Phase II NIAC Grant

FINAL REPORT

BIO-NANO-MACHINES FOR SPACE APPLICATIONS

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List of Contributors

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Overview

This Phase II NIAC grant started on September 1, 2004. The project goals included:

a) The identification and study (computationally and experimentally) of protein and DNA configurations that can be used as bio-nano-machine components.

b) The design of two macro-scale devices for space application that will be using bio-nano-component assemblies. These devices are:

   b.1) The *Networked TerraXplorer* (NTXp) that is a long and light-weight network of channels containing millions of bio-nano-robotic elements with ultra-enhanced sensing and signaling capabilities for the detailed mapping and exploration of very large planetary surfaces.

   b.2) The *All Terrain Astronaut Bio-Nano Gears* (ATB) that will serve as an extra layer of shield on the astronaut providing early detection and protection against dangerous and harmful environments or aiding in healing damages caused to the astronaut.

During the two year period for the project, the following research activities took place and are described in detail in this report:

List of Research Activities
1. Establishment of the design requirements for the NTXp and the ATB due to the planetary environmental conditions
2. Identification and characterization of the bionano machine components for use in space
3. Initiation of the system level design of NTXp
4. Detailed description of the design and control principles of bionanorobotic systems. This chapter on bionanorobotic systems was written to serve as our guideline for the design and control of all bionano components for the NTXp and the ATB and will be included in the final report of this project.
5. Identification and characterization of four bionano machine components for use in space.
6. Sensor-signaling dynamics.
7. Description of the computational framework for the optimization of the bio nano components.
8. Setup of the computational studies on the effect of UV radiations of the bio nano components. Ab initio studies on small atomic systems and amino acids
9. Effect of temperature on bio-nano-components.
10. Effect of temperature on the atomic level.
11. Nanofluidics actuator for NTXp transport mechanism.
12. Design of ATB and radiation responsive molecular assembly for astronauts.
13. Listing of future work and goals of this non invasive design for detecting space radiations.
14. A brief overview of the world's toughest bug which can sustain very high degree of radiations.

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Chapter 1: Bionanorobotics - A Field Inspired by Nature

1.1 Introduction

The underlying principle of biomimetics deals with the understanding, conceptualization and mimicking nature's way of handling various problems and situations. Nature has inspired mankind for ages and has been a key source from which we can learn and adapt. Natural processes are extremely efficient in terms of energy and material usage and provide us with many inspiring and thought provoking designs and principles. This chapter discusses biomimetics at the nano scale, where we talk about nanorobotics and its design principles which are inspired by nature's way of doing things at that scale.

Figure 1-1 describes the biomimetics principle and details the various aspects of mimetics. It explains the mimetics at two levels when nano scale is considered. One is the "*machine nano mimetics*" principle meaning the creation of nano-machine components inspired by the equivalent machine components at the macro-scale and the other is the "*bio nano mimetics*" principle where biological entities such as proteins and DNA are used to create the nano-machine components. The field of nanorobotics hence encapsulates these two mimetic principles, and inherits their various characteristics, design logic and advantages.

Nanotechnology can best be defined as a description of activities at the level of atoms and molecules that have applications in the real world. A nanometer is a billionth of a meter, that is, about 1/80,000 of the diameter of a human hair, or 10 times the diameter of a hydrogen atom. The size-related challenge is the ability to measure, manipulate, and assemble matter with features on the scale of 1-100nm. In order to

achieve cost-effectiveness in nanotechnology it will be necessary to automate molecular manufacturing.

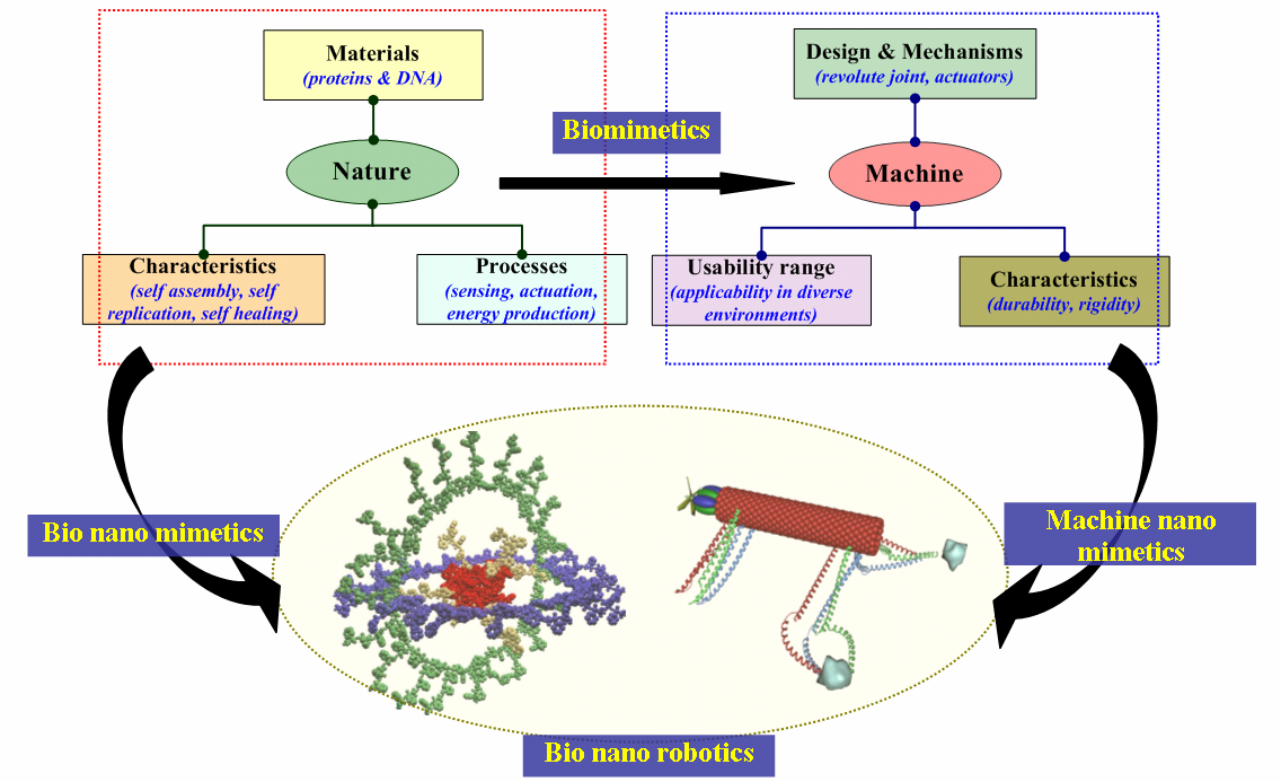


Figure 1-1: *Biomimetics – bio nanorobotics, inspired by nature and machine.*

The engineering of molecular products needs to be carried out by robotic devices, which have been termed *nanorobots* [Freitas, 1999, 2003]. A nanorobot is essentially a controllable machine at the nano meter or molecular scale that is composed of nano-scale components and algorithmically responds to input forces and information. The field of nanorobotics studies the design, manufacturing, programming and control of the nano-scale robots.

This review focuses on the state of the art in the emerging field of nanorobotics and its applications and discusses in brief some of the essential properties and dynamical laws which make this field more challenging and unique than its macro scale counterpart. This chapter is only reviewing nano-scale robotic devices and does not include studies related

to nano precision tasks with macro robotic devices that usually are also included in the field of nano-robotics (e.g. ATMs and other forms of proximal probe microscopy).

Nanorobots would constitute any active structure (nano scale) capable of actuation, sensing, signaling, information processing, intelligence, and swarm behavior at nano scale. These functionalities could be illustrated individually or in combinations by a nano robot (swarm intelligence and co-operative behavior). So, there could be a whole genre of actuation and sensing or information processing nano robots having ability to interact and influence matter at the nano scale. Some of the characteristic abilities that are desirable for a nanorobot to function may include:

i. *Swarm Intelligence* – decentralization and distributive intelligence
ii. *Self assembly and replication* – assemblage at nano scale and *'nano maintenance'*
iii. *Nano Information processing and programmability* – for programming and controlling nanorobots (autonomous nanorobots)
iv. Nano to macro world *interface architecture* – an architecture enabling instant access to the nanorobots and its control and maintenance

There are many differences between macro and nano-scale robots. However, they occur mainly in the basic laws that govern their dynamics. Macro scaled robots are essentially in the Newtonian mechanics domain whereas the laws governing nanorobots are in the molecular quantum mechanics domain. Furthermore, uncertainty plays a crucial role in nanorobotic systems. The fundamental barrier for dealing with uncertainty at the nano scale is imposed by the quantum and the statistical mechanics and thermal excitations. For a certain nano system at some particular temperature, there are positional uncertainties that can not be modified or further reduced [Drexler, 1992].

The nanorobots are invisible to the naked eye, which makes them hard to manipulate and work with. Techniques like Scanning Electron Microscopy (SEM) and Atomic Force Microscopy (AFM) are being employed to establish a visual and haptic interface to enable us to sense the molecular structure of these nano scaled devices. Virtual Reality (VR) techniques are currently being explored in nano-science and bio-technology research as a way to enhance the operator's perception (vision and haptics) by approaching more or less a state of 'full immersion' or 'telepresence'. The development of nanorobots or nano machine components presents difficult fabrication and control challenges. Such devices will operate in microenvironments whose physical properties differ from those encountered by conventional parts. Since these nano scale devices have not yet been fabricated, evaluating possible designs and control algorithms requires using theoretical estimates and virtual interfaces/environments. Such interfaces/simulations can operate at various levels of detail to trade-off physical accuracy, computational cost, number of components and the time over which the simulation follows the nano-object behaviors. They can enable nano-scientists to extend their eyes and hands into the nano-world, and they also enable new types of exploration and whole new classes of experiments in the biological and physical sciences. VR simulations can also be used to develop virtual assemblies of nano and bio-nano components into mobile linkages and to predict their performance.

Nanorobots with completely artificial components have not been realized yet. The active area of research in this field is focused more on molecular machines, which are thoroughly inspired by nature's way of doing things at nano scale. Mother Nature has her own set of molecular machines that have been working for millions of years, and have

been optimized for performance and design over the ages As our knowledge and understanding of these numerous machines continues to increase, we now see a possibility of using the natural machines, or creating synthetic ones from scratch, using nature's components. This chapter focuses more on molecular machines and explores various designs and research prevalent in this field. The main goal in the field of molecular machines is to use various biological elements — whose function at the cellular level creates motion, force or a signal — as machine components. These components perform their preprogrammed biological function in response to the specific physiochemical stimuli but in an artificial setting. In this way proteins and DNA could act as motors, mechanical joints, transmission elements, or sensors. If all these different components were assembled together in the proper proportion and orientation they would form nano devices with multiple degrees of freedom, able to apply forces and manipulate objects in the nanoscale world. The advantage of using nature's machine components is that they are highly efficient and reliable.

Nanorobotics is a field which calls for collaborative efforts between physicists, chemists, biologists, computer scientists, engineers and other specialists to work towards this common objective. *Figure 1-2* details the various fields which come under the field of bio nanorobotics (this is just a representative figure and not exhaustive in nature). Currently this field is still developing, but several substantial steps have been taken by great researchers all over the world who are contributing to this ever challenging and exciting field. The ability to manipulate matter at the nano scale is one core application for which nanorobots could be the technological solution. A lot has been written in the literature about the significance and motivation behind constructing a nanorobot.

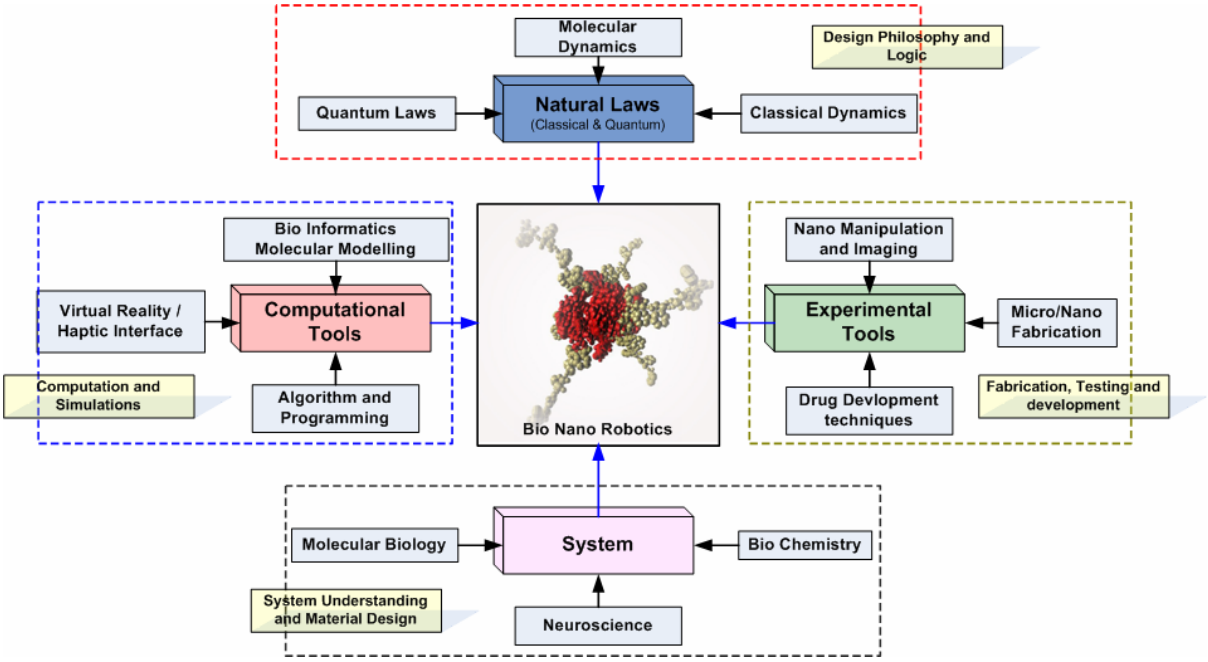


Figure 1-2: *Bio nanorobotics – a truly multidisciplinary field.*

The applications range from medical to environmental sensing to space and military applications. Molecular construction of complex devices could be possible by nanorobots of the future. From precise drug delivery to repairing cells and fighting tumor cells; nanorobots are expected to revolutionize the medical industry in the future. These applications come under the field of nanomedicine [Freitas, 1999, 2003] which is a very active area of research in nanotechnology. These molecular machines hence form the basic enablers of future applications.

In the next section, we shall try to understand the principles, theory and utility of the known molecular machines and look into the design and control issues for their creation and modification. A majority of natural molecular machines are protein-based which involve using the exact replica of nature's mechanism, while the DNA-based molecular machines use the basic properties of DNA to design various synthetic mechanisms (which might not be present in the nature). Nature deploys proteins to perform various

cellular tasks – from moving cargo to catalyzing reactions, while it has kept DNA as an information carrier. It is hence understandable that most of the natural machinery is built from proteins. With the powerful crystallographic techniques available in the modern world, the protein structures are clearer than ever. The ever increasing computing power makes it possible to dynamically model protein folding processes and predict the conformations and structure of lesser known proteins [Rohl *et al*, 2004]. All this helps unravel the mysteries associated with the molecular machinery and paves the way for the production and application of these miniature machines in various fields including medicine, space exploration, electronics and military.

1.2 Biomolecular Machines: Background and Significance

1.2.1 Significance

The recent explosion of research in nanotechnology, combined with important discoveries in molecular biology have created a new interest in biomolecular machines and robots. The main goal in the field of biomolecular machines is to use various biological elements — whose function at the cellular level creates motion, force or a signal, stores information — as machine components. These components perform their preprogrammed biological function in response to the specific physiochemical stimuli but in an artificial setting. In this way proteins and DNA could act as motors, mechanical joints, transmission elements, or sensors. If all these different components were assembled together in the proper proportion and orientation they would form nanodevices with multiple degrees of freedom, able to apply forces and manipulate objects in the nanoscale world. The advantage of using nature's machine components is that they are highly efficient [Kinosita *et al*.], and reliable. Just as conventional macro machines are

used to generate forces and motions to accomplish specific tasks, bio nanomachines can be used to manipulate nano-objects, to assemble and fabricate other machines or products, to perform maintenance, repair and inspection operations.

Such bionanorobotic devices will hopefully be part of the arsenal of future medical devices and instruments that will: 1) perform operations, inspections and treatments of diseases inside the body, and 2) achieve ultra-high accuracy and localization in drug delivery, thus minimizing side effects. *Figure 1-3* shows an idealized rendition of a biomolecular nanorobot repairing an infected cell in a blood vessel.

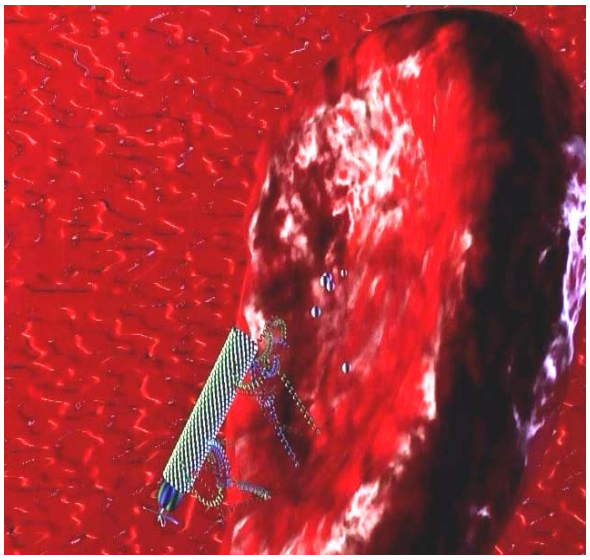


Figure 1-3: *A "nanorobot" flowing inside a blood vessel, finds an infected cell. The nanorobot attaches to the cell and projects a drug to repair or destroy the infected cell.*

The bionanorobot will be able to attach to the infected cell alone, and deliver a therapeutic drug that can treat or destroy just the infected cell, sparing the surrounding healthy cells. Development of robotic components composed of simple biological molecules is the first step in the development of future biomedical nanodevices. Since the planned complex systems and devices will be driven by these components, we must first develop a detailed understanding of their operation. From the simple elements such as

structural links to more advanced concepts as motors, each part must be carefully studied and manipulated to understand its functions and limits.

Figure 1-4 lists the most important components of a typical robotic system or machine assembly and the equivalence between macro and potential bionanocomponents. Beyond the initial component characterization is the assembly of the components into robotic systems.

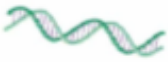
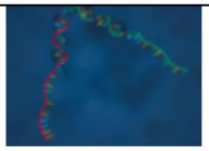
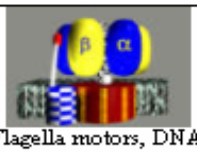
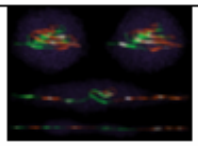
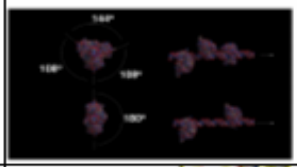
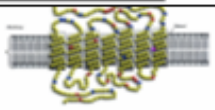
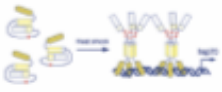
COMPONENT	MACROROBOTS	BIONANOROBOTS
Structural Elements- Links	Metal, Plastic Polymer	DNA  Nanotubes 
Joints	Metal, Plastic Polymer Revolute  Prismatic  Spherical  Cylindrical 	 DNA hinge Molecular bonds, synthetic joints
Actuators	Electric Motors, Pneumatic motors, Hydraulic motors, Smart material based actuators	 ATPase protein Flagella motors, DNA actuators, Viral Protein Motors etc
Transmission Elements Springs (Metal, Polyvinyl) Bearings Gears	 	 β Sheets Molecular camshaft design [Smith, 2001] 
Sensors	Light sensors, force sensors, position sensors, temperature sensors	 Rhodopsin,  Heat Shock Factor

Figure 1-4: *Macro and Bionano Equivalence of Robot Components*

Figure 1-5 shows one such concept of a nano-organism, with its 'feet' made of helical peptides and its body using carbon nanotubes while the power unit is a biomolecular motor. For this phase to be successful, a library of biological elements of every category must be available. At that point, conventional robotics can be used as a guide for fabrication of bionanorobots that function in the same manner. There will be systems that have mobile characteristics to transport themselves, as well as other objects, to desired locations. Some bionanorobots can be conceived as able to manufacture additional elements and various structures. There may also be robots that not only perform physical labor, but also sense the environment and react accordingly. There is no doubt that biomedical applications will be both a driving force and a beneficiary of these developments.

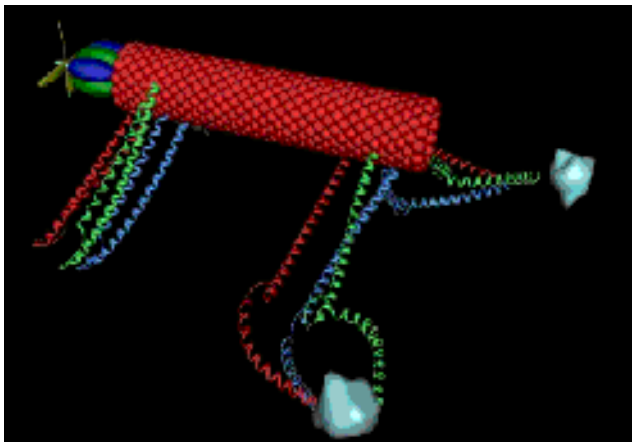


Figure 1-5: *The biological elements will be used to fabricate robotic systems. A vision of a nano-organism: carbon nanotubes form the main body; peptide limbs can be used for locomotion and object manipulation, a biomolecular motor located at the head can propel the device in various environments.*

1.2.2 Brief Review of Biomolecular Machines

While the majority of the prior research in this field has largely focused on biomolecular motors, several other nano-components such as sensors and even assemblies of components in the form of mechanisms have been studied. In the macroscopic world, what we understand by a 'motor' is a machine capable of imparting motion associated by the conversion of energy. Biomolecular Motors have attracted a lot of attention recently because: 1) they operate at high efficiency, 2) some could be self-replicating and hence cheaper in mass usage, and 3) they are readily available in nature [Boyer, 1998]. A number of enzymes function as nanoscale biological motors, such as kinesin [Block, 1998; Schnitzer and Block, 1997], RNA polymerase [Wang et al, 1998], myosin [Kitamura et al 1999], and adenosine triphosphate (ATP) synthase function as nanoscale biological motors [Montemagno and Bachand, 1999; Bachand and Montemagno, 2000; Soong et al, 2000; Noji et al, 1997; Yasuda et al, 1998; Walker, 1998].

1.2.2.1 The ATPase Motor

One of the most abundant rotary motors found in life forms is F0F1 ATP synthase, commonly known as the "ATPase Motor". Oxidative Phosphorylation was demonstrated over 50 years ago as an important process by which our bodies capture energy from the food that we eat. The mechanism of this process was not known until 1997, when Boyer and Walker described the key role that ATP plays in the process [Boyer, 1998; Walker, 1998]. Noji et al published the structural and performance data of the ATPase motor in 1997 [Noji et al, 1997; Yasuda et al, 1998]. According to this study, the γ subunit, which is about 1 nm in diameter, rotates inside the F1 subunit, which is about 5 nm in diameter,

to produce approximately 40 pN-nm of rotary torque. Montemagno and his group were the first to indicate that the rotation of the γ subunit of the ATPase motor could be mechanically useful based on fabricated nanomechanical inorganic devices which could be compatible with the force production and dimensions of the molecular motors [Montemagno and Bachand, 1999; Bachand and Montemagno., 2000; Soong et al, 2000]. Frasch's group is currently studying the binding of metals to amino acids of the motor protein. These experiments are providing new insights into the means by which the energy obtained from the hydrolysis of ATP can be converted into the physical action of pumping a proton in a unilateral direction [Frasch, 2000].

1.2.2.2 Kinesin and Myosin

Motor proteins are tiny vehicles that transport molecular cargoes within cells. These minute cellular machines exist in three families: the kinesins, the myosins and the dyneins [Farrell et al, 2002]. Conventional kinesin was found to be a highly processive motor that could take several hundred steps on a microtubule without detaching [Block et al, 1990; Howard et al, 1989], whereas muscle myosin has been shown to execute a single "stroke" and then dissociate [Finer et al, 1994]. A detailed analysis and modeling of these motors has been done [Vale and Milligan, 2000]. Hackney's group has concentrated upon the usage of ATP energy by motors like kinesin, myosin, dynenin and related motor families [Hackney, 1996]. Unger's group is currently working towards developing a microtubule-kinesin system as a biological linear-motoric actuator. Their work is aimed at producing force multiplication by parallel action of numerous single driving units as well as a more efficient means for system control [Bohm et al, 1997]. Other researchers have discovered a new member of the myosin-V family (Myo5c) and

have implicated this myosin in the transport of a specific membrane compartment [Mehta et al, 1999]. The role of ATP hydrolysis in kinesin motility has also been recently described [Farrell et al, 2002].

1.2.2.3 The Flagella Motors

Escherichia coli and similar organisms are equipped with a set of rotary motors only 45 nm in diameter. Each motor drives a long, thin, helical filament that extends several cell body lengths out into the external medium. In addition to rotary engines and propellers, E. coli's standard accessories include particle counters, rate meters, and gearboxes, and thus have been described as a nanotechnologist's dream [Berg, 2000]. Berg developed one of the earliest models for the rotary motor [Berg, 1974]. Improved models came in 1992 [Ueno et al, 1992; Ueno et al, 1994]. Flagella motor analysis coupled to real-time computer assisted analysis of motion has also been performed [Khan et al, 1998]. Researchers in Japan have applied crystallographic studies in order to understand the molecular structure of flagella motors as well as that of kinesin [Namba and Vonderveczt, 1997]. Finally, Hess' group is attempting to build a nanoscale train system, complete with tracks, loading docks and a control system. Since motor proteins are a thousand times smaller than any man-made motor, they aim to utilize them in a synthetic environment as engines powering the nanotrains [Hess and Vogel, 2001].

1.2.2.4 Other Motors and Mechanisms

In addition to work on naturally existing motors, considerable effort is also being applied to develop synthetic molecular motors. The structure of the ATP synthase, a rod rotating inside a static wheel, suggests the use of rotaxanes as potential artificial models

for natural motors [Harada, 2001]. Rotaxanes are organic compounds consisting of a dumbbell-shaped component that incorporates one or more recognition sites in its rod section and is terminated by bulky 'stoppers', encircled by one or more ring components. The possibility of manufacturing specific forms of rotaxane and creating molecular motors capable of guided rotary motion and the possibility of fueling such a motor by light, electrons and chemical energy has been proposed [Schalley et al, 2001].

Schemes for using pseudorotaxanes, rotaxanes and catenanes as molecular switches to perform chemical, electrochemical and photochemical switching, and controllable molecular shuttles have also been proposed recently in the literature [Balzani et al, 1998]. Molecular shuttles have been reported using α-cyclodextrin – a parent of rotaxanes and catenanes [Harada, 2001]. A light-driven monodirectional rotor made of helical alkene, with rotation around a central carbon-carbon covalent bond due to chirality has been reported [Koumura et al, 1999]. Another simple way to convert chemical energy into mechanical motion in a controlled fashion is by using a metal ion which can be translocated reversibly between two organic compartments with the change of its ionization state, controllable by redox reaction or pH change [Amendola et al, 2001]. Motility of unicellular organisms like vortecellids reminds us of energy storage and release by mechanical springs on a macromolecular scale. Spring-like action has been observed in sperm cells of certain marine invertebrates during fertilization. Springs and supramolecular ratchets by actin polymerization have yet to be built in vitro, but they theoretically can be generalized, as recently demonstrated [Mahadevan and Matsudaira, 2000].

1.2.2.5 DNA-Based Molecular Nanomachines, Joints and Actuators

Several researchers are exploring the use of DNA in nanoscale mechanisms. DNA is small, relatively simple and homogeneous, and its structure and function is well understood. The predictable self-assembling nature of the double helix makes it an attractive candidate for engineered nanostructures. This property has been exploited to build several complex geometric structures, including knots, cubes and various polyhedra [Seeman, 1998]. Mathematical analyses of the elastic structure of DNA using energy minimization methods have been performed to examine its molecular stability, wherein short DNA strands were treated as an elastic rod [Tobias et al, 2000]. Initial experiments on DNA visualization and manipulation using mechanical, electrical, and chemical means have been underway for a decade [Yuqiu et al, 1992; Hu et al, 2002]. A dynamic device providing atomic displacements of 2-6 nm was proposed in [Mao et al, 1999], wherein the chemically induced transition between the B and Z DNA morphologies acts as a moving nanoscale device. A method for localized element-specific motion control was seen in the reversible transition between four stranded topoisomeric DNA motifs (PX and JX2) thereby producing rotary motion [Yan et al, 2002]. A very important, though simple DNA machine that resembles a pair of tweezers has been successfully created, whose actuation (opening and closing) is also fueled by adding additional DNA fuel strands [Yurke et al, 2000].

