Physics 407 Project Overview

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Abstract. There is currently no cure for AD and no reliable method for early detection. The disease is characterized by plaques and fibrils formed in the brain. However, these plaques are not direct indicators of the disease, demanding further research into the aggregation process and its role. We use a small-angle x-ray scattering technique to obtain structural (and temporal) information of aggregated proteins associated with Alzheimer's, specifically α-syn A532 and tau 383 0N4R.

1. Overview of SAXS

Small-angle x-ray scattering is a technique used to understand structures on a nanometer scale. It measures elastic scattering at small angles $(0.1-10^{\circ})$ corresponding to intermolecular spacing between 1-100nm. X-rays are used for this application because their wavelength is of the same order as these structures' intermolecular spacing. The intensity of the scattered X-rays are measured on a CCD camera.

When an electromagnetic wave is incident on a charged particle, the particle experiences a force $F = q(E + v \times B)$. This causes the electron to oscillate and radiate a spherical wave. The scattered waves can interfere either constructively or destructively, creating a characteristic pattern corresponding to the intermolecular structure. The measured interference pattern is indicative of the intermolecular spacing of the sample.

The scattering vector q, (see fig. 2) is $\vec{q} = \vec{k} - \vec{k_0}$ where $\vec{k_0}$ is the incoming vector and $\vec{k}$ is the scattered vector. The wavenumber k is defined as $k = \frac{2\pi}{\lambda}$ and represents radians per unit distance. This results in an expression for the scattering vector or momentum transfer $q = \frac{4\pi \sin(\theta)}{\lambda}$.

The resulting scattering peaks from a structure are given by Bragg's law, $n\lambda = 2dsin\theta$ where d is periodic spacing in some structure. Therefore, the scattering angle off of the sample, 2θ, is inversely proportional to the repeating distance, d. Rearranging Bragg's law in terms of d and substituting the expression for momentum transfer results in an expression for the corresponding intermolecular spacing terms of the scattering vector $\vec{q}$ (see eq. 1.1)

$$d_{bragg} = \frac{2\pi}{q_{peak}}. \tag{1.1}$$

Therefore a larger q [1/nm] corresponds to a larger scattering angle. The purpose of the q range is to get a representation independent of distance.

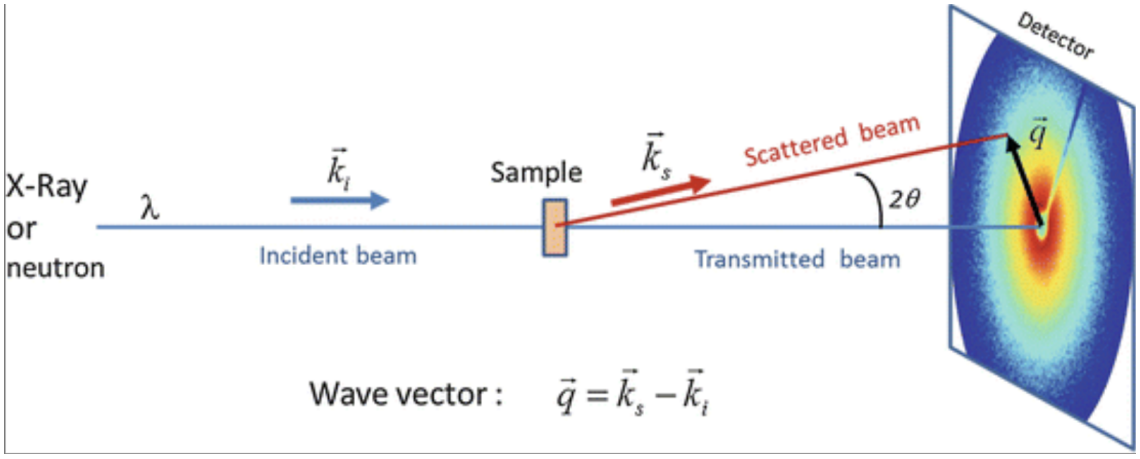

Figure 1. Schematic of incoming wave $\vec{k_0}$, scattered wave $\vec{k}$ and resulting $\vec{q}$ vector $\vec{k} - \vec{k_0}$.- not my figure)

