Supporting Information

Glutathione-adaptive peptide amphiphile vesicles rationally designed using positionable disulfide-bridges for effective drug transport

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1. Techniques and Instruments

Peptide amphiphiles (PAs) were sequenced using CEM Focused Microwave™ Synthesis System, Discover (CEM corporation, Matthews, USA) and purified using YL9100 high pressure liquid chromatography (HPLC, Young Lin Instrument Co., Ltd., Anyang, Republic of Korea) equipped with a C18 reverse phase chromatographic column. Mass spectrometry was performed on a Bruker Ultraflextreme matrix-assisted laser desorption/ionization time-of-flight/time-of-flight (MALDI-TOF/TOF) mass spectrometer (Bruker Daltonik GmbH, Bremen, Germany) using a matrix, α-cyano-4-hydroxycinnamic acid dissolved in acetonitrile:water = 1:1 mixed solution (0.1% trifluoroacetic acid). Ultraviolet-visible (UV-vis) absorption spectra were recorded from a UH-5300 UV-visble spectrometer (Hitachi High Technologies Corporation, Tokyo, Japan). Fluorescence spectra were obtained from FS-2 fluorescence spectrometer (Scinco, Seoul, Republic of Korea) and F-7000 fluorescence spectrophotometer (Hitachi High-Technologies Corporation, Tokyo, Japan). Fourier transform infrared (FTIR) spectra were obtained from a FTS-175C (Bio-Rad Laboratories, Inc., Cambridge, MA, USA). Transmission electron microscopy (TEM) images were taken from 300 kV and 120 kV TEM (JEM-3011 HR and JEM-1400, respectively, JEOL Ltd., Tokyo, Japan). The secondary structure was determined by a Jasco J-810 circular dichroism (CD) spectropolarimeter (Jasco Inc., Tokyo, Japan). Dynamic light scattering (DLS) experiment was performed using ELS-Z (Otsuka Electronics, Osaka, Japan).

2. Characterization

TEM A drop of PA solution was placed on a formvar/carbon-coated copper grid and allowed to evaporate under ambient conditions. When sample was stained, a drop of uranyl acetate solution (2 wt%) was placed onto the surface of the sample-loaded grid. The sample was deposited about 3 min at least, and excess solution was wicked off by filter paper. The specimen was observed with a JEOL-JEM-3011 HR operating at 300 kV and JEM-1400 operating at 120 kV. The data were analyzed using Gatan Digital Micrograph software (Gatan Inc., Pleasanton, CA, USA) and Simple Measure software (JEOL Ltd., Tokyo, Japan).

Cryogenic TEM (cryo-TEM) Cryo-TEM experiments were performed with a thin film of aqueous PA

solution (3 μL) transferred to a lacey supported grid by plunge-dipping method. The thin aqueous films were prepared at ambient temperature and with humidity of 97–99% within a custom-built environmental chamber in order to prevent an evaporation of water from sample solution. The excess liquid was blotted with filter paper for 2–3 sec, and the thin aqueous films were rapidly vitrified by plunging them into liquid ethane (cooled by liquid nitrogen) at its freezing point. Cryo-TEM imaging was performed using a JEM-3011 HR (JEOL Ltd., Tokyo, Japan) operating at 300 kV equipped with a Gatan 626 cryo-holder (Gatan Inc., Pleasanton, CA, USA). The data were analyzed using Gatan Digital Micrograph software (Gatan Inc., Pleasanton, CA, USA).

FTIR spectroscopy FTIR measurements were performed using ZnSe pellet. The spectra of disulfide crosslinked PA solution in the presence of 1 mM or 10 mM glutathione (GSH) were recorded in the range of 2600–2500 cm^{-1}.

CD Spectroscopy CD spectra of PAs were measured using a Jasco J-810 spectro-polarimeter. Spectra were monitored from 190 nm to 260 nm using a 1.0 cm light path-length cuvette at 20 ℃, and scans were repeated three times and averaged.

Grazing Incidence Small-Angle X-ray Scattering (GISAXS) and Wide-Angle X-ray Scattering (WAXS) spectroscopy GISAXS and WAXS experiments were performed on the PLS-II 9A beamline at Pohang Accelerator Laboratory (PAL, Pohang, Republic of Korea). X-rays generated from the in-vacuum undulator were monochromated by Si(111) double crystals and were focused on the detector position using K_B-type mirror system. The operating conditions were set at a wavelength of 1.119 Å. The thin films were as-cast from a PA solution on silicon substrates. Before casting of each PA solution, the Si-wafer was cleaned by sonication in chloroform, acetone, and isopropanol, respectively, for 20 min each. The cleaned substrate was transferred in piranha solution and heated for 1 h at 100 ℃.

