Combined Influence of Ectoine and Salt:

Spectroscopic and Numerical Evidence for

Compensating Effects on Aqueous Solutions

SUPPLEMENTARY MATERIAL

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Experimental Methods

The following materials were used as received without further purification: Ectoine (purity > 99%) from Biotop (Germany), high purified water for chromatography (Lichrosolv) and deuterium oxide (Magnisolv 99.96% for NMR) from Merck (Germany), and NaCl from Sigma-Aldrich (Germany). The solutes were weighted within the cuvette on a Sartorius balance A200S and 1 mL purified water was added to obtain the correct molality. For the combined measurements of ectoine and NaCl, the NaCl was weighted separately and added

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to the solution.

Confocal Raman measurements were carried out using a confocal Raman microscope (Witec alpha300R) equiped with a 20x Zeiss EX Epiplan DIC objective (working distance 3mm, numerical aperture 0.4), an Excelsior double diode laser (wavelength 488nm, power 29.9mW), spectrometer UHTS-300-VIS (grid of 1800 gratings/mm) and thermoelectrical cooled CCD-camera Andor DV-401A-BV-532 (at -62∘ C). Laserpower was checked by Coherent Lasercheck device and temperature by an Extech RH520. Directly before the Raman measurements the cuvettes with solutions were vortexed for 20s. The spectra were recorded with an integration time of 5s and 10 accumulations each. Incident light was linear polarized. The temperature during the measurement was 26± 2∘ C. Measurements were taken in random order to avoid systematical errors originating from sources as unstable laser power or temperature change. After the measurement spectra were background subtracted within Witec Project 2.08 software using a polynomial of first order and noise threshold of zero. To compare Raman intensities, the frequency dependence of the spectra has to be taken into account.[1] Therefore all spectra were scaled by the equation given by McCreery[2] to account for the intensity dependence on vibrational frequency (ν_{vib}):

$$I_{Raman}(\nu_{vib}) \propto I_{Raman} \cdot \nu_0(\nu_0 - \nu_{vib})^3, \tag{1}$$

with I_{Raman} as Raman intensity, ν_0 as wavenumber of the exciting laser and ν_{vib} as vibrational wavenumber. Following this the spectra were normalized with respect to the area of the OH(OD) stretching region. This included the fitting procedure with fityk software[3] using fixed peaks and a Levenberg-Marquardt algorithm. The spectral range used for fitting of the H_2O spectra was limited to 3060-3700cm^{-1} to exclude the ectoine contribution. The D_2O system was included in this work as the spectra are related to the H2O spectra solely through a reduce-mass factor. Since there is no overlap exists with the ectoine features the fitting was performed within the full range of the DO stretching of 2100-2800cm^{-1}range.

The fitting residuals were below 3% for all spectra.

Simulation Details

In order to obtain valid first hydration shell configurations around ectoine for the electronic DFT calculations, we performed MD simulations with the software package GROMACS 4.5.5[4,5]. We simulated a single ectoine molecule with the force field presented in Ref. 6 in combination with the SPC/E water model[7]. Simulations with force fields from reference 6 are in accordance with experimental findings[6,8,9] and have further been validated in our group by comparing to experimental dielectric spectra (data not shown). The cubic simulation box with the dimensions $(2.67224 \times 2.67224 \times 2.67224)$ nm^3 and one ectoine was filled with 618 water molecules. Electrostatic interactions have been calculated by the Particle Mesh Ewald method[10]. The time step was $\delta t = 1$ fs and the temperature was kept constant by a Nosé-Hoover thermostat[11] at 300 K with a thermostat relaxation time of 0.5 ps. Furthermore we used the Parrinello-Rahman barostat[12] with a relaxation time of 1 ps to adjust a constant pressure of 1 bar. All bonds have been constrained by the LINCS algorithm[13]. After energy minimization, we conducted a constant pressure and constant temperature (NpT) equilibration run of 5 ns and a NpT production run of 5 ns. The evaluation of the radial distribution function for the center-of-masses between ectoine and water molecules indicates a first hydration shell radius of 0.6 nm (position of the first minimum) which corresponds to a hydration number of 9 water molecules around ectoine. We took an arbitrary snapshot of the system with one ectoine molecule and all 9 water molecules within a distance of 0.6 nm as the initial configuration for the DFT calculations. Hydrogen atoms were added manually and the structure of the cluster was minimized with respect to its total energy. The DFT calculations have been carried out with the freely available quantum chemistry program ORCA 2.9.1[14,15]. We chose the gradient-corrected GGA BLYP functional[16,17] with dispersion interactions[18] for all molecules, expanded the wavefunction into the def2-TZVPP

