Nano Focus

Single nanotube endocytosis imaged in 3D using a plasmonic ruler

▲arbon nanotubes (CNTs) exhibit - unique optical and electrical properties. Profiting from this behavior, a group of researchers comprising G. Hong, J.Z. Wu, J.T. Robinson, H. Wang, B. Zang, and H. Dai from Stanford University has used the intrinsic ultrasensitive near-infrared (NIR) fluorescence enhancement property of CNTs to facilitate a unique three-dimensional (3D) method of imaging the cellular internalization of single molecules (i.e., endocytosis). These results were published in the February 28 issue of Nature Communications (DOI:10.1038/ncomms1698), and are based on the design of a "plasmonic ruler" comprising a single CNT near a plasmonic substrate made of gold islands (Au/Au film). The gold film exhibits a plasmonic resonance peak at a wavelength of ~800 nm and enhances the NIR fluorescence of the PEGylated CNT. The researchers also report an exponential decay in the fluorescence enhancement with distance of the PEGylated CNT from the plasmonic substrate. The addition of PEG helped prevent the CNT from being quenched by the metal substrate. This distance-dependent fluorescence enhancement was then used to create a working demonstration of a 3D tracking system with high temporal and sub-10 nm axial resolution.

The researchers then employed their plasmonic ruler to track cellular internalization of individual single-walled CNTs. Trypsinized U87-MG cells were stained with highly diluted single-walled



Single nanotube imaging on cell membrane on a plasmonic gold substrate at 37°C. (a–c) Photoluminescence (PL) images of a single carbon nanotube (CNT) overlaid with optical images of a cell (outlined by the dashed line) at three time points: 20 s, 73.3 s, and 263.3 s after the incubation temperature stabilized at 37°C. (d) Two-dimensional trajectory of the single CNT moving on the cell membrane during endocytosis. (e) PL intensity (black squares) of this single nanotube plotted as a function of time and fitted to a first-order exponential decay. (f) Schematic of a single nanotube being endocytosed by the invagination of cell membrane and probed by the ultrasensitive "nanoscopic ruler" of the plasmonic Au substrate.

carbon nanotubes (SWCNTs) with cell targeting ligands at a low temperature. The stained cells were then cast onto the plasmonic gold substrate, and the plasmonically enhanced fluorescence of a SWCNT undergoing the process of endocytosis was imaged successfully by analyzing the near-infrared-II (1.1-1.7 um wavelength, the so-called second window of near-infrared) emission spectra. Upon identification of a single CNT using the emission spectra, the incubation temperature was increased and stabilized at 37°C and the fluorescence of the SWCNT during endocytosis was tracked at 0.3 frames per second. The figure depicts an example of the SWNCT endocytosis imaging and tracking process on a plasmonic gold substrate. The resultant endocytosis of the SWCNT was found to be clathrin-dependent.

This 3D imaging system exhibits high temporal and axial resolution with the ability to track ~10 nm scale transmembrane displacements. However, in the current setup, the plasmonic enhancement property is limited to a distance of 20 nm from the gold substrate. The researchers predict that this limitation can be overcome by exploring different combinations of fluorophores and plasmonic substrates, which will lead to the creation of plasmonic rulers of varying lengths able to probe an extended range of nanoscale molecular motions.

Rufina S.A. Sesuraj

Nano Focus

Plasmonic gold nanotubes serve as sensitive refractiveindex reporters when suspended in solution

Of the many potential applications of plasmonic metallic nanostructures, their use as refractive-index reporters has proven particularly successful. This behavior can be optimized when resonances occur in the near-infrared region, and through the use of hollow nanostructures, which experience reduced plasmonic damping and exhibit stronger surface plasmon resonance (SPR) signals than their solid counterparts. Many applications also demand that such structures can be homogeneously suspended in solution.

As reported in the March 27 issue of *Chemistry of Materials* (DOI: 10.1021/ cm203184d; p. 963), C. Bridges, P. DiCarmine, and D. Seferos of the University of Toronto in Canada describe the first-known successful synthesis of solution-suspendable gold nanotubes.



Extinction spectra of gold nanotubes that are 55 ± 7 nm in diameter and aligned in an AAO template (a). Cartoon depiction (b) and SEM image of aligned nanotubes (c). Extinction spectra of gold nanotubes suspended in D₂O (length = 258 ± 42 nm; width = 55 ± 7 nm) (d), accompanied by their TEM (e, f) images. Scale bars are 100 nm. Reproduced with permission from *Chem. Mater.* **24** (6) (2012), DOI: 10.1021/cm203184d; p. 963. ©2012 American Chemical Society.

The synthesis was accomplished by deposition of metals at the base of the template followed by electro-polymerization of a sacrificial hydrophobic polymer rod. Following collapse of the polymer core due to hydrophobic effects, a gold shell was deposited, and all sacrificial materials were then removed to yield hollow gold nanotubes. The synthesized nanotubes were ≈ 260 nm in length, ≈ 55 nm wide, and ≈ 15 nm thick, where the dimensions could be tailored according to the pore size and deposition time.

A series of spectroscopic experiments were performed to determine the SPR behavior of the nanotubes. Investigation of the nanotubes in water, *in situ* in the template showed a single peak corresponding to the transverse SPR at 553 nm, as compared with 574 nm for a similarly sized gold nanorod. Alternatively, suspension of the nanotubes in D_2O resulted in transverse and longitudinal SPR modes at 527 nm and 1210 nm, respectively, together with a weak quadrupolar mode at ~700 nm. In a final experiment, glycerol was added incrementally to a colloidal suspension of the nanotubes, and it was observed that the sensitivities of the longitudinal and transverse SPRs were approximately double those of solid nanorods.

The Seferos group said this discovery may open the door to further development and study of hollow nanoparticles, particularly in applications that require a high sensitivity to subtle changes in refractive index, such as sensing and imaging. Also of potential interest are the photothermal heating properties of these hollow nanostructures.

Anthony S. Stender



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