SUPPORTING INFORMATION

# Controlled apoptosis by a thermally toggled nanoscale amplifier of cellular uptake

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**Materials.** Oligonucleotides for recombinant cloning were purchased from Integrated DNA Technologies (Coralville, IA). Restriction enzymes, calf intestinal alkaline phosphatase, and their respective buffers were obtained from New England Biolabs (Ipswich, MA) and T4 DNA Ligase was purchased from Invitrogen (Grand Island, NY). EB5α competent cells for cloning and BL21 competent cells for protein expression were purchased from Edge BioSystems (Gaithersburg, MD). Terrific Broth dry media was obtained from MO BIO Laboratories (Carlsbad, CA) and kanamycin was purchased from EMD Chemicals (Rockland, MA). 4-20% Tris-HCl Ready Gels and 10-20% Mini-PROTEAN Tris-tricine gels were purchased from Bio-Rad Laboratories (Hercules, CA). Alexa Fluor 488 C5-maleimide, Alexa Fluor 594 wheat agglutinin, Hoechst 33342, and EnzChek® Caspase-3 Assay Kit #2 were obtained from Invitrogen (Grand Island, NY). Sephadex® G-25 desalting columns were acquired from GE Healthcare (Pittsburgh, PA). Anotop low protein binding 0.02 μm pore filters were purchased from Whatman (Piscataway, NJ). 8-well chambered coverglass for confocal microscopy was purchased from Electron Microscopy Sciences (Hatfield, PA). Furin enzyme was purchased from New England Biolabs (Ipswich, MA). BH3 peptide corresponding to residues 71-89 of the Bak protein was acquired from AnaSpec (Fremont, CA) or Bio Synthesis (Lewisville, TX). Cell titer 96 aqueous non-radioactive cell proliferation reagent was purchased from Promega (Madison, WI). BCA assay kit was acquired from Pierce Biotechnology (Rockford, IL).

**Cell culture.** HeLa cervical cancer cells were obtained from ATCC (Manassas, VA) and were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 1000 mg/L glucose, 3.7 g/L sodium bicarbonate, 1 mM sodium pyruvate, 0.1 M

non-essential amino acids, 100 units/mL penicillin, and 100 μg/mL streptomycin. Cells were maintained at 37 °C and 5% $CO_2$.

**Recombinant design of elastin-like polypeptide diblock copolymers ($ELP_{BC}$s).** $ELP_{BC}$s were synthesized with recursive directional ligation by plasmid reconstruction (PRe-RDL).[1] This technique allowed modular synthesis of $ELP_{BC}$ genes that could be easily appended with sequences that encode various N-terminal and C-terminal peptides for functionalization of $ELP_{BC}$s with a peptide drug and cell-penetrating peptide (CPP) motifs. The hydrophobic $ELP_{BC}$ domain was composed of the sequence $(VGVPG)_{40}$, while the hydrophilic $ELP_{BC}$ domain was composed of the sequence $(AGVPGGGVPG)_{30}$. Genes encoding these domains were fused to create a $(VGVPG)_{40}$-$(AGVPGGGVPG)_{30}$ diblock gene that was further modified at the N-terminus to include codons that encode a peptide that contains a unique reactive residue for fluorophore conjugation or a peptide drug, and at the C-terminus to include codons that encode a CPP. $ELP_{BC}$s used to evaluate cellular uptake all contained an N-terminal MGCGWPG peptide sequence, where the C provided a reactive residue for fluorophore conjugation by maleimide chemistry and the W provided an optically active residue for peptide quantification by UV-VIS absorption. The C-terminus of each $ELP_{BC}$ incorporated a short, flexible linker (PGGS) followed by a CPP sequence. $ELP_{BC}$s used for the delivery of peptide drug were functionalized at the N-terminus with the BH3 peptide (MGQVGRQLAIIGDDINRRY) derived from residues 71-89 of the Bak protein[2,3] with or without a furin cleavable peptide linker (RVRR) between the $ELP_{BC}$'s hydrophobic terminus and the BH3 peptide.[4-6] As the critical micelle temperature (CMT) of the $ELP_{BC}$ was already carefully tuned to the desired temperature of ~40 ºC, it was important to avoid an excessively hydrophobic cleavable peptide that could potentially lower the CPP-

$ELP_{BC}$'s CMT, which dictated our choice of the protease cleavable RVRR linker over more hydrophobic alternatives. CPP-$ELP_{BC}$s appended to BH3 through this cleavable linker are denoted CPP-$ELP_{BC}$-cBH3, whereas a non-cleavable control, lacking the RVRR linker, is denoted CPP-$ELP_{BC}$-BH3. An additional control was synthesized to include only the RVRR linker sequence, without the BH3 peptide drug. The genetic design of $ELP_{BC}$s is shown in detail in Figure S1. All $ELP_{BC}$ genes were synthesized in a pET24a+ expression vector encoding kanamycin resistance.

