doi:10.2533/chimia.2017.243 Chimia 71 (2017) 243–245

FH HES **Universities of Applied Sciences**

Fachhochschulen – Hautes Ecoles Spécialisées

Engineering Nanosized Organosilica for Molecular Recognition and Biocatalysis Applications

M. Rita Correro[a], Yves Dudal[b], Philippe F.-X. Corvini[a], and Patrick Shahgaldian*[a]

*Correspondence: Prof. Dr. P. Shahgaldian, E-mail: patrick.shahgaldian@fhnw.ch, [a]University of Applied Sciences and Arts Northwestern Switzerland, School of Life Sciences, Institute of Chemistry and Bioanalytics, Gründenstrasse 40, CH-4132 Muttenz, [b]INOFEA AG, Hochbergerstasse 60c, CH-4057 Basel

Abstract: A series of synthetic nanomaterials capable of molecular recognition and/or biocatalysis have been produced by exploiting the self-sorting, self-assembly and polycondensation of organosilane building blocks around protein templates. The established methodology allows for the production of thin organosilica layers of controlled thickness, down to nanometer precision. Fully synthetic virus recognition materials have been shown to specifically bind their target virus down to picomolar concentrations. The shielding of natural enzymes allowed producing nanobiocatalysts functioning under harsh operational conditions.

Keywords: Molecular recognition · Nanobiocatalysts · Nanoparticles · Organosilica · Self-assembly

Introduction

The outstanding properties of biological systems originate predominantly from their capability to assemble, with a meticulous proficiency, molecular building blocks through non-covalent interactions to generate sophisticated functional structures and compartments. While self-assembly exploits a set of basic rules that are known in and of themselves, these interactions interplay in such a complex, intricate and synergistic manner that they result in tremendous efficiency.[1,2]

Living organisms, from almost all taxonomic groups, possess the ability to produce solid, inorganic matter with complex shapes and textures, remarkable mechanical properties and outstanding structural hierarchies.[3] As these materials often surpass the efficiency of their artificial equivalents, mimicking their biosynthesis offers myriad opportunities to develop synthetic strategies to produce novel functional nanomaterials.[4]

In our efforts to design functional nanomaterials, we have developed a chemical strategy that exploits the ability of silane building blocks to self-assemble around protein templates prior to polycondensation yielding stable and covalent organosilica shells. We have applied this strategy to the design of virus recognition nanomaterials and nanobiocatalysts.

Nanomaterials for Molecular Recognition of Viruses

In our efforts to develop recognition nanomaterials endowed with specific virus recognition properties, we have created a method to generate chemical imprints of viruses at the surface of silica nanoparticles (SNPs).[5–7] In order to establish the proof of concept, we used plant viruses that can be produced in large quantities. Briefly, the method developed consists in first cross-linking the virus at the surface of SNPs. This step is followed by a controlled organosilica layer growth at the surface of the nanoparticles; this is achieved by incubating the SNPs in a mixture of organosilanes (OSs) in buffered conditions. Based on their chemical functionality, OSs sort at the surface of the virion capsids, which act as templates, before undergoing a polycondensation reaction to yield a stable organosilica layer. The conditions of the polycondensation reactions (temperature, buffer, pH, OS concentrations) were finely optimized so as to reach a precise control over the thickness of the organosilica layer produced, down to the nanometer precision. Virions were consequently removed by acidic treatment under ultrasound and imaged by field emission scanning electron microscopy (FE-SEM), Fig. 1. Using an enzyme-linked immunosorbent assay (ELISA), we have demonstrated that virus-imprinted nanoparticles selectively bind their template virus in water and in serum down to picomolar concentrations.[6]

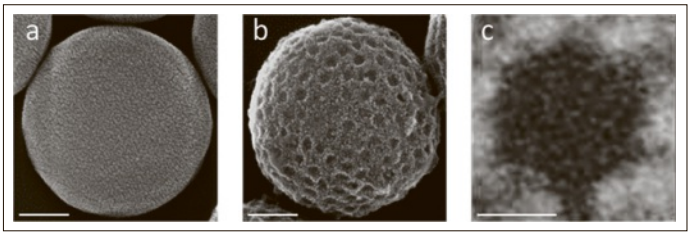

Fig. 1. Representative FE-SEM micrographs. (a) SNP obtained with the Stöber reaction serving as carrier material; (b) virus-imprinted nanoparticle obtained after layer growth and virus removal; (c) single virus imprint in which the shape of the template virus originating from the icosahedral shape of the template virus could be distinguished. Scale bars represent: a,b, 100 nm and c, 10 nm. Reprinted from ref. [6], Copyright 2013 Nature Publishing Group.

