

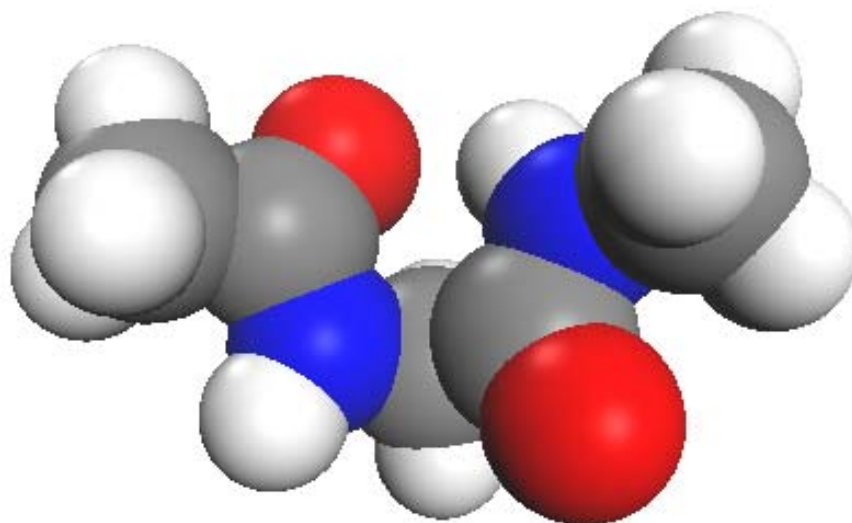
# Hydration water dynamics near biological surfaces: an experiment using the NCNR Disk Chopper Spectrometer

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## Abstract

Time-of-flight neutron spectroscopy will be used to probe water dynamics close to the surface of a small hydrophilic peptide. This experiment illustrates the important technique of quasielastic neutron scattering (QENS). We shall discuss all aspects of the experiment, from sample preparation and the choice of instrumental setup through to data treatment and interpretation of results.



## I. Introduction

In many real life situations water molecules are confined near surfaces, or contained within small cavities and are integral to most biological macromolecules. The interfacial water plays a particularly crucial role in determining the dynamics and conformations of proteins. It is known that protein dynamics are not fully activated until a critical level of hydration water is present and the dynamics are strongly correlated with enzymatic activity. Understanding the dynamical properties of hydration water is important to gain insight into microscopic mechanisms behind protein functionality.

To illustrate the difference in water dynamics between bulk water and that next to a hydrophilic biological surface we will measure the water dynamics in a N-acetyl-glycine-methylamide (NAGMA) solution where the peptide has been deuterated.

We will be using the Disk Chopper Spectrometer at the National Institute of Standards and Technology Center for Neutron Research to probe local dynamics of water adsorbed near the surface of this small peptide. After reading the background material you should be able to choose an appropriate spectrometer configuration to probe the temperature dependent diffusional dynamics present in the NAGMA:H<sub>2</sub>O system.

The neutron has several properties that enable scattering experiments to measure properties of materials that other techniques can measure with much less precision or not at all. Neutrons with wavelengths on the order of interatomic spacings also possess energies on the same order as those characteristic of phonons and intermolecular interactions; for example, a 1.8 Å neutron has an energy of ~25 meV (~200 cm<sup>-1</sup>) and speed ~2200 ms<sup>-1</sup>. This means that structural and temporal information can be measured simultaneously.

The reader is reminded that the scattering of neutrons is usually treated as the sum of two parts, known as *coherent* and *incoherent* scattering. To understand why such a separation is performed recall that the strength of the scattering from nuclei of the same element can vary (and generally does vary) with spin and/or isotopic species. Hence when a neutron is scattered by a collection of nuclei the interference between the different scattered waves is normally neither complete nor completely absent. For this reason the *double differential cross section* [ $d^2\sigma/d\Omega d\omega$ ], which describes the probability that neutrons are scattered into solid angle  $d\Omega$  and energy transfer window  $d(\hbar\omega)$ , is normally separated into two terms. The first term is the coherent part, which contains all of the interference effects such as Bragg scattering and small angle scattering. The second term is the incoherent scattering, which represents the scattering from individual nuclei and is approximately isotropic. For a single element [ $d^2\sigma/d\Omega d\omega$ ] can be expressed as

$$\frac{d^2\sigma}{d\Omega d\omega} = \frac{1}{4\pi} \frac{k_f}{k_i} [\sigma_{\text{coh}} S(\mathbf{Q}, \omega) + \sigma_{\text{inc}} S_{\text{inc}}(\mathbf{Q}, \omega)] \quad (1)$$

where  $k_i$  and  $k_f$  are the magnitudes of the initial and final neutron wavevectors,  $\sigma_{\text{coh}}$  and  $\sigma_{\text{inc}}$  are the coherent and incoherent scattering cross sections, and  $S(\mathbf{Q}, \omega)$  and  $S_{\text{inc}}(\mathbf{Q}, \omega)$  are the corresponding scattering functions which depend only on the momentum transfer

