Single-Molecule Electronic Detection Using Nanoscale Field-Effect Devices

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ABSTRACT

Traditionally, biomolecular systems have been studied in ensemble. While much can be determined with ensemble measurements, scientific and technological interest is rapidly moving to single-molecule techniques, which rely primarily on fluorescent markers and advanced microscopy techniques. In this paper, we describe recent work using nanoscale transistors based on carbon nanotubes as charge-sensitive detectors. We show carbon nanotubes can be used for ensemble studies through sidewall adsorption. Sensitivity can be greatly enhanced though an engineered defect in the nanotube. Biomolecular interactions are characterized by random-telegraph-noise response, which can be analyzed to study single-molecule kinetics and thermodynamics.

Categories and Subject Descriptors

J.3. [**Computer Applications**]: Life and Medical Sciences – *Biology and genetics.*

General Terms

Design

Keywords

Single-molecule sensing, carbon nanotube transistors

1. INTRODUCTION

Traditionally, bimolecular systems are studied in ensemble either on surfaces or in bulk solution. Assays are one of the most common applications. Surface-based assays include DNA microarrays to identify target gene expression profiles [1] and common enzyme-linked immunosorbent assays (ELISA) to identify proteins [2]. Optical techniques, usually based on fluorescence detection, are most commonly used for these studies, requiring labeling of the molecules in question, using microscopy to capture emitted photons, and employing CCD and CMOS imagers to convert these photonic signals back to electronic form. Interactions between biomolecular species can be studied with fluorescence resonance energy transfer (FRET) in which interacting species are labeled with a donor-acceptor pair [3].

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Direct electronic interfaces have been pursued for surface-based assays, most based on electrochemical detection of target species. In this case, integrated CMOS electronics have been a useful means to support a large number of capture probe sites on a single immobilization substrate [4-8]. CombiMatrix is one example of a company that attempted to commercialize electrochemical-based DNA assays based on CMOS active substrates [9]. These commercialization efforts have not been successful, however, because of the lack of real advantages in either sample preparation or sensitivity of detection over fluorescence-based techniques. One recent commercial success of CMOS-based biochemical analysis is the Ion Torrent sequencing platform, recently acquired by Life Technologies, which uses CMOS-integrated pH sensors for ensemble sequencing-by-synthesis [10, 11].

While much can be determined with ensemble measurements, scientific and technological interest is rapidly moving to single-molecule techniques, which have become an important tool for studying biomolecular systems *in vivo* and *in vitro*. Single-molecule measurements can probe microscopic behavior of folding assembly, and dynamics which are not possible with ensemble measurements. Single-molecule sensors represent the ultimate limit of sensitivity and eliminate the need for amplification in many assay protocols. Most single-molecule techniques also rely on light as an intermediary between the biological and the solid-state. Noise-limited bandwidths of detection are limited by the relatively long times required to integrate collected photons in CCD or CMOS imaging detectors. Advances in optical detection generally involve development of better fluorescent probes and improved microscopy techniques, including recent developments in super resolution microscopy [12].

Alternative methods to probe single molecule systems include force spectroscopy techniques, including those based on optical tweezers [13] and atomic force microscopy [14]. More recently, nanopores have become another single-molecule measurement technique for analyzing biomolecular polymers, such as DNA [15]. In this case, molecules translocate through a small "hole," typically less than 5 nm in a thin silicon nitride membrane. When the molecule enters the pore, a fraction of the ionic current through the pore is blocked. The length of time the current is blocked contains information about the length of the biopolymer while the extent of blocking contains information about the molecule itself.

Nanoscale transistors in electronics applications are approaching single-molecule dimensions, leading to the possibility for direct field-effect detection using sensitivity to charge on the target molecule. In particular, transistors based on carbon nanotubes are an important emerging technology [16]. Carbon nanotubes can be used for ensemble detection because adsorbed charged molecules

cause a field effect and electrostatically gate the channel. Since carbon nanotubes have all atoms at the surface, it is possible to covalently bind probe molecules directly to the device channel [17], which is not possible with CMOS transistors. These resulting field-effect sensors have two essential attributes for detection, transistor action in the sensing platform through field-effect and a very localize region of sensitivity in the transistor detector.

