

## Structural evidence for solvent-stabilisation by aspartic acid as a mechanism for halophilic protein stability in high salt concentrations

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### Methods

Standard parameters used for the EPSR simulations of aspartic acid are shown in Tables 1, 2 and 3. The final composition of the simulation boxes used for the EPSR runs is shown in table 4.

**Table 1** Salt and water reference parameters (Taken from Mancinelli *et al*<sup>1</sup>)

	E (kJ/mol)	$\sigma$ (Å)	M	Q(e)
Ow	0.6500	3.17	16	-0.8476
Hw	0	0	2	0.4138
Cl	0.5700	4.19	36	-1
K	0.5100	2.94	39	1

**Table 2** Aspartic acid reference parameters (Taken from Jorgensen *et al*<sup>2</sup>)

	E (kJ/mol)	$\sigma$ (Å)	M	Q(e)
C <sub>1</sub>	0.4932	3.75	12	0.700
C <sub>2</sub>	0.4973	3.90	12	0
C <sub>3</sub>	0.4142	3.80	12	0.300
H <sub>1</sub>	0	0	2	0
H <sub>2</sub>	0	0	2	0.330
O <sub>1</sub> and bO <sub>1</sub>	0.8786	2.96	16	-0.800
N1	0.71128	3.25	14	-0.300

**Table 3** Aspartic acid bond lengths

Bond	Length (Å)
C <sub>1</sub> -C <sub>1</sub>	1.5914
C <sub>1</sub> -C <sub>3</sub>	1.5547
C <sub>2</sub> -C <sub>3</sub>	1.5349

<b>C<sub>1</sub>-O<sub>1</sub></b>	1.2636
<b>C<sub>2</sub>-H<sub>1</sub></b>	1.0975
<b>C<sub>3</sub>-H<sub>1</sub></b>	1.1000
<b>C<sub>3</sub>-N<sub>1</sub></b>	1.5040
<b>N<sub>1</sub>-H<sub>2</sub></b>	1.0163

**Table 4** Simulation boxes used in aspartic acid (ASP) studies.

	<b>ASP in low salt</b>	<b>ASP in high salt</b>
<b>[KCl] (M)</b>	0.25	2.50
<b>Number K+ Cl-</b>	40	400
<b>[ASP] (M)</b>	0.10	0.10
<b>Number ASP</b>	16	16
<b>[KOH] (M)</b>	0.12	0.12
<b>Number K+ OH-</b>	20	20
<b>Number H<sub>2</sub>O</b>	8888	8888
<b>Atomic number density</b>	0.0995	0.0960
<b>Temperature</b>	298K	298K

## Results

### Contributions of aspartic acid to the neutron diffraction data.

In order to determine the contribution of aspartic acid to the total scattering data solutions where measured with and without aspartic acid. The data for solutions without aspartic acid were subtracted from the aspartic acid containing solutions (fig S.I.1). The resulting residuals are the contributions of aspartic acid to the diffraction.

