Energy Transfer

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Nanoscale Integration of Sensitizing Chromophores and Porphyrins with Bacteriophage MS2**

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Photosynthetic systems integrate broad-spectrum light-harvesting arrays with reaction centers that generate a charge separation upon photoexcitation.[1,2] To optimize energy and electron transfer, the distances between the individual components are established with nanometer precision by self-assembling scaffolds of multiple membrane-bound proteins.[3] Numerous investigations have sought to mimic this arrangement of chemical moieties for energy-conversion applications,[4] commonly with porphyrins as the final electron-transfer components. For example, porphyrins have been arranged into nanometer-scale arrays through the use of covalent linkages,[5–7] hydrogen bonds, and metal coordination.[8,9] In terms of polymeric structures, dendritic architectures have been used with particular success for the construction of porphyrin-based photocatalytic materials.[10–16]

As larger and increasingly complex systems are developed, it becomes difficult to mimic the long-scale rigidity necessary for optimal energy transfer. It also becomes synthetically cumbersome, although not impossible, to install large numbers of porphyrins and other components while maintaining control of positioning and solubility properties. An alternative and potentially highly efficient synthetic strategy is provided by the self-assembling proteins that comprise viral capsids, as their multiple subunits can be modified with chemoselective bioconjugation reactions to position synthetic groups in specified locations. With a view toward the development of photocatalytic or chemically catalytic materials, porphyrins have been templated noncovalently by the surface of bacteriophage M13 to enable sensitization by tryptophan residues in the coat proteins,[17] iron porphyrins have been captured by using poly(His) tags introduced into the cowpea mosaic virus coat protein,[18] and the coat proteins of the tobacco mosaic virus (TMV) have been used to arrange both fluorescent dyes[19] and porphyrins[20] within the RNA-binding channel of the virus.

An important next step in this field is the attachment of several different types of groups to the protein scaffolds; this goal requires multiple site-selective bioconjugation reactions. Herein we demonstrate this concept by using a multistep synthetic protocol to arrange fluorescent dyes and a zinc porphyrin on the surface of bacteriophage MS2. This positioning enables energy transfer and sensitization of the porphyrin at previously unusable wavelengths, as demonstrated by the ability of the system to effect a photocatalytic reduction reaction at multiple excitation wavelengths.

The protein shell of bacteriophage MS2 is comprised of 180 identical monomers arranged in a hollow shell with a diameter of 27 nm. The protein subunits can be expressed recombinantly in *Escherichia coli* and are obtained as fully assembled monodisperse capsids that are stable from pH 3 to pH 10 and at temperatures approaching 60 °C.[21–23] A total of 32 holes of 1.8 nm in diameter enable appreciably large reagents to enter the structures for interior surface modification.[21,24] To create photocatalytic materials, we chose to attach donor chromophores to the interior surface of MS2 and to position zinc porphyrins capable of electron transfer on the exterior (Figure 1 a). Such an arrangement would function by fluorescence resonance energy transfer (FRET) through the 2 nm thick protein shell. Zinc porphyrin photocatalysts have been used in a variety of energy-conversion systems[25–27] owing to their ability to transfer electrons to carbon nanotubes or fullerenes,[28,29] or to electron carriers (such as methyl viologen, MV^{2+}) that can be used to produce H_2, NADH, NADPH (the reduced forms of nicotinamide adenine dinucleotide and nicotinamide adenine dinucleotide phosphate, respectively), and lactic acid.[27,30,31]

We targeted the interior of the capsid by changing the native N87 residue into a cysteine residue and modifying it with maleimide-containing dyes (Figure 1 b). We chose dyes that absorb at wavelengths at which the porphyrin does not, and with emission spectra that overlap with a particular porphyrin absorbance band. Alexa Fluor 350 (**1**) was chosen to sensitize the porphyrin Soret band at 424 nm, and Oregon Green 488 (**2**) was selected to target the first porphyrin Q band at 557 nm (Figure 2). Treatment of the capsids with the maleimide dyes (20 equiv) for 2 h at room temperature resulted in complete modification of the monomers (180 dyes per capsid), as determined by ESIMS (see Figure S2 in the Supporting Information). Furthermore, the small Stokes shift of **2** enables FRET to occur between apposed donor dyes, as the distance between adjacent C87 residues (0.9–3.7 nm)[22] is below the Förster radius of 4.0 nm for FRET between two molecules of **2**.[19] The two native cysteine residues of the capsid proved unreactive towards the modification conditions (see Figure S7 in the Supporting Information).

