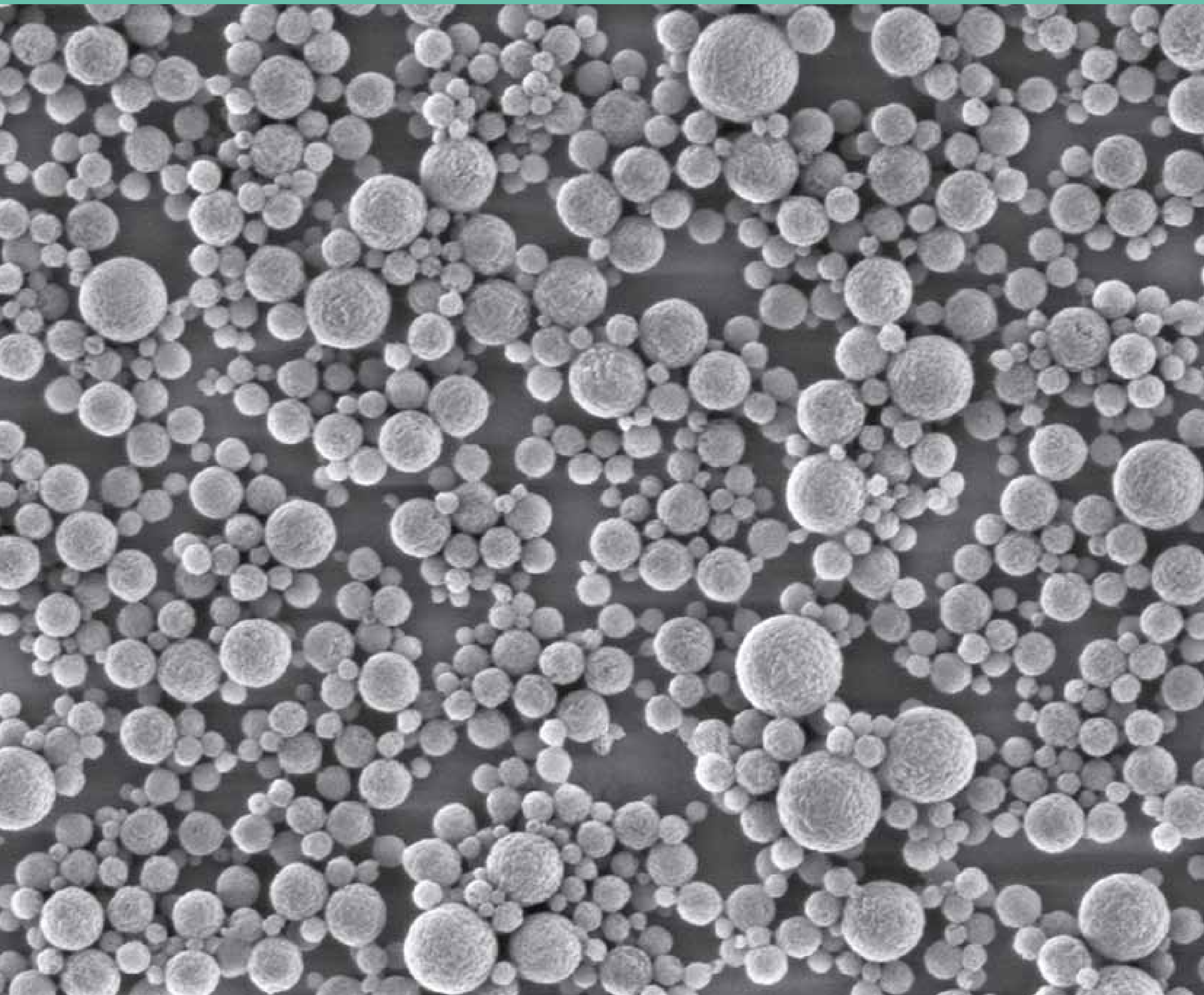
Fraunhofer IGB

FRAUNHOFER INSTITUTE FOR INTERFACIAL ENGINEERING AND BIOTECHNOLOGY IGB

NANOCYTES® – TAILOR-MADE PARTICLES AS SPECIFIC ADSORBERS



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NANOCYTES®

TAILOR-MADE PARTICLES AS SPECIFIC ADSORBERS

A key task in many processes in chemistry, pharmaceutics and biotechnology is the specific separation of molecules from mixtures, either to obtain or purify substances or to remove troublesome associated substances. Nanoscopic molecularly imprinted polymers (NanoMIPs) act as artificial receptors and are outstandingly suitable as adsorbers for the solution of these problems. The nanoparticles specifically recognize biomolecules and active ingredients such as amino acids, peptides and proteins, low-molecular compounds or substances such as toxins and endocrinal substances.

The range of future fields of application of these new materials is almost unlimited. Over and above the use of synthetic adsorbers for selective purification and separation processes, the NANOCYTES® technology also offers solutions for diagnostic applications in medical technology and for analytical questions.

Molecular recognition function based on Nature's model

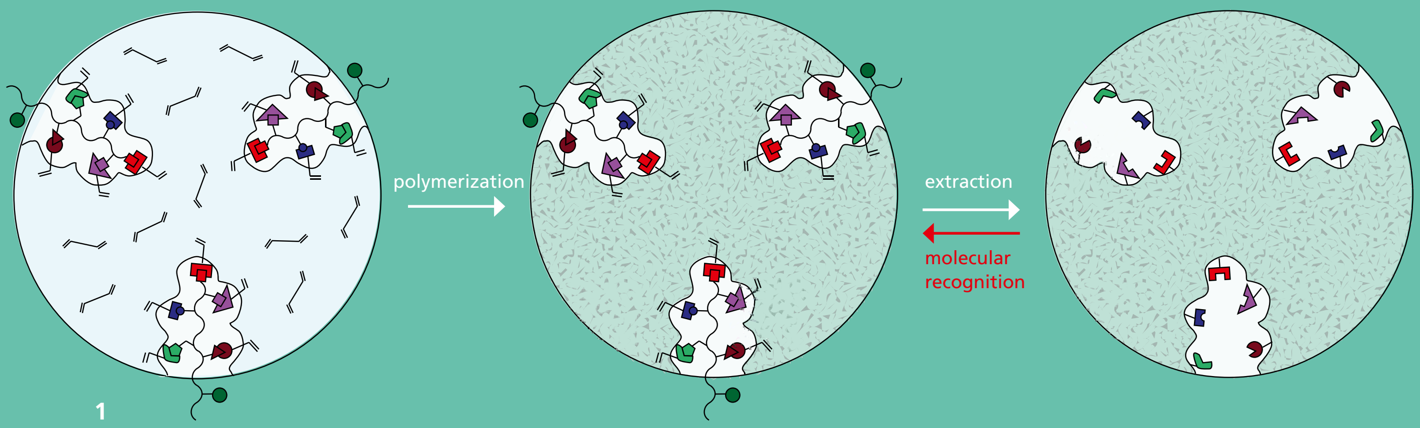
In nature, information is transmitted by contact between substances. Hormones dock onto specific receptors and trigger precise signals. Or harmful substances are recognized, masked and then removed in an organism by antibodies.

These molecules behave like a lock and key, but with an induced fit consisting of flexible modules. Spatially defined chemical structures fit together like the positive and negative imprint of the same chemical pattern. Nature has extended this principle to a fascinating range of applications. Because of the limited stability of natural receptors, new ones have to be produced constantly – a massive hindrance for technical applications.

Stable nanostructured synthetics

On the basis of the biomimetic principle of molecular imprinting, molecule-specific recognition tags are generated by mapping certain structures in the form of a chemical negative imprint in synthetics. Thus robust and stable "artificial antibodies" become available. These molecularly imprinted polymer nanoparticles emulate the biological systems in their functionality. However, their stability is far superior to that of biomolecular receptors such as antibodies; they can therefore be used in technical processes without any problems. In addition, the stability of these synthetic receptors guarantees a high degree of regenerability and reusability. This fact makes their utilization in a large number of technical applications attractive from the ecological and economic viewpoint.

