Molecular deuteration for neutron scattering contrast and other scientific purposes

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9am Friday 16 September 2018
AONSO Neutron School

Science. Ingenuity. Sustainability.

Contents

- National Deuteration Facility
  - Access
- What is deuterium; deuterium and neutron scattering
- Contrast labelling with deuterium for neutron scattering
- Deuteration for neutrons. NR, SANS, nX, nBS;
  Deuteration for other purposes. NMR. MS
- Case studies.
  - ScsB low res domain movement
  - nX ChoX; Rubisco
- How to deuterate samples
  - Chemical synthesis
  - Biological deuteration

National Deuteration Facility
What do we do?

- Support externally driven research to use the national facilities

- Produce the labelled materials.

- Usually in full intellectual collaboration from design to publication.

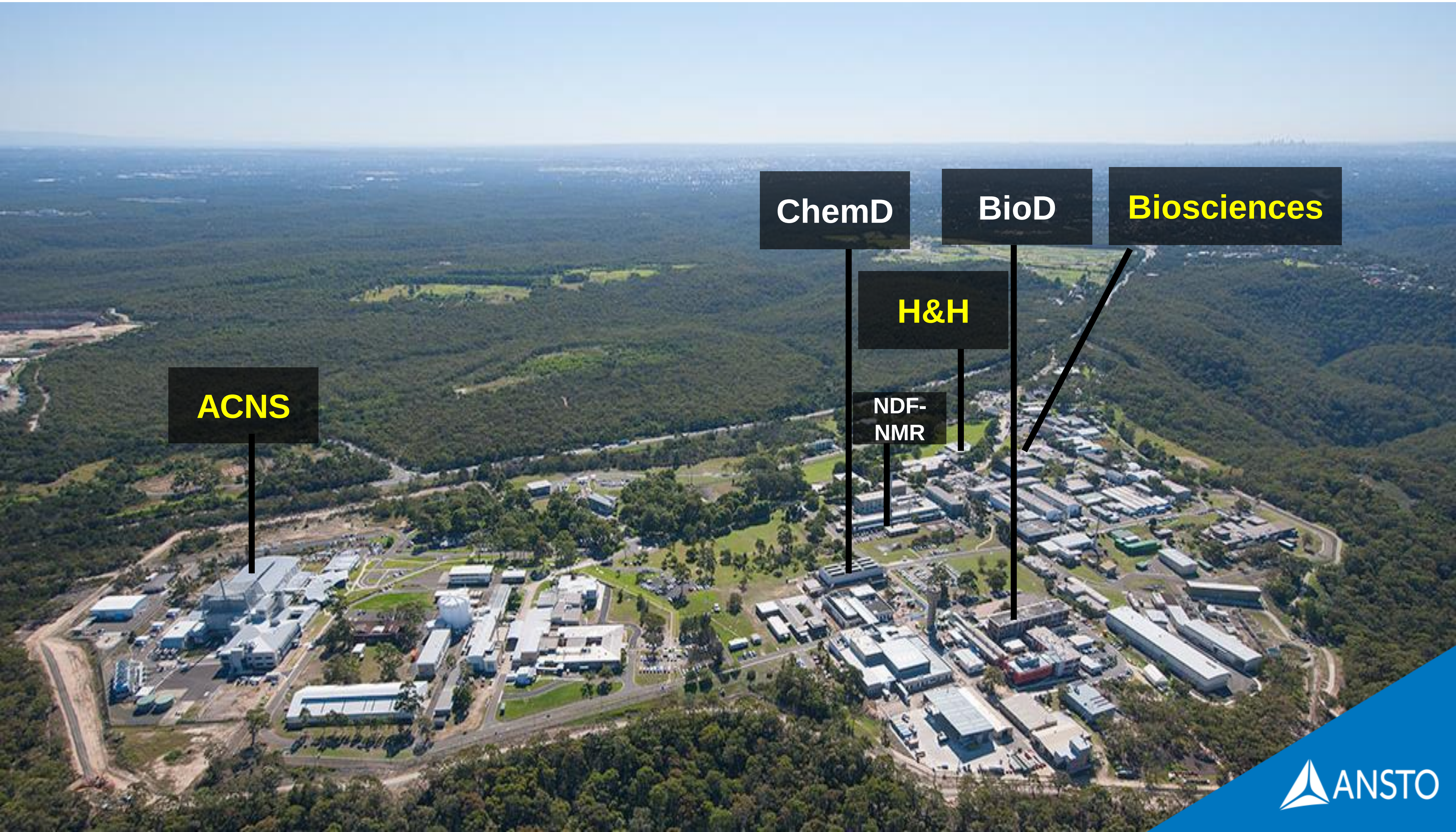
Facility-based science:

Is the method a good way to answer a good question.

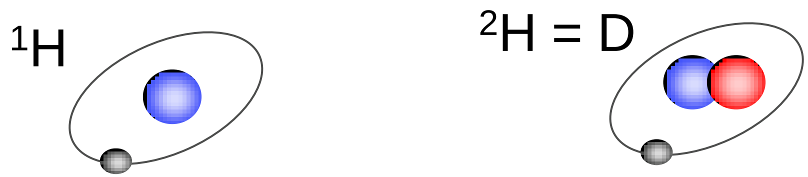
- http://www.ansto.gov.au/ndf

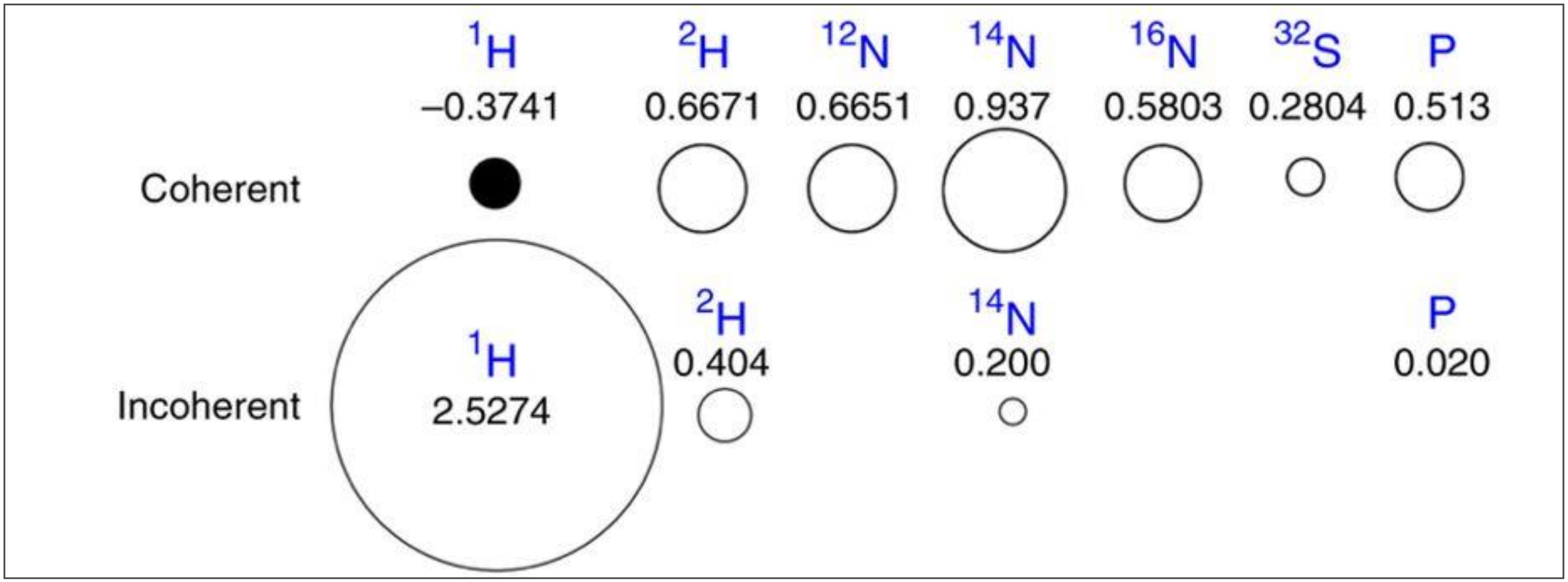
The National Deuteration Facility is partly supported by the National Collaborative Research Infrastructure Strategy – an initiative of the Australian Government.

NDF and the Major Internal Stakeholders



What is deuterium





Coherent and incoherent neutron cross-sections of the 'biological' elements (σc, displayed as circles) and their respective neutron-scattering lengths (bc, 10−12 cm; where σc = 4πbc2) (ref. 50). 1H has a negative coherent scattering length (represented as a black circle), as compared with deuterium and the other commonly occurring biological isotopes. Coherent scattering arising from correlated distances within a particle's volume produces a scattering profile from which structural information can be extracted. Conversely, incoherent neutron scattering cannot be used to extract shape/structural information and contributes to a SANS profile as 'noise' across all angles. 1H has a considerable incoherent scattering length, the effect of which is demonstrated by the SANS scattering from lysozyme in 100% (vol/vol) 1H2O (left), which is considerably noisier than the same sample collected in 100% (vol/vol) 2H2O (right). SANS data were collected on the Quokka-SANS instrument at ANSTO96 using the same neutron wavelength, exposure times, detector distances, instrument geometry, sample path length and protein concentration.

Jeffries, et al. Nature Protocols volume 11, pages 2122–2153 (2016)

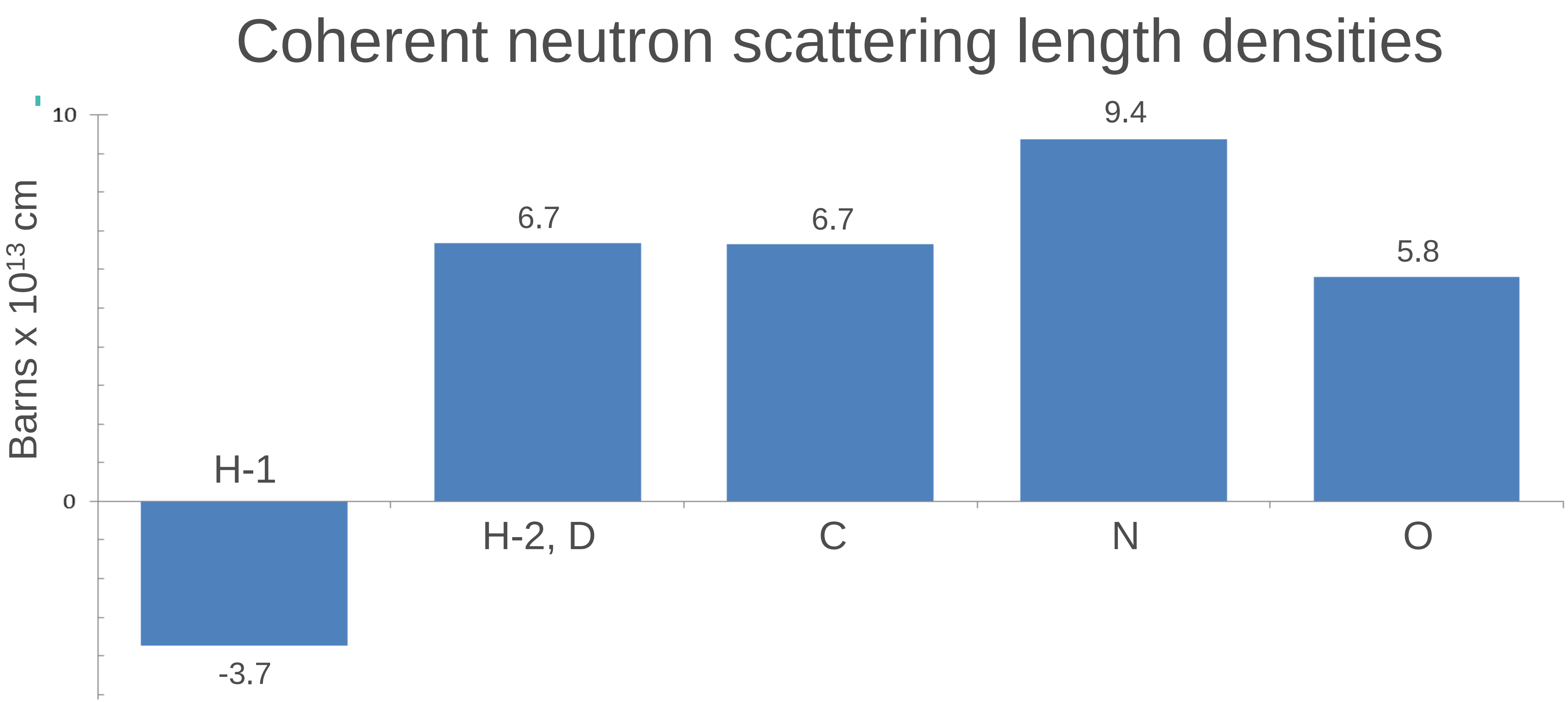
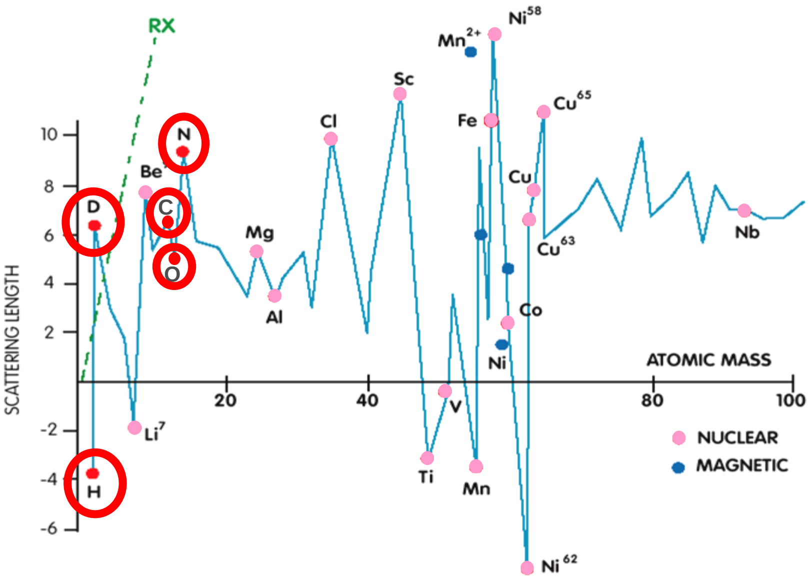


Figure 1. Coherent neutron scattering lengths. H-1 has a neutron scattering length density (nSLD) of opposite sign and approximately half the magnitude of carbon. H-2, or D, has a very similar nSLD to carbon. Nitrogen and oxygen have similar positive nSLDs. Not shown are incoherent scattering lengths densities. H-1 has a very large incoherent nSLD, while the other nuclei have small to negligible incoherent nSLDs. Incoherent nSLD produces isotropic signal that serves helpful purpose and contributes noise. Data taken from Engelman and Moore, 1972

Contrast labelling with deuterium for neutron scattering

Hydrogen and deuterium provide strong neutron scattering contrast

Neutron scattering by element



Biological macromolecules

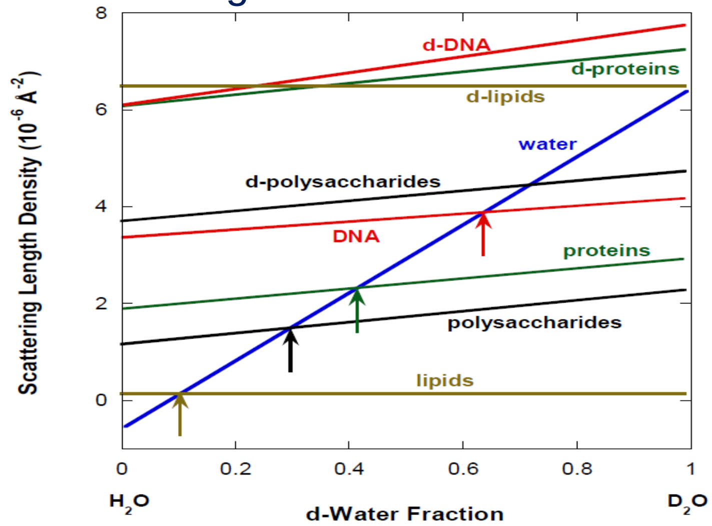


Figure 16: Average scattering length densities for DNA, proteins, lipids and polysaccharides as well as deuterated DNA, deuterated proteins, deuterated lipids and deuterated polysaccharides following H/D exchange in H_2O (left) or D_2O (right). Arrows mark the D_2O/H_2O contrast match conditions.

136 J.K. Krueger et al.

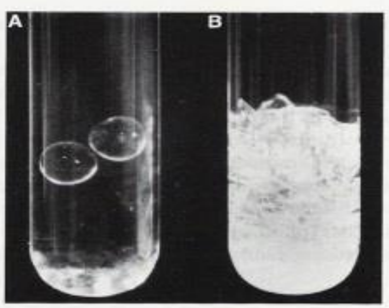


Fig. 8.3. Two tubes containing Pyrex beads in glass wool and solvent: (A) Refractive index of solvent matches that of glass wool. (B) Refractive index of solvent is different to that of glass wool or Pyrex beads and scattering from the glass wool dominates (reproduced with permission of D.M. Engelman)

When the monster came,
Lola remained undetected.
Harold, of course,
was immediately devoured.

