Supporting Information

Co-Delivery of Protein and Small Molecule Therapeutics using Nanoparticle-Stabilized Nanocapsules

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Table S1. Size and zeta potential of arginine-functionalized gold nanoparticles (Arg-AuNP) were measured by DLS at 5mM PB, pH=7.4.

Name	Hydrodynamic diameter (nm)	Zeta potential (mV)
Arg-AuNP	16 ± 8	43 ± 11

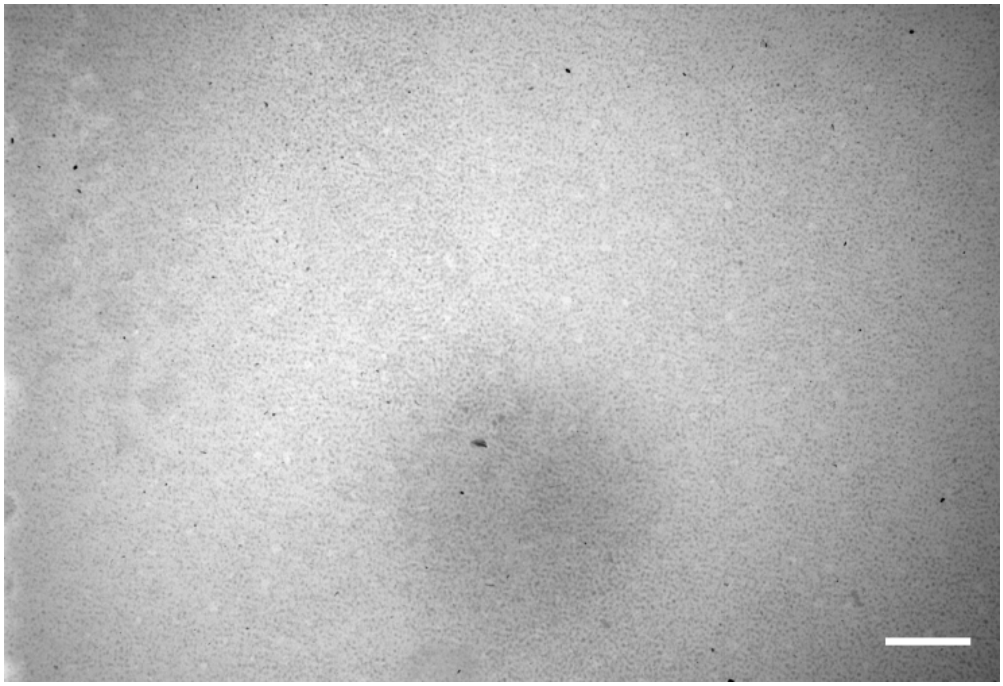

Figure S1. TEM image of Arg-AuNP. The TEM image confirmed 2 nm core diameters of gold nanoparticles (Arg-AuNP). Scale bar: 100 nm.

Table S2. Characterization of NPSCs. Size and zeta potential of NPSCs were measured by DLS.

	PTX	CASP3	TF	Hydrodynamic diameter (nm)	Zeta potential (mV)
NPSC	-	-	-	109 ± 58	-59 ± 9
NPSC-PTX	+	-	-	107 ± 52	-53 ± 7
NPSC-TF	-	-	+	123 ± 64	-52 ± 12
NPSC-CASP3	-	+	-	129 ± 50	-54 ± 14
NPSC-PTX-TF	+	-	+	140 ± 50	-49 ± 14
NPSC-PTX-CASP3	+	+	-	142 ± 64	-55 ± 9

TEM sample preparation

TEM samples were prepared by drying a drop (10 µL) of the NPSC solution on a copper grid coated with amorphous carbon. For protein-stabilized capsules, 20 µL uranyl acetate solution (2 wt% in water) was added to the copper grid. After 1 min, the grid was blotted with a piece of filter paper. The grid was finally dried overnight at room temperature before TEM imaging.[1]

