OAK RIDGE National Laboratory

Crystallography of Dynamically Polarized Proteins with Polarized Neutron Beams

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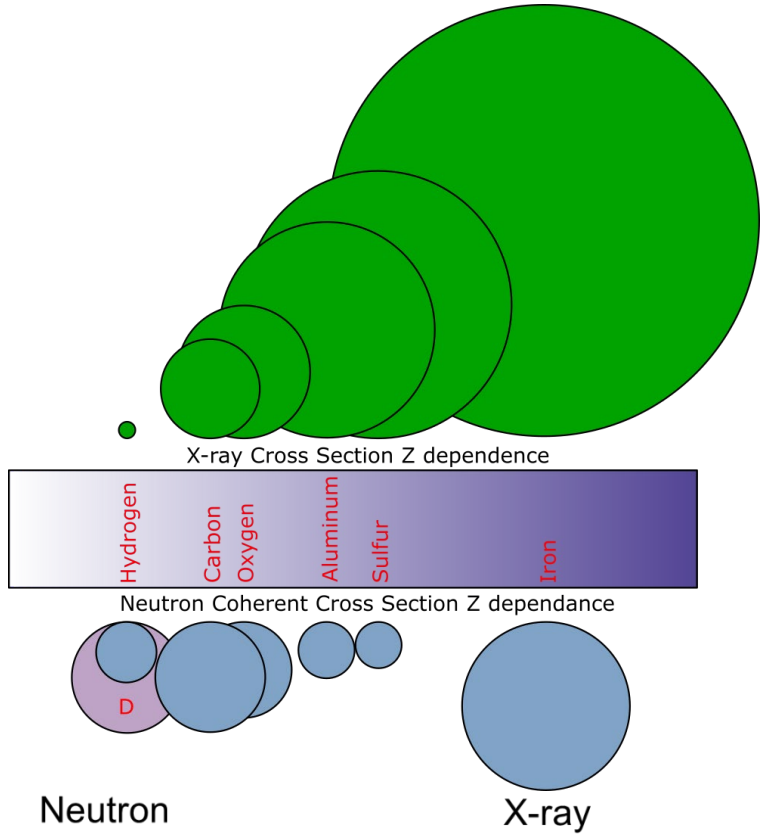
Neutron Technologies Division

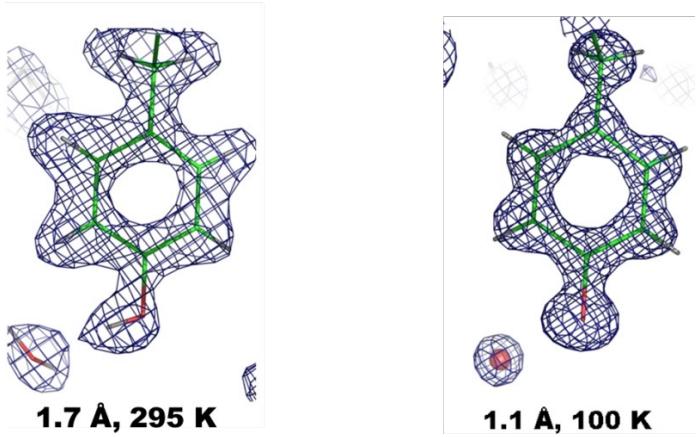
ORNL is managed by UT-Battelle, LLC for the US Department of Energy

U.S. DEPARTMENT OF ENERGY

Motivation:
Neutron protein crystallography

- Usually, macromolecular crystallography uses X-ray facilities to measure molecular structure
  - Modern light sources have incredibly high flux
- Using neutrons for crystallography has pros/cons:
  - Sensitivity of the neutron cross section to lighter elements (especially hydrogen)
  - Large incoherent cross section of hydrogen
  - Sensitivity to isotopes (deuteration is very powerful *where it is possible)*
  - Comparatively low flux





Gardberg *et al.* (2010) *Acta Cryst.* D**66**:558-567

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- Neutron Scattering is a unique experimental tool for determining the location of key hydrogen atoms and water molecules in biological macromolecules


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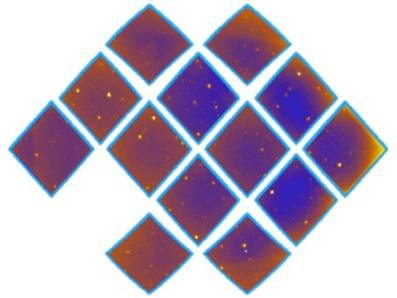
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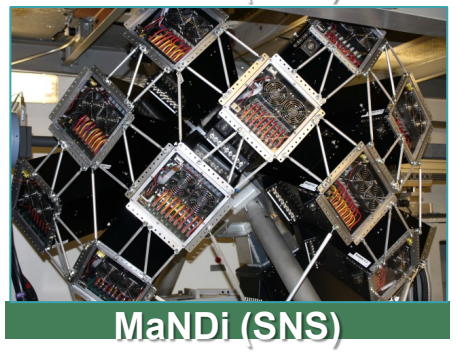
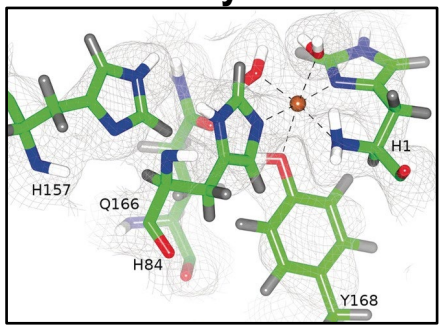
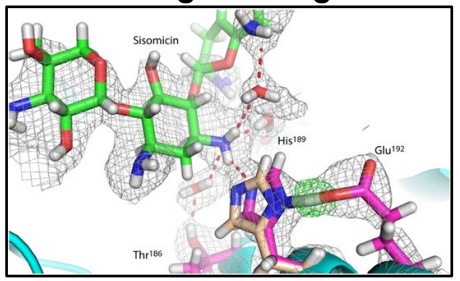

MaNDi (SNS)

Catalysis



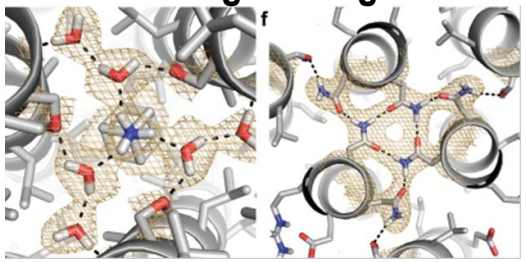
O'Dell, Ange Chem. 2017

Drug binding



Kumar, Sci Adv. 2018

Bioengineering



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- Neutron Scattering is a unique experimental tool for determining the location of key hydrogen atoms and water molecules in biological macromolecules but use is slowed by requirement for huge crystals. Which can be challanging/ or impossible to produce

X-ray Crystals <0.001mm^3
Neutron Crystals >0.1mm^3


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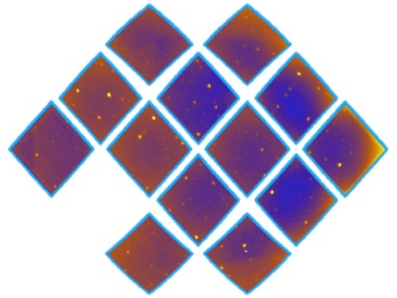
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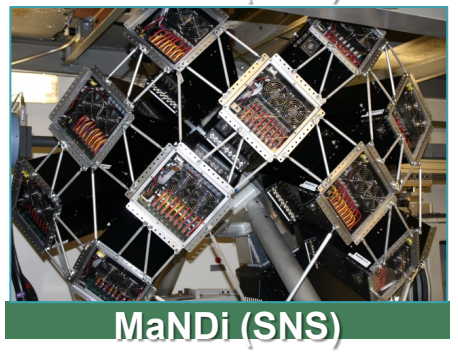
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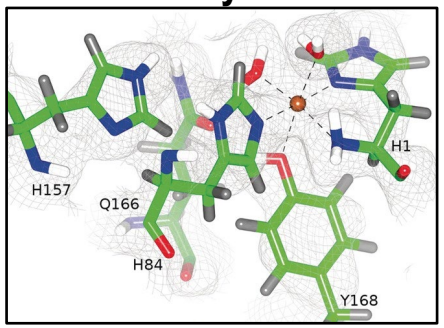

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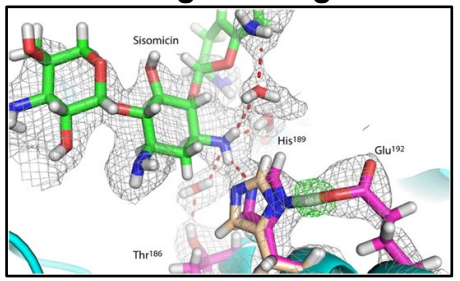

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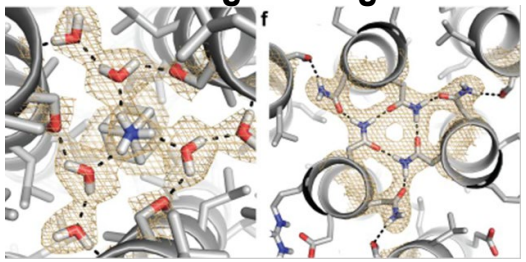


