Sequence Control

Sequence-Controlled Polymers

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New Insights into Poly(lactic-*co*-glycolic acid) Microstructure: Using Repeating Sequence Copolymers To Decipher Complex NMR and Thermal Behavior

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A Facile Procedure for Controlling Monomer Sequence Distribution in Radical Chain Polymerizations

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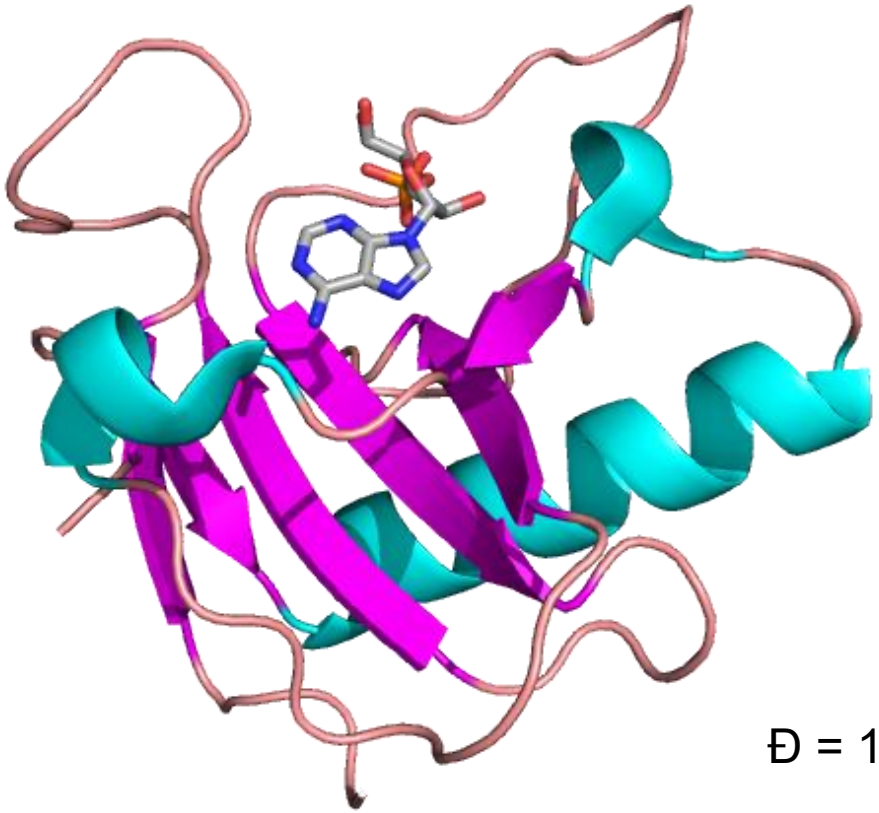
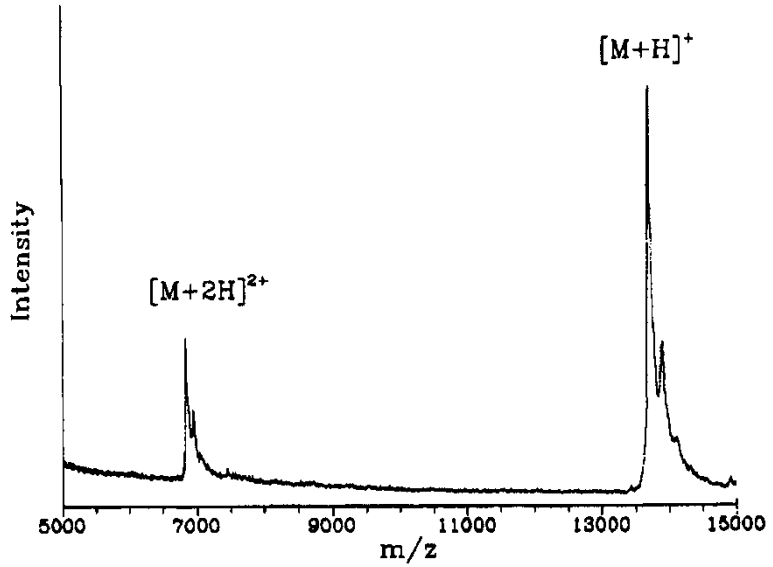
Questions

- How is monomer sequence controlled in DNA and protein biosynthesis?
- What are factors that limit control over mononer sequence in conventional chain and step polymerization reactions ?
- What are limitations of the approaches towards sequence controlled addition and condensation polymers that have been described in the articles you have studied ?

Biological Polymers

Proteins: Perfect control of chain length and sequence





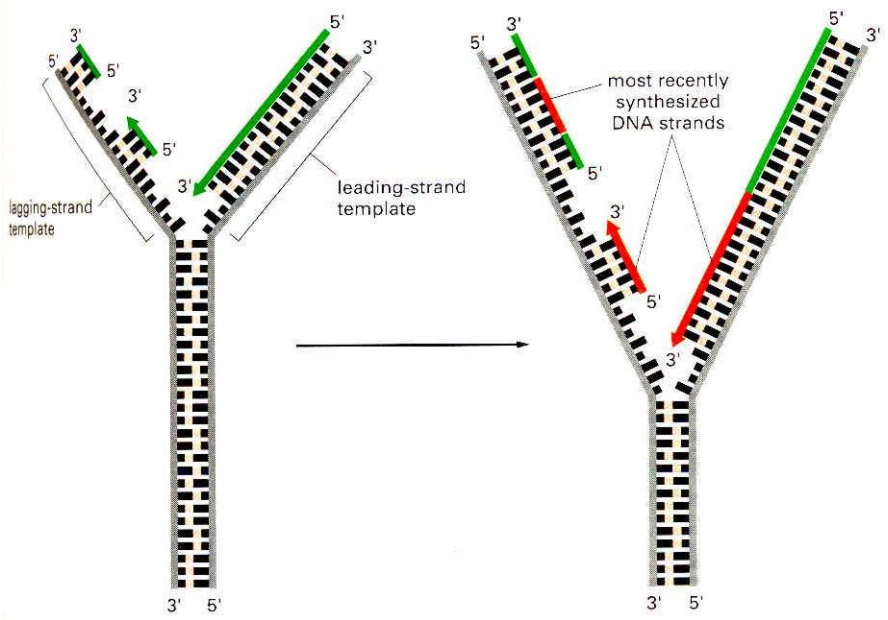
(Anal. Chem. **1990**, 62, 1836)

