The architectural change of the shell forming block from linear to V-shaped accelerates micellar disassembly but slows the complete enzymatic degradation of the amphiphiles

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Supporting information

Instrumentation and materials

Instrumentation:

HPLC: All measurements were recorded on a Waters Alliance e2695 separations module equipped with a Waters 2998 photodiode array detector. All solvents were purchased from Bio-Lab Chemicals and were used as received. All solvents are HPLC grade. **^{1}H and ^{13}C NMR**: spectra were recorded on Bruker Avance I and Avance III 400MHz or 100MHz spectrometers as indicated. Chemical shifts are reported in ppm and referenced to the solvent. The molecular weights of the PEG-dendron hybrids were determined by comparison of the areas of the peaks corresponding to the PEG block (3.63 ppm) and the protons peaks of the dendrons. **GPC**: All measurements were recorded on Viscotek GPCmax by Malvern using refractive index detector and PEG standards (purchased from Sigma-Aldrich) were used for calibration. **Fluorescence spectra**: Measurements were recorded on an Agilent Technologies Cary Eclipse Fluorescence Spectrometer using quartz cuvettes or for the CMC measurements: measurements were recorded on a TECAN Infinite M200Pro device. **MALDI-TOF MS**: Analysis was conducted on a Bruker AutoFlex MALDI-TOF MS (Germany). α-Cyano-4-hydroxycinnamic acid matrix was used. **High resolution MS**: Analysis was conducted on Autospec HRMS (EI) Micromass (UK) or Synapt High Definition MS (ESI), Waters Inc. (USA). **DLS**: All measurements were recorded on a Cordouan technology VASCOγ – particle size analyzer. **TEM**: Images were taken by a Philips Tecnai F20 TEM at 200kV. **SAXS**: Data was collected at beamline B21, in the "Diamond Light Source" (Diamond, Oxfordshire). Using an Eiger detector at a sample to detector distance of 3.7 m and a wavelength of 1.0 Å, with a range of momentum transfer (q) between 0.026 and 3.4 nm^{-1}. **Microwave:** Reactions were performed using Biotage® Initiator.

Materials:

Poly (Ethylene Glycol) methyl ether (5kDa and 10kDa), 2-mercaptoethanol (98%), cystamine dihydrochloride (96%), hexanoic acid (99.5%), 2,2-dimethoxy-2- phenylacetophenone (DMPA, 99%), 4-(Dimethyl amino)pyridine (DMAP, 99%), Propargylamine (98%), sodium ascorbate (98%) Porcine liver esterase (PLE) and Sephadex® LH20 were purchased from Sigma-Aldrich. Propargyl bromide (80% in toluene), allyl bromide (99%), chlorotriphenylmethane (Trt-Cl, 98%), para-nitrophenol (99%), triethylsilane (98%), N,N'- dicyclohexylcarbodiimide (DCC, 99%), and Anhydrous K_2CO_3 (99%) were purchased from Alfa Aesar. 3,5-dihydroxy benzoic acid was purchased from Apollo scientific. Potassium hydroxide, cupric sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$, 98%), Sodium azide (99%) and N,N-Diisopropylethylamine (DIPEA, 99%) were purchased from Merck. Silica Gel 60Å, 0.040-0.063mm, NaOH and all solvents were purchased from Bio-Lab and were used as received. Deuterated solvents for NMR were purchased from Cambridge Isotope Laboratories (CIL), Inc.

Synthesis and characterizations

Synthesis of linear and V-shaped PEG-dendron hybrids based on four end-groups

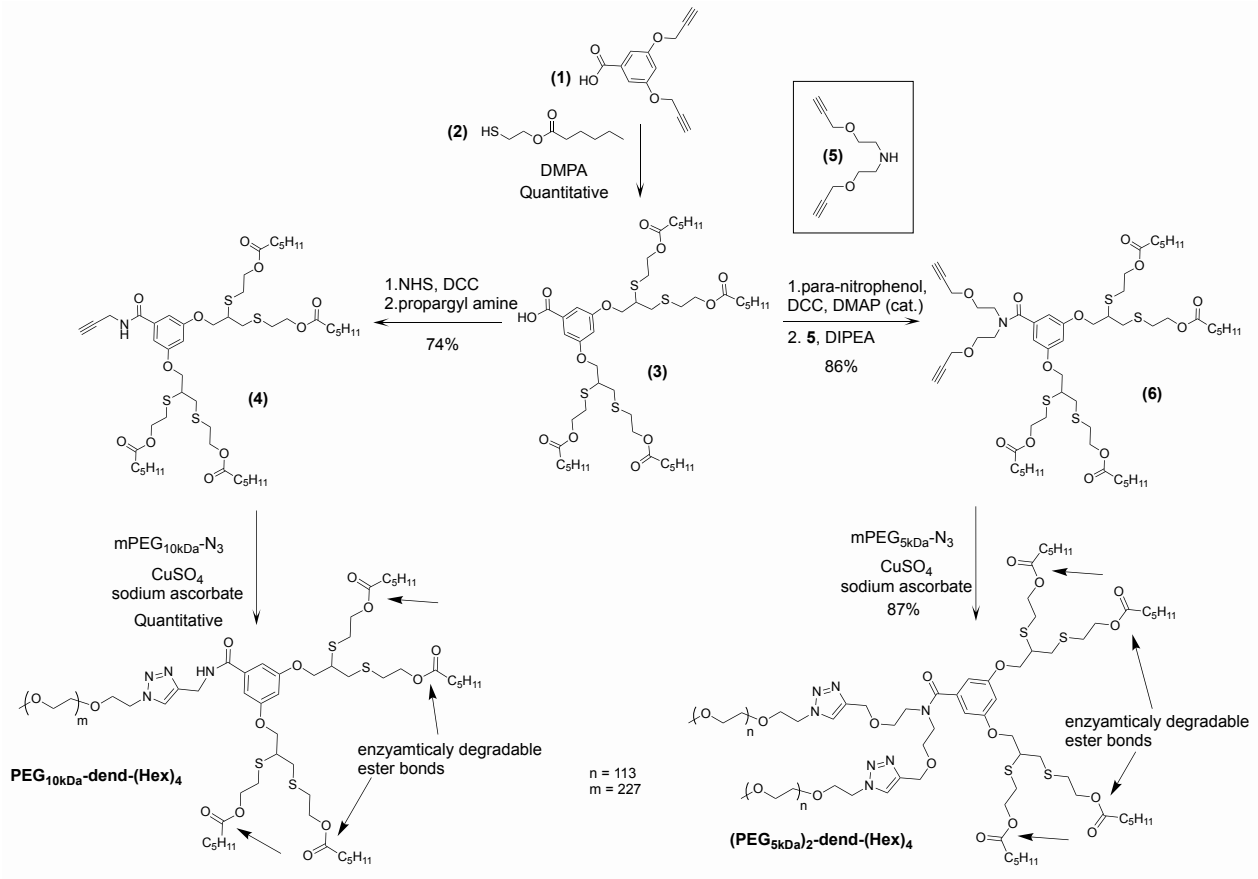

***Figure S1**: Synthetic route of Linear and V-shaped hybrids with four end-groups.*

Compound 3:

250 mg of compound **1**[1] (1.1 mmol) were dissolved in DMF (0.5mL). Compound **2**[2] (1.53 gr, 8.7 mmol) and DMPA (28mg, 0. 11 mmol) were added to the solution. The solution was purged with nitrogen for 15 minutes and then placed under UV light at 365nm for 2 hours. The crude mixture was loaded on silica column using gradient of DCM (100%) to MeOH:DCM (5:95 v/v). The fractions contained the compound were combined and evaporated to obtain 1.0 g oily product (quantitative yield). ^{1}H-NMR (400 MHz, $CDCl_3$) : δ 7.26-7.25 (m, 2H, Ar-***H***), 6.74 (t, *J = 2.3 Hz*, 1H, Ar-***H***), 4.36 – 4.11 (m, 12H, Ar-O-C***H***$_2$-+ S-CH_2-C***H***$_2$-O-), 3.23 (q, *J = 5.9 Hz*, 2H, -C***H***-S-), 3.06-2.88 (m, 8H, -C***H***$_2$-S-), 2.81 (t, *J = 6.9 Hz*, 4H, -C***H***$_2$-S-), 2.31 (t, *J = 7.6 Hz*, 8H, -O-CO-C***H***$_2$-$(CH_2)_3$-CH_3), 1.62 (qui, *J=7.32 Hz*, 8H, -O-CO-CH_2-C***H***$_2$-$(CH_2)_2$-CH_3), 1.36-1.25 (m, 16H, -O-CO-CH_2-CH_2-$(CH_2)_2$-CH_3), 0.89 (t, *Z= 6.9 Hz*, 12H, -O-CO-$(CH_2)_5$-C***H***$_3$). HRMS (EI, negative mode): calculated mass $C_{45}H_{74}O_{12}S_4$ $[M-H]^-$: 933.3985; found: 933.3977.

Compound 4:

140 mg of compound **3** (0.15mmol) were dissolved in 1 ml DCM. 19.2 mg (0.17 mmol) of NHS were separately dissolved in minimal amount of DCM and was combined with the former solution. Then the solution was cooled to 0°C with ice bath and 34 mg (0.17mmol) of DCC, which was dissolved in 0.3 ml of DCM, was added. The reaction was stirred for 1 hour in room temperature. After confirming full conversion by HPLC the urea by-product was filtered using cotton topped with little amount of celite. Next, 19µl of propargylamine (0.3 mmol, 2eq) was added to solution and was stirred overnight in room temperature. The crude mixture was loaded on silica column using gradient of Ethyl acetate: hexane (30:70 v/v) to (40:60 v/v). The fractions contained the compound were combined and evaporated to dryness to obtain 108 mg colorless thick oil (74% yield). ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.03 (d, *J = 2.2 Hz*, 2H, Ar-***H***), 6.92 (t, *J = 5.2 Hz*, 1H, amide), 6.62 (t, *J = 2.2 Hz*, 1H, Ar-***H***), 4.29 – 4.17 (m, 14H, Ar-O-C***H***$_2$-+ S-CH_2-C***H***$_2$-O- + HC≡C-C***H***$_2$-), 3.21 (qui, *J = 6.1 Hz*, 2H, -C***H***-S-), 3.13 – 2.78 (m, 12H, -C***H***$_2$-S-), 2.33-2.29 (m, 8H, -O-CO-C***H***$_2$-$(CH_2)_3$-CH_3), 2.25 (t, *J = 2.5 Hz*, 1H, ***H***C≡C-CH_2-), 1.66-1.60 (m, 8H, -O-CO-CH_2-C***H***$_2$-$(CH_2)_2$-CH_3), 1.40 – 1.24 (m, 16H, -O-CO-CH_2-CH_2-$(CH_2)_2$-CH_3), 0.89 (t, *J = 6.9 Hz*, 12H, -O-CO-$(CH_2)_5$-C***H***$_3$). ^{13}C-NMR (101 MHz, CDCl3): δ 173.81, 166.66, 159.63, 136.22, 106.47, 104.85, 69.84, 63.57, 63.21, 45.63, 34.94, 34.31, 31.66, 31.39, 30.52, 29.82, 24.71, 22.41, 14.02, 0.11. HRMS (EI, positive mode): calculated mass of $C_{48}H_{78}NO_{11}S_4$ $[MH]^+$: 972.4452; found: 972.4455.

mPEG$_{10kDa}$-dend-(Hex)$_4$

100 mg of MeO-PEG_{10kDa}-N_3 (10 µmol) was dissolved with 300µL of DMF (using mild heating). 38 mg of Compound **4** (39 µmol) were separately dissolved with minimal amount of DMF and combined with the former solution. 2.5 mg of $CuSO_4 \cdot 5H_2O$ (10µmol) and 4.0 mg of sodium ascorbate (20µmol) were added to the solution. The solution was purged with nitrogen for 15 min and the reaction was performed using the microwave at 80°C for 1.5 hours. The salts were filtered through PTFE syringe filter. The filtered solution

was further purified by LH20 column using methanol as solvent. The fractions that contained the product were unified. MeOH was evaporated with evaporator, and dried under high vacuum obtaining white solid (105 mg, quantitative yield) ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.75 (s, 1H, H-triazole), 7.38 (t, J = 5.1 Hz, 1H, amide), 6.97 (d, J = 2.1 Hz, 2H, Ar-H), 6.54 (t, J = 2.0 Hz, 1H, Ar-H), 4.63 (d, J = 5.4 Hz, 2H, mPEG-O-C***H***$_2$-CH_2-N-triazole), 4.46 (t, J = 5.1 Hz, 2H, mPEG-O-CH_2-C***H***$_2$-N-triazole), 4.34 – 4.02 (m, 12H, Ar-O-C***H***$_2$-+ S-CH_2-C***H***$_2$-O-), 3.82-3.38 (m, 980H, PEG backbone), 3.32 (s, 3H, C***H***$_3$-O-PEG-), 3.15 (qui, J = 6.1 Hz, 2H, -C***H***-S-), 3.02 – 2.69 (m, 12H, -C***H***$_2$-S-), 2.23-2.24 (m, 8H, -O-CO-C***H***$_2$-$(CH_2)_3$-CH_3), 1.55 (qui, J = 7.2 Hz, 8H, -O-CO-CH_2-C***H***$_2(CH_2)_2$-CH_3), 1.38 – 1.14 (m, 16H, O-CO-CH_2-CH_2(CH_2)$_2$-CH_3), 0.82 (t, J = 6.9 Hz, 12H, O-CO-CH_2-$CH_2(CH_2)_2$-C***H***$_3$).^{13}C-NMR (101 MHz, $CDCl_3$): δ 199.97, 199.22, 188.01, 159.67, 159.34, 123.60, 106.37, 100.50, 77.15, 70.71, 63.54, 63.23, 50.41, 45.69, 35.04, 34.31, 31.41, 30.56, 24.73, 22.43, 14.04. GPC (DMF + 25 mM NH_4Ac): Expected Mn = 11.0 kDa; experimental M_n = 10.7 kDa; D_M= 1.05. MALDI-TOF MS: molecular ion centered at 10.9kDa.

