Published in final edited form as:

Biomacromolecules. 2019 February 11; 20(2): 1018–1027. doi:10.1021/acs.biomac.8b01631.

Chemoselective, Post-Polymerization Modification of Bioactive, Degradable Polymers

Joshua M. Fishman[†], Daniel B. Zwick[‡], Austin G. Kruger[†], Laura L. Kiessling^{*,†,‡,§}

[†]Department of Chemistry, University of Wisconsin – Madison, Madison, WI 53706

[‡]Department of Biochemistry, University of Wisconsin – Madison, Madison, WI 53706

[§]Department of Chemistry, Massachusetts Institute of Technology, Cambridge, MA 02139

Abstract

Degradable polymers promote sustainability, mitigate environmental impact, and facilitate biological applications. Tailoring degradable polymers is challenging because installing functional group-rich side chains is difficult when the backbone itself is susceptible to degradation. A convenient means of side chain installation is through post-polymerization modification (PPM). In functionalizing polyoxazinones, a class of degradable polymers generated by the ring-opening metathesis polymerization (ROMP), we predictably found PPM challenging. Even the versatile azide–alkyne cycloaddition click reaction was ineffective. To solve this problem, we screened PPM reactions whose efficiencies could be assessed using photochemistry (excimer formation). The mildest, pH-neutral process was functionalization of a ketone-containing polymer to yield either oxime (acid labile)- or alkyoxylamine (stable)-substituted polymers. Using this approach, we equipped polymers with fluorophores, reporter groups, and bioactive epitopes. These modifications imbued the polymers with distinctive spectral properties and biological activities. Thus, polyoxazinones are now tunable through a modular method to diversify a macromolecule's function

Graphical Abstract

*Corresponding Author: kiesslin@mit.edu.

Supporting Information. Synthesis of small molecules **5-6**, **9-11**, **13**, **17**, polymers **12**, **14-16**, **18-21**, Table S1, Figures S1-S2, cell culture conditions, microscopy procedures, and NMR characterization. This material is available free of charge via the Internet at <http://pubs.acs.org>.

Keywords

Ring-opening metathesis polymerization; post-polymerization modification; degradable polymer; polyoxazinone

INTRODUCTION

Most materials possess backbones that are not readily degraded; therefore, they result in refuse accumulation.^{1–4} Polymers that can undergo degradation can mitigate environmental impact, and they have advantages for biological applications. The identification of new degradable materials is therefore valuable; yet the⁵ challenge is to ensure that degradable polymers possess properties consummate to their extant non-degradable counterparts, including control over structure and function.

A handful of polymerization reactions can yield degradable polymers with control over molecular weight and dispersity. For example, the anionic ring-opening polymerization (ROP) of cyclic monomers with hydrolyzable linkages such as esters, carbamates, and phosphoesters can introduce degradable functionality into a linear polymer backbone.⁶ Still, ROP must be conducted under stringently dry conditions, the monomers must be of ultra-high purity, and the monomers cannot contain functionality susceptible to transesterification or termination reactions during polymerization. Chemoselective “click” reactions⁷ can enable polymers generated by ROP to be modified via post-polymerization modification (PPM). Although multiple click reactions have been used in PPM to generate specialty non-degradable polymers, many of these functionalization reactions can lead to backbone degradation.^{8, 9} To mitigate side reactions, click PPM reactions on ROP backbones must be optimized to favor efficient conjugation over degradation.

An alternative approach is to employ a living radical copolymerization (LRP) of functionalized monomers. For example, reversible addition-fragmentation chain transfer (RAFT) polymerization can be used to intersperse ester linkages within a poly(vinyl acetate) backbone.¹⁰ While RAFT polymerization is more air and moisture tolerant than anionic ROP and can proceed in aqueous media,^{11–13} the cyclic ketene acetal co-monomers need to introduce degradable linkages require specialty handling.⁶ Additionally, these cyclic monomers suffer from low incorporation into the polymer chain^{14–16}, with the exception of specific monomer pairs.^{17, 18} We therefore examined alternative methods to generate degradable, functionalized polymers.

Like ROP and RAFT, the ring-opening metathesis polymerization (ROMP) can be a living polymerization that affords defined polymers that can be tailored for multiple desired applications.^{19, 20} Metathesis has multiple benefits^{21, 22}: catalysts can polymerize monomers that possess polar functional groups, the reaction can occur at room temperature, and the polymerization kinetics are fast. These features obviate the need for hot plates or Schlenk lines, which has democratized the use of ROMP and yielded scaffolds that can be tailored for materials,^{23–26} electronic,^{27, 28} and biological applications.^{29–36} Indeed, ROMP can accommodate functionality incompatible with other polymerization methods. Additionally, the molecular weight, dispersity, and backbone architecture of ROMP products

are all controllable through judicious choice of polymerization conditions.^{37, 38} Pragmatically, ruthenium catalysts are air and moisture compatible; however, with few exceptions,^{39–44} they afford polymers consisting of backbones that are not degradable. We sought to use ROMP to generate degradable materials with diverse functionality.

To this end, we reported that bicyclic oxazinones undergo ROMP to afford polyoxazinones,³⁹ which are stable yet degradable under acidic or basic conditions. We diversified the polyoxazinone backbone by generating a monomer library with various hydroxamic ester substituents (Figure 1A). The monomers were synthesized in a two-step sequence starting from a range of *O*-functionalized hydroxylamine precursors.⁴⁵ This approach requires that each unique monomer be accessed separately.

The efficiency of producing tailored polyoxazinones via the *grafting-through* approach previously described could be improved using PPM. A *grafting-from* PPM strategy is advantageous for generating bioactive polymer probes because fluorophores and biological ligands are often costly to produce and only available in milligram quantities. Furthermore, PPM enables the rapid synthesis of polymers decorated with multiple functional moieties with control and standardization of polymer chain length between samples. Such features are important when testing differences in bioactivity due to ligand type or density.^{46–58}

We envisioned an effective PPM strategy would streamline the synthesis of bioactive polyoxazinones (Figure 1B). Since we previously demonstrated that polyoxazinones degrade when the pH is below 4.5 or above 10,³⁹ what was needed was an efficient chemoselective reaction occurring at neutral pH values. Several transformations failed, but oxime formation was effective. Reaction of ketone-bearing polymers with alkoxyamines afforded high degrees of conjugation without decomposition. The efficiency of conjugation was assessed by quantifying excimer fluorescence—a rapid measure of the extent of modification. The synthetic strategy was used to generate cell permeable polymers and synthetic antigens, highlighting the utility of functionalized polyoxazinones.

MATERIALS AND METHODS

All commercially available reagents were purchased from Sigma-Aldrich (St. Louis, MO). The fluorescent dye, Alexa Fluor 488 C5-aminooxyacetamide bis(triethylammonium) salt, was purchased from Invitrogen (Grand Island, NY) as a hydroxylamine derivative. Monomers **1** and **2**, polymers **3** and **4**, compound **9a**, and the metathesis catalyst were synthesized as described previously.^{22, 39, 59} Tetrahydrofuran (THF) was distilled over sodium/benzophenone. Dichloromethane and triethylamine were distilled over calcium hydride. Dimethylsulfoxide (DMSO), *N,N*-dimethyl formamide (DMF), diethyl ether, hexanes, ethyl acetate (EtOAc), and methanol were used as received. All reactions were run under an inert atmosphere of N₂ unless otherwise specified. Reactions were stirred using Teflon coated magnetic stir bars. All glassware and stir bars were stored in oven before use.

