

# Chemical Deuteration at the European Spallation Source, part of the broader Deuteration Network (DEUNET)

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The chemical deuteration laboratory is one of the three pillars of the European Spallation Source (ESS) Deuteration and Macromolecular Crystallisation (DEMAX) platform for users of the ESS neutron instruments. DEMAX will begin responding to service requests in early 2019 in order to be well-prepared for the installation and commissioning of the ESS instruments and subsequent user program. The chemical deuteration laboratory will offer, in the first instance, saturated fatty acids, fatty alcohols, alkyl halides, oleic acid and a limited range of surfactants and glycerol- and phospholipids.

The ESS chemical deuteration laboratory is using known chemical deuteration methodologies to produce deuterated molecules, as well as establishing **new biochemical methods** to enable a broader suite of molecules to be synthesised with greater efficiency. Some of the results of both the chemical and the biochemical syntheses at ESS will be presented.

The ESS DEMAX platform operates within the broader Deuteration Network (DEUNET), a collaboration between several laboratories with expertise in deuteration techniques, including the deuteration laboratories at J-PARC Centre/CROSS. This collaboration is initially funded by SINE2020, a European project funded by the Horizon 2020 program. Some recent results from the European partner laboratories will also be presented.

# Recent research progress in heterogeneous platinum group metal-catalyzed H–D exchange reactions

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The utility of deuterium (D)-labeled compounds has been expanding in various fields in the past few decades, such as elucidation of reaction mechanisms, deuterated semiconductor devices, and heavy drugs and so on. Therefore, the development of easy and efficient D incorporation method to various substrates is quite desirable.

The hydrogen(H)–D exchange reactions based on the C–H activation is a strong and straightforward tool to introduce deuterium atoms into target compounds. We have developed platinum group metal on carbon-catalyzed H–D exchange methods of various aromatic and aliphatic compounds using D<sub>2</sub>O as a deuterium source in the presence of hydrogen gas (H<sub>2</sub>) as an activator of the metal catalyst. Recently, we have also developed alternative deuterium labeling methods using *in situ* generated H<sub>2</sub> based on the platinum (Pt)-catalyzed dehydrogenation of 2-propanol. Since Pt/C could be effectively activated by quite small amount of *in situ* generated H<sub>2</sub>, the H–D exchange of acrylic and methacrylic acid derivatives also smoothly proceeded without the reduction of alkene functionalities. Furthermore, Pt or iridium on carbon-catalyzed deuteration of aromatic rings in D<sub>2</sub>O and 2-propanol-mixed solvent at room temperature have also been accomplished.

# Chemical Deuteration of Small Organic Molecules and its Application to Neutron Studies

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Isotopic labelling of organic molecules with the stable and non-radioactive isotope, deuterium (<sup>2</sup>H), using chemical processes is effective to enhance contrast and reduce incoherent background of the organic samples in neutron scattering experiments. The Japan Proton Accelerator Research Complex (J-PARC), Comprehensive Research Organisation for Science and Society (CROSS), Japanese Atomic Energy Agency (JAEA), and National Institutes for Quantum and Radiological Science and Technology (QST) have cooperated to promote the deuteration activities at J-PARC. Recently, Parr Reactor system which is used in metal-catalysed H/D exchange reactions and liquid chromatography–mass spectrometry (LC-MS) were installed and their test operation has been performed for the chemical deuteration research.

We have not only developed a simple and effective method for the deuteration of small hydrocarbon molecules, ionic liquids and monomers, on gram scale starting from their protonated versions but also we have installed and developed the methods of deuteration level analysis using LC-MS and elemental analysis techniques. In this presentation, we will show the summary of deuteration works and the results of practical application of the deuterated compounds to neutron studies.

# Biological deuteration and support for macromolecular crystallization at the European Spallation Source

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DEMAX is the Deuteration and Macromolecular Crystallography support lab for soft matter and life science users of the European Spallation Source (ESS). DEMAX has three support pillars and will be available to all users of ESS instruments. This presentation will focus on support for biological deuteration and large protein crystal growth. For these biological support functions we have established a close partnership with Lund University's Lund Protein Production Platform ([LP3](#)), a cross-faculty support lab for the production and crystallization of proteins, both unlabeled and labeled (<sup>13</sup>C, <sup>15</sup>N, <sup>2</sup>H) using bacteria, insect cells, and yeast. DEMAX and LP3 are co-located in the Biology Department of LU. DEMAX will offer deuterated biomass production of yeast and bacteria and are also growing algae in heavy water to be used for making rich deuterated media. The LP3 and DEMAX share a state-of-the-art crystallization laboratory, fully equipped with a variety of crystallization robots and set-ups for everything from small volume high-throughput screening to fine-screening and large volume crystal optimization. We have access to a Mosquito with LCP, Opticrys, Oryx8, Dragonfly and TECAN screen formulators, and crystallization plate storage and UV imaging. The second half of the talk will feature a science highlight from a recent research project.

