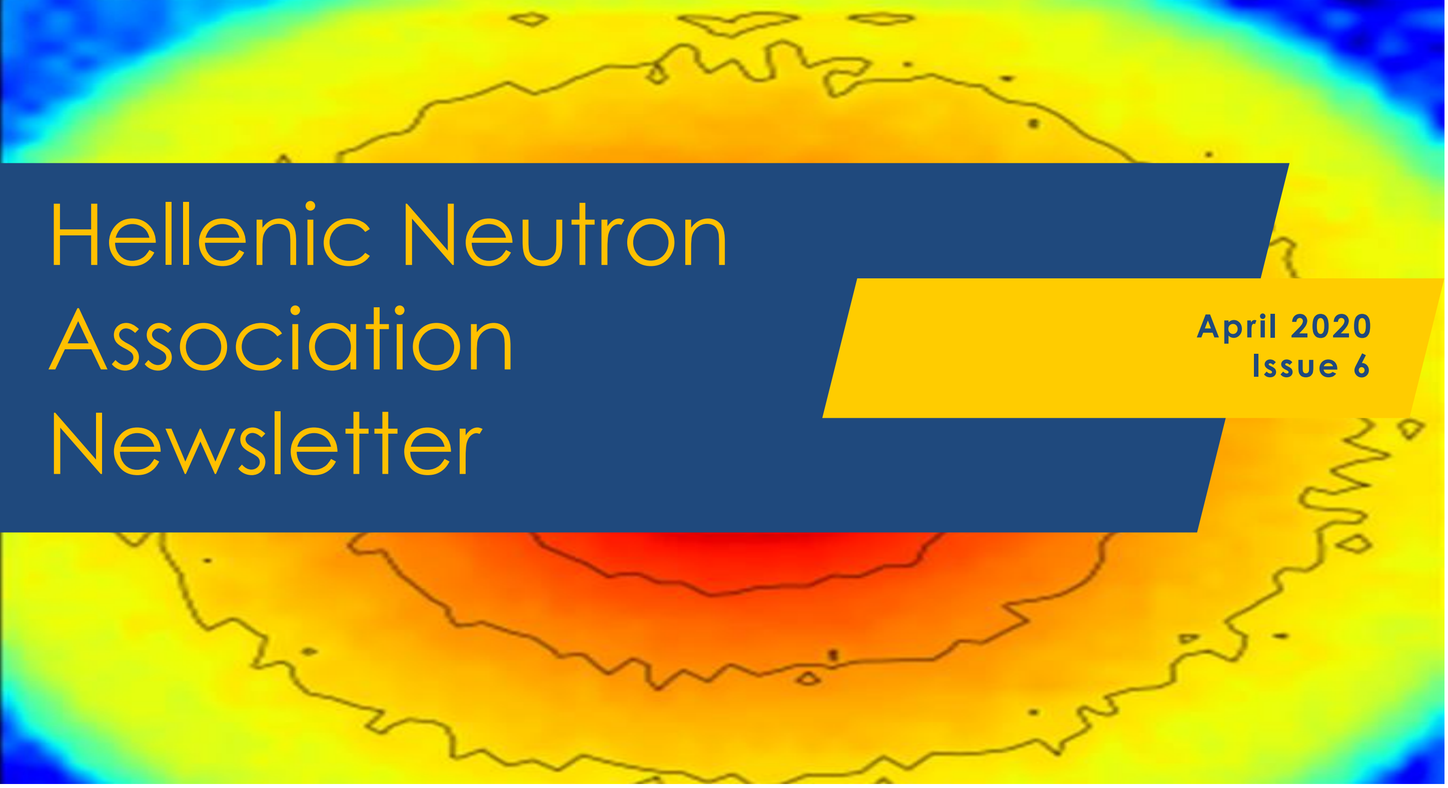
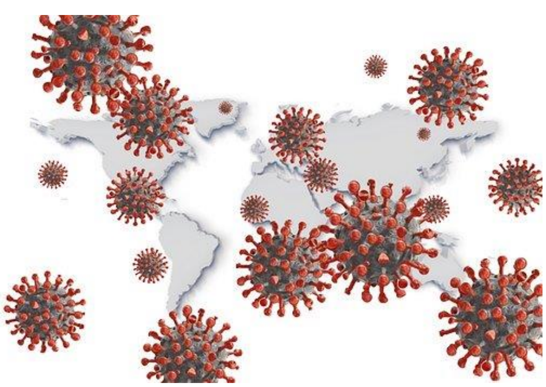


