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Technical Note

USING MICROSCALE THERMOPHORESIS TO EASILY MEASURE BINDING AFFINITY

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Abstract: While it's very common for biologists and chemists to test whether or not two molecules interact with each other, it's much more useful to gather information on the nature of that interaction. How strong is it? How long will it last? What does that mean for its biological function?

One way to answer these questions is to study affinity. Binding affinity is defined as the strength of the binding interaction between a single biomolecule to its binding partner, or ligand, and it can be quantifiably measured, providing information on whether or not molecules are interacting, as well as assigning a value to the affinity.

When measuring binding affinity, there are several parameters to look at, but the dissociation constant (K_d), which defines the likelihood that an interaction between two molecules will break, is a very common measurement. The smaller the dissociation constant, the more tightly bound the ligand is, and the higher the affinity is between the two molecules.

Keywords: Binding Affinity; Binding Interaction; Ligand; K_d; Biomolecule

Introduction

Binding affinity is a useful metric in both academia and industry. Academic researchers can study binding affinity to learn about structural biology, structure-function relationships, and the intermolecular interactions that drive biological processes. On the other hand, drug developers study binding affinity to identify high-affinity molecules that bind to drug targets selectively and specifically. In this case, affinity can guide decisions about the biological relevance of a particular molecule, such as whether the molecule under investigation warrants further screening or characterization.

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Measuring binding affinity can be useful in research for

- Characterizing receptor binding properties
- Measuring interactions with antibodies
- Analyzing protein complexes
- Investigating enzyme inhibition
- Observing molecular transport processes
- Mapping epitopes
- Optimizing leads
- Pursuing fragment-based lead discovery
- Measuring the effects of buffers, solutions, and concentration on binding affinity

What is MicroScale Thermophoresis?

MicroScale thermophoresis (MST) is a methodology that quantitatively examines molecular interactions in solution at the microliter scale. It measures binding interactions utilizing the characteristics of thermophoresis combined with Temperature Related Intensity Change (TRIC). Thermophoresis–

–the movement of molecules in the presence of a thermal gradient. TRIC is the quenching of a fluorophore when subjected to a thermal gradient. MST is influenced by a molecule's size, charge, hydration shell and the effects of TRIC. Altogether, the data generated results in a more specific and robust measurement of binding interactions and modifications. The method works equally well in standard buffers and biological liquids like blood or cell-lysate. MST provides information regarding the binding affinity, stoichiometry, competition, and enthalpy of two or more interacting proteins.

How does MicroScale Thermophoresis work?

MST is performed in-solution in thin, glass capillaries that can hold low volumes (microliters) thus providing close-to-native conditions (immobilization-free in any buffer, even in complex bioliquids). Typically one of the binding partners is fluorescently labeled (although a label-free MST detection system is available) and the change in fluorescence signal is detected. When performing an MST experiment, a microscopic temperature gradient is induced by an infrared laser, and the directed movement of molecules or thermophoresis is detected and quantified. The signal generated depends on three parameters that typically change upon interaction. Thus, the thermophoresis signal is plotted against the ligand concentration to obtain a dose-response curve, from which the binding affinity can be deduced.

