

Supporting Information

Porphyrin-Based Probe for Simultaneous Detection of Interface Acidity and Polarity during Lipid-Phase Transition of Vesicles

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Contents

Synthetic protocol of GPP (2)	p. S3–S5
Table S1 Acid/base pK_a values for GPP (2) at various temperature and bulk dielectric constant of buffer solutions in the presence and absence of acetone.	p. S6
Table S2 Acid/base pK_a values for GPP (2) at various temperature and bulk dielectric constants in the presence amphiphilic self-assembled systems	p. S7
Figure S1 UV-vis absorption spectra of GPP (2) in buffer containing low amount of acetone.	p. S8
Figure S2 UV-vis absorption spectra of GPP (2) in buffer containing methanol or ethanol.	p. S9
Figure S3 UV-vis absorption spectra of porphyrin (1) in buffer containing methanol or acetone.	p. S10
Figure S4 Temperature-dependent UV-vis absorption spectra of GPP (2) in mixed solvents.	p. S11
Figure S5 pH-dependent UV-vis absorption spectra of GPP (2) in buffer containing acetone.	p. S12
Figure S6 pH- and temperature-dependent UV-vis absorption spectra of GPP (2) in buffer containing acetone	p. S13
Figure S7 DLS measurement showing particle size distribution profile of DMPG LUV	p. S14
Figure S8 Cryo-transmission electron microscope (TEM) image of DMPG LUV	p. S15
Figure S9 NOESY spectrum of the mixture of GPP (2) and DMPG LUV at 20 °C.	p. S16
Figure S10 UV-vis absorption spectra of GPP (2) in presence of various concentration of self-assembled system.	p. S17
Figure S11 pH-dependent UV-vis absorption spectra of GPP (2) in the presence of SDS micelle at various temperatures.	p. S18

Figure S12 pH-dependent UV-vis absorption spectra of GPP (**2**) in presence of DMPG LUV at various temperatures.

p. S19

References

p. S20

Synthetic protocol of GPP (2)

Synthesis of 4-aceoxybenzaldehyde (6). In a 200-mL round-bottom flask, 4-hydroxybenzaldehyde (**5**, 2.44 g, 20 mmol) was suspended in CH₂Cl₂ (50 mL). On the addition of trimethylamine (2.22 g, 22 mmol), the suspension turned into a clear pale yellow solution. To the solution was added DMAP (0.12 g, 1 mmol) and acetic anhydride (2.64 g, 22 mmol). The solution was stirred at room temperature for 45 min. After the complete of reaction was confirmed by silica TLC (eluent: hexane/AcOEt = 1/1), the solution was diluted with CH₂Cl₂. The solution was washed with water (x 2), 0.1 M HCl (x 2) and 5% NaHCO₃ subsequently before dried over Na₂SO₄. The solvent was evaporated to yield **6** as colorless oil (3.3 g, quant). ¹H NMR (400 MHz, CDCl₃, TMS) δ_H: 9.998 (s, 1H, -CHO), 7.927 (dd, AA'BB', 2H, J_{AB} = 8.7, J_{AA'} = 4.1 Hz, J_{AB'} = 2.8 Hz, 2,6-phenyl protons), 7.284 (AA'BB', 2H, J_{AB} = 8.7, J_{AA'} = 4.1 Hz, J_{AB'} = 2.8 Hz, 3,5-phenyl protons) and 2.370 (s, 3H, -OAc); ¹³C NMR (100 MHz, CDCl₃, TMS) δ_C: 190.74, 168.70, 154.47, 133.68, 130.79, 122.18, 20.98; HR-MS (EI, positive): [M⁺] for C₉H₈O₃; Calcd. 164.0473; Found 164.0470.

Synthesis of 5,10,15,20-tetrakis(4-acetoxyphenyl)porphyrin (7). In a 100-mL round-bottom flask equipped with a condenser, compound **6** (0.473 g, 2.88 mmol) was suspended in propionic acid (15 mL). Pyrrole (0.193 g, 2.88 mmol, 200 μL) was added at 80 °C, and the solution was refluxed for 30 min with avoiding light. After the resultant deep purple solution was cooled to room temperature, porphyrin deposited from the solution was collected by suction. The purple solid was rinsed with cold MeOH till washing solution became colorless. After the solid was rinsed with hexane, the solid was dried *in vacuo* to yield **7** as purple powder (0.128 g, 21% yield). ¹H NMR (400 MHz, CDCl₃, TMS) δ_H: 8.879 (s, 8H, β-protons), 8.215 (d, 8H, J = 8.5 Hz, 2,6-phenyl protons), 2.510 (s, 3H, -OAc) and -2.838 (br, 2H, inner-NH); ¹³C NMR (100 MHz, CDCl₃, TMS) δ_C: 169.68, 150.79, 139.65, 135.29, 120.34, 119.08, 21.21 (α- and β-Carbons in pyrrole rings were not detected under the measurement conditions); HR-MS (MALDI; matrix: DCTB, positive): [M⁺] for C₅₂H₃₈N₄O₈; Calcd. 846.2684; Found 846.2685.

