Non-uniform Photoinduced Unfolding of Supramolecular Polymers Leading to Topological Block Nanofibers

Keigo Tashiro, Kosuke Katayama, Kenta Tamaki, Luca Pesce, Nobutaka Shimizu, Hideaki Takagi, Rie Haruki, Martin J. Hollamby, Giovanni M. Pavan,* and Shiki Yagai*

Abstract: Synthesis of one-dimensional nanofibers with distinct topological (higher-order structural) domains in the same main chain is one of the challenging topics in modern supramolecular polymer chemistry. Non-uniform structural transformation of supramolecular polymer chains by external stimuli may enable preparation of such nanofibers. To demonstrate feasibility of this post-polymerization strategy, we prepared a photoresponsive helically folded supramolecular polymers from a barbiturate monomer containing an azobenzene-embedded rigid π-conjugated scaffold. In contrast to previous helically folded supramolecular polymers composed of a more flexible azobenzene monomer, UV-light induced unfolding of the newly prepared helically folded supramolecular polymers occurred nonuniformly, affording topological block copolymers consisting of folded and unfolded domains. The formation of such blocky copolymers indicates that the photoinduced unfolding of the helically folded structures initiates from relatively flexible parts such as termini or defects. Spontaneous refolding of the unfolded domains was observed after visible-light irradiation followed by aging to restore fully folded structures.

Introduction

Modern preparation methods of polymer has enabled us to synthesize a variety of polymers with unique primary and higher order structures.[1] Such well-designed synthetic polymers are useful not only as functional soft materials wherein their collective behaviors are important, but also as more single-chain nanomaterials that can function like biomacromolecules.[2] Especially block copolymerization techniques allow the synthesis of polymers with distinct structural domains (topologies) in a main chain like proteins, which is crucial to develop polymers as discrete nanomaterials.[3] For supramolecular polymers (SPs), an emerging noncovalent counterparts of polymers,[4] the construction of such “topological” block or “topologically” blocky supramolecular copolymers is particularly challenging because of inherently dynamic nature in monomer binding. In other words, the monomers affording different higher-order structures generally have different molecular structures, and accordingly it may be difficult to keep them connected in a thermodynamically stable state through non-covalent interactions (hetero-recognition). In fact, while several groups have recently reported elegant examples of block or blocky SPs based on thermodynamic[5] or kinetic approaches,[6] all of them have one-dimensionally extended structures. As an exceptional example, we recently reported block supramolecular copolymers consisting of helically folded extended domains by kinetically controlled gradient supramolecular copolymerization of the two molecules with similar chemical structures but affording SPs with distinct higher order structures.[7] However, bottom-up design of such topological block SPs remains a formidable challenge from the viewpoint of the above compatibility between higher-order structures and hetero-recognition, which will limit monomer design to those leading to analogous one-dimensional structures.

[1] K. Tashiro, Prof. S. Yagai
Institute for Global Prominent Research (IGPR), Chiba University
1–33 Yayoi-cho, Inage-ku, Chiba 263–8522 (Japan)
E-mail: yagai@faculty.chiba-u.jp
K. Katayama, K. Tamaki
Division of Advanced Science and Engineering, Graduate School of Science and Engineering, Chiba University
1–33 Yayoi-cho, Inage-ku, Chiba 263–8522 (Japan)
L. Pesce, Prof. G. M. Pavan
Department of Innovative Technologies, University of Applied Sciences and Arts of Southern Switzerland
Via La Santa 1, 6962 Lugano-Viganello (Switzerland)
N. Shimizu, H. Takagi, R. Haruki
Photon Factory, Institute of Materials Structure Science, High Energy Accelerator Research Organization
Tsukuba 305–0801 (Japan)
M. J. Hollamby
School of Physical and Geographical Sciences, Keele University
Keele, Staffordshire ST55BG (UK)

Prof. G. M. Pavan
Department of Applied Science and Technology, Politecnico di Torino
Corso Duca degli Abruzzi 24, 10129 Torino (Italy)
E-mail: giovanni.pavan@polito.it
Prof. S. Yagai
Department of Applied Chemistry and Biotechnology, Graduate School of Engineering, Chiba University
1–33 Yayoi-cho, Inage-ku, Chiba 263–8522 (Japan)

Supporting information and the ORCID identification number(s) for the author(s) of this article can be found under: https://doi.org/10.1002/anie.202110224. The computational material (i.e. simulation and data analysis) has been published on Zenodo. URL: https://zenodo.org/record/5025104

