Supplementary Information

Micellar and vesicular nanoassemblies of triazole-based amphiphilic probes triggered by mercury(II) ions in a 100% aqueous medium

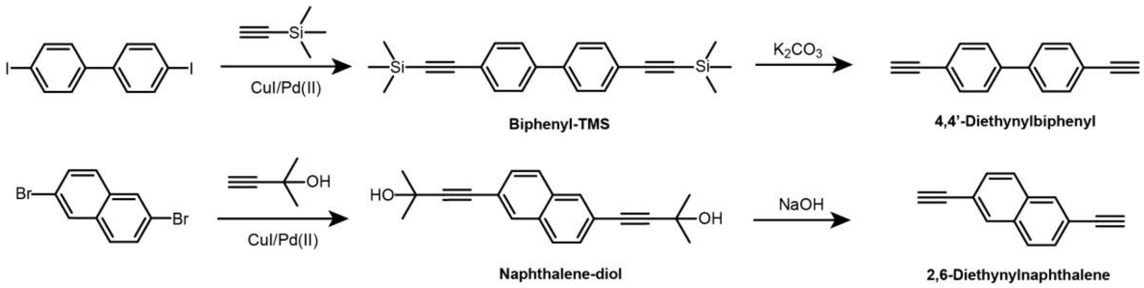
Inhye Kim,[a] Na-Eun Lee,[a] Yoo-Jeong Jeong,[a] Young-Ho Chung,[b] Byoung-Ki Cho,*[c] and Eunji Lee*[a,b]

Correspondence to: eunjilee@cnu.ac.kr and chobk@dankook.ac.kr

[a]Graduate School of Analytical Science and Technology, Chungnam National University, Daejeon 305-764, Republic of Korea
[b]Division of Life Science , Korea Basic Science Institute, Daejeon 305-806, Republic of Korea
[c]Department of Chemistry, Dankook University, Gyeonggi-Do, 448-701, Republic of Korea

Materials and Instruments. All commercially available reagents were reagent grade and used without further purification. $Hg(ClO_4)_2$, $CuSO_4$, $ZnSO_4$, $CaCl_2{\cdot}2H_2O$, $Pb(NO_3)_2$, $Al(NO_3)_3{\cdot}9H_2O$, and $MgSO_4$ were purchased from Daejung Chemicals, Sigma-Aldrich, and KANTO. 1,4-Diethynylbenzene was purchased from Sigma-Aldrich, and used as received. ^1H and ^{13}C NMR spectra were recorded from $CDCl_3$ and D_2O solution on Varian 200, JEOL JNM-AL400, Bruker AM500 spectrometers and Bruker AVANCE DRX 300 NMR spectrometer. The purity of the products was checked by thin layer chromatography (TLC; Merck, silica gel 60). The compounds were purified by column chromatography (silica gel) and prep-HPLC (Japan Analytical Instrument). Mass spectrometry was performed on a Bruker Ultraflextreme MALDI-TOF/TOF mass spectrometer. UV-Vis absorption spectra were obtained by using a Shimadzu UV-1800 spectrometer. The fluorescence spectra were obtained from a Perkin Elmer LS-55 fluorescence spectrophotometer. Dynamic light scattering (DLS) measurements were performed using ELS-Z (Otsuka Electronics, Japan). Fourier transform infrared (FTIR) spectra were obtained from a FTS-175C (Bio-Rad Laboratories, Inc., Cambridge, U.S.A.). Transmission electron microscopy (TEM) images were taken from 300 kV in-situ and 120 kV TEM (JEM-3011 HR and JEM-1400, respectively). The energy dispersive X-ray (EDX) spectrum and HAADF-STEM-EDS mapping images were obtained with a JEOL JEM-2100F FE-TEM equipped with a Link analytical system.

Synthesis of amphiphiles 1, 2 and 2N.



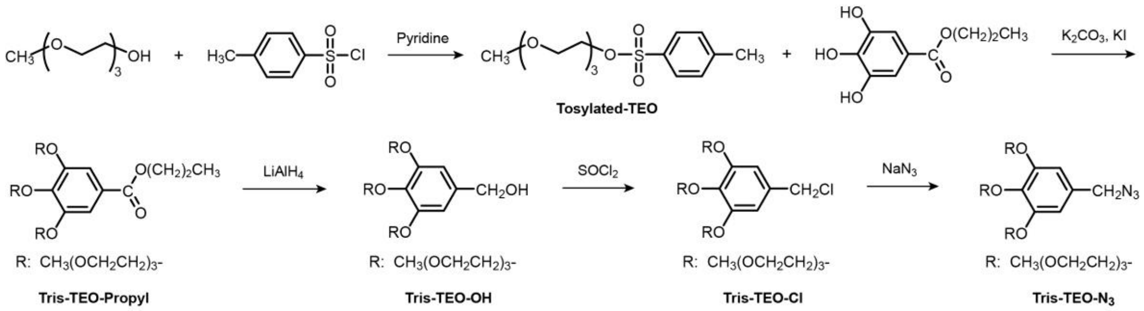
Scheme S1. Synthesis of diethynylene aromatic precursors.

