Electronic Supplementary Information

Self-assembly of Photoresponsive Azo-containing Phospholipids with a Polar Group as the Tail

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1. Characterization of N6, M6, and N3

NMR spectra were measured in CD$_3$OD solutions. $^1$H NMR spectra were recorded on a JEOL JNM-LA400 at 400 MHz. The chemical shifts of the $^1$H NMR signals were referenced to Me$_4$Si as the internal standard ($\delta = 0.00$) and are expressed as chemical shifts in ppm ($\delta$), multiplicity, coupling constant (Hz), and relative intensity. Elemental analyses were carried out by the Elemental Analysis Laboratory, Institute of Multidisciplinary Research for Advanced Materials, Tohoku University, using a Yanako MT-6 CHN Corder. The compounds N6, M6 and N3 were determined as follows:

**N6**: M.p. = 253.8-254.9 °C. 1H-NMR (400 MHz, CD$_3$OD), $\delta$ (TMS, ppm): 8.39 (d, J = 9 Hz, 2H), 8.02 (d, J = 9 Hz, 2H), 7.96 (d, J = 9 Hz, 2H), 7.09 (d, J= 9 Hz, 2H), 4.28-4.23 (m, 2H), 4.11 (t, J = 6 Hz, 2H), 3.94 (tt, J = 6, 6 Hz, 2H), 3.62-3.60 (m, 2H), 3.21 (s, 9H), 1.87-1.81 (m, 2H), 1.73-1.66 (m, 2H), 1.54-1.48 (m, 4H). Elemental analysis: Calculated for C$_{23}$H$_{33}$N$_4$O$_7$P (+2H$_2$O) (%): C, 50.73; H, 6.85; N, 10.29. Found (%): C, 50.74; H, 6.62; N, 10.27.

**M6**: M.p. = 239.7-241.9 °C. 1H-NMR(400 MHz, CD$_3$OD), $\delta$ (TMS, ppm): 7.85 (d, J = 3 Hz, 2H), 7.83 (d, J = 3 Hz, 2H), 7.05 (d, J =3 Hz, 2H), 7.02 (d, J =3 Hz, 2H), 4.26-4.22 (m, 2H), 4.07 (t, J = 6 Hz, 2H), 3.93 (tt, J = 6, 6 Hz, 2H), 3.78 (s, 3H), 3.62-3.59 (m, 2H), 3.21 (s, 9H), 1.85-1.81 (m, 2H), 1.71-1.67 (m, 2H), 1.55-1.50 (m, 4H). Elemental analysis: Calculated for C$_{24}$H$_{36}$N$_3$O$_6$P (+2H$_2$O) (%): C, 54.46; H, 7.62; N, 7.94. Found (+2H$_2$O) (%): C, 54.44; H, 7.56; N, 7.93.

**N3**: M.p. = 243.1-244.8 °C. 1H-NMR (400 MHz, CD$_3$OD), $\delta$ (TMS, ppm): 8.39 (d, J = 9 Hz, 2H), 8.02 (d, J = 9 Hz, 2H), 7.98 (d, J = 9 Hz, 2H), 7.13 (d, J= 9 Hz, 2H), 4.28-4.23 (m, 4H), 4.12-4.08 (m, 2H), 3.59 (t, J = 4 Hz, 2H), 3.19 (s, 9H), 2.17-2.14 (m, 2H). Elemental analysis: Calculated for C$_{20}$H$_{27}$N$_4$O$_7$P (+1.5H$_2$O) (%): C, 48.68; H, 6.13; N, 11.35. Found (%): C, 48.63; H, 6.16; N, 11.25.
2. Crystal data and X-ray single crystal structure of N3

For the evaluation of the self-assembling structure of N6 and N6/M6 mixtures, x-ray single crystal structure analysis of N3 was carried out. The single crystal was obtained by slow cooling of aqueous hot solution of N3 containing acetonitrile. The crystal data were collected by a R-AXIS RAPID diffractometer using multi-layer mirror monochromated Cu-Kα radiation at 90 K, and the crystal structure was analyzed using the SHELXL within the OLEX-2 GUI for modelling the molecular structures. The refined data for N3 are as follows: Triclinic, space group P-1 (No. 2), (C_{20}H_{27}N_{4}O_{3}P)·3H_{2}O; a = 8.3108(3), b = 11.6756(3), c = 25.7039(8) Å, α = 81.273(6)°, β = 88.301(6)°, γ = 83.153(6)°; V = 2447.51(14) Å³; Z = 4; R = 0.1060, wR₂ = 0.2386.

