Supporting Information:

A New Nanotechnology Technique for Determining Drug Efficacy using Targeted Plasmonically Enhanced Single Cell Imaging Spectroscopy (T-PESCIS)

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SUPPORTING FIGURES

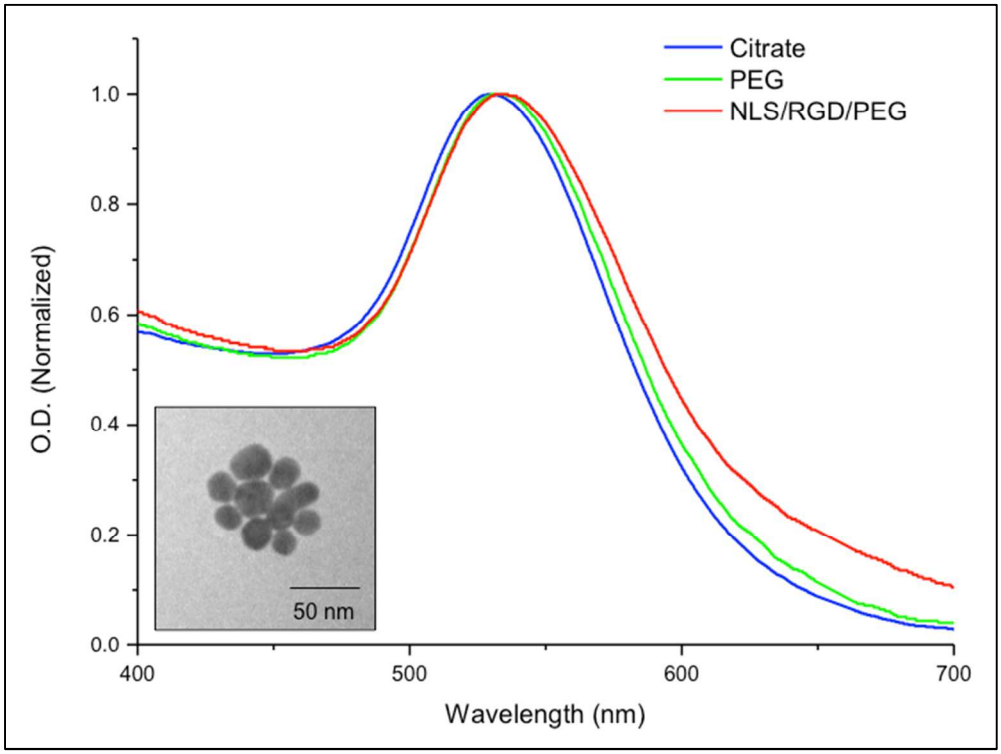

Figure S1. Characterization of targeted AuNSs. UV-Vis spectrum of AuNSs before (blue) and after conjugation to mPEG-SH (green), and NLS and RGD peptides (red) in aqueous solution. There was a slight red shift in the SPR peak indicating successful conjugation. The inset shows a TEM micrograph of 24 ± 3 nm citrate stabilized AuNSs.

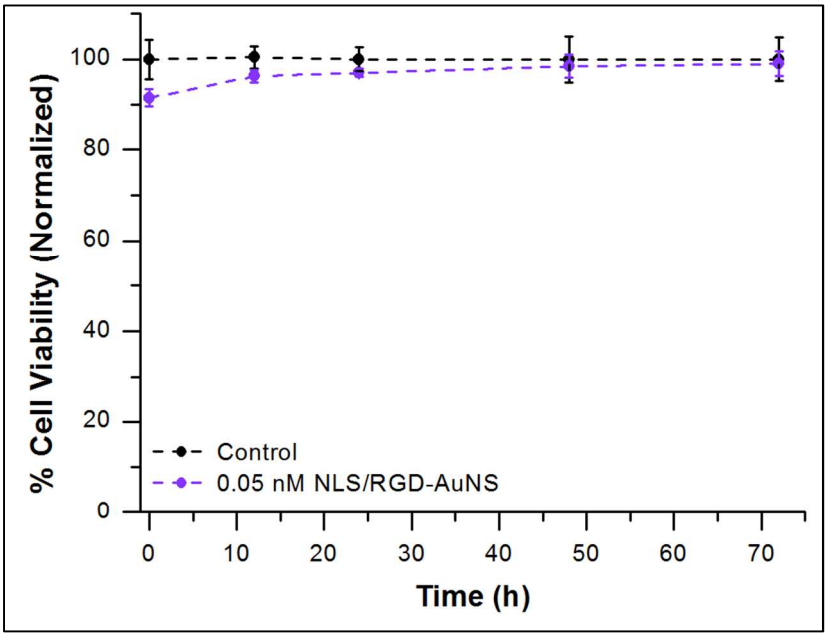

Figure S2. Cell viability of HSC-3 cells that were untreated (black) or treated with 0.05 nM NLS/RGD-AuNSs (purple) for 72 h. The treatment with nuclear-targeted gold nanospheres did not induce a detectable change in viability.