The point defect in the nanotube device is created by electrochemical oxidation [17]. This defect controls electronic transport and is extremely sensitive to local charge density. For the case of DNA, probe DNA is covalently attached to the defect, and the binding of complementary DNA target molecules results in two-level fluctuations in the tube conductance [18]. These transistors represent an important potential sensing platform for label-free single-molecule science with potential biotechnology applications.

In Section 2, we describe the fabrication of carbon nanotube devices and show how they can be used for ensemble measurements. In Section 3, we introduce a method to controllably create defects in the nanotube and dramatically improve the sensitivity to the single-molecule level. We describe the resulting data from these sensors in Section 4, how the data is analyzed, and similarities with other physical systems. Noise in the nanotube device is discussed in Section 5, and in Section 6, we discuss how these sensors may be "scaled up" with CMOS co-integration.

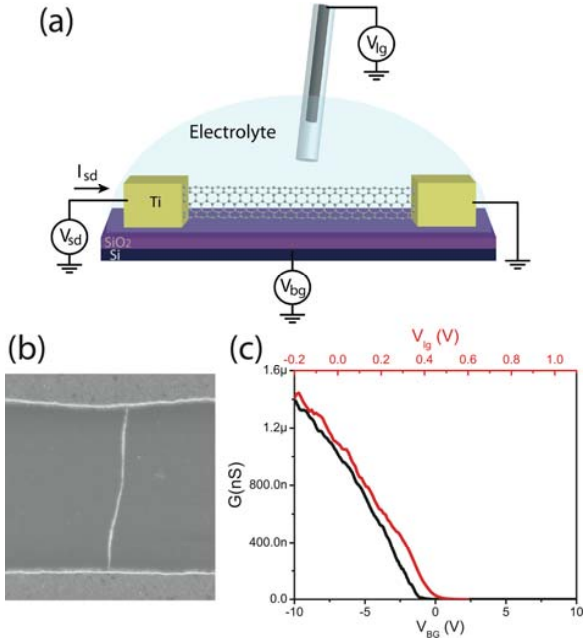

Figure 1. (a) Setup, (b) SEM image of carbon nanotube device, (c) Current as a function of back gate (black) and liquid gate (red)

2. DEVICE FABRICATION

2.1. General Setup

Chemical vapor deposition is used to grow carbon nanotubes approximately 1.4-1.6 nm in diameter on silicon wafers with a thin 300 nm grown silicon oxide layer. Photolithography is used to pattern titanium electrodes on top of the grown nanotubes to make source and drain contacts separated by about 2.5µm. From a single carbon nanotube, 20-30 individual devices are routinely created, which are then wire-bonded and epoxy encapsulated for the following biological experiments. The basic setup is shown in Fig. 1a. When a small bias is applied between the source and drain electrodes, a current can be measured through the individual carbon nanotube, shown in Fig. 1b.

The carrier density in the nanotube is controlled through a global back gate. The carbon nanotube can also be gated through the liquid electrolyte by controlling the electrolyte potential with an Ag/AgCl or platinum pseudo reference electrode [19]. Mobile ions in the electrolyte can cause an electric field at the nanotube liquid interface and induce carriers in the nanotube channel. The amount by which the potential drops is given by the Debye length, which is about 0.7nm for the buffer used here (1X PBS, pH=7.4). Because of the much larger capacitance (C_{lg}/C_{BG}~10), the semiconducting nanotube, shown in Fig. 1c can be turned off at much smaller voltages with the electrolyte gate than with the back gate.

2.2. Ensemble Measurements

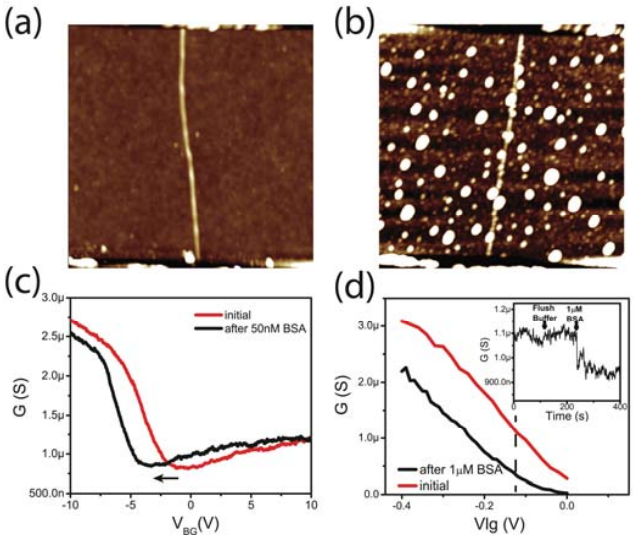

Figure 2. AFM image of pristine device (a), device in 50nM BSA solution for 1h (b), back gate sweep before and after BSA immersion and electrolyte gate sweep (d). Inset shows real time BSA detection.

