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Article

Delivery of Gold Nanoparticles Inside Carbon Nanotubes by Oligonucleotides

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Abstract

Delivery of gold nanoparticles with 2 nm or so in diameter inside multiwall carbon nanotubes (MCNTs) by oligonucleotides was performed under the condition of 400 k and 3 bar for 20 min. The Au-oligo-CNT complexes were first purified via 1% agarose gel electrophoresis and then analyzed via high resolution transmission electron microscopy (HR-TEM) and energy dispersive X–ray spectroscopy (EDX). The results showed that the excess of oligonucleotides, Au nanopartilces and the Au-oligo hybrids attached to the outside walls of CNTs could be removed away by agarose gel electrophoresis. HR-TEM and EDX results demonstrated that 2% or so Au-oligo hybrids were successfully delivered inside MCNTs. In contrast, few Au nanopartilces were observed to locate inside CNTs under identical experimental conditions. This is the very first confirmation that oligonucleotides can be used to deliver Au nanoparticles inside MCNTs. The van der Waals attraction between CNT and Au-oligo hybrids is likely the main driving force for this phenomenon. This phenomenon has potential applications in future nanotechnology such as molecular electronics, biochemical sensors, nano-devices, gene storage and delivery systems.

Keywords: gold nanoparticles, carbon nanotube, oligonucleotide, encapsulation
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1. Introduction

Carbon nanotubes (CNTs) were discoveried in 1991 [1]. Since its discovery, CNTs' many unique properties such as mechanical, chemical and physical properties have been found gradually [2-4], CNTs have become highly attractive materials for applications in various fields including molecular electronics [5-7], field emission devices [8] and bioengineering [9,10]. So far many novel properties of CNTs have been being explored by attaching molecules outside [11,12] or encapsulating molecules inside CNTs [13-15]. Functionalization of CNTs with biomolecules such as DNA, RNA and protein has potential broad application. For example, DNA has been widely utilized for nanowire fabrication [16], nanowire template formation [17], and the synthesis of chain-like arrangements of metal and semiconductor clusters [18]. DNA and peptide molecules can increase CNTs' solubility [19,20] and can also be used to sort CNTs [21,22].

In our previous studies, we found that DNA oligonucleotides can be inserted spontaneously into CNT in water by molecular dynamics simulation [23], our experiments also have confirmed Pt–labelled DNA molecules can be encapsulated inside CNTs [24].

In the present study, we reported the experiment observation of delivery of Au nanoparticles inside CNT by using oligonucleotides as carrier. Au nanopartilces with the diameter of 2 nm or so can bind with oligonucleotides strongly, then mixed with MCNTs, and then were treated for 20 min under the condition of 400K and 3 bar. Au-oligo-CNT complexes were purified via agarose gel electrophoresis, HR-TEM and EDX were used to confirm the presence of Au-oligo hybrids inside CNTs. This experimental condition and method are likely generally applied to bring metal nanoparticles inside nanotubes or other nano-porous materials. The Au-oligo-CNT complexes have potential applications in future nanotechnology such as biological sensors, nano-devices and gene storage and delivery systems.

2. Experimental Section

2.1 Materials and reagents

The c-myc oligonucleotides of 15bp in length with

thiol group at 5'-terminal end (The sequence: 5'-Thiol-AAC-GTT-GAG-GGG-CAT-3') were synthesized in MWG company and diluted in ion-free water to a concentration of 100μg ml^{-1}. Multiwall carbon nanotubes (MWCNT) 40-70 nm in outer diameter, 10-20 nm in the diameter of the inner hole and 200-500 nm in length were purchased from Nanostructured & Amorphous Materials (Los Alamos, NM). All chemical reagents in experiments were purchased from Sigma Chemicals, Inc.. The gel electrophoresis device was purchased from Bio-Rad, Inc., and MinElute gel extraction kit was purchased from QIAGEN Company.

