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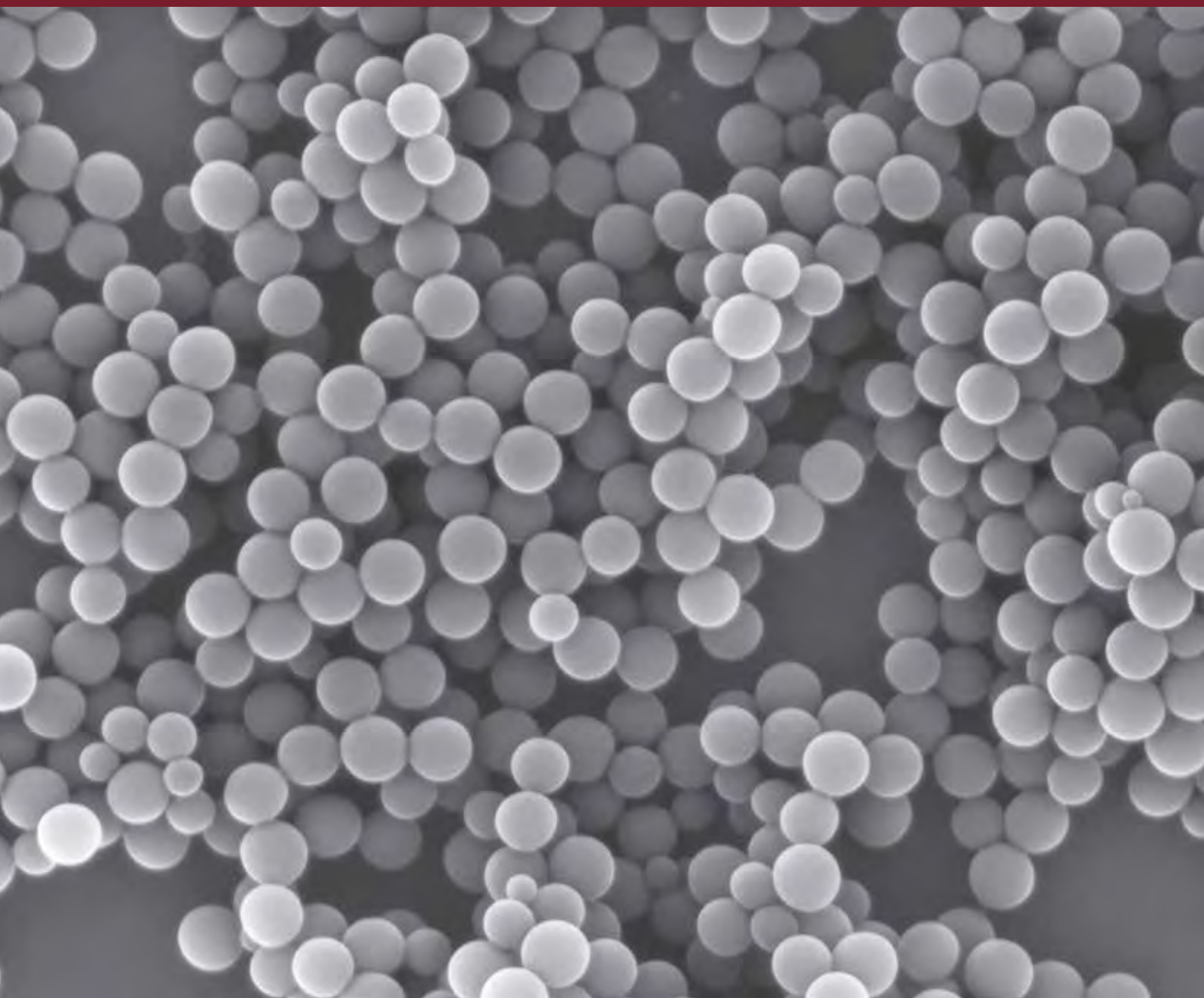
NANOCYTES®
CUSTOMIZED CORE-SHELL PARTICLES FOR CHEMISTRY, MEDICINE, PHARMACY AND THE ENVIRONMENT



