Development and Study of Synthetic Polypeptides for Biomaterial Applications

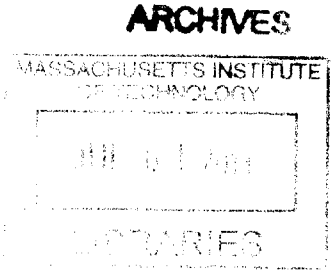
by

Shannon Marie Morey

B.S. Chemistry
Michigan State University, 2010

Submitted to the Department of Chemistry
in Partial Fulfillment of the Requirements for the Degree of

Master of Science in Chemistry



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May 28, 2013

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Abstract

Creating new scaffolds for cells is critical to the development of new tissue engineering techniques. In this work, the synthesis of new polypeptide systems is discussed. These systems are intended for the formation of hydrogels which can then be used as cell substrates. Attempts at using the clickable synthetic polypeptide poly(γ-propargyl L-glutamate) (PPLG) to form a self-assembly amphiphilic system is discussed, as is the formation of potentially amphiphilic block copolymers with PPLG. The synthesis of a hydrolytically stable synthetic polypeptide with click functionality is also investigated. Additionally, the creation of a polypeptide system with two functionalities available for orthogonal click chemistry is discussed.

Thesis supervisor: Paula T. Hammond

Title: David H. Koch Professor in Engineering

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Introduction

Organ Transplants and Tissue Repair

Tens of thousands of people in the United States are on organ transplant waiting lists. According to the U.S. Department of Health and Human Services, at the end of 2011, 54,599 people were waiting for kidney transplants, 1,353 for pancreas transplants, 12,905 for liver transplants, 183 for intestine transplants, 2,208 for heart transplants, and 1,323 for lung transplants.[1] This totals more than 72,500 transplants needed for these six organs. Additionally, the number of patients on kidney transplants waiting lists has continued to grow with an average increase of 2.7% over the last five years. Each year only approximately 17,500 kidney transplants are performed. The number of patients on the waitlist is almost three times as large as the number of transplants performed annually.[1] Given these grim statistics, scientists and engineers have focused great efforts on developing alternatives to organ transplants. In addition to the need for organ transplants, there are other types of injury or degredation of bodily tissues that we currently have very limited treatments for. This includes peripheral nerve regeneration, a complex problem involving regenerating axons, avoiding the formation of scar tissue, and the need to direct the growth of the nerve being formed.[2]

In recent years, tissue engineering has provided the potential to replace or repair damaged tissue and has emerged as a very active area of research.[3] However, the inherent complexity of biological systems poses barriers to advancement in tissue engineering. The material surrounding cells, the extracellular matrix (ECM), sends a number of physical and chemical cues to the cell and is integral to cellular development. In order to control these cues and manipulate cell behavior, we must develop scaffolds

that effectively mimic the ECM.[4] A successful biomaterial mimic of the ECM must provide a three-dimensional area through which cells can grow.[5] It must also provide the necessary structural support to allow cells to grow and proliferate. Additionally, most cells must adhere to their surroundings in order to survive, thus it is necessary for a material imitating the ECM to provide this adhesion. The introduction of different bioactive factors, chemical functionalities, and physical characteristics into synthetic scaffolds allows for control of the cell environment thereby controlling cellular characteristics.[2] By developing a series of novel scaffolds for cellular growth, we seek to facilitate the creation of a diversity of cell structures.

Hydrogels

Among the most promising materials that have been developed for the construction of cellular scaffolds are hydrogels – polymer networks that form gels upon the absorption of water.[6] Hydrogels demonstrate several characteristics that emulate the ECM. They are hydrophilic, but not water soluble, because of the crosslinks between polymer chains. They have a high water content, sometimes absorbing as much as 1000 times their dry weight, and they are very permeable to oxygen and nutrients.[3,6] They are biocompatible and can be designed to have a wide range of mechanical and structural properties (e.g. stiffness and permeability), depending on their composition.[3,6] As effective mimics of the ECM, hydrogels are useful platforms for tissue engineering.

Hydrogels can be produced from numerous synthetic or natural materials. Collagen, agarose, hyaluronic acid, fibrin, alginate, gelatin, and chitosan are common natural materials employed to produce hydrogels.[7] Hydrogels composed of natural

materials such as collagen and gelatin have ready-made sites for cell adhesion and cell signaling, thus given them an advantage over synthetic gels.[7,8] In fact, some natural materials used for hydrogels, such as collagen, hyaluronic, acid, and fibrin, are produced from parts of mammalian ECM. Other natural polymers used from hydrogels such as alginate and agarose can be obtained from algae. These natural materials tend to be non-toxic and have an inherent biocompatibility.[7] However, hydrogels based on natural materials often suffer from inconsistent composition batch to batch due to difficulties in purification and can have limited structural stability forcing scientists to introduce additional chemical crosslinks or create hybrid materials. This can limit their usefulness for tissue engineering.[3,7]

Synthetic polymers provide a channel for homogenous production of hydrogels and also allow greater control over the mechanical and chemical properties of the hydrogel. A number of neutral and ionic polymers (and derivatives of these polymers) have been used extensively for synthetic hydrogel systems including poly(hydroxyethyl methacrylate) (PHEMA), poly-(ethylene glycol) (PEG), poly(vinyl alcohol) (PVA), poly(acrylic acid) (PAA), poly(methacrylic acid) (PMMA), and polyacrylamide (PAAm). PEG has been approved by the US Food and Drug Administration for use in a number of medical applications and does not create an immune response when introduced into the body making it a highly favored material for biomaterials. However, introducing new functionalities into PEG systems and other synthetic polymer hydrogel systems can be difficult and they often lack stable secondary structures that are of benefit to the hydrogel.[9]

Some of the most promising synthetic hydrogels are based on synthetic polypeptides. The use of synthetic polypeptides creates inherent biocompatibility and the ability to control secondary structure while still giving the consistency of a synthetic polymer. Polypeptides with exact sequences can be produced using solid-phase peptide synthesis, which involves the protection and deprotection of amino acids attached to a solid support. However this is an expensive, time consuming, and often low yield procedure with an upper bound on how long the peptide chains can be.[10]

N-Carboxyanhydrides and Synthetic Polypeptides

Long polypeptide chains can be created without direct control of sequence using the ring opening polymerization of *N*-carboxyanhydrides (NCAs). These polypeptides can be easily synthesized and a wide range of functionalities can be introduced during their production through the use of both natural and unnatural amino acids.[9] NCAs are typically formed through phosgenation, a process that must be highly controlled due to the inherent dangers of phosgene gas. Phosgene is a colorless gas that has been used as chemical warfare agent since World War I. Phosgene causes suffocation by attacking pulmonary alveoli which help gases exchange between the blood and air.[11] Typically, diphosgene, a liquid, or triphosgene, a solid, is used because they are more easily handled, but some confuse this with being much safer than phosgene gas. In fact, in both cases the headspace of a vial containing these substances also contains phosgene gas and the two easily breakdown to form the gas.[12]

Since Deming developed a pseudo-living polymerization of NCAs, the field has grown steadily. The diversity and capabilities of synthetic polypeptides has allowed them to make important contributions to a number of biomaterial applications. Many

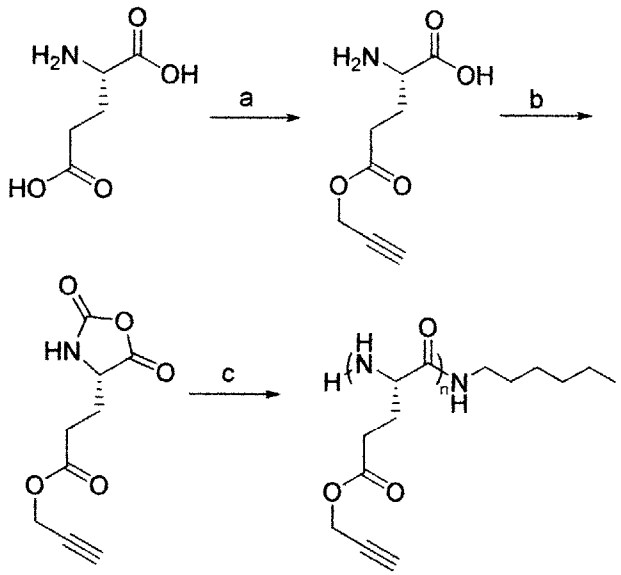
scientists have focused on making polypeptide membranes for drug delivery or gene delivery.[9,13] Others have been developing polypeptide-based adhesives mimicking natural adhesives produced by marine organisms.[9] Additionally, polypeptides are being investigated for their anti-microbial properties.[14,15] Finally, one of the most intriguing uses for polypeptides is to form hydrogel systems. As mentioned above, synthetic polypeptide hydrogels have numerous advantages over other types of hydrogel systems currently being employed and developed. The focus of this work is to elucidate new polypeptide systems based on the advantages click chemistry gives to these materials.

