

CMC – Structure Initiative Protocols

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Isothermal titration calorimetry (ITC)

ITC is used mainly to measure affinity for protein-protein and protein-ligand interactions. In an ITC experiment aliquots of a titrant (protein, peptide or small molecule typically at ≥ 0.5 mM) are injected into the cell containing protein solution (typically 20 to 100 μ M). Upon each titration the amount of heat released or absorbed is measured. In addition to the equilibrium dissociation constant (K_d), ITC titration allows to determine the number of binding sites, binding enthalpy and entropy. In contrast to BIAcore ITC is a truly equilibrium solution method where K_d is measured for native proteins. No labeling or immobilization is required. Also, ITC is not limited by the ligand or protein size. It is relatively artifacts-free, and is not affected by the optical properties of the samples. The only major disadvantage of ITC is that it requires relatively high concentrations of samples.

SAMPLE GUIDELINES:

No buffer or salt limitations. Except NO REDUCING AGENTS (DTT, etc) or unstable chemicals. Typically, common organic solvents (DMSO etc.) can be used. Importantly, the buffer composition should be EXACTLY THE SAME for the titrant and cell solutions. Good starting point is to use 50-100 μ M protein (2mls per titration) and 0.5-1 mM titrant (800 μ l per experiment). Typical ITC experiment consists of two titrations (titrant against protein and titrant against buffer).