Synthesis of 5,10,15,20-tetrakis(4-hydroxyphenyl)porphyrin (8). In a 100-mL round-bottom flask equipped with a condenser, compound **7** was suspended in a mixed solvent of H₂O (3 mL) and EtOH (1 mL). The addition of conc. HCl (1 mL) made a green suspension. The suspended solution was refluxed for 1.5 h. After the residual of the starting material was confirmed on a silica TLC plate (eluent: CHCl₃/MeOH = 5/1), H₂O (3 mL), EtOH (1 mL) and conc. HCl (1 mL) were further added. After refluxed for 2 h, the green suspension was cooled to room temperature. The reaction mixture was diluted with water and neutralized with 2 M NaOH to adjust pH = 8 (checked by pH test paper). During this procedure, the suspension turned to be purple in color. The solution was stirred for 30 min in the dark, and AcOEt (50 mL) was added. The mixed solution was vigorously stirred for 30 min and transferred into a separatory funnel. The organic phase (purple in color) was separated and dried over Na₂SO₄. After the solvent was evaporated, the formed purple solid was suspended in CH₂Cl₂ and collected by suction. The solid was subsequently rinsed with CH₂Cl₂ and hexane to yield porphyrin **8** as purple solid (90 mg, 88% yield). ¹H NMR (400 MHz, DMSO-*d*₆, TMS) δ_H: 9.971 (s, 4H, phenol-OH), 8.855 (s, 8H, β-protons), 7.988 (d, 8H, J = 8.0 Hz, 2,6-phenyl protons), 7.206 (d, 8H, J = 8.0 Hz, 3,5-phenyl protons), -2.907 (br, 2H, inner-NH); ¹³C NMR (100 MHz, CDCl₃, TMS) δ_C: 157.16, 135.96, 131.51, 119.64, 114.13 (α- and β-Carbons in pyrrole rings were not detected under the measurement conditions); HR-MS (MALDI; matrix: DCTB, positive): [M⁺] for C₄₄H₃₀N₄O₄; Calcd. 678.2262; Found 678.2262.

Synthesis of 2,3,4,6-tetra-*O*-acetyl-1-*O*-(3-chloropropyl)- β -D-glucopyranoside (10**).** In a 200-mL three-neck flask with a dropping funnel, 1,2,3,4,6-penta-*O*-acetyl-D-glucopyranoside (**9**, 3.90 g, 10 mmol) and 3-chloropropanol (1.88 g, 20 mmol) were dissolved in dry CH₂Cl₂ (25 mL) under a N₂ atmosphere. The solution was cooled with an ice bath (below 5 °C). To the solution was added BF₃•Et₂O (7.1 mL, *ca.* 5 equiv.) dropwise. The solution was stirred in the ice bath for 1 h and at room temperature for 12 h. After neutralized by trimethylamine (5.05 g, 50 mmol) slowly, the solution was concentrated and AcOEt (100 mL) was added. The solution was washed with water (x 3), 5% NaHCO₃ (x 2) and brine. The organic phase was dried over Na₂SO₄. The solvent was evaporated and the residue was subjected to a silica gel chromatography with elution of hexane/AcOEt = 1/1. The product was detected on a TLC plate stained in Von's reagent. After evaporation of solvent, compound **10** was obtained as colorless viscous oil (1.90 g, 45% yield). ¹H NMR (400 MHz, CDCl₃, TMS) δ _H : 5.227 (dd, *J* = 9.5 Hz, 9.5 Hz, 1H, sugar-H3), 5.097 (dd, *J* = 9.5 Hz, 9.5 Hz, 1H, sugar-H4), 5.002 (dd, *J* = 9.5 Hz, 8.0 Hz, 1H, sugar-H2), 4.522 (d, *J* = 8.0 Hz, 1H, sugar-H1), 4.283 (dd, *J* = 4.8 Hz, 14.5 Hz, 1H, sugar-6H_a), 4.153 (dd, *J* = 2.6 Hz, 12.5 Hz, 1H, sugar-6H_b), 4.010 (ddd, *J* = 5.1 Hz, 5.0 Hz, 9.5 Hz, 1H, sugar-5H), 3.716 (m, 2H, -OCH₂CH₂CH₂Cl), 3.618 (dd, *J* = 5.1 Hz, 6.8 Hz, 2H, -OCH₂CH₂Cl), 2.104 (s, 3H, -OAc), 2.074 (s, 3H, -OAc), 2.040 (s, 3H, -OAc), 2.024 (s, 3H, -OAc), 1.980 (m, 2H, -OCH₂CH₂CH₂Cl); ¹³C NMR (100 MHz, CDCl₃, TMS) δ _C : 171.39, 171.03, 169.94, 101.75, 73.38, 72.48, 71.91, 69.03, 67.08, 62.57, 42.02, 32.83, 21.44, 21.34, 21.31, 21.30; HR-MS (MALDI; matrix: DCTB, positive): [M+Na⁺] for C₁₇H₂₅ClO₁₀Na; Calcd. 447.1028; Found 447.1024.

Synthesis of tetraacetylglucose-pendant porphyrin **11.** In a 100-mL two-neck flask with a condenser and a CaCl₂-tube, porphyrin **8** (0.25 g, 0.45 mmol) was dissolved in dry DMF (20 mL) under a N₂ atmosphere. To the solution were added glucose derivative **10** (1.26 g, 3.0 mmol), KI (0.743 g, 4.48 mmol) and K₂CO₃ (1.86 g, 13.5 mmol). The reaction mixture was stirred at 60 °C for 13 h. The product formation was confirmed by TLC (elution: CHCl₃/MeOH = 10/1). Insoluble materials were removed by filtration and rinsed with CH₂Cl₂ on a filter paper. The mother liquor was concentrated using a rotary evaporator (65 °C, 35 mbar). Water was added the residue and filtered. The resultant purple gummy material was dissolved in AcOEt. The solution was washed with brine and dried over Na₂SO₄. After removal of the salt, silica gel (5 g) was added to the solution, and the solvent was slowly evaporated to have porphyrin pre-absorb onto silica. The porphyrin-absorbed silica was load onto a silica gel equilibrated with hexane/AcOEt = 1/1 (column volume (CV) = 65 mL). The porphyrin component was washed on silica with hexane/AcOEt = 1/1 (2CV) to remove excess the glucose derivative. After that the porphyrin was eluted with hexane/AcOEt = 1/4. Purple fraction was collected and the solvent was evaporated. Porphyrin **11** was obtained by addition of hexane as purple solid (0.62 g, 61% yield). ¹H NMR (600 MHz, CDCl₃, TMS) δ _H : 8.843 (s, 8H, β -protons), 8.115 (d, *J* = 8.8 Hz, 8H, 2,6-phenyl protons), 7.273 (d, *J* = 8.8 Hz, 8H, 3,5-phenyl protons), 5.275 (dd, *J* = 9.5 Hz, 9.5 Hz, 4H, sugar-H3), 5.153 (dd, *J* = 9.5 Hz, 9.5 Hz, 4H, sugar-H4), 5.098 (dd, *J* = 9.5 Hz, 8.0 Hz, 4H, sugar-H2), 4.650 (d, *J* = 8.0 Hz, 4H, sugar-H1), 4.320 (m, 12H, -OCH₂CH₂CH₂OPh and sugar-6H_a), 4.206 (m, 8H, -OCH₂CH₂CH₂OPh and sugar-6H_b), 3.919 (m, 4H, -OCH₂CH₂CH₂OPh), 4.010 (ddd, *J* = 9.5 Hz, 4.4 Hz, 2.2 Hz, 4H, sugar-5H), 2.262 (m, 2H, -OCH₂CH₂CH₂OPh), 2.130 (s, 3H, -OAc), 2.079 (s, 3H, -OAc), 2.053 (s, 3H, -OAc), 2.038 (s, 3H, -OAc), -2.271 (br, 2H, -inner-NH); ¹³C NMR (150 MHz, CDCl₃, TMS) δ _C : 170.77, 170.36, 169.47, 169.41, 158.68, 135.63, 134.68, 119.70, 112.65, 101.10, 72.82, 71.87, 71.37, 68.40, 66.79, 64.34, 61.95, 29.59, 20.82, 20.72, 20.66, 20.64 (α - and β -Carbons in