$\hbar Q$  (or wave vector transfer  $Q$ ) and the energy transfer  $\hbar\omega$ . (Note that in general  $Q$  is a vector but since we shall be working with a liquid, which has no preferred orientation, all that need concern us in this experiment is the magnitude of the vector.) The most important incoherent scatterer is hydrogen for which  $\sigma_{\text{inc}} = 80.3$  barns/atom whereas  $\sigma_{\text{coh}}$  is only 1.76 barns/atom (1 barn =  $10^{-24}$  cm<sup>2</sup>). Since the incoherent scattering cross section of hydrogen is much larger than those of almost all other nuclei, it is often reasonable (as a first approximation) to neglect the coherent scattering in systems that contain a relatively large fraction of hydrogen atoms.

Can you explain the usefulness of deuteration, given that  $\sigma_{\text{coh}}$ (deuterium) and  $\sigma_{\text{inc}}$ (deuterium) are 5.6 barns/atom and 2 barns/atom, respectively?

*Elastic neutron scattering* is scattering with no change in neutron energy, i.e. with  $\hbar\omega = 0$ , and *inelastic neutron scattering* is scattering with a change in neutron energy, i.e. with  $\hbar\omega \neq 0$ . On the other hand, *quasielastic neutron scattering* (QENS) involves the Doppler-like broadening of otherwise elastically scattered neutrons due to reorientational or diffusive motions of atoms in the target material. Thus QENS is a special kind of inelastic neutron scattering. In this experiment you will use neutron scattering to measure QENS spectra close to room temperature and interpret the resulting data.

We shall first describe the sample to be used for the experiment. The next section gives a brief discussion of the spectrometer as well as matters to be considered in choosing the incident wavelength for this experiment. We then describe the reduction of the data to obtain the scattering function, and we follow with some words about the scattering that is expected for these measurements. This then sets the scene for the analysis and discussion of the experimentally measured scattering function.

## II. The sample

Prior to the experiment a 1.5M  $\text{CD}_3\text{CONHCD}_2\text{CONHCD}_3$  (N-acetyl-glycine-methylamide, NAGMA) solution will have been loaded into an annular aluminum cell. The aluminum cell will be mounted in a closed-cycle helium refrigerator to allow for temperature control. Through the night we shall collect data at temperatures and wavelengths decided by the experimental group.

Given that the molecular mass of NAGMA is 138.2 g, and the volume of the aluminum can is 1.5 ml, how much NAGMA must be used to obtain a 1.5 M solution?

Why do we typically use aluminum for sample containers and cryostat windows?

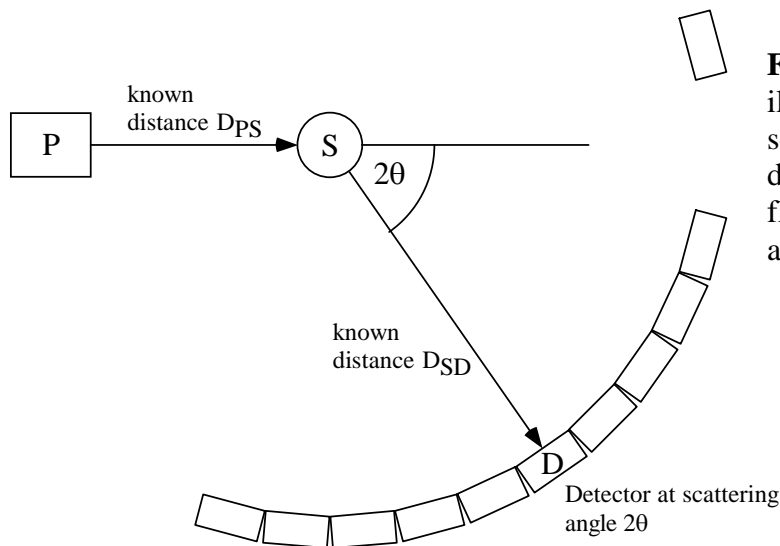
Apart from indium, what materials might be used to seal sample containers?

