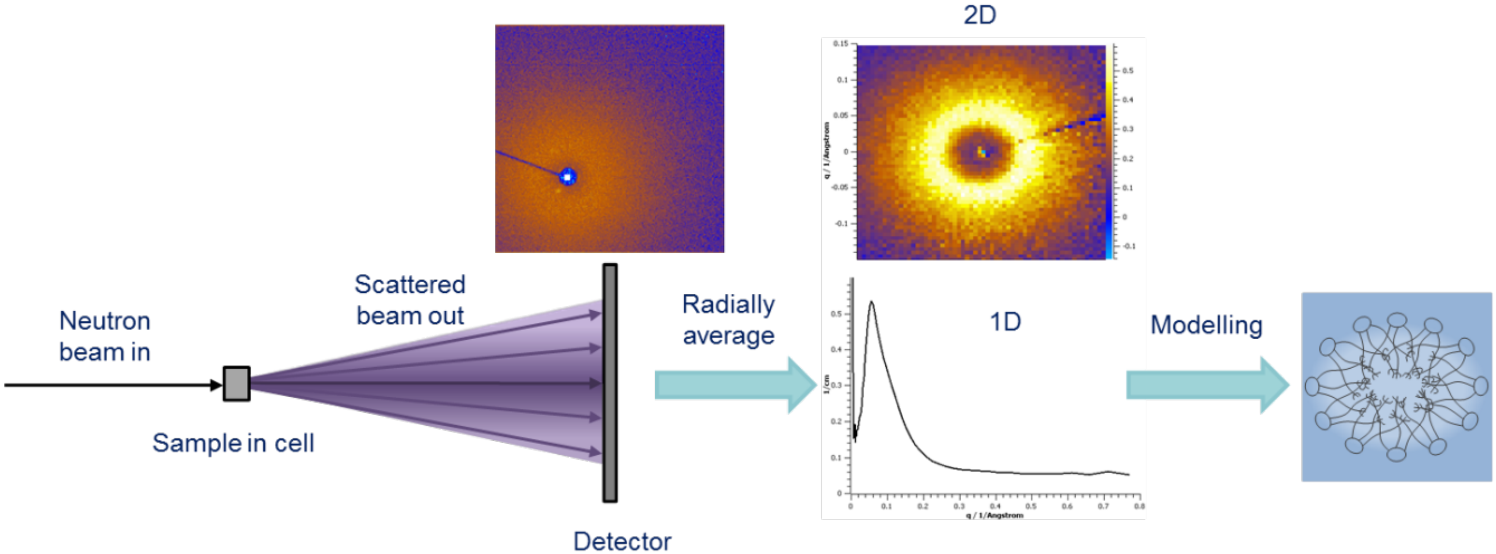
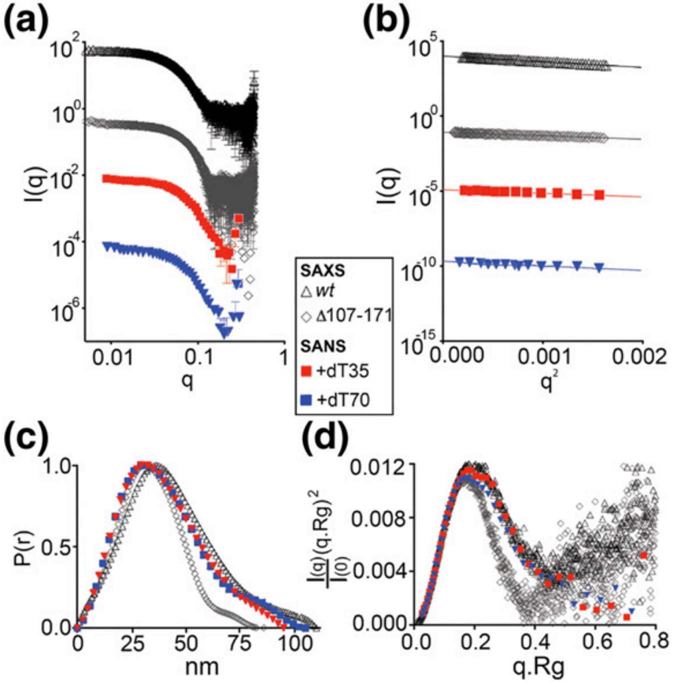
Very wide-angle scattering of neutrons to look at the solvent layer of proteins

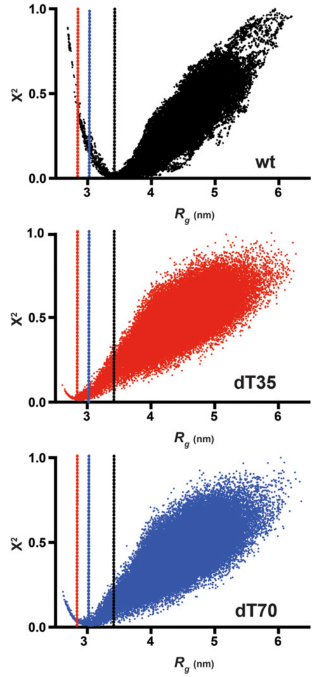
David J. Scott

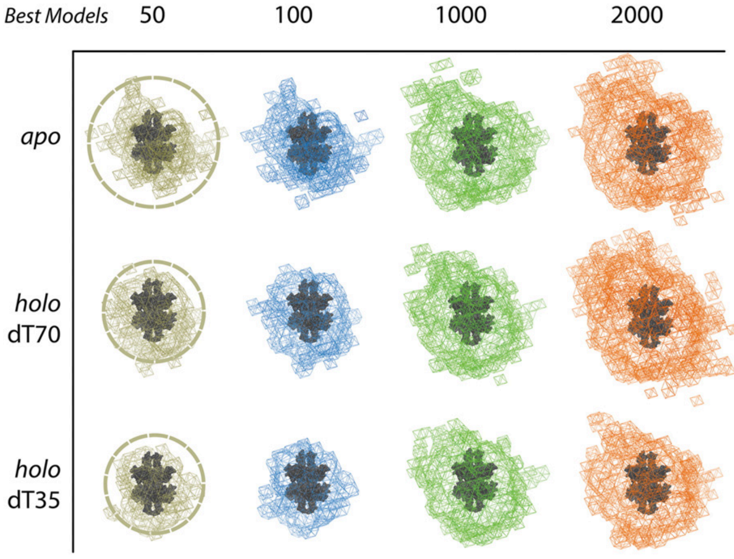
University of Nottingham/Research Complex at Harwell

Neutron scattering









Wider and yet wider angles

The Solvent Layer in Neutron Scattering

Protein hydration in solution: Experimental observation by x-ray and neutron scattering

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Proc. Natl. Acad. Sci. USA 95 (1998) 2269