NANOCYTES®

CUSTOMIZED CORE-SHELL PARTICLES FOR CHEMISTRY, MEDICINE, PHARMACY AND THE ENVIRONMENT

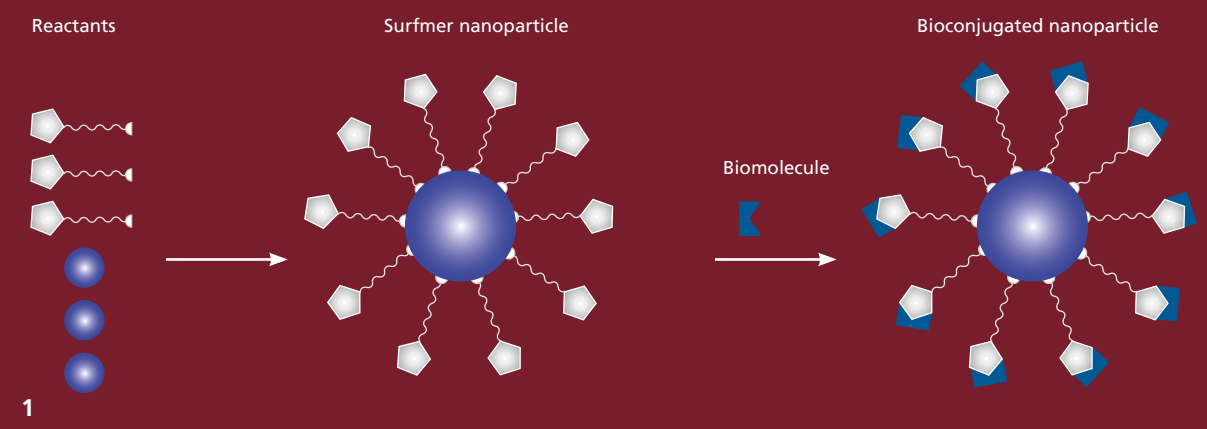
In nanobiotechnology the "biofunctional" surfaces, i.e. those provided with biologically active molecules, have a very special significance. As the "skin" of a material, instrument or piece of equipment they exercise a function in contact with biological environments. For example, they fish certain molecules out of their environment, receive signals or stimulate a reaction. Such biofunctionality is far more than mere compatibility – the surface communicates on a molecular level! The applications of customized core-shell particles – from medical diagnostics by way of therapeutic methods in medicine to the specific removal of individual active substances from the environment – open up new possibilities for society.

Core-shell nanoparticles are composite materials consisting of at least two different components. At the Fraunhofer IGB these nanoparticles with a diameter from as smallest 30 nanometers as well as microparticles up to several 100 micrometers are produced from organic and inorganic materials. The focus is on the design of the surface, for example by binding biological molecules. However, the cores can also be given additional functions.

The Fraunhofer IGB has developed biological-synthetic hybrid particles that simulate the conditions on cell surfaces. On the surface of these cell-mimetic i.e. cell-imitating nanoparticles, membrane proteins are bound in such a way that their biological properties are fully preserved. The basis of these NANOCYTES® is formed by chemically customized nanoparticles that are produced, as required, from silicon oxide and organic materials or from various polymers. The surface of the tiny particles can be modified depending on the application, so that various biomolecules can be coupled to them.

The focal points of our research work are the development of biodegradable and biocompatible nano- and microparticles as well as the production of specific receptor nanoparticles and customized 3D microarrays for research and diagnostics.

1 *Suspension of colored nanoparticles.*



NANOCYTES® PROCESSING – ORGANIC NANOPARTICLE CORES

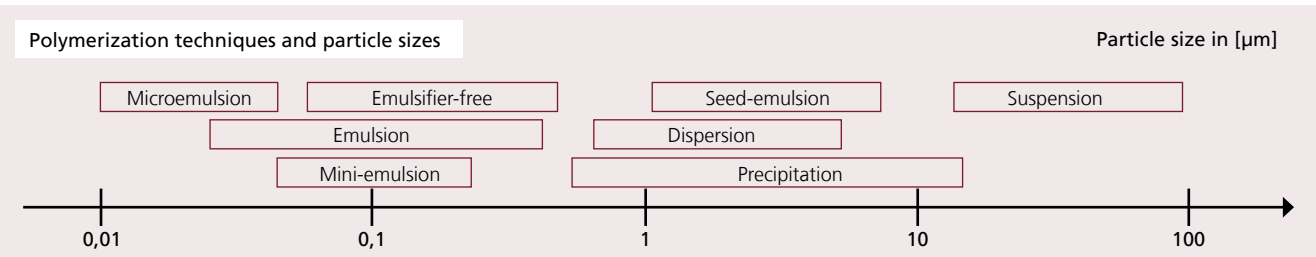
At the Fraunhofer IGB we produce nano- and microparticles – for individual customers – with cores of organic material made from commercially available polymers or customized polymer materials by means of various polymerization techniques such as mini-emulsion or dispersion polymerization. The particle sizes that can be achieved range from a few nanometers up to several 100 micrometers. The diagram below gives an overview of the methods that are available.

