

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

Klara H Malinowska¹ and Michael A Nash^{1,2,3}



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.

Addressees

¹ Lehrstuhl für Angewandte Physik and Center for Nanoscience, Ludwig-Maximilians-Universität, 80799 Munich, Germany

² Department of Chemistry, University of Basel, 4056 Basel, Switzerland

³ Department of Biosystems Science and Engineering, Eidgenössische Technische Hochschule (ETH-Zürich), 4058 Basel, Switzerland

Corresponding author: Nash, Michael A (michael.nash@lmu.de)

Current Opinion in Biotechnology 2016, 39:68–75

This review comes from a themed issue on **Nanobiotechnology**

Edited by **Michael Nash** and **Oded Shoseyov**

<http://dx.doi.org/10.1016/j.copbio.2016.01.007>

0958-1669/© 2016 Elsevier Ltd. All rights reserved.

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].

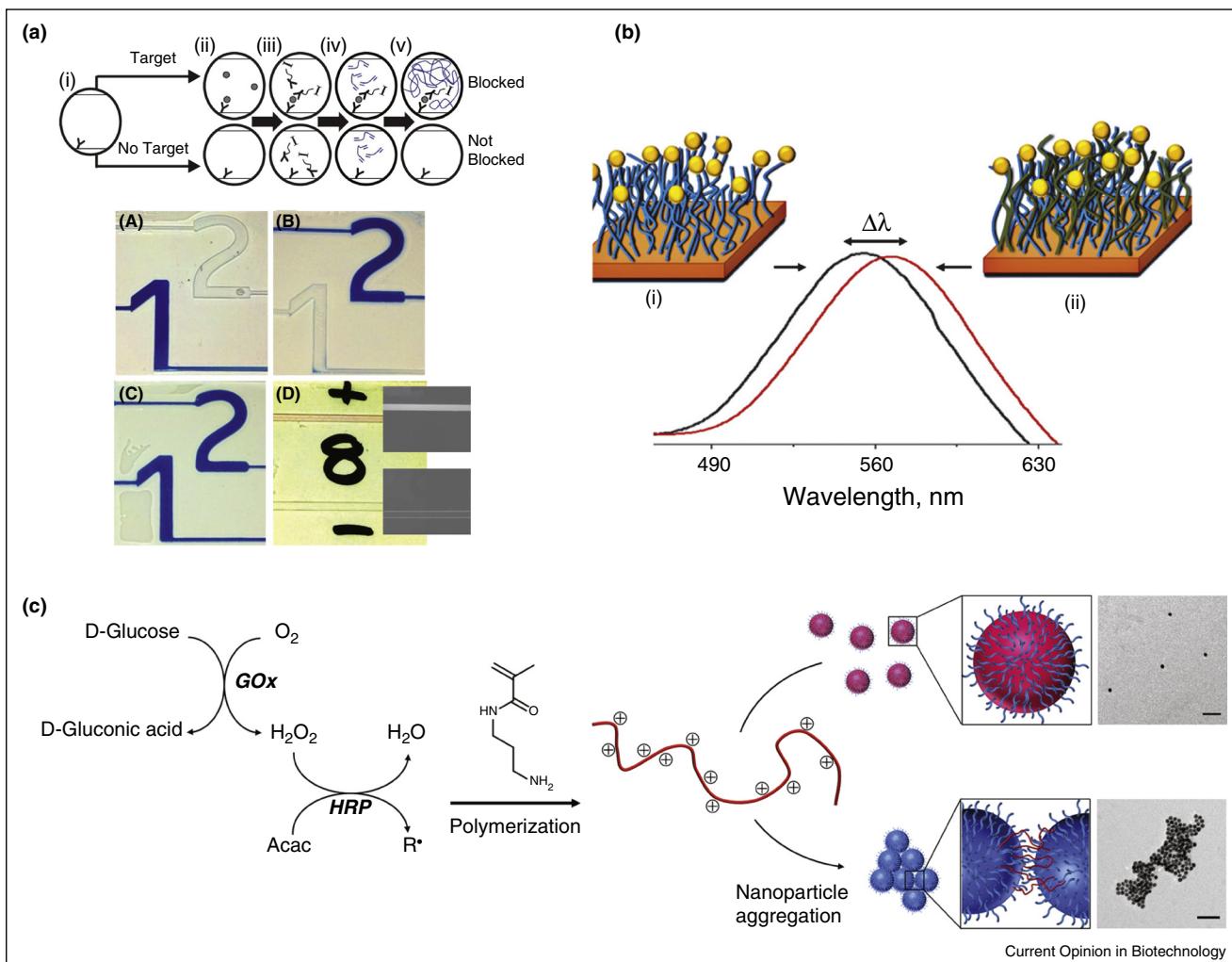
One of the recent trends includes the use of PBA with plasmon-based detection. For example, when immobilized at a glass surface, gold nanoparticles adhered to a poly(2-vinylpyridine) film shifted their absorbance band

