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Progress in cytochrome P450 active site modeling

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Abstract

Models capable of predicting the possible involvement of cytochromes P450 in the metabolism of drugs or drug candidates are important tools in drug discovery and development. Ideally, functional information would be obtained from crystal structures of all the cytochromes P450 of interest. Initially, only crystal structures of distantly related bacterial cytochromes P450 were available—comparative modeling techniques were used to bridge the gap and produce structural models of human cytochromes P450, and thereby obtain some useful functional information. A significant step forward in the reliability of these models came four years ago with the first crystal structure of a mammalian cytochrome P450, rabbit CYP2C5, followed by the structures of two human enzymes, CYP2C8 and CYP2C9, and a second rabbit enzyme, CYP2B4. The evolution of a CYP2D6 model, leading to the validation of the model as an in silico tool for predicting binding and metabolism, is presented as a case study.

Keywords: Cytochromes P450; Drug binding; Drug discovery; Drug metabolism; Structural models; Predictive tools

Cytochromes P450 (P450s, CYPs)[1] constitute a large superfamily of heme-containing monooxygenases responsible for the oxidative metabolism of a wide variety of structurally different endogenous and exogenous compounds. Seven of the 57 known human isoforms of P450s are responsible for more than 90% of the metabolism of all pharmaceuticals in current clinical use: CYP1A2, CYP2C9, CYP2C18, CYP2C19, CYP2D6, CYP2E1, and CYP3A4. Some of these isoforms (CYP2D6 [1,2]; CYP2C9 [3–7]) display polymorphisms which can result in the poor metabolism of drugs. A major advance in drug development would therefore be the availability of a tool to predict whether or not a drug candidate will interact with the P450s in general, and if so which isoform(s) the drug candidate will preferentially interact with in particular. This could reduce significantly the failure rate in clinical trials by identifying potential problems at an early stage of development and consequently reduce the time and money required to bring a new drug to market.

The 3D-structure of a protein can provide valuable insight into its function. Ideally, experimental techniques such as X-ray crystallography, nuclear magnetic resonance (NMR) spectroscopy, and electron microscopy are used to determine the 3D-structure of proteins. Unfortunately, the vast majority of proteins are currently not amenable to these techniques as they are difficult to crystallize, insufficiently soluble, or too large for NMR studies. Alternative methods have been developed to determine the 3D-structure, one such technique is comparative (or homology) modeling.

It has been observed that proteins with similar amino acid sequences have a tendency to adopt similar 3D-structures [8]. Therefore, it is possible to predict the 3D-structure of a protein based solely on knowledge of its amino acid sequence and the 3D-structures of proteins with similar sequences. Although these models will

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[1] *Abbreviations used:* CYP, cytochrome P450; NMR, nuclear magnetic resonance; RMSD, root mean square deviation.

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be inherently less accurate than those derived experimentally, they are invaluable as they provide testable hypotheses in the absence of experimental data.

Up until recently, structural models of human P450s were based on the known, distantly related, bacterial P450s. The recent determination of the crystal structure of the more closely related rabbit CYP2C5 [9] has improved the reliability of comparative models for human P450s [10], and the availability of crystal structures of human P450s (currently CYP2C9 [11,12] and CYP2C8 [13]) will likely help to further improve these models.

This review gives an overview of the techniques used for modeling the three-dimensional structures of human P450s, presenting the development and validation of a model of CYP2D6 as a case study.

Methodologies for comparative modeling

Homology model building

Comparative modeling is a predictive technique, involving the generation of the 3D-structure of a (target) protein for which the amino acid sequence, but not the 3D-structure, is known. To generate a structure for the target protein the technique uses (i) the amino acid sequence of the protein and (ii) the 3D structure(s) of proteins with similar amino acid sequence (structural templates). A detailed analysis of comparative modeling as applied to the cytochromes P450 is not addressed here but is available elsewhere (e.g. [14]).

The results of comparative modeling are critically dependent on (i) the choice of structural template(s) and (ii) the alignment of the amino acid sequences of the target protein and the templates [14]. Programs for comparative modeling use one of two approaches—either a fragment-based stepwise approach, utilized in programs such as SWISSMODEL [15] and COMPOSER [16,17], or a single-step approach utilized in programs such as Modeller [18], our preferred method.

Model quality

Comparative modeling is normally used to predict the structure of a protein where no experimentally derived structure is available, and consequently direct comparison between a model and a crystal structure is not possible. To assess the quality of a model, it is therefore necessary to subject the model to a number of complementary, yet independent, tests. Our methods of choice are outlined below (see, e.g. [14] for a detailed discussion).

Two types of structural checks are generally used to assess the quality of a model: stereochemical quality and sidechain environment. A third structural check can also be used: the root mean square deviation (RMSD) between the main chain atoms of the model and those of the most homologous template.

It has been shown that the RMSD between main chain atoms in the template with the highest homology to the target and the model is a good method of validation [19]. This builds on earlier work [8,20] which showed that for two proteins there was a relationship between the percentage sequence identity and the RMSD of the main chain atoms in homologous regions. Examples of programs employed to check stereochemical quality of the models include PROCHECK [21], PROVE [22] and WhatIf? [23]. Amino acid environment can be assessed using complementary programs such as Errat [24] and Verify 3D [25].

It is important to stress that results from all of these validation procedures must be put into context by direct comparison with the results achieved by the templates used to generate the target model. It is unreasonable to expect a model to perform better than any of the templates it was based upon, and as such comparable values between the templates and the models for these validations are indicative of a valid model.

Model accuracy

The above programs assess the quality of a model by comparing its properties with the properties of a large number of crystal structures taken from the PDB [26]. The tests can show a model has similar properties to known crystal structures but they cannot give an indication as to how accurately a model represents the target protein. The function of a protein depends critically on its 3D structure (e.g. [27]), and therefore prediction and/or rationalization of experimental results can be used to check the accuracy of a model.

The binding mode of a ligand in complex with its receptor can be predicted using molecular docking. An important aspect of any docking method is an energy function that is capable of predicting binding modes. In general, a very large number of possible ligand–receptor orientations are generated, these are then scored using the energy function and the orientation with the most energetically favorable value is chosen. The most energetically favorable value of the function should correspond to the preferred binding mode of the ligand. In some applications, an estimate of binding affinity is used as the energy function [28–30]. In most docking applications, the ligand is docked into a receptor with known 3D-structure. It is also possible to dock ligands into homology models and by comparing the predicted binding modes and binding affinities with experimental data an assessment of the accuracy of the models can be made.

The subject of molecular docking has been widely researched and there are a large number of programs available, including AutoDock [31], DOCK [32,33],