Biological Targeting of Plasmonic Nanoparticles Improves Cellular Imaging via the Enhanced Scattering in the Aggregates Formed.

*Mena Aioub[‡], Bin Kang[‡†], Megan A. Mackey, and Mostafa A. El-Sayed**

Laser Dynamics Laboratory, School of Chemistry and Biochemistry, Georgia Institute of Technology, Atlanta, GA

[‡] Equal Contribution

† Present Address: College of Material Science and Technology, Nanjing University of Aeronautics and Astronautics, Nanjing, 210016, P.R. China

*Corresponding author: Mostafa A. El-Sayed, Department of Chemistry and Biochemistry, Georgia Institute of Technology, 901 Atlantic Drive, Atlanta, GA, 30332-0400, Adjunct Professor: Department of Chemistry, Faculty of Science, King Abdulaziz University, Jeddah, Saudi Arabia. E-mail: melsayed@gatech.edu

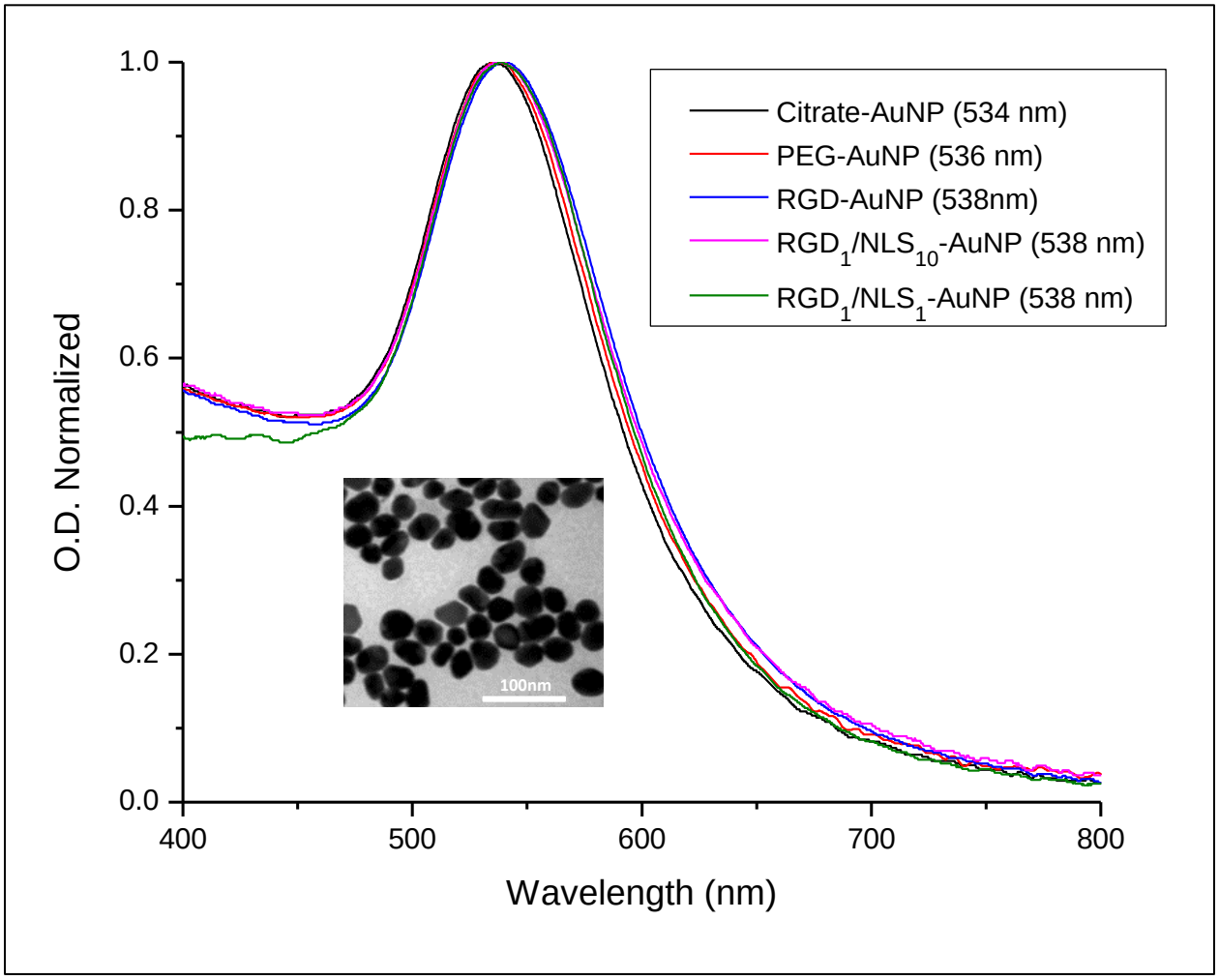

Figure S1. Characterization of targeted AuNPs. UV-Vis spectrum of AuNPs before (black) and after conjugation with mPEG-SH_{5000} (red), and varying molar ratios of RGD and NLS peptides (blue, pink, and green) in aqueous solution. There was a slight red shift in the plasmon peak indicating successful conjugation. The inset shows a TEM micrograph of 31 ± 4 nm citrate capped AuNPs.

