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# Sequence-Defined Polymers via Orthogonal Allyl Acrylamide Building Blocks

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**S** *Supporting Information*

**ABSTRACT:** Biological systems have long recognized the importance of macromolecular diversity and have evolved efficient processes for the rapid synthesis of sequence-defined biopolymers. However, achieving sequence control via synthetic methods has proven to be a difficult challenge. Herein we describe efforts to circumvent this difficulty via the use of orthogonal allyl acrylamide building blocks and a liquid-phase fluorous support for the de novo design and synthesis of sequence-specific polymers. We demonstrate proof-of-concept via synthesis and character-ization of two sequence-isomeric 10-mer polymers. ^{1}H NMR and LCMS were used to confirm their chemical structure while tandem MS was used to confirm sequence identity. Further validation of this methodology was provided via the successful synthesis of a sequence-specific 16-mer polymer incorporating nine different monomers. This strategy thus shows promise as an efficient approach for the assembly of sequence-specific functional polymers.

Spatial control of a monomer sequence along a polymer backbone is essential to the complex self-assembly of proteins and nucleic acids. To achieve macromolecular diversity, biological systems have evolved extremely efficient processes for the rapid synthesis of sequence-defined biopolymers virtually error free. Similarly, achieving primary sequence control using synthetic monomers, for which we have a nearly unlimited toolbox, should facilitate control over structural properties such as folding, self-assembly into nanostructures, structural stimuli response, and formation of catalytic sites. These structural properties will invariably determine bulk material properties including solubility, conductivity, elasticity, nonfouling, bio-compatibility, and catalytic performance.[1] Understanding sequence–structure–material property relationships is of para-mount importance toward our ability to carry out predictive bottom-up materials design and fabrication. Progress toward this goal requires the development of reliable methods for achieving precise polymeric sequence control.

Synthetic approaches should enable large-scale production of sequence-controlled polymers with massive structural diversity. Synthetic efforts toward controlling polymer sequence, i.e. the placement of different monomeric groups in a predefined order along a polymer chain, can be grouped into two categories: (i) one-pot synthesis approach and (ii) supported synthesis approach. In the one-pot approach, different monomers are introduced and allowed to react to near full conversion, with no purification steps. Two strategies that fall under the umbrella of

this approach are templated reactions and sequential living radical polymerization. Templated reactions involve the spatial arrangement and subsequent ligation of monomers on a preorganized template[2−4] or the sequential use complementary templates to facilitate monomer ligation.[5,6] Sequential living radical polymerizations involve precise tuning of monomer reactivity (or relative reactivity) and reaction time to achieve well-defined multiblock copolymers.[7−10] The latter offers operationally simple conditions, with a trade-off of more limited sequence control.

In contrast to the one-pot approach, the supported synthesis approach involves the iterative addition and washing of monomers to a tethered solid or liquid support. This approach was first developed for the synthesis of peptides[11] and nucleic acids[12] and involves extensive monomer protection and deprotection chemistries. Recently, alternative strategies that circumvent the need for protecting groups via the use of orthogonal chemical reactions have been developed. Notable examples include the submonomer method for peptoid syn-thesis,[13,14] thiolactone aminolysis,[15] and the Passerini three-component reaction.[16] Inspired by the promise of these

**Scheme 1. Fluorous Assisted Sequence Control via Allyl Acrylamides and Dithiols**
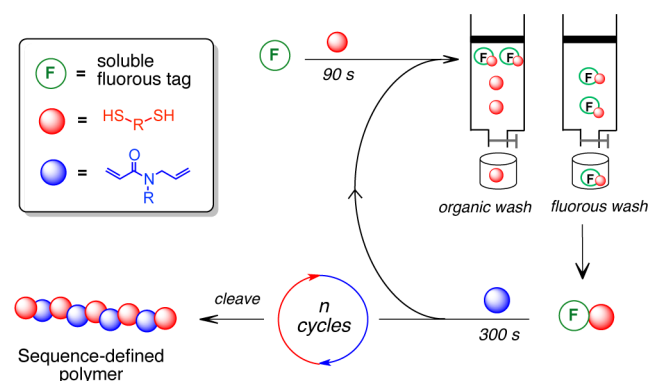


approaches and the need for increased backbone diversity, we report an efficient strategy for the assembly of sequence-defined polymers via unique allyl acrylamide building blocks with orthogonal reactive sites coupled with a powerful fluorous separation technology (Scheme 1). The latter allows us to decouple monomer reaction from purification by performing

monomer addition in solution and polymer purification on a supported solid phase.

