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Defining the Field of Sequence-Controlled Polymers

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1.1 Introduction

This book is fully devoted to an exploding area in fundamental and applied polymer science, namely the synthesis, characterization, and utilization of sequence-controlled polymers (SCPs) [1]. This topic is at the same time very old and very new: old because monomer sequence regulation is a central concept in biology and has therefore been thoroughly studied in biochemistry and biophysics for several decades. Thus, efficient methods are available for the synthesis and characterization of sequence-defined biopolymers such as deoxyribonucleic acid (DNA), ribonucleic acid (RNA), and proteins [2]. In comparison, however, the development of man-made polymers with controlled monomer sequences is a much more recent area of research. As pointed out in early essays and reviews [3–5], very little research efforts have been devoted to that topic until about 10 years ago. However, the subject has recently gained significant academic attention, in particular during the last 5 years. This recent emerging trend is actually what is described in the present book, and all the authors of the following chapters have been actively participating in the development of that new discipline. Yet, it should be clearly stated that the field of SCPs was not started 5 years ago. Studies on monomer sequences began with the development of the first synthetic copolymers and can therefore be tracked back to the early days of polymer science [6, 7]. However, as described in a recent historical summary [8], the subject has been stuck for more than 50 years and was mostly limited to the synthesis of classical copolymers such as statistical, alternating, periodic, and block copolymers. More complex degrees of sequence regulation were regarded by the polymer science community as difficult to achieve or as the preserve of biochemists. This state of mind is exactly what has changed during the last few years, which have witnessed the emergence of a large number of original ideas and concepts for making SCPs [1]. As a consequence, the description of monomer sequences in man-made polymers has started to get outdated in classical textbooks. In particular, many copolymers that have been reported in recent years are difficult to describe using current polymer nomenclature and

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terminology [9]. In this context, the objective of the present volume is to define, rationalize, and categorize that burgeoning new field of research.

As a key to decode the present volume, the reader should always keep in mind that the field of SCPs is actually a hybrid discipline located at the interface between biology and polymer science, as depicted in Figure 1.1. Biological polymers, such as nucleic acids and proteins, are indeed archetypal examples of macromolecules with perfectly controlled monomer sequences. Thus, one important trend in the field consists in using biological principles to synthesize SCPs. Protein engineering [10] and DNA-templated polymerizations [11] are good examples of strategies following this school of thought (see Chapters 3 and 4 of this volume for details). However, a second important trend in the field of SCPs aims to synthesize non-natural macromolecules that are as structurally defined as biopolymers [4]. In such approaches, man-made chemistry concepts are used to synthesize the polymers. These tools can be classical polymerization approaches such as chain-growth and step-growth polymerizations but also tools that are imported from other areas of chemistry such a solid-phase synthesis and molecular machines [12, 13]. Solid-phase iterative chemistry was first introduced for the chemical synthesis of biopolymers such as proteins [14, 15] and nucleic acids [16, 17]. However, it was later demonstrated that such approaches can also be used for preparing a wide variety of synthetic sequence-defined polymers (see Chapters 5–7 for details), which includes peptide–polymer bio-hybrid structures [18], peptidomimetics such as peptoids [19], as well as fully unnatural macromolecules such as information-containing polymers [20, 21]. Yet, such multistep growth approaches remain overall experimentally demanding as well as time consuming, and consequently more straightforward sequence-controlled polymerization strategies have been also investigated in the past years. For instance, living or pseudo-living chain-growth polymerizations such as carbanionic polymerization, cationic polymerization, and controlled radical polymerization have been extensively studied for monomer sequence regulation (see Chapters 8–11 for details). Original step-growth polymerization concepts have also been recently examined for the preparation of periodic SCPs (see Chapter 12 for details). Furthermore, efficient chemical transformations known as “click” reactions, which have become quite popular in synthetic polymer chemistry during the last decade [22, 23], have also been shown to be useful tools for the preparation and modification of sequence-controlled macromolecules (see Chapter 13).

This recent progress in the synthesis of SCPs has also raised a large number of new questions and challenges in the broad field of polymer science. Indeed, the emerging domain of SCPs is not restricted to polymer synthesis but also opens up new directions in polymer physics, analytical chemistry, engineering, materials science, and nanotechnology. For instance, the characterization of SCPs requires the development of new analytical approaches [24]. Besides standard polymer analytics such a nuclear magnetic resonance spectroscopy and size-exclusion chromatography, SCPs call for more specific sequencing methods that permit the full characterization of monomer sequences (see Chapter 16). As highlighted in Figure 1.1, engineering aspects will also probably play an increasing role in the field of SCPs in the near future. Indeed, although SCPs are

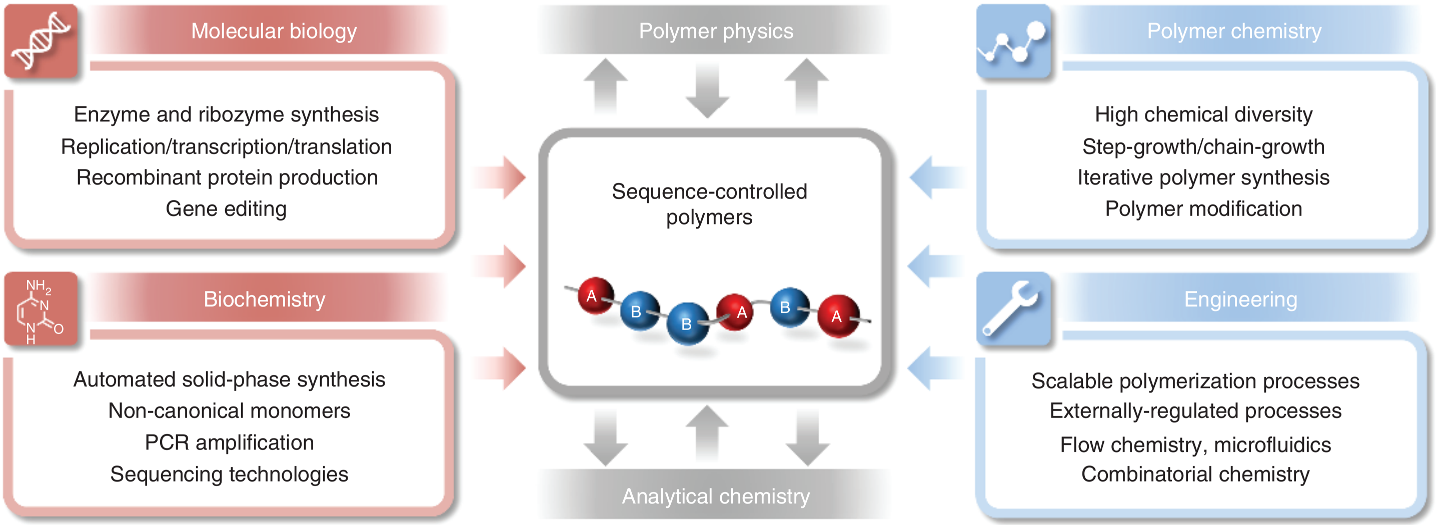

Figure 1.1 The field of sequence-controlled polymers is at the crossroads between biology (red) and polymer science (blue). One trend in the field consists in importing sequence-defined biopolymers in materials science and nanotechnology applications, whereas the opposite one consists in rendering synthetic macromolecules as structurally defined as natural ones. In that regard, sequence-controlled polymers constitute a new category of macromolecules that fills the gap between evolution-optimized biopolymers and conventional plastics.

