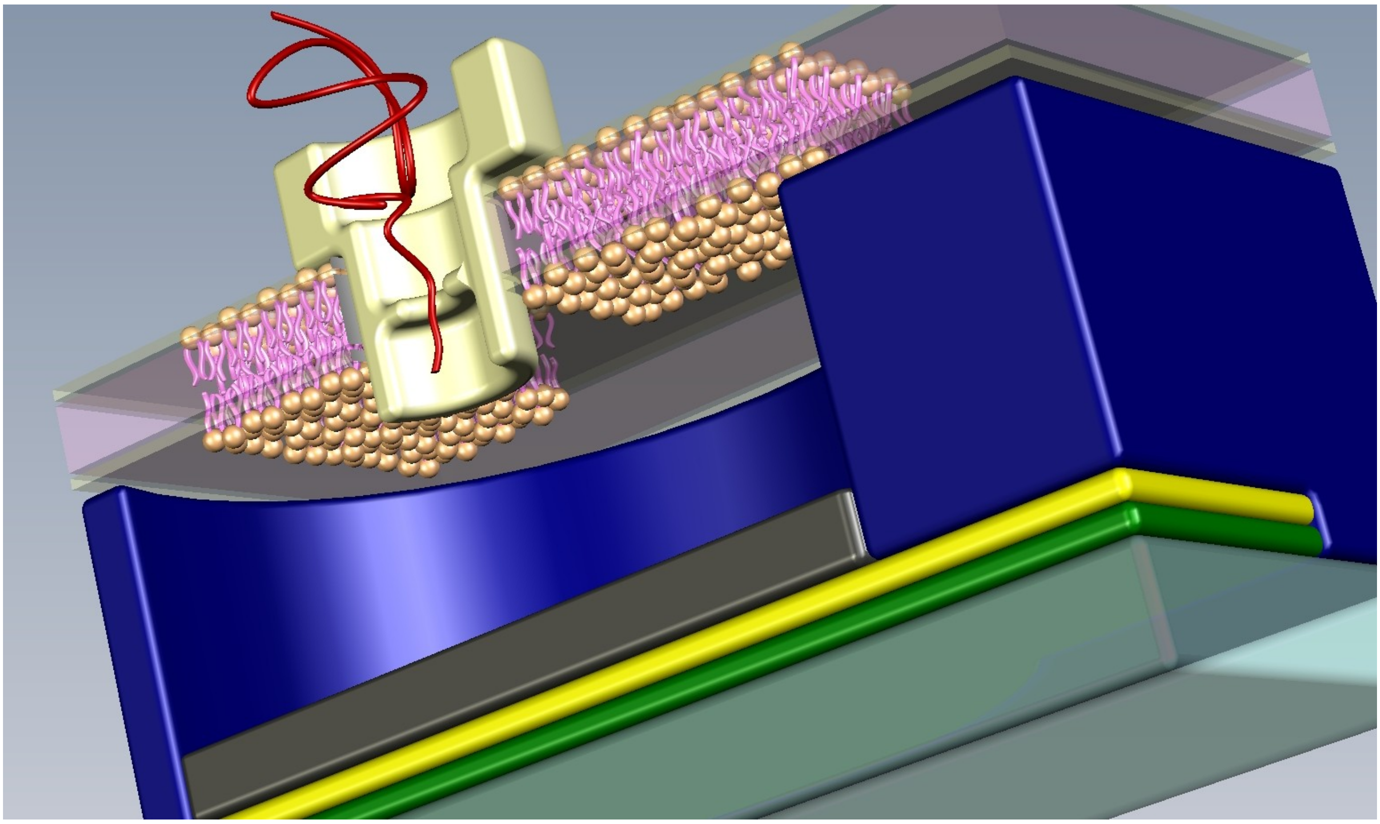
Biological nanopores on chips for determining the properties of molecules

Up until now scientists have needed devices the size of rooms to measure polymer units. Researchers led by Prof. Dr. Jan C. Behrends and Dr. Gerhard Baaken from the University of Freiburg have developed a chip the size of a fingertip containing biological nanopores that determine molecule size with great precision. Developing this new system, which is a combination of biological and micro-technical components, involved a great deal of technical skill. The system has the same level of sensitivity as a chromatography device, but is much easier to handle and is also cheaper than the large devices. The chip has also the potential to be used for sequencing genes and for analysing other molecule classes.



Principle of a chip with a biological nanopore for determining the chain length of polymer molecules.