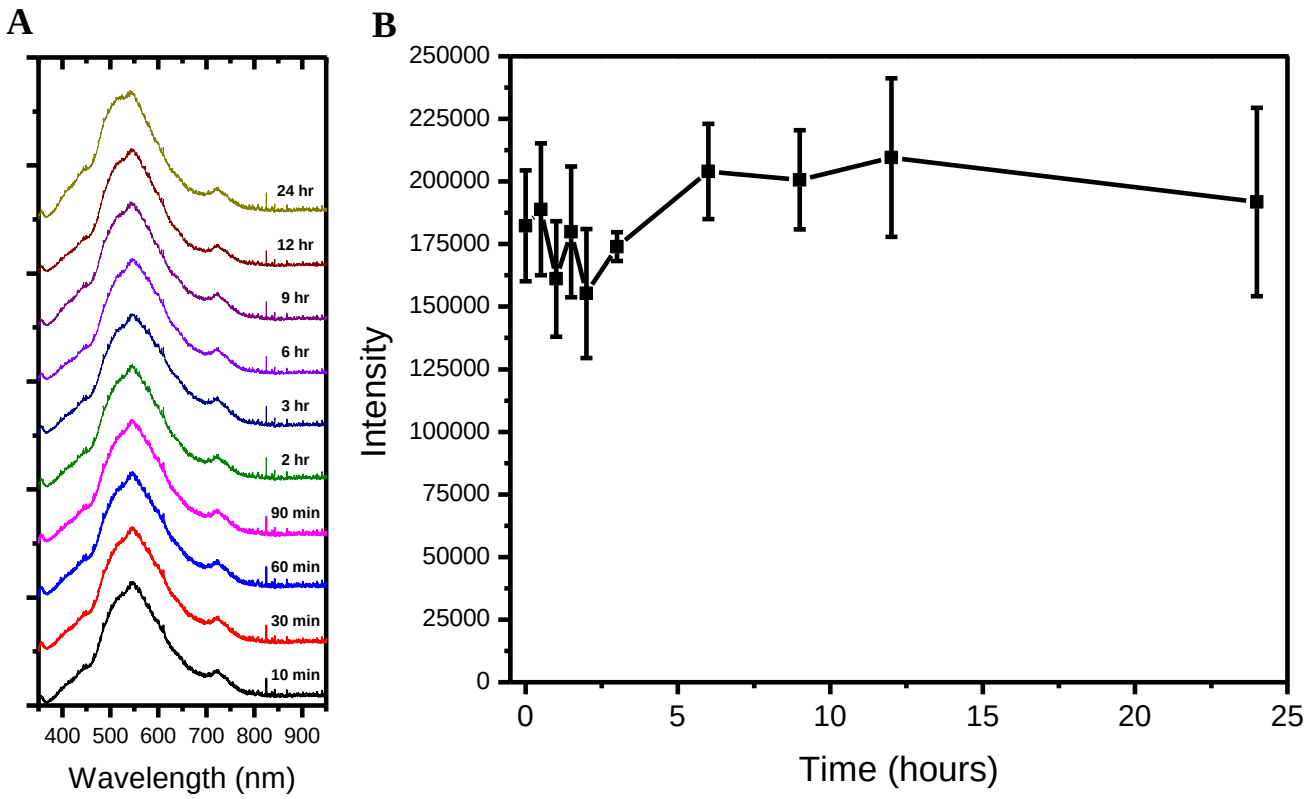

Figure S2. (A) Rayleigh scattering spectra of HSC-3 cells without AuNP treatment show no change with time. (B) Scattering intensity of untreated cells does not change significantly with time ($p = 0.1388$).