not foreseen to be used as large-scale commodity polymers, the development of automated protocols and combinatorial approaches seem mandatory for facilitating their synthesis and processing. Last but not least, SCPs have already opened up many new opportunities for the design of applied polymer materials. The range of applications of SCPs is particularly broad and includes areas that are as diverse as data storage, catalysis, anti-counterfeiting technologies, information technologies, microelectronics, automotive industry, nanomedicine, and biotechnology (see Chapter 15).

The field of SCPs is therefore an exciting multidisciplinary domain with its own terminology, topics, challenges, and frontiers. In order to guide the readers through this new scientific universe, this chapter provides a succinct general overview about SCPs. In particular, the following sections introduce, in a few words, the main aspects and topics that will be developed in more detail throughout the whole book. At first, some general terms and definitions are briefly presented. Afterward, the different mechanisms, either biological or man-made, that permit the synthesis of SCPs are presented and categorized. Biological sequence-regulated polymerizations that occur in living organisms are briefly described, but the reader is guided to specialized biology textbooks for more detailed information [2]. Man-made approaches are presented more comprehensively and ranked in subcategories. Finally, some general considerations about the sequencing and properties of SCPs are presented at the end of this chapter. Overall, this introductory chapter should serve as a useful starting point for understanding what SCPs are.

1.2 Glossary

As mentioned in the introduction, the field of SCPs is still relatively new and therefore definitions and terminologies are still malleable. However, it is noteworthy that traditional polymer vocabulary is not sufficient for describing the different kinds of SCPs that have been reported in recent years [9]. For instance, only standard copolymers such as block, alternating, random, statistical, and periodic copolymers are described in official IUPAC polymer terminology [25]. This language comes mostly from chain-growth copolymerizations (i.e., reactivity ratios driven copolymerizations) and is limited for describing other situations. Thus, some broader definitions are proposed below. These definitions are not yet official (unless noted) and may still evolve.

Sequence-controlled polymer. A sequence-controlled polymer is a macromolecule in which monomer sequences are controlled to some degree. This control can be absolute but is not necessary. For example, an alternating copolymer prepared by radical chain-growth polymerization is a sequence-controlled polymer, although it is also a nonuniform polymer in which chains have different lengths and slightly different compositions.

Sequence-defined polymer. *Syn. sequence-specific polymer, sequence-ordered polymer.* A sequence-defined polymer is a uniform macromolecule with an exact chain length and a perfectly defined sequence of monomers. In other words, each monomer unit is at a defined position in the chain. Sequence-defined polymers constitute therefore a subclass of the field of sequence-controlled polymers, as proposed in Figure 1.2.

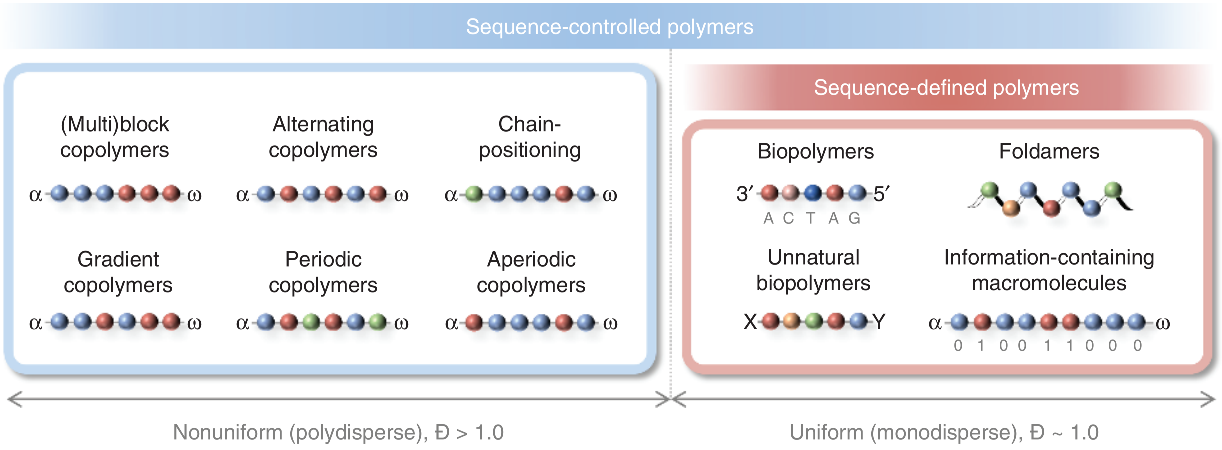

Figure 1.2 Schematic representation of the meaning of the terms “sequence-controlled polymers” and “sequence-defined polymers.” “Sequence-controlled polymers” is a broad term that denotes all kind of polymers in which sequences are controlled to some degree. “Sequence-defined polymers” is a more restrictive term that denotes uniform polymers with perfectly controlled monomer sequences. A sequence-defined polymer is also sequence-controlled. A sequence-controlled polymer is not necessarily sequence-defined.

Primary structure. *Syn. microstructure. [IUPAC] In the context of macromolecules such as proteins, constitutional formula, usually abbreviated to a statement of the sequence and if appropriate cross-linking of chains* [26]. Additional information from the author: although traditionally belonging to the glossary of biology, the term "primary structure" may also be used to denote the sequence distribution of non-natural sequence-defined polymers.

Periodic copolymer. *[IUPAC] A copolymer consisting of macromolecules comprising more than two species of monomeric units in regular sequence* [25]. Additional information from the author: a periodic copolymer is a macromolecule containing minimal motifs that repeat periodically in the chain. For example, polymers with repeating motifs $[AB]_n$ (i.e., alternating), $[ABC]_n$, or $[ABCD]_n$ are periodic copolymers. A periodic copolymer can be uniform (i.e. sequence-defined) or non-uniform (i.e. sequence-controlled).

