(54) Title: SEPARATION OF CARBON NANOTUBES DISPERSED BY NUCLEIC ACIDS

Dispersion of Carbon Nanotubes by Single-stranded DNA

(57) Abstract: Carbon nanotubes dispersed by a stabilized solution of nucleic acid molecules formed separated nanotube-nucleic acid complexes and were separated according to standard chromatographic methods, including gel electrophoresis, two phase solvent systems and ion exchange chromatography.
TITLE
SEPARATION OF CARBON NANOTUBES DISPERSED BY NUCLEIC ACIDS

This application claims the benefit of United States provisional application 60/450,590. Filed February 27, 2003, United States provisional application 60/428087, filed November 21, 2002, and United States provisional application 60/432804, filed December 12, 2002.

FIELD OF INVENTION

The invention relates to methods of separating dispersed carbon nanotubes for use in nanodevices. More specifically, nucleic acid molecules have been found to disperse bundled carbon nanotubes and to form separated nanotube-nucleic acid complexes followed by separation according to standard chromatographic methods.

BACKGROUND

Carbon nanotubes (CNT) have been the subject of intense research since their discovery in 1991. CNT's possess unique properties such as small size and electrical conductivity, which makes them suitable in a wide range of applications, including use as structural materials in molecular electronics, nanoelectronic components, and field emission displays. Carbon nanotubes may be either multi-walled (MWNTs) or single-walled (SWNTs), and have diameters in the nanometer range.

Depending on their atomic structure CNT's may have either metallic or semiconductor properties, and these properties, in combination with their small dimensions makes them particularly attractive for use in fabrication of nano-devices. A major obstacle to such efforts has been the diversity of tube diameters, chiral angles, and aggregation states in nanotube samples obtained from the various preparation methods. Aggregation is particularly problematic because the highly polarizable, smooth-sided fullerene tubes readily form parallel bundles or ropes with a large van der Waals binding energy. This bundling perturbs the electronic structure of the tubes, and it confounds all attempts to separate the tubes by size or type or to use them as individual macromolecular species. One method to disperse these aggregates is by the use of stabilized solutions of nucleic acid molecules as described in USSN 60/432804 and USSN 60/428087 incorporated herein by reference.

Co-incident with the problem of dispersion of aggregated carbon nanotubes is the problem of separation. Because most populations of nanotubes are aggregated it has been very difficult to obtain discreet
populations of nanotubes that have a uniform length, diameter, chirality or other physical properties. The ability to perform such separations will be essential if carbon nanotubes are to be effectively used in electronics and other applications at the nano-scale.

Jiang et al (Journal of Colloid and Interface Science (2003), 260(1), 89-94 have demonstrated that populations of nanotubes may be dispersed in surfactant and separated by precipitation however no attempt was made to characterize the fractions on the basis of common physical or chemical parameters.

The problem to be solved, therefore, is to provide a method for the facile and inexpensive separation of dispersed carbon nanotubes into populations having discreet characteristics. Applicants have solved these problems through the use of stabilized solutions of nucleic acid molecules that have the ability to disperse and solubilize carbon nanotubes, resulting in the formation of nanotube-nucleic acid complexes. Applicants have discovered a method for the separation of these nucleic acid associated carbon nanotubes based on common chromatographic means. Although complexes of nucleic acids and carbon nanotubes are known, the present complexes are new, in that the association between the nanotube and nucleic acid is non-covalent and not through the interaction of specific functionalized groups.

**SUMMARY OF THE INVENTION**

The invention relates to methods for the separation of dispersed carbon nanotubes (CNT's). The CNT's of the invention are typically dispersed by contacting them with solutions of stabilized nucleic acids where the nucleic acid becomes associated with the CNT. The separation methods include gel electrophoresis, two phase separations and ion exchange chromatography.

Accordingly the invention provides a method for the separation of a carbon nanotube-nucleic acid complex comprising:

a) providing a carbon nanotube-nucleic acid complex in solution comprising an unfunctionalized carbon nanotube bound to a nucleic acid molecule wherein the solution comprises a densifying agent;

b) loading the carbon nanotube-nucleic acid complex solution of step (a) on to an electrophoresis gel; and

c) separating the loaded complexes of (b) by applying an electric field to the gel.
In an alternate embodiment the invention provides a method for the separation of a carbon nanotube-nucleic acid complex comprising:

a) providing a carbon nanotube-nucleic acid complex in and aqueous solution comprising an unfunctionalized carbon nanotube bound to a nucleic acid molecule;

b) adding a substantially water-miscible organic solvent to the dissolved complexes of (a) whereby a certain size fraction of the complexes are precipitated; and

c) collecting the complex precipitate of step (b).

Similarly the invention provides a method for the separation of a carbon nanotube-nucleic acid complex comprising:

a) providing a carbon nanotube-nucleic acid complex in an aqueous solution comprising an unfunctionalized carbon nanotube bound to a nucleic acid molecule wherein the nucleic acid portion of said complex is comprised of at least 50% hydrophobic nucleotides;

b) applying the solution of (a) to an ion exchange media wherein the carbon nanotube-nucleic acid complex becomes associated with the ion exchange media; and

c) eluting the carbon nanotube-nucleic acid complex from the ion exchange media into discreet fractions.

In an alternate embodiment the invention provides a population of carbon nanotubes separated by the method of any one of Claims 1, 4 and 5.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 is a graph of optical density measurements to illustrate the carbon nanotube dispersion efficiency by a variety of sequences.

Figure 2 is a graph of optical density measurements illustrating the dispersion efficiency of a particular sequence as a function of duration of sonication and with addition of formamide.

Figure 3 is a graph of optical density measurements comparing the dispersion efficiency of carbon nanotubes by ssDNA (Figure 3A) versus surfactant (Figure 3B).

Figure 4 is a graph of optical density measurements to illustrate the carbon nanotube dispersion efficiency by RNA.

Figure 5 is a photograph illustrating immobilization of DNA dispersed carbon nanotubes through biotin-streptavidin interaction. Figure 5A, shows the use of ssDNA for carbon nanotube dispersion is biotinylated N90; where
as in Figure 5B, ssDNA used for carbon nanotube dispersion is unmodified N90.

Figure 6A is an image of gel electrophoresis separated carbon nanotubes. Figure 6B and 6C are optical absorption spectra obtained from carbon nanotubes eluted from fraction 1 and 2, respectively.

Figure 7A is an elution profile of (C/T)60 dispersed carbon nanotubes running through an anion exchange column.

Figure 7B is optical absorption spectra obtained from carbon nanotubes eluted from fraction 39, 41 and 43, respectively, as discussed in Example 10.

Figure 8 is an optical absorption spectrum obtained from carbon nanotubes eluted from an anion exchange column.

Figure 9 is an optical absorption spectrum obtained from carbon nanotubes eluted from an anion exchange column from fractions 35, 40 and 50 as discussed in Example 14.

Figure 10 is an electronmicrograph taken via atomic force microscopy of an isolated carbon nanotube wrapped with DNA.

Figure 11 is an electronmicrograph taken via atomic force microscopy showing different modes of DNA wrapping on carbon nanotubes.

DETAILED DESCRIPTION OF THE INVENTION

The invention relates to methods for the separation of dispersed carbon nanotubes (CNT's). Typically the CNT's are dispersed by contacting the bundled nanotubes with a solution of stabilized nucleic acid molecules. Separation proceeds either by gel electrophoresis, two phase solvent separation or ion exchange chromatography.