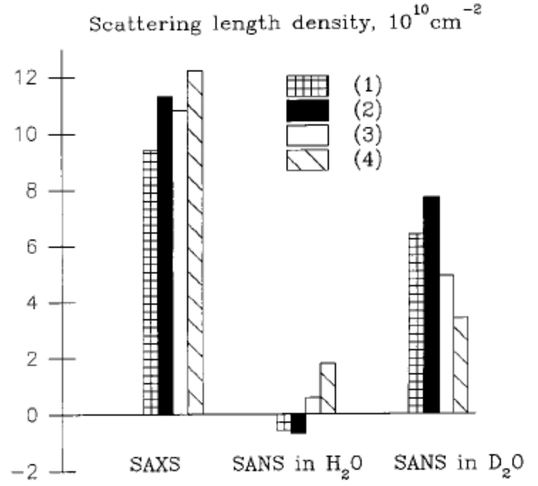
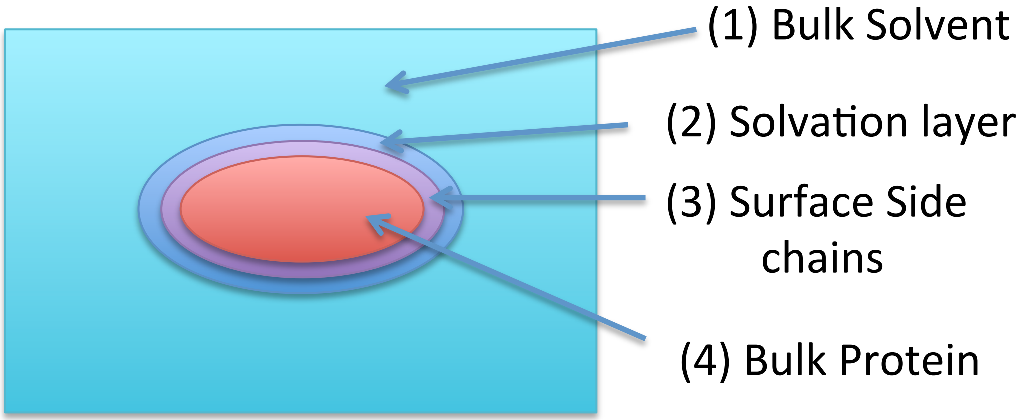


FIG. 1. Relationship among the scattering densities of the bulk solvent, protein, and protein–solvent interface for x-rays and neutrons in H_2O and D_2O. (1) Bulk solvent, (2) a shell with density 20% above that of the bulk solvent, (3) mobility of the side chains on the protein surface; scattering density in the interface is drawn in the middle between those of protein and of bulk; (4) protein. Scattering density of protein in D_2O is larger than that in H_2O because of H/D exchange.



(1) Bulk Solvent

(2) Solvation layer

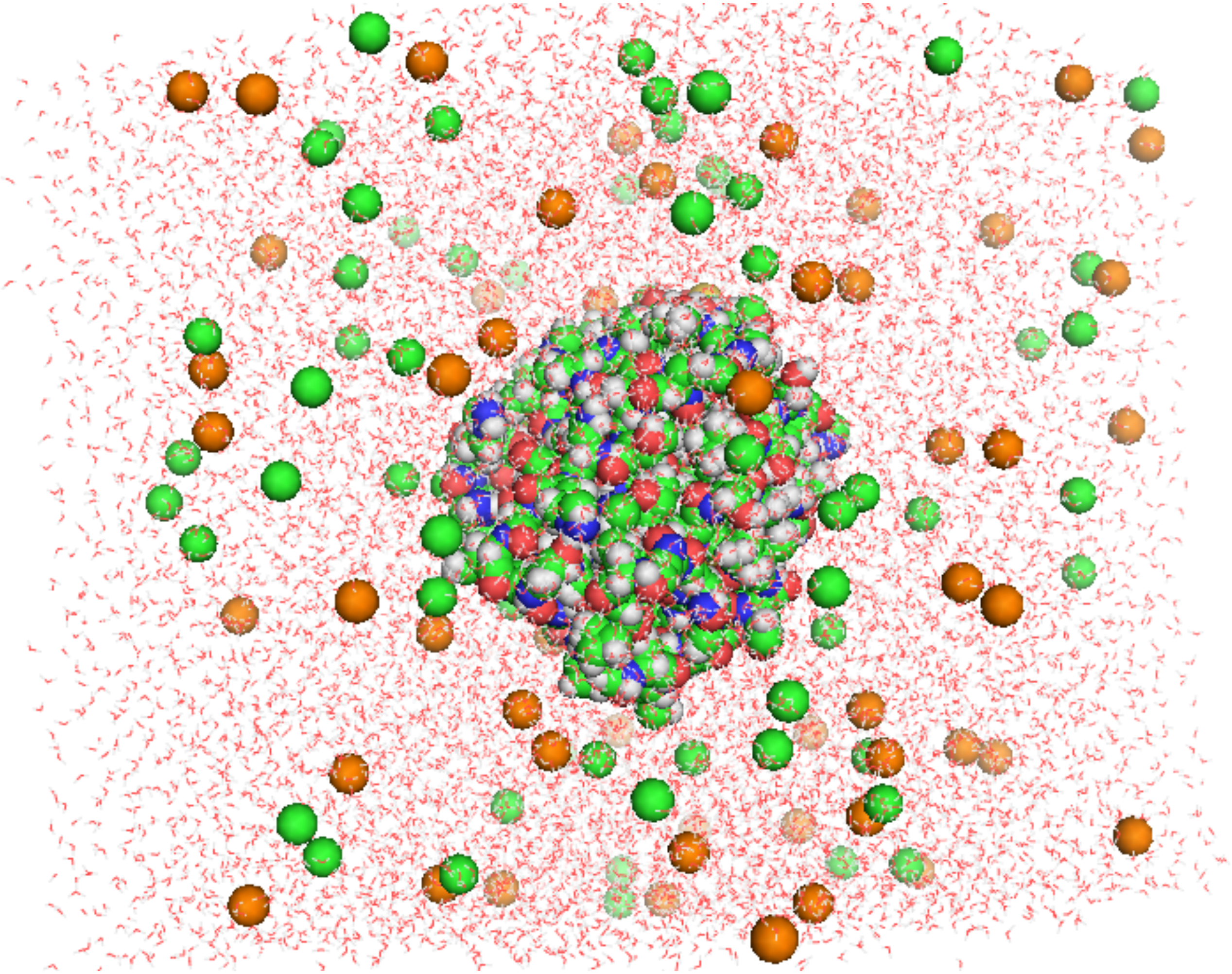
(3) Surface Side chains

(4) Bulk Protein

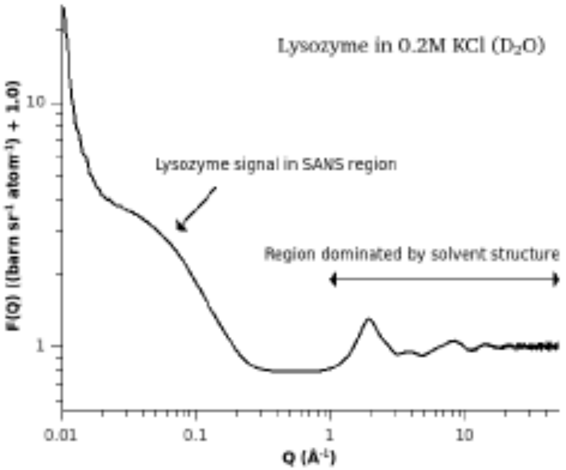
- Assumes a dense first layer of solvent only
- SAXS data analysis needs this layer present
- In SANS, this layer is contrast dependent