2. DATA ACQUISITION (FOR PROVIDED DATA)

2.1. **Experimental Setup.** Previous measurements of powdered silver behenate (AgBe) and glassy carbon were taken. These measurements were performed using SAXSpace (Anton Paar, Ashland, VA). The incident beam had $\lambda = 0.154\ nm$ (CuK_α) and was configured to be monochromatic and point collimated. The scattered rays are recorded on a CCD camera (2084 x 2084 pixels). The sample was placed in a matrix holder (see Fig. 2). Measurements were taken at 110, 207 and 305 mm from the detector. A blank image, known as the dark current image, is taken before turning on the beam. Following the dark current acquisition, the beam is turned on, and an image of the empty sample container is taken at a given exposure. Next, the sample is placed in the container, and an image is taken at the same exposure used for the empty container.

For the silver behenate measurement, we used an acquisition time of 200 seconds and obtained three frames of dark current, blank and sample. The program automatically downsampled the image by a factor of 4. Each measurement is stacked by adding or averaging the three frames. These steps were repeated for sample-to-detector distances at 305, 207 and 107 mm. Different sample-to-detector distances allow us to probe different q ranges. Different q ranges give information about different intermolecular spacings.

For each distance, the background-corrected sample is found by subtracting I_{dc} (dark current intensity values) from the initial sample I_s. The background-corrected sample holder (matrix) is found by the same method. The background-corrected sample is divided by the sample transmission ratio T_s, and the background-connected matrix is divided by the matrix transmission T_m, where T is the ratio of the transmitted versus incident photons for a specific measurement. The process is described by (eq. 2.1):

$$\Delta I(q) = \frac{I_s(q) - I_{dc}}{T_s} - \frac{I_m(q) - I_{dc}(q)}{T_m} \tag{2.1}$$

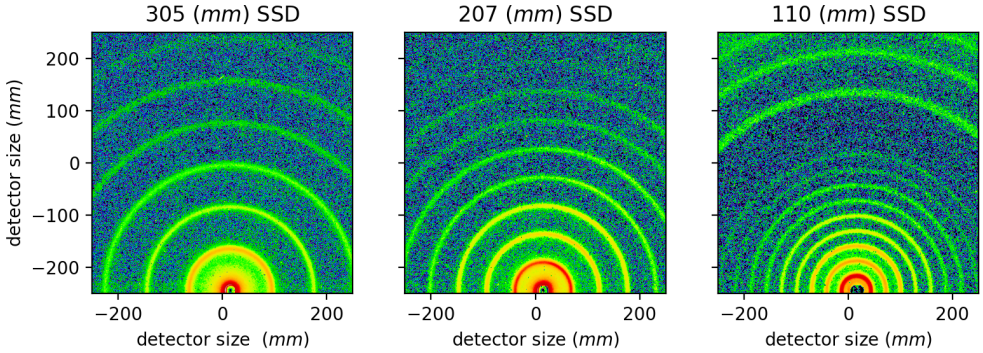

Figure 2. 2d scattering pattern for silver behenate (AgBe) after background correction for sample-to-detector distances of 305, 207 and 110 mm.

The 2d scattering patterns for silver behenate (see fig. 2) are shown above for varying sample to detector distances. The 1d scattering profile (see fig. 3) is obtained by taking a radial average of the scattering curves. The repeated diffraction peaks are obtained and converted into the corresponding distance. Note that these images are on a log scale for increased visibility. The transmission was estimated from the ratio of the intensities at $q = 0$.

