Experimental Report

Proposal: **8-03-773** **Council:** 4/2012

Title: Interaction between scFv antibodies and a biocompatible synthetic polymer

This proposal is a new proposal

Researh Area: Biology

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Samples: natural and deuterated antibody scFv
poly(vinyl pyrrolidone)

Instrument	Req. Days	All. Days	From	To
D11	1	1	08/07/2013	09/07/2013

Abstract:

We wish to investigate the interaction between a recombinant antibody of human origin (scFv) and a synthetic polymer in free solution using SANS. Both normal and deuterated scFv will be employed. The polymer, polyvinyl pyrrolidone (PVP) is a standard antigen employed in animal studies. The aim of the experiment is to quantify previous observations, by antibody phage display and by DLS, of binding in this system. Measurements will be made of the radius of gyration and molecular weight of the PVP solutions without and with the scFv, both in the deuterated and in the natural form, at different concentrations. Since scFv tends to form large clusters in solution, use of its deuterated form, which is nearly matched by the D2O, also appreciably simplifies the data analysis.

Preliminary report of the SANS measurements on 8 July 2013 on D11

Interaction between scFv antibodies and a biocompatible synthetic polymer

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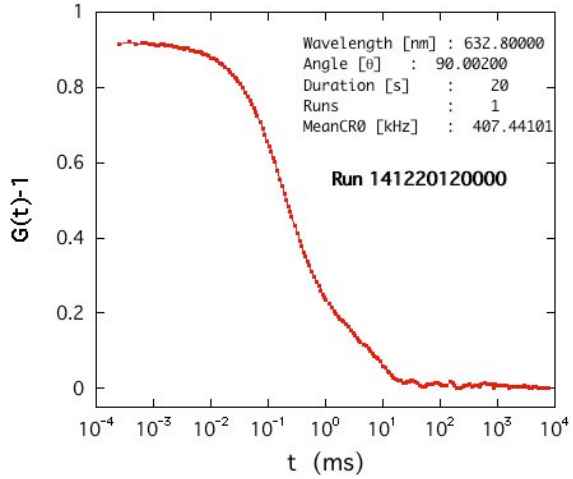
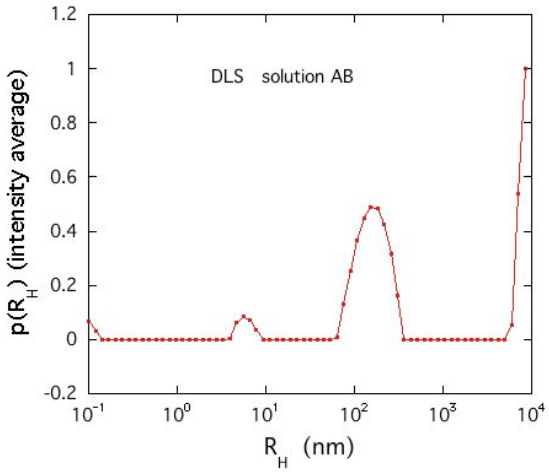
Background DLS measurements





Figures 1a and b. DLS intensity correlation function G(t)-1 from solution of scFv AB in water, measured at 90º, and its Laplace Transform(CONTIN). This is the unweighted relative amplitude $p(\tau)$ of the characteristic relaxation times τ. The relationship between τ and R_H the hydrodynamic radius of freely diffusing particles is $\tau=6\pi\eta R_H/kTq^2$, so $p(R_H)=p(\alpha\tau)$, where $\alpha=kTq^2/6\pi\eta$

In Figure 1b the non zero values of $p(R_H)$ at 10^{-1} nm and at $\sim 10^4$ nm are noise. The peak at 5 nm is the AB monomer and that at 150 nm is due to aggregates. For the *number* average of the particle size probability, divide $p(R_H)$ by $R_H{}^n$, where n is the fractal dimension of the protein. Taking $n\sim2.5$, we get Figure 2 (the position of the maximum does not change if n=3).

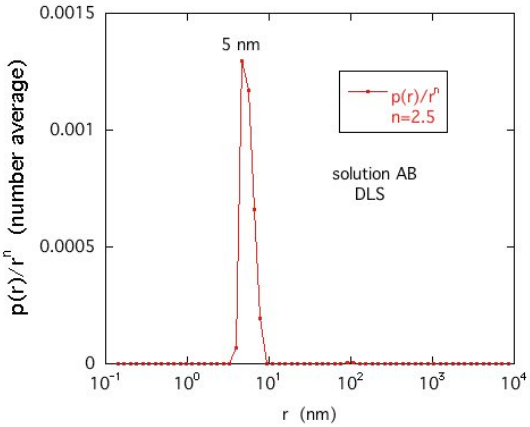


Figure 2. Number average distribution of DLS response from Figure 1b. Taking the number average enhances the signal of the monomers at the expense of that of the aggregates.

The DLS data suggest that the hydrodynamic radius of the monomer is about 5 nm. So we may expect $R_G\sim4$ nm, i.e., $qR_G=1$ when $q\approx0.025$ Å^{-1}. The SANS measurements covered the range $0.0045 \le q \le 0.25$ Å^{-1}.