Applications of Deuteration

- SANS and Reflectometry: Contrast for scattering.
- Neutron crystallography
- Neutron backscattering; neutron imaging; infrared spectroscopy
- NMR (nuclear Magnetic Resonance)
  Reduction of ^{1}H signal for large proteins.
  ^{15}N and ^{13}C labelling.
  Amino acid specific labelling for answering specific questions in a well characterised large protein complex.
- Labelling for mass spectroscopy

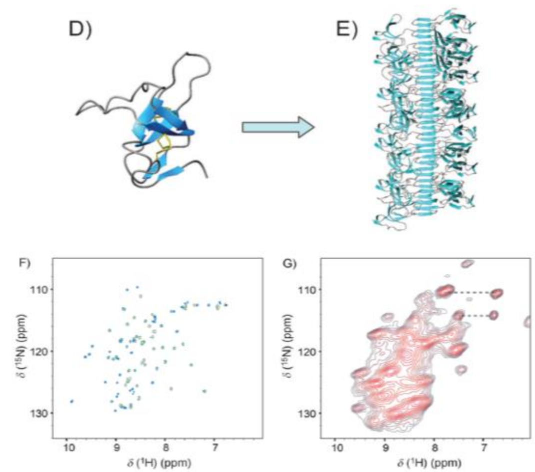
Angewandte Chemie

Amyloids

DOI: 10.1002/anie.201205625

Solid-State NMR Spectroscopy of Functional Amyloid from a Fungal Hydrophobin: A Well-Ordered β-Sheet Core Amidst Structural Heterogeneity**

Vanessa K. Morris, Rasmus Linser, Karyn L. Wilde, Anthony P. Duff, Margaret Sunde, and Ann H. Kwan**

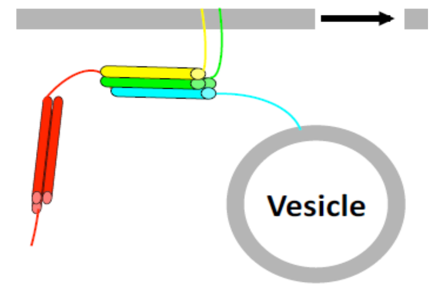


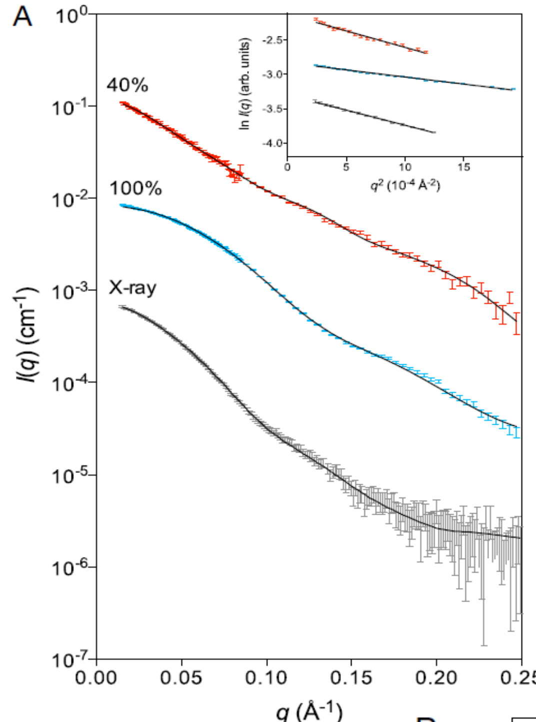
Is deuteration required?

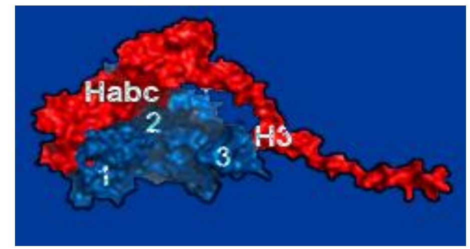
- SANS with contrast variation: Yes, for protein to protein contrast.
- Neutron reflectometry: “Very useful” to “yes”.
- Neutron crystallography: No. It has advantages.
  - Reduces noise / increases signal-to-noise / reduces required crystal size / reduces required beamtime
  - Produces density maps that are human-intuitive. CH_2 groups, positive density for ^{12}C, negative for ^{1}H, at moderate resolution they bleed into each other, cancelling, producing density gaps.

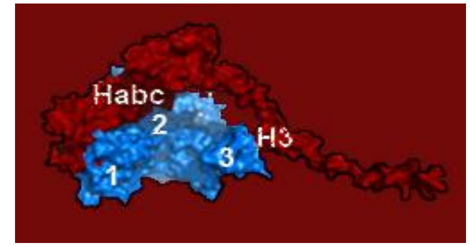
Deuteration in Structural Biology

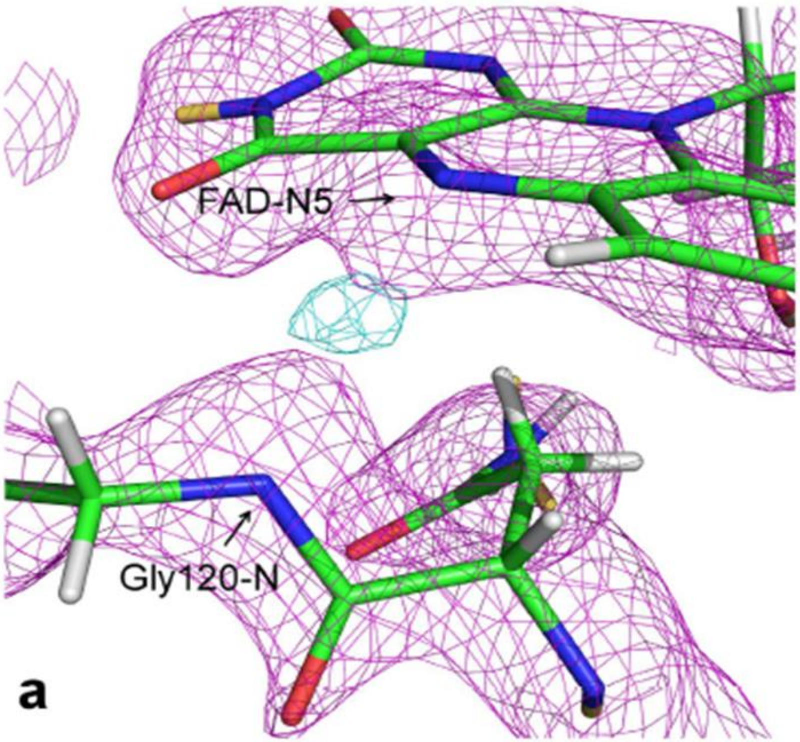
- SANS and Reflectometry: Contrast for scattering.
- Neutron crystallography

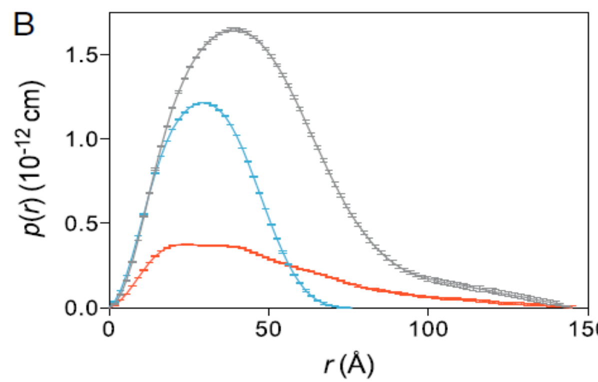






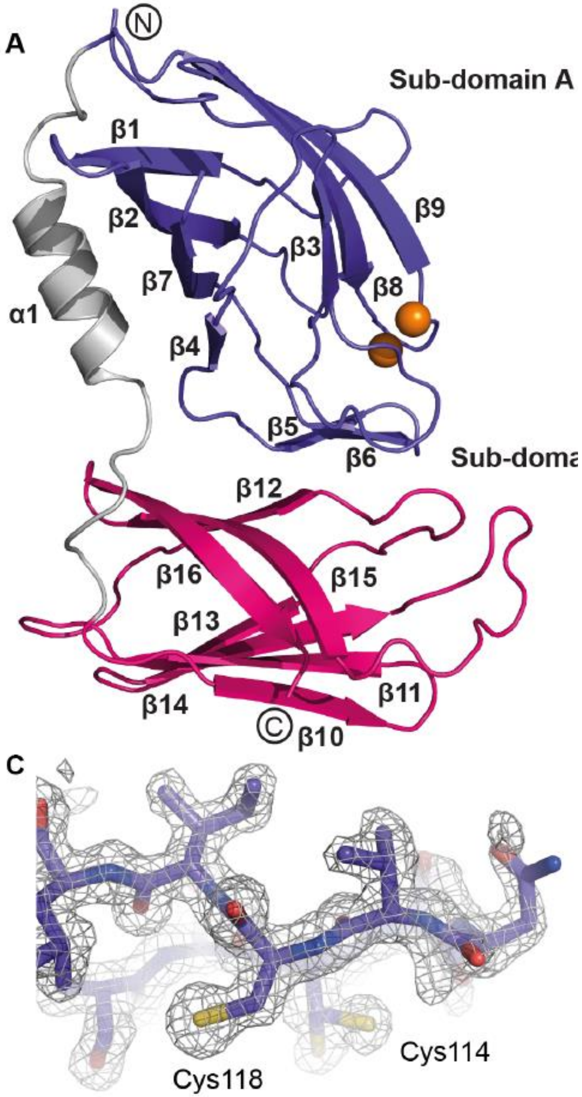


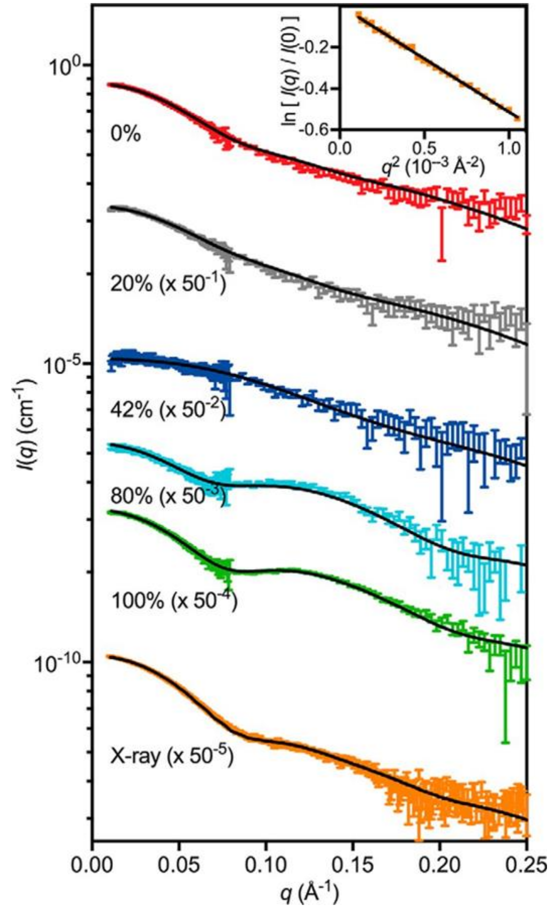


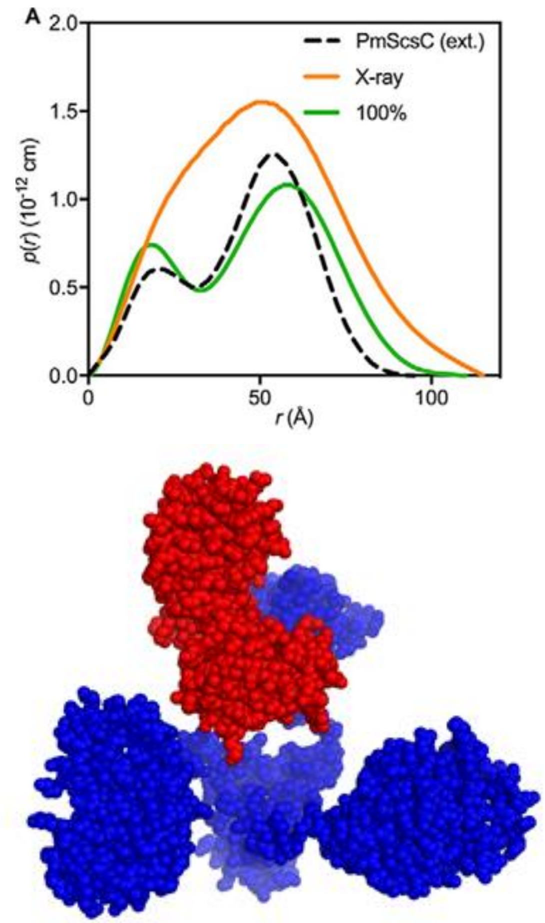


Characterising a complex involved in copper-resistance of human pathogens, *Proteus mirabilis and Salmonella enterica*

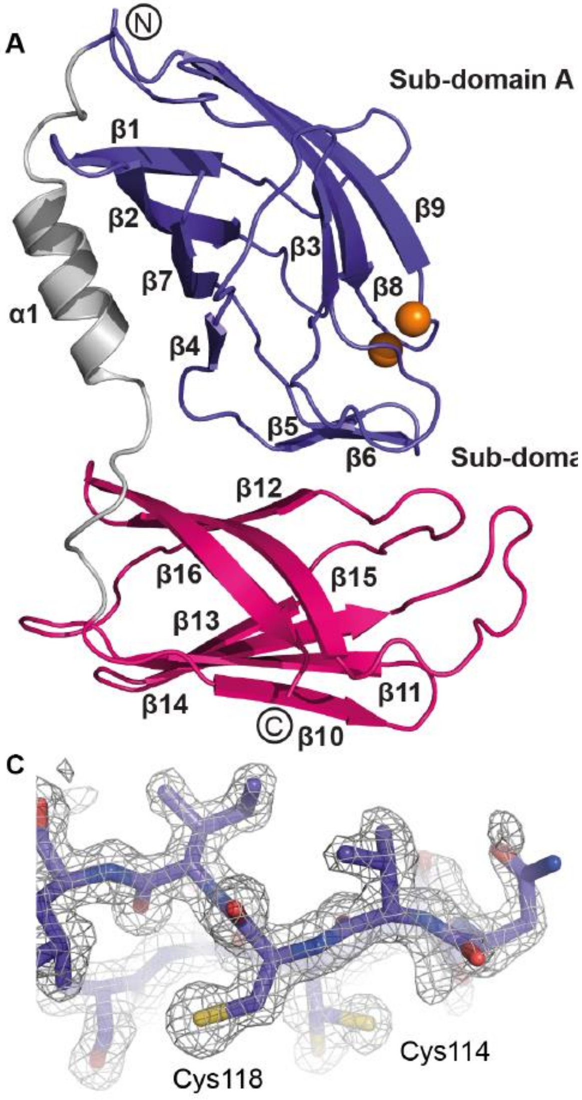
Furlong et al. *Journal of Biological Chemistry.* 2018



