# DNA/RNA purified single walled carbon nanotubes on self-assembled networks

J.C.G. Jeynes, E. Mendoza *, D. Chow, J. McFadden and S.R.P Silva
Nano-Electronics Centre, Advanced Technology Institute,
University of Surrey, Guildford, Surrey, GU2 7XH. United Kingdom
* Email: e.mendoza@surrey.ac.uk

## ABSTRACT

Single walled carbon nanotubes (SWCNT) have attracted a great interest due their extraordinary properties that envisage their use for a wide range of applications [reference physical properties]. However, these properties are controlled by the chirality of the SWCNTs. Unfortunately, the growth processes available to-date produce SWCNTs with different chiralities. Also, the SWCNTs are produced together with a relatively high quantities of impurities such as amorphous carbon and metallic catalyst particles. Indeed, the purification and manipulation remains problematic, hindering some of the possible applications of these materials. In this paper, the purification of SWCNTs with biological polymers is presented. The results shown that DNA and RNA effectively purify SWCNT from the "soot" obtained during the growth process. The results show how effectively total genomic RNA (tgRNA) purifies SWCNT. Atomic force microscopy (AFM) studies reveal how nucleic acids wrap around SWCNTs forming RNA-CNT composites. Moreover, when a RNA-CNT solution is dried on a hydrophilic surface, SWCNTs are found lying or embedded on a self assembled two dimensional RNA network. Using tgRNA is not only a cheap and effective method of solubilising and purifying CNTs but offers a first step towards the self-assembly of CNTs from solution. Furthermore, tgRNA networks could be a convenient method of electrically linking individual RNA functionalised CNTs over a surface which could prove useful for RNA or DNA biosensors.

***Keywords***: carbon nanotubes, DNA, RNA, biosensor

## 1 Introduction

Single walled carbon nanotubes (SWCNTs) have been under intense study due to their excellent thermal, electronic and mechanical properties which make them ideal candidates for applications in many disciplines ranging from electronics to biosensing. However, SWCNTs have major shortcomings, namely they are not soluble in aqueous solutions, have no functional groups and are produced with many imperfections such as catalyst particles and amorphous carbon. Much work has been dedicated to solubilising and purifying SWCNTs in many different types of organic solvents, surfactants and biomolecules. Recently, short strands (oligonucleotides) of deoxyribose nucleic acid (DNA) have been used to purify SWCNTs [1] and even to separate them out according to their chirality [2]. Based on simulations, it was proposed that the bases on the nucleotides wrap around to the outside of the tube wall interacting via $\pi$-stacking providing a unique way to separate out CNT from impurities.

It has also been shown that uridine, a specific nucleotide of ribose nucleic acid (RNA)(so called poly(rU)), wraps around SWCNTs in an indentical manner to short oligos of DNA [3]. Single molecules of the poly(rU)-CNT composites have been visualize using RNA fluorescent dyes [4] and have been transported inside living cells for use in possible drug delivery applications [5].

In this report, it is shown how both DNA and RNA form self-assembled networks when dried on mica and how they can purify and solubilise SWCNT. The study focuses on the novel use of total genomic RNA (tgRNA) to simultaneously form self-assembled networks and purify SWCNT. There are some advantages that tgRNA has over poly(rU) and DNA oligos, namely it is cheaper and readily forms networks. The networks are important because they have the potential to create an ordered array of CNT while also linking all the individual CNT over a surface.

Similar networks have been described before but with DNA [6]. It was shown that the height and mesh diameter of the network could be controlled by post-treatment with ethanol and the type of DNA that was used. Moreover the networks exhibited ohmic behavior giving about $1M\Omega$cm resistivity [7]. If the RNA networks also show similar ohmic behavior, this could be a strategy to electrically network the CNT together making a CNT-RNA functionalized active surface which could be used for nucleic acid biosensors. RNA networks have also been described where specific motifs can make "jigsaw puzzles" with almost infinite possibilities of network sizes and shapes [8]. Packaging RNA (pRNA) from a virus has also been used to make nanostructures [9]. However, to our knowledge this is the first instance where tgRNA has been shown to form networks.

