Layered assembly of cationic and anionic supramolecular polymers

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1 Abbreviations

AFM Atomic force microscope
APTES (3-Aminopropyl)triethoxysilane
CEP-Cl 2-Cyanoethyl N,N-diisopropylchlorophosphoramidite
DCE 1,2-Dichloroethane
DCM Dichloromethane
DIPEA N,N-Diisopropylethylamine
DLS Dynamic light scattering
Et$_3$N Triethylamine
EtOAc Ethyl acetate
MeOH Methanol
RT Room temperature
TEM Transmission electron microscope
THF Tetrahydrofuran
TLC Thin-layer chromatography
General methods

All chemicals and solvents were purchased from commercial suppliers and used without further purification. 3,6-Dibromophenanthrene (2) was prepared according to literature. All NMR spectra were measured on a Bruker Avance III HD (300 MHz) spectrometer. Mass spectra were obtained on a Thermo Fisher LTQ Orbitrap XL with nano Electrospray Ionization (ESI) from the Analytical Research and Services of the University of Bern. Absorption spectra were measured on an Agilent Cary 100 spectrophotometer using quartz cuvettes with an optical path of 1 cm. Fluorescence spectra were recorded on a Cary Eclipse fluorescence spectrophotometer setting the excitation and emission slits to 5 nm. The supramolecular polymer formation of N-Phe₃ (vesicles) was performed via thermal disassembly and reassembly process. N-Phe₃ in ethanol was added to a solution of 10 mM sodium acetate buffer (pH 4.7) and 10 vol% ethanol. The sample was heated to 70 °C to fully disassemble the N-Phe₃ and then cooled to 20 °C with a controlled rate of 0.33 °C/min in an Eppendorf Thermomixer Compact to form the N-Phe₃ vesicles. The formed vesicles were stable for at least 24 h. The formation of Py₃-nanosheets was achieved in 10 mM sodium phosphate buffer (pH 7.1), 10 mM sodium chloride and 10 vol% ethanol with a controlled cooling rate of 0.5 °C/min from 70 °C to 20 °C in a Cary 100 spectrophotometer equipped with a Peltier thermostat. Atomic force microscopy (AFM) measurements were performed on a Nanosurf FlexAFM instrument in tapping mode under ambient conditions. Tap190-Al-G cantilevers from Budget-Sensors were used with a resonant frequency of 190 kHz, a force constant of 48 N/m and a tip radius of 10 nm. Mica sheets (Glimmer “V1”, 20 mm x 20 mm, G250-7, Plano GmbH) were used as substrates. The graphical illustration and evaluation of the AFM measurements was done with the softwares Nanosurf C300 and SPIP. The mica sheets were APTES-modified according to published procedures. For every sample preparation, 30 μl of the sample solution was pipetted onto the mica sheet. After 10 minutes of adsorption time, the solution was sucked with a KIMTECH® precision wipe and the mica was rinsed with Milli-Q water (1 ml), then dried under an argon stream. For the electrostatic layer assembly, the two molecules N-Phe₃ and Py₃ had to be preassembled as previously described. Py₃-sheets were adsorbed on APTES-modified mica, the mica was washed and dried as previously described, and afterwards the second layer, positively charged N-Phe₃-vesicles, was adsorbed on top and treated like before. Transmission electron microscopy (TEM) was performed on a Tecnai Spirit instrument with an operating voltage of 80 kV and with either an Olympus-SIS Veleta CCD camera or FEI Eagle CCD camera. The N-Phe₃ was self-assembled as previously described, and a carbon-coated copper grid (300 Mesh, Agar Scientific) was used as a substrate. The samples were prepared as followed: 5 μl of the supramolecular polymer was dropped onto the grid, waited for 10 min, and the solution was blotted. Afterwards the grid was washed by dipping into 20 μl of Milli-Q water and blotting two times. The last step was the staining of the sample with a 0.5% aqueous uranyl-acetate solution. This was done according to the washing procedure: the sample was dipped twice into 20 μl of the uranyl-acetate solution (0.5%). DLS measurements were carried out on a Zetasizer Nano instrument (Malvern Instruments) by using the standard operation procedure for particle size determination. Pre-washed plastic cuvettes were used for the measurements. The equilibration time at 70 °C was 5 minutes, before cooling to 20 °C. The zeta potential and the size distribution measurements were determined by nanoparticle tracking analysis (NTA) with a ZetaView (software version 8.05.05 SP2, ParticleMetrix, Inning am Ammersee, Germany) equipped with a 488 nm laser, zeta potential and temperature control units. Measurements were performed at 20 °C, a camera sensitivity of 85, and 100 1/ms shutter value.

