Measuring Single-Wall Carbon Nanotubes with Solid-State Nanopores

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Introduction

Solid-state nanopores represent an emerging technique for single-molecule detection and analysis[1, 2]. Essentially, the technique utilizes a single, nanometer-scale aperture fabricated in an otherwise impermeable membrane as the sole pathway between two reservoirs of ionic solution. Application of a voltage across this membrane sets up an electric field that is highly localized to the pore which leads to electrophoretically driven transport of charged molecules through the pore to the opposite chamber (Fig. 1a). During this translocation process, the presence of the passing molecule temporarily affects the flow of ions through the pore, resulting in a measurable change in trans-membrane conductance. The characteristics (Fig. 1a, inset) of this transient change (amplitude ΔG, duration Δt) are indicative of properties of the molecule being transported (diameter, length).

Thus far, this measurement system has been utilized almost exclusively for investigating biological molecules like DNA[3,4], RNA[5] and proteins[6,7], wherein measurements did yield structural information useful for rapid screening applications. However, there is great potential for similar analysis of non-biological nanoparticles that has not yet been explored. One particular nano-object that is especially intriguing is the single-wall carbon nanotube (SWNT): a single sheet of graphene formed into a continuous cylinder (Fig. 1b). For this class of particle, diameter is dictated by the length of the wrapping vector and the angle at which the graphitic lattice wraps around the perimeter of the tube. These parameters have been found to be tied intrinsically to the nanotube electrical transport properties, which can range from metallic to semiconducting with a moderate band gap[8,9]. For this reason, the proposition of characterizing SWNT by size at the single nanotube level is attractive. Here, we present an exploratory study detailing the first measurements of SWNT transportation through solid-state nanopores.

Methods

Common microfabrication techniques[10] are used to produce free-standing silicon nitride membranes of 5 μm diameter and 20 nm thickness supported in commercially available silicon wafers. Next, such a membrane is inserted into a transmission electron microscope, where exposure with the highly focused electron beam is used to locally ablate the membrane, resulting in a single nanopore of controllable dimensions[11] (Fig. 2, left). For the experiments described here, nanopores with diameters of 18-20 nm were used. Samples are stored in 50% ethyl alcohol prior to use to maintain cleanliness. For translocation measurements, a chip is first cleaned with acetone and ethyl alcohol and dried under nitrogen flow before being treated with an oxygen plasma for 30 sec. The chip is then immediately loaded into a Perspex flow cell (Fig. 2), which contains two chambers with access to either side of the membrane. Measurement buffer is introduced onto both sides of the chip. High molar salt conditions are used here in order to emphasize size discrimination rather than charge discrimination in the measurements[12]. Electrical contact is established with each compartment using Ag/AgCl electrodes and current is measured using a patch-clamp amplifier (Axon 200B, Axon Instruments).

A given nanopore is first measured for low noise (<20 pA RMS) and linear current-voltage (I-V) curves, ensuring the absence of nanobubbles[13] or excessive contamination. Samples not meeting these standards can be treated with brief exposure to 1 M NaOH (~3 min) or rinsed in the flow cell with a cycle of water, ethyl alcohol and water before being reintroduced to measurement solution. Nanopore devices still outside the acceptable range after these treatments are abandoned in favor of a new chip; even fresh nanopores can be unsuitable for measurements due to unidentified causes. With the handling procedure described here, roughly 80% of all nanopores produced are suitable for measurement.

Target nanotube material is added to one (*cis*) chamber of the flow cell and a voltage is applied between the other (*trans*) chamber and the *cis* chamber (Fig. 2, right). Ionic current through the nanopore is recorded at 200 kHz and low-pass filtered at 100 kHz prior to digitization. Deviations from the baseline current greater than a set threshold level (4.5 times the average RMS noise level) are identified in real-time and trigger the segment of data to be recorded. In this way, translocation events are saved and long stretches of baseline are disregarded. Resultant events are then fit with a square pulse to characterize their average depth and duration.

Two main challenges exist for the measurement of carbon nanotubes with this measurement scheme. First, SWNT have no inherent charge associated with them, nullifying the electrophoretic mechanism of transport through the nanopore. Second, SWNT are hydrophobic, making them insoluble in the aqueous ionic solutions typically used in nanopore measurements. Both of these issues can be addressed simultaneously by water-solubilization techniques[14,15]. Essentially, these rely on the addition of amphiphilic molecules, which can wrap around SWNT and make them water soluble. Amphiphilic molecules with inherent charge give a net

charge to the encapsulated nanotube structure, thereby allowing electrodynamic processes to drive their motion.

