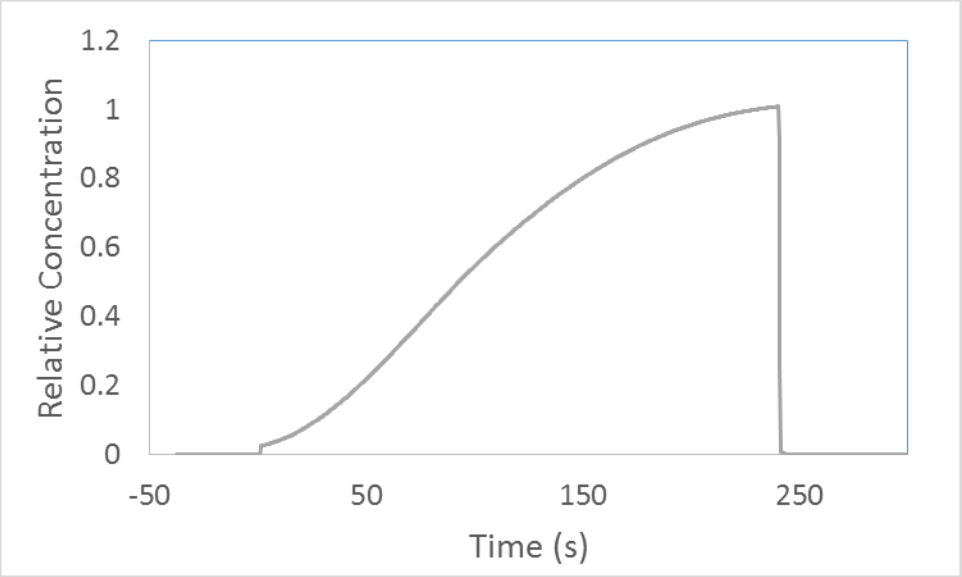

Figure 2B: By subtracting the blue curve from the orange curve in Fig. 2A, binding of ANALYTE only is shown. So two NeXtStep injections (Fig. 2A) are performed to obtain the data show in Figure 2B.

The NeXtStep in Direct Competition Fragment Assays:

- Derive full kinetics and affinity data in the presence of a competitor molecule with a single NeXtStep injection which covers a wide concentration range and one dissociation value.
- Simpler to use and to optimize workflows than the competition: competitor instrumentation requires that multiple concentrations are repeatedly injected to get full affinity and kinetics.
- Simpler to use and to optimize fragment screening campaigns compared to the competition: NeXtStep rapidly characterizes analyte binding modes against a control molecule.

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Materials and Methods:

Carbonic anhydrase II (CAII) was immobilized on a COOHV sensor chip via amine coupling and the Pioneer *FE* system was primed in HEPES buffered saline, pH 7.4, with 0.005% Tween-20 (HBST). 24 fragment actives were previously identified from the Maybridge Ro3 1,000 compound library using Pioneer *FE* and its integrated actives selection. These fragments were prepared in two sample solutions, one in buffer and another in buffer with 100 µM Furosemide. NeXtStep injections (50 µL/min) were performed on each analyte sample by dispersing the buffer solutions with buffer and the Furosemide solutions with Furosemide (100 µM). Control injections of buffer and Furosemide were also performed for double referencing. Data analysis was performed using Qdat (SensíQ Technologies, Inc.) and results are shown in Figures 3 - 6.

Results:

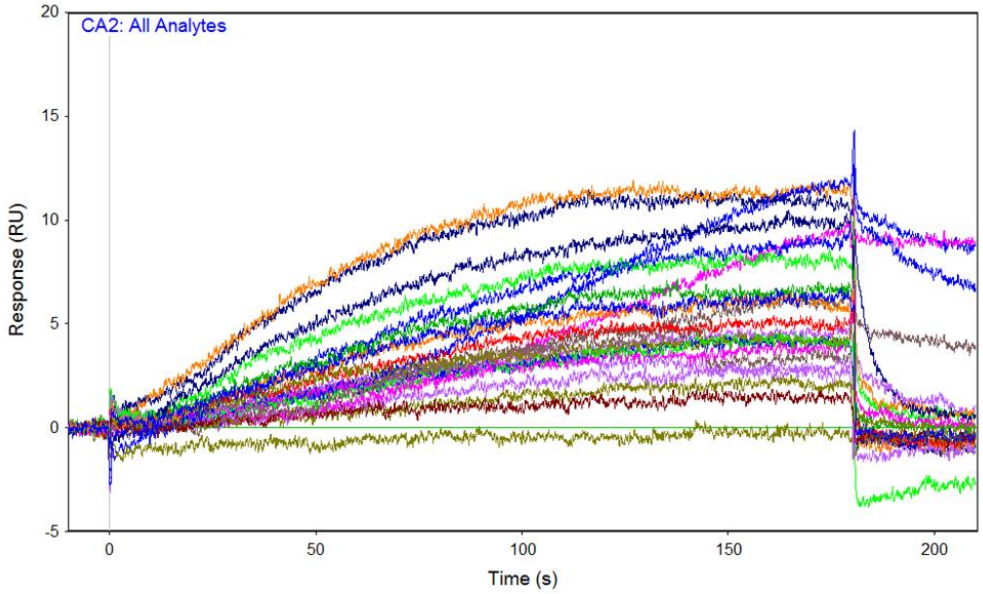

Fig 3A: Fragment actives injected using NeXtStep in buffer binding CAII.

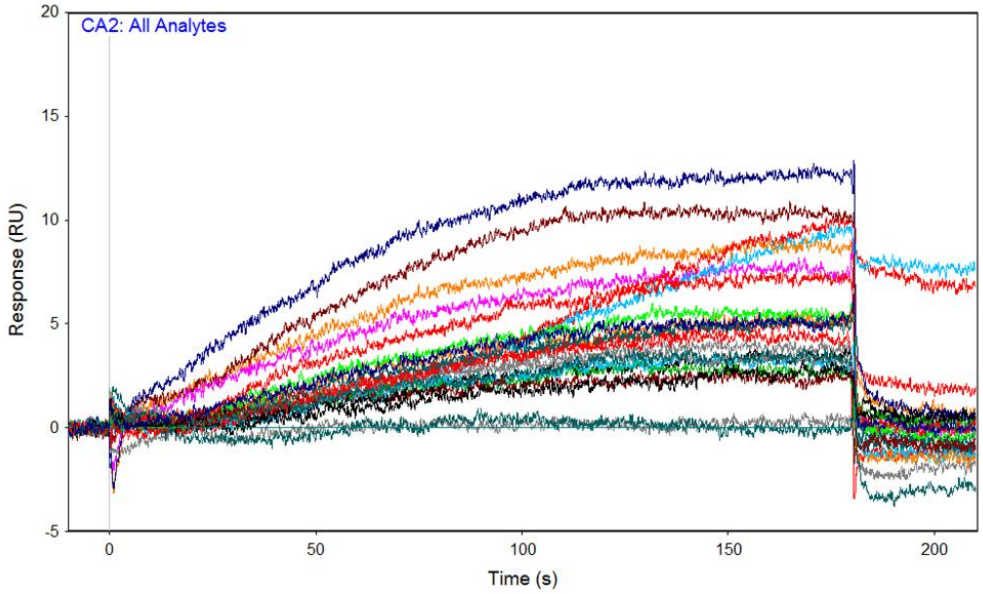

Figure 3B: Fragment actives injected with NeXtStep in the presence of Furosemide binding CAII.

Figures 3A and 3B: Shows the complementary results of analytes binding CAII (3A) and analytes binding CAII in the presence of Furosemide (3B).

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As stated previously, Pioneer with NeXtStep Injection technology has the unique ability to determine full kinetics and affinity in the presence of a competitor molecule from a single injection. Figure 4 shows one binding mode represented by comparing the binding of an analyte in the presence and absence of control. When a fragment binds the target protein away from the active site, the binding will be unchanged in the presence of the control. Fragment actives are therefore promptly binned without further screening cycles typical of other vendor SPR instrumentation.

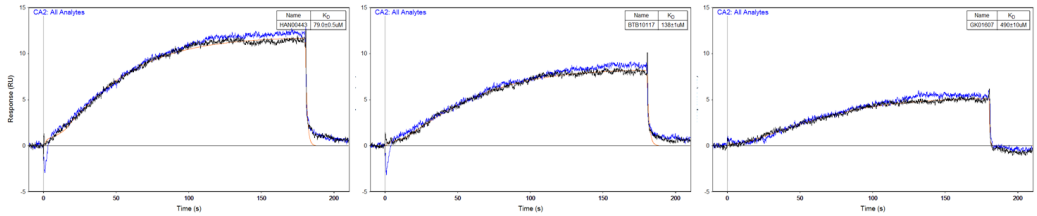

Figure 4: Fragments with a unique binding site show an unchanged response in the presence (blue curves) and absence (black curves) of a control molecule. K_D data derived from a single NeXtStep injection (model fit in orange).