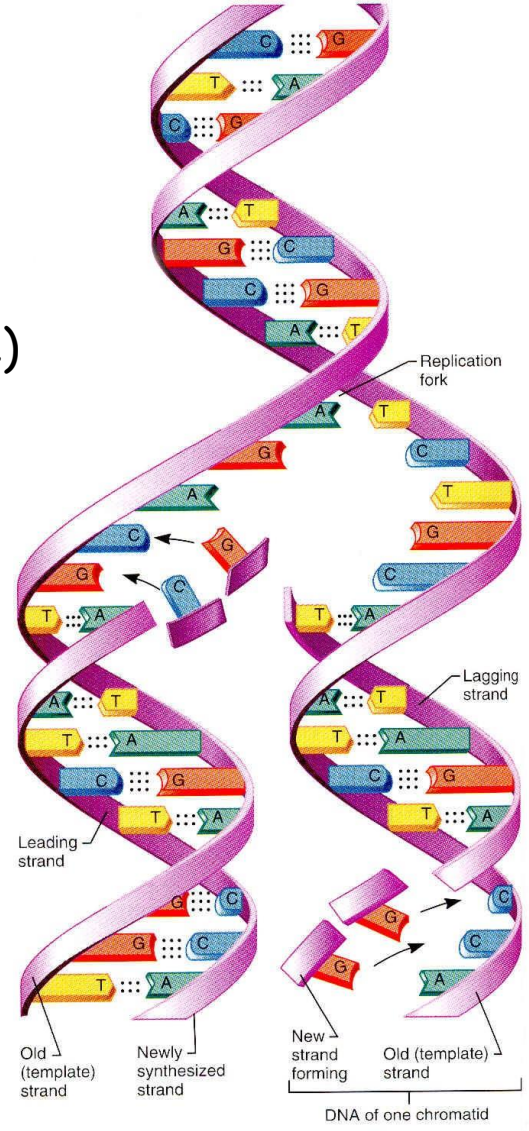
Đ = 1.0

Ribonuclease A: molecular weight = 13 690,29 gr/mol
124 amino acids (degree of polymerization = 124)

DNA Replication

- Unwinding of DNA helices from the nucleosome
- Untwisting of the DNA double helix (helicase)
- Each single strand acts as a template for a new strand
    - Leading strand (5' → 3'; DNA polymerase)
    - Lagging strand (Fragment condensation; DNA ligase)
- DNA replication is semiconservative
  (one „old" and one „new" strand)





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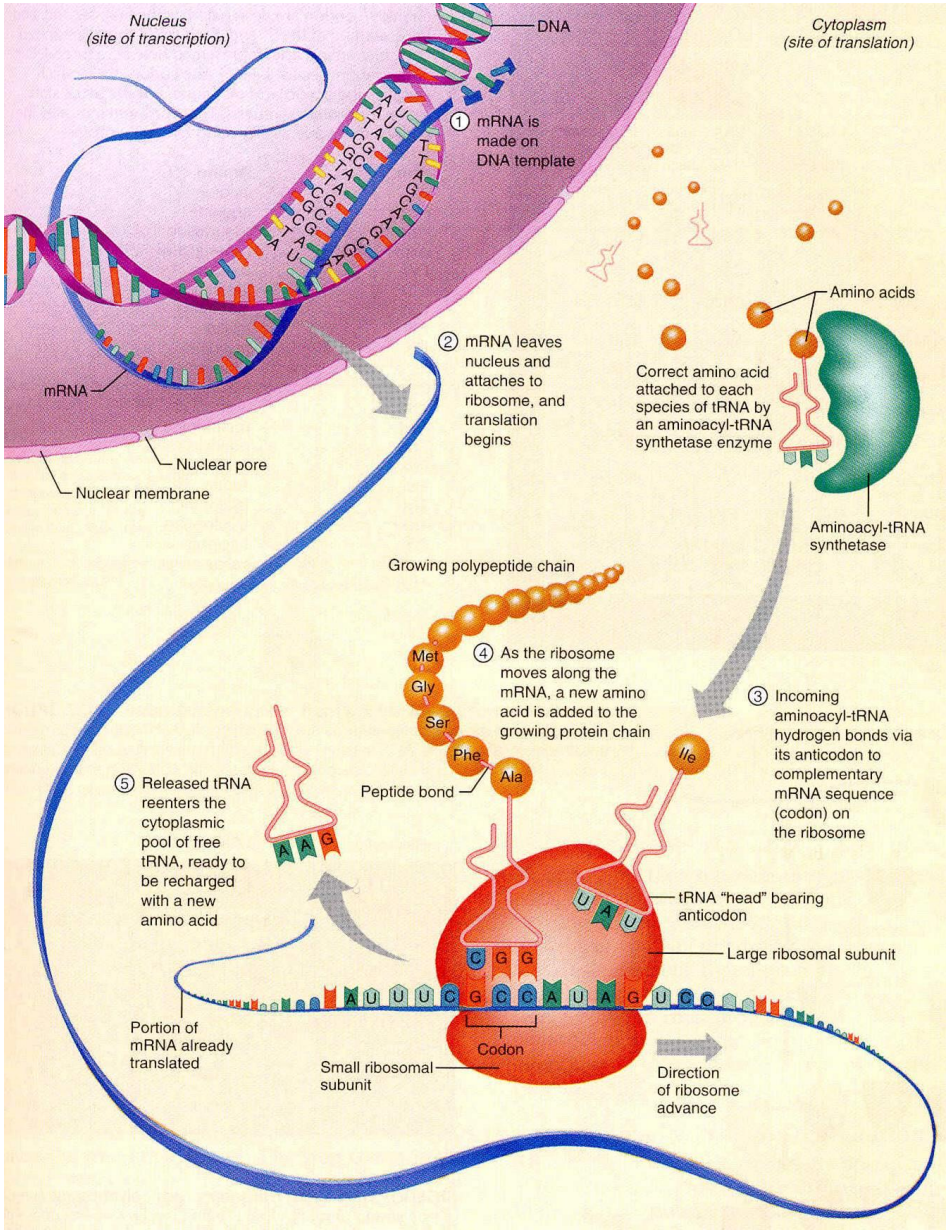
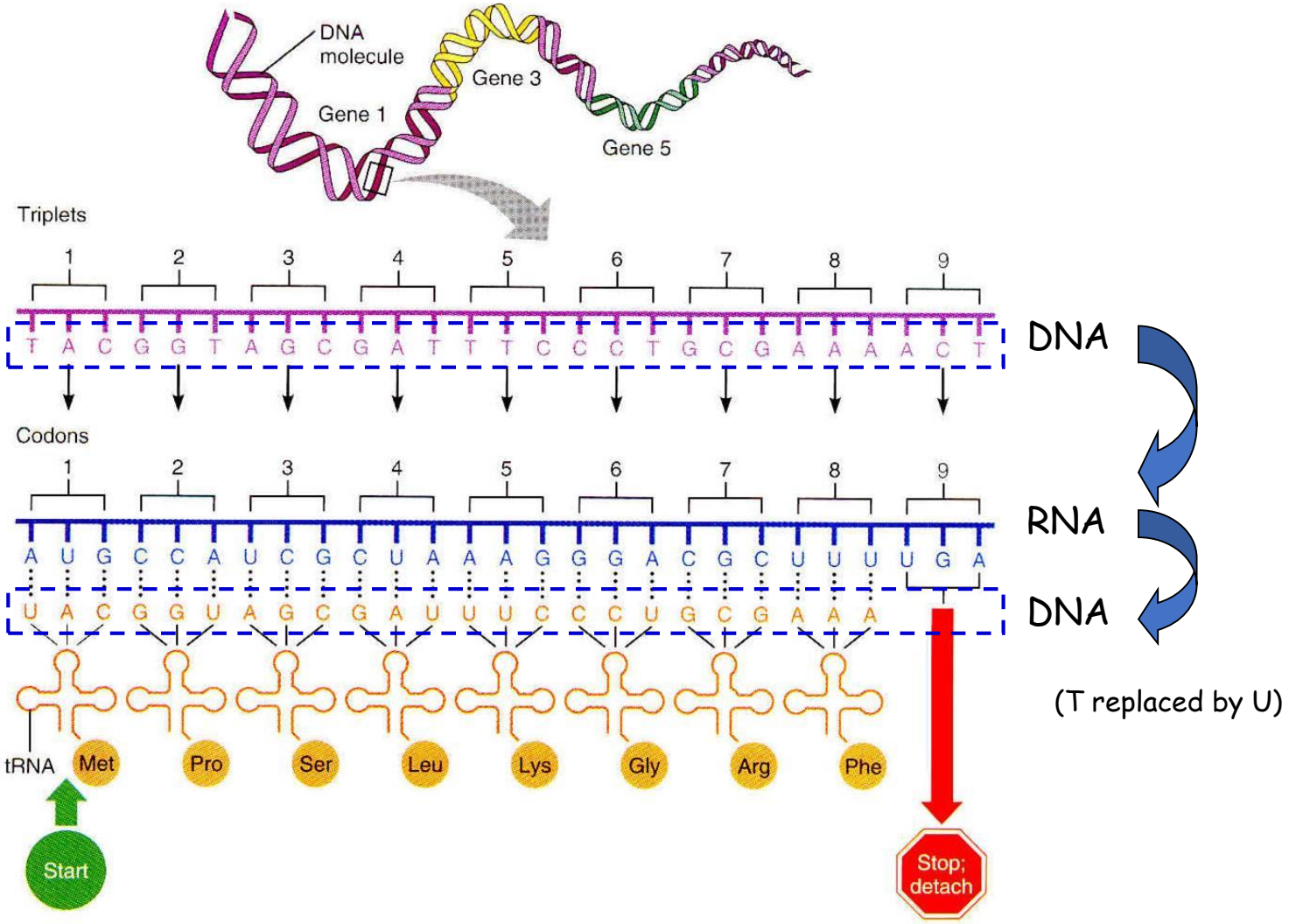
Protein Biosynthesis



