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Supporting Information

A water-soluble metal–organic complex array as a multinuclear heterometallic peptide amphiphile that shows unconventional anion dependency in its self-assembly

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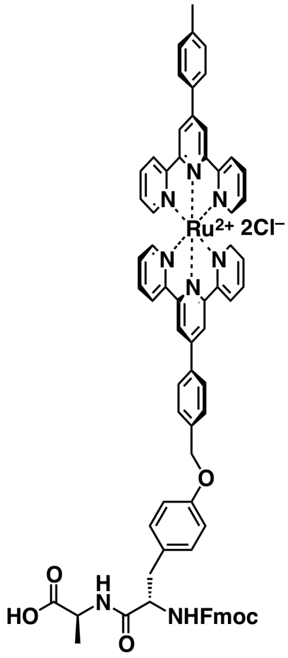
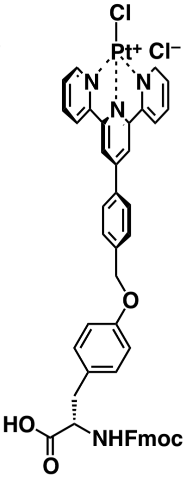
1. General methods

CH_2Cl_2, THF and DMF were treated with the Grubbs apparatus prior to use. Other chemicals and solvents were purchased from TCI, Kanto Chemical, Aldrich, Merck, or Watanabe Chemical Industries and used as received. ^{1}H and ^{19}F NMR spectra were recorded on a JEOL model AL300 spectrometer operating at 300 and 282 MHz, respectively. Chemical shift values in ^{1}H or ^{19}F NMR spectra were determined using the non-deuterated solvent residue or C_6F_6 (δ –164.9) as an internal reference, respectively. Matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry was performed on a Shimadzu-Kratos AXIMA-CFR$^+$ using dithranol or CCA as a matrix. Electronic absorption spectra were recorded on a JASCO model V-650DS spectrophotometer. Reverse-phase HPLC was performed using a JASCO model PU-2080 Plus equipped with a variable-wavelength UV/Vis detector UV-2070 Plus. Circular dichroism (CD) spectra were recorded on a JASCO Type J-820 spectropolarimeter. Dynamic light scattering (DLS) and zeta potential data were obtained on a Beckman Coulter model Delsa$^{\text{TM}}$Nano C. Transmission electron microscopy (TEM) was performed on a JEOL model JEM 2100F operating at 200 kV. The cryogenic TEM experiments were performed with a thin film of sample solution (3 μL) transferred to a lacey supported grid. The thin sample films were prepared under controlled temperature and humidity conditions (97–99 %) within a custom-built environmental chamber in order to prevent evaporation of solvent from sample solution. The excess liquid was blotted with filter paper for 2–3 seconds, and the thin films were rapidly vitrified by plunging them into liquid ethane (cooled by liquid nitrogen) at its freezing point. The grid was transferred, on a Gatan 626 cryoholder, using a cryo-transfer device and transferred to the JEOL–JEM HR2100 TEM. Direct imaging was carried out at a temperature of approximately –175 °C and with a 120 kV accelerating voltage, using the images acquired with a Dual vision 300 W and SC 1000 CCD camera (Gatan, Inc.; Warrendale, PA). The data were analyzed using Digital Micrograph software.

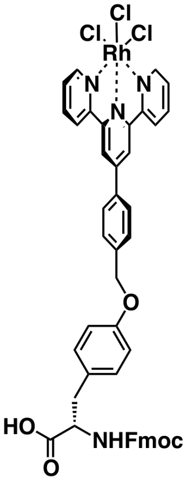
2. Synthesis



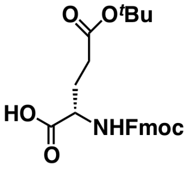
Ru monomer



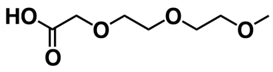
Pt monomer



Rh monomer



Glu



TEG acid

Ru, Pt and Rh monomers were prepared as reported (*S1, S2*), while Fmoc- and side-residue ^{t}Bu-protected (*L*)-glutamic acid (Glu) as well as 2-[2-(2-Methoxyethoxy)ethoxy]acetic acid (TEG acid) were purchased and used as received.

Protocol a: Fmoc deprotection.

Typically, to a CH_2Cl_2 (1 mL) suspension of resin (as-received or metal complex loaded NovaSyn TG Sieber resin, 20 µmol) were added DMF (3 mL) and piperidine (1 mL) under N_2 atmosphere and the mixture was stirred for 2.5 h at 25 °C. After the solution was removed by filtration, the resin was washed with MeOH (3 mL, 3 min stirring) and CH_2Cl_2 (3 mL, 3 min stirring) alternately three times, and with CH_2Cl_2 (3 mL, 3 min stirring) four times. The solution part of the reaction mixture separated from the resin was combined with solutions used for washing of the resin and diluted with CH_3CN to a volume of 50 mL. The amount of the deprotected Fmoc moiety was calculated absorption spectroscopically, by using the molar absorptivity for piperidine–dibenzofulvene adduct (6234 at 299 nm; *S1–S3*).

