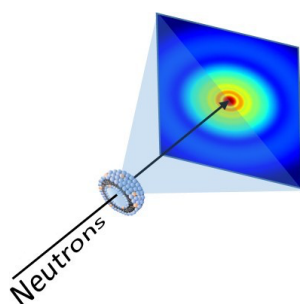


# Hellenic Neutron Association Newsletter



## Editorial

*We are in the second year of continuous effort to raise awareness about the research carried out using neutrons and to bring closer the relevant scientific community. In the current newsletter several scientific highlights are presented where Small Angle Neutron Scattering elucidates the structure of "Soft Matter" systems. We hope you enjoy reading them!*

— Dr. Konstantina Mergia (NCSR Demokritos)

## Research Note: Small Angle Neutron Scattering on Soft Self-Assembled Synthetic, Biological and Hybrid Nanostructures \* †

by DR. ARISTEIDIS PAPAGIANNPOULOS ‡

### Introduction

Small angle neutron scattering (SANS) is an established and powerful tool for studying matter at the nanoscale. It exploits the interaction of thermal neutrons with atomic nuclei to extract in a non-invasive manner information about

density/concentration fluctuations in the sample due to the specific overall atomic arrangement. SANS is applied to a wide range of research fields such as polymer science, biotechnology, food science, semiconductors, fuel cells, etc. In the field of soft matter (i.e. solutions of synthetic and organic polymers, foods, amphiphilic vesicles, hydrogels etc.) it has a long tradition going back to the fundamental studies on proteins in solution [1] and other macromolecular systems [2].

### SANS principles

As a collimated monochromatic neutron beam passes through a ma-

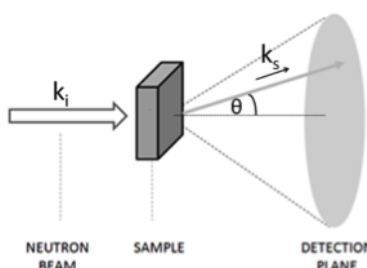
terial the neutrons are scattered from the atomic nuclei. The intensity of the interaction between a thermal neutron and a nucleus is described by the nucleus characteristic scattering length. As different isotopes have different neutron scattering lengths, this is exploited by the extremely important feature of contrast variation and contrast matching. For example the scattering length of hydrogen ( $H$ ) is very different from that of its chemically equivalent deuterium ( $D$ ). It is therefore common practice to use deuterated water ( $D_2O$ ) as a solvent for experiments in aqueous solutions with normal hydrogenated biological and synthetic polymers instead of  $H_2O$ . The intensity of scat-

\*The reported SANS experiments were performed on the KWS-2 high intensity/wide- $q$  small angle neutron diffractometer at the research reactor FRM II (Jülich Centre for Neutron Science) and the small angle scattering instrument V4 at the reactor BERII (Helmholtz-Zentrum Berlin).

†The synthetic polymers of the referred works were synthesized at the Theoretical and Physical Chemistry Institute of the National Hellenic Research Foundation.

‡Theoretical and Physical Chemistry Institute, National Hellenic Research Foundation, 48 Vassileos Constantinou Avenue, 11635 Athens, Greece

tered neutrons is usually measured by a two-dimensional detector. For an isotropic system it is azimuthally integrated to the one-dimension scattering function  $I(q)$ . The scattering wave vector  $\vec{q}$  has an amplitude equal to  $q = 4\pi/\lambda \sin \theta/2$  where  $\lambda$  is the wavelength of the neutrons and  $\theta$  the scattering angle. It is defined by the difference between the scattered and incident wave vector i.e.  $\vec{q} = \vec{\kappa}_s - \vec{\kappa}_i$ . The  $q$  range ( $10^{-2} \text{ nm}^{-1}$  to  $1 \text{ nm}^{-1}$ ) is covered by utilizing small angles in comparison to other neutron techniques and long neutron wavelengths.



Schematic illustration of the scattering setup in a SANS experiment.