Information Transfer During Protein Biosynthesis



Synthetic Polymers are Mixtures!

Chain length heterogeneity:

- Due to statistical variations in the polymerization process, polymers even in their purest form, are usually mixtures of molecules of different molecular weights
- Both the average molecular weight and the molecular weight distribution are needed to fully characterize a polymer

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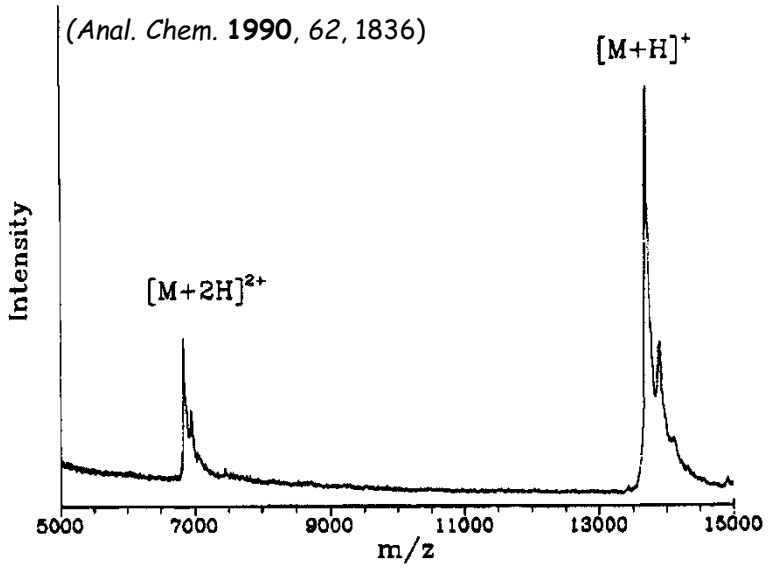
MALDI TOF mass spectra of a natural polymer (protein) vs. that of a synthetic polymer

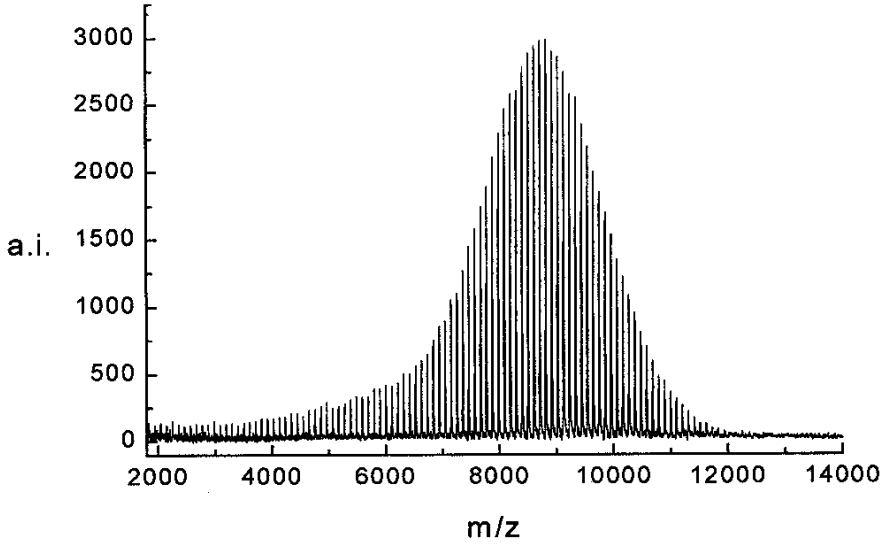
Ribonuclease A:

(Anal. Chem. **1990**, *62*, 1836)



Polystyrene (M_n = 9000 Da):

(Acta Polymer. **1998**, *49*, 272)



Monomer sequence distribution in copolymerizations:

Step copolymerization

- Polymerization is usually carried out to 100 % conversion

  Copolymer composition = composition of the monomer feed

- Most step polymerizations are equilibrium reactions, i.e. the initially obtained copolymer composition is rapidly changed by equilibration („chain scrambling")

