CORE
Metadata, citation and similar papers at core.ac.uk
Provided by University of Strathclyde Institutional Repository

Repurposing Biocatalysts to Control Radical Polymerizations

Kyle J. Rodriguez, Bernadetta Gajewska, Jonas Pollard, Michela M. Pellizzoni, Csaba Fodor[†], Nico Bruns*

Adolphe Merkle Institute, University of Fribourg, Chemin des Verdiers 4, 1700 Fribourg, Switzerland.

Reversible-deactivation radical polymerizations, Controlled Radical Polymerizations, Enzymes, ATRPases, Biosensing, Green Chemistry, ATRP, RAFT

ABSTRACT: Reversible-deactivation radical polymerizations (controlled radical polymerizations) have revolutionized and revitalized the field of polymer synthesis. While enzymes and other biologically derived catalysts have long been known to initiate free radical polymerizations, the ability of peroxidases, hemoglobin, laccases, enzyme-mimetics, chlorophylls, heme, red blood cells, bacteria, and other biocatalysts to control or initiate reversible-deactivation radical polymerizations has only been described recently. Here, the scope of biocatalytic atom transfer radical polymerizations (bioATRP), enzyme-initiated reversible addition–fragmentation chain transfer radical polymerizations (bioRAFT), biocatalytic organometallic mediated radical polymerizations (bioOMRP), and biocatalytic reversible complexation mediated polymerizations (bioRCMP) is critically reviewed and the potential of these reactions for the environmentally friendly synthesis of precision polymers, for the preparation of functional nanostructures, for the modification of surfaces, and for biosensing is discussed.

Biologically derived catalysts, such as enzymes or their cofactors, represent an attractive alternative to conventional polymerization catalysts because they are non-toxic, biodegradable, and derived from sustainable resources. Moreover, enzymes can display high stereo-, regio-, or chemo-selectivity,[1] while working under mild conditions. They have been extensively explored for the *in vitro* synthesis of polymers,[1-3] *e.g.* by ring opening polymerization (ROP)[4-5] or polycondensation.[6-7] Several enzymes can also mediate free radical polymerizations.[8-9] For example, laccases use oxygen to create radicals on phenols, which then undergo radical coupling polymerization.[10] This reaction has been used since ancient times to create traditional Japanese lacquerware from the sap of the lacquer tree *Rhus vernicifera* that contains the monomers and the enzyme.[11] Enzymatic radical polymerizations are also involved in the biosynthesis of lignin[12] and of melanin.[13] Not surprisingly, radical-producing enzymes have also been explored in synthetic polymer chemistry, for example to polymerize vinyl monomers (*e.g.* acrylates and acrylamides),[8, 14] anilines,[15] phenols[2, 16-17] and lignols.[3, 18-19] While peroxidases and other heme proteins, as well as laccases, can initiate free radical polymerizations using peroxides and oxygen, respectively,[8-9] until recently it was unknown that biocatalysts can also control or initiate radical polymerizations in very similar ways to conventional catalysts for reversible-deactivation radical polymerizations (also termed controlled radical polymerizations (CRPs).[20-23]

Here, we review the nascent field of biocatalytic controlled radical polymerizations (bioCRP) and critically discuss the potential of these novel enzymatic polymerizations in applications such as polymer synthesis, development of functional nanostructures, and biosensing.

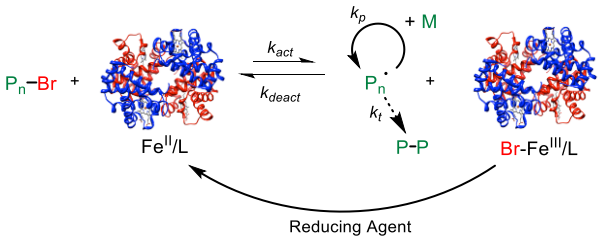


Figure 1. Mechanism of bioATRP using hemoglobin as the *ATRPase* catalyst under ARGET ATRP conditions.

Enzyme-catalyzed ATRP. The first evidence of the catalytic activities of enzymes in an ATRP-like manner were found simultaneously by our group[24-26] and by di Lena and coworkers.[27-28] We polymerized *N*-isopropylacrylamide (NIPAM),[24-25] methyl ether poly(ethylene glycol) acrylate (PEGA)[25] and methyl ether poly(ethylene glycol) methacrylate (PEGMA)[25] under activator regenerated by electron transfer (ARGET) ATRP conditions using horseradish peroxidase (HRP),[24] hemoglobin (Hb),[25] and the erythrocyte fraction of full human blood[25] as catalysts (Figure 1). We termed this novel activity of these promiscuous enzymes *ATRPase activity*. Over the last years, we could show that laccase polymerizes *N*-vinlyimidazole,[29] that HRP-catalyzed ATRP can be confined into polymersomes[30] and that it can be carried out in protein nanoreactors.[31] Moreover, we have investigated surface-initiated ATRP mediated by hemoglobin.[32] These and other examples will be discussed in more detail below to illustrate the main conceptual ideas of this Viewpoint. Di Lena and coworkers first performed a laccase-initiated free radical polymerization of PEGMA from a typical ATRP initiator which they controlled by the addition of a conventional RAFT agent.[27] They also synthesized PEGMA brushes onto the surface of crosslinked polystyrene microparticles by laccase-

catalyzed bioATRP. Unfortunately, the resulting polymer brushes were not characterized in depth so that it cannot be judged which level of control was achieved.[27] Then, they carried out the ATRP-like controlled radical polymerization of PEGA, which was catalyzed by laccase, catalase, and HRP.[28]

The discovery of ATRPase activity of metalloproteins inspired research groups worldwide to explore bio-catalyzed ATRP or enzyme-initiated RAFT polymerizations. Matyjaszewski and coworkers confirmed that catalase can synthesize narrowly dispersed polymers.[33] Proof that surface-initiated bioATRP can result in narrowly dispersed polymers came from the group of Ko.[34] They used laccase, HRP, and catalase to control the grafting-from polymerization of NIPAM from the surface of lignin nanofibers that were modified with an ATRP initiator. Electrospun lignin fiber mats could be modified by PNIPAM brushes that were more than 100 nm thick, which had monomodal molecular weight distributions and dispersities as low as 1.3. Chanana and coworkers immobilized HRP on gold nanoparticles to be able to recycle the enzyme after bioATRP.[35] The catalyst-coated nanoparticles could be recovered and reused in multiple polymerizations. However, relatively broad molecular weight distributions were obtained. Sun *et al*. introduced the method of electrochemically mediated ATRP to the field of biocatalytic ATRP. Using hemoglobin simultaneously as the polymerization catalyst and as the template, they synthesized protein-imprinted polymers (acrylamide crosslinked by bis-acrylamide) on electrodes that were modified with ATRP initiators.[36-37] The aim was to generate electrochemical sensors for the protein (*vide infra*).

Enzyme-mimetic ATRP catalysts. It is very likely that activation and deactivation reactions involved in bioATRP take place at the metal center of the enzymes. In the case of HRP and Hb, the prosthetic group is heme, *i.e.* an iron protoporhyrin IX complex. Metalloporphyrins have been used as catalysts in a variety of polymerizations for years.[38-41] To the best of our knowledge, the first work on photopolymerizations catalyzed by iron-porphyrins had been reported in 1974.[42] PEGylated heme complexes were used as catalysts for free radical polymerization in the early 2000s.[43-44] Inspired by the discovery of ATRPase activity of heme proteins, Kadokawa and coworkers[45] as well as Matyjaszewski and coworkers[33, 46-47] developed reaction conditions that allowed the use of hemes as catalytic species for ATRP of NIPAM[45] and PEGMA.[33, 46] As heme is not soluble in water, mixtures of DMF and water were used.[33, 45-46] Furthermore, PEG was conjugated to heme to increase its solubility.[33, 46-47] The out of plane vinyl double bond of heme was hydrogenated to avoid the catalyst copolymerizing with the monomer.[33, 46-47] Addition of halogen salts proved key to improve the degree of control in the reaction, possibly because the heme catalysts have a low halidophilicity.[33, 47]

The group of Tang decorated a heme with six amino acid residues rendering it water soluble. With this enzyme mimetic catalyst, they could synthesize block copolymers via ARGET ATRP of PEGMA, methacrylic acid, HEMA and glycidyl methacrylate from a polycaprolactone macroinitiator that had been synthesized by enzymatic ROP.[48] The same peroxidase-mimetic catalyst was also used to grow poly(*N*,*N*-dimethyl aminoethyl methacrylate) on mesoporous silica nanoparticles,[49] and to synthesize poly(caprolactone)-*b*-poly(glycidyl methacrylate) block copolymers as precursors for amphiphilic non-viral gene vectors.[50]

