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Insights into the structure and function of membrane proteins from anomalous scattering of light atoms

More than a third of all proteins contain metal ions [1] with crucial roles in stabilizing structure or enabling a specific function. Identifying metal ion-binding sites is important for understanding the biological functions of proteins and further helps in designing potent therapeutics.

Membrane proteins have important roles to play in shuttling a variety of ligands and ions across lipid bilayers. The anomalous scattering properties of these ions can be harnessed to confirm their identity and location, to elucidate the mechanism of action of membrane proteins and to perform structure determination by experimental phasing. However, experimentally identifying and locating metal ions, such as calcium and potassium, in protein structures, can be challenging, since their X-ray absorption edges are inaccessible to most beamlines.

The unique wavelength range of the macromolecular crystallography beamline I23 at Diamond Light Source [2] allows identification and location of metal ions and lighter atoms of biological relevance (Ca, K, S, P and Cl) using X-ray anomalous scattering analysis. In a typical experiment, anomalous datasets are collected at two wavelengths, above and below the ion or element absorption edge, and then processed to calculate phased anomalous difference Fourier maps. The difference in anomalous peak heights between these two datasets allows the direct identification and visualisation of the ion in the protein structure.

The efficacy of this technique will be demonstrated using studies on membrane proteins investigating the gating mechanism of transporters, ion occupancy status through channels and multiple ion identification. Examples of experimental phasing of membrane proteins using sulfur single-wavelength anomalous dispersion will also be highlighted.

[1] Ibers J. A., Holm R. H. Modeling coordination sites in metallobiomolecules. Science, 1980, 209(4453):223–35.

[2] Wagner A., Duman R., Henderson K., Mykhaylyk V. In-vacuum long-wavelength macromolecular crystallography. Acta Crystallogr D Struct Biol, 2016, Mar;72(Pt 3):430-9.

Primary author: DUMAN, Ramona (Diamond Light Source, UK)

Co-authors: EL OMARI, Kamel (Diamond Light Source); ORR, Christian (Diamond Light Source); MYKHAYLYK, Vitaliy (Diamond Light Source); WAGNER, Armin (Diamond Light Source)

Presenter: DUMAN, Ramona (Diamond Light Source, UK)