

Supporting Information

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SI Methods

Synthesis of Peptide Conjugated Gold Nanoparticles (Satellite Nanoparticles). Biotinylated peptide (6 pmol) was added to the gold nanoparticle solution (3 nM) and reacted overnight. Then, 10^5 molar excess of $\text{HOOCCH}_2(\text{OCH}_2\text{CH}_2)_6\text{S-S}(\text{OCH}_2\text{CH}_2)_6\text{-CH}_2\text{COOH}$ was added to the solution and further reacted for 2 h. Peptide-conjugated nanoparticles were separated by agarose gel electrophoresis at 100 V for 1 h and isolated. Centrifugation of the nanoparticle solution at 1800 RCF for 10 min yielded red pellets. Clear supernatant was decanted and the nanoparticle pellet was resuspended in T100 buffer (10 mM Tris, pH 8.0, 100 mM NaCl) to a final concentration of 5 nM.

Synthesis of Neutravidin (Ntv)-Coated Gold Nanoparticles (Core Nanoparticles). A 10^5 molar excess of $\text{HOOCCH}_2(\text{OCH}_2\text{CH}_2)_6\text{S-S}(\text{OCH}_2\text{CH}_2)_6\text{-CH}_2\text{COOH}$ and biotin- $\text{CH}_2(\text{OCH}_2\text{CH}_2)_6\text{S-S}(\text{OCH}_2\text{CH}_2)_6\text{-CH}_2\text{-biotin}$ mixture (25:1 molar ratio) was added to a gold nanoparticle solution (3 nM) and reacted for 2 h. The nanoparticles were separated by gel electrophoresis as described above. A 10,000 equivalent amount of succinylated Ntv was then added to the nanoparticle solution. After an overnight reaction at 4 °C, nanoparticles were isolated via centrifugation at 1800 RCF for 10 min and dissolved in T100 buffer. The excess amount of Ntv was removed by repeated washing with T100 buffer (3 times) by using a Centricon (Amicon). The final concentration of Ntv-coated nanoparticles was adjusted to 1 nM.

1. Heerdt BG, Houston MA, Mariadason JM, Augenlicht LH (2001) Dissociation of staurosporine-induced apoptosis from G₂-M arrest in SW620 human colonic carcinoma cells: Initiation of the apoptotic cascade is associated with elevation of the mitochondrial membrane potential (1.1_m). *Cancer Res* 60:6704–6713.

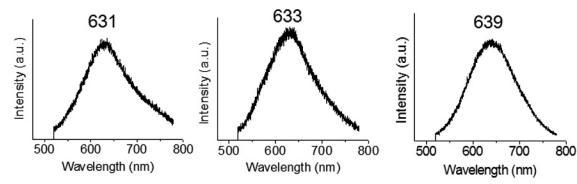


Fig. S1. Representative scattering spectra of single crown nanoparticles.

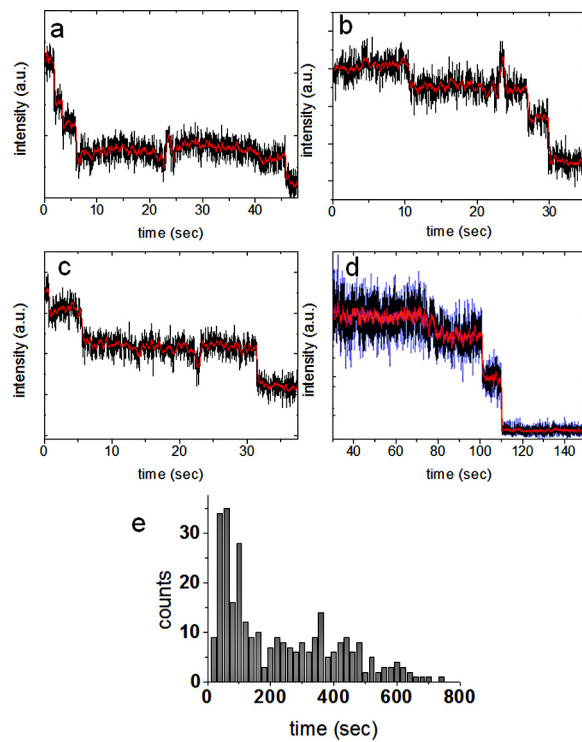


Fig. S2. (a–d) Representative single crown nanoparticle trajectories and (e) histogram of cleavages ($n = 300$) during the experimental time course.