Aperiodic copolymer. Aperiodic copolymers are copolymers in which the monomer sequence distribution is not regular but follows the same arrangement in all chains [9, 27]. An aperiodic copolymer shall be distinguished from random or statistical copolymers, in which sequences are uncontrolled. For example, a non-natural information-containing macromolecule contains, in most cases, an aperiodic sequence-coded message.

Block copolymer. *[IUPAC] A copolymer that is a block polymer. In the constituent macromolecules of a block copolymer, adjacent blocks are constitutionally different, that is, adjacent blocks comprise constitutional units derived from different species of monomer or from the same species of monomer but with a different composition or sequence distribution of constitutional units* [25]. Additional information from the author: multiblock copolymers contain several successive blocks that are arranged in a defined block sequence.

Gradient copolymer. A gradient copolymer is a macromolecule in which comonomer distribution follows a gradual change in composition along the chains. Gradient copolymers are often nonuniform macromolecules prepared by a controlled or living chain-growth process [28]. Although rarely described in the literature, sequence-defined uniform gradient copolymer may also be conceived and achieved.

Irregular copolymer. *Related terms: random copolymers, statistical copolymers.* The term irregular copolymer could be used to denote all copolymers in which monomer sequences are uncontrolled. It could include different types of uncontrolled sequences that follow Bernoullian or Markovian statistics. It shall, however, be noted that the official IUPAC term "irregular polymer" depicts very different situations that are related to the polymer architecture [25].

Sequencing. The term "sequencing" denotes an analytical technique that permits the comprehensive deciphering of monomer sequences of a sequence-defined polymer [24]. It should be noted that sequencing methods are restricted to the analysis of uniform sequence-defined polymers. Although non-official, the term "sequence analysis" is proposed for the analytical characterization of nonuniform sequence-controlled polymers.

1.3 Sequence Regulation in Biopolymers

Sequence control is a key feature in biology. Indeed, in DNA the genetic information is stored in controlled A, T, G, and C nucleotide sequences, following Watson–Crick principles [29]. In addition, the high complexity and advanced properties of protein-based biomaterials are determined by the primary structure of sequence-defined peptide chains [30, 31]. The polymerization mechanisms that lead to monomer sequence regulation in biological polymers are complex and enabled by large biocatalysts such as enzymes and ribozymes. The reader is therefore guided to comprehensive biology textbooks for detailed information on these processes [2]. A condensed description of biological polymerizations is described in the following subsections.

1.3.1 Nucleic Acids

Two main types of nucleic acids are found in biology, DNA and RNA. The former is found in cell nuclei and stores the genome of living organisms, whereas the latter has different types and functions, such as messenger RNA (mRNA), ribosomal RNA (rRNA), or transfer RNA (tRNA). Sequence-defined nucleic acids are synthesized *in vivo* via two main polymerization processes, namely DNA replication (DNA→DNA) and transcription (DNA→mRNA). Small noncoding RNAs are also obtained via other mechanisms, which are outside the scope of this chapter [2]. Replication is a template polymerization in which the sequence-defined double-stranded DNA is copied into two new nascent strands, namely the leading and the lagging strands. The synthesis of the leading strand is an interesting and inspirational mechanism for the field of SCPs [3]. In this part of the replication process, the nascent sequence-defined DNA strand is formed with the help of DNA polymerases, which catalyze the formation of phosphodiester bonds between the growing 3′-OH terminus of the leading DNA chain and a nucleoside triphosphate monomer. In particular, the polymerase provides an optimal environment for confining the template strand, the leading nascent strand, and the upcoming monomer, thus enabling sequence regulation. In particular, this confined environment prevents any uncontrolled polymerization of the nucleoside triphosphate and also allows error correction in the rare event of an erroneous monomer incorporation. Furthermore, this synthesis is directional, and after each monomer attachment, the polymerase walks along the template strand, thus allowing its sequence to be accurately copied. The DNA→mRNA transcription mechanism is overall very similar to leading strand synthesis in replication but involves specific RNA polymerases that unwind double-stranded DNA and promotes phosphodiester bond formation [2]. Furthermore, the monomers are not deoxyribose nucleoside triphosphates as in DNA replication but ribose-based equivalents.

1.3.2 Proteins

Proteins are sequence-defined biopolymers that are built using 22 different α-amino acid monomers (21 in eukaryotes). The primary structure of proteins (i.e., the sequence of amino acids in a peptide chain) influences chain-folding into defined secondary, tertiary, and eventually quaternary structures, which govern their biological properties and functions. In biological organisms, proteins are synthesized via the translation process (mRNA→proteins), in which the nucleotide sequences of mRNA are transcripted into amino acid sequences [32]. From a polymer chemistry point of view, translation is probably the most achieved polymerization mechanism identified to date, since it allows information transfer between two totally different types of polymeric backbones, namely from a poly(phosphodiester) to a polyamide. This fascinating mechanism is enabled by the ribosome, which is a large and complex catalytic nucleoprotein. In particular, the ribosome is a machinery that provides an ideal confined environment for bringing together an mRNA template chain, a growing protein chain, and tRNAs, which are molecular adaptors allowing translation. Indeed, tRNAs contain both a nucleic acid an anticodon trimer that can bind to a codon on the mRNA template and a polymerizable amino acid monomer. After installation of a tRNA on the mRNA template, the ribosome catalyzes the reaction of the amino acid with the nascent protein and liberates afterward the adaptor. Following the basic codon/anticodon rules of the genetic code, the information of the mRNA strand is translated into a protein primary structure. Furthermore, after each amino acid attachment, the ribosome moves forward on the mRNA template, thus allowing complete translation of the genetic sequence.

