Detailing protein solvation shell in an aqueous mixture: a SANS study

WATER INTERFACES IN PHYSICS, CHEMISTRY AND BIOLOGY: A MULTI-DISCIPLINARY APPROACH

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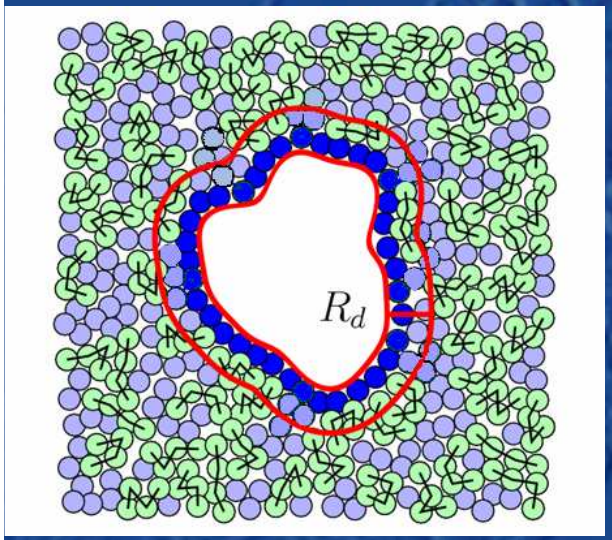
Outlook

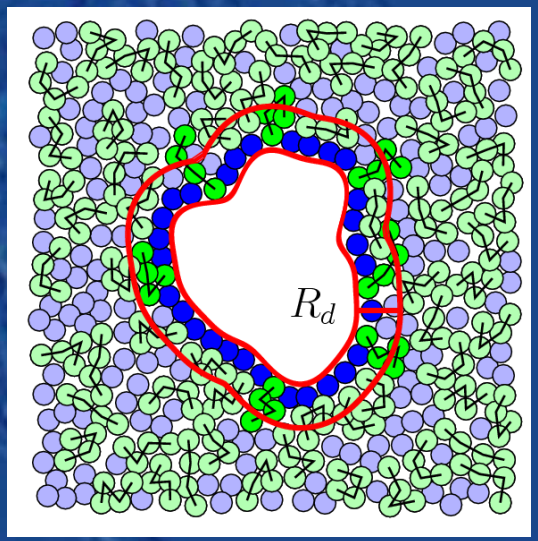
- Small Angle Neutron Scattering
- Protein-solvent interface in water mixtures
- Thermodynamic model for preferential hydration
- SANS data Global fit
- Lysozyme in presence of glycerol and urea: comparing results

Protein-solvent interface in water mixtures

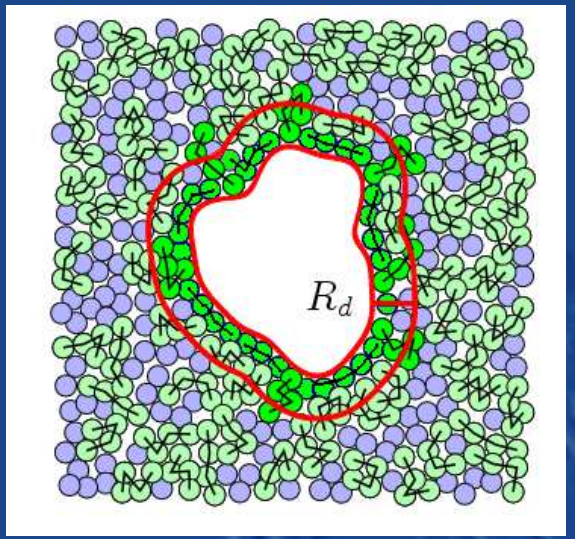
Just water



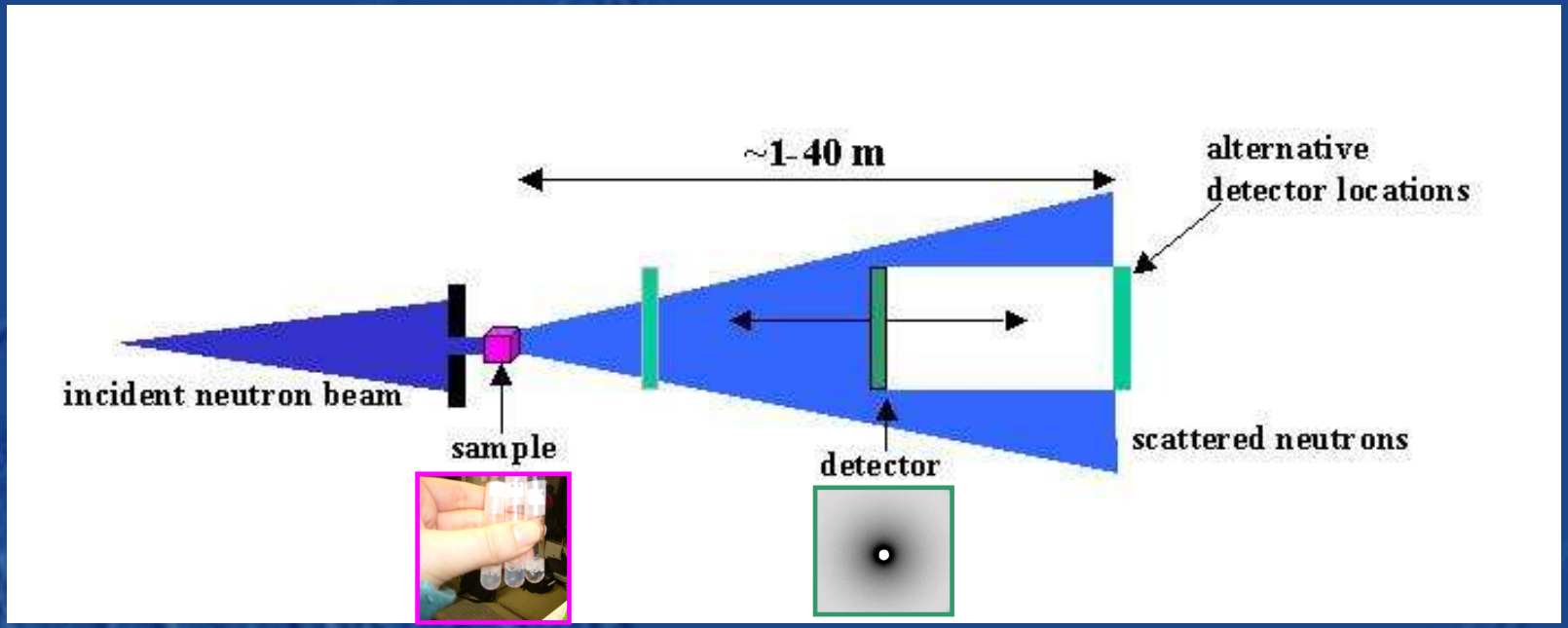
Water & cosolvent



Just cosolvent



Small Angle Neutron Scattering



Despite SANS is a low resolution technique, we have many advantages:

