Experimental report

09/02/2016

Proposal: 9-10-1329 **Council:** 10/2012

Title: Co-adsorption vs. complexation of protein and surfactant in the tubular pores of SBA-15 silica

Research area: Soft condensed matter

This proposal is a new proposal

Main proposer: Gerhard FINDENEGG

Experimental team: Bhuvnesh BHARTI
Jens MEISSNER
Gerhard FINDENEGG

Local contacts: Viviana CRISTIGLIO

Samples: dodecylpyridinium chloride/C17H39ClN
Mesoporous silica/SiO2
Protein/Lysozyme

Instrument	Requested days	Allocated days	From	To
D16	5	5	21/02/2013	26/02/2013

Abstract:

The interaction of surfactants with adsorbed protein layers is of practical relevance in the context of surface cleaning and the removal of protein from the surface of porous separation membranes. Here we propose to investigate the assembly of the cationic surfactant DPC in the cylindrical pores of SBA-15 (pore width ~ 8 nm) in the presence of pre-adsorbed lysozyme. We will address the question how the processes of co-adsorption, complexation, or displacement of proteins with surfactants are affected by confined geometry of the narrow pores. In preceding studies we have used SANS to determine the morphology of surfactant aggregates adsorbed in the periodic cylindrical pores of SBA-15 silica. The scattering data were analyzed on the basis of a sum of two independent contributions: Bragg scattering (from the ordered pore lattice) and diffused scattering (from spatial correlations) among surfactant aggregates. A similar model will be used to analyze the changes induced by the presence of preadsorbed protein in the pores. From our earlier experience we expect that instrument D16 is ideally suited for studying the co-assembly of lysozyme with the cationic surfactant DPC in nanopores.

Experimental Report on Neutron Scattering Experiments at Institute Laue-Langevin

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Instrument	D16
Dates of experiment:	From : 21/02/2013 To : 25/02/2013
Experimental team: Names	Addresses
Gerhard H. Findenegg	Institute of Chemistry, Stranski Laboratory, Sekretariat TC7, Technical University Berlin, Strasse des 17. Juni 124, 10623 Berlin
Jens Meissner	Institute of Chemistry, Stranski Laboratory, Sekretariat TC7, Technical University Berlin, Strasse des 17. Juni 124, 10623 Berlin
Bhuvnesh Bharti	Department of Chemical and Biomolecular Engineering, North Carolina State University, 911 Partners Way, Raleigh, NC, 27695-7508, USA
Local Contact:	Viviana Cristiglio

Experimental report text body

We have investigated the co-adsorption of lysozyme with cationic surfactants in the cylindrical pores of SBA-15, a periodic mesoporous silica material that constitutes arrays of cylindrical pores (diameter 8 nm) disposed side-by-side in a 2D hexagonal lattice. The pore walls of SBA-15 are hydrophilic and become negatively charged in aqueous media at pH > 5, due to the deprotonation of surface silanol groups. Lysozyme is a globular protein (isoelectric point pI = 11) which carries a net positive charge below pH 11. It is well-established that lysozyme is adsorbed in the pores of SBA-15 and that its adsorption level increases with increasing pH up to nearly pH 11 [1]. Dodecylpyridinium chloride (DPC) and hexadecylpyridinium chloride (CPC) are cationic surfactants which are also strongly adsorbed in the pores of SBA-15. The purpose of this project has been to study the co-adsorption and interaction of lysozyme with these cationic surfactants in the pores of SBA-15.

Earlier we have developed a formalism for analyzing small-angle diffraction/scattering (SAND/SANS) data from assembly structures of surfactants in periodic mesoporous matrices like SBA-15 [2]. This formalism was applied to nonionic surfactants [2-4] and the cationic surfactant DPC [5]. Instrument D16 was found to be well suited for such measurements when the collimation is adjusted for maximum resolution [5]. For surfactant studies much information can be extracted from the scattering profile I(q) of matrix-solvent contrast matched samples. In this case the surfactant aggregates are measured against a uniform scattering background. In the case of proteins a complication arises from their high SLD ($\rho_n = 2.5\times10^{10}$ cm^{-2} for lysozyme), causing a low contrast against silica ($\Delta\rho_n \approx 1\times10^{10}$ cm^{-2}), which is further decreased in H_2O/D_2O mixtures by hydrogen/deuterium exchange of lysozyme. For this reason measurements on proteins must be made at full contrast in pure D_2O. Preliminary SAND/SANS data for lysozyme at full contrast in SBA-15 (obtained in an earlier beam time at D16) indicated that when the concentration of lysozyme in the pores is gradually increased the intensity of the prominent 10 Bragg peak first increases and later decreases. This decrease can be attributed to a decreasing contrast of the solution in the pores against the silica matrix, but the origin of the initial increase in intensity is not fully understood.

