Chapter-2
2.0 Drug Designing

2.1 Activity of Drug

The human biological system, even the part which is already understood, is very complex. Pharmaceutical drugs can be classified in different ways: By the protein they bind to, by their chemical structure or by their pharmacological effect. Drugs often act on enzymes, which may then perform a reaction or which are hindered at performing a reaction, or may also act on receptors, which respond to a signal or are blocked to respond.

2.2 Action of Drugs on Enzyme

Enzymes are proteins that act as biological catalysts in the human body. Many complex reactions required by our metabolism do not take place spontaneously under physiological conditions. Enzymes have the ability to decrease the activation energy and consequently allow these reactions to take place at a lower temperature, as illustrated in Fig. 2.1. Enzymes have an active site, to which substrates can bind. These substrates are the reactants of the catalytic reaction which is performed by the enzyme. After this reaction all products are released and the enzyme doesn't alter during or after the process, is ready to repeat the cycle. Such an enzyme activity is illustrated in Fig. 2.2. Enzymes are very important for cell metabolism. They are specifically adapted to fulfill certain tasks in the cell and thus keep the cell maintaining. Usually, one enzyme participates only in one chemical reaction. The specificity of the enzyme originates from its unique three dimensional conformations (Patrick, 1995). Many enzymes, which are present in human cells, cannot be found in bacteria and viruses or vice versa. The cells of bacteria and human cells seem to be quite different.

This fact is also one of the starting points for eliminating, for example, dangerous bacteria in the human body. Using specific tools (drugs), the cell processes of unwanted bacteria can be disturbed and the cells can be killed by disrupting their metabolism. Penicillin, for example, acts just in that way. It blocks enzymes, which bacterial cells need to maintain the bacterial membrane (Stahl, 2003). If their activity is disrupted, the bacterial cell cannot maintain itself and is stopped from multiplying itself. Because of these reasons it is important in drug discovery to investigate and understand the metabolism and mechanism of these alien intruders in the human body, unwanted cells in the biological system, and then search for strategies. If specific enzymes are recognized as an integral part
of a disease, they can be structurally resolved and one can search for drugs, that would stop these enzymes to work.

Fig.2.1: Illustration of a chemical reaction with and without enzyme. The red curve illustrates a reaction without any enzyme activity. As illustrated, if an enzyme acts catalytically at the chemical reaction (blue curve), the necessary activation energy to start the reaction is significantly lower.

Fig.2.2: Illustration of a chemical reaction involving an enzyme. After a substrate has bound (first and second picture from the left) a chemical reaction takes place. After the reaction is completed, the products are released and the enzyme is ready for the next reaction (first and second picture from the right).

Fig.2.3 illustrates this idea. If a drug has a stronger affinity to bind to the enzyme than the natural substrate, the catalytic reaction can be stopped. Because the substrate does not have the enzyme as a partner no chemical reaction can take place. If this chemical reaction is important for the cell metabolism or for cell functions, the drug will lead to biological effects. The described process and the
illustration are only abstract and exemplary. There are different ways to hinder an enzyme at working (B'ohm et. al., 2002).

Fig. 2.3: Illustration of drug activity upon an enzyme. Because of the bound drug (left picture), the natural substrate can not bind and the enzymatic process is stopped (right picture).

2.3 Drugs acting at protein receptors

The endocrine and the nervous system are the two important control systems of the human body. Receptors are an integral part of both systems. The endocrine system directly uses chemical molecules as their messengers to cells. These molecules should only deliver their message to those cells they were sent to. Due to receptors, the addressee, i.e. the recipient cell can be identified. Likewise, receptors are also important for the nervous system. Nervous cells can not communicate directly with each other or with other cells (muscles or glands) by electric potentials. They are interconnected by chemical synapses. However, instead of communicating through electric impulses as signals are transmitted in neurons, they use neurotransmitters. An electric impulse causes many neurotransmitters to be released from the axon terminal to the synapse. Receptors situated at the outside of the cell membrane of dendrites, have the task to identify these molecules and transmit the message accordingly; they are an essential part of the biological signal transduction.