**A**


**B**

(M) G Q V G R Q L A I I G D D I N R R Y R V
ATG GGT CAG GTG GGC CGT CAG CTG GCG ATT ATC GGC GAT GAT ATT AAC CGT CGC TAT CGT GTG

R R G C G W P G (V G V P G V G V P G V G
CGC CGT GGC TGC GGG TGG CCG GGC (GTG GGT GTT CCG GGC GTA GGT GTC CCA GGT GTG GGC

V P G V G V P G V G V P G)$_8$ (A G V P G G G
GTA CCG GGC GTT GGT GTT CCT GGT GTC GGC GTG CCG GGC)$_8$ (GCA GGT GTT CCG GGT GGC GGT

V P G A G V P G G G V P G A G V P G G G
GTG CCG GGC GCA GGT GTC CCG GGT GGC GGT GTG CCG GGC GCA GGT GTC CCG GGT GGC GGT

V P G A G V P G G G V P G A G V P G G G
GTT CCG GGC GCA GGT GTC CCG GGT GGC GGT GTG CCG GGC GCA GGT GTT CCG GGT GGC GGG

V P G)$_5$ A G V P G G G V P G A G V P G G G
GTG CCG GGC)$_5$ GCA GGT GTT CCG GGT GGC GGT GTG CCG GGC GCA GGT GTC CCG GGT GGC GGT

V P G A G V P G G G V P G A G V P G G G
GTG CCG GGC GCA GGT GTC CCG GGT GGC GGT GTT CCG GGC GCA GGT GTC CCG GGT GGC GGT

V P G A G V P G G G V P G P G G S R R R
GTG CCG GGC GCA GGT GTT CCG GGT GGC GGG GTG CCG GGG CCG GGC GGT AGC CGC CGT CGT

R R R R R . .
CGC CGC CGT CGC CGT TGA TAA

**C**

Leader

(M) G C G W P G
ATG GGC TGC GGG TGG CCG GGC

$ELP_{BC}$

(V G V P G V G V P G V G V P G V G V P G
(GTG GGT GTT CCG GGC GTA GGT GTC CCA GGT GTG GGC GTA CCG GGC GTT GGT GTT CCT GGT

V G V P G)$_8$ (A G V P G G G V P G A G V P G
GTC GGC GTG CCG GGC)$_8$ (GCA GGT GTT CCG GGT GGC GGT GTG CCG GGC GCA GGT GTC CCG GGT

G G V P G A G V P G G G V P G A G V P G
GGC GGT GTG CCG GGC GCA GGT GTC CCG GGT GGC GGT GTT CCG GGC GCA GGT GTC CCG GGT

G G V P G A G V P G G G V P G)$_5$ A G V P G
GGC GGT GTG CCG GGC GCA GGT GTT CCG GGT GGC GGG GTG CCG GGC)$_5$ GCA GGT GTT CCG GGT

G G V P G A G V P G G G V P G A G V P G
GGC GGT GTG CCG GGC GCA GGT GTC CCG GGT GGC GGT GTG CCG GGC GCA GGT GTC CCG GGT

G G V P G A G V P G G G V P G A G V P G
GGC GGT GTT CCG GGC GCA GGT GTC CCG GGT GGC GGT GTG CCG GGC GCA GGT GTT CCG GGT

G G V P G
GGC GGG GTG CCG GGG

## C-cont.

Arg$_5$

**P G G S R R R R R . .**

CCG GGC GGT AGC CGC CGT CGC CGT CGC TGA TAA

Arg$_8$

**P G G S R R R R R R R R . .**

CCG GGC GGT AGC CGC CGT CGT CGC CGC CGT CGC CGT TGA TAA

TAT

**P G G S Y G R K K R R Q R R R . .**

CCG GGC GGT AGC TAT GGC CGT AAA AAA CGT CGT CAG CGT CGC CGT TGA TAA

RTAT

**P G G S Y G R G G R R G R R R . .**

CCG GGC GGT AGC TAT GGC CGT GGC GGT CGT CGT GGC CGT CGC CGT TGA TAA

Non-functionalized control

**P G G S . .**

CCG GGC GGT AGC TGA TAA

## D

cBH3

**(M) G Q V G R Q L A I I G D D I N R R Y R V**

ATG GGT CAG GTG GGC CGT CAG CTG GCG ATT ATC GGC GAT GAT ATT AAC CGT CGC TAT CGT GTG

**R R G C G W P G**

CGC CGT GGC TGC GGG TGG CCG GGC

BH3

**(M) G Q V G R Q L A I I G D D I N R R Y G C**

ATG GGT CAG GTG GGC CGT CAG CTG GCG ATT ATC GGC GAT GAT ATT AAC CGT CGC TAT GGC TGC

**G W P G**

GGG TGG CCG GGC

RVRR

**(M) G R V R R G C G W P G**

ATG GGT CGT GTG CGC CGT GGC TGC GGG TGG CCG GGC

**D-cont.**

ELP$_{BC}$

(V G V P G V G V P G V G V P G V G V P G
(GTG GGT GTT CCG GGC GTA GGT GTC CCA GGT GTG GGC GTA CCG GGC GTT GGT GTT CCT GGT