1.4 Bio-Inspired Sequence-Regulated Approaches

As described in Section 1.3, evolution has selected optimal polymerization processes for the preparation of sequence-defined nucleic acids and proteins. Thus, an obvious strategy for preparing artificial SCPs consists in exploiting these biological mechanisms. For instance, a large number of studies have been devoted to artificial replication processes. These studies are depicted in detail in previous reviews [1, 11] as well as in Chapter 3 of this book. Recently, Holliger and coworkers have described non-natural genetic polymers that can replicate [33], evolve, and catalyze simple reactions [34]. Such polymers contain artificial backbones that differ from the deoxyribose and ribose backbones of DNA and RNA. However, it was demonstrated that these polymers can perform enzymatic replication and, in particular, be copied from a DNA template and even transcribe back their sequence information to a DNA chain [33]. In addition, nucleotides containing non-natural nucleobases have been extensively studied in *in vitro* enzymatic replication [1, 35]. Romersberg and coworkers have reported recently that non-natural base pairs can be recognized by the biological replication machinery [36]. These examples show that biology can be tweaked for the preparation of non-natural SCPs. However, the aforementioned studies deal with synthetic biology and are a little removed from the classical field of polymer science. Therefore, enzyme-free artificial replication has also been widely investigated for the preparation of synthetic sequence-defined polymers

[11]. Although a large number of studies have turned out to be unsuccessful [3], promising results have been obtained by Liu and coworkers, for example, for the enzyme-free, DNA-templated synthesis of sequence-defined peptide nucleic acids [37]. More recently, the same group has described the enzyme-free translation of DNA templates into fully synthetic, sequence-defined polymers [38]. This interesting example emphasizes that bio-inspired (but non-biological) mechanisms can be successfully developed for the preparation of SCPs. Besides replication, the complementarity of DNA strands is also an interesting feature for preparing SCPs. For instance, Turberfield and coworkers have studied self-recognizing DNA supports for programming and guiding the synthesis of non-natural, sequence-defined segments [39, 40].

Ribosomal synthesis is also a tool and an inspiration for the field of SCPs. For instance, protein-based SCPs can be synthesized *in vivo* in bacteria. In such approaches, a gene coding for a specific protein is included in bacterial DNA using recombinant DNA techniques. This gene is then expressed by the ribosomal machinery of the bacteria, and the targeted protein polymer is afterward extracted. This interesting methodology is described in reviews [10] and in Chapter 4 of this book. Most of the pioneering studies describing the relevance of tailored proteins made by genetic engineering in materials science have been reported by Tirrell and coworkers [41–43]. In particular, they have shown that non-canonical amino acids can be utilized by bacterial ribosomes, thus allowing the synthesis of non-natural, protein-based SCPs [44]. Yet, these approaches remain so far strictly restricted to α-amino acid-based proteins. In this context, Suga and coworkers have studied the ribosomal compatibility of other monomers such as β-amino acids [45, 46]. Although still preliminary, such approaches could allow ribosomal synthesis of non-natural SCPs. Another interesting challenge is the development of ribosome-inspired synthetic processive catalysts [47]. A first convincing proof of concept was recently published by Leigh and coworkers [48, 49], who described a rotaxane-based molecular machine allowing sequence-defined synthesis.

1.5 Sequence Regulation in Synthetic Macromolecules

Traditionally, synthetic polymerization methods are split in two main categories: that is, step-growth and chain-growth polymerization. However, in recent years, multistep growth approaches in which polymers are prepared using stepwise reactions have become more and more prominent in polymer chemistry, in particular for the synthesis of SCPs. Thus, in a recent review [13], synthetic polymerizations have now been ranked in three main categories, as depicted in Figure 1.3. This new chart will be followed in the present section for describing chemical approaches that have been reported in recent years for the synthesis of SCPs. In particular, these approaches will be presented starting with the methods leading to the less uniform polymers (i.e., step growth that typically leads to $Đ \sim 2$) and finishing with the one leading to uniform structures (i.e., multistep growth that typically leads to $Đ \sim 1$).

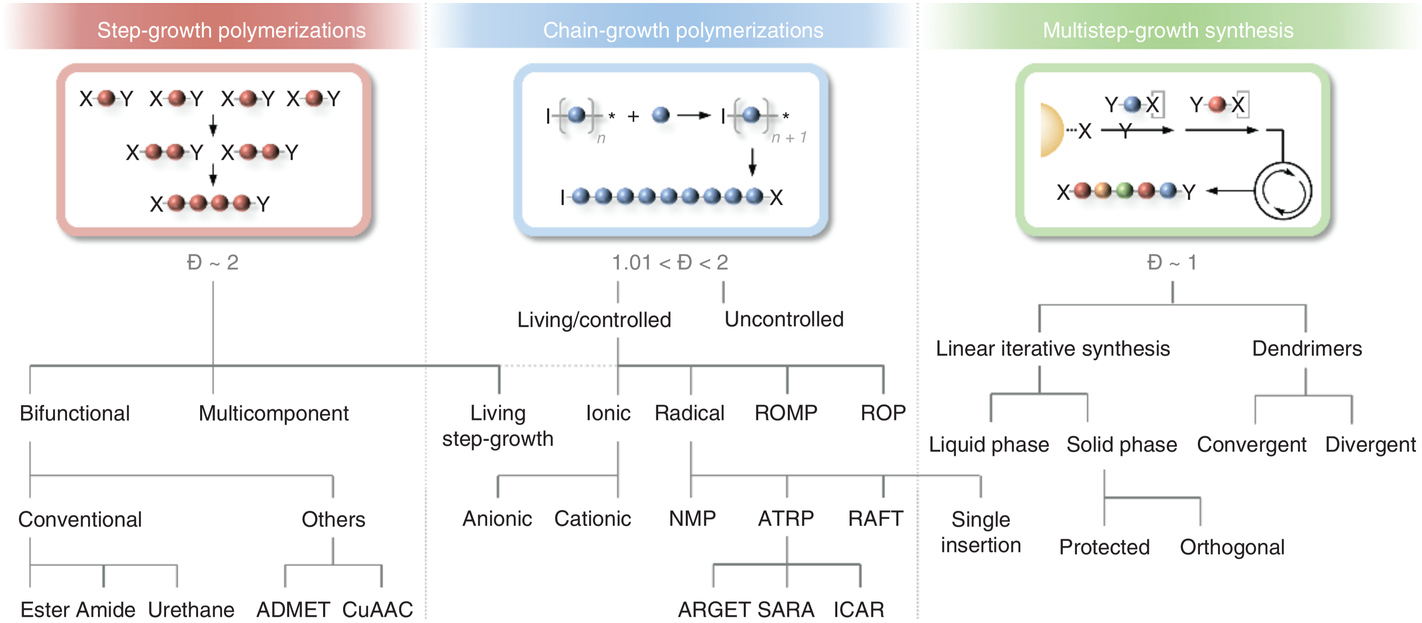

Figure 1.3 Synthetic polymers can be prepared via a step-growth, chain-growth, or multistep-growth mechanism [13]. Definition of the acronyms: acyclic diene metathesis (ADMET), activators regenerated by electron transfer (ARGET), atom transfer radical polymerization (ATRP), copper-catalyzed azide–alkyne cycloaddition (CuAAC), initiators for continuous activator regeneration (ICAR), nitroxide-mediated polymerization (NMP), reversible addition–fragmentation chain-transfer polymerization (RAFT), ring-opening polymerization (ROP), supplemental activator and reducing agent (SARA).