To reduce the data we will need a detector normalization file obtained using a sample of vanadium metal, plus a “dark count” run with the beam closed (a type of background). These runs will have been performed before the start of the summer school since there will not be time to complete them during the school.

Why do we use vanadium to normalize the data from different detectors? Hint:  
 $\sigma_{\text{coh}} = 0.02$  barns/atom,  $\sigma_{\text{inc}} = 5.19$  barns/atom.

### III. The spectrometer

We shall be performing this experiment using the Disk Chopper Spectrometer (DCS), which is a so-called “direct geometry” (fixed incident energy) time-of-flight spectrometer. In this type of instrument (figure 2) bursts of monochromatic neutrons strike the sample at equally spaced times. The energies of the scattered neutrons are determined from their arrival times at the detectors, since we know when the pulses were created as well as the distances  $D_{\text{PS}}$  from the pulsing device to the sample and  $D_{\text{SD}}$  from the sample to the detectors. There are two ways to produce a monochromatic pulsed beam at a steady state neutron source. One method is to use a single crystal to monochromate the white beam and a mechanical “chopper” to pulse it; the other method is to use multiple choppers, such as the seven (!) choppers of the DCS.



**Figure 1.** A schematic illustration of the scattering geometry for a direct geometry time-of-flight spectrometer such as the DCS.

A monochromatic pulsed beam of neutrons can in principle be created using two choppers. How does that work? Can you think why more than two choppers might be needed and/or desirable?

Given the initial and final energies of the neutrons,  $E_i$  and  $E_f$ , the energy transfer  $\hbar\omega = E_i - E_f$  is trivially obtained. Knowing the scattering angle  $2\theta$  we can also calculate the magnitude of the momentum transfer to the sample,  $\hbar Q$ :

$$(\hbar Q)^2 = 2m_n \left[ E_i + E_f - 2\sqrt{E_i E_f} \cos 2\theta \right] \quad (2)$$

where  $m_n$  is the mass of the neutron. (This follows from the definition  $\vec{Q} = \vec{k}_i - \vec{k}_f$  and the relationship between the magnitude of a neutron's wave vector,  $k$ , and its energy  $E$ :  $E = \hbar^2 k^2 / 2m_n$ .)

The data acquisition system separately accumulates neutron counts for each of the 913 DCS detectors. Furthermore the time between pulses,  $T$ , is normally divided into 1000 time channels of equal width  $\Delta t = 0.001T$  and each neutron event in a given detector is stored in one of these time channels according to its time of arrival at the detector. Thus the data acquisition system generates a two-dimensional array of counts  $I(i,j)$  as a function of detector index  $i$  and time channel index  $j$ . This array is accumulated in a "histogramming memory" which is resident in the data acquisition computer and reflected to the instrument computer. At the end of each run cycle the array is saved, along with other pertinent information, to the hard disk of the instrument computer.

With the sample environment mounted on the spectrometer, we can control and monitor the temperature remotely. We must also set the incident wavelength, together with a few other parameters such as the "master speed" of the choppers. The choice of wavelength is critical to the experiment and several factors must be considered. These include intensity at the sample (which peaks, remaining roughly constant, between  $\sim 2.5$  and  $\sim 4.5$  Å, see Appendix A), the width of the elastic energy resolution function (which roughly varies as  $1/\lambda^3$ ), the available  $Q$  range (which varies as  $1/\lambda$ ), and concerns about "frame overlap" problems. A related consideration is the available range of energy transfers in sample energy gain (neutron energy loss).

What is the maximum theoretical sample energy gain that can be measured when the incident energy is  $E_i$ , and how long would it take to measure the intensity of neutrons scattered with this change in energy?

Overnight you will collect data. You will need to define a "sequence" consisting of several "runs". The sequence may also include a change in sample temperature or incident wavelength. Each run is divided into a set of "cycles" of relatively short duration, typically 10 to 15 minutes. At the end of each cycle the temperature is recorded and the data are backed up to the disk. Having defined the runs we shall start the

overnight sequence of measurements. Next day we shall stop the measurements and start into the data reduction.

In the experimental runs we shall collect intensity histograms  $I(i,j)$  for the sample at temperatures and wavelengths of your choice. Using previously acquired intensity histograms for a vanadium sample and for a “dark count” run with the beam shutter closed, we shall reduce the data to obtain the scattering function.