One-dimensional conductors such as single-walled carbon nanotubes act as high-gain field-effect sensors, in which the conductance varies strongly with local charge density. There has been significant work in using carbon nanotube field-effect devices as ensemble sensors. However, these experiments, conducted either with specific adsorption and coated nanotubes [20] or non-specific adsorption with pristine nanotubes [21] have not demonstrated sufficient sensitivity to detect single biomolecules with the highest reported sensitivity for DNA detection (14 pM) [21].

As an example of ensemble sensing, we have used bovine serum albumin (BSA). The pristine nanotube in the atomic force microscope (AFM) image (Fig. 2a) is immersed in 50 nM BSA in 1X PBS solution for 1h. As shown in the AFM image afterwards (Fig 2b), the proteins tend to stick preferentially to the carbon nanotube. We have measured a device before and after this exposure using the back gate in air and the adsorbed protein cause

a doping of the channel, resulting in a threshold shift of -3V. A shift in the same direction was also observed in a device under aqueous buffer. Fig. 2d demonstrates that the Ag/AgCl electrolyte gate response is shifted by around -120mV when exposed to 1 μM BSA buffer. This can also be seen in real time in the inset of Fig. 2d. Here, we have held the device under a constant bias and observed the conductance. The device shows no response when only buffer is flushed in but a clear drop when the device is immersed in BSA solution.

3. NANOTUBE FOR SINGLE-MOLECULE DETECTION

2.1. Electrochemical Oxidation

The previous experiment has shown that a carbon nanotube can be used for label-free detection of biomolecules but the sensitivity is still far away from single-molecule resolutions. In order to

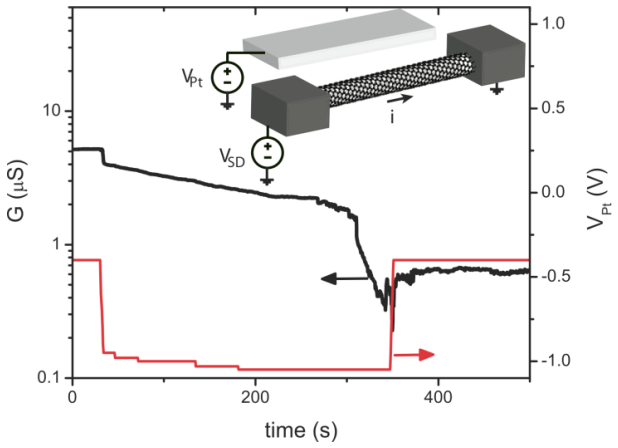

Figure 3. Conductance controlled real-time oxidation of carbon nanotube

increase the sensitivity of the device, we create a point defect in the carbon nanotube [17], limiting the region of charge sensitivity to the region around the defect. This is done by conductance-controlled electrochemical etching of the tube in 1 M sulfuric acid using a platinum electrode, followed by a 45 s exposure to 6.5 mM potassium permanganate to create a defect with a carboxylic acid functional group. A typical conductance-based oxidation of the carbon nanotube is shown in Fig. 3. As the electrolytic gate voltage applied to the platinum electrode is changed below a threshold voltage (around -1V), the conductance shows a very slow decrease, followed by an abrupt jump. A Labview program detects this jump and immediately ramps up the platinum voltage in order to avoid further oxidation. While holding the potential constant, the device is now immersed in 6.5 mM potassium permanganate to create the carboxylic acid functional group.

In order to characterize the location of the defect, scanning gate microscopy (SGM) is used, which uses the biased tip of an atomic force microscope as a very localized gate. Fig. 4a shows an SGM image overlaid with a topography image of a pristine semiconducting nanotube device. The Schottky barriers at the contacts (darker areas) can clearly be seen when the tip is biased with a -5 V bias. With the presence of a point defect, the sensitivity is then localized to a very small region around the center of the nanotube (Fig 4b). We further confirm this localization by a coupling reaction with gold-labeled streptavidin; a gold nanoparticle can be found around the region of highest sensitivity, indicating that the defect is both localized and chemically reactive (Fig 4c).