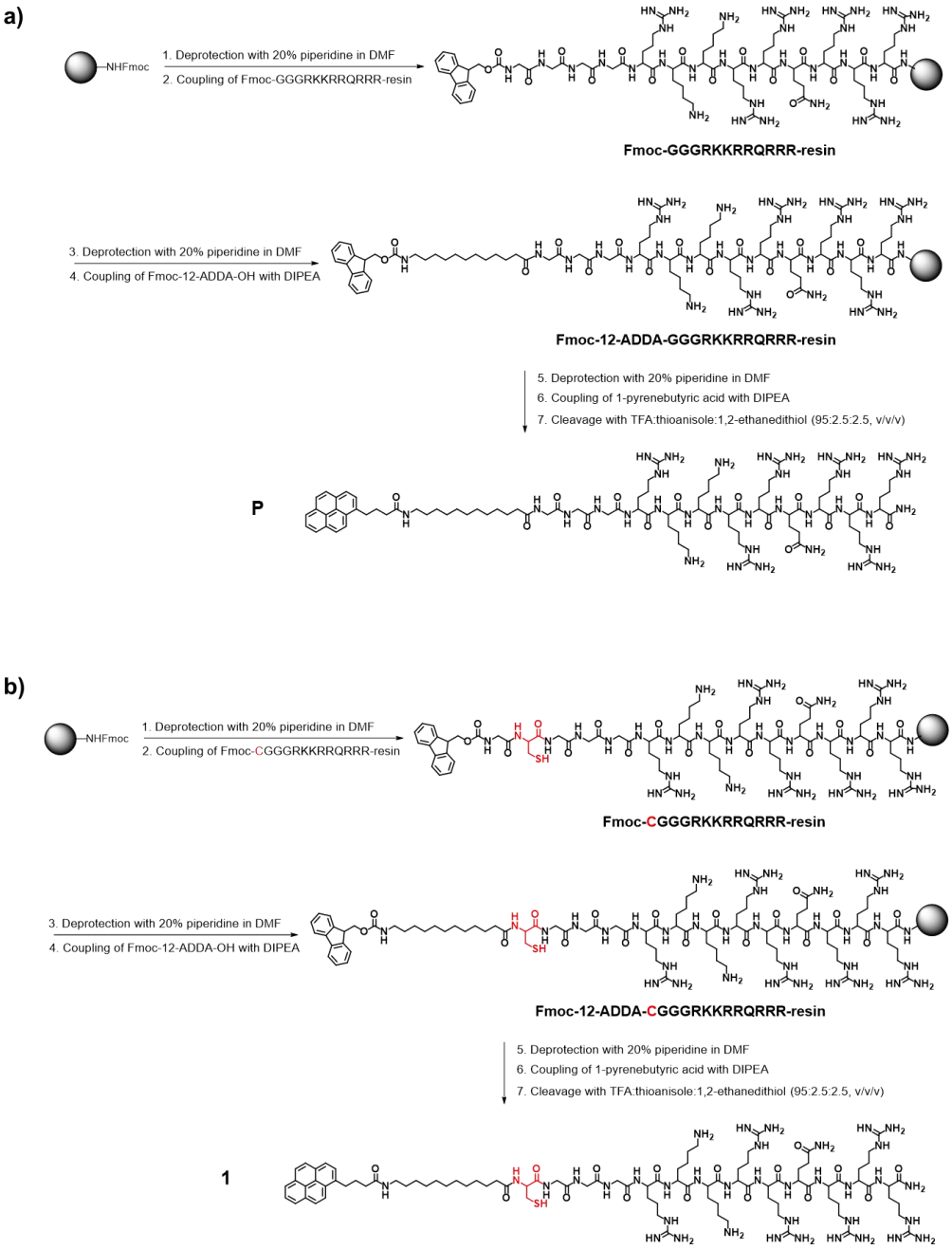

Figure S1. Synthesis of PAs: a) **P** and b) **1**. **2** and **1-1** were prepared by same procedure.

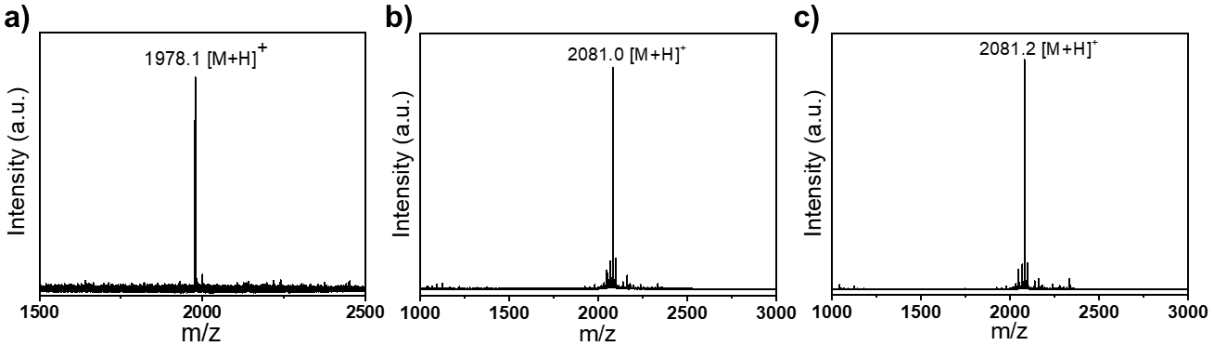

Figure S2. MALDI-TOF/TOF mass spectra of PAs: a) **P**, b) **1**, and c) **2**.