Synthesis of Polymer 12. To a stirring solution of **11** (60 mg, 0.20 mmol) in tetrahydrofuran (0.1 mL) under a blanket of argon was added a 1 M solution of chlorodicyclohexyl borane in hexanes (20 μ L) and then ruthenium carbene catalyst in chloroform (0.1 mL) (Note: polymer

chain length is dictated by the ratio of monomer to catalyst). The solution was stirred for 30 min at rt. The reaction was quenched with ethyl vinyl ether (0.1 mL) and 4 drops of methanol and the resulting mixture was stirred overnight (in cases where needed 0.1-0.2 mL chloroform was also added to resolubilize any precipitated polymers). The solution was triturated into diethyl ether (30 mL) and the solids were collected by centrifugation. The solids were then dissolved in minimal chloroform and re-triturated until all of the unreacted monomer was removed as determined by NMR (usually achieved within 3 rounds). Polymer **12** was isolated as a white solid. ¹H-NMR (CDCl₃, 500 MHz): δ 8.03-7.70 (m, 2H), 7.60-7.30 (m, 2H + end group Ph Hs), 6.30-5.60 (m, 2H), 5.55-5.12 (m, 1H), 5.12-4.60 (m, 2H), 4.40-3.85 (m, 1H), 2.67-2.40 (m, 3H), 1.35-0.92 (m, 6H). Note: Monomers were purified before each polymerization.

General procedure for the synthesis of oxime-conjugated polymer

Polymer **12** (n eq. wrt acetophenone) was taken up in a solution of hydroxylamine ligand (1.5n eq) in DMSO so the final concentration of acetophenone was 40 mM. Pyridine (8n eq) was added and the solution was stirred at rt for 24 h. Benzaldehyde (1.5n eq) was added and the solution was stirred for 1 h (benzaldehyde caps any unreacted hydroxylamine and aids in polymer purification in next step). The solution was triturated into 1.25 mL diethyl ether and the solids were collected by centrifugation. Trituration was repeated until all small molecules were removed as determined by NMR.

Cell Culture

A20 and A20/2J cells that had been stably transfected with DNP/TNP-specific IgM to generate the A20/2J HLTNP cell line were provided by A. Ochi (University Health Network, Toronto, ON, Canada).^{6,7} A20/2J HLTNP cells were cultured in RPMI medium 1640 supplemented with 2 mM L-glutamine, 10% FBS, 50 μM 2-mercaptoethanol, 100 U mL⁻¹ penicillin and 100 U mL⁻¹ streptomycin.

Procedures for the Internalization of Polymer Probes

18-21 by A20 and A20 HL Cells. A20 and A20HL cells were resuspended at 1.5×10^6 cells/ml in 1% BSA/RPMI and incubated for 30 min in the presence of 8.8 μg/ml Dylight 549 conjugated transferrin (Jackson Immunoresearch) at 37 °C. Cells were pelleted and resuspended on ice using the previous buffer, prechilled. Cell surface BCR was labeled using 15 μg/ml Alexa Fluor 647-conjugated goat anti-mouse IgM mu chain-specific Fab fragment for 20 min on ice. Cells were then pelleted and resuspended in PBS, pH 7.4 supplemented with 1 mM CaCl₂ and 0.5 mM MgCl₂ at 37 °C. Cells were then treated with synthetic antigens at 5 μg/mL at 37 °C and samples were placed on ice after 30 min. Cells were visualized in no. 1.5 borosilicate eight-well chambered coverglass (Nunc). Images were collected on a Nikon A1R confocal microscope using a 60× objective. Intensity and co-localization analysis was performed using ImageJ software (National Institutes of Health, NIH). The Pearson's Coefficient was calculated for individual cells using the colocalization threshold plugin in ImageJ. Images were despeckled to remove noise.

RESULTS

Click azide-alkyne cycloaddition (CuAAC) was problematic for polyoxazinone PPM

The CuAAC has been useful in generating tailored materials.⁶⁰ With its high chemoselectivity and occurrence at neutral pH, CuAAC seemed a reasonable choice for polyoxazinone functionalization. The precursor was not generated from an azide-substituted monomer, as azide groups can react with ruthenium catalysts, but we could access azide-substituted polymers through halide-substituted polymers.⁶¹ We previously reported the synthesis of bicyclic oxazinone monomer **1** bearing an alkyl halide (Scheme 1).³⁹ Monomer **1** was subjected to ROMP and the resulting polymer was exposed to sodium azide to yield polyoxazinone **4**, a polymer poised for PPM via CuAAC. We anticipated this reaction would be successful, as similar processes had been used to modify non-degradable ROMP polymers.^{48, 50, 52, 53, 62} However, polymer **4** was not transformed to the desired polymer but rather to an insoluble gel (Scheme 1). Gel formation was unexpected as the polymer product generated by a different protocol was soluble. Specifically, we had previously converted monomer **1** to the corresponding azide and appended a peracetylated propargyl mannoside using the click reaction to afford monomer **2**. Monomer **2** was successfully polymerized to afford well behaved, protected glycopolymer **3**. Thus, the polymer expected from CuAAC on **4** should be soluble, but it was not. The insolubility of **4** appeared to arise from its ability to chelate to intermediate organometallic complexes formed during copper-mediated azide-alkyne cycloaddition. Specifically, when polymer **4** was stirred under conditions amenable to CuAAC, but without the alkyne present, no gelation or side reactions were observed. Thus, while CuAAC can be used for monomer functionalization, it was ineffective for PPM (Scheme 1).

Copper-free azide-alkyne cycloaddition occurs with the use of strained alkynes.^{63, 64} Indeed, exposure of polymer **4** to commercially available azadibenzocyclooctyne-acid (ADBCO-A) led to a non-crosslinked polymer with >90% of the azide moieties converted to the expected triazole product (see Supporting Information). However, the use of ADBCO-A affords a polymer product with large, hydrophobic groups; even with free acids present the product was insoluble in aqueous solutions. Additionally, the polymer product from the strain-promoted click reaction increased in dispersity from 1.4 to 2.1, which is undesirable when precisely defined multivalent biological probes are needed. Therefore, we searched for a useful alternative chemoselective handle.

