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Enzyme- and affinity biomolecule-mediated polymerization systems for biological signal amplification and cell screening

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Enzyme-mediated polymerization and polymerization-based signal amplification have emerged as two closely related techniques that are broadly applicable in the nanobio sciences. We review recent progress on polymerization systems mediated by biological molecules (e.g., affinity molecules and enzymes), and highlight newly developed formats and configurations of these systems to perform such tasks as non-instrumented biodetection, synthesis of core–shell nanomaterials, isolation of rare cells, and high-throughput screening. We discuss useful features of biologically mediated polymerization systems, such as multiple mechanisms of amplification (e.g., enzymatic, radical chain propagation), and the ability to localize structures at interfaces and at cell surfaces with microscopic spatial confinement. We close with a perspective on desirable improvements that need to be addressed to adapt these molecular systems to future applications.

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Introduction

Polymers play a central role in many aspects of our modern society, ranging from consumer goods to industrial strength materials to biotechnology and pharmaceutical products. At the most basic level, polymers are created through a process of polymerization entailing the formation of chemical linkages between monomer units. Classically, polymerization reactions have been performed using organic polymer chemistry which often times requires the use of harsh solvents and environmentally questionable compounds. Given the multitude of environmental pressures facing mankind today, researchers have made a concerted effort to develop green methods for producing polymers. Ideally, new environmentally compatible processes would not compromise on material performance, but could be carried out under mild conditions and with reduced waste streams.

It is within this context of green chemistry that enzyme-mediated radical polymerization is appreciated as a valuable approach to producing synthetic polymers. Enzymes are desirable as polymerization catalysts due to their ability to perform high stereo- and regioselective reactions. As early as 1951, the concept of using enzymes to produce initiators for free-radical chain propagation polymerization was known, when xanthine oxidase was used to polymerize methyl methacrylate [1]. In the 1980s Klibanov *et al.* showed that horseradish peroxidase (HRP) could be used to polymerize phenol compounds from coal-conversion waste waters, and thereby perform environmental remediation [2].

Currently, a range of enzymes are commonly used in the bulk synthesis of phenolic and acrylic-based polymers [3], including peroxidases (e.g., horseradish or soybean peroxidases), oxidases, and laccases. Prominent examples include initiation of radical polymerization by glucose oxidase [4,5] and sarcosine oxidase [6], biocatalytic atom-transfer radical polymerization (ATRP) [7,8], enzyme-mediated reversible addition fragmentation chain transfer (RAFT) [9,10], and enzyme mimetic-catalyzed ATRP [11]. We caution the reader that the numerous examples of enzyme-mediated and affinity biomolecule-mediated polymerization systems are too broad and varied to provide a complete overview of the relevant literature in a single focused review article, therefore the references in this article are not comprehensive. We also caution the reader to take note of the difference between enzyme-mediated polymerization and polymerization systems where a radical initiator (typically photoinitiator) is conjugated to an affinity biomolecule. Both such approaches fall under biologically mediated polymerization, and are discussed in this article. Several relevant related reviews are also provided in Refs. [3,12–25].

Here we focus on two aspects which demonstrate the utility of biologically mediated polymerization systems: (1) the high signal-to-noise ratio due to multiple amplification mechanisms (i.e., enzymatic amplification and amplification through chain-propagation), and (2) the ability to localize the formation of polymeric structures

through molecular recognition events. The first aspect (i.e., multiple amplification mechanisms) is a direct result of the nature of polymerization-based systems. When enzymes are used to generate free radicals, the signal generation benefits from enzymatic turnover, as well as from the fact that a single free radical initiation event is sufficient to polymerize hundreds or thousands of monomer units, effectively amplifying the signal. The second aspect (i.e., microscale spatial localization) works through the localization of catalysts and initiators at interfaces, for example at the surfaces of cells [26••] or cellulose nanocrystals [27••]. As we outline below, both multi-mode signal amplification and microscale spatial localization enable new types of nanobio systems to be developed for applications including biosensing, high-throughput screening and chemical imaging.

