Life science using neutrons

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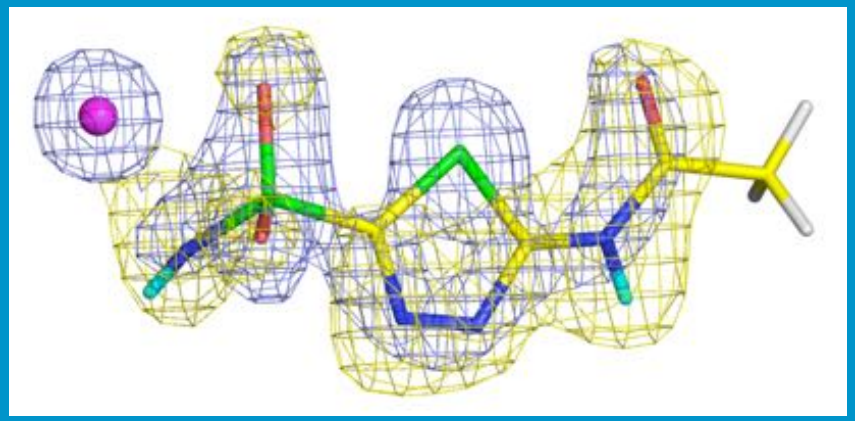
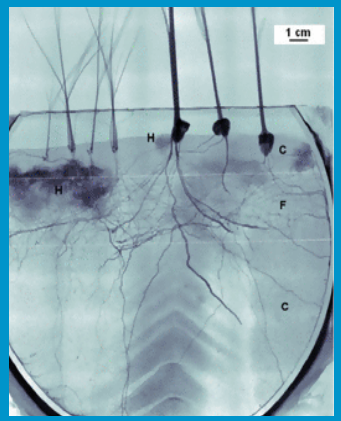
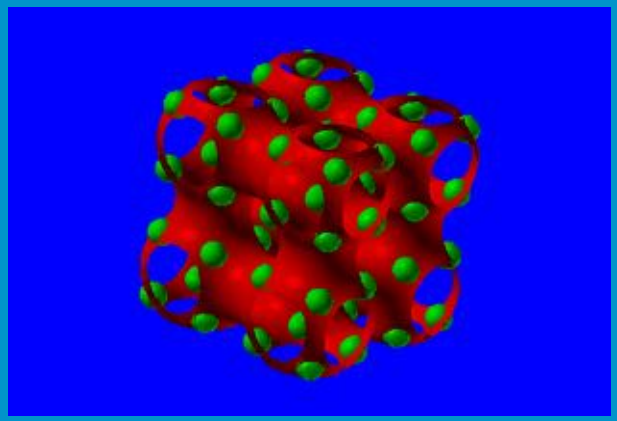




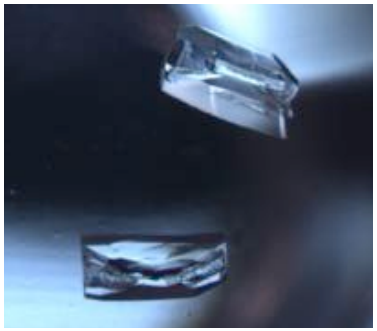
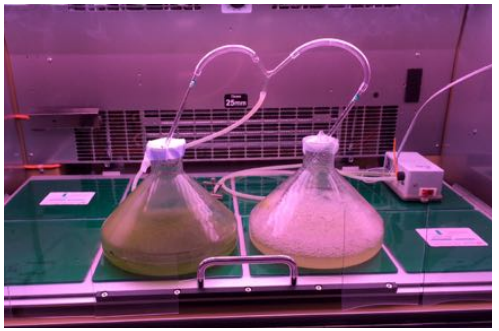
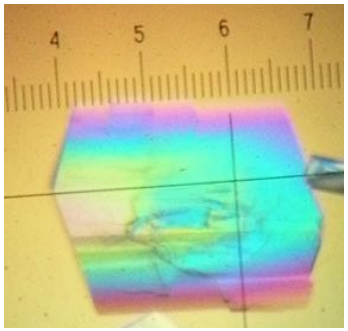


Dr. Zoë Fisher – Scientific Activities Division

Group Leader for Deuteration & Macromolecular Crystallization (DEMAX) Platform

Life science is a growing sector

- Life science and soft matter is a fast growing sector in neutron science.
- Historically it has been hampered by lack of dedicated instruments, support facilities, and by technical difficulties related to sample preparation (large samples, specific (D) labeling, D_2O toxicity).
- Most N-facilities have user support labs to overcome some of these issues (deuteration – chemical, biological)

Life science using neutrons as a tool

Several areas where neutrons are a very good – and complementary - tool:

- To determine the 3-D atomic (crystal) structures of macromolecule (eg. protein or DNA): the structure of the molecule is related to its function. Enzymes are bio-catalysts and “seeing” their insides allows us to understand how they work .
- Sometimes biological systems are large, complex, and dynamic (i.e. not well-ordered enough to form a crystal) – then we need small angle scattering to see larger “shapes” but at lower resolution. Dynamic data: complex formation, changes.
- For layers – like lipid bilayers, cell membranes we can use reflectometry to tell us about the thickness and behaviour of the membranes under a variety of conditions (temperature, pH, salt concentration etc.)
- Imaging with neutrons gives contrast that lets us “see” special features that are not possible with optical or X-ray techniques.

