Thermo-responsive Coiled Coil Peptide-polymer Grafts

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Supporting Information Placeholder

ABSTRACT: Alkyl halide side groups are selectively incorporated into monodispersed, computationally designed coiled coil-forming peptide nanoparticles. Poly 2-(dimethylamino)ethyl methacrylate (PDMAEMA) is polymerized from the coiled coil periphery using photoinitiated atom transfer radical polymerization (photoATRP) to synthesize well-defined, thermo-responsive star copolymer architectures. This facile synthetic route is readily extended to other monomers for a range of new complex star-polymer macromolecules.

Peptide–polymer conjugates are an emerging class of biomaterials that combine the sequence-to-structure fidelity and the encoded functionalities associated with biological materials (peptides and proteins) with the stability and processability associated with synthetic polymers[1-8]. Coiled-coil peptide bundle units, or 'bundlemers', consist of individual peptide strands that are encoded via their primary amino acid sequence to form helical structures that subsequently assemble into well-defined, monodisperse nanoparticles.[9] Bundlemers can be viewed as modular monomeric building blocks for the creation of more complex hierarchical macromolecular architectures.[10-12] Previous research has depicted bundlemers as an ideal substrate for bundlemer-polymer conjugates, since biophysical evidence supports that coiled coil bundles retain their oligomerization structure after terminal[13-15] or side-chain[16, 17] polymer conjugation.

Bundlemer-polymer conjugates combine a well-defined core structure with tunable polymer interactions. Early work on coiled-coil polymer grafts examined the effects of polymer conjugation (i.e., using grafting-to approach) on coiled coil oligomerization state and stability[16, 18-22]. Some of the well-known drawbacks of the grafting-to approach include the need for a large excess of polymer, difficulties in purification, and steric hinderance lowering probability that complementary functional groups will meet. These issues may be overcome by a 'grafting-from' approach, where polymerization occurs from the substrate.

Here, a halide side group was selectively incorporated into a bundlemer-forming peptide sequence to create a thermo-responsive bundlemer–polymer conjugate using atom transfer radical polymerization (ATRP) (illustrated in **Figure 1**). ATRP is a reversible deactivation radical polymerization (RDRP) strategy that utilizes a metal complex to mediate radical growth of polymer chains via the temporary transfer of the halide to the metal center[23, 24]. By controlling this transfer process, the chains can be polymerized simultaneously at nearly the same rate to produce a low dispersity product. There are several advantages of the grafting-from strategy, such as compatibility with a wide range of monomers, easy incorporation of the initiating species, and widely available and relatively inexpensive reagents. Additionally, the use of oxygen tolerant activator regeneration ATRP approaches, such as photoinduced ATRP (photoATRP)[25, 26], increases the applicability of ATRP for new applications, such as modification and stabilization of biomolecular materials[27-30].

As illustrated in **Figure 1B**, bundlemer grafts of poly 2-(dimethylamino)ethyl methacrylate (PDMAEMA) were synthesized via a grafting-from approach utilizing photo-ATRP. PDMAEMA is a water-soluble polymer that exhibits a lower critical solution temperature (LCST) and exhibits an extended-to-globular chain transition in responsive to a range of pH, ionic strength, and temperature conditions. While there is a growing body of work utilizing ATRP to graft polymer from proteins[31-36], to our knowledge, this is the first example to synthesize coiled-coil peptide–polymer conjugates using ATRP. The unique control over the macromolecular architecture is enabled by the use of solid phase peptide synthesis (SPPS)[37, 38], which allows for the exact placement of polymer initiating sites on the bundlemer exterior. Additionally, since ATRP is only initiated from specific sites on the bundlemer surface, challenges associated with separation of products from polymer biproducts are mitigated.

