Supporting Information

Sensitive and Selective Plasmon Ruler Nanosensors for Monitoring the Apoptotic Drug Response in Leukemia

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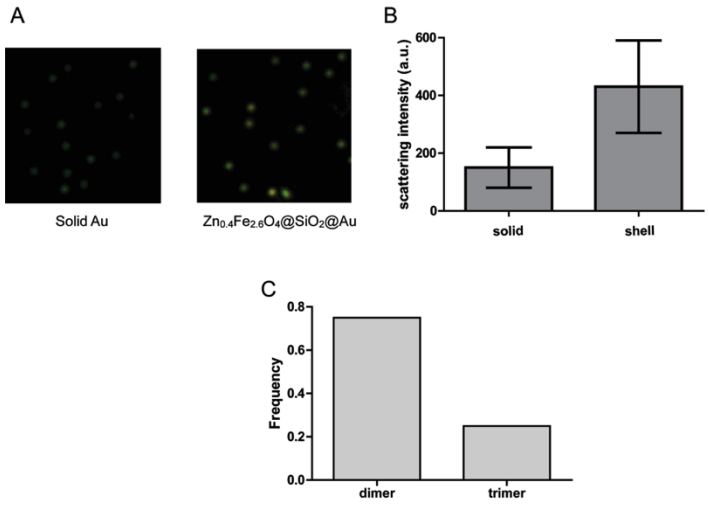
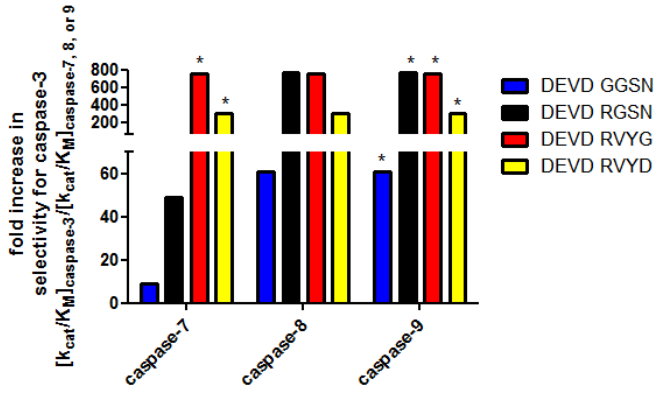
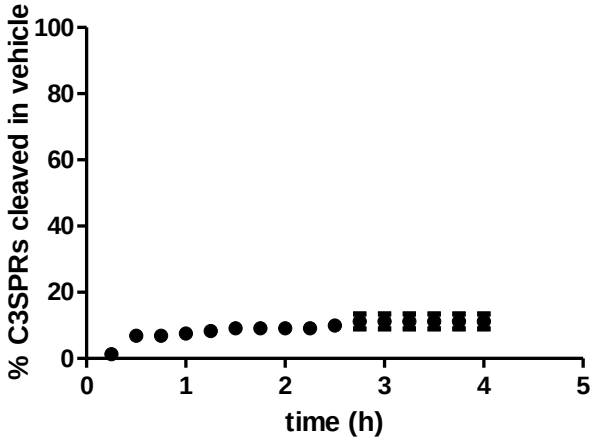


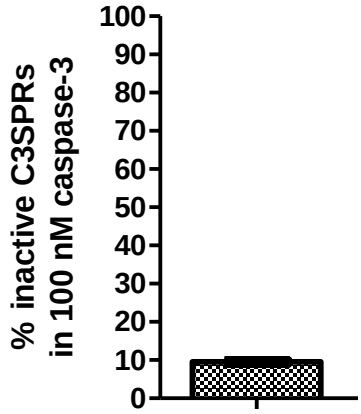
Supplementary Figure 1. Characterization of a caspase-3 selective plasmon ruler (C3SPR). (A) Dark-field microscopy images of solid Au nanoparticles (left) *versus* $Zn_{0.4}Fe_{2.6}O_4$@ SiO_2@Au core-shell nanoparticles (right) and (B) resulting light scattering intensities show a ~3-fold increase with the $Zn_{0.4}Fe_{2.6}O_4$@ SiO_2@Au core-shell nanoparticles. (C) Following synthesis of the C3SPRs, yield consisted of 75% dimers and 25% trimers by TEM.



