

New Title: Observation of correlated x-ray scattering at atomic resolution

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Abstract

1 Introduction

In a pioneering paper, Kam [1] showed that correlated x-ray scattering (CXS) from an ensemble of randomly oriented particles could in principle reveal information about the internal structure of the particles beyond usual small and wide angle solution scattering measurements. The extraction of such information in the absence of an ordered system (e.g. a crystal) can be beneficial in biological studies, as many biological processes are inherently disordered (e.g. proteins in solution).

In order to gauge the feasibility of Kam’s method at atomic length scales, and to assess the difficulties associated with such a sensitive measurement, we conducted experiments measuring CXS from silver nanoparticle (NP) solutions at wide angles. Crucially, each measurement was conducted on an ensemble of NPs oriented randomly in three dimensions, extending previous work done in two dimensions [2], at small angles in 3-dimensions [3-4], and on single particles in 3-dimensions [5-6].

By successfully measuring CXS signal from an ensemble of silver NPs, we have demonstrated the effectiveness of Kam’s method given the current advances in x-ray technology. This experiment will serve as a benchmark for future experiments involving much weaker scatterers, such as proteins. It is our hope that the refinement of our analysis techniques on a well known sample such as silver NPs will help facilitate the extension of CXS to studies of biomolecules in solution.

2 Theory

Let $S(\vec{q}, \omega)$ represent the structure factor of an oriented particle in solution, i.e.

$$S(\vec{q}, \omega) = \left| \int \rho(\hat{R}(\omega) \cdot \vec{r}) e^{i\vec{q} \cdot \vec{r}} d\vec{r} \right|^2$$

where ρ is the electron density, ω is a triple of Euler angles, and $\hat{R}(\omega)$ is a 3-dimensional rotation operator.

Kam showed that the correlation function

$$C(\vec{q}_1, \vec{q}_2) \equiv \int S(\vec{q}_1, \omega) S(\vec{q}_2, \omega) d\omega \tag{1}$$

may be extracted from a CXS measurement where one repeatedly records snapshots of a solution of N identical particles, with each snapshot representing a unique ensemble of particle orientations. Kam argued (neglecting inter-particle scattering interference) that averaging correlations of intensity fluctuations on each snapshot would result in the construction of (1) , i.e.

$$\langle \delta n_k(\vec{q}_1) \delta n_k(\vec{q}_2) \rangle_k \Rightarrow C(\vec{q}_1, \vec{q}_2) \quad (2)$$

where $n_m(\vec{q})$ is the total photons scattered from all particles into momentum transfer vector \vec{q} on snapshot m and $\delta n_k(\vec{q}) = n_k(\vec{q}) - \langle n_k(\vec{q}) \rangle_k$.

For the case of silver nanoparticles we can represent them by the simplest model consisting of a face-centered-cubic lattice cutoff by a spherical boundary. The scattering can then be represented by reciprocal lattice vectors cutting the Ewald sphere and giving rise to Bragg peaks broadened owing to the the finite size of the nanoparticles. Hence, each snapshot records a series of “powder rings” corresponding to the different vectors of the reciprocal lattice, and the different particle orientations. We denote the magnitude of the strongest Bragg ring by $|\vec{q}_{111}|$ which is equal to the space diagonal of the reciprocal lattice body-centered-cubic primitive cell, $|\vec{q}_{111}| = \sqrt{3}a/2 = 2.66\text{\AA}^{-1}$. Each NP has 8 reciprocal lattice points at this magnitude. Using the Scherrer formula relating the width of a Bragg peak to the particle size (our NP distribution was peaked at 20 nm) , along with geometrical arguments discussed below, we find that $\approx 8.3\%$ of silver NP orientations will contribute to the first powder ring. Call this set of orientations $\Omega_{111,1}$. The sub-population of NP orientations in $\Omega_{111,1}$ that simultaneously subtend a second Bragg peak on the Ewald sphere at $|\vec{q}_{111}|$ will give rise to an auto-correlation signal along the $|\vec{q}_{111}|$ powder ring. Our best estimates suggest that $\approx 0.17\%$ of orientations in $\Omega_{111,1}$ satisfy this criterion, or roughly 1.1% of all NP orientations. Call this set of orientations $\Omega_{111,2}$. We arrive at these numbers by tracing out volumes of rotation of the reciprocal lattice points and determining which fractions intersect the Ewald sphere. To determine $\Omega_{111,1}$ we rotate the reciprocal lattice points over all possible orientations. To determine $\Omega_{111,2}$ we rotate the reciprocal lattice about one of the 8 vectors with magnitude $|\vec{q}_{111}|$, hence fixing 1 lattice point on the Ewald sphere and rotating until another intersects. These arguments are simple in nature, and deserve to be properly verified. We simulated these volumes of intersection and found $\Omega_{111,1}$ and $\Omega_{111,2}$ to represent 8.8% and 1.3% of all NP orientations respectively, in good agreement with our geometrical arguments [figure ?].