Protocol b: Loading of metal complex monomers, Glu or TEG acid to the resin.

Typically, to a CH_2Cl_2 suspension (0.5 mL) of the resin (20 μmol) subjected to Protocol a were added one of the loading substrate (40 μmol), HBTU (22.7 mg, 60 μmol), DMSO (4.5 mL) and *i*Pr_2NEt (50 μL) in this order under N_2 atmosphere and the mixture was stirred for 12 h at 25 °C. After the solution was removed by filtration, the resin was washed with DMSO (3 mL, 5 min stirring) three times, MeOH (3 mL, 3 min stirring) and CH_2Cl_2 (3 mL, 3 min stirring) alternately three times, and with CH_2Cl_2 (3 mL, 3 min stirring) three times. The solution part of the reaction mixture separated from the resin was combined with DMSO solutions used for washing of the resin and diluted with CH_3CN to a volume of 50 mL. The loading amount of the reactant to the resin was evaluated through the following Fmoc deprotection.

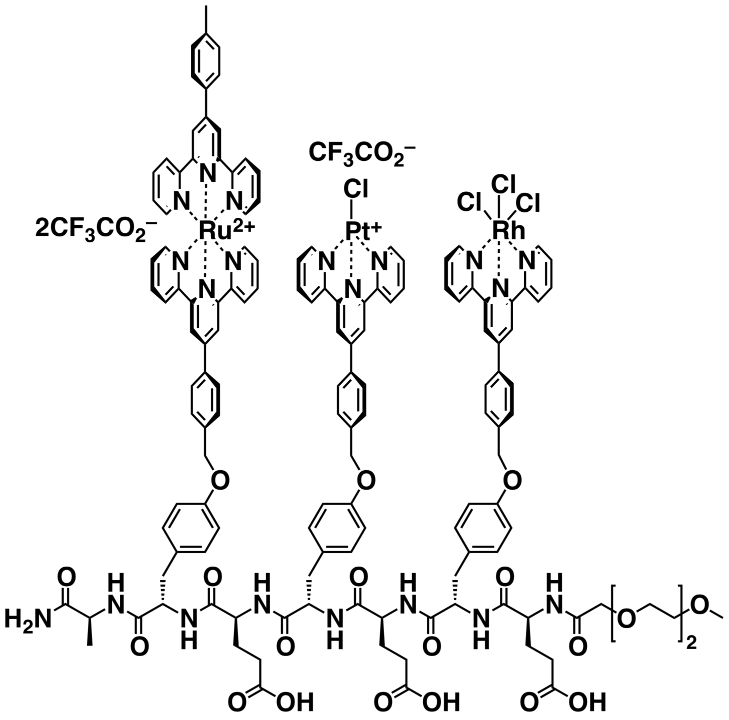
Protocol c: Capping of unreacted –NH_2 terminal on the resin.

Typically, to a CH_2Cl_2 suspension (1 mL) of the resin (20 μmol) subjected to Protocol b were added CH_2Cl_2 (3 mL), benzoic anhydride (1.5 mmol) and *N*-methylimidazole (*N*-MeIm; 0.12 mL, 1.5 mmol) under N_2 atmosphere and the mixture was stirred for 2 h at 25 °C. After the solution was removed by filtration, the resin was washed with CH_2Cl_2 (3 mL, 3 min stirring) and MeOH (3 mL, 3 min stirring) alternately three times, and with CH_2Cl_2 (3 mL, 3 min stirring) three times.

Protocol d: Cleavage from the resin.

Typically, resin (20 μmol) with metal–organic complex arrays was washed with Et_2O (4 mL, 5 min stirring) three times, dried under vacuum and swelled with CH_2Cl_2 (1 mL, 5 min stirring). To the CH_2Cl_2 suspension was added a mixture of CF_3CO_2H (0.1 mL), Et_3SiH (20 μL) and 1,2-dichloroethane (1.9 mL), and the mixture was stirred for 1 h at 25 °C. After the solution was removed, the resin was washed with 1,2-dichloroethane three times. Treatment of the resin with a new CF_3CO_2H/Et_3SiH/1,2-dichloroethane mixture was repeated until the solution after stirring became visually colorless. All of the solutions were combined together and evaporated to dryness to leave residue containing a cleaved mixture of metal–organic complex arrays. At the end of Protocols a–c small amount of resin was subjected to similar conditions and the resulting solution was analyzed by mass spectrometry to check the production of targeted arrays.