As there are six lysine residues on each monomer, modification methods based on *N*-hydroxysuccinimide

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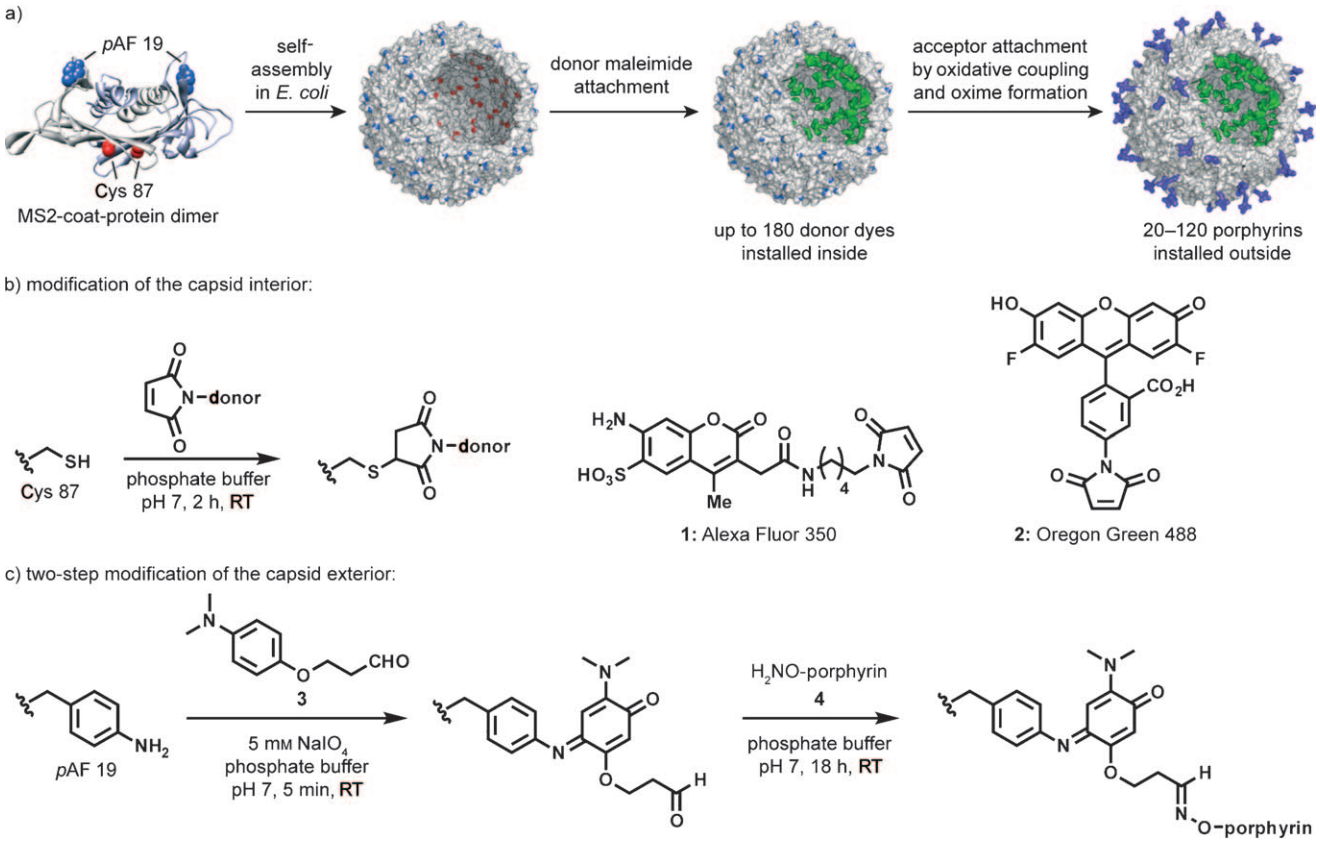
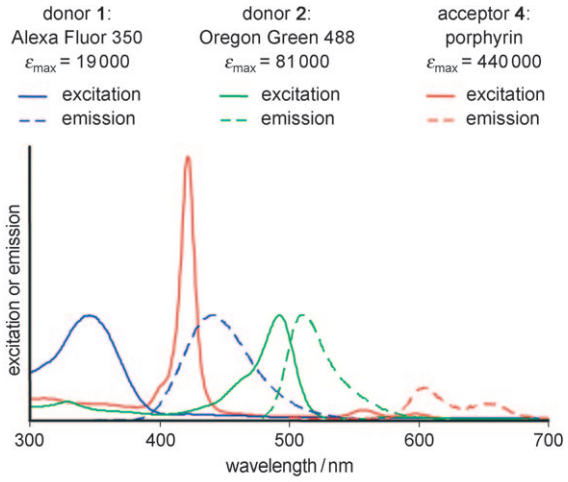