#### **IV. Data reduction**

In this section we shall indicate some of the more important steps in the data reduction process. We shall go into greater detail in our discussions at the time that the data reduction takes place.

The measured scattering in a sample run includes contributions from the sample itself, from its container and the sample environment, and from the time-independent background. Before doing any data analysis we need to subtract the time independent background from each of the runs. We also need to subtract scattering from the container.

Where does the time independent background come from?

Neglecting effects such as self-shielding and multiple scattering the scattering in detector  $i$  and time channel  $j$  may be related to the corresponding double differential cross section  $[d^2\sigma/d\Omega dt]_{ij}$  (note that this is per unit time, not energy) in the following fashion:

$$I(i, j) = \frac{I_{BM}}{\eta_{BM}} \cdot \left( \frac{d^2\sigma}{d\Omega dt} \right)_{ij} \cdot \Delta\Omega \Delta t \cdot N_m \cdot \eta_{ij} \quad (3)$$

where  $\Delta\Omega$ , the solid angle subtended by detector  $i$ , and  $\Delta t$ , the width of time channel  $j$ , are (for these measurements) independent of  $i$  and  $j$  respectively,  $N_m$  is the number of sample molecules in the beam,  $\eta_{ij}$  is the efficiency of detector  $i$  for neutrons detected in time channel  $j$ , and  $I_{BM}$  and  $\eta_{BM}$  are respectively the counts and the efficiency of the beam monitor (situated upstream of the sample).

Since we are not trying to extract an absolute cross section we can neglect the multiplicative constants in the above equation, but we should not ignore the detector efficiency function  $\eta_{ij}$ . Since all of the detectors are to first order identical it is not unreasonable to treat  $\eta_{ij}$  as the product of two terms, a function  $\eta_{i0}$  which represents the efficiency of detector  $i$  for elastically scattered neutrons and a detector-independent function  $f_j$  that describes the energy dependence of the efficiency of the detectors. The correction for differences in detector response, i.e. the determination of  $\eta_{i0}$ , is performed using the results of a measurement with a vanadium sample.

The correction of the data for the energy dependence of the efficiency is achieved by calculation, knowing the various factors that affect the probability that a neutron is absorbed within a detector.

What are these factors? Can you write an expression for the efficiency of a detector, assuming its cross section is rectangular?

To improve statistics we sometimes define several detector “groups”, each of which includes detectors within a specified range of angles. The differential cross section  $[d^2\sigma/d\Omega dt]$  for all detectors in a group will be summed and divided by the number of detectors in the group. Having obtained a quantity proportional to  $[d^2\sigma/d\Omega dt]$  we must now compute  $[d^2\sigma/d\Omega d\omega]$  and finally  $S_{inc}(Q, \omega)$ . Since a neutron’s energy  $E$  is related to its time-of-flight  $t$  over a fixed distance as  $E \propto t^{-2}$ , it follows that  $dE \propto t^{-3} dt$ . Hence

$$\frac{d^2\sigma}{d\Omega d\omega} \propto \frac{d^2\sigma}{d\Omega dE_f} = \left( \frac{d^2\sigma}{d\Omega dt} \right) \left( \frac{dt}{dE_f} \right) \propto \left( \frac{d^2\sigma}{d\Omega dt} \right) t^3 \quad (4)$$

To obtain  $S_{inc}(Q, \omega)$  we simply divide by  $k_f$  (see eq. 1). Equivalently we multiply by another factor of  $t$ .

If a system in thermodynamic equilibrium can exist in a number of thermodynamic states and we consider two such states separated by an energy difference  $\hbar\omega$ , the probability that the system is in the lower energy state is greater by a factor  $\exp(\hbar\omega/kT)$  than the probability that it is in the higher energy state. From this it can be shown that for systems in thermodynamic equilibrium the scattering function  $S_{inc}(Q, \omega)$  satisfies the so-called “detailed balance” relationship:  $S_{inc}(-Q, -\omega) = \exp(-\hbar\omega/kT) S_{inc}(Q, \omega)$ . Since we shall be fitting the data to a theoretical form that is symmetric in  $\hbar\omega$  we shall first “symmetrize” the experimental  $S_{inc}(Q, \omega)$  by multiplying it by  $\exp(-\hbar\omega/2kT)$ .