In this disclosure the following terms and abbreviations may be used for the interpretation of the claims and specification.

"cDNA" means complementary DNA
"PNA" means peptide nucleic acid
"SEM" means scanning electron microscopy
"ssDNA" means single stranded DNA
"tRNA" means transfer RNA
“CNT” means carbon nanotube
“MWNT” means multi-walled nanotube
“SWNT” means single walled nanotube
“TEM” means transmission electron microscopy
The term "carbon nanotube" refers to a hollow article composed primarily of carbon atoms. The carbon nanotube can be doped with other elements, e.g., metals. The nanotubes typically have a narrow dimension (diameter) of about 1-200 nm and a long dimension (length), where the ratio of the long dimension to the narrow dimension, i.e., the aspect ratio, is at least 5. In general, the aspect ratio is between 10 and 2000.

As used herein a "nucleic acid molecule" is defined as a polymer of RNA, DNA, or peptide nucleic acid (PNA) that is single- or double-stranded, optionally containing synthetic, non-natural or altered nucleotide bases. A nucleic acid molecule in the form of a polymer of DNA may be comprised of one or more segments of cDNA, genomic DNA or synthetic DNA.

The letters "A", "G", "T", "C" when referred to in the context of nucleic acids will mean the purine bases adenine (C₉H₆N₆) and guanine (C₅H₅N₅O) and the pyrimidine bases thymine (C₅H₆N₂O₂) and cytosine (C₄H₅N₃O), respectively.

The term "peptide nucleic acids" refers to a material having stretches of nucleic acid polymers linked together by peptide linkers.

As used herein the term "stabilized solution of nucleic acid molecules" refers to a solution of nucleic acid molecules that are solubilized and in a relaxed secondary conformation.

The term "nanotube-nucleic acid complex" means a composition comprising a carbon nanotube loosely associated with at least one nucleic acid molecule. Typically the association between the nucleic acid and the nanotube is by van der Waals bonds or some other non-covalent means.

The term "binding pair" refers to chemical or biopolymer based couples that bind specifically to each other. Common examples of binding pairs are immune-type binding pairs, such as antigen/antibody or hapten/anti-hapten systems. Suitable binding pairs glutathione-S-transferase/glutathione, 6X histidine Tag/Ni-NTA, streptavidin/avidin, S-protein/S-peptide, cutinase/phosphonate inhibitor, antigen/antibody, hapten/anti-hapten, folic acid/folate binding protein, and protein A or G/immunoglobulins. Another example of a binding pair is a negatively charged phosphate backbone of a nucleic acid molecule, with the second member being a positively charged surface.

The term "ligand" or "reactive ligand" will refer to one member of a binding pair which has been incorporated into the nucleic acid analyte and
may include but is not limited to antibodies, lectins, receptors, binding proteins or chemical agents.

The term “agitation means” refers to a devices that facilitate the dispersion of nanotubes and nucleic acids. A typical agitation means is sonication.

The term “denaturant” as used herein refers to substances effective in the denaturation of DNA and other nucleic acid molecules.

The term “solid support” means a material suitable for the immobilization of a nanotube-nucleic acid complex. Typically the solid support provides an attachment of a member of a binding pair through which the complex is capture and immobilized.

The term “hybridization domain” refers to a specific portion of a nucleic acid molecule that is designed to hybridize to a complementary strand of another nucleic acid molecule. “Hybridizable” nucleic acids may be used to immobilize or direct placement of nanotube-nucleic acid complexes in the fabrication of nano-devices.

A nucleic acid molecule is “hybridizable” to another nucleic acid molecule, such as a cDNA, genomic DNA, or RNA, when a single stranded form of the nucleic acid molecule can anneal to the other nucleic acid molecule under the appropriate conditions of temperature and solution ionic strength. Hybridization and washing conditions are well known and exemplified in Sambrook, J., Fritsch, E. F. and Maniatis, T. Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor (1989), particularly Chapter 11 and Table 11.1 therein (entirely incorporated herein by reference). The conditions of temperature and ionic strength determine the “stringency” of the hybridization. Stringency conditions can be adjusted to screen for moderately similar fragments, such as homologous sequences from distantly related organisms, to highly similar fragments, such as genes that duplicate functional enzymes from closely related organisms. Post-hybridization washes determine stringency conditions. One set of preferred conditions uses a series of washes starting with 6X SSC, 0.5 % SDS at room temperature for 15 min, then repeated with 2X SSC, 0.5 % SDS at 45°C for 30 min, and then repeated twice with 0.2X SSC, 0.5 % SDS at 50°C for 30 min. A more preferred set of stringent conditions uses higher temperatures in which the washes are identical to those above except for the temperature of the final two 30 min washes in 0.2X SSC, 0.5 % SDS was increased to 60°C. Another preferred set of highly
stringent conditions uses two final washes in 0.1X SSC, 0.1 % SDS at 65°C. Hybridization requires that the two nucleic acids contain complementary sequences, although depending on the stringency of the hybridization, mismatches between bases are possible. The appropriate stringency for hybridizing nucleic acids depends on the length of the nucleic acids and the degree of complementation, variables well known in the art. The greater the degree of similarity or homology between two nucleotide sequences, the greater the value of Tm for hybrids of nucleic acids having those sequences. The relative stability (corresponding to higher Tm) of nucleic acid hybridizations decreases in the following order: RNA:RNA, DNA:RNA, DNA:DNA. For hybrids of greater than 100 nucleotides in length, equations for calculating Tm have been derived (see Sambrook et al., supra, 9.50-9.51). For hybridizations with shorter nucleic acids, i.e., oligonucleotides, the position of mismatches becomes more important, and the length of the oligonucleotide determines its specificity (see Sambrook et al., supra, 11.7-11.8). In one embodiment the length for a hybridizable nucleic acid is at least about 10 nucleotides. Preferable a minimum length for a hybridizable nucleic acid is at least about 15 nucleotides; more preferably at least about 20 nucleotides; and most preferably the length is at least 30 nucleotides.

Furthermore, the skilled artisan will recognize that the temperature and wash solution salt concentration may be adjusted as necessary according to factors such as length of the probe.


Dispersion of Carbon Nanotubes.

The invention provides utilizes a population of dispersed carbon nanotubes. Typically these are dispersed by contacting the nanotubes with a stabilized solution of nucleic acid molecules.

Carbon Nanotubes
Carbon nanotubes of the invention are generally about 0.5-2 nm in diameter where the ratio of the length dimension to the narrow dimension, i.e., the aspect ratio, is at least 5. In general, the aspect ratio is between 10 and 2000. Carbon nanotubes are comprised primarily of carbon atoms, however may be doped with other elements, e.g., metals. The carbon-based nanotubes of the invention can be either multi-walled nanotubes (MWNTs) or single-walled nanotubes (SWNTs). A MWNT, for example, includes several concentric nanotubes each having a different diameter. Thus, the smallest diameter tube is encapsulated by a larger diameter tube, which in turn, is encapsulated by another larger diameter nanotube. A SWNT, on the other hand, includes only one nanotube.


Additionally CNT’s may be grown via catalytic processes both in solution and on solid substrates (Yan Li, et al., Chem. Mater.; 2001; 13(3); 1008-1014); (N. Franklin and H. Dai Adv. Mater. 12, 890 (2000); A. Cassell et al. J. Am. Chem. Soc. 121, 7975-7976 (1999)).