Synthesis of Biphenyl-TMS. 4,4'-Diiodobiphenyl (2.0 g, 4.93 mmol), ethynyltrimethylsilane (2.80 mL, 19.70 mmol), copper(I)iodide (0.0067 g, 0.035 mmol), and $PdCl_2(PPh_3)_2$ (0.012 g, 0.017 mmol) were dissolved in 20 mL of dry triethylamine and 10 mL of tetrahydrofuran (THF). The mixture was heated at 60 ^{o}C overnight with stirring under N_2 atmosphere. After cooled to room temperature, the solvent was removed by a rotary evaporator. And then the mixture was extracted with deionized water and dichloromethane. The dichloromethane layer was washed with deionized water several times, and dried over $MgSO_4$. Residual metal catalysts were removed by passing through celite several times. The solvent was removed by a rotary evaporator, and the crude product was then purified by silica gel column chromatographies using *n*-hexane as the eluent, to yield 1.60 g (94.0%). ^{1}H-NMR ($CDCl_3$, δ, ppm): 7.56 (s, Ar-*H*), 0.24 (s, $Si(CH_3)_3$).

Synthesis of 4,4'-Diethynylbiphenyl. Biphenyl-TMS (1.60 g, 4.62 mmol) and K_2CO_3 (12.7 g, 92.3 mmol) were dissolved in 50 mL of dry THF and 50 mL of dry methanol. The reaction mixture was stirred for 3 hours at room temperature under N_2 atmosphere. The solvent was removed by a rotary evaporator. And then the mixture was extracted with deionized water and dichloromethane, and dried over $MgSO_4$. The solvent was removed by a rotary evaporator, and the crude product was then purified by the silica gel column chromatography using hexane as the eluent, to yield 0.87 g (93.2%). ^{1}H-NMR ($CDCl_3$, δ, ppm): 7.56 (s, Ar-*H*), 3.15 (s, Ar-C≡C*H*).

Synthesis of Naphthalene-diol. 2,6-Dibromonaphthalene (1.0 g, 3.50 mmol) and 2-methylbut-3-yn-2-ol (5.0 mL, 14.0 mmol) were dissolved in 30 mL of triethylamine. Then, to the solution, catalytic amounts of copper(I)iodide and $PdCl_2(PPh_3)_2$ were added. The mixture was heated at reflux for 3 days with stirring under N_2 atmosphere. After cooled to room temperature, triethylamine was removed by extraction with a diluted HCl solution and dichloromethane. The dichloromethane layer was washed with deionized water several times, and then dried over $MgSO_4$. After removing dichloromethane by a rotary evaporator, the resulting mixture was purified by the several recrystallizations using *n*-hexane, to yield 0.55 g (55%). ^{1}H-NMR ($CDCl_3$, δ, ppm): 7.99 (s, Ar-*H*), 7.75 (d, *J* = 8.4 Hz, Ar-*H*), 7.54 (d, *J* = 8.4 Hz, Ar-*H*), 1.65 (s, $C(CH_3)_2OH$).

Synthesis of 2,6-diethynylnaphthalene. Naphthalene-diol (0.55 g, 1.88 mmol) and sodium hydroxide (0.75 g, 18.8 mmol) were dissolved in 30 mL of toluene. The reaction mixture was heated to reflux for 4 hours under N_2 atmosphere. After cooling to room temperature, the solvent was removed by a rotary evaporator. The resulting mixture was extracted with deionized water and dichloromethane. The dichloromethane layer was washed with deionized water two times, and dried over $MgSO_4$. After removing dichloromethane by a rotary evaporator, the resulting compound was purified by a silica gel column chromatography using hexane as the eluent, to yield 0.20 g (60%). ^{1}H-NMR ($CDCl_3$, δ, ppm): 7.99 (s, Ar-*H*), 7.75 (d, *J* = 8.4 Hz, Ar-*H*), 7.54 (d, *J* = 8.4 Hz, Ar-*H*), 3.18 (s, Ar-C≡C*H*).



Scheme S2. Synthesis of azide-terminated hydrophilic dendron (Tris-TEO-N_3).