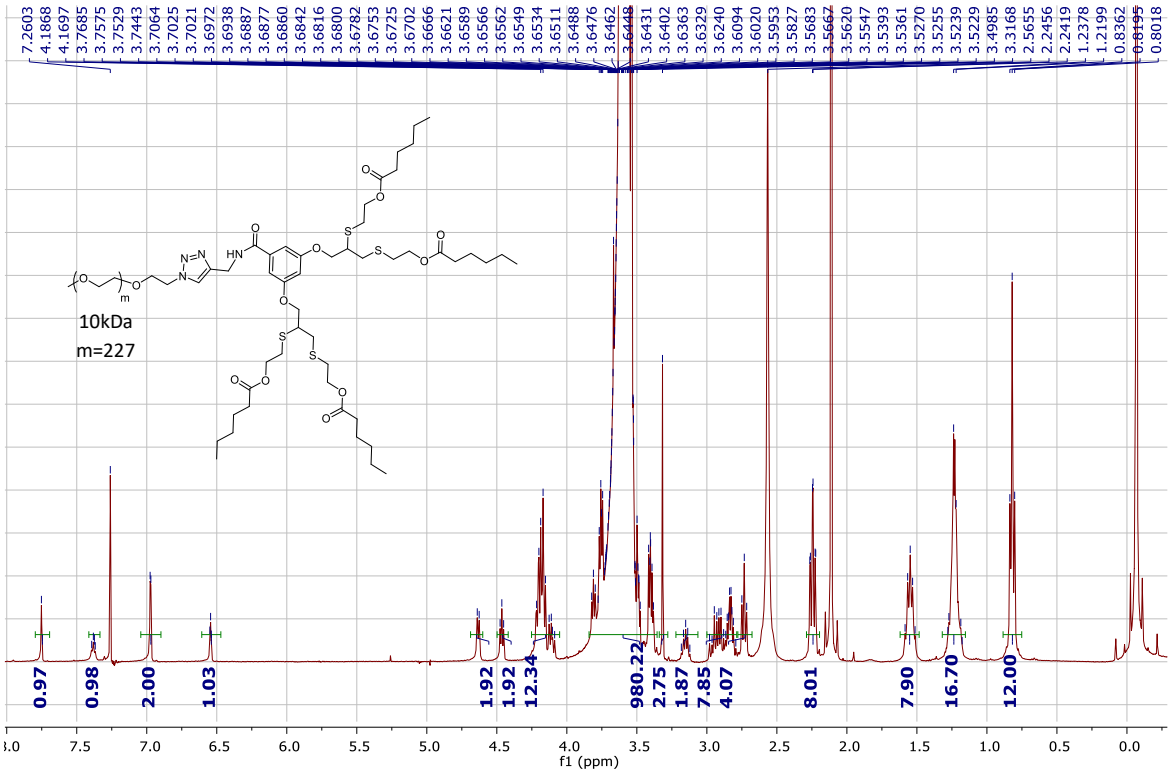

***Figure S2**: ^{1}H-NMR spectrum of Linear hybrid with four end-groups PEG_{10kDa}-dend-$(Hex)_4$ in $CDCl_3$.*

Compound 6:

239 mg of compound **3** (0.26 mmol), 39 mg of p-nitrophenol (0.28 mmol) and 3 mg of DMAP (25 µmol) were dissolved in 2 ml of THF. Then the solution was cooled to 0°C with ice bath and 58 mg of DCC (0.28 mmol), which was dissolved in 2 ml of DCM, was added. The reaction was stirred for 1 hour in room temperature. After confirming full conversion by HPLC, the urea by-product was filtered using cotton topped with little amount of celite. Solvents were evaporated. The solid was re-dissolved in 600µl of DMF,

236 mg compound **5**[3] (1.3mmol) were dissolved in 1 ml of DMF and was added to the former solution. Then 353µl of DIPEA (2 mmol) were added and the reaction was stirred at 40°C for 24 hours. The solvents were evaporated using evaporator and high vacuum. Crud was re-dissolved in DCM and was loaded on silica column. The product was purified using gradient of Ethyl acetate:Hexane (started from 20:80 and raised gently until 40:60). The fractions that contained the product were unified. Solvents were evaporated with evaporator, and dried under high vacuum obtaining 244 mg of yellowish oily product (86% yield).^{1}H-NMR (400 MHz, $CDCl_3$): δ 6.57 (d, *J* = 2.1 Hz, 2H, Ar-H), 6.47 (d, *J* = 2.0 Hz, 1H, Ar-H), 4.32 – 4.04 (m, 16H, Ar-O-C***H***$_2$-+ S-CH_2-C***H***$_2$-O-, HC≡C-C***H***$_2$-O-CH_2-CH_2-N-), 3.75 (d, broad, 4H, HC≡C-CH_2-O-C***H***$_2$-CH_2-N-), 3.56 (s, broad, 4H, HC≡C-CH_2-O-CH_2-C***H***$_2$-N-), 3.24 – 3.13 (m, 2H, -C***H***-S-), 3.05 – 2.82 (m, 8H, -C***H***$_2$-S-), 2.76 (t, *J* = 6.7 Hz, 4H, -C***H***$_2$-S-), 2.46 (s, 2H, **H**C≡C-), 2.27 (t, *J* = 7.5 Hz, 8H, -O-CO-C***H***$_2$-$(CH_2)_3$-CH_3), 1.69 – 1.48 (m, 8H, -O-CO-CH_2-C***H***$_2$$(CH_2)_2$-$CH_3$), 1.34 – 1.18 (m, 16H, O-CO-CH_2-CH_2(CH_2)$_2$-CH_3), 0.85 (t, *J* = 6.9 Hz, 12H, O-CO-CH_2-$CH_2$$(CH_2)_2$-C***H***$_3$).^{13}C-NMR (101 MHz, $CDCl_3$): δ 173.69, 159.60, 138.79, 106.16, 102.92, 99.52, 69.93, 63.49, 63.22, 58.49, 45.72, 35.12, 34.31, 31.43, 30.57, 24.73, 22.44, 14.04. HRMS (EI, positive mode): calculated mass for $C_{55}H_{87}NO_{13}S_4Na$ $[MNa]^+$: 1120.4952; found: 1120.4954.

$(mPEG_{5kDa})_2$-dend-$(Hex)_4$

163 mg $mPEG_{5kDa}$-N_3 (32 µmol) were dissolved in 1ml of DMF (using mild hitting) then 15.5 mg of compound **6** (14µmol) which was dissolved in minimal amount of DMF, was added to the flask. 3.5 mg of $CuSO_4 \cdot 5H_2O$ (14 µmol) and 5.6 mg of sodium ascorbate (28 µmol) were added to the solution. The solution was purged with nitrogen for 15 min and the reaction was performed using microwave at 80°C for 1.5 hours. The salts were filtered through PTFE syringe filter. The filtered solution was first purified by LH20 column using methanol as solvent. The fractions that contained the product were unified. MeOH was evaporated with evaporator. In order to isolate the product from the access of mPEG5kDa-N_3, prep-HPLC with wide-pore C18 column was used. The product was eluted in 50% ACN. After the prep, ACN was evaporated and the residue was extracted with 3x50ml of DCM and then washed 3x50ml of Brine. The organic phase was dried using Na_2SO_4 and evaporated to obtain white solid product (137 mg, 87% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.73 (s, 2H, H-triazole), 6.58 – 6.44 (m, 3H, Ar-H), 4.68 – 4.45 (m, 8H, -N-CH_2-C***H***$_2$-O, triazole-C***H***$_2$-NH-CO), 4.38 – 4.02 (m, 12H, Ar-O-C***H***$_2$-+ S-CH_2-C***H***$_2$-O-), 3.85 – 3.45 (m, 1188H, PEG-backbone), 3.36 (s, 6H, C***H***$_3$-O-PEG), 3.19 (qui, *J* = 6.0 Hz, 2H, -C***H***-S-), 3.03 – 2.82 (m, 8H, -C***H***$_2$-S-), 2.79 (t, *J* = 6.8 Hz, 4H, -C***H***$_2$-S-), 2.29 (t, *J* = 7.5 Hz, 8H, -O-CO-C***H***$_2$-$(CH_2)_3$-CH_3), 1.60 (qui, *J* = 7.4 Hz, 8H, -O-CO-CH_2-C***H***$_2$$(CH_2)_2$-$CH_3$), 1.38 – 1.13 (m, 16H, O-CO-CH_2-CH_2(CH_2)$_2$-CH_3), 0.87 (t, *J* = 6.8 Hz, 12H, O-CO-CH_2-$CH_2$$(CH_2)_2$-C***H***$_3$). ^{13}C NMR (101 MHz, $CDCl_3$): δ 190.38, 184.27, 173.67, 159.62, 144.64, 138.78, 124.00, 106.01, 77.49, 77.18, 76.86, 70.69, 63.23, 59.14, 45.71, 35.06, 34.28, 31.78, 31.41, 30.55, 29.81, 24.71, 22.43, 14.04. GPC (DMF + 25 mM NH_4Ac): Expected Mn = 11.2 kDa; experimental M_n = 10.6 kDa; D_M = 1.03. MALDI-TOF MS: molecular ion centered at 11.3 kDa.

