Supporting Information

Tunable enzyme responses in amphiphilic nanoassemblies through alterations in unimer-aggregate equilibrium

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1. General Methods

All the reagents were from commercial source and used as received. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker DPX-400 MHz NMR spectrometer using the residual proton resonance of the solvent as the internal standard. All molecules without characterization data mentioned below were synthesized through well-established synthesis procedures previously reported by our group. [1–3] UV-vis absorption spectra were obtained by a Carry 100 Scan spectrometer. Fluorescence spectra were recorded on a PerkinElmer LS 55 spectrofluorimeter. Mass spectrometric data were collected by Capillary LC (Thermo Dionex Ultimate 3000)-ESI-MS (Bruker AmaZon quadrupole ion trap).

Dynamic Light Scattering (DLS) Study: For the DLS measurements, the sizes of each solution were recorded overtime by a Malvern Nanozetasizer ZS90 with a 637-nm laser source with non-invasive backscattering technology detected at 173^{o} using disposable sizing cuvette.

Transmission Electron Microscope (TEM) Study: The same sample for DLS measurement was dropped onto carbon-coated copper grid. The grid was dried by slow evaporation in air, and then dry separately in a vacuum overnight. Images were recorded on a JEOL-2000FX electron microscopy operated at 200 kV and at a nominal magnification of 5000X. At least 10 locations on the TEM grid were examined.

Calculation of critical aggregation concentration (CAC): A stock solution (1 mM) of oligomer micelle was prepared was diluted into various solutions of different concentrations. The concentration range of polymer was maintained from 0.2 mM to 0.001 mM. Nile Red was encapsulated to the micelle by adding 10 µL of Nile Red stock solution (20 µM in acetone). All the micelle solutions were kept uncapped overnight to evaporate the acetone. Then emission spectrum was recorded for each solution and emission maxima of each spectrum were plotted as a function of the concentration of each oligomer. The inflection point of the plot was taken as CAC of each oligomer.

DiI encapsulation: Oligomeric amphiphile solutions in phosphate buffer were stirred at room temperature and DiI stock solution (1 mg/mL in acetone, 5 wt% to oligomers) was added in each solution. The solutions were stirred for 8 h in room temperature, open to the atmosphere allowing the organic solvent to evaporate, and then filtered through hydrophilic membranes with pore size of 0.45 µm to remove unencapsulated DiI.

Guest release study: DiI-encapsulated oligomeric amphiphile solutions were treated with esterase. The absorption spectra of DiI were recorded overtime.

The % release of DiI was calculated by using the following equations:

% Release of DiI = $(I_t-I_0)/I_t*100$

Where I_0 =the highest absorbance of DiI

I_t = the highest absorbance of DiI at each time point

Aggregation number: The aggregation number of micelles were calculated by using the following equation[4,5], [η] is the intrinsic viscosity of micelle solutions, c is the micelle concentration, R_h is radius of micelle aggregates, M is the unimer molecular weight. Relative viscosity of micelle solution was measured at different concentrations by Ubbelohde viscometer and was then converted to inherent viscosity. By plotting η_{inh} vs concentration, the value of intrinsic viscosity [η] was the y-intercept. After determination of intrinsic viscosities of dimers and pentamers, calculation using the following equation provided the range of aggregation numbers 2.6*10^5~ 4.2*10^3.

$N_{ag} = 10\pi \times N_A \times R_h^3/3[\eta] \times M$.

2. Synthetic procedures

General procedures for synthesis of molecule **b**: Oligoamine (1 eq.) was dissolved in dry tetrahydrofuran (THF), trienthylamine (2 eq. for 1 amine group) was added to the solution and stirred for 15 minutes at 0°C. A solution of benzoyl chloride molecule **1a** (1.2eq for one amine group) in THF was added to the mixture dropwise and then stirred at room temperature overnight. Solvent was evaporated and then redissolved in dichloromethane, then washed with water for three times. The organic layer was dried over Na_2SO_4 and evaporated to dryness. The crude product was purified by silica gel column chromatography.