© 2021 The Authors. Angewandte Chemie International Edition published by Wiley-VCH GmbH. This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.
We envisage that post-polymerization structural transformation,\cite{8} as has already been applied for covalent polymers, would be another strategy to prepare topological block SPs. As a basis that can verify this idea, we invoke the photoresponsive of monomer \(1\) we have reported in 2017 (Figure 1a).\cite{9} This barbiturated azobenzene monomer forms helically folded SPs (SP\(_{\text{fold}}\)) in nonpolar media through the formation of six-membered hydrogen-bonded rosettes (Figure 1b). The helically folded structure is a result of continuous generation of intrinsic curvature upon stacking of the rosettes\cite{10} with translational and rotational displacements.\cite{11} One of the unique features of the SP\(_{\text{fold}}\) of \(1\) is unfoldability to linearly extended structures by UV-light, which is due to the perturbation of the intrinsic curvature by the generation of sterically demanding cis-azobenzene units.\cite{12} Importantly, our AFM study showed that the photoinduced unfolding proceeded uniformly throughout the entire fiber, providing unfolded SPs with curvature (SP\(_{\text{unfo}}\)) as intermediate structures (Figure 1c). The uniform unfolding suggests that the SP fiber of \(1\) is flexible enough to allow deformation of the curvature by cis-azobenzene units even in tightly folded internal domains.

Based on the above mechanism, we expected that SP\(_{\text{fold}}\) consisting of a more rigid SP fiber allows “non-uniform” photoinduced unfolding, and can provide blocky structures as an intermediate state (Figure 1d). We thus designed and synthesized new monomer \(2\) in which azobenzene unit was embedded in a rigid \(\pi\)-conjugated backbone (Figure 1a). As we will show in this paper, SP\(_{\text{fold}}\) of \(2\) shows significant resistance to the photoinduced structural deformation at ambient temperature. At high temperature, however, photo-induced unfolding proceeds non-uniformly, leading to topological block SPs consisting of folded and unfolded domains as intermediate structures. Our molecular dynamics simulations demonstrate that photoisomerization of azobenzene units occurred throughout entire main chains, while high inner rigidity led the cooperative unfolding.

**Results and Discussion**

Monomer \(2\) was synthesized according to the procedure described in the Supporting Information, and characterized by \(^1\)H- and \(^13\)C-NMR spectroscopies, and APCI-MS spectrometry. \(^1\)H NMR demonstrated that the as-synthesized molecule \(2\) was >99.9\% trans-isomer (Figure S4a). Upon cooling a hot MCH solution of monomeric \(2\) (\(c = 10\) \(\mu\)M) from 373 to 308 K and subsequently heating at a rate of 1.0 K min\(^{-1}\), a change of an absorption shoulder at 465 nm was reversibly observed, indicating aggregation at low temperatures (Figure 2a). When the temperature-dependence of this new band (\(\lambda = 465\) nm) was monitored as \(\alpha_{\text{agg}}\), cooperative (nucleation-elongation) supramolecular polymerization\cite{13} was observed for both cooling and heating curves but with different critical temperatures (\(T_c/\) and \(T_e/\), Figure 2b). A gradual increase of \(\alpha_{\text{agg}}\) above \(T_c/\) upon cooling is probably related to pre-nucleation including conformational change of the \(\pi\)-conjugated system such as planarization.\cite{14} The thermal hysteresis in our system is mainly caused by the formation of diverse hydrogen bonding species during

---

**Figure 1.** a) Molecular structures 1 and 2. b) Formation mechanism of the SP\(_{\text{fold}}\). c,d) Photo-induced unfolding processes of SP\(_{\text{fold}}\) composed of 1 (c) and 2 (d).
These are not the final page numbers!
In line with this, AFM visualized that the SP\textsubscript{unfo} lack any trace of helically folded domains. Importantly, unlike to \textit{1},\textsuperscript{[9]} unfolding up to linearly extended fibers lacking intrinsic curvature was not observed for \textit{2} even after further prolonged UV-light irradiation. AFM analysis of the SP\textsubscript{unfo} revealed the presence of curvature with $r_{av}$ of 10.6 ± 0.3 nm, which is almost comparable to that of SP\textsubscript{fold} (Figure S7b). This result also reflects the rigid molecular structure of \textit{2}, by which the curvature of SPs becomes persistent to the internal perturbation induced by the photoisomerization of the azobenzene unit.

