

Fine structural tuning of the assembly of elastin-collagen-peptide conjugates with drug loading and tuning of molecular interactions

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1. Solid-phase peptide synthesis

All peptides were synthesized via solid-phase peptide synthesis methods (SPPS) as described in the main content. 4-azidobutanoic acid was introduced to the N terminus of the CLP via amidation reaction. An alkyne group from propargyl glycine was attached to the C-terminus of the ELP domain via SPPS. The facile conjugation of ELP and CLP via CuAAC “click” reaction was conducted as described in the main content (Fig. 1). The molecular weight of CLP, ELPs & ELP-CLP conjugates was verified via electrospray ionization mass spectrometry (ESI-MS) After purification with reverse-phase HPLC, conjugates with purity greater than 95% were obtained.

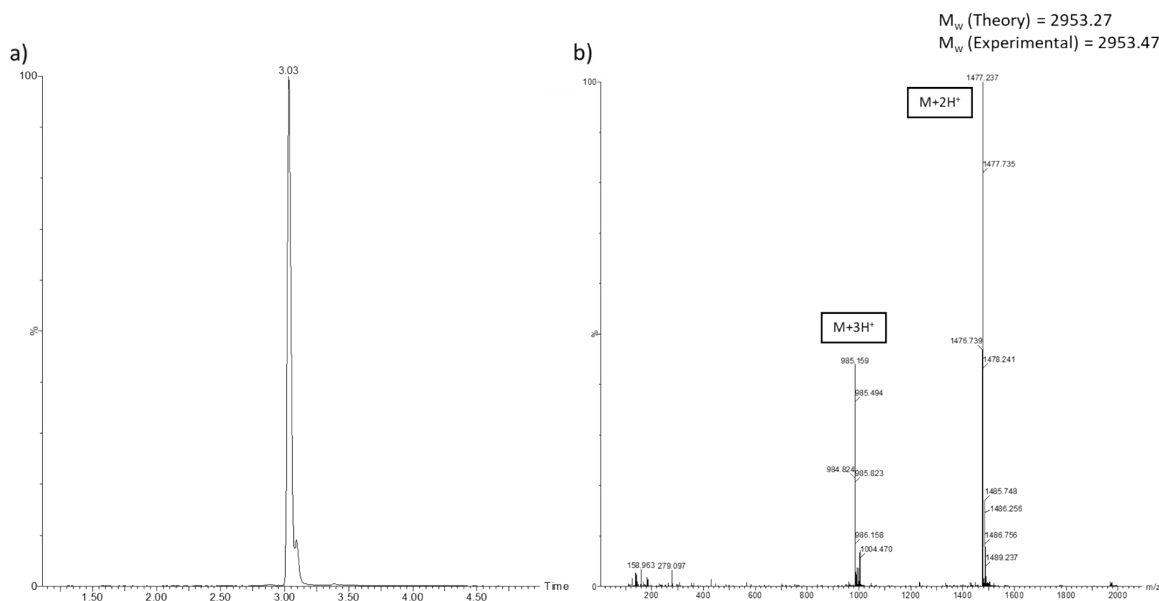


Figure S1. LC-MS of the purified Y₆: a) UPLC trace; b) ESI-MS spectrum.

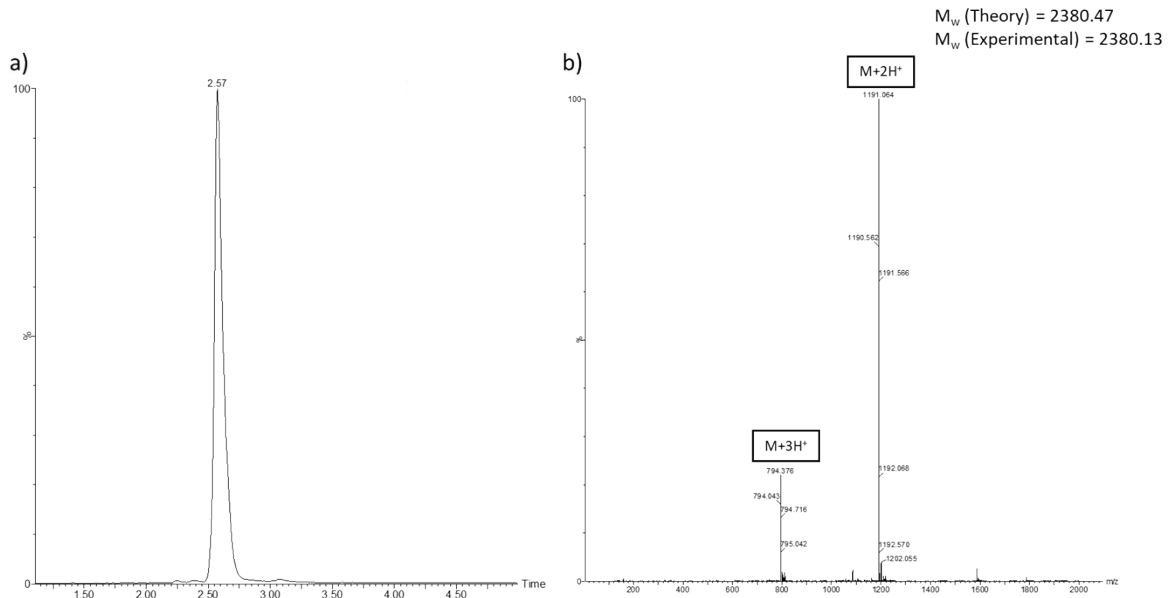


Figure S2. LC-MS of the purified G_8 : a) UPLC trace; b) ESI-MS spectrum.

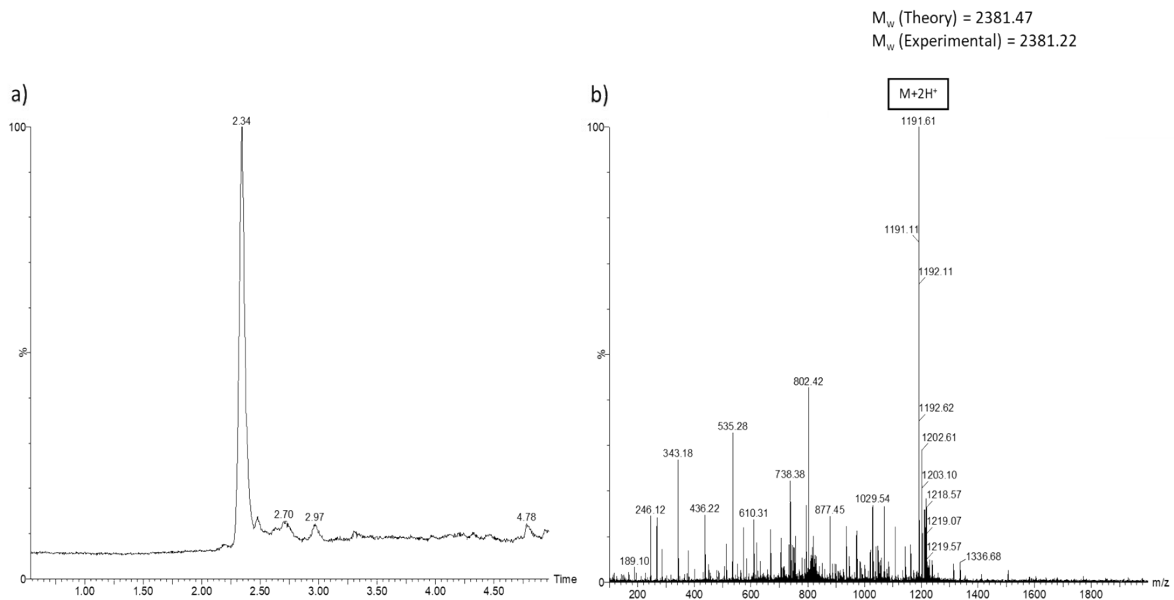


Figure S3. LC-MS of the purified G_8 -COOH: a) UPLC trace; b) ESI-MS spectrum.