1 *Composite membrane.*



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THE PRINCIPLE OF THE MOLECULAR IMPRINTING OF POLYMER NANOPARTICLES

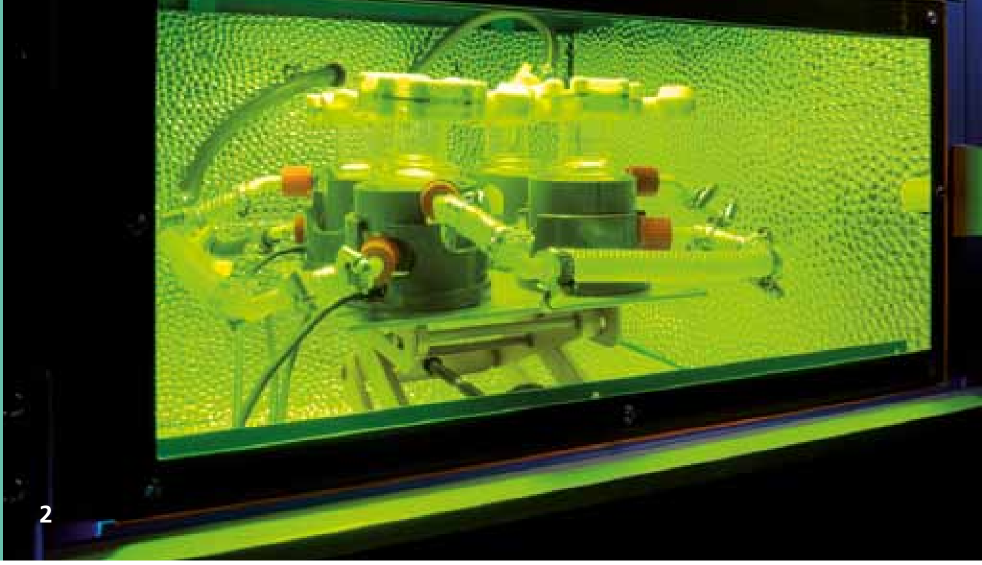
Molecular imprinting of polymer nanoparticles is a process in which a polymerizable mixture – consisting of functional monomers and crosslinkers – with non-polymerizable target molecules is transformed into NanoMIPS, nanoscopically-sized polymer beads. Here the target molecules act as molecular stamps, so-called templates. When in each case they have been removed from the polymer network following successful polymerization, they leave behind both specific spatial-physical and chemical imprints in the synthetic surface of the polymer nanoparticles. As a result of this, specific recognition tags for the target molecules are produced in the nanostructured polymers – artificial receptor nanoparticles are created.

Template target molecule fragments are used in the imprinting process for the production of selective nanoparticles for the molecular recognition of large biomolecules or complex compounds. Here the areas in the molecule are selected that are specifically and spatially easily accessible for the target molecule. The binding to the selective nanoparticle matrix is carried out later via the corresponding fragment of the complete molecule.

Selective nanoparticles by means of mini-emulsion polymerization

At the Fraunhofer IGB molecularly imprinted polymer nanoparticles are represented by means of mini-emulsion polymerization with typical particle sizes ranging from 50 to 300 nm. This process is a heterophase polymerization in which, under the influence of high shearing forces, the use of surfactants as well as co-stabilizers, two non-miscible liquid phases are emulsified to become homogeneous and stable. The nanodroplets produced in this way from the monomer, the template molecule and the osmotic reagent form nanoreactors in which the polymerization takes place. This results in polymer nanoparticles that are direct reproductions of the emulsion droplets as regards their size and morphology. This technology of the synthesis of molecularly imprinted polymer nanoparticles, patented by the Fraunhofer IGB, has – compared with the widely used methods for the synthesis of polymer nanoparticles such as emulsion polymerization or precipitation polymerization – the advantage that the synthesis, although complex, is effected in one stage with a quantitative yield and independently of the diffusion of the educts.