Is symmetrization of  $S_{inc}(Q, \omega)$  likely to be a larger effect at low or high temperatures?

Having reduced the experimental data to a symmetrized scattering function it is time to interpret those results.

## V. Theory

A common way of expressing  $S_{inc}(\vec{Q}, \omega)$  is in terms of the *intermediate self scattering function*  $I_s(\vec{Q}, t)$ , which is the space Fourier transform of the time-dependent pair distribution function  $G_s(\vec{r}, t)$ :

$$S_{\text{inc}}(\vec{Q}, \omega) = \frac{1}{2\pi} \int I_s(\vec{Q}, t) e^{-i\omega t} dt \quad (5)$$

An illustrative model for  $G_s(\vec{r}, t)$  (though inappropriate in the context of the present experiment) is that of simple Brownian diffusion, where times of observation are much longer than typical times between collisions. Fick's Law governs this type of diffusion:

$$\frac{\partial}{\partial t} G_s(\vec{r}, t) = D \nabla^2 G_s(\vec{r}, t), \quad (6)$$

where  $D$  is the diffusion constant. A solution to this equation is given by a self-correlation function of the form

$$G_s(\vec{r}, t) = \frac{\exp(-r^2 / 4Dt)}{(4\pi Dt)^{3/2}}, \quad (7)$$

the space Fourier transform of which is

$$I_s(\vec{Q}, t) = \exp(-Q^2 Dt). \quad (8)$$

Since this represents an exponential decay in time, the time Fourier transform yields a Lorentzian lineshape:

$$S_{\text{inc}}(\vec{Q}, \omega) = \frac{1}{\pi} \left( \frac{DQ^2}{(DQ^2)^2 + \omega^2} \right) \quad (9)$$

that is centered at zero energy transfer and has a full width at half maximum height (FWHM),  $\Gamma$ , given by

$$\Gamma = 2DQ^2. \quad (10)$$

In the current situation, we should distinguish two types of water motion: rotational and translational. This can be described as a convolution of the rotational [ $S_{\text{inc}}^{\text{rot}}(Q, \omega)$ ] and translational [ $S_{\text{inc}}^{\text{trans}}(Q, \omega)$ ] scattering functions with an overall Debye-Waller factor, convoluted with the instrumental resolution,  $R$ :

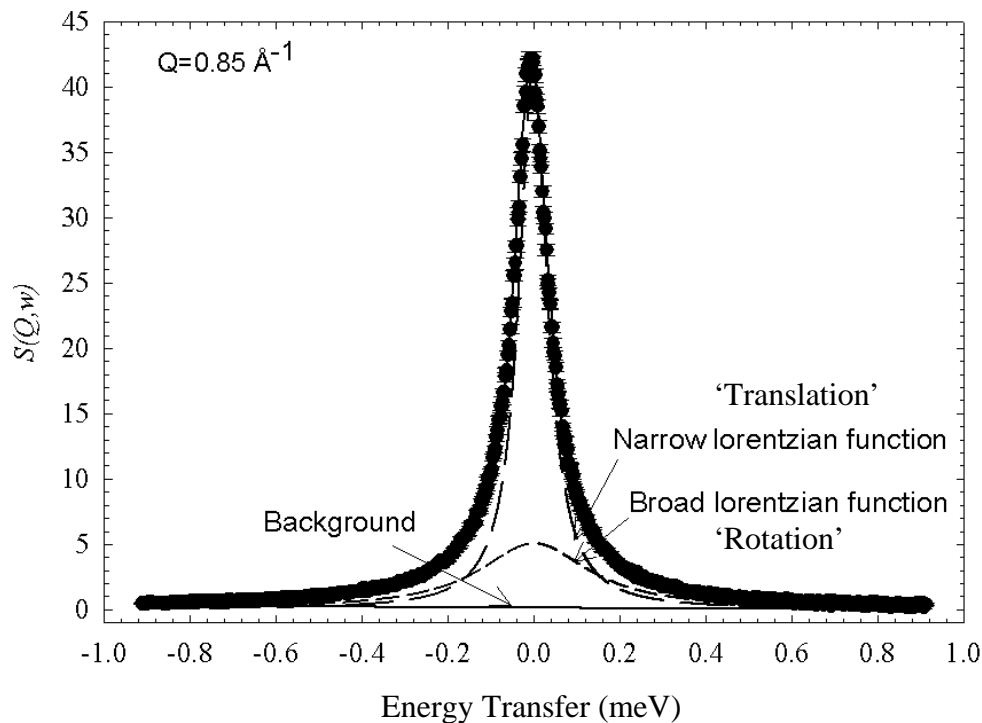
$$S_{\text{inc}}(Q, \omega) = e^{-\frac{1}{3}Q^2 \langle u^2 \rangle} S_{\text{inc}}^{\text{trans}}(Q, \omega) \otimes S_{\text{inc}}^{\text{rot}}(Q, \omega) \otimes R \quad (11)$$