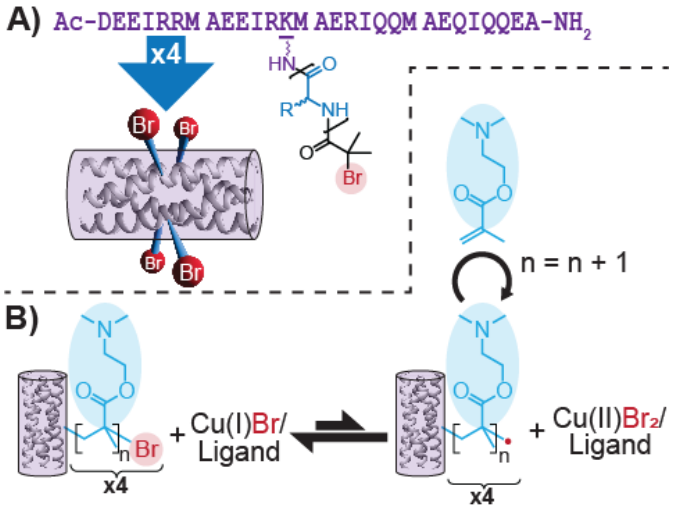

Figure 1: Schematic of bundlemer macroinitiator and subsequent grafting-from process. A) The bundlemer forming peptide amino acid sequence (denoted as BNDLE in the text) was synthesized with a halide terminated amino acid side chain to produce a tetramer ATRP macroinitiator. B) Bundlemer-DMAEMA grafts are formed via the introduction of the ATRP activator (i.e., Cu(I)Br) to produce a four-armed star-polymer architecture.

The bundle forming peptide used in this work was computationally designed *de novo* to form an antiparallel homotetrameric coiled coil. Generally, this peptide sequence follows a seven amino acid repeat pattern, where the first and fourth amino acids are alanine and isoleucine, which ultimately reside in the hydrophobic core of the bundlemer. The other amino acids are generally charged and were selected through an energy minimization routine outlined elsewhere[39, 40]. The coiled coil sequence chosen for this work (shown in **Figure 1A**) has been thoroughly investigated in other studies[41-43]. Specifically, the tetrameric oligomerization state of this computational designed peptide bundle has been confirmed in past works through biophysical techniques such as analytical ultracentrifugation[41] and small angle neutron scattering[43], demonstrating that tetramers still form even at high peptide concentrations or with added salts. All prior work on this bundle sequence suggests remarkable stability with respect to changes in temperature, pH, and ionic strength. This stability enables the bundlemer to be viewed as a well-defined nanoparticle with exact display of chemistry defined by the primary sequence of the peptide, which can be used as a substrate from which one can perform polymerization.

The peptide was synthesized via microwave SPPS, where one of the amino acids protecting groups was selectively removed and converted to an alkyl halide initiator. Specifically, a select lysine residue was added to the peptide, which contained an alloc protecting group that was selectively removed on resin using Pd to reveal a free amine (see **SI** for details). The amine is used as a branch point for subsequent modification and functionalization. While the middle of the sequence was selected (i.e., position 13), SPPS allows for easy placement of the alkyl halide at any amino acid position in the sequence that resides on the exterior of the coiled coil. Initially, the alkyl halide initiator was added directly onto the lysine using 2-bromoisobutyrl bromide (BIBB). The peptide was liberated from the resin by acid treatment, which additionally removes the remaining amino acid protecting groups. After purification via HPLC and lyophilization, the coiled coil formation and aqueous solution stability of the purified peptide was assessed via circular dichroism (CD).

The addition of the BIBB initiator negatively affected the thermal stability of the bundlemer as demonstrated by a decrease helicity, characterized by a maximum at 195 and minima at 208 and 222 nm in the CD spectra[44] (cf. **Figure 2A&B**), and decreased melting temperature (see **SI**). Specifically, the coiled coil undergoes an alpha helical to Gaussian coil transition at 57.3 ± 0.7 °C, as determined by the inflection in the sigmodal fit of the MRE versus temperature at 222nm. The direct addition of the BIBB initiator to the lysine side group replaces the ammonium charged side group for a relatively hydrophobic tertiary alkyl halide species. This substitution is speculated to negatively impact the assembly of the bundlemer, which is driven by hydrophobic interactions. While the bundlemer does maintain the coiled coil structure at room temperature, it is critical that the halide is readily accessible to participate in the photo-ATRP reaction. The synthetic route of the peptide readily enables further modification to the amine branch point to incorporate additional amino acid functionality.

Improved thermal stability of the coiled coil was achieved by the inclusion of a lysine within the branch, which preserved the original positive charge and disfavors interactions of the branch side group with the hydrophobic core of the coiled coil. This new bundlemer construct, which also included a glycine spacer in peptide the branch (i.e., BNDLE-KG-Br), resulted in similar CD spectra to the original bundlemer structure shown in **Figure 2C** (see SI for other glycine-glycine-Br branch point modifications). The peptide remained assembled even after being held for several hours at 90 °C. Given the stability and halide accessibility of the bundlemer, BNDLE-KG-Br was selected for all subsequent photo-ATRP reactions.