Receptors, which are proteins, situated in a cell membrane, fulfill different tasks:
- Information exchange between cells
- Regulation of the ion flow in ion channels
- Regulation of protein synthesis due to binding to DNA
The tasks of various receptors are so different from each other, that they can only be described in an exemplary and abstract way. Fig.2.4 displays a possible signal pathway of hormones. When a hormone finds its corresponding addressee, then it can bind tightly to the receptor, as illustrated in the picture. In doing so the receptor alters its conformation slightly and a secondary messenger inside the cell is released. Such a signal could activate, for example, another receptor or enzyme, which could finally result in a new synthesis of proteins. In contrast to the interaction with enzymes (see Fig.2.2), messengers acting on receptors do not change their chemical structure upon binding. Usually the binding energy is also not very strong. After some time the messenger is released from the receptor and drifts away. Receptors are essential for inter-cell communications, because polar compounds cannot cross the lipid bilayer of the membrane (Bohm et. al., 2002) and consequently no direct interaction within the interior of the cell is possible. In the following, two other examples illustrate the way signals are processed by the receptor. Fig.2.5 displays a possible receptor-induced ion-channel opening. Before a messenger molecule binds to the receptor, the relaxed receptor structure hinders ions to flow through the channel. When the receptor structure is ‘activated’ due to the binding of the molecule, the receptor conformation changes which leads to an un-blocking of the ion-flow. Sometimes the conformational change of the receptor can involve large parts of the structure, but often slight changes of the receptor structure, for example changes in the side-chain conformation, can have such an effect. This process could also happen in the reverse order. Due to the binding messenger, the ion-channel may get blocked. Again, if one can design drugs that fit tighter into the receptor pocket than a respective messenger, the receptor can be successfully hindered to alter its conformation. In our example consequently the ion-channel will not open due to the blocking of the opening-mechanism. Many drugs work in such a way. Anesthetics, for example, reduce the excitability of nerves in this manner (Bohm et. al., 2002). So the patient does not feel the pain anymore.
**Fig. 2.4:** Schematic representation of the signal pathway of hormones. If a cell has the corresponding receptor to a hormone, then it can bind tightly to the receptor and a signal is transferred into the cell (here to the cell core).

**Fig. 2.5:** Schematic illustration of how an ion-channel is opened by an induced conformational change of a receptor. Before the messenger binds to the receptor, the receptor structure hinders ions to flow through the ion channel. After the messenger has bound, the receptor changes its conformation, which leads, in this example, to an opening of the ion-channel.

**Fig. 2.6** illustrates how an enzyme in the cell interior is activated by a signal from the cell exterior. A messenger molecule binds to a receptor and induces a conformational change of the receptor. In this example the new receptor conformation also induces directly a conformation change of a neighboring enzyme. Before the messenger is bound, the enzyme is 'switched off'; the enzyme is inactive. Consequently no bio catalytic reaction could start.
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Fig. 2.6: Sketch of how an induced conformational change of a receptor structure enables enzymatic reactions to take place. Before a messenger is being bound to a receptor, the active site of a neighboring enzyme in the cell interior is closed. If the messenger is bound to the receptor, the receptor changes its conformation and induces an additional conformational change of the enzyme. As a result the active site of the enzyme opens and substrates in the cell interior can start a catalytic reaction.

Due to the altered enzyme conformation, substrates can now bind to the active site of the enzyme and start the catalytic reaction. The resulting products may lead to further reactions. Mechanisms, following the abstract scheme of figure 2.4, controlled by many different types of receptors are abundant in different human cells. For instance, the class of G-protein coupled receptors (GPCRs) act in a similar way. However, instead of a direct enzyme alteration, the receptor activates other proteins which then activate an enzyme for catalytic reactions. GPCRs are involved in many different stimulus-response pathways from inter-cellular communication to physiological senses. Now drugs can be used to influence the signaling pathway of messengers. They can hinder directly or indirectly a messenger from binding to the receptor and thus disconnecting the signal-pathway. Now drugs can be used to influence the signaling pathway of messengers. They can hinder directly or indirectly a messenger from binding to the receptor and thus disconnecting the signal-pathway.

2.4 Bioavailability:

It is crucial that a drug binds strongly to the receptor, but no matter how well a substance binds, it is useless, if it cannot be transported to the receptor. The field of pharmacokinetics describes mathematically if, in which concentration and how
quick a drug gets to the target location and how and when it is removed from the human organism (Böhm et. al., 2002).

The most important processes in pharmacokinetics are abbreviated by the acronym LADME:

- Liberation (L): If the drug is in a solid phase, it first has to get dissolved.
- Absorption (A): To enter the blood stream the drug has to cross different barriers by passive diffusion. These barriers are usually membranes in the duodenum. Highly polar and large molecules have difficulties crossing the lipid bilayer membranes.
- Distribution (D): The bloodstream will not distribute the medicament equally in the human body. Because of different barriers in the human organism (for example the blood-brain-barrier), a drug may not reach some parts of the human organism. Consequently, the drug concentration will be higher in some regions (organs) than in others. Additionally, the molecule distribution often also depends on how good a drug flows in the blood. Molecules that are too large will have difficulties to flow quickly to the target and the chances of degeneration are higher.
- Metabolism (M): There are special enzymes in the human body that detect alien substances and convert them into products which are easy to excrete.
- Excretion (E) describes the process of how the drug leaves the human body.