Hellenic Neutron Association Newsletter

April 2020
Issue 6

INSIDE THIS ISSUE

“Neutron, as a probe, may prove useful in the battle to unveil the morphology and functionality of the virus and contribute to the discovery of new drugs and therapies”



Editorial

By Dina Mergia (NCSR Demokritos, Greece)

We hope this newsletter reaches you in a place of safety and health. The beginning of 2020 found the whole globe confronted with an invisible enemy, SARS-CoV-2, which has created exceptional circumstances for our lives. Neutron, as a probe, may prove useful in the battle to unveil the morphology and functionality of the virus and contribute to the discovery of new drugs and therapies. How neutrons can contribute in the fight against COVID-19 is described in the first article.

Also, an article is presented describing the use of neutron techniques in the investigation of the structural properties of porous materials and pore-confined fluids. These materials find applications in a range of fields varying from catalysis and gas storage to oil recovery and carbon dioxide sequestration.

We hope you enjoy reading them in the new format of our newsletter!

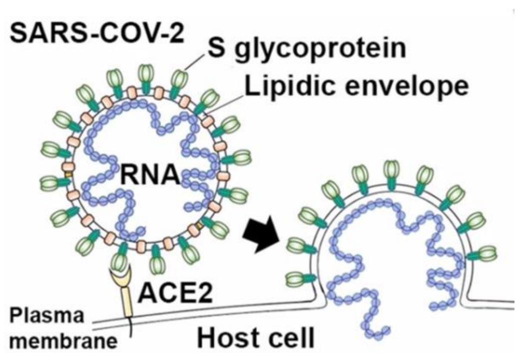
How neutrons will contribute in the fight against Covid-19

By the editorial board [largely based on the article published on the webpage of the League of Advanced Neutron Sources (LENS)]

Our newsletter is published in a time where probably the daily life of most colleagues reading it has been affected in some form by the Corona-virus (SARS-CoV-2) pandemic that broke out in late 2019. Despite that we are only about 4 months after the discovery of the virus, there is already an impressive amount of information gathered thanks to the combined effort of the global scientific community that is aiming to the discovery of drugs that may impede the virus's effects and ultimately to the development of a vaccine. Here, we present a brief description of the virus's characteristics and argue that neutrons will potentially play an important role in providing insights into the morphology and functionality of the virus and its constituent parts.

"We argue that neutrons will potentially play an important role in providing insights into the morphology and functionality of the virus and its constituent parts."

Enveloped viruses interact with human cells through specific binding interactions between glycoproteins on their envelope and receptors on the cell surface. In particular SARS-CoV-2 has trimeric protein 'spikes' (S-protein) on their surface that have attained an ability to attach to a receptor protein that is widely prevalent on cells of the human respiratory system: the ACE2 receptor. Binding S-protein trimmers to the ACE2 receptor triggers further conformational changes, which mediate fusion between viral and cell membranes that finally lead the viral-RNA in the inside of the human cell.

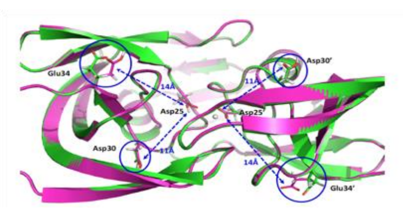


Schematic diagram of SARS-COV-2 virus and its entry into a host cell (taken from "Quel rôle pour la cristallographie dans la lutte contre le Covid-19?")

Modern analytical tools such as synchrotron X-ray radiation, cryo-electron microscopy and neutron scattering are indispensable for important insights into the morphology and functionality of viruses. Following the urgency of the pandemic situation, structural biology groups from around the world have tried to provide critical high-resolution information about the main molecules involved during the infection process. Indicatively, the S-protein has been characterized by cryo-EM by two groups in the USA [Walls et al., **Cell** (2020) 180,1 and Wrapp et al., **Science** (2020) 367, 1260] while the structure of the human ACE2 receptor has also been solved by cryo-EM in China, in complex with the receptor-binding domain of the viral S-protein [Yan et al., **Science** (2020) 367, 14444]. Neutron scattering's particular role here is to provide unique information on the chemistry of enzymatic reactions that often involve proton transfer. Recent studies on HIV-1 protease, an enzyme essential for the life-cycle of the HIV virus, perfectly illustrate the case.