3 Methods

A snapshot in (2) is supposed to represent an ensemble of particles frozen at an instant in time, however exposure times are finite. Therefore, the sample was cooled to 100 Kelvin using a nitrogen cryo jet to ensure that the particles remained immobilized during each exposure. This also ensured that any heating due to the x-ray beam was minimal. We had an estimated 10^9 20 nm NPs per snapshot, but we observed significant numbers of NPs that were up to 50 nm in size. The NPs were held in colloidal suspension with glycerol-based antifreeze used to prevent the formation of solvent crystals at the low temperature. By monitoring the intensity at constant scattering angle one can check for sample damage and diffusion.

To house the solutions we used kapton capillaries with a 500 and 600 μm inner and outer diameter respectively. Kapton scatters into relatively lower angles as does glycerol, hence we did not anticipate corrupting our silver nanoparticle signal with scattering from the kapton or glycerol. The experiment was conducted at the micro-crystallography beamline (12-2) at SSRL. Samples were prepared a day early and stored in a liquid nitrogen bath. Samples were loaded and oriented in the X-ray beam using the Stanford Automated Mounting System (SAM) , controllable from the experimental hutch. Using a liquid nitrogen-cooled double crystal monochromator we tuned the beam energy to 17 keV. The beam was focused down to about $20 \times 50 \mu\text{m}^2$ using Rh coated Kirkpatrick-Baez mirrors. Snapshots were recorded on a Dectris Pilatus 6M pixel detector. Our goal was to record as many snapshots as possible, each one representing a different ensemble of particle orientations frozen in time. The sample holder was equipped to automatically rotate the capillary about its longitudinal axis. The capillary was oriented such that its longitudinal axis was perpendicular to the beam (Figure needed) and then it was rotated through a 150 degree angle. The photon counts were read out and reset every 0.7 second and every 0.3 degrees of rotation, giving us 500 shots per 150 degree rotational scan. This was deemed an optimal timing to simultaneously maximize signal and minimize damage and heating. The beam was shining on the capillary for the duration of the scan, hence the center of the capillary was constantly heated. However, this effect was negligible given the path length of the beam through the sample (500 μm). Between scans we moved the capillary longitudinally, so as to always probe different regions of the sample, and hence different ensembles of particle orientations.

The intensity fluctuation correlations in (2) were constructed by auto- and cross- correlating the powder rings on each snapshot. For example, assuming an area detector perpendicular to the x-ray beam defined by the coordinates (q^\perp, ϕ) , the auto-correlation function of the q_{111} powder ring

$$\begin{aligned} \langle \delta n_k(\vec{q}_{111,1}) \delta n_k(\vec{q}_{111,2}) \rangle_k &= \langle \delta n_k(q_{111}, \phi_1, \theta_{111}) \delta n_k(q_{111}, \phi_2, \theta_{111}) \rangle_k \\ &\Rightarrow \left\langle \int_0^{2\pi} \delta n_k(q_{111}^\perp, \phi) \delta n_k(q_{111}^\perp, \phi + \Delta \phi) d\phi \right\rangle_k \end{aligned} \quad (3)$$

should exhibit CXS peaks at values of $\Delta \phi = \phi_1 - \phi_2$ corresponding to the geometrical separation of two NP Bragg reflections at q_{111} .