Spin Dependence of Neutron Scattering

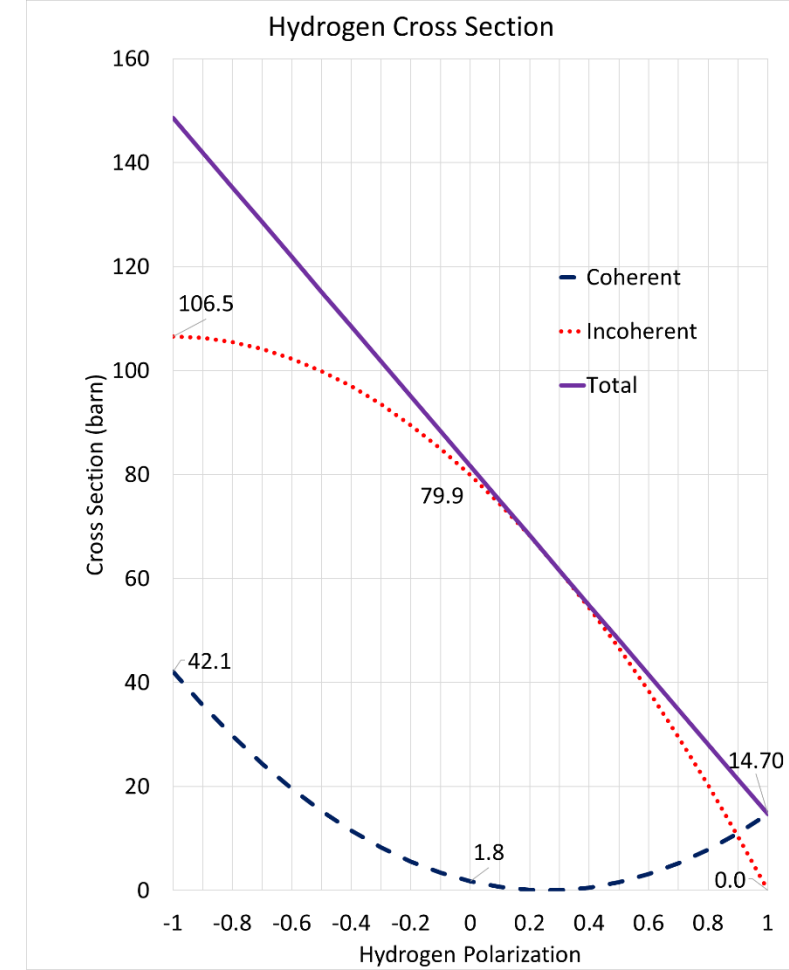
- For a lattice of identical atoms with non-zero spin, the incoherent and coherent cross section for neutron scattering has a dependence on the spin alignment of the neutron and the struck nucleus
- Control over spin orientation gives control over scattering.
- Neutron Polarization is well developed
  - Supermirror polarizers
  - 3He filters
- Nuclear Polarization is more challenging

$$\left(\frac{d\sigma}{d\Omega}\right)_{inc} = \frac{b^2}{4}\{I(I+1) - pPI - P^2I^2\};$$

$$\left(\frac{d\sigma}{d\Omega}\right)_{coh} = \left\{b_0^2 + bb_0IpP + \frac{I^2P^2b^2}{4}\right\};$$

Spin Dependence of Neutron Scattering from Hydrogen

- Hydrogen is a special case
  - The spin dependence of the hydrogen cross section is very large
  - Looking for hydrogen locations is a primary motivation for Neutron Protein Crystallography
- Nuclear incoherent scattering can be removed entirely (true for any nucleus)
- Coherent scattering can be increased by a factor of 7 (or 20)
- An increase in signal to noise enters **squared** into the calculation figure of merit
  - Factor of 10 in signal to noise is a factor of 100 in flux/sample size/data collection time
- The hydrogen nucleus is *very* polarizable via Dynamic Nuclear Polarization, DNP



Coherent, incoherent and total scattering cross section of hydrogen as a function of the proton polarization for fully polarized neutrons.

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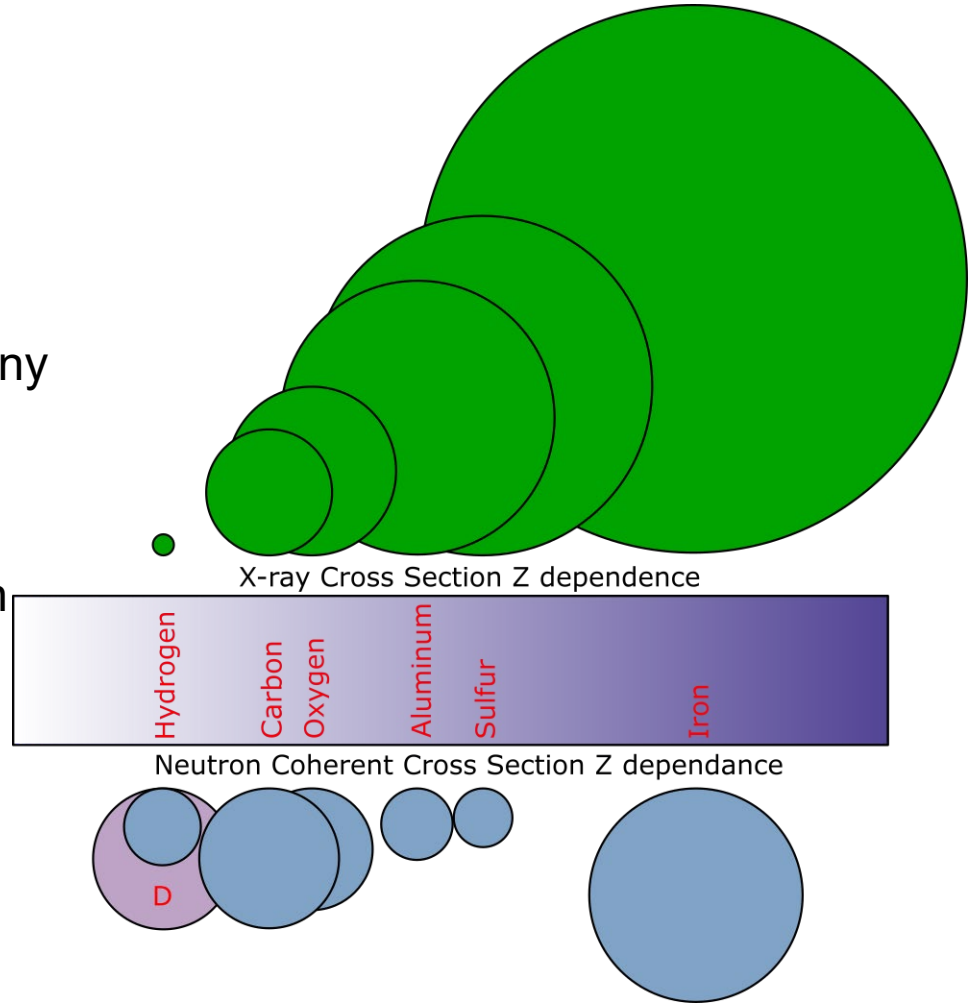

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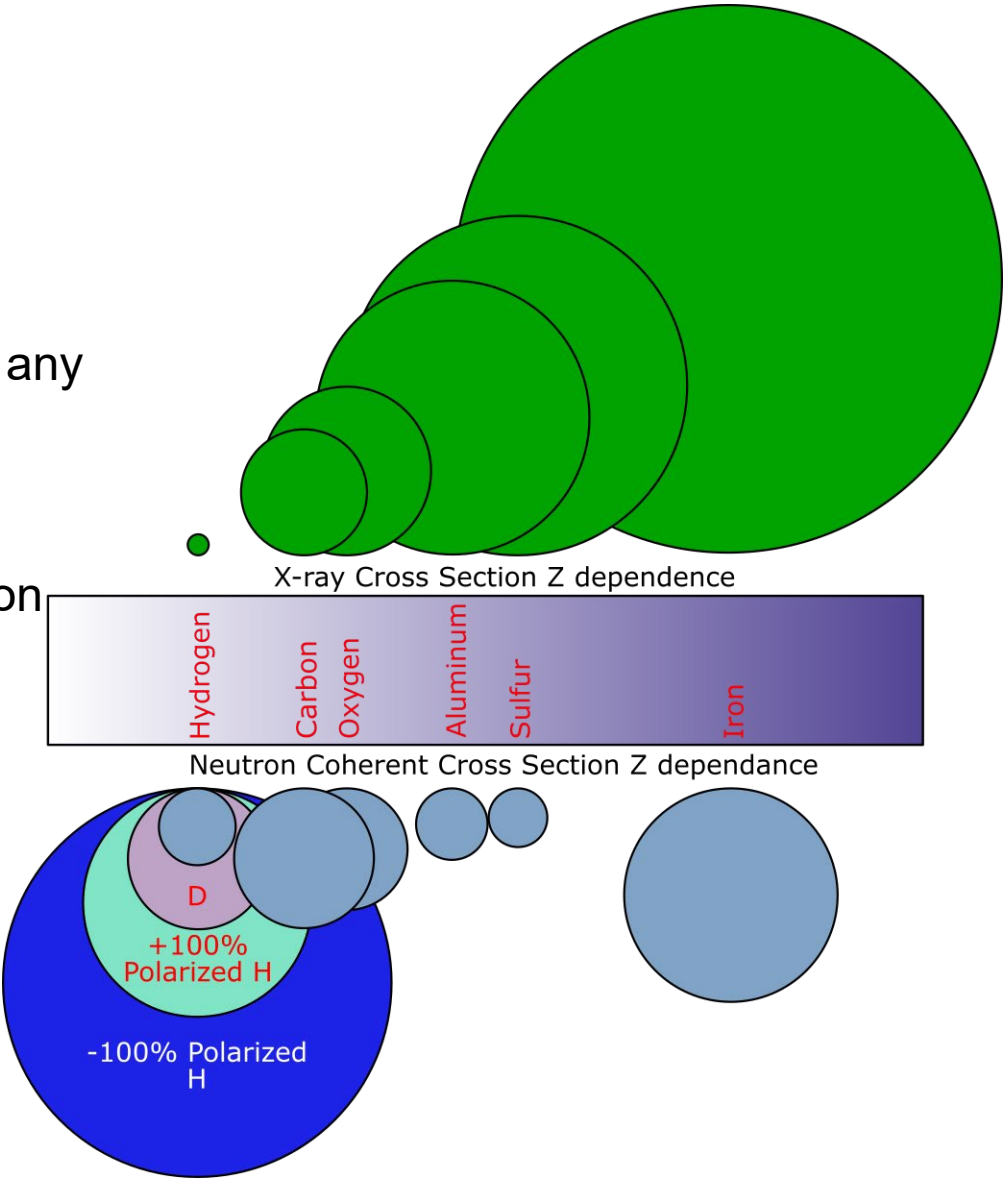
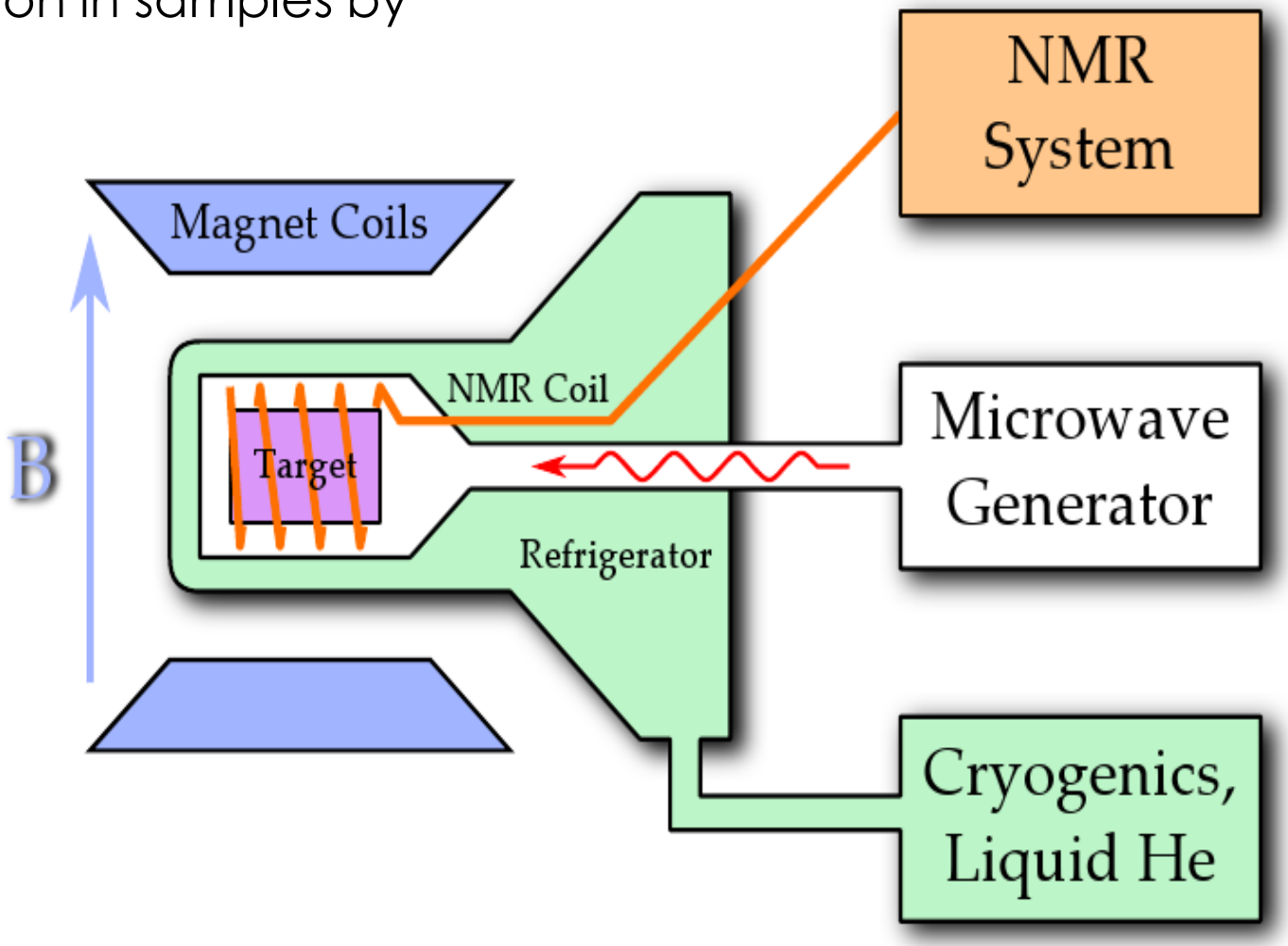