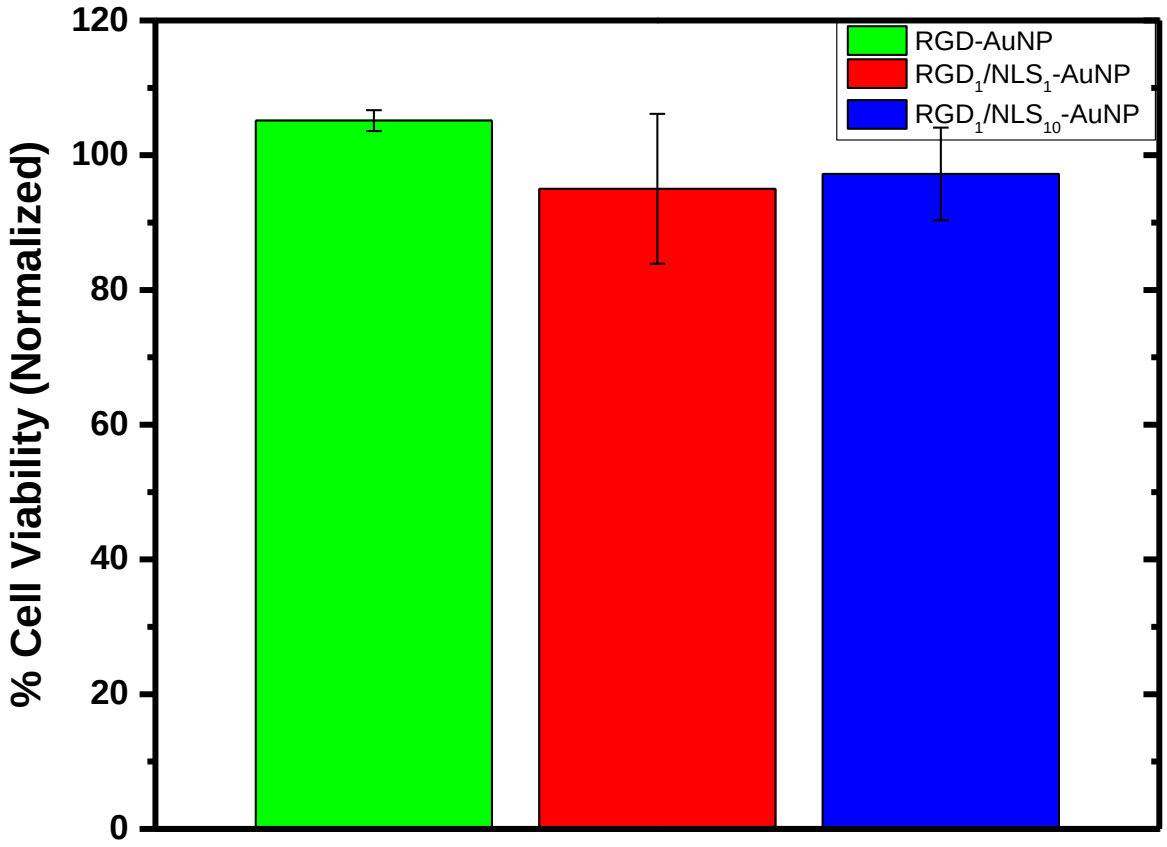

Figure S3. Viability of HSC-3 cells after 24 hour incubation with targeted AuNPs. No statistically significant cell death was observed from treatment with the three AuNP designs.

