Electronic Supplementary Material (ESI) for Analytical Methods.

Supporting Information

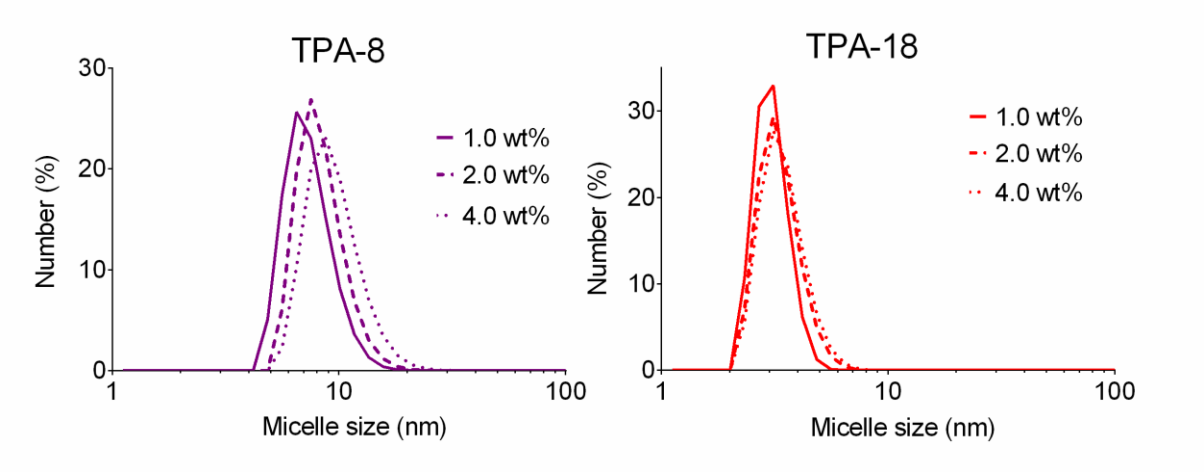


Figure S1. Representative diagrams for the size distribution of micelles formed by a previously reported TPA (TPA-8) and a newly prepared TPA (TPA-18) obtained by dynamic light scattering (DLS). These agents were used at 1.0 wt%, 2.0 wt%, and 4.0 wt % for comparison. Scattered light intensity depending on the time scale of micelle movements was analyzed. These compounds form a single set of micelles.

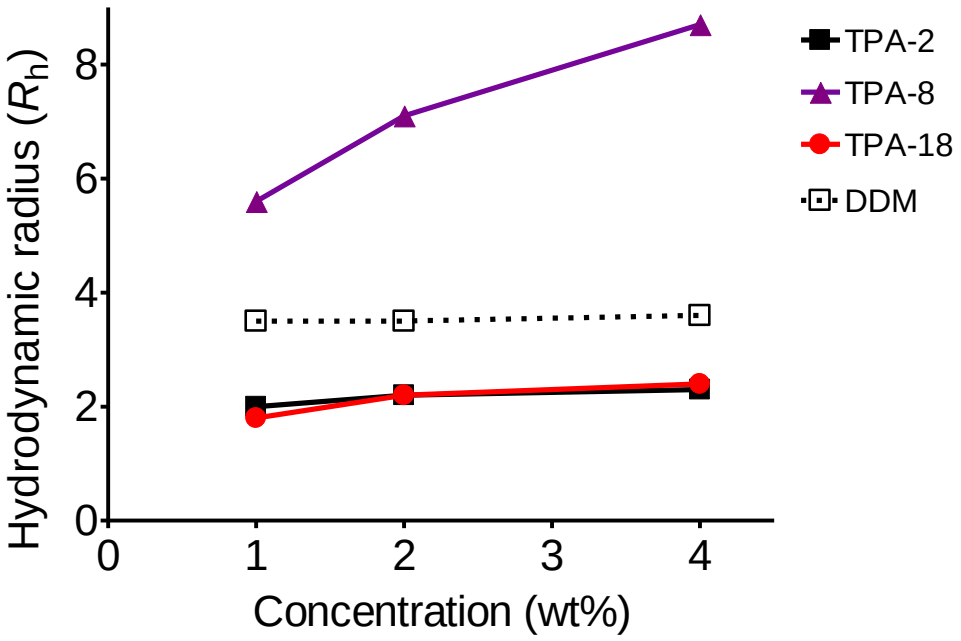


Figure S2. Variations in the hydrodynamic radii (R_h) of tripod amphiphiles (TPA-2, TPA-8, and TPA-18) and DDM. Each agent was evaluated at three different concentrations (1.0 wt%, 2.0 wt%, and 4.0 wt %). Branched amphiphiles such as TPAs tended to increase in micelle size, whereas linear amphiphiles (e.g., DDM) showed little change in the micelle size, as the detergent concentration increased. Please see Table 2 in the main text for details.

