ESRF	**Experiment title:** XRS study of the influence of TMAO and urea on the structure of water	**Experiment number**: SC-3814
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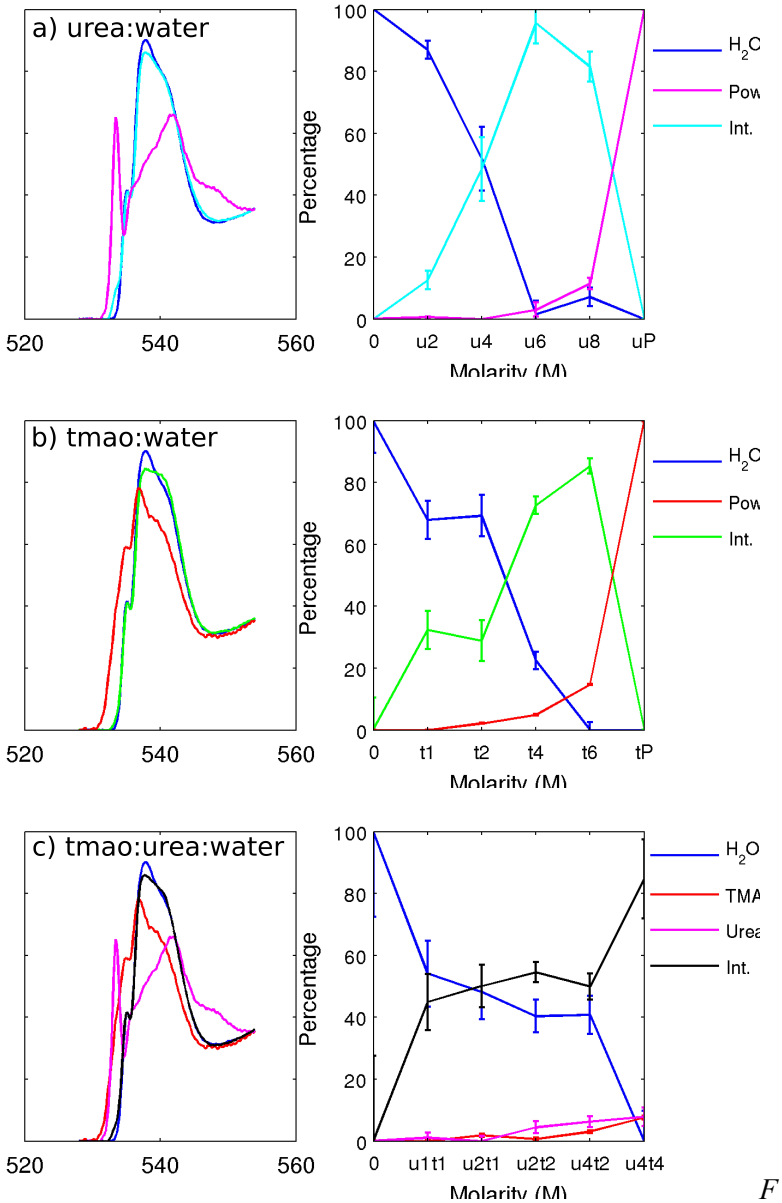
Report: The purpose of the experiment SC-3814 was to study the the microsolvation structure and bonding topology of water upon solvation of the naturally occurring osmolytes trimethylamine N-oxide (TMAO) and urea. Whereas the protein stabilizing effect of TMAO and protein destabilizing effect of urea are well studied and documented [Wang1997,Bennion2004,Krywka2008], the underlying mechanisms that lead to this stabilizing/destabilizing effect on proteins, nucleic acids, and polymers are vividly debated (e.g. see discussion in [Hunger2012]).

In essence, two different scenarios have been proposed over the past years, a direct interaction between the osmolytes and the macromolecule [Street2006,Meersman2009,Meersman2013] and an indthe microsolvation structure and bonding topology of water upon solvation of the naturally occurring osmolytes trimethylamine N-oxide (TMAO) and urea.irect mechanism mediated by the osmolyte-water interaction [Bennion2004,Hunger2012,Sarma2013,Canchi2013].

In an effort to understand the influence of these osmolytes on the microscopic structure of water and therefore the role of water in the described stabilizing and destabilizing properties, we measured the oxygen K-edge for series of concentrations of aqueous TMAO, aqueous urea, and mixtures of the two.

To distill the effects due to changes in the hydration of the solutes and changes in the structure of the solvent, both of which yield an oxygen K-edge signal due to the presence of oxygen in

the osmolytes and the water solvent, we analyzed these concentration series using constrained non-negative matrix factorization (CNNMF) [Niskanen2015a,Inkinen2015,Niskanen2015b]. The results of the decompositions are shown in Fig. 1.



igure 1: Results of the CNNMF analysis of a) the concentation series of urea:water, b) series of TMAO:water, and c) the ternary urea:TMAO:water mixtures.

Within this CNNMF procedure, we constrained the component spectra of the respective references TMAO (powder sample), urea (powder sample), and pure water, whereas we let one component vary freely to best describe the spectral changes observed along the concentration range. This free spectrum (denoted "int." in Fig. 1) gives access to the information sought: TMAO influences the water H-bond network the strongest, evidenced by the suppression of the main-edge feature, whereas urea and mixtures of urea and TMAO have smaller influence. In the presence of both solutes their mutual effect seems to be counteracted upon as evidenced by the plateau in the CNNMF coefficients (right panel of Fig. 1 c)).

These preliminary results are currently refined and can be found in [Sahle2016].

[Wang1997] A.J. Wang, et al. Biochemistry 36, 9101 (1997). **[Bennion2004]** B.J. Bennion et al. Proc. Natl. Acad. Sci. USA 101, 6433-6438 (2004). **[Krywka2008]** C. Krywka et al. ChemPhysChem 9, 2809-2815 (2008). **[Hunger2012]** J. Hunger et al., J. Phys. Chem. B 116, 4783-4795 (2012). **[Street2006]** T.O. Street et al., Proc. Natl. Acad. Sci. USA 103, 13997 (2006). **[Meersman2009]** F. Meersman et al. Biophys. J. 97, 2559 (2009). **[Meersman2013]** F. Meersman et al. Phys. Chem. Chem. Phys. 13, 13765 (2013). **[Sarma2013]** R. Sarma et al., J. Phys. Chem. B 117, 677-689 (2013). **[Canchi2013]** Canchi, Deepak R., and Angel E. Garca. "Cosolvent effects on protein stability." Annual review of physical chemistry 64 (2013): 273-293. **[Niskanen2015a]** J. Niskanen et al. The Journal of Physical Chemistry B 11732-11739 (2015). **[Inkinen2015]** J. Inkinen et al. Scientific reports 5 (2015). **[Niskanen2015b]** J. Niskanen et al. Scientific reports (accepted 2015). **[Sahle2016]** in prepariation.