Figure 1. Modification of N87C T19*p*AF MS2. a) Two mutations (N87C and T19*p*AF) were introduced into subunits of the MS2 coat protein. After capsid formation in *E. coli*, the interior and exterior surfaces were differentially modified in a multistep sequence. b) The interior of the capsid was modified at C87 (red) with either Alexa Fluor 350 (**1**) or Oregon Green 488 (**2**) maleimide dyes. Up to 180 copies of each chromophore were installed. c) The exterior of the capsid was modified first through an oxidative coupling reaction to attach aldehyde **3** to the *p*AF19 groups (blue), and subsequently with the aminooxy-containing porphyrin **4** to form stable oxime linkages. This design enables energy transfer from the dye inside the capsid to the porphyrin on the outside through FRET.

esters[24] would not be suitable for the installation of a single porphyrin per capsid monomer. Instead, we targeted the artificial amino acid *p*-aminophenylalanine (*p*AF), which we previously showed[32] can be incorporated into MS2 capsids by using the amber-codon-suppression technique developed by Schultz and co-workers.[33,34] The N87C/T19*p*AF MS2 double mutant was used in all experiments described herein and was obtained by recombinant expression in *E. coli* that possessed the appropriate tRNA and aminoacyl tRNA synthetase with a yield of 10 milligrams per liter of culture.

Previously, we reported the coupling of phenylenediamine derivatives to *p*AF in 30–60 min with aqueous periodate.[32,35] We have since found that *N*,*N*-dimethylanisidine derivatives couple to this residue with equivalent chemoselectivity in only a few minutes (see Figure S8 in the Supporting Information). This process enables the rapid installation of up to 180 copies of aldehyde **3** on the capsid exterior (Figure 1c). We subsequently modified the aldehyde with the aminooxy-containing porphyrin **4**, the synthesis of which was based on previously reported procedures,[20,36,37] in concentrations ranging from 20 μM to 2.5 mM. The degree of porphyrin attachment relative to the donor dye (Figure 3c) was determined by comparing the extinction coefficients of the Soret band of **4** with the absorbance maximum of donor **1** or **2**. Capsids without aldehyde **3** showed only minimal noncovalent association of the porphyrin (see the Supporting Information). Although the porphyrins can also be attached to *p*AF in a single step, we chose instead to develop this two-step bioconjugation procedure to enable the future attachment of metal catalysts that would be sensitive to the periodate used in the oxidative coupling reaction. In the doubly modified capsids, the dyes were positioned at a distance of 2.4–3.1 nm from the porphyrins (as measured from the crystal structure).[22] The capsids remained intact and water soluble throughout all synthetic steps, as determined by size-exclusion chromatography and transmission electron microscopy (see Figures S3 and S4 in the Supporting Information).