pyrrole rings were not detected under the measurement conditions). After confirmation of NMR spectral information, this material was used for next reaction as is.

Synthesis of glucose-pendant porphyrin (GPP) 2. In a 100-mL round-bottom flask equipped with a CaCl₂-tube, tetraacetylglucose-pendant porphyrin **11** was dissolved in mixed solvent of MeOH (30 mL) and THF (7 mL). To the solution was added 28% NaOMe in MeOH (0.3 mL), and the solution was stirred at room temperature for 12 h. After neutralized by AcOH (checked pH by wet pH test paper), the solvent was evaporated. The residue was dissolved in pyridine and passed through a Sephadex LH-20 gel column with elution of pyridine/MeOH = 1/3. Purple band was collected and the solvent was evaporated. Addition of toluene afforded purple solid. The solid was collected by suction and suspended in toluene. After the suspension was stirred for 1 h, residual pyridine was removed by azeotrope with toluene three times. The solid was rinsed with CH₂Cl₂ and dried *in vacuo*. Porphyrin **2** was obtained as purple powder (320 mg, 74% yield). ¹H NMR (600 MHz, DMSO-*d*₆, residual protons in solvent) δ_H : 8.854 (s, 8H, β-protons), 8.095 (d, *J* = 8.3 Hz, 8H, 2,6-phenyl protons), 7.362 (d, *J* = 8.8 Hz, 8H, 3,5-phenyl protons), 5.139 (d, *J* = 4.8 Hz, 4H, -OH at sugar-H2), 5.000 (d, *J* = 4.8 Hz, 4H, -OH at sugar-H3), 4.957 (d, *J* = 4.8 Hz, 4H, -OH at sugar-H4), 4.563 (t, *J* = 6.0 Hz, 4H, -OH at sugar-H6), 4.361 (t, *J* = 5.4 Hz, 8H, -OCH₂CH₂CH₂OPh), 4.248 (d, *J* = 7.6 Hz, 4H, sugar-H1), 4.069 (dt, *J* = 6.6 Hz, 7.8 Hz, 4H, -OCH_aCH₂CH₂OPh), 3.771 (dt, *J* = 6.6 Hz, 7.8 Hz, 4H, -OCH_bCH₂CH₂OPh), 3.714 (dd, *J* = 5.4 Hz, 1.2 Hz, 4H, sugar-H6_a), 3.489 (dd, *J* = 6.0 Hz, 12 Hz, 4H, sugar-H6_b), 3.169 (m, 8H, sugar-H3 and sugar-H5), 3.097 (m, 4H, sugar-H4), 3.031 (ddd, 4H, *J* = 4.8 Hz, 7.6 Hz, 7.9 Hz, sugar-H2), 2.167 (m, 8H, -OCH₂CH₂CH₂OPh), -2.905 (br, 2H, inner-NH); ¹³C NMR (150 MHz, DMSO-*d*₆, solvent) δ_c : 158.73, 149.65, 135.43, 133.45, 119.80, 113.05, 103.80, 76.98, 76.77, 73.60, 70.12, 65.54, 64.91, 61.14, 29.46 (α- and β-Carbons in pyrrole rings were not detected under the measurement conditions); HR-MS (MALDI; matrix: DCTB, positive): [M⁺] for C₈₀H₉₄N₄O₂₈; Calcd. 1558.6049; Found 1558.6049.

Table S1. Acid/base pK_a values for GPP (**2**) at various temperature (T) and bulk dielectric constant ($\kappa(b)$) of buffer in the presence and absence of acetone.

		T (°C)	pK_a^a		$\kappa(b)^b$
			450 nm	685 nm	
Acetone% (w/w) in buffer	0	25	4.20 ± 0.02	4.20 ± 0.03	78.5
	28.0	25	4.10 ± 0.02	4.10 ± 0.03	62.0
	31.0	25	4.07 ± 0.02	4.07 ± 0.03	60.6
	31.0	35	3.94 ± 0.02	3.93 ± 0.03	58.1
	31.0	40	3.88 ± 0.02	3.90 ± 0.03	56.7
	37.0	25	3.90 ± 0.02	3.85 ± 0.02	56.4
	42.5	25	3.68 ± 0.02	3.67 ± 0.03	53.0
	60.0	25	3.35 ± 0.02	3.34 ± 0.03	42.0

^a pK_a values were estimated from the absorption intensities at ~450 nm and ~685 nm for GPP. ^b ref. 1

Table S2. Acid/base pK_a values for GPP (**2**) at various temperature (T) in the presence amphiphilic self-assembled systems.