In order to apply VIPs to medical diagnostics, we have adapted this approach to virus-like particles (VLPs).[5] VLPs, being deprived of genetic material, are non-infectious and can be produced in large quantities using established molecular biology methods. However, the implementation of the technique to VLPs turned out to be problematic as the high density of amine functions present at the surface of the virions strongly promoted the polycondensation of OSs on this surface. This issue was circumvented by using a supramolecular additive, namely citrate, which was demonstrated to overlay the surface charge of the virus. This allowed, by controlling the concentration of citrate, to control the polycondensation reaction of OSs.

Nanomaterials for Biocatalysis

Enzymes are able to catalyze a large variety of chemical reactions with outstanding selectivity and efficiency in their 'physiological' environment. However, their implementation in industrial processes is made problematic by the limited stability

of most enzymes in non-physiological conditions. Besides enzyme engineering approaches, a variety of chemical strategies aimed at improving the resistance of enzymes to harsh conditions has been developed.[8,9]

In this context, we have established a synthetic method to produce hybrid organic/inorganic nanobiocatalysts displaying an unprecedented resistance to a series of physical and chemical stress conditions.[10,11] As model enzyme, we used a β-galactosidase enzyme (β-gal). After binding the β-gal enzyme at the surface of SNPs using a homobifunctional crosslinker (*i.e.* glutaraldehyde), a protective organosilica layer was produced by the polycondensation of OS building blocks ((3-aminopropyl) triethoxysilane and tetraethylorthosilicate). We observed that the catalytic activity of the enzyme was substantially diminished immediately after the synthesis of the organosilica layer. However, this activity was recovered after conserving the particles at room temperature for few hours. In order to understand this phenomenon, we have carried out a careful characterization of the particles during this 'curing' phase measuring catalytic constants and analyzing the particles by means of atomic force microscopy. Remarkably, force-distance measurements allowed to demonstrate that, during the curing phase, the organosilica layer underwent a chemical rearrangement causing a layer softening. We have demonstrated that this phenomenon was concomitant to the recovery of the enzymatic activity, and that it is triggered by the presence of the immobilized enzyme. Indeed, no such softening is observed when the particles are deprived of enzyme. Additionally, we have shown that the shielded β-galactosidase possesses remarkable stability against stresses of different nature (*i.e.*, pH, temperature, chaotropes, ultrasounds and protease). The successful shielding of a series of different natural enzymes (*i.e.*, acid phosphatase, laccase, alcohol dehydrogenase, aspartate aminotransferase) and of an artificial metalloenzyme imine reductase[12] confirmed the versatility of the synthetic strategy developed.

As a further improvement of this enzyme protection method, we modified the method so to enhance the number of interactions points between the surface of the immobilized enzyme and the organosilica layer; this was expected to improve the stability of the shielded enzyme (Fig. 2). Different mixtures of OSs building blocks sharing chemical similarities with natural amino acids were used.[13] In more detail: the hydroxyl group of hydroxymethyl-triethoxysilane mimics polar amino acids (*e.g.* serine, threonine); propyl-triethoxysilane and benzyl-triethoxysilane serve as aliphatic and aromatic building blocks mimicking leucine and phenylalanine, respectively. We have demonstrated that increasing the complexity of the protection layer allowed for significant improvement of the protection effect against thermal and chaotropic stresses.

Merging Molecular Recognition and Biocatalysis

Merging the strategies established for the development of materials for molecular recognition and for biocatalysis, we have developed a novel method for molecular detection of a target virus.[7] The nanomaterial consists of an enzyme immobilized onto SNP covered with an organosilica shell, and sequentially enclosed in a virus-recognition layer produced by surface imprinting (Fig. 3a). The detection principle is based on the following hypothesis: *a)* The enzyme preserves its biocatalytic activity after shielding; *b)* The recognition layer prevents the substrate uptake by the enzyme, with exception of the imprints; *c)* The binding of the target virus impedes the substrate to reach the catalytic site of the enzyme. We confirmed the detection principle by measuring a loss of catalytic activity when the substrate was added after incubation with the target virus.

