Using SANS to Investigate Chaining of Bio-Magnetic Nanoparticles

1. Introduction

Senior Scientific, LLC uses 25 nm superparamagnetic magnetite (Fe_3O_4) nanoparticles (NPs) [1] for early detection of cancer via magnetic relaxometry (MRX) [2]. The basic idea is that the NPs are coated with a proprietary, biocompatible, amphiphilic polymer coating containing antibodies that will attach to the receptors of specific, pathogenic cells of interest. After a short period of magnetization using a small, applied magnetic field, the superparamagnetic NPs will magnetically relax in the absence of an applied field at different rates depending on whether they are bound or unbound to local cells. Specifically, unbound NPs relax via Brownian motion (physical rotation of the NP) while bound NPs relax via the slower route of Néel relaxation (reorientation of the electron orbits within the NP), Figure 1. The differences in the relaxation of the NPs' remanence fields are measurable using a SQUID sensor array.

Ultimately, the sensitivity of the technique depends largely on the properties of the nanoparticles (low size/shape dispersity, high magnetism), as well as the specificity and stability of the nanoparticle-antibody conjugate. It also depends on knowing whether the NPs are in fact fully separated (the so-called dilute limit) or interact with one another.
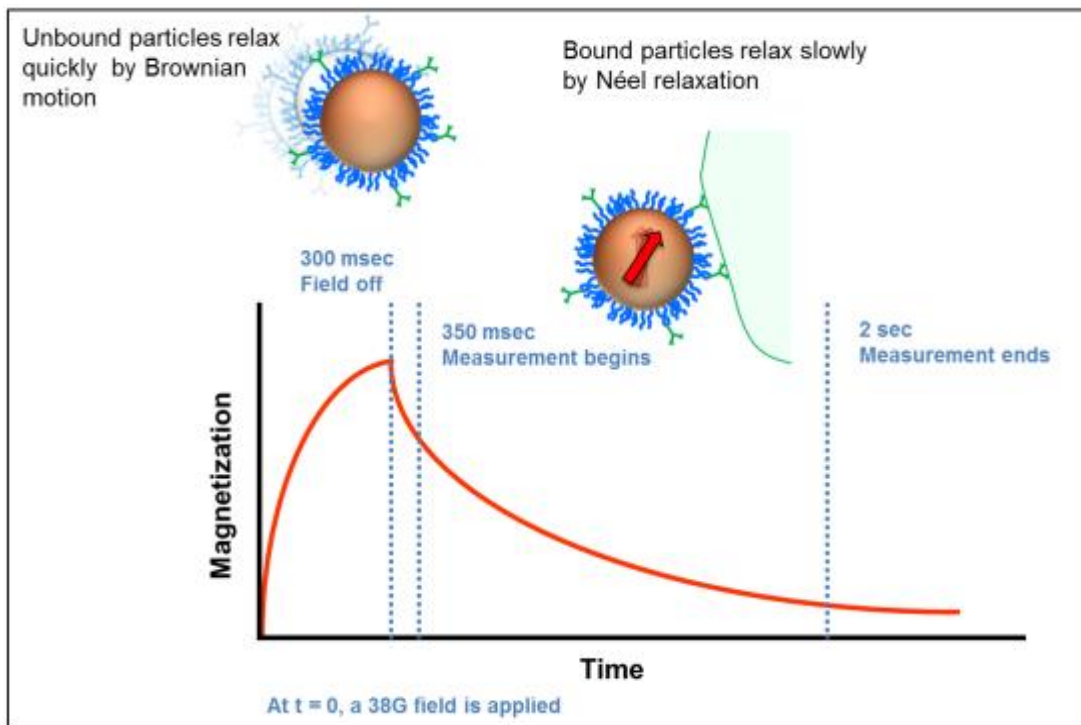

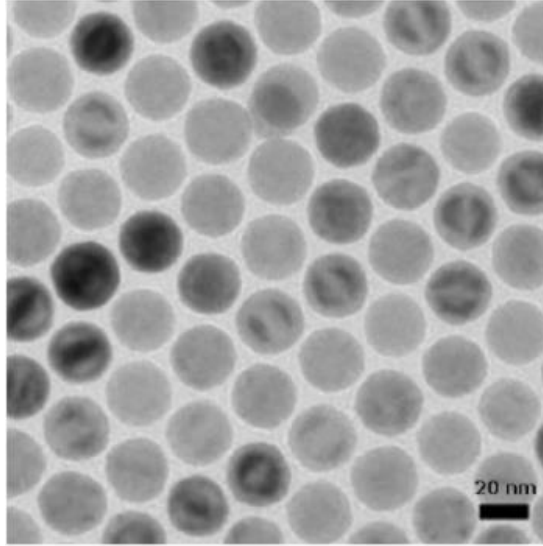Figure 1. SQUID relaxometry measurement.