		T (°C)	pK_a^a		$\kappa(b)^b$
			450 nm	685 nm	
Self assembly	SDS	20	–	4.83 \pm 0.03	80.4
		25	–	4.79 \pm 0.03	78.5
		35	–	4.66 \pm 0.03	76.8
		40	–	4.58 \pm 0.03	73.1
	DMPG	20	4.70 \pm 0.02	4.71 \pm 0.03	80.4
		25	4.74 \pm 0.02	4.74 \pm 0.03	78.5
		30	4.87 \pm 0.02	4.88 \pm 0.03	76.8
		35	4.98 \pm 0.02	4.99 \pm 0.03	75.0
		40	5.02 \pm 0.02	5.03 \pm 0.03	73.1

^a pK_a values were estimated from the absorption intensities at ~450 nm and ~685 nm for GPP. ^b ref. 1.

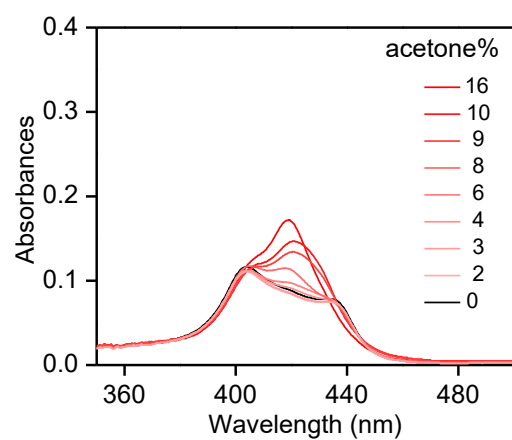


Figure S1. UV-vis absorption spectra of GPP (**2**) (2.5 μM) in 10 mM citrate/phosphate buffer containing different acetone% (*w/w*) (2–16), pH 6.0 at 25 °C. The spectrum in the absence of acetone is depicted by black.

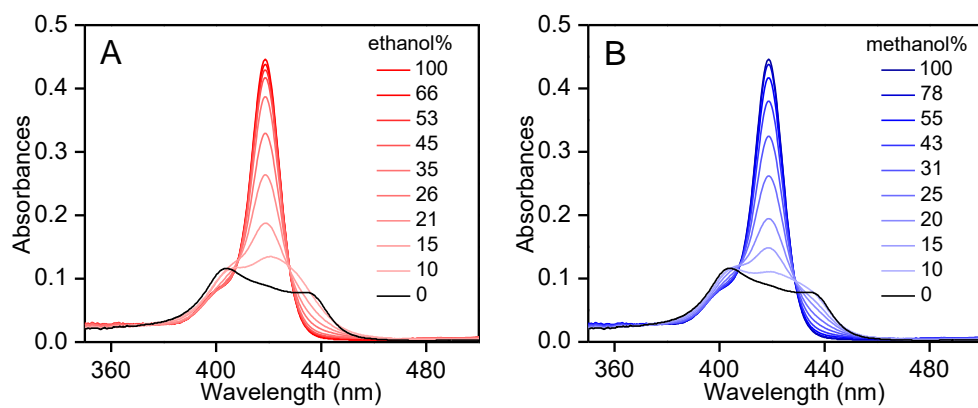


Figure S2. UV-vis absorption spectra of GPP (**2**) (2.5 μM) in 10 mM citrate/phosphate buffer containing various amount of (A) ethanol% and (B) methanol% (*w/w*) at pH 6.0 at 25 $^{\circ}\text{C}$.

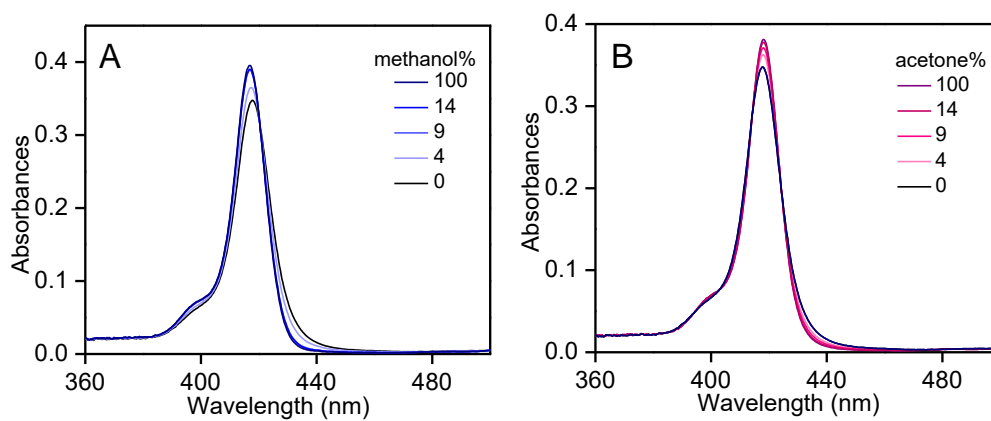


Figure S3. UV-vis absorption spectra of cationic porphyrin (**1**) (2.5 μM) in buffer containing various amount of (A) methanol% and (B) acetone% (w/w) at pH 7.0 at 25 °C. The spectra in the absence of acetone are depicted by black.

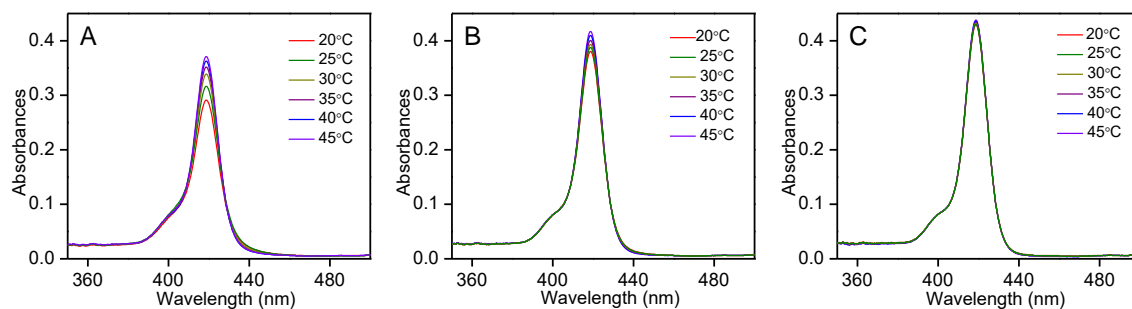


Figure S4. Temperature-dependent UV-vis absorption spectra of GPP (2) (2.5 μM) in 10 mM citrate/phosphate buffer containing various amount of acetone% (*w/w*) at pH 6.0: A, 24%; B, 33%; and C, 50%.

