Applications of Orthogonal "Click" Chemistries in the Synthesis of Functional Soft Materials

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1. Introduction

Applications for advanced functional soft materials that possess precisely engineered properties and functional groups have been expanding significantly with the development of nanotechnology and the growing need to address resource, health, and energy issues. Demand in a range of industrial and research settings are increasing the necessity to construct soft material systems with precise control over architecture, domain size, functionality, polarity, solubility, and reactivity. In addition to these materials and structural requirements, researchers have also sought to prepare these systems via high yielding, simple covalent chemistry. Much of the inspiration for constructing such complex, multifunctional materials can be found in a combination of fundamental organic chemistry, biology, and bioconjugation chemistry; these motivations in tandem with the recent advances in controlled polymerization techniques have created a growing research area with concentration on robust, efficient, and orthogonal (REO) approaches for new soft material preparation.

The aim of this review is to summarize and highlight the rapidly expanding area of applied REO chemistry for the preparation and functionalization of soft materials. The emphasis of this review is centered on soft materials chemistry, with a primary interest in functional polymer preparation and the modification of two-dimensional polymer surfaces and three-dimensional polymer objects of nanoscopic dimensions. The exploitation of this chemistry for the orthogonal modification of biological entities, such as peptides, proteins, enzymes, and viral systems, will also be

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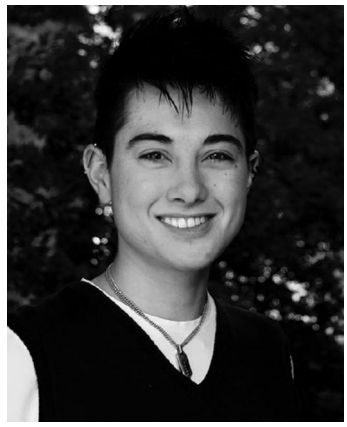

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Karen L. Wooley received a B.S. in Chemistry from Oregon State University in 1988 and then studied under the direction of Professor Jean M. J. Fréchet at Cornell University, obtaining a Ph.D. in polymer/organic chemistry in 1993. She then began an academic career as an Assistant Professor of Chemistry at Washington University in St. Louis, Missouri, and was promoted in 1999 to Full Professor with tenure. In 2006, Karen was installed as a James S. McDonnell Distinguished University Professor in Arts & Sciences at Washington University and was granted a joint appointment in the School of Medicine, Department of Radiology, in 2007. In 2009, she moved to Texas A&M University, as the W. T. Doherty-Welch Chair in the Department of Chemistry. Research interests include the synthesis and characterization of degradable polymers, unique macromolecular architectures, and complex polymer assemblies, and the design and development of well-defined nanostructured materials. Karen currently serves as an Editor for the *Journal of Polymer Science, Part A: Polymer Chemistry*.

Andreas M. Nyström received his M.Sc. in Chemical Engineering and Polymer Technology in 2002 from the Royal Institute of Technology (KTH) in Stockholm, Sweden, after which he continued with his Ph.D. in dendrimer chemistry under the guidance of Prof. Anders Hult at KTH. In 2006, Andreas graduated with a Ph.D. (engineering) and joined the group of Prof. Karen L. Wooley at Washington University in Saint Louis for a two-year postdoctoral position, financed by a distinguished assistant professor and postdoctoral grant from Knut and Alice Wallenberg foundation. In 2009, Andreas joined the faculty at Karolinska Institute of Medicine as an assistant professor working with polymeric nanosystems for cancer therapy and detection.

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addressed. Not included in this review is the preparation of inorganic and inorganic hybrid materials and their modification, as well as the functionalization of soft materials via noncovalent strategies.

This review is divided into several sections that highlight applications of orthogonality for a wide range of soft material syntheses. We begin with aspects of control over individual polymers in solution and then continue to the use of REO chemistries for modification of three-dimensional objects, two-dimensional substrates, and biological entities. The majority of the discussion involves demonstrations of REO chemistry for the preparation of well-defined, discrete macromolecules or nanoscale structures, and there are commonalities in the types of reactions employed throughout the various materials preparation and functionalization examples.

Section 2 highlights the synthesis and functionalization of polymers of varying architectures, including linear, graft, star, and branched structures, with emphasis on differentiation of regions within the macromolecular frameworks. An initial focus on linear polymer preparation via functional initiators that allow for control and further modification of chain ends (especially in conjunction with controlled polymerization techniques) leads next to control over the backbone composition and properties by the preparation of monomers bearing both polymerizable groups and functional groups. Stepwise and simultaneous modification reactions that allow for further introduction of functionality permit the preparation of such materials and are an important step in the realization of designer polymers for advanced applications. Block copoly-

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Craig J. Hawker was born in 1964 in Toowoomba, Australia, and is currently the Director of the Materials Research Laboratory at the University of California, Santa Barbara, where he is also a Professor in the Materials, Chemistry and Biochemistry departments. After undergraduate education at the University of Queensland, he completed a Ph.D. in 1988 at Cambridge University (supervisor Professor A. R. Battersby). Shifting to the world of polymer chemistry, he undertook a postdoctoral fellowship with Prof. Jean Fréchet at Cornell University from 1988 to 1990 and then returned to the University of Queensland as a Queen Elizabeth II Fellow from 1991 to 1993 before spending 12 years as a research staff member at the IBM Almaden Research Center. His research has focused on the interface between organic and polymer chemistry with emphasis on the design, synthesis, and application of well-defined macromolecular structures in biotechnology, microelectronics, and surface science.

mer synthesis and functionalization through the utilization of end groups as well as side groups of the polymer chain have also been driven by robust and efficient chemistry. The preparation of such materials is key to developing new applications in research areas such as electronics and biomedicine, where precise control and placement of functionality is of critical importance for their successful utilization. With an emphasis on the introduction of functionality, secondary modification reactions on materials as a strategy for the incorporation of functional groups in an orthogonal manner are examined. Similarly, the application of orthogonal chemistry in the area of polymeric nanosystems such as branched, dendritic, and star copolymer preparation, dendronized polymers, and block−graft copolymers are described.

Nanoparticle preparation and functionalization has enabled significant breakthroughs, and the modification of these structures with biologically active components is a particular highlight (section 3). Such efforts are the emphasis of numerous research groups worldwide and may be important in the development of new, safe, and effective detection systems and pharmaceutics. The application of orthogonal strategies for the construction of two-dimensional surfaces and the modification of such surfaces and interfaces further demonstrates the scope of this chemistry (section 3). Such orthogonality allows for the creation of patterned and nonpatterned surfaces with precise control over the number and nature of functional groups. The ultimate test of these concepts is the orthogonal functionalization of biological systems (section 4) where peptides, proteins, enzymes, and viral particles or containers have been modified with polymeric materials, resulting in synthetic polymer−biological chimeras.

The significant breadth and depth of this research clearly demonstrates the increasing importance of the development of complementary methods for synthesizing large complex molecules as the diversity in function and chemical nature of advanced materials grows. Success in this area will necessitate that robust, efficient, and orthogonal (REO) techniques be used for the synthesis of highly modular materials, permitting the properties of a material to be studied over a continuum of functionalities, architectures, and molecular size. The applications for these materials and techniques span the range of soft materials from medical imaging and drug delivery to energy capture and storage. The fundamental and practical achievements of the past decade will likely continue to contribute to increasing quality of life across the globe as they satisfy a diverse spectrum of needs.

This review aims to highlight the important advances made predominantly in the last 10 years in the area of soft material preparation and orthogonal functionalization with key findings and directions selected to illustrate the underlying potential, current challenges, and future directions. The most well-known of REO reactions, the Cu-catalyzed azide/alkyne cycloaddition (CuAAC) reaction, will be discussed along with other increasingly popular REO reactions, such as thiol−ene, oxime, Diels−Alder, and pyridyl disulfide reactions, as well as select examples of Michael additions and activated ester couplings (Figure 1). These REO reactions satisfy the basic philosophy of "click" chemistry, being selective and high yielding under mild conditions with little or no byproduct. In addition, section 2 includes a discussion of controlled polymerization techniques because, while they may not appear to strictly fit the description of REO chemistry, they provide efficient routes to complex polymeric structures and are often orthogonal with each other and many other REO reactions. Ultimately, this review will attempt to demonstrate that because of the challenges associated with chemical reactions on macromolecular scaffolds, nanostructures, surfaces, or biological substrates, including the large number of sites for functionalization, the large size of the molecular structure or material, and the various types and numbers of other chemical subunits, the development of REO chemistry has been critical to the production of well-defined and multifunctional systems that are either fully synthetic or synthetic−biologic hybrids.

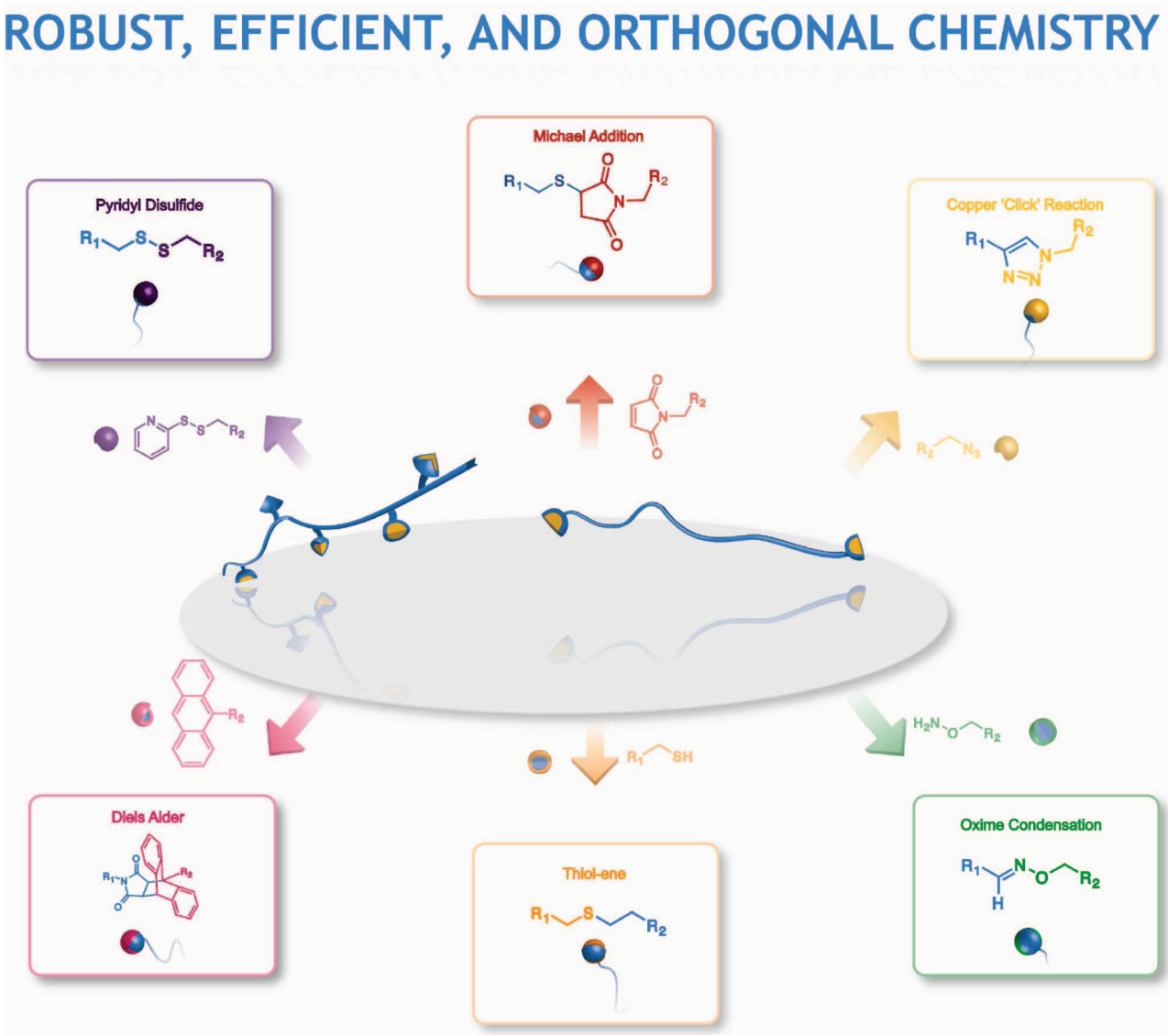


Figure 1. This review highlights examples employing click chemistry for the preparation and functionalization of complex polymer materials, three-dimensional substrates, two-dimensional surfaces, and biological systems. The types of chemical reactions include those that involve reactive functional groups, which undergo efficient, orthogonal couplings (REO chemistry), selectively and in high yield with limited byproduct.

2. Polymers from REO Chemistries

2.1. Chain End-Functional Homo- and Copolymers

2.1.1. Mono-End-Functional Polymers

End-functional polymers constitute one of the simplest functional macromolecular architectures, containing only one functional group at a single polymer chain end. Despite this simplicity, end-functional polymers are a vital starting point for the synthesis of a wide diversity of functional soft materials, including a significant number of more complex polymeric structures. For example, two functionalized polymers each possessing one chemically unique end-group capable of reacting only with the chain end on the other polymer allow for covalent coupling to afford a diblock copolymer.[1] Similarly, end-functional homopolymers are also instrumental in the synthesis of multiblock copolymers, graft copolymers, star-shaped architectures, and cross-linked networks.[2] The success of soft material synthesis through the use of end-functional polymers is predicated upon the high fidelity of end group incorporation followed by highly efficient and specific modification and coupling reactions. Using literature examples, we will demonstrate the potential for robust, efficient, and orthogonal click reactions to construct a variety of interesting or useful functional mono-end-functional materials, with functional initiator or post-polymerization modification strategies (Figure 2). Due to the special demands placed upon reactions occurring at a polymer chain end, attention will be paid to functionalities that offer the highest degree of reactivity and efficiency, while also being compatible with current controlled polymerization methods.

In general terms, the use of a well-defined initiator in controlled polymerizations not only affords control over molecular weight and polydispersity but also leads to one chain end being derived from the initiator and the other from termination of the propagating chain end or from modification of the mediating radical group if it is a living free radical process.[3–11] As a result, two distinct end groups are typically obtained, the α-chain end from the initiator and the ω-chain end from the propagating species. Ultimately, the presence

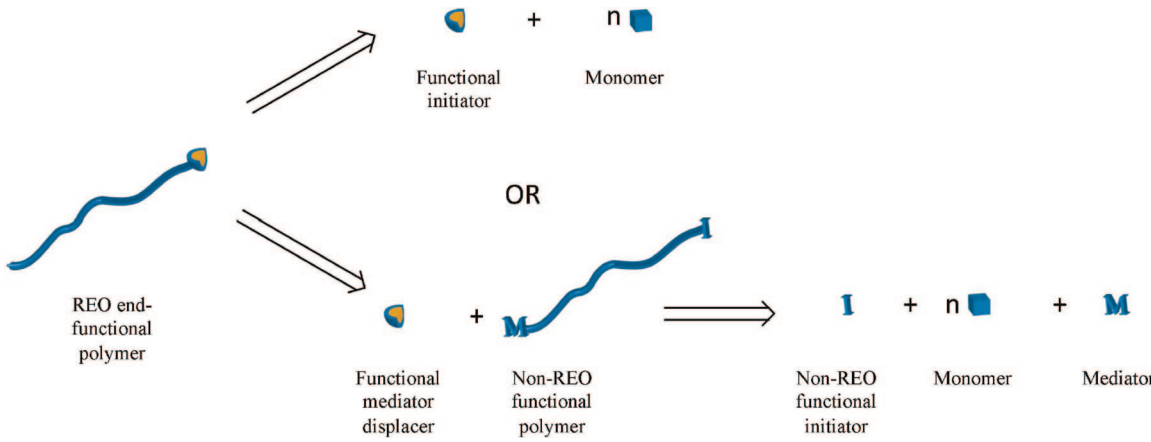
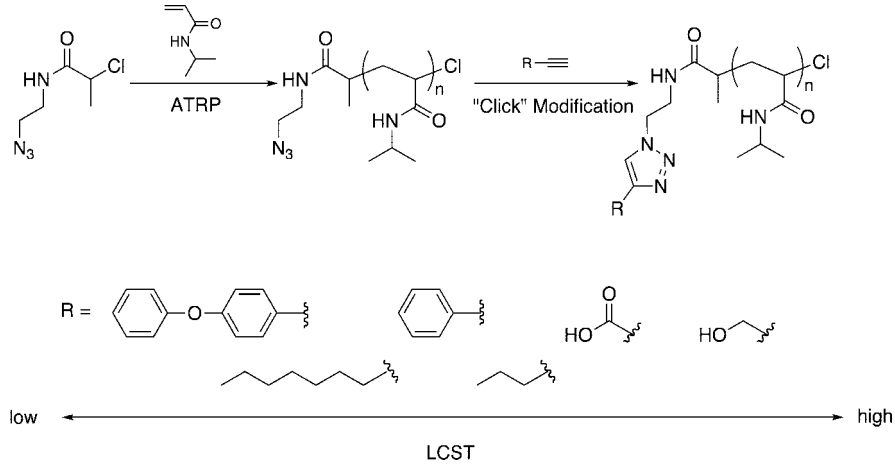


Figure 2. Retrosynthetic analysis for a homopolymer containing a single REO reactive group at one chain end. In this example, a mediator represents some species that controls polymerization at the active end of a polymer chain (e.g., halogen in ATRP, nitroxyl in NMRP, catalyst in ROP/ROMP), and a "nonfunctional" initiator is one that does not include a moiety capable of undergoing a REO transformation.

Scheme 1. Modification of the Chain Terminus of P(NIPAM) Polymers Derived from Functional Initiators and Postpolymerization CuAAC Chain End Modification[a]



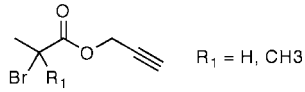
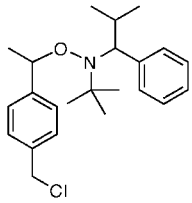
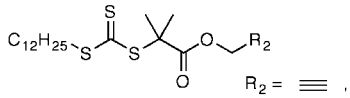
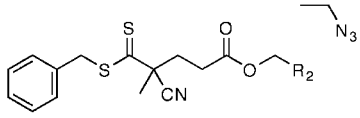
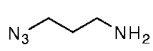
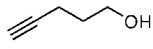
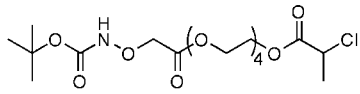
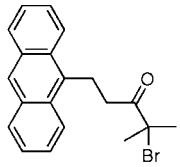
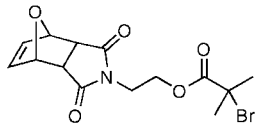
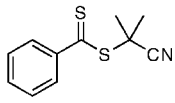
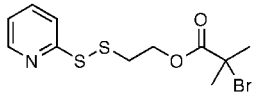
[a] This study revealed significant effects of only changing the end group on the LCST, which varied by over 10 °C.[37]

of well-defined, if not functional, chain ends allows for the manipulation of the polymer chain ends by design[12] with many examples of functional initiators being used in atom transfer radical polymerization (ATRP),[13–15] reversible addition−fragmentation chain transfer (RAFT) polymerization,[16–19] nitroxide-mediated polymerization (NMP),[20,21] and ring-opening polymerization (ROP)[22–24] to give a desired end-group directly. In addition to initiators bearing reactive moieties, chemical modification of the ω-chain end can introduce a chemical handle for postpolymerization incorporation of other polymer chains and small molecules. Both methods are effective for producing polymers with functional chain ends and, if employed in conjunction with one another, can lead to telechelic polymers with unique reactive groups at both the α- and ω-chain termini.

Polymerization initiators with pendant chemical handles are often the most simple and efficient way to incorporate a desired chain end. Assuming chemical orthogonality of the handle relative to the polymerization technique and employment of a polymerization method that displays living character, functional initiators can offer (near) quantitative introduction of a wide variety of reactive groups. Indeed, the breadth of chemical groups that have been introduced at the chain ends through the use of functional initiators in conjunction with modern controlled polymerization techniques is staggering and ranges from the incorporation of small functional groups to be used in subsequent reactions, such as organic azides,[25] to significantly more complex moieties, such as peptides or multiple hydrogen-bonding groups.[13] It is important to note that functional initiators have been employed for the synthesis of polymers by ATRP,[26–49] NMP,[48,50–53] and ROP,[2,53–68] and functional handles have been incorporated into chain transfer agents (CTAs) for use in RAFT polymerizations (see Table 1).[38,69–75] There have also been examples of modified ring-opening metathesis polymerization (ROMP) catalysts that can provide chain end functionality, but in this case, a significant synthetic cost in the design of the catalyst/initiator is required.[76] Many of the functional initiators and transfer agents incorporate groups at the chain terminus that can be used subsequently for many of the fundamental REO reactions discussed in this review, and the synthesis of complex polymeric topologies often arise from linear polymers bearing functional chain ends derived from tailored initiators.

One of the most popular initiator-based approaches to obtain end-functional polymers involves the combination of functional initiators with ATRP.[77] Some early examples of azide- or alkyne-functionalized initiators for ATRP were documented by the groups of Haddleton,[43] van Hest,[1] and Matyjaszewski.[29] A simple transformation involving poly-

Table 1. Examples of Well-Studied Initiators for Incorporation of Different REO Functional Groups Using a Variety of Controlled Polymerization Techniques

Initiator Structure	Polymerization Method	Deprotection or Modification Required?	ROE Rxn	Ref.
	ATRP	NO	CuAAC	1,13,28-31
	NMP	YES	CuAAC	50,52,299
	RAFT	NO	CuAAC	74,75
	RAFT	NO	CuAAC	61,69
	ROP	NO	CuAAC	58,67,100
	ROP	NO	CuAAC	32,59,64
	ATRP	YES	Oxime	39,40
	ATRP	NO	Diels-Alder	32-35, 45, 49,178,179
	ATRP	NO, YES	Diels-Alder, Maleimide	32-35,42-45, 47,178, 179
	RAFT	YES	Thiol-ene	181
	ATRP	NO	Pyridyl Disulfide	98,103

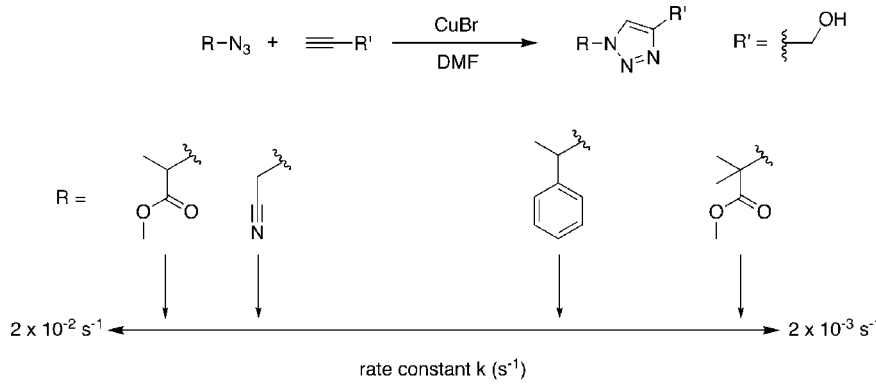
mers with end-functional REO handles is the covalent attachment of small molecules, which many research groups have demonstrated through the addition of chromophores, allowing the efficiency of reaction and level of chain end incorporation to be determined.[38] A recent example from Narumi et al. demonstrates the potential power of attaching small molecules to the chain end of a well-defined polymer.[37] In this study, an azide-functionalized ATRP initiator was used to grow poly(*N*-isopropylacrylamide) (P(NIPAM)), which traditionally has an lower critical solution temperature (LCST) of approximately 32 °C. By attaching small molecules with varying polarities to the end of the P(NIPAM)

Table 2. Small Molecule Agents for Introduction of REO Functional Groups at the ω-Polymer Chain End by Either Termination of Controlled Polymerization or Postpolymerization Modification[a]

Modifier Structure	Polymerization Method	Functional Group Installed	Modifier or Terminator	REO Rxn	Ref.
NaN_3	ATRP	Azide	Modifier	CuAAC	1,13,27-29, 33,36,48,54, 56,63,79-90
	ROMP	Aldehyde	Terminator	Oxime	97
	ROP	Maleimide, Alkyne, etc	Terminator	Michael addn, Click, etc.	182
$R-NH_2$	RAFT	Thiol	Modifier	Thiol-ene	181

[a] In the final example, the amine used to free a thiol by aminolysis is not incorporated into the polymer end group.

Scheme 2. Small-Molecule Azides Representative of Acrylic, Cyanoacrylic, Styrenic and Methacrylic Polymer Chain Ends Are Used to Probe Reactivity of Azide Terminal Polymers Made by ATRP and Halogen Displacement[78]



via copper-catalyzed alkyne−azide cycloaddition (CuAAC) reactions, the authors were able to modify the LCST of the polymer over a temperature range of 10 °C, which is significant given the high molecular weight of the polymer chain (Scheme 1). The high efficiency of the CuAAC modification was determined using NMR spectroscopy and matrix-assisted laser desorption−ionization time-of-flight (MALDI-TOF) mass spectrometry. By use of a functional initiator, a series of polymer samples could be prepared that differed only in the chemical moieties present at the chain end, eliminating the need to create a series of initiators and also removing the inherent variability in molecular weight and polydispersity that occurs between different polymerization reactions.

Another common method for adding chain-end functionality to linear polymers involves modification of the ω-chain end of the polymer, through termination of the living polymer chain with a functional terminating agent or by chemically altering the dormant mediating radical functionality, present at that chain end (Table 2). This method potentially provides a simpler alternative for achieving a single functional handle at one chain end because it does not require a unique initiator or catalyst to be prepared. Additionally, this methodology can be combined with a functional initiator leading to telechelic polymers bearing a unique functional group at each chain end.

While a variety of chemical groups associated with REO transformations have been incorporated using this general strategy, by far the most well-known and commonly used approach is the displacement of the terminal halogen atom of polymers prepared by ATRP with sodium azide.[1,13,27–30,33,36,48,54,56,63,79–89] This method offers an extremely simple route to high-fidelity end-functional polymers for subsequent modification by CuAAC. The influence of the polymeric repeat unit on the efficiency of chain end modification was recently probed by the Matyjaszewski group through a study using azido-based model compounds to compare the rate of the functionalization reaction with the nature of the monomer unit from which the terminal halogen was displaced.[78] While simplified schematics might imply that all azide-terminated polymer chains are equal and some reports have hailed this technique as a general method for derivatizing polymer chain ends, it is important to consider the inherent differences in reactivity and rate based upon the structure of the azide. Scheme 2 shows the different model compounds that were chosen to represent and study styrenic, acrylic, methacrylic, and acrylonitrile-based polymers. This study showed that the rate of triazole formation depends on both the electronic properties of the azide and the steric environment. A significant outcome of this study is the demonstration that monomer choice must be carefully considered if CuAAC is to be used for the synthesis of functional polymeric materials. For example, Scheme 2 illustrates that the methacrylate-type

model compound undergoes reaction with a rate constant that is ca. 10 times smaller than that of the acrylate-type model compound, which would suggest that replacement of a poly(methyl methacrylate) (PMMA) chain end would reach 90% conversion ten times slower than would a poly(methyl acrylate) (PMA) chain end. In addition to the monomers used, the same authors have also studied the effect of different CuAAC catalysts on reaction performance at polymer chain ends.[90] It was determined that aliphatic amine ligands produced significant rate enhancements compared with pyridine-based ligands and that tridentate ligands increased the rate of reaction relative to tetradentate ligands. Finally, noncoordinating solvents were found to give greater rate enhancements compared with coordinating solvents.

Another polymerization technique that has benefited from REO reactions in diversifying the range of end-functional polymers available is ring-opening metathesis polymerization (ROMP). ROMP is an effective and prevalent technique used to produce well-defined macromolecules from strained cyclic olefin monomers. The Grubbs group demonstrated early in this decade that chain transfer agents were a simple and successful way to make end-functional and telechelic polymers via ROMP.[91–94] These methods simply and effectively yield functional chain ends but do so at the price of polydispersity and control over the polymerization process. More traditional strategies such as the use of chemically tailored catalysts are challenging due to the synthetic cost of making a variety of complex organometallic compounds.[76] These factors highlight the importance of ω-functionalization and the potential of REO chemistry. In 2006, Hilf et al. presented the sacrificial block approach to obtain low PDI and end-functional ROMP polymers, wherein a block copolymer is grown *in situ*, followed by controlled degradation of the second block to give an alcoholic chain end. While effective, this approach requires several extra synthetic steps to achieve a single alcohol moiety at the chain terminus.[95,96] A more recent report from the same authors provided a straightforward approach that would allow for extensive REO chemical transformations for chain end modification. By termination of a ROM polymerization with a vinylene carbonate, a Fischer-type carbene is linked to the polymer chain end by a carbonate linkage, which decomposes into an aldehyde chain by loss of CO_2.[97] The high degree of aldehyde incorporation at the chain end was confirmed by MALDI-TOF mass spectrometry and NMR spectroscopy. To further demonstrate the utility of this technique, the authors then chemically derivatized the aldehyde with 2,4-dinitrophenylhydrazine, indicating that, at a minimum, 97% of the polymer chains carried the desired end group. This approach provides a functional handle at the chain end for future functionalization of metathesis polymers through formation of REO linkers such as hydrazones or oximes, dramatically increasing the range of unique end-functional polymers available via ROMP.

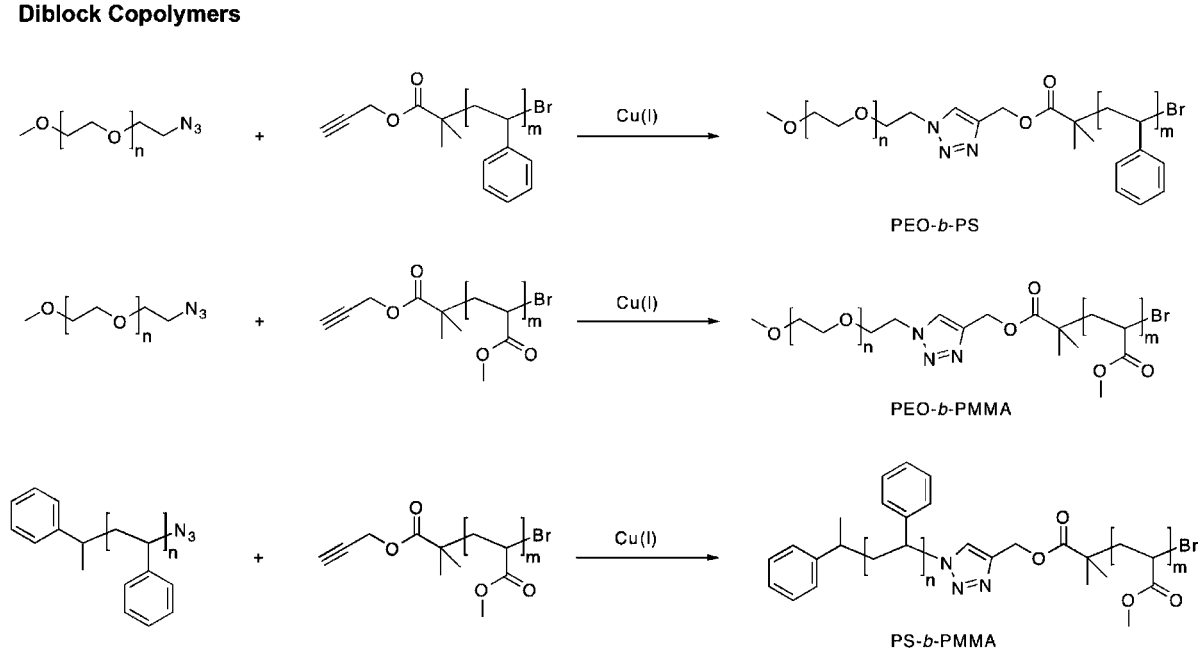
Another area of interest for polymers possessing a chain end functional group capable of undergoing REO chemical transformations is to exploit the fidelity and efficiency of the coupling step to construct a block copolymer by attachment of a second polymer chain. CuAAC has been used extensively in the synthesis of AB diblock copolymers, as well as more complex block copolymer structures, with great success.[1,13,26,32,50,56,58,99–102] One of the first examples of REO chemistry being used to create well-defined block copolymers by the joining of two end-functional homopolymers came from the group of van Hest, when they used alkyne-bearing ATRP initiators as well as azide displacement to create a series of complementary CuAAC-functional PMMA, polystyrene (PS), and poly (ethylene glycol) (PEG) homopolymers, which could be coupled to give diblock copolymers in high yields (Scheme 3, upper portion).[1] In a similar vein, Thayumanavan and co-workers prepared a series of diblock copolymers through the reaction of a thiol-terminated polymer with a pyridyl disulfide-terminated polymer, to form, in excellent yield, the corresponding disulfide-linked diblock copolymer (Scheme 3, lower portion).[98] The authors took advantage of a functional ATRP initiator to synthesize homopolymers containing a pyridyl disulfide group at a single chain end. For one of the homopolymers, the pyridyl disulfide was reduced by dithiothreitol (DTT) to afford a thiol, which was isolated and allowed to undergo reaction with the other homopolymer still bearing a pyridyl disulfide moiety. Using this approach, block copolymers with molecular weights of up to 20 kDa were made with low polydispersities and minimal homopolymer impurities. One benefit to this approach is that the disulfide linkage between the two blocks allows for selective cleavage of the block copolymers to recover the respective homopolymers under reducing conditions. More recently, Thayumanavan and Russell used this approach to make nanoporous thin films, where the walls of the pores are lined with thiol functionalities. The authors then demonstrated the thiol groups' utility by coating the pore walls with gold, providing a simple and mild route to a unique polymer−gold composite nanostructure.[103]

2.1.2. Telechelic Macromolecules

Telechelic polymers, specifically polymers bearing functional groups known to participate in REO transformations at both chain ends, are synthetically difficult to prepare but offer great opportunities for the creation of more complex polymeric architectures. Telechelic polymers can generally be classified into two structural types, the first has the same functional group at both chain ends (i.e., homobifunctional), while for the second class, the functional group at the α-chain end differs from that present at the ω-chain end (i.e., heterobifunctional). Three basic strategies have been developed for the preparation of these systems. The first involves the use of a bifunctional initiator, which, after polymerization and termination or chain end modification, affords a homobifunctional telechelic polymer (Figure 3, middle portion). Another method, though not utilized as extensively as the two alternative methods discussed here, has potential to be an interesting future technique for creating homobifunctional telechelic materials; this approach mandates the use of functional initiators, leaving the desired reactive group at the α-chain end followed by combination of the ω-chain end of two polymers, resulting in a telechelic polymer whose molecular weight is twice that of the original end-functional polymer (Figure 3, lower portion). The final method is the most obvious and perhaps the most effective method for synthesizing telechelic polymers. It employs a functional initiator during polymerization followed by either quenching the polymerization with a terminating agent or replacement of the dormant modifier with a reactive moiety to achieve a heterobifunctional polymer (Figure 3, upper portion).

In 2005, the Matyjaszewski group presented a simple yet elegant approach to making telechelic polystyrene through azide displacement of the terminal ATRP halogen atom.[104] In this

Scheme 3. Formation of Diblock Copolymers by CuAAC and Cleavable Diblock Copolymers Using Disulfide Chemistry[1,98]



publication, Gao et al. utilized a difunctional initiator to grow polystyrene in both directions from a central core and upon displacement of the bromine atoms with sodium azide, propargyl alcohol was placed onto both chain ends of the polymer by CuAAC to give a bis(hydroxyl-terminated)polystyrene derivative (middle panel approach of Figure 3). Interestingly, the authors found that for a single polymer chain, the rate of reaction of the first chain end is 3 orders

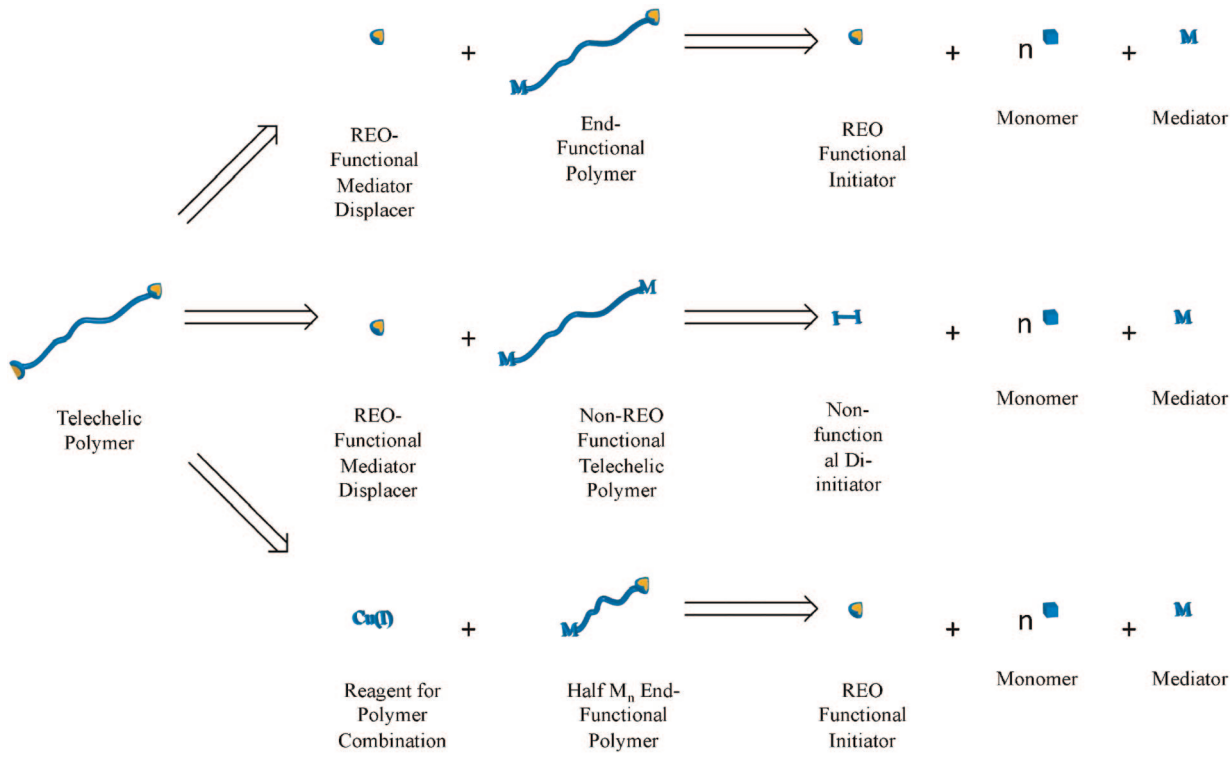
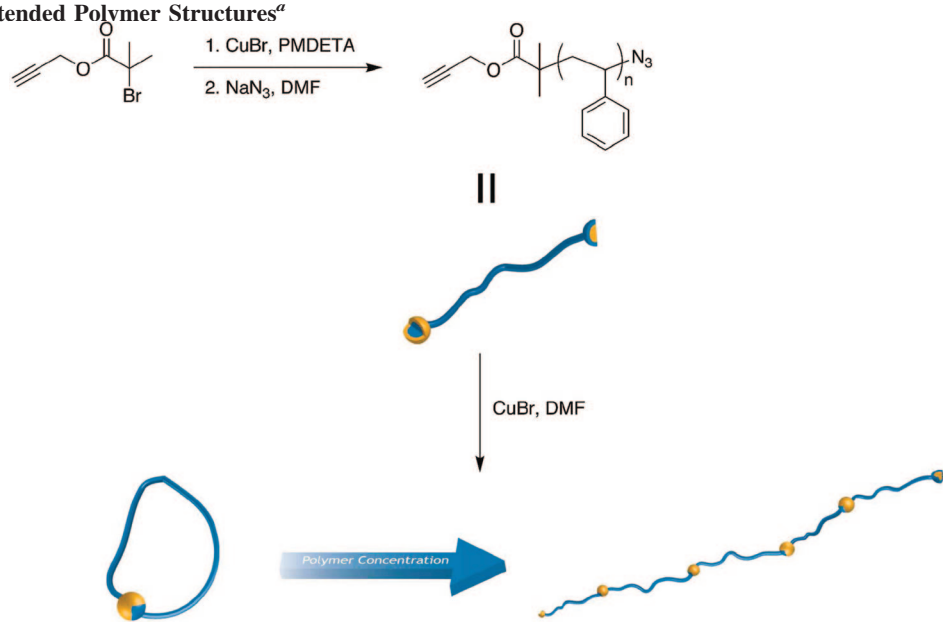


Figure 3. Retrosynthetic analysis for a homopolymer containing REO reactive groups at both chain ends. The upper method can produce heterobifunctional materials, while the bottom two methods can only successfully make homobifunctional polymers. In this example, a mediator represents some species that controls polymerization at the active end of a polymer chain (e.g., halogen in ATRP, nitroxyl in nitroxide-mediated radical polymerization (NMRP), catalyst in ROP/ROMP), and a "nonfunctional" initiator is one that does not include a moiety capable of undergoing an REO transformation. In the case of ATRP, the halogen, which serves as the mediator, can be replaced by a nucleophile to give some useful end-group that can undergo an REO transformation (e.g., organic azide).

of magnitude higher than the reaction rate for the second chain end. Regardless, they were able to install an alcohol group at 97% of both chain ends after 8 h of reaction time. This work has served as a template for the synthesis of telechelic materials by a difunctional initiator plus REO reaction approach.

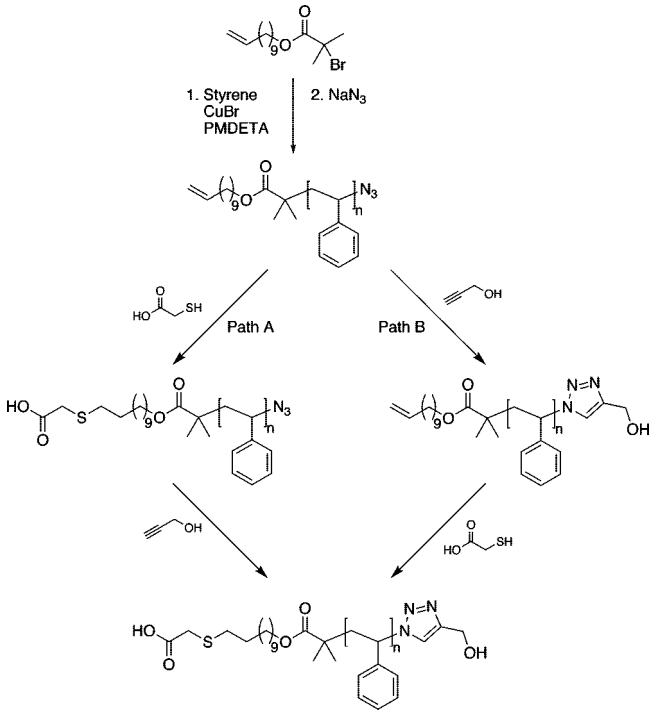
The second approach to homobifunctional materials was used by Kopping et al. to synthesize polystyrene bearing aminooxy groups[40] at both termini.[39] In this report, end-functional polystyrene was dimerized by coupling of the ω-chain ends by atom transfer radical coupling (ATRC) (lower panel approach of Figure 3). This technique takes advantage of the halogen atom in a different manner, by altering atom transfer reaction conditions to encourage radical−radical combination of two polymer chains. The authors were able to demonstrate high reaction efficiency both in the polymer−

Scheme 4. Production of Heterobifunctional, Telechelic Polystyrene, Followed by Application of CuAAC Conditions, Affords Macrocyclic or Extended Polymer Structures[a]



[a] The polymer concentration determines whether the major product is cyclic polymer or chain-extended materials by a step growth mechanism.[28,29]

Scheme 5. Orthogonal Functionalization of the α- and ω-Chain Ends of a Polystyrene Derivative Using Orthogonal Thiol−Ene and CuAAC Chemistries[46]



polymer coupling step and during deprotection of the *N*-hydroxyphthalimide end group to give telechelic aminooxy polystyrene. Finally, a high yield of oxime coupling at both chain ends was demonstrated by reaction with a model aldehyde. Since this report, there have been other examples of telechelic materials by a similar chain−chain coupling of end-functional materials by ATRC.[105–108]

The final approach to constructing telechelic polymers is the most versatile with respect to creating soft materials of increasing complexity (upper panel of Figure 3). The use of functional initiators in combination with replacement or substitution of dormant chain ends provides a two-step route to either homobifunctional or heterobifunctional materials. The combination of CuAAC and ATRP, in particular, has made excellent use of this technique for the preparation of a variety of complex and functional polymeric architectures from heterobifunctional telechelic materials. The general approach employs an alkyne-functionalized initiator to synthesize a polymer bearing an alkynyl group at one end and a halogen at the opposite end. By subsequent performance of a nucleophilic displacement of the terminal halogen with sodium azide, a heterobifunctional telechelic polymer is obtained in two steps (Scheme 4). In an early report using this approach, Tsarevsky et al. synthesized short polystyrene chains with alkynyl and azido end-groups. These polymer chains were then treated as AB monomers by subjecting them to CuAAC conditions.[29] Upon reaction, significant polymeric growth was observed as M_w increased from 2 kDa to greater than 100 kDa. There was, however, a small and constant percentage (approximately 7%) of what appeared to be unreacted starting material, which the authors attributed to the formation of cyclic products. As such, Grayson et al. used comparable telechelic polymers for the deliberate synthesis of cyclic polymers by click cycloaddition.[28] Telechelic polystyrene bearing an alkyne at one chain end and an azide at the other was prepared using the same synthetic strategy, after which it was subjected to CuAAC conditions but under significantly more dilute reaction concentrations than those used by Tsarevsky and co-workers. In order to avoid excessive amounts of solvent, a continuous addition method was used so that the concentration of unreacted functional groups would remain low. In this way, the authors obtained, in excellent yield, cyclic polystyrene made from polymers with molecular weights of up to 4 kDa. MALDI-TOF mass spectrometry was used to demonstrate that the observed decrease in hydrodynamic volume by GPC was due to an architectural change (linear random coil to macrocyclic), as the MALDI-TOF-measured molecular weight and polydispersity of the polymer sample remained unchanged after reaction.