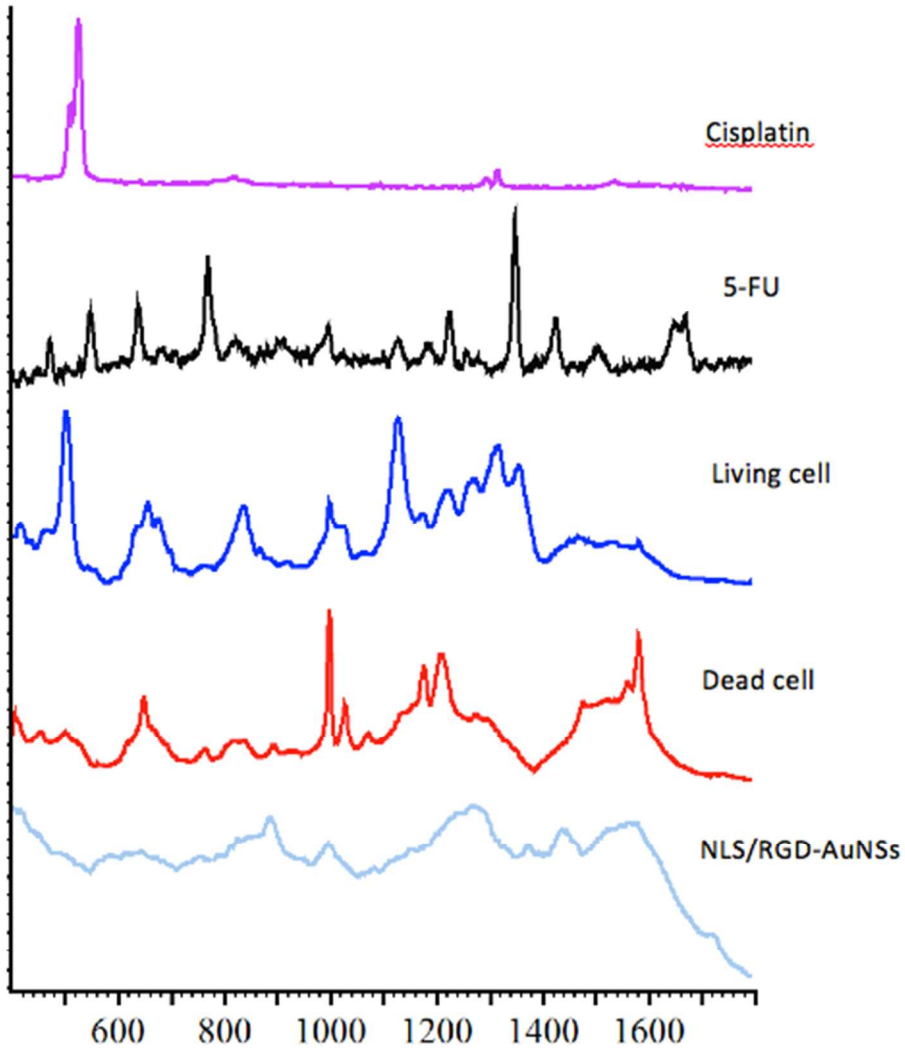

Figure S3. SERS spectra of cisplatin (purple), 5-fluorouracil (black), living cells (blue), dead cells (red), and NLS/RGD-AuNSs.

