

Thermodiffusion as a Probe of Protein Ligand Binding

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Molecular recognition via protein-ligand interactions is of fundamental importance to numerous processes in living organisms. The behaviour of biomolecules in a temperature gradient, known as thermodiffusion or thermophoresis, changes when a ligand binds. Microscale thermophoresis (MST) uses this sensitivity of the thermophoretic response to access information on binding dynamics, although the physicochemical processes are still unclear [1]. Additionally, thermophoresis is promising as a tool to gain information on the hydration layer and how it changes due to complex formation. We use infra-red thermal diffusion forced Rayleigh scattering (IR-TDFRS) in a temperature range from 10 to 50°C to investigate the thermodiffusion properties. In previous studies [2] we used cyclodextrin-aspirin as a model system for complexes and showed that the temperature dependence of the thermodiffusion behaviour is sensitive to solute-solvent interactions. Now we shift our focus to the protein streptavidin (SA) and its biotin complex. Similar to the cyclodextrins, formation of the SA-biotin complex leads to a weaker temperature sensitivity of the thermodiffusion behaviour, although the effect is more pronounced. This indicates a less hydrophilic complex. To quantify the influence of structural fluctuations and conformational motion of the protein on the entropy change of its hydration layer upon ligand binding, we combine quasi-elastic incoherent neutron scattering (QENS) and isothermal titration calorimetry (ITC) data. As the QENS measurements are only possible in heavy water, the ITC need to be performed in heavy water as well in order to gain a better understanding of the hydration layer. The aim of this work is to develop a microscopic understanding of the correlation between the strength of solute-solvent interactions and the thermophoretic behaviour.

[1] M. Jerabek-Willemsen *et al.*, J. Mol. Struct. (2014).

[2] D. Niether *et al.*, Langmuir 33(34), 8483-8492 (2017).

