CHAPTER 2

LITERATURE REVIEW

Nanotechnology is an interdisciplinary field which combines physics, engineering, biology and chemistry. The term “nanotechnology,” has been derived from the Greek word nanos, or “dwarf,” which generally refers to engineering and manufacturing at the nanometer length scale i.e. between 1 and 100 nanometers. Nature is best nanotechnology example from cell to molecule being of nanodimensions. The concept of the nanoscale can be very well imagined using the Figure 2.1 which is self explanatory.

Feynman, a Noble laureate, is widely acclaimed to be the father of nanotechnology. In 1959, Feynman gave a lecture in which he conceptualized the idea of nanotechnology (Feynman, 1960). The man made products that we manufacture or buy are made by cutting or joining or pushing piles of atoms together in a bulky, imprecise manner. Manipulating individual atom of an object and placing them in a pattern to produce a desired structure is the basic idea of nanotechnology. If that happens, almost everything, including medicine, computers and cars, can be designed and constructed differently.
Feynman presented a talk entitled “There’s Plenty of Room at the Bottom” and he proposed using machine tools to make smaller machine tools, which, in turn, would be used to make still smaller machine tools, and so on all the way down to the molecular level (Feynman, 1960). He suggested that such nanomachines, nanodevices and nanorobot ultimately could be used to develop a wide range of atomically precise microscopic instrumentation and manufacturing tools. His lecture is published in the book called “Miniaturization” (Feynman, 1961). The vision of nanotechnology was born, though the term nanotechnology was coined by Taniguchi in 1974. The Tokyo Science University Professor defined nanotechnology as a technology mainly consisting of the processing of, separation, consolidation, and deformation of materials by one atom or one molecule (Taniguchi, 1974). Nearly two decades after Feynman’s lecture, K. Eric Drexler was hit upon the same idea. In 1986 he wrote a popular book titled “Engines of Creation” in which he outlined some of the concepts of nanotechnology (Drexler, 1986). Drexler described it as the knowledge and means for designing, fabricating and employing molecular scale devices by the manipulation and placement of individual atoms and molecules. A nanodevice can be defined as nanoscale objects capable of performing simple tasks (Astier et al, 2005).

The research at this level demands high quality tools and equipments. Recent years have seen the development of a number of technologies that allow us to observe the behavior of individual molecules. A new era of biomechanical studies has been associated with the development of optical and mechanical probes that are sensitive enough to make measurement of single biological molecules (Wendel et al, 1996; Mansoori, 2002). The micromanipulation of biomolecules have been extensively studied by using Microneedle (Kishino et al, 1988), Optical tweezer (Finer et al, 1994) and Scanning probe microscopes (Chih et al, 2007). The major milestones achieved in this field are shown in Figure 2.2.
In the coming decades, nanotechnology could make a supercomputer so small it could barely be seen in a light microscope. Inexpensive and lightweight materials 50 times stronger than structural steel may give us easy personal access to space. New fabrics could be embedded with billions of tiny motors, sensors, and even computers, allowing our clothing to react instantly and intelligently to our ever-changing surroundings. Low cost solar cells and batteries could replace coal, oil and nuclear fuels with clean, cheap and abundant solar power. Medicine will be revolutionized by nanodevices that can interact with and repair individual living cells, leading to cures for most diseases, probably including aging. Nanorobot with sizes comparable to bacteria could provide many novel capabilities through their abilities to sense and act in microscopic environment where nanorobot inside the body can diagnose and treat the disease (Couvreur et al., 2006; Leary et al., 2006). Clean factories could prevent new pollution, and remediate old pollution, that is traditionally caused by conventional manufacturing processes. The natural molecular machines therefore form the basic enablers of future nanodevices (Morries et al., 2001; Mallik et al., 2004). All these applications are only possible if researchers and technologists of interdisciplinary fields sit together and put their effort in a common direction of the development of nanomachines.
2.1 Muscle and Nanomotors

The muscles are the very important part of the living system. The diameter of muscle cells is typically 10 – 100 μm and the length can range from less than a millimeter to a centimeter.