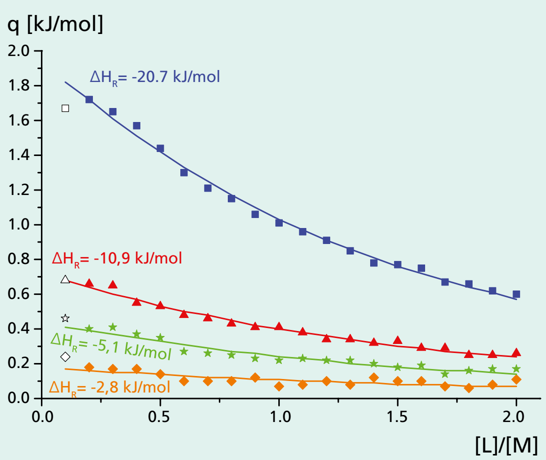
Besides the classical mini-emulsion polymerization, which is used for the molecular imprinting of hydrophobic molecules, the process can be carried out in an inverse mini-emulsion polymerization. Nanoparticles for the molecular recognition of hydrophilic molecules, for example peptides and proteins, can be produced using this technology.



Structure-selective nanoparticles – specific adsorbers for amino acid derivates

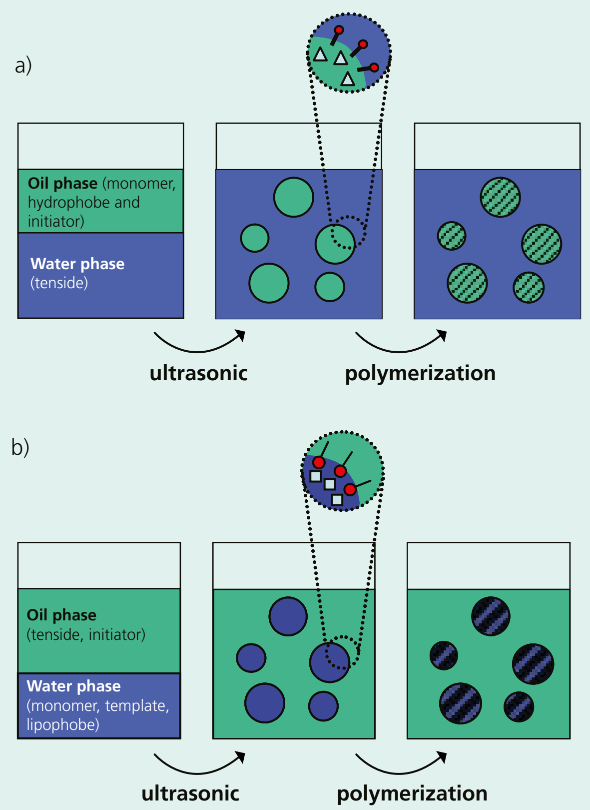
At the Fraunhofer IGB selective nanoparticles, among others, are successfully represented for the molecular recognition of amino acid derivates, for example Boc-L-phenylalanine anilide (L-BFA). By means of a photo-chemically induced co-polymerization in the presence of the target molecule L-BFA, stable, coagulate-free molecularly imprinted poly(ethylene glycol dimethacrylate co-methacrylic acid) particles are produced. The selectivity of the nanoparticles was demonstrated by examining of the interaction between the synthetic receptor and various ligands by means of HPLC and microcalorimetry.

Verification of the structure selectivity of L-BFA imprinted polymer nanoparticles by means of micro-calorimetry.



- ■ L-BFA to L-BFA-NanoMIPs
- ▲ L-Boc-Tryptophan to L-BFA-NanoMIPs
- ★ L-Boc-Phenylalanine to L-BFA-NanoMIPs
- ◆ L-Boc-Tyrosine to L-BFA-NanoMIPs

Process steps in classical (a) and inverse (b) mini-emulsion polymerization.