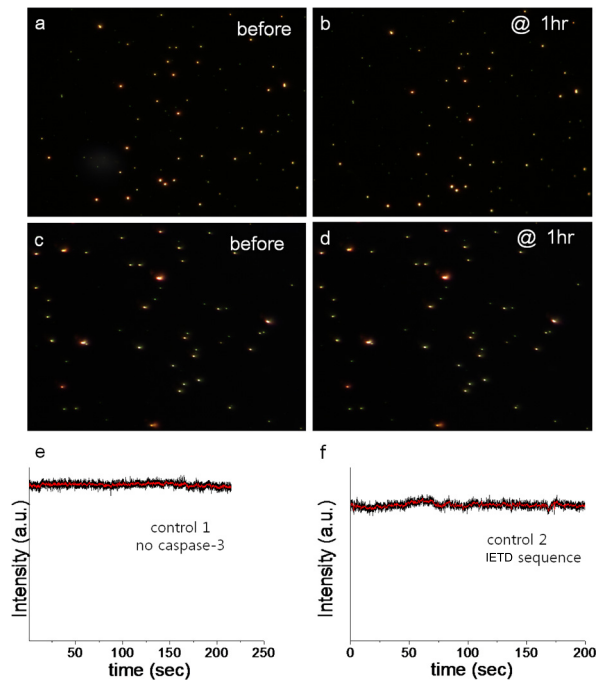


Fig. S3. Control experiments for detection of in vitro caspase-3 activity (*a* and *b*) Scattering images of crown nanoparticles that were not treated with caspase-3. (*c* and *d*) Scattering images of crown nanoparticles that are linked by a peptide containing the sequence IETD, instead of DEVD. Although it is reported that that Ac-IETD-AFC can be cut by caspase-3, its cleavage activity is almost negligible at the very low concentration of caspase-3 that we used (250 ng/mL or 4.31 nM). No changes in color and intensity were observed for these controls. (*e* and *f*) Representative single particle trajectories of the non-treated crown nanoparticles linked by peptides containing the sequence DEVD (*e*) and caspase-3 treated crown nanoparticles linked by peptides containing the sequence IETD (*f*).

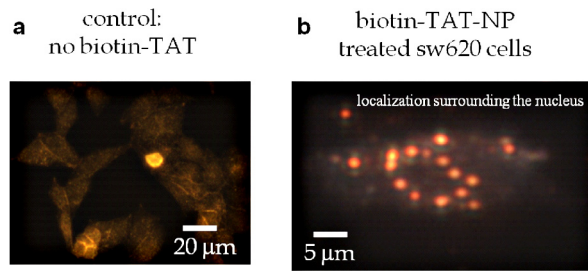


Fig. S4. Delivery of crown nanoparticles inside cells. (a) Dark field microscope images of HeLa cells incubated with unmodified crown nanoparticles (b), SW620 cells incubated with TAT conjugated nanoparticles. Localization of nanoparticle scattering surrounding the nucleus indicates the nanoparticles are either in the cytosol or in endosomes rather than in surface membrane.

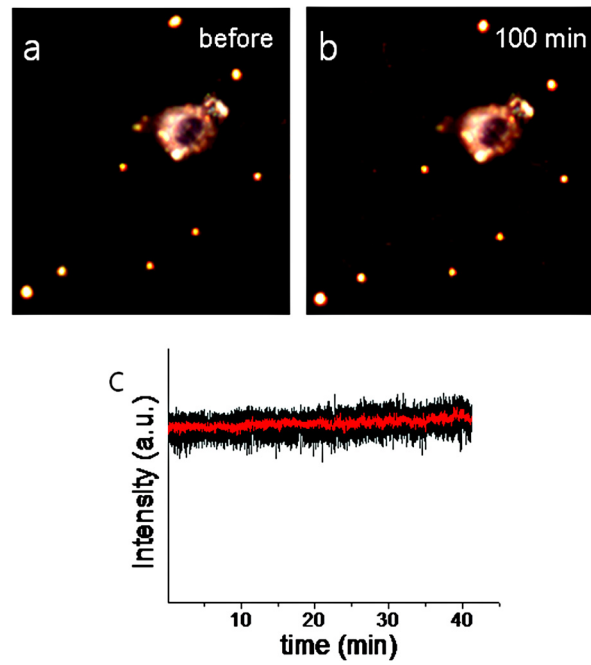


Fig. S5. Behavior of crown nanoparticles without TAT modification that have nonspecifically bound to the cell culture plate. (a and b) Scattering images show nonspecifically bound crown nanoparticles before (a) and 100 min after (b) the addition of TNF- α /CHX to SW620 cells. (c) A representative single particle trajectory of the nonspecifically bound crown nanoparticles.

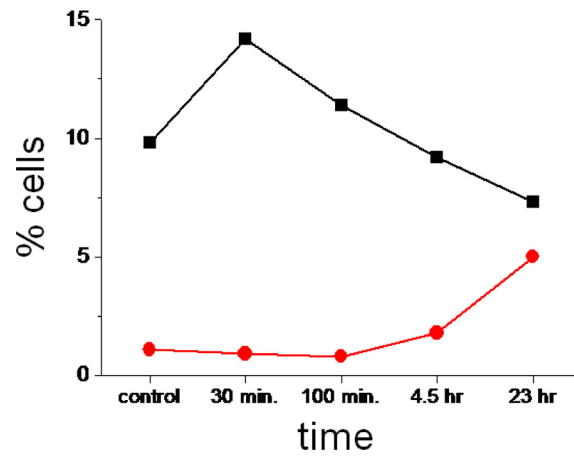


Fig. 57. Percentage of cells within region II (black line) and within region III (red line) of Fig. 3D in the main text.

