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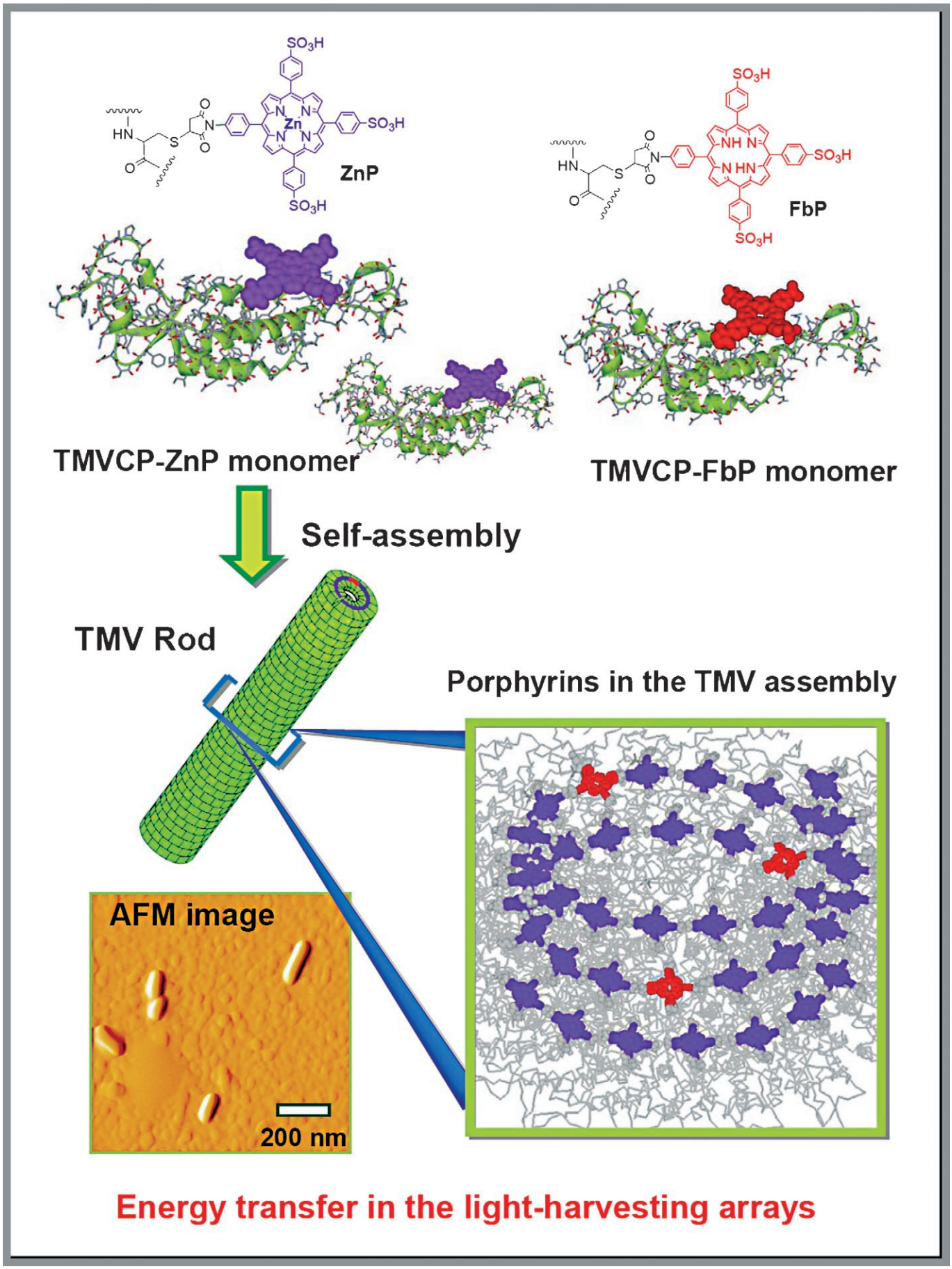