Other approaches for quasi-biocatalytic controlled radical polymerizations are the use of metal-organic frameworks to immobilize heme,[51] or to create enzyme mimetic polymerization catalysts by single polymer chain folding.[52] In the latter case, the resulting globules are stable against degradation by hydrolytic enzymes and can possess a higher temperature stability than proteins. Pomposo and coworkers created copper-containing single chain globules from a copolymer of PEGMA and 2-acetoacetoxy ethyl methacrylate as mimics of laccase. They used them as a catalyst for the ARGET ATRP of PEGMA with a good degree of control.[53]

RAFT polymerizations initiated by biocatalysts. Enzymes are also used to initiate RAFT polymerizations. Unlike bioATRP, where the enzyme is responsible for the activation and deactivation steps of the reaction, bioRAFT only requires the enzyme to generate a radical for the initiation step. In contrast to the first example by di Lena who used ATRP initiators to start RAFT polymerizations,[27] An and coworkers,[54,55] Konkolewicz and coworkers,[56-57] as well as Tan, Zhang, and coworkers[58] relied on the classic reaction of HRP with hydrogen peroxide and acetylacetone (ACAC; pentan-*2,4*-dion) as a mediator to create ACAC radicals (Figure 2). A variety of monomers, including dimethylacrylamide,[54, 56-57] 2-hydroxy ethylacrylate,[54] PEGA,[54, 56] PEGMA,[54] *N*-vinylpyrrolidone,[54] NIPAM,[56] and 2-hydroxypropyl methacrylate[58] were polymerized with good degrees of control. Block copolymers[54, 56, 58] and protein-polymer conjugates[56] were synthesized, and enzymatic RAFT polymerization was used to prepare nanostructures such as worms and vesicles by polymerization-induced self-assembly (PISA).[58] Moreover, the cascade reaction of glucose oxidase and HRP allowed to initiate the RAFT polymerizations by conversion of glucose and oxygen.[54, 58] In a similar approach, Yang and coworkers utilized the peroxidase-like activity of a bovine serum albumin/copper phosphate hybrid nanoflower to catalyze the polymerization of DMAEMA and PEGMA via a RAFT

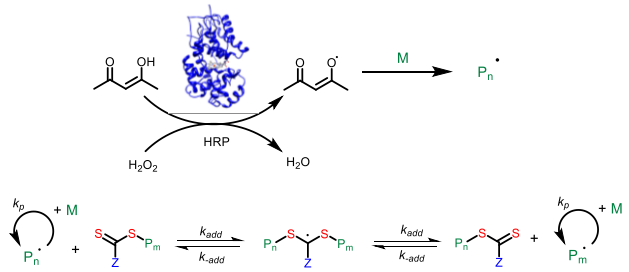


Figure 2. Mechanism of bioRAFT using horseradish peroxidase to catalyze the reaction of H_2O_2 and ACAC to generate ACAC radicals that initiate RAFT polymerizations.

mechanism initiated by ACAC radicals.[59] Very recently, Qiao and coworkers initiated RAFT polymerizations by the conversion of hydrogen peroxide into reactive hydroxyl radicals, catalyzed by Hb and ovine blood.[60]

Chlorophyll a and bacteriochlorophyll are naturally abundant porphyrin-magnesium complexes. They were used as biocatalysts to mediate photoinduced electron transfer (PET) during initiation of RAFT polymerizations by the group of Boyer.[61-62] This allowed for the polymerization of a wide variety of monomers, including acrylates, methacrylates and acrylamide in an '*on*' and '*off*' manner dictated by visible[61-62] and near infrared light.[62] Chlorophylls represent a green alternative to expensive

and potentially toxic iridium or ruthenium catalysts that are conventionally used for such kinds of PET-RAFT polymerizations.

Biocatalytic organometallic mediated radical polymerizations (bioOMRP). Vitamin B_{12} is a cobalt(III) complex with octahedral geometry. It is also known as cobalamin. Di Lena and coworkers reduced the derivative hydroxocobalamin (vitamin B_{12a}) to produce a catalyst that lead to a linear growth of molecular weight in a conventionally initiated radical polymerization of 2-hydroxyethyl acrylate.[63] However, the polymer´s dispersity increased from 1.4 to 3.3 over the course of the reaction. The catalyst controlled the polymerization though a reversible deactivation by coupling mechanism. In contrast, the methacrylate PEGMA underwent catalytic chain transfer.

Biocatalytic reversible complexation mediated polymerizations (bioRCMP). One can veer from metal-containing biocatalysts and focus on organic biomolecules as candidates for CRP catalysts. Goto and coworkers reported the controlled radical polymerization of methacrylates and acrylates using choline iodide, acetylcholine iodide, and butyrylcholine iodide as

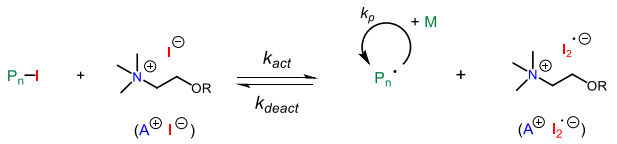


Figure 3. Mechanism of bioRCMP using choline derived alkyl iodide catalysts.

catalysts for reversible complexation mediated polymerizations (Figure 3).[64] These molecules are derived from a vitamin-like nutrient and a neurotransmitter. Therefore, they should be nontoxic and affordable catalysts for the synthesis of well-defined polymers and block copolymers.

Advantages of biocatalytic controlled radical polymerizations. The abovementioned examples demonstrate that enzymes, their cofactors, and enzyme-mimetic catalysts can control ATRP or initiated RAFT polymerizations. However, it is difficult for a novel type of catalyst to outperform conventional polymerization catalysts and initiating systems that have been explored and optimized in thousands of research papers over the last two decades. The main impact of biocatalytic polymerizations is therefore not to provide yet another set of catalysts, but to tackle those kinds of reactions and systems where the special properties of the biomolecules provide an intrinsic advantage. The following paragraphs will discuss such cases and will hopefully stimulate scientific curiosity and creativity amongst our readers. The list is by no means exhaustive and we are convinced that many exciting and unexpected features of biocatalytic controlled radical polymerizations are yet to be demonstrated.

Controlled radical polymerization of difficult monomers. While CRPs are conducted widely in academic laboratories and for the synthesis of some industrial products, several important monomers can still not be polymerized by conventional CRP methods in a controlled way, or can only be polymerized by one method and not another, *e.g.* by RAFT but not by ATRP. Biocatalysts can open new routes for the polymerization of such difficult monomers and therefore expand the toolbox of synthetic polymer chemistry. For example, poly(*N*-vinylimidazole) (PNVIm), its cationic derivatives, and its copolymers have great potential as gene delivery vectors,[65-66] as laundry formulation ingredients,[67] or as polymerized ionic liquids (PILs).[68] However, the full potential of this polymer is not unlocked, because it is difficult (and even considered "*impossible*"[69]) to polymerize this monomer in a controlled way by ATRP.[70] One reason for this problem is that the polymer strongly complexes copper ions and therefore removes the metal from conventional ATRP catalysts. We could show that laccase from *Trametes versicolor* can overcome this limitation by catalyzing the polymerization of NVIm under ARGET ATRP conditions. Polymers with dispersities as low as 1.07 were synthesized (Figure 4).[29] In the enzyme, the copper ions are embedded within its three-dimensional structure and are not released to the polymer. Thus, laccase-catalyzed polymerization of NVIm represents a good pos-

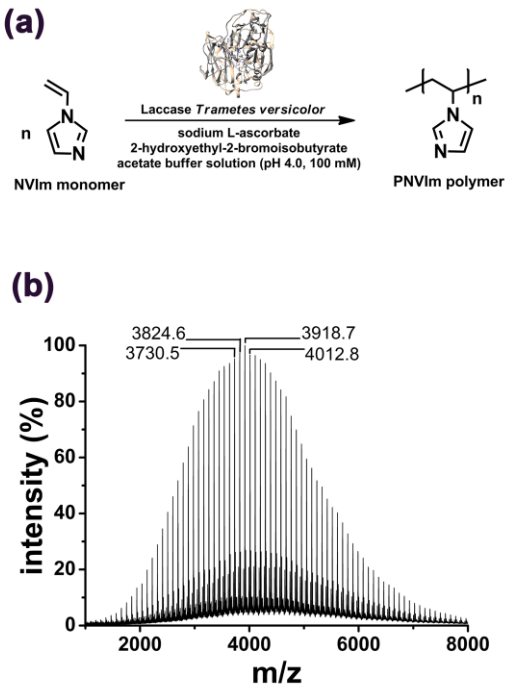


Figure 4. a) Controlled radical polymerization of NVIm by laccase; b) MALDI mass spectrum of a PNVIm (M_n= 4300 g mol^{-1}, Đ = 1.07) synthesized by laccase-catalyzed CRP. Adapted from Ref. 29 with permission from the Royal Society of Chemistry. Copyright 2016.

sibility to synthesize PNVIm-based building blocks for nanostructures and gene-delivery systems and as precursors for PILs.