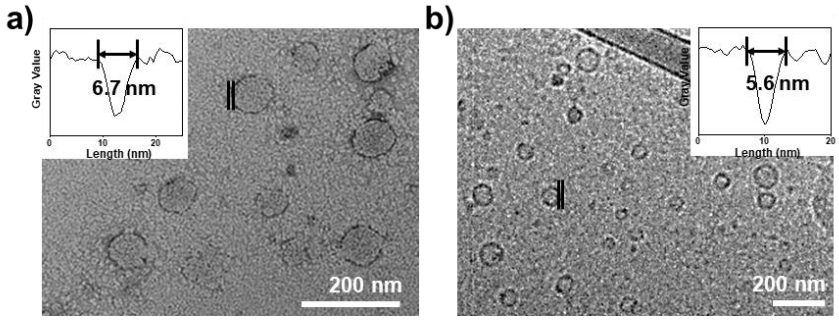

Figure S3. a) Negatively stained TEM image of self-assembled **P** (0.15 mM) with 2 wt% uranyl acetate. Inset of a): density profile showing the thickness of vesicle wall observed by TEM. b) Cryo-TEM image of self-assembled **1** (0.1 mM) in water. Inset of b): density profile showing the thickness of vesicle wall observed by cryo-TEM.

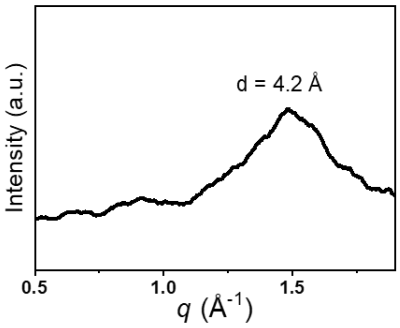

Figure S4. WAXS pattern of a thin film as-cast from PA solution of **2**, indicating the mutual interaction between peptide backbones.[S1]

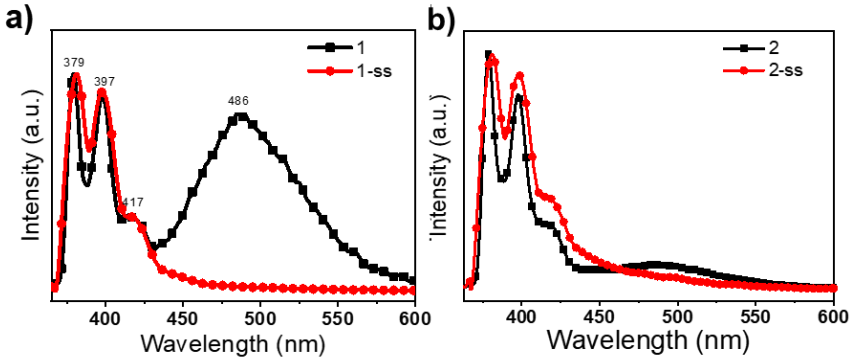

Figure S5. Fluorescence emission spectra of a) **1**, **1-ss**, b) **2**, and **2-ss** (λ_{ex} = 340 nm).

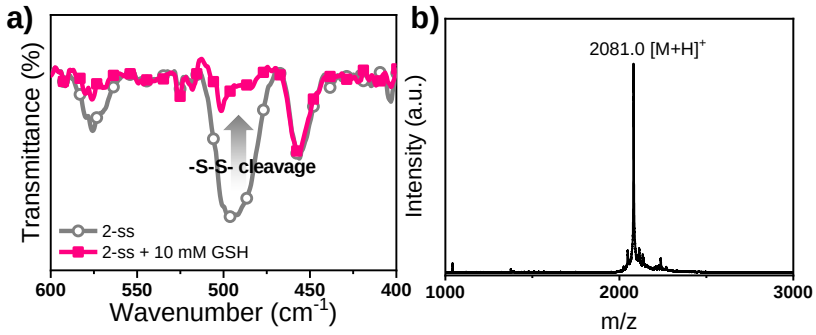

Figure S6. FTIR spectra and MALDI-TOF/TOF mass spectrum of **2-ss** after treatment of 10 mM GSH, confirming the reductin of disulfide bonds of **2-ss**.

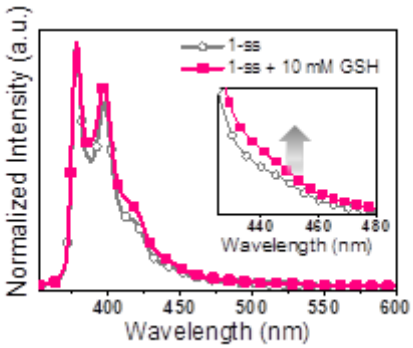

Figure S7. Fluorescence spectrum (λ_{ex} = 340 nm) of **1-ss** before and after treatment of 10 mM GSH.

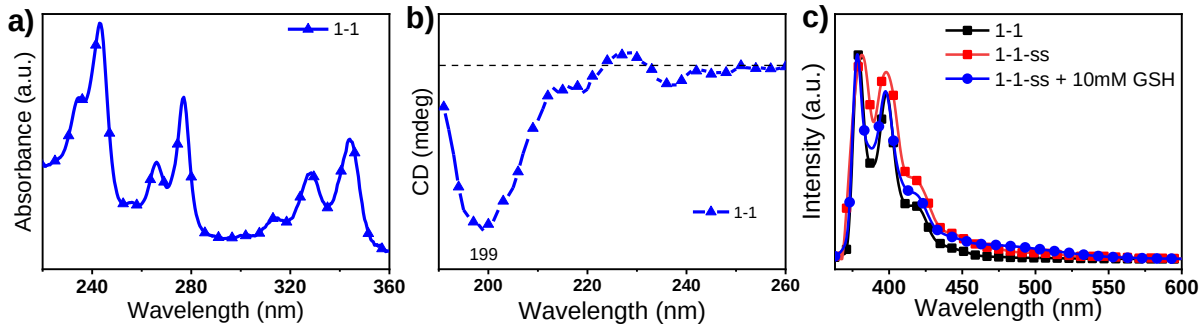

Figure S8. a) Absorption and b) CD spectra of **1-1** in aqueous solution (0.005 wt%). c) Emission spectra (λ_{ex} = 340 nm) of **1-1** and **1-1-ss** before and after addition of 10 mM GSH in aqueous solution (0.005 wt%).

