Supporting information for:

Protein-Triggered Supramolecular Disassembly: Insights Based on Variations in Ligand Location in Amphiphilic Dendrons

Diego Amado Torres,† Matteo Garzoni,§ Ayyagari V. Subrahmanyam,† Giovanni M. Pavan,§* and S. Thayumanavan†*

†Department of Chemistry, University of Massachusetts, Amherst, MA 01003, United States
§Department of Innovative Technologies, University of Applied Science of Southern Switzerland, Manno 6928, Switzerland

AUTHOR EMAIL ADDRESS thai@chem.umass.edu; giovanni.pavan@supsi.ch

General:

Materials and Methods:

All chemicals and solvents were purchased from commercial sources and were used as such, unless otherwise mentioned. 1H NMR spectra were recorded on a 400 MHz Bruker NMR spectrometer using the residual proton resonance of the solvent as the internal standard. Chemical shifts are reported in parts per million (ppm). When peak multiplicities are given, the following abbreviations are used: s, singlet; bs, broad singlet; d, doublet; t, triplet; m, multiplet. 13C NMR spectra were proton decoupled and recorded on a 100 MHz Bruker spectrometer using the carbon signal of the deuterated solvent as the internal standard. Fluorescence spectra were recorded using a JASCO FP-6500 spectrofluorimeter. FAB-MS spectra were measured on a JEOL JMS700. MALDI-TOF spectra were measured on a Bruker Omniflex. IR spectra were measured on a Bruker Alpha-P FT-IR.

Encapsulation of Nile Red:

To encapsulate Nile red in a dendrons’ aggregate 2 mL 50 µM solutions of each dendron were prepared and stirred at 5 °C for 12 hours. Small amounts of Nile red were mixed with the dendron solutions and these heterogeneous mixtures sonicated for 4 hours. The solutions were stirred at room temperature until temperature equilibration and then stirred at 5 °C for 16 hours. Then, the samples were diluted to 25 µM and stirred at 5 °C for 12 hours more. After that, each sample was filtered through a
syringe filter (0.22 µm). The 25 µM dendron solutions were thus ready for CAC measurement and dye release.

**Determination of CACs:**

The CACs of the dendrons in aqueous solutions were determined using the fluorescence intensity of Nile red ($\lambda_{em}= 615$ nm), as shown in Figure S1. 1 mL of 25 µM dendron solution as prepared above was transferred to a cuvette where its concentration was varied by replacing a measured volume of this solution with the same volume of water. An emission spectrum ($\lambda_{ex}= 550$ nm) was recorded for each concentration of the dendron and a decreasing fluorescence intensity was obtained from each spectrum. When the concentration of the dendron was below the CAC the change in fluorescence intensity became smaller each time. The intensity values were plotted against the concentration of dendron to get a curve (ideally sigmoidal). From the curve, the point where the best fitted horizontal and vertical lines merge was taken as the CAC.

![Figure S1. CAC plots based on nile red fluorescence for dendron assemblies a) G1-F, b) G1-P, c) G2-F, d) G2-M, e) G2-P.](image)

**Dye Release upon Exposure to an Increasing Concentration of Protein:**

A 500 µL solution of dendron 25 µM encapsulating Nile red was exposed to 2 µM proteins (extravidin, α-chymotrypsin, pepsin, myoglobin) by adding 2 µL of protein solution from a stock 500 µM. After mixing well, the emission spectrum of Nile red was recorded ($\lambda_{em}= 615$ nm, $\lambda_{ex}= 550$ nm). Consecutive additions of 2 µL of protein 500 µM were made every two minutes, recording fluorescence after each time until completing 14 µM of protein in the 500 µL of dendron solution. Temperature was maintained at 25 °C. A control experiment to test stability of encapsulation over time was made by exposing the assemblies only to the buffer solution (HEPES 25 mM, pH 7.4) as shown in Figure 3c. A control experiment exposing 25 µM solutions of analog dendrimers G1 and G2, with PEG instead of ligand, to increasing concentration of...
extravidin was made to test the non-specificity of the assemblies lacking biotin towards the protein, as shown in Figure 3d.

**DLS Measurements to Monitor Disassembly:**

The size of the dendrimeric assemblies and their change in size, when exposed to proteins, was measured by dynamic light scattering. For that, 12 µM solutions of the dendrons were prepared in water. Initial size of the assemblies was measured in 1 mL solution. Then, the assemblies were exposed to 2 µM protein and after mixing well, DLS was measured. The results for disassembly in presence of extravidin, interactions with α-chymotrypsin, pepsin, myoglobin, and control dendrons (PEG instead of ligand) are shown in Figure 2. The proteins' sizes are shown in Figure S2. All measurements were made in a Malvern Zeta-sizer.

![Figure S2. Size of the Proteins at 20 µM.](image)

**Dye Release upon a Single Exposure to 14 µM of Protein:**

A 500 µL solution of dendron encapsulating Nile red was exposed to protein 14 µM (extravidin, α-chymotrypsin, pepsin, myoglobin) by adding 14 µL of protein solution from a stock 500 µM. After mixing well, the emission spectrum of Nile red was recorded ($\lambda_{em}$ = 615 nm, $\lambda_{ex}$ = 550 nm) every 15 minutes during the first hour, and then every hour during six hours. Release profiles over time for α-chymotrypsin, pepsin, and myoglobin are shown in Figure S3. Temperature was maintained at 25 °C.