Utilizing a functional initiator to modify the α-chain end, while also terminating or modifying the ω-chain end, is a method that can be extended to synthesize both homobifunctional and heterobifunctional materials. One advantage of heterobifunctional polymers is the option to attach different functional groups to each chain end and to fully exploit this strategy; orthogonal functional groups that undergo different REO transformations allow optimal structural control. Following this strategy, either a one-pot, single-step reaction or a two-step reaction sequence leads to a polymer with unique functionalities at each chain end. An example of the potential for this approach is the two-step orthogonal functionalization of both ends

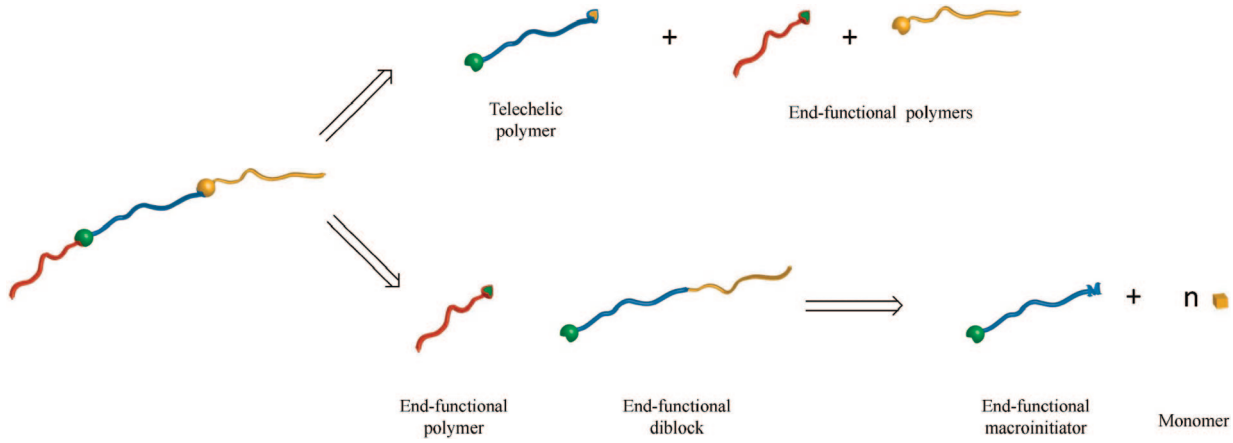
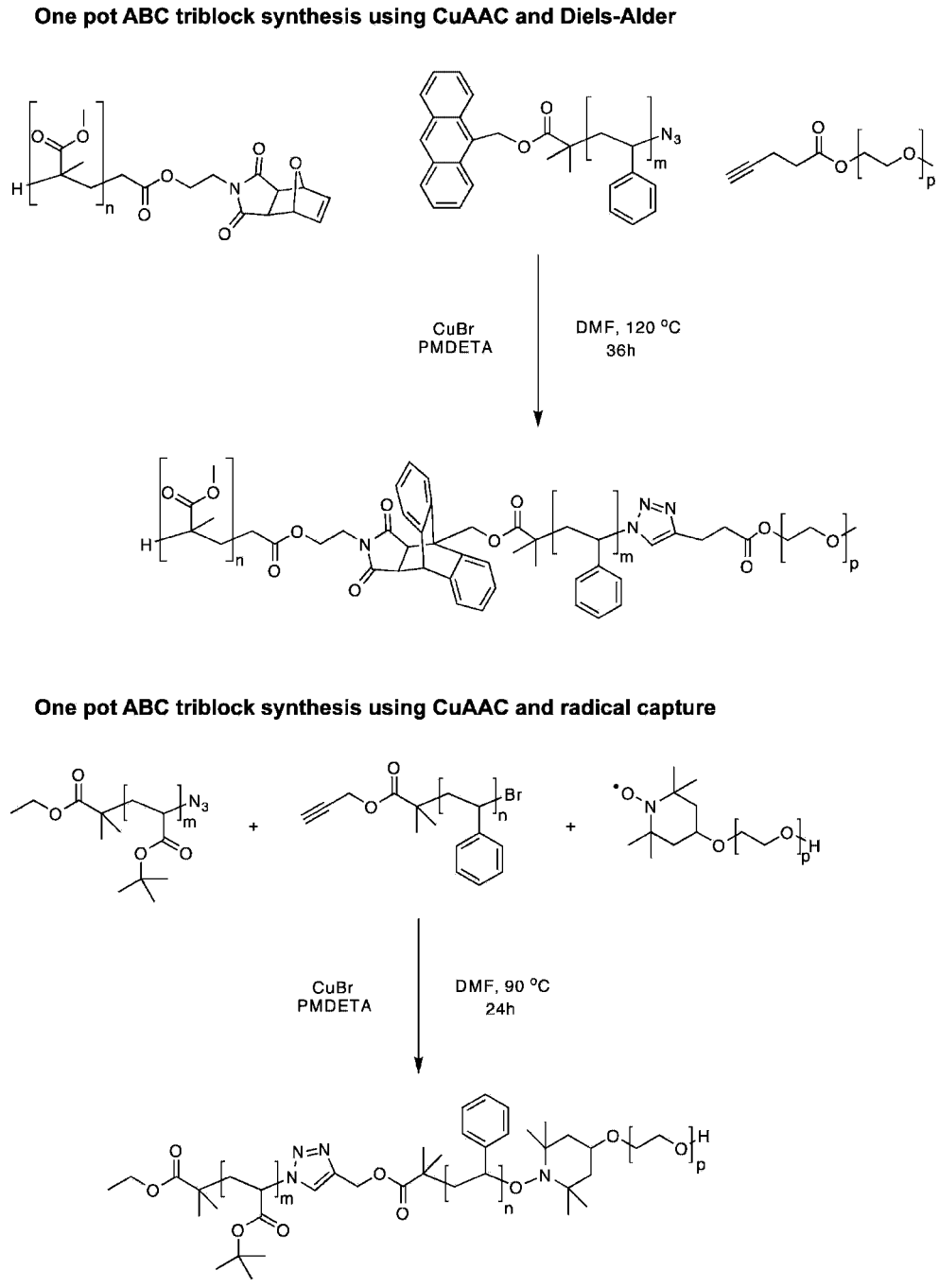


Figure 4. Retrosynthetic analysis for ABC triblock copolymers using REO chemistry by either a one-pot coupling of three unique mono- or heterobifunctional end-functionalized homopolymers (upper pathway) or a stepwise growth and coupling of a homopolymer and diblock copolymer, each having a single complementary reactive chain end (lower pathway).

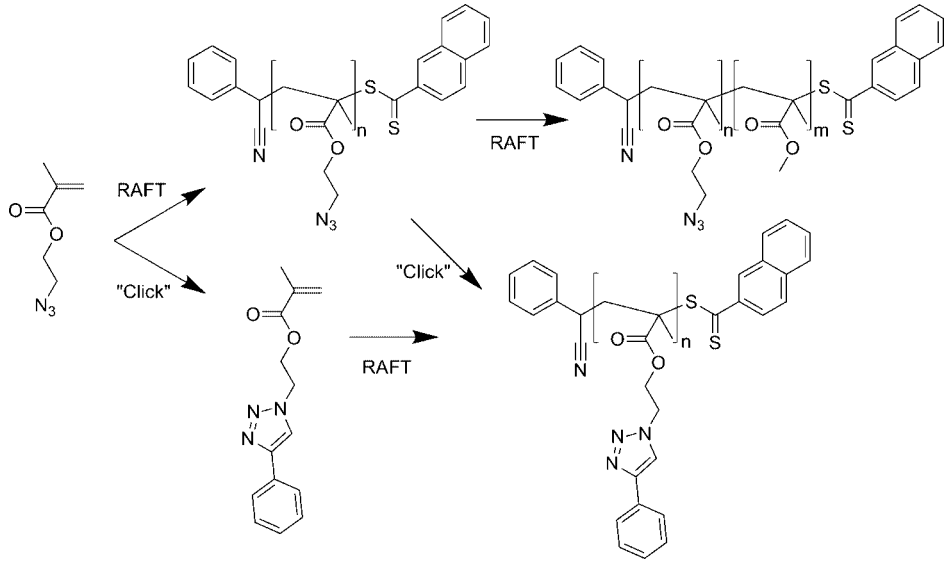
Scheme 6. Orthogonal Strategies Based on CuAAC Chemistry for the Preparation of ABC Triblock Copolymers in One Pot from Two Mono-End-Functional and One Heterobifunctional Telechelic Homopolymers[32,41]



of a block copolymer by Campos (Scheme 5).[46] By polymerizing styrene from an alkene-containing initiator by ATRP and subsequently displacing the bromine chain end with sodium azide, Campos and co-workers were able to test the efficiency of thiol−ene coupling using a variety of thiols and reaction conditions (thermal and photochemical), while also demonstrating orthogonal reactivity and quantitative functionalization of the chain ends by thiol−ene and CuAAC click chemistries.

In an extreme case, the two functionalities introduced at the chain ends of the heterobifunctional macromolecule can be polymeric blocks, which allows for the construction of triblock copolymers from end-functionalized linear polymer precursors (Figure 4). To illustrate this strategy, CuAAC has been used in combination with nitroxyl radical capture to synthesize ABC triblock copolymers in a one-pot reaction at low temperatures.[41] In this example, the CuAAC is used in conjunction with radical formation by atom transfer radical coupling conditions, where the radicals are captured by nitroxyl functional polymer to give a triazole linkage connecting the A and B blocks, and an alkoxyamine connecting the B and C blocks (Scheme 6, lower portion). This reaction sequence is possible due to the dual role of the copper(I) catalyst, which is active for both the CuAAC and the formation of radicals by bromine abstraction from ATRP (macro)initiators. Similarly, CuAAC has been combined with Diels−Alder cycloaddition of anthracene and a tricylic furan derivative to afford an ABC triblock copolymer in a one-pot reaction (Scheme 6, upper portion).[32]

Scheme 7. Demonstration of the Effectiveness of CuAAC Both before and after Polymerization[110]



2.2. High-Fidelity Functional Monomers

While chain-end functionalization of polymers offers routes to macromolecules decorated with functional groups at the backbone termini, greater levels of functional group incorporation is often desired. This is often possible through the copolymerization of functionalized monomers, but the incompatibility of many reactive functional groups with a variety of polymerization conditions is often a considerable synthetic challenge. As a result, facile methods for postpolymerization functionalization through REO chemistry is becoming an attractive alternative and offers a number of advantages compared with copolymerization approaches. REO reactions are ideal for side chain functionalization of polymers, because of the need for high yields to accomplish transformation of a majority of the monomeric repeat units.

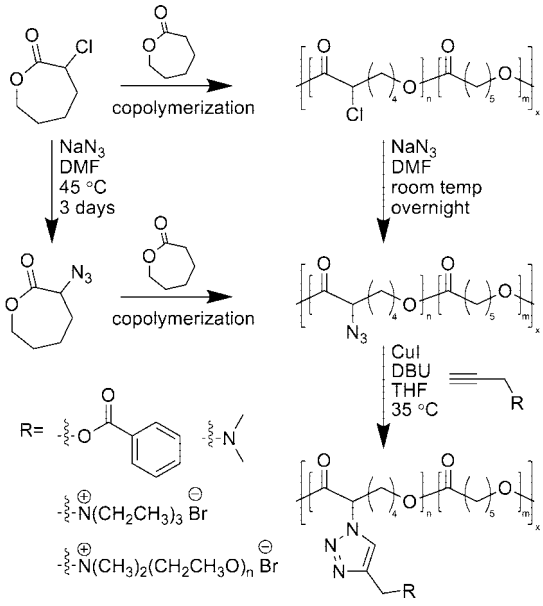
2.2.1. 1,3-Dipolar Cycloaddition

Due to the initial focus on CuAAC click chemistry, backbone-functionalized polymers bearing multiple alkyne and azide moieties have become synthetic targets for any number of applications. Unprotected alkynes often undergo radical side reactions, so protection is typically necessary in order to successfully incorporate them into copolymer structures. Sumerlin et al. initially observed a broadening of molecular weight distributions upon attempting to polymerize propargyl methacrylate, which ultimately required protection with a trimethylsilyl group.[109] Due to the extra step required for deprotection, the alkyne group was interchanged for an azide functionality and 3-azidopropyl methacrylate (AzPMA) was found to be tolerant of ATRP conditions, producing polymers with PDI < 1.4. CuAAC functionalization was then carried out on the 3-azidopropyl-containing homopolymer, with reaction efficiencies of >95% being observed for the attachment of several functional groups along the backbone. The synthesis of amphiphilic diblock copolymers of AzPMA with 2-dimethylaminoethyl methacrylate (DMAEMA) was also demonstrated, proving both the living nature of the polymerization and the ability to polymerize azido-substituted monomers by RAFT polymerization.[110] In order to confirm retention of the azido group through polymerization, Li et al. carried out the click reaction with phenyl acetylene on both the polymeric derivatives and the small molecule monomer (followed by polymerization), which gave identical materials by NMR, DSC, and IR, demonstrating that there was no measurable loss in efficiency when the click reaction was performed on the polymer (Scheme 7).

The ready availability of azide salts and the high reactivity of N_3^- in nucleophilic displacement reactions have also prompted a growing effort in preparing azide-functionalized polymers through postpolymerization strategies, analogous to the synthesis of end-functionalized, ATRP-derived materials (*vide supra*). For example, Tunca has developed a facile synthesis of copolymers of styrene and *p*-chloromethylstyrene that can easily be converted into random heterograft copolymers[111] and again demonstrates the orthogonality of the CuAAC reaction coupled with its efficiency in preparing advanced macromolecular architectures from simple building blocks. Another example of the postpolymerization introduction of the azide moiety is found in polyester materials derived from ROP. Easily synthesized α-chloro-ε-caprolactone can be reacted with sodium azide either prior to polymerization or afterward in order to prepare click-able materials (Scheme 8).[112] It was found that introduction of the azide group to the polymer backbone was complete within 24 h, while preparation of the azido monomer took three days at elevated temperatures. These materials were then functionalized with propargyl benzoate using water-free conditions developed in order to prevent transesterification and the associated increased molecular weight distributions. Catalysis by CuI/DBU in THF proved to be highly efficient leading to complete functionalization in only 2 hours at 35 °C.

The compatibility of alkynes with ring-opening polymerization, also allows alkyne groups to be easily carried through ROP, and this is exemplified with Emrick's incorporation of alkyne moieties into δ-valerolactone.[113] Although the polymerization of this monomer is slower than for ε-caprolactone, it was reported that the two monomers could be copolymerized in any ratio, with the alkyne group then providing a more direct route to peptide-functionalized polyesters that does not require the use of protecting group chemistry. Significantly, the peptide-functional materials had PDIs as low as 1.07. Additionally, Emrick and co-workers also reported that PEG could be grafted onto the polyester backbone with a high degree of efficiency using CuAAC.

Scheme 8. Click-able Polyesters with a Number of Functional Groups Introduced[112]



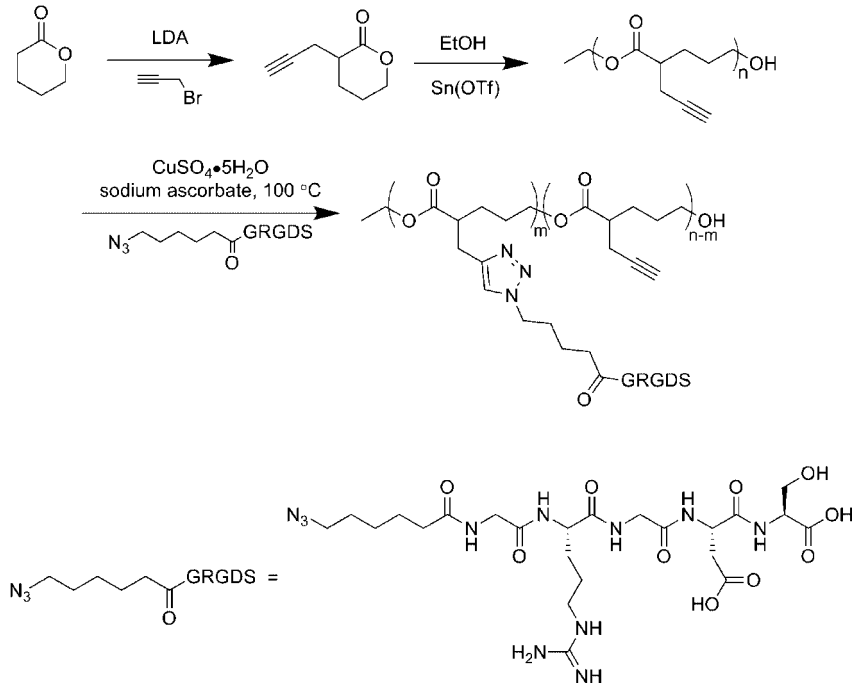
These results helped bolster claims that the advantages of click chemistry permit advances in biomaterials synthesis with the toxicity of required copper catalysts abated through standard purification techniques (Scheme 9).

Several earlier approaches had also focused on incorporating the alkyne moiety into monomers for click functionalization, with the first being polyvinylacetylene.[114] This simple material could be efficiently converted to dendronized polymers through reaction with azide-functionalized benzyl ether dendrons of various generation numbers, with G1 and G2 quantitatively reacting and G3 reacting up to 98%. This coupling-to strategy appears to reach a steric limit at G4, with the level of reaction dropping significantly. Although this report demonstrated the potential to build interesting, complex materials from simple building blocks, the authors also sought to broaden the scope of the CuAAC reaction and to understand the orthogonality of the system when applied to backbone functionalization. In a subsequent article, the syntheses of styrenic, acrylic, and acrylamide monomers containing tetramethylsilane (TMS)-protected alkyne groups were reported.[115] These monomers were each incorporated into various copolymers with other reactive functional groups with tandem or simultaneous reactions being performed. For example, reaction of an alkyne-functionalized polystyrene derivative with a linker unit containing both an azide group and a nucleophilic amine in the presence of an active ester in one-pot gave the corresponding derivative in which the linker group was attached to the backbone by triazole formation while having also undergone an amidation reaction, thus demonstrating the utility of developing orthogonal methods for building functional molecules. To date, there has been a diverse collection of CuAAC-based functionalization reactions for backbone derivatization that have built upon the pioneering work mentioned above, and the interested reader is directed to a number of specific reviews on the use of CuAAC for polymer functionalization.

2.2.3. Thiol−Ene

The success of the CuAAC reaction has prompted an evaluation of other chemistries that fulfill the basic requirement of a click reaction. A prominent example is the reaction of thiols with unactivated alkenes, sometimes in the presence of either a thermal or photosensitive radical initiator, commonly referred to as thiol−ene chemistry. This reaction proceeds in extremely high yields, with good tolerance for functional groups, and can often be done without solvent, simplifying purification and making the reaction very user-friendly. Although the reaction has long been known for the uncontrolled vulcanization of rubbers, it has recently been employed in the synthesis of far more well-defined materials, and recent methods have demonstrated its utility in functionalizing many different polymers. These materials have ranged from commodity polymers, such as polybutadiene[116]

Scheme 9. Alkyne-Functional Polyesters Allow for Incorporation of Biologically-Active Peptides[113]



and polybutadiene block copolymers,[117] to methacrylate and styrenic based systems and poly(ethylene glycol).[118,119]

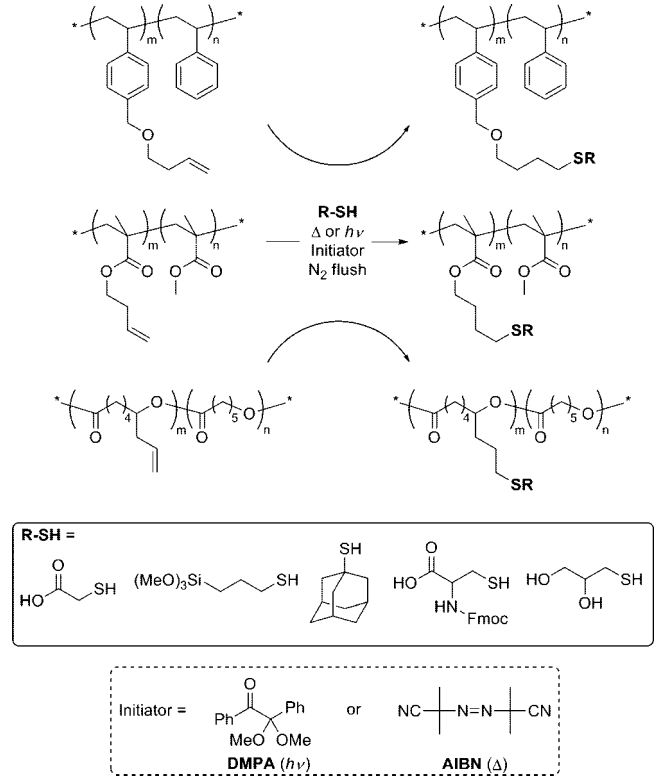
One of the early attempts to prove the modern utility of thiol−ene reactivity on linear polymers was that of Brummelheis et al.[120] This report demonstrated the rapid conversion of fairly simple, inexpensive, commodity polybutadiene into a series of highly complex functional polymers through two different routes: either a functional thiol would be introduced onto the polymer backbone, or a thiol would be used to introduce an alcohol group that could then be functionalized with various acid chlorides. Through the functional thiol route, they demonstrated efficient coupling of a number of different functional groups including phenols, pyridine, and carbazole. This was done with a greatly improved protocol, using UV light to activate the thiol mixture, eliminating the need for large excesses of the thiol and high temperatures. There have been numerous other reports[121] of polybutadiene modification with various thiols leading to the introduction of a broad range of functionalities, though one potential drawback of using radical reactions to functionalize these materials arises from the close proximity of the alkenes, which results in a highly complex mixture of cyclic repeat units.[121]

Following these reports, interest has rapidly developed in exploiting this reaction for the synthesis of more sophisticated materials from controlled polymerization methods, and a number of alkene-functional monomers were synthesized to this end. Campos et al. reported[46] the synthesis of styrenic, methacrylic, and caprolactone monomers, each bearing alkene functionalities that could be combined with functional initiators and postpolymerization modifications to further increase the range of orthogonal groups that can be built into these systems. Polymers were made by ROP, RAFT, or ATRP, and both photochemical and thermal radical conditions were tested to explore the efficiency of the thiol−ene reaction (Scheme 10). It was found that employing photochemical conditions generally leads to faster and more complete reaction. Additionally, the orthogonality of the azide functional group during the thermal thiol−ene reaction was demonstrated.

Expanding on this work, Ma et al. studied a series of monomers containing alkenes and their employment for the synthesis of homopolymers, block copolymers, and random copolymers.[122] By tuning the block length and composition of these materials, incorporating reactive fluorinated styrenic monomers and maleic anhydride, they demonstrated the synthesis of highly functionalizable systems capable of nanoscale organization into micelles. These materials provide an excellent example of the modular nature of polymers formed with reactive groups for REO chemistry. The maleic anhydride units allow functionalization with an amine, while also giving hydrophilic character, and the alkene functional units remain available for functionalization by thiol−ene reaction.

Cyclic alkenes are also interesting as candidates for thiol−ene modification, as ring strain can significantly increase the energetic favorability of the reaction. To this end, Ma et al. demonstrated the synthesis of well-defined polymers with cyclohexenyl side groups by RAFT.[123] Again, the polymerization conditions gave specificity in polymerizing the styrenic group rather than the cyclic alkene even at high conversions, with the living nature of the polymerization apparent from the low MW distributions and retention of the chain transfer group at the chain end. The functionalization of this polymer with dodecylthiol required only 30 min of UV irradiation in the presence of photoinitiator DMPA in order to reach 85% conversion. Precipitation was the only workup required, and the functional polymer was recovered in high yield. The development of thiol−ene chemistry as an orthogonal partner to the original CuAAC click reaction opens up the variety of functional materials with well-defined structures that are readily obtainable and provides significant motivation for the development of other orthogonal reaction partners.

Scheme 10. Thiol−Ene Modification of Structurally Diverse Polymers with Various Thiols[46]

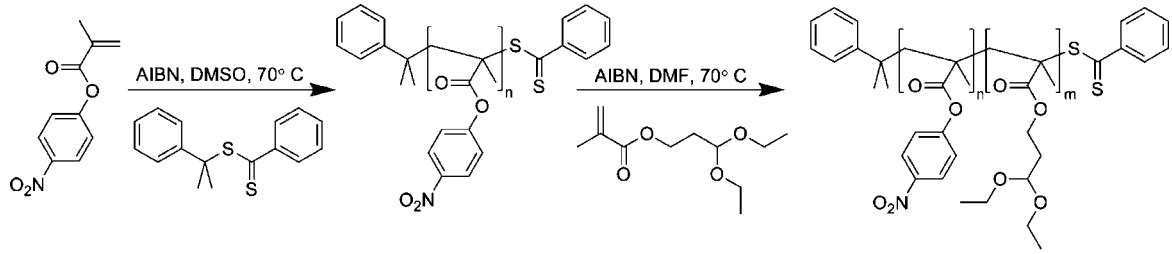


2.2.4. Carbonyl-Based Strategies

Ketones and aldehydes are capable of undergoing facile reaction with a variety of nucleophiles, most often resulting in reversible bond formation. However condensation with hydrazines or alkoxyamines provides bonds much more stable than traditional systems such as imines. The stability of the hydrazine and alkoxyamine adducts coupled with the stabilization of imines by reduction has prompted many of these reactions to be explored for the REO functionalization of a wide variety of materials.

In the late 1990s, use of oxime chemistry began to rise in popularity for immobilizing biological molecules. As tools became available for introducing the necessary functional groups into biological molecules of interest, more well-defined materials were required, and in 2005, Christman and Maynard reported polymer films synthesized with acetal side chains.[124] These acid-sensitive groups could be cleaved in order to produce aldehydes, which were shown to form stable oxime linkages with a range of functional units. Rather than attempt to control the placement of the polymer or the protected aldehydes in the polymer, a simple photoacid-generator strategy was used. This strategy allowed for selective deprotection in areas that were not covered by a photomask. In a further expansion of this work, it was

Scheme 11. Synthesis of an Orthogonally Reactive Block Copolymer by RAFT[128]



demonstrated that the remaining acetals could be subsequently deprotected and allowed to react with a different aminooxy compound in order to eliminate any nonspecific protein interactions.[125] These surfaces were then used to immobilize the protein ANTRX-1, an anthrax toxin receptor, and it was demonstrated that the protein held its native conformation and was able to bind to the anthrax toxin protective antigen.

Following this report, a number of papers have demonstrated the utility of oxime formation in the synthesis of functional polymers. For example, methacrylate polymers[126] and copolymers[127] containing 4-nitrophenyl esters and acetal-protected aldehydes were prepared by RAFT with control over the ratio of each repeat unit and associated control over the molecular weights and PDIs (Scheme 11).[128] The aldehyde units allowed for the introduction of functional groups as simple as benzyloxyamine or as complex as RGD peptide sequences. In a subsequent report, conditions were also optimized for polymerization of the acetal monomer by ATRP,[129] which offers a straightforward functional initiator synthesis to be able to incorporate orthogonal functionality also on the chain ends.

Controlled synthesis of polyesters through ring-opening polymerization offers access to polymers that offer tunable degrees of degradability, crystallinity, biocompatibility, and mechanical properties. As with other REO chemistries, interest has developed in the synthesis of monomers that would allow site-specific integration of functionality into well-studied polymer backbones in a modular fashion. Jérôme and co-workers reported the synthesis of PCL bearing hydroxyl groups along the backbone,[130] which could then be cross-linked or further functionalized. Taking advantage of this prior work, the ketone-based polymer precursors were identified as ideal candidates for oxime and hydrazone functionalization. One of the first reports employing oxime formation was reported by Taniguchi et al. in 2005[131] who transformed this simple linear material into a comb polymer by grafting aminooxy-terminated PEG using relatively low temperatures and no catalyst. Expanding on this work, Van Horn explored the functionalization of these materials with multiple functional aminooxy compounds both simultaneously and in sequential reactions. Functionalization with a number of different alkoxyamines and a model hydrazine compound showed the difference in reactivity of various alkoxyamines while also highlighting a difference in the stability of hydrazone moieties relative to ketoxime ether groups. Additionally, these studies demonstrated that there are different synthetic benefits depending upon whether a one-pot or a sequential set of reactions is employed. This strategy of functionalizing the same unit with different groups was extended and exploited through the formation of vesicles derived from poly(ethylene glycol)-*b*-poly(vinylbenzaldehyde) that were subjected to sequential reactions to attach hydrazine-functional fluorescein dyes followed by cross-linking of the nanostructure with a low molecular weight diamine.[132] Sodium cyanoborohydride was used to reduce the resulting imine and hydrazone moieties. The one-pot reaction was effective in this case, because the hydride reagent countered the reversibility in the reactions. This strategy was also aided by the hydrazone and imines bonds having similar degrees of reversibility compared with the oxime case.

As demonstrated previously, the reverse strategy, reaction of an amine-containing backbone with a ketone/aldehyde functionality, is also possible and has a number of advantages since many small molecule drugs contain carbonyl moieties. In this case, hydrazone linkers are attractive, due to their reversible nature, and drug release can be accelerated in the acidic environments of tumors. Work by Etrych[133] and Chytil[134] demonstrated that doxorubicin could be effectively conjugated to polymer backbones with exposed hydrazine groups and that release could be influenced by changing the properties of the polymer though introduction of hydrophobic moieties. It is also possible to incorporate alkoxyamines as backbone functional groups, and in light of recently developed methods for affixing aldehydes and ketones to proteins, this has also been proven to be a useful strategy.[135]

Analogous to Maynard's difunctional polymers, Yang and Weck developed a series of ROMP-based polymers that could be functionalized simultaneously through both a click cyclization and a condensation reaction.[136] Impressively, the only postpolymerization modification necessary was the introduction of the azide unit through displacement of a halide substituent, which occurred quantitatively as measured by NMR, and the unprotected aldehyde-functional norbornene was incorporated without any difficulties. The authors also synthesized and polymerized a ketone-functional monomer in place of the aldehyde in order to improve the solubility of the polymer. As a prime display of the orthogonality of the two reactions, the authors were able to click a nucleotide onto the azide, biotinylate the ketone, and recover the functionalized product in 96% yield after this one-pot process.

2.3. Graft and Block Graft Copolymers

In analogy with star polymers, a topic discussed later in this review, graft copolymers consist of polymer chains covalently attached to a core; however in this case the core is not a central unit but a linear polymer backbone. Brush and block graft copolymers comprise an important category of three-dimensional macromolecular architectures with many examples detailing their single molecule visualization, and these polymers have attracted significant attention due to their unique properties.[137–139] Block graft copolymers can be prepared through one of three general synthetic methods: "grafting from", "grafting through," and "grafting onto" (Figure 5). Previous reviews have focused on controlled

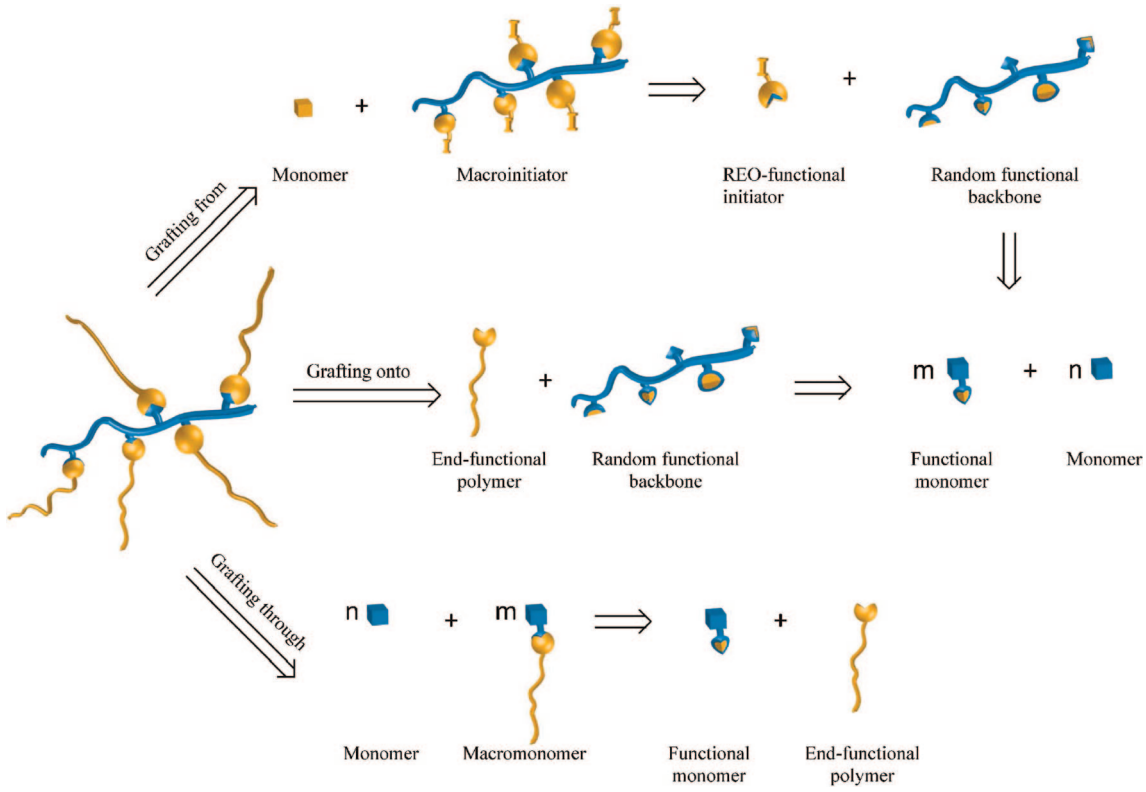


Figure 5. Retrosynthetic analysis for graft copolymers made using REO chemistry by the "grafting from" (upper), "grafting onto" (middle), or "grafting through" (lower) approaches.

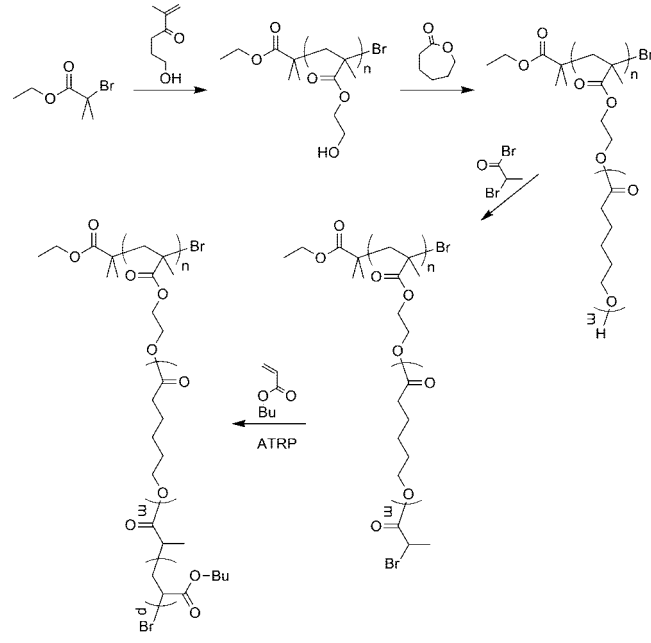
polymerization methods for their preparation, including "grafting from" and "grafting through" strategies by anionic polymerization techniques[137] as well as controlled radical polymerization methods such as NMP,[3] ATRP,[4] and RAFT.[140,141] As such, this portion of this review will highlight developments in the synthesis of block graft copolymers where multiple functionalities are present in the polymeric grafts and where orthogonal chemistries and polymerization techniques are employed in the creation of macromolecular combs and brushes using the "grafting from" and "grafting through" techniques. Ultimately, this section will concentrate on recent advances using the "grafting onto" approach and the employment of REO chemistries[142,143] for the conjugation of polymeric grafts onto a backbone to construct multifunctional block graft copolymer architectures.

2.3.1. "Grafting from" Strategy

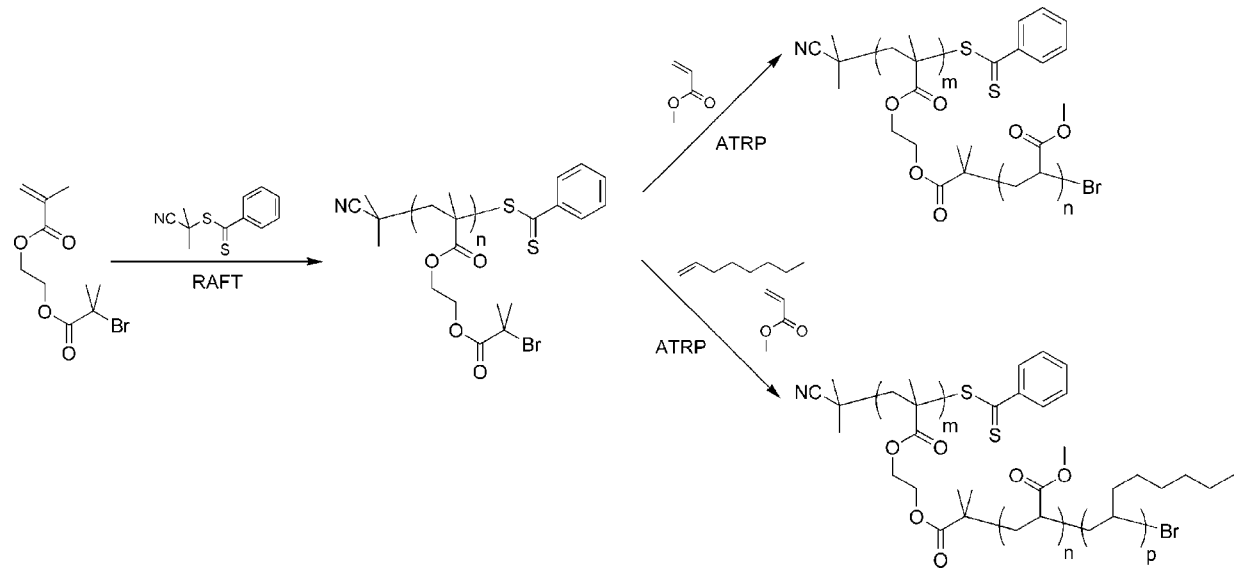
The "grafting from" method incorporates active sites along the backbone from which polymerization can be initiated (Figure 5, upper). Polymerization from these initiating sites results in the formation of polymeric grafts to afford the graft copolymer. The "grafting from" approach has been used extensively in the synthesis of macromolecular combs and brushes. Herein we highlight a few recent examples that employ the "grafting from" technique using orthogonal polymerization chemistries for the synthesis of macromolecules with multifunctional grafts.

Numerous graft copolymers have been synthesized using only a single polymerization method and the "grafting from" approach.[4,144,145] However, there are significantly fewer examples that employ two or more different polymerization techniques for the synthesis of block graft copolymers using a "grafting from" strategy. In one study, a core−shell brush block copolymer with a poly(ε-caprolactone) (PCL) core and a poly(*n*-butyl acrylate) (PBA) shell was prepared via the "grafting from" approach and a combination of ATRP and ring-opening polymerization (ROP).[146] The successful synthesis of a brush macromolecule with PCL-*b*-PBA copolymer side chains was achieved by initiating ROP of ε-caprolactone (CL) from a poly(2-hydroxyethyl methacrylate) (PHEMA) backbone. The ends of the PCL grafts were further functionalized with an ATRP initiator and this new multifunctional macroinitiator was used to grow the PBA block segments by ATRP (Scheme 12). The resulting core−shell

Scheme 12. Synthesis of a Block Graft Copolymer with PCL-*b*-PBA Side Chains from a PHEMA Backbone[146]



Scheme 13. Synthesis of a Polymer Brush with PMA and P(MA-*co*-Oct) Grafts Using Sequential RAFT and ATRP Polymerizations[147]



brush block copolymer was comprised of a crystalline PCL core and an amorphous PBA shell.

The "grafting from" approach has also been employed in the synthesis of brush copolymers with statistical copolymer side chains. Klumperman et al. reported the successful homopolymerization of 2-(2-bromoisobutyryloxy)ethyl methacrylate (BIEM), an acrylate possessing an ATRP initiator side group.[147] Polymerization of BIEM was accomplished using RAFT polymerization, after which poly(methyl acrylate) (PMA) and poly(methyl acrylate-*co*-octene) (P(MA-*co*-Oct)) grafts were grown from the PBIEM macroinitiator via ATRP to yield densely grafted brushes with homopolymer and statistical copolymer side chains, respectively (Scheme 13). Protection of the ATRP initiator on the BIEM side chain was unnecessary because the conditions used in the RAFT polymerization of the graft copolymer backbone were tolerant of the reactive bromide on the ATRP initiator side chain. Consequently, by using RAFT polymerization, the direct polymerization of BIEM was achieved, and subsequent growth of polymeric side chains could be achieved without the need for deprotection or further functionalization reactions.

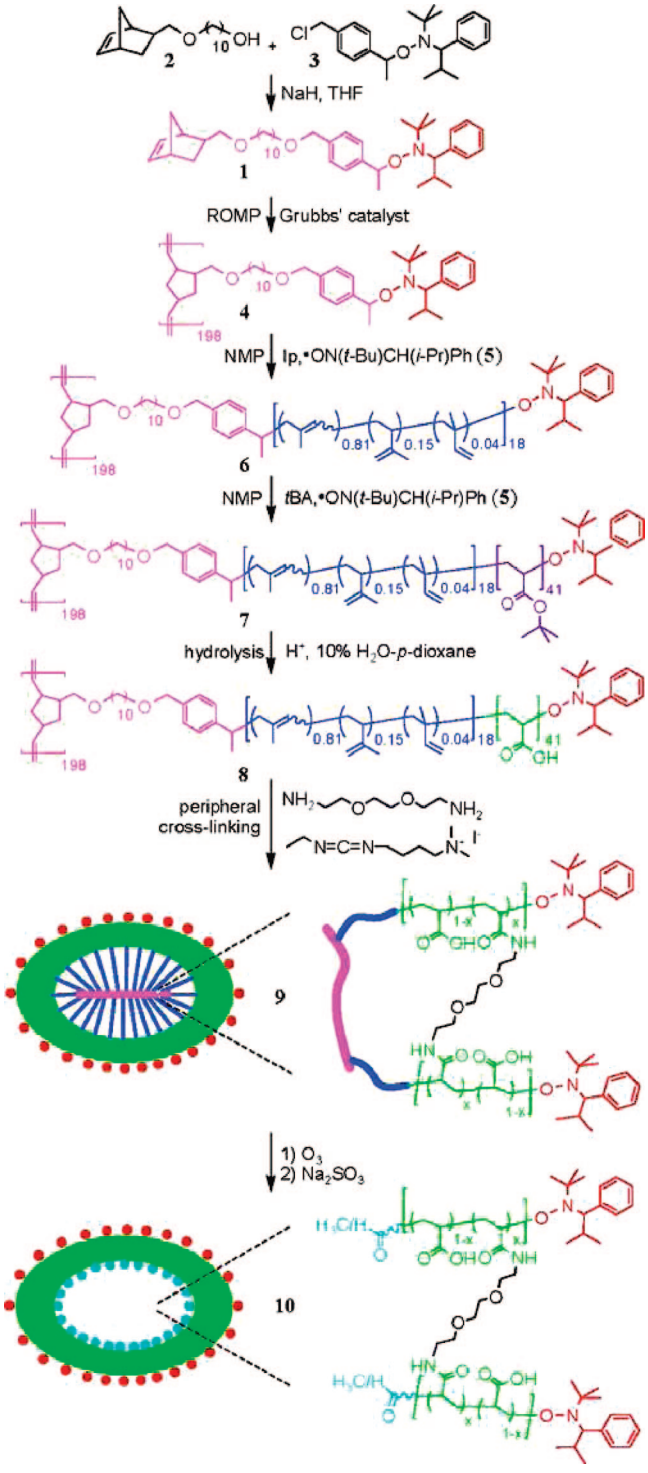
The Wooley group reported another example that employs orthogonal polymerization techniques in the synthesis of block graft copolymers. With the "grafting from" technique, a combined NMP−ROMP initiator−monomer, *inimer*, possessing norbornene and alkoxyamine functionalities was employed in the preparation of a graft copolymer by ring-opening metathesis polymerization (ROMP) and NMP.[148,149] Using this methodology, they prepared a core−shell brush copolymer using a tandem synthetic strategy in which a polynorbornene (PNb) backbone with pendant alkoxyamine moieties was grown via ROMP and then the resulting polymer was used as a polyfunctional NMP macroinitiator from which isoprene (Ip) and *tert*-butyl acrylate (*t*BA) were polymerized sequentially to give a core−shell brush block copolymer with PIp-*b*-P*t*BA grafts (Scheme 14). This core−shell brush block copolymer architecture was transformed into an amphiphilic core−shell nanostructure by cleavage of the *t*BA groups to give acrylic acid residues, which was followed by peripheral cross-linking of the acrylic acid groups using amidation chemistry. This process resulted in the fabrication of shell-cross-linked unimolecular nanoparticles. To demonstrate the versatility of this system, the PIp block could be selectively degraded by ozonolysis to yield a hollow nanostructure (Scheme 14).[148] The core−shell brush copolymer with PIp-*b*-P*t*BA possessed a core composed of PNb-*g*-PIp, a region with ubiquitous alkenyl groups. To further extend the utility of this brush copolymer system, selective cross-linking could also be confined to the core domain through the use of sulfur monochloride, yielding a core-cross-linked nanostructure.[149]

The utility of combining ROMP and controlled radical polymerization techniques for the synthesis of brush block copolymers was further demonstrated through the one-pot synthesis of a core−shell brush block copolymer using tandem ROMP and RAFT polymerization.[150] A polyfunctional RAFT agent was prepared *in situ* by ROMP of a norbornene-functionalized RAFT agent using Grubbs' catalyst; upon addition of azobisisobutyronitrile (AIBN), styrene (S), and maleic anhydride (MAn), the growth of P(S-alt-MAn)-*b*-PS polymer grafts and the completion of a core−shell brush copolymer was achieved (Scheme 15). Hydrolysis of the internal maleic anhydride units converted the structure to an amphiphilic core−shell nanoparticle. Significantly, the high degree of compatibility between controlled radical polymerizations and ROMP, the high functional group tolerance of ROMP when initiated by ruthenium-based catalysts, and the breadth of monomers and comonomers that can be incorporated using RAFT polymerization illustrate the power and potential of using robust, efficient, and orthogonal chemistry for the design and synthesis of a diverse range of functional macromolecular architectures.