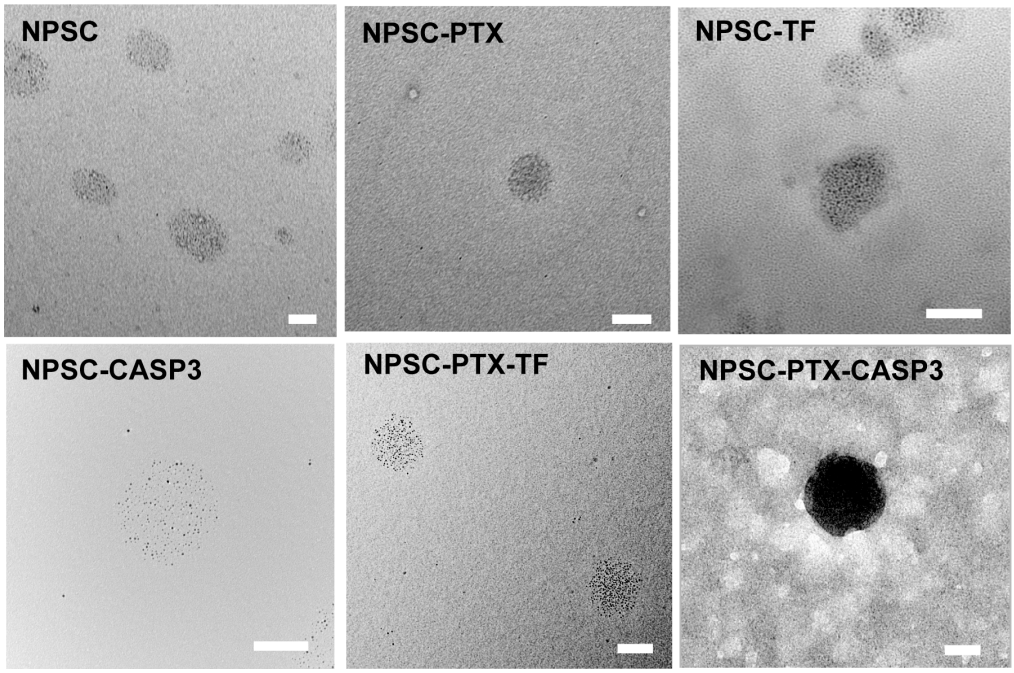

Figure S2. TEM images of different NPSCs. Scale bar: 100 nm.

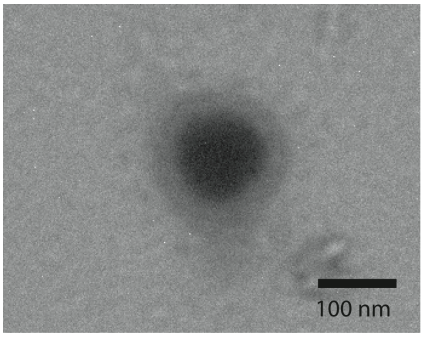

Figure S3. NPSC-CASP3 was stained with uranyl acetate (2% (v/v) in MilliQ water). The halo around the AuNP core indicates the addition of protein to the surface. The average thickness of the protein shell of NPSC-CASP3 is 21 nm ± 1 nm (sample number = 5).

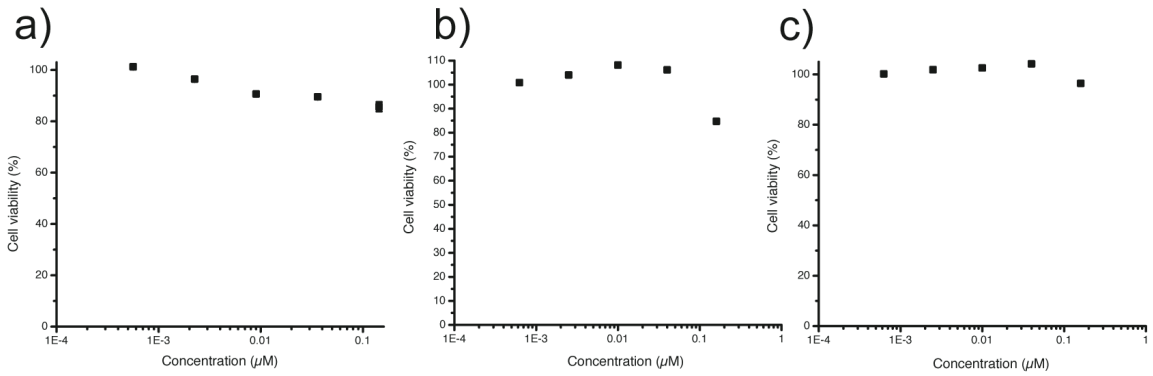

Figure S4. Cell viability studies with (a) Arg-AuNP, (b) CASP3, and (c) transferrin. Cell viabilities with different concentrations were measured with an Alamar blue assay.