Surfactant-wrapped SWNT

The first solubilizing agent investigated is the bile-acid detergent sodium cholate (SC, 98%, Sigma Aldrich), chosen for its affinity with SWNT in previous treatments[16] and for its anionic composition. For this preparation, starting SWNT material is formed through the electric arc discharge method (P2 nanotubes, Carbon Solutions, Inc.). This is added to 70 mL of 1% w/v sodium cholate (SC) aqueous solution at a loading of 2 mg mL^{-1} and treated with horn ultrasonication (Fisher Scientific model 500 Sonic Dismembrator) for 1 h at 55 W using an immersed tip extension to effectively disperse bundled material. The resultant solutions are ultracentrifuged in a SW 32 rotor (Beckman Coulter) for 30 minutes at 32 krpm to remove large aggregates. The top 75% of the supernatant is retained as a solution nominally rich in individual SWNT.

When added to a typical measurement solution containing 1 M KCl, 10 mM Tris-HCL (pH 8.0), and 1 mM EDTA, this material is observed to quickly sediment out of solution. This is due to competition between ions in the detergent structure (Na+) and in the solute (K+), causing fast dissociation of SC from the SWNT. Therefore, for these measurements, the KCl in the measurement solution is replaced with 1 M NaCl, which is expected to function very similarly in nanopore experiments. Under these conditions, SWNT material indeed stays suspended on the order of hours. Though it is difficult to gauge SWNT concentration after the loss of material in the preparation step, the final concentration following dilution in measurement solution contains approximately 10 μg/ml.

Control experiments are first performed on measurement solution that contains SC only. At a concentration of 100 μg/ml (greater than the presumed background in the diluted final measurement solution), a voltage applied across the nanopore at either polarity results in no observable events, as expected. However, after exchanging for measurement solution that contains SC-encapsulated SWNT, transient spikes in the conductance $G=I_{meas}/V_{appl}$ are measured upon the application of +100 mV to the *trans* chamber (Fig. 3a). These events are easily resolved above the noise and correspond to translocation of SWNT through the nanopore. However, further inspection of the measurements reveals non-ideal aspects of the state of the material.

Fig. 3b shows examples of individual recorded events, demonstrating both simple, square-pulse-like events of different depths (top, middle) as well as events with substructure (bottom). This observation is made clearer by looking at a histogram of all recorded conductance data points relative to the baseline for 614 individual events (Fig. 3c). The plot reveals multiple populations of quantized conductance

blockade levels (Fig. 3c inset), reminiscent of measurements on double-strand DNA (dsDNA)[3,4]. In that case, the existence of multiple populations was explained by folding of the molecule; doubly folded DNA would result in a blockage of the measured conductance twice as large as linearly translocating DNA. However, this is not a valid explanation here. The very high stiffness of SWNTs results in a persistence length of about 800 nm[17] – far too large to allow a folded tube to pass through a constriction of only 20 nm. It is also notable that in previous dsDNA experiments, partially-folded molecules were found to enter the pore almost exclusively with the folded end first; that is, the deep portion of events with two internal levels would occur first. With SC-SWNT, events with multiple levels appear not to be biased towards this orientation (i.e. Fig. 3b, bottom). This is a further argument against folding in the present case.

The measurements are much more easily explained by bundling of multiple nanotubes. Although the initial treatments are performed to create separated nanotubes in solution, there is apparently some re-agglomeration of the tubes. This is likely a result of the high ionic strength of the measurement solution. While like-charged materials normally repel each other, under 1 M salt conditions, the layer of counterions surrounding the encapsulated SWNT screens this effect and allows for increased interactions among individual tubes. The high salt also interferes with the ability of SC to solubilize the tubes in general, as evidenced by the observation that sedimentation of material occurs over a period of days. The multiple populations see in Fig.3c are therefore the result of different numbers of individual SWNT bundled together, encapsulated in SC (Fig. 3c, blue). The substructure observed in individual events is then readily explained by the aggregation of tubes with different lengths.

In order to apply the nanopore system to single-molecule SWNT translocation, tubes ideally are to be analyzed on an individual basis. As such, SC is an nonideal solubilizing agent and must be replaced by one with higher salt stability.