1.2.3 Nanosensors

The technology of nanosensing is also under development. For example, silicon probes with single walled carbon nanotube tips are being developed [http://www.media.mit.edu/nanoscale/]. For sensing certain analytes, genetically

engineered versions of pore-forming proteins like Staphylococcus aureus alpha-hemolysin are also being studied [Kasianowicz and Bayley, 2003]. Efforts to detect biological warfare agents, like cholera toxins, by utilizing their ability to bind to a bilayer membrane in the presence of gangliosides are another example [Plant and Silin, 2003]. Light sensors could be made using certain photoreceptive polypeptides containing azobenzene or spyropyran units as they respond to light or dark environmental conditions by undergoing conformational change, e.g. transition from random coil to a α-helix [Pieroni et al, 2001]. An optical DNA biosensor platform has been reported using etched optical fiber bundles filled with oligonucleotide-functionalized microsphere probes [Ferguson et al, 1996]. Finally, work is in progress to develop sensors for brain implantation, which would foretell the development of a stroke and be useful for perioperative on-line monitoring during coronary by-pass surgery [Manning and McNeil, 2001].

In addition to many of the examples mentioned above which generally correspond to one degree of freedom rotary actuators, there are many other machine elements, the functional capabilities of which have not yet been represented by biomolecular elements. In addition, the assembly of different molecules in a multi-degree of freedom machine or the formation of hybrid systems composed of biomolecules and synthetic non-organic elements has not yet been explored. In this context, our long term goal is to identify novel biomolecules that can be used as different types of machine components and to assemble them into controlled multi-degree of freedom systems using organic and synthetic non-organic parts.

1.3 Design and Control Philosophies for Nanorobotic Systems

The design of nanorobotic systems requires the use of information from a vast variety of sciences ranging from quantum molecular dynamics to kinematic analysis. In this chapter we assume that the components of a nanorobot are made of biological components, such as proteins and DNA strings. So far, there is no particular guideline or a prescribed manner that details the methodology of designing a bio-nanorobot. There are many complexities that are associated with using bio components (such as protein folding and presence of aqueous medium), but the advantages of using these are also quite considerable. These bio components offer immense variety and functionality at a scale where creating a man made material with such capabilities would be extremely difficult. These bio components have been perfected by nature through millions of years of evolution and hence these are very accurate and efficient. As noted in the review section on Molecular Machines, F_1-ATPase is known to work at efficiencies which are close to 100%. Such efficiencies, variety and form are not existent in any other form of material found today. Another significant advantage in protein-based bio nano components is the development and refinement over the last 30 years of tools and techniques enabling researchers to mutate proteins in almost any way imaginable. These mutations can consist of anything from simple amino acid side-chain swapping, amino acid insertions or deletions, incorporation of non-natural amino acids, and even the combination of unrelated peptide domains into whole new structures. An excellent example of this approach is the use of zinc to control F_1-ATPase, which is able to rotate a nanopropeller in the presence of ATP. A computational algorithm [Hellinga and Richards, 1991] was used to determine the mutations necessary to engineer an allosteric zinc-binding site into

the F_1-ATPase using site-directed mutagenesis. The mutant F_1-ATPase would rotate an actin filament in the presence of ATP with average torque of 34 pN nm. This rotation could be stopped with the addition of zinc, and restored with the addition of a chelator to remove the zinc from the allosteric binding site [Liu *et al*, 2002]. This type of approach can be used for the improvement of other protein-based nanocomponents. These bio components seem to be a very logical choice for designing nanorobots. In addition since some of the core applications of nanorobots are in the medical field, using bio-components for these applications seems to be a good choice as they both offer efficiency and variety of functionality. This idea is clearly inspired by nature's construction of complex organisms such as, bacteria and viruses which are capable of movement, sensing and organized control. Hence our scope would be limited to the usage of these bio components in the construction of bio-nanorobotics. A roadmap is proposed which details the main steps towards the design and development of bio-nanorobots.

1.3.1 The Roadmap

The roadmap for the development of bio-nanorobotic systems for future applications (medical, space and military) is shown in *Figure 1-6*. The roadmap progresses through the following main steps:

Step 1: Bio Nano Components

Development of bio-nano components from biological systems is the first step towards the design and development of an advanced bio-nanorobot, which could be used for future applications (see *Figure 1-7*). Since the planned systems and devices will be composed of these components, we must have a sound understanding of how these behave and how they could be controlled. From the simple elements such as structural

links to more advanced concepts such as motors, each component must be carefully studied and possibly manipulated to understand the functional limits of each one of them. DNA and carbon nanotubes are being fabricated into various shapes, enabling possibilities of constructing newer and complex devices.

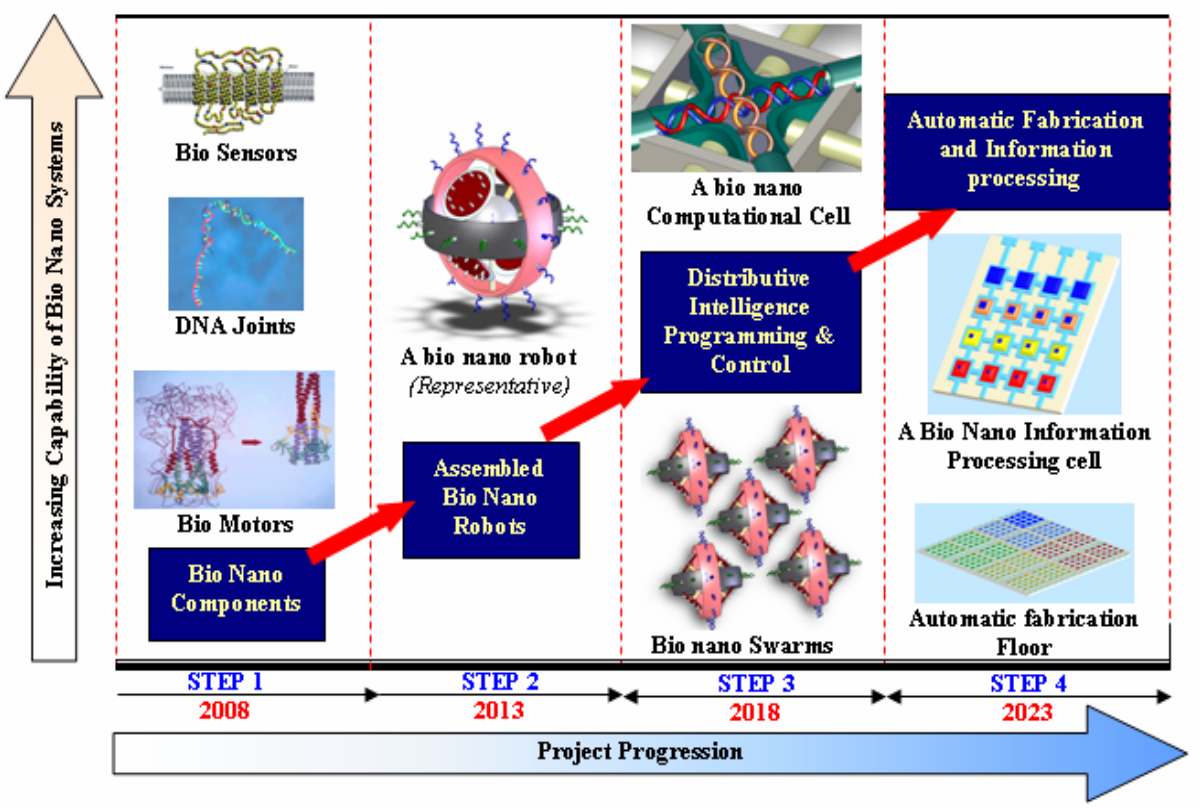


Figure 1-6: *The Roadmap, illustrating the system capability targeted as the project progresses.*

These nano-structures are potential candidates for integrating and housing the bio-nano components within them. Proteins such as *rhodopsin* and *bacteriorhodopsin* are a few examples of such bio-nano components. Both these proteins are naturally found in biological systems as light sensors. They can essentially be used as solar collectors to gather abundant energy from the sun. This energy could either be harvested (in terms of proton motive force) for later use or could be consumed immediately by other components, such as the ATP Synthase nano rotary motor. The initial work is intended to

be on the bio-sensors, such as heat shock factor. These sensors will form an integral part of the proposed bio-nano assemblies, where these will be integrated within a nano structure and will get activated, as programmed, for gathering the required information at the nano scale. Tools and techniques from *molecular modeling* and *protein engineering* will be used to design these modular components.

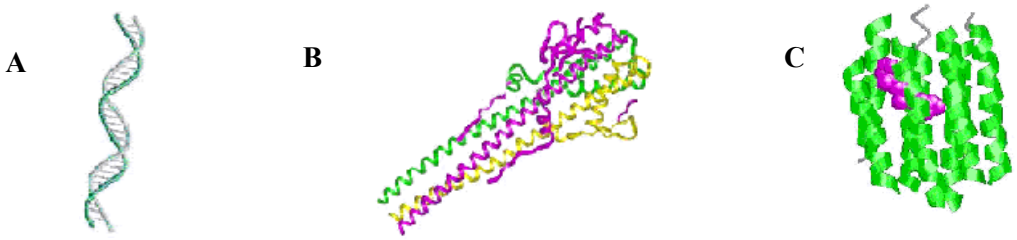


Figure 1-7 (Step 1): *Understanding of basic biological components and controlling their functions as robotic components. Examples are:* ***(A)*** *DNA which may be used in a variety of ways such as a structural element and a power source;* ***(B)*** *Hemagglutinin virus may be used as a motor;* ***(C)*** *Bacteriorhodopsin could be used as a sensor or a power source.*

Step 2: Assembled Bio Nano Robots

The next step involves the assembly of functionally stable bio-nano components into complex assemblies. Some examples of such complex assemblies are shown in *Figure 1-8*. This figure shows a conceptual representation of *modular organization* of a bio-nanorobot. The modular organization defines the hierarchy rules and spatial arrangements of various modules of the bio-nano-robots such as: the inner core (the brain / energy source for the robot); the actuation unit; the sensory unit; and the signaling and information processing unit. By the beginning of this phase a "*library of bio-nano components*" will be developed, which will include various categories such as, actuation, energy source, sensory, signaling etc. Thereafter, one will be able to design and develop such bio-nanosystems that will have enhanced mobile characteristics, and will be able to

transport themselves as well as other objects to desired locations at nano scale. Furthermore, some bio-nanorobots need to assemble various bio-components and nano-structures, including *in situ* fabrication sites and storage areas others will manipulate existing structures and maintain them. There will also be robots that not only perform physical labor, but also sense the environment and react accordingly. There will be systems that will sense an oxygen deprivation and stimulate other components to generate oxygen, creating an environment with stable homoeostasis.

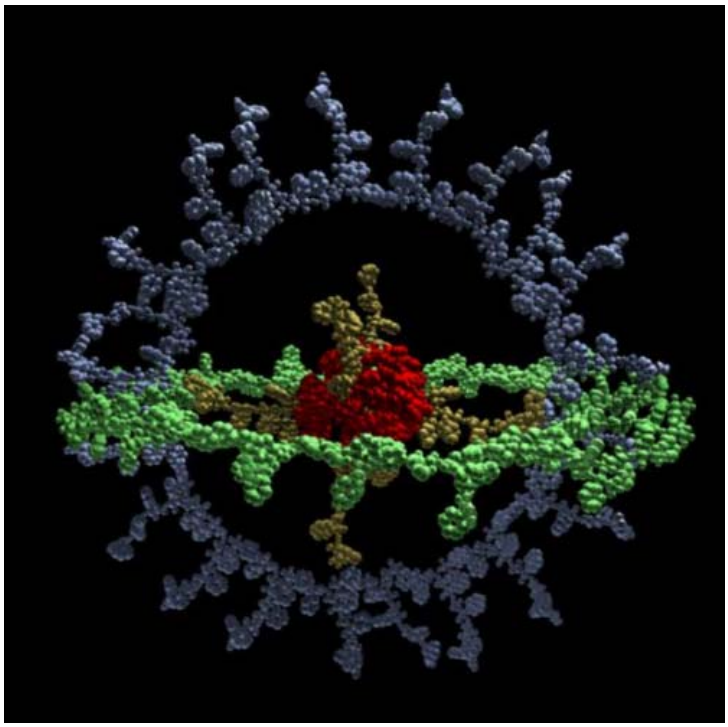


Figure 1-8 (Step 2): *Modular Organization concept for the bio-nano robots. Spatial arrangements of the various modules of the robots are shown. A single bio nano robot will have actuation, sensory and information processing capabilities.*

Step 3: Distributive Intelligence, Programming and Control

With the individual bio-nanorobots capable of basic functions, we would now need to develop concepts that would enable them to collaborate with one another to develop "colonies" of similar nanorobots. This design step could lay the foundation towards the

concept of *bio-nano swarms* (distributive bio-nanorobots) (see *Figure 1-9A*). Here work has to be done towards the control and programming of such swarms. This will evolve concepts like distributive intelligence in the context of bio nanorobots. Designing swarms of bio-nano robots capable of carrying out complex tasks and capable of computing and collaborating amongst them will be the focus of this step. Therefore, the basic computational architectures needs to be developed and rules need to be evolved for the bionanorobots to make intended decisions at the nano scale.

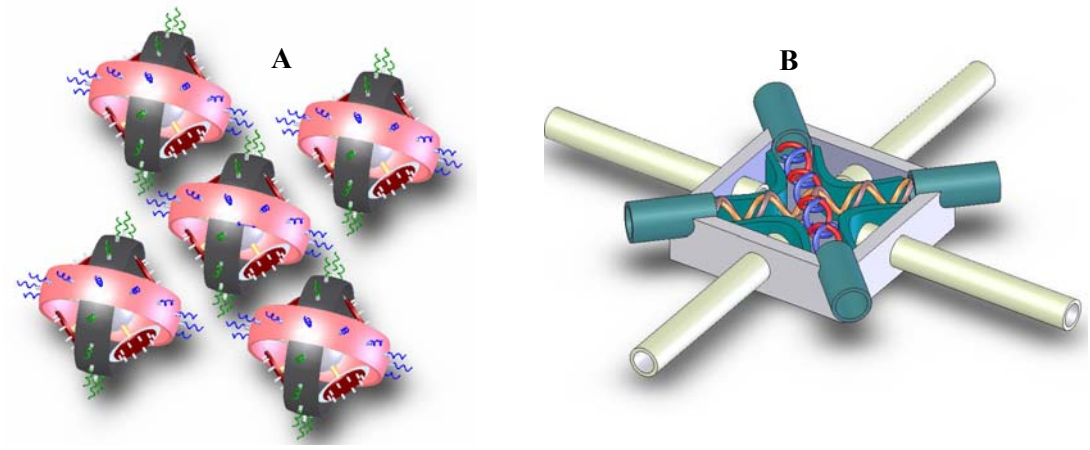


Figure 1-9 (Step 3): (A) *Basic bio nano robot forming a small swarm of five robots. The spatial arrangement of the individual bio nano robot will define the arrangement of the swarm. These swarms could be re-programmed to form bindings with various other types of robots. The number of robots making a swarm will be determined by the mission. Such swarms will attach additional bionanorobots at run time and replace any non functional ones.* ***(B)*** *A basic bio-nano computational cell. This will be based on one of the properties of the bio molecules, which is "reversibility".*

To establish an interface with the macro world, the computers and electronic hardware have to be designed as well. *Figure 1-10* shows the overall electronic communication architecture. Humans should be able to control and monitor the behavior and action of these swarms. This means that basic computational capabilities of the swarms will need

to be developed. A representative computational bio-nano cell, which will be deployed within a bio-nano-robot, is shown in *Figure 1-9B*. This basic computational cell will initially be designed for data retrieval and storage at the nano scale. This capability will enable us to program (within certain degrees of freedom) the swarm behavior in the bio-nano robots. We will further be able to get their sensory data (from nano world) back to the macro world through these storage devices. This programming capability would control the bio-nano robotics system and hence is very important.

Step 4: Automatic Fabrication and Information Processing Machines

Specialized bio-nanorobotic swarms would need to be designed to carry out complex missions, such as sensing, signaling and data storage. The next step in nanorobotic designing would see the emergence of automatic fabrication methodologies (see *Figure 1-11, which only shows the floor concept of assembling bionanorobots*) of such bio-nano robots *in vivo* and *in vitro*. Capability of information processing will be a key consideration of this step. This would enable bio-swarms to have capability of *adjusting* based on their interacting environment they will be subjected to. These swarms could be programmed for more than one energy source and hence would have an ability to perform in an alternate environment. Energy management, self-repairing, and evolving will be some of the characteristics of these swarms.

1.3.2 Design Architecture for the Bio-Nanorobotic Systems

a) Modular Organization: Modular organization defines the fundamental rule and hierarchy for constructing a bio-nanorobotic system. Such construction is performed through *stable integration* (energetically in the most stable state) of the individual *'bio-modules or components'*, which constitute the bio-nanorobot. For example, if the entity

ABCD, defines a bio-nanorobot having some *functional specificity* (as per the Capability Matrix defined in *Table 1*) then, A, B, C, and D are said to be the basic bio-modules defining it.

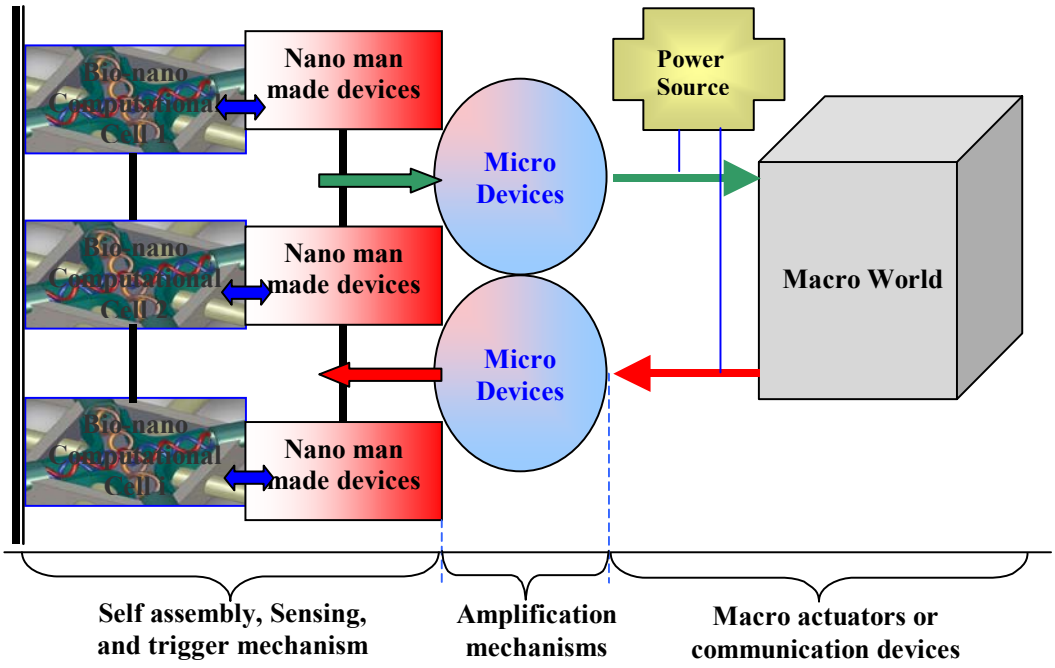


Figure 1-10: *Feedback path from nano to macro world route.*

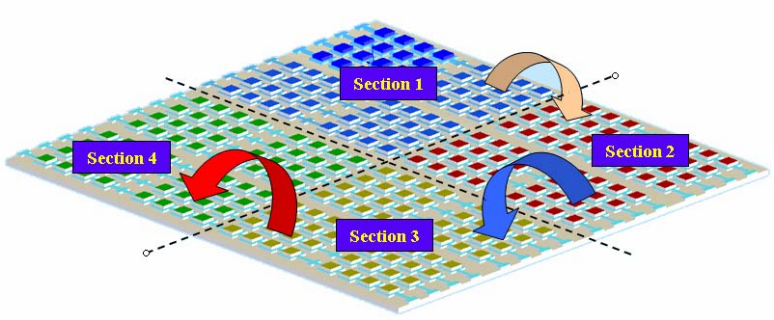


Figure 1-11 (Step 4): *An automatic fabrication floor layout. Different color represents different functions in automatic fabrication mechanisms. The arrows indicate the flow of components on the floor layout.* ***Section 1→*** *Basic stimuli storage – Control expression;* ***Section 2→*** *Bio molecular component manufacturing (actuator / sensor);* ***Section 3→*** *Linking of bio-nano components;* ***Section 4→*** *Fabrication of bio-nano robots (assemblage of linked bio-nano components).*

Functionality	Bio Nano Code	Capabilities Targeted	General Applications
Energy Storage and Carrier	**E**	Ability to store energy from various sources such as, Solar, chemical for future usage and for its own working	Supplies the energy required for the working of all the bio-chemical mechanisms of the proposed bio-nano-robotic systems
Mechanical	**M**	Ability to precisely move and orient other molecules or modules at nano scale. This includes ability to mechanically bind to various target objects, and carry them at desired location.	1. Carry moities and deliver them to the precise locations in correct orientations. 2. Move micro world objects with nano precision.
Input Sensing	**S**	Sensing capabilities in various domains such as, chemical, mechanical, visual, auditory, electrical, magnetic	Evaluation and discovery of target locations based on either chemical properties, temperature or others characteristics.
Signaling	**G**	Ability to amplify the sensory data and communicate with bio-systems or with the micro controllers. Capability to identify their locations through various trigger mechanisms such as fluorescence	Imaging for Medical applications or for imaging changes in Nano Structures
Information storage	**F**	Ability to store information collected by the sensory element. Behave similar to a read - write mechanism in computer field	1. Store the sensory data for future signaling or usage 2. Read the stored data to carry out programmed functions. 3. Back bone for the sensory bio-module 4. Store nano world phenomenon currently not observed with ease
Swarm Behavior	**W**	Exhibit binding capabilities with "similar" bio-nano robots so as to perform distributive sensing, intelligence and action (energy storage) functions	All the tasks to be performed by the bio-nano robots will be planned and programmed keeping in mind the swarm behavior and capabilities
Information Processing	**I**	Capability of following algorithms (Turing equivalent)	Programmable
Replication	**R**	Replicate themselves depending on the situation and requirement	1. Replicate by assembling raw components into nanorobots, and programming newly-made robot to form swarms that form automated fabricators. 2. Assemble particular bio-modules as per the demand of the situation, consistent with the Foresight Guidelines for safe replicator development [Foresight Institute, 2000]

Table 1: *Defining the Capability Matrix for the Bio-Modules*

The basic construction will be based on the techniques of molecular modeling with emphasis on principles such as *Energy Minimization* on the hyper surfaces of the bio-modules; *Hybrid Quantum-Mechanical and Molecular Mechanical* methods; *Empirical Force field* methods; and *Maximum Entropy Production* in least time. Modular organization also enables the bio-nanorobots with capabilities such as, organizing into

swarms, a feature, which is extremely desirable for various applications. *Figures 1-12A & B* show the conceptual representation of Modular Organization. *Figure 1-12C* shows a more realistic scenario in which all the modules are defined in some particular spatial arrangements based on their functionality and structure. A particular module could consist of other group of modules, or sub modules.

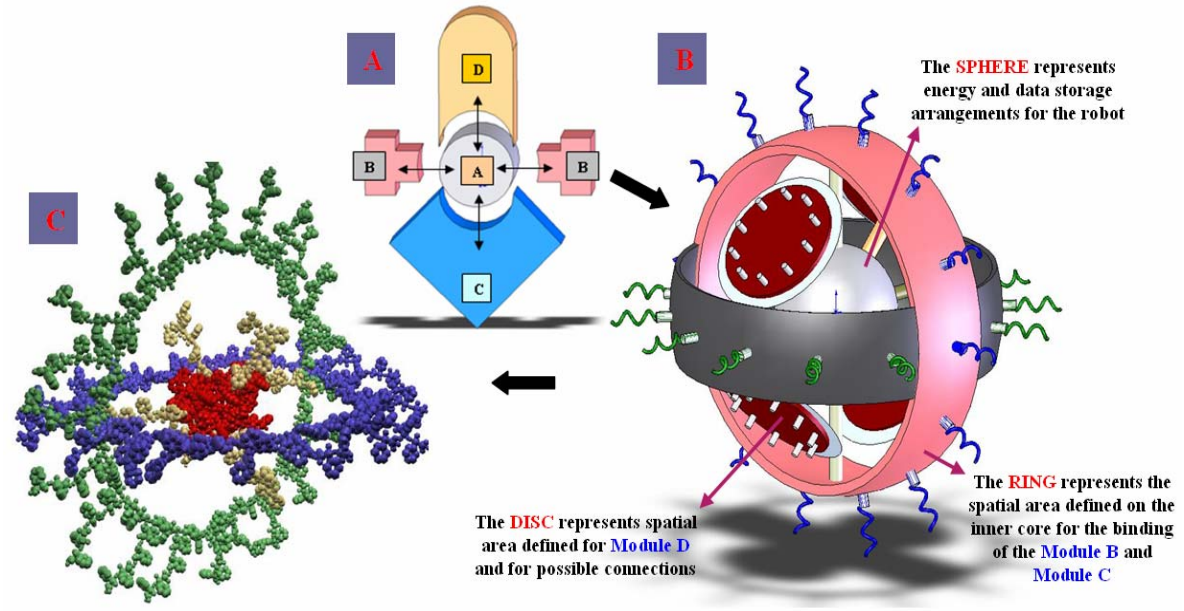


***Figure 1-12: (A) A** Bio-Nano-Robotic Entity 'ABCD', where A, B, C and D are the various Bio Modules constituting the bio-nano-robot. In our case these bio modules will be set of stable configurations of various proteins and DNAs. **(B)** A Bio-Nano-Robot (representative), as a result of the concept of Modular Organization. All the modules will be integrated in such a way so as to preserve the basic behavior (of self-assembly and self organization) of the bio-components at all the hierarchies. The number of modules employed is not limited to four or any number. It's a function of the various capabilities required for a particular mission. **(C)** A molecular representation of the figure in part B. It shows the red core and green and blue sensory and actuation bio-modules.*

The concept of *Bio Nano Code* has been devised, which basically describes the unique functionality of a bio nano component in terms of alphabetic codes. Each Bio Nano Code represents a particular module defining the structure of the bio nano robot. For instance, a code like **E-M-S** will describe a bio nano robot having capabilities of energy storage, mechanical actuation and signaling at the nano scale. Such representations will help in general classifications and representative mathematics of bio nanorobots and their swarms. *Table 1* summarizes the proposed capabilities of the bio-modules along with their targeted general applications. The Bio Nano Code **EIWR || M || S || FG** representing the bio nano system shown in *Figure 1-12 B* which could be decoded as shown in *Figure 1-13*.

b) The Universal Template – Bio Nano STEM System: The modular construction concept involves designing a universal template for bio-nano systems, which could be 'programmed and grown' into any possible Bio Nano coded system. This concept mimics the embryonic stem cells found in the human beings, that are a kind of primitive human cells which give rise to all other specialized tissues found in a human foetus, and ultimately to all the three trillion cells in an adult human body. Our Bio Nano Stem system will act in a similar way. This universal growth template will be constituted of some basic Bio Nano Codes, which will define the Bio-Nano-STEM system. This STEM system will be designed in a manner that could enable it to be programmed at run-time to any other required bio-module. *Figure 1-14* shows one such variant of the Bio-Nano STEM system, having the Bio Nano Code: EIWR || M || S || FG and having enhanced sensory abilities.

