JOURNAL OF THE AMERICAN CHEMICAL SOCIETY

Subscriber access provided by TULANE UNIVERSITY

Communication

Phototriggered dynamic and biomimetic growth of chlorosomal self-aggregates

Shogo Matsubara, and Hitoshi Tamiaki

J. Am. Chem. Soc., **Just Accepted Manuscript** • DOI: 10.1021/jacs.8b13056 • Publication Date (Web): 09 Jan 2019

Downloaded from http://pubs.acs.org on January 9, 2019

Just Accepted

“Just Accepted” manuscripts have been peer-reviewed and accepted for publication. They are posted online prior to technical editing, formatting for publication and author proofing. The American Chemical Society provides “Just Accepted” as a service to the research community to expedite the dissemination of scientific material as soon as possible after acceptance. “Just Accepted” manuscripts appear in full in PDF format accompanied by an HTML abstract. “Just Accepted” manuscripts have been fully peer reviewed, but should not be considered the official version of record. They are citable by the Digital Object Identifier (DOI®). “Just Accepted” is an optional service offered to authors. Therefore, the “Just Accepted” Web site may not include all articles that will be published in the journal. After a manuscript is technically edited and formatted, it will be removed from the “Just Accepted” Web site and published as an ASAP article. Note that technical editing may introduce minor changes to the manuscript text and/or graphics which could affect content, and all legal disclaimers and ethical guidelines that apply to the journal pertain. ACS cannot be held responsible for errors or consequences arising from the use of information contained in these “Just Accepted” manuscripts.

ACS Publications

is published by the American Chemical Society. 1155 Sixteenth Street N.W., Washington, DC 20036

Published by American Chemical Society. Copyright © American Chemical Society. However, no copyright claim is made to original U.S. Government works, or works produced by employees of any Commonwealth realm Crown government in the course of their duties.

Phototriggered dynamic and biomimetic growth of chlorosomal self-aggregates

Shogo Matsubara, and Hitoshi Tamiaki*

Graduate School of Life Sciences, Ritsumeikan University, Kusatsu, Shiga 525-8577, Japan

Supporting Information Placeholder

ABSTRACT: Supramolecular polymerizations mimicking native systems, which are step-by-step construction to form self-aggregates, were recently developed. However, a general system to successively and spontaneously form self-aggregates from monomeric species remains challenging. Here we report a photoinduced supramolecular polymerization system as a biomimetic formation of chlorophyll aggregates which are the main light-harvesting antennas in photosynthetic green bacteria, called "chlorosomes". In this system, inert chlorophyll derivatives were UV-irradiated to gradually produce active species through deprotection. Such active monomers spontaneously assembled to form fiber-like chlorosomal self-aggregates in a similar manner as a dynamic growth of natural chlorosomal self-aggregates. The study would be useful for elucidation of the formation process of the chlorosomal aggregates and construction of other supramolecular structures in nature.

Supramolecular polymerization has been investigated in the last decade for the step-by-step construction of biological systems.[1–5] Most of the supramolecular aggregates were quickly prepared by dispersing a large amount of self-assembling species (active species) in poor solvents at controlled temperature and concentration. Particularly, the preparation of aggregate nuclei known as seeds is important for spontaneous supramolecular polymerization.[1,3,4] Supramolecular structures in nature are gradually formed by assembling a small amount of spatiotemporally biosynthesized active species in which seeds are produced in limited amounts.

Here, we report a spatiotemporally controlled supramolecular polymerization system whose conditions are similar to those used in biosystem preparation; that is based on the protection of active species and photoinduced deprotection of the inactive species. Temporally inactive species prepared by photoremovable protection of active species were illuminated with light to generate self-assembling species (photodeprotection) to form its self-aggregates, where an active monomer was gradually supplied as in native chlorosome systems. In this study, we focused on constructing a special light-harvesting antenna in a green photosynthetic bacterium called "chlorosome"[6–13] and mimicked its natural biological supramolecular system.