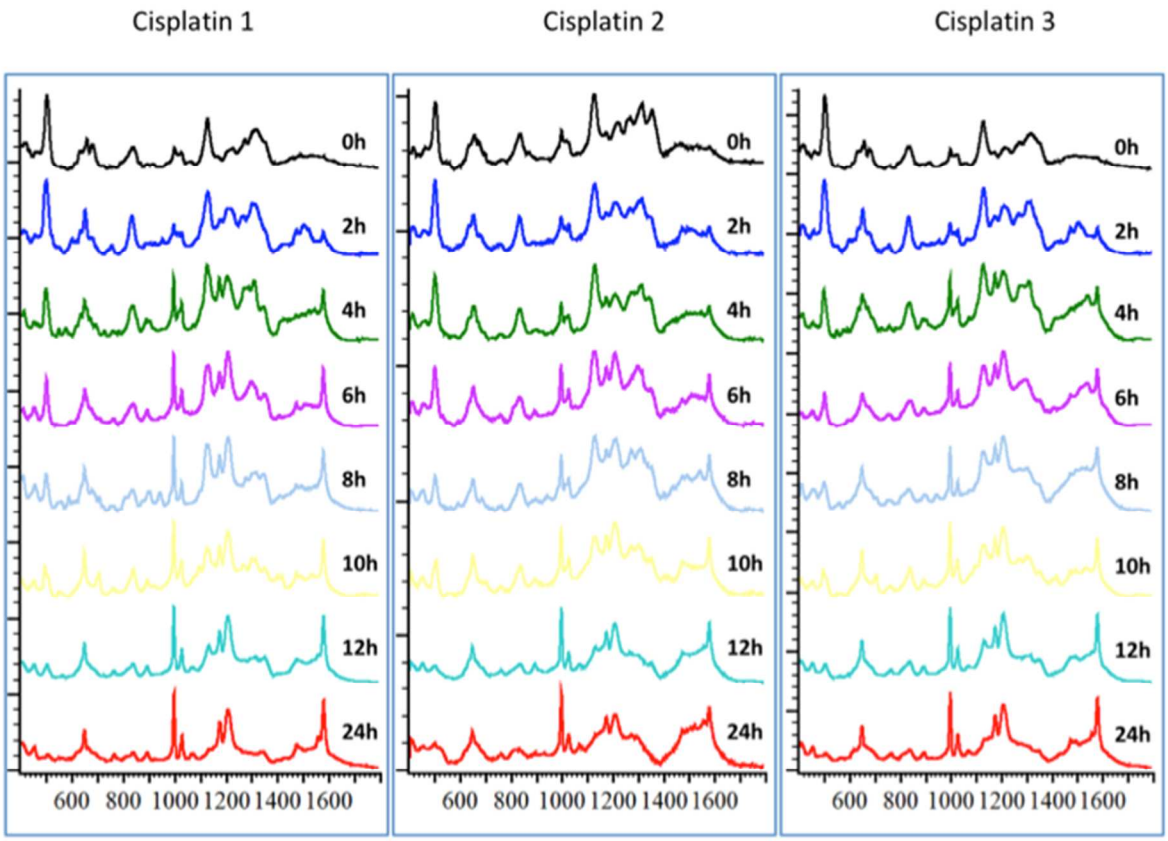

Figure S4. Raman spectra of HSC-3 cells pretreated with 0.05 nM NLS/RGD-AuNSs for 24 h and then treated with 100 μM cisplatin for 24 h. Spectra are from 3 independent experiments indicating the reproducibility in recording the Raman spectra as a function of time after cisplatin treatment.

