# Polymersome nanoreactors as artificial organelles

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Compartmentalisation is one of the techniques that cells adopt to enable a high level of control over (bio-)chemical processes, for instance the order in which enzymes react. In many cases, the compartment also serves to protect the cell from the action of its degrading contents, as is the case with lysosomes. It furthermore serves as a scaffold for the precise positional assembly of enzymes that work together in a multistep cascade reaction.

In an effort to mimic these complex enzyme systems, many studies concerning enzyme encapsulation or assembly have been reported in the literature. The focus of this research initially was on phospholipid liposomes. However, the relative fragility of liposomes limits their potential applicability. Like liposomes, polymersomes are spherical aggregates that contain a bilayer architecture. They are formed by the self-assembly of amphiphilic block copolymers in an aqueous environment and their polymeric bilayer shows a greater stability, mainly due to the lower critical aggregation concentration of amphiphilic macromolecules. A drawback of polymersome membranes is their low permeability even to water, which hampers application as nanoreactors.

To overcome this problem, block copolymers have to be used that give an intrinsically porous bilayer when self assembled. One such copolymer is PS-PIAT. On dispersal in water it forms porous polymersomes that possess a relatively high degree of Small molecules can move across their membranes while larger molecules, such as proteins, cannot.

In a first line of research, we have positioned enzymes at three different locations on these polymersomes, namely, in their lumen (glucose oxidase, GOx), in their bilayer membrane (Candida antarctica lipase B, CalB) and on their surface (horseradish peroxidase, HRP, see figure 1). The surface coupling was achieved by ‘click’ chemistry between acetylene-functionalised anchors on the surface of the polymersomes and azido functions of HRP, which were introduced by using a direct diazo transfer reaction to lysine residues of the enzyme [1].

To determine the encapsulation and conjugation efficiency of the enzymes, they were decorated with metal-ion labels and analysed by mass spectrometry. This revealed an almost quantitative immobilisation efficiency of HRP on the surface of the polymersomes and a more than statistical incorporation efficiency for CalB in the membrane and for GOx in the aqueous compartment. The enzyme-decorated polymersomes were studied as nanoreactors in which glucose acetate was converted by CalB to glucose, which was oxidised by GOx to gluconolactone in a second step. The hydrogen peroxide produced was used by HRP to ABTS to $ABTS^{\cdot+}$ (figure 2). Kinetic analysis revealed that the reaction step catalysed by HRP is the fastest in the cascade reaction.

In a second line of research, artificial organelles were created. For this purpose we modified the outer surface of the polymersome nanoreactors with cell-penetrating peptides, in particular the tat sequence. As a result, the polymersomes obtained the property to enter cells. Enzymes which were encapsulated in the polymersomes could be transported into mammalian cells and perform their biological activity in a living system. This was demonstrated via the introduction of HRP, which could neutralize hydrogen peroxide and therefore protect the cell against oxidative stress (figure 3) [2].

## References

[1] S. F. M. van Dongen, M. Nallani, J.J. L. M. Cornelissen, R. J. M. Nolte, J. C. M. van Hest Chemistry - a European Journal **15** (2009) 1107-1114.

[2] S. F. M. van Dongen, W. P. Verdurmen, R. J.R.W. Peters, R. J.M. Nolte, R. Brock, and J. C.M. van Hest. Angew. Chem. Int. Ed Engl **49** (2010) 7213-16

## Figures

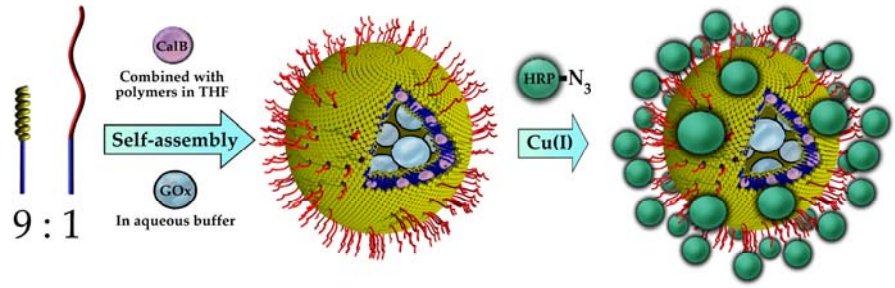


***Figure 1.*** *Positional assembly of enzymes in a polymersome.*

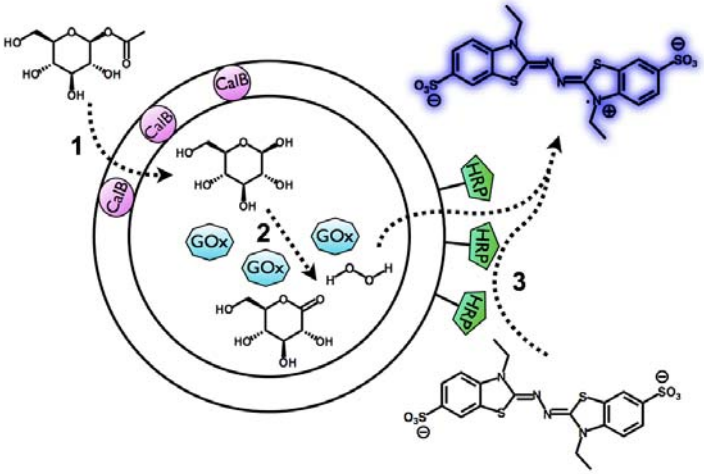


***Figure 2.*** *Schematic representation of the multistep reaction. 1) Monoacetylated Glucose is deprotected by CalB, which is embedded in the polymersome membrane. 2) In the inner aqueous compartment, GOx oxidises glucose to gluconolactone, providing a molecule of hydrogen peroxide. 3) Hydrogen peroxide is used by HRP to convert ABTS to ABTS$^{+}$. HRP is tethered to the polymersome surface.*

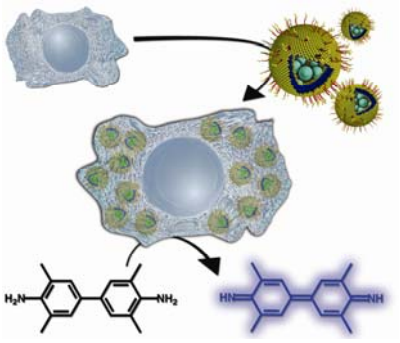

***Figure 3.*** *Schematic representation of the introduction of polymersomes into cells. Polymersomes filled with HRP and functionalized on the periphery with cell penetrating peptides are taken up by mammalian cells. They display their activity as artificial organelles by the oxidation of TMB with hydrogen peroxide*