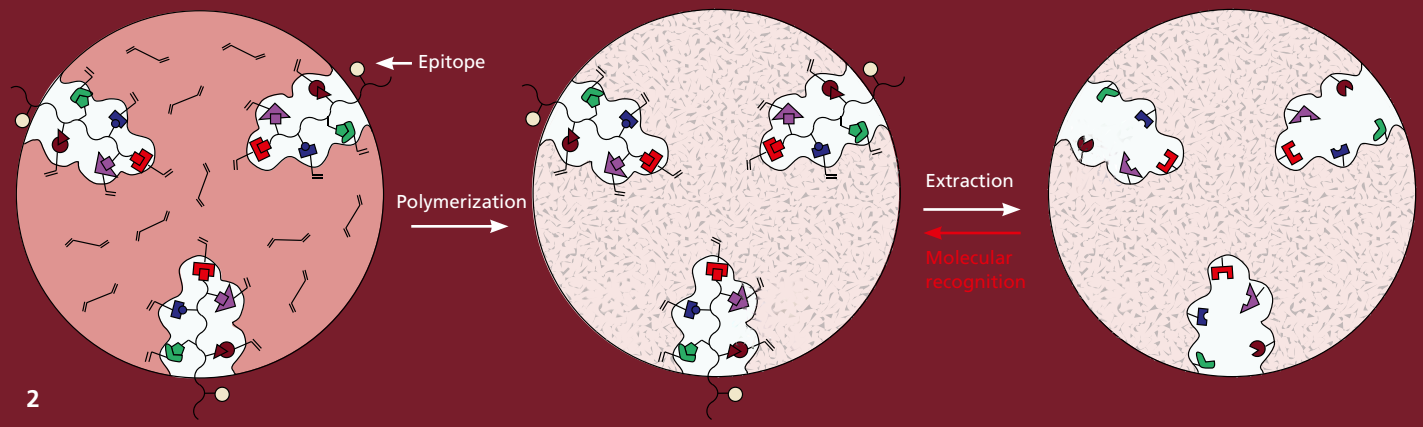
Surfmer nanoparticles

With modularly constructed active ester surfmers (surfmer = surfactant monomer) we make available a molecule class that provides three completely different reactivities in one single molecule. Thus the surfmers combine the function of a nanoparticle-stabilizing emulsifier, the function of polymerizability by means of a radical chain reaction as well as an activated ester group that is stable with regard to polymerization and storage. In conditions that are easy to realize, the activated esters can be used for the further covalent anchoring of components such as biomolecules or for the covalent crosslinking of nanocomposites.

With these surfmers we can selectively produce nanoparticles with controlled adjustable properties in one step by means of emulsion polymerization. Typical particle diameters of co-polystyrene or methyl methacrylate nanoparticles range from 80 to 200 nanometers. The nanoparticles carry a defined number of anchor sites for further chemical functionalizations and implementations, for example for the immobilization of biomolecules.

Our surfmer-based technology permits the one-stage production of nanoparticles with customized anchor sites for biomolecules. In this way the elaborate routine technical production methods used so far can be replaced by several different process steps. The surfmer technology particles also enable the production of beads many times smaller than those that are currently used for the immobilization of biomolecules.