nearly physiological conditions

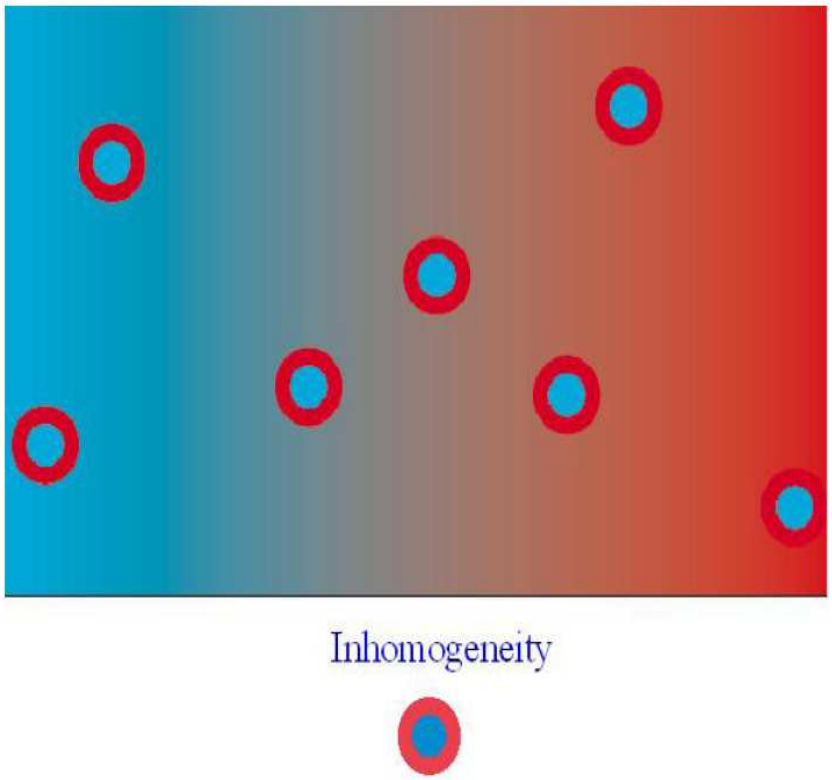
detail the bound water

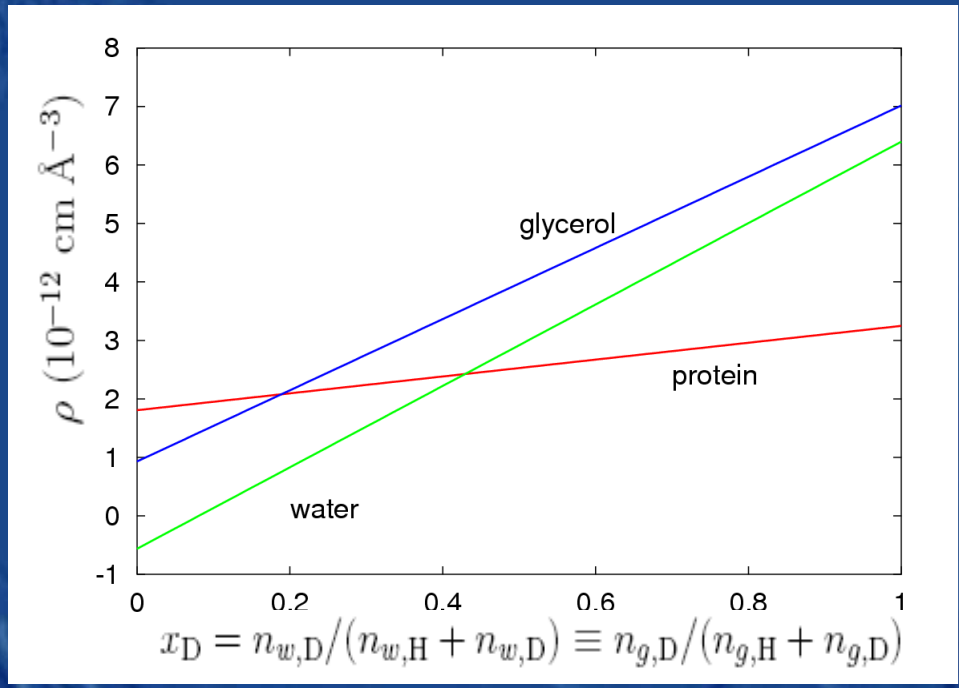
measure interactions

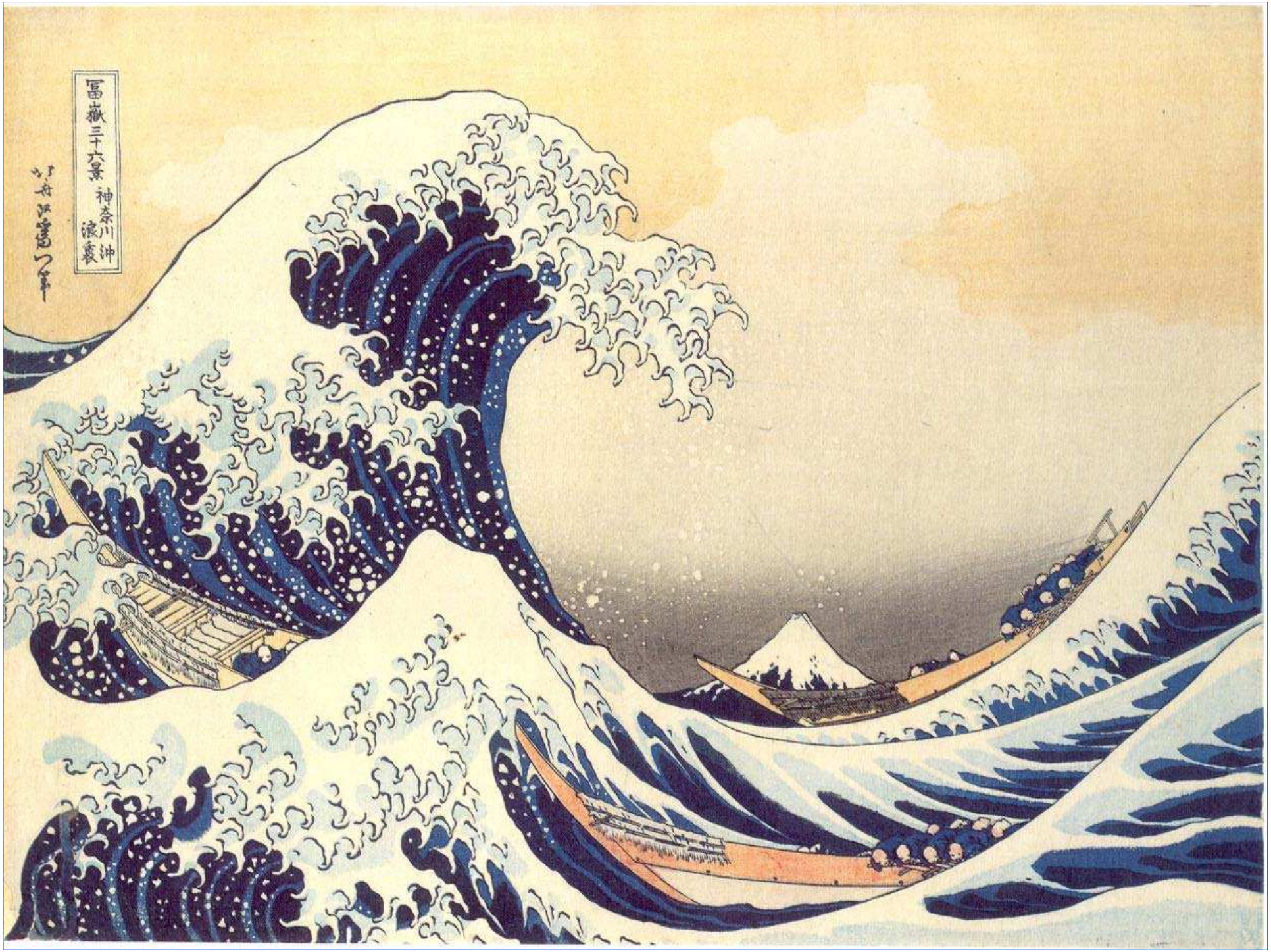
statistical "ensemble" over all particles