Life science research using neutrons

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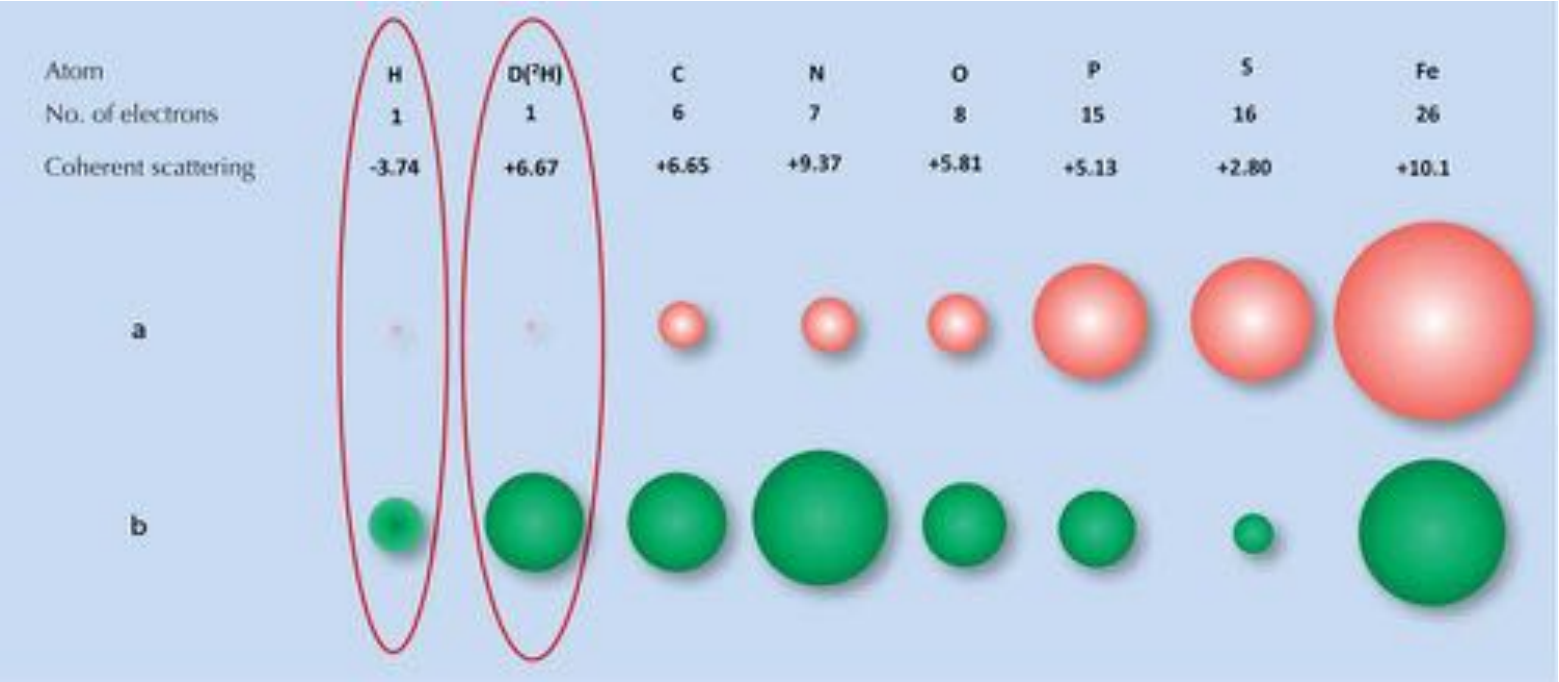
- Neutrons are a useful tool for the study of biological macromolecules:

- ❖ They have wavelengths appropriate to inter-atomic distances
- ❖ They have energies comparable to molecular motions
- ❖ They interact weakly with materials, and can penetrate into the bulk
- ❖ They are non-destructive at thermal energies
- ❖ Most important: *they see a completely different* ***contrast*** *compared to x-rays* (with appropriate isotope labelling).

Neutron & X-ray scattering cross sections

H atoms make up *~50% of atoms of biological macromolecules* (lipids, proteins, nucleic acids, carbohydrates).
Also: protein crystals are composed of 30-70% water.

Neutron scattering lengths for different atom types found in biological materials:



Purpose of deuteration depends on technique

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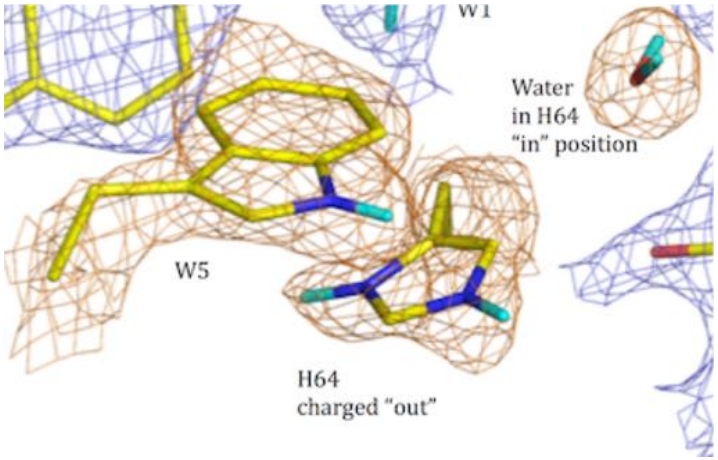
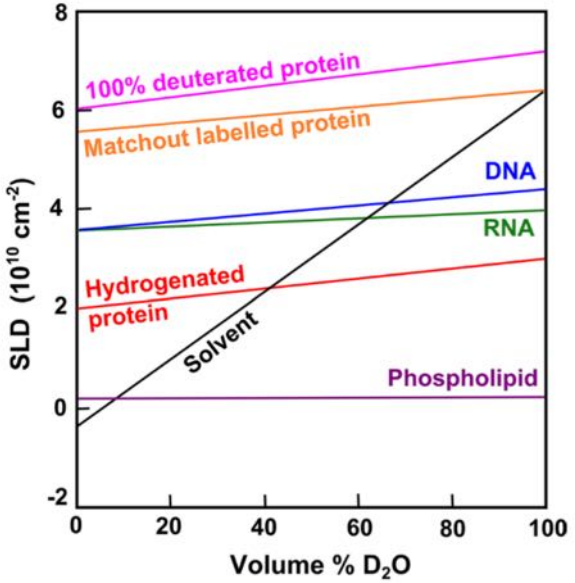
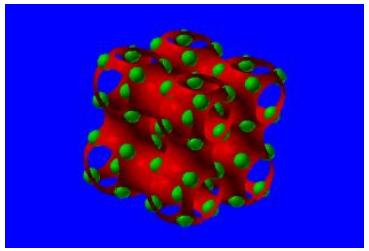
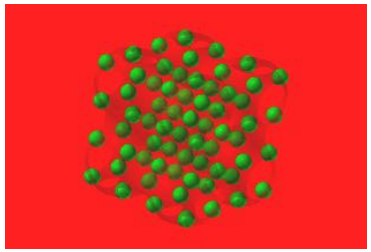
Localization of hydrogen atoms in macromolecular structures



