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SUNFEST REU Program

# CARBON NANOTUBE-BASED BIODETECTION OF TRIIODOTHYRANINE

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## ABSTRACT

We report biodetection of the thyroid hormone triiodothyranine (T3) using carbon nanotube field-effect transistors. T3 molecules bind non-covalently the semiconducting nanotube and donate electrons to the nanotube lattice, thereby altering its electronic characteristics via chemical doping. The effect is semi-reversible by rinsing the transistors with the T3 solvent without the biomolecule. The nanotube transistors are found to have no electrical response to the solvent itself. The results demonstrate that T3 can have an electrostatic effect on a one-dimensional system and may suggest an electrical interaction between T3 and DNA.

**TABLE OF CONTENTS**

# 1. INTRODUCTION

Since their discovery in 1996[1], it has been understood that the mechanical, thermal, and electronic properties of carbon nanotubes distinguish them as one of the most interesting materials on earth. Not only are nanotubes the strongest material ever discovered, they also are among the best conductors of heat and electricity. As condensed matter science and engineering has increasingly moved toward the "nano-" world, carbon nanotubes have become one of the most widely researched nanomaterials.

As laboratory methods become more refined, it is becoming easier to isolate and manipulate single-walled carbon nanotubes (SWNTs) as opposed to the bundles and multi-walled nanotubes (MWNTs), the form in which they were originally dis covered. This improvement is particularly favorable for studying and engineering electronic transport of nanotubes since transport measurements of bundles and MWNTs are difficult to check against theory and SWNTs represent the smallest possible scale for electronic devices. The versatility of SWNTs has been exploited in several different applications. SWNTs have been incorporated into prototypes of field effect transistors,[2] atomic force microscopy tips,[3] and chemical sensors.[4] Recently, SWNTs have been applied as key components in nanoscale biodetectors.[5]

Carbon nanotube-based biodetection of various enzymes and proteins has been reported in the literature. We choose to use the hormone triiodothyranine (T3) as a target molecule for our experiments – a brief background illuminates our motivation. T3 is a hormone produced in the thyroid gland that is essential to human development and metabolism. Deficiency during fetal development results in permanent mental impairment to the child. In adults, symptoms of deficiency are severe but can by treated through drug therapy. T3 takes its active form after entering the cells nucleus and is known to interact with DNA, but it is not known through what biological mechanism information is transferred between T3 and DNA. Furthermore, the structure of the T3 molecule raises a question – Why are the iodine atoms necessary? T3 is formed, in part, by two benzene rings decorated with three iodine atoms. The role of the iodine atoms is not understood, especially since there are so many iodine-deficient areas in the world whose population is unable to produce enough thyroid hormone. Why hasn't another substituent replaced the iodine atom evolutionarily?

One suggested answer is that iodine is essential to the *electrostatic* interaction between T3 and DNA. Unfortunately, the electronic properties of DNA are not well understood, and transport measurements are still in their infancy. Carbon nanotubes, though, provide a possible model for DNA as a 1-dimensional conductor, at least to see if the T3 molecule as a whole has some electronic properties.

The motivation for this project, then, is twofold. First, biodetection of T3 by SWNTs will increase the capability of nanoscale biosensors and confirm carbon nanotubes as useful biosensor elements. At the same time we will learn something about the role of thyroid hormone in the body, and whether or not it can act as an electrostatic gate or a dopant for DNA.