General procedures for synthesis oligomers: The mixture of oligomeric acetylene compound **b** (1.0 eq.), azide **1c** (2 eq. for 1 acetylene group), $CuSO_4 \cdot 5H_2O$ (0.5 eq.) and sodium ascorbate (0.5 eq.) in THF/H_2O (1:1) solvent mixture was heated at 50 °C for 24 h. The reaction progress was monitored by TLC. After completion of the reaction, the reaction mixture was partitioned between ethyl acetate and saturated aqueous NH_4Cl solution. The aqueous layer was extracted twice with ethyl acetate and the combined organic layer was dried over Na_2SO_4 and evaporated to dryness. The crude product was purified by silica gel column chromatography.

Characterizations for oligomers

2-EG5: Yield: 94%. ^{1}H NMR (400 MHz, $CDCl_3$, TMS): δ (ppm) 7.63 (s, 2H), 7.55 (d, *J* = 8.8 Hz, 2H), 6.99-6.94 (m, 4H), 6.59-6.55 (m, 6H), 6.17 (s, 2H), 5.80 (s, 4H), 5.15 (s, 2H), 5.01 (s, 2H), 4.27 (t, *J* = 5 Hz, 4H), 4.01-3.54 (m, 40H), 3.36 (s, 6H), 3.05 (s, 4H), 2.41-2.35 (m,10H), 1.89 (m, 4H), 1.63 (m,8H), 1.33 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 171.98, 171.36, 160.92, 159.43, 154.89, 152.37, 138.18, 125.92, 115.18, 113.18, 112.95, 105.89, 105.53, 103.38, 84.70, 71.91, 70.73, 70.56, 70.54, 70.48, 69.52, 67.63, 61.82, 59.01, 44.51, 37.92, 33.68, 29.79, 25.86, 23.90, 18.70. MALDI-ToF m/z 1618.593 ($C_{80}H_{106}N_8O_{26}$+Na^+ requires 1617.738).

3-EG5: Yield: 90%. ^{1}H NMR (400 MHz, $CDCl_3$, TMS): δ (ppm) 7.69-7.64 (m, 3H), 7.55 (d, *J* = 8.8 Hz, 3H), 6.98-6.94 (m, 6H), 6.56-6.52 (m, 9H), 6.17 (s, 3H), 5.80 (s, 6H), 5.18-4.92 (m, 6H), 4.31-4.27 (m, 6H), 4.12-3.52 (m, 62H), 3.36 (s, 9H), 3.05-2.99 (d, *J* = 13.6 Hz, 3H), 2.65 (s, 3H), 2.40-2.38 (m, 15H), 1.89 (m, 6H), 1.63 (m, 12H), 1.33 (m, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 171.97, 160.90, 160.03, 159.87, 159.40, 159.23, 154.84, 152.42, 137.67, 125.93, 115.15, 113.16, 112.88, 105.51, 103.34, 84.67, 71.86, 70.68, 70.50, 70.43, 69.47, 67.71, 61.91, 58.97, 50.33, 33.65, 29.74, 25.84, 23.87, 18.68. MALDI-ToF m/z 2414.982 ($C_{120}H_{158}N_{12}O_{39}$+$Na^+$ requires 2414.107).

4-EG5: Yield: 76%. ^{1}H NMR (400 MHz, $CDCl_3$, TMS): δ (ppm) 7.72 (m, 4H), 7.55 (d, *J* = 4.4 Hz, 4H), 7.01-6.94 (m, 8H), 6.59-6.53 (m, 12H), 6.19 (s, 4H), 5.80 (s, 8H), 5.31-5.02 (m, 8H), 4.32 (m, 8H), 4.13-3.54 (m, 80H), 3.37 (s, 12H), 3.05 (m, 4H), 2.64 (m, 3H)2.42-2.39 (m,20H), 1.89 (m, 8H), 1.71 (m, 16H), 1.33 (m, 8H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 171.99, 160.91, 159.86, 154.86, 152.41, 125.93, 115.16, 113.16, 112.90,

103.36, 103.17, 84.68, 71.88, 70.68, 70.59, 69.48, 67.59, 58.99, 50.07, 33.87, 29.80, 25.83, 23.89, 18.69. MALDI-ToF m/z 3211.185 ($C_{160}H_{210}N_{16}O_{52}$+$Na^+$ requires 3210.476).