## VI. Data analysis

We will take a few detours on the route to obtaining a symmetrized form of the experimental scattering function,  $S_{\text{inc}}(Q, \omega)$ .

The next step will be to fit the  $S_{\text{inc}}(Q, \omega)$  data in the tunneling regime as a function of  $Q$ . We suggest that you try fitting each  $Q$  group to a series of phenomenological Lorentzians and possibly a background function, and then extract the fitted parameters.





**Figure 2.**

Representative QENS data fit to a broad and narrow Lorentzian function, representing the rotational and translation components of the water molecules, and a background function.

If you have taken data at higher temperatures, use the isotropic rotational diffusion model described in the previous section (i.e. an elastic delta function and just *one* broader Lorentzian) to fit the ‘empty sample’ background subtracted data as a function of  $Q$ . In an actual experiment the scattering function is broadened with the instrumental resolution function so the model function must be numerically convoluted with the instrumental resolution function. Having fitted the experimental data to the model, the next step is to make plots of the Lorentzian line parameters as functions of  $Q$ .

- (1) How well does the single Lorentzian fit?
- (2) How do the Lorentzian parameters behave?
- (3) Can you extract a diffusion constant or correlation time?
- (4) If you have access to data at other temperatures, can you extract an activation energy?

## VII. Concluding remarks

In section V we discussed a scattering function that corresponds to a very simple model of rotational and translational diffusive motions. The situation can be more complicated when a system displays multiple types of reorientational motion, or more than one

rotational axis. If the various motions are uncoupled, the intermediate scattering function is a product of the individual intermediate scattering functions so that the scattering function is a convolution of the scattering functions for the individual motions, as we have seen. The situation simplifies considerably if additional motions occur on very different time scales. Motions that are much slower than the time scale represented by the instrumental resolution show up as elastic scattering. On the other hand motions that are much faster give rise to an essentially flat background. Different instruments, with different dynamical windows and different resolution capabilities, are needed to observe such motions. For example motions that are too slow to see using the DCS may well show up if the sample is put on the backscattering spectrometer. Conversely motions that are fast by DCS standards can usefully be studied using the FANS spectrometer.

This experiment was initially performed on the DCS (C. Malardier-Jugroot, M. E. Johnson, R. K. Murarka and T. Head-Gordon, *Phys. Chem. Chem. Phys.* (2008) **10**, 4903) with addition information and figures courtesy of Cecile Malardier-Jugroot.

### **VIII. General references**

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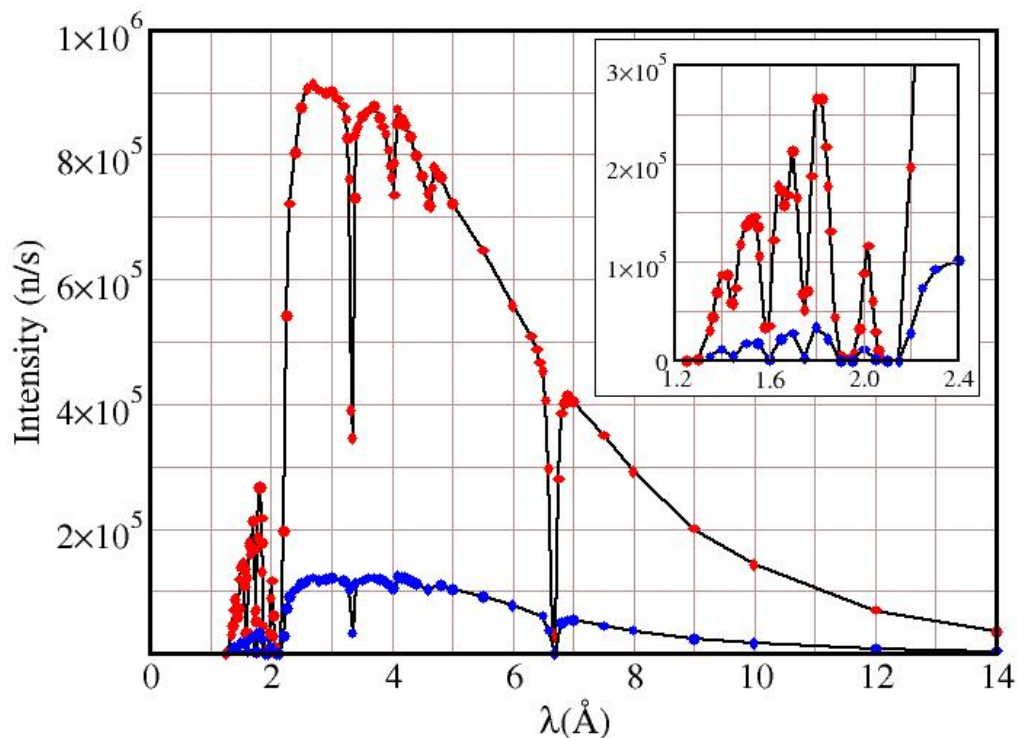
## Appendix A. Instrument Characteristics for the Disk Chopper Spectrometer