V G V P G)$_8$ (A G V P G G G V P G A G V P G
GTC GGC GTG CCG GGC)$_8$ (GCA GGT GTT CCG GGT GGC GGT GTG CCG GGC GCA GGT GTC CCG GGT

G G V P G A G V P G G G V P G A G V P G
GGC GGT GTG CCG GGC GCA GGT GTC CCG GGT GGC GGT GTT CCG GGC GCA GGT GTC CCG GGT

G G V P G A G V P G G G V P G)$_5$ A G V P G
GGC GGT GTG CCG GGC GCA GGT GTT CCG GGT GGC GGG GTG CCG GGC)$_5$ GCA GGT GTT CCG GGT

G G V P G A G V P G G G V P G A G V P G
GGC GGT GTG CCG GGC GCA GGT GTC CCG GGT GGC GGT GTG CCG GGC GCA GGT GTC CCG GGT

G G V P G A G V P G G G V P G A G V P G
GGC GGT GTT CCG GGC GCA GGT GTC CCG GGT GGC GGT GTG CCG GGC GCA GGT GTT CCG GGT

G G V P G
GGC GGG GTG CCG GGG

Arg$_5$

P G G S R R R R R . .
CCG GGC GGT AGC CGC CGT CGC CGT CGC TGA TAA

Arg$_8$

P G G S R R R R R R R R . .
CCG GGC GGT AGC CGC CGT CGT CGC CGC CGT CGC CGT TGA TAA

Non-functionalized control

P G G S . .
CCG GGC GGT AGC TGA TAA

**Figure S1**. Genetic design of ELP$_{BC}$s. (A) The modular design of all CPP-ELP$_{BC}$s included a therapeutic domain (green), hydrophobic ELP domain (purple), hydrophilic ELP domain (blue), and CPP domain (red). (B) An example of a complete gene is provided for Arg$_8$-ELP$_{BC}$-cBH3, whose cleavable peptide linker sequence is underlined. (C) CPP-ELP$_{BC}$s designed for evaluation of controlled cellular uptake consisted of a therapeutic domain containing a cysteine for conjugation of fluorophore (a model cargo molecule - green), an ELP$_{BC}$ whose hydrophobic (purple) and hydrophilic (blue) domains were optimized for its desired thermal properties, and a

CPP domain (red) encoding a flexible linker appended with $Arg_5$, $Arg_8$, TAT, RTAT, or not functionalized to serve as a control. (D) CPP-$ELP_{BC}$s designed for evaluation of controlled cytotoxicity by delivery of drug cargo were composed of a therapeutic domain (green) containing a BH3 peptide drug appended to the ELP via a cleavable RVRR linker (cBH3), a BH3 peptide drug without a cleavable linker (BH3), or the cleavable linker alone, an $ELP_{BC}$ composed of a hydrophobic (purple) and hydrophilic (blue) domain, and a CPP domain (red) encoding a flexible linker appended with $Arg_5$, $Arg_8$, or not functionalized to serve as a control.

**Expression and purification of $ELP_{BC}$s.** BL21(DE3) *Escherichia coli* were transformed with a plasmid encoding one of the $ELP_{BC}$ genes and grown for 24 h in Terrific Broth media. Cells were isolated by centrifugation at 3,200 RPM at 4 ºC and lysed by sonication in cycles of 10 sec on and 20 sec off for a total sonication time of 3 min. DNA contaminants were condensed by the addition of 10% w/v polyethylenimine and cellular debris was pelleted by centrifugation at 14,000 RPM at 4 °C. ELP purification then proceeded by inverse transition cycling, as follows.[7] The supernatant from the previous step was collected and warmed to 37 °C before crystalline NaCl was added (approximately 1.5 M) to induce ELP aggregation. The ELP coacervate was collected by centrifugation above the transition temperature (this step is termed a hot spin). The supernatant was discarded and the ELP pellet was resuspended in chilled (4 ºC) phosphate buffered saline (PBS). Soluble contaminants were then removed by centrifugation at 4 °C (this step is termed a cold spin). The contaminant pellet was discarded while the supernatant was removed and subjected to another hot spin. Five cycles of alternating hot and cold spins were performed to achieve purified $ELP_{BC}$ products, as evaluated by SDS-PAGE using 4-20% Tris-HCl gels stained with 0.5 M $CuCl_2$. SDS-PAGE analysis confirmed the size and purity of CPP-

$ELP_{BC}$s (Figure S2A). As is typical of ELPs, this CPP-$ELP_{BC}$ library migrated with an apparent molecular weight (MW) that is slightly greater than what is expected from its calculated MW.[8,9] CPP-$ELP_{BC}$s with BH3 peptide drug cargo, and their controls, were synthesized, expressed, and purified as described above. The correct size and purity of CPP-$ELP_{BC}$ drug conjugates were confirmed by SDS-PAGE analysis (Figure S2B).

