Electronic Supplementary Information (ESI)

Palladium- or Proton-induced Submicro Spherical Aggregation of Macrocyclic Amphiphiles in Aqueous Solution

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Materials and methods

All solvents, organic and inorganic reagents are commercially available, and were used without further purification. A macrocyclic ligand L1 and Pd(en)(NO3)2 were synthesised according to previously reported procedures. Unless otherwise noted, reactions were performed under aerobic condition.

NMR spectroscopic measurements were performed using Bruker DRX 500 and AVANCE 500 (500 MHz for 1H; 125 or 126 MHz for 13C) spectrometers. The chemical shifts were reported in parts per million (ppm) on the δ scale, and were calibrated as below; CDCl3: tetramethylsilane (Si(CH3)4) = 0 ppm for 1H, CDCl3 = 77.16 ppm for 13C; DMSO-d6: CHD2SOCD3 = 2.50 ppm for 1H; D2O: HDO = 4.70 ppm for 1H. The multiplicity of each signal for 1H NMR was indicated by s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), brs (broad-singlet) and brt (broad-triplet). UV-vis spectroscopy was performed using a HITACHI U-3500 spectrophotometer. Electrospray ionization-time-of-flight (ESI-TOF) mass spectra were recorded on a Micromass LCT spectrometer. FT-IR spectra were obtained with a JASCO FT/IR 4200 spectrometer using a ZnSe ATR method. Elemental analyses were performed on a YANAKO MT-6. Melting point was measured by a YANAKO MP-500D apparatus. Molecular modeling was performed by a Spartan ’08 program. The modeling structures were displayed using a Mercury program. Dynamic light scattering (DLS) measurements were performed using a Malvern Zetasizernano-ZS instrument equipped with a He-Ne laser operating at 4 mW power and 633 nm wavelength, and a computer-controlled correlator, at a 173° accumulation angle. Measurements were carried out in a polystyrene cuvette at 25 °C. The data were processed using Dispersion Technology software version 5.10 to give the particle size distribution and average particle sizes. Transmission electron microscope (TEM) images were obtained using a JEOL JEM-1400 microscope without staining. Scanning TEM (STEM) images and energy dispersive X-ray spectroscopy (EDS) mapping were obtained using a JEOL JEM-2100F microscope. Static light scattering (SLS) measurements and angle-dependent DLS measurements were performed using an OTSUKA ELECTRONICS DLS8000 instrument equipped with a He-Ne laser (633 nm wavelength). Atomic force microscope (AFM) images were observed by a SII SPI4000 probe station with a SPA300HV unit using a DFM mode.
**Synthetic procedures and physical properties**

**Macrocycle L2.** To a solution of 4,5-dimethyl-o-phenylenediamine (10.0 g, 73.5 mmol) in degassed THF (40 mL) was added a solution of terephthalaldehyde (9.85 g, 73.4 mmol) in degassed THF (68 mL). The mixture was stirred at 50 ºC for 18 h, then the resulting orange precipitate (6.05 g) was collected by suction, washed with THF (5 mL × 4), dried in vacuo. To a suspension of LiAlH₄ (4.79 g, 126 mmol) in dry THF (90 mL) was added dropwisely the suspension of the resulting orange precipitate (6.03 g) in dry THF (1.1 L), and the mixture was heated at reflux for 2 h. After careful quenching by dropwise addition of H₂O (5 mL), 1.0 M KOH aq (5 mL) and H₂O (10 mL) sequentially, the mixture was heated at reflux for 1 h. After filtration, the filtrate was evaporated, and the residue was dried in vacuo to obtain a crude red solid (6.18 g). The crude (4.84 g) was dissolved in 5 M HCl aq (640 mL). After filtration, the solution was neutralized with 1 M Na₂CO₃ aq, and then the resulting pale yellow solid was collected by suction. The solid was washed with CH₃OH to afford L2 (1.05 g, 1.47 mmol, 6.0%) as a pale yellow solid.

¹H NMR (500 MHz, CDCl₃, 300 K): δ 7.33 (s, 12H), 6.63 (s, 6H), 4.24 (s, 12H), 3.39 (brs, 6H), 2.21 (s, 18H).

¹³C NMR (125 MHz, CDCl₃, 293 K): δ 138.8, 135.4, 128.5, 127.1, 114.2, 49.0, 19.5.

ESI-TOF mass: m/z calcd for [C₄₉H₅₄N₆ + Na] 737.37; found: 737.36.


FT-IR (ATR): 3309, 2918, 2855, 1520, 1443, 1259, 1115, 999, 849 cm⁻¹.

M.p.: >130 ºC (dec.).

