Pioneer and Pioneer FE systems

Get one step ahead in biological characterization and fragment screening

FORTÉBIO

High-performance binding kinetics and fragment screening analysis

Pioneer and Pioneer FE systems provide high quality kinetics and affinity measurements in a single injection in a matter of minutes. Using surface plasmon resonance (SPR) technology, they rapidly characterize a wide variety of biomolecular interactions, from small molecule fragments to biologics, without compromising sensitivity. Pioneer systems provide rapid affinity data directly from primary screens, significantly reducing the time and cost associated with secondary screening while enabling the prompt identification of lead candidates.

Make decisions earlier

- OneStep® gradient injection acquires reliable affinity (K_D) and kinetic (k_a, k_d) data directly from primary screens.
- Perform kinetic screening of 768 small-molecule fragments in a single experiment in 24 hours.
- Obtain detailed, high-quality kinetic characterization of 64 samples in 29 hours.
- Exquisite sensitivity with minimal baseline noise and drift for high affinity binding pairs
- Integrated, industry-proven data analysis software based on Scrubber and Clamp platforms.
- Completely automated for 72 hours of unattended run time

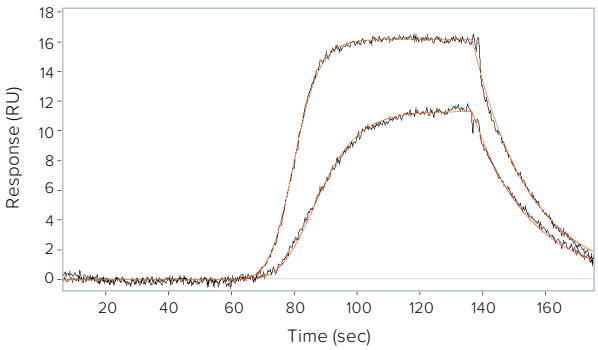

Figure 1: Kinetic analysis of a molecular interaction using OneStep Injections at two top concentrations.

New gold standard in fragment screening

There are many technical challenges in fragment screening that primarily stems from the need to analyze a large number of low molecular weight compounds (< 300 Da). Furthermore, the affinities of these interactions can range between 10 µM to 10 mM whereby analysis of these fragments at concentrations above K_D becomes a challenge due to poor solubility of these compounds in aqueous buffers. Additionally, in traditional single concentration primary screens, decisions have to be made on small square shaped response curves where kinetics are poorly defined.

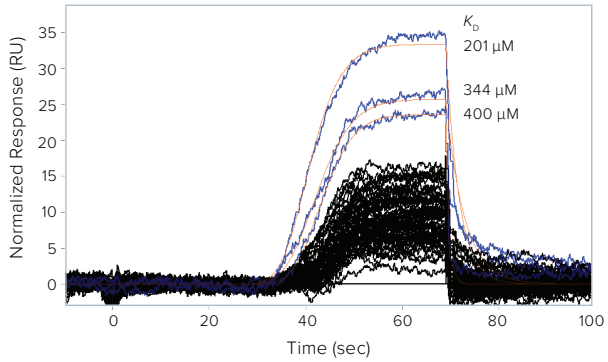

Figure 2: Fragment screen using OneStep. Actives (blue curves) are shown analyzed (model fit in orange) with the real-time isotherm affinity model.

The Pioneer FE system changes the fragment-based drug discovery paradigm with OneStep injection technology where a single analyte injection is sufficient to measure kinetic and affinity values accurately. In contrast to traditional fixed concentration injections (FCI), OneStep provides better resolved kinetic traces compared to square-shaped traces due to the generation of a continuous concertation gradient 3–4 orders of magnitude during the OneStep process (Figure 2). Therefore OneStep screening can derive kinetics and affinities from single point primary screens, eliminating the need for a secondary characterization screen providing significant time savings (Figure 3).