Polynorbornene substrates bearing N-hydroxy succinimidyl esters can be elaborated to generate compositionally complex macromolecules (Scheme 2A).^{32, 46, 65} Polymers bearing succinimidyl esters can undergo facile functionalization by PPM, as amine-containing ligands can be appended via amide bond formation. To capitalize on this strategy, we targeted the synthesis of bicyclic oxazinone monomer containing a succinimidyl ester (Scheme 2B). Starting with O-(carboxymethyl) hydroxylamine, the carboxylic acid was esterified with an allyl group and then acylated with bromoisobutryl bromide (BIBB) to afford hydroxamic ester **5**. Bicyclic oxazinone **6** was subsequently generated using an aza-[4+3] cycloaddition with furan followed by allyl ester removal. The latter process could be conducted in the presence of the bicyclic olefin using tetrakis(triphenyl-phosphine)

palladium and morpholine to yield carboxylic acid **6**. Attempted esterification with N-hydroxyl succinimide afforded the desired monomer **7** in low yield. We surmise that the modest yield of **7** results from the production of ketene **8**. This ketene intermediate could participate in side reactions, such as a cycloaddition with the bicyclic olefin, to erode yields. This mode of ketene formation has been observed in other systems in which a carboxylic acid with an electron withdrawing heteroatom at the alpha carbon was activated for esterification.⁶⁶ We therefore pursued an alternative approach.

Synthesis of an alkoxyamine-reactive polyoxazinone

Macromolecules have been modified previously using chemoselective oxime formation. In one manifestation, proteins engineered to contain the unnatural amino acid *p*-acetyl phenylalanine react selectively with hydroxyalmines.^{67–69} Inspired by these studies, we focused on synthesizing a polyoxazinone displaying an acetophenone derivative. Compound **9** is readily accessible in gram quantities (Scheme 3);⁵⁹ therefore, we were attracted to it as a building block to assemble acetophenone-containing bicyclic oxazinones and polyoxazinones.

The oxime PPM strategy was readily implemented. The required bicyclic oxazinone monomer was assembled via a 1,3 dipolar cycloaddition between compound **10** and furan. Compound **10** was accessed from acetophenone. After installation of the protected alkoxyamine, the ketone was converted to a 1,3-dioxane **9**. Carbonyl protection was used to avoid self-condensation upon liberation of the free hydroxylamine. The resulting compound was acylated to generate the α -bromo hydroxamic ester, and the dioxane group was removed using mild conditions (catalytic iodine) to afford ketone **10**. Mild conditions were critical as α -bromohydroxamic esters rapidly decompose to acrylamide *N*-oxides in acidic or basic solutions. Finally, oxazinone monomer **11**, displaying an acetophenone moiety, was successfully generated from an *aza*-[4+3] cycloaddition with furan.

Our initial attempts to polymerize monomer **11** using a solvent system of tetrahydrofuran (THF) and chloroform afforded polyoxazinone **12**, but conversion was unexpectedly low (Table 1, entry 6). Substrates that contain hydroxamic esters can be problematic for such metathesis reactions because the precursors and their polymer products can chelate the metallocarbene catalyst to stymy turnover.^{39, 70} One potential solution is to add a Lewis acid that binds to polar groups and thereby frees the carbene catalyst to mediate metathesis.⁷⁰ To this end, we added chloro-dicyclohexylborane (10 mol% BCy₂Cl) to the reaction mixture. The result was increased polymer conversion and good molecular weight control over a range of monomer to catalyst loading ratios (Table 1).

Post-polymerization modification of a ketone-substituted polyoxazinone

We next investigated methods to elaborate acetophenone-functionalized polymer **12**. Non-degradable polynorbornene-derived polymers containing ketone functionality have been decorated through formation of acyl hydrazones,^{48, 71} but these linkages are acid labile, which would result in the release of the bioactive group from the polymer backbone. For bioactive polymers to function as probes, they must be stable. We turned to oxime linkages because they are more hydrolytically stable than acyl hydrazones at physiological pH.⁷² To

generate these polymers, we envisioned exposing alkoxyamines to ketone-containing polymers.

The conjugation reactions of alkoxyamine ligands to ketone-bearing biomacromolecules, such as proteins, are typically conducted in acetate buffer.^{69, 73} The low aqueous solubility of polyoxazinone **12** and the acid lability of the oxazinone linkages of the polymer backbone render these conditions incompatible. We therefore used an oligoethylene glycol ligand **14** terminated with a hydroxylamine to screen alternative conditions (Figure 2). With a dimethyl sulfoxide and pyridine co-solvent, we used an excess of alkoxyamine **13** (1.5-2.0 eq) to produce oxime conjugate **14**.⁷⁴ Proton NMR signals indicative of aryl ketones were present in the starting material ($H^a = \delta$ 7.8 ppm; $H^b = \delta$ 2.6 ppm), and within 24 hours of functionalization, signals consistent with oxime **14** ($H^a = \delta$ 7.6 ppm; $H^b = \delta$ 2.2 ppm) were detected (Figure 2). Analysis of the resulting sample with gel permeation chromatography (GPC) revealed a shift in retention time to a shorter elution volume (Figure S1) consistent with the expected increase in polymer mass upon modification. These data indicate that hydroxylamine ligands can be attached with negligible backbone decomposition and with remarkably high conjugation efficiency.

We also verified the post-polymerization modification of **12** by exploiting the differences in optical properties of proximal chromophores. These spectroscopic changes can be observed using a fluorimeter, instrumentation that is present in most biological laboratories. Specifically, we exposed the ketone-bearing polymer to *O*-(4-pyrenylbutyl) hydroxylamine to afford pyrene-substituted polymer **16** (Figure 3). When two pyrene derivatives are proximal, they give rise to spectra indicative of excimer formation.⁷⁵ Excimer formation manifests as a broad fluorescent emission band centered at 480 nm, and the ratio of excimer to monomer emission can be quantified [Ex(480)/Ex(378)]. When polymer **16** was exposed to 50 μ M pyrene, an emission band at 480 was observed leading to an exciplex ratio of 0.79, a value indicative of excimer formation (Figure 3). Pyrene concentrations up to 500 μ M gave similar Ex(480)/Ex(378) ratios. In contrast, in this concentration range, neither free hydroxylamine-pyrene ligand nor model “1-mer” **17** afforded an exciplex band. Thus, excimer formation from polymer **16** results from a high local concentration of the pendant residues on the polymer backbone. The consistent excimer ratios for **16** are indicative of efficient oxime formation. Therefore, exciplex formation can be used to qualitatively confirm PPM in a simple and rapid test.

Tailoring polyoxazinone activity using PPM

An advantage of PPM is that it enables the attachment of multiple, pendant groups in a single step. This feature enables polymer SAR: differentially modified polymers can be readily compared as their backbones are identical. A wide range of different moieties, including ligands and fluorophores or other reporter groups, can be appended through PPM.⁹ Bioactive ROMP polymers have been used as probes to optimize and understand the mechanisms by which multivalent ligands affect cell surface receptor signaling and function.^{30, 76} Polyoxazinones, however, have not been assessed for biological activity. We therefore synthesized polymer **18**, which bears the immunogenic epitope 2,4-dinitrophenol (DNP) and

the water-soluble fluorophore AlexaFluor488 (AF488) (Figure 4), and tested this agent as an antigen.

A DNP-responsive B cell line was used to assess the ability of polymer **18** to activate immune signaling. Antigen activation and internalization by B lymphocytes are processes critical for immunity, as they can elicit antibody production. B cell responses are activated upon antigen engagement of a membrane-bound immunoglobulin receptor termed the B cell receptor (BCR). A B cell line (A20HL)^{77–79} with a DNP-binding BCR can be activated by multivalent displays of DNP epitopes to mediate antigen endocytosis.^{31, 32, 52, 65}

Specifically, upon antigen engagement with the BCR, both the ligand and receptor are internalized and rapidly directed to early endosomal compartments.^{80, 81} We therefore tested whether the polar DNP-functionalized polyoxazinones could behave as antigens.

