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Supporting Information

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Self-Assembly of Cytotoxic Peptide Amphiphiles into Supramolecular Membranes for Cancer Therapy

*R. Helen Zha, Shantanu Sur, and Samuel I. Stupp**

PA Solution	Primary Structures	Diameter [nm]
5 mM K_3	cylinder	10.0
5 mM KLAK	sphere	8.6
4.5 mM K_3/0.5 mM KLAK	cylinder, sphere	9.2 (cylinder), 9.4 (sphere)
4.5 mM K_3	cylinder	10.0
0.5 mM KLAK	sphere	6.4
4 mM K_3/1 mM KLAK	sphere	11.6
4 mM K_3	cylinder	10.0
1 mM KLAK	sphere	6.3
3.5 mM K_3/1.5 mM KLAK	sphere	10.9
3.5 mM K_3	cylinder	10.0
1.5 mM KLAK	sphere	7.7
2.5 mM K_3/2.5 mM KLAK	sphere	11.5
2.5 mM K_3	cylinder	9.7
2.5 mM KLAK	sphere	7.8

Figure S1. Nanostructure morphology and size of pure and co-assembled PA solutions as determined by SAXS. Scattering profiles were fit to a core-shell cylinder model or a polydisperse core-shell sphere model using IGOR Pro software (WaveMetrics, Inc) with Irena SAS macros (provided by Argonne National Labs Advanced Photon Source) and NCNR Analysis macros (provided by NIST Center for Neutron Research). K_3 PA cylinders were observed to be monodisperse and maintain a constant diameter with changing concentration. KLAK PA spheres were observed to be highly polydisperse and increase in diameter as concentration increases. Mixtures containing 10% KLAK PA (5 mM PA total) exhibited a mixture of cylindrical and spherical morphologies, while mixtures with 20% KLAK PA or higher exhibited predominantly spherical morphology by SAXS analysis.