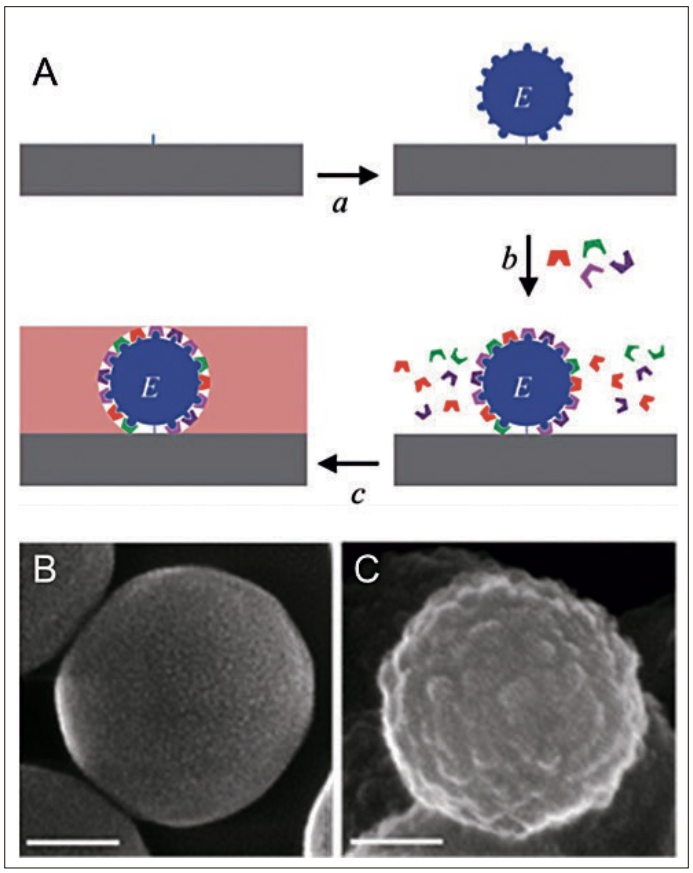

Fig. 2. Schematic representation of the chemical strategy of enzyme shielding (A) and representative SNPs micrographs (bottom). Top: amino-modified SNP surface (in grey) is reacted with glutaraldehyde (not shown); the enzyme (*E*) is anchored at the surface of SNPs (*a*) and incubated with a mixture of different silanes (*b*); the sorting and polycondensation of OSs around the enzyme yields a stable, covalent and protective layer. Bottom: representative FE-SEM micrographs of a Stöber SNP before (B) and after (C) the growth of the protective layer.

In summary, the controlled growth of an organosilica layer with a nanometer-precision at the surface of nanoparticles where proteins (*e.g.* enzymes) or protein assemblies (*e.g.* viruses, virus-like particles) are immobilized allowed the development of functional nanoparticulate materials with potential applications in molecular recognition and catalysis. Work is currently underway to implement the technology for different applications including proteomics, production of biologics, targeted drug delivery, biocatalysis in organic media, to name but a few.

Acknowledgments

The authors gratefully acknowledge the funding sources which supported this work: the Swiss Commission for Technology and Innovation (KTI/CTI), the Swiss Nanoscience Institute (SNI), the Swiss state secretariat for education, research and innovation (SERI) and INOFEA AG.

Received: March 10, 2017

[1] Y. Lee, 'Self-assembly and nanotechnology', John Wiley & Sons, Hoboken, **2008**.
[2] B. W. Niham, P. Lo Nostro, 'Molecular Forces and self-assembly', Cambridge University Press, New York, **2010**.
[3] Y. Dauphin, in 'Encyclopedia of inorganic and bioinorganic chemistry', Ed. R. B. King, John Wiley & Sons, Hoboken, **2011**, p. 391.
[4] C. S. S. R. Kumar, 'Biomimetic and bioinspired nanomaterials', Wiley-VCH, Weinheim, **2010**.

