A new approach for structure analysis of two-dimensional membrane protein crystals using X-ray powder diffraction data

Integral membrane proteins (IMPs) are involved in such neurological processes as memory and fine motor control. Their dysfunctions are implicated in many diseases including schizophrenia, Alzheimer's and Parkinson's diseases. To date, however, only approximately thirty unique structures of IMPs have been solved to atomic resolution, compared to more than 3000 unique crystal structures for soluble proteins, because of the intrinsic difficulty in extracting these proteins from the membrane and manipulating them to produce three-dimensional crystals for structural analysis [1]. The majority of known IMPs are much more likely to form two-dimensional (2D), rather than three-dimensional (3D) crystals during the crystallization process, which limits the possibility of obtaining their molecular structures using the standard methods of protein crystallography. Powder diffraction methods are, in contrast, not critically sensitive to the quality and dimensions of crystals, and this suggests their use in the structure analysis of 2D crystals. The application of powder diffraction methods for the structure analysis of proteins, however, is still regarded as mostly intractable because of the large number of unresolved (overlapping) reflections.

We have developed a novel approach for the structure analysis of membrane protein crystals using X-ray powder diffraction data [2]. This approach allows severely or completely overlapping reflections to be decomposed using intensity ratios of resolved reflections and a *priori* information, such as unit cell parameters, the crystallographic symmetry group, and the chemical composition of the sample. The approach not only allows the intensity of the individual components of the diffraction pattern to be extracted, but also furnishes initial estimates of the phases for subsequent refinement.

Our method is based on the fact that the electron density distribution of proteins is quite uniform at a low resolution, and scattering from a protein molecule comprised of discrete atoms is almost indistinguishable from that of a homogeneous and continuous envelope. The initial low-resolution shape of the molecule, therefore, can be considered as a solid ellipsoid, Fig. 1. In such a representation, the origin and the orientation of the low-resolution shape of the molecule are characterized by three parameters, R, α , and β. The first two parameters define the origin of the molecule in the asymmetric unit, and the last one defines the orientation of the molecule. For low-resolution structure analysis, therefore, the orientation and origin of the molecule, rather than the actual distribution of electron density inside the molecular envelope, is the dominant factor affecting the amplitude of the structure factor. In this way, different positions and orientations of the molecule give different ratios of intensities for the pair of Bragg reflections. The measured ratios of reflections that can be resolved experimentally, therefore, are used to refine the position, and orientation of low-resolution molecular structures within the unit cell, leading to the resolution of the remaining overlapping reflections. The molecular model is then made progressively more sophisticated as additional diffraction information is included in the analysis.

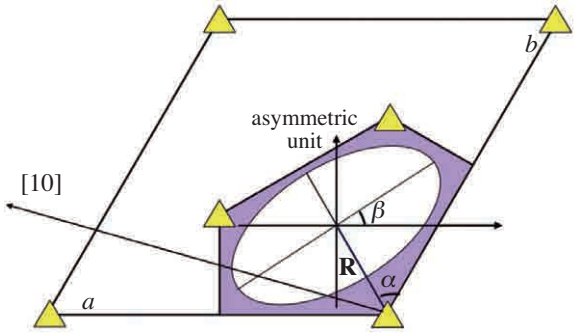

Fig. 1. The model of the molecular envelope located in the asymmetric unit of the *p3* symmetry unit cell.

The ability of the method to decompose completely overlapping reflections was first tested with the diffraction data simulated using the results presented in [3]. Figure 2 shows the reconstructed and simulated molecular form factor (MFF) of a bacteriorhodopsin molecule (bR). MFF characterizes the scattering from the individual objects (molecular clusters) which make up periodic crystal. One can see that the magnitudes and phases of calculated and simulated MFF are in good agreement.

