Polymer Sequencing

DOI: 10.1002/anie.201406766

Reading Polymers: Sequencing of Natural and Synthetic Macromolecules

*Hatice Mutlu and Jean-François Lutz**

mass spectrometry · nanostructures · NMR spectroscopy · polymers · sequence determination

The sequencing of biopolymers such as proteins and DNA is among the most significant scientific achievements of the 20th century. Indeed, modern chemical methods for sequence analysis allow reading and understanding the codes of life. Thus, sequencing methods currently play a major role in applications as diverse as genomics, gene therapy, biotechnology, and data storage. However, in terms of fundamental science, sequencing is not really a question of molecular biology but rather a more general topic in macromolecular chemistry. Broadly speaking, it can be defined as the analysis of comonomer sequences in copolymers. However, relatively different approaches have been used in the past to study monomer sequences in biological and manmade polymers. Yet, these "cultural" differences are slowly fading away with the recent development of synthetic sequence-controlled polymers. In this context, the aim of this Minireview is to present an overview of the tools that are currently available for sequence analysis in macromolecular science.

1. Introduction

The full mapping of the human genome, reported in 2001,[1] and the more recent results of the ENCODE project[2] have revolutionized the field of genomics. The scientific impact of these findings is enormous, and will probably strongly influence 21st century research and technology.[3] However, none of this would have been possible without the development of reliable methods which allow sequencing (i.e., reading monomer sequences) of biopolymers. The roots of the field of macromolecular sequencing can be traced back to the early 1940s with the work of Bergmann and Niemann,[4] Perutz,[5] and Sanger,[6] on protein structure. It is, however, classically admitted that the report of Sanger, describing the primary structure of insulin, is the earliest example of a deciphered macromolecular sequence.[7] Some decades later, Sanger et al.[8] and Maxam and Gilbert[9] described methods for DNA sequencing which paved the way to modern genomics. Both protein and DNA sequencing have forever changed the face of molecular biology.

However, fundamentally speaking, macromolecular sequencing is not only a matter of biology but rather a matter of chemistry, and more specifically of polymer chemistry. Indeed, putting aside its biological implications, the field of sequencing can be simply described as the analysis of monomer sequences in macromolecules.

However, historically, biological and man-made polymers have been studied separately in two different disciplines. Consequently, the analytical tools which have been developed for the characterization of biomacromolecules are often very different from those used for the analysis of synthetic polymers. For example, the size of biopolymers is classically assessed by gel electrophoresis, whereas synthetic polymers are more commonly analyzed by gel permeation chromatography. Yet, the historical and cultural barriers between disciplines are slowly disappearing as modern synthetic polymers have recently become almost as structurally complex as biopolymers. Over recent years, numerous examples of synthetic polymers with controlled primary[10] and secondary[11] structures have been reported. For instance, the recent emergence of the field of sequence-controlled polymers[12] has opened up interesting perspectives in applied polymer science.[13] It was, for example, recently suggested that synthetic polymers can be used to store molecular information.[14]

[*] Dr. H. Mutlu, Dr. J.-F. Lutz
Precision Macromolecular Chemistry, Institut Charles Sadron, UPR22-CNRS
23 rue du Loess, BP 84047, 67034 Strasbourg Cedex 2 (France)
E-mail: jflutz@unistra.fr

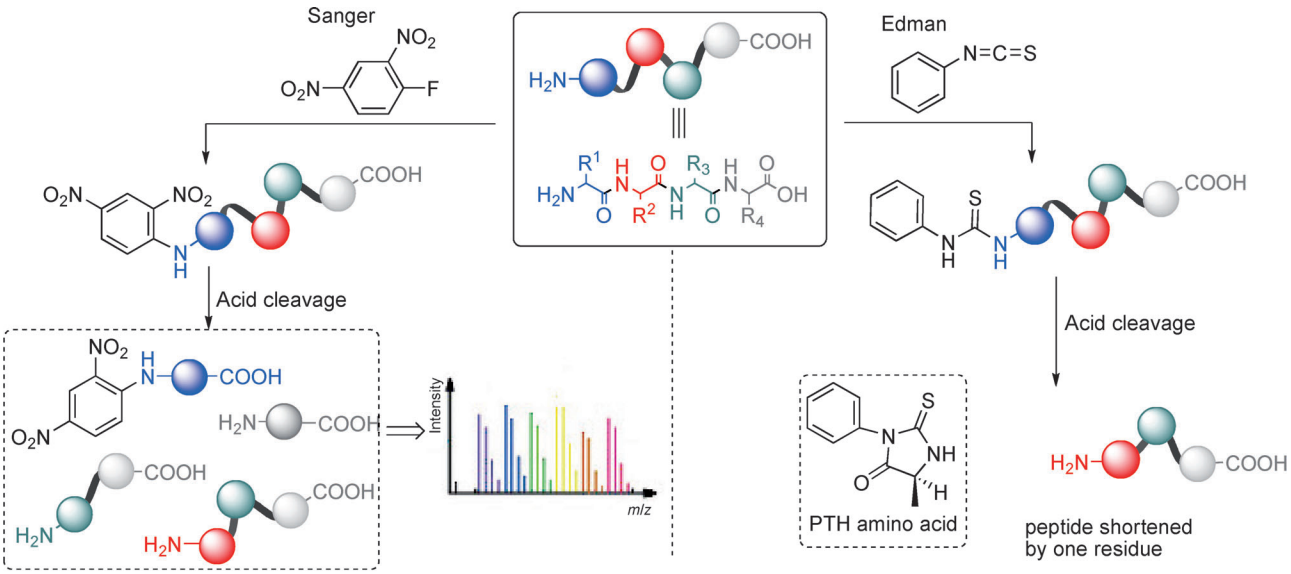

Figure 1. Sanger (left) and Edman degradation (right) for peptide sequencing. Both methods rely on the modification of the N-terminal residue with an identifiable marker under alkaline conditions to facilitate sequencing. Sanger sequencing utilizes 2,4-dinitrofluorobenzene to react with the N-terminal residue, while the Edman approach is based on using phenylisothiocyanate. The advantage of the Edman process is that sequencing occurs inward from the N-terminus, thus the rest of the peptide remains intact. Therefore, the entire sequence of reactions can be repeated over and over to obtain the sequences of the peptide.

In this context, the analysis of comonomer sequences in synthetic copolymers has recently sparked renewed interest in conventional polymer science.[15] However, the tools that are currently used for such a purpose are far from being as elaborate as those developed for biological sequencing. During the last few decades, the sequences of synthetic copolymers have been mainly studied using ^{1}H and ^{13}C NMR spectroscopy.[16] Although interesting for the description of simple copolymers such as statistical or alternating copolymers, classical NMR measurements are probably not sufficient for the full sequencing of more complex primary structures. In fact, some of the approaches used for protein and DNA sequencing may also be adequate for studying manmade macromolecules. Thus, the present Minireview describes the main methods which have been developed in the past for the analysis of synthetic and biological macromolecules. In particular, the Minireview is organized into two main sections so as to compare the parallel developments of both disciplines. It should be, however, clearly stated that the present Minireview is not meant to be fully comprehensive. Many specific aspects of protein and DNA sequencing have been described in previous reviews and are therefore not described in detail in the present text.[17] Thus, the present Minireview was conceived to be a concise introduction to the important field of macromolecular sequencing.