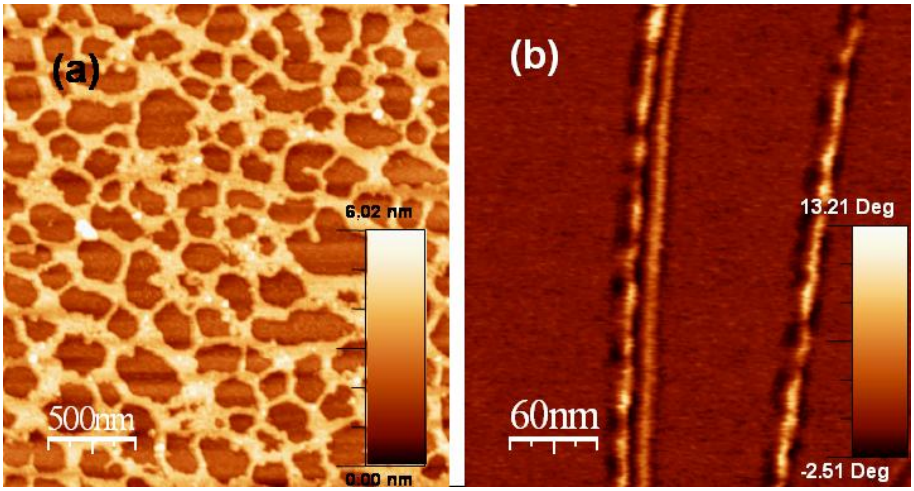


Figure 1: (a) Topographic AFM tapping mode image of a self-assembled DNA network on mica (b) Phase contrast AFM Tapping mode image of an oligonucleotide (d)T30 wrapped carbon nanotubes

## 2 Experimental

The tgRNA-CNT composites were fabricated according to a modified version from Zheng *et al* [1]. To disperse the tubes, HiPCO produced SWCNT tubes (Sigma) were sonicated in eppendorf tubes in an ice-waterbath (3W) with total genomic RNA(Sigma)in deionised water. The solution was then centrifuged to ensure that all the insoluble particles were spun out of the solution. The supernatent was then gently pipetted off so that the pellet was not disturbed and then run through a YM-100 column (Milipore) to concentrate up the solution of RNA-CNT composites. The DNA-CNT composites were made in the same way apart from an oligonucleotide of thirty thymines (d)T30 was used instead of tgRNA. The oligo was purchased from Sigma-genosys.

The DNA networks were created on mica using both $\lambda$-DNA and plasmid DNA pUC19 (New England Biolabs). The samples were left to dry in air on mica after it had been treated with magnesium acetate and then was washed with deionised water.

AFM images were collected using a Dimension 3100 SPM (Veeco) and analysed using the freeware software WSxM from Nanotec Electronica. The composite solution was pipetted onto a small piece of freshly cleaved mica and left to air dry in an airflow cabinet to prepare nucleic acid-CNT composite samples.

## 3 Results and Discussion

In previous reports it was shown that poly(dG)-poly(dC), poly(dA)-poly(dT), poly(dA-dT)-poly(dA-dT) and poly(dG-dC)-poly(dG-dC) DNA forms self-assembled two-dimensional networks over the surface of mica [6], [7]. Here, similar networks are shown but using virus and plasmid DNA molecules which in solution are a Watson-Crick double stranded mixture of the four nucleotides adenine, guanine, cytosine and thymine (Figure 1(a)). The height of the network is between 2.0-3.5 nm which is higher than the 0.5-1.2nm height associated with ds-DNA by AFM [10], [11]. Therefore, the DNA is probably forming triple-stranded associations when it dries onto the mica.