  „random" copolymer, i.e. purely statistical comonomer sequence

The situation, however, can be different for **chain copolymerization**

- Copolymer composition and comonomer sequence depend on the relative concentrations of both monomers and their relative reactivities

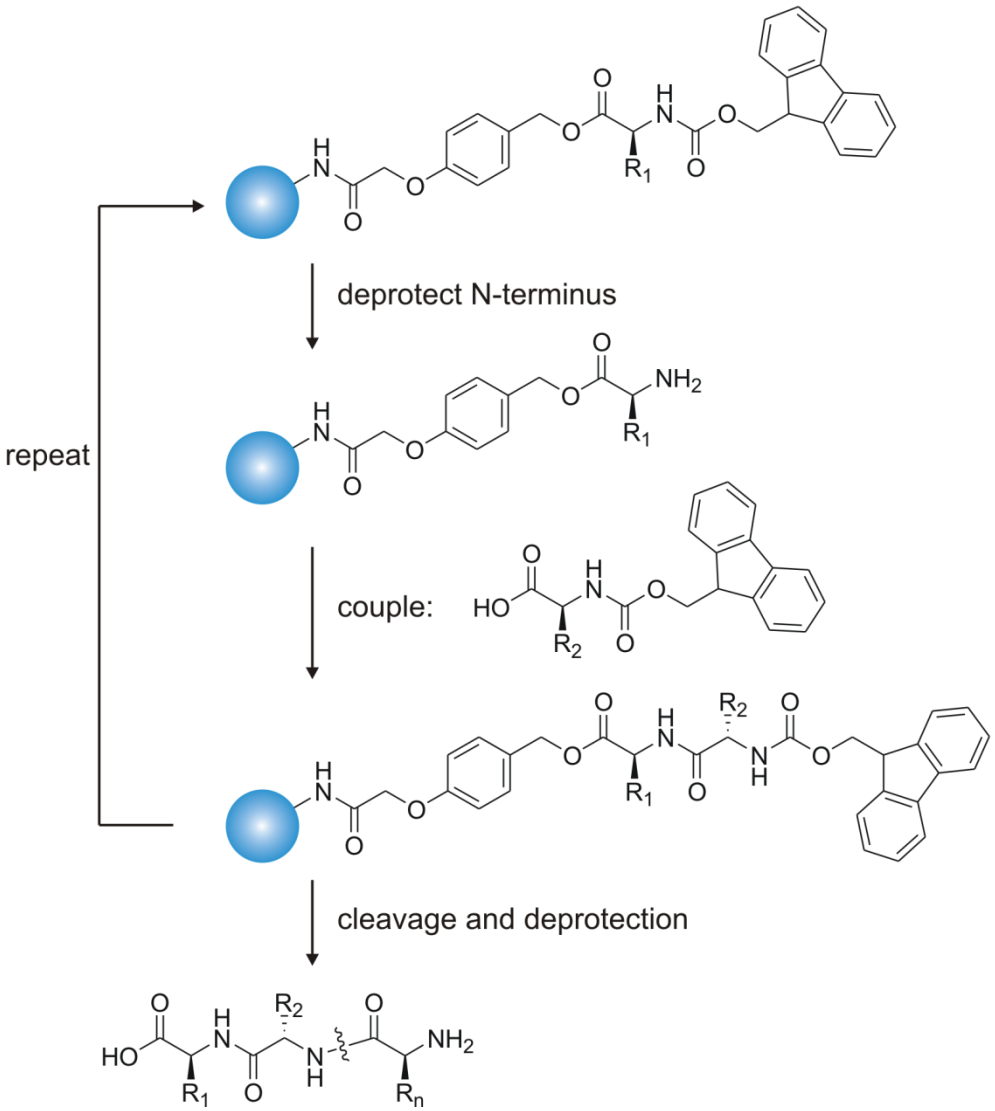
Copolymer composition, Comonomer sequence } { Relative monomer concentrations, Relative reactivities

This implies that analysis of copolymer composition can provide information about monomer reactivity

Improving Control over Sequence

- Solid phase peptide synthesis
- Addition polymers
- Condensation polymers

Solid Phase Peptide Synthesis



☺ Perfect control of monomer sequence (primary structure)

☺ Uniform chain lengths

☹ Multistep synthesis

☹ Limited molecular weight (total yield = [yield per step]^n)

Automated solid phase peptide synthesis

Chemspeed PSW1100 Peptide Synthesizer

R. B. Merrifield, *J. Am. Chem. Soc.* **1963**, *85*, 2149
Nobel Prize in Chemistry 1984

Addition Polymers

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A Facile Procedure for Controlling Monomer Sequence Distribution in Radical Chain Polymerizations

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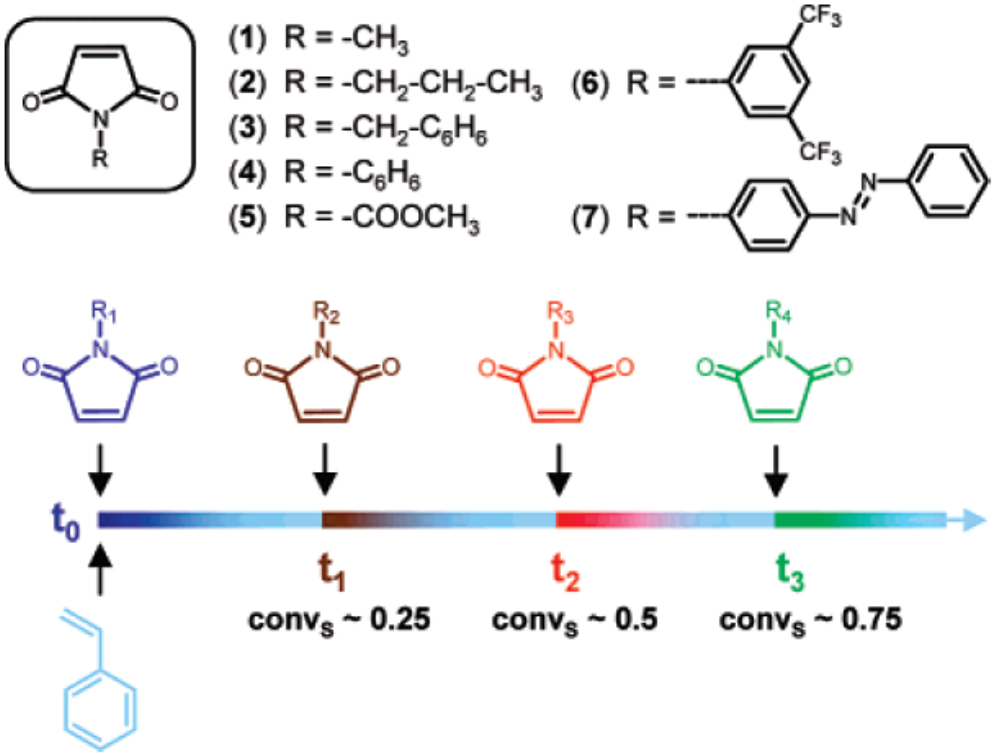
Would this also work with conventional free radical polymerization ?