Based on the information about the single components, the present experiment was devoted to a study of co-adsorption of lysozyme with the cationic surfactants DPC or CPC in SBA-15. Samples covering a wide range of protein and surfactant concentrations in contrast-matching H_2O/D_2O were prepared in the following way: A known amount of SBA-15 was dispersed in the solvent and adjusted to pH 9.5 by adding aliquots of NaOH (aq). Stock solutions of lysozyme and

surfactant in H_2O/D_2O were also prepared. Aliquots of these stock solutions were then added to the silica dispersion to reach the following overall concentrations of protein and surfactant:

Lysozyme:	0, 2.0, 3.9, 5.9, and 8.3 mg/mL
DPC:	15, 29, 59, and 98 mM
CPC:	7, 13, 20, and 25 mM

In all cases protein was added first and the silica was equilibrated with the protein before adding the surfactant. After final equilibration the supernatant solution was removed and the silica slurry was transferred into specially designed SANS sample cells made from aluminum with quartz glass windows (sample width 1 mm, sample volume V_{cell} = 0.1 cm^3. Typically, the sample cells contained 30 mg of SBA-15 with an associated mesopore volume of ca. 30 µL (pore liquid) and 55 µL of extra-particle liquid (bulk solution), in which the concentration of protein and surfactant was much lower than in the pore liquid. SANS measurements were performed in the q range 0.04 – 0.32 nm^{-1}. Most samples with the shorter-chain surfactant (DPC) were studied in a q range up to 0.65 nm^{-1}.