"Synchrotron X-ray radiation, cryo-electron microscopy and neutron scattering are indispensable for important insights into the morphology and functionality of viruses."

Treating the disease and stopping the virus: Proteases are like biological scissors that cleave polypeptide chains at precise locations. If the cleavage is inhibited, for example, by appropriate anti-retroviral drugs, then so-called poly-proteins remain in their original state and the machinery of virus replication is blocked. For the treatment to be efficient this inhibition has to be robust—that is, the drug occupying the active site should be strongly bound to the backbone of the protease. In this way the likelihood that treatments can be effective in the long run, despite mutations of the enzyme, is increased. Neutron crystallography data adds supplementary structural information to X-ray data by providing key details regarding hydrogen atoms, critical players in the binding of such drugs to their target enzyme through hydrogen bonding, and revealing important details of protonation and hydration. In this way neutron crystallography data can help towards the design of more effective medications.



Proton transfer and drug binding details revealed in neutron diffraction studies of wild-type and drug resistant HIV-1 protease. *CREDIT*: ILL/Methods in Enzymology

High-resolution X-ray data on the protease of SARS-CoV-2 are already available and efforts are being deployed to obtain crystals for neutron crystallography studies. Proteases are, however, not the only proteins where neutron crystallography can provide essential information. For example, virus spike proteins responsible for mediating the attachment and entry into human cells are of great relevance for developing therapeutic defence strategies against the virus. Here neutron crystallography can provide unique information about the precise coupling mechanism of the virus and the receptor proteins of the cell membrane.

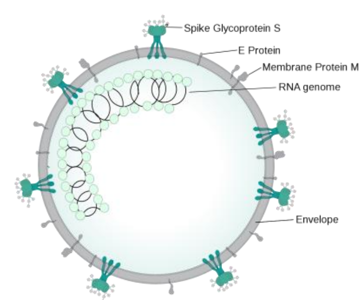
The big picture and the moving picture: When it comes to studying the function of larger biological complexes such as assembled viruses, small angle neutron scattering becomes an important analytical tool. The technique's capacity to distinguish specific regions (RNA, proteins and lipids) of the virus—thanks to advanced deuteration methods—enables researchers to map out the arrangement of the various components, contributing invaluable information to structural studies of SARS-CoV-2.

While NMR and cryo-electron microscopy provide the detailed atomic-resolution structure of small biological assemblies, neutron scattering allows researchers to pan back to see the larger picture of full molecular complexes at lower resolution. Neutron scattering is also uniquely suited to determining the structure of functional membrane proteins in physiological conditions. Neutron scattering will therefore make it possible to map out the structure of the complex formed by the SARS-CoV-2 spike protein—the protein surrounding the virus—and its human receptor.

A full understanding of the virus's life cycle requires the study of the interaction of the virus with the cell membrane and the mechanism it uses to penetrate the host cell. Covid-19 is one of those viruses, like HIV, possessing a viral envelope composed of lipids, proteins and sugars. By providing information on its molecular structure and composition, the technique of neutron reflectometry helps to elucidate the precise mechanism the virus uses to penetrate the cell. This may be by direct fusion of the virus membrane with the external cell membrane, or with the plasma membrane, or, in the case that the virus is internalized, by endocytosis.

Finally, we should not forget that viruses in their physiological environments are highly dynamic systems. Knowing how they move, deform and cluster is essential to the optimisation of diagnostic and therapeutic processes. Neutron spectroscopy, which is ideally suited to follow the motion of matter from the small chemical group to large macromolecular assemblies, is the tool of choice to provide this information.

"Assembling the data from all of these neutron-based analyses of the coronavirus will be essential to control its spread and limit its societal impact over the long term."



Schematic diagram of the coronavirus particle. *CREDIT*: **Binte Altaf**

Neutron scattering methods for investigating the structural properties of porous materials and pore-confined fluids

By Dr Konstantinos L. Stefanopoulos (NCSR Demokritos, Greece)

In this study we will mainly focus on the benefits of utilising elastic neutron scattering techniques such as *neutron diffraction*, *neutron total scattering*, *small-angle neutron scattering (SANS)* and *ultra-small-angle neutron scattering (USANS)* to investigate the structural properties of porous materials as well as the location, the structure and the phase behavior of fluids confined within their pores. In general neutron scattering, compared to the complementary X-ray scattering and porosimetry techniques, has the following advantages:

"One of the main advantages of neutrons, in comparison with X-rays, is their high penetration capability because they have no charge and interact with atomic nuclei with relatively weak interactions."