Caspase-3 (CASP3) expression and purification

The full-length human *CASP3* gene in the pET23b vector[2] (supplied by Addgene) was used for the expression of recombinant CASP3 in BL21(DE3) *E. coli*. Briefly, the cells were harvested after a three-hour IPTG induction to achieve an enzymatic self-activation of CASP3. The supernatant of lysate was loaded onto a 5 mL HiTrap Ni-affinity column (GE Healthcare) followed by a washing step using 50 mM sodium phosphate (pH 8.0), 300 mM NaCl, and 50 mM imidazole. The CASP3 protein was eluted with a buffer containing 50 mM sodium phosphate (pH 8.0), 300 mM NaCl, and 250 mM imidazole. The eluted fraction was 7-fold diluted into 25 mM Tris pH 8.0 and 5 mM DTT and loaded onto the 5 mL Macro-Prep High Q column (Bio-Rad Laboratories, Inc.). The column was developed with a linear NaCl gradient elution and the CASP3 was finally eluted in 25 mM Tris pH 8.0, 120 mM NaCl and 5 mM DTT. The eluted protein was stored at -80 °C in the elution buffer conditions. The active form of CASP3 was analyzed by SDS-PAGE to be ~98% pure (Figure S5). Further ESI-MS analysis was carried on to confirm the mass (Table S4).

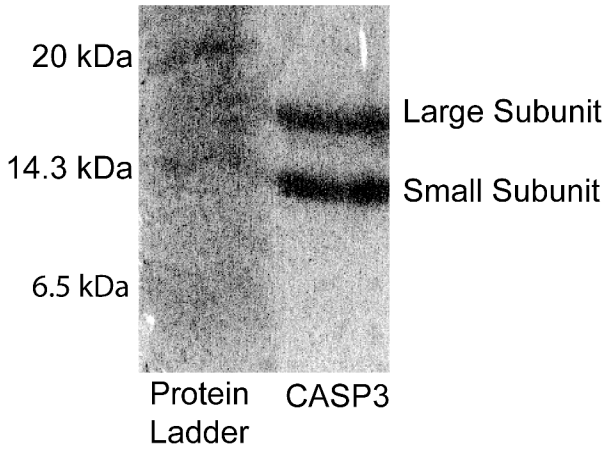

Figure S5. SDS-PAGE of CASP3.

Table S3. Molecular weight of CASP3. The molecular weight of CASP3 was identified by mass spectrometry.

	Prediction	**Mass spect.**
Large subunit	16.6 kDa	16.6 kDa
Small subunit	13.0 KDa	13.0 KDa

Isoelectric point of CASP3: The isoelectric point of mature CASP3 (pI=6.7) was calculated using following sequences for the large and small subunits respectively.

Large subunit:

SGISLDNSYKMDYPEMGLCIIINNKNFHKSTGMTSRSGTDVDAANLRETFRNLKYEVRN
KNDLTREEIVELMRDVSKEDHSKRSSFVCVLLSHGEEGIIFGTNGPVDLKKITNFFRGDR
CRSLTGKPKLFIIQACRGTELDCGIETD

Small subunit:

SGVDDDMACHKIPVEADFLYAYSTAPGYYSWRNSKDGSWFIQSLCAMLKQYADKLEF
MHILTRVNRKVATEFESFSFDATFHAKKQIPCIVSMLTKELYFYHLEHHHHHH

(1) Wang, Y., Fang, J., Cheng, D., Wang, Y., and Shuai, X. (2014) A pH-sensitive micelle for codelivery of siRNA and doxorubicin to hepatoma cells. *Polymer 55*, 3217-3226.
(2) Zhou, Q., Snipas, S., Orth, K., Muzio, M., Dixit, V. M., and Salvesen, G. S. (1997) Target protease specificity of the viral serpin CrmA. Analysis of five caspases. *J. Biol. Chem. 272*, 7797-7800.