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# The Dynamic Nuclear Polarization Program at ORNL

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The ORNL High Flux Isotope Reactor's IMAGINE instrument facilitates a wide array of single crystal macromolecular diffraction experiments. Notably, the neutron scattering experiments performed with this single-crystal diffractometer provide atomic-resolution insights into protein structure. The sensitivity of neutron macromolecular crystallography can be significantly enhanced by leveraging the spin dependence of the neutron scattering cross section of hydrogen. At ORNL, a proof-of-concept system employing Dynamic Nuclear Polarization (DNP) of samples has demonstrated a significant improvement in the signal-to-noise ratio of neutron diffraction data. Current efforts are focused on developing: 1) a highly polarized neutron beam with a high efficiency flipper; 2) a next-generation SiPM-based neutron detector array; and 3) a DNP apparatus consisting of a 5T Helmholtz magnet and a dry 1K $^4$He refrigerator. The progress and future prospects of constructing a new DNP-enhanced beamline will be presented.

*The 20th International Workshop on Polarized Sources, Targets, and Polarimetry (PSTP 2024)*
*22–27 September, 2024*
*Jefferson Lab, Newport News, USA*

*Speaker





PoS(PSTP2024)036

## 1. Introduction

The precise determination of the hydrogen atom locations inside large protein remains one of the central problems in structural biology. The primary approach for obtaining diffraction data, X-ray crystallography, has certain limitations when it comes to visualizing hydrogen atoms. Despite the very high flux of modern X-ray facilities, the X-ray diffraction sensitivity to lighter elements is low as the X-ray scattering cross-section drops significantly for low-Z atoms, especially hydrogen. In contrast, neutron macromolecular crystallography (NMC) relies on scattering by atomic nuclei, which is characterized by a larger neutron scattering length, and enables precise determination of hydrogen atoms. However, the major restricting factor of NMC is the relatively modest neutron flux, which remains sub-optimal even at the highest-intensity neutron sources worldwide. This limitation primarily impacts the size of the protein crystals that can be used with this procedure, as crystals larger than 0.1-1.0 $mm^3$ need to be grown. In addition to the need for larger sample volumes and longer measurement times, the NMC signal is also affected by a substantial incoherent scattering background, which can limit diffraction peak resolution and overall data quality. Sample deuteration is commonly employed to address this issue, given the higher coherent scattering cross-section of deuterons. However, deuterium labeling tends to be significantly more expensive, and the incorporation of deuterium atoms into molecules remains a technically challenging process. An alternative strategy to mitigate these complications involves leveraging the spin degrees of freedom of neutrons and protons in hydrogen [1].

## 2. DNP neutron macromolecular crystallography

The total neutron scattering cross section can be expressed in terms of the coherent and incoherent components. The useful signal is provided by the coherent contribution, which gives access to the spatial correlation between scattering centers and, consequently, provides information on the molecular structure of the protein. The incoherent contribution represents an isotropic background that determines the signal-to-noise ratio of the data and affects the resolution of the diffraction images. Both components display significant, varying dependencies on the spin polarization of the neutrons, the nuclear polarization of the sample, and their relative orientation. This dependence can be written [2] as

$$\left(\frac{d\sigma}{d\Omega}\right)_{\text{coh}} = b_0^2 + bb_0IpP + \frac{b^2I^2P^2}{4} \tag{1}$$

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

where $b_0$ ($b$) represents the spin independent (dependent) part of the scattering amplitude, $p$ ($P$) is the neutron (nuclear) polarization, and $I$ is the spin of the nucleus. As shown in Fig. 1, controlling the polarization magnitude and orientation allows for the reduction of the incoherent background. In the ideal case, when $p = 1$ and $P = 1$, it is possible to achieve the complete background suppression. A similar effect occurs in the anti-aligned spin configuration, leading to a more than

20-fold increase in the coherent cross section compared to the unpolarized configuration, while the incoherent component rises by only about 30%. An infrastructure that enables such beam-sample spin manipulation leads to an order-of-magnitude increase in the signal-to-noise ratio of the data and opens up the possibility of new classes of neutron spin-flip difference experiments.

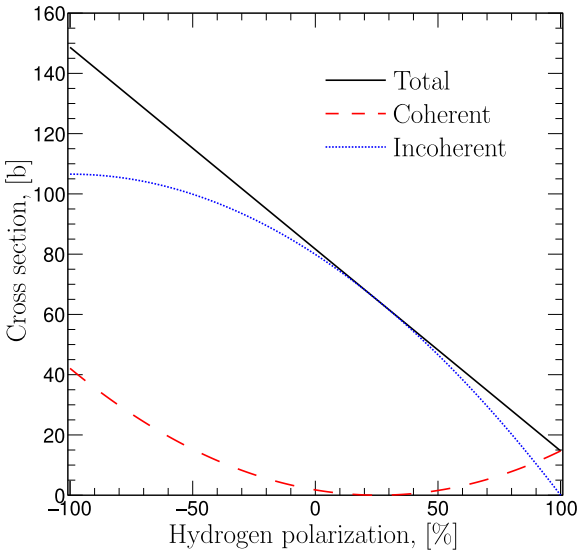


**Figure 1:** Coherent, incoherent and total scattering cross section of hydrogen as a function of the proton polarization for fully polarized neutrons.

