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# Determination of Biomolecular Interactions Using Microscale Thermophoresis

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**Abstract :** Characterization of biomolecular interactions, such as protein-protein, protein-nucleic acid or protein-small molecule, provides critical insights into cellular processes and is essential for the development of drug diagnostics and therapeutics. Here we present a novel, label-free, and tether-free technology to analyze picomolar to millimolar affinities of biomolecular interactions by Microscale Thermophoresis (MST). The entropy of the hydration shell surrounding molecules determines thermophoretic movement. MST exploits this principle by measuring interactions using optically generated temperature gradients. MST detects changes in the size, charge and hydration shell of molecules and measures biomolecule interactions under close-to-native conditions: immobilization-free and in bioliquids of choice, including cell lysates and blood serum. Thus, MST measures interactions under close-to-native conditions, and without laborious sample purification. We demonstrate how MST determines the picomolar affinities of antibody::antigen interactions, and protein::protein interactions measured from directly from cell lysates. MST assays are highly adaptable to fit to the diverse requirements of different and complex biomolecules. NanoTemper´s unique technology is ideal for studies requiring flexibility and sensitivity at the experimental scale, making MST suitable for basic research investigations and pharmaceutical applications.

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