

Video Article

Small and Wide Angle X-Ray Scattering Studies of Biological Macromolecules in Solution

Li Liu¹, Lauren Boldon¹, Melissa Urquhart¹, Xiangyu Wang¹

¹Department of Mechanical, Aerospace, and Nuclear Engineering, Rensselaer Polytechnic Institute

Correspondence to: Li Liu at llue@rpi.edu

URL: <https://www.jove.com/video/4160>

DOI: [doi:10.3791/4160](https://doi.org/10.3791/4160)

Keywords: Bioengineering, Issue 71, Biophysics, Structural Biology, Physics, Molecular Biology, Mechanical Engineering, Nanotechnology, Small angle X-ray scattering, wide angle X-ray scattering, X-ray, biological macromolecules

Date Published: 1/8/2013

Citation: Liu, L., Boldon, L., Urquhart, M., Wang, X. Small and Wide Angle X-Ray Scattering Studies of Biological Macromolecules in Solution. *J. Vis. Exp.* (71), e4160, doi:10.3791/4160 (2013).

Abstract

In this paper, Small and Wide Angle X-ray Scattering (SWAXS) analysis of macromolecules is demonstrated through experimentation. SWAXS is a technique where X-rays are elastically scattered by an inhomogeneous sample in the nm-range at small angles (typically 0.1 - 5°) and wide angles (typically > 5°). This technique provides information about the shape, size, and distribution of macromolecules, characteristic distances of partially ordered materials, pore sizes, and surface-to-volume ratio. Small Angle X-ray Scattering (SAXS) is capable of delivering structural information of macromolecules between 1 and 200 nm, whereas Wide Angle X-ray Scattering (WAXS) can resolve even smaller Bragg spacing of samples between 0.33 nm and 0.49 nm based on the specific system setup and detector. The spacing is determined from Bragg's law and is dependent on the wavelength and incident angle.

In a SWAXS experiment, the materials can be solid or liquid and may contain solid, liquid or gaseous domains (so-called particles) of the same or another material in any combination. SWAXS applications are very broad and include colloids of all types: metals, composites, cement, oil, polymers, plastics, proteins, foods, and pharmaceuticals. For solid samples, the thickness is limited to approximately 5 mm.

Usage of a lab-based SWAXS instrument is detailed in this paper. With the available software (e.g., GNOM-ATSAS 2.3 package by D. Svergun EMBL-Hamburg and EasySWAXS software) for the SWAXS system, an experiment can be conducted to determine certain parameters of interest for the given sample. One example of a biological macromolecule experiment is the analysis of 2 wt% lysozyme in a water-based aqueous buffer which can be chosen and prepared through numerous methods. The preparation of the sample follows the guidelines below in the Preparation of the Sample section. Through SWAXS experimentation, important structural parameters of lysozyme, e.g. the radius of gyration, can be analyzed.

Video Link

The video component of this article can be found at <https://www.jove.com/video/4160/>

Protocol

1. Preparation of the Sample

1. Use a needle to remove some of the sample from the sample container.*
2. Use the needle to fill the capillary (maximum diameter of 2.2 mm) with sample. The capillary must be filled between 2 and 3 cm from the bottom.
3. Close the capillary by melting wax on its tip.
4. Unscrew the vacuum sample holder from the system.
5. Take the capillary by the fused end (wax end) and insert the un-fused end into the sample holder.
6. Place the holder back into the system and screw in place.

* Solid samples (including powder samples) can be directly placed in the sample holder (no capillary necessary), whereas liquid samples must be placed in a capillary.

Start-Up of the SWAXS Machine

2. Source Cold Startup Procedure

1. Close the shutter by pushing the safety shutter button.
2. Switch the main power "ON" by pushing the green on button.

3. Verify the Emergency "Shut-Off" button is in the extended position.
4. Switch the main power on by pushing the green button.
5. Verify the interlock LED light is green, indicating all interlocks are ok.
6. Wait for the touch screen to load. Once it has loaded, press "R6" on the menu.
7. Turn the Standby key to the "ON" position.
8. Turn the X-ray Key to the "ON" position. The red X-ray light should light up.
9. Wait for the touch screen to read standby levels (30 kV and 0.4 mA).
10. Turn the Standby Safety Key to the "OFF" position. Press the "Start Cycle" button on bottom of the touch screen.
11. Wait for the touch screen to read nominal power (50 kV and 1 mA). If a different power level is desired, enter the configuration screen by pressing "R4" on the touch screen and entering the desired settings.