2.3.2. "Grafting through" Strategy

In the "grafting through" strategy, preformed macromonomers are polymerized to produce the graft copolymer (Figure 5, middle). The macromonomers are typically polymeric or oligomeric chains with a polymerizable end group; thus the grafts are composed of the macromonomer chain segment, and the backbone is formed *in situ*. The grafting through strategy has been used to synthesize graft copolymers using a variety of controlled polymerization methods. Conse-

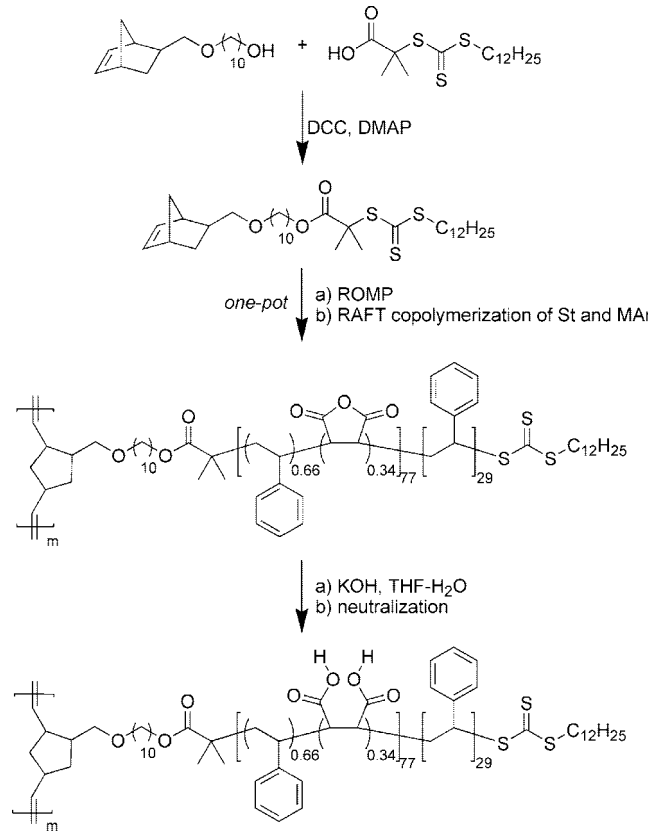
Scheme 14. Fabrication of a Shell-Cross-Linked Molecular Brush and a Hollowed Nanostructure via a Core−Shell Brush Block Copolymer Prepared via ROMP of Inimer 1, Followed by Growth of Polymer Grafts by NMP from the Multifunctional Macroinitiator 4[a]



[a] Reproduced with permission from ref 148. Copyright 2006 American Chemical Society.

quently, only a few recent examples using the "grafting through" process in conjunction with REO chemistries and combinations of orthogonal polymerization techniques will be highlighted. The grafting through method is complicated by the need to ensure that there is a specific polymerizable chain end functionality and the steric effects that can inhibit effective polymerization of high molecular weight or bulky

Scheme 15. One-Pot Synthesis of a Core−Shell Graft Block Copolymer via ROMP of an Inimer and then RAFT Grafting from the Multifunctional Macroinitiator[150]

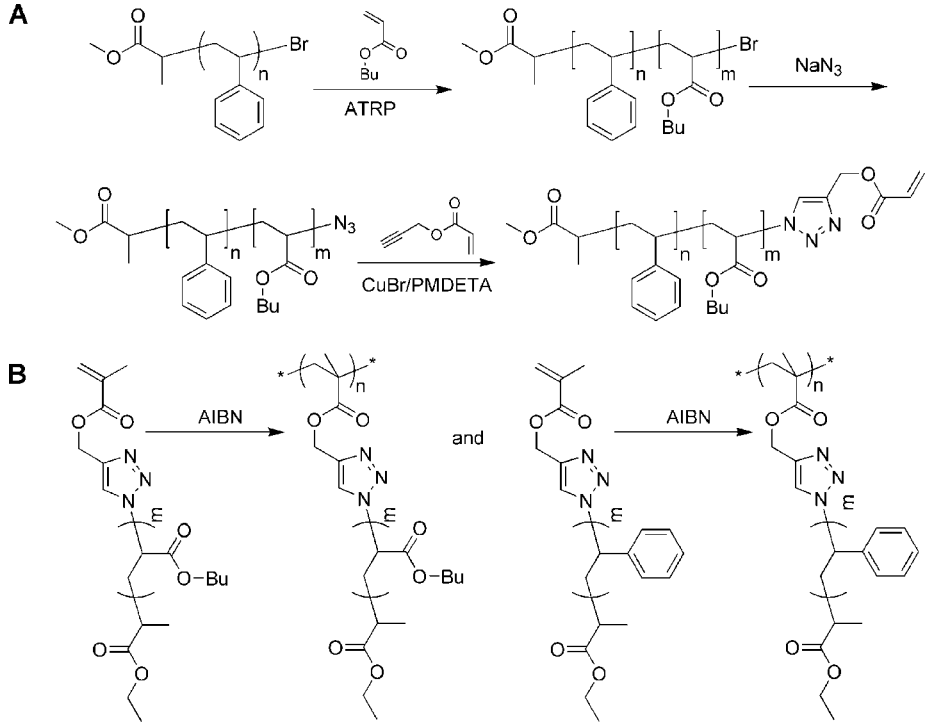


macromonomers; REO chemistry is important due to its ability to minimize or mitigate these factors.

ATRP has been used in the synthesis of a variety of graft copolymers via the "grafting through" approach both to grow the polymeric side chain macromonomers and to establish the graft copolymer backbone. In order to obtain macromonomers that can subsequently be polymerized by controlled radical polymerization techniques, postpolymerization modification is typically necessary. For example Mueller et al. used a hydroxyl-terminated ATRP initiator that, after polymerization, could be reacted with methacryloyl chloride to yield a polymerizable macromonomer,[151] and Muehlbach et al. employed nucleophilic substitution with methacrylic acid to convert the halogen end groups from ATRP to methacrylate end groups.[152] While postpolymerization modification and subsequent growth of the graft copolymer backbone by ATRP efficiently yielded densely grafted polymer brushes in these studies, the reactions used to incorporate a methacrylate moiety were not tolerant of a wide variety of functional groups and would not be feasible for use with polymers containing hydroxyl- or carboxy-functional monomer units.

In an effort to extend the use of ATRP in the synthesis of graft copolymers by the "grafting through" technique, the Summerlin group reported a versatile and efficient route for the synthesis of macromonomers using ATRP and CuAAC click chemistry.[81] In this work, polystyrene (PS), PBA, and polystyrene-*block*-poly(*n*-butyl acrylate) (PS-*b*-PBA) were all prepared by ATRP, and the resulting bromine end group was converted to an azide by reaction with sodium azide. The resulting azido-terminated polymers were allowed to undergo a Huisgen 1,3-dipolar cycloaddition with an alkyne-

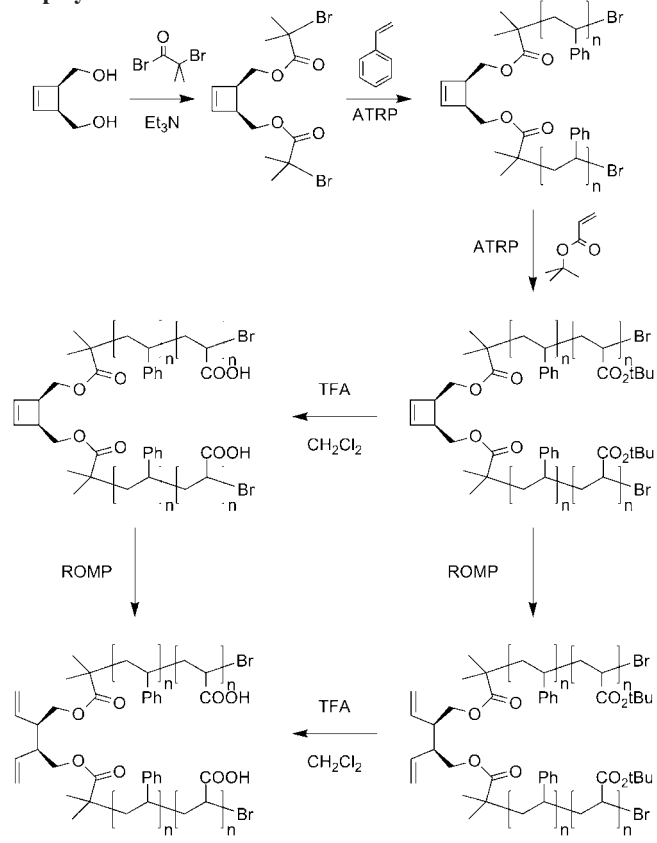
Scheme 16. Synthesis of (A) PS-*b*-PBA Macromonomer Using ATRP and CuAAC and (B) Graft Copolymers Achieved by the Conventional Radical Polymerization of PBA and PS Macromonomers[81]



containing acrylate to complete the synthesis of the macromonomers (Scheme 16A). This approach presents a general strategy for the synthesis of many different types of macromonomers containing diverse functionalities due to the inherent orthogonality of the click cycloaddition reaction. Consequently, it can be employed to make a wide diversity of macromonomers from almost any polymer or copolymer synthesized by ATRP. Finally, to demonstrate the polymerizability of the resulting macromonomers, the homopolymerization of methacrylate-functionalized PS and PBA using conventional radical polymerization was performed to yield densely grafted brush copolymers via the "grafting through" technique (Scheme 16B). This work focuses on efficient synthesis of macromonomers using REO chemistries and also demonstrates that the macromonomers made can be effectively used in the synthesis of densely grafted brush copolymers.

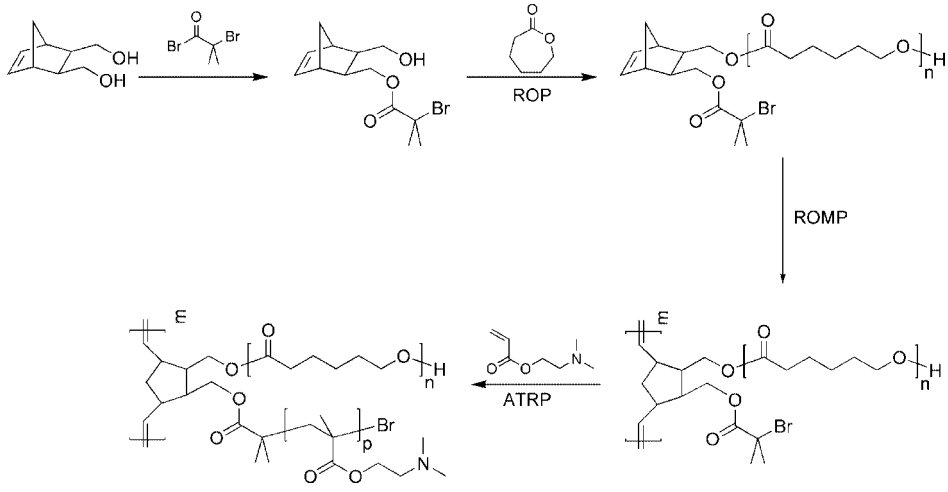
Similar to several "grafting from" examples presented above, block graft copolymers have also been prepared by combinations of ROMP and controlled radical polymerization in the opposite sequence, involving synthesis via the "grafting through" of ROMP macromonomers. One specific example exploited a combination of ATRP and then ROMP to prepare an amphiphilic core−shell graft copolymer system[153] with the macromonomer being prepared by the sequential ATRP of styrene and *tert*-butyl acrylate from a cyclobutenyl-functionalized initiator to give polystyrene-*b*-poly(*tert*-butyl acrylate) (PS-*b*-P*t*BA) (Scheme 17). The desired amphiphilic graft block copolymer, polybutadiene-*graft*-(polystyrene-*b*-(poly acrylic acid)) (PBD-*g*-(PS-*b*-PAA)), was obtained by two different sequences, the first involved the removal of the *tert*-butyl groups by treatment with trifluoroacetic acid to yield polystyrene-*b*-poly(acrylic acid) (PS-*b*-PAA) macromonomer that was polymerized by ROMP in emulsion to yield PBD-*g*-(PS-*b*-PAA) (Scheme 17). The second sequence involved ROMP of the PS-*b*-P*t*BA macromonomer followed

Scheme 17. Synthesis of a Block Graft Copolymer by ATRP and then ROMP (Direct Pathway Downward), Followed by Acidolysis to Release an Amphiphilic Graft Block Copolymer[a]



[a] A similar product was afforded by acidolysis at the macromonomer stage followed by ROMP under emulsion polymerization conditions.[153]

Scheme 18. Synthesis of a Densely Grafted Amphiphilic Brush Block Copolymer Employing Both the "Grafting from" and "Grafting through" Strategies[154]



by acidolysis of the *tert*-butyl groups to yield an amphiphilic PBD-*g*-(PS-*b*-PAA) graft copolymer (Scheme 17).

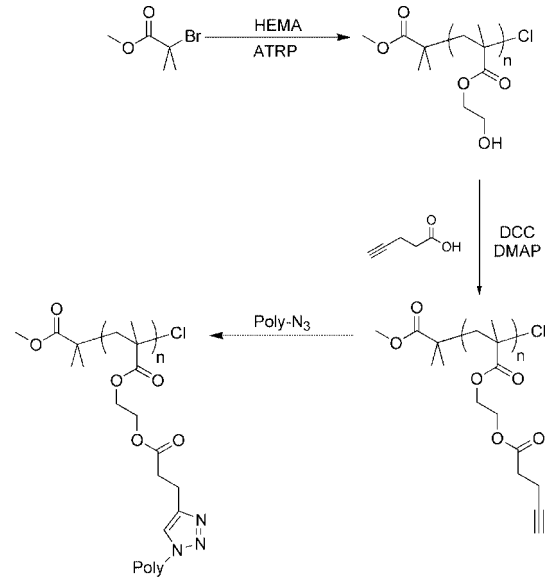
The "grafting through" approach has also proven effective for the synthesis of heterografted brush macromolecules, and the synthesis of a well-defined amphiphilic heterograft copolymer made by applying complementary polymerization techniques using both the "grafting from" and "grafting through" approach has been reported.[154] ROP of ε-caprolactone (CL) was initiated by 2-hydroxymethyl-3-(2-bromoisobutyroxymethyl)-5-norbornene (NBE−OH/Br) to generate a norbornene-*graft*-poly(ε-caprolactone)/Br (NBE-*g*-PCL) macroinimer (Scheme 18). The "grafting through" strategy was subsequently employed to construct the polymer backbone via ROMP of the norbornene functionality, after which poly((2-dimethylamino)ethyl methacrylate) grafts were grown by a "grafting from approach," from the backbone by ATRP (Scheme 18). Like the other syntheses described, this study takes advantage of the functional group tolerance of ROMP and combines it with two other controlled polymerization methods, ROP and ATRP, to yield functional, complex, and well-defined materials.

2.3.3. "Grafting onto" Strategy

Due to the steric congestion that occurs when trying to couple polymer chains onto a backbone, the grafting density of polymeric brushes made using the "grafting onto" technique is typically low (Figure 5, lower portion). One strategy used to increase the grafting density is the employment of highly efficient organic chemistries that will maximize the extent of reaction between the polymeric grafts and the backbone. CuAAC, in particular, has been widely used as an efficient "grafting-onto" route to graft polymers. When triazole formation is employed, the alkyne can reside either on the polymer backbone or on the chain end of the polymer chain being coupled to the backbone. The Matyjaszewski group has reported the synthesis of a fully alkyne-modified linear backbone by esterification of the hydroxyl side chains on PHEMA with pentynoic acid. The alkyne-functionalized backbone was then reacted with azide-terminated poly(ethylene oxide), polystyrene, poly(*n*-butyl acrylate), and poly(*n*-butyl acrylate)-*b*-PS to produce a series of block graft copolymers (Scheme 19).[155]

In another study, a polymer chain with alkyne moieties presented along the backbone was obtained by RAFT

Scheme 19. Synthesis of Block Graft Copolymers via a Combination of ATRP for the Construction of the Backbone and the Functional Grafting Polymer Chains, Followed by CuAAC Click Chemistry for the Grafting onto Strategy[155]

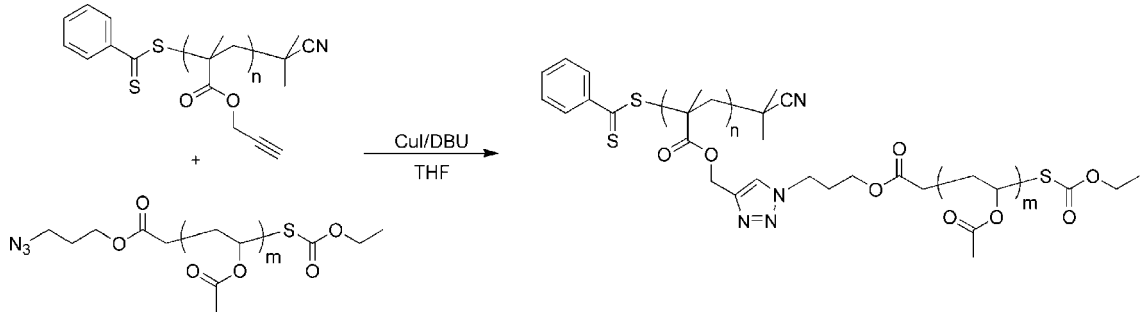


Poly = PEO, PS, PBA, PBA-*b*-PS

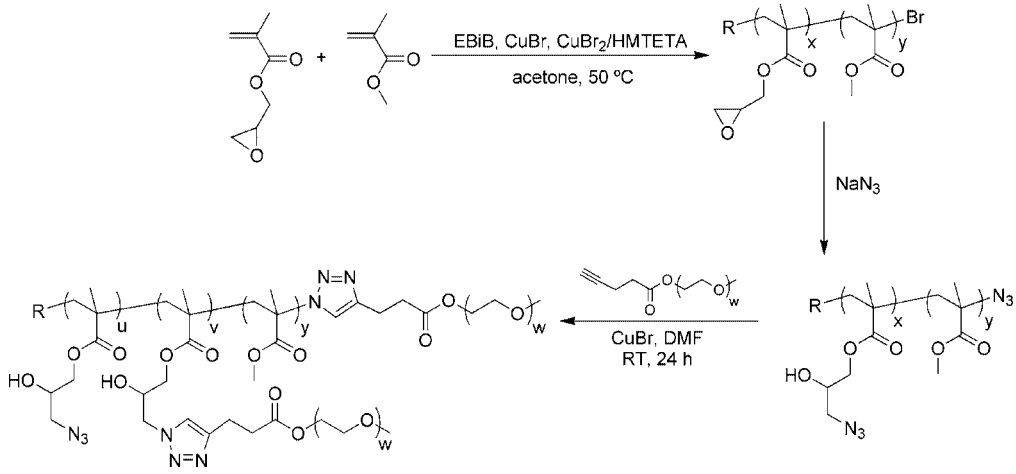
polymerization of a TMS-protected propargyl methacrylate monomer and subsequent removal of the TMS group with tetrabutylammonium fluoride (TBAF).[156] Independently, vinyl acetate was polymerized using an azide-functionalized xanthate to control chain growth by macromolecular design via the interchange of xanthates (MADIX). The azide-terminated poly(vinyl acetate) (PVA) and the alkyne-functionalized backbone were allowed to undergo reaction in the presence of copper iodide, by which formation of 1,2,3-triazoles resulted in a brush copolymer with PVA grafts (Scheme 20).

In order to present the azide on the polymer backbone for the subsequent click conjugation of polymer side chains, it is feasible, though hazardous, to directly polymerize an azide-containing monomer. To avoid polymerization of an azide-containing monomer, ATRP was used to generate a statistical copolymer of glycidyl methacrylate and methyl acrylate, yielding a polymer backbone with pendant epoxide groups. Postpolymerization modification of the epoxide-functional-

Scheme 20. Copolymer Prepared Using RAFT, MADIX, and CuAAC Cycloaddition[156]



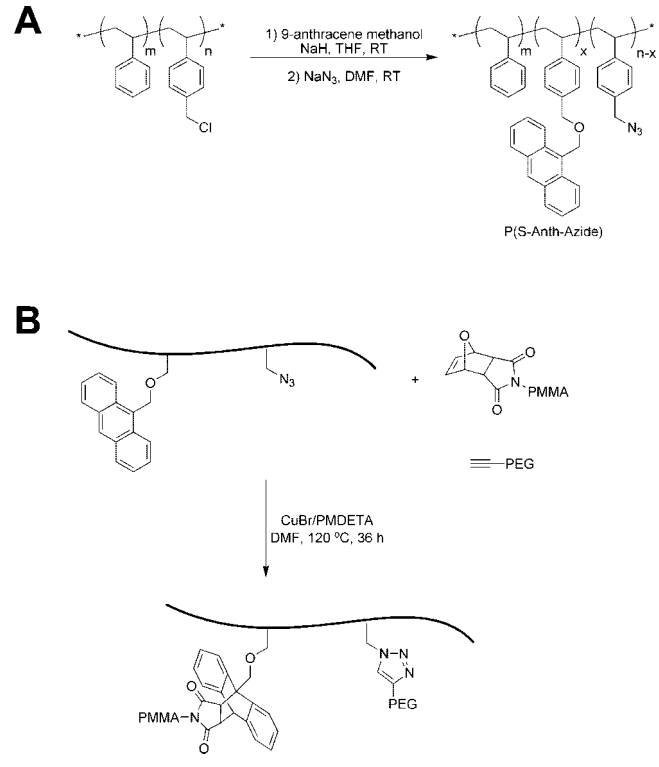
Scheme 21. Synthesis of a Low-Density Grafted Brush Copolymer Using CuAAC Click Chemistry and the "Grafting onto" Approach[157]



ized polymer affords the desired side chain azide groups without the inherent danger of working directly with an azido monomer (Scheme 21).[157] The resulting polymer was then allowed to react with poly(ethylene oxide) methyl ether pentynoate to yield a loosely grafted polymer with a hydrophobic backbone and hydrophilic PEO side chains by employing two consecutive click reactions, the opening of an epoxide by sodium azide and the subsequent CuAAC of an alkyne-terminated PEO (Scheme 21).

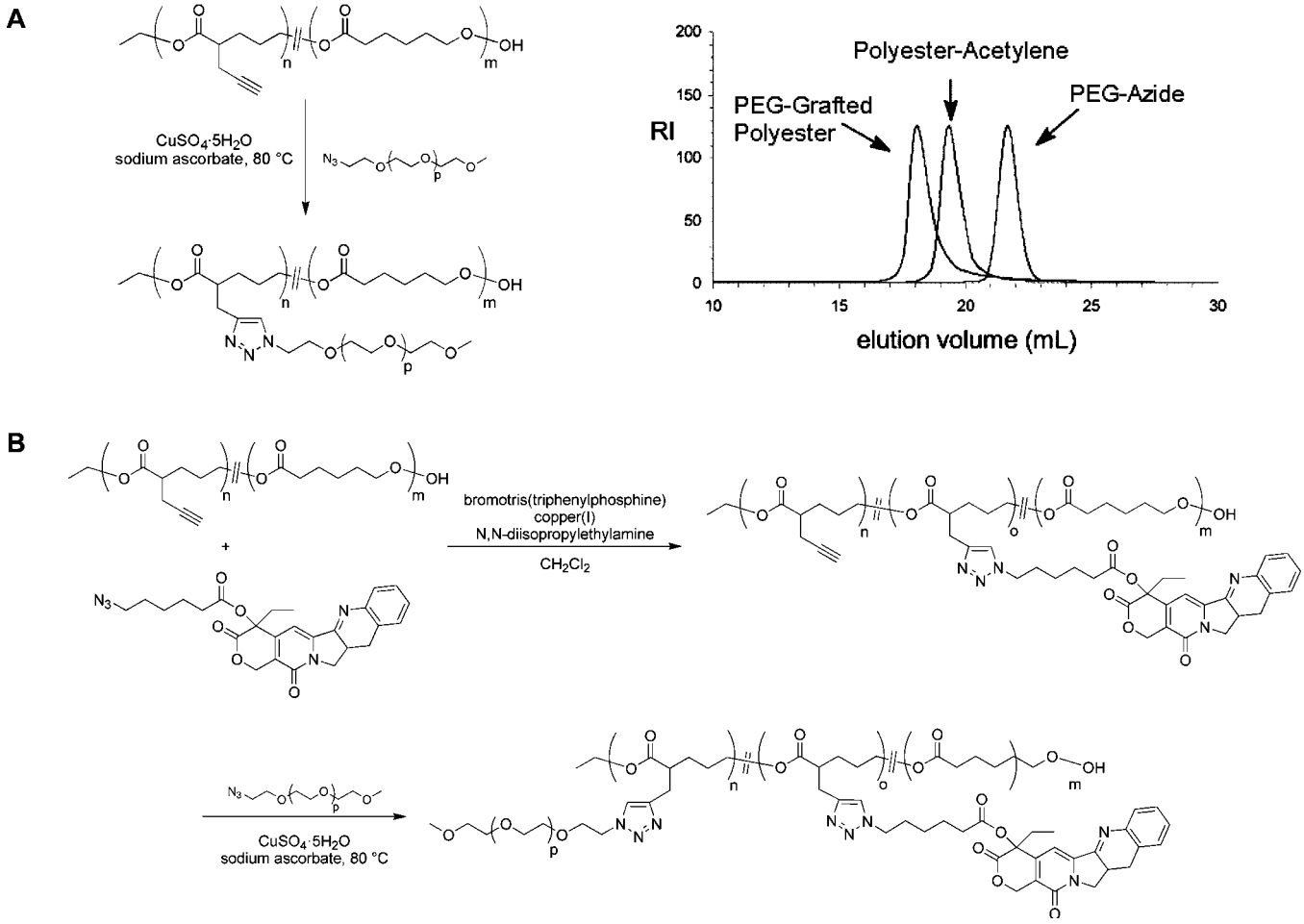
Block graft copolymers with one type of polymeric side chain have effectively been made using the "grafting onto" approach, but this technique has also been used to prepare heterograft copolymers by employing two different click reactions.[111] To illustrate this point, a random copolymer of styrene and 4-chloromethyl-styrene was synthesized by NMP, and the chloromethyl groups were replaced with anthracene and azide moieties by reacting the copolymer with anthracene methanol and sodium azide, respectively (Scheme 22A). To obtain a heterograft copolymer, a maleimide end-functionalized poly(methyl methacrylate) (MI-PMMA), which reacts with the anthracene in a Diels−Alder cycloaddition reaction, was made by ATRP with a maleimide-functionalized ATRP initiator. Separately, an alkyne end-functionalized PEG, which selectively reacts with the pendant azide group, was prepared via an esterification reaction with 4-pentynoic acid. An anthracene- and azide-functionalized PS copolymer was then combined in a flask with MI-PMMA, alkyne-functionalized PEG, CuBr, and *N*,*N*,*N*′,*N*′,*N*-pentamethyldiethylenetriamine (PMDETA) and allowed to undergo reaction at 120 °C for 36 h to yield a graft copolymer with PMMA and PEG side chains (Scheme 22B). The use of the Diels−Alder and CuAAC chemistries in a one-pot reaction is a recurring theme and further confirms the orthogonality and selectivity

Scheme 22. (A) The Synthesis of Anthracene- And Azide-Functionalized PS Derivatives and (B) the One-Pot Preparation of PS-*g*-(PMMA-PEG) Heterograft Copolymers Using Two Different Click Reactions[111]



of these two reactions while also demonstrating that the one-pot reaction employing double click reactions offers a

Scheme 23. Alkyne-Functional Polyesters Allow for (A) the Incorporation of Biologically Stealth PEG Grafts and (B) the Preparation of PEG-Grafted Polyester−Camptothecin Conjugates by a CuAAC "Grafting onto" Approach[114,159a]



[a] GPC reproduced with permission from ref 113. Copyright 2005 American Chemical Society.

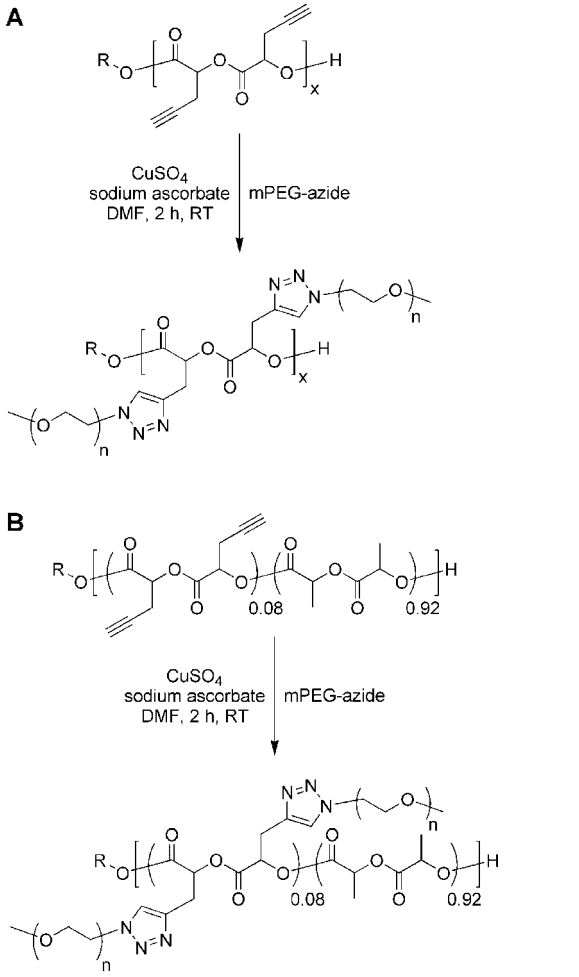
versatile and straightforward method for the preparation of heterograft copolymers.

The compatibility of REO chemistries with a wide range of functional groups also allows for the preparation of graft copolymers containing a variety of functional units.[158] The Emrick group has exploited the CuAAC reaction between an azide and an alkyne to generate multifunctional aliphatic polyesters bearing drug molecules, peptides, and poly(ethylene glycol) (PEG) grafts.[113,159] To achieve the synthesis of PEG-grafted aliphatic polyesters, ring-opening polymerization was used to polymerize α-propargyl-δ-valerolactone with ε-caprolactone; the resulting statistical copolymer was reacted with azide-functionalized PEG to yield an amphiphilic graft copolymer. The grafting of azido-PEG groups to the backbone of the polymer was conducted in water, with high reaction temperatures being necessary for complete triazole formation; this conjugation method resulted in the incorporation of multiple PEG grafts causing a significant increase in molecular weight, while the reaction conditions were mild enough to avoid any substantial degradation of the polyester backbone based upon GPC analysis (Scheme 23A). Significantly, the biocompatibility of the PEG-grafted materials showed minimal essential medium (MEM) testing and hemolysis testing, which demonstrates a level of biocompatibility comparable to PEG itself. To further extend the utility of aliphatic polyesters in biomedical and therapeutic applications, poly((α-propargyl-δ-valerolactone)-*co*-(ε-caprolactone)) was functionalized with both azide-functionalized PEG and an azide-functionalized camptothecin derivative in a sequential set of reactions to generate a water-soluble polyester−camptothecin conjugate (Scheme 23B).

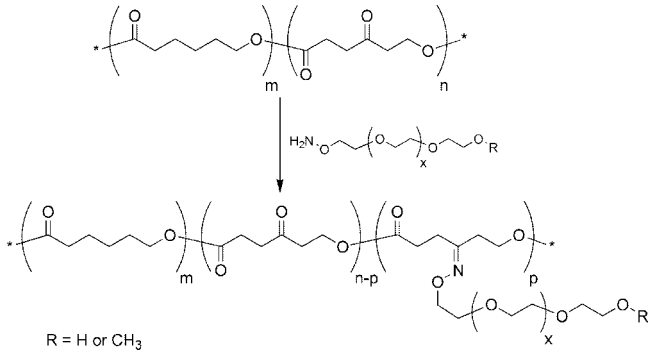
The water solubility of commonly used, polyester-based biomaterials has also been controlled and modified using click chemistry and click-able lactide monomer units.[160] In order to provide a functional handle for postpolymerization modification, 3,6-dipropargyl-1,4-dioxane-2,5-dione (PGL), an acetylene-functionalized glycolide monomer, was polymerized and copolymerized with lactide to yield a polyglycolide homopolymer and copolymer, respectively. These polymers can act as platforms upon which additional chemical functionality or polymeric grafts can be incorporated via triazole formation. This particular strategy provides a single monomer that allows for the incorporation of numerous functionalities onto a polyester substrate, while avoiding backbone degradation. To impart water solubility, both the PPGL homopolymer and the PPG-PLA copolymer were functionalized with azide-terminated PEO to generate amphiphilic graft copolymers (Scheme 24).

In addition to the alkyne/azide click reaction, oxime bond formation has also proven useful in both the functionalization of polyesters and the construction of graft copolymers from polyesters. Poly(ε-caprolactone-*co*-2-oxepane-1,5-dione) (P(CL-*co*-OPD)), an aliphatic polyester with reactive ketones presented along the polyester backbone,[161] has been used for

Scheme 24. Synthesis of (A) PPGL-*g*-PEO Homopolymer and (B) (PPG-*g*-PEO)-*co*-PLA using CuAAC Chemistry[160]



Scheme 25. Preparation of PCL-*g*-PEO Using Ketoxime Ether Formation[131]



reaction with aminooxy groups, leading to facile and orthogonal incorporation of multiple functionalities, while minimizing exposure to reaction conditions that could lead to premature degradation.[162,163] Using a "grafting onto" approach, hydrophilic polymers with an aminooxy-terminus can be employed as nucleophiles to obtain amphiphilic graft copolymers via reaction with the ketones on the hydrophobic P(CL-*co*-OPD) backbone. Mayes et al. have used ketoxime ether formation for the chemoselective incorporation of PEO grafts ranging from 150 Da to 2 kDa[131] and have also used these graft copolymers to create functional biocompatible surfaces[164] (Scheme 25).

2.4. Star Polymer Synthesis and Functionalization

Many of the principles elucidated in section 2.5.1 for dendrimers are equally valid for star-shaped polymers, which consist of linear chains linked to a central core and, in general, have a lower functional group density compared with dendrimers.[137] Similarly, the synthesis and derivatization of star polymers allows for the construction of multifunctional single-molecule materials on the nanoscale that have well-defined chemical compositions and significant potential for orthogonal functionalization. While these are widely studied, the current discussion will specifically address the incorporation of functionality into star polymers via various synthetic techniques and will concentrate on the use of highly efficient and selective reactions for the synthesis and functionalization of star polymers, with a special emphasis on the construction of miktoarm systems.

2.4.1. Star Polymer Synthesis

In general, star polymers are grown by one of three different strategies: the "core first" approach, where the arms of the star are grown from a multifunctional initiator; the "arm first" technique, which involves the coupling of preformed macroinitiators (MI) or macromonomers (MM) with a cross-linking agent to form the core of the star; and the "coupling to" method, where preformed polymer chains are conjugated onto a multifunctional core (Figure 6). Controlled polymerization techniques have been widely employed in the synthesis of star polymers using each of these techniques, and reviews on anionic polymerization,[137] NMP,[3] ATRP,[4] and RAFT[140,141] have documented their use in the preparation of star topologies.

As previously mentioned, the "core first" approach employs a multifunctional core from which polymerization is initiated to form the arms of the star. As such, dendrimers,[165,166] hyperbranched polymers,[167] cyclodextrins,[168] and calixarenes[169,170] have all been used as scaffolds for the growth of polymeric arms. In the "core first" method, functionality is incorporated into the structure through the use of functional monomers, and multifunctionality has typically been introduced by growing block copolymer arms.[167,171–173] The same is generally true with respect to the synthesis of stars by the "arm first" technique, where prefunctionalized monovinyl comonomers and divinyl cross-linking agents are used during the core formation process.[174,175]

In direct contrast, for the "coupling to" method, the variety of star polymers that can be synthesized is limited by the efficiency and selectivity of the reactions used in the conjugation step. Consequently, the emphasis now being placed on robust, efficient, and orthogonal (REO) chemistries[142] or click reactions[143] has resulted in an increase in the number and types of star polymers that can be synthesized using a "coupling to" approach. As expected, there are a variety of examples that employ the CuAAC reaction between an azide and an alkyne to conjugate linear polymer chains onto multifunctional cores to produce well-defined star polymers.

A seminal example of this strategy involved the synthesis of three- and four-arm stars using ATRP and click cycloaddition.[86] In this example, polystyrene (PS) was synthesized by ATRP using ethyl 2-bromo isobutyrate (EBiB) as the initiator and CuBr/PMDETA as the catalyst system. The resulting bromine chain ends were converted

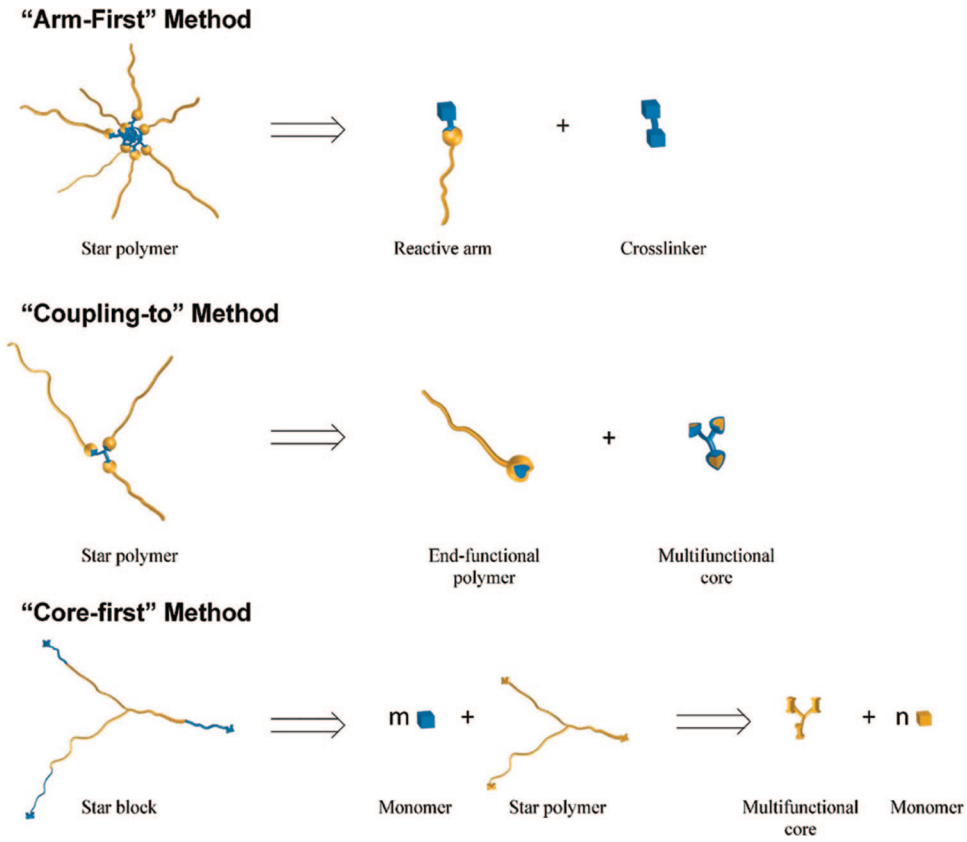


Figure 6. Illustration of the three general strategies for star polymer synthesis.[83]

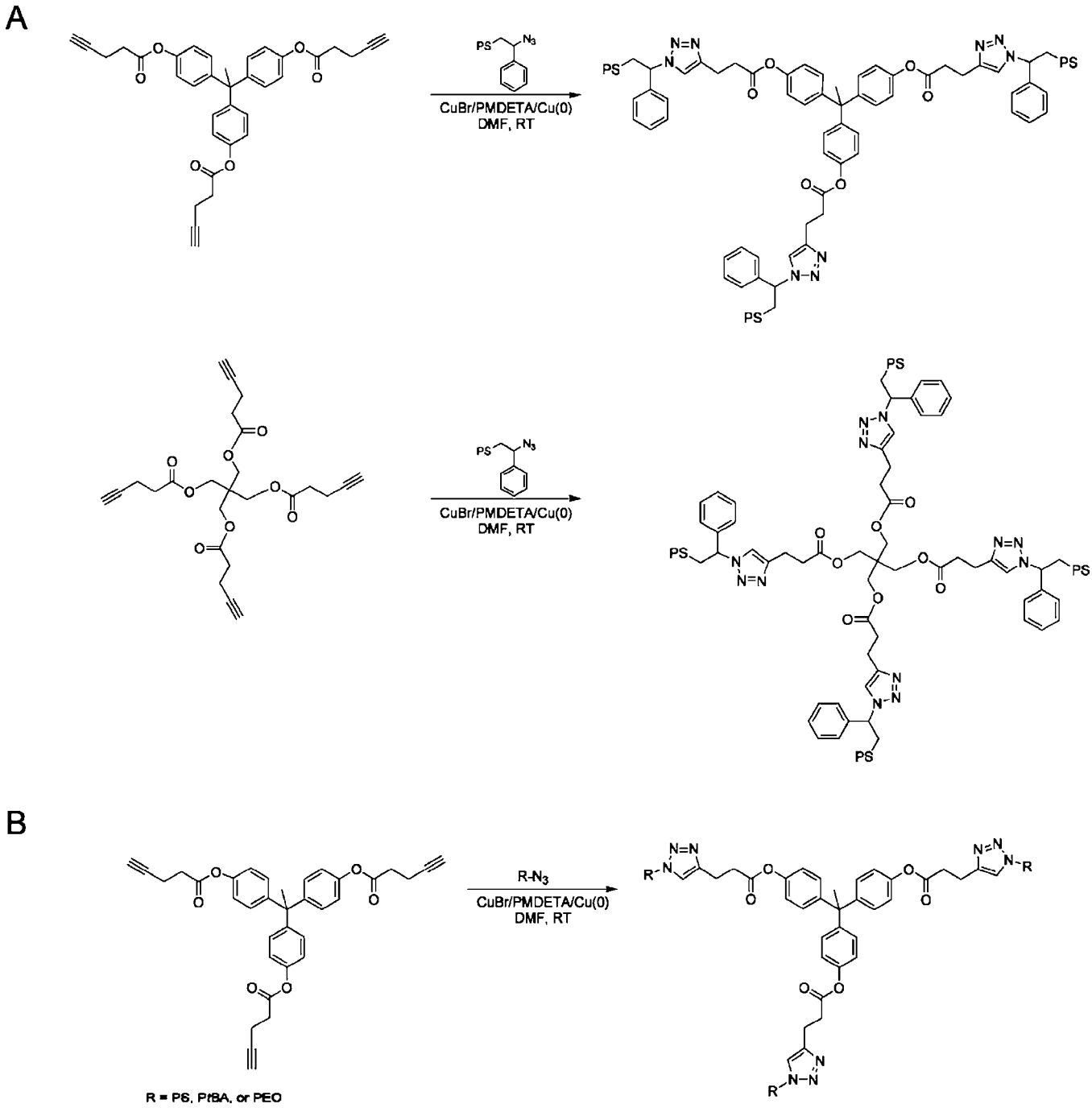
into azide groups by nucleophilic substitution with sodium azide. Subsequently, the azido-functionalized PS was reacted with trialkyne- and tetraalkyne-containing coupling agents to produce three- and four-armed PS star polymers, respectively (Scheme 26A). In this study, it was found that when the molar ratio of alkynyl groups to azido groups was 1:1, triazole formation was very efficient and resulted in a 90% yield and 83% yield of three- and four-arm star polymers, respectively. In a similar study, the effectiveness of click chemistry in the synthesis of star polymers using the "coupling to" method was again demonstrated with a series of three-arm star polymers prepared by reacting a trialkyne-containing core with azido-PS, azido-poly(*tert*-butyl acrylate) (P*t*BA), and azido-poly(ethylene oxide) (PEO) (Scheme 26B).[176] The application of the copper-catalyzed dipolar cycloaddition was found to be highly efficient, as 87% of the PS chains reacted to give a three-arm PS star. The efficiency of the click reaction was also observed in the construction of three-arm PEO and P*t*BA star polymers, which had respective formation efficiencies of 82% and 85%.