…the major advantage in our case is the contrast variation

$$\Delta\rho = \rho_{protein} - \rho_{solvent}$$



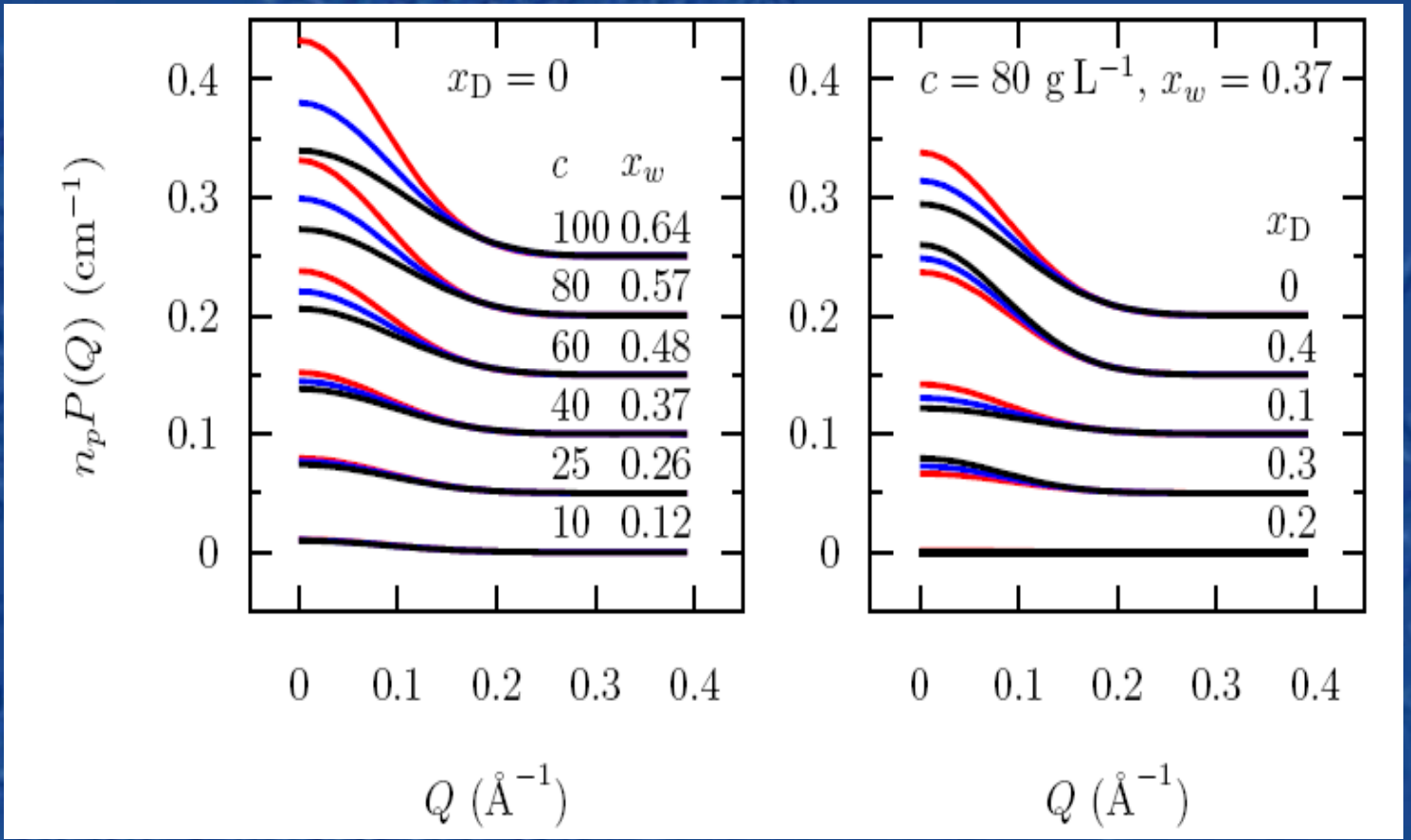



富嶽三十六景
神奈川沖
浪裏

Numerical simulation of SANS curves, calculated in the approximation $S_M(Q)=1$.

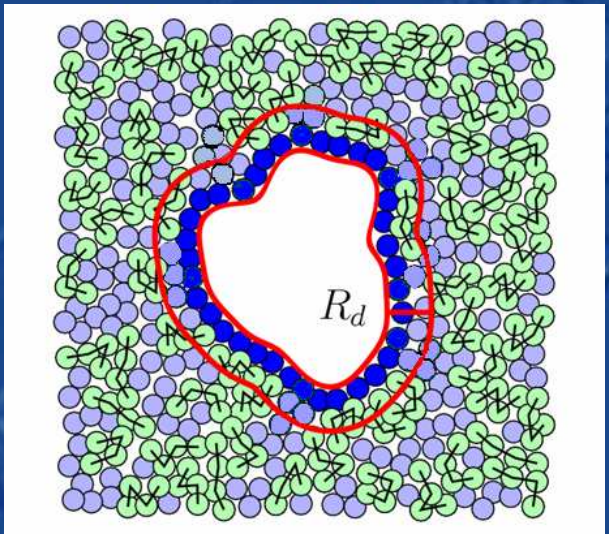
$$\frac{d\Sigma}{d\Omega}(Q) = n_p P(Q) S_M(Q)$$

$$\frac{x_{w,l}}{x_w} = 1,\ 1.2,\ 1.4$$

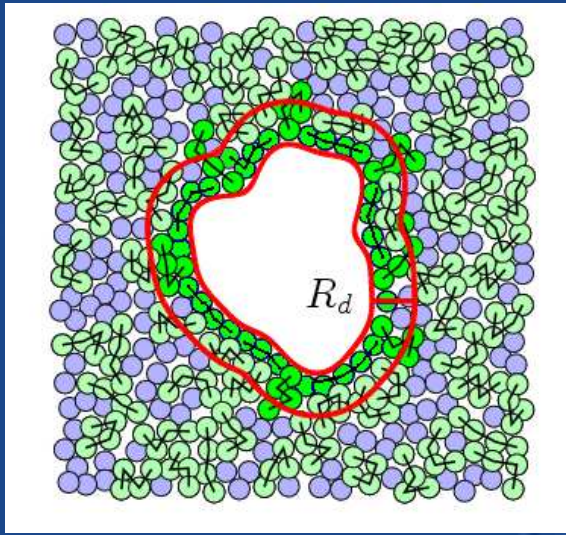


Protein-solvent interface in water mixtures

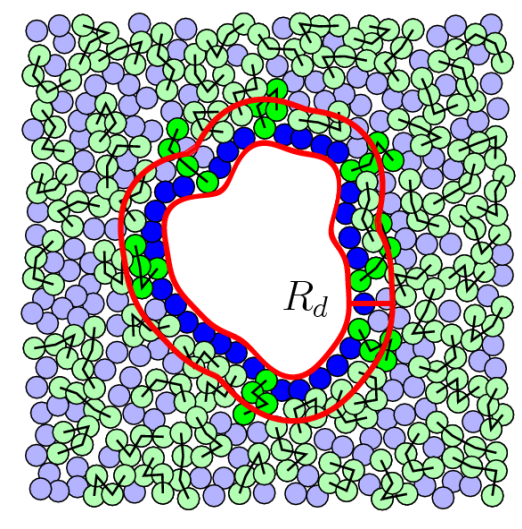
Just water



Just cosolvent



Thermodynamic equilibrium



Scaled representations of a solvated lysozyme molecule based on PDB structure (6LYZ).

, water molecules in the bulk and in the first solvation layer.

, glycerol molecules in the bulk in contact with the protein.

Thermodynamic model* for preferential hydration

$$c_l + w_b \rightleftharpoons c_b + w_l$$

c: cosolvent
w: water
l: local domain
b: bulk

$$K = \frac{x_{w,l}}{1 - x_{w,l}} \frac{1 - x_{w,b}}{x_{w,b}}$$

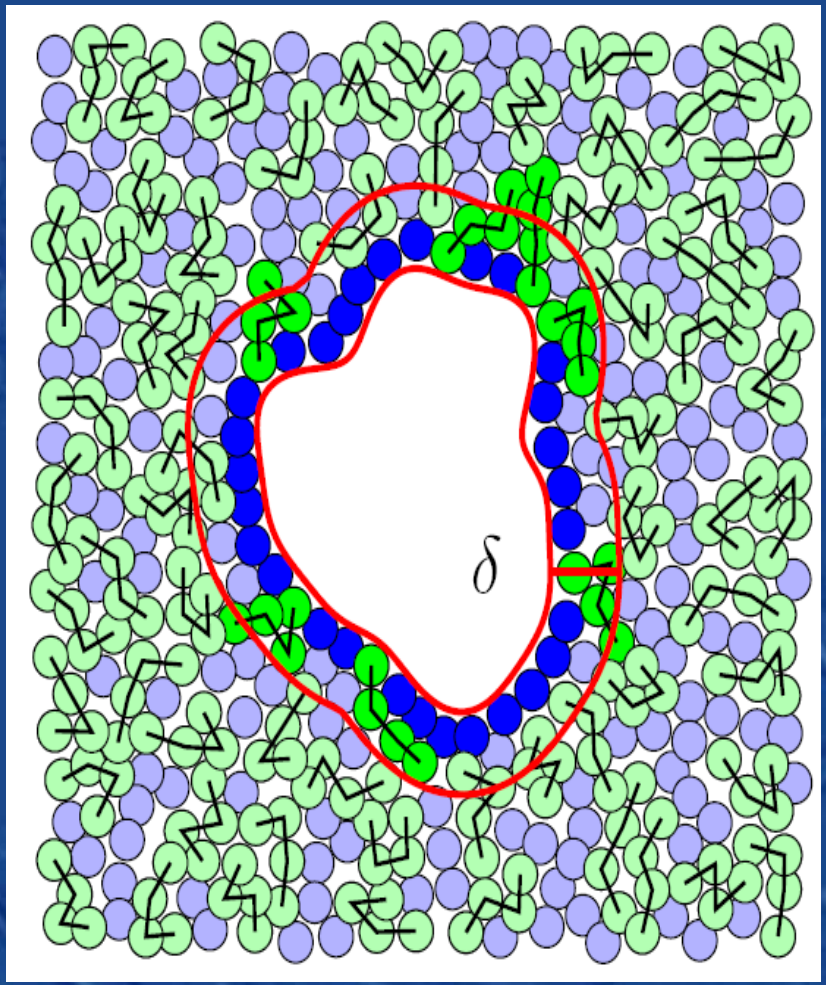
$x_{w,i}$ water molar fraction in the *i*-th domain