## 2. BACKGROUND INFORMATION

### 2.1 Carbon Nanotube Physics

Nanotubes are of a family of molecules including diamond, graphite, and buckminsterfullerene (C60). Structurally, a SWNT is equivalent to a sheet of graphene rolled up to form a cylinder. Experimentally, SWNTs used in our laboratory are 1-3 nm in diameter and as long as several hundred μm. A class of SWNTs can be uniquely defined by how its hexagonal graphene lattice orients itself with respect to the axis of the tubes. Specifically, a pair of indices, (n,m), known as the wrapping vector indicates the chirality of the nanotube in terms of the unit vectors of graphite (see Figure 1).[6]
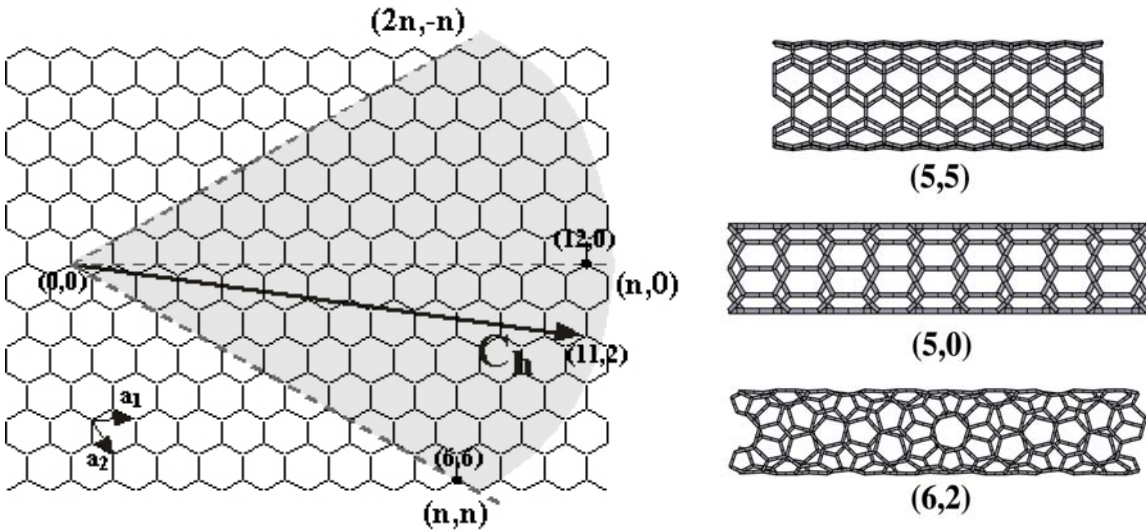


**Figure 1** (Left) The wrapping vector (n,m) specifies the so-called chirality of the nanotube. These coordinates define not only the circumference and geometry of the nanotube, but also its electronic properties. (Right) Three examples of SWNTs.

One reason carbon nanotubes have warranted so much investigation is their electronic versatility. SWNTs can behave as metals or semiconductors, depending on their chirality. The band structure of graphene is interesting in that it touches periodically at six Fermi points making it a zero-gap semiconductor. This six-fold symmetry is a result of the hexagonal carbon lattice. The hexagon in reciprocal space that is formed by the Fermi points is known as the Brillouin zone. When the sheet is wrapped up to form a tube, waves become confined along the circumference of the cylinder, and a boundary condition is imposed. Only certain energy states, corresponding to waves that satisfy this condition, are allowed in that dimension. The effect in k-space is that the Brillouin zone becomes sliced along at discrete $k_y$ values. If the allowed states intersect with the Fermi points, the conduction and valence bands are continuous, and electronically, the nanotube behaves as a metal. Conversely, if the allowed states miss the singularities, the nanotube acts as a semiconductor. Experimentally, the range of resistances of semiconducting SWNTs is ~0.3-100 MΩ, while that for metallic SWNTs is about 10-500kΩ. In practice, resistance depends not only on the chirality of the nanotube, but also on the nature of the electrical contacts to the tube and the environment of the nanotube.