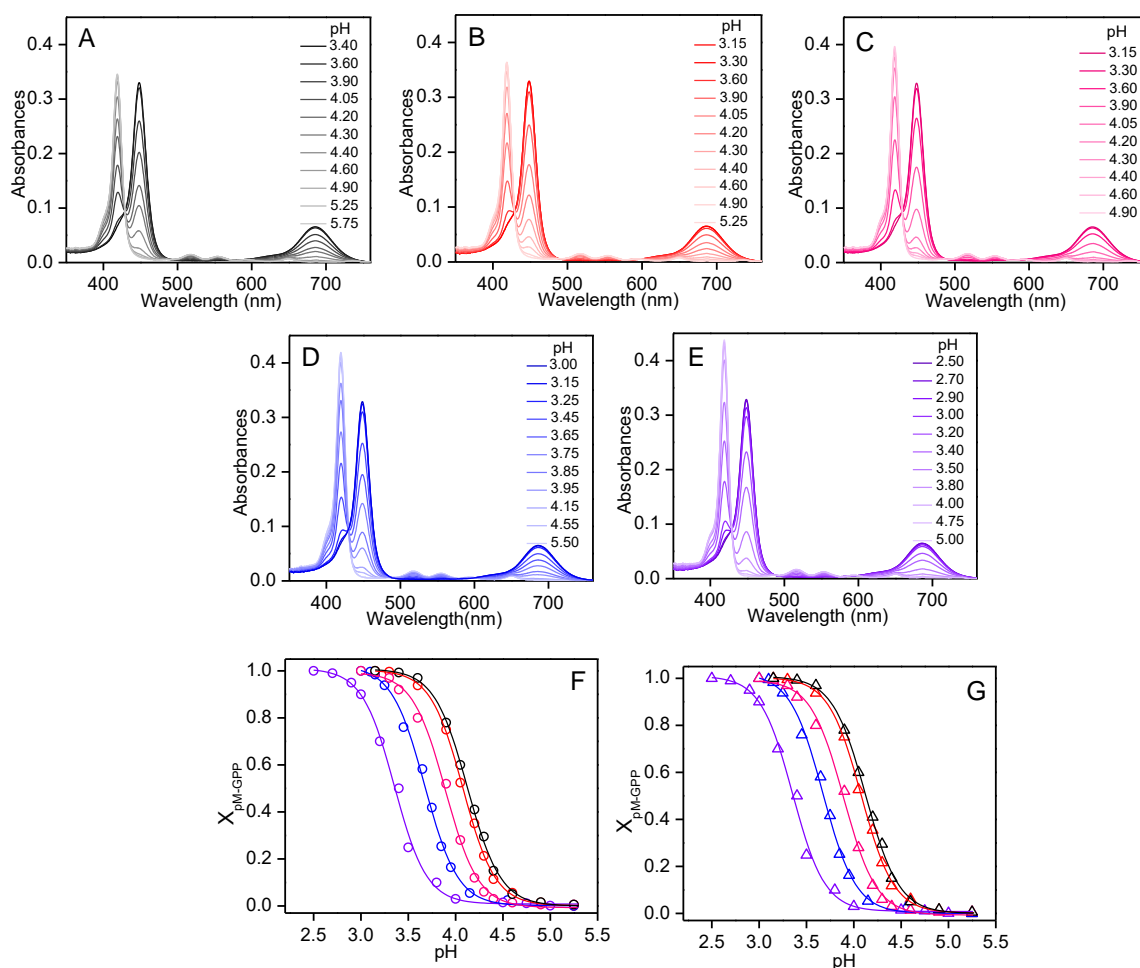


Figure S5. pH-dependent UV-vis absorption spectra of GPP (2) (2.5 μ M) in 10 mM sodium citrate/sodium phosphate buffer containing various amount of acetone% (w/w) at 25 $^{\circ}$ C: A, 28% (dielectric constant: 62.0); B, 31% (dielectric constant: 60.5); C, 37% (dielectric constant: 56.4); D), 42.5% (dielectric constant: 53.0) and E, 60% (dielectric constant: 42.0). (F,G) p_{M} -GPP mole-ratio ($X_{\text{pM-GPP}}$) analyzed from the intensity at \sim 450 nm (graph F) and \sim 685 nm (graph G) vs. pH in the buffer containing different wt% of acetone (Black: 28%; Red: 31%; Pink: 37%; Blue: 42.5% and Violet: 60%).

