



NEUROTRANSMITTER RECEPTOR AND TRANSPORTER STRUCTURES

Dahl S. G.

Department of Medical Biology, Faculty of Health Sciences

Abstract. Transporter proteins in biological membranes may be divided into channels, which function as selective pores that open in response to a chemical or electrophysiological stimulus, and active carrier proteins using an energy producing process to translocate a substrate against a concentration gradient. Secondary active transporters use the movement of a solute down a concentration gradient to drive the translocation of another substrate across a membrane and against a concentration gradient. The secondary transporters include the neurotransmitter:sodium symporters (NSS family), glucose transporters, and the *Escherichia coli* lactose permease symporter (Lac Permease). Secondary transporters are very distinct in terms of sequence and function, and transport a wide variety of substances including the monoamine neurotransmitters dopamine, serotonin and noradrenalin. Members of the NSS family are the target of many currently used psychotropic drugs and several substances of abuse. The serotonin transporter (5HTT) and the norepineprine transporter (NET) are the main sites of action of antidepressant drugs, and the dopamine transporter (DAT) is the main site of action of cocaine and other substances of abuse. The rational design of novel drugs interfering with monoamine reuptake is limited by the scarcity of structural information about these transporter proteins. We and others have therefore used molecular modeling techniques to generate 3-dimensional (3D) models of monoamine transporters, based on homology with bacterial membrane transporters and information obtained from site directed mutagenesis studies. 3D modeling of monoamine transporters relies on the availability of experimental 3D structures that may be used as templates. When the crystal structure of a bacterial homologue of the human monoamine neurotransmitter transporters from *Aquifex aeolicus* (LeuTAA) was reported in 2005, this represented a breakthrough in the structural/functional analysis of monoamine transporters, and provided the possibility of using a homology modeling approach to generate 3D molecular models of NSS family members. These models have demonstrated how transporters may undergo substantial conformational changes during the transport cycle. When performing docking studies on such models, the structural flexibility of transporters should therefore be considered. The conformational flexibility of membrane transporters suggest that different transporter inhibitors may bind to different conformations of the transporter. Computational methods based on accurate molecular transporter models represent a useful tool in the discovery of safer and more efficient drugs acting on membrane transporters.

Keywords: molecular structure, receptors, transporters, neurotransmitters, drug targets

Molecular drug targets

Analysis of the human genome sequence has indicated that human cells contain 600-1500 different potential drug targets [1]. The largest number of potential drug targets were found for enzymes

(47%) and G-protein coupled receptors (30%). Similar results had previously been found by analysis of the molecular targets for all existing therapeutic drugs described in a standard textbook [2]. The composition of potential drug targets in the human genome, and the targets for existing drugs, showed that less than 4% were transporters. Although relatively few of the current therapeutic drugs have a transporter protein as molecular site of action, transporters may still have many interesting potential therapeutic applications as drug targets. It is interesting to note

Svein G. Dahl

Department of Medical Biology, Faculty of Health Sciences, University of Tromsø, Norway
email: svein.dahl@uit.no

that two of the most widely prescribed drugs in the world, fluoxetine and omeprazole, both have a carrier transporter protein as site of action.

In the TC classification system [3], transporter proteins in biological membranes are divided into two main classes: channels and carriers. *Channels* function as selective pores that open in response to a chemical or electrophysiological stimulus, allowing movement of a solute down an electrochemical gradient, while *active carrier proteins* use an energy producing process to translocate a substrate against a concentration gradient.

Three groups of transporters have particular interest as drug targets [4]:

- The major facilitator superfamily (MFS), which contain almost 4000 different proteins transporting sugars, drugs, neurotransmitters, metabolites, amino acids, peptides, organic and inorganic anions and many other substrates.
- The ATP binding cassette (ABC) superfamily, which plays an important role in multidrug resistance to cancer chemotherapy.
- The neurotransmitter: sodium symporter (NSS) family, which includes the molecular targets for some of the most widely used psychotropic drugs.

Neurotransmitter transporters

The functional mechanism of NSS transporters involves three subsequent steps:

1. *Binding* of neurotransmitters, ions, neurotoxins and drugs.
2. *Translocation* of neurotransmitters, ions and neurotoxins through membrane.
3. *Release* of neurotransmitters, ions, neurotoxins into the presynaptic neuron.

All transporters have a recognition site for a particular substrate. The recognition site has the potential to be used as molecular target for drugs inhibiting or enhancing the transporter function. So far, drugs acting on active carrier proteins are inhibiting transporter function [5].