Click chemistry is the term for a set of chemical reactions that meet certain criteria. The reaction must be able to be performed in fairly innocuous conditions. Most click reactions can occur at room temperature and sometimes they do not require an inert atmosphere. Additionally, the reaction must be simple to purify and the byproducts of the reaction should not be highly toxic. The reaction should also not be limited to a few particular chemicals, but be able to be applied to numerous materials with the same functional groups. Probably the most important feature of a click reaction is the high reaction yield. The reaction that best exemplifies these characteristics is the 1,3-dipolar Huisgen cycloaddition of an alkyne and azide to form a triazole, made famous by Sharpless.[16]

We are developing synthetic polypeptide systems for the production of a "toolkit" of hydrogels to control the mechanical and chemical cues cells receive during development. By changing the properties of these polypeptides, we will be able to tune the physical and chemical characteristics of the hydrogel systems. In this way, we will be able to control the development of cells grown on the hydrogel scaffold.

Poly(γ-propargyl L-glutamate)

Previously, Amanda Engler of the Hammond lab reported the synthesis of poly(γ-propargyl L-glutamate) (PPLG) using the ring opening polymerization of the NCA of γ-propargyl L-glutamate, shown in Scheme 1.[17] The pendant alkyne group provides a convenient functionality for click chemistry. Using the 1,3-dipolar Huisgen cycloaddition of an alkyne and azide to form a triazole, Engler was able to attach a variety of functionalities, including long polyethylene glycol (PEG) chains, thiols, and amines, with extremely high yields (upwards of 95%) showing that a number of interesting and useful functionalities could be introduced to one simple polypeptide backbone.[15,17]



Scheme 1. The formation of PPLG from L-glutamate. Reagents and conditions: (a) propargyl alcohol; Si(CH3)3Cl, 36 hours, room temperature; (b) EtOAc, triphosgene, 5 hours, 80° C; (c) DMF, n-hexylamine, 72 hours; room temperature.

Engler determined that the grafted polypeptides had a very stable α-helical secondary structure. She was also able to demonstrate the applications of the grafted polymers for controlled drug delivery and as antimicrobials.[13,15] However, with the wide functionality that can be obtained by grafting azides onto PPLG, its uses are not limited to these applications.

We have identified a novel application for clickable synthetic polypeptides - as convenient polymer backbones that would allow us to create a large series of hydrogels with diverse properties to serve as cellular scaffolds. Using our polypeptide systems, current Hammond group members are already addressing some of the major concerns in hydrogel systems, mainly the ability to independently tune permeability and stiffness. This is a concern in hydrogels used to study stem cell differentiation, because without being able to independently tune these two parameters one cannot determine which parameter is affecting cell development. As mentioned above, grafted PPLG has an α-helical structure, which gives it rigid rod characteristics. However, if one synthesizes a polypeptide with a 50:50 mixture of L- to D-glutamate randomly dispersed (PPDLG), it forms a random coil, which is much more flexible. It is possible to form hydrogels with both of these polypeptides using chemical crosslinkers. PPLG and PPDLG with the same degree of polymerization and crosslink density have similar swelling and permeability, but have large differences in stiffness.[18] Using these systems we can create a variety of hydrogels with the same chemical characteristics, but a wide variety of physical properties which will allow us to elucidate the effect of stiffness in a three dimensional cellular environment.

Self-Assembling Hydrogels

The PPLG and PPDLG hydrogels currently being synthesized in the Hammond lab use a chemical crosslinker, specifically *N*-(*p*-Maleimidophenyl)isocyanate (PMPI), that can be toxic to cells. Additionally, synthesis takes place in organic solvent. This solvent is difficult to completely remove and replace with aqueous buffer solution to produce a biologically compatible material. I investigated ways to create a synthetic

polypeptide system that will self-assemble in water to form a hydrogel, thereby eliminating the need for harsh chemical crosslinkers and crosslinking in organic solvent.

Self-assembling hydrogels have other advantages over their chemically crosslinked relatives - they allow for greater control of chain length and three-dimensional structure.[19] There are many ways to create a self-assembling hydrogel and nature has created many methods of self-assembly.[20,21] Systems using polypeptides typically take advantage of the ability of polypeptides to form stable α-helices or β-sheets.

Researchers have begun using α-helices that self-assemble into coiled coil structures to form hydrogels. When the coils form, they are hydrophobic on one side and hydrophilic on the other. In fact, the coils are distorted leading to a periodicity of 3.5 amino acids per turn.[21]

One way to create a self-assembling material is to create a triblock polypeptide with the ends being positively charged and the middle uncharged. This creates a facially amphiphilic polypeptide that then form dimers which align with each other forming β-sheets due to hydrogen bonding.[21]

Another way to create β-sheets is to synthesize a polypeptide that will result in a β-hairpin. By introducing a β-hairpin into these systems researchers have been able to create hydrogels that were based on supramolecular forces and led to the formation of the gel to be highly reversible depending on conditions. Others have also created hydrogels based on amyloid fibers which are naturally occurring in patients with some degenerative diseases such as Alzheimer's.[21]

One can create a self-assembled hydrogel is the use of an amphiphilic polypeptide composed of alternating groups that can form a β-sheet with a hydrophilic face and a hydrophobic face. These β-sheets interact to form fibrillar structures in the presence of inorganic salts, thereby forming hydrogels, as shown in Figure 1.[19,22,23]

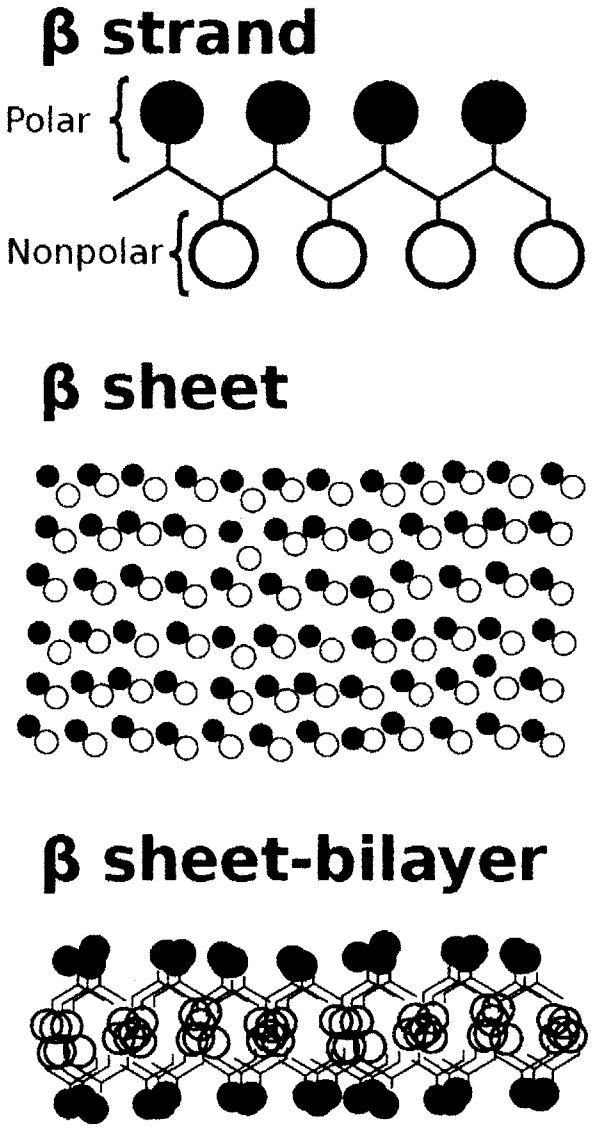
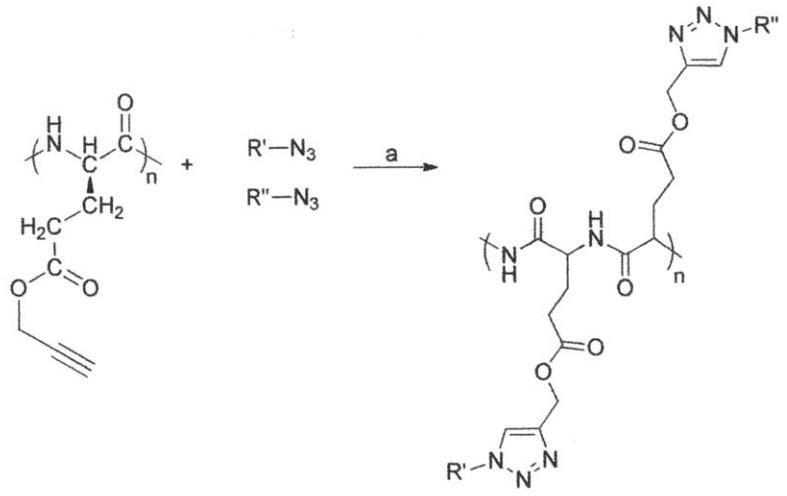


Figure 1. The self-assembly of amphiphilic polymers can occur through the formation of β-sheets. First the polymer organizes itself such that the nonpolar moieties are on one side of the molecule and the polar moieties are on the other. This then assembles into a β-sheet which can assemble into a β-sheet bilayer.