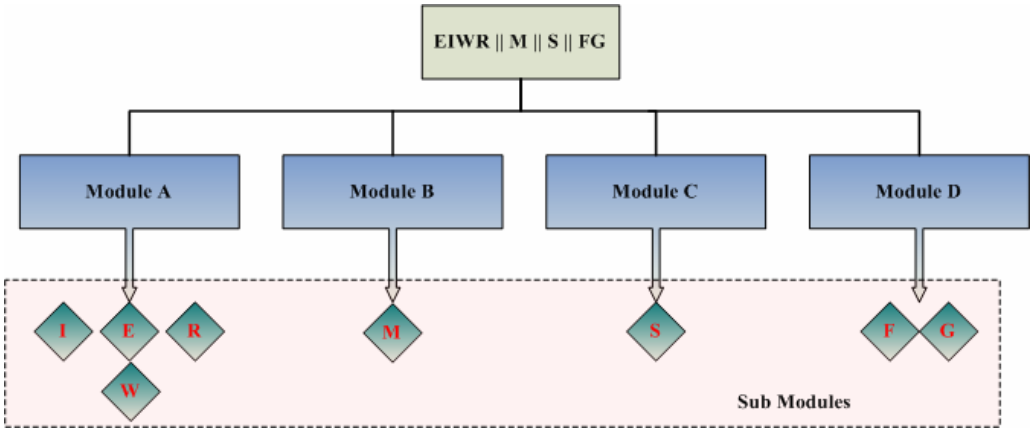


Figure 1-13: *Showing the bio nano code and the fractal modularity principle. The letter symbols have the values specified in Table 1. The "||" symbol integrates the various bio-modules and collectively represents a higher order module or a bio-nano robot*

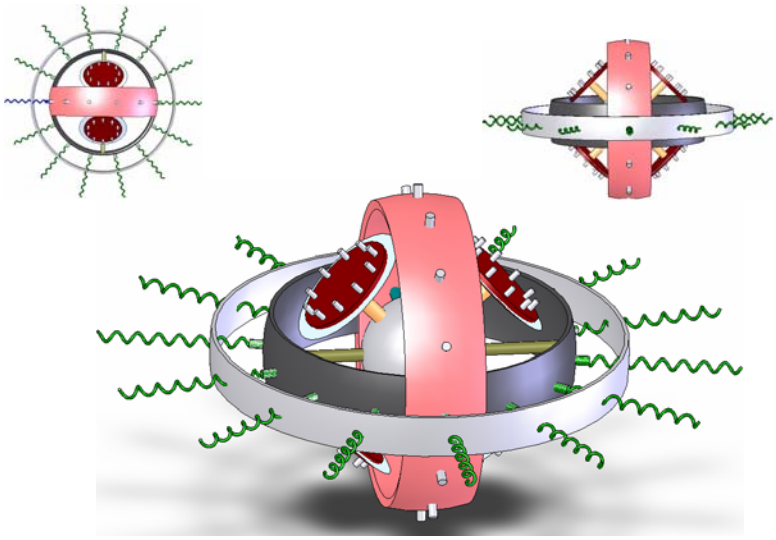


Figure 1-14: *Shows a variant of the initial bio nano STEM system (figure 1-12), fabricated with enhanced bio nano code S, which defines it as a bio nano robot having enhanced sensory capabilities. The other features could be either suppressed or enhanced depending upon the requirement at hand. The main advantage of using Bio Nano Stem system is that we could at run-time decide which particular type of bio-nano-robots we require for a given situation. The suppression ability of the bio nano Stem systems is due to the property of "**Reversibility**" of the bio components found in living systems.*

c) Information Processing – Memory Storage and Programming: Capability of information processing is one of the most novel features of the proposed bio-nano system. Here we underline some design aspects on memory storage mechanisms and bio-nano intelligence. The main hypotheses considered for designing such a mechanism are: **i)** *reversibility of the bio-chemical reactions and molecules,* and **ii)** Functions arises from *conformations.* The basic storage and retrieval mechanism is represented in *figure 1-15* below.

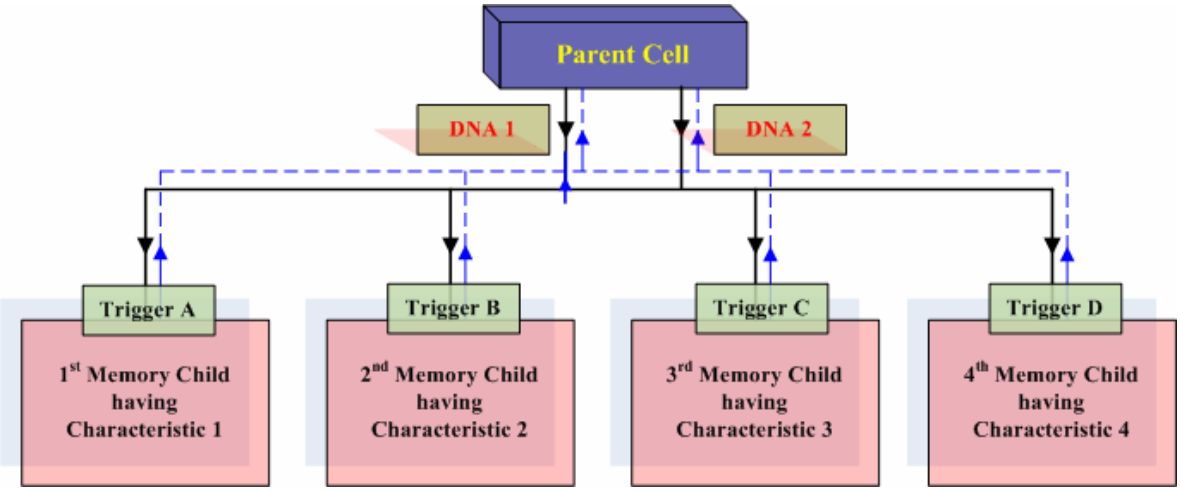


Figure 1-15: *Showing the Bio Nano Code and the Fractal Modularity Principle. The dotted line represents the "reversibility" of the bio-chemical reaction.*

A "*Parent Cell"* contains two stable structure of DNAs (DNA 1 and DNA 2) which bind within themselves four trigger ions or similar bio-molecule (Trigger A, Trigger B, Trigger C and Trigger D). These specific numbers of bio-components are used only for illustration purposes, exact number would vary upon the required bio-chemical reactions. Additional four different ions (Ion A, Ion B, Ion C & Ion D) are defined, which would react with the stable conformations of the two DNAs in the parent cell. The working principle is illustrated in the following equations (these equations are representative of the plausible bio-chemical reactions):

$$IonA^{+} + (DNA_{1a} + DNA_{1b}) \underset{fully\ reversible\ reaction}{\overset{Presence\ of\ a\ Field\ Gradient}{\rightleftharpoons}} (DNA_{1^{*}}) + Trg_{A}$$

$$IonB^{+} + (DNA_{1a} + DNA_{1b}) \underset{fully\ reversible\ reaction}{\overset{Presence\ of\ a\ Field\ Gradient}{\rightleftharpoons}} (DNA_{1^{**}}) + Trg_{B}$$

$$IonC^{+} + (DNA_{2a} + DNA_{2b}) \underset{fully\ reversible\ reaction}{\overset{Presence\ of\ a\ Field\ Gradient}{\rightleftharpoons}} (DNA_{2^{*}}) + Trg_{C}$$

$$IonD^{+} + (DNA_{2a} + DNA_{2b}) \underset{fully\ reversible\ reaction}{\overset{Presence\ of\ a\ Field\ Gradient}{\rightleftharpoons}} (DNA_{2^{**}}) + Trg_{D}$$

$$4\ Ions\ (Input) \underset{Generates}{\overset{Reacts\ With}{\rightleftharpoons}} Bio\_Chemical\_Center \underset{Reacts\ With}{\overset{Generates}{\rightleftharpoons}} 4\ Triggers$$

Where, the four basic ions are represented by: $IonA^{+}; IonB^{+}; IonC^{+}; IonD^{+}$ and the DNAs are represented by the following two symbols (for j = 1, 2); $DNA_{ja}; DNA_{jb}$ .The 'a' & 'b' sub script represents one of the chains of the double helix of the DNA.

The trigger ions are designed to bind with the child DNAs so as to change their conformations. When these trigger ions are released, altered conformations of the DNAs are left in the parent cell. These new conformations should also be stable at those ionic concentrations and field gradients. These released trigger ions flows into the adjacent "*memory child"* cells thereby changing the conformation their DNAs.

Hence, this change in conformation of the DNAs of the child cell along with the parent cell DNAs constitutes the data storage (and retrieval) mechanism (as shown in *figure 1-16*). Four Memory child cells are defined in accordance with the possible number of trigger ions generated as a result of the biochemical reactions in the Parent Cell DNAs. This *change in conformation* will act as the memory storage mechanism for

us. This change in the conformation of the DNAs has to be long-term and stable and at the same time reversible under the application of specific conditions (ions, temperature, pressure and pH value of the environment).

While *storing* the data, the ions (A, B, C & D) are passed through the ion channels connected with the parent cell. *Retrieval* of the data is the reversible process where the trigger ions are flowed through the ion channels of the memory child cells towards the parent cell (Mechanism shown in *figure 1-17*). Hence a reversible mechanism is established between the Parent Cell and the Memory child cells. Hence, this efficient information storage device could be used to program the behavior of the bio-nano system which will embed this in its *"inner core"* module.

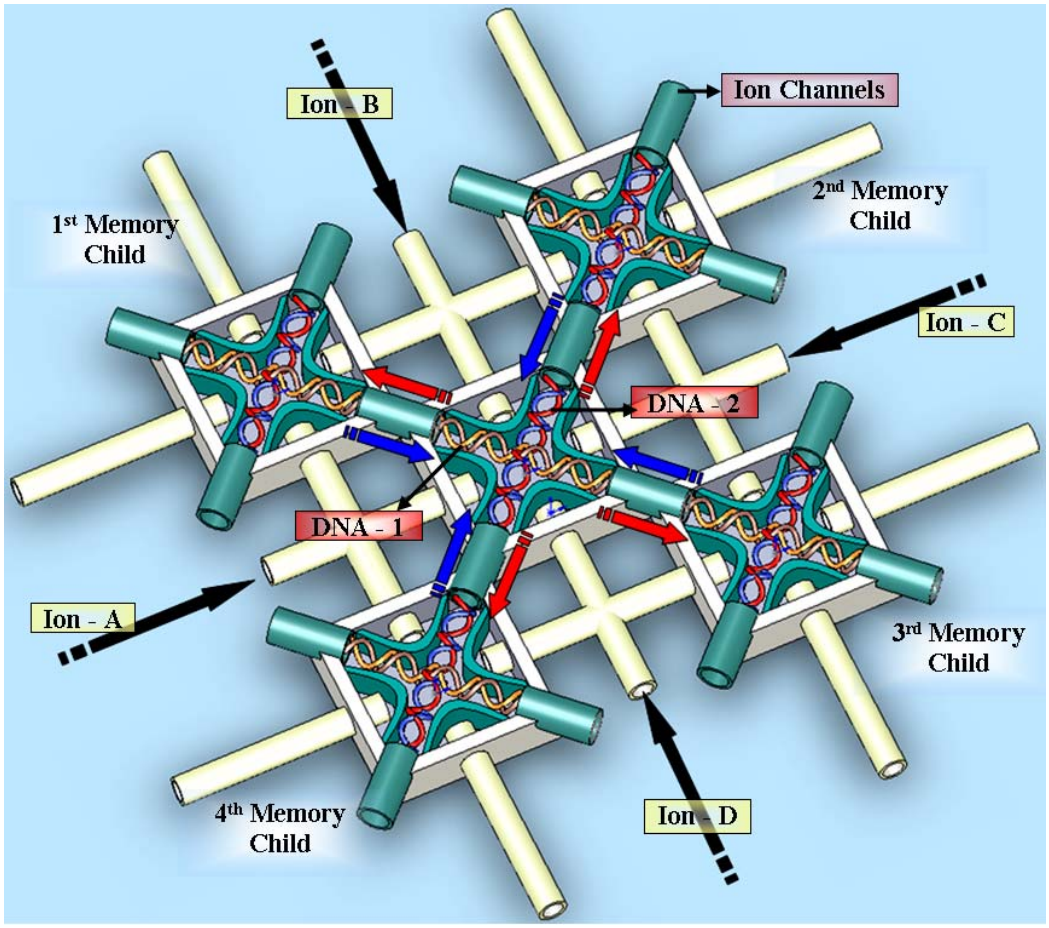


Figure 1-16: *Basic Information Processing Bio Nano Cell*

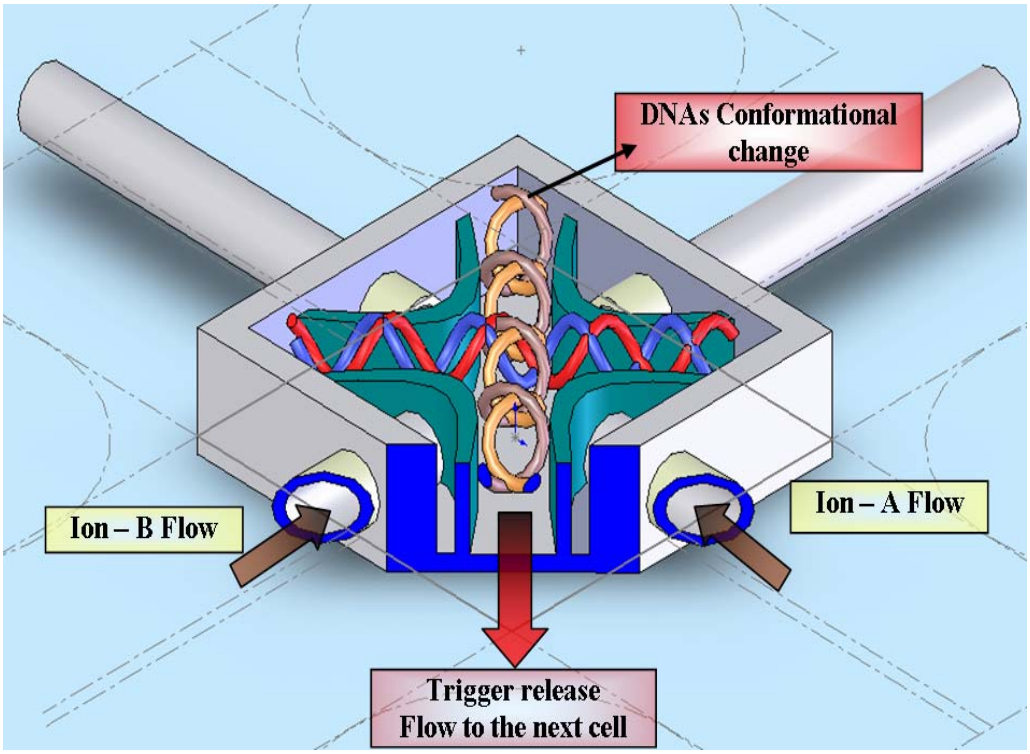


Figure 1-17: *Trigger Release Mechanism - reversible reaction*

d) Bio Nano Intelligence: Integrating bio-nano information storage and programming capability with the functionality of growth and evolution, lays the foundation of Bio-nano intelligence. What exactly is intelligence and how we can simulate intelligence is still an open area of research. Programming, learning and hence evolving could be one combination of events which can quantitatively describe intelligence. Therefore, we equate bio nano intelligence with the ability to "grow" coupled with basic information processing capabilities. *Figure 1-18* depicts our basic concept. The bio-nano computational cells are embedded along the two axes. These axes have different functionalities. Axis I, is the axis where the information is filtered and various components are sequentially isolated and stored in the bio-nano computational cell. The stimuli obtained by activation of the first block, triggers a reaction, which activates the subsequent blocks along the Axis I. The number of blocks would depend upon the bio

chemical reaction employed. But there would be facility of increasing this axis at runtime.

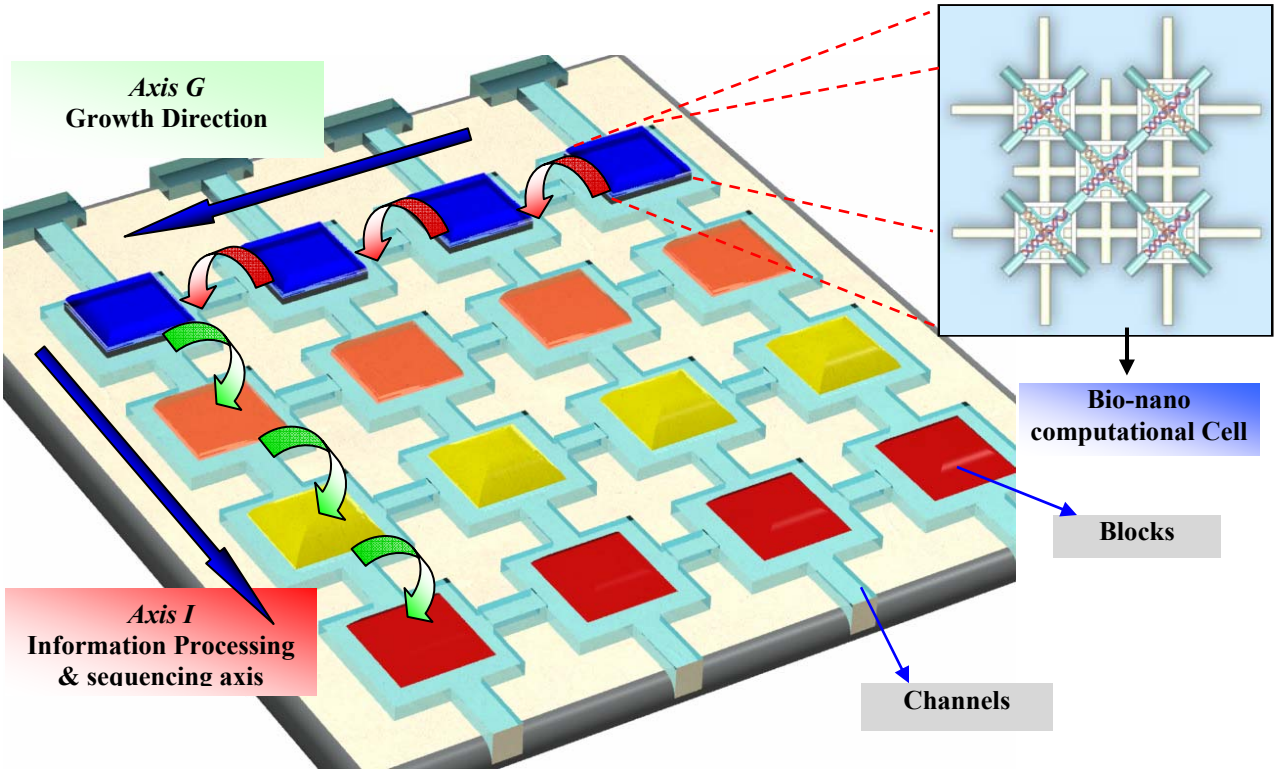


Figure 1-18: *Mechanism for achieving bio-nano intelligence*

Axis G is the Growth Direction. Along this axis copy of the initial data is made. Once a row is finished along the Axis I, the Axis G elements are activated. And again the sequencing along the Axis I would start. A single stimulus could trigger multiple outputs through this mechanism. Axis G would also enable parallel computations, thereby accelerating the response to a given input. This is typical of an intelligent living system, where the past stimuli are responded to faster when they felt. Ions play a very crucial role in triggering and controlling the biochemical reaction in our scheme and hence, form the basic element in concept of *"experience gathering"*. This capability coupled with the above explained advanced information processing mechanism would constitute bio-nano intelligence in our proposed systems. **Ion types** could be related to the ***variations*** in

behavior and **Ion Concentration** could be related to the ***intensity*** of the behavior of a bio-nano robotic system.

1.4 Self Replication – mimetics a novel property of living systems

1.4.1 Significance

Mimetics of self replication, as exhibited by nature, would influence any nano level application. We would need an army of nanorobots (or living systems), mass-produced via techniques of self-replication (or life), to carry out many meaningful tasks at the nano scale. These applications involving nanorobots demand that these machines are manufactured in millions or billions and in a time frame reasonable for a particular application. One of nature's noblest properties is that of life. It is how nature progresses through its environment ever adapting and evolving. Though philosophically, what life means and what constitutes it is not very clear, but what's clear is how nature propagates itself with time and survives, every day and every moment! This is one attribute of nature which is of prime importance to us, as researchers of science and engineering, and which, if understood, would bring a unique revolution, which in a way would change the course of our lives.

The concept of self-replicating mechanisms (SRM) or mimetics of life is not new [Freitas and Merkle, 2004]. We are perfect examples of these kinds of systems. We are wounded and our internal mechanisms heal it, with some differences in some cases. Taking the example of the wound and its healing process, we move ahead and try and analyze how we can achieve such behaviors in the mechanisms, which we design. Core to the concept of self-replication lays the basic material (DNA/RNA) which undergoes such activity. For the current scenario, we hardly know why these materials behave in this fashion, but what we know is how they behave and this creates the stepping stone for us

to move ahead in our quest. Before getting into some of the possible designs, a brief discussion on the application of such mechanisms is necessary. Why after all we need living systems or self-replications? Where they would be best suited?

1.4.2 Applications

a) Consider that your application depends upon a particular part, a mechanical, an electrical or any other physical, biological or chemical element. And in the process, some element fails, or starts developing problems. We will need some self-rectifying mechanisms within our application to detect such changes and rectify them. It's similar to our wound example. We can think of many applications where we would desire such behavior. Given some initial material feedstock it would be desired that the self-replicating mechanisms would rectify the problems. Having said that we can classify the self-replicating mechanisms in the order we classify our main mechanisms or machines.

- Mechanical self-replicating mechanisms.
- Electrical self-replicating mechanisms.
- Chemical or biological self-replicating mechanisms.

There could be several other classifications, which would depend upon the way applications are classified. Numerous other examples of the applications, which could follow the lines of our wound example, could be thought of. It just depends upon the extent of our imaginations. For example, suppose that we build some SRM which mimics the living system and its function is to detect a crucial defect in a mechanical element and when detected mend that defect. So if we are able to device such mechanisms, it could significantly enhance the lives and performance of the system. The system in this example would be designed and constructed to work at nano scale, and therefore they

would have an ability to detect very slight defects and would start working towards rectifying it.

b) Remote Applications would also benefit from SRM systems. Maintaining these applications requires a constant human interaction. If these systems mimic living system's coded logic and goals then it would be able to perform optimally with minimal human interventions. For example, deep space explorations would require circuits, machines, equipments to adjust and adapt with time and as per the conditions they would be subjected to.

c) Applications at the nano scale. This category of applications would be most influenced by our bio mimetic systems because they could lay the foundations of nano devices that have the capability of manipulating molecular matter.

In the following section we would attempt to define some of the guidelines and working philosophies for designing and fabricating such replicating systems. The details are the thoughts and ideas of the authors and are not verified or supported by experimental facts.

1.4.3 The Design of Life Mimetic Systems

The design of life mimetic systems requires new innovative materials to be designed that behave in the same fashion as the one designed by nature. These new materials are termed as "*intrinsic materials*" from here on.

1.4.3.1 Intrinsic Material

Unique arrangements of the constituent atoms of intrinsic materials would give rise to:

- unique potential field surface around them
- unique charge distribution

- unique internal energy gradients

It is through these internal energy gradients that two particular intrinsic materials would interact with each other. Hence the behavior of the intrinsic material would be a direct function of its internal energy gradients.

1.4.3.2 Interaction Laws

The final objective of any two interacting intrinsic materials would be to achieve the inherent balance of the resulting system (termed as *self-balancing*). This would translate to achieving minimum energy gradients in all the directions for all the interacting materials subjected to external potentials and stochastic environment. The final system would then be defined by its new achieved internal energy gradients and other inherent distributions. These intrinsic energy gradients would also be constantly influenced or varied by the external fields and potentials. The true statistical nature of these variations is one of the important factors to study and understand intrinsic materials.

1.4.3.3 Self-Balancing

It implies that the materials considered would tend to align with their intrinsic energy gradients and would try to minimize the instantaneous imbalance. The classical instance of self-replication via energy-minimized self-assembly was first demonstrated in the late 1950s. The canonical example of this approach is called the Penrose Blocks (Penrose, 1957, 1958). The unbalance and the property of self-balancing are similar in essence to what is postulated by the law of entropy. This concept of self-balancing is motivated from the law of maximum entropy production, which says that a system follows a path which minimizes the potential or maximizes entropy at the maximum rate [Archives of Science, 2001].

1.4.3.4 Growth and its Growth Limit

An intrinsic material needs to possess property of growth (an important variable for replication). This property of growth only occurs when the system is provided with some energy maybe in the form of additional intrinsic material or external potential gradients. Growth cannot happen in isolation. This implies that in the process of self-balancing, it is possible that the particular configuration of the intrinsic material is stable up to a particular level. This level would be governed by the strength of the intrinsic potential gradients for that intrinsic material and the extrinsic fields. Therefore, growth implies that the intrinsic material can alter its internal distribution of atoms without disturbing its self-balance or creating energetically unfavorable intrinsic potentials or dynamics. But this growth can only be achieved to a particular extent, beyond which the system tends to divide by following the paths which might be defined by the laws of maximum entropy production and free energy minimizations. And this particular limit of growth is termed as Reproductive Limit. Tracking such paths by carrying out quantum evolution at a system level could be one of the challenges of this field and might hold potential to explain such behavior.

1.4.3.5 Self-Filtering and Self Healing

The concept of replication further demands that the materials thus designed should exhibit the property of self-filtering and self-healing. Self-filtering implies that the material involved in the systems exhibiting self replication will not allow any kind of growth pattern but only a particular one. This particular growth pattern (which inherently depends on the interactions of the components) will determine which material it binds to

and to which it doesn't. Hence such systems will only interact with certain intrinsic materials.

In the process of this growth, a readjustment of the intrinsic material occurs. As the addition of further intrinsic material takes place, the whole system tries to align itself towards the most dynamical stable state subject to certain laws, which are not totally understood (energy minimization, maximized entropy). This adjustment goes on with the self-filtering process. If at any incremental stage system doesn't find the kind and type of material it is adaptable for, it wouldn't bind and probably in due time reject that particular material and in the end wouldn't have any growth. By rejection, it might imply that either the system doesn't align or bind with the material or marks it as an unstable configuration and seeks for the opportunity to replace it immediately. Hence it acts as a self-healer.

1.4.4 Self Replication – A Gedankenexperiment

Let us try to replicate a system, based on the properties described above. Consider a system A, consisting of some intrinsic materials (*Figure 1-19*). The system A is completely defined by the way these intrinsic materials are associated and aligned within it. According to the above properties of self-replicating mechanisms, it is observed that, the intrinsic materials of the system could be broken down into further fundamental intrinsic materials.

Any stable configuration of the individual intrinsic material is in sync with the property of self-balancing. Now when a particular intrinsic material (say 1) gets in an interactive distance of another intrinsic material (say 2), then these two intrinsic materials try to form another subsystem A1 within the super system A, following the property of self balancing. These two intrinsic materials combined will have some other function of

intrinsic energy gradient and could be the sum of the individual intrinsic energy gradients of the intrinsic materials and the applied external fields.

Now this argument could be extended to the situation when the third intrinsic material (say 3) comes into the picture. This intrinsic material 3 would not only interact with intrinsic material 1 but also with intrinsic material 2. Finally, a system A comes into generation, because of self-balancing acts of these three intrinsic materials. The configuration they achieve becomes highly stable for that particular situation. Now let's introduce more *energy* to the system A. It would be in the form of introducing intrinsic materials or applying external gradients to the system A or both. *Figure 1-20* explains the concept.