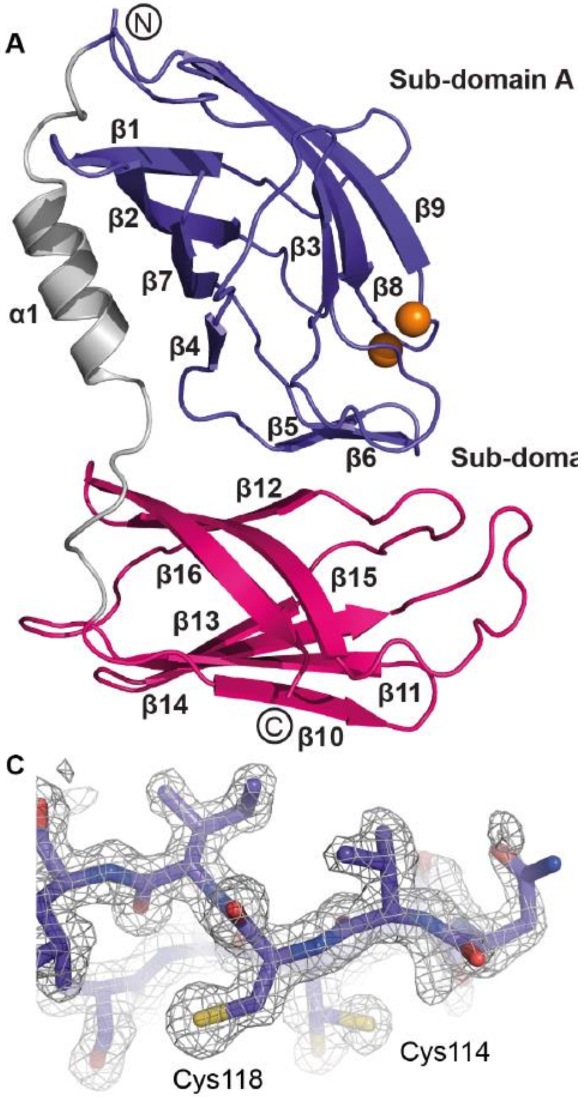




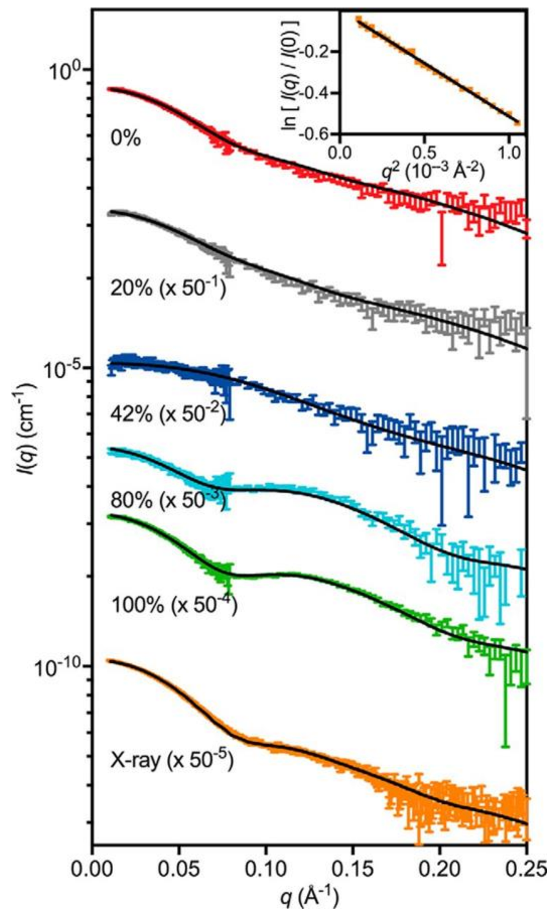
X-ray crystallography:
A new protein structure (1.5 Å)

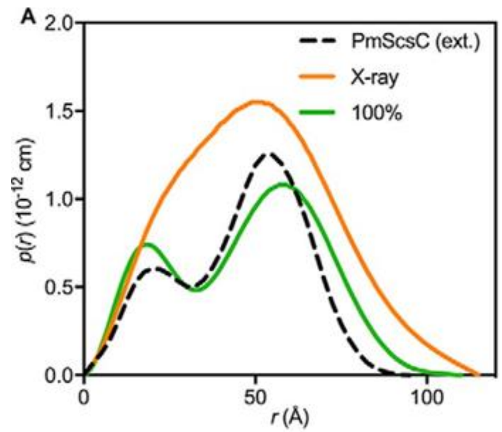


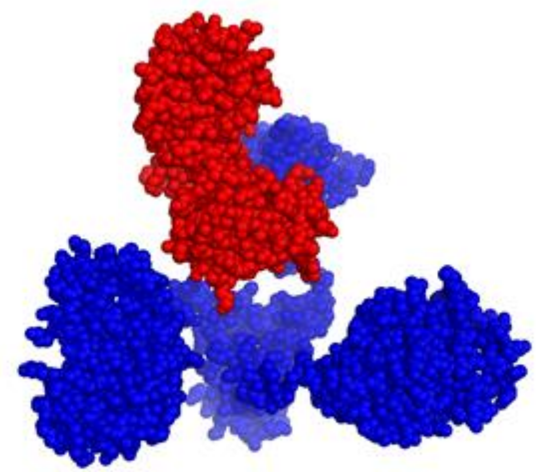
X-ray crystallography: A new protein structure (1.5 Å)



SANS with contrast variation on that protein with partner (trimer)





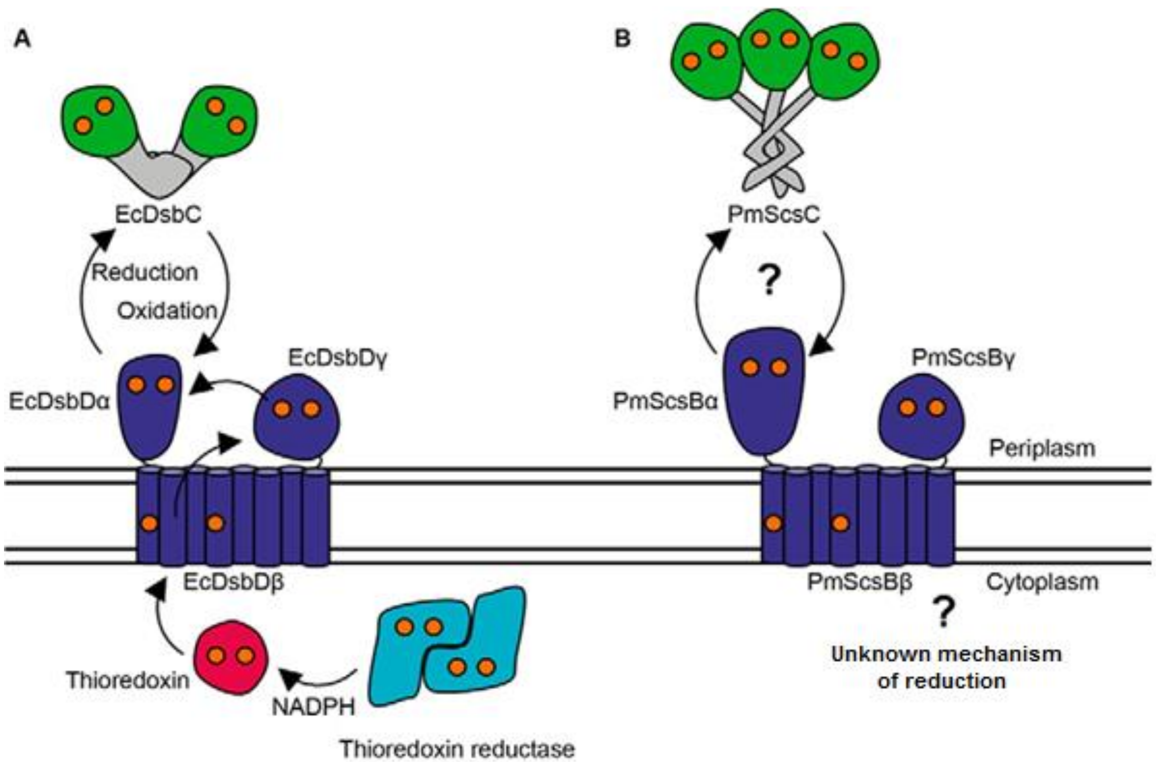


Cross-membrane cystine reduction

Related systems, all connected to bacterial virulance, evolved to different tasks

E. coli: Disulfide bond forming ("DsB") proteins redox cycle across the membrane. Well characterised.

Salmonella enterica, and Proteus mirabilis, have similar proteins, with some strikin similarities, but serving as suppressors of copper sensitivity ("Scs"). Also seen in *Caulobacter crescentus.*



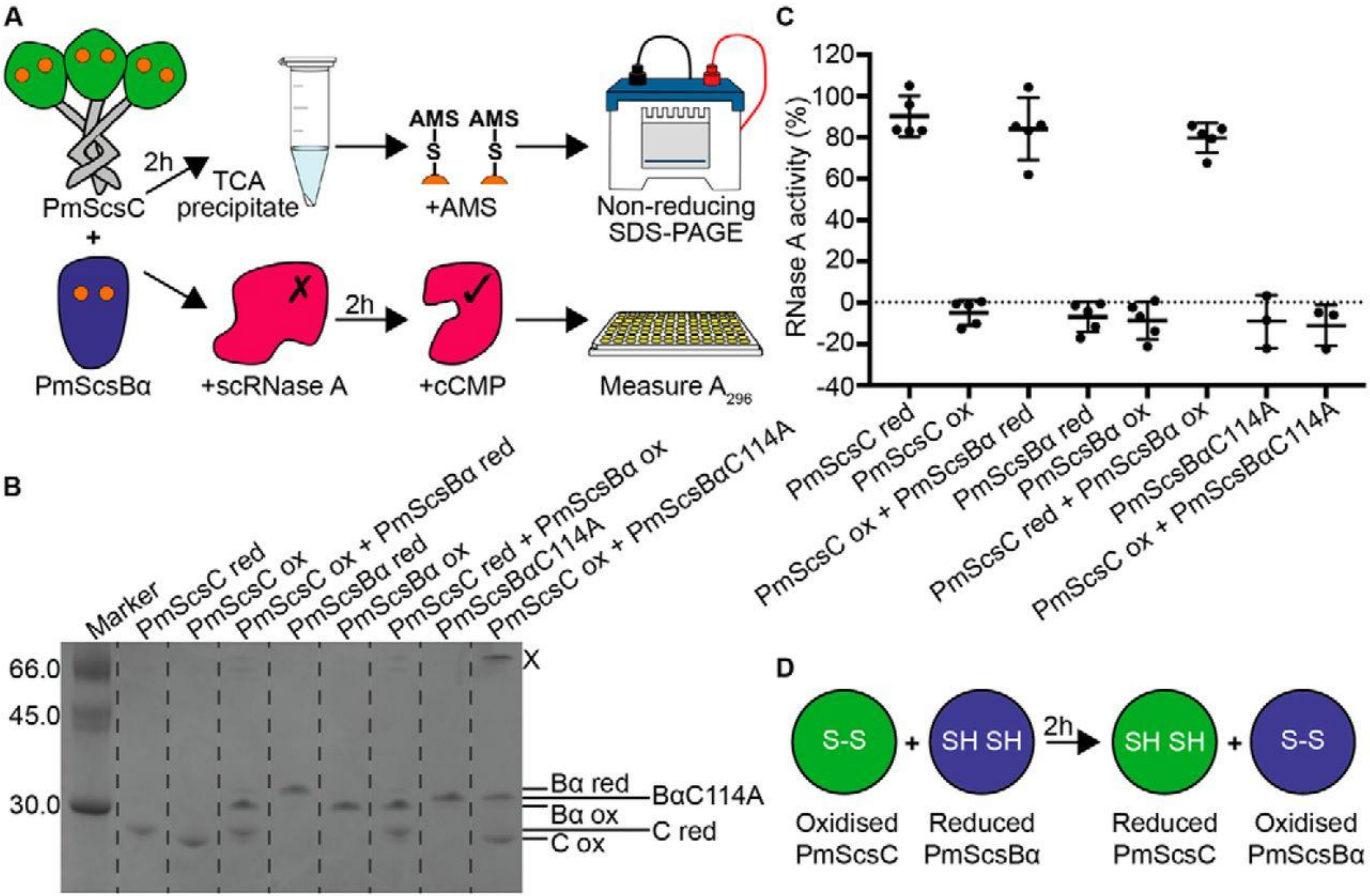
PmScsBα crystallised.

DALI structure-based homology search reveals new relationships

PmScsC, trimeric binding partner, not yet.

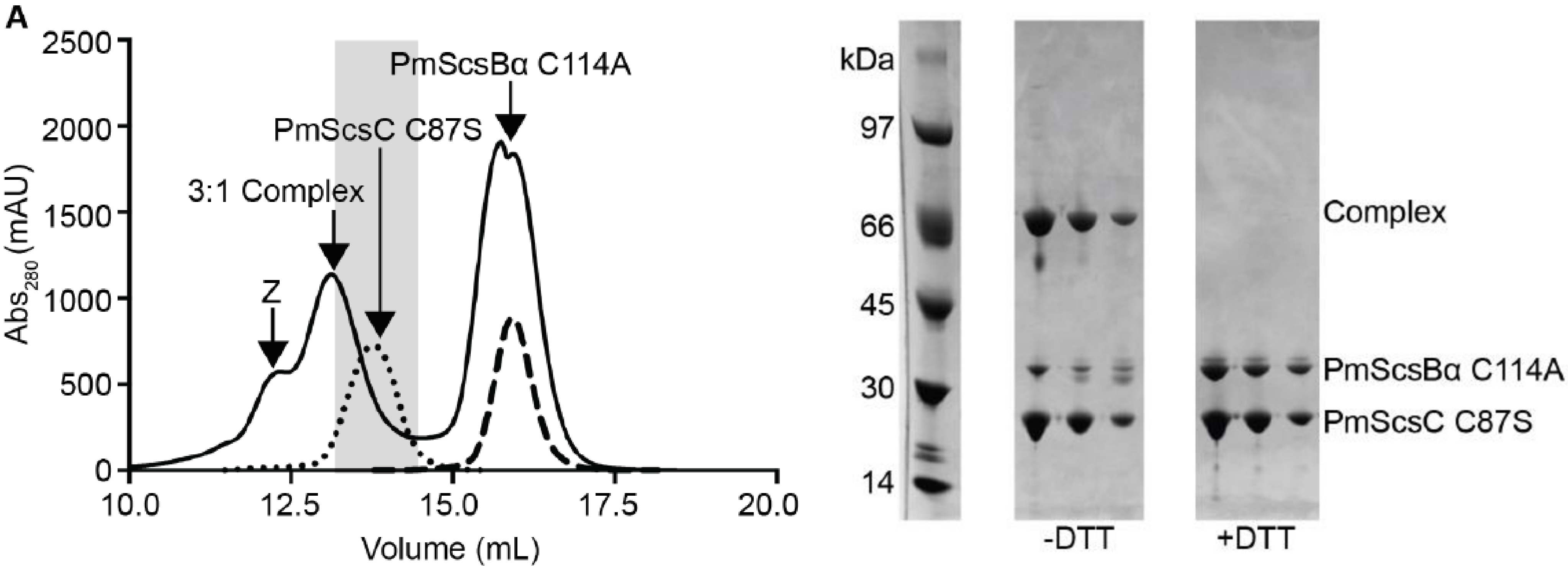
- Careful assays demonstrate one-directional reduction transfer.
- Mutagenesis identifies Cys residues critical to activity.
  And an inactive stable complex over gel filtration.

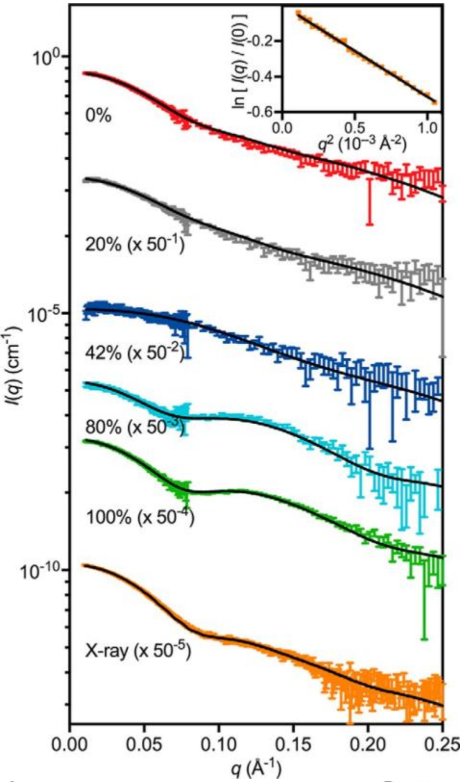
PmScsBα reduces and activates PmScsC.



Emily J. Furlong et al. J. Biol. Chem. 2018;293:5793-5805

PmScsBα C114A binds tightly to PmScsC C87S, 1:3.

