Minireview: Molecular Structure and Dynamics of Drug Targets

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The concepts of receptors and drug targets

The British physiologist, J. N. Langley, postulated as early as in 1878 that "drug molecules need to reach tissue cells in the target organ in order to produce a therapeutic effect" [1], and later, in 1905, the existence of a *"receptive substance"* [2]. Ever since then, and until the introduction of molecular biology in pharmacological research around 1980–82, "drug receptors" remained a theoretical concept, useful in explaining quantitative dose-response relationships. With the introduction of molecular cloning techniques, receptors emerged as real molecules: proteins embedded in cell membranes. As a result of this development, the earlier "receptor" concept had to be replaced with the modern concept of "drug targets", which may be divided into:

- Enzymes
- Membrane proteins
  - Receptors
    - G protein coupled
    - Ligand gated ion channels
    - Kinase linked
  - Ion channels
  - Transporters
- Nuclear receptors.

The DNA sequencing of the entire human genome led to identification of many previously unknown proteins which may represent potential drug targets.

In order to fully understand the functional mechanisms of a known or novel potential drug target, it is crucial to know its 3-dimensional molecular structure. This may be determined experimentally by X-ray crystallography, NMR spectroscopy or electron microscopy, and computationally by structural bioinformatics and molecular modelling. When the structure of a drug target is known, computer programs can be used to predict ligand-target binding affinities and to search for novel drug candidates.

3-Dimensional structures of membrane proteins

Ion channels, active carrier proteins (transporters) and G protein coupled receptors, all membrane proteins, represent important classes of current and potential new drug targets. Membrane proteins have proven extremely difficult to purify and crystallize due to their amphipathic surface, with a hydrophobic area in contact with membrane phospholipids and polar surface areas in contact with the aqueous phases on both sides of the membrane. Still, a small but increasing

number of membrane proteins have now been crystallised and their structure determined at atomic resolution. These structures provide templates for molecular modelling of potential new drug targets. Molecular modelling, combined with site-directed mutagenesis studies, has provided valuable information about drug-receptor and drug-transporter interactions.

However, although membrane proteins represent one third of the proteins coded for in the human and other genomes, out of the more than 42000 entities deposited in the PDB database, *only ~ 0.3% are unique structures of membrane proteins*.

Protein modelling

As demonstrated by Hopkins and Groom [3], more than 95 % of current drug targets are proteins. Modelling of drug-target interactions and subsequent molecular events therefore implies, in most cases, modelling of 3-dimensional protein structures.

3-Dimensional protein models may be constructed from their secondary structure, i.e. their amino acid sequence, based on a 3-dimensional template protein, using molecular modelling methods. The template usually is a protein with known 3-dimensional structure, which is known or postulated to have 3-dimensional structure similar to that of the modelled protein. The molecular modelling methods include:

- Quantum mechanics calculations
- Molecular mechanics calculations
- Potential energy minimisations
- Simulations
- Conformational analysis
- Computer graphics

The accuracy of protein models constructed by such methods depends on how accurately the template protein structure has been determined, the structural and functional resemblance between the template protein and the modelled protein, and how well their amino acid structures may be aligned.

Modelling of drug-target interactions and the subsequent chain of events

When a drug interacts with its molecular target, this leads to a chain of events at the molecular level, cellular level, organ/physiological system level, and whole body level. Ultimately, this results in a therapeutic effect and possible adverse effects. In principle, modelling may be performed at each of these levels.

Drug-target interactions

Molecular modelling of drug-target interactions may describe:

- Docking of the drug into the binding site
- Binding of the drug to the binding site
- Target modification (activation/inactivation):
  - Conformational changes leading to *activation* of the target
    Receptor agonist stimulation
    Ion channel opening
  - *Inactivation* of target
    Receptor antagonism
    Transporter inhibition
    Ion channel blocking
    Enzyme inhibition

Modelling of the subsequent chain of events
An example of modelling of a cellular signal transduction step was reported by Bonacci et al. [4], who performed virtual docking of a small-molecule library to G protein $\beta\gamma$ subunits mediating protein interactions. From this model, the authors hypothesised that differential targeting of this surface could allow for selective modulation of $G\beta\gamma$ subunit functions, which was experimentally verified.