Biodegradable particles

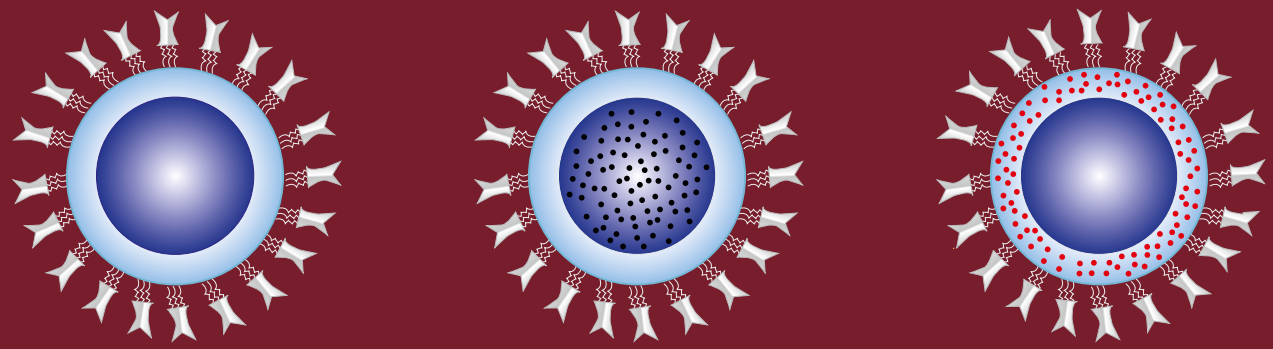
Commercially available biodegradable linear polyesters often yield insufficient properties to bind active substances in such a way that they are suitable for a controlled release. That is why new polymer matrix systems – biodegradable and biocompatible block copolymers – with improved properties and various molecular weights are being developed at the Fraunhofer IGB. By selecting suitable polymer systems, for example modified polylactides, we adapt the nanoparticles to the individual applications in accordance with the customer's wishes and specifications.

Molecularly imprinted nanoparticles

On the principle of molecular imprinting, molecule-specific identification tags are generated by mapping certain structures in the form of a chemical stability negative imprint in synthetic materials. In this way "artificial receptors" become accessible at low cost. However, their chemical stability and also their thermal stability is superior to that of biomolecular receptors such as antibodies, so that they can be used in numerous technical processes.

Molecularly **I**mprinted **P**olymers (**MIP**s) are produced by polymerizing a functional monomer and a crosslinking agent in the presence of a molecule that serves as a template. By means of self-organization the growing polymer framework adapts to the molecular pattern of the template and forms a negative imprint of the template molecule. After the polymerization the template molecule is extracted from the polymer framework. The imprints remaining in the polymer have a stable form because of the high degree of crosslinking of the polymer. As a result of the geometric orientation of the functional groups of the polymer and their interactions with the template molecule (for example via hydrogen bridges), the MIPs can bind the template molecule in a specific way.

1 *Diagram of surfmer particles with binding of biomolecules.*
2 *Diagram of the principle of the molecular imprinting of nanostructured polymers.*



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NANOCYTES® PROCESSING – INORGANIC PARTICLE CORES WITH A FUNCTIONAL SHELL

At the Fraunhofer IGB we also develop nanoparticles with inorganic core material. Metals, metal oxides or ceramic materials can be used for this purpose. The shell, on the other hand, consists of an organic substance and provides a supramolecular environment that is designed especially for the interaction with other organic molecules.

Silica nanoparticles

A widespread method for the production of cores is the use of organic silanes for the synthesis of spherical silica nanoparticles. Using the sol-gel method, silica (SiO_2) particle cores with diameters of 30 nanometers up to 1 micrometer are synthesized at the Fraunhofer IGB. During the synthesis, functional elements such as colorants, fluorescent colorants or polymers such as polyethylene glycol (PEG) can be enclosed in the core or in the core shell. Independently of this, the particle surface is functionalized with organic anchor sites that permit a coupling of further functional or biofunctional building blocks (see the section "Functional Shell"). Unmodified silica cores, or cores with ionizable groups, form suspensions in aqueous media that are stable over long periods of time.

Magnetite nanoparticles

Superparamagnetic nanoparticles with a diameter of less than 15 nanometers are produced by the precipitation of iron salts in a basis environment (hydrophilic/hydrophobic magnetite) and thermal decomposition (hydrophobic magnetite) of organic iron compounds. If the particle surfaces carry carboxy groups, the particles can be easily derivatized and used for biomedical applications.

