Journal of Structural Biology xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Journal of Structural Biology

journal homepage: www.elsevier.com/locate/yjsbi

# Calculation of the cross-sectional shape of a fibril from equatorial scattering

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ARTICLE INFO

*Article history:*
Received 20 March 2017
Received in revised form 9 May 2017
Accepted 10 May 2017
Available online xxxx

Ths paper is written in celebration of the occasion of Donald L.D. Caspar's 90th Birthday.

*Keywords:*
Fiber diffraction
Small angle X-ray scattering
SAXS
Tobacco Mosaic Virus
TMV
Cross-section reconstruction
Aβ amyloid fibrils

ABSTRACT

An alternate formulation of helical diffraction theory is used to generate cross-sectional shapes of fibrous structures from equatorial scattering. We demonstrate this approach with computationally generated scattering intensities and then apply it to scattering data from Tobacco Mosaic Virus (TMV) and *in vitro* assembled fibrils of $A\beta_{40}$ peptides. Refining the cross-sectional shape of TMV from SAXS data collected on a 26 mg/ml solution resulted in a circular shape with outer diameter of ~180 Å and inner diameter of ~40 Å consistent with the known structure of TMV. We also utilized this method to analyze the equatorial scattering from TMV collected by Don Caspar from a concentrated (24% ~295 mg/ml) gel of TMV as reported in his Ph.D. thesis in 1955. This data differs from the SAXS data in having a sharp interference peak at ~250 Å spacing, indicative of strong interparticle interactions in the gel. Analysis of this data required consideration of interatomic vectors as long as 2000 Å and resulted in generation of images that were interpreted as representative of local organization of TMV particles in the sample. Peaks in the images were separated, on average by about 250 Å with a density consistent with Caspar's original measurements. Analysis of SAXS data from Aβ fibrils resulted in a cross-sectional shape that could be interpreted in terms of structural models that have been constructed from ssNMR and cryoEM. These results demonstrate an unexpected use of the small-angle region of fiber diffraction patterns to derive fundamental structural properties of scattering objects.



## 1. Introduction

Don Caspar has provided intellectual leadership to the Structural Biology community for over 60 years. He has been credited with coining the term 'Structural Biology' and the laboratory he established with Carolyn Cohen and Susan Lowey, at Children's Hospital in Boston may have been the first to be named 'Laboratory for Structural Biology'. His influence has gone far beyond the impact of his publications and is deeply embedded in the many personal interactions he has had through the years with essentially everyone in the field. Our understanding of the molecular basis of cell behavior can be traced back to his contributions to our understanding of the interactions among biological macromolecules. His early studies, on Tobacco Mosaic Virus (TMV) and Tomato Bushy Stunt Virus (TBSV) motivated the generalizations put forward in the theory of 'quasi-equivalence' communicated by Don and Aaron Klug in 1962 (Caspar and Klug, 1962) and established an intellectual foundation for understanding the structural basis of many cellular functions.

Don was introduced to TMV as a child by Isadore Fankuchen, a family friend, who, at the time was studying TMV structure with Bernal (Bernal and Fankuchen, 1941). Don has never lost interest. He did his Ph.D. thesis on TMV (Caspar, 1955), and the seminal results of that work were published (Caspar, 1956) back to back with a paper by Rosalind Franklin (Franklin, 1956). Comparison of his cylindrically averaged electron density map with that of the re-assembled TMV protein (minus RNA) carried out by Rosalind, provided the location of the RNA, deeply embedded in the protein coat. In this paper we compare small-angle X-ray diffraction data extracted from Don's Ph.D. Thesis with recent solution scattering data from dilute solutions of TMV and use these data sets to derive novel information about the packing of TMV particles in the concentrated gels[1] used in his early experiments. This work is based on a re-formulation of fiber diffraction theory that suggests a

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[1] The samples of TMV used for fiber diffraction have almost universally been referred to as "gels" but may more accurately be described as "sols". Given the historic use (or misuse) of the term "gel", we continue to use it here, to maintain historic continuity, confident that its use will cause no confusion.

http://dx.doi.org/10.1016/j.jsb.2017.05.003
1047-8477/

novel approach to calculation of the cross-sectional shapes of fibrillar objects.

Development of the theory of X-ray scattering by helical objects was motivated in large part by the studies of DNA. Crick and Cochran independently developed formulations that were merged and formalized by Vand and published in 1952 (Cochran et al., 1952). Over the next 5 years these were systematized into a widely used formalism (Klug et al., 1958). Here, we show that an alternate formulation of helical diffraction theory can be used as a basis for calculating the cross-sectional shapes of fibrils from small-angle X-ray diffraction data when the helical symmetry of the fiber is such that the small angle data is uniquely due to equatorial scattering. This approach utilizes algorithms analogous to those currently used for reconstruction of three-dimensional shapes of macromolecules from small angle X-ray scattering (Svergun, 1999; Svergun et al., 2001; Konarev et al., 2001; Franke and Svergun, 2009; Olek et al., 2014; Badger et al., 2016; Roig-Solvas et al., 2016). We demonstrate this approach using computationally generated data and then apply it to X-ray scattering data from TMV and Aβ amyloid fibrils.

