JANUARY 2019, NO 4

Hellenic Neutron Association Newsletter

Editorial

Dear colleagues, the editorial board of the HENA newsletter wishes you a happy and very successful new year! 2019 started with the kick off meeting of the BrightnESS2, a project funded by Horizon 2020, with 15 participating research organisations and facilities from 11 different countries aiming at strengthening neutron scattering in Europe and creating an ecosystem for sustainable neutron science with the European Spallation Source under construction in Sweden. Within Greece, discussions have been initiated with the General Secretariat of Research and Technology for the participation of Greece in existing neutron sources. Members of our society are invited to submit their ideas in order to formulate the policy of our society towards this end. The suggestions received will be circulated to all members of our society for a fruitful dialog. In the near future a meeting will be organized to discuss the issue. Also the Greek Crystallographic Association will be contacted for co-organizing the meeting including also a discussion for joining a Synchrotron source. In the current newsletter two research highlights are presented elucidating the use of small angle neutron scattering technique at grazing and normal incidence for the study of interfaces and membrane protein structures.

— Dr. Konstantina Mergia (NCSR Demokritos)

Research Note: Probing close to the interface using grazing-incidence small-angle neutron scattering *

by PROF. VASSILIOS KAPAKLIS †

Interfaces and structures forming in the vicinity of them, are beyond doubt of paramount importance in physics, chemistry, biology and materials science. As such, a vast arsenal of experimental techniques has been developed to tackle the rather tough challenge of characterizing structures close to interfaces. These span from microscopy [1,2], spectroscopy [3], ion beam probes [4] to scattering methods, employing x-rays [5,6], and neutrons [7,8].

In the case the interfaces studied, involve buried, solid/liquid or soft matter related interfaces, neutron scattering holds an advantage. The possibility to enhance the sensitivity using isotopic labelling and contrast variation, combined with the very high penetration depth, valid for neutrons, offers unique opportunities for advanced interface characterization. In the specific case of grazing-incidence small angle neutron scat-

*The author is indebted to Shirin Nouhi, Maja Hellsing and Adrian R. Rennie for the fruitful collaboration and development of the presented projects. The Swedish Research Council is acknowledged for financial support, along with Institut Laue-Langevin and NCNR for beamtime allocation. The support from staff at NG3 SANS, NIST and D22, ILL is also greatly acknowledged.

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tering (GISANS), the probing depth can be tuned and targeted to volumes of interest close to an interface [6]. These facts raise the rather obvious follow up question, stating: "*to which extend does this tuning apply and how can one design, perform experiments and evaluate the related data, with respect to realistic conditions and available instruments?*".

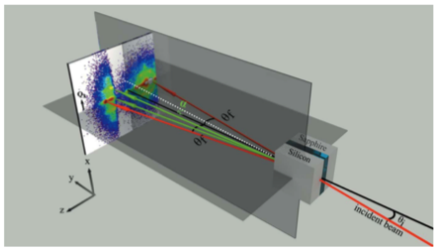

Figure 1. Typical geometry for a GISANS experiment [7], for a sample confined between two solid crystals (Silicon and Sapphire). Scattering patterns can be recorded, related to a beam transmitted through the sample and one scattered ($\theta_i = \theta_f$). Reproduced with permission of the International Union of Crystallography.

In an attempt to shed more light on this question [9], we have recently endeavored into a study of a system, which can be considered as a prototype, consisting of charge stabilized colloidal polystyrene particles [10]. These have been shown to form extended crystal structures, against solid interfaces [11], up to more than 10 cm^2. Using this approach, we have explored the current limits of GISANS characterization, along with the ability to control the probing depth. The investigated samples have been characterized with a variety of other techniques [12], meaning we can directly review and evaluate the GISANS results in comparison to them.