Antidepressants and certain CNS stimulant drugs exert their action by binding to a transporter protein and thereby inhibit its functioning. These drugs are believed to stay in their binding site and inhibit the conformational movements of the transporter which are required for its function, and are not transported through the membrane [6-9].

Molecular modeling in drug discovery

The three dimensional (3-D) molecular structure of a transporter protein contains information about

- the shape and electrostatic potentials of the active site,
- possible ligand binding affinities,

- evolutionally relationships within the protein family.

As described in an example from the Novartis Institutes for Biomedical research, drug discovery has entered the post-genomic era and the process now usually starts with the identification of a drug target which may be used for screening of a large number of compounds [10]. Once a compound with desired biological effects is identified, molecular modeling techniques, based on the 3-D structure of the drug binding site, may be used as guide in medicinal chemistry efforts to optimize the compound and develop a lead compound with optimal biological properties, which may be used for further testing and eventually enter into clinical trials.

The cost of discovery and development of a new drug is currently estimated to ~ 800 000 000 USD, and increasing, with a time frame of 8 - 12 years or longer. Optimizing the drug discovery process is therefore of crucial importance.

At the compound optimization stage, molecular modeling techniques have proven to be extremely useful and are now being commonly used in all research-based pharmaceutical industries.

Membrane proteins as drug targets

Transporters and G protein coupled receptors (GPCRs) have as a common molecular structural feature that both are *membrane proteins*.

Out of the more than 73 800 3-dimensional protein structures currently deposited in the PDB database [11], only ~ 0.4% are unique structures of membrane proteins, although membrane proteins represent one third of the proteins coded for in the human and other genomes. The reason for the small number of known 3-D structures of membrane proteins is that these proteins are difficult to produce in sufficiently large quantities, purify and crystallize [4].

Molecular modeling has therefore proven to be a particularly useful technique to study the 3-D structures and conformational dynamics of membrane proteins. Molecular modeling of a protein requires a *template structure*, as indicated in Figure 1. A structural template is a 3-dimensional molecular structure of another protein which may be assumed to have a similar overall 3-D architecture.

The results of such modeling calculations are dependent on the quality of an amino acid sequence alignment between the two proteins, and on the accuracy with which the template structure has been determined:

- Low-resolution templates provide low-accuracy protein models,
 - Using high-resolution crystal structures as template provides more accurate protein models.
- Our first 3-D model of a transporter protein [12]

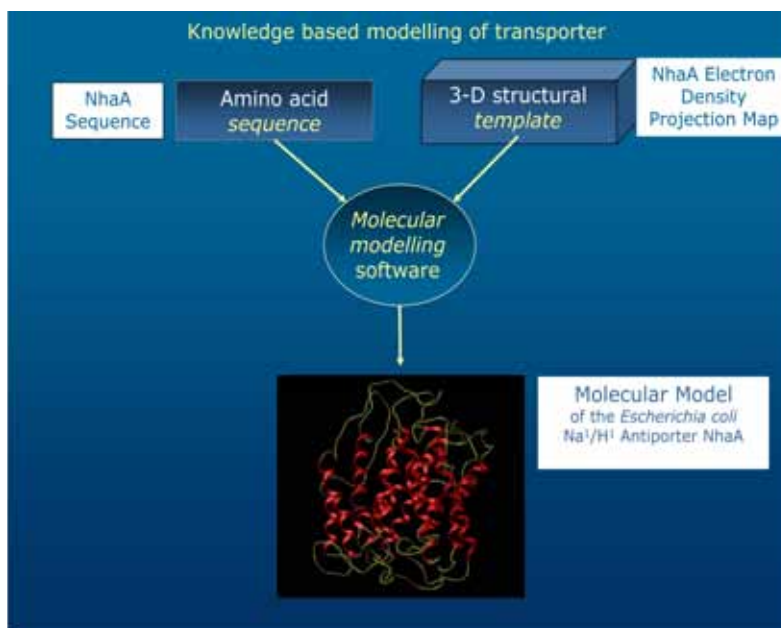


Figure 1. Procedure used to model the molecular structure of the NhaA transporter [12]

used an electron density map of the *Escherichia coli* Na⁺/H⁺ antiporter (NhaA) [13] as a structural template, as illustrated in Figure 1.

In 2005 the crystal structure of LeuT_{Aa}, determined by x-ray crystallography at atomic resolution, was reported. LeuT_{Aa} is a bacterial homolog of the NSS transporters, and its crystal structure represented a breakthrough for modeling of NSS transporters. We and others have used the LeuT_{Aa} structure as a template for modeling of the transporters for noradrenaline (NET), dopamine (DAT) and serotonin (SERT). All our transporter models showed a generally electronegative molecular surface at the extracellular side, and positive electrostatic potential at the intracellular surface [14].