![Figure S3. Release profile of 25 µM dendron assemblies when exposed to 14 µM solution of a) α-chymotrypsin, b) pepsin, and c) myoglobin.](image)
**Encapsulation Stability Test based on Crosslink Density:**

Three different solutions of nanogels with 0%, 20%, and 50% crosslink densities encapsulating nile red were prepared in a concentration of 1 mg/mL. The initial fluorescence was measured. Then, 1 mL of these solutions was exposed to 0.5 mg/mL myoglobin and the fluorescence recorded for 50 min. The concentration of myoglobin was increased to 1.0 mg/mL, 1.5 mg/mL, and 2.0 mg/mL measuring fluorescence for each change in myoglobin concentration for a few minutes. The final plots of time vs quenching percentage are shown in Figure S4a. When the nanogels were exposed to a high concentration of myoglobin, the change in quenching percentage was also high, Figure S4b.

![Figure S4](image.png)

Figure S4. a) Quenching of nile red (NR) fluorescence encapsulated in nanogels (NG, 1 mg/mL) at different crosslink densities and increasing concentration of Myo, b) quenching after a single exposure to Myo (5.5 mg/mL), c) Stern-Volmer plot for G1-F + NR at 25 °C and 38 °C vs increasing concentration of Myo, d) Stern-Volmer plot for G2-F + NR at 25 °C and 38 °C vs increasing concentration of Myo.

**Stern-Volmer Plots for G1-F and G2-F:**

Two samples of dendron solution 25 µM in 500 µL encapsulating nile red, at temperatures of 25 and 38 °C, were exposed to increasing concentrations of myoglobin. The value of the initial fluorescence of the dendrons (F₀) divided by the fluorescence of the same dendrons in presence of the myoglobin (F), in increasing concentration Vs the metalloprotein concentration was plotted in Figure S4c,d.
Synthetic Procedures for Dendrons G1 & G2:

Compounds G1-F, G2-F, 1, 4, 7, and the biotin ligand moiety 6 were synthesized based on known procedures.¹

General Procedure for the Synthesis of Dendritic Compounds:

To a solution of the biaryl monomer 1 (1.0 equiv.) and the appropriate bromobenzyl compound (1.0-3.0 equiv.) in anhydrous acetone, was added K₂CO₃ (3 equiv.) and 18-crown-6 (0.1 equiv.). The reaction mixture was refluxed under argon atmosphere for 12-24 h (12 h for G1 and 24 h for G2). The progress of the reaction was monitored by TLC. After completion of the reaction, acetone was evaporated and the crude reaction mixture was partitioned between ethyl acetate and water. The aqueous layer was extracted three times with ethyl acetate and the combined organic layer was dried over Na₂SO₄. Afterwards, the solution was filtered and evaporated to dryness. The crude product was purified by silica gel column chromatography, CombiFlash.

General Procedures for Incorporation of Biotin-azide to the Dendron using “Click” Chemistry:

Procedure A: To a solution of dendritic acetylene compound (1.0 equiv.), and biotin-azide 6 (2 equiv.) in THF, was added the same volume of aqueous CuSO₄.5H₂O (0.2 equiv.) and sodium ascorbate (0.2 equiv.) in such a way that the final solution THF/H₂O was in a ratio 1:1. The reaction was heated at 50 °C for 24 h to 60 h, depending on dendron generation. After completion of the reaction, NH₄Cl solution was added to the reaction mass and then, extracted with ethyl acetate three times. The organic layers were collected and dried over anhydrous Na₂SO₄, filtered, concentrated, and the product purified by silica gel column chromatography, CombiFlash.

Procedure B: A mixture of the dendritic acetylene compound (1.0 equiv.), biotin azide 6 (3.0 equiv.), CuSO₄.5H₂O (1.0 equiv.) and sodium ascorbate (1.0 equiv.) in DMSO solvent was heated at 50 °C for 24-32 h (24h for G1 and 32h for G2). The progress of the reaction was monitored by TLC. After completion of the reaction, the reaction mixture was portioned between dichloromethane and saturated aqueous NH₄Cl solution. The aqueous layer was extracted twice with dichloromethane and the combined organic layer was dried over anhydrous Na₂SO₄ and evaporated to dryness. The crude product was isolated by silica gel column chromatography, CombiFlash.

Synthesis of periphery 2:

To a stirring solution of 2b[¹] (1.8 g, 5.65 mmol) in dichloromethane (20 mL) was added PBr₃ (1 mL, 11.31 mmol) under argon atmosphere at room temperature. The reaction was monitored using TLC. After
complete disappearance of the starting material the remaining PBr₃ was quenched by slow addition of a saturated NaHCO₃ solution. The resulting mixture was extracted with dichloromethane (3x50 mL). The combined organic layer was dried over anhydrous Na₂SO₄ and concentrated using vacuum. The crude compound was purified by column chromatography CombiFlash (1:4 Ethyl acetate, Hexanes) to afford 2 (1.65 g, 76%). ¹H NMR (CDCl₃-400MHz) δ 6.57 (t, J = 1.9 Hz, 2H), 6.49 (t, J = 2.2 Hz, 1H), 4.67 (d, J = 2.3, 2H), 4.41 (s, 2H), 3.92 (t, J = 6.5 Hz, 2H), 2.53 (t, J = 2.3 Hz, 1H), 1.79-1.72 (m, 2H), 1.48-1.21 (m, 14 H), 0.87 (t, J = 6.8 Hz, 3H). ¹³C NMR (CDCl₃-100MHz) δ 162.4, 160.5, 158.8, 139.8, 108.6, 107.7, 101.9, 78.4, 75.9, 68.32, 56.0, 33.7, 32.0, 29.7, 29.5, 29.5, 29.3, 26.1, 22.8, 14.3.

