From ideas to innovative Biotech Products

Bio-Engineered Materials
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The RFA “Health and Performance” consists of 5 Research Modules one of which is “Bio-Engineered Materials”.

Biotechnology plays an important role in research on materials in the context of life sciences. It allows the synthesis, optimization and functionalization of (macro) molecules that can be used to solve health and performance related problems. Empa uses this technology to develop new biocatalysts for sustainable chemical processes. Microbiological and (bio)chemical procedures are applied to synthesize bio-based and bio-degradable polymers such as polyhydroxyalkanoate (PHA) bio-polyesters. Biopolymers are further modified and functionalized, or are blended with suitable additives and processed using various techniques to enable novel applications through “green” materials.

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Biopolymers

- We apply the principles of modern macromolecular engineering to the preparation of biocompatible and biodegradable polymer-based materials with well-defined 2D and 3D arrangements for custom-made applications.

- By precisely controlling the polymers’ average molecular weights, molecular weight distribution, functionality, composition, and topology, we are able to finely tune the properties of the final material and thus to meet the specific needs of our academic and industrial research partners.

- Molecules from renewable resources are employed whenever possible. These include: enzymes as catalysts; plant oils, amino acid and sugar derivatives as monomers; water as solvent; biological polymers such as PHAs, proteins, etc. as building blocks; hydroxyapatite, cellulose etc. as fillers.

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Isolated and purified PHAs

Biodegradable flipflops
Biopolymers - Polymer therapeutics

We design and prepare new macromolecular architectures functioning as inert drug delivery systems or as bioactive pharmaceuticals in their own right.

Polymer matrices (hydrogels, fibers, foams etc.), with or without the ability to release bioactive molecules, polymer–drug and polymer–protein conjugates, polymer complexes (polyplexes) for DNA/RNA delivery, and polymer assemblies (micelles, vesicles, etc.) as encapsulating agents are typical examples of the first class of compounds.

Polymer sequestrants to remove ions, toxins, etc. from the body, polymers with hemostatic properties, as well as polymers acting as multivalent ligands for activating given cellular responses or inhibiting the binding of pathogens are examples of the second class of compounds.
Examples of polymer delivery systems for therapeutic applications

- **Red circles** = drug
- **Blue circles** = tumor-targeting moiety

- **Dendritic polymer** (1-15 nm)
- **Polymer-drug conjugates** (5-15 nm)
- **Polymer-protein conjugates** (10-20 nm)
- **Polymer-DNA complexes** (20-40 nm)
- **Polymer micelles** (20-150 nm)
- **Nanogels** (20-200 nm)
In addition to drugs, micro- and nano-sized polymer assemblies can be used as convenient encapsulating agents for other active ingredients such as agrochemicals, food additives, flavors and fragrances. Our research moves also in this direction. The key advantages that these polymeric systems offer include: (i) sustained delivery, i.e., release over extended periods of time; (ii) shielding of sensitive ingredients from the surrounding environment; (iii) isolation of the unpleasant taste or odor of some additives; and (iv) protection of particularly volatile compounds.

Many drugs, agrochemicals, food additives, flavors and fragrances share two important features: (i) they are “small”, (usually) hydrophobic organic molecules; and (ii) they come into contact with living organisms and the environment. Hence, several delivery systems originally designed for drugs can be adapted to encapsulate and release, in a controlled way, these other molecules as well.
Agrochemicals

Microcapsules
**Biopolymers - Bionanocomposites**

We make use of nano-sized fillers based on montmorillonite and hydroxyapatite (inorganic), cellulose (organic) or polymer particles (organic/inorganic) to improve the chemical (resistance, degradability, etc.) and/or the physical (mechanical, thermal, barrier, etc.) properties of biopolymers. To this end, not only we use the fillers as received by the providers but also we modify their surface via polymer grafting in order to (i) improve the interaction with the matrix, (ii) optimize the dispersion within the matrix, and (iii) minimize their aggregation.