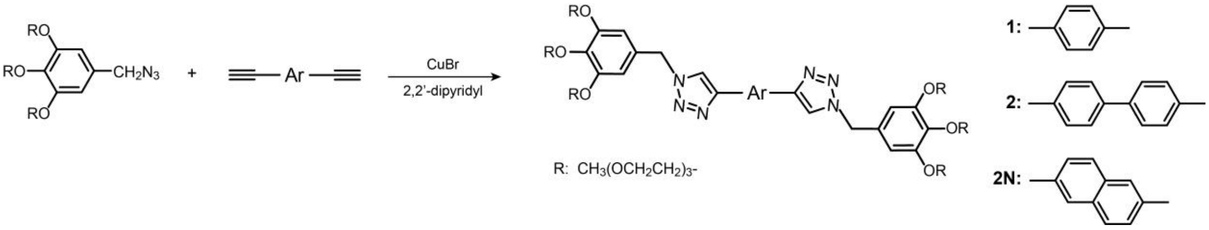
Synthesis of Tosylated-TEO. Tri(ethylene glycol) methyl ether (100 g, 609 mmol), 4-toluenesulfonyl chloride (9.31 g, 48.8 mmol), and pyridine (98.1 mL, 1218 mmol) were dissolved in 500 mL of dry dichloromethane. The reaction mixture was stirred for 2 days at room temperature under N_2 atmosphere. The pyridine was removed by extraction with a diluted HCl solution. The dichloromethane layer was washed with deionized water several times, and then dried over $MgSO_4$. After removing dichloromethane by a rotary evaporator, the resulting compound was purified by sequential silica gel column chromatographies from dichloromethane to dichloromethane:methanol = 8:1 solvent mixture as the eluents, to yield 150 g (77.0%). ^{1}H-NMR ($CDCl_3$, δ, ppm): 7.78 (d, *J* = 8.0 Hz, Ar-*H*), 7.35 (d, *J* = 8.0 Hz, Ar-*H*), 4.16 (t, *J* = 5.2 Hz, CHCH_2OTs), 3.42-3.68 (br, CH_2CH_2O), 3.37 (s, CH_3O), 2.45 (s, Ar-CH_3).

Synthesis of Tris-TEO-Propyl. Propylgallate (3.7 g, 17.5 mmol), Tosylated-TEO (22.3 g, 70 mmol), K_2CO_3 (12.1 g, 87.5 mmol), and KI (2.91 g, 17.5 mmol) were dissolved in 65 mL of methyl ethyl ketone. The reaction mixture was heated at reflux for 26 h under N_2 atmosphere. The reaction mixture was extracted with ethyl acetate and deionized water three times, and then dried over $MgSO_4$. After removing organic solvents using a rotary evaporator, the resulting mixture was purified by sequential silica gel column chromatographies from dichloromethane:ethyl acetate = 4:1 solvent mixture to ethyl acetate:methanol = 30:1 solvent mixture as the eluents, to yield 7.49 g (65.8%). ^{1}H-NMR ($CDCl_3$, δ, ppm): 7.23 (s, Ar-*H*), 4.10-4.25 (br, C*H*$_2$OAr and ArCOOC*H*$_2$), 3.42-3.90 (br, C*H*$_2$C*H*$_2$O), 3.37 (s, C*H*$_3$O), 1.73 (m, COOCH$_2$C*H*$_2$), 0.98 (t, *J* = 7.4 Hz, COOCH$_2$CH$_2$C*H*$_3$).

Synthesis of Tris-TEO-OH. Tris-TEO-Propyl (4.38 g, 6.73 mmol) was dissolved in 15 mL of THF, and LiAlH$_4$ (0.38 g, 10.1 mmol) was slowly added to the solution. The reaction mixture was stirred for 24 h, and quenched by adding water dropwise. The precipitate was filtered off, and the transparent solution was dried over MgSO$_4$. After removing organic solvents using a rotary evaporator, 4.45 g (84.6%) of a colorless oil was obtained as the product. ^{1}H-NMR (CDCl$_3$, δ, ppm): 6.63 (s, Ar-*H*), 4.57 (d, *J* = 6.0 Hz, ArC*H*$_2$OH), 4.14 (m, C*H*$_2$OAr), 3.42-3.90 (br, C*H*$_2$C*H*$_2$O), 3.37 (s, C*H*$_3$O), 2.03 (t, *J* = 6.0 Hz, Ar-CH$_2$O*H*).

Synthesis of Tris-TEO-Cl. Tris-TEO-OH (4.11 g, 6.91 mmol) and a catalytic amount of *N,N'*-dimethylformamide (DMF) were dissolved in 41 mL of dichloromethane. To the solution, 0.70 mL (9.68 mmol) of SOCl$_2$ was added carefully. The reaction mixture was stirred for 30 min, and then volatile compounds were removed using a rotary evaporator. The residual mixture was extracted with dichloromethane and deionized water three times. After removing dichloromethane using a rotary evaporator, 4.14 g (97.9%) of a colorless oil was obtained as the product. ^{1}H-NMR (CDCl$_3$, δ, ppm): 6.62 (s, Ar-*H*), 4.57 (s, ArC*H*$_2$Cl), 4.14 (m, C*H*$_2$OAr), 3.42-3.90 (br, C*H*$_2$C*H*$_2$O), 3.37 (s, C*H*$_3$O).