**Synthesis of 3:**

A mixture of compound 1 (0.25 g, 0.04 mmol), compound 2 (0.14 g, 0.04 mmol), K₂CO₃ (0.08 g, 0.06 mmol) and 18-crown-6 (0.01 g, 0.004 mmol) in acetone (15 mL) was refluxed for 12 h. Upon evaporation of the solvent, the mixture was dissolved in water and extracted three times with ethyl acetate. The combined extracts were dried over anhydrous Na₂SO₄. The crude product obtained upon evaporation of solvent was purified by silica gel column chromatography to afford compound 3 (0.079 g, 25%). ¹H NMR (Acetone d₆-400MHz) δ 6.78-6.69 (m, 4 H), 6.51-6.50 (m, 3 H), 6.40 (d, J = 2.36, 1H), 5.01 (s, 2H), 4.78 (d, J = 2.4 Hz, 2 H), 4.63 (m, 2 H), 4.04 (t, J = 4.68 Hz, 2 H), 3.99 (t, J = 6.48 Hz, 2H), 3.91 (t, J = 6.28 Hz, 2 H), 3.65 (t, J = 4.56 Hz, 2 H) 5.53-3.52 (m, 11 H), 3.44-3.42 (m, 2H), 3.26 (s, 3H), 3.05 (t, J = 2.36, 1H), 2.84 (d, J = 12.8, 3H), 1.80-1.73 (m, 2H), 1.64-1.57 (m, 2H), 1.51-1.45(m, 2H), 1.42-1.19 (m, 26H), 0.92-0.83 (m, 6H); ¹³C NMR (Acetone d₆-100MHz) δ 161.4, 159.9, 159.9, 158.2, 158.1, 157.9, 157.8, 144.3, 141.2, 137.3, 119.6, 112.4, 112.3, 109.9, 107.2, 106.8, 104.8, 104.6, 101.4, 79.7, 77.0, 72.6, 71.4, 71.4, 71.1, 71.1, 71.0, 70.2, 70.1, 69.5, 69.2, 68.6, 64.8, 64.7, 58.7, 56.3, 32.6, 32.6, 30.3, 30.3, 30.3, 30.1, 30.1, 26.8, 23.3, 23.3, 14.4, 14.3.
Synthesis of 5:

According to the general procedure for synthesis of dendritic compounds, the biaryl mono-G1-propargyl monomer 3 (0.13 g, 0.116 mmol) was reacted with the bromomethyl compound 4 (0.06 g, 0.127 mmol) to give compound 5 (0.12 g, 72%). $^1$H NMR (Methanol d$_4$-400MHz) $\delta$ 6.69-6.67 (m, 2H), 6.65-6.51 (m, 7H), 6.46 (t, $J = 2.2$ Hz, 1H), 6.42 (t, $J = 2.2$ Hz, 1H), 4.99-4.97 (m, 4 H), 4.69 (d, $J = 2.36$ Hz, 2 H), 4.60 (s, 2H), 4.10-4.08 (m, 2H), 4.02-4.0 (m, 2H), 3.94-3.79 (m, 10H), 3.61-3.39 (m, 38H), 2.91 (t, $J = 2.4$ Hz, 1H), 1.78-1.70 (m, 4H), 1.59-1.12 (m, 44H), 0.92-0.82 (m, 9H); $^{13}$C NMR (Acetone d$_6$-100MHz) $\delta$ 168.5, 167.4, 165.3, 159.4, 159.1, 156.6, 154.8, 150.7, 144.2, 140.8, 136.1, 133.6, 125.5, 124.4, 110.9, 110.4, 107.3, 106.3, 102.5, 100.5, 83.4, 83.0, 78.7, 77.1, 74.1, 71.6, 70.3, 70.2, 69.9, 67.7, 65.8, 58.0, 55.5, 34.7, 31.7, 31.5, 30.1, 25.9, 23.0, 22.4, 20.8, 13.4, 12.3. MALDI-ToF m/z expected for C$_82$H$_{130}$O$_{19}$: 1420.9; found 1442.6 for C$_82$H$_{130}$O$_{19}$+Na$^+$. 