B cells were treated with polymer **18** at physiological temperature (37 °C) and monitored using fluorescence microscopy. The presence of intracellular fluorescent puncta indicates the polymer was internalized (Figure 5A). Polymer fluorescence was coincident with that due to the BCR, an observation consistent with BCR-mediated internalization (Figure 5B). The internalized polymer and BCR also co-localized with the early endosomal marker transferrin, indicating that the polymeric antigen undergoes trafficking to relevant sites within the immune cells (Figure 5C). Finally, internalization was ablated at 4 °C, a finding consistent with uptake via BCR-mediated endocytosis (Figure 5A). Taken together, the uptake and trafficking of polymer **18** is consistent with it activating physiological processes involved in B cell-mediated immunity.

We carried out additional experiments to assess whether the oxazinone backbone could engage in off-target biological interactions. When DNP-specific B cells were exposed to fluorophore-labeled polymer **19**, which bears non-binding oligoethylene glycol groups no internalization was observed (Figure S2a). Similarly, mannose-substituted polymer **20** was not taken up at either temperature. (Figure S2b) These results indicate the groups displayed on the polyoxazinone backbone, not the backbone itself, are essential for recognition and therefore polymer endocytosis.

We also have been interested in synthesizing polymers that penetrate cell membranes. One solution to this problem is to conjugate a binding epitope to the polymer that can trigger receptor-mediated endocytosis, as described above for the BCR. This strategy can enable cell type-specific uptake; however, compounds that exploit generic uptake mechanisms are also being sought. One such strategy is to incorporate a transporter domain or a protein transduction domain mimic.^{82–85} Short arginine-rich amino acid sequences that can complex with negatively charged sulfated glycosaminoglycans (GAGs) found on the cell surface can promote uptake of compounds to which they are attached.^{86–88} While the mechanisms underlying internalization are still being elucidated, the process is typically energy-dependent and relies on deformations in the curvature of the membrane.⁸⁹ We previously showed ROMP of norbornenes functionalized with an oligoguanidinium artificial transduction domain (ATD) are internalized by cells.^{61, 89, 90} Because uptake and trafficking can be sensitive to molecular features, we tested whether the polyoxazinone backbone can be taken up by cells. Ketone-containing polymer **12** was decorated with guanidinium groups

to afford polymer **21** (Figure 4). Polymer **12** is relatively short because longer oligoguanidinium species are not readily internalized.^{61, 84}

Polyoxazinone **20** was generated with an ATD using a guanidinium-functionalized hydroxylamine (Figure 4). The fluorophore AF488 also was conjugated to visualize internalization. The cell-permeability of the polymer was tested in both A20HL B cells, which express a DNP-specific BCR, and in A20 cells, which do not. Internalization was observed in both cell lines. While uptake occurred readily after 30 min at 37 °C, none was observed at 4 °C (Figure 6). The temperature-dependence of polymer internalization is consistent with an active mode of cell penetration.⁹⁰ These data indicate that polyoxazinones can be functionalized to promote either receptor-mediated endocytosis or ATD-facilitated uptake highlighting the utility of this backbone and the advantages of PPM.

DISCUSSION

ROMP combines chain length control, fast polymerization kinetics, and tolerance to air and moisture during polymerization. This polymerization method also provides access to synthetic scaffolds that can be elaborated to afford biologically active polymer probes. The use of ROMP to generate polyoxazinones demonstrated that ROMP can afford degradable polymers with tailored functionality from a single monomer. Still, our previously reported *grafting-through* synthesis of polyoxazinones required quantities of highly functionalized monomers that can be difficult to obtain and that limit comparisons between polymers bearing different epitopes.

PPM facilitates the synthesis of functionalized backbones from ROMP. Non-degradable ROMP scaffolds have been elaborated using click handles, such as activated esters,^{31, 32, 46, 50, 65} azides,^{48, 52, 53, 62} α -chloroacetamides,⁶¹ maleimides,⁵⁰ and ketones.^{48, 71} We found that we could decorate polyoxazinones from the polymerization of ketone-containing monomer **11**, which is readily accessible through a dipolar cycloaddition. The resulting clickable polyoxazinone scaffold represents a new reactive and degradable scaffold.

Despite the existing collection of chemoselective reactions, the problems we encountered equipping the polyoxazinone backbone with an orthogonally reactive group highlight the intrinsic challenges in modifying degradable backbones for biological studies. We avoided bio-orthogonal reactions that yield a hydrophobic linker, as their application could dramatically and unpredictably influence the properties of the polymers.⁹¹ The CuAAC click reaction has the advantage that the resulting linker is small. Though the CuAAC has been used on some degradable scaffolds, the reaction can be problematic for substrates with a high density of heteroatoms. In our backbone, these heteroatoms are intrinsic to the polymer's ability to undergo degradation, but they apparently suppress the desired click reaction. Attempts to install a succinimidyl ester resulted in monomer decomposition, presumably through ketene formation. Ultimately, we utilized a hydrazone linkage. The neutral conditions used for its introduction promoted desired transformation over backbone degradation and facilitated the transformation of polymers into biologically active multivalent probes.

We verified the successful PPM of polyoxazinones through both NMR and fluorescence spectroscopy. The latter could detect the conjugation of a pyrene fluorophore, as pyrene exciplex formation was induced.⁹² We anticipate that this approach can be used generally to monitor the efficiency of PPM modification.⁹²

An obstacle to polymer-based therapeutics and polymer probes is accessing the cell's interior.^{93, 94} We used oxime formation to generate polymers with biologically relevant functionality and reporter groups. This synthetic strategy afforded compounds that undergo cellular uptake either through either receptor-mediated endocytosis or using an artificial transduction domain. To this end, polyoxazinones that can engage the BCR undergo endocytosis by B lymphocytes and thereby function as synthetic antigens. Furthermore, the polymer was routed to endosomal compartments that can lead to antigen processing. Alternatively, the conjugation of guanidinium groups to the polyoxazinone backbone enabled uptake into cells, presumably through a process similar to that employed by protein-transduction domains. Thus, the heteroatom-rich polyoxazinone scaffold is compatible with cellular uptake. The ability to functionalize polyoxazinones opens up avenues to create a new class of polymer probes that can traverse membranes.

