Experimental Report

Proposal: **9-13-520** **Council:** 10/2012

Title: Protein aggregation under Constrainment

This proposal is a new proposal

Researh Area: Soft condensed matter

Main proposer: **KLOESGEN Beate**

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Samples: monoolein / C21H40O4
lysozymw
lysozyme
Na2SO4
NaI
H2O, D2O

Instrument	Req. Days	All. Days	From	To
D11	4	2	11/03/2013	13/03/2013

Abstract:

The proposal involves the study of protein aggregation under volume constraints. Lysozyme as a test protein will dissolved in aqueous conditions within the open cages of a lipid cubic phase of monoolein, and the aggregation state will be studied as a function of temperature for three different types of solution conditions: pure water, and solutions of Na2SO4 or NaJ as examples for kosmotropic resp. chaotropic salt solutions. The two different hydration conditions within the salt solutions will modulate the details of the cubic phase by widening / narrowing its pore radii, thus as well modifying the aggregation conditions of the dissolved macromolecule. Contrast matching methods will give access to clearly distinguishing scattering contributions due to the structure of the cubic phase from the SANS signal of the dissolved macromolecules, allowing to extract the details of the composite system in separate subsequent experiments. D11, with its high flux, is a perfect choice for that kind of experiment.

Proposal Number 9-13-520

Protein aggregation under Constraint

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Introduction

The peculiar polymorphism of the aqueous mono-olein (MO) exhibits a wealth of appearances [1] comprising flat lamellar phases, crystalline and fluid, micellar and hexagonal phases, and as well several curved lamellar phases controlled by bending elasticity. We were especially interested in the Im3m cubic phase when hosting the soluble test protein lysozyme (Fig.1). Two major points were investigated: (1) the impact of the excluded volume constraint on the aggregation conditions of the protein when embedded into the cubic phase [2] under conditions that do not yet initiate dimer formation and (2) the stability of the cubic phase upon the presence of the protein in its aqueous cages and pores [3]. In a first set of experiments we sought to approach these details by use of contrast enhanced SANS.

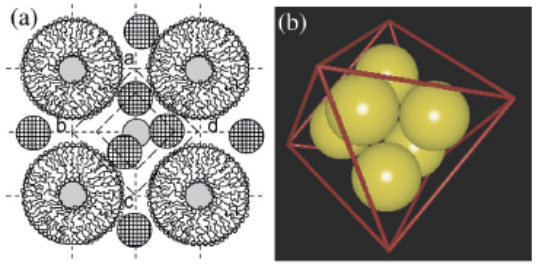

Fig. 1: Schematics showing the configured entrapment of the dissolved test protein, lysozyme (3.4nm), within the pore cages of the cubic structure Im3m (~124nm) of a monoolein host , in 2D- and 3D- projection (from [2]).

Experimental Details

Samples. Aqueous solutions of lysozyme or MO were prepared at varying concentrations and for different aqueous contrast conditions such that the match point of the single component solutions, their initial structure and, for lysozyme, the aggregation state could be measured. Mixed systems were prepared under MO-matching conditions in order to enhance contrast of the dilute lysozyme solution within the MO cubic phase. NaCl was added in varying concentration to explore the electrostatic impact on lysozyme aggregation.

Beamline settings. Data were collected at λ=6Å from detector distances of 1.2m, 4.0m, and 20m to cover a q-range from 0.003 $Å^{-1}$ – 0.5 $Å^{-1}$.

Results

The match point of the aqueous cubic pure MO-phase was found to be at a scattering length density (SLD) of ~$0.486 \cdot 10^{-6}$ A^{-2} (corresponding to a 15/85 mixture of D_2O/H_2O (mol/mol)), and the one of fully hydrated lysozyme was obtained as SLD≈$2.89 \cdot 10^{-6}$ A^{-2} (~1:1 D_2O/H_2O). MO prepared in 15% D_2O did though not fully remove intensity contributions from the cubic phase (see Fig.2).

