Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics.
This journal is © the Owner Societies 2016
CREATED USING THE RSC LaTeX PCCP ARTICLE TEMPLATE – SEE www.rsc.org/electronicfiles FOR DETAILS

Electronic Supporting Information: Atomistic insights into urea-protein interactions in solution.

Nicola Steinke,[a] Richard J. Gillams,[a] Luis Carlos Pardo,[b] Christian D. Lorenz[c] and Sylvia E. McLain[a]*

1 Neutron Diffraction

As mentioned in the main text isotopically substituted yet chemically identical samples were prepared at a 1:4:58 ratio of GPG: urea : water. Table 1 gives a summary of these samples. Please notice that the isotopical substitution was only done for the exchangeable hydrogens (Hn1, Hn3, HT1, HT2, Hu and Hw; see Table 2 and Fig. 1

Sample	GPG + urea + water
1	100%H
2	64%H 36%D GPG
3	50%H 50%D GPG
4	64%H 36%D GPG
5	100%D

Table 1 List of samples used for collection of neutron diffraction data.

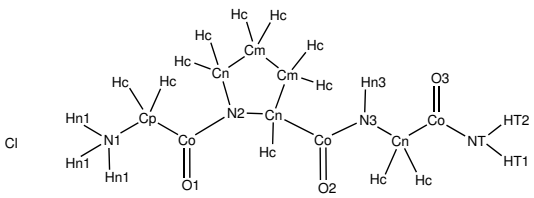

Fig. 1 Full GPG labelling.

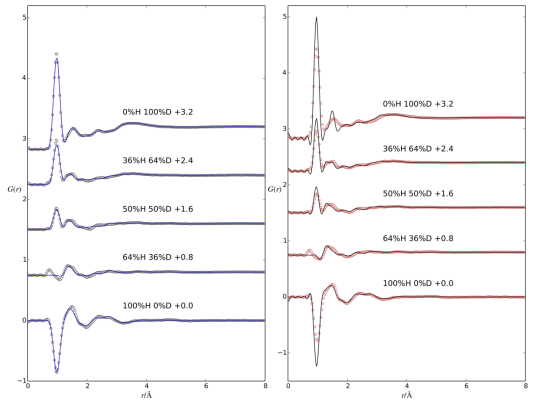

Fig. 2 Sum of radial distribution function ($G(r)$) from EPSR for MD (right)and EPSR (left). The coloured dots correspond to the data, the black lines to the individual simulations.

2 EPSR

An EPSR simulation was set up with 20 GPG, 20 chlorine, 80 urea and 1160 water molecules. An experimentally determined atomic density of 0.10175 atoms $Å^{-3}$ was used. Fig 1 shows the detailed GPG atom labelling. The initial parameters were determined by MD and a CHARMM forcefield and are shown in Table 2. To limit the number of atom labels, similiar atoms were grouped together (e.g. Hc, Co...). The measured $F(Q)$ compared with MD and EPSR simulations can be found in the main text. The related fourier-transformed $G(r)$ are in Fig. 2.

3 Additional RDFs

Figure 3 shows the Ow-Hw and Hw-Hw $g(r)$s for water-water interaction in addition to the Ow-Ow $g(r)$ in the main text. These functions are shown for the present solutions for gpg:water in the absence of urea,[1], for pure water[2] and for urea:water solutions at approximately the same concentration as the urea:water concentration in the present system.[3]

Figure 4 shows $g(r)$s for water and urea hydrogen bonding interactions to the hydrogens (bound to nitrogens) on GPG (Fig. 1, main text). The coordination numbers for these functions, each taken at the first peak minimum are listed in Table 3.

[a] *Department of Biochemistry, University of Oxford, Oxford OX1 3QU, UK*
[b] *Departament de Física i Enginyeria Nuclear, Escola Tècnica Superior d'Enginyeria Industrial de Barcelona (ETSEIB), Universitat Politècnica de Catalunya, 08028 Barcelona, Catalonia (Spain)*
[c] *Department of Physics, King's College London, London SE1 9NH, UK*
**Email:sylvia.mclain@bioch.ox.ac.uk*
† Electronic Supplementary Information (ESI) available

Atom Label	ε (kJ mol^{-1})	σ (Å)	q (e)
N1	0.83680	3.2963	-0.300
Hn1	0.19246	0.4000	0.330
Cp	0.23012	3.8754	0.130
Hc	0.92050	2.3520	0.090
Co	0.37656	3.5636	0.530
O1	0.50208	3.0291	-0.530
N2	0.83680	3.2963	-0.270
Cn	0.15481	3.9645	0.000
Cm	0.23012	3.8754	-0.180
O2	0.50208	3.0291	-0.530
N3	0.83680	3.2963	-0.490
Hn3	0.19246	0.4000	0.310
O3	0.50208	3.0291	-0.530
NT	0.83680	3.2963	-0.620
HT1	0.19246	0.4000	0.320
HT2	0.19246	0.4000	0.300
Cl	0.62760	4.0447	-1.000
Hu	0.19246	0.4000	0.340
C	0.29288	3.5636	0.600
Nu	0.83680	3.2963	-690
Ou	0.50208	3.0291	-0.580
Hw	0.19246	0.4000	0.417
Ow	0.63639	3.1507	-0.834

Table 2 The parameters used in the EPSR simulations of the GPG/urea/water samples. The first 17 atom types belong to GPG. For urea and water the second letter of the atom label (u, w) indicates the molecule to which the atom belongs.

