**Nano-scale enzyme membrane reactors for compartmentalized multienzyme syntheses**

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**Highlights**

* Vesicles made from amphiphilic block-copolymers were used as reaction compartments.
* Inserting porins into the polymer membrane created nano-scale enzyme membrane reactors.
* Enzymes in the vesicle lumen were spatially separated from inhibitors in the bulk phase.
* New strategies to augment the catalyst loading of the vesicles are under investigation.

**1. Introduction**

When looking for examples of complex biocatalytic systems, one has not to look far. Already the estimated 3.72\*10^13 cells of a human [1] depict a perfect example in which nature designed an astonishing biocatalyst network. In natural cells, a vast array of chemical reactions takes place simultaneously. Two fundamental principles allow these reactions to proceed efficiently: compartmentalization and selective mass transport. By spatial separation of enzymes and control over the reacting compounds, highly productive reaction cascades can be established.

Figure 1: Nano-scale enzyme membrane reactor

To make use of these principles in bioprocess engineering, nano-compartments made from self-assembling, amphiphilic block-copolymers can be used to mimic these principles of cells and to improve biocatalytic reactions. By entrapping enzymes in vesicles with a selectively permeable membrane, nano-scale enzyme membrane reactors (nano-EMRs) can be created (Figure 1). The spatial separation of incompatible reactions can lead to more efficient one-pot multi-enzyme syntheses by avoiding cross-inhibitions and undesired side-reactions.

**2. Methods**

The triblock-copolymer poly(2-methyloxazoline)15-*b*-poly(dimethylsiloxane)68-*b*-poly(2-methyl-oxazoline)15 (PMOXA-PDMS-PMOXA) is ideally suited for the formation of selectively permeable nano-EMRs because natural (or engineered) channel proteins can be embedded in functional form into this polymer membrane. The polymer vesicles are formed by injecting a 20 % (w/v) polymer solution in ethanol into a stirred tank reactor containing an aqueous phase [2]. Upon vigorous stirring for 1- 3 hours (depending on the composition of the aqueous phase), vesicles with a narrow size distribution (polydispersity index < 0.25) are obtained.

To turn the vesicles into nano-EMRs, enzymes are encapsulated during vesicle formation. Additional enzymes can be immobilized on the surface of pre-formed vesicles by using genetically modified enzymes [3]. By equipping them with membrane anchoring domains, the enzymes of interest can associate with the polymer membrane. In the process of nano-EMR assembly, this is achieved by simply combining the different components. Enzymes with membrane anchoring domains and channel proteins spontaneously integrate into the nano-EMR membrane as the integration minimizes hydrophobic-hydrophilic interactions with the surrounding aqueous phase.

**3. Results and discussion**

Using the three-step enzymatic synthesis of CMP-*N*-acetylneuraminic acid as an example, it was demonstrated that the nano-EMR technology can be applied to avoid cross-inhibitions mediated by low molecular mass compounds in cascade reactions. This synthesis suffers from a strong inhibition of the first enzyme in the cascade, the *N*-acylglucosamine 2-epimerase (AGE), by CTP, which is a substrate of the third enzyme, the CMP-sialic acid synthetase (CSS). The implementation of a highly specific mass transport over the compartment boundaries was demonstrated by using the engineered channel protein OmpF G119D. This porin was the key element for the spatial separation of two incompatible reactions since it allows for the free diffusion of the substrates of the AGE, but fully excludes the larger substrates of the CSS thereby abolishing their inhibitory effects. It was shown that the compartmentalization of the enzymes enables synthesis of CMP-*N*-acetylneuraminic acid, whereas no product was formed with the free enzymes [4].

One of the major limitations of the nano-EMR technology is the low biocatalyst loading of the vesicles due to the statistical encapsulation of the enzymes during the nano-EMR formation. Thus, several strategies to augment the biocatalyst loading are currently under investigation.

**4. Conclusions**

The nano-EMRs are new tools to tackle the incompatibility challenge in enzymatic cascade reactions. Further developments of this exciting technology are needed to improve their operational performance, e.g. by increasing the enzyme concentration in the vesicle lumen.

**References**

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