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A. Ligand-Receptor Interactions: From Understanding to Design

Organized by: Prof. Gerd Folkers*, ETH Zürich
Chairperson: Prof. Gerd Folkers, ETH Zürich

The ultimate step in drug action is its interaction with the biological target. This process governed by physicochemical forces, should be as specific as possible. High specificity paired by high affinity is one of the main pillars of a safe drug. In a rational approach the close integration of biophysical analytics, molecular biology and functional genomics and computational chemistry leads to a deep understanding of ligand–protein interaction at the molecular level. Switching forth and back between the virtual and real world, modern design processes allow for the creation of sophisticated ligands for the benefits of future therapeutic intervention. This situation was reflected in the talks of four speakers of the morning session at the symposium having the focus on medicinal chemistry.

In his compelling style *Hugo Kubinyi* gave a comprehensive overview on ligand–receptor interactions. Besides teaching the basics, numerous examples coming from his rich and life-long experience in drug design pointed out both scope and limitations of the actual approaches in drug design.

Osman Güner from Accelrys was not able to come but was substituted by the European representative *Remy Hofmann*, who has longstanding experience in computational chemistry, especially focusing in the 3D-QSAR and pharmacophore description. His talk summarized the current approaches and provided insight in future developments which could soon facilitate categorization processes based on pharmacophoric properties for huge compound databases.

In the third talk, *Leonardo Scapozza* from the Institute of Pharmacy of the ETHZ delivered a fascinating and exciting research case from his lab. Based on the highly integrated technology mentioned above he designs therapeutic genes for application in cell-based therapy. By use of these 'genetic switches' one might be able in future to control graft-*versus*-host reactions after tissue transplants. Not only the protein, designed to avoid immune recognition and delivered *via* stem-cells, but also the adequate ligand has been designed to specifically interact on demand.

The end of the session was marked by *Wolfgang Jahnke*, showing us the exciting new applications of nuclear magnetic resonance methodology. Especially for detailed studies in the life sciences – recently acknowledged by the Nobel price granted to Kurt Wüthrich – NMR seems to develop as the ultimate technology.

Keywords: Antigen presentation · Biomolecular NMR · Cell-based therapy · Computational chemistry · Computer-assisted ligand design · Database mining · Drug design · Drug targets · Genetic switch · Ligand binding mode · Ligand-protein interactions · NMR screening · Pharmacophore · Reporter ligand · Stem cells · Structure-based drug design · Suicide gene · Therapeutic gene · Virtual libraries

Understanding Ligand-Receptor Interactions

Hugo Kubinyi
University of Heidelberg and BASF AG, D-Ludwigshafen, Germany
E-Mail: Kubinyi@t-online.de

Drugs interact with macromolecular targets, in most cases with soluble, membrane-bound or membrane-imbedded proteins, *e.g.* enzymes, receptors, ion channels, transporters, and signal proteins. Already more than 100 years ago, Emil Fischer stated "*to use a picture, I would like to say that enzyme and glucoside have to fit like a lock and a key, in order to exert a chemical action on each other*". Neglecting for a moment the flexibility of a binding site, this statement is still true; however, it constitutes only part of the truth: in addition to geometric fit, a ligand must have comple-

*Correspondence: Prof. Dr. G. Folkers
Department of Applied BioSciences
Institute of Pharmaceutical Sciences
ETH Zürich
Winterthurerstrasse 190
CH-8057 Zürich
Tel.: +41 1 635 60 60
Fax: +41 1 635 68 84
E-Mail: folkers@pharma.anbi.ethz.ch

mentary surface properties to its binding site. Most important are neutral and charged hydrogen bonds and hydrophobic interactions.

Whereas hydrophobic interactions, driven by entropy, always increase affinity, proportional to the interacting surface area but at the cost of decreasing solubility of a ligand, hydrogen bonds need separate consideration. They are important for ligand recognition and ligand orientation within the binding site but their contribution to affinity cannot be predicted with sufficient accuracy. Sometimes they increase affinity by up to some orders of magnitude, sometimes they are even detrimental for ligand binding. An increase in affinity will result if the hydrogen bonds between a ligand and its binding site are 'stronger' (*i.e.* geometrically more favorable) than the hydrogen bonds of the polar groups of the free, un-liganded partners to the surrounding water molecules; otherwise, affinity will be reduced.