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

Figure 1

Enzyme-mediated polymerization systems for signal amplification. **(a)** Molecular schematic depicting a competitive enzyme-linked immunoassay with polymerization-based detection. A simple visual readout of polymerized hydrogel in a microchannel provides a non-instrumented readout for a protein biosensor. Re-printed from Berron *et al.* [36] with permission from RSC. **(b)** The optical resonance of gold nanoparticles in response to polymer formation provides an additional non-linear signal amplification mechanism to further boost sensitivity in polymerization-based biosensors. Reprinted from Recco *et al.* [35] with permission from Wiley-VCH & ChemPubSoc Europe. **(c)** Enzyme-mediated polymerization of a cationic polymer was used to induce flocculation of a gold nanoparticle solution resulting in a color change that was measured photometrically. Reprinted from Gormley *et al.* [39^{**}] Copyright 2014 American Chemical Society.

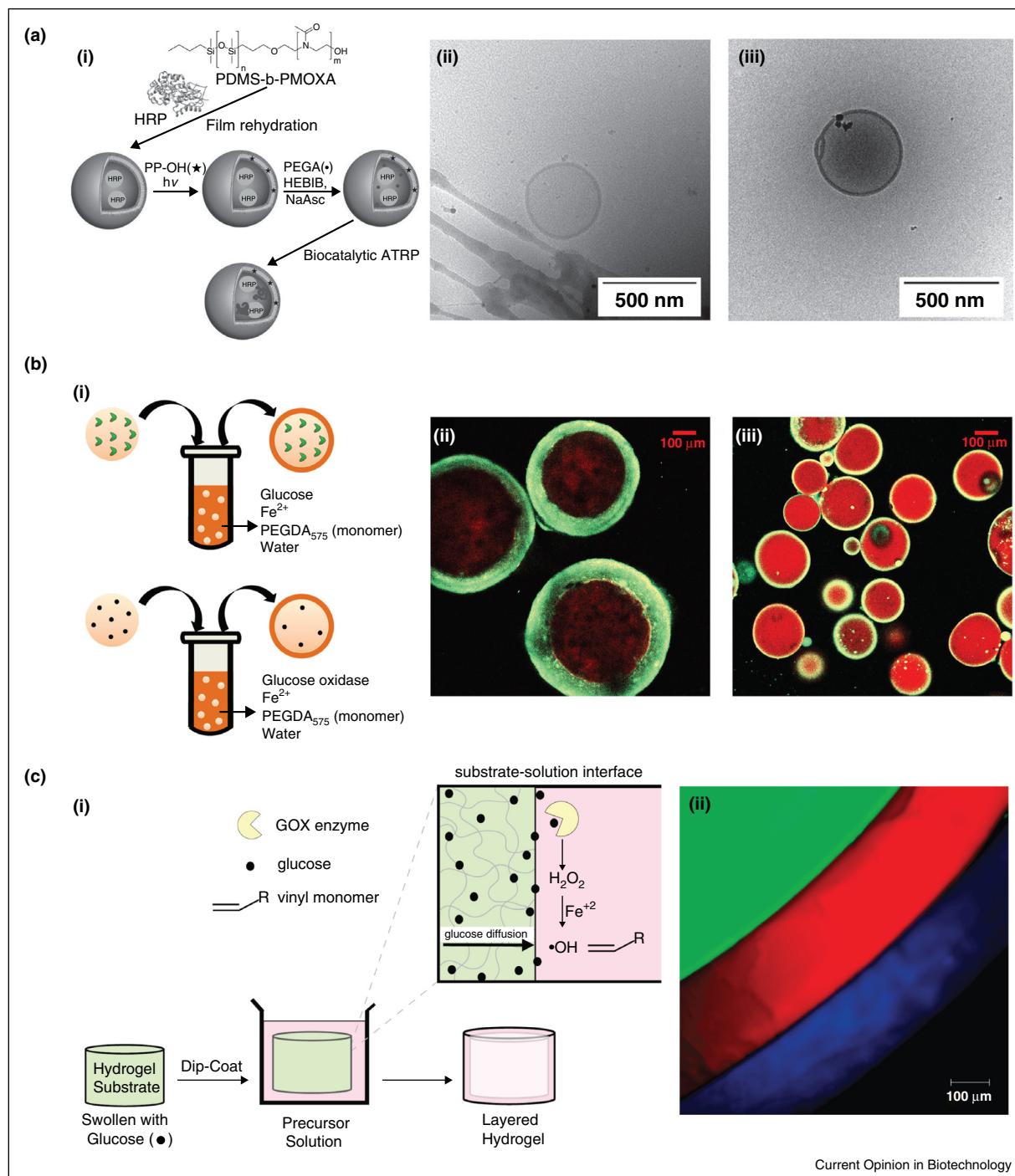
polymerization of lactones within the polymer vesicle interiors [48]. These demonstrations of nanomaterials synthesis suggest future opportunities in designing and controlling soft materials at the nanoscale aided by enzyme-mediated polymerization systems.