Figure 2. Transmission electron microscopy image of iron oxide nanoparticle cores with an average diameter of 25 nm. Figure reproduced from [1].

The aim of this experiment is to characterize the physical and magnetic structure of Fe_3O_4 nanoparticles suspended in H_2O (or D_2O). In addition, a goal is to determine if magnetic and/or steric interactions among the nanoparticles in the colloid, which has a concentration of 2 mg/ml, gives rise to the formation of any chains, clusters or agglomerates.
Using SANS your task is to:

1) Characterize the polymer shell thickness and scattering length density by nearly-contrast matching an aqueous solution to the metal-oxide nanoparticle cores
2) Learn to recognize the telltale signs of particle agglomeration and quantify the degree to which a system of polymer-coated magnetic nanoparticles intrinsically chain/agglomerate
3) Determine how any NP ensemble responds to an applied magnetic field (0-1.5 T).

2. Why Use SANS?

Small-angle x-ray scattering (SAXS) and small-angle neutron scattering (SANS) provide similar information regarding the macroscopic measurement of scattering cross-section, $d\Sigma / d\Omega(q)$. Yet, neutron scattering distinguishes itself in several ways: 1) strong hydrogen scattering cross-section, 2) sensitivity to hydrogen-deuterium substitution, 3) ability to contrast match many samples to solution based on hydrogen-deuterium content, and 4) sensitivity to magnetism. The former properties make neutrons ideal for study of many biological and polymer systems; the latter makes neutrons ideal for studying magnetic systems. Additionally, the manner in which the neutron spin processes upon scattering allows both the magnitude and *orientation* of sample magnetic moments to be precisely determined in PASANS (polarization analyzed small-

angle neutron scattering) experiments [3-5] – to be briefly discussed, though not performed during this summer school.

3. The SANS Instrument

SANS instrument NG7 is optimized to cover a q-range of 0.008 nm^{-1} to 7 nm^{-1}, which translates to features sizes below 1 nm and up to 500 nm. Recall that q (sometimes denoted Q) = 2π sin(α) /λ ≈ 2π/distance, where α is the scattering angle on the detector with respect to the transmitted neutron beam center. The neutron wavelength (λ) may be tuned between 0.5 nm and 2 nm with a wavelength spread between 11% and 22% full-width half-maximum.
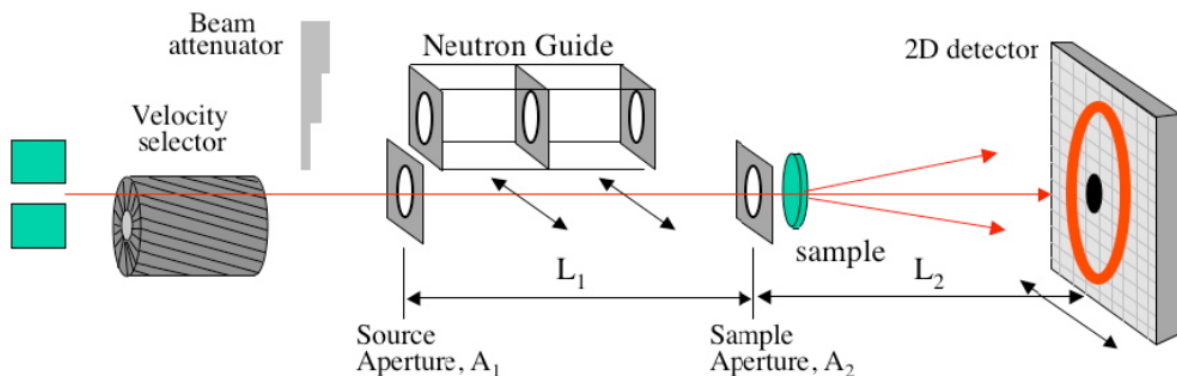

Figure 3 shows a schematic of the 30 meter SANS beamline at the NCNR.