Synthesis of Tris-TEO-N$_3$. Tris-TEO-Cl (4.14 g, 6.76 mmol) and NaN$_3$ (4.40 g, 67.6 mmol) were dissolved in 15 mL of DMF. The reaction mixture was heated at 100 ^{o}C for 3 days under N$_2$ atmosphere. The reaction mixture was extracted with dichloromethane and deionized water three times, and then dried over MgSO$_4$. After removing dichloromethane using a rotary evaporator, the resulting mixture was purified by a silica gel column chromatography to dichloromethane:methanol = 50:1 as the eluent, to yield 2.61 g (62.3%). ^{1}H-NMR (CDCl$_3$, δ, ppm): 6.54 (s, Ar-*H*), 4.10-4.25 (br, C*H*$_2$OAr and ArCOOC*H*$_2$), 4.14 (m, C*H*$_2$OAr), 3.42-3.90 (br, C*H*$_2$C*H*$_2$O), 3.37 (s, C*H*$_3$O).



Scheme S3. Synthesis of ABA-type amphiphiles **1**, **2** and **2N**; aromatic moiety is denoted by **Ar**.

Synthesis of amphiphiles 1, 2 and 2N. 1, **2**, and **2N** were synthesized using a similar procedure. A representative example is described for **1**. In a glove box, 1,4-diethynylbenzene (0.2 g, 1.58 mmol), Tris-TEO-N_3 (2.16 g, 3.16 mmol), CuBr (1.13 g, 7.9 mmol), and 2,2'-dipyridyl (1.23 g, 7.90 mmol) were dissolved in deoxygenated anhydrous THF. The reaction mixture was degassed by performing freeze-pump-thaw cycle three times, and allowing the reaction mixture to equilibrate to room temperature. The reaction mixture was allowed to stir for 12 h at room temperature. The solvent was removed using a rotary evaporator, and the residue was purified by a silica-column chromatography using ethyl acetate:methanol = 20:1 as the eluent, to yield 1.33 g (62%) of the product. ^{1}H-NMR (500 MHz, $CDCl_3$, δ, ppm) : 7.88 (s, Ar-*H*), 7.73 (s, triazole), 6.58 (s, trialkoxybenzenyl protons), 5.45 (s, -NCH_2(phenyl)), 4.13 (m, CH_2OAr), 3.50-3.90 (br, CH_2CH_2O), 3.36 (s, CH_3O). ^{13}C-NMR (125 MHz, $CDCl_3$, δ, ppm): 153.2, 147.8, 130.3, 129.8, 126.1, 119.5, 108.3, 69.1-72.4 (carbons of TEO chains), 59.0 (CH_3O), 54.4 (benzylic carbon). M_w/M_n (GPC) = 1.02.

Compound 2. Yield: 42%. ^{1}H-NMR (500 MHz, $CDCl_3$, δ, ppm) : 7.90 (d, *J* = 8.4 Hz, Ar-*H*), 7.73 (s, triazole), 7.68 (d, *J* = 8.4 Hz, Ar-*H*), 6.58 (s, trialkoxybenzenyl protons), 5.47 (s, -NCH_2(phenyl)), 4.15 (m, CH_2OAr), 3.50-3.90 (br, CH_2CH_2O), 3.36 (s, CH_3O). ^{13}C-NMR (125 MHz, $CDCl_3$, δ, ppm): 153.1, 147.8, 140.2, 139.0, 129.9, 129.7, 127.3, 126.1, 119.6, 108.1, 69.1-72.4 (carbons of TEO chains), 58.9 (CH_3O), 54.3 (benzylic carbon). M_w/M_n (GPC) = 1.02.

Compound 2N. Yield: 44%. ^{1}H-NMR (500 MHz, $CDCl_3$, δ, ppm) : 8.32 (s, Ar-*H*), 7.92 (s, Ar-*H*), 7.81 (s, triazole-*H*), 6.60 (s, trialkoxybenzenyl protons), 5.48 (s, -NCH_2(phenyl)), 4.15 (m, CH_2OAr), 3.50-3.90 (br, CH_2CH_2O), 3.36 (s, CH_3O). ^{13}C-NMR (125 MHz, $CDCl_3$, δ, ppm): 153.1, 148.1, 139.0, 133.2, 129.9, 128.8, 128.2, 124.4, 124.2, 119.9, 108.1, 69.1-72.4 (carbons of TEO chains), 58.9 (CH_3O), 54.3 (benzylic carbon). M_w/M_n (GPC) = 1.02.