Given the ability of the SANS technique in the elucidation of the structure of "Soft Matter" systems, in the following we present a series of relevant applications involving the study of polymer solutions and also the interaction of polymers with protein molecules.

#### Interactions between self-assembled amphiphilic nanoparticles and proteins

The size and shape of particles is determined by measurements at sufficiently low solution concentrations so that the inter-particle interactions are negligible. For spherically symmetric particles

$$I(q) = N_p \left\{ 4\pi \int_0^R (\rho(r) - \rho_{D_2O}) r^2 \frac{\sin qr}{qr} dr \right\}^2$$

where  $R$  is the particle radius,  $N_p$  the number of particles per unit vol-

ume and  $\rho(r)$  the distribution of the scattering length density.

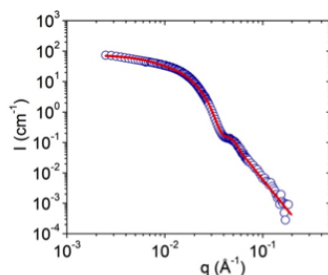


Figure 1. SANS profile from PnBA94-b-PAA170 3 mg/ml at pH 7 with 0.01 M NaCl in  $D_2O$ . The red line is the best fit with a micellar core-shell model. Adapted with permission from [3]. Copyright 2016 American Chemical Society.

The experimental data of Fig. 1 show that poly(n-butyl acrylate)-b-poly(acrylic acid) (PnBA-b-PAA) diblock copolymers self-assemble into spherical core-shell micelles with a compact hydrophobic core (PnBA blocks) with radius of  $R_c = 10.8 \pm 0.4 \text{ nm}$  and a hydrated hydrophilic shell (PAA blocks) of radially decreasing volume fraction profile with radius  $R_m = 58 \pm 1 \text{ nm}$ .

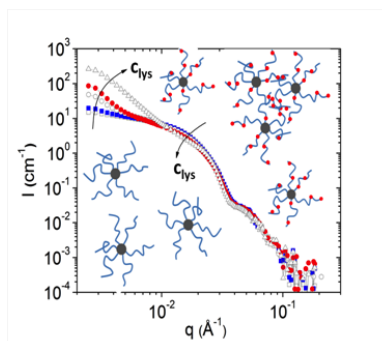


Figure 2. SANS from lysozyme/PnBA94-b-PAA170(0.6 mg/ml) at pH 7 0.01 M NaCl with mass ratios of lysozyme/polymer: 0/10 ( $\square$ ), 0.5/10 (blue  $\blacksquare$ ), 1/10 ( $\circ$ ), 2/10 (red  $\bullet$ ), and 4/10 ( $\triangle$ ). Adapted with permission from [3]. Copyright 2016 American Chemical Society.

When the oppositely charged globular protein lysozyme is added to the solution a strong increase of  $I(q)$  at low  $q$  reveals that micelles form large intermicellar aggregates (Fig. 2). The mild but systematic decrease of  $I(q)$  at intermediate  $q$  is a modulation of the micellar form factor and it

is related to the shell of the micelles i.e. lysozyme globules attach mostly on the periphery of the micelles leading to bridging between micelles [3]. Protein loading is electrostatically driven as it weakens by an increase in the salt concentration in solution. This work can find applications in protein/nanoparticle interactions for drug and protein delivery purposes.

#### Self-organization in polyelectrolyte/protein complexes tuned by hydrophobic content

Two poly(chloromethyl styrene) polymers (QIm-PCMS) of different quaternization degrees (QIm-PCMS1 at 73% and QIm-PCMS2 at 100%) were used to elucidate the role of hydrophobic interactions on oppositely charged polyelectrolyte/protein complexes [4]. In Fig. 3 the probed length scales are relevant to the size of bovine serum albumin (BSA). In the absence of BSA the profiles are practically identical because counterion condensation along the polyelectrolyte chains dominates the effective charge fraction. In the presence of BSA the enhancement of scattering at low  $q$  is more pronounced for the polyelectrolyte with higher hydrophobic content (QIm-PCMS1). Circular dichroism experiments showed that the alpha helix of BSA is denatured upon interaction.