2. Biopolymer Sequencing

2.1. Protein Sequencing

Chain-cleavage methods are classically used for protein sequencing. In such approaches, the chemical modification of the end of a polymer chain (i.e., C- or N-terminus) is often required. The early methods of protein sequencing reported by Sanger and Tuppy,[7] and Edman[18] are both based on N-terminus modification (Figure 1). Approaches employing C-terminus modification have been also considered but turned out to be much more challenging.[19] As shown on the left of Figure 1, in the Sanger approach the peptide N-terminus is

Hatice Mutlu, born in 1981 in Bulgaria, received her BS in Chemistry in 2005 from Marmara University in Istanbul. She continued her education in polymer chemistry under the guidance of Prof. Selim Küsefoglu at Bogaziçi University in Istanbul, and received a MS in Chemistry in 2008. She obtained her Ph.D. in 2012 from Karlsruhe Institute of Technology in the group of Prof. Michael A. R. Meier. She has recently joined the group of Jean-François Lutz as a postdoctoral fellow.

Jean-François Lutz is CNRS research director, deputy director of the Institut Charles Sadron, and head of the Precision Macromolecular Chemistry Group. He received a Ph.D. degree from the University of Montpellier in 2000 and a habilitation degree from the University of Potsdam in 2009. He is author/inventor of approximately 150 publications and patents. In 2008 he received the prize of the polymer division of the French Chemical Society. He is also an ERC laureate since 2010.

first modified with dinitrofluorobenzene. Afterwards, the peptide is cleaved by chemical or enzymatic degradation, and the fragments are analyzed and deciphered. In this strategy, the dinitrofluorobenzene labelling of the chain-end helps in sorting out the peptide decryption. Indeed, the dinitrophenol-labeled terminal fragment can be identified by its characteristic migration rate in thin-layer chromatography or paper electrophoresis. In the Edman approach (Figure 1, right), the N-terminus of the peptide is modified by phenylisothiocyanate. This modification triggers a chain-end cyclization which leads to the cleavage of the terminal amino acid of the chain, and is then analyzed using liquid chromatography. Afterwards, the procedure is repeated until all amino acids are identified. Thus, in terms of polymer chemistry, the Edman method can be described as a stepwise chain-end degradation process (see Section 3.4 for some analogies in synthetic polymer science). To improve the identification of the Edman degradation products, liquid chromatography was later replaced by mass spectrometry (MS) based on optical detection.[20] Shortly thereafter, the gradual improvement of MS/MS techniques laid the groundwork for protein sequencing by MS,[21] thus providing access to the first multiple protein sequences performed in the 1980s.[22] Over the course of time, various developments in terms of protein sequencing have been exploited, such as ladder sequencing,[23] a method which relies on controlled stepwise chemical degradation at the amino terminus followed by analysis through matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) to result in a mass spectrum which provides information on the sequence and identities of amino acid residues, both unmodified and modified. The rapid expansion in this field made the tandem mass spectrometry based proteomics the method of choice for protein sequencing. Additionally, a number of groups have used biological and artificial nanopores to study protein structure and the potential applications of nanopores in protein analysis (see Section 2.2.3 for additional information about these techniques and their use in nucleic acids sequencing).[24] As mentioned in the introduction, the field of protein sequencing has become a very broad area of research. For more detailed information, the readers are guided to recent comprehensive reviews on the topic.[25]

2.2. DNA Sequencing

Given its crucial implications in genomics and biotechnology, DNA is nowadays the most studied polymer in the field of macromolecular sequencing. However, the development of fast and efficient DNA sequencing methods has required decades of research, and an in-depth understanding of DNA chemistry and physicochemistry. The Sanger method reported in 1977 has dominated the field of DNA sequencing for more than 25 years.[8] However, it has been supplanted over the years by faster and cheaper methodologies, including next-generation (next-gen) techniques and nondestructive approaches (i.e., methods that do not degrade the polymer analyte) such as nanopore analysis. This section of the Minireview describes briefly the chronological evolution of the methods used for nucleic acid sequencing. It should, however, be emphasized that there is only a limited range of chemical strategies capable of sequencing DNA, with DNA polymerases lying at the heart of most current technologies.[26]

2.2.1. Historical Methods

Starting in the early 1970s, Sanger introduced the use of DNA polymerases for DNA sequencing.[27] In such approaches, the polymerases are used to prepare incomplete copies of a given sequence that can be analyzed. This was accomplished with in vitro radioactive labelling of a small specific region, the primer, of an unlabelled DNA molecule, thus yielding DNA polymerase primed by synthetic oligonucleotides. This approach was completed in 1975 by the introduction of the combination of "plus" and "minus" sequencing methods which involve specific nucleotide addition to extend the primer oligonucleotide, thus creating a radioactive complementary copy of a defined part of the template DNA, followed by removal of excess triphosphates to assess specific sequences.[28] However, this technique had some disadvantages. For instance, it was difficult to assess the length of homopolymer runs, thus leading to unreliable results for longer sequences. Building upon this early work, Sanger and co-workers developed a new breakthrough method for sequencing oligonucleotides based on DNA sequencing with chain-terminating inhibitors.[8] Indeed, this method was quite similar to the plus and minus, with the advantage of not requiring the preliminary extension of the primer oligonucleotide. This optimized version has revolutionized the field of genomics and was initially referred to as the chain-termination method or the dideoxynucleotide method. In a few words, it consists of a catalyzed enzymatic reaction which polymerizes the DNA fragments complementary to the template DNA of interest (unknown DNA). Although in the same year, Gilbert and Maxam[9] introduced a chemical approach for DNA sequencing which is based on ability of hydrazine, dimethyl sulphate (DMS), or formic acid to specifically modify bases within the DNA molecule (Figure 2), the enzymatic method was used for genomic research as the main tool to generate the fragments necessary for sequencing, regardless of the sequencing strategy. It should be also mentioned that the introduction of fluorescently-labelled chain terminators[29] (or so called dye-terminator sequencing) greatly contributed to the throughput, thus enabling the completion of the Human Genome project. In spite of that, the need and potential for higher-throughput sequencing technologies was inevitable.

2.2.2. Next-Generation DNA Sequencing

Generally speaking, next-generation sequencing (also called next-gen sequencing or NGS) mainly refers to DNA sequencing methods which arose during the last two decades, after the capillary sequencing methods which relied upon Sanger sequencing.[17a] NGS methods are defined as highly parallelized processes which enable the sequencing of thousands to millions molecules at once. Whereas, NGS will not be covered in detail here, interested readers are directed to

