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Pharmaceutical Chemistry at the ETH Zürich

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Abstract: Pharmaceutical Chemistry at the ETH Zürich is devoted to research on structure–activity relationships. The combination of biophysical methods, like protein crystallography, mass spectrometry and NMR, with theoretical approaches such as QSAR studies and molecular mechanical as well as quantum mechanical molecular modeling yield insight into ligand–protein recognition and has enabled a more fundamental understanding of ligand binding and transformation in several classes of proteins. The main biological targets are and have been, kinases, major histocompatibility proteins, peptide hormones and most recently glycoproteins. In parallel the results fostered the improvement and new development of appropriate software tools for molecular design studies, the most recent of which is the automated integration of tautomeric states in docking.

Keywords: Ligand–protein interaction · Molecular design · Pharmaceutical chemistry · Structure–activity relationships

From 'Key and Lock' …

For more than 100 years we have been using the metaphor of the key in the lock to describe the interaction of a drug with its target. The times when Emil Fischer coined the key- and lock paradigm were times in which mechanical engineering evolved very rapidly and successfully and hence many terms and metaphors used in other disciplines were inspired by mechanical devices and principles. The metaphoric description of mutual recognition of two molecules leading to an optimized chemical derivative of the one was one of the most successful working hypotheses in research.

It was Emil Fischer's contemporary Paul Ehrlich who fundamentally contributed to the concept of pharmaceutical chemistry. His conceptual definition of drug selectivity and a biological unit named 'receptor' which selects for its ligands and then transmits a signal into the target cell represents the other giant's shoulder that drug discovery rests upon up to the present date.

… and the 'Magic Bullet '…

However, Ehrlich's 'magic bullet', which enters the body, exerts its therapeutic action, and then exits without leaving any sign of harmful interaction, has never been found and still remains the ideal that we are all searching for. In the times of Paul Ehrlich, chemistry was probably the paradigmatic and most successful science. Making new molecules meant also making new drugs and the triumphal processions of Salvarsan and Aspirin began. Pharmaceutical chemistry was close to synonymous with synthetic chemistry. Otherwise, it was analytics; the fundamental and traditional expertise of the pharmacists. Later on, new technologies and mainly the emergence of computer technology and its co-evolution with molecular life-sciences made the biosciences and pharmaceutical chemistry in particular an attractive and rapidly expanding field of research – with surprisingly low interest from the pharmacists themselves. Medicinal chemistry was born and was soon used as a synonym for pharmaceutical chemistry. Only few universities, mainly in the USA, were bold enough and prepared to integrate the 'new' contents into their curricula of pharmaceutical education. European universities reacted more conservatively.

The new driving force for the development of pharmaceutical chemistry were the researchers in pharmaceutical industry rather than in academia. ETH Zürich was clearly one of the exceptions, since in the early sixties Jakob Büchi and Xavier Perlia published a book on 'Grundlagen der Arzneimittelforschung und der synthetischen Arzneimittel' featuring structure–-activity relationships as a central topic of research and education in pharmaceutical chemistry.

… to 'Computer Aided Drug Design' and the Challenge of 'Multiple Binding Mode'

Structure–activity relationships and in particular ligand–protein interactions are the main focus of research today. In our group, we use the methods and tools of theoretical chemistry and bioinformatics in combination with biophysical analytics and *in vitro* assays for biological activity to describe structure–activity relationships on the molecular level.

While nowadays even in industry structure-based drug design (SBDD) has become a standard procedure, the drug design field has its origins in the property description of the ligand only. Today, we have broad access to analytical tools which are able to yield three-dimensional information about the protein targets of drugs at atomic

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resolution. X-ray crystallography and more recently nuclear magnetic resonance spectroscopy are the dominant technologies which provide the 3D information necessary for the understanding of interaction processes and subsequently the design of new ligands.

Combined with force field calculations and sophisticated graphics software, the structure determination methods allow for a pictorial representation of the drug molecule's interaction with its molecular targets, mostly protein surfaces.