Results and Discussion

Amphiphilic PPLG-based Polypeptides with Random Dispersion of Functionalities

Based on the amphiphilic β-sheet formation model, I created a set of amphiphilic polymers utilizing the click functionality of our PPLG system by grafting on two different azides, one with a terminal hydrophilic group, the other with a terminal hydrophobic group (Scheme 2). Synthesis of PPLG was conducted as previously reported.[17] The first set of azides I used were 2-(2-azidoethoxy)ethanol and 1-azido-2-(2-methoxyethoxy)ethane which led to methoxy and hydroxy terminal groups. Grafting efficiency on polymers with was determined by NMR to be 96% as calculated by comparing the addition of the functional groups to the polymer backbone. A representative spectrum is shown in Figure 2 using a polymer with a degree of polymerization of 55. The addition of methoxy group to the backbone was found to be approximately 28/55, while the addition of the hydroxyl group was found to be approximately 25/55. The different between the two is likely due to deuterium exchange with the hydroxyl group and not a large difference in actual grafting efficiency. This can also account for the less than 100% grafting efficiency. If we are unable to detect some of the hydroxyl groups attached, we could not be able to find a grafting efficiency of 100%. The propargyl peak after grafting is difficult to separate out from other peaks in the same region on the NMR spectrum, because it has diminished greatly in size from the ungrafted polymer.



Scheme 2. Huisgen "click" reaction of two azides onto the PPLG backbone. R' is a hydrophobic functional group while R'' is a hydrophilic functional group; Reagents and conditions: (a) CuBr, N,N,N',N',N"-pentamethyldiethylenetriamine, DMF, room temperature, 12 hours. The grafted groups are randomly dispersed on the polymer backbone.

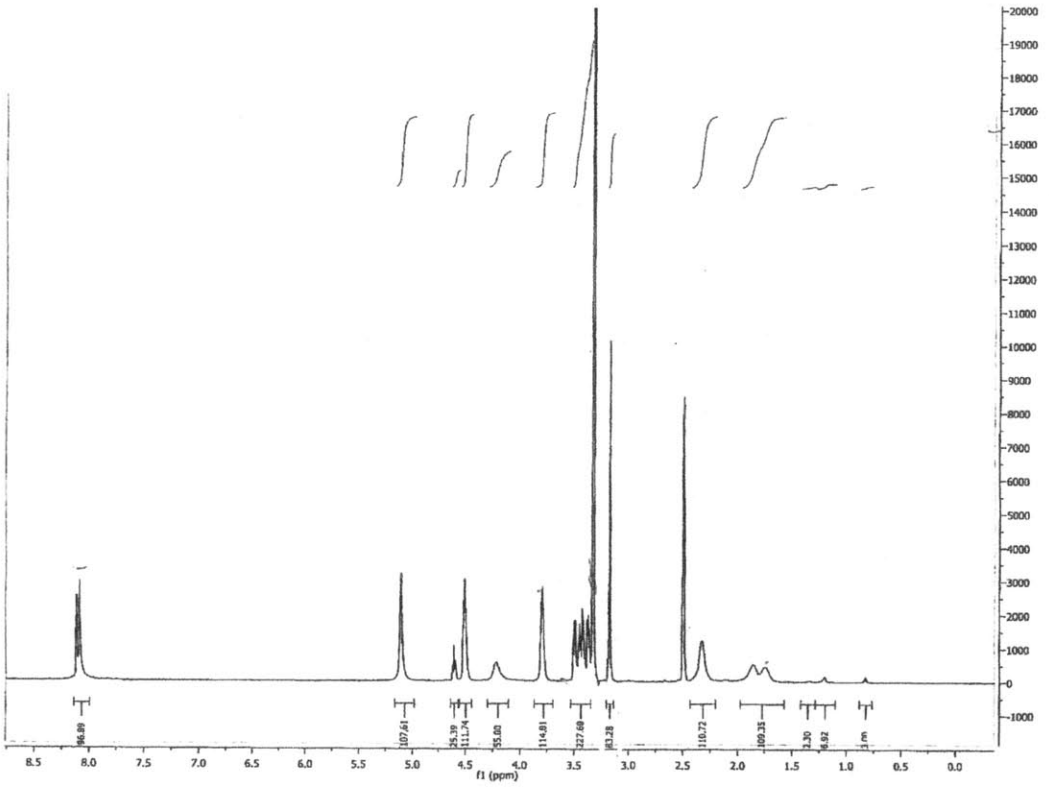


Figure 2. NMR of a grafted PPLG with a degree of polymerization of 55 as determined by comparing the initiator peak at 0.75 ppm with the side chain methoxy and hydroxyl peaks at 3.2 ppm and 4.6 ppm respectively.

Using circular dichroism, the grafted polypeptide was found to be α-helical at a temperature range from 25° C to 90° C (Figure 3).

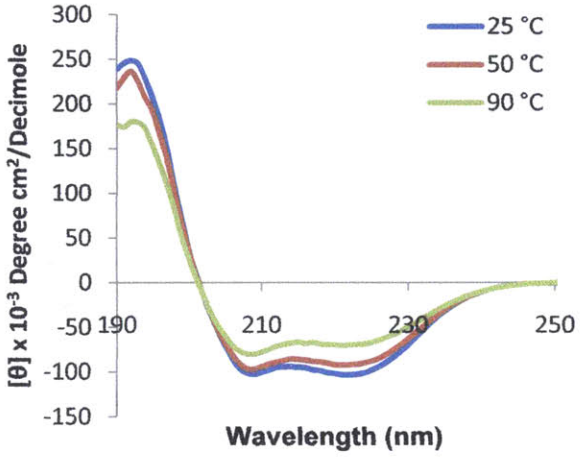


Figure 3. Circular dichroism spectra for the dual grafted PPLG showing the characteristic line shape for α-helical secondary structure at 25, 50, and 90 °C.

Based on the gelation of other polypeptide systems reported, I attempted to create gels at 1, 2, and 3 weight percent of polymer in aqueous solutions with sodium chloride concentrations of 20 mM, 150 mM, and 400 mM,[24] However, the polypeptide displayed no signs of gelation – the viscosity of the material did not appear to increase over the course of 24 hours. Based on the α-helical nature of the grafted PPLG and its inability to gel, we concluded that the difference in hydrophilicity was not great enough to induce β-sheet formation.

I then prepared a series of polypeptides with various ratios of methylamine, dimethylamine, benzyl, and hydroxy terminal groups in order to study how differences in grafting group hydrophilicity would affect self-assembly. These polypeptides precipitated during the click reaction and were insoluble in common solvents including water, methanol, ethanol, dimethylformamide and dimethylsulfoxide. We concluded that

due to the random distribution (as opposed to strict alternation) of grafting groups along the polymer backbone, we could not obtain the β-sheets necessary for hydrogel formation.

Block Co-polymers with PPLG

Block copolymers are also able to form numerous self-assembling structures including vesicles, micelles, and hydrogels.[19] Because block copolymers combine two or more different chemical structures with largely varying properties into one long molecule, they can form many basic structures include spheres, cylinders, gyroids, and lamella.[25] I focused on the synthesis of an amphiphilic triblock copolymer of the form BAB where the B block is a hydrophobic polypeptide and the A block is a hydrophilic polypeptide. For these types of block copolymers in an aqueous solution, the A block will be soluble, while the B blocks aggregate together. This aggregation allows for self-assembly into hydrogels.[26]

I synthesized BAB triblock copolymers of the form poly(γ-benzyl L-glutamate)-*block*-poly(γ-propargyl L-glutamate)-*block*-poly(γ-benzyl L-glutamate) (Figure 4). The poly(γ-benzyl L-glutamate) (PBLG) acts as the hydrophobic portion of the block copolymer, while the PPLG block allows for the addition of numerous hydrophilic functionalities via click chemistry.[17]

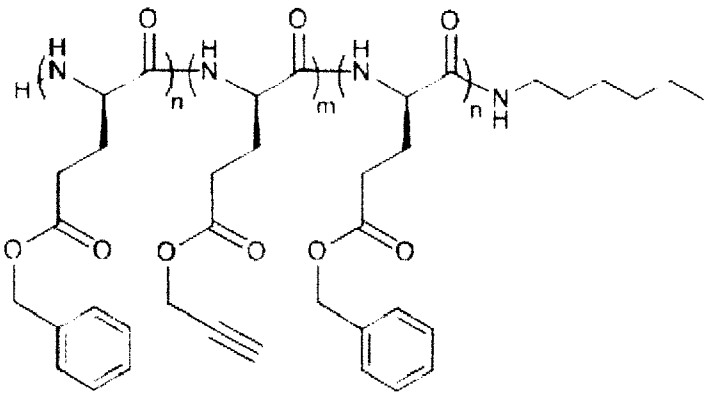


Figure 4. Triblock copolymer structure, synthesized using the sequential addition of NCA monomer.