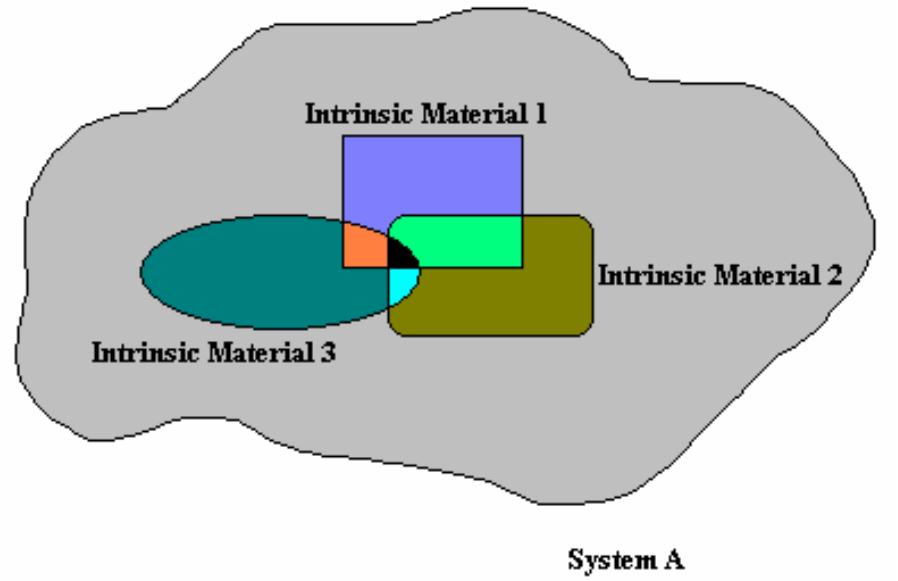


Figure 1-19: *System of intrinsic materials – a Self replicative system A.*

Here because of the process of self-filtering, copies of intrinsic material 1, 2 and 3 are introduced. The property of self-balancing comes into dominance and the systems tries to adjust itself into the most stable state. As defined earlier, the initial state is the most stable

state; following is what happens to the system A. Two sub systems within the main systems are made as shown in *Figure 1-21*.

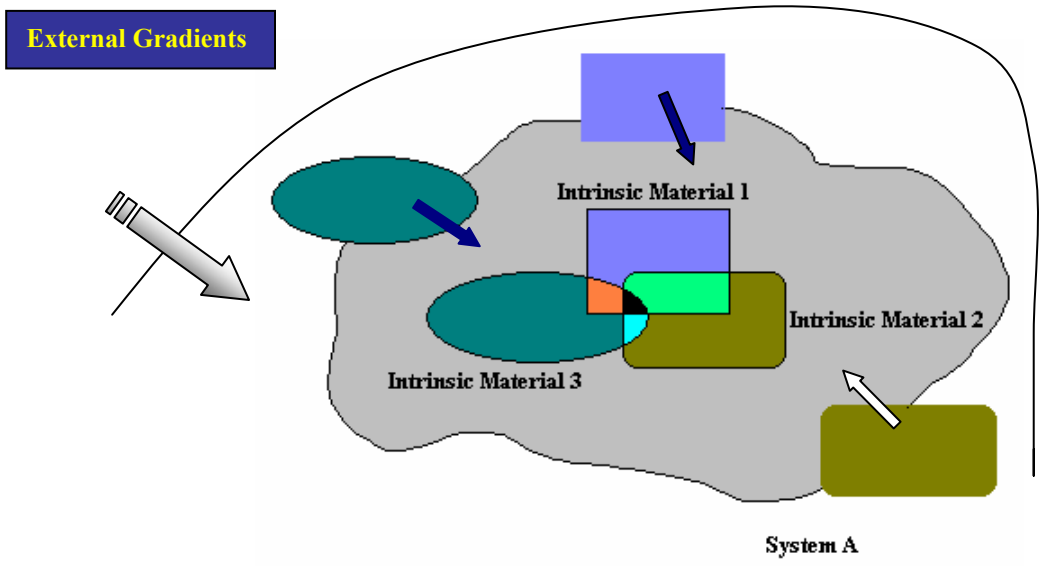


Figure 1-20: *Energy being added to the self replicative system A in the form of newer intrinsic material (1, 2 and 3) and external gradient (this external gradient is applied either to aid the interaction between the intrinsic materials or to impart a particular dynamics to the system for favorable environment for the interaction).*

The alignment of *subsystems A1* and *A2* is similar to the one of the initial system, that is, *A*. Please note that such system is possible, because we can control the external parameters, namely, extrinsic gradients and the intrinsic material introduced. The triangles drawn in the figure above shows the configuration of the intrinsic materials of *subsystems A1* and *A2*. The dotted lines, depict the interaction between the old intrinsic materials and the new ones and the possible configuration that could be achieved.

Now because the external gradients are still applicable a unique instability in the system occurs. The system tries to self-balance and in the process leads to its most stable configurations, which was its initial one (the initial configuration, system A). *Figure 1-22* explains the concept. In the end, the original *system A* replicates into *system B* and *system*

C. Both these new systems, have the same functionalities as defined by the original system (A), because the have received the same configuration and the same intrinsic materials.

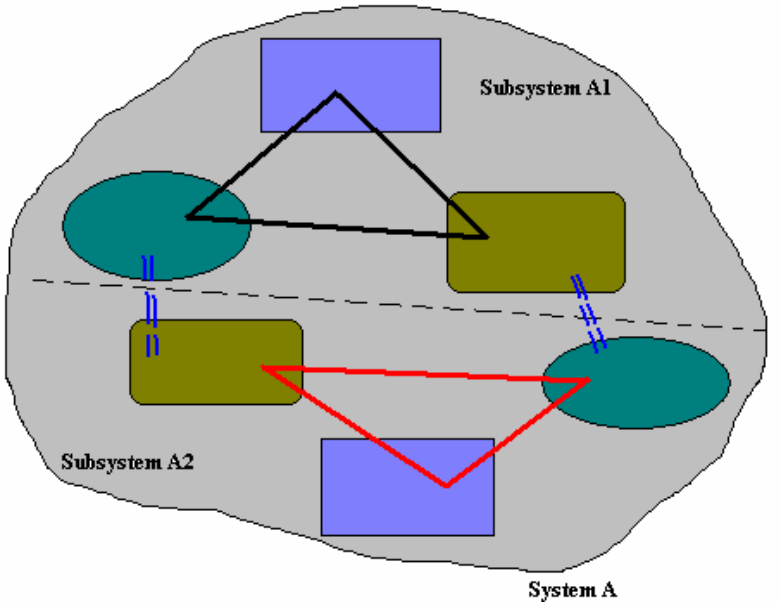


Figure 1-21: *Creation of stable subsystems within the original system A (which as a whole is marginally unstable under the external gradients and two independently stable subsystems). This step is the most crucial in the process of attaining a self replication super system. This demands a unique selection of such replication intrinsic materials in the initial place, namely, 1, 2 and 3.*

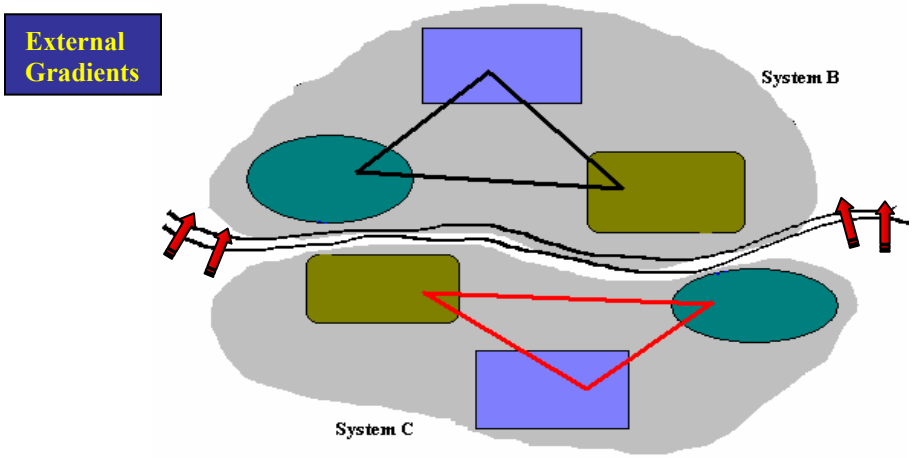


Figure 1-22: *Replicating stage of the system A into system B and system C. System B and C which could be called the child systems are similar to system A in function and its configurations.*

1.4.5 Design Parameters for Self-Replicating Systems

Following are the various design parameters that need to be considered while designing a self-replicating system.

1.4.5.1 Selection of Intrinsic Materials

This is, of course, the most important parameter in designing the desired system. The obvious choices would be biomaterials and chemicals found in the human body, which have exhibited self-replication. Their choice is mainly because of their availability and the fact that they themselves are the materials resulting from a replication process. This doesn't limit the selection of other replication materials. Also a lot of data on nature's biomaterials is available from the experiments performed in the field of biology and genetic engineering. The field of nanotechnology is the biggest area where the concept of self-replication system would be a success and the biomaterials could be managed at that scale.

1.4.5.2 Defining the External Gradient Parameters

It is extremely important to define the external gradient parameters within which our system needs to perform. Our choice of the intrinsic materials would be greatly impacted by their behavior. Also their sensitivities to these external gradients need to be calculated so as to fine-tune our system.

1.4.5.3 Generating Stable Alignment and Internal Gradients

Selection of the appropriate intrinsic materials for our system implies that we need to also select: the appropriate internal gradient functions and the alignment generated by these intrinsic materials. We need to calculate the most stable configuration for our system at no external gradient levels and then fine tune our alignment as it is applied. Application of these external gradients could generate a situation where no stable

configuration is possible within our operating conditions. This calls for adding some further intrinsic materials to the system, which would help us to get to the stable configuration (closure engineering) [Robert et al, 2004]. This variation of the intrinsic gradient in this manner is termed as *intrinsic variational gradients* to distinguish it from the inherent intrinsic gradients generated due to the intrinsic materials.

The parameters mentioned above create the foundation for the development of mathematics for this field. To create any system with self replicating mechanism we need to first find out its most stable state, then we need to calculate its behavior in the extrinsic gradients and then we need to excite it with energy and supply of intrinsic materials so that it replicates. Though these methodologies are not verified, further research in this area is carried on by the authors and their collaborators.

1.5 Conclusions

Bio-mimetic and its principles would greatly influence the field of nanorobotics and nanotechnology. The way nature is designed and the way natures solves its problems is of great interest to us because they allow us to understand basic principles that would pave to practical nanotechnology.

The recent explosion of research in nanotechnology, combined with important discoveries in molecular biology have created a new interest in bio nanorobotic systems. The preliminary goal in this field is to use various biological elements — whose function at the cellular level results in a motion, force or signal — as nanorobotic components that perform the same function in response to the same stimuli — but in an artificial setting. This way proteins and DNA could act as motors, mechanical joints, transmission elements, or sensors. Assembled together, these components would form nanorobots with

multiple degrees of freedom, with the ability to apply forces and manipulate objects at the nano scale, and transfer information from the nano- to the macro scale world.

The first research area is in determining the structure, behavior and properties of basic bio-nano components such as proteins. Specific problems include the precise mechanisms involved in molecular motors like ATP Synthase, and of protein folding. The next step is combining these components into complex assemblies. Next concepts in control and communication in swarms need to be worked out. Again, we plan to follow nature's path, mimicking the various colonies of insects and animals, and\transforming principles learned to our domain. Since it would require specialized colonies of nanorobots to accomplish particular tasks, the concepts of co-operative behavior and distributed intelligence need to be developed, possibly by using knew hierarchical and other techniques.

Principles like self replication are the ones of greatest importance for the field of nano robotics. It is this life mimetic which will enable us to design and fabricate the future nanorobots having immense capabilities and potential. These would require innovative materials (intrinsic materials) and fabrication methodologies, with due regard to well-known manufacturing- and applications-related safety concerns. The safety issue is of paramount importance in this field for researchers and scientists. The proposed bionanorobots would be completely controlled molecular devices and are far from being dangerous to society. Though these devices would have many unique capabilities, which are not seen currently, they are harmful as projected in science fiction movies and books. There is an increasing need for educating the community about the exact nature of this

research and its essential differences with the projections of the science fiction community.

Chapter 2: Bio nano Machines for Space

2.1 Introduction

In this chapter we will list some of the requirements for designing bio nano machines for space. These bio nano machines are utilized to further design some macro level space applications, namely, NTXp (***N***etworked ***T***erra***Xp***lorers) and ATB (***A***ll ***T***errain ***B***io nano) Gears for astronauts. Further chapters will talk about these space applications at a greater length. We have identified Mars as a representative working environment for our bio-nano based space devices. Hence, all our design and analysis will be based on Martian environmental conditions that are listed below. We also explain how these conditions need to be taken into account in our designs.

2.2 Design Requirements Due to Planetary Environmental Conditions

Atmosphere: The atmosphere of Mars is quite different from that of Earth. It is composed primarily of carbon dioxide with small amounts of other gases [Hamilton 2001, JPL]. The six most common components of the atmosphere are: Carbon Dioxide (CO_2): 95.32% ; Nitrogen (N_2): 2.7%; Argon (Ar): 1.6%; Oxygen (O_2): 0.13%; Water (H_2O): 0.03%; Neon (Ne): 0.00025 %. The Martian air contains only about 1/1,000 as much water as our air. *Therefore, we need to develop bio nano robots which can utilize the carbon-di-oxide for energy production. For instance, certain microorganisms called methanogens could grow at low pressures and other conditions similar to that on Mars. Methanogens need hydrogen and carbon dioxide as the raw materials to produce energy.*

Temperature: The average recorded temperature on Mars is -63° C (-81° F) with a maximum temperature of 20° C (68° F) and a minimum of -140° C (-220° F) [Hamilton 2001]. *Therefore, the operating temperature range for the bio nano systems has to be*

between -140 to 20 degree centigrade. With the use of unique thermally insulating skins we could narrow down this range further but need to find the exact figures.

Pressure: Barometric pressure varies at each landing site on Mars on a semiannual basis. Carbon dioxide, the major constituent of the atmosphere, freezes out to form an immense polar cap, alternately at each pole [Hamilton 2001, JPL]. The carbon dioxide forms a great cover of snow and then evaporates again with the coming of spring in each hemisphere. When the southern cap was largest, the mean daily pressure observed by Viking Lander 1 was: as low as 6.8 millibars; at other times of the year it was as high as 9.0 millibars. The pressures at the Viking Lander 2 site were 7.3 and 10.8 millibars. In comparison, the average pressure on Earth is 1000 millibars. *Therefore, the material of the NTXp has to be strong enough to sustain the internal force generated due to the higher inside pressure. The designed bio nano system has to be able to survive in as low pressures as possible. In addition a transport mechanism has to be designed which transfers the outside particles, or sensed parameters, in the NTXp's "skin" layer.*

Topography of Mars: The full range of elevations on Mars is about 19 miles (30 kilometers) which is one and a half times that found on Earth [Hamilton 2001, JPL]. An interesting aspect of Mars' topography, is the striking difference between the planet's low and smooth Northern Hemisphere and the heavily cratered Southern Hemisphere, which sits, on average, about three miles (five kilometers) higher than the north. In addition, many geographic features show a resemblance to drainage systems on Earth, where water acts at slow rates over long periods of time. As on Earth, the channels here merge together to form larger channels (see *Figure 2-1A*) [Hamilton 2001]. *Therefore, the*

topography of Mars will help us to select the length scales of NTXp and identify possible areas where NTXp could be deployed.

Local Dust Storm: Local dust storms are relatively common on Mars [Hamilton 2001]. They tend to occur in areas of high topographic and/or high thermal gradients (usually near the polar caps), where surface winds would be strongest. *Figure 2-1B* [Hamilton 2001] shows a storm which is several hundreds of kilometers in extent and is located near the edge of the south polar cap. *Therefore, weather conditions such as winds and dust storms have to be taken into account in our design as the NTXp could be easily carried away by the storms and could be submerged into it and hence disrupt contact with the mission center.*

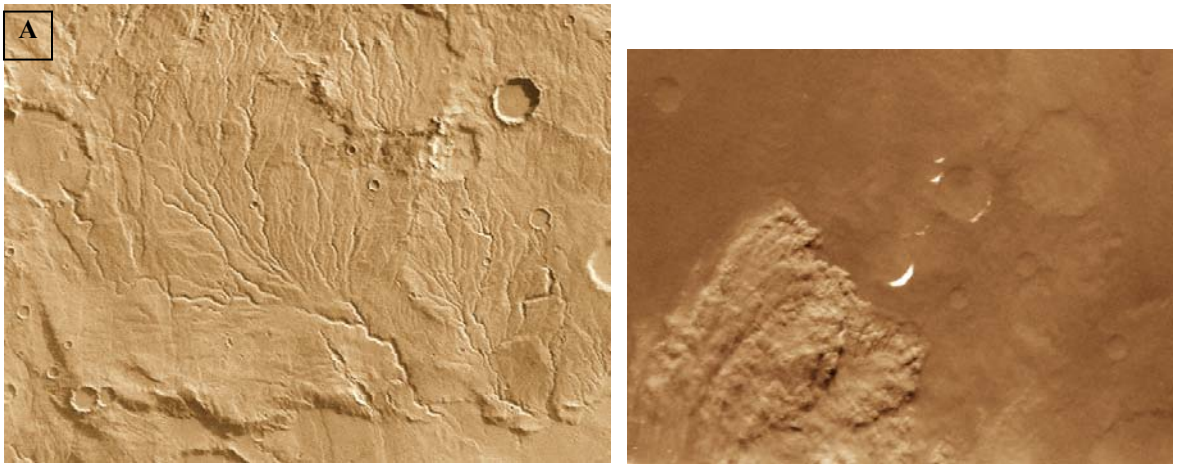


Figure 2-1A: Topography of Mars *- This figure shows the drainage like system on the Mars surface. This kind of site is important for us as it might support evidence for finding water on the surface of Mars. This condition is important for deciding the scale of NTXp as its scale is dependent on the topography of the surface we want to sense.* ***Figure 19 B: Local Dust Storms*** *- The local dust storms can inhibit the sensing capabilities of the NTXp. Hence the weight and the overall structure of the NTXp has to be such that it minimizes these effects*

Radiation: The main source of radiation at the Martian surface is ultraviolet (UV) radiation between the wavelengths of 190 and 300 nm [Muller et al]. UV-radiation can be lethal. It is absorbed by nucleic acids (i.e. DNA) and activates the chemical formation of various adjuncts that inhibit replication and transcription of DNA. In the absence of an ozone layer, organisms can only escape the lethal affects of UV-radiation by living in protected habitats. Even those surface organisms which have efficient DNA and cellular repair enzymes would probably perish. Also, due to the continuous bombardment of the Martian surface with UV-radiation the topmost layer of the regolith is thought to contain strong oxidants which are damaging for cellular components. *Therefore, this parameter suggests that the skin of NTXp has to be radiation resistant and resistant to oxidants present on the surface of the mars. This aspect is very important for the design for our bio nano devices.*

2.3 Identification of Bionano Machine Components for Use in Space

In this section we present possible biological elements that can be used as bionano machine components in space devices that can withstand the harsh planetary environmental conditions. In general there is a positive correlation between the degree of stability (such as thermophilicity, ability to survive in harsh environment) of a particular source organism and the degree of stability (such as thermostability) of both their intracellular and extracellular proteins [Herbert et al, 1992]. Thermostability of the protein is defined as the Hence, we have identified various micro organisms (extreme organisms), which exist in extreme environmental conditions. In the next few months we will isolate some of their biological components that could be used as sensor elements, actuators, end-effectors, signaling modules and information processing modules. some of

the biological elements that could be considered for further studies are thermally stable enzymes listed in *Table 2-1*.

Enzymes	Organisms
Pullulanase	Pyrococcus furiosus
RNA Polymerase	Thermoplasma acidophilum Sulfolobus acidocaldarius Thermoproteus tenax Desulfurococcus mucosus
Succinate thiokinase	Thermoplasma acidophilum
Sulphur oxygenase	Acidianus brierleyi
Topoisomerase I	Sulfolobus acidocaldarius
Transglucosylase	Desulfurococcus mucosus

Table 2-1: *Thermostable enzymes listed with their source organisms*

These are archaeobacterial enzymes on which there are publications surveying some of their structural as well as functional data. These enzymes are targeted because they are pretty accessible and their models could be used for investigating protein thermostability in general, which is our primary design requirement. Proteins from thermophiles are characterized with more charged residues, particularly in the exposed surfaces, with more salt bridges, and hence are more accessible on average as compared to those in proteins from mesophiles. The tabulated thermostable enzymes provide many clues for working towards designing thermostable proteins. The molecular dynamics result from these enzymes would help us create better mutants (and obtain better analytical models) for stable bio nano components. *Table 2-2* presents various micro organisms (extreme organisms), which exist in extreme environmental conditions [Herbert et al 1992, Reysenbach et al 2001, Horikoshi et al 1998, 1999].

Eukaryotes in extreme environments	Definition	Operating Regime	Name of the organisms
Thermophiles	Mico organisms which can exist at higher temperatures	The range is pretty broad. The limit of life is expected to be around 140 degree centigrades.	1. *Cyanidium caldarium* - its optimal growth temperature was 45ºC and the maximum temperature at which growth occurred was 57ºC. 2. Cells like the archaean Pyrococcus grow above 100ºC.
Psychrophiles	Micro organisms which can exist at colder temperatures	Water is the solvent for life and must be present in a liquid state for growth to occur. This sets a practical lower limit for growth slightly below 0ºC.	*Cold Shock protein* - CspA a major cold shock protein of E Coli. *Cold-acclimation protein (CAPs)*- a second group of protein that are involved in the low-temperature growth of psychrophilic bacteria and yeasts. *(Pseudomonas syringae)* Ice-nucleating proteins forms ice crystals on leaves and flowers (-2 to -5 C). *(Pseudomonas, Erwinia, Xanthomonas)*
Acidophiles	Micro organisms which can exist in acidic environment (pH 3 or less). The internal pH of acidophiles has been measured between 5 to 7 C.	less than pH of 3.	*T. ferrooxidans, Acontium cylatium, Cephalosporium spp., and Trichosporon cerebriae*
Alkalophiles	A micro organism whose optimum rate of growth is observed at least two pH units above neutrality or above 9 pH.	9 - 11 pH	*Spirulina, B. alcalophilus*
Xerophiles	A micro organism which can survive in driest environments.		*Metallogenium, Pedomicrobium,* and *lichens such as Rhizocarpon geographicum, Aspicilia cinerea , Caloplaca saxicola*
Radiation resistant organisms	Which can sustain ionizing radiations	When exposed to 1.5 million rads of ionizing radiation (a dose 3000 times higher than would kill organisms from microbes to humans), Deinococcus repaired the damage to its shattered DNA in a matter of hours.	*1. Deinococcus radiodurans 2. Halobacterium* - a master of the complex art of DNA repair. This bacteria has survived normally-lethal doses of ultraviolet radiation (UV), extreme dryness, and even the vacuum of space. Evolving to cope with a salty lifestyle could explain why Halobacterium is so good at surviving radiation and other ravages.

Table 2-2: *List of various extremophiles and their operating regimes.*

Chapter 3: Various Bio-nano components

3.1 Introduction

In this section some bionano components are described which have potential of being utilized for the proposed bionano space systems. These mechanisms have a control mechanism, an *external dependency*, which triggers them. For instance, in the case of Viral Protein Linear (VPL) motors, its mechanism is controlled by the change in the pH of its medium. The mechanisms considered here for all the identified bionano components exhibit mechanical behavior. The change in the external environment triggers changes in the bionano component in the form of their conformation or variations in the pattern of their self-assembly with other interacting components and their environment. These changes demonstrate motion and a desired trajectory of a specific part of the bionano component within the context of the nano-world workspace.

Another feature of these mechanisms pronounced by these bionano components is the ***"reversibility"***. This feature is an integral part of any desired bionano component. Reversibility gives these bionano components ability to be utilized as machine components in the nano-world workspace. This repeatability is much desired for our bionano systems. The future step is the synchronization of these individual components to demonstrate a useful output in the nano workspace. Stochastic and other less understood bionano phenomena's are some of the major hurdles for us in designing these components.

3.2 Proposed bionano components of interest from literature

3.2.1 Peptide Nanotubes

a) Structure: The peptide nanotubes are made from two types of amino acid residues:

a) L-amino acid residues

b) D-amino acid residues

L-type amino acid residues occur naturally in the nature, but D-type does not. D-type is synthetically produced. L-type and D- type are symmetric in mirror image.

Trigger mechanism – Variations in the acidity of the peptide solution

Changing the acidity of the peptide solution results in the self-assembly of L-amino residues and the D-amino acid residues in an alternating sequence to form a cyclic ring called cyclic peptide. These cyclic peptides are formed in a pair of two rings. These two rings form one unit in this system. Each of these units combines together by means of hydrogen bonding and form hollow tubular structures. The structure thus formed is called peptide nanotube. *Figure 3-1A* shows the top view of a cyclic peptide ring structure. The self-assembly of the individual units (pair of two rings) could be seen in the *figure 3-1B*.

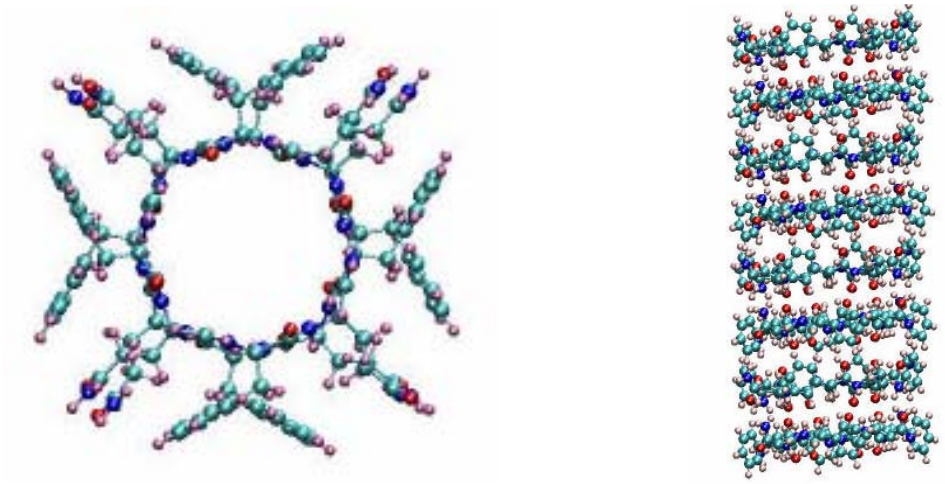


Figure 3-1 (A) [Lewis Group 2004]***:*** *the top view of the cyclic peptide ring: L-Gln & D-Phe.* ***(B)*** [Lewis Group 2004] *the self-assembled structure of the individual units to form a hollow tubular structure through hydrogen bonding.*

The peptide nanotubes are thought to have many properties which would be useful for our bio nano space systems. These peptide nanotubes can be used to channel ions [Ghadiri et al 2001, Braha et al 1997] through it, forming a device which passes only certain size specific ions. These could be used as channels to trigger ions and transporting them in our proposed bio-nano device ***"basic information processing bio nano cell"*** which is explained later in the thesis. The passage of these ions can also be based on their *chemical affinity* and their *behavior* with respect to the amino acids and the hollow tubular passage thus created. Thus, we can achieve ion type and ion size variations which are much desired for the design of bio nano computational cell.

Other applications are also suggested in the literature for the proposed peptide nanotubes. These are electrically conductive [Fukasaku et al 1997] and photoresponsive materials. These can also be used as nanoscale fluidic transport system and also provide closed chambers for molecular reactions [Eisenberg et al 1998, Sigler et al 1998].

b) Future research on peptide nanotubes:

Most of the current research on peptide nanotubes has been done either find out their electrical properties or their transmembrane transport behavior. We propose to further analyze the peptide nanotubes as effective "channels" in the design of proposed bionano computational cell, and from the mechanical perspective i.e. treating them as structural elements for bio-nano robotic systems. The self-assembly mechanism of the nanotubes can be used to generate motion and forces in the direction perpendicular to the plane of the rings. For example, a membrane in an aqueous solution can be moved up and down by the assembly and the subsequent salvation of the nanotubes in the solution. *Figure 3-2* below shows an analogy between a tentacle manipulator [Walker, Clemson University]

and a peptide nanotube [Tarek et al 2003]. The assembly and salvation of peptide nanotubes can be triggered by changing the pH of their solution.