Synthesis of G1-P:

According to the general procedure for click chemistry, the compound 5 (0.04 g, 0.0028 mmol) was reacted with biotin azide 6 (0.02 g, 0.0056) to give G1-P dendron (0.03 g, 70%). $^1$H NMR (Methanol d$_4$-400MHz) ; 8.22 (s, 1H), 6.81-6.72 (m, 3H), 6.70-6.63 (m, 5H), 6.62-6.53 (m, 2H) 6.47-6.43 (m, 1H), 5.25-5.15 (m, 2H) 5.10-4.95 (m, 4H), 4.64 (s, 2H), 4.57 (t, $J = 4.8$ Hz, 2H), 4.52-4.40 (m, 1H), 4.29-4.22 (m, 1H), 4.13-4.11 (m, 3H), 4.04-4.03 (m, 2H), 4.0-3.96 (4H), 3.93-3.87 (m, 3H), 3.86-3.82 (m, 2H), 3.81-3.78 (m, 3H), 3.69-3.41 (m, 41H), 3.35-3.29 (m, 2H), 3.27-3.26 (m, 6H), 3.13-3.08 (m, 1H), 2.98-2.82 (m, 2H).
2.72-2.62 (d, J = 12.4 Hz, 1H), 2.17-2.14 (t, J = 7.2 Hz, 2H), 1.82-1.71 (m, 4H), 1.67-1.52 (m, 6H), 1.51-1.18 (m, 44H), 0.2-0.82 (m, 9H); $^1^3$C NMR (CDCl$_3$-100MHz) δ 160.4, 160.0, 158.9, 157.4, 157.2, 157.0, 142.0, 139.4, 135.9, 119.3, 112.1, 111.0, 106.1, 105.6, 104.4, 104.2, 102.1, 100.8, 94.6, 77.2, 71.9, 70.8, 70.6, 70.5, 69.9, 69.7, 69.6, 68.8, 68.0, 67.4, 65.4, 59.0, 55.9, 31.9, 30.9, 29.5, 29.4, 29.3, 29.1, 26.0, 25.9, 22.6, 14.1. MALDI-ToF m/z expected for C$_{96}$H$_{154}$N$_6$O$_{22}$S: 1777.34; found 1799.15 for C$_{96}$H$_{154}$N$_6$O$_{22}$S + Na$^+$, 1777.14 for C$_{96}$H$_{154}$N$_6$O$_{22}$S + H$^+$.  

**Synthesis of AB$_2$ Dendron-mono-alkylated Scaffold 8:**

According to the general procedure for synthesis of dendritic compounds, the biaryl monomer 1 (0.072 g, 0.115 mmol) was reacted with the bromomethyl compound 7 (0.192 g, 0.115 mmol) to give the mono alkylated product 8 (0.061 g, 19%). $^1$H NMR (CDCl$_3$ – 400 MHz); δ 6.79-6.62 (m, 6H), 6.60-6.51 (m, 9H), 6.44-6.42 (m, 2H), 5.02 (s, 2H), 4.92 (s, 4H), 4.69 (s, 2H), 4.14-4.06 (m, 8H), 3.94-3.87 (m, 12H), 3.78-3.44 (m, 70H), 3.35 (s, 6H), 3.33 (s, 3H), 3.31 (s, 3H), 1.79-1.72 (m, 8H), 1.69-1.58 (m, 4H), 1.46-1.38 (m, 4H), 1.37-1.15 (m, 48H), 0.91-0.79 (m, 12H); $^{13}$C NMR (CDCl$_3$-100MHz) δ 160.5, 160.1, 159.3, 159.1, 157.3, 157.2, 157.0, 141.9, 139.5, 138.4, 135.6, 119.8, 119.2, 110.3, 109.3, 106.3, 105.8, 105.2, 104.4, 103.6, 100.9, 72.0, 70.9, 70.7, 70.6, 70.5, 69.8, 68.9, 68.2, 67.5, 65.9, 65.6, 59.1, 32.0, 31.7, 29.7, 29.7, 29.5, 29.4, 29.3, 26.2, 26.1, 22.8, 15.4, 14.2. MALDI-ToF m/z expected for C$_{124}$H$_{202}$O$_{33}$: 2221.91; found 2259.66 for C$_{124}$H$_{202}$O$_{33}$ + K$^+$, 2243.69 for C$_{124}$H$_{202}$O$_{33}$ + Na$^+$, 2221.73 for C$_{124}$H$_{202}$O$_{33}$ + H$^+$. 

S8
Synthesis of G1-Bromide (Propargyl at Focal Point) 9:

To a stirring solution of a G1 bearing a propargyl unit and a hydroxymethyl unit at the focal point (0.53 g, 0.373 mmol) in dichloromethane (20 mL) was added PBr$_3$ (0.3 g, 1.12 mmol) under argon atmosphere at room temperature. The reaction was monitored using TLC. After complete disappearance of the starting material the remaining PBr$_3$ was quenched by slow addition of saturated NaHCO$_3$ solution. The resulting mixture was extracted with dichloromethane (3x50 mL). The combined organic layer was dried over anhydrous Na$_2$SO$_4$, filtered and concentrated using vacuum. The crude compound was purified by column chromatography using CombiFlash (1:4 Ethyl acetate, Hexanes) to afford 9 (0.432 g, 78%). $^1$H NMR (Methanol d$_4$ – 400 MHz); δ 6.89 (s, 1H), 6.76 (s, 1H), 6.58-6.53 (m, 7H), 6.42-6.40 (m, 2H), 4.96 (s, 4H), 4.57-4.55 (m, 4H), 4.47 (s, 1H), 4.08-4.06 (m, 4H), 3.94-3.78 (m, 11 H), 3.74-3.54 (m, 31 H), 3.53-3.46 (m, 5H), 2.91 (t, J = 2.3 Hz, 1H), 1.80-1.69 (m, 5H), 1.61-1.16 (m, 53H), 0.95-0.84 (m, 9H). MALDI-ToF m/z expected 1483.79 for C$_{82}$H$_{129}$BrO$_{18}$; found 1483.51 for C$_{82}$H$_{129}$BrO$_{18}$ + H$^+$. 