Figure 2.3: Myosin motor molecules and actin filaments are the most basic constituents of the muscle tissue. They form overlapping filaments in the cells that slide past one another to make the muscle contract or expand.
About 80 percent of the muscle cell is occupied by cylindrical rods of protein and are known as myofibrils. Many myofibrils, each about 1µm in diameter, are contained within the cross section of a single muscle cell. Myofibrils are the structures that are responsible for muscle contraction. The most distinctive feature of myofibrils is their banded appearance; the dark bands correspond to higher density of protein. The entire repeating structure of the alternating light and dark band from one Z-disc to the next is known as sarcomere (refer Figure 2.3). The banded appearance of the sarcomere is produced by hundreds of protein filaments bundled together in a highly ordered fashion. The two main types of filament are: (i) thick filaments, about 15 nm in diameter, are made mostly of myosin motor; (ii) thin filaments, about 8 nm in diameter, consist mostly of actin motor. Both these types of filaments together produce relative motion in the presence of other types of proteins also which help to hold them in correct arrangement and regulate the process of contraction. Arrays of thin and thick filaments overlap in the sarcomere in a manner to produce relative motion. The thick filaments come within about 13 nm of the adjacent thin filament which is close enough for the formation of cross-bridges between the myosin heads belonging to the thick filament and actin molecules constituting the thin filaments.

Huxley and Hanson proposed the sliding filament hypothesis of muscle contraction (Huxley, 1953; Hanson et al, 1953). It was acknowledged that Myosin molecules are arranged in such a way on the thick filament that their heads point away from the mid-zone towards either end of the filament. According to this hypothesis, it is the sliding of the thick and thin filaments past each other that leads to the contraction of the muscle. This theory was formulated clearly and quantitatively in another classic paper of A.F. Huxley in 1957 (Huxley, 1957). In 1969, H.E. Huxley proposed the myosin “lever arm” hypothesis (Huxley, 1969). This model was developed further and formulated quantitatively by A.F. Huxley and Simmons in 1971 (Huxley et al., 1971). The cross bridge action was explained in 2000 by Huxley A.F. (Huxley, 2000). Muscle contraction is achieved by sliding two kinds of filaments past one another. One of these filaments is made of myosin motor; the other is called actin motor. The two kinds of filaments overlap in the muscle cells to maximize their interactions. The forces and movements produced by a single cycle are very small, but the combination of millions of myosin molecules acting simultaneously amplifies the effect by many orders of magnitude. The interaction between the two motors allow studying these motors for the load transportation (Howard et al., 2001, Goldstein et al., 2001).
From the structure analysis of these motors, it is well established that head of the motor domain provides the motion along the track while tail leads to binding of the cargos (Hess et al., 2001, Mallik et al., 2004).

2.2 Bio-nanomotors

Biomolecular motors are the biological nanomachines which convert the chemical energy to mechanical energy in the presence of ATP. In cells, various types of linear motors like actin-myosin, kinesin-microtubule, dynein-microtubule and rotary motors like ATPase, bacterial flagella motors exist which helps to perform various functions e.g. muscle contraction, transport of small vesicles in a cell etc (Berg, 2003).

2.2.1 Actin and Myosin Motors

The actin myosin nano motors are associated in the process of force generation during muscle contraction or expansion. In addition, these machines transport cargos along the actin filaments. Actin was discovered by Straub in 1942. In 1960 Emmeline J. Hanson observed the fine structure of the thin actin filaments for the first time. The crystal structure of G-actin was solved in 1990 by Kabsch and colleagues (Kabsch, 1990). In the same year, a model for F-actin was proposed by Holmes and colleagues (Holmes et al, 1990).

Myosin molecules were first sighted through electron microscope protruding out from thick filaments and interacting with the thin actin filaments in late 1950s (Huxley H.E. 1953; Hanson and Huxley, 1953; Huxley H.E. 1957). Muscle myosin II’s are the most extensively studied molecular motor. Myosin molecule has a size of about 520 KD including two 220 kD heavy chains and light chains of sizes between 15 and 22 kD (Weeds et al, 1971).

The movement of the actin-myosin system is made possible by the hydrolysis of ATP where ATP acts as fuel to the system. Earlier, it was known that ATP plays a role in myosin related muscle movement along actin (Huxley H.E., 1969). However, the exact mechanism was unknown, which was explained later in 1971 by Lymn and Taylor (Lymn and Taylor, 1971). The cycle starts with the binding of the actin filament with the myosin head. Only one motor head is
able to connect to the actin filament at a time, the other head remains passive. As soon as energy is released, the lever arm swings counter clockwise due to a conformational change that pushes the actin filament down by about 10 nm along its longitudinal axis (Jontes et al, 1995; Irving et al, 1995; Baker et al, 1998; Houdusse et al, 1999; Veigel et al, 1999; Corrie et al, 1999; Vale et al, 2000; Forkey et al, 2003). The myosin motor then dissociates from the actin filament, and a new cycle starts. The schematic representation of the actin filament moving over the myosin is shown in the Figure 2.4.