# Porphyrin Light-Harvesting Arrays Constructed in the Recombinant Tobacco Mosaic Virus Scaffold

**Masayuki Endo,* Mamoru Fujitsuka, and Tetsuro Majima*[a]**

*Dedicated to Professor Isao Saito on the occasion of his 65th birthday*

**Abstract:** We have demonstrated the construction of multiple porphyrin arrays in the tobacco mosaic virus (TMV) supramolecular structures by self-assembly of recombinant TMV coat protein (TMVCP) monomers, in which Zn-coordinated porphyrin (ZnP) and free-base porphyrin (FbP) were site-selectively incorporated. The photophysical properties of porphyrin moieties incorporated in the TMV assemblies were also characterized. TMV–porphyrin conjugates employed as building blocks self-assembled into unique disk and rod structures under the proper conditions as similar to native TMV assemblies. The mixture of a ZnP donor and an FbP acceptor was packed in the TMV assembly and showed energy transfer and light-harvesting activity. The detailed photophysical properties of the arrayed porphyrins in the TMV assemblies were examined by time-resolved fluorescence spectroscopy, and the energy transfer rates were determined to be $3.1–6.4\times10^{9}\,s^{-1}$. The results indicate that the porphyrins are placed at the expected positions in the TMV assemblies.

**Keywords:** energy transfer · light-harvesting systems · porphyrinoids · self-assembly · tobacco mosaic virus

## Introduction

Self-assembled multichromophore systems have recently become one of the most important issues in the fields of materials science and nanotechnology.[1] Molecular components self-assemble to form a unique architecture possessing high functionality, for example, light-harvesting protein complex 2 is one of the most sophisticated protein-assisted multichromophore systems, in which the protein subunits control the coordination of multiple bacteriochlorophylls into a π-stacked ring shape.[2] Therefore, the rational design of the functional repeating units is important for the resulting structures and performance of the self-assembled materials. Protein architectures such as large protein assemblies[3] and virus shells[4] have recently been used for the preparation of nanoscale materials. Tobacco mosaic virus (TMV) is also one of the most characterized protein architectures, and the nanostructures are easily controlled by pH values of the solution. Under basic conditions (pH 8) TMV remains as monomers, which then self-assemble into double-layered disk-like structures under neutral conditions (pH 7), and multilayered rod-like structures under acidic condition (pH 5.5).[5] TMV has been widely employed as a template for functionalization with inorganic and organic molecules both at the inner cavity and the outer surface.[6] We recently constructed a TMV–assisted self-assembled chromophore system in the TMV structure, the inner cavity of which was used for assembling pyrenes.[7] Among the integration of chromophores, porphyrin derivatives have widely been used because of their advantageous properties such as a visible-light-absorbing nature and stability, and their photophysical properties are easily changed by metal coordination. Multi-porphyrin molecular systems have been investigated not only as energy- and electron-transfer systems, but also for mimicking light-harvesting systems.[8–9] In the present study, we employed a Zn-coordinated porphyrin (ZnP) and a free-base porphyrin (FbP) to construct a spatially controlled self-assembled energy-transfer system in the TMV structures. A multiple ZnP antenna was used to transfer the collected energy to an FbP acceptor by using the energy gradient as investigated in the various systems.[8] Therefore, the precise-

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ly controlled arrangement of porphyrin moieties finally shows unique photochemical properties, such as light-harvesting activity. TMV assemblies were recently used as a scaffold for modification of three different dyes for a light-harvesting system.[11] We also designed and constructed three-dimensionally organized porphyrin arrays utilizing the intrinsic self-assembling property of TMV and its nanostructures by employing the strategies developed in the TMV–pyrene assemblies.[7]

Herein, we prepared the ZnP- and FbP-attached TMV coat protein (TMVCP) monomers as building blocks, and the porphyrin derivatives were organized into double-layered disk-type TMV assemblies at pH 7.0 and multi-layered rod-type ones at pH 5.5 by self-assembly. TMV assemblies containing randomly distributed ZnP donors and FbP acceptors were used for analysis of the energy transfer and light-harvesting activity. We examined the self-assembled TMV structures by atomic force microscopy (AFM), and the photochemical properties of the porphyrins in the TMV assemblies by steady-state spectroscopic analysis. The detailed photophysical properties of the organized porphyrins were investigated by time-resolved fluorescence spectroscopy to determine the energy transfer rates in the TMV system.