1.5.1 Step-Growth Polymerizations

In step-growth polymerizations, multifunctional monomers are reacted to afford linear, cyclic, or cross-linked architectures. For example, as shown in Figure 1.3, bifunctional monomers containing two reactive functions X and Y usually lead to linear polymers. Alternatively, two monomers XX and YY can be copolymerized. Step-growth polymerizations are overall easy methods but lead generally to a relatively poor control of chain length and molecular weight distribution. In terms of monomer sequence regulation, such polymerizations are also limited by their simple mechanism. Yet, step-growth polymerizations are very interesting options for the preparation of periodic copolymers. Indeed, in many cases, it is possible to include long functional spacers between the functionalities X and Y. Hence, the step-growth polymerization of sequence-controlled telechelic oligomers allows the synthesis of high molecular weight macromolecules containing periodically repeating motifs. For instance, using such strategies, a given functional group can be included periodically in a polymer chain and the distance between the functional sites can be precisely controlled. Among the pioneers in that area, Wagener and coworkers have described the acyclic diene metathesis (ADMET) synthesis of precision polyolefins containing periodically spaced alkyl branches or polar groups [50–52]. Similar concepts have also reported by our group [53] as well as that of Du Prez [54] using step-growth “click” polymerizations based on either CuAAC or thiol–ene chemistry. Furthermore, step-growth strategies are practical tools to polymerize functional, sequence-defined oligomers. For example, Kamigaito and coworkers have synthesized periodic ABCC-periodic vinyl copolymers using metal-catalyzed step-growth radical polymerization [55]. A comparable strategy was investigated by Meyer and coworker for the synthesis of sequence-controlled poly(lactic-*co*-glycolic acid) (PLGA) [56]. In this, approach, oligomers containing defined lactic/glycolic acid minimal sequences and reactive alcohol (X) and carboxylic acid (Y) terminal functions were polymerized, thus affording periodic aliphatic polyesters.

1.5.2 Chain-Growth Polymerizations

In chain-growth polymerizations, the growing polymer chains containing at least one active center react with the monomers one by one and incorporate them successively [13]. Chain-growth polymerizations can proceed via radical, anionic, cationic, or ring-opening mechanisms, as shown in Figure 1.3. Although monomers are incorporated successively in these polymerizations, the process is usually performed in one pot, and therefore chain-growth copolymerizations are usually not sequence-regulated [4]. When two monomers are copolymerized in a chain-growth process, the primary structure of the formed copolymer depends on the initiation/propagation kinetics (i.e., living, controlled, or steady-state) and on the difference in reactivity between the monomers, which is expressed by the reactivity ratios. In most known cases, the reactivity of the comonomers is not markedly different, and therefore statistical copolymers with irregular primary structures are formed. However, in some particular cases, such as the radical copolymerization of styrene (donor monomer) and maleic anhydride (acceptor monomer), sequence-regulated alternating copolymers are formed [57, 58]. Yet, as highlighted in earlier reviews [8], the mechanism of chain-growth copolymerizations had already been known in the 1940s [59, 60], and since then very little progress had been made for several decades. As a result, until very recently, the main types of copolymers that were prepared by chain-growth copolymerizations were statistical, alternating, and block copolymers [4]. This situation has changed with the development of living polymerizations [61] and, in particular, with the widespread utilization of controlled radical polymerization methods [62, 63]. Indeed, in a living or controlled/living chain-growth mechanism, all chains are initiated and grow simultaneously. As a consequence, in copolymerizations, the difference in reactivity of the comonomers can be "written" in the microstructure of the formed chains [64]. The first example of that was the description of gradient copolymers by Matyjaszewski *et al.* [28]. Since then, more precise sequence regulation has been reported. For instance, in 2007, our group introduced a facile concept for placing functional monomers at precise chain locations in chain-growth radical copolymerizations [65]. In this approach, donor and acceptor comonomers are used. However, instead of using equimolar amounts of comonomers as in standard alternating radical copolymerizations, a discrete amount of the acceptor comonomer (typically an N-substituted maleimide) is copolymerized with a large excess of the donor monomer (generally styrene). As a consequence of the controlled/living mechanism and of the marked reactivity differences of the comonomers, the N-substituted maleimide is usually incorporated into relatively narrow regions of the growing chains [64]. Thus, using time-controlled monomer additions, it is possible to prepare complex functional microstructures using this simple methodology [66–68]. Although not leading to perfectly defined microstructures [69], such easy sequence-controlled copolymerizations open up interesting avenues for the design and folding of tailored functional polymers [70–73]. Donor and acceptors comonomers have also been used for preparing other types of SCPs by controlled radical copolymerization. For instance, Kamigaito and coworkers have described the synthesis of AAB-periodic polymers by copolymerization of limonene and N-substituted maleimides [74, 75]. Periodic copolymers can also be prepared by chain-growth cyclopolymerization of monomers containing minimal AB- or ABA-sequence motifs, as elegantly proposed by Ouchi and coworkers [76, 77]. Another interesting recent trend in the field of SCPs is the development of multiblock copolymers [78], as described in Chapter 14 of this book. Although for several decades the synthesis of block copolymers has been mainly limited to diblock or triblock copolymers, it has been shown in recent years that longer block sequences can be prepared. In particular, the groups of Coates [79], Perrier [80, 81], and Haddleton [82, 83] have made significant progress in that domain.

Attempts have also been made to achieve monomer single insertion in chain polymerizations. In such strategies, the chain-growth process is decomposed into a multistep growth polymerization (see Section 1.5.3), as underlined in Figure 1.3. The earliest example was reported by Higashimura and coworkers, who described the successive insertion of four vinyl ether monomers in cationic polymerizations [84]. Attempts have also been made subsequently in controlled radical polymerizations, although they are limited so far to a very small number of monomer insertions [85, 86]. Junkers and coworkers partially bypassed that problem by implementing an inline chromatography purification protocol that permits the separation of sequence-defined oligomers from polydisperse mixtures [87]. Using this approach, the synthesis of sequence-defined, acrylate-based pentamers has been reported [88]. Sawamoto and coworkers have also investigated advanced chemical concepts for regulating single monomer insertion in radical polymerizations. For instance, they have studied innovative template initiators that permit the recognition and selective chain-incorporation of specific monomers [89, 90]. However, this elegant chemistry is limited so far to low molecular weight models. More recently, they have described the iterative radical addition of monomer carrying bulky side chains [91]. This process allows successive monomer insertions but requires three relatively time-consuming chemical steps for each monomer attachment. In a very recent publication, these authors have also depicted an elegant stepwise cyclopolymerization mechanism, inspired by ribosomal chemistry [92]. This process was, however, only validated for a small number of iterations.