1 *Diagram showing the principle of the molecular imprinting of polymer nanoparticles.*

2 *Miniaturized and parallelized glass reactors to optimize the production of nanoparticles.*

3 *UV-initiated mini-emulsion polymerization in the immersion lamp reactor.*

CUSTOMIZED RECEPTOR NANOPARTICLES AND THEIR FIELDS OF APPLICATION

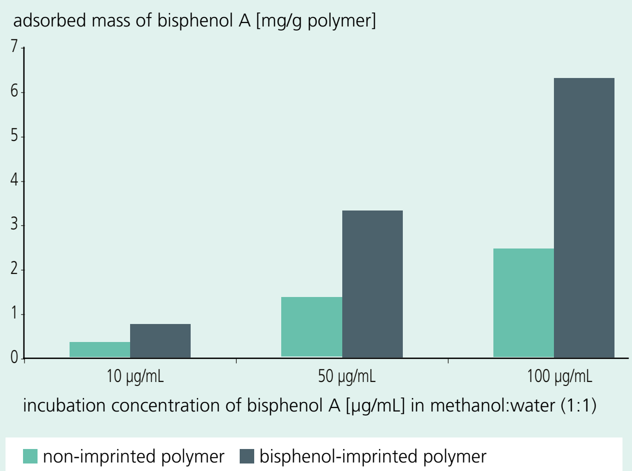
Molecularly imprinted polymer nanoparticles in their function as artificial receptors are suited to use in a large number of different technical applications. Synthetic receptors for insulin purification, selective adsorbers for the removal of environmental pollutants from wastewater as well as for the separation of valuable minor components are being developed in current research projects at the Fraunhofer IGB.

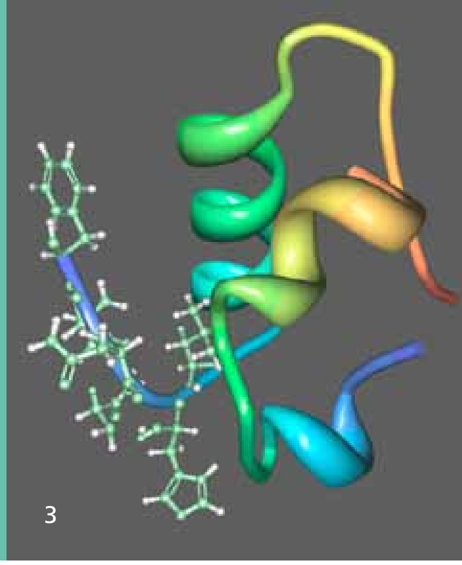
Synthetic adsorbers for removing environmental pollutants

Water is the basis of all life on earth, clean and perfectly hygienic water is therefore the precondition for human health. In recent years we have seen an increased emission of hormonally ("endocrine") active substances into the environment. Via the wastewater, for example, these substances reach the purification plants where they are not – or not effectively – broken down, even in the biological stages. They have even been detected in drinking water. Endocrine active substances interfere with the hormonal systems of humans and animals, and can lead to negative effects on health, in particular to reproductive disorders.

At the Fraunhofer IGB our approach is therefore to produce selective adsorbers to remove endocrine active substances. These compounds include alkyl phenols in industrial detergents and shampoos, polychlorinated biphenyls (PCB) in sealants and hydraulic oil, phthalates as softeners (plasticizers) in plastics, pesticides and bisphenol A, which is mainly used in large quantities in the plastics and paper industry. Bisphenol A (BPA) has an estrogenic action. It has already been detected in the sewage sludge of German communal purification plants, in landfill leachate and also in drinking water. At the Fraunhofer IGB we develop nano- and microstructured NanoMIPs, for example to deal with the widely used substance bisphenol A. In a project funded by the European Union, which is being carried out in cooperation with the University of Stuttgart, we were able to demonstrate, by means of model solutions, the specificity of the molecularly imprinted polymers.

Adsorbed mass of bisphenol A in bisphenol-imprinted and non-imprinted polymer nanoparticles.





