SUNFEST

NSF REU Program
Summer 2006

FORMING VESICLES FROM CARBON NANOTUBES

NSF Summer Undergraduate Fellowship in Sensor Technologies
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ABSTRACT

Carbon nanotubes (CNTs) are essentially layers of graphite seamlessly wrapped into cylinders. They have shown great potential in a wide variety of applications. One prospect in bioengineering is to utilize CNTs for drug delivery by forming micelles or vesicles from aligned tubes. These structures would essentially be spherical nanocontainers with short carbon nanotube arrays forming the wall of the chamber. The nanotubes that constitute these arrays can be made amphiphilic by functionalizing only one end of the tube with a hydrophilic molecule, since CNTs are naturally hydrophobic. Amphiphilic molecules naturally configure so that their nonpolar ends are away from aqueous solution (in the core of the structure) while the polar ends are next to the aqueous solution (on the outside surface). Nanotubes that have been functionalized with a polar molecule on one end could be influenced to aggregate into such arrangements by altering the dimensions of the tubes, since short and straight amphiphilic nanotubes have a good chance of forming micelles spontaneously. The nanotubes would mimic the behavior of phospholipids in water, self-assembling into micelles in aqueous solutions. If successfully developed, these micelles could be used for transport of poorly soluble drugs, such as many anticancer agents. Vesicles, which have a bilayer membrane of nanotubes, could also be formulated, and could be used to transport water-soluble drugs since both the inner and outer walls would be hydrophilic.

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1. INTRODUCTION

In recent years, new drug delivery systems have been developed that are more efficient, safer, and more beneficial than the traditional forms of drug administration. Such new approaches have allowed novel therapies to be established that can be used to treat conditions that have been previously incurable. One of the most prominent drug delivery methods is the entrapment of drugs in small spherical containers called vesicles which can then be injected into the bloodstream of a patient. This mechanism can be used to deliver medicine directly to the site in need of treatment, and has been specifically tailored to target cancerous tumors.

Vesicles can be made of substances such as proteins, carbohydrates, and synthetic polymers, but vesicles made from lipids, called liposomes, are by far the most widely studied. These structures are generally very tiny, their dimensions being on the nanoscale. They exhibit remarkable properties such as biocompatibility, degradability, and nontoxicity that allow them to travel with ease throughout a patient's body. However, existing vesicles are not without their problems, such as the poor stability of liposomes in vivo [17]. In this paper, carbon nanotubes will be introduced as a possible alternative in creating vesicles to transport drugs, instead of lipids and polymers.

The goal of this project was to investigate how an artificial vesicle could be developed from carbon nanotubes in order to be used to encapsulate and transport drugs within a patient's body. Nanotubes would need to align parallel to each other (just as lipids do) to make up the membrane. In order for this to be achieved, the carbon nanotubes would need to mimic the behavior of phospholipids, which make up naturally occurring vesicles found in cells. Phospholipids are amphiphilic molecules, that is, they have a hydrophobic (water-fearing) region and a hydrophilic (water-loving) region. Because the two ends of phospholipids have different polarities, they spontaneously assemble into different phases when placed in aqueous solutions so that they hydrophilic ends are near the water and the hydrophobic ends are away from the water. To form such phases from carbon nanotubes, they too must be made into amphiphilic molecules so that self-assembly is made possible. This can be achieved by functionalizing one end of the nanotubes with a hydrophilic molecule, since carbon nanotubes are intrinsically hydrophobic.

Once carbon nanotubes have been functionalized on one end with a polar molecule, experiments can be run to determine what types of conditions are most preferable for the nanotubes to assemble into aggregates such as bilayers, micelles, and vesicles. The dimensions of the nanotubes as well as their concentration in solution can be altered in order to observe how these variables affect what phase the nanotubes will form in aqueous solutions. Also, different surfactants can be added to the solution to better disperse the nanotubes and establish whether they aid the formation of aggregates from carbon nanotubes.

2. BACKGROUND

2.1 Micelles and Vesicles

Naturally occurring micelles and vesicles are made up of phospholipids, which are amphiphilic molecules consisting of a water-loving (hydrophilic) region and a water-fearing (hydrophobic) region. These amphiphilic molecules spontaneously aggregate into structures such as micelles, inverse micelles, bilayers, and vesicles when placed in aqueous solutions such as water in order to keep the hydrophobic regions hidden from water and the hydrophilic regions near water. These types of formations are commonly found in animal cells: a bilayer forms the cell membrane as well as organelle membranes, and vesicles are used to transport substances in and out of the cell, typically to either excrete waste or to import nutrients. Because of the tiny size of typical vesicles inside a cell (about 20-50nm), and their inherent function to hold and transfer various materials, they are the perfect candidates for drug deliver systems [0].

Employing micelles and vesicles made of various materials as drug carriers is currently an area of intense research. These types of structures have many attractive properties such as good biocompatibility, small size, and high stability both in vitro and in vivo [1]. They can also be made to have other, more specific, characteristics including slow degradability or reaction to certain stimuli (temperature, pH, etc.). The carriers should also be long circulating, so that they can "slowly accumulate in pathological sites with affected and leaky vasculature (such as tumors, inflammations and infracted areas) via the enhanced permeability and retention effect (EPR) and enhance drug delivery in these areas" [3]. In this way micelles and vesicles can be used to transport drugs, such as anti-cancer agents, to a target site such as a tumor.

Since micelles have an interior that is hydrophobic, they can be used to carry many poorly soluble pharmaceuticals. If the drug to be transported is hydrophilic, liposomes (artificial vesicles) can be created. These structures have a bilayer membrane, so that hydrophilic tails make up both the outside and inside surfaces while the hydrophobic regions are trapped in the middle of the membrane. Images of a micelle and a liposome are shown below:

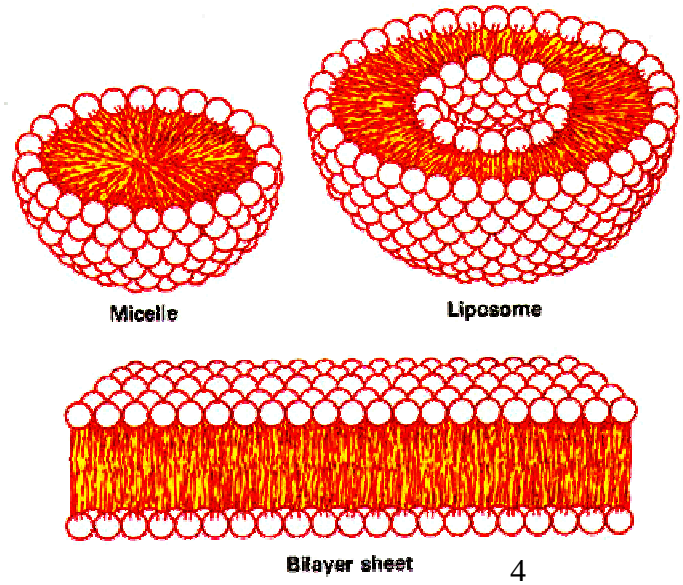


Figure 1 – Illustration of the different phases of amphiphilic molecules

Amphiphilic molecules can also spontaneously form straight bilayer sheets, also shown above. Which of these three configurations are actually formed when amphiphilic molecules are placed in water depends on several conditions, the main ones being amphiphilic molecule concentration and the actual dimensions of the molecules.

Although the molecules used to form micelles are amphiphilic, they will still disperse in water in very low concentrations. The onset of formation of micelles is called the *critical micelle concentration* (CMC); when the concentration is increased above this value, the number of micelles formed increases. When the concentration reaches very high values, an inverted micelle phase can be formed, in which the hydrophobic regions are actually on the outside of the sphere and the little water present in solution is actually trapped inside the core of the micelle [0].

The shape and dimensions of the amphiphiles have a lot do with what type of phase will be formed in an aqueous solution. When the cross-section of the hydrophilic region of the amphiphile is large compared to that of the hydrophobic area, the hydrophobic regions will bundle in the core of the micelle since it is easy for them to fit all together, and a regular micelle will be formed. When the two regions are close to the same size, it is likely that a bilayer will be formed because it is not easy for either portion to cramp together in the center. If the hydrophilic part of the amphiphile is too small, an inverted micelle may form (as mentioned above) since the hydrophilic parts can fit together in the core while the larger hydrophobic regions will be on the outside of the micelle [30].

Both the concentration and shape of the amphiphiles in an aqueous solution tie in with the energy considerations that really determine which phase is most stable in a particular environment. Forming micelles reduces entropy, an unfavorable operation, since order is being made from a disorganized solution. However, it is favorable when there is minimal energy present, which occurs when a micelle is formed since the hydrophobic regions are away from water while the hydrophilic parts are near the water. Therefore, the respective amounts of entropy and energy must be balanced for micelles, or other aggregates, to form in a given situation [0].

By experimenting with different concentrations of amphiphilic molecules and by varying their dimensions, certain conditions can be established that would be preferable for the formation of vesicles (for the transport of hydrophilic drugs) or micelles (for hydrophobic drugs) in aqueous solutions. One anti-cancer drug that can be potentially used is y-Secretase Inhibitor IX, which is hygroscopic, meaning it attracts moisture [Appendix A]. In this case, a vesicle would be needed to hold and transport this drug since it would behave like a hydrophilic substance. However, many other anti-cancer agents are intrinsically hydrophobic, so micelles that could be formed to carry such substances would also be of great benefit [1].