Scheme 1. Concept of the Sequential Atom Transfer Radical Copolymerization of Styrene and Various N-Substituted Maleimides



Why Styrene and Maleimides ?

Macromolecules **1990,** *23,* 4508–4513

Radical Polymerization of *N*-(Alkyl-substituted phenyl)maleimides: Synthesis of Thermally Stable Polymers Soluble in Nonpolar Solvents

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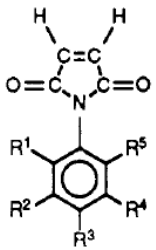
Received February 5, 1990; Revised Manuscript Received April 3, 1990

Chart I



RPhMI

RPhMI	R^1	R^2	R^3	R^4	R^5
1	H	H	H	H	H
2a	CH_3	H	H	H	H
2b	C_2H_5	H	H	H	H
2c	i-C_3H_7	H	H	H	H
2d	CH_3	H	H	H	CH_3
2e	C_2H_5	H	H	H	C_2H_5
2f	i-C_3H_7	H	H	H	i-C_3H_7
2g	CH_3	H	CH_3	H	CH_3
2h	Cl	H	H	H	H
3a	H	CH_3	H	H	H
3b	H	CF_3	H	H	H
3c	H	C_2H_5	H	H	H
3d	H	CH_3	H	CH_3	H
4a	H	H	CH_3	H	H
4b	H	H	C_2H_5	H	H
4c	H	H	n-C_4H_9	H	H

Table II
Radical Copolymerization of RPhMI (M_2) with St or MMA (M_1) in Benzene at 60 °C

M_1	M_2	r_1	r_2	$1/r_1$	Q_2[a]	e_2[a]
St	1	0.07	0.01	14		
	2d	0.14	0.08	7.2		
	2e	0.19	0.02	5.3		
MMA	1	0.91[b]	0.30[b]	1.10	1.29	1.54
	2a	2.22	0.09	0.45	0.55	1.65
	2d	3.62	0.07	0.28	0.33	1.57
	2e	4.78	0.05	0.21	0.25	1.59
	2f	10.9	0.02	0.092	0.11	1.56
	2h	2.10	0.10	0.48	0.58	1.64
	3a	1.18	0.22	0.84	1.00	1.56
	3b	1.57	0.10	0.64	0.81	1.75
	3d	1.63	0.28	0.61	0.65	1.30
	4a	0.83[b]	0.34[b]	1.20	1.40	1.53
	5[c]	1.35	0.24	0.71	0.80	1.44
	6[d]	2.50	0.17	0.40	0.44	1.35

[a] With $Q_1 = 0.74$ and $e_1 = 0.40$ for MMA. [b] Recalculated by the nonlinear least-squares method from the data in the literature.[7] [c] *N*-Cyclohexylmaleimide.[15] [d] N-Unsubstituted maleimide.[21]

Monomer Reactivity Ratios and Copolymerization Behavior

Monomer reactivity ratios: $r_1 = k_{11}/k_{12}$ and $r_2 = k_{22}/k_{21}$

Alternating copolymerization: $r_1 r_2 = 0$ (r_1, $r_2 < 1$)

- Extreme alternating behavior: r_1 and r_2 are zero and $\frac{d[M_1]}{d[M_2]} = 1$

$$F_1 = 0.5$$

The copolymer has a perfect alternating structure irrespective of the comonomer feed composition

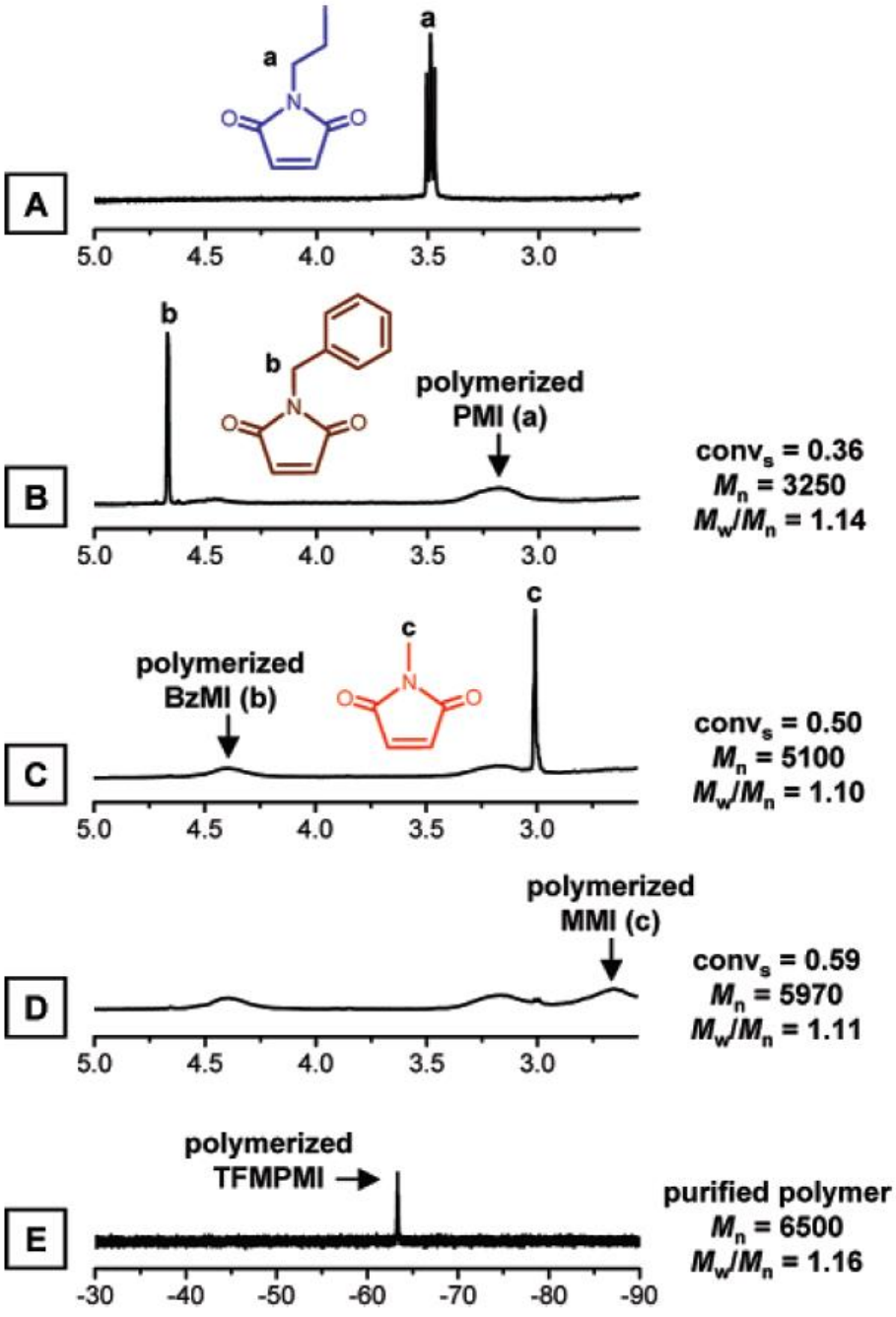
- Moderate alternating behavior occurs when:
  (i) both r_1 and r_2 are small ($r_1 r_2$ very small, close to 0)
  (ii) one r value is small and the other is zero ($r_1 r_2 = 0$)