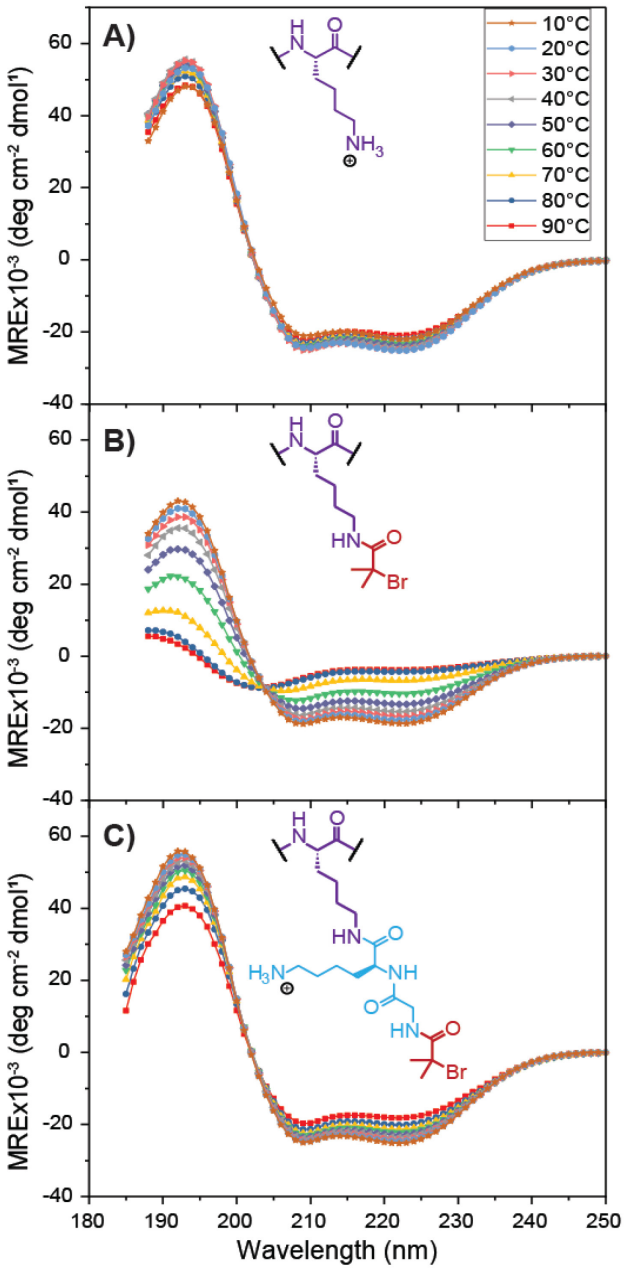

Figure 2: Sequence and CD spectra of BNDLE peptide with various side chains, measured over a range of temperatures to demonstrate its melting behavior and overall stability. A lysine is modified at position 13, resulting in CD spectra of A) the unmodified BNDLE, B) BNDLE-Br, and C) BNDLE-KG-Br.

A grafting-from ATRP approach was performed on the assembled bundlemer under aqueous conditions, which is similar to strategies in which protein–polymer conjugates are generated to maintain biological function[33, 45-47]. Within an aqueous medium, there are several mechanisms associated with a loss of the ATRP control, which leads to poor dispersity[27, 48-51]. Since bromide anions have a high degree of solubility in water, the reversible dissociation of the halide anion from the deactivating complex (i.e., $Cu(II)Br_2$ in **Figure 1B**), can lower the concentration of deactivating species and lead to uncontrolled polymerization. The addition

of halide salts suppresses this dissociation[25, 49, 50, 52]. Additionally, activator regenerative ATRP methods can be implemented to reduce the Cu(II) species back into the Cu(I) species, providing a competing reaction to Cu(II) dissociation. Here, photoATRP is utilized, which additionally enables the reaction to be more oxygen tolerate, to not require exogeneous radicals or high temperatures, and to be turned on and off (i.e., temporal control).[25, 28, 49, 52-54]