# Protein Deuteration for Neutron Scattering

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Neutrons provide unique tools to investigate structures and dynamics of biological macromolecules. The information available with the neutron techniques includes, for example, the positions of the hydrogen atoms by neutron crystallography, the *in situ* structural information by small-angle neutron scattering (SANS), and the dynamics of the molecules by quasielastic neutron scattering (QENS). Protein deuteration makes significant contributions to usefulness of these techniques applied to the protein studies. In neutron crystallography, the problem of cancellation of the density due to the negative density of hydrogens can be avoided, and the background mainly due to the incoherent scattering from hydrogens is reduced. In SANS, the technique of selected deuteration is applied to obtain the structural information on the selected component within the complex. The QENS measurements on the macromolecular complex consisting of the deuterated components and the usual protonated component provide the information on the dynamics of the protonated component within the complex. Perdeuteration of the proteins is required for neutron crystallography and QENS, while for SANS, the proteins deuterated to various degrees can be utilized. This is because the scattering length density of D<sub>2</sub>O solvent matches to that of the proteins with the deuteration level at about 80%. There are also various SANS techniques using the proteins deuterated to various degrees. It is thus useful to control the degrees of deuteration in producing the deuterated proteins.

Based on the technique of protein deuteration using algal peptone, we have developed a technique of producing the proteins at various deuteration levels. In this talk, I will describe a simple technique to produce such deuterated proteins in detail.

# Optimized Strategies for Perdeuteration and Crystallization of Human Carbonic Anhydrase for Neutron Crystallography

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Neutron protein crystallography benefits from using fully deuterated protein by improved signal-to-noise ratio and reduced background from hydrogen incoherent scattering. In our study we use carbonic anhydrase IX (CA IX) as a model for deuterated protein expression and crystallisation. Full-length native CA IX is a multi-domain membrane protein that is insoluble and difficult to express or crystallise. To prepare crystals we are using the soluble catalytic domain of CA IX with 6 surface mutations - CA IX surface variant (CA IX SV). Expression media and cell culture conditions were optimized to produce high yields of deuterated protein. The CA IX SV was then structurally characterized by X-ray, tested for deuterium incorporation by mass spectrometry, temperature stability, and propensity to crystallize. Using unlabeled carbon source and recycled heavy water, we were able to get 65–77% deuterium incorporation, sufficient for most neutron-based techniques, and in a very cost-effective way. Expression of protein and cell culture conditions will be further optimized by using rich algal peptone media at J-PARC labs. The crystallization of CA IX SV, was enabled by the use of microseed matrix screening (MMS), commercial screens, and refinement screens. These results demonstrate potential of MMS in growth of large crystals for neutron studies and provide the basis for developing strategies for macromolecular crystallization.

# Methyl Configurations Observed by Neutron Crystallography in Perdeuterated Proteins

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More than 20 neutron crystal structures of perdeuterated proteins have been deposited in PDB database by 2017. The deposited number of crystal structures is estimated to be only 20% of total crystal structures solved by protein neutron crystallography. Although perdeuteration costs much to get deuterated chemicals and heavy water or commercially available medium for bacterial cultivation, crystallization and so on, the perdeuteration in protein crystallography gives the three advantages as follows; 1) no cancellation of contour maps by negative scattering length of hydrogen atoms, 2) higher contour map levels of deuterium than hydrogen atoms due to longer scattering length for structural analysis, and 3) reduction of background noise and enhancement of reflection signals with better S/N ratio for experimental measurement.

Here, I would like to focus on the methyl configuration in proteins, because the cancellation of contour maps should adversely affect to the observation for methyl groups that include hydrogen atoms. The cancellation will cause the position of hydrogen atoms to be ambiguous. The perdeuteration is needed to replace hydrogen atoms with deuterium atoms in the methyl groups. Recently, two neutron diffraction data sets for T4 lysozyme have been collected at both room and cryo temperatures using perdeuterated protein crystals to 2.1 and 1.7 Å resolution, respectively. As a result in joint refinement using X-ray and neutron diffraction data, many differences of the methyl configurations are observed between the two conditions. It appears that the methyl configurations are sensitive depending on its environment. In addition, differences from methyl configurations are not always explained by steric hindrance between atoms. These observations may provide a new insight into the analyses for protein dynamics and by quantum chemical calculations. In this meeting, I would like to discuss about importance for direct observations of the methyl configuration in proteins.