Utilisation of advanced sample environment: One of the main advantages of neutrons, in comparison with X-rays, is their high penetration capability because they have no charge and interact with atomic nuclei with relatively weak interactions. This means that neutron scattering experiments in transmission mode provide information about the bulk of the sample rather than its surface. For X-rays, typical sample thickness varies from 0.01 to 1 mm, whereas for neutrons sample thickness can be up to about a few cm. The neutron penetration capability makes then easier to utilise advanced sample environment such as gas handling equipment, cryostats, furnaces and high-pressure sample containers for monitoring *in situ* various processes such as sorption on porous media.

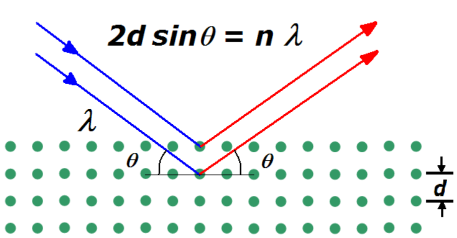
Detecting open and closed porosity: Gas sorption by volumetric or gravimetric methods and mercury porosimetry are widely used techniques for investigating the structural properties of the pore network in porous solids such as pore volume, pore size distribution, surface area etc. Their main limitation, however, is that they are invasive providing, thus, information only about the accessible (*open*) pores. On the other hand, neutron scattering techniques such as SANS and USANS can only "see but not destruct" the pores and they can also "monitor" the changes in the sorption behavior of fluid molecules confined in pores of various sizes (from nanometer- to micrometer- length scales) and topology. Furthermore, neutrons have also the ability to "see" the pores that are inaccessible to the invading fluid (*closed* pores). To this end, these methods are very effective particularly for materials with complex pore architecture and *closed porosity* like sedimentary rocks.

"Neutrons have also the ability to "see" the pores that are inaccessible to the invading fluid (closed pores)."

In general, the main disadvantages of utilising neutron scattering are the relatively low neutron flux and the high cost of the neutron beam. Moreover, getting access to neutron sources (nuclear reactors or accelerators) is neither an easy nor a quick procedure because the beam time is allocated on a very competitive basis after a peer-review process in which experimental proposals are evaluated by scientific panels commonly twice a year.

In the following we will present briefly in a non-technical but in a rather qualitative way the benefits of utilising neutron diffraction, neutron total scattering and SANS-USANS techniques to probe the structural properties of porous materials and pore-confined fluids.

Neutron diffraction



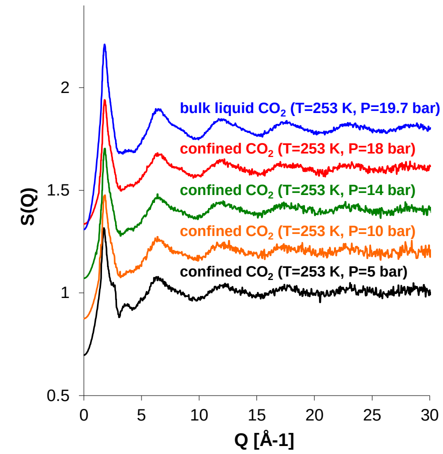
The diffraction technique is routinely used to determine the phase and the atomistic structure of crystalline materials. Both x-ray and neutron diffraction experiments usually consider only the *Bragg scattering* to reveal the long-range order of the atomic structure, represented by the unit cell. Neutron powder diffraction is a powerful tool especially for the structural study of crystalline porous materials that contain light atoms (such as H, D, Li, C, N, O). The reason is that light elements may be poorly located in X-ray diffraction experiments especially when high-Z elements are also present. Another advantage of neutron diffraction is that the Bragg reflections have similar intensities over the whole pattern while in X-ray diffraction patterns the intensity of Bragg peaks decreases with the increasing 2ϑ angle.