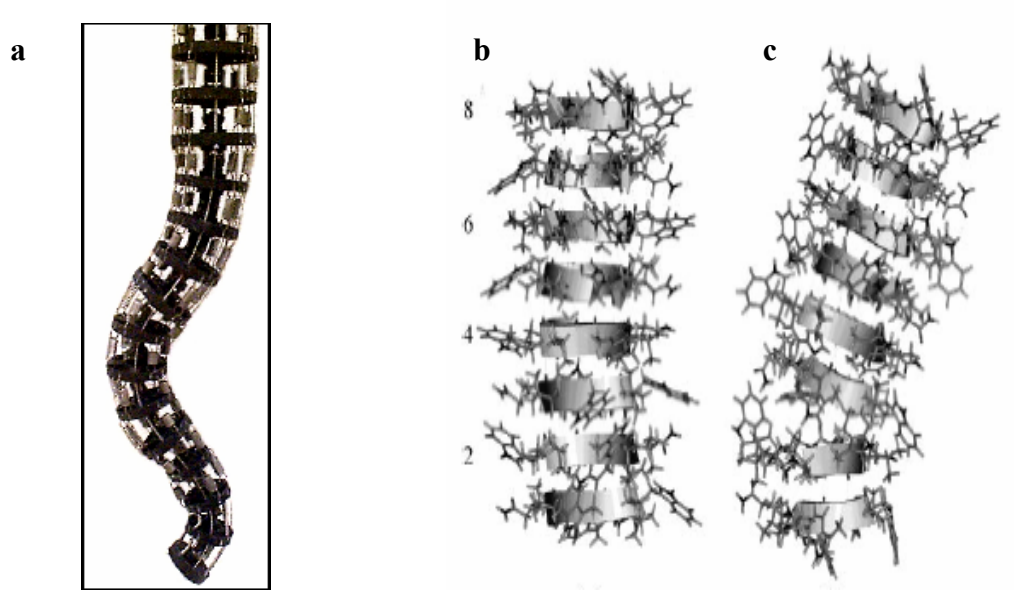


Figure 3-2 (a): *[Walker, Clemson University] A conventional tentacle manipulator;* ***(b)*** *[Tarek et al 2003]****:*** *Arrangement of an eight cyclo peptide nanotube where the rings are flat and perfectly stacked at t=1ns; in* ***(c)*** *[Tarek et al 2003]****:*** *the nanotube exhibits a kink between the second and the third ring at t=8ns.*

3.2.2 GCN4 Nano-Hinge

A nano-hinge is equivalent to a macro-scale hinge in its desired motion and mechanical behavior. A nano-hinge is proposed [Sharma et al] which will have a capability to act as a nano-mechanical hinge and would have an ability to power a nano-scale gripper for transporting matter at nano scale. This peptide has two main chains which are highly stable motifs. The nano-hinge operates when it is triggered by the changing pH of its solution and which creates an effective electrostatic repulsion between the specific elements of the two chains.

Trigger mechanism – induced Electrostatic repulsion through variations in pH

The GCN4 Nano-Hinge was chosen for performing molecular dynamics studies and for further deriving preliminary results though its mutations. This peptide has been further engineered to obtain the characteristic motion of a nano-hinge through optimized mutations [Sharma et al]. Following paragraph describes the steps and the methodology employed for some of the preliminary results through simulations performed in the molecular dynamics software *NAMD* [Sharma et al]:

a) Simulations

Step 1: Preparation of simulation (Figure 3-3)

- The two coils of the GCN4 Leucine Zipper were isoloated from each other to facilitate the attachment of six Histidines residue (HIS Tags) at their terminals.
- After the HIS Tags were attached to each end, they were put together to form the dimer coiled-coil orientation (*Figure 3-3b).*
- Since the HIS Tags were oriented anti-parallel they were manually pulled up to face each other in order to maximize the electrostatic forces *(Figure 3-3c)*.
- All the Histidines were protonated to simulate the lower pH of the environment and thus positive charge was added on their suraface. This was done during the MD preparation by changing the protonation state of the Histidines in the GCN4 pdb file.
- Since the dimer structure was externally modified due to pulling it might contain some residual forces or improper bonds etc. Therefore the molecule was subjected to a small energy minimization routine (~10 picoseconds (ps)) in vacuum to bring

it back to a more stable state wherein the molecule is relaxed. It is done in MD by systematically varying the positions of atoms and calculating the energy.

- The molecule was next solvated in a water sphere of 40 A (angstroms) diameter and was then ready for simulations.

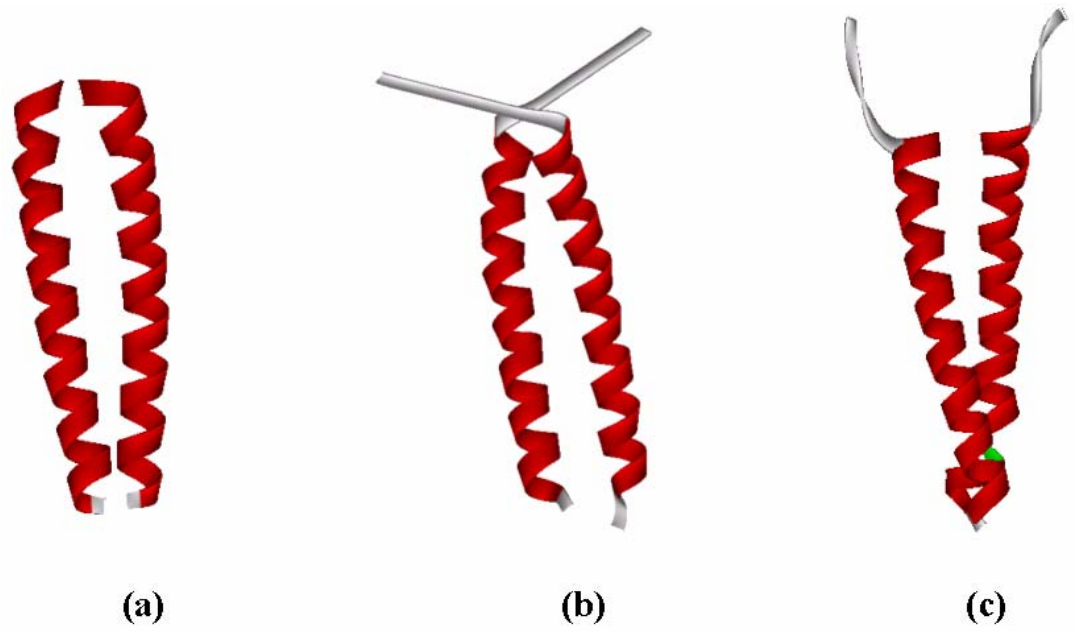


Figure 3-3(a): *Native GCN4 dimer;* ***(b):*** *GCN4 dimer with the HIS Tag attached to the two terminuses. Note the anti-parallel alignment of the tags;* ***(c):*** *HIS Tag pulled up using a small Steered Molecular Dyamics simulation.*

Step 2: Molecular Dynamics (MD) Simulations

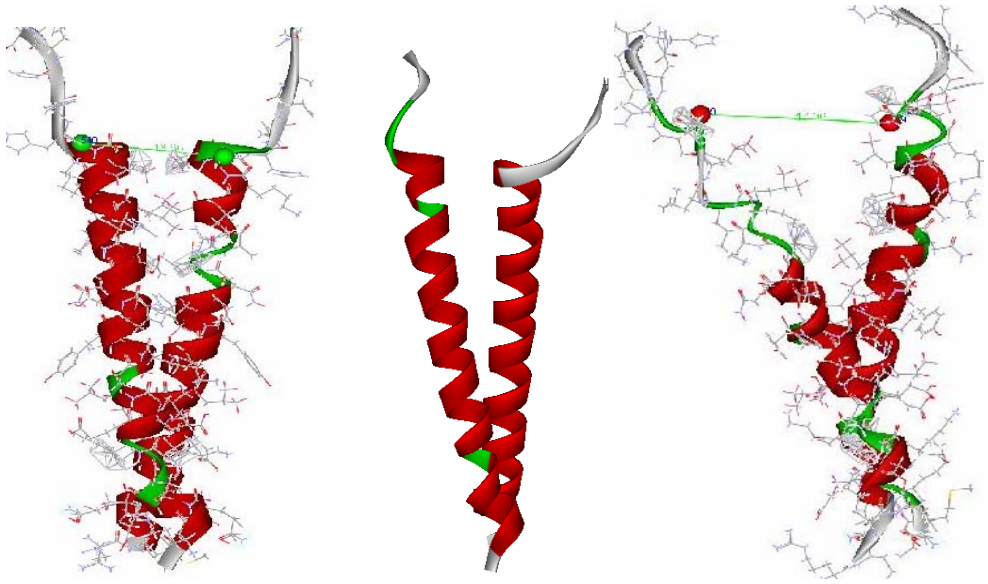
The parameters used for the MD simulation of the Nano-Hinge in water are:

a) Temperature: 300 K

b) Runtime: 200 ps (picoseconds)

b) Results: No noticeable deflection was observed in the hinge after 200 ps (picoseconds) in water [Sharma et al]. In order to verify the electrostatic repulsion phenomenon of HIS tag, the same simulation was performed in vacuum instead of water. The reason for this is that the dielectric constant of vacuum is 80 times less than that of

water and thus the electrostatic forces are 80 times larger in vacuum. Also the molecule was freer to move in vacuum. *Figure 3-4* below shows the result of a 40 ps (picoseconds) simulation in vacuum. A deflection of around 4 A (angstroms) was observed.



(a) (b) (c)

Figure 3-4(a): *Initial GCN4 coil with HIS tag;* ***(b)*** *After a 200 ps simulation in water (Note: Side chains are not shown in Fig b). The deflection was less than 2 A;* ***(c)*** *After a 40 ps simulation in vacuum. The total deflection was around 4 A leading us to believe that the electrostatic forces are indeed pushing the molecule apart but (as shown in (b)) are perhaps not strong enough to overcome both the steric hindrances of the water molecules and the interchain hydrophobic interactions.*

c) Future research: This component is well suited for the proposed bio nano space applications. Higher deflections for the nano-hinge have to be obtained for its use as nano gripper or hinge. This could be made possible through the mutations of the side chains and performing quantum - molecular dynamic simulations. For the space related bionano systems this could also be used for transporting other bio-components from one place to

other within a defined nano-workspace. Also the mechanical power obtained through these nano-hinges could be used to drive nano - channels or devices.

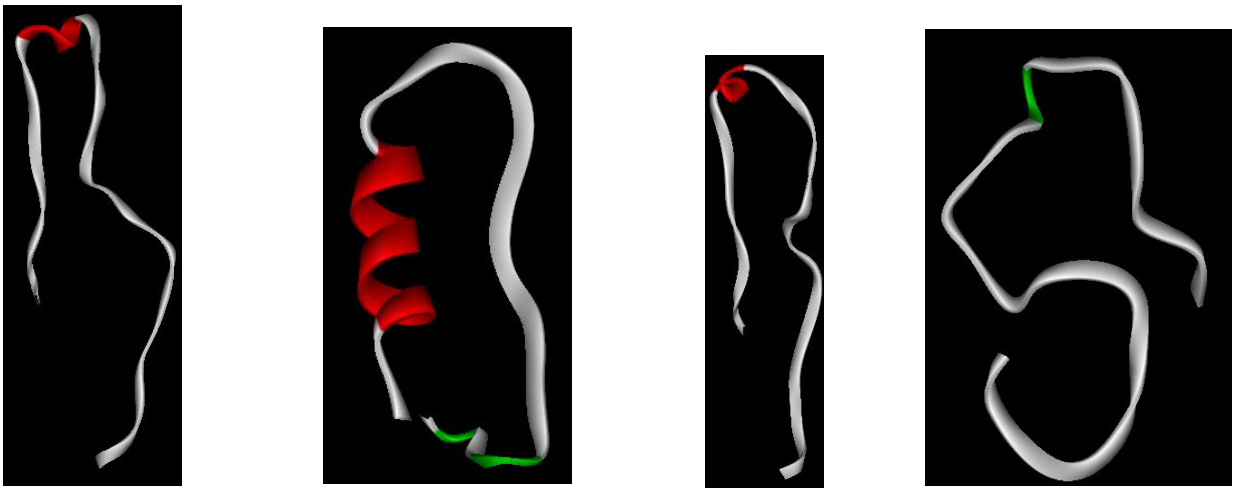
3.2.3 Viral Protein Linear actuator

In order to infect new cells, several viruses employ proteins on their surface that undergo changes in their structural conformation in order to promote the fusion of the viral membrane with the cellular membrane. This change is due to the pH change associated with the vicinity of the cell. Given similar conditions, it is proposed in this project to use this conformational change to produce VPL actuator. This actuator is one of the sections of the HA trimer protein. Computational and experimental studies have been performed on VPL.

Trigger mechanism –Variations in pH of the solution

a) Computational Studies: The computational work consisted of the development of algorithms and programs to perform the molecular dynamic and kinematic simulations of the proposed Viral Protein Lineal (VPL) Nano-motors. To predict and characterize the dynamic performance of the proposed VPL motors (i.e. energy and force calculation) we performed Molecular Dynamic (MD) Simulations that are based on the calculation of the free energy that is released during the transition from native to fusogenic state. We used the MD software called CHARMm (Chemistry at Harvard Molecular Mechanics). Loop36 peptides were modeled individually as well as trimers. The effect of pH was simulated by protonating 10 titrable amino acids (Glutamic Acid - GLU, Aspartic Acid - ASP, and Histidine - HIS). MD simulations ranging from 10 ps to 1 ns were performed before and after protonation of HIS, ASP and GLU. The peptides were also mu-tated by replacing GLY22 by ALA (G22A) in line with the experimental observations. Targeted Molecular Dynamics (TMD) and Steered Molecular Dynamic (SMD) simulations were

also per-formed to design hybrid simulations that will lead to the desired conformations and will follow a feasible trajectory. We can then calculate the forces, velocities and displacements in order to characterize the motor. Molecular kinematic simulations have also been developed to study the geometric properties and conformational space of the VPL motors. The kinematic analysis was based on the development of direct and inverse kinematic models and their use towards the workspace analysis of the VPL motors. An example simulation is shown in *Figure 3-5* where a loop36 monomer was pulled apart in SMD and subjected to MD with and without protonation in order to see the effect of protonation. Additional information on our computational studies on the VPL nanomotors can be found in [Dubey, 2004] and in http://www.bionano.neu.edu.



a ***b*** ***c*** ***d***

Figure 3-5: ***(a)*** *Unprotonated Loop36 monomer pulled by SMD;* ***(b)****when subjected to MD simulations regains its helical character and comes back to a state similar to the crystal structure;* ***(c)*** *defined state at high pH;* ***(d)*** *Loop36 monomer does not revert back to its stable conformation with partial alpha-helical character when protonated.*

The following table describes the mutation locations for the peptide:

Position in Peptide	Native Amino Acid	Mutated Amino Acid
4	Glutamate (E)	Glutamine (Q)
8	Glutamate (E)	Glutamine (Q)
11	Histidine (H)	Glutamine (Q)
14	Glutamate (E)	Glutamine (Q)
16	Glutamate (E)	Glutamine (Q)
19	Glutamate (E)	Glutamine (Q)
21	Glutamate (E)	Glutamine (Q)
22	Glycine (G)	Alanine (A)
28	Glutamate (E)	Glutamine (Q)
32	Glutamate (E)	Glutamine (Q)

Table 3-1: *Site-directed mutations of loop 36*

3.3 Effect of temperature on bio-nano-components

Studying the effect of temperature on nano components is a very challenging problem. We can possibly study this at two levels based on the level of theories used. Various ab initio techniques can be employed to calculate various thermodynamic properties of a decent sized molecular system. Some of the properties that can be calculated are:

i) thermal energy correction

ii) heat capacity at constant volume

iii) entropy

For a fermionic system, which is the point in the case, there exists zero-point motion and is a quantum effect. This basically arises because of Pauli's principle. Therefore, even at zero degree Kelvin, any two particles cannot occupy the single energy states and hence there is a distribution achieved which occupies many lowest possible energy states. And hence thermal energy corrections have to be considered for accurate calculations.

a) ***Bio nano component level:*** to the study the effect of temperature on the whole protein or peptide.

b) ***Atomic level:*** to study the fundamental concepts of thermodynamics and its unification with Quantum mechanics and hence study the effect of temperature on an individual atom and further scale to a molecular level.

3.3.1 Bio nano component level

Studies were carried out to understand the temperature effects on **GCN4 Nano-Hinge** bio-component. Temperature plays an important role in all molecular processes. It affects the behavior of the protein-based systems as it indicates energy import into the system from the surrounding media. Normally, the simulations are carried out at 273.15K. Here we want to see the effect of temperature variations on the dynamics of the protein. We will perform strand-alone temperature variation simulations as well as allow temperature variations in conjunction with protonation, mutation, and explicit solvent. These simulations would help us find the *optimal* operation temperature for the proteins.

Simulations were carried out at the following temperatures: i) 263K → below the freezing point of water. This lower temperature range is a design requirement for our space related applications.

ii) 323K

iii) 343K

iv) 363

v) 423 → this is the highest temperature at which we simulated our system. This temperature naturally falls beyond the physiological performance range of the wild type protein.

In this analysis we calculated the following parameters:

a) RMSD values of the co-ordinates of the residues in the peptide

b) Free energy of the protein along the simulation temperatures

GCN4 Nano-hinge peptide has two main chains which are highly stable motifs. The nano-hinge operates when it is triggered by the changing pH of its solution and which creates an effective electrostatic repulsion between the specific elements of the two chains.

a) RMSD of the individual residues inside the peptide

i) 263K: At this temperature we see the least amount of vibrations of the residues from their initial position and orientation (*figure 3-6**).

ii) 323K: Physiologically this is the temperature where the protein could be towards the range of instability. The vibrations in the protein increases and this could be evident throughout the structure. The helical structure starts to loosen up then its initial form *(figure 3-7)*.

iii) 343K: This temperature range sees increasing vibration in the whole protein molecule. The stability of the protein is tremendously affected here *(figure 3-8)*.

iv) 363K: This temperature sees the various vibrational modes of the protein. The portions of the protein which were static even at 343K starts vibrating and hence marks the instability of the protein *(figure 3-9)*.

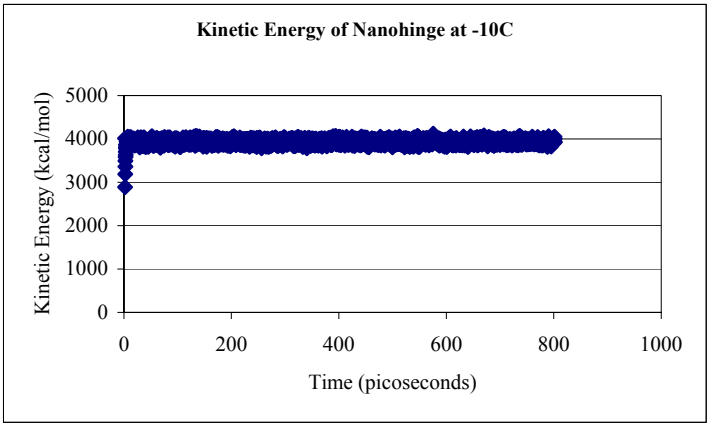
v) 423K: This temperature sees very pronounced vibrations all the protein structure. The most remarkable point here is the *rmsd* value of 4.64 angstroms for residue number 0. These values indicate the instability of the protein because of the disassociation of the protein's structure due to pronounced vibrations *(figure 3-10)*.

b) Free energy of the protein along the simulation temperatures

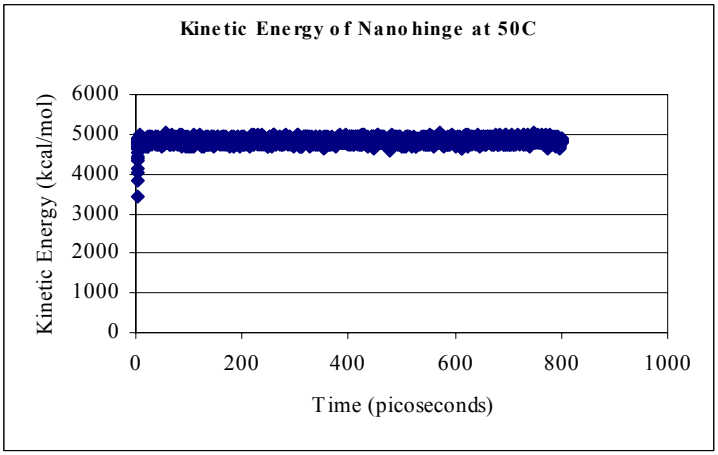
The following figure *(figure 3-11)* shows the kinetic energy change in the protein as the temperature is changed.

** Figures 3-6 to 3-10 are attached in Appendix 1*

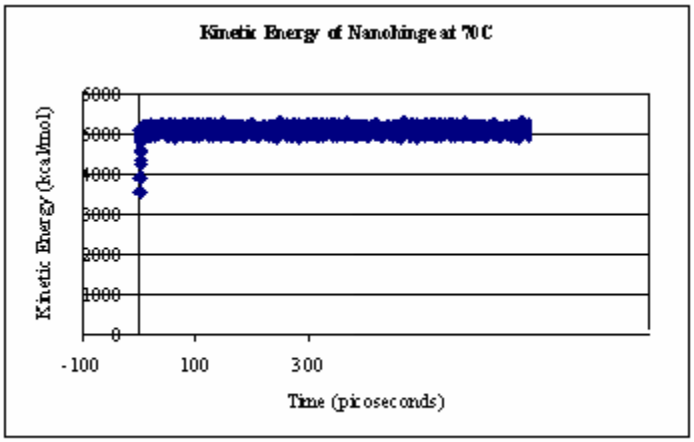
a


Kinetic Energy of Nanohinge at -10C
Kinetic Energy (kcal/mol)
5000
4000
3000
2000
1000
0
0
200
400
600
800
1000
Time (picoseconds)

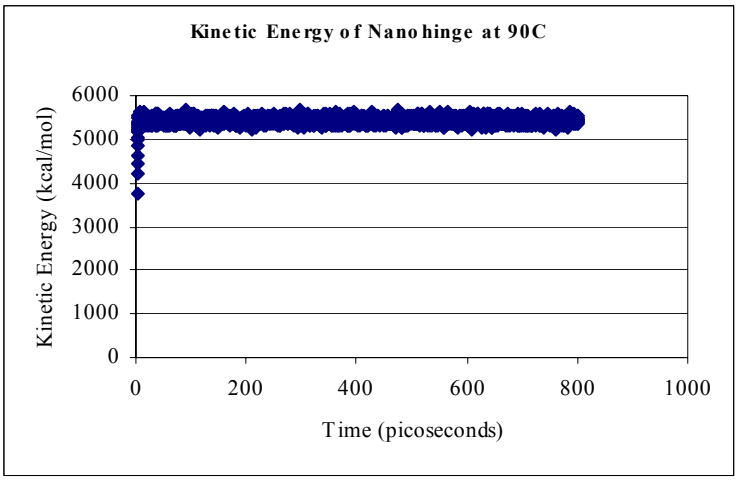
b


Kinetic Energy of Nanohinge at 50C
Kinetic Energy (kcal/mol)
6000
5000
4000
3000
2000
1000
0
0
200
400
600
800
1000
Time (picoseconds)

c


Kinetic Energy of Nanohinge at 70C
Kinetic Energy (kcal/mol)
6000
5000
4000
3000
2000
1000
0
-100
100
300
Time (picoseconds)

d



e

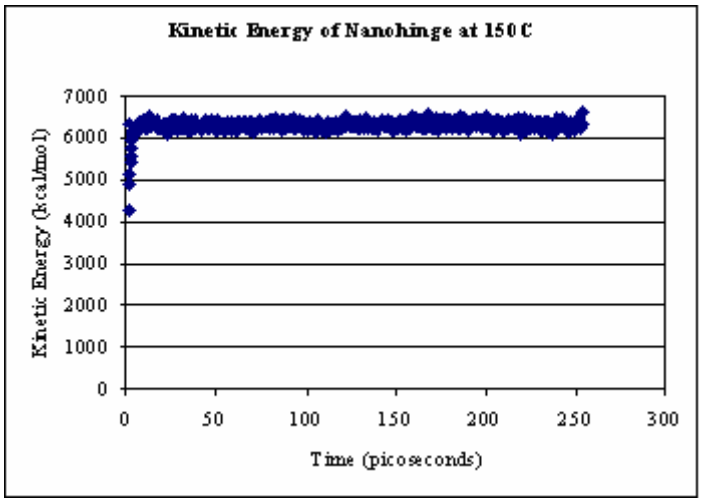


Figure 3-11: *As the temperature of the system is increased the energy increases proportionally. The kinetic energy of the system is composed of momentum of the particles, and hence the velocities. Higher the temperature, implies higher are the velocities of the particles and hence higher vibrations and higher energy. Here a constant pressure is assumed and the simulation system maintains the temperature throughout the simulation time frame. The energy of the system at 150 degrees is around 6500 kcal/mol whereas the energy of the system at negative 10 degrees is only 4000 kcal/mol. This shows 62.5 % increase from the initial value. Here we observe a constant step increase of 500 kcal/mol energy as the temperature is increased in the given interval.*

3.3.2 Atomic level

Understanding the effect of temperature on an individual atom is one of the important steps towards learning the effects of temperature on the whole molecule. This section describes some of the new theories in the field of unified quantum mechanics and thermodynamics. The focus for us here is to leverage these theories and apply it to the whole protein system, which might give us much valuable insights in designing bionano machines. This section presents some of the work done by professor Elias P. Gyftopoulos and his colleagues and group.

The foundations of quantum mechanics and thermodynamics postulate many unique concepts. Some of the concepts are as follows:

a) We know that the normalized wave function $\Psi(x)$ [Gyftopoulos et al 2003, Messiah 1999], where *x* is the spatial coordinates of the system, represents the dynamical state of the system. The probabilities associated with the measurements are derived from $\Psi(x)$. Associated with the wave function, we can also utilize another notation, that is of Dirac ket $|\psi\rangle$. The probability function is given by:

$$|\psi(x)|^2 = \psi(x,t)^*\psi(x,t) \; or \; \langle\psi|x\rangle\langle x|\psi\rangle = |\langle x|\psi\rangle|^2$$

Zero entropy non equilibrium state in quantum mechanics can be characterized by a time dependent wave function such as:

$$\psi(x,t) = \sum_{n=1}^{\infty} a_n \varphi_n(x) \exp(-i2\pi\varepsilon_n t/h)$$

The wave function can also be written in the form of the density operator ρ or statistical operator. The density operator is a *positive definite*, *Hermitean operator* of *trace equal to unity*.

$$\rho > 0; \quad \mathrm{Tr}\rho = 1; \quad \rho \geq \rho^2$$

In the matrix form and in energy eigenfunction representation, the ij-element of the matrix is given as follows for all *i* and *j*:

$$\rho_{ij} = a_i \exp(+i2\pi\varepsilon_i t/h) a_j \exp(-i2\pi\varepsilon_j t/h)$$

The unified quantum theory and thermodynamics says that: "*the laws of physics apply only to density operators '* ρ *' that can be represented by a homogeneous ensemble of identical systems, identically prepared. Homogeneous is an ensemble in which the probabilities of results of measurements on any member are represented by the same density operator* ρ *as those on any other member" (ref: Gyftopoulos et al.2003).*

Gyftopoulos et al. proved that of all the expressions available for entropy S, only one satisfies the necessary criteria given by the relation $S = -k\mathrm{Tr}[\rho \ln \rho]$ provided that ρ is represented by a homogeneous ensemble. These authors give a special meaning to the interpretation of ρ as its relation to the entropy of the system. If ρ is interpreted as the shape of the constituents of the system, then entropy of the system could be a measure of shape with variable values, from 0 to maximum for each set of energy, amount of constituents and parameter values. Therefore, based on the value of the ρ we can interpret entropy S as follows:

- if ρ is a projector (wave function), then S =0
- if ρ is not a projector but corresponds to a state which is not stable equilibrium then S has a positive value smaller than the largest possible value for the given parameters and values

- and, if ρ corresponds to the unique stable equilibrium state, then S has the largest possible value of all entropies of the system with a given value of energy, amounts of constituents and parameters.

The conclusion of the unified theory is that the entropy S [Gyftopoulos et al. 2003], of the system is a measure of the shape of the state of the system and is a fundamental property of a matter. This entropy as per the theory is given by the density operator of the system.

The interpretation of this theory might be extremely useful for studying the shapes of the proteins and their dynamics. As per this theory one can visualize the spontaneous increase of entropy in a process (irreversible) as an inherent behavior of the system (or a protein) to optimize its shape with respect to the internal and external forces until the system achieves the largest value of the entropy S possible, for a given value of the energy, amount of constituents and parameters.

Chapter 4: Computational Framework

4.1 Introduction

Herein we describe a computational framework which we will employ for the design and optimization of the bio-nano components. Through optimizations and various design algorithms, we will identify best mutations that will facilitate survival and performance of the bio-nano component in the planetary conditions. For carrying out this task we are designing a software architecture whose function will be to generate the best possible mutants given a particular objective function and the operating conditions. For this we will be utilizing the open source code of the software NAMD (molecular dynamics software) and other protein conformation predicting software packages, such as Rosetta. This framework is shown in *figure 4-1*.