2.2 Sample preparation

MCNTs were oxidated by refluxing them in Nitric Acids [25] for 24 h so that two terminal ends of the WCNTs can be opened. The oxidated MCNTs were then washed with ion-free water, filtered and dried at room temperature, and finally suspended in ion-free water to a concentration of 10 mg ml^{-1}. Colloidal Au nanoparticles with 2 nm or so in diameter were fabricated according to the procedure documented in the literature [26-28]. The oligonucleotides of 15bp in length can bind with Au nanoparticles convalently via Thiol group [29]. The Au-oligo hybrids were added in MWCNT solution at equal volume, fully mixed, treated for 20 minutes under 400K and 3 Bar. Next, 1% agarose gel electrophoresis was used to remove the excess of Au nanoparticles, oligonucleotides and Au-oligo hybrids attached to the outside wall of CNTs by the following procedure: 1% agarose gel was prepared according to the method described in Molecular Cloning [30]. Au-oligo–CNT complexes were added into sample wells of agarose gel, and run for 3-4 hours under 60 V and 30 mA, and then were stained with EB dye for 20 min. The gel was observed under UV light and white light so that the concrete position of CNTs in the gel lanes can be recognized. Next, the gels with CNTs were excised with a clean sharp scalpel before weighed in a colorless tube. The purification process was performed according to MinEluteTM Handbook manual. Au-oligo-CNTs adhered to membrane are extracted in aceton solution [24].

2.3 Characterization of Au-oligo-CNTs complexes

Purified MWCNTs were coated over holey carbon cooper grids for SEM observations. The Au oligo-CNT complexes (both before and after electrophoresis) were spread over holey carbon copper grids, and dried for 24 hours. The samples were then observed. When the Au nanoparticles were found to locate inside the CNT, the sample was observed by gradually increasing the magnification to 20 nm and simultaneously tilting the holder at different angles. If the Au nanoparticles can be observed to locate inside CNT at any angle, we finally consider that the Au nanoparticles locate inside CNT and imaged using a Philips CM 200 microscope under an excitation voltage of 200 KeV, the same samples were subjected to EDX analysis with 1.2nm probe size in a VG 501 scanning transmission microscope at an accelerating voltage of 100 kV using a probe size of 30 angstroms and with an acquisition time of 60 seconds.

3. Result and Discussion

3.1 Preparation and characterization of open oxidized MCNTs and Au-oligo hybrids

As shown in Fig. 1A, the oxidated MCNTs are completely dispersed and free of contamination, their