DNP

- Produces very high nuclear polarization in samples by using a combination of:
  - High Magnetic field
    - Superconducting magnets
      - 5T or 2.5T being the most common
  - Low Temperature
    - 1K using a ^{4}He evaporation refrigerator
    - 300mk using a dilution refrigerator
  - Microwaves
    - Near the electron Larmor frequency
  - Prepared sample material
  - NMR system for polarization measurement



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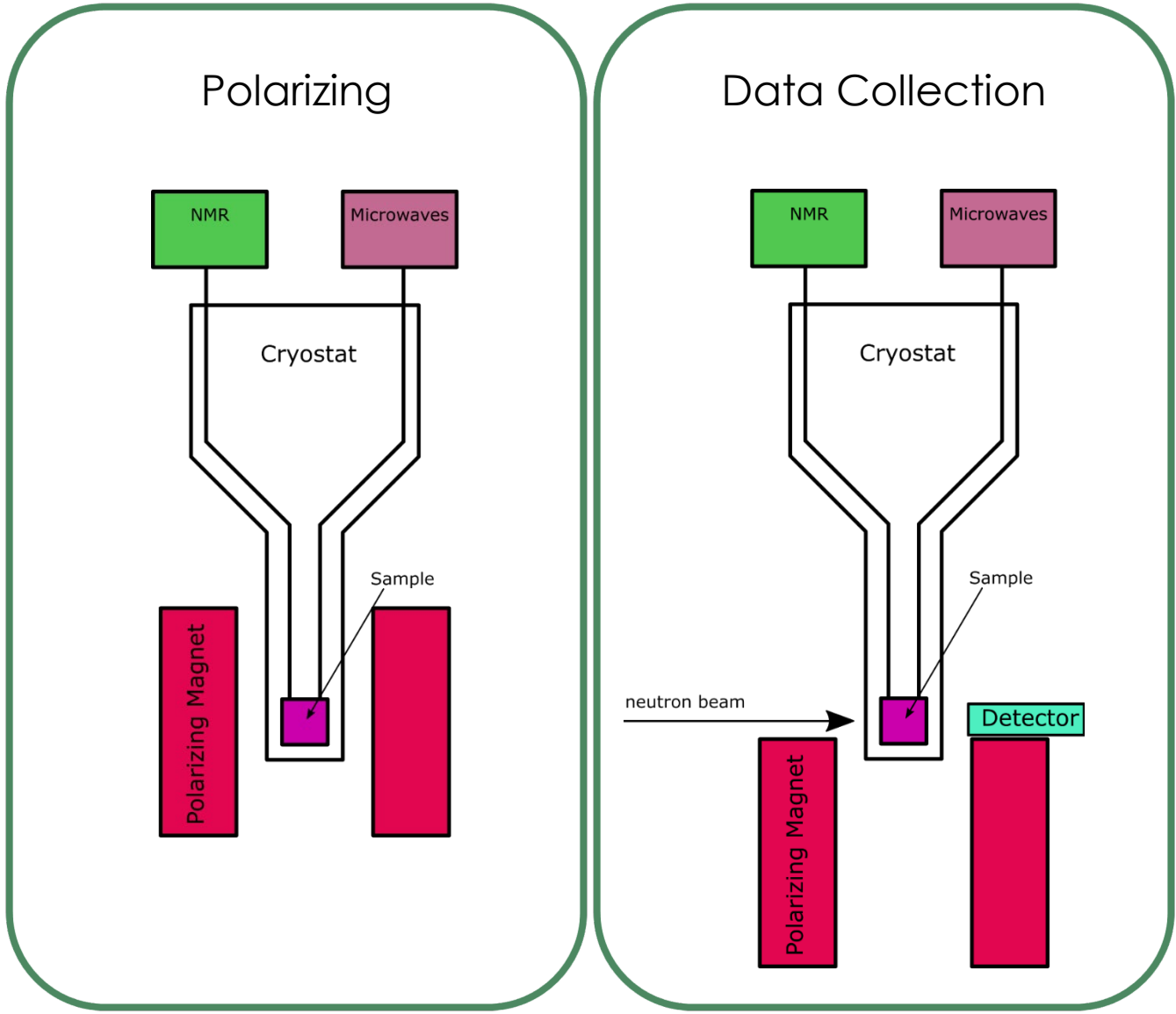
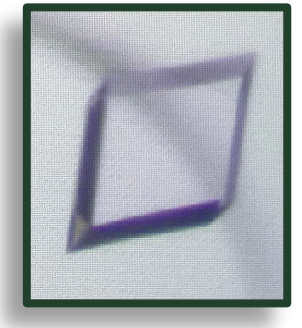
DNP – Frozen Spin Mode

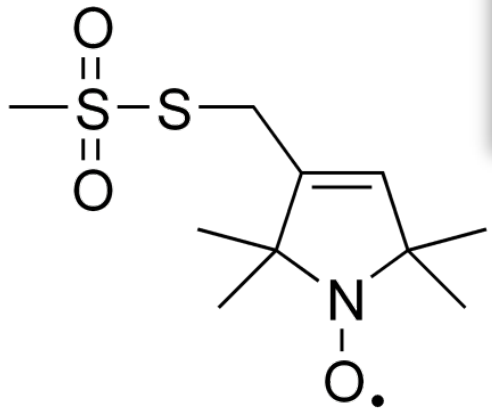
- Starts out as a normal DNP system
  - Spins and polarized through DNP at a high field and reasonably low temperature (300mK)
- Once the target is polarized, the temperature is greatly decreased
  - Final operating temperature ideally <100mK
- Nuclear spin relaxation time can become very long (tens to thousands of hours)
  - Microwaves no longer necessary
- Magnet can be moved out of beam
  - No Need for a custom, split magnet
  - Need dilution refrigerator
  - Time dependent decay to poalrization



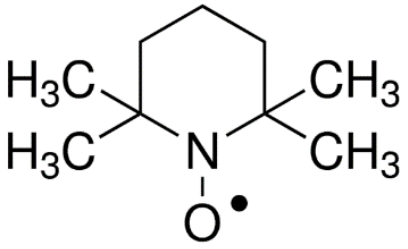
Paramagnetic Labeling in Protein Crystals



- Strategies
  - Site specific
  - Non-specific
- Site specific labeling (Intrinsic)
  - Mutagenesis for intrinsic/site specific labels
  - Spin-labeled mutants T4 lysozyme constructed, expressed, crystallized
  - X-ray and first neutron structures determined
- Non-specific
  - Crystals soaked in a solution with paramagnetic label
  - Crystals grown in a solution with paramagnetic label