This project's goal was to learn more about the conditions that would allow carbon nanotubes to form such aggregates as micelles and vesicles. The notion of utilizing micelles and/or vesicles to transport various drugs has been investigated rather

thoroughly, but most of these structures have been formulated from polymers, not carbon nanotubes. In these cases, polymer monomers have been made into amphiphilic molecules so that they will aggregate into polymeric micelles in aqueous solutions, with their hydrophobic regions in the core of the micelle and their hydrophilic tails toward the water [3]. These polymeric monomers are made to mimic the behavior of phospholipids, which is what will be attempted here with carbon nanotubes. Since carbon nanotubes are intrinsically hydrophobic, hydrophilic molecules will need to be attached at one end of each nanotube to make them into amphiphilic molecules. If this can be accomplished, the nanotubes will function in a self-assembling system when placed in aqueous solution by forming spontaneously under the right conditions [3, 4].

Carbon nanotubes are more suitable materials for this purpose than are polymers or other molecules for a few reasons. The intrinsic properties of nanotubes provide one advantage, and the mechanisms of drug release from the vesicle/micelle once it arrives at its target site provide another. The small size of carbon nanotubes allows them to form vesicles/micelles that could travel anywhere throughout the body without much obstruction. Since carbon nanotubes "are of the size where cells do not recognize them as harmful intruders", they are able to enter cells with ease [19]. The dimensions of most cells are in the micrometer range and the space within them is very congested, so ideal drug delivery systems must be in the nanometer range in order to properly interact with or enter a cell [20]. Also, nanotubes are very stiff and exceptionally strong, yet have remarkable flexibility and can resist fracture [19]. The rigidity of their structure would make aggregates formed from them very stable, with the ability to withstand harsh conditions present within the body [18, 33].

However, some may argue that these properties can be accomplished with certain types of polymers, or combinations of polymers. So the deciding factor is really how these micelles/vesicles will rupture at the target site and release their contents. The structures that have currently been made from polymers either degrade over time, which means micelles/vesicles that have not reached the target site will deliver their contents wherever they are in the patient's body, or are ruptured by a change in pH. This can be harmful for the patient's body since the pH balance of the blood is altered as a result [4]. Vesicles or micelles that are made from carbon nanotubes, however, can be made to rupture when a specific stimulus is applied, such as increased temperature [30]. This will avoid the problem of vesicles decomposing during delivery and releasing drugs that could be toxic. The heat can be applied directly to the tumor region using an infrared light, which is not harmful to the patient's body since skin and other biological systems are transparent to it [10]. This technique is also beneficial since it does not require the pH balance of the patient's body to be modified. Because carbon nanotubes intrinsically absorb infrared light, they can be optically stimulated to carry out different functions [10]. In this case, the carbon nanotubes would be heated up and caused to disassemble by the infrared light source, but no harm would be done to the healthy regions of the patient's body.

It is clear that if vesicles can be developed from carbon nanotubes, there will be many opportunities to make advances in drug delivery systems. Transporting

pharmaceuticals in vesicles made of nanotubes will provide more precision in controlling the structure and organization of drug-delivery mechanisms [20]. But forming these vesicles may be a difficult task, since the properties of carbon nanotubes are still being thoroughly investigated. In order to understand how to approach the venture of formulating vesicles or micelles, the structure and characteristics of carbon nanotubes must first be recognized.

2.2 Carbon Nanotubes

A carbon nanotube is essentially a sheet of hexagonally arranged carbon atoms (a sheet of graphite, or graphene sheet) rolled seamlessly into a cylinder, as pictured below:

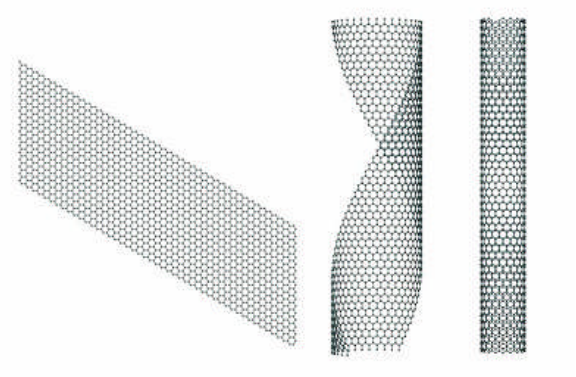


Figure 2 – Illustration of a graphene sheet being rolled into a carbon nanotube

A graphene sheet can be rolled into a cylinder in several ways, leading to different orientations of the lattice with respect to an arbitrary tube axis. These varying configurations are referred to as chiralities, and there are three types that nanotubes are generally grouped in: armchair, zigzag, and chiral nanotubes [33]. Carbon nanotubes (CNTs) can also be pictured as a hollow sphere of carbon atoms, or fullerene, that has been stretched along one axis to produce a cylinder that is capped with a hemisphere on each end. The three different types of nanotubes, along with their respective fullerenes, are shown below:

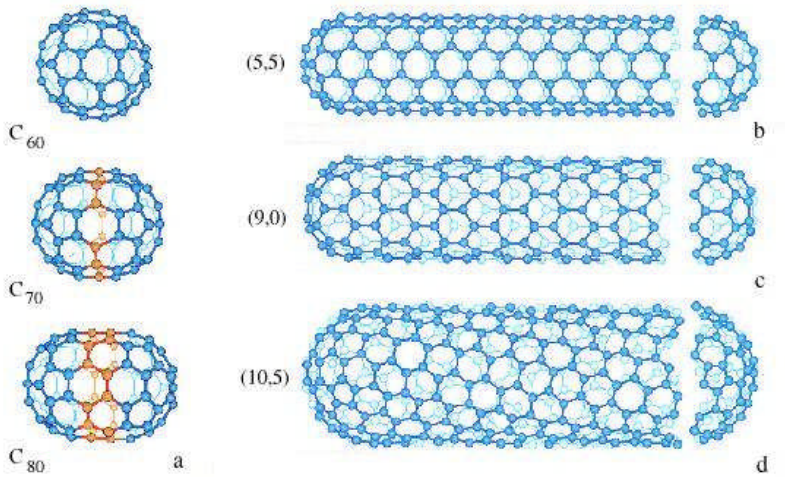


Figure 3 – Image of armchair, zigzag, and chiral nanotubes (top to bottom on the right) and their corresponding fullerene forms (top to bottom on the left)

The varyin erties change as ıchair

nanotubes are metallic, whereas zigzag and chiral nanotubes can be either metallic or semiconducting. The configuration of a nanotube can be defined in terms of an integer pair (*n*, *m*), which characterizes the tube's direction and diameter by the following equations:

$$d = [a \sqrt{(m^2 + mn + n^2)}] / \prod$$

$$\theta = \arctan [-(\sqrt{3}n) / (2m + n)]$$

where d is the diameter, a is the lattice constant in the graphene sheet and θ is the chiral angle of the carbon nanotube. The relation between n and m describes the three categories of nanotubes: 1) armchair → $n = m$ and chiral angle is 30 degrees, 2) zigzag → $n = 0$ or $m = 0$ and chiral angle is 0 degrees, and 3) chiral → other values of n and m and chiral angles between 0 and 30 degrees [33, 34]. Example integer pairs for each of the nanotube types are shown in Figure 3.

Besides the varying chiralities and diameters of nanotubes, there are also two major types of CNTs: single-walled nanotubes (SWNTs) and multi-walled nanotubes (MWNTs). Single-walled nanotubes consist of only one graphene cylinder, while multi-walled nanotubes have many concentric graphene cylinders nestled within one another and can have diameters much larger than single-walled tubes [23]. SWNTs typically have a diameter of about 1.4 nm, which is very close to the diameter of an ideal (10, 10) carbon nanotube. The spacing between the concentric layers in a MWNT is about 0.34 nm, which is close to the interlayer distance in graphite [18, 19, 33]. Examples of single-walled and multi-walled nanotubes are pictured below to allow for contrast:

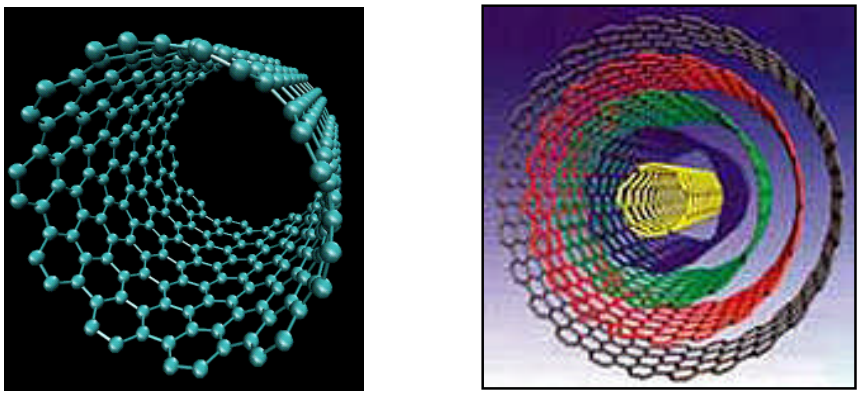


Figure 4 – Cross-sectional images of a SWNT (left) and a MWNT (right)

Both single-walled and multi-walled nanotubes are insoluble in most solvents due to the great hydrophobicity of carbon [6]. Also, strong van der Waals forces between adjacent nanotubes compel them to bunch together in clusters or ropes [2, 22, 36]. Hence, both types of CNTs will bundle together in solution, especially if the solution is polar or aqueous [7]. In order to allow the manipulation of nanotubes, many steps have to be taken to disperse the nanotubes since they will clump together otherwise. Dispersion in various solvents is an important step to enhance the performance of CNTs, and usually

involves techniques such as ultrasonication and centrifuging [2]. Other mechanisms have been described that use polymers to "wrap carbon nanotubes and render them soluble in water or organic solvents", but this technique is not used in this project [5].