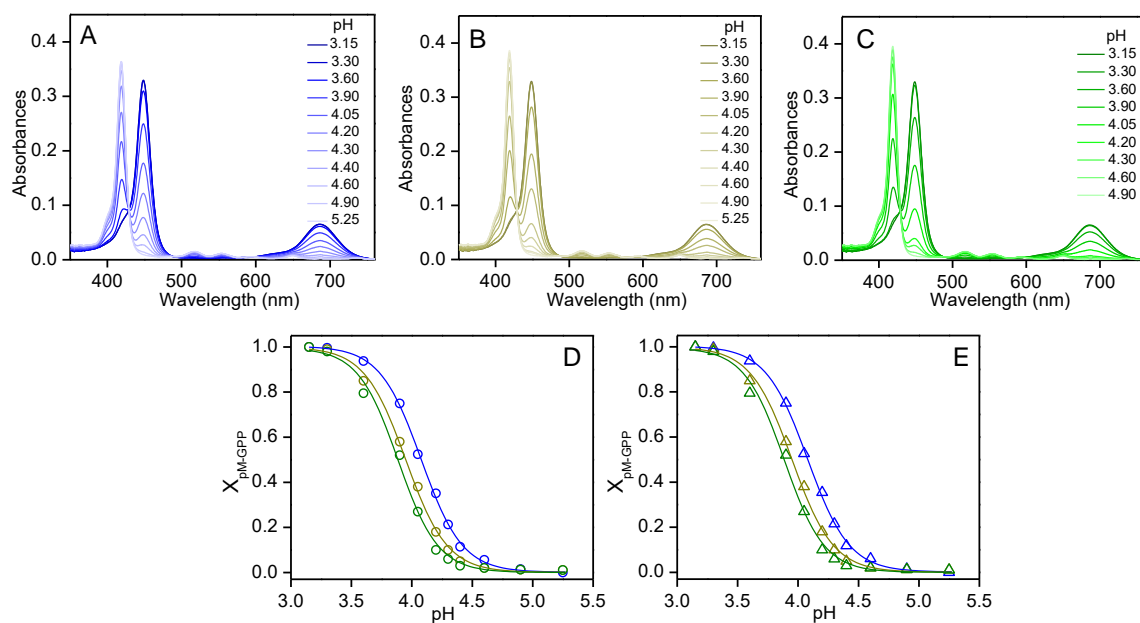


Figure S6. pH-dependent UV-vis absorption spectra of GPP (**2**) (2.5 μ M) in 31% (w/w) acetone/buffer medium at various temperature: A, 25 °C; B, 35 °C and C, 40 °C. (D,E) p_M-GPP mole-ratio (X_{pM-GPP}) analyzed from the intensity at ~450 nm (graph D) or ~685 nm (graph E) vs. pH in the mixed medium (Blue: 25 °C; Dark yellow: 35 °C; Green: 40 °C).

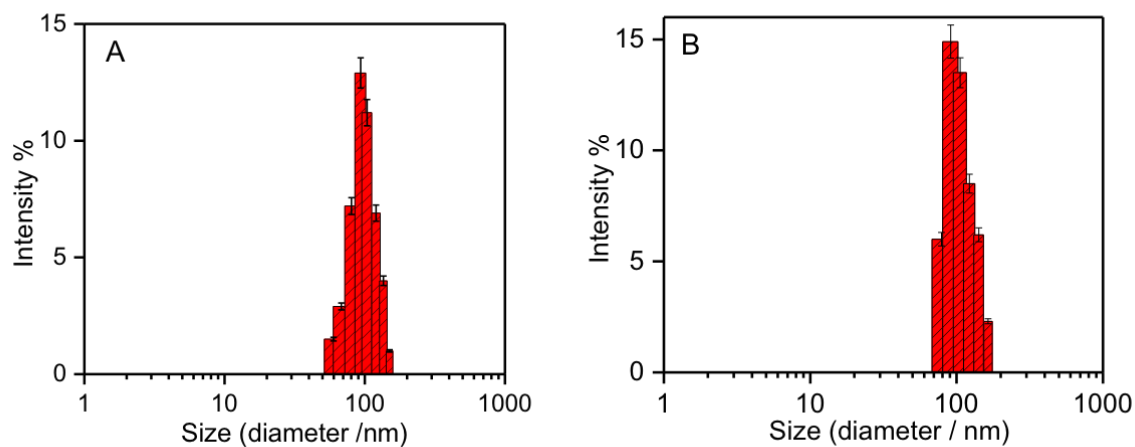


Figure S7. DLS measurement showing particle size distribution profile of DMPG LUV (lipids, 50 μ M) at different pH: (A) pH 4.0 and (B) pH 7.0. Each of these spectra is an average of 48 scan. Standard deviations for measurements taken from five independent experiments are depicted by error bars.

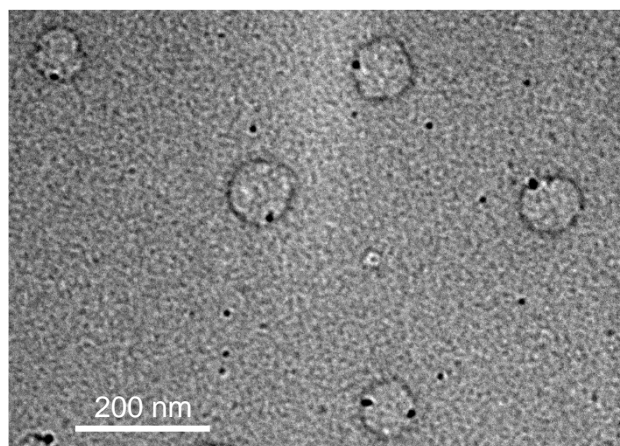


Figure S8. Cryo-transmission electron microscope (TEM) image of DMPG LUV.