Measurements.

Preparation of aqueous solution of amphiphiles. Self-organized samples were prepared by dissolving amphiphiles in aqueous solution. The samples were left in a 1 mL glass vial and sealed with parafilm at room temperature for a day to allow aging.

Dynamic light scattering (DLS). Dynamic light scattering (DLS) experiments were performed with the aqueous solution of **2** and **2N** (20 μM) at a scattering angle of 90° at 25 °C, using a He-Ne laser operating at 632.8 nm. The hydrodynamic diameters were determined from the DLS autocorrelation functions by the cumulants and the CONTIN methods using the software provided by the manufacturer.

Nuclear magnetic resonance (NMR). ^{1}H and ^{13}C NMR spectra were recorded on Varian 200, JEOL JNM-AL400, Bruker AM500 spectrometers and Bruker AVANCE DRX 300 NMR spectrometer. using a solution of molecule **1**, **2**, and **2N** in $CDCl_3$ and D_2O with a concentration of 1 wt%. The solutions of **2**-Hg^{2+} and **2N**-Hg^{2+} were prepared by addition of 1 equiv. Hg^{2+} to the aqueous solution of **2** and **2N**.

Transmission electron microscopy (TEM). A drop of each sample in aqueous solution was placed on a formvar/carbon-coated copper grid and allowed to evaporate under ambient conditions. When sample was stained, a drop of uranyl acetate solution (2 wt%) placed onto the surface of the sample-loaded grid. The sample deposited about 1 min at least, and excess solution was wicked off by filter paper. The specimen was observed with a JEOL-JEM-3011 HR operating at 300 kV and JEM-1400 operating at 120 kV. The data were analyzed with Gatan Digital Micrograph and JEOL Simple Measure programs.

Cryogenic TEM (cryo-TEM). Cryo-TEM experiments were performed with a thin film of aqueous solution of sample (3 μL) transferred to a lacey supported grid by plunge-dipping method. The thin aqueous films were prepared at ambient temperature and with humidity of 97-99% within a custom-built environmental chamber in order to prevent evaporation of water from sample solution. The excess liquid was blotted with filter paper for 2-3 sec, and the thin aqueous films were rapidly vitrified by plunging them into liquid ethane (cooled by liquid nitrogen) at its freezing point. The specimen was observed with a JEOL-JEM-3011 HR operating at 300 kV. The data were analyzed with Gatan Digital Micrograph program.

Fourier transform infrared spectroscopy (FTIR). Samples were prepared by dissolving **2** and **2N** in water at a concentration of 5 μM and then added 1. equiv. Hg^{2+} to the aqueous solution of **2** and **2N**. After that, several drops of solution were coated on an ZnSe pellet.

Measurement for critical micellar concentration (CMC) of aromatic amphiphiles.[S1] Fluorescence measurements were carried out on a Perkin Elmer LS-55 Fluorescence Spectrometer with excitation wavelength of 340 nm and the emission spectrum was recorded from 370 nm to 600 nm. The fluorescence intensities of the peaks at ~392 nm (I_{III}) and ~378 nm (I_{I}) were extracted from the spectra and the $I_{\mathrm{III}}/I_{\mathrm{I}}$ value vs. concentration of the solution was used for CMC determination. The relative intensity of two emission peaks (I_{392}/I_{378}) of pyrene was highly sensitive to the medium polarity.

Metal ion binding study by UV-Vis and fluorescence spectrometer. To examine optical properties upon the complexation of metal ions with **1**, **2** and **2N**, a variety of metal ions, including Hg^{2+}, Cu^{2+}, Zn^{2+}, Ca^{2+}, Pb^{2+}, Al^{3+}, and Mg^{2+}, were added to aqueous solutions of amphiphiles **1**, **2** and **2N**, respectively. All spectra were recorded from 200 nm to 900 nm with a 1.0 cm light path length of cuvette.

Determination of binding stoichiometry (Job's Plot).[S2] The binding stoichiometry of amphiphile-metal ion complexes was determined by Job's plot analysis. The fluorescence intensities at 385 nm and 411 nm were plotted against molar fraction of **2** and **2N,** respectively, under a constant total concentration. When the emission intensity reaches a maximum point, the molar fraction represents the binding stoichiometry of amphiphile-metal ion complexes.