Nucleic Acid Molecules

Nucleic acid molecules of the invention may be of any type and from any suitable source and include but are not limited to DNA, RNA and peptide nucleic acids. The nucleic acid molecules may be either single stranded or double stranded and may optionally be functionalized at any point with a variety of reactive groups, ligands or agents. The nucleic acid molecules of the invention may be generated by synthetic means or may be isolated from nature by protocols well known in the art (Sambrook supra).

It should be noted that functionalization of the nucleic acids are not necessary for their association with CNT’s for the purpose of dispersion. Functionalization may be of interest after the CNT’s have been dispersed and it is desired to bind other moieties to the nucleic acid or immobilize the carbon
nanotube-nucleic acid complex to a surface through various functionalized elements of the nucleic acid. As used herein nucleic acids that are used for dispersion, typically lack functional groups and are referred to herein as “unfunctionalized.”

Peptide nucleic acids (PNA) are particularly useful in the present invention as they possess the double functionality of both nucleic acids and peptides. Methods for the synthesis and use of PNA’s are well known in the art, see for example Antsypovitch, S. I. Peptide nucleic acids: structure Russian Chemical Reviews (2002), 71(1), 71-83.

The nucleic acid molecules of the invention may have any composition of bases and may even consist of stretches of the same base (poly A or polyT for example) without impairing the ability of the nucleic acid molecule to disperse the bundled nanotube. Preferably the nucleic acid molecules will be less than about 2000 bases where less than 1000 bases is preferred and where from about 5 bases to about 1000 bases is most preferred. Generally the ability of nucleic acids to disperse carbon nanotubes appears to be independent of sequence or base composition, however there is some evidence to suggest that the less G-C and T-A base-pairing interactions in a sequence, the higher the dispersion efficiency, and that RNA and varieties thereof is particularly effective in dispersion and is thus preferred herein.

Nucleic acid molecules suitable for use in the present invention include but are not limited to those having the general formula:

1. \( \text{An wherein } n = 1 - 2000; \)
2. \( \text{Tn wherein } n = 1 - 2000; \)
3. \( \text{Cn wherein } n = 1 - 2000; \)
4. \( \text{Gn wherein } n = 1 - 2000; \)
5. \( \text{Rn wherein } n = 1-2000, \) and wherein R may be either A or G;
6. \( \text{Yn wherein } n = 1 - 2000, \) and wherein Y may be either C or T;
7. \( \text{Mn wherein } n = 1 - 2000, \) and wherein M may be either A or C;
8. \( \text{Kn wherein } n = 1 - 2000, \) and wherein K may be either G or T;
9. \( \text{Sn wherein } n = 1 - 2000, \) and wherein S may be either C or G;
10. \( \text{Wn wherein } n = 1 - 2000, \) and wherein W may be either A or T;
11. \( \text{Hn wherein } n = 1 - 2000, \) and wherein H may be either A or C or T;
12. \( \text{Bn wherein } n = 1 -2000, \) and wherein B may be either C or G or T;
13. \( \text{Vn wherein } n = 1 - 2000, \) and wherein V may be either A or C or
G;

14. Dn wherein n = 1 - 2000, and wherein D may be either A or G or T; and
15. Nn wherein n = 1 - 2000, and wherein N may be either A or C or T or G;

In addition the combinations listed above the person of skill in the art will recognize that any of these sequences may have one or more deoxyribonucleotides replaced by ribonucleotides (i.e., RNA or RNA/DNA hybrid) or one or more sugar-phosphate linkages replaced by peptide bonds (i.e. PNA or PNA/RNA/DNA hybrid).

The invention provides methods for the separation of nucleic acid associated CNT's on the basis of ion exchange chromatography. In these instances it is preferred if the composition of the nucleic acids be of a length that ranges form about 10 bases to about 100 bases where a length of about 30 to about 60 bases is preferred. Additionally, it will be preferred if the base composition of the nucleic acid favors hydrophobic bases such as guanine and thymine. Nucleic acids having a base composition of at least 50% guanine are suitable where nucleic acids of the general formula (G/T)n where n=5 – 50 are preferred.

Ligand Incorporation

It is contemplated that ligands may be introduced into the nucleic acid molecules in a variety positional orientations and numbers. For example ligands can be incorporated into one or both strands of a duplex nucleic acid analyze. Positionally, ligands can be incorporated either at the 5' or 3' ends of the analyze or incorporated on internal bases within the nucleic acid sequence, but incorporation internally is generally preferred.

The method of incorporation of the ligand into the nucleic acid sequences may be accomplished either by chemical or enzymatic means, or by direct incorporation of ligand labeled bases into the target sequence. In a preferred approach, ligand incorporated sequences are prepared using ligand labeled bases or primers during polymerase chain reaction. Ligand incorporation can be accomplished either through the incorporation of primers modified with ligand(s) or by using ligand labeled dNTPs. Ligand labeled primers can be prepared using standard oligonucleotide cyanooethyl phosphorimidate chemistry by substituting selected bases with ligand modified phosphorimidate bases during primer synthesis. Alternatively, if primers are prepared with modified bases containing a linkable molecular
spacer, the ligands can be chemically linked to the spacer after primer synthesis. Another method would make use of ligand labeled dNTPs or amino modified dNTPs which can be incorporated into a target nucleic acid sequence during the amplification procedure.

There are several advantages to synthesis of ligand incorporated nucleic acid sequences by PCR. For example, where labeled primers are used, it is possible to control both the positioning and number of ligands within one or both strands of the target sequence by the appropriate placement of the ligand in the primers.

Of particular interest in the present invention is the incorporation of binding pairs into the nucleic acid molecule. Where a nucleic acid is associated with a carbon nanotube, the use of specific binding pairs enables the placement of the nucleic acid portion at a specific point of attachment and facilitates the rationale design of nano-devices and structures. Members of specific binding pairs suitable for use in practicing the invention can be of the immune or non-immune type. Immune specific binding pairs are exemplified by antigen/antibody systems or hapten/anti-hapten systems. The antibody member, whether polyclonal, monoclonal or an immunoreactive fragment thereof, of the binding pair can be produced by customary methods familiar to those skilled in the art. The terms “immunoreactive antibody fragment” or “immunoreactive fragment” refer to fragments which contain the binding region of the antibody. Such fragments may be Fab-type fragments which are defined as fragments devoid of the Fc portion, e.g., Fab, Fab’ and F(ab’)2 fragments, or may be so-called “half-molecule” fragments obtained by reductive cleavage of the disulfide bonds connecting the heavy chain components of the intact antibody. If the antigen member of the specific binding pair is not immunogenic, e.g., a hapten, it can be covalently coupled to a carrier protein to render it immunogenic.

Non-immune binding pairs include systems wherein the two components share a natural affinity for each other but are not antibodies. Exemplary non-immune binding pairs are biotin-avidin or biotin-streptavidin, folic acid-folate binding protein, complementary probe nucleic acids, Proteins A, G, and immunoglobulins, etc. Also included are non-immune binding pairs which form a covalent bond with each other: exemplary covalent binding pairs include sulfhydryl reactive groups such as maleimides and haloacetyl derivatives and amine reactive groups such as isothiocyanates, succinimidyl

Binding pairs particularly suitable or use in the present invention include but are not limited to immune-type binding pairs, such as antigen/antibody or hapten/anti-hapten systems as well as glutathione-S-transferase/glutathione, 6X histidine Tag/Ni-NTA, streptavidin/biotin, S-protein/S-peptide, cutinase/phosphonate inhibitor, antigen/antibody, hapten/anti-hapten, folic acid/folate binding protein, and protein A or G/immunoglobulins. Binding pairs may also include charged moieties as for example, a negatively charged phosphate backbone of a nucleic acid molecule, with the second member being a positively charged surface.