DNA-wrapped SWNTs: $(AT)_{15}$

The second class of solubilizing agent investigated is small (15 base) single-strand DNA (ssDNA) oligomers. These are chosen because of recent demonstrated success in SWNT wrapping[15,18] and because of the known negative charge of the DNA phosphate backbone. Furthermore, DNA is very stable in conditions of high ionic strength, making solutions stable for long periods of time. Preparation[19] of DNA-wrapped SWNT begins with a mixture of 0.1 M NaCl solutions of DNA oligomers (1.0 mg mL^{-1}) and SWNTs (~0.2 mg mL^{-1}). These are dialyzed in a 3.5-5.0 kDa membrane (Spectrum Laboratories, Inc.) in a bath of 0.1 M NaCl for 3 days to remove the surfactant slowly while the DNA oligomers encapsulate the SWNTs. Subsequently, the solutions are further dialyzed in a 100 kDa membrane for 7 days to remove any excess DNA and iodixanol. Here, DNA oligomers with sequence $(AT)_{15}$ are chosen first because of the proven high affinity of alternating purine-pyrimidine nucleotides

to nanotubes[18]. However, as will be shown below, this choice creates unintended complications.

$(AT)_{15}$–wrapped SWNTs are added to a measurement solution containing 1 M KCl, 10 mM Tris-HCL (pH 8.0), and 1 mM EDTA. Additionally, 0.01% (v/v) POP-6 (Applied Biosystems) is added to this solution in order to prevent sticking to the nanopore[20]. The concentration of SWNTs in this case is ~300 μg/mL. When this material is added to the *cis* side, conductance blockades are again observed. A typical measurement trace is shown in Fig. 4a, yielding events that are easily resolvable above the noise. Interestingly, these occur when *negative* voltage is applied to the *trans* chamber; that is, the negatively charged SWNTs appear to translocate counter to the direction of electrophoresis. A possible explanation for this is presented in the following section.

The histogram of all measured data points for 2463 individual events (Fig. 4b) again shows multiple quantized populations. $(AT)_{15}$-SWNT is found to be much more stable in ionic solutions - it was found to remain suspended for days - so it is unlikely that the DNA encapsulation layer is unwrapped to allow for tube-tube interaction and then reformed around the bundle. Instead, the broad histogram can be explained by DNA interactions. Because $(AT)_{15}$ oligomers were used here, complex hybridization events can occur between DNA wrapped around different SWNTs, where part of an AT oligomer on one tube can hybridize to a TA counterpart on an oligomer on an adjacent tube. In this way, bundles of DNA-wrapped SWNTs can be formed through mutual coupling of the wrapping oligomers. This indicates that the material in a form is not conducive to serial molecular characterization.

DNA-wrapped SWNTs: $(AC)_{15}$

In order to address the difficulty of bundling between individual SWNTs, the oligomer sequence is switched from $(AT)_{15}$ to $(AC)_{15}$. This allows for similar SWNT wrapping, but removes the possibility of cross-hybridization as adenine does not form a natural hybridization pair with cytosine (like it does with thymine). Material is prepared in the same manner as described above and added to final measurement solution with the same concentration of components, except that the POP-6 is replaced by 0.01% (v/v) Tween-20 to reduce interaction with the interior of the nanopore. When material is added to the *cis* chamber, events are observed upon the application of -100 mV to the *trans* chamber; once again, counter to the expected direction of electrophoresis (Fig. 5a). In this case, however, the histogram of all measured conductance data points (462 events) reveals only a single population (Fig. 5b). This indicates that the recorded events indeed correspond to the passage of *individual* DNA-wrapped SWNTs through the nanopore.

A possible explanation for the unexpected directionality of travel is the effect of electroosmosis The same electric field that is responsible for electrophoresis has an effect on the counterion cloud surrounding charged objects in ionic solution, like the

DNA-wrapped SWNT and the nanopore itself. This can set up a net flow that opposes the direction of the electrophoretic force. A recent report[21] demonstrated that the magnitude of this electroosmotic force can be equivalent or even greater than electrophoresis, causing translocation events to occur in the non-intuitive direction. Future experiments and modeling will investigate this aspect further, but sufficient controls are performed here in order to confirm that measured events are caused by DNA-SWNTs. For instance, clean measurement buffer without nanotube material causes no events in either direction over a period of minutes. The addition of $(AC)_{15}$ oligomers at a concentration of 100 nM (in case the DNA dissociates from the SWNT) similarly causes no events in either direction. When DNA-wrapped SWNT are added to the *cis* chamber and +100 mV applied to the *trans* chamber, no events are observed over a period of minutes. Upon switching to -100 mV, however, transient conductance blockades are immediately observed.