Solubilization of carbon nanotubes in different solutions can be achieved by functionalization with certain molecular groups or by adding surfactants to the solution [9, 31, 32]. Functional groups such as amines, alkylaryl amines, or carboxylic acids allow CNTs to be dissolved in various solvents [5, 21]. Numerous surfactants can be used to produce "stable aqueous dispersions" of carbon nanotubes by "coating the nanotubes and increasing the surface interaction between the nanotubes and the water" [32]. Sonication can also be used in combination with functionalization and surfactants in order to maximize the dispersion of CNTs in solution. Improved solubility of carbon nanotubes increases their ability to be processed and allows their unique properties to be coupled with other materials to produce innovative applications in a variety of fields [21]. Functionalization of nanotubes and coating with surfactants can also be used to achieve biocompatibility, which is especially important for applications such as drug delivery systems that will take place in vivo [8].

3. ONE-END FUNCTIONALIZATION

In order for carbon nanotubes to have the ability to self-assemble in aqueous conditions, a hydrophilic region must be introduced to one end of the nanotube so they become amphiphilic molecules. Since spontaneous self-aggregation is best known in phospholipids, carbon nanotubes will be used to mimic the unique behavior of these molecules.

Phospholipids are made up of two fatty acid tails, which are hydrophobic, and phosphate head, which is hydrophilic. This structure makes the molecule amphiphilic, and they naturally assemble to keep their tails away from water and their heads near water [0, 4]. To give carbon nanotubes the same feature, a hydrophilic molecule (or numerous molecules) needs to be attached to one end to mimic the phospholipids head, and the nanotube itself will act as the hydrophobic tail. The structure of a phospholipid is compared to a theoretical model of a functionalized nanotube below:

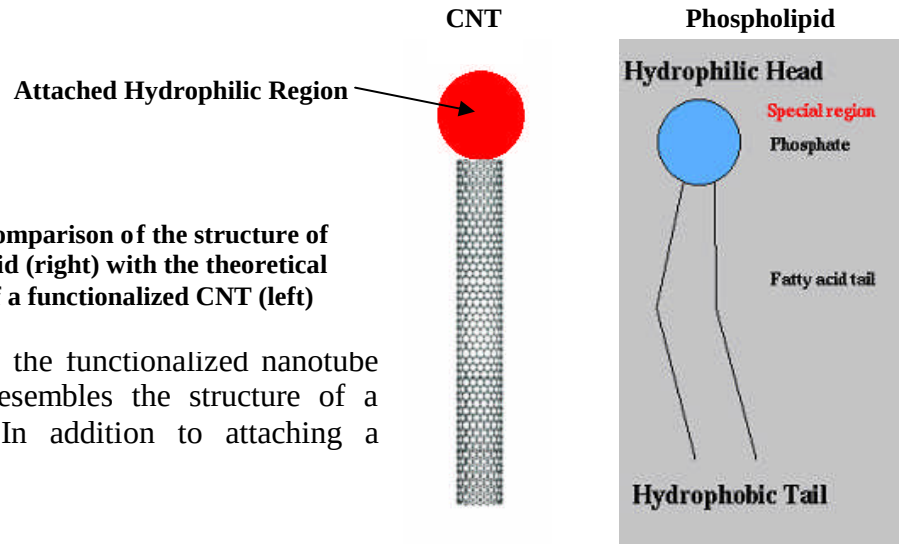


Figure 5 – Comparison of the structure of a phospholipid (right) with the theoretical structure of a functionalized CNT (left)

Clearly, the functionalized nanotube very closely resembles the structure of a phospholipid. In addition to attaching a

hydrophilic group to one end of the tube, the nanotubes should also be as straight as possible in order for them to align next to each other when forming a micelle, vesicle, or bilayer. Because nanotubes ordinarily "have very small diameters and large length, they have very high aspect ratios" and are therefore "susceptible to structural instability" [19]. This property (along with the strong van der Waals attractions) causes CNTs to bend and twist around each other, resembling thin hairs that have been entangled into networks [22]. Hence, if straight nanotubes are desired, the CNT dimensions need to be modified to lower the aspect ratio by increasing the diameter and shortening the length [16]. Specifying the length and diameter of carbon nanotubes is still a methodology that is being perfected, so it is difficult to control the exact proportions of the nanotubes. Nanotubes can be cut into smaller portions by utltrasound, but the broken pieces are not uniform in length and would not be well-suited to form aggregates such as micelles and vesicles [18]. In order to obtain carbon nanotubes of uniform dimensions, they are grown in parallel, aligned arrays on a substrate. This method allows more precision in controlling the length and diameter of the nanotubes. Specifically, the size of the catalyst particle at the tip of the nanotube determines the diameter of the tube, and the growth process can simply be stopped when the desired length is reached [29]. An image of a carbon nanotube array is shown below:

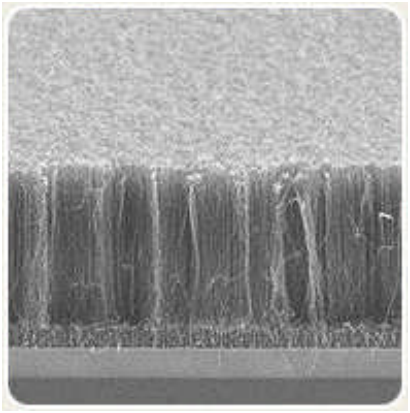


Figure 6 – An aligned array of carbon nanotubes grown on a silicon substrate

Since nanotube dimensions are on such a tiny scale, it becomes very difficult grow arrays of tubes that have a length smaller than about 1 micron (1000nm), purely because it becomes challenging to work with precision. The diameters, however, can be substantially smaller, ranging anywhere from 5-100nm, depending on the size of the catalyst particle used to grow each tube [29]. In order to acquire straight and uniform nanotubes, the diameter to length ratio should be relatively small so that the tubes are short and wide. Since the smallest length that can be obtained from the process is 1000nm, the diameter was chosen to be about 100nm, so that a ratio of 1:10 would be produced. Making the nanotubes short and thick would ensure that they would be straight and also would make them better able to align next to each other in different patterns [16]. Also, let it be clarified that the CNTs used will be multi-walled nanotubes since the diameter necessary is quite large and could not be achieved with single-walled tubes [23]. This is also an advantage because a tube with more walls has superior resistance to bending than one with just one wall [12].

The next step was to determine what molecule, or molecules, to attach to the ends of the nanotubes. The conditions that had to be met were that the molecule be hydrophilic and that it would bind relatively easily to the carbon structure of the CNTs. Numerous hydrophilic molecules were found that have been frequently attached to carbon nanotubes for various purposes. These included the hydroxyl group (-OH), the carboxyl group (-COOH), poly(*m*-aminobenzene sulfonic acid) (PABS), and polyethylene glycol (PEG) [3, 5, 8]. Since both PABS and PEG molecules are polymers, it was decided that they should not be used because their effects on how the nanotubes would aggregate together were unknown. The hydroxyl group was also disregarded due to the fact that this molecule reacts so readily with water and might separate from the carbon nanotubes when they are placed in aqueous solution. Therefore, it was decided that the carboxyl group would be used to functionalize the ends of the nanotubes because of its hydrophilic properties, easy attachment to CNTs, and its frequent and customary applications.

Nanotubes that have been functionalized on just one end with a hydrophilic molecule, such as carboxylic acid (COOH) are not readily available. Because the chemistry behind one-end attachment is rather complex (the process is described in the next section), the decision was made to purchase nanotubes that have already been functionalized and put into solution. Many commercial companies were contacted; the majority of them did not know how to approach the task of attaching a molecule on just one end of the nanotube. Certain companies did offer functionalization, but this was usually just the addition of molecules all over the nanotubes – sidewalls as well as ends – accomplished simply by immersing the nanotubes in different substances, such as acids [11, 21]. Finally, a vendor, NanoLab, Inc., was found that was willing to perform a technique that would leave only one set of ends of the CNTs functionalized with carboxyl groups. The price quotations for the nanotubes can be found in Appendix B, and two 15x15mm2 arrays (to be dispersed in distilled water) were ordered based on the third quotation (page 31) to take advantage of the discount offered when numerous arrays were ordered.

3.1 Chemical Process

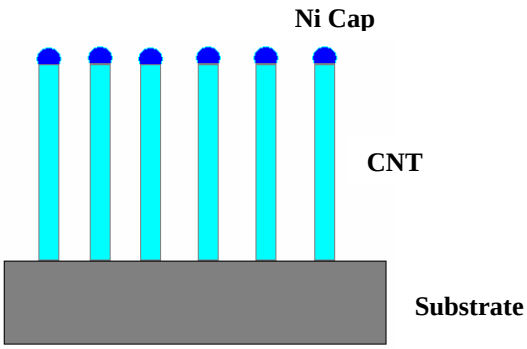
The process to be performed by NanoLab, Inc. to functionalize just one end of the nanotubes with carboxyl groups is a unique technique that has not been done prior to this order. Because of the novel nature of the procedure, the lead time offered was very long (8-10 weeks). Unfortunately, this means that the nanotubes will arrive after the time allotted for this research project has passed.