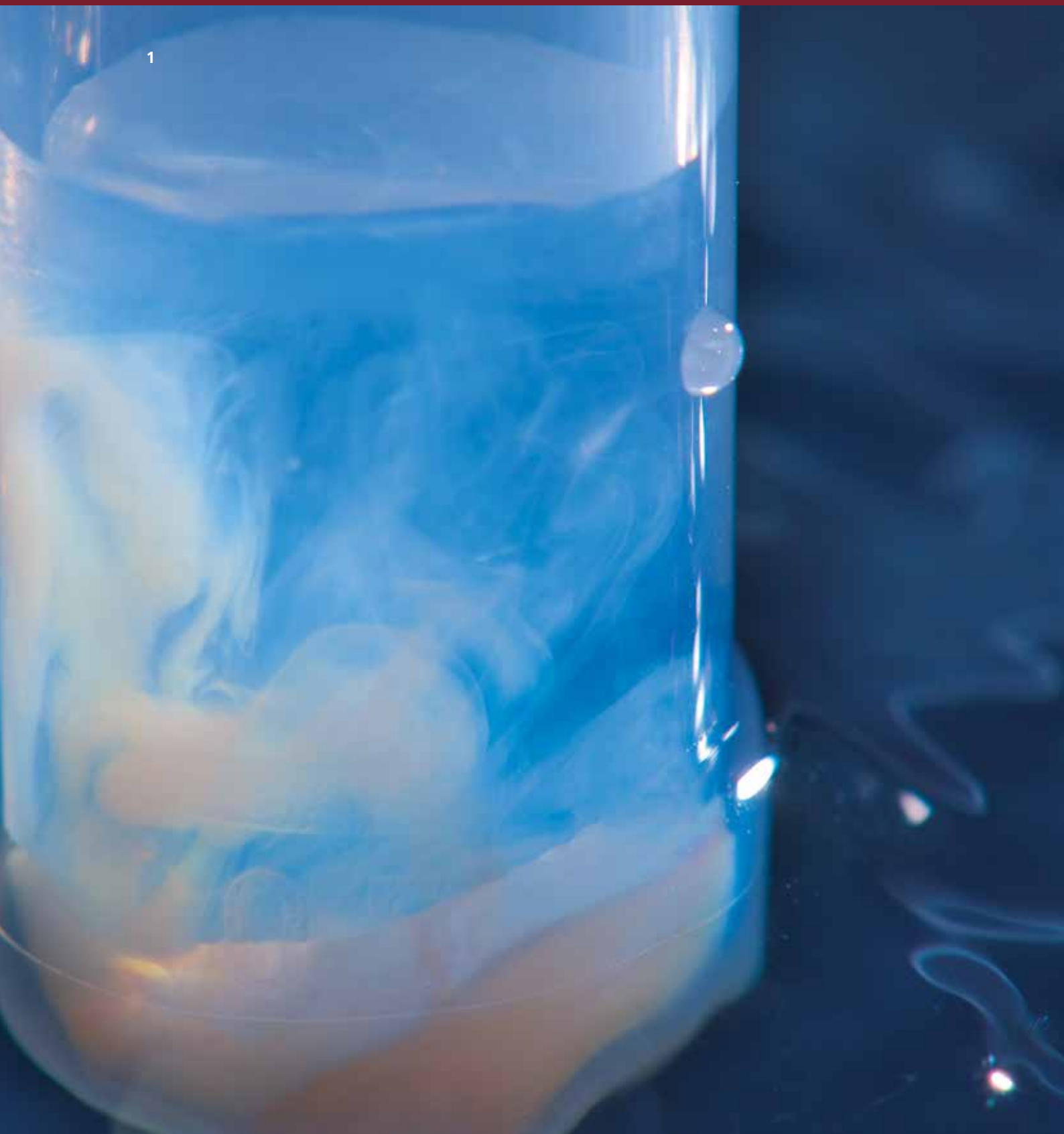
Functional shells

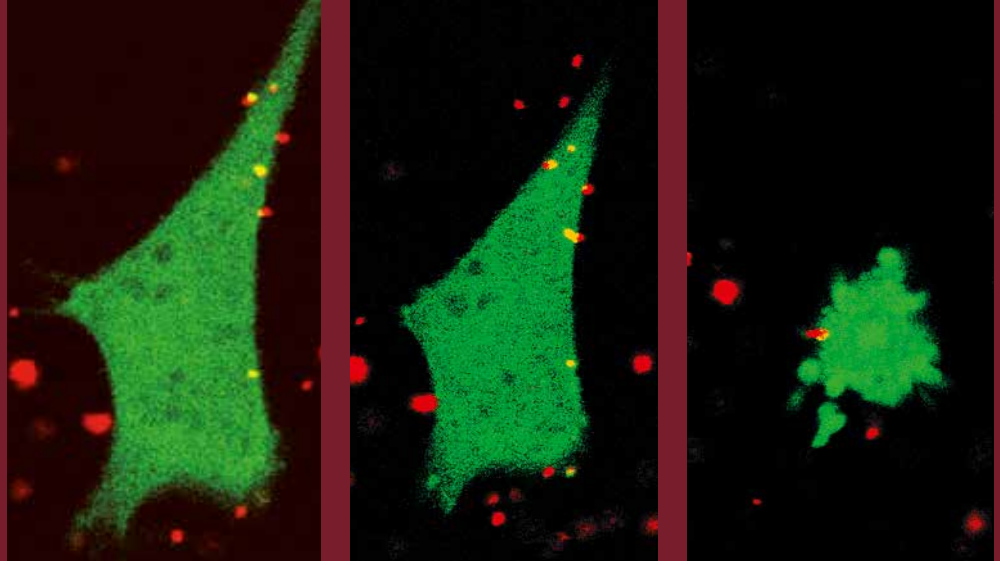
In addition to their functionalization in the core, the inorganic particles are additionally provided with an organic shell that can be customized for the coupling of further functional modifications. As a standard procedure at the Fraunhofer IGB, nanoparticles are produced with hydroxy-, amino-, carboxy-, or epoxy-functional shells. The functionalization with ionizable groups creates electrically charged particle surfaces to which biopolymers such as DNA charged via electrostatic interactions can be bound. Streptavidin particles are used for the coupling of biotinylated ligands. By means of modified linker chemistry reactive particle surfaces can be provided that selectively bind, for example, thiol or amino groups and can thus be used for the targeted binding of proteins. Antibody-functionalized nanoparticles offer a surface for the binding of specific ligands. By immobilizing bioactive proteins, cell-mimetic particles can be generated. These imitate cell-membrane-based ligands and can be used for the selective activation of signal cascades (see NANOCYTES® Applications – Cell-mimetic Nanoparticles").

1 *NANOCYTES®: Particles are provided with functional cores, shells and surfaces.*

2 *Nanoparticles with polymer shell and magnetizable core (magnetite).*


50 nm





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NANOCYTES® APPLICATIONS – CELL-MIMETIC PARTICLES

Multifunctional particles are interesting tools both for fundamental research and also for clinical developments. This is made clear by the example of the TNF-NANOCYTES®: The cytokine **T**umor **N**ecrosis **F**actor α (TNF-α) occurs in nature both in a solution and as a component of the outer membrane of cells. The dissolved form of the cytokine has a different effect from the bound form. Dissolved TNF-α can only activate one of two TNF receptors and thus pass on only a certain signal to the cell, whereas membrane-bound TNF can activate two different receptors. For a long time researchers only had the dissolved molecule available for experiments, so only one signal path could be investigated.