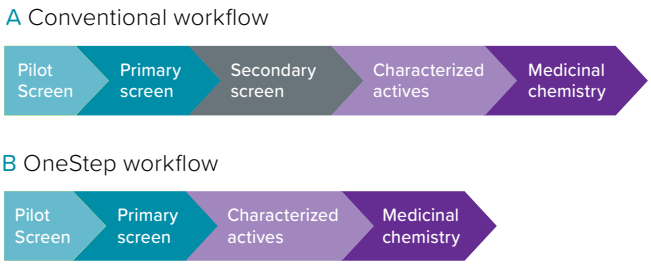

Figure 3: (A) Conventional fragment screening workflow. An initial screening process is followed by a secondary screening process where samples have to be prepared at different concentrations and analyzed separately to allow for affinity characterization and fragment hit confirmation. (B) Optimized workflow using diSPR. Initial compound screening is followed by a specificity test analysis that leads to full characterization of the identified hits. The selected compounds can then be used in various applications in medicinal chemistry.

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. Like OneStep, NeXtStep™ Injections enable full kinetic analysis in a single injection, allowing site-specific competition to be clearly seen as a modulation of binding in the presence of the competitor molecule.

RAPID DATA ANALYSIS

Data analysis of SPR-based fragment screens can be cumbersome and require days of an analyst's time for a single plate screen. Qdat analysis software uses a unified approach for selection of actives from non-actives and allows normalizing of screening data across different days or instruments, so an entire screen campaign can be compared. This integration results in a major reduction in post processing time, from hours/days to seconds/minutes. The software provides models incorporating kinetics, mass transport corrections, and multi-site binding as required to fit the interaction. Primary screening data are ready for K_D analysis without having to perform laborious, time consuming, and potentially error-prone secondary screening.

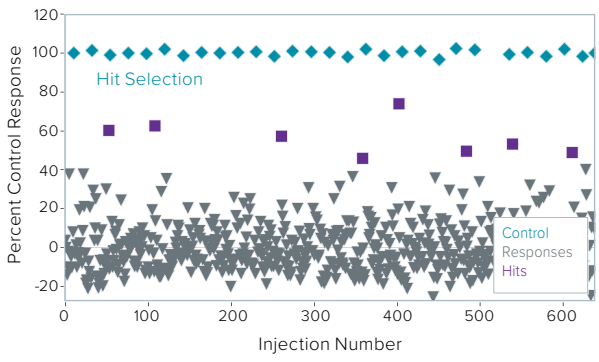

Figure 4: Qdat software has an integrated actives selection tool for automatic identification of actives from screening data. Actives identified in the selection process are then analyzed for kinetics and affinity.

OneStep Injection advantage

In conventional SPR analysis, a standard injection (FCI) provides a flow of uniform analyte concentration. Pioneer systems use OneStep Injection technology to diffuse the compound solution into a moving stream of buffer to create a concentration gradient during the injection. With this dynamic approach, an analyte titration of three orders of magnitude can be recorded in one continuous injection. This eliminates the need to prepare a full dilution series required with other SPR instrumentation. It also changes the paradigm for fragment screening as affinity (K_D) analysis can be performed on primary screening data. Additionally, diffusion coefficients of analyte molecules can be estimated, allowing aggregation information about the molecules being tested to be collected.

Protein-small molecule interactions

Interactions between proteins and small molecules are at the center of academic and drug development research. The exquisite sensitivity of the Pioneer systems coupled with novel surface chemistries allow kinetics measurements on molecules as small as 70 Da. Quantitative data at this level enables researchers to gain more information about new drug candidates.

Antibody characterization and aggregation

The Pioneer platform's superior sensitivity with baseline noise at <0.035 RU and minimal drift enable calculation of reliable kinetic and affinity values for high affinity binders. As the biopharmaceutical industry moves towards generating very high affinity molecules, Pioneer systems provide an indispensable solution with the ability to differentiate between molecules with very slow off-rates down to 10^{-6} s^{-1}.