The intensity of the scattering on the detector after background correction in the SANS experiment is given by

$$I_{meas} = \phi A dT (\frac{d\Sigma}{d\Omega}) \Delta \Omega \varepsilon t$$

where

ϕ is the number of neutrons per second per unit area incident on the sample

A is the sample area

d is the sample thickness

T is the sample transmission

$\Delta\Omega$ is the solid angle over which scattered neutrons are accepted by the analyzer

ε is the detector efficiency

t is the counting time

The aim of the SANS experiment is to obtain the differential macroscopic scattering cross-section $d\Sigma/d\Omega$ from I_{meas}. Data reduced in this way is said to be on "absolute scale" and should be easily comparable from one neutron scattering facility to another.

4. Planning The Experiment

4a. Scattering Length Density

In order for there to be small-angle scattering, there must be scattering contrast, in this case between the nanoparticles, oleic acid coating, and solvent. The scattering is proportional to the scattering length density (abbreviated SLD or symbolized as ρ) *squared*. SLD is defined as

$$\rho = \frac{1}{V} \sum_{i}^{n} b_i$$

where V is the volume containing n atoms, and b_i is the (bound coherent) scattering length of the ith atom in V. V is usually the molecular or molar volume for a homogeneous phase in the system of interest.

Neutrons are scattered either through interaction with the nucleus (nuclear scattering, N) or through interaction between the unpaired electrons (and hence the resultant magnetic moment, M) and the neutron spin. Hence, Fe_3O_4 has both a nuclear SLD and a magnetic SLD and will display both nuclear and magnetic contrast.

Nuclear SLDs can be calculated from the above formula, using a table of the scattering lengths [6] for the elements, or calculated using the interactive SLD Calculator available at the NCNR's web pages [7]. Magnetic SLD can be calculated using the following formula

$$\rho_m = M \left(in \ \frac{A}{m} \right) x \ 2.853 \ x \ 10^{-6} \ \frac{m}{A \ \text{Å}^{-2}}$$

Some handy magnetic conversions are:

$$\frac{A}{M} = 1000 \frac{emu}{cc}; \ \mu_B = 9.274 \ x \ 10^{-21} \ emu$$

The magnetic saturation of *bulk* Fe_3O_4 is 513 emu/cc. Note that nanoparticles may vary from bulk due to dislocations and from the effect of surface termination sites. Table 1 provides some useful SLDs for our experiment.

Material (bulk)	Chemical Formula	SLD_nuclear (Å^{-2})	SLD_magnetic (Å^{-2})
Water	H_2O	-5.605 x 10^{-7}	0
Heavy water	D_2O	6.35 x 10^{-6}	0
Magnetite	Fe_3O_4	6.91 x 10^{-6}	1.46 x 10^{-6}
Oleic Acid	$C_{18}H_{34}O_2$	7.81 x 10^{-8}	0

Table 1. Nuclear and magnetic scattering length densities of interest

4b. Sample Thickness

Given the calculated sample contrast, how thick should the sample be? Recall that the scattered intensity is proportional to the product of the sample thickness, d_s, and the sample transmission, T. It can be shown that the transmission, which is the ratio of the transmitted to the incident beam intensity, is given by

$$T = e^{-\Sigma_t d_s}$$

where $\Sigma_t = \Sigma_c + \Sigma_i + \Sigma_a$ (the sum of the coherent, incoherent, and absorption macroscopic cross sections). The absorption cross section, Σ_a , can be accurately calculated from tabulated absorption cross sections of the elements (and isotopes) if the mass density and chemical composition of the sample are known. The incoherent cross section, Σ_i ,can be estimated from the cross section tables for the elements as well, but not as accurately as it depends on the atomic motions and is, therefore, temperature dependent. The coherent cross section, Σ_c , can also only be estimated since it depends on the details of both the structure and the correlated motions of the atoms in the sample. This should be no surprise as Σ_c as a function of angle is the quantity we are aiming to measure!