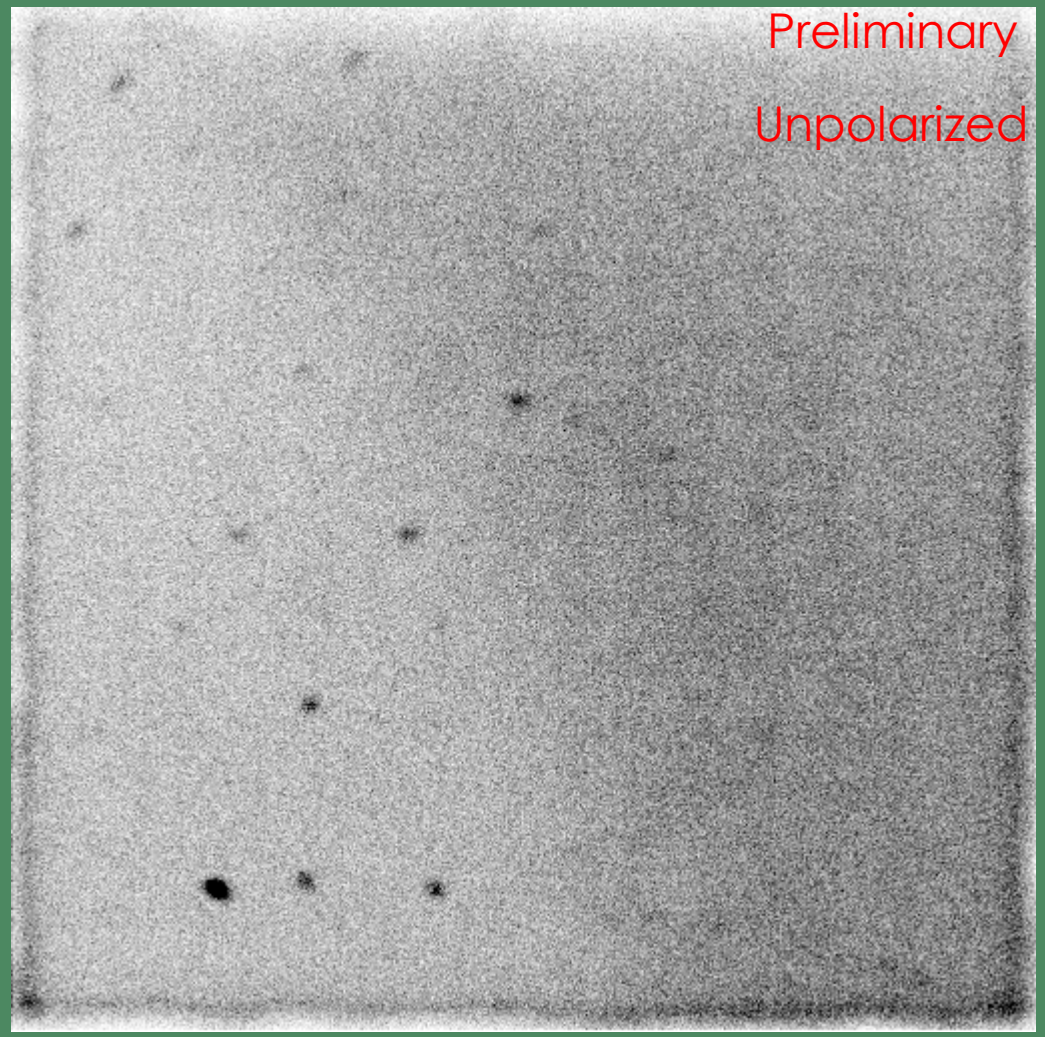
MTSL



TEMPO

T4 Lysozyme Results

- Doped with TEMPO
- Large crystals
  - ~0.900 mm on edge
- Single prototype anger camera
- Short hold times in “frozen spin” mode
  - ~120-240 min T_1
  - Low temperatures
    - ~200 mK
- Measured diffraction pattern change
- Enhancements of 2-3 in integrated diffraction pattern for anti-aligned spins
  - The enhancement of individual reflections depends varies depending on the relative contribution of hydrogen
- Consistent with maximum polarizations of around 50%



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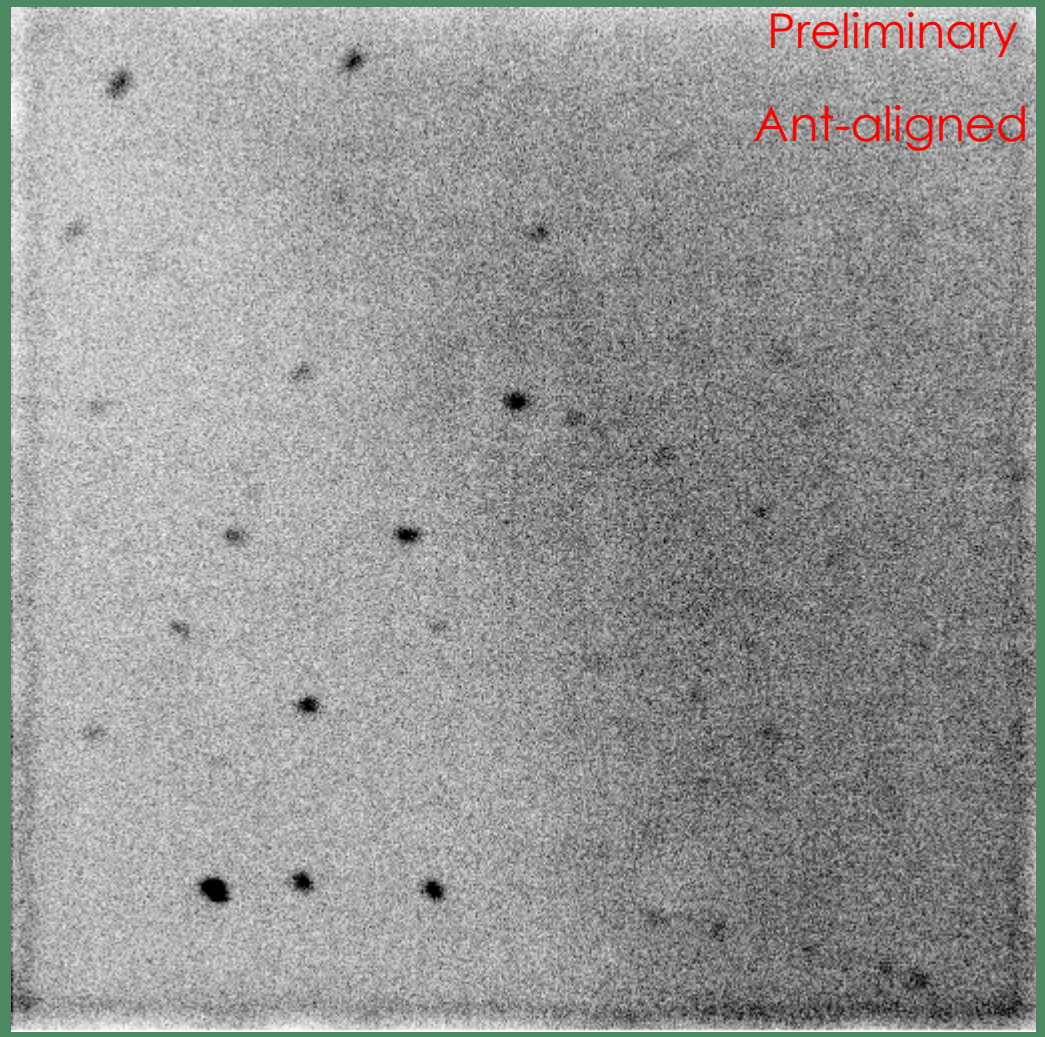

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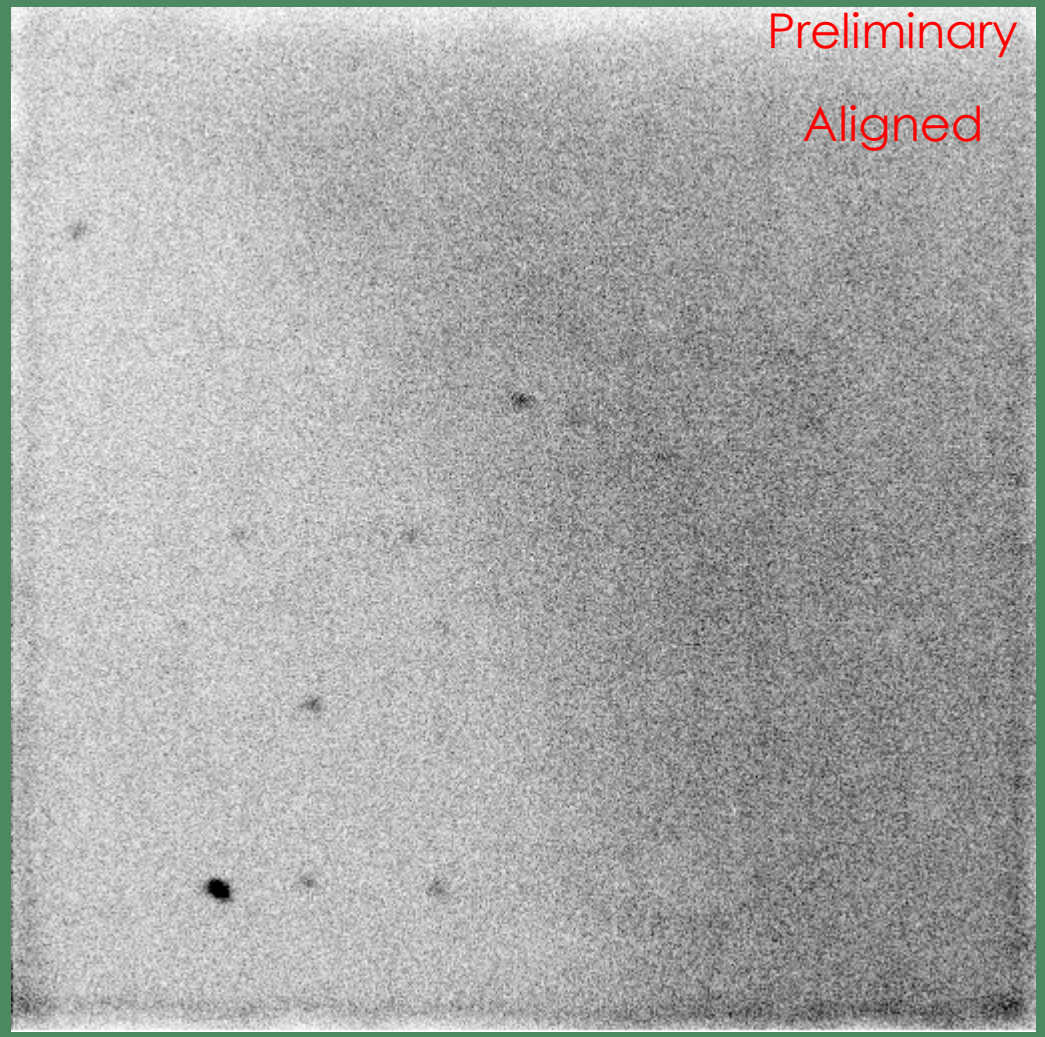