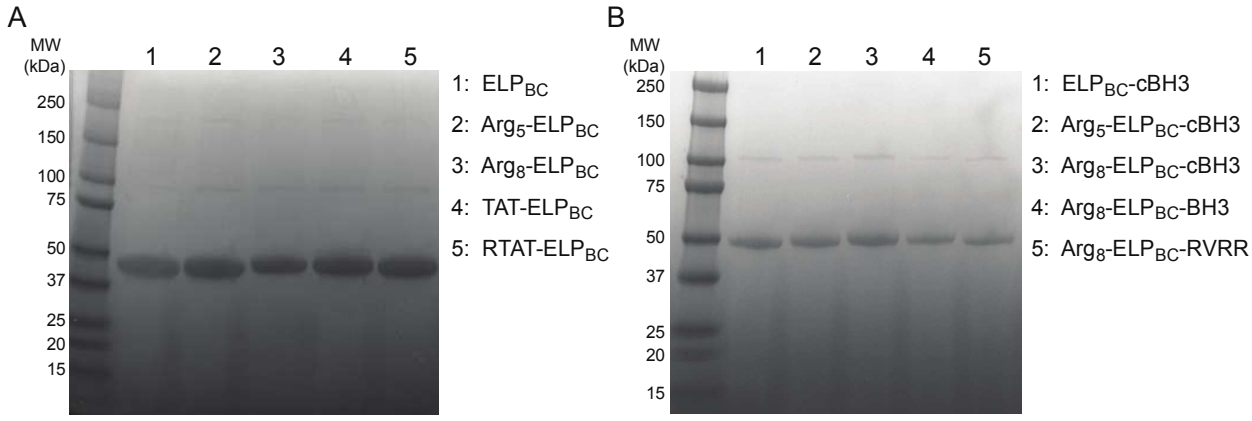


**Figure S2**. SDS-PAGE analysis of purified CPP-$ELP_{BC}$s. (A) The expected molecular weights of the CPP-$ELP_{BC}$s varied slightly due to CPP functionalization and were, by lane: 1) 39.72 kDa, 2) 40.05 kDa, 3) 40.97 kDa, 4) 41.26 kDa, and 5) 41.05 kDa. (B) The expected molecular weight of the CPP-$ELP_{BC}$ drug carriers varied slightly by CPP, drug, and linker functionalization and were, by lane, 1) 42.32 kDa, 2) 43.10 kDa, 3) 43.57 kDa, 4) 43.00 kDa, and 5) 41.60 kDa.

**Fluorescent labeling of $ELP_{BC}$s.** Purified $ELP_{BC}$s were solubilized in 15 mM $NaPO_4$/3 mM TCEP HCl at pH 7. Alexa Fluor 488 C5-maleimide was resuspended in DMSO and added to the $ELP_{BC}$ solution, while shaking. The reaction was allowed to proceed at room temperature for 2 h, while rotating, after which the ELP aggregation was triggered by the addition of heat and NaCl. The ELP was collected by centrifugation at 14,000 RPM at 45 ºC. Free fluorophore in the supernatant was discarded and the ELP pellet was resuspended in PBS. Residual free fluorophore

was removed by size exclusion chromatography on a sephadex desalting column. Labeling efficiency was determined by absorption at 494 nm and 280 nm using the following equation: percent labeling = $(Abs_{494}/71{,}000)/((Abs_{280}-0.11*Abs_{494})/\varepsilon_{ELP})$, where 0.11 is the correction factor for the contribution of Alexa Fluor 488 absorption at 280 nm and $\varepsilon_{ELP}$ is the extinction coefficient of the ELP. Unlabeled ELP was added to the fluorescently labeled ELP solutions to achieve 40% or 50% labeling efficiency for evaluation of cellular uptake by confocal microscopy and flow cytometry, respectively.

**Thermal characterization of $ELP_{BC}$s.** Each CPP-$ELP_{BC}$ was characterized by temperature-regulated turbidimetry and dynamic light scattering (DLS). Turbidimetry measurements were acquired on a Varian Cary 300 Bio UV-Visible spectrophotometer. Optical density (OD) was measured at 350 nm as temperature was increased by 1 °C/min for CPP-$ELP_{BC}$ samples at 15 μM in PBS. A moderate increase in optical density (OD at 350 nm < 0.1) indicated the transition from soluble unimer to micelle and a drastic increase in optical density indicated aggregation of $ELP_{BC}$ micelles into micron-size coacervates. Turbidimetry thus allowed for approximation of the CMT and the temperature at which complete aggregation occurred. The temperature-controlled self-assembly of $ELP_{BC}$s at 15 μM in PBS was confirmed with DLS measurements taken at 37 °C and 42 °C on a Wyatt Technology DynaPro temperature controlled microsampler, after passing the sample through a 0.02 μm filter.