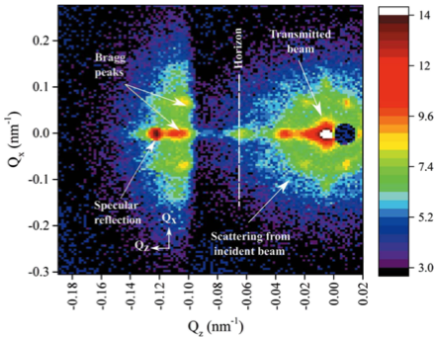

Figure 2. A typical detector image from an experimental geometry, shown in Figure 1. The 2D detector area can be divided into two regions. The first one relating to the transmitted beam (right side) and a second one, around the specularly reflected beam (left side). Scattering around the transmitted beam originates mostly from the bulk of the sample, whereas scattering around the reflected beam from the volume close to the solid/liquid interface [7]. Reproduced with permission of the International Union of Crystallography.

In GISANS, the penetration depth of the neutron beam is given by [9]

$$z_{1/e} = \frac{\sqrt{2}\lambda}{4\pi\left\{\left[(\theta_i^2-\theta_c^2)^2+(\frac{\lambda\mu}{2\pi})^2\right]^{1/2}-(\theta_i^2-\theta_c^2)\right\}^{1/2}}$$

where $z_{1/e}$ is the penetration depth, λ the neutron wavelength, μ the attenuation coefficient, θ_i the incidence angle and θ_c the critical angle for the measured interface. This equation is valid when we assume that the scattered beam angle θ_f is equal to θ_i. Theoretically, the combination of all these parameters defines precisely the penetration depth $z_{1/e}$ and the volume close to the interface contributing to the measured scattering intensities. In reality, things are somewhat more complicated, as all instruments have given resolutions [9, 13]. More specifically, all neutron scattering instruments do not actually have "monochromatic" beams, but a rather broad wavelength distribution around the set wavelength, which is further accompanied by a range of incident angles - angular dispersion. This fact has stark implications on the scattering recorded in GISANS. Shorter wavelengths penetrate the sample well below the critical angle for the set wavelength, giving rise to scattering, while the mean wavelength is still totally reflected (see Figure 3). This results in a smearing of all recorded intensities close to the critical angle, making the interpretation of experiments that aim in characterization of the volume very close to the interface, challenging.

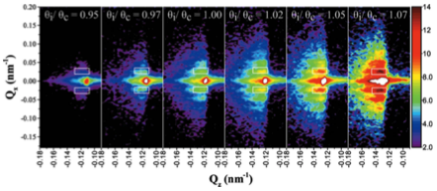

Figure 3. Detector images from a GISANS experiment, involving a charge-stabilized polystyrene colloid against a Silicon surface. Significant scattering for incidence angles θ_i well below the critical angle θ_c is observed [9]. Reproduced with permission of the International Union of Crystallography.

To examine and elaborate further on this smearing effect, we have proposed a very simple and adaptive model, for the estimation of the scattered intensities near the critical angle in GISANS experiments [9]. Our model accounts for the real instrumental resolutions (for both wavelength and incident angles), which enter as adjustable parameters. Assuming, a scattering length density profile for the sample, it is now possible to compute expected scattering intensity values for Bragg peaks, similar to those shown in Figure 3, by a simple convolution function [9].

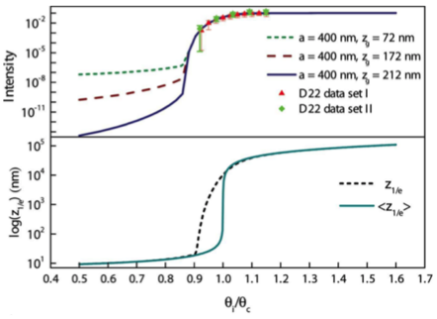

Figure 4. Relative GISANS intensities calculated using the model described in the main text (polystyrene particles on a lattice, where a is the lattice paramater). Lines stand for model calculations and points to experimental data from Nouhi et al. [9]. Top panel: GISANS intensities for a charge-stabilized polystyrene colloid at a Silicon interface. The various lines shown calculations for different separations z_g from the interface. Bottom panel: Penetration depths with (dashed line) and without (solid line) wavelength spread. Reproduced with permission of the International Union of Crystallography.