The ring opening polymerizations of NCAs are pseudo-living polymerizations – they will continue to polymerize until no more monomer is present or the polymerization is quenched by another reagent or impurity. Therefore, in order to synthesize a block copolymer one can add different NCA monomers sequentially. I added γ-benzyl L-glutamate N-carboxyanhydride (BLG-NCA) and monitored its consumption by gel permeation chromatography (GPC). When all of the BLG-NCA was consumed, I added γ-propargyl L-glutamate N-carboxyanhydride (PLG-NCA). Following the consumption of the PLG-NCA, I added another quantity of BLG-NCA in order to create the triblock copolymer.

The polymerization of the PLG-NCA often experiences early termination due to impurities. With this in mind, I synthesized a relatively small triblock copolymer of the form $PBLG_{10}PPLG_{10}PBLG_{10}$ in order to probe how the impurities in PLG-NCA would affect the synthesis of a triblock copolymer.

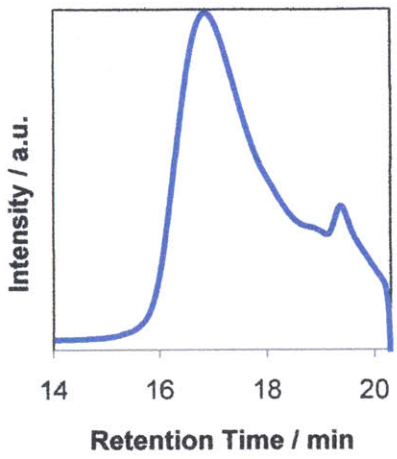
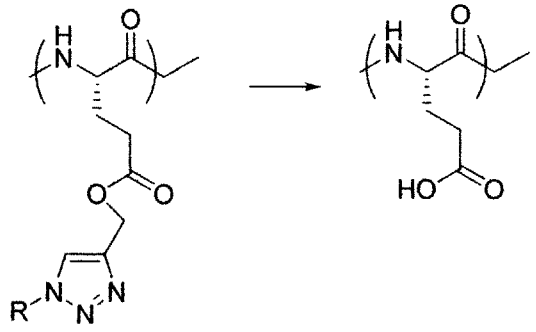


Figure 5. Gel permeation chromatography trace of $PBLG_{10}PPLG_{10}PBLG_{10}$ showing the broad peak and small peak at about 19 minutes indicating unreacted monomer.

Using GPC, I found the polydispersity index (PDI) of this polymer to be 1.23. This number is larger than we would prefer – a polymer composed of a single molecular weight would have a PDI of 1. Additionally, the GPC trace shows a small peak indicative of remaining NCA monomer (Figure 5). I determined the ratio of BLG-NCA to PLG-NCA incorporated to be 21:10 by NMR analysis. The large PDI, extra peak in the GPC trace, and a ratio of BLG-NCA to PLG-NCA larger than 20:10 indicates possible contamination from diblock copolymer and the quenching of NCA monomer by impurities. Because the triblock $PBLG_{10}PPLG_{10}PBLG_{10}$ polypeptide does not have a large difference in molecular weight from a diblock $PBLG_{10}PPLG_{10}$ copolymer it was difficult to determine if the triblock is contaminated with diblock and if so how much diblock is present. In order to make these block copolymer systems more versatile, we decided to develop polymers with alternative click functionalities thereby allowing for the facile synthesis of a large variety of block copolymers.[27] This work is described later.

Hydrolytically Stable Polypeptide with Click Functionality

Another concern regarding the use of PPLG for hydrogels is its stability in water. The grafted groups are connected to the polymer backbone using an ester linkage, which is hydrolysable as shown in Scheme



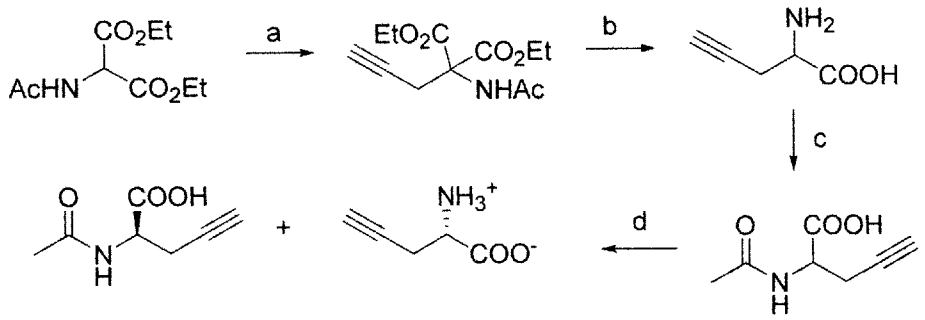
Scheme 3. The hydrolysis of grafted PPLG can readily occur in aqueous solution.

The hydrolysis of this ester bond is dependent on the pH, the identity of the grafted group, and whether PPLG has been connected to other polymers. Hydrolysis occurs faster at a higher pH and complete hydrolysis can occur in two days at pH 11.[13] Diblock copolymers of the form PPLG-*block*-PEG are more hydrolytically stable than those composed solely of PPLG due to the formation of micelles which shield the grafted PPLG from water.[13] Additionally, PPLG-*block*-PEG polymers with grafted diethylamine are less stable than those grafted with diisopropylamine.[13] When conducting long term cell studies on hydrogels it is necessary for the gel to be stable over the course of the experiment; therefore we are developing a hydrolytically stable synthetic polypeptide with click functionality by creating a polymer without the hydrolyzable ester functionality.

Poly(α-propargyl L-glycine)

I investigated poly(α-propargyl L-glycine) as a possible hydrolytically stable synthetic polypeptide. As commercially available α-propargyl L-glycine is expensive, I

designed a synthesis using microwave assisted reactions and the enzymatic resolution of α-propargyl D,L-glycine to reduce cost and provide a convenient means of production (Scheme 4).[28-31] First a propargyl group is added to diethyl acetamidomalonate by adding propargyl bromide in acetonitrile with the reaction assisted by microwave radiation at 140 °C. The product from this reaction is then used to form α-propargyl glycine by addition of hydrochloric acid. The α-propargyl glycine must then be separated into L and D amino acid by first acylating it and then using an enzymatic reaction employing acylase for separation. The microwave assisted reactions and acylation (using purchased propargyl glycine) were successfully performed, however the yield was such that prevented further syntheses.



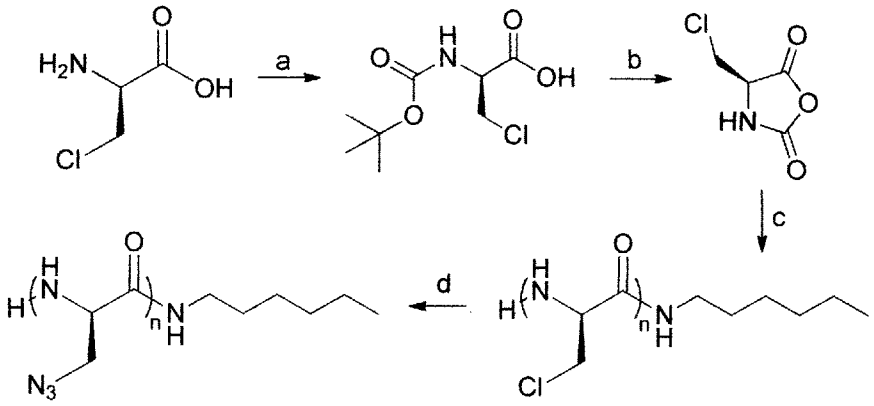
Scheme 4. Synthesis of α-propargyl L-glycine from diethyl acetamidomalonate. Reagents and conditions: (a) propargyl bromide, MeCN, Cs_2CO_3, MW, 140 °C, 10 minutes (b) 6M HCl, MW, 90 °C, 10 minutes (c) NaOH, acetyl chloride, 2 hours (d) acylase (aq).

Using purchased α-propargyl L-glycine, I prepared the α-propargyl L-glycine NCA, using the same reaction conditions as the previously published synthesis of the γ-propargyl L-glutamate NCA, but with low yield.[17] I polymerized this NCA to form poly(α-propargyl L-glycine). Using the same polymerization concentrations as in PPLG synthesis, the poly(α-propargyl L-glycine) product precipitated out of solution before the reaction had gone to completion.[17] After investigating numerous solvents (dimethylformamide, dimethyl sulfoxide, tetrahydrofuran, etc.), solvent mixtures, and

additives (lithium salts), the polymerization could not be increased beyond ten units as calculated by GPC. This size of polymer is too small to be of significant use. We therefore began investigating alternative hydrolytically stable polymers.

Poly(β-azido L-alanine)

I also investigated the synthesis of azide functionalized polypeptides, including poly(β-azido L-alanine), shown in Scheme 5.[32-34] Azide substitution after polymerization ensures that azido functionalized compounds are not exposed to acidic conditions which could lead to the formation of the highly explosive hydrazoic acid.



