Oral presentation

Quaternary Symmetry of a Prokaryotic Potassium Channel in Both the Open and Closed Conformation: Conclusions from an information-theoretic analysis of digital TEM images

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Information-theoretic methods for objective classifications of digital images and electron diffraction spot patterns into projected Laue classes and plane symmetry groups [1, 2] as well as 2D point symmetry groups [3] have recently been developed. The objectivity of these crystallographic symmetry classifications is ensured by the selections of the best geometric models for complementing aspects of the periodic signal from a crystal in its image and/or diffraction data on the basis of geometric Akaike Information Criteria [4].

Two experimental transmission electron microscope (TEM) images of the cyclic nucleotide-modulated potassium channel MloK1 from bacterium mesorhizobium loti were downloaded from the EM Data Bank [5]. Both of these images had been recorded by other authors with a large under-focus of the objective lens at a specimen goniometer tilt of zero degrees. Due to the very low electron dose during the recording of the TEM images, they are as noisy as such images typical are in structural biology studies. One of these images is from the potassium channel in its open conformation and the other in its closed conformation. The plane symmetry of this potassium channel is in zero-tilt TEM images generally believed to be *p4gm* [6-8], which results in 3D point symmetry group *4* as the quaternary point symmetry for the membrane protein complex itself in both conformations. A structural dynamics model for the opening and closing mechanisms of this potassium channel was accordingly restricted to this particular 3D point symmetry [8].

The information-theoretic analysis [2] implies, however, that imposing this restriction is not warranted on the basis of symmetry classifications of the experimental TEM images that rely exclusively on the experimental data itself. Fittingly, a 2020 review [9] of the evolution of data standards for cryo-EM structures concluded that "as currently practiced, the procedure is not sufficiently standardized: a number of different variables (e.g. … threshold value for interpretation) can substantially impact the outcome".

The Kullback-Leibler best projected Laue class and plane symmetry group in these two TEM images are objectively only *2mm* and *p2gg* [2]. This fact assigns 3D point group *2* and a strong four-fold pseudosymmetry to the quaternary structure of this membrane protein complex in both conformations. This is quantified by the Akaike weights [1] for the *4mm* classification (which is theoretically less sensitive to small sample movements during TEM imaging than the *p4gm* classification). In other words, the numerically processed structural information in the digital TEM images supports the conclusion that the membrane protein complex features a two-fold rotation axis more strongly than the alternative that there is a four-fold rotation axis. A TEM image/electron density map data supported model mechanism for the opening and closing of this particular potassium channel that is restricted to four-fold rotation symmetry (as the one in [8]) has, accordingly (at the present time), less experimental support than an alternative mechanism that is restricted to incorporate two-fold rotation symmetry only.

Experimental TEM images of this membrane protein that are less noisy and/or sets of translation averaged complex Fourier coefficients of the direct space image intensity that were recorded from much larger sample areas (or several samples) may allow for more definitive quantifications of the genuine symmetries and pseudo-symmetries of this membrane protein in the future. Experimental high-spatial-resolution electron diffraction patterns of this membrane protein could also be useful for such quantifications when they are classified with the information theoretic method [3]. With the *objective* crystallographic symmetry quantification method demonstrated on experimental TEM images, the modest suggestion is made to do away with the subjectivity in structural biology that comes with arbitrarily set symmetry interpretation thresholds.

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