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Perspective

DEER-Refinement of X-Ray Crystal Structures to Guide Medicinal Chemistry

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INTRODUCTION

For Structure-based Drug Discovery to guide Medicinal Chemistry in real time, the underlying crystal structures must be reliable and representative of the relevant molecular conformation of the protein being targeted by the small molecule. While X-ray crystallography continues to be the gold standard for high resolution structural studies, crystallisable forms of the protein may not always coincide with the desired biologically relevant conformation [1]. The origins of these structural mismatches are often attributed to intrinsic physical constraints on the protein brought about by crystal contacts and packing forces within the lattice, inevitably influencing the resulting structure and accounting for differences from the lowest energy equivalent NMR structures [2]. This is seen particularly in the positions of polar side chains [3] and surface loops [4] and in the relative arrangements of domains, for example variation in the Cε3-Cε4 interdomain angle of IgE Fc seen in different crystal structures according to the packing forces acting on the Cε3 domain in different crystal forms [5].

Orthogonal biophysical techniques such as DEER (double electron-electron resonance) spectroscopy can be used to complement X-ray crystallography, to enable the generation of useful working models of structures, into which virtual fragments and leading compounds can be docked to gain a more accurate picture and understanding of the options available for rational elaboration.

DEER is a powerful technique, which enables precise intramolecular distance measurements to be obtained, in a close-to-native solution environment (before flash freezing). Typically, pairs of cysteine residues are introduced at specific points on a protein andare labelled with the nitroxide spin label, MTSL (1-oxyl-2,2,5,5-tetramethyl-pyrroline-3-methyl) methanesulfonothioate).In a Q-band spectrometer the spin echo decay from one label is modulated by the dipolar interaction with the second label, leading to an oscillating echo decay, the period of which is related to the distance between the two labels. The technique is well suited to assess distances in the range 2-8nm,with some2.5nmolesof protein consumed in each distance determination [6]. Distances between spin labels are determined with reference to the inherent flexibility of the MTSL linker [7-9] and presented as histogram plots. Any shifts in these average distances, for example brought about by small molecule-stabilisation of different conformers, can be readily determined. A distance network can be established from a library of paired cysteine mutants of the target protein to provide validation and refinement of crystal structuresand valuable insight into conformational change, biological function and molecular mechanism [10,11]. A major advantage of the method is the ability to probe local sites at specific points of interest within complex systems for precise structural information. In addition, distance measurements can be made between monomers in, for example dimeric [12] or, trimeric [13] conformations by labelling just one residue per monomer to ascertain relative positions and orientation.

The general applicability of combining X-ray crystallography with DEER for proteins and protein complexes, with no upper size limitation, is attractive, and the extra degree of structural refinement is especially important for those protein targets with important dynamic character (often non-enzyme pocket targets).

Simultaneous incorporation ofcomplex and highly coupled DEER-derived measurements to generateaccurate working models presents a significant computational challenge, and restrained ensemble molecular dynamics simulation [14] has been applied and successfully developed [15] to address this issue. Parallel molecular dynamics simulations are carried out in the presence of a biasing potential whichimposes the DEER distance distributions onto the system and enforce the ensemble average of a given property towards the experimentally derived value [16]. In this way, existing crystal structures can be both validated, if the distance measurements coincide, or refined by incorporation of the experimentally determined values, to generate working model structures.

While DEER has become established as a valuable tool in structural refinement and conformer definition, it is now poised to make a major contribution to rational small molecule drug discovery, particularly when targeting protein-protein interactions at allosteric sites, by enhancing the quality and specificity of both virtual screening and chemical elaboration.

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CONFLICTS OF INTEREST

ADGL holds shares and share options in UCB.

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