The rest of this paper will discuss what is to be done to continue this project once the functionalized nanotubes have arrived. The recommended methods and materials to be employed when this project continues were derived from other research endeavors that were either related to working with carbon nanotubes or with amphiphilic molecules that can act as models of nanotube behavior in aqueous solutions.

In order to attach carboxyl groups (COOH) to just one end of the nanotube array, NanoLab, Inc. will take the following measures:

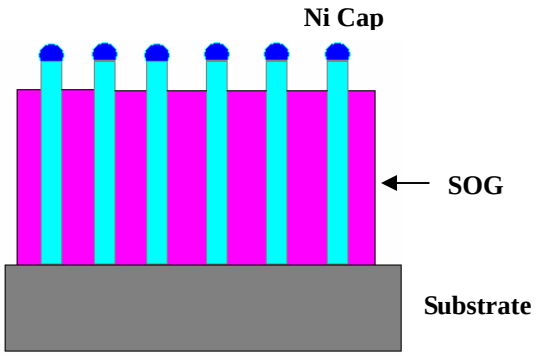
1. Grow the nanotubes aligned on a substrate

This is achieved by placing nickel dots on a chromium coated silicon wafer, which will act as the substrate. The nickel catalyst will get lifted up and stay at the tip of the growing nanotube. The dot size determines the diameter of the nanotube, which will be between 50-150nm. The growth time determines the length, and at the end there will be a cap of nickel covered by a closed-end nanotube.



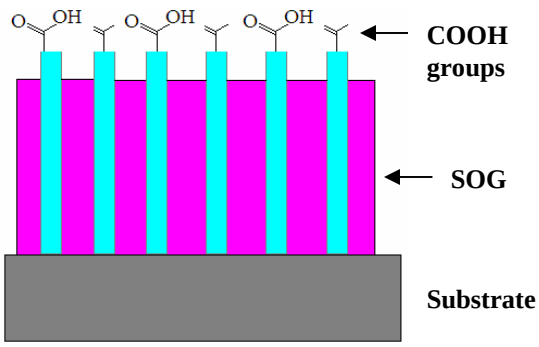
2. Fill in a matrix between the nanotubes, leaving only the tips exposed

To avoid functionalizing the sidewalls, the array will be filled with spin-on-glass (SOG) to cover the entire length of the tubes. The surface will then be polished with hydrofluoric acid (HF) to expose just the tips of the nanotubes.



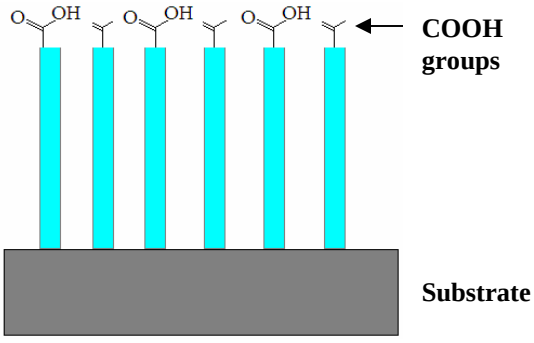
3. Functionalize the tips

To attach carboxyl groups to the tips, the array will be exposed to nitric acid (HNO_3) or sulfuric acid (H_2SO_4), which would open up the nanotube ends, remove the nickel, and leave -COOH groups at the tips.



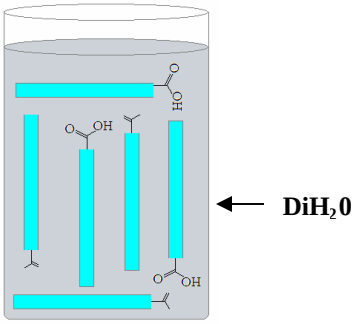
4. Dissolve the matrix, leaving a free-standing nanotube array

The sealed array would be placed in hydrofluoric acid (HF) to etch away the spin-on-glass matrix and leave the nanotubes functionalized and standing aligned on the substrate.



5. Strip the nanotubes from the substrate

The nanotubes need to be cleaved from the substrate, and put into distilled water. The end result of this detailed process will be nanotubes in surfactant-free solution (distilled water) that have carboxyl groups attached on one end.



A very similar approach to functionalizing nanotube arrays is discussed in one source, but with the goal of attaching nucleic acids to the nanotubes instead of carboxyl acid groups [14]. In this examination, multi-walled carbon nanotubes were also grown in an array using metal catalyst particles at the tips, and spin-on-glass (SOG) was deposited within the gaps between the aligned nanotubes. The researchers found that the "SOG film provided structural support to the carbon nanotubes, enabling them to retain their vertical configuration" during "oxidative treatment for the opening of closed CNT ends" and also made the nanotubes more compatible for coupling chemistry in aqueous solutions [14]. This demonstrates that the spin-on-glass will not only keep the sidewalls from getting functionalized in this current experiment, but will also ensure the mechanical stability of the nanotube array, which can often collapse during various treatments [14].

3.2 Confirmation of Functionalization

Once the carbon nanotube order has arrived, the presence of carboxyl groups (COOH) on the ends of the CNTs must be confirmed. This can be accomplished by using Fourier Transform InfraRed (FTIR) spectroscopy, which is used to identify organic materials by measuring their absorption of various infrared light wavelengths [28]. Different materials can be identified by comparing the spectrum of an unknown substance to that of a known, and preferably similar, substance. In this experiment, it would be preferable to compare the spectrum of the functionalized carbon nanotubes to that of plain carbon nanotubes to see if the carboxyl group peak can be observed. It would also be advantageous for the two types of nanotubes to have been prepared by the same method, so that peaks that occur as a result of amorphous carbon or impurities will appear on both spectra. Absorption bands in the frequency range of 4000-1500cm^{-1} are typically due to functional groups.

More specifically, the carboxyl group (COOH) has three different types of bonds, C-O, C=O, and O-H, and therefore has three different peaks that will appear. The C-O peak will typically appear in the 1260-1000 cm^{-1} region, the C=O in the 1760-1670 cm^{-1} region, and the O-H in the 3000-2500 cm^{-1} region. Besides frequency, peaks can also be classified by their intensity and shape. For example, the C-O and C=O bonds have strong peaks, and the O-H bond has a broad peak. Below is an example of an FTIR spectrum of 2-bromobutanoic acid, a carboxylic acid:

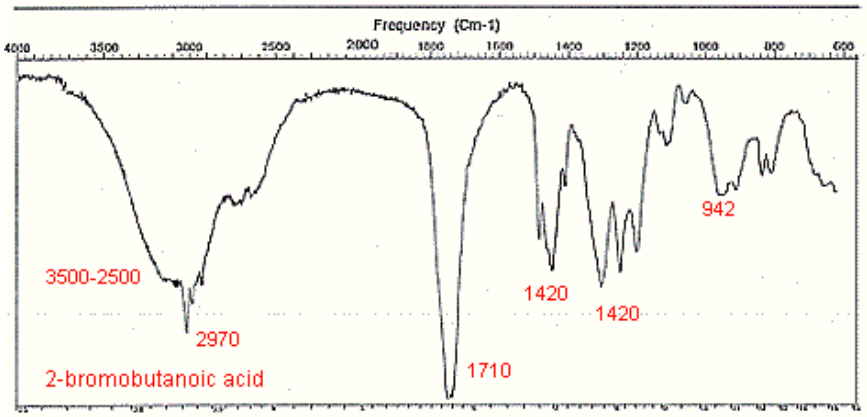


Figure 7 - FTIR spectrum of 2-bromobutanoic acid showing the peaks for COOH bonds

Although FTIR spectroscopy can be used to detect types of bonds, it is very difficult to quantify results with this method. For example, it would be very useful in this experiment if the number of carboxyl groups on each end of the nanotubes could be determined. But this would be nearly impossible to do with FTIR because the only way concentration can be ascertained is if it is compared to an established curve of known concentration of the same material.

In order to get quantitative results, a method called thermogravimetric analysis (TGA) could be used to assess the mass-fractions of different materials. This type of testing determines changes in the weight of a sample as a function of either time or temperature. The TGA technique would allow the evaluation of the number of carboxyl groups that are attached at the terminus of each nanotube, which would provide information as to what the dimensions of the hydrophilic region are like in comparison to the hydrophobic region of the tubes. One source mentions using acid-base titrations to determine the concentration of carboxyl groups attached to carbon nanotubes, which can also be investigated when doing this experiment [11]. This information would assist in determining what type of phase the nanotubes would form most readily in aqueous solution, and will be discussed further in Section 5.

4. DISPERSION

As previously mentioned, carbon nanotubes naturally clump together into ropes or bundles due to their hydrophobicity and strong van der Waals interactions [24]. Dissolution of CNTs is almost always necessary to conduct proper chemical reactions and for utilization of the tubes [23]. In order to disperse individual carbon nanotubes in solution, the “thermodynamic drive towards bundling must be overcome” [24]. This is commonly done by either ultrasonicating to physically disperse the CNTs or by coating the tubes with surfactants to increase their solubility in solution. Surfactants provide an additional repulsive force to separate the nanotubes and prevent aggregation while sonication acts as an “external mechanical energy” source which helps to overcome the attractive van der Waals forces [13].

