JOINT INSTITUTE FOR NUCLEAR RESEARCH
Frank Laboratory of Neutron Physics (FLNP)

FINAL REPORT ON THE START PROGRAMME

Small Angle Scattering Study of Nanosystems

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Contents

1) Abstract

Small-angle neutron scattering (SANS) is a fundamental technique for studying condensed matter, with the analysis of biological macromolecules and nanoparticle systems being among its most relevant applications. This is due to its ability to provide detailed structural information about molecules in their native environment. This report will highlight several key aspects: the importance of proper sample preparation, the necessity of having monodisperse samples and the influence of the solvent on sample properties. Sample concentrations will be estimated using UV-vis spectroscopy, and the results will be compared with data obtained from small-angle neutron scattering experiments performed on the YuMO spectrometer. For raw data processing, specialized software packages will be used, including SAS, SasView, Raw BioXTAS, ATSAS (Primus), and Origin 2025. Finally, the structural features obtained will be analyzed and compared with findings reported in the scientific literature.

2) Introduction

Nanosystems are promising for advanced applications in therapy, diagnosis, and bioimaging. In medicine, drug delivery systems are among the most powerful tools because they encapsulate drugs and deliver them to specific target sites in the body. These systems include a wide variety of morphologies and structures, such as micelles, vesicles, and polymeric nanoparticles, which typically encapsulate enzymes and form interactions with proteins. These systems feature complex, hierarchically organized nanostructures across multiple length scales, which complicates their characterization, especially when attempting to understand the interactions and macromolecular conformations[1].

Small-angle scattering is fundamental for the study of condensed matter. One of the main fields covered by this technique is the study of biological macromolecules and nanoparticle systems[2]. Small-angle neutron scattering (SANS) allows the characterization, in a non-destructive way, of small particles (1–100 nm), providing statistical data representative of the whole sample. One of the major advantages of this technique is that it can provide structural information about molecules in their native medium. This benefit arises from scattering at small angles, which yields information about structural inhomogeneities in electron density and certain characteristic dimensions[2].

Additionally, this method allows researchers to obtain low-resolution structural information on the shape and internal structure of particles, even in the absence of crystals[2].

The principle of the technique is as follows: a monochromatic beam of thermal neutrons is directed at a sample, and the neutrons are scattered by the atomic nuclei. The neutron also interacts with the spin of the sample, which is why the neutron scattering length consists of two terms:

$$f_n = f_p + f_s$$

Here, f_s carries structural information only if the neutron spins in the incident beam and the nuclear spins in the sample are oriented. If they are not, the scattering yields only a flat incoherent background[2].

In the case of f_p this value does not increase monotonically with the atomic number; instead, it is sensitive to the isotopic content. This is why the scattering cross-section of nuclei from isotopes of the same element can vary significantly. Consequently, isotopic substitution can be used to enhance contrast[3].

The orientations of the particles lead to rotational averaging, which yields a one-dimensional (1D) scattering profile: I (q) versus q, as is expressed in *Figure 1*. Here "q" depends on the half of the scattering angle (**θ)** and on the wavelength of the radiation (λ)[4].

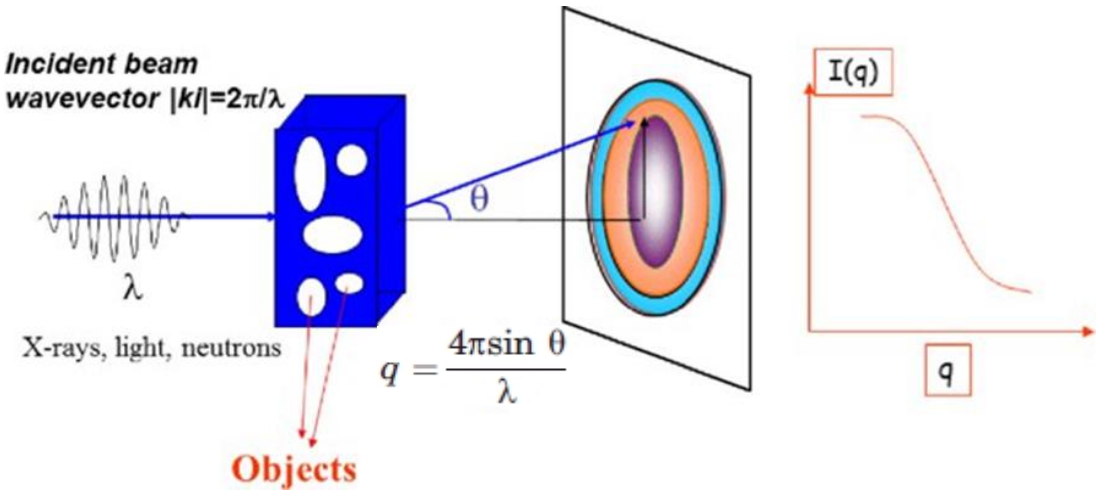


***Figure 1. Principle of the Small Angle Neutron Scattering experiment*[5]**

This technique provides important information about biomolecular complexes, assemblies of proteins, membranes, and dynamic systems. Although SANS experiments require relatively simple sample preparation, it is fundamental to have monodisperse particles in solution. This is because the random orientation of particles in solutions leads to spherical averaging of the single-particle scattering, yielding a one-dimensional scattering pattern, especially for the interpretation of the scattering curves in terms of three-dimensional (3D) structure[2,4].

These advances have opened exciting opportunities to learn about the size, shape, folding, recognition, flexibility, and disorder of soluble single or multidomain proteins. Moreover, SANS can be judiciously combined with contrast matching for two component systems[6].

In structural biology, SANS is mostly used to determine structural parameters of biomolecules, such as the radius of gyration [Rg], scattering volume, interatomic distance distribution P(r) or aggregation parameters[7].

To obtain information about the structural characterization and parameters of the biomolecular components within an assembly, it is necessary to manipulate the neutron scattering contrast. This is achieved through solvent exchange (H_2O/D_2O), primarily due to the different neutron scattering characteristics of ^1H(hydrogen) and ^2H (deuterium)[8].

Ferritin is a highly spherical shape large protein consisting of 24 ferritin polypeptides and comprises central part containing iron-based molecules. The apoferritin is a protein complex which is a ferritin shell (without iron core). Apoferritin may encapsulate small molecules, making it an interesting stable system for drug delivery. SAS is an important approach that could be used to determine structural parameters of apoferritin under native conditions[8].

Bovine serum albumin (BSA) is a globular protein composed of 585 amino acid residues, including 35 cysteines that form 17 disulfide bridges. These bridges contribute to the protein's relatively high stability[9]. Due to its ability to bind and transport various molecules, such as fatty acids, dyes, metals, amino acids, and pharmaceutical compounds, BSA is frequently employed in drug delivery systems as a carrier. It is also widely used in studies examining drug-surfactant interactions[9,10].

a) Project goals

The goal of this project is to acquire fundamental knowledge in sample preparation, understand the required conditions for analysis, and learn data processing using specialized software tools such as SAS, SasView, and ATSAS. The study will focus on the analysis of apoferritin and bovine serum albumin (BSA), proteins of significant interest in nanosystems and drug delivery applications. To address sample heterogeneity and interparticle interactions, it will be employ size-exclusion chromatography (SEC). Additionally, UV-visible spectroscopy measurements will be performed prior to small-angle scattering (SAS) experiments to determine sample concentrations using UV extinction coefficients. These measurements are essential for calculating the molecular weight of scattering species via the I(0) parameter. All experiments will be conducted at the Frank Laboratory of Neutron Physics, Joint Institute for Nuclear Research, in Dubna, Russia.

b) Scope of work

- Preparation of samples of protein solutions for small-angle neutron scattering (change of water buffer with the deuterated PBS buffer).
- Estimation of protein concentration in solution.
- Performing the experiment on a small angle scattering spectrometer
- Primary processing of raw data,
- Analysis of small angle scattering curves and obtaining structural characteristics of nanosystems.

