



Structural analysis of crystals is aiding in pharmaceutical research

## Multifaceted **impact**

*Crystals reveal the spatial structure of chemical compounds. Pharmaceutical researchers at Bayer HealthCare are using crystals and state-of-the-art computer technologies to help decipher the molecular mode of action of new substances and develop innovative medications.*

The delicate latticework floats gently in space. A short while later it changes shape, the surface becoming smooth and shiny. Rounded hillocks now protrude outward, while fissures retreat into the depths. Anyone who tries to reach out and touch this floating object of several cubic meters in size will find themselves grasping at thin air, however. This fascinating spectacle is not the brainchild of a modern-day magician, but of the scientist Dr. Alexander Hillisch, the Head of the Medicinal Chemistry Department at Bayer HealthCare (BHC) and responsible for Computational Chemistry at the Wuppertal site. Hillisch is presenting the structural elucidation results for a protein molecule in the three-dimensional

viewing room at the Bayer HealthCare research center in Wuppertal. Special glasses make onlookers feel as if they are seeing a stereoscopic structure. The protein that Hillisch is projecting into space is an exact three-dimensional representation of Factor Xa (pronounced "ten a") on which the anticoagulant currently under development (see *research 18*) has docked.

"In the process of developing rivaroxaban, we have already passed the stage in which structural elucidation of proteins plays an important role, but spatial models such as this one are now providing us with valuable information on the structure-activity relationships between target molecules and new

pharmacological active substances at a very early phase in many projects centering around the search for active ingredients," says Hillisch, explaining the importance of protein structures and his department, which includes more than just molecule designers. Before they can begin with their task, however, the crystallographers must get to work. Crystals reveal the spatial structure of chemical compounds, thus pointing the way for future optimizations in medication development.

In addition to high-tech equipment, crystallographers require experience and good instincts to help them solve their tasks. And sometimes a little bit of luck as well. After all, it is impossible to



Three-dimensional research: (from left) Dr. Alexander Hillisch, Dr. Axel Harrenga and Dr. Tobias Marquardt in front of the computer simulation of a molecular structure that provides valuable information for development of new active ingredients. Transferring crystals into special nutrient solutions (picture above) also helps in this process.

predict if and when the molecules of a dissolved substance will form a regular latticework of crystals or a shapeless mass. "Crystal growing is still subject to the principle of trial and error," says Dr. Axel Harrenga, a biochemist at Bayer HealthCare, who grows and analyzes what are known as co-crystals together with his colleague Dr. Tobias Marquardt. It is a complex undertaking. "Crystals only form from a supersaturated solution and in a specific concentration range typical for the substance," explains Harrenga.

The crystals formed by proteins are not the size of rose quartz or amethyst, however. To the pharmaceutical researchers, large crystals are those with edges averaging 0.2 millimeters in length, made up of trillions of identical molecules. In the ideal crystal lattice, the space between each of these molecules is the same.

And each of their atoms is likewise the same distance from its counterparts in neighboring molecules. These distances must be measured in order to decode the molecular structure. Measuring techniques for molecules have nothing to do with measuring tapes and yardsticks, however. Instead, the researchers use x-ray diffraction analysis to chart the crystal lattice and develop a structural model.

X-rays have a specific wavelength which approximately corresponds to the distances between the individual atoms

in a crystal. The rays are thus ideal for use in structural elucidation. If they come into contact with the electrons of an atom while passing through the crystal, they are refracted. This creates diffraction reflections that are registered by a detector.

### Molecular structure as a starry nighttime sky

The result is a specific diffraction pattern for each individual substance. This pattern does not reveal structural formulas like those in chemistry textbooks, however. To non-experts, it looks more like a picture of the starry nighttime sky. But experienced structural analysts such as the researchers at Bayer HealthCare can use what others see as scattered points of light to determine the three-dimensional structure of a molecule with absolute certainty. "The position of the reflections reveals what is known as the crystallographic space group, or the precise arrangement of the molecules in a crystal. How intense the reflections are depends on the contents of the crystal, i.e. the proteins and their structure," says Marquardt, explaining the two key analysis criteria. Scientists have to rely on high-performance computers when conducting analyses, however. A useful structural model requires up to one million measuring points. Based on the exact position and respective intensity of these points, the computer calculates

an initial structural model which scientists then test for plausibility.

Scientists worldwide have meanwhile decoded the spatial structure of more than 40,000 proteins, including more than 40 percent of the target proteins of interest to BHC. After analysis, the active ingredient and protein complexes supply a three-dimensional image of the structural bond between an active ingredient and the target protein in an organism. This forms an important basis for future research on active ingredients. Via molecule design on a computer screen, a structural model can pave the way for future optimizations in the synthesis laboratory. "Structural elucidation findings and their computer-based interpretation have led to the discovery and improvement of the properties of a number of promising substances in our active ingredient research," says department head Hillisch.



[www.che.utoledo.edu/mcl.htm](http://www.che.utoledo.edu/mcl.htm)

*The Macromolecular Crystallization Laboratory explains all about protein crystals.*