

# **Nanomotor-Enabled pH-Responsive Intracellular Delivery of Caspase-3: Toward Rapid Cell Apoptosis**

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## **SUPPORTING INFORMATION**

### **1. Supporting Videos description**

### **2. Supporting Figures**

### **3. Supporting Table**

### **4. References**

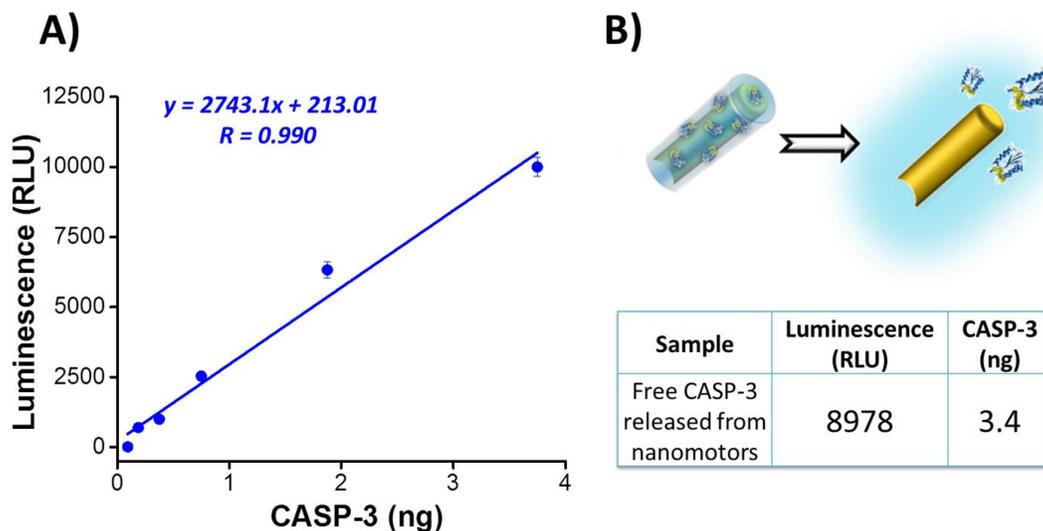
## 1. Supporting Videos description

**Supporting Video S1.** US-propelled pH-responsive polymer-CASP-3@AuNW approaching an AGS cell (US field: 6 V and 2.56 MHz).

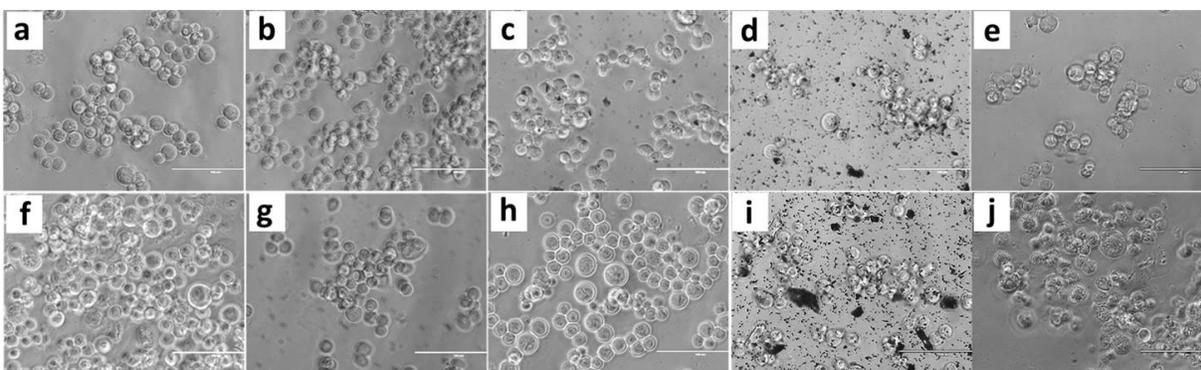
**Supporting Video S2.** Comparison of US-propulsion of AuNWs and pH-responsive polymer-CASP-3@AuNWs (US field: 6 V and 2.56 MHz).

**Supporting Video S3.** Dense aggregation of AGS cells (light spheres) and pH-responsive polymer-CASP-3@AuNWs (black rods) attached to the surface of the cells and inside the cells due to the US field (6 V and 2.56 MHz).

