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Project Officer: Dr. Richard Gordon / Dr. John Miller

**Novel Nanoparticles for Ultrasensitive Detection
and Spectroscopy**

Shuming Nie

**Indiana University
P. O. Box 1847, Bloomington, IN 47402-1847**

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MDvorscak
Mark P. Dvorscak
(630) 252-2393
E-mail: mark.dvorscak@ch.doe.gov
Office of Intellectual Property Law
DOE Chicago Operations Office
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Goals and Accomplishments

The long-term objective of this DOE project was to develop a new class of metal nanoparticles with novel optical properties for ultrasensitive detection and spectroscopy. The specific aims were (i) to prepare and screen colloidal silver and gold nanoparticles with novel optical properties; (ii) to characterize the intrinsic size-dependent properties of these nanoparticles and to identify the factors responsible for efficient optical enhancement; (iii) to determine the characteristics and origins of intermittent photon emission in single metal particles; and (iv) to develop methodologies for enriching these novel particles and for fabricating thin nanoparticle films. In the following we describe the major accomplishments of this project.

(a) First observation of efficient Raman enhancement and intermittent light emission in single gold nanocrystals. This result was published as a full paper in *J. Am. Chem. Soc.* (Krug, Wang, Emory, and Nie, 121, 9208-9214, 1999). We reported two fundamental observations on the size-dependent optical properties of colloidal gold nanoparticles. First, faceted gold nanocrystals in the size range of 63 ± 3 nm was found to be highly efficient for surface-enhanced Raman scattering (SERS). These nanocrystals were identified from a heterogeneous population for large optical enhancement at 647-nm laser excitation. Second, spatially isolated single gold particles were shown to emit Stokes-shifted Raman photons in an intermittent on and off fashion. In contrast to population-averaged studies, blinking surface-enhanced Raman scattering is a signature of single-particle (or even single-molecule) behavior. By directly measuring optical enhancement and time-resolved emission on single nanoparticles, this work opens new possibilities in studying the mechanisms of SERS, in developing metal tips for near-field optical microscopy, and in designing new nanostructured materials.

(b) Preparation of enriched nanoparticle thin films for ultrasensitive detection and spectroscopy. This part was published in *Chemistry of Materials* (Maxwell, Emory, and Nie 2001, 13, 1082-1088.) This work represents a practical application of our recent discovery that a rare population of metal colloidal nanoparticles is extremely efficient for surface-enhanced Raman scattering. We showed that this enormous enhancement effect allows detection and spectroscopy of single molecules at room temperature. A major problem, however, is that only ca. one percent of the particles are optically active in the standard silver colloids. We have overcome this problem by using nanomembrane filtration method. We have succeeded not only in enriching the optically active particles but also in preparing thin nanoparticle films for practical applications. With these nanopore membranes, size-selective filtration separates the optically active particles from the inactive ones. The enriched fractions are then used to prepare thin nano films. We found that the nanoparticulate films are highly efficient for surface optical enhancement, show excellent reproducibility, and should find use in ultrasensitive spectroscopy.

(c) New insights into the fundamental mechanisms of single-molecule and single-nanoparticle SERS (Doering and Nie, *J. Phys. Chem.* 2002, 106, 311-317). We developed an integrated flow injection and ultrasensitive optical imaging / spectroscopy

system for examining the roles of surface sites and chemical enhancement in single-molecule and single-particle SERS. A key feature was that colloidal silver nanoparticles were immobilized on a glass surface inside a microflow device, and that single-particle SERS signals were observed in real time while the immobilized particles are treated by chemical reagents in the flow cell. Surface plasmon resonance (SPR) imaging indicates that the electromagnetic properties of the immobilized particles remain essentially unchanged after chemical treatment. Thus, the observed SERS spectral changes come primarily from chemical enhancement at surface active sites. Our experimental data reveal that three halide ions (Cl^- , Br^- , and I^-) have a substantial activating effect, while other ions such as citrate, sulfate and fluoride have little or no effect on single-particle SERS. A "quenching" effect is observed for thiosulfate ions, which completely destroys the SERS activity. The chemical enhancement factors achieved on single particles are estimated to be about 10^2 to 10^3 .

(d) Development of self-assembled nanoparticle probes for detection and recognition of biomolecules. In this effort, we developed nanoparticle-based probes that are able to recognize and detect specific DNA sequences and single-base mutations in a homogeneous format. The core of this nanoprobe is a 2.5-nm colloidal gold nanocrystal that functions both as a structural scaffold and an energy acceptor (quencher). Attached to this core are oligonucleotide molecules labeled with a thiol group at one end and a fluorescent dye at the other. A surprising finding is that these oligo molecules spontaneously assemble into a constrained arch-like conformation on the particle surface. The "arch" sequence is exposed and is available for target binding, which causes the constrained structure to open and the fluorophore to separate from the particle surface. In comparison with molecular beacons and other homogeneous probes, these nanoparticle biosensors offer unique advantages and capabilities, and represent a first step in developing smart nanostructures with integrated sensing, targeting, and therapeutic functions.

List of Publications Acknowledging DOE Support (total of 11)

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- (2) W. Chan, D. J. Maxwell, X. Gao, R. E. Bailey, and M. Y. Han, "Luminescent Quantum Dots for Multiplexed Biological Detection and Imaging," *Current Opinion in Biotechnology* **2002**, 13, 40-46.
- (3) W. E. Doering and S. Nie, "Examining the roles of active sites and chemical enhancement in surface-enhanced Raman scattering of single molecules," *J. Phys. Chem.* **2002**, 106, 311-317.
- (4) W. Chan, X. Gao, and S. Nie, "Semiconductor quantum dots as multicolor and ultrasensitive biological labels," an invited chapter in: *Colloids and Colloid*

Assemblies: Synthesis, Modification, Organization, and Application, Wiley-VCH Publishers, **2002**.

- (5) D. J. Maxwell, S. R. Emory, and S. Nie, "Nanostructured thin-film materials with surface-enhanced optical properties," *Chemistry of Materials* **2001**, 13, 1082-1088.
- (6) J. R. Taylor, M. M. Fang, and S. Nie, "Probing Specific Sequences on Single DNA Molecules with Bioconjugated Fluorescent Nanoparticles," *Analytical Chemistry* **2000**, 72, 1979-1986.
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- (11) J. T. Krug, J. D. Wang, S. R. Emory, and S. Nie, "Efficient Raman Enhancement and Intermittent Light Emission Observed in Single Gold Nanocrystals," *J. Am. Chem. Soc.* **1999**, 121, 9208-9214.