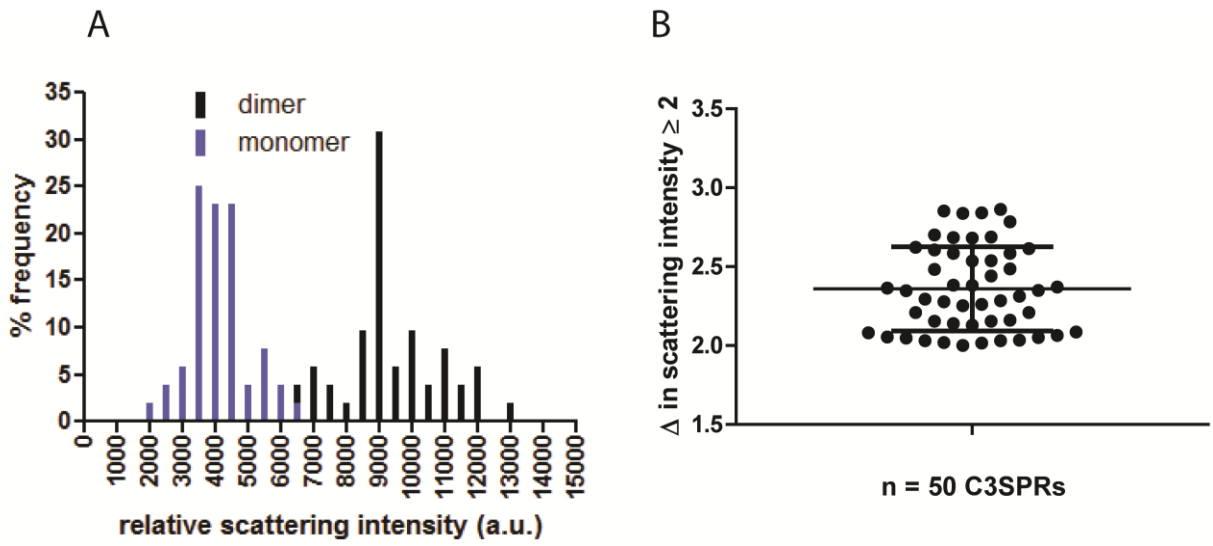
Supplementary Figure 2. Caspase-3 selectivity among DEVD-containing fluorescent peptides flanked by donor/quencher moieties. Selectivity was measured by calculating the ratio of the catalytic efficiencies of caspase-3 over that of caspase-7, -8, or -9 for a given peptide. *Indicates conditions where selectivity is above the reported value due to the absence of activity of either caspase-7 or -9 in the fluorescence assay.



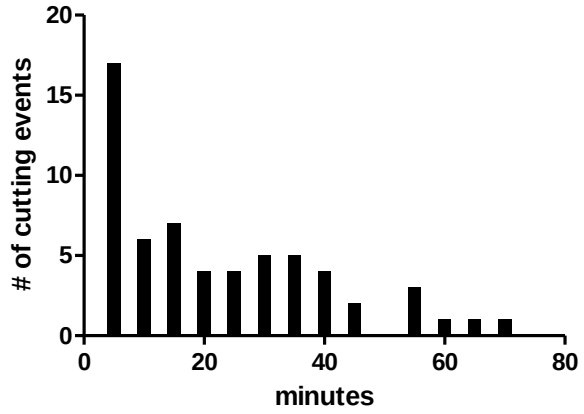
Supplementary Figure 3. C3SPRs indirectly responded to vehicle treatment (10%) and value was subtracted from nanoparticle activity measurements reported in Figures 2 and 3.



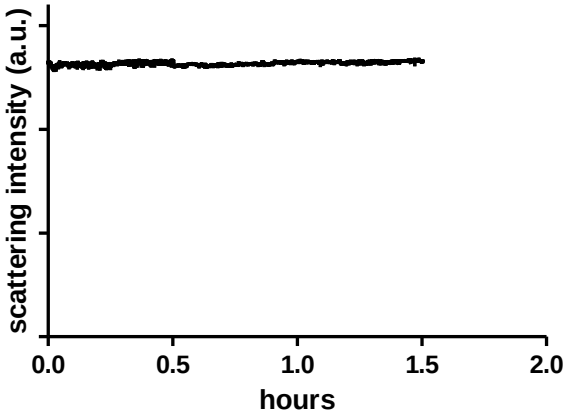
Supplementary Figure 4. C3SPRs treated with excess caspase-3 (100 nM) to quantify fraction of inactive sensors. 9.5 +/- 0.7% of the C3SPRs are inactive in a given batch of nanoparticles.