Nowadays, there is no drug development where molecular graphics, computational chemistry and structure-based design are not involved. The success of SBDD is well documented [1][2] and has contributed to the introduction of more than 50 compounds into clinical trials. New HIV inhibitors as well as the new nonsteroidal anti-inflammatory drugs may be taken as examples [3]. We are however still far away from the 'magic bullet' and from a prediction of drug action in molecular detail. The reasons for this are on the one hand individuality-based influences on the fate of a drug molecule such as individual absorption, individual target regulation and individual metabolism, and on the other hand a fundamental lack of understanding of target/receptor thermodynamics. To the present day, single 'crystallographic' water molecules and their roles in the binding site have hardly been studied. The same holds true for counter ions and catalytic elements as well as movements of 'un-structured' regions in the target protein, which are controlled by the molecular environment and in particular by the presence of cellular membranes and/or glycosylation.

Our present research focuses on the physico-chemical interpretation of the pictorial representation of drug action at the molecular level. This approach includes the following methods in which our group has been specializing over the years:

- SBDD-guided mutation and structure determination of the target proteins [4–6];
- Exploration of the ligand binding process by molecular dynamic and quantum chemistry methodology, especially Carr-Parinello approaches [7–12];
- Simulation of ligand binding by computational chemistry (docking) and introduction of improved modeling tools as for the inclusion of tautomers [13][14] or the conformational activation processes of the protein targets [15][16];
- Biophysical experiments, preferably X-ray crystallography, NMR spectroscopy, and calorimetry to test the models hypotheses [17–19].

The following examples elucidate the approaches taken in the Pharmaceutical Chemistry group and how we intend to contribute to the understanding of ligand–protein interactions.

The recent work of Christian Klein on methionine aminopeptidases (MetAPs) [12] unequivocally shows the importance of the insights generated by quantum chemistry for the understanding of complex protein–ligand interaction and the design of new ligand classes. The enzyme family of MetAPs plays a central role for *in vivo* protein synthesis as they remove the starter methionine from newly synthesized proteins. The natural product fumagillin (Fig. 1), an epoxide, covalently modifies one of the histidines in the active site of the eukaryotic methionine aminopeptidase II (MetAP-II) [20–23] and other MetAPs. Fumagillin inhibits the growth of vessels in tumors, and a derivative of the compound has been evaluated in clinical trials as an anticancer drug. The antiangiogenic effect of fumagillin and other inhibitors of MetAP-II have been attributed to the inhibition of the Ets-1 transcription factor expression and the activation of the p53 pathway [24][25]. Furthermore, MetAPs have the potential to become the target proteins of antibacterial substances because the MetAP functionality is essential for cell growth and bacteria possess only one of two known MetAP subtypes [26]. We have performed a computational study of different protomeric states of the methionine aminopeptidase active site using a combined quantum-mechanical/molecular mechanical simulation approach. The aim of this study was to clarify the native protonation state of the enzyme, which is needed for the development of novel irreversible inhibitors that can possibly be used as antiangiogenic and antibiotic drugs. For that purpose we used virtual screening and other drug design methods. The results of the simulations indicated that two protonation states are possible without

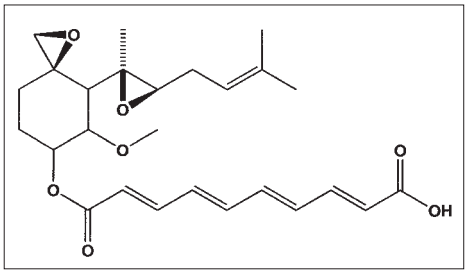

Fig. 1. Fumagillin

disturbing the overall geometry of the active site (Fig. 2). We then experimentally verified the presence of the two protonation states by studying the substrate hydrolysis and inhibitor binding reactions at different pH values and came to the conclusion that one protomeric state is relevant for inhibitor binding, whereas the other is relevant for substrate hydrolysis. This result has implications for the development of other inhibitors of this class of enzymes and adds a new perspective to the pharmacological properties of the antiangiogenic drug fumagillin, which is an irreversible inhibitor of the human methionine aminopeptidase type II.