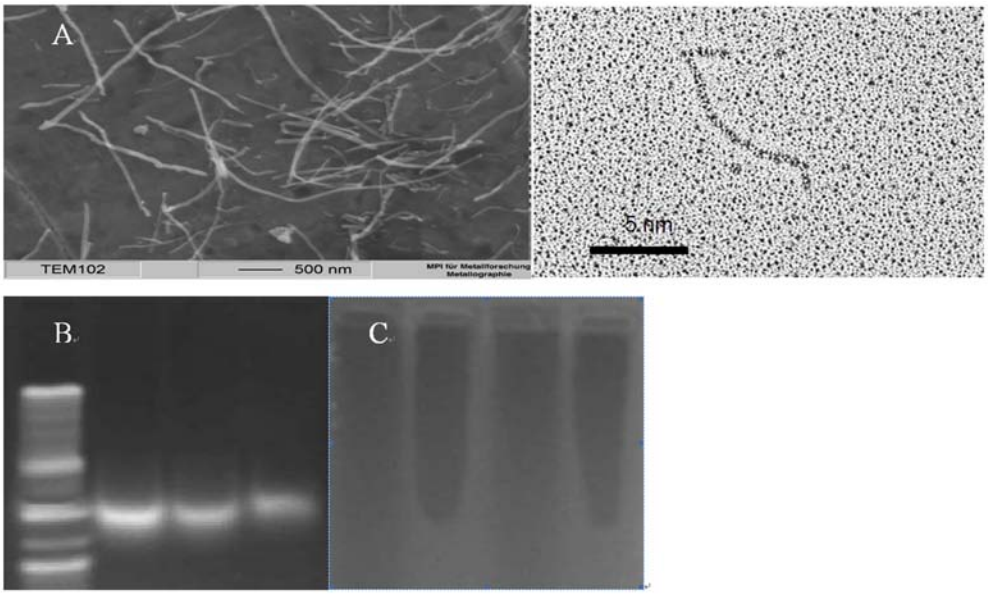

Fig. 1 SEM image of carbon nanotubes, DNA-CNTs and electrophoresis images of DNA-CNTs in 1% agarose gel under 60 V and 30 mA A: SEM image of multiwall carbon nanotubes showing their tubular structure and smooth surfaces; **B**: SEM image of oligonucleotide DNA molecules wrapping around CNTs; **C**: Electrophoresis image of the mixture of DNA and CNTs under UV lamp, showing that DNA molecules moved ahead of CNTs and reached the lower bottom of the gel lanes. The leftmost lane is the DNA molecular marker; **D**: Electrophoresis image of the mixture of DNA and CNTs under white lamp, showing that carbon nanotubes exhibited gradient distribution in each lane.

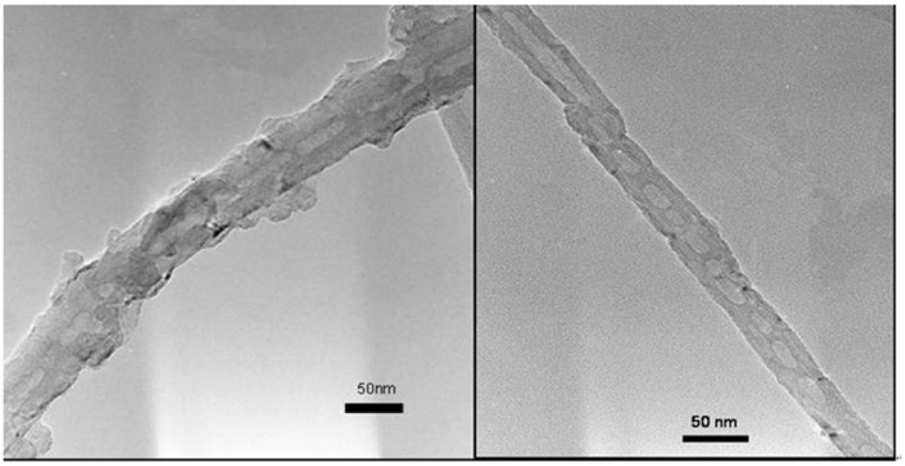

Fig. 2 HR-TEM images of DNA-MWCNTs before and after electrophoresis. A. HR-TEM image of DNA-MWCNT before electrophoresis, showing that DNA molecules wrap around the outside wall of a CNT; B. HR-TEM image of DNA-MWCNT after electrophoresis, showing that DNA molecules attached on the outside wall of the CNT have been removed.

surface are very smooth. HR-TEM observation showed that almost 60% of the MCNTs had open ends (caps removed). 15bp of oligonucleotides with thiol group can bind with Au nanoparticles covalently, and form Au-oligo hybrids with 5 nm or so in diameter. Under the observation of HR-TEM, Au-oligo hybrids looked like ball particles.