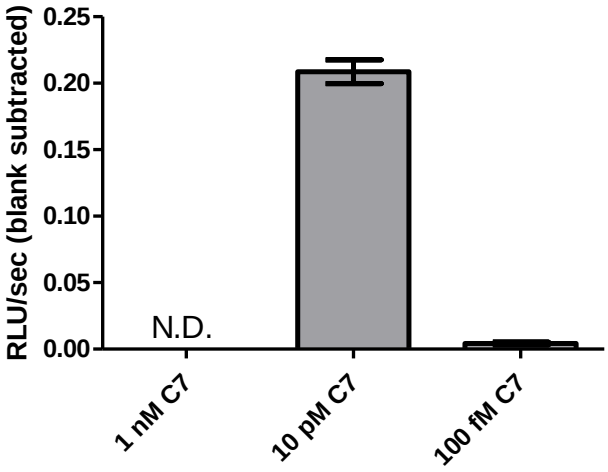
Supplementary Figure 5. Dimers and cleaved monomeric product are distinguishable under dark-field microscopy *via* scattering intensity. 50 C3SPRs (A) have an initial and final relative scattering intensity upon exposure to caspase-3. (B) The change in scattering intensity greater than or equal to 2 shows a transition from dimer to monomer under microscopy conditions. Average = 2.4 +/- 0.3.



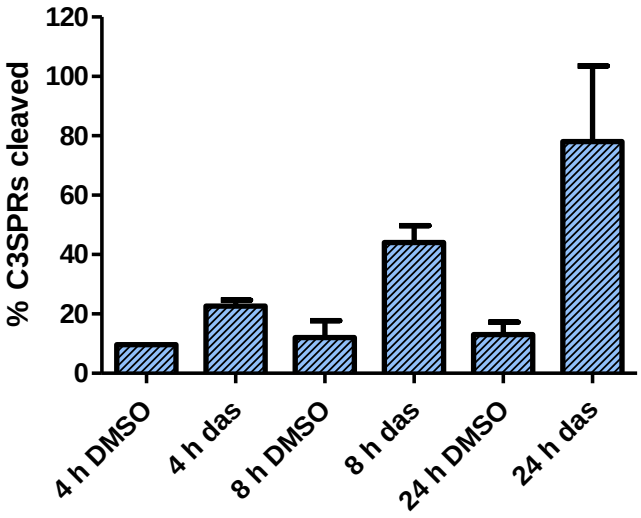
Supplementary Figure 6. Number of cutting events plotted as a function of time upon exposure to 1 nM caspase-3 using C3SPRs.



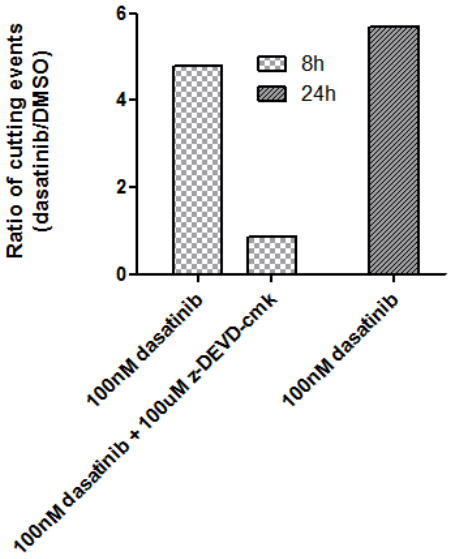
Supplementary Figure 7. A representative single particle trace of a C3SPR upon treatment with 5 nM caspase-7. Approximately 1 per 100 C3SPRs responded to caspase-7 treatment among a population of particles imaged under dark-field microscopy.