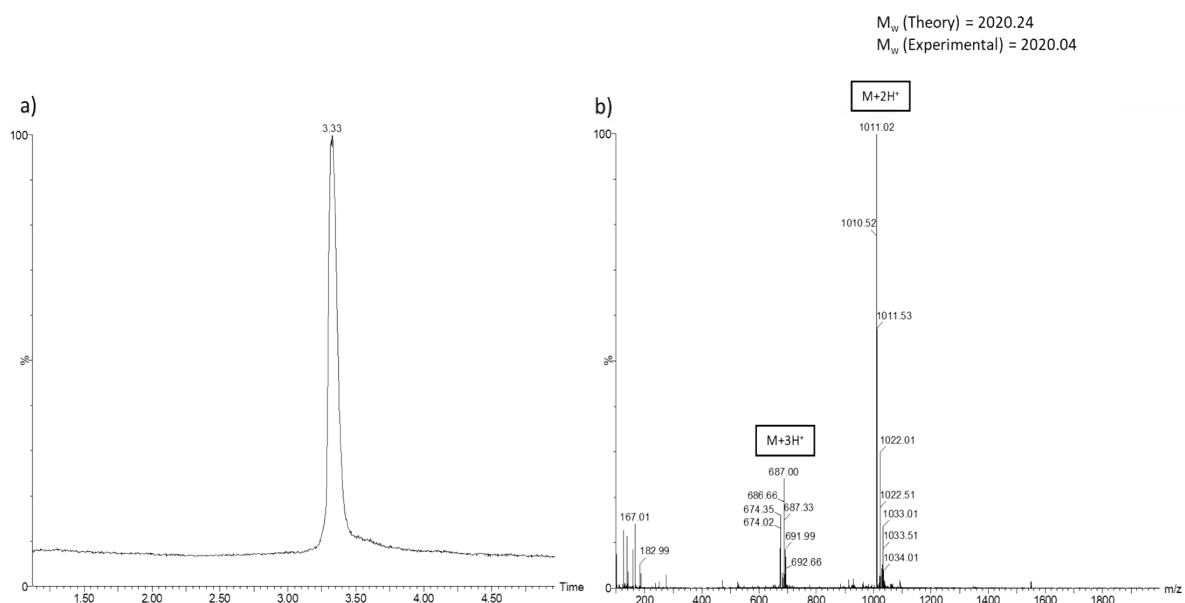


Figure S4. LC-MS of the purified W_2F_2 : (a) UPLC trace; (b) ESI-MS spectrum.

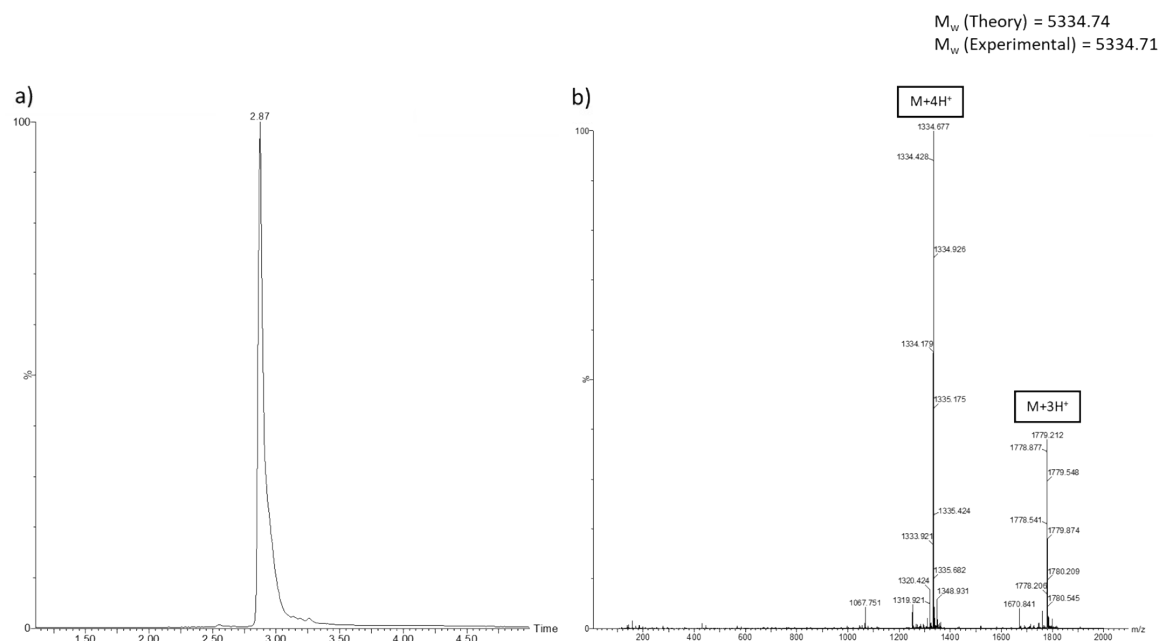


Figure S5. LC-MS of the purified Y_6-G_8-COOH : (a) UPLC trace; (b) ESI-MS spectrum.

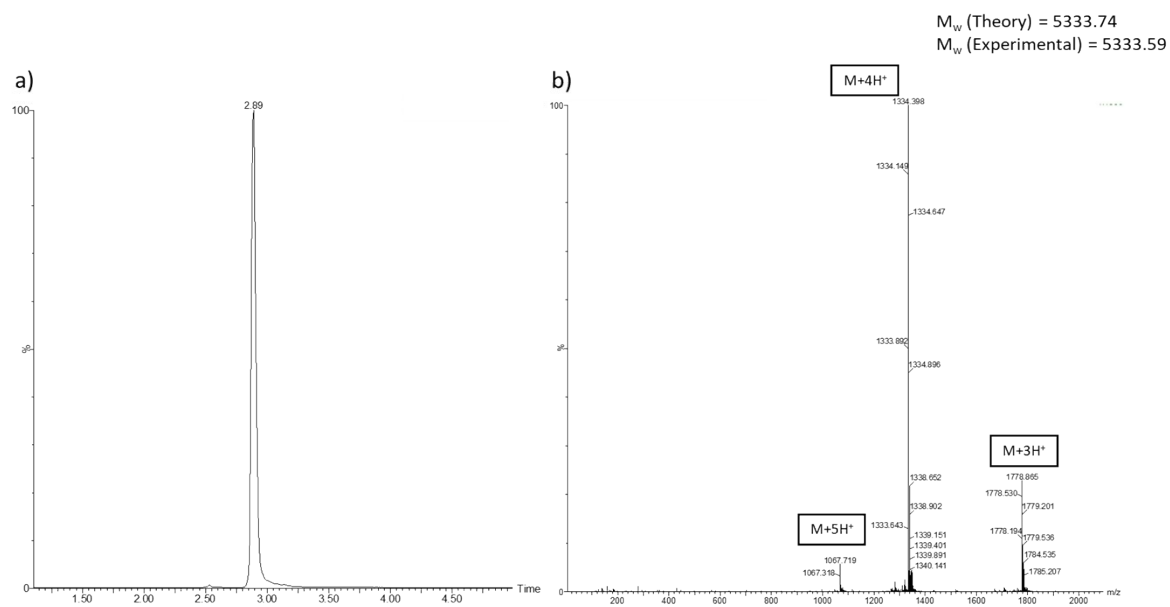


Figure S6. LC-MS of the purified Y_6-G_8 : a) UPLC trace; b) ESI-MS spectrum.

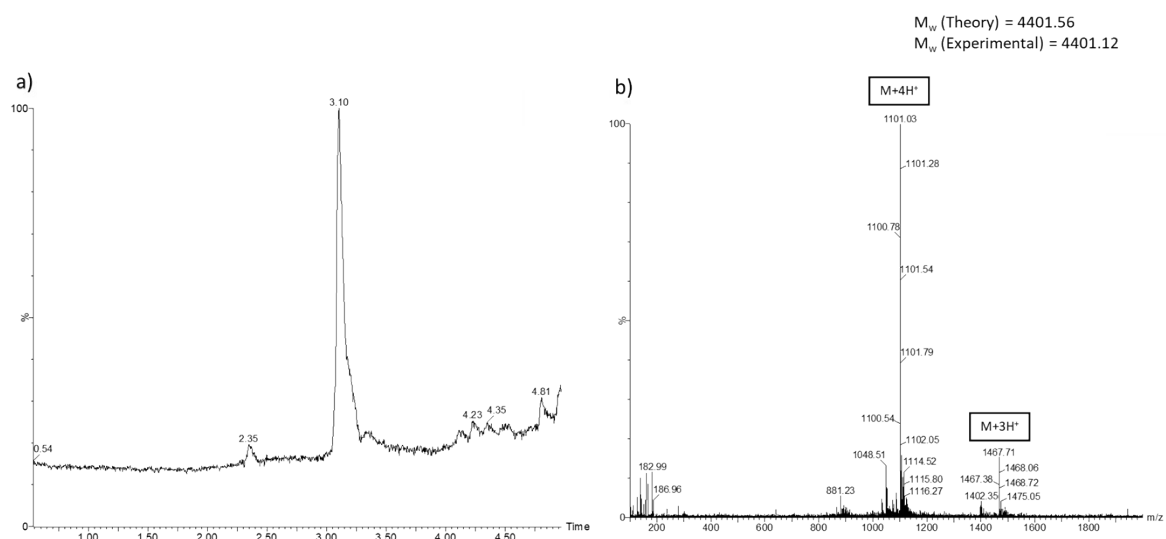


Figure S7. LC-MS of the purified $W_2F_2-G_8$: (a) UPLC trace; (b) ESI-MS spectrum.