It is suggested here that when the functionalized carbon nanotubes arrive, a small portion should be taken out and sonicated while the rest of the functionalized nanotube solution be stored in a safe spot. The sonicated portion can then also be divided into different amounts, one of which will be used for FTIR in order to confirm that the carboxyl groups are indeed attached. The other samples should be sonicated for different amounts of time and observed, to see what the optimal sonication time is for maximum dispersion of tubes. As a side note, if there are impurities in the solution when the functional nanotubes arrive, they can be removed by centrifugation. This process is commonly used to remove graphitic particles or other constituents larger than 500nm from solution [13].

Since the goal of this experiment is to form vesicles or micelles from carbon nanotubes, it would be preferable to achieve good dispersion with just the sonicating technique and to avoid using surfactants. The reason for this is that surfactants

themselves are amphiphilic molecules that will strive to self-assemble into micelles or other such aggregates when placed in aqueous solutions [25]. This is precisely why they work well to separate nanotubes and make them more soluble – they surround each nanotube with the hydrophobic regions near the tube and the hydrophilic regions away from the tube to form elongated micelles that surround the entire length of the CNT [31].

While this is a useful outcome when solubilizing non-functional carbon nanotubes, this experiment makes the matter a bit more complex since the nanotubes themselves have been made into amphiphilic molecules. Since it is desired that the nanotubes aggregate together into certain structures, it cannot be known what type of effect the surfactant molecules will have on their self-assembly since they will coat the entire perimeter of the nanotubes [25]. Although the surfactant molecules will help to separate individual nanotubes, they might also prevent the tubes from aligning parallel to each other to form bilayers and vesicles. Also, since there will be a hydrophilic region on the functionalized nanotubes, it is not clear how the surfactants will behave in those areas, as they might induce unwanted chemical reactions [13]. Another concern is that the nanotubes will be made entirely hydrophilic when the surfactant molecules surround them, since all the hydrophilic regions from the surfactant will be facing the outside. This is illustrated below:

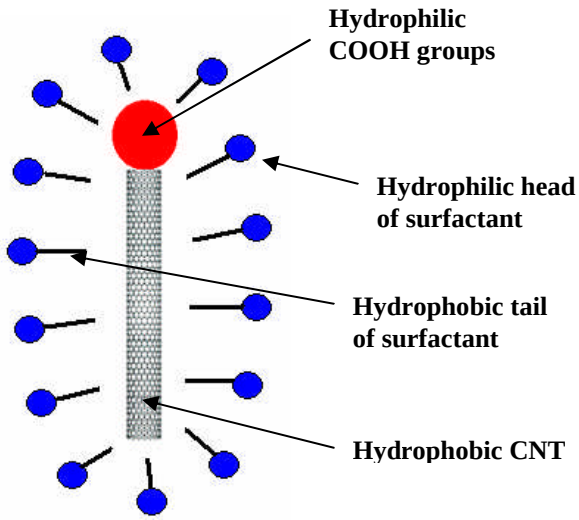


Figure 8 – Demonstration of how amphiphilic surfactant molecules surround a functional nanotube. Since all of the hydrophilic surfactant heads are on the outside, this could potentially make the entire nanotube hydrophilic

If the functional nanotubes are made entirely hydrophilic, they will no longer have the amphiphilic properties needed for self-assembly into aggregates such as micelles, vesicles, or bilayers. Hence, the attachment of the carboxyl groups to single ends of the nanotubes will have just been a wasted effort since they will still not display amphiphilic properties. There is no way to know exactly how the surfactant molecules will affect the nanotubes' ability to self-aggregate except to run experiments and observe the results. For these reasons, it is proposed that ultrasonication of the nanotubes be performed numerous times first to establish whether that process is substantial for dispersing the nanotubes. If it is found that the nanotubes cannot be separated well by sonication alone, then different surfactants can be tested to see how they influence the behavior of functional carbon nanotubes. If surfactants are indeed utilized, there might

also be a need for the added complication of removing the surfactant after processing so they do not interfere with the nanotube self-assembly [15]. In summary, surfactants should not be used unless it has been determined that they do not agitate the amphiphilic characteristics of the functional CNTs, which are vital in order for the nanotubes to spontaneously assemble into formations when placed in aqueous solution.

5. PHASE DETERMINATION

Probably the vaguest aspect of this research project is how to establish what phase (micelle, vesicle, bilayer, or other) will be formulated when the amphiphilic nanotubes are placed in aqueous solution. There has been no prior research conducted on making carbon nanotubes amphiphilic or how these nanotubes will assemble in water. Therefore, alternate amphiphilic molecules must be researched and it must be assumed that carbon nanotubes will act similarly when in the same environment. By far the most well-known amphiphilic molecules are phospholipids, which make up the cell membrane as well as many other cell organelles. In order to understand the conditions required for functional nanotubes to self-assemble, phospholipids will be used as models of their behavior. It should be understood that the way in which the amphiphilic nanotubes function might vary from how phospholipids function since phospholipids are much smaller (~5-10nm in length) than the nanotubes used here (~100nm in length), and they have some differing characteristics due to their chemical composition [0]. Nevertheless, assuming that the nanotubes will act similarly to the way phospholipids act in specific situations is the only way to establish certain parameters for experimentation. The following information about amphiphilic molecules was derived from sources discussing phospholipid self-assembly and behavior.

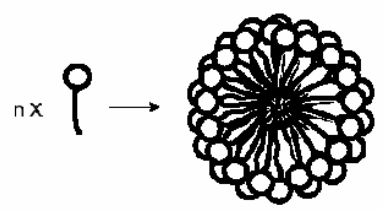
There are two main variables that determine what phase amphiphilic molecules will fall into when they exist in aqueous solution: the concentration of the amphiphile and the shape of the amphiphilic molecules. The concentration ascertains whether or not the molecules will aggregate at all, while the dimensions of the molecules determine which phase will most likely be formed – micelle, inverted micelle, or bilayer. One may notice that a vesicle is not included in the phase options; this is because a vesicle is formed by bending an already existing bilayer, meaning that a bilayer would have to be formed in solution first. It may be possible to create recipes for specific aggregates by altering the concentration and shape of the amphiphilic nanotubes during experimentation and analyzing the way in which they assemble.

5.1 Concentration

The concentration of amphiphiles in a given aqueous solution is an important factor in concluding whether or not a phase will form. The effect of varying concentration is mainly analyzed by discussing the onset of formation of micelles in solution. The reason for this is that micelle formation is affected primarily by concentration while the formation of a bilayer is affected by the shape of the molecule. To clarify, a bilayer will only form when the appropriate concentration for micelle formation is reached, but the shape of the amphiphilic molecules prefers a bilayer. So when the concentration to form

micelles is discussed, one can think of it as the concentration necessary to form either the micelle or bilayer phase [0].

When the concentration of amphiphilic molecules in solution is below the *critical micelle concentration* (CMC), the molecules form a single layer on the liquid surface and disperse throughout the solution. At the critical micelle concentration, the molecules will organize into spherical micelles, with the hydrophobic tails on the inside and the hydrophilic heads on the outside, as shown here [0].



Micelle

Figure 9 – A spherical micelle formed from *n* amphiphilic molecules

The CMC depends on the chemical composition of the amphiphilic molecules, mainly on the ratio of the head area and the tail length. As the concentration of amphiphilic molecules is increased beyond the CMC, more spherical micelles will be formed and the amphiphiles might also start to form elongated cylindrical micelles, also called hexagonal micelles, shown below [30]. If the concentration is slightly further increased, there is an interesting effect: the number of micelles will increase but the sizes of the micelles stay pretty constant and are only weakly affected [0].

Cylindrical micelle

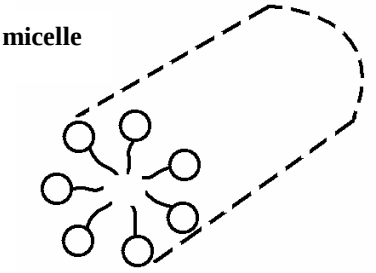
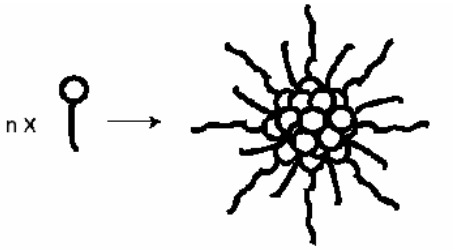


Figure 10 – A cylindrical micelle formed from amphiphilic molecules

Increasing of the concentration still further will lead to increasingly more micelles formed until such a high concentration of amphiphiles is reached that inverted micelles will begin to be formed. At this point the amount of amphiphilic molecules is so high that the aqueous solution is actually trapped in the interior of the micelle, and the micelle is formed with the hydrophobic regions on the outside and the hydrophilic heads on the inside [0, 30]. A spherical inverted micelle is illustrated below.



Inverted micelle

Figure 11 – An inverted spherical micelle formed from *n* amphiphilic molecules

Finally, when the amphiphile concentration is kept extremely high, inverted cylindrical micelles may also form in addition the spherical inverted ones. Again, the aqueous solution will actually be trapped on the inside of the structure, next to the hydrophilic heads, since there will be far more amphiphiles than water molecules in solution [30].