Based on Table 2 (*"List of various extremophiles and their operating regimes"*, Report 1) we need to study effect of some of the external conditions on the protein based bio-nano components. This study is carried out with the following strategy:

a. ***Identification*** of the protein from the mentioned organisms is characterized with respect to the following three main parameters (representing the space environment):

i) The *high temperature variations* – this includes not only the conditions of very high and very low temperature but also the situation of high temperature variations.

ii) The *dry conditions* – this implies the conditions where minimum amount of solvent is required. The solvents could be replaced by gels in the space environments.

iii) The *space radiations* – this implies the condition where the bio nano machines would be subjected to the UV and other kinds of radiations.

b. ***Stability analysis*** for the proteins for all the space conditions have to be first performed individually. The stability of the protein components in the complex environment where all the constraints will act at the same time depends in a very complex way to its sequence. The individual stability analysis is important to carry out to get a complete insight into the dynamics and the response of the protein component with respect to the external conditions. The following describes the stability functions for three main parameters, temperature (temp), dry, and radiations.

$$S_{\text{dry}} = f(x_1^{\,a}; y_1^{\,b}; \ldots; t)$$
$$S_{\text{temp}} = g(x_2^{\,j}; y_2^{\,i}; \ldots; t)$$
$$S_{\text{radiations}} = h(x_3^{\,v}; y_3^{\,u}; \ldots; t)$$

The overall system stability of the bio-nano component would then be a function of all of the above individual stability functions. Therefore, S_{net} is a complex function such as:

$$S_{\text{net}} \propto F(S_{dry}^{\beta}; S_{temp}^{\nu}; S_{radiations}^{\lambda}; t)$$

c. ***The Variational dynamics*** – the dynamics of the basic bio nano component depends upon certain external parameters, such as, temperature, pH, other bio chemical molecules, light etc. This module focuses on defining and modeling the dynamics of the bio nano components subject to these Variational parameters.

Variational dynamics would include time-dependent quantum mechanical models and theories which would play a vital role in studies related to dynamics of a protein, its interaction with the medium while in transition, its interaction with the other bio-components and modules. While a protein transits from initial conformational state to the subsequent states while reaching the final state, each instant has to be clearly understood

and defined. We propose to utilize bio-nano components as robotic elements, and hence we require the detailed dynamics of that system and its behavior.

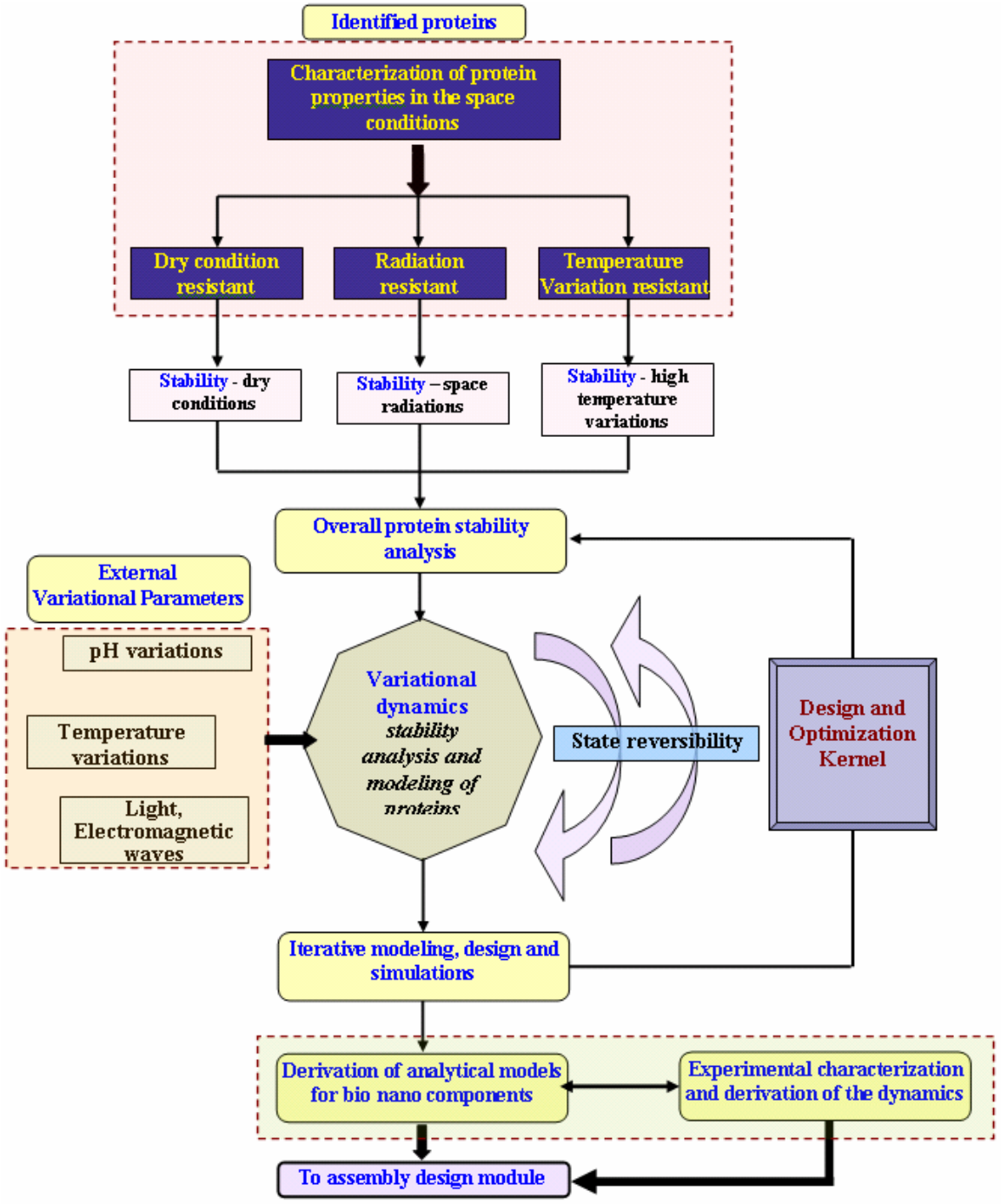


Figure 4-1: *Computational framework for the design and optimization of the bio nano components*

These Quantum-Classical and Quantum-Stochastic Molecular Dynamic Models (QCMD) would help us computationally form formal models for predicting the dynamical and interactive behavior of the large and complex molecular systems for large time scales [Bala et al 1996]. In QCMD models there exists a coupling of equation which considers two subsystems, quantum subsystem and classical subsystem. Schrödinger wave equation governs the quantum part and the classical molecular dynamics governs the classical subsystem [Bala et al 1996]. The variations in the position and momentum of the classical particles in the system are evaluated using the ψ, wave function of the quantum particles and using the potential energies of the classical and quantum regions. This results in including another force term in the classical equation due to quantum effects through extended Hellmann-Feynman theorem.

The dynamics of the proteins or their complex assemblies should be such that they follow a reversible path given particular stimuli and energy for such transformations. This reversibility constraint further imposes stricter restrictions on us in terms of predicting the more accurate dynamics of the system. To restore the reversibility all the interactions and the potentials have to be reversed, or there could be another path which would lead to the exact initial state. These studies are of paramount importance to us and would give us more insights into how the system could be designed and further optimized.

Figure 4-2 below describes the "reversibility dynamics" of the molecular system in context of variational dynamics. These dynamical predictions would describe two distinct paths, the forward path (trajectory) and the backward path. The dynamics of one complete cycle of forward and backward path demonstrated by the bionano system would

determine the actual behavior of the system as a robotic element for one cycle. QCMD methods would help us develop predictions which would involve and interactions and potentials while these paths are pursued by the system. These interactions could be in the form of variations in the pH of the environment or in terms of potential fields such as space radiations on the system. Both the forward and the backward path of the system would be different in the terms of co-ordinates of the atoms and the energy required for traversing the path. Also these nano-robotic elements could be used as actuation or structural elements, and hence the effect of variations of these loads and the number of interacting elements has to be taken into account while performing the predictions for the system. The conditions along the forward and the backward path could be very different.

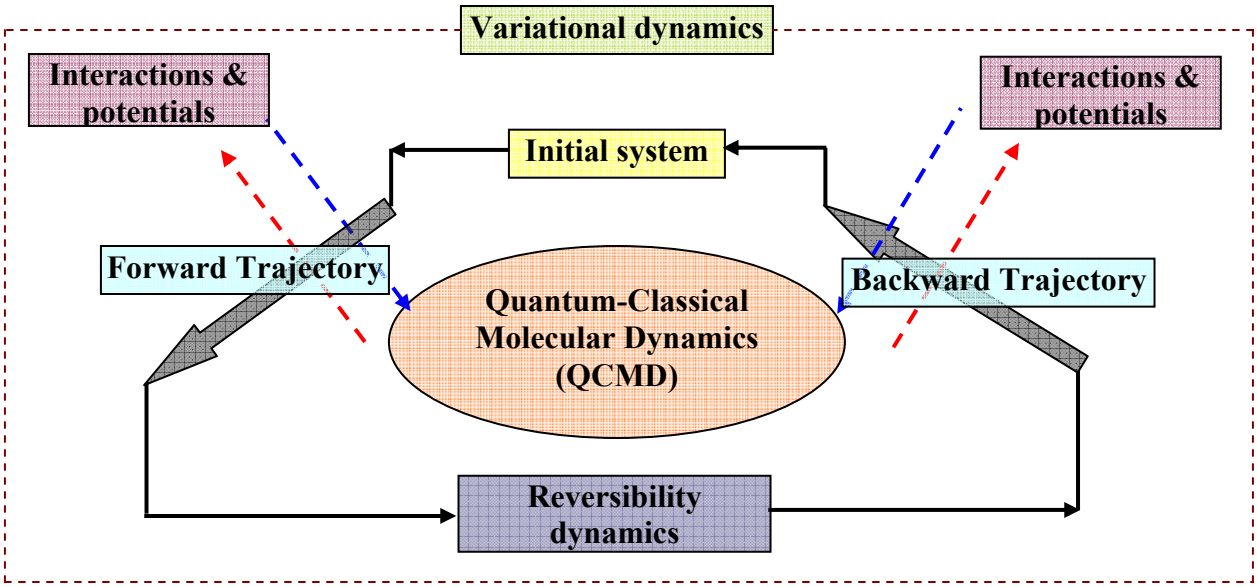


Figure 4-2: *Reversibility dynamics in context of Variational dynamics*

d. ***Design and Optimization Kernel*** (DOK) – This module will design the most favored and stable conformation of the protein for the particular simulation parameters. This module will work with the overall design process of Variational dynamics to generate the protein dynamics.

e. ***Derivation of Analytical models*** for the protein dynamics – this will entail derivation of the protein dynamics, its working limits and its error rates. This will also generate many useful design points which would be utilized in the designing of the protein assemblies.

f. ***The experimental models*** – this would be guided by the Derivation of the analytical model module. Also this will provide with many valuable models and results which will verify the analytical models generated by the derivation module. Both the analytical model and experiment models will act as an input to the next step in our design process – *the assembly modules*.

4.2 Design and Optimization Kernel (DOK)

A) The objective of this kernel is to generate stable confirmation of the protein bio component and optimize it based on some defined objective functions. The objective function could be different depending upon the design goals we have at our hands. For example, if our design goal is to achieve linear actuation of the protein module, then the objective function would be:

"Achieve *maximum conformational change of the protein module in a particular direction. The maximum conformational change would be calculated as the distance between the base or anchor point and the end effector point. These points would be defined in the objective function with respect to the co-ordinates of the amino acids."*

Figure 4-3 details the structure of the **DOK, design and optimization kernel.**

B) This architecture for DOK has many modules, such as, an expert system, an engine or user interface, an algorithm module etc. These are explained in more details in the following paragraphs:

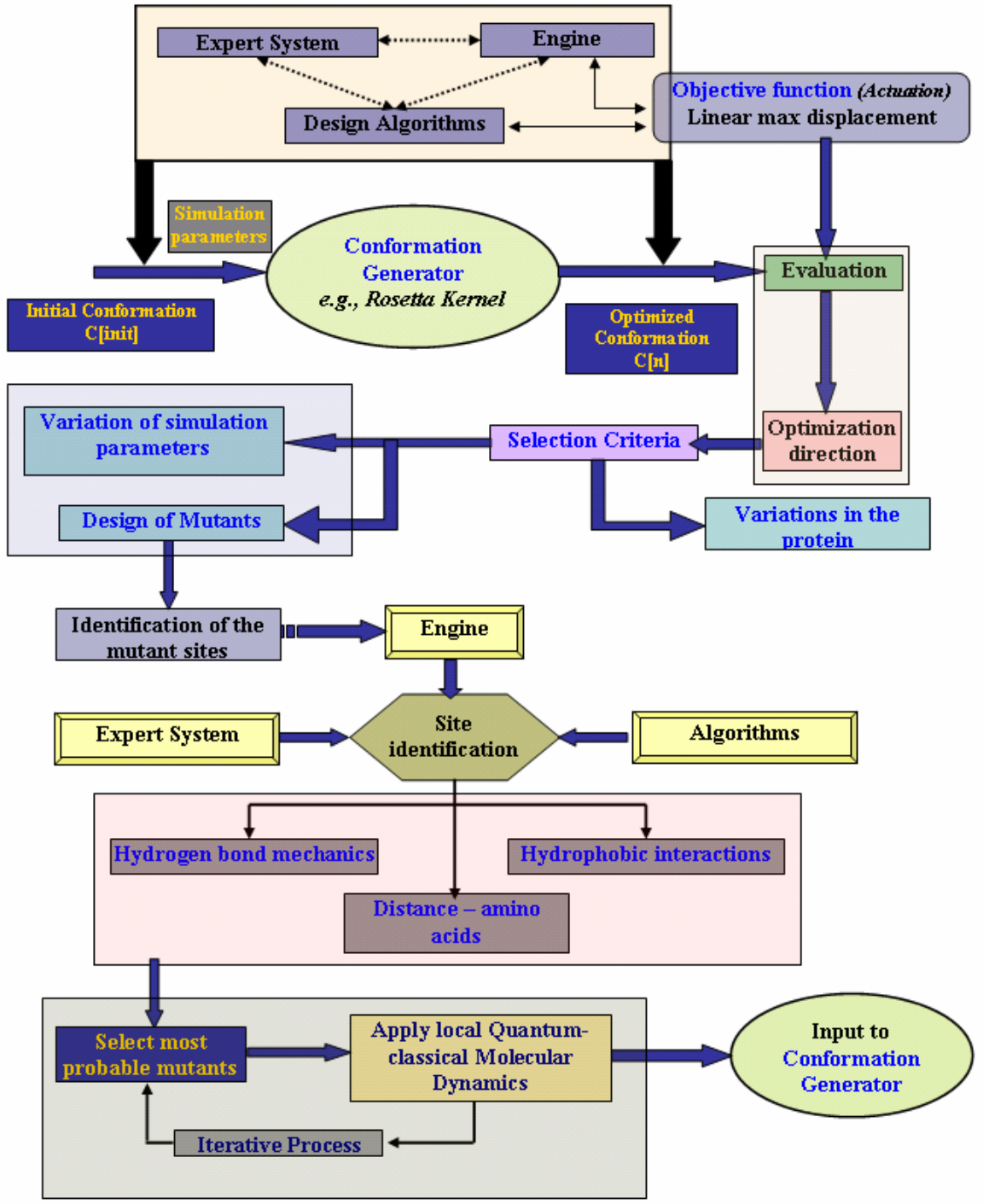


Figure 4-3: *Architecture of design and optimization kernel, the DOK*

i) **An expert system** – this will guide the optimization and design process through the captured expert knowledge in the field of biology and bio chemistry. This will guide to select the best possible mutants for the present protein in consideration.

ii) **An engine** – this is the shell or user interface which will command the whole operation. Some of its functions are:

a. Communicate with the user and provide the overall interface.

b. The main program will run through this module interacting with the Expert system and the algorithm module.

c. The input to the Conformation Generator and the output from it would be guided by this engine. The output would be evaluated with the defined objective function of the particular operation.

d. The mutant selection and the local Molecular dynamics module will be operated by it.

e. The simulation parameters would also be governed by this module.

iii) **Algorithm module** – this will present all the logic and rules which will try to generate the most optimized protein bio component for the given parameters. This will also embed the local molecular dynamics algorithms which would use NAMD (molecular dynamics software) libraries and files.

iv) **Conformation Generator** – this is a software to predict the most probable conformation of the protein given its primary structure and simulation parameters. Presently the libraries to employ under this are from the Rosetta software. This software is assumed to generate the best possible conformation of the tertiary structure of the proteins currently. Further this software would enable us in the assembly of the protein components.

C) The methodology of DOK explains how this framework would work and provides details through an example:

i) An *objective function* is defined for a particular process, for example actuation. Then an initial protein sequence is assumed (the best possible solution from literature research).

ii) The *initial conformation matrix C[init]* is fed into the Conformation generator module.

iii) An *output conformation matrix C[n]* is obtained. *n* represents the number of steps in the design and optimization process.

iv) This conformation matrix is subjected to the objective function by the engine. For the above defined objective function, the evaluator function will calculate the distance between the base point and the end effector point of the protein conformation.

v) The selection criterion will then execute, which will decide that either the protein selected should be changed drastically (like consider new protein) or to trigger "design of mutants" or "variations of simulation parameters" module.

vi) If the "variations of simulation parameters" module is selected by the system then the following parameters would be changed in the conformation generator:

- pH
- Temperature
- Pressure
- Solvent
- Other interacting biomolecules

vii) If the "design of mutants" module is selected then the engine will trigger the following process:

- Identification of the mutant sites

- The expert system will guide on the probable set of mutants and the sites
- The algorithm module will process the information and generate a few set of mutants which are selected by the local MD process.
- These selected set will then be fed by the engine to the conformation generator for the optimized results.

viii) This process will run till the objective function is achieved. The derived protein sequence and the structure then at hand will be the most optimized solution for the particular simulation parameters.

4.3 Computational studies on the effect of space radiations on bionano system

In this section we will setup the computational platform for evaluating the effect of *space radiations* on the bionano robotic systems. *Figure 4-4* describes the setup of the system. Radiations can produce many effects in a molecular system. It can break bonds, change the structure, destroy the amino acid residues, form other bonds and render bionano system with inability to perform its functions. In our case the bionano system has to perform dynamical tasks. So the effect of space radiations on the structure and condition of the bionano system is only one part, we have to further evaluate these effects on the dynamics of the whole system.

Space radiations are form of electromagnetic radiations. Maxwell's fundamental equations describe the evolution of the electric and magnetic fields $\boldsymbol{E}$ and $\boldsymbol{H}$ in the presence of a distribution of electric charge density and electric current density. These equations are coupled with Lorentz equations which can then determine the motion of an electric charge in the presence of an electromagnetic field [Messiah 1999].

Coupling of radiations to the atomic system [Messiah 1999]**:** To begin the study we will first focus on a very simple atomic system of ***A*** electrons and a nucleus of charge ***–Ae,*** where ***e*** is the charge of an electron. Now we have to define the Hamiltonian for Radiation coupled to this set of atomic system. The total Hamiltonian can be written down as:

$$H \equiv H_{RAD} + H_{ATOM} + H^{"}$$

Here the H_{RAD} is the Hamiltonian of the free-radiation; H_{ATOM} is the Hamiltonian of the atom itself (in the coulomb field of its nucleus and interaction with one another) and $H^{"}$ is the term coupling the electrons of the atom with the radiation.

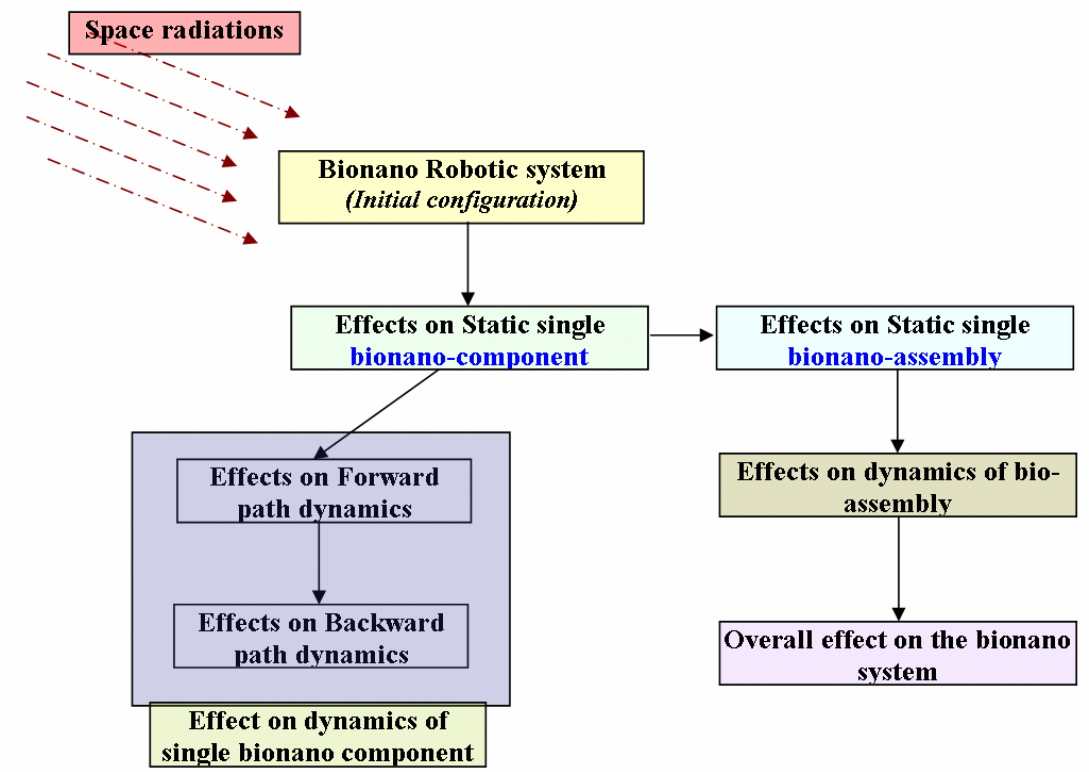


Figure 4-4: *The flow of computational studies on the effect of space radiations on the bionano system*

Further $H^{"}$ is the sum of A coupling terms H_n:

$$H^{"} = \sum^{A} H_n$$

Here H_n is the Dirac Hamiltonian of the nth electron in the presence of the electromagnetic field. Now we can treat this problem either considering the electromagnetic radiations classically or treating the $H^{"}$ as a small perturbation. Now we will further treat this problem as a case of inelastic scattering treating this as a quantum-electrodynamical system.

The steps in this study are shown in *figure 4-4* above. First a static bionano system would be considered which exhibits no dynamical behavior. The results obtained from this would then be taken into account to treat the system as dynamic with consideration to both forward and backward paths. Once the results are obtained for a single bionano component these would then be extended to a full bionano assembly and a bionano system.

4.4 Atomistic level computational techniques

Some of the basic theories which are potentially applied for studying the biological components and their interaction with their environment are described in this section. Our research methodology is to develop computational framework for studying the dynamic behavior and perform stability analysis of bio-nano components which comprises mainly of peptides and proteins. One of the steps of the computational framework is to perform ab initio calculation to a specific part of the bio-nano component. These ab initio calculations are computationally very intensive and are usually coupled with other semi-empirical and classical techniques. These approaches though very intensive in their

implementations are more accurate than the conventional classical approaches. Hence initial studies are conducted on these ab initio techniques and these would further be employed to form the computational framework and hence engineer bio-nano components which are stable in space environments. **Density functional theory** [Parr 1989] is one such ab initio approach which we would study and employ for our system. Also studied in this section is the **SCF (Self consistent field) Hartree Fock Approximation** [Szabo 1989, Levine 2000, Messiah 1999] which forms the core of the computational techniques.

1) Density Functional Theory (DFT) is a functional based method. It is used to calculate the ground state energy of multi electron system. Core to this functional methodology is the *Local Density approximation* – In this approximation, electronic properties are determined as functionals of the electron density by applying locally relations appropriate for a homogeneous electronic system. Hohenberg and Kohn (HK) developed these on the following two fundamental theorems:

a) 1st Hohenberg – Kohn theorem: It asserts that the density of any system determines all "ground-state" properties of the system, i.e., $E = E(\rho)$, where ρ is the ground state density of the system. It therefore, legitimizes the use of electron density $\rho(r)$ as the basic variable. Since ρ determines the number of electrons, it follows that $\rho(r)$ also determines the ground-state wave function ψ and all other electronic properties of the system.

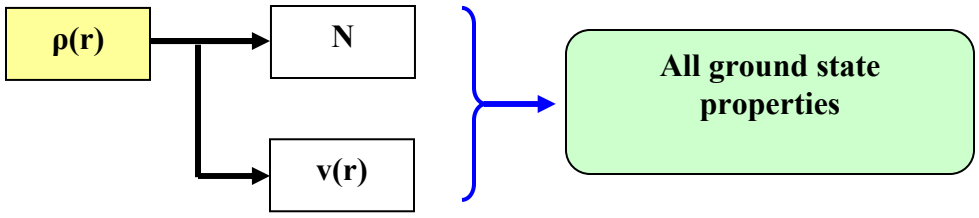


Figure 4-5: *Flow chart of the HK theorems*

In this flow chart (*figure 4-5*), N is the total number of electrons and v(r) is the external potential applied on the system.

b) *2nd Hohenberg – Kohn theorem:* This theorem provides the energy variational principle for the above calculated energy functional E(ρ). It basically states that if **ρ'** is not the ground state density functional of the system, then

$$E(\boldsymbol{\rho}') \geq E(\boldsymbol{\rho}^0)$$

Here, $\boldsymbol{\rho}^0$ is the ground state density of the system. The energy functional is defined as follows:

$$E(\rho) = T(\rho) + V_{ne}(\rho) + V_{ee}(\rho)$$

Here, **T** is the kinetic energy component, $\mathbf{V_{ne}}$ is the electron-nucleus component, and $\mathbf{V_{ee}}$ is the electron-electron repulsion component.

One of the basic advantages of the DFT is that no matter how large the system is, it has only three variables, x, y, and z. In QM wave function ψ of N electron system has 3N variables. Hence it reduces the computational efforts by a large amount. But this theory is accurate for ground state only. The extension to higher states is not obvious and not fully defined. We would use these basic methods for calculating some of the basic properties of the bio-nano robotic elements, such as, nanoGripper. Density functional theory and other molecular orbital algorithms could be used to simulate bio-nano components for various conditions. These algorithms would give us atomic level details on the various effects on the molecules. Using these computational techniques one can potentially calculate many ground state properties, such as vibrational energies of the molecules. Some of the software where these methods are implemented is Gaussian, DFT++ (C++

based DFT software framework) and other basic quantum mechanics routines. We would be using Gaussian and DFT++ to model our bio-nano systems as per our framework.

The interaction of the atoms with the electromagnetic waves is studied by the quantum electrodynamic (QED) theories. This theory determines very precisely the effect of these fields on the electrons. Application of these theories is a very complex task when considered to be applied for biological components, such as, proteins.