Having established a method by which to translocate $(AC)_{15}$-SWNTs, we now turn to the analysis of this data. Fig. 6a shows the average conductance blockade ΔG for all 462 recorded translocation events. The distribution has a center at 1.49 nS and a width (FWHM) of 0.68 nS. Although the physical diameter distribution that this corresponds to is difficult to quantify, it is possible to use previous measurements on biomolecules like ssDNA[22], dsDNA[4] and RecA-coated dsDNA[23] as a metric. By comparing the known cross-sectional areas of these materials to the measured conductance blockade they cause upon translocation and applying a linear fit to them (Fig. 6b), we estimate the $(AC)_{15}$-SWNT diameter as about 2.5 nm. This fits quite well with expectations when one considers the size of the raw SWNT material alone (1.3-1.7 nm) and a thin DNA layer surrounding it.

The dwell time distribution of events (Fig. 6c) yields a Δt of 53 (+42, -24) μs, where the +42/-24 μs values denote the width of the distribution. Fig. 6d shows the length distribution of SWNT used here, as measured by AFM[24]. The shapes of Fig.6c and 6d are gratifyingly similar. The data of Fig.6d indicate a contour length of 0.4 (+0.4, -0.2) μm. An equivalent length of dsDNA under similar solvent conditions yields a dwell time of ~300 μs[25], meaning that $(AC)_{15}$-SWNT appear to translocate significantly faster than the biopolymer. This surprising finding may be attributed to a lower viscous drag force on the nanotube caused by its high persistence length, which would make a lagging entropic coil impossible[25] It is also interesting to note that both distributions follow log-normal distributions. This may add further support to the notion that translocation measurements can be used to characterize the SWNT length.

First experiments towards diameter-specific nanotube characterization

As an initial investigation into the efficacy of this method to characterize individual SWNTs based on their diameters, raw nanotube material is treated prior to $(AC)_{15}$ wrapping in order to enrich the fraction of tubes of a single diameter. In this case,

raw SWNT formed with the CoMoCAT method (Southwest Nano Technologies) is treated with the bulk method of density gradient ultracentrifugation[16, 26]. The result is a mixture of material composed of ~60% (6,5) nanotubes (as judged from fits to the absorption spectrum), which have a well-defined diameter[9] of 0.75 nm but the same length distribution as above.

Nanopore translocation of this material results in the same types of conductance blockade events observed previously. However, a histogram of average event depth for 457 events (Fig. 7a) reveals a distribution with a center at 1.38 nS and a FWHM of 0.58 nS. A fit to the dwell time distribution (Fig. 7b) yields a Δt nearly identical to that of untreated SWNT at 45 (+55, -27) μs. Both of these observations support the supposition that a mixture of nanotubes enriched in (6,5) SWNT is being translocated. Because of their smaller than average diameter, material rich in this particular type of SWNT is expected to result in a smaller average conductance blockade than that of non-enriched material, which is indeed observed. Furthermore, because the length distribution is unchanged relative to previous material, the dwell times is expected to be equivalent, which again is the case. These measurements, therefore, suggest that high-resolution SWNT sorting on diameter with the nanopore technique may be possible in future experiments.

Conclusions

Solid-state nanopores can be used to translocate SWNT in a manner very similar to previous experiments that have largely concentrated on biopolymers. In order to successfully perform this type of measurement, two main challenges had to be overcome: SWNT must be made soluble in a suitable ionic solution and a charge must be added to the normally neutral particles, making them susceptible to electrical forces. Here, we showed that this is accomplished best by wrapping individual SWNTs in charged amphiphilic molecules.

We showed that the anionic detergent sodium cholate can be used for this purpose, but that strong ionic conditions of the measurement solution facilitate tube-tube interactions to result in bundles of multiple SWNTs rather than the ideal of individual tubes. We next showed that DNA oligomers of sequence $(AT)_{15}$ can be used in a similar fashion, but that cross-hybridization between the nucleotide partners adenine and thymine result in agglomeration of multiple SWNTs. Finally, we showed that coating with oligomers of sequence $(AC)_{15}$ results in well-dispersed, individual tubes that translocate serially in a well-defined way. These translocating nanotubes yield a single distribution in both the conductance blockade ΔG and dwell time Δt, with values that correspond well to the expected properties of the starting material.