The use of the CuAAC cycloaddition reaction was also extended to the synthesis of star-shaped polyesters.[177] Poly(ε-caprolactone) (PCL) was synthesized using unprotected 5-hexyn-1-ol as an initiator and tin octanoate as the catalyst, thus incorporating the alkyne moiety at the PCL chain end. The resulting alkyne-functionalized PCL was reacted with heptakis-azido-β-cyclodextrin in the presence of copper sulfate and sodium ascorbate under microwave irradiation at 100 °C to yield a seven-armed PCL star (Scheme 27).

While the copper-catalyzed Huisgen reaction has received significant attention in the area of polymer synthesis, the Diels−Alder cycloaddition between anthracene derivatives and maleimides has recently been described as a click-type reaction and has been employed in the synthesis of diblock,[178] triblock,[32] and graft copolymers.[45,111] Three-arm stars were also prepared by coupling polymeric arms onto a trifunctional core using this specific Diels−Alder chemistry.[179] In this approach, protected maleimide-functionalized ATRP initiators were used to synthesize poly(methyl methacrylate) (PMMA) and P*t*BA; additionally, a furan-protected maleimide was conjugated to poly(ethylene glycol) (PEG) through an esterification reaction to produce a library of three polymers with protected maleimide end groups. The resulting maleimide-functionalized polymers could then be conjugated to a trifunctional core bearing three anthracene moieties to give PEG_3, $PMMA_3$, and $PtBA_3$ star polymers (Scheme 28) The high yields, 82%, 89%, and 93% yield for the PEG_3, $PMMA_3$, and $PtBA_3$ star polymers, respectively, indicates that the Diels−Alder reaction between furan-protected maleimides and anthracene derivatives is a robust and efficient method for the synthesis macromolecular architectures.

The groups of Barner-Kowollik and Stenzel also reported the synthesis of star polymers by the "coupling to" technique using a Diels−Alder reaction for the conjugation of linear polymer chains onto a diene-containing core.[180] While the prior example employed a Diels−Alder reaction between an anthracene moiety and a maleimide functionality, this study employed a hetero-Diels−Alder reaction to facilitate star polymer synthesis by exploiting the end group of polymers synthesized by RAFT polymerization. The polymeric arms of the star were grown by RAFT polymerization of styrene using benzyl (diethoxyphosphoryl)dithioformate as a chain transfer agent. The electron-withdrawing nature of the diethoxyphosphoryl group activates the thiocarbonyl end group on the PS as a reactive

Scheme 26. Examples of (A) Three- And Four-Arm Star Polymers Made by Click Coupling of Azido-Terminated Polystyrene onto a Central Tri- or Tetraalkynyl Core, Respectively, and (B) Three-Arm Stars Made by Independent Coupling of Several Types of Azido-Terminated Polymers to a Trialkynyl Core[86,176]

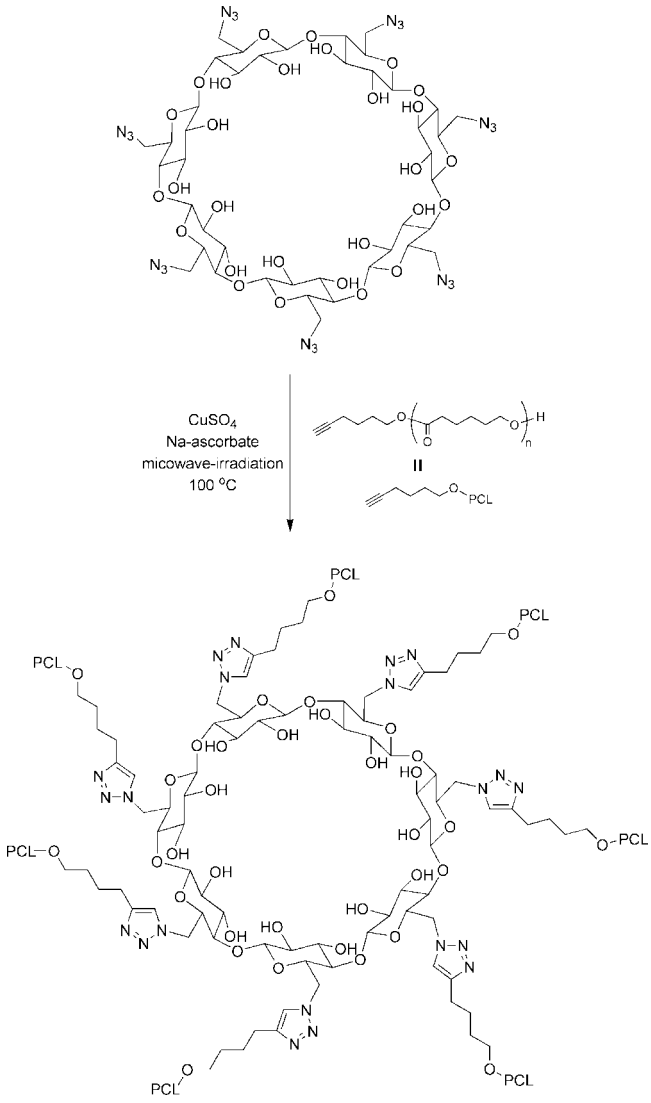


heterodienophile. Consequently, it was then used in a Diels−Alder reaction with multidiene coupling agents to yield two-, three-, and four-arm PS polymers with a star topology (Scheme 29). While previously described methods often required postpolymerization modification of the chain end prior to conjugation, this method is, in some ways, simpler because no modifications were necessary prior to the Diels−Alder coupling between the linear polymer arms and the multifunctional core.

In addition to cycloaddition chemistry, another reaction receiving significant attention due to high selectivity and reactivity is the Michael addition of a thiol to an alkene. Recently, the convergent synthesis of a three-arm star polymer involving a RAFT-synthesized precursor was reported.[181] A linear homopolymer of poly(*N*,*N*-diethylacrylamide) (PDEAm) was synthesized by RAFT polymerization under standard conditions using 1-cyano-1-methylethyldithiobenzoate as the chain transfer agent followed by reduction of the thioester and subsequent Michael addition of the thiol-terminated polymers to trimethylolpropane triacrylate to yield the desired three-arm PDEAm star polymer (Scheme 30). Since the "coupling to" method for constructing polymers with a star topology requires efficient chemical transformations that are tolerant of multiple functional groups, Michael reactions provide another approach for the facile synthesis of complex polymeric materials.

In an effort to further develop the use of highly efficient and orthogonal reactions in the synthesis of star polymers

Scheme 27. An Example of Click Chemistry to Make Star-Shaped PCL via Grafting of Alkynyl-Terminated PCL to a Cyclodextrin Core Bearing Seven Azides[177]

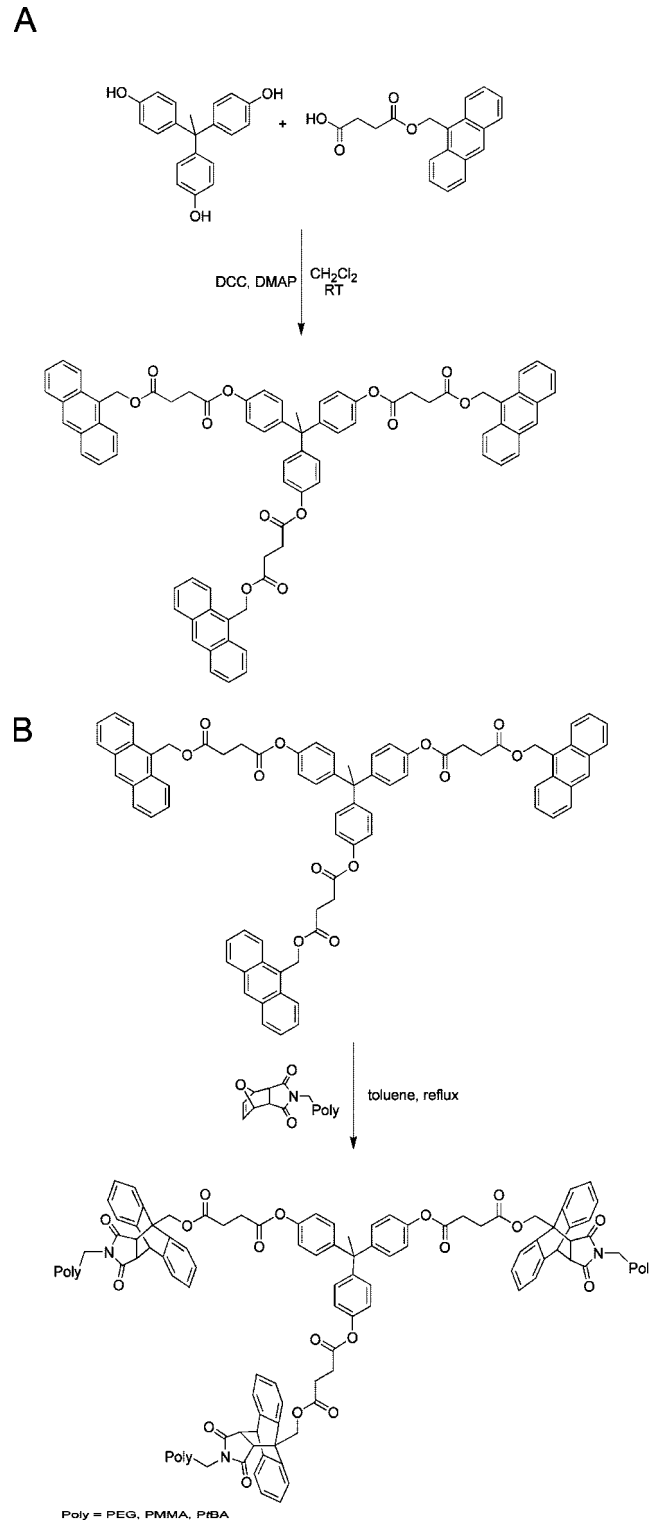


by the "coupling to" method, the Tunca group reported the one-pot synthesis of three-arm star block copolymers using two click reactions.[33] PS was initially synthesized using an anthracene-functionalized ATRP initiator, and the bromine chain end was then converted to an azide to give a heterobifunctional α-anthracene−ω-azide PS derivative. In analogy, PMMA was synthesized by ATRP using a protected maleimide initiator, and PEG was functionalized with a protected maleimide through ester bond formation. To complete the one-pot synthesis of star block copolymers, a trialkyne functional linking agent was combined with α-anthracene−ω-azide PS and either maleimide-functionalized PMMA or PEG to give PS-*b*-PMMA and PS-*b*-PEG three-arm star polymers, respectively (Scheme 31). The use of a one-pot methodology clearly demonstrated the orthogonality of the Diels−Alder and Huisgen cycloadditions.

2.4.2. Chain End Functionalization of Star Polymers

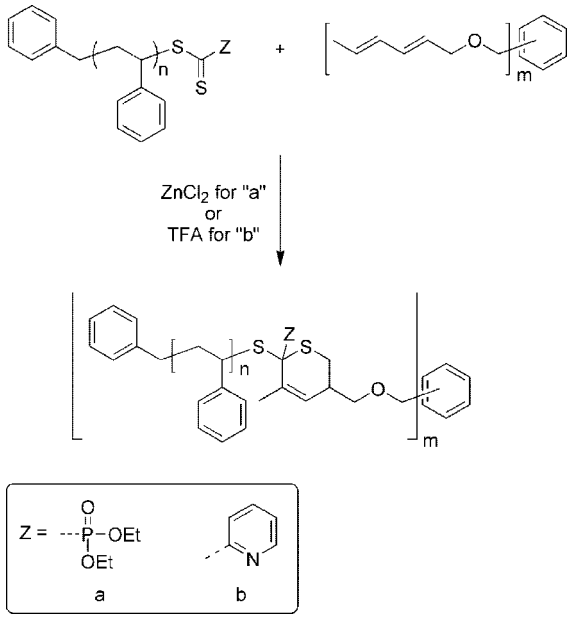
In parallel with dendrimer chemistry, the strategies used for functionalization of the chain ends of star polymers, like the chemistries used for synthesizing star polymers via the "coupling onto" approach, must have a high degree of reactivity and selectivity. A particularly elegant example of

Scheme 28. Construction of Three-Arm Star Polymers Using an Anthracene−Maleimide Based Diels−Alder Click Reaction[179]

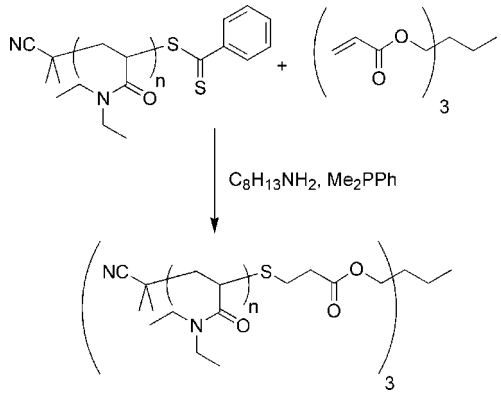


a one-pot synthesis of chain end functional, stereoregular, star-shaped poly(lactide) (PLA) with three or six arms was recently reported by Dove and co-workers.[182] Initial ring-opening polymerization of lactide was initiated by 1,1,1-tris(hydroxymethyl)ethane and an aluminum−salen complex to yield three-arm PLA star polymers, and as part of the one-pot synthesis, the polymerization was quenched with either a furan-protected maleimide-functionalized acid chloride or hexanoyl chloride to give star-shaped PLA with click

Scheme 29. Use of RAFT Polymerization and Subsequent Hetero-Diels−Alder Reaction To Construct Two-, Three-, and Four-Arm Star Topologies[180]



Scheme 30. Star Polymer Synthesis Utilizing Michael Addition of a Dithioester-Terminated Linear Polymer to a Multifunctional Diene Core[181]



functional handles at the chain termini (Scheme 32). The alkyne-terminated PLA was reacted with an azido-PEO via a CuAAC reaction, while the maleimide-terminated PLA was functionalized with thiophenol through Michael addition (Scheme 32).

Significantly, the thiol−maleimide reaction gave quantitative chain end conversion, while the cycloaddition reaction between an azide and alkyne only proceeded to 40% conversion before degradation of the polyester was observed. To further extend the utility of the one-pot synthesis, the star-shaped PLA was also quenched with the chain transfer agent 4-(chlorocarbonyl)benzyldodecyl trithiocarbonate; the resulting PLA was chain extended with styrene to yield star-shaped block copolymers with a degradable PLA core (Scheme 33).

While CuAAC did not prove to be the most successful conjugation method for functionalizing the chain ends of PLA, it has been used effectively for the addition of polymer chains to a three-arm star polymer. A three-arm PS star was synthesized using ATRP and the "core first" technique with the benzylic bromide chain ends being transformed to azides and subsequent reaction with alkyne-terminated PEO produced a (PS-*b*-PEO)$_3$ three-arm star block copolymer via click cycloaddition (Scheme 34).[83]

Another example that demonstrated the preparation of star block copolymers employed the "arm first" methodology and a Diels−Alder cycloaddition reaction to synthesize a multiarm, core-cross-linked star with PS-*b*-PMA or PS-*b*-P*t*BA arms.[35] Polymerization of styrene was initiated with an anthracene-functionalized ATRP initiator, and a multiarm star presenting anthracyl moieties on the periphery was achieved by cross-linking the dormant linear chains with divinylbenzene. The resulting multiarm PS star could then be reacted with either PMMA or P*t*BA chains bearing furan-protected maleimide groups, employing *in situ* deprotection, to yield a multiarm star block copolymer (Scheme 35).

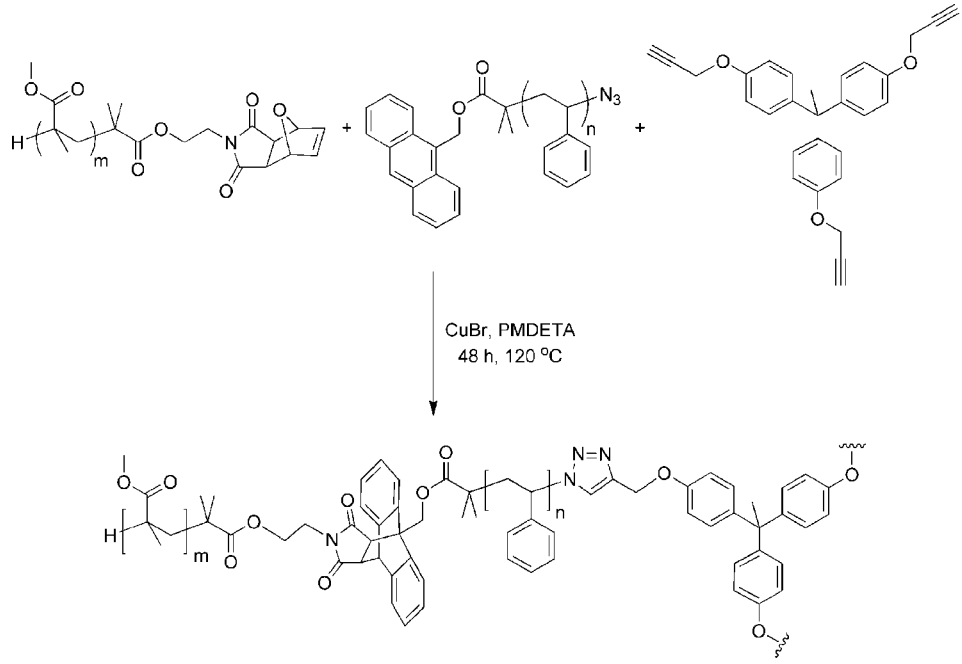
2.4.3. Synthesis of Miktoarm Star Polymers

End group functionalization and the growth of block copolymer arms provide two strategies for the incorporation of functionality into polymers with star topologies. Another approach is the preparation of miktoarm or heteroarm star copolymers that contain two or more different types of chemically unique arms connected to a central core (Figure 7). These unique macromolecular architectures are particularly interesting since a star-shaped architecture composed of arms with different chemical and physical characteristics imbues the resulting star polymer with distinctive properties.[137]

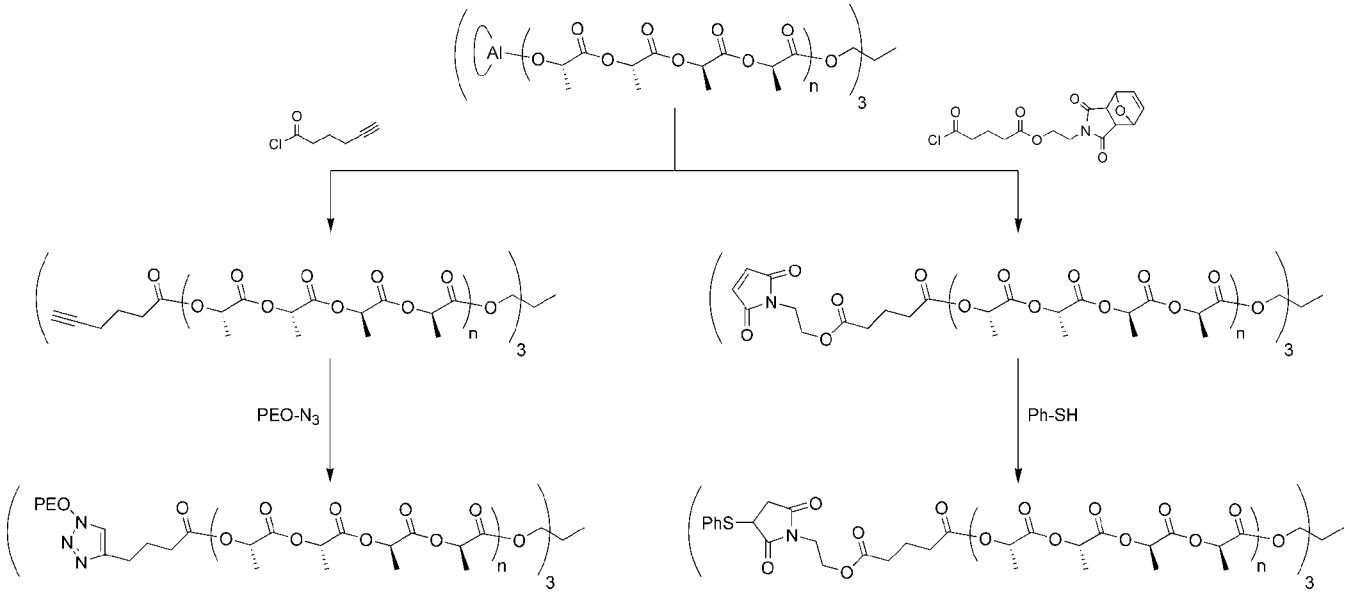
The three basic strategies for constructing star polymers, "core first," "arm first", and "coupling onto", have all been employed in the synthesis of miktoarm stars. However, the synthetic approaches that exemplify the spirit of this review involve the construction of miktoarm stars using a multifunctional initiator, where orthogonal polymerization methods are employed to grow unique polymeric arms, combined with a "coupling" approach, where linear polymers or block copolymers are connected at a central point using efficient and orthogonal chemical conjugations.

Using a "core-first" or "core-out" approach, the groups of Hedrick and Miller employed a miktofunctional initiator for the synthesis of a star polymer with alternating PCL and PMMA arms via ROP and ATRP.[183] The synthetic approach for construction of the initiator involved the coupling of a protected hydroxyl group to be used for the initiation of ROP and an activated bromide for subsequent ATRP to a trifunctional core (Scheme 36). To achieve an alternating miktoarm star, the polymerizations were performed using two different sequences. The first involved polymerization of MMA from a silyl ether protected initiator followed by deprotection to expose hydroxyl functionalities that were then used to initiate the ROP of CL (Scheme 36, route A). In this sequence, the polymerization of MMA resulted in a well-defined three-arm PMMA star, and deprotection of the silyl ether groups was achieved without degradation. From the hydroxyl-functionalized PMMA macroinitiator, PCL arms were grown via ROP to yield the alternating six-arm miktostar. The second sequence involved deprotection of the silyl ether groups prior to polymerization, after which ROP of CL and ATRP of MMA were performed consecutively (Scheme 36, route B). The initial deprotection resulted in low yields of the miktoinitiator, a result attributed to the presence and proximity of reactive bromide moieties in the small molecule, because the deprotection of the PMMA macroinitiator described in the previous sequence occurred in near quantitative yields. ROP of CL was performed

Scheme 31. One-Pot Synthesis of a Three-Arm Star Block Copolymer through Two Selective and Sequential Click Reactions[33]



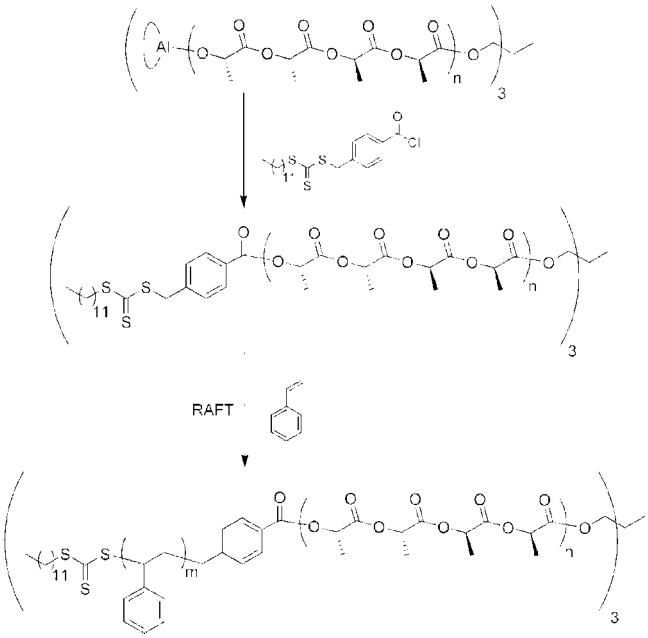
Scheme 32. One-Pot Synthesis of Chain End Functional, Stereoregular, Star-Shaped Poly(lactide) and Subsequent Chain End Functionalization via 1,3-Dipolar Cycloaddition and Michael Addition Chemistry[182]



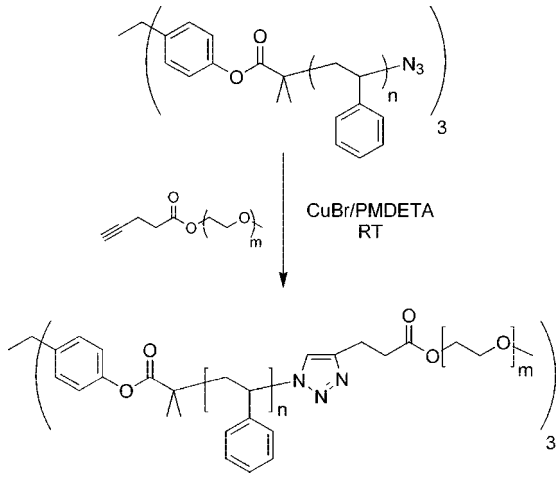
followed by ATRP of MMA to yield the six-arm miktostar polymer demonstrating both the tolerance of an ATRP initiator to ROP conditions and the orthogonality of these two polymerization methods.

The synthesis of ABC miktostars using the "core-out" approach and three different controlled polymerization methods was reported in two different studies. Zhao and coworkers reported the sequential synthesis of a PCL/PMMA/PS miktoarm star from a trifunctional initiator using ROP, ATRP, and NMP to prepare each respective arm (Scheme 37A).[184] While any of the polymerization methods could technically be performed first, Zhao et al. started with the ROP of CL using triethyl aluminum and the trifunctional initiator because living ROP requires anhydrous conditions that are much more easily accomplished using small molecules than polymer-containing components. After the growth of the PCL arm, ATRP was used to grow the PMMA arm due to the stability of the NMP initiator under standard ATRP conditions. The final arm was made by the NMP of styrene to yield a star with three unique polymeric arms. Similarly, Tunca et al. prepared a three-arm star containing PCL, PS, and P*t*BA arms via sequential ROP, NMP, and ATRP (Scheme 37B).[185] The groups of Tunca and Zhao both demonstrated the syntheses of ABC-type miktoarm star terpolymers using three different controlled polymerization methods that, when performed in the proper order, allow for the polymerization of each arm in the presence of initiators reserved for subsequent polymerization reactions. The employment of miktoinitiators provides a general strategy for the synthesis of a variety of well-defined miktoarm star polymers and clearly demonstrates the range of complex structures that can easily be prepared if the orthogonality of polymerization techniques and initiators with respect to each other is known.

Scheme 33. Chain End Functionalization with a RAFT Chain-Transfer Agent and Chain Extension with Styrene To Yield Star-Shaped Block Copolymers[182]



Scheme 34. Synthesis of PS-*b*-PEO Three-Arm Star Block Copolymers by a Combination of the "Core First" and "Coupling to" Methods[83]



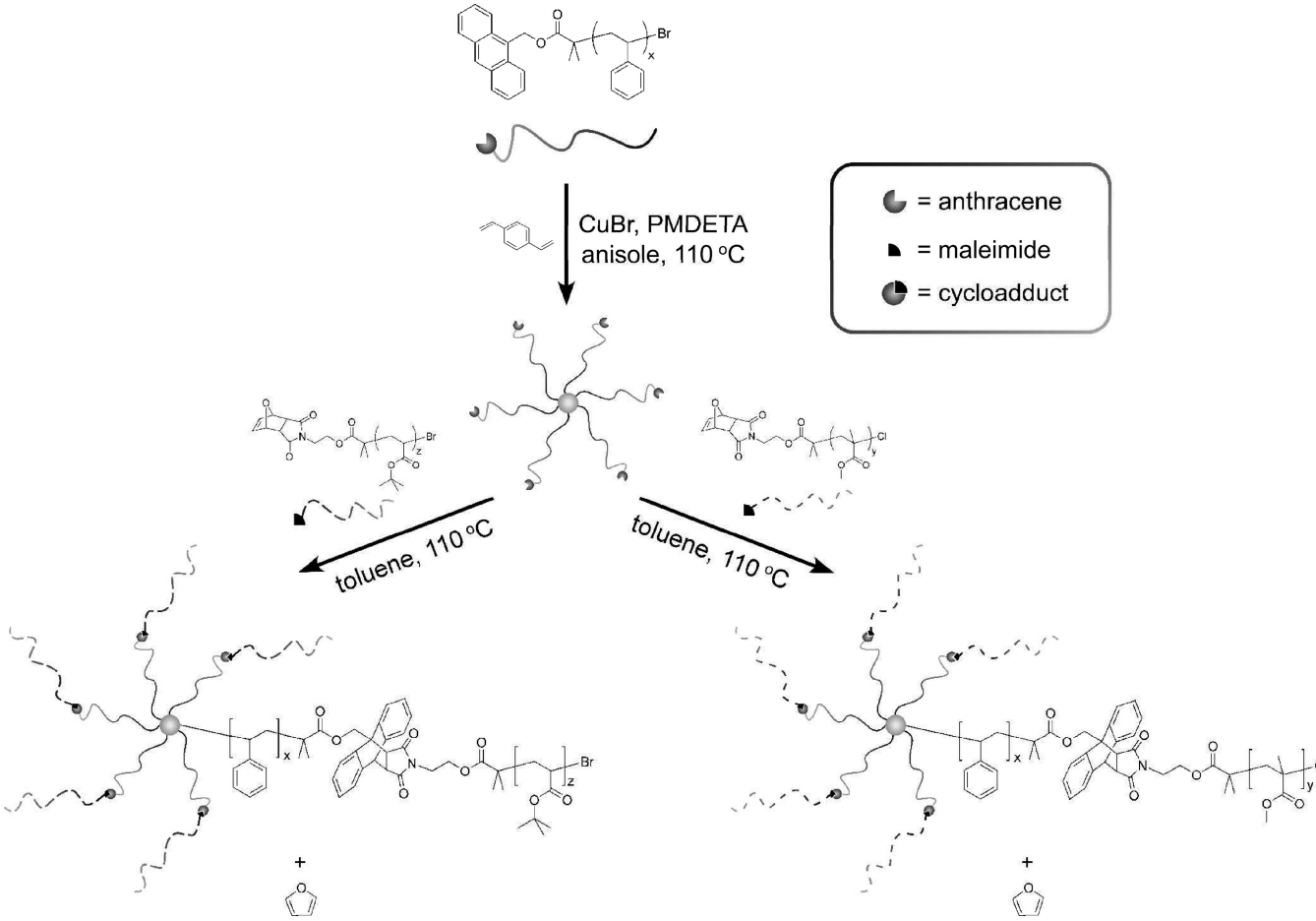
The synthesis of ABC-type heteroarm star terpolymers made through the combination of controlled polymerization methods and CuAAC chemistry using a "coupling" strategy has also been explored. In this case, the Tunca group employed a bifunctional ATRP and NMP initiator with a central alkyne moiety to initiate polymerization of methyl methacrylate and styrene, respectively. The desired ABC star terpolymers were then constructed by coupling either azido-terminated PEO or azido-terminated P*t*BA to the alkyne-containing PMMA-*b*-PS (Scheme 38). Interestingly, no significant reaction was observed at the propargyl unit during the two sequential living free radical procedures.[186]

The use of complementary polymerization techniques and REO chemistries for the construction of complex star topologies was advanced through the implementation of a one-pot synthesis of an ABC three-arm star by ROP, ATRP, NMP, and the click [3 + 2] cycloaddition.[53] In this study, a bifunctional initiator, 2-(hydroxymethyl)-2-methyl-3-oxo-(2-phenyl-2-(2,2,6,6-tetramethylpiperidin-1-yloxy)ethoxy) propyl pent-4-ynoate, ε-caprolactone, styrene, and either azido-terminated PEG or PMMA were combined in a single flask and were heated in the presence of CuBr, PMDETA, and tin octanoate at 125 °C for 48 h (Scheme 39A). This reaction yielded either PEG−PCL−PS or PMMA−PCL−PS miktoarm star terpolymers in a one-pot, one-step synthesis. While the GPC traces of the terpolymers were monomodal, a low molecular weight tail was observed in the PEG−PCL−PS terpolymer suggesting that there may be star polymers present with shorter PCL and PS arms relative to the PEG arm. In an attempt to resolve this problem, a one-pot, two-step synthesis was performed to construct comparable star terpolymers (Scheme 39B). In this reaction, the bifunctional initiator, ε-caprolactone, styrene, and tin octanoate were combined in a single flask and heated at 125 °C for 20 h to yield the alkyne-functionalized PCL-*b*-PS *in situ*. A solution of either azido-PEG or azido-P*t*BA was then added to the reaction flask followed by the addition of CuBr and PMDETA to catalyze 1,2,3-triazole formation. The resulting PEG−PCL−PS or P*t*BA−PCL−PS three-miktoarm star terpolymers afforded GPC traces that were monomodal and that did not display any low molecular weight tailing. The one-pot synthetic method clearly demonstrates the utility of REO chemistries and also provides an approach for the efficient and straightforward synthesis of ABC three-arm star polymers. It should be noted, however, that while this technique is promising, it does have some limitations; specifically polymerization conditions that could cause star−star coupling and the possibility of inefficient click coupling at lower initiator concentration.

While the synthesis of three-arm miktostars is becoming more prevalent, the synthesis of miktoarm stars bearing four or more unique polymeric arms is rare, due to the complexity of the synthetic methodologies that must be implemented. However, by employing a strategy similar to that used in the synthesis of ABC miktoarm terpolymers, the production of ABCD four-miktoarm star quarterpolymers was completed using a combination of ROP, NMP, ATRP, and click coupling.[88] In this synthesis, a PCL-*b*-PS copolymer with an azide at the junction was a synthesized in a one-pot, two-step reaction; the first step involved the growth of PS and PCL from a bifunctional initiator using ROP and NMP, respectively; the second step involved transformation of a central reactive bromide into an azide (Scheme 40A). A second block copolymer was made by coupling a carboxylic acid-functionalized PEG onto an alkyne-functionalized ATRP initiator; this PEG-macroinitiator was used to initiate ATRP polymerization of MMA or *t*BA to form PEG-*b*-PMMA or PEG-*b*-P*t*BA with an alkyne at the junction point (Scheme 40B). To obtain the desired ABCD four-miktoarm star polymers, the azide-functionalized PCL-*b*-PS copolymer was reacted with either the alkyne-containing PEG-*b*-PMMA or PEG-*b*-P*t*BA in the presence of CuBr or CuCl and PMDETA to yield PCL−PS−PEG−PMMA and PCL−PS−PEG−P*t*BA heteroarm star polymers via click cycloaddition chemistry (Scheme 40C).

To further illustrate the utility of combining different, yet compatible, polymerization strategies with REO coupling approaches, ABCD star polymers with arms consisting of PS, PEO, PCL, and PMA have been assembled.[187] A PS-*b*-

Scheme 35. Synthesis of Multiarm Star Block Copolymers Using the Diels−Alder Click Reaction between Anthracene and Maleimide Derivatives[35]



PCL copolymer with an alkyne at the junction was synthesized by growing PS by RAFT polymerization followed by functionalization with 2-hydroxyethyl-3-(4-prop-2-ynyloxy)phenyl) acrylate (HEPPA), a non-homopolymerizable cinnamate with alkyne and hydroxyl moieties, to yield a PS−HEPPA conjugate; this conjugate was then used as a macroinitiator for the ROP of CL to give PS−HEPPA−PCL (Scheme 41). A PMA arm was grown from the PS−HEPPA−PCL macroinitiator by RAFT polymerization to yield a PS−PCL−PMA three-arm star with an alkyne present at the junction of the three arms (Scheme 41). In the final step of the synthesis, an azido-PEO was conjugated to the three-

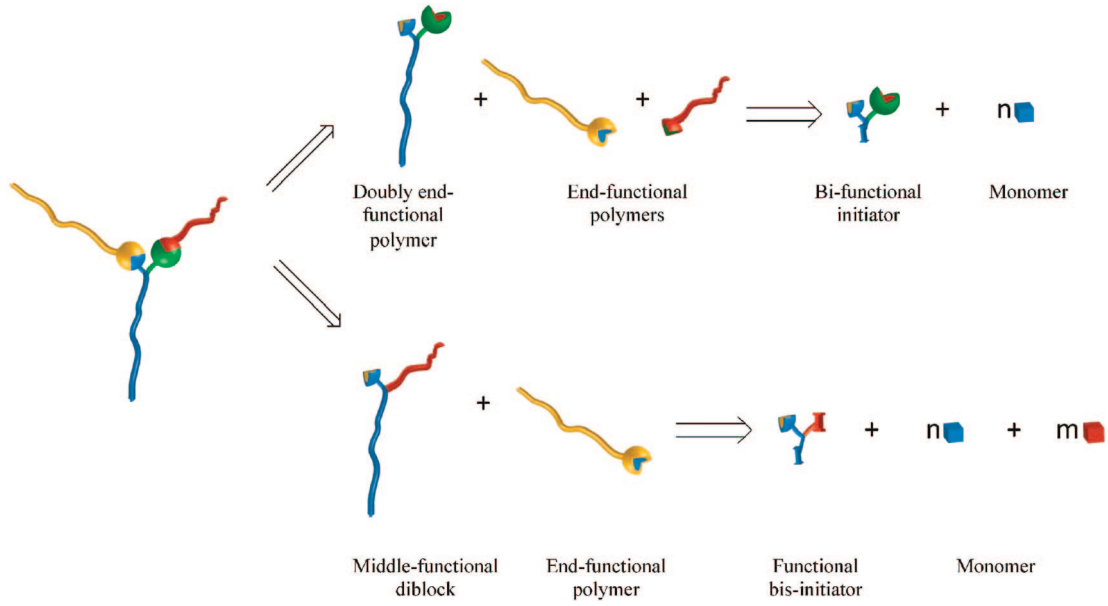
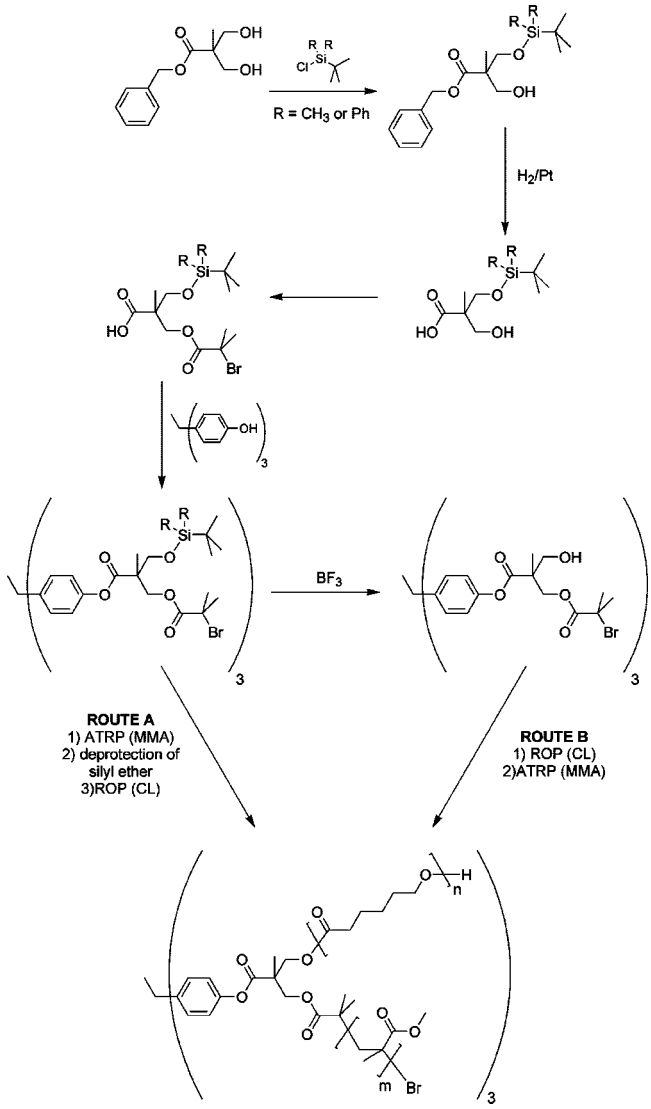


Figure 7. Retrosynthetic analysis for miktoarm star polymers prepared using REO chemistry.

Scheme 36. Synthetic Routes To Produce an Alternating, Six-Arm PCL−PMMA Miktoarm Star Polymer[183]



arm star by click cycloaddition to produce a four-miktoarm star quarterpolymer (Scheme 41).

2.5. Branched Systems

2.5.1. Dendritic Polymers

The area of dendrimer chemistry has been extensively reviewed over the past decade;[188–203] however their monodispersity and large number of functional end groups makes them an excellent test vehicle for developing and exploiting REO chemistry. In order to focus on the potential for click reactions in dendrimer chemistry, we have chosen to focus on the recent applications of orthogonal chemistry that extend beyond the standard surface/focal point modification and global functionalization of dendrimers and dendrons that have been previously reported.[188–203] More consideration will be given to examples that include the fabrication of asymmetric dendrimers carrying two or more functional groups that can be addressed independently. The interest in such structures is rapidly increasing in the area of nanomedicine where accurate control over the placement of radioisotopes, near-IR dyes, etc. for detection and the overall three-dimensional shape for regulating biodistribution is proving to be critical for multimodal, targeted imaging, which necessitates the need for effective conjugation strategies.

The group of Fréchet has pioneered the construction of advanced multifunctional dendrimers with orthogonal functional units for biomedical applications.[204,205] The term "bow-tie dendrimers", has been developed for these systems with the earliest examples being polyester-based and diverse in their applicability owing to their compositions, including a Janus-type structure, being functionalized on one molecular face with latent hydroxyl groups and on the other with *p*-nitrophenyl carbonates that could be reacted independently. Such orthogonally functionalizable dendritic polyester structures have been further refined since the first published examples and now encompass a range of advanced macromolecular constructs that are helping to define many important structure/property principles in nanomedicine (Scheme 42).[206,207]

The groups of Hawker[208] and Malkoch[209] have also utilized polyester-based dendrimers as scaffolds for preparing orthogonally functionalized dendrimers, again exploiting combinations of peripherally addressable hydroxyl and acetylene[208] or hydroxyl and azide groups (Scheme 43). These functional groups have been further derivatized with sugars and optical probes in good yields[208] and have also allowed the development of accelerated one-pot divergent growth strategies for dendrimers via ester formation and 1,3-dipolar cycloaddition reactions.[209]

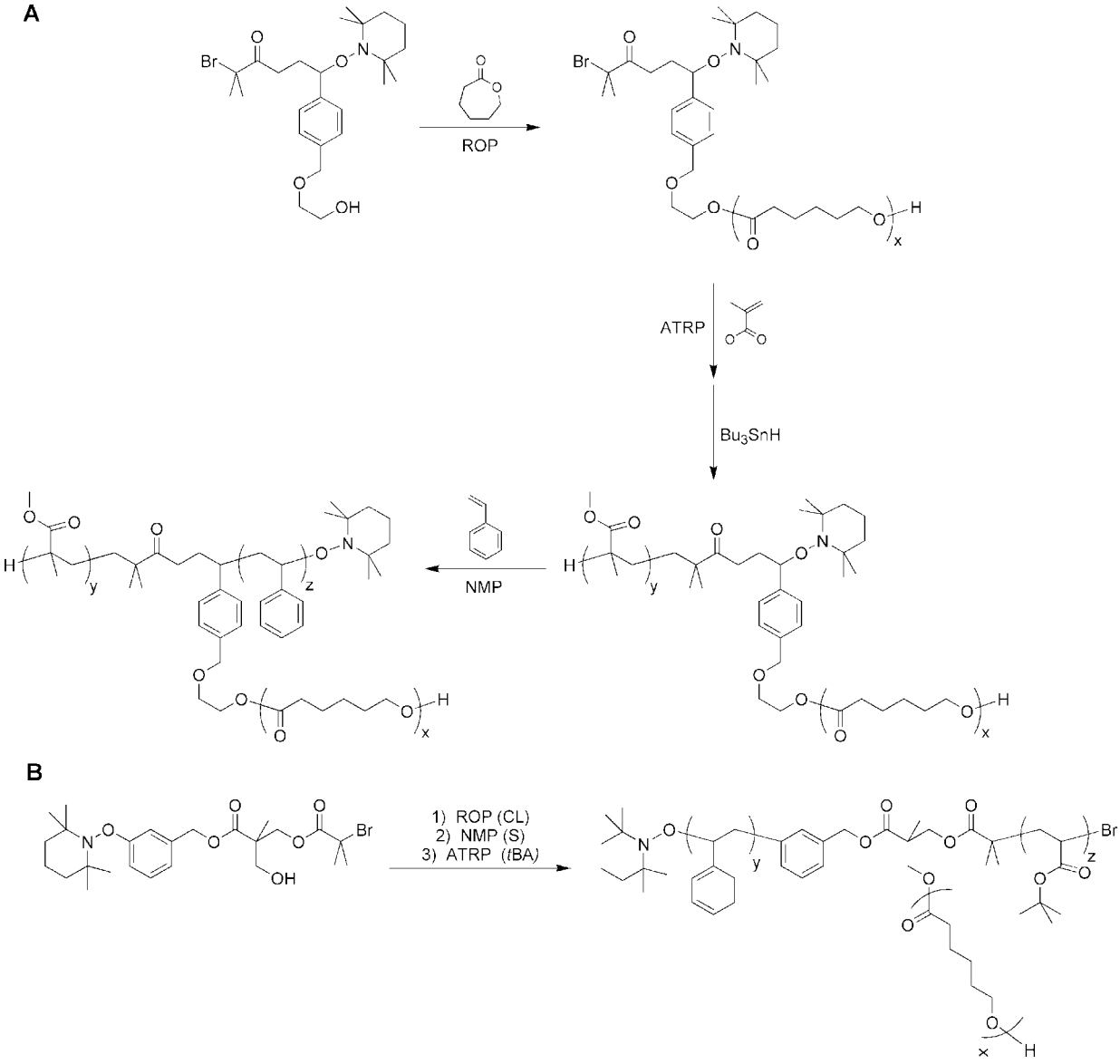
Orthogonally functionalized dendrimers have also been prepared based on a polyamide skeleton, built in a selective manner to present either aldehydes or a combination of azides and aldehydes in the shell of the dendrimer (Scheme 44).[210] These groups were further probed by conjugation with propargyl glycine and biotin hydrazide with yields of 97%, suggesting that these dendrimers undergo facile functionalization with large, highly functional units. Modification of polyamidoamine (PAMAM) dendrimers with cyclic RGD ligands and chelators through oxime bond formation has also been reported recently.[211] In this case, the modifications were performed sequentially, utilizing aldehyde functional peptides and gadolinium chelators. By this method, the exact placement of each unit is not controlled and the number of units conjugated is only defined by the stoichiometry of the reaction.