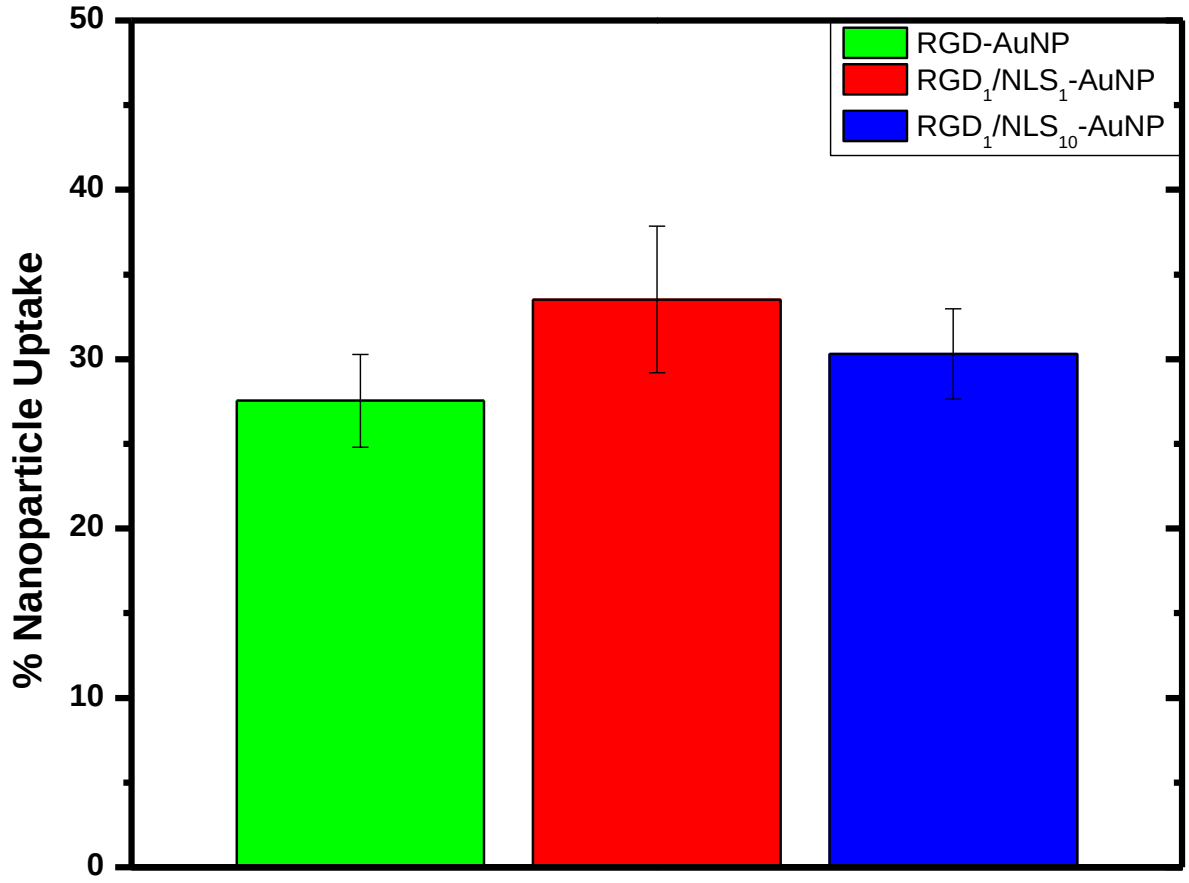

Figure S4. Cellular uptake of targeted AuNPs by HSC cells after 24 hours, shown as percentage of AuNPs taken up ± s.e.m. No statistically significant difference in uptake was observed for the three AuNP designs.

