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Deducing the symmetry of helical assemblies: Applications to membrane proteins

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ABSTRACT

Helical reconstruction represents a convenient and powerful approach for structure determination of macromolecules that assemble into helical arrays. In the case of membrane proteins, formation of tubular crystals with helical symmetry represents an attractive alternative, especially when their small size precludes the use of single-particle analysis. An essential first step for helical reconstruction is to characterize the helical symmetry. This process is often daunting, due to the complexity of helical diffraction and to the low signal-to-noise ratio in images of individual assemblies. Furthermore, the large diameters of the tubular crystals produced by membrane proteins exacerbates the innate ambiguities that, if not resolved, will produce incorrect structures. In this report, we describe a set of tools that can be used to eliminate ambiguities and to validate the choice of symmetry. The first approach increases the signal-to-noise ratio along layer lines by incoherently summing data from multiple helical assemblies, thus producing several candidate indexing schemes. The second approach compares the layer lines from images with those from synthetic models built with the various candidate schemes. The third approach uses unit cell dimensions measured from collapsed tubes to distinguish between these candidate schemes. These approaches are illustrated with tubular crystals from a boron transporter from yeast, Bor1p, and a β-barrel channel from the outer membrane of *E. coli*, OmpF.

1. Introduction

The analysis of helical diffraction from biological assemblies dates back to the original studies of DNA (Franklin and Gosling, 1953; Watson and Crick, 1953; Wilkins et al., 1953) and the folding of polypeptides into an α-helix (Pauling et al., 1951; Perutz, 1951). Those structures were solved by building models based on intensity profiles from X-ray fiber diffraction patterns, and this analysis not only laid the mathematical foundation for helical diffraction theory (Cochran et al., 1952) but also gave rise to Fourier-Bessel reconstruction methods for 3D reconstruction from electron micrographs (DeRosier and Klug, 1968). Since then, electron microscopy (EM) has been a major tool in studying the structural properties of helical assemblies that either appear naturally in a biological context, or are reconstituted *in vitro* (Egelman, 2015).

There are many examples of naturally occurring helical assemblies, such as actin (Galkin et al., 2015), myosin filaments (Woodhead et al., 2005), microtubules (Li et al., 2002), amyloid fibrils (Schmidt et al., 2015), bacterial flagella (Trachtenberg and DeRosier, 1992), tobacco mosaic virus (Jeng et al., 1989), and filamentous bacteriophages (Wang et al., 2006). Helical assemblies formed by reconstitution of purified proteins include the capsid protein of polyoma virus (Baker et al., 1983), dynamin (Low et al., 2009; Zhang and Hinshaw, 2001), BAR proteins interacting with lipid bilayers (Frost et al., 2008) and integral membrane proteins embedded within such bilayers (Coudray et al., 2013; Toyoshima and Unwin, 1990; Zhang et al., 1998). In the case of membrane proteins, the helical symmetry offers a practical advantage over the alternative planar morphology of conventional 2D crystals formed, for example, by bacteriorhodopsin (Henderson and Unwin, 1975) and light-harvesting complex II (Wang and Kühlbrandt, 1991). In particular, molecules within helical assemblies adopt a complete range of orientations, thus overcoming a major technical hurdle in the analysis of planar crystals by eliminating the need to collect images of tilted specimens. Furthermore, the helical assembly overcomes size limitations of single-particle analysis, which is currently impractical for many membrane proteins due to their small size.

Various reconstitution techniques have been used to produce helical assemblies of membrane proteins. For example, helical arrays of Ca^{2+}-ATPase (Young et al., 1997) and the ABC transporter

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MsbA (Ward et al., 2009) were produced by using BioBeads to rapidly remove detergent, helical arrays of the polytopic mitochondrial outer membrane protein TspO were grown more slowly in Slide-A-Lyzer dialysis cassettes (Pierce) (Korkhov et al., 2010a), and the membrane domain of human erythrocyte band 3 was crystallized in dialysis buttons (Yamaguchi et al., 2010). In recent years, our laboratory has developed a pipeline for automating crystallization screens using 96-well microdialysis plates, which yielded helical crystals for a large number of different membrane protein targets (Lasala et al., 2015).

Although there are different approaches for 3D reconstruction of helical assemblies, they all rely on an initial assignment of the helical symmetry, which is often a difficult process (Egelman, 2014). The conventional parameters used to describe a simple, one-start helix are its rise and the number of subunits per turn (Diaz et al., 2010). However, membrane proteins typically adopt more complex helical assemblies making it more convenient to define an indexing scheme that is comparable to indexing diffraction patterns from 2D and 3D crystals (Toyoshima, 2000). In the case of planar 2D crystals, assignment of indices to diffraction spots is relatively straight-forward and leads directly to an estimate of the size and shape of the unit cell. In contrast, the cylindrical geometry adopted by helical assemblies makes it difficult to establish the number of unit cells that are wrapped around the cylinder (Fig. 1) and thus the parameters that govern the helix (Egelman, 2014). Moreover, the diameter of helical assemblies produced by reconstituted membrane proteins is often variable, thus generating a number of different helical symmetries within a given sample, all of which are derived from the same underlying lattice. In such cases, it is necessary to segregate the helical crystals into classes according to their distinct symmetries. The determination of helical symmetry is facilitated by graphical software tools like Windex (Ward et al., 2003), EMIP (Diaz et al., 2010) and SPRING (Desfosses et al., 2014). However, given ambiguities in this process, it is important to define a set of approaches that can be used not only to establish, but also to validate the indexing scheme, which, if not correct, leads to incorrect structures and inappropriate interpretations of molecular function.