Another example for difficult to polymerize monomers in ATRP is methacrylic acid, as many conventional ATRP catalysts do not perform well in acidic conditions. Remarkably, PEGylated hemin was found to be a good catalyst for the ATRP of this monomer,[47] because the enzyme-mimetic catalyst withstands the acidic conditions and because the iron-based catalyst, in contrast to conventional copper-based catalysts, suppresses chain-end cyclization of brominated-terminated chains.

Route towards metal-free polymer products. Conventional ATRP requires heavy metal complexes as catalysts; they can be toxic and are often tedious to remove from the polymer. Although modern developments in catalyst design and reaction processes have greatly reduced the concentration of catalysts needed to achieve a good degree of control,[20] traces of the metal in the polymer can deteriorate the material properties. Residual catalyst traces still represent a major problem for the use of

ATRP-derived polymers in biomedical and in electronic applications.[20] Enzymes can be easily removed from solution because of their distinct physicochemical properties. Many methods are routinely used in biochemistry labs. For example, proteins can be precipitated by addition of water-soluble organic solvents[71] or by ammonium sulfate,[72] and thereby separated from polymers.[73-74] Moreover, proteins can be isolated by dialysis, spin ultracentrifugation, and by size exclusion, affinity, or ion exchange chromatography.[75] The effectiveness of such methods to purify polymers after a biocatalytic polymerization was demonstrated recently.[29] After the laccase-catalyzed controlled radical polymerizations of NVIm, a few simple purification steps, including precipitation of the enzyme, quantitatively removed the catalyst from PNVIm, even though the polymer strongly complexes copper ions. The metal ion content of the polymer was below the detection limit of inductively coupled plasma optical emission spectrometry (ICP-OES) which can detect copper as low as 9.1 ppb in the dry mass of the polymer. Thus, enzymes represent a viable way to generate ATRP-derived polymers that are free of metal traces.

Enzymatic deoxygenation. Traditionally, CRPs are conducted under stringent oxygen-free conditions because oxygen inhibits radical polymerizations. It is well known that in nature deoxygenation occurs through the enzymes glucose oxidase (GOx)[76] and pyranose oxidase (P2Ox).[77] The research groups of Stevens,[78-79] Matyjaszewski and Russell,[80] as well as An,[81-82] have pioneered the utilization of nature's own deoxygenating processes for radical polymerizations. GOx and P2Ox were employed to deoxygenate RAFT polymerizations[58, 78-79, 81, 83] and ATRP.[80] Highlighting one of these works, An and coworkers generated a suite of multi-block and ultrahigh-molecular-weight (UHMW) polymers using a variety of monomers: *N*,*N*-dimethylacrylamide (DMA), 4-acryloylmorpholine (AM), PEGMA, and PEGA.[81] An elegant, dual enzyme cascade catalysis was designed to first deoxygenate the reaction vessel by P2Ox, which produced H_2O_2 as a byproduct, followed by the HRP-catalyzed generation of ACAC radicals to initiate the RAFT polymerization (Figure 5). The multiblock polymers had good sequence definition up to 10 blocks, and UHMW polymers with molecular weights up to 2.3×10^6 g mol^{-1} were achieved.

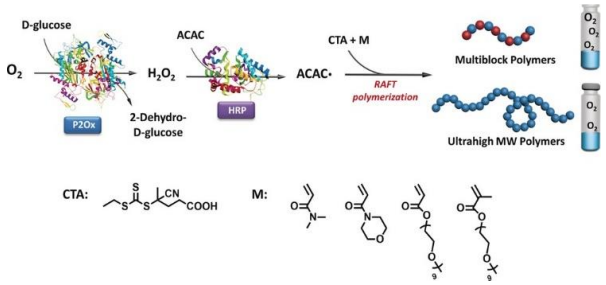


Figure 5. Schematic representation of P2Ox-HRP cascade catalysis of oxygen-tolerant bioRAFT polymerization and chemical structures of the chain-transfer agent (CTA) and monomers (M). Adapted from Ref. 81 with permission from John Wiley and Sons. Copyright 2017.

Immobilized biocatalysts. In order to make biocatalysis efficient and cost-effective, the catalysts should be recycled after a biotransformation. To this end, enzymes are often immobilized, *e.g.* on porous microbeads.[84-86] The catalysts can then be recovered from a reaction mixture by simple filtration or centrifugation. For bioATRP, the first step in that direction represents the immobilization of HRP on gold nanoparticles, as reported by Chanana and coworkers.[35] Although gold nanoparticles have useful properties (*e.g.* because their plasmonic properties allow for an easy and fast detection of their aggregation), they are certainly not the best choice to improve the costs of biocatalysis. Enzymes on superparamagnetic iron oxide nanoparticles, as reported by the same group, could be an alternative.[87] Given the fact that enzyme immobilization is well-established, microporous supports, polymeric microbeads, or other carriers will have to be tested for the immobilization of ATRPases and RAFT-initiating enzymes.

Polymerizations in spatial confinements. Because of their large size, enzymes can be physically entrapped into nanoreactors,[88-90] such as block copolymer vesicles, nanostructured polymeric materials, polymer microcapsules, or the pores of inorganic mesoporous materials. Moreover, the rich, yet very defined distribution of functional groups on the surface of proteins allows to covalently conjugate them into various cavities. In addition, they can be directly engineered into hollow proteinaceous nanoobjects, such as virus-like particles or other protein cages.[91-92] Therefore, enzymes are ideally suited to confine chemical reactions into nanoscale reaction compartments. A rich body of literature describes intriguing biocatalytic nanoreactors.[88-92] They have led to improved catalytic properties in demanding environments, altered or switchable substrate selectivity and the possibility of establishing efficient cascade reactions by close spatial proximity of several different enzymes. Nanoreactors can also be beneficial for controlled radical polymerization reactions, as chain growth reactions in confined reaction spaces improves the control over the molecular mass distribution and reduces the chance of termination reactions, therefore increasing the degree of chain end functionality.[93] To demonstrate that enzymatic ATRP can be conducted in nanoreactors, HRP was chemically conjugated into the cavity of the protein cage thermosome.[31] It is a chaperonin with approx. 16 nm diameter that usually helps to refold unfolded proteins and therefore has pores that are large enough to allow macromolecules to leave the cage. BioATRP within the protein nanoreactor resulted in more narrowly dispersed polymers compared to polymers synthesized by the same enzyme in solution, indicating that the confined space within nanoreactors can enhance the polymerization. HRP was also encapsulated into polymersomes.[30] Polymerization of PEGA by bioATRP within the vesicles transformed the nanoreactors from hollow spherical objects to polymersomes that were filled with the hydrophilic polymer. Thus, bioATRP allows packing polymers into polymersomes. The resulting nanostructures mimic cell organelles. They have a semipermeable membrane that encloses a dense and viscous interior, similar to the cytosol. Thus, they could be used as model systems to study enzymatic reactions in crowded microenvironments. Moreover, polymerization of monomers with side chains that allow attaching drug molecules or magnetic resonance imaging contrast agents could lead to drug delivery vehicles and imaging probes with a very high loading density of cargo.