Organic synthesis

3.1 N-Phe₃

3,6-Dibromophenanthrene (2)

The commercially available 4,4’-dibromo-trans-stilbene (1) (2.5036 g, 7.41 mmol) and Iodine (0.3638 g, 1.43 mmol) was dissolved in toluene (4 l). The solution was irradiated with a mercury medium pressure UV lamp for 70 hours while air was bubbled through. The reaction was monitored by ¹H NMR. Water (1 l) and an excess of Na₂S₂O₃ were added to remove the iodine (color change from pink to beige). The organic phase was evaporated under reduced pressure. The obtained yellow solid was dissolved in boiling DCM (99 ml), methanol (60 ml) was added, and the solution was kept for 2 days in
the fridge. The white crystals were filtered off and washed with cold DCM (7 ml). The filtrate was evaporated and recrystallized again. The combined fractions of compound 2 were obtained in 70% yield (1.7460 g). $^1$H NMR (300 MHz, CDCl$_3$) δ 8.69 (d, J = 1.6 Hz, 2H), 7.79 – 7.65 (m, 6H).

2-(5-(6-(5-hydroxypent-1-yn-1-yl)phenanthren-3-yl)pent-4-yn-1-yl)isoindoline-1,3-dione (3) and

5,5'-[(phenanthrene-3,6-diyl)bis(pent-4-yn-1-yl)]isoindoline-1,3-dione (3) and

5,5'-[(phenanthrene-3,6-diyl)bis(pent-4-yn-1-yl)]isoindoline-1,3-dione (3) and

5,5'-[(phenanthrene-3,6-diyl)bis(pent-4-yn-1-ol) (4) (4)

To a degassed solution of 3,6-Dibromophenanthrene 2 (1.0016 g, 2.98 mmol) in dry THF (33 ml) and Et$_3$N (16.5 ml) under an argon atmosphere, Pd[P$_2$Ph$_3$]Cl$_2$ (116.7 mg, 0.166 mmol), CuI (51.9 mg, 0.273 mmol), 4-Pentynyl-phthalimide (635.9 mg, 2.98 mmol) and 4-Pentyn-1-ol (275.5 µl, 2.98 mmol) was added. The black solution was refluxed overnight. The mixture was filtered, the filtrate was washed with citric acid (10%) and saturated NaHCO$_3$ solution. The solution was dried over Na$_2$SO$_4$ and concentrated in vacuo. The crude products 3 and 4 were separated by flash column chromatography on silica gel (hexane/EtOAc 3:1). Compound 3 was isolated as an orange-yellowish solid (437.1 mg, 31%). Compound 4 was isolated as an orange solid (164.7 mg, 16%). $^1$H NMR (300 MHz, CDCl$_3$) δ 8.64 (s, 1H), 8.51 (s, 1H), 7.84 – 7.68 (m, 4H), 7.65 (s, 2H), 7.62 – 7.41 (m, 4H), 3.98 – 3.86 (m, 4H), 2.71 – 2.54 (m, 4H), 2.10 (p, J = 6.9 Hz, 2H), 1.96 (p, J = 6.6 Hz, 2H), 1.80 (p, J = 7.3 Hz, 2H), 1.80 (p, J = 7.0 Hz, 2H), 1.74 – 1.68 (m, 2H), 1.63 – 1.47 (m, 2H), 1.37 – 1.28 (m, 2H), 0.96 (t, J = 6.9 Hz, 2H), 0.87 (t, J = 6.9 Hz, 2H), 0.71 (t, J = 7.3 Hz, 2H), 0.69 (t, J = 7.0 Hz, 2H), 0.68 (t, J = 7.0 Hz, 2H), 0.67 (t, J = 6.9 Hz, 2H). $^{13}$C NMR (75 MHz, CDCl$_3$) δ 168.68, 133.96, 132.29, 131.42, 129.71, 128.43, 127.06, 126.42, 123.30, 122.16, 90.42, 81.68, 62.04, 49.71, 47.16, 31.67, 31.44, 27.61, 21.38, 17.68, 16.30. HRMS-NSI (m/z): [M+H]$^+$ calcd for C$_{33}$H$_{32}$NO$_3$: 472.1907, found: 472.1895. 4: $^1$H NMR (300 MHz, CDCl$_3$) δ 8.69 (s, 2H), 7.77 (d, J = 8.2 Hz, 2H), 7.67 (s, 2H), 7.63 – 7.53 (m, 2H), 3.90 (t, J = 6.2 Hz, 4H), 2.64 (t, J = 6.9 Hz, 4H), 1.94 (p, J = 6.6 Hz, 2H), 1.74 – 1.68 (m, 2H), 1.63 – 1.47 (m, 2H), 1.37 – 1.28 (m, 2H), 0.96 (t, J = 6.9 Hz, 2H), 0.87 (t, J = 6.9 Hz, 2H), 0.71 (t, J = 7.3 Hz, 2H), 0.69 (t, J = 7.0 Hz, 2H), 0.68 (t, J = 7.0 Hz, 2H), 0.67 (t, J = 6.9 Hz, 2H). $^{13}$C NMR (75 MHz, CDCl$_3$) δ 131.54, 129.78, 129.61, 128.62, 127.15, 126.28, 125.74, 125.62, 123.30, 122.16, 90.33, 81.83, 62.04, 49.71, 47.16, 31.67, 31.44, 27.61, 21.38, 17.68, 16.30. HRMS-NSI (m/z): [M+H]$^+$ calcd for C$_{33}$H$_{32}$NO$_3$: 434.1693, found: 434.1703.