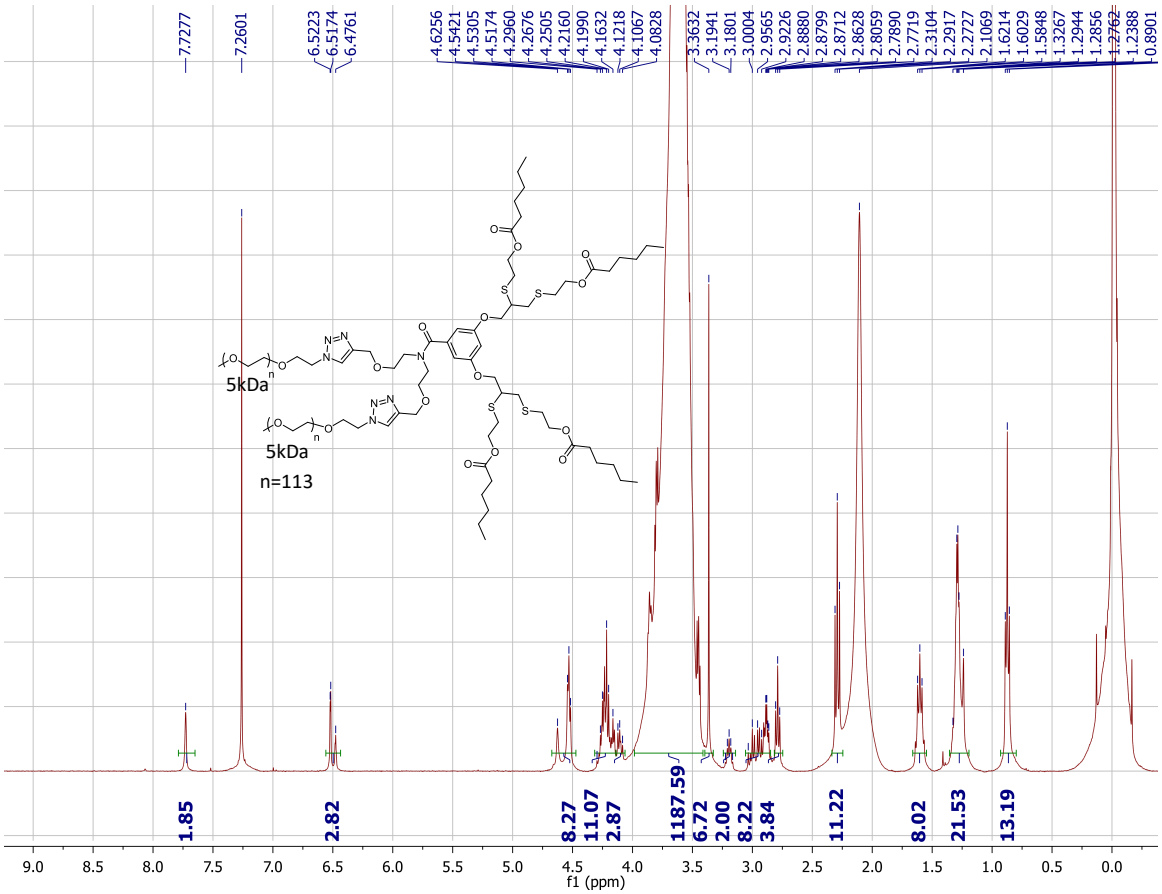

Figure S3: *^{1}H-NMR spectrum of V-shaped hybrid with four end-groups $(PEG5kDa)_2$-dend-$(Hex)_4$ in $CDCl_3$.*

Synthesis of linear and V-shaped PEG-dendron hybrids based on one end-group

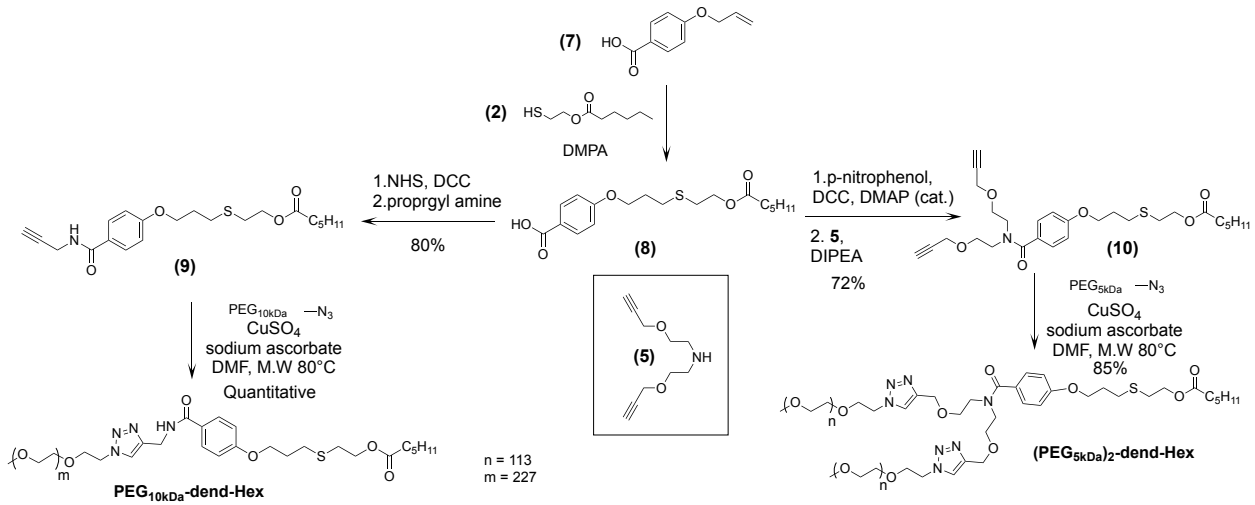

Figure S4: *Synthetic route of Linear and V-shaped hybrids with one end-group.*

Compound 8:

445 mg of compound **7**[4] (2.5 mmol) were dissolved in DMF (0.7mL). 1.3 gr of compound **2**[2] (7.4 mmol) and 63 mg DMPA (0.25 mmol) were added to the solution. The solution was purged with nitrogen for 15 minutes and then placed under UV light at 365nm for 2 hours. The crude mixture was loaded on silica column using gradient of DCM (100%) to MeOH:DCM (5:95 v/v). The fractions contained the compound were combined and evaporated to obtain the product 870 mg (quantitative yield) as oil. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.03 (d, *J = 8.9 Hz*, 2H, Ar-H), 6.93 (d, *J = 8.9* Hz, 2H, Ar-H), 4.23 (t, *J = 6.9* Hz, 2H, S-CH_2-C***H*****$_2$**-O-CO), 4.13 (t, *J = 6.0 Hz*, 2H, Ar-O-C***H*****$_2$**-), 2.79 – 2.74 (m, 4H, -C***H*****$_2$**-S-), 2.31 (t, *J = 7.6* Hz, 2H, CO-C***H*****$_2$**-$(CH_2)_3$-CH_3), 2.10 (qui, *J = 6.4 Hz*, 2H, -O-CH_2-***CH*****$_2$**-CH_2-S), 1.62 (qui, *J = 7.6 Hz*, 2H, CO-CH_2-C***H*****$_2$**$(CH_2)_2$-CH_3), 1.34 – 1.25 (m, 4H, CO-CH_2-CH_2(C***H*****$_2$**$)_2$-CH_3), 0.88 (t, *J = 6.9* Hz, 3H, CO-$(CH_2)_4$-C***H*****$_3$**). ^{13}C NMR (101 MHz, $CDCl_3$): δ 173.76, 170.50, 163.36, 132.46, 121.73, 114.33, 66.43, 63.30, 34.32, 31.41, 30.77, 29.24, 28.87, 24.72, 22.42, 14.01, 0.11. HRMS (EI, Negative mode): calculated mass for $C_{18}H_{25}O_5S$: 353.1423 $[M-H]^-$; found: 353.1426.

Compound 9:

274 mg of compound **8** (0.77 mmol) and 99 mg of NHS (0.86 mmol) were dissolved in 1.5 ml of DCM. Then, the solution was cooled to 0°C with ice bath and 175 mg (0.85 mmol) of DCC, which was dissolved in 1ml of DCM, was added. The reaction was stirred for 1.5 hours in room temperature. After confirming full conversion by HPLC, the urea by-product was filtered using cotton topped with little amount of celite. Next, 99µl of propargylamine (1.5 mmol) were added to solution and was stirred overnight in room temperature. The crude mixture was loaded on silica column using gradient of Ethyl acetate: hexane (20:80 v/v) to (30:60 v/v). The fractions contained the compound were combined and evaporated to dryness to obtain 242 mg of the product as colorless thick oil (80% yield).^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.84 – 7.68 (m, ,2H, Ar-***H***), 7.01 – 6.86 (m, 2H, Ar-***H***), 6.20 (t, *J = 5.6 Hz*, 1H, amide), 4.30 – 4.20 (m, 4H, Ar-O-C***H*****$_2$**, HC≡C-C***H*****$_2$**-), 4.12 (t, *J* = 6.0 Hz, 1H, S-CH_2-C***H*****$_2$**-O-), 2.80 – 2.756 (m, 4H, -C***H*****$_2$**-S-), 2.39 – 2.25 (m, 3H, ***H***C≡C-CH_2-, CO-C***H*****$_2$**-$(CH_2)_3CH_3$), 2.10 (qui, *J* = 6.5 Hz, 2H, S-CH_2-C***H*****$_2$**-CH_2-O), 1.68 – 1.59 (m, 2H, CO-CH_2-C***H*****$_2$**-$(CH_2)_2CH_3$), 1.38 – 1.24 (m, 4H, CO-CH_2-CH_2-(C***H*****$_2$**$)_2CH_3$), 0.91 (t, *J* = 7.0 Hz, 3H, CO-$(CH_2)_4$C***H*****$_3$**). ^{13}C-NMR (101 MHz, $CDCl_3$) δ 173.76, 166.65, 161.80, 128.97, 126.22, 114.45, 79.79, 71.92, 66.37, 63.30, 49.30, 34.31, 34.08, 31.40, 30.76, 29.87, 29.28, 28.88, 25.74, 25.06, 24.72, 22.42, 14.01. (EI, positive mode): calculated mass for $C_{21}H_{29}NO_4SNa$ (MNa^+): 414.1715; found: 414.1714.

mPEG$_{10kDa}$-dend-Hex

150 mg of MeO-PEG_{10kDa}-N_3 (15µmol) was dissolved with 300µL of DMF (using mild heating). 29 mg of Compound **9** (74µmol) were separately dissolved with minimal amount of DMF and combined with the former solution. 3.8 mg of $CuSO_4{\cdot}5H_2O$ (15µmol) and 5.9 mg of sodium ascorbate (30µmol) were added to the solution. The solution was purged with nitrogen for 15 min and the reaction was performed using microwave at 80°C for 1.5 hours. The salts were filtered through PTFE syringe filter. The filtered solution was further purified by MeOH based LH20. The fractions that contained the product were unified. MeOH

was evaporated with evaporator and dried under high vacuum obtaining the product as white solid (150 mg, quantitative yield). ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.78 - 7.75 (m, 3H, Ar-***H***+ triazole-***H***), 6.97 (s, 1H, amide-***H***), 6.89 (d, *J = 8.8 Hz*, 2H, Ar-***H***), 4.69 (d, *J = 4.8 Hz*, 2H, triazole-C***H₂***-NH-CO), 4.52 (t, *J = 5.1 Hz*, 2H, -N-C***H₂***-CH_2-O), 4.22 (t, *J = 6.9 Hz*, 2H, S-CH_2-C***H₂***-CO), 4.08 (t, J = 6.1 Hz, 2H, Ar-O-C***H₂***-CH_2-CH_2-S), 3.88 – 3.84 (m, 2H, -N-CH_2-C***H₂***-O), 3.82 – 3.44 (m, 1063H, PEG backbone), 3.37 (s, 3H, C***H₃***-O-PEG), 2.77 – 2.73 (m, 4H, S-C***H₂***), 2.30 (t, *J = 7.5 Hz*, 2H, CO-C***H₂***-$(CH_2)_3CH_3$), 2.07 (qui, *J = 6.2 Hz*, 2H, O-CH_2-C***H₂***-CH_2-S), 1.61 (qui, *J = 7.5 Hz*, 2H, CO-CH_2-C***H₂***$(CH_2)_2CH_3$), 1.38 – 1.19 (m, 4H, CO-CH_2-CH_2(CH_2***H₂***$)_2CH_3$), 0.88 (t, *J = 6.9 Hz* ,3H, CO-$(CH_2)_4$-C***H₃***). ^{13}C-NMR (101 MHz, $CDCl_3$): δ 144.96, 144.73, 129.05, 126.64, 123.58, 114.29, 70.68, 66.34, 63.29, 59.14, 50.42, 35.59, 34.30, 31.39, 30.73, 29.80, 29.30, 28.88, 24.71, 22.79, 22.41, 14.01, 0.11. GPC (DMF + 25 mM NH_4Ac): Expected Mn = 10.4 kDa; experimental M_n = 10.5kDa; D_M = 1.04. MALDI-TOF MS: molecular ion centered at 10.3kDa.