Our approach for achieving synthetic sequence control involves the design of a unique monomer with two orthogonal reactive sites to the same nucleophile. The monomer framework includes a reactive acrylamide group that can undergo phosphine-catalyzed Michael addition with thiols, the desired functional group and a reactive allyl group that can undergo photoinitiated thiol–ene "click" addition. These reactions were chosen due to their rapid solution kinetics at room temperature and pressure.[17] Since both reactive ends of the monomer undergo orthogonal reactions with thiols, we reasoned that a dithiol molecule could be used as a comonomer without the need for protective groups. The allyl acrylamide monomer can be synthesized in two steps from a plethora of primary amines or halides (Figure 1) and tolerates many functional groups. For this
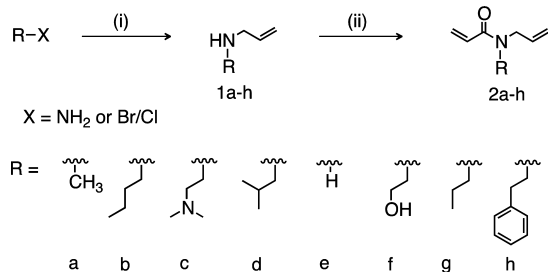


**Figure 1.** (i) For X = NH$_2$; K$_2$CO$_3$, allyl bromide (0.2 equiv). For X = Br/Cl; K$_2$CO$_3$, allyl amine (5 equiv). (ii) Acryloyl chloride (1 equiv), Et$_3$N, CH$_2$Cl$_2$.

proof-of-concept study, we synthesized eight different allyl acrylamide monomers **2a**−**h** and utilized 1,3-propanedithiol as the comonomer. In our hands, the phosphine-catalyzed Michael addition of N-allyl-N-methylacrylamide, **2a** (Figure 1), with 1,3-propanedithiol is complete in 540 s (Figure S1), and the photoinitiated thiol–ene reaction of N-allyl-N-methyl-3-(octyl-thio)acrylamide (SI, page 8) with 1,3-propanedithiol is complete in 90 s (Figure S2).

We employed fluorous tags as a liquid phase reaction support in order to combine the advantages of both solution-phase and solid-phase iterative syntheses. Fluorous tags are removable perfluorocarbon alkyl chains that are soluble in common organic solvents, yet selectively partition onto a fluorous solid phase.[18] Assembling our sequence-defined polymers on fluorous tags allows us to perform monomer addition in solution while simultaneously benefiting from rapid fluorous solid phase extraction (FSPE) for purification. The advantages of using a fluorous liquid support include homogeneous reaction conditions, fast solution phase kinetics, and reaction monitoring via common spectroscopic techniques. Fluorous tags are also inert to most common reaction conditions and are commercially available. We employed a fluorous tag with an acid cleavable Boc functionality to initiate our proof-of-concept studies.

To verify that the reaction kinetics remain rapid in the presence of bulky fluorous tags and test the efficiency of FSPE, we initiated the synthesis of a short oligomer with N-allyl-N-methylacrylamide, **2a**, and 1,3-propanedithiol (Figures 2 and S3−S5). The thiol–ene reaction of 1,3-propanedithiol (5-fold excess) with a fluorous Boc protected allyl amine was complete in 90 s and provided the monosubstituted product exclusively, as determined via ^{1}H NMR (Figure 2). Additional experiments that employed a fluorous tagged allyl amine to dithiol ratio of 1:2, 1:1,
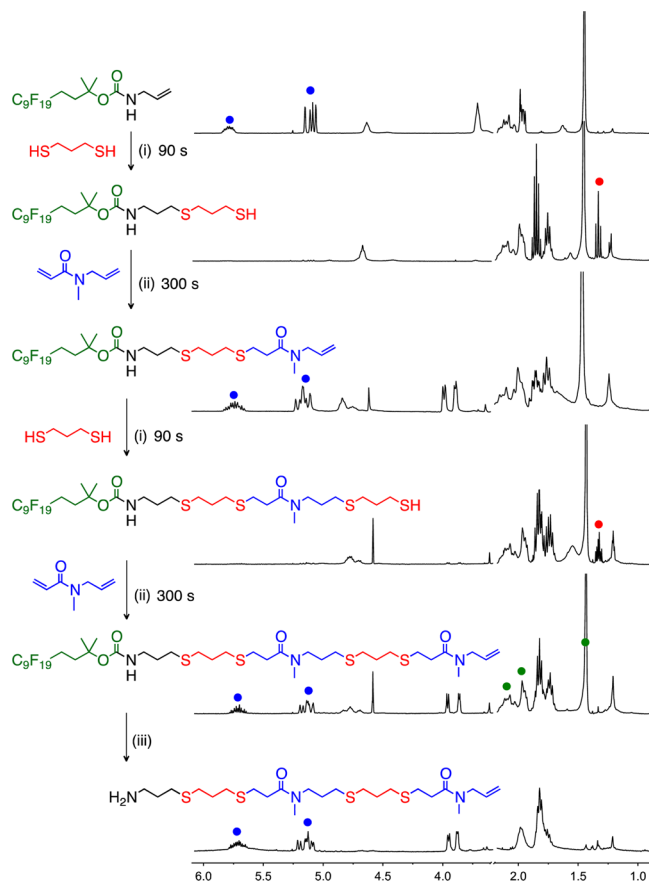


