Invited Lecture

Verification in microED structure solution in ARCIMBOLDO and SHELX

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Electron diffraction (MicroED/3DED) can provide the three-dimensional atomic structures of molecules from previously unamenable samples1. Despite its transformative potential, MicroED is subject to the crystallographic phase problem. For macromolecules, MR solution has been considerably simplified by the availability of accurate and complete predictions. Especially for some types of versatile structures models may be misleading or unavailable. So, for peptidic structures, where MicroED has revealed novel structures of naturally occurring peptides, synthetic protein fragments, and peptide-based natural products1.

In the presence of limited data and complete prior models, as validation heavily relies on stereochemistry of the resulting model, the questions arise whether errors in the model are going unnoticed and in extreme cases, whether the determination has a chance to contribute information beyond the starting model. In other words, is every crystallographic determination we deposit truly an experiment?

We are proposing an alternative verification approach based on the discrimination among competing hypotheses. We started developing verification in the case of coiled-coils, setting up variations of our best solutions incorporating the ways in which wrong solutions had been seen to deviate2.

In the case of MicroED peptidic structures phased with ARCIMBOLDO3, a fragment-based approach combining Phaser4 and SHELXE5 to structure determination, enforcing stereochemical constraints through libraries of competing small model fragments allowed to discern congruent motifs in solution space.

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[2] Caballero, I., Sammito, M., Millán, C., Lebedev, A., Soler, N. & Usón, I. (2018). ARCIMBOLDO on coiled coils. Acta Crystallogr. D74, 194-204.

[3] Millán, C, Sammito, M. & Usón, I. (2015). Macromolecular ab initio phasing enforcing secondary and tertiary structure. IUCrJ 2, 95-105.

[4] McCoy, A.J., Oeffner, R. D., Wrobel, A. G., Ojala, J. R. M., Tryggvason, K., Lohkamp, B. & Read, R. J. (2017). Proc. Natl. Acad. Sci. USA 114: 3637-3641.

[5] Usón, I. & Sheldrick, G.M. (2024). Acta Crystallogr. D80: 4-15.