Where is it Heading? Single-Particle Tracking of Single-Walled Carbon Nanotubes

Michael S. Strano* and Hong Jin

Department of Chemical Engineering, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

ABSTRACT Single-particle tracking of individual single-walled carbon nanotubes (SWNTs) using their near-infrared band gap fluorescence is a powerful tool for understanding how these Brownian rods diffuse and interact with various molecular force potentials, including living systems. Pioneered by the Weisman laboratory at Rice University, the method is one of the only available to study single SWNT molecules in solution over extended periods since SWNTs have no apparent irreversible photobleaching threshold at moderate fluence and no intrinsic blinking mechanism. Recent progress by Tsyboulski *et al.* shows how real-time measurement of rotational and transitional diffusivities can provide information about rod length and mechanical properties. Recently, Jin *et al.* used single-particle tracking to map the trajectories of SWNTs as they are incorporated into and expelled from NIH-3T3 cells in real time. The technique has provided the first evidence of nanoparticle exocytosis in this case and demonstrates an expulsion rate that closely matches the endocytosis rate. The ability to track and to analyze single molecules in this way may lead to new technologies that utilize as their platform a single, freely diffusing nanotube.

Expectations for nanotechnology are exceedingly high, but in fact, there is much work to do in understanding the basic properties of nanomaterials. If we are to ultimately arrive at Feynman's miniature version of Maxwell's demon[1] and other autonomous nanomachines, we need to understand how nanometer-sized objects of various geometries and chemistries passively diffuse, orientate, and interact with other materials. In the case of single-walled carbon nanotubes (SWNTs), the discovery of near-infrared (NIR) photoluminescence in 2002 has greatly increased our ability to study their dynamics at the single-molecule level.[2] As a molecular fluorophore, SWNTs have an interesting combination of properties. Unlike organic dyes, the conjugated sp^2 carbon bonds in a carbon nanotube are chemically quite stable and the one-dimensional (1D) quantum confinement creates an unusual, low energy band gap in the NIR. This chemical stability translates into a high degree of photostability, as there is no apparent irreversible photobleaching observed for SWNTs, even at moderate fluence, and single-molecule spectroscopy, even for extended periods, is now routine.[3] This is not the case for even the most photostable quantum dots in solution, which tend to show moderate bleaching and an intermediate blinking that confounds continuous tracking.[4] Originally, the SWNT fluorescence quantum yield was estimated at 0.1% for ensembles that included defective species and debris,[5] but better sample preparation and experimental conditions have since yielded larger estimates, approaching 10%.[6] The transition dipole is polarized along the axial direction, making it relatively easy to determine the orientation of the rod.

The photostable single-walled nanotube is perfect for long-term single-particle tracking studies, enabling new phenomena to be observed and studied, including the observation of nanotube exocytosis from cells.

In terms of applications, our laboratory has been interested in using SWNTs as optical biosensors.[7−10] We and others have demonstrated utility for intracellular imaging[11−14] due to their stable NIR photoluminescence[15] and environmental sensitivity.[16] For the former, our laboratory has focused particularly on sensors based on photoluminescence quenching, enhancement,[7] and solvatochromic shifting.[7−10,17] Single-particle tracking (SPT) is a technique for studying the diffusion of small molecules both computationally[18,19] and experimentally.[20−22] However, the use of fluorophores constrains the observation window during real-time tracking due to photobleaching. The photostable SWNT is perfect for long-term SPT studies, enabling new phenomena to be observed and studied. As an illustration of this, we have recently applied SPT to SWNT−cell interactions using SPT in the NIR and were able to observe the exocytosis process.[14] In a simpler set of experiments, SPT provides a wealth of information about diffusional properties *via* analysis of the particle mean square displacement, among other metrics.

See the accompanying Article by Tsyboulski *et al.* on p 1770.

*Address correspondence to strano@mit.edu.