It is worthy to note that the above transformation from topological block SPs to SP\textsubscript{unfo} proceeded while keeping the constant amount of cis-isomer (21%) as is evident from no absorption change upon UV-light irradiation at 323 K (Figure 3a). Namely, the \textit{trans}—\textit{cis} photoisomerization of the azobenzene units drives the unfolding of the main chain, but the generated \textit{cis}-isomers are smoothly reconverted thermally to the \textit{trans}-isomers.

Thermodynamic parameters of the SP\textsubscript{fold} and the photo-generated SP\textsubscript{unfo} were estimated from the thermal dissociation experiments using UV–Vis spectroscopy in order to gain insight of the impact of temperature on the unfolding. The non-sigmoidal thermal dissociation curves of SP\textsubscript{fold} and SP\textsubscript{unfo} composed of \textit{2}, in which all azobenzenes were \textit{trans}-isomers, recorded at several concentrations ($c = 10, 15, 20,$ and 25 $\mu\text{M}$), could be fitted with a nucleation-elongation model (Figure S12),\textsuperscript{[9]} and the resulting elongation temperature ($T_{e}$)
were used to make the modified van’t Hoff plot (Figure S13).[20] The changes of standard enthalpy ($\Delta H^\circ$), entropy ($\Delta S^\circ$), and Gibbs free energy ($\Delta G^\circ$) obtained from the plot were summarized in Table 1. Both $\Delta H^\circ$ and $\Delta S^\circ$ of SP_unfold are significantly smaller than those of SP_fold, which is ascribable to the stabilization of helically folded structures by inter-loop van der Waals interactions between alkyl chains.[18a,21] The difference of $\Delta G^\circ$ values ($= \Delta G^\circ (\text{SP_unfold}) - \Delta G^\circ (\text{SP_fold})$) were 10.3 and 2.7 kJ mol$^{-1}$ at 308 and 323 K, respectively. Such large change of $\Delta G^\circ$ against temperature is because of entropic effect. As described above, the no unfolding proceeded under UV-light irradiation at 308 K. These results indicate that the entropic effect is also important factor although main driving force of the unfolding is the large structural change of rosettes. Indeed, the photo-unfolded SP_unfold spontaneously refolded into SP_fold upon aging at 308 K for 18 h, supporting the drastic impact of entropic effect (Figure S14).

To obtain a molecular-level insight into the observed inhomogeneous photoinduced unfolding of the SP fiber of 2, we conducted high-resolution molecular simulations for polymeric rosette stacks of 1 and 2 in native unperturbed conditions as well as upon transition of the excited azobenzene units. Recently, molecular models of a photo-responsive supramolecular tubule allowed to observe that the isomerization of the azobenzene units contained in the self-assembling monomers under UV-light irradiation occurs and proceeds at defective sites in the tubule structure.[106] Less ordered domains and defects, which are in principle unavoidably present in soft self-assembled materials such as SPs, constitute spots where the transitions of excited units are more probable to occur.[116] The cascade of isomerization that tends to localize in proximity of such less defined domains in the assemblies may then provoke a non-uniform isomerization of excited groups over time, and a cooperative response by the assembly structure.

Here we used fully atomistic molecular dynamics (MD) simulations to obtain insights on the mechanism by which the azobenzene isomerization occurs in the SPs. First, we built atomistic models of SP fibers of 1 (SP1) and 2 (SP2), composed of 192 monomers initially arranged in 32 perfectly pre-stacked rosettes. These initially extended fiber models have been then equilibrated via 1 μs of MD simulation in explicit MCH solvent at 297 K (see Supporting Information for details on the models and simulations). From these MD runs, we obtained equilibrated structures of SP1 and SP2 (Figures 4a,b). While the two fibers start from the same configuration, SP2 was observed to be slightly longer and more persistent at the MD equilibrium than SP1, consistent with a higher level of internal rigidity of SP2. We analyzed the environment that surrounds the azobenzene units in the two ordered-domain fibers represented by our models. We calculated the distributions of the azobenzene units in the two equilibrated fiber sections in terms of contacts between them (only azobenzene units) and contacts with the entire surrounding monomers in SP1 and SP2 (Figure 4c,d). The average contact values for the azobenzene units in the two fibers are identified by the black lines in the distribution plots. The slightly lower average values and the broader distribution obtained for SP1 compared to SP2 suggests a slightly tighter and more ordered packing of the azobenzene units in SP2 compared to SP1. This can be related to the higher atomic density surrounding the azobenzene units, which is related to their shorter radial distance from the center of the fiber in SP2. For such geometrical reasons, in SP1, this provokes a slightly reduced stacking between the azobenzene units compared to SP2.