In this report, we start by reviewing the conventional approach to indexing, which relies on the position and phase of the layer lines composing the diffraction pattern. Because this approach leads to ambiguity for the relatively wide tubes formed by membrane proteins, we go on to describe alternative approaches designed to identify and validate the correct scheme. These alternatives include summing diffraction patterns from multiple tubes, analyzing the 2D lattice of collapsed tubes, comparing diffraction patterns from experimental images with those from synthetic images, and comparing density maps from tubes with related helical symmetries. We illustrate the utility of these approaches by analyzing images of two different membrane proteins: the boron

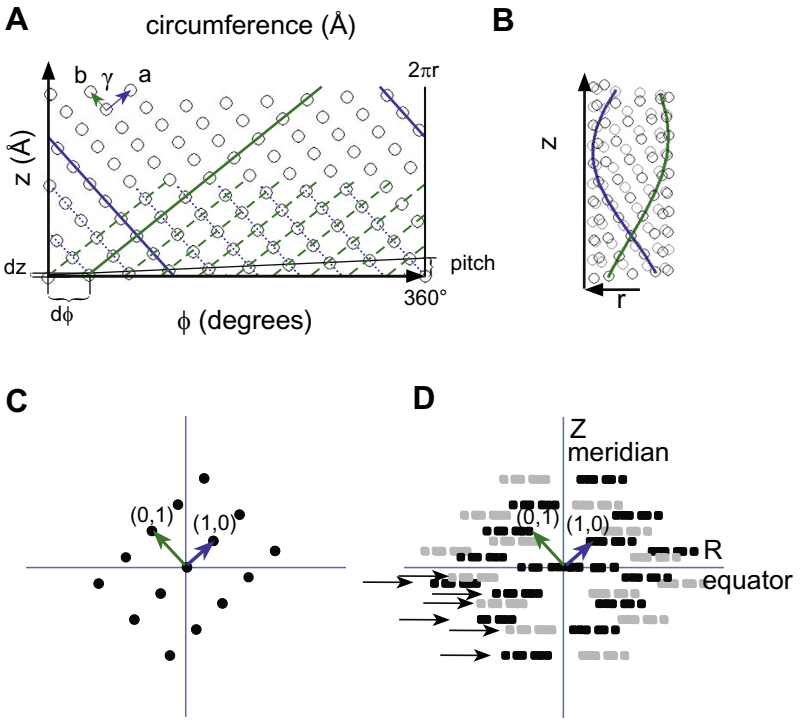

Fig. 1. Relationship between a planar, 2D lattice and a helical assembly. (A) The surface of a helical assembly is composed of a planar lattice, which is characterized by unit cell axes *a* (blue) and *b* (green) and the intervening angle γ. The horizontal axis corresponds to the circumference of a cylinder, which can be measured either with azimuthal coordinates (ϕ = 0–360°) or a linear dimension (0–2πr, r is the radius) and is depicted as a vector extending from one point in the lattice to another. These two points coincide when the assembly is rolled into a cylinder. The vertical axis corresponds to the longitudinal axis of the cylinder, which has a linear dimension. (B) The same lattice as in A, but rolled up into a cylinder. The solid blue and green lines correspond to those in A and follow a helical path around the surface of the cylinder. Each of these lines belongs to a helical family, which are drawn as parallel, dotted lines in A. The start numbers for each family correspond to the number of parallel lines that cross the circumferential vector, which in this case are 10 for the green helices and 9 for the blue helices. The Bessel orders for these helical families are therefore −9 and 10, with the sign reflecting the handedness of the helices. (C) Mock diffraction pattern from the planar lattice in A showing the principal (1,0) and (0,1) reflections. This pattern of reflections is consistent with Bragg's law that describes how the lattice lines drawn in A generates this diffraction pattern. (D) Mock diffraction pattern from the helical lattice in B. Each discrete reflection in C generates a Bessel function, depicted as a series of three amplitude peaks drawn in black. In addition, the helix generates mirror symmetry about the meridional axis (Z), resulting from the superposition of near and far sides of the assembly. This mirror symmetry gives rise to the amplitude peaks drawn in grey. Together, the black and grey amplitudes constitute a layer line. The rise dz and the azimuthal angle dϕ are shown in panel A, which characterize a one-start helix running through all of the points in the lattice. This one-start helix has a pitch, indicated on the right in panel A, and the distance between the corresponding layer line (with a Bessel order of one) and the equator will equal the reciprocal of this pitch. Such a one-start helix is sometimes referred to as a "genetic helix" and is useful for iterative real-space helical reconstruction methods.