DOI: 10.1002/marc.

Communication

Wavelength Selective Folding of Single Polymer Chains with Different Colors of Visible Light\textsuperscript{a}

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Photochemistry allows chemists to exert control over chemical reactions with spatiotemporal precision. Furthermore, light holds the potential to not only gate when and where, but also which reaction takes place. Herein, two photocycloaddition reactions, which are initiated by different colors of visible light, are utilized to control the intramolecular crosslinking of single polymer chains. Irradiation with blue light (\(\lambda_{\text{max}} = 470\) nm) triggers a [2+2] photocycloaddition inducing an initial intramolecular crosslinking reaction, whereas subsequent irradiation with violet light (\(\lambda_{\text{max}} = 415\) nm) induces a [4+4] photocycloaddition, fully compacting the dual photoreactive polymer into a single-chain nanoparticle (SCNP). Importantly, both crosslinked states are accessible under ultra-mild conditions requiring nothing but two different colors of visible light. The reported strategy of wavelength selective crosslinking degrees provides great potential to be translated into materials applications for the remote control of mechanical properties.

\textsuperscript{a} Supporting Information (bold) is available online from the Wiley Online Library or from the author.
\[ \lambda_{\text{max}} = 470 \text{ nm} \]

\[ \lambda_{\text{max}} = 415 \text{ nm} \]

*Elution Time / min*
1. Introduction

Since the work of Staudinger heralded the era of polymer chemistry,[1] macromolecular sciences flourished on all levels from fundamental research to everyday applications.[2] The resulting scientific progress has enabled control over chain lengths[3–7] and monomer sequences of synthetic polymers,[8–11] and even given rise to uniform sequence defined polymers.[12–14] Compared to the structures of nature’s precision macromolecules of peptides and nucleic acids, these scientific achievements took place at the level of primary structures.[15] While perfectly defined primary structures are prerequisite in nature, the actual function of proteins is associated with the precise folding of biopolymers into 3D architectures.[16] To elevate synthetic macromolecular architectures from primary structures to higher order 3D structures, research efforts – among other approaches[17] – began to crosslink and fold synthetic polymers intramolecularly into so called single-chain nanoparticles (SCNPs).[18,19] The required intramolecular crosslinking of single polymer chains can be carried out using a broad synthetic toolbox, spanning both covalent and non-covalent chemistry.[20]

In contrast to the controlled hierarchical folding observed in nature, the majority of folding chemistries applied in the field of SCNPs requires the addition of catalysts and reagents, or harsh reaction conditions (high temperature[21] or highly energetic irradiation[22,23]) to enable one distinct folding pathway. Hierarchical folding of single polymer chains transitioning through defined intermediates is scarce, even though it is critical to gain an in-depth understanding of the folding process and its impact on the resulting macromolecular architecture. To enable such a controlled stepwise folding of SCNPs, orthogonal chemistry allowing to trigger selectively one crosslinking reaction is key. Such an orthogonality was achieved using non-covalent recognition sites, as utilized in the pioneering work of Meijer and Palmans,[24,25] to stepwise fold[26] or unfold[27] single polymer chains. However, a major motivation for research in the field of SCNPs results from their application as catalytic nanoreactors,[25,28–30] inspired by the unmatched catalysis of enzymes. By replacing the non-
covalently driven folding with covalent bond formations, the final polymeric architectures of the SCNPs becomes more robust towards their chemical and physical environment including solvents and temperature.

Sparing the usually required addition of reagents or catalysts,[31,32] we report the first additive-free stepwise folding of single polymer chains based on covalent bond formations. Direct remote control over the stepwise folding is enabled solely by two different colors of light (Fig. 1).

2. Results and Discussion

2.1. Synthesis of the Dual Wavelength Responsive Polymer

To enable a light-gated stepwise crosslinking of single polymer chains, the [2+2] photocycloaddition of styrylpyrene[33,34] was combined with the [4+4] photocycloaddition of anthracene.[23] The chemical orthogonality of [2+2] and [4+4] photocycloaddition was recently demonstrated on the basis of a photoreactive polymer, which could be either intramolecularly cross-linked or intermolecularly ligated.[35] In contrast to previously reported anthracene monomers for SCNP folding,[23,35] the length of the linker was significantly increased to provide sufficient freedom for a selective intramolecular reaction of the anthracene containing monomer (M1, Fig. 1 violet). The anthracene containing M1 was copolymerized with a styrylpyrene containing methacrylate based monomer (M2, Fig. 1 blue)[36] and MMA using reversible addition–fragmentation chain transfer (RAFT) polymerization.[4] The obtained terpolymer (P1, $M_n = 6,200 \text{ g mol}^{-1}$, $D = 1.31$, Fig. S18) contained equal numbers of anthracene and styrylpyrene units (on average 5 photoreactive units each per chain, see SI section 2).
2.2. Wavelength Selective Activation of the Styrylpyrene Folding Points