Previous studies demonstrated that the degrees of molecular crowding in the environment surrounding the azobenzene units can have an important effect on how the excited chromophores isomerize in the stack: It can occur randomly if the crowding effect is reduced whereas it can occur in spatially localized manner if the crowding effect is dominant.[116] To obtain indications on how the isomerization most likely accumulate in the stack, we used previously validated atomistic model of excited azobenzenes.[22] We investigated the isomerization of the azobenzene units that are extreme in the obtained azobenzene crowding distributions—i.e., those azobenzene groups which are more loosely (blue points, slightly lower contacts than the average) or more tightly (green points in the distributions, slightly higher contacts than the average) packed in the assembled fibers. The MD simulations showed that the azobenzene units having the highest number of contacts, if excited, have a characteristic isomerization timescale that is just ~5 times slower than that of the azobenzene units with the lowest number of contacts (Figures 4e,f). The Poisson distributions related to the azobenzene units with higher contacts (Figure 4e,f: solid curves) are translated on the right (slower kinetics) compared to those of the loosely packed azobenzene units (dashed curves). Black lines in Figures 4e,f identify the characteristic transition times calculated by the cumulative Poisson fits of azobenzene transitions occurring in such molecular models of ordered helical sections of the SPs. In all cases, the MD simulations provided characteristic transition timescales for excited azobenzene units below the nanosecond scale. In this model of an ordered section of SP1, the characteristic isomerization timescale for the excited azobenzene units was found to be in the order of picoseconds, very similar to that expected for free/disassembled azobenzenes in solution.[23] This suggests that in SP1, the eventual presence of defects or less ordered domains than the ones present in such ordered models (likely present in the real systems in such soft SP fibers), will have little impact on how the transitions accumulate in the assembly: i.e., in all cases the isomerization would occur randomly along SP1. This is consistent with the rather uniform reorganization of SP1 upon light excitation seen in the experiments. On the other hand, the isomerization in SP2 occurred with a slower characteristic timescale (i.e., they are less probable) within the ordered section model (Figure 4 e vs. Figure 4 f). This suggests that, in SP2, defects or

| Table 1: Changes of standard enthalpy ($\Delta H^\circ$), standard entropy ($\Delta S^\circ$), and Gibbs free energy ($\Delta G^\circ$) of SP_unfold and SP_fold of 2. |
|----------------|----------------|----------------|----------------|
|                | $\Delta H^\circ$ [kJ mol$^{-1}$] | $\Delta S^\circ$ [J mol$^{-1}$ K$^{-1}$] | $\Delta G^\circ$ at 323 K [kJ mol$^{-1}$] | $\Delta G^\circ$ at 308 K [kJ mol$^{-1}$] |
| SP_unfold     | $-114.8$       | $-222.8$       | $-36.1$        | $-46.2$        |
| SP_fold       | $-53.6$        | $-57.6$        | $-33.3$        | $-35.9$        |
less ordered domains where the azobenzene units are less tightly packed than in these ordered SP sections (and where the isomerization may occur faster), constitute spots along these assemblies where the isomerization are more favored to accumulate. This is consistent with the cooperative and non-uniform reorganization of SP2 upon light excitation seen in the experiments. Finally, starting from a preequilibrated structures of the SP fibers (Figure 4a,b), we also carried out MD simulations where 20% (experimentally observed value at PSS) of the trans-azobenzene units in SP1 and SP2 were converted into cis-isomers (Figure 4g,h: cis isomers in green). Such 20% cis-SP models were then simulated via MD. Analysis of the global SP structures during the simulations demonstrates that, following to the azobenzene isomerization, the fiber length of SP1 changed over time (Figure 4i: cyan vs. blue curves). Conversely, the structure of SP2 remained substantially unchanged (Figure 4j: orange vs. red curves). This observation reflects very well the higher rigidity of SP2, and provides a rational explanation for the experimentally observed non-uniform and cooperative UV-induced unfolding of the SP\textsubscript{add} of 2, which afforded topological block SPs.