Determination of the association constants. The association constants for [**2** + Hg^{2+}] complex with 1 : 1 stoichiometry[S3,S4] and [**2N** + Hg^{2+}] complex with 1 : 2 binding stoichiometry[S5] can be calculated by non-linear curve fitting of the fluorescence titration profile.[S3-S5]

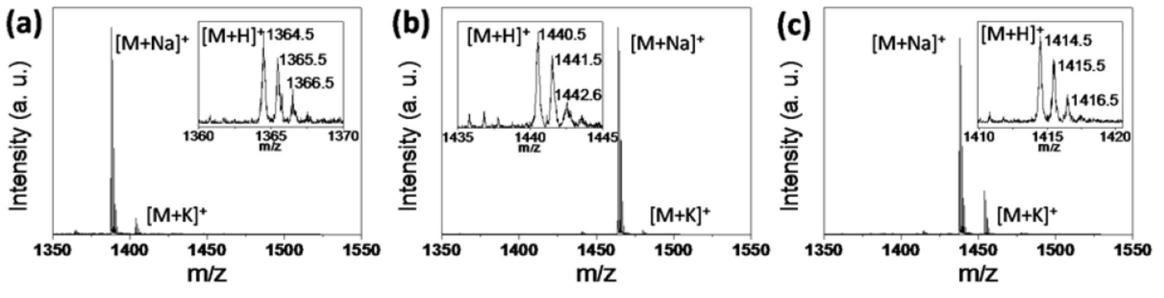


Fig. S1 MALDI-TOF/TOF MS spectra of molecule (a) **1**, (b) **2**, and (c) **2N**.

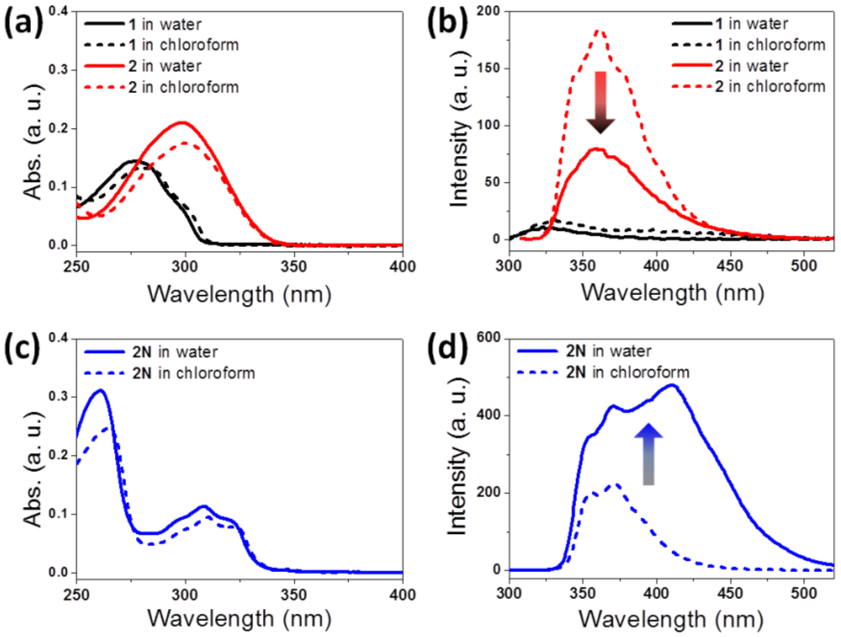


Fig. S2 (a,c) UV-Vis absorption spectra and (b,d) fluorescence spectra of molecules **1, 2** and **2N** in an aqueous solution (solid line) and chloroform (dashed line).

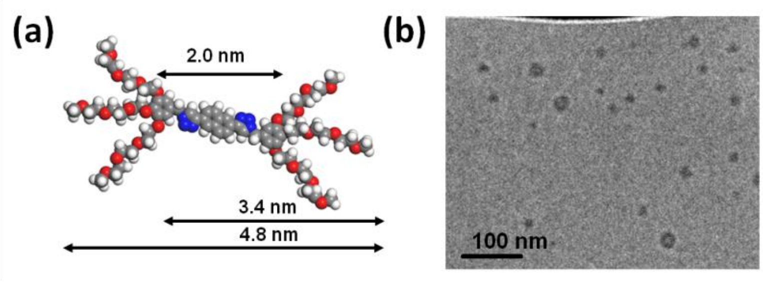


Fig. S3 (a) Molecular length of **2N** calculated by CPK modeling. Extended molecular length is about 4.8 nm. (b) Cryo-TEM image of aqueous solution of **2N**, showing the vesicular structure.