Microscale localization and cell screening

Spatial localization is a feature of enzyme-mediated and PBA systems that is beginning to be exploited by several groups. Since many of the polymers created through enzyme-mediated polymerization reactions are not soluble (e.g., cross-linked gels), the reaction product will precipitate immediately or shortly after formation. This

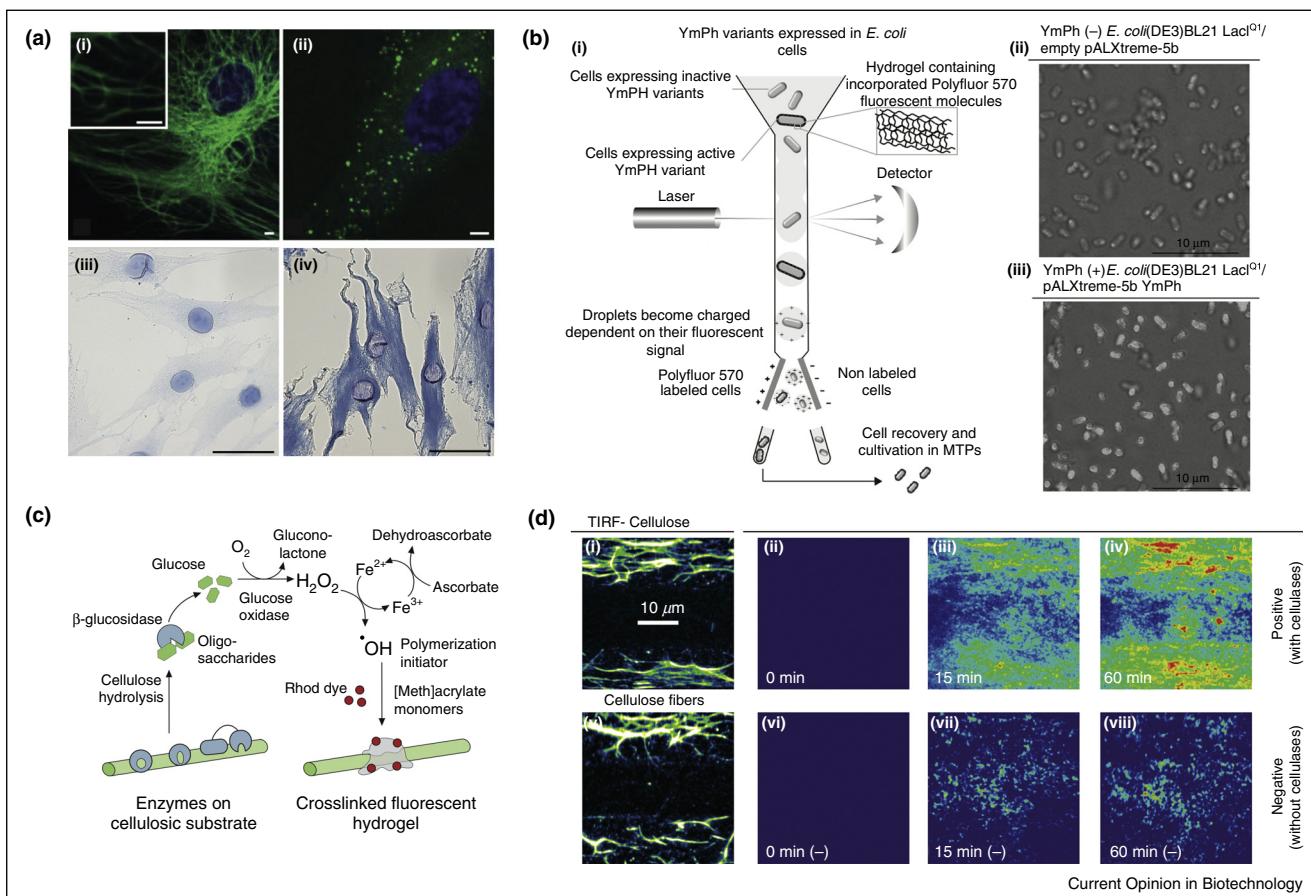
feature can be used to create large polymeric structures that are spatially restricted to locations of enzymatic activity, for example at crystalline–liquid interfaces [27^{**}] or cell surfaces [53]. The ability to confine polymerization reactions at interfaces has been exploited in a series of novel applications, including cellular coating, cell immunostaining, and time-resolved imaging of cellulose hydrolysis. Microscale localization is also inherently a mechanism at work in the core–shell particle formation reactions described above [41,43].

