The TSA – global fitting for the masses, using MicroCal PEAQ-ITC Analysis Software

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ITC can determine stoichiometry n, binding enthalpy ΔH, and dissociation constant K_D for protein-protein interactions. For an ideal measurement – where all independent factors are perfectly known and controlled – these quantities can be calculated from a single titration. However, largely due to the unknown active concentration of the binding partners ([Syr] and [Cell]), this is rarely the case in reality.

It has therefore become good practice to perform biological triplicates and fitting globally. When ideal fit parameters (n, ΔH, K_D) are shared among replicates and only [Syr] and [Cell] are allowed to vary, random errors should average out.

Unfortunately, a function for performing a global fit is neither available in common ITC-analysis software, nor easily established in-house. To remedy this, we have developed an alternative method to fit data globally. Using MicroCal PEAQ-ITC Analysis Software, we "shift" titration curves three times. This results in identical shared fit parameters (n, ΔH, K_D) and corrected [Syr] and [Cell]. A subsequent Support Plane Analysis [1] evaluates the robustness of the resulting quantities.

The "Triple-Shift" [2] is an easy to understand and perform method that only requires MicroCal PEAQ-ITC Analysis Software and Excel, thereby lowering the barriers of entry for newcomers in the world of ITC data handling.

[1] G. Kemmer, S. Keller, *Nature Protocols* **2010**, *5*, 267–281.

[2] S. Fuchs, S. C. Hansen, M. Markones, E. V. Mymrikov, H. Heerklotz, C. Hunte, *Scientific Reports* **2018**, *8*, 14837.