(<http://www.ncnr.nist.gov/instruments/dcs>)

The white beam from the cold neutron source is cleaned of high energy neutron and gamma ray contamination using an “optical filter”. This is basically a bent guide which ensures that there is no line of sight from the source to points beyond the local shutter. A cooled graphite filter removes short wavelength ( $\sim 0.5 \text{ \AA}$ ) neutrons that remain in the beam, permitting measurements at wavelengths down to roughly  $1.5 \text{ \AA}$ .

A clean, pulsed, monochromatic neutron beam is produced using seven disk choppers. Chopper speeds may be varied from 1200 to 20000 rpm. The pulsing and monochromating choppers have three slots of different widths. In principle this permits three choices of intensity and resolution at a given wavelength and master chopper speed.

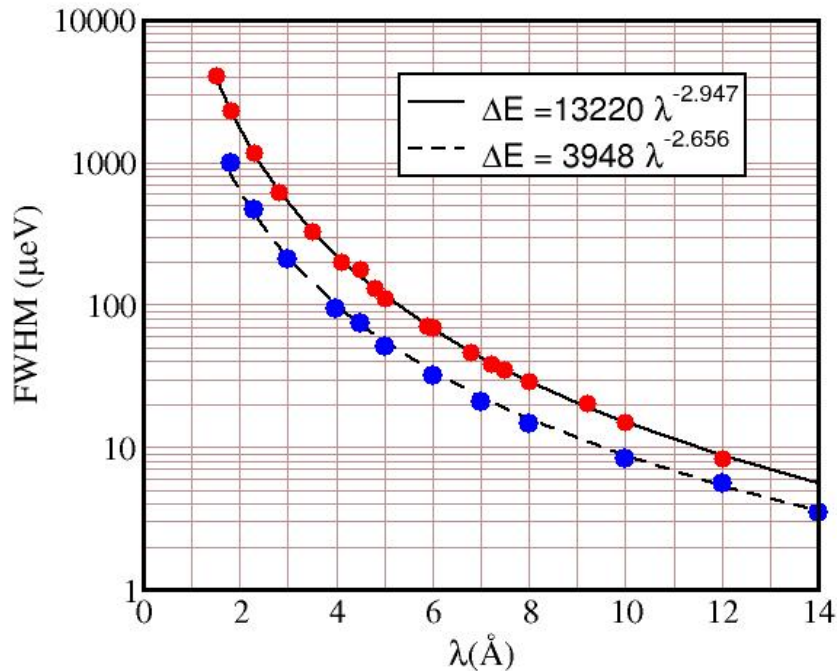
The measured intensity at the sample is reproduced below. Red and blue points (upper and lower plots) correspond to measurements using different chopper slot widths.



Why are there dips in the measured flux at wavelengths near 3.335 and 6.67  $\text{\AA}$ ?  
What's going on around 2  $\text{\AA}$ ?

The resolution of the instrument is approximately triangular and essentially independent of beam height (10 cm) but depends on the width of the beam. Hence samples should ideally be tall and thin rather than short and fat.

The measured elastic energy resolution, for the same choices of chopper slot width as in the intensity plot above, is shown in the figure below. Lines represent fits to the measurements.



An oscillating radial collimator, inside radius 200 mm, outside radius 300 mm, blade separation  $2^\circ$ , is used to reduce the scattering from sample environment structures.