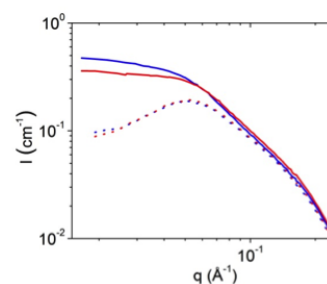


Figure 3. SANS from BSA(4mg/ml)/QIm-PCMS(16mg/ml) complexes (black= QIm-PCMS1 and red= QIm-PCMS2). Dotted lines are the profiles of the corresponding pure polymer solutions. Reproduced from [4] with permission from the PCCP Owner Societies.

This work demonstrated the ability to tune the polyelec-

trolyte/protein interactions and polyelectrolyte inter-chain associations by modifying the hydrophobic content of the polyelectrolytes [4].

### Hierarchical structure in polysaccharide solutions

SANS has the capability to resolve morphology in multiple length scales. The case of polysaccharide xanthan, a short-side-chain high molecular weight natural polyelectrolyte and an important food biopolymer that creates complex self-similar viscoelastic fluids in water [5] offers a great example. Fig. 4 reveals self-organization at three hierarchical levels. Scattering from large length scale structures ( $> 100nm$ ) reveals that the double-helix associations cause inter-chain contacts that are responsible for the complex rheological behaviour [6].

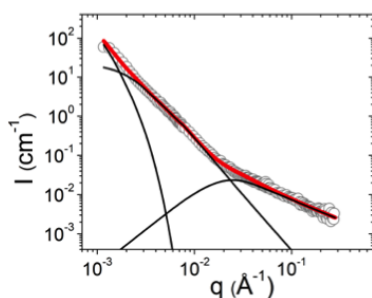


Figure 4. SANS from xanthan solutions at  $8mg/ml$  in  $D_2O$ . The red line is the best fit with a three-level hierarchical model. The separate contributions are shown with black lines. Adapted from [6] with permission.

At intermediate length scales the structure factor of the semidilute solution of xanthan is acquired. At short length scales (high  $q$ ), defined by the extracted correlation length  $\xi = 11 \pm 1nm$ , the power-law of rigid rods  $I(q) \sim q^{-1}$  is obtained. This is the signature of the local helical morphology of xanthan which is stabilised by its side-chains. The molar mass per unit length of xanthan can be estimated at this regime at  $77 \pm 4gmol^{-1}\text{\AA}^{-1}$  and corresponds to single-stranded helix conformation.

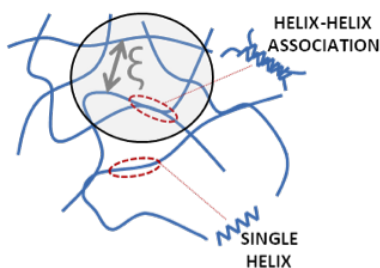


Figure 5. Schematic illustration of xanthan semidilute solutions based on SANS measurements.

A schematic representation of the hierarchical organization in xanthan extracted by SANS is shown in Fig. 5. This work described the morphology and dynamics of xanthan solutions in an attempt to explain the morphological origins of their viscoelastic properties and can be generalized to other polysaccharides [6].

### Conclusion

The motivation of this research comes from the need to understand the morphology/mechanical properties relation in complex fluids e.g. food hydrocolloids, to control interactions in polyelectrolyte/protein complexation and to elucidate the physical chemistry of protein or drug encapsulation and delivery at the nanoscale. The advances on the use of SANS, as an invaluable tool towards this scope, have been demonstrated. This research note may find use as a reference for future developments within the Hellenic Neutron Association.

### Acknowledgements

The author would like to thank Dr. Eleni Vlassi, Mr. Konstantinos Sotiropoulos, Dr. Aurel Radulescu and Dr. Stergios Pispas for fruitful collaboration in the last four years.