basis set[19], and performed a SCF geometry optimization of 100 cycles for both systems. The Raman spectrum was then calculated from the derivative of the polarizability with respect to the normal modes of the system.

Ab initio molecular dynamics (AIMD) simulations were performed for both a 64-molecule neat water system, and a 1.6 M ectoine aqueous solution (87 water molecules and three ectoine molecules). The QUICKSTEP[20] module of the CP2K program package was used to perform the ab initio MD simulations. The BLYP density funcitonal was used together with an effective, pairwise additive potential accounting for dispersion interactions[18]. The Kohn-Sham orbitals were expanded into a dual-ζ basis set (with polarization functions) optimized for calculations in the liquid phase[21] and the electronic density was expanded into an auxiliary basis set of plane waves with a kinetic energy cutoff of 400 Ry. Initial geometries were generated as described at the beginning of this section (bulk structures). We used the SPC/E force field and an ectoine force field constructed from atom types of the all-atom variant of the 'Optimized Potentials for Liquid Simulations'[22] using ACPYPE[23]. This force field reproduces the experimental mass density of 1.6 M ectoine solution, and the solution structure compares well with simulations using the united-atom force field. The resulting side lengths of the cubic cells are 1.24138 nm for neat water, and 1.46024 nm for 1.6 M ectoine solution. The obtained geometries were re-equilibrated for more than 20 ps with the AIMD methodology and a canonical sampling through velocity rescaling thermostat[24]. Afterwards the thermostat was switched off and the simulations were continued in the microcanonical ensemble. Data was collected for $\approx$ 100 ps for 1.6 M ectoine solution and $\approx$ 200 ps for pure water. In both cases heavy water was used.

References

(1) Eysel, H. H.; Bertie, J. E. Raman intensities of liquids: Precise relative measurement of some potential secondary standards, and an interim scale of absolute intensities for

blue and green excitations. *J. Raman Spectrosc.* **1988**, *19*, 59–64.

(2) McCreery, R. L. *Handbook of Vibrational Spectroscopy*; John Wiley & Sons, Ltd, 2006.

(3) Wojdyr, M. Fityk : a general-purpose peak fitting program. *J. Appl. Crystallogr.* **2010**, *43*, 1126–1128.

(4) van der Spoel, D.; Lindahl, E.; Hess, B.; Groenhof, G.; Mark, A. E.; Berendsen, H. J. C. GROMACS: Fast, flexible, and free. *J. Comp. Chem.* **2005**, *26*, 1701–1718.

(5) Pronk, S.; Páll, S.; Schulz, R.; Larsson, P.; Bjelkmar, P.; Apostolov, R.; Shirts, M. R.; Smith, J. C.; Kasson, P. M.; van der Spoel, D.; Hess, B.; Lindahl, E. GROMACS 4.5: a high-throughput and highly parallel open source molecular simulation toolkit. *Bioinformatics* **2013**, *29*, 845–854.

(6) Smiatek, J.; Harishchandra, R. K.; Rubner, O.; Galla, H.-J.; Heuer, A. Properties of Compatible Solutes in Aqueous Solution. *Biophys. Chem.* **2012**, *160*, 62–68.

(7) Berendsen, H. J. C.; Grigera, J. R.; Straatsma, T. P. The missing term in effective pair potentials. *J. Phys. Chem.* **1987**, *91*, 6269–6271.