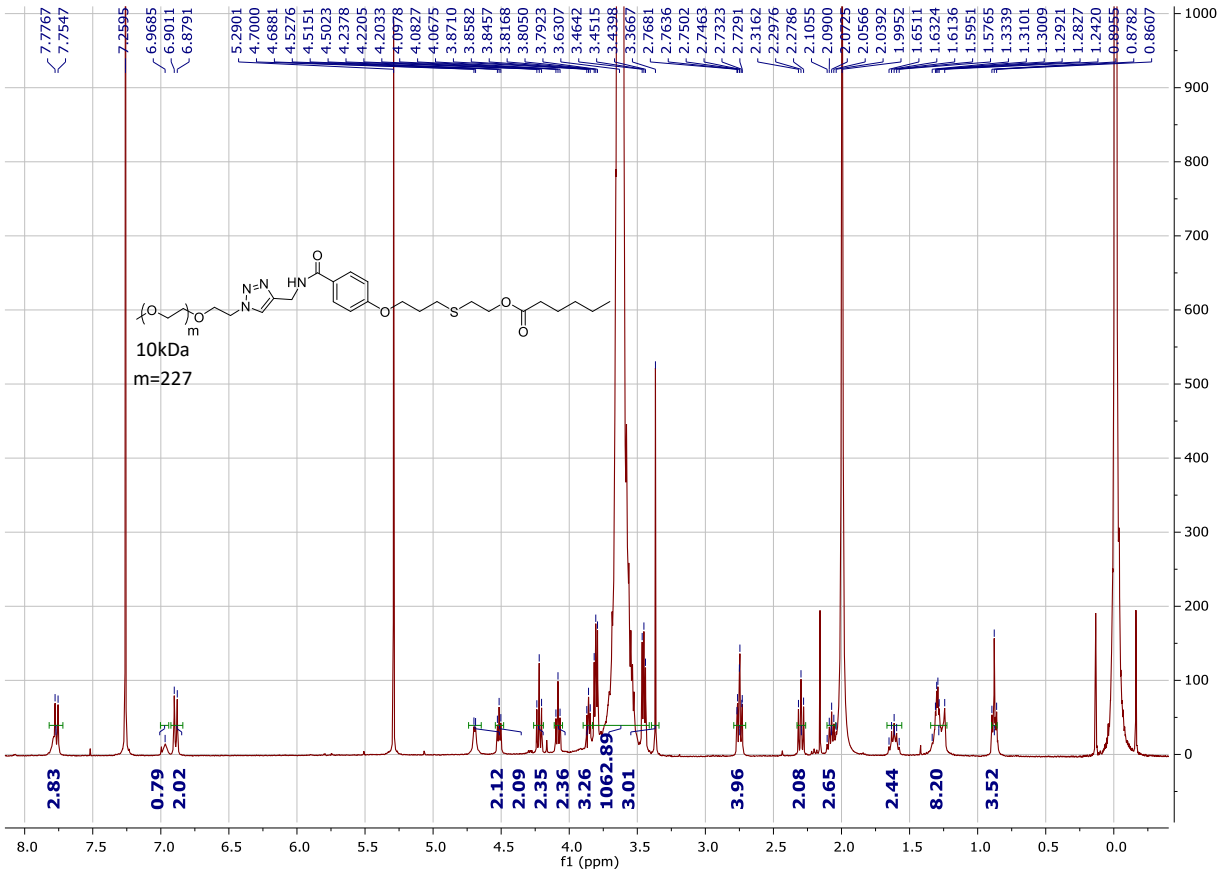

Figure S5: *^{1}H-NMR spectrum of Linear hybrid with one end-group PEG10kDa-dend-Hex in $CDCl_3$.*

Compound 10:

130 mg of compound 8 (0.37 mmol), 56 mg of p-nitrophenol (0.4 mmol) and 4.5 mg of DMAP (37 µmol) were dissolved in 2 ml of THF and DCM mixture (1:1). Then the solution was cooled to 0°C with ice bath and 83 mg of DCC (0.4 mmol), which was dissolved in 0.5 ml of DCM, was added. The reaction was stirred for 3 hours in room temperature. After confirming full conversion by HPLC, the urea by-product was

filtered using cotton topped with little amount of celite. Solvents were evaporated and the crude mixture was loaded on silica column washed with Ethyl acetate and Hexane (EA:Hex 25:75 v/v) . The fractions contained the compound were combined and evaporated. Next, the solid was re-dissolved in 600µl of DMF, 100 mg compound **5**[3] (0.55 mmol) were dissolved in 1 ml of DMF and added to the former solution. Then 315 µl of DIPEA (1.8 mmol) were added and the reaction was stirred at 40°C for 50 hours. The solvents were evaporated using evaporator and high vacuum. The crude product was re-dissolved in DCM and was loaded on silica column. The product was purified using gradient of Ethyl acetate:Hexane (started from 20:80 and raised gently until 50:50). The fractions that contained the product were unified. Solvents were evaporated with evaporator and dried under high vacuum obtaining 137 mg of yellowish oily product (72% yield).^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.38 (d, *J = 8.7 Hz*, 2H, Ar-H), 6.88 (d, *J = 8.7 Hz*, 2H, Ar-H), 4.24 (t, *J = 6.9 Hz*, 2H, S-CH_2-C***H₂***-CO), 4.15 (s broad, 4H, HC≡C-C***H₂***-O), 4.07 (t, *J = 6.0 Hz*, 2H, Ar-O-CH_2-), 3.69 (d broad, 8H, O-C***H₂***-C***H₂***-N), 2.88 – 2.69 (m, 4H, S-C***H₂***-), 2.43 (t, *J = 2.3 Hz*, 2H, ***H***C≡C-), 2.32 (t, *J = 7.6 Hz*, 2H, CO-C***H₂***-$(CH_2)_4$-CH_3), 2.08 (qui, *J = 6.5 Hz*, 2H, S-CH_2-C***H₂***-CH_2-O), 1.67 – 1.59 (m, 2H, CO-CH_2-C***H₂***$(CH_2)_2$-CH_3), 1.34 – 1.28 (m, 4H, CO-CH_2-CH_2(C***H₂***$)_2$-CH_3), 0.89 (t, 3H, CO-$(CH_2)_4$-C***H₃***). ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.75, 172.34, 159.73, 129.09, 128.94, 114.31, 77.45, 77.13, 76.81, 74.71, 69.79, 68.20, 66.25, 63.33, 58.41, 34.31, 31.40, 30.75, 29.36, 28.94, 24.72, 22.42, 14.01. (EI, positive mode): calculated mass for $C_{28}H_{39}NO_6SNa$ [MNa^+]: 540.2390; found: 540.2388.

<u>(mPEG$_{5kDa}$)$_2$-dend-Hex</u>

138 mg mPEG$_{5kDa}$-N_3 (27.5 µmol) were dissolved in 1ml of DMF (using mild hitting) then 5.7 mg of compound **10** (11µmol), which were dissolved in minimal amount of DMF, were added to the flask. 2.7 mg of $CuSO_4$·$5H_2O$ (11 µmol) and 4.4 mg of sodium ascorbate (22 µmol) were also added to the solution. The solution was purged with nitrogen for 15 min and the reaction was performed using microwave at 80°C for 1.5 hours. The salts were filtered by nylon syringe filter. The filtered solution was first purified by MeOH based LH20. The fractions that contained the product were unified. MeOH was evaporated. In order to isolate the product from the access of mPEG5kDa-N_3 prep-HPLC was performed using wide-pore C18 column. The product was eluted in 50% ACN. After the prep, ACN was evaporated and the residue was extracted and washed with 3x50ml of DCM and 3x50ml of Brine. The organic phase was dried using Na_2SO_4 and evaporated to obtain 99 mg white solid product (85% yield). ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.71 (s, 2H, triazole-***H***), 7.30 (d, *J = 8.7 Hz*, 2H, Ar-***H***), 6.84 (d, J = 8.7 Hz, 2H, Ar-***H***), 4.60 – 4.51 (m, 8H, N-C***H₂***-CH_2-O + tiazole-C***H₂***-O), 4.21 (t, *J = 6.9 Hz*, 2H, S-CH_2-C***H₂***-O-CO), 4.04 (t, *J = 6.0 Hz*, 2H, Ar-O-C***H₂***-), 3.85 (t, *J = 5.1 Hz*, 4H, N-CH_2-C***H₂***-O), 3.81 – 3.43 (m, 1009H, PEG backbone), 3.36 (s, 6H, CH_3-O-) 2.74 (t, *J = 6.9 Hz*, 4H, S-C***H₂***), 2.44 – 2.22 (m, 2H, CO-C***H₂***-$(CH_2)_3$-CH_3), 2.05 (qui, *J = 6.4 Hz*, 2H, O-CH_2-C***H₂***-CH_2-S), 1.61 (qui, J = 7.4 Hz, 2H, CO-CH_2-C***H₂***$(CH_2)_2CH_3$), 1.34 – 1.25 (m, 4H, CO-CH_2-CH_2(C***H₂***$)_2$-CH_3), 0.87 (t, J = 6.7 Hz, 3H, CO-$(CH_2)_4$-C***H₃***). ^{13}C-NMR (101 MHz, $CDCl_3$): δ 172.26, 159.70, 144.69, 129.03, 123.88, 114.28, 77.45, 74.09, 71.78, 68.41, 67.18, 66.24, 64.47, 63.75, 63.27, 59.11, 50.32, 34.28, 31.36, 30.70, 29.77, 29.34, 28.90, 24.68, 22.39, 22.39. GPC (DMF + 25 mM NH_4Ac):

Expected Mn = 10.6kDa; experimental Mn = 10.5 kDa, D_M = 1.04. MALDI-TOF MS: molecular ion centered at 10.4kDa.

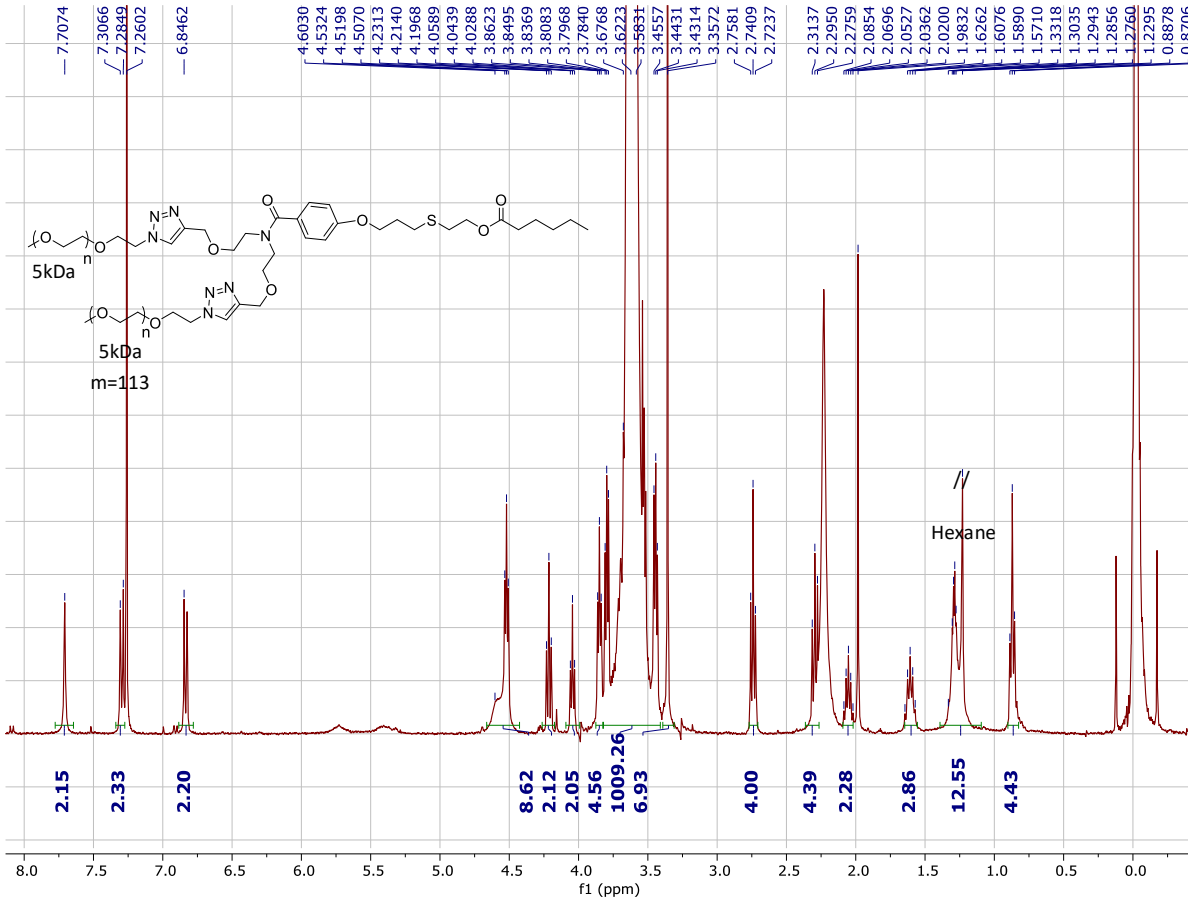

Figure S6: *^{1}H-NMR spectrum of V-shaped hybrid with one end-group $(PEG_{5kDa})_2$-dend-Hex in $CDCl_3$.*

Gel Permeation Chromatography (GPC)

measurements Instrument: Viscotek GPCmax (Malvern)

Columns: 2 x PSS GRAM 1000Å + 1 x PSS GRAM 30Å Columns

Temperature: 50 °C

Mobile phase: DMF + 25 mM NH_4Ac

Flow rate: 0.5 mL/min

Needle wash: DMF

Detector: Refractive index Detector temperature: 50 °C

Run time: 90 minutes

Sample preparation: Typically, the PEG compound was dissolved using gentle heating in the mobile phase to afford a final concentration of 10 mg/mL. Samples were filtered through 0.22 µm PTFE syringe filter.