**Figure 2.** Assembly of a test oligomer. (i) fluorous polymer: 1,3-propanedithiol (1:5), 2,2-dimethoxy-2-phenyl acetophenone (DMPA), $hv$ (20 mW/cm$^2$), MeOH; (ii) fluorous polymer: **2a** (1:2), Me$_2$PhP, MeOH; (iii) 50% trifluoroacetic acid (TFA) in CH$_2$Cl$_2$. Fluorous tag is highlighted in green. Blue dots, olefin protons; green dots, CH$_2$CH$_2$C-(CH$_3$)$_2$ protons on fluorous tag; and red dots, SH proton. Full spectra (with omitted sections 2.5−3.5 ppm) are available in Figure S5.

and 1:0.5 demonstrated that, for fluorous-tagged substrates, the use of more than a 2-fold excess of dithiol is necessary to push the reaction to completion in 90 s (Figure S6A). In addition, the monosubstituted product appears to be the dominant species even at a low ratio of 1:0.5 (Figure S6B). The progress of the thiol–ene reaction was followed via the appearance of the thiol peak (red dot) at ~1.33 ppm and the disappearance of the olefin peaks (blue dots) at ~5.14 and 5.75 ppm (Figure 2). The phosphine-catalyzed Michael addition proceeded slightly faster on fluorous-tagged substrates and was complete in just 300 s (Figure S7). The Michael addition reaction progress was monitored via the disappearance of the thiol peak (red dot) and the reappearance of the olefin peaks (blue dot). The wash and elute steps, which take place between each monomer addition, were performed over a prepacked mini-fluorous silica column in ~5 min. These prepacked columns can be regenerated and reused up to 10 times. As such, the total time for monomer or comonomer addition is roughly 10−15 min. After this first round of monomer addition, we continued oligomer synthesis with another round of dithiol and N-allyl-N-methylacrylamide addition. Again, both reactions proceeded smoothly as determined by ^{1}H NMR (Figure 2) and mass spectroscopy (Figure S8). Finally, we performed an acid deprotection to cleave the desired oligomer off the fluorous support. The structure of the final product was confirmed by ^{1}H NMR via the

disappearance of the fluorous Boc groups (green) at 1.46, 1.98, and 2.11 ppm respectively (Figure 2) and by liquid chromatography/mass spectrometry (LCMS) (Figure S8).