3.2 Agarose gel electrophoresis.

1% agarose gel electrophoresis was used to remove the Au-oligo hybrids attached to the outside walls of the MWCNTs. Under the application of a constant electric field, molecules with negative charges move towards the positive electrode, and those molecules with positive charges move towards the negative electrode, different charges result in different mobilities during the process. The agarose gel acts as a molecular sieve [31]. Fig. 1B is the result of 1% agarose gel electrophoresis under UV lamp, showing that Au-oligo hybrids moved faster than CNTs on gel lanes, Fig. 1 C is the result of electrophoresis under white light, showing that CNTs exhibit gradient distribution on gel lanes. No mobility was observed of pure CNTs in sample well. Fig. 2A and Fig. 2B are the HR-TEM images of Au-oligo–CNT complexes before and after electrophoresis, respectively. A lot of complexes wrapped the outside of CNT as shown in Fig. 2A, the CNT's surface was very smooth after electrophoresis as shown in Fig. 2B. This electrophoresis result clearly showed that those excess composition such as Au nanopartilces, oligonucleotides and Au-oligonucleotide hybrids attached to the outside of CNTs can be removed away by agarose gel electrophoresis. These observations can be explained as follows. The negatively charged Au-oligo hybrids and the Au-oligo-CNT complexes move towards the positive electrode. Different Au-oligo-CNT complexes have different sizes and amounts of charge, and hence result in different mobilities over the electrophoresis lanes [32]. The Au-oligo-CNT complexes continue to move until a critical time when the Au-oligo hybrids attached to the outside of the MCNTs become separated from the MCNTs. Afterwards, the Au-oligo molecules move away from the electrically neutral MWCNTs which no longer respond to the applied electric field. In contrast, Au-oligo hybrids inside CNTs can not be easily removed via electrophoresis. This provides a basis to purify Au-oligo-CNT complexes.

3.3 HR-TEM observation and EDX analysis

HR-TEM was used to investigate whether Au-oligo hybrids had been inserted inside CNTs, and EDX was conducted to analyze the elemental composition of the same samples. Fig. 3B is a HR-TEM image of the end section of a MWCNT taken at 300 KeV, indicating the location of a Au nanoparticle inside CNT. In order to confirm the observed Au nanoparticle locate inside CNT, we consecutively observed the Au nanoparticle at different angles by tilting the sample holder. We found that the Au nanoparticle always located inside a CNT at different angles. Fig. 3E is an EDX analysis spectrum obtained with a 1.2 nm probe focused in the vicinity of the gold particle, indicative of the presence of Au, C, N, O and P lines (peaks). The C, N, O and P lines in the spectrum are indicative of the DNA phosphate backbone structure, confirming the presence of Au-oligo hybrids inside the MWCNT. The carbon peak has the highest amplitude due to the presence of the nanotubes and the holey carbon film from the copper grid sample holder. Fig. 3A is another HR-TEM image showing an Au nanoparticle approachs the open end of a CNT.

Fig. 3C is another HR-TEM image of Au nanoparticles inside a MWCNT. Fig. 3F is the corresponding EDX spectrum indicating the presence of C, N, O, P and Au in the linear strand of Au nanoparticles. Fig. 3 D is another HR-TEM image of Au –oligo–CNT complexes, showing that some Au-oligo hybrids attached to the outside of CNTs, three Au-oligo hybrids located inside CNTs as