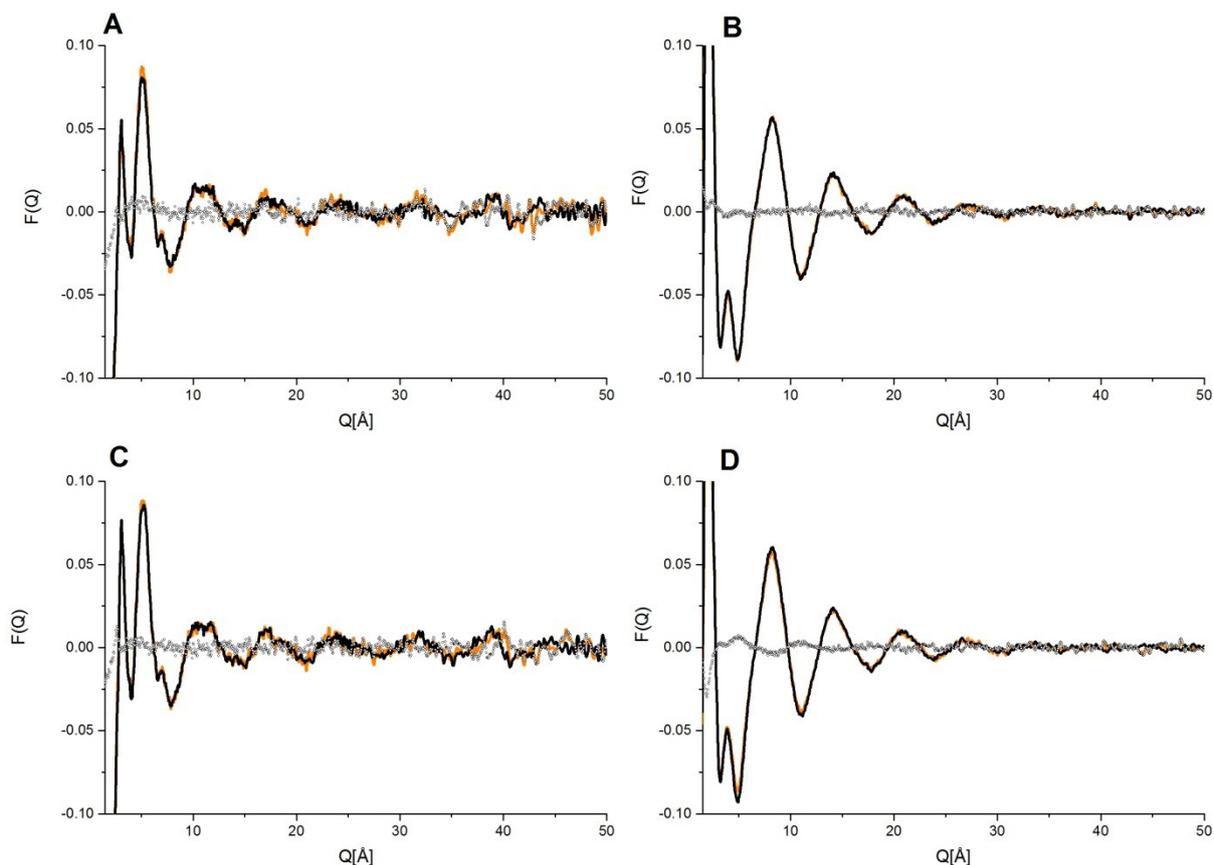


Figure S1: Measured diffraction data of samples without ASP (orange lines) and samples containing ASP (black lines) the difference between the data is indicated by black hollow spheres. Shown for high salt concentrations in A) H<sub>2</sub>O, B) D<sub>2</sub>O and low salt concentrations in C) H<sub>2</sub>O and D) D<sub>2</sub>O, respectively

The quality of the fit to all of the EPSR simulations can be compared visually by plotting the sum of the residuals squared,

$$\sum_Q [D_i(Q) - F_i(Q)]^2$$

over all datasets as a function of  $Q$ . The plots are shown below for diffraction data with ASP and an EPSR simulation with ASP (Figure S2) and diffraction data with ASP and an EPSR simulation without ASP for both the high and low salt concentration mixtures (Figure S3). In both graphs, the high salt concentration mixture is on the left and low salt concentration is on the right. Again, this shows that the EPSR simulation with ASP provides the better fit to the experimental data with ASP.