After verification of the reaction kinetics in the presence of fluorous tags, we proceeded with the synthesis of two 10-mer sequence isomeric polymers, isomer 1 and isomer 2 (ISO1 and ISO2, Figure 3a). Both polymers were synthesized with the same
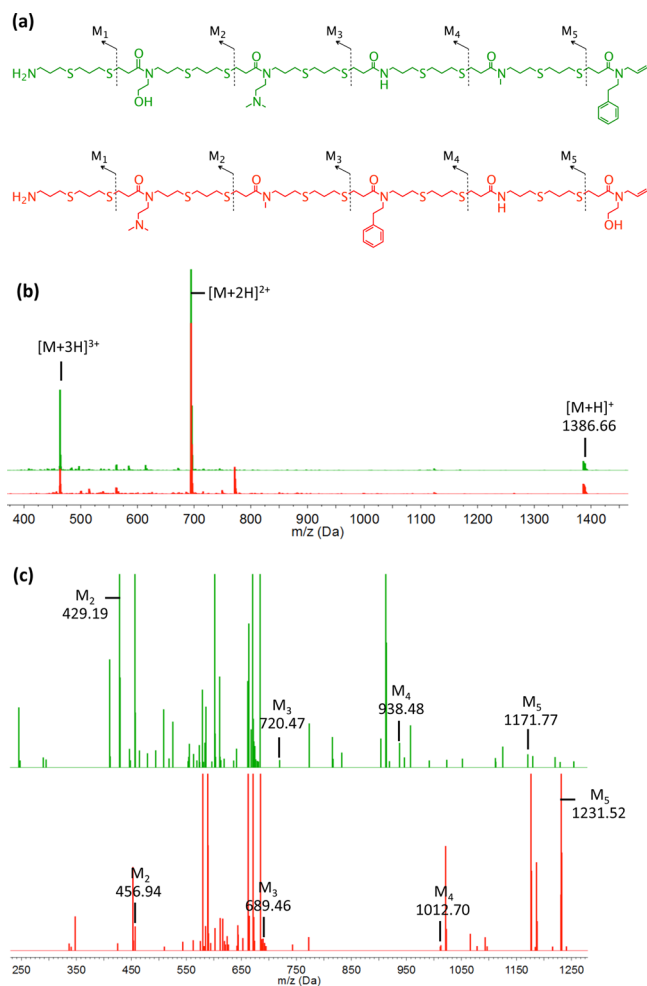


**Figure 3.** (a) Chemical structure of two polymeric sequence isomers (ISO1-green and ISO2-red). (b) MS spectra of ISO1 and ISO2. (c) Tandem MS spectra of parent ion $[M + 2H]^{2+}$ showing sequence specific fragmentation patterns. M ions are annotated.

allyl acrylamide monomers (**2a, 2c, 2e, 2f,** and **2h**) and 1,3-propanedithiol as the comonomer but have different sequences (Figure 3a). The synthesis of ISO1 was followed and confirmed at each step with $^1$H NMR and LCMS (Figures S9–S15). ISO2 on the other hand was synthesized in one setting without spectroscopic stepwise confirmation in less than a day with pre- and postcleavage yields of 72% and 68% respectively. As shown in Figure S16A and B, the $^1$H NMR of fluorous tagged and cleaved ISO1 and ISO2 are nearly identical. Minor differences in peak positions are possibly due to the differences in the relative positions of the functional groups. ISO1 and ISO2 were analyzed and purified via reversed phase chromatography and eluted at 17.8 and 17.1 min respectively (Figure S17). Further structural confirmation of both isomers was obtained via LCMS. The observed parent ions of the two sequence isomers were identical at 1386.66 Da (Figure 3b) and matched the theoretical value of

1386.62 Da. The multiply charged $[M + 2H]^{2+}$ and $[M + 3H]^{3+}$ ions for both isomers were also identical.

Sequence identity was elucidated via a tandem MS (MS/MS) experiment on one isotope of the $[M + 2H]^{2+}$ ion. Cleavage of the carbon−sulfur bond $\beta$ to the carbonyl to yield a thiol-containing $[M + 2]^+$ fragment ion was the most prominent and consistent fragmentation mechanism. All expected M ions, with the exception of $M_1$ due to its mass being below the detection limit, were detected in the MS/MS fragmentation spectra (Figure 3c; see Figures S18, S19 and Tables S1, S2 for full fragmentation analysis). Moreover, none of the M ions of ISO1 could be detected in the fragmentation profile of ISO2, and vice versa. Identification of the desired M ions as well as differences in the fragmentation pattern of the two sequence isomers confirms their chemical identity and sequence specificity.

