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# 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.**

polymers | automation | continuous flow chemistry | unimolecular macromolecules | sequence-controlled polymers

The automation of chemical synthesis provides nonexperts with access to enabling technologies that revolutionize entire fields of scientific inquiry. For example, automation of DNA sequencing (1) provided a user-friendly technology that helped unlock how hereditary information is passed between generations, eventually enabling the Human Genome Project (2). Likewise, automating processes for solid-phase synthesis (SPS) provided routine access to biological polymers such as peptides (3), nucleic acids (4), and polysaccharides (5) for a variety of fundamental and applied explorations in biotechnology, medicinal chemistry, and immunology.

Sequence-defined synthetic polymers hold immense potential for applications in a wide range of fields, including biomedicine, nanotechnology, and high-density information storage (6). The automated synthesis of perfect polymers would provide these materials to those best equipped to exploit their potential. Despite their promise, the efficient and scalable syntheses of unimolecular, sequence-defined, and nonbioressemblant polymers remains a major challenge for chemical synthesis (7). Herein, we report a general strategy for the scalable, semiautomated synthesis of macromolecules with uniform mass and precisely defined primary sequence by integrating iterative exponential growth (IEG) (8), an underused methodology in synthetic polymer chemistry, with modern multistep continuous flow synthesis (9).

Chemical methods for the synthesis of unimolecular, sequence-defined synthetic polymers typically rely on either co-opting biological machinery—for example, in PCR (10) or in vitro protein expression (11, 12)—or using SPS (13). Although these powerful methods have enabled transformative advances in biotechnology and have recently been revisited for the synthesis of sequence-defined synthetic polymers (14–16), alternative strategies are needed that provide a general solution for access to sequence-defined synthetic polymers in a scalable and sustainable manner. In contrast, step- or chain-growth polymerization methods that use differences in monomer reactivity, living polymerizations,

monomer design, and/or templating strategies to impart sequence regulation have been recently reported (17–23). These scalable methods, however, still rely on stochastic monomer addition and thus do not generate unimolecular macromolecules.

IEG, pioneering by Whiting and coworkers for the synthesis of unimolecular samples of polyethylene (24, 25), is a method where a common starting material is partitioned in half, with each half undergoing two separate and complementary deprotections, followed by coupling of the two halves to give a new molecule with approximately twice the original molecular weight (Fig. 1) (8). Because each doubling of molecular weight requires three different reactions, this method is exceedingly laborious when done under standard batch conditions and thus has not been broadly adopted for routine polymer synthesis.

To overcome the challenges associated with IEG, we envisioned a union of IEG with continuous flow chemistry (Flow-IEG). Continuous flow methods can have considerable advantages in terms of mass and heat transfer, reproducibility, throughput, and telescoping reactions (9, 26, 27). We sought to leverage these advantages by merging the multistep flow methods being developed for organic and medicinal chemistry (28) with the capabilities of polymer synthesis in-flow (29, 30). The automated and exacting control over mixing, thermal conditions, in-line purification, and residence time make continuous flow methods ideal for IEG, as optimized conditions can be repeatedly applied to the iterative coupling of polymer building blocks in a continuous system.

The Flow-IEG system reported herein performs three reaction steps and an in-line purification in a total residence time of under 10 min. The optimized Flow-IEG system generates unimolecular

## 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.

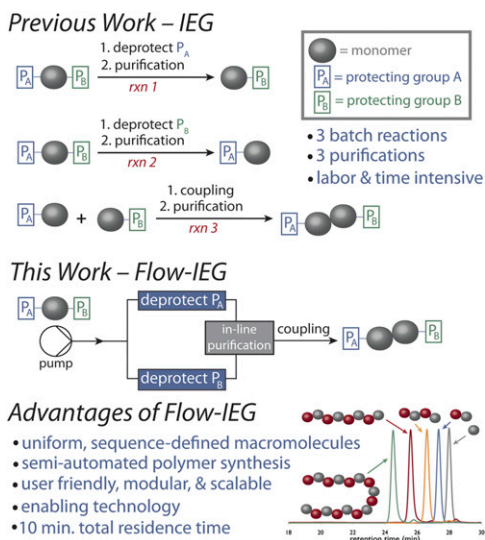
Author contributions: F.A.L., J.A.J., and T.F.J. designed research; F.A.L. performed research; F.A.L., J.A.J., and T.F.J. analyzed data; and F.A.L., J.A.J., and T.F.J. wrote the paper.