CONCLUSIONS

The covalent functionalization of macromolecules using PPM is a powerful means of synthesizing functional materials. The identification of new degradable polymer scaffolds amenable to PPM facilitates the production of polymer probes. Our studies show that polyoxazinones can be used as biological probes; their activities are similar to existing polynorbornene or polycyclooctene ROMP scaffolds, but they can be degraded. Our conditions for polyoxazinone elaboration do not require large quantities of the modifying agent. Additionally, the acid-labile oxime bond that results could be converted to a non-hydrolyzable alkoxylamine through reduction. Because PPM is exceptionally valuable when tailoring a scaffold for biological applications, we generated polymers decorated with biologically relevant moieties, including fluorophores, reporter groups, and bioactive epitopes. These modifications imbued the polymers with distinctive spectral properties and biological activities. In this way, the properties of polyoxazinones are now tunable through covalent PPM, an economical and modular method to diversify a macromolecule's function. We anticipate these findings will facilitate the generation of degradable polymers for diverse applications in materials science and biology.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGMENT

This research was supported by the NIH (R01 AI055258) and the NSF (CHE-1151002). The UW-Madison Chemistry NMR facility is supported by the NSF (CHE-9208463 and CHE-9629688) and NIH (1s10 RRO 8389-01). The UW-Madison Soft Materials Laboratory is supported by the NSF UW-Nanoscale Science and Engineering Center (NSEC, DMR-0425880) and UW Materials Research Science and Engineering Center (MRSEC, DMR-0520527).

REFERENCES

1. Thompson RC; Moore CJ; vom Saal FS; Swan SH, Plastics, the environment and human health: current consensus and future trends. *Philos. Trans. R. Soc., B* 2009, 364 (1526), 2153–2166.
2. Barnes DKA; Galgani F; Thompson RC; Barlaz M, Accumulation and fragmentation of plastic debris in global environments. *Philos. Trans. R. Soc., B* 2009, 364 (1526), 1985–1998.
3. Eriksen M; Labretton LCM; Carson HS; Thiel M; Moore CJ; Borerro JC; Galgani F; Ryan PG; Reisser J, Plastic pollution in the world's oceans: more than 5 trillion plastic pieces weighing over 250,000 tons afloat at sea. *PLoS One* 2014, 9 (12), e111913/1–e111913/15, 15 pp. [PubMed: 25494041]
4. Cozar A; Echevarria F; Gonzalez-Gordillo JJ; Irigoien X; Ubeda B; Hernandez-Leon S; Palma AT; Navarro S; Garcia-de-Lomas J; Ruiz A; Fernandez-de-Puelles ML; Duarte CM, Plastic debris in the open ocean. *Proc. Natl. Acad. Sci. U. S. A.* 2014, 111 (28), 10239–10244. [PubMed: 24982135]
5. Song JH; Murphy RJ; Narayan R; Davies GBH, Biodegradable and compostable alternatives to conventional plastics. *Philos. Trans. R. Soc., B* 2009, 364 (1526), 2127–2139.
6. Nuyken O; Pask SD, Ring-opening polymerization - an introductory review. *Polymers (Basel, Switz.)* 2013, 5 (2), 361–403, 43 pp.
7. Kolb HC; Finn MG; Sharpless KB, Click chemistry: diverse chemical function from a few good reactions. *Angew. Chem., Int. Ed.* 2001, 40 (11), 2004–2021.
8. Iha RK; Wooley KL; Nystrom AM; Burke DJ; Kade MJ; Hawker CJ, Applications of Orthogonal “Click” Chemistries in the Synthesis of Functional Soft Materials. *Chem. Rev. (Washington, DC, U. S.)* 2009, 109 (11), 5620–5686.
9. Patterson DM; Nazarova LA; Prescher JA, Finding the Right (Bioorthogonal) Chemistry. *ACS Chemical Biology* 2014, 9 (3), 592–605. [PubMed: 24437719]
10. Hedin GG; Bell CA; O'Reilly RK; Dove AP, Functional Degradable Polymers by Radical Ring-Opening Copolymerization of MDO and Vinyl Bromobutanoate: Synthesis, Degradability and Post-Polymerization Modification. *Biomacromolecules* 2015, 16 (7), 2049–2058. [PubMed: 25997518]
11. McCormick CL; Lowe AB, Aqueous RAFT Polymerization: Recent Developments in Synthesis of Functional Water-Soluble (Co)polymers with Controlled Structures. *Accounts of Chemical Research* 2004, 37 (5), 312–325. [PubMed: 15147172]
12. Kope M; Krysz P; Yuan R; Matyjaszewski K, Aqueous RAFT Polymerization of Acrylonitrile. *Macromolecules* 2016, 49 (16), 5877–5883.
13. Hoff EA; Abel BA; Tretbar CA; McCormick CL; Patton DL, Aqueous RAFT at pH zero: enabling controlled polymerization of unprotected acyl hydrazide methacrylamides. *Polymer Chemistry* 2017, 8 (34), 4978–4982.
14. Wickel H; Agarwal S, Synthesis and Characterization of Copolymers of 5,6-Benzo-2-methylene-1,3-dioxepane and Styrene. *Macromolecules* 2003, 36 (16), 6152–6159.
15. Kobben S; Ethirajan A; Junkers T, Synthesis of degradable poly(methyl methacrylate) star polymers via RAFT copolymerization with cyclic ketene acetals. 2014, 52 (11), 1633–1641.
16. Tardy A; Honoré J-C; Siri D; Nicolas J; Gimes D; Lefay C; Guilleaume Y, A comprehensive kinetic study of the conventional free-radical polymerization of seven-membered cyclic ketene acetals. *Polymer Chemistry* 2017, 8 (34), 5139–5147.
17. Hill MR; Guégain E; Tran J; Figg CA; Turner AC; Nicolas J; Sumerlin BS, Radical Ring-Opening Copolymerization of Cyclic Ketene Acetals and Maleimides Affords Homogeneous Incorporation of Degradable Units. *ACS Macro Letters* 2017, 6 (10), 1071–1077.
18. Hill MR; Kubo T; Goodrich SL; Figg CA; Sumerlin BS, Alternating Radical Ring-Opening Polymerization of Cyclic Ketene Acetals: Access to Tunable and Functional Polyester Copolymers. *Macromolecules* 2018, 51 (14), 5079–5084.
19. Ivin KJ; Mol IC; Editors, Olefin Metathesis and Metathesis Polymerization, Second Edition Academic: 1996; p 496 pp.
20. Handbook of Metathesis. by Grubbs RH. Wiley VCH: Weinheim. 2003 1156 pp (3 volumes). \$260 ISBN 3-527-30616-1. *Org. Process Res. Dev.* 2004, 8 (Copyright (C) 2012 American Chemical Society (ACS). All Rights Reserved.), 294.