GPC traces of hybrids with four end-groups

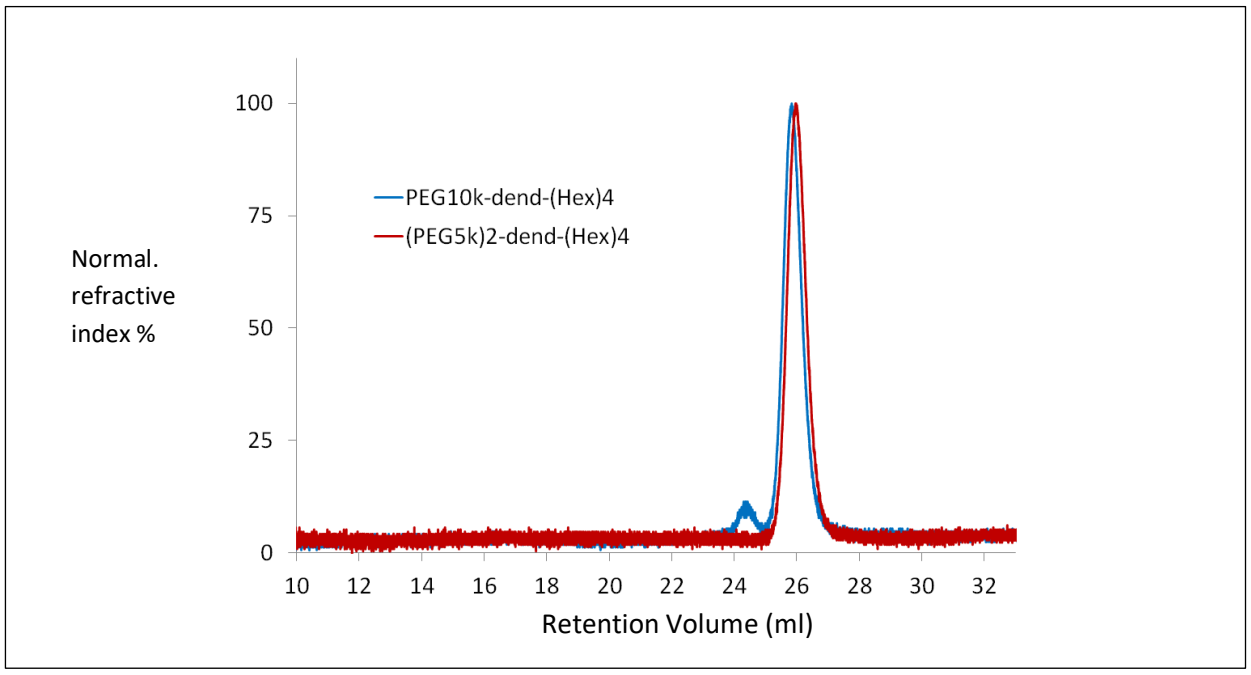

***Figure S7**: GPC overlay of the synthesized PEG-dendron hybrids with four end-groups: V-shaped hybrid $(PEG_{5kDa})_2$-dend-$(Hex)_4$ (Mn = 10.6 kDa D_M = 1.03), Linear hybrid PEG_{10kDa}-dend-$(Hex)_4$ (Mn = 10.7 kDa D_M = 1.05).*

GPC traces of hybrids with one end-group (G0 hybrids)

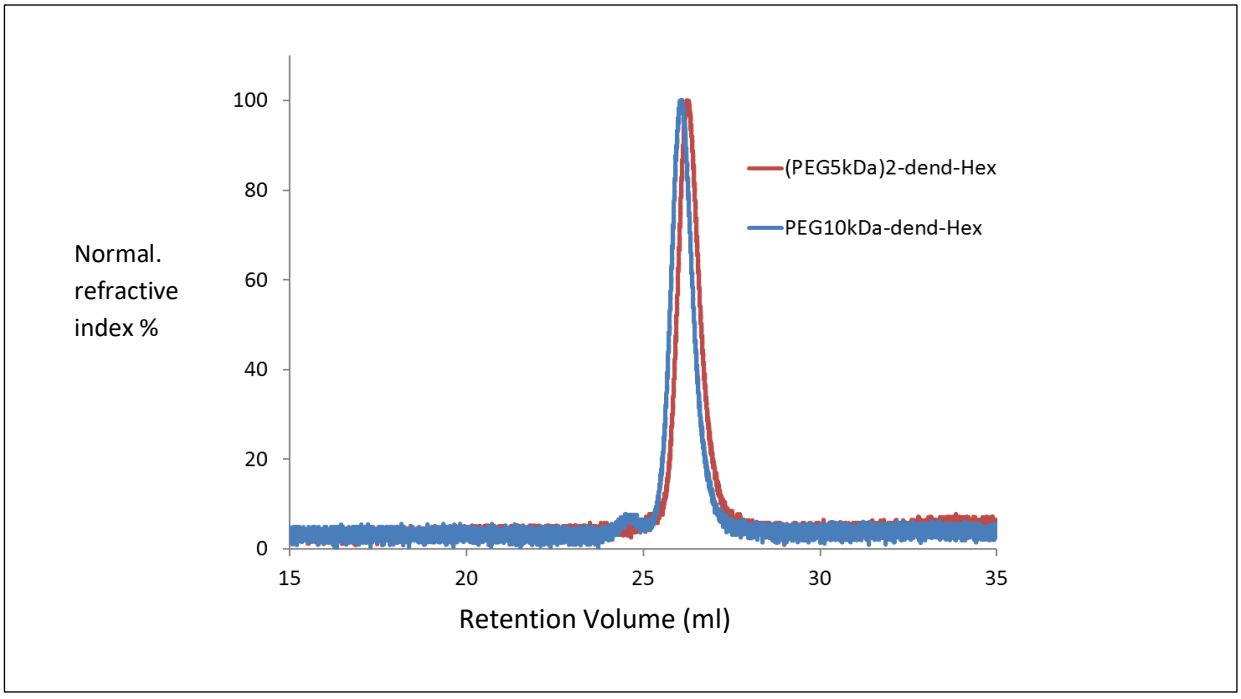

***Figure S8**: GPC overlay of the synthesized PEG-dendron hybrids with one end-group: V-shaped hybrid $(PEG_{5kDa})_2$-dend-(Hex) (Mn = 10.5 KDa D_M = 1.04), Linear hybrid PEG_{10kDa}-dend-(Hex) (Mn = 10.5 kDa D_M = 1.04).*

MALDI measurements

MALDI for hybrids with four end-groups

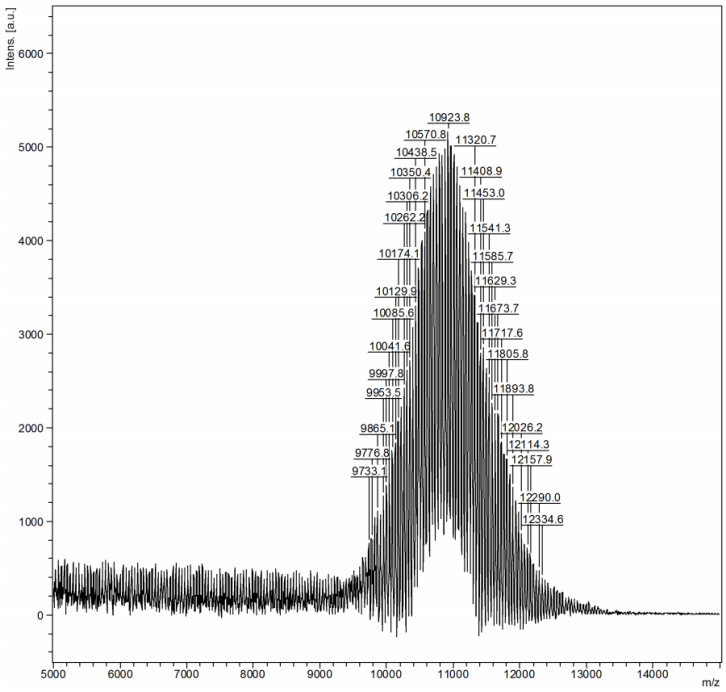

***Figure S9**: MALDI measurement of Linear hybrid PEG_{10kDa}-dend-$(Hex)_4$. Mn = 10.9 kDa, Expected Mn = 11.0 kDa.*

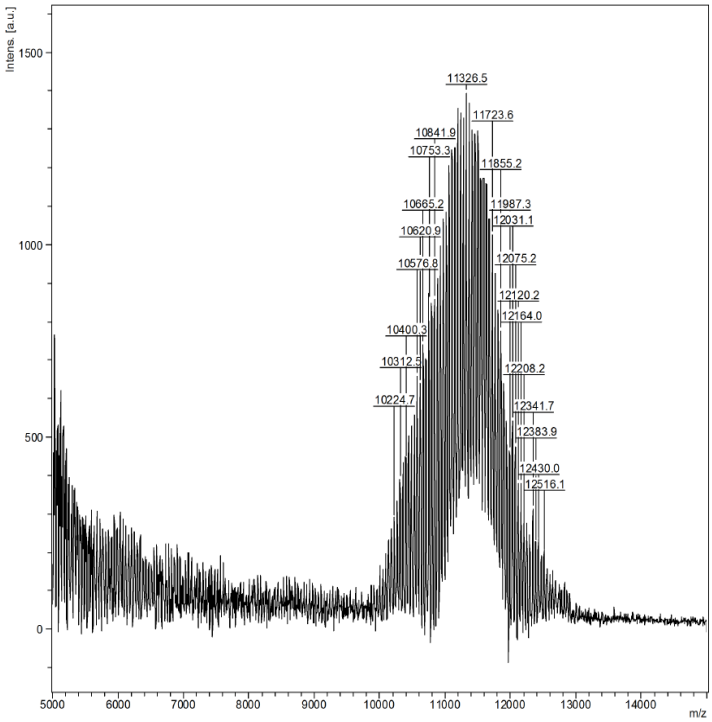

***Figure S10**: MALDI measurement of V-shaped hybrid $(PEG_{5kDa})_2$-dend-$(Hex)_4$. Mn = 11.3 kDa, Expected Mn = 11.2 kDa.*

MALDI for hybrids with one end-group

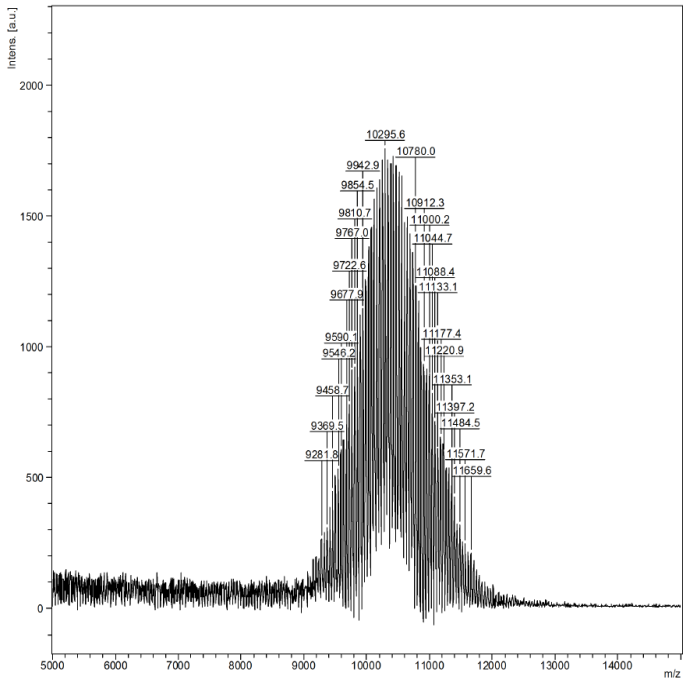

Figure S11*: MALDI measurement of Linear hybrid PEG_{10kDa}-dend-Hex. Mn = 10.3 kDa, Expected Mn = 10.4 kDa.*