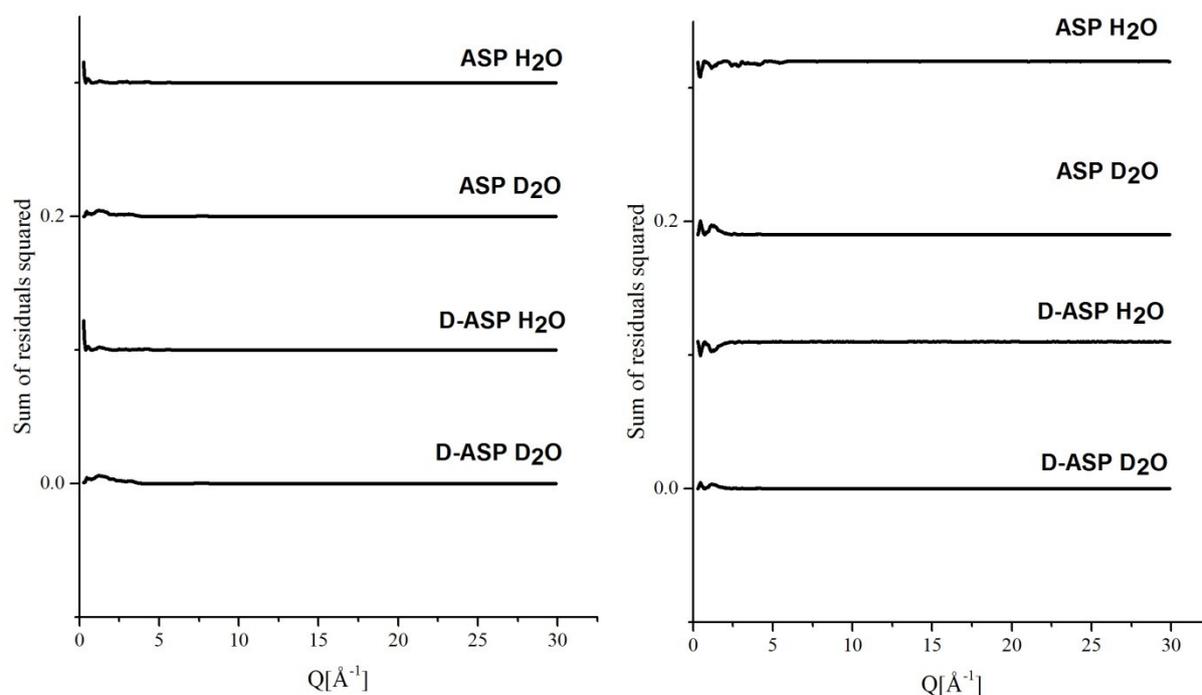


Figure S2: The sum of residuals squared as a function of  $Q$  for the 4 EPSR simulations with ASP for high salt concentration (left) and low salt concentration (right) when compared with the experimental data with ASP.

