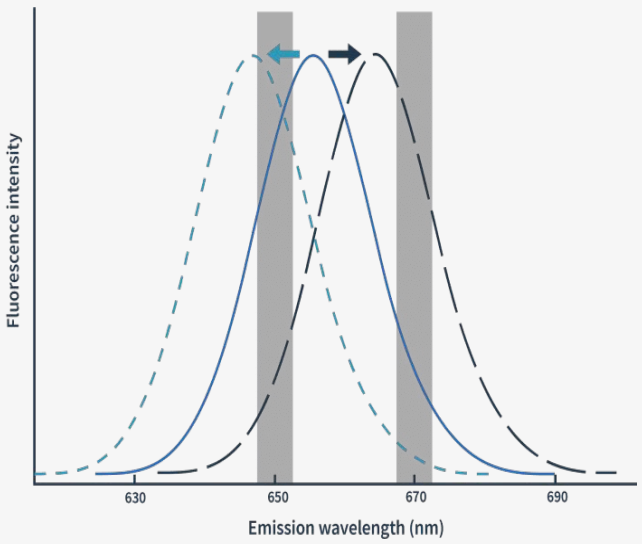
Spectral Shift

Spectral Shift is a fluorescence-based biophysical technique used for quantifying the strength of molecular interactions.

Spectral Shift builds upon the principle that the chemical environment around a fluorophore bound to a target molecule changes when the target molecule interacts with a ligand, causing a subtle wavelength shift in the emission spectrum of the fluorophore in isothermal conditions.

During a Spectral Shift measurement, a ratiometric measurement is reported by recording the emission fluorescence simultaneously at two preselected wavelengths.



Adapted from Langer et al, DOI: 10.1089/adt.2021.133

Upon binding to a ligand, the fluorophore bound to a target molecule undergoes a spectral shift relative to their known peak emission wavelength (solid line) leading to a decrease in the fluorescence at 650 nm (dotted line) and an increase in the fluorescence at 670 nm (dashed line) or vice versa.

Tackle your challenging interactions with Spectral Shift

The notion of spectral shift isn't new, but Dianthus and Monolith are the only instruments that use it to derive affinity constants. Shifts in the emission spectrum are observed and measured in solution from a mixture of target and ligand in microwells or glass capillaries.

Spectral Shift successfully measures challenging interactions when other methods fail or give you low-quality data. In particular, interactions involving:

- Multimeric complexes like the ones observed in degraders' ternary complexes
- Intrinsically disordered proteins
- Covalent ligands
- Molecules that aren't amenable to immobilization to biosensor

Use Spectral Shift in drug development and basic research

The affinity between two molecules or binding partners tells you how tightly they bind to each other. Affinity measurements are reported as the affinity constant, equilibrium dissociation constant, or K_d. The K_d and affinity are inversely related. The K_d value is related to the concentration of one of the binding partners, so the lower the K_d value — lower concentration expressed in molar values — the higher the affinity between the two molecules.

Scientists use Spectral Shift during drug discovery and also for basic research projects. Some examples are:

- Single-dose screening of small molecules or fragments
- Dose-response for hit validation and hit-to-lead optimization, SAR, or post-HTS triage

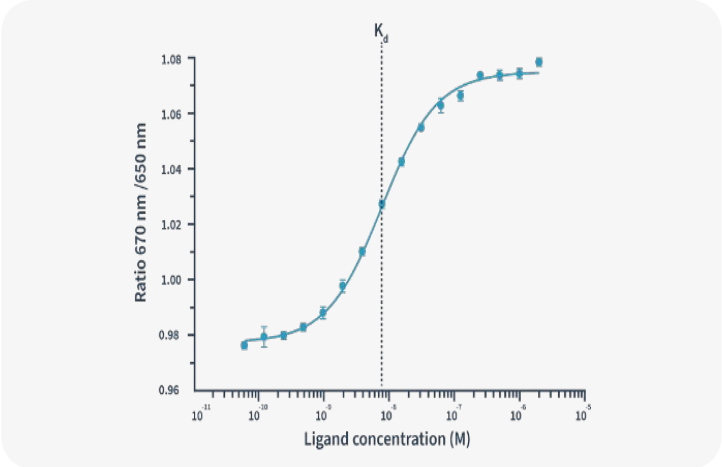
- Characterization of protein degraders' binary and ternary complexes
- Study of protein-protein interactions that regulate cellular processes like cell signaling

Get affinities with high precision by detecting subtle shifts in the emission spectrum

In a Spectral Shift assay, the molecule you label with the fluorophore is called a target. The other binding partner — another protein, nucleic acid sequence, small molecule, or fragment — is called a ligand.

To calculate the K_d, you mix together a constant amount of the fluorescently labeled target with a dilution series of a ligand. The ratio of the fluorescence intensities recorded in an isothermal environment at 670 and 650nm is plotted against the logarithmic ligand concentration. The K_d is determined from the binding curve using the law of mass action.

NanoTemper instruments resolve even sub-nanometer spectral shifts of the emission wavelength maximum — too low to be measured with a conventional plate reader or fluorimeter — so you can derive binding affinities with high precision.



The affinity constant K_d is derived by plotting the ratio of the fluorescence signal detected simultaneously at 670 and 650 nm against the ligand concentration on a logarithmic scale.