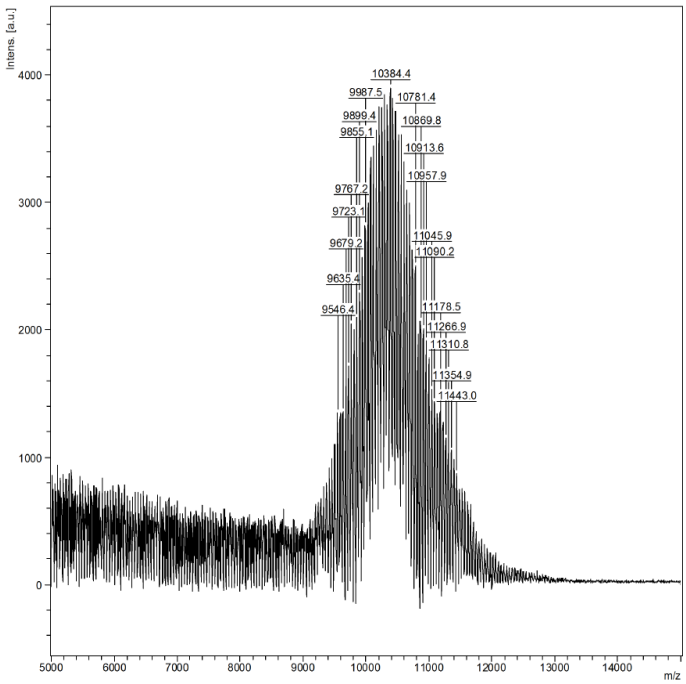

Figure S12*: MALDI measurement of V-shaped hybrid $(PEG_{5kDa})_2$-dend-Hex. Mn = 10.4 kDa, Expected Mn = 10.6 kDa.*

Critical Micelle Concentration (CMC) Measurements

Instrument: TECAN Infinite M200Pro device

Excitation wavelength: 550nm

Emission intensity scan: 580-800nm

Diluent solution preparation:

Nile Red stock solution (0.88 mg/ml in ethanol) was diluted into phosphate buffer Selin (0.1M, pH 7.4) to give a final concentration of 1.25 µM.

CMC measurement for all hybrids: A 400µM solution of the hybrid was prepared in diluent. Solution was vortexed and sonicated for 15 minutes until clear. This solution was repeatedly diluted by a factor of 1.5 or 2 with the diluent. 100µL of each solution were loaded onto a 96 wells plate. A fluorescence emission intensity was performed for each well. The maximum emission intensity at about 630nm was plotted vs. hybrid's concentration. The cross-section between low emission samples and the sudden rise in fluorescence was used in order to determine the CMC value of the examined hybrid. All measurements were repeated 3 times.

CMC of hybrids with four end-groups

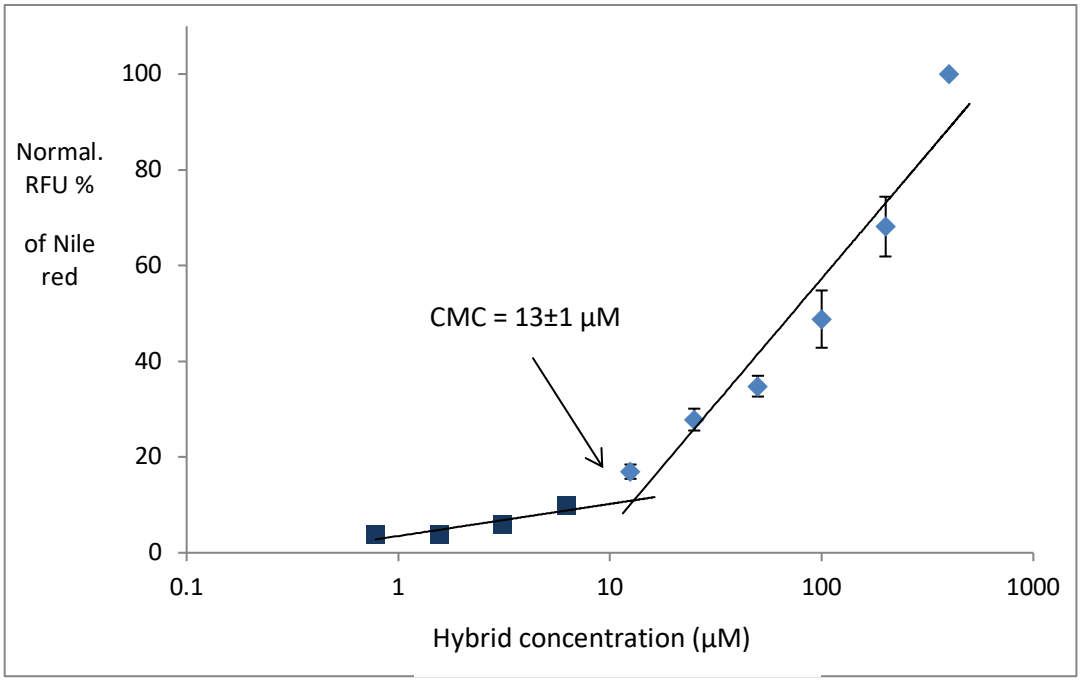

***Figure S13**: CMC measurement of Linear hybrid PEG_{10kDa}-dend-$(Hex)_4$ using Nile red technique. Maximum emission of each sample was plotted vs. the concentration of the hybrid.*

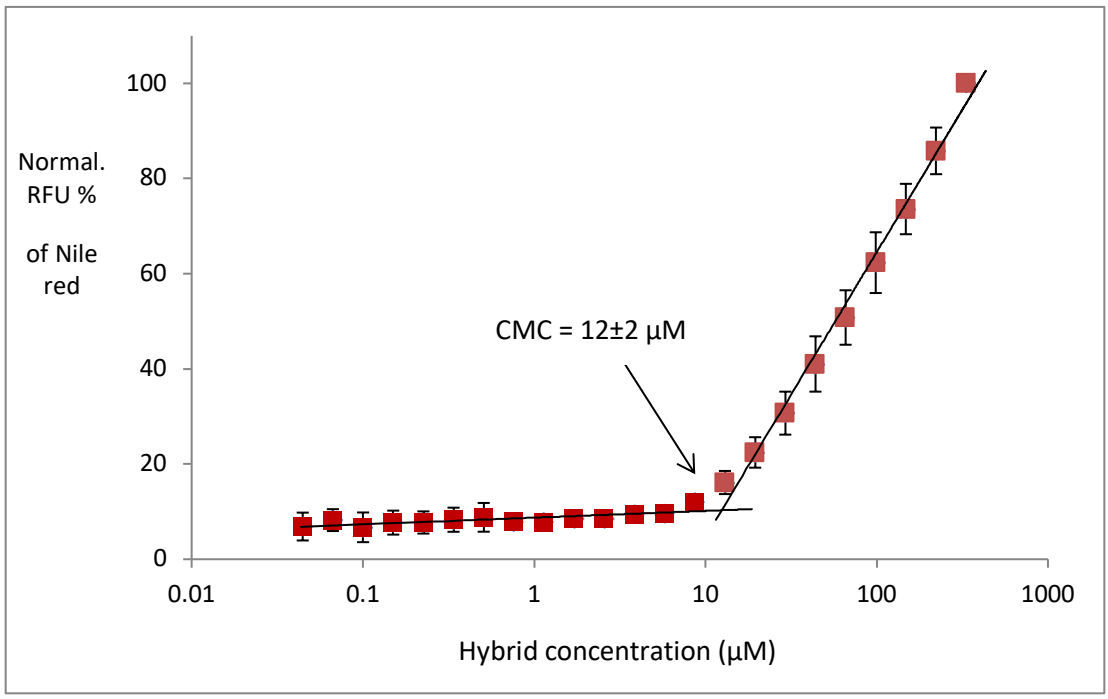

Figure S14: *CMC measurement of V-shaped hybrid $(PEG_{5kDa})_2$-dend-$(Hex)_4$ using Nile red technique. Maximum emission of each sample was plotted vs. the concentration of the hybrid.*

CMC of hybrids with one end-group

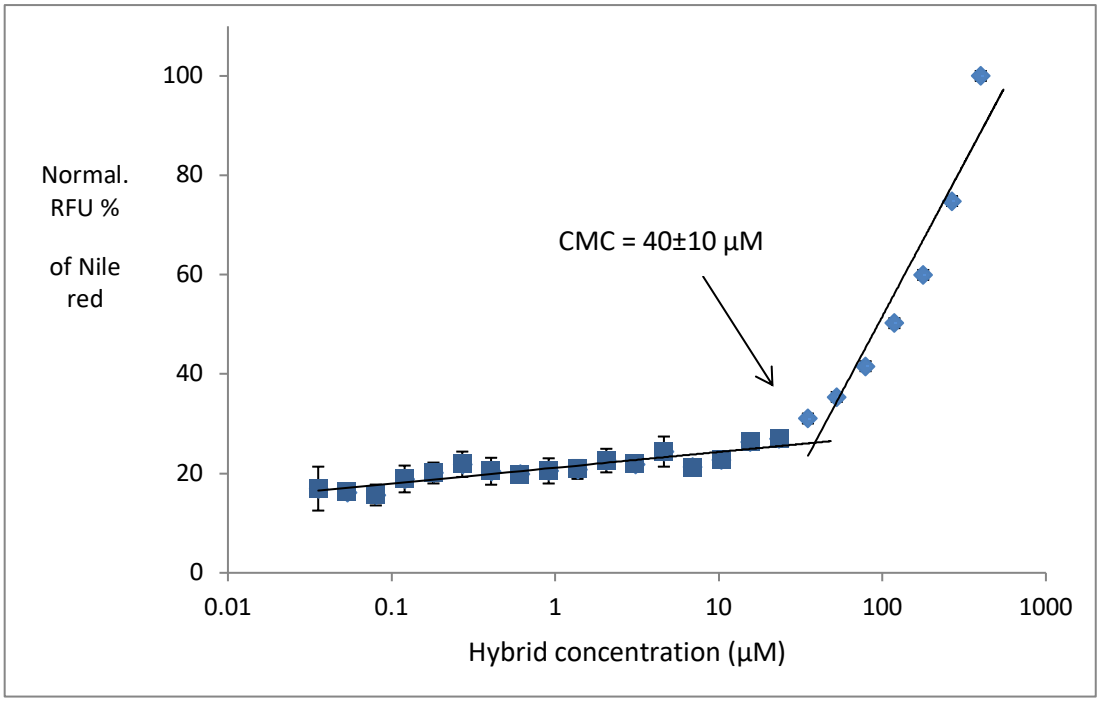

Figure S15: *CMC measurement of Linear hybrid PEG_{10kDa}-dend-Hex using Nile red technique. Maximum emission of each sample was plotted vs. the concentration of the hybrid.*