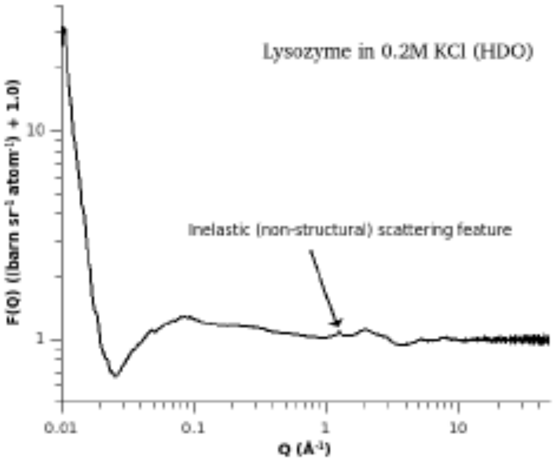
Water, water, everywhere and ignored by just about everyone…

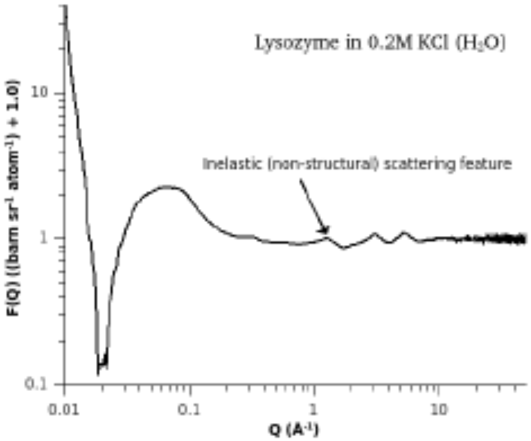
Test system: good old lysozyme

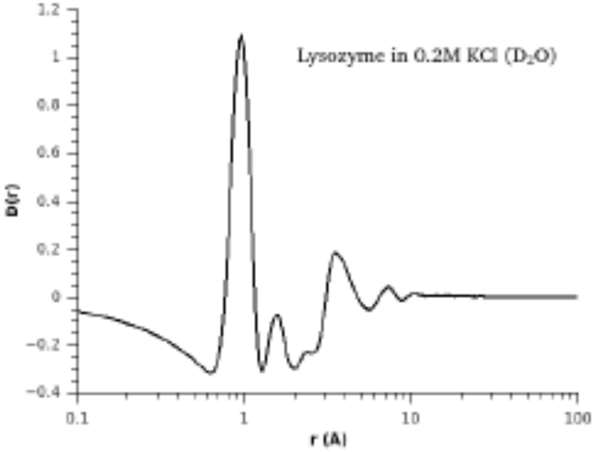


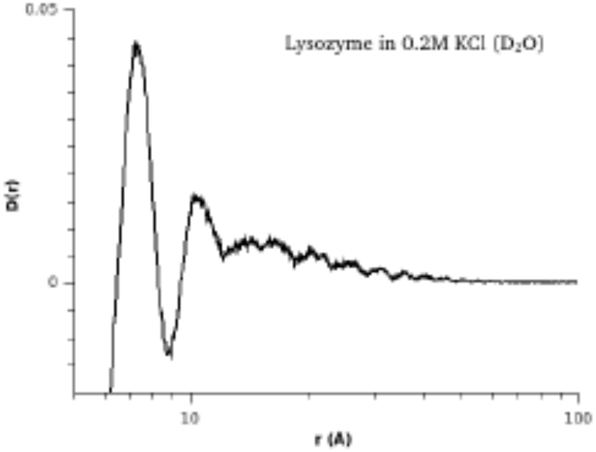
Data


Lysozyme in 0.2M KCl (D_2O)
Lysozyme signal in SANS region
Region dominated by solvent structure
F(Q) ((barn sr^{-1} atom^{-1}) + 1.0)
10
1
0.01
0.1
1
10
Q (Å^{-1})

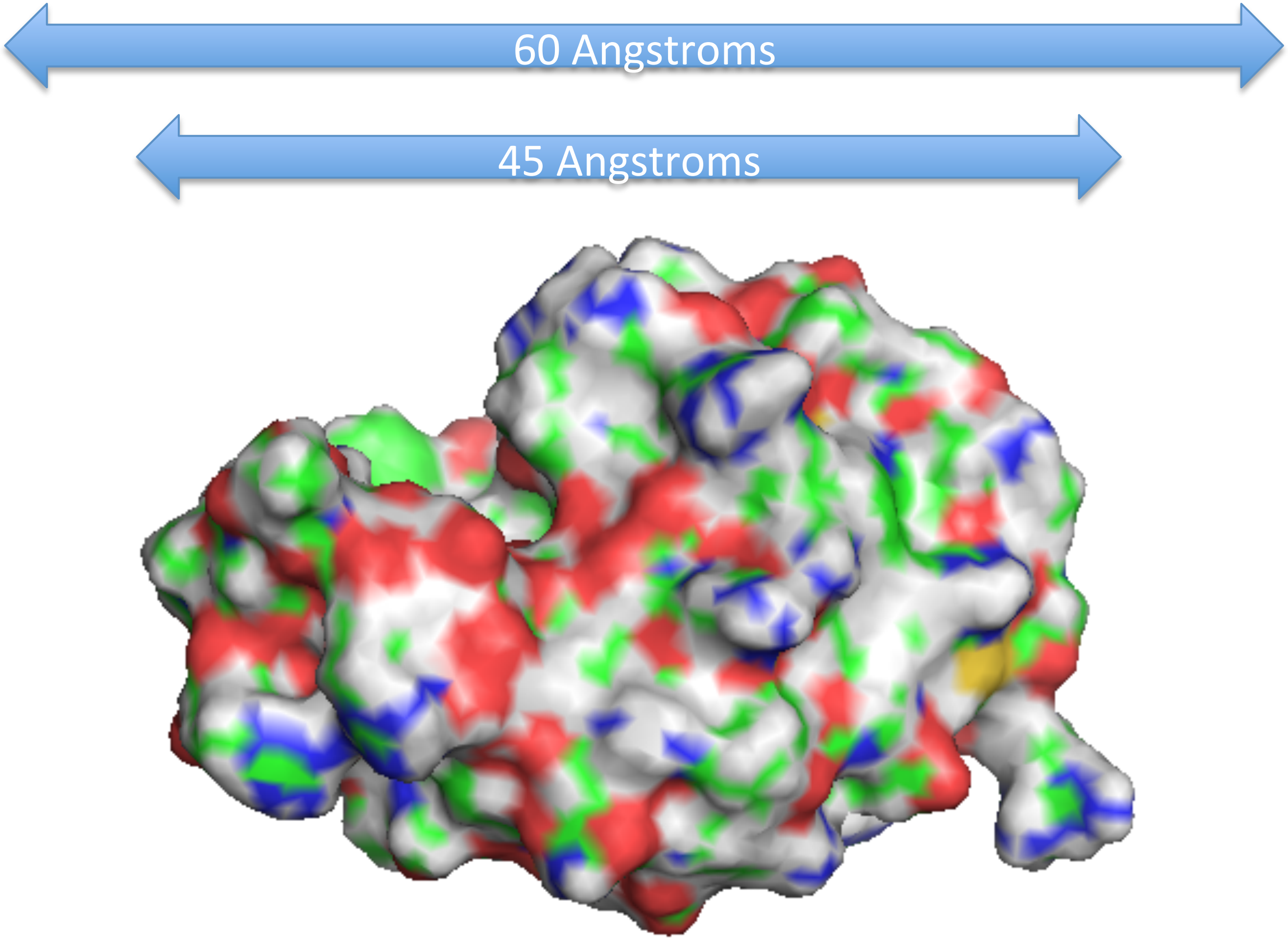

Lysozyme in 0.2M KCl (HDO)
Inelastic (non-structural) scattering feature
F(Q) ((barn sr^{-1} atom^{-1}) + 1.0)
10
1
0.01
0.1
1
10
Q (Å^{-1})