The PVP was in two forms,
1) free polymer of weight-average molecular weight M_w=360 kDa, 0.8 mg in 0.6 ml D_2O, and
2) γ-ray cross-linked PVP gels (made in Budapest by K. László), swollen in D_2O
In both cases the polymer concentration was just above 1%. For the uncross-linked polymer, this is above the coil overlap concentration $c^*\sim 3M_w/(4\pi R_g^3)$, where R_g is the radius of gyration of the polymer coil, i.e., the polymer solution is in the de Gennes semi-dilute concentration range. So the polymer solutions are appreciably more viscous than pure water.
The AB samples were also in two forms, deuterated scFv, AB_D (1.7 mg, Deuteration Laboratory), and protonated AB_H (0.6 mg, LIPhy). The limited amount available, a consequence of aggregation during the preparation, proved to be something of a challenge.
Sample backgrounds were subtracted using an approximation that accounts for incoherent scattering:

$$S(q)= I_s(q)/T_s - I_b(q)T_s(1\text{-}T_b)/[T_b(1\text{-}T_s)] \qquad (1)$$

where $I_s(q)$, $I_b(q)$, and T_s, T_b are respectively the normalised scattering intensities of the sample and the background, and the corresponding transmissions.

Results

The SANS measurements from the solutions of AB were in fact challenging. The signal from AB_D in a mixture of 67% D_2O and 33% H_2O was too weak to be seen, and the signal from AB_H in D_2O was also weak, because of the low concentration, but it showed excess intensity at low q, characteristic of clusters. The effect on the polymer solutions and gels is more visible.

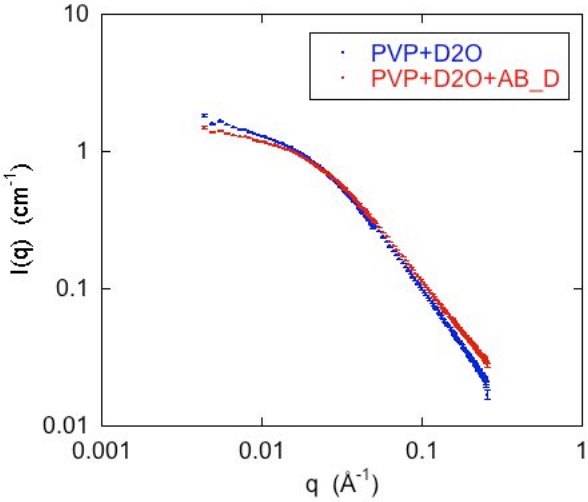


Figure 3. SANS response of PVP solution in D_2O (blue curve) and in D_2O with AB_D. (There was not enough AB_H to prepare the equivalent solution.) These curves are described approximately by the expression $I(q)=I(0)/[1+(q\xi)^2]^d$,
where according to the de Gennes scaling theory, the fractal dimension d=3/5.

In the low q region, where, if no clusters are present, a plateau is expected, the scattering intensity of a polymer solution is

$$I(0) = (\rho_p\text{-}\rho_s)^2 kTc/(\partial\Pi/\partial c), \qquad (2)$$

where $(\rho_p\text{-}\rho_s)^2$ is the contrast and Π is the osmotic pressure. Figure 3 shows that the addition of the (invisible) AB_D *decreases* the low q intensity.
The same behaviour is seen with the gels (Figure 4). At low q the intensity is depressed when either AB_H or AB_D is added. The slope of the response at higher q is close to the predicted

value -1.6. In gels the low q region is usually dominated by scattering from static concentration inhomogeneities caused by the non-uniform tension in the network chains around the cross-links. In spite of this extra (static) scattering, the change in the thermodynamic fluctuations is clearly visible.

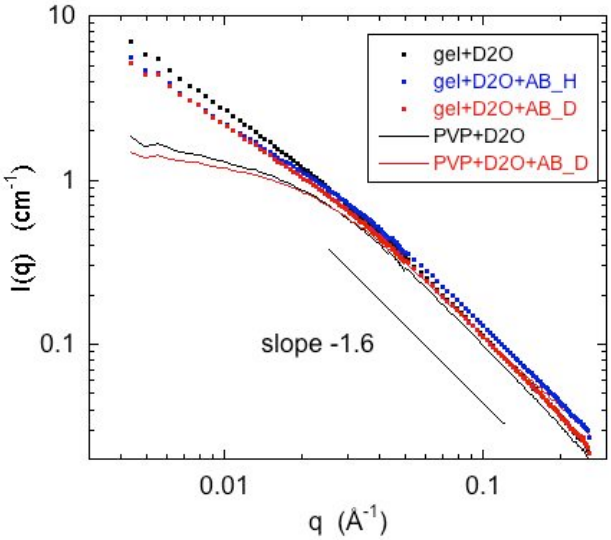


Figure 4. Scattering response of gels (symbols) and of solutions (lines). In both cases the addition of AB_H and AB_D reduces the intensity at low q .

General remarks

1) If the polymer and antibody are independent of each other, then their scattering intensities at low q (the thermodynamic response region) should be additive. This is not the case.
2) If the polymer and antibody attract each other, then the low q intensity would increase, because the osmotic pressure decreases.
3) If the interaction between the polymer and antibody is repulsive, then the osmotic pressure increases and the intensity decreases.

Provisional Conclusion

The SANS data point to scenario 3. This is also consistent with the DLS measurements, which showed that, within experimental error, neither the hydrodynamic radius of the PVP molecules in solution nor their scattering intensity changed when the solution of AB was added.
Thermodynamically, this is not perhaps too surprising, because in general different polymers repel each other in solution. The opposite is rare. But this case is special: the AB is a compact molecule and consequently its entropy of configuration is lower than that of an extended polymer.
The above conclusion may be too simple-minded. We cannot exclude the possibility of associations among a minority of molecules. Compensation effects can sometimes play dastardly tricks, and our measurements may not be sensitive enough to detect them.

Outlook

The findings are provisional, and will be backed up by DLS measurements of AB_H in the PVP gels.
The gel option overcomes some of the difficulties that are encountered in DLS (and which we encountered in the preliminary measurements) due to the presence of clusters diffusing in the solution: in a gel, large clusters are immobilised.