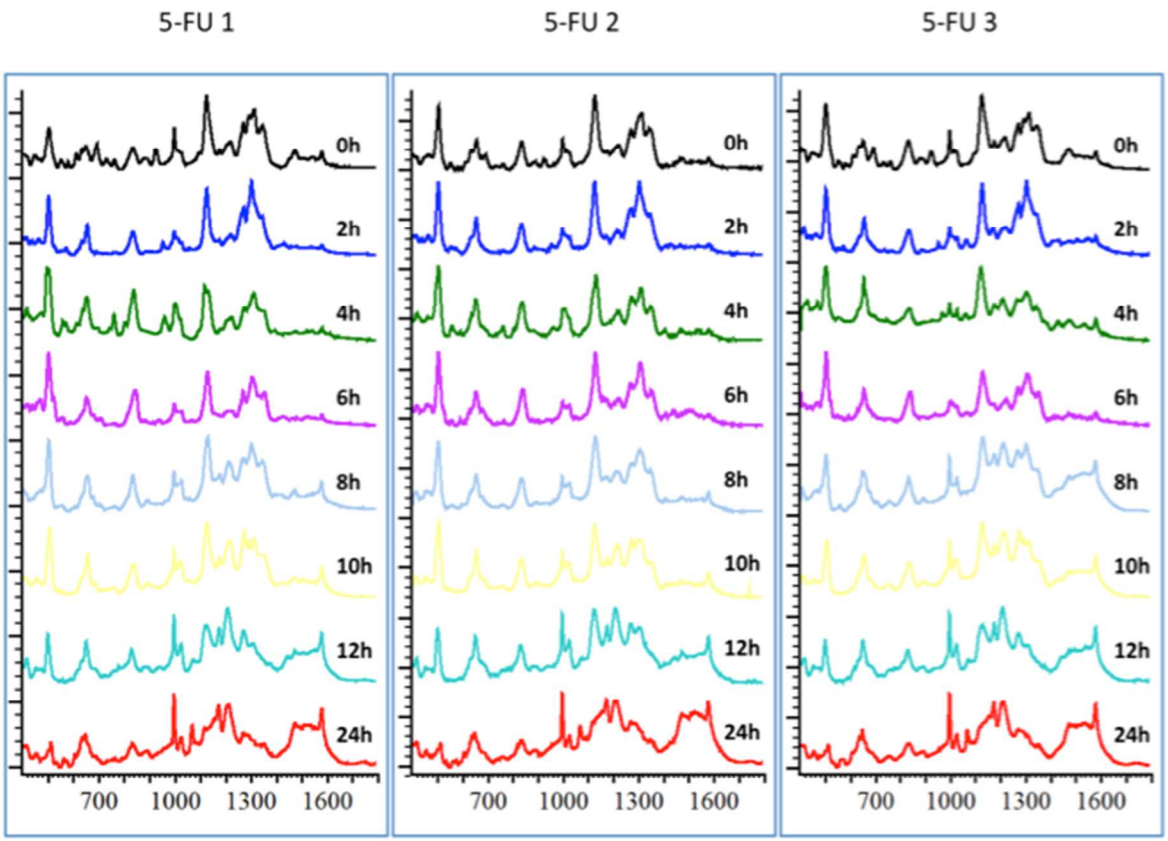

Figure S4. Raman spectra of HSC-3 cells pretreated with 0.05 nM NLS/RGD-AuNSs for 24 h and then treated with 100 μM 5-fluorouracil for 24 h. Spectra are from 3 independent experiments indicating the reproducibility in recording the Raman spectra as a function of time after 5-fluorouracil treatment.

EXPERIMENTAL PROCEDURES

AuNS Synthesis. Citrate stabilized AuNSs were synthesized using a modified Turkevich method.[1] A 95 mL solution of 1.6 mM $HAuCl_4$ was heated to 100 °C under stirring. After the gold solution reached 100 °C, 5 mL of 18.0 mM trisodium citrate was added. Once the color change from colorless to red occurred, stirring and heating were discontinued and the solution was allowed to cool to room temperature. AuNSs were cleaned via centrifugation at 6,000 rpm for 15 min and DI water was used for redispersion. TEM analysis showed an average nanosphere diameter of 24 ± 3 nm and UV-Vis spectroscopy showed a surface plasmon resonance peak at 530 nm (Figure S1). TEM micrographs were obtained on a JEOL 100CX-2 transmission electron microscope and ImageJ was used to calculate the average particle diameter.

PEG_{5k} Conjugation. PEGylation of the purified AuNSs was done to reduce particle aggregation and prevent non-specific binding between proteins when AuNSs are used in biological environments.[2] A 30% surface coverage of PEG ligands on the surface of the AuNSs was achieved using a 1.0 mM solution of mPEG-SH 5000 (Laysan Bio, Inc.) prepared in DI water. The PEG-AuNS solution was allowed to incubate at room temperature with gentle agitation overnight. Excess PEG ligands were removed using centrifugation (6,000 rpm, 15 min) and the PEG-AuNSs were redispersed in DI water. The success of PEG functionalization was determined using UV-Vis Spectroscopy (Figure S1).