Nucleic Acid Stabilization

Once the nucleic acid molecule has been prepared it must be stabilized in a suitable solution. It is preferred if the nucleic acid molecules are in a relaxed secondary conformation and only loosely associated with each other to allow for the greatest contact by individual strands with the carbon nanotubes. Stabilized solutions of nucleic acids are common and well known in the art (see Sambrook supra) and typically include salts and buffers such as sodium and potassium salts, and TRIS (Tris(2-aminoethyl)amine), HEPES (N-(2-hydroxyethyl)piperazine-N'-(2-ethanesulfonic acid), and MES (2-(N-Morpholino)ethanesulfonic acid. Preferred solvents for stabilized nucleic acid solutions are those that are water miscible where water is most preferred.

Once the nucleic acid molecules are stabilized in a suitable solution they may be contacted with a population of bundled carbon nanotubes. It is preferred, although not necessary if the contacting is done in the presence of an agitation means of some sort. Typically the agitation means employs sonication for example, however may also include, devices that produce high shear mixing of the nucleic acids and nanotubes (i.e. homogenization), or any combination thereof. Upon agitation the carbon nanotubes will become dispersed and will form nanotube-nucleic acid complexes comprising at least one nucleic acid molecule loosely associated with the carbon nanotube by hydrogen bonding or some non-covalent means.

The process of agitation and dispersion may be improved with the optional addition of nucleic acid denaturing substances to the solution.

Common denaturants include but are not limited to formamide, urea and guanidine. A non-limiting list of suitable denaturants may be found in Sambrook supra.
Additionally temperature during the contacting process will have an effect on the efficacy of the dispersion. Agitation at room temperature or higher was seen to give longer dispersion times whereas agitation at temperatures below room temperature (23°C) were seen to give more rapid dispersion times where temperatures of about 4°C are preferred.

Recovery of Dispersed Nanotubes

Once the nanotube-nucleic acid molecule complexes are formed they must be separated from solution. Several methods are available for separation including gel electrophoresis and liquid chromatography. Where the nucleic acid has been functionalized by the addition of a binding pair for example separation could be accomplished by means of immobilization thought the binding pair as discussed below. However, where the nucleic acid has not been functionalized an alternate means for separation must be found.

Gel Electrophoresis

Applicants have provided a novel separation method involving either gel electrophoresis chromatography or a phase separation method that is rapid and facile and permits the separation of nanotube-nucleic acid complexes into discreet fractions based on size or charge. These methods have been applied to the separation and recovery of coated nanoparticles (as described in 10/622889 incorporated herein by reference) and have been found useful here.

Gel electrophoresis is a commonly used method in biochemistry and molecular biology to separate macromolecules such as proteins and nucleic acids. The gel serves as a sieving medium to separate the macromolecules on the basis of size. In the present invention, the gel can be made from agarose or polyacrylamide. Methods for preparing suitable gels are well known and exemplified in Sambrook, supra, particularly Chapter 6 (entirely incorporated herein by reference). Suitable agarose gels have an agarose concentration between 0.6 and 6% (weight per volume), while suitable polyacrylamide gels have an acrylamide concentration between 3.5 and 20% (weight per volume). It is well know in the art that the concentration of the gel to be used depends on the size of the molecules being separated. Specifically, higher gel concentrations provide better separation for smaller molecules, while lower gel concentrations are used to separate larger molecules. The gel concentration to be used for a given nanoparticle
fractionation can be determined by routine experimentation. The preferred gel of the present invention is a 1% or lower agarose gel.

In order to determine the average particle size of the complexes a densifying agent may be added to an aqueous solution of the complexes. The purpose of densifying agent is to increase the specific gravity of the nanoparticle solution to facilitate loading of the solution into the gel. Suitable densifying agents are well known and include, but are not limited to, glycerol, sucrose, and Ficoll (a nonionic, synthetic polymer of sucrose, approximate molecular weight of 400,000, available from Sigma, St. Louis, MO). The complex solution is then added to the wells in the gel. The complexes migrate according to their apparent molecular weight and size of any particular complex may be determined by using molecular weight standards.

Alternatively the complexes may be separated by two phase separation methods. In this method nanotube-nucleic acid complexes in solution are fractionated by adding a substantially water-miscible organic solvent in the presence of an electrolyte. The amount of the substantially water-miscible organic solvent added depends on the average particle size desired.

A substantially water-miscible organic solvent is herein defined as an organic solvent that dissolves completely in water up to a concentration of at least 80% by volume. Suitable organic solvents include, but are not limited to, methanol, ethanol, isopropanol, dimethyl sulfoxide, tetrahydrofuran, dimethylformamide, dioxane, and acetone. Suitable organic solvents also include mixtures of organic solvents that are completely miscible with each other and that result in a mixture which is a substantially water-miscible organic solvent. Examples of mixed solvents include, but are not limited to, ethyl acetate and methanol; ethyl acetate and ethanol; ethyl acetate and isopropanol; ethyl acetate and acetone; ethyl acetate, dimethylformamide and dimethyl sulfoxide; and ethyl acetate, tetrahydrofuran, and dioxane. The preferred organic solvent is methanol or ethanol. The electrolytes that can be used include, but are not limited to, sodium chloride, sodium phosphate, sodium citrate, sodium acetate, magnesium sulfate, calcium chloride, ammonium chloride, and ammonium sulfate. The divalent metal ion salts appear to work better with nanoparticles that are stabilized with mixed coatings, such as poly(ethylene glycol) and glutathione, than with nanoparticles that are stabilized with single component coatings. The preferred electrolyte is sodium chloride.
Typically, the substantially water-miscible organic solvent is added to give a concentration of about 5% to 10% by volume to precipitate out the largest particles. The complexes are collected by centrifugation or filtration. Centrifugation is typically done using a centrifuge, such as a Sorvall® RT7 PLUS centrifuge available from Kendro Laboratory Products (Newtown, CT), for about 1 min at about 4,000 rpm. For filtration, a porous membrane with a pore size small enough to collect the complex size of interest can be used. Optionally, sequential additions of the substantially water-miscible organic solvent are made to the complex solution to increase the solvent content of the solution and therefore, precipitate out complexes of smaller sizes.

Ion Exchange Chromatography

Alternatively it has been found that ion exchange chromatography is a suitable method for separation of the nucleic acid associated CNT’s as well as providing the added advantage of separation on the basis of uniform diameter and chirality of the CNT, or on the basis of whether the CNT’s are substantially metallic or semiconducting in nature.

A variety of ion exchange media are suitable for this mode of separation including, anion exchange resins such as choestyramine, diethylaminoethyl cellulose, diethylaminoethyl sephadex, and diethylaminoethyl sepharose resins, as well as cation exchange resins such as cellulose phosphate, CM cellulose, CM sephadex and dowex resins, where any strong ion exchange media having been functionalized to contain quarterized polyethyleneimine groups is preferred. Elution of ion exchange column is typically effected with salt solutions (i.e., NaCl, NH₃SO₄, NaSCN) according to increasing gradients. Generally the elution solutions are buffered and range in pH from about 5.0 to about 8.0 where about 7.0 is preferred. Methods of adjusting gradients for optimal separation as well as defining the most appropriate ion exchange media are well known in the art and are generally addressed in Randall, Cynthia, HPLC (2nd Edition) (2001), 287-313.