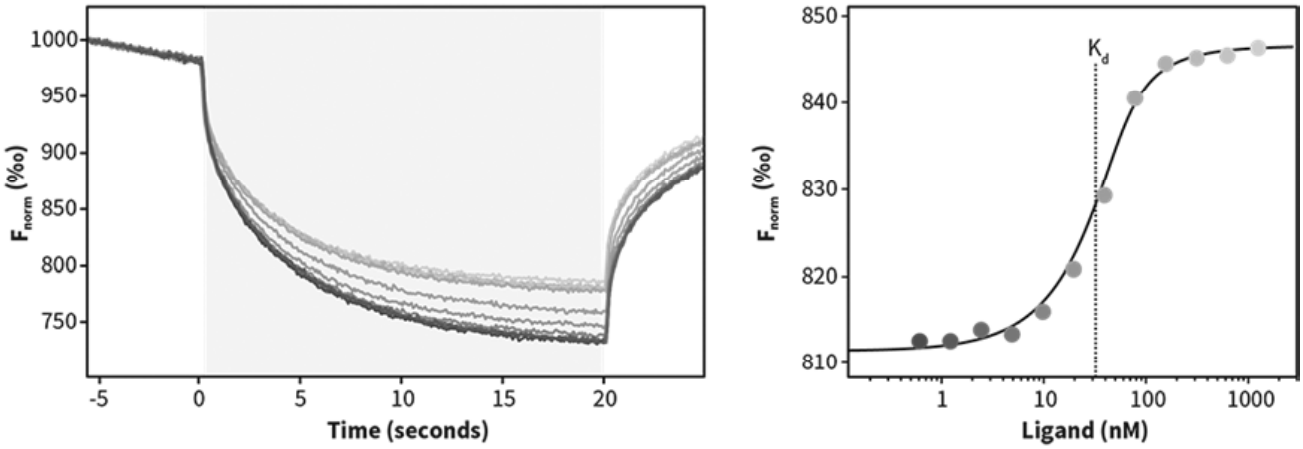

Figure 1: **Strength of interactions between a fluorescently labeled or intrinsically fluorescent sample and a binding partner (or ligand) is measured while a temperature gradient is applied (gray box) over time (left figure). From this, binding affinity (K_d) is automatically calculated from a fitted curve that plots normalized fluorescence against concentration of ligand (right figure)**

MicroScale Thermophoresis can measure the affinity of an antibody to an antigen or Fc receptor

Determining the affinity of an antibody to its antigen, a carrier protein or an Fc receptor with MicroScale Thermophoresis (MST) is often performed during the development of biotherapeutics. Here, the interactions between the Fc segment or the Fab domains of therapeutic antibodies - Herceptin (Trastuzumab) and Humira (Adalimumab) - with their respective binding partners/antigens are examined.

The Advantages of using MicroScale Thermophoresis

It is an in-solution method in which binding partners being studied are not immobilized on a biosensor or solid surface. With this technology, binding affinity is determined using very small amounts of sample. Results are measured in minutes. It's also very flexible, meaning you can look at molecules of all weights and in all sorts of buffers––ideal for investigating sensitive molecules that need specific buffer conditions, or for looking at interactions in close-to-native conditions. A label-free option is available.

Monolith systems use MicroScale Thermophoresis

The Monolith systems from NanoTemper Technologies use MST technology to quickly and precisely generate Kd values using very little sample. Both capillaries and labeling kits are offered for optimizing assays on any of the systems.