Synthesis of Ru-Pt-Rh triad 1



a1) *Fmoc deprotection*: As-received NovaSyn TG Sieber resin (130 mg, 27.6 μmol –NH_2 group) was Fmoc-deprotected.

b1) *Loading of* Ru monomer: 70.2 mg, 54.4 μmol added, 20.2 μmol loaded.

c1) *Capping of unreacted –NH_2 terminal on the resin*.

a2) *Fmoc deprotection.*

b2) *Loading of* Glu·H_2O: 44.7 mg, 100.9 μmol added, 19.3 μmol loaded.

c2) *Capping of unreacted –NH_2 terminal on the resin*.

a3) *Fmoc deprotection.*

b3) *Loading of* Pt monomer: 36.7 mg, 37.0 μmol added, 15.6 μmol loaded.

c3) *Capping of unreacted –NH_2 terminal on the resin.*

a4) *Fmoc deprotection.*

b4) *Loading of* Glu·H_2O: 34.6 mg, 78.1 μmol added, 12.9 μmol loaded.

c4) *Capping of unreacted –NH_2 terminal on the resin*.

a5) *Fmoc deprotection.*

b5) *Loading of* Rh monomer: 24.0 mg, 25.7 μmol added, 11.4 μmol loaded.

c5) *Capping of unreacted –NH*$_2$ *terminal on the resin.*

a6) *Fmoc deprotection.*

b6) *Loading of* Glu·H_2O: 25.2 mg, 56.8 μmol added, 9.7 μmol loaded.

c6) *Capping of unreacted –NH*$_2$ *terminal on the resin.*

a7) *Fmoc deprotection.*

b7) *Loading of* TEG acid: 17.2 mg, 96.8 μmol added.

d) *Cleavage from the resin.*

The cleavage mixture from the resin was evaporated to dryness, and the residue was dissolved in DMSO (0.5 mL). To the solution was added excess amount of EtOAc, and the resultant suspension after standing for 1 h was centrifuged. The supernatant was removed by decantation, and the residue was subjected to the re-precipitation process again. To the residue was added MeOH (1 mL), and the suspension was sonicated for 5 minutes and centrifuged. The supernatant was removed by decantation, and the residue was subjected to the washing process with MeOH again. The residue was dried under reduced pressure to afford Ru-Pt-Rh triad **1** as red solid (7.3 mg, 2.2 μmol, 8%). ^{1}H NMR (300 MHz, DMSO-d_6) Broadening and poor resolution of the spectrum hampered the signal assignments; ^{19}F NMR (283 MHz, DMSO-d_6) δ –77.4 (s, $CF_3CO_2^-$). MALDI-TOF-MS (dithranol) *m*/*z* calcd. for $C_{140}H_{130}Cl_2N_{20}O_{20}PtRhRu$ ($[M–2Cl–3CF_3CO_2]^+$) 2881.69, found 2880.17. UV-vis (CH_3CN, 25 ºC): $\varepsilon_{490} = 29000\ M^{-1}cm^{-1}$.