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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.

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

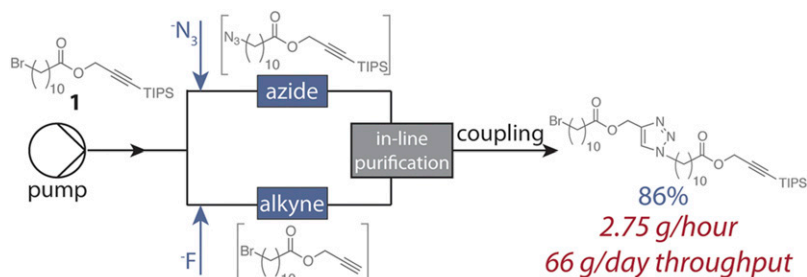
The copper catalyzed azide–alkyne cycloaddition (CuAAC) is an ideal candidate for Flow-IEG systems because of its efficiency, chemoselectivity, and simple preparation of coupling partners, as demonstrated by Drockenmuller and coworkers (32). Monomer **1** was thus designed to contain the requisite masked functional groups for IEG: a triisopropylsilyl (TIPS) protected alkyne and an alkyl bromide (Fig. 2). Initial investigations demonstrated that the tetrabutylammonium salts of the both azide (TBAA) 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  $130^\circ\text{C}$  with a residence time ( $t_R$ ) of 5 min, and the silyl deprotection reached full conversion with a  $t_R$  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.]

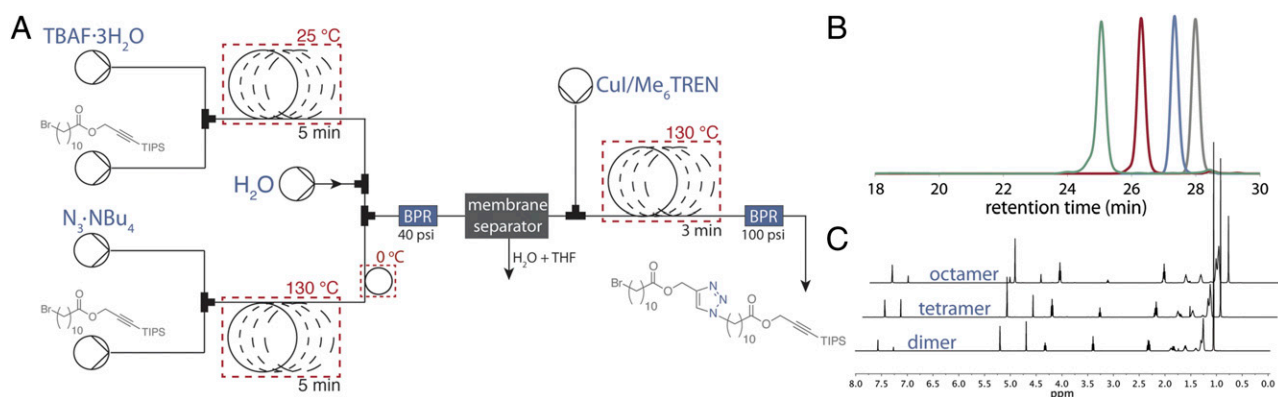
Having optimized the preparation and in-line purification of the azide and alkyne coupling partners, we turned our attention to joining them via CuAAC with a copper–ligand combination that was both highly active and completely soluble under the reaction conditions (34). After evaluating several conditions, we discovered that a 0.1 M solution of CuI with 1.1 equivalents of the ligand tris[2-(dimethylamino)ethyl]amine ( $\text{Me}_6\text{TREN}$ ) provided a CuAAC that reached full conversion in 3 min at  $130^\circ\text{C}$  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.

Critical to the success of Flow-IEG is its ability to produce high-molecular-weight species. Therefore, the purified dimer of monomer **1** was reintroduced in the Flow-IEG system. (For reactions generating larger oligomeric species, chlorobenzene was found to provide superior solubility while having no observable effect on reactivity.) The dimer was converted to the tetrameric polyester via Flow-IEG in 87% isolated yield; subsequently, Flow-IEG transformed the tetramer into the octamer in 78% isolated yield. Because these long-chain aliphatic esters are prone to crystallization, solubility became a significant challenge in this system when attempting to make structures larger than the octamer. This rapid construction of large unimolecular species required only three runs through the Flow-IEG system; including solution preparation, running the Flow-IEG system, purification, and isolation, the entire semiautomated process can be easily completed in a routine 8-h work day. Therefore, octamer synthesis by a skilled practitioner would require only 3 workdays. In contrast, nine reaction and purification steps would typically be required using traditional batch chemistry. The polyester octamer with a molecular weight of 2,317 g/mol was synthesized by Flow-IEG in an isolated yield of 58% from **1**.