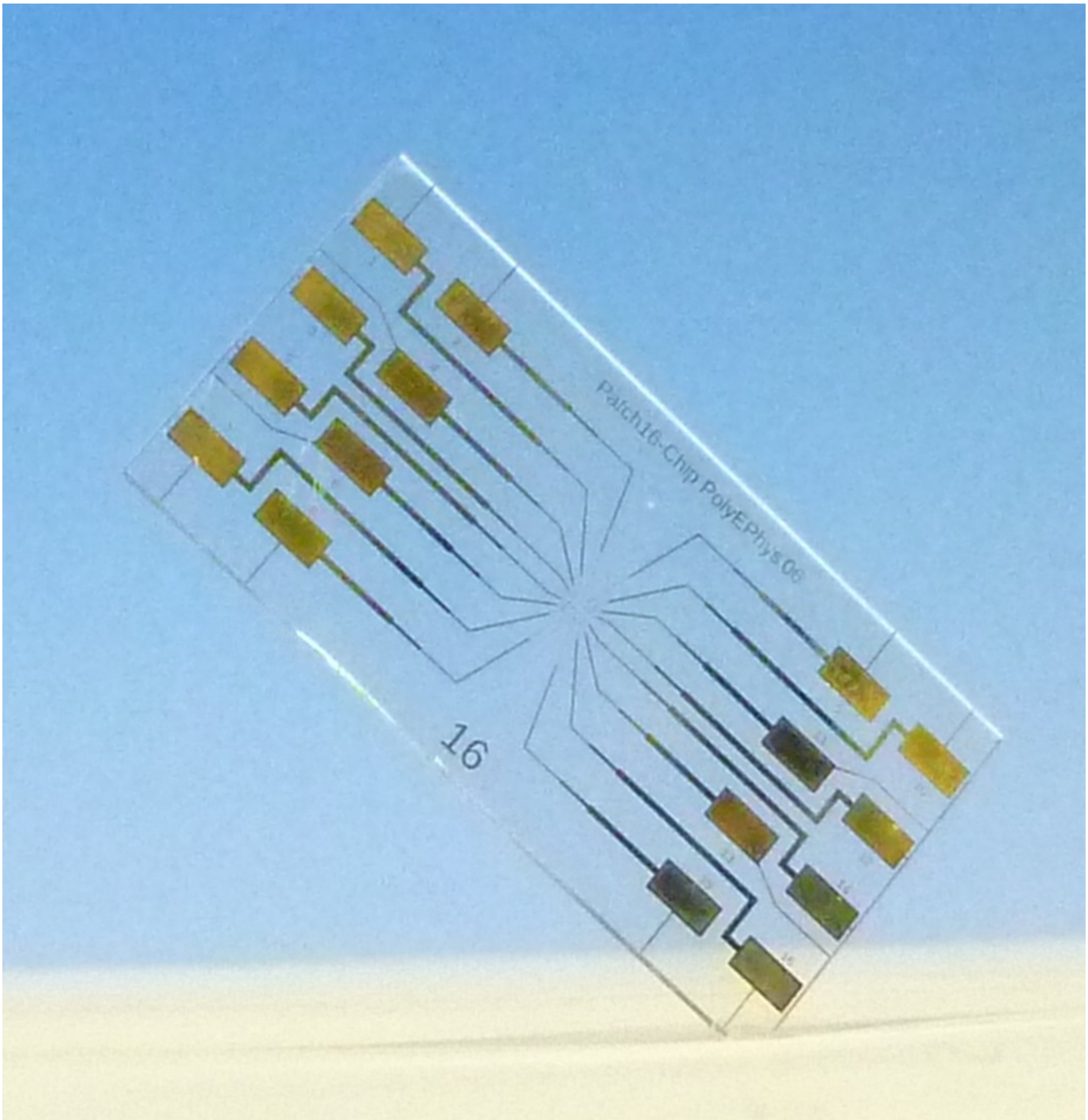
The ingredients of creams and lotions would separate if they did not contain polyethylene glycol