The capability to translocate SWNTs opens the door for single-molecule sorting based on diameter, which relates directly to their electronic properties. As a first

step, we performed nanopore measurements on SWNT pretreated to contain a high percentage of one type of tube: (6,5) nanotubes of 0.75 nm diameter. Here, translocations yielded a distribution of conductance blockade levels that is smaller and narrower, but with a dwell time distribution indistinguishable from untreated SWNT. These data support the idea that translocation of the small-diameter material causes a resolvable statistical difference in size differentiation when compared to non-enriched SWNT.

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20. In control experiments, clean solutions with POP-6 added are found to cause translocation events. These were very brief (<10 μs) and more shallow (<1 nS) than SWNT events, and thus do not affect the interpretation of the results. Still, in subsequent measurements, POP-6 is replaced to avoid complications due to spurious events.
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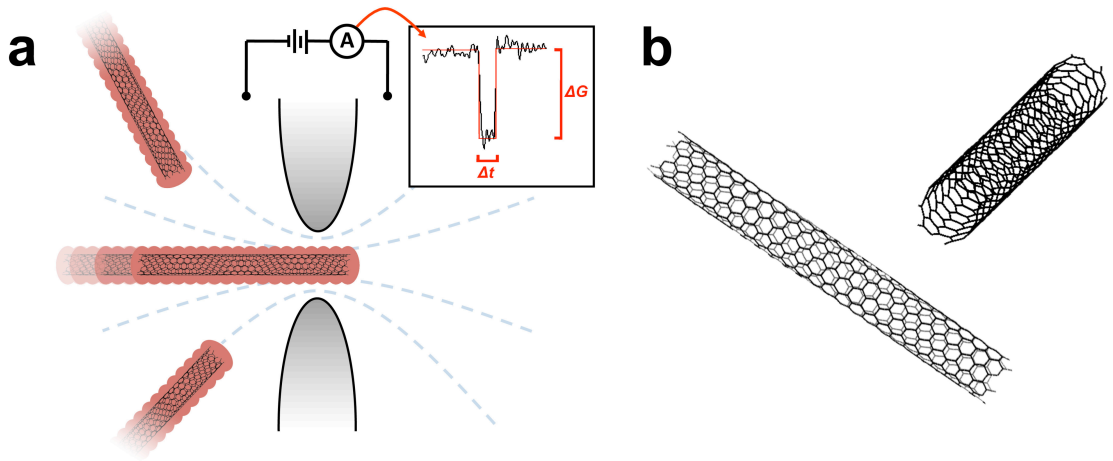

Figure 1 (a) Solid-state nanopore translocation, in which charged particles are threaded through an aperture one at a time, resulting in measurable blockades in conductance (inset). (b) Single-wall carbon nanotube structure, showing the cylindrically-wrapped graphene lattice of bare nanotubes.