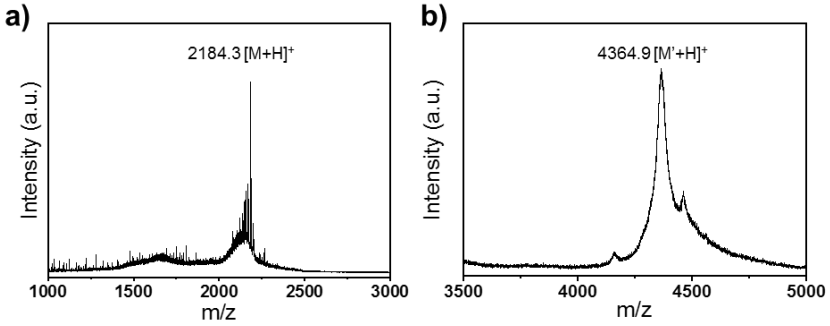

Figure S9. MALDI-TOF/TOF mass spectra of a) **1-1** and b) **1-1-ss**. The molecular weight of **1-1-ss** was confirmed by MALDI-TOF/TOF mass spectrometry: *m/z* 4364.9 (4364.5 calculated m/z for $[M'+H]^+$, M': molecular weight of dimerized **1-1** by formation of two disulfide linkages).

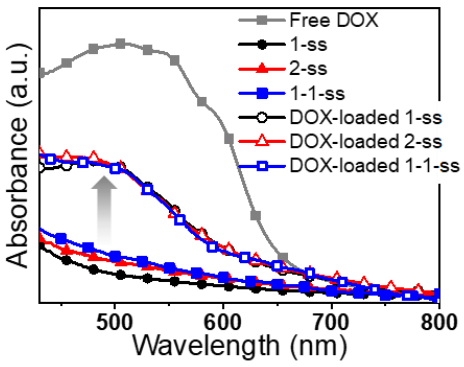

Figure S10. Absorption spectra showing the DOX-loading capability of PA nanostructures in water. Absorbance of free DOX was measured in chloroform.

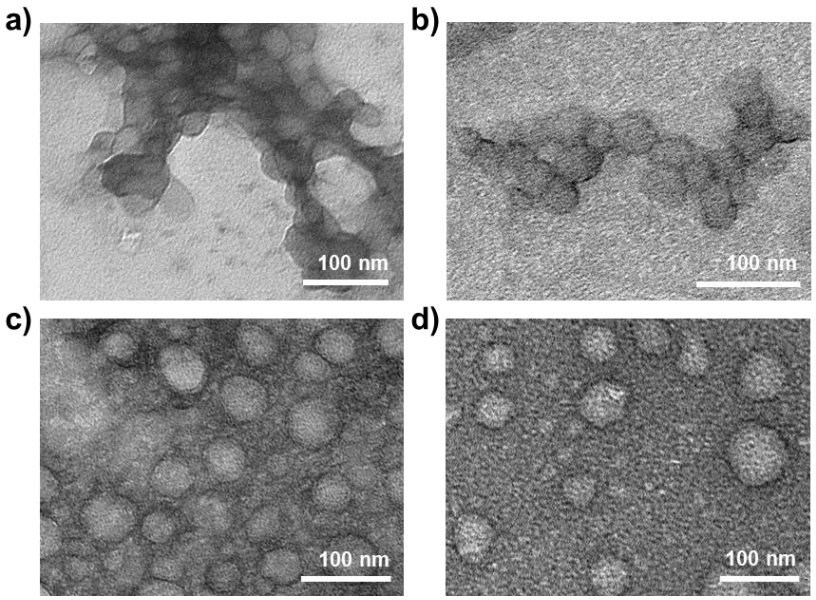

Figure S11. Negatively stained TEM images of PA nanostructures with 2 wt% uranyl acetate after loading of DOX: a) **P**, b) **1-ss**, c) **2-ss**, and d) **1-1-ss**.