Lysozyme in 0.2M KCl (H_2O)
Inelastic (non-structural) scattering feature
F(Q) ((barn sr^{-1} atom^{-1}) + 1.0)
10
1
0.1
0.01
0.1
1
10
Q (Å^{-1})


Lysozyme in 0.2M KCl (D_2O)
D(r)
1.2
1
0.8
0.6
0.4
0.2
0
−0.2
−0.4
0.1
1
10
100
r (Å)


Lysozyme in 0.2M KCl (D_2O)
D(r)
0.05
0
10
100
r (Å)

Unaccounted distances



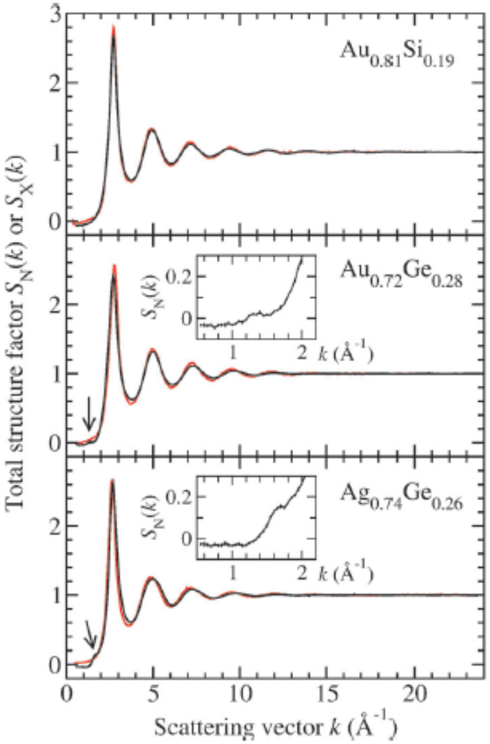
60 Angstroms

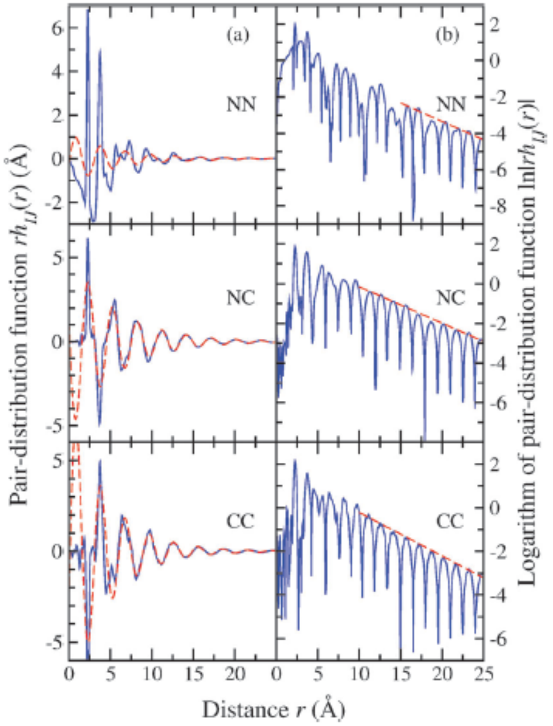
45 Angstroms

Empirical Potential Structure Refinement

- Instead of refining against Chi2, refine against the energy of the system.
- This gives more realistic and less “averaged” partial distribution functions.
- Using different neutron constrasts allows refinement against experimental data using reverse MCMC.
- Produces physically realistic distributions against experimental data and validated against many different molecular systems.

Applications to Small molecules



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Identifying and characterising the different structural length scales in liquids and glasses: an experimental approach

Cite this: *Phys. Chem. Chem. Phys.*, 2013, **15**, 15286

Philip S. Salmon* and Anita Zeidler*

Application to large molecules

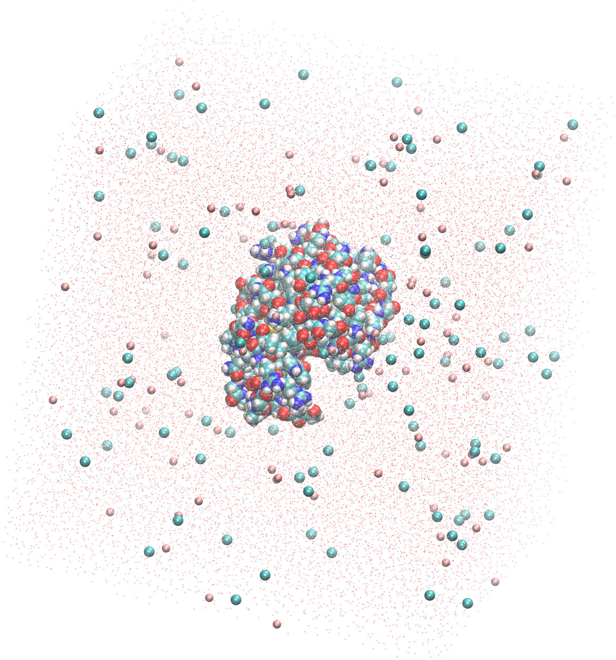
- Amino acids
- Dipeptides
- Small peptides
- Micellar system

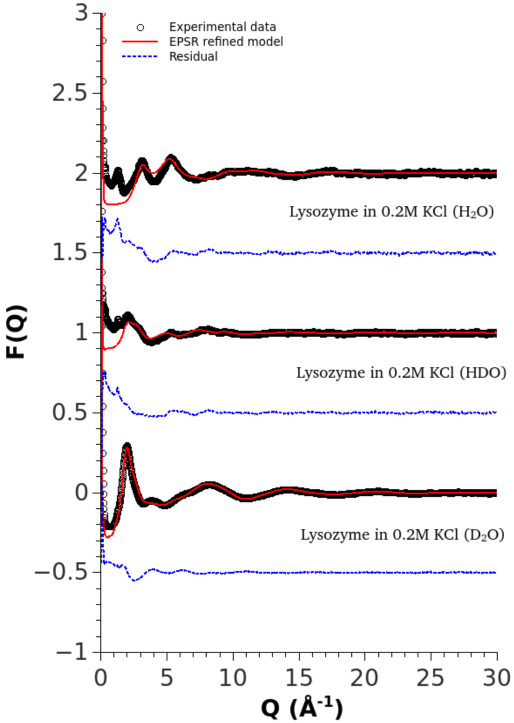
Structure Factor Information

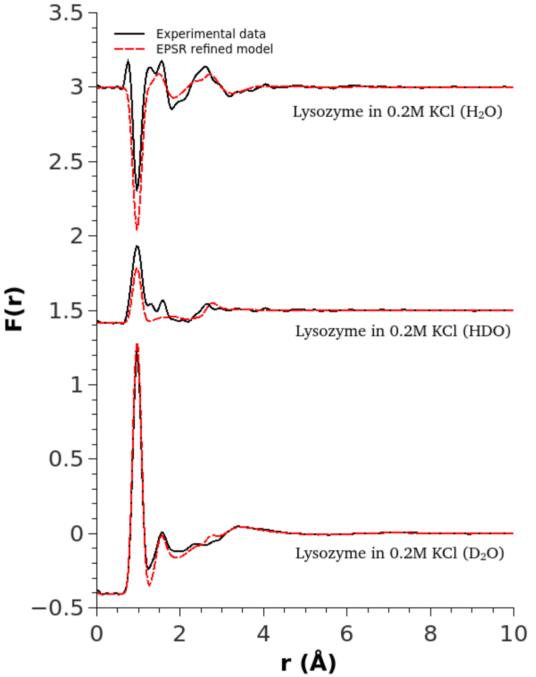
$$F\left(Q\right)=\sum_{\alpha,\beta\geq\alpha}\left(2-\delta_{\alpha\beta}\right)c_{\alpha}b_{\alpha}c_{\beta}b_{\beta}\left(S_{\alpha\beta}\left(Q\right)-1\right)$$