To detect FRET from the dyes inside the capsids to the porphyrins on the outside, we obtained excitation spectra for the conjugates by monitoring the porphyrin fluorescence (Figure 4). The capsids showed porphyrin fluorescence upon excitation of donor **1** or **2**, which indicated that energy transfer did indeed occur through the protein shell. A greater excitation contribution was observed at the donor wavelength relative to the intensity of the porphyrin excitation band for systems with fewer porphyrins per donor. This result indi-



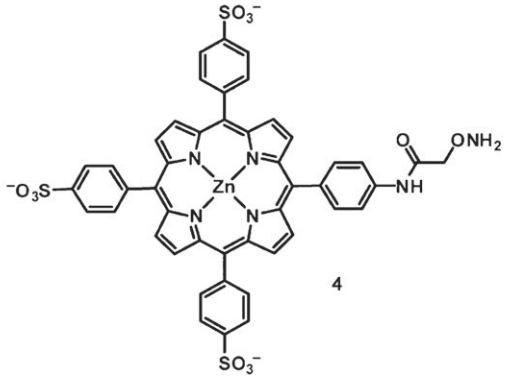
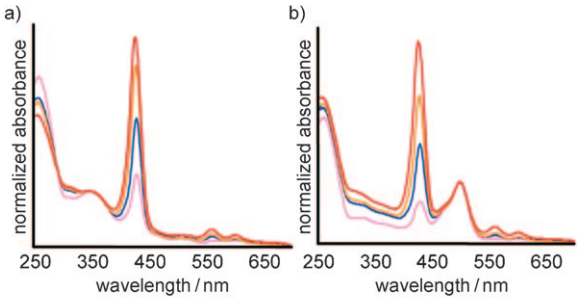


Figure 2. Comparison of the excitation and emission spectra of the chromophores used in this study. FRET is possible owing to the overlap of the Alexa Fluor 350 emission band with the porphyrin Soret band and the overlap of the Oregon Green 488 emission band with the first porphyrin Q band.

cated that a greater number of dyes contributed to the fluorescence of any single porphyrin.

The energy transfer was further confirmed by monitoring the quenching of the donor emission by the porphyrin. The emission spectra upon excitation at either 350 nm (for **1**) or 500 nm (for **2**) are shown in Figure 5 a,b. The normalized spectra for capsids containing either donor **1** or donor **2** (as well as aldehyde **3**) but lacking acceptor **4** are also shown. The decreasing donor emission as well as the increasing porphyrin fluorescence emission in Figure 5 c,d further demonstrated energy transfer.

We next investigated the electron-transfer ability of the porphyrin, because this property provides a more relevant way to use the transferred energy. The excited state of zinc porphyrins can reduce the methyl viologen dication (MV^{2+}) to its radical cation ($MV^{+\bullet}$), the formation of which can be monitored on the basis of its absorbance at 605 nm.[27,30] By the inclusion of 2-mercaptoethanol as a sacrificial reductant, the porphyrin cation that is generated can be reduced to close the catalytic cycle (Figure 6 a). Because the peak of the solar spectrum occurs at around 500 nm,[38] we used capsids containing donor **2** for these investigations and illuminated them with light of wavelength 505 nm from a home-built light-



c)

Color	[4]	Alexa Fluor 350 (**1**) **1/4**	Alexa Fluor 350 (**1**) **4**/capsid	Oregon Green (**2**) **2/4**	Oregon Green (**2**) **4**/capsid
	20 μM	8.5:1	21:1	8:1	23:1
	100 μM	4.5:1	40:1	3.3:1	55:1
	500 μM	3:1	60:1	2:1	90:1
	2.5 mM	2.5:1	72:1	1.6:1	113:1

Figure 3. UV/Vis spectra of the a) MS2–Alexa Fluor–porphyrin and b) MS2–Oregon Green–porphyrin systems. The spectra are normalized at the absorbance maximum of the donor dye. c) The ratio of the donor to the porphyrin was calculated by comparing the absorbance ratios and using the extinction coefficients listed in Figure 2. The number of porphyrins per capsid was calculated by assuming complete dye conversion and dividing the total number of monomers (180) by the number of dyes per porphyrin.