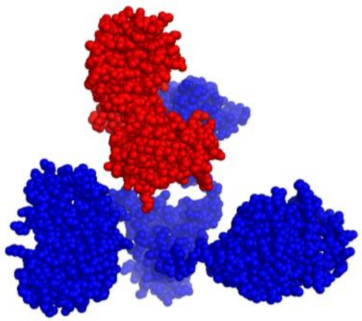


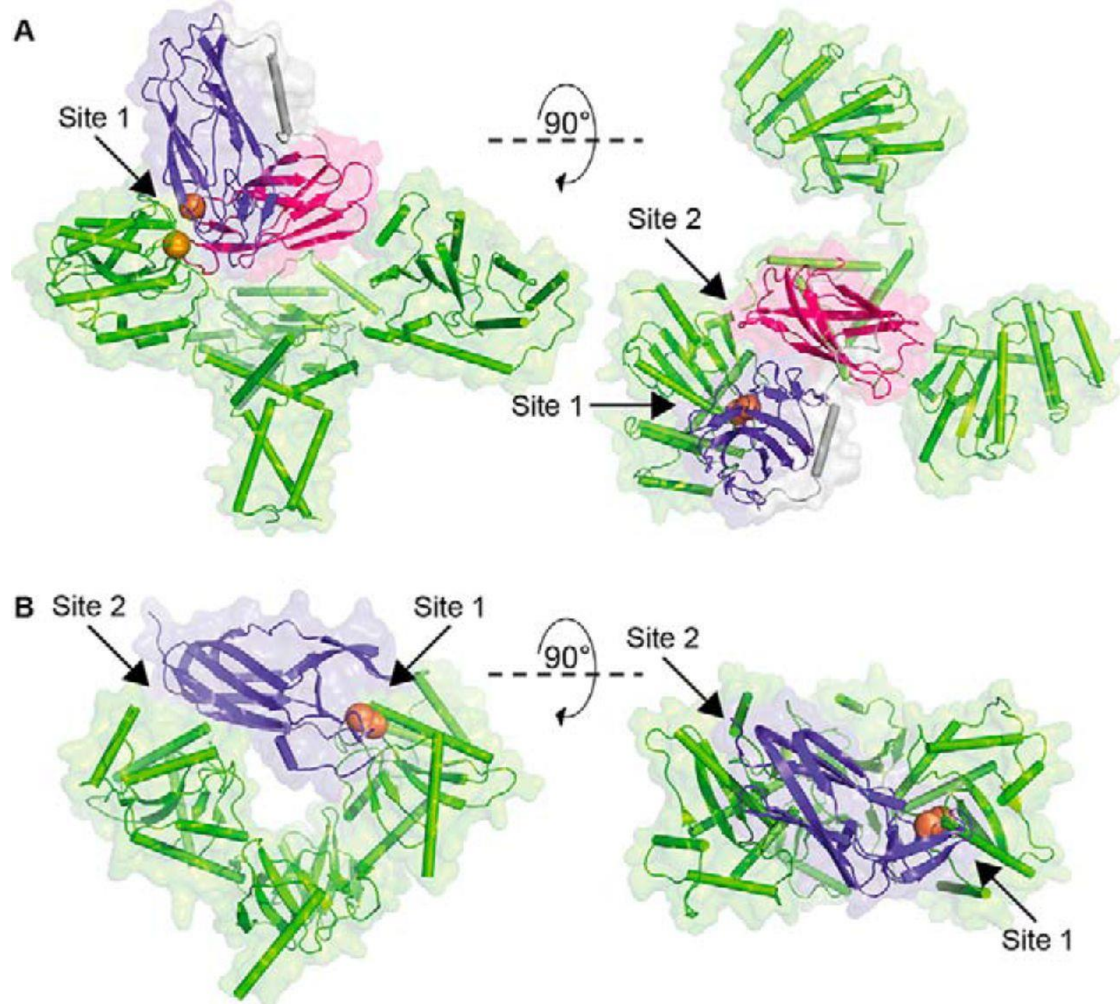


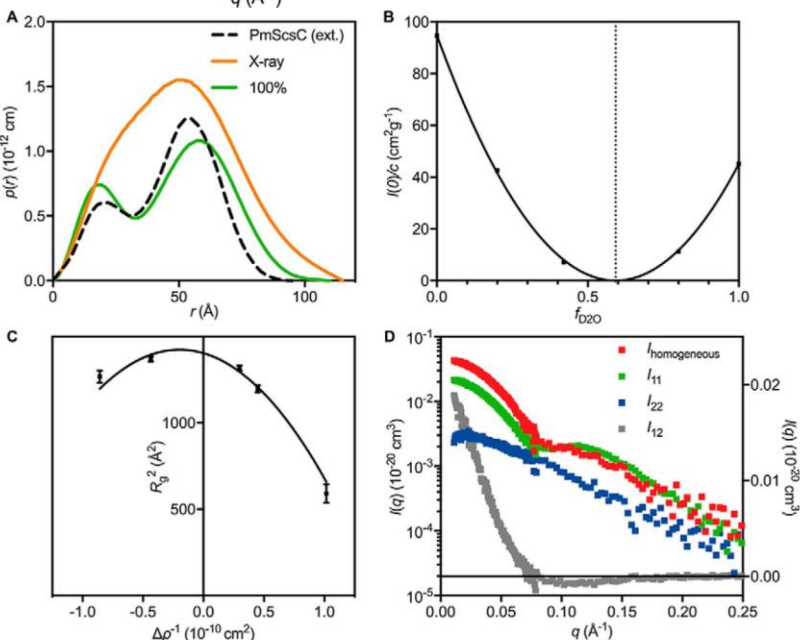
PmScsBα C114A (monomer) is deuterated, match point 100% D_2O

PmScsC C87S (trimer) is unlabelled, match point 42% D_2O

SAXS and SANS defines the low resolution structure;
Modelling used to dock the crystal structure to a homology model.



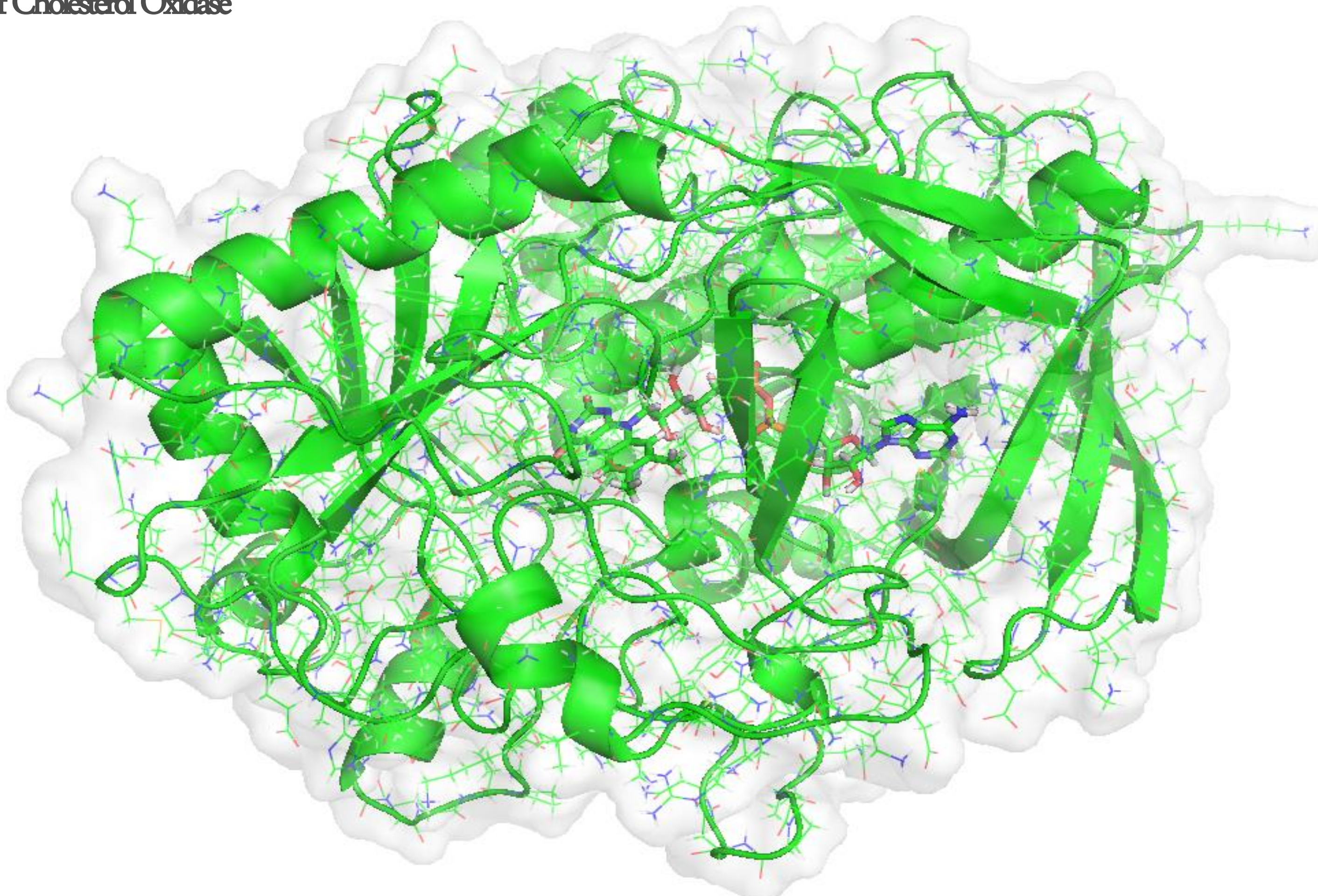




Neutrons and deuteration for atomic resolution.

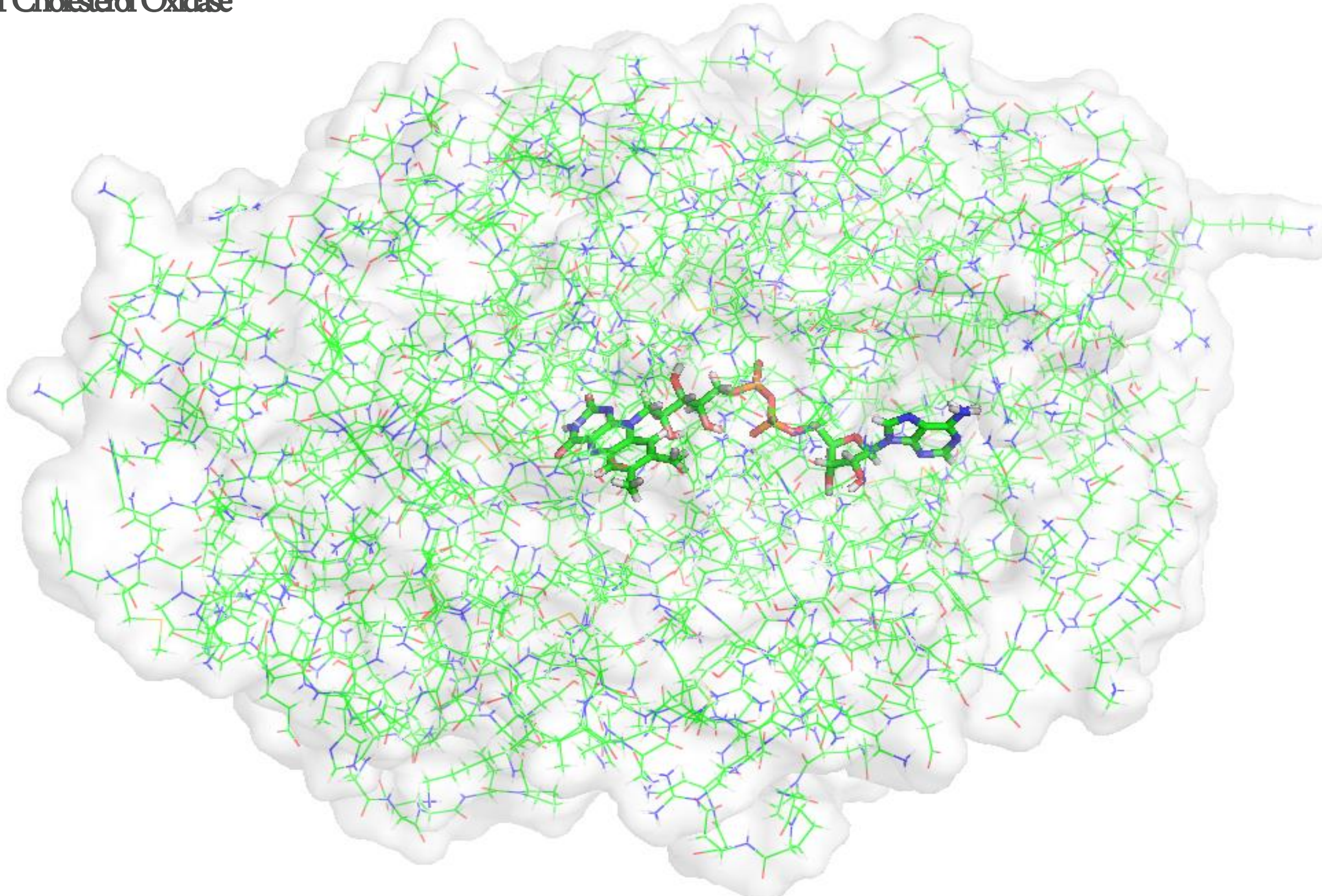
Neutron crystallography

Neutron Crystallography of Cholesterol Oxidase



ANSTO

Neutron Crystallography of Cholesterol Oxidase



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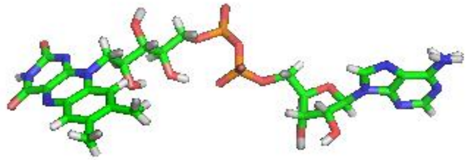

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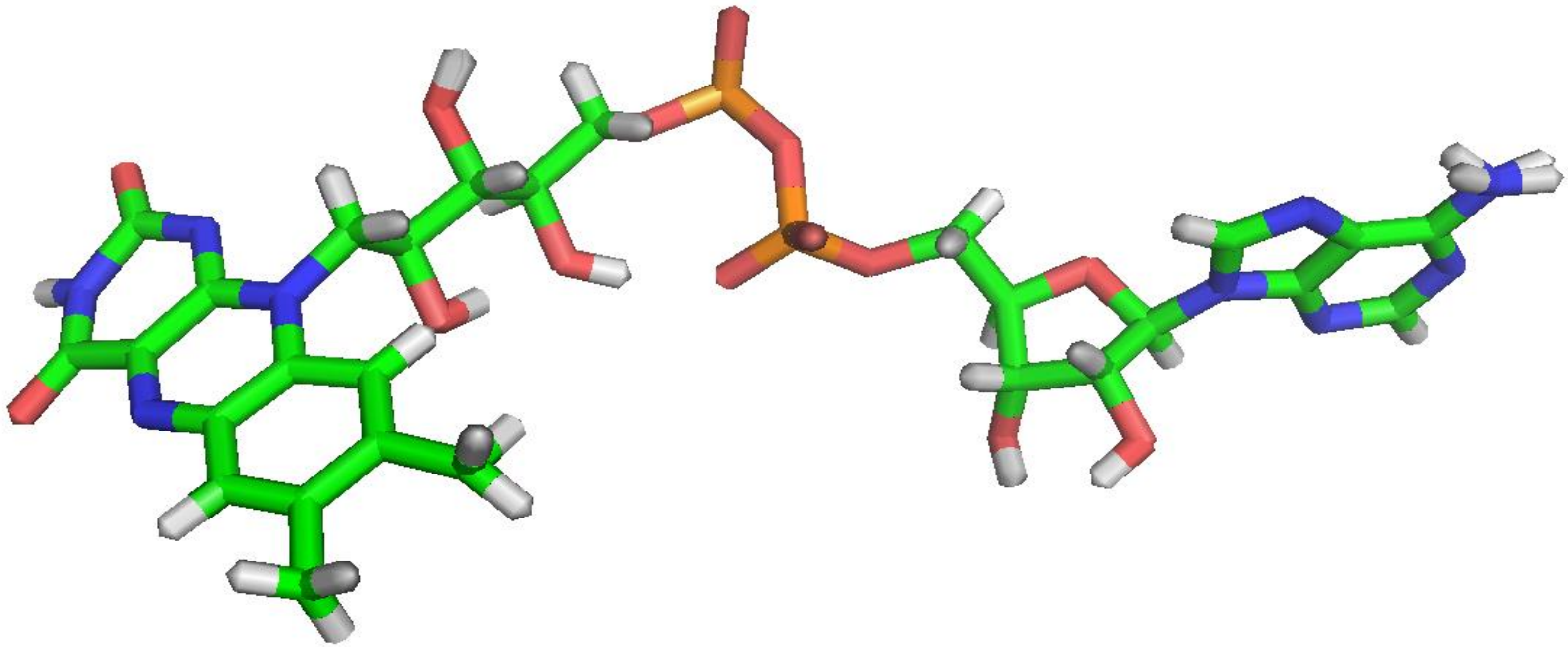
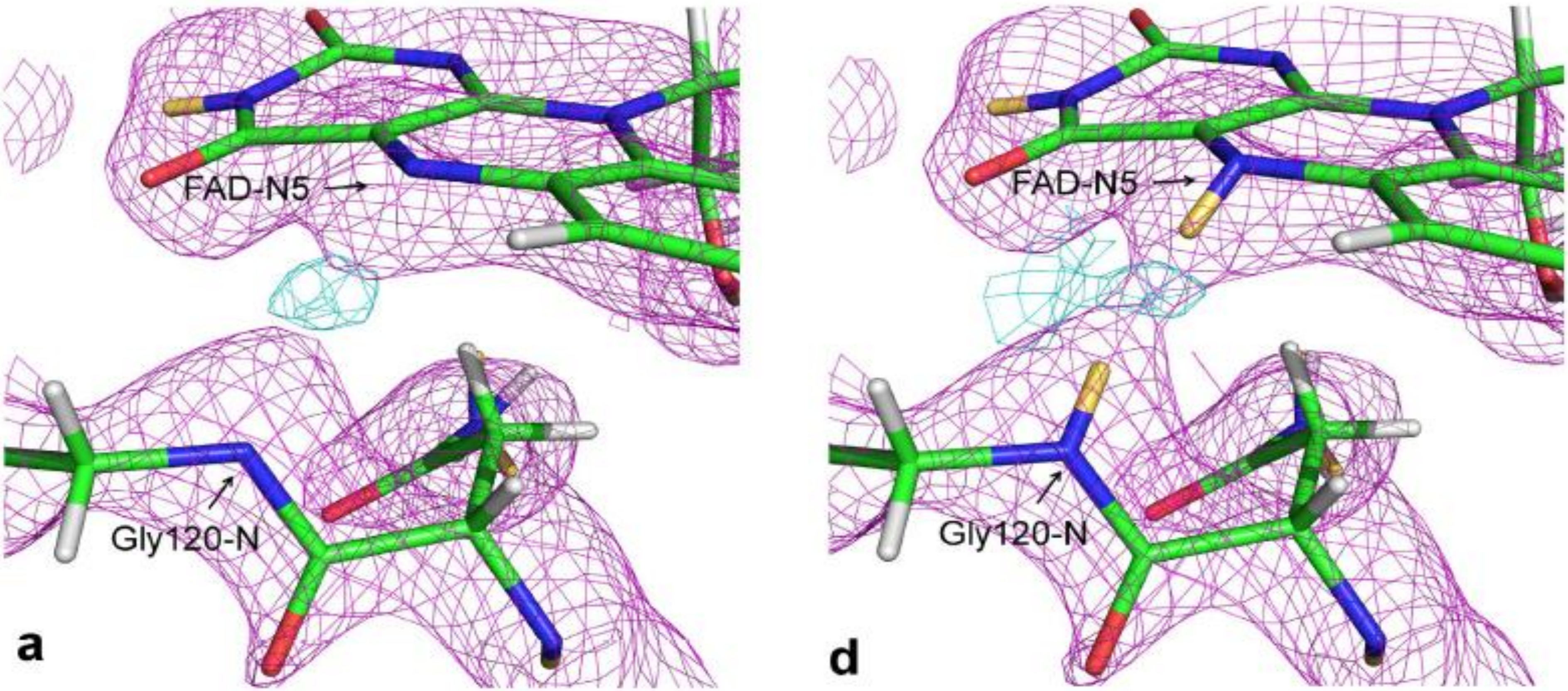
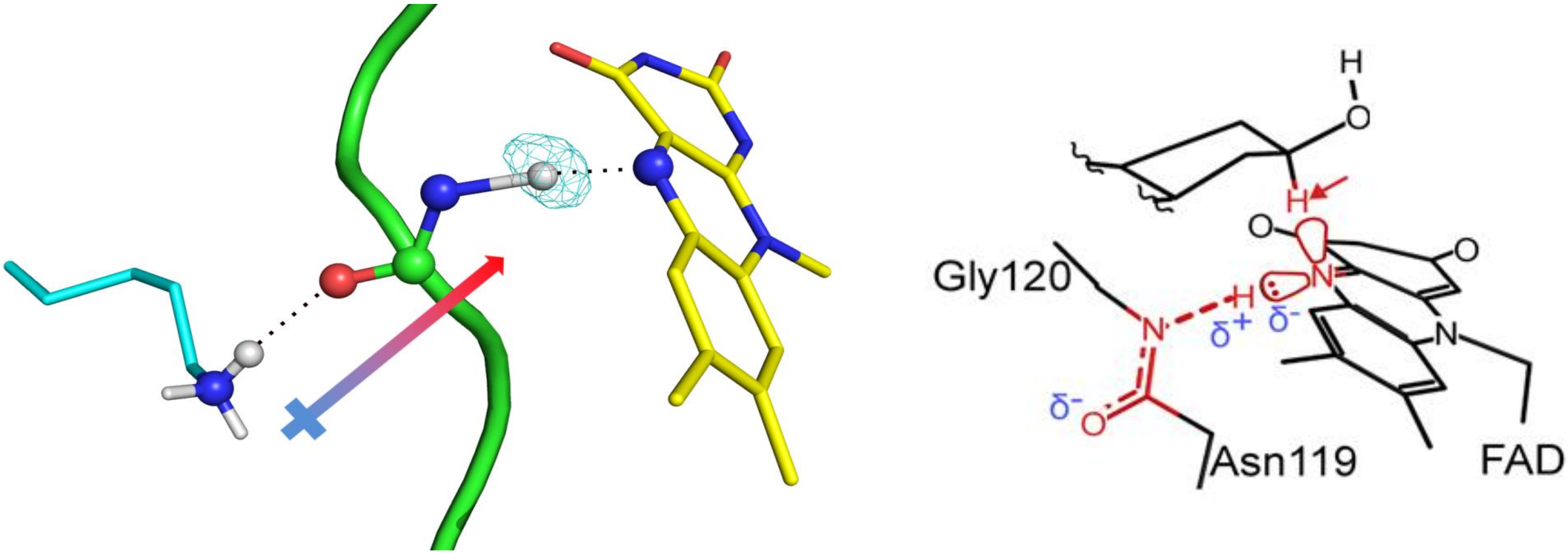