Can you explain how the radial collimator works, and why it is oscillated?

There are 913 six atmosphere  $^3\text{He}$  detectors covering an essentially continuous solid angle of  $\sim 0.65$  steradians and arranged in three banks:

- Middle bank detector scattering angles range from  $-30^\circ$  to  $-5^\circ$  and from  $+5^\circ$  to  $+140^\circ$
- Upper and lower bank angles range from  $-30^\circ$  to  $-10^\circ$  and from  $+10^\circ$  to  $+140^\circ$

The flight distance from sample to detectors is 4010 mm. The flight chamber is purged with argon.

Why is the flight chamber purged with argon?

## Appendix B. Possible Experiments on the Disk Chopper Spectrometer

Phenomena that can be investigated include:

- Translational and rotational diffusion processes, where scattering experiments provide information about time scales, length scales and geometrical constraints; the ability to access a wide range of wave vector transfers, with good energy resolution, is key to the success of such investigations
- Low energy vibrational and magnetic excitations and densities of states
- Tunneling phenomena
- Low Q powder diffraction

Research areas include:

- **Chemistry** --- e.g. clathrates, molecular crystals, fullerenes
- **Polymers** --- bound polymers, glass phenomenon, confinement effects
- **Biological systems** --- protein folding, protein preservation, water dynamics in membranes
- **Physics** --- adsorbate dynamics in mesoporous systems (zeolites and clays) and in confined geometries, metal-hydrogen systems, glasses, magnetic systems
- **Materials** --- negative thermal expansion materials, low conductivity materials, hydration of cement, carbon nanotubes, proton conductors, metal hydrides

## Appendix C. Some useful properties and relationships

### Neutron properties

Mass:	$1.660 \times 10^{-24}$ g
Electric charge:	0
Spin:	$\frac{1}{2}$
Magnetic moment:	-1.913 nuclear magnetons

### Exact relationships

$$\lambda = \frac{h}{mv} \qquad E = \frac{1}{2}mv^2 \qquad k = \frac{2\pi}{\lambda}$$

### Approximate relationships

$$E[\text{meV}] = \frac{81.8}{(\lambda[\text{\AA}])^2}; \quad v[\text{mm}/\mu\text{s}] = \frac{3.956}{\lambda[\text{\AA}]}; \quad E[\text{meV}] = 2.07(k[\text{\AA}^{-1}])^2; \quad 1 \text{ meV} = 8.1 \text{ cm}^{-1}$$

### Appendix D. Spin Incoherence

The strength of the scattering of a neutron by a nucleus, i.e. the neutron scattering length, depends on the spin of the compound nucleus. For an isotope with nuclear spin  $I$  the combined “nucleus + neutron” spin,  $I'$ , has two possible values,  $I^+ = I+1/2$  and  $I^- = I-1/2$ , with which we associate two possible scattering lengths  $b^+$  and  $b^-$ . Each of the possible values of the combined spin has  $2I'+1$  possible spin states, i.e.  $2(I+1/2)+1 = 2I+2$  and  $2(I-1/2)+1 = 2I$  states respectively, for a total of  $4I+2$  spin states.

If the neutron and nuclear spins are randomly orientated, all states are equally probable, and the probabilities of the combined + and - spin states are  $p^+ = (I+1)/(2I+1)$  and  $p^- = I/(2I+1)$  respectively.

The mean scattering length,  $\langle b \rangle$ , and the mean of the scattering length squared,  $\langle b^2 \rangle$ ,

$$\langle b \rangle = p^+ b^+ + p^- b^- \quad \text{and} \quad \langle b^2 \rangle = p^+ (b^+)^2 + p^- (b^-)^2$$

are used to calculate the coherent and incoherent bound cross sections. These cross sections are defined as follows:

$$\sigma_{\text{coh}} = 4\pi \langle b \rangle^2 \quad \text{and} \quad \sigma_{\text{inc}} = 4\pi (\langle b^2 \rangle - \langle b \rangle^2).$$

Working through the numbers for hydrogen and deuterium is instructive. The relevant scattering lengths for hydrogen are  $b^+ = 1.086 \times 10^{-12}$  cm and  $b^- = -4.751 \times 10^{-12}$  cm, whereas the values for deuterium are  $b^+ = 0.951 \times 10^{-12}$  cm and  $b^- = 0.095 \times 10^{-12}$  cm.