5-EG5: Yield: 78%. ^{1}H NMR (400 MHz, $CDCl_3$, TMS): δ (ppm) 7.73 (m, 5H), 7.53 (d, *J* = 4.2 Hz, 5H), 6.97-6.94 (m, 10H), 6.52 (m, 15H), 6.16 (s, 5H), 5.80 (s, 10H), 5.29-4.95 (m, 10H), 4.29 (m, 10H), 4.13-3.54 (m, 128H), 3.37 (s, 15H), 2.96(m, 4H), 2.61 (m, 4H), 2.39-2.36 (m,25H), 1.89 (m, 10H), 1.68 (m, 20H), 1.34 (m, 10H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 171.99, 160.90, 160.05, 159.42, 154.85, 152.42, 137.73, 125.96, 115.15, 113.16, 112.89, 105.85, 103.37, 84.75, 71.88, 70.50, 69.50, 67.72, 58.99, 33.68, 29.67, 25.99, 23.89, 18.71. MALDI-ToF m/z 4007.328 ($C_{200}H_{262}N_{20}O_{65}$+$Na^+$ requires 4006.845).

P-EG5: Yield: 80%. ^{1}H NMR (400 MHz, $CDCl_3$, TMS): δ (ppm) 7.71, 7.53, 6.94, 6.45, 6.13, 5.78, 5.07, 4.91, 4.25, 3.60-3.33, 2.38, 1.83, 1.62, 1.31. ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 172.02, 160.87, 159.98, 159.44, 154.83, 152.50, 137.69, 126.04, 115.12, 113.10, 112.83, 105.15, 103.38, 84.71, 71.87, 70.46, 70.45, 62.32, 67.57, 58.97, 49.92, 33.66, 29.82, 25.83, 23.89, 18.69. THF GPC: Mw 12 kDa, PDI 1.08.

2-EG8: Yield: 92%. ^{1}H NMR (400 MHz, $CDCl_3$, TMS): δ (ppm) 7.63 (s, 2H), 7.54 (d, *J* = 4.4 Hz, 2H), 6.95 (m, 4H), 6.59-6.52 (m, 6H), 6.17 (s, 2H), 5.80 (s, 4H), 5.15 (s, 2H), 5.01 (s, 2H), 4.28 (t, J = 3.6 Hz, 4H), 4.11-3.53 (m, 60H), 3.36 (s, 6H), 3.05 (s, 3H), 2.71(s, 2H), 2.41-2.37 (m,10H), 1.89 (m, 4H), 1.69 (m, 8H), 1.33 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 171.98, 160.92, 159.91, 159.42, 154.87, 152.39, 138.16, 125.92, 123.11, 115.17, 113.17, 112.92, 105.86, 105.51, 103.36, 103.21, 84.67, 71.90, 70.70, 70.52, 70.46, 69. 50, 67.60, 61.96, 59.01, 49.99, 33.67, 29.84, 29.68, 25.80, 23.89, 18.69. MALDI-ToF m/z 1882. 356 ($C_{92}H_{130}N_8O_{32}$+Na^+ requires 1881.879).

