Resolving the Phase Ambiguity of Single-Wavelength Anomalous Dispersion

This report features the work of Chun-Jung Chen and his co-workers published in Acta Cryst. ***D70****, 2331 (2014).*

X-ray protein crystallography remains a predominant method in the community to determine the three-dimensional structures of biological macromolecules. Despite great progress towards its automation and efficiency, phasing massive diffraction reflections remains a critical step for the determination of structures. The importance of the single-wavelength anomalous dispersion (SAD) method using sulfur and various heavy atoms in proteins in the phasing purpose has increased because protein crystals are typically damaged by synchrotron radiation during the long duration of data collection with the commonly used multiple-wavelength anomalous-dispersion method.[1,2] Two possible phase solutions (ϕ_1 and ϕ_2) generated from two symmetric phase triangles in the Harker construction for the SAD method cause the well-known phase ambiguity (Fig. 1).

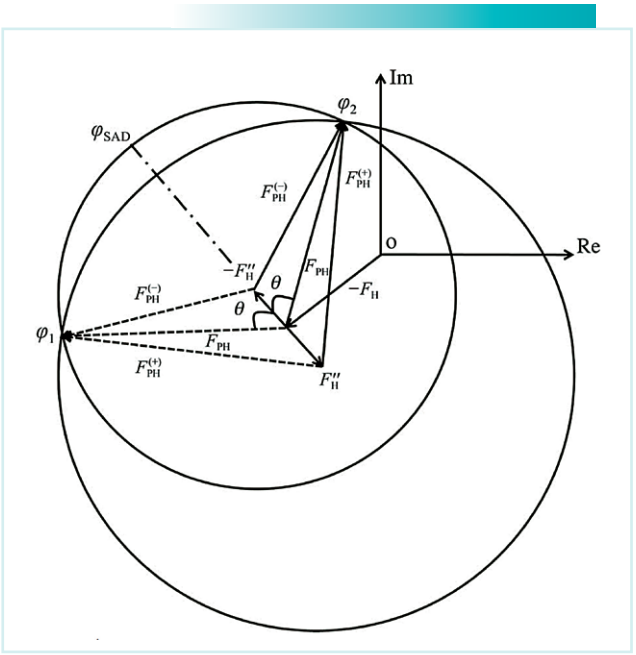

Fig. 1: Harker construction for SAD phasing. The contribution of heavy atoms to a structure factor consists of a normal part, F_H, and an anomalous part, F''_H. The structure factor F_{PH} is a normal part and F^+_{PH} and F^-_{PH} are anomalous parts from a protein crystal containing heavy atoms. (Reproduced from Ref. 3)

In 2014, a research team of Chun-Jung Chen from the Life Science Group, NSRRC, developed a novel algorithm to optimize the initial phases from the SAD.[3] This so-called direct phase-selection method greatly enhances the success of the subsequent electron-density modification, model building and structure determination of biological macromolecules, which will benefit the community of structural biology.

This newly developed algorithm can effectively improve the initial phases from the general SAD method with sulfur or heavy atoms using a novel direct phase-selection method based on a θ_{DS} list; θ_{DS} is the angle between the initial SAD phase and the preliminary DM phase, differing from previously reported methods. The authors demonstrated that this method of phase selection can resolve the phase ambiguity and improve the phases from SAD with increased effectiveness in combination with *RESOLVE* or *DM* utilizing only simple solvent flattening without phase combination and a FOM cutoff. They tested several experimental SAD data sets with sulfur or metal (Zn, Gd, Fe and Se) atoms as the anomalous scatterers, which were collected at protein crystallography beamlines, including **BL13B1** and **BL15A1** at the TLS and **BL12B2** and **BL44XU** at SPring-8. All results showed superior phases to generate electron-density maps with an enhanced quality and model building with greater completeness.

Their work showed that a high percentage correct for the SAD phases occurs at angle θ_{DS} in a range 35–145° in region 1 or 2, respectively (Fig. 2). The "θ_{DS} list" from the smallest to the largest angles can be generated. Reflections from the θ_{DS} list with angle θ_{DS} between 35° and 145° are selected, which have a large probability