To further test the durability and scope of this synthetic method for attaining sequence control, we synthesized a 16-mer polymer (Figure 4a) consisting of all eight allyl acrylamide
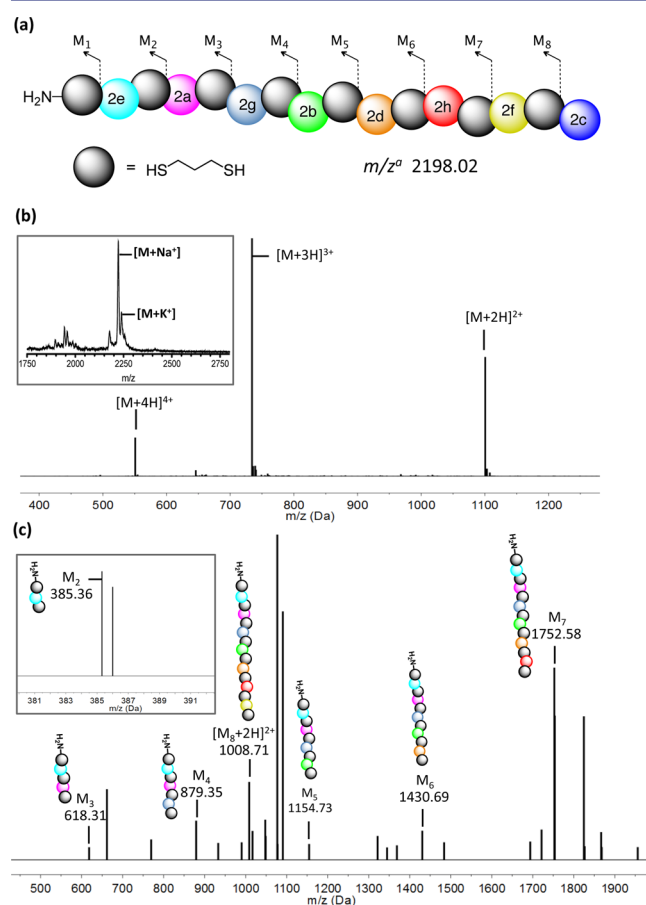


**Figure 4.** (a) Schematic representation of 16-mer polymer. (b) LCMS and MALDI (inset) of 16-mer polymer. (c) Tandem MS spectra of 16-mer polymer. Inset shows $M_2$ fragment. M ions are annotated. $^a$ m/z ratio for monoisotopic species.

monomers (Figure 1) and 1,3-propanedithiol as the comonomer. LCMS of the final cleaved product only showed the multiply charged $[M + 2H]^{2+}$, $[M + 3H]^{3+}$, and $[M + 4H]^{4+}$ ions as the parent $[M + H]^+$ ion (2198.02 Da) was beyond the instrument detection limit. The total parent mass of the polymer was confirmed by MALDI (Figure 4b inset, $[M + Na]^+$ and $[M + K]^+$ ions). The $^1$H NMR of the full product (Figure S20) is also in agreement with the proposed structure. The observed peaks in

the MS/MS fragmentation pattern of the $[M + 2H]^{2+}$ ion matched those of the assigned M ions in the polymer structure (Figure 4c; see Figure S21 and Table S3 for full fragmentation analysis) thus confirming the molecular sequence of the 16-mer product. Although the $C_9F_{19}$ fluorous tag employed in this work was capable of separating the 16-mer polymer from its byproducts, we do anticipate a decrease in recovery as the organic to fluorine ratio increases. However, the latter can be circumvented with the use of larger fluorous tags.

In conclusion, we have described the development of new functional allyl acrylamide monomers coupled with an innovative fluorous-mediated methodology for the rapid and efficient assembly of sequence-defined polymers. We have shown evidence of sequential polymer assembly via $^1$H NMR and LCMS. The sequences of our prepared polymers were confirmed via tandem MS fragmentation analysis. The ease of our monomer synthesis along with the rapid polymer assembly should facilitate exploration of a wide variety of monomer combinations and functional polymer structures that may lead to the discovery of advanced materials. Additionally, all the polymers generated by our coupling strategy have an amine and alkene as orthogonal terminal functionalities, which make them attractive for postsynthetic modification of proteins, nucleic acids, nano-particles, and other biomaterials. Precise control over the polymer sequence holds great potential for mediating a high level of control over the chemical and physical properties of materials, which can span the molecular to the macromolecular scale. As such, our future studies will focus on expanding the number and types of available building blocks as well as the synthesis of longer functional sequence-specific polymeric structures. We anticipate these polymers will find use in sequence-specific self-assembly, stimuli-responsive materials, controlled drug delivery, and much more.

## ASSOCIATED CONTENT

### Supporting Information

Experimental procedures, details on the synthesis of monomers and polymers, and additional spectroscopic data such as $^1$H NMR and LCMS are included in the Supporting Information. This material is available free of charge via the Internet at http://pubs.acs.org.

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## ACKNOWLEDGMENTS

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