3-EG8: Yield:69%. ^{1}H NMR (400 MHz, $CDCl_3$, TMS):δ (ppm) 7.69-7.67 (m, 3H), 7.55 (d, *J* = 4.4 Hz, 3H), 6.98-6.95 (m, 6H), 6.56-6.51 (m, 9H), 6.17 (s, 3H), 5.80 (s, 6H), 5.18-4.92 (m, 6H), 4.29 (m, 6H), 4.12-3.52 (m, 98H), 3.36 (s, 9H), 3.05-2.99 (d, J = 13.2 Hz, 3H), 2.65 (s, 3H), 2.40-2.35 (m, 15H), 1.89 (m, 6H), 1.63 (m, 12H), 1.34 (m, 6H).^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 171.96, 160.90, 160.04, 159.40, 154.84, 152.41, 137.91, 125.93, 115.15, 113.16, 112.88, 105.86, 103.34, 84.67, 71.87, 70.68, 70.49, 69.48, 67.69, 61.86, 58.98, 50.23, 33.65, 29.76, 25.82, 23.87, 18.68. MALDI-ToF m/z 2810.593 ($C_{138}H_{194}N_{12}O_{48}$+$Na^+$ requires 2810.342).

4-EG8: Yield: 77%. ^{1}H NMR (400 MHz, $CDCl_3$, TMS): δ (ppm) 7.69 (m, 4H), 7.53-7.51 (d, *J* = 4.2 Hz, 4H), 6.96-6.93 (m, 8H), 6.53-6.50 (m, 12H), 6.15 (s, 4H), 5.79 (s, 8H), 5.01-4.95 (m, 8H), 4.27 (m, 8H), 4.13-3.54 (m, 120H), 3.36 (s, 12H), 2.97 (s, 3H), 2.71-2.58 (m, 3H), 2.39-2.34 (m, 20H), 1.89 (m, 8H), 1.65 (m, 16H), 1.33 (m, 8H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 172.07, 160.86, 160.05, 159.86, 154.85, 152.40, 137.67, 125.95, 115.14, 113.14, 112.88, 105.48, 103.35, 84.69, 71.88, 70.68, 70.50, 69.50, 67.71, 59.00, 53.51, 46.06, 33.91, 29.66, 26.06, 25.88, 18.69. MALDI-ToF m/z 3738.234 ($C_{184}H_{258}N_{16}O_{64}$+$Na^+$ requires 3738.788).

5-EG8: Yield: 82%. ^{1}H NMR (400 MHz, $CDCl_3$, TMS): δ (ppm) 7.73 (m, 5H), 7.55-7.53 (d, *J* = 4.2 Hz, 5H), 7.00-6.95 (m, 10H), 6.51 (m, 15H), 6.16 (s, 5H), 5.80 (s, 10H), 5.19-4.96 (m, 10H), 4.29 (m, 10H), 4.08-3.51 (m, 166H), 3.36 (s, 15H), 2.96(m, 3H), 2.61 (m, 3H), 2.40 (m,25H), 1.88 (m, 10H), 1.65 (m, 20H), 1.31 (m, 10H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 172.01, 160.92, 160.07, 159.44, 154.87, 152.43, 125.97, 115.17, 113.18, 112.91, 106.20, 103.39, 84.75, 71.90, 70.52, 69.52, 67.61, 59.02, 33.70, 29.69, 25.95, 23.91, 18.73. MALDI-ToF m/z 4667.821. ($C_{230}H_{322}N_{20}O_{80}$+$Na^+$ requires 4667.235).

3. Supplementary figures

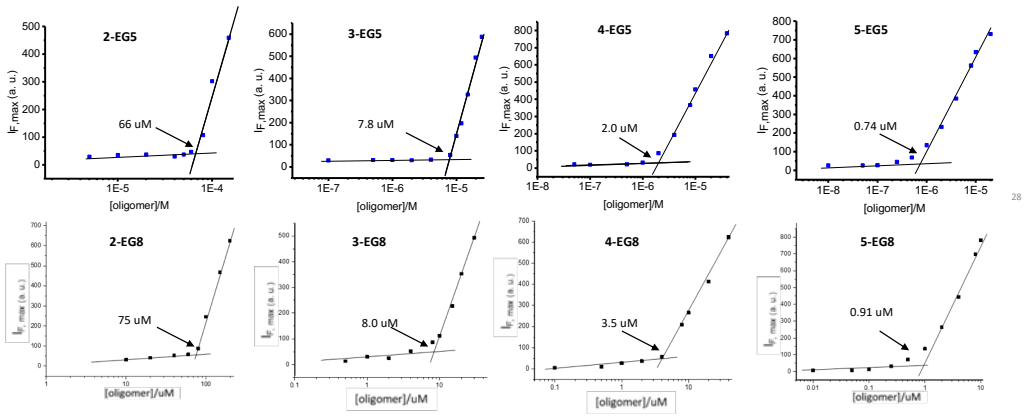

Figure S1: Critical aggregation concentration (CAC) of oligomeric assemblies.