As illustrated by this example, the complexity of intracellular signalling systems represents a challenge in their modelling. Furthermore, cellular signalling systems are highly dynamic. Including the time dimension therefore represents a challenge in modelling mechanisms of cellular systems.

G-protein coupled receptors (GPCR) and transporter proteins
G-protein coupled receptors are the molecular targets for about 45% of current therapeutic drugs, and about 30% of all potential drug targets in the human genome [3]. Their secondary structures (amino acid sequences) have been determined by molecular cloning, which has shown that all have a 7 transmembrane alpha-helical (7 TMH) structure. The detailed 3-dimensional structures have been experimentally determined by x-ray crystallography for rhodopsin [5] and for the b2 adrenergic receptor [6].

Molecular modelling of GPCRs, performed by many different research groups and virtually all medium- and large-size pharmaceutical companies, have provided a wealth of information concerning their detailed 3-dimensional structures, receptor mechanisms of antagonist and agonist actions, signal transduction and G-protein coupling.

Although only about 4% of molecular targets for currently used drugs are transporters, these represent an important class of biologically active molecules which are involved in a large number of different cellular processes.

Transporter proteins in biological membranes may be divided into channels and carriers [7, 8]. Channels function as selective pores that open in response to a chemical or electrophysiological stimulus, allowing movement of a solute down an

electrochemical gradient. Active carrier proteins use an energy producing process to translocate a substrate against a concentration gradient. Three groups of carrier transporters have particular interest as drug targets: The major facilitator superfamily which includes almost 4000 different proteins transporting sugars, polyols, drugs, neurotransmitters, metabolites, amino acids, peptides, organic and inorganic anions and many other substrates, the ABC (ATP binding cassette) superfamily which plays an important role in multidrug resistance in cancer chemotherapy, and the neurotransmitter: sodium symporter (NSS) family which includes the molecular targets for some of the most widely used psychotropic drugs [8].

We have used molecular modelling methods, based on crystal structures of related proteins, to construct 3-dimensional models of various neurotransmitter receptors and transporters [7, 8]. These models have been used to study their structural properties, functional mechanisms, and the molecular mechanisms of drug action. The results demonstrate the large structural flexibility of such proteins, with substantial movements and conformational changes taking place during substrate translocation.

Molecular dynamics of drug targets: The living "molecules of life"

Studies of the molecular dynamics of biologically active molecules have demonstrated that such molecules are indeed as alive as the organisms in which they act [9]. A rigid-structure "lock and key" concept does not adequately describe drug-target interactions, since all such functional mechanisms require motion at the molecular level.

Time scale definitions:

- milliseconds (ms) 10^{-3} s
- microseconds (μs) 10^{-6} s
- nanoseconds (ns) 10^{-9} s
- picoseconds (ps) 10^{-12} s
- femtoseconds (fs) 10^{-15} s

The time scale of protein dynamics may be classified (time scales in parentheses) as:

- "long" (ms, μs)
- short (ns, ps, fs)

The following methods have been used to study protein dynamics:

- Laser, IR spectroscopy (fs, ps)
- NMR spectroscopy (μs, ms)
- Computer simulations (fs, ps, ns)

Computer simulation of proteins and other macromolecules, which is the most widely used method to study their molecular dynamics, requires relatively high-performing computers.

Conclusions

- Transporters and G-protein coupled receptors are membrane proteins. Their molecular structures may be modelled from crystal structures of homologous proteins.
- Transporters and G-protein coupled receptors have a dipolar electrostatic structure: Negative outside and positive inside the cell membrane. Electrostatic charges pull drugs and neurotransmitters, which are protonated and positively charged at pH 7.4, into the primary receptor/transporter binding site.
- Receptors and transporters have flexible structures and their function requires motion: In order to explain their molecular mechanisms, both the target protein and the ligand must be regarded as highly flexible entities.
- Ligand interactions may lead to changes in
  - molecular conformations
  - electrostatic fields of functionally important protein domains.
- High-resolution crystal structures used as templates provide more accurate protein models than those constructed from low-resolution protein templates.
- Previous 3-dimensional GPCR models have been corroborated by reported crystal structures of rhodopsin [5] and a $beta_2$ adrenergic receptor [6].

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