ESRF	**Experiment title: Modulation of the protein – protein interaction by natural cosolvent mixtures encountered at extreme environmental conditions**	**Experiment number**: SC-4268
Beamline: ID02	**Date of experiment**: from: 04.05.2016 to: 06.05.2016	**Date of report**: 07.03.2017
Shifts: 6	**Local contact(s)**: Johannes Möller	*Received at ESRF:*

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Report:

The aim of the experiment was to investigate the influence of biologically relevant solution conditions, including amino acids (glycine, sarcosine, □□alanine, betaine, alanine, myo-inositol, taurine and proline), urea and the compatible osmolyte trimethylamine-N-oxide (TMAO), on the pressure dependent intermolecular interaction potential of proteins in highly concentrated aqueous lysozyme solutions. In most marine organisms, small organic molecules like amino acids, methylamines and urea are accumulated to high concentrations by cells as cellular adaption to physical stresses such as low or high temperature and high pressure. Compatible (kosmotropic) osmolytes – especially TMAO and other methylamines – enhance the stability of proteins and compensate for destabilizing effects of salt ions, high temperature, high hydrostatic pressure and chaotropic agents such as urea. It has been shown that the osmolyte compositions in cells of deeper-living species differ significantly from shallow-water species. Particularly, a correlation of TMAO and habitat depth in the deep ocean within and among species was found.[1,2]

In this experiment we reveal the protein – protein interaction potential in 10 wt.-% lysozyme solutions containing complex organic osmolyte mixtures as found in muscles of marine animals (shrimps, skates, crabs) from shallow habitats, bathyal (1800-2000 m) and abyssal (2850 m) trawl sites.[2] By using biologically relevant solvent mixtures, we go one step further towards a better understanding of ‘real’ biological intracellular fluids as they appear

in Nature, and investigate in detail the differential effects of these cosolvents. SAXS measurements were performed at ESRF beamline ID02 at an incident photon energy of 16 keV and a sample-to-detector distance of 2 m with a custom-built high pressure cell.[3] Pressure series between 1 bar and 4 kbar were be carried out in 250 bar steps for different organic osmolyte compositions in 10 (w/v) % aqueous lysozyme solutions. To guarantee a constant pH 7 over the whole pressure range, 25mM Bis-Tris buffer was used.

The protein-protein interaction potential is modelled in the framework of the Derjaguin-Landau-Verwey-Overbeek (DLVO) theory by the sum of a hard sphere potential, a repulsive screened Coulomb potential and an attractive Yukawian type part. Leaving the first two parts unaltered, for lysozyme solutions a nonmonotonic dependence of the attractive potential with rising pressure was found.[4,5] In the pure buffer solution, the amplitude, J, of the attractive part of $V(r)$ shows a minimum at a pressure of 2 kbar, which may be attributed to pressure dependent changes of the solvent structure, specifically, a collapse of the second hydration shell of water.

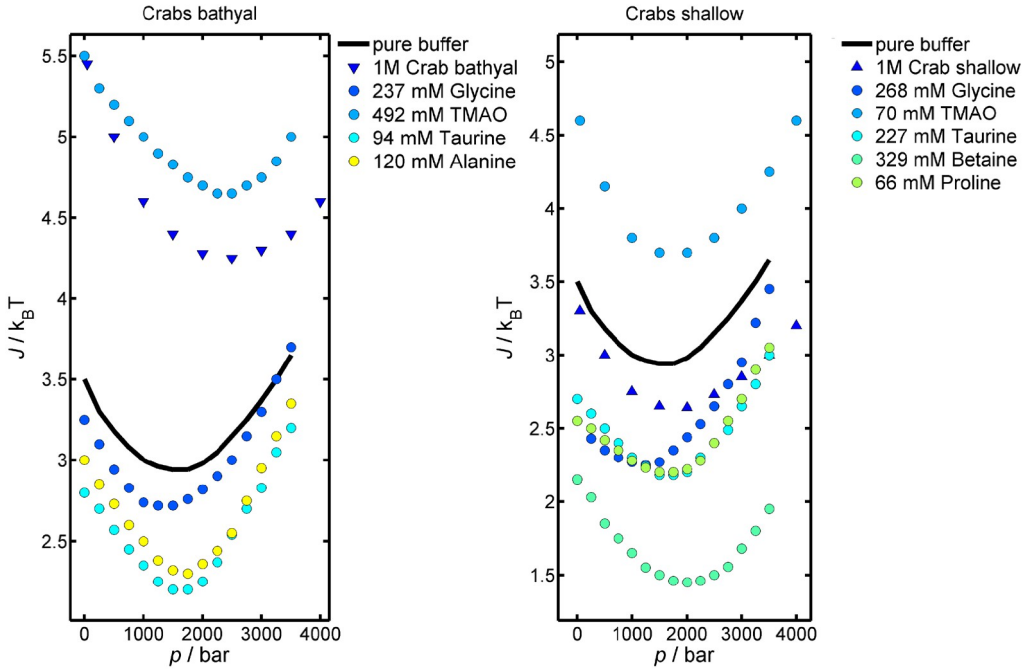

Figure 1. The experimental determined strength, $J(p)$, of the attractive Yukawian type part of the protein-protein interaction potential, $V(r)$, of a 10 (w/v)% lysozyme solution at 25°C and pH 7 for 1M osmolyte mixtures and the main components for crabs from shallow habitats (227mM taurine, 38 mM alanine, 66 mM proline, 329 mM betaine, 268 mM glycine and 70 mM TMAO) and bathyal trawl sites (94 mM taurine, 120 mM alanine, 24 mM proline, 35 mM betaine, 237 mM glycine, 492 mM TMAO) compared with the pure buffer scenario.

In Figure 1, the pressure dependence of $J(p)$ is presented exemplarily for 1 M of the osmolyte mixtures found in muscles of crabs as well as for its constituents. As clearly seen. Despite the fact that all these cosolvents are compatible, i.e. stabilize proteins against unfolding, they and also the two nature-mimicking osmolyte mixtures have a significant and different effect on the magnitude of J and, in some cases, also on its pressure dependence. For example, the effect of glycine on the intermolecular interactions is markedly different from that of TMAO, which leads to a pronounced increase of attractivity.

The protein-protein interactions in the 1 M deep-sea osmolyte mixture are clearly dominated by the strong kosmotropic agent TMAO. Conversely, the osmolyte mixture found for the shallow crabs reveals a similar protein-protein interaction potential as observed in pure buffer solution.

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