The potential of enzyme-mediated polymerization for cell encapsulation was first explored by Johnson *et al.* [54] who

Figure 2

(a) Synthesis of multi-layer nanomaterials via enzyme-mediated polymerization. (a, i) Synthetic route to filling self-assembled, enzyme-loaded polymersomes with polymers. (a, ii–iii) Photo-permeabilized PMOXA-b-PDMS polymersomes before (ii), and after (iii) HRP-catalyzed ATRP within the polymersomes. Reprinted from Dinu *et al.* [50] with permission from Wiley–VCH. **(b)** Formation of core–shell particles by interfacial radical polymerization. (b, i) Spatial organization of the initiating components in the hydrogel core and the bulk media prior to interfacial polymerization, with either glucose or GOx incorporated in the core. (b, ii–iii) Fluorescence images of the coated hydrogel cores for two concentrations of iron (Fe^{2+}) in the precursor solution. Reprinted from Shenoy *et al.* [43] Copyright 2013 American Chemical Society. **(c)** Generation of a three-dimensional layered hydrogel using GOx-mediated interfacial polymerization. (c, i) A cylindrical cross-linked core hydrogel substrate (green), pre-swollen with glucose (●), is immersed into an aqueous precursor solution (pink) that contains components necessary for initiating polymerization. (c, ii) Fluorescence images of a multilayer cylindrical hydrogel cross-section. Reprinted from Johnson *et al.* [42] Copyright 2010 American Chemical Society.

Figure 3



Overview of microscale localization by polymerized films. Prominent examples include immunostaining, cellular coating/labelling and cellulose degradation imaging. **(a)** Human endothelial cells stained for vimentin (1:50 000) (a, i) and von Willebrand factor (a, ii) using fluorescent polymerization-based amplification. Scale bars are 5 µm. Reprinted from Avens *et al.* [57], with permission from SAGE. (a, iii–iv) Localization of nuclear pore complex (iii) and vimentin (iv) in human dermal fibroblasts using bright-field microscopy with dyed polymer as a stain. Scale bars are 50 µm. Reprinted from Lilly *et al.* [58]. **(b)** Flow cytometer sorting principle of the fur-shell screening technology using a glucose phosphatase/GOx-coupled reaction to initiate radical polymerization. Reprinted from Pitzler *et al.* [26••] with permission from Elsevier. **(c)** Overview of hydrogel reagent signaling (HyReS) system for detecting and imaging the degradation of cellulosic substrates sing enzymatic polymerization. **(d)** Time-lapse TIRF imaging of cellulose hydrolysis by *T. reesei* cellulases (i–iv) in comparison to negative control (v–viii) using HyReS. (c–d) Reprinted from Malinowska *et al.* [27•] with permission from Wiley and ChemPubSoc Europe.

showed that fibroblasts could be encapsulated in a PEGTA₂₀₀₀₀ matrix formed through GOx-mediated redox polymerization. In this implementation, all components were premixed in bulk, encapsulating multiple cells into a polymeric block while maintaining cell viability. Single-cell encapsulation via the same GOx/Fe(II) polymerization pathway was later demonstrated by Pitzler *et al.*, who performed a hydrogel-based flow cytometry directed evolution and screening study to optimize hydrolytic enzymes [26••]. Here, a fluorescent PEG-based hydrogel shell was formed around *E. coli* cells expressing functionally active phytase (YmPh) converting glucose-6-phosphate into D-glucose. The reaction product diffused out of the cells, resulting in locally initiated polymerization via reaction with GOx in the medium. A YmPh mutant library was sorted using flow cytometry by selecting for cells with the

highest fluorescent gel signal (see Figure 3b). A large increase in specific activity in a single round of evolution was achieved. This targeted coating technique was further extended to other hydrolytic enzymes (e.g., cellulase, lipase, and esterase) in follow up work [55].