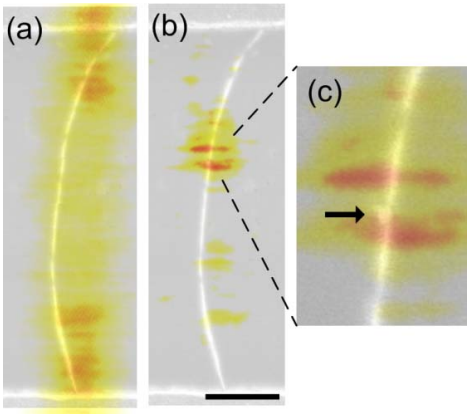

Figure 4. Combined SEM/SGM image before (a), after oxidation (b) and after coupling (c). Scale bar is 500nm.

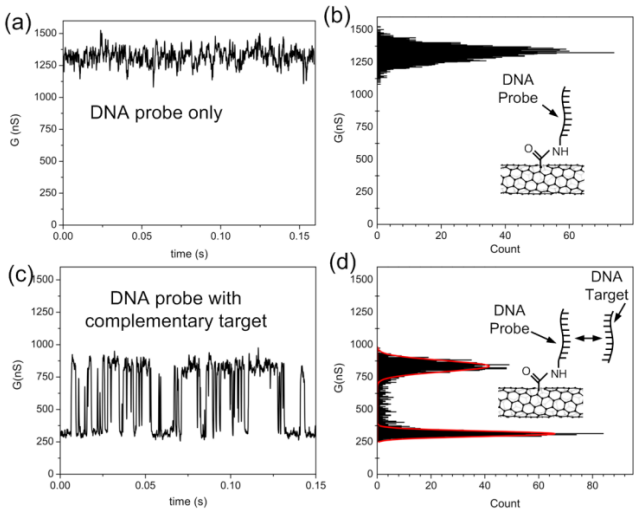

Figure 5. Real time conductance data of probe only (a) and with complementary target DNA (c) and conductance histograms (b,d)

4. MEASUREMENT DATA

4.1. Analyzing random telegraph noise

We have used this single-molecule sensor to study DNA hybridization. A 10-mer DNA probe with an amine group at the 5' end (NH_2-5'-GTGAGTTGTT-3') is attached to the freshly created defect in the nanotube by a standard amine to carboxylic acid coupling reaction assisted by 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and sulpho-N-hydroxysuccinimide (sulpho-NHS). After thoroughly rinsing the device with deionized water, the conductance is monitored in phosphate buffered saline buffer (1X PBS). No particular features in the conductance are observed and the device is dominated by

flicker (1/f) noise as shown in Fig. 5a. The histogram of the conductance is fit to a single Gaussian shown in Fig. 5b.

After exposure to 1 μM complementary DNA concentration at 28°C, the device shows two-level fluctuations as shown in Fig. 5c, which can also be clearly seen in the corresponding histogram in Fig. 5d. We have carefully analyzed the kinetics[22] and have developed a model in which the low-conductance state represents a device with DNA in the duplex form and the high-conductance state with only DNA probe attached.

In addition to the two-level fluctuations, there is a small decrease in the overall conductance level with the addition of target DNA, which we attribute to non-specific adsorption to the sidewall of the nanotube. From this model, we can now extract the DNA melting curve (Fig 6a) by taking the ratio of the areas under the Gaussian fits to the histograms in Fig. 4d. The melting point measured with the nanotube conductance on a single DNA molecule is slightly lower (29.4°C) than the measurements in solution by UV-Vis spectroscopy (36.2°C not shown here). However, this observation is not unusual for surface-based hybridization and has also been observed for DNA linked to gold nanoparticles [23]. The fit uses the Langmuir isotherm $K=\alpha/(1-\alpha)C$ where C is the concentration of target DNA together with the thermodynamic relationship $-RT\ln(K)=\Delta H°-T\Delta S°$. We have also extracted the kinetics of both the high and low states, shown in the Arrhenius plot in Fig. 6b.