MALDI-TOF mass spectrometry

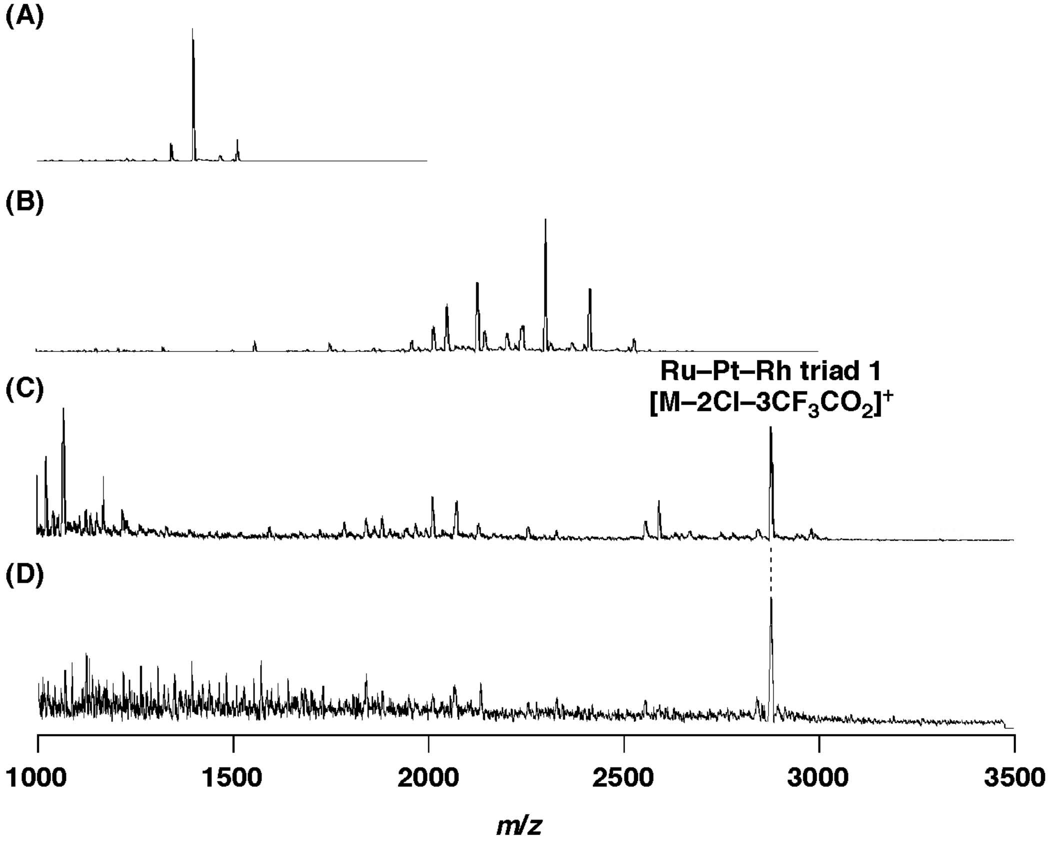


Fig. S1. MALDI-TOF mass spectra of as-cleaved from the resin samples after synthetic steps (A) c2), (B) c4) and (C) d) as well as (D) that of **1** after purification.

^{1}H NMR spectroscopy

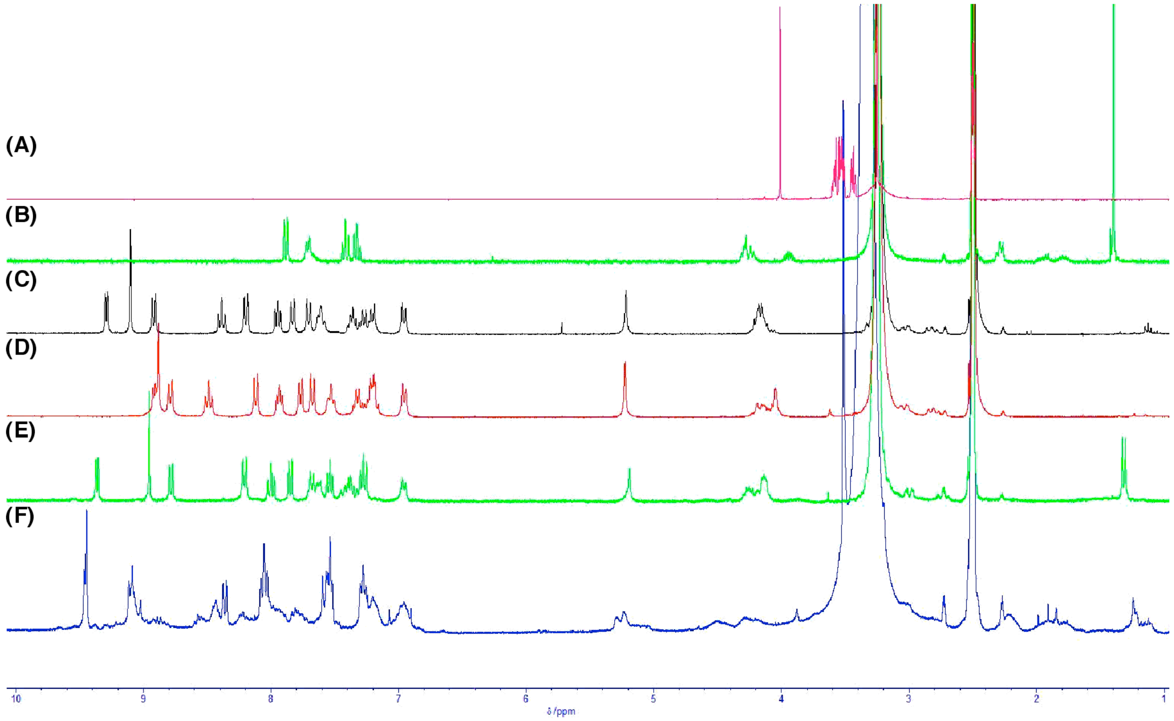


Fig. S2. ^{1}H NMR spectra of (A) TEG acid, (B) Glu, (C) Rh monomer, (D) Pt monomer, (E) Ru monomer and (F) **1** in DMSO-d_6 at 40 °C.

Reverse-phase HPLC

Column:	YMC-Triart C18 (250 x 4.6 mm)
Eluent:	CH_3CN
Flow rate:	0.5 mL/min
Detection:	280 nm