Surface-initiated biocatalytic controlled radical polymerizations. Surface-initiated polymerizations allow to modify surfaces with a thin layer of polymers in so-called "*grafting from*" polymerizations, *e.g.* to produce biomaterials.[94] Enzymatic controlled radical polymerizations on surfaces have been reported by di Lena,[27] Ko,[34] Chen,[95] and by our group in collaboration

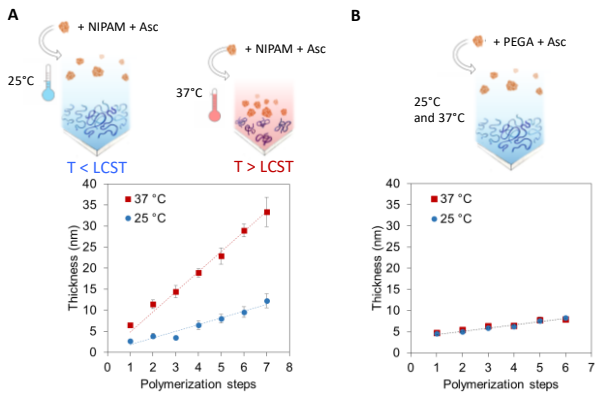


Figure 6. Surface-initiated bioATRP catalyzed by Hb: Control of polymer brush growth through the affinity of the surface towards proteins. a) Linear increase in thickness of PNIPAM films upon repetitive bioATRP at 25 °C, where the polymer chains are hydrated and protein repellent, and at 37 °C, where the polymer chains are collapsed and protein adsorbent; b) Linear, but small growth of protein repellent PPEGA films at 25 °C and 37 °C. Adapted from Ref. 32 with permission from the American Chemical Society. Copyright 2017.

with Benetti.[32] An obvious advantage of such polymerizations is that the surface only gets in contact with a biological benign molecule, which could be beneficial for the creation of biocompatible surfaces. However, this biocompatibility remains yet to be demonstrated. A unique feature of proteins compared to other polymerization catalysts is that their interaction with surfaces can be finely tuned, as surfaces can be designed to be protein repellent or protein adherent. It can therefore be expected that the affinity of a surface towards proteins plays an essential role in such polymerizations and that it should be possible to influence the course of surface-initiated polymerizations in unique ways by engineering the interactions between the surface and the biomolecules. To demonstrate this concept, we conducted an in-depth study of surface-initiated enzymatic ATRP using Hb as the catalyst.[32] PNIPAM brushes with switchable bioaffinity as well as protein-repellent PPEGA brushes were synthesized. Both polymers formed homogeneous thin films. When reinitiated by hemoglobin at 25 °C, the thickness of PPEGA and of PNIPAM brushes increased only slightly, even though their chain end fidelity was high. However, when PNIPAM grafting was stimulated at 37 °C, above the polymer´s lower critical solution temperature (LCST), a significant increase in thickness was observed due to hemoglobin's adsorbtion on the propagating polymer chains in their collapsed hydrophobic state. When brought back to 25 °C, the protein layer could be detached from the brush and renewed in a subsequent heating step. Based on this principle, a multistep SI-bioATRP protocol was developed that allowed for a linear growth of PNIPAM brushes in 4 nm steps up to at least 30 nm dry thickness, suggesting the absence of irreversible termination reactions and a high degree of control over the polymerization (Figure 6). Moreover, multi-block copolymer brushes consisting of one PPEGA and five PNIPAM blocks were synthesized. Thus, the growth of the polymer brushes could be tuned by the hydrophobicity of the polymer layer and, therefore, the tendency of the protein to weakly adsorb to the polymer surface.

Exploiting the catalytic activity of biocatalysts for biosensing. An intriguing opportunity for biosensing and diagnostic purposes is to use the catalytic activity of ATRP-active biomolecules to prepare biosensors, such as molecularly-imprinted polymer networks (MIPs) on electrodes. When an imprinted protein is present in a sample, the pores in the polymer get blocked and therefore the peak current in differential pulse voltammetry measurements decreases. To prepare such biosensors, hemoglobin-catalyzed eATRP of acrylamide and bisacrylamide on planar[36] and dendritic gold electrodes[37] was carried out. The protein not only acted as the catalyst but also as a template for the MIPs. As a result, hemoglobin-selective, highly sensitive biosensors with a lower limit of detection of 3.2×10^{-14} mg mL^{-1} and an impressive linear range (spanning protein concentrations over 12 orders of magnitude) were achieved. The dendritic gold electrodes had better performance characteristics than the planar electrodes because of the higher surface area of the nanodendrites.

Controlled radical polymerizations with living cells. A yet largely unexplored aspect of biocatalytic CRPs is that they can be conducted with living systems, therefore bridging polymer chemistry into biology. First examples are bioATRP[25] and bio-RAFT[60] catalyzed by red blood cells (*vide supra*), but the stability of the cells during polymerization remains an issue.[60] The electroactive bacteria *Shewanella oneidensis* has been explored by Keitz and coworkers as a living reduction system to regenerate copper-complex-based ATRP catalysts through an extracellular electron transfer process (Figure 7).[96] These reports show that cells cannot only act as catalysts to initiate and control radical polymerizations, but can also control other important aspects of CRPs. Full cell biocatalysis could therefore be developed as a means to produced well-defined polymers by fermentations, or to prepare polymers in, on, or around cells, *e.g.* as novel therapeutic approach or biosensing mechanism.

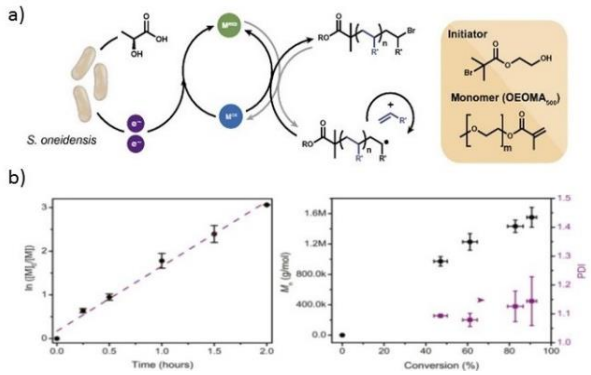


Figure 7. *S. oneidensis* as living reductant for conventional ATRP. a) Reaction scheme; b) Reaction kinetics and evolution of molecular weight and dispersity during the ATRP of PEGMA catalyzed by tris(2-pyridylmethyl)amine copper(II). Adapted from Ref. 96 with permission from the National Academy of Sciences. Copyright 2018.

Perspectives and outlook. Many interesting and important aspects of biocatalytic controlled radical polymerizations have not yet been investigated. The first advantage that comes to mind when discussing enzymes for any kind of biotransformation is that they are natural, non-toxic, and biodegradable compounds that are derived from renewable resources. Thus, they are generally considered to be green, environmentally friendly catalysts.[3] While this statement is true in many cases, the equation *"enzyme = green"* is too simplistic and the whole process of a reaction, including the used solvents, the atom efficiency of the reaction, the energy and efforts required to produce the enzymes, the recyclability of the catalyst, etc. has to be

taken into account in a holistic evaluation of the environmentally friendliness of a given biocatalysis.[97] Unfortunately, this is seldom done in academic research. It remains yet to be determined how green biocatalytic controlled radical polymerizations really are. The fact that most enzymes are non-toxic compounds allows to assume that the materials prepared by biocatalytic polymerizations are suitable for biomedical applications or for food contact, even if traces of the catalysts remain within the polymer. However, this has also not been investigated. Studies that compare, *e.g.* the cytotoxicity of polymeric materials synthesized by bioATRP to those synthesized by copper-complex-mediated ATRP would be highly desirable.