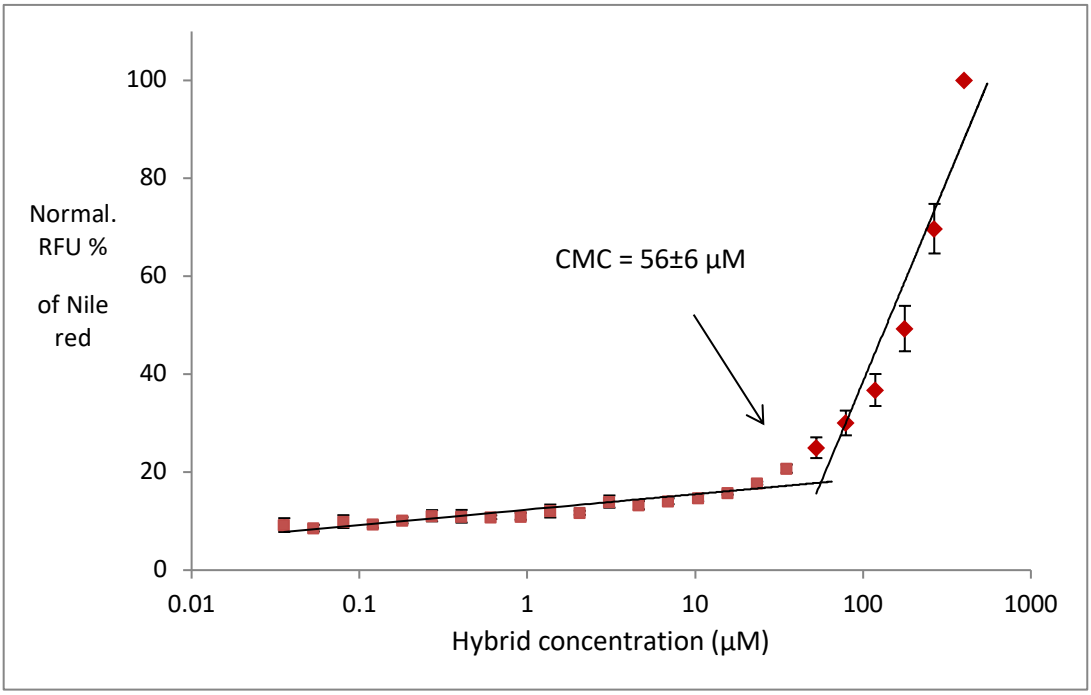

***Figure S16**: CMC measurement of V-shaped hybrid $(PEG_{5kDa})_2$-dend-Hex using Nile red technique. Maximum emission of each sample was plotted vs. the concentration of the hybrid.*

Dynamic Light Scattering (DLS)

Instrument: Corduan Technology VASCOγ particle size analyzer

Parameters for measurement of samples:

- Time interval: 10-15 µsec
- Number of channels: 100-650
- Laser Power: 50-100%

General sample preparation:

The PEG-dendron hybrid was directly dissolved in phosphate buffer saline (pH 7.4) to obtain a final concentration of 100 µM. Solution was vortexed and sonicated for 5-15 min. Then, solution was filtered through 0.22 µm nylon syringe filter and immediately measured using the specified parameters with 10 repetition for each measurement. Micellar diameters (Dh) were recorded in nm as a function of volume % by SBL analysis.

Transmission electron microscopy (TEM)

General sample preparation: Compound was dissolved in phosphate buffer saline (pH 7.4) to afford a final concentration of 100µM. 5 µL sample solution were dropped cast onto carbon coated copper grids and inspected in a transmission electron microscope (TEM), operated at 200 kV (Philips Tecnai F20). The excessive solvent of the droplet was wiped away using a solvent-absorbing filter paper after 1 min and the

sample grids were left to dry in air at room temperature for 5 minutes. This procedure was repeated 3 times. After the third cycle the sample grids were left to dry in air at room temperature overnight.

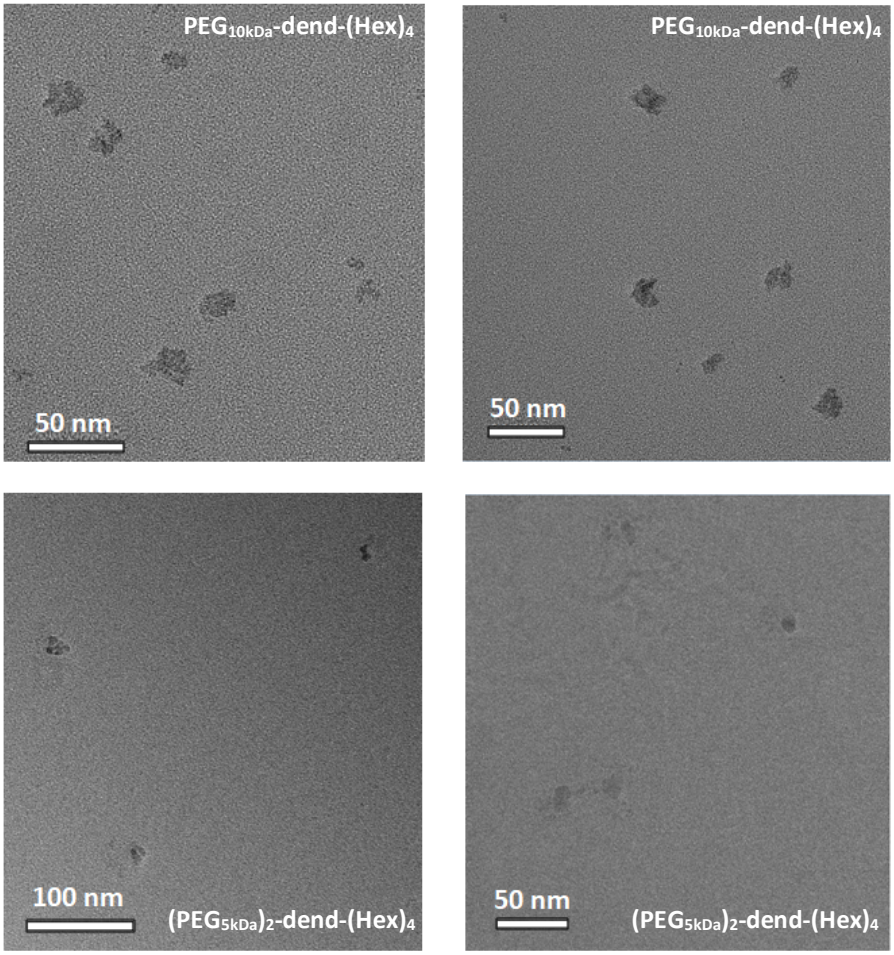

Figure S17*: TEM images of micellar solutions of the two amphiphiles: Top - Linear hybrid PEG_{10kDa}-dend-$(Hex)_4$; Bottom- V-shaped hybrid $(PEG_{5kDa})_2$-dend-$(Hex)_4$. Scale bars, 50 nm or 100nm.*

Small-angle X-ray scattering (SAXS)

SAXS Data collection:

Data was collected at beamline B21, in the "Diamond Light Source" (Diamond, Oxfordshire). Using an Eiger detector at a sample to detector distance of 3.7 m and a wavelength of 1.0 Å, with a range of momentum transfer (q) between 0.026 and 3.4 nm^{-1}.

Each sample was measured at a rather low concentration (≅2.5-5mg/ml) and extrapolated to zero concentration, so no significant inter-micelle interactions are present. Samples were automatically loaded onto a continuous flow cell capillary by a liquid handling robot, for measurement. For analysis, the scattering curves were subtracted from measurements of matching buffer solution.

SAXS data analysis:

Background-subtracted SAXS curves were fitted using "Primus" software[5] to derive a pair-distance distribution (r) such that its Fourier-transform $\mathrm{I(q)} = \mathrm{I_0} \int \mathrm{P(r)}\,\mathrm{sin(qr)} / \mathrm{qr}\,\mathrm{dr}$ fits the measured intensity $\mathrm{I(q)}$, with $\mathrm{I_0}$ being the forward scattering intensity.

Radius of gyration (Rg) was derived from each pair-distance distribution with the relation using the relation: $Rg^2 = \int r^2\ P(r)dr$ as shown in Figure s18.[6]

We estimated micelles' mass from the extrapolated forward scattering intensity ($\mathrm{I(q \to 0) = I_0}$) and absolute intensity scattering value.[6] Following, the molecular weight can be estimated by

$M = I_0(\frac{N_A}{c\Delta\rho_M^2})$,

where N_A is the Avogadro number, c the concentration and $\Delta\rho_M$ the scattering contrast per mass.
Dendrimer electron density was approximated to be equal to PEG electron density. Therefore, the mass calculation takes into account the ratio in electron density contrast with the solvent, water, and PEG. PEG 10.000 (227 repeating units, M = 10kDa) has $N_{el} = 5458$ electrons. The resulting scattering length per mass is $\rho_{PEG,M} = \frac{N_{el}}{M} \cong 3.2868 * 10^{23}\frac{e}{g}$. The scattering contrast per mass is given by: $\Delta\rho_M = (\rho_{PEG,M} - \rho_{Water}\nu)r = 1.4896 * 10^{10}$ cm/g, where $\nu = 0.82580$ cm^3/g is the partial specific volume of one PEG molecule, $\rho_{water} = 0.334\frac{e}{\text{Å}^3}$ is the number of electrons per volume of water[7], and $r = 0.28179 * 10^{-12}$cm is the scattering length of one electron.
Aggregation numbers (AN) were calculated by dividing the measured micelle weight by the molecular weight of the specific dendrimer molecule. Confidence intervals were propagated from the error estimation for I_0 by "Primus" software.[5]

We estimated micelles' densities by $d = \frac{AN}{\frac{4}{3}\Pi R_g^3}$, where AN is the number of monomers per-micelle divided by the volumes of globular shape with the micellar measured-radius $V = \frac{4}{3}\Pi R_g^3$. The resulting densities are $d = 6.88\ and\ 7.74\ \left[\frac{\text{polymers}\times 10^{-3}}{\text{nm}^3}\right]$ (for the linear and V-shaped hybrids, respectively).

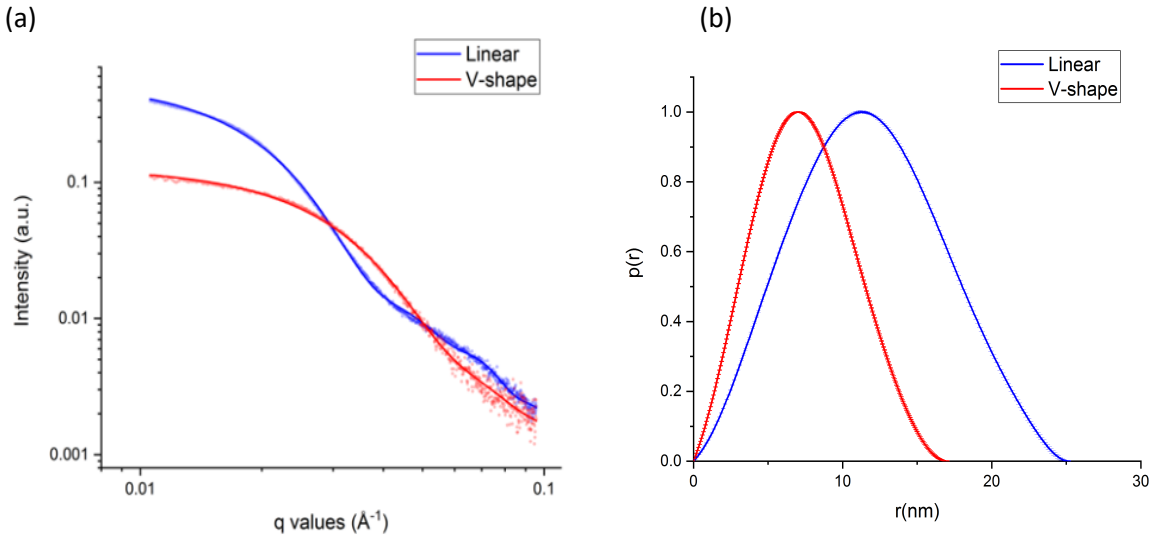

***Figure S18**: (a) Small-angle scattering data and (b) Pair-distance-distributions functions. Data was fitted using "Primus" for Linear hybrid PEG_{10kDa}-dend-$(Hex)_4$ (blue) and V-shaped hybrid $(PEG_{5kDa})_2$-dend-$(Hex)_4$ (red). The Fourier transformed pair-distance-distributions (solid lines) overlay the measured curves (symbols).*

Drug Encapsulation

The tested hybrid was dissolved in DCM (1 mg/ml). 1 ml of hybrid solution was mixed with 0.5 ml drug solution (CPT/PTX [0.5 mg/ml in DCM]). DCM was removed in vacuum forming a thin film, which was further dried on high vacuum for 30 minutes. Then, 1 ml of PBS was added and the solution was stirred vigorously and placed in an ultrasonic bath for 30 minutes (initial concentrations: [hybride]=1mg/ml; [drug]=0.25mg/ml. Undissolved drug was filtered off using 0.45µm nylon filter and the clear solution was analyzed by HPLC (CPT: λ=360 nm; PTX: λ=240 nm).