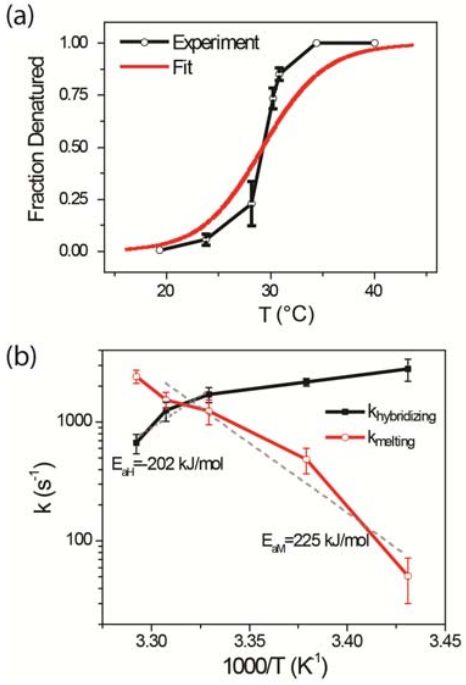

Figure 6. (a) Melting curve; and (b) Arrhenius plot

4.1 Data analysis

The random telegraph noise observed here matches similar physical behavior observed in other contexts, providing the opportunity to leverage data analysis techniques previously employed. In particular, random telegraph signal is a "noisy" background general result from single-molecule FRET studies [24] and have been generally analyzed using hidden Markov model techniques [25]. We employ such data analysis to analyze the time-domain data such as that shown in Fig. 5c.

Random telegraph noise is an emerging problem in nanoscale CMOS devices. In this case, the fact that these devices are scaled to nanometer dimension means that trapping phenomena at the silicon-dielectric interface can be dominated by one (or few) traps [26]. Various data analysis techniques have been developed in this context, including time lag plots (TLP) [27], which are two dimensional histograms of the conductance at consecutive data points. Fig. 7a shows the single state in the TLP with probe DNA only. Fig. 7b shows the two states after adding complementary DNA target.

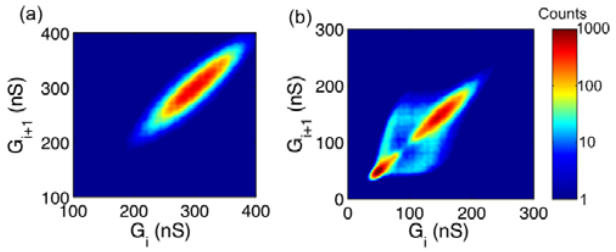

Figure 7. Time lag conductance plot (a) before and (b) after exposure to complementary DNA.

5. NOISE ANALYZIS

5.1 Flicker noise

As was previously shown, having the channel completely exposed to the environment is advantageous because molecules can be directly tethered to it, creating a very sensitive device. However, it also implies that any small fluctuating changes in the environment will affect the electronic transport. In order to improve the sensor,

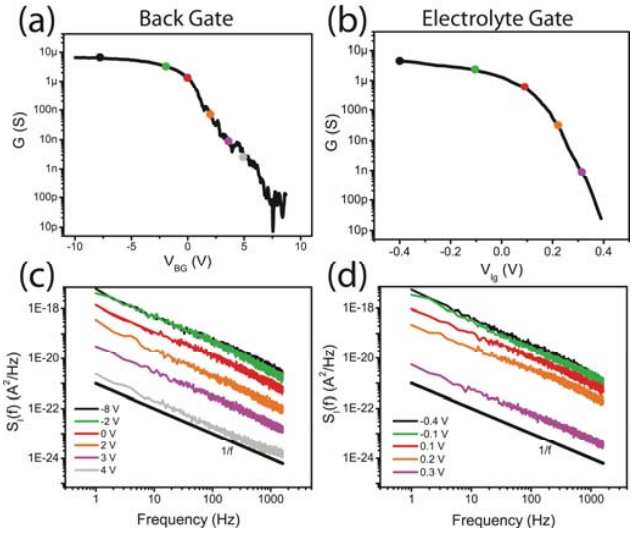

Figure 8. Gate sweep with back gate (a) and electrolyte gate (b) and corresponding flicker noise (c) and (d).

it is crucial to better understand the underlying noise. Fig. 8 shows the noise power spectral density of the carbon nanotube current as a function of bias both for the back gate configuration and the

electrolyte gate. Both experiments show that the noise is mostly flicker or 1/f noise and that the noise amplitude is very similar.