5-(6-(5-aminopent-1-yn-1-yl)phenanthren-3-yl)pent-4-yn-1-ol (5)

A solution of compound 3 (212.8 mg, 0.451 mmol) and hydrazine-monohydrate (0.18 ml, 3.610 mmol) in THF (9 ml) was stirred at 40 °C, under argon atmosphere overnight. The grey precipitation was filtrated off and the filtrate was concentrated in vacuo. The crude mixture was diluted with DCM (50 ml) and sat. aq. NaHCO$_3$ solution (50 ml), sat. aq. NaHCO$_3$ souteion (50 ml), and concentrated in vacuo. The solution was dried over MgSO$_4$ and concentrated in vacuo. The crude product was purified by flash column chromatography on silica gel (hexane/EtOAc 3:1). The product was gained as a white yellowish fluffy solid (120.8 mg, 41%). $R_f$ = 0.3 (hexane/EtOAc 2:1). $^1$H NMR (300 MHz, CDCl$_3$) δ 9.92 (s, 2H), 8.69 (s, 2H), 7.78 (d, J = 8.2 Hz, 2H), 7.68 (s, 2H), 7.62 – 7.52 (m, 2H), 2.88 – 2.80 (m, 8H). $^{13}$C NMR (75 MHz, CDCl$_3$) δ 131.35, 129.59, 129.46, 128.46, 128.96, 126.18, 122.02, 90.42, 81.68, 61.73, 41.38, 32.48, 31.45, 17.06, 16.16. HRMS-NSI (m/z): [M+H]$^+$ calcd for C$_{24}$H$_{22}$NO: 342.1852, found: 342.1849.

5,5'-[(phenanthrene-3,6-diyl)bis(pent-4-ynal) (6)

The procedure was carried out analog to reference.[3] Dess-Martin Periodinane (DMP) (817.2 mg, 1.926 mmol) was dissolved in DCM (9 ml) and placed under argon. Compound 4 (299.8 mg, 0.876 mmol) was dissolved in DCM (9 ml) and was added to the DMP solution. The reaction mixture was stirred at RT for 1 hour. The reaction mixture was diluted with DCM (50 ml) and washed with sat. aq. NaHCO$_3$ solution containing 12.5 g Na$_2$S$_2$O$_3$ (50 ml), sat. aq. NaHCO$_3$ souteion (50 ml) and water (50 ml). The organic phase was washed over MgSO$_4$ and concentrated in vacuo. The crude product was purified by flash column chromatography on silica gel (hexane/EtOAc 3:1). The product was gained as a white yellowish fluffy solid (120.8 mg, 41%). $R_f$ = 0.3 (hexane/EtOAc 2:1). $^1$H NMR (300 MHz, CDCl$_3$) δ 9.92 (s, 2H), 8.69 (s, 2H), 7.78 (d, J = 8.2 Hz, 2H), 7.68 (s, 2H), 7.62 – 7.52 (m, 2H), 2.88 – 2.80 (m, 8H). $^{13}$C NMR (75 MHz, CDCl$_3$) δ 131.35, 129.59, 129.46, 128.46, 128.96, 126.18, 122.02, 90.42, 81.68, 61.73, 41.38, 32.48, 31.45, 17.06, 16.16. HRMS-NSI (m/z): [M+H]$^+$ calcd for C$_{24}$H$_{22}$NO: 342.1852, found: 342.1849.
MHz. CDCl$_3$ δ 200.99, 132.09, 130.20, 129.08, 127.68, 126.78, 122.24, 89.16, 82.53, 43.24, 13.41. HRMS-NSI (m/z): [M+Na]$^+$ calcd for C$_{24}$H$_{18}$O$_2$Na: 361.1199, found: 361.1198.

