Protein-polymer nanoreactors and processors act as artificial organelles

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Abstract

The combination of biological molecules and synthetic polymer carriers/templates represents a very promising approach for development of efficacious therapies with minimum side effects, diagnostic methods featuring significantly higher sensitivity and selectivity, and personalized diagnostics and therapeutics via theragnostic approaches. In this respect, suitable amphiphilic block copolymers self-assemble into in aqueous media into vesicles with membranes mimicking biological membranes. The properties of such vesicles can be extensively controlled via chemical composition, molecular weight and the hydrophilic-to-hydrophobic block length ratio of the polymers, and have the advantage of superior stability and robustness. The combination with suitable biological molecules (proteins, enzymes, DNA, peptides) introduces other well-defined functions, such as molecular recognition, cooperation, and catalytic activity.

We exploited the concept of bio-synthetic combination to develop antioxidant nanoreactors that encapsulated superoxide dismutase/mimics in the aqueous cavities of vesicles generated by the self-assembly of poly(2-methyloxazoline)-b-poly(dimethylsiloxane)-poly(2-methyloxazoline), PMOXA-PDMS-PMOXA copolymers.[1,2] By synthesizing appropriately functionalized polymers (e.g. biotin, antibody) we successfully immobilized the nanoreactors on solid support to follow the folding/unfolding of single proteins, and to monitor enzymatic reactions down to the scale of a few molecules.[3] A step further in obtaining multifunctionaliy, is to co-encapsulate enzymes that act in tandem inside the polymer cavity: cascade reactions can therefore take place *in situ*.[4]

Here we present antioxidant processors designed by simultaneous co-encapsulation of enzymes and channel proteins (Figure).[5] Cascade reaction of co-encapsulated superoxide dismutase and lactoperoxidase allowed for a complete detoxification of superoxide radicals and related H_2O_2. The polymer membrane was selectively controlled by insertion of channel proteins, which allowed the exchange of substrates and products with the environment, supporting the *in situ* activity of the enzymes. In addition, the detection of superoxide radicals and related H_2O_2 was based on a fluorescent product of the second enzyme that strongly favored a dual application of the processor: in biosensing and detoxification of reactive oxygen species. By changing the enzyme/combination of enzymes either to hemoglobin, or to superoxide dismutase - catalase, we enlarged the detoxification approach to other free radicals species, such as nitrogen reactive species, or combination of oxygen and nitrogen reactive species.

Inside cells nanoreactors and processors preserved their integrity over more than 48hours, and did not present toxicity in that interval. After cellular uptake, the nanoreactors/processors retained their function over extended periods of time, thus acting as artificial organelles that continuously exchange molecular information with the host cell. This opens new avenues in protein therapy as well as intracellular sensing approaches.

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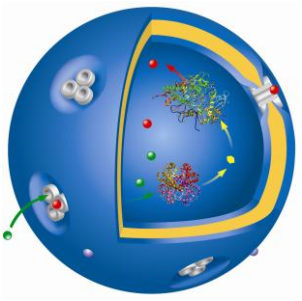

Figure. Schematic representation of an antioxidant processor based on the co-encapsulation of a combination of enzymes inside polymer nanovesicles.