MicroScale Thermophoresis

MicroScale Thermophoresis, or MST, is a fluorescence-based biophysical technique used for quantifying the strength of molecular interactions.

MST 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 variation in the intensity of the fluorescence.

During MST measurements a brief and precise laser-induced temperature increase is applied to amplify the change in fluorescence intensity which is related to the amount of ligand bound.

MST has been used in drug discovery for over 10 years

MicroScale Thermophoresis (MST) is a technology pioneered by NanoTemper more than 10 years ago for measurements in solution from a mixture of target and ligand in the glass capillaries used in the Monolith instrument.

Since the change in fluorescence is dependent on the overall chemical environment, one needs to label the target molecule with a fluorophore sensitive to these changes. And any binding partner or ligand works, including proteins, nucleic acids, or small molecules.

MST has been used successfully to measure interactions with molecules like:

- Membrane proteins
- Intrinsically disordered proteins
- DNA aptamers
- Fragments

Use MST to directly measure the strength of a molecular interaction

The affinity between two molecules 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 and so the lower the K_d value — lower concentration expressed in molar values — the higher the affinity between the two molecules.

Scientists working in drug development use MST and Spectral Shift to tackle challenging interactions with a versatile tool perfect for working with a wide variety of projects.

The technologies are also used for the characterization of protein degraders' binary and ternary complexes.

Get affinities and insights into ligand-induced aggregation

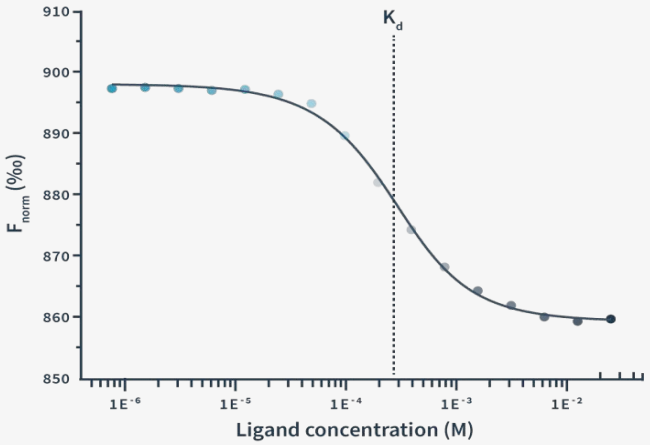
In an MST assay, the molecule you label with the fluorophore is called a target. The other binding partner — another protein, nucleic acid sequence, small molecule, or fragment — is called a ligand.

To calculate the K_d, a constant amount of the fluorescently labeled target is mixed with a dilution series of a ligand. The recorded changes in fluorescence are plotted against the logarithmic ligand concentration to build a binding curve.

The K_d is determined from the binding curve using the law of mass action.

Additionally, MST gives you information about ligand-induced aggregation. Aggregation is very common, especially with insoluble fragments and small mole-

cules with hydrophobic substitutions, and it’s often the reason why other technologies can’t determine binding affinities, but they never identify the cause of their failure.



The dose-response curves are obtained by plotting the F_{norm} values (the ratio between the fluorescence values after and prior to the laser activation) against the ligand concentration. The data is fitted with a dose-response model that describes the law of mass action.