Together with research scientists from the University of Stuttgart (Professor Pfizenmaier and Professor Scheurich, Institute of Cell Biology and Immunology) TNF–α was attached to nano- and microparticles developed at the Fraunhofer IGB especially for this purpose. These so-called cell-mimetic core-shell particles act like cells that carry the cytokine TNF on the outer shell.

In the cell experiment the TNF-NANOCYTES® develop their full efficacy, which is otherwise only shown by the membrane-bound cytokine. This was made verifiable using specially prepared cells that react to the activation of the TNF receptor-2 by programmed cell death (apoptosis) and thus demonstrate the activation (illustration above). After TNF-NANOCYTES® have docked onto the TNF receptors-2, the program for apoptosis is started in the cells. With the help of the TNF-NANOCYTES® both TNF-α-mediated signal paths can now be studied. At the same time, the example of the TNF-NANOCYTES® shows clearly that NANOCYTES® can improve the efficacy of biopharmaceuticals by permitting a formulation that is inspired by nature.

1 *Dissolving a particle pellet.*

2 *TNF-NANOCYTES®: use of cell-mimetic particles (red) for the selective activation of signal cascades in cells (green). The cell death occurs after a short time.*

NANOCYTES® APPLICATIONS – ENZYME IMMOBILIZATION

Enzymes are versatile biocatalysts that are being used to an increasing extent in various branches of industry. However, the technical use of an enzyme is often restricted by insufficient long-term stability in real process conditions and by difficulties with recycling. These weak points can be circumvented by an immobilization. In addition, they offer the possibility of influencing catalytic properties of the enzyme and of avoiding protein contamination in the product.

Our NANOCYTES® technology consists of the coupling of biomolecules such as peptides, antibodies or enzymes to particular systems in the nanometer range. New types of hybrid systems can thus be used for the production of immunotoxin and fluorescent conjugates or as biosensors. Here, the fundamental properties and advantages of the conjugates are based on their small size and the resulting volume/surface effect. For customized applications we develop bioconjugation strategies. By means of customized particle surfaces and the choice of suitable coupling strategies enzymes can be immobilized, at the same time retaining their full activity on particle surfaces.