Starting from the 3D structure of a biological target, structure-based and computer-aided design techniques are powerful tools for the discovery of new drugs. High-affinity ligands can be designed, using programs like GRID, LUDI, FlexX, LigandFit, and others. The recent development of potent neuraminidase inhibitors as flu remedies is just one success story in rational drug design. Recently experimental and *in silico* methods have been developed to assemble low-affinity fragments within a binding site to high-affinity ligands. SAR by NMR, the dynamic assembly of ligands, and the combinatorial design of ligands 'from bits and pieces' illustrate into which direction computer-assisted ligand design will develop in the very next future.

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Chemical-Feature Based Pharmacophores as Useful for Lead Identification: Some Recent Applications

Rémy Hoffmann[a], Thierry Langer[b]*
[a]Accelrys SARL, Parc Club Orsay Université, 20, rue Jean Rostand, F-91898 Orsay Cédex, France
[b]University of Innsbruck, Institute of Pharmaceutical Chemistry, Innrain 52a, A-6020 Innsbruck, Austria
*E-Mail: remy@accelrys.com

The concept of pharmacophores was introduced at the beginning of the 20th century by Paul Ehrlich. Classically, the constitutive elements of a pharmacophore model (also called pharmacophoric points) are atoms, chemical groups, bonds. These points are connected together by geometric constraints (distances, angles, torsions, plane orientation). In an extension of this concept, the classical pharmacophoric points can be replaced by chemical features (donors, acceptors, ….). These features are meant to mimic the general types of interactions encountered in ligand–protein interactions.

Pharmacophores have been – and still are – intensively used in drug design projects. Recently there has been an increased interest in their use for virtual library focusing.

Pharmacophores are mostly qualitative, but some methods allow the generation of quantitative pharmacophore models. They can be derived from a set of ligands, but the information extracted from protein active sites (incorporating bound ligands) constitutes another possible source to derive pharmacophore models.

Pharmacophores models are used to identify new potential leads in database mining experiments.

Some of the results have been reviewed, obtained during the past years using the pharmacophore perception on different targets, using both ligand-based as well as feature-based approaches.

Design of Genetic Switches

Leonardo Scapozza, Regula Johner, Patrick Ballmer*
Institute of Pharmaceutical Sciences, Department of Applied BioSciences, ETH, CH-Zürich
*E-Mail: Leonardo.Scapozza@pharma.anbi.ethz.ch

Leukemia is a disorder of the hematopoietic stem cells. By far the only proven curative therapy is allogeneic stem cell transplantation but because of several limitations only a few percent of newly diagnosed leukaemia patients are eventually cured by allogeneic stem cell transplantation (SCT). In this context to increase the number of patients able to profit from SCT, T-cell-based immunotherapy is used. Infusion of donor T-lymphocytes following allogeneic SCT is associated with long-term remission. Furthermore, T-lymphocytes infusion shows effects against solid tumors such as breast cancer. However, T-lymphocytes infusion therapy is limited by the risk of graft *versus* host disease (GvHD). To control the GvHD effect donor T-lymphocytes are transduced with a suicide gene *e.g.* Herpes simplex virus thymidine kinase (HSV1 TK) gene acting as genetic switch. The transduced cells are sensitive to ganciclovir and thus they can be eliminated in the event of GvHD by using ganciclovir (GCV). This approach presents two major clinical limitations a) incompatibility between treatment of cytomegalovirus infections with GCV and HSV1 TK transduced lymphocytes, b) immunogenicity of HSV1 TK expressed in the cell. These issues have been addressed using an interdisciplinary approach combining bioinformatics and molecular modeling with protein engineering and medicinal chemistry designing the lock and creating the correspondent keys. To solve the treatment incompatibility a new suicide gene producing a tailor-made HSV1 TK protein for a new prodrug has been developed. This new HSV1 TK no longer accepts GCV. Its effectiveness has been proven using cell assays. To address the immunogenicity issue a bioinformatic tool for predicting MHC-class I epitope considering the antigen presentation pathway has been developed. The prediction has been tested experimentally using immunogenicity tests. By means of prediction new genetic switches have been designed.

NMR in Drug Discovery

Wolfgang Jahnke
Novartis Pharma AG,
Central Technologies, CH-4002 Basel
E-Mail:
Wolfgang.Jahnke@pharma.novartis.com

Biomolecular NMR spectroscopy is a powerful tool to characterize molecular interactions, and is increasingly being recognized for its potential to identify or optimize lead candidates for a particular drug target. NMR can speed up and improve the drug discovery process by a variety of contributions:

- Validation of hits obtained from high-throughput screening or virtual screening is an important task of NMR. NMR provides a robust and reliable assay for the detection of molecular interactions between protein and ligand. Apart from hit validation or rejection, the binding site and possibly the binding mode can be rapidly elucidated. Competition experiments with known ligands can be carried out, and dissociation constants can be measured.
- If no lead compounds are available, or if diversity considerations favor the introduction of novel scaffolds, NMR can be used to screen compound libraries for affinity to the protein target. This can result in novel lead compounds, or in alternative fragments that can be incorporated in an existing lead to improve its affinity, selectivity, or pharmacological properties.
- High-resolution structures or docking models of protein targets, in apo form or complexed to lead compounds, can be obtained by NMR. The obtained precise structural information can be used to guide chemistry efforts in optimizing the lead compound, or to form a basis for *in silico* docking of virtual libraries.