Neutrons enable contrast variation through selective deuteration of materials (SANS, NR):







Contrast variation

When the monster came,
Lola remained undetected.

Harold, of course, was
immediately devoured.

The Far Side (G Larson)

Small angle neutron scattering (SANS)

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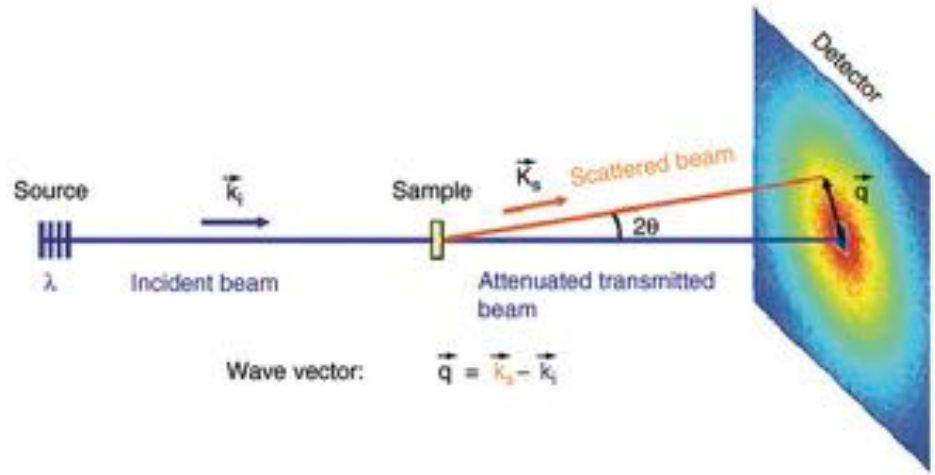
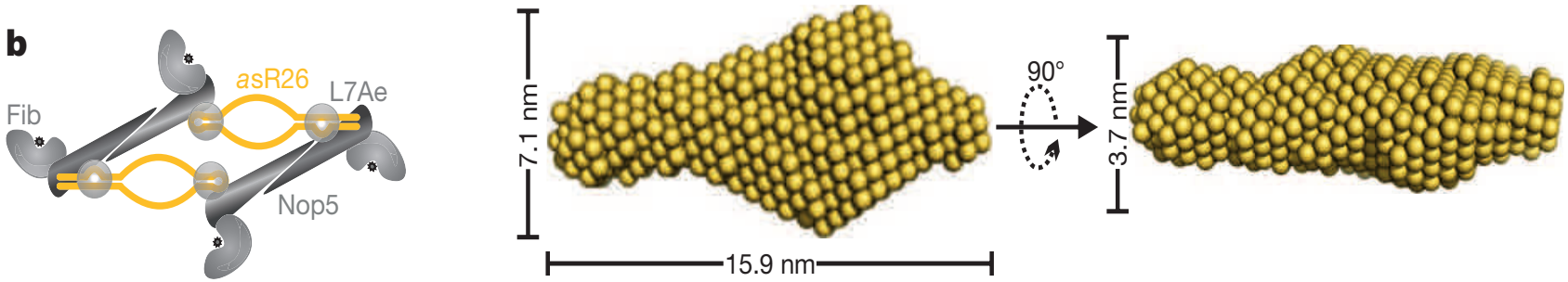
- SANS used to study materials in 1 - 1000 nm length scale (can be done on solids, solutions, powders, crystals).
- Large, complex biological systems that are dynamic and inherently flexible are very well suited to SANS.
- Employ contrast matching: selective perdeuteration strategies of macromolecules in combination with SANS measurements in different ratios of D_2O/H_2O.
- Mask parts of a large complex while highlighting molecules/areas of interest.
- Information: size, structure, and dynamic behavior of molecules in a complex while being able to change the environment.