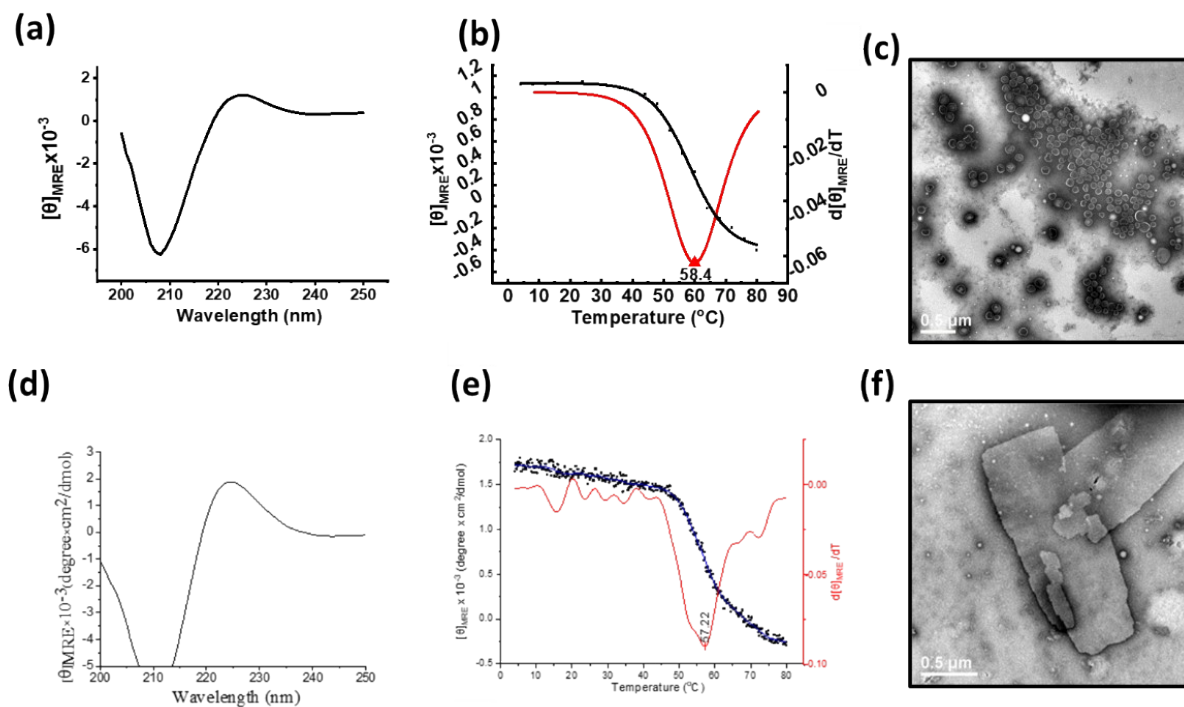


Figure S8. CD spectra and TEM measurements for $W_2F_2-G_8$ and Y_6-G_8 . CD spectra showing representative full-wavelength scans for the (a) Y_6-G_8 ; (d) $W_2F_2-G_8$; CD spectroscopy data at 224nm for the thermal unfolding profile for the (b) Y_6-G_8 ; (e) $W_2F_2-G_8$, the first derivative of the unfolding curve with respect to temperature is shown in red; TEM images of nanostructures self-assembled from water solutions of (c) Y_6-G_8 ; (f) $W_2F_2-G_8$ at 37 °C after staining with 2% PTA.

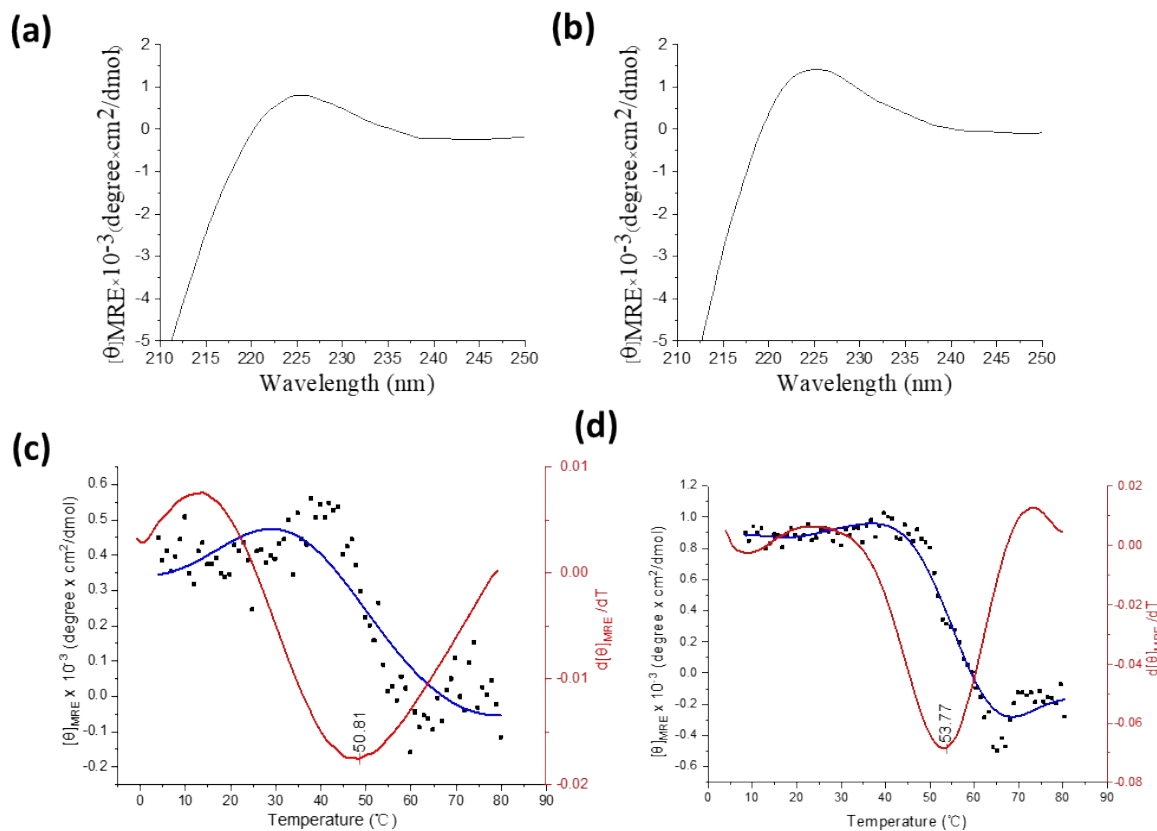


Figure S9. CD spectra showing the wavelength scans for a) Dex-CF/W₂F₂-G₈ b) Dex-CF+W₂F₂-G₈ suggest folded triple helical structure. Thermal unfolding profile for c) Dex-CF/W₂F₂-G₈ d) Dex-CF+W₂F₂-G₈. Samples were mixed in solution at a 1:10 mass ratio of Dex to peptide.