## Results and Discussion

**Design and preparation of TMVCP–porphyrin conjugates**: Porphyrin derivatives were incorporated at the 127 position of the TMVCP monomer, which is located near the viral RNA binding site (Figure 1).[5,12] Because we used a recombinant TMVCP monomer, the RNA binding site should be a cavity to allow for modification with a relatively large, as reported in a recent study.[11] For incorporation of porphyrin moieties into the TMV monomers with a cysteine residue and a maleimide group, we introduced double-mutation to change the intrinsic Cys27 of the TMVCP monomer to alanine to prevent the undesired coupling reaction,[7] and the Asn 127 of the TMVCP C27A mutant was then mutated to cysteine. Porphyrin derivatives were incorporated to this 127 position by a maleimido–thiol coupling reaction (Figure 1b). Expression and purification of the recombinant TMVCP and mutants were carried out according to the previously reported method.[7] Selective introduction of the porphyrin moiety into the N127C mutant was carried out by treatment with porphyrin–maleimide derivatives in a solution at pH 8.0. The TMVCP–porphyrin conjugates were identified by MALDI-TOF mass spectroscopy (Figure S1 in the Supporting Information).

**Nanostructures of TMV–porphyrin assemblies**: We examined the nanoscale structures of the TMV–porphyrin assemblies by atomic force microscopy (AFM). The porphyrin-attached TMVCP monomers were assembled to form TMV supramolecular structures at pH 5.5. The porphyrin-attached monomers formed rod structures with lengths ranging from

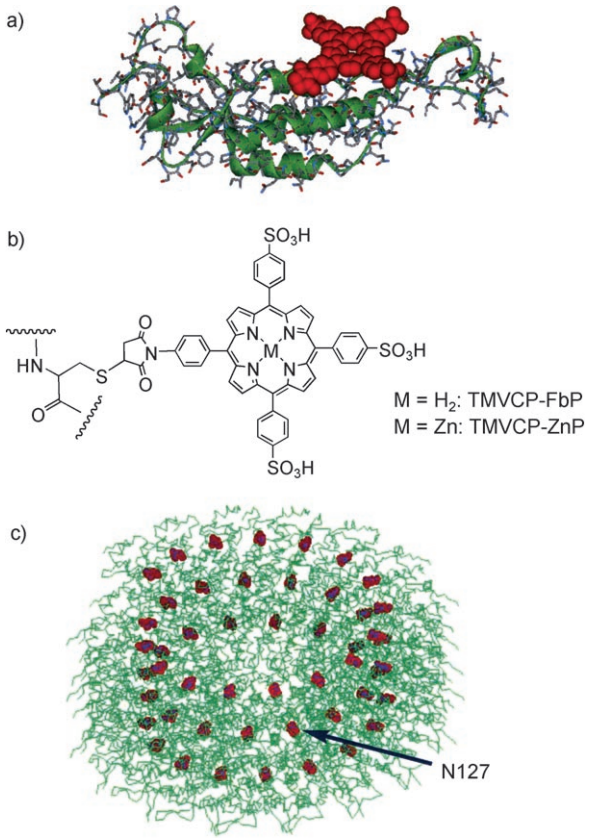


Figure 1. Structure of tobacco mosaic virus (TMV) and TMV coat protein monomer (TMVCP) conjugated with a porphyrin derivative. a) Structure of TMVCP–porphyrin conjugated monomer; the porphyrin moiety was introduced at the 127 position. b) Porphyrin derivatives introduced to the cysteine residue of a TMV monomer through maleimido–thiol coupling. c) Crystal structure of the TMV assembly; amino acid N127 is shown in red.[12]

90 to 160 nm and a height of about 17 nm, which corresponds to the diameter of the native TMV rod assembly (18 nm; Figure 2). These results indicate that the porphyrin modification does not prevent the self-assembly process of TMV monomers and the characteristic rod-structure formation. At pH 7, the TMV assembly should form a double-layered disk structure, and the assembled structures were observed to be approximately 4 nm in height by AFM analysis (Figure S2 in the Supporting Information), which is close to the height of the native double-layered disk structure (4.6 nm). The results show that formation of the disk- and rod-type nanostructure containing the porphyrin derivatives can be controlled by changing pH values similar to the native TMV assemblies.

**Interaction of porphyrins in the TMV assemblies**: We examined the photophysical properties of porphyrin derivatives in the TMV assemblies. To examine the energy transfer from ZnP to FbP, we carried out the formation of TMV assemblies with mixtures of the TMVCP–ZnP and TMVCP–FbP monomers by changing the content of the TMVCP–FbP monomer to 10, 15, 20, 33, and 50%. TMVCP–ZnP

