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Preface

When Hermann Staudinger won the 1953 Nobel Prize in Chemistry for introducing the concept of macromolecules, he emphasized that “macromolecular compounds include the most important substances occurring in nature such as proteins, enzymes, the nucleic acids, besides the polysaccharides (...), as well as rubber, and lastly the large number of new, fully synthetic plastics and artificial fibers” (Staudinger, 1953). The naturally occurring polymers are synthesized by the biochemical machinery of living cells. Not surprisingly, a plethora of enzymes can catalyze polymerization reactions in which low molecular weight monomers are linked into high molecular weight macromolecules. These reactions are essential for life, and the resulting polymers are major building blocks of living matter. Importantly, many enzymes have been found to be promiscuous with respect to the reactions they can catalyze so that many non-natural polymerizations can nowadays also be catalyzed by enzymes.

Since Staudinger’s time, synthetic macromolecules have become indispensable materials that everyone uses on a daily basis, ranging from plastics in packaging, to light-weight high performance materials in the automotive and the aerospace sector, or as materials for biomedical applications. At the same time, the sheer volume in which plastics are produced and scattered across our planet make synthetic macromolecules a global environmental threat if not properly discarded and recycled. While most synthetic polymers and plastics are produced by chemical means, it is feasible to synthesize polymers via biocatalytic pathways, employing either enzymes or whole cells as polymerization catalysts. There are many reasons to use nature’s catalysts for polymerizations, some of the most prominent being the potential environmental benefit that most enzymes are non-toxic and biocompatible, or that they allow to produce polymers with chemo- or regioselectivity. Moreover, some biologically derived polymers such as polyhydroxyalkanoates (PHAs) are biodegradable polymers that can play a role in tackling the plastic waste crisis. Biocatalysis in polymer science is a highly active and burgeoning field, as exemplified by multitude of scientific papers being published each year (Douka, Vouyiouka, Papaspyridi, & Papaspyrides, 2018; Hollmann & Arends, 2012; Jiang & Loos, 2016; Kobayashi, Uyama, & Kadokawa, 2019; Loos, 2010; Rodriguez et al., 2018; Shoda, Uyama, Kadokawa,

Kimura, & Kobayashi, 2016), and by the implementation of biotechnological routes toward monomers and polymers in industry, e.g., for the synthesis of PHAs or the synthesis of lactic acid. We therefore decided to collate important methods for the biocatalytic synthesis of polymers, monomers, and the modification of polymers by enzymatic means in this volume of *Methods in Enzymology*. The chapters are organized into six sections that cover five classes of polymers, that is polyesters, DNA, polysaccharides and glycomonomers, vinyl polymers, and poly(aromatics), as well as the use of enzymes to modify the surface of polymeric materials. The type of polymerizations includes polycondensations and ring-opening polymerizations (ROPs), whole-cell polyester synthesis, free radical and controlled radical chain growth polymerizations, as well as radical addition polymerizations.

The first part of this volume is dedicated to polyesters, which are an important class of technical relevant polymers. In Chapter 1, Zhao reviews the methods of lipase-catalyzed polymerizations to polyesters in a variety of non-aqueous media that include organic solvents, ionic liquids, and supercritical CO₂. The reactions include polycondensations and ROPs. In Chapter 2, Pellis and Gardossi provide protocols for the enzyme catalyzed solvent-free synthesis of polyesters in a thin film method under reduced pressure, using immobilized cutinase and lipase. They highlight the importance of computational methods to design and optimize experimental conditions. These methods are the visualization of protein structures and their active sites in three-dimensional models, as well as design of experiment (DoE). An advantage of enzymatic polymerizations over their chemical counterparts is that enzymes can discriminate between similar functional groups on the monomers, thereby selectively reacting a subset of them in the polymerization reaction. This is demonstrated in Chapter 3 by Kressler and coworkers who prepare polyesters that feature hydroxyl groups on their backbone by lipase-catalyzed polycondensations. The subsequent chemical modification of some of these functional groups with hydrophobic fatty acids yields amphiphilic polymers. They can self-assemble in water into microparticles or stabilize cubosomes. Polyesters are not only readily accessible by enzymatic means, but also occur naturally in many organisms such as in plants or bacteria. Some bacteria use PHAs as carbon and energy storage. These polyesters are biodegradable and possess many desirable properties. Fermentation routes have been established to produce PHAs with tunable mechanical properties, even on the industrial scale. Zinn and coworkers provide state-of-the-art methods for the

biotechnological production of PHAs in Chapter 4. In Chapter 5, Lee and coworkers review the possibility to synthesize the technologically important polymer poly(lactate) (PLA) by fermentative approaches. To this end, microbial strains that produce PHAs were hijacked and reengineered to produce PLA, feeding on simple carbon sources such as glucose.