(PEG), a compound composed of repeating ethylene glycol units; the macromolecule is used in tablets as a drug carrier and enables the continuous release of the pharmaceutical substances contained in the formulation. Every year, hundreds of tons of polymers are produced worldwide. The production of pharmaceutical substances, amongst other things, is subject to strict regulations and chain length and mass distribution of products must be precisely controlled. Up until now, liquid and gas chromatography techniques have been indispensable tools for determining the size and sequence of the individual polymer units. However, chromatography devices are expensive and tend to fill entire rooms, which is why researchers and companies around the world have been working on the development of smaller and less expensive tools. “It would be good to have methods that are easy to apply, cheap and accurate, especially for molecules that need to be tested frequently,” said Prof. Dr. Jan C. Behrends from the Institute of Physiology at the University of Freiburg. Working in cooperation with the Laboratory for Chemistry and Physics of Interfaces led by Prof. Dr. Jürgen Rühe in the Department of Microsystems Engineering (IMTEK), Behrends has brought the fields of biology and microsystems engineering together. This has resulted in the development of a chip the size of a fingertip on which the researchers arranged biological nanopores that serve as sensors for determining the size of molecules; the nanopores were then combined with a system of microelectrodes.

A treasure trove with huge technical potential

Biological pores are channel proteins contained within cells. Their function is to transport ions and other molecules across membranes. Bacteria contain many transmembrane proteins, which differ from each other in pore diameter and other molecular characteristics. They are an absolute treasure trove for researchers who are able to technically exploit their potential. The chip developed by Behrends and Dr. Gerhard Baaken, a scientist in Dr. Rühe’s department, consists of a glass plate with sixteen tiny cavities arranged on an area of one square millimetre. Each cavity is covered with a tiny synthetic cell membrane containing an alpha haemolysin molecule, a protein produced by staphylococci to destroy red blood cells in infected organisms. If a polymer gets into one of the channel proteins, the channel is blocked for a fraction of a second, which in turn leads to changes in the membrane’s ability to transport ions. The researchers can therefore use special microelectrodes for measuring changes in the system’s electrical conductivity. “Our measuring method is extremely sensitive and even allows us to detect the presence of a single molecule and derive the number of units of the polymer trapped in the channel,” said Baaken.

The theory behind the innovative chip sounds rather simple. However, the development of the chip has not been a trivial matter. “The work relating to the technical aspects of the chip amounted to an art in itself,” said Behrends. He has been concentrating on merging biology and technology for more than ten years and has achieved good results. While working at the University of Munich, one of his many achievements involved transferring the patch clamp method to a planar chip. When he moved to Freiburg around three years ago, he met Prof. Rühe from the IMTEK whose openness and expertise has contributed enormously to the development of the innovative chip. Meeting Prof. Rühe and starting a collaborative project with Rühe’s research group was an enormous piece of good luck for Behrends. He is full of praise for Baaken’s interdisciplinary commitment and in particular for his achievements in establishing the technology behind their joint chip project. Baaken has been intellectually as well as physically connected with Rühe’s laboratory at IMTEK and Behrends’ Institute of Physiology for around three years now. The stability of the silver electrodes in the chip matrix was one of the major challenges the researchers faced. “To ensure that electrodes do not polarize over time and become instable, the material used needs to be highly dense and have a relatively large surface,” said Baaken explaining that they had to produce a surface with nanoscopic pores as well as