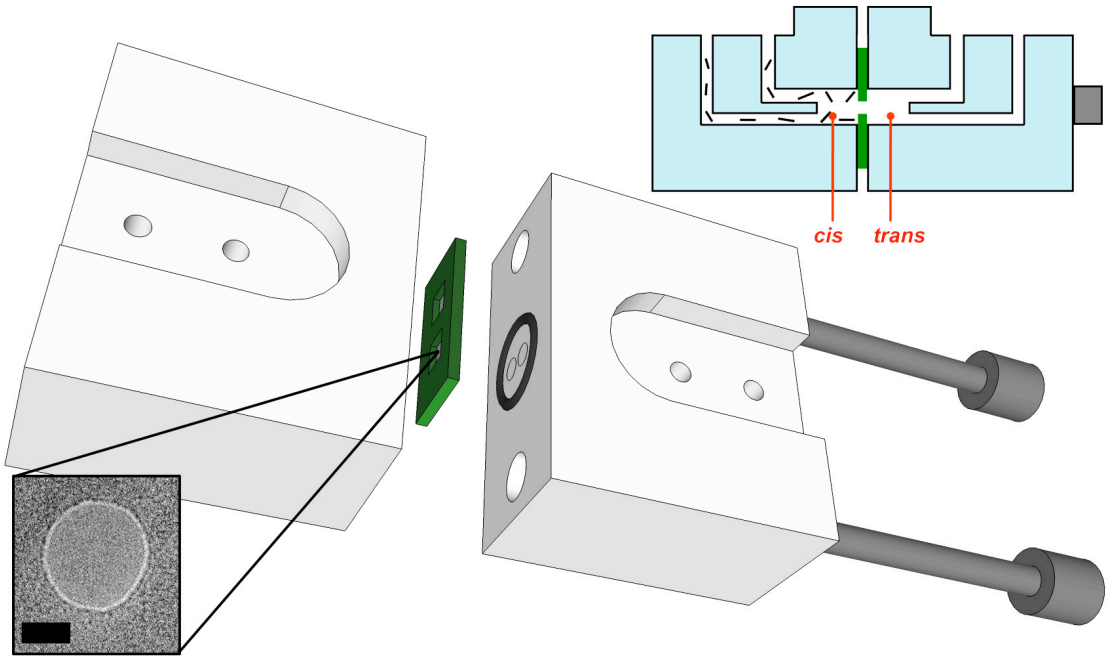

Figure 2 Flow cell used for translocation measurements through a solid-state nanopore. Inset: transmission electron micrograph of a typical pore (scale bar 10 nm). Upper right: side view of the assembled flow cell, showing the *cis* and *trans* chamber separated by a single nanopore.

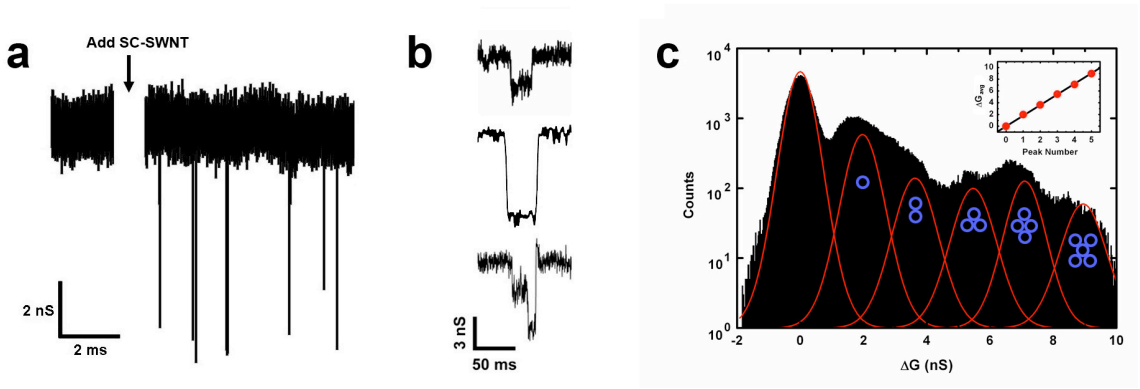

Figure 3 Translocation of sodium cholate-wrapped SWNTs. (a) Measured conductance trace showing translocation events upon addition of SWNT. (b) Three typical events, indicating passage of an individual SC-SWNT (top), a two-tube bundle (middle) and an individual tube partially bundled at one end (bottom). See text for details. (c) Histogram of all measured conductance points, showing multiple populations corresponding to different bundle sizes (blue). The distribution is fit with a series of peaks with equal width. Inset: centers of fits to subpopulations, showing a linear relationship between the average conductance-blockade depth and the peak number.