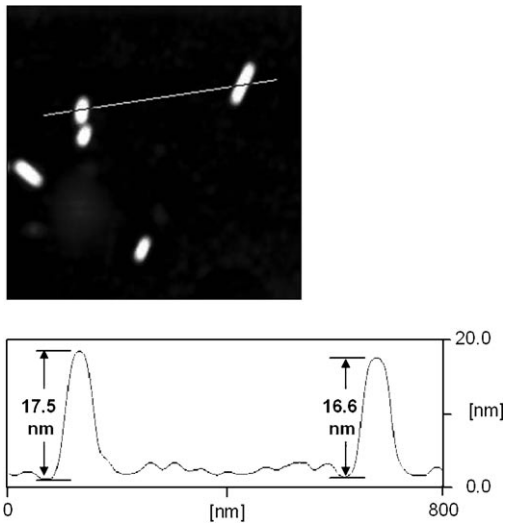


Figure 2. AFM image of TMV–ZnP assemblies at pH 5.5. Top: Image size 1×1 μm. Bottom: Sectional analysis of the line in a.

and TMVCP–FbP monomers were mixed at pH 8 to give a random distribution of the monomers; the pH was then lowered to form ZnP/FbP-mixed TMV assemblies. A porphyrin derivative is a well-known chromophore, the interaction of which can be detected by monitoring UV/Vis and fluorescence spectra.[8,13–15] When the double-layered disks and multi-layered rods are formed, the neighboring porphyrin moieties should be located so as to form helical array structures. In the UV/Vis spectra of the TMV–ZnP assemblies at pH 7, the Soret band of the TMV–ZnP assembly was 427 nm, which did not show any peak shift from that of the TMVCP–ZnP monomer (427 nm; Figure 3 and Figure S3 in the Supporting Information). The TMV–ZnP assembly at pH 5.5 showed a similar tendency. These results indicate that the porphyrin moieties in the TMV assemblies are separated without formation of strong aggregates.[16] In the cases of the TMV assemblies at pH 7, by increasing the content of FbP, the peaks gradually shifted to a shorter wavelength without causing aggregation of the porphyrins. In the case of the 50%-FbP/ZnP–TMV assembly at pH 5.5, a shoulder at a shorter wavelength was observed compared to the spectrum of the corresponding content of FbP at pH 7, indicating a slight porphyrin aggregation under these conditions. However, porphyrins in the TMV assemblies containing a lower content of FbP were basically located in well-separated places.

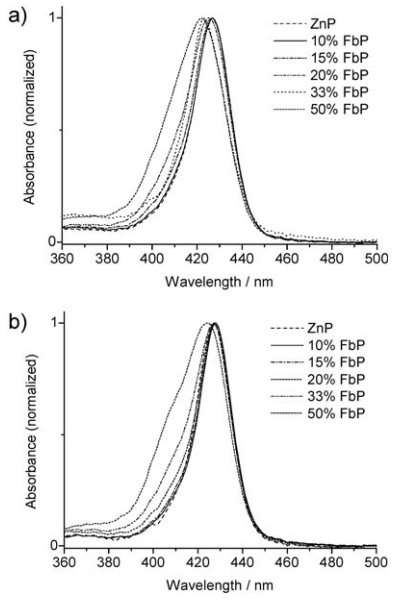


Figure 3. UV/Vis spectra of the mixture of TMVCP–ZnP and TMVCP–FbP at a) pH 7.0 and b) pH 5.5.

**Energy transfer and light-harvesting activity of porphyrins in the TMV assemblies**: We next examined the fluorescence spectra of ZnP/FbP-mixed assemblies at pH 7.0 and 5.5 by changing the contents of FbP from 10 to 50% (Figure 4).