A single chlorosome is constructed from *J*-aggregates of bacteriochlorophyll-*c, d,* or *e* (BChl-*c, d,* or *e*) molecules (Fig. 1a, left),[7,9,12] and the aggregates are tube-like (a 5–30 nm diameter) or lamellar structures.[10,11,13] Chlorosomal self-aggregates were able to be prepared *in vitro* by self-assembly of BChl-*c, d,* or *e* isolated from natural chlorosomes and their model molecules such as **Zn-HM** (Fig. 1a, middle).[8,14–18] However, directly forming a tube-like structure as in a natural chlorosome is difficult because of the spontaneous formation of amorphous particle-like aggregates. The predominant preparation of tube-like chlorosomal aggregates was required to stand aggregates in a solution for over 1 week as described in previous reports.[14–18] These studies indicated that the conventional method as shown in Fig. 1b preferentially induced the formation of kinetically trapped metastable aggregates, whereas tube-like aggregates are thermodynamically stable.

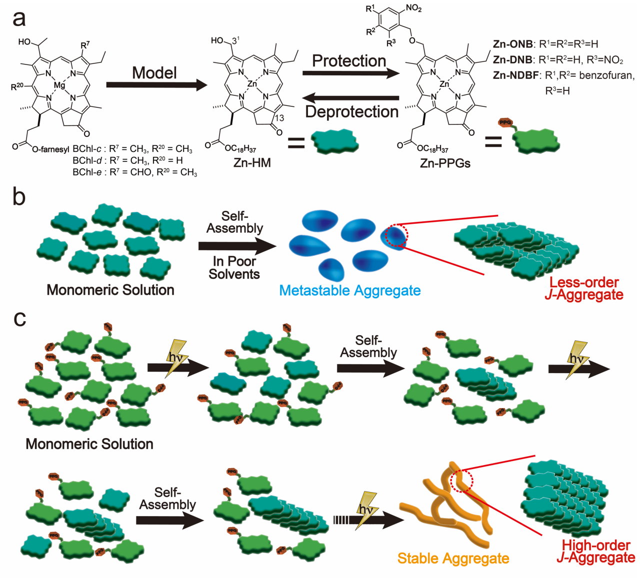

Figure 1. Schematic chlorophyll self-assembly. (a) Chemical structures of natural BChl-*c*, *d*, and *e* molecules (left), zinc Chl derivative possessing the 3-hydroxymethyl group **Zn-HM** as a synthetic model (middle), and its protected compounds with a photoremovable group **Zn-PPGs** (right). (b) Self-assembly of synthetic **Zn-HM** to form metastable aggregates. (c) Photoinduced deprotection of **Zn-PPGs** and successive self-assembly of **Zn-HM** to directly form stable nanofibers with a high-order *J*-aggregate.

To directly construct tube-like chlorosomal aggregates, we propose a biomimetic system that gradually supply self-assembling chlorophyll (Chl) molecules (Fig. 1c) by photolysis of Chl derivatives (Fig. 1a, right) possessing a photoremovable protecting group. Photoremovable protection has been utilized in various systems including fluorescence emission imaging and drug delivery systems.[19–22] Because hydrogen bonding between a hydroxy group at the 3^1-position and a keto carbonyl group at the 13-position as well as coordination of the hydroxy group with a central metal are essential for construction of *in vivo* and *in vitro* chlorosomal *J*-aggregates.[8,9,11,13] Chl derivatives protected at the 3^1-hydroxy group **Zn-PPGs** (**Zn-ONB**, **Zn-DNB**, and **Zn-NDBF**) cannot assemble to form their chlorosomal self-aggregates. **Zn-PPGs** were irradiated with UV-light to generate **Zn-HM**, which could self-assemble to form chlorosomal aggregates.