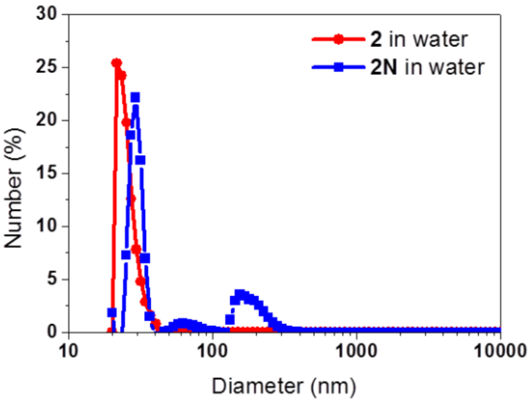


Fig. S4 Hydrodynamic diameters of **2** and **2N** in water.

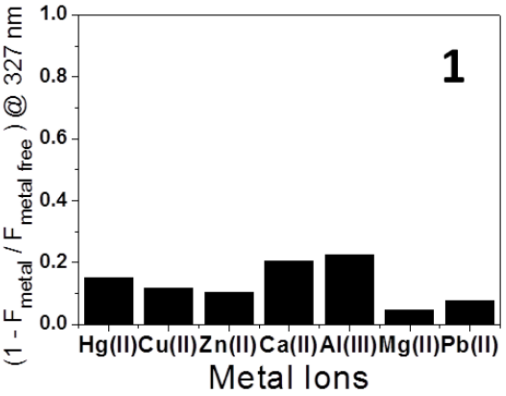


Fig. S5 Fluorescence intensity ratios (1-$F_{\text{metal}}/F_{\text{metal free}}$) for **1** at 327 nm ($\lambda_{\text{ex}}$ = 276 nm) in the presence of 100 equiv. of specified metal ions.

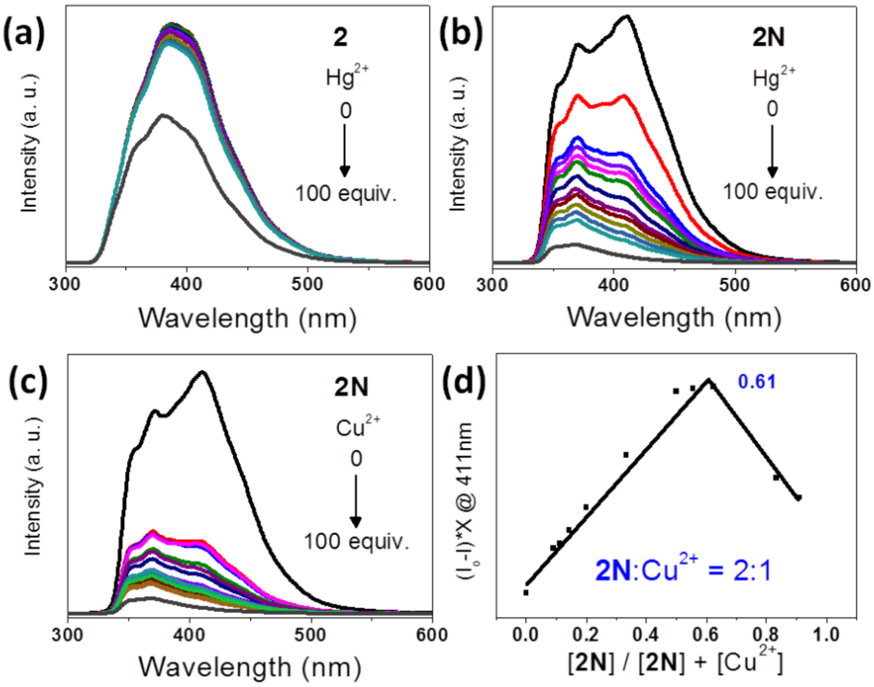


Fig. S6 (a-c) Emission spectra changes of aqueous solution of **2** and **2N** (5 μM) upon addition of metal ions (Hg^{2+} or Cu^{2+}). (d) Job's plot showing 2:1 complex of **2N**:Cu^{2+}.

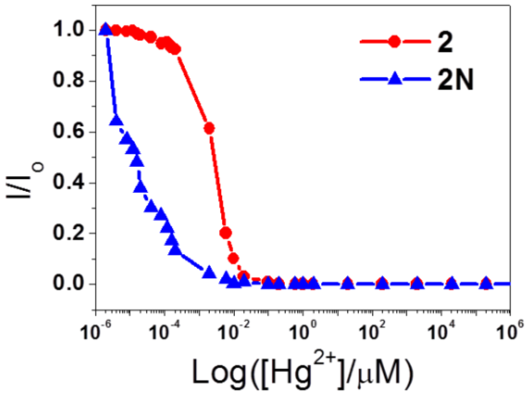


Fig. S7 The plot of PL intensity as a function of the concentration of Hg^{2+} (λ_{em}=385 and 411 nm for **2** and **2N**, respectively).

