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Distinguishing Between Quaternary Symmetries and Pseudo-symmetries in a Prokaryotic Potassium Channel in Both the Open and Closed Conformation

Peter Moeck*

Department of Physics, Portland State University, Portland, Oregon, USA
* Corresponding author: pmoeck@pdx.edu

Information theory based methods for objective classifications of digital images and electron diffraction spot patterns into two dimensional (2D) Bravais lattice types [1], projected Laue classes and plane symmetry groups [2,3], as well as 2D point symmetry groups [4] 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 2D periodic signal in the image and/or diffraction data on the basis of geometric Akaike Information Criteria [5]. As recently reviewed [6], these particular crystallographic image classification techniques are the only ones that can be considered to be objective, i.e. researcher and arbitrary thresholds independent.

Digital input images are in the objective projected Laue class and plane symmetry group classification techniques [2,3] considered to consist of the pixel-wise sums of more or less Gaussian distributed noise and an unknown underlying signal that is strictly 2D periodic. Structural defects in the molecular (or atomic) 3D array, instrumental image recording noise, slight deviations from zero-tilt conditions in transmission electron microscopy (TEM), and small inaccuracies in the algorithmic processing of the image data all contribute to one generalized noise term. Geometric Akaike weights that represent the probabilities of particular crystallographic symmetry models to be the Kullback-Leibler best model in a set of alternative models of the experimental data are also obtained with the information-theoretic techniques.

These techniques are analytic in nature rather than based on machine learning (ML). This enables them to deal with all types of pseudo-symmetries as well as with the well-known symmetry inclusion relations, i.e. subgroups and supergroups [7]. Machine learning systems have so far ignored pseudo-symmetries in crystallographic symmetry classification studies. Traditional translation-equivariant ML systems are rather ineffective when symmetry inclusion relations are at the core of a classification problem, see expanded arXiv versions of [3,6]. Equivariant ML systems that capture the geometry/symmetry of Euclidian spaces are expected to perform better than traditional ML systems [8] in such tasks.

Two experimental TEM images of the cyclic nucleotide-modulated potassium channel MloK1 from bacterium mesorhizobium loti were downloaded from the EM Data Bank [9]. Both of these images had been recorded by others with a strong under-focus of the objective lens at a goniometer tilt of zero. Due to the very low electron dose during the recording of the TEM imaging, they are as noisy as it is typical in structural biology studies. One of these images is from the potassium channel in its open 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* [10-12], 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 of the opening and closing mechanisms of this potassium channel was accordingly restricted to this particular 3D point symmetry [12]. As the analysis results below show, imposing this restriction is

not warranted on the basis of a symmetry classification of the downloaded experimental TEM images that relies exclusively on the experimental data itself. Fittingly, a 2020 review [13] 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"* [14].

The Kullback-Leibler best projected Laue class and plane symmetry group in these two TEM images are *objectively* only *2mm* and *p2gg*. This fact assigns 3D point group *2* and a strong four-fold pseudo-symmetry to the quaternary structure of this membrane protein complex in both conformations. This pseudo-symmetry is quantified by the Akaike weights 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 experimental 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. An image/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 [12]) 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 image intensity that were recorded from larger sample areas and several samples may allow for more definitive quantifications of the genuine symmetries and pseudo-symmetries 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 theory based method [4]. With the new crystallographic symmetry quantification method demonstrated on experimental TEM images, the suggestion is made to do away with the subjectivity in structural biology that comes with arbitrarily set symmetry interpretation thresholds.

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