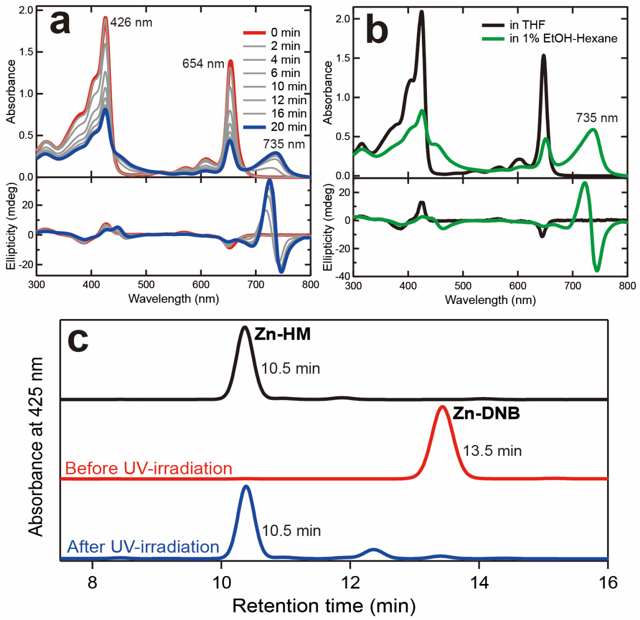

Figure 2. Photoinduced self-assembly of chlorophyll derivatives. (a) UV-vis absorption and CD spectral change of **Zn-DNB** (20 μM) in 1%(v/v) EtOH-hexane with UV-irradiation (red to blue). (b) UV-vis absorption and CD spectra of **Zn-HM** (20 μM) in tetrahydrofuran (THF; black) and 1%(v/v) EtOH-hexane (green). (c) HPLC profiles of **Zn-HM** (upper), **Zn-DNB** before UV irradiation (middle), and **Zn-DNB** after UV irradiation for 28 min (lower); HPLC conditions: Cosmosil 5C$_{18}$AR-II 3.0ϕ × 150 mm, acetonitrile : acetone : pyridine : H_2O = 75.6 : 18.9 : 0.5 : 5, 1.0 mL/min.

Zn-DNB possessing a dinitrobenzyl group[21,22] showed the highest deprotection yield in the three **Zn-PPGs** as shown in Supplementary Information. The photoinduced self-aggregation behaviour of **Zn-DNB** was investigated using UV-visible (vis) absorption and circular dichroism (CD) spectra. **Zn-DNB** was dissolved in a 1%(v/v) EtOH-hexane solution to give two sharp peaks called Soret and Qy absorption bands at 426 and 654 nm, respectively (Fig. 2a, upper, red). UV-irradiation (250–280 nm) to the **Zn-DNB** solution triggered the appearance of red-shifted Soret and Qy bands at 450–500 and 735 nm, respectively, where CD signals were visible (Fig. 2a, gray and blue). The resulting absorption peaks and CD signals are comparable to those of **Zn-HM** self-aggregates (**Agg**$_{\textbf{HM}}$) in 1%(v/v) EtOH-hexane (Fig. 2b, green),[15,23] demonstrating that **Zn-DNB** was photo-deprotected by UV-irradiation to produce **Zn-HM** which self-assembled to form chlorosomal self-aggregates (**Agg**$_{\textbf{UV-1}}$). The result of reverse-phase high performance liquid chromatography (RP-HPLC)-mass spectrometry (MS) analysis also indicated that **Zn-DNB** was deprotected to generate **Zn-HM**. Before photoirradiation, a single HPLC peak of **Zn-DNB** was observed at 13.5 min (Fig. 2c, red). During irradiation, an arising 10.5-min peak was assigned to **Zn-HM** by comparison with the authentic sample (Fig. 2c, black and blue).