- (1) L.A. Feigin, D.I. Svergun, Structure Analysis by Small-Angle X-Ray and Neutron Scattering, Springer 1987
- (2) J.S. Higgins, H.C. Benoit, Polymers and Neutron Scattering, Oxford Science Publications 1997.
- (3) A. Papagiannopoulos, A. Meristoudi, S. Pispas, A. Radulescu, Biomacromolecules, 17 (2016) 3816-3827.

(4) A. Papagiannopoulos, E. Vlassi, S. Pispas, C.J. Jafta, Physical Chemistry Chemical Physics, 19 (2017) 18471-18480.

(5) A. Papagiannopoulos, K. Sotiropoulos, S. Pispas, Food Hydrocolloids, 61 (2016) 201-210.

(6) A. Papagiannopoulos, K. Sotiropoulos, A. Radulescu, Carbohydrate Polymers, 153 (2016) 196-202.

## PhD Report: Nanoporous alumina membranes for the confinement of condensed matter

by ANASTASIA CHRISTOULAKI §

Nanoporous materials are suitable templates for the study of polymers in confined dimensions. The walls of the porous template impose a topological constrain for the available conformations that a polymer chain can explore. As polymers are governed by entropic forces this barrier is expected to affect the conformation and the dynamics of the chains in comparison to bulk conditions. Moreover, porous templates bear an electrical charge in polar solvents like water, due to the dissociation of chemical surface groups. This property makes these templates appropriate systems for studying interactions of charged surfaces with charged polymers such as polyelectrolytes (PEs). Understanding the behavior of charged polymers inside such nanochannels helps in designing a large number of technological and medical applications related to the modification of a porous surface, such as in filtration devices and biosensors. Throughout the literature few experimental studies deal with the confinement of PEs from which original physical behaviors can emerge. For example, by Small Angle Neutron Scattering (SANS) measurements on a disordered porous network (Vycor glass) of  $7\text{\AA}$  in pore diameter it was found that a PE acquired a more stretched conformation inside the channels [1]. The advantage of SANS measurements is the implementation of the contrast vari-

§Sorbonne Universités, UPMC Université Paris 06, CNRS UMR 8234, Laboratoire PHENIX, Case 51, 4 place Jussieu, F-75005 Paris, France

ation method. By adjusting the ratio of deuterated to hydrogenated solvent that wets the matrix, the porous template can become "invisible" to neutrons and such ratio is the so called matching point. As a consequence, only the contribution from the polymer can be detected, from which its radius of gyration and persistence length can be extracted.

In this doctoral scientific project, sodium polystyrene sulfonate (NaPSS), a well-known model polyelectrolyte is studied in nanoporous alumina membrane (nPAM). The nPAM consists of an ordered porous network of parallel channels with pore diameters lying in the nanometer scale. The membranes are synthesized electrochemically by anodization of aluminum. The synthesis involves the immersion of an Al foil inside an electrolyte, usually consisting of oxalic, phosphoric or sulfuric acid and the application of a constant voltage. As seen in Figure 1a, the pores are self-ordered in hexagonal lattice and they are enclosed by a cell made from aluminum oxide. The pore structure can be extracted from the SEM image by applying a Fast Fourier Transform (FFT). This operation provides the structure factor that can be fitted with a hexagonal lattice model (Figure 1b). The thickness of the membrane can reach up to a hundred of microns and at the end each pore is closed by nanometric barrier type oxide, which is called the barrier layer. Anodization is a low-cost synthesis and provides the possibility to tune the geometrical characteristics of the membranes such as, the pore diameter  $D_p$ , interpore distance  $D_{int}$  and thickness by modifying the conditions of the anodization (electrolyte, voltage). For example, a universal law exists for the dependency of the  $D_{int}$  on the applied voltage  $V$  according to:

$$D_{int} = kV$$

$$2.3 < k < 2.5 \text{ nm/V}$$

The exact mechanism of pore ini-

tiation and self-ordering remains still in debate but it has experimentally been observed that the pores initially form in less ordered positions on the aluminum foil but as they grow in thickness they experience stresses and compressive forces that lead them to take a closed pack hexagonal organization at deeper distances. To obtain an ordered membrane through its whole thickness a two-step anodization is applied. A first a film of several microns in thickness is synthesized and chemically dissolved afterwards. The dissolution will not affect the aluminum foil which is now imprinted by an ordered network of dimples. Then, a second step anodization under the same conditions as the first is performed leading to the growth of ordered pores.