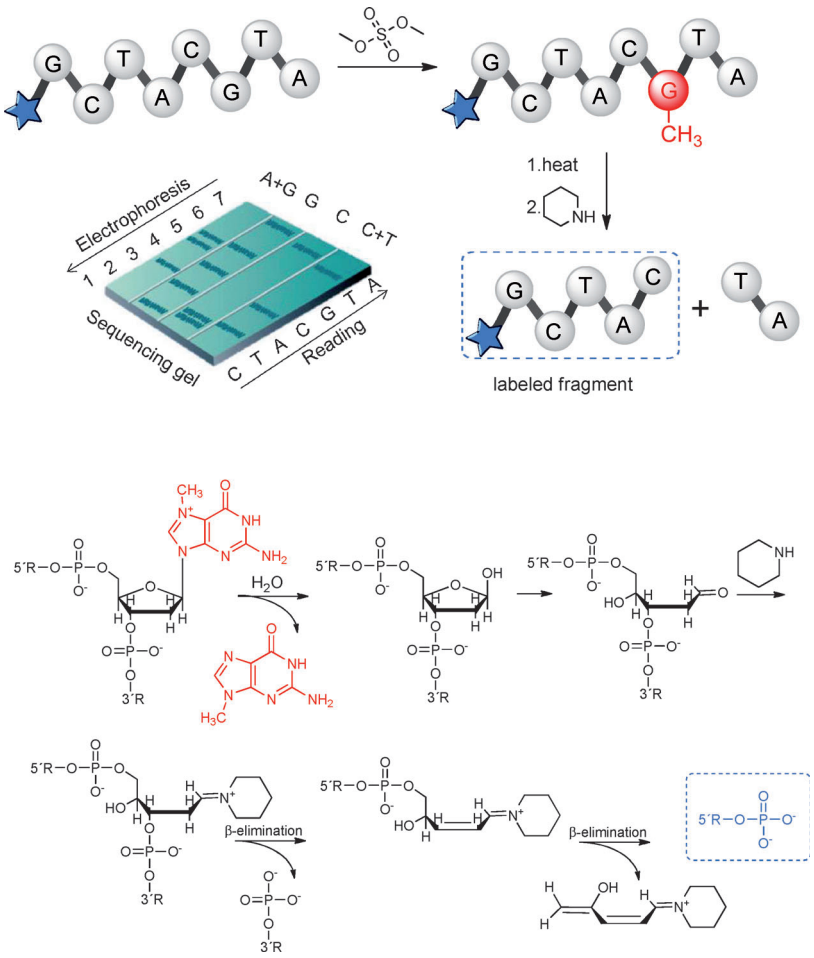

Figure 2. Maxam–Gilbert chemical degradation. This approach is based on radioactive labeling of double-stranded DNA fragments. Thus, it employs a set of base-specific cleavage reactions to generate the necessary fragments that are subsequently separated by electrophoresis. The specificity resides in the first reaction with either hydrazine, dimethyl sulphate, or formic acid, which react with only a few percent of the bases. For example, as exemplified in this figure, dimethyl sulphate reacts specifically with the nitrogen of G, thus resulting in a positive marker in the G-lane of the electrophoresis.

a comprehensive review by Mardis and co-workers,[17a] and it should be mentioned that Margulies et al. were the ones who introduced the first widely adopted NGS approach based on pyrosequencing technology.[30] During the last few years, NGS methods have been evolved from a second-generation platform (which essentially uses DNA synthesis chemistry as employed by the traditional Sanger's sequencing) to a third-generation and finally to a fourth-generation (4G) sequencing machine, which is also known as nanopore technologies.

2.2.3. Nanopore Sequencing

Nanopore sequencing, also sometimes referred to as a fourth-generation sequencing technology, is based on the analysis of a sequence-defined biopolymer through a biological or synthetic pore (Figure 3). Almost two decades ago, Kasianowicz and colleagues showed experimentally that single-stranded DNA molecules can be driven through a biological nanopore channel (i.e., α-hemolysin in that specific study) embedded in a lipid bilayer. When moving through the pore, the DNA analyte induces a blockade in the channel current (Figure 3).[31] Early studies have shown that

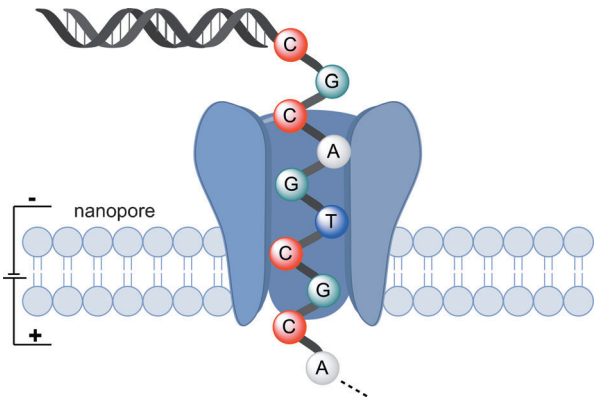

Figure 3. DNA is sequenced by threading it through a microscopic pore embedded in a membrane. Within the nanopore characteristic disruptions of current result from combinations of bases, then the order and identity of bases are identified by the way they affect ions flowing through the pore from one side of the membrane to the other.

DNA/RNA homo- and copolymers could be readily differentiated by this biological nanopore mainly by comparing

their characteristic plot features, such as current blockage amplitude.[32] In addition, numerous studies involving single-stranded DNA and double-stranded DNA sequencing[33] showed that silicon-based solid-state nanopores could also be used for similar experiments as a versatile alternative to the biological ones. Moreover, Wanunu and Meller have shown that a great diversity of functions can be given to a solid-state nanopore by chemically modifying the surface.[34b] More specifically, by employing chemically modified solid-state nanopores they were able to perform experiments which pointed out that it is even possible to distinguish between 3′ or 5′ end translocations of identical DNA molecules.[34] Recently, it was shown that nonsilicon-based solid-state nanopores can also serve as channels for the sequencing of single-stranded DNA molecules.[35] Along these pioneering experiments, Meller and co-workers have reported proof-of-principle of an optipore technique capable of a multicolor readout of DNA sequence.[36] Comprehensive information about the progress made in nanopore-based sequencing and detection of nucleic acids can be found in a recent review by Long et al.[17c]

3. Sequencing of Synthetic Copolymers

Analytical approaches currently employed for studying monomer sequences in synthetic polymers are rather different from those used in biopolymer sequencing. These differences are, in part, due to the fact that synthetic polymers, synthesized by chain-growth and step-growth polymerization techniques, are polydisperse samples. Consequently, such macromolecules are analyzed in general as whole and average expressions are used to describe their molecular structure (e.g., the degree of polymerization (*DP*), comonomer composition, dyad and triad fractions). This description differs markedly from perfectly defined biopolymers which can be characterized more accurately. However, recent developments in sequence-controlled polymerizations have narrowed the gap between natural and synthetic macromolecules.[12] In this context, the present section describes current tools for analysis of synthetic copolymer sequences and their probable evolution over the next few years.

3.1. Sequence Analysis by NMR Spectroscopy

NMR spectroscopy is certainly the most widespread method for the analysis of monomer sequences in synthetic copolymers.[16] Significant developments in that area of research have been reported by Bovey and co-workers who described early methods for ^{1}H, ^{13}C, and two-dimensional NMR analysis of comonomer sequences and tacticity.[37] Indeed, the NMR resonance of a given monomer unit in a polymer chain is directly influenced by the molecular structure of its close neighbors.[16] Depending on the field strength of the NMR spectrometer and on the structure of the analyzed copolymer, dyads, triads, tetrads, pentads, and in some rare cases higher-order neighboring effects, up to octads, can be observed and quantified by integration. For instance, by knowing the relative abundances of all comonomer dyads in a copolymer (e.g., dyads AA, AB, and BB in an AB copolymer), number-average sequence fractions can be determined.[38] However, NMR sequence analysis remains general and gives limited information about sequence distribution along polymer chains (e.g., gradients, clusters, long-range sequence order).

One possibility to describe more precisely comonomer sequence distribution along chains is to monitor by NMR spectroscopy the chain incorporation of comonomers during polymer synthesis. It should be however specified that such an analysis is usually restricted to controlled/living polymerizations, in which all polymer chains grow at the same kinetic rate. Indeed, in such polymerizations, comonomer kinetic consumption is directly encrypted in the microstructure of the formed copolymers. For instance, NMR kinetic monitoring has been used for the microstructural characterization of alternating[39] block[40] and gradient[41] copolymers prepared by controlled radical copolymerization. Recently, our group has shown that NMR monitoring can also be used to characterize complex aperiodic comonomer patterns obtained by sequence-controlled copolymerization.[13a,42] These measurements allow a description of microstructure, a description not attainable by conventional NMR analysis of the final polymer isolated after synthesis. Furthermore, it is also possible to follow the formation of comonomer sequences using in situ NMR characterization. For example, Frey and co-workers have recently studied the microstructure of gradient polyethers by in situ ^{13}C NMR spectroscopy.[43]