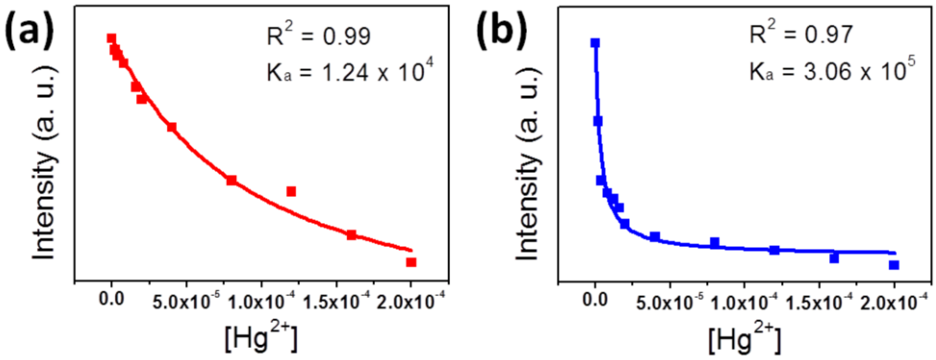


Fig. S8 Determination of the association constant (K_a) for **2** and **2N** (5 μM) in the presence of Hg^{2+} by a non-linear fitting curve method with the emission intensity profiles at 385, 411 nm for **2** and **2N**, respectively.[S3-S5]

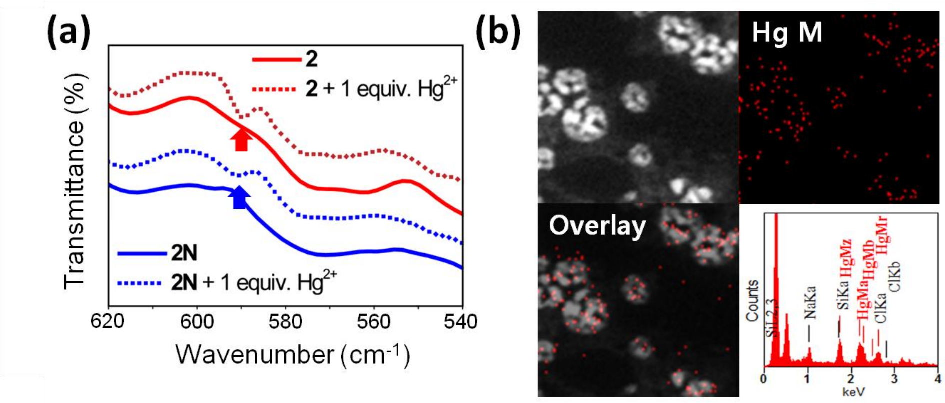


Fig. S9 (a) FTIR spectrum of **2** and **2N** in the absence and presence of Hg^{2+}. New peaks appeared at 587 cm^{-1} due to Hg-N stretching vibrations. (b) HAADF-STEM-EDS mapping images of hybrid vesicles of **2N** containing Hg^{2+}.

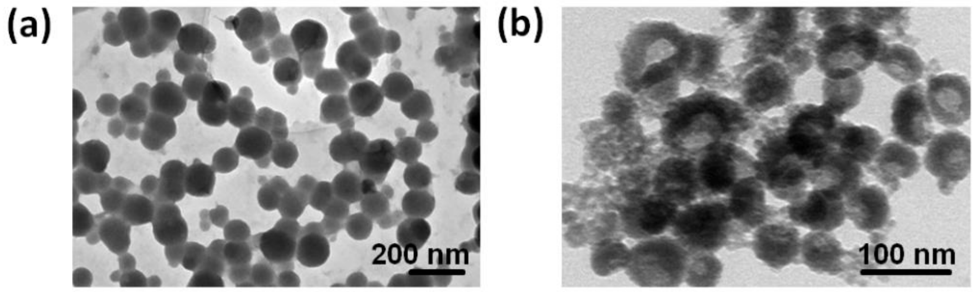


Fig. S10 Non-stained TEM images of the aqueous solution of **2N** (a) before and (b) after addition of Cu^{2+}.

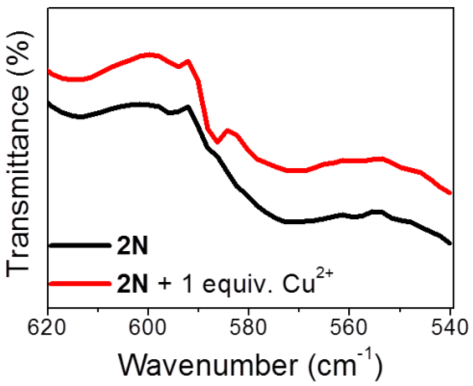


Fig. S11 FTIR spectra of **2N** upon addition of 1 equiv. of Cu^{2+}.

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