## 2. Supporting Figures



**Figure S1.** Estimation of CASP-3 loading on the nanomotors. (A) Calibration of CASP-3 obtained by using the Caspase-Glo<sup>®</sup> 3/7 Assay, and (B) calculated amount of CASP-3 from enzyme-loaded nanomotors (mean value calculated from the average of 3 measurements)



**Figure S2.** Optical microscope images corresponding to untreated cells (a), and cells treated with: only US (b), US-powered-polymer-AuNWs (no CASP-3) (c), static-polymer-CASP-3@AuNWs (d), CASP-3 released from motors and static conditions (e), 3.4 ng of free CASP-3 under static conditions (f), CASP-3 released from motors and US conditions (g), 3.4 ng of free CASP-3 under US conditions (h), US-powered-polymer-CASP-3@AuNWs (i) and Triton X-100 (j).

### 3. Supporting Table

**Table S1.** Comparison of CASP-3-based apoptosis strategies.

Strategy	CASP-3 amount	Apoptosis time	Apoptosis, %	Reference
Lipid-mediated intracellular delivery	3.3 ng	o/n	58.0	1
BioPorter™ Protein delivery reagent	250 ng	2 days	80.0	2
Intracellular delivery with NP-Stabilized Nanocapsules	$7.5 \times 10^8$ ng	1 h	72.0	3
Intracellular delivery by PLGA-CNTs conjugates	3,000 ng/mL	3 days	70.0	4
Intracellular delivery by cell-penetrating Poly(disulfide)s	50 nM	2 h	50.0	5
Intracellular delivery based on reversible and specific assembly of His-tagged CASP-3 onto soluble Ni-immobilized polymer	1,536 ng	1 day	80.0	6
Au-nanosurface energy transfer probe for real-time monitoring of cell apoptosis	3.75 ng	3 h	70.0	7
Intracellular delivery by US-propelled polymer/CASP-3@AuNWs	3.4 ng	5 min	80.0	This work

## 4. References

1. Zelphati, O.; Wang, Y.; Kitada, S.; Reed, J. C.; Felgner, P. L.; Corbeil, J. Intracellular Delivery of Proteins with a New Lipid-Mediated Delivery System. *J. Biol. Chem.* **2001**, *276*, 35103–35110.
2. Zassler, B.; Blasig, I. E.; Humpel, C. Protein Delivery of Caspase-3 Induces Cell Death in Malignant C6 Glioma, Primary Astrocytes and Immortalized and Primary Brain Capillary Endothelial Cells. *J. Neurooncol.* **2005**, *71*, 127–134.
3. Tang, R.; Kim, C. S.; Solfiell, D. J.; Rana, S.; Mout, R.; Velázquez-Delgado, E. M.; Chompoosor, A.; Jeong, Y.; Yan, B.; Zhu, Z. J.; Kim, C.; Hardy, J. A.; Rotello, V. M. Direct Delivery of Functional Proteins and Enzymes to the Cytosol Using Nanoparticle-Stabilized Nanocapsules. *ACS Nano* **2013**, *7*, 6667–6673.
4. Cheng, Q.; Blais, M. O.; Harris, G.; Jabbarzadeh, E. PLGA-Carbon Nanotube Conjugates for Intercellular Delivery of Caspase-3 into Osteosarcoma Cells. *PLoS One* **2013**, *8*, e81947.
5. Fu, J.; Yu, C.; Li, L.; Yao, S. Q. Intracellular Delivery of Functional Proteins and Native Drugs by Cell-Penetrating Poly(disulfide)s. *J. Am. Chem. Soc.* **2015**, *137*, 12153–12160.
6. Postupalenko, V.; Desplancq, D.; Orlov, I.; Arntz, Y.; Spehner, D.; Mely, Y.; Klaholz, B. P.; Schultz, P.; Weiss, E.; Zuber, G. Protein Delivery System Containing a Nickel-Immobilized Polymer for Multimerization of Affinity-Purified His-Tagged Proteins Enhances Cytosolic Transfer. *Angew. Chem., Int. Ed.* **2015**, *54*, 10583–10586.
7. Li, Y.; Li, P.; Zhu, R.; Luo, C.; Li, H.; Hu, S.; Nie, Z.; Huang, Y.; Yao, S. Multifunctional Gold Nanoclusters-Based Nanosurface Energy Transfer Probe for Real-Time Monitoring of Cell Apoptosis and Self-Evaluating of Pro-Apoptotic Theranostics. *Anal. Chem.* **2016**, *88*, 11184–11192.