PEOPLE

MARIE CURIE ACTIONS

Intra-European Fellowships (IEF)

PIEF-GA-2009-237110

"ThermosomeNanoReact"

PERIODIC REPORT

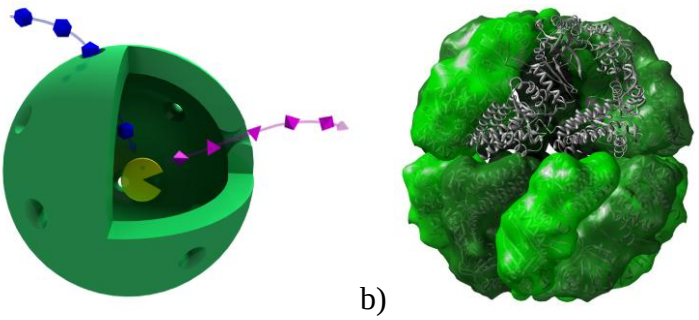
Reporting period: March 2009 – February 2011

ThermosomeNanoReact

Publishable summary

Chemical reactions can be confined to nanoscale compartments by encapsulating catalysts in hollow nanoobjects. Such reaction compartments effectively become nanoreactors when substrate and product are exchanged between bulk solution and cavity (Figure 1a). Nanoreactors hold promise for applications ranging from selective and size-constrained organic and polymer synthesis to biomedical advances (e.g., artificial organelles, biosensing) and as analytical tools to study reaction mechanisms. To the best of our knowledge, no nanoreactor has been reported yet that responds to adenosine 5′-triphosphate (ATP) or its analogues, although it is well-known that ATP is the fuel that can power large conformational movement within protein nanomachines. ATP is Nature`s ubiquitous energy currency, and is also readily available for *in vitro* experiments. Thus, an ATP responsive nanoreactor could find application ranging from biosensors that monitor ATP levels, to localized ATP-triggered drug production and delivery or as an ATP-addressable device in nanotechnology, e.g. to carry out polymerization reactions in constrained reaction volumes.

We have introduced the thermosome, a group II chaperonin from the archaea *Thermoplasma acidophilum*, as an ATP-responsive nanoreactor. It is a protein cage consisting of 16 subunits that form a spherical, hollow complex approx. 16 nm in diameter. Each half sphere of the thermosome encloses a cavity of about 5 – 9 nm in diameter (Figure 1b). We encapsulated the enzyme horse radish peroxidase (HRP) into this protein cage and explored the influence of the conformational state of the nanoreactor, which can be controlled by the addition of ATP and its analogues, on the activity of the biocatalyst. Locking the thermosome into its closed conformation efficiently stops substrate conversion by HRP, most likely because substrates and products cannot enter or leave the nanoreactor. Apart from this ATP-controllable gating, the thermosome enjoys another advantage compared to established nanoreactor systems. It possesses gated pores that are large enough to allow macromolecules to enter and leave its cavity. Thus, it is an ideal nanoreactor to synthesize polymers in its interior, providing the possibility of releasing the formed macromolecule into solution. This has been achieved by entrapping an organometallic catalyst for atom transfer radical polymerization (ATRP) in the thermosome.



a) b)

Figure 1. a) Schematic of a nanoreactor, where substrate and product are exchanged between bulk solution and cavity. b) Structure of the thermosome in its closed conformation. The outer diameter of the protein nanoreactror is approx. 15.8 nm. (Reproduced with permission from K. Renggli et al, *Adv. Funct. Mater.* **2011**, *21*, 1241–1259. Copyright 2011 Wiley-VCH Verlag GmbH & Co. KGaA)

During the course of this project, we developed a method to conjugate ATRP catalysts to proteins. This allowed us to attach the catalyst to the inner wall of the thermosome, effectively localizing the polymerization in the cavity of the protein cage. The polymers obtained within the thermosome are substantially smaller than the polymers obtained with conventional catalysts in solution. Moreover, they have a much narrower distribution of molecular weights. These findings indicate two effects of the confined reaction space. On the one hand, the polymerization is more controlled, i.e. less side reactions take place, on the other hand, the reaction is slower (e.g. due to diffusion limitations) or stops earlier (due to size constrains in the nanoreactor).
The developed method is very versatile and allows conjugating ATRP catalysts to other proteins as well. The scope of protein-catalyst conjugates was demonstrated by the removal of the toxic transition metal catalysts from the polymer product by methods known from the toolbox of biochemistry, e.g. by selective protein precipitation. Such an approach did significantly lower the copper concentration in the product as compared to conventional copper-based ATRP catalysts and their removal strategies.

The thermosome, as an ATP-responsive nanoreactor, could find application ranging from biosensors to monitor ATP levels, to localized ATP-triggered drug production and delivery and as an ATP-addressable device in nanotechnology, e.g. to investigate single enzyme kinetics. To the best of our knowledge, we are the first to report ATRP catalysts conjugated to proteins as well as ATRP carried-out in protein nanoreactors. Such systems permit a drastic reduction in residual copper content in polymers synthesized by ATRP and allow synthesizing well-defined polymers, because ATRP in a nanoreactor enhances the degree of control of aqueous ATRP.