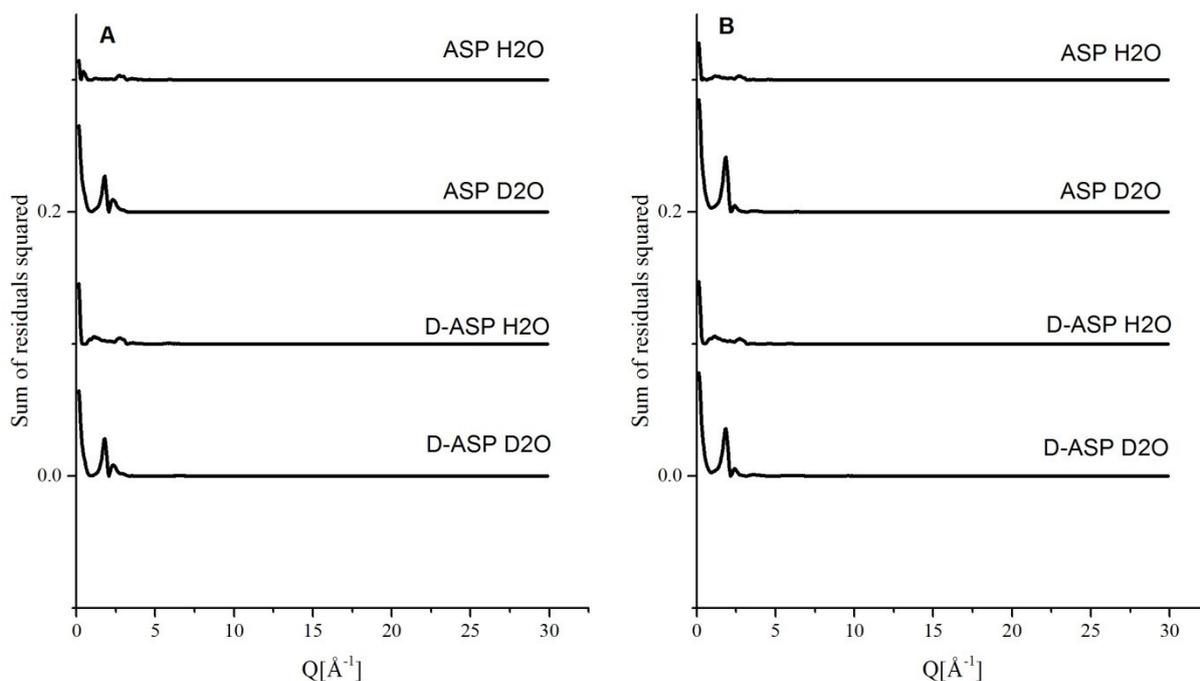
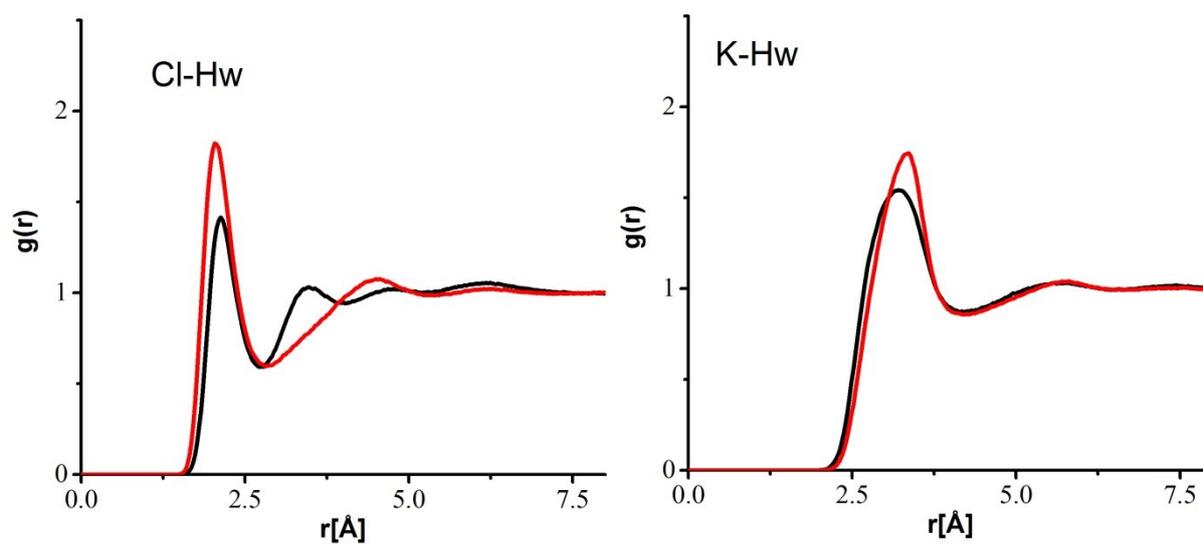


Figure S3: The sum of residuals squared as a function of  $Q$  for the 4 EPSR simulations without ASP for high salt concentration (left) and low salt concentration (right) when compared with the experimental data with ASP.

### Ion-hydration

The ion-water RDFs for high and low salt solutions are shown in Fig. S4.



**Figure S4:** Site-site RDFs of the hydrogen from water (Hw) around chloride (CL) and potassium (K) ions at low (red) and high (black) salt conditions.

## References

1. R. Mancinelli, A. Botti, F. Bruni, M. A. Ricci and A. K. Soper, *The journal of physical chemistry. B*, 2007, **111**, 13570-13577
2. Jorgensen WL, Maxwell DS, Tirado-Rives J. *Journal of the American Chemical Society*. **1996**;118(45):11225-36.