## 2. Materials and methods

### 2.1. Alternate formulation of helical diffraction theory

Franklin and Klug (1955) showed that on layer line, $l$, in scattering from an object with axial repeat distance, $c$, the cylindrically averaged intensity is given by:

$$I_l(R) = \left\langle F^2\left(R, \psi, \frac{l}{c}\right)\right\rangle = \sum_n |G_{n,l}(R)|^2 \tag{1}$$

where $l$ is the layer line number, $c$ the axial repeat,

$$G_{n,l}(R) = \sum_j f_j J_N(2\pi R r_j) \exp\left(i\left(-n\phi_j + 2\pi l \frac{z_j}{c}\right)\right), \tag{2}$$

and the sum is over all atoms in the repeating unit of the structure with scattering factors $f_j$ and positions $(r_j, \phi_j, z_j)$. In this notation, scattering on the equator of a fiber pattern from a helical object is represented as an expansion of Bessel function terms,

$$\langle F^2(R, \psi, 0)\rangle = \sum_n |G_{n,0}(R)|^2, \tag{3}$$

where

$$G_{n,l}(R) = \sum_j f_j J_N(2\pi R r_j) \exp(-in\phi_j) \tag{4}$$

The $J_0$ term represents the cylindrically symmetric part of the structure, and higher order terms, $J_n$, are limited to multiples of the rotational symmetry of the object. For instance, since TMV has 49 subunits in 3 turns of its primary helix, the projection of the electron density along the particle axis will have 49-fold rotational symmetry and include terms with $n = 0, \pm 49, \pm 98, \ldots$. Bessel functions are essentially zero for values of their argument less than $n + 2$, meaning that to a resolution of ~10 Å, the cross-sectional shape of the axial projection of TMV is essentially circularly symmetric.

For objects that have lower order rotational symmetry, this formulation is not always the most convenient. An alternative formulation, based on the Debye formula (Debye, 1915) widely used in analysis of small angle X-ray solution scattering (SAXS) may be more useful. The Debye formula states that the solution scattering from a macromolecule can be expressed as

$$I(q) = \sum_i \sum_j f_i f_j \frac{\sin(r_{ij} q)}{r_{ij} q} \tag{5}$$

where $r_{ij}$ is the distance between atoms $i$ and $j$ and $q$ is the momentum transfer ($q = 2\pi R$). Using an analogous formulation (see Appendix), the scattering on the equator of a fiber diffraction pattern can be expressed as (Oster and Riley, 1952; Inoyue et al., 1993; Zhang et al., 2016).:

$$I_{eq}(q) = \sum_i \sum_j f_i f_j J_0(p_{ij} q) \tag{6}$$

where $p_{ij}$ is the projection of the distance between atom $i$ and atom $j$ onto a plane perpendicular to the fiber axis. This formulation can be generalized to all layer lines (Zhang et al., 2016).

The close correspondence of Eq. (6) to Eq. (5) strongly suggests that it can be used as a basis for reconstructing cross-sectional shapes of fibers analogous to the approach used to reconstruct three-dimensional shapes of macromolecules from SAXS data. An approach analogous to this utilized a neutron-scattering contrast series to calculate fiber cross-sectional shape (Whitten et al., 2008). Here we demonstrate the results of this approach using computationally generated models and then apply it to scattering from TMV and amyloid fibrils assembled from $A\beta_{40}$ peptides.

### 2.2. Guinier plot for fibrous assemblies

It is well known (Putnam et al., 2007) that at very small angles, solution scattering from globular macromolecules can be approximated as

$$\ln(I(q)) \sim \ln(I(0)) - \frac{R_g^2 q^2}{3} \tag{7}$$

where $R_g$ is the radius of gyration of the object. This approximation is valid in the small angle (Guinier) regime, often quoted as being limited to $qR_g < 1.3$ for globular proteins or $qR_g < 0.8$ for elongated structures (Putnam et al., 2007).

An analogous formulation can be made for scattering from fibrous structures. This formulation has been used rarely and there is some confusion in the literature as to its application. The confusion arises due to the limitation of small angle scattering to the equatorial plane in scattering from a helical object. If intensity is measured from a completely disoriented, dilute solution of fibrous materials, the small angle scattering measured corresponds to intensities on the equatorial plane being distributed over a three-dimensional volume in reciprocal space. To recover equatorial intensities from data collected in this way, it is essential to multiply by a geometric correction factor proportional to q. This is implicit in the radius of gyration of cross section formula oft quoted (Table 1 in Putnam et al. (2007)). However, if equatorial intensities are collected from a highly oriented fiber, gel or liquid crystal, taking disorientation into account (Makowski, 1978), this geometric correction is not required. As detailed in the appendix, for equatorial scattering from a fiber,

$$\ln(I_{eq}(q)) \sim \ln(I_{eq}(0)) - \frac{R_{xc}^2 q^2}{2} \tag{8}$$

where $I_{eq}(q)$ is the equatorial scattering intensity and $R_{xc}$ is the cross-sectional radius of gyration.

### 2.3. Cross-section reconstruction algorithm

Analogous to the widely used algorithms for generating three-dimensional shapes from SAXS data (Svergun, 1999; Svergun et al., 2001; Konarev et al., 2001; Franke and Svergun, 2009), we developed a highly efficient algorithm for computing two-dimensional electron density distributions consistent with equatorial scattering in a fiber diffraction pattern.

Please cite this article in press as: Roig-Solvas, B., Makowski, L. Calculation of the cross-sectional shape of a fibril from equatorial scattering. J. Struct. Biol. (2017), http://dx.doi.org/10.1016/j.jsb.2017.05.003