## 2.2 Triiodothyranine

Thyroxine is a hormone produced in the thyroid gland.  Its most obvious structural feature is the iodine atoms that bond specifically to the benzene rings.  Immediately after it is synthesized in the thyroid, four iodine atoms line the molecule and are subsequently removed by a series of deiodinations.  After deiodination, the iodine atoms proceed to the urine unless the thyroid recycles them.   That is, they play no other physiological role.  The most active form of thyroxine is T3 (see Figure 3), which is known to alter gene expression via a receptor molecule in an interaction with DNA.  It is not understood how this interaction is carried out but the presence of the iodine atoms suggests that perhaps the T3 molecule induces charge transfer to the DNA, thereby altering its conductivity.
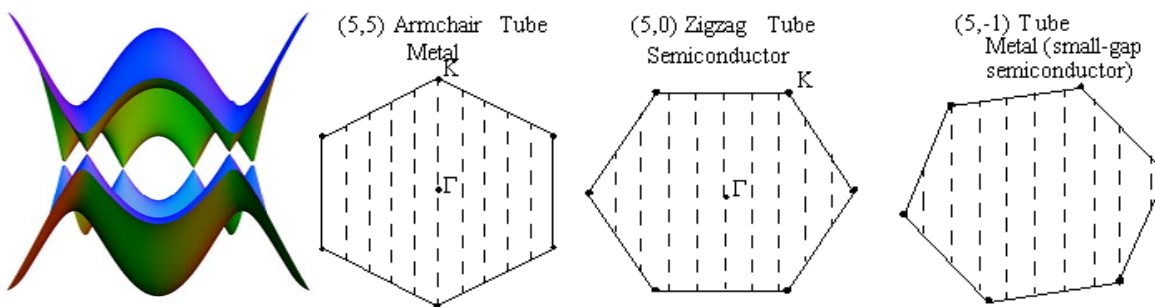


**Figure 2**  (Left) A diagram of the 3D conduction and valence membranes of graphene.  Those points at which the two membranes touch are the Fermi points.  Together, the Fermi points form the vertices of the Brillouin zone. (Right) Only certain states, represented by the dotted lines, are allowed when the boundary condition for the cylindrical nanotube is applied.  If the lines pass through the Fermi, point, the tube is a metal.  Otherwise the tube is a semiconductor.

An *in situ* experiment that can properly model the environment in which DNA is biologically functional has yet to be designed.  A more robust 1D system for which the electronic properties are better understood is the SWNT.  Additionally, SWNTs are a particularly relevant model for DNA the aromatic ring structure of the carbon nanotube resembles the pi-stack geometry of DNA.

## 3.  THE CARBON NANOTUBE BIOSENSOR

The geometry of the carbon nanotube biosensor looks exactly like that of the carbon nanotube field effect transistor (FET).  Nanotubes are grown via chemical vapor deposition (CVD) similar to the procedure described by Hafner et al.[7]  Twenty drops of a 100 mg/L catalyst solution containing particles of iron nitrate, $Fe(NO_3)_3 \cdot 9(H2O)$, in isoproponal are spun onto a p-doped silicon wafer covered with 400 nm of silica. The wafers are heated to 900° C in an Ar atmosphere and growth is then carried out for 15 minutes flowing 3500 sccm $CH_4$ and 450 sccm $H_2$. The wafers are then cooled flowing Ar.

Electrical contacts of the nanotubes are made using a novel electron beam lithography technique and thermal evaporation.  Several hundred pairs of source and drain leads, 10 nm Cr followed by 30 nm Au, with a gap of ~1 μm between them, are patterned at random on the substrate wafer.  We are able to control the nanotube density on the wafer so that we consistently get ~10-12 circuits with one semiconducting SWNT
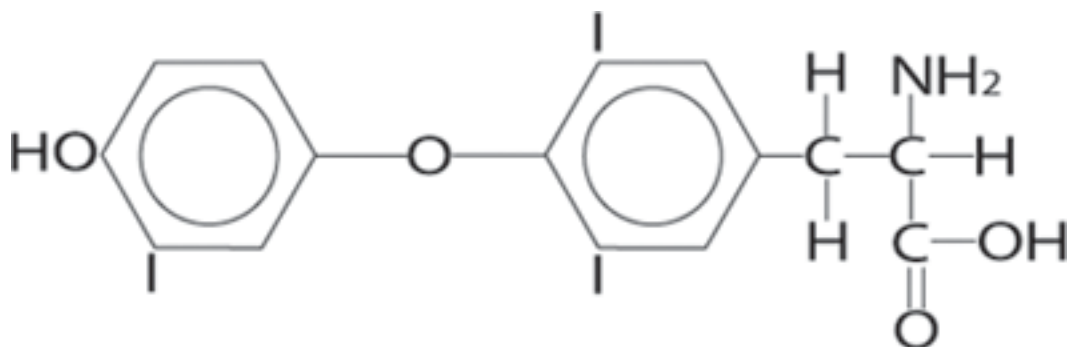
**Figure 3**  The structure of the T3 molecule.