While techniques for achieving high polarization of neutron beams are well-established, polarization of protons within hydrogen presents more challenges. The method of dynamic nuclear polarization is used to obtain very high nuclear polarizations. In DNP, nuclear spins are polarized in the presence of suitable paramagnetic centers, which are added in specific amounts to the sample. Under high magnetic field and low temperature conditions, the polarization of these unpaired electrons is transferred to hydrogen protons via microwave irradiation at the appropriate frequency.

To integrate DNP capabilities, a new NMC facility has been approved for construction at ORNL's High Flux Isotope Reactor (HFIR). This initiative brings the IMAGINE-X upgrade, which will replace the existing IMAGINE beamline that currently houses a Quasi-Laue neutron diffractometer primarily used for protein crystallography research. By adding DNP capabilities, IMAGINE-X will provide researchers with a powerful tool for exploring the detailed structural and functional properties of biological macromolecules, advancing the frontiers of structural biology and related fields.

## 3. Proof-of-concept DNP system

A prototype DNP apparatus designed for use on the IMAGINE beamline was built to test the feasibility of exploiting modern DNP technologies in the NMC environment, as well as to establish appropriate experimental protocols and highlight remaining technological challenges. The detailed system overview and its operation are given in [3, 4]. The system included all components required for DNP (refrigerator, magnet, microwave, and NMR system) and was operated in frozen spin mode to maximize detector acceptance.

A vertically movable, warm bore, cryogen-free magnet was used to provide a 5T magnetic field with a homogeneity region better than $\sim 10^{-4}$ (2 cm DSV). A commercially available cryogen-free dilution refrigerator with a nominal cooling power of 12 mW (400 µW) at 1 K (100 mK) incorporated a custom-designed sample space and sample loading system. While the refrigerator was capable of reaching a base temperature of 7 mK, with sample space modifications, the actual observed sample temperatures varied between 200 and 250 mK. A 300 mW (at 140 GHz) solid-state microwave source was equipped with a copper-nickel tube waveguide and a sheet of brass as a 90° microwave reflector for sample irradiation. This amount of power was sufficient, as the sample size does not normally exceed $1\text{mm}^3$. However, NMR measurements that monitor sample polarization are extremely challenging for samples of this size. A Liverpool Q-meter [5], coupled with a factor of 500 amplification pre-amplifier card, was used for polarization measurements. The extraction of the calibration constant was hindered by significant uncertainties associated with the thermal equilibrium (TE) polarization measurements. The maximum achieved polarization of approximately 50% was estimated through a series of polarization measurements on larger samples obtained by freezing a solution with the same chemical composition as the crystals used for diffraction imaging. Despite difficulties with thermal equilibrium (TE) calibration, the enhanced polarization signals and corresponding polarization decay were routinely monitored during the data taking period.

A $\sim 0.6\text{mm}^3$ T4-lysozyme crystal was chemically doped in a solution containing 100 mM hydroxy-TEMPO and flash-frozen in liquid nitrogen. Special protocols were developed for sample transfer and loading, as the sample must remain below around 146 K to avoid ice formation and disruption of the crystal lattice. The sample was then DNP-polarized at about 1 K in a 5 T field. The irradiation typically took more than 8 hours, after which the temperature of the sample was lowered as much as possible to maximize the spin-lattice relaxation time, $T_1$. The magnet could then be removed from the beamline while maintaining the sample in the 0.5 T fringe field (Fig. 2).

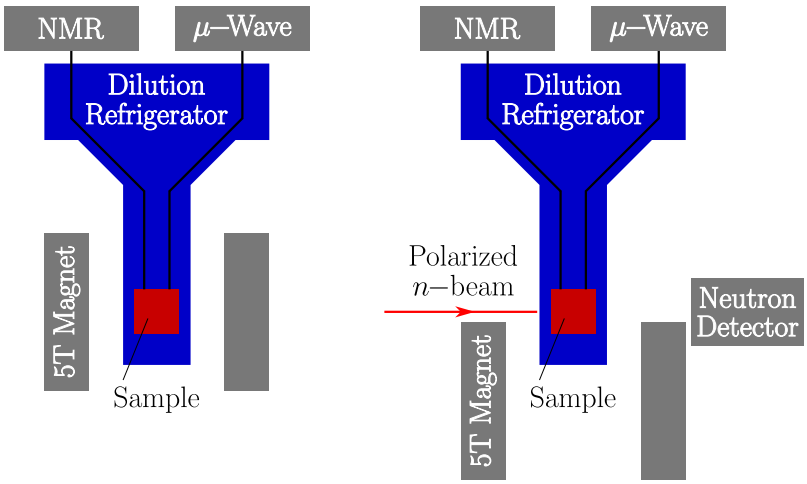


**Figure 2:** Schematic of a frozen spin DNP system for NMC. Left: the system in polarizing mode in a 5 T field; Right: the system in frozen spin mode, with sample aligned in the neutron beam.