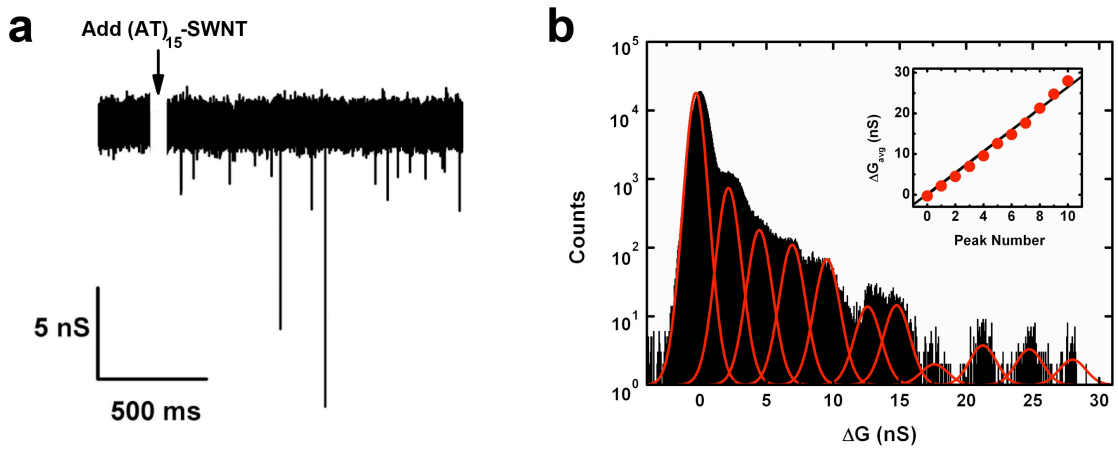

Figure 4 Translocation of $(AT)_{15}$-wrapped SWNTs. (a) Conductance trace showing translocation events upon addition of $(AT)_{15}$-SWNT. (b) Histogram of recorded conductance, showing up to ten subpopulations resulting from bundling due to to AT-TA hybridization between individual wrapped SWNTs. Inset: centers of fits to subpopulations, showing a linear relationship between the average conductance-blockade depth and the peak number.

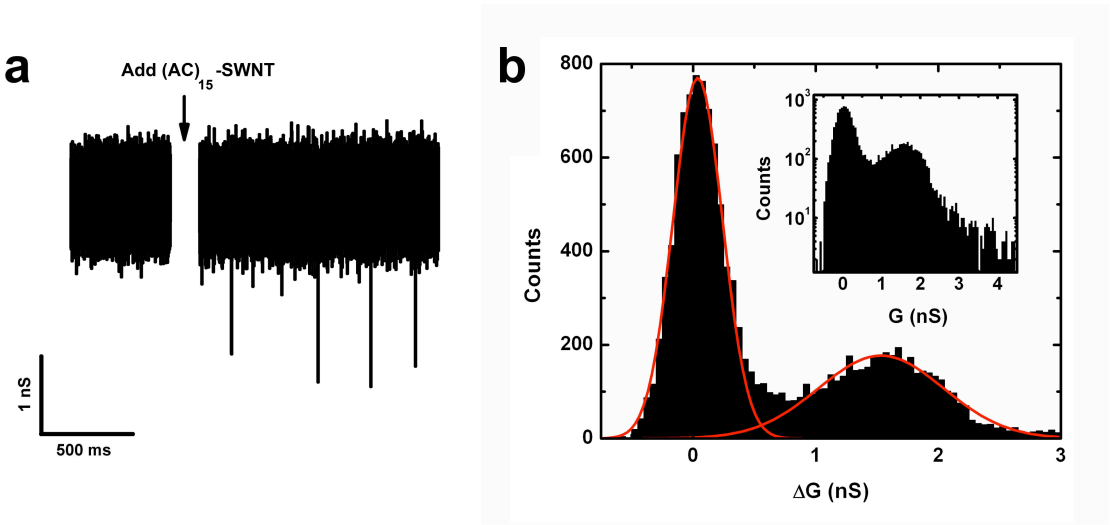

Figure 5 Translocation of $(AC)_{15}$-wrapped SWNTs. (a) Conductance trace showing translocation events upon addition of $(AC)_{15}$-SWNT. (b) Histogram of the recorded conductance, showing a single population outside of the baseline value at 0. Inset: semi-log plot of the same data.