3) Methodology

a) Reagents

- Apoferritin in storage buffer (50% glycerol and 0.075 M NaCl)
- Deuterium D_2O
- Bovine serum albumin (BSA)
- Bicinchoninic acid solution (B9643)
- Copper (II) sulfate pentahydrate (4% solution)

b) Methods

The experimental workflow comprised several key analytical steps to characterize protein samples and prepare them for structural analysis.

i) Size exclusion chromatography (SEC) for apoferritin

Size exclusion chromatography (SEC) was employed to assess the monodispersity of apoferritin and characterize its molecular size distribution under native conditions.

Prior to SEC analysis, a buffer exchange step was performed to remove glycerol and transfer the sample into the running buffer: 5 mM Na_2HPO_4 pH 7.3. This exchange was carried out using centrifugal ultrafiltration (4696xg – 10 minute - 8°C), following the general methodology described by Zabelskii et al. (2018)[7], with minor modifications.

The SEC separation was performed using a column, directly coupled to the sample-inlet valve of the BM29 sample capillary system. The running buffer was filtered through a 0.22 µm membrane filter and degassed to prevent bubble formation and ensure stable baseline readings.

The column was equilibrated with two column volumes of running buffer at a flow rate of 0.5 ml/min at room temperature. Baseline stability was monitored via UV absorbance at 280nm until a flat, noise-free signal was achieved.

A volume of 500 µL of the apoferritin solution was injected using a Hamilton syringe. The sample was then separated using an isocratic elusion at a flow rate of 1mL/min. Fractions of 500 µL each were collected in individual tubes. Elution progress was monitored by UV absorbance at 215nm, 260 nm, 280 nm and 350 nm. Fractions showing a clear peak at 280 nm indicative of protein content and apoferritin monomers, were identified and pooled into a Falcon tube.

Since the final SEC fractions were in PBS prepared with H_2O, a final buffer exchange into PBS prepared with D_2O was necessary. This exchange was performed three times via centrifugal ultrafiltration (4696xg – 10 minute - 8°C), a filter membrane of 100KDa was used, since apoferritin has a molecular weight of 450 – 500 KDa. After ultrafiltration, the concentrated sample was resuspended in PBS (D_2O) to the desired final volume.

ii) Protein quantification

(1) UV-vis spectroscopy

This method involves measuring apoferritin concentration using the extinction coefficient method. The concentration of apoferritin was determined using UV visible spectroscopy at a wavelength of 280 nm. Apoferritin is composed of 24 subunits, comprising both heavy and light chains, each with distinct extinction coefficients:

- Heavy chain: 0.755 $(mg/mL)^{-1} \cdot cm^{-1}$
- Light chain: 0.729 $(mg/mL)^{-1} \cdot cm^{-1}$

For this analysis, the extinction coefficient corresponding to the light chain was selected. This coefficient indicates that an absorbance of 0.729 at 280 nm corresponds to a protein concentration of 1 mg/mL (assuming a path length of 1 cm). A cuvette with a path length of 0.2 mm was employed.

(2) Bicinchoninic acid (BCA) protein assay

The bicinchoninic acid (BCA) protein assay was performed to determine the concentration of apoferritin and bovine serum albumin (BSA). This colorimetric method relies on the reduction of Cu^{2+} to Cu^{+} by protein in an alkaline medium, followed by the formation of a purple-colored complex between Cu^{+} and BCA, which can be quantified spectrophotometrically[11].

First, a working BCA reagent was prepared by combining 2500 µL of BCA solution and 50 µL of $CuSO_4$ solution. The mixture was thoroughly mixed until a homogeneous solution was obtained. We evaluate four samples of BSA (5 mg/mL in PBS with D_2O, 2 mg/mL in PBS with D_2O, 5 mg/mL in PBS with H_2O and 2 mg/mL in PBS with H_2O) and three samples of apoferritin (10 mg/mL, 5 mg/mL and 1 mg/mL). Each sample (10 µL) was mixed with 200 µL of the freshly prepared BCA working reagent. After mixing, the reaction tubes were incubated at 37°C for 30 minutes to allow the color development[11].

To determine protein concentrations, a calibration curve was constructed using BSA standard solutions at the following concentrations: 0 mg/mL, 0.2mg/mL, 0.4mg/mL, 0.6 mg/mL, 0.8 mg/mL and 1 mg/mL. The same procedure was applied to the standards as to the samples[11].

In all cases, following incubation, the absorbance of each solution was measured at 562 nm using a UV-Vis spectrophotometer. The resulting absorbance values were used to interpolate protein concentrations from the BSA standard curve[11].

iii) Sample preparation for small-angle neutron scattering (SANS)

Samples were prepared for small-angle neutron scattering (SANS) measurements using standard quartz cuvettes with a 1mm path length. The preparation protocol followed a systematic approach to ensure accurate background subtraction. For each sample, a matching solvent cuvette was

prepared to serve as a background reference. For samples dispersed in PBS D_2O, a reference cuvette containing pure PBS D_2O was prepared and for samples dispersed in PBS H_2O, a corresponding reference cuvette with PBS H_2O was used. Additionally, separate cuvette containing pure D_2O was prepared to enable assessment of solvent contributions. All cuvettes were carefully filled to avoid air bubbles and ensure uniform sample distribution. The filled cuvettes were then sealed with parafilm to prevent evaporation or contamination prior to measurement. SANS experiments were conducted using the YuMO spectrometer in a two-detector configuration mode, allowing for extended-range coverage and enhanced data quality.

iv) Processing of the raw data

For the treatment of raw scattering data and extraction of the sample minus buffer 1D profiles from SANS experiments, it was employed SAS analysis software. The resulting spectra were calibrated to an absolute intensity scale using a vanadium scatterer as a standard. To determine key structural parameters such as, including volume fraction, particle radius, and thickness, it was used SasView, software employed for data modeling and analysis. SAXS data processing was performed using Raw BioXTAS. For more advanced and comprehensive data processing, we leveraged the ATSAS software. Graphical representations and data visualization were generated using Origin 2025.

4) Results and discussion

a) Apoferritin quantification using extinction coefficient

The measured absorbance value was A_{280} = 0.272, so using the light-chain extinction coefficient and accounting for the actual path length, the apparent concentration was calculated (Equation 1) as:

$$Concentration = \frac{A_{280}}{\varepsilon * l} \qquad (1)$$

Where:

- A_{280} = measured absorbance
- ε = extinction coefficient - 0.729 (mg/mL) $^{-1}$·cm^{-1}
- l = path length

$$Concentration = \frac{0.272}{0.729 * 0.02}$$
$$Concentration = 0.372\ mg/mL$$

Since the sample had been measured in a cuvette of 0.02 cm, the apparent concentration was multiplied by the dilution factor to obtain the final concentration:

$$Concentration = 0.372 \frac{mg}{mL} * 50 = 18.63\ mg/mL$$

The UV-Vis analysis yielded a final apoferritin concentration of 18.63 mg/mL, based on this concentration, serial dilutions were performed to prepare apoferritin 10 mg/mL, 5 mg/mL and 1 mg/mL for subsequent assays.

b) Apoferritin Bicinchoninic acid (BCA) protein assay

Figure 2 shows the results of the BSA calibration curve and the apoferritin quantification.