Monitoring micelle disassembly with Nile Red fluorescence

Instrumentation:

Monitoring of micelle disassembly rate by enzymes was performed using an Agilent Technologies Cary Eclipse Fluorescence Spectrophotometer.

Instrument Method**:**

Excitation: 550nm

Emission scan: 580-800nm

Excitation and Emission slits width: 10nm-15nm

Scan rate: 620nm/min

Temperature control: 37°C

Sample preparation and measurement:

PEG_{10kDa}-dend-$(Hex)_4$ and $(PEG5kDa)_2$-dend-$(Hex)_4$ were separately dissolved in PBS (pH 7.4) to give final concentration of 100µM. For each hybrid 10mL of the solution were accurately measured and 4.5µL of Nile Red stock solution (0.88mg/mL in Ethanol) were added to give a final concentration of 1.25µM. The solutions were mixed using vortex and then were sonicated for 10-15 minutes. 650µL of each hybrid solution containing Nile Red were accurately transferred to separate quartz cuvettes for reference measurements without PLE enzyme (control) and with presence of PLE.

For micelle degradation in the presence of PLE enzyme:

6.3µL of PLE enzyme stock solution (0.28µM in PBS pH 7.4) were added to 650µL of each solution of the tested hybrid to give final PLE concentration of 2.7nM. Repeating fluorescence scans were performed every 15 minutes, measurements were repeated 3 times.

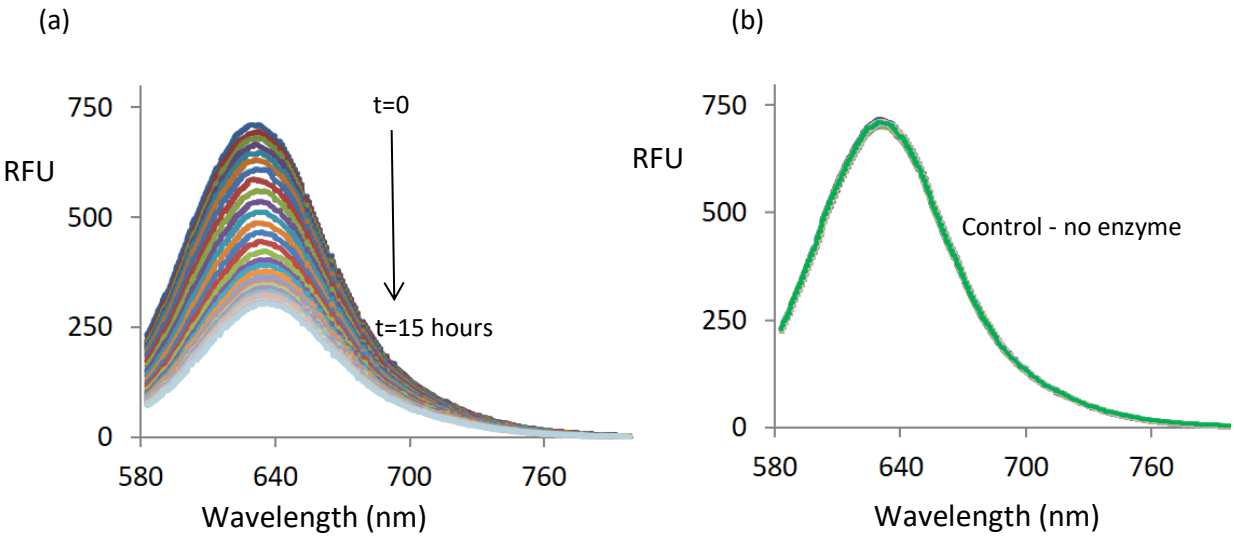

Figure S19: *Fluorescence emission spectra of Nile Red (1.25µM) in the presence of Linear hybrid PEG_{10kDa}-dend-$(Hex)_4$ [hybrid] =100µM; with presence [PLE=2.7 nM] (a) or absence (b) of PLE enzyme over 15 hours.*

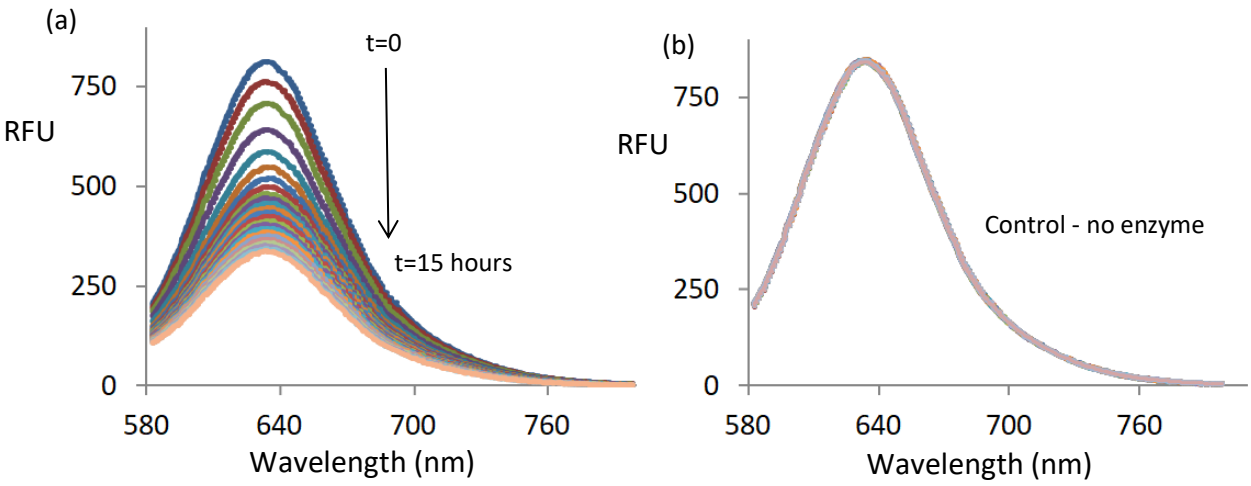

Figure S20: *Fluorescence emission spectra of Nile Red (1.25µM) in the presence of V-Shaped hybrid* $(PEG_{5kDa})_2$*-dend-*$(Hex)_4$ *[hybrid] =100µM; with presence [PLE=2.7 nM] (a) or absence (b) of PLE enzyme over 15 hours.*

HPLC monitoring of enzymatic degradation

HPLC measurements

Instrument: Waters Alliance e2695

Column: Aeris WIDEPORE, C4, 3.6µm, 150x4.6mm

Column temperature: 30°C

Sample temperature: 37°C

Solution A: 0.1% $HClO_4$:ACN 95:5v/v

Solution B: 0.1% $HClO_4$:ACN 5:95v/v

Solution C: THF

Flow rate: 1.0mL/min

Detector: Waters 2998 photodiode array detector

Sampling rate: 2 points/sec

Gradient program for 30 minutes injection:

Time [minutes]	Sol. A [%]	Sol. B [%]	Sol. C [%]
0.0	95	0	5
1.0	95	0	5
20.0	0	95	5
23.0	0	95	5
23.1	95	0	5
30.0	95	0	5

General Sample Preparation for enzymatic degradation monitoring by HPLC

Hybrids based on four end-groups

Solid hybrids compounds were separately dissolved in PBS (pH 7.4) to give the desired concentration of 100µM and 1.25µM Nile red (Nile Red stock solution 0.88mg/mL in Ethanol was diluted directly to the hybrid's solutions). Each solution was mixed using vortex and sonicated for 10-5 minutes. 1500µL of the hybrid solution were transferred to a proper HPLC vial. Injection 30µl to the HPLC as t=0 injection was performed. PLE enzyme stock solution in PBS buffer pH 7.4 was added to give final concertation of 2.7 nM and mixed using vortex. Enzymatic degradation was monitored by repeating injections from the same vial over time. Measurements were repeated 3 times.

Hybrids based one end-group

Solid hybrids compounds were separately dissolved in PBS (pH 7.4) to give the desired concentration of 15µM which is under the hybrid's CMC and 1.25µM Nile red (Nile Red stock solution 0.88mg/mL in Ethanol was diluted directly to the hybrid's solutions). Each solution was mixed using vortex and sonicated for 10-5 minutes. 1500µL of the hybrid solution were transferred to a proper HPLC vial. Injection of 100µl to the HPLC as t=0 injection was performed. PLE enzyme stock solution in PBS buffer pH 7.4 was added to give final concentration of 54 pM and mixed using vortex. Enzymatic degradation was monitored by repeating injections from the same vial over time. Measurements were repeated 3 times.

HPLC overlay of hybrids based on one end-group

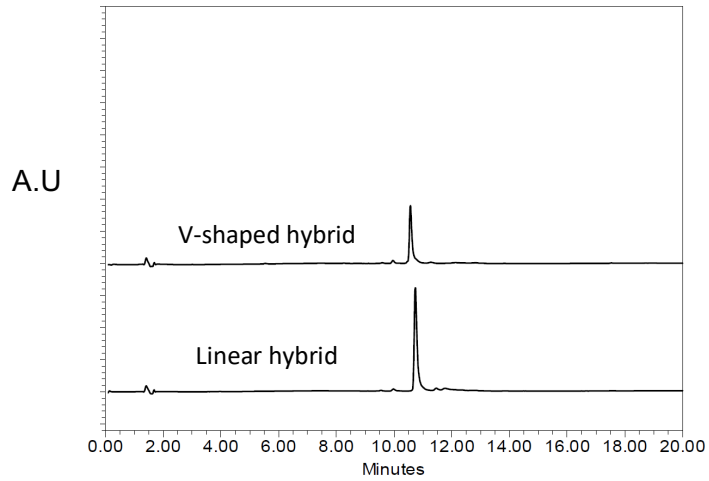

Figure S21: *HPLC overlay of hybrids based on one end-group: Linear hybrid PEG_{10kDa}-dend-Hex (Bottom) and V-shaped hybrid $(PEG_{5kDa})_2$-dend-Hex (Top). λ = 250 nm.*

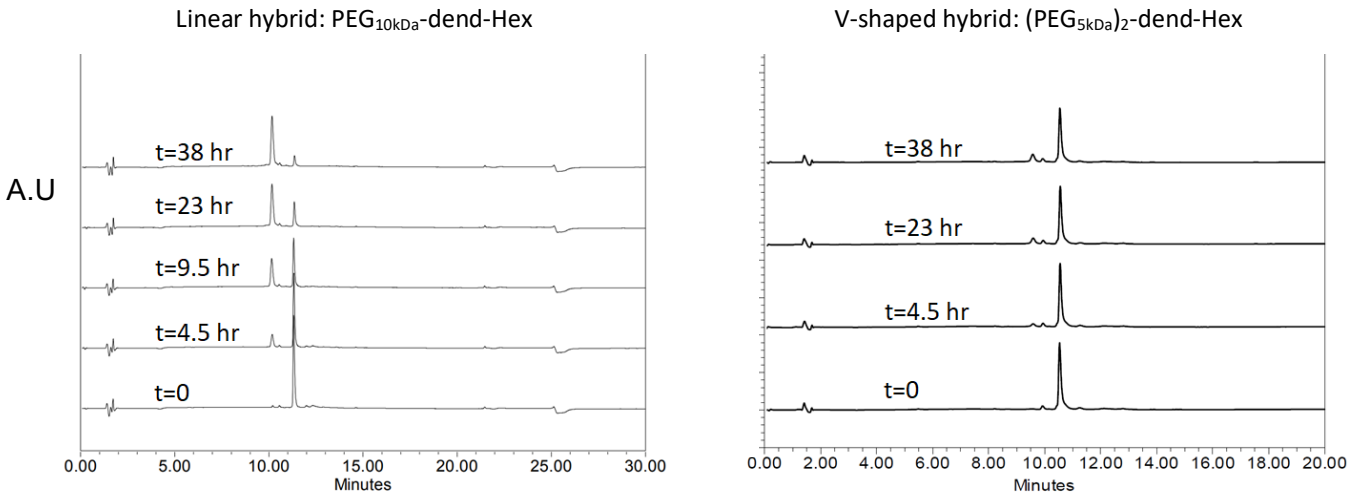

Figure S22: *HPLC overlay of hybrids based on one end-group under their CMC during incubation with PLE enzyme: (Left) Linear hybrid PEG_{10kDa}-dend-Hex 15µM in presences of PLE 54 pM; (right) V-shaped hybrid $(PEG_{5kDa})_2$-dend-Hex 15 µM in presences of PLE 54 pM; λ = 250 nm.*

References

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