Optimizing Gold-Silica Nanostars for Multiplexed Surface Enhanced Resonance Raman Spectroscopy Mapping

Michael B. Fenn, Niksa Roki, Jose Gomez-Feria Ferreiro
Department of Biomedical Engineering, Florida Institute of Technology, Melbourne, FL 32901.

Introduction: Surface Enhanced Raman spectroscopy (SERS) and Surface Enhanced Resonance Raman Spectroscopy (SERRS) mapping of cancer cells tagged with SERS/SERRS nanoprobes provides a highly sensitive (i.e., sub-fM limit of detection) and specific, photo-bleaching free means for cellular sensing. A wide variety of SERRS reporter molecules exist for highly multiplexed analysis using a single excitation source due to each reporter's unique spectral pattern. Maximizing signal intensity due to surface plasmon resonance (SPR) of the nanoprobes is required for achieving maximum sensitivity, and thus robust techniques are needed to maintain a constant SPR during nanoprobe fabrication. We have synthesized silica-encapsulated gold nanostars with highly tunable SPRs for optimization of sensitivity and reproducibility for combination of SERS/SERRS reporters with a 785nm near-infrared laser. Furthermore, we studied silica functionalization and subsequent cyclo-RGDf/k peptide conjugation for SERS/SERRS mapping of integrins expressed by breast and melanoma cells in vitro.

Materials and Methods: Gold nanostars of varying size (80-100nm diameter) were synthesized from spherical gold nanoparticles (~10nm) in DMF and polyvinylpyrrolidone (PVP) by addition $AuCl_4$. Modulation of the SPR was performed by varying Au seed/$AuCl_4$ ratios and seed addition timing. Reporters investigated include fluorescein isothiocyanate (FITC), Rhodamine B isothiocyanate (RITC), malachite green isothiocyanate (MGITC), malachite green oxalate (MG Ox), 5(6)-carboxy-x-rhodamine (ROX), IR-780, IR-820, IR-895, IR-1061. Nanostars were encapsulated in a 10nm thick silica shell and functionalized with thiols or amines. Thiols were conjugated with maleimide-mPEG/carboxylic acid blends, and amines conjugated with diacid-PEG/mPEG-acid for coupling to cyclo-RGDf/k peptide via carboiimide chemistry. UV-vis, TEM and DLS were used for nanoparticle characterization. SERS/SERRS measurements were performed on a Renishaw InVia Raman microscope system with a 785nm diode laser and 63x objective and nanoprobes at equivalent concentrations.

Results and Discussion: We have developed a highly tunable synthesis for producing silica-coated gold nanostars for SERS/SERRS mapping of integrins *in-vitro*. We found that 'curing' the nanoparticles prior to the addition of $AuCl_4$ provides a linear relationship of [$AuCl_4$] to SPR in the range λ = 720-850nm. Nanoprobes with SPRs blue shifted (~720nm), red shifted (~850), and at the laser line (~785nm) were prepared with each reporter. It was found that reporter adsorption red or blue shifts the plasmon in a reporter-specific manner prior to silica encapsulation. This demonstrates the importance of precise SPR tuning in order to maintain resonance with the 785nm laser line in order provide the highest SERS/SERRS signal intensity and reproducibility. Unique nanoprobe spectra peak patterns of the nine reporters combined with 785nm SPR nanostars are shown in Figure 1(a.-i.), thus demonstrating the multiplexing capability. IR-780/785 SPR nanoprobes yielded the highest intensity SERRS signal ($p < 0.05$), followed by IR-1061, IR-895 and MGITC, and IR-820 the lowest. Figure 1(j.) shows a TEM image of IR-780 nanoprobes with SPR of 780nm and shell thickness of 10.4 +/- 2.0 nm. Figure 1(k.) shows a fluorescence image overlay with the SERRS map of the cRGDf/k-conjugated nanoprobes targeting MDA-MB-231 breast cancer cells. **Conclusion:** Optimization of SERS/SERRS nanoprobes will provide a highly sensitive, nondestructive technique for improving the understanding of cancer etiology and diagnosis. **References:** Moore, Nature biotechnology 2004; 1133-8.

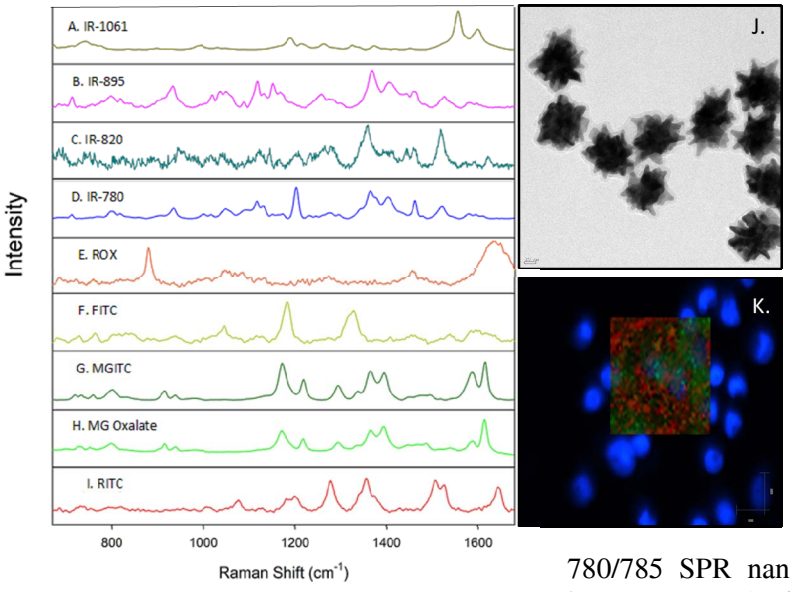

Figure 1. A.-I.) SERS/SERRS spectra of the nine reporters, J.) TEM image of IR-780 nanoprobes (scale bar 50nm), K.) SERS map of MDA-MB-231 cells dosed with IR-780 nanoprobes targeted with cRGDf/k peptide.