This is in accordance with the general 'positive inside' rule for membrane proteins [15]. A similar bipolar charge distribution was observed for the first model of a G protein coupled receptor [16], and for all our subsequent and more accurate GPCR models.

Crystal structures of several different GPCRs have since been reported, and used as templates for modeling of other receptors within the GPCR superfamily. These crystal structures include visual rhodopsin [17], beta1 adrenergic [18], beta2 adrenergic [19], adenosine A2a [20,21], dopamine D3 [22] and a chemokine receptor [23].

Psychotropic drugs and neurotransmitter molecules are protonated and positively charged at pH 7.4. The bipolar charge distribution of neurotransmitter receptors and transporters strongly indicate that electrostatic charges contribute to pulling the drugs and neurotransmitters into the primary receptor/transporter binding site.

Molecular dynamics of drug-target interactions

Biologically active molecules generally have more or less flexible structures and their biological function always requires some kind of motion.

The time span of protein dynamics is usually divided into

- "long scale" (ms, μ s)
- "short scale" (ns, ps, fs)

Protein dynamics may be studied by the following methods:

- Laser and IR spectroscopy (fs, ps)
- NMR spectroscopy (μ s, ms)
- Computer simulations (fs, ps)

Molecules in solution change their conformations so fast that up to 5 years ago, no experimental method had been able to record the process, and molecular dynamics (MD) simulations [24] were the only way to study such movements in detail. Recently, spectroscopic methods have been able to record molecular conformational changes on a ps time scale [25].

We and others have used MD simulations to study the internal movements of GPCR and transporter molecules, and the dynamics of their ligand interactions. MD simulations consume substantial computer power, and in our initial simulations with a model of the dopamine D2 receptor, which were carried out on a Cray supercomputer, only the ligand was allowed to move while the receptor model was held in a fixed conformation [16]. The first MD studies of a GPCR and ligands where both the receptor and the ligands were allowed to move, were performed with a model of the 5-HT2 serotonin receptor and an antagonist, ritanserin [26].

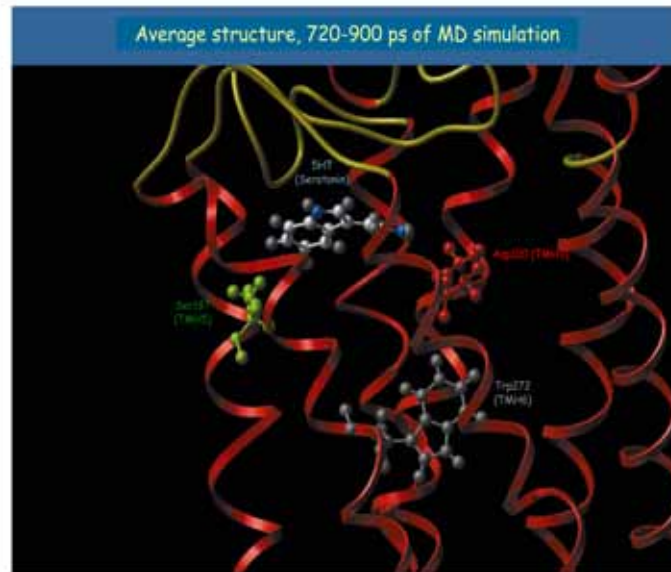


Figure 2. Average position of the serotonin molecule in the 5-HT₄ serotonin receptor during the final 180 ps of a 900 ps molecular dynamics simulation.

We have also performed simulations with the neurotransmitter in a postulated binding site, and Figure 2 shows the average position of the neurotransmitter during the final 180 ps of a 900 ps MD simulation of serotonin in a 5-HT₄ serotonin receptor, performed on a Hewlett Packard 'Super-Dome' computer. The serotonin molecule showed substantial movements during the simulation, but the protonated nitrogen molecule stayed in the vicinity of an aspartic acid residue, Asp 100 in transmembrane helix no 3, which has a negative charge.

Conclusions

Monoamine transporters are membrane proteins, and their molecular structures may be modeled from crystal structures of homologous proteins. Such models provide useful tools in the discovery and development of new drugs.

G protein coupled receptors and neurotransmitter transporters all have a dipolar structure with negative electrostatic potentials at the outside of the cell membrane and positive potentials inside the cell membrane. Psychotropic drugs and neurotransmitters are protonated and positively charged at pH 7.4 and it is likely, therefore, that electrostatic charges contribute to pulling the drugs and neurotransmitters into the primary receptor/transporter binding site.

The transporter proteins have particularly flexible structures, and their function requires substantial motions of the membrane spanning parts of the protein.

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