5,5’-(((phenanthrene-3,6-diylbis(pent-4-yne-5,1-diyl))bis(azanediyl))bis(pent-1-yne-5,1-diyl))bis(phenanthrene-6,3-diyl))bis(pent-4-yn-1-ol) (N-Phe$_3$)

The procedure was carried out analog to reference.$^{[4]}$ A solution of 5 (20.2 mg, 0.0591 mmol) and Na(OAc)$_3$BH (25.5 mg, 0.12 mmol) in CHCl$_3$ (6 ml) was placed under argon and a solution of 6 (10.0 mg, 0.0296 mmol) in CHCl$_3$ (3 ml) was added dropwise. The reaction was stirred overnight. The reaction mixture was diluted with CHCl$_3$ (15 ml) and washed twice with 2 M K$_2$CO$_3$ solution. The CHCl$_3$-phase was dried over K$_2$CO$_3$ and concentrated in vacuo. The crude product was purified by preparative TLC (DCM/MeOH 95:5 + 1% Et$_3$N) and yielded N-Phe$_3$ as a white-yellowish solid (22.8 mg, 78%). $R_f$ = 0.22 (DCM/MeOH 95:5+1% Et$_3$N). HRMS-NSI (m/z) [M+H]$^+$ calcd for C$_{72}$H$_{65}$N$_2$O$_2$: 989.5041, found: 989.5042.
Scheme S1. The synthesis of the 1,6-dipentynyl linked pyrene trimer with phosphodiester-bridges (Py₃).

5,5′-(Pyrene-1,6-diyl)bis(pent-4-yn-1-ol) (8)

Compound 8 was synthesized according to literature.[6] 1,6-Dibromopyrene (7) (1.0201 g, 2.833 mmol) was dissolved in anhydrous THF (23 ml) and degassed Et₃N (14 ml) under an argon atmosphere and heated to 84 °C. 4-Pentyn-1-ol (1 ml, 10.8 mmol) was added, followed by the catalysts Pd[PPh₃]₂Cl₂ (52 mg, 0.074 mmol) and CuI (10 mg, 0.053 mmol). The reaction mixture was refluxed at 84 °C overnight. TLC (DCM/MeOH 92:8) showed the
disappearance of starting material 7. The reaction mixture was cooled to RT, before it was diluted with DCM (100ml), and filtrated through Celite 503. The filtrate was washed once with 10% citric acid (100ml), once with aq. sat. NaHCO₃ (100ml), dried over Na₂SO₄, filtrated, and concentrated under reduced pressure. The residue was purified by a flash column chromatography on silica gel (DCM/toluene/MeOH 88:10:2). Compound 8 could be isolated as a pure yellow compound (437.4 mg, 42%). Rf = 0.23 (DCM/MeOH 97:3). ¹H NMR (300 MHz, CDCl₃) δ 8.52 (d, J = 9.1 Hz, 2H), 8.13 – 8.02 (m, 6H), 3.96 (t, J = 6.2 Hz, 4H), 2.79 (t, J = 7.0 Hz, 4H), 2.04 (p, J = 6.6 Hz, 4H).