Editor(s): Swadesh, Joel K. Publisher: CRC Press LLC, Boca Raton, Fla.

Analysis of Separated Nanotube-Nucleic Acid Complexes

Solution characterization of singly dispersed CNT by optical spectroscopy provide a convenient tool to analyze detailed composition of bulk CNT samples, yielding distributions in both tube diameter and chiral angle (O’Connell, M. J. et al., Science 297, 593-596 (2002); Bachilo, S. M. et al., Science 298, 2361-2366 (2002)). The simplest theoretical treatments (Saito, R. et al., Physical Properties of Carbon Nanotubes (Imperial College
Press, London, 1998)) predict that the optical transition wavelengths of semiconducting and metallic nanotubes depend linearly on diameter according to the relations

\[ \lambda_{S1} = \frac{hc}{} \sqrt{2} a_{cc} \gamma_0 \]

\[ \lambda_{S2} = \frac{hc}{} \sqrt{4} a_{cc} \gamma_0 \]

\[ \lambda_{M1} = \frac{hc}{} \sqrt{6} a_{cc} \gamma_0 \]

where \( d \) is the tube diameter, \( a_{cc} \) is the C-C bond distance, \( \gamma_0 \) is the interaction energy between neighboring C atoms, \( h \) is the Planck constant and \( c \) the speed of light. Although deviations from such simple description are predicted by theory and observed experimentally, they nevertheless provide a guidance to decipher information obtained from optical absorption spectroscopy.

The ability to wrap or adsorb a charged molecule (e.g., ssDNA) onto a nanotube (or any nanowire) provides materials and methods by which one can separate the latter according to electronic structure and diameter. The energetics of binding can lead to specificity in several ways. For example, van der Waals interactions, hydration and entropic terms likely depend on tube diameter, and possibly on chirality. Charges on the molecule polarize the tube. This last effect is particularly sensitive to the electronic structure of the tube; metals are far more polarizable than semiconductors. This fact has two consequences: (a) the binding of the molecule to metallic tubes is stronger than to semiconductors, and (b) the resulting electric field outside the hybrid is weaker when the core is metallic than when it is a semiconductor. Both can be used to selectively separate tubes by electronic structure. In the following sections we quantify these ideas. Experimentally, it can be demonstrated that one can use ion-exchange liquid chromatography to separate CNT’s according to their diameters and electronic properties.

**Immobilization / Association of Nanotube-Nucleic Acid Complexes**

Once formed the dispersed and/or separated nanotube-nucleic acid complexes containing ligands or binding pairs may be either immobilized on a solid substrate or rationally associated with other complexes in a process of nano-device fabrication.
For example, where the nucleic acid molecule has been functionalized with a first member of a binding pair, it may be immobilized on a solid support containing a second member of the binding pair. Solid supports suitable for such purposes are common and well known in the art and include but are not limited to, silicon wafers, synthetic polymer supports, such as polystyrene, polypropylene, polyglycidylmethacrylate, substituted polystyrene (e.g., aminated or carboxylated polystyrene; polyacrylamides; polyamides; polyvinylchlorides, etc.), glass, agarose, nitrocellulose, nylon, nickel grids or disks, silicon wafers, carbon supports, aminosilane-treated silica, polyllysine coated glass, mica, and semiconductors such as Si, Ge, and GaAs. Method for incorporating binding pair members onto the surface of solid supports is also and advanced art (see for example, *Immobilized Enzymes*, Inchiro Chibata, Halsted Press, New York (1978) and Cuatrecasas, *J. Bio. Chem.*, 245: 3059 (1970)).

Preferred binding pairs for immobilization of the present complexes are biotin/streptavidin or biotin/avidin.

Alternatively it will be possible to immobilize the complexes of the invention by direct interaction between nucleic acid molecules. For example, nucleic acid molecules in the dispersion sample may be chosen or designed to incorporate a specific hybridization domain that will hybridize with a specific complementary sequence. Nucleic acids having the complement sequence to the hybridization domain may be placed on the surface of the support and the complex captured by hybridization. Immobilization of nucleic acids to a solid support is common and well known in the art and may be accomplished for example using ultraviolet irradiation, baking, capillary transfer or vacuum transfer. Examples of nucleic acid immobilization on nitrocellulose and other suitable supports are given in Kalachikov, S. M., et al., *Bioorg. Khim.*, 18, 52, (1992) and Nierzwicki-Bauer, et al., *Biotechniques*, 9, 472, (1990).

It will be appreciated by the person of skill in the art that the above mentioned interactions that enable the immobilization of the complexes of the invention may be equally employed to associate individual complexes with other complexes in a specific fashion. For example, a biotin containing complex may associate with a streptavidin containing complex or the hybridization domain of one nucleic acid molecule may be designed to bind to a similar domain on another complex.

In this fashion complexes may be rationally associated or immobilized to facilitate device fabrication.
Metallization of Associated or Immobilized Complexes

Where it is desired to use the complexes of the invention, either immobilized or associated in a particular conformation, as electrical conductors, the complexes can be metallized according to methods well known in the art. So for example it is well known in the art to sputter or evaporatively coat nanoscale structures with metals such as gold or platinum, respectively, in order to stabilize and image the structures (Schnur, J.M. et al. (1987) *Thin Solid Films* 152, 181-206; Markowitz, M. et al. (1992) *Thin Solid Films* 224, 242-7), raphidosomes (Pazirandeh, M. et al. (1992) *Biomimetics* 1, 41), DNA (Braun, E. et al. (1998) *Nature* 391, 775-8) and microtubules (Kirsch, R. et al. (1997) *Thin Solid Films* 305, 248-253).

Metallized nanotube-nucleic acid complexes of the invention will be useful as nanowires or molecular interconnects in the fabrication of nanodevices. It will be expected for example that these metallized structures could be arrayed in a crossed arrangement, where the distance between adjacent complex can be controlled by the potential difference between them, then the array could be used as a non-volatile memory device similar to that proposed by Leiber and collaborators (Rueckes T. et al. (2000). *Science* 289, 94-97) for carbon nanotubes. Semiconducting complexes could find use in 3-terminal gated devices which can be used directly as switches, amplifiers or logic gates. By linking the metallized complexes with organic semiconductors, it may be possible to develop 2-terminal switching devices, showing, for example, negative differential resistance (e.g. Fan et al. (2002) *JACS* 124, 5550-5560). Other possible applications include point sources for emission in field-emission display devices and as conductive inclusions in conductive coatings.

**EXAMPLES**

The present invention is further defined in the following Examples. It should be understood that these Examples, while indicating preferred embodiments of the invention, are given by way of illustration only. From the above discussion and these Examples, one skilled in the art can ascertain the essential characteristics of this invention, and without departing from the spirit and scope thereof, can make various changes and modifications of the invention to adapt it to various uses and conditions.

**GENERAL METHODS**

The meaning of abbreviations is as follows: "h" means hour(s), "min" means minute(s), "sec" means second(s), "d" means day(s), "mL" means milliliters, "L" means liters.