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



**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.



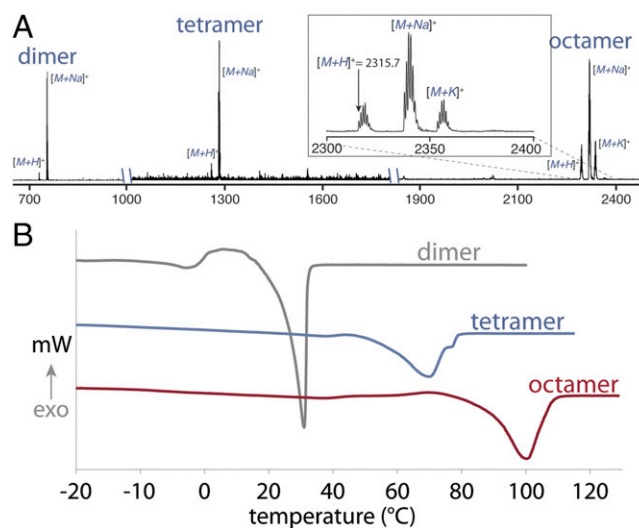
**Fig. 3.** A machine for automated IEG. (A) Schematic of the Flow-IEG system detailing reaction times and flow sequence. (B) SEC traces of unimolecular macromolecules derived from **1**. (C) <sup>1</sup>H-NMR spectra of the unimolecular macromolecules derived from **1**.

thermal gravimetric 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 polystyrene standards, thus corroborating the unimolecular nature of the polymers. <sup>1</sup>H-NMR proved diagnostic for tracking the growth of Flow-IEG derived polymers. The unique resonances of the polymer end groups were clearly visible by <sup>1</sup>H-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]<sup>+</sup>) at 2,315.7 Da, but also displays the characteristic isotopic fine structure associated with a species of the correct chemical formula (C<sub>121</sub>H<sub>204</sub>BrN<sub>21</sub>O<sub>16</sub>Si).

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 (*T<sub>g</sub>*) and the crystallization behavior of these oligomers. The *T<sub>g</sub>* 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 (*T<sub>m</sub>*) 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 *T<sub>m</sub>* to 70.0 °C for the tetramer and 100.4 °C for the octamer. This dramatic increase in *T<sub>m</sub>* even at low molecular weights demonstrates not only chain growth, but also the strong propensity of these polymers to crystallize and quickly approach the *T<sub>m</sub>* 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. 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]<sup>+</sup> 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.