T4 Lysozyme Results

- Measured diffraction pattern change
- Indexed and integrated using LAUEGEN (CCP4)
- Enhancements of 2-3 in integrated diffraction pattern for anti-aligned spins
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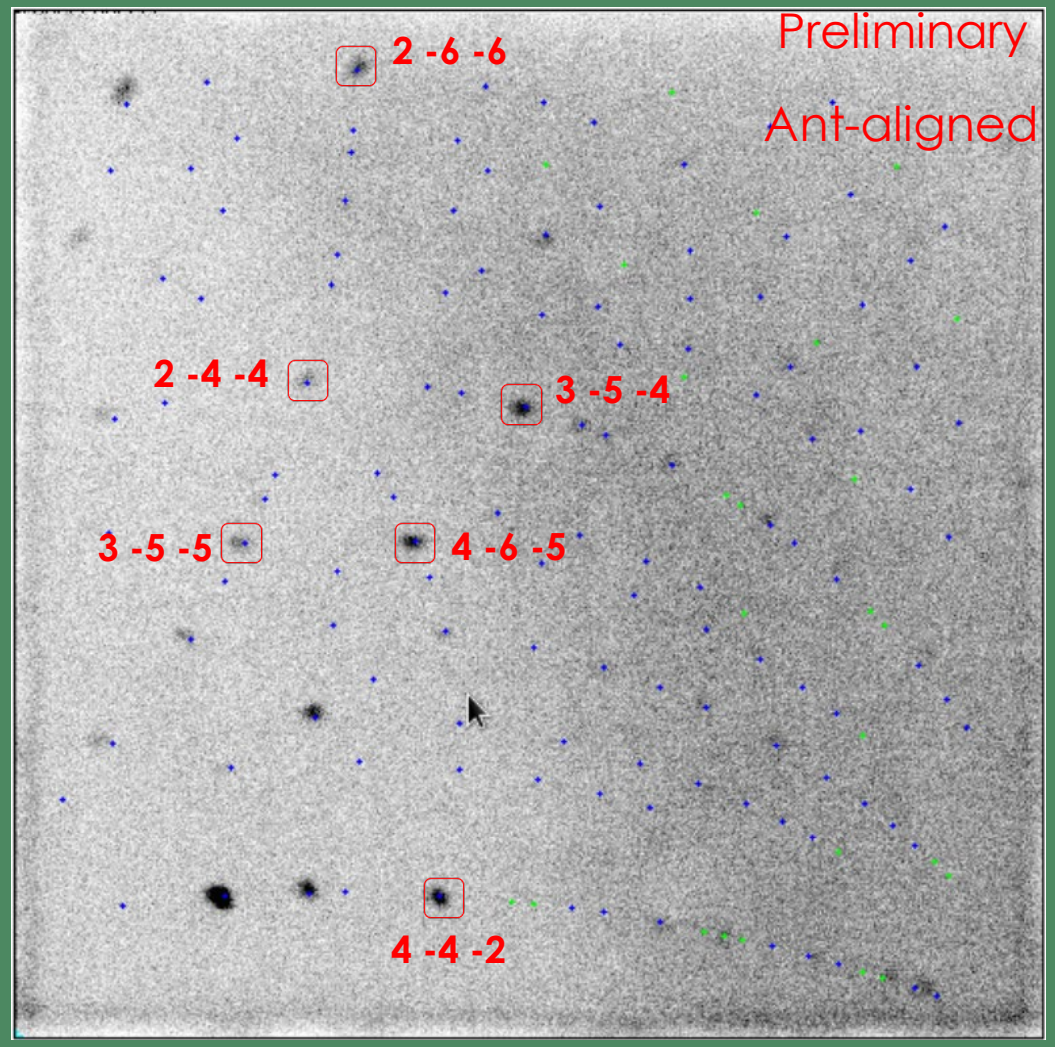
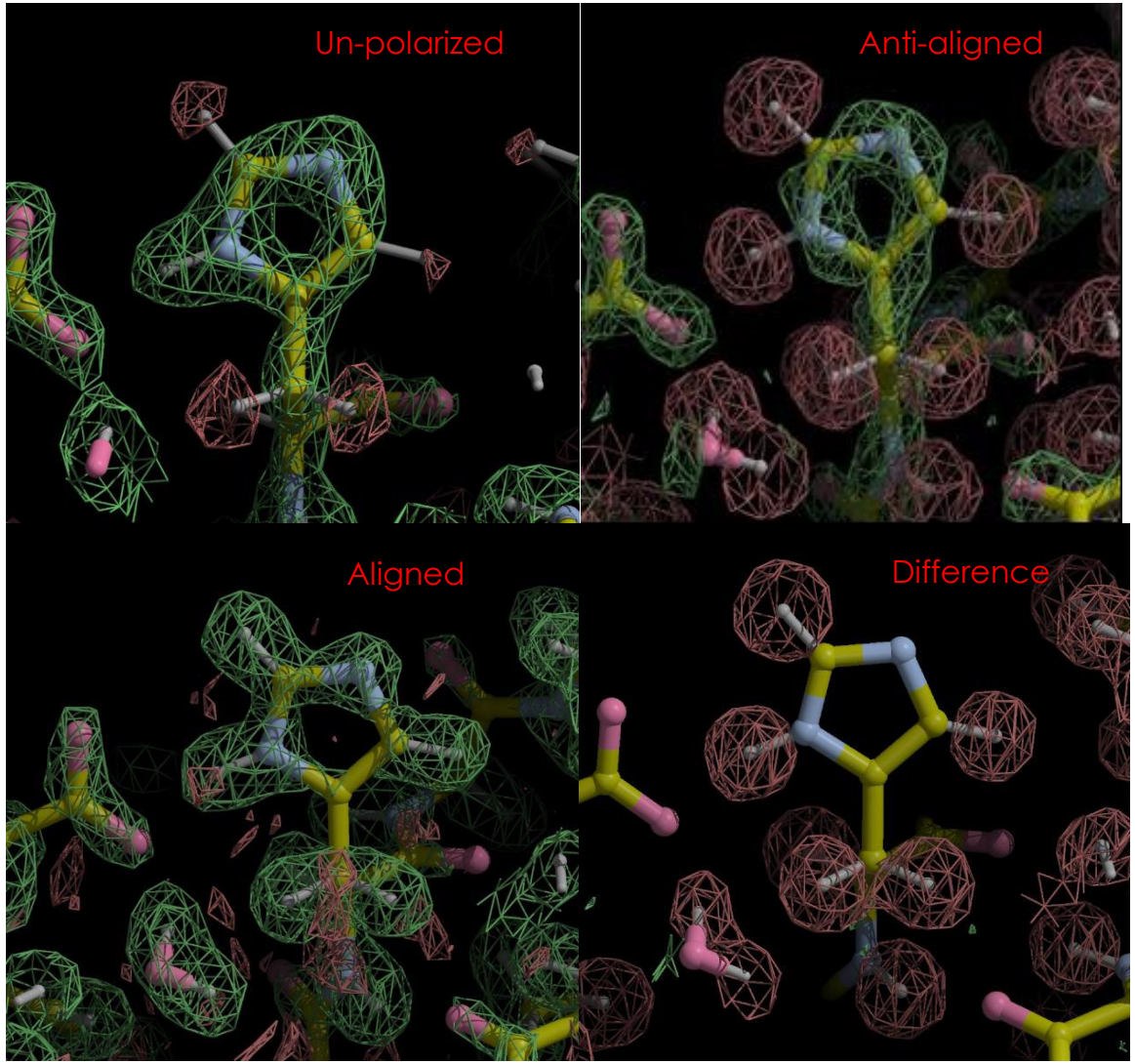
HKL	UnPolarized	Pol-Positive	Pol-Negative
2 -4 -4	170 (25)	235 (39)	28 (24)
4 -4 -2	549 (58)	1064 (82)	250 (38)
3 -5 -4	479 (37)	833 (48)	405 (32)
3 -5 -5	255 (28)	265 (41)	201 (28)
4 -6 -5	406 (31)	849 (48)	167 (32)
2 -6 -6	152 (28)	592 (27)	162 (15)



Difference Measurements

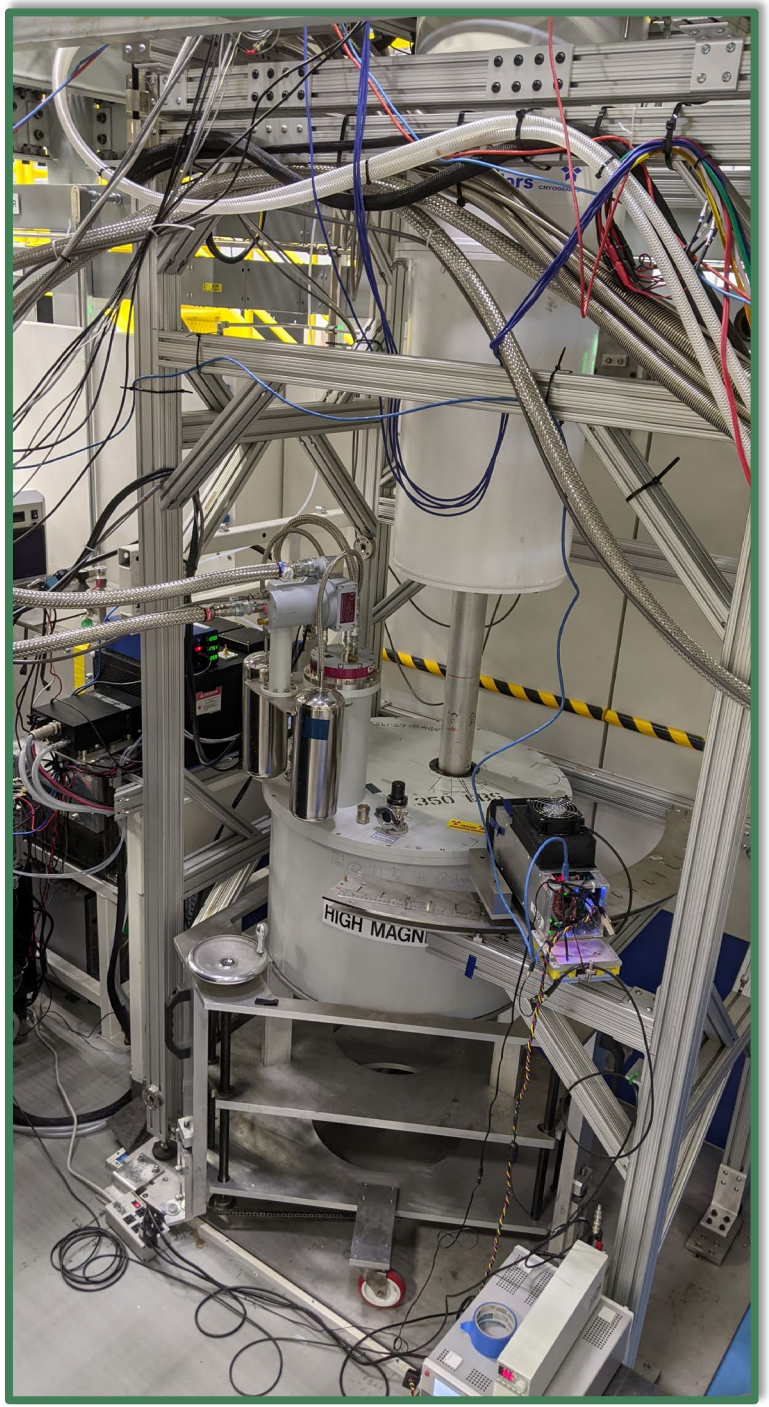
- For hydrogen $b = -3.7423 + 14.56 \times P_n \times P_H$
- Comparison to Deuteration
  - The unpolarized coherent scattering length of hydrogen is -3.74 fm
  - The unpolarized coherent scattering length of deuterium is 6.674 fm
- Polarization
  - 100 % positive polarization: 10.82 fm
  - 100% negative polarization: -18.3 fm
- All that is required is to change the microwave frequency to change polarization sign
  - Field remains constant
  - Temperature remains constant
- Adiabatic Fast Passage or neutron spin flipper can reverse polarization more quickly
- Only thing that changes is the cross section for the nuclei, and that changes in a predictable manner