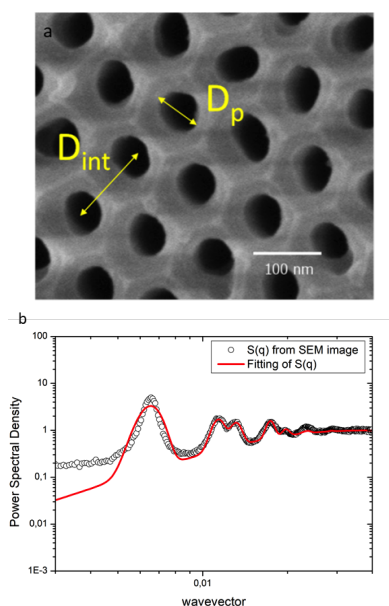


Figure 1. a) Top view of a nPAM prepared in 0.3M oxalic acid at 40V, observed by Scanning Electron Microscope (SEM). b) Structure factor extracted from a SEM image of a porous alumina membrane.

SANS is a very powerful technique to access both structure and composition of nPAM. First measurements with SANS were performed on the membrane ("PAXY" spectrometer, Laboratoire Léon Brillouin, LLB, CEA, Saclay) in order to access the geometrical characteristics averaged in the membrane's volume

and find the appropriate volume ratio of deuterated/hydrogenated water ( $D_2O/H_2O$ ) that corresponds to the contrast matching point. Contrast variation is a method which can give information on the composition of the membrane and also helps to overcome the effect of multiple scattering which is very common in porous systems. The existence of ordering throughout the membrane's volume is reflected on the SANS spectrum by the appearance of characteristics peaks (Figure 2). The spectrum is obtained by measuring the nPAM inside a quartz cell filled with a mixture of  $D_2O/H_2O$  with the neutron beam parallel to the pore axis. In this case the pores are the scattering object with scattering length density (SLD) given by the mixture of  $D_2O/H_2O$  in use and the porous mesh is the solvent. The scattering intensity is given by the following expression:

$$I(q) = \phi V \Delta\rho^2 P(Q) S(q)$$

where  $\phi$  is the volume fraction of the scattering object,  $V$  is the volume of the scattering object,  $\Delta\rho$  is a contrast term which is equal to the difference between the scattering length density of the solvent and the scattering object,  $P(q)$  is the form factor related to the shape of the object and  $S(q)$  is the structure factor which is given by the pore positions. At a first glance on the curve of Figure 2 one can observe several peaks appearing in the region of low and middle scattering vector  $q$ . The first peak  $q_1$  appearing at the low  $q$  region is coming from the contribution of the structure factor and corresponds to the interpore distance  $D_{int}$ . A value of 100nm can be calculated by converting the reciprocal dimensions into real space dimensions via the relation:

$$D = \frac{2\pi}{q}$$

The next peaks appearing at the middle  $q$  region are a combination of the structure factor and the form factor. Moreover, the scattering intensity in the high  $q$  region decays with a



power law of  $q^{-3}$ , which is a characteristic scattering from aligned cylinders in respect to the beam. Several samples in different volume fractions of D<sub>2</sub>O were measured but matching of the membrane was not possible. One explanation is that the membrane's pore walls are not homogeneous in composition. In the literature it is reported that the pore walls of the alumina membrane consist of a double layer that differs in composition [2]. During the synthesis of the membranes anions coming from the electrolyte, for example oxalates in the case of anodization in oxalic acid, are incorporated into the oxide walls. The amount and spatial extent of this contamination differs between samples prepared at different anodization conditions. Another cause of heterogeneity is that the alumina starts to form aluminum hydroxide in the presence of water. Such compositional differences affect greatly the scattering intensity and their spatial extent can be determined by fitting the spectra with an appropriate model. We are currently working on the fitting of the scattering spectra with a model that consists of aligned cylindrical core/shells for the  $P(q)$  to account for the contamination effects and a hexagonal lattice for the  $S(q)$  [3]. To constrain the fitting parameters, for example the inter-pore distance and its distribution or the size of the ordered domains can be extracted from the structure factor of a SEM image. In conclusion, nPAMs are promising and versatile membranes for confinement studies. Their characterization with SANS can give information on their structure. Their complex composition can be studied with SANS and is essential before using them as host a matrix for condensed matter.