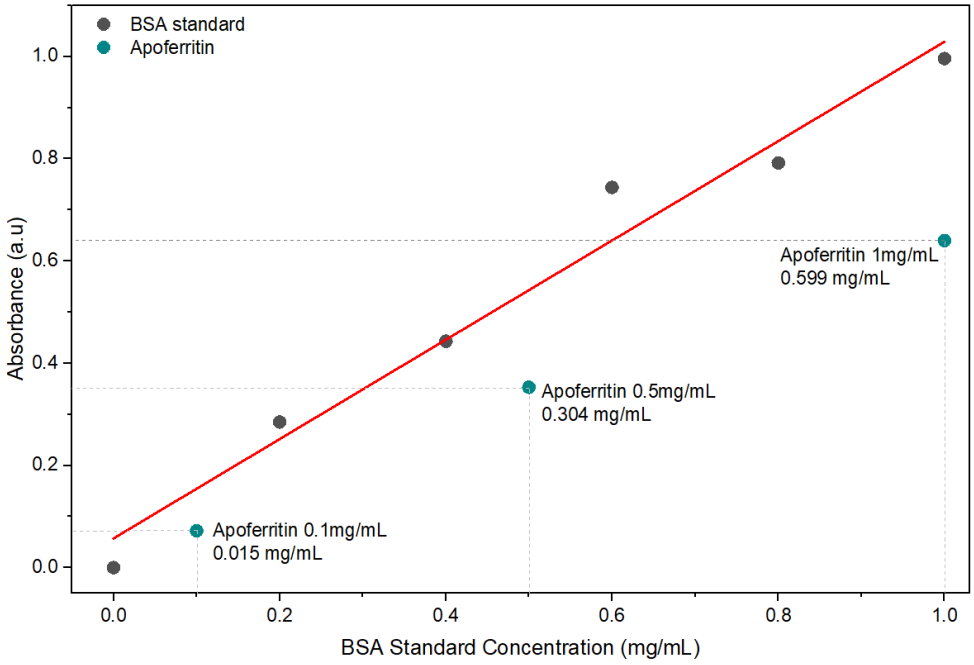


Fig 2. BSA standard curve and apoferritin concentration calculation

Although sample dilutions were performed to ensure that absorbance values fell within the calibration curve range, the concentrations derived from the calibration curve were lower than expected. It is possible see that the calculated concentration of apoferritin 5 mg/mL was 50.8 % for apoferritin concentrations of 10 mg/mL However, the sample with 1 mg/mL of apoferritin yielded a recovery rate of only 2.5 % (respect apoferritin 10mg/mL) This outcome is inconsistent, as a minimum recovery rate of 10 % would be anticipated. The discrepancy may be attributed to the limited sensitivity of the BCA method when analyzing highly diluted samples.

c) Small-angle scattering results from apoferritin

Figure 3 displays the results obtained from the SANS experiment conducted on our three apoferritin concentrations. We observed that the spectra for the 10 mg/mL and 5 mg/mL concentrations exhibited good statistical quality. However, the spectrum for the 1 mg/mL apoferritin sample was somewhat affected by background noise, which compromised the statistical reliability of the data. To validate our findings, the same samples were analyzed using small-angle X-ray scattering (SAXS) at the Shanghai Synchrotron Radiation Facility (SSRF). The results are presented in Figure 4. The high intensity of the synchrotron light enabled us to detect very weak signals with high sensitivity and low noise, particularly for the 1 mg/mL apoferritin sample. We therefore decided to compare the results obtained from both SANS and SAXS techniques. Figure 5 shows a combined plot of the SAXS and SANS data, generated using the PRIMUS software. To investigate the correlation between our results and the sample concentrations, we employed the “scale” tool, as illustrated in Figure 6.