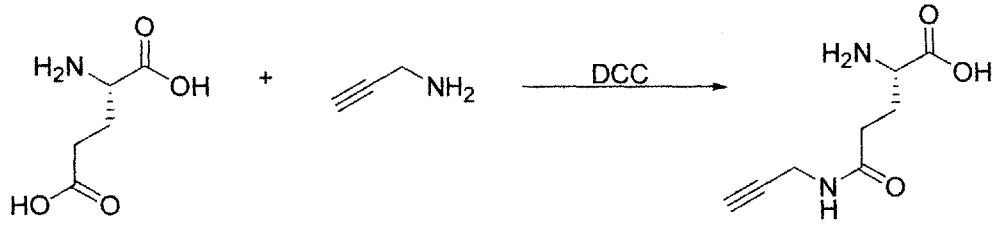
Scheme 5. Synthesis of poly(β-azido L-alanine). Reagents and conditions: (a) 1. NaOH, dioxane/water di-*tert*-butyl carbonate, $KHSO_4$, 0 °C, 2 hours (b) 1. *tert*-butylchlorodimethylsilane, triethylamine, ethyl acetate 0 °C 2. oxalyl chloride, DMF, chloroform(c) hexylamine, DMF, 72 hours (d) sodium azide, DMF, 60 °C, 2 days

I successfully synthesized the β-chloro L-alanine NCA; however, the purification of this NCA proved difficult and yields were low enough to prevent the investigation of its polymerization characteristics. Additionally, the formation of polymer with such a high density of azido groups could have proven explosive and therefore was not pursued.

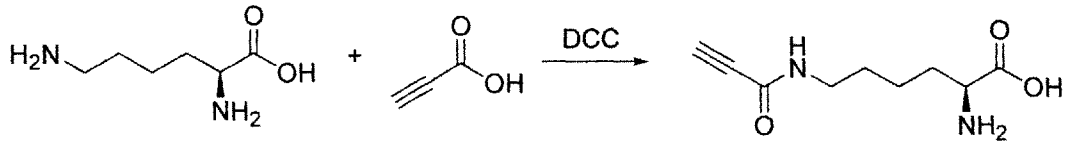
Amide-based Hydrolytically Stable Polypeptides

An amide bond is far more hydrolytically stable than an ester bond. We therefore decided to take the general PPLG structure, but replace the ester linkage with an amide linkage. Amide linkages can be created using N,N'-dicyclohexylcarbodiiimide (DCC). DCC is a commonly used to form amide linkages.[35]

In the synthesis of PPLG, the side chain carboxyl group is far more reactive than the carboxyl group on the peptide backbone. This allows for the esterification of the side chain carboxyl group without need to protect the backbone carboxyl. I investigated whether it was necessary to use protected amino acids to form an amino acid with an amide linkage exclusively on the side chain. I initially performed a DCC reaction on unprotected L-lysine and L-glutamate, reaction schemes shown in Scheme 6 and Scheme 7 Both reactions were done at room temperature under argon for 24 hours.



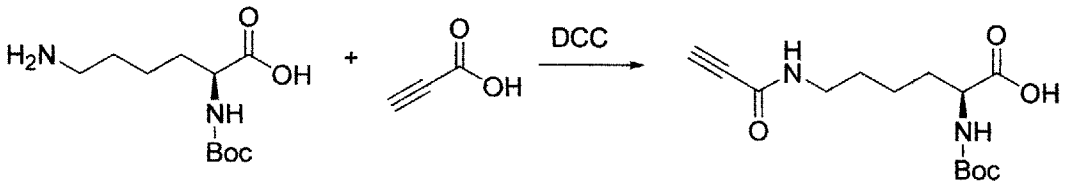
Scheme 6. The addition of propargyl amine to L-glutamic acid via DCC coupling. Conditions: room temperature, 24 hours, in MeCN.



Scheme 7. The addition of propynoic acid to L-lysine acid via DCC coupling. Conditions: room temperature, 24 hours, in MeCN.

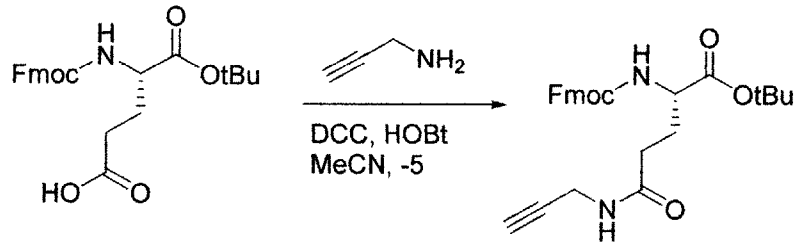
The product of these reactions were very impure and removal of byproducts proved difficult with amide coupling occurring non-selectively.

I then attempted to synthesize an amide linked L-lysine using a partially protected amino acid, as shown in Scheme 8. Again, this reaction proved to have too many byproducts to be viable. Again, amide bonding occurred non-selectively.



Scheme 8. The addition of propynoic acid to protected L-lysine acid via DCC coupling. Conditions: room temperature, 24 hours, in MeCN.

I then moved to using L-glutamic acid with the amide protected by an Fmoc group and the backbone carboxyl protected by a t-butyl group, N-α-Fmoc-L-glutamic acid α-t.-butyl ester. This allows for the exclusive formation of an amide bond between the propargyl amine and the side chain carboxyl group. Additionally, we found that upon addition of a catalytic amount of 1-hydroxybenzotriazole reaction yields increased (Scheme 9).



Scheme 9. The addition of propargyl amine acid to L-lysine acid via DCC coupling aided by1-hydroxybenzotriazole

This reaction has been performed successfully as shown by the comparison of the starting material NMR (Figure 6) and the product NMR (Figure 7).

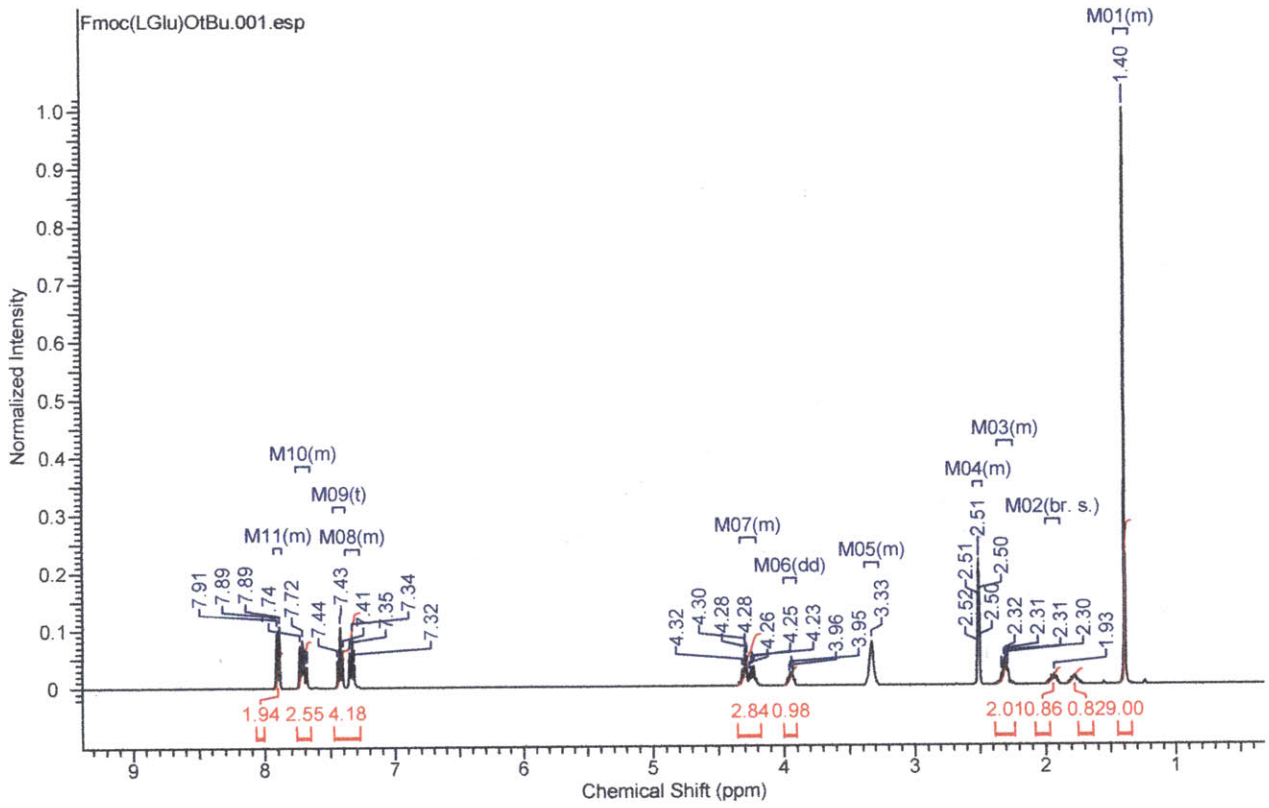


Figure 6. NMR spectrum of purchased N-α-Fmoc-L-glutamic acid α-t.-butyl ester.