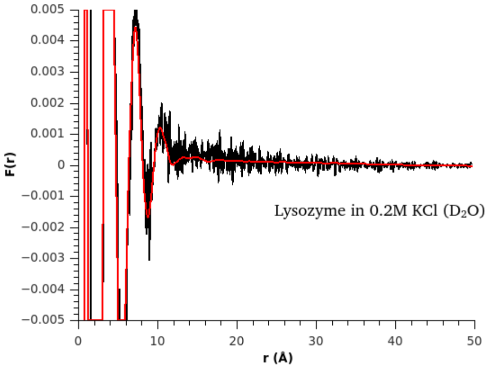
$$S_{\alpha\beta}\left(Q\right)=1+\frac{4\pi\rho_{0}}{Q}\int_{0}^{\infty}rG_{\alpha\beta}\left(r\right)\sin\left(Qr\right)dr$$

Data fitting

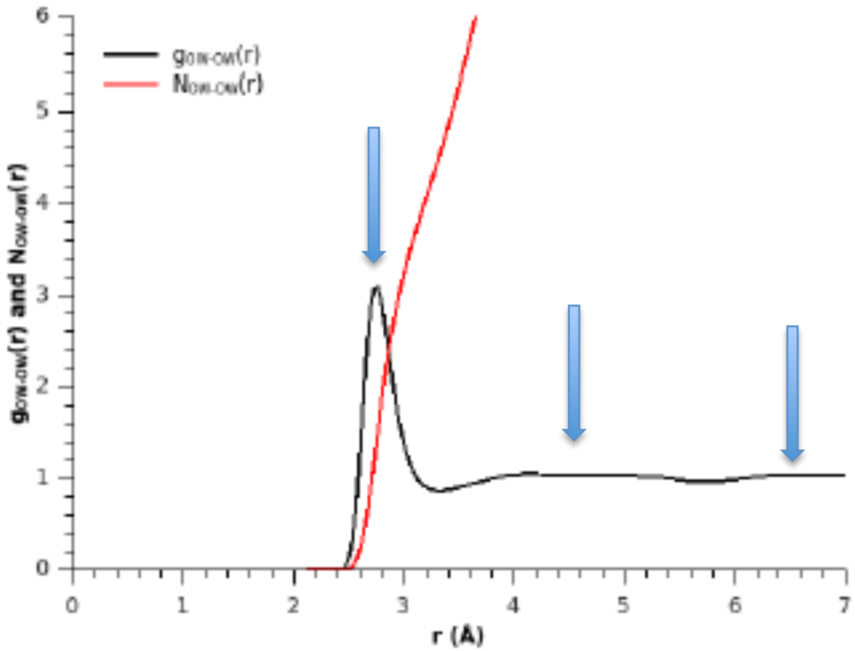


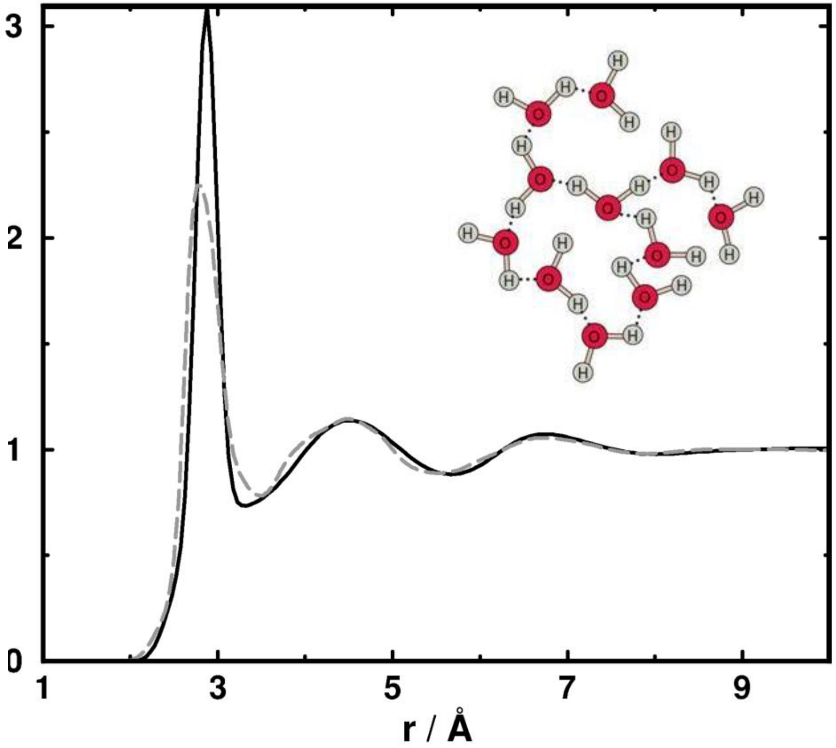






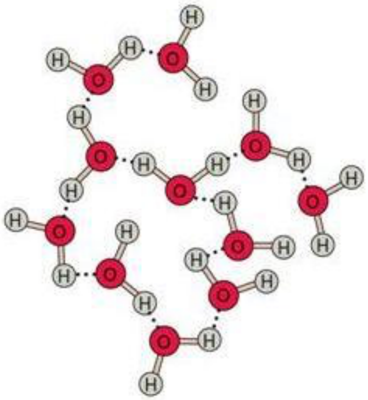
Water-water interactions

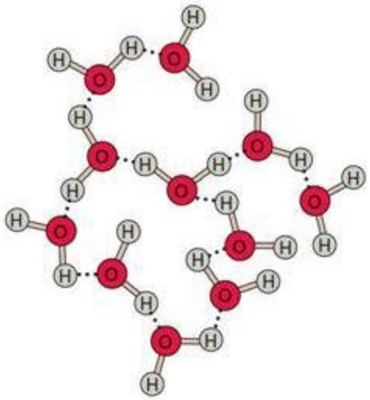


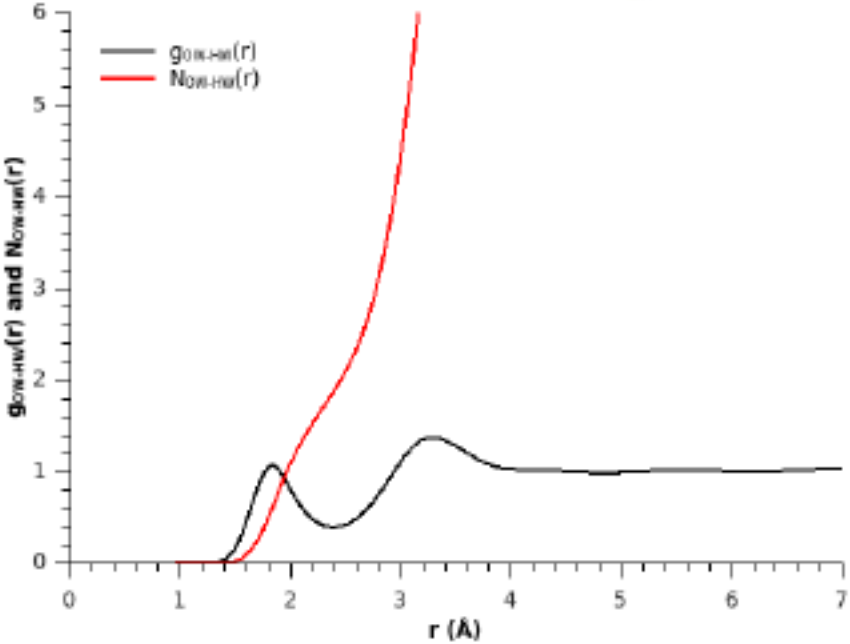


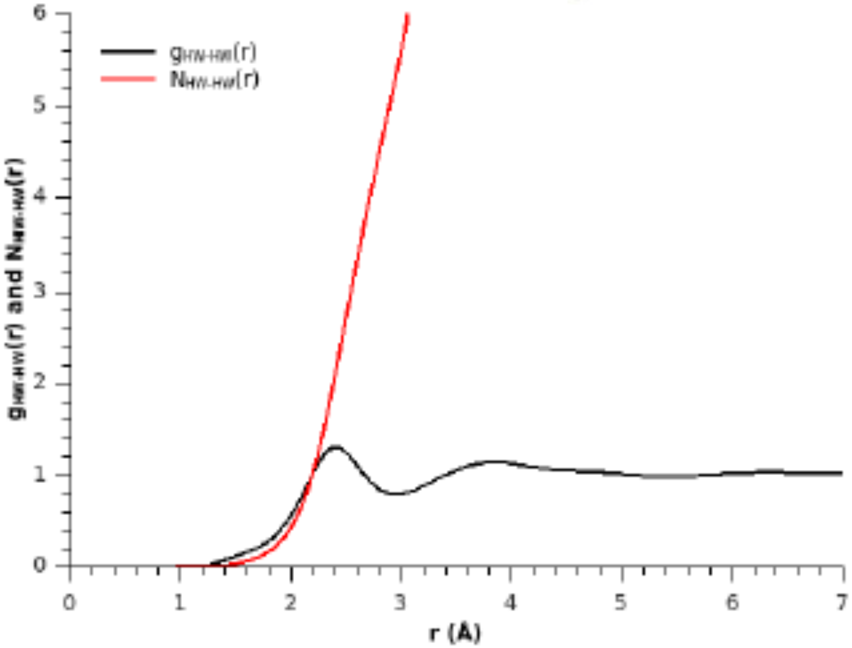
1. A. K. Soper, F. Bruni, and M. A. Ricci, J. Chem. Phys. 106, 247 (1997).
2. 2. A. K. Soper and J. Turner, Int. J. Mod. Phys. B 7, 3049 (1993).

