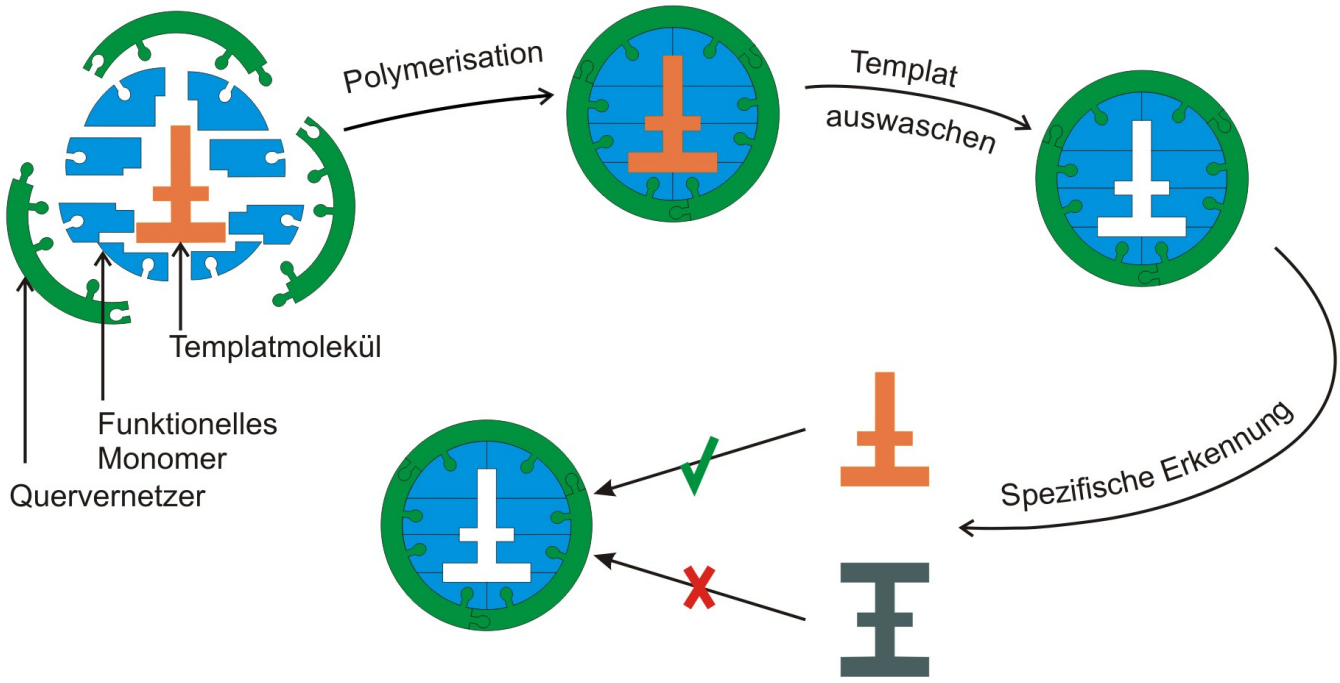
New analysis tools involving molecularly imprinted polymers

The demand for made-to-measure analytical methods is increasing as interest in the biological production of materials and pharmaceuticals increases. It is extremely important economically for bioindustry to know the production time and quantity of products that result from specific fermentation conditions. Inspired by natural molecular processes, researchers from Tübingen and Stuttgart are working on new, label-free analytical methods that make it possible to monitor the entire process.

Biotechnology plays an increasing role in industrial production. Fine chemicals as well as active pharmaceutical ingredients are produced in large bioreactors, known as fermenters, using unicellular organisms. The growing economic success of biological production has given rise to larger production facilities with increasingly complex process management. Physical parameters such as temperature and pressure, the addition of nutrients and the separation and purification of products needs to be optimised. This is why the entire fermentation process needs to be backed up by sophisticated process analytics. The control of fermentation can be improved by molecular bionics, which is a topic being dealt with by a team at the Tübingen-based Institute of Physical and Theoretical Chemistry led by Prof. Dr. Günter Gauglitz. The Tübingen team works closely with a team of researchers led by PD Dr. Günter Tovar from the Institute for Interfacial Engineering, IGVT, at the University of Stuttgart.