Molecular read-outs can also be used in NMR spectroscopy to decipher comonomer sequences of complex macromolecules. For instance, noncovalent interactions such as hydrogen bonds or π–π stacking can be used to associate molecules to particular microstructural environments and help to resolve sequences. For example, structurally defined cleftlike molecules containing two parallel subunits capable of interacting with substrates, so-called molecular tweezers, have been recently developed by Colquhoun and co-workers to analyze polyimide sequences.[44] These authors have shown that complementary π–π stacking has significant potential for the development of molecular-level information processing. It was first shown that electron-rich tweezer-type molecules can bind selectively to specific chain motifs containing 13 aromatic rings (sequence SIS in Figure 4a).[44a] Such a supramolecular association leads to a shift in imide resonance in ^{1}H NMR spectroscopy. Thus, the tweezers can be used to detect and quantify SIS sequences in complex copolymers. Indeed, in the absence of the tweezers, the imide groups of a copolymer usually lead to a unique NMR signal, whereas in the presence of the tweezers the SIS signal is split off from the main imide peak and can be therefore integrated. In a more recent publication, it was reported that sequence selectivity in binding can be achieved through the introduction of minor variations in the tweezer structure.[15b] For instance, a specific tweezer was also found for the sequence ISI (Figure 4b). Thus, different tweezers can be used to finely analyze the sequences of a given copolymer by NMR spectroscopy. Besides the tweezers developed by Colquhoun and co-workers, other types of supramolecular read-outs can be used

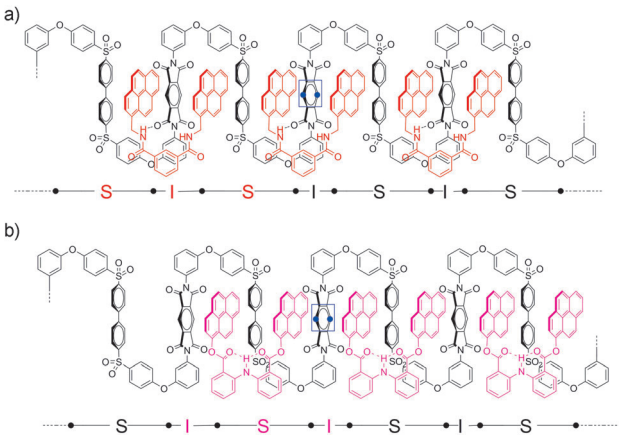

Figure 4. Recognition of specific polyimide sequences by supramolecular tweezers: a) tweezer that specifically binds to the sequence SIS. b) tweezer that specifically binds to the sequence ISI. The letters I and S represent a diarylpyromellitimide unit and a bis(arylsulfonyl)-4,4-biphenylene unit, respectively. Reprinted with permission from Ref. [15b], copyright Macmillan Publishers 2010.

in NMR spectroscopy. For instance, Ramkumar and Ramakrishnan have recently shown that supramolecular intercalators can be used to study the sequences of donor–acceptor macromolecular foldamers.[45]

3.2. Kerr Effect

For several decades ^{13}C NMR spectroscopy in solution has been used as premier experimental means for elucidating the microstructure of synthetic copolymers. However, as highlighted in Section 3.1, NMR spectroscopy is generally only sensitive to relatively local, short-range microstructures.[38b] The sensitivity is often limited to stereosequences at (or below) the pentad or hexad levels, and comonomer sequences at the diad or triad levels, which at best do not extend along the polymer backbone beyond nanometer length. For detailed microstructure analysis, one needs not only to identify such constituent short-range microstructures, but it is also necessary to locate their positions along the copolymer chains. For this aspect, additional assessment techniques may be of benefit. For instance, as a potentially sensitive means to resolve much longer-range microstructures and to probe the complete macromolecule, electrical birefringence—the Kerr effect—measured in dilute polymer solutions under a strong electric field can be addressed.[46] In fact, Tonelli and coworkers have demonstrated the efficiency of this method as a result of the fact that the Kerr coefficient—the molar Kerr constant—of polymers depends on the overall polarizability tensor or changes of the magnitude and/or orientation of its overall dipole moment vector with respect to the direction of its maximum polarizabity, thus enabling the whole characterization of a polymer chain microstructure.[46,47] In most of the reported studies, Tonelli and colleagues have focused on the molar Kerr constant of diverse copolymers of styrene with different microstructures.[48] However, this analysis is not restricted to such copolymers. In a recently published perspective article, Tonelli notes that homo- and copolymers with a wide variety of chemical structures could be analyzed using Kerr effect measurements.[46] It was pointed out that this macroscopic property of the polymer could be determined even in cases where the difference between the birefringence induced by the applied electric field and contributed by the polymer solute is small. Thus, it should be emphasized that the characterization of complete polymer microstructures appears feasible by Kerr-effect measurements. Although currently underexplored, this technique will probably lead to interesting developments in the future for the sequence-analysis of synthetic polymer materials.

3.3. Mass Spectrometry

Over the last twenty years, soft ionization mass spectrometry has played an increasingly important role in polymer analysis. In particular, MALDI-TOF MS has opened up many opportunities for macromolecular structure characterization.[49] For instance, the relevance of MALDI-TOF MS for comonomer sequence analysis was recognized during the early developments of this technique. Interesting thoughts about MALDI-TOF sequence analysis can be found in early reviews by Montaudo and Nielen.[50] In fact, many early examples of comonomer sequences analysis by MALDI-TOF MS were reported by the group of Montaudo, who has studied, for example, the comonomer sequences of polyesters,[51] polyamides,[52] and poly(ester amides).[53] However, it should be noted that in most of these works small oligomers, obtained by chemical degradation (see Section 3.4) or post-source decay fragmentation of longer chains, were studied by MALDI-TOF MS.

As discussed in Section 2.1, tandem mass spectrometry (i.e., MS/MS and MS^n) is a useful technique for the sequence analysis of macromolecules. Detailed information about the utilization of these methods for the characterization of synthetic polymers can be found in recent comprehensive reviews.[55] The sequencing of non-natural polymers by MS/MS has been reported. For instance, Liskamp and co-workers have described the sequence analysis of monodisperse peptoids.[56] Longer sequences were afterwards studied using MS/MS by Zuckermann and co-workers.[54] For example, Figure 5 shows the MS/MS sequencing of a 10-mer peptoid. In this case, the oligomer was attached to a brominated moiety, which serves as an isotope tag and simplifies MS/MS analysis. Very recently, Leigh and co-workers described a fascinating example of an artificial molecular machine which allows synthesis of monodisperse sequence-defined oligopeptides.[13 h] In particular, the authors have used tandem mass spectrometry to confirm the synthesis of a peptide tetramer with this machine.[57] The utilization of MS/MS methods for the characterization of comonomer sequences in more complex polydisperse samples has also been reported.[58] For instance, Studer and co-workers have recently examined the comonomer sequences of well-defined copolymers synthesized by nitroxide mediated polymerization.[59] Substantial information about the copolymers microstructure was obtained in this study. This example is particularly interesting

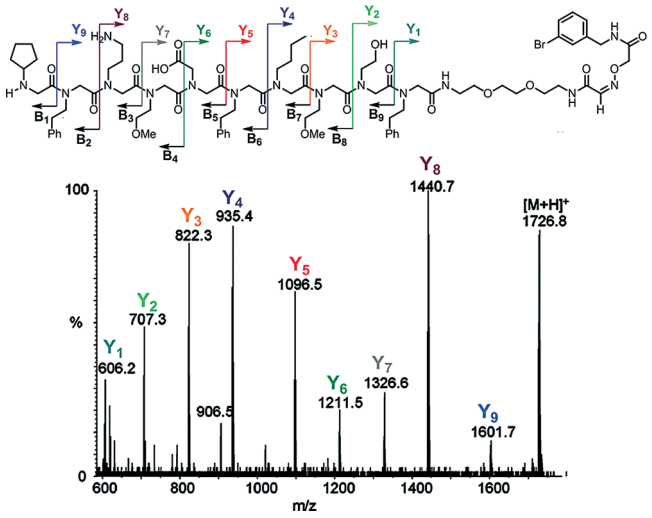

Figure 5. Tandem mass spectrometry (MS/MS) sequencing of a peptoid decamer containing an oligo(ethylene glycol) spacer and a halogen mass tag that facilitates MS analysis. Redrawn with permission from Ref. [54]. Copyright ACS 2006.