Data from about 15,496 shots was collected and analyzed. A bicubic interpolation algorithm was used to convert the cartesian pixel lattice to polar coordinates for calculation of equations like (3).

4 Results

In order to eliminate any source of systematic noise in the intensity measurement we applied a binary filter to the data: intensities greater than a chosen threshold were set to unity, the rest were set to zero (Figure 1). The resulting auto correlations averaged to display a pair of peaks corresponding to the double Bragg scattering discussed above rising above the background due to the UDS events (fig 2)

The signal/noise in these measurements results both from the intrinsic UDS noise and from the Poisson statistics of photon scattering. To gauge the relative magnitudes of these sources of noise we took advantage of the photon counting capability of the Pilatus detector.

The total elastic scattering integrated over all angles is $n_{\text{scatteredphotons}} \simeq \Phi N \sigma_{\text{nanoparticle}}$ (up to a constant of $\mathcal{O}(1)$). Here N is the number of particles in the beam Φ is the x-ray fluence and σ_{NP} is the coherent part of the x-ray scattering cross section for a nanoparticle (calculated from the atomic cross section taken from tables.) Integrating the photon counts over all 5 Bragg rings which are accessed by the 17keV x-rays, we get a total scattered photon count of order 10^9 photons per shot. The x-ray fluence was $2 \cdot 10^{12}$ photons into a focal spot of $20 \times 50 \mu\text{m}^2$ for a 0.5 second exposure. From the tabulated coherent cross section, and putting in 260,000 silver atoms/NP we estimate a scattering rate of 1.6 photons per NP per shot. We conclude that there were of order $6.3 \cdot 10^8$ NPs in the beam.

From the estimates of the fraction of NPs giving CDS events given above, we conclude that the observed correlated scattering results from $\approx 0.1\%$ of the total scattering, giving an order of magnitude estimate of 10^6 CDS events per shot, thus showing that Poisson noise is not limiting the determination of the correlator profile in our simple case. Since our experiments show that averaging over a few thousand shots is adequate to separate the CDS events from the UDS background we conclude that the main impediment to accurate measurements of the correlated scattering comes from systemic errors resulting from anisotropy artifacts induced by the detector system, which we have been able to partially overcome by the nonlinear filtering to a binary signal.

5 Discussion

As originally shown by Kam, the correlator for scattering from an arbitrary molecule averaged over random orientations, $C(\vec{q}_1, \vec{q}_2, \psi)$, with ψ the angle between the scattering vectors, may be expanded in a series of Legendre polynomials in ψ whose coefficients may be directly calculated from the scattering structure factors of the molecule $F(\vec{q}) = \sum_i f_i(q) \exp(i\vec{q} \cdot \vec{r}_i)$ where \vec{r}_i are the atomic positions and $f_i(q)$ the atomic x-ray form factors. Hence accurate measurement of C in the 3-dimensional $\{\vec{q}_1, \vec{q}_2, \psi\}$ space can lead to constraints which can be placed on the atomic positions, thus giving a route to iterative refinement of a given model (ref Brunger).

Our results show that it is possible to obtain atomic scale information on the internal structure, for the very simple example of a silver nanoparticle, for a bulk sample containing of order 10^8 identical but randomly oriented particles. These results suggest that it should be feasible to obtain more detailed atomic scale constraints on models of more complex biomolecules by measuring scattering using x-ray pulses from xFELs (refs Hajdu, Chapman - Spence). Such measurements have the potential to scatter many more photons/molecule, yielding more detailed q_1, q_2, ψ information on the correlators.

6 References

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