2) SCF (Self consistent field) Hartree Fock Approximations: This is the most reliable approximation method used in the literature (together with Configuration Interaction approach). This method gives energy predictions of the ground state energies most close to the actual measurable ground state energies.

$$\mathbf{E_{ground\ state} <= E_{HF}}$$

Where,

$\mathbf{E_{ground\ state}}$ is the measured actual value of the energy

$\mathbf{E_{HF}}$ is the energy calculated by Hartree Fock method

The Hartree-Fock Approximation is the basis for all ab initio techniques involving the optimization of molecular structure, and uses a Slater Determinant formed from a basis set of spinorbitals:

$$\psi_{so} = \frac{1}{\sqrt{n!}} \begin{vmatrix} \phi_i(1) & \phi_j(1) & \cdots & \phi_k(1) \\ \phi_i(2) & \phi_j(2) & \cdots & \phi_k(2) \\ \vdots & \vdots & \ddots & \vdots \\ \phi_i(n) & \phi_j(n) & \cdots & \phi_k(n) \end{vmatrix}$$

ψ_{so} describes the molecular wavefunction in terms of (atomic) spinorbitals, $\varphi_i(j)$, under a normalization coefficient $1/\sqrt{(n!)}$; i is the spinorbital index, j is the electron index and n is

the number of spinorbitals. The approximation has the effect of antisymmetrising the electrons with respect to exchange in accord with the *Pauli Principle* for *fermions*, particles with half integer spins, and uses spinorbitals chosen such as to meet the *orthonormality condition*. The Slater Determinant is then used as a trial wavefunction to be solved variationally:

$$E \geq \frac{\langle \psi_{trial} | H | \psi_{trial} \rangle}{\langle \psi_{trial} | \psi_{trial} \rangle}$$

$$H = T_e + V_{Ne} + V_{ee} + V_{NN}$$

$$h_i = -\frac{1}{2}\nabla_i^2 - \sum_a \frac{Z_a}{R_a - r_i} = T_e + V_{Ne}$$

$$g_{ij} = \frac{1}{|r_i - r_j|} = V_{ee}$$

h_i, the *core hamiltonian*, describes the motion of the electron i in the static field of nuclei, thus the attractive force it experiences in their presence, but omits the interaction with all other electrons. g_{ij} describes the electron-electron repulsion of i in the averaged field of all electrons $j \neq i$ (the ***Mean Field Approximation***). It is implicit that as the electronic wavefunction is found in a static nuclear configuration, the *Born-Oppenheimer Approximation* must hold. ***H*** can be found from the following equation:

$$H = \sum_{i=1}^{n} h_i + \sum_{i=1}^{n} \sum_{j>i}^{n} g_{ij} + \sum_{a} \sum_{b>a} \frac{Z_a Z_b}{|R_a - R_b|}$$

Where,

$$\sum_{i=1}^{n} \sum_{j>i}^{n} g_{ij} - \sum_{i=1}^{n} \sum_{j>i}^{n} (J_{ij} - K_{ij})$$

$\boldsymbol{J_{ij}}$ and $\boldsymbol{K_{ij}}$ are defined as the *Coulomb* and *Exchange* operators, whose matrix elements may be defined as follows:

$$J_{ij} = \langle \phi_i(1)\phi_j(2)\ldots\phi_n(n)|g_{ij}|\phi_i(1)\phi_j(2)\ldots\phi_n(n)\rangle = \langle \phi_i(1)\phi_j(2)|g_{ij}|\phi_i(1)\phi_j(2)\rangle$$

$$K_{ij} = \langle \phi_i(1)\phi_j(2)\ldots\phi_n(n)|g_{ij}|\phi_j(1)\phi_i(2)\ldots\phi_n(n)\rangle = \langle \phi_i(1)\phi_j(2)|g_{ij}|\phi_j(1)\phi_i(2)\rangle$$

The above theory is mostly exact and hence hartree fock gives a very good approximation. The only considerable draw back of this approach is the *mean field approximation*. No electronic correlations are considered and hence this method doesn't give the appropriate solutions for larger systems. Two approaches deals with the concept of **electron correlations**:

a) Configuration Interaction (CI)

b) Moeller-Plesset perturbation theory (MP)

Electron interactions are much more specific than just mean field approximations, and include Pauli repulsions as well as electrostatic ones. This is taken care by utilizing excited electronic configurations in the calculations. Computationally, MP correlation is less laborious than CI, and thus has displaced the CI type in many ab initio calculations, where the computational labor is already high.

3) Quantum Monte Carlo methods: Monte Carlo method is used for n-dimensional integrations which is otherwise extremely expensive to carry out with other methods. Therefore an accurate algorithm integrated with the molecular dynamics approach seems to be the choice for carrying out simulations of larger biomolecular systems. This approach is rather statistical in nature as it is based on the probability distribution functions, but far much faster for larger systems. *Appendix-2* shows an application of basic quantum Monte Carlo method. VMC (Variational Monte Carlo) method is implemented in FORTRAN 77 for hydrogen atom its energies are obtained. Hydrogen molecule is further studied through the solution of Schrödinger Wave Equation. This is

implemented in FORTRAN 77 and the detailed codes are shown in Appendix-3. The basic computational flow for the problem is detailed in *figure 4-6* below. The calculated ground state energy of H2 molecule by this algorithm is *-4.477 eV* which is in very good agreement with the experimental value.

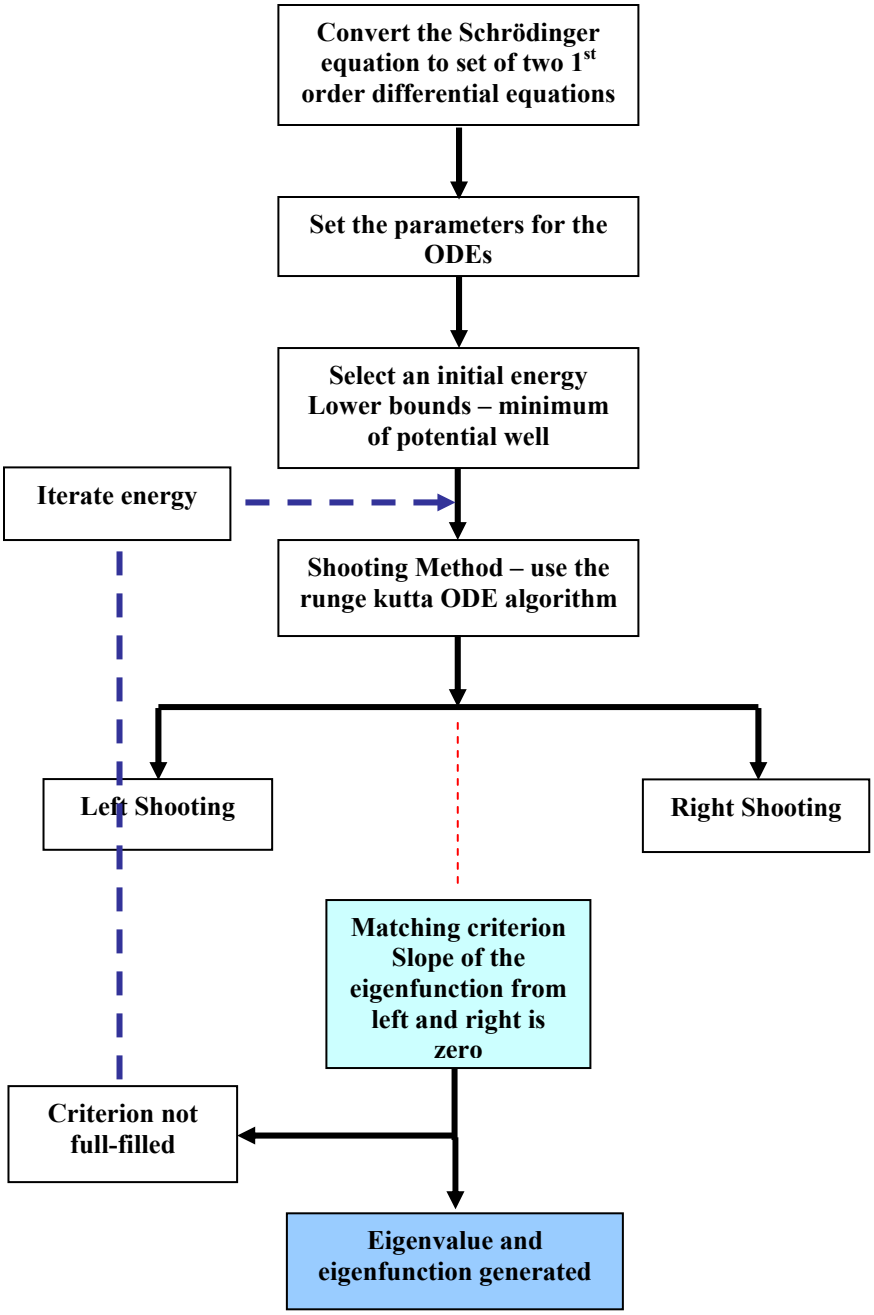


Figure 4-6: *Flow chart of the algorithm to calculate the eigenvalues of the vibrational modes of the H2 molecule.*

Chapter 5: Networked TerraXplorers (NTXp)

5.1 Introduction

In this section we present the *Networked TerraXplorer* (NTXp) and the initial design steps that have been undertaken during the first two months of the project. Some of the design methodologies and principles are presented here. In these two months we have worked on a possible design and developed an animation to show the concept.

5.2 Concept Overview

Mapping and surveying a vast planetary terrain is a very difficult task. Some of the difficulties faced are limited area of landing for sophisticated planetary probes and rovers. It has been estimated that only a very small percentage of a planet's area is suited for landing. The planetary terrains and the atmospheric conditions pose a lot of difficulties for surface as well as air probes. Hence, only limited mobility could be achieved. Also, the investment required will be enormous for designing such rovers with capabilities of exploring the vast and difficult terrains.

Networked TerraXplorers is a concept shown in *Figure 5-1* in which we exploit various advantages of the proposed bio-nano robotic systems such as their extremely light weight; low cost of manufacturing; mass scale production and bulk usage (billions) and their ability to self-assemble and self – organize.

NTXp is a network of channels containing the bio nano robots having the enhanced sensing and signaling capabilities. This essentially is a static device, which could be easily projected onto a planetary surface, which we intend to explore. The length of this device could be in miles, and yet it will be very light in weight. These could be easily packaged into small volumes appropriate for space missions. Also the power

consumption for this device will be considerably less. The main consumption of power will be to maintain gradients for transporting the bio-nano components inside these channels and for signaling and communicating with the main receiver.

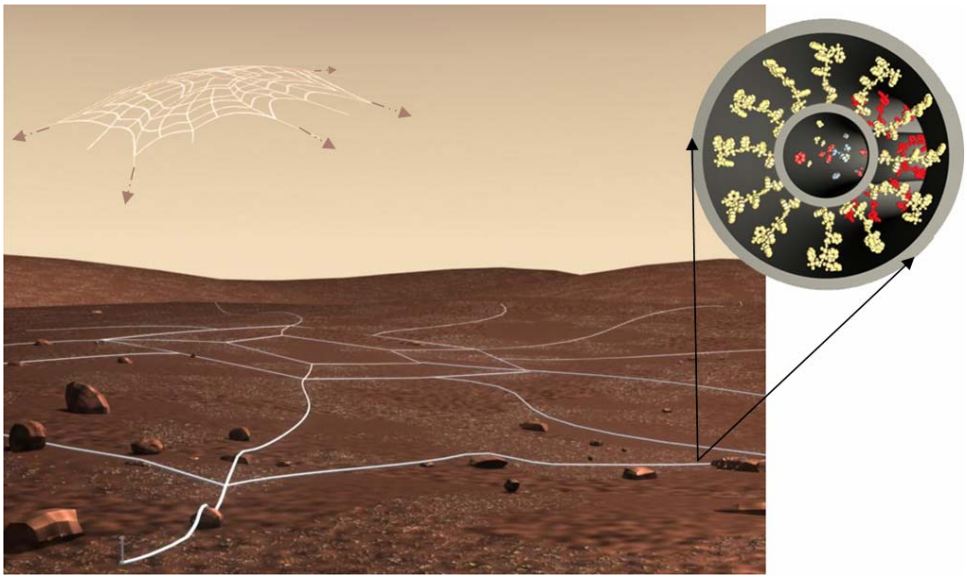


Figure 5-1: *A realistic scenario where the Networked TerraXplorers (NTXp) are employed. These meshes will be launched through the parachute and these will be spread open on the target surface. These NTXps could be launched in large quantities (hundreds) and hence the target terrain could be thoroughly mapped and sensed. A single NTXp could run into miles and when integrated with other NTXps could cover a vast terrain.*

The bio-nano robots will move inside the channels of the network and will have *'limited'* window of interaction, through special valves with the outside environment. They will interact with the outside terrain and chemically sense the presence of water or other targeted resources / minerals as it is shown in *Figure 5-2*. They will also act like a position sensor on the surface of the terrain enabling it with the capability to map the terrain geometrically. They will communicate with their main nodes and will pass the

information about the terrain through them to the main receiver (which could be an altitude orbiter). These networks could be spread throughout the terrain irrespective of the topological constraints. Their mass production will be cheap as compared to any technology available now, which could be used to map a terrain. Furthermore, these networks could be used by future rovers or human explorers for tracing out the vast terrain and thereby guiding them to the direction they should follow. Discovery of caves and low-lying surfaces could be possible with the help of these networks. This information will be very crucial for future human explorations to any planetary terrains.

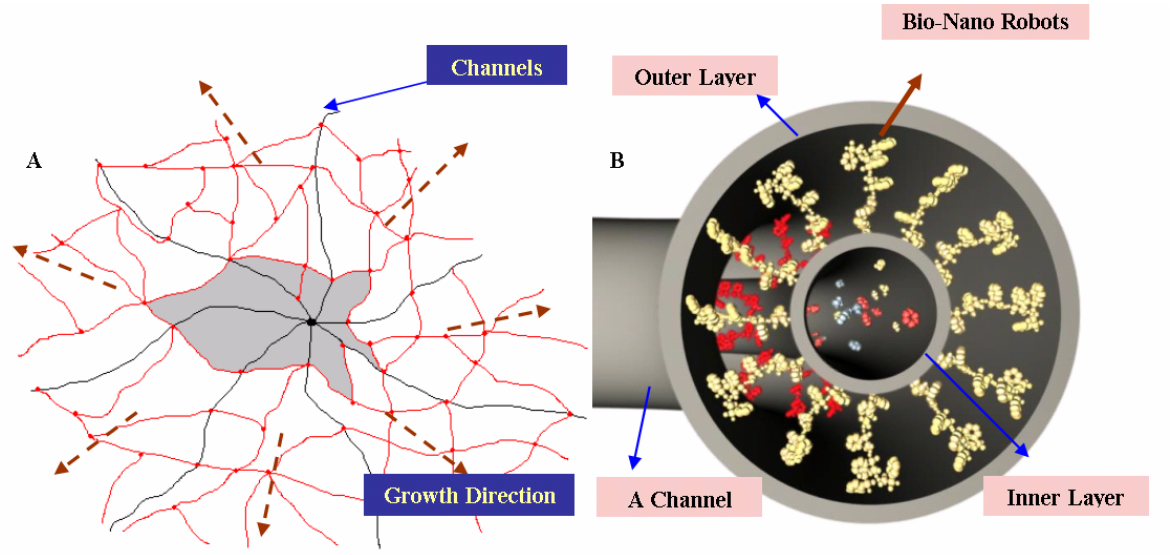


Figure 5-2: (A): *A Networked TerraXplorers (NTXp). These meshes will grow "internally" as directed by the dotted arrows towards the area of interest. It will then evaluate the region and map it as per the sensory and signaling capabilities it inherits through the bio-nano robots. The black central node represents the central processor, which will be responsible for integrating the entire mesh and will have the capability of communicating with the orbiting satellite or other receiving stations.* ***(B)*** *Shown is the cross-section of the channel of the NTXp. The channel will be designed in various layers*

(two layers are shown). The inner layer will be responsible for the transportation of the bio-modules required by the basic bio-nano robots. The outer layer will sense the environment and will signal the findings back to the central unit responsible for communicating and maintaining through inner layer which will also carry signaling bio-nano robots.

The micro channels will be designed in various layers, making it ready for the harsh conditions on the planetary surface, such as: Radiations, Temperature, pressure etc. The various layers will have specific functions and will contain specifically designed bio-nano robots for those layers. These bio-nano robots will be enhanced in signaling and sensory capabilities (bio-nano code EGS, with E for energy storage and carrier, G for signaling and S for sensory capabilities). One material under considerations for being used as the outer skin of the network could be *polyethylene polymer*. This will protect the bio-nano robots from the harmful radioactive radiations.

Sustaining such map will not be energetically expensive and NTXps will have extremely low energy to mass requirements. The network and the bio-nano robots inside the channels could be sustained for long periods of time and hence will enable the NTXp with a long mission life, which is much desired for such exploration missions. These Networked Channels will be able to grow "internally" and will be able to move its internal contents in a specific direction (depending upon the direction of the target). It could also be triggered as that it concentrates internally towards a particular location of interest so as to generate higher capabilities. These networks will be designed to be integratable with similar networks. Once these networks are integrated the whole terrain could be mapped by a single "super network".

NTXps could be designed in another two variants *Macro level* (as described above) and *Micro level*. The design of these variants would be based on their level of reach. Macro level variant could be miles long and integrated to a unitary central unit, whereas the micro variant would be only few millimeters (or smaller) in dimensions. The Micro variant (as shown in the *figure 5-3* below) called as *Micro Networked TerraXplorers*, is the extension of the NTXp concept. In this concept, the bio-nano robots would perform sensory function, but the signaling function would be performed by the "communication microchip" integrated with the bio-nano system. This microchip would signal the data gathered to the central receiver. This Micro NTXp would be very small in size (few mms or smaller) and could be sprayed from the air borne rover or orbiter to the desired location. These devices would form a sensory network amongst them and would act in collaborative and distributive way. The following figure describes the concept of this device:

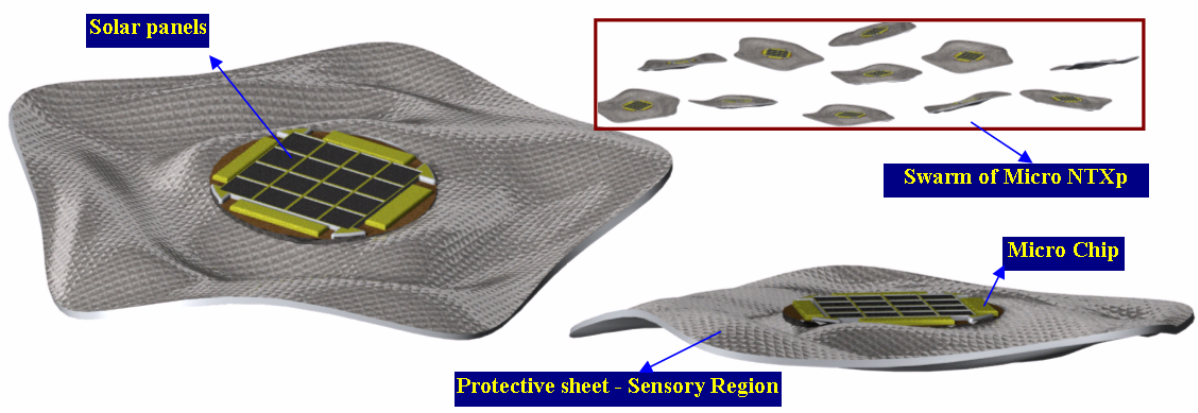


Figure 5-3: *Micro Networked TerraXplorers*

The bio-nano robots would sense and then trigger the micro processor with the findings. These micro networks could be dynamic in nature as these could be easily

flown by the high surface winds and currents and in effect would let us know more about the inside details of the structure of the storms and winds on the planet.

5.2 System Level Design of NTXp

The design of the NTXp is based on the design of root architecture in plants [Berntson, 1997]. Particularly, an important mechanism of the roots of the plants is the *Mineral Uptake* mechanism. Minerals enter separately in the roots of the plants. Even when no water is being absorbed, minerals enter freely. Minerals can enter against their concentration gradient; that is, by *active transport*. Plants absorb their nutrients in inorganic form. For examples: nitrogen enters as nitrate (NO_3^-) or ammonium ions (NH_4^+); phosphorus as PO_4^{3-}; potassium as K^+; calcium as Ca^{2+}. Based on these observations the following design characteristics, shown schematically in *Figure 5-4*, have been proposed for the NTXp:

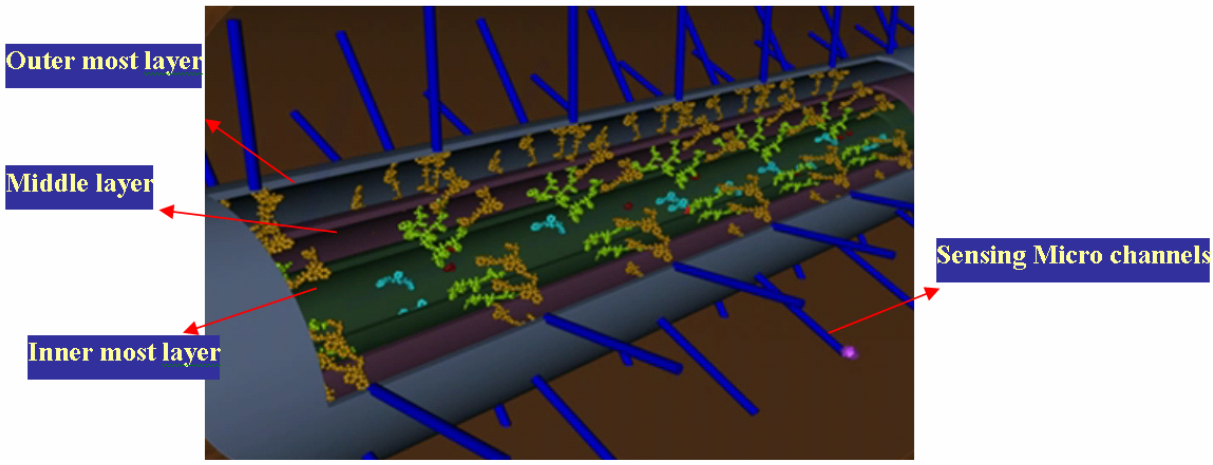


Figure 5-4: *The 3tier architecture of NTXp:outer layer, middle layer and the inner layer.*

1. Creation of inorganic external micro channels that will be responsible for interacting with the outside environment and transport the sensed information to the inner sensory layer.

2. Presence of charges (H+) on the NTXp surface that can initiate reactions with the planetary environment.

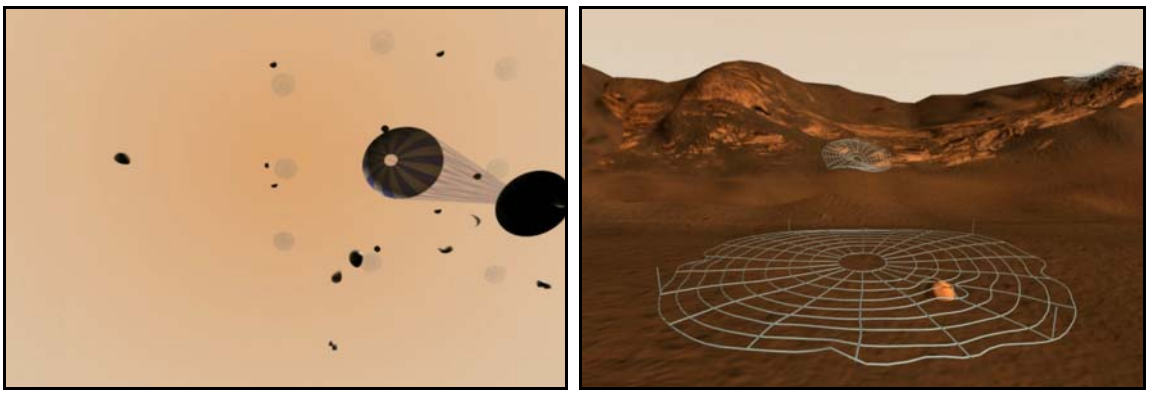
3. Existence of an external insulating and radiation resistive layer that would shield the inner bio modules from radiations and temperatures.

4. Establishment of an intermediary exchange layer that will have small tubular structure for enabling active transport of ions or charges across. This layer will enable the connection between the inorganic micro channels and the bio-nano sensory module.

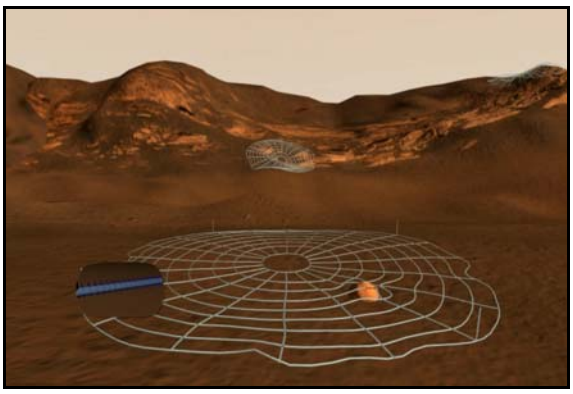
5. Creation of an inner sensing layer that will sense the absorbed constituents and transfer the information of the measured parameters to the signaling module.

5.3 Deployment Scenario for NTXp

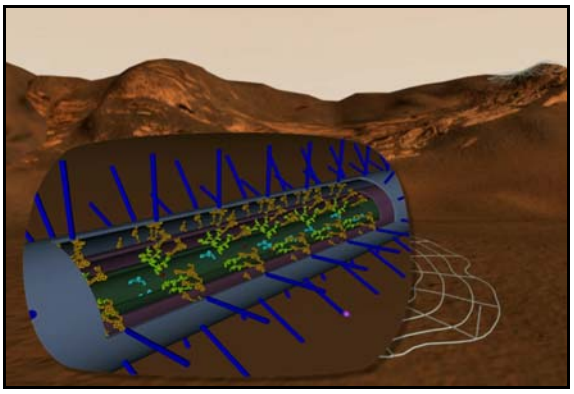
An animation was created that presents the deployment scenario of NTXp. The animation can be downloaded from http://www.bionano.neu.edu/mru.html . Representative pictures from this animation are presented in *Figure 5-5*.



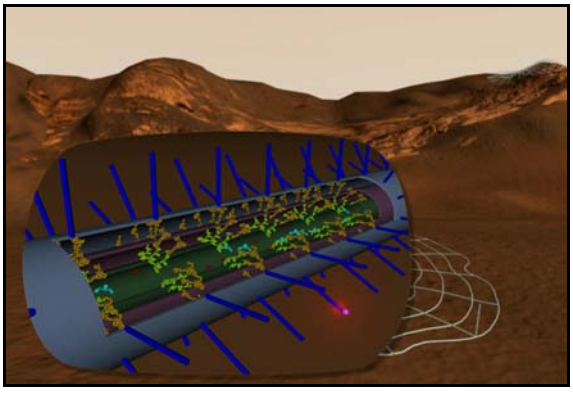
(A) (B)



(C)



(D)



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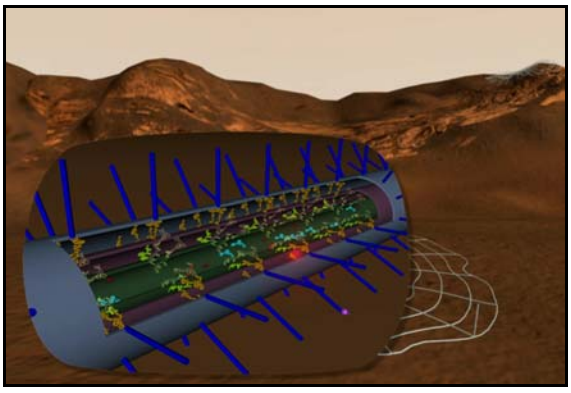

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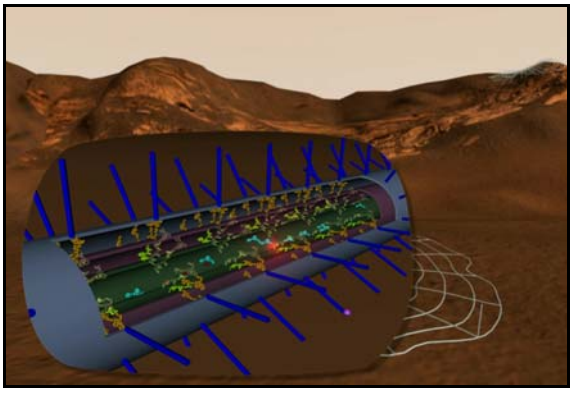

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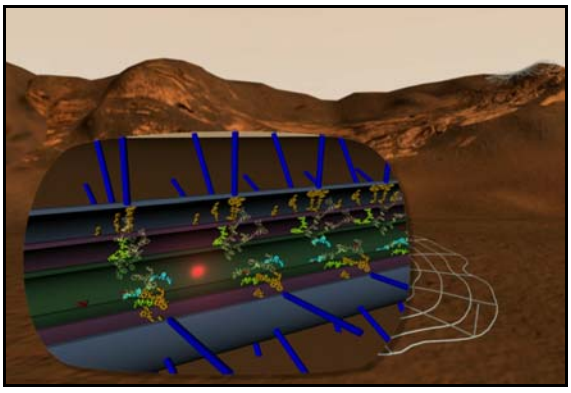
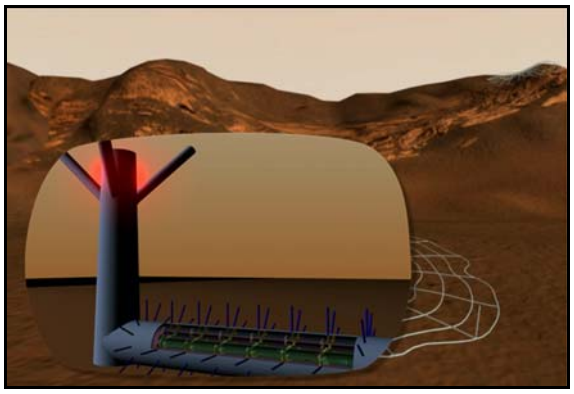


(H)



(I)

Figure 5-5: (A): *A scenario of deployment of NTXps from a parachute. Multiple devices which will have an ability to communicate with each other will be deployed on the same time so that they cover a vast area of the planetary surface;* ***(B):*** *Shows the NTXp on the planetary surface. The deployment of these devices doesn't assume any topography or surface geometry of the landing site. This is one of the main advantages of using such devices. A web kind of architecture is defined for NTXp to maximize the probability of sensing in a defined area.* ***(C):*** *A section of one of the channels of the NTXp is zoomed in to show the inner details of the device;* ***(D):*** *This shows the three layer architecture of the NTXp with the above detailed properties. Shown here are micro channels (shown in blue) which protrude from the NTXp surface. These micro channels are the elements through which the surface sensing is carried out;* ***(E):*** *A scenario is described in which one of the micro channels senses a particular element on the planetary surface. A glow is shown in the figure which is analogous to the sensing process and flow of the information;* ***(F):*** *The sensed information (in terms of some parameters) is passed to the outer sensory layer whose function is to connect to the middle sensory layer which is responsible for sensing a defined parameter, say water;* ***(G):*** *The sensed parameters are converted to the signaling parameters and now this information is passed to the inner most layer which is*

responsible for storing this information and passing it to the main transmitter; ***(H):*** *Shown is the information storage module which is taking the information to the transmitter for communicating with the main station;* ***(I):*** *Shown here is the transmitter which converts the sensed parameters to electrical signals and finally transmits to information to the main station or communicates with the other NTXp devices.*

5.4 Sensor – signal dynamics

Sensor signaling dynamics would be used in the both of the proposed space applications, *Networked TerraXplorer* (NTXp) and *All Terrain Astronaut Bio-Nano Gears* (ATB). Sensor signaling architecture should be capable of converting the sensed parameter to a parameter which could be used for signaling. This could either be in the form of flow of electrons, or variations in the concentration of ions and their gradients. The signaling module is partially responsible for amplification too. The interface between the sensors and the signaling module, namely the *sensor-signaling dynamics* has to be defined. Need a real life example to define the architecture mathematically. The concept of modularity has to be kept in mind while designing the system architecture. *Figure 5-6* describes the overall sensor-signaling architecture and the various components of it.