**General Procedure for Dispersion of Carbon Nanotubes by Nucleic Acid**

The single wall carbon nanotubes were made by the HiPCO process, either purified or unpurified, and were purchased from CNI (Houston, Texas). The materials were used as received without further modification. Single-stranded DNA (ssDNA) oligonucleotides were purchased from Integrated DNA Technologies, INC (Coralville, IA). Yeast tRNA was purchased from Sigma (St. Louis, MO). RNA homopolymers poly(A), poly(C) and poly(U) were purchased from Amersham Biosciences (Piscataway, NJ). In a typical experiment, 10 mg of CNT were suspended in 10 mL of 1XSSC buffer (0.15M NaCl, 0.015M sodium citrate), then sonicated for 2 min. with a TORBEPO 130-Watt Ultrasonic Processor (Cole-Parmer Instrument Company, Vernon Hills, IL). Nucleic acids were dissolved in H2O to give a final concentration of 10 mg/mL. 50 μL of the CNT suspension and 5 μL of 10 mg/mL nucleic acid solution were added to 200 μL of H2O to give a final volume of 255 μL. The mixture was sonicated for 3 min., followed by 90 min of centrifugation at 16,000g (Biofuge fresco, Kendro Laratory Products, Newtown, CT). The supernatant was then removed for spectroscopic measurement. Absorption spectra from 400 nm to 900 nm were recorded using Ultrospec 3300 UV-Vis spectrophotometer (Amersham Biosciences, Piscataway, NJ). The 730 nm peak was taken as a measure of the yield of the dispersion process.

**EXAMPLE 1**

Dispersion of Carbon Nanotubes by Single-stranded DNA: Sequence Dependence

In this experiment, purified single wall carbon nanotubes (HiPCO) were used. Following the procedure described above, the dispersion efficiency of a variety of sequences was examined. The sequences used include:
(N)90: where N stands for a random mix of G, A, T and C, the total length is 90 bases;
(C/A)90: where C/A stands for a random mix of C and A, the total length is 90 bases;
(G/A)90: where G/A stands for a random mix of G and A, the total length is 90 bases;
(G/T)90: where G/T stands for a random mix of G and T, the total length is 90 bases;
(C/T)90, 60, 30: where C/T stands for a random mix of C and T, the total length is 90, 60 and 30 bases, respectively;
C60: a homopolymer of C with 60 bases long;
T60: a homopolymer of T with 60 bases long.
Results from above sequences are shown in Figure 1. In general, the less G-C and T-A base-pairing interactions in a sequence, the higher the dispersion efficiency.

EXAMPLE 2
Dispersion of Carbon Nanotubes by Single-stranded DNA: Effect of Sonication

In this experiment, purified single wall carbon nanotubes (HiPCO) were used. Following the procedure described above, the dispersion efficiency of a particular sequence (C/T)30 as a function of duration of sonication examined. As shown in Figure 2, the longer the sonication, the more the dispersed CNT. This was found to be true for other sequences tested when sonication time is shorter than 15 minute. Beyond 15 min. duration, there is no further improvement in dispersion efficiency.

EXAMPLE 3
Dispersion Carbon Nanotubes by Single-stranded DNA: Effect of Denaturants

In this experiment, purified single wall carbon nanotubes (HiPCO) were used. Following the procedure described above, the dispersion efficiency of a particular sequence (C/T)30 in the absence and presence of nucleic acid denaturant formamide. As shown in Figure 2, denaturant improves the final yield by a factor of 3 for this particular sequence. Other experiments showed that denaturants (such as formamide and urea) in general improve dispersion efficiency by 2 to 3 fold.

EXAMPLE 4
Dispersion of Carbon Nanotubes by Single-stranded DNA vs. SDS
In this experiment, purified single wall carbon nanotubes (HiPCO) were used. Two dispersion tests were set up in this Example, following general procedures described above. In the first one, (C/T)30 DNA was used. In the second sample, no DNA was used and 175 μl of H₂O plus 25 μl of 10% SDS (sodium dodecyl sulfate, purchased from Sigma, St. Louis, MO) aqueous solution were added in place of 200 μl of H₂O. As shown in Figure 3, the yield from DNA-dispersed CNT was at least five times higher that from SDS-dispersed CNT. Moreover, the degree of dispersion by DNA is higher that by SDS, as judged by the line-width of absorption peaks.

**EXAMPLE 5**

**Dispersion of Carbon Nanotubes by RNA**

In this experiment, unpurified single wall carbon nanotubes (HiPCO) were used. Dispersion experiments were performed as described above, except that RNA (poly A, poly C, poly U and yeast tRNA) were used. The results shown in Figure 4 indicate that RNA can also disperse CNT.

**EXAMPLE 6**

**Dispersion Carbon Nanotubes by Single-stranded DNA: Effect of Sonication Temperature**

In this experiment, unpurified single wall carbon nanotubes (HiPCO) were used. Following procedure described above, we have examined dispersion efficiency of a particular sequence (C/T)30 as a function of sonication temperature. In the first test, sonication was done as described in above. In the second test, sample was prepared the same way as the first test, except that sample was held in an ice-water bath during sonication. The yield of carbon nanotube dispersion from the second test was determined to be four times higher than the first test. This temperature effect was found to be true for other sequences as well.

**EXAMPLE 7**

**Precipitation of DNA and RNA Dispersed Carbon Nanotubes**

In this experiment, both purified and unpurified single wall carbon nanotubes (HiPCO) were tested. Dispersion experiments were performed as described above. In a typical experiment, 200 μl of either DNA or RNA dispersed carbon nanotube solution at concentration of ~ 0.1 mg/ml were mixed with equal volume of ethanol. Immediately after mixing, aggregation of carbon nanotube in solution was visible. After 1 min centrifugation at 16,000g (Biofuge fresco, Kendro Laporatory Products, Newtown, CT), the supernatant
solution became clear and all the carbon nanotubes went to the pellet. It was found that the pellet could be re-suspended into aqueous solution again.

**EXAMPLE 8**

**Immobilization of DNA Dispersed Carbon Nanotubes through Biotin-Streptavidin Interaction**

In this experiment, unpurified single wall carbon nanotubes (HiPCO) were used. Dispersion experiments were performed as described in above. Two ssDNAs were tested: 1) random 90-mer (N90 hereafter); 2) random 90-mer with biotinylated 5’ end (biotin-N90 hereafter). Streptavidin-coated agarose beads were purchased from Sigma (St. Louis, MO), and were used as received. Two samples were prepared. Sample 1: 100 μl of the agarose beads were mixed with 100 μl of N90 dispersed carbon nanotube (~ 0.1 mg/ml). After 30 min of incubation, the beads were spun down and washed with 0.5 mL of 1x SSC solution for 3 times. Sample 2: same as Sample 1, except biotin-N90 dispersed carbon nanotubes were used. After washing, one could notice that the pellet from Sample 2 was darker than Sample 1, indicating carbon nanotube binding to the beads. The two samples were analyzed by SEM (Scanning Electron Microscopy). The results shown in Fig. 5A and 5B confirmed that biotin-N90 but not N90 dispersed carbon nanotubes were trapped on the surface of the beads.

**EXAMPLE 9**

**Separation of Different DNA-Carbon Nanotube Species Using Gel Electrophoresis**

The purpose of this Example is to demonstrate that DNA-dispersed carbon nanotubes can be electrophoretically separated according to their diameters.