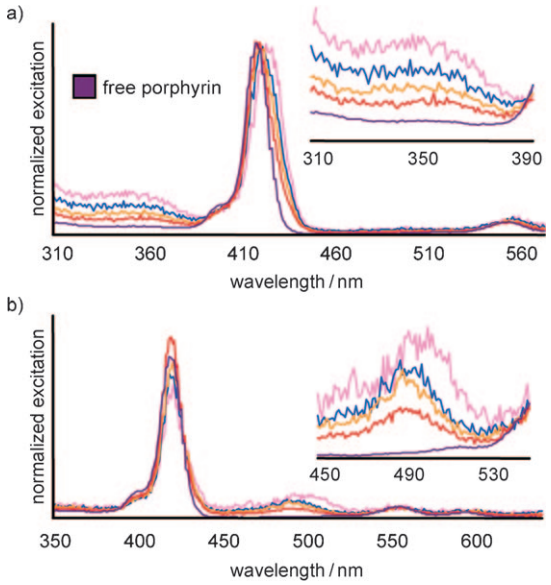


Figure 4. Excitation spectra of conjugates a) MS2–**1**–**4** and b) MS2–**2**–**4**. The insets show expansions of the areas of maximum donor excitation. The MS2–**1**–**4** spectra were normalized at the porphyrin Soret band, and excitation was monitored at the first porphyrin emission band at 602 nm. The MS2–**2**–**4** spectra were normalized at the first porphyrin Q band, and excitation was monitored at the second porphyrin emission band at 655 nm to minimize any residual fluorescence emission from the Oregon Green dye. The colors correspond to the chromophore ratios listed in Figure 3 c. The slight red shift observed at lower modification levels is possibly due to coordination of the two surface-accessible exterior lysine residues to the zinc center.

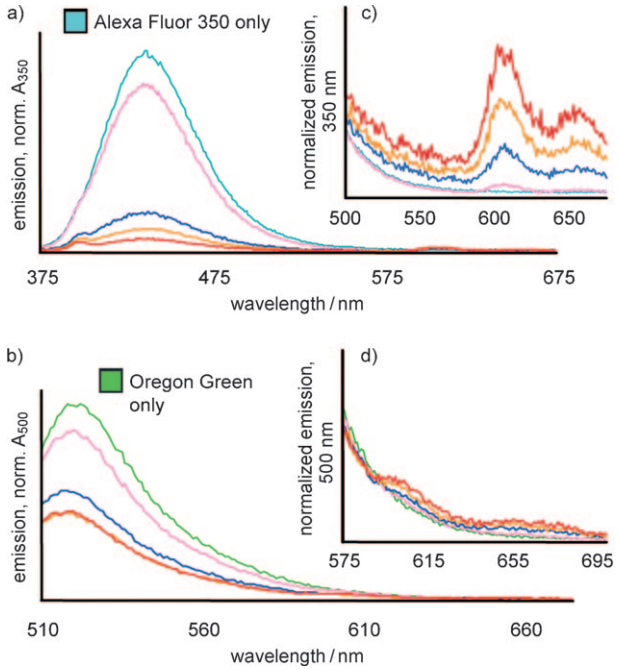


Figure 5. Emission spectra upon donor excitation for the systems a) MS2–**1**–**4** and b) MS2–**2**–**4**, with emission normalized with respect to the donor absorbance. Increased amounts of porphyrin result in greater dye quenching owing to energy transfer. The insets show the emission of the porphyrin in systems c) MS2–**1**–**4** (emission normalized at 350 nm) and d) MS2–**2**–**4** (emission normalized at 500 nm); the emission of the porphyrin is normalized at the donor emission maximum to demonstrate the relationship between donor quenching and acceptor emission. The two porphyrin emission bands at 602 and 655 nm are clearly visible.