Sophisticated examples of orthogonally functionalized dendrimers, possessing up to six different functional groups that can all be addressed independently, have recently been presented by Simanek et al.[212] These dendrimers, as well as "less" functional melamine-based dendrimers (Scheme 45),[212–216] may allow for the construction of complex dendritic macromolecules carrying a multitude of units with controlled placement, multiplicity, and function, one of the grand challenges in this area.

2.5.2. Dendronized Polymers

Dendronized polymers represent a hybrid architecture that combines linear and dendritic units to create three-dimensional macromolecular architectures that can adopt extended rod-like conformations due to the steric crowding imposed by the dendrons attached to the linear polymer backbone. Their synthesis (Figure 8) and applications have been covered in a number of earlier reviews,[200,201,217–223] and this discussion will be limited to highlighting advances in the synthesis of these complex macromolecules involving the application of covalent orthogonal chemistry.

Scheme 37. Syntheses of ABC Miktoarm Stars Employing ROP, NMP, ATRP and a "Core-First" Approach[184,185]

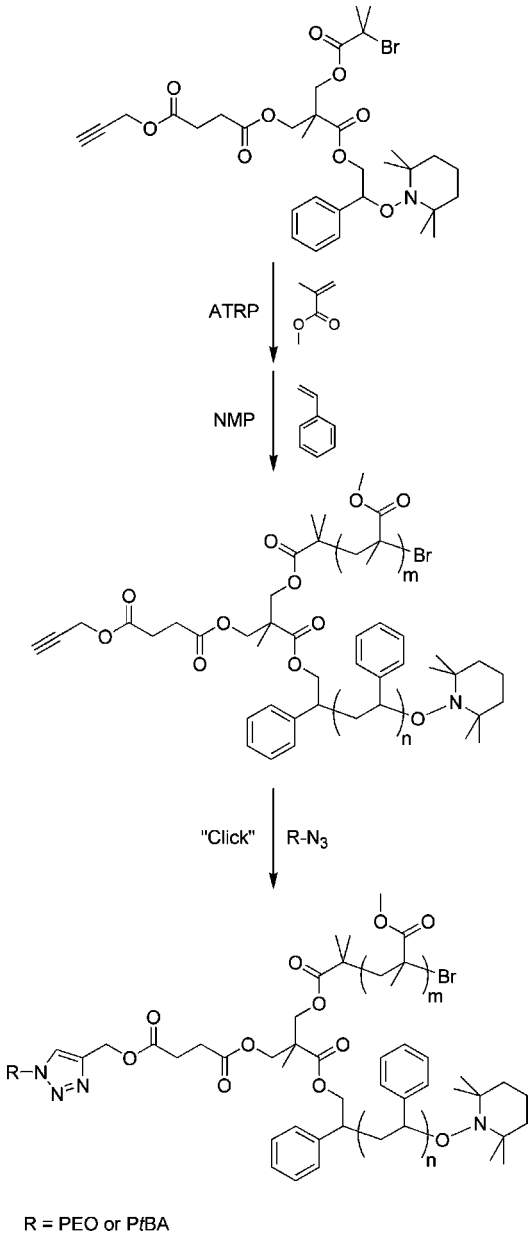


Following an early report by Fréchet and Hawker,[114] the same team published the synthesis of ultrahigh molecular weight doubly dendronized polymers based on CuAAC chemistry (Scheme 46).[224] This synthesis demonstrated the power of divergent growth approaches to achieve high molecular weight systems, as well as the efficiency and high yield that can be obtained with click chemistry. Starting with poly(*p*-hydroxystyrene) and dendronizing the initial backbone polymer up to the third generation with bisMPA-based dendrons results in eight chain end hydroxyl groups per repeat unit. This extremely high concentration of hydroxyl groups was then functionalized with 4-pentynoic acid to give a three-dimensional macromolecular object, functionalized radially with alkyne groups. By exploitation of the CuAAC reaction to overcome steric congestion, coupling of G3 polyether dendrons gave pseudo-G6 dendronized polymers with molecular weights in excess of 1 000 000 Da. Size exclusion chromatography−multiangle laser light scattering (SEC−MALLS), data, in addition to NMR and IR characterization, provided strong evidence for the effective coupling of the dendrons in this densely functionalized polymer using CuAAC.

Recent reports in which the click cycloaddition has been used to synthesize other complex dendronized systems have included Voit's synthesis of dendronized block copolymers from 4-hydroxystyrene precursors with a high degree of control over the block length and PDI through nitroxide-mediated polymerization.[225] They expanded this work to include poly(propargyloxystyrene) by using the TMS protecting group and demonstrated that the diversity of structure can be expanded by using click chemistry to further modify the block of poly(propargyloxystyrene). In addition to functionalizing this block with bulky groups, such as 1-adamantane azide,[226] they have reported the preliminary synthesis of diblock copolymers including a block of dendronized polymer prepared using CuAAC via the "grafting onto" approach (Scheme 47).[227]

Dendrons can also be placed specifically at the polymer chain ends through click chemistry, using methods described in section 2.1. In one elegant example, Gillies et al.[228] prepared a diblock copolymer poly(butadiene)-*b*-poly(ethylene glycol) with a single azide moiety on the free end of the PEG chain. These polymers form vesicles in aqueous solutions, with the azide group at the surface and available

Scheme 38. Click Coupling of PS-*b*-PMMA at an Alkyne Site Located between the PS and PMMA Chain Segments, With Either Azido-PEO or Azido-P*t*BA To Give ABC Miktoarm Star Copolymers[186]



for coupling to a dendron labeled with rhodamine derivatives (Figure 9). The use of a chain end functionalized dendrimer allows for a significant number of dye molecules to be covalently linked to the vesicle through a relatively small number of azide groups presented on the surface.

An interesting perspective on the synthesis of dendronized polymers through click chemistry comes from Chow et al.,[229] who utilized monomers containing hydrocarbon-based dendrons derived from Meldrum's acid. Opening of the Meldrum's acid core with a nucleophilic linker molecule introduces an azide moiety, and the resulting carboxylic acid can be coupled to install an alkyne, creating an AB monomer. In order to achieve a high degree of polymerization, these monomers were polymerized in the presence of copper sulfate and sodium ascorbate for 4 days and isolated by precipitation or extraction (Scheme 48). The resulting polymers are interesting not just for their architecture but also for their physical properties. The triazole backbone of the polymers gives high polarity and hydrogen bonding, while the dendrons provide for solvent entrapment. Interestingly, although the G2-based polymers formed organogels, G1 and G3 polymers did not; the authors suggested that the second-generation derivatives have the correct balance of hydrogen bonding and solvent entrapment for physical cross-linking to occur (Figure 10).

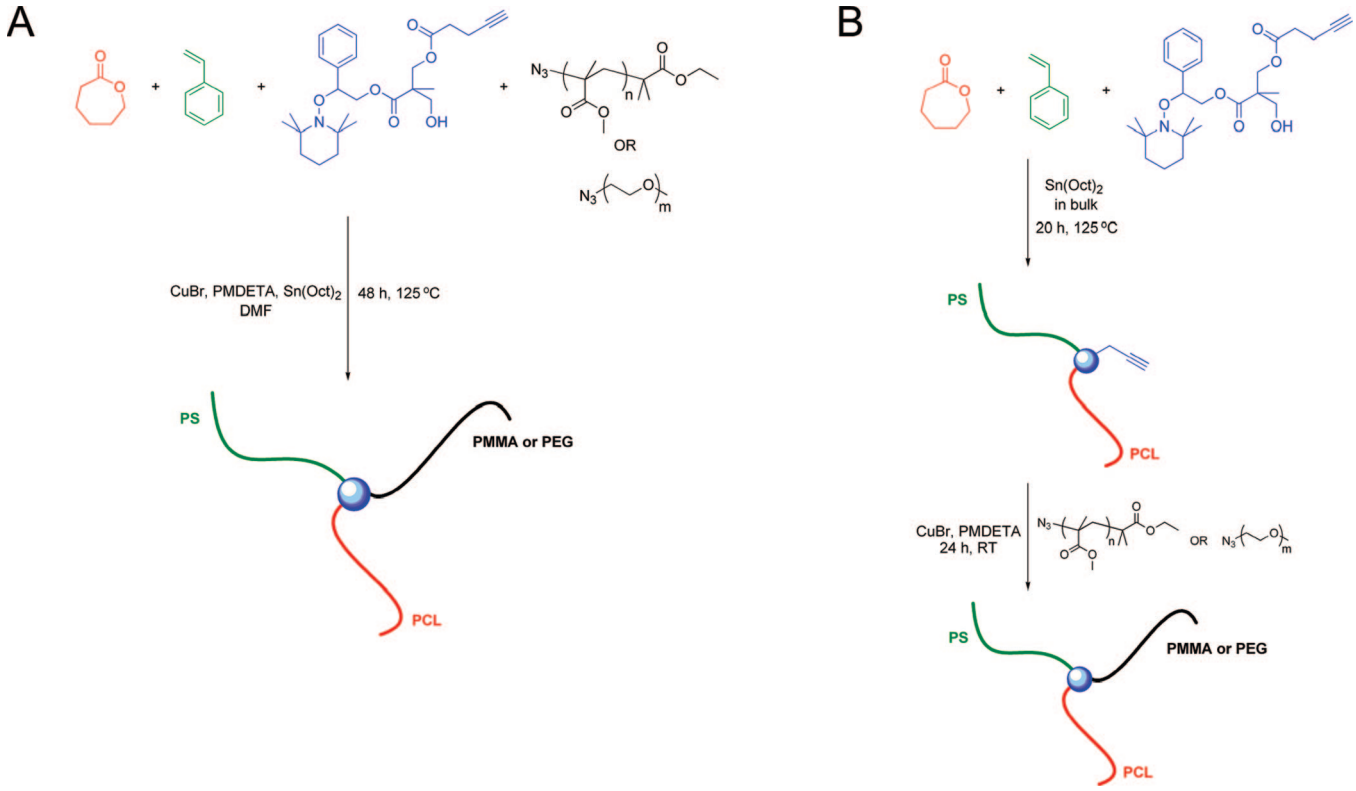
2.6. Cross-Linked Systems

Traditional cross-linked systems have relied on an excess of potentially reactive/cross-linking sites to achieve an infinite network, which negates inherent deficiencies in the cross-linking reaction as well as the loss in efficiency as reactive groups become sterically constrained during network formation. However, the high efficiency of REO reactions has allowed for a much greater degree of control over the network-forming reactions to be achieved and has led to the synthesis of ideal networks that can attain superior mechanical properties compared with conventional networks. In addition, the orthogonality that is by definition an important feature of REO reactions allows for the introduction of varied functional groups or materials into cross-linked systems and gels, both pre- and post-cross-linking (Figure 11).

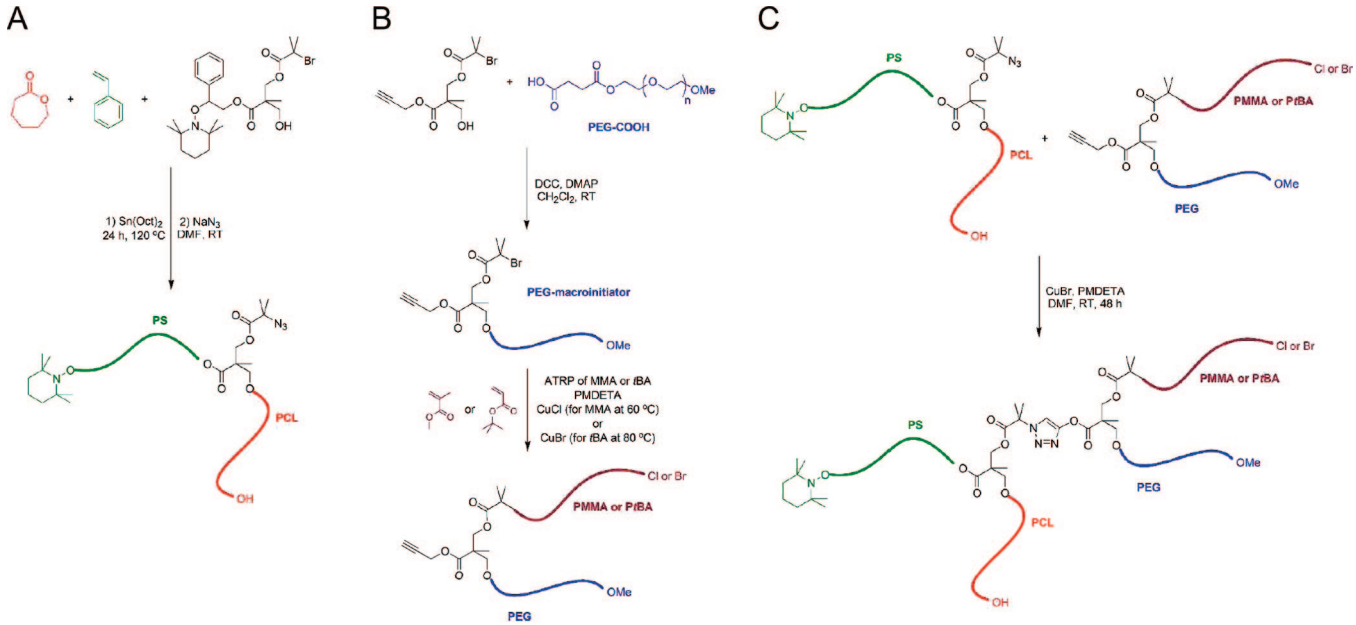
The most widely used REO chemistry for the formation of cross-linked networks is thiol−ene coupling, and its use has been extended to hydrogels,[230] thin films,[231,232] lithographic applications[233–235] and bulk materials. During the last several years, Hoyle has published an impressive body of work regarding the use of thiol−ene chemistry to make cross-linked networks with improved physical and mechanical properties.[231,236–251] These studies have addressed the energetics[251] and kinetics[236,242] of cross-linking, and the effects of alkene,[237,238,245,250] thiol,[252] and photoinitiator[237,238] structure on the mechanical properties of networks formed by thiol−ene chemistry. The groups of Anseth and Bowman have also taken advantage of the inherent versatility of thiol−ene chemistry to make cross-linked, degradable networks in a facile and highly efficient manner for use in biomedical applications.[233,253–262] In these studies, PEG-based hydrogels are prepared by cross-linking telechelic diallyl PEG chains with tri- or tetrathiols. These gels are designed to be biodegradable through the inclusion of ester groups either in the thiol-cross-linker or as short blocks of poly(lactide) between the PEG chain and the olefin end groups. These gels have been used to encapsulate cells and study the effect of network structure and chemical modification on cell−materials interactions.[260,261,263] The authors have also demonstrated that these gels can be formed in the presence of cells without adverse effects on the cell viability.[261] The groups of Bowman and Anseth have also recently explored thiol−yne chemistry for use in network formation[264] with the major difference being that the alkyne can react with two thiols (once as an alkyne, again as a vinyl sulfide), leading to more densely cross-linked networks compared with standard thiol−ene systems. As a result, the thiol−yne networks showed significantly higher glass transition temperatures and rubbery moduli than similarly prepared thiol−ene networks.

The CuAAC reaction has also been used successfully in the formation of gels and other cross-linked networks.[265–270] In 2006, Malkoch and co-workers exploited the click properties of this chemistry in the formation of hydrogels with tunable mechanical properties by cross-linking a dialkynyl PEG with a tetraazide (Scheme 49).[271] Network formation was reported to take place in less than 30 minutes

Scheme 39. (A) A One-Pot, One-Step Technique for the Preparation of Three-Miktoarm Star Terpolymers, and (B) a One-Pot, Two-Step Method for the Preparation of Three-Miktoarm Star Terpolymers[53]

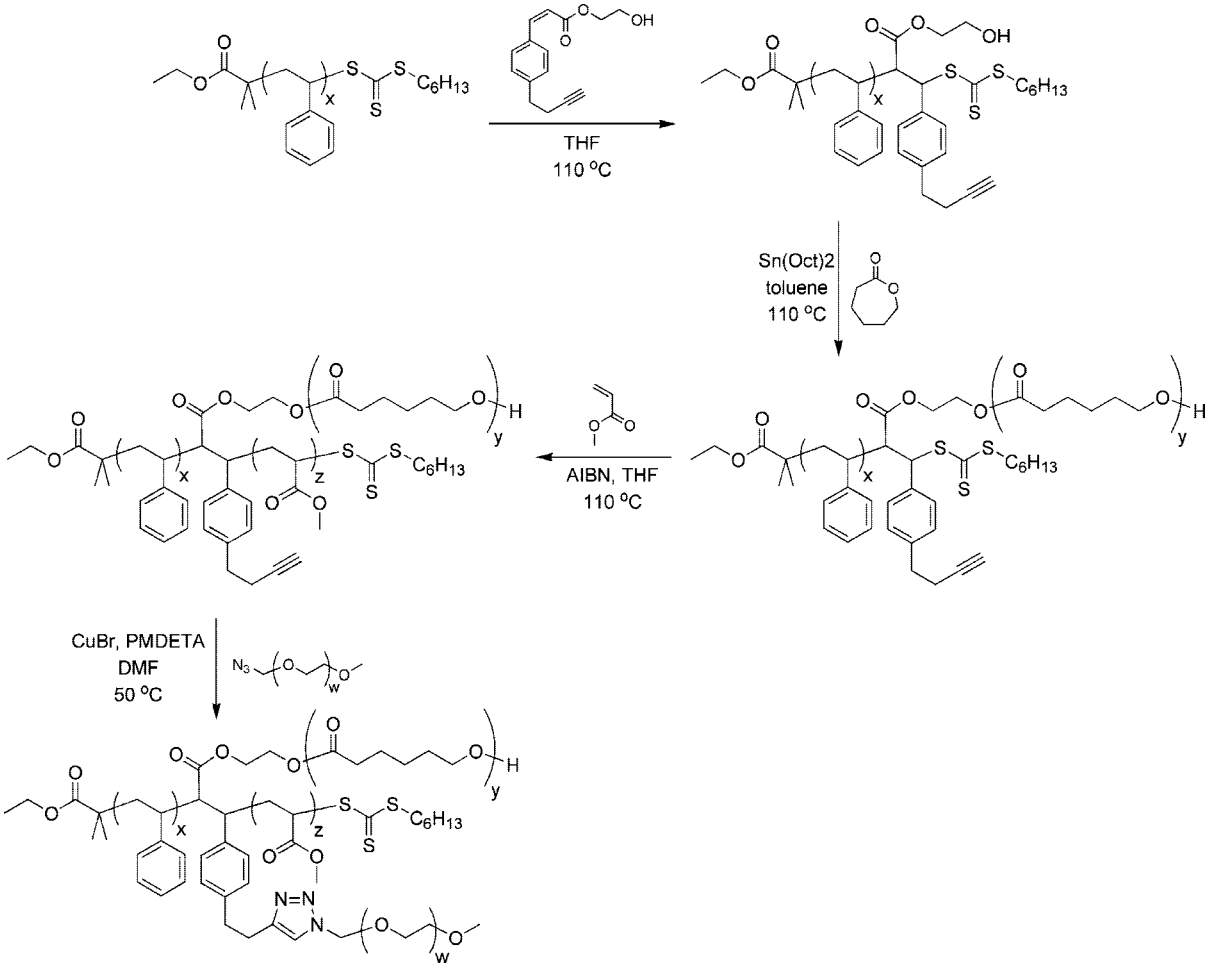


Scheme 40. Preparation of (A) a PS-*b*-PCL Copolymer with an Azide at the Junction Point, (B) PEG-*b*-PMMA or PEG-*b*-P*t*BA with an Alkyne at the Junction Point, and (C) PCL−PS−PEG−PMMA and PCL−PS−PEG−P*t*BA Heteroarm Star Polymers via Click Cycloadditions[88]



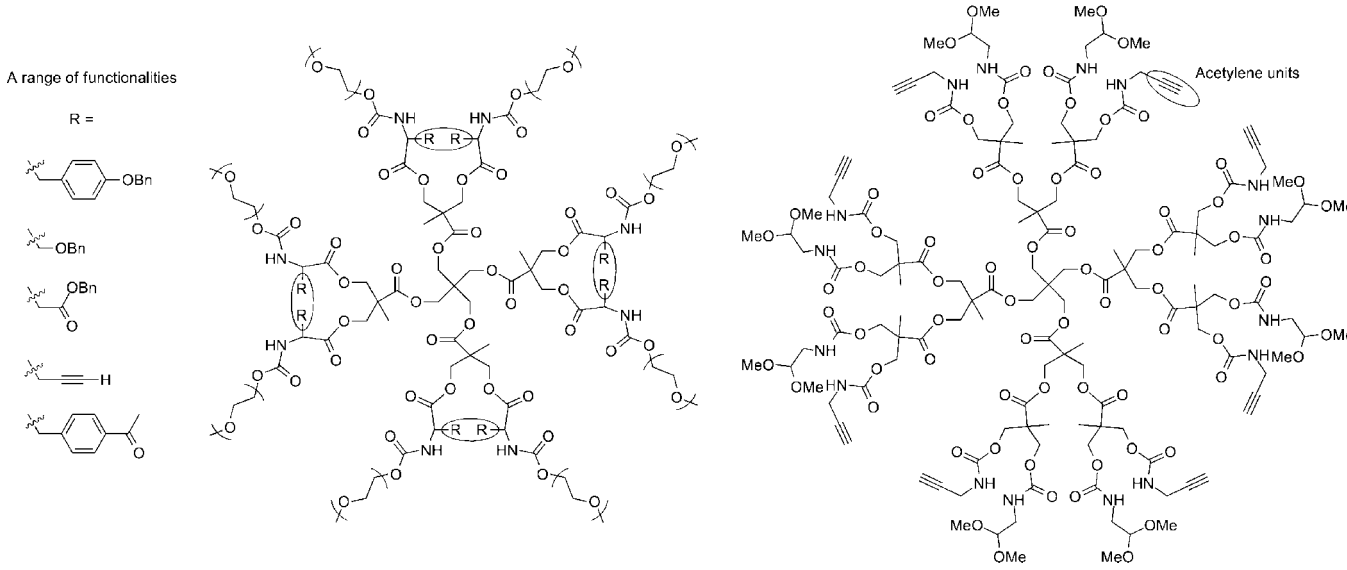
under standard CuAAC conditions, and in under 1 minute when subjected to microwave irradiation. The percentage of unreacted chain ends was determined to be less than 0.2% by using small molecule fluorophores in a subsequent click post-cross-linking reaction, and it was shown that various fillers (e.g., titanium dioxide nanoparticles) could be incorporated into the gels without adversely affecting the extent of cross-linking. The most remarkable aspect of this study was the resulting mechanical properties of the hydrogels, which were shown to withstand higher applied stresses as well as greater elongation before failure than analogous gels prepared by photopolymerization of acrylates. For example, in a comparison of gels made from PEG with molecular weights around 10 kDa, the click gels extended to 1550% of their original dimensions before breaking, which was 10-fold greater than the acrylate-based gel. The authors also demonstrated the facile removal of copper to allow for use of the hydrogels in biological systems. More recently, Anseth

Scheme 41. An ABCD Four-Arm Star Quarterpolymer Made Using a Combination of RAFT Polymerization, ROP, and Click Coupling[187]



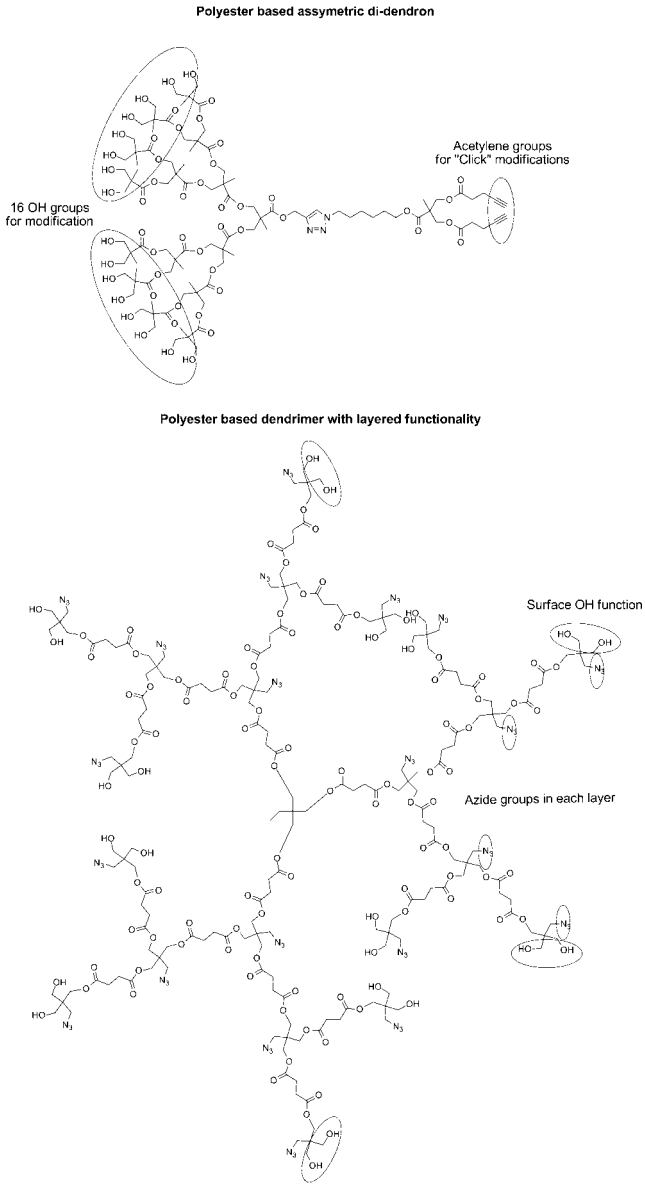
Scheme 42. Examples of Multifunctional Polyester Dendrimer Constructs for Nanomedical Applications[206,207]

Polyester based dendrimers with PEG grafts and a multitude of functional groups



and co-workers have prepared PEG-based hydrogels by CuAAC using a cross-linker that contained several pendant alkenes.[272] The alkenes, inert under click conditions, were then used in a subsequent modification step to pattern peptides on the hydrogel surface by photochemical thiol−ene coupling using the native thiol from cysteine residues. Without taking advantage of the orthogonality of CuAAC and thiol−ene, it would be difficult to realize ideal network formation followed by micrometer-scale covalent patterning of peptides. Diaz and co-workers have also exploited the

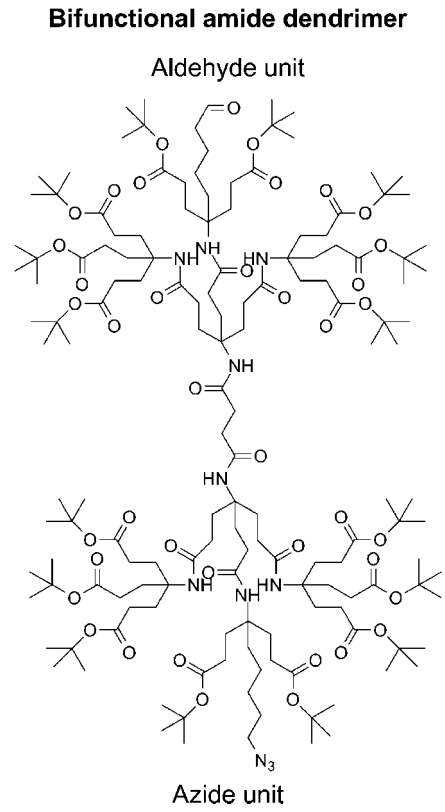
Scheme 43. Asymmetrically-Functionalized Polyester Dendrimers (Top)[208] and Multifunctional Polyester Dendrimers (Bottom) That Allow for Modification via Orthogonal Chemistry[209]



unique features of the CuAAC reaction to successfully stabilize organogels formed by supramolecular interactions.[273]

CuAAC has also been used to form and study degradable model networks as part of an ongoing study by Turro and co-workers. In their initial publication, Johnson et al. reported the formation of networks by cross-linking diazido poly(*t*-butyl acrylate) with either tri- or tetra-alkynyl molecules.[84] An interesting structural feature in this system is that diazido poly(*t*-butyl acrylate) was obtained from a difunctional ATRP initiator containing an internal alkene followed by azide displacement of the bromine chain ends. As a result, each polymer contained an alkene at the midpoint of the backbone. After network formation by CuAAC, ozonolysis was then employed to degrade the network into soluble polymeric byproducts by selective cleavage of these alkenes. The soluble products were then studied by FTIR/size-exclusion chromatography (SEC). For an ideal network with 100% efficient cross-linking, the expected product would be a three-arm star polymer with a molecular weight 150% that of the original diazido poly(*t*-butyl acrylate). The results from SEC

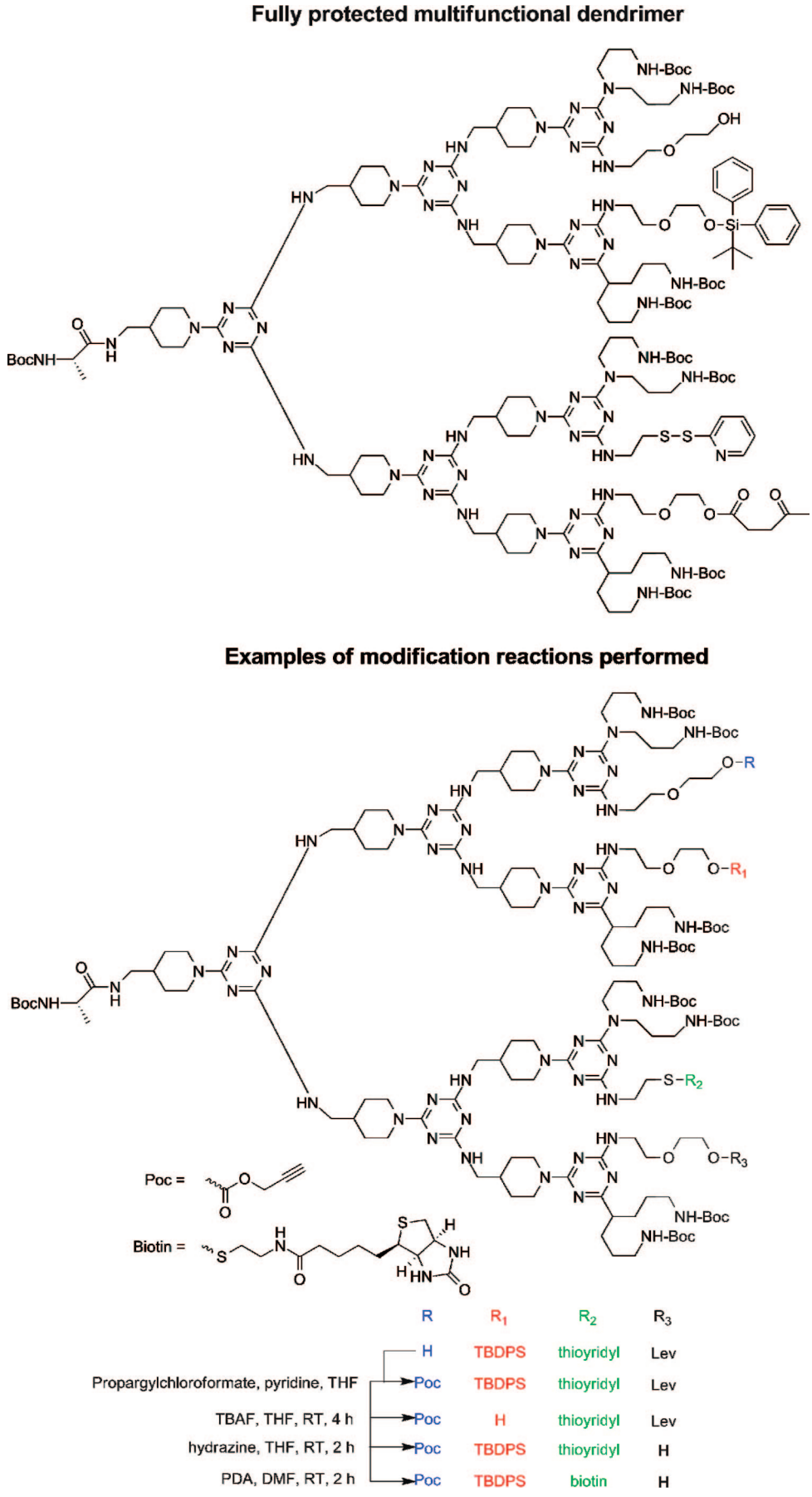
Scheme 44. Orthogonally-Functionalized Dendritic Polyamides[210]



showed a majority of the desired three-arm star polymer but also lower molecular weight peaks that corresponded to unreacted polymer. The authors noted the difficulty in controlling the exact stoichiometry for cross-linking because of the polydispersity of the polymer samples. The same authors extended this concept to photodegradable networks by synthesizing an ATRP initiator containing a 1,3-dimethylnitrobenzyl moiety, which allows degradation by 350 nm light (Scheme 50).[85] The versatile and orthogonal nature of this chemistry also permits alternative strategies to be explored. The study by Johnson et al. provides an excellent example by extending the same principle to strain-promoted azide−alkyne cycloaddition for cross-linking by using fluorinated cyclooctyne reagents in combination with tetra-azido four-arm star polymers.[274]

As demonstrated previously, one major advantage of using REO chemistries for the formation of cross-linked networks is the high tolerance to other functional groups, which allows for the incorporation of active moieties that significantly impact the properties of the final material. To illustrate this feature, the groups of Grubbs and Kornfield have reported the use of CuAAC to cross-link polymers containing mesogenic groups leading to a gel displaying liquid crystalline properties (Scheme 51).[276] Cyclooctene was derivatized with either one or two mesogenic groups, followed by its polymerization by ROMP using a dibrominated chain transfer agent. The resulting telechelic polymer contained mesogenic groups spaced regularly along the backbone and bromine atoms at both chain ends. Following nucleophilic displacement with sodium azide, the diazido poly(cyclooctene) was cross-linked using tripropargylamine under CuAAC conditions. It was found that more mechanically constrained samples (dependent on cross-link density and swelling ratio) showed suppressed electro-optic response compared with less

Scheme 45. Examples of Multifunctional Dendrimers from the Group of Simanek[212]



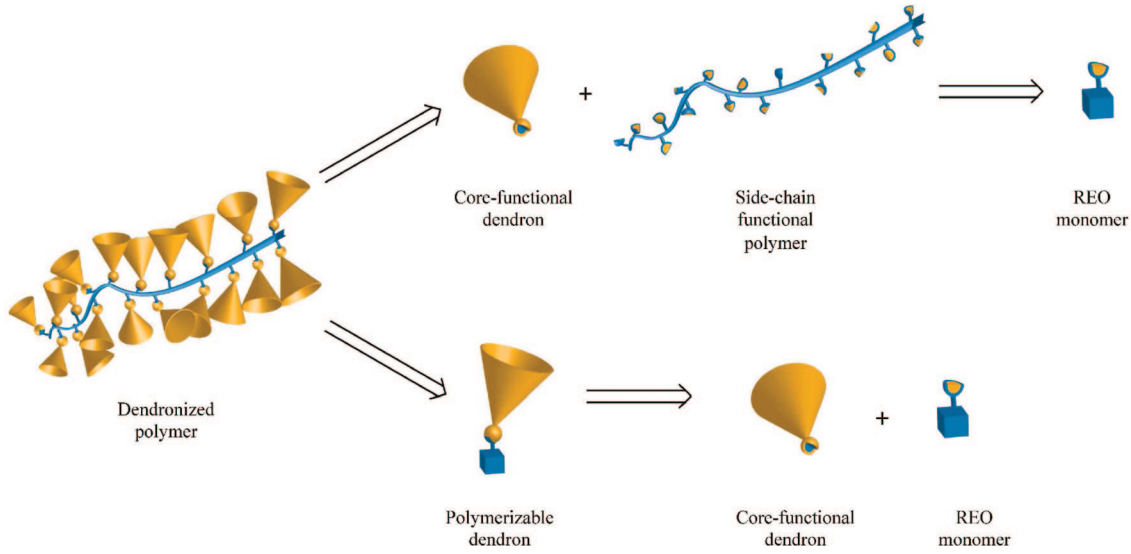
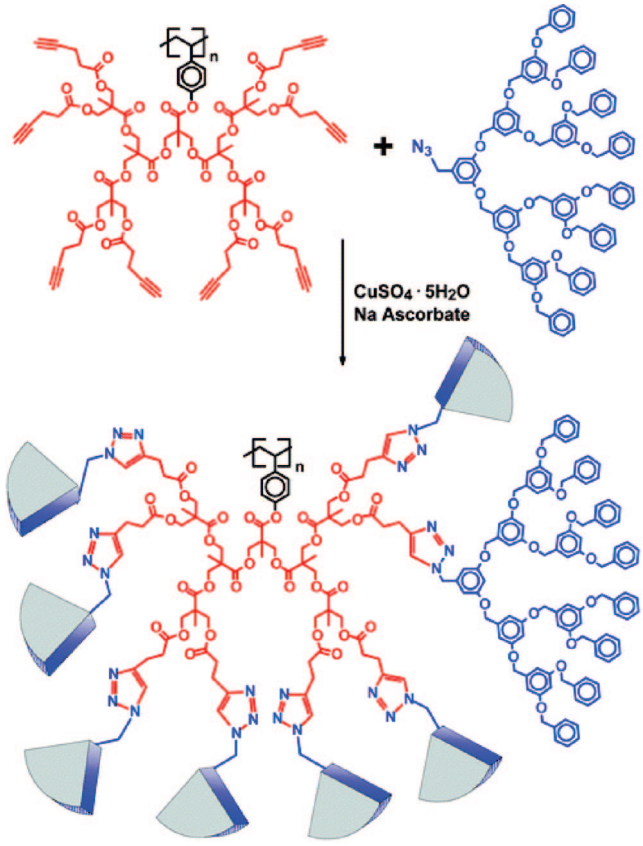


Figure 8. The two main synthetic strategies for preparation of dendronized polymers.

Scheme 46. Synthesis of a Doubly-Dendronized Polymer through Click Cycloaddition[a]



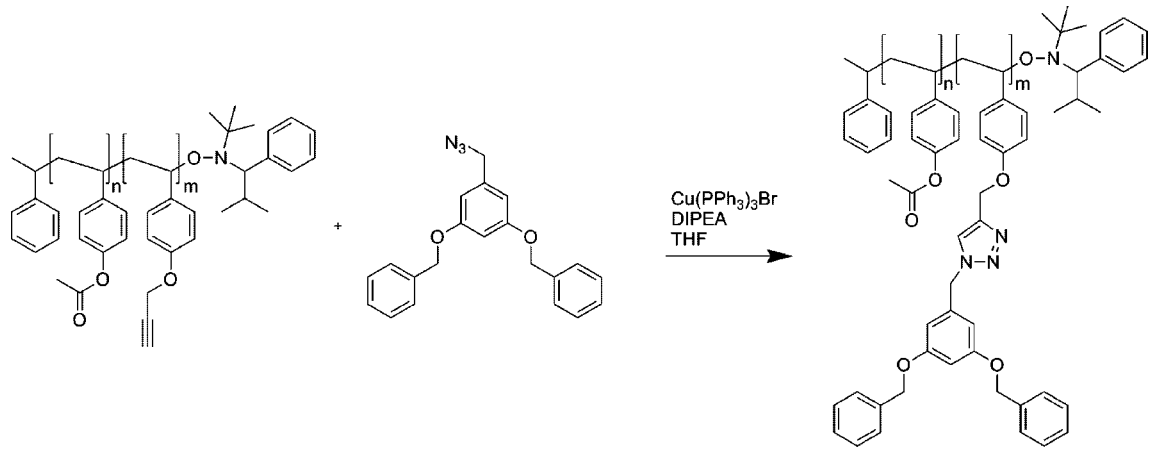
[a] Reproduced with permission from ref 224. Copyright 2005 The Royal Society of Chemistry.

constrained samples[277–280] with gels formed by CuAAC showing a lower threshold response than gels made by uncontrolled radical polymerization. The authors attribute this to a more regular network structure that avoids regions of high cross-link density, which can decrease sample alignment due to mechanical constraint.

In another example of incorporating functional moieties into a network, Jérôme[281] described the synthesis of biodegradable amphiphilic networks with pH responsive properties by a combination of ROP and CuAAC cross-linking. ROP of α-chloro-ε-caprolactone followed by azide displacement was used to produce poly(α-azido-ε-caprolactone). Before being used to cross-link dialkynyl PEG, the poly(caprolactone) sample was functionalized with *N*,*N*-dimethylprop-2-yn-1-amine along the backbone in various loadings using CuAAC. The cross-linking reaction was done in the same reaction vessel by simply adding the functional PEG to the reactor and allowing gelation to occur. The resulting gels were amphiphilic in nature, and pH-dependent release of a guest was demonstrated using a model dye.

Other examples using REO chemistry to prepare cross-linked networks with improved properties have centered on Diels−Alder cycloadditions. By careful choice of the diene and dienophile, systems showing thermally reversible bonding[282] have been developed, which allow for the self-healing of cracks or fractures by a simple heating and cooling cycle. The heating cycle serves to initiate retro-Diels−Alder reactions leading to a decrease in cross-link density with the cross-links being reformed in a less mechanically strained and more energetically favorable configuration upon cooling. A 2002 report by Wudl and co-workers made use of a tetrafuran and a trismaleimide for network formation leading to a thermoset with excellent mechanical properties.[283] The authors initiated crack propagation and subsequent mechanical failure in the networks, after which they demonstrated remending of the structural failure by heating to temperatures between 120 and 150 °C for several hours. The mended networks were then subjected to mechanical testing, where the site of the mechanical failure was able to bear 80% of the stress of the original sample even after three cycles of breaking and remending. Subsequent reports from the Wudl group improved this system by altering the cross-linking groups so that networks could be made colorless, in the absence of solvent,[284] and prepared through a one-component system (Scheme 52). The latter systems exploit a cyclic tethered dicyclopentadiene adduct,[285] which upon heating undergoes a retro-Diels−Alder reaction giving free cyclopentadiene and effectively forming an AB monomer. The cross-linking of these linear polymers occurs simultaneously when a free cyclopentadiene unit reacts with the norbornyl alkene to give a cyclopentadiene trimer resulting in a cross-link. These Diels−Alder-based self-healing networks have a number of advantages over other strategies, including no

Scheme 47. Block Copolymer Containing Dendronized Blocks[227]



need for catalyst and the potential to undergo many cycles of mechanical failure and repair.

A fundamentally important outcome of this preliminary work on the synthesis of cross-linked networks using REO chemistry is focus on functionalized materials. Not only does the REO chemistry allow for the introduction of a wide range of "active" groups into a three-dimensional network, but the stability of the reactive groups used in the cross-linking chemistry permits secondary functionalization reactions to be performed after cross-linking. These features, coupled with the formation of ideal networks, strongly suggest a number of advantages for these materials compared with those prepared using traditional strategies.

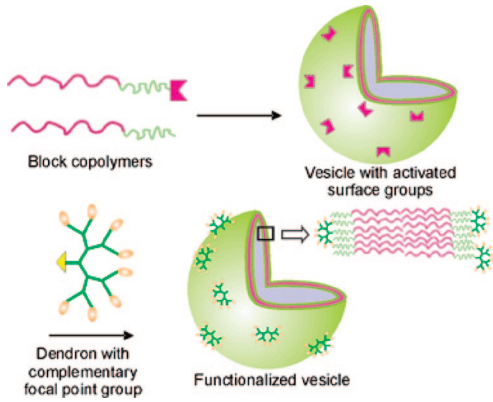
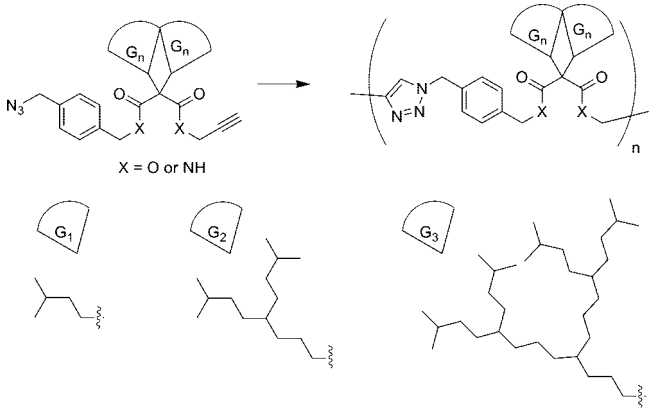


Figure 9. Conjugation of functional dendrons to a vesicle surface. Reproduced with permission from ref 228. Copyright 2007 The Royal Society of Chemistry.