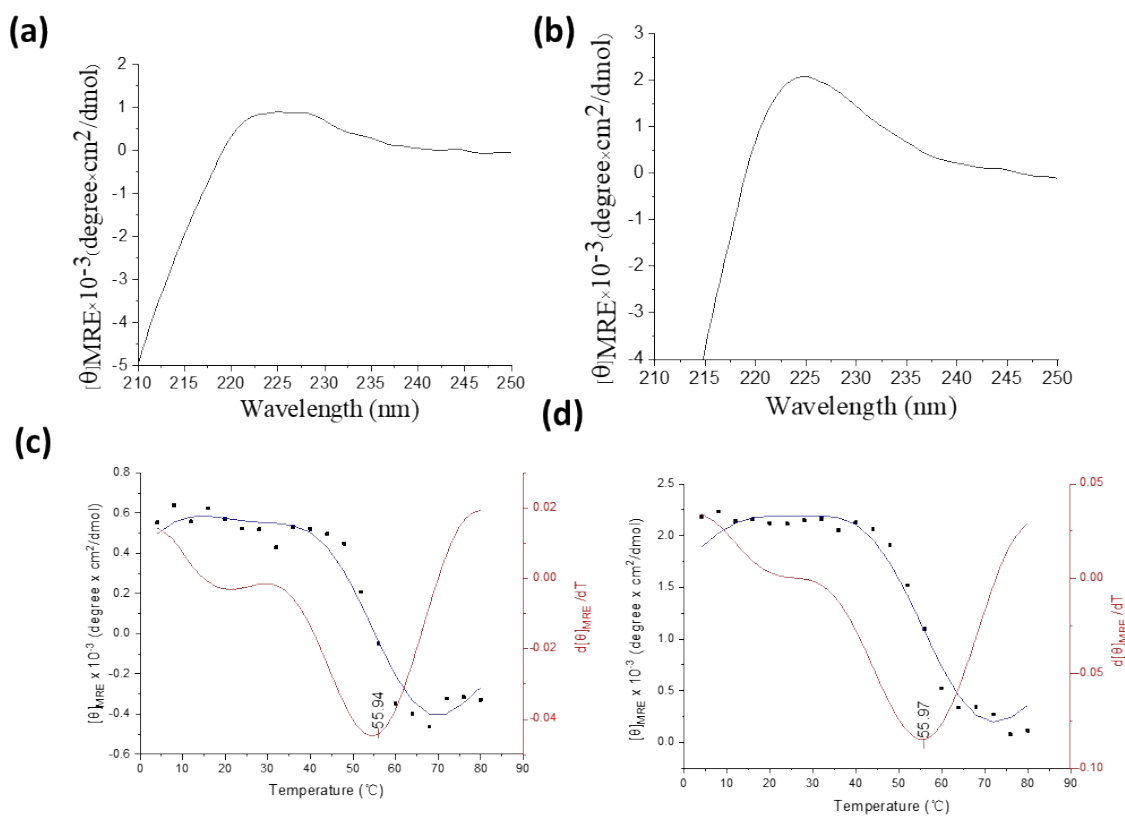


Figure S10. CD spectra showing the wavelength scans for a) Dex-CF/W₂F₂-G₈ b) Dex-CF+W₂F₂-G₈ suggest folded triple helical structure. Thermal unfolding profile for c) Dex-CF/W₂F₂-G₈ d) Dex-CF+W₂F₂-G₈. Samples were mixed in solution at a 1:6 mass ratio of Dex to peptide.

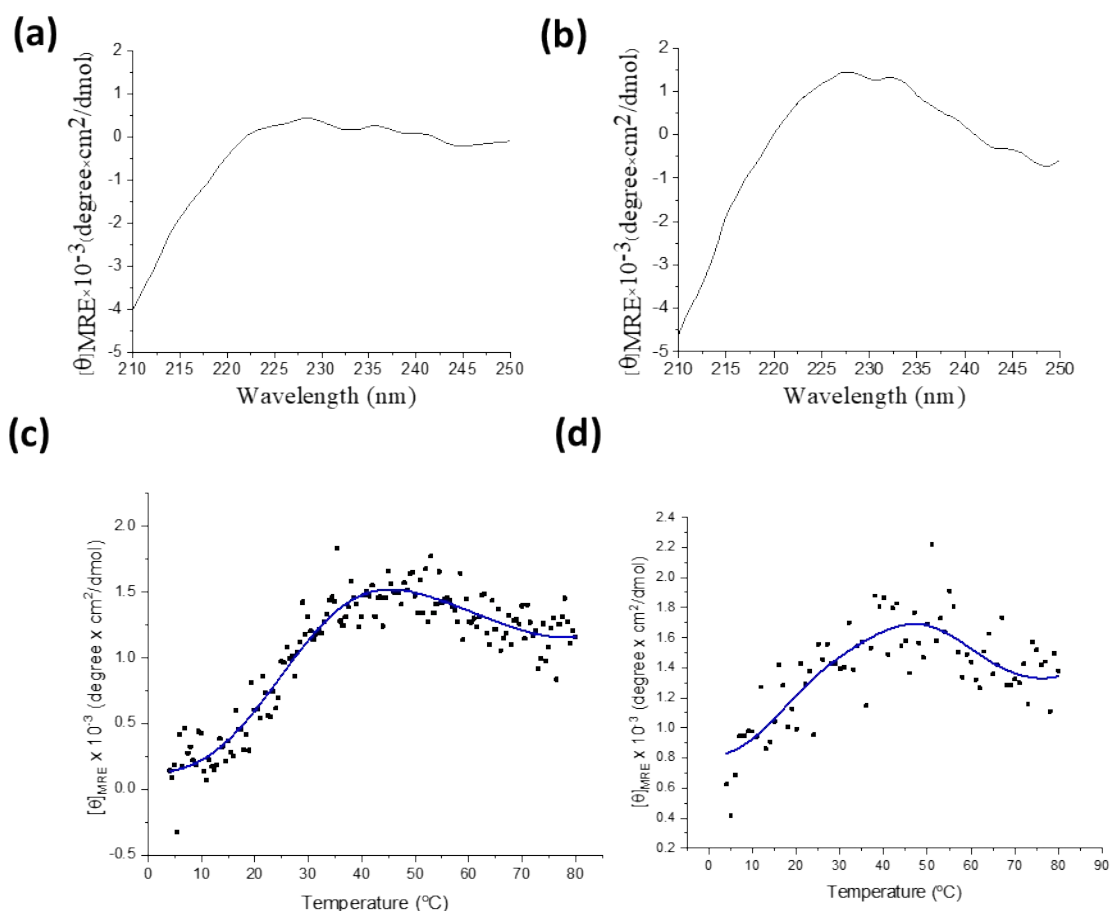


Figure S11. CD spectra showing the wavelength scans for a) Dex-CF/W₂F₂-G₈ b) Dex-CF+W₂F₂-G₈ suggest folded triple helical structure. Thermal unfolding profile for c) Dex-CF/W₂F₂-G₈ d) Dex-CF+W₂F₂-G₈. Samples were mixed in solution at a 1:1 mass ratio of Dex to peptide.

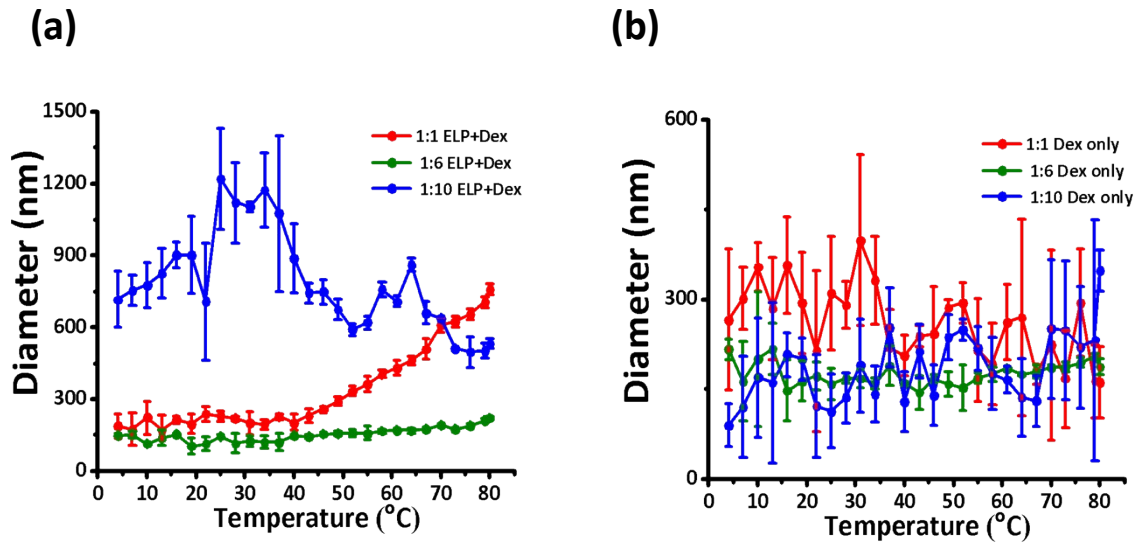


Figure S12. Dynamic light scattering results indicating the hydrodynamic diameters of nanostructures as a function of temperature upon heating. a) Dex-CF + ELP peptides mixed in mass ratio of 1:1 (0.25 mg Dex-CF), 1:6 (0.042 mg Dex-CF) and 1:10 (0.025 mg Dex-CF.); b) Drug only samples with Dex-CF concentrations equivalent to the Dex-CF:ELP samples; 1:1 (0.25 mg Dex-CF), 1:6 (0.042 mg Dex-CF) and 1:10 (0.025 mg Dex-CF).