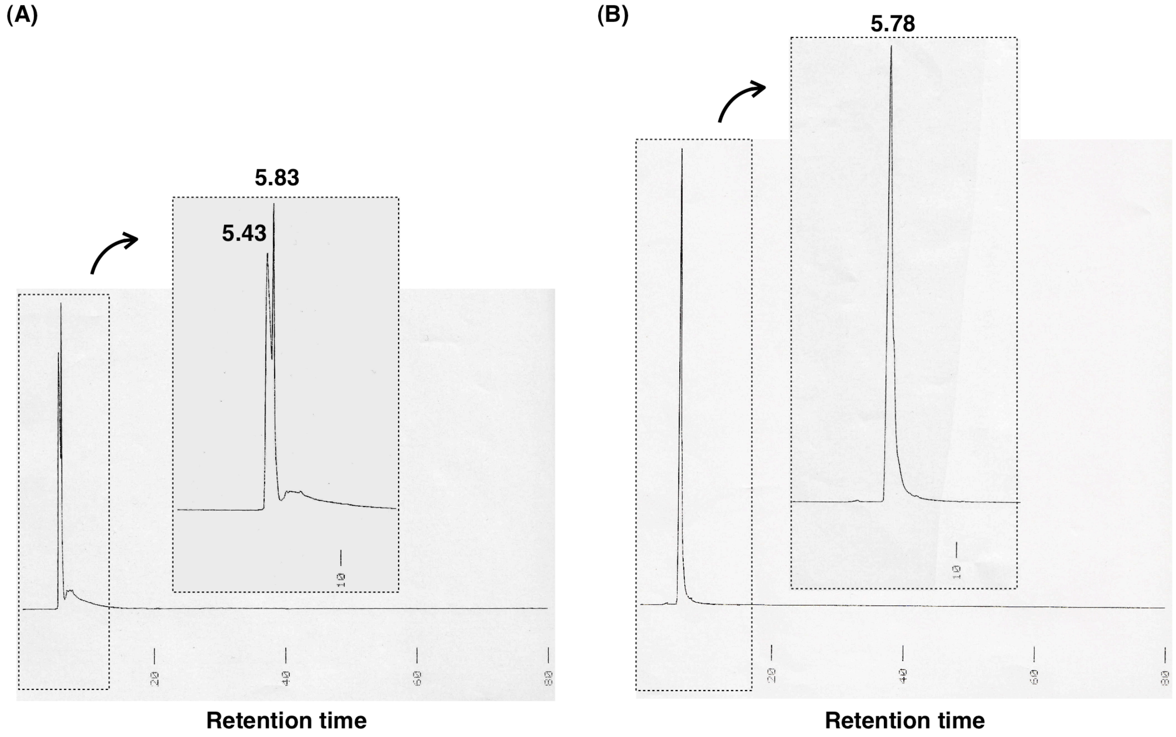
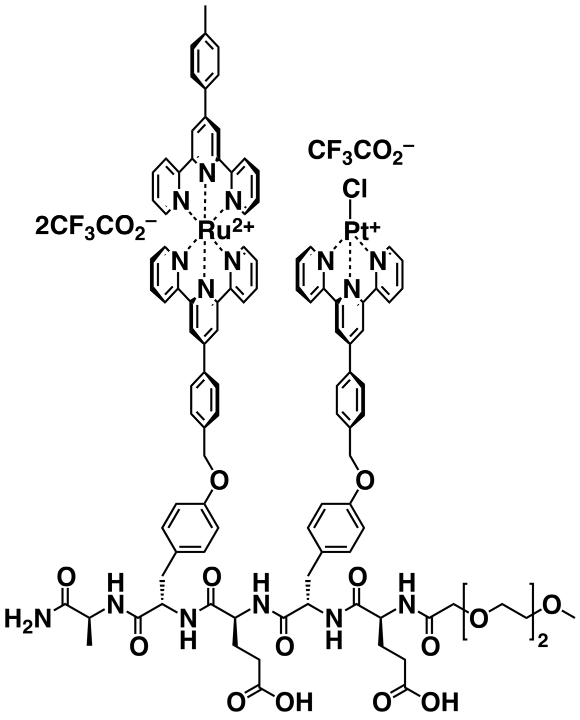


Fig. S3. Reverse-phase HPLC chromatograms of (A) as-cleaved from the resin sample after synthetic step d) and (B) **1** after purification. Samples were dissolved in CH_3CN containing 20 mM of $NaOCOCF_3$ and subjected to the analysis.

Synthesis of Ru-Pt dyad 2



a1) *Fmoc deprotection*: As-received NovaSyn TG Sieber resin (134.1 mg, 27.5 μmol –NH_2 groups) was Fmoc-deprotected.

b1) *Loading of* Ru monomer: 67.2 mg, 52.1 μmol added, 18.1 μmol loaded.

c1) *Capping of unreacted –NH_2 terminal on the resin*.

a2) *Fmoc deprotection.*

b2) *Loading of* Glu·H_2O: 40.1 mg, 90.4 μmol added, 17.7 μmol loaded.

c2) *Capping of unreacted –NH_2 terminal on the resin.*

a3) *Fmoc deprotection.*

b3) *Loading of* Pt monomer: 35.0 mg, 35.3 μmol added, 10.9 μmol loaded.

c3) *Capping of unreacted –NH_2 terminal on the resin.*

a4) *Fmoc deprotection.*

b4) *Loading of* Glu·H_2O: 24.1 mg, 54.3 μmol added, 10.8 μmol loaded.