Inverted cylindrical micelle

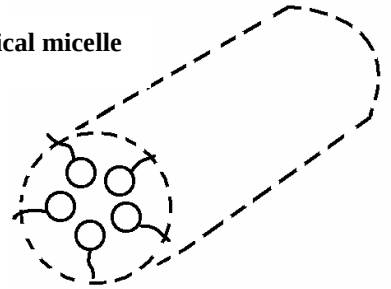


Figure 12 – An inverted cylindrical micelle formed from amphiphilic molecules

The amphiphilic molecules exemplified here have only one hydrocarbon tail, whereas phospholipids have two hydrophobic tails. They were illustrated in this way because the functional nanotubes that this project is concerned with will be more similar to a lipid with just one hydrophobic tail, since the CNT itself will function as the single hydrophobic region. The difference between single- and double-chain molecules is that single-chain amphiphilic molecules have a higher critical micelle concentration than double-chain molecules. The reason for this is that the double-chain molecules have twice as many carbon atoms for the same length. Since they have a larger hydrophobic region than single-chain molecules, they have a higher tendency to form aggregates at low concentrations, and therefore have lower CMC values. By the same principle, CMC values decrease as the lengths of the hydrophobic regions are increased in either single- or double-chain amphiphiles [0]. In this case, since the functional carbon nanotubes will act like single-chain lipids, the critical micelle concentration is expected to be higher than that of double-chain lipids or other amphiphilic molecules.

5.2 Amphiphilic Molecule Dimensions

How amphiphilic molecules aggregate depends on the way in which they are able to align with each other, which depends on their spatial geometry [30]. The ratio of the hydrophilic head cross-sectional area to the cross-sectional area of the hydrophobic tail plays a major role in determining whether the amphiphilic molecules will form micelles, bilayers, or inverted micelles [0]. The size of the respective regions can vary due to hydrophilic head group size, the number of hydrocarbon chains present, and sometimes attractive or repulsive forces between adjacent head groups can also have an effect. Another important factor that influences what phase is most favorable in a given circumstance is temperature. When the temperature of a solution is increased, the motion and mobility of the hydrocarbon chains increases, which results in the hydrophobic region becoming larger than when the temperature was at a lower value. Hence, if a solution contained amphiphilic molecules that were forming micelles because the hydrophilic region was bigger than the hydrophobic, and then the temperature was increased, the phase would most likely change to the bilayer formation since the area of the hydrophobic region has increased [30].

When the head cross-sectional area is large compared to that of the hydrophobic region, the hydrophobic tails get cramped together in the center and a micelle is formed. This usually occurs if the hydrophobic chains are short or if there is just one hydrocarbon tail. In these cases the molecule resembles a wedge shape with the hydrophilic region as the wide portion and the hydrophobic region at the tip [0, 30]. It is easily visualized that the smaller hydrophobic regions will gather in the center of the micelle while the hydrophilic regions stay on the outside, as picture below.

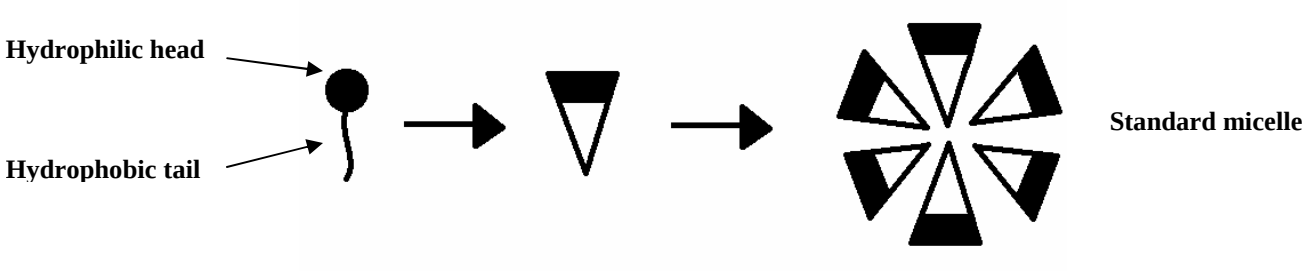


Figure 13 – A micelle formed from amphiphilic molecules with large hydrophilic regions (black) compared to the hydrophobic portions (white)

When the cross-sectional areas of the hydrophilic and hydrophobic regions are about equal, the amphiphilic molecules line up in parallel to each other to form a bilayer structure. These molecules have hydrocarbon tails that take up about the same width as their hydrophilic heads, and so resemble cylinders. The hydrophobic tails are again shielded from the water by being on the inside of the bilayer, while the hydrophilic heads are on the perimeter of the bilayer, next to the water. An illustration of this is shown:

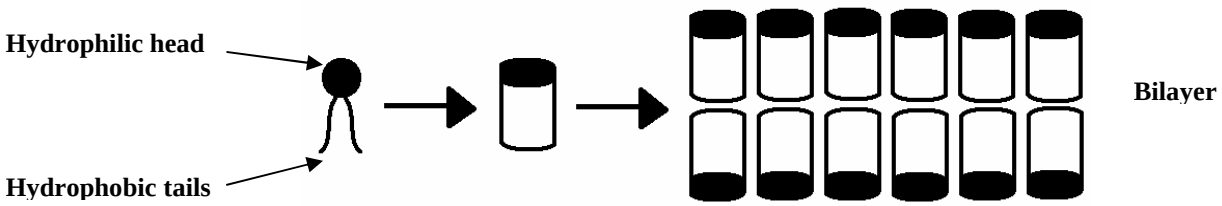


Figure 14 – A bilayer formed from amphiphilic molecules with hydrophilic (black) and hydrophobic (white) regions of about the same size

If the cross-sectional area of the nonpolar hydrocarbon chains is larger than that of the polar (hydrophilic) head, the amphiphilic molecules will again look like wedges, but now with the hydrophobic region as the wide part and the hydrophilic region as the tip. According to the structures of the miolecules, the wedges will want to align so that the hydrophilic tips are in the center of the micelle and the wide hydrophobic parts are on the outside, thus forming an inverse micelle [0]. However, if these molecules are located in aqueous solutions, this formation will be against energy considerations since the

nonpolar regions would be placed near water, a very detrimental state. Therefore, if an inverse micelle is to be formed, a very high concentration of amphiphilic molecules is needed in addition to the reverse wedge shape. This way the water molecules can be trapped within the nonpolar interior of the micelle, while the hydrophobic regions stay on the outside next to the hydrophobic portions of the neighboring inverse micelles. Hence, a high amphiphilic concentration is needed so that all of the aqueous solution present can be confined within the structures.

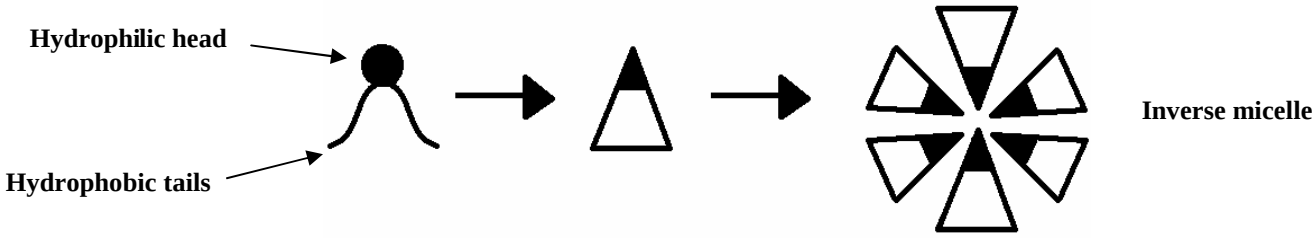


Figure 15 – An inverse micelle formed from amphiphilic molecules with small hydrophilic regions (black) compared to the hydrophobic regions (white)

Since it would be quite difficult to try to alter the dimensions of the functional nanotubes once they arrive, only the concentration of the nanotubes could be experimented with in this project. However, determining the amount of carboxyl groups on the ends of the nanotubes will be of great benefit since that will help to determine the ratio between the hydrophilic and hydrophobic cross-sectional areas. Although the ratio could not be modified, it would still be of great use to know which phase would be favored by the geometry of the molecules alone. During experimentation, the concentration of nanotubes in solution should be altered and the samples observed in an attempt to find an optimal concentration to form each of the phases.

6. EXPERIMENTAL VARIABLES

6.1 Nanotube Concentration

As mentioned, experiments should be run at various nanotube concentrations in order to establish whether there is a critical micelle concentration (CMC) for the functionalized nanotubes. Optimal concentrations for the formation of bilayers or inverted micelles may also be found, in which case specific recipes can be developed for each type of phase.

One difficulty that arises is how to measure the concentration of nanotubes when they are in solution. Their size is so small that traditional methods of computing the quantity present in solution do not suffice. In one source, optical absorption spectroscopy is discussed as a technique to determine nanotube concentration. This approach involves recording the absorbance of light as a function of CNT concentration, which allows the amount of nanotubes to be measured even when dispersed in aqueous solution [35].

There have also been studies done to assess how nanotubes behave in relation to each other when placed in water, which would be a useful point to understand when trying to pick appropriate conditions for CNTs to self-assemble. One source discusses the forces that are present between two nanotubes in water, and suggests there are maximum and minimum attractive forces depending on the specific distance present between the carbon nanotubes [7].