3. Chiller Procedure

1. Turn the power switch to the "ON" position on the temperature control panel. A control light and the LED temperature display will light up.
2. Turn the power switch to the "ON" position on the chiller.
3. Wait until the temperature display reads "OFF." This is standby mode.
4. Press and hold the enter button (return symbol) for about 4 sec or until the temperature display indicates an actual temperature.
5. To change the temperature, press the up or down arrows. The set value will be shown on the temperature display for approximately 8 sec before it will return to the actual value.
6. Press the enter button once the desired set value is displayed. This will store that value.
7. Wait until the actual temperature reaches the desired set value.

NOTE: If at any time the error "E 01" shows up on the temperature display, follow the Chiller Shutdown instructions, refill the bath unit by pouring purified water into the tank fill, and then perform the chiller Startup procedure again.

4. Shutdown of Chiller

1. Press "enter" for approximately 4 sec.
2. Turn the power switch to the "OFF" position.

5. Turning on the Vacuum

1. Ensure the vacuum door is secured in place.
2. Turn the Vacuum 1 and 2 knobs to the "ON" positions.
3. Wait until the VAP5 vacuum gauge reads a vacuum level less than 1.5 mbar.

6. Detector System Setup

1. To set up the gas pressure, verify the Main Pressure Gauge of the reduction valve is at least 10 bar.
2. Open the Main Valve.
3. Slowly open the Operating Pressure Valve until the pressure reaches 8 bar at the Operating Pressure Gauge.
4. Open the Second Main Valve
5. To adjust gas flow, open the Main Pressure valve by turning the knob to the vertical position on the Gas Control panel.
6. Adjust the flow rate with the needle valve until the gauge reads approximately 8 bar.
7. Turn the Main Power switch to the "ON" position. The LED light should light up.
8. To adjust the high voltage, activate the high voltage by turning the switch to the "ON" position. The LED light should light up.
9. Slowly turn the voltage control knob until approximately 3.5kV is reached on the gauge. DO NOT EXCEED 4kV.

7. Calibration

1. The experimental steps above are performed with a sample of known peaks.
2. ASA 3.3 is used to obtain graph of intensity vs. channel with 5 peaks.
3. Go to Menu →Options→Enter channels and corresponding intensities.
4. Go to ASA3, "TPF" tab first.
5. Change filter, top left *1 mm Nickel (beam filter)*.
6. Set position 32,000 (range 28,000 to 32,000, critical region around 28,000) → go to position, make sure vacuum is below 5 mbar, make sure the detector is reading between 1k -10K.
7. Run for 10 sec for approximately 52 counts per sec.
8. Change the energy windows, (or measure the energy window with the sample only, not including the filter case). Set the final run for 10 sec.
9. Find the location of the primary beam (channel 233 for example).
10. For other runs, change filter again to *2 mm Tungsten (W, beam stopper)* and start from 30,000.
11. Find the location of all other peaks of the samples.
12. Use ImageJ-macro software to calibrate SAXS - making transitions from intensity vs. channel to intensity vs. q (or d spacing) (**Figure 1**).
13. Use ImageJ-macro software to calibrate WAXS - making transitions from intensity vs. channel to intensity vs. q (or d spacing) (**Figure 2**).

8. Software Procedure

1. Use the software ASA 3.3, to collect the live data: Live Data→ TPF tab→ Save file.
2. For EasySWAXS, click on the Device Settings tab.
3. Enter the "a", lambda, and d values, as well as the center of gravity.
4. Select Point Collimation.
5. Select Globular particle type (unless it is a known to be a rod of flat shaped particle).
6. Click on the Guinier-Plot tab. An I vs. q^2 graph will be displayed.
7. Drag the vertical lines to surround a section of approximately linear slope.
8. At the bottom of the screen, an R value will be displayed. Next to this there is a validation requirement.
9. Continue to drag the vertical lines closer together until the validation value is between 1 and 2. This is subjective. The R value, or Radius of Gyration, obtained is an estimate.