It has also been shown that short oligonucleotides can solubilise and purify SWCNT by wrapping it and interacting by $\pi$-stacking [1], [2]. Here a SWCNT is shown with (d)T30 oligonucleotide wrapping around it. The wrapping can be clearly seen by the "bobbled" appearance of the tube. This is emphasised by the smooth tube next to it which is not wrapped with DNA. This result is in good agreement with those previously reported [1], [2].

A drop of RNA-CNT composite solution that has not been concentrated up by spinning through a YM-100 column is shown (Figure 2(A)). Here, the RNA network can be clearly seen with just a few RNA-CNT composites embedded in it and lying on top of it. Analysis of these images reveal that the RNA network is fairly uniform with a height of about 2.5nm. This is higher than a single molecule of double stranded (ds) DNA (0.5-1.2nm)[10], [11] which indicates that a few RNA molecules are bunching to form the network. However, it is suprising how uniform the network is when one considers that tgRNA consists of many different types of RNA, some being single stranded messenger RNA (mRNA) while others having 3-D tertiary structures such as ribosomal RNA (rRNA) and transfer RNA (tRNA). Clearly on drying, many of these molecules lose their tertiary structures and are amalgamated into the network.

This same process occurs with concentrated RNA-CNT composites (Figure 2(B)) with the difference that many more composites become embedded into the net-

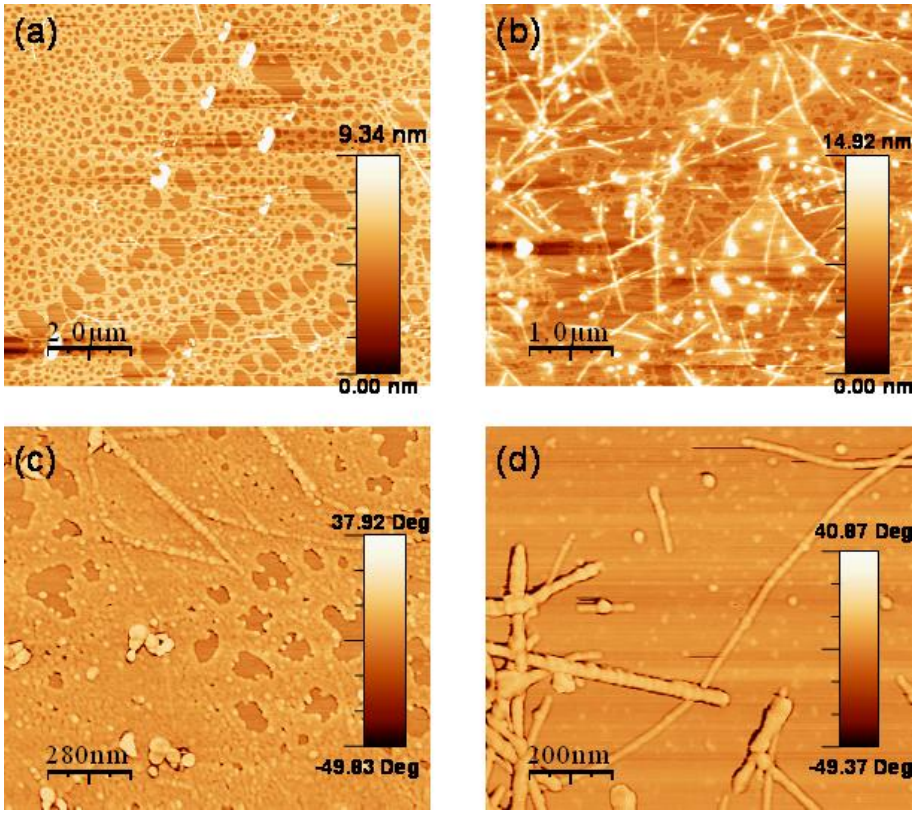


Figure 2: (a) Topographical tapping mode AFM images of non-concentrated RNA-CNT composites clearly showing the RNA network (b) Concentrated RNA-CNT composites embedded in an RNA network (c) Magnified phase contrast image of the RNA-CNT composite embedded in the RNA network (d) Phase contrast image of RNA-CNT composites where the RNA network has been washed away.