Water-water interactions

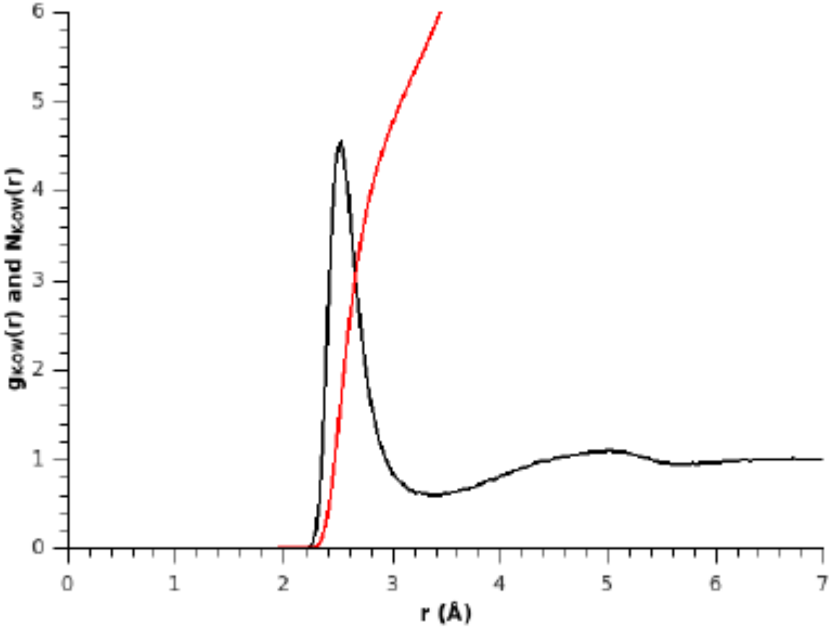


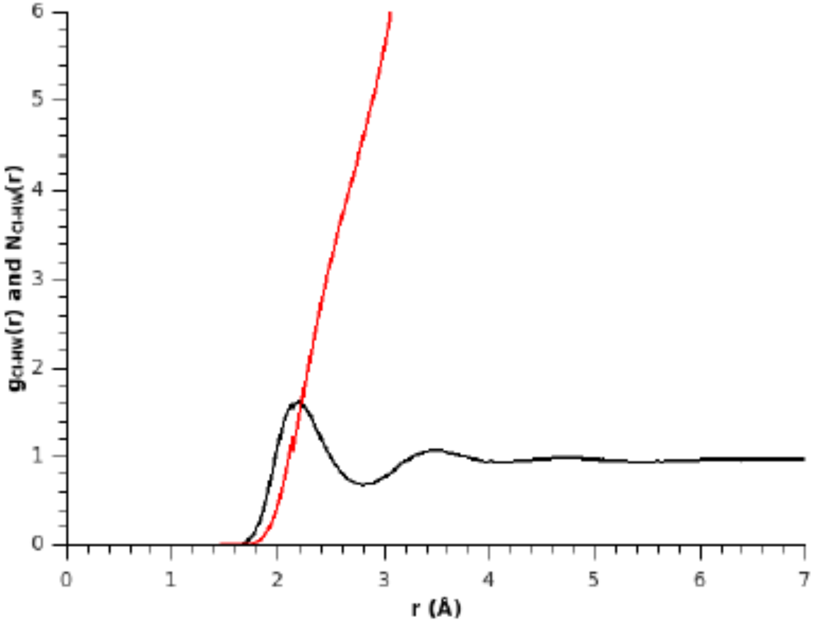


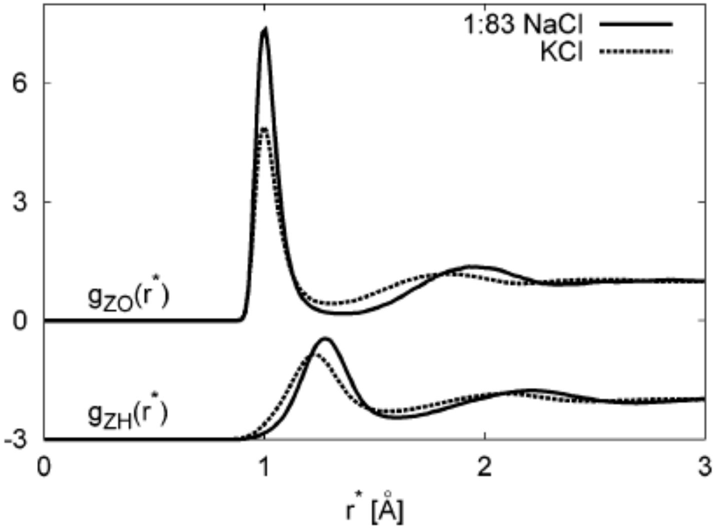


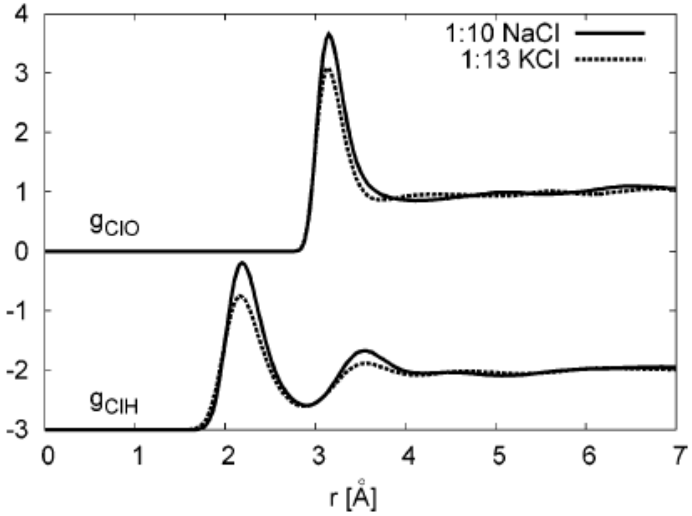


Salt hydration



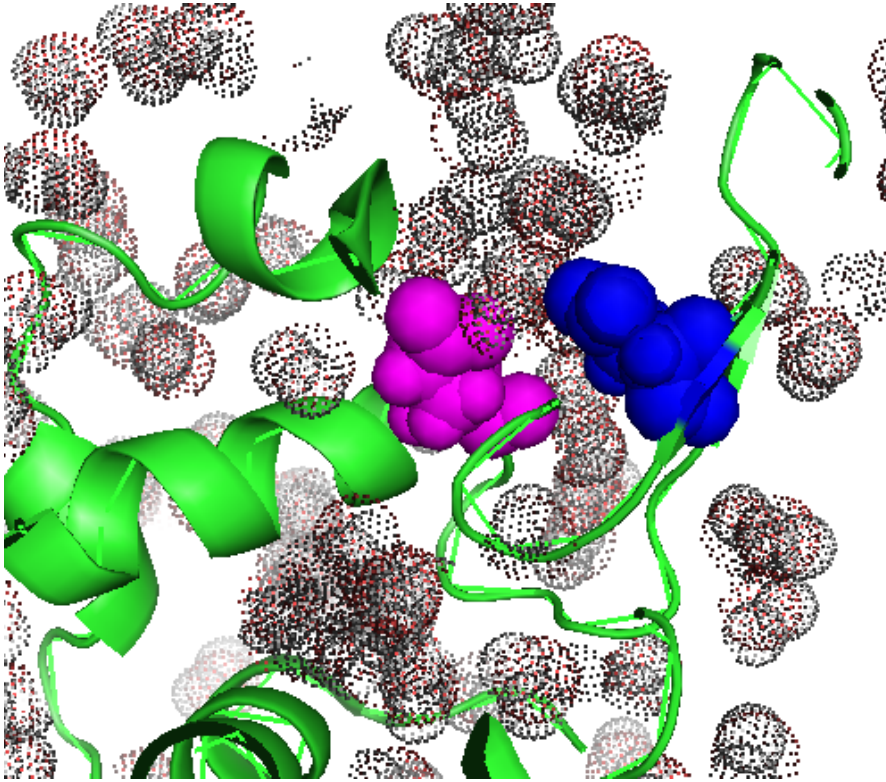




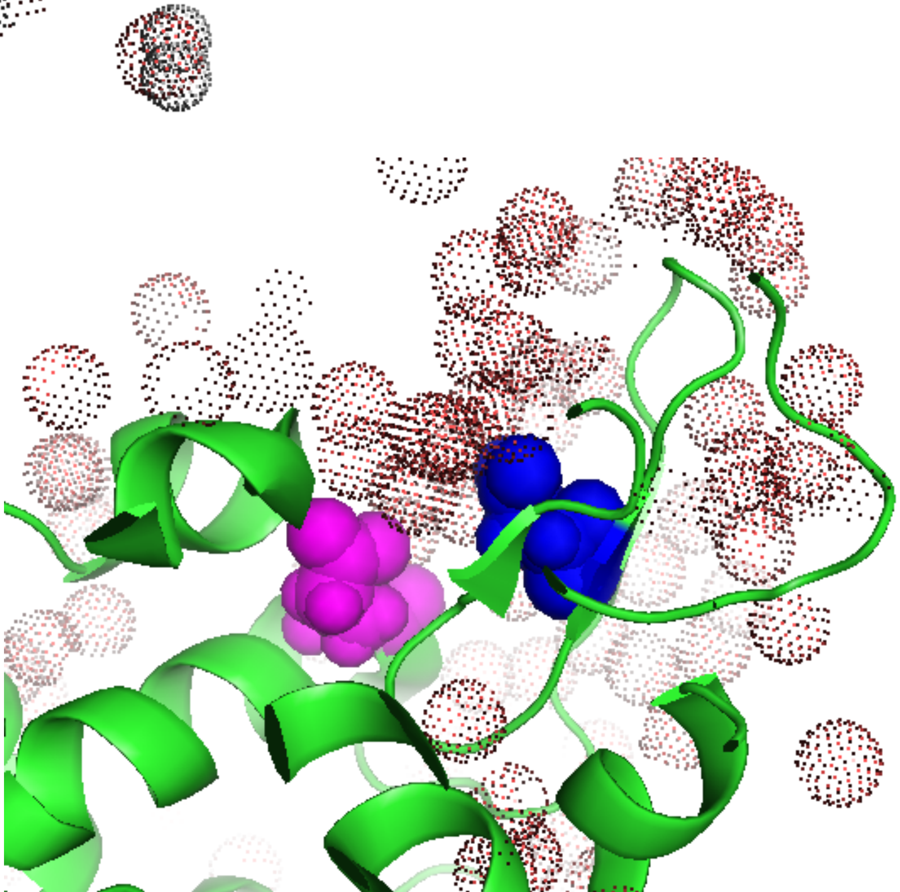


Mancinelli *et al*. (2007) *J. Phys. Chem. B* **111** 13570-13577

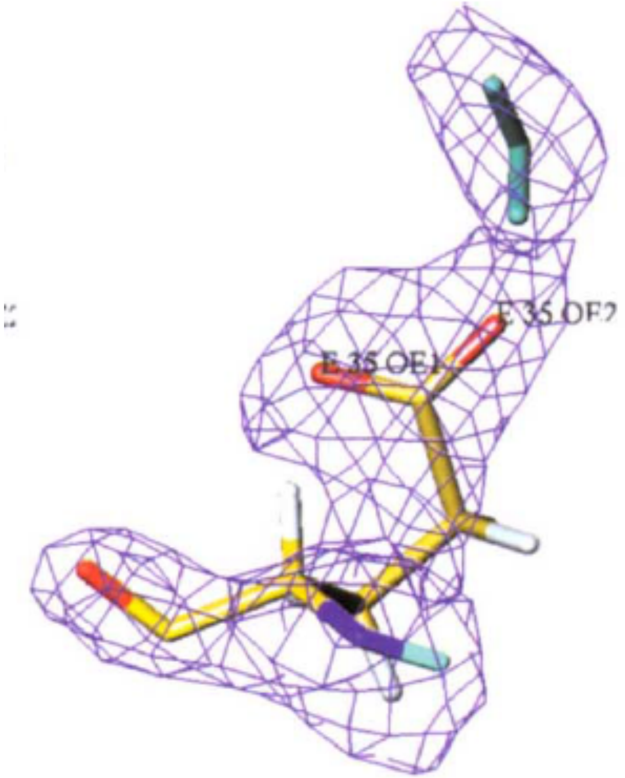
Water is present in the active site