Calculated maps, A) un-polarized; B) fully polarized aligned; and C) anti-aligned, 4) difference maps (simulated at 1.5 Å)

Recent Results

- New samples tested
  - Different proteins (MalE2)
  - Different dopants (co-crystalized)
  - Both worked!
- "Fast" flipping of neutron spin
  - In-situ 3He filter was used as polarizer
  - Adiabatic fast passage of 3He used to flip polarization
  - Completely Isomorphic difference measurements
  - Transmission monitor
  - Poor 3He performance due to fringe field
  - Can be improved by moving polarization upstream
- *almost Ready to be redeployed



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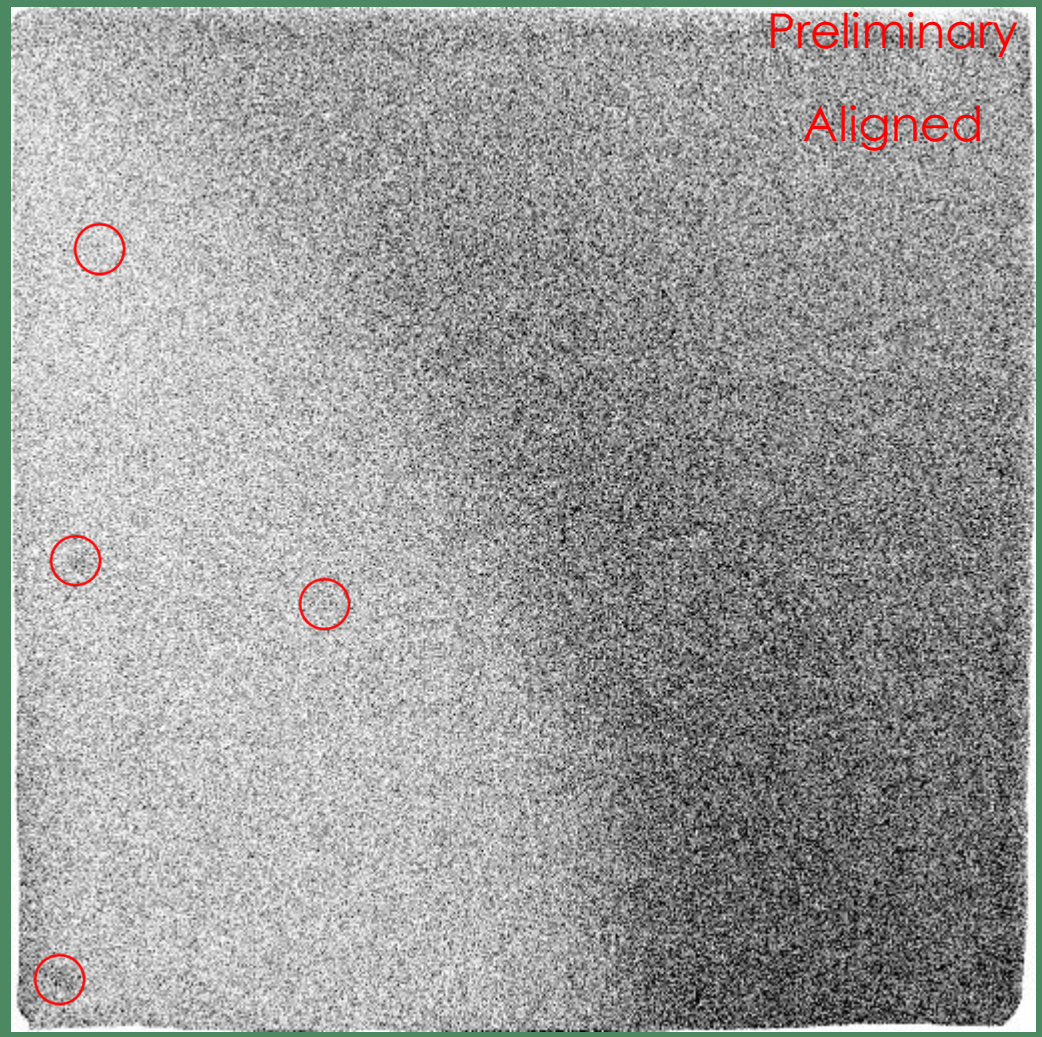

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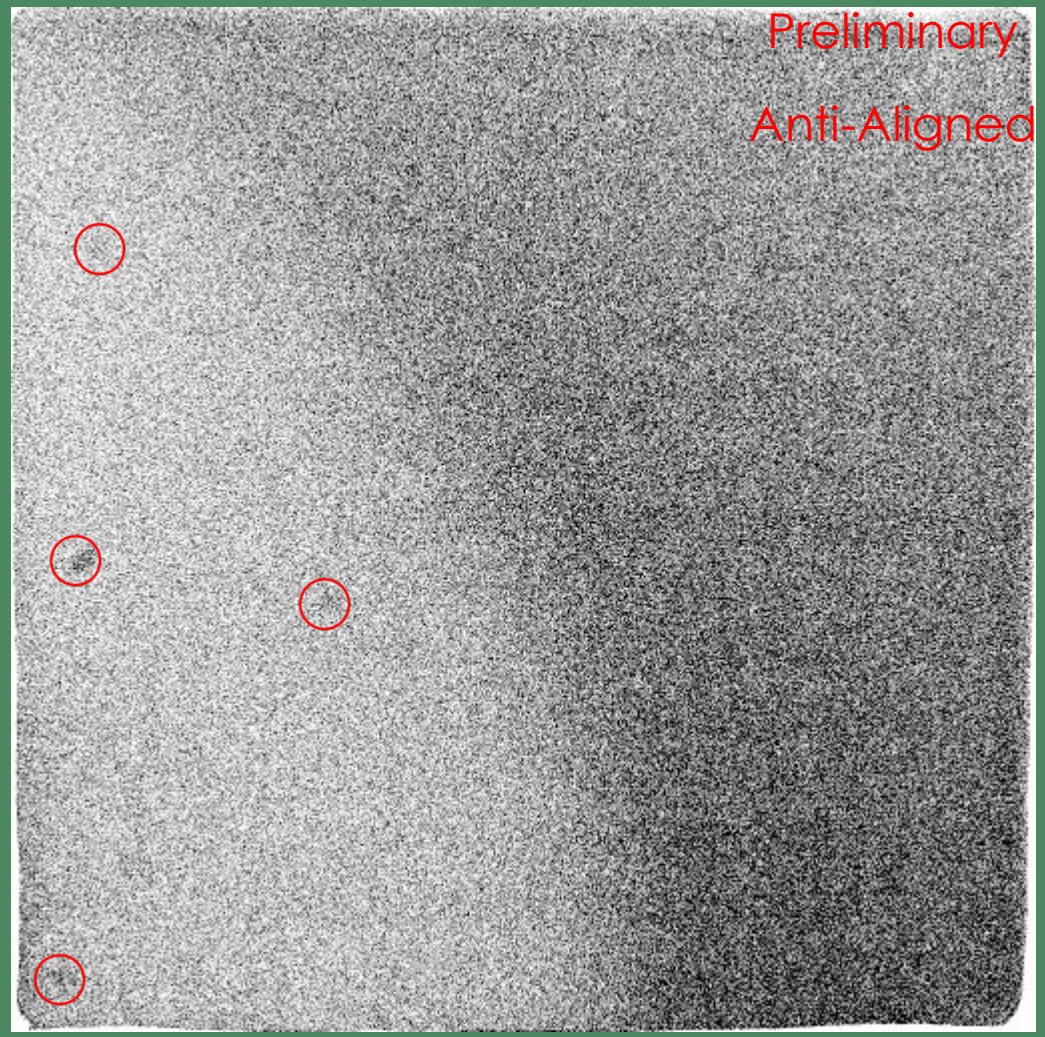
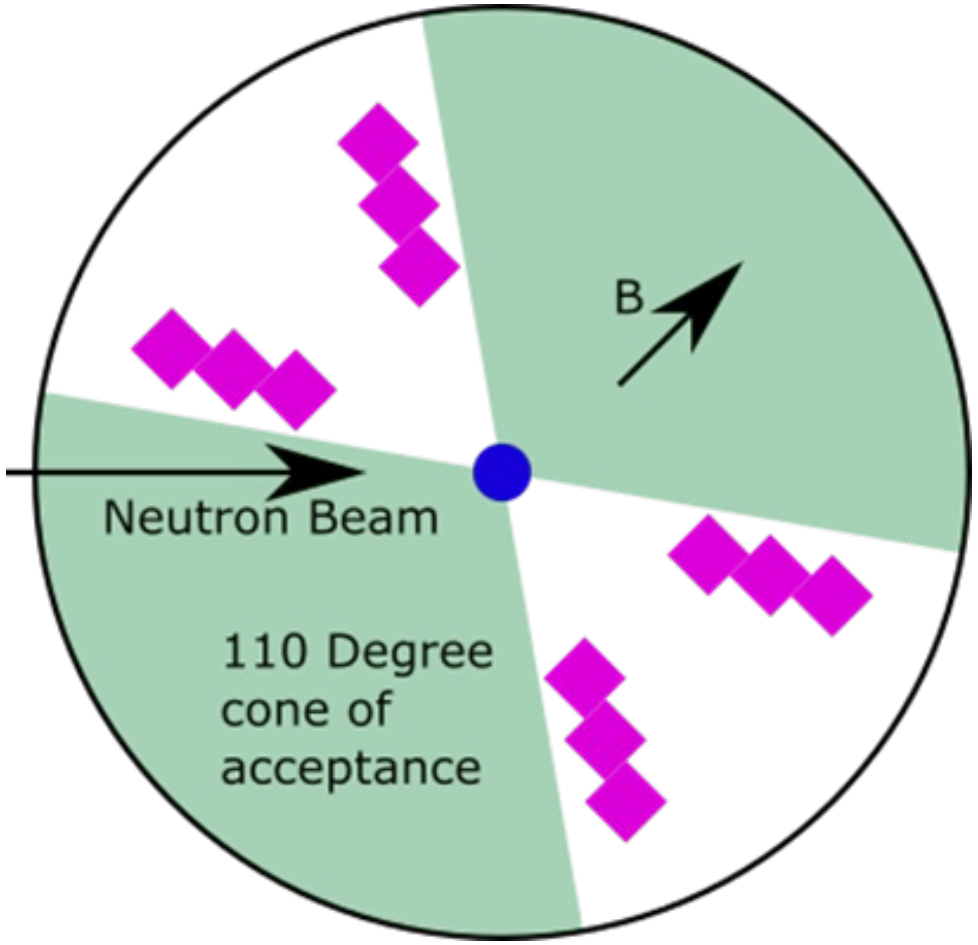