In another approach to cell sorting, Romero *et al.* developed a method that they called antigen-specific lysis, where a polymer shell was used to protect antigen-positive cells from lysis agents, allowing for enrichment of minority cell populations from blood [56••]. In this application, eosin conjugated antibodies were used to localize photoinitiators onto CD45 antigen-positive cells. Polymer coatings were formed upon photoirradiation of the cells with 530 nm light. Only cells that were antigen positive and therefore encapsulated in PEG polymer were able to

survive the subsequent lysis treatment. Incorporation of a UV-degradable PEG monomer enabled removal of the PEG coating after the sorting procedure using UV light. This report did not directly use enzyme-mediated polymerization, however, the localization aspect of PBA was crucial for the system to function. Enzyme-mediated polymerization could conceivably be used with similar effect.

The unique spatial resolution of enzyme polymerization can further be exploited to label cellular structures within tissues. In this regard, Avens *et al.* developed a fluorescent polymerization-based amplification approach for cell immunostaining [57]. Cells were fixed and stained with primary antibodies against membrane pore complex proteins, vimentin or von Willebrand factor, followed by labelling with biotinylated secondary antibodies. Streptavidin coupled to eosin was then added and bound to biotin. A mixture of PEG diacrylate monomers, a co-initiator (*N*-methyldiethanolamine), and polystyrene fluorescent nanoparticles (NPs) was added. Upon visible light irradiation, polymerization was initiated and the growing hydrogel entrapped the fluorescent NPs, anchoring them to the cell surfaces. Entrapment of multiple NPs per recognition site generated strong fluorescent signal comparable to that obtained by enzymatic tyramide signal amplification approach. The signal to noise ratio and signal localization was superior for the PBA approach, which is not prone to nonspecific staining in presence of endogenous enzymes. Lilly *et al.* presented a similar immunostaining technique, but instead of adding fluorescent NPs during the polymerization step, they performed staining with Evans Blue dye after polymerization was completed. This way they achieved a colorimetric staining method that allowed bright field observation of both the spatial distribution of protein expression and cell morphology (see Figure 3a) [58].

For our own part, in our group we employed enzyme-mediated polymerization using the GOx/Fe(II) redox system to detect and localize cellulose hydrolysis on micropatterned cellulose substrates [27^{••},59]. This method allowed for total internal reflection fluorescence (TIRF) microscopy of biomass degradation in real time. In the one-pot detection scheme, glucose was produced through the synergistic activity of endo/exoglucanases and beta-glucosidase. The glucose was then converted into hydroxyl radicals using GOx/Fe(II) and used to initiate PEG hydrogel crosslinking. In addition to PEG, a small amount of Rhodamine methacrylate was also incorporated into the gels, increasing the local density of fluorophores while the sample fluorescence was recorded using time-resolved TIRF microscopy. This procedure effectively visualized hotspots of glucose production from biomass decomposition (see Figure 3c). Our approach was also further adapted into a sensitive assay for quantifying synergy and thermostability of cellulases and multi-enzyme cellulosome complexes [59]. The work

combined autofluorescence of biomass disks with PBA. Attenuation of cellulose autofluorescence due to an increase in turbidity and light scattering from the polymerized hydrogel was used as the detection signal. The assay has several advantages over existing cellulase assays, including being rapid, one-step and label free. The PEG hydrogel was formed as a thin film onto the cellulose disks, and was found to exhibit controlled microscale localization down to a length scale of a few μm .

Conclusions

We have provided an overview of enzyme-mediated polymerization and affinity protein-mediated PBA. We described the numerous successful implementations of enzyme-mediated polymerization in several areas including biosensing, nanomaterials synthesis, and cell encapsulation/screening.

Several key challenges remain which if solved could enable new platforms or assays. Minimizing or restricting the diffusion distances of radicals in systems relying on microscale localization could improve the spatial resolution that is ultimately achievable, ideally down into the nanoscale regime. Effectively dealing with oxygen inhibition of polymerization could address a limiting factor in signal generation. Furthermore, polymerization-based signal generation or localization is generally an irreversible reaction. This irreversibility could limit the reusability of any fluidic devices that are to be used in biosensing with PBA. For cellular encapsulation, interactions between the encapsulating gel and the biological machinery of the cell will have to be considered, along with any deleterious effects on cell viability and proliferation rates. Also many applications are likely to require disentangling cells from the gel matrices. As we have already seen above, photodegradable PEG is useful in this regard, but other solutions not relying on UV light could provide greater flexibility for a range of scenarios. Further work to address these challenges will aim to bring enzyme-mediated polymerization and PBA systems into the mainstream toolbox to address major hurdles in diverse fields from nanobio sciences, to materials chemistry and bioengineering.

Acknowledgments

The authors acknowledge support from Society in Science—The Branco Weiss Fellowship (to MAN) administered by the ETH Zürich and from the Excellence Cluster Nanosystems Initiative Munich (NIM).