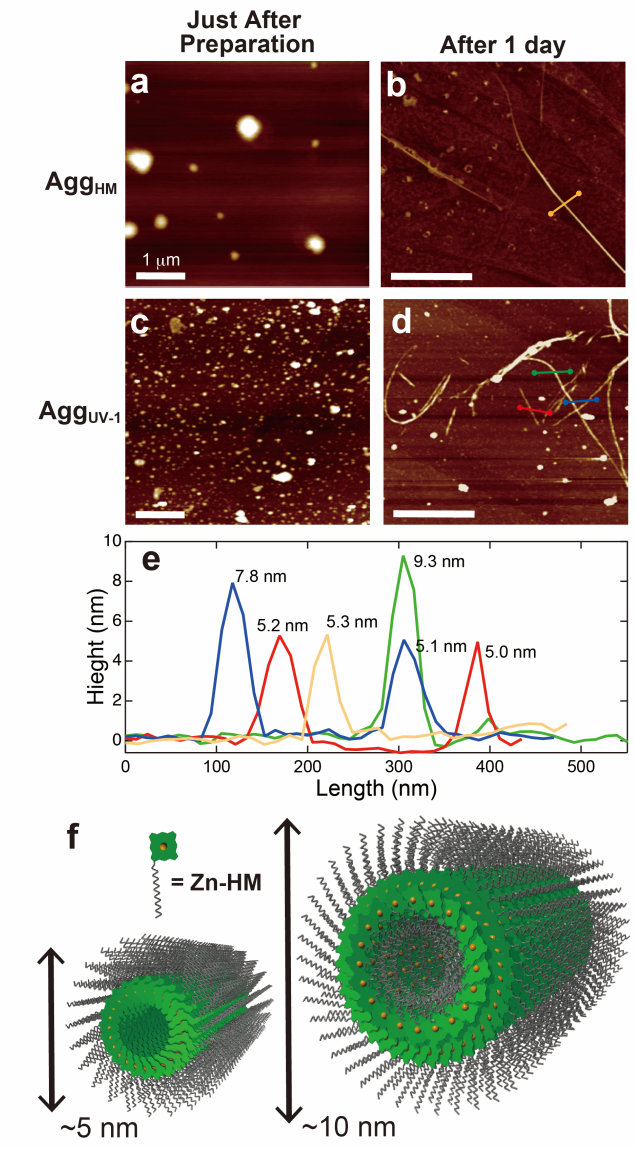

Figure 3. AFM images of (a) **Agg**$_{\textbf{HM}}$ just after preparation and (b) 1 week, and (c) those of **Agg**$_{\textbf{UV-1}}$ just after UV-irradiation for 20 min and (d) after standing the solution for 1 day on an HOPG substrate; all the scale bars are 1 μm. (e) Height profiles along yellow, green, red, and blue lines in Figs. 3b and 3d. (f) Schematic tube-like monolayer (left) and bilayer structures (right) of **Zn-HM** self-aggregates.

To observe the structural morphology of self-aggregates **Agg**$_{\textbf{HM}}$ and **Agg**$_{\textbf{UV-1}}$, we analyzed their solids prepared by drop-casting the sample solutions on a highly oriented pyrolytic graphite (HOPG) substrate by atomic force microscopy (AFM) measurements. Just after preparation, **Agg$_{\mathrm{HM}}$** was the principal nanoparticles present with 300–600 (NP$_{\mathrm{large}}$) and 100–200 nm (NP$_{\mathrm{medium}}$) diameters (Fig. 3a), and nanofibers (NFs) were partially observed after standing in the solution for 1 week (Fig. 3b). This transformation indicated that NP and NF were thermodynamically metastable and stable aggregates, respectively, which agrees with previous studies.[15] One more month later, numerous NFs were observed (Fig. S11a), showing that metastable NPs slowly transformed into more stable NFs. In contrast to **Agg$_{\mathrm{HM}}$**, **Agg$_{\mathrm{UV\text{-}1}}$** just after preparation (20-min illumination) formed small nanoparticles (NP$_{\mathrm{small}}$) with a <50 nm diameter (Fig. 3c). Interestingly, transformation into NF with an accompanying decrease in NP$_{\mathrm{small}}$ was observed after standing for 1 day (Fig. 3d), and more NFs were observed after 1 week (Fig. S11c); this transformation was much faster than that of **Agg$_{\mathrm{HM}}$**. The diameter of these NFs was 5 or 10 nm (Fig. 3e). Based on previously reported data for self-aggregates of **Zn-HM**,[15] these NFs were tube-like monolayer or bilayer aggregates (Fig. 3f).