Biomolecular NMR is a rapidly developing field with a high demand in innovation. Two major method developments are:

- NMR identification of compounds with binding affinity to a target ('NMR screening') generally suffers from the need for large amounts of target protein. By utilizing paramagnetic probes as spin labels, we can reduce protein requirements for NMR screening by one or two orders of magnitude (SLAPSTIC) [1], and form a robust and sensitive assay for second-site NMR screening [2].
- NMR reporter screening changes the principle of NMR screening by observing not the binding of a test compound itself, but its ability to displace a known "reporter ligand" by competition for the same binding site. By doing so, also strongly bound ligands can be detected by NMR screening, and the throughput of NMR screening is increased by an order of magnitude [3][4].

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B. Structure-Properties-Relationships and Kinetics

Organized by: Manfred Kansy*, F. Hoffmann-La Roche, CH-Basel
Chairperson: Manfred Kansy, F. Hoffmann-La Roche, CH-Basel

Biological affinity screening itself is often not the time limiting step in modern drug discovery and development. Factors like solubility, absorption, biodegradation or toxicity are equally important and have to be optimized as early as possible in the discovery development cascade. The afternoon session in medicinal chemistry of the r+d in life science Congress focused on that specific topic.
The gastrointestinal absorption of an orally administered drug for example is one of the key factors for its bioavailability. Today HT-screening as well as theoretical methods are used to determine the potential of a compound to permeate a membrane *in vivo*. *Pierre-Alain Carrupt*, University of Lausanne, focused in an excellent overview on structural aspects influencing passive absorption, and his efforts to develop a computational tool for permeability prediction. *Alex Avdeef*, pION Inc. Boston, one of the leading experts in the development of HT-system for physicochemical profiling gave an overview on the status of passive permeability screening systems. Today's methods allow the simulation of sink conditions and pH effects in HT-mode.
In the third talk, *Luc Balant*, University of Geneva, took a new direction in explaining the complex relationships in pharmacokinetics and the importance of the underlying factors in early drug optimization. The html-based lecture was well received and showed how complex relationships can be explained in an easy way. Finally *Gerd Klebe*, University of Marburg, gave some insight in computational approaches to functionality among proteins. His exciting lecture described how ligand-receptor interaction is tackled, and demonstrated how the integrated database Relibase can be used to query simultaneously ligand and protein information.

Keywords: Absorption ADME · Artificial sink conditions · BBB permeation · Bioinformatics · Biopharmaceutics · Blood-brain barrier · Clinical pharmacology · Double sink · Functional similarity · Molecular fields · PAMPA · Permeation · Physicochemical transport model · Relibase · Skin permeation · Structural genomics

Pharmacokinetics

Luc P. Balant
Department of Psychiatry,
University of Geneva
E-Mail: luc.balant@medecine.unige.ch

Pharmacokinetics is the study of the time course of drug and metabolite concentrations in different fluids, tissues, and excreta of the body, and the mathematical relationships required to develop models to interpret such data. The goals of pharmacokinetics are as diverse as the disciplines that have come to apply its principles to their efforts. These disciplines include the clinical sciences, particularly clinical pharmacology, drug metabolism, molecular biology, biopharmaceutics, pharmacology, statistics, and toxicology among others. The ultimate goal of pharmacokinetics, to some, is the elucidation of relationships between pharmacological or toxicological response and concentrations of drugs or their metabolites in body fluids. To others, the study of the kinetics of absorption (A), distribution (D), metabolism (M), or excretion (E) of drugs or chemicals may further enhance their understanding of the basic mechanisms underlying these processes. To still others, pharmacokinetics offers an approach to improve and optimize the therapeutic management of individual patients.

In recent times, pharmacokinetics has also been increasingly used to improve the processes involved in drug discovery and development. During drug discovery, pharmacokinetic principles may help select better drug candidates. During drug development, it helps, in addition to the above mentioned objectives, to detect and control variability factors that may affect the behavior of drugs in the organism (ADME) and thus influence their therapeutic, as well as their unwanted effects.