Thermal properties of CPP-$ELP_{BC}$ drug conjugates were characterized by temperature-regulated turbidimetry and DLS to confirm that functionalization with peptide drug and cleavable peptide linker did not perturb the temperature-controlled self-assembly of CPP-$ELP_{BC}$s (Figure S3A-D). Turbidimetry, in addition to DLS measurements, confirmed that the appended

peptide drug and linker did not perturb the thermal properties, such that these constructs existed as unimers at 37 °C, and self-assembled into micelles at 42 °C. CPP-$ELP_{BC}$s with drug and linker were, however, more prone to low levels of aggregation, where aggregates of several hundred nanometers were apparent at low mass percentage at both thermal conditions. These constructs were thus filtered prior to characterization and *in vitro* testing.

An additional control was synthesized in which $Arg_8$-$ELP_{BC}$ was appended with the cleavable RVRR peptide linker alone, to confirm that this linker sequence did not contribute significantly to the cytotoxic action of the drug carrier in the absence of BH3 peptide drug. This $Arg_8$-$ELP_{BC}$-RVRR construct was characterized with temperature-regulated turbidimetry (Figure S3E), which confirmed that functionalization with the linker alone did not perturb the desired thermal properties of the $Arg_8$-$ELP_{BC}$-RVRR control. DLS further confirmed the temperature-dependent self-assembly of this construct, which existed as a unimer at 37 °C ($R_H$ = 7.7 ± 0.3 nm) and a micelle at 42 °C ($R_H$ = 24.4 ± 1.2 nm).