Figure 2.4: Schematic representation of actin filaments sliding over myosin rails

### 2.2.2 Actin Myosin Motility Experiments

In order to study the molecular motors in-vitro, the necessary environmental conditions has to be fulfilled, hence a term motility assay has been developed. The first quantitative movement of myosin motor along actin filament in vitro was made by Sheetz and Spudich (Sheetz et al, 1983). Fluorescently labeled phalloidin was a key feature in the development of the in vitro motility assay (Yanagida et al 1984; Kron & Spudich, 1986), in which single actin filament are observed sliding across a myosin coated surface. Yanagida et al observed single fluorescent actin filaments in solution by using a video light microscope. Kron & Spudich (1986) showed that fluorescently labeled actin filaments could be seen to move in presence of ATP in a
unidirectional manner over a glass surface to which myosin filaments had been attached. The myosin motors have been immobilized on the base surface like glass and actin filament which are fluorescently labeled slides over myosin in the presence of ATP fuel (Kron and Spudich, 1986, Kron et al., 1991; Spudich, 1994). The in-vitro motility assay is a powerful tool for studying the function and properties of myosin (Kron et al., 1986; Toyoshima et al., 1987). The working stroke has been reported in the range between 5-25 nm while max force ranges from 1-5 pN by the actomyosin system (Simmon et al., 1996; Mehta et al., 1997; Gulford et al., 1997).

There are two geometries used for in-vitro motility assays: (i) the gliding assay and (ii) the bead assay. In the gliding assay, the motors themselves are fixed to a substrate and the filaments are observed under an optical microscope as they glide along the motor coated surface. In the bead assay, the filaments are fixed to a substrate. Small plastic or glass beads, whose diameters are typically of the order of 1-3 μm, are coated with the motors. These motors move along the fixed filaments carrying the bead as their cargo. The movements of the beads are recorded optically.

As the myosin molecules are arranged randomly on the surface, the actin filaments moves over the myosin molecules in some random fashions. This can be avoided by using tracks on the glass slides. In order to create grooves for guidance of myosin induced actin filament motility, electron beam lithography can be used. Constraining actomyosin motility along very narrow predefined grooves opens up possibilities for future attempts to further control and direct motility in nanotechnological applications.

2.2.3 Other Motility Experiments

Kinesin motors are involved in cellular cargo transport along microtubules as opposed to actin in the case of myosin (Brady et al., 1985; Block et al., 1995; Howard, 1996). It is the most abundant motor in the cell. Kinesin move from minus end to plus end of the microtubules. Like myosin, Kinesin is also an ATP-driven motor. One unique characteristic of kinesin family of proteins is their processivity—they bind to microtubules and literally “walk on it” for many enzymatic cycles before detaching (Berliner et al, 1995; Vale et al, 1996). Also, each of the globular heads/motor domains of kinesin is made of one single polypeptide unlike myosin (heavy and light chains and dynein heavy, intermediate, and light chains). Kinesin is able to take about 100 steps before detaching from the microtubule (Block et al, 1990; Vale et al, 1996) while moving
at 1000 nm/sec and exerting forces of the order of 5 to 6 pN (Hunt et al, 1994; Svoboda et al, 1994; Rice et al., 1999).

The Dynein superfamily of proteins was introduced in 1965. Dyneins are also involved in cargo movement (Lye et al, 1987; Schroer et al, 1989; Hirokawa et al, 1990; et al, 1993 king et al ,2000). Because dynein is larger and more complex structure as compared to other motor proteins, its mode of operation is not as well known. However, very recently, Burgess et al. (Burgess et al, 2003) have used electron microscopy and image processing to show the structure of a dynein at the start and end of its power stroke, giving some insight into its possible mode of force generation.

Moreover, DNA has found use in not only mechanochemical, but also in nanoelectronic systems as well. A DNA double-helical molecule is about 2 nm in diameter and has 3.4-3.6 nm helical pitch. Furthermore, double-stranded DNA has a respectable persistence length of about 50 nm which provides it enough rigidity to be a candidate component of molecular machinery (Robinson et al, 1987; Smith et al, 1996; Seeman et al, 2002; Seeman, 2003).