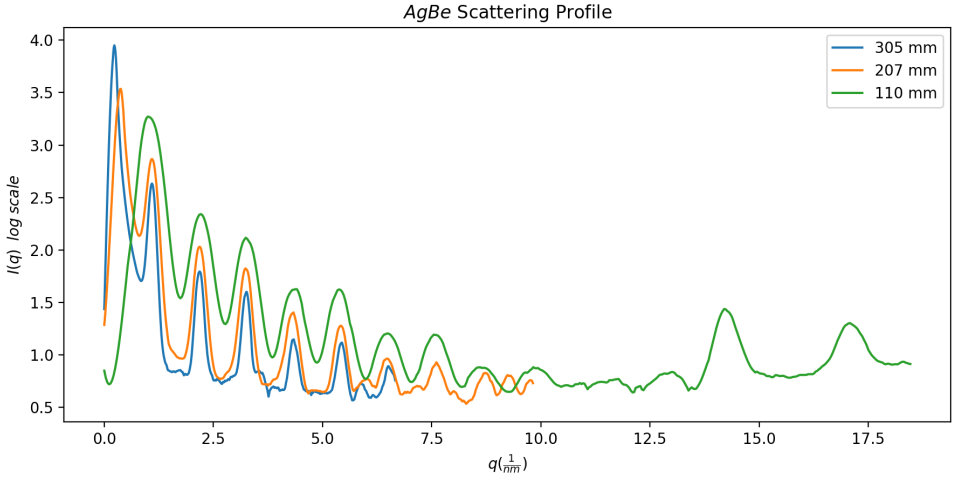

Figure 3. 1d scattering pattern after radial averaging of fig. 2 for sample-to-detector distances of 305, 207 and 110 mm.

3. Experimental Procedure for Phys 407

SAXS is a powerful method for quantifying the structure of proteins. It can provide valuable low-resolution information of structural changes of folded proteins offering insight into their responses to various external conditions. SAXS provides information about the

Bragg Peak Number	$q_{peak}\left(\frac{1}{nm}\right)$	spacing (nm)
1st	1.09	5.76
2nd	2.17	2.89
3rd	3.26	1.92
4th	4.33	1.43
5th	5.44	1.15

Table 1. The first 5 q_{max} values and the corresponding distances, d_{bragg}, for AgBe . The Bragg distance is found using eq. 1.1.

protein's size through the radius of gyration R_g and pair-wise distance $P(r)$ offering insight into the aggregated protein's disordered nature.

Certain environmental conditions cause proteins to aggregate, which results in neuropathologies such as dementia. Beta amyloid's aggregation has been extensively studied using SAXS (Dahal et al., 2017) Meanwhile, structural properties of fibrillated proteins such as tau and α-syn have been studied to a lesser extent.

The function of tau is to oversee the microtubules. However, in Alzheimer's, tau tends to aggregate and form helical filaments (PHF). Along with beta-amyloid, tau is a hallmark indicator of Alzheimer's. Conversely, α-syn is associated more strongly with Parkinson's disease and other forms of dementia with Lewy bodies. However, α-syn has been found in a majority of autopsied AD brains (Twohig & Nielsen, 2019), thus motivating this research.

3.1. **Sample Preparation.** We will be using a powdered version of α-syn A53T (Sigma Aldrich) and a powdered version of tau 383 (r Peptide). Generally, to resolve a sample in SAXS, it is necessary to have a sample to solution ratio of at least 0.5 mg/ml. To induce fibrillation, the sample can be heated, shaken, or put in a higher pH solution. The ideal temperatures, shaking procedures, and pH are to be determined. Alternatively, fibrillation can be induced if the sample is at a supercritical concentration (8mg/ml for α-syn) (Giehm et al., 2011).

3.2. **Data Collection.** Measurements of AgBe will be taken to ensure the device is working properly and obtain the center coordinates necessary for radial averaging. All measurements will be done on the same device and follow the same procedure specified in section 2. However, significantly more frames will be taken per sample and at a higher exposure. We will start by taking 300 frames at 60 seconds of exposure and vary the exposure and frames depending on the scattering intensity. Once the data are corrected for background scattering and dark current (eq. 2.1) a radial transform will be applied, and the scaling will be adjusted in terms of q.