5-(6-(5-Hydroxypent-1-ynyl)pyren-1-yl)pent-4-yn-1-yl acetate (9)

Compound 8 (152.6 mg, 0.416 mmol) was dissolved in pyridine (4.2 ml) under argon. A 2M solution of acetic anhydride (0.2 ml, 0.416 mmol) in pyridine was added dropwise over ten minutes to the dissolved compound 8. The reaction mixture was stirred at RT for 2 hours until TLC (hexane/EtOAc 3:2) showed mainly the mono-acetylated product (9) and some unreacted starting material 8. The reaction mixture was diluted with DCM (10ml) and washed once with aq. 0.5 M HCl (10ml), once with aq. sat. NaHCO₃ (10ml) and once with brine (10ml). The organic phase was dried over MgSO₄, filtrated, and concentrated under reduced pressure. The residue was purified by a flash column chromatography on silica gel (hexane/EtOAc 3:2). Compound 9 could have been isolated as a pure, yellow solid (41.1 mg, 24%). Rf = 0.43 (DCM/MeOH 97:3)
4  NMR spectra

4.1  N-Phe₃

Figure S1. ¹H NMR of compound 2 in CDCl₃.

Figure S2. ¹H NMR of compound 3 in CDCl₃.
Figure S3. $^{13}$C NMR of compound 3 in CDCl$_3$.

Figure S4. $^1$H NMR of compound 4 in CDCl$_3$. 
Figure S5. $^{13}$C NMR of compound 4 in CDCl$_3$.

Figure S6. $^1$H NMR of compound 5 in CDCl$_3$. 


Figure S7. $^{13}$C NMR of compound 5 in CDCl$_3$.

Figure S8. $^1$H NMR of compound 6 in CDCl$_3$. 
Figure S9. $^{13}$C NMR of compound 6 in CDCl$_3$.

4.2 Py$_3$

Figure S10. $^1$H of compound 8 in CDCl$_3$. 
Figure S11. $^1$H NMR of compound 9 in CDCl$_3$.

Figure S12. $^{13}$C NMR of compound 9 in CDCl$_3$. 
Figure S13. $^1$H NMR of compound 10 in CDCl$_3$.

Figure S14. $^{13}$C NMR of compound 10 in CDCl$_3$. 
Figure S15. $^{31}$P NMR of compound 10 in CDCl$_3$.

Figure S16. $^1$H NMR of compound 11 in CDCl$_3$. 
Figure S17. $^{31}$P NMR of compound 11 in CDCl$_3$. 
5 MS spectra

5.1 N-Phe$_3$

Figure S18. Mass spectrum of compound 3.

Figure S19. Elemental composition of compound 3.
Figure S20. Mass spectrum of compound 4.

Elemental composition search on mass 343.1703

| m/z   | Theor. Mass (ppm) | Delta (%) | Composition |
|-------|-------------------|-----------|-------------|
| 343.1703 | 343.1693 | 0.33 | C_{11}H_{20}O_{8} |
| 343.1669 | 343.1701 | -1.14 | C_{11}H_{20}O_{8}Na |
| 343.1805 | 343.1701 | -29.60 | C_{11}H_{20}O_{8}Na |
| 343.1677 | 343.1685 | 0.50 | C_{11}H_{20}O_{8}N |
| 343.1543 | 343.1577 | 35.70 | C_{11}H_{20}O_{8}NNa |

Figure S21. Elemental composition of compound 4.
Figure S22. Mass spectrum of compound 5.

Elemental composition search on mass 342.1049

| m/z   | Theo. Delta (ppm) | Composition |
|-------|-------------------|-------------|
| 337.1049 | 342.1049          |             |
| 342.1352 | 0.68 C26H45N17O12M |             |
| 342.1399 | 3.04 C26H45N17Na  |             |
| 342.1423 | 6.15 C26H45N17O11Na |         |
| 342.1426 | 6.95 C26H45N17O4  |             |
| 342.1874 | -7.09 C26H45N17O4Na |         |
| 342.1815 | 10.07 C26H45N17Na |             |
| 342.1812 | 10.00 C26H45N17O4Na |         |
| 342.1807 | -11.01 C26H45N17O4Na |         |
| 342.1982 | 15.90 C26H45N17O4Na |         |
| 342.1899 | -14.12 C26H45N17O4Na |         |

Figure S23. Elemental composition of compound 5.
Figure S24. Mass spectrum of compound 6.

**Elemental composition search on mass 361.1190**

| m/z     | Theor. Mass | Delta (ppm) | Composition          |
|---------|-------------|-------------|----------------------|
| 361.1190| 361.1199    | -0.01       | C18H12O2Na           |
|         | 361.1201    | -3.25       | C18H12O2Na           |
|         | 361.1186    | 3.41        | C18H12O2Na           |
|         | 361.1163    | 4.17        | C18H12O2Na           |
|         | 361.1223    | -6.97       | C18H12O2Na           |
|         | 361.1249    | -10.83      | C18H12O2Na           |
|         | 361.1244    | -12.65      | C18H12O2Na           |
|         | 361.1143    | 15.31       | C18H12O2Na           |
|         | 361.1268    | -15.51      | C18H12O2Na           |

Figure S25. Elemental composition of compound 6.
Figure S26. Mass spectrum of compound N-Phe$_3$.

