3D GPCRs – a platform for the discovery of new drugs

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G-protein coupled receptors (GPCRs) are complex, membrane-embedded proteins, responsible for communication between the cell and its environment. These receptors are involved in a wide variety of body systems and processes, and control the physiology of almost all major organs in the body. Moreover, GPCRs are important in major diseases such as hypertension, cardiac dysfunction, depression, anxiety, obesity, inflammation and pain. This puts them amongst the most important drug targets for pharmacological intervention via activation or blockage of their action. Thus, while GPCRs are only a small subset of the human genome (2%-3%), they constitute about 50% of the drug targets that are of interest to the pharmaceutical industry. The success of GPCRs as drug targets over the past 20 years has spawned significant imitations in certain categories – such as beta-blockers in the treatment of hypertension. Hence – according to some estimates – GPCRs account for 50% of all drugs on the market, and 30% of the top 50 best-sellers, including such well-known brands as Claritin®, Zyprexa®, Zantac® and Cozaar®.

Drug discovery

GPCRs, being lucrative drug targets, attract a significant portion of the drug discovery effort worldwide. In a typical drug discovery programme, between 300,000 and 500,000 compounds are screened by High-Throughput Screening (HTS), yielding hits at the μM affinity level (1-30 μM), requiring – in addition to the HTS robotics – the synthesis, purchase, registration, storage and handling of hundreds of thousands of compounds. The best hits undergo a ‘hit to lead’ process in which medicinal chemistry is applied to convert the ‘hit’ into a ‘lead compound’ with <1μM affinity, involving the synthesis of as many as 1,000 compounds. Subsequent lead optimisation requires the synthesis of 200 to 2,000 compounds by a large team of medicinal chemists, culminating in an ‘early development candidate’ (EDC) which is ready for preclinical development. Overall, this discovery process – up to an EDC – will typically take two to seven years and cost $12-$50 million.

A new evolving paradigm for drug discovery is to use the 3D structure of the drug target to guide the
drug discovery process in what is often called ‘structure based drug discovery’ or ‘rational drug design’. This approach relies on the fact that interactions of molecules within the human body take place in three-dimensions (3D). Drug molecules bind to the functional site of the target protein, competing with the natural ligands and inducing (agonists) or inhibiting (antagonists) its activity. Since this interaction takes place in 3D, knowledge of the 3D structure should shed light on the parameters that drive the drug–target interaction, leading to a more rational and more focused approach to discovering and optimising drug molecules.

3D structure of GPCRs

Structurally, it is known that all GPCRs consist of seven transmembrane helices joined together by three extracellular and three intracellular loops. Of this structure, it is the transmembrane region that is of paramount importance for drug discovery. Site-directed mutagenesis studies have clearly shown that small organic compounds (that is, most drug compounds) bind primarily in a cleft formed by the portions of the transmembrane domains of the protein facing the extracellular milieu. The loops and N-terminal domains are involved in the binding of physiological peptide and protein ligands, but play only a minor role in the binding of drugs.

3D drug discovery for GPCRs

In the absence of experimentally determined 3D structures, the application of structure-based drug discovery becomes much more challenging, as the whole process relies on the quality of the computational 3D models. Hence, until recently, success in this field has been limited. However, with the introduction of advanced modeling algorithms – such as the PREDICT algorithm – and careful integration of numerous computational tools into a single streamlined process (Figure 1), structure-based drug discovery for this important class of drug targets has become a reality.

In the absence of x-ray structures, the first step is to generate computationally a high-quality 3D model of the target GPCR. This model is then used for 3D screening of large virtual compound libraries culminating in the identification of a small number of ‘virtual hits’ – that is, a selected set of compounds which are predicted to have the highest likelihood for binding to the target receptor. These virtual hits are purchased or synthesised, and then tested in biological binding assays to verify their potency. Since the confirmed hits are usually at the ‘lead compound’ level, they are directly subjected to a combined computational-experimental lead optimisation process, which is driven by the 3D structure of the target GPCR. Overall, this combined process can dramatically reduce the time and cost as well as increase the success rates in the preclinical phases of development.

3D models of GPCRs

A common method for modeling protein structure is through homology modeling; this models the structure of a new protein, based on its sequence homology to a set of proteins with previously solved 3D crystallographic structures. However, because only one non-druggable GPCR 3D structure has been solved so far, the utility of this approach to druggable GPCR is limited. To date, the only validated GPCR modeling methodology that does not rely on the rhodopsin structure is Predix’s PREDICT™ algorithm (2, 3). The PREDICT algorithm searches through the receptor’s conformation space,
In silico 3D screening is a method by which large virtual compound libraries – of the order of hundreds of thousands to millions of compounds – are ‘docked’ into the 3D protein binding-site in an attempt to identify novel compounds that bind to the drug target. The docking process assigns each compound with a score that characterises its fit to the binding pocket. These scores are then used to rank the library accordingly. The top few percentiles of the ranked library are typically selected for experimental testing. Before screening for new binders, the potential of the 3D model to be successful in this task is evaluated by calculating ‘enrichment factors’. This is done by virtually screening a random drug-like compound library – say of 10,000 compounds – to which a small number of known ligands are added. The rate at which the virtual screening procedure identifies the known binders from the background of random compounds relative to simple random picking (no enrichment) is denoted as the ‘enrichment factor’. The results show that the PREDICT 3D GPCR models yield enrichment factors ranging from 20-fold to 350-fold better than random – enrichment factors similar to those obtained when using high-resolution x-ray structures.