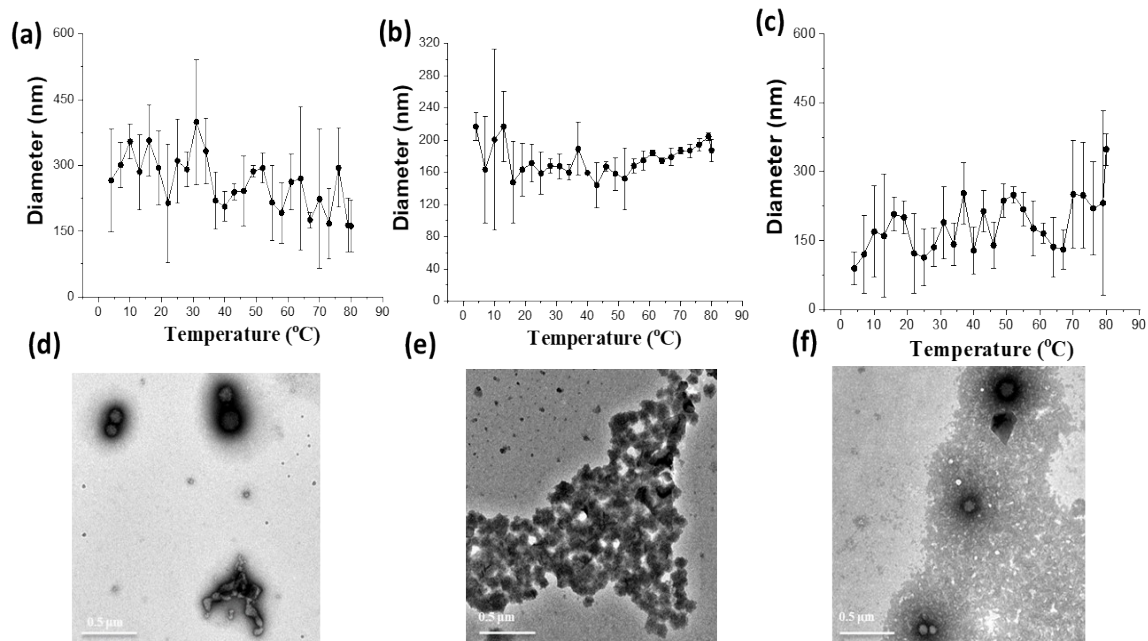


Figure S13. DLS measurements and TEM for Dex-CF-CF-only controls. a) D_h of Dex-CF only as a function of temperature upon heating (0.25 mg Dex-CF in 0.5 mL DI H₂O). b) Hydrodynamic diameters of Dex-CF only as a function of temperature upon heating (0.042 mg Dex-CF in 0.5 mL DI H₂O). c) Hydrodynamic diameters of Dex-CF only as a function of temperature upon heating (0.025 mg Dex-CF in 0.5 mL DI H₂O). d) TEM image for Dex-CF only solution (0.25 mg Dex-CF in 0.5 mL DI H₂O) after staining with 2% PTA at 37 °C. e) TEM image for Dex-CF only solution (0.042 mg Dex-CF in 0.5 mL DI H₂O) after staining with 2% PTA at 37 °C. f) TEM image for Dex-CF only solution (0.025 mg Dex-CF in 0.5 mL DI H₂O) after staining with 2% PTA at 37 °C.

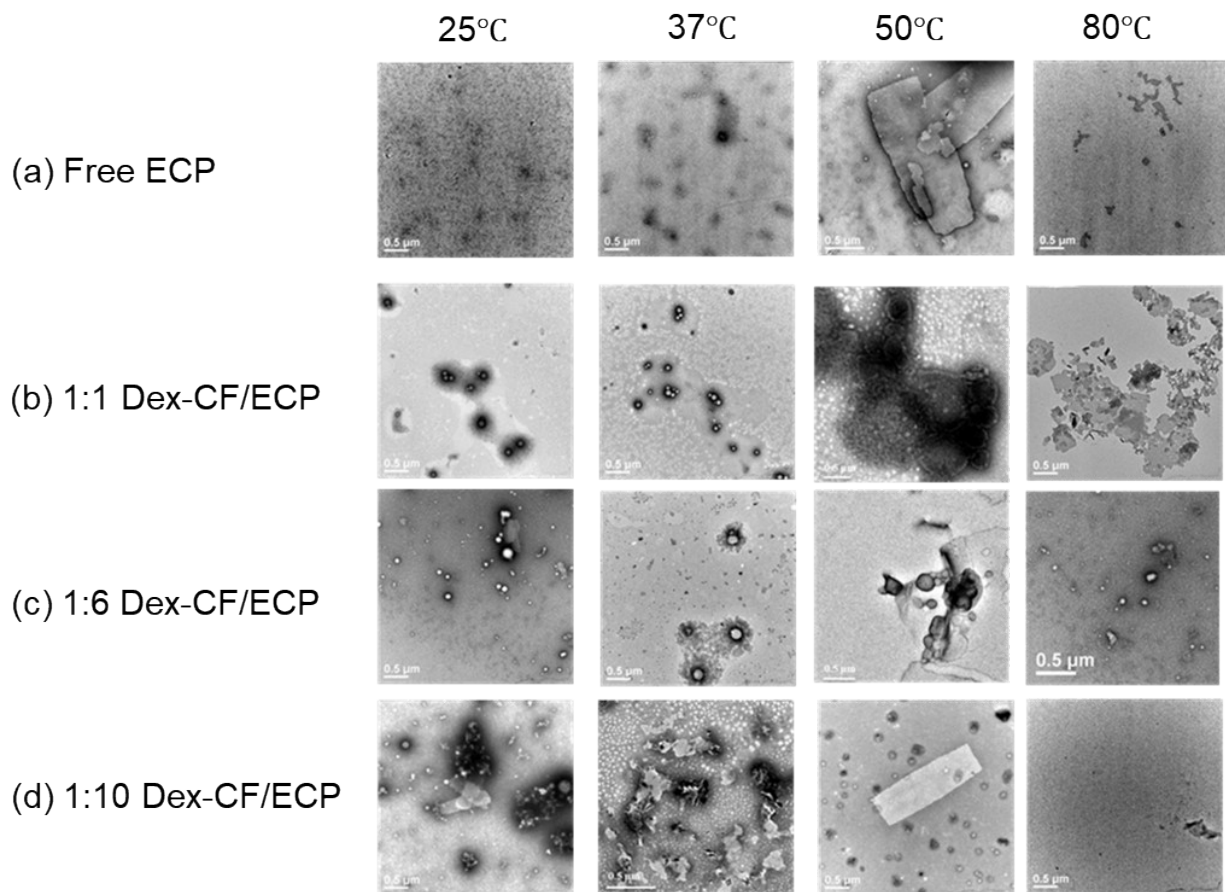


Figure S14. TEM images of nanostructures self-assembled from water solutions of Dex-CF/W₂F₂-G₈ samples at various temperatures, after staining with 2% PTA. a) W₂F₂-G₈ bioconjugates without drug loading. b) Dex-CF/W₂F₂-G₈ samples mixed in solution at a 1:1 mass ratio of Dex to peptide. c) Dex-CF/W₂F₂-G₈ samples mixed in solution at a 1:6 mass ratio of Dex to peptide. d) Dex-CF/W₂F₂-G₈ samples mixed in solution at a 1:10 mass ratio of Dex to peptide.