for every ~150 pairs of leads we write, while 30+ other pairs will have metallic SWNTs or multiple SWNTs completing a circuit, and the remaining pairs will be empty leads. The p-doped silicon serves as a backgate separated by the insulating silica.

The semiconducting SWNTs are then externally contacted and characterized.  The response of the conductance of a semiconducting SWNT to changes in the backgate voltage is the most important characteristic of the FET to our biodetection scheme.  A simplified classical explanation for this response is as follows.  For a p-type semiconducting SWNT, one where holes carry the current, applying a negative voltage to the gate attracts more holes to the nanotube, making it easier to carry current, and thereby increasing the conductance.  On the other hand, positive gate voltages will deplete positive charges, leaving none to carry current, and the current will be suppressed.  The gate, then, acts like a rheostat for the current where for a given bias voltage we can turn the current through the SWNT on and off. This is a transistor, called a field effect transistor because the electric field between the backgate and the nanotube is responsible for the changes in conductance of the tube.  An n-type material exhibits the opposite gate response, since the current is carried with electrons. All of the devices we measured were p-type semiconducting SWNTs, with the exception of one that was ambipolar, with n-carriers conducting slightly better than p-carriers at saturation.
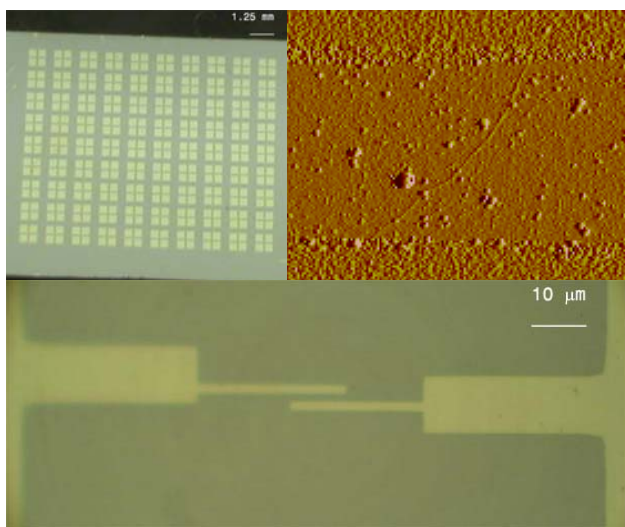


**Figure 4**  (Top Left) 180 FET devices patterned using ebeam lithography on ~1cm$^2$ chip.  (Bottom) Close-up.  (Top Right) AFM image of SWNT connecting leads.  The spacing between the leads is 1 μm.

T3 is applied locally to the FETs in solution through a microcapillary tube (World Precision Instruments, Inc.).  Drops of ~10 nL-0.1 μL of T3 solution can be held over the device for an indefinite amount of time by applying a back pressure in the capillary equal to the vapor pressure of the drop.