The scattered intensity is proportional to d_sT and hence

$$I_{meas} \propto d_s e^{-\Sigma_t d_s}$$

which has a maximum at $d_s = 1/\sum_t$ and implies an optimal transmission at $1/e = 0.37$. The sample thickness at which this occurs is known as the "1/e" length, which is about 1.55 cm for D_2O and only 0.175 cm for H_2O [7]. However, one must be also be wary of multiple scattering for transmissions less than ≈ 0.90.

4c. SASCALC

SASCALC is a tool built into the SANS IGOR reduction package that allows different beamline configurations to be simulated, helping users to select an ideal balance between desired q-range and maximum beam intensity. Since we are looking for interactions among 25 nm nanoparticles, two example configurations for this experiment would be:

```
Configuration #1
Source Aperture Diameter =             5.08 cm
Source to Sample =              1262 cm
Sample Aperture to Detector =   1160 cm
Beam diameter =                 7.46 cm
Beamstop diameter =             3.00 inches
Minimum Q-value =               0.0040 1/Å (sigQ/Q = 30.4 %)
Maximum Horizontal Q-value = 0.0290 1/Å
Maximum Vertical Q-value =       0.0290 1/Å
Maximum Q-value =               0.0410 1/Å (sigQ/Q = 5.5 %)
Beam Intensity =                493476 counts/s
Figure of Merit =               1.78e+07 Å^2/s
Attenuator transmission =       0.0108 = Atten # 5
***************** NG7 *** NG7 *****************
Sample Aperture Diameter =             1.27 cm
Number of Guides =              2
Sample Chamber to Detector =    1100.0 cm
Sample Position is              Huber
Detector Offset =               0.0 cm
Neutron Wavelength =            6.00 Å
Wavelength Spread, FWHM =       0.115
Sample Aperture to Sample Position =   5.00 cm
Lenses are OUT
```

```
Configuration #2
Source Aperture Diameter =              5.08 cm
Source to Sample =                     487 cm
Sample Aperture to Detector =          440 cm
Beam diameter =                        7.35 cm
Beamstop diameter =                    3.00 inches
Minimum Q-value =             0.0113 1/Å (sigQ/Q = 28.4 %)
Maximum Horizontal Q-value = 0.0769 1/Å
Maximum Vertical Q-value =          0.0769 1/Å
Maximum Q-value =             0.1086 1/Å (sigQ/Q = 5.5 %)
Beam Intensity =               2405548 counts/s
Figure of Merit =              8.66e+07 Å^2/s
Attenuator transmission =      0.00216 = Atten # 7
***************** NG7 *** NG7 *****************
Sample Aperture Diameter =             1.27 cm
Number of Guides =                     7
Sample Chamber to Detector =           380.0 cm
Sample Position is                     Huber
Detector Offset =                      0.0 cm
Neutron Wavelength =                   6.00 Å
Wavelength Spread, FWHM =              0.115
Sample Aperture to Sample Position =   5.00 cm
Lenses are OUT
```

We may optimize these further and/or run an additional, higher-q configuration.

4d. Magnetic field

In neutron scattering only the component of a magnet moment oriented perpendicular to the scattering wave vector, q, participates in scattering [3]. Thus, if an applied magnetic field (H) is set along X and the detector is in the X-Y plane, magnetic moments oriented along Y or Z (M_Y, M_Z) may be detected if measuring along the X-direction, while magnetic moments oriented along X or Z (M_X, M_Z) may be detected if measuring along the Y-direction (Figure 4).
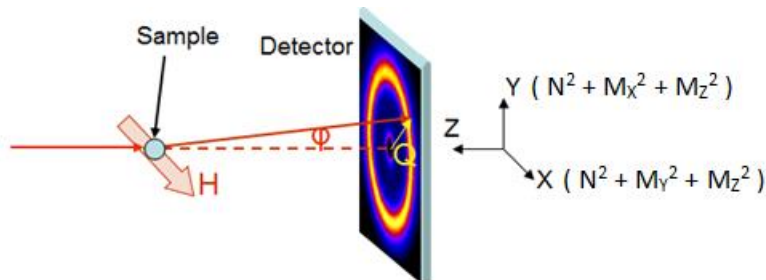
Figure 4. Scattering with an applied magnetic field, H, set along the X-direction. Note how only the components of the sample magnetism perpendicular to q (noted on right hand side) may be seen in the scattered intensity.