Scheme 48. Step Polymerization of Hydrocarbon-Based Dendritic Monomers through CuAAC Chemistry[229]



3. Surface and Interface Modification

3.1. Three-Dimensional Nanoparticle Systems

The orthogonal functionalization of polymeric nanoparticles and micelles is an important initial goal for the development of targeted drug delivery systems, contrast agents, and many other complex biomedical, electronic, or optical applications. In particular, nanomedical applications require the ability to control the number and spatial location of multiple functional groups while also tailoring surface properties in order to realize disease-specific delivery, achieve enhanced cell penetration, and achieve effective drug or gene delivery, as well as *in vivo* and *in vitro* detection capabilities.[286–293]

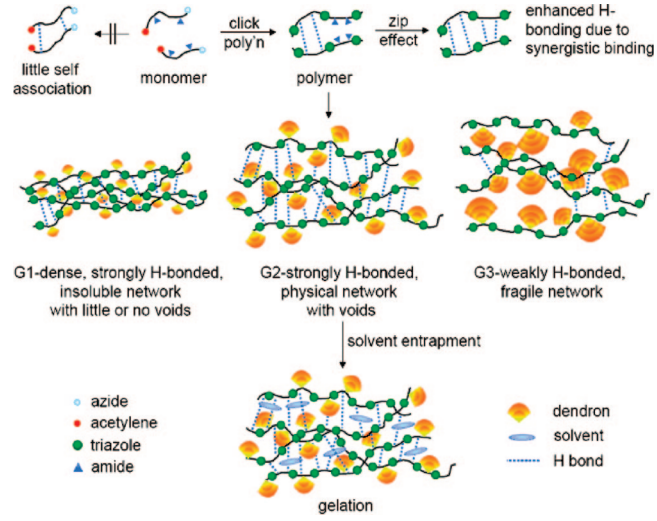


Figure 10. Gelation model for amphiphilic, dendronized polymers. Reproduced with permission from ref 229. Copyright 2008 Wiley-VCH.

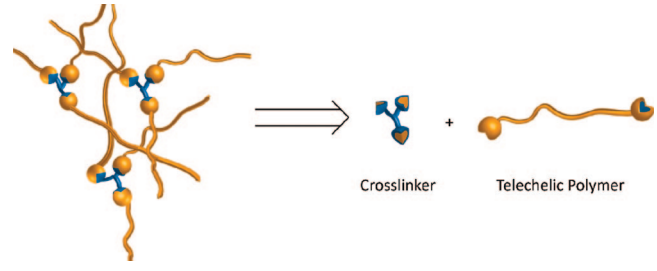
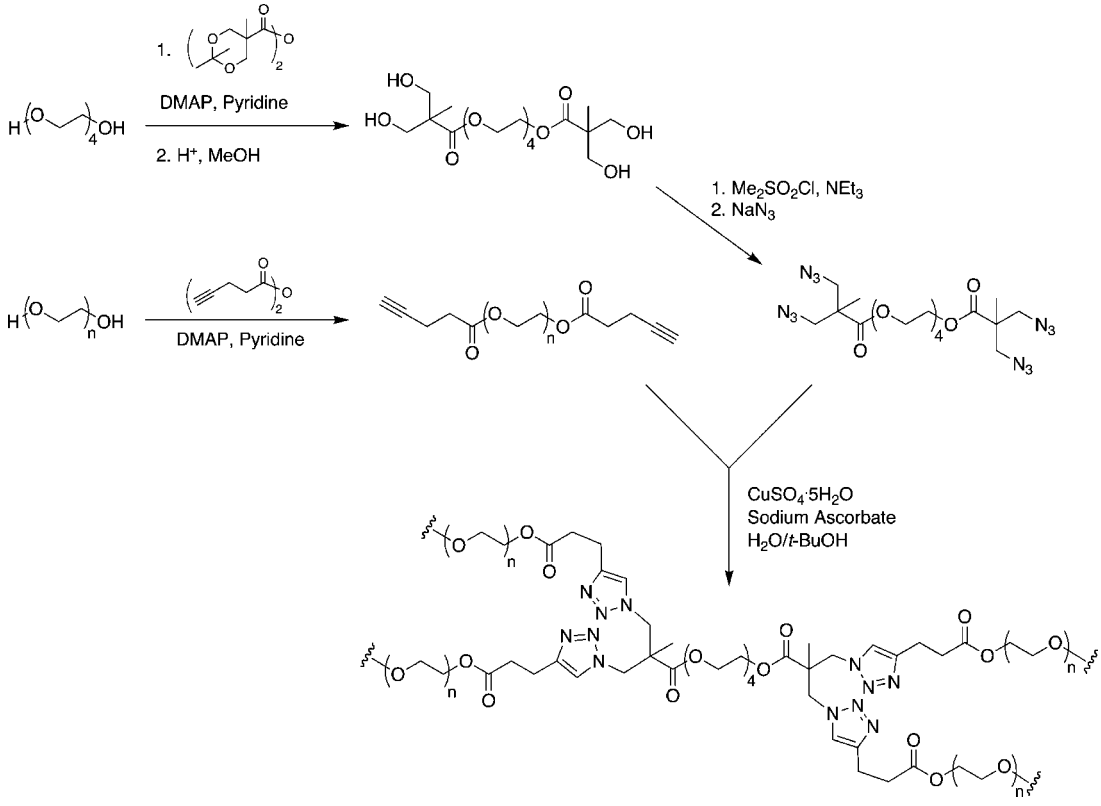
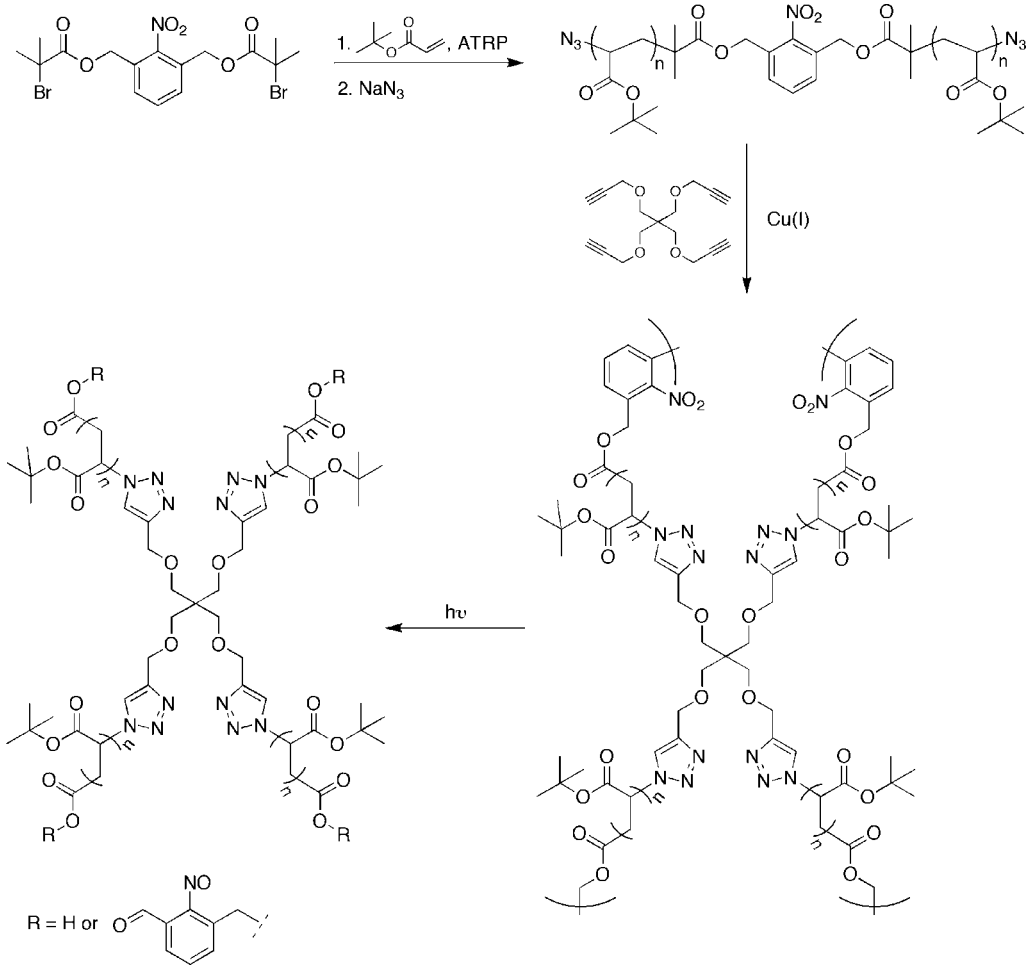


Figure 11. Retrosynthetic analysis for well-defined networks made by cross-linking telechelic polymers with multifunctional cross-linkers using REO reactions.

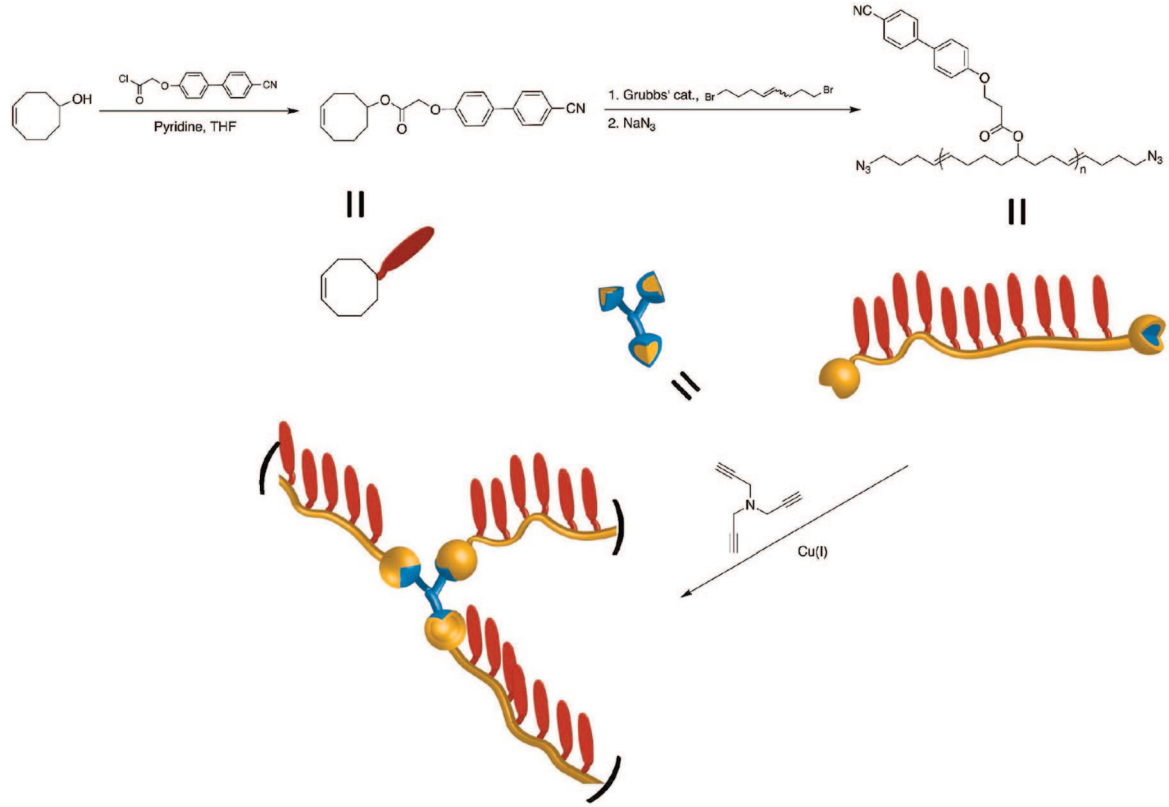
Scheme 49. The Synthesis and Formation of PEG-Based Hydrogels Using CuAAC as the Cross-Linking Reaction[271]



Scheme 50. Synthesis and Degradation of Photodegradable Model Networks Made by ATRP and CuAAC[84,85,275]



Scheme 51. Chemical Strategy for Construction of Liquid Crystalline Networks Cross-Linked in a Well-Defined Manner from Metathesis-Based Telechelic Polymers Containing Either One or Two Mesogens per Repeat Unit[276]



As a result, the covalent functionalization of preformed soft nanoparticles, micelles, and liposomes from polymeric precursors is a major challenge and opportunity for REO chemistry. To allow focus, this section will emphasize the modular functionalization of polymeric nanoparticles. While also relevant in a larger context, the use of REO strategies for the modification of metal/organic hybrid particles including carbon nanotubes, fullerenes, silica and metal oxide nanoparticles, and quantum dots have been extensively reviewed previously and will not be further explored herein.

3.1.1. 1,3-Dipolar Cycloadditions

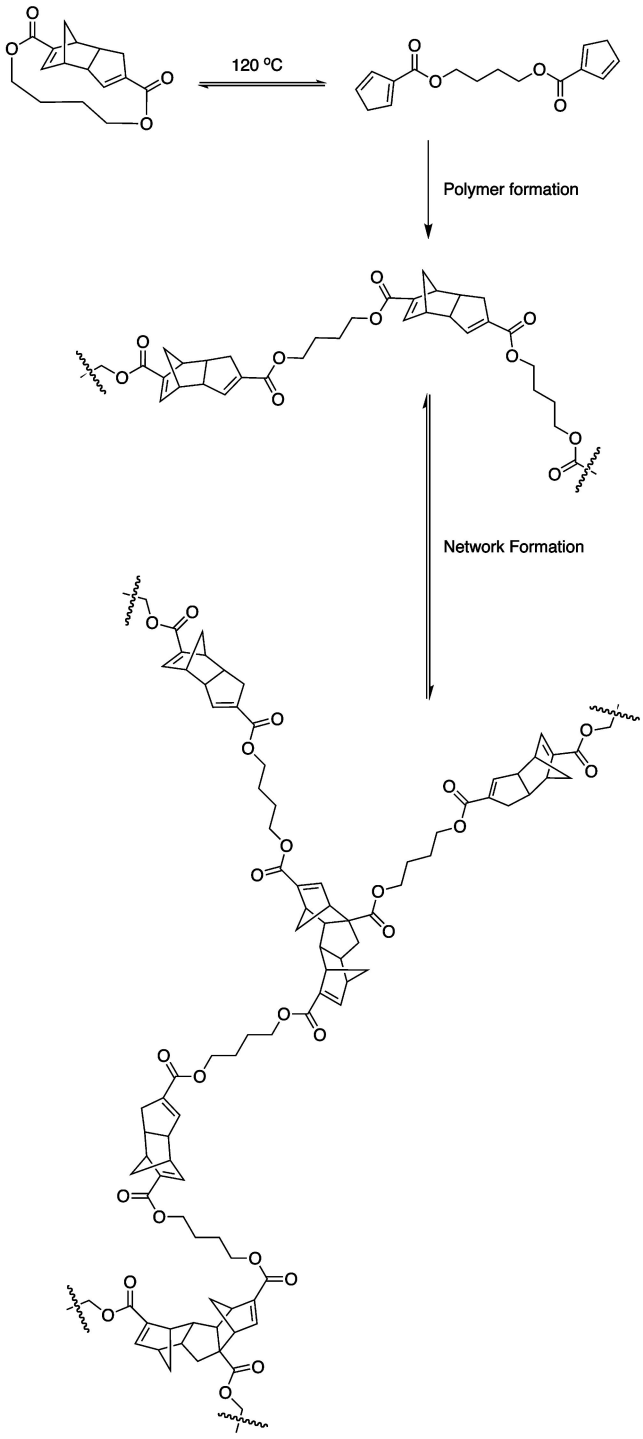
The utilization of 1,3-dipolar cycloadditions, such as CuAAC, for the functionalization of polymeric nanoconstructs is a rapidly expanding area, as indicated by the multitude of papers reported over the last 5 years. The potential to functionalize orthogonally in a regiospecific manner, to conduct the functionalization reactions in water, and to tolerate biologically relevant groups such as peptides, proteins, and a multitude of functional handles suggests that the 1,3-dipolar cycloadditions are an essential member of the nanoscale manipulation toolbox.[275,294–296]

The groups of Wooley and Hawker reported shell cross-linked knedel-like nanoparticles that were prepared with a mixture of azide/COOH or alkyne/COOH groups on the surface of the particle via transformation of the preformed nanostructure utilizing carbodiimide-based amidation reactions.[297] These functionalities could be selectively modified by reaction with the corresponding azido/alkynyl fluorescein dye while retaining the COOH for further amidation-based modifications. In addition, the selective introduction of azido groups in the hydrophobic core of the nanoparticle through a functional monomer strategy was illustrated[297] and allowed for orthogonal 1,3-dipolar cycloadditions in the core and amidation based strategies in the shell of the nanoparticle (Scheme 53). In an extension of this work, azido functional dendrimers of different generations were utilized for shell-cross-linking where residual azide functionalities could be utilized in a subsequent reaction with fluorescent alkynes in the shell.[298] This ability to control the reactivity of nanoparticles and the location of functional groups within a three-dimensional structure is at the heart of the click chemistry philosophy, as has been exemplified in the studies above and in the selective functionalization of nanoparticle cores[299] or surfaces via reaction at the chain end sites of block copolymer components.[300]

In a recent example, regioselective and orthogonal functionalization of polymeric core−shell nanoparticles was achieved via an initial Michael addition reaction to selectively functionalize the interior of a poly(dimethylacrylamide) (PDMA) nanoparticle with fluorescein units, followed by 1,3-dipolar cycloaddition to couple azido groups in the poly(NIPAM) shell of the nanoparticle with dansyl units (Scheme 54).[301]

CuAAC chemistry has also been utilized for the modification of liposome surfaces by the introduction of alkynes in the shell through functional phospholipid units.[302] Regioselective reaction via copper-mediated cycloadditions was demonstrated by the creation of a fluorescence resonance energy transfer (FRET) pair in the lipid shell while preserving the internal double bonds of the lipid bilayer. In addition, bioactive mannose groups have been introduced on the surface of polymeric liposomes that selectively bind concanavalin A.[303] Polymersomes prepared from poly(styrene)-*b*-poly(acrylic acid) block copolymers bearing an ω-azido chain end have been utilized to introduce either dansyl chromophores, biotin groups, or enhanced green fluorescent protein (EGFP) on the surface of the nanostructure, allowing

Scheme 52. Formation of Self-Reparable Networks Using Reversible Diels−Alder Reactions for Cross-Linking from a Single Component System[285]



for further modification of the PAA groups in the shell (Scheme 55).[304] Similarly, polymersomes of poly(styrene)-*b*-poly(ethylene glycol) bearing ω-alkyne on the PEG chain were modified with the catalytically active CalB enzyme.[305]

3.1.2. Aldehyde-Based Conjugation Strategies

The success of orthogonal functionalization strategies using CuAAC chemistry has led to a number of other REO transformations being examined. A prime example is the introduction of aldehyde functionalities, presented regioselectivly on polymeric nanoconstructs, which provide for a convenient and potentially orthogonal handle for modification reactions. Methods for introducing carbonyl moieties into linear polymers and block copolymers are well-established in the literature and the option of creating either stable linkages via reductive amination or pH-sensitive linkages via hydrazones provide additional tunable handles in controlled release applications.[289,306,307]

An early example of polymeric micelles carrying aldehyde functional groups in the shell was reported by Kataoka et al.[308,309] The block copolymer of poly(ethylene glycol) and poly(lactic acid) was prepared by sequential anionic polymerization from a protected aldehyde initiator and, in a later study, was also terminated with a vinyl group.[310] Core cross-linked nanoparticles could then be prepared by selectively cross-linking the core while the aldehyde functionalities in the shell were found to be stable and could be reacted via hydrazide chemistry with good fidelity and high coupling yields (Scheme 56). The group of Kataoka and others have since then demonstrated the successful conjugation of a multitude of functionalities via this approach such as mannose[311] and lactose,[312] phenylalanine and tyrosyl-glutamic acid (Tyr-Glu),[313] and tyrosine and Tyr-Glu,[314] demonstrating the versatility of this strategy.[315]

However, there are few examples of fully orthogonal modifications based on these systems. One such orthogonal example is the preparation of folate receptor-targeted micelles composed of poly(ethylene glycol)-*b*-poly(aspartate) for pH-activated delivery of chemotherapeutics such as adriamycin (doxorubicin).[316,317] The polymer precursors of these micelles were prepared via a combination of reductive amination chemistry and pH-sensitive hydrazone formation through selective protection/activation chemistry and represent a sophisticated example of REO chemistry (Scheme 56).

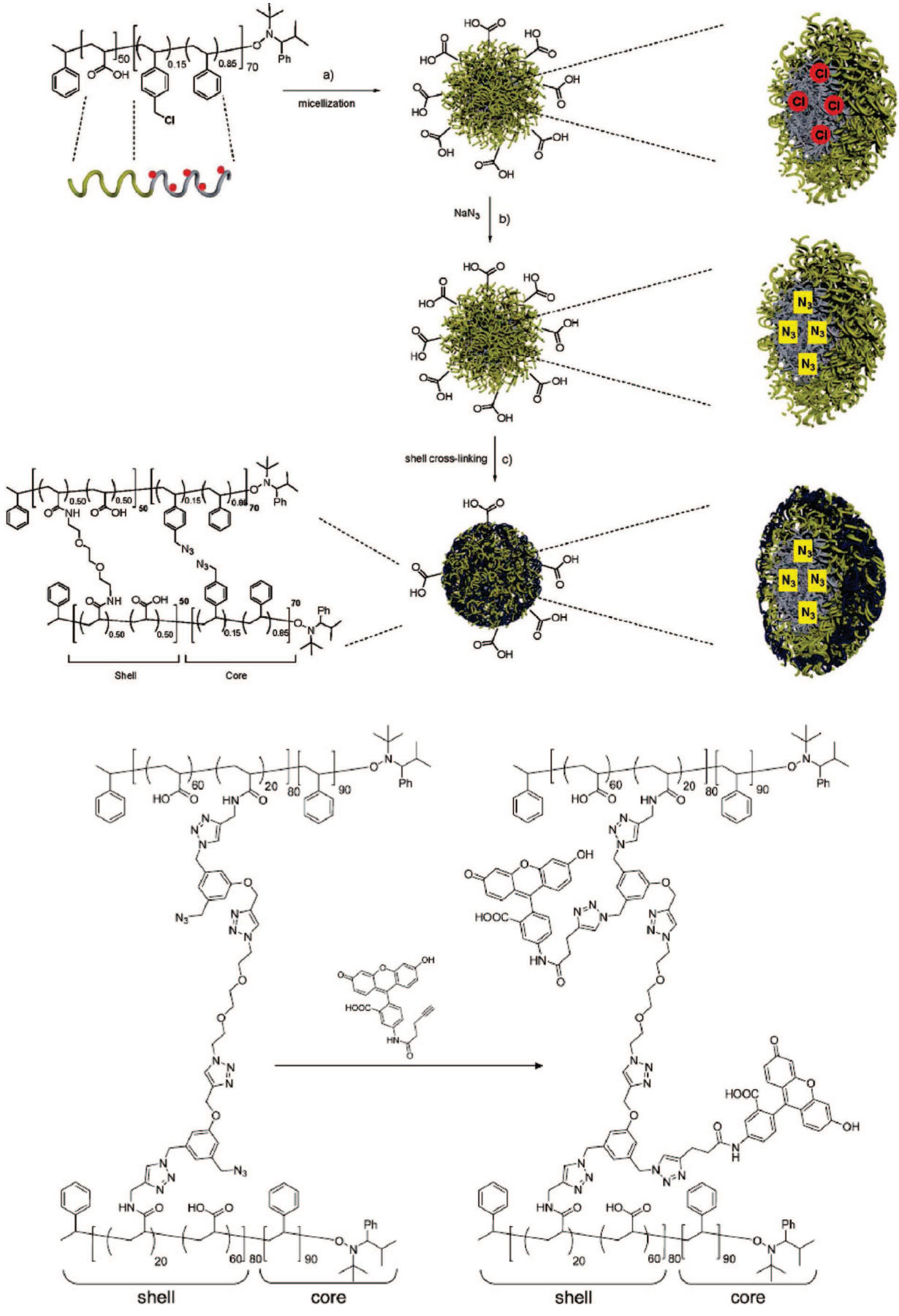
Another example of reductive amination-based functionalization was reported by Stuart et al., who utilized a mixed micellar preparation method for the ionic assembly of PAA and a controlled amount of poly(*N*-methyl-2-vinyl pyridinium iodide)-*b*-poly(ethylene oxide) bearing either aldehydes or hydroxyls on the ω-PEO end.[318] The aldehydes were then utilized for introduction of a lyzosome enzyme to the surface of the micelle (Scheme 57).

Cross-linked nanocages presenting aldehyde functionalities are another type of nanoconstruct that has been prepared and selectively reacted in an orthogonal manner.[319] The Wooley group has reported nanocages derived from shell cross-linked nanoparticles where the internal polyisoprene chains were excavated via ozonolysis and the residual carbonyls on the inside of the structure were orthogonally functionalized by Schiff-base chemistry.[320,321] These structures also contained carboxylic acid groups in the periphery that were available for further carbodiimide-based coupling chemistry in the shell domain (Scheme 58).

3.1.3. Michael Addition and Thiol-Based Strategies

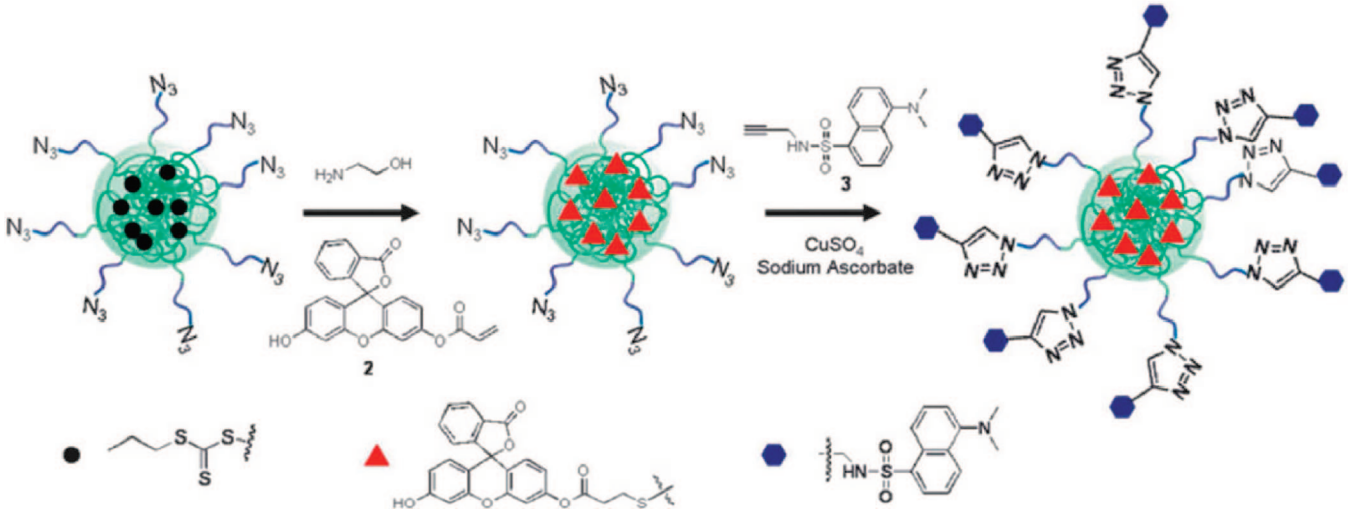
Latent thiol-bearing polymers or peptides are typically facile to synthesize, especially by RAFT polymerization methods,[322] as well as other techniques,[323] and represent a functional group that is attractive from a REO perspective for bioconjugation reactions. The work of Allen et al. on thiol functional liposomes represents one of the first examples for which thiol groups were used for conjugation of maleimide-functional antibodies to the termini of poly(ethylene glycol) chains presented on the outside of a liposome wall.[324] Several examples utilizing this construction methodology are

Scheme 53. Examples of Shell Cross-Linked Knedel-Like (SCK) Nanoparticles Functionalized via Combinations of 1,3-Dipolar Cycloadditions and Carbodiimide-Based Amidations[297,298a]



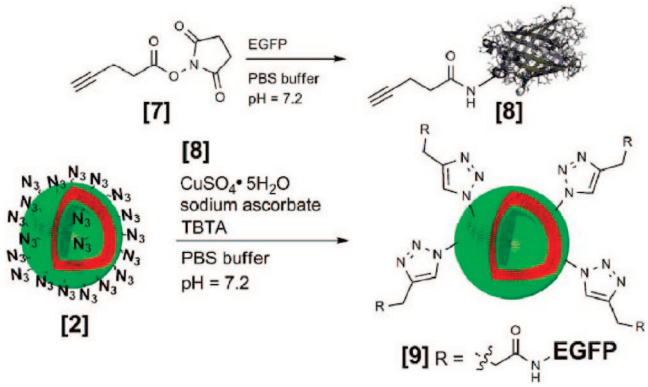
[a] Reproduced with permission from refs 297 and 298. Copyright 2005 American Chemical Society.

Scheme 54. Combination of Michael Additions and 1,3-Dipolar Cycloadditions[301a]



[a] Reproduced with permission from ref 301. Copyright 2008 The Royal Society of Chemistry.

Scheme 55. Example of Click Modifications of a Polymersome to Couple Enhanced Green Fluorescent Protein Units and Produce Hybrid Synthetic−Biologic Nanostructures[304a]



[a] Reproduced with permission from ref 304. Copyright 2007 The Royal Society of Chemistry.

presented in this section to highlight the potential of this conjugation reaction for nanoparticle functionalization.

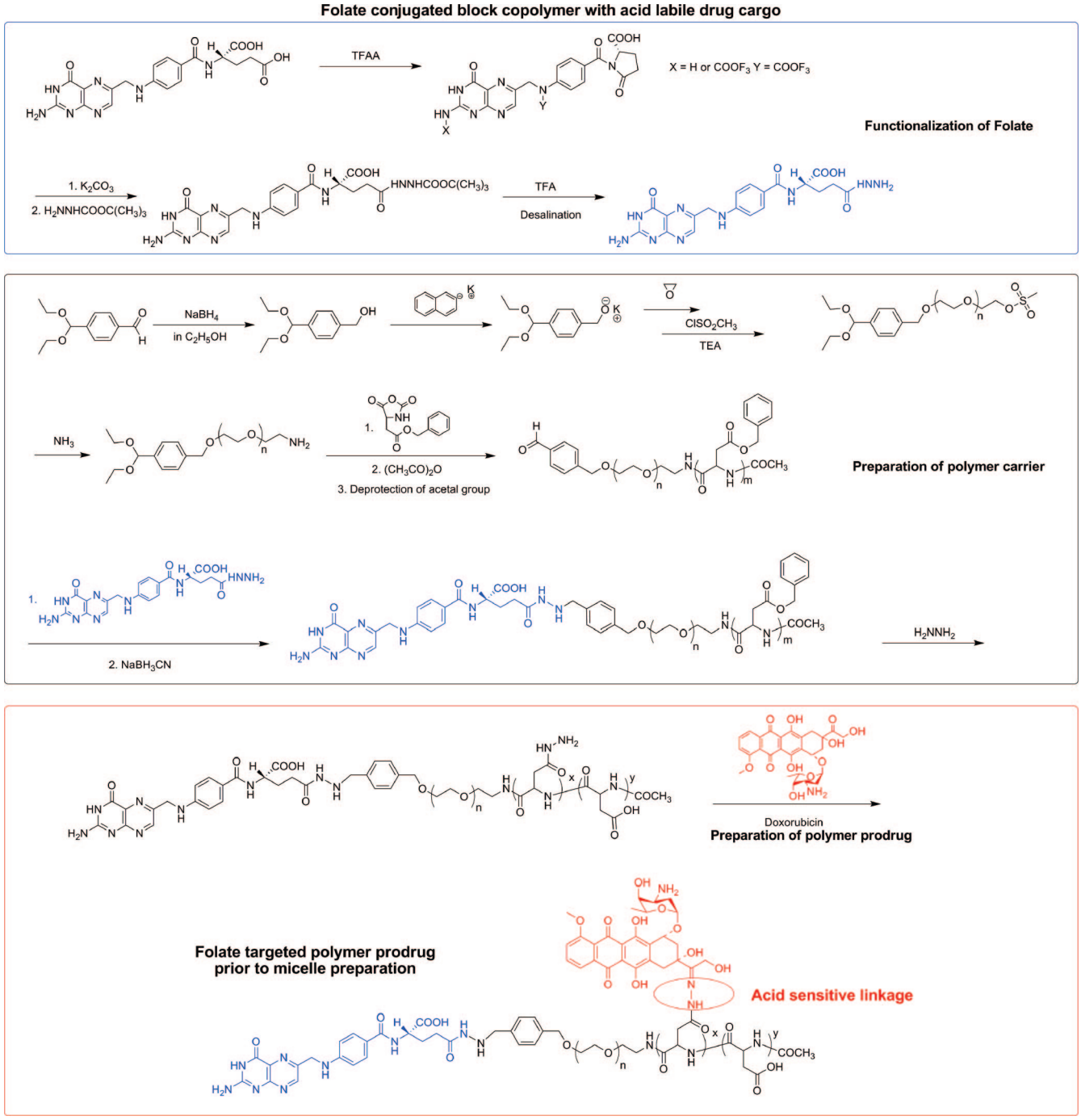
The group of Kataoka utilized reversible S−S conjugations to prepare polymeric micelles bearing glutathione-cleavable antisense oligodeoxynucleotide (ODN) in the cores of the micelles and aldehydes for potential Schiff-base conjugations in the shells. As a control micelle, noncleavable thio−maleimide-type conjugations were used to prepare polymer−antisense conjugates.[325] It was found that the reversible conjugation of the antisense sequence improved the transfection efficiency compared with the noncleavable system. Variations and improvements of this system for ODN delivery have also been reported, including core cross-linked micelles, approaches to modulate charge, and methods of thiol incorporation, suggesting that the system could be a versatile transfection agent for ODNs (Scheme 59).[326–329]

Thiol functionality has also been introduced on PLA nanoparticles via modification reactions of the COOH end group of the PLA chain with cystamine. Upon cleavage of the disulfide, micelles with active thiols were generated and used for conjugation to maleimide functional proteins bearing an avidin unit that could be utilized for further biotin/avidin recognition binding.[330,331] Similarly, Wooley utilized protected thiol functionalities presented at the periphery of a PEG spacer on a copolymer-based nanoparticle and investigated the conjugation of maleimido-functional BSA to the surface of the nanoparticle. This approach allowed for the placement of the biologically active units on the surface of a PEG brush layer on the core−shell polymer nanoparticles, while maintaining the potential for selective modification of the poly(acrylic acid) subsurface shell of the nanoparticle via amidation type chemistry.[332] An elegant example of the functionalization of liposomes was also reported by Schuber et al., in which the pH controlled reactivity of maleimido groups and bromo acetyl groups could be utilized for the immobilization of different thiol-functionalized peptides on the surface of liposomes by sequential reactions.[333,334] Other illustrative examples of nanostructure transformation via REO conjugation strategies include maleimide-functional micelles prepared from poly(ε-caprolactone)−PEG where the PEG functionalized with maleimido groups is used to introduce RGD peptides as a binding motif on the nanoparticle surface.[335] Allyl functional PEO block copolymer micelles have also been utilized for postfunctionalization of polymeric nanostructures utilizing different SH-bearing small molecules to introduce COOH or NH_2 functionality on the surface via thiolene coupling.[336] Also, several examples of Michael additions to liposomes have been reported, where maleimido-modified antibodies were conjugated via thiol functional liposomes with preservation of immunoactivity,[337–339] as well as the reverse strategy,[340] and via disulfide formation.[341] Several examples of reversibly cross-linked nanoparticles based on S−S formation have also been reported recently, including the preparation of subshell cross-linked micelles for chemotherapeutic delivery,[342] shell cross-linked polymer nanoparticles,[343] and core cross-linked polymer micelles.[15] Clearly, the functionalization of well-defined polymer nanoparticles to effect function is a highly active area of current research.

3.1.4. Diels−Alder Chemistry

Diels−Alder reactions, specifically the [4 + 2] cycloaddition reaction between a diene and a dienophile, are effective modification reactions that are both tolerant toward other

Scheme 56. Example of Aldehyde-Based Strategies for Preparation of Folate Receptor-Targeted Polymeric Micelles with pH-Sensitive Drug Cargos[317]

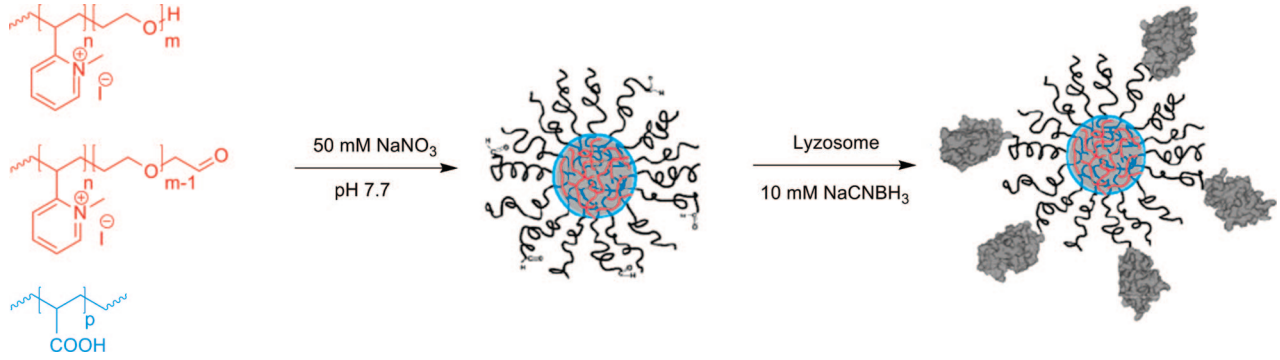


functional groups and accelerated in water.[344,345] The examples of utilizing Diels−Alder-type conjugations for orthogonal functionalization of nanoconstructs are, however, few, which is surprising given the efficiency and orthogonality of this process. In fact, the high fidelity and accelerated kinetics often observed in water imply that this type of functionalization strategy may become useful in the preparation of nanomedical constructs in combination with other chemistries.

One recent example of Diels−Alder functionalization utilized polymeric micelles derived from random graft copolymers presenting pendant furan groups as the reactive group on the surface of the nanostructure.[346] These pendant furan groups on a PEG spacer have been utilized for conjugation of maleimide functional anti-HER-2 antibodies (Fc-specific modification) orthogonally to the surface of the micelle while allowing further chemistry to take place on the COOH groups of the main chain polymer.[346] The conjugation proceeded with good coupling efficiency, and the immunotargeted micelles retained full immunoactivity after conjugation (Scheme 60).

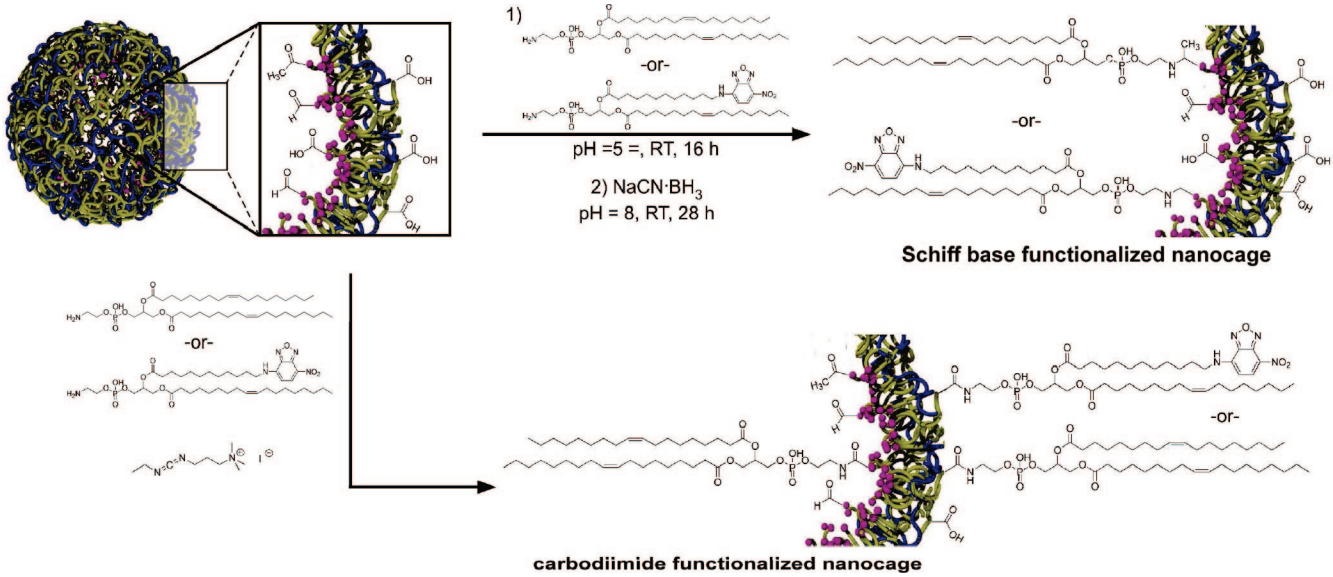
In summary, the wide variety of uses envisaged for nanoparticles coupled with their high degree of functionality represent a significant opportunity for the development and exploitation of REO-type conjugations. Using an array of different chemistry, multifunctional nanoobjects can now be obtained with a degree of control over the number and placement of functional groups within the nanoobject. In the area of selective functionalization of biological systems, a main focus has been on the precise introduction of cystine

Scheme 57. Example of a Mixed Micelle Preparation Strategy for Introducing Orthogonal Functional Groups[318a]



[a] Reproduced with permission from ref 318. Copyright 2007 American Chemical Society.

Scheme 58. Nanocage Modification Using Amidation and Reductive Amination Chemistries (Reproduced with Permission from Ref 321; Copyright 2005 Elsevier)



fragments or azido groups in peptides, proteins, and reduced antibodies, through genetic modifications and synthetic chemistry, which suggests a greater use of these strategies for nanoparticle functionalization with complex biomacromoleclues.

3.2. Two-Dimensional Substrates

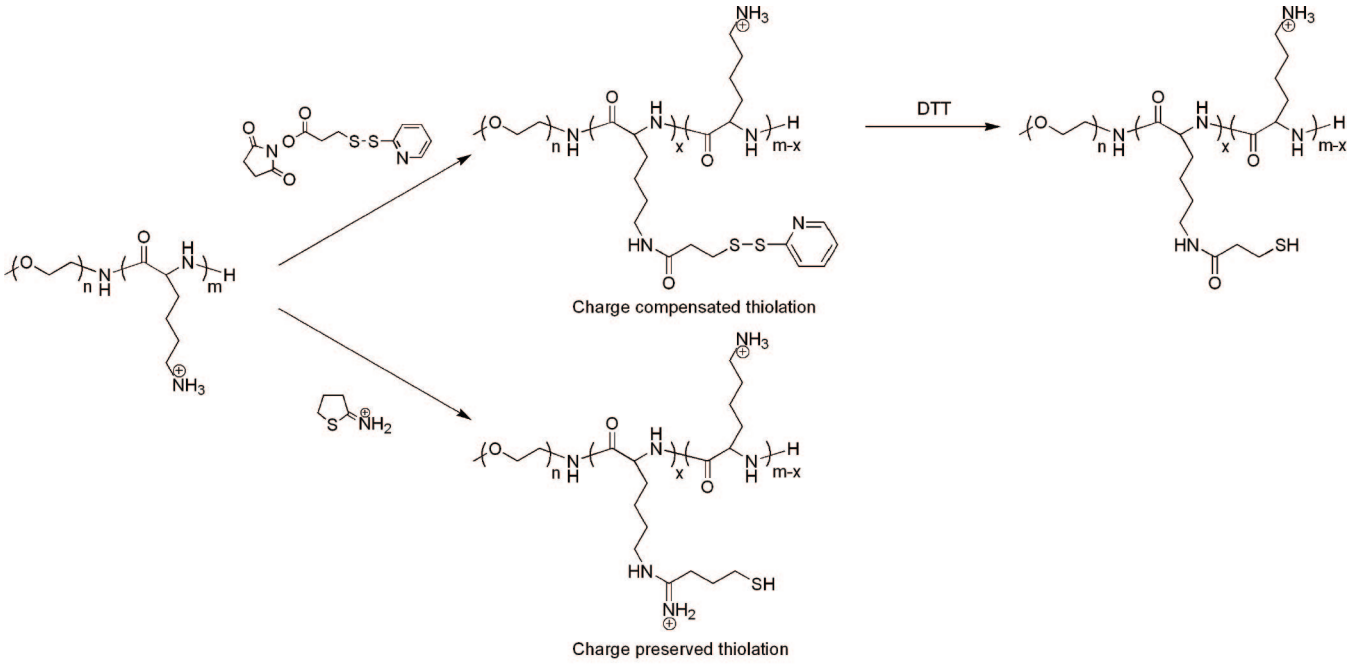
The derivatization of two- and three-dimensional substrates is an important topic in the construction of functional materials because surfaces and interfaces provide sites for immobilization events and are involved in any contact between objects, yet these regions are often locations of incomplete chemical reaction due to a number of factors, such as sterics. Efficient and orthogonal functionalization of surfaces/interfaces, therefore, presents a major challenge that can be addressed by REO chemistries. In terms of surface functionalization or modification, the field can generally be split into two categories, the functionalization of two-dimensional surfaces and the functionalization of solid, three-dimensional particles or substrates (*vide supra*). While the functionalization of three-dimensional inorganic nanoparticles is of immense interest and importance to the field of materials science, it falls outside the general scope of this review, which instead focuses on 2-D surfaces. The functionalization of two-dimensional substrates is essential for the construction of patterned surfaces and microarrays, and the use of orthogonal chemistries is particularly useful for the surface immobilization of biological molecules and synthetic macromolecules. To be generally applicable for use in the creation and preparation of functional surfaces, specifically those involving biomolecules and biopolymers, the chemistries employed should occur rapidly and selectively and be tolerant of the variety of functionalities often found in biological molecules.