As an example, metal-organic frameworks (MOFs) have recently attracted a lot of interest in various fields, such as gas storage and separation, sensor, catalysis, energy storage and biological engineering. MOFs are crystalline porous materials composed of metal-based building units coordinated to organic bridging ligands to form a three-dimensional network with uniform pore system including channels and cages. Neutron diffraction offers unique possibilities for determining key features of their complex structural characteristics. Furthermore, when carrying out *in situ* neutron diffraction and gas sorption, valuable information can be deduced about the precise locations of the guest gas molecules within the pore cavities and their interactions with the crystalline framework [1].

Neutron total scattering

The atomic arrangement in real crystals exhibits always deviations from the ideal periodicity due to thermal vibrations, dislocations etc. This type of "weak disorder" gives rise to diffuse scattering resulting in a reduction in the Bragg scattering. When only Bragg scattering is analysed, for example by Rietveld refinement, the diffuse scattering is simply assumed as background. Disordered materials however, such as liquids and porous solids, exhibit short-range or limited-range crystalline structure. This means that the correlations between the disordered structural features are contained in the diffuse scattering. The structural information can therefore be revealed only by the measurement of the total scattering (Bragg scattering and diffuse scattering). There is in principle no difference between a neutron total scattering instrument and a neutron powder diffractometer. They are both used to measure the scattering intensity as a function of the momentum transfer, $\underline{Q}$. However, for total scattering measurements, the instrument should have the capability to collect data over a very large *Q* range with sufficient resolution. In addition, significant neutron flux and low background are also required in order to measure the weak signal from diffuse scattering with adequate statistics.

Again, neutron total scattering is very powerful for the structural investigation of disordered systems containing light elements. Furthermore, isotopic substitution can also be applied to elucidate complex liquid structures (bulk or confined) such as ionic liquids, battery electrolytes and also to investigate interactions in electrolyte solutions, pharmaceutical solutions, surfactant solutions, etc.



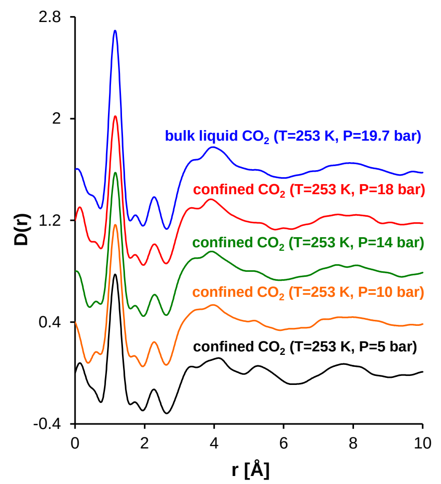
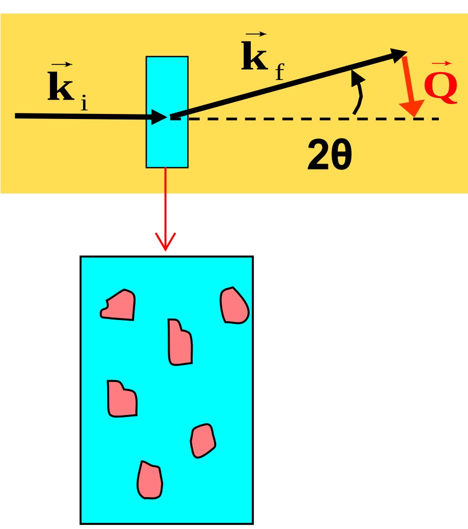


Fig.1. Top: Total scattering structure factors, S(Q), and **Bottom**: Differential correlation functions, D(r), for bulk liquid and sorbed CO_2 in the cylindrical pores of MCM-41 along an isotherm at 253 K. Data from Ref. 2.

> "When fluids are confined in nanoscale pores, the combination of solid-fluid interactions and finite volume can significantly alter their structural and dynamic properties and strongly influence their phase behavior."



Sample can be a crystal, solid, powder or an aqueous solution comprising of inhomogenieties with length scale 1 nm – 10 μm.