1.5.3 Multistep Growth Polymerizations

In multistep growth approaches, uniform polymers are prepared using stepwise chemical steps [13]. For instance, the successive attachment of bifunctional monomers containing two reactive functions X and Y allows the synthesis of uniform polymers ($Đ \sim 1$) [1]. In order to prevent these monomers from polymerizing via a step-growth process (see Section 1.5.1), one of both reactive functions of the monomer is temporarily protected. For instance, a simple strategy to synthesize uniform polymers is iterative exponential growth, in which a bifunctional XY monomer containing a protected X function is reacted with its analog containing a protected Y function [93]. After coupling, the formed adduct is divided in two batches, which are selectively deprotected on one terminus and coupled again. The process is then repeated a certain number of times until a desired length is reached. In other words, the chain length of the polymer is multiplied by 2 at each stage of the process (i.e., going through successive dimer, tetramer, octamer, 16mer, 32mer stages). Although simple and scalable, this method remains, however, mostly limited to the synthesis of periodic primary structures. For attaining a wider variety of sequences (e.g., aperiodic coded sequences), iterative approaches in which the monomers are attached one by one are used, as depicted in Figure 1.3. In such approaches, a growing polymer chain containing a reactive X group is reacted with a bifunctional XY monomer containing a protected X function. Hence, monomers can be successively attached using stepwise coupling/deprotection cycles. Since monomers are assembled following a predetermined order, such a stepwise synthesis is therefore a powerful strategy for preparing sequence-defined polymers. Such approaches have been initially developed for the chemical synthesis of biopolymers, in particular peptides [3]. Yet, stepwise synthesis is somewhat difficult when performed in solution, because it requires intensive purifications after each monomer attachment. One important breakthrough in the field was the introduction by Merrifield of solid-phase synthesis, which greatly simplifies the purification in multistep growth approaches (see Chapter 5) [14, 15]. In this approach, the chains are grown on cross-linked polymer beads that can be filtered. Thus, after each chemical step, the growing polymers can be easily separated from the excess reactants. Similar results can also be obtained with other types of supports, such as controlled-pore glass, solid nanoparticles, soluble polymer supports, or fluorescent tags. Although multistep growth synthesis has been predominantly studied for peptides and oligonucleotides, it allows also the preparation of non-natural sequence-defined polymers. For instance, such approaches have been utilized for the design of a wide variety of non-natural bio-oligomers, including peptidomimetics and foldamers [3]. More recently, these strategies have also been investigated for preparing a broader variety of synthetic polymers [94], including, for example, non-natural information-containing macromolecules (see Section 1.7) [20]. For example, phosphoramidite chemistry, which was initially developed for the synthesis of oligonucleotides [17], is also a practical chemistry for preparing synthetic sequence-defined polymers, as recently demonstrated by Sleiman and coworkers [95] and by our group [96, 97]. Using this approach, our group has reported the automated synthesis of uniform, sequence-defined chains containing more than 100 monomer units [98], thus demonstrating that multistep growth approaches are not restricted to oligomers. Yet, it should be clearly understood that such long chains can be obtained only if the yield of each individual reaction is near-quantitative. Thus, highly efficient reactions should be selected for optimal multistep growth synthesis. Another important parameter in those syntheses is time. Indeed, coupling steps that require hours or days for reaching high yields are unpractical for the synthesis of long polymers. Thus, chemical reactions that can be completed in less than 1 h are somewhat preferred. For example, an automated coupling/oxidation/deprotection phosphoramidite cycle usually takes ~5–10 min. Thinking further out into the future of the field, ultrafast or microwave-assisted reactions, which would permit the coupling steps to proceed in less than 5 min, would be very interesting options. Significant efforts have also been made for avoiding the use of protecting groups in iterative syntheses. For instance, interesting orthogonal iterative approaches have been recently reported. They are described in detail in recent reviews [12, 94] as well as in Chapter 6 of this book. The first example of an efficient orthogonal iterative process was the so-called submonomer strategy reported by Zuckermann *et al.* for the synthesis of peptoids (see Chapter 7) [99]. More recently, related approaches have been reported by our group as well as by the groups of Alabi [100], Du Prez [101, 102], and Meier [103, 104]. For example, our group described the orthogonal synthesis of oligo(triazole amide)s [105, 106], oligo(alkoxyamine amide)s [107, 108], oligo(alkoxyamine phosphodiester)s [109], and oligocarbamates [110]. These examples illustrate that a wide variety of uniform synthetic polymers can now be synthesized by iterative chemistry, thus opening interesting new perspectives for the design of new types of functional macromolecules (see Section 1.8).

1.6 Characterization of SCPs

Standard polymer analytical methods that are used for the characterization of homopolymers and simple copolymers are not always sufficient for examining sequence-controlled microstructures. For instance, a diblock copolymer structure can be relatively easily evidenced using size-exclusion chromatography and NMR, but this analytical combination cannot be used for characterizing more complex sequence-defined polymers. In a recent review, we proposed different meanings for the terms “sequence analysis” and “sequencing” [24]. The former denotes the examination of sequence tendencies in polydisperse polymers (e.g., analysis of dyads and triads by ^{13}C NMR), while the latter refers to comprehensive deciphering of sequence-defined information, as performed in proteomics and genomics. For several decades, only sequence analysis tools, in particular NMR [111, 112], have been used in polymer science for the characterization of copolymers [7]. However, in very recent years, some sequencing methods have also been screened for characterizing non-natural, sequence-defined macromolecules. It should be mentioned that several analytical techniques that are used for the sequencing of proteins and nucleic acids utilize biological reactions (e.g., polymerase chain reaction or PCR amplification) that cannot be applied to synthetic polymers [24]. However, some techniques are suitable for both biological and abiotic polymers (Figure 1.4). For instance, the most suitable tool for reading non-natural sequences is probably tandem mass spectrometry (MS/MS). In MS/MS measurements, macromolecules are fragmented by collision-induced dissociation, and the mass differences between the fragments are used to decode the monomer sequences. In close collaboration with the group of Charles, we have shown in recent years that MS/MS is a very practical method to read the sequences of a wide variety of non-natural information-containing polymers [110, 115–117]. In particular, it was observed that the MS/MS of synthetic polymers is often even easier than that of biopolymers since the macromolecular structure of non-natural analytes can be optimized to favor fragmentation and sequencing [109, 110, 118]. The recent findings are summarized in Chapter 16 of this book.