9. Source Shutdown Procedure

1. Verify the safety and fast shutters are closed.
2. Turn the Standby key to the "ON" position.
3. Wait for the touchscreen to read standby levels.
4. Press R5 on the touchscreen.
5. Reduce the current to 0 A.
6. Reduce the voltage to 0 A.
7. Turn the X-ray key to the "OFF" position.
8. Switch the main power "OFF" by pushing the red off button.

Representative Results

SAXS and WAXS altogether can provide structural information of the sample through the following parameters: the radius of gyration, particle size and shape, solution structure factor, specific inner surface and pore size, lattice type and dimension, and electron density. SAXS and WAXS can also be applied to the study of protein dynamics¹.

The structural information of SWAXS experiments is obtained by comparing the experimentally detected spectra and the computational results of the system. The computational results were calculated in the software with a reasonable effective potential $V_{eff}(r)$ developed from statistical mechanics models, such as the Ornstein-Zernike (OZ) integral equation theory (an example of such analysis may be seen from Ref. ²).

As part of the data analysis methods, models for the SWAXS absolute intensity $I(q)$ will need to be developed in the software for study, where the scattering intensity, $I(q)$, is a function of the momentum transfer in reciprocal space, the scattering vector $q=4\pi \sin(\theta/2)/\lambda$. q is a scalar quantity which is connected to the scattering angle, θ , and the wavelength of the radiation, λ . q lies in the range of 0.03 - 0.6 Å⁻¹ in a typical SAXS experiment with a selected sample-to-detector distance. The size of the region investigated in real space is related to q by $r=2\pi/q$, and lies in the range 11-2000 Å³. WAXS, on the other hand, can resolve spacing larger than 3.3 Å. $I(q)$ depends on the atomic features and the position of the atomic scattering centers. In the SWAXS experiment, first the measured intensity vs. channel must be calibrated to intensity vs. q or spacing d (Figure 1 and Figure 2). Then the software may be utilized to analyze the structural information.

An example of the SAXS analysis of the lysozyme in 2 wt% water based aqueous buffer is shown in Figure 3. The value for radius of gyration obtained and shown in Figure 3 compares nicely to the expected value of approximately 1.44 nm⁴. More examples of how to apply SAXS to biological macromolecules may be found from Refs. ⁵⁻¹². An example of the WAXS analysis of the liposome dispersed in aqueous solution is shown in Figure 4. The equally spaced peaks decreasing with increasing q , lends the liposome in the water based aqueous solution sample to a lamellar structure. With each lamellae, there is a decrease in the scattering that will occur.