because it shows that MS/MS sequence analysis is not restricted to polymers containing heteroatoms in their backbones (e.g., polyesters or polyamides) but can also be used to study polymers with C–C backbones synthesized by controlled radical polymerization. It seems therefore obvious that mass spectrometry will play an important role in future sequence analytics. For additional thoughts on the topic, the reader is guided to an excellent recent review by Altuntas and Schubert.[60]

3.4. Cleavage/Depolymerization

As shown in the first part of this Minireview, chain cleavage is an efficient approach for macromolecular sequencing. Both the Edman method[18] (Figure 1b) and the Maxam–Gilbert approach[9] are based on the controlled chemical degradation of macromolecules. Such cleavage strategies are indeed also relevant for studying comonomer sequences in synthetic copolymers. In fact, such degradation approaches were widely used during the early days of polymer science (i.e., before the widespread utilization of NMR and MS methods) to study the composition and comonomer sequences of copolymers. For instance, oxidative, enzymatic, hydrolytic, and thermal degradation mechanisms have been examined to analyze sequences.[61] Some of these early studies have been summarized in an interesting highlight published by Harwood about 50 years ago.[62] Additionally, a broad body of literature already exists on polymer degradation because the stability, chemical degradability, and biodegradability of synthetic macromolecules have been studied for decades.[63] Generally speaking, linear polymer backbones do degrade by two main mechanisms: 1) random chain scission and 2) chain-end depolymerization (i.e., unzipping). Both mechanisms are interesting in the context of polymer sequencing. For example, chemically- or enzymatically-induced random chain scission was used for the sequence analysis of natural polyesters such as poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate).[51] In such approaches, the copolymer is first chopped into small oligomers which are afterwards analyzed by NMR or MS methods.[64] Comparable strategies can also be developed for unnatural polyesters. For instance, it was recently shown that the degradation profile of aliphatic copolyesters strongly depends on sequence distribution.[65] Likewise, Meyer and co-workers have reported that alternating and random poly(lactic acid-*co*-glycolic acid) copolymers exhibit dramatically different hydrolysis behaviors.[66] This recent work confirms that main-chain degradation is a very useful approach for the analysis of sequences in synthetic polymers. Another interesting example was recently published by Aoshima and co-workers.[67] In this work, alternating polymers of a conjugated aldehyde and an alkyl vinyl ether were degraded into low-molecular-weight compounds which were isolated and characterized.

Perhaps even more interestingly, unzipping mechanisms could be used for the sequencing of synthetic macromolecules. Some examples of copolymer thermal unzipping have been reported.[68] However, these experiments are usually performed at high degradation temperatures, and unzipping processes often coexist with random chain cleavage and other side reactions.[69] In general, copolymers do not depolymerize in a controlled fashion as some homopolymers do.[70] Nevertheless, over recent years, it was shown that chain-end depolymerization can proceed in a more controlled manner if appropriate conditions are used.[71] For instance, interesting deconstruction mechanisms have been described in the emerging context of self-immolative polymers.[72] The degradation of these polymers is triggered by a specific chemical reaction and proceeds afterwards sequentially. The kinetics of depolymerization of such macromolecules could be possibly slowed down to analyze their monomer sequences. A first step in that direction was recently reported by Li and co-workers.[73] By performing time-dependent ^{1}H NMR measurements during of degradation of polyesters obtained by a Passerini multicomponent polymerization and a subsequent hydrogenation, it was observed that the depolymerization can occur in a controllable unzipping manner by an intramolecular cyclization yielding cyclic small molecules. Of course, such unzipping approaches would be more relevant if monodisperse sequence-defined analytes could be synthesized, but as shown in many recent studies,[12] that goal is not too far away.

3.5. Nanopore Analysis of Synthetic Polymers

As highlighted in Section 2.2.3, nanopore sequencing is now a popular method for the sequence analysis of biomacromolecules. However, although significant research effort has been devoted to nucleic acids, very few studies have been dedicated to the nanopore analysis of nonbiological macromolecules. So far, most of the reported studies have focused on poly(ethylene glycol) (PEG).[74] About two decades ago, Bezrukov, Kasianowicz, and co-workers[74a] showed that water-soluble PEG chains could be detected in the aqueous

cavity of staphylococcus aureus α-toxin using ionic-current blockade methods. In this work, the experiments were performed with PEG macromolecules in a range of low-molecular masses (the number average molecular weight, $M_n < 10$ kDa) and at relatively low mass concentration ($c \leq 15\,\%$), in a dilute regime where the chains are not entangled. Afterwards, PEG single-chain analysis in α-hemolysin nanopores was also studied experimentally and theoretically.[75] In particular, the parallel high-resolution chip-based technology introduced by the group of Behrends allows refined analysis of the molecular-weight distribution of PEG samples.[76] Besides PEG, a few polyectrolytes, such as dextran sulphate[77] and polystyrene sulfonate,[78] have been studied in protein nanopores. However, nanopore studies of synthetic water-soluble polymers remain limited, so far, to linear homopolymers. Recently, some theoretical studies have addressed the behavior of more complex macromolecules in confined nanopores. For instance, Sakaue and Brochard-Wyart have recently described the behavior of branched polymers in pores.[79] Perhaps more importantly, Muthukumar and co-workers have examined the pore translocation of heterogeneous copolymers containing charged and uncharged blocky sequences.[80] In particular, it was shown that the probability and the rate of translocation strongly depend on the copolymer microstructure. These interesting results suggest that a refined analysis of synthetic comonomer sequences may be achievable in nanopores.

4. Outlook

Important progress has been made during the last 80 years for the analysis of comonomer sequences in synthetic and natural macromolecules. However, there is a true difference between sequencing and sequence distribution analysis. Monodisperse sequence-defined polymers such as proteins and DNA can be fully sequenced (i.e., comprehensive deciphering of comonomer sequences from one chain end to the other). In contrast, polydisperse polymer samples, in which all chains have slightly different primary structures, can only be described by an average of comonomer distribution. Nevertheless, as shown in this Minireview, the analogies between protein sequencing, DNA sequencing, and conventional polymer analytics are evident. For instance, the strategies developed during the early days of macromolecular science for analyzing sequences in proteins[6] and synthetic polymers[61a] present obvious similarities. In fact, as underlined in this Minireview, biopolymers and synthetic polymers have many analytical tools in common. Yet, the fact that synthetic copolymers cannot be fully sequenced as are biopolymers is probably due to two main reasons. First, the range of available synthetic copolymers is broad and diverse.[81] Thus, for a given type of copolymer, a few sequence analysis studies are usually available, whereas, in comparison, thousands of publications have been devoted to proteins. The second crucial point is that the primary structure of most synthetic copolymers is ill-defined. Full copolymer sequencing would only make sense if monodisperse information-containing copolymers could be prepared.[15a] Recent progress in the field of sequence-controlled polymers clearly shows that a new era in synthetic polymer science has emerged.[12a] In this challenging and exciting context, new options need to be urgently considered for the sequencing of synthetic copolymers. In fact, some recent approaches described in the third section of this Minireview suggest that full sequencing of synthetic macromolecules is most probably attainable. For instance, tandem mass spectrometry, chemical cleavage, and nanopore analysis are promising methods for non-natural polymer sequencing. It should, however, be noted that some synthetic polymers might be easier to sequence than others. For instance, atactic polymers with fully carbonated backbones, prepared using popular controlled radical polymerization methods, are probably not ideal structures for sequencing. Yet, polymer chemistry offers many other options for creating readable macromolecular analytes. We therefore hope that the present text will inspire our peers to pursue further research in the field of macromolecular sequencing.