*Correspondence: Dr. M. Kansy
F. Hoffmann-La Roche Ltd.
Pharma Research Basel Discovery PRBD-CS
Molecular Properties
CH-4070 Basel
Tel.: +41 61 688 58 74
Fax: +41 61 688 74 08
E-Mail: Manfred.kansy@roche.com

Molecular Fields and Permeation: A Deeper Description of Molecular Structures

Pierre-Alain Carrupt
Institut de Chimie Thérapeutique, BEP,
Université de Lausanne,
CH–1015 Lausanne
E-Mail: pierre.alain.carrupt@ict.unil.ch

One method to investigate interactions between a molecule and its environment is the generation of Molecular Interaction Fields (MIFs). These fields describe the variation of interaction energy between a target molecule and a chemical probe moved in a 3D grid constructed around the target. Since the information contained in 3D molecular fields is related to the interacting partners, the amount of information in MIFs is clearly greater than that in one-

or two-dimensional computed molecular descriptors. In this context, the VolSurf method able to transform the information present in 3D-MIFs into a limited number of quantitative descriptors was used to correlate 3D molecular structures of a large and heterogeneous set of compounds with pharmacokinetic data, such as skin or BBB permeation.

Two kinds of MIFs were used in combination with VolSurf, namely GRID which is based on the total energy of interaction between a probe and a target molecule, and the MxPs, namely the **M**olecular **H**ydrogen-**B**onding **P**otentials (**MHBPs**) and the **M**olecular **L**ipophilicity **P**otential (**MLP**) which are able to compute the interactions between a target molecule and its environment based on fragmental or atomic physicochemical values. The **MHBPs** compute the 3D hydrogen bonding potentials of small molecules and biological macromolecules, while the **MLP** computes their 3D lipophilicity properties.

The combination of MIFs produced by GRID and MxPs procedures and VolSurf has led to a powerful method able to correlate 3D molecular structures BBB permeation. Similarly, combining MxP and Grid fields gave a good PLS model to discriminate between good and poor skin and permeants. These approaches afford a better understanding of the molecular features needed to enhance skin and BBB permeation and generate QSAR models useful for the *in silico* screening of pharmacokinetics properties of drug candidates.

High-Throughput Permeability and Membrane Retention Measurement Using Artificial Phospholipid Membranes

Alex Avdeef
pION INC, 5 Constitution Way,
Woburn, MA 01801 USA
E-Mail: aavdeef@pion-inc.com

In this presentation, the new PAMPA (parallel artificial membrane permeability assay) developments emerging from our laboratory were summarized. Our search for the minimalist physicochemical transport model [1–3] has involved the study of over 60 lipid systems (some neutral, some anionic) at pH 7.4, and several of these systems over a range of pH from 3 to 10. We have considered artificial sinks in the acceptor compartments (serum proteins, surfactants), and artificial solubilizers in the donor compartments (bile acids, cosolvents). The effects of solute retention by the membrane, the unstirred water layer, buffers (conditioning pH gradients in the unstirred water layer), solute charge and membrane charge, and general pH conditions on permeability have been critically studied. New equations have been derived to handle the variety of new experimental conditions. Double-sinks created by pH gradients and sink-inducing surfactants have revealed promising transport models for the gastrointestinal tract and blood-brain barrier absorptions.

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Computational Approaches to Match Functional Similarity among Proteins

Gerhard Klebe
Institute of Pharmaceutical Chemistry,
University of Marburg,
D-35039 Marburg, Germany
E-Mail: klebe@mailer.uni-marburg.de

In recent times several genome projects have been accomplished. This provides us with the sequence and overall function of the genes involved. Subsequently it is hoped to determine by means of powerful bioinformatic tools the gene variants that contribute to various multifactorial diseases in humans or genes that exist in certain infectious agents but not in humans. As a consequence, this will allow us to define the most appropriate levels for intervention with new drugs. Structural genomics is a follow-up, combined with new techniques to validate the therapeutic relevance of such newly discovered targets. Accordingly, it can be expected that in the near future we will witness a substantial increase in novel putative targets for drugs along with their structural characterization. Are we prepared to exploit this tremendous flood of information to efficiently discover and develop new drugs?

The explosion in protein sequence and structural information demands new computational tools for the storage and subsequent retrieval of structural data. We have embarked on the development of the integrated database Relibase that allows to query simultaneously ligand and protein information. Protein function is almost invariably linked with the specific recognition of substrates and endogenous ligands in given binding pockets; proteins of related function should therefore share comparable recognition pockets. We have developed a new algorithm based on the placement of physicochemical descriptors assigned to the exposed binding-site residues that can be used to retrieve common sub-structures and thereby related binding pockets independent of any given sequence or fold homology.