Crystals of large macromolecular complexes often diffract quite poorly, typically having high solvent content, relatively feeble lattice contacts, quite weak subunit associations, and somewhat flexible interdomain linkages. Although resolution may be limited to $d_{\text{min}} > 7-8$ Å, the diffraction amplitudes should suffice, in principle, to specify conformational torsion angles; however, at such ultralow resolution, realizing and maintaining a suitable model within the radius of refinement convergence is a challenge. Important insights into biological processes may be obtained, but only if structural validity can be assured.

Having successfully refined a four-copy structure of Hsp70 DnaK in the S-state at 7.7 Å resolution as rigid bodies (Wang et al., Mol. Cell 81, 3919, 2021), we set out to refine a crystal structure of ryanodine receptor RyR1 at 8.0 Å resolution by having multiple quasi-rigid bodies to comprise the 5037 residues in each protomer of the RyR1-tetramer as complexed with calstabin. After molecular replacement from a 65%-complete cryo-EM model at 3.6 Å resolution (des Georges et al., Cell 167, 145, 2016), the structure was refined from a single rigid-body ($R_{\text{free}} = 0.53$), through five linked rigid bodies ($R_{\text{free}} = 0.47$), and finally as 18 linked domains ($R_{\text{free}} = 0.43$) identified in the cryo-EM analysis and then sub-divided as dictated by $(F_o-F_c)$ difference map and the $R_{\text{free}}$ analysis. We then turned to AlphaFold, presuming that the process had stalled due to incompleteness and uncertainty in the initial model. Trials showed that AlphaFold-predicted domains reduced $R_{\text{free}}$ when fitted into crystal density. We then systematically identified such AlphaFold-modeled domains and obtained substantial improvement ($R_{\text{free}} = 0.38$). Further improvement followed after Rosetta refinement using tight restraints in the `phenix.rosetta_refine` module ($R_{\text{free}} = 0.35$).

Finally, after grid-search optimization of the solvent mask, we obtained $R/R_{\text{free}} = 0.293/0.338$ for a model that comprises one protomer (4439 of 5037 RyR1 residues (88% complete), the 107-residue FKBP12.6, and an Au$_{102}$ gold cluster). Its geometry is excellent (97.4% favored Ramachandran angles with only 5/4546 outliers, 0 rotamer outliers, and $|\text{Rama-Z}| = 0.09$). The crystal structure replicates RyR1 as associated into sarcoplasmic-reticulum arrays, which are implicated in cooperative Ca$^{2+}$ release. We further tested this quasi-rigid-body AlphaFold/Rosetta-aided low-resolution structure (QARLS) refinement procedure in applications to the DNA-dependent protein kinase catalytic subunit (DNA-PKcs) at 6.6 Å resolution (PDB: 3kgv) and to a coat nucleoporin complex (CNC) structure at 7.4 Å resolution (PDB: 4xmn). These tests validate QARLS as a robust procedure for refining ultralow-resolution crystal structures. QARLS-like procedures should be useful in cryo-EM and cryo-ET analyses as well; however, the current lack of an $R_{\text{free}}$-like process complicates the monitoring of effectiveness for molecular microscopy.