Suitable photoATRP reaction conditions were determined to polymerize 2-(dimethylamino)ethyl methacrylate from the bundlemer macroinitiator (see **SI** for details). Specifically, a solution was prepared consisting of 2.5 mM peptide, 2.5 mM copper (II) bromide, 10 mM tris(2-pyridylmethyl)amine (TPMA), and 75 mM sodium bromide. TPMA was used as a copper ligand and, as discussed, sodium bromide was added to suppress the dissociation of the Cu(II)Br_2 species. TPMA is a tripodal ligand that contains three picolyl substituents that help stabilize the copper in the deactivated state, thus leading to better polymerization control as compared to other ligands[27, 53-56]. Monomer amounts were added based on desired degree of polymerization (50, 100, or 200 eq.). The reaction was performed in D_2O to make subsequent NMR analysis easier. The samples were exposed to 365 nm light at 2.5 mW cm^{-2} intensity to initiate the polymerization via the photoreduction of Cu(II) to Cu(I). (See SI for details about optimization of the stoichiometric amounts of reagents). The polymerization proceeded for 2 hours, reaching approximately 50% conversion (determined by NMR, see SI). While conversions as high as 90% were attainable at reaction times of 4-5 hours, lower conversions led to a lower molecular weight dispersity.

The target molar masses of 4, 8, and 16 kDa were selected to be similar to the peptide, twice the peptide, and four times the peptide (i.e., similar to the bundlemer) molecular weight, respectively. Upon optimization of reaction times, polymer conjugates having number average molar masses of **3.7, 8.0, 15.6** kDa were obtained (determined by NMR, see **SI**). The control reaction, where a peptide without a halide initiating species was used (unmodified BNDLE), exhibited no polymerization. After polymerization, dialysis, and lyophilization, the bundlemer–polymer conjugate exhibited the higher-order coiled coil structure at room temperature, as determined by the minima at 208 and 222 nm in the CD spectroscopy (**Figure 3A**).

The polymer grafts were uniform in size as indicated by LC-MS and GPC. The purified peptide exhibited a single MW species (see **SI**). To isolate the molecular weight of the polymer product, the peptide was digested utilizing acid hydrolysis as described by Murata et al[33] (also see **SI** for details). GPC of the polymers, using 0.5wt% LiBr in dimethylacetamide as eluent and relative to a PMMA standard, reveals an estimated dispersity of around 1.3 (see **Figure 3B** and **SI**). This data indicates that the molecular weight can be rationally tuned by the stoichiometry of monomer and the reaction time, which ultimately influences the bundlemer–PDMAEMA star responsiveness.

At temperatures above the LCST, the PDMAEMA grafted chains become dehydrated and collapse into core-shell nanoparticles that precipitate to cause a phase change within the solution. The LCST of PDMAEMA is dependent on the temperature, pH, and ionic strength of the solution, as well as the molecular weight and concentration of the sample. Literature values for its LCST are well-documented[33, 57-60] and tend to be approximately 50 °C at pH 8.0. As the LCST of PDMAEMA decreases with increasing molecular weight, the phase change behavior can be controlled through molecular design of the conjugate, namely the original monomer–bundlemer stoichiometry and reaction time.