Elemental composition search on mass 989.5042

| m/z  | Theo. Mass (ppm) | Composition |
|------|------------------|-------------|
| 989.5042 | 0.11 C$_{15}$ H$_{26}$ O$_2$ N$_2$ | | 989.5042 | -1.51 C$_{15}$ H$_{26}$ N$_4$ Na |
| 989.5047 | 2.56 C$_{15}$ H$_{26}$ O$_2$ N$_2$ Na | | 989.5041 | 2.83 C$_{15}$ H$_{26}$ O$_2$ N$_2$ |
| 989.5049 | 4.19 C$_{15}$ H$_{26}$ O$_2$ N$_2$ | | 989.5000 | 5.26 C$_{15}$ H$_{26}$ O$_2$ N$_2$ |
| 989.5102 | 6.09 C$_{15}$ H$_{26}$ O$_2$ N$_2$ Na | | 989.5102 | -6.09 C$_{15}$ H$_{26}$ O$_2$ N$_2$ Na |
| 989.5115 | 6.42 C$_{15}$ H$_{26}$ O$_2$ N$_2$ | | 989.5115 | -7.44 C$_{15}$ H$_{26}$ O$_2$ N$_2$ |
| 989.5126 | -5.52 C$_{15}$ H$_{26}$ O$_2$ N$_2$ | | 989.5126 | -6.52 C$_{15}$ H$_{26}$ O$_2$ N$_2$ |

Figure S27. Elemental composition of compound N-Phe$_3$. 

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**Figure S26.** Mass spectrum of compound N-Phe$_3$.

**Figure S27.** Elemental composition of compound N-Phe$_3$. 

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**Table:**

| m/z  | Theoretical Mass (ppm) | Composition              |
|------|------------------------|--------------------------|
| 989.5042 | 0.11 C$_{15}$ H$_{26}$ O$_2$ N$_2$ | |
| 989.5047 | -1.51 C$_{15}$ H$_{26}$ N$_4$ Na | |
| 989.5049 | 2.56 C$_{15}$ H$_{26}$ O$_2$ N$_2$ Na | |
| 989.5000 | 4.19 C$_{15}$ H$_{26}$ O$_2$ N$_2$ | |
| 989.5102 | 5.26 C$_{15}$ H$_{26}$ O$_2$ N$_2$ Na | |
| 989.5115 | 6.09 C$_{15}$ H$_{26}$ O$_2$ N$_2$ Na | |
| 989.5126 | 6.42 C$_{15}$ H$_{26}$ O$_2$ N$_2$ | |
Figure S28. Mass spectrum of compound 9.

Elemental composition search on mass 409.1701

| m/z  | Theo. Mass (ppm) | Delta | Composition |
|------|------------------|-------|-------------|
| 408.1701 | 408.1701 | 0 | C_35H_2OS_3Na |
| 408.1700 | 408.1700 | -0.25 | C_34H_2O |
| 408.1675 | 408.1675 | 6.40 | C_47H_17ClN_2O_3 |
| 408.1651 | 408.1651 | 12.30 | C_35H_15ClN_1O_3Na |

Figure S29. Elemental composition of compound 9.
Figure S30. Mass spectrum of compound 10.

Elemental composition search on mass 767.3816

| m/z   | Theo. Mass (g/mol) | Delta (g/mol) | Composition       |
|-------|--------------------|---------------|-------------------|
| 767.3816 | 767.3926            | -1.20         | Cs3H10O6Na2F2      |
| 767.3816 | 767.3950            | -4.40         | Cs3H10O6NaF2       |
| 767.3816 | 767.3951            | -4.40         | Cs3H10O6NaF2       |

Figure S31. Elemental composition of compound 10.
Figure S32. Mass spectrum of compound 11.