As can be seen in Figure 4 (bottom panel) the considerations con-

cerning the wavelength and angular spread, explain why we record scattering intensities below the critical angle (Figure 3). Furthermore, to the best of our knowledge, our model simulates quantitively for the first time GISANS data (Figure 4, top panel). When looking closer, it becomes clear that the wavelength spread induces limitations on the ability to tune precisely the penetration depth. For our sample and given the instrumentation parameters, it is not possible to resolve spacings between our colloid crystal and the interface, in the range of 0 to 250 nm. This happens mainly, because the wavelength spread is so significant that the intensity smearing near the critical angle makes any distinction impossible. Independently, quartz crystal microbalance with dissipation measurements (QCM-D) have shown that the particles are close to the interface but not bound to it. The spacing between the first layer of particles and the interface lies in the range of 20-100 nm, which proved not possible to resolve using GISANS. For the simulated intensities, a difference varying the spacing is only observable well below the critical angle. This region suffers though from the fact, that it would yield intensities orders of magnitude below what is measurable today, at state-of-the-art GISANS instruments.

GISANS is nonetheless a powerful technique for the study of interfaces. As seen here, structure characterization up to several micrometers is possible. The evanescent wave, extending only a few tens of nm from the interface, probes a very small volume, giving weak scattering compared to that of the transmitted beam. Even though the accurate characterization of the near interface volume can be challenging, our work has provided a generic model that can be applied to any instrument and sample, providing a tool for the quantitative analysis of GISANS data, but also the design and optimization of an experiment. Hopefully in the near future, new neutron sources with higher flux and improved instrument design (small wavelength and angular distributions) will enable an improved and more extended application of GISANS.

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Research Note: Low resolution membrane protein structure from SANS

by Dr. Alexandros Koutsioumpas ‡

Solution small angle scattering of X-rays (SAXS) and neutrons (SANS) represent established methods for the determination of the overall size, form and interaction between particles dissolved in a solvent. In the previous issue of the HENA newsletter, the general principles of the technique and also applications to polymer and nanoparticles systems have been showcased in the short article contributed by Dr. A. Papagiannopoulos.

Small angle scattering is also well suited for low-resolution investigations of biological molecules in solution. Experimental data from dilute solutions of proteins, nucleic acids and of their complexes, contain information about the overall size and shape of the molecules at a typical resolution of 1-2nm (the interested reader may refer to excellent reviews [1,2] of the topic). Despite that the accessible resolution from small-angle scattering is much lower than the typical resolution of crystallographic investigations, the fact that there is no need for crystallization of the sample and also the ability to perform measurements in solution in near physiological conditions, makes the technique quite useful in a wide range of biophysical studies.

In practice in studies involving the use of biological small-angle scattering (bio-SAS) we encounter one of the two following general scenarios a) an all-atom structure of a molecule

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or molecular complex is available by crystallographic input or by a structure prediction methodologies and we want to verify that the structure in solution is compatible or b) there is none or limited information about the structure of the system and only a small-angle scattering curve (scattered intensity I as a function of momentum transfer Q) is available. In the latter case that will be considered in this short article, we need to solve what we call the "inverse problem" that is extracting the maximum amount of structural information from the available scattering data.

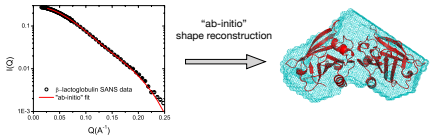

Figure 1. Shape reconstruction from bovine β-lactoglobulin SANS data using the program DENFERT [4]. On the right the obtained molecular envelope (cyan) is superimposed with the crystallographic cartoon representation of the dimer.