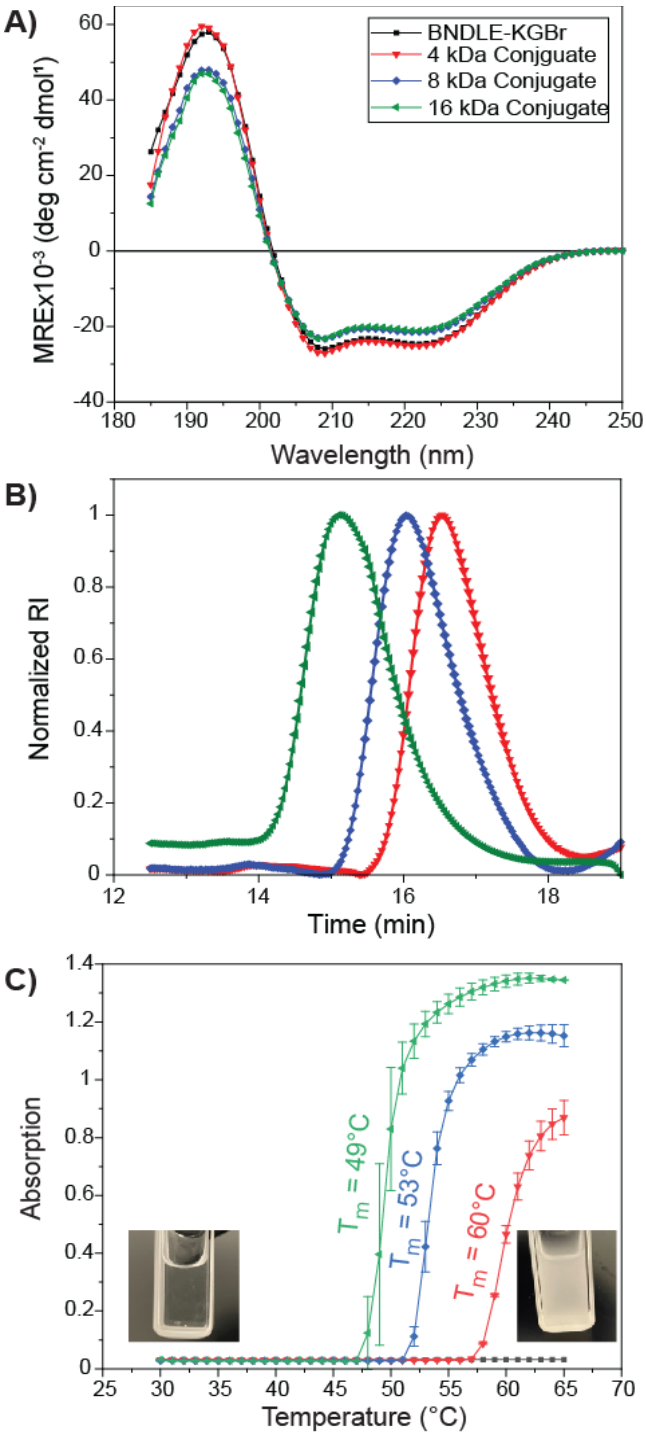

Figure 3: Characterization of the bundlemer-PDMAEMA conjugate. A) Room temperature CD spectra indicates the preservation of the coiled coil structure after polymerization. B) GPC of the cleaved polymer shows three distinct molecular weights having a narrow dispersity of approximately 1.3. C) Light absorption at 490 nm confirms the presence of a molecular weight dependent LCST for the bundlemer-polymer conjugates. The reported T_m are the inflection point in the data and the images are of a specimen before and after phase separation.

The LCST of each conjugate was determined by turbidity measurements by monitoring the absorbance of visible light as a function of temperature (see **Figure 3C**). As expected, increasing the polymer conjugate chain length decreased the LCST of the solution. This study demonstrates the conjugates' responsiveness to changes in solution conditions, and the ability to control the archi-

tectures behavior simply by increasing the solution temperature. Moreover, this transition is reversible, with a decrease in temperature causing the chains to expand and resolubilize in their aqueous environment, as shown in the **SI**. CD spectroscopy reveals that bundlemer-polymer conjugates remain assembled throughout this heating and cooling cycle (see **SI**). The reversible nature of the LCST can be exploited for future functionalities, such as purification, and demonstrates success in imparting specific features of the synthetic polymer onto the bundlemer material.

The ability to introduce synthetic polymer side chains rationally and robustly into peptidic materials allows for an immense expansion in peptide design possibilities. While we selected DMAEMA in this study, the versatility of this surface-initiated photo-ATRP approach to bundlemer-polymer grafts is readily extended to a wide range of aqueous vinyl free-radical polymerizing systems, such as oligoethylene glycol methacrylate and *N*-Isopropylacrylamide. The combination of being able to place ATRP initiators at precise positions along the bundlemer periphery with extensive chemical diversity and interaction provided by synthetic polymers, enables new materials discovery and fundamental exploration of polymer interactions on the surface of a nanoparticle. Although there has been much work in the incorporation of RDRP initiators into proteins, their incorporation into peptide-based nanomaterials is relatively unexplored. The ability to precisely incorporate ATRP initiators into coiled coil peptide bundles coupled with the development of more user-friendly forms of ATRP, should lead to a surge of studies using this method to modify bundles or other peptidic structures to contain synthetic moieties.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website.

Materials, methods, and experimental procedures, synthesis and characterization of peptide macro-initatior and resulting bundlemer star copolymers, circular dichroism data, and additional LCST measurements (PDF)

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Notes

The authors declare no competing financial interests.

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