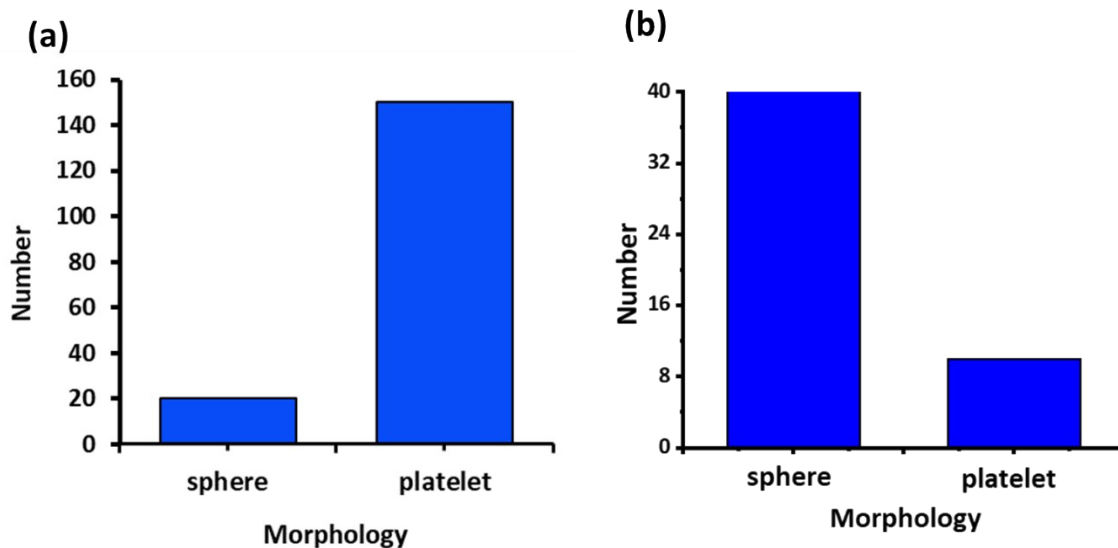


Figure S15. Quantified morphology distribution based on analysis of TEM images. (a) Y_6-G_8 at pH 7.0 with a total of 170 measurements of ECnVs taken from three grids; (b) 1:10 Dex-CF/ECnV after washing at 37°C with a total of 50 measurements of ECnVs taken from one grid.

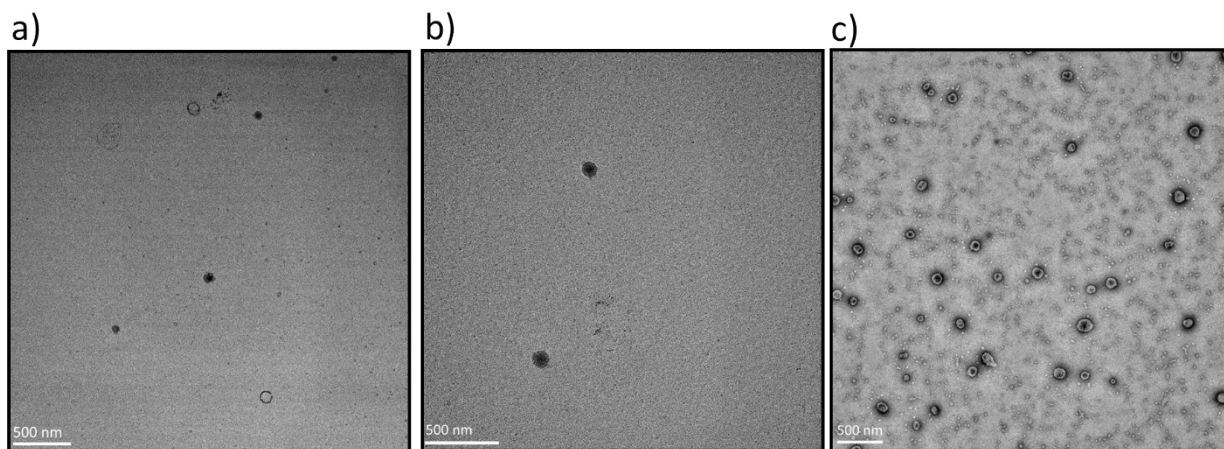


Figure S16. Transmission electron microscopy images for F-containing ECnPs with various compositions and at various pH conditions at 37 °C. a) F_6-G_8 at pH 5.0; b) F_6-G_8 at pH 7.0; c) F_6-G_8 at pH 9.0. Scale bars = 500 nm.

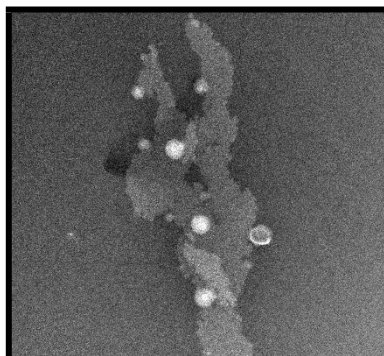


Figure S17. TEM images of F₂Y₄-G₈ at room temperature showing a mixture of morphologies of vesicles and platelets. Stained with 1% PTA,

2. The calculation of the concentration of negatively charged Y₆-G₈

Based on the K_a value of the phenol side chain of Y (3.16e⁻¹¹), the relative ratio between the negatively charged and the neutral Y is calculated based on the Henderson–Hasselbalch equation:

$$\frac{Y^-}{Y} = 10^{pH} \times 3.16 \times 10^{-11} \quad (1)$$

where ‘Y⁻’ represents negatively charged Y, ‘Y’ represents none charged Y, and pH represents the pH of the solution in which the ECnV is dissolved.

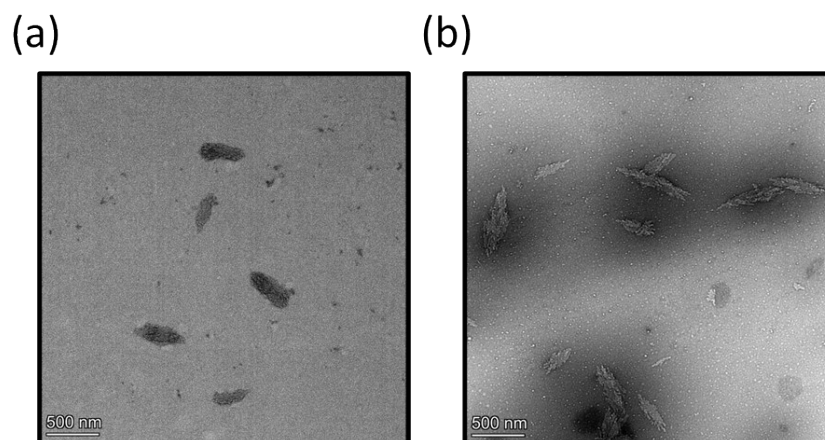


Figure S18. TEM images of Y₆-G₈ at room temperature showing the reversibility of the ECP morphologies at different pH values. a) pH=7.0; b) pH=9.0 to pH 5.0. Stained with 1% PTA, scale bars = 500 nm.