In a second binding mode, when a fragment binds to the active site, the NeXtStep response will decrease in the presence of the control. The decrease in signal is readily identifiable in the Figure 5 examples. Further screening becomes a rate-limiting and unnecessary step when using NeXtStep (only available on SensíQ SPR instrumentation).

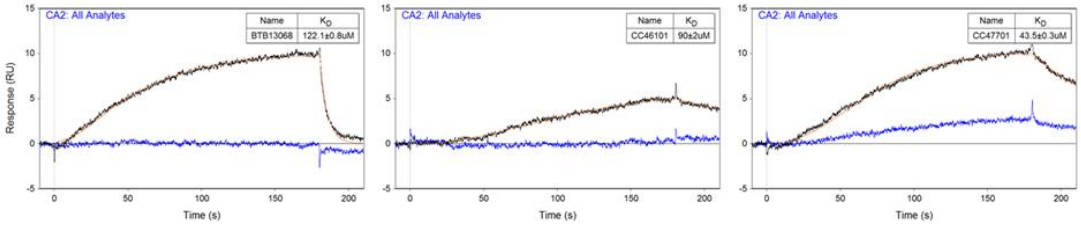

Figure 5: Fragments which bind active site show a decreased NeXtStep response in the presence of the control allowing for prompt identification.

What happens when fragment binding cooperates with the control molecule? In a third binding mode shown in Figure 6, fragments which display complementary binding with the control will show an increased NeXtStep binding response in the presence of the control. This binding mode would likely be overlooked using a fixed concentration competition assay.

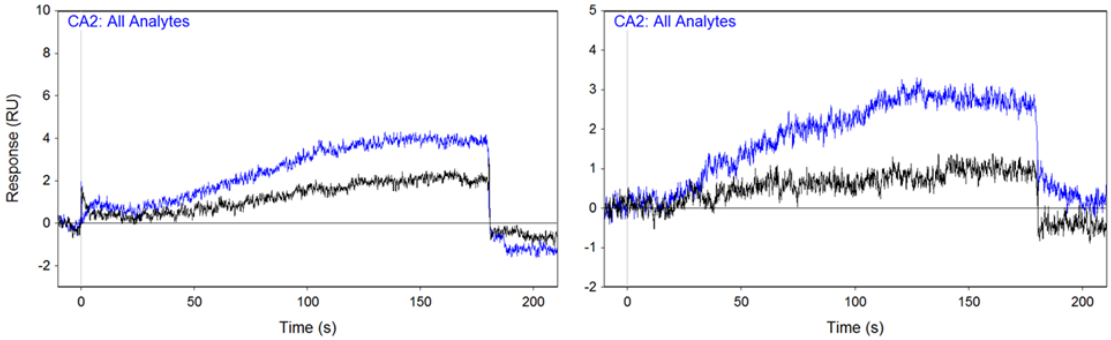

Figure 6: Fragments which bind cooperatively or potential activators will show increased binding in the presence of the control.

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Conclusions and Future Directions:

Figure 7: diSPR® enabled fragment screening uses NeXtStep to further characterize hits for binding site specificity.

SensíQ's patented gradient injection methods provides K_D's from single injections, allowing SensiQ instruments to eliminate secondary screens such as one competitor's "Affinity Screen". No other SPR providers have gradient injection technology. SensiQ Pioneer FE customers have demonstrated that a combination of excellent sensitivity, OneStep® gradient injections and the integrated Active Selection software provides superior results over other SPR suppliers. Now SensíQ has developed a new binding competition assay method for rapidly screening fragments, NeXtStep. Like OneStep®, NeXtStep enables full kinetic analysis in single competition injection and competition is clearly seen as a lack of binding in the presence of the competitor. Taken together, OneStep® and NeXtStep enable straightforward fragment screening workflow optimization and thus a faster drug discovery process (Fig. 7).

The NeXtStep in Direct Competition Fragment Assays:

- Derive full kinetics and affinity data in the presence of a competitor molecule with a single NeXtStep injection which covers a wide concentration range and one dissociation value.
- Simpler to use and to optimize workflows than the competition: competitor instrumentation requires that multiple concentrations are repeatedly injected to get full affinity and kinetics.
- Simpler to use and to optimize fragment screening campaigns compared to the competition: NeXtStep rapidly characterizes analyte binding modes against a control molecule.

SensíQ Technologies - Providing the NeXtStep in SPR-based fragment screening.

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F+ 1.405.235.8608

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