“At present, the progress of fermentation is almost exclusively determined from the pH value and from CO2 concentrations, occasionally also from the concentration of glucose. The fermentation products cannot be directly measured online during the fermentation process, which takes usually several days, by removing and subsequently analysing the samples,” said Gauglitz explaining the current situation, which he hopes to change. He hopes to be able to determine in a timely manner how the product develops in the fermenters, the point at which a maximum product yield is achieved, the point at which the added substances have been metabolised and when it is best to terminate the production process.

Live monitoring of the ups and downs of product formation



During the production process, the polymers are given the ability for the molecular recognition of particular analytes.
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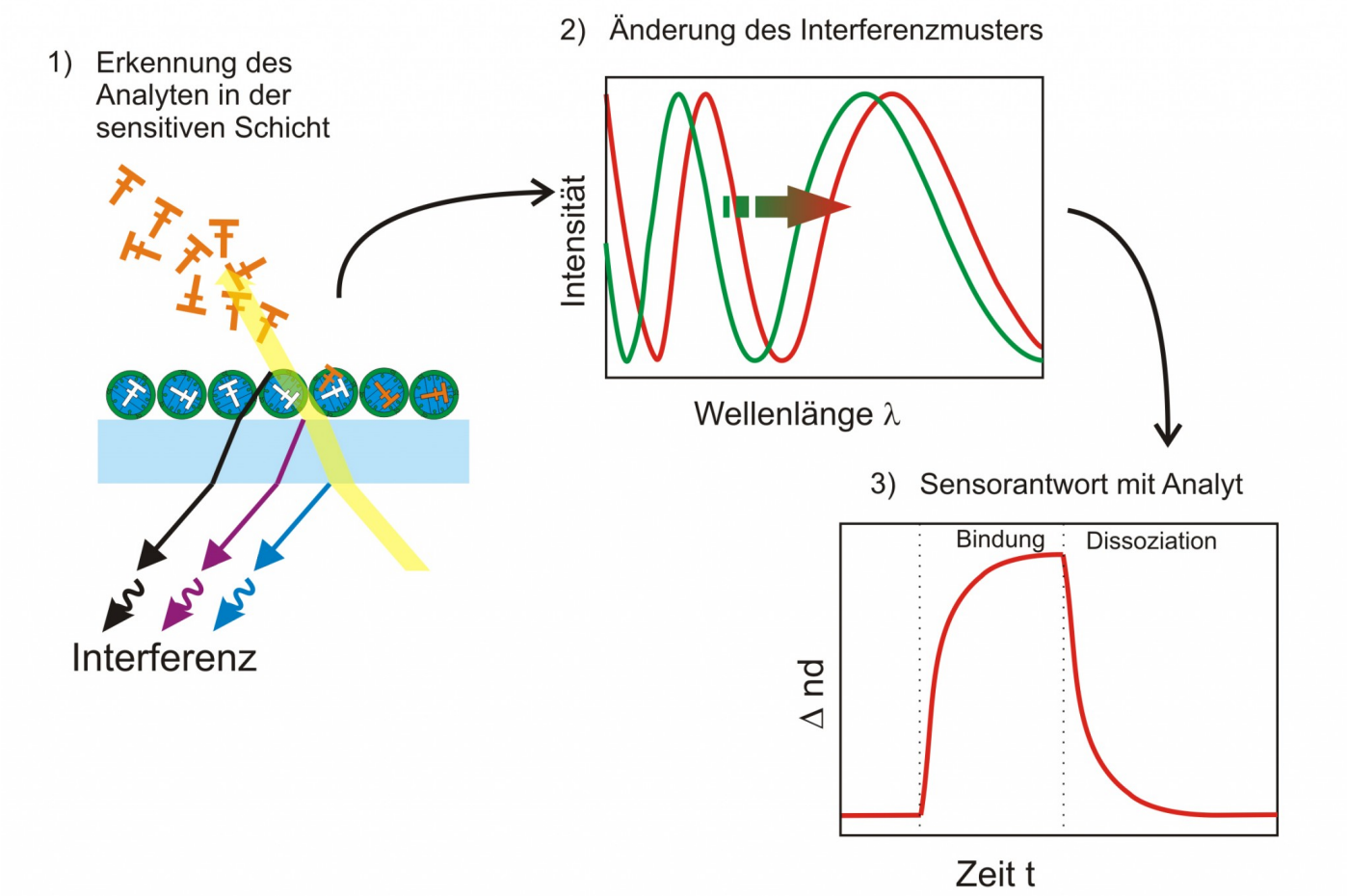
In theory, several methods are available to analyse the products, for example through their reaction with antibodies. “However, antibodies have the disadvantage that they deteriorate under fermentation conditions and that they can only be applied in specific concentration ranges,” said Gauglitz who, along with his project partners from the IGVT, bases his research on the natural binding and imprinting mechanisms on the molecular level. The researchers use proteins and peptides as well as nucleic acids and carbohydrates for their investigations. Tovar’s group of researchers intends to use molecularly imprinted polymers to which the fermentation products to be analysed can bind specifically though reversibly. This is made possible by adding the analyte during the polymerisation process. The art of producing molecularly imprinted polymers depends on choosing conditions that prevent the analyte from binding firmly to the emerging polymer. The researchers hope to avoid the use of covalent bindings as much as possible, as this will enable them to remove the analyte by adding solvents. On the other hand, polymerisation must be carried out in such a way that only the specific analyte, and no other molecule that might potentially be present in the fermentation broth, can bind. The entire process is a tricky interplay of molecular interactions that need to be carefully balanced.