$$x_{w,l} = n_{w,l} / n_p m$$

m: number of sites

if K>1 preferential interaction with water

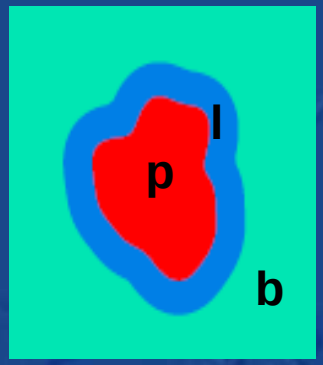
if K<1 preferential interaction with cosolvent



* J. A. Schellmann, Biophys. J. 85, 108 (2003).

SANS data Global fit

Three phase Form Factor



$$P(Q) = (\rho_p - \rho_b)^2 V_p^{\,2} P_{pp}(Q) + (\rho_l - \rho_b)^2 V_l^{\,2} P_{ll}(Q) + 2(\rho_p - \rho_b)(\rho_l - \rho_b) V_p V_l P_{pl}(Q) +$$

$$\rho_i = \frac{x_{w,i}(a_w - a_c) + a_c}{x_{w,i}(\nu_{w,i} - \nu_{c,i}) + \nu_{c,i}} \qquad i=b,l$$

$\nu_{w,i}, \nu_{c,i}$ partial molecular volumes of water and cosolvent in the *i*-th domain

a_w, a_c scattering lengths of water and cosolvent at x_D

$$K = \frac{x_{w,l}}{1 - x_{w,l}} \frac{1 - x_{w,b}}{x_{w,b}}$$

Effective Structure Factor

Under the Random Phase Approximation (RPA)

$$u_C(r) = \frac{Z^2 e^2}{\varepsilon (1 + \kappa_D R)^2} \frac{\exp[-\kappa_D (r - 2R)]}{r}$$

$$u_A(r) = -2JR \frac{\exp[-(r - 2R)/d]}{r}$$

Effective Structure Factor

Under the Random Phase Approximation (RPA)

$$S_M(Q) = \frac{S_0(Q)}{1+\beta n_p S_0(Q)[U_C(Q)+U_A(Q)]}$$

$$[S_0(Q)]^{-1} = 1 - \frac{12\eta[\eta(3-\eta^2)-2]}{(1-\eta)^4}\frac{j_1(2RQ)}{2RQ}$$

$S_0(Q)$ structure factor relative to the hard sphere potential

$\beta=1/k_BT$

U_C Fourier transform of the screened Couloumbic potential

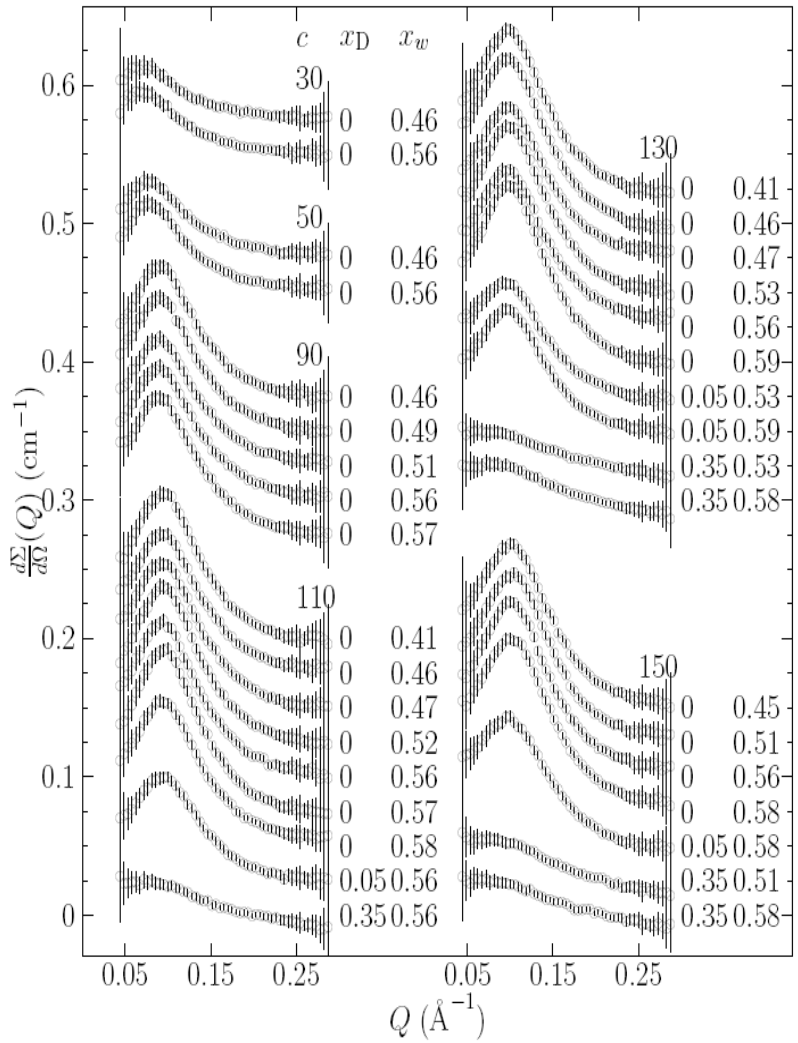
U_A Fourier transform of the attractive potential

η protein volume fraction

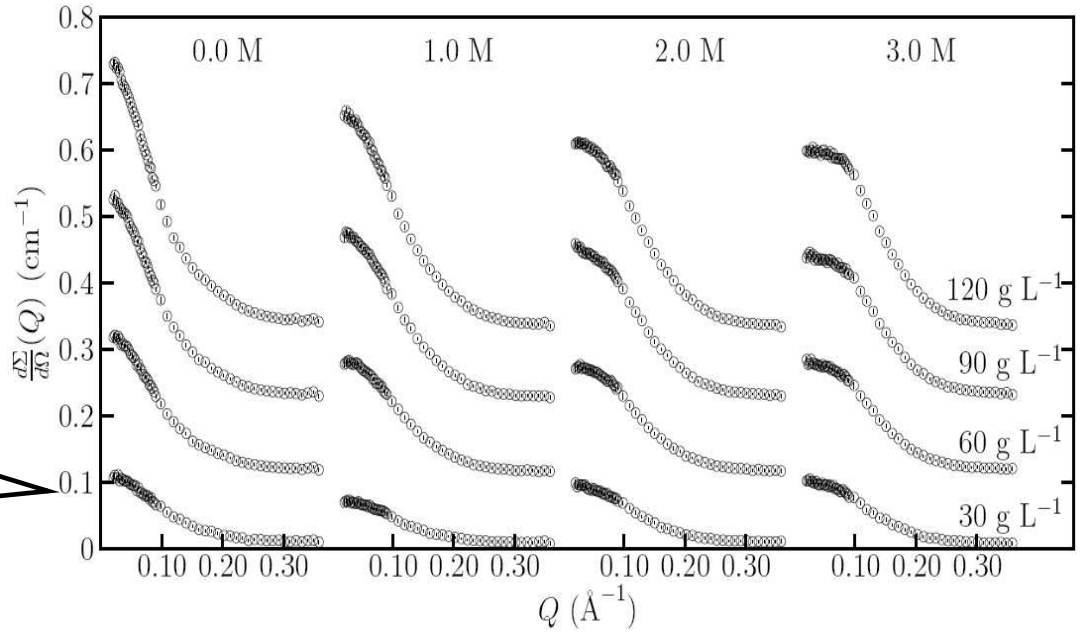
$$u_C(r) = \frac{Z^2e^2}{\varepsilon(1+\kappa_D R)^2}\frac{\exp[-\kappa_D(r-2R)]}{r}$$