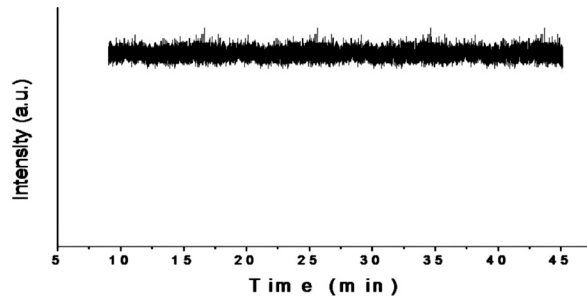


Fig. S9. Trajectory of a single crown nanoparticles located inside vehicle treated SW620 cells.

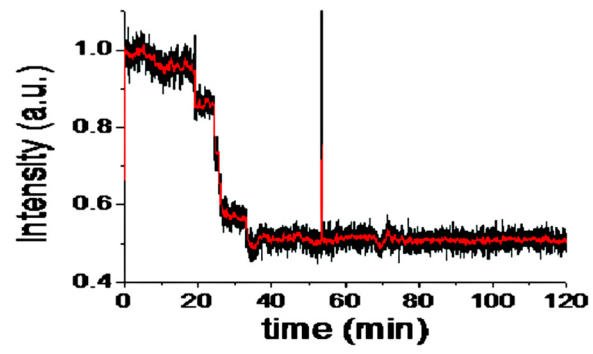


Fig. S10. A trajectory of a single crown nanoparticle located inside TNF- α /CHX treated SW620 cells for two hours.

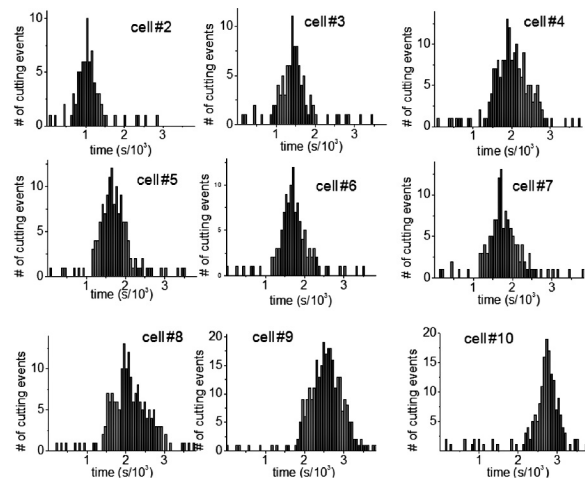
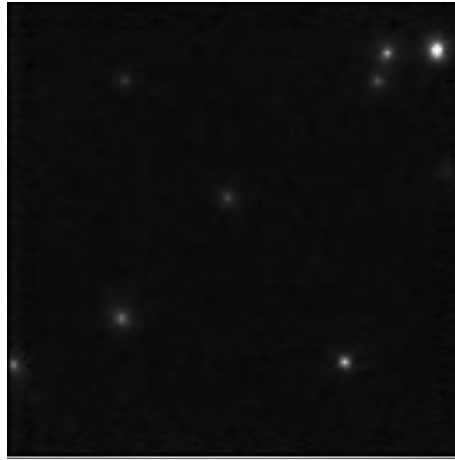
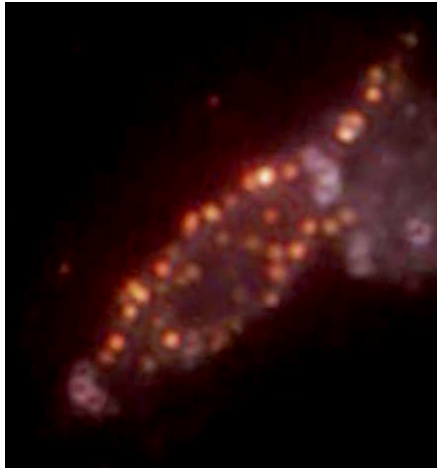


Fig. S11. Cleavage statistics of nanoparticles located inside 10 individual cells from the same group. For cell #1, see Fig. 4C.



Movie S1. In vitro imaging of enzymatic cleavages of crown nanoparticle plasmon rulers.

[Movie S1 \(MP4\)](#)



Movie S2. Continuous in vivo imaging of caspase-3 activation in SW620 cells using crown nanoparticle plasmon rulers (2 h).

[Movie S2 \(MP4\)](#)