The second important advantage of biocatalysis is that enzymes often display a high stereo-, regio-, or substrate selectivity,[1] thus enabling syntheses that are not possible with conventional catalysts. The available data on bioATRP and bioRAFT does not hint towards a stereo- selectivity induced by the biocatalysts. The resulting polymers are atactic, judging from their NMR spectra. This is not surprising, because the radical chain growth reactions of ATRP and RAFT polymerizations usually do not happen on the catalyst itself but free in solution.[98] Thus, the role of the bioATRP catalyst is to activate and deactivate the chain ends, but not to guide monomers in a specific orientation to the growing chain. Nevertheless, in-depth and systematic studies on the selectivity of biocatalytic controlled radical polymerizations have still to be carried out. It could be, *e.g.* that bulky asymmetric monomers interact with the active site of enzymes so that the enzyme can induce stereo-, regio-, or substrate selectivity. Moreover, it might be possible to induce chemo selectivity between different (macro)initiators by fine tuning the interactions of the initiator with the enzyme, *e.g.* at hydrophobic secondary binding sites, in analogy to what has been reported for the processive polymerization of carbohydates.[99-100]

As enzymes are water-soluble, it is straightforward to synthesize water-soluble polymers with them. However, enzymes are also stable and active in non-aqueous media such as organic solvents.[73-74, 101-103] Enzymatic CRPs in non-aqueous conditions would greatly expand the scope of the polymers that can be biocatalytically synthesized. An alternative is to conduct dispersion polymerizations,[58] or to use enzymatic cofactors, such as modified hemes, which can be directly applied in organic solvents such as anisol[33]

Systematic studies to understand mechanistic details or the structure-activity relationships for biocatalysts in controlled radical polymerizations have not been reported, except for the seminal work on CRP-catalysis by heme derivatives.[46] Computational simulations could be useful to model the interaction of the catalysts' active site with the growing polymer chains. Similarly, the parameter space for these reactions is huge and involves, *e.g.* the pH, temperature, reagent concentration, influence of salts and buffers, choice of reducing agent and type, and biological source of metallo-enzymes. Only a few combinations of parameters were explored to date and we are far from a predictive understanding of such parameters on the performance of biocatalysts in controlled radical polymerizations. A first detailed investigation of the influence of the concentration of the various reagents in HRP-initiated RAFT polymerizations showed *e.g.* that an optimum RAFT agent concentration is essential for well-controlled polymerizations, as a too high concentration of the RAFT agent inhibits the activity of HRP.[57] Similar detailed studies are urgently required for other biocatalytic CRPs in order to increase their performance and to understand the underlying chemistry.

While it is very tempting to reduce the catalytic activity of enzymes to their active site or even their prosthetic group, this is an oversimplified approach. The protein structure gives a defined steric environment around the metal centers and it provides an intricate network of hydrogen bonds, electrostatic interactions, and hydrophobic interactions that bring the redox active metal centers into the right reduction potential.[104-105] Moreover, amino acids can participate directly in electron transfer reactions to and from the metal center.[105] While this makes biocatalysts more complex to understand than conventional metal complex catalysts, it also offers the tremendous opportunity to engineer the enzyme towards, *e.g.* higher catalytic turnover, higher or different selectivity, or an increased thermal stability.[102, 106-107] Biocatalytic controlled radical polymerizations have so far only been carried out with off-the-shelf enzymes and the opportunities of protein engineering still wait to be exploited for the benefit of radical polymerizations.

Building off of the natural promiscuity of enzymes and the ability to construct tailor-made active sites and secondary coordination spheres, one can imagine that these biocatalysts can be used beyond the scope of the above-mentioned polymerization techniques. These newly designed enzymes could potentially be applied to other CRP methods as well as other atom transfer techniques, *e.g.* atom transfer radical addition (ATRA) and atom transfer radical coupling (ATRC).

Conclusions. The discovery that certain metalloproteins catalyze ATRP by reversible dehalogenation and halogenation reactions has shown that radical polymerizations can not only be initiated by biomolecules, but also controlled by them. As a consequence, various biocatalytic controlled radical polymerizations have been developed in recent years. In this Viewpoint, we have highlighted those reactions and applications that demonstrate the advantages that arise from using biocatalysts instead of conventional polymerization systems. It is, however, also clear that significant research efforts will be necessary to further improve the performance of biocatalysts, *e.g.* to enhance the degree of control in the polymerizations and to gain a fundamental understanding of the involved reactions. Nevertheless, the field of biocatalytic controlled radical polymerizations is still in its infancy and offers significant chances and opportunities, especially if the unique properties of bio(macro)molecules are exploited in creative ways.

AUTHOR INFORMATION

Corresponding Author

* nico.bruns@unifr.ch

Present Addresses

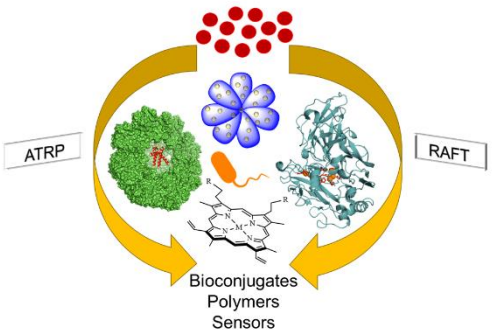
[†]Department of Physical Chemistry, University of Basel, Mattenstrasse 24a, BPR 1096, 4058 Basel, Switzerland.

ACKNOWLEDGMENT

The authors thank the Swiss National Science Foundation (grants No. PP00P2_144697, PP00P2_172927) and Sciex-NMS[ch] (BioCRP, 13.085) for financial support.

REFERENCES

TOC Image:



1. Loos, K., *Biocatalysis in Polymer Chemistry*. Wiley-VCH: Weinheim, Germany, 2010.
2. Kadokawa, J.-I.; Kobayashi, S., Polymer Synthesis by Enzymatic Catalysis. *Curr. Opin. Chem. Biol.* **2010**, *14* (2), 145-153.
3. Shoda, S.-I.; Uyama, H.; Kadokawa, J.-I.; Kimura, S.; Kobayashi, S., Enzymes as Green Catalysts for Precision Macromolecular Synthesis. *Chem. Rev.* **2016**, *116* (4), 2307-2413.
4. Albertsson, A. C.; Srivastava, R. K., Recent Developments in Enzyme-catalyzed Ring-opening Polymerization. *Adv. Drug Delivery Rev.* **2008**, *60* (9), 1077-1093.
5. Spinella, S.; Ganesh, M.; Lo Re, G.; Zhang, S.; Raquez, J. M.; Dubois, P.; Gross, R. A., Enzymatic Reactive Extrusion: Moving Towards Continuous Enzyme-catalysed Polyester Polymerisation and Processing. *Green Chem.* **2015**, *17* (8), 4146-4150.
6. Uyama, H.; Kobayashi, S., Enzymatic Synthesis of Polyesters via Polycondensation. In *Enzyme-Catalyzed Synthesis of Polymers*, Kobayashi, S.; Ritter, H.; Kaplan, D., Eds. Springer-Verlag Berlin: Berlin, 2006; Vol. 194, pp 133-158.
7. Tang, L.; Navarro, L. A.; Chilkoti, A.; Zauscher, S., High-Molecular-Weight Polynucleotides by Transferase-Catalyzed Living Chain-Growth Polycondensation. *Angew. Chem., Int. Ed.* **2017**, *56* (24), 6778-6782.
8. Hollmann, F.; Arends, I. W. C. E., Enzyme Initiated Radical Polymerizations. *Polymers* **2012**, *4* (1), 759-793.
9. Wang, X.; Chen, S.; Wu, D.; Wu, Q.; Wei, Q.; He, B.; Lu, Q.; Wang, Q., Oxidoreductase-Initiated Radical Polymerizations to Design Hydrogels and Micro/Nanogels: Mechanism, Molding, and Applications. *Adv. Mater.* **2018**, *30* (17), 1705668.
10. Riva, S., Laccases: Blue Enzymes for Green Chemistry. *Trends Biotechnol.* **2006**, *24* (5), 219-226.
11. Uyama, H.; Kobayashi, S., Enzymatic Synthesis and Properties of Polymers from Polyphenols. *Adv. Polym. Sci.* **2006**, *194*, 51-67.
12. Hatfield, R.; Vermerris, W., Lignin Formation in Plants. The Dilemma of Linkage Specificity. *Plant Physiol.* **2001**, *126* (4), 1351-1357.
13. d'Ischia, M.; Wakamatsu, K.; Napolitano, A.; Briganti, S.; Garcia-Borron, J. C.; Kovacs, D.; Meredith, P.; Pezzella, A.; Picardo, M.; Sarna, T.; Simon, J. D.; Ito, S., Melanins and Melanogenesis: Methods, Standards, Protocols. *Pigment Cell Melanoma Res.* **2013**, *26* (5), 616-633.
14. Hollmann, F., Enzymatic Polymerization of Vinyl Polymers. In *Biocatalysis in Polymer Chemistry*, Loos, K., Ed. Wiley-VCH: Weinheim, 2010; pp 143-164.