Concept for Future DNP Crystallography System

- 1K/5T system
- Cryogen free, superconducting 5T Helmholtz Coil
  - ~2π Acceptance for scattered neutrons
- Cryogen free 1K recirculating 4He refrigerator
  - ~200mW @ ~1K
  - Large sample space to accommodate goniometer
- SiPM based anger cameras (or similar)
  - Necessary due to large magnetic field
- Transmission Polarizer
  - To preserve neutron optics
- Broadband flipper
  - For pulsed or white beam, depending



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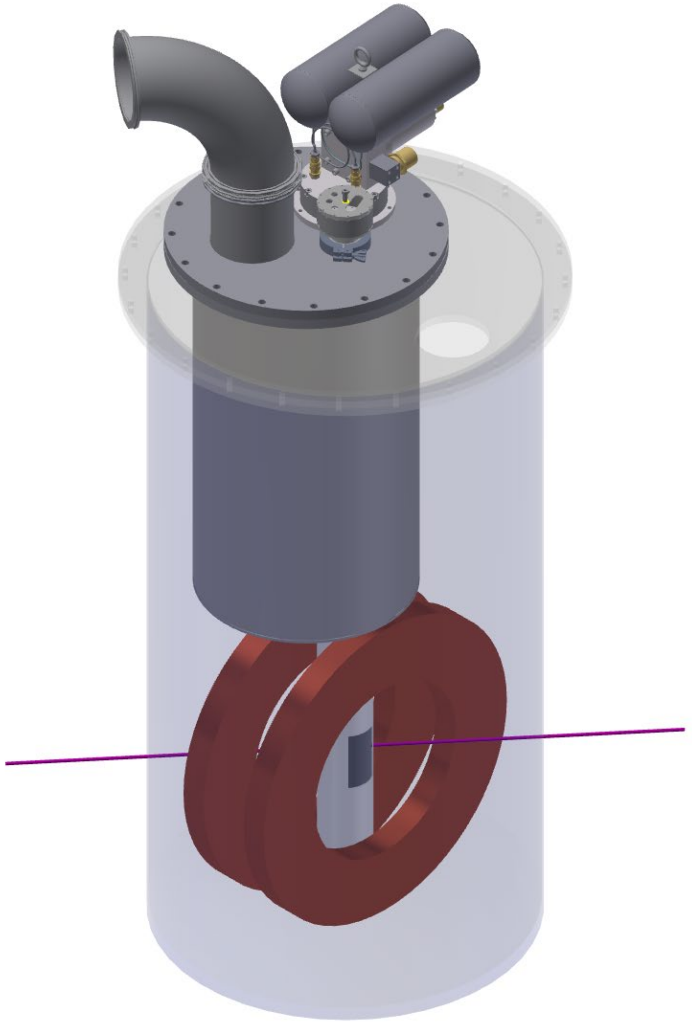

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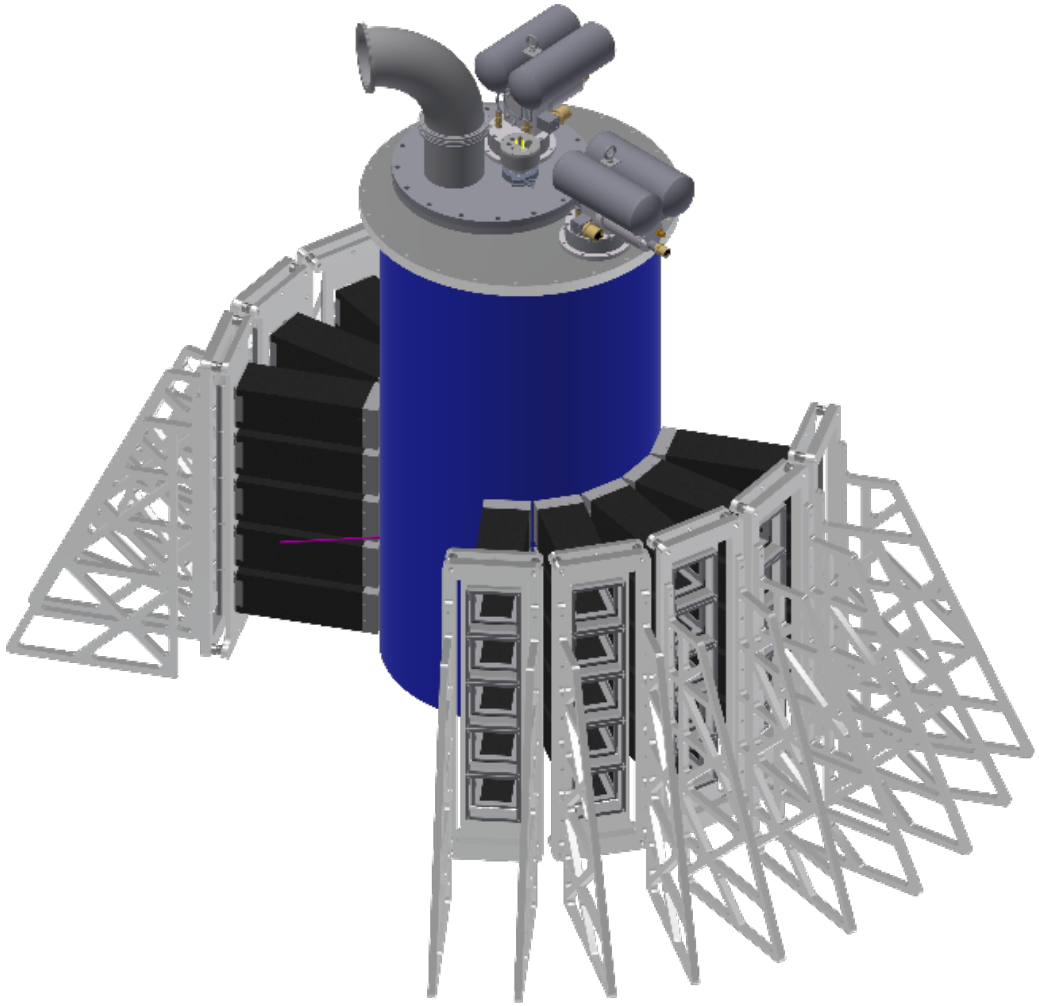

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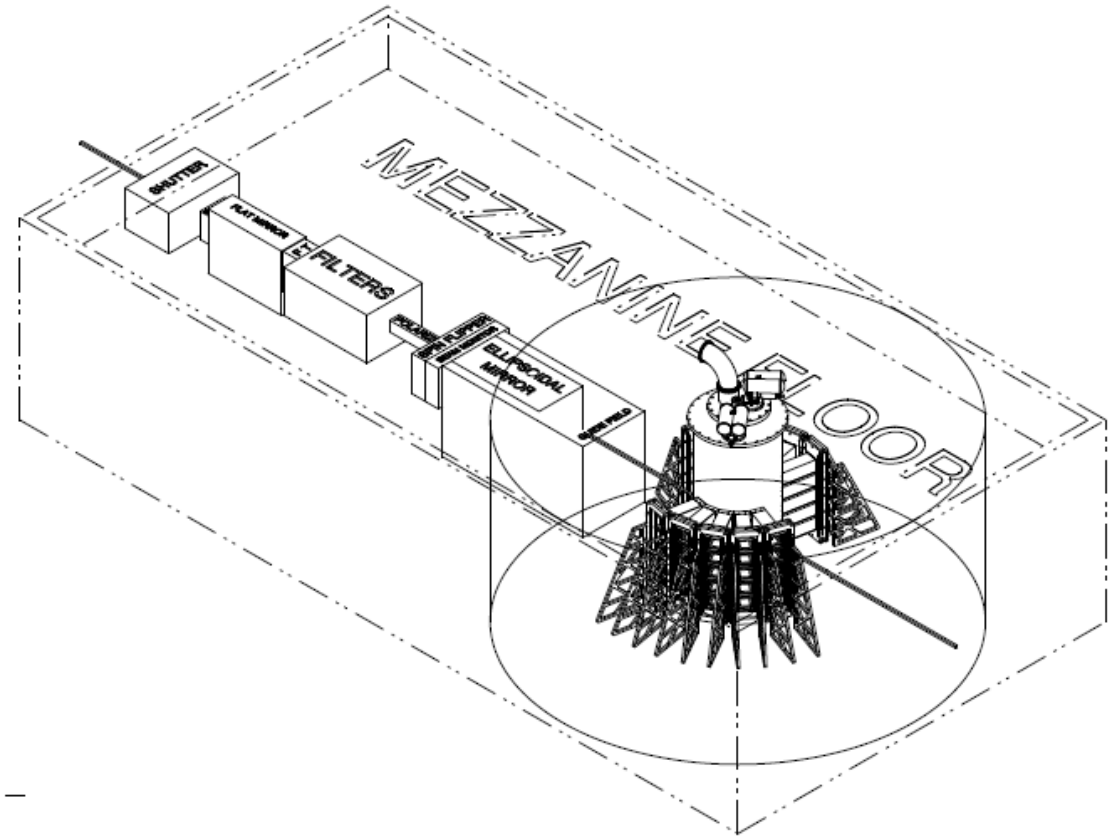