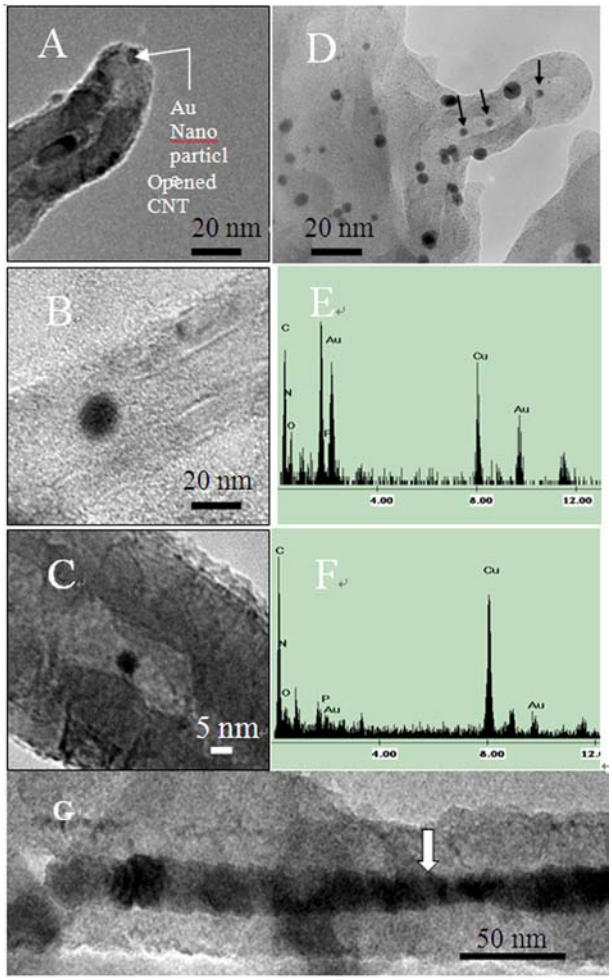

Fig. 3. HRTEM images and EDX spectra of carbon nanotubes filled with Au-oligo hybrids A. Image of an Au labelled DNA oligonucleotide near the entrance of a nanotube obtained at the voltage of 100 KeV; B. TEM image of a gold nanoparticle inside a nanotube obtained at a voltage of 300 KeV; C. The TEM image of a gold nanoparticle inside a nanotube obtained at a voltage of 100 KeV; D. TEM image of Au nanoparticles labelled with oligonucleotides located inside a CNT, as indicated by arrows; E. The EDX analysis showing the presence of C, N, O, P and Au lines (peaks) in the vicinity of the gold particle; F. The EDX spectrum of Au-DNA inside the carbon nanotube at a voltage of 100 KeV, showing the presence of C, N, O, P and Au lines in the vicinity of the gold particle. G. The TEM image of Au-oligonucleotides DNA inside CNT.

indicated by arrows. In our experiments, a lot of Au–oligo hybrids were randomly unexpectedly observed to locate inside a CNT under identical experiment condition as shown in Fig. G. In contrast, we also finished the control experiments by using CNT and Au nanoparticles as research targets, showing that few Au nanoparticles were found to locate inside CNT under identical experimental condition. These observations showed that oligonucleotides can be used to deliver Au nanoparticles inside CNT.

3.4 Mechanism of delivery of Au nanopartilces inside CNTs

Our previous molecular dynamics simulations have demonstrated that oligonucleotide DNA molecules can be encapsulated inside a CNT, and the van der Waal's force has been identified to be the dominant driving force for the encapsulation phenomenon [23]. A small reduction in the van der Waals force dramatically slows down and even stops the encapsulation process. The encapsulation process strongly depends on the diameter and length of the CNT. Our previous experiments also have confirmed that Pt-labeled DNA molecules can be filled inside CNTs under the condition of 400 K and 3 Bar [24]. These results highly indicate that DNA molecules are likely potential carrier to deliver metal nanoparticles inside CNTs.

The experiments reported here have demonstrated that Au-oligo hybrids can be delivered inside CNTs under the condition of 400 K and 3 Bar. In contrast, few pure Au nanoparticles can be observed to locate inside CNTs under identical experiment condition.

At room temperature and pressure conditions, oligonucleotide DNA molecules have the following characteristics [33]: they are highly hydrophilic, negatively charged, have the capability of forming a hydration layer with water molecules, have strong cohesive ability, and they can bind with CNTs via static electronic attraction or van der Waal's forces and increase the water solubility of CNTs. The van der Waal's force is very weak compared with the π-bond and static electronic attraction between CNT and oligo DNA molecules at room temperature and pressure conditions, and it can not drive the encapsulation of Au-oligonucleotide hybrids inside CNTs. Conversely, Au-oilgo DNA molecules mainly attach to the outside of CNTs.