21. Nguyen ST; Johnson LK; Grubbs RH; Ziller JW, Ring-opening metathesis polymerization (ROMP) of norbornene by a Group VIII carbene complex in protic media. *J. Am. Chem. Soc.* 1992, 114 (Copyright (C) 2012 American Chemical Society (ACS). All Rights Reserved.), 3974-5.
22. Love JA; Morgan JP; Trnka TM; Grubbs RH, A practical and highly active ruthenium-based catalyst that effects the cross metathesis of acrylonitrile. *Angew Chem Int Ed Engl* 2002, 41 (Copyright (C) 2012 U.S. National Library of Medicine.), 4035-7.
23. Robertson NJ; Kostalik HAIV; Clark TJ; Mutolo PF; Abruna HD; Coates GW, Tunable High Performance Cross-Linked Alkaline Anion Exchange Membranes for Fuel Cell Applications. *J. Am. Chem. Soc.* 2010, 132 (Copyright (C) 2012 American Chemical Society (ACS). All Rights Reserved.), 3400-3404.
24. Steinbach T; Alexandrino EM; Wahlen C; Landfester K; Wurm FR, Poly(phosphonate)s via Olefin Metathesis: Adjusting Hydrophobicity and Morphology. *Macromolecules* (Washington, DC, U. S.) 2014, 47 (15), 4884–4893.
25. Sutthasupa S; Shiotsuki M; Sanda F, Recent advances in ring-opening metathesis polymerization, and application to synthesis of functional materials. *Polym. J. (Tokyo, Jpn.)* 2010, 42 (12), 905–915.
26. Kratz K; Xie W; Lee A; Freeman BD; Emrick T, Phosphorylcholine-Substituted ROMP Polyolefin Coatings Provide Fouling Resistance to Membrane Materials. *Macromol. Mater. Eng.* 2011, 296 (12), 1142–1148.
27. Feast WJ, Application of ROMP in the synthesis of functional polymers: Electro-active polymeric materials. *NATO Sci. Ser, II* 2002, 56 (Ring Opening Metathesis Polymerisation and Related Chemistry), 177-183.
28. Gibbs JM; Park S-J; Anderson DR; Watson KJ; Mirkin CA; Nguyen ST, Polymer-DNA Hybrids as Electrochemical Probes for the Detection of DNA. *J. Am. Chem. Soc.* 2005, 127 (4), 1170–1178. [PubMed: 15669856]
29. Mortell KH; Weatherman RV; Kiessling LL, Recognition Specificity of Neoglycopolymers Prepared by Ring-Opening Metathesis Polymerization. *J. Am. Chem. Soc.* 1996, 118 (Copyright (C) 2012 American Chemical Society (ACS). All Rights Reserved.), 2297-8.
30. Kiessling LL; Gestwicki JE; Strong LE, Synthetic multivalent ligands in the exploration of cell-surface interactions. *Curr. Opin. Chem. Biol.* 2000, 4 (Copyright (C) 2012 American Chemical Society (ACS). All Rights Reserved.), 696-703.
31. Puffer EB; Pontrello JK; Hollenbeck JJ; Kink JA; Kiessling LL, Activating B cell signaling with defined multivalent ligands. *ACS Chem. Biol.* 2007, 2 (Copyright (C) 2012 American Chemical Society (ACS). All Rights Reserved.), 252-262.
32. Courtney AH; Puffer EB; Pontrello JK; Yang Z-Q; Kiessling LL, Sialylated multivalent antigens engage CD22 in trans and inhibit B cell activation. *Proc. Natl. Acad. Sci. U. S. A.* 2009, 106 (Copyright (C) 2012 American Chemical Society (ACS). All Rights Reserved.), 2500-2505.
33. Baessler KA; Lee Y; Sampson NS, β 1 Integrin Is an Adhesion Protein for Sperm Binding to Eggs. *ACS Chem. Biol.* 2009, 4 (Copyright (C) 2012 American Chemical Society (ACS). All Rights Reserved.), 357-366.
34. Smith D; Pentzer EB; Nguyen ST, Bioactive and Therapeutic ROMP Polymers. *Polym. Rev.* (Philadelphia, PA, U. S.) 2007, 47 (Copyright (C) 2012 American Chemical Society (ACS). All Rights Reserved.), 419-459.
35. Lienkamp K; Madkour AE; Musante A; Nelson CF; Nusslein K; Tew GN, Antimicrobial Polymers Prepared by ROMP with Unprecedented Selectivity: A Molecular Construction Kit Approach. *J. Am. Chem. Soc.* 2008, 130 (Copyright (C) 2012 American Chemical Society (ACS). All Rights Reserved.), 9836-9843.
36. Lee S-G; Brown JM; Rogers CJ; Matson JB; Krishnamurthy C; Rawat M; Hsieh-Wilson LC, End-functionalized glycopolymers as mimetics of chondroitin sulfate proteoglycans. *Chem. Sci.* 2010, 1 (Copyright (C) 2012 American Chemical Society (ACS). All Rights Reserved.), 322-325.
37. Slugovc C, The ring opening metathesis polymerisation toolbox. *Macromol. Rapid Commun.* 2004, 25 (14), 1283–1297.
38. Bielawski CW; Grubbs RH, Living ring-opening metathesis polymerization. *Prog. Polym. Sci.* 2007, 32 (1), 1–29.

39. Fishman JM; Kiessling LL, Synthesis of functionalizable and degradable polymers by ring-opening metathesis polymerization. *Angew Chem Int Ed Engl* 2013, 52 (19), 5061–4. [PubMed: 23568693]
40. Mallick A; Xu Y; Lin Y; He J; Chan-Park MB; Liu X-W, Oxadiazabicyclooctenone as a versatile monomer for the construction of pH sensitive functional polymers via ROMP. *Polymer Chemistry* 2018, 9 (3), 372–377.
41. Fraser C; Hillmyer MA; Gutierrez E; Grubbs RH, Degradable Cyclooctadiene/Acetal Copolymers: Versatile Precursors to 1,4-Hydroxytelechelic Polybutadiene and Hydroxytelechelic Polyethylene. *Macromolecules* 1995, 28 (21), 7256–7261.
42. Chang C-C; Emrick T, Functional Polyolefins Containing Disulfide and Phosphoester Groups: Synthesis and Orthogonal Degradation. *Macromolecules* 2014, 47 (4), 1344–1350.
43. Gutekunst WR; Hawker CJ, A General Approach to Sequence-Controlled Polymers Using Macrocyclic Ring Opening Metathesis Polymerization. *Journal of the American Chemical Society* 2015, 137 (25), 8038–8041. [PubMed: 26053158]
44. Steinbach T; Alexandrino EM; Wurm FR, Unsaturated poly(phosphoester)s via ring-opening metathesis polymerization. *Polymer Chemistry* 2013, 4 (13), 3800–3806.
45. Jeffrey CS; Barnes KL; Eickhoff JA; Carson CR, Generation and Reactivity of Aza-Oxyallyl Cationic Intermediates: Aza-[4 + 3] Cycloaddition Reactions for Heterocycle Synthesis. *J. Am. Chem. Soc.* 2011, 133 (Copyright (C) 2012 American Chemical Society (ACS). All Rights Reserved.), 7688–7691.
46. Strong LE; Kiessling LL, A General Synthetic Route to Defined, Biologically Active Multivalent Arrays. *J. Am. Chem. Soc.* 1999, 121, 6193–6196.
47. Pontrello JK; Allen MJ; Underbakke ES; Kiessling LL, Solid-phase synthesis of polymers using the ring-opening metathesis polymerization. *J Am Chem Soc* 2005, 127 (42), 14536–7. [PubMed: 16231882]
48. Yang SK; Weck M, Modular Covalent Multifunctionalization of Copolymers. *Macromolecules* (Washington, DC, U. S.) 2008, 41 (2), 346–351.
49. Allen MJ; Wangkanont K; Raines RT; Kiessling LL, ROMP from ROMP: A New Approach to Graft Copolymer Synthesis. *Macromolecules* 2009, 42 (12), 4023–4027. [PubMed: 20161406]
50. Schaefer M; Hanik N; Kilbinger AFM, ROMP Copolymers for Orthogonal Click Functionalizations. *Macromolecules* (Washington, DC, U. S.) 2012, 45 (17), 6807–6818.
51. Jung H; Carberry TP; Weck M, Synthesis of First- and Second-Generation Poly(amide)-Dendronized Polymers via Ring-Opening Metathesis Polymerization. *Macromolecules* 2011, 44 (23), 9075–9083.
52. Mangold SL; Carpenter RT; Kiessling LL, Synthesis of Fluorogenic Polymers for Visualizing Cellular Internalization. *Org. Lett.* 2008, 10 (14), 2997–3000. [PubMed: 18563907]
53. Binder WH; Kluger C, Combining ring-opening metathesis polymerization (ROMP) with Sharpless-type “click” reactions: An easy method for the preparation of side chain functionalized poly(oxynorbornenes). *Macromolecules* 2004, 37 (25), 9321–9330.
54. Mansfeld U; Pietsch C; Hoogenboom R; Becer CR; Schubert US, Clickable initiators, monomers and polymers in controlled radical polymerizations – a prospective combination in polymer science. *Polymer Chemistry* 2010, 1 (10), 1560–1598.
55. Cairo CW; Gestwicki JE; Kanai M; Kiessling LL, Control of multivalent interactions by binding epitope density. *J Am Chem Soc* 2002, 124 (8), 1615–9. [PubMed: 11853434]
56. Gama CI; Tully SE; Sotogaku N; Clark PM; Rawat M; Vaidehi N; Goddard WA 3rd; Nishi A; Hsieh-Wilson LC, Sulfation patterns of glycosaminoglycans encode molecular recognition and activity. *Nat Chem Biol* 2006, 2 (9), 467–73. [PubMed: 16878128]
57. Gestwicki JE; Cairo CW; Strong LE; Oetjen KA; Kiessling LL, Influencing receptor-ligand binding mechanisms with multivalent ligand architecture. *J Am Chem Soc* 2002, 124 (50), 14922–33. [PubMed: 12475334]
58. Kiessling LL; Grim JC, Glycopolymer probes of signal transduction. *Chem Soc Rev* 2013, 42 (10), 4476–91. [PubMed: 23595539]
59. Terent’ev AO; Krylov IB; Sharipov MY; Kazanskaya ZM; Nikishin GI, Generation and cross-coupling of benzyl and phthalimide-N-oxyl radicals in a cerium(IV) ammonium nitrate/N-hydroxyphthalimide/ArCH₂R system. *Tetrahedron* 2012, 68 (50), 10263–10271.