Transfer of a drug from the solvent phase into the bound state within a 'protein phase' is associated with a fundamental change of the environment. The drug molecule loses the water shell and interacts with a mixture of hydrophilic and hydrophobic surfaces in the active or binding site of the target. Consequences are pka shifts and additional stabilization modes and hence energy states, besides the ground state, that may contribute to the 'bound state' of the ligand. In a collaboration of the groups of Gerd Folkers and Leonardo Scapozza, Pavel Pospisil and Patrick Ballmer have designed a piece of software named **AGENT** for the treatment of tautomers in docking procedures. **AGENT2** generates tautomers

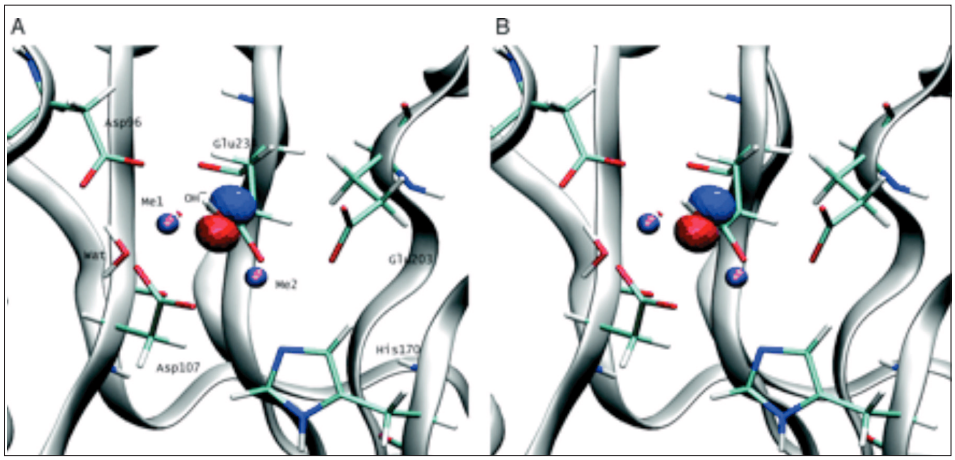

Fig 2. Final structure of the active site with one hydroxide ion and one water molecule coordinated to the metals (Me1, Me2, *purple spheres*). The HOMO-1 orbital located at the bridging hydroxide ion is also shown (*red* and *blue* clouds). The cut-off for the visualization of the HOMO-1 electron density was 0.1 e/au3 (electron per cubic atomic units).

