FINAL PUBLISHABLE SUMMARY

Enzymatic catalysis is one of the key processes for biochemical conversion in nature and of utmost importance for any form of life. The fundament for this kind of conversion is the enzyme itself, which is a very specialized protein targeting another molecule, peptide, or protein in a very specific way to convert it into a product with a minimum of activation energy. The latter point is very critical as it allows biochemical reactions at physiological conditions. This specialization to physiological conditions is, although beneficial for natural processes, very often a drawback for technological processes. Enzymes, being proteins, in most cases cannot resist serious deviations in temperature or pH values and degrade. This operating window of the enzymes limits their flexibility for ex vivo application in technical synthesis, food processing, etc., seriously.

Inorganic nanoparticles (NPs) are considered to be a very promising alternative to enzymes as they might increase the operating window for the catalytic reaction. Such nanomaterials show in most cases much lower sensitivity to the processing conditions and may be a promising substitute for organic enzymes. Indeed, some research is already being performed to evaluate the potential of inorganic NPs as enzyme analogues. The research direction resembles a sort of molecular biomimetics, since inorganic phases are supposed to mimic biomaterials (enzymes) in their function. Few examples of enzyme-mimicking inorganic NPs have been investigated until now, with the most prominent particles consisting of Fe_3O_4, CeO_2, or Pt.

The investigation of inorganic enzyme analogues is a newly developing research field with a tremendous potential for future applications, either of medical or of technological nature. However, a serious lack of knowledge about the reactivities and the activities is still present. Therefore, this project aims to go far beyond the current state of the art. The specific objectives of the project are defined as follows:

a) Gaining knowledge on enzyme-analogue catalytic reactions of various inorganic NPs

The investigation should give a broader picture of potential inorganic substitutes to real enzymes and their difference in catalytic activity.

b) Understanding the differences in kinetics the two types of catalysts exhibit

Good comparability should allow tuning the inorganic enzymes by changing the physical and chemical properties, like size, surface structure, surface termination, etc.

c) Approach to application fields of inorganic enzymes

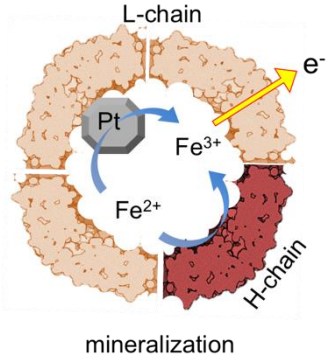
The work that was performed in the course of the project included the establishment of synthetic strategies for inorganic nanoparticles consisting of metals, metal oxides or metallic alloys. The syntheses were performed in two ways, a wet-chemical route that involves mineralization of the inorganic nanoparticles on biological nanoscale templates and a vacuum-based processing technology called atomic layer deposition. Among the synthesized nanoparticles those consisting of noble metals were identified to be more susceptible to catalytic reactions, thus more attention was paid to the wet-chemical synthetic procedure that allows a more straightforward synthesis of noble metal nanoparticles.

The synthesized nanoparticles have been evaluated for their catalytic activity. The main focus was on the most promising 3 types of reactions, namely the catalase, superoxide dismutase and ferroxidase mimicking reactions. Detailed characterization of the catalytic activity, including the tunability, inhibition and restorability of the reactivity was performed. [1-3] The results showed that such particles indeed broaden the applicability range of such enzyme-like reactions towards operational temperatures and pH values that are commonly not acceptable for purely organic enzymes. In some cases the catalytic activity was even enhanced once the operational conditions went beyond the physiological conditions. A synergistic effect was identified where upon enclosure of nanoparticles in protein cages an enzymatic reaction could be induced that is not intrinsic for the protein cage.

The investigated hybrid materials are considered to be of great promise for future biomedical applications. The protein shell provides a protection for the embedded nanoparticles, but also shows further functionalities such as mediation of electron transfer and may serve as vector for a delivery of nanomaterials to various cells.

Therefore the further work focused on the control of redox-chemical and biochemical parameters that enable the use of the hybrid nanoparticles in various applications. On the one hand, a detailed analysis of the protein characteristics that constitute the used protein cage (apoferritin), were investigated and lead to interesting observations. The protein cage may be constituted by two different types of proteins, the so-called H-chain and the L-chain proteins. In the course of this project some of the functions of those proteins have been investigated. A very interesting functionality relates to the coupling of chemical redox-reactions inside the cavity and outside the cavity of the cage. Such processes require transport of an electron through the protein shell, which can be achieved only with the L-chain protein, while the H-

chain protein is responsible for the ferroxidase enzymatic reaction. With a series of experiments we found that the L-chain protein is electron conducting, but does not have ferroxidase activity. This activity can be introduced by growing platinum nanoparticles inside the cavity of the protein and in this way construct a hybrid enzyme analogue. [3]



Schematic of a platinum-apoferritin hybrid nanoparticle with the metal fulfilling the same ferroxidase catalytic reaction as the H-chain protein within the composite. Figure from Ref. [3]

Another interesting functionality is the possibility to deliver the cargo of the protein cage to either the cellular cytoplasm or the nucleus. [4, 5] The cargo may consist of drugs, nanoparticles or even small interference RNA. The latter is of particular interest for silencing specific genes of the DNA and can be transported directly to the nucleus of a cell by programming the protein shell in its composition. The developed toolbox of delivery can be further optimized for a combined delivery system for artificial enzymes, drugs or RNA and combinations thereof, which can play a role in various future therapies.
Those findings encourage further investigation, especially of hybrid bio-inorganic nanoparticles as semi-artificial agents for delivery and enzymatic reactivity within cells. Tuning of the reactivities towards protective or de-protective functionalities within cells may have considerable impact for diverse therapies and therefore will be investigated in more detail in future. The obtained results promise great potential for innovation both from the scientific and the technological aspect. Initiated collaborations with biomedical institutions will be further intensified for more in-depth investigation and optimization towards the most promising application fields.

[1] U. Carmona, L. Zhang, L. Li, W. Münchgesang, E. Pippel and M. Knez, *Chem. Commun.*, **2014**, 50, 701.
[2] L. Li, L. Zhang, U. Carmona and M. Knez, *Chem. Commun*., **2014**, 50, 8021.
[3] U. Carmona, L. Li, L. Zhang and M. Knez, *Chem. Commun.*, **2014**, 50, 15358.
[4] L. Zhang , et al., *Adv. Healthcare Mater*. **2015**, 4, 1305.
[5] L. Li, et al., *Biomaterials* **2016**, 98, 143.