60. Anastasaki A; Nikolaou V; Nurumbetov G; Wilson P; Kempe K; Quinn JF; Davis TP; Whittaker MR; Haddleton DM, Cu(0)-Mediated Living Radical Polymerization: A Versatile Tool for Materials Synthesis. *Chem Rev* 2016, 116 (3), 835–77. [PubMed: 26226544]
61. Kolonko EM; Pontrello JK; Mangold SL; Kiessling LL, General Synthetic Route to Cell-Permeable Block Copolymers via ROMP. *J. Am. Chem. Soc.* 2009, 131 (21), 7327–7333. [PubMed: 19469577]
62. Johnson JA; Lu Y-Y; Burts AO; Lim Y-H; Finn MG; Koberstein JT; Turro NJ; Tirrell DA; Grubbs RH, Core-Clickable PEG-Branch-Azide Bivalent-Bottle-Brush Polymers by ROMP: Grafting-Through and Clicking-To. *J. Am. Chem. Soc.* 2011, 133 (Copyright (C) 2012 American Chemical Society (ACS). All Rights Reserved.), 559–566.
63. Agard NJ; Prescher JA; Bertozzi CR, A Strain-Promoted [3 + 2] Azide–Alkyne Cycloaddition for Covalent Modification of Biomolecules in Living Systems. *Journal of the American Chemical Society* 2004, 126 (46), 15046–15047. [PubMed: 15547999]
64. Dommerholt J; Rutjes FPJT; van Delft F. L. J. T. i. C. C., Strain-Promoted 1,3-Dipolar Cycloaddition of Cycloalkynes and Organic Azides. 2016, 374 (2), 16.
65. Courtney AH; Bennett NR; Zwick DB; Hudon J; Kiessling LL, Synthetic Antigens Reveal Dynamics of BCR Endocytosis during Inhibitory Signaling. *ACS Chem. Biol.* 2014, 9 (1), 202–210. [PubMed: 24131142]
66. Snider BB; Hui RAHF; Kulkarni YS, Intramolecular [2 + 2] cycloadditions of ketenes. *Journal of the American Chemical Society* 1985, 107 (7), 2194–2196.
67. Wang L; Zhang Z; Brock A; Schultz PG, Addition of the keto functional group to the genetic code of *Escherichia coli*. *Proc. Natl. Acad. Sci. U. S. A.* 2003, 100 (1), 56–61. [PubMed: 12518054]
68. Gauthier MA; Klok H-A, Peptide/protein-polymer conjugates: synthetic strategies and design concepts. *Chem. Commun. (Cambridge, U. K.)* 2008, (23), 2591–2611.
69. Lu H; Wang D; Kazane S; Javahishvili T; Tian F; Song F; Sellers A; Barnett B; Schultz PG, Site-Specific Antibody-Polymer Conjugates for siRNA Delivery. *J. Am. Chem. Soc.* 2013, 135 (37), 13885–13891. [PubMed: 23924037]
70. Vedrenne E; Dupont H; Oualef S; Elkaïm L; Grimaud L, Dramatic Effect of Boron-Based Lewis Acids in Cross-Metathesis Reactions. *Synlett* 2005, 2005 (04), 670–672.
71. Yang SK; Weck M, Covalent and orthogonal multi-functionalization of terpolymers. *Soft Matter* 2009, 5 (3), 582–585.
72. Kalia J; Raines RT, Hydrolytic Stability of Hydrazones and Oximes. *Angewandte Chemie International Edition* 2008, 47 (39), 7523–7526. [PubMed: 18712739]
73. Tsai AS; Brasse M. I.; Bergman RG; Ellman JA, Rh(III)-Catalyzed Oxidative Coupling of Unactivated Alkenes via C–H Activation. *Organic Letters* 2010, 13 (3), 540–542. [PubMed: 21175143]
74. Beak P; Basha A; Kokko B; Loo D, The geometry of displacements at nonstereogenic atoms: the formal displacement of alkoxide from alkoxyamines by organolithium reagents. *Journal of the American Chemical Society* 1986, 108 (19), 6016–6023. [PubMed: 22175366]
75. Winnik FM, Photophysics of preassociated pyrenes in aqueous polymer solutions and in other organized media. *Chem. Rev.* 1993, 93 (2), 587–614.
76. Kiessling LL; Gestwicki JE; Strong LE, Synthetic multivalent ligands as probes of signal transduction. *Angew. Chem., Int. Ed.* 2006, 45 (15), 2348–2368.
77. Liu F-T; Bohn JW; Ferry EL; Yamamoto H; Molinaro CA; Sherman LA; Klinman NR; Katz DH, Monoclonal dinitrophenyl-specific murine IgE antibody: preparation, isolation, and characterization. *J. Immunol.* 1980, 124 (6), 2728–37. [PubMed: 7373045]
78. Peacock JS; Barisas BG, Antigen-specific mouse lymphocyte stimulation by DNP-conjugated T-independent antigens studied by photobleaching recovery. *J. Supramol. Struct. Cell. Biochem.* 1981, 17 (1), 37–49. [PubMed: 6172591]
79. Sato K; Ochi A, Inhibition of B-cell receptor-antigen complex internalization by FcγRIIB1 signals. *Immunol. Lett.* 1998, 61 (2,3), 135–143. [PubMed: 9657266]
80. Bryant P; Ploegh H, Class II MHC peptide loading by the professionals. *Curr. Opin. Immunol.* 2004, 16 (1), 96–102. [PubMed: 14734116]