Changes in the gate response of a device during and after application of T3 shed the most light on how the T3 molecule interacts with the nanotube. The hysteresis in the gate curves can be explained by charge injection from the nanotube to the substrate (For an example of this hysteresis, see Figure 6).[8] At large negative gate voltages, positive
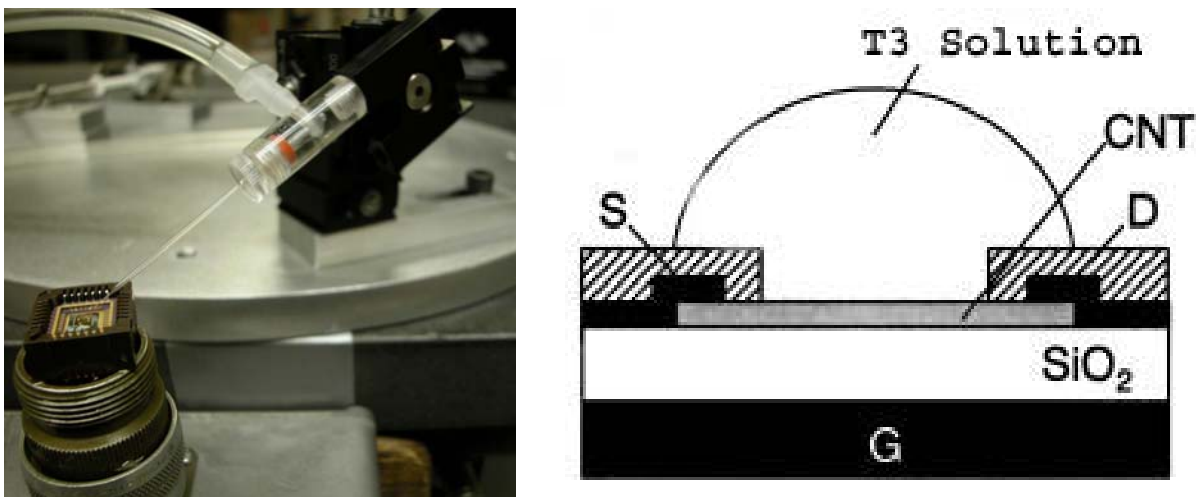


**Figure 5** (Left) Microcapillary apparatus designed to apply drops as small as 10 nL to SWNT biosensor devices. The capillary delivers solution to the FET, which is connected to the electronics through the socket. (Right) Schematic of nanotube biosensor.

charges become trapped in the substrate, and so when the voltage is ramped back up, the effective gate voltage that the nanotube sees is more positive than the applied $V_{Gate}$. For large positive values of $V_{Gate}$, the opposite effect occurs. A simpler form of data is measures the current as a function of time while applying, removing, and rinsing the drop.

## 4. SOLVENT CONTROL EXPERIMENTS

Three solvents are known to dissolve T3 well: NaOH in water, NaOH and Ethanol in water, and Dimethyl Formamide (DMF). The first is favorable in terms of T3 solubility, but for biosensing, doping should occur regardless of the choice of solvent. Also, it is important to rule out the possibility of electronic response due to the solvent by itself, or at least to understand the response in order to subtract it from the signal due to the solubilized T3. To that end, several control experiments were conducted.

Drops of solution were applied over the biosensors while the current through the nanotube was recorded. 10 V Gate sweeps were taken before the experiment, while the drop was applied, and after it had evaporated. Typical bias voltages for conductance and gate measurements ranged from about 0.1 to 0.5 V.

Deionized water ($\rho \sim 18$ M$\Omega$-cm), by itself, increases the current through the nanotube by <.001%, and the effect is reversible. That is, the conductance due to the water is negligible. Up to a molarity of 1 mM, NaOH in water performs like DI water – it does not alter significantly either the conductance of the nanotube or its gate characteristics (See Figure 6). At higher concentrations a change in conductance is observed, but it is predictable and repeatable. Presumably, there is a threshold concentration of NaOH for which the ions begin to contribute to the conductance. NaOH