Received: July 1, 2014
Revised: July 24, 2014
Published online: October 3, 2014

[1] a) International Human Genome Sequencing Consortium, *Nature* **2001**, *409*, 860 – 921; b) J. C. Venter, et al., *Science* **2001**, *291*, 1304 – 1351.
[2] J. R. Ecker, W. A. Bickmore, I. Barroso, J. K. Pritchard, Y. Gilad, E. Segal, *Nature* **2012**, *489*, 52 – 55.
[3] E. S. Lander, *Nature* **2011**, *470*, 187 – 197.
[4] M. Bergmann, C. Niemann, *J. Biol. Chem.* **1938**, *122*, 577 – 596.
[5] M. F. Perutz, *Nature* **1942**, *149*, 491 – 494.
[6] F. Sanger, *Nature* **1948**, *162*, 491 – 492.
[7] F. Sanger, H. Tuppy, *Biochem. J.* **1951**, *49*, 481 – 490.
[8] F. Sanger, S. Nicklen, A. R. Coulson, *Proc. Natl. Acad. Sci. USA* **1977**, *74*, 5463 – 5467.
[9] A. M. Maxam, W. Gilbert, *Proc. Natl. Acad. Sci. USA* **1977**, *74*, 560 – 564.
[10] a) R. N. Zuckermann, J. M. Kerr, S. B. H. Kent, W. H. Moos, *J. Am. Chem. Soc.* **1992**, *114*, 10646 – 10647; b) C. Y. Cho, E. J. Moran, S. R. Cherry, J. C. Stephans, S. P. Fodor, C. L. Adams, A. Sundaram, J. W. Jacobs, P. G. Schultz, *Science* **1993**, *261*, 1303 – 1305; c) L. Hartmann, H. G. Börner, *Adv. Mater.* **2009**, *21*, 3425 – 3431.
[11] a) D. J. Hill, M. J. Mio, R. B. Prince, T. S. Hughes, J. S. Moore, *Chem. Rev.* **2001**, *101*, 3893 – 4012; b) E. Yashima, K. Maeda, H. Iida, Y. Furusho, K. Nagai, *Chem. Rev.* **2009**, *109*, 6102 – 6211; c) G. Guichard, I. Huc, *Chem. Commun.* **2011**, *47*, 5933 – 5941.
[12] a) J.-F. Lutz, M. Ouchi, D. R. Liu, M. Sawamoto, *Science* **2013**, *341*, 1238149; b) N. Badi, J.-F. Lutz, *Chem. Soc. Rev.* **2009**, *38*, 3383 – 3390; c) J.-F. Lutz, *Polym. Chem.* **2010**, *1*, 55 – 62.
[13] a) S. Pfeifer, J.-F. Lutz, *J. Am. Chem. Soc.* **2007**, *129*, 9542 – 9543; b) S. Ida, T. Terashima, M. Ouchi, M. Sawamoto, *J. Am. Chem. Soc.* **2009**, *131*, 10808 – 10809; c) S. Pfeifer, Z. Zarafshani, N. Badi, J.-F. Lutz, *J. Am. Chem. Soc.* **2009**, *131*, 9195 – 9196; d) K. Satoh, et al., *Nat. Commun.* **2010**, *1*, 6; e) Y. Hibi, M. Ouchi, M. Sawamoto, *Angew. Chem.* **2011**, *123*, 7572 – 7575; *Angew. Chem. Int. Ed.* **2011**, *50*, 7434 – 7437; f) M. Zamfir, J.-F. Lutz, *Nat. Commun.* **2012**, *3*, 1138; g) P. Espeel, L. L. G. Carrette, K. Bury, S. Capenberghs, J. C. Martins, F. E. Du Prez, A. Madder, *Angew. Chem.* **2013**, *125*, 13503 – 13506; *Angew. Chem. Int. Ed.* **2013**, *52*, 13261 – 13264; h) B. Lewandowski, G. De Bo, J. W. Ward, M. Papmeyer, S. Kuschel, M. J. Aldegunde, P. M. E. Gramlich, D. Heckmann, S. M. Goldup, D. M. D'Souza, A. E. Fernandes,

D. A. Leigh, *Science* **2013**, *339*, 189 – 193; i) J. Niu, R. Hili, D. R. Liu, *Nat Chem.* **2013**, *5*, 282 – 292; j) T. G. W. Edwardson, K. M. M. Carneiro, C. J. Serpell, H. F. Sleiman, *Angew. Chem.* **2014**, *126*, 4655 – 4659; *Angew. Chem. Int. Ed.* **2014**, *53*, 4567 – 4571; k) S. C. Solleder, M. A. R. Meier, *Angew. Chem.* **2014**, *126*, 729 – 732; *Angew. Chem. Int. Ed.* **2014**, *53*, 711 – 714.

[14] a) J.-F. Lutz, *Acc. Chem. Res.* **2013**, *46*, 2696 – 2705; b) T. T. Trinh, L. Oswald, D. Chan-Seng, J.-F. Lutz, *Macromol. Rapid Commun.* **2014**, *35*, 141 – 145.

[15] a) H. Colquhoun, J.-F. Lutz, *Nat. Chem.* **2014**, *6*, 455 – 456; b) Z. Zhu, C. J. Cardin, Y. Gan, H. M. Colquhoun, *Nat Chem.* **2010**, *2*, 653 – 660; c) Z. Zhu, C. J. Cardin, Y. Gan, C. A. Murray, A. J. White, D. J. Williams, H. M. Colquhoun, *J. Am. Chem. Soc.* **2011**, *133*, 19442 – 19447.

[16] a) J. C. Randall, *Polymer Sequence Determination*: *Carbon-13 NMR Method*, Academic Press, New York, **1977**; b) F. A. Bovey, P. A. Mirau, *NMR of Polymers*, Academic Press, San Diego, **1996**.

[17] a) D. C. Koboldt, K. M. Steinberg, D. E. Larson, R. K. Wilson, E. R. Mardis, *Cell* **2013**, *155*, 27 – 38; b) C.-S. Ku, D. H. Roukos, *Expert Rev. Med. Devices* **2013**, *10*, 1 – 6; c) Y.-L. Ying, J. Zhang, R. Gao, Y. T. Long, *Angew. Chem.* **2013**, *125*, 13392 – 13399; *Angew. Chem. Int. Ed.* **2013**, *52*, 13154 – 13161.

[18] P. Edman, *Acta Chem. Scand.* **1950**, *4*, 283 – 293.

[19] A. S. Inglis, *Anal. Biochem.* **1991**, *195*, 183 – 196.

[20] a) H. M. Fales, Y. Nagai, G. W. Milne, H. B. Brewer, Jr., T. J. Bronzert, J. J. Pisano, *Anal. Biochem.* **1971**, *43*, 288 – 299; b) H. R. Schulten, B. Wittmann-Liebold, *Anal. Biochem.* **1976**, *76*, 300 – 310.

[21] a) K. Biemann, *Int. J. Mass Spectrom.* **2007**, *259*, 1 – 7; b) C. K. Barlow, R. A. J. O'Hair, *J. Mass Spectrom.* **2008**, *43*, 1301 – 1319.

[22] a) K. Biemann, S. A. Martin, *Mass Spectrom. Rev.* **1987**, *6*, 1 – 75; b) D. F. Hunt, W. M. Bone, J. Shabanowitz, J. Rhodes, J. M. Ballard, *Anal. Chem.* **1981**, *53*, 1704 – 1706; c) D. F. Hunt, J. R. Yates III, J. Shabanowitz, S. Winston, C. R. Hauer, *Proc. Natl. Acad. Sci. USA* **1986**, *83*, 6233 – 6237.

