CELL-FREE BIOCATALYTIC MODULES FOR BIOTRANSFORMATION OF ORGANIC WASTE

Solid-binding peptides (SBPs) are short amino acid sequences that act as molecular linkers to direct the orientated immobilization of biomolecules onto solid matrices. Silica-based materials are suitable matrices for enzyme immobilization in industrial processes. We have exploited the property a SBP that binds to materials that contain silica and have constructed a library of functional fusion proteins displaying binding affinity to this material whilst retaining high levels of enzyme activity.

Cell-free biocatalysis offers a versatile platform for the biomanufacturing of bulk or specialty chemicals due to the flexibility in assembling enzymes from different organisms in synthetic reaction pathways. Current challenges of this approach include costly enzyme preparation, low enzyme stability and efficient enzyme recycling. To overcome these challenges, we implement a molecular toolbox that facilitates the construction of biocatalytic modules with predefined functions and catalytic properties. The toolbox is comprised of three interchangeable building blocks: (i) low-cost inorganic matrices (e.g., silica, zeolite), (ii) matrix-specific SBPs and (iii) thermostable enzymes. The rational combination of these building blocks allows for flexibility and a ‘pick and mix’ and “re-use” approach with multiple biocatalytic modules available for the assembly of natural and non-natural pathways.

Individual immobilized enzymes can be rationally combined to assemble recyclable and product-specific reactions.

DEVELOPMENT OF PROTEIN-BASED NANOCOMPARTMENTS FOR DRUG DELIVERY

Compartmentalisation is an important organisational feature of life that allows otherwise incompatible biochemical processes to function cohesively within a cell. It occurs at varying levels of complexity, from eukaryotic organelles and bacterial microcompartments, to viral capsids and even the molecular reaction chambers formed by enzyme assemblies. Encapsulins are a newly reported class of protein-based nanocompartments produced in bacteria and archaea. They are typically composed of multiple copies of a single protein subunit, which self-assemble with precision to form hollow cage-like nanostructures that are uniform in composition, size and morphology.

Encapsulins have been recently used to encapsulate foreign cargo, such as recombinant proteins and inorganics. In addition, the external and internal surfaces of these nanocompartments can be easily genetically engineered to display short peptide sequences that can further enhance their functionality. Accordingly, encapsulins represent a promising alternative to the lipids,
polymer, and inorganic-based compartments that are currently used as vehicles for the encapsulation and targeted delivery of therapeutics. We apply synthetic biological techniques to modify encapsulins that can be loaded with a drug and then upon reaching their biological target be activated to disassemble and release the drug, thus providing both spatial and temporal control of drug delivery in vivo.

**PRODUCTION OF BIOACTIVE COMPOUNDS FROM PARAMYLON**

Paramylon is the storage polysaccharide of the flagellated protist *Euglena gracilis*. It is a high molecular weight polymer consisting of β-1,3-glucans units, deposited as granules in the cytoplasm of *E. gracilis*. β-1,3-glucans were reported to have bioactivity in mammals, like anticholesterol, immunostimulating, antiinflammatory, antimicrobial, antitumor, hepatoprotective, antidiabetic and antihypoglycemic activities, making them ideal candidates for nutraceuticals. It is assumed that large β-1,3-glucans first must be broken down to become bioactive. Even though the structure of paramylon is not complex, little is known about the mechanism of its degradation via enzymatic hydrolysis. So far, just one enzyme from *E. gracilis* is known to degrade paramylon to a certain degree. The fact that paramylon needs to be pretreated in vitro for an effective hydrolysis most probably indicates the involvement of more than one enzyme. This study aims to elucidate the enzymatic degradation pathways of paramylon to facilitate the biotechnological production of bioactive health-enhancing compounds for potential industrial applications.

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