Characterizing the affinity and aggregation profile of antibody interactions is of great importance in application areas such as biotherapeutic discovery, development and quality control. OneStep Injections can simultaneously detect both antibody aggregation and perform full kinetic characterization in a single standard experiment. As the analyte concentration gradient is generated with OneStep, analyte diffusion coefficients can be calculated which provides a rapid and reliable assessment of aggregation formation. There appears to be a linear relationship between diffusion coefficients derived from OneStep Injections versus aggregate composition as determined by SEC in the same sample. This novel capability of OneStep injection technology will directly impact many applications in drug development by allowing process optimization previously not possible.

Key features

- Automatic analyte titration over 3 orders of magnitude
- K_D from single gradient injections
- Two 384-well microplates for samples
- Three sensing channels
- Real time reference curve subtraction
- High mass transport
- Detection down to 70 Da
- Wide variety of surface chemistries
- Sophisticated, easy-to-use data analysis software

Pioneer specifications

Refractive index range	1.33–1.40
Short term noise	< 0.1 RU
Long term noise	< 0.3 RU
Molecular weight cutoff	< 70 Da
Working ranges	k_a $10^2 - 10^8$ $M^{-1}s^{-1}$ k_d $10^{-6} - 0.1$ s^{-1}
Sample capacity	2 sample racks
Sample configuration	96 vial, deep well and PCR formats, 384 well microplates, custom high volume
Sample temperature control	4 to 40°C (max 15° below ambient)
Sample loading	Automatic
Number of channels	3
Flow path	1, 1–2, 1–2–3, 3, 3–2, 3–2–1
Flow channel volume	< 90 nL
Channel-channel dead volume	< 20 nL
Real time reference curve subtraction	Yes
Injection volume	2–700 µL
Injection rise and fall time	< 0.75 second @ 25 µL/min
Simultaneous injections	Yes, dual sample loops
Gradient injections	Yes
Flow rate	0.1–150 µL/min
Inline buffer degassing	Yes
System temperature control	4–40°C (Max 15° below ambient)
Variable data rate	1–20 Hz
Automation capabilities	> 72 hour unattended operation
Qdat hit selection feature	No

Pioneer FE specifications

Refractive index range	1.33–1.40
Short term noise	< 0.035 RU
Long term drift	< 0.3 RU/min
Molecular weight cutoff	< 70 Da
Working ranges	k_a $10^2 - 10^9$ $M^{-1}s^{-1}$ k_d $10^{-6} - 2.5$ s^{-1} K_D ~$10^{-3} - 10^{-12}$ M Concentration ~$10^{-3} - 10^{-12}$ M
Sample capacity	2 sample racks, 2 reagent racks
Sample configuration	96 vial, deep well and PCR formats, 384-well microplates, custom high volume
Sample temperature control	4 to 40°C (max 15° below ambient)
Sample loading	Automatic
Number of channels	3
Flow path	1, 1–2, 1–2–3, 3, 3–2, 3–2–1
Flow channel volume	< 90 nL
Channel-channel dead volume	< 20 nL
Reference curve subtraction	Yes
Injection volume	2–700 µL
Injection rise and fall time	< 0.75 second @ 25 µL/min
Simultaneous injections	Yes, dual sample loops
Gradient injections flow rate	Yes, OneStep, NeXtStep 0.1–200 µL/min
Inline buffer degassing	Yes
System temperature control	4–40°C (Max 15° below ambient)
Variable data rate	1–40 Hz
Automation capabilities	> 72 hour unattended operation
Qdat hit selection feature	Yes

SENSOR CHEMISTRIES

Immobilization by amine coupling	COOH1, CDH, CDL, PCH
Streptavidin-biotin based immobilization	SADH
Affinity capture surface for histidine-tagged proteins	HisCap

FORTÉBIO

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