**Conclusion**

We have demonstrated a novel strategy to synthesize topological block supramolecular polymers consisting of two distinct higher-order structures, helically folded and unfolded domains. Our strategy is based on post-polymerization photoinduced unfolding of helically folded supramolecular nanofibers consisting of six-membered hydrogen-bonded rosettes of barbiturates with azobenzene photoswitches. To achieve non-uniform unfolding of helically folded structures upon isomerization of internal azobenzene units, the previously designed flexible monomer structure was modified to a more robust structure, by which supramolecular nanofibers became more curvature-persistent toward azobenzene isomerization. This monomer-based improvement in the robustness of supramolecular polymer chains has been reproduced by MD simulations of fibers consisting of the previous flexible and the current robust monomers upon isomerization of their azobenzene units. The improved robustness of supramolecular polymer chains realized non-uniform unfolding of helically folded structures into fully unfolded structures through intermediate topological block structures. Owing to the improved curvature-persistence, spontaneous re-folding of the fully unfolded structures has been observed unlike the
These are not the final page numbers!
Prabhu, S. Datta, T. Aizawa, S. Yagai, Chem. Eur. J. 2020, 26, 8997 – 9004.
[16] a) D. Bochicchio, S. Kwangmettatam, T. Kudernac, G. M. Pavan, ACS Nano 2019, 13, 4322 – 4334; b) D. Bochicchio, M. Salvalaglio, G. M. Pavan, Nat. Commun. 2017, 8, 147; c) L. Albertazzi, S. van der Zwaag, C. M. A. Leenders, R. Fitzner, R. W. van der Hofstad, E. W. Meijer, Science 2014, 344, 491 – 495.
[17] a) T. Fukushima, K. Tamaki, A. Isobe, T. Hirose, N. Shimizu, H. Takagi, R. Haruki, S. Adachi, M. J. Hollamby, S. Yagai, J. Am. Chem. Soc. 2021, 143, 5845 – 5854; b) K. Tamaki, T. Aizawa, S. Yagai, Chem. Commun. 2021, 57, 4779 – 4782.
[18] a) K. Aratsu, R. Takeya, B. R. Pauw, M. J. Hollamby, Y. Kitamoto, N. Shimizu, H. Takagi, R. Haruki, S. Adachi, S. Yagai, Nat. Commun. 2020, 11, 1623; b) S. Datta, Y. Kato, S. Higashiharaguchi, K. Aratsu, A. Isobe, T. Saito, D. D. Prabhu, Y. Kitamoto, M. J. Hollamby, A. J. Smith, R. Dalgliesh, N. Mahmoudi, L. Pesce, C. Perego, G. M. Pavan, S. Yagai, Nature 2020, 583, 400 – 405; c) M. J. Hollamby, K. Aratsu, B. R. Pauw, S. E. Rogers, A. J. Smith, M. Yamauchi, X. Lin, S. Yagai, Angew. Chem. Int. Ed. 2016, 55, 9890 – 9893; Angew. Chem. 2016, 128, 10044 – 10047.
[19] M. M. J. Smulders, M. M. L. Nieuwenhuizen, T. F. A. de Greef, P. van der Schoot, A. P. H. J. Schenning, E. W. Meijer, Chem. Eur. J. 2010, 16, 362 – 367.
[20] P. van der Schoot, Theory of Supramolecular Polymerization. In Supramolecular Polymers, 2nd Edition (Ed: A. Ciferri), Taylor & Francis, London, U. K., 2005, pp. 77 – 106.
[21] D. D. Prabhu, K. Aratsu, Y. Kitamoto, H. Ohchi, T. Ohba, M. J. Hollamby, N. Shimizu, H. Takagi, R. Haruki, S. Adachi, S. Yagai, Sci. Adv. 2018, 4, eaat8466.
[22] a) L. Pesce, C. Perego, A. Grommet, R. Klajn, G. M. Pavan, J. Am. Chem. Soc. 2020, 142, 9792 – 9802; b) J. W. Fredy, A. Mendez-Ardoy, S. Kwangmettatam, D. Bochicchio, B. Matt, M. C. A. Stuart, J. Huskens, N. Katsonis, G. M. Pavan, T. Kudernac, Proc. Natl. Acad. Sci. USA 2017, 114, 11850 – 11855.

Manuscript received: July 30, 2021
Revised manuscript received: September 1, 2021
Accepted manuscript online: October 8, 2021
Version of record online: ▶ ▶ ▶ ▶ ▶ ▶
Helically folded supramolecular polymers with a high internal rigidity undergo non-uniform unfolding upon UV-irradiation, affording topological block nanofibers consisting of two distinct domains with helically folded and unfolded structures.