![Fig. S1 ¹H NMR spectrum of L2 (500 MHz, CDCl₃, 300 K).](image-url)
Fig. S2 $^{13}$C NMR spectrum of L2 (125 MHz, CDCl$_3$, 293 K).

Fig. S3 (a) HSQC and (b) HMBC of L2 ($^1$H: 500 MHz, $^{13}$C: 126 MHz, CDCl$_3$, 300 K).

[Pd$_3$L1(en)$_3$](NO$_3$)$_6$·3.5H$_2$O. To a solution of L1 in CHCl$_3$ (2.0 mM, 1.26 mL, 2.52 µmol, 1.00 equiv.) was added a solution of Pd(en)(NO$_3$)$_2$ in CH$_3$CN (14 mM, 1.26 mL, 17.7 µmol, 7.00 equiv.) gently at room temperature. The solution was left to stand for 14 h. A colourless precipitate was collected by centrifugation, washed twice with CH$_3$CN (2 mL), and then dried in vacuo to afford [Pd$_3$L1(en)$_3$](NO$_3$)$_6$·3.5H$_2$O as a colorless solid (2.47 mg, 1.57 µmol, 63%).

ESI-TOF mass: $m/z$ calcd for [C$_{48}$H$_{66}$N$_{12}$Pd$_3$ + NO$_3$ − 3H]$^{2+}$ 594.62; found 594.67.

Elemental analysis: calcd for [C$_{48}$H$_{73}$N$_{18}$O$_{21.5}$Pd$_3$ ([Pd$_3$L1(en)$_3$](NO$_3$)$_6$·3.5H$_2$O)]: C 36.83, H 4.70, N 16.11; found: C 37.22, H 4.71, N 15.72.

FT-IR (ATR): 3224, 3107, 1338, 1145, 1059, 824, 760, 668 cm$^{-1}$.

M.p.: > 190 °C (dec.).
Fig. S4 $^1$H NMR spectrum of [Pd$_3$L$_1$(en)$_3$](NO$_3$)$_6$ (1.0 mM) (500 MHz, D$_2$O, 10 mM DNO$_3$, 300 K).

Fig. S5 ESI-TOF MS spectrum of acidic aqueous solution ([HNO$_3$] = 10 mM) of [Pd$_3$L$_1$(en)$_3$](NO$_3$)$_6$.

The ratio of L$_1$ and [Pd(en)]$^{2+}$ moieties was further confirmed from the $^1$H NMR spectra of a digested solution of [Pd$_3$L$_1$(en)$_3$](NO$_3$)$_6$ in DCl-DMSO-$d_6$. To a solution of the resulting colourless solid in DMSO-$d_6$ (1.0 mM, 0.50 mL, 0.5 µmol) was added a solution of DCl in DMSO-$d_6$ (1.0 M, 30 µL, 30 µmol, 60 equiv.) to decompose the complex. The resulting solution was analysed by $^1$H NMR measurement to estimate the molar ratio of both protonated ligands, ethylenediamine/L$_1$ to be 3.0 ± 0.1.

Fig. S6 $^1$H NMR spectrum of digested [Pd$_3$L$_1$(en)$_3$](NO$_3$)$_6$ (500 MHz, DMSO-$d_6$–DCl, 300 K).

[Pd$_3$L$_2$(en)$_3$](NO$_3$)$_6$. To a solution of L$_2$ in CHCl$_3$ (2.0 mM, 2.08 mL, 4.16 µmol, 1.00 equiv.) was added a solution of Pd(en)(NO$_3$)$_2$ in CH$_3$CN (10 mM, 2.08 mL, 20.8 µmol, 5.00 equiv.) gently at room temperature. The solution was left to stand for 17 h. A colourless precipitate was collected by
centrifugation, washed three times with CH$_3$CN (2 mL), and then dried in vacuo to afford [Pd$_3$L$_2$(en)$_3$](NO$_3$)$_6$·8H$_2$O as a colorless solid (4.08 mg, 2.36 µmol, 57%).

ESI-TOF mass: m/z calcd for [C$_{54}$H$_{78}$N$_{12}$Pd$_3$ + NO$_3$ − 3H]$^{2+}$ 636.66; found 636.72.

Elemental analysis: calcd for [C$_{54}$H$_{94}$N$_{18}$O$_{26}$Pd$_3$ ([Pd$_3$L$_2$(en)$_3$](NO$_3$)$_6$·8H$_2$O)]: C 37.48, H 5.47, N 14.57; found: C 37.73, H 5.49, N 14.26.

FT-IR (ATR): 3217, 3106, 1338, 1059, 856, 824, 668 cm$^{-1}$.

M.p.: > 185 °C (dec.).