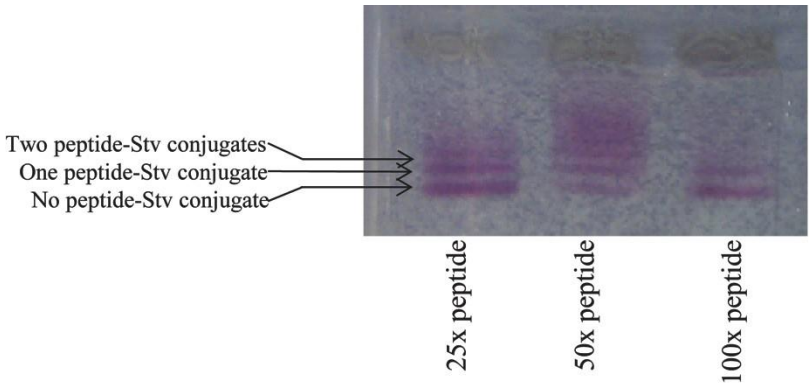
Supplementary Figure 8. Sensitivity of the Caspase-Glo® 3/7 reagent. Optimal concentration range of caspase-7 detection *via* Caspase-Glo® 3/7 after a kinetic run is in the picomolar range. 1 nM caspase-7 produced no fit and was outside of the dynamic range of the luminescence assay.



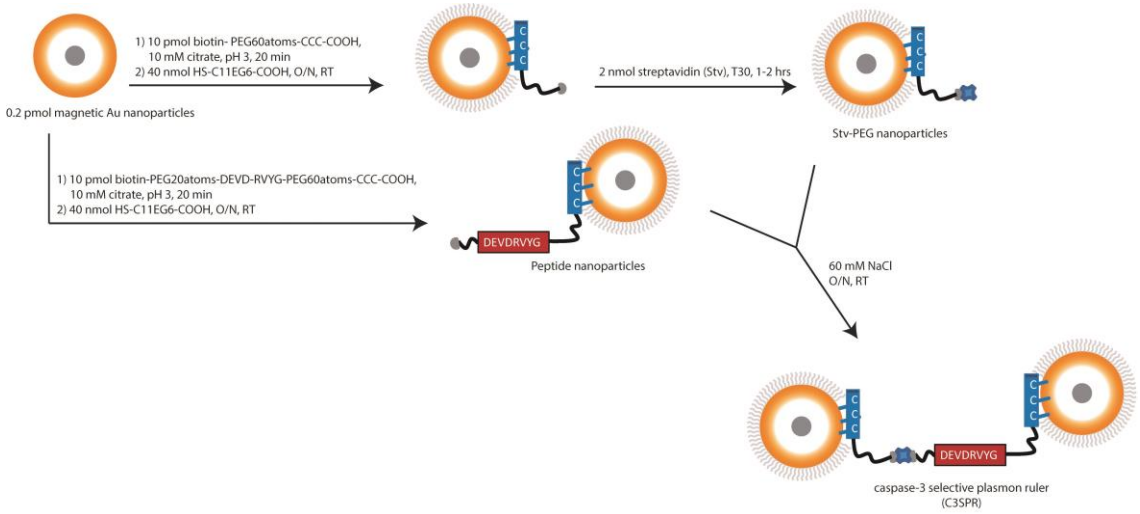
Supplementary Figure 9. C3SPR responsiveness to K562 vehicle- and drug-treated lysates across time series. Increasing caspase-3 activity is maintained at longer drug exposures.



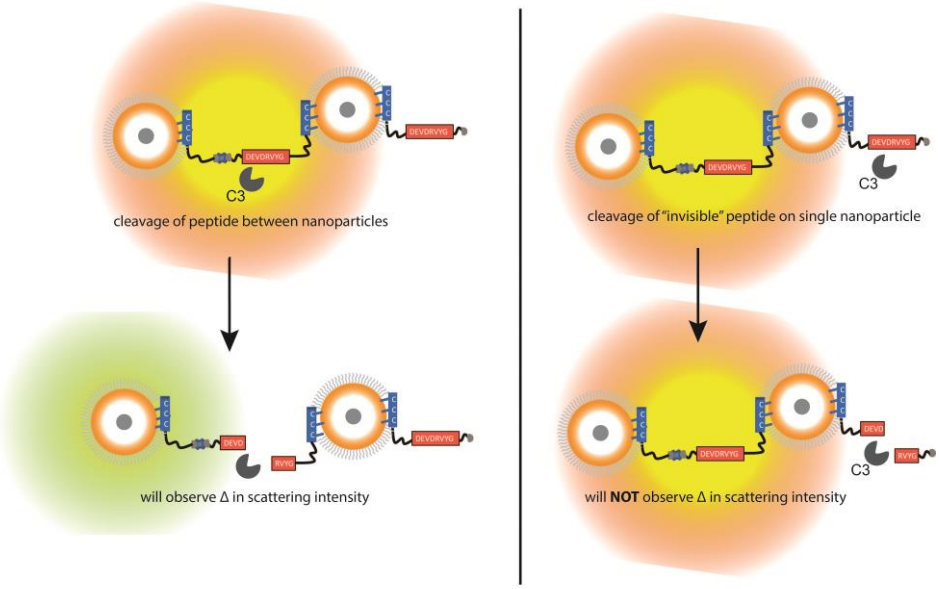
Supplementary Figure 10. Ratio of cutting events observed per K562 lysate condition (10 ng total protein applied per chamber) in 8 and 24 hour samples. Cutting events were determined by analyzing whole single particle trajectories using methods described in [1,2].