Crystallographic data of N3 has been deposited with Cambridge Crystallographic Data Center, deposition no. CCDC 2003927.

Figure S1 shows a molecular structure (ORTEP) and the single crystal structure of N3 viewed along a-, b-, and c-axes.
**Figure S1.** ORTEP diagram (thermal ellipsoids drawn at 50% probability) and an X-ray single crystal structure of N3.
Figure S2 (a), (b), and (c) shows the single crystal structure of N3, high lightened water positions in the crystal, viewed along b-, a-, and c-axes, respectively. Compound N3 forms tilted bilayer structure through H-aggregation of the Az moieties. Yellow-colored spheres in the Figures represent water molecules in the crystal. The water molecules were occupied interlayer between the bilayers of the N3 molecules. Figure 2(d) shows the part of the crystal structure of N3 focusing on the phosphocholine-moiety to clarify the formation mechanism of the stacked bilayer of N6. The magenta-colored part represents the phosphocholine-moiety of N3. The part bended away from the long axis of the Az skeleton to increase the affinity for the water layer through hydrogen bonding. The bending behavior of the phosphocholine moiety might enable phospholipids without a long alkyl tail to form a bilayer-type self-assembled structure through H-aggregation. Actually, it is impossible for the hydrophilic parts amphiphiles with only ammonium moieties but no phosphocholine moiety to bend in this way.
Figure S2. An X-ray single crystal structure of N3.

(C_{20}H_{27}N_{4}O_{7}P)·3H_{2}O;
a = 8.3108, b = 11.6756, c = 25.7039 Å,
α = 81.273 °, β = 88.301 °, γ = 83.153 °
3. Calculation of dipole moment of azobenzene derivatives by Spartan

![Image](image.png)

**Figure S3.** The dipole moment of Az compounds calculated by Spartan

4. Consideration about sheet-like bilayer structure formation by van der Waals forces.

![Image](image.png)

**Figure S4.** A schematic representation of the packing of lipid molecules with a polar head and a hydrophobic tail.
5. Effect of hydrophilic moiety on the formation of $H$-aggregates

As shown in Figure S5(a), the blue shift was not observed when the concentration of NAz6TM increased. It suggested that the NAz6TM molecules in water did not assembled into aggregation even at a higher concentration. As shown in Figure S5(b), the same situation was also found in the mixture of NAz6TMA and MAz6TMA in water.

|            | R    | n   |
|------------|------|-----|
| MAz6TMA    | CH3O | 6   |
| NAz6TMA    | NO2  | 6   |

**Figure S5.** Absorption spectra of (a) NAz6TMA; (b) a mixture of NAz6TMA and MAz6TMA ($R = 50/50$) in water
6. Changes in absorption spectra and surface tension by the change in the concentration of the mixture of N6/M6 in water (R = 50/50)

As shown in Figure S6(a), an absorption band at approximately 362 nm was observed at lower concentration in water (e.g. $c = 0.05$ mM). The wavelength of the absorption band was shifted to shorter wavelength (329 nm) by increasing the concentration (e.g. $c = 1.0$ mM). The blue shift of this band with increasing concentration was due to the formation of $H$-aggregates, and the driving force is furtherly enhanced by the stacking of the Az moieties. Figure S6(b) shows the changes in $A_{362}/A_{329}$ and the surface tension as a function of concentration of N6/M6 mixtures in water. An abrupt change in $A_{362}/A_{329}$ as well as the surface tension was both observed at approximately 0.1 mM. These results indicate that self-assembly behavior of these molecules in $H$-aggregation occurs at concentrations higher than 0.1 mM of N6/M6 mixture in water.

Figure S6. Changes in absorption spectra (a) and surface tension and $A_{372}/A_{350}$ as a function of concentration of the mixture of N6 and M6 ($R = 50/50$) (b) in water.
7. \textit{\textsuperscript{1}H}NMR characterization to form self-assembled vesicles in water