Assuming that the nuclear scattering is isotropic, this gives a way to separate nuclear and magnet scattering contributions. The measured scattering intensity, I, is proportional to the squared sum of the spatial nuclear (N^2) and magnetic (M^2) Fourier transforms, defined as

$$N, M_J(q) = \sum_K \rho_{N,M_J}(K)e^{iq \cdot R_K}$$

where J is any Cartesian coordinate, $\rho_{N,M}$ is the nuclear or magnetic scattering length density, and R_K is the relative position of the Kth scatterer. Note that because we can only measure the absolute value of the Fourier Transform squared in a scattering experiment, rather than the complex components of the Fourier Transform itself, we lose *phase* information. The result is that we may not be able to uniquely distinguish between a family of curves that model our data – a fact that should be kept in mind during data fitting and analysis.

5. <u>Data Collection and Reduction</u>

The SANS IGOR Analysis package [8] will be used to properly scale and reduce your data onto an absolute scale. In order to do this, you will need to collect several types of data files. Transmissions are collected with the beam stop removed (translated to the side) in order to survey the unscattered neutron beam; these are taken with a series of attenuating filters to insure this main neutron beam isn't so intense so as to burn the detector. Scattering files, on the other hand, make use of a beam stop that blocks this main beam in order to emphasize the weaker sample scattering observed at higher angles. In this case, a sufficiently large beam stop (1" to 4" possible depending on detector distance and beam collimation) is chosen so that attenuating filters are typically not required. Note that a transmission file will be used to define the beam center (from which Q is calculated), while a scattering file will be used to align the beam stop with respect to the main beam.

An abbreviated list of the data reduction steps includes:

5.1   Sample scattering files will be acquired at several detector distances to cover an optimal q-range

5.2   Corresponding transmission files of the sample (NPs + solvent + sample holder), empty (sample holder only), and open beam will be used to determine [and correct for] absorption.

5.3   The "open" transmissions described above will additionally be used for absolute-scale intensity normalization.

5.4   "Empty" scattering files will be taken to remove the scattering resulting from the sample holder + any main beam spillover (around the beam stop)

5.5   Background scattering files will be taken using a beam block in order to remove electronic noise and spurious neutron scattering from nearby experiments

5.6   Sector cuts which consist of a summation of angles centered parallel and perpendicular to the applied magnetic field will be performed

5.7   Data sets from different detector distances and angles will be joined to create a single data file for each sample condition + angle (*i.e.* parallel or perpendicular to the applied field)