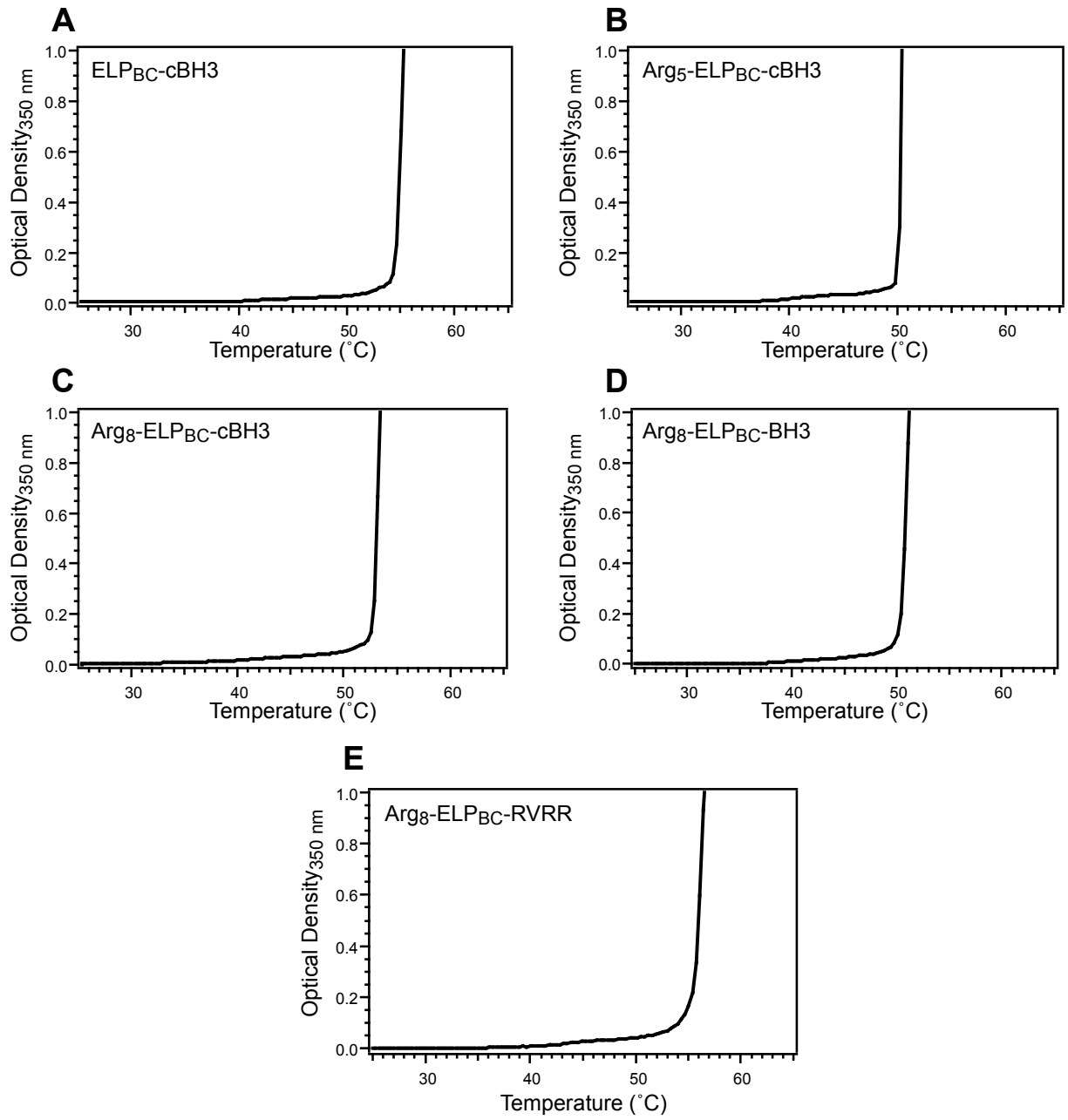


**Figure S3.** Thermal characterization of CPP-ELP$_{BC}$ drug conjugates and controls. Samples at 15 μM in PBS were characterized by temperature-regulated turbidimetry. Changes in optical density of (A) non-functionalized ELP$_{BC}$-cBH3, (B) Arg$_5$-ELP$_{BC}$-cBH3, (C) Arg$_8$-ELP$_{BC}$-cBH3, (D) Arg$_8$-ELP$_{BC}$-BH3, and (E) Arg$_8$-ELP$_{BC}$-RVRR confirmed that functionalization with BH3 peptide drug and/or cleavable linker, did not perturb the tuned thermal properties that allowed CPP-ELP$_{BC}$s to exist as unimers at 37 °C and self-assemble into micelles at 42 °C.

**Confocal microscopy.** HeLa cells were seeded at $2x10^4$ cells per well on 8-well chambered coverglass and allowed to adhere for 24 h. Cells were washed with PBS and incubated with 15 μM of 40% Alexa Fluor 488-labeled CPP-$ELP_{BC}$ in serum-free DMEM (SF-DMEM) for 1 h at 37 °C or 42 °C. Peptide solutions were removed and cells were incubated in 5 μg/mL Alexa Fluor 594 wheat agglutinin and 2 μM Hoechst 33342 in Hank's balanced salt solution (HBSS) for 10 min. Cells were washed 3 times with PBS and imaged immediately on a Leica SP5 confocal microscope. Images were acquired with a 63x water immersion objective. Signal from the CPP-$ELP_{BC}$ represents the maximum projection through the z-dimension of the cell.

**Flow cytometry.** HeLa cells were seeded at $5x10^4$ cells per well on 12-well tissue culture treated polystyrene plates. Cells were allowed to adhere for 24 h before the cells were washed with PBS and incubated with 10 μM of 50% Alexa Fluor 488-labeled CPP-$ELP_{BC}$s in SF-DMEM. Following a 1 h incubation at 37 °C or 42 °C, the cells were washed with PBS and gently removed from the wells with the addition of 0.05% trypsin/EDTA. Cells were collected, washed once with complete DMEM, and twice more with PBS prior to immediate analysis with a BD LSRII flow cytometer. Healthy cells were gated by forward and side scatter and the median fluorescence of each cell population was corrected to an untreated cell control. Cellular fluorescence was averaged over 3 experiments and normalized to cells treated with non-functionalized $ELP_{BC}$ at 37 °C.

**Furin cleavage.** Furin reaction buffer was prepared with 100 mM HEPES containing 0.5% Triton®X-100, 1 mM $CaCl_2$, and 1 mM 2-mercapto-ethanol at pH 7.5. CPP-$ELP_{BC}$s were

incubated with 2 units of Furin in reaction buffer for 6 h at 37 °C. The reaction was mixed 1:1 with tricine sample buffer containing 2-mercaptoethanol and heated to 95 °C for 2 min. Reaction products were separated on a SDS-PAGE 10-20% Tris-tricine gel and stained with $CuCl_2$ to visualize the ELP by negative staining (minimally charged ELPs do not stain well with conventional coomassie blue staining), washed with water, and subsequently stained with coomassie blue to visualize the released BH3 peptide (Figure S4). Although the arginine-rich CPPs of $Arg_5$-$ELP_{BC}$-cBH3, $Arg_8$-$ELP_{BC}$-cBH3, and $Arg_8$-$ELP_{BC}$-BH3 are competitive substrates for furin cleavage,[4] they did not interfere with BH3 release and did not appear as cleavage products.