6.2 Types and Amount of Surfactant

If using various amounts of nanotubes in combination with ultrasonication still does not show any useful results, different surfactants can be used to see if they facilitate the self-aggregation of the nanotubes. Although it is not clear whether the surfactant molecules will aid in assembly of the tubes or actually interfere with it, this is another variable that can be tested to see if there are any beneficial outcomes. Several surfactants that have been used successfully with carbon nanotubes are Triton X-100 [25], poly(methyl 2-methylpropenoate) (PMMA) [6], Gum Arabic (GA) [22], dimethylformamide (DMF) [28], and sodium dodecyl sulfate (SDS) [31]. Each of these, and other surfactants, can be examined to see what type of effects they have on the self-assembly properties of the functional CNTs.

7. VISUALIZATION

One of the biggest challenges that arises when working with nanotubes is how to visualize them. Because of the tiny size of carbon nanotubes, traditional methods using microscopes do not have enough resolution to examine the CNTs closely. For this reason, two alternative techniques for visualizing the functional carbon nanotubes are discussed here.

7.1 Electron Microscopy

The most common approach that many researchers utilize to visualize carbon nanotubes is electron microscopy, of which there are several forms. This category includes scanning electron microscopy (SEM), transmission electron microscopy (TEM), freeze-fracture electron microscopy, and many variations of each of them. However, preparation for each of these techniques is intensive, and the sample to be investigated must be fixed according to specific standards for each type of equipment. For example, SEM and TEM both require that samples must be dehydrated and fixed by either chemical or physical methods so that they can withstand a high vacuum environment. Since this research project is based on observing the behavior of carbon nanotubes while in aqueous solution, both of these techniques will not be successful since it is impossible to investigate CNTs in solution using either of these methods [27].

The only possible electron microscopy technique that could be applied to this project is the freeze-fracture method, in which a sample (which could be a solution) is frozen by liquid nitrogen and then cracked to expose the interior region of the sample. This approach would work in this case because the nanotubes and their aqueous

surroundings would be frozen together in one block, and the nanotube positions could be examined when the sample is fractured. The advantage of this process is that the resolution that can be achieved by electron microscopy is very high, to about 0.2 nanometers, so the nanotubes could be observed very closely. However, the disadvantage is that the sample must be frozen, so it is not possible to detect how the nanotubes move around or interact with each other in real time. In order to examine these dynamic properties of nanotubes, another technique, fluorescence microscopy, is introduced.

7.2 Fluorescence

Staining with fluorescent molecules, or fluorophores, allows carbon nanotubes to be distinctly visualized in solvents using simple light microscopes [27]. Although optical microscopes typically only have a resolution of about 200 nanometers, fluorescence of the nanotubes greatly eases their observation and manipulation, which enhances the visualization significantly [26]. This process allows nanotubes to be observed in real time under a simple microscope, so the motion and assembly of nanotubes can be assessed, and even recorded with video enhancement. Also, the conditions in which the nanotubes are present can be altered during observation so the effects can be immediately monitored. For example, the sample on the slide can be diluted with a drop of water while it is on the microscope and the reaction of the nanotubes can be immediately analyzed.

Two specific fluorophores have been proven to successfully stain carbon nanotubes in a study that tested six common dyes. The two that labeled the CNTs best were 3,3'-Dihexyloxacarbocyanine iodide, DiOC6(3), and 1,1’-Dihexadecyl-3,3,3’,3’-tetramethylindocarbocyanine perchlorate, $DiIC_{16}(3)$. A suggested explanation of the success of these two fluorophores in comparison to others was that they had long nonpolar, hydrocarbon chains that could interact with the hydrophobic nanotubes. The structures of both molecules are shown below [26].

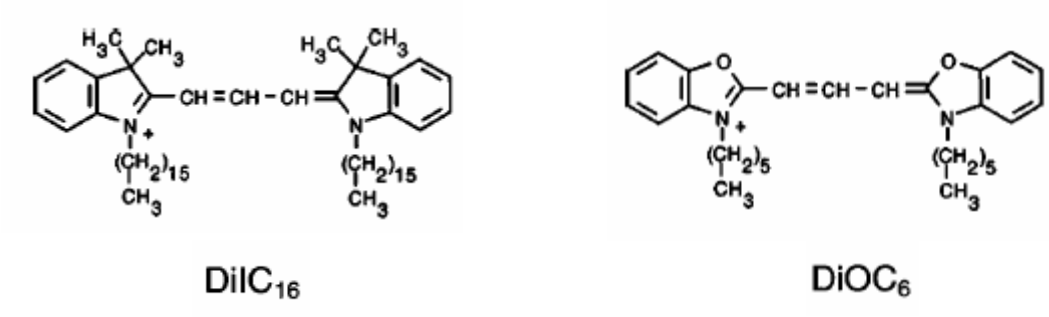


Figure 16 – An illustration of the chemical structures of two common fluorophores that successfully label CNTs

Although fluorescence microscopy does provide the opportunity to study the dynamics of carbon nanotubes by giving them fluorescent tags, there are several problems that may arise with this technique. The resolution with an optical microscope is always much worse than with electron microscopy methods. This may not be a dilemma

at first because the nanotubes that are being worked with are relatively large in size (~100nm in diameter and 1.5 microns in length), but if this type of research continues on to work with smaller nanotubes, visualization of them may not be possible with fluorescence microscopy. Another issue that may arise is how these molecules will affect the ability of the nanotubes to assemble into phase formations. Just like with the surfactant molecules, the response of the functional nanotubes to the fluorophore molecules cannot be predicted. The only way to find out how the nanotubes will be affected is to visualize two identical samples using both fluorescence microscopy and freeze-fracture electron microscopy to assess whether the fluorophore molecules have any impact on the self-aggregation of the functional CNTs.

8. CONCLUSIONS and RECOMMENDATIONS

In summary, this paper has discussed the topics that need to be addressed when attempting to form various phases, such as micelles, vesicles, and bilayers, from nanotubes that have been made amphiphilic. Once they arrive, the functionalized carbon nanotubes will be analyzed under diverse conditions, for instance, with different amounts of surfactant present in the water and different nanotube concentrations. Throughout experimentation with different variables, it would be worthwhile to determine whether there are optimal conditions for these nanotubes to form certain aggregates. Ideally, a sort of recipe could be developed for each type of phase so that they could be easily produced when desired. Things such as the type and amount of surfactant that is best to use would also be beneficial information to obtain. It would also be valuable to establish a critical micelle concentration for nanotubes that have been functionalized at one end with carboxyl groups, since nothing of the sort has yet been investigated. There may also be other concentration values that lead to bilayers or inverse micelles that would be useful to determine. Correlations between nanotube concentration and/or amount of surfactant in solution could be developed to show which aggregates form in which situations. This type of assay will illustrate which, if any, circumstances allow the nanotubes to form micelles, vesicles, bilayers, or other such aggregates most readily.

Another very important issue that should be investigated is how to make a carbon nanotube bilayer form into a vesicle. The only mechanisms currently known to form vesicles or liposomes are to alter the conditions of pre-existing bilayers. For example, if excess water is added to a solution that contains a lipid bilayer formation, the end areas are able to detach and round off to form a sealed spherical liposome [30]. After a bilayer has been successfully formed from CNTs, this will be the key step in forming a carrier for pharmaceuticals. The sort of information that could be acquired from this research project will surely lead to huge advances in the biomedical and drug delivery fields.

9. ACKNOWLEDGEMENTS

I would like to thank my advisor, Dr. Jennifer Lukes of the University of Pennsylvania, for all of her advice and encouragement. I would also like to thank Dr. Jan Van der Spiegel of the University of Pennsylvania for coordinating and supervising the SUNFEST program. Finally, I would also like to thank the National Science Foundation

for their ongoing support of the SUNFEST program, which presents undergraduate students with the opportunity to do valuable research.

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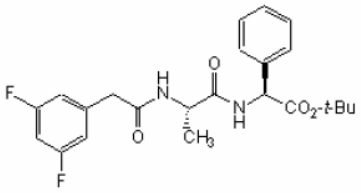
APPENDIX A:

CALBIOCHEM®

Data Sheet 565784 Rev. 23-November-04 JSW

γ-Secretase Inhibitor IX in Solution
Cat. No. 565784

Description:	A cell-permeable dipeptide that inhibits γ-secretase activity and suppresses Aβ production (Aβ total IC_{50} = 115 nM; $Aβ_{42}$ IC_{50} = 200 nM). Reported to be functionally active in both HEK293 cells overexpressing human APP751 and neuronal cultures, however, it does not affect the secretion of amyloid-b precursor protein (APP). Effective in acutely lowering Ab in APPV717F-transgenic mice. Also effective in inhibiting the production of an ~ 6 kDa C-terminal cleavage product (CTFg) in HEK293 cells stably transfected with the Swedish mutant, $βAPP_{695}$. Reported to reduce extracellular Aβ plaques and intracellular Aβ accumulation in 3xTgAD transgenic mice.
Form:	Liquid. Supplied as a 25 mM (5 mg/462 µl) solution of γ-Secretase Inhibitor IX (Cat. No. 565770) in DMSO. Packaged under inert gas.
Molecular Weight:	432.5
Molecular Formula:	$C_{23}H_{26}F_2N_2O_4$
Structure:	
Purity:	≥95% by HPLC
Storage:	REFRIGERATOR (+4°C). Protect from light. Hygroscopic. Following initial use, aliquot and refrigerate (+4*deg;C). This product is stable for 2 years as supplied.
Toxicity:	MSDS available upon request.
References:	Oddo, S., et al. 2004. *Neuron* **43,** 321. Dovey, H.F., et al. 2001. *J. Neurochem.* **76,** 173. Vandermeeren, M., et al. 2001. *Neurosci. Lett.* **315,** 145.