**Fig. S7** ESI-TOF MS spectrum of acidic aqueous solution ([HNO$_3$] = 10 mM) of [Pd$_3$L$_2$(en)$_3$](NO$_3$)$_6$.

The ratio of L$_2$ and [Pd(en)]$^{2+}$ moieties was further confirmed from the $^1$H NMR spectra of a digested solution of [Pd$_3$L$_2$(en)$_3$](NO$_3$)$_6$ in DCl-DMSO-$d_6$. To a solution of the resulting colourless solid in DMSO-$d_6$ (1.0 mM, 0.50 mL, 0.5 µmol) was added a solution of DCl in DMSO-$d_6$ (1.0 M, 30 µL, 30 µmol, 60 equiv.) to decompose the complex. The resulting solution was analysed by $^1$H NMR measurement to estimate the molar ratio of both protonated ligands, ethylenediamine/L$_2$ to be 3.4 ± 0.4.

**Fig. S8** $^1$H NMR spectrum of digested [Pd$_3$L$_2$(en)$_3$](NO$_3$)$_6$ (500 MHz, DMSO-$d_6$-DCl, 300 K).

$^1$H NMR spectra of 4 in D$_2$O only or acidic D$_2$O (DNO$_3$ = 10 mM)

S6
[Pd₃L₂(en)₃](NO₃)₆·8H₂O (1.0 mg, 0.60 µmol) was dissolved in D₂O (0.6 mL) degassed by passing a nitrogen gas for 1 min. After 10 min, ¹H NMR measurement was carried out (Fig. S9a). Then, a D₂O solution of DNO₃ (1.3 M, 4.6 µL, 6.0 µmol) was added to the solution, and ¹H NMR measurement was immediately conducted (Fig. S9b). The resulting spectra did not change after 1 h.

Fig. S9 ¹H NMR spectra of [Pd₃L₂(en)₃](NO₃)₆ (1.0 mM) (500 MHz, D₂O, 292 K) (a) without DNO₃ and (b) with DNO₃ ([DNO₃] = 10 mM).

**UV-vis absorption measurements of 4**

[Pd₃L₂(en)₃](NO₃)₆·8H₂O (0.58 mg, 0.34 µmol) was dissolved in DMSO (1.0 mL) degassed by the freeze-pump-thaw method. Similarly, [Pd₃L₂(en)₃](NO₃)₆·8H₂O (0.61 mg, 0.35 µmol) was dissolved in acidic water (1.0 mL, [HNO₃] = 10 mM) degassed by the freeze-pump-thaw method. Both solutions were analysed by UV-vis absorption spectrosopies at 25 °C with a 0.1 cm-cell.

**DLS measurements of 3 and 4**

[Pd₃L₁(en)₃](NO₃)₆ (0.81 mg, 0.539 µmol) was dissolved in 10 mM HNO₃ aq (0.540 mL, 1.00 mM), and then filtered with a disc filter (0.45 µm, PTFE hydrophilic). DLS was measured for the resulting solution after 1 d (Fig. S10a).

[Pd₃L₂(en)₃](NO₃)₆·8H₂O (7.68 mg, 4.44 µmol) was dissolved in 10 mM HNO₃ aq (0.444 mL, 10.0 mM). A part of this solution was diluted with 10 mM HNO₃ aq into 1.0 mM. The solution was centrifuged to remove dusts (6200 ± 20% rpm, 10 min). DLS was measured for the resulting solution after 2 h (Fig. S10b).
Fig. S10 DLS profiles for aqueous solutions ([HNO$_3$] = 10 mM) of (a) 3 ([3] = 1.0 mM) and (b) 4 ([4] = 1.0 mM).

**TEM measurements of 3 and 4**

[Pd$_3$L1(en)$_3$(NO$_3$)$_6$] (0.13 mg, 0.086 µmol) was dissolved in 10 mM HNO$_3$ aq (86 µL, 1.0 mM). 2–3 µL of this solution was put on the TEM grid, and then lyophilized to conduct TEM measurements (Fig. S11a).

[Pd$_3$L2(en)$_3$(NO$_3$)$_6$·8H$_2$O] (0.49 mg, 0.28 µmol) was dissolved in 10 mM HNO$_3$ aq (94 µL, 3.0 mM) and then centrifuged (6200 ± 1340 rpm, 10 min). A supernatant (2–3 µL) was put on the TEM grid, and then lyophilized to conduct TEM measurements (Fig. S11c).

Fig. S11 TEM images of aggregates formed from (a) 3 and (c) 4, and size distributions of aggregates observed on their TEM measurements for (b) 3 and (d) 4.

