Scalable synthesis of sequence-defined, unimolecular macromolecules by Flow-IEG

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We report a semiautomated synthesis of sequence and architecturally defined, unimolecular macromolecules through a marriage of multistep flow synthesis and iterative exponential growth (Flow-IEG). The Flow-IEG system performs three reactions and an in-line purification in a total residence time of under 10 min, effectively doubling the molecular weight of an oligomeric species in an uninterrupted reaction sequence. Further iterations using the Flow-IEG system enable an exponential increase in molecular weight. Incorporating a variety of monomer structures and branching units provides control over polymer sequence and architecture. The synthesis of a uniform macromolecule with a molecular weight of 4,023 g/mol is demonstrated. The user-friendly nature, scalability, and modularity of Flow-IEG provide a general strategy for the automated synthesis of sequence-defined, unimolecular macromolecules. Flow-IEG is thus an enabling tool for theory validation, structure–property studies, and advanced applications in biotechnology and materials science.

Significance

Automated chemical processes, such as DNA sequencing and nucleic acid and peptide synthesis, have transformed the fields of genetics and biotechnology. There is no analogous automated or semiautomated process, however, to provide unimolecular, sequence-defined synthetic polymers to those interested in studying them. The combination of multistep continuous flow chemistry and polymer synthesis by iterative exponential growth (Flow-IEG) enables the semiautomated synthesis of perfect polymers reported herein. The user-friendly nature, scalability, and modularity of Flow-IEG provides a general strategy for the automated synthesis of sequence and architecturally defined, uniform macromolecules. We envision this polymer synthesis machine will serve as an enabling tool for both fundamental explorations and advanced applications in biotechnology, medicinal chemistry, and materials science.


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polymers of molecular weight >4,000 g/mol in a scalable fashion. Further, because the Flow-IEG system is designed to allow “plug-and-play” monomer selection (31), the semiautomated synthesis of sequence and architecturally defined, unimolecular polymers is demonstrated, and their structure–property relationships are compared.

**Design and Optimization of the Flow-IEG System**

The copper-catalyzed azide–alkyne cycloaddition (CuAAC) is an ideal candidate for Flow-IEG systems because of its efficiency, chemoselectivity, and simple preparation of coupling partners, as demonstrated by Drockenmüller and coworkers (32). Monomer 1 was thus designed to contain the requisite masked functional groups for IEG: a triisopropylsilyl (TIPS) protected alkyne and an alkyl bromide (Fig. 2). Initial investigations demonstrated that the tetraalkyldimethylammonium salts of both azide (TBAAm) and fluoride (TBAF) were excellent reagents to unveil the azide and alkyne derivatives of 1, respectively. Optimization (SI Materials and Methods) identified conditions that provided chemoselective conversion of 1 into the requisite azide and alkyne coupling partners over a range of substrate concentrations. Specifically, azide substitution reached full conversion in flow at 130 °C with a residence time ($t_R$) of 5 min, and the silyl deprotection reached full conversion with an $t_R$ of 5 min at room temperature. To quench and remove excess or unreacted fluoride and azide reagents immediately before the CuAAC, we incorporated an aqueous workup into the continuous system by taking advantage of an in-line, membrane-based liquid–liquid separator developed by Jensen and coworkers (33). Its straightforward implementation, small footprint, and integrated pressure control allows the use of excess fluoride and azide reagents to ensure complete conversion without deleterious cross reactivity in subsequent steps. [The aqueous workup also serves to remove tetrabutylammonium salts and tetrahydrofuran (THF), thus both purifying the flow stream and concentrating the coupling partners into toluene before the CuAAC reaction.]

Having optimized the preparation and in-line purification of the azide and alkyne coupling partners, we turned our attention to joining them via CuAAC with a copper–ligand combination that was both highly active and completely soluble under the reaction conditions (34). After evaluating several conditions, we discovered that a 0.1 M solution of CuI with 1.1 equivalents of the ligand tris[2-(dimethylamino)ethyl]amine (Me$_2$TREN) provided a CuAAC that reached full conversion in 3 min at 130 °C with only 3 mol% copper loading. These conditions provided excellent chemoselective reactivity at a variety of substrate concentrations. Optimizing other system design elements, such as cooling the flow stream containing the azide and quenching the silyl deprotection before mixing further, prevented deleterious cross-reactivity. The optimized end-to-end Flow-IEG system (Fig. 3A) affects three reactions and an in-line purification in a total average $t_R$ of only 10 min. This system design generally provided coupled product in high conversion with no high-molecular-weight impurities present. Collection of the material, solvent removal, and chromatography provides the pure coupled product of 1 in 86% isolated yield over the three reaction steps.

Full characterization of these oligomeric species was accomplished by $^1$H-NMR, $^{13}$C-NMR, size exclusion chromatography (SEC), matrix-assisted laser desorption ionization (MALDI) MS,

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**Fig. 1.** Flow-IEG improves on previous work to enable the synthesis of unimolecular polymers by conducting multiple reactions and purifications in a continuously flowing system.