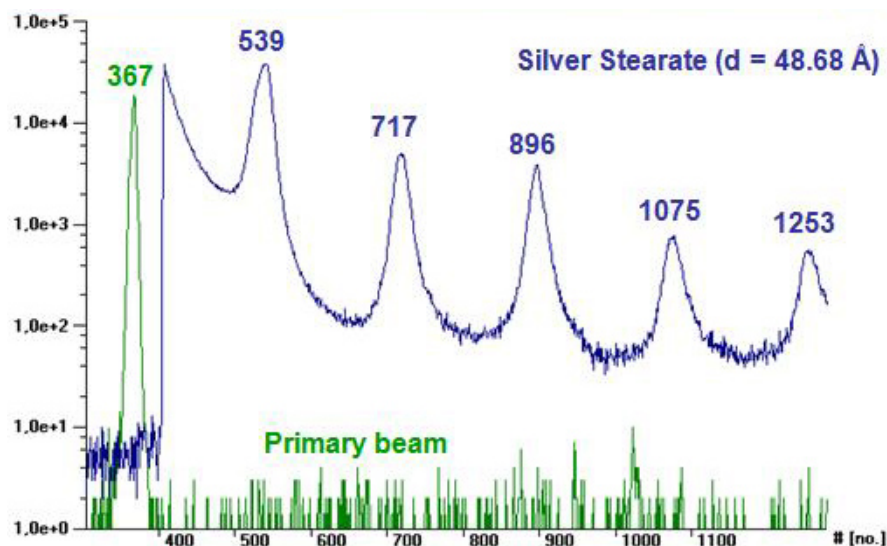


Figure 1. The SAXS Calibration with ImageJ-macro software. The sample used is silver stearate with d spacing 48.68 Å. The primary beam is located at channel 367 and the five major peaks (or lattice parameters of the sample) are located at 539, 717, 896, 1075, and 1253 channels, respectively.

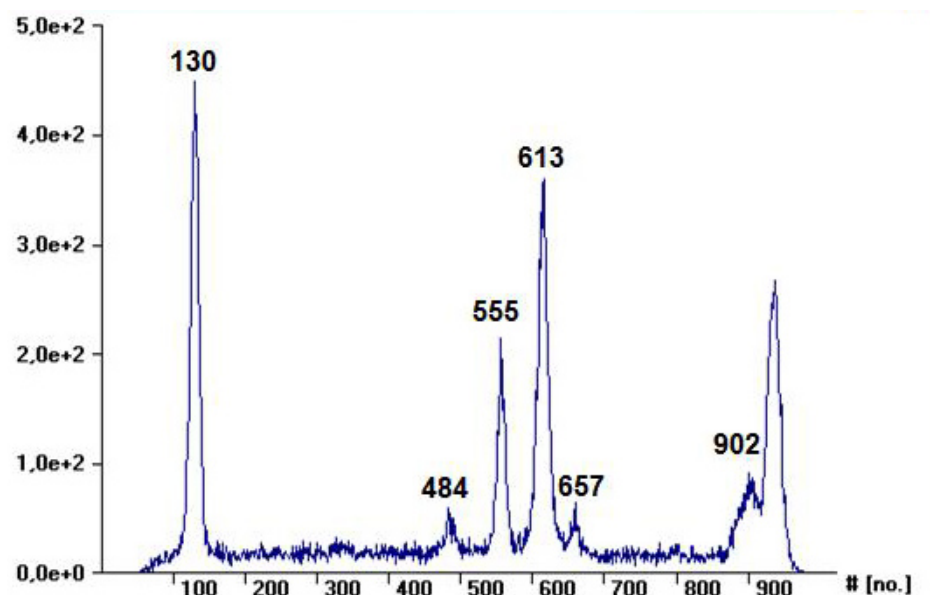


Figure 2. The WAXS-Calibration with the ImageJ-macro software. The sample used is Para-Bromo Benzoic Acid powder. The six major peaks (or lattice parameters of the sample) are located at 130, 484, 555, 613, 657, and 902 channels, respectively.