The ATP synthase is a rotary motor found in all the organisms. The flow of protons provides the energy for the synthesis of ATP. Extensive study of ATP synthase was done on bacteria (Ducan et al., 1996). The bacterial flagella motor utilizes the energy from ATP hydrolysis to produce rotational motion. It is more powerful than other molecular motors, yet no device has been developed due to the difficulty of isolating the motor outside the cell (Ryu et al., 2000)

2.2.4 Thermal Brownian Molecular Motors

Objects under a microscope appear to be undergoing continuous random motion-jiggling. This is due to the constant bombardment of a microscopic object by atoms and molecules. Atoms and molecules are always in constant motion and they collide frequently with other larger objects, making the object, look like it is jiggling around by itself. One cannot see the atoms bombarding the larger object. Due to imbalance in impacts, hence the object moves one way and then another in an apparent random motion. Brownian motion was first described by the botanist Robert Brown in 1828. The Brownian motion was first perceived as uninterrupted and irregular
swarming motion. The motion was ascribed to the thermal molecular motion of the liquid. Einstein in 1905 was the first to formulate a correct picture of the entire problem. But when trap is there, the Brownian motion will be around the mean position forming a envelop around the mean position. The Brownian motion of the particles was studied by the Nakroshis et al (2002). In this study, Boltzmann’s constant $k$ was measured by observing the Brownian motion of polystyrene spheres in water.

2.3 Object tracking software

A number of methods have been used for tracking single particles. All include two basic steps. The first is segmentation, in which multiple particles in a field of view are identified and discriminated. Subsequently, an algorithm tracks the particles individually to monitor their displacement between successive video frames. Tracking algorithms used to date have included cross correlation of subsequent images (Gelles et al., 1988; Kusumi et al., 1993; Guilford and Gore, 1995), calculating the center-of-mass (centroid) of the object of interest and directly fitting Gaussian curves to the intensity profile (Anderson et al., 1992; Schutz et al., 1997). Many laboratories develop custom-written computer programs for analyzing the data, and incorporate additional thresholds and filters to improve the consistency of their results. Finding the best algorithm for use under these conditions, and knowing its limitations, is vital.

2.4 Instruments for Nanotechnology

The invention of the optical microscopes in the seventeenth century made it possible to have a glimpse of the world of micro-organisms like bacteria, etc. (Chowdhury D. et al., 2008). But, it is impossible to see a molecule directly under an optical microscope of conventional design because nature has imposed a limit on the resolution that can be achieved with these optical instruments. The optical microscopes merely enhance the power of our visionary perception. Therefore, in principle, it should be possible to achieve higher resolution if X-rays or γ-rays are used for imaging although we can no longer use our eyes as detector. Furthermore, in principle, it is possible to reconstruct the shape of an object, without seeing or touching it, by throwing balls at it from all sides and, then, analyzing the way the balls are scattered by the object. A
sufficiently high resolution microscope can be constructed if a charged particle is selected and it is accelerated to the required momentum by applying an external electric field. Electrons are most convenient for this purpose; an electron beam can be easily bent and focused using a suitable magnetic field configuration. Electron microscopy is one of the most powerful experimental techniques for determination of the structures of molecular machines.

For visualization of the conformational changes or movements of the molecule under investigation in a single molecule experiment, a prior attachment of a label to the molecule is essential. Fluorescence microscopy provided a glimpse of single molecules. Imaging a fluorescently labeled molecular motor in real time enables us to study its dynamics. The term “fluorescence” was first used by George Gabriel Stokes in 1852, after observing the strong response of fluorspan to ultraviolet light. But, it was until 1903 when Koeler and Wohn Rohr built an ultraviolet microscope with quartz optics. Fluorescence is a molecular phenomenon in which a substance absorbs light of some color and almost instantaneously radiates light of another color, one of lower energy and thus longer wavelength. This process is known as excitation and emission. If the investigated specimen does not fluoresce naturally, a fluorescent label can be attached to it. For example, non-fluorescent actin filaments can be labeled with a fluorescent dye called rhodamine phalloidin. This molecule absorbs most at 557 nm, whereas the emission maximum is at 576 nm.