Peptide Conjugation. Once the PEG-AuNSs were rid of excess PEG ligands and impurities, particles were conjugated with RGD and NLS peptides using a previously established method.[3-5] Custom peptides with C-terminal amidation were purchased from GenScript. Using a 5.0 mM solution of RGD (CGPDGRDGRDGRDGR) peptides and a 5.0 mM solution of NLS

(GGVKRKKKPGGC) peptides a molar excess of 10^4 and 10^5, respectively, were added to the PEG-AuNS solution. The solution was allowed to sit overnight while shaking and the peptide-AuNSs were cleaned via centrifugation (6,000 rpm, 15 min). Purified particles were redispersed in DI water and the conjugation was confirmed using UV-Vis Spectroscopy. To achieve the desired concentration of 0.05 nM NLS/RGD-AuNSs, peptide-particles solutions were diluted in DMEM cell culture medium.

Cell Culture. Human oral squamous carcinoma (HSC-3) cells were cultured using Modified Eagle's Medium (DMEM) (Corning Cellgro) supplemented with 4.5 g/L glucose and sodium pyruvate, 10% v/v fetal bovine serum (FBS) (Corning Cellgro) and 1% v/v antimycotic solution (Corning Cellgro). Cell cultures were maintained at 37 °C in a 5% CO_2 humidified incubator.

Cell Viability Assays. HSC-3 cells were grown for 24 h in 96-well plates and then incubated with 0.05 nM NLS/RGD-AuNSs diluted in culture medium (DMEM). After 24 h incubation, the nanoparticle solutions were replaced with the desired concentration of either cisplatin (Sigma) or 5-fluorouracil (Sigma). Following 72 h drug treatment, a XTT solution (Biotium, Inc.) that was prepared in culture medium (DMEM) was added. To determine the EC_{50} for each drug, concentration data from three independent experiments were averaged and fit to a sigmoidal growth function, and the EC_{50} value was generated from the fit using Origin 8.0 (Origin Lab, Corp.). The quality of fit for the cisplatin and 5-fluorouracil curves were $R^2 = 0.94$ and $R^2 = 0.95$, respectively. To determine the cell viability over time, the prepared XTT solution was added after 0, 12, 24, 48, and 72 hrs. To determine the ET_{50} for each drug, time data from three independent experiments were averaged and fit an exponential function, and the ET_{50} was calculated from the exponential fit generated from Origin 8.0 (Origin Lab, Corp.). The quality of fit for the cisplatin and 5-fluorouracil exponential curves were $R^2 = 0.97$ and $R^2 = 0.99$,

respectively. Cells for both viability experiments were measured between 4 h and overnight using a Biotek Synergy H4 Multi-Mode Plate Reader. Absorbance measurements were taken at 450 and 690 nm. The cell death enhancement (CDE) factor is the ratio of the half-times of 5-fluorouracil and cisplatin.

TPESCIS Spectroscopy and Anticancer Drug Treatment. Briefly, HSC-3 cells were cultured on 18 mm coverslips in culture medium (DMEM) for 24 h. Cells were then treated with 0.05 nM NLS/RGD-AuNSs for 24 h. After particle treatment, the coverslips were placed in a homemade live cell chamber that maintained stable humidity, 37 °C temperature and 5% CO_2 concentration, and inserted into the TPESCIS set-up (Figure 1). Drugs solutions (100 μM) diluted in culture medium were injected into the live cell chamber using an auto-injection system and Rayleigh and Raman spectra were obtained over 24 h. The Raman system utilized a 785 nm excitation laser, an inverted microscope with a 50x objective lens fitted with filters to prevent signals from the laser and Rayleigh scattering. The pretreatment with NLS/RGD-AuNSs allowed for acquisition times for well-resolved spectra to be less than 10s using the extended scan mode while the spectra mapping mode required < 1s. Raman spectra from three independent experiments were averaged and normalized to the most intense band. Normalized intensities of 500, 1000, and 1585 cm^{-1} bands were plotted against time and fit to a sigmoidal growth curve using Origin 8.0 (Origin Labs, Corp.). The ET_{50} values were calculated from the sigmoidal growth curve equation. The quality of the fits for cisplatin and 5-fluoruracil at 500 cm^{-1} is $R^2 = 0.94$ and $R^2 = 0.70$, respectively. Fits for the band at 1000 cm^{-1} had a quality of $R^2 = 0.99$ for cisplatin and $R^2 = 0.88$ for 5-fluorouracil. The band at 1585 cm^{-1} showed a quality of fit of $R^2 = 0.96$ for cisplatin and $R^2 = 0.92$ for 5-fluorouracil. The cell death enhancement (CDE) factor is the ratio of the ET_{50} values of cisplatin and 5-fluorouracil.

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