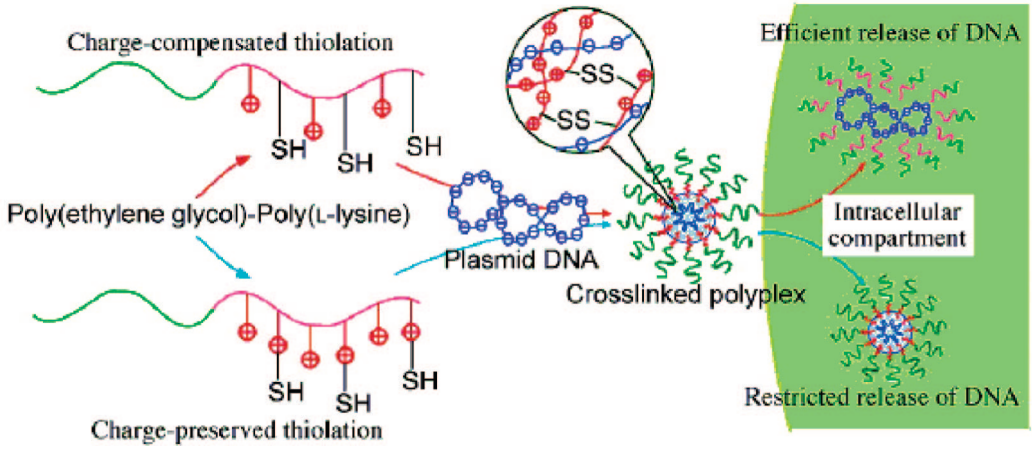
3.2.1. 1,3-Dipolar Cycloadditions

The use of the copper-catalyzed 1,3-dipolar CuAAC cycloaddition reaction has found particular utility in the modification of self-assembled monolayers (SAMs), with many studies employing click cycloadditions to selectively and efficiently derivatize a planar substrate. The formation of stable, 1,2,3-triazole linkers has also proven to be a successful technique for the immobilization of biological molecules. Since the use of click chemistry in surface modification is ubiquitous and since this topic has also been addressed in recent reviews,[295,347–349] we have herein included a limited selection of seminal and recent papers discussing the use of the Huisgen 1,3-dipolar cycloaddition reaction in the modification of two-dimensional surfaces.

Initial reports by Collman et al. employing CuAAC for surface derivatization involved the decoration of a SAM made from azidoundecanethiol and decane thiol with acety-

Scheme 59. Examples from Kataoka et al. on Preparing Thiol-Functionalized Cationic Poly(L-lysine) Segments within Block Copolymers with Poly(ethylene oxide) (Upper Portion) and Their Transformation into Disulfide Crosslinked Polyplexes for Delivery of Plasmid DNA (Lower Portion)[326,329a]





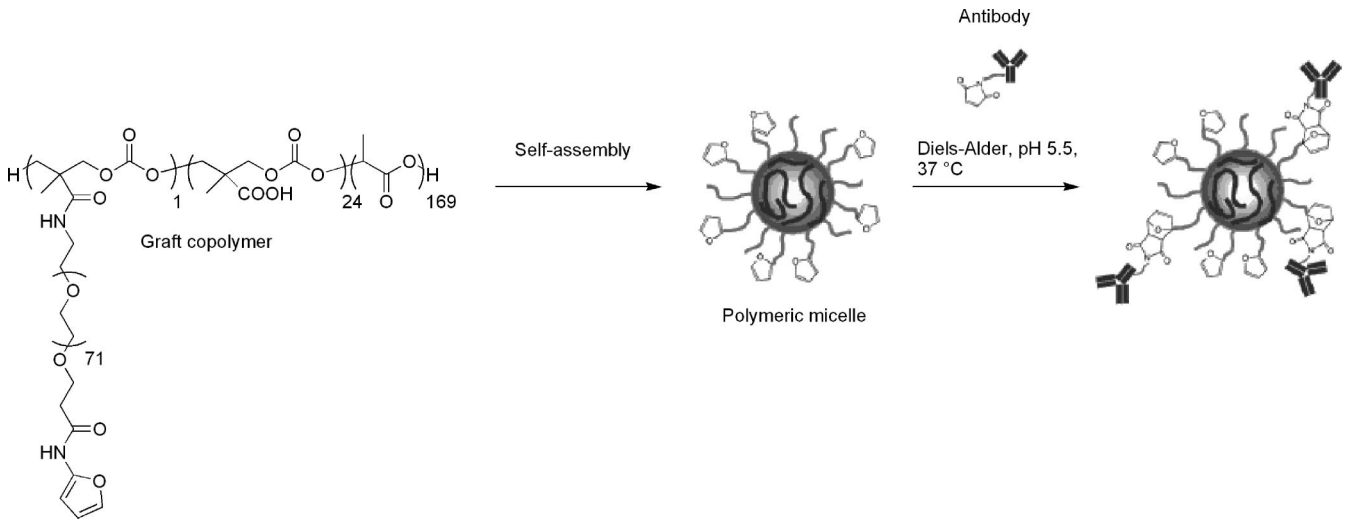
[a] Reproduced with permission from ref 329. Copyright 2004 American Chemical Society.

lene-possessing, redox-active ferrocene molecules.[350,351] This method of modification allowed for determination of the surface coverage by the employment of a redox-active probe, while the consumption of the azide could be monitored by IR spectroscopy. This series of experiments clearly demonstrated that surfaces presenting organic azides provide an excellent handle for surface modification and functionalization. In addition to SAMs constructed from organic azides, alkyne-functionalized surfaces have also been used as a platform for click modification, with Drockenmuller and coworkers reporting the grafting of various brushes to an alkyne-functionalized SAM.[352] In this report, a silicon substrate was "passivated" via vapor deposition to yield a SAM presenting alkyne functionalities; ω-azido polymers (PEG-N_3, PMMA-N_3, and PS-N_3) were then grafted onto the surface via the copper-catalyzed Huisgen 1,3-dipolar cycloaddition to yield well-defined polymer brushes with a thickness of ca. 6 nm and grafting densities of ca. 0.2 chains/nm^2. The employment of this approach, due to the tolerance and selectivity of the click cycloaddition, provides a general method for densely grafting a variety of polymer brushes bearing a diversity of functional groups onto a surface using a "grafting onto" approach.

While CuAAC chemistry has been shown to be effective for the covalent attachment of small molecules and polymers to a surface, its orthogonal reactivity and tolerance of functionalities commonly found in biomolecules makes it an outstanding reaction for the covalent immobilization of biological molecules to two-dimensional surfaces. Early examples involving the biological modification of surfaces via click cycloaddition include the immobilization of acetylene-functionalized oligonucleotides on an azide-presenting SAM[353] and the conjugation of azido sugars onto an alkyne-functionalized SAM[354] to fabricate biologically active surfaces in a highly efficient and straightforward manner.

In addition to small biological molecules and oligomers, the click cycloaddition has also recently proven useful for the chemoselective immobilization of proteins to a surface.

Scheme 60. Diels−Alder-Based Functionalization of a Polymeric Nanoparticle with Antibodies[346a]



[a] Reproduced with permission from ref 346. Copyright 2007 Wiley-VCH.

Chaikoff et al. reported the modification of a surface with biotin, carbohydrates, and proteins through the use of sequential Diels−Alder and azide alkyne cycloadditions.[355] In this example an α,ω-poly(ethylene glycol) (PEG) linker with alkyne and cyclodiene terminal groups was reacted with an *N*-(ε-maleimidocaproyl)-functionalized glass slide through a Diels−Alder reaction to give a PEGylated surface presenting surface alkyne moieties. Then, azide-containing biotin, lactose, and recombinant thrombomodulin proteins containing an S-tag were conjugated to the surface via 1,3-dipolar cycloadditions. To ascertain their availability and activity, the surfaces were incubated with a fluorescein isothiocyanate (FITC)-labeled streptavidin, a FITC-labeled lectin from *Arachis hypogaea*, and a FITC-labeled S-protein, respectively (Figure 12). This report provides a method for the immobilization of functionally complex molecules onto a solid substrate through an efficient linking strategy using two reactions that are orthogonal to the functionalities present in biomolecules.

Another challenge in the creation of functional protein microarrays that can be addressed by REO chemistry is the site-specific, covalent immobilization of proteins onto a solid surface. Lin and co-workers examined this issue by covalently attaching a maltose binding protein (MPB) with an alkyne at the C-terminus to an azide-functionalized surface via 1,2,3-triazole formation in the presence of copper(I).[356] Biotinylated maltose was then used as a probe to interact with the surface bound MPB, followed by fluorescence visualization with streptavidin-Cy3, which demonstrated that the chemoselective attachment of the protein to the surface by triazole formation was successful (Figure 13a). To compare the effect of a site-specific immobilization to a random immobilization of MPB, the same alkyne-functionalized MBP was immobilized on an *N*-hydroxysuccinimide (NHS)-functionalized surface by random amide bond formation, and by use of the same method to probe protein activity, it was determined that the random conjugation resulted in a reduced activity while the site-specific immobilization provided for higher protein binding affinity based upon fluorescence (Figure 13b). Since the crystal structure indicates that seven out of 41 arginine and lysine residues on the protein surface reside close to the maltose binding site, these residues may react with the NHS-presenting surface, causing partial or complete blocking of the maltose binding site

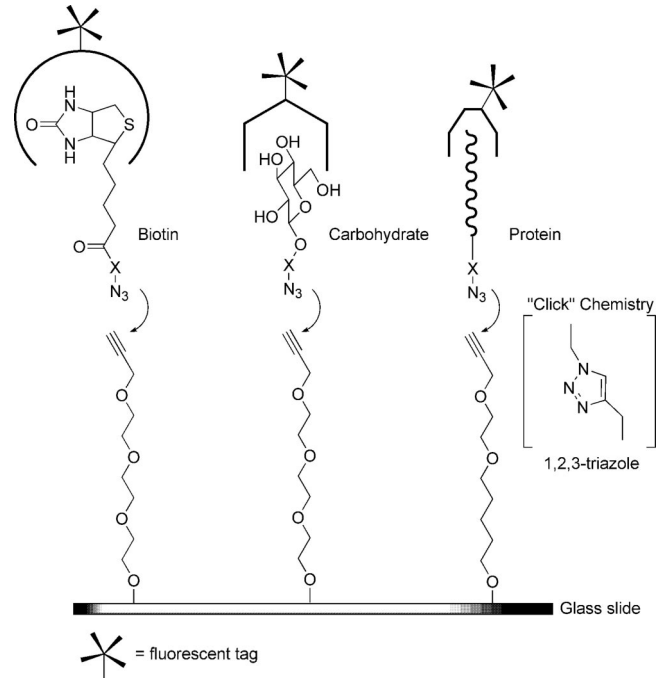


Figure 12. Schematic illustration of test substrates produced by sequential Diels−Alder and azide−alkyne cycloadditions of azide-bearing biotin, carbohydrates, and proteins, and the resultant interactions with fluorescently tagged proteins to demonstrate not only that substrates were covalently attached to the surface but that upon conjugation, the ligands retained bioactivity postconjugation.[355]

(Figure 13c). Through this study, Lin et al. demonstrated the utility of an orthogonal functionalization method for the site-specific immobilization of a MBP that maintained protein orientation on a two-dimensional surface. Additionally, this study, through a comparison of site-specific and nonspecific protein attachment, also clearly illustrated that the orientation of the protein on a substrate is essential for preserving high levels of bioactivity.

3.2.2. Thiol−Maleimide Conjugations

While the copper-catalyzed 1,3-dipolar cycloaddition has recently been employed to create and modify functional surfaces, thiol−maleimide conjugation strategies have also

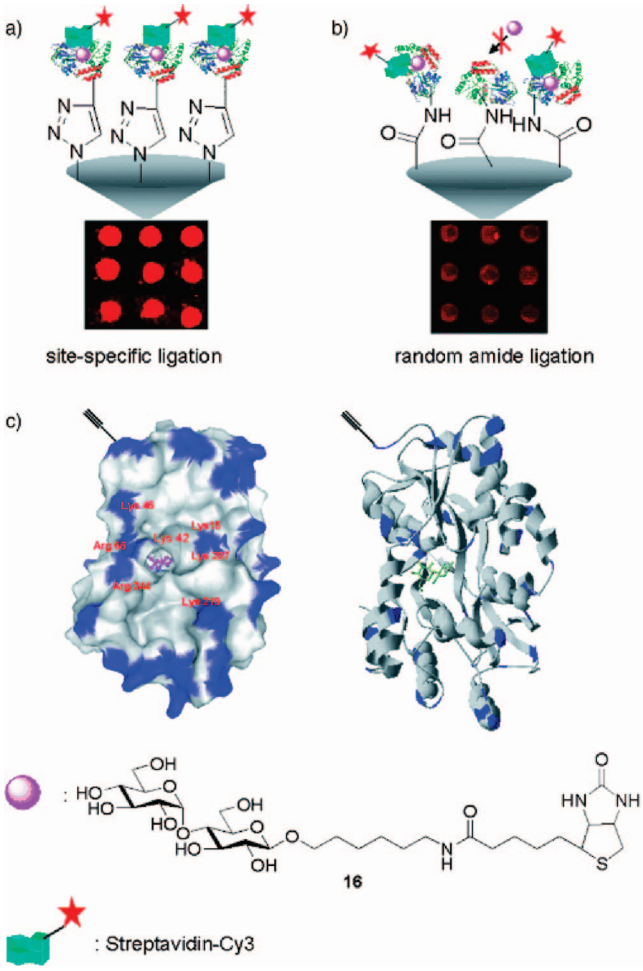


Figure 13. Fabrication of a protein microarray by (a) site-specific covalent bond (1,2,3-triazole) formation and (b) random amide bond formation, contrasting the differences in fluorescence obtained after incubation with biotinylated maltose and a fluorescently tagged streptavadin. Panel c shows the X-ray structure of MBP with blue regions representing lysine and arginine residues that may react with the NHS-decorated surface. Reproduced with permission from ref 356. Copyright 2006 Wiley-VCH.

found universal application in the area of surface functionalization through the selective reaction of maleimide groups with thiol functionalities.[349,357] A general and often employed method for constructing maleimide-presenting SAMs involves immersing gold-coated surfaces into a mixture containing two disulfides, one presenting a terminal maleimide group and the other presenting oligo(ethylene glycol) groups. After construction of the SAM, the maleimide groups can be selectively reacted with thiol-bearing ligands to generate a covalently functionalized surface (Scheme 61).[358]

In a key study reported by Mrksich and co-workers, maleimide-terminated SAMs were used for the immobilization of thiol-terminated ligands and subsequently for the preparation of peptide and carbohydrate arrays.[358] After construction of the SAM, the maleimide groups were chemoselectively reacted with a thiol-functionalized mannose and a CGGRGDS-NH_2 peptide to construct carbohydrate and peptide arrays, respectively. In this study, the immobilized ligands were found to participate in biospecific interactions with proteins and enzymes while the penta(ethylene glycol) groups on the SAM inhibited the nonspecific adsorption of these proteins onto nonfunctionalized regions of the surface. Using a comparable SAM assembly and ligand conjugation strategy, Magnusson et al. recently reported the use of a maleimide-functionalized SAM for the construction of a peptide array containing the *N*-formyl-Met-Leu-Phe (fMLF) peptide, a peptide known to trigger chemotaxis and calcium-dependent oxidative metabolism in neutrophils.[359] The fMLF peptide retained bioactivity after surface immobilization, and assays revealed that the surface-bound peptides were still able to rapidly trigger neutrophil activation, even after conjugation to a planar surface.

Due to both its selectivity and efficiency, this conjugation method has also been used to decorate surfaces with oliogonucleotides and has been employed in the functionalization of carbon nanotubes with DNA,[360] as well as the immobilization of oligodeoxyribonucleotides onto glass and silica substrates.[361] Another example using a thiol−maleimide conjugation of DNA to a SAM was reported by the group of Castner. Castner et al. conjugated thiol-terminated single-stranded DNA (ssDNA) to a SAM made in a single step by solution self-assembly of maleimide−ethylene glycol−disulfide onto gold.[362] This strategy proved useful because the maleimide reacted selectively with the thiol end group on the DNA while the ethylene glycol SAM helped to minimize nonspecific adsorption onto the surface. In this study, the array was able to capture target DNA from biologically complex samples such as blood serum. The array, however, still requires optimization, because some nonspecific adsorption onto the sensing surface limited the detection efficacy of the array.

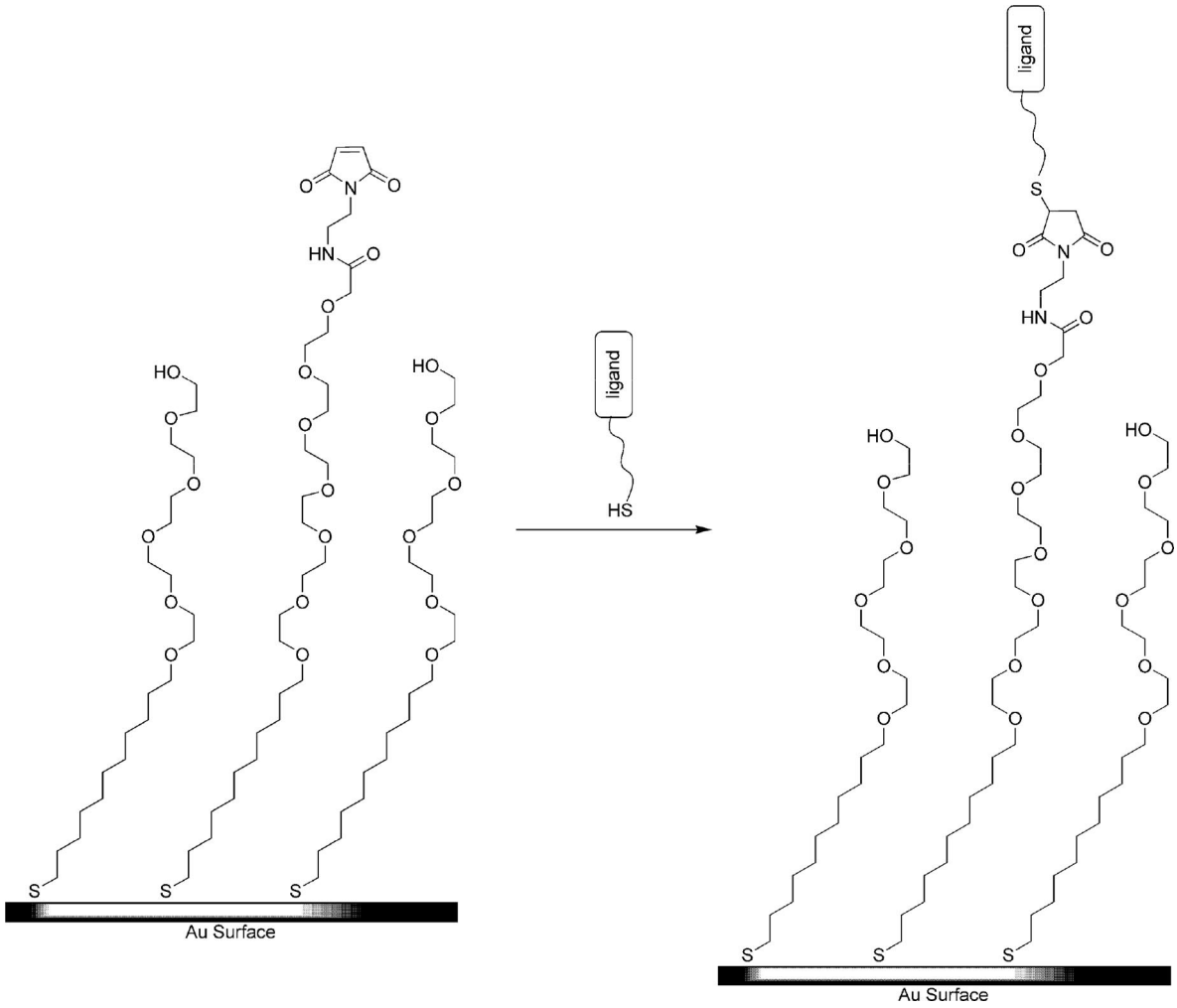
In a recent report, Brozik and co-workers also described the use of thiol−maleimide chemistry to create a series of functional two-dimensional surfaces; in this case, maleimide-activated diazonium salts were used to functionalize an electrode surface with both biologically active and redox-active molecules.[363] A maleimide-active surface was prepared in a single step through the electrodeposition of *N*-(4-diazophenyl)maleimide tetrafluoroborate on both gold and carbon surfaces. The resulting SAM was then reacted with thiol-bearing ferrocene and cytochrome *c* to create redox-active and biologically active surfaces, respectively (Scheme 62A). Additionally, *N*-phenylmaleimide diazonium was reacted with a ferrocene-modified, thiol-terminated ssDNA prior to deposition to create a diazonium active conjugate; this conjugate was then deposited onto an electrode surface to achieve the direct immobilization of DNA and to create a ssDNA-functionalized surface (Scheme 62B).

3.2.3. Oxime Formation

As noted previously, another REO coupling strategy receiving considerable attention is the use of oxime formation to immobilize and organize biomolecules onto a two-dimensional surface. While the reaction between an aminooxy substituent and an aldehyde or ketone moiety is not necessarily a novel chemical transformation (it has previously been used to covalently attach small molecules,[364] peptides,[364,365] and gold nanoparticles[366] to surfaces), it is receiving significant attention for the creation of patterned surfaces through a combination of photochemistry and electrochemistry and is also being actively employed in the site-specific immobilization of proteins.

In a particularly elegant set of studies, the group of Yousaf has generated a variety of functionalized and bioactive surfaces employing oxime formation on a hydroquinone-containing SAM. In one such example, a redox-active hydroquinone monolayer was electrochemically oxidized to the benzoquinone, which was subse-

Scheme 61. Structure of a Self-Assembled Monolayer Used to Immobilize Thiol-Terminated Ligands[a]



[a] The maleimide reacts selectively with thiol groups in a contacting solution while the oligo(ethylene glycol) groups are present to minimize non-specific adsorption of proteins and peptides onto the monolayer.[358]

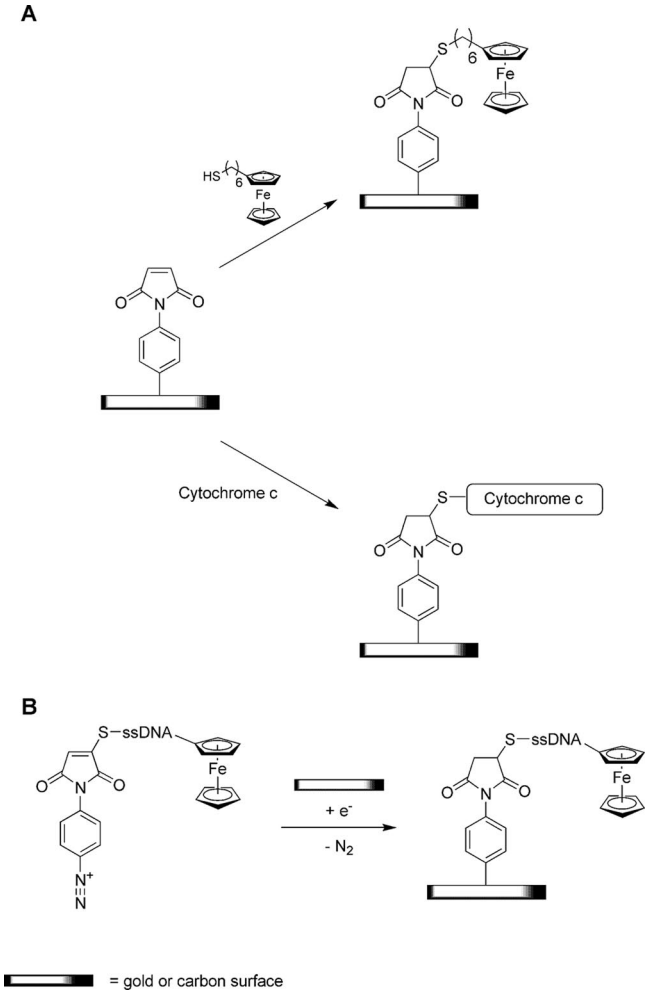
quently reacted with aminooxy-containing compounds including aminooxy acetic acid, rhodamine-oxyamine, and aminooxy-functionalized peptides to form the corresponding oxime (Scheme 63).[367]

To demonstrate the utility of this methodology for the covalent conjugation of bioactive molecules to a surface, aminooxy-terminated FLAG peptides and RGD-oxyamine peptides were conjugated to the surfaces, and both peptides maintained their bioactivity after conjugation. In an extension of this work, Yousaf et al. generated complex patterned surfaces through a combination of photochemical lithography and chemoselective oxime formation.[368] In this study, mixed monolayers presenting nitroveratryloxycarbonyl (NVOC)-protected hydroquinones were photochemically deprotected to afford hydroquinone moieties; subsequent oxidation of the hydroquinone generated the corresponding quinone, which was allowed to undergo chemoselective ligation with aminooxy-terminated ligands (Figure 14A). To demonstrate both the utility and generality of this method, the NVOC protecting group was removed using UV illumination through a photomask to reveal the hydroquinone in specific regions of the monolayer, and after oxidation, the patterned surface was reacted with aminooxy-bearing ligands including rhodamine, Alexa Fluor 488, and the GRGDS peptide (Figure 14B). Through this study, Yousaf and co-workers demonstrated a methodology that allowed for control of ligand density on complex patterned surfaces and also demonstrated that it is possible to covalently immobilize and pattern multiple ligands on a surface through a combination of photolithography, electrochemistry, and chemoselective reactions.

While previous work in the Yousaf group focused upon SAMs presenting hydroquinone moieties that were functionalized by oxime formation after oxidation, Yousaf et al. also reported another example exhibiting the effectiveness of oxime formation and the functionalization of aminooxy-presenting SAMs.[369] In this example, an NVOC-oxyamine-terminated SAM was photochemically deprotected to afford an aminooxy group that was then allowed to undergo reaction with a series of ketone-functionalized ligands (Figure 15). In this study, the newly deprotected aminooxy groups were allowed to react with carbonyl-bearing molecules including ferrocene, fluorescent dyes, and the GRGDS peptide to create redox-active, fluorescent, and bioactive surfaces respectively.

Oxime formation can also be employed for the surface immobilization and patterning of proteins, with the Maynard group reporting the effective combination of photolithography and oxime formation to generate biotin presenting SAMs that can subsequently be used to pattern proteins onto a planar surface.[125] To achieve a patterned biotinylated surface, a film of poly(3,3′-diethoxypropyl methacrylate) (PDEPMA) was spin-coated onto a silicon wafer, and upon exposure to

Scheme 62. (A) 6-Ferrocenyl-1-hexanethiol and (B) Cytochrome *c* Protein Functionalization of Phenylmaleimide Thin Films and (C) Direct Functionalization of a Surface with Phenylmaleimide Diazonium Conjugated to Ferrocene-Labeled ssDNA via a Thiol Michael Addition[363]



UV light through a mask, the acetal groups were site specifically converted to aldehydes; subsequent reaction with a biotinylated aminooxy compound to form an oxime linkage afforded a patterned biotinylated surface (Scheme 64). This patterned film was exposed to UV light to remove any remaining acetals, and aminooxy-terminated PEG was covalently attached to the background aldehyde moieties, while fluorescently labeled streptavidin was noncovalently attached at the biotinylated sites to generate a streptavidin-patterned surface capable of suppressing nonspecific binding (Scheme 64). After conjugation of streptavidin, a wide range of biotinylated proteins can be immobilized onto the surface making this a general method for patterning and assembling proteins in two dimensions.

In addition to patterning streptavidin through a noncovalent interaction with a biotinylated surface, Maynard et al. also employed oxime formation for the direct and site-specific immobilization of streptavidin.[370] A copolymer made from 2-hydroxymethyl methacrylate and a Boc-protected aminooxy tetra(ethylene glycol) methacrylate was spin coated onto a silicon wafer, and the Boc groups were removed by photoacid generator-based photolithography to reveal aminooxy groups that were subsequently reacted with a N-terminated α-ketoamide modified streptavidin to afford a surface patterned with proteins having controlled orientations (Scheme 65).

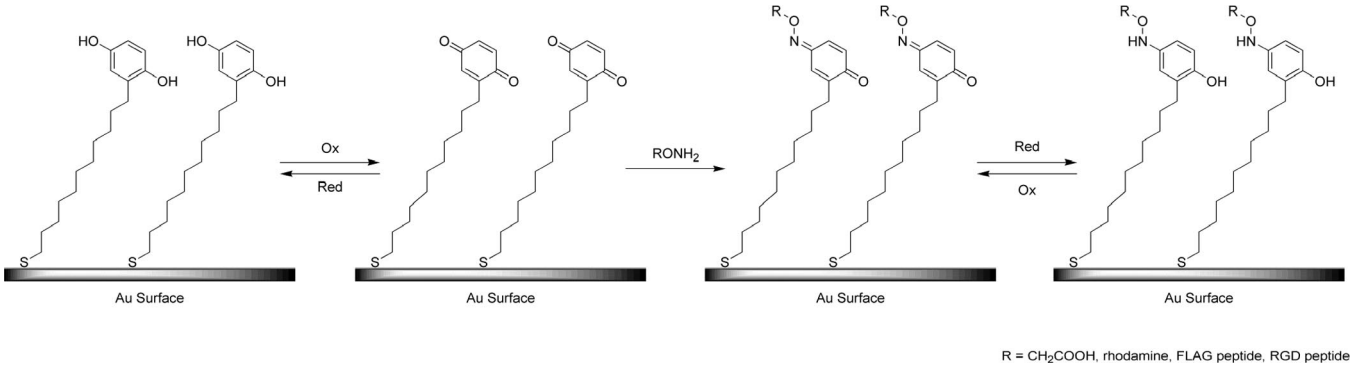
3.2.4. Multifunctional Surfaces

While chemoselective reactions can be employed singularly for the creation of functional arrays and bioactive surfaces, the selective patterning of a surface with multiple ligands or biomolecules can be achieved through the employment of multiple chemoselective chemistries that display orthogonal reactivity. Recently, two studies have reported the synthesis of functional patterned surfaces using two or more orthogonal conjugation strategies.

Gleason and co-workers reported the synthesis of nanopatterned multifunctional surfaces, where one nanodomain contained an acetylene group for derivatization through click chemistry and the other domain contained pendant amines that could be functionalized with *N*-hydroxysuccinimide (NHS)-containing compounds.[371] To obtain a surface with amine and acetylene functionalities, a poly(allylamine) (PAAm) film was deposited and cross-linked using plasma-enhanced chemical vapor deposition, after which a poly(propargyl methacrylate) layer was added by initiated chemical vapor deposition to form a bilayer. A nanopatterned surface displaying amine and acetylene groups was achieved by capillary force lithography of the bilayer, and the self-sorting of two fluorescent dyes, an azide-bearing rhodamine and an NHS-functionalized fluorescein, was performed on the patterned surface in a one-pot functionalization reaction (Figure 16). This method, due to the ease of fabrication and the orthogonality of the two reactive groups, provides a potential platform for the synthesis of multicomponent bioactive arrays.

In another example employing multiple orthogonal reactions, Maynard and co-workers used a combination

Scheme 63. The Redox-Active Hydroquinone Monolayer Undergoes Electrochemical Oxidation to the Benzoquinone and Then Undergoes a Chemoselective Reaction with an Aminooxy-Containing Compound To Yield the Corresponding Oxime[367]



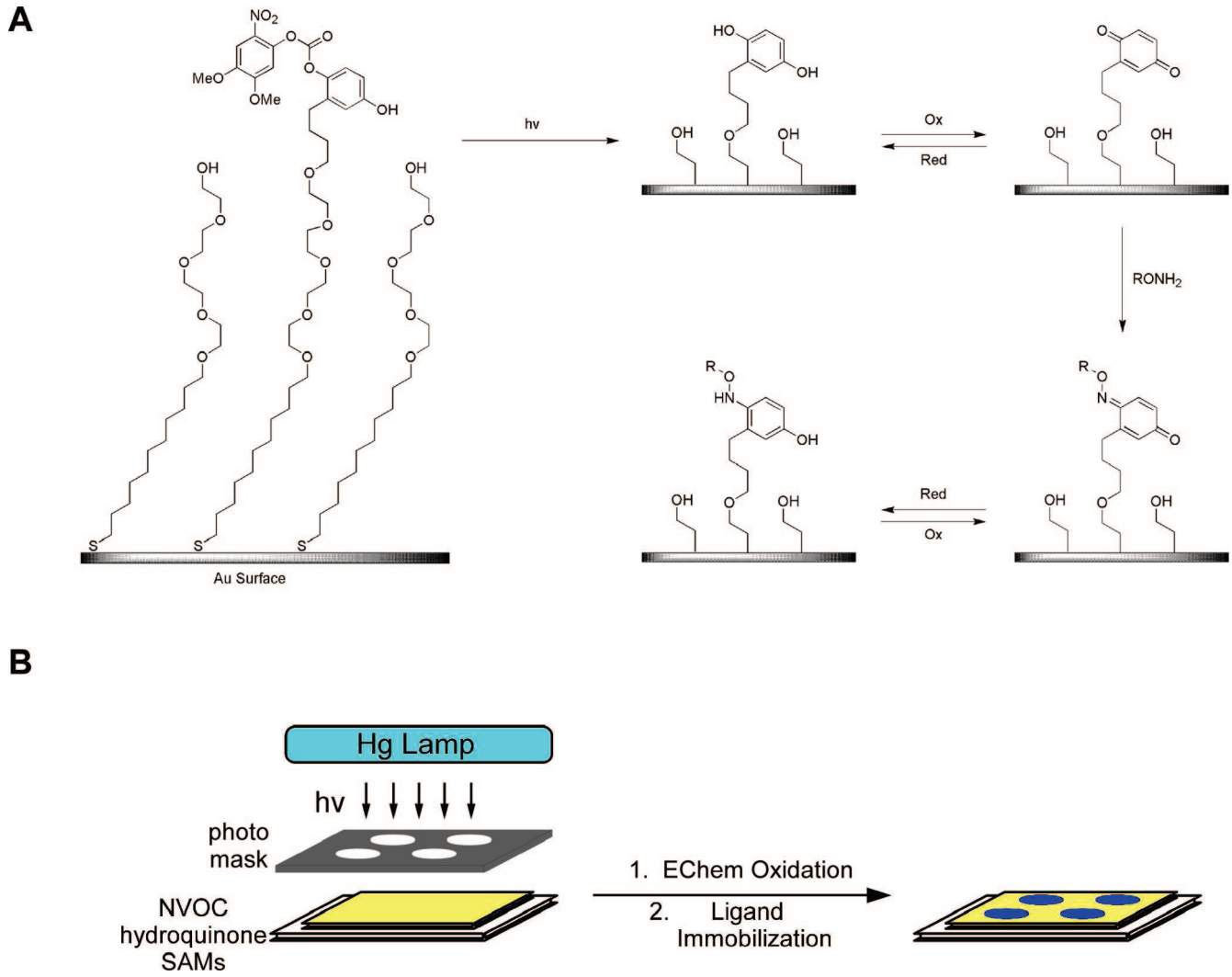


Figure 14. (A) Mixed monolayers presenting NVOC-protected hydroquinone and tetra(ethylene)glycol are illuminated with UV light to afford the hydroquinone. Subsequent oxidation of the hydroquinone produces the corresponding benzoquinone, which can then undergo reaction with aminooxy-terminated ligands. (B) A photochemical strategy for generating patterns and gradients of immobilized ligands onto an electroactive monolayer.[368]

of electron beam lithography and orthogonal chemistries to precisely pattern proteins onto a two-dimensional surface.[372] Eight-arm poly(ethylene glycol)s having biotin, maleimide, aminooxy, or nitrilotriacetic acid end groups were cross-linked on a silicon surface using electron beam lithography to prepare protein-reactive PEG hydrogels with micrometer-sized domains presenting each unique functional group (Figure 17a). Proteins with a biotin binding site, a free cysteine, an N-terminal oxoamide, and a histidine tag were incubated with the functional surface to afford a patterned array displaying four unique proteins. Streptavidin was immobilized through a noncovalent interaction with the biotinylated portions of the surface (Figure 17b). Bovine serum albumin (BSA) was covalently immobilized on the surface through a thiol Michael addition reaction with the PEG−maleimide and the sulfhydryl group of the free cysteine present in BSA (Figure 17c). Oxime formation was employed in the conjugation of myoglobin as the PEG−aminooxy domain was reacted with a modified myoglobin displaying an α-oxoamide at the N-terminus (Figure 17d). Finally, histidine-tagged calmodulin was immobilized on the surface via a nickel−histidine affinity interaction with the nickel(II) being coordinated by the nitrilotriacetic acid functionality (Figure 17e). In this elegant study, using two orthogonal noncovalent interactions and two chemoselective reactions, Maynard et al. were able to efficiently and selectively construct a patterned surface bearing four proteins using four distinctive and chemically unique methods for immobilization.

4. Functionalization of Biological Systems

The specific functionalization of biological molecules has become an area of significant academic and industrial interest in recent years, driven in part by the success of REO strategies in these transformations. As previously described, a number of the defining characteristics of a REO reaction (quantitative yield, mild reaction conditions, compatibility with functional groups, etc.) are especially central to their use in functionalizing biological systems, because these molecules are typically only available in limited amounts and the high degree of functionality with associated instability to elevated temperatures demands a benign, orthogonal process. The examples discussed below will not only illustrate these points but also demonstrate the modularity inherent in REO processes and the potential for the functionalized biomolecules in a variety of applications.

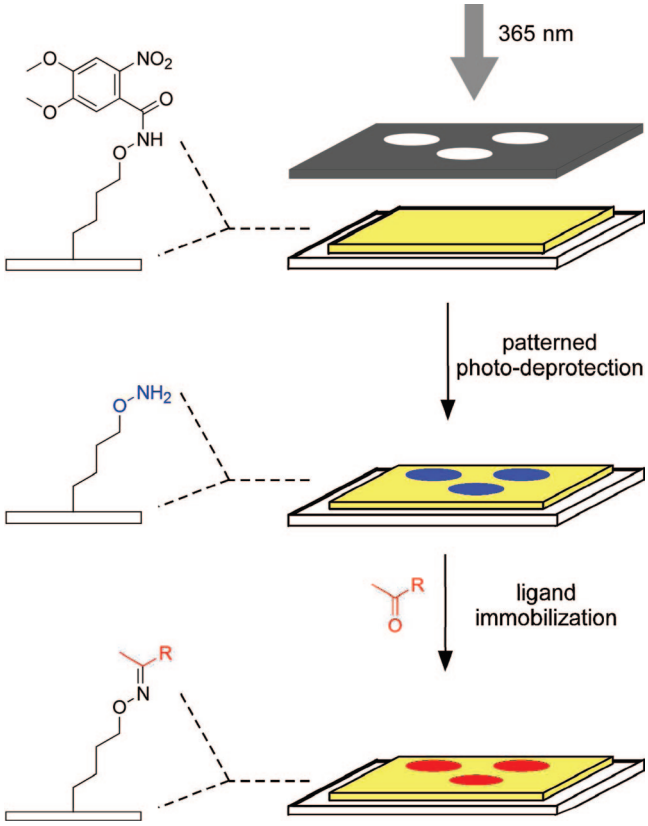


Figure 15. Depiction of the patterned photodeprotection of aminooxy-terminated SAMs and the subsequent functionalization with ketone-containing ligands to generate a functional patterned surface.[369]

4.1. Viral Particles

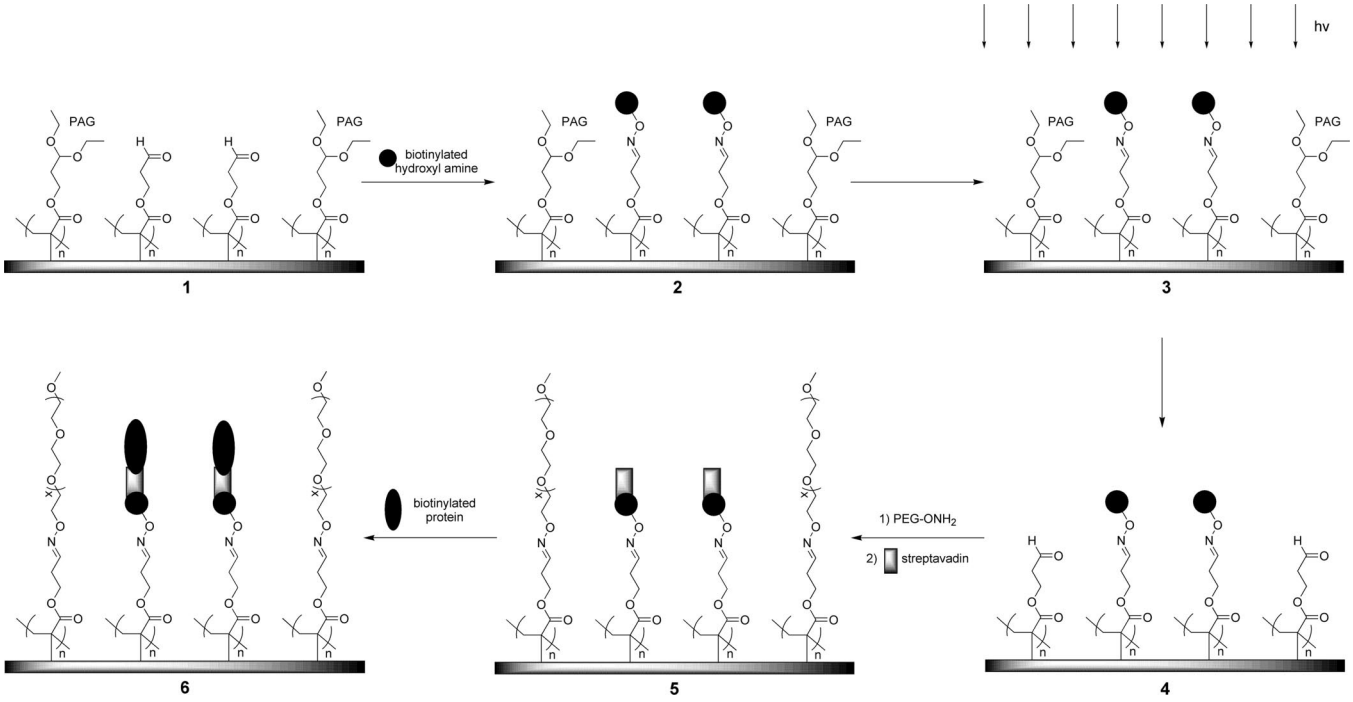
Viral particle constructs, self-assembled from individual protein units, represent an interesting class of nanoscale building blocks for the development of advanced soft materials for applications in materials science and medicine. Through chemical or genetic modifications, a multitude of strategies for the regioselective functionalization of viral particles have been demonstrated. The application of these materials and their preparation have been well-reviewed in recent years,[373–380] and therefore, this section will highlight a few important examples of orthogonal chemical modification of viral particles.