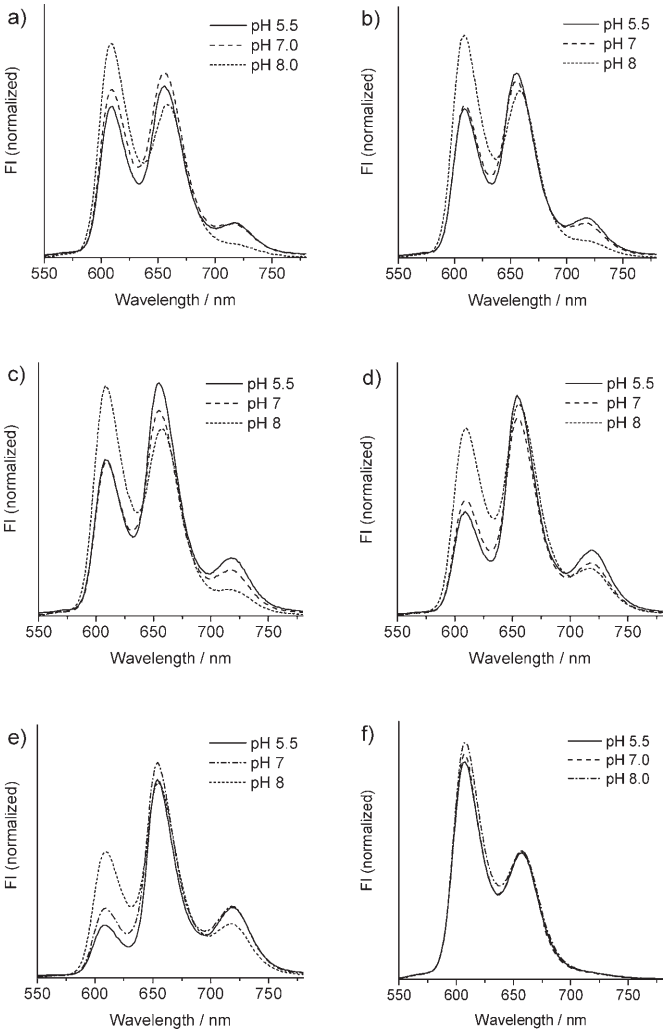


Figure 4. Fluorescence spectra of ZnP/FbP-mixed TMV assemblies at pH 7.0 (solid line) and 5.5 (dashed line) and the mixtures of monomers (pH 8.0, broken line). FbP content in the ZnP/FbP TMV assemblies: a) 10%; b) 15%; c) 20%; d) 33%; e) 50%. f) TMV–ZnP assemblies. Excitation wavelength was 424 nm.

  

The 424 nm wavelength was used mainly for exciting the ZnP moieties. In the cases of TMV–ZnP assemblies at pH 7.0 and 5.5, emission bands (610 and 665 nm, respectively) assigned to ZnP in the singlet excited state were observed. The fluorescence intensities at pH 7 and 5.5 decreased by 5–7% compared to that of the monomer at pH 8 (Figure 4 f), indicating an environmental change around the ZnP after self-assembly. When ZnP/FbP-mixed TMV assemblies containing 10% FbP were employed, the intensities of the ZnP emissions (610 nm) were reduced compared to that of the corresponding mixture of the monomers (10% FbP, pH 8.0; Figure 3a). Simultaneously, the intensities of the FbP emissions (720 nm) were enhanced relative to those of the corresponding mixture of the monomers (10% FbP, pH 8.0). These results suggest that the energy transfer from ZnP to FbP occurs when the TMVCP-porphyrin monomers self-assemble to form the specific TMV structures. Fluorescence spectra of TMV assemblies containing 15–50% FbP showed a similar decrease in ZnP emission and an increase in FbP emission. Quenching yields of ZnP emission and enhancements of FbP emission are summarized in Table 1.

Table 1. Quenching yields of ZnP emission at 610 nm and enhancements of FbP emission at 720 nm under the pH 7.0 and 5.5 conditions.

| FbP content [%][a] | pH 7.0 ZnP[b] quenching | pH 7.0 FbP[b] enhancement | pH 5.5 ZnP[b] quenching | pH 5.5 FbP[b] enhancement |
|---|---|---|---|---|
| 10 | 0.24 | 4.1 | 0.32 | 4.5 |
| 15 | 0.32 | 3.1 | 0.33 | 3.8 |
| 20 | 0.33 | 1.9 | 0.33 | 2.6 |
| 33 | 0.39 | 1.4 | 0.45 | 1.9 |
| 50 | 0.45 | 1.4 | 0.58 | 1.4 |

[a] TMVCP–FbP content (%) in the ZnP/FbP-mixed TMV assemblies. [b] Quenching yields of ZnP emission at 610 nm and enhancements of FbP emission at 720 nm were calculated from the steady state fluorescence spectra of the ZnP/FbP-mixed TMV assemblies at pH 7.0 and 5.5 by comparison with those of the corresponding FbP content of the TMVCP monomer mixtures (pH 8).