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest
- of outstanding interest

1. Parravano G: **Chain reactions induced by enzymic systems.** *J Am Chem Soc* 1951, **73**:183–184.
2. Klibanov AM, Tu TM, Scott KP: **Peroxidase-catalyzed removal of phenols from coal-conversion waste waters.** *Science* 1983, **221**:259–261.

3. Hollmann F, Arends IWCE: **Enzyme initiated radical polymerizations.** *Polymers* 2012, **4**:759-793.
4. Iwata H, Hata Y, Matsuda T, Ikada Y: **Initiation of radical polymerization by glucose-oxidase utilizing dissolved-oxygen.** *J Polym Sci A Polym Chem* 1991, **29**:1217-1218.
5. Berron BJ, Johnson LM, Ba X, McCall JD, Alvey NJ, Anseth KS, Bowman CN: **Glucose oxidase-mediated polymerization as a platform for dual-mode signal amplification and biodetection.** *Biotechnol Bioeng* 2011, **108**:1521-1528.
6. Tsujimoto T, Uyama H, Kobayashi S: **Polymerization of vinyl monomers using oxidase catalysts.** *Macromol Biosci* 2001, **1**:228-232.
7. Sigg SJ, Seidi F, Renggli K, Silva TB, Kali G, Bruns N: **Horseradish peroxidase as a catalyst for atom transfer radical polymerization.** *Macromol Rapid Commun* 2011, **32**:1710-1715.
8. Silva TB, Spulber M, Kocik MK, Seidi F, Charan H, Rother M, Sigg SJ, Renggli K, Kali G, Bruns N: **Hemoglobin and red blood cells catalyze atom transfer radical polymerization.** *Biomacromolecules* 2013, **14**:2703-2712.
9. 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**:7792-7802.
10. Chapman R, Gormley AJ, Herpoldt K-L, Stevens MM: **Highly controlled open vessel RAFT polymerizations by enzyme degassing.** *Macromolecules* 2014, **47**:8541-8547.
11. 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**:42728-42735.
12. Sarac AS: **Redox polymerization.** *Prog Polym Sci* 1999, **24**: 1149-1204.
13. Kobayashi S, Ritter H, Kaplan D: *Enzyme-Catalyzed Synthesis of Polymers.* Springer Science & Business Media; 2006.
14. Lou X, He P, Okelo GO, He L: **Radical polymerization in biosensing.** *Anal Bioanal Chem* 2006, **386**:525-531.
15. Scrimin P, Prins LJ: **Sensing through signal amplification.** *Chem Soc Rev* 2011, **40**:4488-4505.
16. Wu Y, Wei W, Liu S: **Target-triggered polymerization for biosensing.** *Acc Chem Res* 2012, **45**:1441-1450.
17. Renggli K, Spulber M, Pollard J, Rother M, Bruns N: **Biocatalytic ATRP: controlled radical polymerizations mediated by enzymes.** *ACS Symposium Series.* 2013:163-171.
18. Bilgic T, Klok H-A: **Surface-initiated controlled radical polymerization enhanced DNA biosensing.** *Eur Polym J* 2015, **62**:281-293.
19. Zhou S, Yuan L, Hua X, Xu L, Liu S: **Signal amplification strategies for DNA and protein detection based on polymeric nanocomposites and polymerization: a review.** *Anal Chem Acta* 2015, **877**:19-32.
20. Kobayashi S, Makino A: **Enzymatic polymer synthesis: an opportunity for green polymer chemistry.** *Chem Rev* 2009, **109**:5288-5353.
21. Gross RA, Ganesh M, Lu W: **Enzyme-catalysis breathes new life into polyester condensation polymerizations.** *Trends Biotechnol* 2010, **28**:435-443.
22. Kadokawa J-I, Kobayashi S: **Polymer synthesis by enzymatic catalysis.** *Curr Opin Chem Biol* 2010, **14**:145-153.
23. Miletic N, Nastasovic A, Loos K: **Immobilization of biocatalysts for enzymatic polymerizations: possibilities, advantages, applications.** *Bioresour Technol* 2012, **115**:126-135.
24. Walde P, Guo Z: **Enzyme-catalyzed chemical structure-controlling template polymerization.** *Soft Matter* 2011, **7**:316-331.
25. Kaastrup K, Sikes HD: **Using photo-initiated polymerization reactions to detect molecular recognition.** *Chem Soc Rev* 2016, **45**:532-545.