ANSTO

Neutrons and deuteration for atomic resolution.
Neutron crystallography

Best used to questions of chemistry, hydrogen atoms.

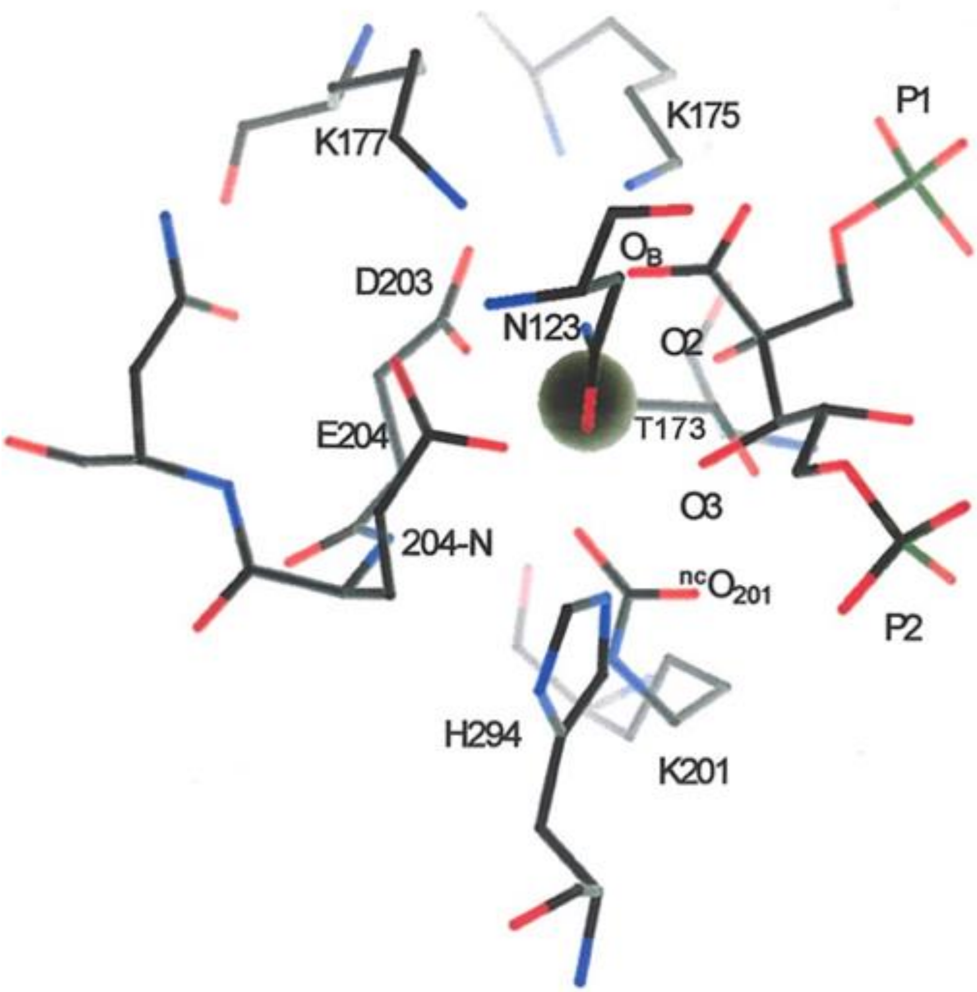


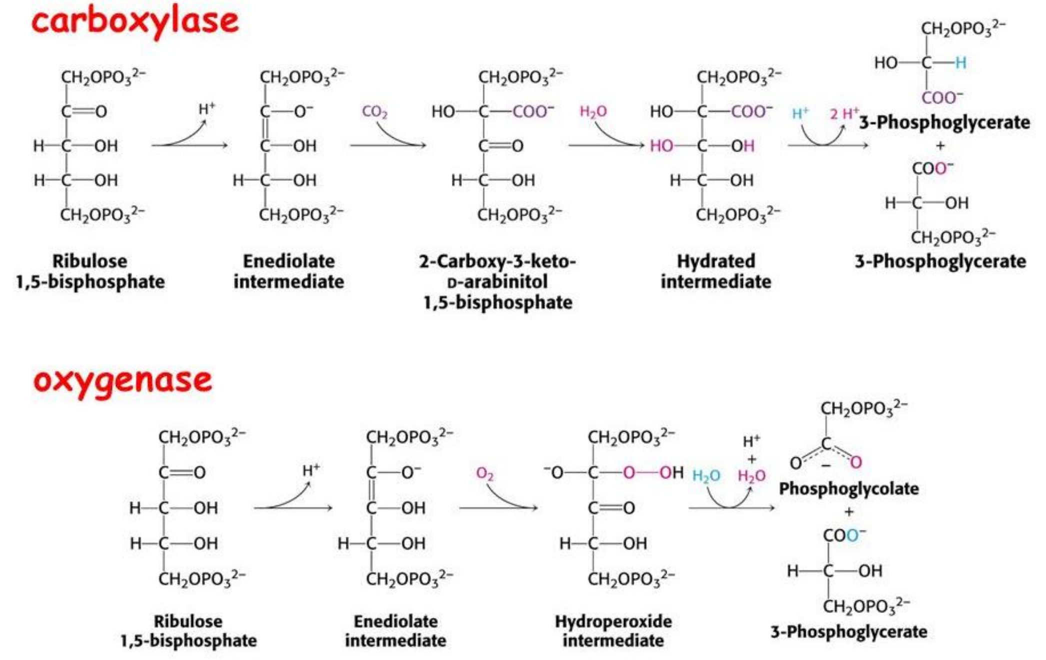
Cholesterol oxidase, Golden *et al* 2017, Scientific reports

Neutrons and deuteration for atomic resolution. Neutron crystallography

Best used to questions of chemistry, hydrogen atoms.

Neutron crystallography of rubisco. Where are the protons?





Neutrons and deuteration for atomic resolution.
Neutron crystallography

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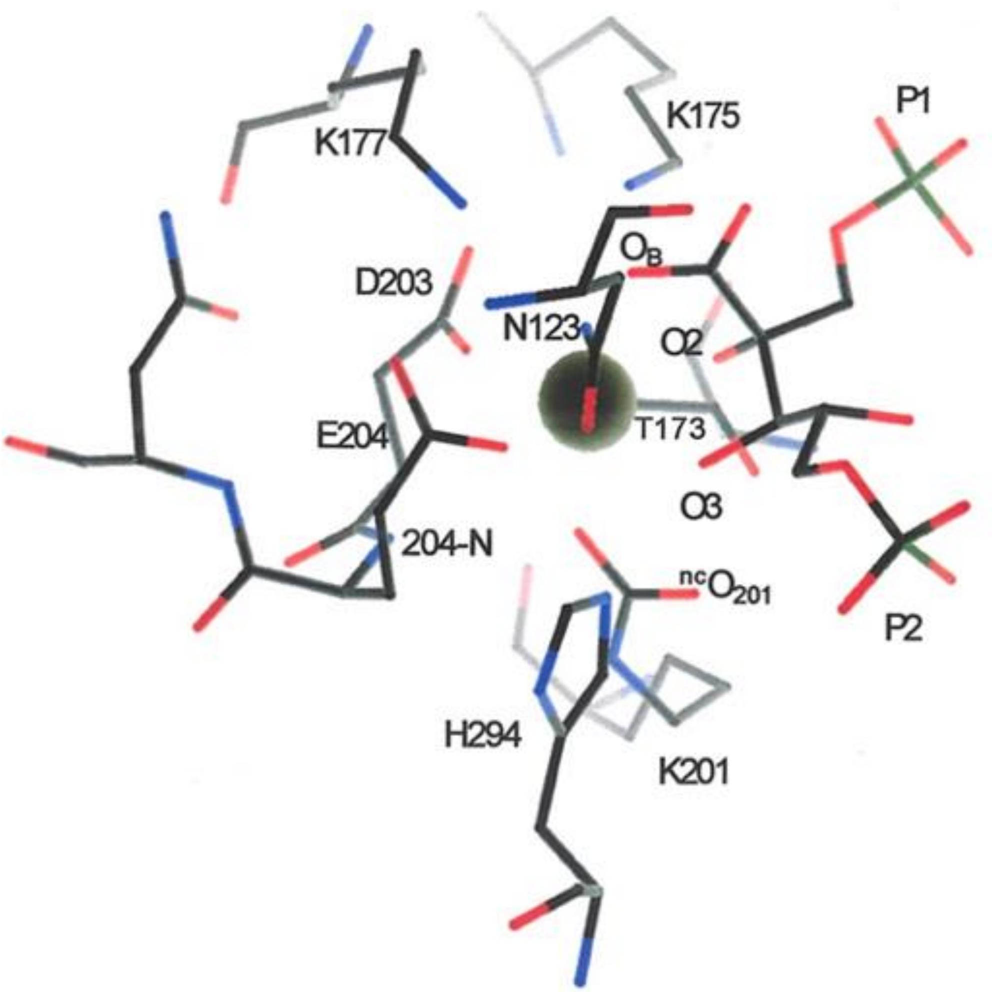
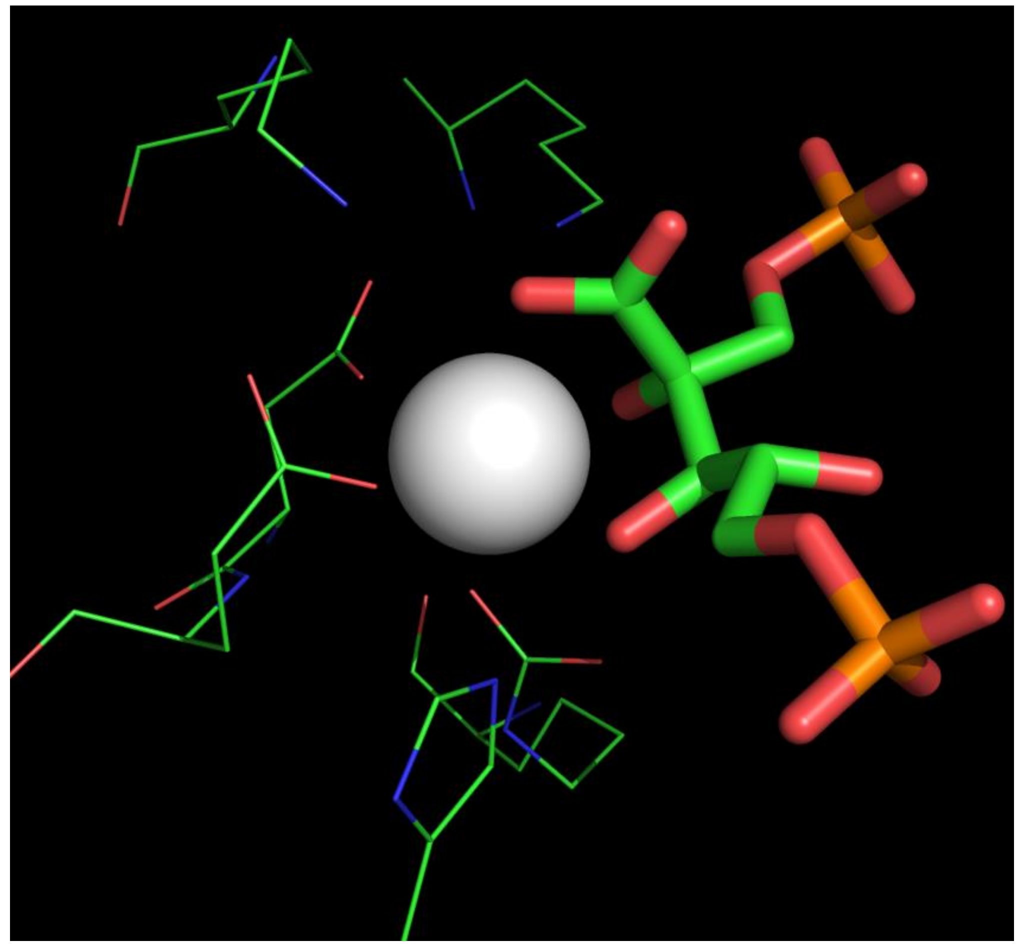
Neutron crystallography of rubisco