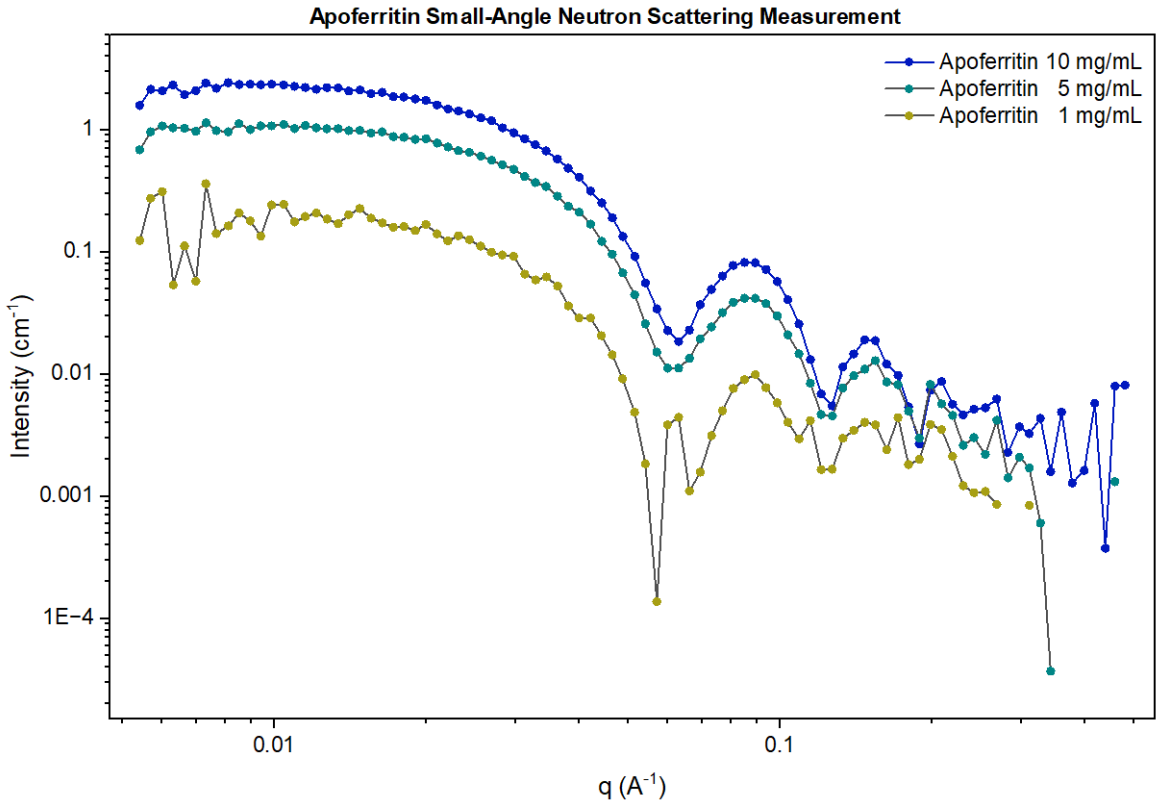


Figure 3. Apoferritin small-angle neutron scattering measurement

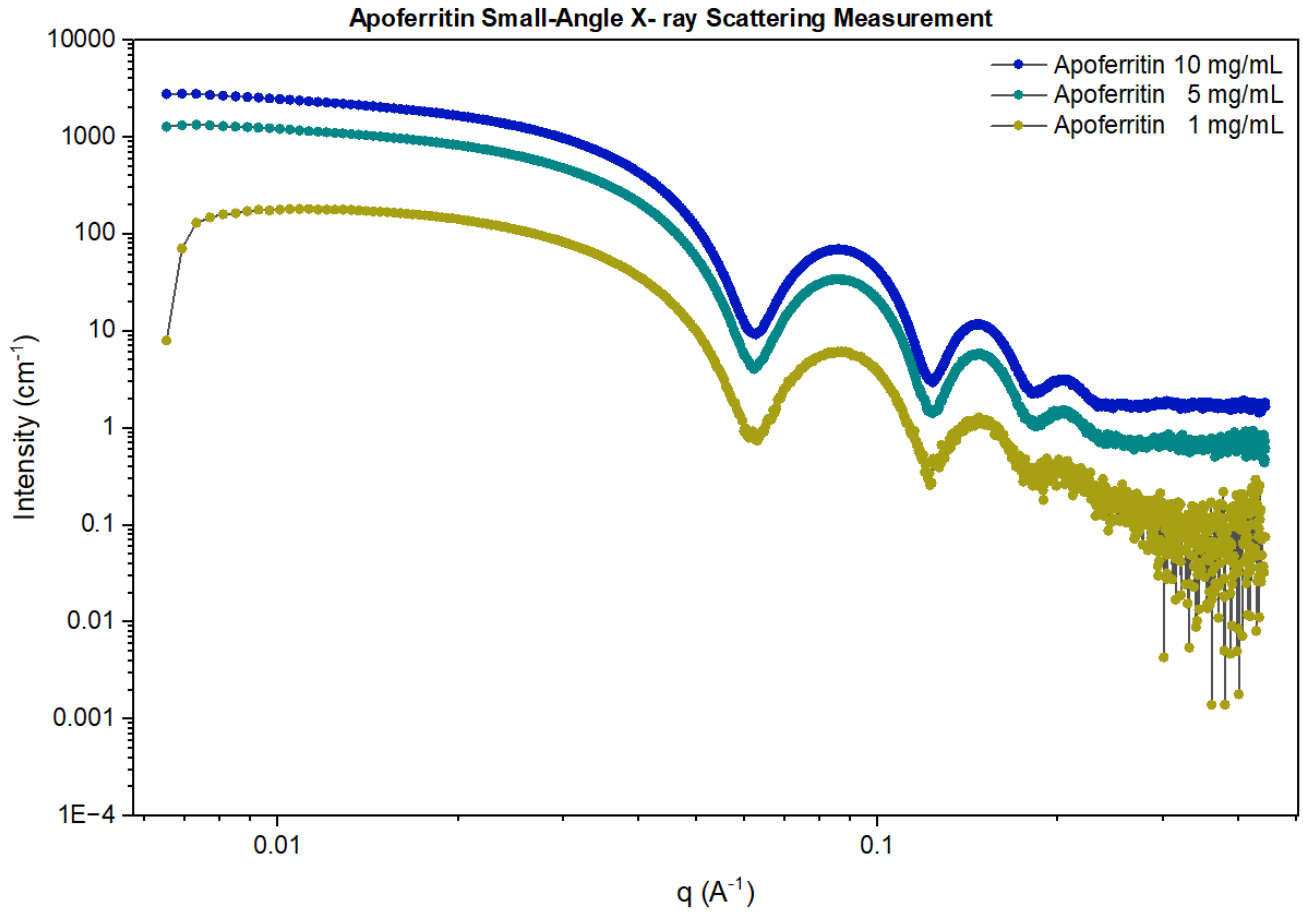


Figure 4. Apoferritin small-angle X-ray scattering measurement

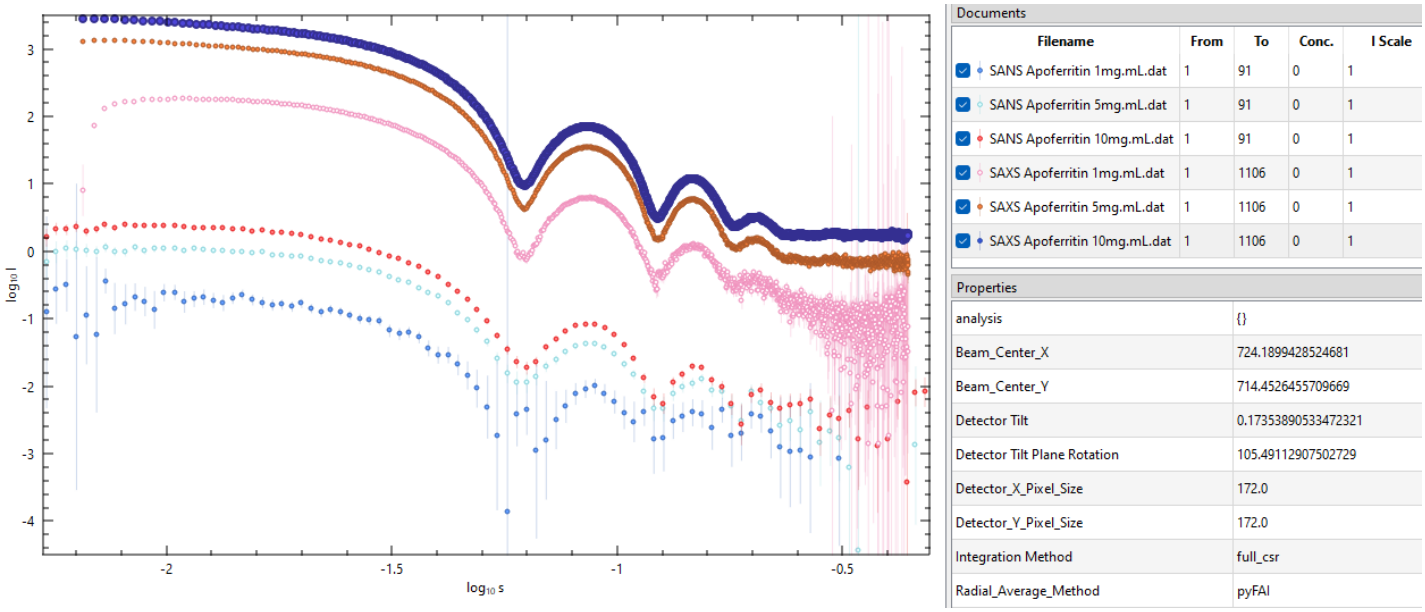
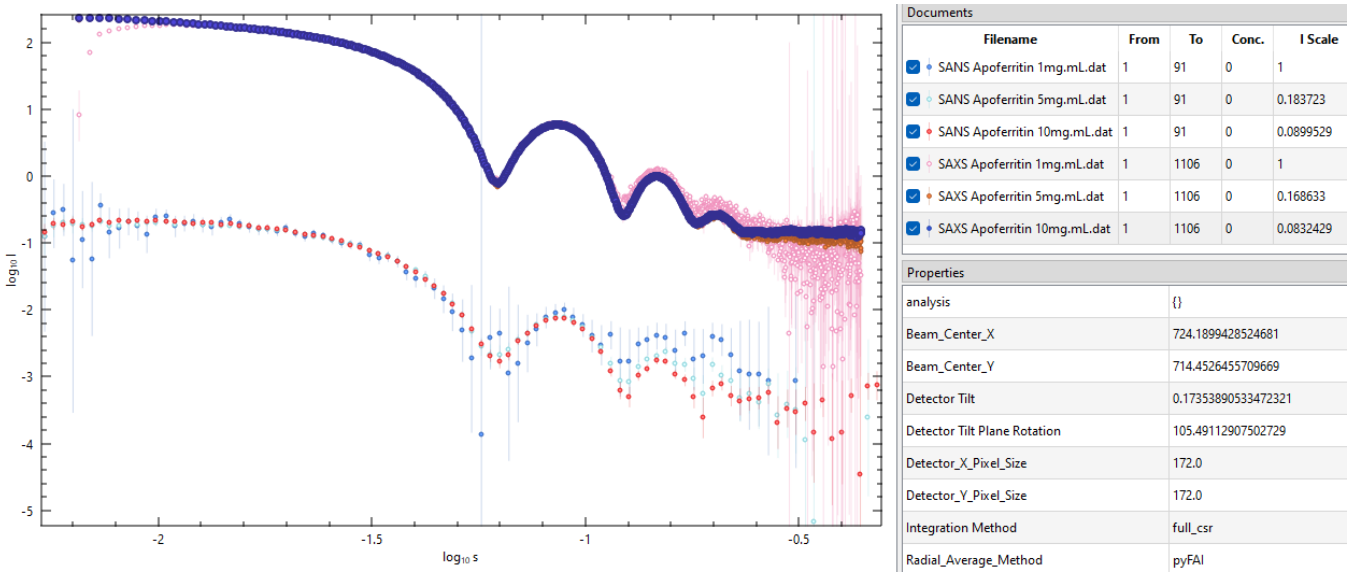