Lysozyme is hydrophilic and bears surface charges. The shape of the hydrated molecule, and even more so the aggregation state, are sensitive to the presence of salt in the aqueous solution (Fig.3) and depend as well on the total concentration of the protein (Fig.4). Preliminary fitting of these data confirms the low resolution solution structure of lysozyme.

The poor matching of MO in 15% D_2O turned out to be a major obstacle in our attempt to detect the presence of the dissolved protein, and possibly study is aggregation state within a cubic host phase of MO (Fig.5).

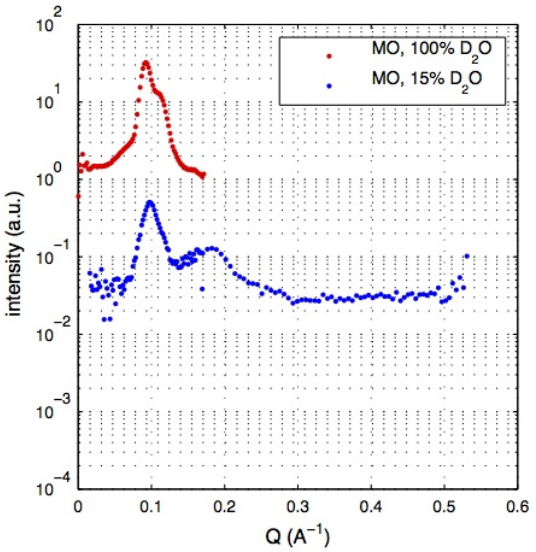

Fig. 2: SANS results from MO on aqueous surroundings (100% D_2O: ●; 15/85, D_2O/ H_2O, (mol/mol): ●). 15% D_2O corresponds to the experimentally determined water matching conditions; the curve demonstrates the imperfect contrast matching.

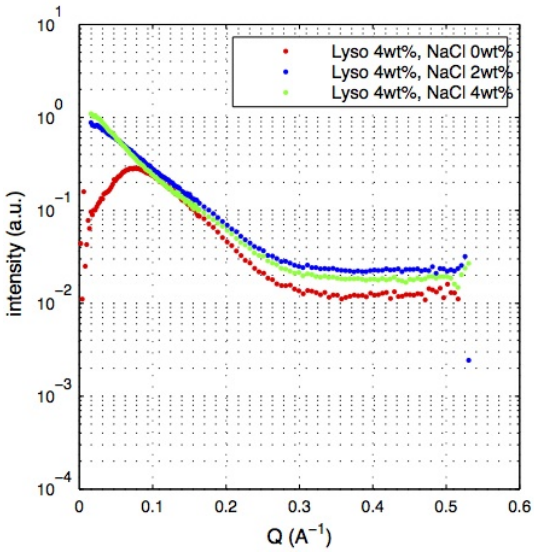

Fig. 3: Lysozyme (4%, by weight) in aqueous surroundings (100% D_2O), dependency of the SANS signal on presence of salt in solution.

The SANS signals obtained with and without lysozyme are very similar, and the residual intensity from the cubic phase dominates the scattering behaviour. The peak positions and the details of the intensity course as a function of q slightly differ from the pure phase. Using SASVIEW for preliminary fitting by use of an FCC model did reproduce the measured intensity quite well for $q<0.3$ Å^{-1} and gave the approximate crystallographic distances for the MO phase, in the absence of lysozyme. The FCC model though has to be discarded for more detailed fitting and must be replaced by a model that is more appropriate to account for the Im3m phase. Still, when trying to fit the mixed system cubic MO/ dissolved lysozyme, the FCC model was less successful to reproduce the scattering intensity in the significant q-range than for the pure system.

The small changes in I(q) in the presence dissolved lysozyme within the cubic phase may originate from (1) either a modification of the cubic host phase or (2) from the SANS scattering of the dissolved protein itself. At the moment, we seek to improve the fitting software such that it allows data fitting for the simultaneous presence of these two structures. The attempt to work with a protein at dilute conditions and at a SLD that is only six times higher than the one of the dominating structure was possibly too challenging for the study. We therefore plan to continue the project, focussing on the modification of the cubic phase alone by the presence of inoculated metallic nanoparticles (Fe). The scattering form these particles will be more pronounced and thus easier to detect. Therefore, their size and as well their impact on the host phase will be easier to assess by SANS. We will use the new system to develop and test the new software model, thus getting ready for turning back to molecular guests of the MO phase in the future.