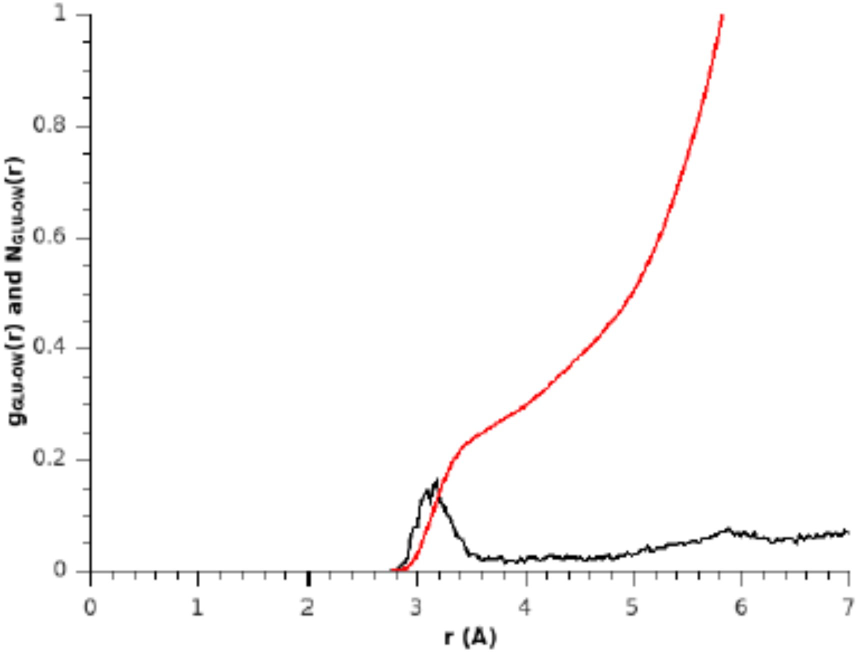


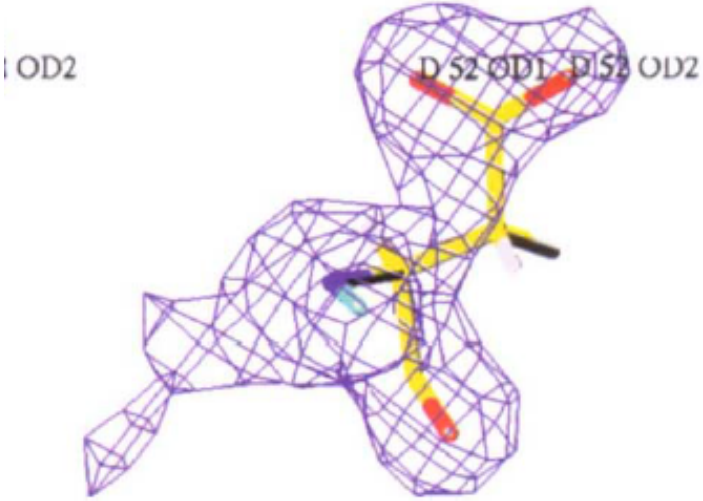
Neutrons

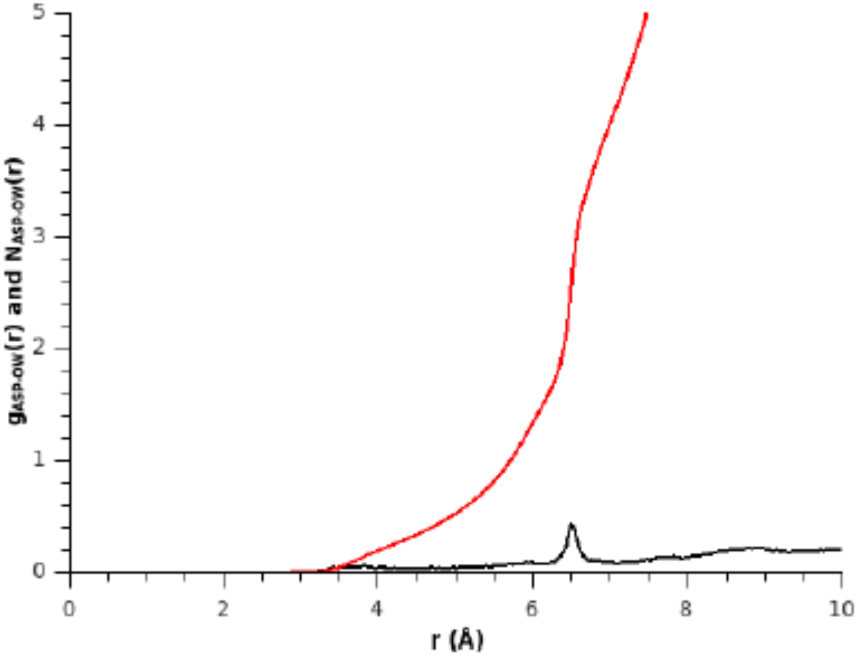


X-rays at 0.65
angstrom resolution


E 35 OE2
E 35 OE1


$g_{GLU\text{-}OW}(r)$ and $N_{GLU\text{-}OW}(r)$
0
0.2
0.4
0.6
0.8
1
0
1
2
3
4
5
6
7
r (Å)


OD2
D 52 OD1
D 52 OD2


$g_{ASP\text{-}OW}(r)$ and $N_{ASP\text{-}OW}(r)$
0
1
2
3
4
5
0
2
4
6
8
10
r (Å)

Data tells about occupation, but not residency

- From NPX and PX we get a static picture
- From MD we get residency
- Need to relate the two.

Comparisons with known data

- Started MD analysis. So far have 1 nsec. Initial analysis shows water is resident around the protein for longer than in the bulk.
- Wealth of hydration probe data and MD analysis
- Incorporation of QENS data to bridge the gap between the static EPSR analysis and the dynamic MD analysis.

Future work

Acknowledgements

- Cameron Neylon
- Daniel Bowron
- Tristam Youngs