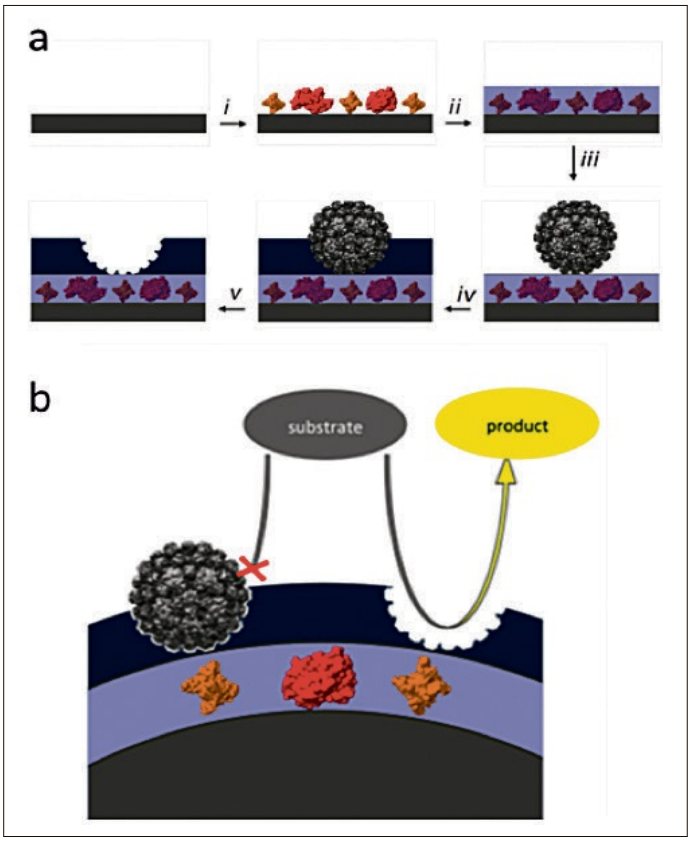

Fig. 3. a) Schematic representation of the synthetic strategy. The catalytic layer is produced by the immobilization of an enzyme (co-immobilized with albumin to ensure colloidal stability) onto SNPs (*i*) and the successive polycondensation of an organosilica shell (*ii*). The detection layer is produced following the sequence: VLP anchoring onto the surface of the catalytic layer (*iii*), synthesis the recognition layer (*iv*), removal of the VLP and formation of the imprints (*v*). b) Principle of detection by catalytic imprinted particles: the enzymatic reaction producing a coloured substrate occurs almost exclusively in the open imprints, the virus-binding event inhibits this reaction. Reprinted with permission from ref. [7], Copyright 2017 Wiley-VCH.

[5] S. Sykora, A. Cumbo, G. Belliot, P. Pothier, C. Arnal, Y. Dudal, P. F. X. Corvini, P. Shahgaldian, *Chem. Commun.* **2015**, *51*, 2256
[6] A. Cumbo, B. Lorber, P. F. X. Corvini, W. Meier, P. Shahgaldian, *Nat. Commun.* **2013**, *4*, 1503
[7] S. Sykora, M. R. Correro, N. Moridi, G. Belliot, P. Pothier, Y. Dudal, P. F.-X. Corvini, P. Shahgaldian, *ChemBioChem* **2017**, DOI: 10.1002/cbic.201700126
[8] U. Hanefeld, L. Gardossi, E. Magner, *Chem. Soc. Rev.* **2009**, *38*, 453
[9] M. R. Correro, S. Sykora, P. F.-X. Corvini, P. Shahgaldian, *Methods Enzymol.* **2017**, in press,
[10] M. R. Correro, N. Moridi, H. Schutzinger, S. Sykora, E. M. Ammann, E. H. Peters, Y. Dudal, F. X. Corvini, P. Shahgaldian, *Angew. Chem., Int. Ed.* **2016**, *55*, 6285
[11] P. Shahgaldian, M. R. Correro, A. Cumbo, P. Corvini, *New Biotechnol.* **2014**, *31*, S43
[12] M. Hestericova, M. R. Correro, M. Lenz, P. F. Corvini, P. Shahgaldian, T. R. Ward, *Chem. Commun.* **2016**, *52*, 9462
[13] M. R. Correro, M. Takacs, S. Sykora, P. F. X. Corvini, P. Shahgaldian, *RSC. Adv.* **2016**, *6*, 89966w