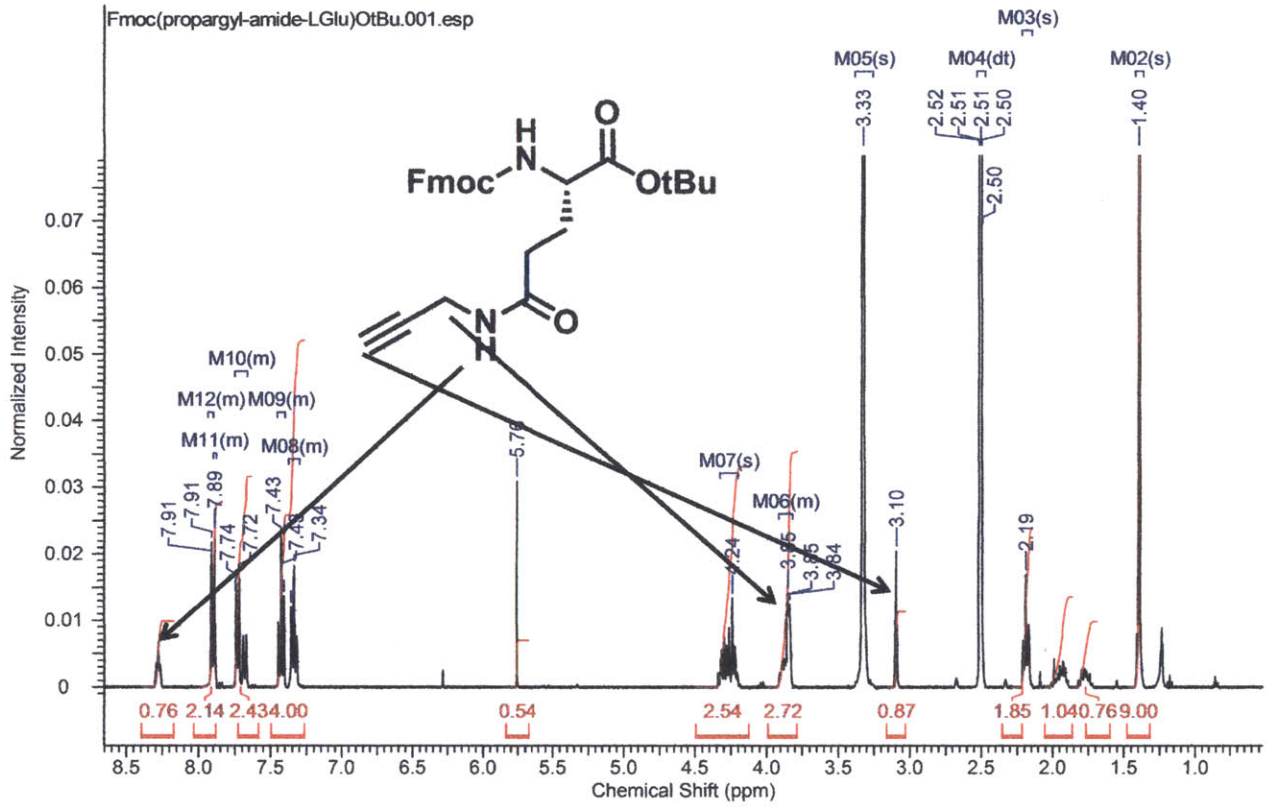
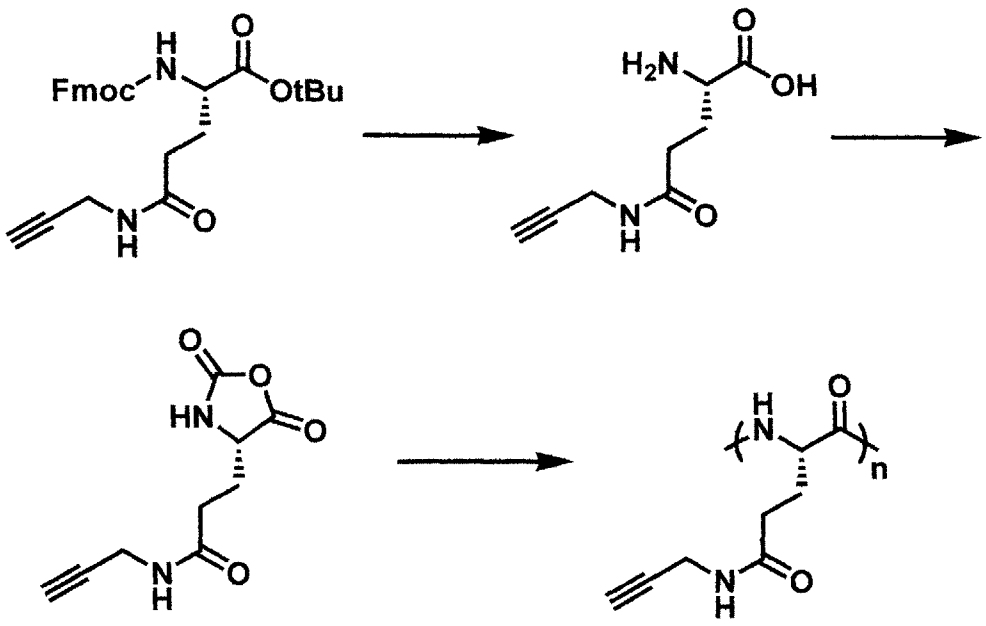


Figure 7. N-α-Fmoc-L-glutamic acid α-t.-butyl ester post DCC coupling with propargyl amine. The peaks associated with the addition of the amine appear in the NMR with the correct integrations.

In order to create a clickable hydrolytically stable polymer, the protecting groups must be removed, the NCA formed, and polymerized (Scheme 10). We predict that similar reactions as those used for the polymerization of PPLG may be employed.[17]



Scheme 10. The general synthesis for creating a hydrolyically stable clickable polypeptide

I have been successful in creating NCAs for two different hydrolytically stable click monomers and a amide linked clickable unnatural amino acid. Although, the glycine-based monomer was unable to polymerize into a large polymer we have found other monomers to create a hydrolytically stable polypeptide. Using the amine-based monomer, we will produce hydrolytically stable polypeptides with click functionality.

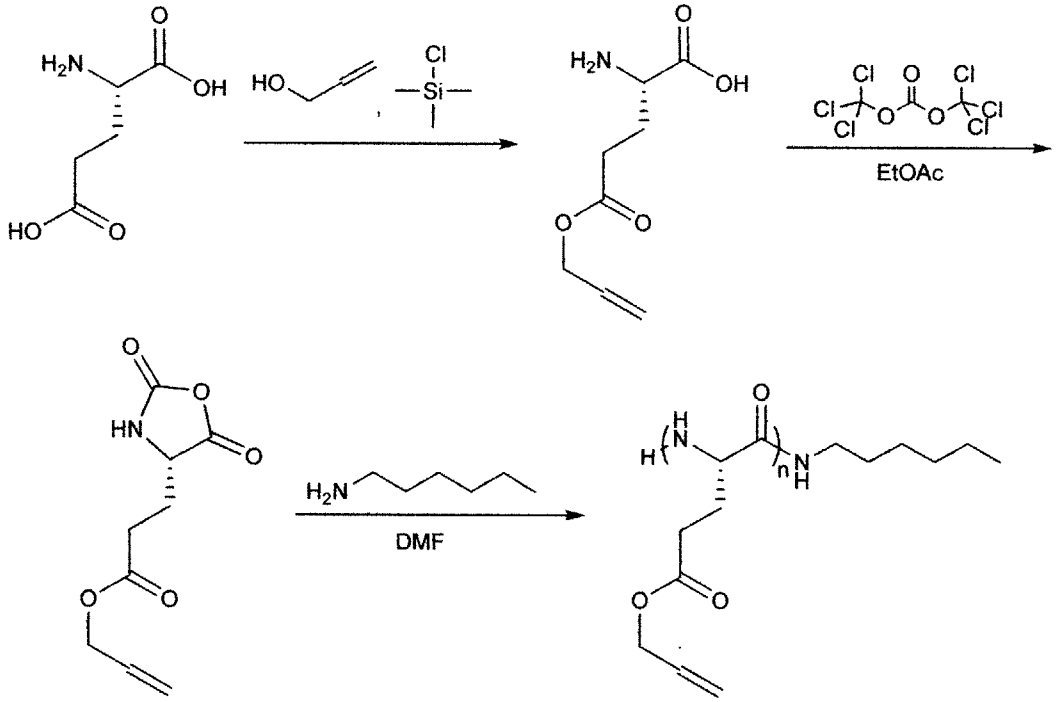
Orthogonal Click Chemistry

In order to further increase the functionality of our PPLG system we wanted to add an orthogonal click group. By adding another click group, we can create novel copolymers – both block and random copolymers.

