J Comput Aided Mol Des (2012) 26:91–92
DOI 10.1007/s10822-011-9537-7

PERSPECTIVE

Frozen out: molecular modeling in the age of cryocrystallography

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Received: 14 December 2011 / Accepted: 16 December 2011 / Published online: 24 December 2011

Abstract As molecular modellers we need to remember that the flexibility of a protein is necessary for it to function. Unfortunately, this flexibility is not readily apparent from the seductive molecular graphics rendering of cryocrystallographic results.

Keywords Protein crystallography · Protein flexibility · Protein function · Molecular graphics · Drug design

Protein crystallography coupled with molecular graphics has provided valuable insight as to the positions on lead molecules that are amenable to substitution and to the character of the preferred substituents [1, 2]. It could be reasonably assumed that structural information of the protein–ligand complex would lead to compounds with improved properties, including increased potency, better ADMET characteristics, etc. However, it has been difficult to predict the affinity and binding pose of newly designed molecules [2]. Indeed, there have been several reports that a structure-based design molecule binds in an orientation different from the known template. For example, the structure–activity relationships of a series of neuraminidase inhibitors was understandable only when crystallography revealed two different binding modes within the series [3]. In addition, docking of small molecule libraries to modeled or experimental structures does not typically identify all high-affinity ligands [4].

An obvious cause of these difficulties is that the inherent flexibility of the protein structure, and its energy landscape, is not readily apparent from the seductive molecular graphics rendering of the crystallographic results.

Protein flexibility as an element of drug design is emphasized in an early review that provided several examples of crystallographic demonstrations that similar molecules invoke different conformational states in their target protein [5]. A comparison of the structures of the complexes of pairs of very similar molecules showed that the water architecture in the binding site was different in 68% of the pairs; the conformations of side-chains changed in 50% of the complexes, but that of the backbones changes in only 7% of the similar pairs [6]. The change in positions of water molecules and side-chains often resulted in a quite different shape of the binding pocket. More recently, demonstrations by NMR [7] and by competitive labeling [8] reveal that structurally similar ligands induce different conformations in the β-adrenergic receptor. Similar results are found for adenosine a2 receptors [9–11].

Protein flexibility is required for function [12]. Enzymes must change conformation to accommodate substrates, to catalyze the reaction, to respond to allosteric modulators, and to expel the products; receptors must change conformation to accommodate and expel ligands and to couple to signaling partners; channels must change conformation in response to their associated gradient; etc. Such dramatic structural changes often are seen in different crystal structures of the same protein.

However, smaller conformational changes may be hidden in the crystallographic data [13, 14]. Although it is often assumed that ligand binding reduces the flexibility of the protein, there are many instances where the opposite is

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true, or indeed where some parts of the binding site are stabilized and others made more mobile [14]. They report different effects on active site mobility in ligand complexes of the same protein, Haemophylis influenza N-acetyl neuramidate lysase.

Limitations of protein crystallography are well documented but often ignored [15, 2]. Beginning in the mid to late 1980s most protein crystallography has been performed at cryogenic temperatures. The development of cryo-techniques has been a boon to crystallography, enabling the determination of large numbers of crystal structures that would not have been attempted without the low-temperature techniques. Cryocrystallography has also made possible the efficient use of high intensity synchrotron sources that would quickly destroy crystals at room temperatures. However, data collection at near liquid nitrogen temperatures is not without affect on the protein–ligand complex being studied. In a comparison of structures solved at cryogenic and room temperature in one case, it was shown that "cryocooling remodels the conformational distributions of more than 35% of the side chains and eliminates packing defects necessary for functional motions" [13]. Matthews and co-workers suggest that intermolecular contacts in crystals constrain the conformation of T4 lysozyme to such an extent that the crystal structure is no longer representative of dynamic behavior in solution [16]. Clearly, cryocrystallographic techniques, while important in collecting high-quality structural data, potentially has subtle effects on the resulting structures, of which down-stream users of the information should be aware.

Of course, a perfect method to calculate the energy of various protein substates would alleviate this problem, but while progress is being made computation is not able to do so at the moment [2]. In the meantime, we suggest that those who use a crystallographic structure to design new molecules use crystallographic B-factors and available computational techniques to identify residues and waters that are most likely to change position on binding a different ligand [17]. Additionally, other biophysical methods such as NMR, competitive covalent modification of residues, and spectroscopic investigations can help understand the relative flexibility of different parts of a protein structure.

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