MS10-O3 AMPLE: exploiting structural bioinformatics developments to extend the reach of molecular replacement to difficult cases

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Molecular Replacement (MR) remains the most popular route to solution of protein crystal structures, accounting for over 70% of recent PDB submissions. Nevertheless, computational structure solution by MR is currently limited in scope by several factors. Most obviously, if the target has a novel fold then, by definition, no crystal structure is available to serve as a search model. This is a particular problem for membrane proteins which are poorly represented in the PDB due to their comparative experimental intractability. Even if the fold of the target can be recognised as familiar then the available structures may be too distantly related and so too structurally divergent to succeed. Coiled-coil proteins, a class with biomedical and biotechnological importance, have their own idiosyncratic difficulties due to promiscuous intra- and inter-chain interactions as well as unpredictable irregularities in secondary structure. AMPLE is a pipeline for unconventional MR which provides a framework to exploit innovations in structural bioinformatics to allow more difficult cases like those above to be addressed. Novel folds have been successfully addressed by ab initio protein modelling with ROSETTA. We show here how a second program QUARK, importantly available as a server, solves a somewhat complementary set of targets [1]. The output from the QUARK server can be input seamlessly to our new AMPLE server. We further illustrate the potential of predicted contacts (evolutionary couplings) to raise the current size constraint on ab initio modelling: larger folds can be now be predicted and the results used in MR. AMPLE also works very effectively for transmembrane proteins, solving cases as large as 223 residues. We report spectacular success in solving coiled-coil targets, reversing the previous perception of their being particularly difficult for MR [2]. AMPLE solved 80% of a large test set of diverse architectures without any requirement to predict oligomeric state. Successes included chain lengths up to 253 residues, cases that diffracted to only 2.9 Å and examples of complexes containing other protein chains or DNA. Finally, we report preliminary data employing AMPLE to generate structurally conserved core search models from computationally-derived flexibility-based ensembles. We suggest that this can enhance the success rate of MR when only distantly homologous structures are available.

1.Keegan et al (2015) Acta D71,338: 2.Thomas et al (2015) IUCR J 2,198

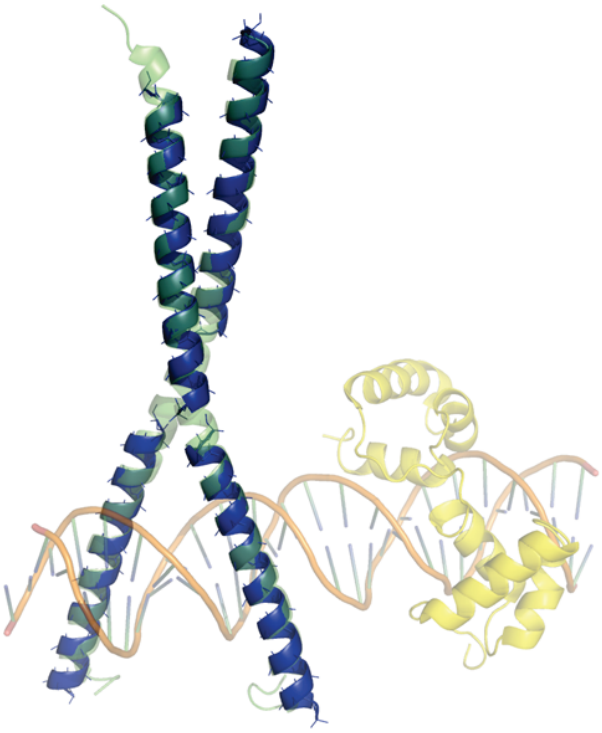

Figure 1. AMPLE solves a protein:DNA complex (1h8a) using the coiled-coil component. Four copies of a truncated search model (blue) allowed fully automated structure solution and required no knowledge of the coiled-coil association state.

Keywords: Molecular Replacement; Structural Bioinformatics; protein modelling; coiled-coil proteins; distant homology.