The copolymer tends towards alternation but is not perfectly alternating

Synthesis of Sequence Controlled St/MI Copolymers

Sequential atom transfer radical copolymerization of Styrene, PMI, BzMI, MMI and TFMPMI. ***(1) Preparation of the initial reaction:*** *N*-Propylmaleimide (112.8 mg, 0.81 mmol, 3 Eq.), copper bromide (38.8 mg, 0.27 mmol, 1 Eq.) and 4,4'-dinonyl-2,2'-bipyridine (220.9 mg, 0.54 mmol, 2 Eq.) were added into a Schlenk tube. The tube was sealed with a septum and subsequently purged with dry argon for a few minutes. Then, 3.1 mL of thoroughly degassed styrene (2.8 g, 27 mmol, 100 Eq.) were added with a degassed syringe. The mixture turned dark brown, indicating complexation of Cu(I)Br and dNBipy. Lastly, (1-bromoethyl) benzene (50 mg, 0.27 mmol, 1 Eq.) was added with a precision syringe. The mixture was heated at 110°C in an oil bath for several hours. ***(2) Controlled Addition of BzMI:*** After 1 hour of polymerization, a thoroughly degassed solution of *N*-benzylmaleimide (151 mg, 0.81 mmol, 3 Eq.) in 0.8 mL of toluene was added with a degassed syringe. Immediately after addition, a polymerization sample was taken with a degassed syringe and analyzed by ^{1}H NMR and SEC. ***(3) Controlled Addition of MMI:*** After 2 hours and 45 minutes of polymerization, a thoroughly degassed solution of *N*-methylmaleimide (90 mg, 0.81 mmol, 3 Eq.) in 0.8 mL of toluene was added with a degassed syringe. Immediately after addition, a polymerization sample was taken with a degassed syringe and analyzed by ^{1}H NMR and SEC. ***(4) Controlled Addition of TFMPMI:*** After 6 hours of polymerization, a thoroughly degassed solution of *N*-[3,5-Bis(trifluoromethyl)phenyl] maleimide (250 mg, 0.81 mmol, 3 Eq.) in 0.8 mL of toluene was added with a degassed syringe. ***(4) Final purification:*** After 21 hours of polymerization, the experiment was stopped by opening the flask and exposing the catalyst to air. Subsequently, the copolymer was purified by selective precipitation in methanol. The purified polymer appeared as a white powder.

J. AM. CHEM. SOC. 2007, *129*, 9542−9543



Scheme 1. Concept of the Sequential Atom Transfer Radical Copolymerization of Styrene and Various N-Substituted Maleimides

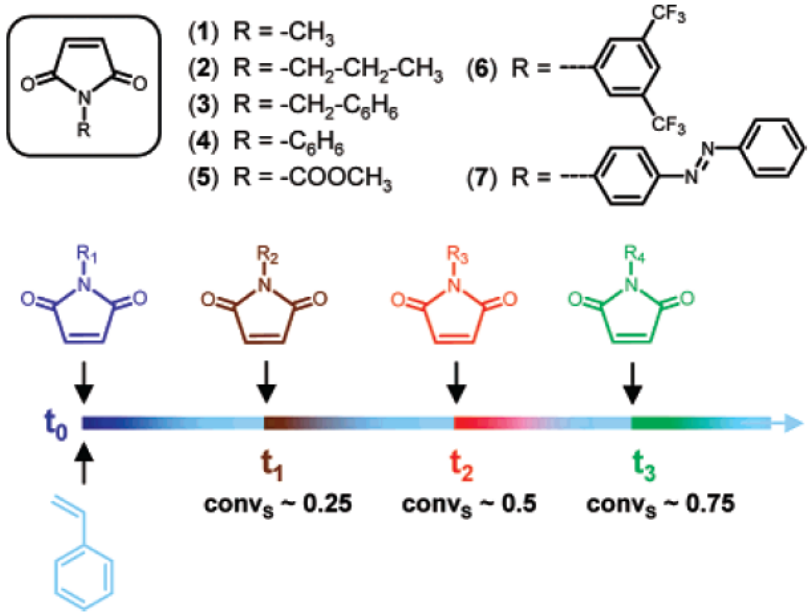
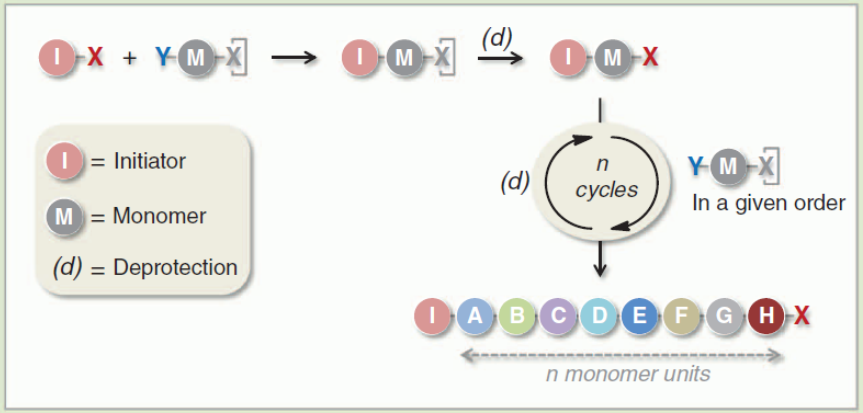


Figure 1. NMR spectra recorded in $CDCl_3$ at different stages of the sequential copolymerization: (A) ^1H spectrum (zoom of the region 2.55−5.0 ppm) of the initial reaction mixture, (B) ^1H spectrum recorded shortly after the addition of BzMI (t = 1 h), (C) ^1H spectrum recorded shortly after the addition of MMI (t = 2 h 45 min′), (D) ^1H spectrum recorded 15 min before the addition of TFMPMI (t = 5 h 45 min′), (E) ^{19}F spectrum recorded for the final purified copolymer (isolated after 21 h of polymerization).

