Utilizing isothermal spectral shift detection to quantify challenging biomolecular interactions with Monolith X

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Abstract

Monolith X is the latest addition to the Monolith product line, combining isothermal spectral shift detection with MST technology to characterize biomolecular interactions in solution. When a target is labelled with a fluorophore it generates a particular emission spectrum, and if a ligand binds to this labelled target, the fluorophore's chemical environment is changed, causing a shift in fluorescence spectra. Monolith X exploits this phenomenon by performing ratiometric measurements at two emission wavelengths of a labelled target in the presence of various concentrations of an unlabelled ligand to derive the affinity constant (K_d) for the interaction.

Isothermal spectral shift detection enables characterization of in solution interactions for a wide range of biomolecules, even for challenging samples such as membrane proteins, intrinsically disordered proteins, and cell lysates. Since the binding partners are in solution, there is no lost activity due to immobilization, and evaluation is size independent. Measurements can be performed in any buffer, including detergents, using low sample volumes and concentrations. The spectral shift analysis also facilitates the evaluation of competition assays and ternary binding events. Monolith X provides a valuable orthogonal method to validate your results from other biophysical methods and to characterize your most challenging interactions.

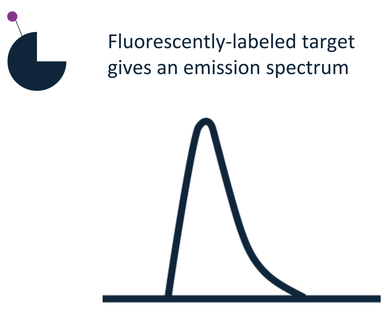
1 – Introduction

Monolith X allows you to characterize your most challenging interactions and provides an orthogonal technique to validate your results from other biophysical methods

- Evaluate the same target classes as other biophysical methods and more
- Use the same buffer composition as your primary method
- Characterize a range of different interactions and sample types: membrane proteins, PROTACs, intrinsically disordered proteins, RNA-based therapeutics, ternary complexes, crude lysates
- Get results in as little as a few seconds
- Measure in solution with no fear of lost activity due to immobilization required by SPR and BLI
- Characterize binding events with a fraction of the sample required by ITC
- Work with detergents or viscous buffers that cause issues for SPR
- Measure the K_d between very different binding partners since evaluation is independent of size and mass

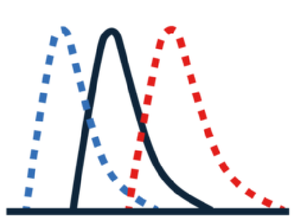
2 – Measuring molecular interactions with Monolith X

How spectral shift detects an interaction at isothermal conditions

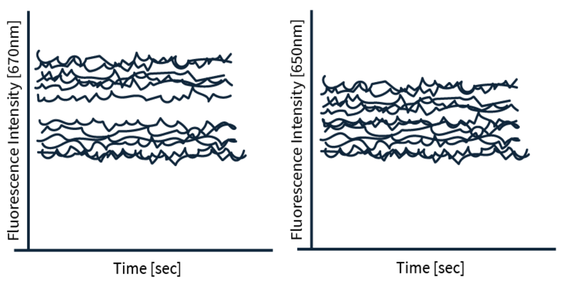


Fluorescently-labeled target gives an emission spectrum

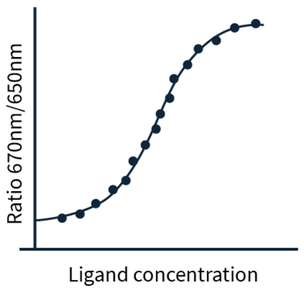
Ligand binding causes blue- or red shift, or broadening of emission peak



Ratiometric measurement provides high quality data to derive a K_d with confidence

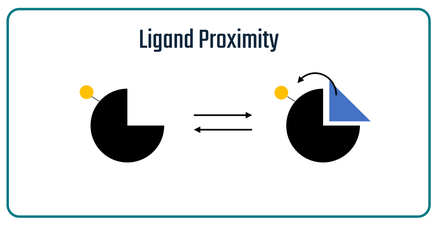


Fluorescence of the target in the presence of varying concentrations of ligand is recorded at 670nm and 650nm for 5 sec

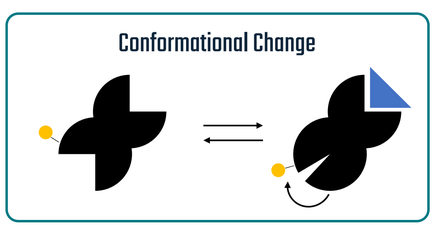


670nm/650nm ratio is calculated and plotted against ligand concetration to derive a K_d for the interaction

Blue- or red-shift is caused by changes in the fluorophore's chemical environment



Ligands that bind close to the fluorophore can directly influence the fluorophore's chemical environment

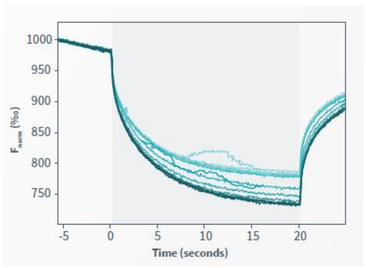


Ligands that bind in a distant position from the fluorophore can lead to ligand-induced conformational changes that affect the chemical environment of the fluorophore

Use MST technology to understand aggregation

Aggregation describes the formation of large clusters of biomolecules. Monolith X uses MST to help identify aggregates by looking for irregularities in fluorescence traces caused by larger particles traversing through the detection volume.