Synthesis of G2 with Propargyl at the Middle Layer 11:

According to the general procedure for the synthesis of dendritic compounds, the dendron-mono-alkylated scaffold 8 (0.07 g, 0.031 mmol) was reacted with the bromo-dendron compound 9 (0.087, 0.059 mmol) to give compound 11 (0.091 g, 80%). $^1$H NMR (Acetone d$_6$ – 400 MHz); δ 7.03-7.0 (m, 1H), 6.92-
6.86 (m, 3H), 6.78-6.59 (m, 21 H), 6.47-6.43 (m, 4H), 5.17-5.10 (m, 4H), 5.07-5.01 (m, 8H), 4.69 (d, J = 2.2 Hz, 2H), 4.67-4.63 (m, 2H), 4.22-4.20 (m, 1H), 4.15-4.04 (m, 13H), 4.12-4.04 (m, 1H), 3.84-3.77 (m, 9H), 3.71-3.40 (m, 97H), 3.29-3.23 (m, 18H), 2.96(t, J = 2.2 Hz, 1H), 1.82-1.71 (m, 8H), 1.65-1.57 (m, 6H), 1.52-1.42 (10H), 1.42-1.17 (m, 88H), 0.92-0.82 (m, 21H); ^13^C NMR (Acetone d6-100MHz) δ 161.4, 161.2, 160.0, 159.9, 157.9, 157.7, 154.6, 140.9, 140.8, 139.6, 119.3, 111.5, 111.3, 111.2, 106.8, 106.5, 105.8, 104.7, 101.4, 101.1, 80.0, 77.1, 72.6, 71.4, 71.3, 71.2, 71.0, 70.3, 70.1, 69.4, 69.3, 69.2, 68.6, 68.4, 58.8, 56.9, 32.7, 32.6, 26.9, 26.8, 23.3, 23.3, 14.4, 14.3. MALDI-ToF m/z expected for C_{206}H_{330}O_{51}: 3623.79; found 3645.49 for C_{206}H_{330}O_{51} + Na^+.

**Synthesis of G2-M:**

According to general procedure for click chemistry, compound 11 (0.017 g, 0.005 mmol) was treated with biotin-azide 6 (0.005 g, 0.014 mmol) to give G2-M dendron (0.0074 g, 40%). ^1H NMR (Methanol d4 – 400 MHz); δ 7.71 (s, 1H), 6.82-6.41 (m, 27H), 5.65-5.58 (m, 4H), 4.68-4.51 (m, 4H), 4.40-4.32 (m, 5H), 4.31-4.23 (m, 5H), 4.18-3.97 (m, 9H), 3.89-3.72 (m, 15H), 3.69-3.63 (m, 12 H), 3.62-3.56 (m, 48 H), 3.55-3.48 (m, 35H), 3.25-3.32 (m, 3H), 2.86-2.72 (m, 4H), 2.41-2.30 (m, 8H), 2.12-1.95 (m, 16H), 1.85-1.62 (m, 33H), 1.60-1.48 (m, 17H), 1.45-1.20 (m, 80H), 1.01-0.85 (m, 21H). ^13^C NMR (Acetone d6-100 MHz) δ 160.6, 160.3, 159.1, 157.2, 157.1, 157.0, 156.5, 144.0, 143.5, 140.0, 138.7, 136.5, 136.3, 119.4, 110.5, 106.0, 105.7, 105.1, 104.5, 104.0, 100.8, 100.7, 100.3, 70.3, 70.2, 70.0, 69.6, 69.5, 69.3, 68.8, 68.7, 68.5, 68.4, 67.7, 67.5, 57.9, 35.2, 31.8, 31.8, 29.6, 29.4, 29.2, 29.2, 28.7, 28.5, 28.4, 28.4, 26.1, 26.0, 25.4,
MALDI-ToF m/z expected for C_{220}H_{354}N_{6}O_{54}S: 3977.49; found 4001.25 for C_{220}H_{354}N_{6}O_{54}S + Na^+; 4017.46 for C_{220}H_{354}N_{6}O_{54}S + K^+.

Synthesis of G1-Bromide (Propargyl at Periphery) 10:

To a stirring solution of 5 (0.324 g, 0.228 mmol) in dichloromethane (10 mL) was added PBr₃ (0.22 mL, 2.28 mmol) under argon atmosphere at room temperature. The reaction was monitored using TLC. After complete disappearance of the starting material, the remaining PBr₃ was quenched by slow addition of saturated NaHCO₃ solution. The resulting mixture was extracted with dichloromethane (3x50 mL). The combined organic layer was dried over anhydrous Na₂SO₄ and concentrated using vacuum. The crude compound was purified by column chromatography, CombiFlash (1:4 Ethyl acetate, Hexanes) to afford 10 (0.076 g, 22%). ^1H NMR (Methanol d₄-400MHz) δ 6.73-6.41 (m, 11 H), 4.95 (s, 4H), 4.67 (d, J = 2.4 Hz, 2H), 4.54 (s, 2H), 4.12-4.06 (m, 2H), 4.05-3.97 (m, 2H), 3.96-3.40 (m, 32H), 2.91 (t, J = 2.3 Hz, 1H), 1.81-1.70 (m, 4H), 1.62-1.11 (m, 42H), 0.95-0.82 (m, 9H); ^13C NMR (Methanol d₄-100MHz) δ 160.4, 160.1, 158.9, 157.5, 157.0, 156.7, 139.8, 130.0, 106.0, 105.6, 100.6, 100.4, 75.5, 71.5, 70.4, 70.3, 70.2, 70.1, 70.0, 69.9, 69.4, 69.2, 67.7, 67.2, 57.7, 55.3, 38.9, 31.7, 29.4, 29.3, 29.3, 29.1, 25.9, 25.8, 22.4, 22.3, 18.7, 13.1, 13.1. MALDI-ToF m/z expected 1483.79 for C₈₂H₁₂₉BrO₁₈; found 1483.51 for C₈₂H₁₂₉BrO₁₈ + H⁺.
Synthesis of G2 with Propargyl at Periphery 12:

According to the general procedure for synthesis of dendritic compounds, the dendron-mono-alkylated scaffold 8 (0.103 g, 0.046 mmol) was reacted with the bromo-dendron compound 10 (0.076 g, 0.051 mmol) to give compound 12 (0.12 g, 71%). \(^1\)H NMR (CDCl\(_3\) – 400 MHz); \(\delta\) 6.73-6.41 (m, 27H), 5.11-4.90 (m, 12 H), 4.73-4.65 (m, 4H), 4.16-4.02 (m, 10H), 3.97-3.81 (m, 20H), 3.77-3.44 (m, 104H), 3.38-3.32 (m, 18H), 2.52 (t, J = 2.2 Hz, 1H), 1.85-1.11 (m, 112H), 0.93-0.81 (m, 21H); \(^{13}\)C NMR (CDCl\(_3\)-100MHz) \(\delta\) 160.5, 160.1, 159.2, 159.1, 158.9, 157.4, 157.3, 157.1, 157.1, 139.7, 139.4, 138.0, 136.3, 136.1, 119.9, 119.6, 110.3, 106.9, 106.3, 105.8, 105.3, 105.2, 104.9, 104.5, 101.2, 100.9, 72.0, 72.0, 70.9, 70.7, 70.7, 70.6, 70.6, 70.5, 70.5, 69.8, 68.9, 68.2, 67.5, 65.4, 59.1, 59.1, 56.0, 32.0, 29.8, 29.7, 29.7, 29.5, 29.4, 29.4, 29.2, 26.2, 26.1, 22.8, 14.2.

Synthesis of G2-P:
According to the general procedure for click chemistry, compound 12 (0.022 g, 0.006 mmol) was treated with biotin-azide 6 (0.006 g, 0.018 mmol) to give (0.013 g, 17%) of G2-P dendron. $^1$H NMR (CDCl$_3$ – 400 MHz); δ 7.78 (s, 1H), 6.82-6.40 (m, 27H), 6.24-6.22 (m, 1H), 5.57-5.56 (m, 1H), 5.32-5.17 (m, 2H), 5.11-4.89 (m, 12H), 4.75-4.66 (m, 2H), 4.60-4.37 (m, 4H), 4.36-4.21 (m, 2H), 4.19-3.32 (m, 155H), 3.26-3.02 (m, 4H), 2.89-2.51 (m, 4H), 2.25-2.15 (m, 2H), 2.10-1.95 (m, 2H), 1.87-1.57 (m, 26H), 1.55-1.11 (m, 88H), 0.95-0.77 (m, 21H). $^{13}$C NMR (Acetone d$_6$–100 MHz) δ 160.9, 160.6, 159.8, 157.9, 157.6, 157.7, 156.5, 144.0, 143.5, 140.2, 138.8, 136.5, 136.3, 119.5, 110.6, 106.2, 105.9, 105.3, 104.7, 104.3, 100.9, 100.8, 100.4, 70.3, 70.2, 70.0, 69.6, 69.5, 69.3, 68.8, 68.7, 68.5, 68.4, 67.7, 67.5, 65.7, 57.9, 35.3, 31.9, 31.8, 29.7, 29.5, 29.3, 29.2, 28.7, 28.5, 28.4, 26.1, 26.0, 25.4, 22.6, 22.5, 13.7, 13.6. MALDI-ToF m/z expected for C$_{220}$H$_{354}$N$_6$O$_{54}$S: 3977.49; found 4001.48 for C$_{220}$H$_{354}$N$_6$O$_{54}$S + Na$^+$; 4017.38 for C$_{220}$H$_{354}$N$_6$O$_{54}$S + K$^+$.

**Computational Methods**

**Molecular Dynamics (MD) Simulation of G2 dendrons in Solution**

The entire simulation work was conducted using the AMBER 12 software.$^2$ The molecular models for G2-P, G2-M, and G2-F dendrons were created and parameterized according to a validated procedure for the simulation of dendritic molecules (and their pegylated derivatives) interacting with biological targets.$^{3-5}$ In particular, G2-P, G2-M, and G2-F dendrons were parameterized with the “general AMBER force field (GAFF)” (gaff.dat).$^6$ The parm99 all-atom force field (leaprc.ff99)$^7$ was used to parameterize all the other standard residues present in the simulated molecular systems.