Small angle neutron scattering (SANS)

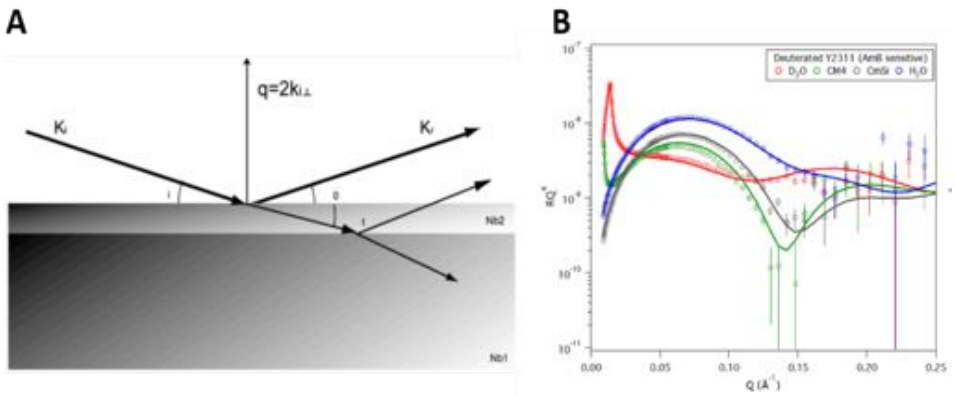


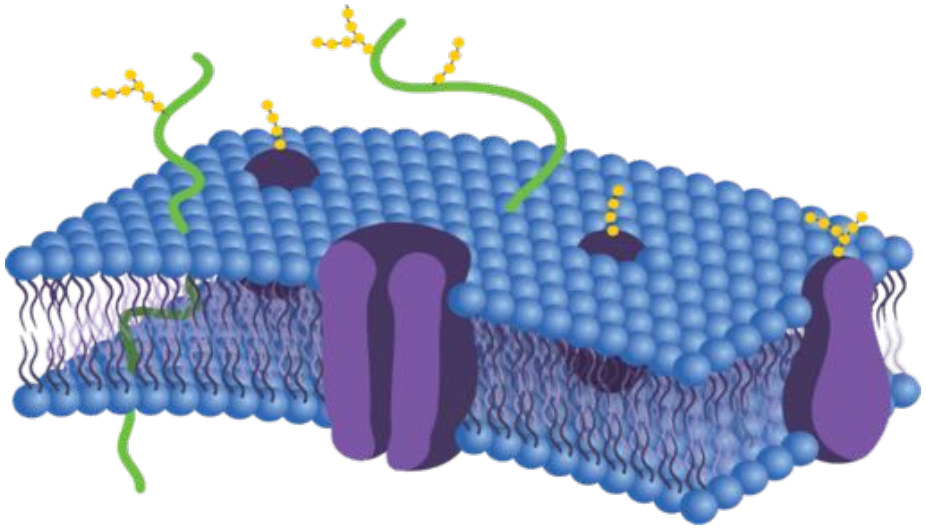
Scheme of a RNA:protein complex explaining the concept of contrast matching in SANS. The data were collected on perdeuterated RNA (yellow) and unlabeled protein (gray). Measurement were done in a mix of D_2O/H_2O that masked the (gray) unlabeled protein. The deuterated RNA scattering dominates the curve. Researchers were able to derive a model of RNA (yellow space-filled model) in the context of the complex from SANS data.

(From Lapinaite et al. (2013) *Nature* **502**, p.519)

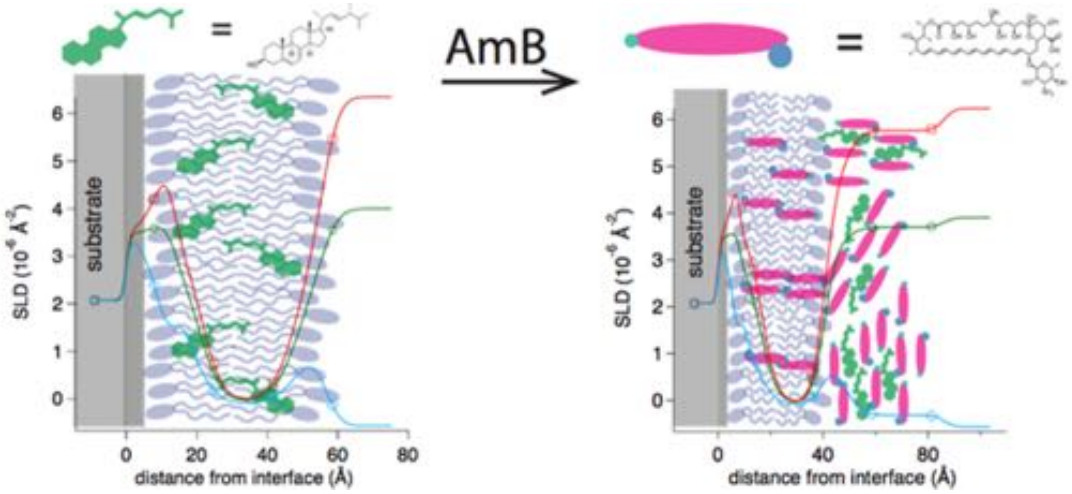
Neutron reflectometry (NR)

- NR is used to study thin films and interfaces on 0.2 to 100 nm scale.
- Measure nuclear scattering length density profiles perpendicular to the membrane surface.
- Gives information about the internal organization of the membrane/interface, thickness, evenness, composition - under different conditions
- Well suited to the study of lipids and biological membranes.





Neutron reflectometry (NR)