### 2.4.1 Optical Tweezer

Even though optical tweezers is a rather young technique, the basic principal behind trapping of particles by light has been known for a long time. It was Kepler long ago when he predicted that Light can exert forces to matter. But in 1970, the initial step towards manipulations of atoms and molecules were demonstrated using intense focused light by Arthur Ashkin (Ashkin, 1970). He noted that optical forces on microscopic object and demonstrated that a laser beam can exert axial forces and push particles across an aqueous chamber (Ashkin, 1971). After a few years in 1986, Ashkin demonstrated trapping of particles using single gradient beam (Ashkin, 1986). After one year he publishes paper on Optical trapping and manipulation of single cells using infrared-laser beams (Ashkin, 1987). In the same year virus and bacteria were trapped and manipulated (Ashkin, 1987). Since then the application of trapping the particles has allowed the multi
discipline researchers to work under one roof in the broad field of nanotechnology. With the introduction of optical tweezers, it is possible to measure forces in the range of 0.01 – 200 pN (Molly et al., 2002). The trapped bead can be moved with constant velocity relative to suspended media with respect to the position of the trap. The bead will be pulled out of the trap along with the bead motion due to the viscous or Stokes’ drag of the fluid on the bead. Trap stiffness tends to be in the range 0.001 to 1 pN nm$^{-1}$. To Calibrate the forces acting on polystyrene bead under optical trap, viscous drag force actin on the bead as well as Brownian motion has to be studied (Scholz et al., 2004).

In early work using the “handles technique,” Block et al. (Block et al., 1990) attached single kinesin motor molecules to spheres and placed them directly onto microtubules where they could be activated by ATP. This new technique greatly improved on earlier in vitro motility assays that used many motors and relied on random diffusion for attachment to filaments. Steve Chu and his group have manipulated single DNA molecules by attaching polystyrene spheres to the ends of a DNA molecule, and measured its elasticity by pulling apart the two spheres and stretching the molecule using optical tweezers (Chu, 1991).

A landmark in the study of single motor proteins came in 1993, when the individual steps taken by the molecular motor, kinesin, were measured as it walked along a fixed microtubule track (Svoboda et al., 1993; Kuo, 1993). In this study, a single kinesin molecule was attached to a plastic microsphere and held in a low stiffness optical tweezers. When presented to a fixed microtubule the kinesin molecule bound and walked along it, taking a succession of tiny (8 nm) steps. Individual steps were identified using an interferometric detection system with sub-nanometer position sensitivity. Finer et al. (1994) studied the interaction of actin with myosin in a dual trap scheme that suspended the actin filament over a single myosin molecule. They observed stepwise motion of 11 nm and forces of 3–4 pN. The non-destructive trait and the versatility of the technique has provided a means of studying molecular mechanical properties (Yin et al., 1995; Wang et al., 1998). A distinct advantage of optical trapping techniques is the degree of control over the applied force. The spring constant of the system can be readily increased or decreased by altering the laser power at the optical focus, providing access to a wide range of loading. Visscher et al. (1998) determined the stalling force for RNA polymerase and
kinesin motion, respectively, by gradually increasing the load on the molecules. This ability to rapidly increase, decrease, or terminate the load gives the tweezers the best time-dependent control of forces applied to single molecules. Brandao et al. (2003) measured the elasticity of red blood cells using optical tweezers. Grier et al. (2003) published a very good review paper on optical tweezers.

2.4.2 Atomic Force Microscope for imaging

Nanotechnology and nanoscience got popular in the early 1980s with developments with the invention of the scanning tunneling microscope (STM). The atomic force microscope was invented five years after the STM was invented. Scanning tunneling microscopy (STM) and atomic force microscopy (AFM) have redefined the concept of microscopy (Binnig et al., 1986). Both techniques are capable of providing topographical information about biomolecular structures that are absorbed at the solid-liquid interface. The atomic force microscope (AFM) is one of the most powerful tools for determining the surface topography of native biomolecules at sub nanometer. Unlike X-ray crystallography and electron microscopy (EM), the AFM allows biomolecules to be imaged not only under physiological conditions, but also while biological processes are at work. The AFM, a descendent of the scanning probe microscopy family, was invented by Binnig et al. (1986) following the advent of the scanning tunneling microscope in 1982 (Binnig et al., 1982). Since this time the AFM has become an established surface analytical technique, capable of high resolution imaging of insulating and conducting surfaces in a variety of environmental conditions, as well as sub molecular imaging of biological material (Hansma et al., 1994). The AFM has a theoretical force resolution of 10^-15 N (Smith, 1995), and employs probes with a tip apex radius of 10-15 nm (Thundat et al., 1992) providing contact regions as small as 10 nm. Atomic force microscope has attracted the attention of biologists, since it theoretically combines the two most important aspects for studying structure-function relationships of biological objects: high-resolution imaging with high signal-to-noise ratio in the molecular/sub molecular range and the ability to operate in aqueous environments, allowing the observation of dynamic molecular events in real-time and under somewhat physiological conditions.
2.5 Bionanodevices