Biosensing and signal amplification

The mechanism of radical polymerization, in which one initiation event leads to inclusion of many monomers into a growing polymer chain, is intrinsically an efficient signal amplification scheme. If initiation is coupled to a molecular recognition event, it provides a means for the development of highly sensitive bioassays. Such systems for biological detection fall under the category of polymerization-based amplification (PBA) [25]. In PBA biosensors, affinity biomolecules (e.g., DNA, antibody) are coupled with photoinitiators to amplify molecular recognition events. A wide range of targets have been detected to date using PBA, including nucleotide [28,29] and protein targets [30–34]. The use of free-radical PBA systems for biosensing applications were reviewed by Lou *et al.* [14], and more recently by Wu *et al.* [16], as well as in the wider context of signal amplification strategies by Scrimin *et al.* [15]. The buildup of polymer in response to a biorecognition event can be detected in various ways, for example by colorimetric [33•], fluorescence [5], and surface plasmon assays [35] (see Figure 1).

Enzyme-mediated polymerization has been implemented to detect proteins in an ELISA-style immunoassay, where glucose oxidase (GOx) was coupled with antigen recognition through a biotin–avidin linkage, triggering redox polymerization in the presence of a Fenton reagent and copolymerizing fluorescent dye [5]. The same principle was used to create capillary-flow microfluidic valves that responded to target antigen by clogging a microfluidic channel via rapidly growing hydrogelation. This stimuli-responsive channel blockage changed the fluid flow in the device and resulted in a binary signal that was read by eye (i.e., non-instrumented detection), a feature advantageous in point-of-use biosensing applications [36].

One of the recent trends includes the use of PBA with plasmon-based detection. For example, when immobilized at a glass surface, gold nanoparticles adhered to a poly(2-vinylpyridine) film shifted their absorbance band in response to GOx/Fe(II)-mediated methyl methacrylate polymerization [35]. Other PBA approaches involving plasmonic detection have included improving the sensitivity of surface plasmon resonance (SPR) biosensing through polymerization [37], and increasing the contrast of SPR-imaging detection with polymerization [38]. In bulk solution, flocculation of gold nanoparticles could also be induced by enzymatic polymerization of polycations. The plasmonic coupling of gold nanoparticles leads to yet another level of non-linear signal amplification in such systems, providing extremely low detection limits, down to parts per billion levels for iron and copper [39••].

Nanomaterials synthesis

Apart from biodetection, enzyme-mediated polymerization systems are powerful bottom-up tools to synthesize functional nanomaterials, particularly core–shell, polymer-grafted and multilayer nanoparticles in an environmentally-friendly and efficient process. Several synthesis methods were designed using HRP [40,41] or GOx [42,43] adsorbed or immobilized within pre-formed particles (see Figure 2). The enzymes trapped at the particle-solvent interface then served as radical-generators, inducing polymerization at the interface and enabling core–shell particle synthesis. Monodisperse polystyrene nanoparticles with diameters ranging from 50 to 300 nm were synthesized by Kohri *et al.* using miniemulsion polymerization with a polymerizable surfactant [44], as well as by heterogeneous, emulsifier-free polymerization in presence of β-diketones as initiators [45]. Miniemulsion polymerization was used with polymerizable surfactants/monomers (surfmers) to create functional polystyrene particles displaying phosphonate moieties that were able to bind calcium and initiate apatite growth [46], or alternatively to attach fluorescence dyes via alkyne/azide click-chemistry [47•]. Particularly, the use of clickable-surfmers allows a multitude of functionalizations through the use of simple, water-based, biocompatible and bioorthogonal conjugation chemistry.

In addition to core/shell and nanoparticle synthesis, another current trend has been the use of polymersomes [48–50], liposomes, and even protein chaperonins [51•] as nanoreactors for enzyme-mediated polymerization reactions. Confinement of polymerization reagents inside of nanoreactors can be used to influence the activity through co-encapsulation of other reactants or crowders that may increase the viscosity or reactivity of compounds [52], providing an added degree of control and in some cases stabilizing enzyme catalysts against denaturation. For example, polymersomes formed from diblock copolymers of poly(dimethylsiloxane)-*block*-poly(2-methyl-2-oxazoline) were used to encapsulate HRP enzymes and polymerize PEG methyl ether acrylate within a confined nanoreactor [50]. In another report, lipase B of *Candida Antarctica* was encapsulated within polystyrene–polyisocyanopeptide polymersomes and used for ring-opening