[23] a) B. Chait, R. Wang, R. C. Beavis, S. B. Kent, *Science* **1993**, *262*, 89 – 92; b) R. Kaufmann, B. Spengler, F. Lutzenkirchen, *Rapid Commun. Mass Spectrom.* **1993**, *7*, 902 – 910.

[24] H. Kumar, Y. Lansac, M. A. Glaser, P. K. Maiti, *Soft Matter.* **2011**, *7*, 5898 – 5907.

[25] a) J. J. Coon, *Anal. Chem.* **2009**, *81*, 3208 – 3215; b) H. Steen, M. Mann, *Nat. Rev. Mol. Cell Biol.* **2004**, *5*, 699 – 711.

[26] J. A. Shendure, et al., in *Curr. Protoc. Mol. Bio*, **2011**, 96:7.1:7.1.1 – 7.1.23.

[27] F. Sanger, J. E. Donelson, A. R. Coulson, H. Kössel, D. Fischer, *Proc. Natl. Acad. Sci. USA* **1973**, *70*, 1209 – 1213.

[28] F. Sanger, A. R. Coulson, *J. Mol. Biol.* **1975**, *94*, 441 – 448.

[29] L. M. Smith, et al., *Nature* **1986**, *321*, 674 – 679.

[30] M. Margulies, et al., *Nature* **2005**, *437*, 376 – 380.

[31] J. J. Kasianowicz, E. Brandin, D. Branton, D. W. Deamer, *Proc. Natl. Acad. Sci. USA* **1996**, *93*, 13770 – 13773.

[32] A. Meller, L. Nivon, E. Brandin, J. Golovchenko, D. Branton, *Proc. Natl. Acad. Sci. USA* **2000**, *97*, 1079 – 1084.

[33] a) J. Li, D. Stein, C. McMullan, D. Branton, M. J. Aziz, J. A. Golovchenko, *Nature* **2001**, *412*, 166 – 169; b) A. J. Storm, C. Storm, J. Chen, H. Zandbergen, J.-F. Joanny, C. Dekker, *Nano Lett.* **2005**, *5*, 1193 – 1197.

[34] a) M. Wanunu, J. Sutin, B. McNally, A. Chow, A. Meller, *Biophys. J.* **2008**, *95*, 4716 – 4725; b) M. Wanunu, A. Meller, *Nano Lett.* **2007**, *7*, 1580 – 1585.

[35] a) H. Liu, J. He, J. Tang, H. Liu, P. Pang, D. Cao, P. Krstic, S. Joseph, S. Lindsay, C. Nuckolls, *Science* **2010**, *327*, 64 – 67; b) C. Y. Lee, W. Choi, J. H. Han, M. S. Strano, *Science* **2010**, *329*, 1320 – 1324.

[36] B. McNally, A. Singer, Z. Yu, Y. Sun, Z. Weng, A. Meller, *Nano Lett.* **2010**, *10*, 2237 – 2244.

[37] a) F. A. Bovey, *J. Polym. Sci.* **1962**, *62*, 197 – 209; b) R. E. Cais, J. H. O'Donnell, F. A. Bovey, *Macromolecules* **1977**, *10*, 254 – 260; c) S. A. Heffner, F. A. Bovey, L. A. Verge, P. A. Mirau, A. E. Tonelli, *Macromolecules* **1986**, *19*, 1628 – 1634; d) F. A. Bovey, *Makromol. Chem. Macromol. Symp.* **1988**, *20 – 21*, 105 – 126.

[38] a) J. C. Randall, *Macromolecules* **1978**, *11*, 592 – 597; b) D. B. Bailey, P. M. Henrichs, *J. Polym. Sci. Polym. Chem. Ed.* **1978**, *16*, 3185 – 3199; c) E. T. Hsieh, J. C. Randall, *Macromolecules* **1982**, *15*, 1402 – 1406; d) T. L. Bluhm, et al., *Macromolecules* **1986**, *19*, 2871 – 2876.

[39] a) B. Kirci, J. F. Lutz, K. Matyjaszewski, *Macromolecules* **2002**, *35*, 2448 – 2451; b) J.-F. Lutz, B. Kirci, K. Matyjaszewski, *Macromolecules* **2003**, *36*, 3136 – 3145.

[40] D. Benoit, C. J. Hawker, E. E. Huang, Z. Lin, T. P. Russell, *Macromolecules* **2000**, *33*, 1505 – 1507.

[41] K. Matyjaszewski, M. J. Ziegler, S. V. Arehart, D. Greszta, T. Pakula, *J. Phys. Org. Chem.* **2000**, *13*, 775 – 786.

[42] D. Chan-Seng, M. Zamfir, J.-F. Lutz, *Angew. Chem.* **2012**, *124*, 12420 – 12423; *Angew. Chem. Int. Ed.* **2012**, *51*, 12254 – 12257.

[43] A. Alkan, A. Natalello, M. Wagner, H. Frey, F. R. Wurm, *Macromolecules* **2014**, *47*, 2242 – 2249.

[44] a) H. M. Colquhoun, Z. Zhu, *Angew. Chem.* **2004**, *116*, 5150 – 5155; *Angew. Chem. Int. Ed.* **2004**, *43*, 5040 – 5045; b) H. M. Colquhoun, Z. X. Zhu, C. J. Cardin, Y. Gan, *Chem. Commun.* **2004**, 2650 – 2652; c) H. M. Colquhoun, Z. Zhu, C. J. Cardin, Y. Gan, M. G. B. Drew, *J. Am. Chem. Soc.* **2007**, *129*, 16163 – 16174; d) H. M. Colquhoun, Z. Zhu, C. J. Cardin, M. G. B. Drew, Y. Gan, *Faraday Discuss.* **2009**, *143*, 205 – 220; e) B. W. Greenland, M. B. Bird, S. Burattini, R. Cramer, R. K. O'Reilly, J. P. Patterson, W. Hayes, C. J. Cardin, H. M. Colquhoun, *Chem. Commun.* **2013**, *49*, 454 – 456.

[45] S. G. Ramkumar, S. Ramakrishnan, *Macromolecules* **2010**, *43*, 2307 – 2312.

[46] A. E. Tonelli, *Macromolecules* **2009**, *42*, 3830 – 3840.

[47] S. N. Hardrict, R. Gurarslan, C. J. Galvin, H. Gracz, D. Roy, B. S. Sumerlin, J. Genzer, A. E. Tonelli, *J. Polym. Sci. Part A* **2013**, *51*, 735 – 741.

[48] a) A. E. Tonelli, *Macromolecules* **1977**, *10*, 153 – 157; b) G. Khanarian, R. E. Cais, J. Kometani, A. E. Tonelli, *Macromolecules* **1982**, *15*, 866 – 869; c) J. J. Semler, Y. K. Jhon, A. Tonelli, M. Beevers, R. Krishnamoorti, J. Genzer, *Adv. Mater.* **2007**, *19*, 2877 – 2883.

[49] G. Montaudo, F. Samperi, M. S. Montaudo, *Prog. Polym. Sci.* **2006**, *31*, 277 – 357.

[50] a) M. S. Montaudo, *Mass Spectrom. Rev.* **2002**, *21*, 108 – 144; b) M. W. F. Nielen, *Mass Spectrom. Rev.* **1999**, *18*, 309 – 344.

[51] G. Adamus, W. Sikorska, M. Kowalczuk, M. Montaudo, M. Scandola, *Macromolecules* **2000**, *33*, 5797 – 5802.

[52] F. Samperi, M. S. Montaudo, C. Puglisi, S. Di Giorgi, G. Montaudo, *Macromolecules* **2004**, *37*, 6449 – 6459.

[53] P. Rizzarelli, C. Puglisi, G. Montaudo, *Rapid Commun. Mass Spec.* **2005**, *19*, 2407 – 2418.