The three G2 dendron models were immerged in a periodic box containing explicit TIP3P water molecules$^8$ by using the *leap* module of AMBER 12. After preliminary minimization, all systems were initially heated for 50 ps of NVT MD simulation to reach the experimental temperature of 25 °C (298 K). During this step the solute was maintained as fixed. All restraints were then removed and all molecular glues were equilibrated by running NPT MD simulations at the experimental temperature of 25 °C and 1 atm of pressure under periodic boundary conditions. After 100 ns of NPT MD simulation all G2-P, G2-M, and G2-F systems were equilibrated. From that moment, the run was switched to NVT, and all dendrons underwent additional 100 ns of MD simulation. The root mean square deviation (RMSD) and radius of gyration ($R_g$) data extracted from the MD trajectories with the *ptraj* module of AMBER 12 were used to verify that each molecule reached the equilibrium with good stability (Figure S5). All equilibration MD runs used a time step of 2 femtoseconds, the Langevin thermostat, and a 10 Å cutoff. The particle mesh Ewald$^9$ (PME) approach was used to treat the long-range electrostatic effects, and the SHAKE algorithm was used on the bonds involving Hydrogen atoms.$^{10}$ All MD simulations were carried out using the *pmemd.cuda* module working on GTX580 GPU cards. The same simulation protocol was adopted also for all the other MD simulations conducted in this work.
Initially, all G2 dendrons were constructed with all PEG chains oriented on one side and all decyl chains on the other side. Two additional G2 molecular models completely decorated with PEG chains and without the biotin ligand were also constructed (Figure S6a,b) with all PEG chains on the same side and all decyl chains on the other side (G2_{sym}), as well as with alternated chains (G2_{asym}). G2_{sym} and G2_{asym} molecular systems were solvated and simulated according to the same procedure adopted for G2-P, G2-M, and G2-F, and the results were compared to be sure to avoid any configuration-dependency issue in the results (Figure S6).

Figure S5. (a) Radius of gyration (Rg) and root mean square deviation (RMSD) data (b) obtained from the MD simulations of the G2 dendrons in solution.

Figure S6. MD simulation of the G2_{sym} (a) and G2_{asym} (b) dendrons in water. Initial starting (a,b) and final (c,d) snapshots (200 ns) are reported. Hydrophobic decyl chains are colored in red, PEG in blue and the dendron scaffold in black. Water Oxygen atoms are represented as transparent cyan spheres.
Figure S7. Comparison between \(G_2^{\text{sym}}\) and \(G_2^{\text{asym}}\) in solution. (a) Radius of gyration \((R_g)\) plots for \(G_2^{\text{sym}}\) (black) and \(G_2^{\text{asym}}\) (red) as a function of simulation time, and average equilibrium solvation energy \(G_{\text{sol}}\) values (measure of hydrophilicity). (b) Radial distribution functions \((g(r))\) for the hydrophilic PEG and hydrophobic decyl groups (continuous and dotted lines respectively). The substantial superposition of these data proof that there is no appreciable difference between \(G_2^{\text{sym}}\) and \(G_2^{\text{asym}}\) in solution.

However, MD simulations of those systems suggested that initial configuration does not have any impact on the shape and equilibrium configuration assumed by such small and flexible molecules in water, in terms of density distribution, radius of gyration, hydrophilicity \((G_{\text{sol}})\) etc. (Figure S3). These findings are consistent with the idea of treating these dendrons as facially amphiphilic structures.

As described in the main paper, we built a simplified model describing the dendron-extravidin interaction as composed of two phases – (i) the unfolding of the biotin ligand to make it available for the protein, and then the (ii) specific biotin-avidin interaction (Figure 4d in the main paper). According to this scheme, we can obtain information on the overall affinity of \(G_2-P\), \(G_2-M\) and \(G_2-F\) for extravidin by evaluating the free energy of the dendron-protein binding process as: \(\Delta A_{\text{bind}} = \Delta A_{\text{specific}} + \Delta A_{\text{unfold}}\), where the specific biotin-avidin affinity is known experimentally (absolute free energy of binding \(\Delta A_{\text{specific}} = -20.4\) kcal mol\(^{-1}\)),\(^{11}\) and it can be considered as a constant for all cases (all dendrons bear the same number of biotin ligands), and \(\Delta A_{\text{unfold}}\) is the free energy necessary to drag out the biotin ligand from its backfolded state and to make it available at the dendron surface. \(\Delta A_{\text{unfold}}\) values for the different cases can be extracted directly from the \(g(r)\),\(^{12}\) and depend on how much the ligand is backfolded within the dendron structure.

In particular, for each point in the \(g(r)\) curves reported in Figure 4c in the main paper it is possible to obtain the related free energy value as: \(A(r) = -k_B T \ln(g(r))\), being the product of the Boltzmann constant \(k_B\) and the temperature \(T\) equal to \(k_B T = 0.593\) kcal mol\(^{-1}\) at room temperature (Figure S8). In this way, if the G2 dendrons are thought of as spheres with radius \(R_g\), \(\Delta A_{\text{unfold}}\) expresses the amount of free energy necessary to unfold the biotin ligand, and to drag it from its most probable location within the dendron structure (the maximum \(g(r)\) point – \(g_{\text{MAX}}(r)\)) to the dendron surface \((g(R_g))\).
The $\Delta A_{\text{unfold}}$ values reported in Table 1 in the main paper were thus calculated as: $\Delta A_{\text{unfold}} = A(g(R_g)) - A(g_{\text{MAX}}(r))$ — i.e., and the free energy variation $\Delta A_{\text{unfold}}$ between the surface value ($A(R_g)$) and the free energy minima ($A_{\text{MIN}}$).