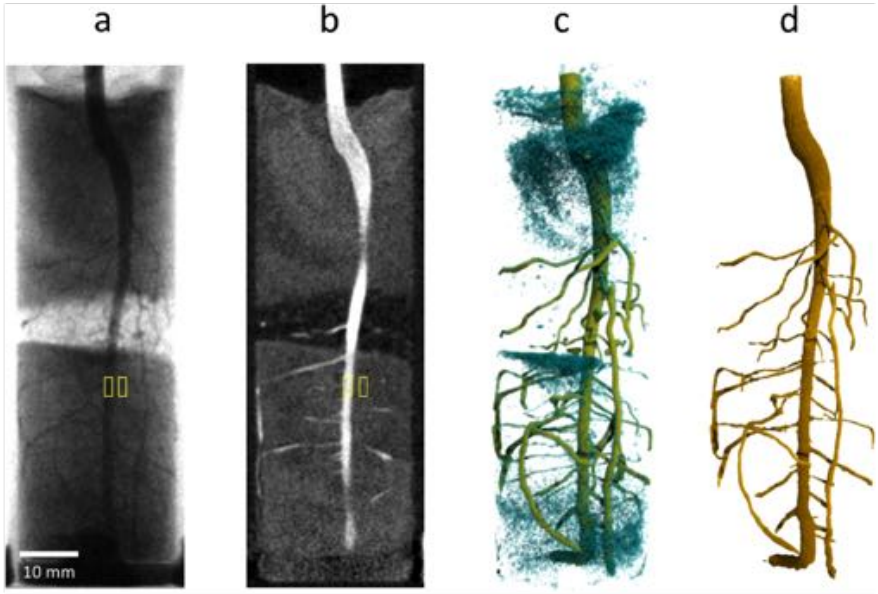


A diagram showing the orientation of potent antifungal drug AmB in relation to the layer thickness and composition of a lipid membrane. The experiments using NR were done on membranes with different lipid components and showed that the efficacy of the drug was strongly dependent on the lipid composition & that it extracts ergosterol. This could help explain why AmB causes toxic side effects in human but also how it works to disrupts fungal membranes.

[From de Ghellinck et al. (2015) *BBA Biomembranes* **1848**, p. 2317]

Neutron imaging

- Biological neutron imaging is not widely done.
- Neutrons have high penetrating power, do not cause radiation damage to biological samples, and are sensitive to the light atoms present in biological organisms.
- Neutron imaging studies looked at living plants and water uptake as it can penetrate the soil and and allow detailed studies of root structure and water uptake.
- Study significant influence that roots have on physical and chemical properties of soil.
- Implications for water use, efficiency, crop production.

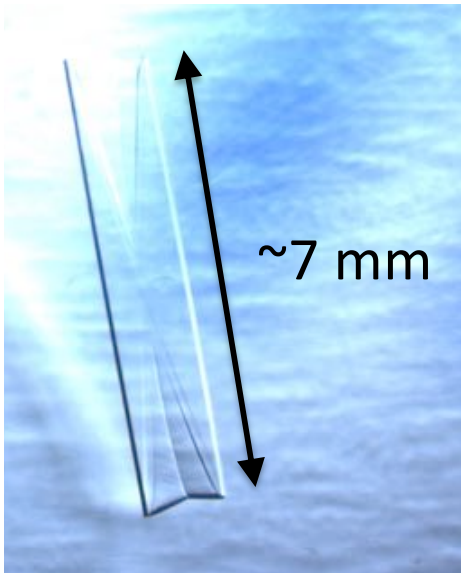


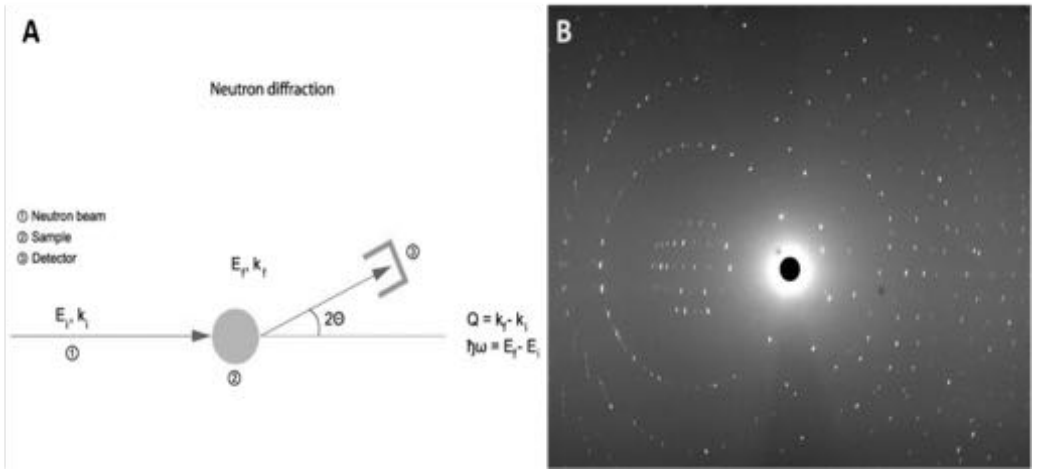
High-speed neutron tomography of lupine root system in sandy soil.

(Tötzke et al. (2019) "What comes NeXT? – High-Speed Neutron Tomography at ILL," Opt. Express 27, p. 28640)

Neutron protein crystallography (NPX)

- NPX is used to determine the atomic crystal structure of protein molecules
- Single crystal Bragg diffraction (can be monochromatic or Laue ~ 0.7 to 7 Å) – same as X-ray diffraction principle.
- Beyond data processing, structure refinement and modeling is similar to how it is done for X-rays.
- Important to remember: Data is weaker (poorer statistics) & takes longer to collect.