To selectively induce the folding of the styrylpyrene units, a solution of P1 in THF (0.5 mg mL\(^{-1}\)) was irradiated with a blue LED (centered at \(\lambda = 470\) nm, emitting between \(\lambda = 440\) nm and \(\lambda = 530\) nm). Upon irradiation, the hydrodynamic volume of the polymer decreases as observed by a shift in the elution volume of the size exclusion chromatography (SEC) trace (Fig. 2 a and b).\(^{[37]}\) After close to 2 h of irradiation, the reduction of the hydrodynamic volume ceases and a plateau is reached. As the shift in elution volume only reveals relative information on the size of the solvated polymer coil, DOSY-NMR was employed to quantify the size reduction in the same solvent based on the hydrodynamic diameter (\(D_H\), SI section 3.3).\(^{[38]}\) After 3 h of irradiation with a blue LED, the \(D_H\) of P1 is reduced from 3.98±0.01 nm to 3.72±0.06 nm upon folding to SCNP1, which corresponds to a reduction of 7\%. While most publications in the field discuss the change of the radius or diameter of the SCNP,\(^{[38]}\) we would like to point out that the compaction of the polymer coil takes place on the 3D scale. The reported reduction in \(D_H\), thus results from a significant reduction of the hydrodynamic volume of 20\%, assuming a spherical conformation of the polymer coil. To combine the macromolecular insight of the single chain folding with
information on the molecular scale, the folding was additionally monitored using UV/vis spectroscopy. Congruent with the SEC results, the absorption maximum ($\lambda_{\text{max}}$) of the polymer at $\lambda = 383$ nm decreases upon blue LED irradiation and reaches a plateau after 2h. While an exact quantification of the number of crosslinks was not possible due the absorption overlap of the styrylpyrene and anthracene (Fig. S1), the formation of the cyclobutane containing [2+2] cycloaddition product can be observed by the two absorption maxima at $\lambda = 333$ and 350 nm which appear upon decoupling of the conjugated systems of pyrene and phenyl group.$^{[33-34,36,39]}$

The formation of the cyclobutane ring can further be observed via the $^1$H-NMR resonances arising between $\delta = 4.75$ and 6.0 ppm (Fig S2).$^{[33]}$ Summarizing the observations on the macromolecular and molecular level, after 2h of irradiation with blue light, the styrylpyrene units accessible for the intramolecular crosslinking reaction are consumed and the partially folded SCNPI is obtained.

![Figure 2.a) Normalized SEC traces of the single chain folding of P1 under blue light irradiation. b) Elution volume maximum of the SEC traces plotted against the irradiation time. c) UV/vis spectra of the single chain folding of P1 under blue light irradiation. d) Absorption maximum ($\lambda_{\text{max}}$) of P1 plotted against the irradiation time.](image)
2.3. Wavelength Selective Activation of the Anthracene Folding Points

To avoid highly energetic UV-light irradiation, which is prone to cause photo-damage to both synthetic and biological soft matter, research efforts have aimed at shifting the absorption of anthracene into the visible light regime through the extension of its conjugated system.\textsuperscript{[40,41]} However, we have recently observed that even the non-extended anthracene that is traditionally dimerized with highly energetic UV-light, can be efficiently brought to reaction with visible light up to 415 nm.\textsuperscript{[42]} In order to induce the second folding step as well with mild visible light, the partially folded \textbf{SCNP1} was irradiated in THF (0.25 mg mL\textsuperscript{-1}) with a violet LED (centered at $\lambda = 415$ nm, emitting between $\lambda = 390$ nm and $\lambda = 440$ nm). The hydrodynamic volume of \textbf{SCNP1} underwent a further decrease as observed by a shift in the SEC trace, affording the fully collapsed \textbf{SCNP2} after the second folding step (Fig. 3 a, b). In the DOSY NMR, the compaction after 1h was attributed to a reduction in the $D_H$ from 3.72±0.06 nm to 3.48±0.06 nm, a further reduction of 7%. The reaction of the anthracene units can be monitored by the decrease of the absorption maximum around 380 nm in the UV/vis spectra and the shift of the proton in 10-position of the anthracene ring.\textsuperscript{[43]} According to the decrease in the UV/vis spectrum, the majority of the anthracene units are reacted within 10 min of irradiation (Fig. 3 c, d). By switching the irradiation wavelengths from blue to violet light, the partially folded \textbf{SCNP1} can thus be efficiently folded into the fully compacted \textbf{SCNP2}. As both photoreactive units have the same abundance within \textbf{P1} according to $^1$H-NMR (Fig. S17), both separate folding steps can induce the same number of crosslinks and leading to a comparable reduction in hydrodynamic volume according to DOSY NMR.
2.4. One Step Folding of P1