15. Guo, Z.; Hauser, N.; Moreno, A.; Ishikawa, T.; Walde, P., AOT Vesicles as Templates for the Horseradish Peroxidase-triggered Polymerization of Aniline. *Soft Matter* **2011**, *7* (1), 180-193.
16. Ayyagari, M.; Akkara, J. A.; Kaplan, D. L., Enzyme-mediated Polymerization Reactions: Peroxidase-catalyzed Polyphenol Synthesis. *Acta Polym.* **1996**, *47* (5), 193-203.
17. Desentis-Mendoza, R. M.; Hernández-Sánchez, H.; Moreno, A.; Rojas del C, E.; Chel-Guerrero, L.; Tamariz, J.; Jaramillo-Flores, M. E., Enzymatic Polymerization of Phenolic Compounds Using Laccase and Tyrosinase from Ustilago maydis. *Biomacromolecules* **2006**, *7* (6), 1845-1854.
18. Greimel, K. J.; Kudanga, T.; Nousiainen, P.; Sipilä, J.; Herrero Acero, E.; Nyanhongo, G. S.; Guebitz, G. M., Two Distinct Enzymatic Approaches for Coupling Fatty Acids onto Lignocellulosic Materials. *Process Biochem.* **2017**, *59*, 111-115.
19. Prasetyo E. N.; Kudanga, T.; Fischer, R.; Eichinger, R.; Nyanhongo G. S.; Guebitz G. M., Enzymatic Synthesis of Lignin–Siloxane Hybrid Functional Polymers. *Biotechnol. J.* **2011**, *7* (2), 284-292.
20. Matyjaszewski, K., Atom Transfer Radical Polymerization (ATRP): Current Status and Future Perspectives. *Macromolecules* **2012**, *45* (10), 4015-4039.
21. Ouchi, M.; Terashima, T.; Sawamoto, M., Transition Metal-Catalyzed Living Radical Polymerization: Toward Perfection in Catalysis and Precision Polymer Synthesis. *Chem. Rev.* **2009**, *109* (11), 4963-5050.
22. Keddie, D. J., A Guide to the Synthesis of Block Copolymers using Reversible-Addition Fragmentation Chain Transfer (RAFT) Polymerization. *Chem. Soc. Rev.* **2014**, *43* (2), 496-505.
23. Tian, X.; Ding, J.; Zhang, B.; Qiu, F.; Zhuang, X.; Chen, Y., Recent Advances in RAFT Polymerization: Novel Initiation Mechanisms and Optoelectronic Applications. *Polymers* **2018**, *10* (3), 318.
24. Sigg, S. J.; Seidi, F.; Renggli, K.; Silva, T. B.; Kali, G.; Bruns, N., Horseradish Peroxidase as a Catalyst for Atom Transfer Radical Polymerization. *Macromol. Rapid Commun.* **2011**, *32* (21), 1710-1715.
25. Silva, T. B.; Spulber, M.; Kocik, M. K.; Seidi, F.; Charan, H.; Rother, M.; Sigg, S. J.; Renggli, K.; Kali, G.; Bruns, N., Hemoglobin and Red Blood Cells Catalyze Atom Transfer Radical Polymerization. *Biomacromolecules* **2013**, *14* (8), 2703-2712.
26. Kali, G.; Silva, T. B.; Sigg, S. J.; Seidi, F.; Renggli, K.; Bruns, N., ATRPases: Using Nature's Catalysts in Atom Transfer Radical Polymerizations. In *Progress in Controlled Radical Polymerization: Mechanisms and Techniques, ACS Symposium Series*, American Chemical Society: 2012; Vol. 1100, pp 171-181.
27. Ng, Y.-H.; di Lena, F.; Chai, C. L. L., Metalloenzymatic Radical Polymerization using Alkyl Halides as Initiators. *Polym. Chem.* **2011**, *2* (3), 589-594.
28. Ng, Y.-H.; di Lena, F.; Chai, C. L. L., PolyPEGA with Predetermined Molecular Weights from Enzyme-mediated Radical Polymerization in Water. *Chem. Commun.* **2011**, *47* (22), 6464-6466.
29. Fodor, C.; Gajewska, B.; Rifaie-Graham, O.; Apebende, E. A.; Pollard, J.; Bruns, N., Laccase-catalyzed Controlled Radical Polymerization of N-vinylimidazole. *Polym. Chem.* **2016**, *7* (43), 6617-6625.
30. Dinu, M. V.; Spulber, M.; Renggli, K.; Wu, D.; Monnier, C. A.; Petri-Fink, A.; Bruns, N., Filling Polymersomes with Polymers by Peroxidase-Catalyzed Atom Transfer Radical Polymerization. *Macromol. Rapid Commun.* **2015**, *36* (6), 507-514.
31. Renggli, K.; Sauter, N.; Rother, M.; Nussbaumer, M. G.; Urbani, R.; Pfohl, T.; Bruns, N., Biocatalytic Atom Transfer Radical Polymerization in a Protein Cage Nanoreactor. *Polym. Chem.* **2017**, *8* (14), 2133-2136.
32. Divandari, M.; Pollard, J.; Dehghani, E.; Bruns, N.; Benetti, E. M., Controlling Enzymatic Polymerization from Surfaces with Switchable Bioaffinity. *Biomacromolecules* **2017**, *18* (12), 4261-4270.

33. Simakova, A.; Mackenzie, M.; Averick, S. E.; Park, S.; Matyjaszewski, K., Bioinspired Iron-Based Catalyst for Atom Transfer Radical Polymerization. *Angew. Chem., Int. Ed.* **2013**, *52* (46), 12148-12151.
34. Gao, G.; Karaaslan, M. A.; Kadla, J. F.; Ko, F., Enzymatic Synthesis of Ionic Responsive Lignin Nanofibres through Surface poly(N-isopropylacrylamide) Immobilization. *Green Chem.* **2014**, *16* (8), 3890-3898.
35. Kreuzer, L. P.; Männel, M. J.; Schubert, J.; Höller, R. P. M.; Chanana, M., Enzymatic Catalysis at Nanoscale: Enzyme-Coated Nanoparticles as Colloidal Biocatalysts for Polymerization Reactions. *ACS Omega* **2017**, *2* (10), 7305-7312.
36. Sun, Y.; Du, H.; Lan, Y.; Wang, W.; Liang, Y.; Feng, C.; Yang, M., Preparation of Hemoglobin (Hb) Imprinted Polymer by Hb Catalyzed eATRP and its Application in Biosensor. *Biosens. Bioelectron.* **2016**, *77*, 894-900.
37. Sun, Y.; Zhang, J.; Li, J.; Zhao, M.; Liu, Y., Preparation of Protein Imprinted Polymers via Protein-catalyzed eATRP on 3D Gold Nanodendrites and their Application in Biosensors. *RSC Adv.* **2017**, *7* (45), 28461-28468.
38. Asandei, A. D.; Moran, I. W.; Brückner, C., *Tetraphenyl Porphyrin Transition Metal Catalysts in the Living Radical Polymerization of Styrene.* 2003; Vol. 44, p 827-828.
39. Dong, H.; Hou, T.; Zhao, Y.; Fu, X.; Li, Y., DFT Study of Cobalt Porphyrin Complex for Living Radical Polymerization of Olefins. *Comput. Theor. Chem.* **2012**, *1001*, 51-59.
40. Hsu, C.-S.; Yang, T.-Y.; Peng, C.-H., Vinyl Acetate Living Radical Polymerization Mediated by Cobalt Porphyrins: Kinetic–Mechanistic Studies. *Polym. Chem.* **2014**, *5* (12), 3867-3875.
41. Shanmugam, S.; Xu, J.; Boyer, C., Exploiting Metalloporphyrins for Selective Living Radical Polymerization Tunable over Visible Wavelengths. *J. Am. Chem. Soc.* **2015**, *137* (28), 9174-85.
42. Tomoyuki, O.; Masahīko, T.; Yoshiaki, I.; Kiichi, T., Photopolymerization of Vinyl Monomers with the System–Tetraphenyl Porphine-Iron(III), Amine, and Carbon Tetrachloride. *J. Polym. Sci. Polym. Lett. Ed.* **1974**, *12* (3), 121-124.
43. Singh, A.; Roy, S.; Samuelson, L.; Bruno, F.; Nagarajan, R.; Kumar, J.; John, V.; Kaplan, D. L., Peroxidase, Hematin, and Pegylated-hematin Catalyzed Vinyl Polymerizations in Water. *J. Macromol. Sci.-Pure Appl. Chem., A* **2001**, *38* (12), 1219-1230.
44. Kadokawa, J.-I.; Kokubo, A.; Tagaya, H., Free-radical Polymerization of Vinyl Monomers using Hematin as a Biomimetic Catalyst in Place of Enzyme. *Macromol. Biosci.* **2002**, *2* (6), 257-260.
45. Yamashita, K.; Yamamoto, K.; Kadokawa, J.-I., Atom Transfer Radical Polymerization of N-isopropylacrylamide by Enzyme Mimetic Catalyst. *Polymer* **2013**, *54* (7), 1775-1778.
46. Smolne, S.; Buback, M.; Demeshko, S.; Matyjaszewski, K.; Meyer, F.; Schroeder, H.; Simakova, A., Kinetics of Fe–Mesohemin–(MPEG500)2-Mediated RDRP in Aqueous Solution. *Macromolecules* **2016**, *49* (21), 8088-8097.
47. Fu, L.; Simakova, A.; Fantin, M.; Wang, Y.; Matyjaszewski, K., Direct ATRP of Methacrylic Acid with Iron-Porphyrin Based Catalysts. *ACS Macro Lett.* **2018**, *7* (1), 26-30.
48. Zhou, H.; Jiang, W.; An, N.; Zhang, Q.; Xiang, S.; Wang, L.; Tang, J., Enzyme Mimetic-catalyzed ATRP and its Application in Block Copolymer Synthesis Combined with Enzymatic Ring-opening Polymerization. *RSC Adv.* **2015**, *5* (53), 42728-42735.
49. Zhou, H.; Wang, X.; Tang, J.; Yang, Y.-W., Surface Immobilization of pH-Responsive Polymer Brushes on Mesoporous Silica Nanoparticles by Enzyme Mimetic Catalytic ATRP for Controlled Cargo Release. *Polymers* **2016**, *8* (8), 277.
50. Wang, X.; Yun, W.; Jiang, W.; Wang, D.; Zhang, L.; Tang, J., An Amphiphilic Non-viral Gene Vector Prepared by a Combination of Enzymatic Atom Transfer Radical Polymerization and Enzymatic Ring-opening Polymerization. *RSC Adv.* **2017**, *7* (16), 9926-9932.
51. Jiang, W.; Pan, Y.; Yang, J.; Liu, Y.; Yang, Y.; Tang, J.; Li, Q., A Peroxidase Mimic with Atom Transfer Radical Polymerization Activity Constructed through the Grafting of Heme onto Metal-Organic Frameworks. *J. Colloid Interface Sci.* **2018**, *521*, 62-68.