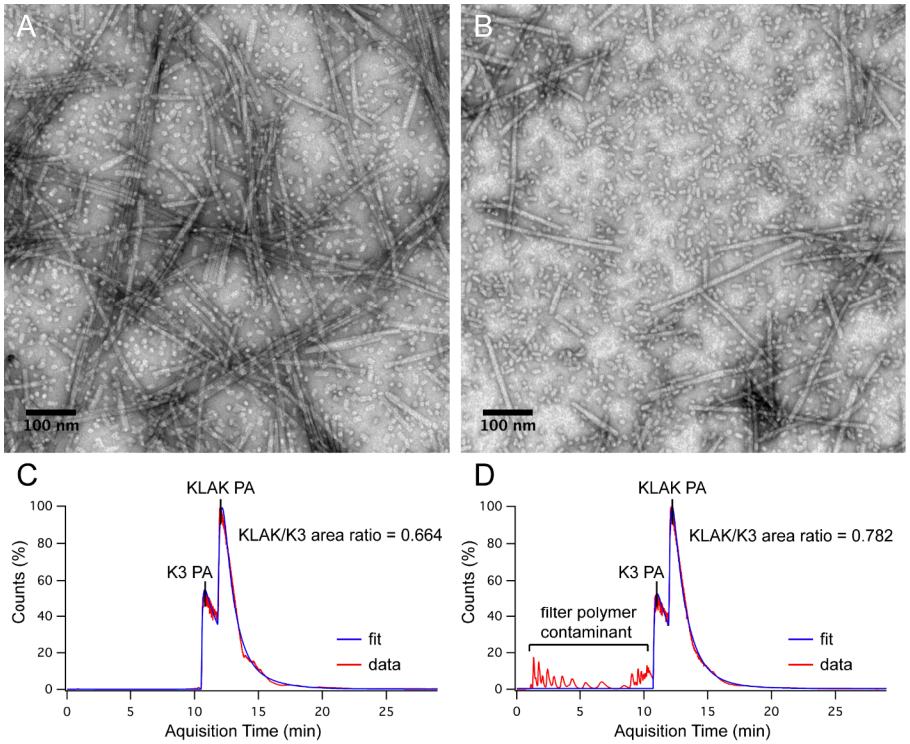

Figure S2. TEM and LCMS analysis of unfiltered (a, c) and filtered (b, d) 20% KLAK PA solution. KLAK PA and K_3 PA were co-assembled as described in section 2.1 and the solution was passed through a 0.2 µm syringe filter three times. TEM imaging was performed as described in section 2.2, and it was observed that filtered solutions (b) were enriched in spherical aggregates compared to unfiltered solutions (a). LCMS analysis was performed with Agilent 6520 Q-Tof mass spectrometer coupled with Agilent 1200 series capillary pumps and DAD detector. Samples were analyzed on a Jupiter C12 Proteo reversed phase column (150X1 mm, 4 µm, 90 Å) at the flow rate of 50 µl/min with a linear gradient from 5% to 70% B over 23 minutes. Solvent A was 0.1% (v/v) formic acid in H2O and solvent B was 0.1% (v/v) formic acid in acetonitrile. Sample elution was monitored at 220 nm. Data was processed with Agilent Mass Hunter Workstation and was then imported into IGOR Pro for multi-peak fitting (c, d). Small, fast eluting peaks seen in the filtered sample were due to polymer contamination from the filter. KLAK PA and K_3 PA peaks were deconvoluted and integrated with IGOR Pro software. Comparing the ratio of peak areas between filtered and unfiltered samples showed that filtering the co-assembled PA solution results in a 17.8% increase in KLAK/K_3 PA ratio, suggesting that spherical nanostructures are KLAK-rich compared to cylindrical nanostructures.

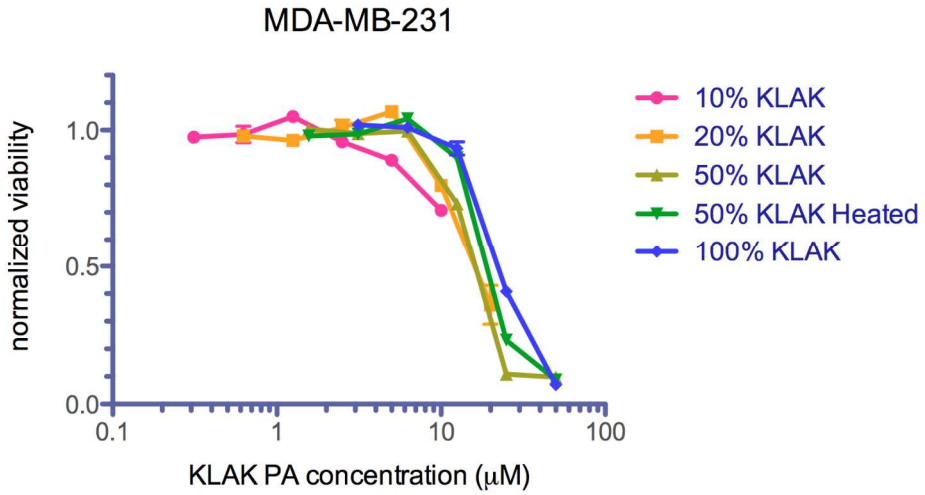

Figure S3. Cytotoxicity of pure and co-assembled KLAK PA in solution. PAs were prepared as described in experimental section and then diluted into cell culture media to achieve the concentrations indicated in the legend. MDA-MB-231 breast cancer cells were plated in flat bottom tissue-culture treated 96 well plates (Corning) at 5000 cells per well and were incubated overnight at 37 °C before media was replaced by 100 μL PA solution. Cell viability was assessed after 24 h by adding 20 μL CellTiter 96 AQueous One Solution MTS Proliferation Assay (Promega) to each well and incubating for 1 h at 37 °C before reading the absorbance at 490 nm using a SpectraMax M5 plate reader (Molecular Devices). Viability is normalized to positive controls (no PA added), and the x-axis shows the concentration of the KLAK PA component. Data points represent mean and standard error for n=3. It can be seen that co-assembling KLAK PA with K_3 PA does not decrease its cytotoxic potency, and KLAK/K_3 PA co-assemblies are in some cases more cytotoxic for the same amount of KLAK PA than pure KLAK PA alone.