**Fig. 2.** Ester monomer 1 was optimized and implemented into the Flow-IEG system, where three reactions and an in-line purification are performed in a continuous system and iterative coupling provides exponential increases in molecular weight. The system has been scaled to provide 2.75 grams of coupled product per hour.
thermogravimetric analysis (TGA), and differential scanning calorimetry (DSC). SEC clearly demonstrates both the growth and purity of the oligomeric species (Fig. 3B). As the substrates grow exponentially in size, the peaks in the SEC shift to shorter retention times while maintaining their narrow and monomodal peak shape. The dispersity (D) of each species is below 1.01 compared with poly styrene standards, thus corroborating the unimolecular nature of the polymers. 1H-NMR proved diagnostic for tracking the growth of Flow-IEG derived polymers. The unique resonances of the polymer end groups were clearly visible by 1H-NMR (at 4.25 and 3.21 ppm), and their integration relative to the polymer backbone and triazole signals after each growth step confirmed the iterative coupling (Fig. 3C). For example, if the integration of the propargylic proton at 4.25 ppm is set to 2.0 protons, the integration of the triazole peak at 7.58 ppm increases from 1.0 to 3.0 to 7.0 protons for the dimer, tetramer, and octamer oligomers, respectively. MS, particularly MALDI, provided valuable information on the unimolecular nature of the polymers. As shown in Fig. 4A, the molecular ion for the dimer, tetramer, and octamer is observed as the single species in each spectrum. A closer look at the octamer (Fig. 4A, Inset) shows not only the correct mass of the molecular ion ([M+H]+) at 2,315.7 Da, but also displays the characteristic isotopic fine structure associated with a species of the correct chemical formula (C121H204BrN21O16Si).

The IEG approach has previously proven valuable in gaining insight into the structure–property relationships of industrially important polymers, such as nylon (35), poly(ethylene terephthalate) (36), and a variety of polyesters (37–39). Accordingly, we used the oligomers made by Flow-IEG to study the evolution of the thermal properties of these unimolecular species. As observed in previous IEG studies, the decomposition temperature of these oligomers increases with increasing molecular weight (TGA in SI Materials and Methods). Most importantly, DSC clearly demonstrates how polymer molecular weight influences both the glass transition temperature (Tg) and the crystallization behavior of these oligomers. The Tg increases significantly with increasing molecular weight, going from −53.1 °C for the dimer to −23.9 °C for the tetramer to −16.5 °C for the octamer, commensurate with Flory–Fox theory (40). Further, as seen in Fig. 4B, the melting temperature (Tm) of these polymers also evolves with increasing molecular weight. Although the dimer is a sticky solid and melts just above room temperature (31.0 °C), increasing the molecular weight exponentially increases the Tm to 70.0 °C for the tetramer and 100.4 °C for the octamer. This dramatic increase in Tm even at low molecular weights dominates not only chain growth, but also the strong propensity of these polymers to crystallize and quickly approach the Tm of the parent polymer, which has been reported to be 112 °C (41). We envision Flow-IEG will serve as an enabling tool for theory validation and future structure–property studies like these on a wide range of materials.

We further envision Flow-IEG can provide a practical means to generate unimolecular polymers in multigram to kilogram quantities, thus differentiating it from SPS. With an eye toward these applications, the Flow-IEG system was scaled to approximately four times the flow rate of the screening reactions, and the reactors were lengthened to maintain the appropriate reaction times. Monomer 1 was coupled at an initial concentration of 0.50 M in toluene to provide the ester dimer. Collection of the product over 1 h of steady-state operation provided 2.75 g of material in 84% isolated yield. Extrapolation of this production rate corresponds to Flow-IEG producing 66.0 g/d (24 kg/y) of coupled product. Further, scaling using known engineering principles will provide significant throughput improvements in future systems (42).

![Fig. 3. A machine for automated IEG. (A) Schematic of the Flow-IEG system detailing reaction times and flow sequence. (B) SEC traces of unimolecular macromolecules derived from 1. (C) 1H-NMR spectra of the unimolecular macromolecules derived from 1.](image)

![Fig. 4. Structure–property relationships of Flow-IEG derived polymers. (A) MALDI MS spectra of the unimolecular copolymers derived from 1. (Inset) Zoom of the octamer spectra demonstrating the mass of the molecular ion [M+H]+ along with its isotopic fine structure. (B) DSC traces demonstrating the increasing melting transitions of the unimolecular oligomers derived from 1. The third heating cycle of the DSC is shown from samples heated at 5 °C/min.](image)
Making Sequence and Architecturally Defined Macromolecules

The Flow-IEG system in Fig. 3A can be adapted to different monomers while maintaining its efficiency and user-friendly nature. To tackle such a challenge, we chose to incorporate monomer 2 into the Flow-IEG system because of its previous utility in IEG and hydrophilic nature (Fig. 5) (32). During the processes of testing each of the three reactions individually with 2, we discovered that azide displacement of the alkyl chloride with TBAA required 7.5 min at 130 °C; thus, the reactor for the azide displacement was lengthened accordingly. To demonstrate the potential of Flow-IEG for sequence-defined polymer synthesis, a perfectly alternating (ABAB)n polymer and its structural isomer, a polymer with the repeat unit (AABB)n, were targeted. These materials demonstrate the power and flexibility of Flow-IEG, allowing the user to combine either two different monomers in the case of the (ABAB)n polymer or two different dimers in the case of the (AABB)n polymer. Further, synthesis of these two structural isomers will probe how sequence influences polymer properties.