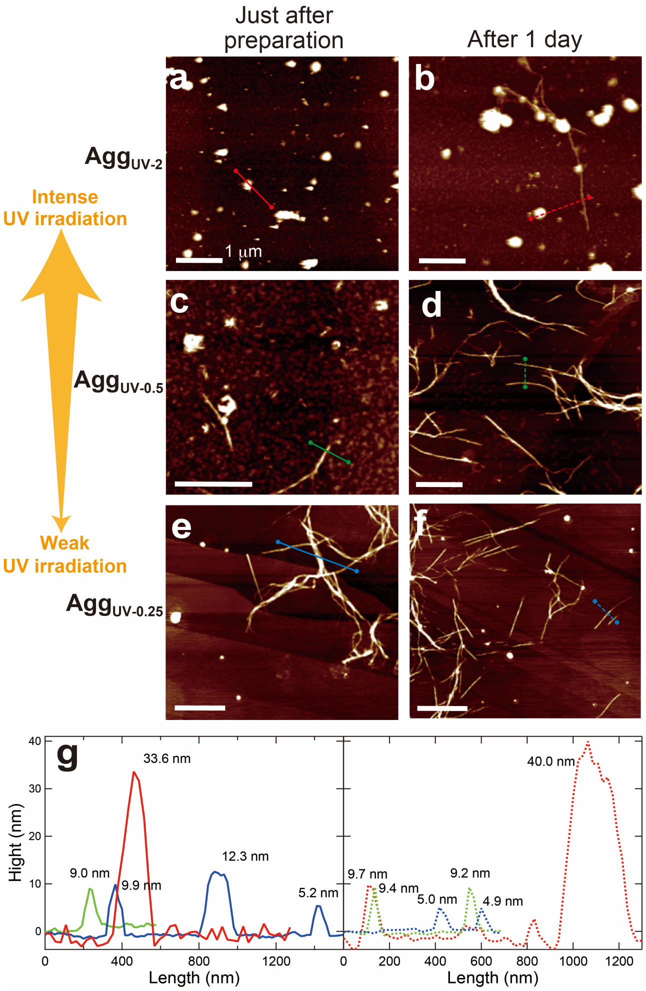

Figure 4. AFM images of (a) **Agg$_{\mathrm{UV\text{-}2}}$**, (c) **Agg$_{\mathrm{UV\text{-}0.5}}$**, and (e) **Agg$_{\mathrm{UV\text{-}0.25}}$** just after irradiation with a UV-light of double, half, and quarter power comparing to the preparation of **Agg$_{\mathrm{UV\text{-}1}}$** for 10, 40, and 80 min, respectively, and (b) **Agg$_{\mathrm{UV\text{-}2}}$**, (d) **Agg$_{\mathrm{UV\text{-}0.5}}$**, and (f) **Agg$_{\mathrm{UV\text{-}0.25}}$** after standing in the solution for 1 day on an HOPG substrate; all the scale bars are 1 µm. (g) Height profiles along solid (just after preparation; left) and broken red, green, and blue lines (after 1 day; right) in Figs. 4a/b, 4c/d, and 4e/f.

Agg$_{\mathrm{HM}}$ was formed by assembling a large number of **Zn-HM** molecules simultaneously, while **Agg$_{\mathrm{UV\text{-}1}}$** was gradually constructed from deprotected **Zn-HM** by UV-irradiation. The aforementioned different behaviours of **Agg$_{\mathrm{HM}}$** and **Agg$_{\mathrm{UV\text{-}1}}$** were attributed to the supply rate of self-assembling **Zn-HM** (active spices). To control the rate, the light intensity was changed and the structural morphology of the obtained self-aggregates was investigated by AFM. UV-irradiation of a **Zn-DNB** solution with a 2-fold higher light intensity more rapidly generated **Zn-HM** aggregates (**Agg$_{\mathrm{UV\text{-}2}}$**) than the aforementioned photoirradiation experiment (Figs. S10c and d). **Agg$_{\mathrm{UV\text{-}2}}$** just after 10-min irradiation formed slightly larger NPs than the corresponding ones in **Agg$_{\mathrm{UV\text{-}1}}$**, to form NP$_{\mathrm{medium}}$ with a 200–300 nm diameter (Fig. 4a). NFs were partially observed in **Agg$_{\mathrm{UV\text{-}2}}$** after 1 day and showed lower transformation into NFs (Fig. 3b) compared to **Agg$_{\mathrm{UV\text{-}1}}$**. After standing for one more week, numerous NFs were observed in **Agg$_{\mathrm{UV\text{-}2}}$** (Fig. S11d), and their transformation into NF from NP was faster than that of **Agg$_{\mathrm{HM}}$**.