There are two different models for the cause of the flicker noise in carbon nanotubes [28]. The first is the empirical Hooge model, which suggest that noise is caused by independent scattering events of charge carriers and therefore scale inversely with the number of charge carriers in the nanotube. The second is the charge-noise model, which is attributed to random fluctuations of charge in the environment of the channel. We have found that the flicker noise can be better fit to the charge-noise model as $S_I \propto 1/C_g^2 (dI/dV_g)^2$. We have plotted the noise spectrum and fitted to the above equation using the gate sweeps in Fig. 8a and b. The only difference between the back gate and the liquid gate are the gate capacitance. We therefore conclude that the liquid itself does not contribute significant noise, which is important for biosensor applications in aqueous environments. Because the electrolyte would screen out fluctuations that are further away than the Debye length, we conclude that the majority of the noise is due to both traps at the interface between the nanotube device and the dielectric substrate on which it is supported and noise due to diffusion or absorption/desorption of biomolecules near the conductance-dominating defect.

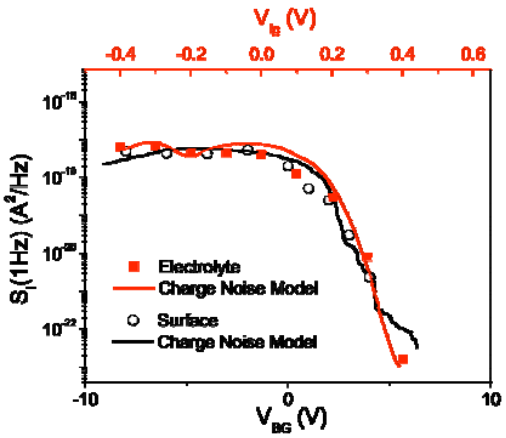

Figure 9. Current noise spectral density at 1Hz showing fit with charge-noise model .

6. SCALE-UP

6.1. CMOS Integration

Scale-up of this platform is on-going and seeks to leverage the integration of nanotube devices with CMOS technology as a post-process [16, 29]. This will allow many such devices to be integrated on the same measurement substrate and allow the reduction in the parasitic capacitance associated with these measurements, dramatically improving measurement bandwidth and reducing amplifier noise. In order to successfully integrate carbon nanotubes with active CMOS substrates, methods have to be developed to controllably transfer the nanotubes since the current growth temperatures of greater than 850 °C are incompatible with CMOS chips. Fig. 10 describes possible integration methods.

Fig. 10a demonstrates a simple technique, which involves spinning on the carbon nanotubes from a suspension. Most of the early research on carbon nanotube relied on spin on nanotubes. The location of the nanotubes is random and unless surfactants are used, the nanotubes tend to bundle up [30]. A more controlled way is to grow carbon nanotubes on solid substrates and then spin on a polymer (such as polymethul methacrylate) that lifts off in strong bases (potassium hydroxide or sodium hydroxide) taking the nanotube with it [31]. This thin film can then be placed on top of the CMOS substrate, transferring all of the nanotubes with it. This is the most controlled way since the density of nanotubes can be adjusted through the growth recipe. Using quartz substrates have shown to result in very dense and highly aligned nanotube arrays, which would be well suited for biosensors [32]. Fig. 10c shows another method using dielectrophoresis. It was demonstrated [33, 34] that by applying and AC voltage between electrodes, nanotubes in suspension will see a force proportional to the gradient of electric fields. The underlying chip could aid in applying the field and stop when a nanotube has bridged the electrodes. In all the above techniques, using an active CMOS chip with a dense electrode array would be advantageous because the chip can be used to locate where a nanotube bridges two contacts and therefore map out the location of nanotube devices.

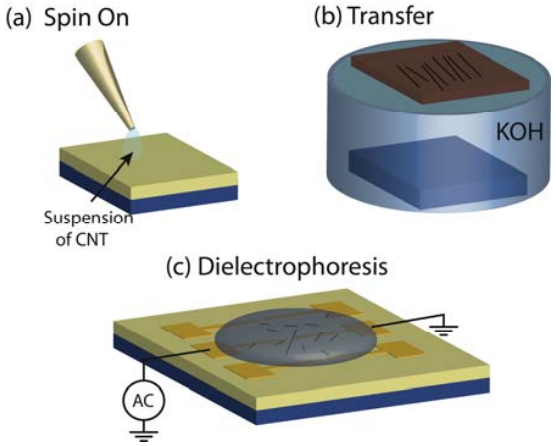

Figure 10. Proposed transfer techniques. (a) Spin on nanotubes from solution, (b) transfer with polymer film from growth substrate and (c) alignment of nanotubes from solution using dielectrophoresis.

7. ACKNOWLEDGMENTS

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