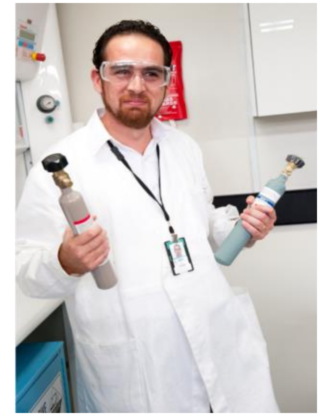
Chemical Deuteration @ NDF-ANSTO

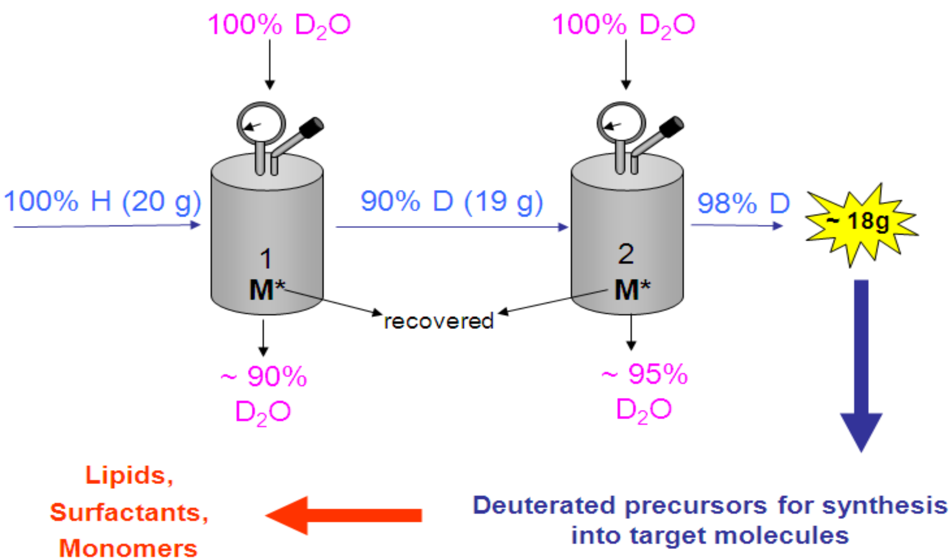
Chemical synthesis in D_2O

Chemical Deuteration
(surfactants, lipids, small molecules)

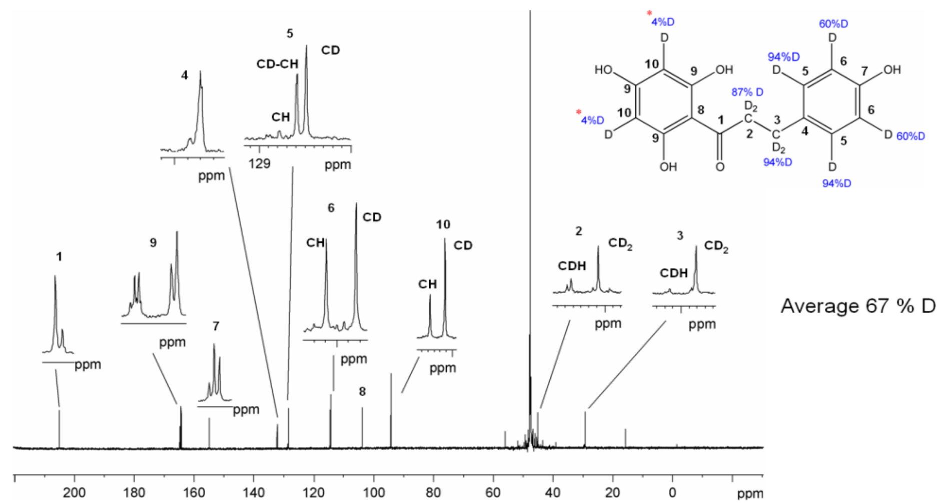
Chemical deuteration

- hydrogen-exchange at high pressure and temperature,
- catalytic exchange,
- synthesis of deuterated precursors.





Phloretin, produced and characterised

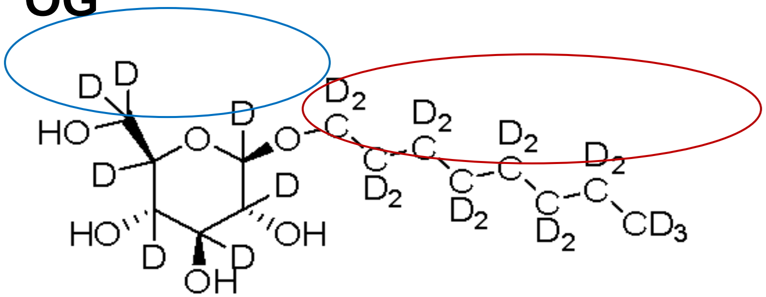


- Deuterated molecules are purified and characterised.

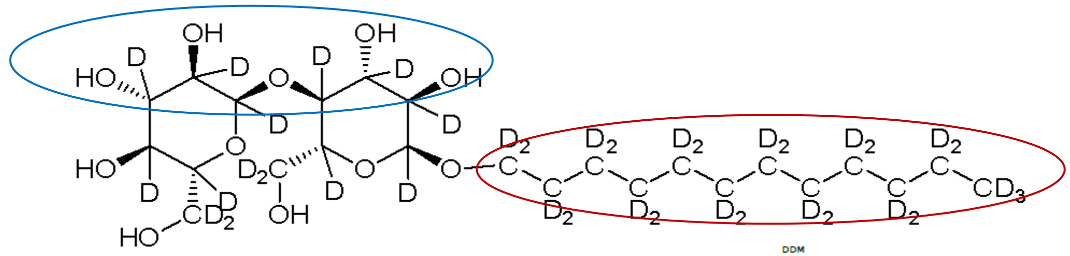
ANSTO

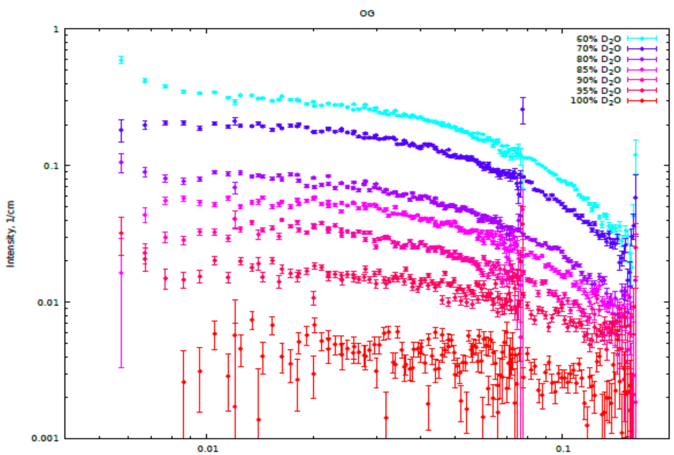
Invisible Detergents for Structural Studies of Integral Membrane protein

OG

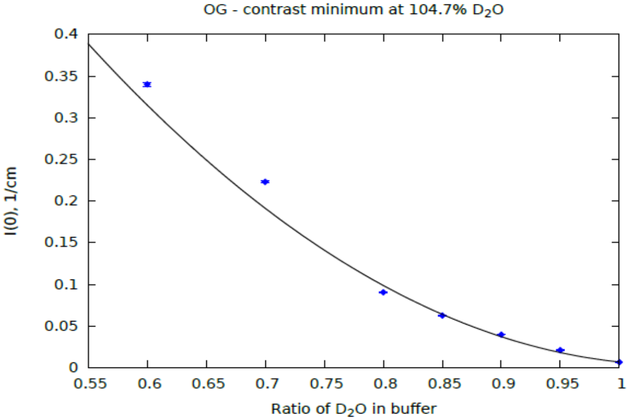


DDM





OG - contrast minimum at 104.7% D_2O

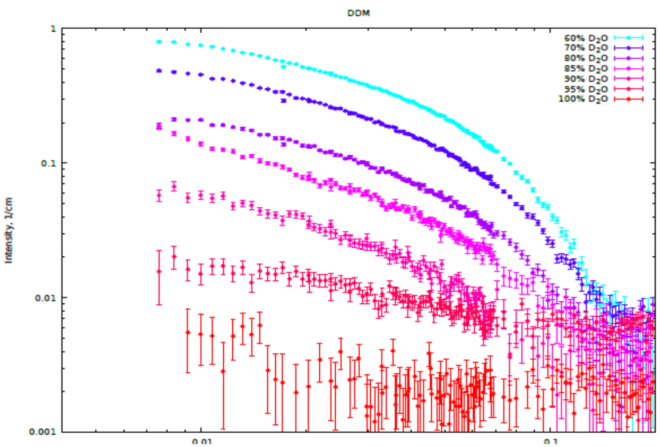


- Specific level of deuteration head and tail

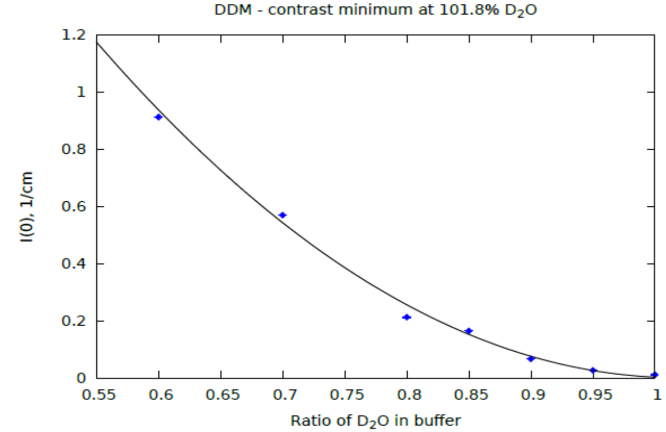
SANS determines structures of Integral Membrane proteins solubilised in detergents.

This is complicated by the large scattering contribution from the detergents.

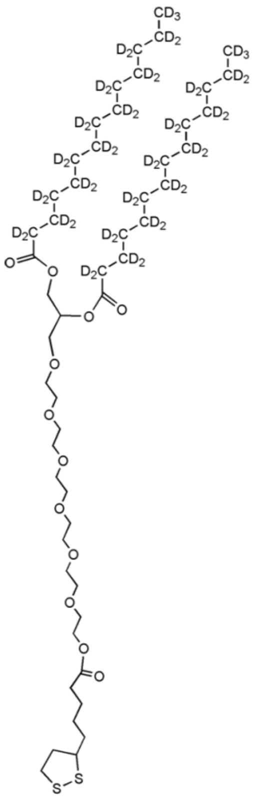
Lise Arleth
Niels Bohr Institute
Copenhagen University

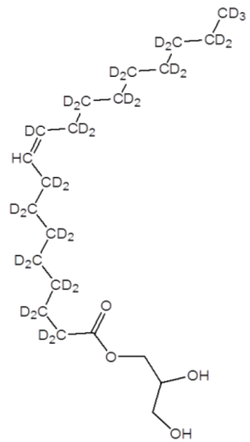


DDM - contrast minimum at 101.8% D_2O

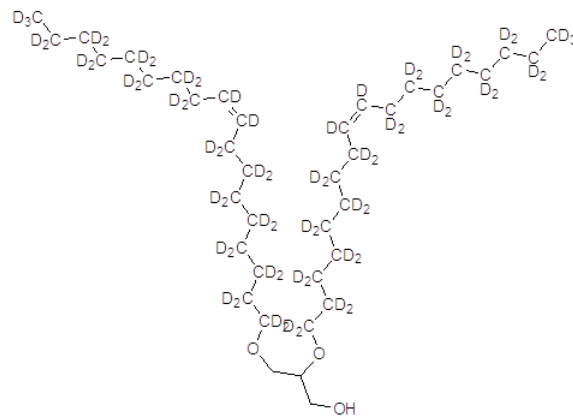


Lipid and tethered lipid derivatives

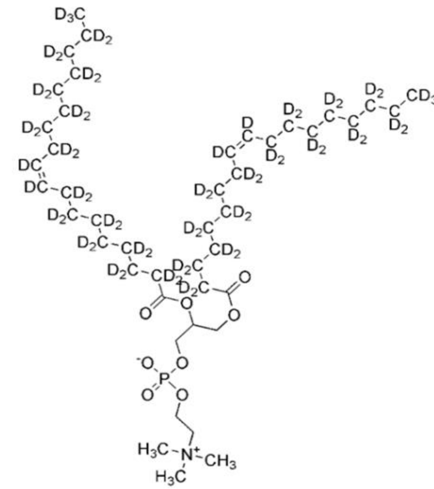




GMO



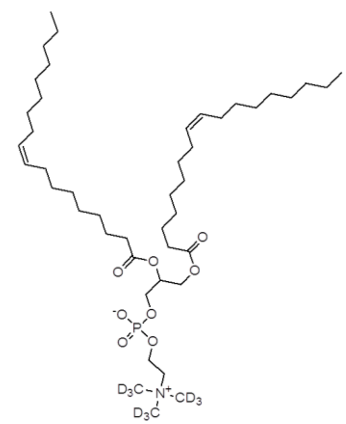
DOG



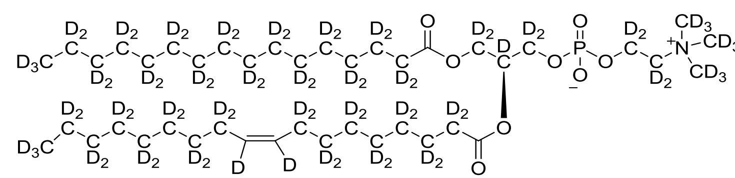
DOPC-deuterated tail

gram scale!

Tethered Lipids



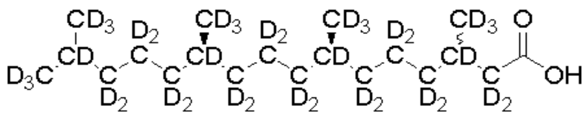
DOPC-deuterated head



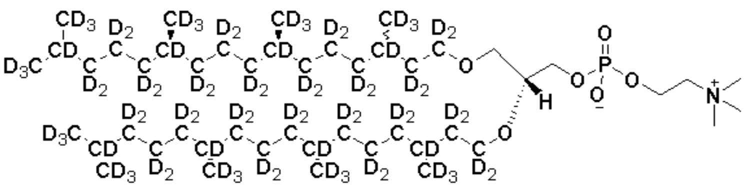
Fully Deuterated POPC

Deuterated Chemicals

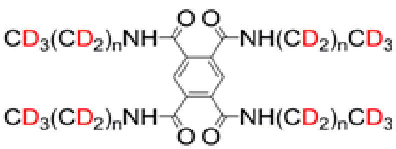
Branched Fatty acids and Lipids



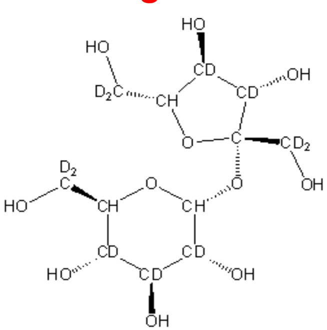
phytanicacid-d_{39}



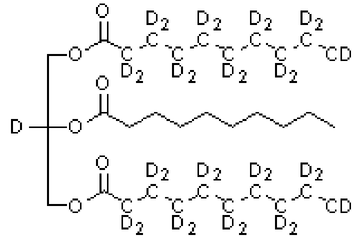
Gelators

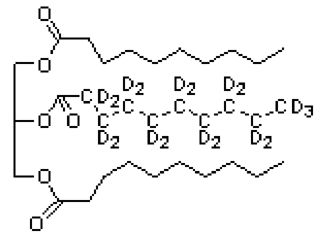


Sugars

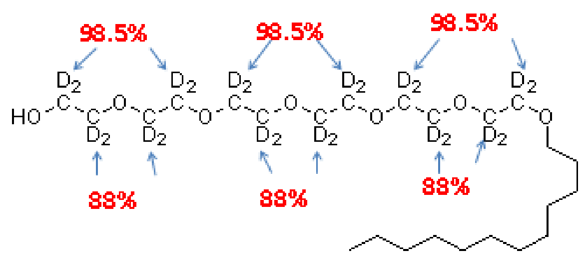


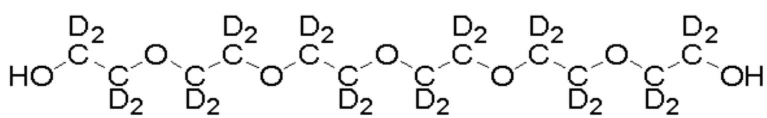
Triglycerides





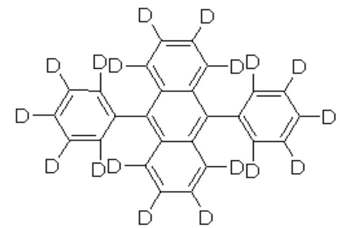
Non-ionic surfactant

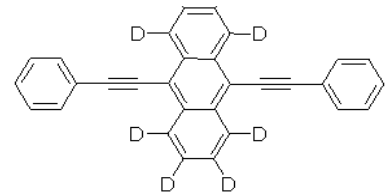


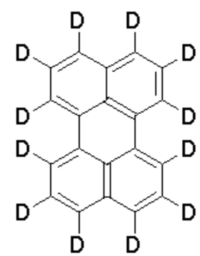


HEG-d_{24}

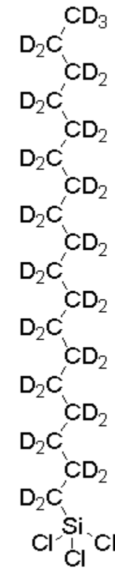
Solar Dyes





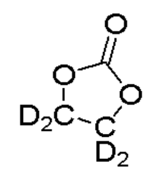


Silyl compounds



OTS-d_{37}

Electrolytes



One standard protein-deuteration protocol

Methods in Enzymology 21 July 2015

CHAPTER ONE

Robust High-Yield Methodologies for ^2H and $^2H/^{15}N/^{13}C$ Labeling of Proteins for Structural Investigations Using Neutron Scattering and NMR