However, under the condition of 400K and 3 Bar, oligo DNA molecules exhibit complete different properties. For example, their kinetic energy increases, and the Brownian motion of oligo DNA molecules become faster. Oligo DNA molecules exhibit a typical hyperchromic effect [34], e.g. their cohesive ability significantly decreases, their floating density increase and their surface tension decrease. In contrast, the properties of Au nanopartilces change very little under identical condition. The pressure of 3 Bar is applied to keep the aqueous solution in the liquid state. These factors result in van der Waal's interaction between DNA molecules and CNTs become more prominent than that interaction under other condition, finally the Au-oligo hybrids are driven towards the interior of the CNTs, resulting in the encapsulation process. The van der Waal's interaction between CNTs and Au-oligo hybrids still constitutes the main driving force for the delivery process.

In the course of experiments, in order to distinguish the Au nanoparticles outside CNT from those inside CNT, we used three methods. First, we used agarose gel electrophoresis to remove Au-oligo hybrids attached to the outside of CNT, then we observed the samples at larger scales such as 200 nm or100 nm. After the Au nanoparticles were found to locate inside the CNT, the sample was observed by gradually decreasing the

scale to 20nm and simultaneously tilting the holder at different angles. If Au nanoparticles actually do not locate inside the CNT, they could be seen to locate the outside of CNT as the holder angle changed. Reversely, if the Au nanoparticles really locate inside CNT, they can be observed to locate inside CNT at any angle. After the Au nanoparticles were always observed to locate inside CNT under these conditions, we finally consider that the Au nanoparticles locate inside CNT and took picture. Finally, the samples were subjected to EDX analysis to confirm the concrete composition of chemistry elements such as C, N, O, P, Au.

All above-montioned results fully demonstrate that oligonucleotide DNA molecules can be used for the delivery of Au nanoparticles inside a carbon nanotube. However, in the experiment, we found that large parts of Au-oligo hybrids attach to the outside wall of CNT, while only 2-3% Au-oligo DNA hybrids were delivered inside CNT, although we also randomly observed that a lot of Au-oligo hybrids thrust inside a CNT. However, the concrete mechanism still needs to be studied. How to establish the controllable condition of delivery of fixed amount of Au-oligo hybrids inside CNT is still a challengable problem we are seeking to solve.

4. Conclusion

DNA-oligo molecules can be used to deliver gold nanoparticles inside carbon nanotubes. Van der Waal's force between Au-oligo hybrids and CNT is main drive force to carry Au-oligo DNA hybrids inside CNT. This seems to be the first report for encapsulating Au clusters inside CNTs. This process may be generally used to insert a variety of nanoparticles into carbon nanotubes. Future work will be directed at chemical, electronic and biological properties of such bio-nano-complexes for potential applications in molecular electronics, chemical sensors, field emission devices and bioengineering.