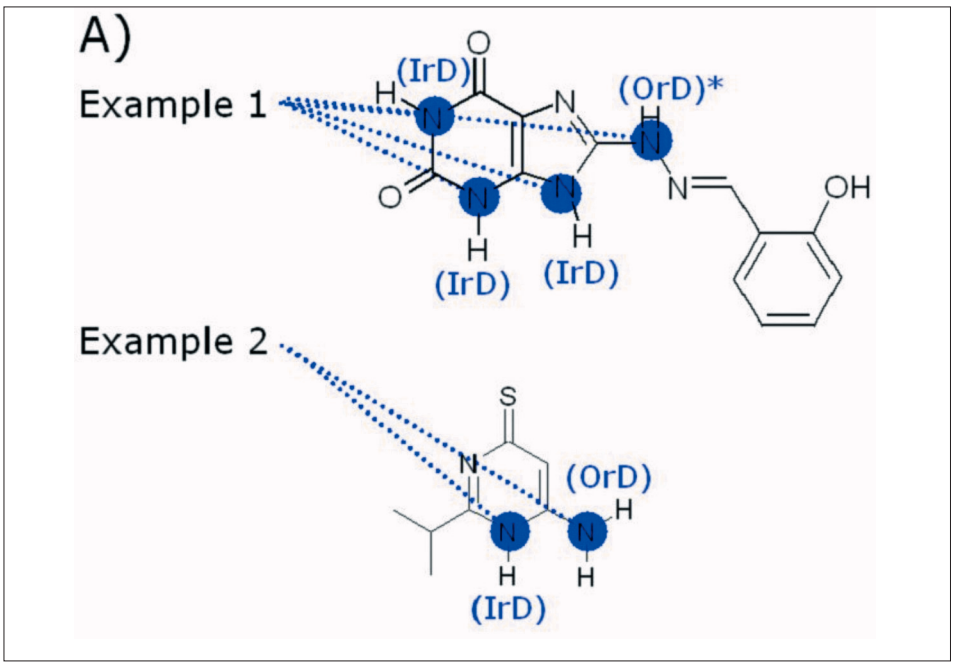

Fig 3. Examples of tautomeric sites created and tautomerized by AGENT: IrD stands for in-the-ring-donor, OrD for out-of-the-ring-donor.

of heterocyclic compounds in a database and predicts their stabilities in terms of energies of formations calculated by MOPAC. The software **AGENT** was inspired by the virtual screening of chemical databases for therapeutically important proteins. Compounds in databases are drawn in their canonical 'ground' states (compounds) rather than in their tautomeric states (tautomers). Tautomers are usually missing in the databases and hence they are not used in molecular docking and virtual screening. **AGENT** is a tool which creates the 'chemically' reasonable tautomers for the corresponding compound entry in the database. Finally, the comparison of calculated energies of tautomers with their parent compounds can predict their relative stability. Screening both the tautomers and their parent compounds together may substantially increase the chance to detect new hits (Fig. 3) [27]27).

In collaboration with Oliver Zerbe, now at the University of Zürich and Annette Beck-Sickinger, now at the University of Leipzig, we have used NMR spectroscopy for the detailed study of membrane interaction of peptidic ligands and its effects on conformational pre-selection of binding geometries [18][28].

An increasingly important subtype of structure–function relationships are the relationships between the glycosylation of a protein and its functions. In fact, there are several examples of therapeutic glycoproteins whose activity and/or pharmacokinetics depend on glycosylation (EPO, TSH, hcG, IgGs). There are however only few systematic investigations of glycosylation–function relationships and the underlying mechanisms. In a collaboration between the groups of Gerd Folkers and Richard D. Cummings, Oklahoma Center for Medical Glycobiology, Oklahoma City, OK, USA, Vivianne Otto and Thomas Schürpf have shown that in some instances, subtle differences in glycosylation of a protein may have a large impact on biological function. We showed that sialylated, complex-type N-glycans strongly enhanced (12-to 26-fold) the ability of soluble intercellular adhesion molecule-1 (sICAM-1) to induce production of the CXC chemokine macrophage inflammatory protein-2 (MIP-2) in mouse astrocytes. In contrast, binding of mouse sICAM-1 to the integrin LFA-1 was not altered by glycosylation [29]. This finding was based on the use of five glycoforms of sICAM-1, namely fully glycosylated sICAM-1 expressed in CHO cells, sICAM-1 lacking either sialic acid or both sialic acid and galactose, as well as two sICAM-1 glycoforms carrying only high-mannose-type N-glycans (Man5 and Man9) (Fig. 4).

Besides the understanding of how glycosylation may influence biological activity, controlling glycosylation both analytically and in biotechnological production processes is of great pharmaceutical relevance. Using sICAM-1 as a model glycoprotein, we are currently enlarging and refining our understanding of how glycosylation influences biological activity and elaborating robust chromatographic and mass spectrometric tools to analyze the types of glycans present (in collaboration with the Zurich Glycomics Initiative 'GlycoInit' of Ari Helenius, Markus Aebi and Peter Seeberger) and the occupancy of glycosylation sites (in collaboration with M. Przybylski, University of Konstanz, Germany).