Anthony P. Duff*,[1], Karyn L. Wilde*, Agata Rekas*, Vanessa Lake*, Peter J. Holden†

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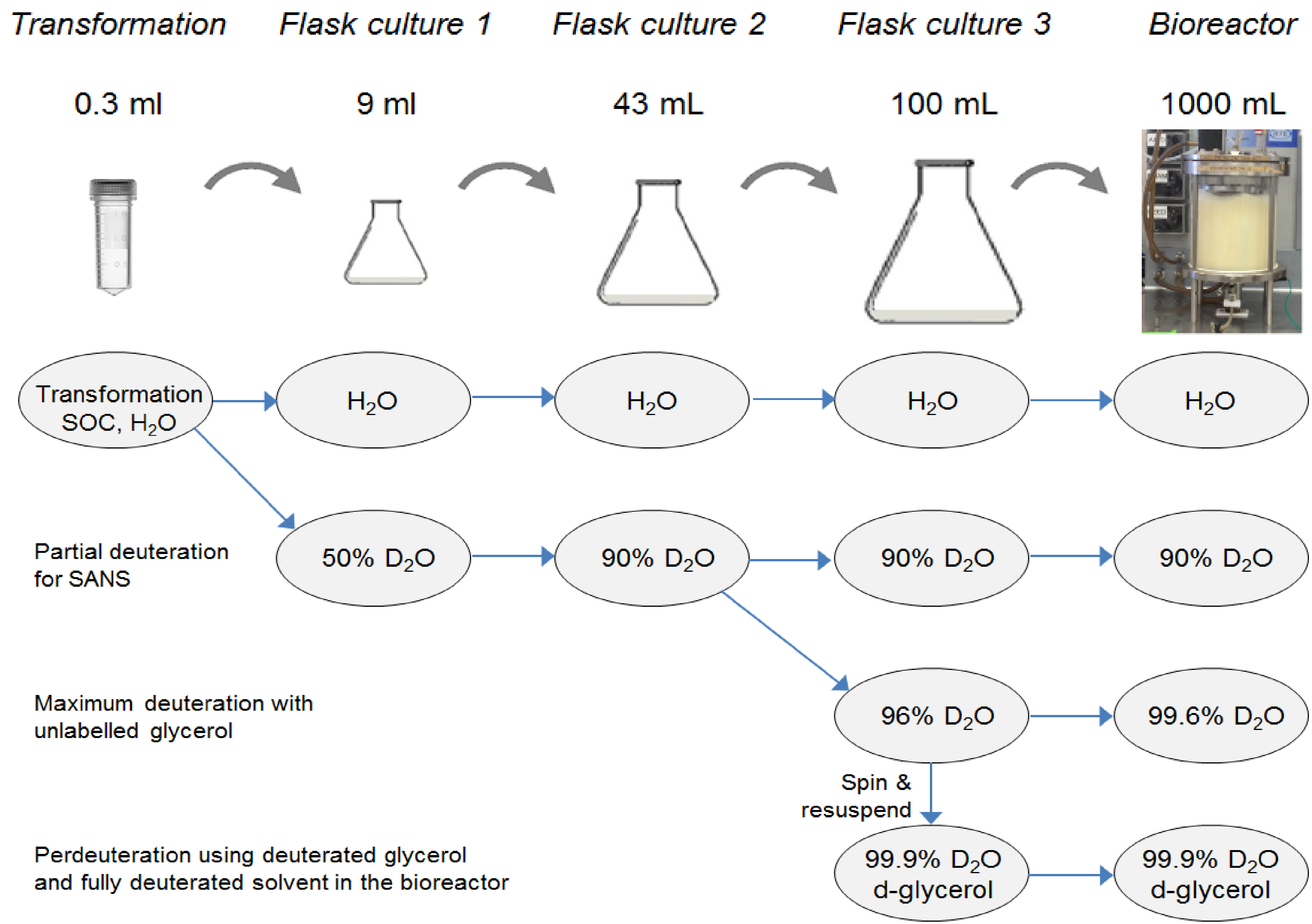
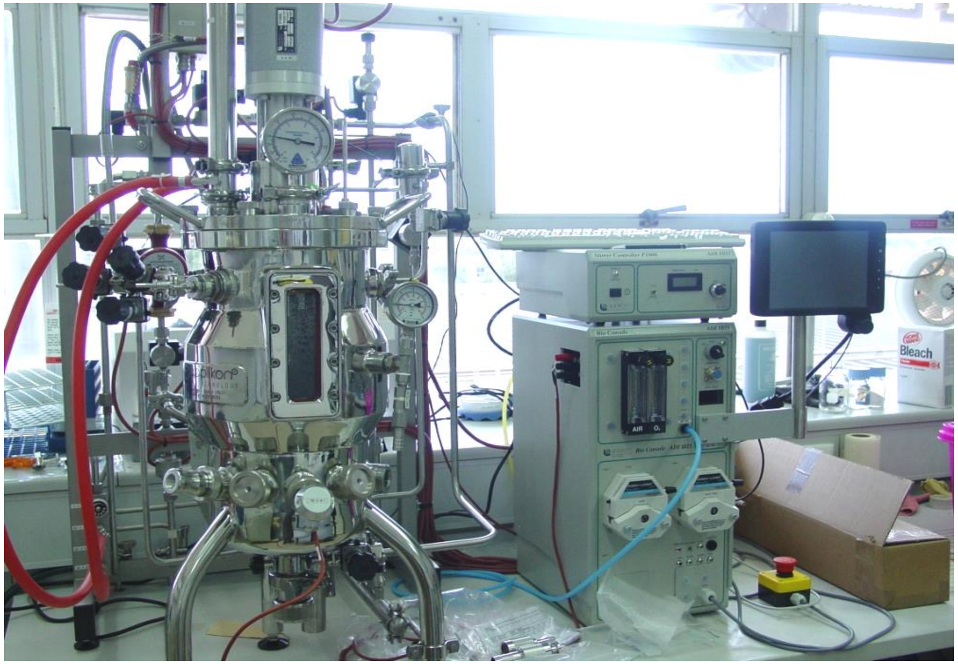
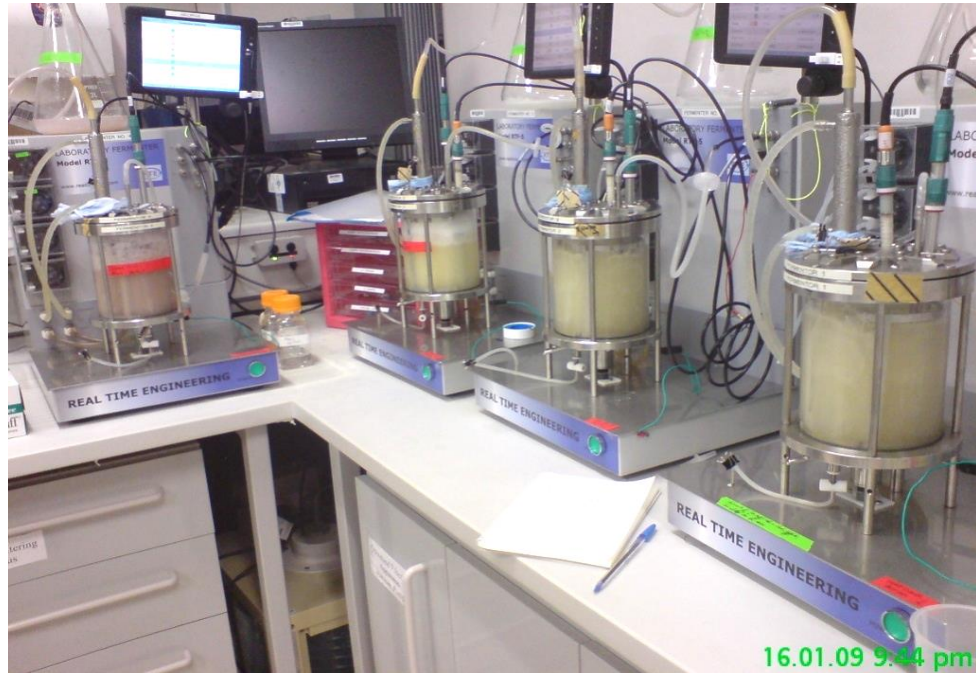
†National Deuteration Facility, Bragg Institute, Australian Nuclear Science and Technology Organisation, New South Wales, Australia
[1]Corresponding author: e-mail address: anthony.duff@ansto.gov.au



Stepped adaptation pathways to deuterated media. The four typical production conditions are indicated. Unlabelled production (top row) provides a test that the protocols utilised will produce induced protein expression by the method and scale intended for labelled production. Varying levels of protein deuteration are achieved by following the branches (arrows) to lower rows.

Bioreactor use





- Airflow,
- Impeller (stirrer) speed
- Temperature
- pH

Typical results (1L, 40 g glycerol)

- 40 – 80 g wet weight
- 50 – 400 mg purified protein

ANSTO

Thank you.

Australian Government

ANSTO

Thank you

Syntaxin and Vesicle Capture

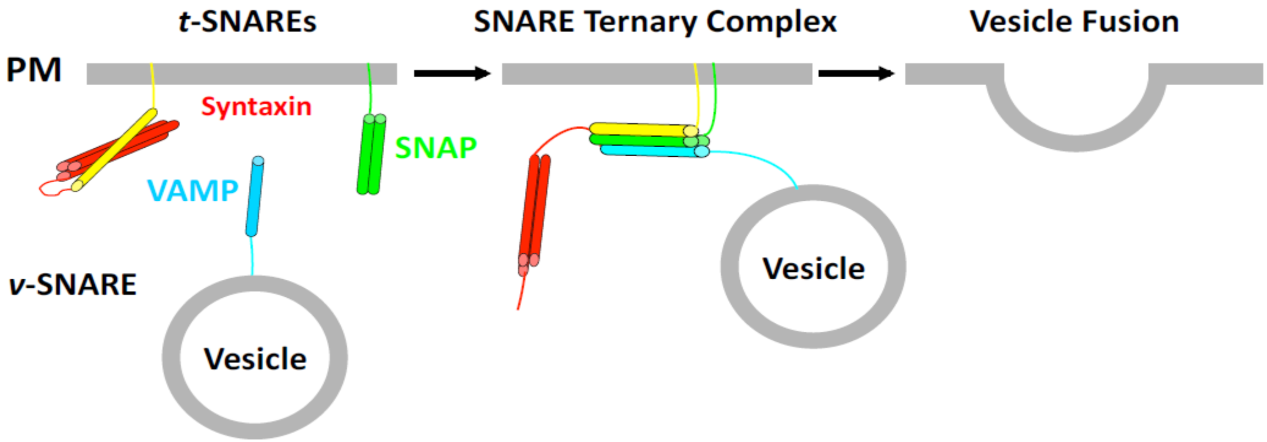


Figure 1 The interaction of three SNARE proteins promotes vesicle docking and fusion. The proteins located at the target membrane (t-SNAREs) are Syntaxin (red and yellow) and SNAP (green). The protein located on the vesicle (v-SNARE) is VAMP (cyan).

PNAS

Low-resolution solution structures of Munc18:Syntaxin protein complexes indicate an open binding mode driven by the Syntaxin N-peptide

Michelle P. Christie[a,1], Andrew E. Whitten[a,1,2], Gordon J. King[a], Shu-Hong Hu[a], Russell J. Jarrott[a], Kai-En Chen[a], Anthony P. Duff[b], Philip Callow[c], Brett M. Collins[d], David E. James[e], and Jennifer L. Martin[a,2]

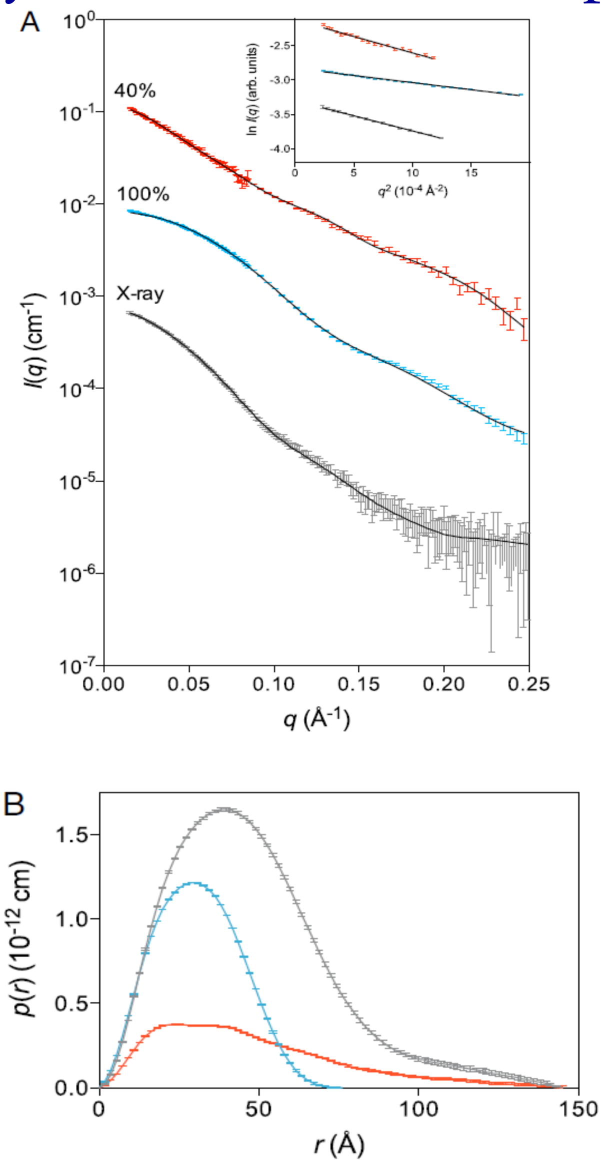
Divisions of [a]Chemistry and Structural Biology and [d]Molecular Cell Biology, Institute for Molecular Bioscience, University of Queensland, St. Lucia, Queensland 4072, Australia; [b]National Deuteration Facility, Australian Nuclear Science and Technology Organisation, Lucas Heights, New South Wales 2234, Australia; [c]Large Scale Structures Group, Institut Laue-Langevin, 3800 Grenoble, France; and [e]Diabetes and Obesity Research Program, Garvan Institute of Medical Research, Darlinghurst, New South Wales 2010, Australia

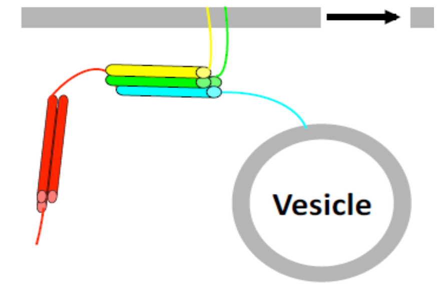
Edited by Axel T. Brunger, Stanford University, Stanford, CA, and approved May 4, 2012 (received for review October 14, 2011)

When nerve cells communicate, vesicles from one neuron fuse with the presynaptic membrane releasing chemicals that signal to the next. Similarly, when insulin binds its receptor on adipocytes or muscle, glucose transporter-4 vesicles fuse with the cell membrane, allowing glucose to be imported. These essential processes require

closed conformation inactivates Sx1a by preventing H3 interacting with SNARE partners, SNAP25 on the plasma membrane and vesicle associated membrane protein 2 (VAMP2, also known as synaptobrevin) on the vesicle membrane. Conversely, when the intramolecular Habc interaction is removed, Sx1a can adopt an

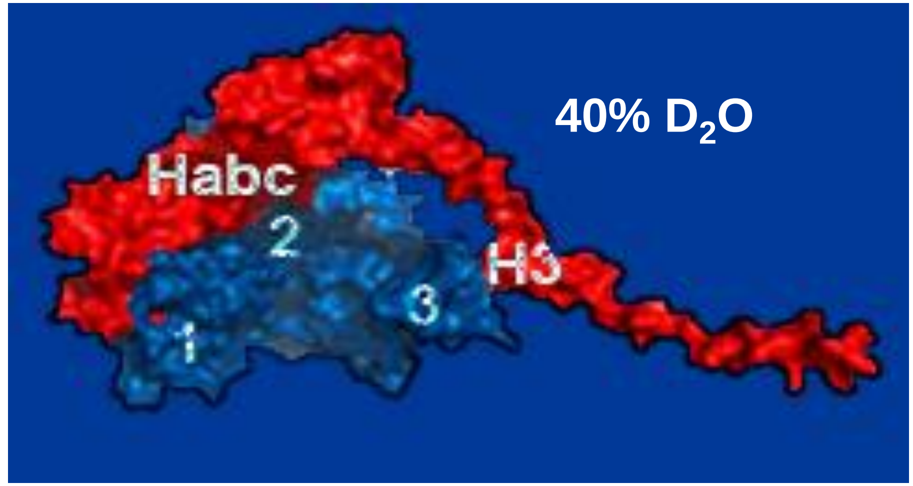
Syntaxin and Vesicle Capture





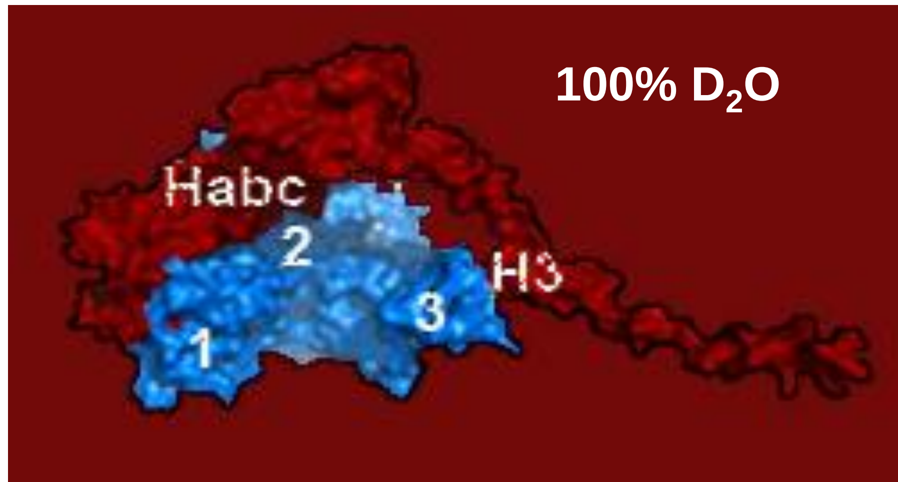
In 40% D_2O:
The relatively straight scattering curve at 40% D_2O (red) indicates that the deuterated syntaxin is elongated.