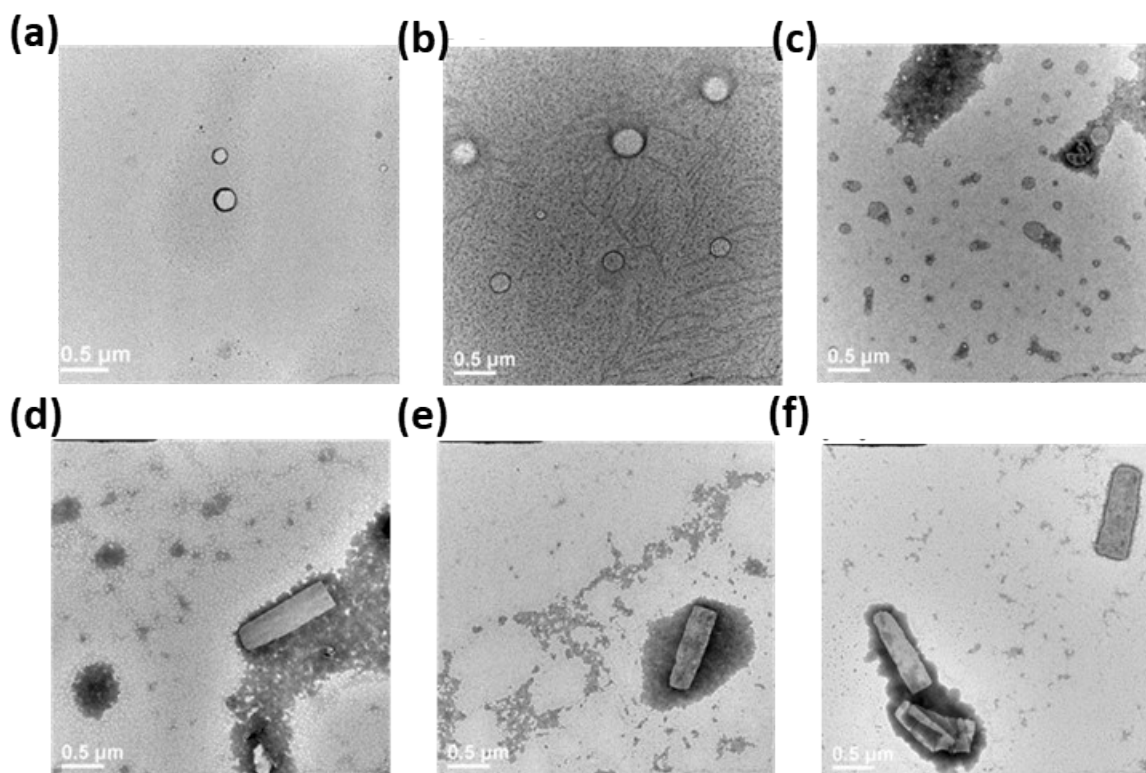


Figure S19. Transmission electron microscopy images of the 1:1 Dex-CF/W₂F₂-G₈ at various stages of drug release. 1% PTA aqueous solution (pH adjusted to 7.0) was used as a negative stain. (a) Dex-CF/ W₂F₂-G₈ samples after drug release at 1 day at 37 °C; (b) at 2 days; (c) at 3 days; (d) at 5 days; (e) at 6 days; and (f) at 7 days (all timepoints at 37 °C). Scale bars = 500 nm.

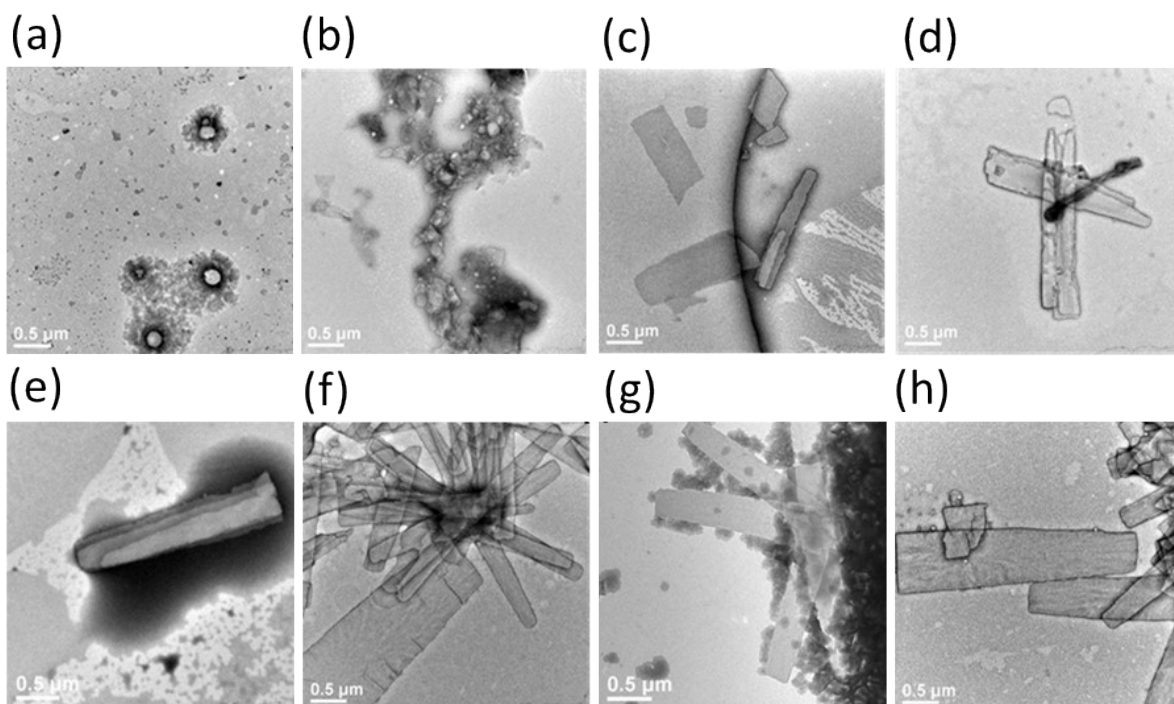


Figure S20. Transmission electron microscopy images of the 1:6 Dex-CF/W₂F₂-G₈ with drug release. 1% PTA aqueous solution (pH adjusted to 7.0) was used as a negative stain. Dex-CF/W₂F₂-G₈ samples after drug release (a) at 0 days; (b) at 1 days; (c) at 2 days; (d) at 3 days; (e) at 4 days; (f) at 5 days; (g) at 6 days; and (h) at 7 days (all timepoints at 37 °C). Scale bars = 500 nm.

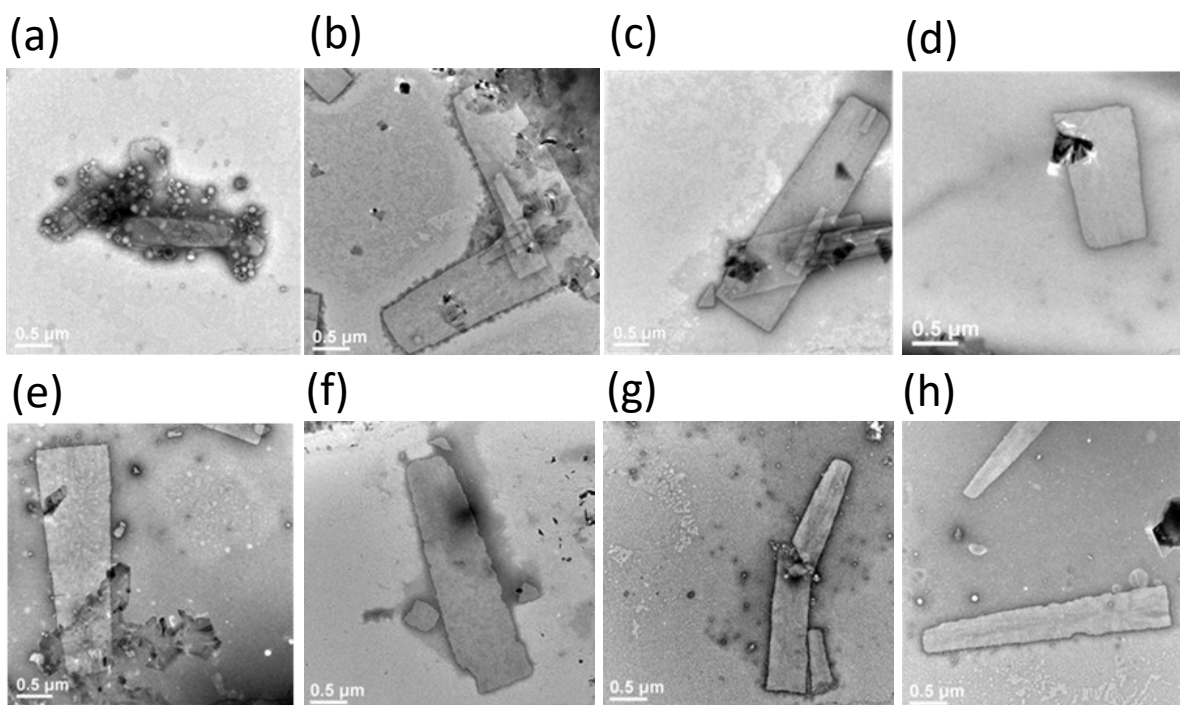


Figure S21. Transmission electron microscopy images of the 1:10 Dex-CF/W₂F₂-G₈ with drug release. 1% PTA aqueous solution (pH adjusted to 7.0) was used as a negative stain. Dex-CF/W₂F₂-G₈ samples after drug release (a) at 0 days; (b) at 1 days; (c) at 2 days; (d) at 3 days; (e) at 4 days; (f) at 5 days; (g) at 6 days; and (h) at 7 days (all timepoints at 37 °C). Scale bars = 500 nm.