$$u_A(r) = -2JR\frac{\exp[-(r-2R)/d]}{r}$$

Lysozyme dissolved in water-glycerol mixtures



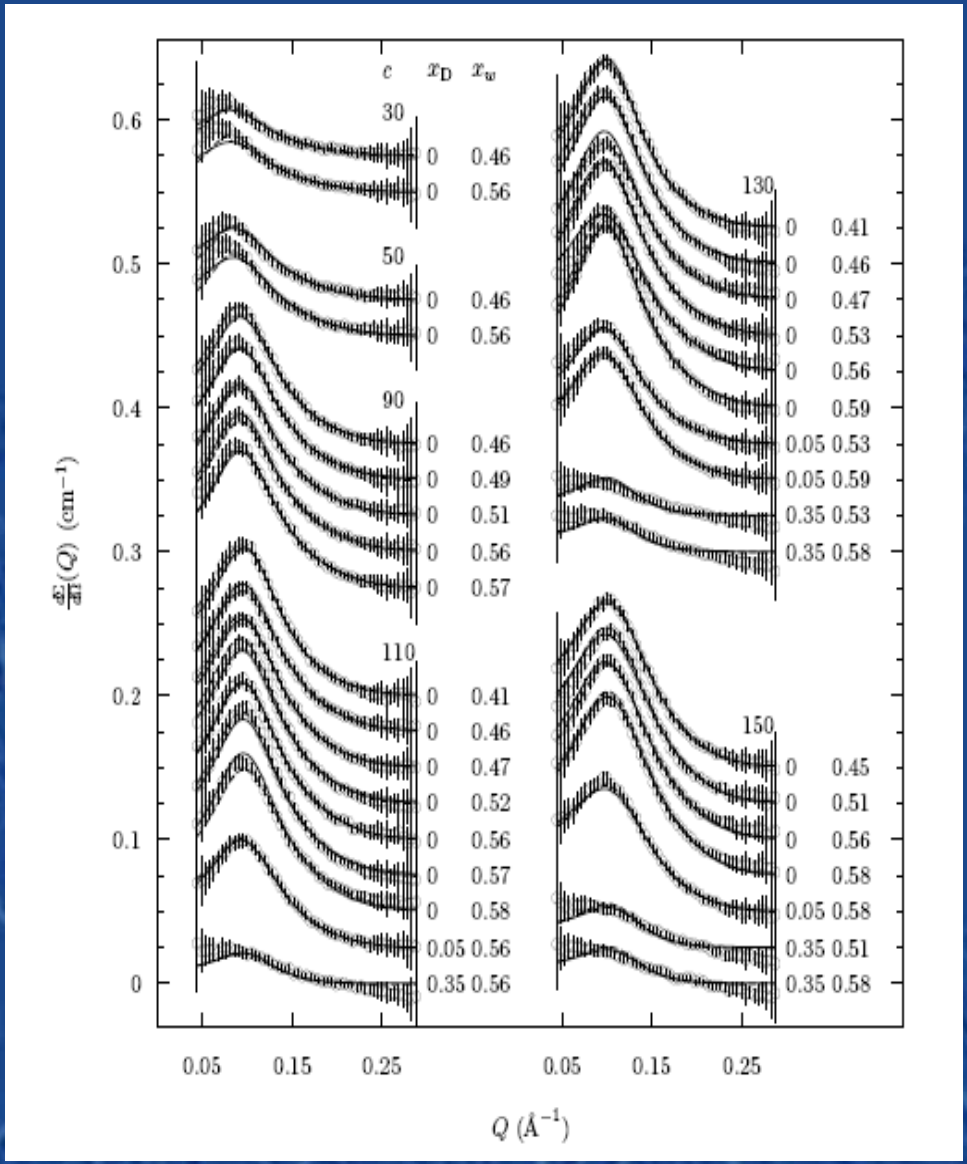
Lysozyme dissolved in water-urea mixtures



SANS data Global fit

Fixed parameters	Fitting parameters
	K
	Z
$\nu_{w,b}$	V_p
$\nu_{c,b}=\nu_{c,l}$	J
	d
	δ_l
	$\nu_{w,l}$

Lysozyme dissolved in water-glycerol mixtures*

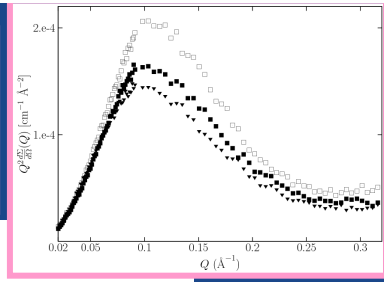


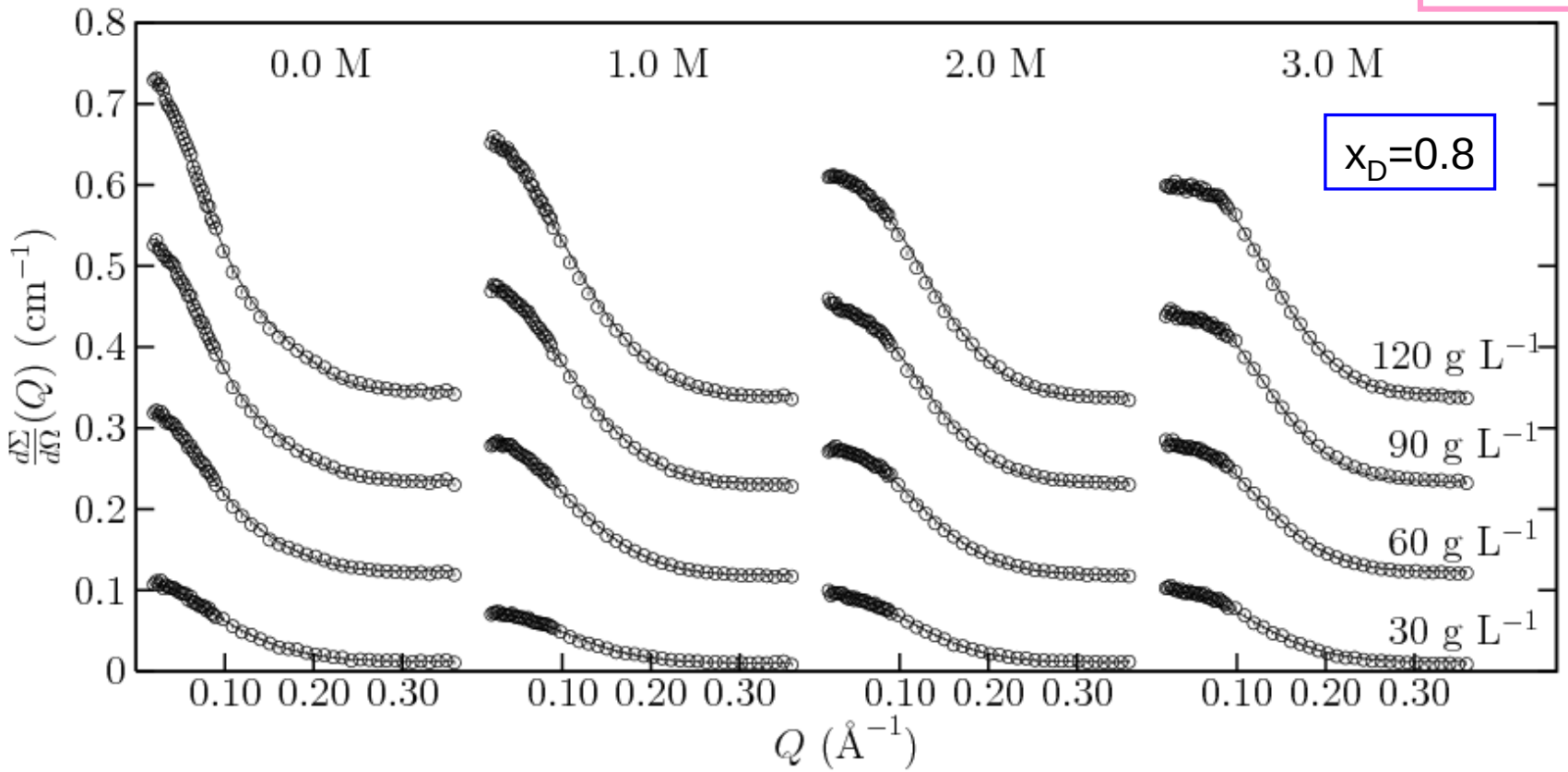
Experimental scattering curves collected at the nuclear reactor of Forschungerszentrum Jülich on 35 different samples. Solid lines correspond to the global fit with K common to all experimental curves.

