A novel method to determine accurately and efficiently the structure of the receptor binding sites in protein-ligand complexes promises to revolutionize drug discovery. Dr Julien Orts and his collaborators at the Swiss Federal Institute of Technology in Lausanne have developed a method that could be a breakthrough in drug discovery.

Proteins are the fundamental building blocks of all living matter, from microscopic viruses and bacteria to highly evolved multicellular organisms, plants, animals, and humans. They play a role virtually in all phenomena associated with life, including providing structural support to individual cells and tissues, enabling motion in complex organisms, producing energy and regulating signalling between cells in the body. Frequently, the physiological function of a protein is modulated by the interaction with small molecules (ligands), which can bind to specific receptor sites in a protein and trigger a response, e.g. in the form of a structural change or of a chemical reaction. For instance, ligands like hormones can promote cell growth by triggering a response, e.g., changes in gene expression.

Proteins are typically large and complex macromolecules, composed of thousands of atoms, which are arranged in chains of subunits, called amino acids. Proteins fold into 3D structures, which are characteristic of each protein and are connected at a fundamental level to the protein's function. Accurate knowledge of a protein's structure is the first step toward understanding how the protein works and how the chemico-physical processes that it carries out can be influenced by means of drugs.

The challenge is to derive, rapidly and reliably, protein-ligand complex structures.

Method

1. Without any protein assignment
2. Without knowing the location of the binding site
3. With receptor side chains and backbone

Experimental inter-molecular NOEs measured on a high field spectrometer: Top: 13C,15N-filtered 2D [1H,1H]-NOESY spectrum showing inter-molecular NOEs between the ligand and unknown methyl groups of HDMX. Bottom: Ligand 1H magnetization auto-relaxation curves (left) and inter-molecular cross-peak build-up curves (right) versus the mixing time of the filtered NOESY experiments.

From drug design to intermolecular communication and biological function via signal transduction.
the whole protein structure from NMR data. NMR drastically reduces the time and effort required to obtain an atomically resolved structure of a binding pocket by using previously determined protein structures, from a couple of months to a couple of days. It can also be partially automated, and therefore it provides a natural tool for high-throughput workflows, which can be used to screen thousands of potential new drugs in sequence and to analyse the nature of their interaction with a target protein.

A typical NMR protein-ligand structure determination requires a preliminary preparation of the sample, in which either the protein or the ligand is isotopically substituted (13C, 15N) or selectively labelled (e.g. isoleucine, laurine and valine methyl labelling). NMR experiments are then used to measure intra-molecular (ligand) and inter-molecular (ligand-protein) atomic distances, which in turn provide a model of the ligand structure in the binding pocket. To understand the exact nature of the ligand-protein interaction, protein structures from existing databases (obtained from X-ray or NMR measurements) are then used as input information. The structures selected can be either that of the protein in the absence of the ligand or those of similar (homologous) proteins. The NMR program then screens all possible assignment groups in the protein and calculates the protein-ligand complex structure for all options. At this stage, it is essential to reduce as much as possible the number of configurations to screen. This can be achieved by initially restricting the assignment groups in the protein to only 3 or 4 relevant ones. False assignments can be ruled out using geometric considerations, based on the knowledge of the input structures. This substantially reduces the calculation time. At the end of this procedure, the resulting complex structures have to be analysed carefully, to detect potential errors arising from the unconstrained relaxation of the protein backbone during the refinement procedure. It is important that a sufficient number of inter-molecular distances are taken into account, typically at least 12 or 15. A high signal-to-noise in the NMR spectra and a good signal resolution are also crucial.

A NEW TOOL FOR DRUG DISCOVERY

The NMR method has been applied successfully to the resolution of various classes of protein-ligand complexes. Several structures containing strong binders or small ligands have been determined with an accuracy of 0.9-1.5 angstroms relative to the reference structure. The applicability of NMR to complexes with ligands in fast exchange or weak affinity binding has also been demonstrated. In the case of the weak affinity binding complex HDM2-4845, a new complex has been characterised, never observed before. The efficiency of NMR is well represented by the complex structure of SJ212-MDMX, which could be resolved at 1.35-angstrom accuracy within a day using a desktop computer. These are a few initial examples of the great potential of NMR in the study of protein-ligand interactions and protein function and they pave the way for its application as a fast, reliable and accurate protocol for drug discovery.

Research Objectives

Dr Orts and his collaborators have developed multidisciplinary approaches to study protein-small molecules complexes using NMR spectroscopy, X-ray crystallography and computational methods.

Personal Response

The accuracy and efficiency of the NMR methods have been documented in a number of protein-ligand complexes. What are the remaining challenges of NMR that need to be addressed in order to make your method a robust and easy to use routine tool for high-throughput molecular screening in drug discovery?

Fragment-based drug discovery is becoming a major approach in both pharmaceutical companies and academic laboratories. Fragment-based methods need fewer compounds to be screened, synthesized and the fragment hits show usually high ligand efficiencies (potency per atom). Currently, no fast and robust NMR method can handle small fragments due to a lack of protons “probes”. We need to develop the current NMR method to create a new approach, that can automatically and simultaneously determine multiple structures of fragment-protein complexes. Having access to the structure of the binding site for each binder allows investigating chemical scaffolds that would otherwise be discarded and to broaden the chemical knowledge as well as the drug-ability of the receptors.

References


Orts, J.; Wälti MA; Manih M, Vera, L, Gossert, AD; Güntert, P; Riek, R. (2016) "NMR-Based Determination of the 3D Structure of the Ligand-Protein Interaction Site without Protein Resonance Assignment." J. Am. Chem. Soc., 138, 4933-4400.


www.researchoutreach.org

Behind the Research

Dr Julien Orts

Laboratory of Physical Chemistry
Swiss Federal Institute of Technology ETH
HCI F217, Vladimir Prelog-Weg 2
8093 Zürich, Switzerland
Bio

Julien Orts graduated in Physics and Biophysics jointly from the Max Planck Institute and the European Molecular Biology Laboratory. He is currently a junior group leader at the ETH Zürich. He develops methods for structure-based drug design and demonstrated for the first time that 3D structure determination of a protein-ligand complex can be achieved from solution NMR data fully automatically.

Collaborators

• Peter Güntert
• May Marsh
• Roland Riek
• Ole Storz
• Felix Torres
• Mariella Aulikki Wälti

ETH
Eidgenössische Technische Hochschule Zürich
Swiss Federal Institute of Technology Zurich

E: julien.orts@phys.chem.ethz.ch
T: +41(0) 44 632 28 64
www.ethz.ch