Supplementary Figure 11. Gel image of magnetic Au nanoparticle-peptide(biotin) conjugation with streptavidin-quantum dot at differing molar concentrations of peptide. 50x molar excess of peptide gave optimal conjugation with either one or two peptide linkers per magnetic Au nanoparticle. Higher order and unconjugated material was removed following subsequent purification steps.



Supplementary Scheme 1. C3SPRs were treated with either biotin- $PEG_{60atoms}$-CCC-COOH or biotin-$PEG_{20atoms}$-DEVD-RVYG- $PEG_{60atoms}$-CCC-COOH, followed by passivation of the surface with HS-$C_{11}EG_6$-COOH. The PEG-conjugated nanoparticles were subsequently reacted with streptavidin. The Stv-PEG and peptide nanoparticles were mixed in a 1:1 ratio and purified to yield the C3SPRs. For clarity, figures are not drawn to scale.



Supplementary Scheme 2. Peptide tethers linked to a single nanoparticle are "invisible" and do not affect scattering intensity readout. Illustrations not drawn to scale.

Supplementary
Table 1. Kinetic parameters of FRET peptides

	Caspase-3			Caspase-7			Caspase-8			Caspase-9			Granzyme-B		
	k_{cat} (s^{-1})	K_M (μM)	k_{cat}/K_M (M^{-1} s^{-1})	k_{cat} (s^{-1})	K_M (μM)	k_{cat}/K_M (M^{-1} s^{-1})	k_{cat} (s^{-1})	K_M (μM)	k_{cat}/K_M (M^{-1} s^{-1})	k_{cat} (s^{-1})	K_M (μM)	k_{cat}/K_M (M^{-1} s^{-1})	k_{cat} (s^{-1})	K_M (μM)	k_{cat}/K_M (M^{-1} s^{-1})
DEVD GGSN	2.27 +/- 0.08	6.5 +/- 0.8	350,000 +/- 40,000	0.63 +/- 0.02	17 +/- 1	37,000 +/- 3000	0.25 +/- 0.01	44 +/- 4	5700 +/- 600	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
DEVD RGSN	2.56 +/- 0.06	9.4 +/- 0.6	270,000 +/- 20,000	0.66 +/- 0.08	120 +/- 20	5500 +/- 1000	0.019 +/- 0.003	54 +/- 10	350 +/- 100	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
DEVD RVYG	2.45 +/- 0.06	6.4 +/- 0.2	380,000 +/- 10,000	N.D.	N.D.	N.D.	0.06 +/- 0.01	120 +/- 30	500 +/- 200	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
DEVD RVYD	2.25 +/- 0.06	4.5 +/- 0.5	500,000 +/- 50,000	N.D.	N.D.	N.D.	0.073 +/- 0.005	46 +/- 5	1600 +/- 200	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
VGPD FGRG	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0.66 +/- 0.02	46 +/- 3	14,000 +/- 900
VGPD FGKK	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0.102 +/- 0.007	85 +/- 10	1200 +/- 200

N.D. = not determined

References

(1) Jun, Y. W.; Sheikholeslami, S.; Hostetter, D. R.; Tajon, C.; Craik, C. S.; Alivisatos, A. P. Continuous Imaging of Plasmon Rulers in Live Cells Reveals Early-Stage Caspase-3 Activation at the Single-Molecule Level. *Proc. Natl. Acad. Sci. U. S. A.* **2009**, *106*, 17735-17740.

(2) Tajon, C.; Jun, Y. W.; Craik, C. S. Single-Molecule Sensing of Caspase Activation in Live Cells *Via* Plasmon Coupling Nanotechnology. *Methods Enzymol.* **2014**, *544*, 271-297.