6 <u>Data Analysis</u>

The SASView package [9] will be used to analyze your reduced data. It provides a variety of useful, built-in model such as core-and-n-shell nanoparticles [10]. Additionally, the package takes care of instrumental q-smearing, sample polydispersity, and allow the user to define and fit variables of interest. Perhaps the best aspect of SASView is that it allows users to construct their own custom models to capture the specific features of their samples. For example, in order to model the core-shell nanoparticles solvated in H_2O under a variety of applied magnetic fields for instance, we have constructed a linear chains model with a Gaussian distribution of chain angles about the applied field direction which allows for the combination of single NPs, dimers, trimers, quadramers, and pentamers. Example fits are shown in Figure 5.
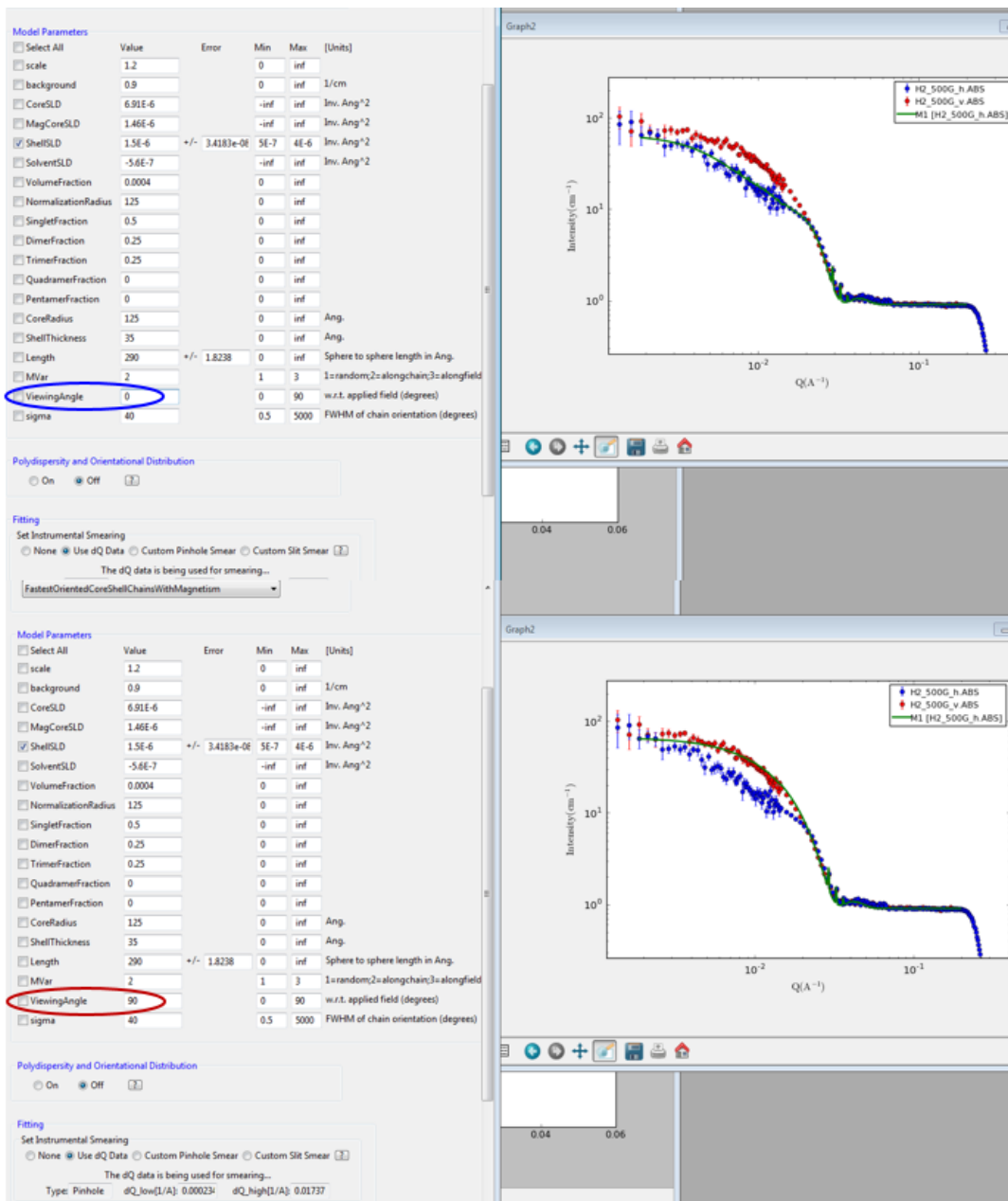
Model Parameters

Select All	Value	Error	Min	Max	[Units]
scale	1.2		0	inf	
background	0.9		0	inf	1/cm
CoreSLD	6.91E-6		-inf	inf	Inv. Ang^2
MagCoreSLD	1.46E-6		-inf	inf	Inv. Ang^2
ShellSLD	1.5E-6	+/- 3.4183e-0E	5E-7	4E-6	Inv. Ang^2
SolventSLD	-5.6E-7		-inf	inf	Inv. Ang^2
VolumeFraction	0.0004		0	inf	
NormalizationRadius	125		0	inf	
SingletFraction	0.5		0	inf	
DimerFraction	0.25		0	inf	
TrimerFraction	0.25		0	inf	
QuadramerFraction	0		0	inf	
PentamerFraction	0		0	inf	
CoreRadius	125		0	inf	Ang.
ShellThickness	35		0	inf	Ang.
Length	290	+/- 1.8238	0	inf	Sphere to sphere length in Ang.
MVar	2		1	3	1=random;2=alongchain;3=alongfield
ViewingAngle	0		0	90	w.r.t. applied field (degrees)
sigma	40		0.5	5000	FWHM of chain orientation (degrees)

Polydispersity and Orientational Distribution
○ On ● Off

Fitting
Set Instrumental Smearing
○ None ● Use dQ Data ○ Custom Pinhole Smear ○ Custom Slit Smear
The dQ data is being used for smearing...