Table S1: Critical aggregation concentration (CAC, mg/mL) of oligomeric assemblies.

Oligomer	CAC (*mg/mL*)	Oligomer	CAC (*mg/mL*)
2-EG5	1.05	2-EG8	1.39
3-EG5	0.18	3-EG8	0.23
4-EG5	0.064	4-EG8	0.14
5-EG5	0.029	5-EG8	0.044

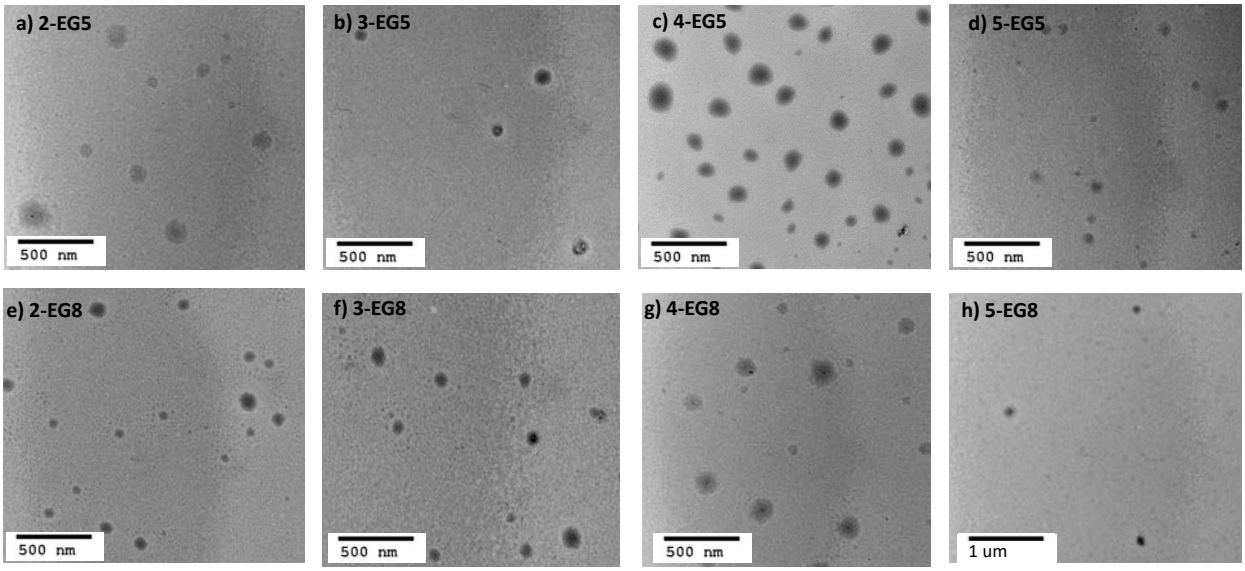

Figure S2: TEM images of oligomeric assemblies.

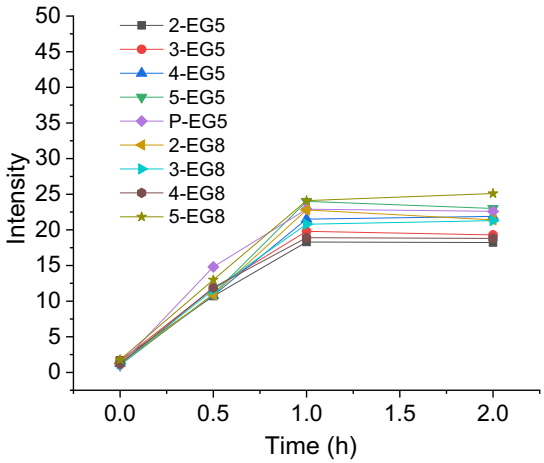

Figure S3. Enzymatic cleavage of covalently attached guest molecules from oligomer amphiphiles with concentration below their CAC. (1.25 uM dimer, 0.83 uM trimer, 0.625 uM for tetramer, 0.50 uM for pentamer and 0.18 uM P-EG5, treated with 0.88 nM esterase)