In order to compare the stepwise folding with a parallel folding, P1 was irradiated directly with a violet LED, triggering both photoreactions and yielding SCNP2’. The concomitant cycloadditions of anthracene and styrylpyrene induced a comparable shift in the SEC elution volume and decrease of UV/vis absorption within 1 h of irradiation (Fig. 4). The UV/vis spectra of the very early stages (< 30 s) display the same ratio of pyrene absorption bands at $\lambda = 333$ and 350 nm to the anthracene band at $\lambda = 380$ nm as the 470 nm styrylpyrene folding (compare Fig. 3c and 4c, Fig. S3). In the later stages, only the anthracene band decreases, whereas the pyrene bands remain as observed for the sequential anthracene folding at 415 nm. While both reactions are initiated under violet light, the styrylpyrene reaction appears to be faster and thus even upon parallel excitation, the earlier stages of the folding are dominated by the styrylpyrene dimerization and the later ones by the anthracene. The final products of both folding pathways
display matching UV/vis and $^1$H-NMR spectra (Fig. S4 & S5), indicating that the product of stepwise and parallel folding contain comparable numbers of close to 5 crosslinking points per chain on average. However, according to DOSY NMR, the $D_H$ of SCNP2’ of 3.62±0.05 nm appears to be slightly larger compared to the stepwise folded SCNP2 ($D_H = 3.48±0.06$ nm). As the timescale of the photoreactions can readily be adjusted through the light intensity and wavelength under entirely identical conditions in closed systems, the developed platform will allow to elucidate in future studies, whether such differences in overall reduction of $D_H$ (13% in SCNP2 vs. 9.0% in SCNP2’) result from the overall faster folding in SCNP2’ or from the loss of the strict separation of both crosslinking steps.

Figure 4. a) Normalized SEC traces of the single chain folding of P1 under violet light yielding SCNP2’. b) Elution volume maximum of the SEC traces plotted against irradiation time. c) UV/vis spectra of the single chain folding of P1 under violet light irradiation. d) Absorption maximum ($\lambda_{\text{max}}$) of P1 plotted against the irradiation time.

3. Experimental Section
Synthetic procedures, additional experimental data and details can be found in the supporting information.

4. Conclusions

A dual photoreactive polymer was developed, which entails equal numbers of anthracene and styrylpyrene units. Upon irradiation with blue light, the styrylpyrene units were selectively dimerized yielding the partially cross-linked SCNP1. By switching the irradiation wavelength to violet light, the dimerization of the unreacted anthracene units was induced to fully cross-linked the single polymer chain into SCNP2. The reported polymer enables facile access to two distinct states of single chain folding – depending exclusively on the color of visible light. Alternatively, direct irradiation of the linear precursor polymer with violet light induces both photoreactions, whereas this folding also appears to be dominated at early stages by the styrylpyrene reaction and later by the anthracene – a result from an expected higher reactivity of the styrylpyrene units under the given irradiation conditions. Such an intramolecular crosslinking that progresses via distinct stages mimics the folding of proteins in nature, which are generally folded in modules and proceed through distinct highly populated intermediates. The developed strategy of remote-control crosslinking degrees can be readily expanded from single polymer chains towards crosslinking densities within materials applications. At stake are materials, whose mechanical properties can be tailored, additive free, and exclusively through the color of light.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

C.B.-K. acknowledges continued support by the Queensland University of Technology (QUT) and the Australian Research Council (ARC) in the form of a Laureate Fellowship enabling his
photochemical research program. C.B-K. additionally acknowledges support by the Karlsruhe Institute of Technology in the context of the STN program of the Helmholtz association. This work was enabled by use of the Central Analytical Research Facility hosted by the Institute for Future Environments at QUT.

Keywords: Single Chain Nanoparticle (SCNP), Photochemistry, Photoligation, Single Chain Folding, Wavelength Selective.

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Remote controlled folding of single polymer chains arises from the combination of two photocycloaddition reactions. Using blue and violet light, a dual photoreactive polymer chain can be intramolecularly cross-linked in two individual steps – additive free and only dependent on the color of light.
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