Life off the grid - case studies and cautionary tales from structural biology in solution

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In recent years, advances in cryo-electron microscopy (cryoEM) have made waves in the field of structural biology, pushing into realms of resolution previously accessible only through the venerable and established tools of macromolecular crystallography. Whether from crystallography or cryoEM, however, it is imperative to examine whether the model is a fair representation of the particle *in vivo*. That is, to check that the act of coaxing a molecule into a crystal or freezing it on a grid has not fundamentally changed its structure.

One means of evaluating this is through the complementary use of so-called "solution" techniques, such as magnetic resonance approaches (NMR, EPR), static and dynamic light scattering and, increasingly, small-angle X-ray scattering (SAXS). Such methods permit access to structural information in an *in vitro* environment that more closely resembles biological conditions, and can be directly compared against high-resolution data.

Here, we present several case studies demonstrating the power of solution analysis to complement and inform high-resolution results. A striking example is provided by a set of related plant coiled-coil resistance proteins, MLA10, Sr33 and Rx, in which a previously published structure of MLA10 was found to be in very poor agreement with solution data from light scattering and SAXS. By solving the solution structure of Sr33 via NMR and recrystallising MLA10, we are able to show that the dimeric conformation of MLA10 observed in the crystal was likely artifactual, and that these proteins exist predominantly as monomers in solution, reconciling longstanding disagreement in the field. Moreover, we show that extended constructs of Sr33 are able to dimerise, with implications for the mechanism of signalling *in vivo* (1).

Other examples include a similar situation regarding the yeast nuclear transport receptor, importin-beta, for which previous studies and simulations have suggested a range of conformations from highly extended to tightly coiled (2, 3). Solution data was consistent with a range of intermediate conformations, suggesting that the spring-like protein shifts within a wide region of conformational space when unbound, but, importantly, even with complementary data this region remains poorly defined. We also examine the flax rust effector protein AvrM, showing via SAXS that virulent and non-virulent mutants of the effector exhibit gross structural differences despite relatively minor sequence substitutions, suggesting that the particle's quaternary structure may play a critical role in host infection and defence.

References

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