NPX gives the ability to “see” Hydrogen atoms – structural enzymology

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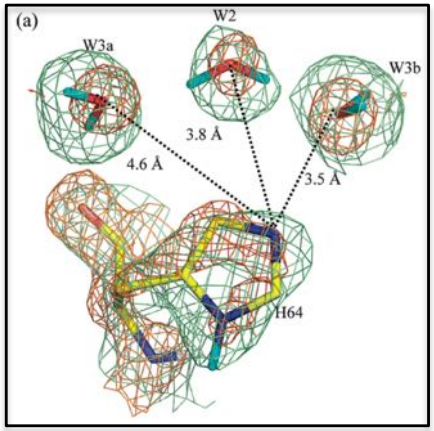
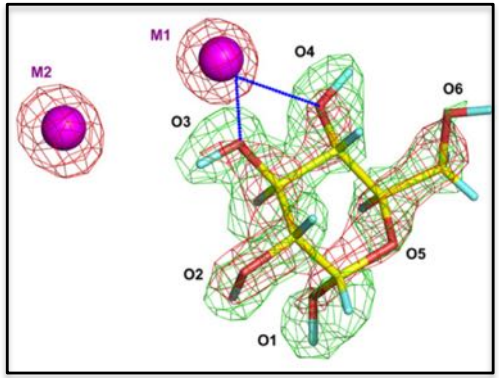
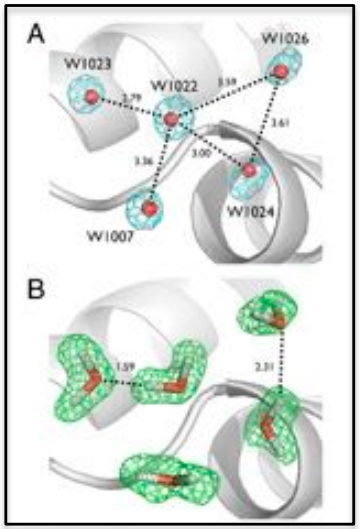
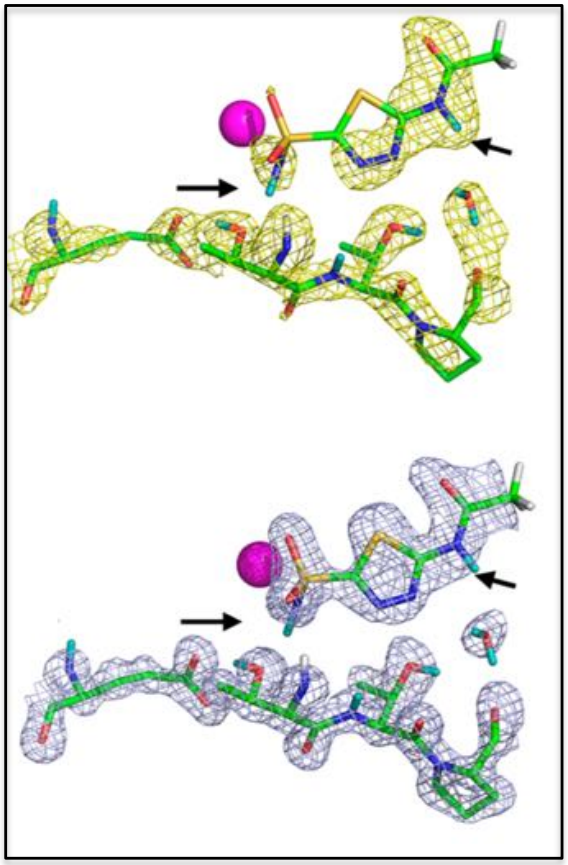
Protonation states, H-bonds, orientation of a.a. residues

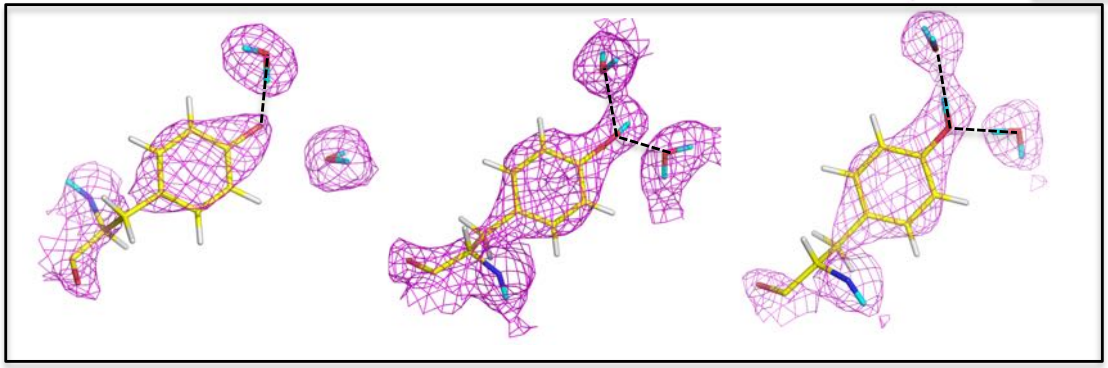
Observe ordered water structures; H-bonded networks











Drug/ligand/inhibitor/substrate binding interactions

Discerning solvent species (D_2O vs OD^- vs D_3O^+)

Biological deuteration – grow cells in deuterated media, extract materials

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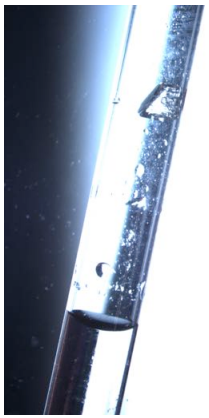
1) Partial deuteration: H/D exchange. Gives 25-30% exchange of labile H (aromatic or aliphatic H will not be replaced)
2) Partial deuteration in vivo – grow cells in recycled D_2O and unlabeled C source (e.g. glycerol). Gives 55-80% D incorporation.
3) Full deuteration (perdeuteration) – use 99.9% D_2O and labeled C. Gives ~99% D incorporation
4) Full deuteration (perdeuteration) – prepare rich media by growing algae n D_2O, prepare hydrolysate and use as media. Gives ~99% D incorporation.