FastestOrientedCoreShellChainsWithMagnetism

Model Parameters

Select All	Value	Error	Min	Max	[Units]
scale	1.2		0	inf	
background	0.9		0	inf	1/cm
CoreSLD	6.91E-6		-inf	inf	Inv. Ang^2
MagCoreSLD	1.46E-6		-inf	inf	Inv. Ang^2
ShellSLD	1.5E-6	+/- 3.4183e-0E	5E-7	4E-6	Inv. Ang^2
SolventSLD	-5.6E-7		-inf	inf	Inv. Ang^2
VolumeFraction	0.0004		0	inf	
NormalizationRadius	125		0	inf	
SingletFraction	0.5		0	inf	
DimerFraction	0.25		0	inf	
TrimerFraction	0.25		0	inf	
QuadramerFraction	0		0	inf	
PentamerFraction	0		0	inf	
CoreRadius	125		0	inf	Ang.
ShellThickness	35		0	inf	Ang.
Length	290	+/- 1.8238	0	inf	Sphere to sphere length in Ang.
MVar	2		1	3	1=random;2=alongchain;3=alongfield
ViewingAngle	90		0	90	w.r.t. applied field (degrees)
sigma	40		0.5	5000	FWHM of chain orientation (degrees)

Polydispersity and Orientational Distribution
○ On ● Off

Fitting
Set Instrumental Smearing
○ None ● Use dQ Data ○ Custom Pinhole Smear ○ Custom Slit Smear
The dQ data is being used for smearing...
Type: Pinhole   dQ_low[1/A]: 0.00023   dQ_high[1/A]: 0.01737

Figure 5. Example fits of Fe_3O_4 core + polymer shell NPs in an oriented linear chain parallel to an applied magnetic field (top) and perpendicular to an applied field (bottom) of 500 G.

Your data will look distinctly different from the NPs solvated in H_2O since (a) the magnetite core will be nearly contrast matched to the D_2O solvent and (b) the incoherent background will be significantly reduced. Your task will be to determine the degree to which your sample chains and whether the polymer shell responds to a change of solvent. Please note that because the data are placed on an absolute scale, calculation of the volume fraction becomes meaningful
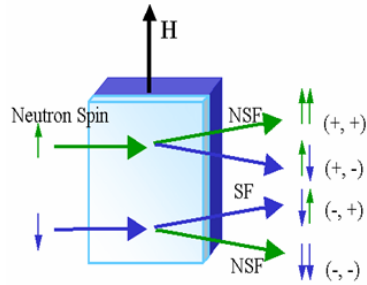
and important for determining accurate scattering length densities. Bulk magnetite has a density of 5.17 g/cc. If there are 2 mg/ml of magnetite nanoparticle cores in the solution this corresponds to a volume fraction of 0.0004. This in turn means that the normalization volume of the sample should correspond to the magnetite radius (125 Å) rather than the core plus polymer shell radius.

References

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[10] ftp://ftp.ncnr.nist.gov/pub/sans/kline/Download/SANS_Model_Docs_v4.10.pdf

Supplementary Information On Spin Selection Rules

The original rule (discussed for unpolarized neutrons in section 4d) that only the component of M ⊥ Q can participate in scattering also holds true for polarized neutrons. Additionally, of this projection of M ⊥ Q, the part that is also || to the neutron polarization axis (defined by H) does not reverse the neutron spin. The remaining projection of M ⊥ Q that is ⊥ to the neutron polarization axis (defined by H) does reverse the neutron spin. These processes are denoted as non spin-flip (NSF) and spin-flip (SF) scattering, respectively. The nuclear scattering does not affect the neutron spin, and so all structural scattering is confined to the NSF scattering. These rules are summarized in Figure 6.

Figure 6. A summary of the nuclear and magnetic spin scattering selection rules.

While it is beyond the scope of this experiment, please note that polarization analysis allows for the measurement of magnetic scattering even if it is (a) dominated by the structural scattering or (b) not aligned with an applied magnetic field, but randomly oriented in space such that its net sum is zero. Please note that an applied magnetic field required to align the neutron spins may be quite small (say 0.005 Tesla or less).