c4) *Capping of unreacted –NH_2 terminal on the resin.*

a5) *Fmoc deprotection.*

b5) *Loading of* TEG acid: 38.3 mg, 216.8 μmol added.

d) *Cleavage from the resin.*

The cleavage mixture from the resin was evaporated to dryness, and to the residue was added CH_2Cl_2 (10 ml). The suspension was sonicated for 10 min and centrifuged after standing for 1 h. The supernatant was removed by decantation, and the residue was dissolved in MeOH (1 ml). To the solution was added excess amount of CH_2Cl_2 and the resultant suspension was sonicated for 10 min and centrifuged after standing for 1 h. The supernatant was removed by decantation, and the residue was washed with MeOH and CH_2Cl_2. The residue was dried under reduced pressure to afford Ru-Pt dyad **2** as red solid (15 mg, 6.1 μmol, 22%). ^{1}H NMR (300 MHz, CD_3OD) δ 1.15–1.35 (d x 2, 3H, Ala-CH_3), 1.80–2.56 (m, 8H, Glu-CH_2CH_2), 2.54 (s, 3H, tolyl-Tpy-CH_3), 2.80–3.20 (m, 4H, Tyr-CH_2), 3.30 (s, 3H, TEG-CH_3), 3.53–3.68 (m, 8H, TEG-OCH_2CH_2), 4.00 (s, 2H, TEG-CO-CH_2), 4.51–4.26 (m, 5H, Ala, Glu and Tyr-CH), 5.14 (s, 2H, OCH_2-Ar), 5.21 (s, 2H, OCH_2-Ar), 6.89–6.99 (m, 4H), 7.16–7.19 (m, 4H), 7.26–7.30 (m, 4H), 7.55–7.58 (m, 6H), 7.66–7.71 (m, 4H), 7.86 (br, 2H), 7.98–8.04 (m, 6H), 8.17–8.24 (m, 4H), 8.41–8.47 (m, 2H), 8.64 (d, 2H), 8.76 (s, 2H, PtTpy), 8.86 (t, 4H), 9.00 (br, 2H), 9.23–9.24 (s x 2, 4H, RuTpy); ^{19}F NMR (283 MHz, CD_3OD) δ –77.1, –76.9 (s x 2, $CF_3CO_2^-$). MALDI-TOF-MS (CCA) *m*/*z* calcd. for $C_{104}H_{99}ClN_{15}O_{15}PtRu$ ($[M-3CF_3CO_2]^+$) 2130.59, found 2130.54. UV-vis (MeOH, 25 ºC): ε_{490} = 29100 $M^{-1}cm^{-1}$.

MALDI-TOF mass spectrometry

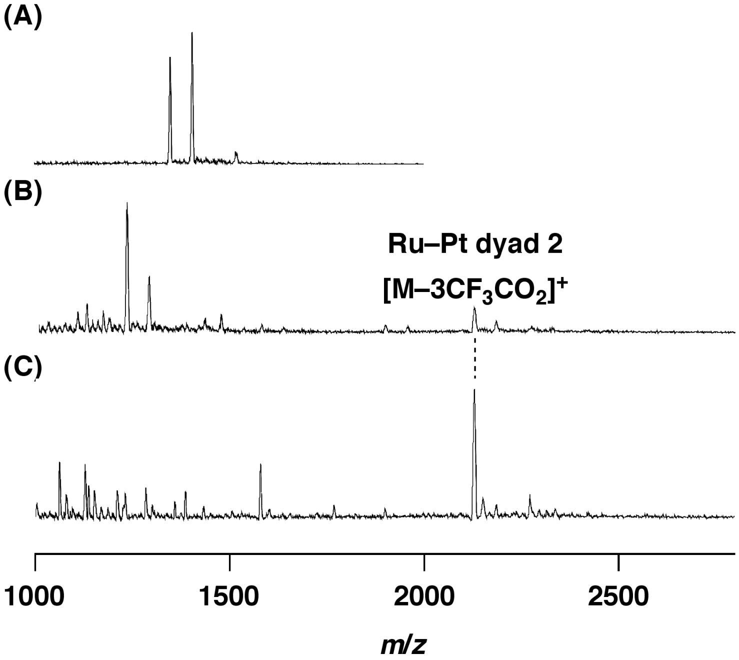


Fig. S4. MALDI-TOF mass spectra of as-cleaved from the resin samples after synthetic steps (A) c2) and (B) d) as well as (C) that of **1** after purification.