AuNP Synthesis

Citrate stabilized AuNPs with an average diameter of 31 nm were synthesized by the reduction of chloroauric acid via sodium citrate. Briefly, 490 ml of a 0.6 mM aqueous solution of chloroauric acid (Sigma-Aldrich) was heated in a 1 L Erlenmeyer flask, with stirring. When the solution reached boiling, 10 ml of a 180 mM sodium citrate (Sigma-Aldrich) in water solution was immediately added. Stirring and heating were discontinued when the solution became a red-wine color and it was allowed to cool to room temperature. TEM images (Figure S1) were taken on a JEOL 100CX-2 transmission electron microscope and the average particle diameter was found to be 31 nm using ImageJ software. UV-Vis spectroscopy showed a surface plasmon resonance peak at 533 nm (Figure S1).

AuNP Functionalization

The citrate capped AuNPs were PEGylated before peptide functionalization to prevent nonspecific interactions in the physiological environment. A 1.0 mM solution of mPEG-SH (MW 5000, Lysan Bio, Inc.) in deionized (DI) water was added to achieve a ~30% surface coverage. The PEG-AuNP solution was shaken overnight at room temperature and unbound PEG was removed by centrifugation (6,000 rpm, 14 minutes). Washed PEG-AuNPs were redispersed in DI water and characterized by UV-Vis spectroscopy (Figure S1). PEG-AuNPs were further conjugated with RGD and NLS peptides following a formerly established method.[1] Briefly, 5.0 mM solutions of NLS (CGGPKKKKRKVGG) in DI water and / or RGD (CGPDGRDGRDGRDGR) in DI water were added to the PEG-AuNPs. A total of 4,000 peptides per PEG-AuNP were added in equal molar amounts of RGD and NLS for RGD_1/NLS_1-AuNPs, a 10:1 ratio of NLS:RGD for RGD_1/NLS_{10}-AuNPs, and only RGD added for RGD-

AuNPs. The peptide-nanoparticle solutions were allowed to shake overnight at room temperature and excess peptides removed by centrifugation (6,000 rpm, 14 minutes). The desired concentration of 0.4 nM AuNPs was achieved by diluting stock AuNPs in cell culture media.

Cell Culture

Human oral squamous carcinoma (HSC-3) cells, a malignant epithelial cell line expressing $\alpha_v\beta_6$ integrins on the cell membrane, were chosen as our cancer cell model.[2] The cells were cultured in Dulbecco's Modification of Eagle's Medium (DMEM, Mediatech) supplemented with 4.5 g/L glucose, L-glutamine, and sodium pyruvate, 10% v/v Fetal Bovine Serum (Mediatech), and 1% v/v antimycotic solution (Mediatech). Cell cultures were kept in a humidified 37°C incubator with an atmosphere of 5% CO_2.

Cell Viability Assay

To assess the viability of HSC-3 cells treated with targeted AuNPs, cells were grown in 96 well plates overnight. The growth media was then removed and replaced with culture media containing 0.4 nM AuNPs. After 24 hour incubation, the AuNP containing culture media was replaced with an XTT solution (Biotium, Inc.) in DMEM. Cells were then incubated with the XTT solution for 4 h and absorbance measurements were taken at 450 nm and 690 nm on a Biotek Synergy H4 Multi-Mode Plate Reader. Cell viabilities are shown as the mean ± s.e.m. of three independent experiments (Figure S3).