Thiol-ene Click Chemistry and Poly(γ-allyl L-glutamate)

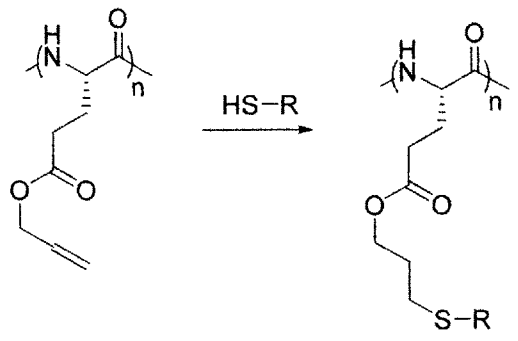
After investigating a number of possible click functionalities to introduce to a polypeptide system, I decided to focus on the click reaction that occurs between thiols and alkenes. Thiols can perform click reactions with a number of functional groups including alkenes, alkynes, epoxides, alkyl halides, and isocyanates.[36] As our PPLG

system already employs alkynes it would not be possible to create a system that has orthogonal click chemistry using an alkyne. However, an alkene can be readily introduced into a synthetic polypeptide by performing an esterification similar to that employed in creating propargyl L-glutamate using allyl alcohol instead of propargyl alcohol. From there, the formation of poly(γ-allyl L-glutamate) (PALG) proceeds as with PPLG (Scheme 11).[17] I performed this reaction successful to form PALG with degrees of polymerization of 10 and 25.



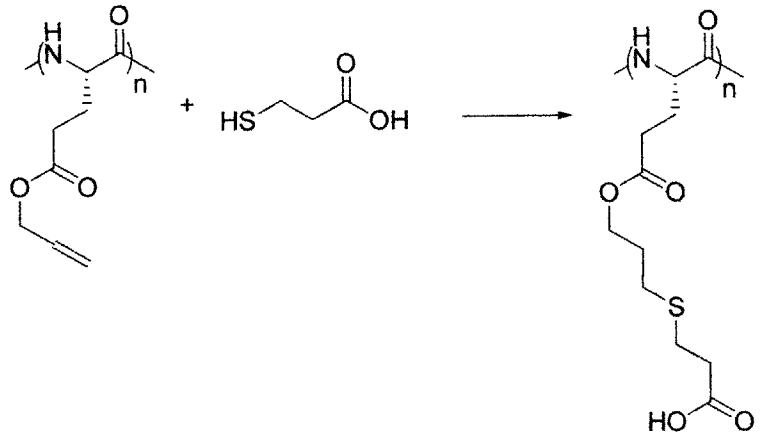
Scheme 11. Synthesis of PALG

I then tested the ability to click thiols onto this polymer (Scheme 12) using UV radiation to initiate the reaction similar to reported methods.[27,36]



Scheme 12. Addition of a thiol to PALG.

I dissolved the polymer in DMF under argon and added a slight excess of 3-mercaptopropionic acid and irradiated with UV light for 12 hours (Scheme 13). DMF was removed under vacuum to yield grafted polymer. Additional aqueous purification proved difficult as the polymer had not fully clicked and was not water soluble.



Scheme 13. Addition of 3-mercaptopropionic acid to PALG.

By comparing the alkene and initiator peaks in the NMR spectra for the ungrafted and grafted PALG I was able to determine the grafting efficiency of the thiol-ene click reaction. A representative set of spectra is show in Figures 8 and 9 with a grafting efficiency of approximately 32%.

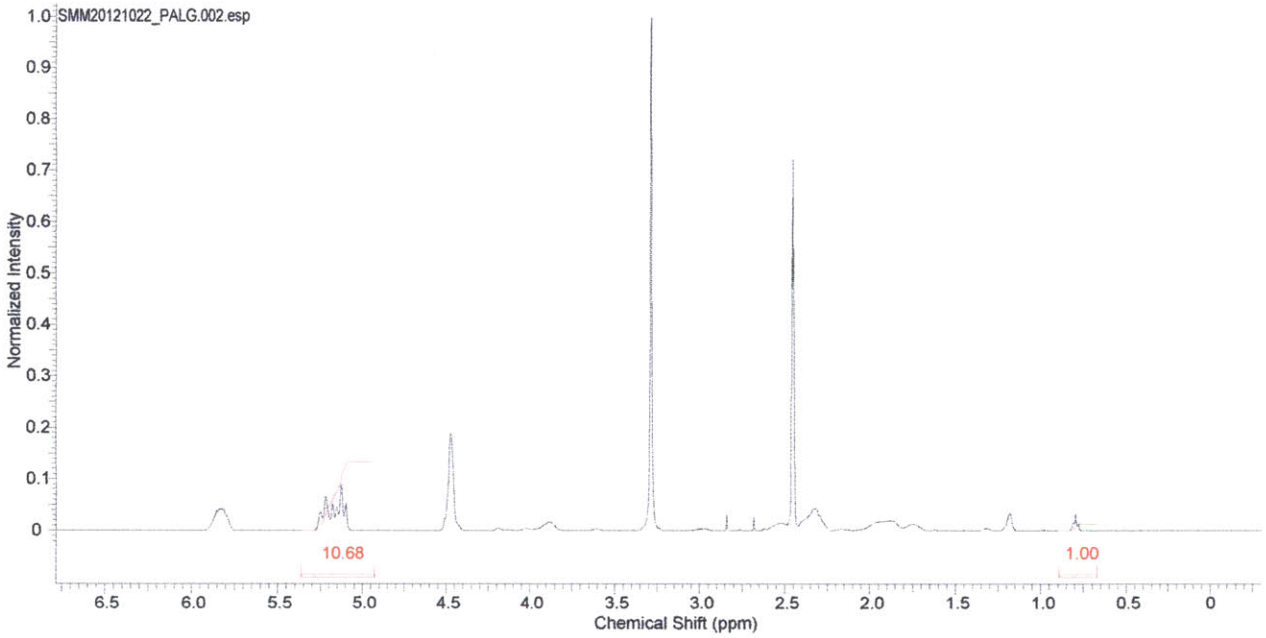


Figure 8. NMR spectrum of PALG with a degree of polymerization of 10 as determined by comparing the initiator peak at 0.75 and the alkene peaks centered at 5.2.

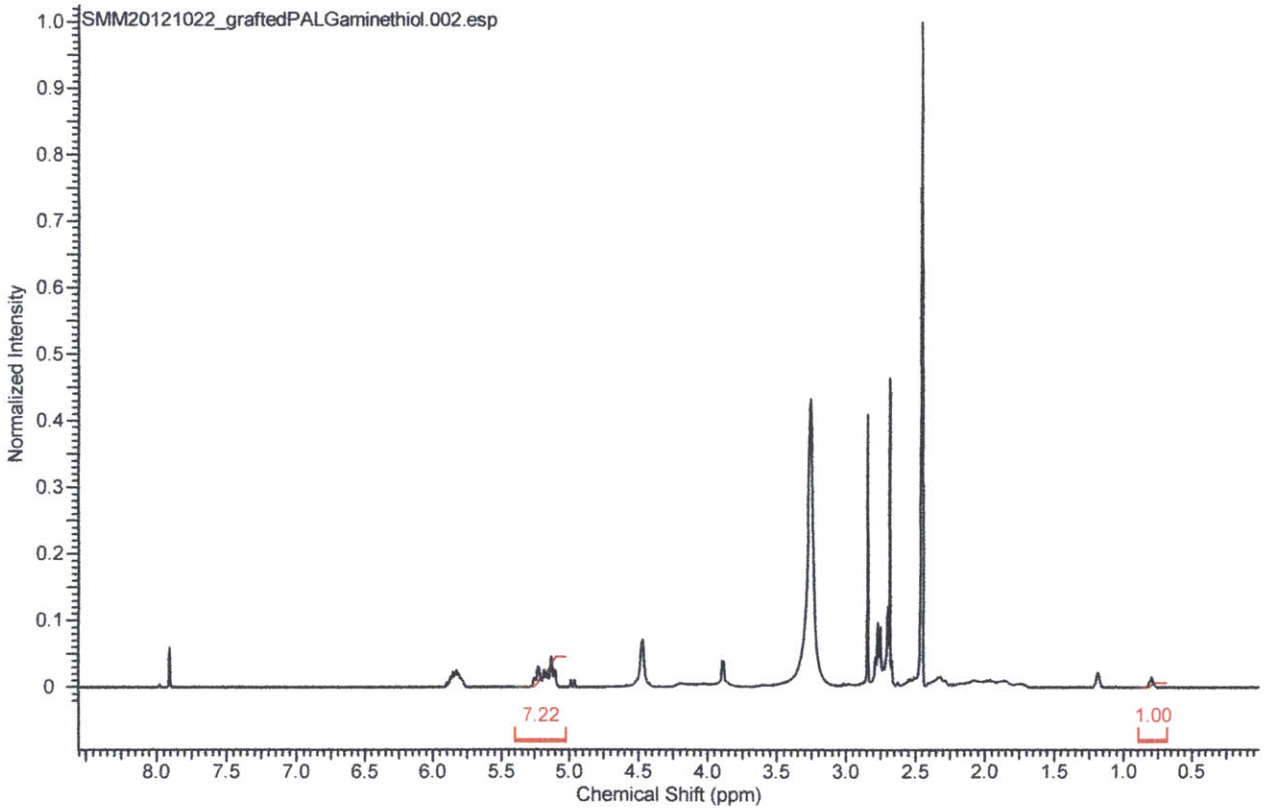


Figure 9. NMR spectrum of PALG with a degree of polymerization of 10 post-grafting. The ratio of initiator peak to alkene peak decreases to 1:7.