## Making Sequence and Architecturally Defined Macromolecules

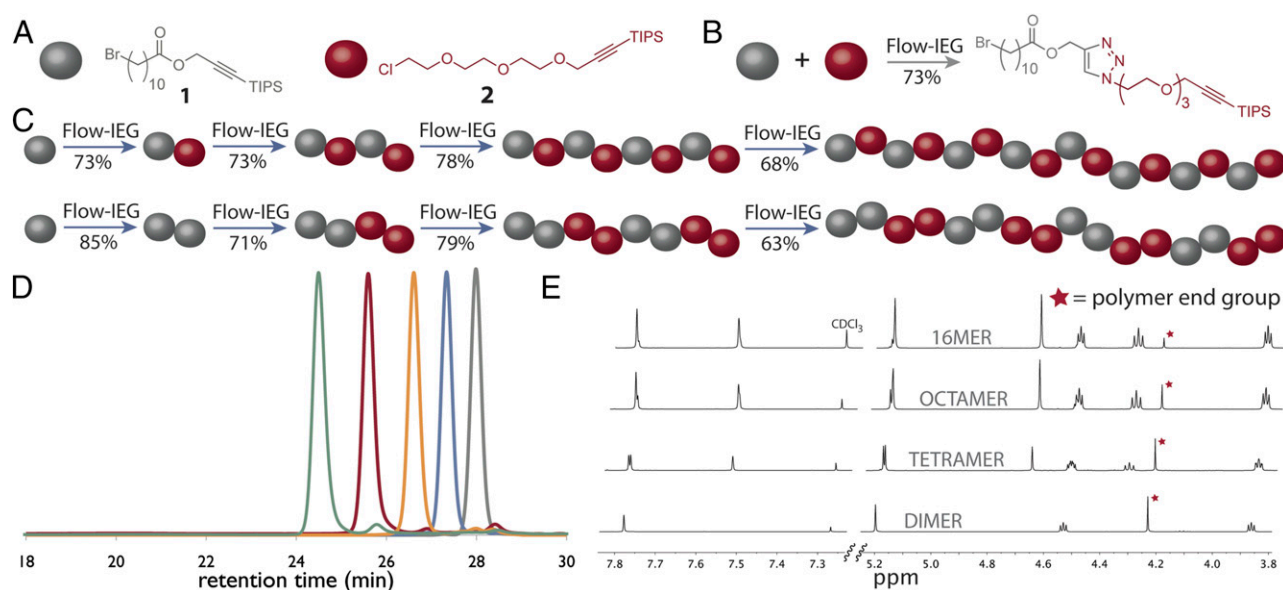
The Flow-IEG system in Fig. 3A can be adapted to different monomers while maintaining its efficiency and user-friendly nature. To tackle such a challenge, we chose to incorporate monomer **2** into the Flow-IEG system because of its previous utility in IEG and hydrophilic nature (Fig. 5) (32). During 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)<sub>n</sub> polymer and its structural isomer, a polymer with the repeat unit (AABB)<sub>n</sub>, 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)<sub>n</sub> polymer or two different dimers in the case of the (AABB)<sub>n</sub> 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 <sup>1</sup>H-NMRs of the perfectly alternating copolymer (ABAB)<sub>n</sub> are shown in Fig. 5D and E, respectively, highlighting both the unimolecular nature and efficient growth of these sequence-defined macromolecules. A closer examination of the <sup>1</sup>H-NMRs provides key structural insights (Fig. 5E). The appearance of a second triazole proton (7.51 ppm) is clearly visible in the aromatic region when two AB-dimers are coupled to make an ABAB tetramer. Further, at 7.78 ppm, two distinct singlets are observed signifying the two chemically inequivalent triazole “end-group” protons in the tetramer product. As the oligomers are coupled to the octamer and subsequently 16mer, the singlets

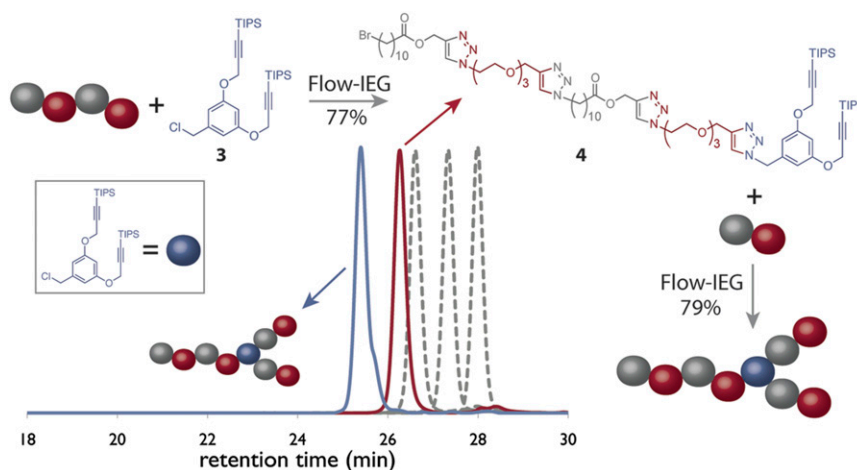
coalesce into a single peak as a result of the triazole protons within the polymer chain having a nearly identical chemical shift and those beginning to dominate the spectrum in longer polymer chains. This same phenomenon can be observed with the peak of the protons between the ester and triazole at 5.20 ppm. Further, the peak assigned to the propargylic protons of the polymer end-group at 4.24 ppm gets smaller compared with the peaks of protons from the polymer backbone as the polymer grows in size, demonstrating the increasing molecular weight of the Flow-IEG products.

Comparing the structure–property relationships of these isomeric copolymers illustrates the important role that sequence plays in influencing polymer properties. The *T<sub>g</sub>*s of the two polymers 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)<sub>n</sub> octamer has a melting transition at 41 °C, whereas the alternating octamer (ABAB)<sub>n</sub> shows no *T<sub>m</sub>* under the same conditions. Comparing the two 16-mers, the (AABB)<sub>n</sub> copolymer has a *T<sub>m</sub>* at 44 °C and the (ABAB)<sub>n</sub> 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 *T<sub>g</sub>* and *T<sub>m</sub>* 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) <sup>1</sup>H-NMR spectra of the perfectly alternating (ABAB)<sub>n</sub> unimolecular copolymer.