We then analyzed the powder diffraction pattern obtained from the 2D crystal of purple membrane (PM), the crystalline form of bR [4]. The integrated intensities of diffraction peaks up to a 4 Å resolution were estimated using the Le Bail analysis, shown in Fig. 3(a). The completely overlapping reflections up to 7 Å resolution were decomposed using the method described previously. Structure factors were then

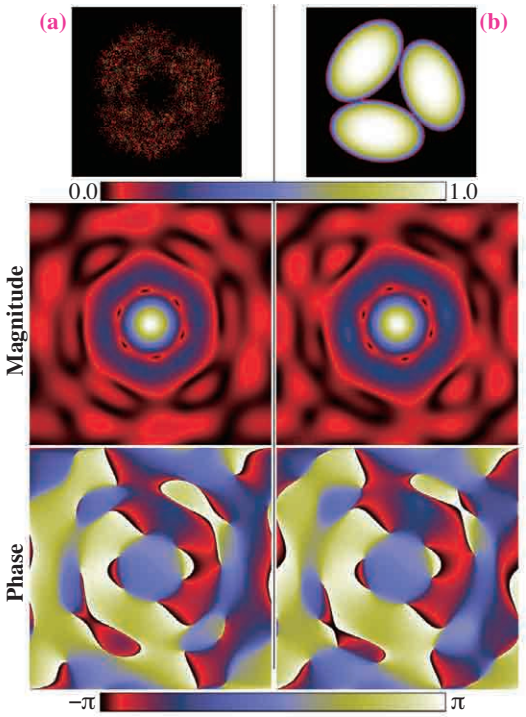

Fig. 2. The normalized molecular form factor of PM **(a)** simulated using discrete representation of the bR molecule, and **(b)** reconstructed using continuous representation of the bR molecule. [2]

analyzed by the maximum entropy method to obtain the 2D electron density map of PM. The resulting low-resolution 2D electron density map of PM is shown in Fig. 3(b). The map clearly indicates the envelope of a bR molecule and the electron density of the transmembrane α-helices. The three helices located in the inner part of the bR molecule have projected density higher than the helices at the outer part of bR.

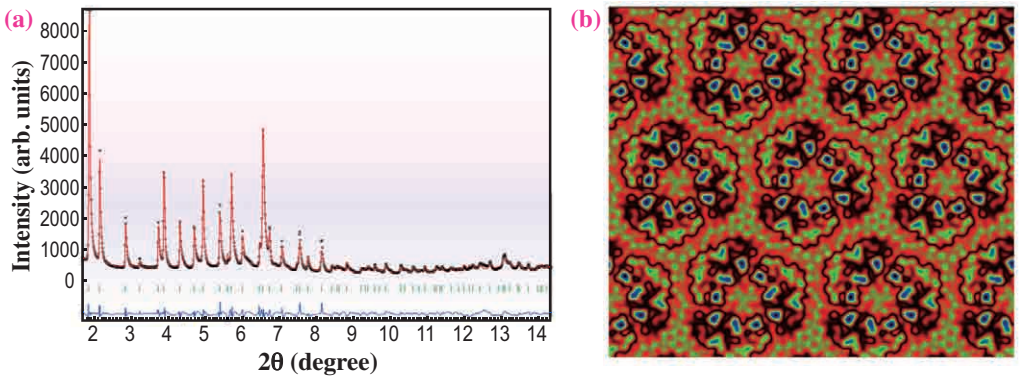

Fig. 3. **(a)** Results of the Le Bail analysis of the powder diffraction pattern obtained from the PM crystals. **(b)** The resulting low-resolution electron density map of bR reconstructed from experimental data. [2]

Ruben A. Dilanian

ARC Centre of Excellence for Coherent X-ray Science, School of Physics, The University of Melbourne, Australia

E-mail: roubend@unimelb.edu.au

References

[1] M. Baker: Nature Meth. **7** (2010) 429.
[2] *R.A. Dilanian, C. Darmanin, J.N. Varghese, S.W. Wilkins, T. Oka, N. Yagi, H.M. Quiney and K. A. Nugent: Protein Science* ***20*** *(2011) 457.*
[3] H. Luecke *et al.*: J. Mol. Biol. **291** (1999) 899.
[4] T. Oka *et al.*: Synchrotron Rad. **13** (2006) 281.