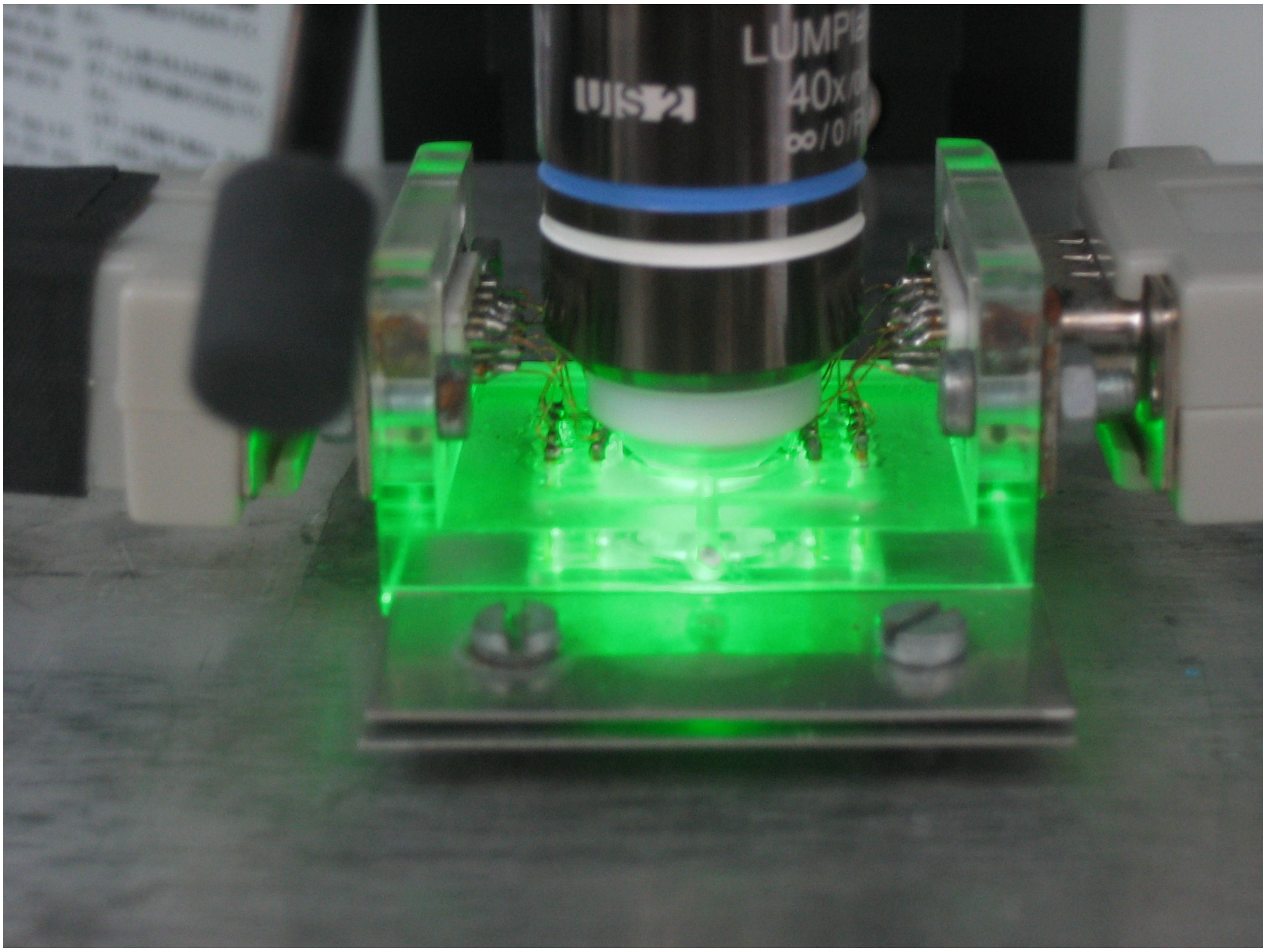


The result of long-term cooperation: the chip is a biohybrid sensor consisting of biological and microtechnical parts. The biological pores are only a few nanometres in diameter and cannot be seen with the naked eye.

finding a reproducible way to do this.

“Bedside” diagnostics and a lot more

Working in a clean-room laboratory at IMTEK, the researchers led by Behrends, Rühe and Baaken have established a serial chip production process that is both cheap and quick. The chip prototype will now be optimised for application in industry. The researchers also believe the chip has great potential in biomedical applications, for example for the identification of small RNAs, which are early biomarkers of cancer and other diseases, in patient sera. The researchers also anticipate that the chip will one day be suitable for mobile, i.e. bedside application. The use of



Measurements are currently carried out with a prototype. However, the serial production of nanopores on a microchip might soon become reality and be used in automated industrial processes.
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antibodies against cocaine and other drugs and medicines could lead to the development of easy-to-use doping and drug tests. And molecular biologists even foresee the possibility of being able to use the chip for analysing the sequence of unknown DNA strands without using markers as well as for the controlled synthesis of pores tailored to different applications, including the analysis of proteins. This would be a revolution for any researchers aiming to specifically interfere with cellular processes. However, for the time being, the chance of such visions becoming reality is still a long way off.

At present, Behrends and Baaken are aiming at something more concrete than far-off visions. They are working to set up a company that they have just spun out from the University of Freiburg. The company will focus on the commercialisation, sale and further development of their multielectrode cavity array technology. The researchers are also aiming to optimise the technology into a platform that will enable analytical methods to be automated, and industry to carry out high-throughput analyses at some stage in the future. “The nice thing about our chip technology is that we are constantly able to increase the number of recording positions,” said Baaken going on to explain “we expect to be able to create up to one thousand measurement points per square centimetre in the future.”

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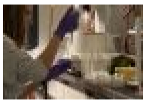

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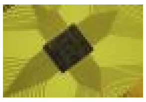

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