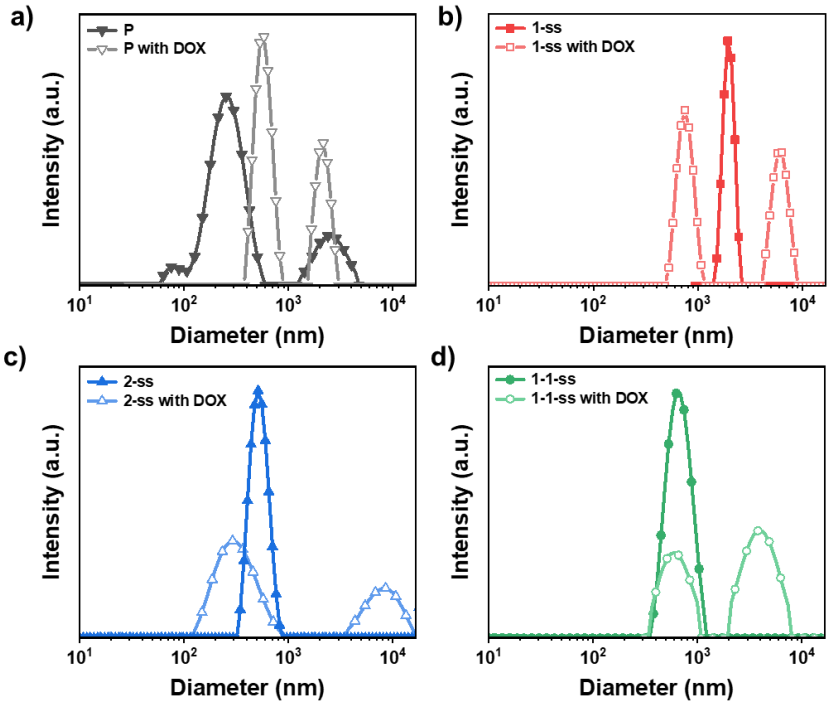

Figure S12. Hydrodynamic diameters of PA nanostructures (0.15 mM) before and after addition of DOX: a) **P**, b) **1-ss**, c) **2-ss**, and d) **1-1-ss**.

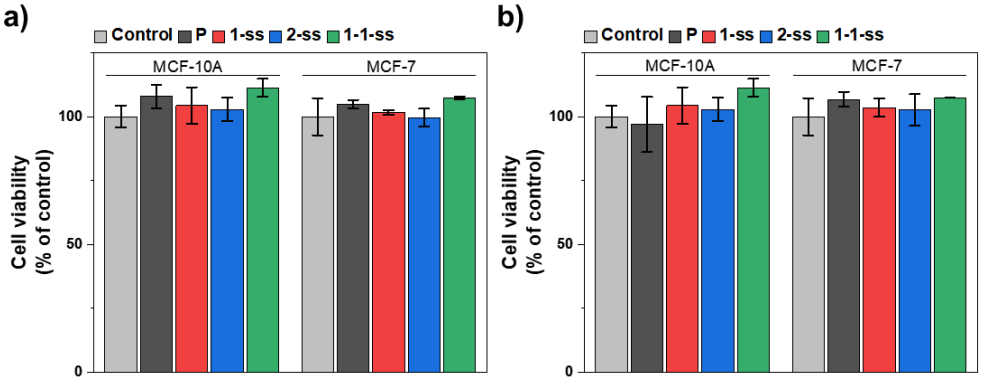

Figure S13. Cell viability test of the self-assembled **P** and **1-ss**, **2-ss**, and **1-1-ss** at a) 25 μM and b) 50 μM on MCF-10A and MCF-7 cells by CCK-8 assay after 24 h treatment.

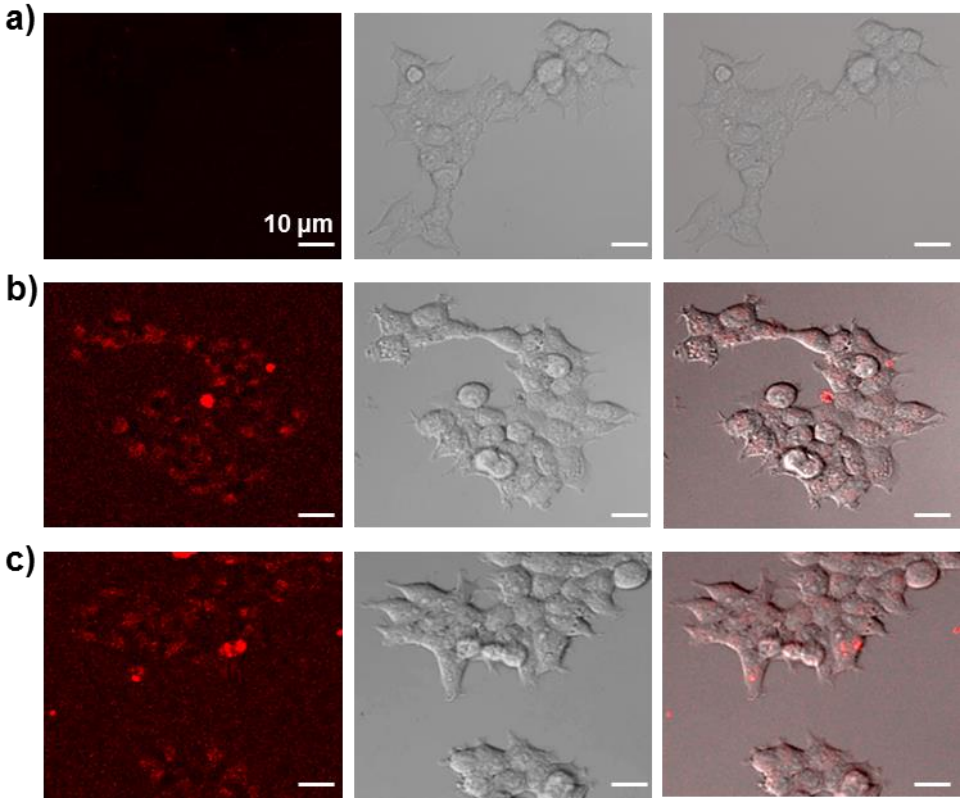

Figure S14. Intracellular delivery of DOX by self-assembled **1-ss** and **2-ss**. CLSM images of MCF-7 cells: a) untreated and treated with DOX-loaded b) **1-ss** and c) **2-ss** (50 μM) after 24 h treatment. Left: DOX colored red, middle: bright field images, and right: merged images. Scale bar = 10 μm.