Condensation Polymers

Iterative Synthesis

See also:
Solid phase peptide synthesis



Box 1. Going the long way: Attaching monomers one by one.
An evident chemical strategy to attain sequence-defined polymers consists in covalently attaching monomers one by one in a given order. Such a concept implies that polymer chain growth has to be regulated in order to avoid repeated incorporation of the same monomer in a given chain. Although different mechanisms of regulation may be considered, the most common approach is using a self-reacting bifunctional monomer XY, in which one of the reactive functions is temporarily protected (i.e., deactivated) as depicted above.

The self-reacting bifunctional monomer XY strategy was developed and optimized for the solution synthesis of oligopeptides (*91*). However, solution approaches are limited and time-consuming because they require purification after each monomer coupling. The field was revolutionized by the development of solid-phase supports by Merrifield (*6*). In this simple approach, the growing oligomers are covalently bound to filterable polymer beads, which greatly improve and facilitate purification protocols. Moreover, the automation of this chemical process has substantially reduced reaction times. The concept was applied for the synthesis of many natural and nonnatural sequence-defined oligomers, including oligopeptides and oligonucleotides (*92*). Such iterative approaches still exhibit some drawbacks. First, in order to avoid substantial sequence defects, the yields of monomer coupling should be very high, particularly if a long polymer chain is targeted. Another limitation is the use of main-chain protecting groups, which imply time-consuming deprotection steps. Protecting groups are currently mandatory in bio-oligomer synthesis, e.g., peptide solid-phase chemistry. However, some nonnatural sequence-defined oligomers can be synthesized in the absence of main-chain protecting groups, for example, if two successive building blocks are used instead of a single XY monomer (*28*, *93*, *94*). An elegant example of that type is the synthesis of peptoids (i.e., oligomers of N-substituted glycines) described by Zuckermann and co-workers (*95*). This approach relies on a "submonomer" strategy. Each N-substituted glycine unit is formed by successive coupling of two submonomer synthons. The backbone chemistry is chemoselective and, therefore, does not require main-chain protecting groups.

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New Insights into Poly(lactic-*co*-glycolic acid) Microstructure: Using Repeating Sequence Copolymers To Decipher Complex NMR and Thermal Behavior

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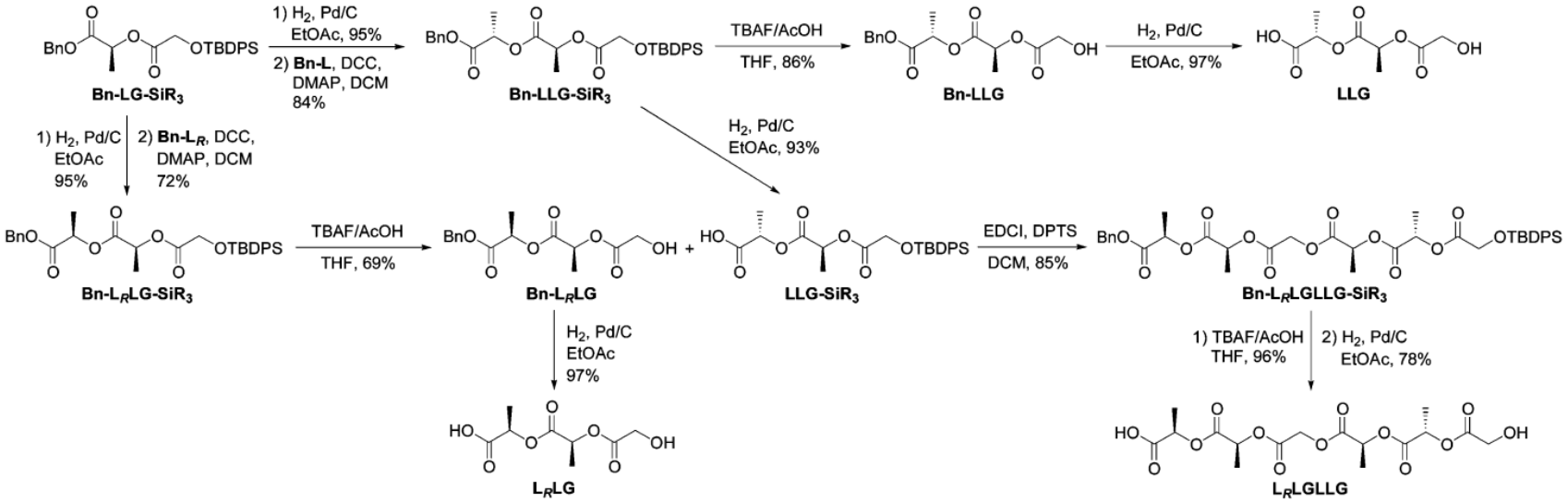
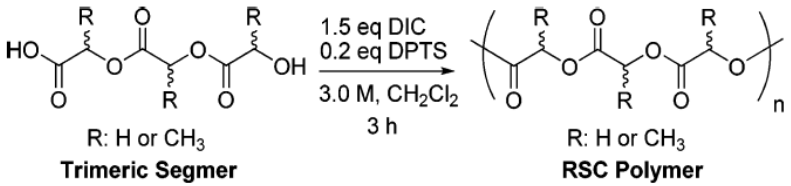
Abstract: Sequence, which Nature uses to spectacular advantage, has not been fully exploited in synthetic copolymers. To investigate the effect of sequence and stereosequence on the physical properties of copolymers, a family of complex isotactic, syndiotactic, and atactic repeating sequence poly(lactic-*co*-glycolic acid) copolymers (RSC PLGAs) were prepared and their NMR and thermal behavior was studied. The unique suitability of polymers prepared from the bioassimilable lactic and glycolic acid monomers for biomedical applications makes them ideal candidates for this type of sequence engineering. Polymers with repeating units of LG, GLG and LLG (L = lactic, G = glycolic) with controlled and varied tacticities were synthesized by assembly of sequence-specific, stereopure dimeric, trimeric, and hexameric segmer units. Specifically labeled deuterated lactic and glycolic acid segmers were likewise prepared and polymerized. Molecular weights for the copolymers were in the range M_n = 12−40 kDa by size exclusion chromatography in THF. Although the effects of sequence-influenced solution conformation were visible in all resonances of the ^{1}H and ^{13}C NMR spectra, the diastereotopic methylene resonances in the ^{1}H NMR ($CDCl_3$) for the glycolic units of the copolymers proved most sensitive. An octad level of resolution, which corresponds to an astounding 31-atom distance between the most separated stereocenters, was observed in some mixed sequence polymers. Importantly, the level of sensitivity of a particular NMR resonance to small differences in sequence was found to depend on the sequence itself. Thermal properties were also correlated with sequence.