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References

1. Iijima S. Helical microtubules of graphitic carbon. *Nature*. 1991; 354(6348):56-58.http://dx.doi.org/10.1038/354056a0
2. Cui DX. Advances and prospects on biomolecules functionalized carbon nanotubes. *Journal of Nanoscience and Nanotechnolgy*. 2007; 7:1298-1314. http://dx.doi.org/10.1166/jnn.2007.654
3. Cui DX, Pan BF, Zhang H, Gao F, Wu R, Wang JP, et al. Self-assembly of quantum dots and carbon nanotubes for ultrasensitive DNA and antigen detection. *Analytical Chemistry*. 2008;80:7996-8001.http://dx.doi.org/10.1021/ac800992m
4. Cui D, Tian F, Kong Y, Gao H. Effects of single wall carbon nanotubes on PCR. *Nanotechnology*. **2004**; 15: 154. http://dx.doi.org/10.1088/0957-4484/15/1/030
5. Pan BF, Cui DX, Xu P, Chen H, Liu FT, Li Q, et al. Design of dendrimer modified carbon nanotubes for gene delivery. *Chinese Journal of Cancer Research*. 2007; 19:1-6.http://dx.doi.org/10.1007/s11670-007-0001-0
6. Huang P, Zhang C, Xu C, Bao L, Li Z. Preparation and characterization of near infrared region absorption enhancer carbon nanotubes hybridmaterials. *Nano Biomed Eng*. 2010; 2:225-230. http://dx.doi.org/10.5101/nbe.v2i4.p225-230
7. Yuan HG, Hu SL, Huang P, Song H, Wang K, Ruan J, et al. Single Walled Carbon Nanotubes Exhibit Dual-Phase Regulation to Exposed Arabidopsis Mesophyll Cells. *Nanoscale Research Letters*. 2010; 6.
8. Pan BF, Cui DX, Ozkan CS, Ozkan M, Xu P, Huang T, et al. Effects of carbon nanotubes on photoluminescence properties of quantum dots. *Journal of Physical Chemistry C*. 2008;112:939-944. http://dx.doi.org/10.1021/jp068920c
9. Cui DX, Tian FR, Coyer SR, Wang JC, Pan BF, Gao F, et al. Effects of antisense-myc-conjugated single-walled carbon nanotubes on HL-60 cells. *Journal of Nanoscience and Nanotechnology*. 2007;7: 1639-1646.http://dx.doi.org/10.1166/jnn.2007.348
10. Cui DX, Zhang H, Sheng J, Wang Z, Toru A, He R, et al. Effects of CdSe/ZnS quantum dots covered multi-walled carbon nanotubes on murine embryonicstem cells *Nano Biomed Eng*. 2010;2:236-244.
11. Chen RJ, Bangsaruntip S, Drouvalakis KA, Wong SKN, Shim M, Li Y, et al. Noncovalent functionalization of carbon nanotubes for highly specific electronic biosensors. *Proc. Natl. Acad. Sci. USA*. 2003;100:4984.http://dx.doi.org/10.1073/pnas.0837064100
12. Xu P, Cui DX, Pan BF, Gao F, He R, Li Q, et al. A facile strategy for covalent binding of nanoparticles onto carbon nanotubes. *Applied Surface Science*.2008;254:5236-5240.http://dx.doi.org/10.1016/j.apsusc.2008.02.082
13. Erlanger F, Chen B, Zhu M, Brus L. Binding of an Anti-Fullerene IgG Monoclonal Antibody to Single Wall Carbon Nanotubes. *Nano Lett*. 2001;1:465.http://dx.doi.org/10.1021/nl015570r
14 Hirahara K, Suenaga K, Bandow S, Kato H, Okazaki T, Shinohara, H, et al. One-dimensional metallofullerene crystal generated inside single-walled carbon nanotubes. *Phys. Rev. Lett.* 2000; 85:5384.http://dx.doi.org/10.1103/PhysRevLett.85.5384
15. Hummer G, Rasalah JC, Noworyta JP. Water conduction through the hydrophobic channel of a carbon nanotube. *Nature*. 2001; 414: 188.http://dx.doi.org/10.1038/35102535
16. Bashir, R. DNA-mediated artificial nanobiostructures: state of the art and future directions. *Superlattices and Microstructures* **2001**, 29, 1. http://dx.doi.org/10.1006/spmi.2000.0904
17. Keren K, Berman RS, Buchstab E, Sivan U, Braun E. DNA-templated carbon nanotube field-effect transistor. *Science* 2003;302:1380.http://dx.doi.org/10.1126/science.1091022
18. Warner MG, Hutchison JE. Linear assemblies of nanoparticles electrostatically organized on DNA scaffolds. *Nature Materials* 2003;2:272.http://dx.doi.org/10.1038/nmat853
19. Shim M, Kam NMS, Chen RJ, Li R, Dai H. Functionalization of carbon nanotubes for biocompatibility and biomolecular recognition. *Nano Lett*. 2002;2;285. http://dx.doi.org/10.1021/nl015692j
20. Chen RJ, Zhang YG, Wang DW, Dai H. Noncovalent sidewall functionalization of single-walled carbon nanotubes for protein immobilization. *J. Am. Chem*. Soc. 2001;123;3838. http://dx.doi.org/10.1021/ja010172b
21. Zheng M, Jagota A, Strano MS, Santos AP, Barone P.et al. Structure-based carbon nanotube sorting by sequence-dependent DNA assembly. *Science*. 2003;302:1545.http://dx.doi.org/10.1126/science.1091911
22. Zheng M, Jagota A, Semke ED, Diner BA, Mclean RS, Lustig SR, et al. DNA-assisted dispersion and separation of carbon nanotubes. *Nature Materials*. 2003;2:338.http://dx.doi.org/10.1038/nmat877
23. Gao H, Kong Y, Cui D, Ozkan OS. Spontaneous Insertion of DNA Oligonucleotides into Carbon Nanotubes. *Nano Lett*. 2003;3: 471.http://dx.doi.org/10.1021/nl025967a
24. Cui D, Ozkan CS, Ravindran S, Kong Y, Gao H.