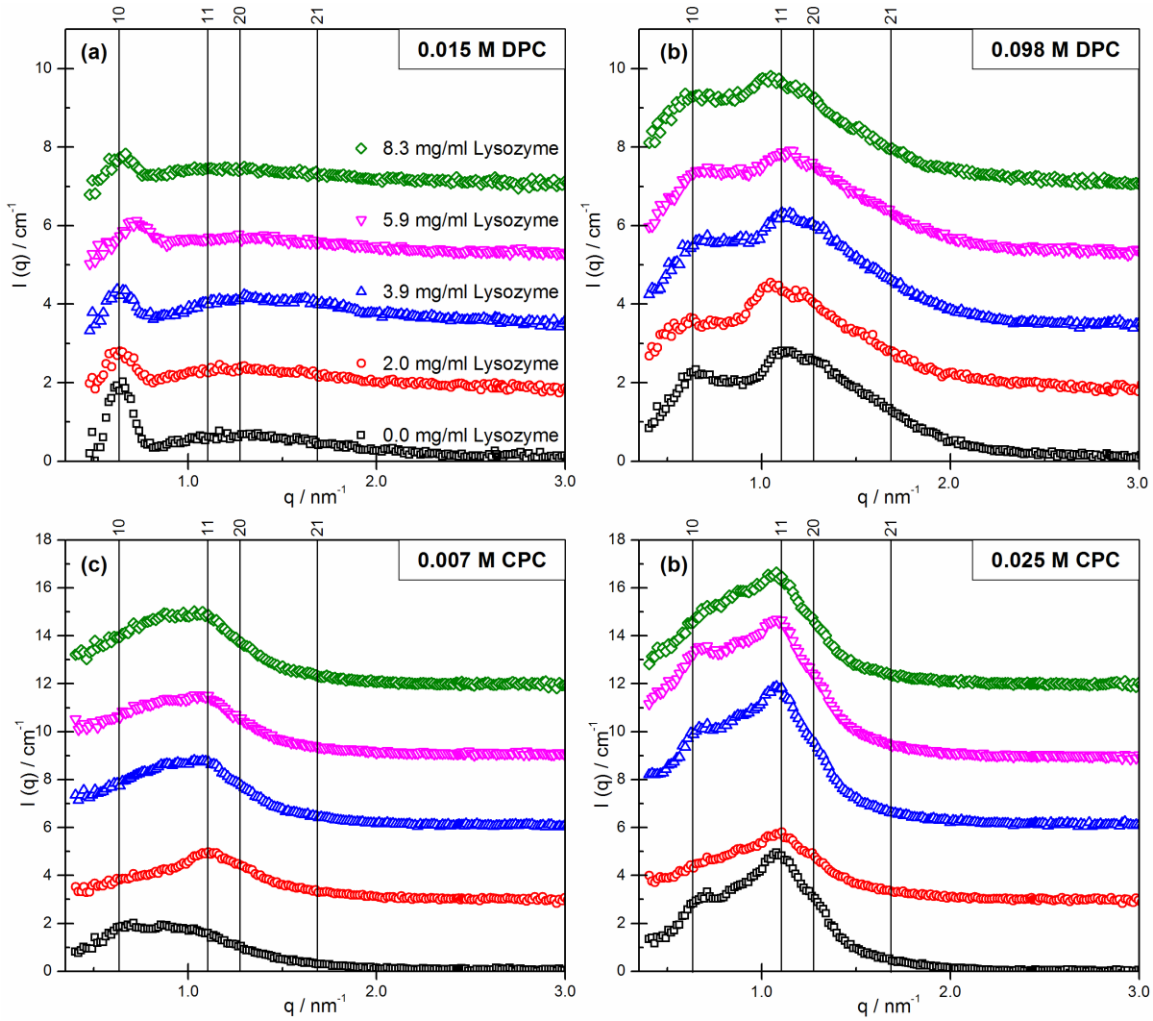

Fig. 1: Experimental scattering profiles *I(q)* for the set of lysozyme concentrations in the presence of the lowest and the highest DPC concentration (15 and 98 mM) are shown in Figure 1(a) and (b). The respective results for CPC at the lowest and highest concentration (7 and 25 mM) are shown in Figure 1(c) and (d).

At the chosen contrast conditions the protein is not directly contributing to I(q). For this reason the I(q) profiles can be interpreted qualitatively by comparison with scattering curves of surfactant in the absence of protein [5]. On this basis the scattering curves of Figure 1 indicate the following behavior: At low surfactant concentration (Fig. 1a) an increase in protein concentration in the pores causes a decrease in surfactant concentration in the pores. This indicates that DPC is adsorbed at those parts of the surface which are not occupied by the protein (co-adsorption regime). For high surfactant concentrations, on the other hand, Fig. 1b indicates that protein is displaced by surfactant in the pores, since the scattering profiles for different protein concentrations all have a similar appearance to that of DPC in the absence of protein. This conclusion is supported by a simulation of I(q) for DPC in SBA-15 based on our quantitative model [5].

This first beam time devoted to a study of protein–surfactant interactions in confined geometry turned out to be quite successful, as it provided an overview of the co-adsorption behavior of the two components for a wide composition range of protein and surfactant, for two surfactants of different chain length (i.e., different level of hydrophobic aggregation). However, a quantitative analysis of these data was hampered by the following experimental weaknesses:

(1) The q resolution of the instrument was not optimized so that the (11) and (20) Bragg peaks of SBA-15 could not be properly resolved.
(2) For samples of low scattering contrast (low surfactant concentration) the sampling time was not sufficient, so that the scattering curves are of no good quality

The chosen method of sample preparation appears to be adequate at low surfactant and protein concentrations. At higher concentrations (particularly in the case of the longer-chain surfactant CPC) the scattering curves of some samples do not fit entirely properly into the sequence of the others. We suspect that this may result from a ‘wrong’ composition in the pores due to incomplete equilibration of the samples. To avoid uncertainties of this kind, samples should be prepared in different ways, viz. pre-adsorption of protein, pre-adsorption of surfactant, and simultaneous addition of the two components. We plan to proceed in this way in the next experiment.

References

[1] J. Meissner, A. Prause, C. Di Tommaso, B. Bharti, G.H. Findenegg, *J. Phys. Chem. C* **2015**, *119*, 2438
[2] D. Müter, T Shin, B. Demé, P. Fratzl, O. Paris, G.H. Findenegg, *J. Phys. Chem. Lett.* **2010**, *1*, 1442
[3] T. Shin, D. Müter, J. Meissner, O. Paris, G.H. Findenegg, *Langmuir* **2011**, *27*, 5252
[4] B. Bharti, M. Xue, J. Meissner, V. Cristiglio, G.H. Findenegg, *J. Am. Chem. Soc.* **2012**, *134*, 14756
[5] B. Bharti, T. Shin, G.H. Findenegg, manuscript in preparation