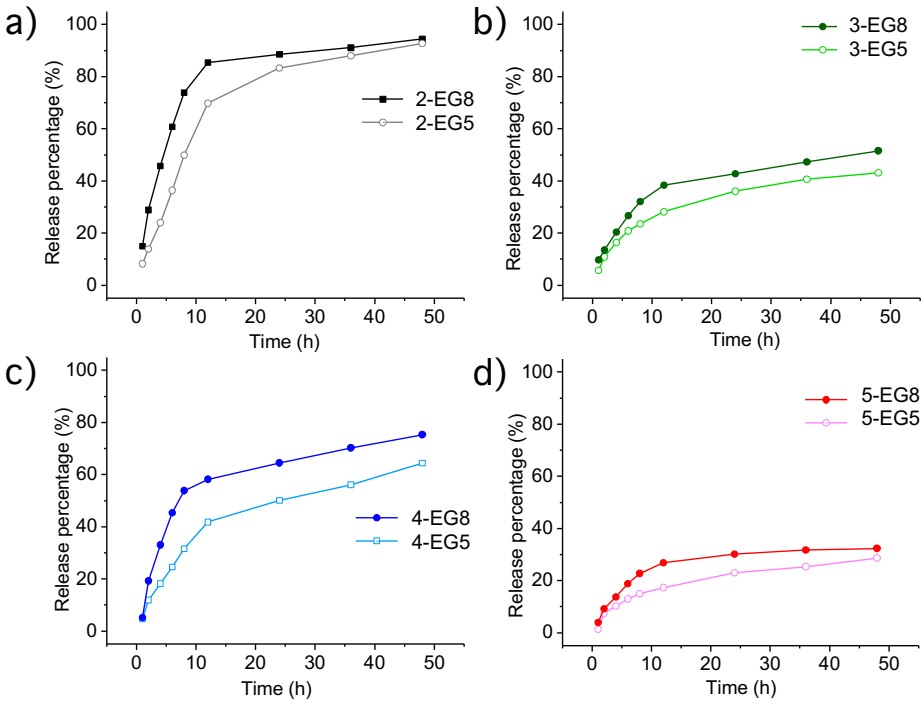

Figure S4: Comparison of non-covalent guest release kinetics between oligomer-PEG and oligomer-OEG.

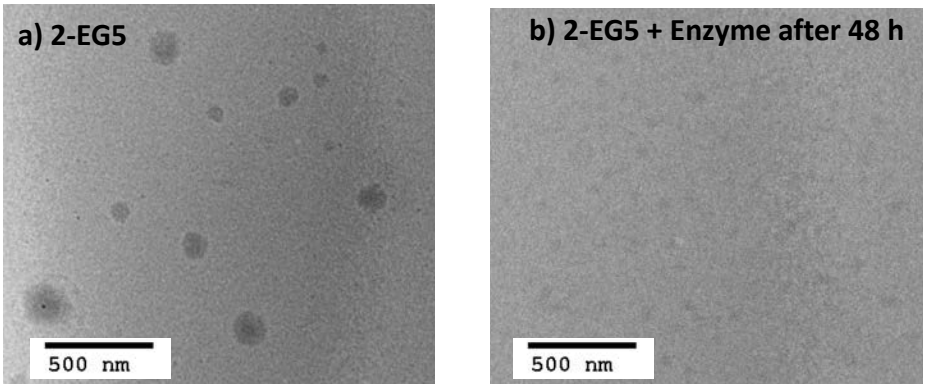

Figure S5: One example of assembly morphological changes in presence of enzyme.

Reference

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