Elemental composition search on mass 1412.4648

| m/z     | Theo. Mass | Delta (ppm) | Composition  |
|---------|------------|-------------|--------------|
| 1412.4649 | 1412.4607  | -2.80       | C_{86}H_{55}O_{12}N_{9}H_{2}F_{2} |
| 1412.4710 | 1412.4667  | -2.98       | C_{84}H_{54}O_{12}N_{8}F_{2} |
| 1412.4813 | 1412.4649  | -2.80       | C_{86}H_{55}O_{12}N_{9}H_{2}F_{2} |

Figure S33. Elemental composition of compound 11.
Figure S34. Mass spectrum of compound Py₃.

6 UV-vis and fluorescence spectra

6.1 N-Phe₃

Figure S35. Absorption spectra of N-Phe₃ in aqueous medium when cooling down from 75 °C to 15 °C in 10 °C steps. A red-shift of 2 nm is observed between 45 °C and 35 °C. Conditions: 1 μM N-Phe₃, 10 mM sodium acetate buffer, 10 vol% ethanol.
Figure S36. Fluorescence spectra of N-Phe$_3$ in aqueous medium measured every 10 °C. Conditions: 1 μM N-Phe$_3$, 10 mM sodium acetate buffer, 10 vol% ethanol, $\lambda_{ex} = 330$ nm.

Figure S37. Excitation spectra of N-Phe$_3$ in aqueous medium measured every 10 °C. Conditions: 1 μM N-Phe$_3$, 10 mM sodium acetate buffer, 10 vol% ethanol, $\lambda_{em} = 387$ nm.
TEM measurements of N-Phe$_3$

Figure S38. TEM measurements of the self-assembled N-Phe$_3$ in aqueous medium. Conditions: 5 μM N-Phe$_3$, 10 mM sodium acetate buffer (pH 4.7), 10 vol% ethanol.
8 DLS measurements of N-Phe₃

Figure S39. Dynamic light scattering (DLS) measurement of the assembled N-Phe₃ in aqueous medium at 20 °C. Conditions: 10 mM sodium acetate buffer (pH 4.71), 10 vol% ethanol, 1 μM N-Phe₃.

Figure S40. DLS measurement of the disassembled N-Phe₃ in aqueous medium at 70 °C. Conditions: 10 mM sodium acetate buffer (pH 4.71), 10 vol% ethanol, 1 μM N-Phe₃.
9  Size distribution measurement

Figure S41. Size distribution profile of the assembled N-Phe₃ (1 μM) in 10 mM sodium acetate buffer (pH 4.7) and 10 % ethanol at 20°C. The mean value is 109.6 nm. The measurement was done with the nanoparticle tracking analysis instrument from ZetaView.

10  Electrostatic assembly experiments

10.1  1. Py₃ + 2. N-Phe₃

Figure S42. AFM measurements of the two layers: 1. Py₃ + 2. N-Phe₃, their height profiles and the deflection scan (right). Conditions: 1st layer 2µM Py₃, 10 mM sodium phosphate buffer (pH 7.1), 10 mM sodium chloride and 10 vol% ethanol, 2nd layer 10 μM N-Phe₃, 10 mM sodium acetate buffer (pH 4.7) and 10 vol% ethanol.

Figure S43. AFM measurement of the two layers: 1. Py₃ + 2. N-Phe₃ and their height profile. Conditions: 1st layer 2µM Py₃, 10 mM sodium phosphate buffer (pH 7.1), 10 mM sodium chloride and 10 vol% ethanol, 2nd layer 10 µM N-Phe₃, 10 mM sodium acetate buffer (pH 4.7) and 10 vol% ethanol.
Figure S44. AFM measurement of the two layers: 1. Py$_3$ + 2. N-Phe$_3$ and their height profile. Conditions: 1$^{\text{st}}$ layer 2µM Py$_3$, 10 mM sodium phosphate buffer (pH 7.1), 10 mM sodium chloride and 10 vol% ethanol, 2$^{\text{nd}}$ layer 10 µM N-Phe$_3$, 10 mM sodium acetate buffer (pH 4.7) and 10 vol% ethanol.

Figure S45. AFM measurement of the two layers: 1. Py$_3$ + 2. N-Phe$_3$ and their height profile. Conditions: 1$^{\text{st}}$ layer 2µM Py$_3$, 10 mM sodium phosphate buffer (pH 7.1), 10 mM sodium chloride and 10 vol% ethanol, 2$^{\text{nd}}$ layer 10 µM N-Phe$_3$, 10 mM sodium acetate buffer (pH 4.7) and 10 vol% ethanol.

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