Besides MS/MS, many other analytical tools have shown promise for characterizing the sequences of non-natural SCPs. For instance, nanopore-based analytics (Figure 1.4) [119], which have been optimized for DNA sequencing during the last few decades [113, 120], can also be interesting tools to read synthetic sequences. In such measurements, sequence-defined polyelectrolytes are subjected to an ionic current, which thread through natural (e.g., porous membrane proteins such as hemolysin or aerolysin) or non-natural (e.g., holes in graphene or molybdenum disulfide flat surfaces) pores of a defined diameter. The interactions of the monomers with the pore lead to voltage variations that can be correlated to the sequence. Such measurements are, however, not trivial, and DNA sequencing required almost 20 years of optimization. This is, in part, due to the fact that DNA nucleobases have closely related molecular structures. In comparison, the sequences of non-natural SCPs could be intentionally engineered to interact to a higher or smaller extent with a pore, thus rendering

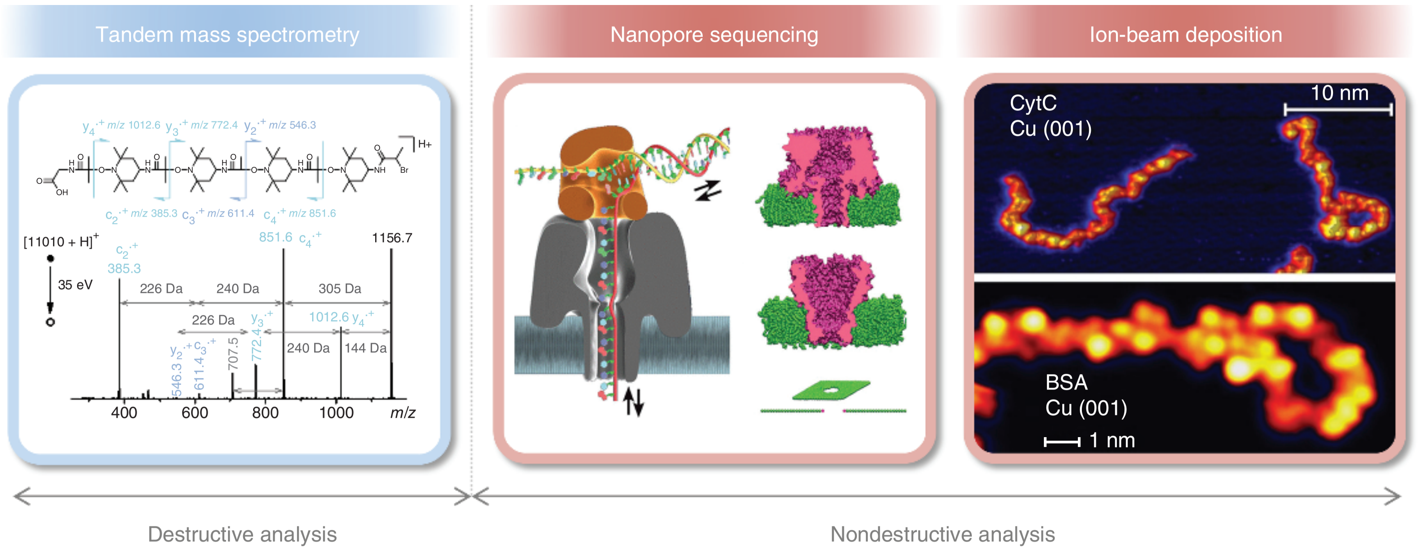

Figure 1.4 Overview of some analytical techniques that are used for the characterization of SCPs. Some of these techniques lead to the destruction of the analytes (shown in blue), while some other are nondestructive (shown in red). The nanopore sequencing scheme is reprinted from Ref. [113]. (Reproduced with permission of Nature Publishing Group.) The scanning tunneling microscopy (STM) images obtained after electrospray ion beam surface deposition are reprinted from Ref. [114]. (Reproduced with permission of American Chemical Society.)

the analytes more "readable" than DNA [97]. Yet, the pore analysis of synthetic polymers is a young discipline, and years of optimization are probably needed until non-natural sequencing will be attained [121, 122]. It has also been shown that electrospray ion beam deposition is a valid method to study intact proteins by scanning tunneling microscopy (Figure 1.4) [114]. Although barely tested, this technique could also be powerful for SCP analysis.

1.7 Impact in Materials Science

Although the field is still relatively young, SCPs have already opened up unprecedented horizons for the design of new materials. These advances and promises are discussed in detail in Chapter 15 of this book. In brief, Figure 1.5 emphasizes the fact that SCPs offer the opportunities to control macromolecular properties at different scales. For instance, at the molecular level, sequence-defined polymers are promising options for the molecular storage of information [20]. Indeed, the monomer units of a polymer can be used as bits, which are basic information units. For instance, binary information can be written and stored in a polymer using two comonomers arbitrarily defined as 0 and 1 bits (Figure 1.4) [21]. This strategy is inspired by the information storage capacity of DNA. It is well known that genetic information is stored in DNA chains in form of a nucleotide sequence. This concept can also be applied to man-made polymers in order to store digital information. Depending on the type of polymer, the size of a monomer unit is generally in the range 2–10 Å. The digital bits in a polymer are therefore much smaller than in a conventional storage medium. In order to create a string of digital information in a polymer chain, each coded monomer unit should be inserted at the right position. Although biopolymerization mechanisms could be, in principle, used to do so, encoded digital sequences are generally obtained by chemical synthesis. For instance, large libraries of information can be produced using parallel synthesis of medium-sized chains. This is actually the strategy used recently by the groups of Church [125] and Goldman [126] to store about a megabyte of data on chemically synthesized DNA. After chemical synthesis, the digitally encoded polymers can be stored at ambient temperature and eventually shipped by post [126]. Various sequencing technologies can then be used to read digital information (see Section 1.6) [24]. Besides storage in DNA, digital information can also be stored in a wide variety of synthetic polymers, as pioneered by our group [106, 107]. Digital polymers are currently studied for different applications including data storage [21, 127], long-term storage [128], and as anti-counterfeiting tags [129, 130].