and Ethanol in water does not perform quite as well; even at .1 mM we observe an electronic response conductance (~30%), but it is predictable as well. Unfortunately, at higher concentrations ≥ 1mM, NaOH/Ethanol irreversibly quenches the gate characteristics for some nanotubes. DMF, a strong organic solvent, performs the worst of the three. In addition to altering the conductance of the nanotube and suppressing its gate characteristics, it also damages our equipment. The apparatus is currently being modified to accommodate DMF. All three solvents are useful to solubilize T3 for biosensing, with NaOH in water performing the best.
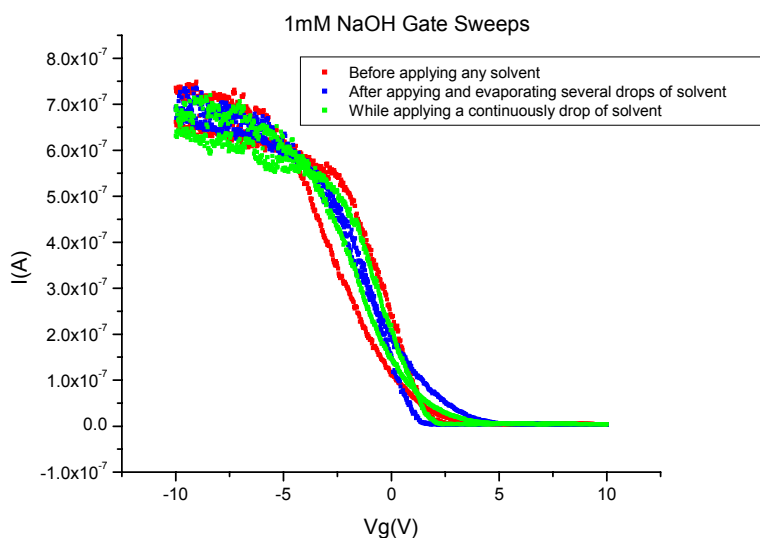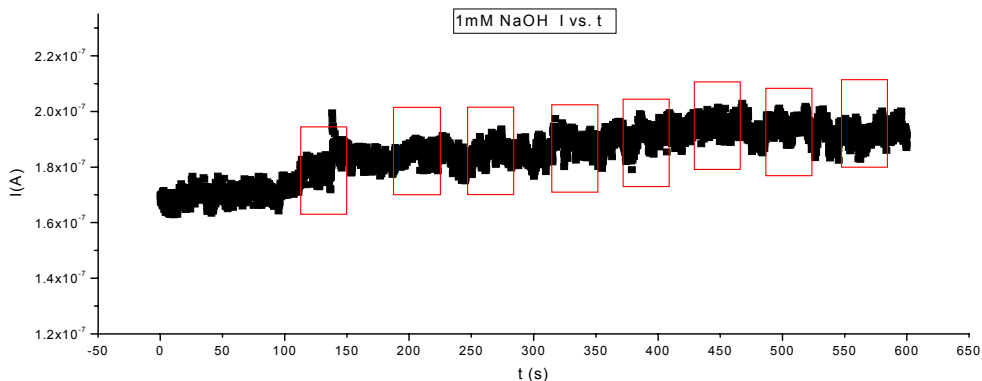




**Figure 6** (Top) Plot of the current through the device as drops of 1mM NaOH were applied to the biosensor. Red boxes are times where drops were applied. (Bottom) Gate sweeps before during and after application of drops. The shifts and variations between the curves are comparable to those observed when adding no drops. $V_{Bias}$=1V for both graphs.

## 5. RESULTS WITH T3

Our procedure for applying T3 is similar to that for applying the control solution. First, several 2V gate sweeps are taken to characterize the device pre-experiment. Then the current is recorded as a drop of solution is applied to the device. Several more 2V

gate sweeps are taken for comparison to the pre-experiment sweeps. If no gate response is observed, the gate voltage is ramped progressively to higher and higher absolute values, as far as ±30V. Then the current is recorded as the drop is removed. Again the range of $V_{Gate}$ is swept in search of a gate response. If none is found, a rinsing is performed by adding and removing several drops of 1mM NaOH without T3 to try to reverse the effect of T3 by diffusing it off of the nanotube.