Sequence Specific Polyesters

Monomer / Segmer synthesis



Polymerization



J. AM. CHEM. SOC. 2010, *132*, 10920–10934

Table 2. PLGA Repeating Sequence Copolymer Characterization Data

Polymer	Repeating Pattern[a]	Yield (%)[b]	THF M_n (kDa)[c]	THF PDI[c]	$CHCl_3$ M_n (kDa)[c]	$CHCl_3$ PDI[c]	$CHCl_3$ Absolute M_n[d]	$CHCl_3$ DP[e,f]
LG		63	27.4	1.3	33.3	1.3	13.4	512 (206)
$L_{rac}G$		52	28.8	1.3	34.3	1.4	---	527
GLG		78	26.2	1.2	36.2	1.4	19.4	577 (309)
$GL_{rac}G$		60	21.4	1.3	27.5	1.4	---	439
LLG		70	41.2	1.2	41.8	1.3	23.1	620 (343)
LL_RG		71	29.0	1.4	42.3	1.3	---	628
L_RLG		59	30.6	1.4	39.8	1.4	---	591
$L_{rac}L_{rac}G$		65	30.5	1.4	35.2	1.3	---	522
$LL_{rac}G$		50	17.8	1.4	19.3	1.6	---	286
$L_{rac}LG$		83	27.4	1.4	40.5	1.4	25.9	601 (384)
$L_{d,rac}LG$		99	32.8	1.3	31.7	1.5	---	468
$LL_{d,rac}G$		62	29.6	1.4	33.7	1.4	---	498
GLG_{d2}		52	15.2	1.4	25.3	1.5	---	400
$GLGL_R$		65	12.3	1.5	21.1	1.4	---	324
$LLGLL_RG$		70	30.0	1.4	32.0	1.5	---	475
L_RLGLLG		63	30.1	1.4	39.8	1.3	---	591

[a] (*S*)-lactic unit, (*R*)-lactic unit, *rac*-lactic unit, glycolic unit, deuterated unit; [b]Isolated after 2x precipitation in MeOH; [c]Determined by SEC relative to PS standards; [d]Determined by SEC-MALLS; [e]DP from SEC data based on number of lactic and glycolic monomers; [f](DP) from SEC-MALLS data based on number of lactic and glycolic monomers.

Monomer Sequence Matters !

Exploiting Sequence To Control the Hydrolysis Behavior of Biodegradable PLGA Copolymers

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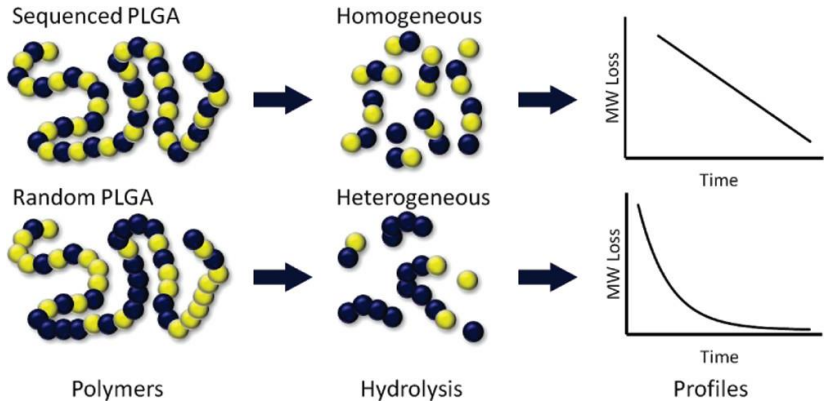

ABSTRACT: Monomer sequence is a potentially powerful but underutilized tool for the control of copolymer properties. Sequence is demonstrated to dramatically affect the hydrolysis profile for the degradation of poly(lactic-*co*-glycolic acid) (PLGA), a member of the most widely used class of biodegradable polymers employed in biomedical applications. The nearly linear molecular weight loss profile and uniform thermal behavior throughout the course of the hydrolysis differ dramatically from the behavior that is exhibited by random copolymer controls with the same comonomer ratio.

J. Am. Chem. Soc. 2011, 133, 6910–6913

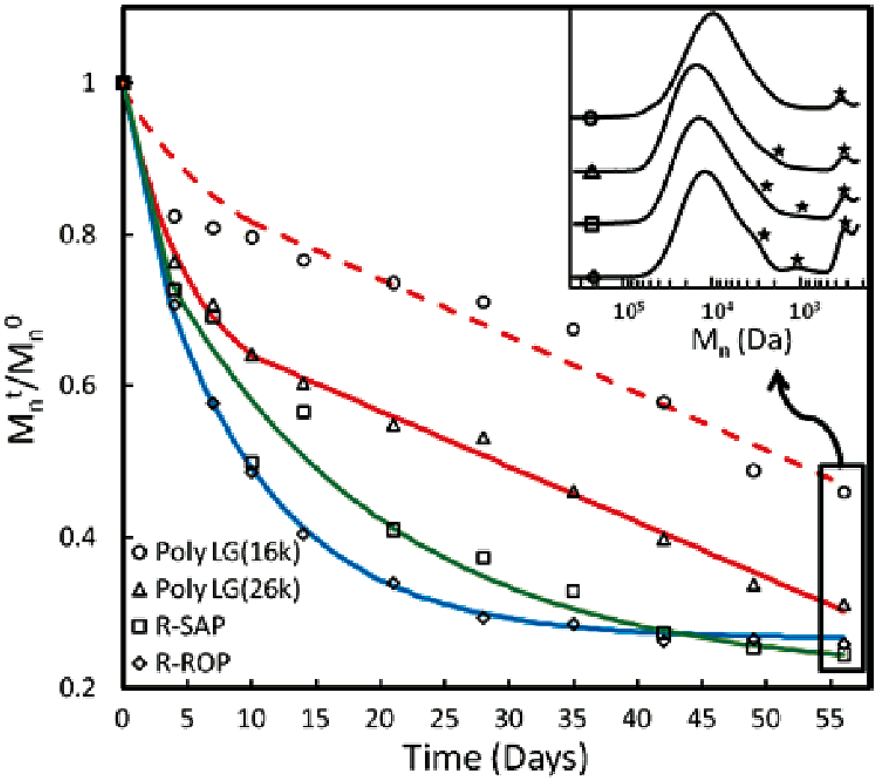

Figure 2. Plot of normalized molecular weight as a function of time for the repeating sequence and random copolymers of poly(lactic-*co*-glycolic acids). Inset: SEC plots for day 56 hydrolysis samples. Asterisks represent low-molecular-weight oligomers.

Learning Objectives

- Understand the mechanistic principles that underlie protein and DNA biosynthesis.
- Understand the limitations of conventional step and chain polymerizations in terms of controlling monomer sequence.
- Be familiar with two basic strategies that provide enhanced control over monomer sequence in synthetic polymers