*R. Sinibaldi, M.G. Ortore, F. Spinozzi, F. Carsughi, H. Frielinghaus, S. Cinelli, G. Onori and P. Mariani. "Preferential hydration of lysozyme in water/glycerol mixtures: a small-angle neutron scattering study". ***Journal of Chemical Physics*, 126, 235101 (2007).**

Lysozyme in presence of urea*

Kratky plots for lysozyme solution (60 mg/ml) with urea from 1 to 3 M



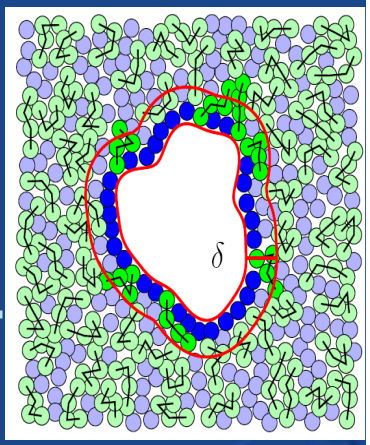


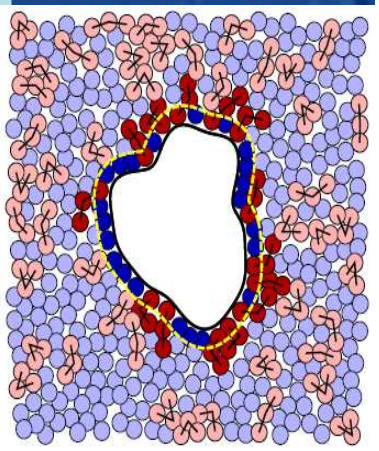
Experimental scattering curves collected at the nuclear reactor of Hahn Meitner Institut in Berlin on 16 different samples. Solid lines correspond to the global fit with K common to all experimental curves.

*M.G. Ortore, R. Sinibaldi F. Spinozzi, F. Carsughi, D. Clemens, A. Bonincontro and P. Mariani. "New insights into urea action on proteins: SANS and Zeta Potential study of lysozyme". Submitted to ***J. Phys. Chem. B***

Comparing results

	V_p (Å^3)	δ_l (Å)*	$\nu_{w,l}$ (Å^3)#	Z (e)	K
Lysozyme with glycerol	17060±70	5.9±0.2	28.81±0.04	9.00 ±0.04	1.87 ±0.03
Lysozyme with urea	16950±100	3.8±0.6	27.5±0.5	2.0→8.0	0.52±0.08



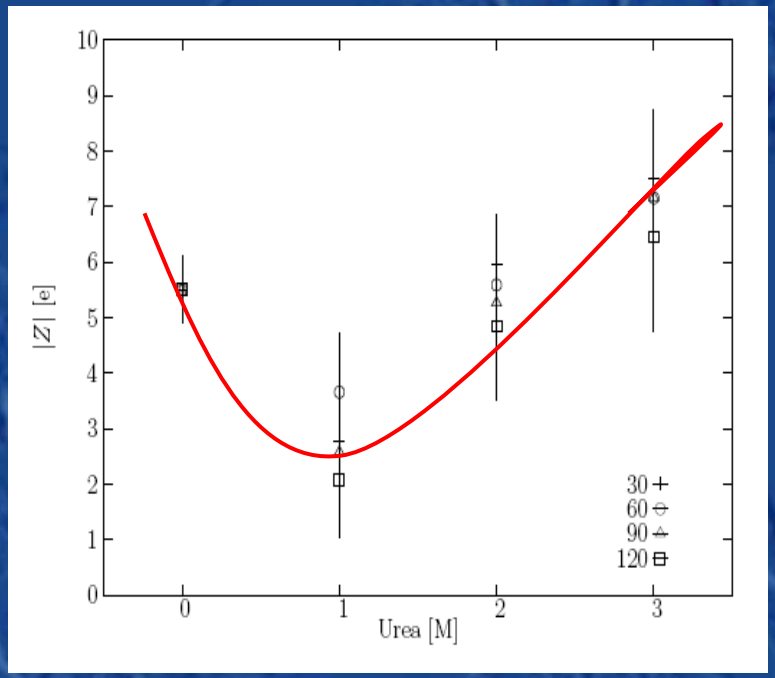


*B. M. Baynes and B. L. Trout, J. Phys. Chem. B **107**, 14058 2003

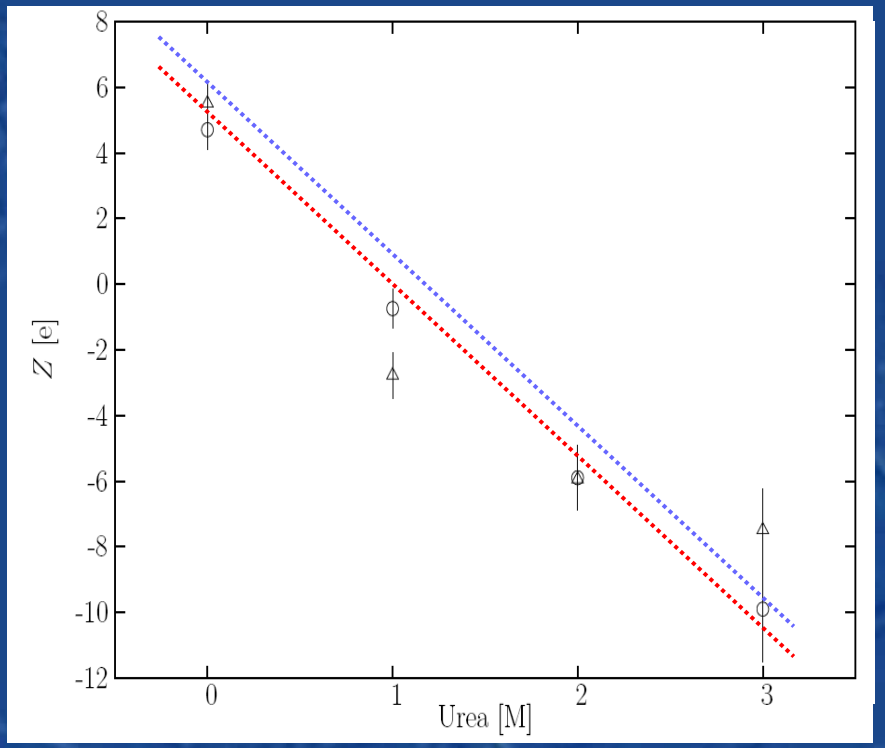
#D. I. Svergun, S. Richards, M. H. J. Koch, Z. Sayers, S. Kuprin and G. Zaccai *Proc. Natl. Acad. Sci. USA* Vol. 95, pp. 2267–2272, 1998