The processing tools we employ include:

- 16 mm co-rotating twin screw extruder (0.1-1 kg/h) with volumetric feeder;
- Several square dies (0.5-2mm x 20 mm); 3 mm strand die;
- Gravimetric feeder (5-50 g/h);
- Compression molder;
- Roll mill
Layered nanocomposites
Microbial Engineering

Microbial engineering uses the capabilities of microorganisms to produce useful biological materials, especially, biopolymers and recombinant proteins. We are active in:

- biosynthesis and downstream processing of proteins, biomolecules and biopolymers;
- applying different culture technologies and efficient product recovery steps to generate safe and reproducible materials at reasonable cost;
- investigation of interactions between microbes and material surfaces.

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- Genetic engineering
- Fermentation
- Downstream processing

Design optimization

- Increase yield
- Increase productivity
- Increase reproducibility
- Reduce cost
- Simplify operation
- Eco-concerns
Microbial Engineering Biopolymer production

Polyhydroxyalkanoates (PHAs) are produced by bacteria and featured with biodegradability and biocompatibility. PHAs are difficult to produce using synthetic chemistry due to their high molecular weight and/or chirality. We focus on:

- production of tailor-made PHAs with or without functional side-chains;
- synthesis of PHAs from cheap carbon sources such as xylose, a major waste product of the paper industry;
- manufacturing PHAs as coatings of metallic bone implants and other medical devices, and as components of artificial tendon;
- production of enantiomerically pure 3-hydroxycarboxylic acids from PHA

We attempt to develop a biotechnological approach for PHA production which offers sustainability from both environmental and economic aspects.
Conversion of polymer to monomers

PHA producing bacteria
Microbial Engineering Recombinant protein production

Recombinant protein production in *E. coli* tends to be more robust, faster and cheaper than in mammalian systems. In our laboratory, we work on:

- production of glycosylated proteins in recombinant *E. coli* at a larger scale. By process optimization, volumetric glycoconjugate yield in bioreactors could be increased 50-fold compared to shake flasks;

- production of a novel tyrosinase. By process optimization, the volumetric yield of tyrosinase in bioreactors was increased 16-fold compared to shake flasks.
Parallel bioreactor system

Overproduced green fluorescent protein
Microbial adhesion and growth on material surfaces have serious economic, environmental and health implications such as biofouling, biocorrosion and infections. We investigate:

- **Textile**: antimicrobial / anti-adhesion effects of textile coatings;
- **Medicals**: antifouling activity of silver coated materials, antibacterial effect of new products in wound management, cleaning efficiency of new formulations of detergents;
- **Household**: test systems to measure biofilm removal in washing machines;
- **Environment**: waste water analyses, antibacterial coatings in the area of plant protection.
Biofilm stained with Concavalin A

Classical antimicrobial test

Biofilm formation in the bioreactor
Biocatalysis

- is a green chemical technology;
- involves the use of enzymes for chemical reactions in diverse synthetic and technological applications;
- works at moderate reaction conditions, and enzymes can be optimized in the laboratory for specific processes by enzyme engineering.

Future trends in enzyme technology target the functionalization of natural and synthetic (bio)polymers, waste water purification and the fabrication of hybrid materials for energy production.
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Biocatalysis Laccases

- are multi-copper oxidases that catalyse the one electron oxidation of a broad range of compounds including substituted phenols, arylamines and aromatic thiols;
- enable controlled, selective and catalytic oxidation reactions;
- are well-suited for technical processes due to their broad substrate range, high specific activity, stability and due to fact that they only need oxygen as co-substrate

Potential applications include organic synthesis, bleaching of textiles and paper or production of wood compound materials.

At Empa we develop novel bacterial laccases for industrial applications using technologies such as directed evolution.
Model of a bacterial laccase active site  
Purified Laccase  
Screening patterns for functional laccases
**Biocatalysis Directed evolution**

Enzymes can be optimized in the laboratory to fulfill specific technical needs such as thermostability, solvent tolerance and high activity. Therefore, the principles of biological evolution are applied to specific target genes. State-of-the art molecular biology tools are used to generate genetic diversity and high throughput screening methods for the selection of improved variants. Empa contributes to projects with industrial partners by:

- developing novel screening methods for enzyme activity in 96-well format;
- designing and constructing random and semi-random mutant gene libraries;
- discovering glycosyl transferase and oxidoreductase variants with increased activity.
improved enzyme variant library

mutagenesis

enzyme

improved enzyme

variant library

screening
Principle of directed evolution (left) and 96-well micro plate for screening of enzyme variants (right)
Biocatalysis Tyrosinases

Tyrosinases are copper containing oxidases that convert phenolic substrates. There are many applications for this class of enzymes in the fields of biocatalysis, biomaterials and biosensors, including:

- the degradation of phenolic waste products;
- bio-sensing of phenolic compounds;
- covalent cross-linking of tyrosyl side chains in polypeptides with nucleophilic groups in polypeptides or other polymers, for example with amino groups in chitosan;
- functionalization of polymeric materials or the gentle formation of stable, active enzyme aggregates.
Model of a bacterial tyrosinase and mechanism of tyrosinase catalyzed protein crosslinking
**Biocatalysis Biosensor Fabrication**

Certain oxidoreductases can be used for the development of novel biosensors. In electrochemical biosensors these enzymes are immobilized by different means onto conducting surfaces. The specific reaction with the molecule of interest consumes or releases electrons directly or indirectly, causing an electrical signal. This allows the selective, qualitative or quantitative detection of specific chemicals in a mixture of substances.

We are currently developing and engineering various oxidoreductases (e.g. P450 enzymes) for the specific detection of natural metabolites for the use in microfabricated sensor devices.
Architecture of a microfabricated electrochemical biosensor
**Biocatalysis** Enzymatic surface functionalization

Enzymes such as laccase or tyrosinase can be used for surface functionalization of inorganic and organic (bio) materials by

- modifying surface exposed functional groups, e.g. for increased hydrophilicity;
- activating and coupling small molecules to the surface e.g. for antimicrobial effects;
- *in situ* polymerizing and crosslinking biopolymers for biocompatible coatings;
- site-specific cross-linking of proteins, peptides and biopolymers to surfaces for obtaining novel bio-hybrid materials.
Lyophilized bacterial laccase

Principle of laccase-catalysed iodination for the protection of wood against microorganisms
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Our aim is to fabricate functional protein-modified surfaces.

- By modifying surfaces with proteins and peptides we produce novel hybrid materials with unique properties that are useful for a wide range of applications, ranging from organic synthesis over biosensing to biomimetic photoelectrochemical cells.

- We employ various chemical and enzymatic immobilization techniques to anchor the selected target molecules on the surface.

**Biocatalysis Protein Immobilization**
Protein immobilization techniques

Green fluorescent protein immobilized on polystyrene beads by sortase
**Biocatalysis Biohybrid Materials**

Enzymatic protein immobilization can be used in combination with organic polymers resulting in semiconductor surfaces functionalized in a well ordered manner. In this way the performance and stability of solar cells for electrochemical water splitting using Solar light can be optimized in environmentally friendly conditions.
Self-organized protein-biopolymer fractal structure on a photo anode
Improved acoustic properties for violins

The method allows improving the acoustic properties of resonance wood in times where it is becoming increasingly difficult to find superior resonance wood due to the impact of global warming. The main objective of this interdisciplinary project is the successful up scaling of our biotechnological process of fungal modification by defining standardized conditions to achieve reproducible results, and by better defining the notion of “resonance wood quality”. If the outcome of the project is successful, in the future, every talented musician will be able to afford a violin with the same tonal quality as an expensive Stradivarius.
Fungal treated Opus 58 at a trade exhibition in Basle
Aspiration of bordered pits in Norway spruce wood is the main reason why the treatability with preservatives is greatly inhibited for increasing the durability against micro-organisms. If this restriction could be overcome wood could be treated more effectively. For this purpose Norway spruce wood is exposed to the white-rot fungus *Physisporinus vitreus* that selectively degrades the pit membranes, without altering the mechanical properties of wood. At present we are undertaking studies to improve the permeability of water-borne wood preservatives or hydrophobic substances applied by brushing, dipping and impregnation.
Heartwood specimens of Norway spruce (top) and White Fir (bottom) impregnated with a 0.1 % aqueous solution of the dye Neolan Glaucin E-A. A significant increase in the uptake of the dye (in kg/m³) is apparent in fungal treated wood specimens.