^{1}H NMR spectroscopy

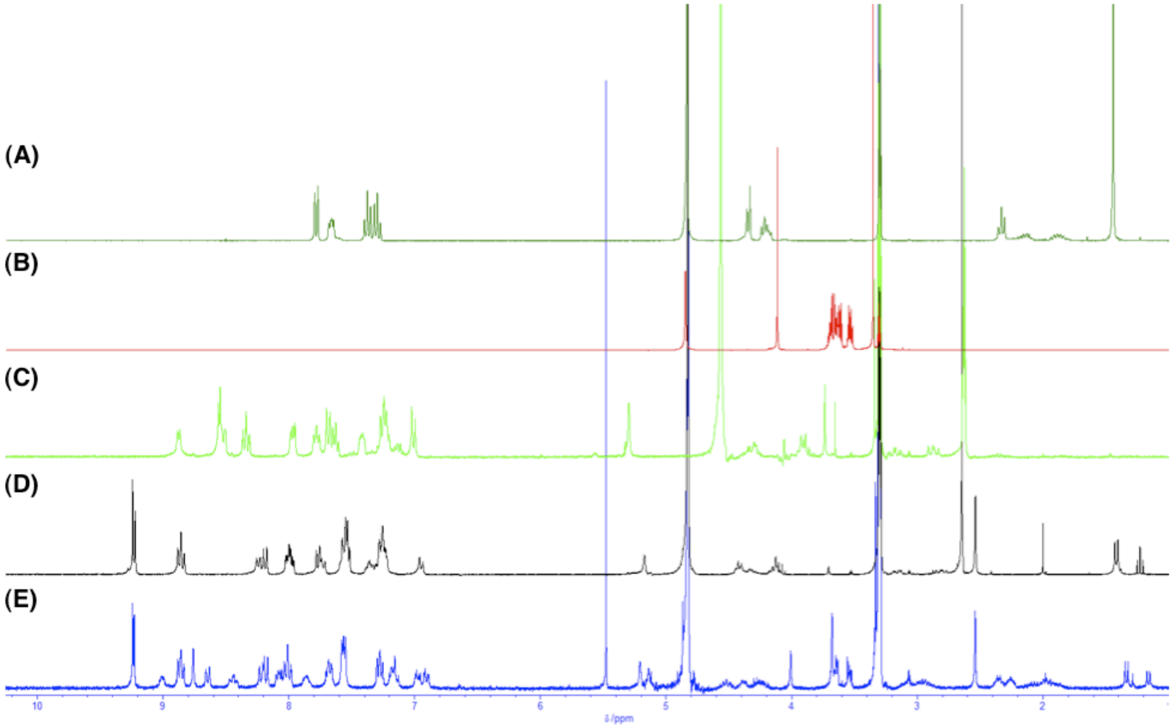


Fig. S5. ^{1}H NMR spectra of (A) Glu, (B) TEG acid, (C) Pt monomer, (D) Ru monomer and (E) **2** in CD_3OD at 25 °C. DMSO-d_6 was added to the CD_3OD suspension of Pt monomer for the dissolution.

3. Self-assembly

Typically, to a CH_3CN solution (100 μL, 500 μM) of Ru-Pt-Rh triad **1** or a suspension of Ru-Pt dyad **2** was added Mili-Q grade water (900 μL) and the resultant solution was sonicated for 1 h. To the solution was added HCl (1 μL, 12 N), NaCl (5.8 mg, 0.1 mmol or 0.58 mg, 0.01 mmol), NaCl (5.8 mg, 0.1 mmol) followed by aqueous NaOH (2.5 μL, 1 mM) or $NaOCOCF_3$ (13.6 mg, 0.1 mmol), and the mixture was incubated at 37 °C for 2 days. For the preparation of a PBS solution of **1**, a CH_3CN solution (100 μL, 500 μM) of **1** was added standard aqueous PBS solution with pH of 7.40 (900 μL). The resultant solution was sonicated for 1 h and incubated at 37 °C for 2 days.

4. Transmission electron microscopy

A carbon coated 200-mesh copper grid (JEOL Cu200) was subjected to glow discharge for 20 seconds before sample adsorption. A sample solution or suspension in the aqueous media was dropped on the grid and left for 30 seconds before removing with a blotter. The grid was immediately rinsed with Mili-Q grade water, allowed to air dry for overnight and subjected to TEM observation. For the preparation of a stained sample, the adsorbed sample on the grid was treated with 1% phosphotungstic acid at pH = 7.0 for 10 seconds, blotted and rinsed with water.

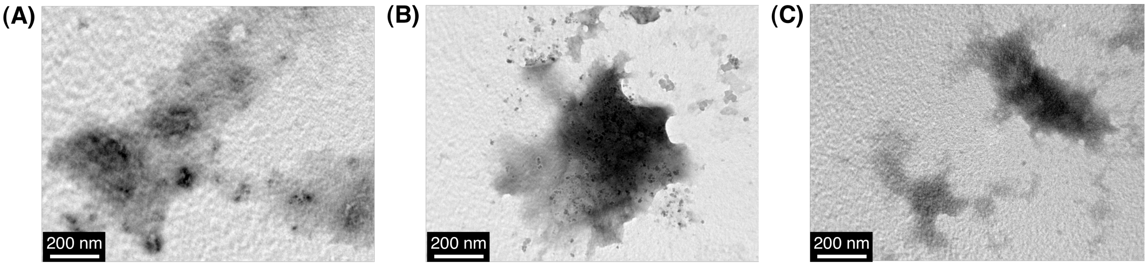


Fig. S6. TEM images of stained **1** aggregated in aqueous media (10% CH_3CN) in the presence of NaCl (A; 10 mM, B; 100 mM) and NaCl/NaOH (C; 100/0.0024 mM).