Lysozyme effective charge in urea-water mixtures

SANS Global fitting results



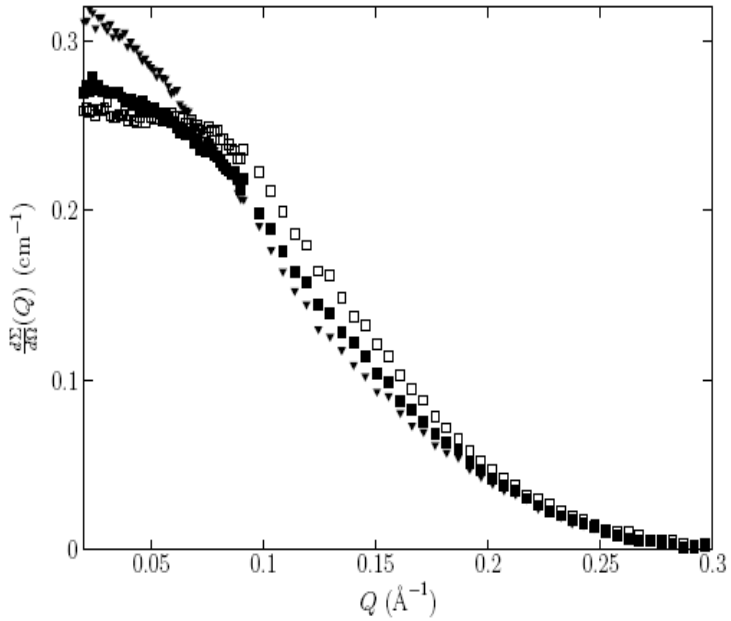
Zeta-potential measurements



Surface charge density $\sigma = \frac{\epsilon\zeta}{4\pi\tau}$

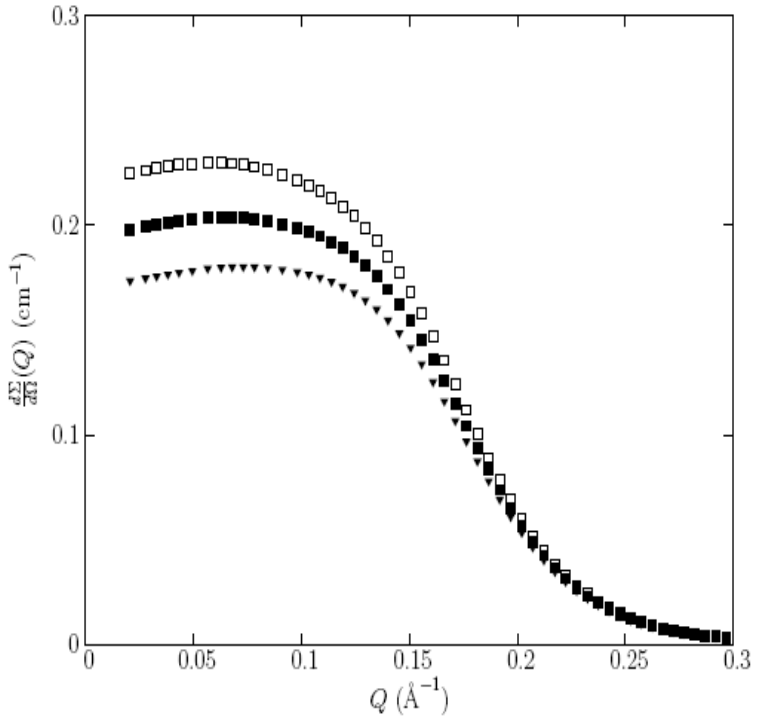
ζ : measured Zeta-potential

τ : thickness of the electric double layer



SANS experimental differential cross sections at c=120gL^{-1} and different amounts of urea in solution.
Symbols refer to different urea concentrations: triangles 1 M, full squares 2 M and empty squares 3 M.

Numerical simulated differential cross sections regarding the same experimental conditions. The scattering length densities of bulk solvent and local domain have been calculated considering the local domain enriched by a 10% of urea with respect to the nominal bulk composition, and assuming a constant protein charge Z = 6 in the coulombic potential and a constant attractive term (J = 3kBT and d = 3℀). Only the dielectric constant was considered to vary with urea concentrations from 78.3 to 82.5 as a function of the urea solvent concentration.



Lysozyme-glycerol

J and *d* parameters, which describe the attractive potential, are smoothly dependent on solvent composition, confirming that glycerol can prevent protein-protein aggregation,

x_w	J (k_BT)	d (Å)
	$I_S = 32 \pm 4$ mM	
0.46	2.36 ± 0.09	5.2 ± 0.2
0.56	3.74 ± 0.09	2.7 ± 0.2

Lysozyme-urea

we considered the depth J to linearly vary with water molar fraction in solution and to be independent on protein concentration: $J = J0 + J_m x_w$.

J decreases adding urea in solution

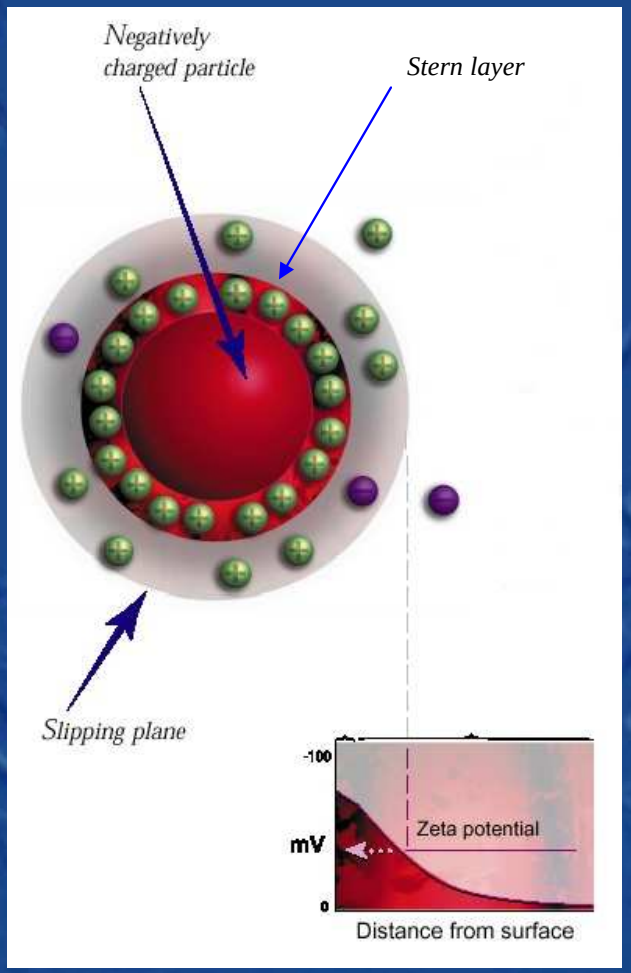
J_0 (k_BT)	J_m (k_BT)	d (Å)
-7.7 ± 0.3	12 ± 2	2.5 ± 0.5

Lysozyme effective charge in urea-water mixture

Zeta-potential measurements

- an electrical double layer exists around charge particles in solution;
- the liquid layer surrounding the particle exists as two parts; an inner region (Stern layer) where the ions are strongly bound and an outer (diffuse) region where they are less firmly associated;
- within this diffuse layer is a *notional* boundary known as the slipping plane, within which the particle acts as a single entity;
- the potential at this boundary is known as **Zeta Potential**, which depends on the protein net charge.

T=13 Angstrom, surface:6500 Angstrom^2



Comparison with literature

The preferential binding coefficient

$$\Gamma_{pj} = \lim_{n_p \to 0} \left(\frac{\partial n_j}{\partial n_p} \right)_{P,T,\mu_j} \qquad \text{j=w,c}$$

$$\Gamma_{pj} = n_j \left(G_{pj} - G_{wc} \right)$$

Kirkwood-Buff integrals

$$G_{ij} = \int_{-\infty}^{+\infty} d\mathrm{r} \left[g_{ij}(\mathrm{r}) - 1 \right]$$

