H/D substitution large effect on protein phase behaviour and the ELCS

Najet Mahmoudi[1,2*], Saskia Bucciarelli[1], Lucía Casal-Dujat[1], Marie Jéhannin[1], Corinne Jud[2] and Anna Stradner[1]

[1] *Physical Chemistry, Department of Chemistry, Lund University, SE-22100 Lund, Sweden*
[2] *Adolphe Merkle Institute (AMI), University of Fribourg, CH-1700 Fribourg, Switzerland*

**najet.mahmoudi@fkem1.lu.se*

Understanding protein stability and behaviour is of central importance in biology, medicine, and chemistry. While solvent isotope effects have already provided important clues about the structural and thermal stability of disparate proteins, the effects of H/D substitution on protein phase behaviour and interactions remain to be elucidated. Here, we report the large effect of solvent isotope substitution on the phase behaviour and interactions of γB-crystallin, a globular eye lens protein interacting via a short-range attractive interaction potential. Using a combination of scattering techniques (small-angle X-ray scattering and static light scattering), we show that the liquid-liquid phase separation critical temperature T_c increases linearly from 276 K in H_2O to 292 K in D_2O (Figure 1).[1] Furthermore, we demonstrate that the phase boundaries and the osmotic compressibility of γB-crystallin scale with the reduced second virial coefficient b_2, quantifying protein–protein interactions, through the extended law of corresponding states (ELCS). This thermodynamic scaling confirms the applicability of the ELCS to the equilibrium properties of colloids with short-range attractions and provides an extension of its predictive power to systems with varying hydrogen isotope content.

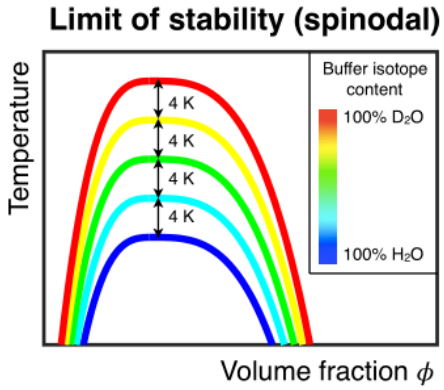

Figure 1. Spinodal of γB-crystallin solutions in solvents with varying hydrogen isotope content. The critical temperature T_c increases by 16 K upon replacing H_2O by D_2O, while the critical volume fraction ϕ_c remains constant.

Acknowledgements We acknowledge the financial support from the Swiss National Science Foundation (SNF, grant 200020-127192), the Swedish Research Council (VR, grants 621-2012-2422 and 2009-6794), the Crafoord foundation (grants 20120619 and 20140756), and the Knut and Alice Wallenberg Foundation (project grant KAW 2014.0052).

[1] S. Bucciarelli, N. Mahmoudi, L. Casal-Dujat, M. Jéhannin, C. Jud and A. Stradner, *The Journal of Physical Chemistry Letters*, 2016, **7**, 1610.