Quenching yields of the ZnP emission gradually increased by increasing the content of FbP, simply because of the increase in the ratio of the acceptor. ZnP emissions in the TMV rod assemblies at pH 5.5 showed larger quenching yields than those in the disk assemblies at pH 7.0. Relatively low quenching yields even at 50% FbP content may be attributed to exciting both ZnP and FbP at 424 nm, which lowered the energy-transfer efficiency. The FbP emission in the ZnP/FbP-TMV rod assembly (pH 5.5) containing 10% FbP was enhanced by 4.5 times relative to that of the corresponding FbP content of TMV monomer mixtures (pH 8.0). The enhancement of the FbP emissions was larger in the assemblies at pH 5.5 than in those at pH 7.0. The ZnP quenching and enhancement of FbP emission occur more efficiently in the TMV rod assemblies at pH 5.5, suggesting that the structures of the TMV disk assemblies (pH 7.0) and rod ones (pH 5.5) affect the arrangement and organization of the porphyrins in the TMV assemblies. We also examined the excitation spectra of FbP/ZnP TMV assemblies using 720 nm (FbP emission) as the emission wavelength (Figures S5 and S6 in the Supporting Information). The excitation peaks of the Soret band were comparable to the absorption peaks of the corresponding FbP/ZnP assemblies (Figure 3), indicating that the energy transfer from ZnP to FbP occurs (Figure S5). Energy transfer was also confirmed by comparison of the excitation and absorption spectra in the Q-band region of 10%-FbP/ZnP assemblies (Figure S6). When the excitation peak of FbP (515 nm) was adjusted to the absorption spectrum, although the excitation peaks around 555 nm corresponding mainly to ZnP did not reach the absorption peak, the intensity increased compared to that of the corresponding peak of the absorption spectrum of the FbP assembly. These results also indicate that the energy transfer takes place in the TMV assemblies of the FbP/ZnP systems.

**Photophysical properties of porphyrins in the TMV assemblies**: To characterize the details of the photophysical properties of the multiple porphyrin moieties in the TMV assembly, we measured the fluorescence lifetimes of ZnP under the pH 7.0 and 5.5 conditions by time-resolved fluorescence spectroscopy. In the cases of the TMV assembly containing only ZnP at pH 7.0 and 5.5, the fluorescence decay curves were fitted to a single exponential (Figure 5), indicating that the self-quenching of ZnP fluorescence does not occur in these assemblies. The results also suggest that the ZnP chromophores would be well separated in the assemblies. We next examined the fluorescence lifetimes of

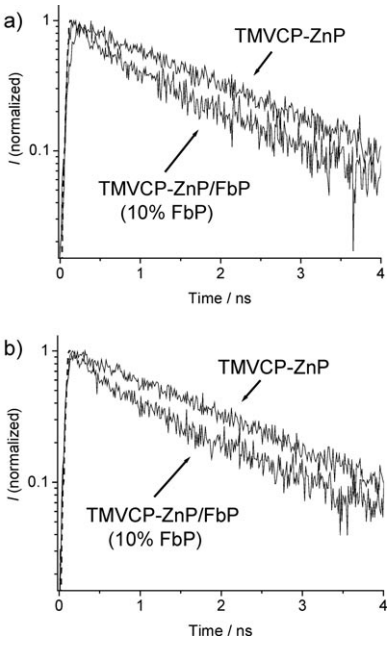


Figure 5. Fluorescence decay profiles of TMVCP–ZnP (0% FbP) assemblies and TMVCP–ZnP/FbP-mixed (10% FbP) assemblies at a) pH 7.0 and b) pH 5.5. Photons of ZnP fluorescence at 590–620 nm were collected for calculation of the decay curves. The dashed lines in the spectra represent the profile of instrument response time to excitation of the laser, which almost overlap the initial rising of the profiles.

  