The hydrogenated Munc18 is matched to the solvent, and the labelled syntaxin dominates the signal

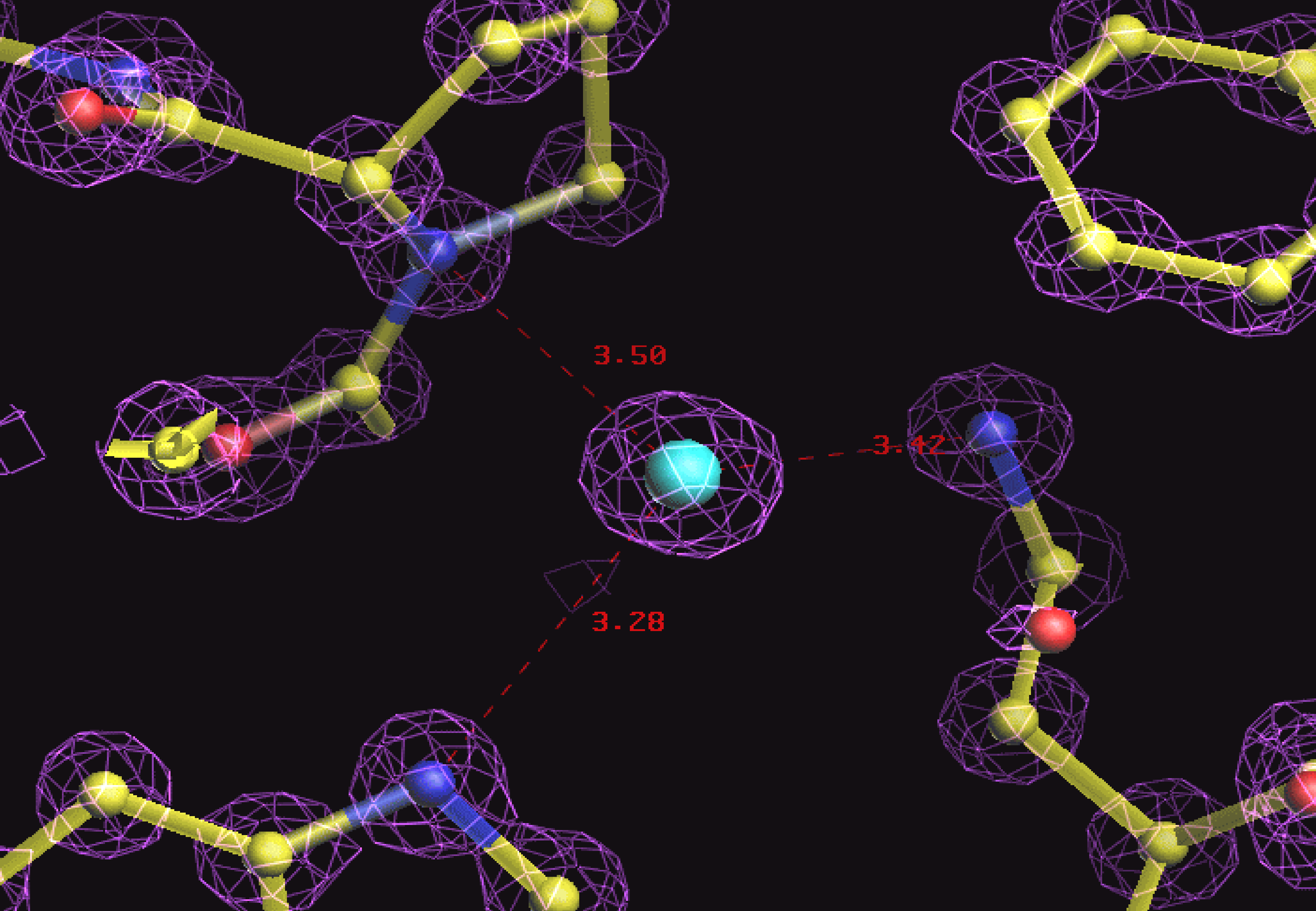


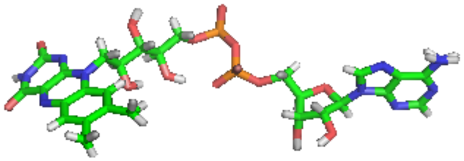
In 100% D_2O:
The much more curved scattering profile at 100% D_2O (blue) indicates that the hydrogenated Munc18 has a compact structure.

The 75% deuterium labelled syntaxin is matched to the solvent, and the hydrogenated Munc18 dominates the signal



ANSTO


3.50
3.42
3.28



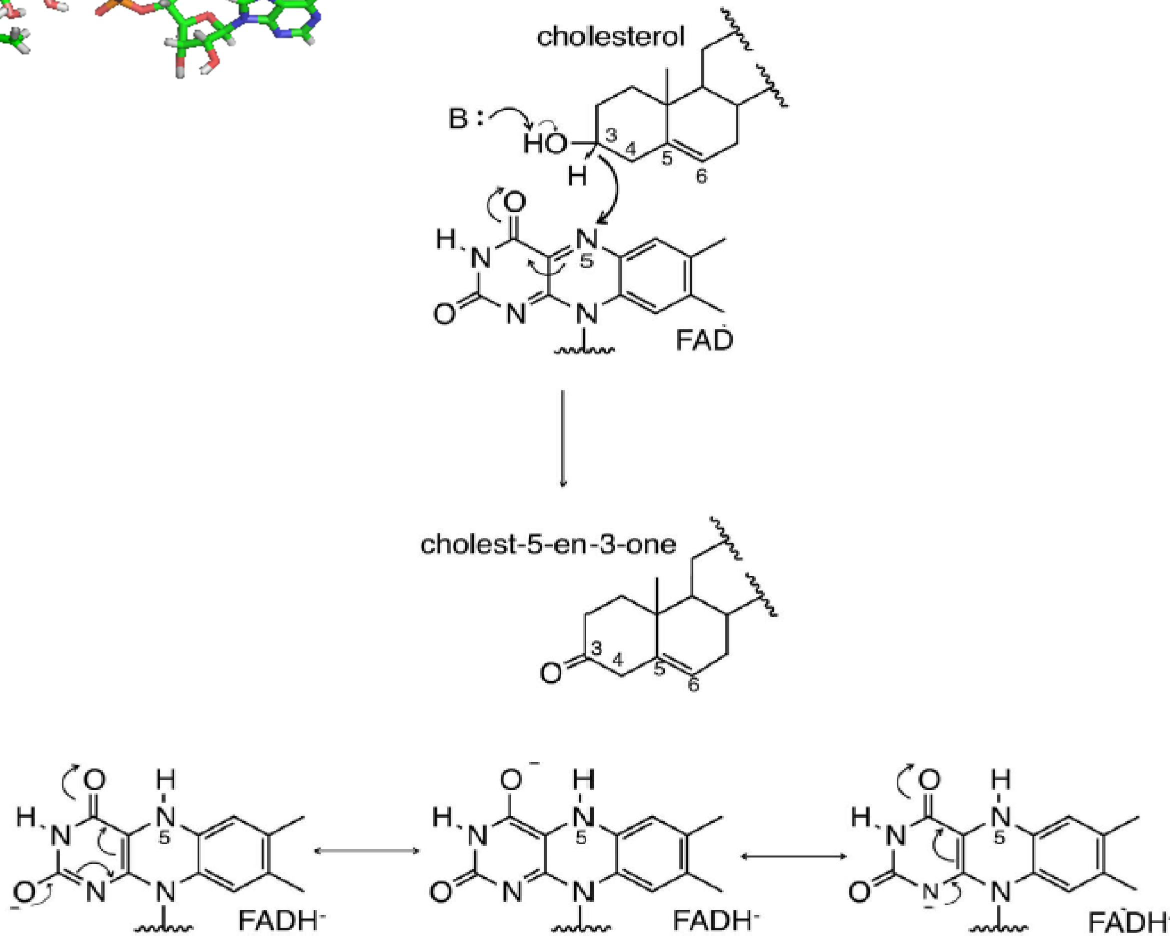
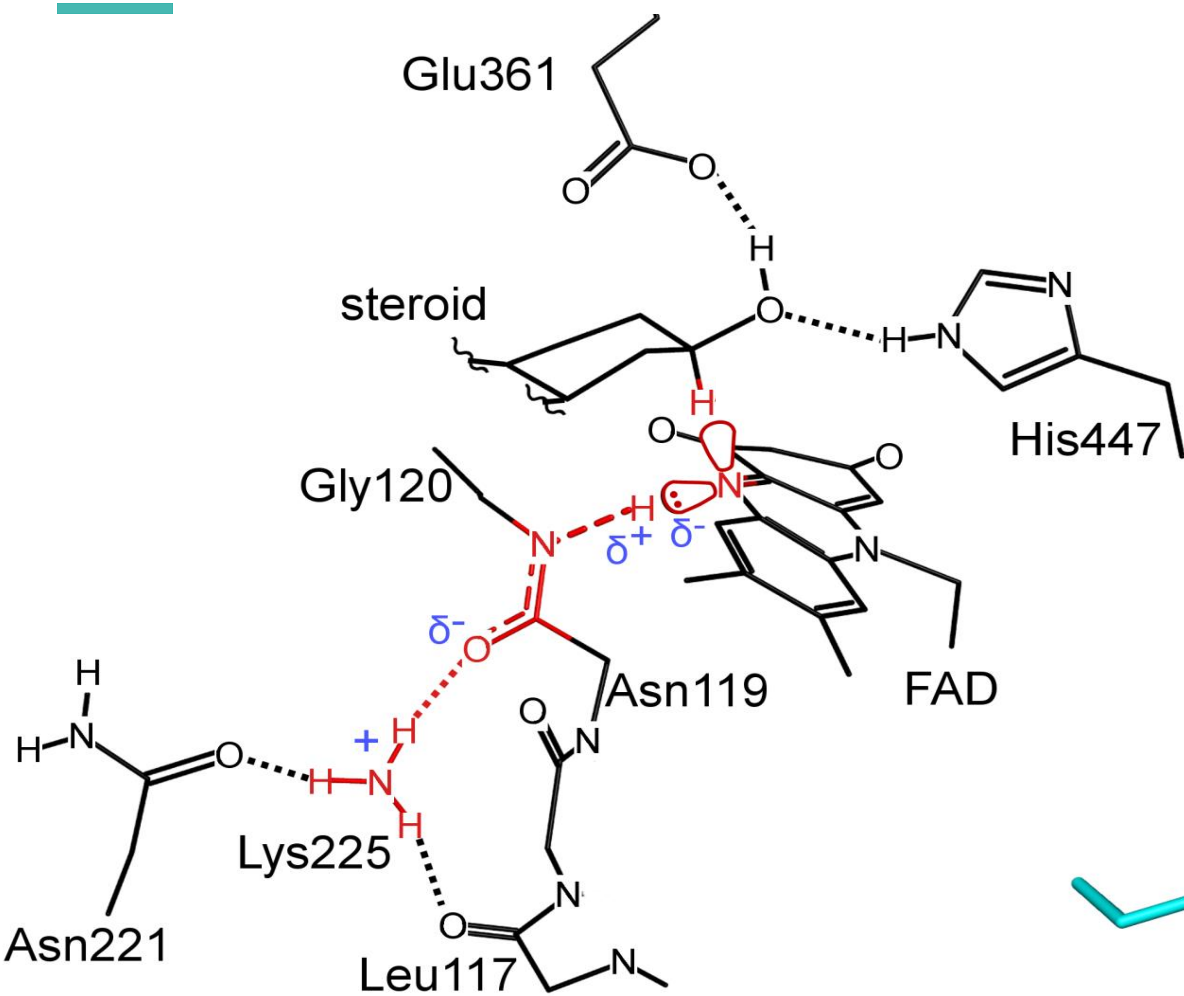
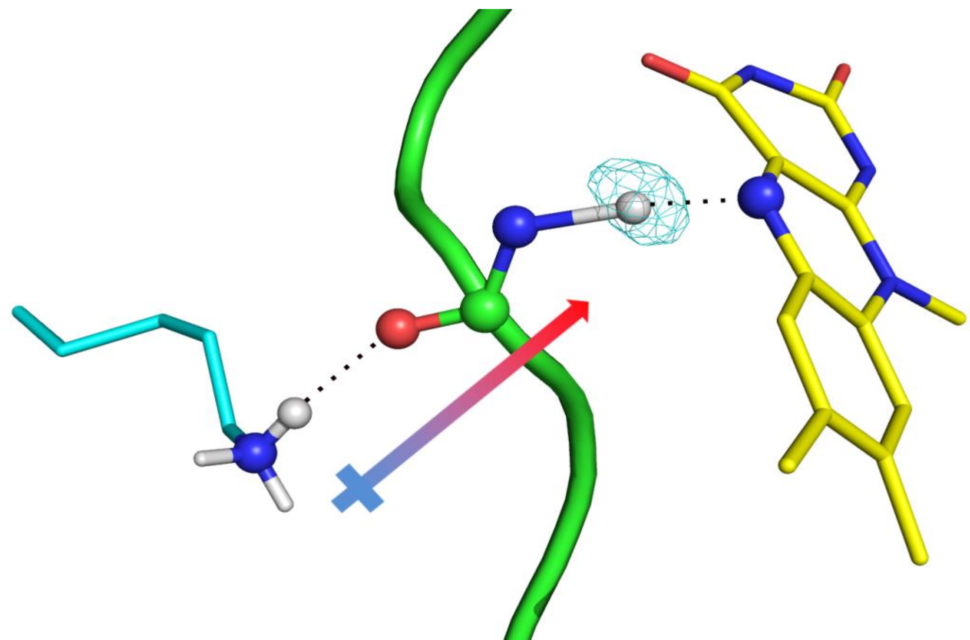


Figure 1. The oxidation reaction catalysed by COx. COx catalyses the oxidation of cholesterol to cholest-5-en-3-one *via* a hydride transfer to N5 of FAD and concomitant reduction of the cofactor. A general base abstracts the substrate hydroxyl proton activating the C3-H bond for hydride transfer to FAD.

Priming the active site for hydride transfer.



- Conserved positively charged residue (Lys225) polarizes Asn119/Gly120 peptide bond and enables elongated N-H bond.
- Gly120-N-H interacts with the FAD-N5 lone pair of electrons to aid in formation of a tetrahedral N5 geometry.
- Orients the receiving orbital of FAD-N5 for optimal alignment with the substrate hydride to be transferred.



Golden, E. *et al.* (2017). *Scientific Reports.*