The solubility of Az-containing phospholipids is poor at room temperature, which resulted in the concentration of samples too low and signals too weak to be detected. Therefore, \textit{\textsuperscript{1}H} NMR measurements were performed at 60 °C in D$_2$O to investigate interactions between N6 and M6 (Figure S7). When measuring M6 in D$_2$O at 60 °C using \textit{\textsuperscript{1}H} NMR, one of sharp peaks was obtained at 7.7 (δ/ppm), corresponding to the symmetrical aromatic hydrogens (-ArH-N=, 4H) (M6-a). Meanwhile, the peak at 7.8 (δ/ppm), was assigned to the aromatic hydrogens (O$_2$N-ArH-, 2H) (N6-a) of N6. However, a relatively broadened and shifted peak was observed at 7.51 (δ/ppm) for the M6-a and N6-a, when incubated N6/M6 mixture in D$_2$O at 60 °C. The reason for the chemical shifts and broadened peaks was that electrostatic interactions was introduced between the groups MeO- and O$_2$N-. Consequently, the change of hydrogen environment drove the occurrence of chemical shifts and broadened peaks.

\textbf{Figure S7.} \textit{\textsuperscript{1}H} NMR of M6 (a), N6 (b) and M6/N6 mixture (R = 50/50, c = 2 mM) (c) in D$_2$O at 60 °C.
8. A plausible self-assembled bi-layer structures by mixing M6 to N6

**Figure S8.** A plausible model for the molecular orientation of N6 and M6 (R = 50/50) in the aggregation.
9. SAXS characterization of freeze dried N6/M6 mixtures in water with different mixing ratio

Figure S9 exhibits SAXS profiles of N6/M6 mixtures prepared by freeze-drying from water dispersions. All the scattering profiles were taken at SPring-8 BL-03XU. Four peaks at 23, 27, 30, 38 Å were mainly observed, and the peak profiles were changed by the change in the N6/M6 mixing ratio. By the comparison with the results shown in Figure 2, the scattering at 38 Å could be assigned as the bilayer thickness of the vesicles. On the other hand, peaks at 30 and 27 Å were due to layer thickness of N6 and M6 crystalline powders. As shown in Figure 2, there was neither phase separation nor recrystallization of N6 and M6. In spite of careful treatment, freeze drying process might influenced the crystal formation. Further, an unassignable broad scattering at 23 Å were sometimes observed.

![SAXS profiles of N6/M6 mixtures prepared by freeze-drying from water dispersions. The total molar concentration of N6 and M6 in the dispersions was fixed to 2 mM.](image)

**Figure S9.** SAXS profiles of N6/M6 mixtures prepared by freeze-drying from water dispersions. The total molar concentration of N6 and M6 in the dispersions was fixed to 2 mM.
10. Changes in DLS profiles of N6/M6 vesicles ($R = 50/50$, $c = 2$ mM) by irradiation with UV light

**Figure S10.** (a) Changes in DLS profiles of N6/M6 vesicles ($R = 50/50$, $c = 2$ mM) by irradiation with UV light (365 nm, 16 mW/cm$^2$); (b) the expanded image of (a).
11. Changes in the absorption spectra of the vesicles as a function of irradiation time of UV light

The N6/M6 mixture (R = 50/50, c = 0.5 mM) in water are vesicles with an average diameter of ca. 500 nm, which has been presented in Figure S11. Then, the changes in the absorption spectra of the vesicles as a function of irradiation time of UV light was detected. As shown in Figure S12, before UV irradiation, there was an absorption band at 340 nm, corresponding to the H-aggregation. The UV irradiation respectively caused the decrease and increase in absorbance at 340 nm and at 450 nm due to the photoisomerization of the Az groups in N6 and M6 from trans-form to cis-one, and the reverse situation was observed upon visible light irradiation less than 30 s.

**Figure S11.** Average mean size of N6/M6 vesicles (R = 50/50, c = 0.5 mM) in water determined by DLS measurement.
Figure S12. Changes in absorption spectra of the N6/M6 mixture ($R = 50/50, c = 0.5$ mM) in water by irradiation of UV light (365 nm, 1 mW/cm$^2$) and following visible light (436 nm, 16 mW/cm$^2$).
12. Changes in DLS profiles of N6/M6 vesicles ($R = 50/50$, $c = 2$ mM) by visible light irradiation

**Figure S13.** Changes in DLS profiles of N6/M6 vesicles ($R = 50/50$, $c = 2$ mM) by visible light irradiation (436 nm, 16 mW/cm²).
13. Changes in absorption spectra of the M6/N6 vesicles ($R = 50/50, c = 0.5$ mM) in water by irradiation of visible light

**Figure S14.** Changes in absorption spectra of the M6/N6 vesicles ($R = 50/50, c = 0.5$ mM) in water by irradiation of visible light (436 nm, 16 mW/cm²)