Gold Nanoparticle Uptake

The percentage of AuNPs taken up by HSC-3 cells was determined using a previously established spectroscopic technique.[1] Briefly, cells were grown in 96-well tissue culture plates overnight. The growth media was then replaced with media containing 0.4 nM AuNPs. After 24 hour incubation with AuNPs, the nanoparticle containing culture media was moved to a new 96-well culture plate. The optical density was measured at a wavelength of 538 nm (Biotek Synergy H4 Multimode Plate Reader). To determine AuNP uptake, the measured optical density of culture media without AuNPs was subtracted from the optical density of the AuNP containing treatment media. This AuNP optical density was then converted to a percentage of that initially added to the cell culture (Figure S4).

Plasmonically-Enhanced Rayleigh-Scattering Imaging Spectroscopy (PERSIS) Technique

The PERSIS system is composed of a home-made live cell incubator, a spectrometer (USB2000+, Ocean Optics), and a dark-field microscope. To obtain scattering spectra, cells were grown overnight on a glass bottom culture dish (MatTek) in DMEM. The growth media was then removed and the cells treated with solutions of 0.4 nM AuNPs in DMEM and immediately moved to the PERSIS live-cell incubator located on the microscope stage. The incubator maintained the cells at 37°C in a humidified atmosphere containing 5% CO_2. The PERSIS system angled a beam of white light onto the sample such that incident light passed through the sample and only scattered light was collected into the 40X microscope objective. The collection area was controlled by a 0.6 mm pinhole and the magnification of the objective, with an absolute collection area of roughly 3 μm x 3 μm (~10 μm^2 collection area), allowing for single cell resolution. A CCD camera was used to capture the true color Rayleigh scattering images from the cells and spectral signals were coupled into an optical fiber and recorded.

Spectral Analysis

Scattering spectra were collected and averaged from 10 different cells and all experiments were performed in triplicate. Reference spectra were taken immediately upon addition of AuNP to cells and subtracted from subsequent time points to remove any scattering from cells or the AuNP/culture medium background. Spectra were then deconvoluted into three Gaussians (Figure 2) using OriginPro 9.0 software (Origin Lab, Corp.). The first Gaussian was fixed at 538 nm to correspond to the plasmon peak of single AuNPs (Figure S1). The peaks due to small and larger AuNP clusters were centered between 636-646 nm and 740-750 nm, respectively, and the best fit integrated to give intensities of the Rayleigh scattered bands. Scattering intensities were fit to a sigmoidal growth curve using a logistic response function with OriginPro 9.0 software. Initial and final scattering values were used to generate the scattering half-times, defined as the time required to reach the center of the fit between these values. The power of the logistic fitting function was assumed to be non-negative and the curve fit was optimized using the Levenberg Marquardt algorithm.

Statistical Analysis

Results are expressed as the mean ± s.e.m. of three independent experiments. Changes in scattering intensity were compared using a linear regression analysis calculator and the changes were not considered statistically significant ($p = 0.1388$). Statistical significance of AuNP uptake was calculated using unpaired *t*-tests (GraphPad Software, Inc.) and the difference in uptake between the AuNPs studied was not found to be statistically significant. The viability of cells treated with targeted AuNPs were also compared to untreated controls using unpaired *t*-tests

(GraphPad Software, Inc.) and differences between the AuNPs studied were not found to be statistically significant.

References

(1) Austin, L. A.; Kang, B.; Yen, C. W.; El-Sayed, M. A. Nuclear Targeted Silver Nanospheres Perturb the Cancer Cell Cycle Differently Than Those of Nanogold. *Bioconjugate Chem.* **2011,** *22*, 2324-2331.

(2) Oyelere, A. K.; Chen, P. C.; Huang, X.; El-Sayed, I. H.; El-Sayed, M. A. Peptide-Conjugated Gold Nanorods for Nuclear Targeting. *Bioconjugate Chem.* **2007,** *18*, 1490-1497.