1) 

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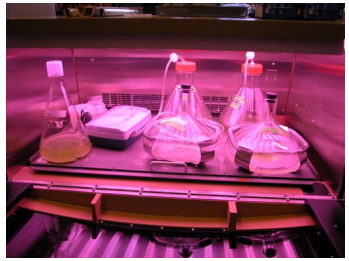
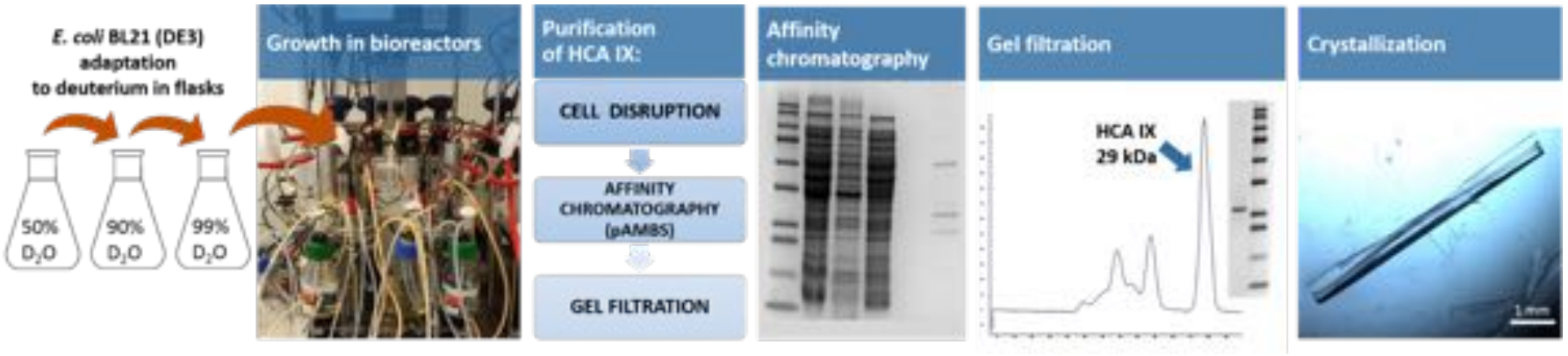
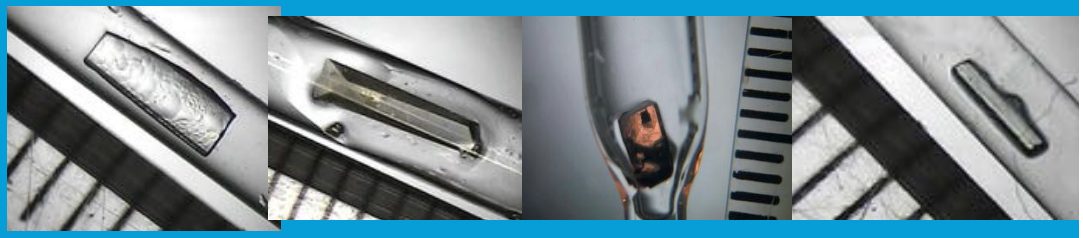
4) 

Biological deuteration (NPX)

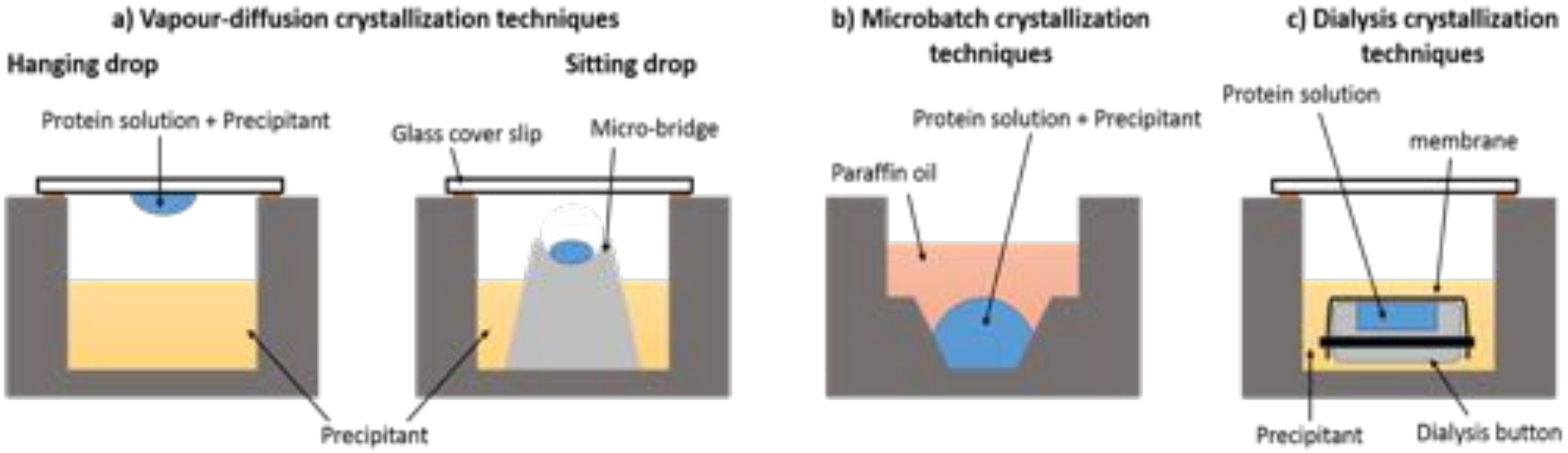
- Bacterial production of proteins is common and cost effective
- You can also grow yeast and algae in D_2O (extract protein, cell lysate, lipids, membranes)
- Need 10 mg – 1000 mg of pure protein/lipid for most neutron experiments (SANS, NSE, NR, NPX)
- For proteins the sample purified & crystallized in H_2O and then exchanged later to re-introduce exchanged D (labile Ds)