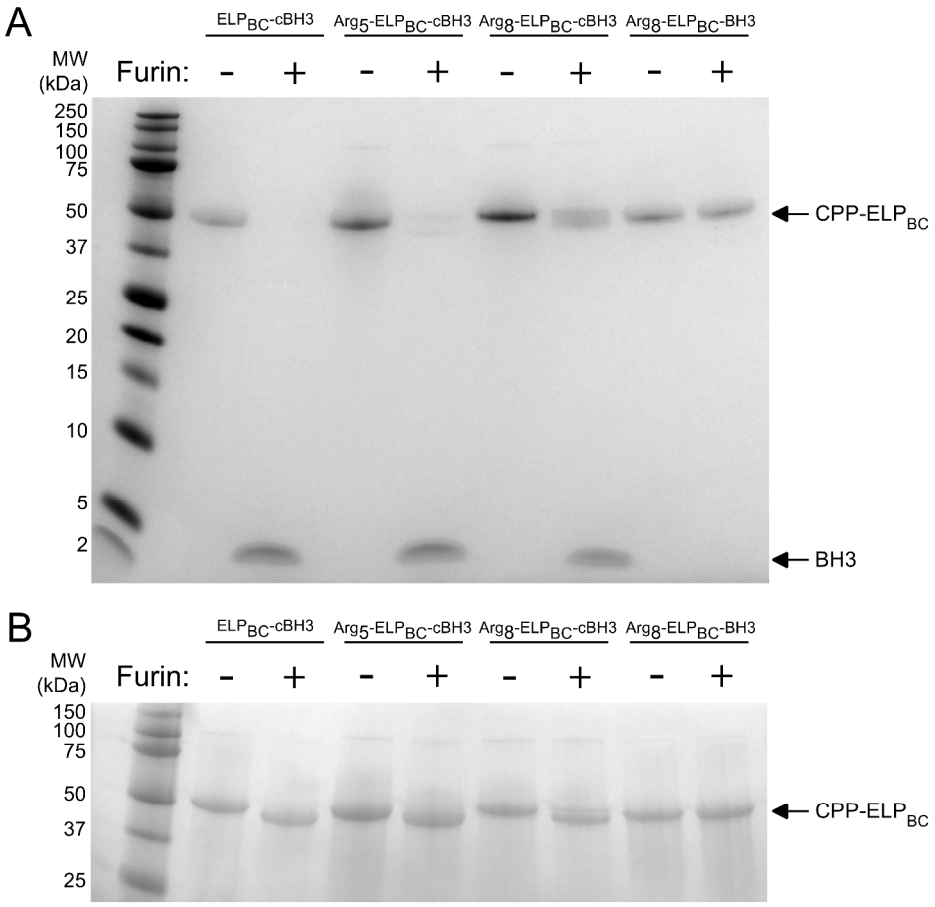

**Figure S4.** SDS-PAGE analysis of free peptide drug release by furin cleavage. A) Coomassie blue staining of cleaved free BH3 peptide (MW: 2.74 kDa, where furin cleaves at the C-terminus of the RVRR linker producing the fragment MGQVGRQLAIIGDDINRRYRVRR) from the CPP-$ELP_{BC}$ (approximate MW: 40 kDa, depending on CPP functionalization). B) $CuCl_2$ staining of CPP-$ELP_{BC}$s with and without furin digestion.

**Cytotoxicity assay.** HeLa cells were seeded at $4x10^4$ cells per well on 96-well tissue culture treated polystyrene plates. Cells were allowed to adhere for 24 h before the cells were washed with PBS and incubated with 15 μM CPP-$ELP_{BC}$ with or without an appended BH3 peptide drug and cleavable peptide linker in SF-DMEM. Cells were incubated for 1 h at 37 °C or 42 °C after which the cells were washed with PBS, provided complete media and returned to 37 °C for 24 h. Surviving cells were quantified by cell titer 96 aqueous non-radioactive cell proliferation (MTS) assay and normalized to untreated cells incubated at 37 °C.

Cytotoxicity of CPP-$ELP_{BC}$s without drug cargo was measured to confirm that the carrier alone did not contribute significantly to cell death. None of the CPP-$ELP_{BC}$s or the $ELP_{BC}$ control showed significantly different cell survival from untreated controls (Figure S5). Additionally, untreated cells incubated at 37 °C or 42 °C showed no significant difference in cell survival, confirming that mild hyperthermia alone had no effect on cytotoxicity in the context of this *in vitro* assay.

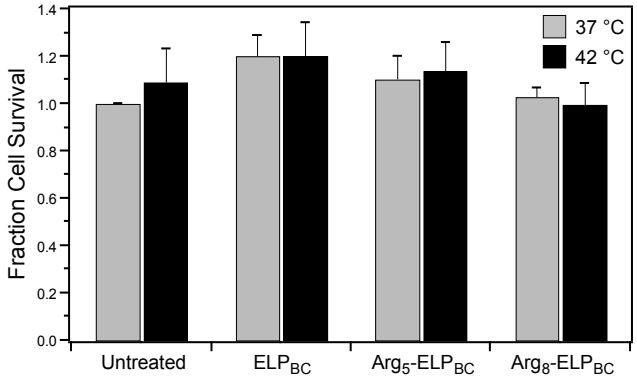


**Figure S5.** Quantification of CPP-ELP$_{BC}$ cytotoxicity without drug cargo. Cell survival was normalized to untreated cells incubated at 37 °C. CPP-ELP$_{BC}$ carriers did not cause cytotoxicity without drug cargo and mild hyperthermia alone did not result in cell death. Data represents mean of 3 experiments ± SEM.