Figure 5. Apoferritin SAXS and SANS results



Documents

Filename	From	To	Conc.	I Scale
SANS Apoferritin 1mg.mL.dat	1	91	0	1
SANS Apoferritin 5mg.mL.dat	1	91	0	0.183723
SANS Apoferritin 10mg.mL.dat	1	91	0	0.0899529
SAXS Apoferritin 1mg.mL.dat	1	1106	0	1
SAXS Apoferritin 5mg.mL.dat	1	1106	0	0.168633
SAXS Apoferritin 10mg.mL.dat	1	1106	0	0.0832429

Properties

analysis	{}
Beam_Center_X	724.1899428524681
Beam_Center_Y	714.4526455709669
Detector Tilt	0.17353890533472321
Detector Tilt Plane Rotation	105.49112907502729
Detector_X_Pixel_Size	172.0
Detector_Y_Pixel_Size	172.0
Integration Method	full_csr
Radial_Average_Method	pyFAI

Figure 6. Apoferritin SAXS and SANS measurement (scale tool)

Based on calculations using the concentration data and the results obtained with the **scale tool**, analysis of the samples via small-angle neutron scattering (SANS) reveals that the concentration of apoferritin at 1 mg/mL corresponds to 8.50 % relative to the 10 mg/mL concentration (set as 100 %), while the 5 mg/mL concentration represents 48.13 % of the 10 mg/mL apoferritin. For the samples measured using small-angle X-ray scattering (SAXS), the 1 mg/mL apoferritin concentration corresponds to 8.29 % relative to 10 mg/mL (100 %), and the 5 mg/mL concentration accounts for 49.38 % of the 10 mg/mL concentration. These results indicate that the data obtained with SANS at the YuMO spectrometer are comparable to those acquired via SAXS at the Shanghai Synchrotron Radiation Facility (SSRF).

With this consistency established, we extracted key structural parameters, including the radius of gyration, porod volume, and distance distribution, as summarized in Table 1. As shown, the radius of gyration for apoferritin at 10 mg/mL and 5 mg/mL obtained via SANS are in good agreement with the corresponding values from SAXS. Furthermore, these gyration radius values (Equation 2) are consistent with those calculated using the spherical shell scattering model in SasView, where we fitted the experimental curves to determine both the radius and the shell thickness. For a spherical shell, the radius of gyration is mathematically related to the inner radius and outer radius (radius + thickness).

$$R_g = \sqrt{\frac{3\,(R_1^5 - R_2^5)}{5\,(R_1^3 - R_2^3)}} \qquad (2)$$

It is worth noting that our radius of gyration values aligns well with those reported by Murugova et al. (2015), who determined a gyration radius of 51.3 Å for apoferritin [8]. Finally, although slight differences in the radius of gyration are observed for the 1 mg/mL apoferritin sample, these discrepancies can be attributed to variations in data processing and experimental noise.

Table 1. Structural parameters of apoferritin derived from both SANS and SAXS measurements

Apoferritin Parameters		SANS						SAXS					
		Apoferritin 10mg/mL		Apoferritin 5mg/mL		Apoferritin 1mg/mL		Apoferritin 10mg/mL		Apoferritin 5mg/mL		Apoferritin 1mg/mL	
VolFraction		0.0020955 ± 3.87E-05		0.0010706 ± 4.02E-05		0.0001482 ± 2.91E-05		0.11174 ± 5.04E+06		0.0983 ± 5.91E+06		0.050182 ± 3.51E+06	
Radius	**R1**	39.629 ± 0.29332		40.185 ± 0.5426		37.915 ± 3.8705		35.595 ± 0.002812		35.592 ± 0.005288		36.5 ± 0.025	
Thickness	**R2**	21.779 ± 0.45424		20.492 ± 0.85409		26.709 ± 5.962		27.421 ± 0.004		27.6 ± 0.00787		26 ± 0.038	
	Radius+Thickness	61.408		60.677		64.624		63.016		63.192		62.5	
Radius of giration (calculated)		**52.42**		**52.12**		**54.05**		**52.34**		**52.46**		**52.23**	
I(s=0)								**2563.02**		**1282.55**		**218.77**	
Radius of giration		**56.17 ± 1.08**		**55.08 ± 1.24**		**61.01 ± 5.9**		**57.07 ± 5.9**		**57.54 ± 0.03**		**57.24 ± 0.07**	
Porod [Da]	**MW [Da]**	**379357**		348713		406023		481231		**526367**		487847	
	smax [A^-1]	0.48152		0.48146		0.48147		0.443459		0.443459		0.443459	
	Volume [A^3]	186546		57389		27356		679442		596282		190217	
MoW[Da]	**MW [Da]:**	**204286**		486886		0		354919		**385463**		383847	
	smax [A^-1]:	0.45955		0.45954		0		0.400358		0.400358		0.400358	
	Q':	0.000068		0.000029		0		0.000035		0.000032		0.000033	
	V':	291701		689069		0		561853		609685		607154	
	Volume [A^3]:	247609		590140		0		430187		467209		465250	
SizeShape [Da]	**MW [Da]**	**443218**		444185		535355		402600		**453072**		435778	
Partial Specific volume	cm^2.g^-1	0.7425		0.7425		0.7425		0.7425		0.7425		0.7425	
Contrast	10^10 cm^-2	2.8086		2.8086		2.8086		2.8086		2.8086		2.8086	
absolute scale	[Da]	3600410		1675570		346193		346193		3600410		346193	
Distance distribution	**Rmax**	**125**		**127**		127.19		138.59		**140**		143	
	Guinier Rq/I(0)	52.68		52.67		54.08		53.1		53.23		53.39	
	p(r)Rq/I(0)	52.57		52.53		53.88		52.74		52.88		53.09	
	Porod volume [A^3]	573831		521320		498867		644014		663273		650269	

d) Bovine serum albumin Bicinchoninic acid (BCA) protein assay

To calculate the concentration of BSA at 2 mg/mL and 5 mg/mL in PBS H_2O and PBS D_2O, it was used the same calibration curve as in Figure 2. The results are detailed in Table 2.