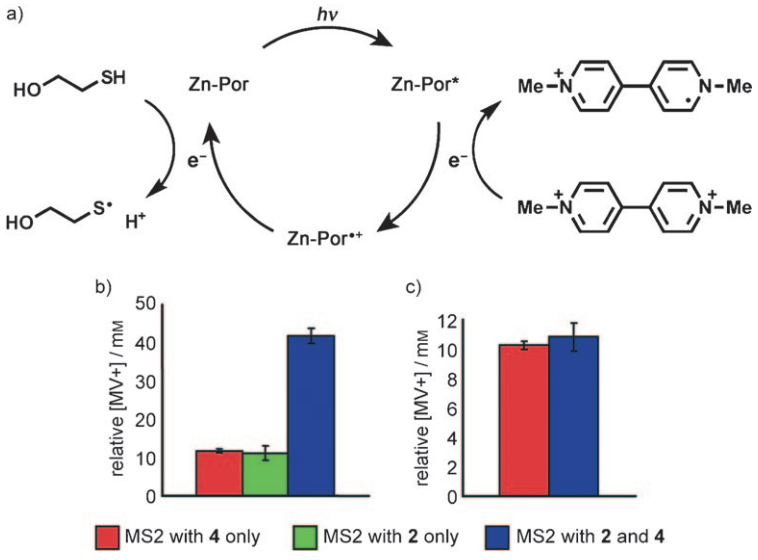


Figure 6. Photoreduction of methyl viologen by a sensitized porphyrin system. a) Catalytic cycle of the photoreduction. b) Relative efficiency of the photoreduction with different systems upon illumination at 505 nm for 15 min. c) Relative efficiency of the photoreduction with different systems upon illumination at 415 nm for 0.5 min. The different reaction times were required as a result of the difference in the extinction coefficients of **2** and **4**. Values correspond to the average and standard deviation for three measurements.

emitting-diode (LED) lamp. We evaluated capsids with porphyrin **4** but no donor (system A), with donor **2** but no porphyrin (system B), and with both donor **2** and porphyrin **4** in a ratio of 3:1 (system C). The solutions also contained MV^{2+} (150 μM) and 2-mercaptoethanol (200 mM). For systems A and C, the porphyrin concentration was approximately 300 nM; all quantities of $MV^{+\bullet}$ produced were normalized to that of system C. Upon illumination at 505 nm for 15 min, system C showed significantly higher $MV^{+\bullet}$ production (134 turnovers per porphyrin) in comparison to system A (38 turnovers per porphyrin; Figure 6b). This 3.5-fold increase in the production of $MV^{+\bullet}$ is due to the sensitization by donor **2** in system B.

Interestingly, system B (which contained donor **2** and aldehyde **3** but no porphyrin) also produced $MV^{+\bullet}$ upon illumination (Figure 6b), but only a quarter of that produced by system C. This result indicated that energy transfer to porphyrin **4** in system C plays a vital role in the electron-transfer process. In control experiments with the free dye **2** (but no MS2 or porphyrin **4**), $MV^{+\bullet}$ was also produced, which confirmed that the dye, rather than the capsids, the oxidative-coupling linkage, or aldehyde **3**, was responsible for the reduction of MV^{2+} in system B.

We also used a commercially available LED lamp with peak emission at 415 nm to irradiate the Soret band of porphyrin **4** directly. Systems A and C both produced similar amounts of $MV^{+\bullet}$ with or without donor **2** (Figure 6c). These experiments thus confirmed that the increased production of $MV^{+\bullet}$ at 505 nm was not a consequence of intrinsic differences in porphyrin activity, but rather that it could be attributed to energy transfer from **2**. All these results indicated that we had indeed sensitized the porphyrin to function catalytically at new wavelengths with our nanoscale integrated system.

In conclusion, we used the rigid protein scaffold of bacteriophage MS2 to construct an integrated, nanoscale system that positions sensitizing dyes and a photocatalytic porphyrin in specific locations. Energy transfer from the inside to the outside of our modified capsids enables sensitization of the porphyrin at previously inaccessible wavelengths and thus broad-spectrum catalytic activity. In principle, our system can be used with any combination of maleimide and aminooxy reagents; therefore, considerable flexibility is possible in the choice of an appropriate donor for a given acceptor. Current efforts are underway to build similar integrated systems with phthalocyanines[39] and ruthenium bipyridine[40] complexes as the active catalysts. As a general method, the integration of two different chemical groups at precisely defined locations demonstrates the power and flexibility of the use of viral capsids as nanoscale scaffolds. This approach will be relevant to both applied and fundamental investigations of complex photocatalytic systems in the future.

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