The toxicity of the cleavable RVRR linker was evaluated with the Arg$_8$-ELP$_{BC}$-RVRR construct (Figure S6). Cell survival with treatment of Arg$_8$-ELP$_{BC}$-RVRR at 42 °C was lower than those cells treated at 37 °C, but this effect was not significant. However, this small, albeit statistically insignificant, effect may suggest a minor role of the RVRR linker in the cytotoxicity of Arg$_8$-ELP$_{BC}$-cBH3, which incorporates this cleavable linker in addition to the BH3 peptide drug.

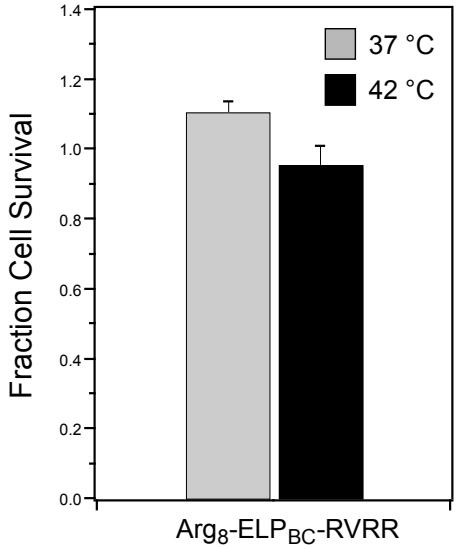

**Figure S6.** Quantification of $Arg_8$-$ELP_{BC}$-RVRR cytotoxicity. Decreased cell survival with $Arg_8$-$ELP_{BC}$-RVRR treatment at 42 °C was not significant compared to cells treated at 37 °C. Data represents mean of 3 experiments ± SEM.

**Caspase-3 activity assay.** HeLa cells were seeded at $5x10^4$ cells per well on 12-well tissue culture treated polystyrene plates. Cells were allowed to adhere for 24 h. The media was removed and cells were washed with PBS prior to the addition of 15μM CPP-$ELP_{BC}$-cBH3 or free BH3 peptide in SF-DMEM. After incubation for 1 h at 37 °C or 42 °C, the cells were washed with PBS, provided complete media, and incubated at 37 °C for an additional 5 h. Cells were then gently removed from the culture surface with 0.05% trypsin/EDTA and analyzed for caspase-3 activity with EnzChek® Caspase-3 Assay Kit #2, following the manufacturer's instructions. Cells were incubated with lysis buffer on ice for 30 min and insoluble cell debris was removed by centrifugation at 5,000 RPM for 5 min. The cell lysate was incubated with Z-DEVD-R110 substrate for 1 h and the fluorescence of the reaction products was evaluated at 520 nm on a Perkin Elmer Victor$^3$ plate reader. Protein content was evaluated in each sample by BCA assay. Caspase-3 activity, as measured by fluorescence from the EnzChek® assay, was corrected to protein content to compensate for slight variations in cell number between samples. Caspase-3 activity was further normalized to the level of caspase-3 activity seen in untreated cells incubated at 37 °C.

The effect of hyperthermia alone on the induction of apoptosis was evaluated by quantification of caspase-3 activity in untreated cells exposed to 37 °C or 42 °C (Figure S7). No significant difference in caspase-3 activity was detected between cells incubated at 37 °C or 42 °C,

confirming that mild hyperthermia alone did not induce apoptosis in the context of this *in vitro* assay.

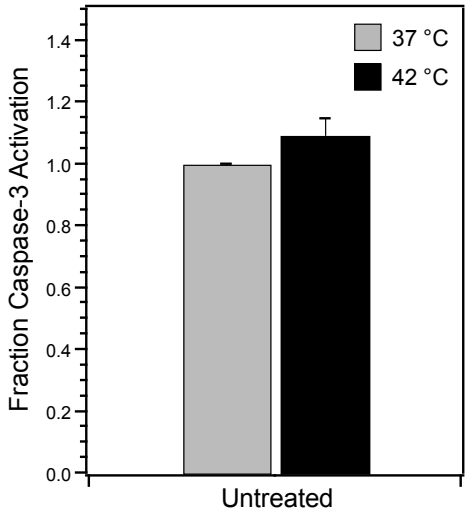


**Figure S7.** Caspase-3 activation in untreated cells. No significant difference in caspase-3 activity was detected between untreated cells incubated at 37 °C or 42 °C. Data represents mean of 3 experiments ± SEM.

**Statistical Analysis.** Data sets including two independent variables (ex. CPP-$ELP_{BC}$ treatment and temperature) were evaluated by two-way analysis of variance (ANOVA) to confirm the significance of the main effects of both ELP treatment and temperature, as well as to confirm the interaction effect between these variables. Bonferroni-corrected post-hoc t-tests were used to identify significant differences between means. Data sets with one independent variable (ex. CPP-$ELP_{BC}$ treatment) were evaluated by one-way ANOVA to confirm a difference in means within the data set and Bonferroni-corrected post-hoc t-tests were used to identify significant differences between these means. All statistical analysis was performed with IBM SPSS Statistics 20 software. Significance was defined at $p$-values<0.05, unless otherwise noted.

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