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APPENDIX B:

QUOTATION

To: Alexsandra, alf8@Lehigh.EDU.
From: Zhongping Huang
Date: 8/15/2006
Re: NanoLab Carbon nanotube arrays

Item	Quantity	Description	Price Each	Total Amount
Functio n-alized low site density of array	1	8x8 mm^2 low site density of carbon nanotube array on 15x15 mm^2, 300 nm chromium-coated Si (400-500 microns thick). Target nanotube diameter ~100 nm, and target length 2 microns. Nanotube site density $5x10^6$ CNTs/cm^2. Seal the low site density of nanotube array with spin-on-glass, sealing height 1 micron. Treat nanotube tips with HF to remove the spin-on-glass from surface, and then with HNO3 and H2SO4 acid to functionalize nanotube tips. After tips functionalized, put the sealed array into HF acid to remove 0.5 micron deep spin-on-glass and remain 0.5 micron thick to support nanotubes in freestanding. Making the functionalized carbon nanotubes into DI water.	$2000.00 $200.00	

Standard lead time is 8-10 weeks after a purchase order is received. The actual lead time could be short than it, depending on concurrent order load. Each array will be accompanied by SEM images verifying length, diameter, etc. Terms: P.O may be faxed to 617 581 6749, Terms Net 30 or credit card. **Shipping and taxes are not included.**

Zhongping Huang, NanoLab, Inc.

QUOTATION

To: Alexsandra, alf8@Lehigh.EDU.
From: Zhongping Huang
Date: 8/15/2006
Re: NanoLab Carbon nanotube arrays

Item	Quantity	Description	Price Each	Total Amount
Function-alized low site density of array	1	15x15 mm^2 low site density of carbon nanotube array on 25x25 mm^2, 300 nm chromium-coated Si (400-500 microns thick). Target nanotube diameter ~100 nm, and target length 2 microns. Nanotube site density $5x10^6$ CNTs/cm^2. Seal the low site density of nanotube array with spin-on-glass, sealing height 1 micron. Treat nanotube tips with HF to remove the spin-on-glass from surface, and then with HNO3 and H2SO4 acid to functionalize nanotube tips. After tips functionalized, put the sealed array into HF acid to remove spin-on-glass. Making the functionalized carbon nanotubes into DI water.	$2600.00	$2600.00
			TOTAL	$2600.00

Standard lead time is 8-10 weeks after a purchase order is received. The actual lead time could be short than it, depending on concurrent order load. Each array will be accompanied by SEM images verifying length, diameter, etc. Terms: P.O may be faxed to 617 581 6749, Terms Net 30 or credit card. **Shipping and taxes are not included.**

Zhongping Huang, NanoLab, Inc.

QUOTATION

To: Alexsandra, alf8@Lehigh.EDU.
From: Zhongping Huang
Date: 8/15/2006
Re: NanoLab Carbon nanotube arrays

Item	Quantity	Description	Price Each	Total Amount
Function-alized low site density of array	2	15x15 mm^2 low site density of carbon nanotube array on 25x25 mm^2, 300 nm chromium-coated Si (400-500 microns thick). Target nanotube diameter ~100 nm, and target length 2 microns. Nanotube site density $5x10^6$ $CNTs/cm^2$. Seal the low site density of nanotube array with spin-on-glass, sealing height 1 micron. Treat nanotube tips with HF to remove the spin-on-glass from surface, and then with HNO3 and H2SO4 acid to functionalize nanotube tips. After tips functionalized, put the sealed array into HF acid to remove spin-on-glass. Making the functionalized carbon nanotubes into DI water.	$2340.00	$4680.00
			TOTAL	$4680.00

Standard lead time is 8-10 weeks after a purchase order is received. The actual lead time could be short than it, depending on concurrent order load. Each array will be accompanied by SEM images verifying length, diameter, etc. Terms: P.O may be faxed to 617 581 6749, Terms Net 30 or credit card. **Shipping and taxes are not included.**

Zhongping Huang, NanoLab, Inc.

APPENDIX C:

Product Name	**DiIC16(3)** [1,1'-Dihexadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate]
Size	100 mg
Catalog #	84905
US$	$95
Molecular Weight	877.8
Spectral Properties	Abs/Em = 549/565 nm
Solvent System	Ethanol
Description	Lipophilic neuronal tracer
References	Cheng Z, et al. (2004). Differential control over postganglionic neurons in rat cardiac ganglia by NA and DmnX neurons: anatomical evidence. Am J Physiol Regul Integr Comp Physiol 286, R625-33; Koo YE, et al. (2004). Real-time measurements of dissolved oxygen inside live cells by organically modified silicate fluorescent nanosensors. Anal Chem 76, 2498-505; Wu CC, et al. (2004). High-throughput morphometric analysis of individual neurons. Cereb Cortex 14, 543-54; Suksaweang S, et al. (2004). Morphogenesis of chicken liver: identification of localized growth zones and the role of beta-catenin/Wnt in size regulation. Dev Biol 266, 109-22; Iriyama A, et al. (2004). Effects of indocyanine green on retinal ganglion cells. Invest Ophthalmol Vis Sci 45, 943-7; Vest RS, et al. (2004). Divalent cations increase lipid order in erythrocytes and susceptibility to secretory phospholipase A2. Biophys J 86, 2251-60; Zhang X and Kirsch LE (2004). Correlation of the thermal stability of phospholipid-based emulsions and the microviscosity measurements using fluorescence polarization. Pharm Dev Technol 9, 219-27; Hefnawy MA and Abou-Zeid AM (2003). Differential adaptation of membranes of two osmotolerant fungi, Aspergillus chevalieri and Penicillium expansum to high sucrose concentrations. Acta Microbiol Pol 52, 53-64; Jordan MB, et al. (2003). Liposomal clodronate as a novel agent for treating autoimmune hemolytic anemia in a mouse model. Blood 101, 594-601; Bianchi L, et al. (2003). A potassium channel-MiRP complex controls neurosensory function in Caenorhabditis elegans. J Biol Chem 278, 12415-24; deAzevedo LC, et al. (2003). Cortical radial glial cells in human fetuses: depth-correlated transformation into astrocytes. J Neurobiol 55, 288-98; Ramani K and Balasubramanian SV (2003). Fluorescence properties of Laurdan in cochleate phases. Biochim Biophys Acta 1618, 67-78.
Storage	4°C

Product Name	**DiOC6(3)** [3,3'-Dihexyloxacarbocyanine iodide]
Size	100 mg
Catalog #	84715
US$	$49
Molecular Weight	572.5
Spectral Properties	Abs/Em = 484/501 nm
Solvent System	DMSO
Description	Most widely used for measuring membrane potential
References	Cheng Z, et al. (2004). Differential control over postganglionic neurons in rat cardiac ganglia by NA and DmnX neurons: anatomical evidence. Am J Physiol Regul Integr Comp Physiol 286, R625-33; Koo YE, et al. (2004). Real-time measurements of dissolved oxygen inside live cells by organically modified silicate fluorescent nanosensors. Anal Chem 76, 2498-505; Wu CC, et al. (2004). High-throughput morphometric analysis of individual neurons. Cereb Cortex 14, 543-54; Suksaweang S, et al. (2004). Morphogenesis of chicken liver: identification of localized growth zones and the role of beta-catenin/Wnt in size regulation. Dev Biol 266, 109-22; Iriyama A, et al. (2004). Effects of indocyanine green on retinal ganglion cells. Invest Ophthalmol Vis Sci 45, 943-7; Vest RS, et al. (2004). Divalent cations increase lipid order in erythrocytes and susceptibility to secretory phospholipase A2. Biophys J 86, 2251-60; Zhang X and Kirsch LE (2004). Correlation of the thermal stability of phospholipid-based emulsions and the microviscosity measurements using fluorescence polarization. Pharm Dev Technol 9, 219-27; Hefnawy MA and Abou-Zeid AM (2003). Differential adaptation of membranes of two osmotolerant fungi, Aspergillus chevalieri and Penicillium expansum to high sucrose concentrations. Acta Microbiol Pol 52, 53-64; Jordan MB, et al. (2003). Liposomal clodronate as a novel agent for treating autoimmune hemolytic anemia in a mouse model. Blood 101, 594-601; Bianchi L, et al. (2003). A potassium channel-MiRP complex controls neurosensory function in Caenorhabditis elegans. J Biol Chem 278, 12415-24; deAzevedo LC, et al. (2003). Cortical radial glial cells in human fetuses: depth-correlated transformation into astrocytes. J Neurobiol 55, 288-98; Ramani K and Balasubramanian SV (2003). Fluorescence properties of Laurdan in cochleate phases. Biochim Biophys Acta 1618, 67-78.
Storage	4°C