Grafting efficiency increased with an increase in the duration of UV radiation; however a grafting efficiency over 70% has not been achieved. Thiol-ene clicks do not typically have grafting efficiencies over 85%.[27] We hope to achieve these efficiencies by increasing the power and duration of our UV radiation.

I also created random copolymers of PALG and PPLG by first forming the NCAs together, by combining allyl L-glutamate and propargyl L-glutamate in a 50:50 mixture to form an NCA mixture which I then polymerized using previously reported methods.[17] This co-polymerization was successful. In attempting to click both an azide and a thiol to this polymer, purification between steps proved difficult due to the "half-grafted" nature of the polymer.

Materials

L-glutamic acid was purchased from Sigma Aldrich and purified via recrystallization. Both α-propargyl L-glycine and N-α-Fmoc-L-glutamic acid α-t.-butyl ester were purchased from Chem-Impex and used without purification. All other reagents were purchased from Sigma Aldrich or Acros Organics and used without purification.

All NMR spectra were obtained on Bruker 400 MHz NMR spectrometer. Circular dichroism was conducted on a Aviv Model 202 and polymer samples (1 mg/mL in water) were passed through a 0.45 μm filter and measured in a quartz cell (1 mm path length, New Era). Ellipticity was measured over the wavelength range of 195 to 250 nm at 25 °C.. All gel permeation chromatography was conducting using a Waters 1525 with refractive index detecto. Polymer solutions at a concentration of 5 mg/mL in DMF were passed through a 0.45 μm filter and run at 75 °C with DMF as eluent.

Conclusions

We are working towards creating a large set hydrogels with a variety of mechanical and structural properties using clickable synthetic polypeptide systems. The physical and chemical characteristics of these hydrogels will be controlled by the addition of different functionalities through the use of click chemistry. This will allow us to create a wide variety of hydrogels based on a few conveniently synthesized polypeptide systems. By using this diverse set of hydrogels as cell scaffolds, we can study how different mechanical and chemical cues affect cell behavior and eventually be able to selectively tune cell development. I investigated the use of PPLG for sel-assembling systems. Currently, we are synthesizing hydrolytically stable clickable polypeptides and polypeptide systems with orthogonal click chemistry capabilities.

Outreach

One of the most important things that scientists can do is communicate their research and love of science to the public. We have a responsibility to help others understand our work and incite a sense of wonder at the natural world. In order to help young students understand science and appreciate what science can do for them and the world at large, I led and participated in several long-term outreach activities during my graduate studies.

Science Fair

Science fair was a major influence on me as a young student just beginning to understand the depth and breadth of current science research. As such I feel it is important to give back to this transformational institution that allows students to

investigate a problem of their choosing using the scientific method, thereby building problem solving and laboratory skills. While I was at MIT, I mentored two science fair students at Braintree High School participating in their school science fair. Additionally, I mentored six students (two groups of three students) at East Boston High School who participated in the East Boston High School Science Fair, Boston Citywide Science Fair, and Massachusetts State Science and Engineering Fair.

I assisted the first group of students in building a 3D printer, specifically, a Makerbot Thing-o-matic. The students constructed the Makerbot using a kit and then investigated different ways to improve the performance of the Makerbot. I was able to discuss polymer science with the students as the Makerbot used the thermoplastic acrylonitrile butadiene styrene as a building material. These students received first place at the East Boston High School Science Fair and the Boston Citywide Science Fair. Additionally, they received an honorable mention at the Massachusetts Science and Engineering Fair and a special award from Intel.

The second group of science fair students I mentored at East Boston High School built a 3D scanner using a portable projector and investigated methods to improve the scanning accuracy. They also incorporated the 3D printer into their project by attempting to take their 3D scans and print miniature versions using the printer. This team won second place at the East Boston High School Science Fair and first place at the Boston Citywide Science Fair.

In addition to mentoring science fair students, I also participated as a judge at the East Boston High School Science Fair and a judge for middle school students at the

Boston Citywide Science Fair. I also write for the Massachusetts State Science and Engineering Fair's blog, Inquiry First.

East Boston Environmental Science Mentoring Program

Many Boston schools do not have potable water, including East Boston High School and the nearby Mario Umana Academy which serves students in grades K-8. Students must bring their own water or use water from 5 gallon water jugs distributed across the school. Students often use small disposable cups provided by the school or bring their own disposable plastic water bottles. East Boston High School and Umana Academy did not provide any means for students to recycle these disposable containers. In the 2012 – 2013 school year myself and Dr. Kristen Cacciatore, a chemistry teacher at East Boston High School, began a recycling program at the Umana Academy with support from the MIT Public Service Center and the MIT Community Service Fund. During this program, East Boston High School students visited the middle school and mentored a class of 6^{th} graders that led the recycling program in the 6^{th}, 7^{th}, and 8^{th} grades. Over the course of two weeks, the students collected over 1,000 water bottles. Additionally, both the high school students and middle school students attended field trips to Beyond Benign, a local environmental chemistry research laboratory and a recycling facility.

East Boston High School AP Chemistry Recycling Program

In 2012, I helped develop an end of term project for the AP Chemistry class at East Boston High School. I led a team of undergraduate and graduate volunteers from Harvard and MIT to assist the AP Chemistry class run a battery recycling campaign with support from the MIT Committee on Race and Diversity. The AP Chemistry class was

able to collect over 1,000 batteries and educate over 250 students on the importance of battery recycling at their kick-off event. This year, I am assisting the AP Chemistry class run a water bottle recycling campaign in conjunction with the East Boston Environmental Science Mentoring Program.

Citizen Schools

Citizen Schools is a nationwide non-profit based that started in Boston in 1995. They focus on extending the learning day for middle school students by providing extra academic support, college and career counseling, and through their apprenticeship program. During a Citizen Schools apprenticeship a professional in a field leads a class of approximately 15 students for 90 minutes once a week for ten weeks, culminating in a presentation known as a WOW!. This "Citizen Teacher" is able to choose from a selection of premade curricula of develop their own.

In the fall of 2012, I developed my own chemistry-based curriculum and taught it to a class of 6^{th} and 7^{th} graders. The class focused on the students performing hands-on science activities that allowed me to cover basic chemistry concepts. My apprenticeship was very well received and was selected for the Citizen Schools Catalyst Launch Event, Fall 2012 Boston WOW! Showcase and the 2013 WOW! Affair. I also assisted with a Spring 2013 Solar Cars apprenticeship.

Chembites

In early 2011, I founded Chembites (www.chembites.org), a blog dedicated to taking recent chemical literature and writing summaries that are accessible to undergraduate students. I have led a team of 15 senior undergraduate and graduate

students writing blog posts. Additionally, I contributed dozens of articles myself and also presented our work at the 2012 Biennial Conference on Chemical Education.

Communicating Science Conference: A Workshop for Graduate Students

I am the co-chair for the 2013 Communicating Science Conference (ComSciCon). This conference is the first science communication conference organized by authors of Chembites and Astrobites and one we hope will become an annual event. The workshop will bring together 50 science graduate students from around the country to improve their communication skills by writing, receiving peer and expert feedback, and participating in panel discussions with journalists, educators, and authors.

Biographical Note

Shannon Morey was born in Flint, MI to parents John and Shellee Morey and lived out her early life in Lum, MI. She attended Imlay City Community Schools from kindergarten through high school. She graduated from Imlay City High School in 2006 having been inspired to pursue the sciences by many wonderful teachers, including Mr. Robert Beebe, Mr. Thomas DeClark, and Mr. Jeffery Gartrell, a recipient of a 2012 MIT Inspirational Teacher Award.

She attended Michigan State University having received an Alumni Distinguished Scholarship award and on the first day of Calculus II met her future husband, Nathan Sanders. A chemistry major throughout her time at Michigan State, Shannon graduated in 2010 with high honors from the College of Natural Science and Honors College. She served for four years as a volunteer with Michigan State University's student-run outreach organization Science Theatre, including two years as its Director. Her work was recognized with the 2009 Michigan State University Student Leader of the Year Award.

In 2010, Shannon came to MIT as National Science Foundation Graduate Research Fellow. As a graduate student in the Department of Chemistry, she has embraced the MIT community as a Graduate Community Fellow for Women's Programs in the Office of the Dean for Graduate Education and as a leader in science outreach and education initiatives throughout the Boston area. She has led education programs in environmental science and mentored science fair projects at East Boston High School for two years, has volunteered as a Citizen Teacher at Dever-McCormack Middle School in Dorchester for two semesters, worked in several Boston-area schools as an instructor for Science From Scientists, and founded a student organization, Chembites, devoted to making cutting-edge research in chemistry more accessible.

After graduation Shannon, she will devote her career to K-12 science outreach and education. She will begin by continuing her work with the Boston-area non-profit organization Science from Scientists. She lives together in the Greater Boston Area with her husband, Nathan Sanders.

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