Table 2. Concentration of BSA according to the bicinchoninic acid method

Sample	Calculated concentration
BSA 2mg/mL – PBS H_2O	2.33 mg/mL
BSA 5mg/mL – PBS H_2O	6.28 mg/mL
BSA 2mg/mL – PBS D_2O	2.27 mg/mL
BSA 5mg/mL – PBS D_2O	4.67 mg/mL

e) Small-angle scattering results from Bovine serum albumin

Figure 7 displays the small-angle neutron scattering (SANS) results obtained for our four bovine serum albumin (BSA) concentrations. The data reveal that the BSA 5 mg/mL–PBS D_2O sample exhibits the highest scattering intensity. Notably, the intensity of the BSA 2 mg/mL–PBS D_2O sample is comparable to that of the BSA 5 mg/mL–PBS H_2O sample. To interpret these results, the data were processed using PRIMUS software, where the “scale” tool was applied to normalize the scattering curves.

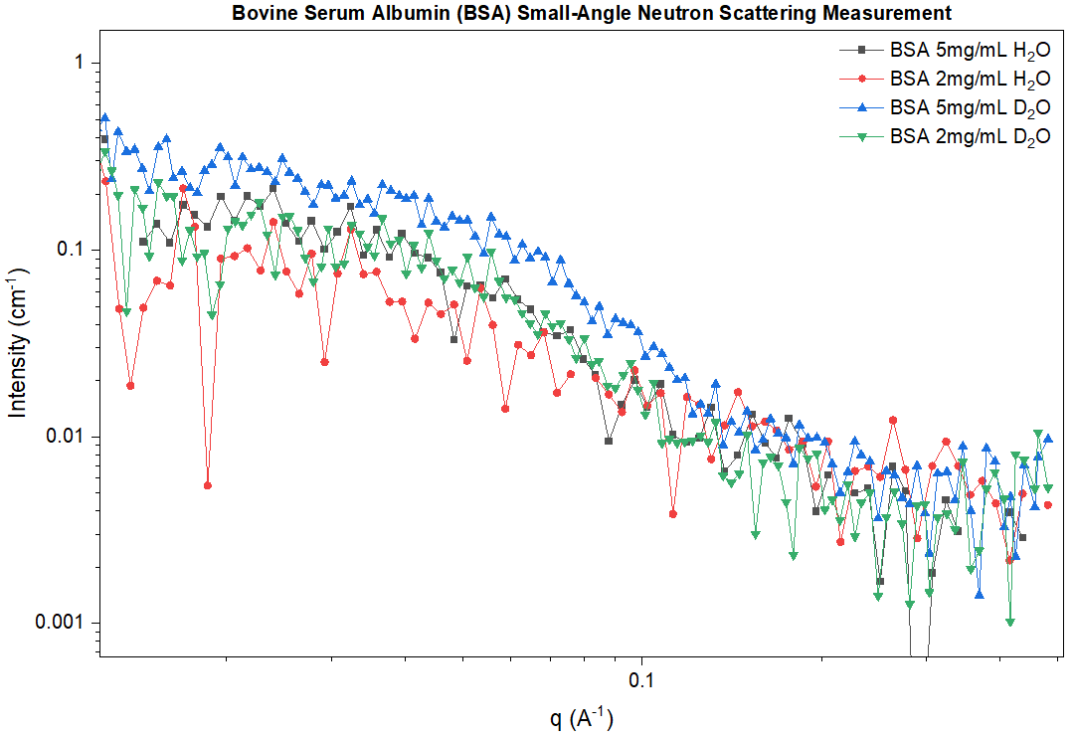


Figure 7. BSA small-angle neutron scattering measurement

Figure 8 presents the scaled SANS results for BSA in D_2O. Based on concentration-dependent analysis and the scaling factors derived from PRIMUS, the BSA 2 mg/mL–PBS D_2O sample shows an intensity corresponding to 53.05 % of the BSA 5 mg/mL–PBS D_2O reference (set at 100 %). This finding aligns reasonably well with the BCA assay quantification, which indicated that the BSA 2 mg/mL–D_2O sample represents 48.61 % of the BSA 5 mg/mL–PBS D_2O concentration. The slight discrepancy between the two methods may arise from the presence of protein

aggregates in the sample, as this BSA preparation was not purified using size-exclusion chromatography.

Figure 9 shows the SANS results for BSA in H_2O. Quantitative analysis using the scaling approach reveals that the BSA 2 mg/mL–H_2O sample exhibits 90.99 % of the intensity of the BSA 5 mg/mL–H_2O reference (100 %). However, this result contrasts sharply with the BCA assay data, which showed the BSA 2 mg/mL–H_2O sample to be only 37.10 % of the BSA 5 mg/mL–H_2O concentration. This significant divergence between SANS and BCA results can be attributed to hydrogen interference in small-angle neutron scattering. Although a PBS H_2O buffer was used and background subtraction was performed, hydrogen atoms in the solvent and protein contribute to additional background scattering. This effect, combined with potential aggregate formation, introduces extra noise into the SANS data, thereby distorting the apparent concentration dependence.

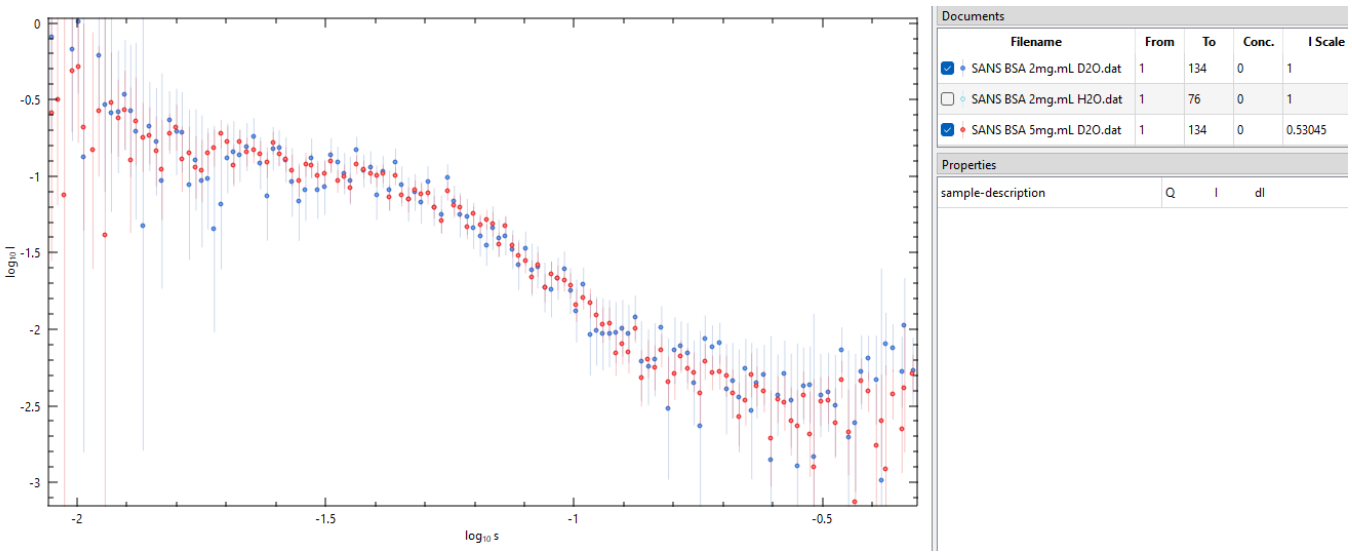


Figure 8. BSA D_2O SANS measurement (scale tool)