Several methods have been developed that attempt to mathematically characterize the LADME processes. Through statistical evaluation of known medicaments the Lipinski rule of five (Lipinski, 2000) characterizes already roughly which compounds have a chance to reach their target and which is not. If difficulties arise by an oral intake of a medicament, other transport ways can be investigated. Some drugs, for example, are injected locally. Presently, even research efforts progress to develop transport vessels, which can bring non-solvable drugs to the target and release them there.

2.5 Brief overview of rational drug discovery

Drugs are rather sparsely distributed in the chemical space of possible candidates (Lipinski, 2000). It would take too much time to blindly test every possible compound in situ. Therefore, different drug discovery strategies were developed and refined over the last 100 years based on the technology and resources of the
time. Modern drug research is often traced back to Emil Fischer. With his metaphor of ‘lock’ and ‘keys’, he gave the field of drug research the direction for the next century: Enzyme and inhibitor ‘must join one another as lock and key to be able to exert a chemical effect’. (Fischer, 1894). Paul Ehrlich’s concept, which he formulated in 1913, was also important for further developments: ‘corpora non agunt nisi fixata’; which means that compounds that do not bind have no biological effect. The strategies of rational drug design can be divided into two different classes: Methods that involve the knowledge of the three-dimensional structure of the active center of enzymes/receptors and strategies that do not require such knowledge of the protein (B’ohm et. al., 2002). The first class could not emerge before the 1970s. With the pioneering work of Max Ferdinand Perutz (Muirhead and Perutz, 1963) and Sir John Cowdery Kendrew (Kendrew et. al.,1958), honored both with the Nobel prize in 1962, methods were developed to determine the three-dimensional structure of macromolecules. These methods progressed with the time and became more and more to a difficult routine. In this section, the main protein strategies are two classes.

2.6 Strategies without knowledge of the protein

In the search for well binding drugs, chemists attempted to extract useful properties of a known binding compound in order to use this knowledge to find a good binding drug. With the analogy of E. Fischer a new ‘key’ should be found by looking at the ‘key’ only. The idea is that all binding compounds to one target are similar in some way. Even though not belonging to the same ‘chemical family’, there is often a similarity in hydrophilic, hydrophobic, aromatic or other properties present. These properties, descriptors, are used to search databases of ligands for similarity. Over time the descriptors became more and more complex: From one dimensional to three dimensional. Now databases are even searched with the help of neural networks (Xu and Agrafiotis, 2002). Especially the pharmacophore model, a three-dimensional descriptor strategy, is widely used. This method uses the three-dimensional orientation of functional groups (hydrophobic, hydrogen binding groups and further) to describe a molecule (B’ohm et. al., 2002) and then search for similarity in a database of compounds. One of its problems lies in sampling: the conformational space of the molecules and aligning it to the pharmacophore. An additional problem is that the search based on similarity applies only well to targets which do not change much their conformation upon
ligand binding. In drug discovery, the search for new types of well binding ligand structure and the optimization of those should be differentiated. When a promising ligand has been found, the newly found ligand structure, the lead structure, can be used as the starting point for further studies to increase the affinity or the transport properties of the ligand to the target. By varying the chemical constituents of the lead structure different new molecules can be synthesized and tested. The optimization of these compounds is not only based on the interaction of the new drug with the protein, but also on an improvement of the drug transport in the human body. Hydrophilic and hydrophobic properties of molecules influence in what concentration they reach certain regions in the human body (B"ohm et. al., 2002). With the method of quantitative structure activity relationship (QSAR) the molecule is decomposed in different groups each contributing differently to a biological activity scale. The method allows to compare different well binding analogs of a new lead structure and to optimize them for transport in the biological system and therefore for the best biological activity.

2.7 Strategies employing knowledge of the protein

Such strategies, also called molecular docking, analyze the interaction properties of a small molecule to a protein. The approaches can be differentiated by the strategy used for sampling different protein-ligand conformations and for estimating how well a ligand can bind. Molecular docking methods have been developed in the last thirty years. In the beginning both protein and ligand were treated as rigid entities and binding was simply based on geometric criteria; the programs searched for shape only (Fradera and Mestres, 2004). But soon the importance of the chemistry in ligand docking (Shoichet and Kuntz, 1993) and also of ligand flexibility was realized and several improved strategies were developed. The Two protein strategies are distinguish between two approaches: Molecular mechanics and fragment based approaches.