UV-irradiation with 0.5-fold intensity for **Zn-DNB** resulted in slower formation of self-aggregates of **Zn-HM** (Fig. S10). Just after 40 min of irradiation, the aggregates (**Agg$_{\mathrm{UV\text{-}0.5}}$**) partially produced NFs (Fig. 4c). Furthermore, reducing UV-irradiation to a light intensity of 0.25-fold afforded **Zn-HM** much more slowly (Fig. S10a) and the aggregates (**Agg$_{\mathrm{UV\text{-}0.25}}$**) were primarily NF forms just after 80-min illumination (Fig. 4e). After standing for an additional day, numerous NFs were observed in **Agg$_{\mathrm{UV\text{-}0.5}}$** and **Agg$_{\mathrm{UV\text{-}0.25}}$** (Figs. 4d and 4f). A rapid supply of **Zn-HM** resulted in promoting the formation of metastable NPs and inhibiting formation of the thermodynamically stable NFs, but a slow supply promoted the formation of NFs and inhibited that of NPs. The formation of NFs was largely dependent on the rate of **Zn-HM** production. We demonstrated that the spatiotemporal control of active species resulted in formation of fiber (tube)-like aggregates mimicking a biological supramolecular system such as a chlorosome.

The optical properties of **Agg$_{\mathrm{UV\text{-}1}}$** prepared by photodeprotection of **Zn-DNB** to **Zn-HM** in 1%(v/v) EtOH-hexane were nearly the same as those of **Agg$_{\mathrm{HM}}$** produced by the quick addition of hexane to an EtOH solution of **Zn-HM** (Figs. 2a and b). This indicated that the local supramolecular structures were identical. Thermodynamically stable NFs were likely formed by gradually elongating a small *J*-aggregate (*J*-aggregate block), which functioned as a nucleus or seed for NFs (Fig. 4). In contrast, metastable NPs (NP$_{\mathrm{small}}$, NP$_{\mathrm{medium}}$, and NP$_{\mathrm{large}}$) were formed by van der Waals and hydrophobic interactions between *J*-aggregate blocks, similar to a polycrystalline made of a large number of single crystals, which was prepared by rapid crystal growth.[24,25]

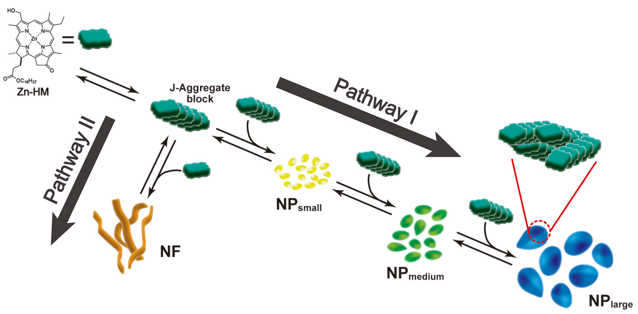

Figure 5. Assembly pathways toward nanofibers and nanoparticles. Pathways I and II were characterized by NP and NF formations, respectively.