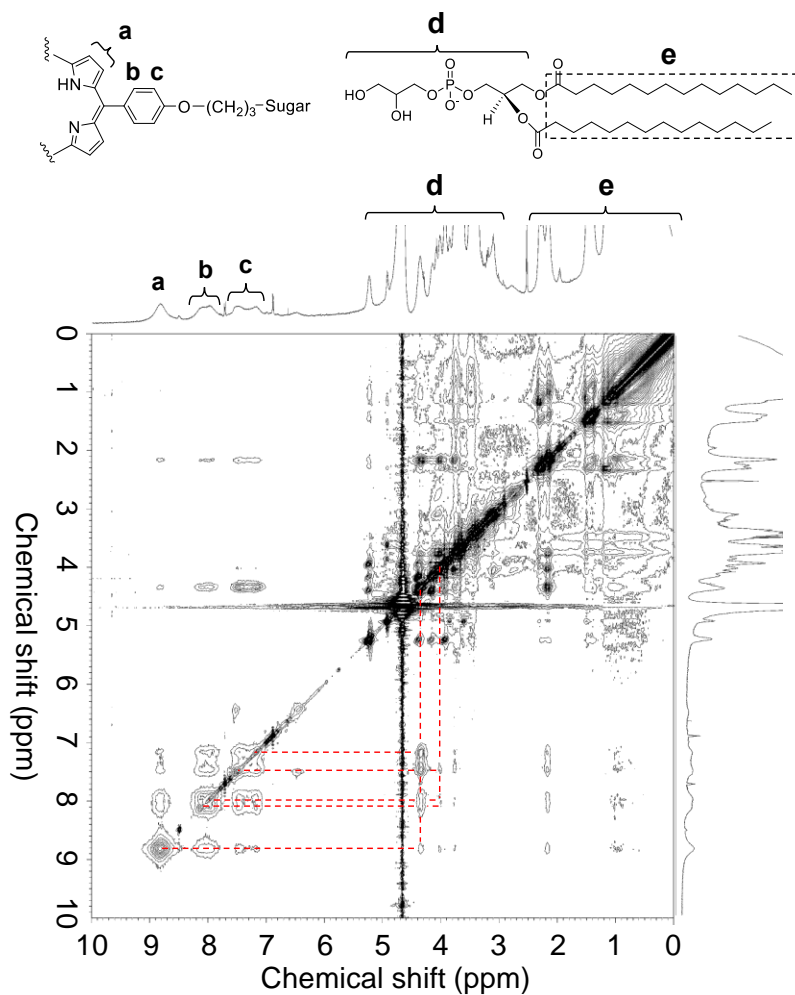


Figure S9. NOESY spectrum of the mixture of GPP (**2**) and DMPG LUV at 20 °C; Conditions: [**2**] = 1 mM, [DMPG LUV (d = 100 nm)] = 8 mM in D₂O containing DMSO-*d*₆ (5% v/v)). The correlations between the protons of *m*-phenyl, *o*-phenyl and porphyrin *p*-positions with those of the head moiety of DMPG are shown by broken red lines.

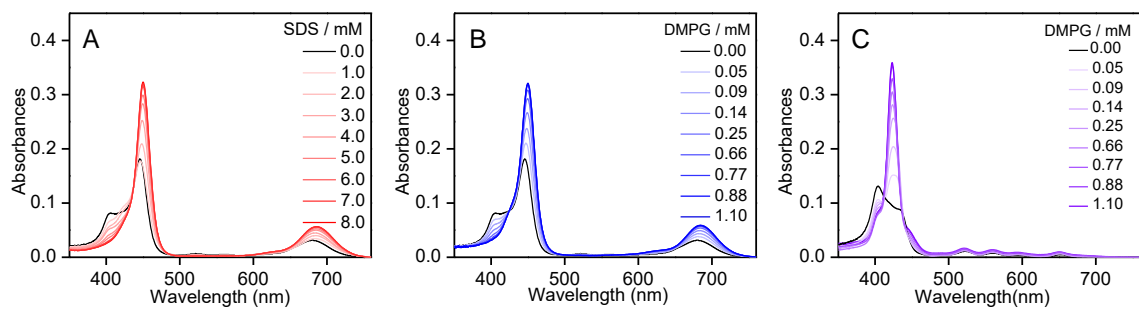


Figure S10. UV-vis absorption spectra of GPP (2) (2.5 μM) in presence of different concentration of amphiphilic molecule in 10 mM sodium citrate/sodium phosphate buffer at different pH: A, SDS micelle (pH 4.2); B, DMPG LUV (pH 4.2) and C, DMPG LUV (pH 6.2).