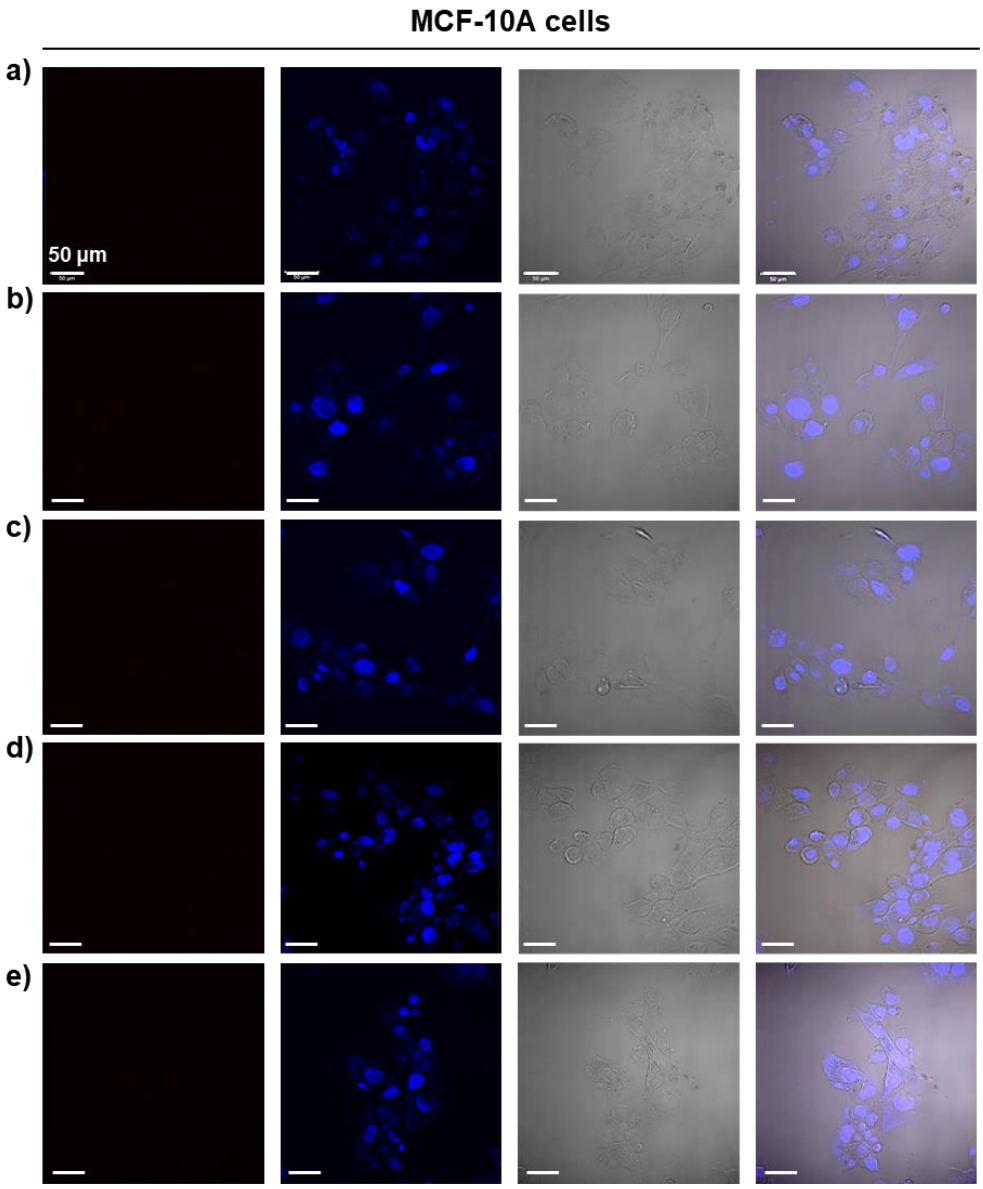

Figure S15. Intracellular delivery of DOX. CLSM images of MCF-10A cells: a) untreated and treated with DOX-loaded b) **P**, c) **1-ss**, d) **2-ss**, and e) **1-1-ss** (50 µM) after 48 h treatment. From left to right: DOX colored red, nuclei stained with DAPI, bright field images, and merged images. Scale bar = 50 µm.