**STEM and EDS measurements of 4**

[Pd$_3$L2(en)$_3$(NO$_3$)$_6$·8H$_2$O] (0.30 mg, 0.17 µmol) was dissolved in degassed 10 mM HNO$_3$ aq (58 µL, 3.0 mM). After 18 h, the resulting solution was centrifuged (6200 ± 1340 rpm, 10 min). A supernatant (1 µL) was put on the hydrophilized TEM grid, and then lyophilized to conduct STEM and EDS measurements.
SLS measurement of 4

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{[\text{Pd}_3\text{L}_2(\text{en})_3](\text{NO}_3)_6\cdot8\text{H}_2\text{O}} \text{ (72.7 mg, 42.0 \text{ \(\mu\)mol}) was dissolved in degassed 10 mM HNO}_3 \text{ aq (5.0 mL, 8.4 mM) and the resulting solution was centrifuged. After 14 h, the differential refractive index of the solution was measured in advance (dn/dC = 2.002 \times 10^{-1} \text{ mL/g}). Then SLS measurements were carried out at different concentrations ([4] = 2.1, 4.2, and 8.4 mM) and scattering angles (30–135°) at 24.9 °C. The collected data were analysed to make a Zimm plot. The weight-average molecular weight (MW) and the radius of gyration (R_G,0) at scattering angle = 0° were estimated to be 2 \times 10^6 \text{ D and 142 nm, respectively, from the extrapolation of the Zimm plot (Fig. S12). These values were evaluated based on the assumed aggregate structure (Fig. S13).}
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Fig. S12  Zimm plot of an aqueous solution ([HNO_3] = 10 mM) of 4.

Fig. S13  Estimation of the number of 4 forming the bilayer of the vesicle with a diameter of 280 nm. The estimated number (ca. 500,000 molecules) is not consistent with the result of Zimm plot (1,600–1,700 molecules), suggesting the low efficiency of the aggregation.
Angle-dependent DLS measurements of 4

$[\text{Pd}_3\text{L}_2(\text{en})_3][\text{NO}_3]_6 \cdot 8\text{H}_2\text{O}$ (72.7 mg, 42.0 µmol) was dissolved in degassed 10 mM HNO$_3$ aq (5.0 mL, 8.4 mM) and the resulting solution was centrifuged. After 11 h, DLS measurements were carried out at different scattering angles (30°–135°) at 24.9 °C (Fig. S14). By extrapolating, $R_{H,0}$ was estimated to be ca. 410 nm.

![DLS measurements of 4](image)

Fig. S14  (a) Diameter and (b) diffusion coefficient estimated from angle-dependent DLS measurements of an aqueous solution ([HNO$_3$] = 10 mM) of 4 ([4] = 8.4 mM). (c) The ratio of $R_{G,0}$ and $R_{H,0}$.

DLS measurements of protonated L1 and L2

To solutions of L1 or L2 in DMSO (0.50 µmol, 0.10 mL, 5.0 mM) was added a solution of 0–10 eq of HCl in DMSO ([HCl] = 100 mM). The resulting solution was diluted with H$_2$O into H$_2$O:DMSO = 9:1, and then filtered with disc filter (0.45 µm, PTFE hydrophilic). After 30 min to 1 d, DLS was measured (Fig. S15).
Fig. S15  DLS profiles for H$_2$O/DMSO (9/1) solutions of L1 or L2 with several amount of HCl. The reason for the aggregation with 0 equiv. of HCl is probably due to slight protonation of amines of L1 or L2 based on the acid-base equilibrium between water and amines in aqueous solution.

**AFM measurements of protonated L1 and L2**

To a solution of L1 in DMSO (0.25 µmol, 50 µL, 5.0 mM) was added a solution of HCl in DMSO (1.5 µmol, 15 µL, 100 mM). A 50 µL of the resulting solution was diluted with H$_2$O (0.45 mL) into H$_2$O:DMSO = 9:1, and then filtered with a disc filter (0.45 µm, PTFE hydrophilic). After 1 h, a droplet of this solution was deposited on a cleaved mica substrate and incubated for several minutes. After removing the bulk solution by spin coating, AFM was measured.

To a solution of L2 in DMSO (2.2 µmol, 440 µL, 5.0 mM) was added a solution of HCl in DMSO (13 µmol, 130 µL, 100 mM). A 50 µL of the resulting solution was diluted with H$_2$O (0.45 mL) into H$_2$O:DMSO = 9:1, and then filtered with a disc filter (0.45 µm, PTFE hydrophilic). After 2 d, a droplet of this solution was deposited on a cleaved mica substrate and incubated for several minutes. After removing the bulk solution by spin coating, AFM was measured.

**References**