**Fig. 6.** Control of polymer architecture is accomplished through the use of monomer **3**. The synthesis by Flow-IEG and SEC traces of the copolymers are shown.

## 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 provides the capacity to produce significant quantities ( $\geq 60$  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 molar 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- $\mu$ L syringes are used for all solvents and reagents except the Me<sub>6</sub>TREN/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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- Prober JM, et al. (1987) A system for rapid DNA sequencing with fluorescent chain-terminating dideoxynucleotides. *Science* 238(4825):336–341.
- Schmutz J, et al. (2004) Quality assessment of the human genome sequence. *Nature* 429(6990):365–368.
- Merrifield RB, Stewart JM, Jernberg N (1966) Instrument for automated synthesis of peptides. *Anal Chem* 38(13):1905–1914.
- Caruthers MH (1985) Gene synthesis machines: DNA chemistry and its uses. *Science* 230(4723):281–285.
- Seeburger PH (2008) Automated oligosaccharide synthesis. *Chem Soc Rev* 37(1):19–28.
- Ouchi M, Badi N, Lutz J-F, Sawamoto M (2011) Single-chain technology using discrete synthetic macromolecules. *Nat Chem* 3(12):917–924.
- Lutz JF, Ouchi M, Liu DR, Sawamoto M (2013) Sequence-controlled polymers. *Science* 341(6146):1238149–1238149.
- Binauld S, Damiron D, Connal LA, Hawker CJ, Drockenmüller E (2011) Precise synthesis of molecularly defined oligomers and polymers by orthogonal iterative divergent/convergent approaches. *Macromol Rapid Commun* 32(2):147–168.
- Ingham RJ, et al. (2015) Organic synthesis: March of the machines. *Angew Chem Int Ed* 54(11):144–148.
- Saiki RK, et al. (1988) Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. *Science* 239(4839):487–491.
- Wang L, Schultz PG (2004) Expanding the genetic code. *Angew Chem Int Ed Engl* 44(1):34–66.
- Johnson JA, Lu YY, Van Deventer JA, Tirrell DA (2010) Residue-specific incorporation of non-canonical amino acids into proteins: Recent developments and applications. *Curr Opin Chem Biol* 14(6):774–780.
- Merrifield RB (1963) Solid phase peptide synthesis: The synthesis of a tetrapeptide. *J Am Chem Soc* 85(14):2149–2154.
- Roy RK, et al. (2015) Design and synthesis of digitally encoded polymers that can be decoded and erased. *Nat Commun* 6:7237.
- Porel M, Alabi CA (2014) Sequence-defined polymers via orthogonal allyl acrylamide building blocks. *J Am Chem Soc* 136(38):13162–13165.
- Solleder SC, Meier MAR (2014) Sequence control in polymer chemistry through the Passerini three-component reaction. *Angew Chem Int Ed Engl* 53(3):711–714.
- Pfeifer S, Lutz J-F (2007) A facile procedure for controlling monomer sequence distribution in radical chain polymerizations. *J Am Chem Soc* 129(31):9542–9543.
- Moatsou D, Hansell CF, O'Reilly RK (2014) Precision polymers: A kinetic approach for functional poly(norbornenes). *Chem Sci (Camb)* 5(6):2246–2250.
- Gody G, Maschmeyer T, Zetterlund PB, Perrier S (2013) Rapid and quantitative one-pot synthesis of sequence-controlled polymers by radical polymerization. *Nat Commun* 4:2505.
- Zhang J, Matta ME, Hillmyer MA (2012) Synthesis of sequence-specific vinyl copolymers by regioselective ROMP of multiply substituted cyclooctenes. *ACS Macro Lett* 1(12):1383–1387.
- Lo PK, Sleiman HF (2009) Nucleobase-templated polymerization: Copying the chain length and polydispersity of living polymers into conjugated polymers. *J Am Chem Soc* 131(12):4182–4183.
- Niu J, Hili R, Liu DR (2013) Enzyme-free translation of DNA into sequence-defined synthetic polymers structurally unrelated to nucleic acids. *Nat Chem* 5(4):282–292.
- Gutekunst WR, Hawker CJ (2015) *J Am Chem Soc* 137(25):8038–8041.
- Paynter OL, Simmonds DJ, Whiting MC (1982) The synthesis of long-chain unbranched aliphatic compounds by molecular doubling. *J Chem Soc Chem Commun* 1165–1166.
- Bidd I, Whiting MC (1985) The synthesis of pure n-paraffins with chain-lengths between one and four hundred. *J Chem Soc Chem Commun* 543–544.
- Longstreet AR, McQuade DT (2013) Organic reaction systems: Using microcapsules and microreactors to perform chemical synthesis. *Acc Chem Res* 46(2):327–338.
- Lévesque F, Seeburger PH (2012) Continuous-flow synthesis of the anti-malaria drug artemisinin. *Angew Chem Int Ed Engl* 51(7):1706–1709.
- Webb D, Jamison TF (2010) Continuous flow multi-step organic synthesis. *Chem Sci (Camb)* 1(6):675–680.