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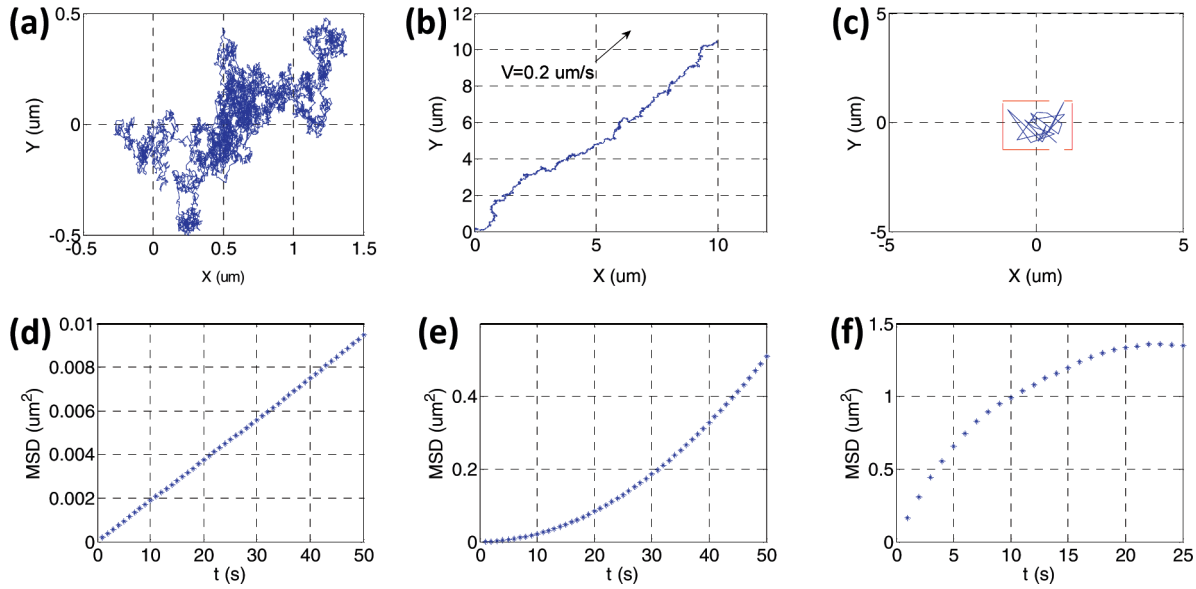

Figure 1. Simulated trajectories starting from (0, 0) of (a) normal diffusion, (b) convective diffusion, and (c) confined diffusion. The corresponding MSD plot is shown in (d) normal diffusion where MSD = $4Dt$, (e) convective diffusion where MSD = $4Dt + (Vt)^2$, and (f) confined diffusion where MSD = $C[1 - A_1\exp(-4A_2Dt/C)]$.

We discuss the basis for this in the next section.

Single-Particle Tracking: A Primer. For nanoparticle diffusion in solvent, in the absence of other forces, the motion of the particle is Brownian as it interacts with the thermal bath. One can experimentally understand the forces acting on the particle through analysis of the mean squared displacement (MSD). The MSD for an individual trajectory is expressed as[19] $\mathrm{MSD}(n\Delta t) = 1/(N - n - 1)\sum_{j=1}^{N-n-1}\{[x(j\Delta t + n\Delta t) - x(j\Delta t)]^2 + [y(j\Delta t + n\Delta t) - y(\Delta t)]^2\}$ where $x(t)$ and $y(t)$ are particle positions at time t, N is the total number of frames, n is the number of time intervals, and j is a positive integer.

Some basic types of motion can be identified using the functional form of the MSD, including normal diffusion (MSD = $4Dt$, where D is the microscopic diffusion coefficient, derived from a two-dimensional random walk[23]), anomalous diffusion (MSD = $4Dt^{\alpha}$, where $\alpha < 1$), directed/convective motion with diffusion (MSD = $4Dt + (Vt)^2$, where V is mean field velocity), and corralled/confined diffusion (MSD = $C[1 - A_1\exp(-4A_2Dt/C)]$, where C is the corral size and A_1 and A_2 are constants determined by the corral geometry).[21] Typical trajectories appear in Figure 1, showing normal (Figure 1a), convective (Figure 1b), and confined diffusion (Figure 1c).

