

# Probing single molecules by Raman spectroscopy

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The ability to probe single molecule or molecular events provides the scientist the ultimate tool to check their theories and comprehend the individual behaviour in the atomic scale. This also means a better understanding of the interactions that distinguish one molecule from another, which is normally averaged in ensemble measurement. Hence the usual assumption that all molecules contribute equally to the behaviour of the system can be directly examined. The advancement in spectroscopic techniques, in recent times, starting from the scanned probe microscopic techniques to the more recent near field optical techniques has extended the boundaries of the molecular detection. There has been a flurry of activity using these techniques for single molecular detection in various laboratories around the world.

The single molecular detection has been achieved earlier in various environments such as low-temperature solids<sup>1</sup>, room temperature liquids<sup>2</sup> and in electrochemical environment<sup>3</sup>. In the February issue of *Science*, Shuming Nie and Steven R. Emory<sup>4</sup> report some exciting new possibilities of using Raman spectroscopy to study the single molecule and single nanoparticle. The method uses the well-known phenomenon of surface enhanced Raman scattering (SERS) and the resonance Raman scattering (RRS) of molecules. The SERS was reported about two decades ago<sup>5</sup> by M. Fleischmann and his group while studying electrochemically roughened silver electrodes adsorbed with pyridine molecules. They found an enhancement of Raman signal intensity by about million times over the intensity for the same concentration of pyridine in the solution. This has opened up a wide field of research both in physics and chemistry of interface and in Raman spectroscopy. By now hundreds of molecules, organic, inorganic, polymeric, ionic and neutral that can adsorb on metals like silver, copper and gold have been studied and SERS has been established as an indispensable tool for surface studies, mostly in electrochemical environment<sup>6,7</sup>. In spite of the large amount of work carried out to understand the processes involved, there still remain several questions which have not been answered satisfactorily till this day. However, the wealth of information provided by this technique is unabated. The resonance Raman scattering occurs when the

radiation excites a molecule at its characteristic absorption frequency, and subsequently leaving the molecule in a vibration state different from the one it started. This amplifies the signal by 5 to 6 orders of magnitude. Nie and Emory have coupled these two phenomena to probe single molecules. They have studied the well-known dye Rhodamine 6G, which has a characteristic absorption band at 530 nm adsorbed on silver sol of average particle diameter 35 nm. However, they find the Raman signals are greatly amplified by only a few of these particles whose dimensions are in a narrow range of 110–120 nm. These particles christened as 'hot particles' when adsorbed with R6G and immobilized on polylysene-coated glass slide, emit very bright stoke shifted light when irradiated with 514.5 nm Ar ion laser. Clear images of the single particle Raman signals were obtained using the evanescent wave near field optical microscope, at a concentration of R6G as low as  $2 \times 10^{-9}$  M. At this low concentration the authors show that each particle contains an average of 1 or 0 R6G molecule and as such the red-shifted light is actually Raman scattering from a single molecule adsorbed on a nanoparticle. Fluorescence is ruled out because they have been able to get such signals from non-fluorescent bio-molecules. Moreover the fluorescence is quenched in this case because of rapid energy transfer from the excited state to the metal surface.

The single particle emission is also found to be strongly polarized unlike the ensemble averaged spectra of the same system which was reported to be strongly depolarized<sup>8</sup>. They were able to isolate two particles and obtain the Raman signals when they are in orthogonal orientation with respect to each other and show that when one is maximally polarized in the s-plane the other is minimally polarized and vice versa when the excitation signal is p-polarized. A similar study with the emission polarized SERS of R6G molecule on a single nanoparticle shows the strong polarization along the molecular axis.

Another interesting observation is the sudden spectral changes observed when a single particle scattering is monitored for a few minutes of illumination. They report frequent changes in both Raman frequency and intensity during this time. These shifts are presumed to be due to changes in the configuration dynamics akin to spectral

diffusion observed in single molecular studies of low temperature solids<sup>1</sup>. The Raman spectra from different molecules have also slightly different vibrational frequency, suggesting each molecule is adsorbed at different sites.

Integrated fluorescent intensity measurements have been made on the R6G molecules adsorbed on glass surface and compared with SERS obtained with the R6G molecules adsorbed on the silver particles at a narrow spectral region where both overlap. The results show that the integrated Raman intensity is at least 4–5 times larger.

The very large enhancement observed in Raman intensity at single molecular level is attributed to the fact that not all particles are 'optically hot'. Only one out of 100 to 1000 particles is effective. Again out of 10,000 surface sites on a hot particle, only one is active, thereby giving an intrinsic enhancement of  $ca 10^7$ .

This work is expected to lead to better understanding of the size-dependent properties of the single organic nanostructures, semiconductors and nanocrystals. SERS of non-fluorescent hemo-proteins, nucleotides and biological compounds can be studied at molecular level. Molecular resonance absorption is not really a pre-requisite for such studies because the large enhancement shown by selected 'hot particles' can be exploited by tailoring the particle sizes using nano fabrication techniques afforded by scanning probe microscopy.

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