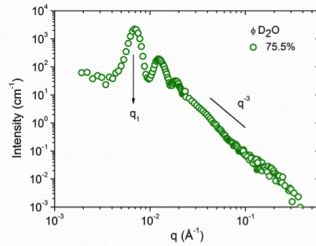


Figure 2. SANS spectrum of a nPAM measured at volume fraction of 75.5% D<sub>2</sub>O. The first peak  $q_1$  corresponds to the  $D_{int}$  inter-pore distance.

- (1) E. P. Gilbert, L. Auvray, and J. Lal, "Structure of Polyelectrolyte Chains Confined in Nanoporous Glass," *Macromolecules*, vol. 34, no. 14, pp. 4942-4948, Jul. 2001.
- (2) F. Le Coz, L. Arurault, and L. Datas, "Chemical analysis of a single basic cell of porous anodic aluminum oxide templates," *Mater. Charact.*, vol. 61, no. 3, pp. 283-288, Mar. 2010.
- (3) A. Christoulaki, A. Chennevière, E. Dubois, N. Jouault, In preparation.
- (4) J. Martín et al., "Direct Observation of Confined Single Chain Dynamics by Neutron Scattering," *Phys. Rev. Lett.*, vol. 104, no. 19, p. 197801, May 2010.

## Useful links

[European Neutron Scattering Association](#)



The European Neutron Scattering Association

The European Neutron Scattering Association (ENSA) is an affiliation of national neutron scattering societies and committees, which directly represent users. The overriding purposes of ENSA are to provide a platform for discussion and a focus for action in neutron scattering and related topics in Europe.

[Neutron News Journal](#)

Neutron News is the most up-to-date resource for the neutron community. Meeting reports provide timely coverage of what the leaders in neutron research are talking about. Scientific research is presented in a broad array of articles and case reports covering topics such as neutron scattering, materials research, site studies, and much more. This magazine is a must read for anyone engaged in neutron research..

[Neutronsources Webpage](#)

[Neutronsources.org](http://Neutronsources.org)

Your entry into the neutron world

Neutronsources.org is an initiative of neutron research facilities and neutron communities around the world. The aim is to provide information material and news on research using neutron beams.

A network of press officers from several neutron centres and associations worldwide is supporting the website coordinator in managing and editing the content published on this platform and ensure the quality of the articles provided. This group works together in regular meetings (online or at the centres) and via email exchanges. For the press officers' contact details click here.

Those who are new in the field can browse the Science with Neutrons page to get to know more about neutrons, their characteristics and applications. Under Resources one can learn about projects and collaborations using neutrons, as well as useful software and tables. On the calendar, you can browse future and past neutron events such as conferences, workshops and schools, including a list of regular events like the European and International Conferences on Neutron Scattering. Have you got a brilliant idea for a research experiment? The website tells you the facilities? deadlines for submission of proposals and also the operating periods. Are you looking for a job or need a change in your career

path? You might find it on the up-to-date job openings.

### Contact with the editorial board

The provisional editorial board welcomes articles and ideas about

the contents of the HENA newsletter from fellow scientists in Greece and abroad. For this purpose please contact:

- Dr. Konstantina Mergia, NCSR Demokritos, Athens  
kmergia[at]ipta.demokritos.gr

- Asst. Prof. Dimitrios Anastasopoulos, Univ. of Patras,  
anastdim[at]physics.upatras.gr
- Dr. Alexandros Koutsioumpas, Maier-Leibnitz Zentrum, Munich,  
a.koutsioumpas[at]fz-juelich.de