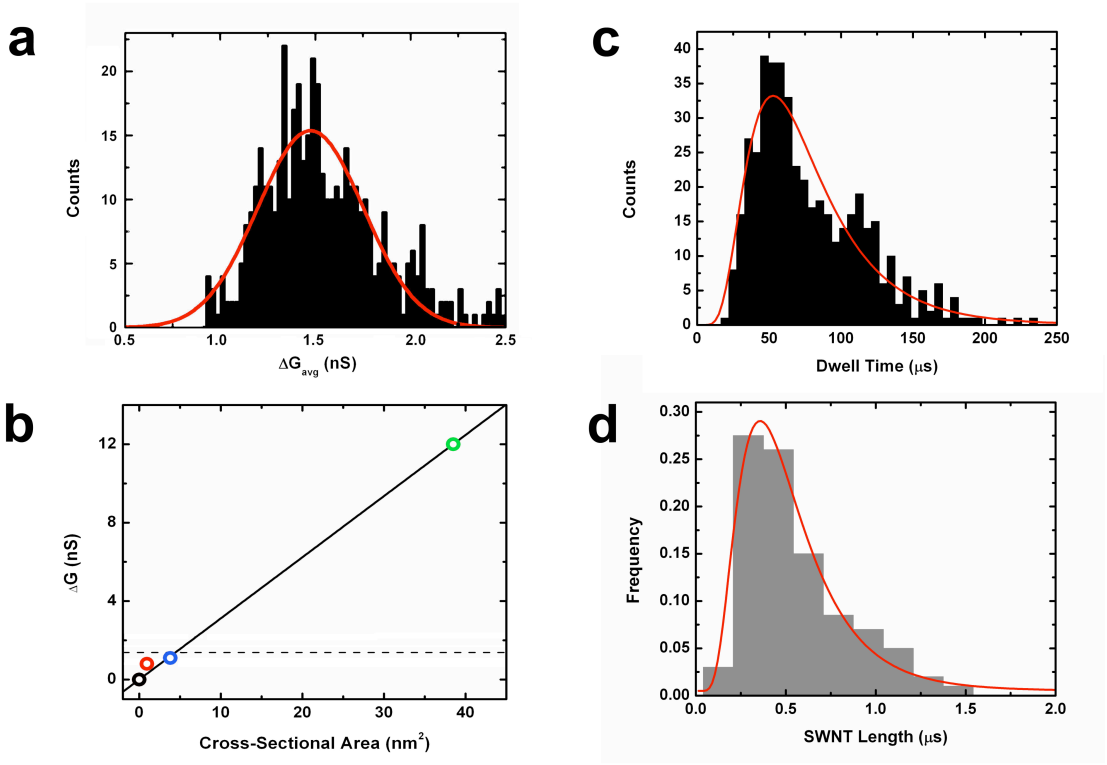

Figure 6 (a) Distribution of average conductance blockade depths measured for 462 $(AC)_{15}$-SWNT translocation events. A Gaussian fit to the data (red) yields an average value of 1.49 nS and a standard deviation of 0.68 nS. (b) Relation of the conductance blockades from past measurements on ssDNA (red), dsDNA (blue) and RecA-dsDNA (green) to their respective cross sectional areas. The black line is a linear fit to the data (slope = 0.31 nS/nm^2) and the dashed line represents the average conductance blockade level measured for $(AC)_{15}$-SWNT in (a). (c) Dwell time distribution for $(AC)_{15}$-SWNT events. The fit denotes a log-normal dependence (Gaussian profile on a log scale). (d) Length distribution of the starting SWNT material as measured by AFM[24]. The fit denotes a log-normal dependence (Gaussian profile on a log scale).

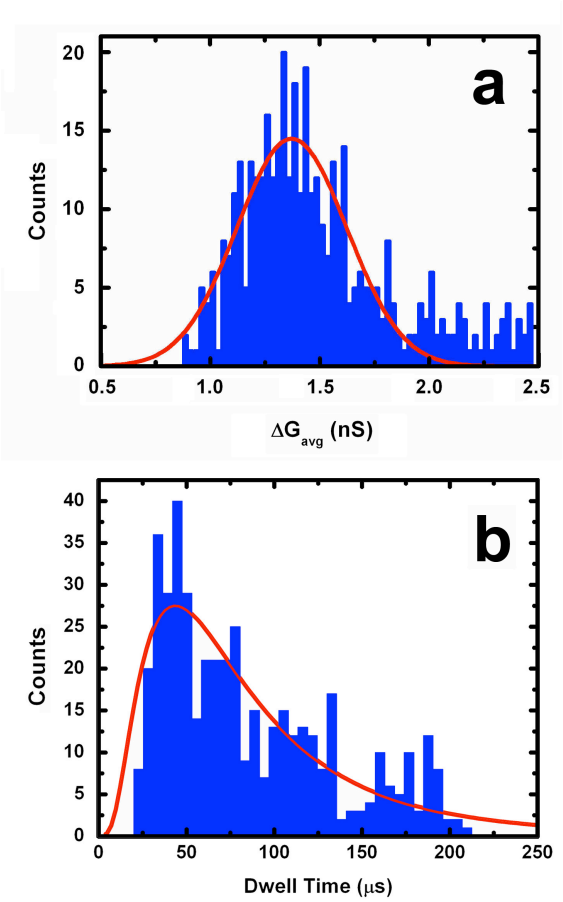

Figure 7 (a) Distribution of average conductance blockade depths for a solution of $(AC)_{15}$-SWNT enriched in (6,5) tubes. A Gaussian fit to the data yields an average value of 1.38 nS and a standard deviation of 0.58 nS. (b) Dwell time distribution for the same material. The fit denotes a log-normal dependence (Gaussian profile on a log scale).