work causing a slight distortion in parts of it. A magnified phase constrast image (Figure 2(C)) relates to the elasticity of the material and emphasizes the difference between the CNT and RNA. Some tubes are seen embedded underneath the network and can just be made out as straight lines, some are partially covered while others are sticking out completely. This image also shows that the wrapping of the RNA around the tube is similar to images of DNA wrapped SWCNT [1], [2]. The mechanism of wrapping is likely to be the same as with DNA nucleotides where the bases interact with the tube wall via $\pi$-stacking. However, it is not clear what type of RNA wraps the tubes. It is more likely to be the single stranded mRNA with weak tertiary structures which have free bases available to interact with tubes.

The RNA network can be washed away with deionised water while the RNA-CNT composites remain intact and adsorbed to the mica (Figure 2(d)). This supports the hypothesis that $\pi$-stacking is attaching the RNA to the CNT rather than just a physical adsorption to a surface which clearly forms the RNA network. Washing away the network also allows more detailed images of the RNA-CNT composites. These have heights ranging from about 2nm to 8nm. There seems to be two categories of tubes which are shown in (Figure 2(d)). One type is about 2-4nm high whereas the other is about 6-8nm high. As the pristine tubes have a mean diameter between 0.7-1.1nm [12], [13] these two categories indicate that there are a few RNA molecules wrapping individual tubes while the tubes with larger diameters are probably a few tubes being wrapped as bundles.

The Raman spectra comparing pristine SWCNT to RNA-CNT composites shows that the RNA purifies the SWCNT from defects and impurities such as amorphous carbon and catalytic particles (Figure 3). It has been shown that there is a strong correlation between the width of the D band and the purity of the sample [14], [15]. The spectra shows that the full-width-at-half-maximum (FWHM) of the D band for the RNA-CNT composites is about 260 $cm^{-1}$ whereas it is about 570 $cm^{-1}$ for the pristine tubes(Figure 3(b)). Moreover, the ratio between the D and G bands also indicate the purity of the tubes with purer samples having a lower ratio [14], [15]. The D:G ratio between the RNA-CNT composite tubes is about 0.14 whereas it is about 0.5 for the pristine

tubes.

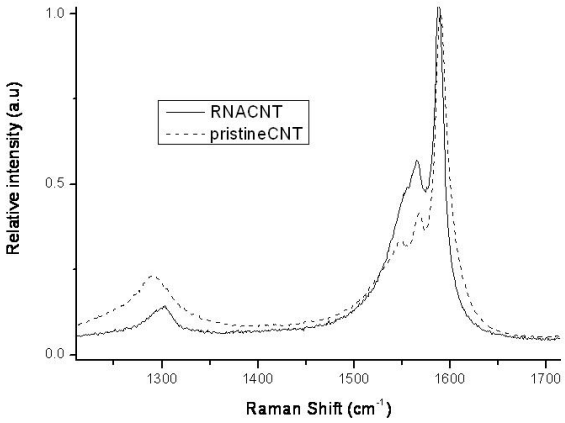


Figure 3: Raman spectra collected at 782nm on a silicon substrate comparing RNA-CNT composites with pristine tubes. The plots were normalised with respect to the amplitude of the $G$ band. It shows that the RNA-CNT has a smaller $D$ band in relation to the pristine tubes indicating that the RNA-CNT sample is more pure.