52. Cole, J. P.; Hanlon, A. M.; Rodriguez, K. J.; Berda, E. B., Protein-like Structure and Activity in Synthetic Polymers. *J. Polym. Sci., Part A: Polym. Chem.* **2016**, *55* (2), 191-206.
53. Sanchez-Sanchez, A.; Arbe, A.; Kohlbrecher, J.; Colmenero, J.; Pomposo, J. A., Efficient Synthesis of Single-chain Globules Mimicking the Morphology and Polymerase Activity of Metalloenzymes. *Macromol. Rapid Commun.* **2015**, *36* (17), 1592-1597.
54. Zhang, B.; Wang, X.; Zhu, A.; Ma, K.; Lv, Y.; Wang, X.; An, Z., Enzyme-Initiated Reversible Addition–Fragmentation Chain Transfer Polymerization. *Macromolecules* **2015**, *48* (21), 7792-7802.
55. Liu, Z.; Lv, Y.; Zhu, A.; An, Z., One-Enzyme Triple Catalysis: Employing the Promiscuity of Horseradish Peroxidase for Synthesis and Functionalization of Well-Defined Polymers. *ACS Macro Lett.* **2017**, *7* (1), 1-6.
56. Danielson, A. P.; Van Kuren, D. B.; Lucius, M. E.; Makaroff, K.; Williams, C.; Page, R. C.; Berberich, J. A.; Konkolewicz, D., Well-Defined Macromolecules Using Horseradish Peroxidase as a RAFT Initiase. *Macromol. Rapid Commun.* **2016**, *37* (4), 362-367.
57. Danielson, A.; Van-Kuren, D.; Bornstein, J.; Kozuszek, C.; Berberich, J.; Page, R.; Konkolewicz, D., Investigating the Mechanism of Horseradish Peroxidase as a RAFT-Initiase. *Polymers* **2018**, *10* (7), 741.
58. Tan, J.; Xu, Q.; Li, X.; He, J.; Zhang, Y.; Dai, X.; Yu, L.; Zeng, R.; Zhang, L., Enzyme-PISA: An Efficient Method for Preparing Well-Defined Polymer Nano-Objects under Mild Conditions. *Macromol. Rapid Commun.* **2018**, *39* (9), 1700871.
59. Wang, X.-H.; Wu, M.-X.; Jiang, W.; Yuan, B.-L.; Tang, J.; Yang, Y.-W., Nanoflower-Shaped Biocatalyst with Peroxidase Activity Enhances the Reversible Addition–Fragmentation Chain Transfer Polymerization of Methacrylate Monomers. *Macromolecules* **2018**, *51* (3), 716-723.
60. Reyhani, A.; Nothling, M. D.; Ranji-Burachaloo, H.; McKenzie, T. G.; Fu, Q.; Tan, S.; Bryant, G.; Qiao, G. G., Blood-Catalyzed RAFT Polymerization. *Angew. Chem., Int. Ed.* **2018**, *57*, 10288-10292.
61. Shanmugam, S.; Xu, J.; Boyer, C., Utilizing the Electron Transfer Mechanism of Chlorophyll a under Light for Controlled Radical Polymerization. *Chem. Sci.* **2015**, *6* (2), 1341-1349.
62. Shanmugam, S.; Xu, J.; Boyer, C., Light-Regulated Polymerization under Near-Infrared/Far-Red Irradiation Catalyzed by Bacteriochlorophyll a. *Angew. Chem., Int. Ed.* **2016**, *55* (3), 1036-1040.
63. Ng, Y.-H.; di Lena, F.; Chai, C. L. L., On the Use of Cob(II)alamin as a Spin Trap in Radical Polymerization. *Macromol. Res.* **2012**, *20* (5), 473-476.
64. Wang, C.-G.; Hanindita, F.; Goto, A., Biocompatible Choline Iodide Catalysts for Green Living Radical Polymerization of Functional Polymers. *ACS Macro Lett.* **2018**, *7* (2), 263-268.
65. Asayama, S.; Nishinohara, S.; Kawakami, H., Zinc-Chelated Poly(1-vinylimidazole) and a Carbohydrate Ligand Polycation Form DNA Ternary Complexes for Gene Delivery. *Bioconjugate Chem.* **2011**, *22* (9), 1864-1868.
66. Allen, M. H.; Day, K. N.; Hemp, S. T.; Long, T. E., Synthesis of Folic Acid-Containing Imidazolium Copolymers for Potential Gene Delivery Applications. *Macromol. Chem. Phys.* **2013**, *214* (7), 797-805.
67. Detering, J.; Schade, C.; Trieselt, W.; Tropsch, J., Use of Vinylpyrrolidone and Vinylimidazole Copolymers as Detergent Additives, Novel Polymers of Vinylpyrrolidone and of Vinylimidazole, and Preparation Thereof. Google Patents: 1998.
68. Yuan, J.; Mecerreyes, D.; Antonietti, M., Poly(ionic liquid)s: An Update. *Prog. Polym. Sci.* **2013**, *38* (7), 1009-1036.
69. Allen, M. H.; Hemp, S. T.; Smith, A. E.; Long, T. E., Controlled Radical Polymerization of 4-Vinylimidazole. *Macromolecules* **2012**, *45* (9), 3669-3676.
70. Green, M. D.; Allen Jr, M. H.; Dennis, J. M.; Cruz, D. S.-D. L.; Gao, R.; Winey, K. I.; Long, T. E., Tailoring Macromolecular Architecture with Imidazole Functionality: A Perspective for Controlled Polymerization Processes. *Eur. Polym. J.* **2011**, *47* (4), 486-496.
71. Englard, S.; Seifter, S., Precipitation Techniques. *Methods Enzymol.* **1990**, *182*, 285-300.