Encapsulation of pt-labelled DNA molecules inside carbon nanotubes. *Mechanics and Chemsitry of Biosystem*. 2004;1(2): 113-121.
25. Bahr JL; Tour JM. Covalent chemistry of single-wall carbon nanotubes. *J. Mater. Chem*. 2002;12:1952.http://dx.doi.org/10.1039/b201013p
26. Harnack O, Ford WE, Yasuda A and Wessels JM. Tris(hydroxymethyl)phosphine-Capped Gold Particles Templated by DNA as Nanowire Precursors. *Nano Lett*. 2002;2:919. http://dx.doi.org/10.1021/nl020259a
27. Christopher FM, Adam TW. DNA-Templated Construction of Copper Nanowires. *Nano Lett*. 2003;3:359.http://dx.doi.org/10.1021/nl034016+
28. Mertig M, Ciacchi L C, Seidel R, Pompe W and De Vita A. DNA as a Selective Metallization Template. *Nano Lett*. 2002;2:841. http://dx.doi.org/10.1021/nl025612r
29. Sato Y, Mizutani F. Formation and characterization of aromatic selenol and thiol monolayers on gold: in-situ IR studies and electrochemical measurements. *Phys. Chem. Phys*. 2004;6;1328. http://dx.doi.org/10.1039/b315034h
30 Sambrook J, Fritsch EF, Maniatis T. Molecular Cloning: A laboratory manual 2 nd ed, Cold Spring Harbor Laboratory Press, USA. 1998.
31. Grossman PD, Soane DS. Capillary electrophoresis of DNA in entangled polymer solutions. *J. Chromatogr*.1991;559:257. http://dx.doi.org/10.1016/0021-9673(91)80076-S
32. Kepka C, Rhodin J, Lemmens R, Tjerneld F, Gustavsson P E. Extraction of plasmid DNA from Escherichia coli cell lysate in a thermoseparating aqueous two-phase system. *J. Chromatogr A*. 2004;1024:95.http://dx.doi.org/10.1016/j.chroma.2003.10.028
33 Cui D, Tian F, Ozkan CS, Wang M,Gao H. Effect of single wall carbon nanotubes on human HEK293 cells. *Toxicology Letters*. 2005;5:73.http://dx.doi.org/10.1016/j.toxlet.2004.08.015
34 Pan BF, Cui DX, Xu P, Ozkan C, Feng G, Ozkan M, et al. Synthesis and characterization of polyamidoamine dendrimer-coated multi-walled carbon nanotubes and their application in gene delivery systems. *Nanotechnology*.2009;20:12http://dx.doi.org/10.1088/0957-4484/20/12/125101