## 4 Conclusions

In conclusion, it has been shown that long molecules of DNA form self-assembled two dimensional networks when dried onto mica and that short DNA oligonucleotides effectively purify and solubilise CNT. We have also demonstrated the novel use of total genomic RNA (tgRNA) to effectively disperse, solubilise and purify single walled carbon nanotubes in water. Using AFM and Raman it is shown that RNA purifies SWCNT "soot". AFM studies reveal that RNA wraps around the tubes leaving individual tubes separated while also sometimes wrapping a few tubes together. It is probable that the RNA binds to the walls of the tubes by $\pi$-stacking and that within the tgRNA mainly the single stranded messenger RNA wraps the tubes. Moreover, tgRNA forms self-assembled two dimensional structures when it is dried over the surface of mica a few molecules high in which RNA-CNT composites become embedded. However, the binding of the RNA to the tubes is much stronger than to the mica, as the network can be washed away just leaving the RNA-CNT composites. An RNA network could be a convenient way of electrically linking individual RNA functionalized CNTs over a surface which could prove useful for RNA or DNA biosensors. Overall, this study has profound implications for the self-assembly, controlled positioning and functionalisation of SWCNT by using nucleic acids. A nucleic acid functionalised electrically active surface will provide an ideal platform for DNA/RNA biosensors.

## REFERENCES


[1] M. Zheng, A. Jagota, E.D. Semke, B.A. Diner, R.S. Mclean, S.R. Lustig, R.E. Richardson, and N.G. Tassi. *Nat. Mater.*, 2(5):338–342, 2003.

[2] M. Zheng, A. Jagota, M.S. Strano, A.P. Santos, P. Barone, S.G. Chou, B.A. Diner, M.S. Dresselhaus, R.S. McLean, G.B. Onoa, G.G Samsonidze, E.D. Semke, M. Usrey, and D.J. Walls. *Science*, 302(5650):1545–1548, 2003.

[3] R. Rao, J. Lee, Q. Lu, G Keskar, K.O. Freedman, W.C. Floyd, A.M. Rao, and P.C. Ke. *Appl. Phys. Lett.*, 85(18):4228–4230, 2004.

[4] Q. Lu, K.O. Freedman, R. Rao, G Huang, J. Lee, L.L. Larcom, A.M Rao, and P.C Ke. *J. Appl. Phys.*, 96(11):6772–6775, 2004.

[5] Q Lu, J. M. Moore, G.Huang, A.S. Mount, A.M.Rao, L.L. Larcom, and P.C. Ke. *Nano Lett.*, 4(12):2473–2477, 2004.

[6] L.T. Cai, H. Tabata, and T. Kawai. *Appl. Phys. Lett.*, 77(19):3105–3106, 2000.

[7] T. Kanno, H. Tanaka, N. Miyoshi, and T. Kawai. *Appl. Phys. Letts.*, 77(23):3848–3851, 2000.

[8] A. Choworos, I. Severcan, A.Y. Koyfman, P. Weinkam, E. Oroudjev, H.G. Hansma, and L. Jaejer. *Science*, 306:2068–2072, 2004.

[9] D. Shu, W.D. Moll, Z.X. Deng, C.D. Mao, and P.X. Guo. parts in nanotechnology. *Nano Lett.*, 4(9):1717–1723, 2004.

[10] Y.L. Lyubchenko and L.S. Shlyakhtenko. *PNAS*, 94(2):496–501, 1997.

[11] T. Thundat, D. P. Allison, and R.J. Warmack. *Nucl. Ac. Res.*, 22(20):4224–4228, 1994.

[12] W. Zhou, Y.H. Ooi, R. Russo, P. Papanek, D.E. Luzzi, J.E. Fischer, M.J. Bronikowski, P.A. Willis, and R.E. Smalley. stability of single wall carbon nanotubes synthesized by the catalytic decomposition of CO. *Chem. Phys. Lett.*, 350((1-2)):6–14, 2001.

[13] M.J. Bronikowski, P.A. Willis, D.T. Colbert, K.A. Smith, and R.E Smalley. *Vacuum Sci. Technol. A*, 19(4):1800–1805, 2001.

[14] A.C. Dillon, T. Gennett, K.M. Jones, J.L. Alleman, P.A. Parilla, and M.J. Heben. *Adv. Mater.*, 11(16):1354–1358, 1999.

[15] A.C. Dillon, P.A. Parilla, J.L. Alleman, T. Gennett, K.M. Jones, and M.J. Heben. *Chem. Phys. lett.*, 401(4-6)):522–528, 2005.