Synthetic adsorbers to recover valuable minor components

The efficient and complete utilization of plant resources makes both ecological and economic sense. Nowadays plants used for the production of biodiesel, such as rape, are already cultivated on a large scale. However, in addition to the triglycerides used for the biodiesel production, rape seed oil contains – like other plant oils – further valuable ingredients. Molecularly imprinted materials can be used to good effect for the specific adsorption of these minor components. At the Fraunhofer IGB we pursue the approach of producing selective adsorbers to obtain α-tocopherol (vitamin E) from vegetable oils. The aim is to extract the valuable α-tocopherol before the vegetable oils are further processed to make biodiesel. The aim here is to develop a new value creation opportunity for small and medium-sized companies whose core business includes the production of phytoextracts.

Synthetic insulin receptors

The treatment of chronic illnesses requires highly efficacious drugs with minimum side effects. The peptide hormone insulin occupies a key role in the therapy of diabetic disorders. As the number of cases of diabetes mellitus is constantly increasing, ever greater quantities of insulin also have to be produced. Today insulin is produced with recombinant microorganisms in large-scale technical installations. In order to be able to use the protein molecules as active agents, they have to be purified in a multi-stage process that is time-consuming and costly. Purification causes up to 50 percent of the production costs of protein therapeutics.

With the help of effective synthetic adsorbers or receptors that bind the insulin reversibly, the purification has been made considerably simpler and, as a result, more cost-effective. For this purpose we are researching the representation of molecularly imprinted nanoparticles that could bind insulin with great specificity and selectivity. The research work is part of the program "New Materials from Bionics" of the Baden-Württemberg State Foundation. It may be a long way till the artificial receptors can be used in purification during insulin production. The current high costs for the purification of insulin and the urgent demand for insulin – in 2007 the insulin market yielded a worldwide turnover of U.S. $5 billion – is an enduring motivation for our research.

1 *Packing materials coated with adsorber particles.*

2 *Demonstration plant for the separation of minor components from vegetable oil.*

3 *3D structure of insulin. The part of the peptide sequence from the N-terminus of the B-chain, which was used for imprinting, is highlighted.*

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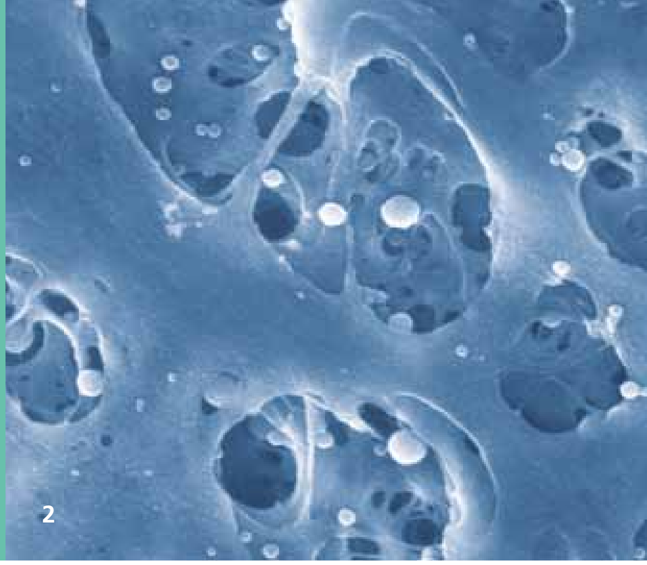

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MOLECULARLY IMPRINTED NANOPARTICLES IN TECHNICAL APPLICATIONS

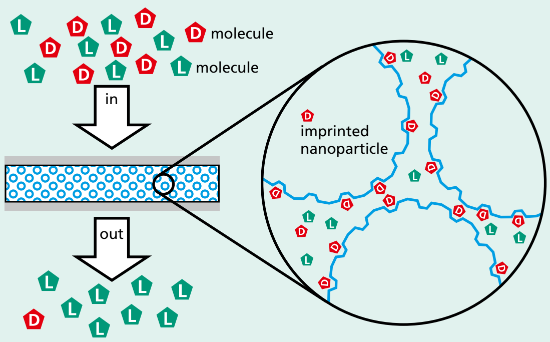
The nanostructured materials created with our new NANOCYTES® technology can be used at a wide variety of processing stages: as a colloid or suspension dispersed in a liquid phase, as a layer in a composite membrane or as an ultrathin matrix on optical sensors or chip surfaces. Also, NanoMIPs can be equipped with magnetizable cores, so that resource-loaded particles can be separated easily by means of magnetic separators. With our technology we make nanoparticles available for use as specific adsorbers in a number of technical applications.