The synthetic sequences and overall yields of Flow-IEG for these materials are summarized in Fig. 5C. Yields remain high over the three-step Flow-IEG sequence, with variations possibly due to fluctuations in the membrane separator operation with the more hydrophilic materials. As a result of the improved solubility of these sequence-defined polymers, hexadecamers could be synthesized from octamers by Flow-IEG, providing access to unimolecular, sequence-defined polymers with molecular weights reaching 4,023 g/mol in >95% purity. The SECs and 1H-NMRs of the perfectly alternating copolymer (ABAB)n are shown in Fig. 5 D and E, respectively, highlighting both the unimolecular nature and efficient growth of these sequence-defined macromolecules. A closer examination of the 1H-NMRs provides key structural insights (Fig. 5E).

Comparing the structure–property relationships of these iso-meric copolymers illustrates the important role that sequence plays in influencing polymer properties. The Tgs of the two poly-mers show similar trends and increase in the progression from tetramer to octamer to hexadecamer (TGA in SI Materials and Methods). Interestingly, the melting behavior is significantly different between the structural isomers. For instance, although the tetramers of the two samples show similar melting behavior, the (AABB)n octamer has a melting transition at 41 °C, whereas the alternating octamer (ABAB)n shows no Tg under the same conditions. Comparing the two 16-mers, the (AABB)n copolymer has a Tg at 44 °C and the (ABAB)n has two melting transitions at 29 °C and 64 °C. All samples that do have crystalline properties exhibit cold crystallization, where crystallization occurs between the Tg and Tm during heating (43). The structure–property relationships of these sequence-defined, unimolecular structural isomers demonstrates the important role that sequence plays in determining properties and how Flow-IEG can be used to evaluate and even tune these physical properties.

Flow-IEG is not only useful for the synthesis of sequence-defined macromolecules but it can also be modified to generate architecturally defined materials. The concept of IEG has many similarities to dendrimer synthesis, and we drew inspiration from the synthesis of triazole-based dendrimers to design monomer 3 (44). The two protected alkynes in 3 provide a branching point within a polymer and, by judiciously choosing when to introduce 3, the branching point can be located precisely within a sequence-defined, unimolecular macromolecule (Fig. 6). This degree of control is necessary for fundamental studies on chain architecture and multivalency.

![Fig. 5. The synthesis and characterization of sequence defined unimolecular polymers by Flow-IEG. (A) Legend for the two monomers used. (B) Example of one round of Flow-IEG incorporating 2. (C) The yields of each Flow-IEG iteration are shown, along with the (D) SECs and the (E) 1H-NMR spectra of the perfectly alternating (ABAB)n, unimolecular copolymer.](https://www.pnas.org/cgi/doi/10.1073/pnas.1508599112)
Conclusions
Flow-IEG is an enabling tool for the semiautomated synthesis of sequence-defined unimolecular macromolecules. The simplicity of Flow-IEG compared with the alternative batch procedures, its capacity for scale-up, and the semiautomated nature of this methodology make it attractive for both exploratory and large-scale applications in polymer chemistry. Specifically, Flow-IEG is one of the only methodologies available for the modular synthesis of unimolecular polymers that has the ability to control both sequence and architecture independently. This advance has important consequences for potential structure–activity screening of synthetic polymers in biomedical applications, as Flow-IEG can provide synthetic polymer libraries where the influences of functional group density, sequence, and chain architecture can be assayed for a desired activity.

Flow-IEG represents a technology for the semiautomated synthesis of sequence and architecturally defined synthetic polymers. Flow-IEG telescopes three reactions and an in-line purification in an uninterrupted system with a total residence time of under 10 min, and a modest scale-up permits the capacity to produce significant quantities (≥60 g/d, as shown herein). Flow-IEG is also an enabling tool for polymer theory validation and structure–property studies on a wide range of materials. Overall, Flow-IEG is a flexible and modular tool empowered by the convergence of multistep continuous flow chemistry and polymer synthesis.

Materials and Methods
All Flow-IEG reactions were performed in the system shown in Fig. 2A. The substrates of interest were dissolved in toluene or chlorobenzene to generate a solution of ∼10 wt%. The TBAA and TBAF were dissolved in THF to generate a solution that contained 1.1 mol equivalents of TBAA and TBAF compared with substrate. Equal flow rates of the TBAA, TBAF, and the substrate solutions were used. All solvent and reagent solutions were degassed by sparging with argon for 20 min before use. Syrris Asia pumps equipped with 50/100-μL syringes are used for all solvents and reagents except the Me6TREN/CuI solution, which used a Harvard PHD2000 pump with an 8-ml stainless steel syringe. The system was allowed to run for 2.5 residence times to reach steady state before collecting and analyzing product composition. Heating the reactors was accomplished by immersing the PFA tubing into an oil bath.

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