Confined fluids have attracted a lot of attention during the last years because their importance for various applications such as catalysis, gas storage, chromatography, oil recovery, carbon dioxide sequestration and many others. From the theoretical point of view, when fluids are confined in nanoscale pores, the combination of solid-fluid interactions and finite volume can significantly alter their structural and dynamic properties and strongly influence their phase behavior. These structural differences can be revealed by combining *in situ* sorption and neutron total scattering (Fig. 1) [2]. Furthermore, the phase behavior of confined fluids can also be observed at various thermodynamic states; for instance, it was observed that during cooling down CO_2 confined in SBA-15 below its bulk triple point the CO_2 molecules, instead of freezing, escaped from the pores (Fig. 2) [3]. It is also noteworthy, that combination of neutron scattering experimental data and atomistic Monte Carlo simulations can provide detailed information concerning the accurate positions of the confined fluids within the pores [4].

Small-angle and ultra-small-angle neutron scattering (SANS-USANS)

Complementary to neutron total scattering that measures interatomic distances, SANS probes the structures at larger length scales, varying from 1 nm to a few hundred nanometers while USANS probes lengths up to a few micrometers (about 10 μm). (U)SANS is an essential tool for the structural characterization of the porous materials by providing information about the geometry and topology of the pores, the interface texture (smooth or rough), the total porosity, the pore size distribution and the specific surface area. One of the benefits of the technique is the utilisation of *contrast-matching SANS*. The fact that H and D have scattering lengths of opposite sign means that by filling the pores using an appropriate mixture of hydrogenous and deuterated solvents (such as H_2O/D_2O) the scattering length density of the porous solid becomes equal to that of the mixture, contrast matching is then attained and the scattering signal is eliminated.

Contrast-matching SANS is a very powerful method for a direct investigation and evaluation of the *closed porosity* in porous media. In practice, the technique is applied by performing SANS on dry samples, where the total scattering comes from all the available pores, including both accessible (open) and inaccessible (closed) pores. As a next step, the accessible pores are saturated with a contrast matching mixture of a hydrogenous and deuterated solvent (for example H_2O/D_2O) and, thus, scattering from open porosity is eliminated. Consequently, the residual scattering signal yields information only about *closed porosity* in the materials.

Melnichenko and coworkers suggested an alternative method of achieving contrast matching in porous media by using non-adsorbing or weakly adsorbing supercritical fluids or gases, such as carbon dioxide CO_2 or deuterated methane, CD_4 (instead of CH_4, for minimising the incoherent background scattering from hydrogen). In a similar way, by measuring the scattering patterns as a function of fluid pressure, when the contrast matching pressure (or *the zero average contrast, ZAC*) is reached, the residual scattering simply indicates the presence of pores inaccessible to CO_2 or CD_4 [5]. Finally, the pore accessibility in confined liquids, such as ionic liquids can also be investigated in a unique way by SANS. The reason is that neutrons have the ability to "see" If the pores are filled with ionic liquid. This is not always the case, however, with gas sorption methods; for instance, there is a possibility that the ionic liquid molecules have also been dispersed around the pore surface preventing, thus, the access to the gas. In this case a negligible uptake from the gas sorption isotherm would erroneously suggest that the pores are empty [6].

Combination of gas sorption and *contrast-matching SANS* in porous materials has also been proved to be an essential tool for elucidating the adsorption mechanism and highlighting also the structural details of the porous matrix. Neutrons provide the unique possibility to obtain structural characteristics, hardly accessible by other analytical techniques. In particular, they allow studying all stages of the fluid adsorption in porous media such as micropore filling, formation of adsorbed layers, and capillary condensation in mesopores.

The complex pore architecture of sedimentary rocks (such as coal, shales, sandstones, carbonates, clays, and others) is a scientifically fascinating subject of major practical importance. The reason is that the microstructure and evolution of porosity plays a crucial role in many geological processes, including the migration and retention of water, gas, and hydrocarbons, as well as CO_2 sequestration and oil recovery. Combined SANS and USANS techniques have been utilised as multi-scale and non-invasive methods for probing the rock microstructure. It has been revealed that sedimentary rocks display the most extensive fractal behavior observed in nature, with self-similarity expanding over at least three orders of magnitude in the length scale. Furthermore, understanding the gas adsorption, pore accessibility and structural properties of confined fluids in sedimentary rocks is of great importance for the design of optimal gas or oil recovery and sequestration projects [7].

"Microstructure and evolution of porosity plays a crucial role in many geological processes, including the migration and retention of water, gas, and hydrocarbons, as well as CO_2 sequestration and oil recovery."