In a bio-SAS curve, the low-Q region contains information about the size of the molecule (or particle). However the intermediate Q-region up to $\approx$ 3nm^{-1} contains information about the overall shape on the molecule. At such a spatial resolution ($2\pi/Q \approx$ 2nm), folded biological molecules like globular proteins and nucleic acids are characterized by a given contrast relative to the solvent (water), since they share common structural motifs. That means that we may approximate biomolecular structures as compact envelopes of uniform contrast relative to the solvent. A way for finding such a molecular envelope is to represent the molecule as a collection of "dummy-atoms" of given contrast in space, and search through a minimization procedure a compact arrangement that reproduces the measured scattering. Algorithms that attempt such shape reconstructions (DAMMIN/IF [3], DENFERT [4]) belong in the class of "ab-initio" shape reconstruction methodologies for low-resolution structure recovery from SAS data. In Fig. 1 we present such a shape reconstruction from SANS data for the dimer of bovine bovine β-lactoglobulin protein.

The above-described ab-initio methods, work in a straightforward way for globular soluble proteins. However such an approach cannot be used in the case of membrane proteins. Due to the special nature of these molecules (large patches of their surface are hydrophobic) there is a need for detergents (or other molecular assemblies such as bicelles, nanodiscs or amphipols) for their solubilization. Even when the scattering signal belonging only to the protein/detergent complex can be measured, the coexistence of the detergent protective belt with solubilized membrane proteins means that extraction of the scattering signal belonging only to membrane proteins is not trivial, as not only the proteins but also detergents contribute to the scattering. Additionally we have to note that due to the "different chemistry" between proteins and the hydrophilic and hydrophobic parts of detergents, their neutron contrast (scattering length density) is quite different, meaning that experimental attempts to contrast match the solvent and the entire detergent molecule are quite challenging.

In a recent paper published at the Biophysical Journal [5], we illustrate that SANS data obtained at different solvent contrasts (i.e. different D2O/H2O ratios) permit, through the use of an appropriate model and minimization procedure, the reconstruction of the low resolution structure of the different parts of detergent/protein complexes (see fig. 2). This model attempts to fit the experimental data using a multiphase "ab-initio" approach that encompasses a set of physical constraints adapted to the anticipated assembly of detergents around the hydrophobic patch of the protein surface.

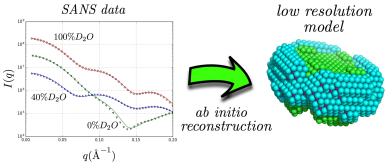

Figure 2. SANS curves of complexes of the membrane protein aquaporin-0 and the detergent n-Dodecyl β-D-Maltoside at various solvent contrasts (left), result of the shape reconstruction (right).

Essentially the membrane protein/detergent complex is represented by "dummy atoms" of three different types (contrasts) that correspond to the protein, detergent hydrophilic heads and detergent hydrophobic tails. A set of physical constraints is applied on the spatial arrangement of each group of "dummy atoms" related to their hydrophobic/hydrophilic nature. Several tests with simulated and experimental data have shown that by acquiring two different solvent contrast SANS curves belonging to protein/detergent complexes, the methodology is able to recover the low-resolution structure of the system. An example of application is illustrated in fig. 2 where the obtained molecular envelope of the filamentous hemagglutinin adhesin b-barrel protein transporter (Fhac) solubilized by n-Octyl β-D-glucopyranoside is superimposed with its known crystallographic structure.

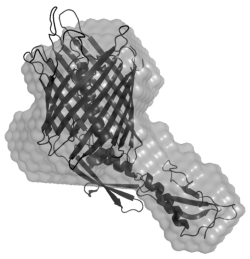

Figure 3. Comparison of the ab initio envelope (light shading) with the crystallographic structure of the Fhac transporter.

The presented methodology coupled with high quality data acquisition at current and future advanced neutron sources provides a powerful new approach for attacking the no-

toriously difficult problem of membrane protein structure determination, especially in the lower molecular weight window that falls outside the current range of cryo-electron microscopy investigations.