72. Wingfield, P. T., Protein Precipitation Using Ammonium Sulfate. *Curr. Protoc. Protein Sci.* **2016**, *84*, A.3F.1-A.3F.9.
73. Paeth, M.; Stapleton, J.; Dougherty, M. L.; Fischesser, H.; Shepherd, J.; McCauley, M.; Falatach, R.; Page, R. C.; Berberich, J. A.; Konkolewicz, D., Approaches for Conjugating Tailor-Made Polymers to Proteins. *Methods Enzymol.* **2017**, *590*, 193-224.
74. Lucius, M.; Falatach, R.; McGlone, C.; Makaroff, K.; Danielson, A.; Williams, C.; Nix, J. C.; Konkolewicz, D.; Page, R. C.; Berberich, J. A., Investigating the Impact of Polymer Functional Groups on the Stability and Activity of Lysozyme–Polymer Conjugates. *Biomacromolecules* **2016**, *17* (3), 1123-1134.
75. Janson, J.-C., *Protein Purification: Principles, High Resolution Methods, and Applications*. 3rd ed.; John Wiley & Sons: Hoboken, New Jersey, USA, 2011.
76. Gibson, Q. H.; Swoboda, B. E. P.; Massey, V., Kinetics and Mechanism of Action of Glucose Oxidase. *J. Biol. Chem.* **1964**, *239* (11), 3927-3934.
77. Giffhorn, F., Fungal Pyranose Oxidases: Occurrence, Properties and Biotechnical Applications in Carbohydrate Chemistry. *Appl. Microbiol. Biotechnol.* **2000**, *54* (6), 727-740.
78. Chapman, R.; Gormley, A. J.; Stenzel, M. H.; Stevens, M. M., Combinatorial Low-Volume Synthesis of Well-Defined Polymers by Enzyme Degassing. *Angew. Chem., Int. Ed.***2016**, *55* (14), 4500-4503.
79. Chapman, R.; Gormley, A. J.; Herpoldt, K.-L.; Stevens, M. M., Highly Controlled Open Vessel RAFT Polymerizations by Enzyme Degassing. *Macromolecules* **2014**, *47* (24), 8541-8547.
80. Enciso, A. E.; Fu, L.; Russell, A. J.; Matyjaszewski, K., A Breathing Atom-Transfer Radical Polymerization: Fully Oxygen-Tolerant Polymerization Inspired by Aerobic Respiration of Cells. *Angew. Chem., Int. Ed.* **2018**, *57* (4), 933-936.
81. Liu, Z.; Lv, Y.; An, Z., Enzymatic Cascade Catalysis for the Synthesis of Multiblock and Ultrahigh-Molecular-Weight Polymers with Oxygen Tolerance. *Angew. Chem., Int. Ed.* **2017**, *56* (44), 13852-13856.
82. Lv, Y.; Liu, Z.; Zhu, A.; An, Z., Glucose Oxidase Deoxygenation−Redox Initiation for RAFT Polymerization in Air. *J. Polym. Sci., Part A: Polym. Chem.* **2017**, *55* (1), 164-174.
83. Gormley, A. J.; Yeow, J.; Ng, G.; Conway, Ó.; Boyer, C.; Chapman, R., An Oxygen-Tolerant PET-RAFT Polymerization for Screening Structure–Activity Relationships. *Angew. Chem., Int. Ed.* **2018**, *57* (6), 1557-1562.
84. Sheldon, R. A., Enzyme Immobilization: The Quest for Optimum Performance. *Adv. Synth. Catal.* **2007**, *349* (8-9), 1289-1307.
85. Tran, D. N.; Balkus, K. J., Perspective of Recent Progress in Immobilization of Enzymes. *ACS Catalysis* **2011**, *1* (8), 956-968.
86. Misson, M.; Zhang, H.; Jin, B., Nanobiocatalyst Advancements and Bioprocessing Applications. *J. Royal Soc. Interface* **2015**, *12* (102), 20140891.
87. Männel, M. J.; Kreuzer, L. P.; Goldhahn, C.; Schubert, J.; Hartl, M. J.; Chanana, M., Catalytically Active Protein Coatings: Toward Enzymatic Cascade Reactions at the Intercolloidal Level. *ACS Catalysis* **2017**, *7* (3), 1664-1672.
88. Renggli, K.; Baumann, P.; Langowska, K.; Onaca, O.; Bruns, N.; Meier, W., Selective and Responsive Nanoreactors. *Adv. Funct. Mater.* **2011**, *21* (7), 1241-1259.
89. Peters, R. J. R. W.; Louzao, I.; van Hest, J. C. M., From Polymeric Nanoreactors to Artificial Organelles. *Chem. Sci.* **2012**, *3* (2), 335-342.
90. Gaitzsch, J.; Huang, X.; Voit, B., Engineering Functional Polymer Capsules toward Smart Nanoreactors. *Chem. Rev.* **2016**, *116* (3), 1053-1093.
91. Rother, M.; Nussbaumer, M. G.; Renggli, K.; Bruns, N., Protein Cages and Synthetic Polymers: a Fruitful Symbiosis for Drug Delivery Applications, Bionanotechnology and Materials Science. *Chem. Soc. Rev.* **2016**, *45* (22), 6213-6249.
92. Aumiller, W. M.; Uchida, M.; Douglas, T., Protein Cage Assembly Across Multiple Length Scales. *Chem. Soc. Rev.* **2018**, *47* (10), 3433-3469.

93. Zetterlund, P. B., Controlled/Living Radical Polymerization in Nanoreactors: Compartmentalization Effects. *Polym. Chem.* **2011**, *2* (3), 534-549.
94. Zoppe, J. O.; Ataman, N. C.; Mocny, P.; Wang, J.; Moraes, J.; Klok, H.-A., Surface-Initiated Controlled Radical Polymerization: State-of-the-Art, Opportunities, and Challenges in Surface and Interface Engineering with Polymer Brushes. *Chem. Rev.* **2017**, *117* (3), 1105-1318.
95. Yang, J.; Lu, S.; Xing, T.; Chen, G., Preparation, Structure, and Properties of Silk Fabric Grafted with 2-Hydroxypropyl Methacrylate Using the HRP Biocatalyzed ATRP Method. *Polymers* **2018**, *10* (5).
96. Fan, G.; Dundas, C. M.; Graham, A. J.; Lynd, N. A.; Keitz, B. K., *Shewanella oneidensis* as a Living Electrode for Controlled Radical Polymerization. *Proc. Natl. Acad. Sci. U.S.A.* **2018**, *115* (18), 4559-4564.
97. Ni, Y.; Holtmann, D.; Hollmann, F., How Green is Biocatalysis? To Calculate is To Know. *ChemCatChem* **2014**, *6* (4), 930-943.
98. Fischer, H., The Persistent Radical Effect: A Principle for Selective Radical Reactions and Living Radical Polymerizations. *Chem. Rev.* **2001**, *101* (12), 3581-3610.
99. May, J. F.; Splain, R. A.; Brotschi, C.; Kiessling, L. L., A Tethering Mechanism for Length Control in a Processive Carbohydrate Polymerization. *Proc. Natl. Acad. Sci. U. S. A.* **2009**, *106* (29), 11851-11856.
100. Levengood, M. R.; Splain, R. A.; Kiessling, L. L., Monitoring Processivity and Length Control of a Carbohydrate Polymerase. *J. Am. Chem. Soc.* **2011**, *133* (32), 12758-12766.
101. Klibanov, A. M., Improving enzymes by using them in organic solvents. *Nature* **2001**, *409* (6817), 241-246.
102. Liszka, M. J.; Clark, M. E.; Schneider, E.; Clark, D. S., Nature Versus Nurture: Developing Enzymes That Function Under Extreme Conditions. *Annu. Rev. Chem. Biomol. Eng.* **2012**, *3* (1), 77-102.
103. Stepankova, V.; Bidmanova, S.; Koudelakova, T.; Prokop, Z.; Chaloupkova, R.; Damborsky, J., Strategies for Stabilization of Enzymes in Organic Solvents. *ACS Catalysis* **2013**, *3* (12), 2823-2836.
104. Rich, P. R.; Iwaki, M., A Comparison of Catalytic Site Intermediates of Cytochrome c Oxidase and Peroxidases. *Biochemistry (Moscow)* **2007**, *72* (10), 1047-1055.
105. Reeder, B. J., The Redox Activity of Hemoglobins: From Physiologic Functions to Pathologic Mechanisms. *Antioxid. Redox Signaling* **2010**, *13* (7), 1087-1123.
106. Bornscheuer, U. T.; Huisman, G. W.; Kazlauskas, R. J.; Lutz, S.; Moore, J. C.; Robins, K., Engineering the Third Wave of Biocatalysis. *Nature* **2012**, *485* (7397), 185-194.
107. Schwizer, F.; Okamoto, Y.; Heinisch, T.; Gu, Y.; Pellizzoni, M. M.; Lebrun, V.; Reuter, R.; Köhler, V.; Lewis, J. C.; Ward, T. R., Artificial Metalloenzymes: Reaction Scope and Optimization Strategies. *Chem. Rev.* **2018**, *118* (1), 142-231.