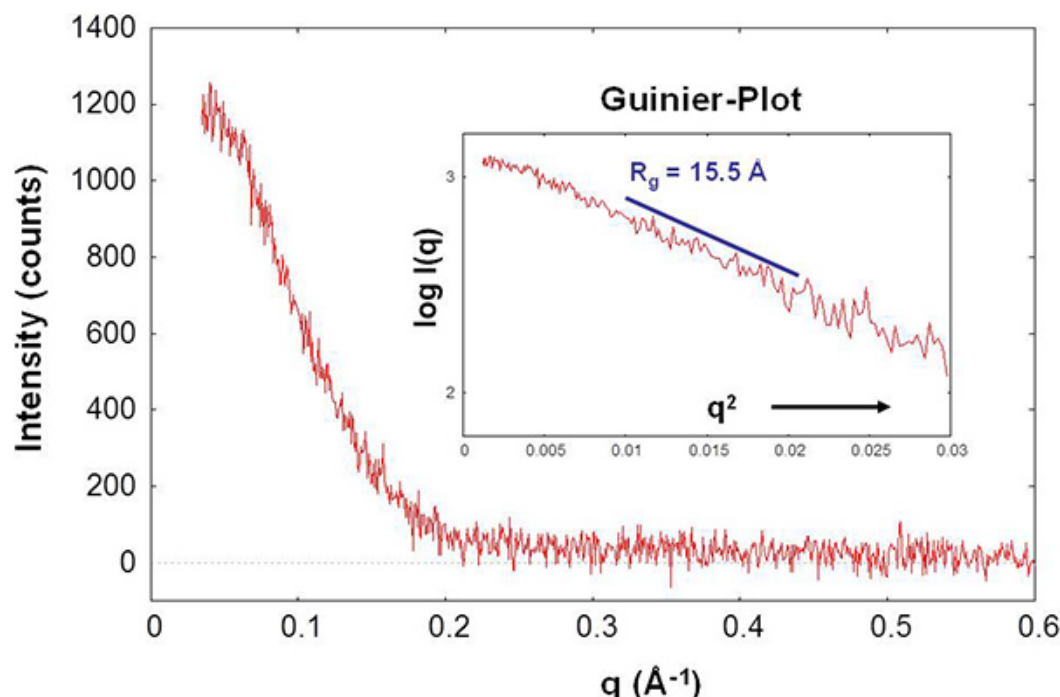


Figure 3. Background-subtracted SAXS raw-data of lysozyme (2 wt%). The Guinier-plot from EasySWAXS software can utilize the very low q part of the raw data to find the radius of gyration.

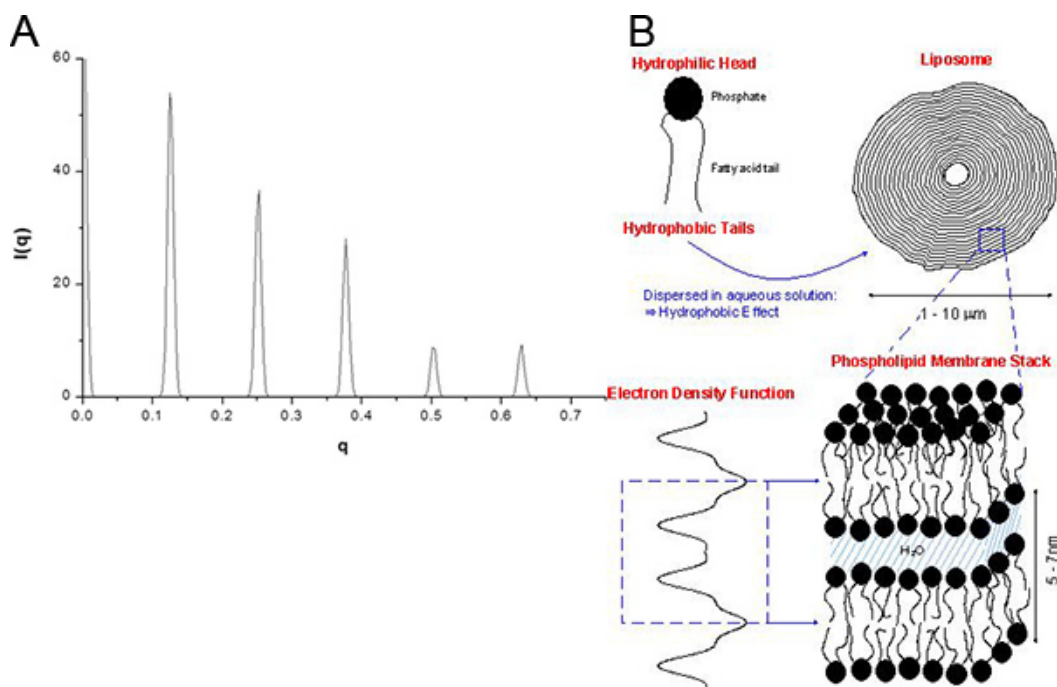


Figure 4. Background-subtracted WAXS raw-data of liposome dispersed in a water based aqueous solution is shown in **Figure 4A**. The schematic diagrams of the structure of liposome (1D lamellar), its hydrophilic head and hydrophobic tail, its phospholipid membrane stack, and its electron density function are shown in **Figure 4B**. [Click here to view larger figure.](#)