Amino- and carboxy-functionalized silica particles were coupled in this way, for example, to various oxidoreductases. By means of linker-mediated synthesis techniques, covalent bonds are generated between the particle surface and the enzyme. Molecular spacers can be created selectively by choosing the appropriate linker molecules. Activity and concentration of the enzymes are determined by means of individually coordinated fluorescence arrays.

The customized anchor sites of polymeric active ester-surfmer particles are particularly suitable for binding biomolecules, since N-nucleophile structural units of the enzymes can be bound here in just one process step. The active ester unit as an anchor group offers optimum reactivity with sensitive biomolecules and at the same time guarantees maximum stability during production, storage and transport.

1 *Fluorescence assays to prove the enzyme activity of glucose oxidase on surfmer nanoparticles.*

2 *Image of the particle size distribution of microparticles by means of light microscopy.*

2

NANOCYTES® APPLICATIONS – DRUG DELIVERY AND DRUG TARGETING

A great challenge in the treatment of diseases is the targeting of active substances to the location of the disease i.e. to a tissue or an organ. Membranes are the most important barriers here that screen the location from the drugs that are to be delivered. A further problem is the breaking down or derivatization of free substances in the body. Such metabolization frequently reduces the targeted effect of the drugs at the location. In addition, incorrectly distributed or modified substances in the body can result in undesired side effects. An already tried and tested way of avoiding these disadvantages is the production of particular drug formulations, whereby the substance is bound into a polymer shell or matrix.

Controlled release

As the carrier polymer core-shell nano- and microparticles control the release of substances intended for a certain effect or drugs. The combination of particles with active protein substances makes it possible, for example, to pursue new drug concepts experimentally. Besides the possibility of protecting sensitive drugs from biodegradation, particular carrier systems can also mediate a targeted release of active substances. Biodegradable compounds are of special interest here, as these are completely metabolized or broken down after their use in the body or in the environment.

At the Fraunhofer IGB customized nano- and microparticles are produced from commercially available and also from tailor-made polymers, depending on the problem involved. By varying the molecular weight and the ratio of the hydrophilic and hydrophobic monomer units we can influence – with individual modifications – the kinetics of the release of encapsulated substances. The functional groups from which biodegradable polymers are composed determine the physical and chemical properties as well as the speed of release and breakdown.

Surface modification – efficient drug targeting

The polymer particles can additionally be functionalized on the surface for an effective targeting of drugs to the location in the body. At the Fraunhofer IGB we modify the surface of nanoparticles using well-established coupling methods via free carboxy groups. By means of carbodiimide and via crosslinkers we successfully bind biomolecules, for example antibodies, to the surface without any loss of activity. The resulting unspecific adsorption is very small. In addition to biodegradable nanoparticles we develop biological-synthetic nanoparticles that simulate the conditions on the surface of the cells.

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2

NANOCYTES® APPLICATIONS – BIOCHIPS

Biochips, or also microarrays, are highly valued tools in the life sciences laboratories. These permit insights into the interaction of substances in the smallest spaces and with a minimum need for samples. A microarray consists of a large number of catcher molecules that – immobilized in the form of tiny dots – bind and make verifiable target molecules from a complex specimen by means of molecular identification. The appropriate chip surface is of decisive importance for the sensitivity and specificity of a biochip.

Multilayer systems consisting of nanoparticles are suitable as a surface for highly complex DNA and protein microarrays. They form three-dimensional reaction spaces and offer a many times greater surface for the binding of catcher and target molecules.

The Fraunhofer IGB develops customized NANOCYTES® surfaces for DNA and protein microarrays as commissioned by the customer. Compared to commercially available amino-functionalized glass substrates, DNA microarrays achieve three times higher fluorescent signals and an improved signal-to-noise ratio on amino-functionalized NANOCYTES® substrates.

NANOCYTES®-based protein microarrays have increased signal intensities, the amplification factor depends on the quality of the catcher molecule used.