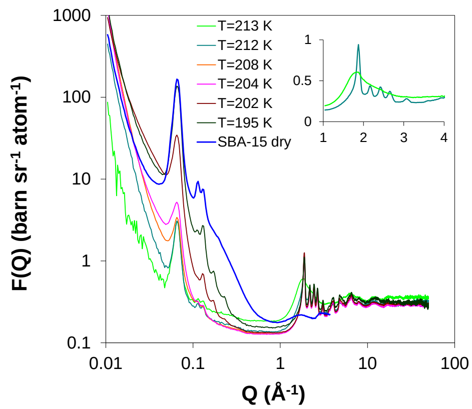
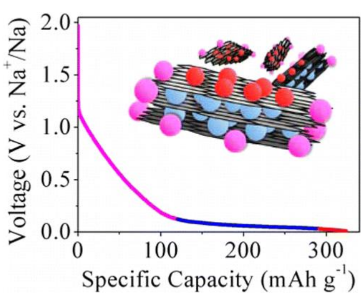


Fig. 2. Neutron scattering profiles of mesoporous SBA-15 filled with CO_2 (T=213 K) during the freezing process. The pattern of dry SBA-15 is also presented for comparison. Inset: Highlight of the liquid-solid transition of the external CO_2. Data from Ref. 3.

As a general remark, there is recently an increased interest in the battery field for the further improvement of lithium-ion rechargeable batteries. Mesoporous carbon could be a candidate as an intriguing anode material because of its higher specific capacity than traditional graphite anode. The development of sodium-ion batteries could also be an alternative for the current lithium-ion technology because of the high abundance and low cost of sodium. The most promising so far anode material for sodium-ion batteries is hard carbon, an amorphous nanoporous material. However, the mechanism for sodium storage in hard carbon remains a mystery. *In situ* or *in operando* neutron scattering techniques during charging/discharging cycles could, thus, provide an in-depth understanding about the storage mechanism by monitoring the structural changes in the porous electrode upon deposition of sodium ions.

"The development of sodium-ion batteries could also be an alternative for the current lithium-ion technology because of the high abundance and low cost of sodium. The most promising so far anode material for sodium-ion batteries is hard carbon, an amorphous nanoporous material."

In conclusion neutron scattering techniques have been proved to be powerful tools for probing structural details of porous materials and pore-confined fluids. In my opinion, nowadays, it is feasible and even more challenging to further utilise the potentiality of neutrons to monitor *in situ* structural changes, phase transitions and molecular interactions in the nanopores during various processes such as sorption, flow, catalytic reactions and by *in operando* charging-discharging batteries.



Schematic of sodium-ion storage mechanism in hard carbon proposed by C. Bommier et al., Nano Lett. 15 (2015) 5888-92.

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Neutron News

The end of 2019 was marked by the end of an era of three European research reactors central to the decades of success in the European neutron landscape, after a period of about 40 years of successful operation:

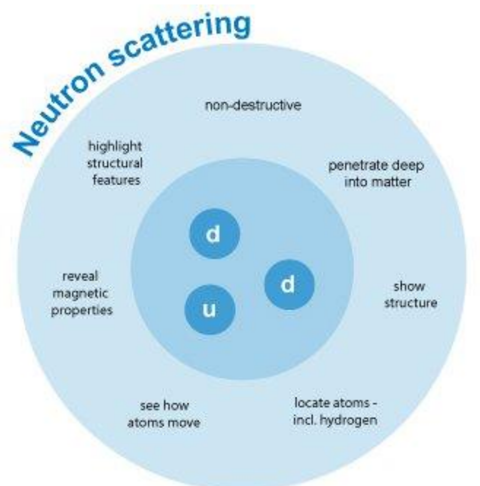
Orphée, operated by the Laboratoire Léon Brillouin (LLB) near Paris,

BER II at the Helmholtz-Zentrum in Berlin, and

JEEP II operated by the Institute for Energy Technology outside Oslo.

Each of these reactors operated as a national facility primarily serving researchers in France, Germany and Norway, but also hosted users of international researchers through trans-national access programmes.

The League of advanced European Neutron Sources, LENS, and the European Neutron Scattering Association, ENSA, warn of a “neutron gap” that could impact scientific research across multiple fields in physics, materials science, chemistry, biology, medical science and engineering. Together, LENS and ENSA are working on a new vision to maintain Europe’s leading position in neutron science. For more information read here.



Editorial board