26. Pitzler C, Wirtz G, Vojcic L, Hitti S, Böker A, Martinez R, • Schwanenberg U: **A fluorescent hydrogel-based flow cytometry high-throughput screening platform for hydrolytic enzymes.** *Chem Biol* 2014, **21**:1733-1742.
- Enzyme-mediated polymerization was used to encapsulate *E. coli* cells in a fluorescent hydrogel, and label them for flow cytometry screening.
27. Malinowska K, Verdoner T, Meinhold A, Milles LF, Funk V, • Gaub HE, Nash MA: **Redox-initiated hydrogel system for detection and real-time imaging of cellulolytic enzyme activity.** *ChemSusChem* 2014, **7**:2825-2831.
- GOx/Fe(II) initiator system was used as a detection method for screening cellulolytic enzymes, and for microscale imaging of cellulose degradation using TIRF microscopy.
28. He P, Zheng W, Tucker EZ, Gorman CB, He L: **Reversible addition-fragmentation chain transfer polymerization in DNA biosensing.** *Anal Chem* 2008, **80**:3633-3639.
29. Qian H, He L: **Surface-initiated activators generated by electron transfer for atom transfer radical polymerization in detection of DNA point mutation.** *Anal Chem* 2009, **81**:4536-4542.
30. Sikes HD, Hansen RR, Johnson LM, Jenison R, Birks JW, Rowlen KL, Bowman CN: **Using polymeric materials to generate an amplified response to molecular recognition events.** *Nat Mater* 2007, **7**:52-56.
31. Avens HJ, Bowman CN: **Development of fluorescent polymerization-based signal amplification for sensitive and non-enzymatic biodetection in antibody microarrays.** *Acta Biomater* 2009, **6**:83-89.
32. Xu L, Yuan L, Liu S: **Macroinitiator triggered polymerization for versatile immunoassay.** *RSC Adv* 2014, **4**:140-146.
33. Badu-Tawiah AK, Lathwal S, Kastrup K, Al-Sayah M, • Christodouleas DC, Smith BS, Whitesides GM, Sikes HD: **Polymerization-based signal amplification for paper-based immunoassays.** *Lab Chip* 2015, **15**:655-659.
- Eosin photo-initiated polymerization-based amplification was used in combination with point-of-use paper devices to detect histidine-rich protein 2 from *Plasmodium falciparum* in human serum.
34. Yuan L, Xu L, Liu S: **Integrated tyramide and polymerization-assisted signal amplification for a highly-sensitive immunoassay.** *Anal Chem* 2012, **84**:10737-10744.
35. Recco LC, Tokarev I, Minko S, Pedrosa VA: **Plasmonic nanobiosensor with chain reaction amplification mechanism.** *Chem Eur J* 2014, **20**:1226-1230.
36. Berron BJ, May AM, Zheng Z, Balasubramaniam V, Bowman CN: **Antigen-responsive, microfluidic valves for single use diagnostics.** *Lab Chip* 2012, **12**:708-710.
37. Liu Y, Cheng Q: **Detection of membrane-binding proteins by surface plasmon resonance with an all-aqueous amplification scheme.** *Anal Chem* 2012, **84**:3179-3186.
38. Hu W, Chen H, Shi Z, Yu L: **Analytical biochemistry.** *Anal Biochem* 2014, **453**:16-21.
39. Gormley AJ, Chapman R, Stevens MM: **Polymerization amplified detection for nanoparticle-based biosensing.** *Nano Lett* 2014, **14**:6368-6373.
- Enzyme-mediated polymerization produced polycations that caused aggregation of gold nanoparticles, resulting in a color change. The sensor system showed parts per billion detection limits for iron and copper.
40. Fukushima H, Kohri M, Kojima T, Taniguchi T, Saito K, Nakahira T: **Surface-initiated enzymatic vinyl polymerization: synthesis of polymer-grafted silica particles using horseradish peroxidase as catalyst.** *Polym Chem* 2012, **3**:1123.
41. Kohri M: **Development of HRP-mediated enzymatic polymerization under heterogeneous conditions for the preparation of functional particles.** *Polym Chem* 2014, **46**:373-380.
42. Johnson LM, DeForest CA, Pendurti A, Anseth KS, Bowman CN: **Formation of three-dimensional hydrogel multilayers using enzyme-mediated redox chain initiation.** *ACS Appl Mater Interfaces* 2010, **2**:1963-1972.