NB-1 At HFIR after upgrade

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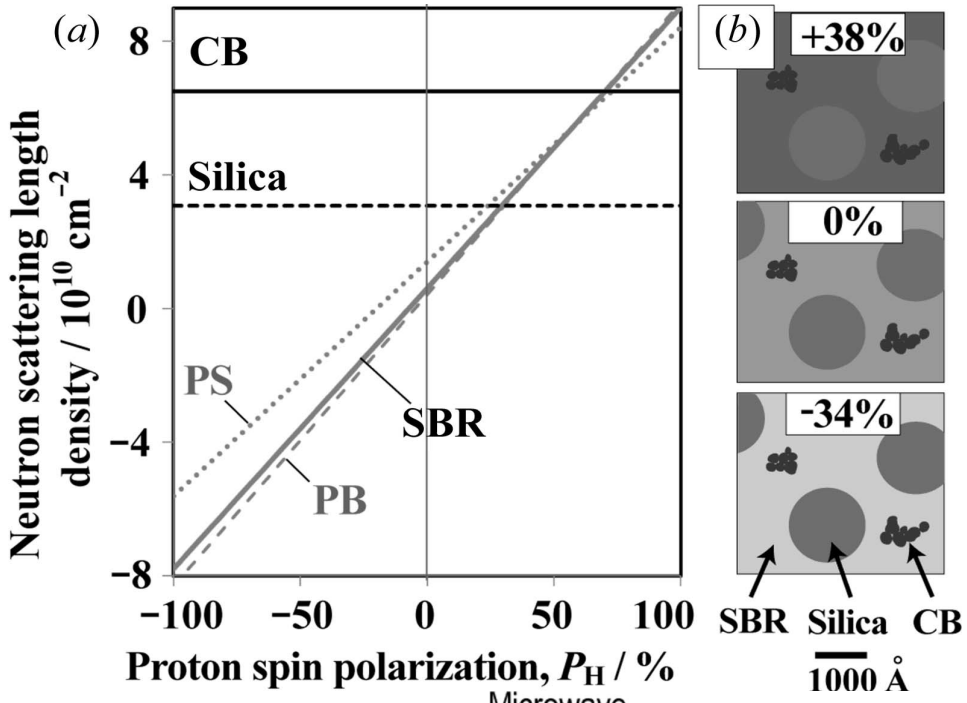
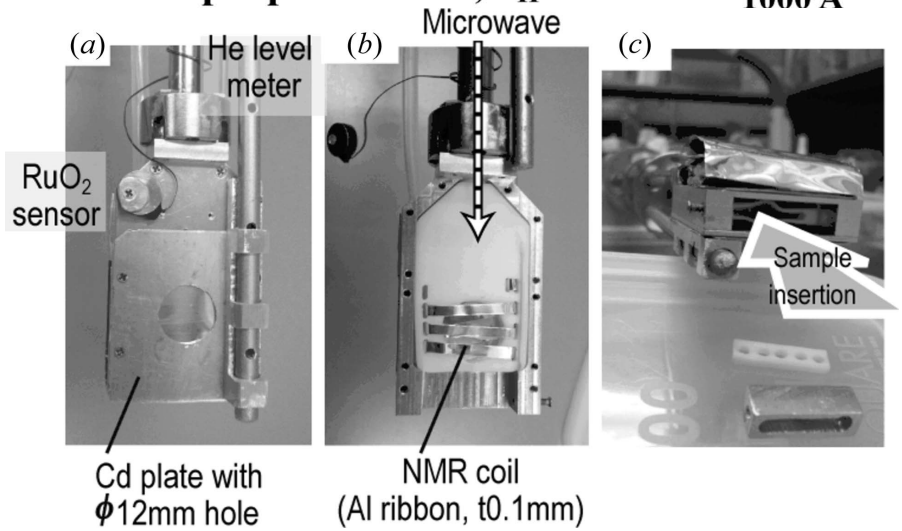
Pioneer at SNS Second Target Station

DNP and Polarized SANS

- Polarized SANS (without polarized sample) a *somewhat* established technique
  - Magnet materials
  - Incoherent separation/subtraction
- Polarized SANS + DNP used for in-situ changes to the to contrast
  - For hydrogen $b = -3.7423 + 14.56 \times P_n \times P_H$
  - Comparison to Deuteration
    - The unpolarized coherent scattering length of hydrogen is -3.74 fm
    - The unpolarized coherent scattering length of deuterium is 6.674 fm
  - Polarization
    - 100 % positive polarization: 10.82 fm
    - 100% negative polarization: -18.3 fm
- Current Programs at J-PARC and JAEA
  - Nice new high field system (very similar to what I described)
    - Noda, Yohei, et al. "First Experiment of Spin Contrast Variation Small-Angle Neutron Scattering on the iMATERIA Instrument at J-PARC." *Quantum Beam Science* 4.4 (2020): 33
  - Difference method extraction of hydrogen structure factor
    - Miura, Daisuke, et al. "Development of spin-contrast-variation neutron powder diffractometry for extracting the structure factor of hydrogen atoms." *Journal of Applied Crystallography* 54.2 (2021).

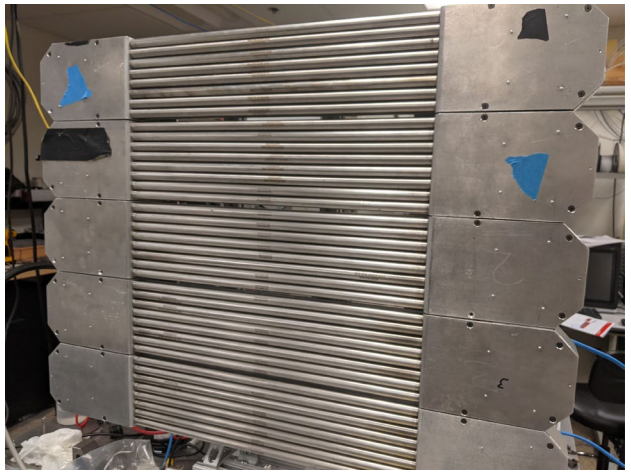


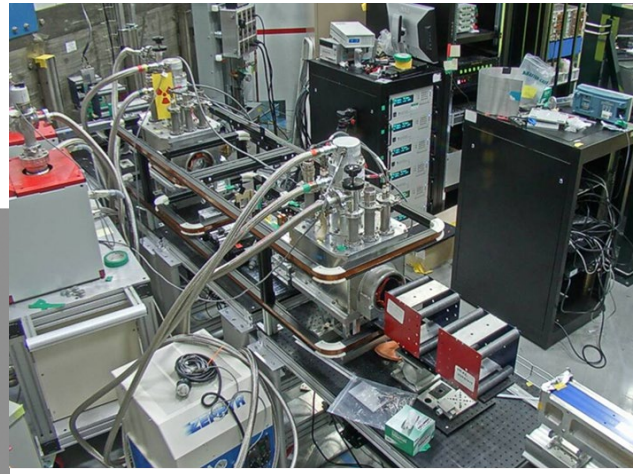


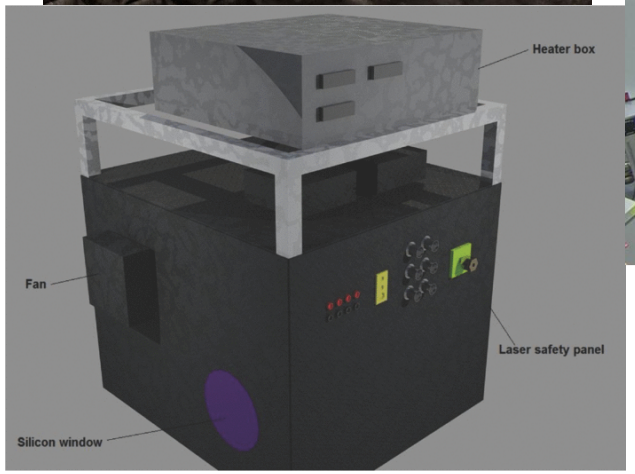
Noda, Yohei, et al. "Contrast variation by dynamic nuclear polarization and time-of-flight small-angle neutron scattering. I. Application to industrial multi-component nanocomposites." *Journal of applied crystallography* 49.6 (2016): 2036-2045.

DNP and Polarized SANS test at HFIR

- CG4B Beamline in the HFIR the cold guide hall
- Polarized monochromatic beam
  - S-Bender polarizer
- Polarized Samples in a 5T field at ~1.4K
  - Horizontal Field magnet (MAG-G)
  - Relatively high nuclear polarization of hydrogen
- Polarization analysis using 3He polarizer
  - Separation of incoherent and coherent scattering
  - Demonstration of nuclear polarization dependence of spin-flip incoherent scattering
    - 2/3-1/3 spin flip to non-spin flip incoherent is only true for unpolarized hydrogen
    - Ratio of spin flip to non-spin flip is dependent on sample polarization
    - Consequences for incoherent subtraction, and for multiple scattering







DNP Enhanced Neutron Scattering

- Advantages for analysis of hydrogenated systems, especially protein crystals:
  - 100 % positive polarization: 10.82 fm
  - 100% negative polarization: -18.3 fm
- Large 10-100-fold increases in S/N of the data for protein crystals.
  - Amplifying the diffracted intensity and minimizing incoherent scattering background
  - Radically smaller protein crystals (< 0.01 mm^3)
  - General – benefits ALL hydrogenated proteins from ANY biological system.
- A new class of perfectly isomorphous, spin-dependent difference experiments
  - Varying the spin-dependent scattering length of hydrogen (from -18.3 to +10.8 fm) in situ, within the same sample, provides new ways to collect, analyze and amplify neutron diffraction data, especially at pulsed neutron sources.
- Polarization analysis + DNP has interesting implications for coherent and incoherent separation in hydrogen scattering
  - Change the ratio of spin flip to non-spin flip scattering
  - Potential for even larger increase in effective signal to noise ratio for hydrogenous materials