

## Temperature-related intensity change

*Temperature-related intensity change*, or TRIC, is a fluorescence-based biophysical technique used for quantifying the strength of molecular interactions.

TRIC 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 TRIC 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.

### TRIC has been used in drug discovery for over 10 years

TRIC is a component of MicroScale Thermophoresis (MST), 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. Alternatively, when measurements are performed in a microwell — the sample vessel used in the Dianthus instrument — the thermophoretic signal is masked and only TRIC is measured.

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.

### TRIC has been used successfully to measure interactions with molecules like:

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

### Use TRIC in drug discovery for primary and affinity screenings

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.

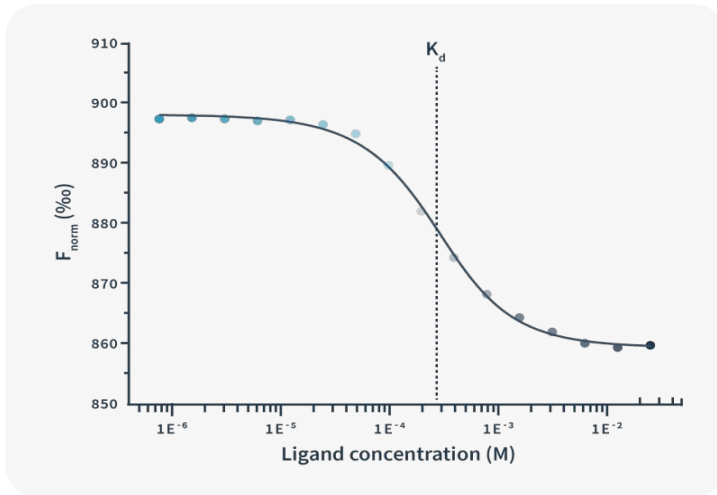
Scientists working in drug development use TRIC and Spectral Shift to screen libraries of small molecules or fragments to find those that bind to a disease-causing protein and inhibit its negative impact at the cellular level. The technologies are also used for the characterization of protein degraders' binary and ternary complexes.

### Get affinities and insights into ligand-induced aggregation

In a TRIC 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, TRIC gives you information about ligand-induced aggregation — probably the most helpful information when TRIC is used as an orthogonal method. Aggregation is very common,

especially with insoluble fragments and small molecules 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.