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National Deuteration Facility (NDF)

Biodeuteration

The National Deuteration Facility (NDF) is the only facility of its type in the Southern Hemisphere. It is partially funded by the National Research Infrastructure for Australia initiative.

NDF provides the facilities and expertise to produce molecules where all or part of the molecular hydrogen is deuterium (^{2}H or D). This enables complex investigations of the relationship between the structure of molecules and their function using neutron scattering, nuclear magnetic resonance and other types of spectroscopy.

This unique facility offers molecular deuteration using both *in vivo* biodeuteration and chemical deuteration techniques.

Biodeuteration

The biological deuteration laboratories are staffed and equipped to undertake molecular biology, biosynthesis, purification and characterisation of deuterated (^{2}H) and multiple labelled biomolecules (^{2}H, ^{15}N, ^{13}C).

Biodeuteration involves the growth of microorganisms in a heavy water (D_2O) culture medium supplemented with either a deuterated or hydrogenated food source, depending on the level of deuteration required for the target biomolecule. The biomass is harvested and the deuterated molecule (such as a protein) is purified and characterised.

Capabilities

NDF Biodeuteration can provide a range of labelled biomolecules including proteins, DNA and biopolymers.

The NDF has developed efficient and reliable high cell density *in vivo* recombinant bacterial methods for protein stable isotope labelling.

Several protein labelling strategies enable the NDF to provide:

- Deuterated (^{2}H) protein, polysaccharides and sterols, 90 - 100% deuteration
- Double- and Triple-labelled protein (^{2}H/^{13}C/^{15}N)
- Selective and methyl labelled protein (^{13}C/^{15}N) +/- ^{2}H background

Biomass or purified material can be provided.

Applications

Biodeuteration can assist in providing valuable information for a wide variety of scientific and technological disciplines including:

- Structural biology (SANS, NR, NMR)
- Biopolymers and biotechnology
- Food science
- Health, pharmaceutical and drug delivery research

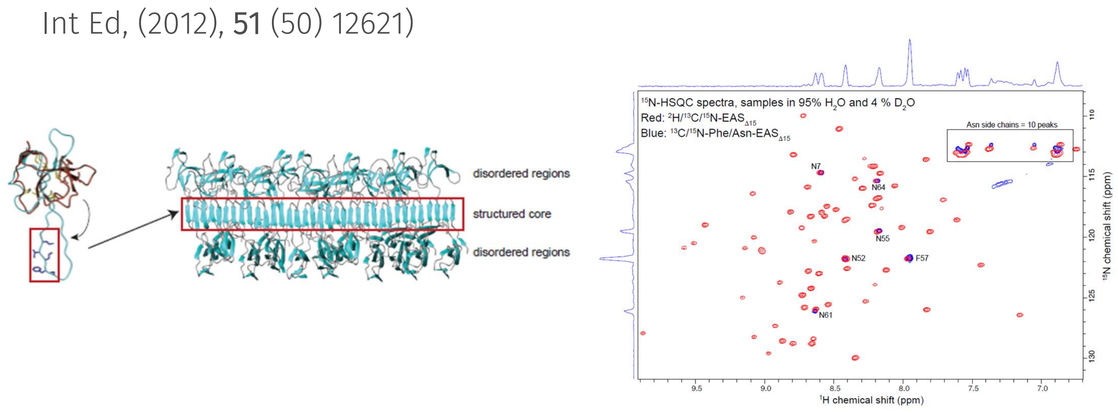
Access

NDF deuteration capabilities are offered through a merit-based proposal program. Accelerated or commercial access can be provided subject to service charges.

Case studies

NMR study of a fungal hydrophobin

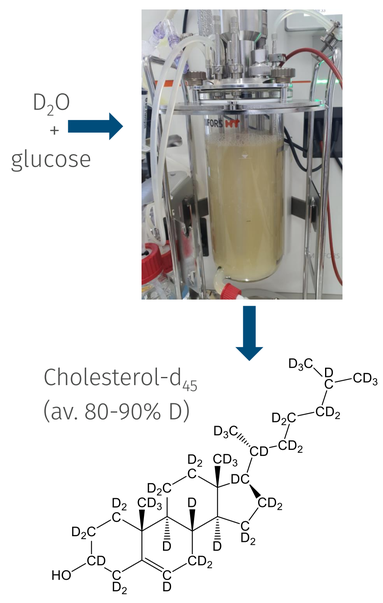
$EAS_{\Delta15}$ is a truncated version of EAS, a fungal hydrophobin from. *Neurospora crassa* – a small highly amphipathic, surface-active protein that self-assembles into monolayers at surfaces and interfaces. The monolayers are composed of laterally-assembled fibrils known as rodlets that are a form of functional amyloid. Multiply labelled $EAS_{\Delta15}$ produced by the NDF was utilised in NMR studies to investigate the molecular structure of $EAS_{\Delta15}$ rodlet assemblies. (V. Morris *et al*. Angewandte Chemie Int Ed, (2012), **51** (50) 12621)

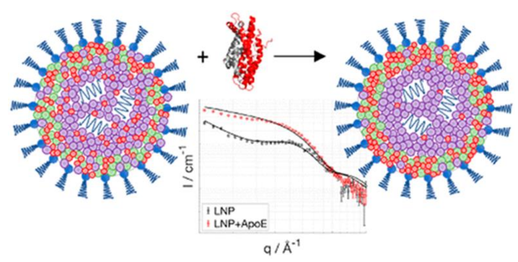


Biosynthesis of deuterated cholesterol

The common yeast (*Saccharomyces cerevisiae*) has been engineered to be a cell factory for biosynthesis of cholesterol.

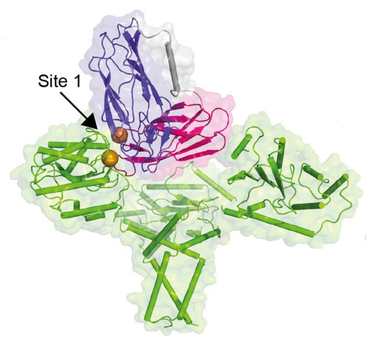
Cholesterol-d_{45} was used for modelling and characterising the structural composition of mRNA containing lipid nanoparticles (LNPs) using small angle neutron scattering (SANS). (Sebastini *et.al.* ACS Nano., (2021), **15** (4) 6709-6722)





SANS study of a protein complex

Disulfide bonds often play a key role in defining a protein's structure and stability and are controlled by a class of proteins called 'Suppressor of Copper Sensitivity' proteins in some organisms. Two forms of the protein bind together and have been characterised by combining X-ray crystallography, SAXS and SANS. Deuteration of one of the proteins was performed at the NDF with SANS studies carried out at the Australian Centre for Neutron Scattering (ACNS). (EJ. Furlong *et al* J Biol. Chem (2018) **293** (16) 5793-5805)



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