The polymers are equipped with a selective synthetic recognition site (imprint) that is sterically and chemically complementary to a particular analyte



Reflectometric interference spectroscopy (RIfS) is a label-free technique for detecting interactions between immobilised molecules.

"We would like to use polymers that are chemically much more inert than antibodies and which are suitable for use in a different concentration range. Our overall aim is to be able to reliably and analytically accompany the entire fermentation process," said Gauglitz. The binding between the product molecule (analyte) and the polymer would then enable the researchers to determine the analyte concentration. In order to achieve this, the researchers will use the direct, label-free analysis technique developed in Tübingen. While the Stuttgart project partners focus mainly on the production of polymers, the researchers from Tübingen concentrate on developing a suitable monitoring method. Gauglitz and his team have already achieved considerable success in the development of analytical methods on the basis of reflectometric interference spectroscopy (RIfS) and now intend to adapt this optical method to the new system used for process analytics. "In the field of process analytics in particular, it makes no sense to label the product molecules and use expensive gold particles, for example. In addition, temperatures might change during the fermentation process, which is why it is also necessary to avoid temperature problems," said Gauglitz explaining why he is looking for label-free reflectometric solutions.

Label-free detection using optical analysis methods

Since the project is still at an early stage, the researchers need to overcome numerous hurdles. "It is worth noting that such an analysis system is not as simple as a Lamda sensor used in cars. The method needs to be adapted to the fermenter fluidics," said Gauglitz highlighting the need to precisely control the selectivity and the reversibility of the polymers. In addition, the researchers need to define the response time of the polymers. Gauglitz believes that the entire process, starting with the binding of the analyte and ending with the measurements, will take no more than five to ten minutes in future.

The project will initially be funded for one year through the Baden-Württemberg "Molecular Bionics" programme. Gauglitz hopes that this will give the teams enough time to take the project as far as being able to file an application for a grant with a longer duration. "At the moment this is still a vision of the future; our fundamental goal is to carry out a feasibility study," said Gauglitz. However, all scientific caution aside, Gauglitz already foresees that the method has the potential to be used in the emerging field of bioindustry and is interested in making contact with potential industrial partners.

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