For all the experiments using T3 so far, we have used the 1mM NaOH solvent. We can vary the concentration of T3 up to a limit of 1mM for this concentration of solvent. For the lowest concentration of T3, 10 μM, no change in conductance is observed when drops are applied other than that associated with the conductance of the water. Additionally, after the drops are applied the gate hysteresis curves do not change more than what is expected due to charge fluctuation in the substrate. For a 100 μM T3 solution, again there is no significant difference in conductance between times when the drop was on and those when it is off, but the gate curves before, during, and after drop are not as stable as they would be without any solution over the time period of the experiment. This may be because T3 molecule promotes additional charge fluctuation around the substrate. Still at this concentration we see no clear detection of T3 attributable to an electrostatic interaction of T3 with the nanotube.

For a concentration of 1 mM T3, though, when the drop is put on, after ~30 seconds, the current is suppressed to that of the transistor's off-state, where it is stable for as long as the drop is applied (See Figure 7). These measurements were taken for four p-type transistors. For three, subsequent gate sweeps out to –30 V show no recovery of on-state current with the drop still applied and after it evaporates. After rinsing, though, gate response is recovered for two of the samples, but the onset of the current turn-on is shifted to ~ -5V. For the fourth device, no rinsing was needed to find the shifted on-state onset, which occurred at ~ -3 V. This suggests that for the three samples for which the on-state disappeared, the on-state was actually shifted to huge negative values past -30 V and rinsing shifted the onset back toward 0 V. For another ambipolar sample, the drop also suppressed the current *to the lowest value of the gate sweep*, not to on-state for positive voltages. Measurements at gate voltages past 2 V have not yet been taken. This sample contradicts the shift theory and suggests that the T3 actually suppresses the gate response completely and when it is rinsed off, the response reappears.

## 6. DISCUSSION AND CONCLUSIONS

Further measurements are warranted. Specifically, it is necessary to ramp the gate voltage lower than –30 V after the T3 is applied to search for the onsets to the FET on-states. If the onsets can always be found, it would be interesting to see whether the amount of shift depends on the concentration of T3, or if there is simply a threshold molarity for which the biodetection is activated. Because iodine, atomic number 53, has an open space for an electron in its valence band, we originally predicted a shift in the gate hysteresis toward the positive. That is, when the iodine binds to the nanotube, it
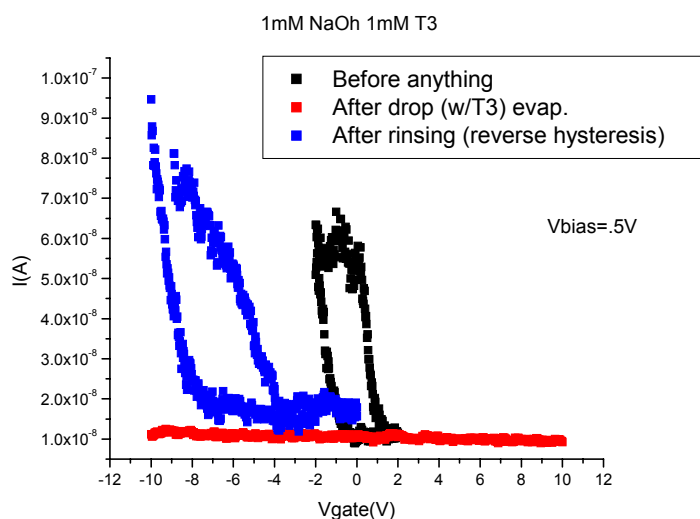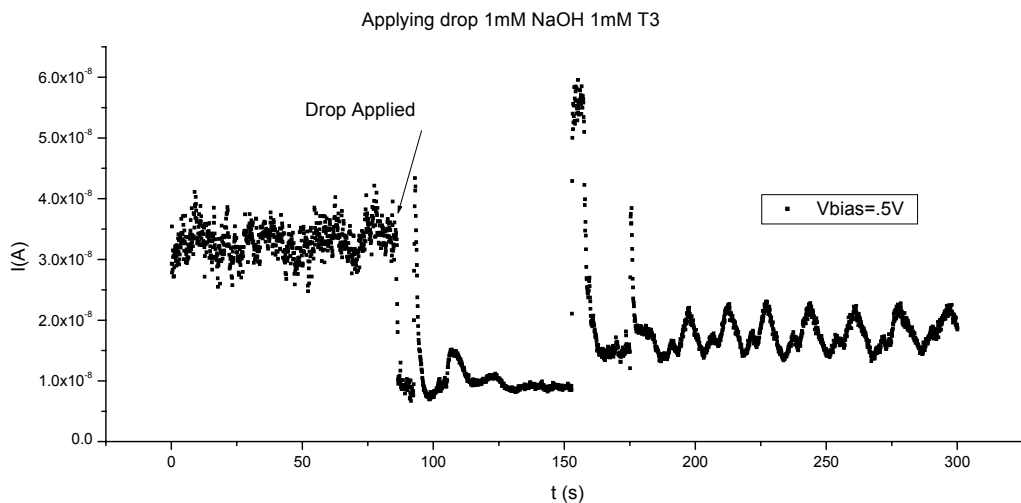
**Applying drop 1mM NaOH 1mM T3**