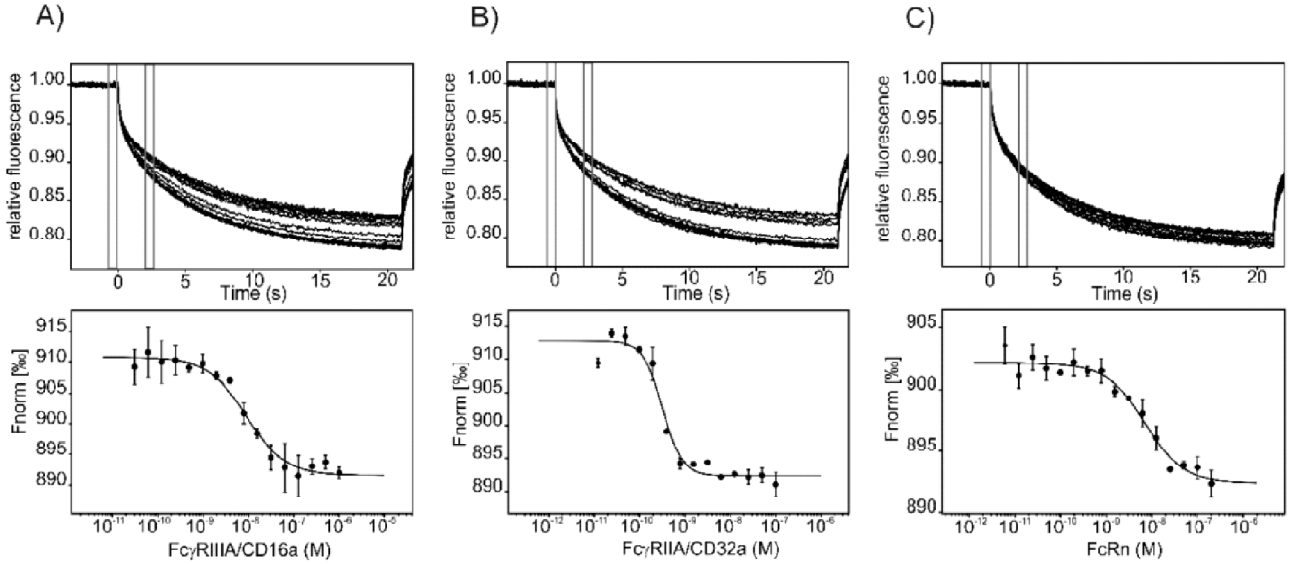

Figure 2: **Binding of (A) Fc RII/CD32, (B) Fc RIIIA/CD16 and (C) FcRn to Humira by MST. The resulting dose-response curves were fitted to a one-site binding model to extract K_d values**

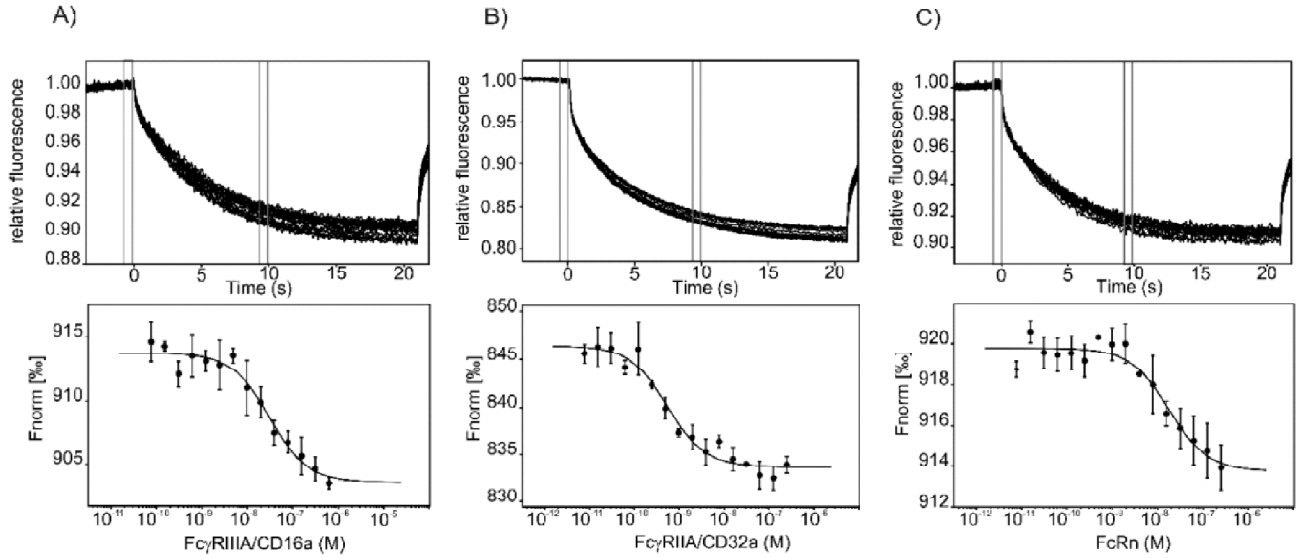

Figure 3: **Binding of (A) Fc RIIIA/CD16, (B) Fc RII/CD32 and (C) FcRn to Herceptin by MST. The resulting dose-response curves were fitted to a one-site binding model to extract K_d values**

Additionally, MO.Control software makes running binding interaction experiments easy—it calculates all the volumes and dilutions needed to run an assay. It guides users through setup, offers real-time optimization tips, and has built-in knowledge-based advice. Binding affinity values are automatically calculated and complete result summaries are instantly generated.

MicroScale Thermophoresis is an alternative technology that can rapidly measure binding affinity for a wide range of sample types and can evaluate difficult targets. For more information on specific applications, visit nanotempertech.com/resources.

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