To estimate the amount of "folded" GPG with and without urea, Fig. 5 shows the intramolecular $g(r)$ between the C-terminal amide cap (HT1) and the first carbonyl oxygen O1. The corresponding coordination numbers in 4 give the fraction of GPG molecules with a closer O1-HT1 distance.

The 'inverse' coordination numbers for Tables 1 & 2 in the main text are showin in Table 5, which were used to calculate the probability ratio ($P^r_{UW_X}$; Table 3 main text) for urea/water preference around peptide bond atoms in GPG.

4 ANGULA

In Fig. 6, 7, 8 the orthonormal coordinate systems are ahown that were assigned to the molecules for the ANGULA analysis.

References

1 S. Busch, L. C. Pardo, W. B. O'Dell, C. D. Bruce, C. D. Lorenz and S. E. McLain, *Phys. Chem. Chem. Phys.*, 2013, **15**, 21023–21033.

2 A. K. Soper, *ISRN Physical Chemistry*, 2013, **2013**, 1–67.

3 A. Soper, Unpublished data courtesy of AK Soper.

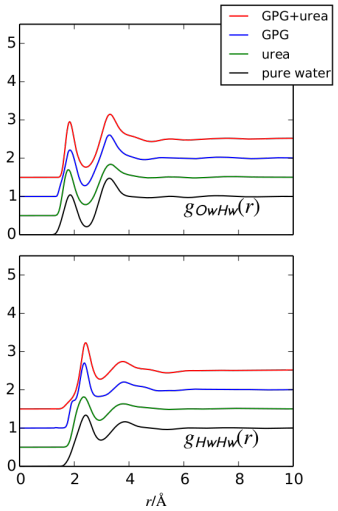

Fig. 3 Radial distribution function ($g(r)$) for water-water interactions not shown in the main text. These are shown for the present solution as well as for previous investigations on GPG in water,[1] pure water[2] and urea in water at approximately the same concentration as for the current solutions.[3]

$g(r)$	EPSR	MD
Hn1–Ow	0.77	0.82
Hn3–Ow	0.78	0.78
HT1–Ow	0.71	0.73
HT2–Ow	0.65	0.60
Hn1–Ou	0.08	0.09
Hn3–Ou	0.06	0.15
HT1–Ou	0.09	0.05
HT2–Ou	0.08	0.07

Table 3 Coordination numbers (n^{β}_{α}) for urea and water around GPG nitrogen atoms from both EPSR and MD. The minima for Hn1 is 2.25 Å and for the other correlations is 2.49 Å.

	EPSR		MD	
r / Å	GPG	GPG + urea	GPG	GPG + urea
0-4	0.11	0.04	0.12	0.24
0-5	0.17	0.07	0.25	0.37

Table 4 Coordination numbers (n^{β}_{α}) for the intramolecular GPG-$g(r)$ in Fig. 5

This journal is © The Royal Society of Chemistry [year]

$g(r)$	r_1/Å	EPSR	MD $_{\text{unf}}$	MD $_{\text{f}}$
Hw–O1	2.52	0.0112	0.0077	0.0066
Hw–O2	2.52	0.0139	0.0117	0.0114
Hw–O3	2.52	0.0152	0.0144	0.0145
Ow–N1	3.60	0.0583	0.0687	0.0694
Ow–N2	6.75	0.4680	0.4520	0.4490
Ow–N3	3.42	0.0164	0.0178	0.0180
Ow–NT	4.26	0.1080	0.1010	0.0942
Ow–Hn1	2.25	0.0133	0.0415	0.0420
Ow–Hn3	2.58	0.0134	0.0129	0.0131
Ow–HT1	2.49	0.0122	0.0098	0.0064
Ow–HT2	2.40	0.0112	0.0089	0.0090
Hu–O1	2.52	0.0075	0.0126	0.0101
Hu–O2	2.52	0.0131	0.0147	0.0146
Hu–O3	2.52	0.0163	0.0158	0.0148
Ou–N1	3.60	0.0750	0.0899	0.0896
Ou–N2	6.75	0.4480	0.5800	0.5820
Ou–N3	3.42	0.0275	0.0307	0.0333
Ou–NT	4.26	0.1050	0.1050	0.0914
Ou–Hn1	2.25	0.0200	0.0628	0.0630
Ou–Hn3	2.58	0.0150	0.0271	0.0284
Ou–HT1	2.49	0.0225	0.0155	0.0082
Ou–HT2	2.40	0.0200	0.0174	0.0170

Table 5 Inverse coordination numbers for GPG atoms around urea and water from both EPSR and MD. For MD are two contact coefficients are listed with folded peptides having an intramolecular distance between the O1 and HT1 atoms less than 4.5 /AA, while unfolded peptides have a distance between O1 and HT1 atoms greater than 4.5 Å. The minima where the CN where taken are also listed.