[54] M. G. Paulick, K. M. Hart, K. M. Brinner, M. Tjandra, D. H. Charych, R. N. Zuckermann, *J. Comb. Chem.* **2006**, *8*, 417 – 426.

[55] a) T. Gruendling, S. Weidner, J. Falkenhagen, C. Barner-Kowollik, *Polym. Chem.* **2010**, *1*, 599 – 617; b) G. Hart-Smith, C. Barner-Kowollik, *Macromol. Chem. Phys.* **2010**, *211*, 1507 – 1529; c) A. H. Soeriyadi, M. R. Whittaker, C. Boyer, T. P. Davis, *J. Polym. Sci. Part A* **2013**, *51*, 1475 – 1505.

[56] a) W. Heerma, C. Versluis, C. G. de Koster, J. A. W. Kruijtzer, I. Zigrovic, R. M. J. Liskamp, *Rapid Commun. Mass Spec.* **1996**, *10*, 459 – 464; b) R. Ruijtenbeek, C. Versluis, A. J. Heck, F. A. Redegeld, F. P. Nijkamp, R. M. Liskamp, *J. Mass Spec.* **2002**, *37*, 47 – 55.

[57] G. De Bo, S. Kuschel, D. A. Leigh, B. Lewandowski, M. Papmeyer, J. W. Ward, *J. Am. Chem. Soc.* **2014**, *136*, 5811 – 5814.

[58] V. Mass, W. Schrepp, B. von Vacona, H. Pasch, *Macromol. Chem. Phys.* **2009**, *210*, 1957 – 1965.
[59] I. C. Wienhöfer, H. Luftmann, A. Studer, *Macromolecules* **2011**, *44*, 2510 – 2523.
[60] E. Altuntaş, U. S. Schubert, *Anal. Chim. Acta* **2014**, *808*, 56 – 69.
[61] a) R. Hill, et al., *Trans. Faraday Soc.* **1939**, *35*, 1073 – 1079; b) K. Tada, et al., *Makromol. Chem.* **1964**, *71*, 71 – 85; c) S. Tsuge, et al., *Macromolecules* **1975**, *8*, 721 – 725.
[62] H. J. Harwood, *Angew. Chem.* **1965**, *77*, 1124 – 1134; *Angew. Chem. Int. Ed. Engl.* **1965**, *4*, 1051 – 1060.
[63] a) C. H. Bamford, C. F. H. Tipper, *Degradation of Polymers*, Elsevier, Amsterdam, **1975**; b) R. Lenz in *Biopolymers I*, *Bd. 107* (Eds.: R. Langer, N. Peppas), Springer, Berlin/Heidelberg, **1993**, pp. 1 – 40.
[64] E. Zagar, A. Kržan, G. Adamus, M. Kowalczuk, *Biomacromolecules* **2006**, *7*, 2210 – 2216.
[65] C. M. Thomas, J.-F. Lutz, *Angew. Chem.* **2011**, *123*, 9412 – 9414; *Angew. Chem. Int. Ed.* **2011**, *50*, 9244 – 9246.
[66] a) R. M. Stayshich, T. Y. Meyer, *J. Am. Chem. Soc.* **2010**, *132*, 10920 – 10934; b) J. Li, R. M. Stayshich, T. Y. Meyer, *J. Am. Chem. Soc* **2011**, *133*, 6910 – 6913; c) J. Li, S. N. Rothstein, S. R. Little, H. M. Edenborn, T. Y. Meyer, *J. Am. Chem. Soc.* **2012**, *134*, 16352 – 16359.
[67] Y. Ishido, A. Kanazawa, S. Kanaoka, S. Aoshima, *Macromolecules* **2012**, *45*, 4060 – 4068.
[68] a) J. K. Haken, T. R. McKay, *Anal. Chem.* **1973**, *45*, 1251 – 1257; b) T. Shimono, *J. Anal. Appl. Pyrolysis* **1979**, *1*, 77 – 84; c) M. S. Choudhary, K. Lederer, *Eur. Polym. J.* **1982**, *18*, 1021 – 1027.
[69] a) N. Grassie, D. R. Bain, *J. Polym. Sci. Part A* **1970**, *8*, 2653 – 2664; b) D. A. Smith, J. W. Youren, *Br. Polym. J.* **1976**, *8*, 101 – 117; c) G. Pan, H. Li, Y. Cao, *J. Appl. Polym. Sci.* **2004**, *93*, 577 – 583.
[70] H. McCormick, *J. Chrom. A* **1969**, *40*, 1 – 15.
[71] G. Schliecker, C. Schmidt, S. Fuchs, T. Kissel, *Biomaterials* **2003**, *24*, 3835 – 3844.
[72] a) A. Sagi, R. Weinstain, N. Karton, D. Shabat, *J. Am. Chem. Soc.* **2008**, *130*, 5434 – 5435; b) G. I. Peterson, M. B. Larsen, A. J. Boydston, *Macromolecules* **2012**, *45*, 7317 – 7328; c) S. T. Phillips, A. M. DiLauro, *ACS Macro Lett.* **2014**, *3*, 298 – 304.
[73] L.-J. Zhang, X.-X. Deng, F.-S. Du, Z.-C. Li, *Macromolecules* **2013**, *46*, 9554 – 9562.
[74] a) S. M. Bezrukov, I. Vodyanoy, R. A. Brutyan, J. J. Kasianowicz, *Macromolecules* **1996**, *29*, 8517 – 8522; b) J. E. Reiner, J. J. Kasianowicz, B. J. Nablo, J. W. F. Robertson, *Proc. Natl. Acad. Sci. USA* **2010**, *107*, 12080 – 12085; c) L. Movileanu, H. Bayley, *Proc. Natl. Acad. Sci. USA* **2001**, *98*, 10137 – 10141.
[75] a) G. Baaken, M. Sondermann, C. Schlemmer, J. Rühe, J. C. Behrends, *Lab Chip* **2008**, *8*, 938 – 944; b) A. G. Oukhaled, A.-L. Biance, J. Pelta, L. Auvray, L. Bacri, *Phys. Rev. Lett.* **2012**, *108*, 088104; c) A. Balijepalli, J. W. F. Robertson, J. E. Reiner, J. J. Kasianowicz, R. W. Pastor, *J. Am. Chem. Soc.* **2013**, *135*, 7064 – 7072; d) M. F. Breton, F. Discala, L. Bacri, D. Foster, J. Pelta, A. Oukhaled, *J. Phys. Chem. Lett.* **2013**, *4*, 2202 – 2208.
[76] a) G. Baaken, N. Ankri, A.-K. Schuler, J. Rühe, J. C. Behrends, *ACS Nano* **2011**, *5*, 8080 – 8088; b) G. Baaken, et al., *Biophys. J.* **2012**, *102*, 28A – 28A.
[77] a) L. Brun, M. Pastoriza-Gallego, G. Oukhaled, J. Mathé, L. Bacri, L. Auvray, J. Pelta, *Phys. Rev. Lett.* **2008**, *100*, 158302; b) G. Gibrat, M. Pastoriza-Gallego, B. Thiebot, M.-F. Breton, L. Auvray, J. Pelta, *J. Phys. Chem. B* **2008**, *112*, 14687 – 14691; c) G. Oukhaled, L. Bacri, J. Mathe, J. Pelta, L. Auvray, *Europhys. Lett.* **2008**, *82*, 48003.
[78] R. J. Murphy, M. Muthukumar, *J. Chem. Phys.* **2007**, *126*, 051101.
[79] T. Sakaue, F. Brochard-Wyart, *ACS Macro Lett.* **2014**, *3*, 194 – 197.
[80] S. Mirigian, Y. Wang, M. Muthukumar, *J. Chem. Phys.* **2012**, *137*, 064904.
[81] J.-F. Lutz, *Nat. Chem.* **2010**, *2*, 84 – 85.