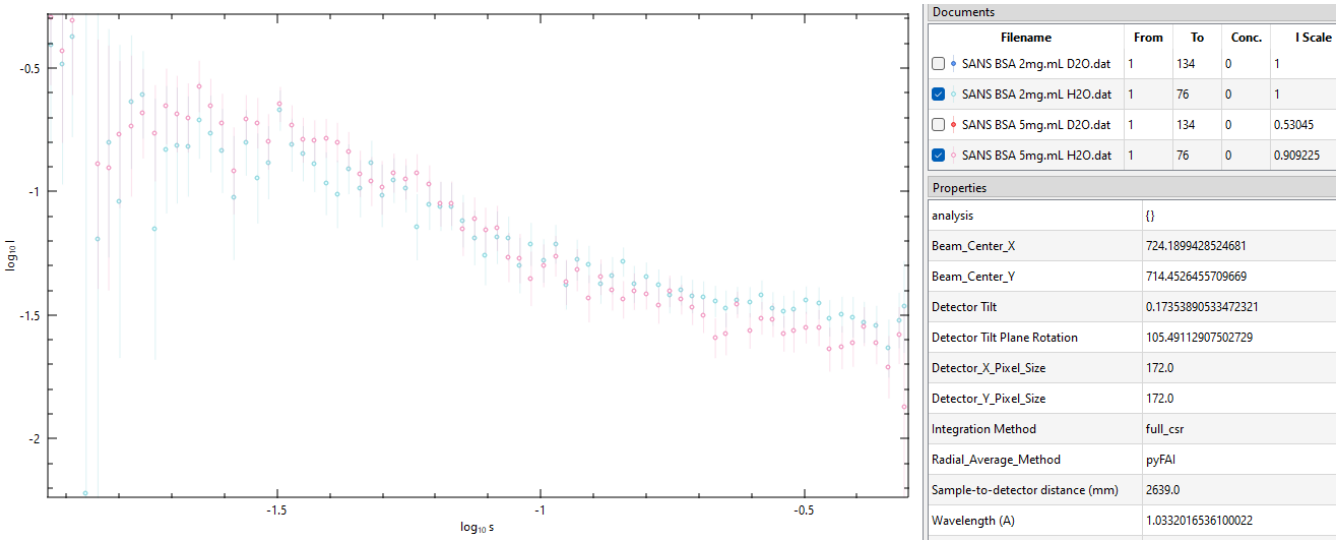


Figure 9. BSA H_2O SANS measurement (scale tool)

Bovine serum albumin (BSA) samples in H_2O were analyzed using small-angle X-ray scattering (SAXS) at the Shanghai Synchrotron Radiation Facility (SSRF). Figure 10 presented the results by employing the Scale tool, and it was determined that the BSA concentration of 2 mg/mL in H_2O corresponds to 38.02 % relative to the 5 mg/mL BSA concentration (set as 100 %). These data are consistent with the results obtained via the BCA method, which showed that the 2 mg/mL BSA sample in H_2O represents 37.10 % of the 5 mg/mL BSA sample in H_2O. The slight discrepancy between the two techniques applied to the same sample can be attributed to the presence of hydrogen. This element has a minimal effect on small-angle X-ray scattering results, which facilitates the analysis of samples in aqueous media. In X-ray scattering, hydrogen exhibits a very low electron density compared to most other elements, rendering its contribution to scattering negligible. Consequently, the presence of hydrogen does not distort the BSA signal.

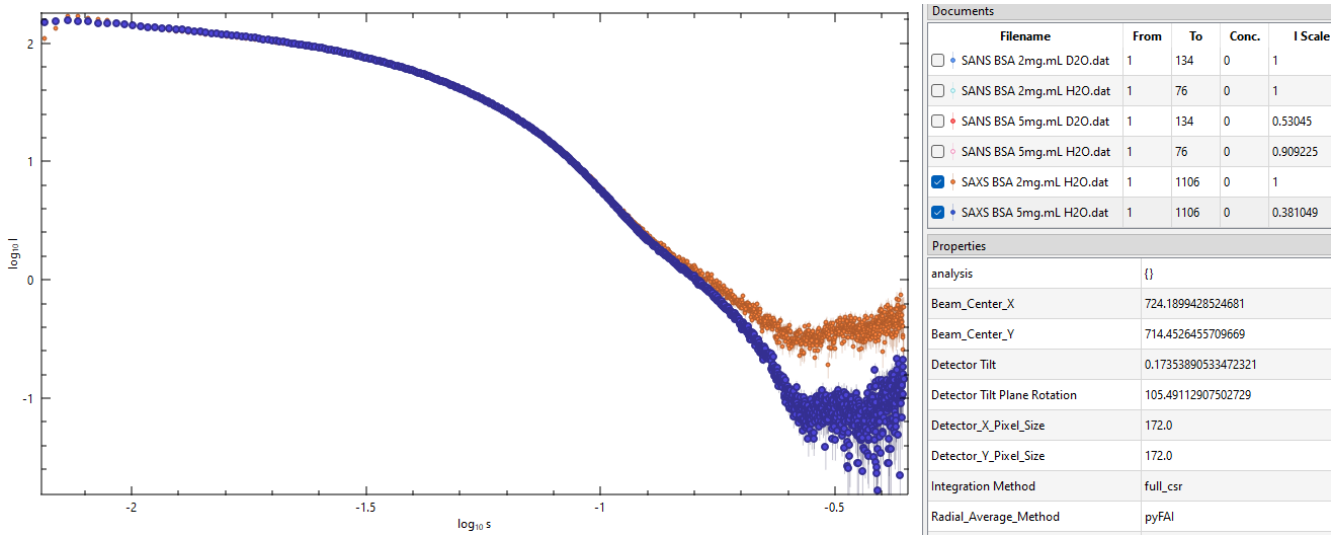


Figure 10. BSA H_2O SAXS measurement (scale tool)

Based on these results, we can affirm that the data obtained using small-angle neutron scattering (SANS) at the YuMO spectrometer for BSA samples prepared in phosphate-buffered saline (PBS) with D_2O are comparable to those derived from the BCA assay. However, for samples prepared in PBS with H_2O, potential interferences may arise, necessitating more careful analysis or the use of solvents with a lower H_2O content. For samples containing H_2O, it is also advisable to consider contrast variation related to the scattering densities of BSA and their solvents by appropriately adjusting the deuteration level of the solvent[12].

Table 3 presents key structural parameters, including the radius of gyration, porod volume, and distance distribution. The radius of gyration values obtained for BSA in D_2O at 2 mg/mL and 5 mg/mL are quite comparable, and they are also in line with the results for BSA in H_2O. However, these values cannot be directly compared with those obtained from SAXS for the BSA sample in H_2O.