ZnP/FbP-mixed assemblies at pH 7.0 and 5.5. Fluorescence decay profiles of ZnP/FbP-mixed assemblies (10% FbP) showed the content of a shorter lifetime component (Figure 5). The fluorescence decay curves were fitted to two components; one was a shorter lifetime component and the other was the usual one of the ZnP fluorescence. The results of the fluorescence lifetimes of ZnP emission in the ZnP/FbP mixed-assemblies at pH 7.0 and 5.5 are summarized in Tables 2 and 3, respectively. Shorter lifetime components only appeared with the addition of the FbP, indicating that the shorter ones are assigned to the energy-transfer terms from ZnP to FbP, which occurs in the timescale of sub-nanoseconds depending on the distance between the chromophores.[8,13,15,17] In the cases of the assemblies at pH 7.0, the lifetimes of the short components were smaller than those at pH 5.5. The short lifetime components at pH 7.0 gradually decreased by increasing the content of FbP, while those at pH 5.5 showed similar values. The results indicate that disk assemblies are sensitive to the ratio of FbP/ZnP, which may affect the structural change in the relatively flexible TMV disk scaffold compared to the TMV rod structures. We estimated the energy transfer rates of ZnP to FbP in the TMV assemblies. In the cases of the TMV–ZnP assemblies containing 10% FbP at pH 7.0 and pH 5.5, the energy-transfer rate constants ($k_{ET}$) were $3.1\times10^9$ and $6.4\times10^9\ s^{-1}$, respectively. These values are comparable to those of the FbP–ZnP systems connected side-by-side with various linkers.[13,14] When the porphyrins are arranged to equally occupy the space, the center-to-center distance between side-by-side porphyrins in the TMV assembly should be 1.7–2.0 nm, which corresponds to energy-transfer rates of $3–6\times10^9\ s^{-1}$.[11,12] On the other hand, the layer-to-layer distance between two porphyrins should be exceed 2.3 nm. In both cases, the energy transfer occurs following the Förster mechanism (dipole–dipole interaction), in which $k_{ET}$ is proportional to the sixth root of the distance between donor and acceptor.[13,17] Therefore, the distance between donor and acceptor is the key factor in the energy transfer. The energy transfer in the layer-to-layer would be disadvantageous because of the longer distance compared to the side-by-side arrangement of the porphyrins. Although we used biexponential fitting for the fluorescence decays, the fittings for the data measured at pH 5.5 seem insufficient according to the $\chi^2$ values for 10% and 50% FbP content (Table 3). The more complicated energy-transfer processes such as layer-by-layer or stepwise energy transfer would exist as minor factors. The faster energy transfer rate constant at pH 7.0 suggests that the relatively flexible structure of the double-layered disk may allow porphyrins to approach the neighboring ones, because the top of the TMV disk is open. In contrast, in the cases of the TMV assemblies at pH 5.5, the porphyrin moieties are packed into the defined positions in the TMV rod assemblies, which keep the porphyrins as separate forms.

Table 2. Photophysical data for the fluorescence of the ZnP of the ZnP/FbP-mixed TMV assemblies at pH 7.

| FbP content [%][a] | $\tau_1$ [ns] | $\tau_2$ [ns] | $f_1$ | $f_2$ | $\chi^2$ |
|---|---|---|---|---|---|
| 10 | 0.14 | 1.63 | 0.19 | 0.81 | 1.16 |
| 15 | 0.16 | 1.62 | 0.26 | 0.74 | 1.10 |
| 20 | 0.17 | 1.66 | 0.36 | 0.64 | 1.11 |
| 33 | 0.21 | 1.63 | 0.25 | 0.75 | 1.19 |
| 50 | 0.21 | 1.72 | 0.24 | 0.76 | 1.09 |

[a] TMVCP–FbP content in the ZnP/FbP-mixted TMV assemblies. [b] TMV–ZnP assembly (0% FbP), $\tau=1.63$ ns, $\chi^2=1.19$, $k=6.1\times10^8\ s^{-1}$.

Table 3. Photophysical data for the fluorescence of the ZnP of the ZnP/FbP-mixed TMV assemblies at pH 5.5.

| FbP content [%][a] | $\tau_1$ [ns] | $\tau_2$ [ns] | $f_1$ | $f_2$ | $\chi^2$ |
|---|---|---|---|---|---|
| 10 | 0.27 | 1.54 | 0.31 | 0.69 | 1.29 |
| 15 | 0.25 | 1.52 | 0.32 | 0.68 | 0.818 |
| 20 | 0.24 | 1.55 | 0.33 | 0.67 | 1.12 |
| 33 | 0.24 | 1.58 | 0.47 | 0.53 | 1.10 |
| 50 | 0.26 | 1.56 | 0.29 | 0.71 | 1.33 |

[a] TMVCP–FbP content in the ZnP/FbP-mixted TMV assemblies. [b] TMV–ZnP assembly (0% FbP), $\tau=1.50$ ns, $\chi^2=1.07$, $k=6.7\times10^8\ s^{-1}$.