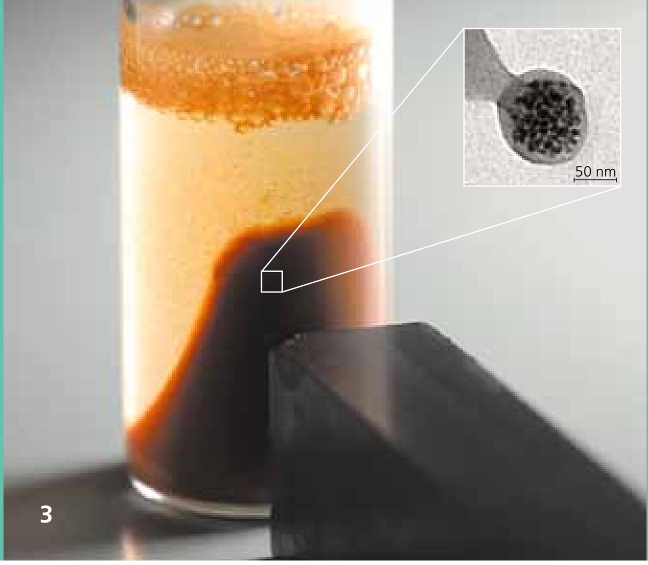
Material-selective nanoparticle composite membranes

A composite membrane for highly selective separation processes has been developed at the Fraunhofer IGB. Here molecularly imprinted nanoparticles function as effective selectors with their large selective surface. These sandwich composite membranes consist of carrier and covering membranes and the selective centerpiece, a layer of molecularly imprinted polymer nanoparticles. The separating capacity and the flow characteristics are adjusted in the membrane by means of the thickness of the layer and the fill level. Decisive advantages of our modular approach are the simple production of the composite membrane, the excellent chemical quality of the selectors and the extremely high surface density of the imprint positions.

The membranes are operated almost continuously or in a circuit. Subsequently, the bound materials can be recovered by means of the appropriate process steps with a separate material flow. The very highly specific surface of this system of approximately 80 m² per g particle permits a high separating capacity even with microscopically thin layers. By modeling the entire separation process we can – an addition to controlled production – also realize a customized conception of selective separation membranes.

Diagram showing the construction and mode of operation of a material-selective composite membrane with molecularly imprinted polymer nanoparticles as selectors.





Selective ultrathin particle layers

The nanostructured polymer particles can be applied to various carrier materials (glass, silicon, polymers) as an ultrathin matrix – for example, with print or masking techniques – for diagnostic and analytical applications. Such microstructured nanoparticle layers form three-dimensional reaction spaces for the binding of analyte molecules with a many times greater surface than the basic area that is occupied. A stable adhesion of the particles to the substrate is guaranteed by means of an adhesion-mediating polyelectrolyte layer. The binding capacity of the layers is adjusted via the packing density of the particle films.

Molecularly imprinted hybrid nanoparticles

A further conception is being pursued at the Fraunhofer IGB in order to widen the technical range of applications of our selective adsorbers. Molecularly imprinted nanoparticles are, for example, being provided with magnetizable cores in order to be able to remove them from a mixture in the purification or separation process – with the bound target molecule – by means of a magnetic separator. The production of the hybrid nanoparticles with particle diameters of 50 to 300 nanometers is effected by copolymerization in the presence of a template molecule and oil acid-layered magnetite particles with diameters of 5 to 10 nanometers.