2.8 Molecular mechanics based approach

At the beginning both ligand and protein were considered as rigid unities. Nowadays it is common to allow for some degree of ligand flexibility during docking, i.e. some inherent adjusting to the protein environment. The protein on the other hand is usually kept rigid. In molecular mechanics (MM) docking approaches the ligand as a whole is simulated. Through a step by step process
the ligand adapts to its environment and the system energy is optimized by changing flexible bonds of the ligand and its orientation. The available methods are distinguished by the scoring function that evaluates the binding energy by a score and the search algorithm. The scoring functions can be classified either in regression-based or interaction-based scoring functions. The former indirectly uses information of known complexes. Through statistical analysis distant-dependent potentials for different atom pairs or functional groups are constructed on the assumption that the individual contributions obey Boltzmann statistics. The frequency with which specific geometry appears is related to the energy of that geometry. The advantage of statistical analysis scoring functions is that relationships can be evaluated, which are either not fully understood or difficult to model in a classical approach. On the other hand docking failures, the reason why some ligands do not bind, may also be more difficult to understand. Interaction-based scoring functions are similar to MM force fields. Both describe the interaction of the ligand with the protein by classical potentials based on physical interactions. The protein-ligand interactions are rooted in physical principles and have problems with features whose origins are not clearly understood. But even in this approach many interaction parameters are usually fitted to experimental data. Using such scoring functions or force fields already solves one problem that pharmacophore models have to face: The ligand deformation is treated in the same way as the interaction of the ligand to the protein. Consequently, it is easier to estimate if the resulting docked geometry is realistic or not. Because the screening programs must be very fast to be applicable, different techniques have been developed to speed up the search for the best protein-ligand conformation. Monte Carlo methods are used frequently as are genetic algorithms, which are used in the program Gold (Jones et al., 1992).

2.9 Fragment based approach

Fragment based approaches use a different technique to sample the huge conformational space of a protein-ligand complex. The ligand is separated into different fragments which are separately docked to the protein and then finally assembled into the whole molecule again (Taylor et al., 2002). Different strategies are used for the reconnection of the broken bonds. One very popular approach is the incremental construction algorithm which is implemented into the
program FlexX (Rarey et al., 1996). This algorithm starts with docking a base fragment to the protein and then sequentially adds the other fragments in energetic favorable directions (Xu and Agrafiotis, 2002). The final protein-ligand conformation is usually evaluated by an empirical scoring function to determine the affinity of the ligand to the protein. This approach does not fully explore conformational space of the ligand. However by concentrating first on a larger or quite characteristic fragment, this strategy has proven to be successful and is, for example, intensively used on supercomputers of the Bayer CropScience Deutschland GmbH. Interestingly, the de-novo ligand design methodology is strongly linked to this approach. Instead of screening a database of ligands, a totally new ligand is constructed by docking fragments of a database to the protein.

2.10 Recent developments
Over the years many rational drug discovery methods have been developed and are being used. Search strategies without the knowledge of the protein structure are still very important. For example, most membrane proteins are difficult to resolve. What is more, drugs can have side-effects, i.e. they may also bind to not designated proteins. The strategies, that compare molecules just by similarity, can help to quickly test drugs for potential unwanted effects. But unfortunately these methods are not working successfully in cases, in which no good binding compound is known, because the potential compounds can not be compared with already known ligands. The major aim of this work is contribute to the molecular docking field. Up to now, conformational changes of the protein structure are usually not considered for docking and are, if considered, still on an experimental footing. The necessity to account for a flexible protein structure is well known and published (Carlson, 2002). The present status regarding protein flexibility can be compared to the time docking of flexible ligands emerged. At the beginning it was computationally too expensive to allow for ligand flexibility. As a consequence, methods were developed to first generate different ligand conformations and to then dock them rigidly to the rigid protein structure (Kearsley et al., 1994).

Now ligand flexibility is standard in most methods but protein flexibility not. As first methods to treat ligand-flexibility one approach to solve this problem is to
possible at the moment, since accurate and affordable simulation strategies are lacking. In this approach, FlexScreen allow for full side chain flexibility (as illustrated in Fig.2.7). By using full sidechain flexibility will enable to describe the majority of conformational changes upon ligand binding.

**Fig.2.7:** Illustration of possible side chain flexibility, as used by our program FlexScreen. FlexScreen allows, for example, the side chain arginine a maximum of three degrees of freedom: three dihedral angles are allowed to be changed. Possible dihedral angles depend on low energy interactions of the side chain with itself and the environment.