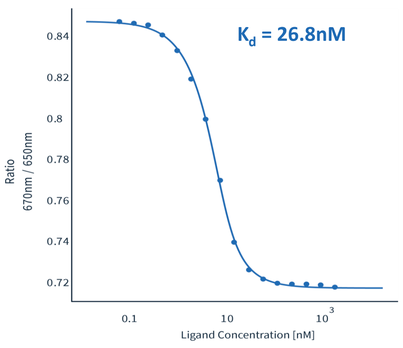
Aggregated biomolecules often lose their functionality, leading to ambiguous results in many biochemical and biophysical assays. Aggregate detection by MST technology provides direct feedback on sample quality and thereby helps to establish optimal assay conditions.



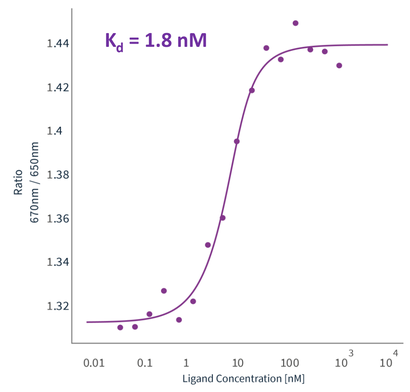
3 – Characterizing challenging interactions with Monolith X

Confirm kinase – small molecule interactions

- SB 239063 is a potent p38 mitogen-activated protein kinase inhibitor with anti-inflammatory activity
- Purified recombinant human p38α was labeled with Nanotemper's red 2nd generation maleimide dye
- 160 µL of 20 nM labeled p38α and 20 µL of 10 µM inhibitor was used for the binding assay run on Monolith X
- Spectral shift analysis shows a K_d of 26.8 nM for its inhibition of human p38α

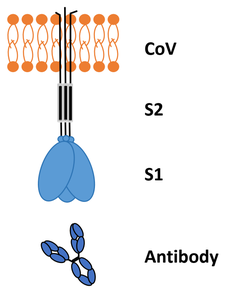


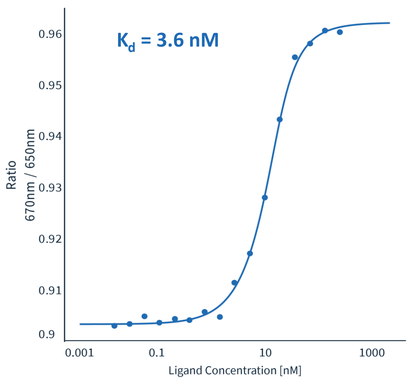
Analyze interactions between membrane proteins and small molecule inhibitors



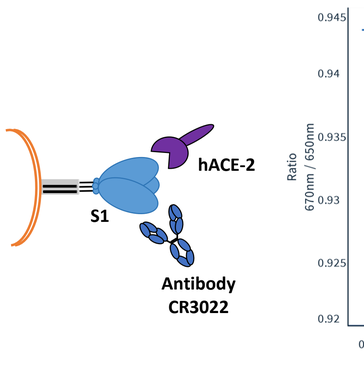
- Glucose transporter 1 (GLUT1) facilitates the transport of glucose across the plasma membranes of mammalian cells
- GLUT1 is an important target in cancer treatment because cancer cells upregulate GLUT1
- BAY-876 is a potent, highly selective GLUT1 inhibitor
- Purified GLUT1 in 0.05% DDM was labeled with Nanotemper's red 2nd generation tris-NTA dye via the protein's His-tag
- Spectral shift analysis shows a K_d of 1.8 nM for the interaction between GLUT1 and BAY-876

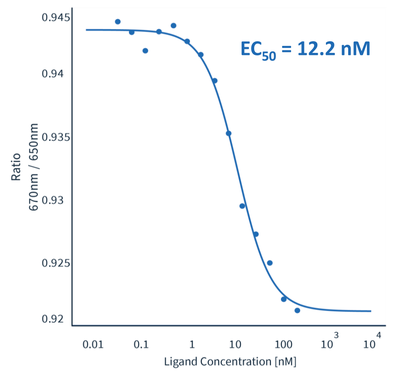
Characterize challenging ternary interactions easily under controlled equilibrium conditions





- CR3022 anti-SARS-CoV-2 antibody was labeled with red 2nd generation NHS dye
- Trimeric SARS-CoV-2 spike protein was used as the unlabeled ligand and titrated
- Spectral shift analysis shows interaction of 500kDa trimeric SARS-CoV-2 spike protein and anti-SARS-CoV-2 antibody (CR3022) with a K_d of 3.6 nM





- Labeled CR3022 anti-SARS-CoV-2 antibody was then mixed with trimeric SARS-CoV-2 spike protein to form a complex
- Unlabeled hACE-2 was used as the ligand and titrated to monitor the ternary interaction
- Trimeric SARS-CoV-2 spike protein in complex with anti-SARS-CoV-2 antibody (CR3022) binds to hACE-2 non-competitively with an EC_{50} of 12.2 nM

Summary

Get results with confidence
Get high signal relative to noise in your data

See the slightest changes in affinities between variants
Ratiometric analysis provides data with high precision

Get a K_d in just a few seconds
In just a few seconds you'll see real-time results

Get feedback on sample quality from MST
Find out if your sample has aggregates or not