Drop Applied

I(A)

6.0x10⁻⁸ ... Vbias=.5V

t (s)

**1mM NaOh 1mM T3**

- Before anything
- After drop (w/T3) evap.
- After rinsing (reverse hysteresis)

Vbias=.5V

I(A)

Vgate(V)

**Figure 7** (Top) Current through the FET biosensor as the 1 mM T3 solution is applied. (Bottom) The gate characteristics were suppressed by T3 and then recovered offset by 6 V toward negative $V_{Gate}$ values by rinsing the T3 off. The hysteresis of the blue curve is not the same hysteresis that can be explained by charge injection: the left curve is taken while the voltage is being ramped down to −10 V and the right curve taken on the way back up.

would accept an electron into its valence shell from the nanotube, making it easier to turn the transistor on. After observing a shift in the opposite direction, we have to modify this theory. Since the T3 molecule is electrically neutral, we have no reason to believe *a priori* that electron donation from the nanotube would be the mechanism for doping as opposed to electron donation from the T3, except for the presence of the iodine atom. It is still possible that the iodine atom is essential to the chemistry of the molecule in order for it to donate n-carriers. Detection experiments could be run on a deiodinated species of thyroxine to test this. We could also try to calibrate the shift with respect to T2 and T4 as well as T3 to see how important the iodine atoms are. On the other hand, perhaps the iodine atoms contribute critically to the electric dipole of T3, and binding to the SWNT with the dipole pointing radially outward away from the tube lowers the valence and

conductance band with respect to the Fermi energy of the metal, thereby increasing the height of the tunneling barrier for holes to get on and off the nanotube.

Experiments using the other two solvents will also contribute to the overall picture.  Crude detection experiments with T3 on bulk material were done several years ago, and it appeared that the effect of T3 changed given a different solvent.[9]  If we can take data that are consistent with this, they would support the charge transfer mechanism for biodetection and would demonstrate the electrical versatility of T3: It can donate holes or electrons depending on its chemical environment.

In conclusion, this experiment shows that it is possible to detect T3 using carbon nanotube sensors.  More interestingly, it has been demonstrated that T3 can be electrically active and can interact electrostatically with a 1-dimensional system.  This may have physiological implications.  Until we can construct a biologically relevant model in which to measure the electrical properties of DNA, carbon nanotubes are the closest we can come.

## 7. ACKNOWLEDGEMENTS

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