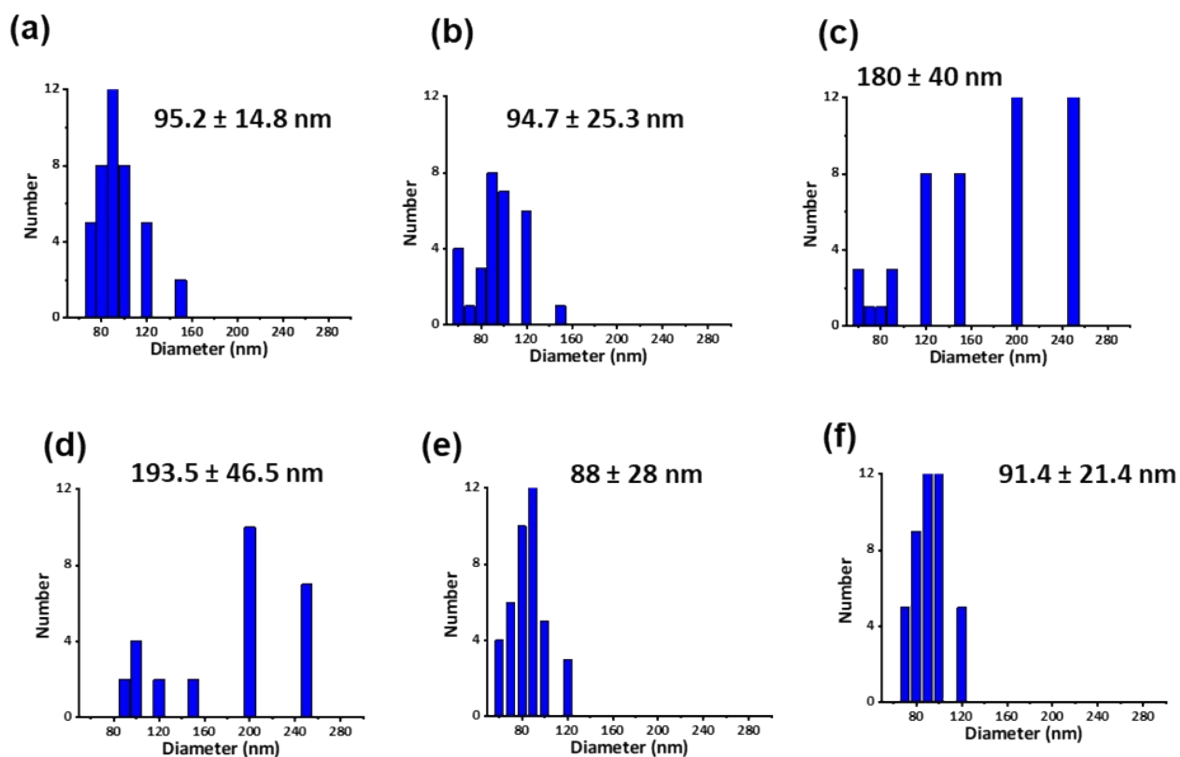


Figure S22. Vesicle diameter measurements for various Dex-CF/W₂F₂-G₈ samples at 37°C. a) 1:1 mass ratio of Dex to peptide at day 0, b) 1:1 mass ratio at day 1, c) 1:1 mass ratio at day 2, d) 1:1 mass ratio at day 3, e) 1:6 mass ratio of Dex to peptide at day 0. (f) 1:10 mass ratio of Dex to peptide at day 0. The average thickness was determined from the TEM images analyzed. The average diameters measured in the TEM images were calculated from 40 measurements of vesicles from one grid.

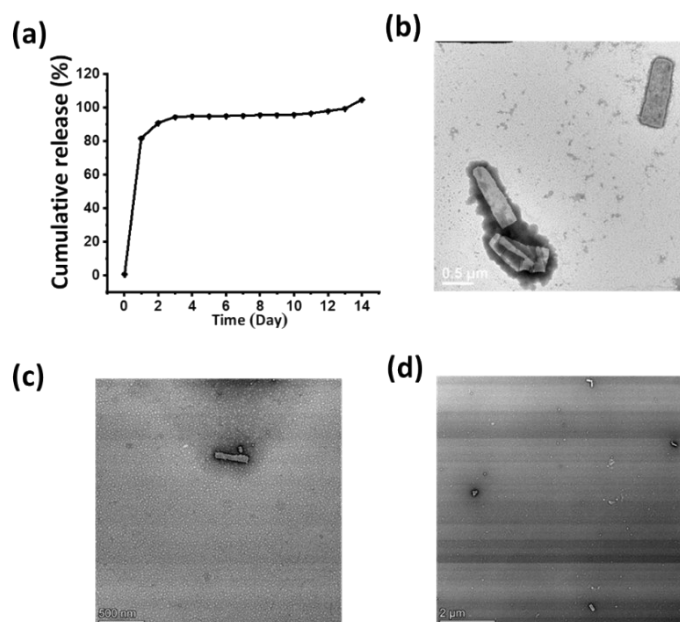


Figure S23. Morphological transition of 1:1 Dex-CF/W₂F₂-G₈ measured by TEM over the 14-day release process. Stained with 1% PTA Scale bars = 500 nm. (a) Release curve (cumulative percentage release (a) at 37 °C over 14 days; (b) Dex-CF/W₂F₂-G₈ after washing at 7 days; (c) at 11 days; (d) at 14 days.

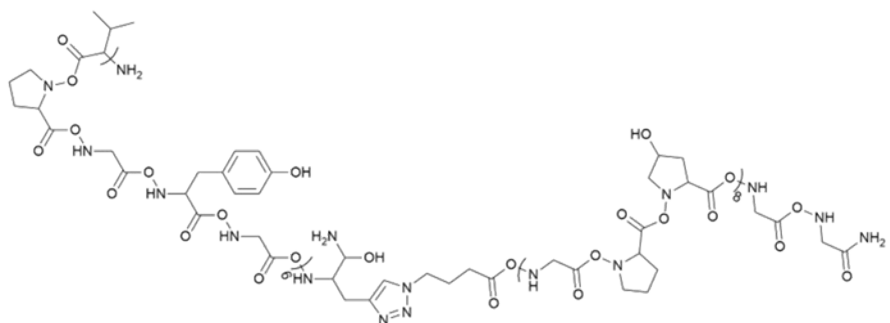


Figure S24 Y₆-G₈ chemical scheme

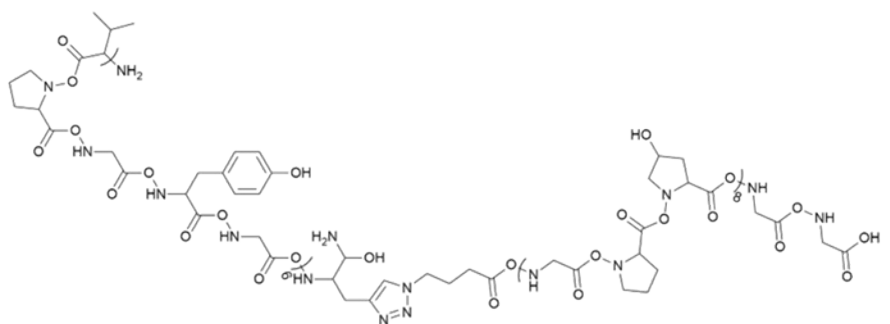


Figure S25 Y₆-G₈-COOH chemical scheme

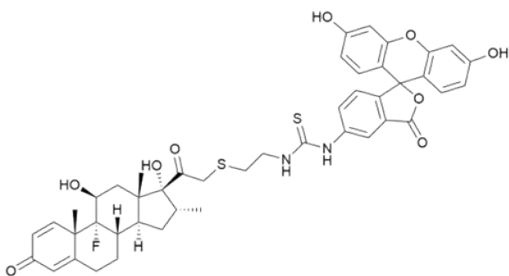


Figure S26 Dex-CF chemical scheme

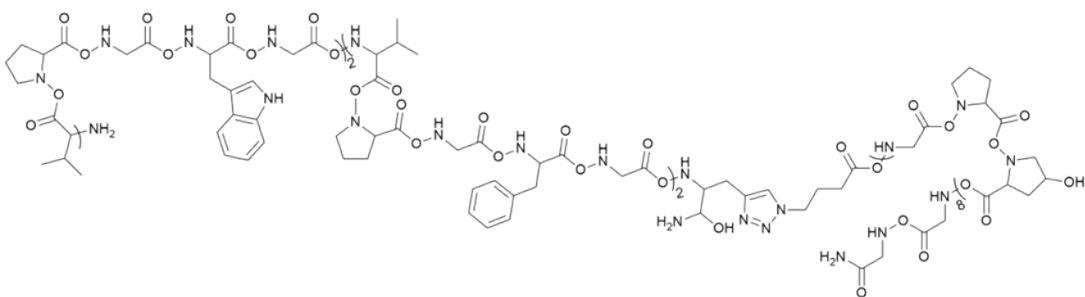


Figure S27 W₂F₂-G₈ chemical scheme