A Golden Molecular Ruler

Coupled Nanoparticles for Nanoscale Distance Measurements

In a collaboration between the LBNL research groups of Paul Alivisatos and Jan Liphardt (Physical Biosciences Division), a new and robust "molecular ruler" based on gold and silver nanoparticles has been developed.

Fluorescence resonance energy transfer (FRET) is widely used in the biological sciences for measuring distances between groups of molecules or complexes. The transfer occurs when the emission spectrum of a laser-excited "donor" dye molecule overlaps the absorption spectrum of an "acceptor" dye molecule 1-10nm away. The efficiency of this transfer is dependent on the distance between the molecules; an analysis of the ratio of the donor emission to the acceptor emission can be used to measure their separation. Changes in FRET can be used to probe dynamic biological processes, even at the single molecule level. However, the dye molecules that are now in this technique suffer from "blinking" and "photobleaching" under laser excitation, which limits the observation time to a few tens of seconds.

The LBNL team overcame this problem by using gold and silver nanoparticles instead of the dye molecules. The electrons in noble metals scatter light though a process known as a "plasmon resonance." The scattering wavelength is dependent on particle size; in fact, this effect has been used for hundreds of years in producing multicolored "stained" glass. Further, it is known, both theoretically and from measurements, that the plasmon resonance wavelength of two nearby identical nanoparticles shifts as a function of the distance between them. In contrast to FRET, this scattering process does not suffer from blinking or bleaching.

The team bound metal nanoparticles to a glass slide, attached short DNA strands of known length, and then a second metal particle to the free end of each strand. Light scattering was measured with a conventional optical microscope. For both gold and silver nanoparticles, the spectral difference between a single isolated particle and a pair of adjacent nanoparticles was easily observed (panel A). The particle complexes were stable and could be monitored continuously for hours. As a qualitative test of this "plasmon" ruler, the interparticle distance was changed by adjusting the ionic strength of the solution. This affects the effective length of the DNA (its rigidity, therefore extension, changes) and therefore the distance between the particles. As expected, the spectrum shifted. The team also used the ruler to monitor the single strand-double strand transition of DNA. The signal from gold nanoparticles tethered at the ends of a piece of single-stranded DNA was monitored as the complementary DNA strand was added and bound. Again, the spectral shift expected from the resulting 2-nanometer increase in distance between the particles—consistent with the fact that the double helix is more rigid than a single strand—was observed.

These new plasmon-based molecular rulers have the potential to become an alternative to dye-based FRET for *in-vitro* single-molecule experiments, especially for applications demanding long observation times without dye bleaching. In addition, analytical bulk assays based on aggregation can now be extended to the single molecule level, enhancing their sensitivity and allowing microfluidic-based parallel processing.

A. P. Alivisatos (510) 642-7371, Biomolecular Materials Program, Materials Sciences Division (510) 486-4755, Berkeley Lab.

C. Sönnichsen, B. M. Reinhard, J. Liphardt, and A. P. Alivisatos, "A molecular ruler based on plasmon coupling of single gold and silver nanoparticles," *Nature Biotech*. 23, 6, 741, (June 2005).

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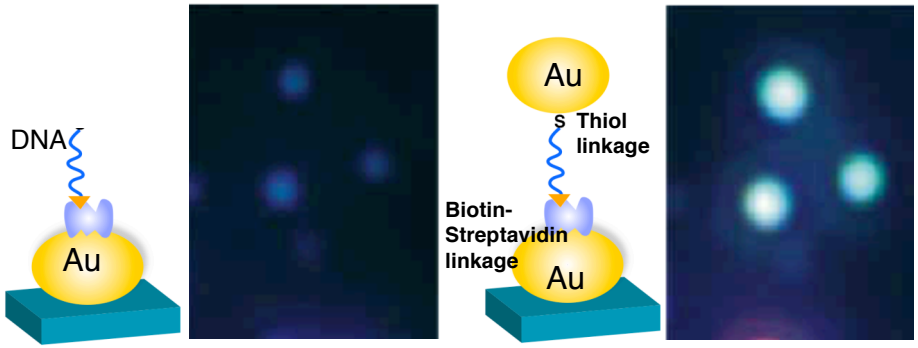
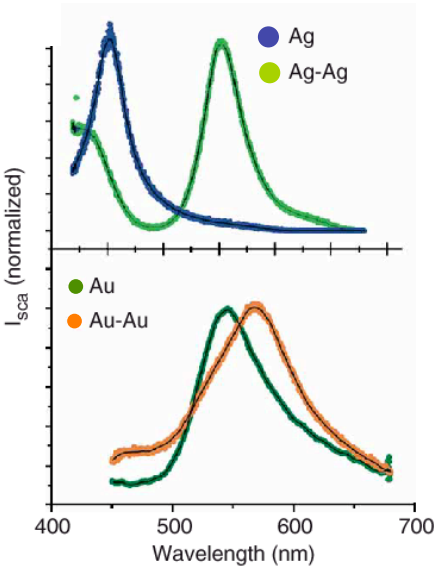
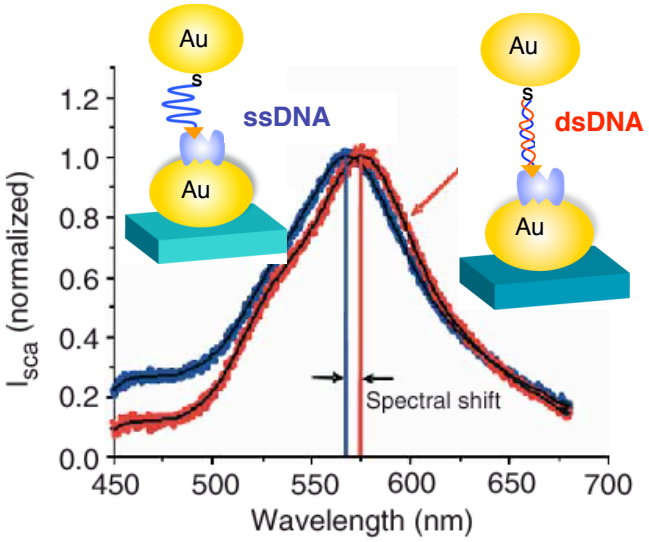


A. Microscope images before (left) and after (right) *attachment of second Au particle clearly differentiate free (blue) and closely bound (blue-green) nanoparticles. Significant spectral shifts (below) are observed for pairs of both silver and gold nanoparticles within 10 nm of each other.*





The time-resolved spectra observed before (red), and after (blue) the attachment of a complementary strand of DNA, which pushes the particles apart by about 2.1 nm.