By rapid supply of **Zn-HM**, self-assembly of *J*-aggregate blocks to construct NPs (Fig. 5, Pathway I) occurred preferentially compared to growth of *J*-aggregates to form NFs. Large NPs constructed just after preparation of $\mathbf{Agg_{HM}}$ and $\mathbf{Agg_{UV\text{-}2}}$ slowly converted to NF over a long time (1 week) through reverse Pathway I to the *J*-aggregate block. Small NPs prepared just after preparation of $\mathbf{Agg_{UV\text{-}1}}$ transformed to NFs for 1 day because they readily disassembled into *J*-aggregate block (disassembly process). A decrease in NP$_{small}$ was accompanied by transformation into NFs (Fig. 3d), while NP$_{large}$ and NP$_{medium}$ continued to be observed without full conversion after standing in the solution for 1 day. In contrast, elongation of the *J*-aggregate block with **Zn-HM** monomers formed NFs (Fig. 5, Pathway II) simultaneously with significantly slow supply of **Zn-HM** by photo-deprotection as in $\mathbf{Agg_{UV\text{-}0.25}}$ (Fig. 4e). Particularly, Pathway II was similar for forming natural chlorosomal self-aggregates by assembling successively biosynthesized BChl molecules, and thus we mimicked the biological supramolecular formation process by controlling the supply rate of the active species. Formation of NFs using the system is the first direct model to mimic the growth of natural chlorosomal self-aggregates, and the model can be used to evaluate the in *vivo* dynamic formation of chlorosomes. The photo-deprotection systems would be useful for producing a variety of supramolecular structures in nature such as protein aggregates and biomembranes, as a photoremovable protecting group can be used to protect amines, amides, thiols, and carboxylic/phosphoric/sulfuric acids as well as any hydroxy groups.

ASSOCIATED CONTENT

Supporting Information.

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

General information, synthesis procedures, spectral data, Figure S1–S11 (PDF).

AUTHOR INFORMATION

Corresponding Author

tamiaki@fc.ritsumei.ac.jp.

ORCID

Hitoshi Tamiaki: 0000-0003-4797-0349

Notes

The authors declare no competing financial interests.

ACKNOWLEDGMENT

We thank Prof. Manabu Abe of Hiroshima University for his valuable advice about photo-removable protecting groups. This work was supported by JSPS KAKENHI Grant Numbers JP18J21058 and JP17H06436.

REFERENCES

[1] Ogi, S.; Sugiyasu, K.; Manna, S.; Samitsu, S.; Takeuchi, M. Living supramolecular polymerization realized through a biomimetic approach, *Nat. Chem.* **2014**, *6*, 188–195.

[2] Kang, J.; Miyajima, D.; Mori, T.; Inoue, Y.; Itoh, Y.; Aida, T. A rational strategy for the realization of chain-growth supramolecular polymerization, *Science* **2015**, *347*, 646–651.

[3] Ogi, S.; Stepanenko, V.; Thein, J.; Würthner, F. Impact of alkyl spacer length on aggregation pathways in kinetically controlled supramolecular polymerization, *J. Am. Chem. Soc.* **2016**, *138*, 670–678.

[4] He, X.; Hsiao, M.; Boott, C. E.; Harniman, R. L.; Nazemi, A.; Li, X.; Winnik, M. A.; Manners, I. Two-dimensional assemblies from crystallizable homopolymers with charged termini, *Nat. Mater.* **2017**, *16*, 481–488.

[5] Besenius, P. Controlling supramolecular polymerization through multicomponent self-assembly, *J. Polym. Sci. A: Polym. Chem.* **2017**, *55*, 34–78.

[6] Holzwarth, A. R.; Griebenow, K.; Schaffner, K. Chlorosomes, photosynthetic antennae with novel self-organized pigment structures, *J. Photochem. Photobiol. A: Chem.* **1992**, *65*, 61–71.

[7] Hildebrandt, P.; Tamiaki, H.; Holzwarth, A. R.; Schaffner, K. Resonance Raman spectroscopic study of metallochlorin aggregates, *J. Phys. Chem.* **1994**, *98*, 2192–2197.

[8] Tamiaki, H. Supramolecular structure in extramembraneous antennae of green photosynthetic bacteria, *Coord. Chem. Rev.* **1996**, *148*, 183–197.

[9] Olson, J. M. Chlorophyll organization and function in green photosynthetic bacteria, *Photochem. Photobiol.* **1998**, *67*, 61–75.

[10] Montaño, G. A.; Bowen, B. P.; LaBelle, J. T.; Woodbury, N. W.; Pizziconi, V. B.; Blankenship, R. E. Lamellar organization of pigments in chlorosomes, the light harvesting complexes of green photosynthetic bacteria, *Biophys. J.* **2004**, *87*, 1165–1172.