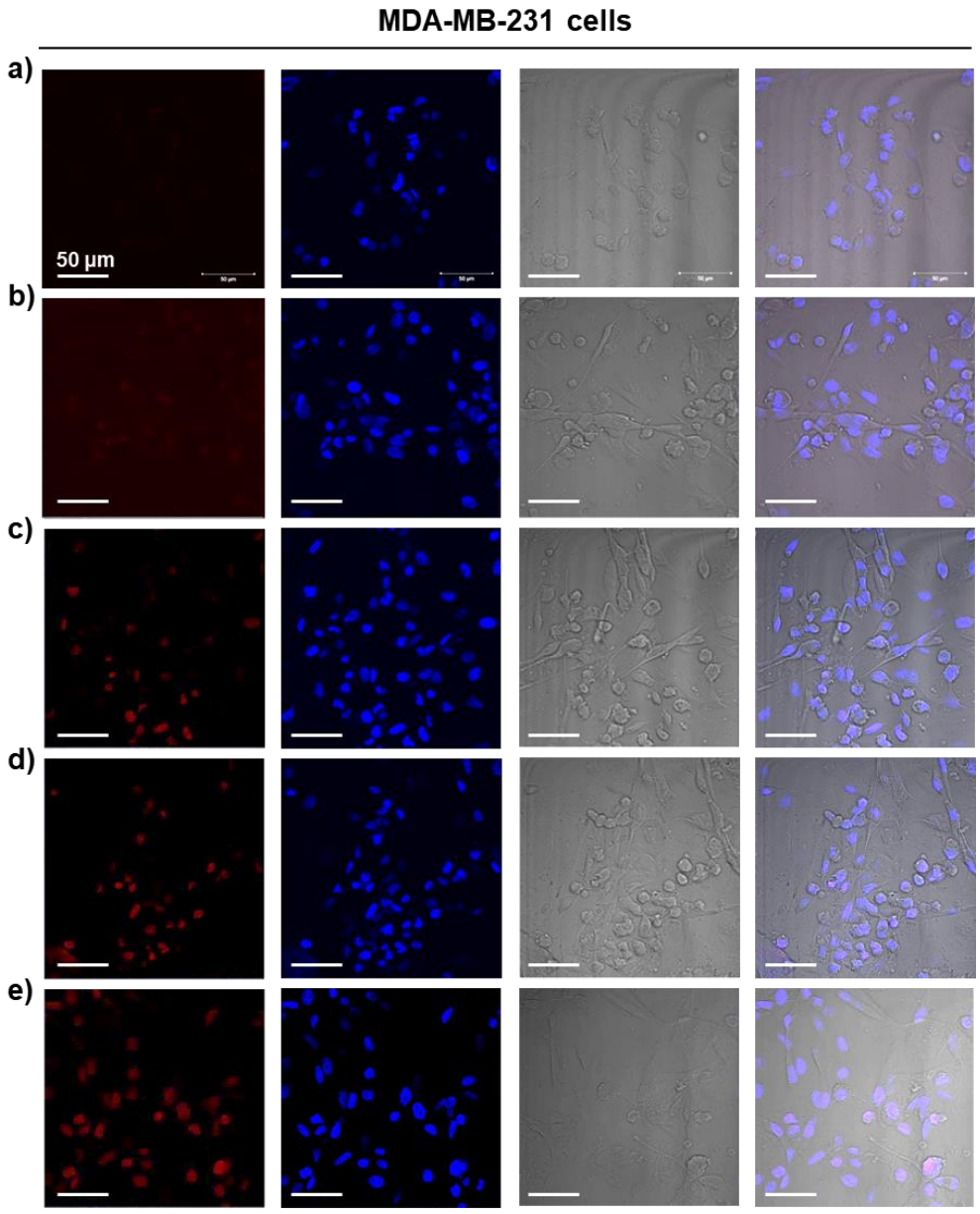

Figure S16. Intracellular delivery of DOX. CLSM images of MDA-MB-231 cells: a) untreated and treated with DOX-loaded b) **P**, c) **1-ss**, d) **2-ss**, and e) **1-1-ss** (50 µM) after 48 h treatment. From left to right: DOX colored red, nuclei stained with DAPI, bright field images, and merged images. Scale bar = 50 µm.