The MSD is a useful way to understand some types of motion, but as others have pointed out, it cannot detect potentially important diffusive regimes, including regions of altered diffusivity (so-called "rafts") or barriers instead of complete confinement.[19] Simple forms of analysis also break down when particles are strongly interacting, and this is important for nanoparticles that are often suspended with surfactants or polymers. Aggregates of the suspending medium (*i.e.*, micelles) may themselves be collision partners in free solution, altering an apparent translation or rotational diffusivity.

Translational and Rotational Dynamics of SWNTs in Aqueous Suspension. Good progress toward the development of the metrology of SWNT dynamics in solution is presented by Tsyboulski *et al.* in this issue of *ACS Nano*.[24] The authors have pioneered the use of NIR fluorescence videomicroscopy, and in this contribution, they characterize the translational and rotational diffusion of SWNT aqueous suspensions. For translational diffusion, the results are not surprising. They confirm that the SWNTs behave as rigid rods, even for relatively long (6 μm) species. The range of diffusion coefficients from 0.3 to 6 $\mu m^2/s$ was in agreement with SWNT lengths of 130 nm to 6 μm.

The study is, however, the first to address seriously the rotation diffusivity of SWNTs. From past work, it is known that photoluminescent emission from SWNTs under these conditions is completely photostable, with no intrinsic blinking observed as in the case of semiconducting nanocrystals.[3] Known quenching molecules include H^+, but relatively low pH values are necessary to observe the phenomena. So for freely translating SWNTs, Tsyboulski *et al.* show that an apparent "blinking" of the emission signal actually provides information about the rotational diffusivity of the SWNTs.[24] The amplitude of the modulation is larger for slowly translating SWNTs, which are longer. The diffusion constant D_{rot} for the rotational diffusion of a rotating rod decreases with increasing length:

$$D_{rot} = \frac{3k_BT}{\pi\eta_s}\cdot\frac{\ln(L/d) - \gamma_r}{L^3}$$

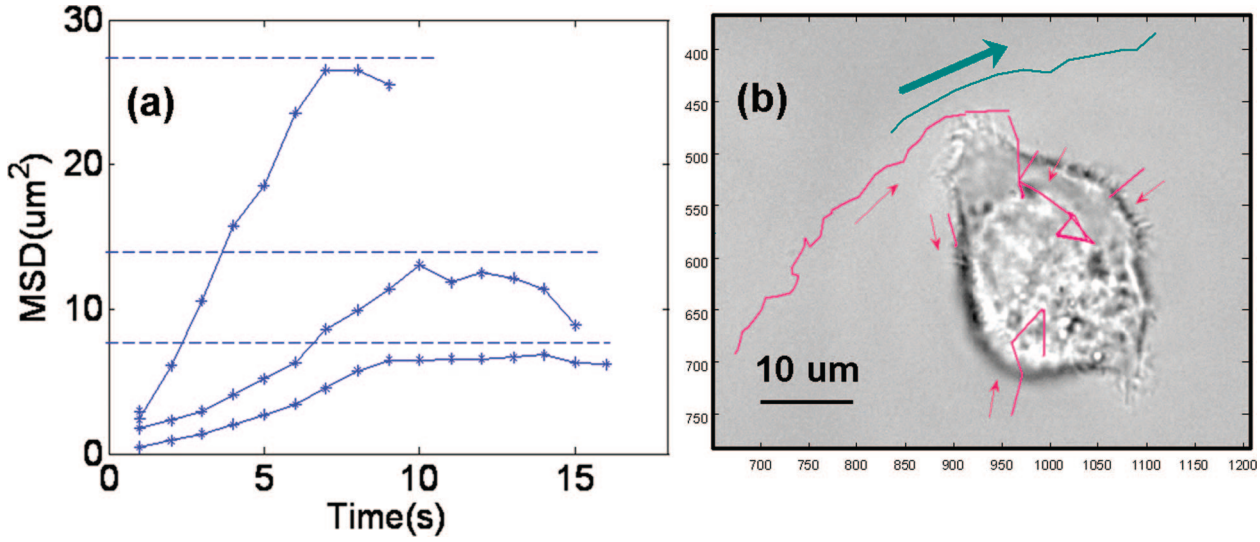

Figure 2. (a) Example MSD plot from single-particle tracking of the interaction of DNA−SWNTs with NIH-3T3 cells on a perfusion stage shows that the type of motion changes from convective to confined. DNA−SWNTs of 5 mg/L were added to the flow (6.65 μL/s) of growth medium (DMEM) and tracked over time. (b) Example trajectories of SWNTs show that the physical picture of (a) is SWNTs adsorbing from the perfusion flow onto the cell membrane, consistent with the MSD feature.

where k_B is the Boltzmann constant, T is the sample temperature, η_s is the solution viscosity, L is the rod length, d is the effective rod diameter, and γ_r is a length-dependent end-correction coefficient.

Faster-diffusing SWNTs (with shorter lengths) have rotational fluctuations that are difficult to resolve for diffusion in the water/surfactant mixture. However, a medium composed of a more viscous 60% glycerin/water mixture amplifies these fluctuations as predicted. This evidence clearly identifies the behavior as Brownian in origin, as well as the fact that the modulation for a conic aperture section appears to scale as

$$\langle I \rangle / I_{max} = \langle \sin^4 \alpha \cos^2 \phi \rangle$$

where $\langle I \rangle$ is the average emission signal of any randomly rotating nanotube over a long time interval, I_{max} is a constant fraction of the maximum emission signal, and α and ϕ are standard spherical coordinates. The analysis offers a way of assessing the translational diffusivity and gradient in rotational motion in real time, for SWNTs in various environments. This should provide a useful set of tools to study the environmental change using SWNTs as sensors.

Single-Particle Tracking of SWNT in Live Cells. What can one do with such information? We recently used essentially the same set of tools to study the endocytosis and trafficking of SWNTs in live NIH-3T3 cells in real time.[14] In this case, even more can be inferred from MSD than translational diffusivities. The MSD is a signature of the transport mechanism and the transport environment.

In this previous study, over 10 000 individual trajectories of nonphotobleaching SWNTs were tracked as they were incorporated into and expelled from NIH-3T3 cells in real time on a perfusion microscope stage for a typical period of 6 h. An analysis of MSD allows the complete construction of the mechanistic steps involved from single duration experiments. We observed conclusive evidence of SWNT exocytosis and showed that the rate closely matches the endocytosis rate with negligible temporal offset. We identified and studied the endocytosis and exocytosis pathway that leads to the previously observed aggregation and accumulation of SWNTs within the cells.

This study would not be possible using the conventional incubation method, where the photobleaching of common utilized fluorophores is a major limitation to extended real-time measurement. Photobleaching constrains the observation window during tracking so that events that occur on the order of several hours normally must be observed through multiple and distinct incubation periods. Tracking the same particle through successive diffusive regimes has distinct advantages. For instance, there is an identifying signature when SWNTs are adsorbed to a cell surface from the perfusion stream. The MSD for this event changes from convective to confined diffusion (Figure 2), as expected.

All of these studies suggest that SPT studies of carbon nanotubes and related nanomaterials will be heavily utilized in future work to answer complex questions in nanotechnology. In addition to sensors, as described above, applications to drug delivery, imaging, and therapeutic agents and studies investigating the environmental fate of carbon nanotubes will all benefit from these techniques. There are still many questions that need to be addressed, such as the experimental measurement and understanding of interparticle potentials for surfactant-wrapped SWNTs in solu-

tion and how their chemically derivatized counterparts translate and rotate. Single-particle tracking has significant advantages for understanding the toxicology of nanomaterials when enzymatic cleavage and quenching complicates the use of organic fluorophore labeling. Further on the horizon, SPT methods are an important tool for designing autonomous and semiautonomous nanomachines, which have the potential to fulfill Feynman's promise.

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