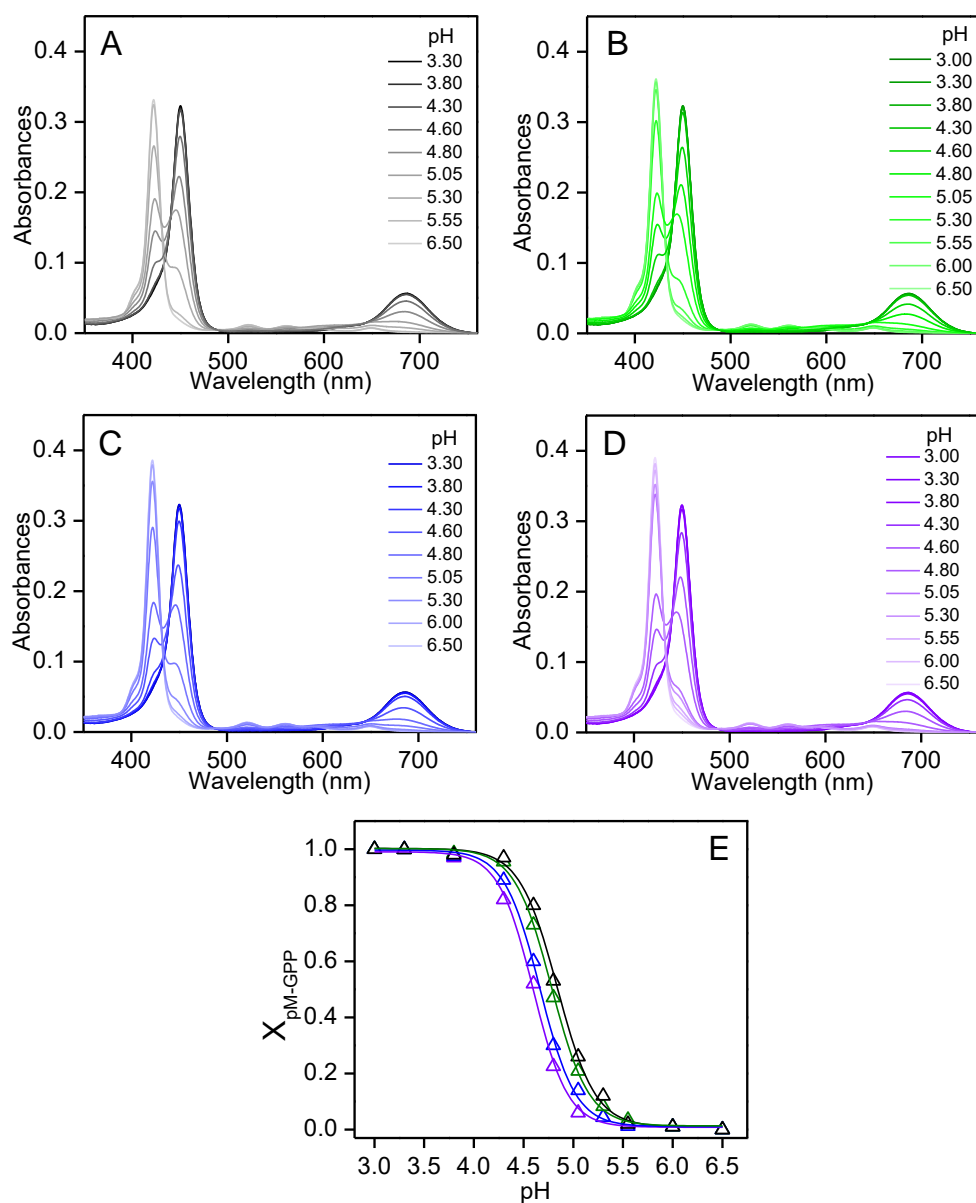


Figure S11. pH-dependent UV-vis absorption spectra of GPP (**2**) (2.5 μM) in 10 mM sodium citrate/sodium phosphate buffer containing SDS micelle (8 mM) at various temperatures: A, 20 °C; B, 25 °C; C, 35 °C and D, 40 °C. (E) $p_{\text{M-GPP}}$ mole-ratio ($X_{\text{pM-GPP}}$) analyzed from the intensity at ~ 685 nm vs. pH (Black: 20 °C; Green: 25 °C; Blue: 35 °C and Violet: 40 °C).

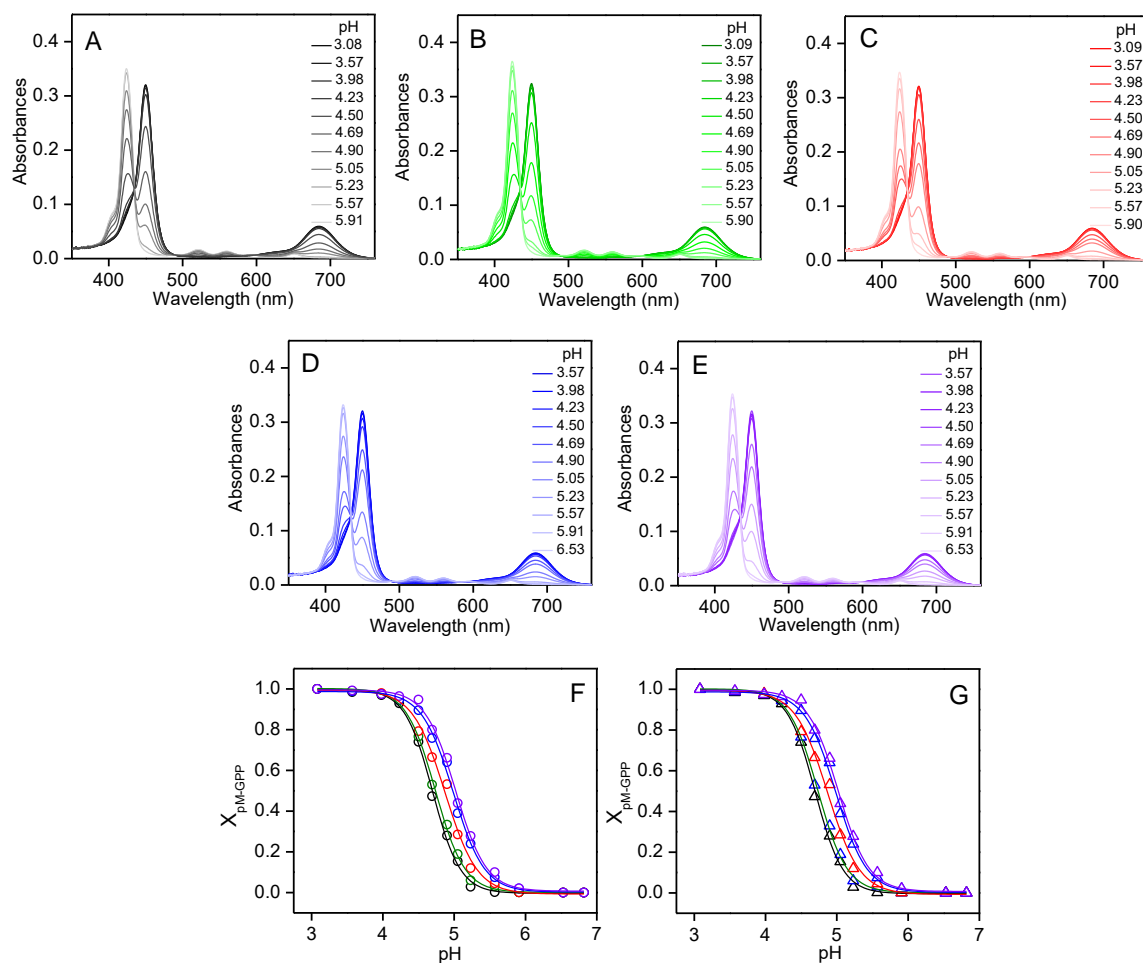


Figure S12. pH-dependent UV-vis absorption spectra of GPP (2) (2.5 μM) in 10 mM sodium citrate/sodium phosphate buffer containing DMPG LUV (1.1 mM) at various temperatures; (A): 20 °C; (B): 25 °C; (C): 30 °C; (D): 35 °C; (E): 40 °C; (F,G) pM-GPP mole-ratio ($X_{\text{pM-GPP}}$) analyzed from the intensity at ~450 nm (graph F) or ~685 nm (graph G) vs. pH at various temperatures (Black: 20 °C; Green: 25 °C; Red: 30 °C; Blue: 35 °C; Violet: 40 °C).

References

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