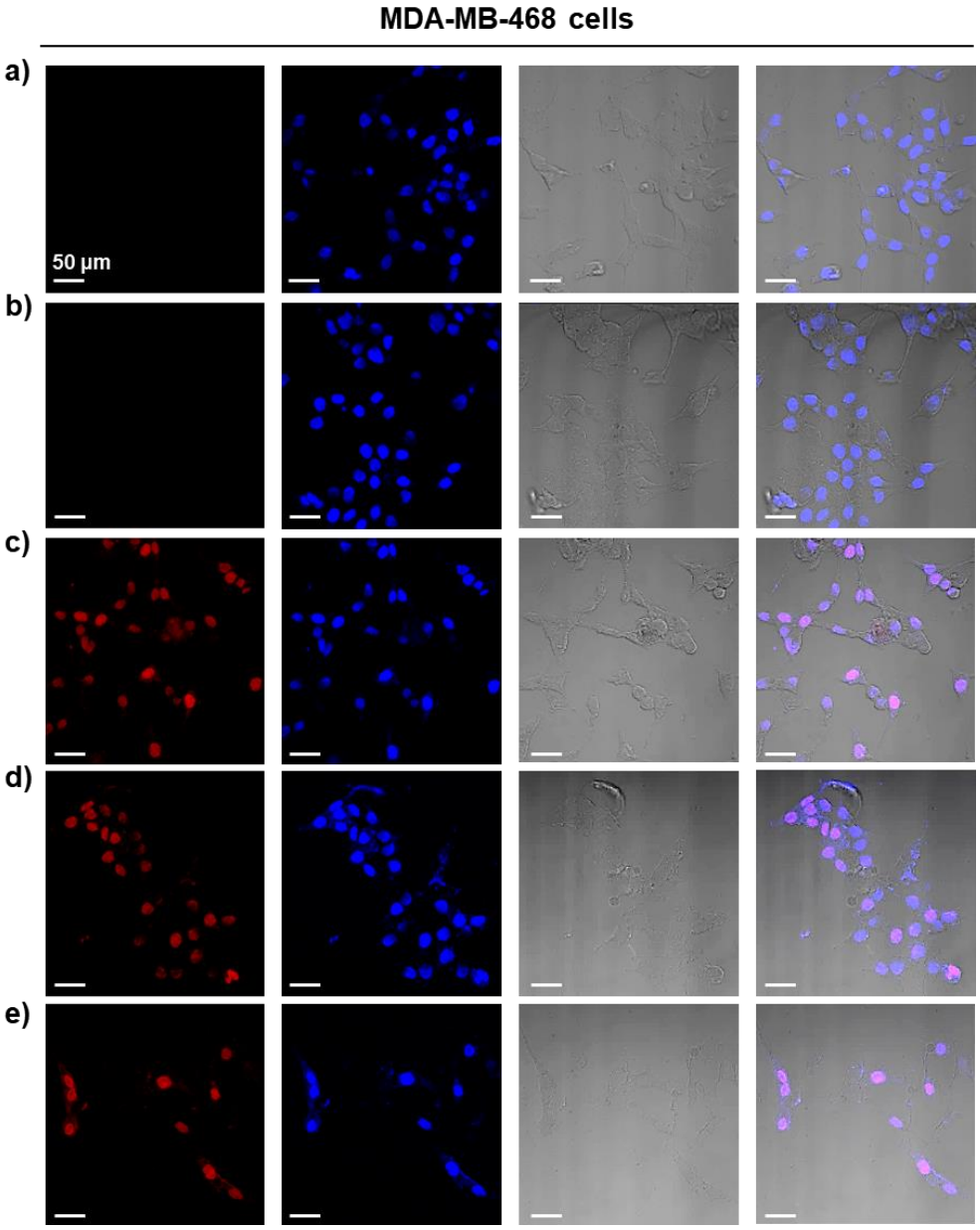

Figure S17. Intracellular delivery of DOX. CLSM images of MDA-MB-468 cells: a) untreated and treated with DOX-loaded b) **P**, c) **1-ss**, d) **2-ss**, and e) **1-1-ss** (50 µM) after 48 h treatment. From left to right: DOX colored red, nuclei stained with DAPI, bright field images, and merged images. Scale bar = 50 µm.

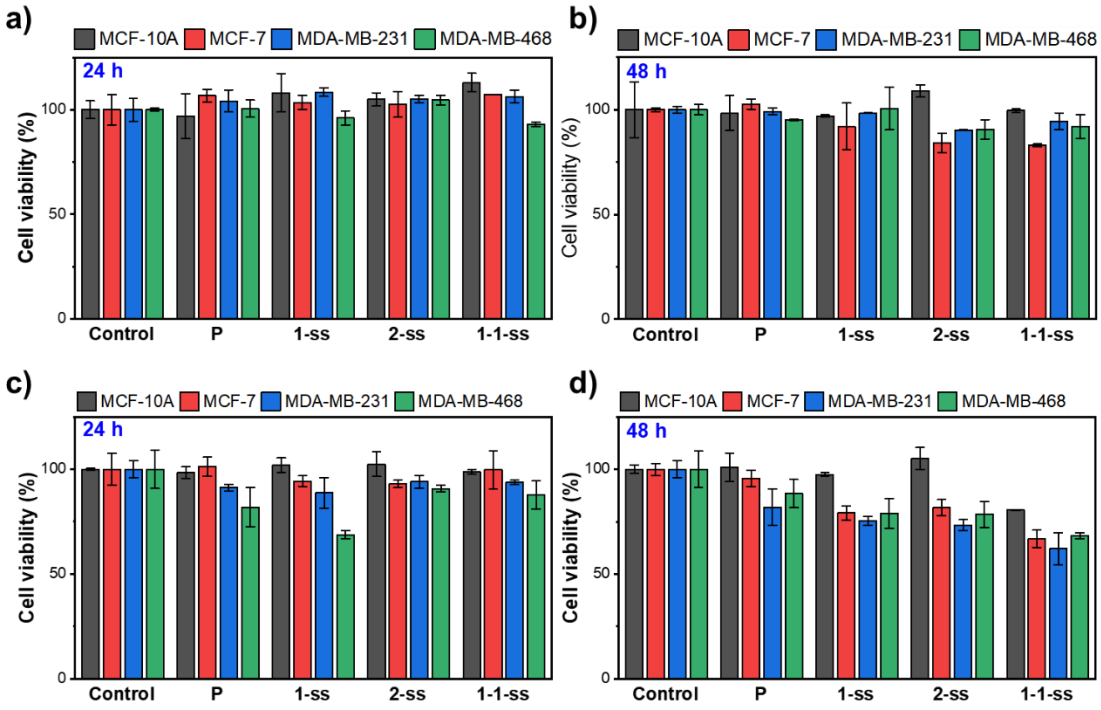

Figure S18. Cell viability of self-assembled **P**, **1-ss**, **2-ss**, and **1-1-ss** (50 μM) against MCF-10A, MCF-7, MDA-MB-231, and MDA-MB-468 cells in the a,b) absence and c,d) presence of DOX, determined by CCK-8 assay after treatment of a,c) 24 h and b,d) 48 h.

Reference

1. R. Verel, I. T. Tomka, C. Bertozzi, R. Cadalbert, R. A. Kammerer, M. O. Steinmetz, B. H. Meier, *Angew. Chem. Int. Ed.* **2008**, *47*, 5842–5845.