Conclusion

The data analysis of the system studied turned out to be quite a challenge. We found small modifications to the structure of the pure cubic phase that may be due to the presence of the lysozyme within the aqueous volumes of the host phase. Final data interpretation requires a modification of the fitting software since the available models so far do not include the simultaneous presence of a crystallographic mesophase like the Im3m phase of the MO and incorporated small scatterers like dissolved proteins. Work is in progress and will be reported later. In the meanwhile we seek to do

experiments in a more simple system in order to acquire a good data set for testing the new fitting tools.

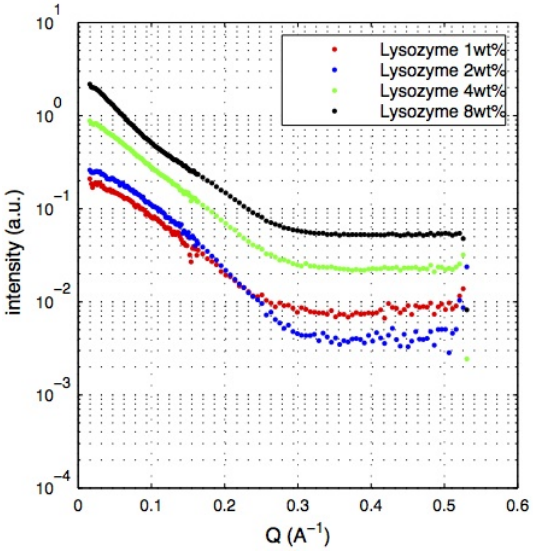

Fig. 4: SANS signal obtained from lysozyme solutions of varying protein concentration (by weight), at fixed salt contents (2%NaCl by weight), in 100% D_2O.

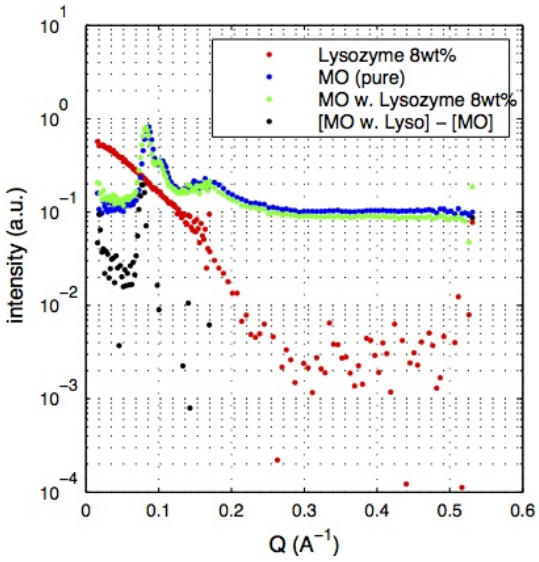

Fig. 5: SANS signal obtained from cubic phase of MO at matching conditions (15% D_2O) with (●) and without (●) lysozyme within the MO-phase. The differential signal (●) is entered as well, and, for comparison, the signal obtained for 8% lysozyme in the same water conditions is entered (●).

References

1. Qiu, H. and M. Caffrey, *The phase diagram of the monoolein/water system: metastability and equilibrium aspects.* Biomaterials, 2000. **21**(3): p. 223-234.
2. Tanaka, S., S.U. Egelhaaf, and W.C.K. Poon, *Crystallization of a globular protein in lipid cubic phase.* Physical Review Letters, 2004. **92**(12).
3. Tanaka, S., S. Maki, and M. Ataka, *Structural transitions of the mono-olein bicontinuous cubic phase induced by inclusion of protein lysozyme solutions.* Physical Review E, 2006. **73**(6).