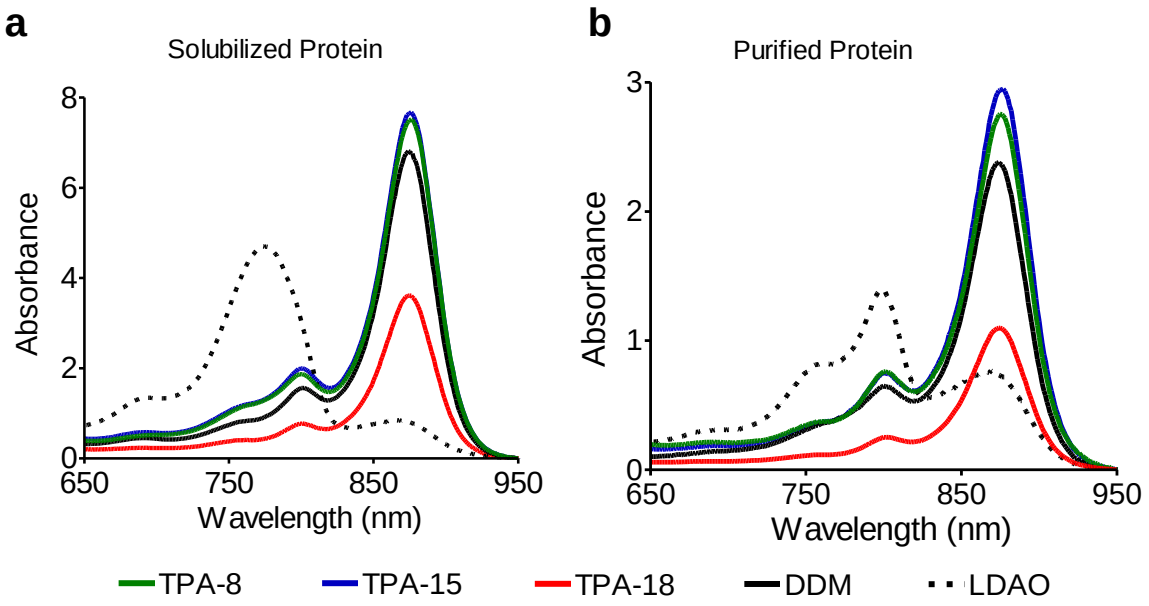
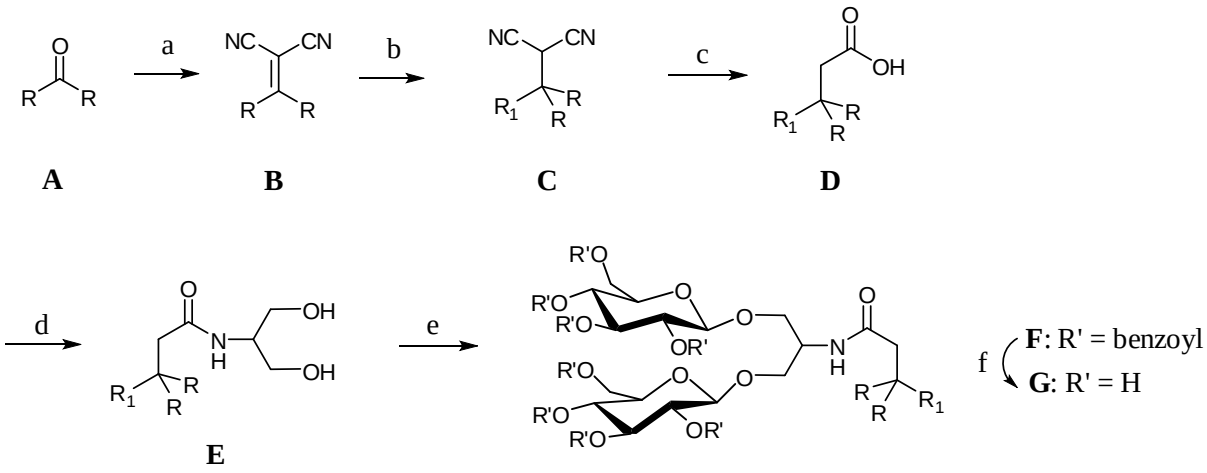


Figure S3. Absorbance spectra of *R. capsulatus* superassembly (a) solubilized, and (b) purified in two previously reported TPAs (TPA-2 and TPA-8), a newly reported TPA (TPA-18), and two conventional detergents (DDM and LDAO). Individual detergents were used at CMC + 1.0 wt% for protein solubilization. For spectroscopic measurements, detergent-solubilized protein portions were separated as supernatants by ultracentrifugation whereas detergent-insolubilized portions were obtained as pellets. Protein purification was conducted by Ni-immobilized affinity column chromatography with elution buffer containing 1×CMC of individual detergents and 1.0 M imidazole. All spectra were measured from 650 nm to 950 nm.