Radial distribution function

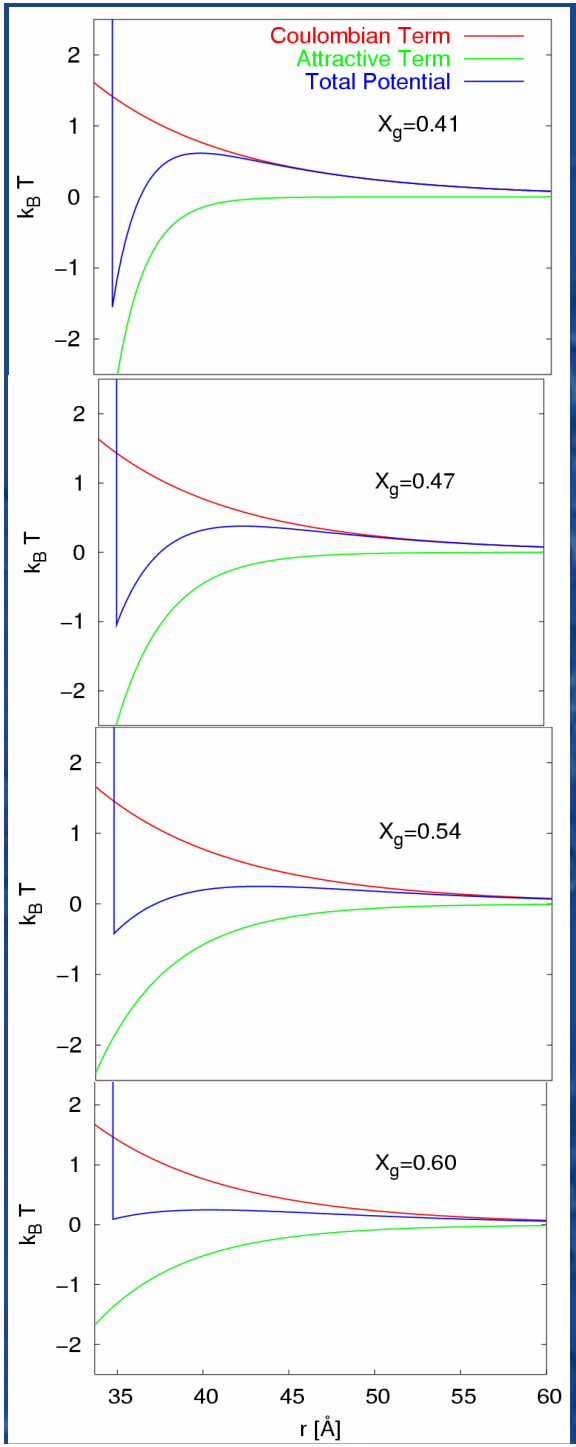
Calculated in the limit $n_p \to 0$

$$\Gamma_{pj} = m x_{j,l} + \frac{x_j}{\nu_{w,b} x_w + \nu_c x_c} \left[\frac{\nu_{w,b} \nu_c}{\nu_{w,b} x_w + \nu_c x_c} - V_p - m \left(\nu_{w,l} x_{w,l} + \nu_c x_{c,l} \right) - k_B T k_T \right]$$

The excess solvation number

$$N_{pj} = n_j G_{pj}$$

Number of displaced molecules *j* when a protein molecule is introduced into the mixed solvent



Protein-Protein interaction varies with the glycerol molar fractio

The structure factor $S_M(Q)$ contains information about the protein-protein correlation. It has been modelized using a three component integrable pair potential including Hard Sphere, Attractive term and Coulombian Term*.

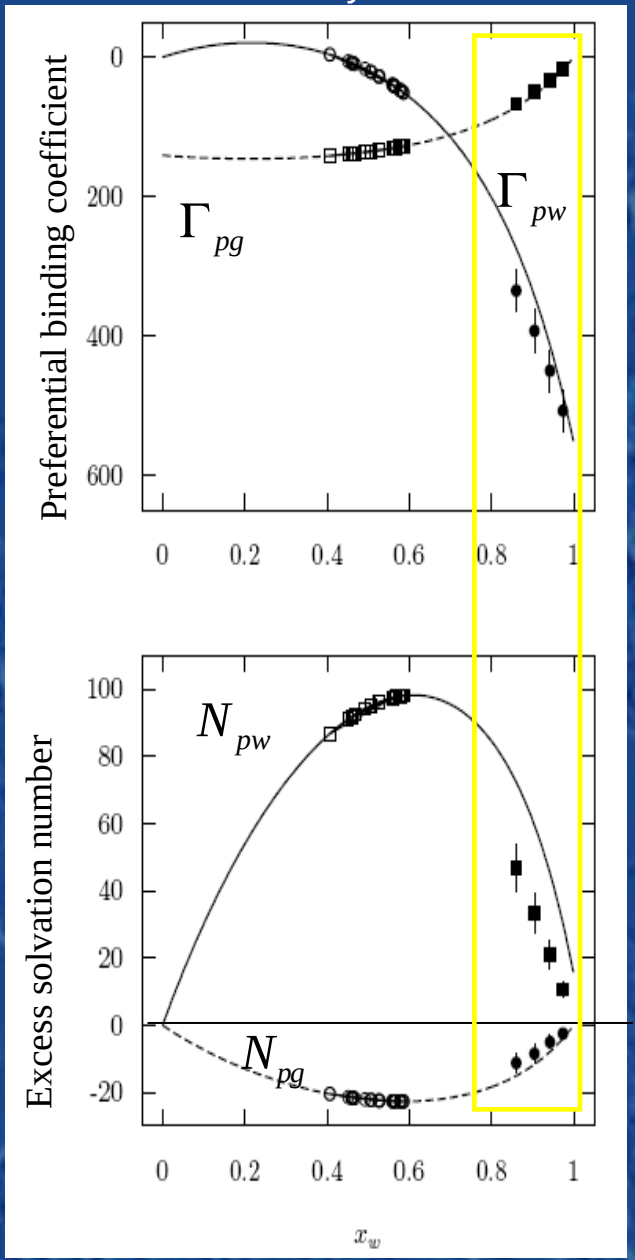
$$u(r) = u_{HS}(r) + u_C(r) + u_A(r)$$

$$u_A(r) = -J\sigma \frac{\exp[-(r-\sigma)/d]}{r}$$

J and **d** are fit parameter that depend on the water molar fraction in solution. **σ** is the protein diameter and correspond to the Hard sphere parameter.

*J.Narayanan X.Y.Liu Biophys. J. 84, 523 (2003).

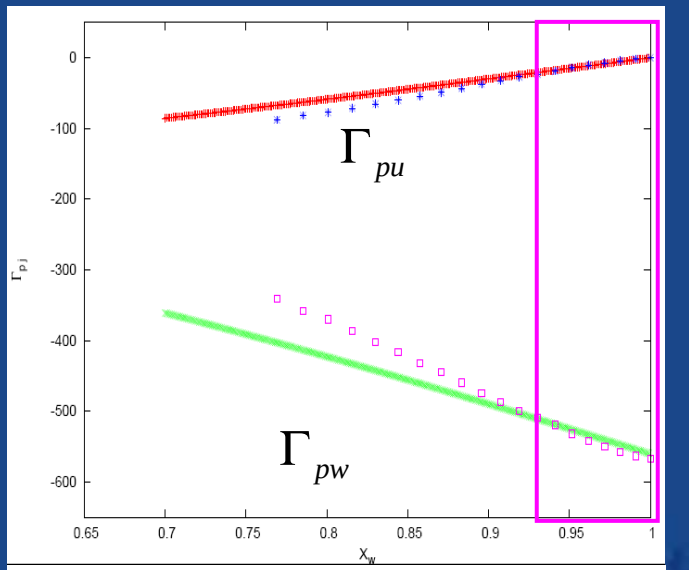
Glycerol in the solvent enhances the amount of water in the solvation layer*.

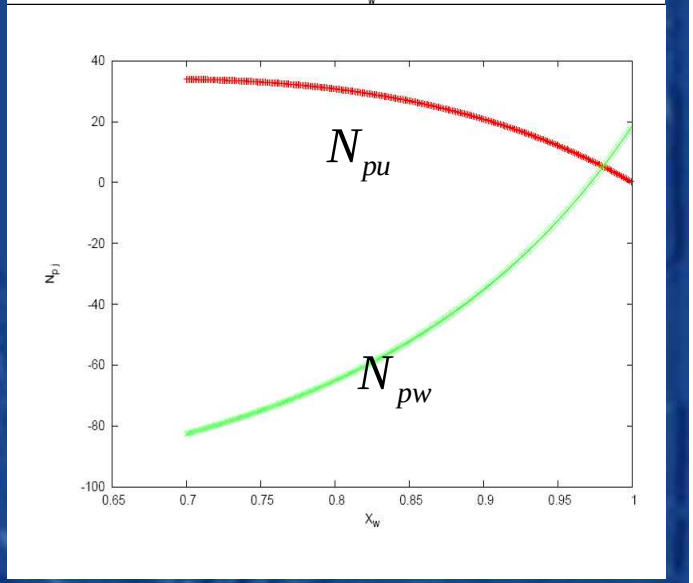


The preferential binding coefficient

$$\Gamma_{pj} = \lim_{n_p \to 0} \left(\frac{\partial n_j}{\partial n_p} \right)_{P,T,\mu_j} \qquad j=w,c$$

Urea accumulates in excess around the peptide, confirming MD and Timasheff literature results #.





*G. Kekko and S.M. Timasheff *Biochemistry* 1981, 20: 4667-4676

S. N.Timasheff and G. Xie *Biophys. Chem.* 2003; 105, 421-448

Concluding remarks

- In-solution SANS technique associated to global fit analysis can *quantitatively* detail the properties of protein solvation shell
- Confim of MD results: thickness of the local domain
- Confim of previous SAXS/SANS results: densier water in the solvation shell
- Perspectives in protein-protein interactions in organic mixtures

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