In this experiment, unpurified single wall carbon nanotubes (HiPCO) and single-stranded DNA T15 were used. Dispersion experiments were performed as described in EXAMPLE 1. 100 microliters of T15 dispersed carbon nanotube at a concentration of ~ 300 micro g/mL was mixed with 10 μL of 50% glycerol, and loaded onto 8 separate wells of a 1% agarose /Tris-Borate-EDTA (TBE) gel (BioWhittaker, Rockland, ME). The gel was immersed in 1X TBE running buffer (89 mM Tris, 89 mM boric acid, 2 mM EDTA, pH = 8.3), and electrophoresis was carried out at a constant voltage of 90 V for 20 min in a Horizon 58 gel box (Life Technologies, Rockville, MD). The gel image was recorded using a HP ScanJet 6300C scanner (Agilent Technologies, Wilmington, DE).
The gel image (one well) resulting from this experiment is shown in Figure 6A. Fraction 1 and 2 of the gel (as labeled in Fig 6A) were cut out and carbon nanotubes trapped inside the gel were eluded by overnight incubation with 0.1 M NaCl solution. Optical absorption spectra (Fig. 6B and C) were then taken for the two eluted fractions. The spectra showed that carbon nanotubes from fraction 1 of the gel have greatly enhanced absorption in the longer wavelength (~ 1500 nm), indicating that bigger diameter carbon nanotubes were enriched in this fraction relative to fraction 2.

EXAMPLE 10
Separation of DNA Dispersed Carbon Nanotubes by Anion Exchange Column Chromatography
This example demonstrates that one can use liquid chromatography to separate DNA dispersed carbon nanotubes. In this experiment, unpurified single wall carbon nanotubes (HiPco) and single-stranded DNA (C/T)60 were used. Dispersion experiments were performed as described in EXAMPLE 1. 500 microliters of (C/T)60- dispersed carbon nanotube at a concentration of ~ 100 micro g/mL was injected into a HQ20 strong anion exchange column mounted on a BioCAD/SPRINT HPLC system (Applied Biosystems, Framingham, MA), and eluted with a linear salt gradient form NaSCN salt gradient in 40 mL volume at a flow rate of 10 mL mL/min (starting solution: 20% 100 mM MES buffer at pH 7, 80% H2O; end solution: 20% 100 mM MES buffer at pH 7, 80% 3M NaSCN). The elution profile was shown in Figure 7A. In Fig. 7B, optical absorption profiles of eluted fractions 39, 41 and 43 were shown. The difference in the absorption profiles indicates that carbon nanotube composition varies from fraction to fraction.

EXAMPLE 11
Dispersion Carbon Nanotubes By Single-Stranded Dna
The procedures used were the same as described above (Example 4) with certain modifications. 1 mg of as-produced HiPco nanotube (Carbon Nanotechnologies Incorporated address, Houston, TX) was suspended in 1 mL aqueous DNA (Integrated DNA technologies, Inc address Coralville, IA) solution (1 mg/ mL ssDNA, 0.1M NaCl, and in some experiments an appropriate pH buffer). The preferred sequence for diameter-dependent separation was GT(n) where n=30 (60 bases in length). The mixture was kept in an ice-water bath and sonicated (Sonics, VC130 PB, Newtown, CT) for 120 min at a power level of 8 W. After sonication, the samples were divided into 0.1 mL aliquots, and centrifuged (Eppendorf 5415C) for 90 min at 16,000g to
remove insoluble material, leaving DNA-dispersed nanotube solutions at a mass concentration in the range of 0.2 to 0.4 mg/mL.

**EXAMPLE 12**

Ion-exchange column separation

A strong anion exchange column HQ20 (Applied Biosystems, Framingham, MA PerSeptive Biosystems is their old name, I changed the name in Ex10 to be consistent.) was chosen because it is packed with resins that are functionalized by quarterized polyethyleneimine, which is expected to bind to the negatively charged phosphate groups of DNA. To increase sample recovery, the top filter of the column was removed. In a typical experiment, a volume of 800 µL of DNA-dispersed carbon nanotubes at a concentration of ~100 µg/mL was injected into the column (4.6 mm by 100mm, 1.8 mL bed volume) mounted on a BioCAD/SPRINT HPLC system (Applied Biosystems), and eluted with a linear salt gradient (0 to 1.8 M NaSCN in 20 mM MES buffer at pH 7) in 80 mL volume at a flow rate of 10 mL/min. Fractions were collected in 0.5 mL aliquots.

**EXAMPLE 13**

Optical Spectroscopy

A common method for determining the chirality and diameter of CNT is optical spectroscopy (O’Connell, M. J. *et al.*, *Science* 297, 593-596 (2002); Bachilo, S. M. *et al.*, *Science* 298, 2361-2366 (2002); (Saito, R. *et al.*, *Physical Properties of Carbon Nanotubes* (Imperial College Press, London, 1998)). These methods were applied herein to determine the diameter and chirality of CNT separated by ion exchange chromatography as described above.

UV-vis–near IR absorption spectra were taken using an optical cell with 10mm path length and 100 µL cell volume. Prior to measurement, fractions were centrifuged using a microcon spin filter YM100 (Millipore), and re-suspended in 100 µL of 0.1 M sodium chloride D2O solution. The starting material and fractions collected from an ion exchange fractionation experiment were measured from 400 nm to 1600 nm (Figure 8). Three regions are identified in Figure 8: $M_{11}$ (400 nm - 600 nm), which correspond to metallic nanotubes; $S_{11}$ (900 nm - 1600 nm) & $S_{22}$ (600 nm - 900 nm), both of which correspond to semiconducting nanotubes. The starting material yielded a spectrum typical of singly dispersed CNT in aqueous solution, with multiple peaks arising from different type of CNTs overlapping across the entire spectrum. In contrast, the spectrum from an early fraction f46 had only
one major peak centered at 980 nm in the S_{11} region, corresponding to the S11 transition from the smallest diameter semiconductor tubes found in HiPCO CNTs. Additionally M_{11} transitions were enhanced, indicating enrichment of metallic tubes. Spectra from later fractions (f48, f50, f52 and f54) showed systematic shift of intensity towards longer wavelength, indicating gradual increase in average tube diameter among these fractions. Simultaneously a decrease in the M_{11} intensities was observed, representing depletion of metallic tubes. Analysis of the spectral data based on Bachilo, supra, indicated that the major peaks in f46, f50 and f54 correlated different semiconducting tubes, having differences in diameter and chirality as shown below in Table 1. In particular fraction 46 contained a narrow distribution of CNT's having a single chirality and diameter, demonstrating that it is possible to separate populations of DNA associated CNT into fractions having discreet distributions based on diameter and chirality.

TABLE 1

<table>
<thead>
<tr>
<th>Fractions</th>
<th>Chirality (n,m)</th>
<th>Diameter (nm)</th>
<th>S11 transition (nm)</th>
<th>S22 transition (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F46</td>
<td>(6,5)</td>
<td>0.76</td>
<td>975</td>
<td>567</td>
</tr>
<tr>
<td>F50</td>
<td>(7,6)</td>
<td>0.89</td>
<td>1122</td>
<td>647</td>
</tr>
<tr>
<td></td>
<td>(8,7)</td>
<td>1.03</td>
<td>1267</td>
<td>728</td>
</tr>
<tr>
<td></td>
<td>(11,3)</td>
<td>1.01</td>
<td>1197</td>
<td>792</td>
</tr>
<tr>
<td>F54</td>
<td>(8,7)</td>
<td>1.03</td>
<td>1267</td>
<td>728</td>
</tr>
<tr>
<td></td>
<td>(9,8)</td>
<td>1.17</td>
<td>1414</td>
<td>809</td>
</tr>
<tr>
<td></td>
<td>(11, 4)</td>
<td>1.07</td>
<td>1372</td>
<td>714</td>
</tr>
<tr>
<td></td>
<td>(13,3)</td>
<td>1.17</td>
<td>1497</td>
<td>760</td>
</tr>
</tbody>
</table>