[11] Ganapathy, S.; Oostergetel, G. T.; Wawrzyniak, P. K.; Reus, M.; Chew, A. G. M.; Buda, F.; Boekema, E. J.; Bryant, D. A.; Holzwarth, A. R.; de Groot, H. J. M. Alternating *syn-anti* bacteriochlorophylls form concentric helical nanotubes in chlorosomes, *Proc. Natl. Acad. Sci. U. S. A.* **2009**, *106*, 8525–8530.

[12] Orf, G. S.; Blankenship, R. E. Chlorosome antenna complexes from green photosynthetic bacteria, *Photosynth. Res.* **2013**, *116*, 315–331.

[13] Günther, L. M.; Jendrny, M.; Bloemsma, E. A.; Tank, M.; Oostergetel, G. T.; Bryant, D. A.; Knoester, J.; Köhler, J. Structure of light-harvesting aggregates in individual chlorosomes, *J. Phys. Chem. B* **2016**, *120*, 5367−5376.

[14] Huber, V.; Sengupta, S.; Würthner, F. Structure–property relationships for self-assembled zinc chlorin light-harvesting dye aggregates, *Chem. Eur. J.* **2008**, *14*, 7791–7807.

[15] Shoji, S.; Hashishin, T.; Tamiaki, H. Construction of chlorosomal rod self-aggregates in the solid state on any substrates from synthetic chlorophyll derivatives possessing an oligomethylene chain at the 17-propionate residue, *Chem. Eur. J.* **2012**, *18*, 13331–13341.

[16] Shoji, S.; Mizoguchi, T.; Tamiaki, H. *In vitro* self-assemblies of bacteriochlorophylls-*c* from *Chlorobaculum tepidum* and their supramolecular nanostructures, *J. Photochem. Photobiol. A: Chem.* **2016**, *331*, 190–196.

[17] Shoji, S.; Ogawa, T.; Hashishin, T.; Ogasawara, S.; Watanabe, H.; Usami, H.; Tamiaki, H. Nanotubes of biomimetic supramolecules constructed by synthetic metal chlorophyll derivatives, *Nano Lett.* **2016**, *16*, 3650–3654.

[18] Ogi, S.; Grzeszkiewicz, C.; Würthner, F. Pathway complexity in the self-assembly of a zinc chlorin model system of natural bacteriochlorophyll J-aggregates, *Chem. Sci.* **2018**, *9*, 2768–2773.

[19] Patchornik, A.; Amit, B.; Woodward, R. B. Photosensitive protecting groups, *J. Am. Chem. Soc.* **1970**, *92*, 6333–6335.

[20] Kaplan, J. H.; Forbush, B. J.; Hoffman, F. Rapid photolytic release of adenosine 5'-triphosphate from a protected analogue: Utilization by the Na:K pump of human red blood cell ghosts, *Biochemistry* **1978**, *17*, 1929–1935.

[21] Cameron, J. F.; Frechet, J. M. J. Photogeneration of organic bases from *o*-nitrobenzyl-derived carbamates., *J. Am. Chem. Soc.* **1991**, *113*, 4303–4313.

[22] Klán, P.; Šolomek, T.; Bochet, C. G.; Blanc, A.; Givens, R.; Rubina, M.; Popik, V.; Kostikov, A.; Wirz, J. Photoremovable protecting groups in chemistry and biology: Reaction mechanisms and efficacy, *Chem. Rev.* **2013**, *113*, 119−191.

[23] Saga, Y.; Nakai, Y.; Tamiaki, H. Temperature-dependent spectral changes of self-aggregates of zinc chlorophylls esterified by different linear alcohols at the 17-propionate, *Supramol. Chem.* **2009**, *21*, 738–746.

[24] Toda, A.; Keller, A. Growth of polyethylene single crystals from the melt: Morphology, *Colloid Polym. Sci*. **1993**, *271*, 328–342.

[25] Yoshikawa, H. Y.; Murai, R.; Adachi, H.; Sugiyama, S.; Maruyama, M.; Takahashi, Y.; Takano, K.; Matsumura, H.; Inoue, T.; Murakami, S.; Masuhara H.; Mori, Y. Laser ablation for protein crystal nucleation and seeding, *Chem. Soc. Rev.* **2014**, *43*, 2147–2158.

Insert Table of Contents artwork here