In order to collect diffraction data, the neutron beam polarization over 96% was achieved using a V-cavity supermirror polarizer. A prototype single-arm detector based on Silicon Photomultipliers

(SiPM) was developed for diffraction image reconstruction and mounted on a theta rotation stage for precise angular positioning between 0° and 60°. Diffraction measurements were performed on a set of protein crystal samples with three different mutual spin orientations: unpolarized, polarized and aligned, and polarized and anti-aligned. By changing the microwave frequency, samples were polarized in both spin-aligned and anti-aligned configurations. Individual data sets were recorded for approximately 0.7 to 3.0 hours (one $T_1$). The resulting averaged polarization was about half of the initial value. An overall three-fold spin-dependent amplification was observed for background-subtracted diffraction peaks when compared between polarized and unpolarized states. Notably, the anti-aligned spin state exhibited nearly twice as many reflections as the unpolarized state. Building on these promising results, the concept for the DNP apparatus was developed with the focus on transforming the IMAGINE beamline into a next-generation, DNP-equipped facility.

## 4. IMAGINE-X DNP-NMC

The proof-of-concept measurements with DNP prototype system provided a number of insights used to improve the final conceptual design of the IMAGINE-X beamline. A critical requirement for maximizing and sustaining sample polarization led to the selection of a continuously pumped DNP system operating at approximately 1 K. Rapid adjustments in spin alignment are crucial for shortening data collection time and mitigating systematic effects in spin-flipped difference measurements.

To produce spin polarized neutron beam, the beamline upgrade will include a transmission polarizer. This polarizer will be mounted on a translation stage, allowing for original unpolarized operation mode at full intensity. This multi-supermirror polarizer with a v-cavity design will have to be compatible with current focusing optics of the IMAGINE-CG4D beamline. The polarizer will be followed by a high efficiency cryogenic spin flipper. The state of the flipper defines the direction of the neutron spin and determines the sign of the beam polarization. This information must be accurately integrated into the data acquisition and data analysis software, ensuring that spin configuration is recorded on an event-by-event basis. Additionally, a neutron spin-transport system will be developed to provide a guide (over 10 G) field with a uniform magnetic field vector along the beam path, ensuring preservation of the neutron spin polarization.

The existing Neutron Image Plate Diffractometer (NIPD) will be replaced with a detector system, consisting of 50 SiPM-based Anger cameras. These cameras will be arranged into two cylindrical banks, each housing 25 detectors. Their positions will be optimized to align precisely with the acceptance range of the DNP system. With a spatial resolution of approximately 0.45 mm and nearly $2\pi$ solid angle coverage, the new detector system will deliver a five-fold improvement in performance for room-temperature measurements compared to the current NIPD setup.

The DNP system will be centered around a cryogen-free 5.2 T split-pair superconducting magnet with a 280 mm cold bore and an open cone angle of ±54°. The outer chamber of the magnet cryostat incorporates a large thinned aluminum section designed to accommodate the incoming neutron beam, which enters at an approximate angle of $\sim 45°$ relative to the magnet's horizontal axis. An integrated cryogen-free, high-cooling power $^4$He evaporation refrigerator will be used, providing a 100 mm diameter cylindrical sample space. Sufficient cooling power is essential for operation in continuous microwave irradiation mode. The necessary infrastructure for the $^4$He

gas recirculation system, dry pumping, and automatic gas handling systems will be constructed on an elevated cryo-platform surrounding the DNP cryostat. Inside the cryostat's sample space, a positioning system will be installed to orient the sample relative to the neutron beam, allowing for the multiple required crystal orientations needed for structure reconstruction. The current design includes a goniometer with three translational and two rotational piezo-electrically activated stages, mounted on a custom insert. An additional insert will be dedicated to positioning the microwave guide and NMR coil as close to the sample as possible. The polarization measurement system will be upgraded with a modern JLab Q-meter [6], potentially improving the quality of TE signals.

## 5. Conclusion and outlook

The ongoing development and integration of the Dynamic Nuclear Polarization techniques on the IMAGINE-X beamline at the High Flux Isotope Reactor represents a significant advancement in biological and chemical neutron crystallography. The new DNP NMC system, equipped with upgraded high-resolution detector cameras, promises to drastically enhance neutron imaging capabilities allowing analysis of hydrogenated crystals with much smaller sizes or at substantially higher data collection rates. Its ability to tune and control the relative strength of hydrogen's coherent scattering opens up new opportunities for spin-dependent measurements and analysis.

## 6. Acknowledgments

This material is based upon work supported by the U.S. Department of Energy, Office of Science, Office of Basic Energy Sciences Biopreparedness Research Virtual Environment program.

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