…and Introducing New Paradigms in Teaching Molecular Life Sciences

Another main focus of our group, besides the research dedicated to protein–ligand interactions, is to elaborate and test novel teaching methods. Collaboration between individuals has been shown to be a successful and powerful activity for learning and problem solving [30]. During collaboration, students discover, construct, and become aware of their own cognitive structures by representing and explaining their concepts and ideas.

Although constructivism and collaborative learning are already important in learning, they are not properly supported by accurate technologies and proper computer

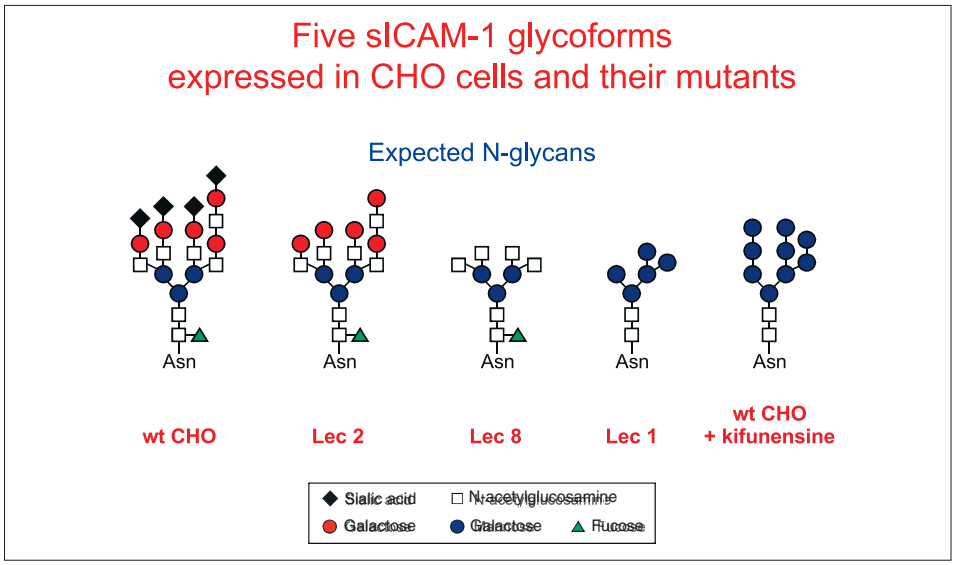

Fig. 4. Five sICAM-1 glycoforms expressed in CHO cells and their mutants

tools. Computer technologies are however important to provide students with the newest results of research and to prepare each student for 'tomorrow's challenge'. All common PCs (personal computers) and notebooks are designed for individual work but not for collaborative work of a whole group. The approach to support collaborative working with a 'personal' computer is wrong. What is needed is a kind of 'team computer'.

Being aware of the necessity of new teaching technology in order to provide students a properly equipped environment for teamwork, a constructivist-learning environment, the so-called *Vireal Lab* (*vir*tual-*real*-*Lab*), has been set up in the library at the Institute of Pharmaceutical Sciences, ETH Zürich. One definition (of such a constructivist learning environment) is: 'A place where learners may work together and support each other as they use a variety of tools and information resources in their pursuit of learning goals and problem-solving activities' [31]. Teamwork is and will remain very important – all modern research in life sciences has switched to teamwork. Only big teams, linked together worldwide, are able to create real breakthroughs in basic and medicinal sciences. Therefore, proper high-tech communication tools are crucial for high-level teaching and research. To prepare students for this working situation, the *Vireal Lab* was established as a completely new scientific environment which combines virtual worlds with the real world of a library.

Vireal Lab is an innovative physical working environment, where high-tech room ware enables all modes of virtual and real collaborative work and learning. High-tech communication roomware are interactive tables and white boards with built-in electronic devices and touch-sensitive surfaces. The latter allow to spontaneously control any computer application and to make annotations using the fingers or a pen. The interactive tables and white boards represent a kind of 'team computer'. Each of them provides easy access to Internet, databases and the local computer network. A large shared, working surface allows everyone to see the object displayed, thus enabling everyone to participate in the problem solving and discussion. For technical details see [32].

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