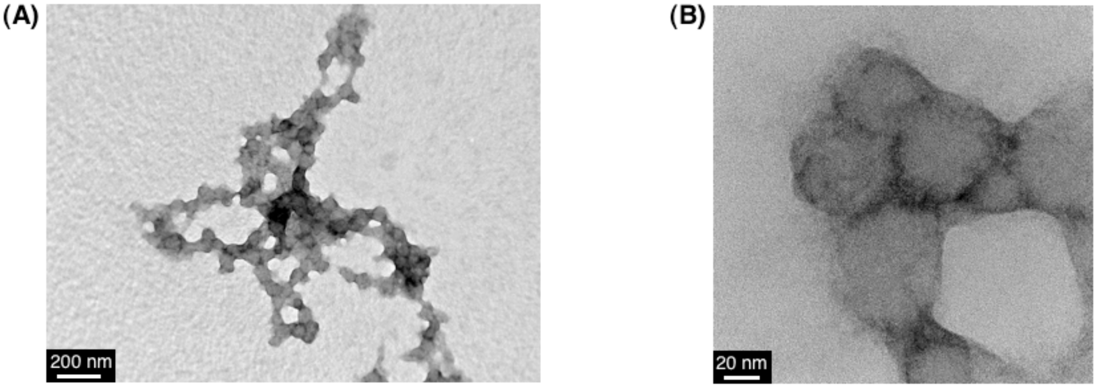


Fig. S7. TEM images of the stained sample prepared from an aqueous Dulbecco's Modified Eagle Medium solution (10% DMSO) of **1** containing fetal bovine serum.

5. Dynamic light scattering

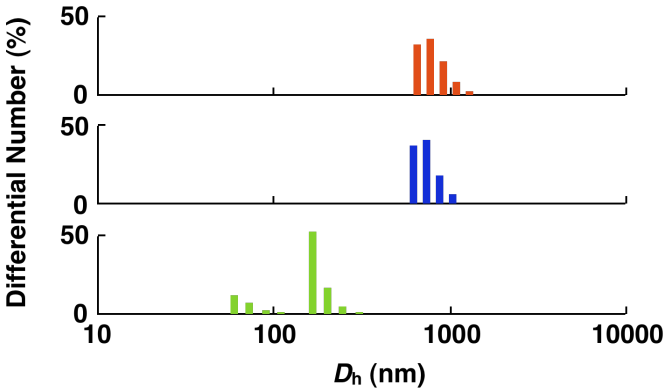


Fig. S8. DLS profiles of **1** in aqueous media (10% CH_3CN) containing NaCl (100 mM; red) or PBS with nile red (1 μM; blue) and that in aqueous Dulbecco's Modified Eagle Medium solutions (10% DMSO) containing fetal bovine serum (green).

Fig. S9. DLS profile of **2** in aqueous media (10% CH_3CN) containing NaCl/NaOH (100/0.0024 mM).

6. CD spectroscopy

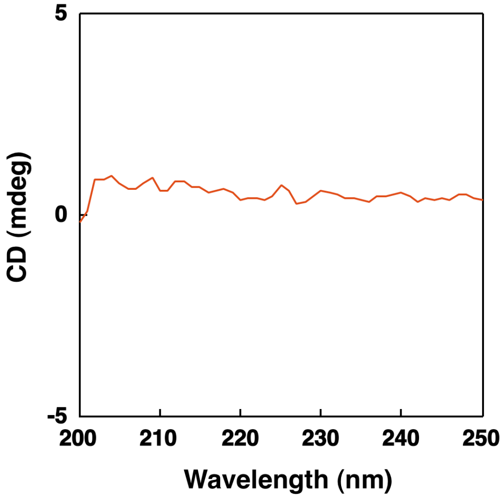


Fig. S10. CD spectrum of assembled **1** in aqueous media (10% CH_3CN) acidified with HCl to pH value of 1.98.

7. References

S1. P. Vairaprakash, H. Ueki, K. Tashiro and O. M. Yaghi, *J. Am. Chem. Soc.* 2011, **133**, 759.

S2. A. M. Fracaroli, K. Tashiro, O. M. Yaghi, *Inorg. Chem.* 2012, **51**, 6437.

S3. K. V. Sajna, A. M. Fracaroli, O. M. Yaghi, K. Tashiro, *Inorg. Chem.* 2015, **54**, 1197.