Discussion

The comparative procedure of the SWAXS system allows for numerous variables to be determined from experimental analysis. The parameters that are attained from the analysis can be used for different purposes according to the sample and experimental setup. SAXS provides information about nano-scale size and shape of the object, whereas WAXS focuses on the atomic and micro-scale structure (e.g. molecular lattice, unit cell dimension symmetry). More specifically, for particles in dilute solutions, SAXS can study the radius of gyration, particle size, and shape; for high-density samples, SAXS may study the structure factor of the solution; for random porous/2 phase systems, SAXS can study

specific inner surface and pore size; and for liquid crystalline samples, WAXS may study lattice dimensions and the unit cell structure. However, a limitation of SWAXS is that a wide range of distribution of particle sizes or polydispersity will severely downgrade the experimental results.

Disclosures

No conflicts of interest declared.

Acknowledgements

We would like to thank Dr. Manfred Kriechbaum of Hecus XRS and the Institute of Biophysics and Nanosystems Research at the Austrian Academy of Sciences in Graz, Austria. LL and XW were supported in part by U.S. Department of Energy, under NERI-C Award No. DE-FG07-07ID14889, and U.S. Nuclear Regulatory Commission, under Award No. NRC-38-08-950. The SWAXS instrument is also supported in part by U.S. Department of Energy, under Award No. DE-NE0000325.

References

1. Bernadó, P. & Blackledge, M., Structural biology: Proteins in dynamic equilibrium. *Nature*. **468**, 1046-1048 (2010).
2. Zhang, F., Skoda, M.W.A., Jacobs, R.M.J., Martin, R.A., Martin, C.M., & Schreiber, F. Protein Interactions Studied by SAXS: Effect of Ionic Strength and Protein Concentration for BSA in Aqueous Solutions. *J. Phys. Chem. B*. **111**, 251-259 (2007).
3. Maranas, J.K. The effect of environment on local dynamics of macromolecules. *Current Opinion in Colloid & Interface Science*. **12**, 29-42 (2007).
4. http://www.bevanlab.biochem.vt.edu/Pages/Personal/justin/gmx-tutorials/lysozyme/10_analysis2.html, 2008-2012 (2012).
5. Stribeck, N. *X-Ray Scattering of Soft Matter*. Springer, Heidelberg, (2007).
6. Mertens, H.D. & Svergun, D.I. Structural characterization of proteins and complexes using small-angle X-ray solution scattering. *J. Struct. Biol.* **172** (1), 128 (2010).
7. Svergun, D.I. Small-angle X-ray and neutron scattering as a tool for structural systems biology. *Biol. Chem.* **391** (7), 737 (2010).
8. Putnam, C.D., Hammel, M., Hura, G.L., & Tainer, J.A. X-ray solution scattering (SAXS) combined with crystallography and computation: defining accurate macromolecular structures, conformations and assemblies in solution. *Quat. Rev. Biophys.* **40**, 191-285 (2007).
9. Bonini, M., Fratini, E., & Baglioni, P. SAXS study of chain-like structures formed by magnetic nanoparticles. *Materials Science & Engineering C-Biomimetic and Supramolecular Systems*. **27** (5-8), 1377-1381 (2007).
10. Falletta, E., Ridi, F., Fratini, E., Vannucci, C., Canton, P., Bianchi, S., Castelvetro, V., & Baglioni, P. A tri-block copolymer templated synthesis of gold nanostructures. *Journal of Colloid and Interface Science*. **357** (1), 88-94 (2011).
11. Glatter, O., Scherf, G., Schillen, K., & Brown, W. Characterization of a Poly(ethylene oxide) Poly(propylene oxide) Triblock Copolymer (EO(27)-PO39-EO(27)) in Aqueous-Solution. *Macromolecules*. **27** (21), 6046-6054 (1994).
12. Mittelbach, R. & Glatter, O. Direct structure analysis of small-angle scattering data from polydisperse colloidal particles. *Journal of Applied Crystallography*. **31**, 600-608 (1998).