3.3. **Data Analysis.** The scattering profile is the Fourier transform of the actual distribution of the sample. To get information about the sample, we can apply an inverse Fourier

transform:

$$P(r) = \frac{r}{2\pi^2}\int_0^\infty q^2 I(q)\frac{sin(qr)}{qr}dq \tag{3.1}$$

This transformation gives us an expression for the pair distance distribution function $P(r)$, which may be used to describe the shape of the macro-molecule.

Because our data is finite, a direct Fourier transform will not be suitable. Alternatively, the $P(r)$ term can be computed by fitting $P(r)$ to the intensity data in eq. 3.2 This method requires knowledge of D_{max}.

$$I(q) = 4\pi\int_0^{D_{max}} q^2 P(r)\frac{sin(qr)}{qr}dr \tag{3.2}$$

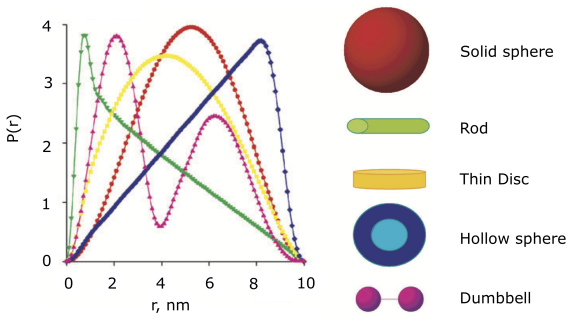

Figure 4. $P(r)$ for different macro-molecule geometries (from BioXTAS - not my figure)

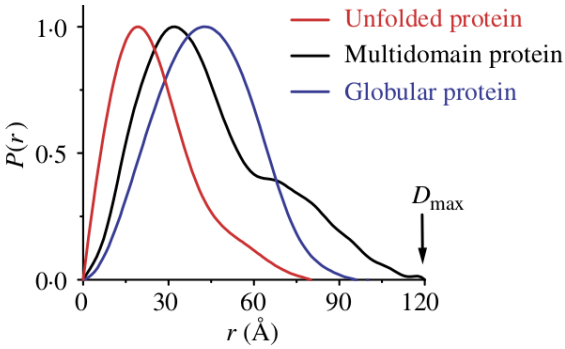

Figure 5. $P(r)$ for different protein types (from BioXTAS - not my figure)

We will obtain a measurement of the sample before aggregation and calculate its pair-distance distribution function. We then aggregate the protein by varying either the pH, temperature or by shaking the sample. We then measure the aggregated sample and obtain the pair distance function. The shape of the resulting $P(r)$ plot will provide information about the level of aggregation in the protein.

The presence of aggregates or fibrils makes this method slightly difficult because D_{max} needs to be determined. Alternative methods such as Guinier analysis may be used to verify the results. Additionally, if time permits, we may also study the time-dependent nature of the aggregation; however, the primary goal for this semester is to determine the level of fibrillation in tau and α-syn and obtain a low resolution render of the protein.

4. Upcoming work

4.1. **Tasks.** This semester I received training in operating the SAXSpace instrument and attempted to take measurements of glassy carbon and AgBe. It turned out there was a malfunction in the X-ray device, so we were delayed. Instead, I analyzed some previously collected data and focused on writing the code that I would need for the following semester. Additionally, I became comfortable with the experimental procedure for scattering profiles and familiarized myself with the device and the analysis techniques.

4.2. **Tentative Schedule and Reports.** Next semester's tentative schedule is to go into the FDA Tuesdays and Thursdays, 5 hours each. Currently, I have a physical lab notebook, which I transcribe to one note. This notebook will be updated biweekly (although I may move my notebook to LaTeX). I will also upload all code on GitHub. Throughout the semester I will be writing a more detailed explanation of the physics of aggregation and the SAXS device, including details on different analysis methods. I am also working on renders and animations of the device that will be included in my final report for physics 407.

5. Acknowledgments

This work was performed under the guidance of Dr. Aldo Badano, Division of Imaging, Diagnostics, and Software Reliability, OSEL/CDRH/FDA. I also want to thank the help of Dr. Eshan Dahal, DIDSR/OSEL/CDRH/FDA for guidance and mentoring in the lab.

References

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