The PREDICT models are then used to screen large virtual libraries in order to identify novel compounds that bind to the target receptor (Figure 3). The process starts with Predix’s virtual library of 1.2 million drug-like compounds assembled from numerous vendors worldwide. Using simple pre-filters, a smaller – yet quite large – ‘profiled library’ of 150,000 drug-like compounds is selected from the large library. These compounds are ‘docked’ into the 3D model of the target GPCR and ranked using several scoring procedures. The 50 to 100 best scorers are the ‘virtual hits’; these are now purchased and tested in experimental binding assays. With validated ‘hits’ showing experimental binding affinities of <5µM, the actual hit rates in these GPCR binding assays is found to be between 10% and 25%. These hit rates should be compared with hit rates obtained by experimental high-throughput screening, which are in the range of 0.1% to 0.01%.

More importantly, in all cases the structure-based in silico screening process yields a diverse set of chemical structures that interact with the target, including many low nM hits. Many of the hits are new chemical entities (NCEs), which offer a promising starting point for lead optimisation. For example, screening the compound library against the dopamine D2 model depicted in Figure 2 led to a selection of 42 ‘virtual hits’. Following experimental binding assays of these compounds, it was found that 7 of these 42 compounds were hits with binding affinities better than 5µM – namely a hit rate of 17%. The best hit discovered in this process was a novel compound with a 58nM affinity to the dopamine D2 receptor.
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3D lead optimisation

Unlike regular HTS, which typically yields μM level hits, the above in silico 3D screening usually yields nM range hits, which can be used as ‘lead compounds’ without having to go through the laborious and time-consuming ‘hit to lead’ process. The 3D Model of the GPCR target is then used for speeding up and focusing the lead optimisation process. To this end, Predix applies its Iterative Computational Experimental Lead Refinement (ICELR-3D™) platform that integrates computational expertise with medicinal chemistry and pharmacology. Beginning with the 3D structure of the target receptor, an integrated suite of computational tools enhances the medicinal chemistry optimisation process and output. These tools enable the creation of virtual focused libraries, estimation of binding affinities, prediction of ADMET properties (including, for example, HERG channel binding and blood-brain barrier penetration) and prediction of cross-reactivity. Using these tools, integrated teams of computational and medicinal chemists efficiently design and synthesise novel compounds for their optimisation into pre-clinical compounds with desired drug-like pharmacological properties.

In this process, the 3D structure of the target receptor is utilised in several ways. First, the 3D structure offers insights into the specific interactions that govern the binding of the lead compound to the receptor (Figure 4). This computational insight speeds up the optimisation process, and efficiently guides the medicinal chemistry effort to reduce the number of compounds that are actually synthesised during the lead optimisation processes. Second, the 3D models aid in providing solutions to target selectivity in compounds by comparing the binding mode of the same compound in several different GPCR models. Finally, the GPCR models also assist in optimising the compound’s pharmacological properties (ADME). Of all the possible compounds that can be designed to optimise a certain ADME property, using the ICELR-3D predictive tools as well as medicinal chemistry practices, the 3D structure ensures that only those compounds that maintain their affinity to the target protein will be synthesised.

Conclusion

Using the integrated structure-based drug discovery process – 3D modeling, 3D screening and 3D lead optimisation – Predix has been able to advance several GPCR drug programmes expeditiously and with minimal resources. Our goal is to require no more than 2-5 medicinal chemists with 1-2 computational chemists per programme, and optimisation time-lines of no more than 6-9 months, reaching the clinic with a new drug candidate within two years from the initiation of a drug programme (compared with up to 7 years in conventional drug discovery). Using this approach, Predix has discovered and developed PRX-00023 – a novel, non-azapirone 5HT1A agonist with improved selectivity, metabolism and toxicology that has shown activity in animal models of anxiety and attention deficit hyperactivity disorder (ADHD). PRX-00023 is scheduled to complete Phase I clinical trials in 2004, only two years after initiation of the in silico work.

Dr Oren M. Becker is a founder and Chief Scientific Officer of Predix Pharmaceuticals. He is a computational biophysicist with over 17 years of experience, focusing on protein structure prediction and in silico drug discovery. Before founding Predix, he held a position as a visiting professor at Harvard University and a professor of chemical physics at Tel Aviv University. Dr Becker received his PhD in Theoretical Chemical Physics from the Hebrew University of Jerusalem. He has published numerous scientific papers and has co-edited a textbook in the field of computational biochemistry and biophysics.

References