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Conferences

European Conference on Neutron Scattering (ECNS 2019)

The European Conference on Neutron Scattering (ECNS 2019) which will take place in St. Petersburg, Russia, July 1-5, 2019. The conference is held every four years under the patronage of the European Neutron Scattering Association (ENSA). Topics that will be covered are directly related with the European Great Challenges.

Synergy of Neutron Scattering and Complementary Tools to Reveal New States of Matter, 4-5 May 2019

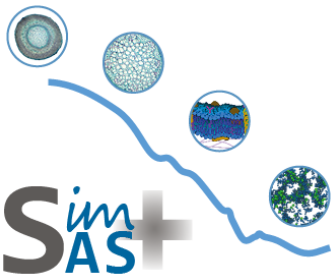
Neutron Scattering (GRS)
Gordon Research Seminar

The Gordon Research Seminar on Neutron Scattering is a unique forum for graduate students, post-docs, and other scientists with comparable levels of experience and education to present and exchange new data and cutting edge ideas.

The 2019 GRS on Neutron Scattering will highlight research being conducted by early career scientists and focused on the neutron scattering technique as a cutting edge tool to probe the structure and dynamic of matter. Oral presentations, poster sessions, and open floor discussions will specifically target on how the neutron scattering can be combined to other experimental, computational and theoretical tools, to provide a unique and powerful approach to reveal new states of matter. This meeting will cover different scientific areas: from soft- and hard-materials to instrumentation and data analysis.

Workshops

SimSAS, 8-11 April 2019



The field of modeling in Soft Matter and Life Sciences is growing tremendously. Yet the gap between the expertise of dedicated research groups in simulation and the knowledge of Small Angle Scattering users is widening.

This CECAM workshop aims at bridging these two worlds by gathering developers of multi-scale simulation methods and expert users of small angle scattering techniques.

The state-of-the-art will be discussed by invited speakers, including their accessibility and limitations. SAS experimentalists from Soft Matter and Life Sciences are encouraged to attend and provide feedback.

Two sessions will be dedicated to parallel working groups to promote collaborations between simulations and Small Angle Scattering communities.

ADD2019, 17-22 March 2019

The ADD2019 School and Conference aim to deepen the understanding and to further the training of the various communities working on real-space data analysis for neutron and x-ray diffraction techniques. Fourier transformation of diffraction data into real-space, traditionally used for the structural determination of liquids and glasses, is now increasingly employed for partially-disordered crystalline powder samples, and most recently for spin-spin correlations in disordered or frustrated magnetic systems (in the case of neutron diffraction).

CanSAS XI Meeting in Freising / Germany

canSAS stands for collective action for nomadic small-angle scatterers. The activities are aimed at improving co-operation between synchrotron and neutron facilities as well as users and manufacturers of laboratory SAXS equipment to advance the technique. Co-operation aims principally to improve shared data handling and analysis tools, provide information resources to the community and to ensure reproducibility and reliability. A current theme is to bring this work to grazing incidence scattering.

The next canSAS workshop will be hosted by JCNS/MLZ from 8 to 10 July 2019 in Freising near Munich, Germany.

People interested in joining these activities are welcome to register now for the meeting and to submit titles/abstracts for short presentations on themes that would promote further co-operation. It is expected that a large part of the time will be devoted to break-out sessions and discussion so that effective work plans emerge.

The organisers look forward to seeing you next July and to holding a productive meeting in Freising.

Neutron Schools

16th Oxford School on Neutron Scattering

Date and Venue: 2-13 September 2019, St. Anne s College, University of Oxford.

About the School: The school is intended primarily for scientists, student and postdoctoral researchers, who are new to the field of neutron scattering. It provides an excellent introduction to the field, which is developed through to its application in contemporary research. Lectures and tutorials covering all aspects of the theory and practice of a variety of neutron scattering techniques will be given by international experts. Students will gain a comprehensive grounding in modern techniques and applications at both steady state and pulsed neutron sources and have the opportunity to hear about the latest research being carried out with the technique.

Useful links

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.

Neutronsources Webpage

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:

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