The second part of this volume shows that also nucleic acids can be used as polymers. Many DNA nanotechnology applications require high molecular weight DNA in amounts that are difficult to synthesize by classical nucleic acid synthesis on solid-phase or on DNA templates. In Chapter 6, Chilkoti, Zauscher, and coworkers comprehensively review the field and methods for the synthesis of high molecular weight single-stranded homo- and co-polynucleotides by terminal deoxynucleotidyl transferase. These polymerizations can be conducted in solution and from surfaces.

The third section of this volume covers enzymatic routes toward polysaccharides and glycopolymers, as well as sugar-containing monomers. In Chapter 7, Kadokawa reviews the methods for α -glucan phosphorylase-catalyzed polymerizations of α -D-glucose 1-phosphate into amylose and into amylose-containing graft polymers. Moreover, the polymerization of amylose-analogs into, e.g., amylosamine, is presented. An alternative strategy toward glycopolymer is to prepare sugar-containing monomers that can then be polymerized by other means. In Chapter 8, Adharis and Loos present various methods for the synthesis of carbohydrate-containing vinyl monomers. These monomers are important building blocks for polymers, since they allow to incorporate renewable biomass into polymers that are synthesized by conventional radical methods. Moreover, the resulting carbohydrate-containing polymers are often bioactive and therefore interesting for biomedical applications.

The fourth section of the volume focuses on radical polymerizations of vinyl monomers (e.g., acrylates, methacrylates, acrylamides, styrene, and styrene derivatives). They are an important class of polymerizations because the resulting polymers are widely used in many applications. Moreover, radical polymerizations are less sensitive to the presence of functional groups and protic solvents than many other types of polymerizations. The simplest form of radical chain growth polymerization is free radical polymerizations in which the radicals react in an uncontrolled way. Chapter 9, authored by Bruns and coworkers, describes the use of horseradish peroxidase (HRP) as a catalyst to initiate free radical polymerizations. The enzymatic polymerization techniques is successfully applied to different vinyl monomers, including 4-acryloylmorpholine, 2-hydroxyethyl methacrylate, and

poly(ethylene glycol) methyl ether acrylate. The advantage of free radical polymerizations lies in its simplicity. However, control over molecular weight and the synthesis of polymers with narrow molecular weight distribution and complex molecular architecture (e.g., block copolymers) is not possible or very difficult to achieve. In contrast, reversible-deactivation radical polymerizations (also termed controlled radical polymerizations) have emerged as powerful methods to obtain precise macromolecules. Controlled radical polymerizations can also be catalyzed or initiated by enzymes. In Chapter 10, Benetti, Bruns and coworkers present their methods for biocatalytic atom transfer radical polymerizations (bioATRP) in solution and from surfaces, which involve reversible halogen transfer between enzymes such as HRP or hemoglobin, and the growing polymer chains. Reversible addition-fragmentation chain transfer (RAFT) polymerization is another very popular controlled radical polymerization. In Chapter 11, Wang and An review the published literature on peroxidase-initiated RAFT polymerization and provide a wealth of experimental protocols for bioRAFT, including homopolymer synthesis, multiblock synthesis, synthesis of high molecular weight polymers, and a protocol for bioRAFT-based polymerization-induced self-assembly.

The next section of the volume is related to the previous one, as it also covers radical polymerizations. However, the enzymatic synthesis of poly(aromatics) is not a chain growth reaction, but an addition polymerization of aromatic radicals that are created by oxidoreductases. In Chapter 12, Romero-García and coworkers review and present methods for the enzymatic synthesis of polyaniline as an archetype for a conductive polymer. The polymerization utilizes the native reactivity of peroxidases in the presence of hydrogen peroxide and can be easily adapted to the synthesis of other conjugated polymers.

The final section and chapter of the volume (Chapter 13) does not cover a polymer synthesis, but instead gives intriguing examples of enzymatic methods to modify and functionalize polymeric materials once they have been synthesized (independent if the synthesis was through an enzymatic route or by conventional chemical means). Guebitz and Ribitsch provide an overview over the possibilities to modify the surface of polyesters such as poly(ethylene terephthalate) (PET) and PLA by treatment with cutinases, lipases, and other enzymes. Moreover, the methods to characterize the resulting polymer samples are summarized and described in detail.

We hope that this methods collection will help colleagues to synthesize useful polymers by biocatalytic means, inspire the reader to elucidate open

scientific and technological questions of enzymatic polymerizations, and motivate the reader to exploit the vast opportunities that this field has to offer. We would like to thank all authors for their excellent contributions to this volume of *Methods in Enzymology* and for their continuous efforts to advance the field of enzymatic polymerizations. We believe that the lessons learned from enzymatic polymerizations will not only open up synthetic pathways to novel materials, but will also contribute to a deeper understanding of the polymerization reactions that happen in nature. As Herman Staudinger concluded his Nobel Lecture: “In the light of this new knowledge of macromolecular chemistry, the wonder of life in its chemical aspect is revealed in the astounding abundance and masterly macromolecular architecture of living matter” (Staudinger, 1953).

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