(a) malononitrile, benzene, 80°C, ~95%; (a) ArMgBr, Cu(I)CN, THF, 0°C; ~60% ; (b) KOH, ethylene glycol, 200°C, ~88%; (c) serinol, EDC • HCl, HOBt, DMF, room temperature, ~92%; (d) perbenzoylated glucosylbromide, AgOTf, CH_2Cl_2, -45°C → room temperature, ~90%; (e) NaOMe, MeOH, room temperature, ~93%.

Scheme S1. General synthetic scheme for previously reported glyco-tripod amphiphiles (e.g., TPA-8). The protocol consists of six synthetic steps: (a) Knoevenagel condensation of a ketone (**A**), (b) Michael addition of an alkylidene compound (**B**), (c) basic hydrolysis of a dinitrile derivative (**C**), (d) amide coupling of a carboxylic acid (**D**), (e) glycosylation of a diol (**E**) and (f) benzoyl group deprotection of a glycosylated compound (**F**). The typical yield of each step was indicated in the footnote of the scheme, giving overall yield of ~ 40%.

Table S1. Effect of buffer composition (pH and/or salt concentration) on critical micelle concentration (CMC; mM) of TPA-18.

Buffer composition[a]	Sodium phosphate (pH 7.0)	HEPES (pH 7.5)	Tris (pH 8.0)
[NaCl] = 0 mM	~8.3	~8.2	~8.3
[NaCl] = 100 mM	~7.9	~7.9	~8.0
[NaCl] = 200 mM	~7.5	~7.7	~7.6

[a] Buffer composition in term of pH and salt concentration. Buffer solutions with pH 7.0, 7.5 and 8.0 were prepared by using 20 mM sodium phosphate, 20 mM HEPES and 20 mM Tris, resepctively.

Protein solubilization, purification and long-term stability assay

Solubilization and purification of the *R. capsulatus* superassembly were performed according to the published protocol.[1] Briefly, specialized photosynthetic membranes called intracytoplasmic membranes were obtained from an engineered strain of *Rhodobacter (R.) capsulatus*, U43[pUHTM86Bgl], lacking the light-harvesting complex II (LHII). To begin the protein solubilization experiment, frozen aliquots of *R. capsulatus* membranes were thawed and homogenized at room temperature for 30 min. The solution was then agitated at 32℃ for 30 min. Subsequently, the solution was treated with CMC+1.0 wt% DDM, LDAO or TPAs (TPA-8, TPA-15 and TPA-18) and incubated for 30 min before ultracentrifugation at 310,000 × *g* at 4℃ for 30 min. The UV-vis spectra of solubilized supernatant and insolubilized pellet were measured over the range from 650 nm to 950 nm. For the protein purification, individual detergents-solubilized samples were transferred into new microcentrifuge tubes including Ni-NTA resin pre-equilibrated in an equal volume of buffer containing 10 mM Tris, pH 7.8, and 100 mM NaCl. After 1-hour incubation at 4℃ under a dark condition, the resins were filtered and washed twice with 0.5 mL of binding buffer (10 mM Tris, pH 7.8 containing the respective detergent at 1×CMC). Each detergent-purified protein solution could be obtained by washing the resins with 0.20 mL of elution buffer containing 1 M imidazole three times (the pH of each solution was readjusted to pH = 7.8) and dilution with 0.4 mL of binding buffer to give 1.0 mL of protein solution. The UV-Vis spectra of these protein samples were measured over the range from 650 nm to 950 nm.

The long-term stability of LHI-RC complexes was measured as follows. DDM-purified protein sample (20 μL) was mixed with amphiphile solution (980 μL) containing individual TPAs (TPA-2, TPA-8, TPA-15, and TPA-18) or conventional detergents (LDAO, OG, and DDM) to reach the final detergent concentration: CMC + 0.04 wt% (low), CMC + 0.2 wt% (medium), or CMC + 1.0 wt% (high). By this dilution, the concentration of DDM became around 0.00044 wt% that is far lower than its CMC value (~0.0087 wt%). UV-Visible spectra of these protein samples in individual agents were measured at regular intervals over 20 days of incubation at room temperature. The integrity of the LHI-RC complexes was assessed by monitoring absorbance at 875 nm (A_{875}).

References

1. P. S. Chae, M. J. Wander, A.P. Bowling, P.D. Laible, S.H. Gellman, *ChemBioChem* **2008**, *9*, 1706-1709.