Figure S8. Free energy data for the biotin ligand within the G2-P, G2-M and G2-F dendrons in solution calculated respect to the distance (r) from the dendron center. (a) Free energy curves obtained from the g(r) plots in the main paper (Figure 4c) calculated with a 0.1 Å grid spacing. (b,c) The same g(r) and related free energy data calculated with a 0.2 Å grid spacing.

**Energetic analysis procedure for the calculation of interaction energies ($\Delta E_{\text{bind}}, \Delta E_{\text{ass}}, \text{etc.}$)**

The following procedure adopted to calculate the binding energy is here explained in detail for the case of the 1:1 G2-P+AVD molecular complex. However, the same approach was used also to calculate all other $\Delta E$ data reported in the paper for all the other simulated complexes (e.g., $\Delta E_{\text{ass}}, \text{etc.}$).

The 1:1 G2-P+AVD molecular complex was created and simulated as described in the main paper. The interaction energy between G2-P and AVD was calculated directly from the MD trajectories according to the MM-PBSA approach\(^\text{13}\) as:

\[
\Delta E_{\text{bind}} = E_{\text{complex}} - (E_{\text{G2-P}} + E_{\text{AVD}}) \quad (S1)
\]

\[
\Delta E_{\text{bind}} = \Delta E_{\text{gas}} + \Delta G_{\text{solv}} \quad (S2)
\]

$\Delta E_{\text{bind}}$ is composed of the total gas-phase in vacuo non-bond energy ($\Delta E_{\text{gas}}$), and of a solvation term ($\Delta G_{\text{solv}} = \Delta G_{\text{PB}} + \Delta G_{\text{NP}}$)\(^\text{14}\) as described in Eq. (S2). The polar component of $\Delta G_{\text{PB}}$ was evaluated using the Poisson-Boltzmann\(^\text{15}\) (PB) approach with a numerical solver implemented in the pbsa program of AMBER 11.\(^\text{16}\) The non-polar contribution to the solvation energy was calculated as $\Delta G_{\text{NP}} = g (\text{SASA}) + b$, in which $g = 0.00542 \text{ kcal/Å}^2$, $b = 0.92 \text{ kcal/mol}$, and SASA is the solvent-accessible surface estimated with the MSMS program.\(^\text{17}\)

The same approach was used to calculate also all other $\Delta E$ data reported in the paper for all the other simulated complexes (e.g., $\Delta E_{\text{ass}}, \text{etc.}$).
Figure S9 reports the plot of the $\Delta E$ data extracted from the MD simulation of the G2-P+AVD molecular system as a function of the simulation time.

Figure S9. Interaction energy ($\Delta E_{\text{bind}}$) between G2-P and AVD data extracted from the MD simulation of the G2-P+AVD molecular system (a) reported as a function of simulation time. (b) Root mean square deviation (RMSD) data as a function of simulation time.

Additional data related to the simulated systems

Figure S10. Root mean square deviation (RMSD) data of the 9G2-P (a) and G2-P+AVD (b) simulated systems as a function of simulation time.
Table S1. Main features of the molecular systems simulated in this study.

| Molecular system | Simulation temperature (°C) | Total number of dendrons in the system | Number of specific interactions | Box volume (Å³) | Number of neutralization Cl⁻ ions in the system [a] | Number of water molecules in the system | Total number of atoms in the system | Simulation time for each MD run (ns) |
|------------------|----------------------------|----------------------------------------|-------------------------------|----------------|-----------------------------------------------|----------------------------------------|-----------------------------------|-----------------------------------|
| G2-P             | 25                         | 1                                      | 0                             | 259478         | 0                                             | 8348                                  | 25682                            | 200                               |
| G2-M             | 25                         | 1                                      | 0                             | 249119         | 0                                             | 8057                                  | 24693                            | 200                               |
| G2-F             | 25                         | 1                                      | 0                             | 243776         | 0                                             | 7841                                  | 24122                            | 200                               |
| G2_sym [b]       | 25                         | 1                                      | 0                             | 286231         | 0                                             | 9133                                  | 28104                            | 200                               |
| G2_asym [b]      | 25                         | 1                                      | 0                             | 290081         | 0                                             | 9071                                  | 27905                            | 200                               |
| G2-P+AVD         | 25                         | 1                                      | 1                             | 725447         | 18                                            | 21548                                 | 72944                            | 200                               |
| 9G2-P            | 25                         | 9                                      | 0                             | 789008         | 0                                             | 24200                                 | 78315                            | 200                               |
| 9G2-P+AVD        | 25                         | 9                                      | 1                             | 1070842        | 18                                            | 31366                                 | 107535                           | 200                               |
| 4G2-P+AVD        | 25                         | 4                                      | 4                             | 931066         | 18                                            | 28653                                 | 93458                            | 200                               |
| 40G2-P+AVD       | 25                         | 40                                     | 4                             | 1870842        | 18                                            | 50534                                 | 189410                           | 150                               |

[a] The overall charge present on the AVD protein tetramer is +18 e. [b] G2_sym and G2_asym molecular systems were simulated to verify the absence of any configuration dependency in the results.

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$\text{CsH}_{12}\text{O}_2$

933.22 g/mol

$\text{^{13}C NMR}$

$\text{H NMR}$
G1-P
$^1$H NMR