Table 3. Structural parameters of BSA obtained with SANS and SAXS

Bovine serum albumin		**SANS**				**SAXS**	
Parameters		**BSA 5mg/mL D_2O**	**BSA 2mg/mL D_2O**	**BSA 5mg/mL H_2O**	**BSA 2mg/mL H_2O**	**BSA 5mg/mL H_2O**	**BSA 2mg/mL H_2O**
Radius of giration		**29.05 ± 0.85**	**29.67 ± 3.01**	**30.13 ± 1.6**	**30.88 ± 2**	**37.79 ± 0.15**	**37.36 ± 0.17**
I(0)		**0.30**	**0.16**	**0.17**	**0.12**	**120.09**	**314.35**
Porod [Da]	**MW [Da]**	**46367**	37562	21626	11873	96957	96474
	smax [A^-1]	0.48126	0.4813	0.4811	0.48109	0.443459	0.443459
	Volume [A^3]	15565	9050	6845	3045	97686	128223
MoW[Da]	**MW [Da]:**	**16660**	10243	26779	6533	81686	123018
	smax [A^-1]:	0.45741	0.45735	0.45775	0.45763	0.400358	0.400358
	Q':	0.00071	0.00105	0.00047	0.00145	0.000147	0.000099
	V':	27878	18853	42105	13637	133974	198699
	Volume [A^3]:	20194	12415	32458	7918	99009	149106
SizeShape [Da]	**MW [Da]**	**51429**	49371	52322	43242	110343	115330
Partial Specific volume	cm^2.g^-1	0.7425	0.7425	0.7425	0.7425	0.7425	0.7425
Contrast absolute scale	10^10 cm^-2 [Da]	2.8086	2.8086	2.8086	2.8086	2.8086	2.8086
Distance distribution	**Rmax**	**106.51**	**98.02**	73.17	68.45	91.77	91.4
	Guinier Rq/I(0)	31.61	30.55	27.44	28.23	32.89	32.86
	p(r)Rq/I(0)	31.67	30.58	27.35	28.21	32.9	32.87
	Porod volume [A^3]	36962	32302	33285	16082	133930	133182

5) Conclusion

During this scientific internship, it was acquired fundamental knowledge about sample preparation for small-angle neutron scattering (SANS). Experimentally, we demonstrated the significant impact of analyzing two types of samples: a monodisperse one (apoferritin) and another prepared by direct weighing and dispersion in PBS (Bovine serum albumin). Software tools such as SAS, SasView, and ATSAS for analyzing SANS data showed that the quality of data obtained with the SANS spectrometer is comparable to results from small-angle X-ray scattering (SAXS) experiments at the Shanghai Synchrotron Radiation Facility (SSRF), provided the sample is monodisperse and prepared with D_2O.

Regarding the bovine serum albumin measurements, it was found that uncertainty about a sample's monodispersity can complicate data processing. Nevertheless, the use of D_2O still proved beneficial in reducing background noise. We also observed experimentally that using 100 % H_2O increases background noise and may obscure the sample signal, ultimately leading to incorrect concentration determination in SANS. This was confirmed by SAXS analysis of a BSA sample in H_2O at the SSRF, where the sample maintained the expected percentage ratio.

In conclusion, this work experimentally demonstrated the critical influence of sample preparation conditions on data processing outcomes. SANS and SAXS curves were successfully analyzed and structural characteristics of apoferritin and BSA were obtained.

6) References

(1) Fanova, A.; Sotiropoulos, K.; Radulescu, A.; Papagiannopoulos, A. Advances in Small Angle Neutron Scattering on Polysaccharide Materials. *Polymers* **2024**, *16* (4), 490. https://doi.org/10.3390/polym16040490.

(2) Svergun, D. I.; Koch, M. H. J. Small-Angle Scattering Studies of Biological Macromolecules in Solution. *Rep. Prog. Phys.* **2003**, *66* (10), 1735. https://doi.org/10.1088/0034-4885/66/10/R05.

(3) Klinkova, A.; Thérien-Aublin, H. *Nanochemistry - Chapter 2: Characterization Techniques*; 2024. https://doi.org/10.1016/B978-0-443-21447-9.00008-4.

(4) Trewhella, J. Recent Advances in Small-Angle Scattering and Its Expanding Impact in Structural Biology. *Struct. Lond. Engl. 1993* **2022**, *30* (1), 15–23. https://doi.org/10.1016/j.str.2021.09.008.

(5) Béchade, J.-L.; Mathon, M.-H.; Carlan, Y. Neutron Analyses for Nuclear Materials: Texture, Residual Stresses and Small Angle Scattering. *The European Physical Journal Conferences*. 2015. https://doi.org/10.1051/epjconf/201510401008.

(6) Barnali, C. Emerging Applications of Small Angle Solution Scattering in Structural Biology. *Protein Sci.* **2015**, *24* (3). https://doi.org/10.1002/pro.2624.

(7) Zabelskii, D. V.; Vlasov, A. V.; Ryzhykau, Y. L.; Murugova, T. N.; Brennich, M.; Soloviov, D. V.; Ivankov, O. I.; Borshchevskiy, V. I.; Mishin, A. V.; Rogachev, A. V.; Round, A.; Dencher, N. A.; Büldt, G.; Gordeliy, V. I.; Kuklin, A. I. Ambiguities and Completeness of SAS Data Analysis: Investigations of Apoferritin by SAXS/SANS EID and SEC-SAXS Methods. *J. Phys. Conf. Ser.* **2018**, *994* (1), 012017. https://doi.org/10.1088/1742-6596/994/1/012017.

(8) Murugova, T.; Vlasov, A.; Ivankov, O.; Rogachev, A.; Ryzhykau, Y. Low Resolution Structural Studies of Apoferritin via SANS and SAXS: The Effect of Concentration. *J. Optoelectron. Adv. Mater.* **2015**, *17* (9), 1397–1402.

(9) Barbosa, L. R. S.; Ortore, M. G.; Spinozzi, F.; Mariani, P.; Bernstorff, S.; Itri, R. The Importance of Protein-Protein Interactions on the pH-Induced Conformational Changes of Bovine Serum Albumin: A Small-Angle X-Ray Scattering Study. *Biophys. J.* **2010**, *98* (1), 147–157. https://doi.org/10.1016/j.bpj.2009.09.056.

(10) Mirizadeh, A.; Hejazi, P. Exploring the Interaction of an Anionic Magnetic Surfactant Nanoparticles with BSA: From Molecular Insights to Macro-Scale Approaches. *Int. J. Biol. Macromol.* **2025**, *329*, 147719. https://doi.org/10.1016/j.ijbiomac.2025.147719.

(11) Sigma - Aldrich. Technical Bulletin - Bicinchoninic Acid Protein Assay Kit, 2025. https://www.sigmaaldrich.com/ES/es/product/sigma/bca1?srsltid=AfmBOoqrgY10asfy1L4l-BrkXcHRjsXVUGgw0gfxJIrKW4Trnek3AKVA (accessed 2025-11-11).

(12) Sonntag, M.; Jagtap, P. K. A.; Simon, B.; Appavou, M.-S.; Geerlof, A.; Stehle, R.; Gabel, F.; Hennig, J.; Sattler, M. Segmental, Domain-Selective Perdeuteration and Small-Angle Neutron Scattering for Structural Analysis of Multi-Domain Proteins. *Angew. Chem. Int. Ed.* **2017**, *56* (32), 9322–9325. https://doi.org/10.1002/anie.201702904.

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