4.1.1. 1,3-Dipolar Cycloaddtions

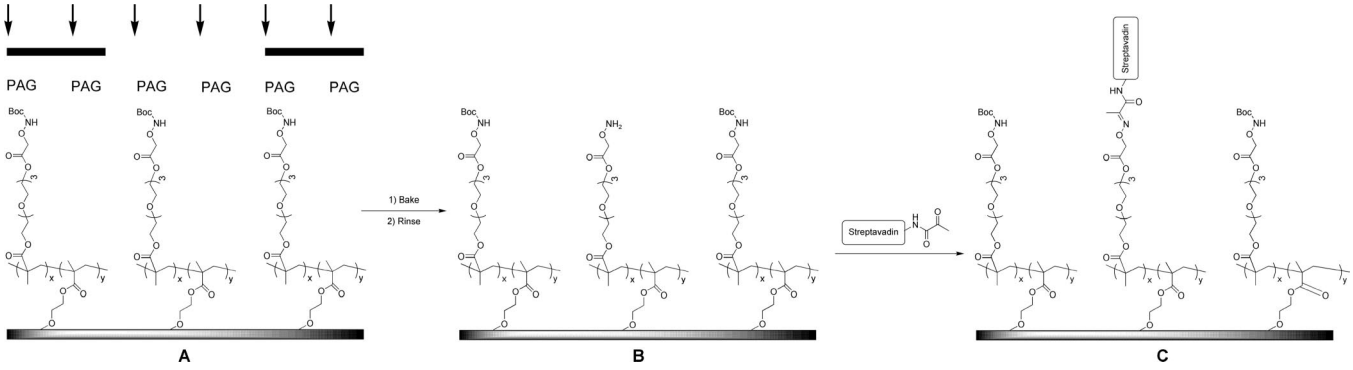
The power of 1,3-dipolar cycloadditions for modifications of viral capsids was first demonstrated by the groups of Finn and Sharpless in 2003.[381] Cowpea mosaic virus (CPMV) served as template for modification, and it was found that the best results were obtained when azide functional viruses were coupled with small molecule alkynes.[381] This work was then expanded to include the orthogonal ligation with a diverse set of functionalities, such as sugars, peptides, proteins, glycopolymers, MRI chelators, and PEGs, with coupling efficiencies of 60−85% using moderate excesses of reagents (Scheme 66).[382–385]

Recently, the same group reported the construction of Gd^{3+} MRI contrast agents by genetic engineering of the bacteriophage Qβ, introducing an unnatural azide-containing amino acid and "clicking" on the chelator.[386] The reaction was found to modulate the surface charge, which resulted in changes in the plasma clearance time of the viral contrast agent. The tobacco mosaic virus has also been utilized as a scaffold for 1,3-dipolar cycloadditions. In this case, the alkyne is introduced by chemoselective modification of the tyrosine

Scheme 64. Following Exposure to UV Light through a Mask, Acetals Were Selectively Deprotected To Afford Aldehydes (1), a Biotinylated Hydroxylamine Was Attached to the Surface through Oxime Formation (2), the Films Were Exposed to UV Light To Remove Additional Protecting Groups (3), and Convert Remaining Acetals to Aldehydes (4), Aminooxy-Terminated PEG Was Allowed to React with the Background Aldehydes while Streptavidin Was Immobilized to the Biotin Patterns (5), and Any Biotinylated Protein Can Then Be Immobilized onto the Streptavidin Foundation (6)[125]



Scheme 65. (A) After Covalently Linking the Copolymer to the Surface, A Photoacid Generator Was Spin-Coated on Top and the Films Were Exposed to UV Light through a Mask, (B) at the Locations Exposed to the UV Light, after Subsequent Acid Exposure, the Boc Protecting Groups Were Removed Affording a Pattern of Aminooxy Functionalities, an (C) an α-Ketoamide Modified Streptavidin Was Immobilized on the Surface through Oxime Formation[370]



residue on the surface of the viral capsid, and other orthogonal functionalities can be brought in as well (Scheme 67).[387]

4.1.2. Aldehyde-Based Conjugation Strategies

In synergy with CuAAC systems, aldehyde-based conjugation strategies have also been demonstrated to be an effective and useful methodology to introduce different functionalities on viral nanostructures. Francis et al. utilized a combination of aldehyde-based (exterior) and COOH (carbodiimide-based) conjugations to selectively and orthogonally modify the tobacco mosaic virus (TMV) with a wide variety of ligands and functional groups including poly(ethylene glycol) on the surface.[388] Combinations of aldehyde-based and carbodiimide-based conjugations for dual surface functionalization have also been utilized to bring in PEGs on the surface of the MS2 viral capsid and fluorescent dyes on the inside as therapeutic mimics.[389] In an extension of this work on regiospecific modification, the MS2 viral capsids were decorated either on the inside or on the outside with chelators for Gd^{3+}, in order to compare their relaxivities and evaluate them as potential MRI contrast agents (Scheme 68).[390,391] Based on this strategy, beneficial effects were observed on selective introduction of the chelators in the interior of the capsid (via more rigid tyrosines), while tissue specific targeting groups could be attached to the exterior.[391]

4.1.3. Michael Addition and Thiol-Based Strategies

Genetic engineering of viral constructs, as well as utilization of naturally occurring cystine residues, have also been widely used for functionalization, though low reactivity or disulfide formation is sometimes an issue. An early example of this strategy was presented by the group of Douglas et al. who engineered the cowpea chlorotic mottle virus (CCMV) (assembled from 180 protein units) with surface-thiol fragments (native form was unreactive) followed by reaction with maleimido-functionalized dyes.[392] A maximum of 30% of these units could be modified with disulfide conjugations with peptides and amidation reactions also being explored.[392] Similarly, the same group genetically modified the small heat shock protein cage from *Methanococcus jannaschii* to express cystine units and probed the reactivity of both SH

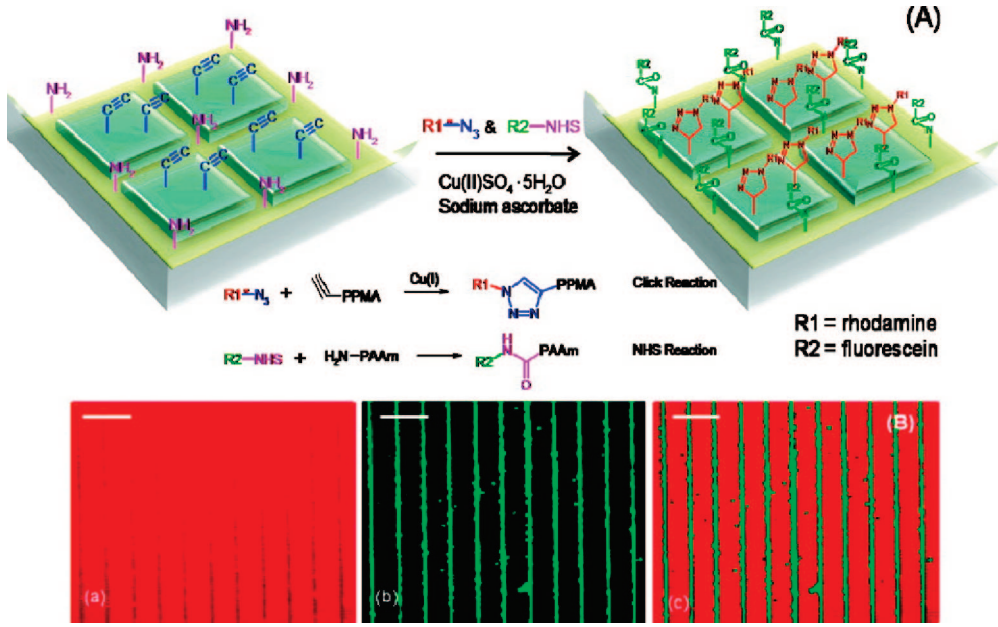


Figure 16. (A) Schematic procedure showing a one-pot functionalization, and (B) fluorescence image of (a) click-functionalized red dye excited at 545 nm, (b) NHS-functionalized green dye excited at 491 nm, and (c) overlapped image of images a and b. Each scale bar represents 30 μm. Reproduced with permission from ref 371. Copyright 2008 American Chemical Society.

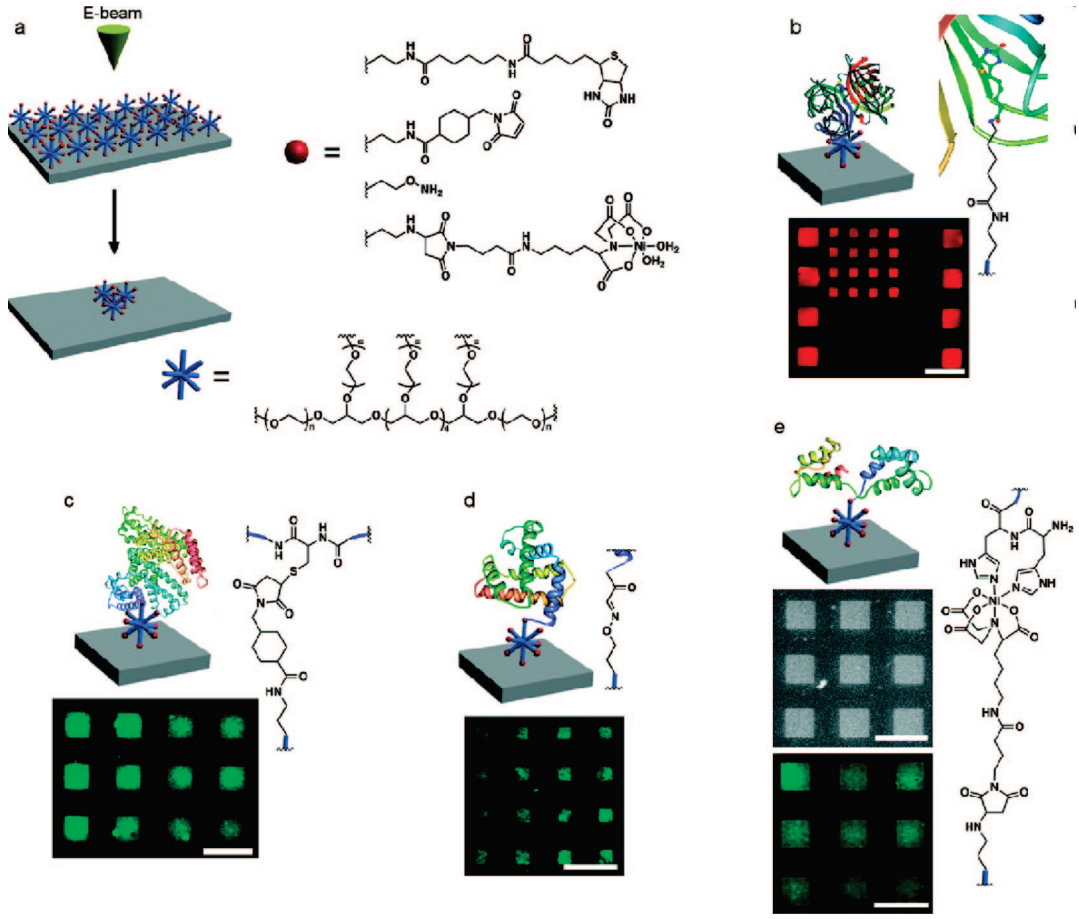


Figure 17. (a) Electron beam cross-linking of end-functionalized eight-arm PEG polymers for protein patterning; each PEG was end-functionalized with one of four protein reactive handles (biotin, maleimide, aminooxy, or nitrilotriacetic acid). (b) Streptavidin was attached to the biotin. (c) Bovine serum albumin was immobilized through a reaction with a cysteine side chain and maleimide. (d) Myoglobin bearing an N-terminal oxo moiety was attached to the surface through oxime formation. (e) Histidine-tagged calmodulin was immobilized through a nickel−histidine affinity interaction using nickel(II) chelated by the nitrilotriacetic acid substituents. Reproduced with permission from ref 372. Copyright 2009 American Chemical Society.

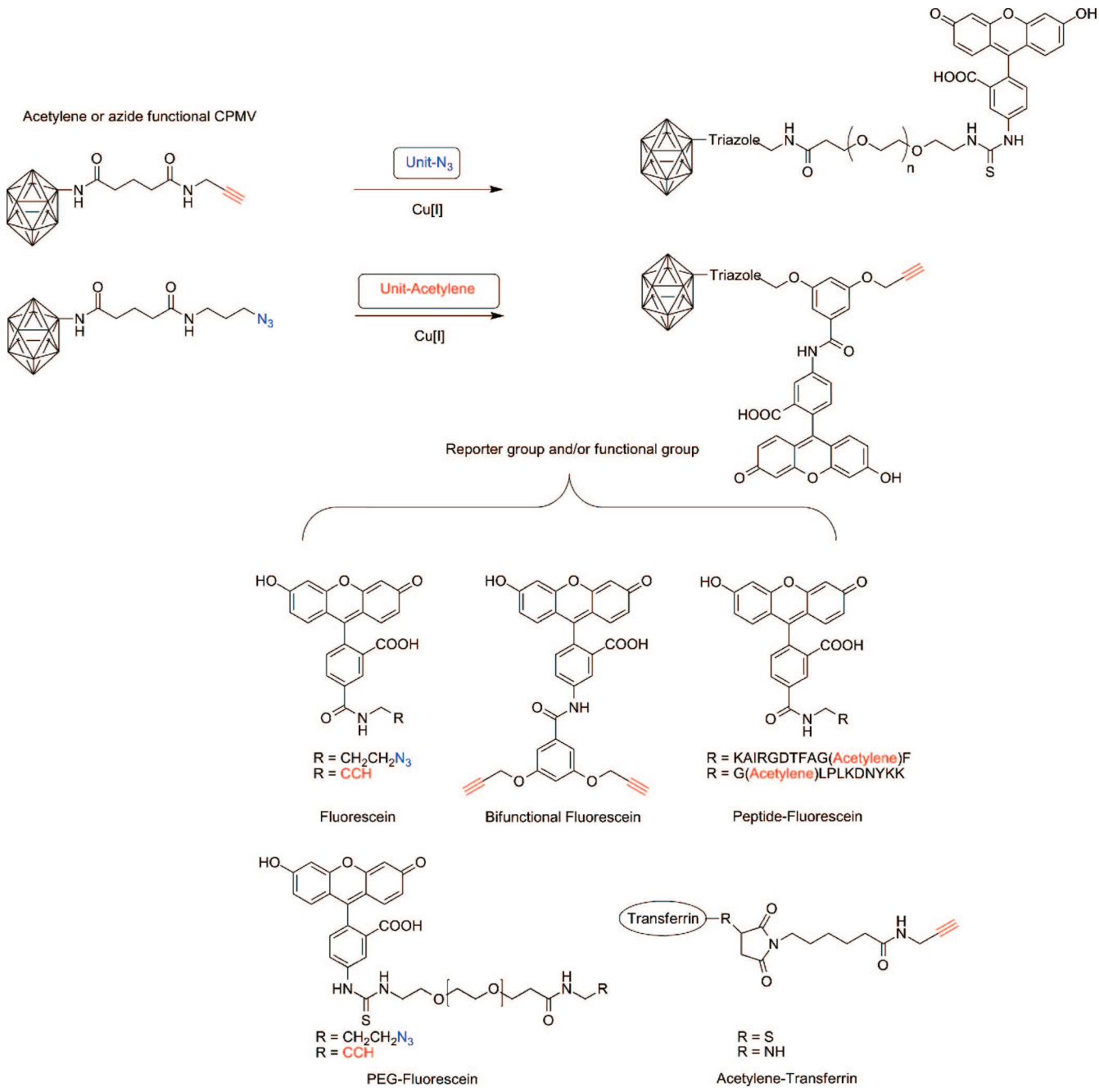
groups and the amine units of the nanocage with fluorophores.[393] Later, it was also demonstrated that the same protein could be internally conjugated with doxorubicin.[394] Douglas et al. have also explored more complex architectures via the disassembly and reassembly of different protein chimeras (DNA binding protein from starved cells of *Listeria innocua*), functionalized via maleimide reactions (internal) and with iodoacetamide functions (external).[395] The group of Finn has also explored thiol units presented selectively on the exterior of CPMV for iodoacetamide-based conjugations with stilbene units for antibody recognition studies.[396] Further, the CPMV unit was functionalized with complementary oligonucleotide segments via maleimido functional segments or amidation-based methods as a means for directing the aggregation behavior of the virus.[397] The ability of REO chemistry to tolerate a wide range of both substrates and functional moieties can be appreciated through many examples, including the conjugation of maleimido-functionalized antibodies[398]and cypate dyes[399] to CPMV or the decoration of tobacco mosaic virus with maleimido-functionalized donor−acceptor-type fluorophores[400] and porphyrins[401] for light-harvesting applications (Scheme 69).

4.1.4. Other Orthogonal Coupling Strategies

A variety of other orthogonal coupling strategies for the functionalization of viral particles have also been developed recently. Francis et al. utilized an internal tyrosine in bacteriophage MS2 for subsequent orthogonal hetero-Diels−Alder modification and reported excellent conversions exceeding 95%.[402] Finn used surface available NH_2 groups to decorate the surface of cowpea mosaic virus with various poly(ethylene glycol)s in an effort to reduce the immunoresponse of systemically delivered viral capsids.[403] Similar approaches have been explored for the addition of MRI chelators to the MS2 viral capsid,[129] and Wang utilized a simple and elegant combination of an activated ester of a terbium complex and amino biotin to create functional turnip yellow mosaic virus (TYMV) for time-resolved fluoroimmuno assays, where the biotin served as a model binding site for proteins (Scheme 70).[404]

More recently, Francis reported the utilization of a novel oxidative coupling strategy that is fully orthogonal to amino acid functionalities as a method for decorating the surface of MS2 viral capsids (Scheme 71).[405] As depicted in the scheme, peptides bearing N-terminal phenyl diamine can be accessed via solid phase synthesis and can be subsequently used for oxidative conjugation with viral capsids bearing engineered phenylalanine groups (introduced via growth media in culture). This methodology may potentially be very useful for the introduction of a range of targeting peptides onto viral particles via nongenetic techniques and demonstrates the power of new orthogonal chemistry in the modification of chemically sensitive biomolecules.

Scheme 66. Functional Assemblies of CPMV Particles and Various Functional Units[382]



4.2. Antibodies, Proteins, and Peptides

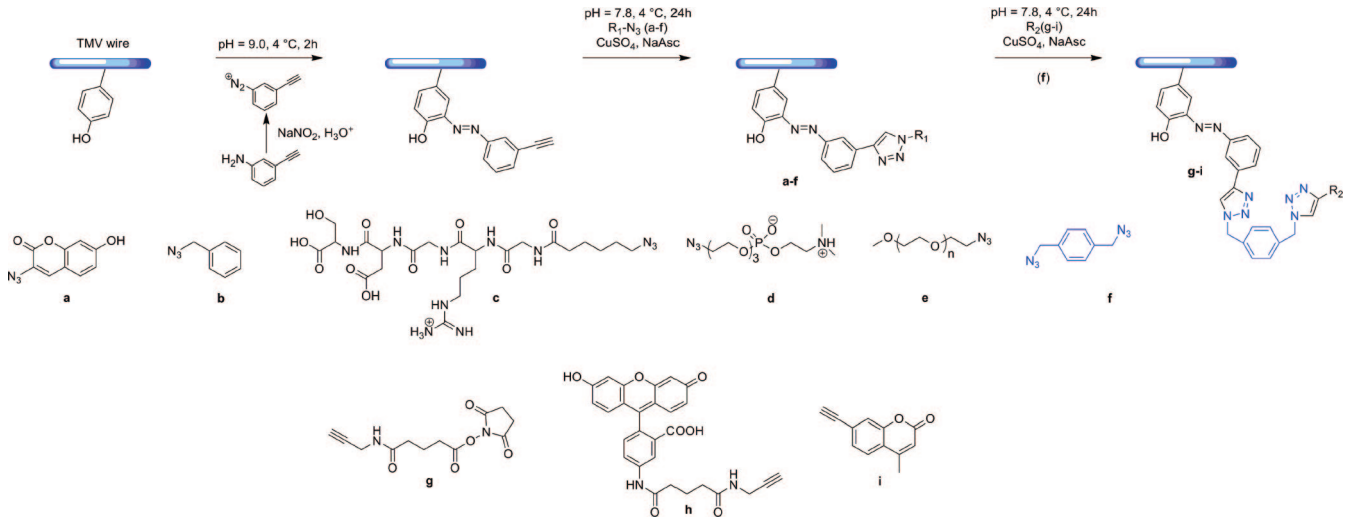
As with viral particles, the covalent modification of biological molecules such as antibodies, proteins, and peptides with synthetic polymers via orthogonal strategies to create biological chimeras is a rapidly expanding area of soft materials chemistry. The utilization of such constructs in the pharmaceutical industry in order to increase the stability, increase the circulation time, and limit toxicity, in combination with research in the emerging areas of nanotechnology and nanomedicine, offers a wide range of new structures and applications. The area has been covered by several recent general reviews,[406–413] as well as reviews focusing on the important preparation of poly(ethylene glycol)−protein or peptide conjugates,[414,415] and we have, therefore, chosen to include a limited selection of examples to further illustrate the unique aspects of REO chemistry that will be critical for future developments in this field.

4.2.1. 1,3-Dipolar Cycloadditions

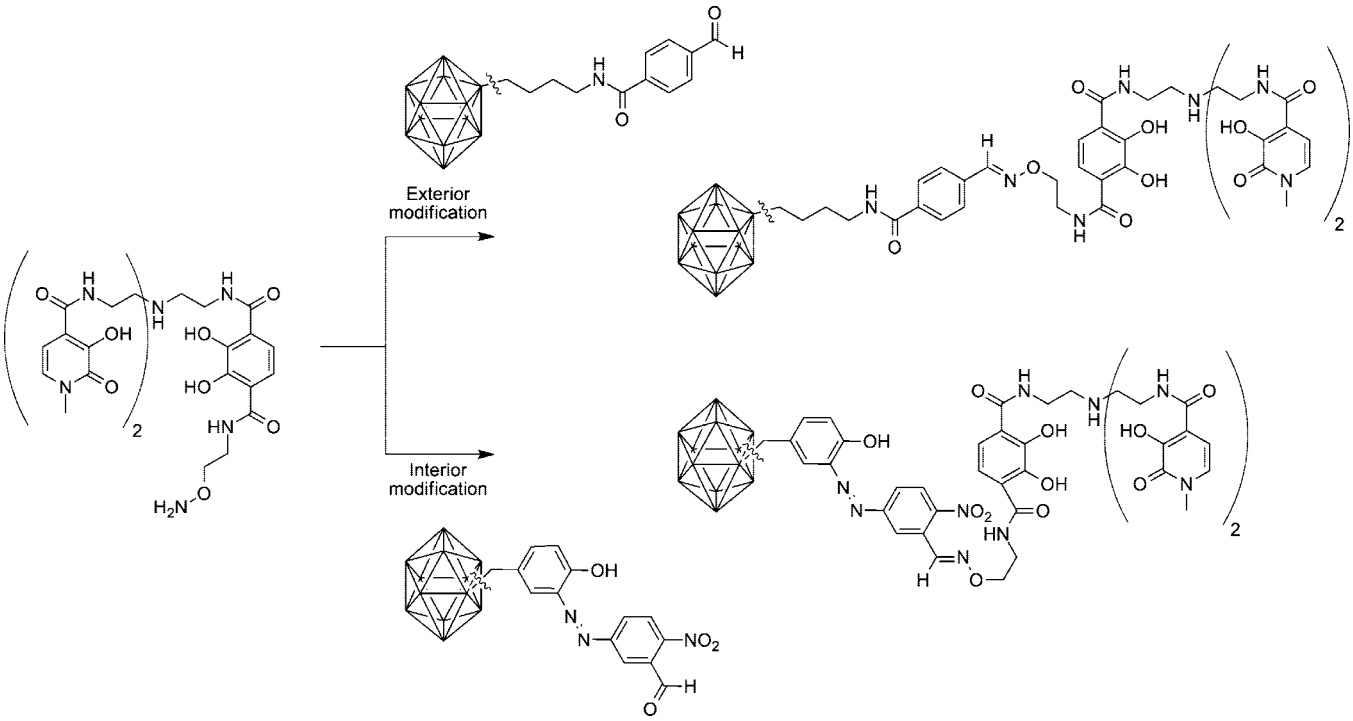
Several recent reviews[77,416–418] have highlighted the importance of 1,3-dipolar cycloadditions for modification of biological entities with polymers and small molecules. The CuAAC reaction's high fidelity and the simplicity of introducing azide or alkyne functional groups in combination with a high tolerance for functionalities present in proteins, peptides, and antibodies make this an attractive method for construction of synthetic polymer-modified biological systems. For example, the group of Schultz incorporated *para*-azidophenylalanine as an unnatural amino acid in yeast and labeled the resulting protein site-specifically with alkyne-functionalized PEG, resulting in a 70−85% conversion of the azides in 24 h.[419]

Proteins with tailored hydrophobicity have also been synthesized by a combination of Michael addition of α-maleimido-functional copolymers to proteins followed

Scheme 67. Examples of 1,3-Dipolar Cycloaddition Modifications of Tobacco Mosaic Virus Wires[387]



Scheme 68. Example of Aldehyde-Based Conjugation Strategies for the Selective Functionalization of the Exterior (K106, K113, and N-Terminus) or Interior (Y85) of MS2 Viral Capsids with 90 Ligands in Both Cases[391]



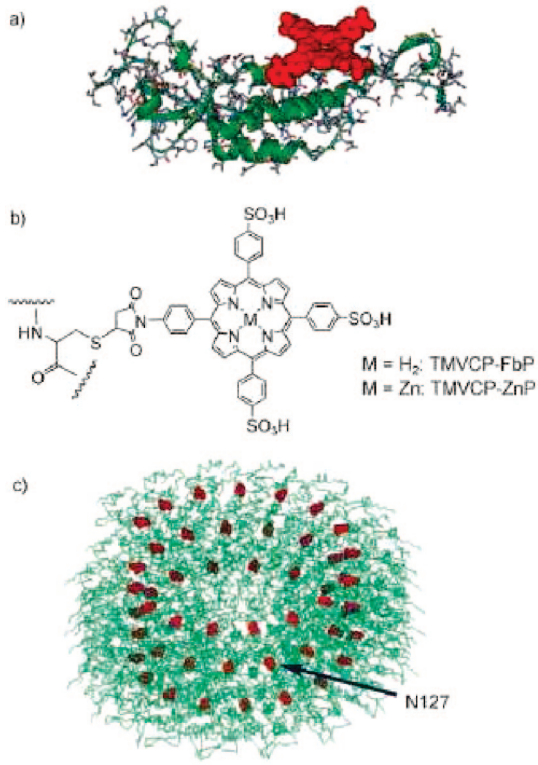
by the introduction of hydrophobic segments via 1,3-dipolar cycloadditions on the pendant alkynes of the copolymer.[44] Following similar approaches, synthetic glycopolymer-modified BSA has also been prepared by combining CuAAC and Michael additions (Scheme 72).[42] Recently, BSA has been modified with P(NIPAM) to create a thermoresponsive protein conjugate that can form nanoparticles above the lower critical solution temperature of P(NIPAM) (Scheme 73).[420] Similar examples with polystyrene have also been presented.[27]

4.2.2. Aldehyde-Based Conjugation Strategies

Ketone- and aldehyde-based conjugation strategies have also been explored for the modification and construction of polymer conjugated proteins. PEG is one of the most studied polymers for this type of conjugation and one of the earliest examples of utilizing an aldehyde functional PEG for conjugation to CD4-IgG was presented in 1994.[421] Other examples include PEG-ylated growth factors,[422] PEG chains linked to the N-terminus of EGFP, PLP-activated myoglobin, RNase A, and thioredoxin,[423] PEGylated lysozymes via iridium-catalyzed transfer hydrogenation of lysine with high fidelity at room temperature and physiological pH,[424] as well as cross-linked polymer−protein gels via genetic modification to introduce aldehydes in the N-terminus of a fluorescent green protein that under shrinking changes fluorescence intensity.[135]

Poly(NIPAM) (prepared via ATRP from functional initiators) conjugated through oxime formation to BSA has been reported,[40,425] as well as poly(methacryloyloxyethyl phosphorylcholine) (poly(MPC)) (functional initiators via ATRP) conjugates with lysozyme, granulocyte colony stimulating factor (G-CSF), and erythropoietin (EPO).[426] Finally, the N-terminal modification of antibodies with PEG via alde-

Scheme 69. Porphyrin-Functionalized Tobacco Mosaic Virus[401a]



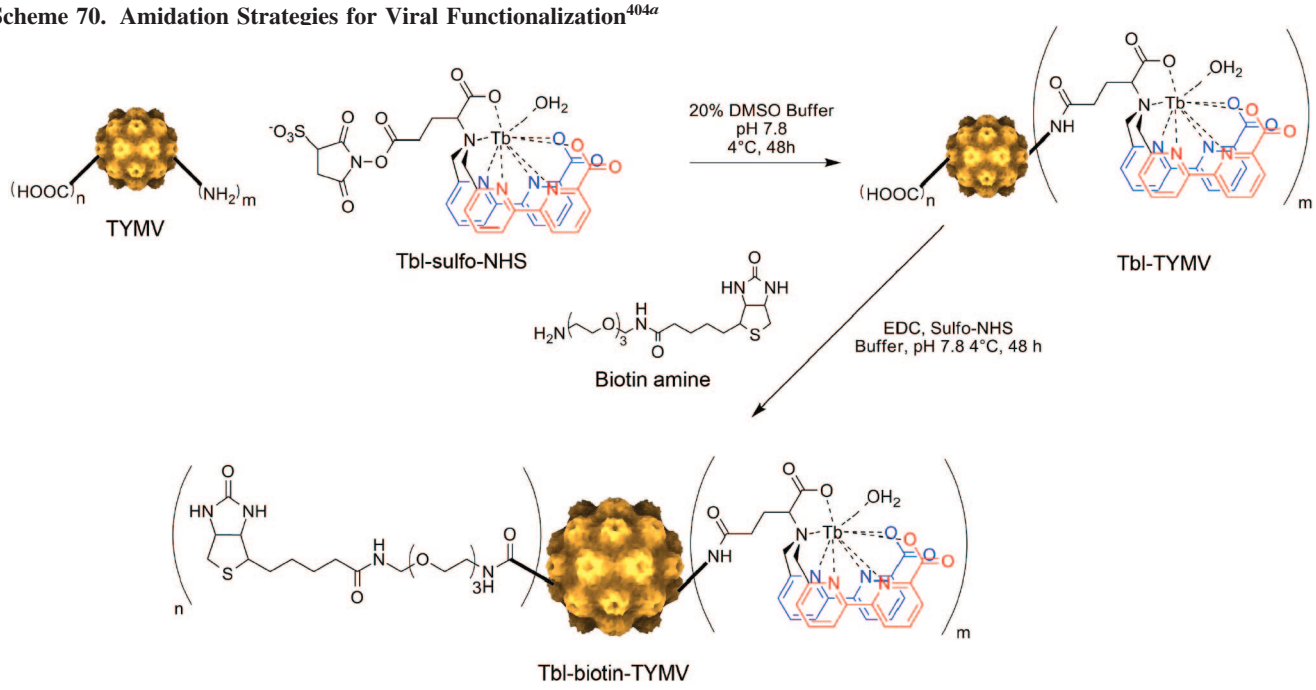
[a] Reproduced with permission from ref 401. Copyright 2007 Wiley-VCH.

hyde-based conjugations have been performed in the presence of pyridoxal-5′-phosphate.[427] Significantly, the mild nature of this conjugation chemistry did not impair the immunoactivity of the tested antibodies and suggests that the method may find a variety of future applications where full retention of biological activity is required.[427]

4.2.3. Michael Addition and Thiol-Based Strategies

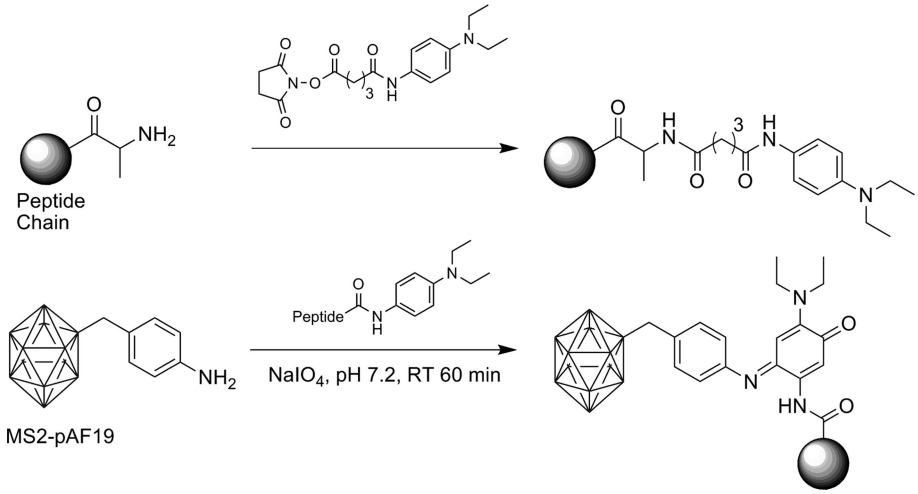
The proven orthogonality of thiol addition to maleimides via Michael reaction, as well as disulfide formation, is extremely well-suited to the preparation of biomolecule conjugates due to the natural occurrence of cystine residues in many protein structures. The efficiency of this chemistry has also been exploited in nonprotein systems, as exemplified by the group of Kiick, who conjugated maleimido-functionalized low molecular weight heparin to star PEGs in order to create noncovalently cross-linked hydrogels,[428] as well as in the preparation of gene delivery vectors based on vinyl sulfone-terminated four-arm PEG conjugated with cysteine-functionalized heparin binding peptides and poly(ethylene imine).[429] Random copolymers of 2-(dimethylamino) ethyl methacrylate (DMAEMA) and aminoethyl methacrylate (AEMA) with the amino groups modified with *N*-succinimidyl 3-(2-pyridyldithio)propionate (SPDP) have also been conjugated to both transferrin (Tf) and the F(ab′) fragment of mAb323/A3 by the formation of reversible disulfide linkages with coupling efficiencies >90%; similarly high coupling efficiencies[430] have been obtained also for maleimido-based strategies. SPDP groups have also been utilized by Maynard in combination with ATRP to prepare poly(hydroxyl ethyl methyl acrylate) (poly(HEMA)) and for conjugation to BSA via formation of reversible disulfide linkages.[431] SPDP is an attractive group for bioconjugations since it releases 2-thiopyridone with an absorption wavelength of 343 nm, which can be used for quantifying the extent of conjugation. Maleimido-functionalized PEGs have also been used for conjugation to recombinant human interleukin-1 receptor antagonist, where modification of native thiols conserved a greater degree of the protein bioactivity compared with random NH_2 modification with PEGs.[432] The group of Ikkala has modified the class II hydrophobin (HFBI) protein and BSA with maleimido-functional dendrons bearing multiple NH groups for high-affinity binding to DNA structures.[433,434] The reactivity of the maleimide unit under radical polymerization conditions necessitates the use of a

Scheme 70. Amidation Strategies for Viral Functionalization[404a]

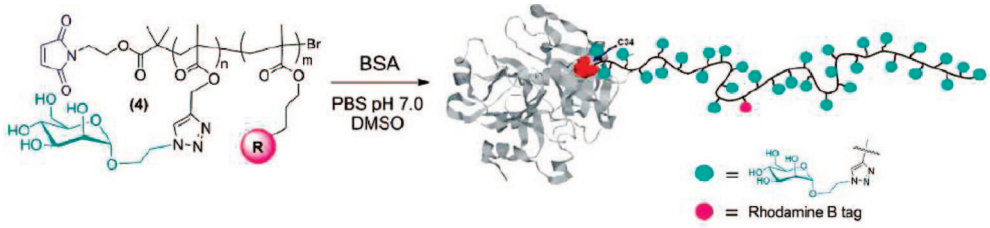


[a] Reproduced with permission from ref 404. Copyright 2007 American Chemical Society.

Scheme 71. Oxidative Coupling of Peptides to the Exterior Surface of MS2 Viral Capsids[405]



Scheme 72. Synthetic Glycopolymers Based on BSA[42a]



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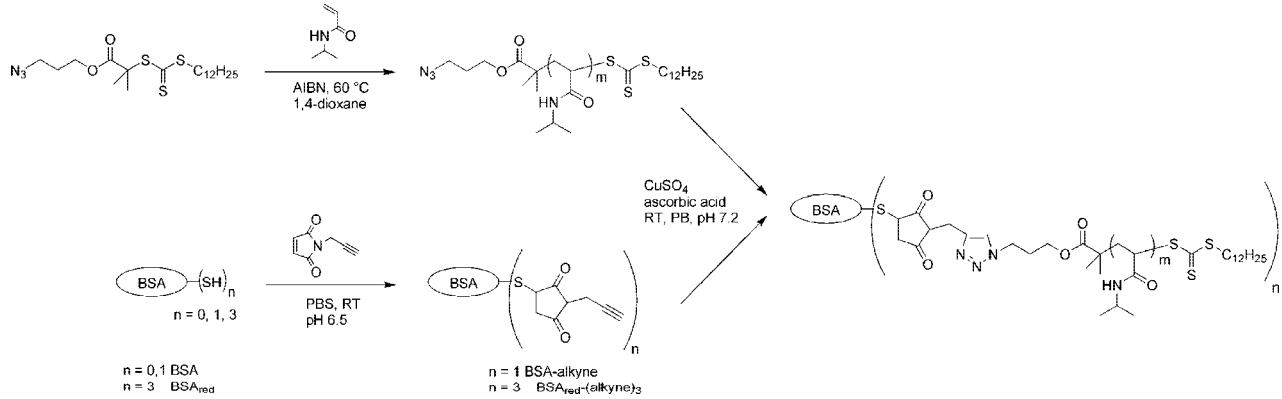
Scheme 73. Thermoresponsive BSA[420]



protecting group strategy, and Haddleton has developed protected maleimido-bearing initiators for ATRP to prepare poly(methoxyPEG) methacrylates and poly(glycerol) methacrylates, for conjugation to BSA and glutathione with high fidelity.[43] These protected initiators are activated via a retro-Diels−Alder reaction prior to conjugation Similarly the group of Maynard synthesized telechelic polystyrene bearing two protected maleimido groups and utilized this polymer for reactions with benzyl mercaptan and *N*-acetyl-L-cysteine methyl ester.[435] An interesting variation on this theme is by Zalipsky, who created cleavable dithiobenzyl (DTB) urethane-linked PEG−lyzosome conjugates capable of cleavage upon exposure to plasma proteins such as albumin and naturally occurring thiols (Scheme 74).[127]

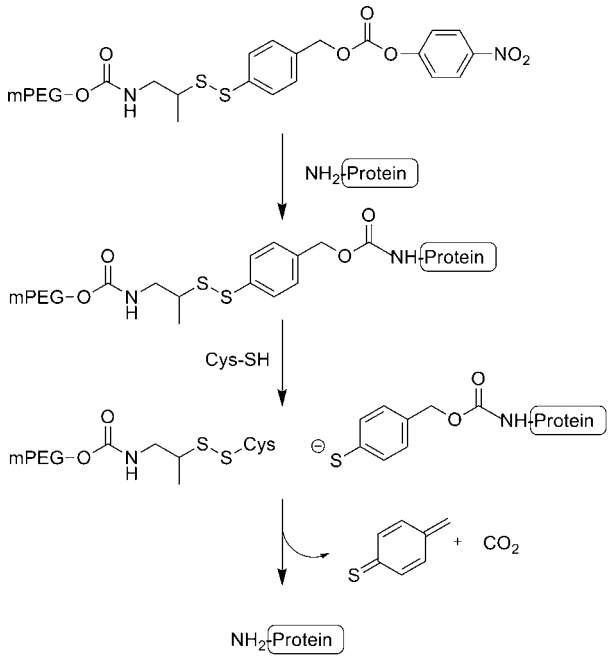
4.2.4. Diels−Alder Chemistry

Polymer modification of biological entities via Diels−Alder strategies is scarce in the literature. The Diels−Alder protection of maleimido groups has been reported, but these end-functional polymers have been utilized for Michael addition reactions.[43,435] However, the use of Diels−Alder chemistry has been demonstrated with diene-modified oligonucleotides that have been successfully modified with PEG and various small molecules via cycloaddition reactions of the diene with maleimido groups (Scheme 75).[436] Significantly, the reactions were done in aqueous solution under mild conditions, with typical conversions around 70−80%.

4.2.5. Grafting Strategies

As noted previously, living free radical procedures are orthogonal to many functional groups associated with REO chemistry, and developments in controlled polymerization techniques, specifically well-defined initiators, have created the possibility of preparing polymeric chimeras by direct polymerization from the biomacromolecule itself.[406] Utilizing this concept, peptide−polymer constructs have been prepared via controlled radical polymerization from solid-phase immobilized peptides bearing initiators for NMP[437,438] and

Scheme 74. PEGylation via the Formation of Thiol Cleavable Conjugates[127]



ATRP.[438,439] Similar strategies have been employed to synthesize peptides bearing initiators for controlled polymerization in solution via ATRP,[14,440–442] as well as RAFT.[443] Solid-phase strategies have also been utilized for the preparation of conjugated polymer−peptide hybrids,[444] various PEG−peptide block copolymers,[445,446] and dendrimer hybrids,[89,447] among many other examples.[406,413]

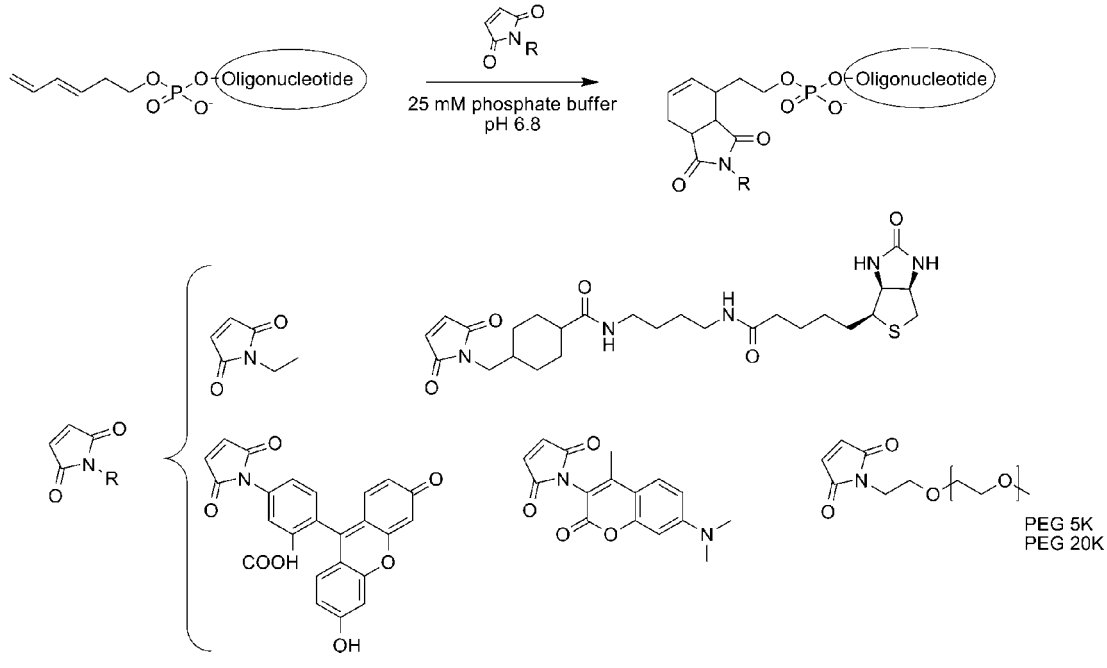
The polymerization from initiator-modified proteins represents a unique example of combining a large number of sequential orthogonal radical reactions that occur under mild conditions during polymerization with more traditional functionalization reactions for obtaining biohybrid polymers. One of the first successful demonstrations of this concept was reported in 2005 by the group of Maynard and involved the preparation of streptavidin with four initiating sites for ATRP. The modified protein was initially prepared by the self-assembly of initiator-bearing biotins with streptavidin and subsequently used as a macroinitiator for the polymerization of NIPAM and ethylene glycol methyl ether methacrylate (EGEMA).[448] The group of Maynard also prepared other poly(NIPAM)-based hybrid materials by modifying specific thiols, such as the Cys-34 of bovine serum albumin (BSA) and Cys-131 of T4 lysozyme V131C with initiating sites for ATRP via maleimido-based conjugation (irreversible) or SPDP (reversible disulfide) chemistry. Significantly, the poly(NIPAM) enzyme conjugate was reported to have complete preservation of bioactivity after polymerization, which is a key requirement for these biohybrid materials.[449] RAFT-based strategies for the preparation of BSA bearing poly(NIPAM) and poly(hydroxyethyl acrylate) (poly(HEA)) at the Cys-34 position have also been reported recently.[450] This strategy utilized a SPDP-functionalized chain transfer agent separated with a PEG spacer for immobilizing the initiating groups.[450]

5. Conclusions and Outlook

Robust, efficient, and orthogonal (REO) chemistries are important for the construction and functionalization of any multifunctional molecular framework, even for relatively small molecules, and they are absolutely critical to the ability to produce well-defined macromolecules and nanostructures. Because of the complexity that is inherently increased as molecular structures grow in size, increasing the numbers of atoms and their combined functional groups, high-yielding chemistries that can be performed independently without interference are required. Many examples of such chemistries and their application to the construction and functionalization of polymers, nanostructures, and surfaces are presented here. Because of the diversity of chemistries and systems included in this review, each is described rather briefly, and the reader is directed to the primary literature for further details.

REO chemistries are considered as a philosophy, including many types of chemistries and not a single particular type of chemical reaction. Traditional organic transformations and

Scheme 75. Diels−Alder Functionalization of Oligonucleotides with PEG and Various Small Molecules[436]



also controlled polymerizations are each considered as REO, provided that they are used in concert to lead to increased sophistication in macromolecular structure and compositional complexity. Organic chemistries and soft materials are the focus of this review, beginning with a demonstration of chain end and side chain compositional control for linear polymers, which then leads to examples of the construction of graft, star, branched, and cross-linked network materials; the functionalization of three-dimensional nanostructures and two-dimensional (patterned) substrates, using small molecule reagents, macromolecules and biological moieties then follows; finally, the functionalization of biological systems and synthetic−biologic hybrids are discussed. Each example was selected based upon its elegant use of REO chemistries.

The review emphasizes techniques for the controlled construction and functionalization of complex materials, where the term complex is defined as having a number of different parts, but of equal consideration should be the potential for selective deconstruction. Reversible assembly/disassembly processes are touched upon in the discussion of self-healing cross-linked networks, but their significance extends far beyond. The programmed deconstruction of a complex molecular object could be important to limit the long-term environmental impact, or it could be used as a functional means to create transformative materials. For instance, multilayered macromolecular architectures or nanostructures can now be produced with each layer contributing a unique property or function, and access to each of those functions could be triggered by a stagewise dismantling of the entire complex unit. It is expected that enhanced modularities in these frameworks will provide for increasingly intelligent materials that perform as discrete molecular devices, capable of highly sophisticated functional operations. REO chemistries will be critical to their construction, incorporation of function, and selective reorganization or deconstruction.

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