Crystallization for NPX

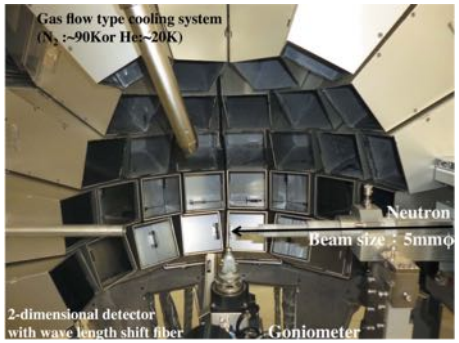


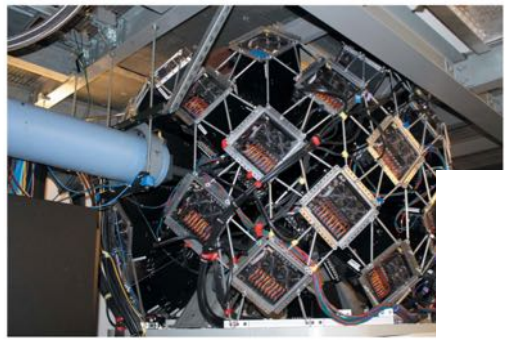
- Large single crystals (>0.5 mm^3 today, in exceptional cases ~0.1 mm^3)
- Methods commonly used: micro & macroseeding, crystal feeding, dialysis, counter-diffusion (capillary) crystallization, large volume sitting drop vapour diffusion, (macro)batch!
- All use *precipitants* of some kind ($(NH_4)_2SO_4$; PEG; NaCl; sodium citrate) to take water away from protein to decrease solubility, in an *ordered* way.

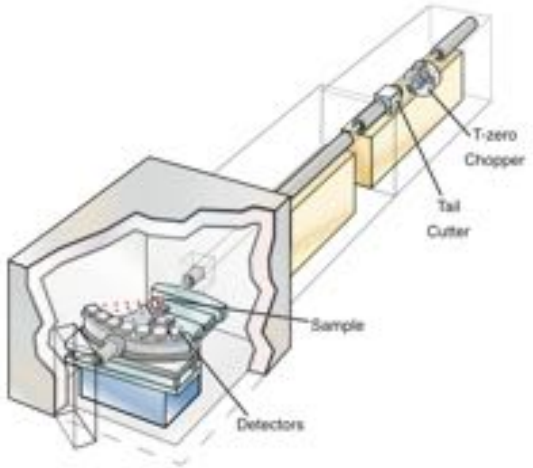


NPX beamlines
[~142 crystal structures/~155 000]

- A few instruments in the world.
- 3 reactor-based: LADI-III (ILL, Grenoble FR); Biodiff (FRM-II, Munich DE), Imagine (HFIR @ ORNL, Oak Ridge TN, USA)
- 2 spallation based: iBIX (JPARC, Tokai, Ibaraki JP), MaNDi (SNS @ ORNL, Oak Ridge TN, USA), *retired: PCS (Lujan @ LANL, Los Alamos NM. USA)*







NMX will be the world's most powerful neutron protein crystallography instrument

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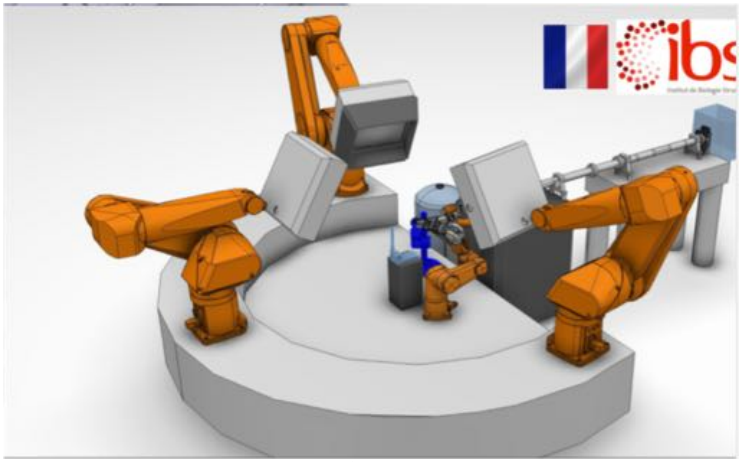
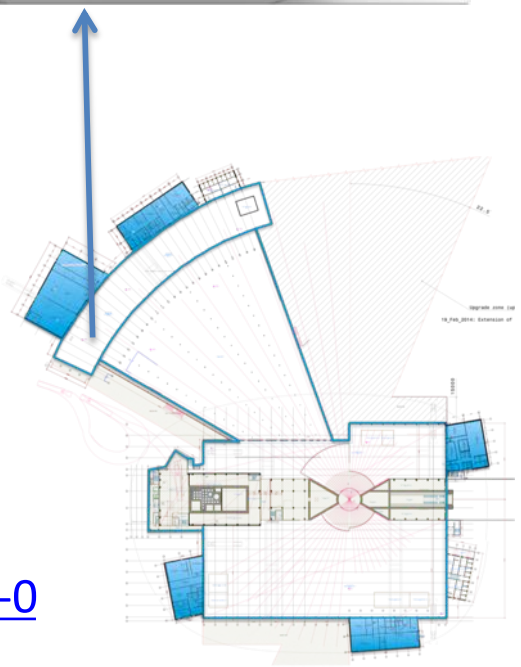
- Much smaller crystals needed (0.2 mm^3 vs 1.0 mm^3)
- Data collection will be faster (days vs. weeks)
- Larger unit cells possible (300 Å vs. 150 Å)
- By making it feasible to collect data from multiple crystals in a few days (eg mutants, or different complexes), NMX will enable systematic studies for structural enzymology, rational drug design.





https://europeanspallationsource.se/macromolecular-diffractometer-0

The ESS is building several instruments, support labs, and sample environments with the life scientist in mind – we expect this user community to grow in the future.

Thank you for you attention! Questions?