Movement is one of life's central attributes from a tiny insect to from the dashing leopard. In an influential paper, published in 1998, Bruce Alberts emphasized that "the entire cell can be viewed as a factory that contains an elaborate network of interlocking assembly lines, each of which is composed of a set of large protein machines" (Albert, 1998). Just like their macroscopic counterparts, molecular machines have an "engine", an input and an output. Some of these machines are analogous to motors whereas some others are like pumps; both linear and rotary motors have been identified. Some motors move on protein filaments whereas others move on nucleic acid strands (i.e., DNA or RNA). Simmel et al (2001) gave the concept of powering nanoactuator using DNA. In spite of the striking similarities, it is the differences between molecular machines and their macroscopic counterparts that makes the studies of these systems so interesting. Biomolecular machines are usually protein or macromolecular complex. These operate in a domain far from thermodynamic equilibrium where the appropriate units of length, time, force and energy are, nano-meter, milli-second, pico-Newton and kBT, respectively (kB being the Boltzmann constant and T is the absolute temperature). The viscous forces and random thermal forces on a nano-machine dominate over the inertial forces. These are made of soft matter and are driven by "isothermal" engines. Howard (2001) discussed about the low reynold number flow for the motor proteins and compared with macroscopic devices. Molecular motors can convert chemical energy directly into mechanical energy. As filamentous actin filaments are typically some μm long, which is many order of the magnitude they are having in lateral direction i.e. diameter. Therefore it may be justified to approximate the filament as a homogeneous elastic rod. A homogeneous elastic material is usually characterized by its Young's Modulus (Landau et al., 1986).

If the input energy directly causes a conformational change of the protein machinery which manifests itself a mechanical stroke of the machine, the operation of the machine is said to be driven by a "power stroke" mechanism. This is also the mechanism used by all man made macroscopic machines. If the machine exhibits "forward" and "backward" movements because of spontaneous thermal fluctuations and energy input is utilized to prevent "backward"
movements, but allow the “forward” movements, the system will exhibit directed, albeit noisy, movement in the “forward” direction.

The feasibility of hybrid organic/inorganic nanodevices has been recently demonstrated and opens up the route towards functional nano-electro-mechanical systems powered by biomolecular motors. Protein molecular motors can potentially be integrated in hybrid nanodevices that use biomolecules and micro/nanofabricated structures, which use molecular motors for sensing, transduction and actuation of biomolecular recognition events. One important class of theoretical nanodevices that has been designed is a gas-powered molecular motor or pump.

The actomyosin system may be used for transportation of cargoes in a “factory on a chip” application or it may be used to power nanomechanical devices (Blackwell et al., 2007). For such purposes, one prerequisite is the control of the directionality of mobile elements (e.g. actin) movement. One way to achieve ordered actomyosin systems in vitro is to utilize lithographical techniques from the microelectronics industry.

The Nanomaterials like carbon nanotubes may be integrated with the Biomotors to conceptualize a nanodevices and drug delivery applications. The carbon nanotubes are the stiffest material having young’s modulus of 1 TPa which is 100 times stiffer than mild steel. There is also preliminary experimental evidence suggesting that carbon nanotubes may act as electromechanical actuators for artificial muscles and that upon fictionalization with suitable bioactive molecules carbon nanotubes may serve as feasible substrates for neuronal growth. But, the dispersion of CNTs is difficult due to high surface interactions between the tubes. Salvetat et al. studied the effect of dispersion of CNTs on the mechanical properties of polymer/CNT composites, and found that poor dispersion and rope-like entanglement of CNTs led to drastic weakening of the composites. But, it is still very challenging to use carbon nanotubes as a drug carrier over the moving with the Biomotors reach at a specific site of disease.

Nanorobot based on Biomotors now seems possible due to the advances made in the field of nanotechnology. Nanorobot need motors to provide motion, pumps to move materials, and power sources to drive mechanical activities. Nanorobot will need to acquire information from their environment to properly execute their assigned tasks. Such acquisition can be achieved
using onboard nanoscale sensors, or nanosensors, of various types which are currently the subject of much experimental research (Chowdhury et al. 2008, Aggarwal et al, 2010).