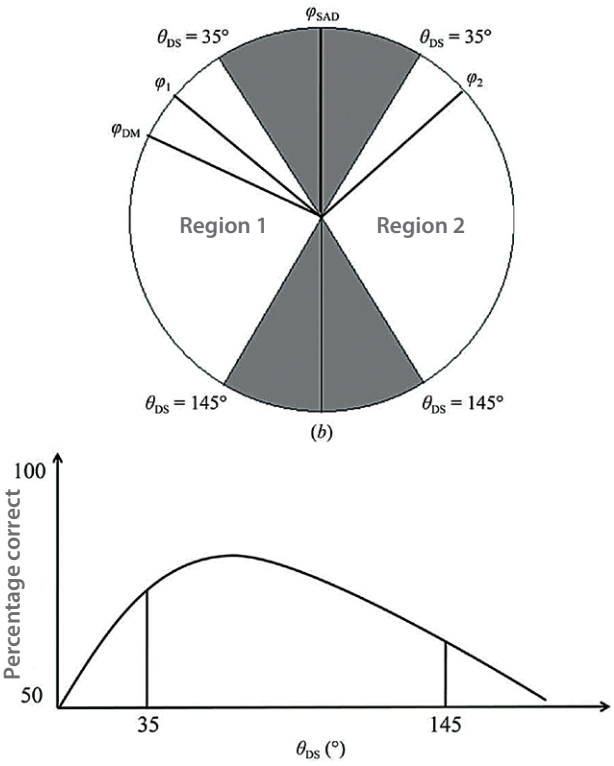

Fig. 2: (upper panel) Diagram of various phases and the range. The circle is divided into grey and white. Angles < 35° and > 145° in the grey zone show a smaller fraction correct in selecting phase φ_1 or φ_2 in region 1 or 2. (bottom panel) Schematic plot of histograms of the fraction correct as a function of angle ϕ_{DS}. (Reproduced from Ref. 3)

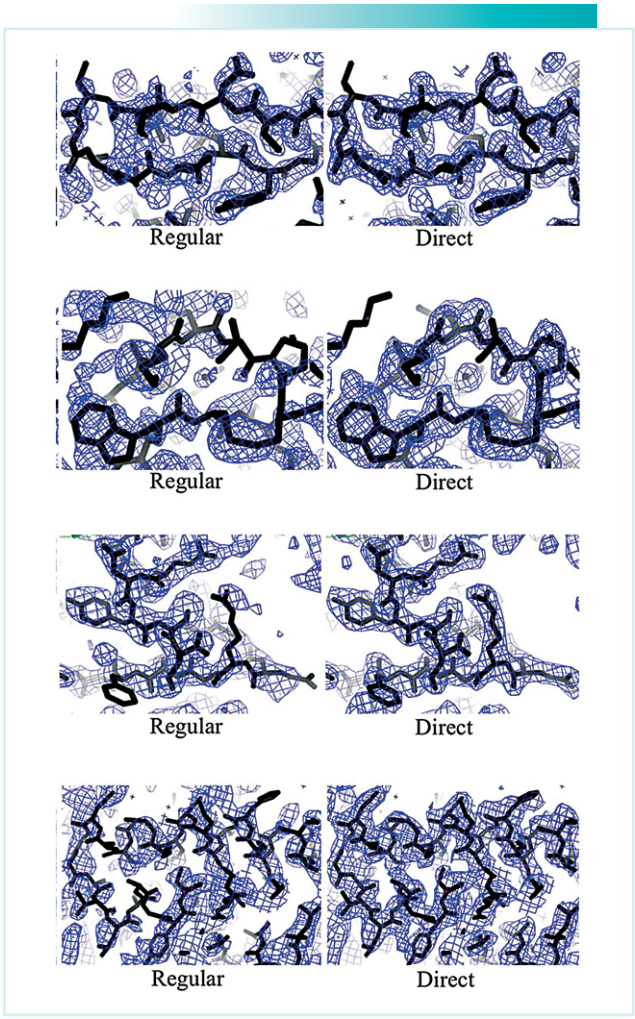

Fig. 3: Electron-density maps of lysozyme_Gd, lysozyme_S, insulin_S and HptB_Se (unknown structure) from the regular method and the direct selection method are shown with the same contour level 1.0σ in blue. The structures are shown as black sticks. (Reproduced from Ref. 3)

of the correct selected phase ϕ_{am}. The selected phase ϕ_{am} is either ϕ_1 or ϕ_2 , depending on the preliminary DM phase ϕ_{DM}^{NHL}. A portion of initial phases ϕ_{SAD} is then replaced correspondingly with these selected phases ϕ_{am}. All reflections with angle ϕ_{DS} in a range 35–145° are selected for an optimized improvement. The reflections with replaced phases (selected phases ϕ_{am}), and the rest with unselected initial phases ϕ_{SAD} are subsequently combined into a new data set with optimum initial phases ϕ_{SAD}^{S}.

A comparison of the conventional maps (the regular maps) and the direct-phase selection maps in all test cases shows that the continuity and completeness of the electron-density maps using the direct-phase selection method are significantly improved, and superior to those of conventional maps (Fig. 3). As a result, the completeness of auto-built residues with main chains and side chains in proteins with the direct-phase selection method is greater than that with regular methods. The map correlation coefficients and mean phase errors are generally improved by 0.05–0.2 and 10–18°, respectively, using the direct-phase selection method with a single cycle utilizing the new selected phases ϕ_{am}(ϕ_1 or ϕ_2), with a greater confidence level, to replace the corresponding initial SAD phases ϕ_{SAD}.

Their new direct phase-selection method provides a powerful protocol with an essential selection step, combined with current software of structure determination, such as *SOLVE/RESOLVE* and *CCP4*, to resolve the initial phase ambiguities of partial reflections for further

density modification. The resulting final DM phases and the electron density maps are improved effectively by the direct-phase selection method, compared with the regular methods, to yield improved statistical indicators of map quality and the completeness of model building for an efficient structure determination.

References

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