Researchers develop chemically specialised germanium surface

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Researchers at the Ruhr Universität Bochum have developed a new method for attaching proteins to the surface of germanium crystals – for the first time also membrane proteins. This enables time-resolved tracking of the interactions between molecules using infrared spectroscopy in a way that is accurate down to atomic resolution.

The method is applied in the EU project "Kinetics for Drug Discovery, K4DD", in which scientists explore the interplay of drugs and their interaction partners. With the new technology, the researchers can also study so-called G-protein-coupled receptors, which are the site of action for many drugs. The team of Prof. Dr. Klaus Gerwert, PD Dr. Carsten Kötting and Jonas Schartner from the Chair of Biophysics reports in the Journal of the American Chemical Society.

Attaching proteins to germanium using electron pair bonds

Using infrared (IR) difference spectroscopy, researchers analyse dynamic processes in proteins. In an earlier study, Bochum's biophysicists already succeeded in binding proteins to germanium surfaces using lipids, thus making them accessible to IR spectroscopy (as reported in September 2012: aktuell.ruhr-uni-bochum.de/pm2012/pm00284.html.de). For this, the researchers shine infrared light into the germanium crystal, which is multiply reflected at its boundary surfaces. Part of the light leaves the crystal and thus reaches

the proteins bound to the surface. Previously, the researchers used hydrophilic interactions between the crystal and lipid – i.e. interactions between polar groups of the molecules – for the bonding. Now they coupled the proteins via an electron pair bond to the germanium. This kind of bond is more stable and works both for soluble and membrane proteins. "Membrane proteins need a kind of soap as an outer shell, a detergent, which washes off a lipid layer. In contrast, our newly developed surface remains stable", Jonas Schartner says.

Chemical modular system

As in a modular system, the researchers placed various molecular layers, one above the other, on the germanium crystal. First, they produced hydroxyl groups on the germanium surface, each consisting of an oxygen atom and a hydrogen atom. The product is referred to as activated germanium. The next layer was formed by a new kind of triethoxysilanes, a hydrocarbon compound, which the RUB team produced itself. The researchers anchored one end of the triethoxysilanes covalently to the germanium, i.e. via an electron-pair bond. They converted the other end into a protein trap. All the proteins that carry a particular adapter, the His-tag, can be attached to this. "There are already a lot of proteins available with this universal adapter" Carsten Kötting says.

Modifying the germanium surface in a controlled manner

Using Fourier transform infrared spectroscopy and X-ray photoelectron spectroscopy (XPS), the researchers kept track of what happened when stacking the different layers on the germanium crystal. Together with Prof. Dr. Martin Muhler and Bastian Mei from the Laboratory of Industrial Chemistry, the biophysicists were able to accurately determine

the atomic composition of the layers with the XPS. Proteins can also be observed on surfaces using other techniques, such as surface plasmon resonance. "With surface plasmon resonance, the gradual modification of the surface is carried out blindly," Jonas Schartner says. "We've observed each modification step live and thus have very good control over the process."

Functional test for the new process successful

A test confirmed that the newly created surface serves its purpose. The researchers equipped the germanium crystal with the switch protein Ras, which plays an important role in carcinogenesis. There they allowed it to interact with a second molecule that switches Ras on and off. These two states – "on" and "off" - were reflected in the infrared difference spectra. With the new method, the RUB team thus successfully made a protein interaction visible. In future, drugs and their receptors are to be put to the test. "Using the conventional surface plasmon resonance method, it is only possible to determine whether an interaction takes place. A special feature of our method is that different types of active substance interactions also lead to differences in the difference spectrum", Jörn Güldenhaupt says. "With this additional information, the mechanism of action can be examined much better. This can be critical in the development of active substances."

More information: J. Schartner, J. Güldenhaupt, B. Mei, M. Rögner, M. Muhler, K. Gerwert, C. Kötting (2013): Universal method for protein immobilization on chemically functionalized Germanium investigated by ATR-FTIR difference spectroscopy, *Journal of the American Chemical Society*, doi: 10.1021/ja400253p

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