EXAMPLE 14

Obtaining Individual (n,m) Enriched Carbon Nanotubes by Anion Exchange Column Chromatography

This example demonstrates that one can use liquid chromatography to obtain individual (n,m) type enriched carbon nanotubes. In this experiment, unpurified single wall carbon nanotubes from Southwest Nanotechnologies (SWeNT, Norman, OK) and single-stranded DNA (GT)30 were used. Dispersion experiments were performed as described in EXAMPLE 1. Anion exchange chromatography separation was done as described in EXAMPLE 10. The SWNT tubes have two major type of semiconducting tubes (6,5) and
(7, 5), as was characterized in the literature (Bachilo et al., JACS, 2003, 125, 11186). In Fig. 9, optical absorption profiles of eluted fractions 35, 40 and 50 were shown. The absorption spectrum of f35 has two dominant peaks at 990 nm and 574 nm. These are close to the literature assignment of 975 nm (E11) and 567 nm (E22) for the (6, 5) tubes, but are red-shifted (Bachilo et al., JACS, 2003, 125, 11186). Similarly, peaks at 928 nm and 702 nm can be assigned to (9, 1) tubes (same diameter as (6, 5)); peaks at ~970 nm (overlapping with 990 nm peak ) and 674 nm can be assigned to (8, 3). By intensity comparison, it is clear that f35 is largely enriched with (6, 5) tubes.

**EXAMPLE 15**

**Atomic Force Microscopy**

The sample solution from Example 11 was deposited onto a piece of mica pre-treated with 1 M MgSO4 solution to enhance DNA adsorption, and rinsed with water and dried prior to measurement. Tapping mode was used to acquire the images under ambient conditions (Digital Instruments Dimension 3100, Woodbury, New York). Figure 10 shows an isolated tube with 1 nm diameter, wrapped by DNA with an average spacing of 11 nm. The width of the tube is 1.9nm and the height is of the CNT/DNA= 2.16nm. Figure 11 shows different modes of DNA wrapping on different CNTs.
CLAIMS

What is claimed is:

1. A method for the separation of a carbon nanotube-nucleic acid complex comprising:
   a) providing a carbon nanotube-nucleic acid complex in solution comprising an unfunctionalized carbon nanotube bound to a nucleic acid molecule wherein the solution comprises a densifying agent;
   b) loading the carbon nanotube-nucleic acid complex solution of step (a) on to an electrophoresis gel; and
   c) separating the loaded complexes of (b) by applying an electric field to the gel.

2. A method according to Claim 1 wherein the electrophoresis gel is comprised of materials selected from the group consisting of agarose and acrylamide.

3. A method according to Claim 1 wherein the densifying agent is selected from the group consisting of glycerol, sucrose, and Ficoll

4. A method for the separation of a carbon nanotube-nucleic acid complex comprising:
   a) providing a carbon nanotube-nucleic acid complex in and aqueous solution comprising an unfunctionalized carbon nanotube bound to a nucleic acid molecule;
   b) adding a substantially water-miscible organic solvent to the dissolved complexes of (a) whereby a certain size fraction of the complexes are precipitated; and
   c) collecting the complex precipitate of step (b).

5. A method according to Claim 4 wherein the substantially water-miscible organic solvent is selected from the group consisting of methanol, ethanol, isopropanol, dimethyl sulfoxide, tetrahydrofuran, dimethylformamide, dioxane, and acetone.
6. A method for the separation of a carbon nanotube-nucleic acid complex comprising:
   a) providing a carbon nanotube-nucleic acid complex in an aqueous solution comprising an unfunctionalized carbon nanotube bound to a nucleic acid molecule wherein the nucleic acid portion of said complex is comprised of at least 50% hydrophobic nucleotides;
   b) applying the solution of (a) to an ion exchange media wherein the carbon nanotube-nucleic acid complex becomes associated with the ion exchange media; and
   c) eluting the carbon nanotube-nucleic acid complex from the ion exchange media into discreet fractions.

7. A method according to Claim 6 wherein the ion exchange media is selected from the group consisting of choestryramine, diethyaminoethyl cellulose, diethyaminoethyl sephadex, diethyaminoethyl sepharose resins, cellulose phosphate, CM cellulose, CM sephadex and dowex resins.

8. A method according to Claim 6 wherein the nucleic acid portion of said complex is from about 5 to about 100 bases in length.

9. A method according to Claim 6 wherein the nucleic acid portion of said complex is at least 50% guanine.

10. A method according to Claim 6 wherein the nucleic acid portion of said complex is defined by the general formula (G/T)n wherein n=5-50.

11. A method according to any of Claims 1, 4 and 6 Wherein the nucleic acid molecules are selected from the group consisting of; single stranded DNA, double stranded DNA, RNA and PNA.

12. A method according to either of Claims 1 and 4 wherein the nucleic acid is from about 10 bases to about 1000 bases in length.

13. A method according to and of Claims 1, 4 and 6 wherein the nucleic acid molecule is selected from the group consisting of:
a. An wherein n = 1 – 2000;
b. Tn wherein n = 1 – 2000;
c. Cn wherein n = 1 – 2000;
d. Gn wherein n = 1 – 2000;
e. Rn wherein n = 1-2000, and wherein R may be either A or G;
f. Yn wherein n = 1 – 2000, and wherein Y may be either C or T;
g. Mn wherein n = 1 - 2000, and wherein M may be either A or C;
h. Kn wherein n = 1 - 2000, and wherein K may be either G or T;
i. Sn wherein n = 1 - 2000, and wherein S may be either C or G;
j. Wn wherein n = 1 - 2000, and wherein W may be either A or T;
k. Hn wherein n = 1 - 2000, and wherein H may be either A or C or T;
l. Bn wherein n = 1 -2000, and wherein B may be either C or G or T;
m. Vn wherein n = 1 - 2000, and wherein V may be either A or C or G;
n. Dn wherein n = 1 - 2000, and wherein D may be either A or G or T; and
o. Nn wherein n = 1 - 2000, and wherein N may be either A or C or T or G.

14. A population of carbon nanotubes separated by the method of any one of Claims 1, 4 and 6.

15. The population of carbon nanotubes according to Claim 14 having a uniform diameter.

16. The population of carbon nanotubes according to Claim 14 having a uniform chirality.
17. The population of carbon nanotubes according to Claim 14 wherein the carbon nanotubes are uniformly metallic.

18. The population of carbon nanotubes according to Claim 14 wherein the carbon nanotubes are uniformly semiconducting.

19. The population of nanotubes according to Claim 14 wherein the nanotubes are metallized.
Figure 1

Dispersion of Carbon Nanotubes by Single-stranded DNA
Figure 2

Sonication time and addition of denaturants on dispersion of carbon nanotubes
Figure 3

Dispersion of carbon nanotubes by ssDNA versus surfactant
Dispersion of carbon nanotubes by RNA
5A. Biotin-N90

Immobilization of DNA Dispersed Carbon Nanotubes through Biotin-Streptavidin Interaction

Figure 5
Figure 8. Optical absorption spectra of CNT separated by anion exchange
Figure 9. Optical absorption spectra of CNT separated by anion exchange
Micrograph of Wrapped Nanotube

Avg spacing = 11 nm
Ht of CNT = 1.08 nm
Width of CNT = 1.9 nm
Ht of CNT/DNA = 2.16 nm
Figure 11
Micrograph of Different Modes of DNA Wrapping on Nanotubes