At the nanoscale, SCPs have also opened up very interesting new avenues for materials design. Indeed, during the last two decades, the field of polymer self-assembly has mostly focused on block copolymers (i.e., architectures containing distinct segments), which has led to interesting bulk or solution nanostructures [131–133]. However, it is noteworthy that the nanoscale morphologies formed by block copolymer assembly are much less controlled than those that can be attained with sequence-defined biological polymers, in particular with proteins. Indeed, as described in Section 1.3.2, the primary structure

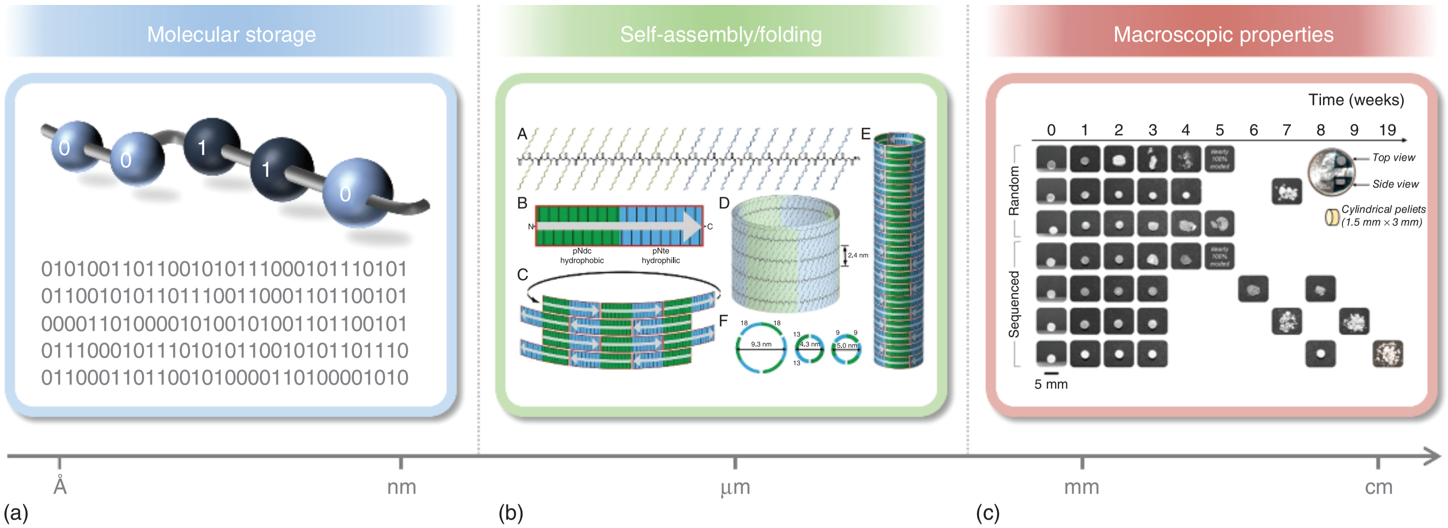

Figure 1.5 SCPs open up interesting properties and applications at all scales. For instance, at the molecular scale (a), sequence-defined polymers can be used to store digitally encoded information. At the nanoscale (b), some sequence-defined polymers may fold and self-organize into defined morphologies that are as sophisticated as those found in nature. The example is highlighted in the green frame [123]. (Sun *et al*. 2016 [123]. Reproduced with permission of Chapter 7 authors.) At the macroscale (c), monomer sequence regulation permits the control of macroscopic behaviors such as the time-controlled degradation of biocompatible polyester pellets. The example is highlighted in the red frame [124]. (Washington *et al*. 2017 [124]. Reproduced with permission of Elsevier.)

of proteins influences their folding and assembly into defined secondary, tertiary, and quaternary structures. Similarly, sequence-defined RNAs fold into precise functional structures, and complementary DNA strands self-hybridize to form a stable double helix. It has been demonstrated in recent years that natural self-assembly principles can be imported into materials science [13]. For instance, man-made materials containing peptide or oligonucleotide segments self-organize into a wide variety of useful morphologies [134, 135]. Following these examples, the next breakthrough in the field of polymer self-assembly will probably be the folding of non-natural, sequence-defined polymers (Figure 1.5). Significant advances in that direction have already been made with foldamers, which are low molecular weight foldable macromolecules [136]. However, the folding of longer SCPs is an underexplored area with great potential for discovery [137–139], in particular for the so-called single-chain technologies [140].

Although SCPs are certainly not foreseen to be commodity polymers, monomer sequence regulation may also influence useful macroscopic properties such as mechanical properties, optical properties, solution behavior, gelation, conductivity, and degradability, just to name a few [1]. Although for many applications the random copolymerization of different comonomers is sufficient to adjust the global properties of the resulting materials, in some cases a finer sequence control seems to have a tremendous impact on the macroscopic behavior. As a single example (see Chapter 15 for more examples), Figure 1.5 compares the swelling and erosion behavior of PLGA pellets based on sequence-controlled or random copolymers [124]. It can be clearly observed that the erosion rate of PLGA with alternating sequences is remarkably lower than that of conventional random PLGA copolymers, even when the overall monomer composition is identical in both types of pellets. Hence, sequence control allows fine-tuning of materials' degradation properties, which is essential for some biomedical applications such as sustained drug delivery or tissue engineering.

1.8 Some Words About the Future

The advances summarized in this chapter indicate that the field of SCPs has grown considerably during the last 5 years and has become a major topic in fundamental polymer science. However, this domain is still in its infancy, and many important aspects remain to be discovered. For instance, the SCPs community is, at the moment, mostly composed of synthetic chemists. As underlined in several chapters of this book, synthetic approaches for preparing SCPs are now numerous and varied, ranging from biological approaches to fully synthetic concepts. Thus, it can be expected that the synthesis of SCPs will be further optimized in the coming years. For instance, progress can be expected regarding (i) the quality of sequence regulation and the reduction of sequence defects, (ii) the attainable length of sequence-defined macromolecules, (iii) the yields and quantity of SCPs that can be produced, and (iv) the speed of synthesis. In particular, it seems obvious that polymer engineering and processing methods will play a significant role for improving all these aspects [87, 98, 141]. Apart from synthesis aspects, SCPs have not yet attracted much attention in polymer theory, physics, and physicochemistry, even though these new polymers raise many questions that are relevant for these domains [142–144]. For example, coding theory, which is commonly used in cryptography, data compression, informatics, and biology, could be helpful to design and optimize new types of functional SCPs such as digital polymers [145, 146]. In addition, the folding and self-assembly of SCPs is an exciting emerging area that surely deserves a deeper understanding of the thermodynamic and kinetic aspects. As highlighted in Section 1.6, major progress has also to be made for the characterization of SCPs. Although very interesting sequencing tools have already been demonstrated [108, 110], progress in that area is still mandatory. Finally, it should also be emphasized that the development of SCPs is still a highly fundamental domain of research. Although some interesting properties and applications have already been identified, industrial applications of SCPs do not exist yet. This implies that cost and structure/properties issues should also be carefully examined in the future. Nevertheless, given the very wide range of opportunities that are offered by SCPs, it can be expected that this new class of polymers will play a major role in future technologies.

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