Poster Presentation

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Rapid molecular replacement of coiled-coil and transmembrane proteins with AMPLE

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Molecular Replacement (MR) is an increasingly popular route to protein structure solution. AMPLE[1] is a software pipeline that uses either cheaply obtained ab inito protein models, or NMR structures to extend the scope of MR, allowing it to solve entirely novel protein structures in a completely automated pipeline on a standard desktop computer. AMPLE employs a cluster-and-truncate approach, combined with multiple modes of side chain treatment, to analyse the candidate models and extract the consensual features most likely to solve the structure. The search models generated in this way are screened by MrBump using Phaser and Molrep and correct solutions are detected using main chain tracing and phase modification with Shelxe. AMPLE proved capable of processing rapidly obtained ab initio structure predictions into successful search models and more recently proved effective in assembling NMR structures for MR[2]. Coiled-coil proteins are a distinct class of protein fold whose structure solution by MR is not typically straightforward. We show here that AMPLE can quickly and routinely solve most coiled-coil structures using ab initio predictions from Rosetta. The predictions are generally not globally accurate, but by encompassing different degrees of truncation of clustered models, AMPLE succeeds by sampling across a range of search models. These sometimes succeed through capturing locally well-modelled conformations, but often simply contain small helical units. Remarkably, the latter regularly succeed despite out-of-register placement and poor MR statistics. We demonstrate that single structures derived from successful ensembles perform less well, and comparable ideal helices solve few targets. Thus, both modelling of distortions from ideal helical geometry and the ensemble nature of the search models contribute to success. AMPLE is a framework applicable to any set of input structures in which variability is correlated with inaccuracy. We also present preliminary data demonstrating structure solution of transmembrane helical structures using Rosetta modelling. We finally consider future sources of starting models which offer the hope that MR with AMPLE, in the absence of close homology between a known structure and the target, may soon be possible with larger proteins.

[1] *J. Bibby, R. M. Keegan, O. Mayans, M. D. Winn & D. J. Rigden, Acta Crystallogr. D Biol. Crystallogr. 2012, 68, 1622–1631,* ***[2]*** *J. Bibby, R. M. Keegan, O. Mayans, M. D. Winn & D. J. Rigden, Acta Crystallogr. D Biol. Crystallogr. 2013, 69, 2194–2201*

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