81. Siemasko K; Clark MR, The control and facilitation of MHC class II antigen processing by the BCR. *Curr. Opin. Immunol.* 2001, 13 (1), 32–36. [PubMed: 11154914]
82. Snyder EL; Dowdy SF, Cell Penetrating Peptides in Drug Delivery. *Pharm. Res.* 2004, 21 (3), 389–393. [PubMed: 15070086]
83. Futaki S, Oligoarginine vectors for intracellular delivery: design and cellular-uptake mechanisms. *Biopolymers* 2006, 84 (3), 241–249. [PubMed: 16333858]
84. Goun EA; Pillow TH; Jones LR; Rothbard JB; Wender PA, Molecular transporters: synthesis of oligoguanidinium transporters and their application to drug delivery and real-time imaging. *ChemBioChem* 2006, 7 (10), 1497–1515. [PubMed: 16972294]
85. Sarapas JM; Backlund CM; deRonde BM; Minter LM; Tew GN, ROMP- and RAFT-Based Guanidinium-Containing Polymers as Scaffolds for Protein Mimic Synthesis. *Chemistry* 2017, 23 (28), 6858–6863. [PubMed: 28370636]
86. Tyagi M; Rusnati M; Presta M; Giacca M, Internalization of HIV-1 Tat requires cell surface heparan sulfate proteoglycans. *J. Biol. Chem.* 2001, 276 (5), 3254–3261. [PubMed: 11024024]
87. Fuchs SM; Raines RT, Pathway for Polyarginine Entry into Mammalian Cells. *Biochemistry* 2004, 43 (9), 2438–2444. [PubMed: 14992581]
88. Poon GMK; Garipey J, Cell-surface proteoglycans as molecular portals for cationic peptide and polymer entry into cells. *Biochem. Soc. Trans.* 2007, 35 (4), 788–793. [PubMed: 17635149]
89. Schmidt NW; Lis M; Zhao K; Lai GH; Alexandrova AN; Tew GN; Wong GCL, Molecular Basis for Nanoscopic Membrane Curvature Generation from Quantum Mechanical Models and Synthetic Transporter Sequences. *J. Am. Chem. Soc.* 2012, 134 (46), 19207–19216. [PubMed: 23061419]
90. Kolonko EM; Kiessling LL, A Polymeric Domain That Promotes Cellular Internalization. *J. Am. Chem. Soc.* 2008, 130 (17), 5626–5627. [PubMed: 18393495]
91. Patterson DM; Nazarova LA; Prescher JA, Finding the right (bioorthogonal) chemistry. *ACS Chem Biol* 2014, 9 (3), 592–605. [PubMed: 24437719]
92. Farhangi S; Duhamel J, Probing Side Chain Dynamics of Branched Macromolecules by Pyrene Excimer Fluorescence. *Macromolecules* 2016, 49 (1), 353–361.
93. Duncan R, Polymer therapeutics at a crossroads? Finding the path for improved translation in the twenty-first century. *J Drug Target* 2017, 25 (9-10), 759–780. [PubMed: 28783978]
94. Atkinson SP; Andreu Z; Vicent MJ, Polymer Therapeutics: Biomarkers and New Approaches for Personalized Cancer Treatment. *J Pers Med* 2018, 8 (1).

A Grafting-through strategy **B Post-polymerization modification (PPM)** **Figure 1.**

ROMP can afford degradable polyoxazinones for tailoring through two strategies: A) *grafting-through* using pre-functionalized monomers or B) post-polymerization modification (PPM) of a reactive polymer precursor.

Figure 2.

Post-polymerization modification of ketone-containing **12** with alkoxyamine **13** affords oxime-substituted polymer **14**. Subsequent chemoselective reduction afforded an alkylamine-displaying polymer **15**. The ¹H-NMR data indicate that the reactions proceeded with high efficiencies.

Figure 3.

Polyoxazinone **16** was generated from exposing polymer **12** to *O*-(4-pyrenylbutyl) hydroxylamine. The resulting polymer **16** displays excimer fluorescence, but monomer **17** does not. Representative UV-Vis and fluorescence spectra are shown at 50 μ M pyrene, a concentration that does not promote solvent-induced dimerization (See Supporting Information).

Figure 4.

Compositions of polyoxazinones modified via PPM with a fluorophore (AAF) and biological recognition elements. χ = molar ratio ligand.

Figure 5.

A20HL B-cells, which express a DNP-binding BCR, were exposed to polymers **18**, **19**, and **20** (Figure 4). A) Fluorescence microscopy images of polymer **18** trafficking at 37 °C, and 4 °C. Markers include Hoechst (nuclear staining), anti-BCR antibody (red) and transferrin (blue) as an early endosomal marker. Arrow indicates co-localization. B) Quantification of uptake data. Left: Polymer co-localization with BCR at 37 °C. Right: Polymer co-localization with early endosomal marker transferrin (Tfr) at 37 °C. The Pearson's coefficient was calculated using at least 25 cells for each condition. * $P=0.0295$, ** $P<0.01$, **** $P<0.0001$ (Mann-Whitney test). Error bars represent the standard error of the mean for one of the 3 independent experiments. For scatter plots of bar graphs shown in B, see the Supporting information.

Figure 6.

Polyguanidinium polymer **21** was internalized by A20 and A20HL B cells at 37 °C but not at 4 °C. Arrows indicate intracellular fluorescence. Total internalized and surface polymer fluorescence was measured using at least 30 cells for each condition and shown as bar and scatter plots. *** $P < 0.001$, **** $P < 0.0001$ (Mann-Whitney test). Error bars represent the standard error of the mean for one of 3 independent experiments.

Scheme 1.

CuAAC affords monomer **2** variants but its application to polymer **4** afforded an insoluble gel.

Scheme 2.

Unexpected instability of monomer bearing an unactivated ester

Scheme 3.
Synthesis of ketone-bearing polymer

Table 1.ROMP of monomer **11**.

[11] ₀ /[cat]	conv (%) ^[a]	M _n ^{GPC} (g/mol) ^[b]	PDI ^[b]
15/1	83	7800	1.3
25/1	87	13700	1.5
50/1	87	16000	1.5
100/1	89	23400	1.4
200/1	84	40000	1.9
200/1 ^[c]	24	25800	2.0

^[a] based on ¹H NMR integrations of monomer olefin signals to polymer olefin signals^[b] calibrated with polystyrene standards, eluted 1.0 mg/mL in THF^[c] without BCy₂Cl