## Conclusion

We have demonstrated the construction of multiple porphyrin arrays in the TMV disk and rod structures by self-assembly of porphyrin-attached TMVCP monomers employed as building blocks. The porphyrin derivatives packed in the TMV assemblies showed energy transfer and light-harvesting activity with mixtures of ZnP donor and FbP acceptor. The results from the energy-transfer rates correlate well to the distance between the porphyrins in the TMV assemblies. The TMV system allows integration of photofunctional molecules and serves to create self-assembled chromophore systems in the TMV supramolecular structures and nanostructures with a high aspect ratio. Although, for chemists, unfamiliar techniques are required for the preparation of protein-functional molecule conjugates, protein architectures built up by programmed self-assembly are valuable for the specific arrangement and integration of target molecules into a unique three-dimensional scaffold. The TMV–porphyrin system can also be expanded to a charge separation system for the extraction of photocurrent by the integration onto the electrodes.

## Experimental Section

**Synthesis of TMVCP–FbP and TMVCP–ZnP conjugates and preparation of TMV assemblies**: The TMVCP C27 A/N127C mutant was prepared according to the previously reported method.[5] For the introduction of porphyrin to the cysteine mutant, the TMVCP monomer (10 μM) in a solution containing 20 mM Tris-HCl (pH 8) and 1 mM DTT was treated with a gel filtration column (BioRad BioSpin P-6) and then reacted with the tris(tetrabutylammonium) salt of 5-(4-maleimidophenyl)-10,15,20-tris(4-sulfonatophenyl)porphyrin (FbP–maleimide) or 5-(4-maleimidophenyl)-10,15,20-tris(4-sulfonatophenyl) zinc porphyrin (ZnP–maleimide) in

  

$CH_3CN$ (50-fold excess) in a 20 mM Tris-HCl (pH 8.0) solution at 30 °C for 2 h. Unreacted porphyrin was removed by a gel filtration column. The porphyrin-modified mutants were analyzed by MALDI-TOF mass spectroscopy. MALDI-TOF MS (positive): *m*/*z* calcd for TMVCP–FbP: 18529; found: 18517; *m*/*z* calcd for TMVCP–ZnP: 18593; found: 18566. For the preparation of ZnP/FbP-mixed TMV assemblies, TMVCP–ZnP and TMVCP–FbP monomers (2 μM) were mixed at pH 8, and then the pH was lowered by phosphate buffer (final 40 mM) to pH 7.0 and pH 5.5, and the samples were left at 4 °C overnight.

**Atomic force microscopy (AFM)**: AFM images were acquired on an atomic force microscope (SPA400-DFM, Seiko Instruments Inc.) in the dynamic force mode. TMVCP–ZnP monomer (2 μM, 30 μL) was dialyzed (3.5 kDa cut-off membrane) against a 40 mM sodium phosphate buffer (300 mL, pH 5.5) at 4 °C overnight. The sample was placed on a freshly cleaved mica plate pre-treated with 0.01 % aminopropyltriethoxysilane, and adsorbed for 5 min at RT. The plate was dried by air blowing.

**UV/Vis and fluorescence spectroscopy**: UV/Vis and fluorescence spectra were acquired on a JASCO V-530 UV/Vis spectrophotometer and a Hitachi 850 spectrofluorometer, respectively. Measurements were carried out at 23 °C in a solution containing TMVCP–porphyrin conjugates (total 2 μM) and 40 mM sodium phosphate buffer for pH 7.0 or pH 5.5 and 40 mM Tris-HCl buffer for pH 8.0.

**Fluorescence lifetime measurements**: Fluorescence decays were acquired by the single photon counting method using a streak scope (Hamamatsu Photonics, C4334-01) equipped with a polychrometer (Acton Research, SpectraPro150). An ultrashort laser pulse was generated with a Ti:sapphire laser (Spectra-Physics, Tsunami 3941 M1BB, fwhm 100 fs) pumped with a diode-pumped solid-state laser (Spectra-Physics, Millnnia VIIIs). For excitation of the sample, the output of the Ti:sapphire laser was converted to SHG (420 nm) with a harmonic generator (Spectra-Physics, GWU-23FL). Measurements were carried out at 23 °C in a solution containing TMVCP–porphyrin derivatives (total 2 μM) at pH 7.0 and 5.5. A 5 ns timescale was used for monitoring, and photos in the wavelength of 590–620 nm were collected for calculation of the ZnP lifetimes. Energy-transfer rate constants ($k_{ET}$) were obtained from the equation, $k_{ET} = 1/\tau_F - 1/\tau_{Zn}$, in which $\tau_F$ and $\tau_{Zn}$ are fluorescence lifetimes of the shorter components in the TMV ZnP/FbP-mixed assemblies and those in TMV–ZnP assemblies, respectively.

## Acknowledgements

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