1 *Special steel module with integrated composite membrane for the use of the membranes in separation processes.*
2 *Polymer membrane with incoporated nanoparticles.*
3 *Nanoparticles with a polymer shell and magnetizable core (magnetite) that are attracted by a magnet (on the right).*
4 *Nanoparticle microarray on silicon chip.*

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ADVANTAGES OF NANOCYTES® TECHNOLOGY

The patented proprietary Fraunhofer technology makes it possible to overcome previous weak points of molecular imprinting such as lack of control over the morphology and the chemical composition of the material produced. With the NANOCYTES® technology synthetic receptors are created with a nanostructure and there is significantly better control of the imprinting process on the polymer surface. Our process also permits molecular imprinting in aqueous systems, which is the precondition for the molecular imprinting of biological macromolecules.

Using the NANOCYTES® technology artificial, nanostructured receptors are created that are predestined for applications in diagnostics, sensorics and downstream processing. The molecularly imprinted polymer nanomonoliths can be modified, for example, with magnetizable cores or colorants, depending on the customer's individual requirements.

Advantages of the NanoMIPs

- High binding capacity
- High degree of selectivity
- High chemical and thermal stability
- Regenerability and reusability
- Cost-efficient production

1 *Atomic force microscopic image of molecularly imprinted nanoparticles.*

Overview of our services

- Development and synthesis of molecularly imprinted nanoparticles
- Conducting feasibility studies
- Surface coatings with nanoparticles
- Conception and manufacturing of selective composite membranes
- Characterization of separation properties
- Development of hybrid materials

Special laboratory equipment

- MALDI-TOF/TOF mass spectrometer (Bruker Ultraflex II)
- Thermogravimetric Analysis (TGA) and Differential Scanning Calorimetry (DSC)
- Simultaneous Thermal Analysis (STA)
- Gel Permeation Chromatography (GPC) with 4 detectors
- Microelectrophoresis (Zetapotential)
- Dynamic Light Scattering (DLS, Nanosizer, measuring range from 0.1 nm to 10 µm)
- Static Light Scattering (SLS, Mastersizer, measuring range from 50 nm to 2 mm)
- Determination of affinity constants by Surface Plasmon Resonance Spectroscopy (Biacore T200 GE Healthcare)
- Ellipsometry
- Microscopy, Scanning Electron Microscopy (SEM)
- Atomic Force Microscopy (AFM)
- Photon Correlation Spectroscopy (PCS)
- Photoelectron Spectroscopy with XPS with EDX
- Mini and Nano-Spray Dryer for the formulation of particles
- High Performance Liquid Chromatography (HPLC)
- Titration Microcalorimetry

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Fraunhofer IGB brief profile

The Fraunhofer IGB develops and optimizes processes and products for the business fields of medicine, pharmacy, chemistry, the environment and energy. We combine the highest scientific standards with professional know-how in our competence areas of Interfacial Engineering and Materials Science, Molecular Biotechnology, Physical Process Technology, Environmental Biotechnology and Bioprocess Engineering, as well as Cell and Tissue Engineering – always with a view to economic efficiency and sustainability. Our strengths are to offer complete solutions from laboratory scale to pilot plant. Customers also benefit from the constructive interplay of the various disciplines at our institute, which opens up new approaches in areas such as medical engineering, nanobiotechnology, industrial biotechnology and wastewater purification. The Fraunhofer IGB is one of 60 research institutes of the Fraunhofer-Gesellschaft, Europe's leading organization for application-oriented research.

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Networking augments expertise

The Fraunhofer IGB works closely with the Institute for Interfacial Engineering IGVT of the University of Stuttgart. This permits continuity of the projects from the basic research to the application. The Fraunhofer IGB is also actively engaged in the Fraunhofer Nanotechnology Alliance, whose main themes are also key issues at the IGB: NANOCYTES® technology – the design of special nanoparticles as carrier substances for biotechnology and medicine – as well as the use of carbon nanotubes for actuatory applications and including multifunctional layers for the automotive sector.

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