(8) Smiatek, J.; Harishchandra, R. K.; Galla, H.-J.; Heuer, A. Low Concentrated Hydroxyectoine Solutions in Presence of DPPC Lipid Bilayers: A Computer Simulation Study. *Biophys. Chem.* **2013**, *180*, 102–109.

(9) Hahn, M. B.; Solomun, T.; Wellhausen, R.; Hermann, S.; Seitz, H.; Meyer, S.; Kunte, H.-J.; Zeman, J.; Uhlig, F.; Smiatek, J.; Sturm, H. Influence of the Compatible Solute Ectoine on the Local Water Structure: Implications for the Binding of the Protein G5P to DNA. *J. Phys. Chem. B* **2015**, *119*, 15212–15220, PMID: 26555929.

(10) Darden, T.; York, D.; Pedersen, L. Particle mesh Ewald: An N log (N) method for Ewald sums in large systems. *J. Chem. Phys.* **1993**, *98*, 10089–10092.

(11) Evans, D. J.; Holian, B. L. The Nose-Hoover thermostat. *J. Chem. Phys.* **1985**, *83*, 4069–4074.

(12) Parrinello, M.; Rahman, A. Polymorphic transitions in single crystals: A new molecular dynamics method. *J. Appl. Phys.* **1981**, *52*, 7182–7190.

(13) Hess, B.; Bekker, H.; Berendsen, H. J. C.; Fraaije, J. G. E. M. {LINCS}: {A} linear constraint solver for molecular simulations. *J. Comput. Chem.* **1997**, *18*, 1463–1472.

(14) Neese, F.; *et. al.*, Orca. *An ab Initio, Density Functional and Semiempirical Program Package version* **2009**, *2*.

(15) Neese, F. The ORCA program system. *WIRes: Comp. Mol. Sci.* **2012**, *2*, 73–78.

(16) Becke, A. D. Density-functional thermochemistry. III. The role of exact exchange. *J. Chem. Phys.* **1993**, *98*, 5648–5652.

(17) Lee, C.; Yang, W.; Parr, R. G. Development of the Colle-Salvetti correlation-energy formula into a functional of the electron density. *Phys. Rev. B* **1988**, *37*, 785.

(18) Grimme, S.; Antony, J.; Ehrlich, S.; Krieg, H. A consistent and accurate ab initio parametrization of density functional dispersion correction (DFT-D) for the 94 elements H-Pu. *J. Chem. Phys.* **2010**, *132*, 154104.

(19) Weigend, F.; Ahlrichs, R. Balanced basis sets of split valence, triple zeta valence and quadruple zeta valence quality for H to Rn: design and assessment of accuracy. *Phys. Chem. Chem. Phys.* **2005**, *7*, 3297–3305.

(20) VandeVondele, J.; Krack, M.; Mohamed, F.; Parrinello, M.; Chassaing, T.; Hutter, J. Quickstep: Fast and accurate density functional calculations using a mixed Gaussian and plane waves approach. *Comput. Phys. Commun.* **2005**, *167*, 103 – 128.

(21) VandeVondele, J.; Hutter, J. Gaussian basis sets for accurate calculations on molecular systems in gas and condensed phases. *J. Chem. Phys.* **2007**, *127*.

(22) Jorgensen, W. L.; Maxwell, D. S.; ; Tirado-Rives, J. Development and Testing of the OPLS All-Atom Force Field on Conformational Energetics and Properties of Organic Liquids. *Journal of the American Chemical Society* **1996**, *118*, 11225–11236.

(23) Sousa da Silva, A. W.; Vranken, W. F. ACPYPE - AnteChamber PYthon Parser interfacE. *BMC Research Notes* **2012**, *5*, 1–8.

(24) Bussi, G.; Donadio, D.; Parrinello, M. Canonical sampling through velocity rescaling. *J. Chem. Phys.* **2007**, *126*.