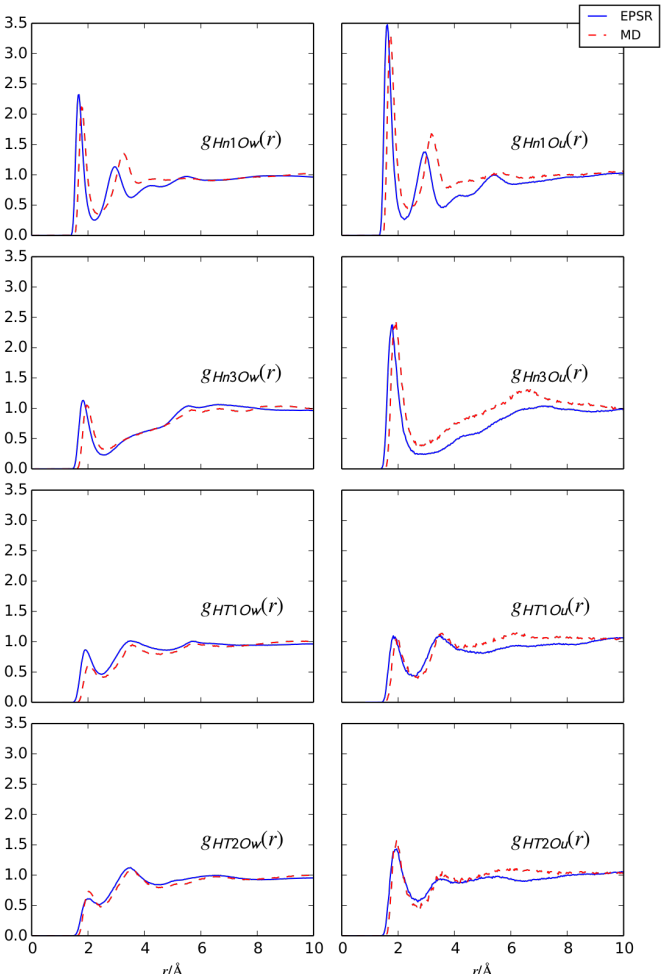

Fig. 4 Radial distribution function ($g(r)$) for GPG peptide bond nitrogens and N terminal nitrogen and Ow/Ou atoms from water and urea, respectively, from MD and EPSR.

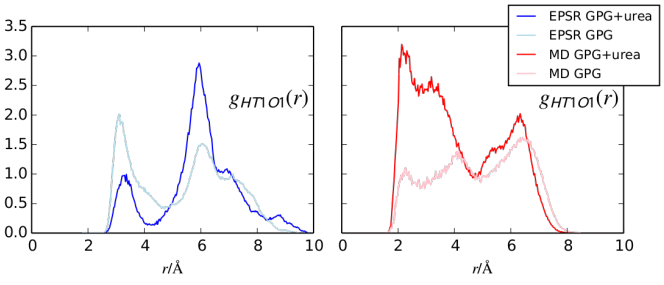

Fig. 5 Radial distribution function ($g(r)$) for GPG peptide bond nitrogens and N terminal nitrogen and Ow/Ou atoms from water and urea, respectively, from MD and EPSR.

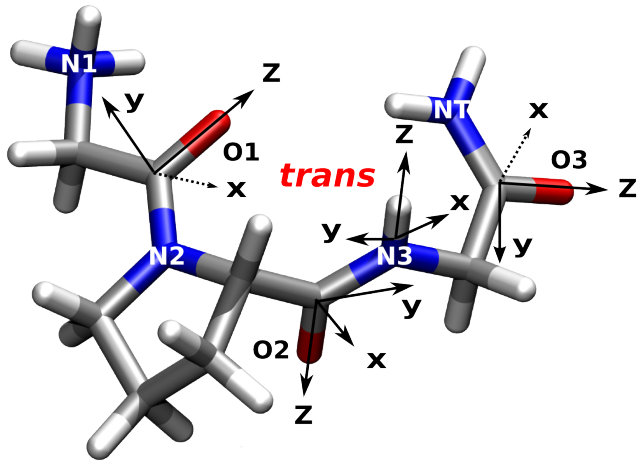

Fig. 6 Orthonormal coordinate systems on each GPG carbonyl group

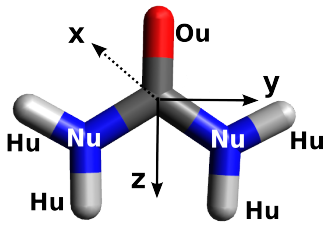

Fig. 7 Orthonormal coordinate systems on urea

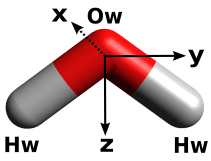

Fig. 8 Orthonormal coordinate systems on water

This journal is © The Royal Society of Chemistry [year]