29. Tonhauser C, Natalello A, Löwe H, Frey H (2012) Microflow technology in polymer synthesis. *Macromolecules* 45(24):9551–9570.
30. Myers RM, Fitzpatrick DE, Turner RM, Ley SV (2014) Flow chemistry meets advanced functional materials. *Chemistry* 20(39):12348–12366.
31. Zhang J, Pesak DJ, Ludwick JL, Moore JS (1994) Geometrically-controlled and site specifically-functionalized phenylacetylene macrocycles. *J Am Chem Soc* 116(10):4227–4239.
32. Binauld S, Hawker CJ, Fleury E, Drockenmüller E (2009) A modular approach to functionalized and expanded crown ether based macrocycles using click chemistry. *Angew Chem Int Ed Engl* 48(36):6654–6658.
33. Adamo A, Heider PL, Weeranoppanant N, Jensen KF (2013) Membrane-based, liquid-liquid separator with integrated pressure control. *Ind Eng Chem Res* 52(31):10802–10808.
34. Presolski SI, Hong V, Cho S-H, Finn MG (2010) Tailored ligand acceleration of the Cu-catalyzed azide-alkyne cycloaddition reaction: Practical and mechanistic implications. *J Am Chem Soc* 132(41):14570–14576.
35. Reddy SS, Dong X, Murgasova R, Gusev AI, Hercules DM (1999) Synthesis and secondary-ion mass spectrometry of linear single oligomers of nylon-6. *Macromolecules* 32(5):1367–1374.
36. Brooke GM, Cameron NR, MacBride JAH, Whiting MC (2002) The synthesis of oligomers related to poly(ethylene terephthalate). *Polymer (Guildf)* 43(4):1139–1154.
37. Williams JB, Chapman TM, Hercules DM (2003) Synthesis of discrete mass poly(butylene glutarate) oligomers. *Macromolecules* 36(11):3898–3908.
38. Takizawa K, Tang C, Hawker CJ (2008) Molecularly defined caprolactone oligomers and polymers: Synthesis and characterization. *J Am Chem Soc* 130(5):1718–1726.
39. Takizawa K, Nulwala H, Hu J, Yoshinaga K, Hawker CJ (2008) Molecularly defined (L)-lactic acid oligomers and polymers: Synthesis and characterization. *J Polym Sci A Polym Chem* 46(18):5977–5990.
40. Fox TG, Flory PJ (1950) Second-order transition temperatures and related properties of polystyrene. I. Influence of molecular weight. *J Appl Phys* 21(6):581–591.
41. Schwartz E, Breitenkamp K, Fokin VV (2011) Synthesis and postpolymerization functionalization of poly(5-iodo-1,2,3-triazole)s. *Macromolecules* 44(12):4735–4741.
42. McMullen JP, Jensen KF (2011) Rapid determination of reaction kinetics with an automated microfluidic system. *Org Process Res Dev* 15(2):398–407.
43. Wunderlich B (2005) *Single Component Materials. Thermal Analysis of Polymeric Materials* (Springer, Berlin), pp 591–704.
44. Wu P, et al. (2004) Efficiency and fidelity in a click-chemistry route to triazole dendrimers by the copper(I)-catalyzed ligation of azides and alkynes. *Angew Chem Int Ed Engl* 43(30):3928–3932.
45. Hoogboom J, Swager TM (2006) Increased alignment of electronic polymers in liquid crystals via hydrogen bonding extension. *J Am Chem Soc* 128(47):15058–15059.