For the production of NANOCYTES®-based biochips nanoparticle surfaces are provided with organic functions or catcher proteins and applied to carrier materials such as glass, silica or polymer foils either over an area or in the form of microdots. The application over an area is carried out automatically by means of piezo-inkjet print technology or by layer-by-layer coating in which polymers with varying charges are applied in layers in thickness of a few nanometers. The nanoparticle microdots are created with the assistance of standard microarrays. This provides the greatest possible flexibility for the production of three-dimensional microarrays in the microfluidic format and in the standard format.

3

NANOCYTES® APPLICATIONS – SYNTHETIC RECEPTORS

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 substances that are also present. Nanostructured molecularly imprinted polymers (NanoMIPs) act as artificial receptors and, as adsorbers, are outstandingly suited to solving these problems. In accordance with the key-lock principle the nanoparticles specifically recognize biomolecules and active substances such as amino acids, peptides and proteins, and also low-molecular compounds or troublesome substances such as toxins and substances with an endocrinal effect.

Separation technology

If the NanoMIPs are used as a polymer particle suspension, they can be given a magnetizable core of magnetite to make separation easier. This permits a quick and easy separation by means of a magnetic separator. A further possibility of using NanoMIPs as a separation tool is to bind the NanoMIPs as a selective element between two membranes. This results in a so-called sandwich composite membrane (Fig. 3) that consists of an overlay and a covering membrane and the selective centerpiece, the layer of molecularly imprinted polymer nanoparticles. NanoMIPs can also be bound directly in polymer membranes as a selective element. In this case they are added directly to the polymer solution during the membrane production by means of phase inversion technology. The polymer solution, which forms the later membrane structure and contains the molecularly imprinted particles, is subsequently cast into the desired form.

Sensor technology

Molecularly imprinted polymers are very well suited for use as an active element in sensor technology. Because of their robustness they can also be employed where the use of biosensors is not possible because of unfavorable conditions (extreme pH values or high temperatures). Chemosensors on the basis of molecularly imprinted polymers are predestined for use in online analytics and can also be produced inexpensively. MIP sensors can be employed for monitoring critical value concentrations in safety technology, in environmental analyses and in process analytics for monitoring chemical reactions.

1 *Surface functionalization of chip surfaces.*
2 *NANOCYTE®-based protein microarray.*
3 *Molecularly imprinted composite membrane.*

OVERVIEW OF SERVICES

On a commission basis or in cooperative research we make available to our customers NANOCYTES® particles that are customized in size and function. These modularly constructed core-shell particles are – like a very variable building block system – suitable for an extremely wide range of applications in research, diagnostics and future therapy as well as in chemistry and the environment.

- Organic and inorganic particle systems
- Development of biocompatible particles
- Development of biodegradable particles
- Loading and encapsulating of particles
- Production of nanoparticles with reactive active ester surface
- Development and synthesis of molecularly imprinted nanoparticles
- Surface coating of micro- and nanoparticles
- Bioconjugation of biomolecules
- Development of hybrid materials
- Formulations of drug matrix systems
- Formulation of special inks for inkjet printing
- Biological analyses and analytics
- Carrying out feasibility studies

Equipment

Biological and polymer analytics

- MALDI-TOF/TOF mass spectrometer (Bruker Ultraflex II)
- Thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC)
- Simultaneous thermoanalysis (STA)
- Gel permeation chromatography (GPC) with 4 detectors

Particle analysis

- Microelectrophoresis (zeta potential)
- Dynamic light scattering (SLS, mastersizer, measuring range 0.1 nm to 10 µm)
- Static light scattering (SLS, mastersizer, measuring range 50 nm to 2 mm)

Surface characteristics and morphology

- Ellipsometry
- Microscopy, scanning electron microscope (SEM), atomic force microscopy (AFM)
- Electron spectroscopy for chemical analysis (ESCA)

Loading of particles

- 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 Institute for Interfacial Engineering and Biotechnology IGB develops and optimizes processes and products in the fields of health, chemistry and process industry, as well as environment and energy. We combine the highest scientific standards with professional know-how in our competence areas – always with a view to economic efficiency and sustainability. Our strengths are offering complete solutions from the laboratory to the pilot scale. Customers also benefit from the cooperation between our five R&D departments in Stuttgart and the institute branches located in Leuna and Straubing. The constructive interplay of the various disciplines at our institute opens up new approaches in areas such as medical engineering, nanotechnology, industrial biotechnology, and environmental technology. Fraunhofer IGB is one of 69 institutes and independent research units of the Fraunhofer-Gesellschaft, Europe's leading organization for applied research.

www.igb.fraunhofer.de

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