43. Shenoy R, Tibbitt MW, Anseth KS, Bowman CN: **Formation of core-shell particles by interfacial radical polymerization initiated by a glucose oxidase-mediated redox system.** *Chem Mater* 2013, **25**:761-767.
44. Kohri M, Kobayashi A, Fukushima H, Kojima T, Taniguchi T, Saito K, Nakahira T: **Enzymatic miniemulsion polymerization of styrene with a polymerizable surfactant.** *Polym Chem* 2012, **3**:900.
45. Kohri M, Uzawa S, Kobayashi A, Fukushima H, Taniguchi T, Nakahira T: **Enzymatic emulsifier-free emulsion polymerization to prepare polystyrene particles using horseradish peroxidase as a catalyst.** *Polym J* 2012, **45**:354-358.
46. Sauer R, Froimowicz P, Schöller K, Cramer J-M, Ritz S, Mailänder V, Landfester K: **Design, synthesis, and miniemulsion polymerization of new phosphonate surfmers and application studies of the resulting nanoparticles as model systems for biomimetic mineralization and cellular uptake.** *Chem Eur J* 2012, **18**:5201-5212.
47. Kohri M, Kobayashi A, Nannichi Y, Taniguchi T: **A green approach for the synthesis of fluorescent polymer particles by combined use of enzymatic miniemulsion polymerization with clickable surfmer and click reaction.** *Trans Mater Res Soc Jpn* 2014, **39**:57-60.
Horseraddish peroxidase-mediated co-polymerization of styrene and clickable surfactants/monomers produced polymeric nanoparticles suitable for modification by click reaction in aqueous buffer at ambient temperature.
48. Nallani M, de Hoog H-PM, Cornelissen JJLM, Palmans ARA, van Hest JCM, Nolte RJM: **Polymersome nanoreactors for enzymatic ring-opening polymerization.** *Biomacromolecules* 2007, **8**:3723-3728.
49. Spulber M, Baumann P, Saxer SS, Pieles U, Meier W, Bruns N: **Poly(N-vinylpyrrolidone)-poly(dimethylsiloxane)-based polymersome nanoreactors for laccase-catalyzed biotransformations.** *Biomacromolecules* 2014, **15**:1469-1475.
50. Dinu MV, Spulber M, Renggli K, Wu D, Monnier CA, Petri-Fink A, Bruns N: **Filling polymersomes with polymers by peroxidase-catalyzed atom transfer radical polymerization.** *Macromol Rapid Commun* 2015, **36**:507-514.
51. Renggli K, Nussbaumer MG, Urbani R, Pfohl T, Bruns N: **• chaperonin as protein nanoreactor for atom-transfer radical polymerization.** *Angew Chem Int Ed* 2013, **53**:1443-1447.
Aspherical protein cage ~16 nm in diameter was used to sequester ATRP within a protein nanoreactor and narrow the molecular weight distribution.
52. Tan C, Saurabh S, Bruchez MP, Schwartz R, LeDuc P: **Molecular crowding shapes gene expression in synthetic cellular nanosystems.** *Nat Nanotechnol* 2013, **8**:602-608.
53. Lilly JL, Romero G, Xu W, Shin HY, Berron BJ: **Characterization of molecular transport in ultrathin hydrogel coatings for cellular immunoprotection.** *Biomacromolecules* 2015, **16**:541-549.
54. Johnson LM, Fairbanks BD, Anseth KS, Bowman CN: **Enzyme-mediated redox initiation for hydrogel generation and cellular encapsulation.** *Biomacromolecules* 2009, **10**:3114-3121.
55. Lülsdorf N, Pitzler C, Biggel M, Martinez R, Vojcic L, Schwaneberg U: **A flow cytometer-based whole cell screening toolbox for directed hydrolase evolution through fluorescent hydrogels.** *Chem Commun* 2015, **51**:8679-8682.
56. Romero G, Lilly JJ, Abraham NS, Shin HY, Balasubramaniam V, Izumi T, Berron BJ: **• Protective polymer coatings for high-throughput, high-purity cellular isolation.** *ACS Appl Mater Interfaces* 2015, **7**:17598-17602.
CD45 antigen-positive cells were tagged with photoinitiator antibodies, and coated with a PEG hydrogel that protected this specific cell subpopulation from lysis buffer. The polymer coating could be removed using further photochemistry to recover the viable enriched cells.
57. Avens HJ, Berron BJ, May AM, Voigt KR, Seedorf GJ, Balasubramaniam V, Bowman CN: **Sensitive immunofluorescent staining of cells via generation of fluorescent nanoscale polymer films in response to biorecognition.** *J Histochem Cytochem* 2011, **59**:76-87.
58. Lilly JL, Sheldon PR, Hoversten LJ, Romero G, Balasubramaniam V, Berron BJ: **Interfacial polymerization for colorimetric labeling of protein expression in cells.** *PLoS One* 2014, **9**:e115630.
59. Malinowska K, Rind T, Verderfer T, Gaub HE, Nash MA: **Quantifying synergy, thermostability, and targeting of cellulolytic enzymes and cellulosomes with polymerization-based amplification.** *Anal Chem* 2015, **87**:7133-7140.