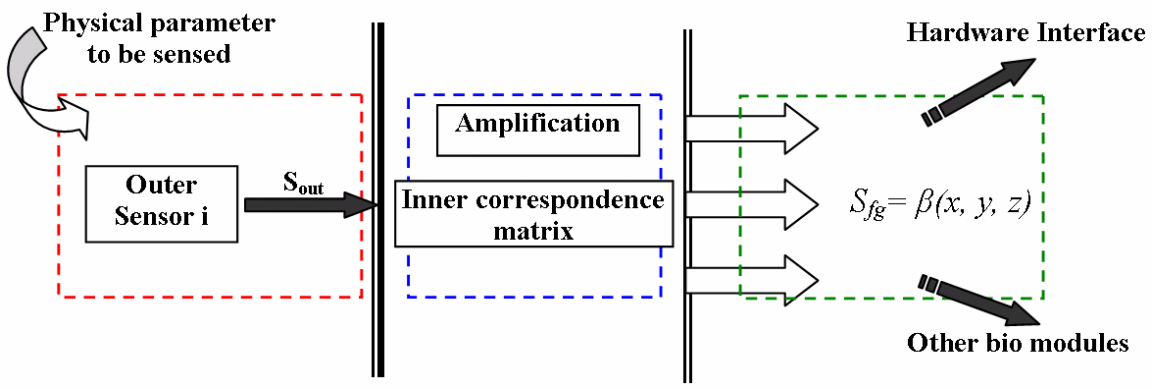


Figure 5-6: *Sensor-signaling architecture*

The following paragraphs explain the architecture in greater details:

1. Let a given physical quantity (say temperature) is sensed by two (or more) parameters, say, f and g. Let the Outer sensor, **i**, senses these two or more parameters. Therefore:

$S_{in}^{i} \rightarrow \{f, g...\}$ is the signal generated per sensor.

Let there be **n** number of sensory components. Therefore, the total signal generated for the outer sensory system = $\sum_{1}^{n} S_{in}^{i}$

Let p be the variable which represents the probability of getting sensory data within the level of confidence desired for the subsequent trigger mechanism (noise, inadequate per sensory signal strength). Therefore, the net output signal from the Outer sensory system is $S_{out}=p\{\sum_{1}^{n} S_{in}^{i}\}$. Hence $S_{out}=p\{\sum_{1}^{n}(a_i f, b_i g)\}$ is the output signal generated by the outer sensory module. The intensity of the output signal is $\left| p\{\sum_{1}^{n}(a_i f, b_i g)\} \right|$

2. The output sensory signal act as an input to the inner correspondence matrix. This matrix does the following function:

a. Amplifies the sensory input. A= $I \left| p\{\sum_{1}^{n}(a_i f, b_i g)\} \right|$. Here the sensory input signal is multiplied by an amplification constant, I.

b. Correspondence Table. This table decodes the input variables, f and g (or more) into pure signaling variables, say, (x, y, z). This decoding is based on the reaction between the sensory signal input and the signaling module and the strength of the signals

required for transmission. This reaction could either be before the amplification or after the amplification.

The initially sensed physical parameter (say temperature) which was sensed by two parameters (f, g) is now corresponded to three signaling parameter (x, y, z). This is shown in *figure 5-7*.

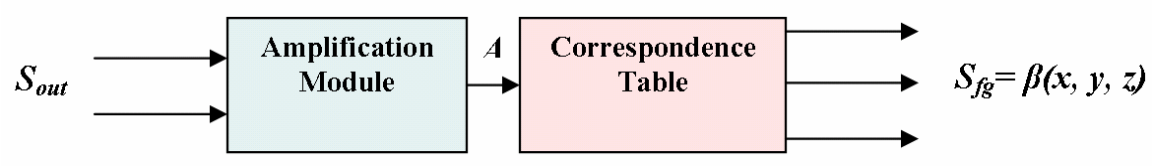


Figure 5-7: *The correspondence relation between the initially sensed parameters and the signaling parameters.*

The search for signaling protein would include therefore the above design parameters. It has to be compatible with the sensory input data and should result in an output signal with sufficient strength. The signaling data could either be transmitted to the memory devices or used to trigger another module, such as actuation, or information processing.

5.5 Micro-Nano fluidics actuator for NTXp transport mechanism

Transporting bio-nano robots inside the NTXp *(figure 5-8)* is one of the biggest challenges in its design. One novel way to achieve the transport behavior is the design of micro-nano fluidic actuators. As seen in *figure 5-8*, the internal and outer layers of the NTXp acts as the electrode surface, producing currents enough for driving the bio-nano robots around the network. One another important consideration while designing the electrode surface would be its reversal of polarity. This would give us forward as well as backward motion of the bionano robots which is pretty important in our scenario.

There are two main designs for this actuator. One is based on micro channels and the other is based on carbon nano tubes. The design would deal with a micro fluidic actuator based on Electro active fluids (EAF). Electro active fluids (EAF) are a promising alternative to smart actuation materials commonly used to derive mechanisms such as electroceramics (EAC), shape memory alloys (SMAs), and electro active polymers (EAP). Electro active fluids are certain *dielectric fluids* that can create a jet flow in response to electrical stimulation. The principle behind an EAF-based fluidic actuator is the electrohydrodynamic (EHD) phenomena. Jet flow is created in the dielectric fluid medium through the interaction of electric fields and flow fields under an applied high voltage. *Figure 5-9* shows the relationship between the generated pressure and the applied voltage to EAF based actuator. This generated pressure is utilized to provide actuation. This actuation would be utilized to pump or transport the bionano robots throughout the NTXp.

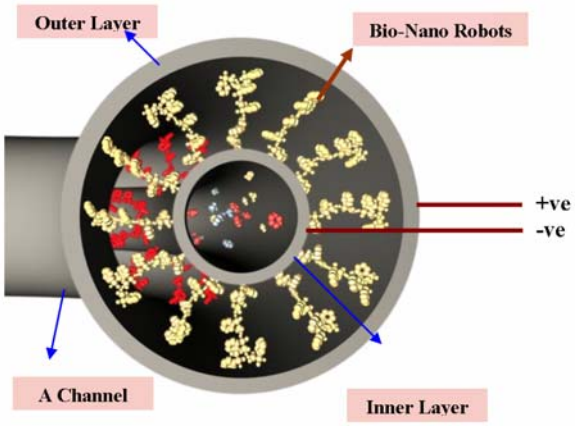


Figure 5-8: *A proposed scenario where the Networked TerraXplorers (NTXp) are employed. These meshes will be launched through the parachute and these will be spread open on the target surface. These NTXps could be launched in large quantities (hundreds) and hence the target terrain could be thoroughly mapped and sensed. A single NTXp could run into miles and when integrated with other NTXps could cover a vast terrain.*

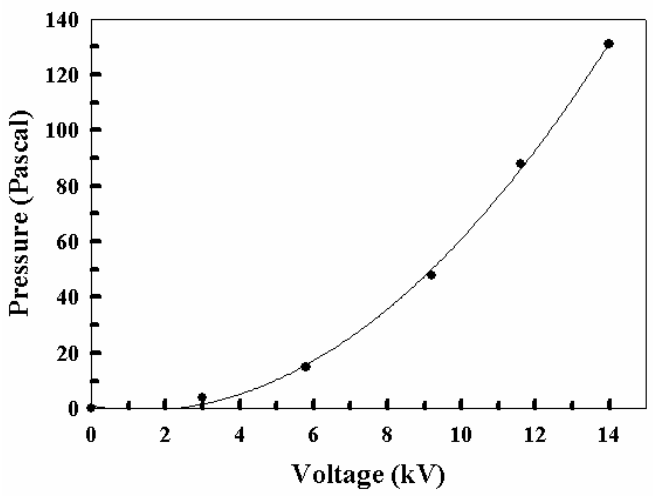


Figure 5-9: *Pressure due to EAF jet versus applied voltage*

The proposed micro-nano fluidics actuator would have a certain force capabilities and ability to move bionano robots. This actuator has to be designed to be bio compatible and would have enough dimensions to transport cell and other big bio-molecules. Before adapting the design to the NTXp, we have to design in detail micro channels based and carbon nanotube based actuators respectively. Once a proof of concept is achieved, this design would be optimized for space environments.

5.5.1 Conceptual design for Micro-Nano fluidics Actuator

Using EAF as the working fluid, the proposed nano actuator has a simple construction that consists of at least one nano electrode pair, which generates the pressure head when connected to a high dc voltage. A much higher pressure can be obtained by simply increasing the number of nano electrode pairs. The result is a force that can rotate a nano shaft, or move nano particles and bio molecules in nano tubes or nano channels.

To study the characteristics of EAF-jet in nano length scale, two conceptual designs are proposed. One of the designs is based on the NEMS (nano channels on a silicon substrate) system. With the current capabilities we propose the dimensions of the nano

channels to be around 50-100 nm. *Figure 5-10* shows the proposed design based on NEMS

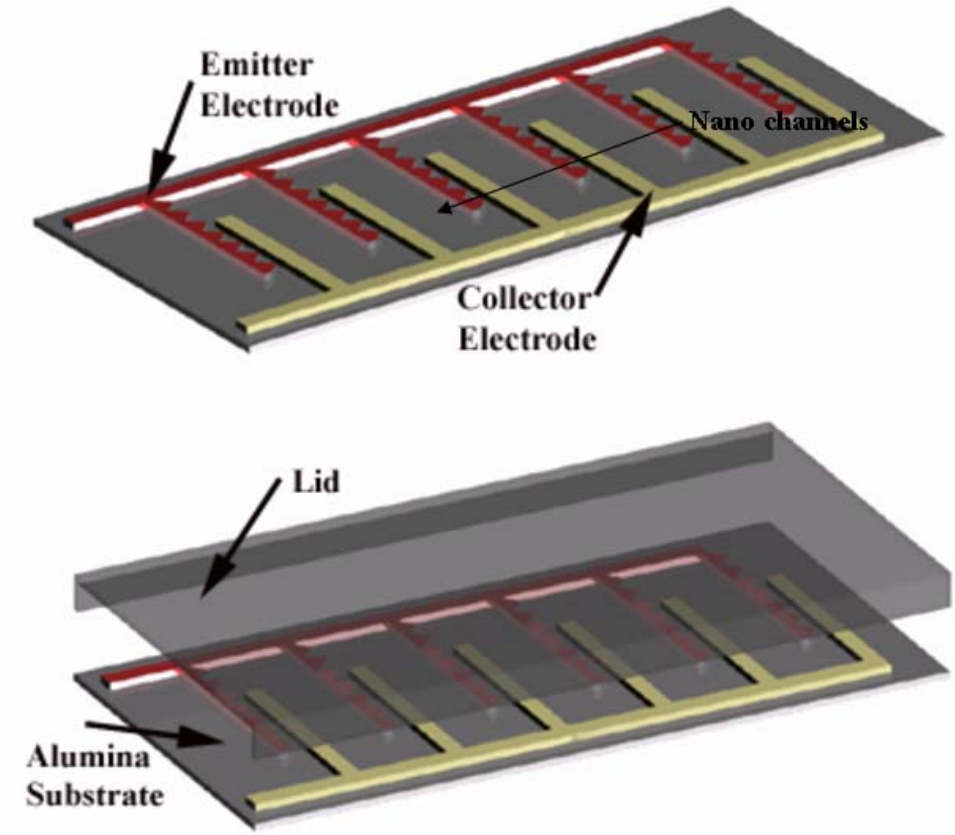


Figure 5-10*: *NEMS based Micro-Nano fluidic actuator

This is a kind of Ion-drag pump for which the driving force is the coulomb's force. The motion of the fluid in this device is induced by the movement of the ions across the applied electric field. This field is imposed using series of pairs of two electrodes:

- ***Emitter***
- ***Collector***

The friction between the moving ions and the working fluids drags the working fluid towards the collector, thus setting the fluid in motion. An EHD pump produces no vibrations; requires low power consumption; are electrically controllable and requires less maintenance. Electrodes are arranged in such a manner that a non-uniform electric

field is formed in the electro active fluid inside the nano channel. Further study would be conducted with micro-force sensors to measure the force (pressure) generated by the fluid jet flow.

Another variant of this linear micro-nano actuator is the rotary actuator. *Figure 5-11* shows the details of this actuator. The emitter electrode and collector electrode are along the circumference of the micro-nano disk and create non uniform electric field which drives the fluid. The rotational field created in the fluidic medium exerts forces on the cylindrical surface where the electrodes are mounted, and hence creates a rotary motion of the actuator.

The other design proposes to use carbon nano tubes (CNT) as the structural elements to which the electrodes are attached and through which the fluid flows. The electrodes in this case are made up of metallic ions attached to the carbon nano tubes through associative elements. *Figure 5-12* shows an electrode pair, +ve and –ve for the proposed concept. Each individual carbon nano tube would be either positive (or negative) through the deposition of the corresponding metallic ions. The electrodes shown would be encapsulated within a bigger diameter carbon nano tube filled with EAF particles. Through the application of some voltage *(which is still to be characterized)* the electrodes will induce non-uniform electric field and thereby generates forces on the EAF particles to produce motion. This motion could be heterogeneous as the electric field is not uniform and the exact forces and dynamics at the nano scale are still undetermined.

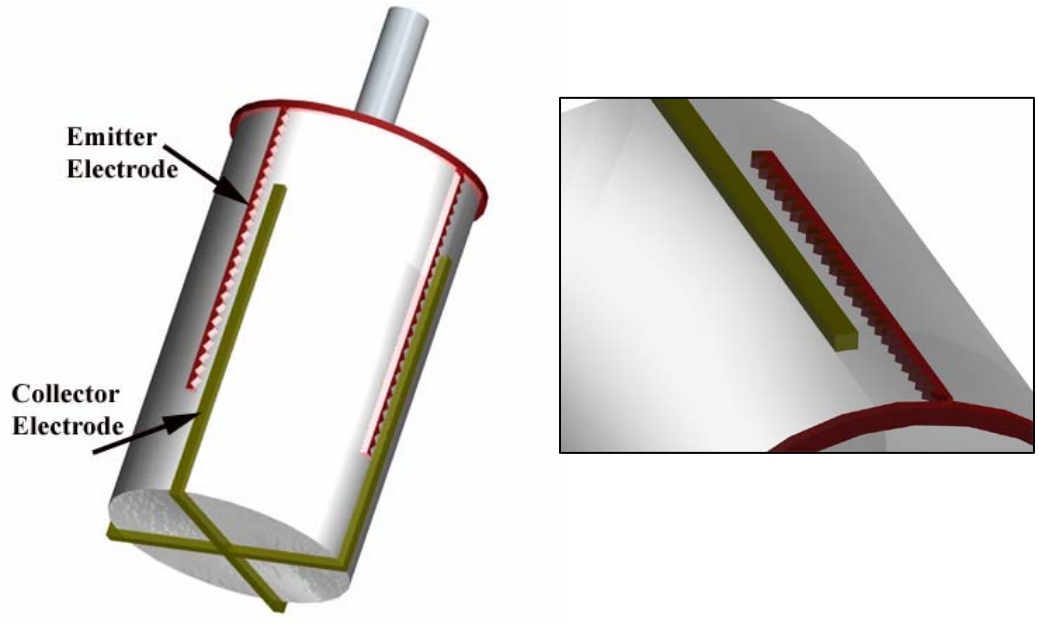


Figure 5-11*: *NEMS based rotary nanofluidic actuator

Patterned growth of carbon nanotubes such as they are like cylindrical units on a substrate has been achieved by researchers [Ajayan et al 2002]. This would help us design the first generation electrodes made out of carbon nanotubes.

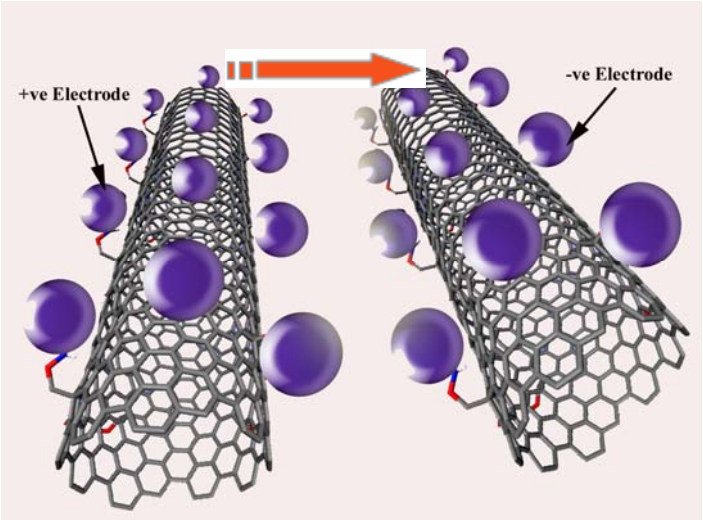


Figure 5-12*: *Carbon nano tube based Nano fluidic actuator

Chapter 6: All Terrain Bio-nano (ATB) gears for Astronauts

6.1 Introduction

To ensure enhanced health management capability for astronauts on future space missions, a multifunction system is needed to perform environment monitoring and serve as early warning and protection system against chemicals, radiations, temperature and pressure for the astronauts. This system will be like an adaptive shield protecting astronauts from possible health hazards. This device will form a complementary layer beneath the current or any other future space suits. The proposed ATB gear is one such concept that has the above-mentioned capabilities and functionalities. It will not only be lightweight but also flexible enough for allowing the astronauts to wear it all the time. The ATB gear shown in *Figure 6-1* is like an extra layer of shield on the human body, which will have the capability of sensing dangerous and harmful environments (such as radiations or chemicals) long before they could significantly harm the human.

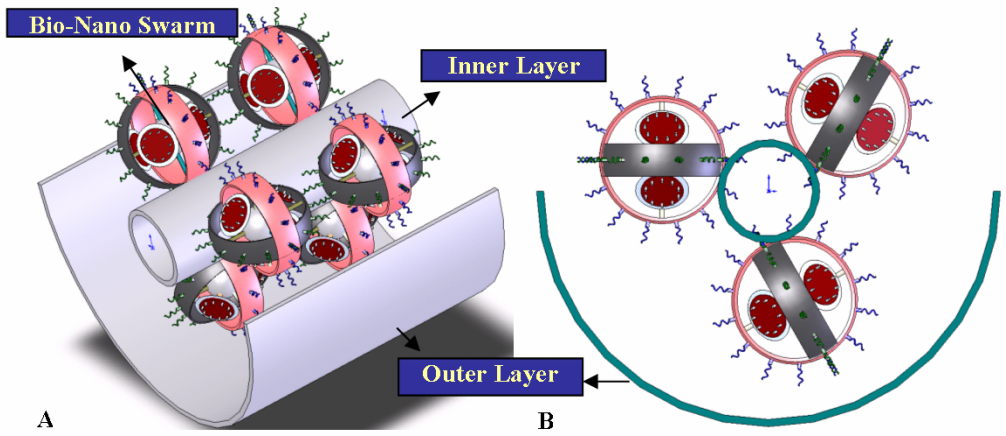


Figure 6-1: (A) *Shows a representative structure of the ATB Gear. Shown is the arrangement of the bio-nano robots inside one of the many channels which forms the ATB gear.* ***(B)*** *The bio nano robots are shown to penetrate the inner layer of the channel for sensory and signaling purposes. The bio nano swarms will have multiple layers having various layers of functionalities.*

These will not only act like early warning and detection systems but will aid in healing through current process and medicines and curing the damages that may be caused to the astronaut. These *"skins"* are meant to be all terrain in the sense that these could be worn at any time and anywhere by the astronaut, either inside the space shuttle or on a planetary terrain. They could further be designed to cover specific areas of interest on the space suit, such as locations where the same may show fragility, and monitor these locations all the times.

The bio nano gear will be made of various micro membranes and surface sheets as it is shown in *Figure 6-2*. Each one of these membranes and sheets will contain swarms of bio-nano robots capable of performing the required sensing and signaling tasks. The Modular Architecture of the bio-nano robots and its design architecture as described before would give us an ability to manufacture and program them for complex tasks. At run-time we could decide the concentration required for the bionano robots required at a particular location along the ATB gear surface. ATB gears could be programmed to form a self-healing layer for the astronaut's space suit. *Figure 6-3* shows various layers of the ATB gear interweaved with the inner layer of the "space suit" for astronauts.

This interweaved concept is designed such that as soon as some breach is detected, the bio nano robots inside these ATB gears will rush to the site and try and seal it, as it is shown in *Figure 6-4*, and block the effects to seep in through. This will give the astronaut valuable time to find a shelter or other alternative solution. This feature could be especially useful for the terrain mobile astronauts who don't have immediate accessibility to the facilities of the main station. The ATB gears hence are made an integral part of the

space suits, to protect and warn the astronaut against any possible health hazard from the outer environment and within.

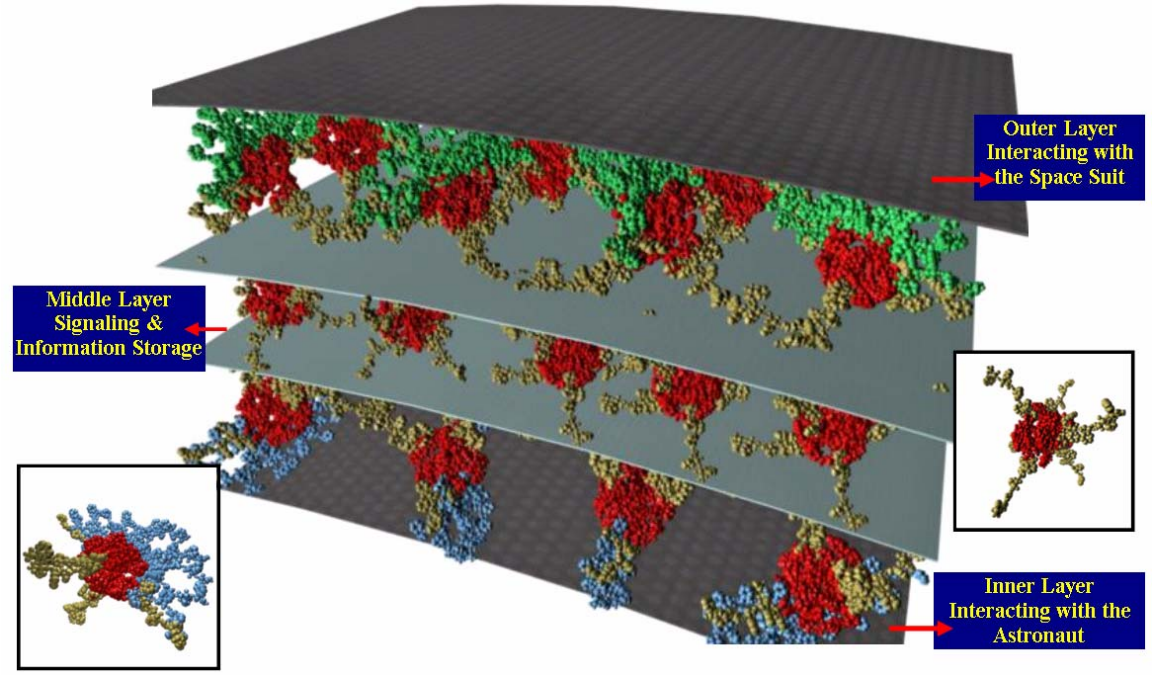


Figure 6-2*: The layered concept of the ATB gears. Shown are three layers for the ATB gears. The inner layer would be in contact with the human body and the outer layer would be responsible of sensing the outer environment. The middle layer would be responsible for communicating, signaling and drug delivery.*

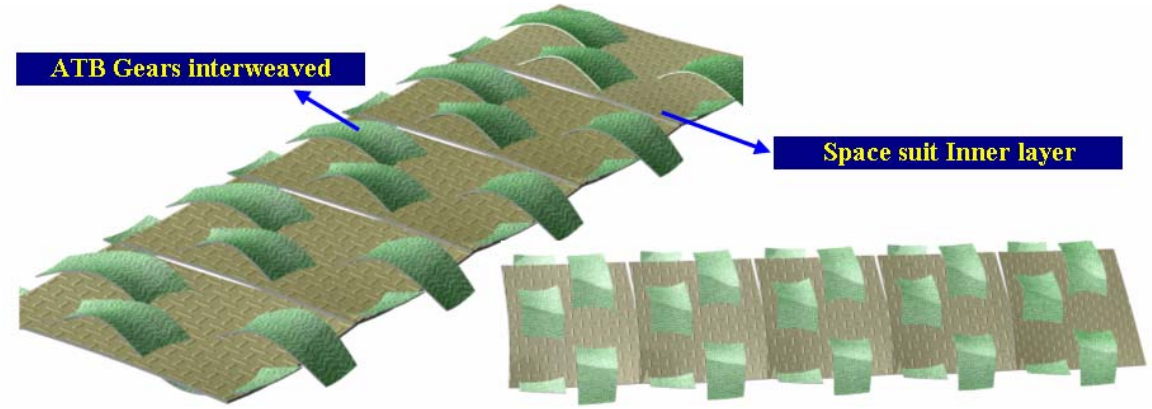


Figure 6-3: *Interweaved Concept for the ATB gears*

Furthermore, in case of a wound, these bio-nano robots will cover the skin of the astronaut so that the intensity of the wound is decreased. They could also be designed to deliver the wound healing drugs (stored inside the bio-nano robots) at the particular location of injury. The bio nano robots will have signaling capabilities that will trigger the flow of excess robots at such sites (stored in a stock on board the astronaut suit) and help accelerate the protection sequence. They can also help in sensing the temperature variations in the astronaut's body and will keep track of it and signal any un-natural event if it crosses any particular range.

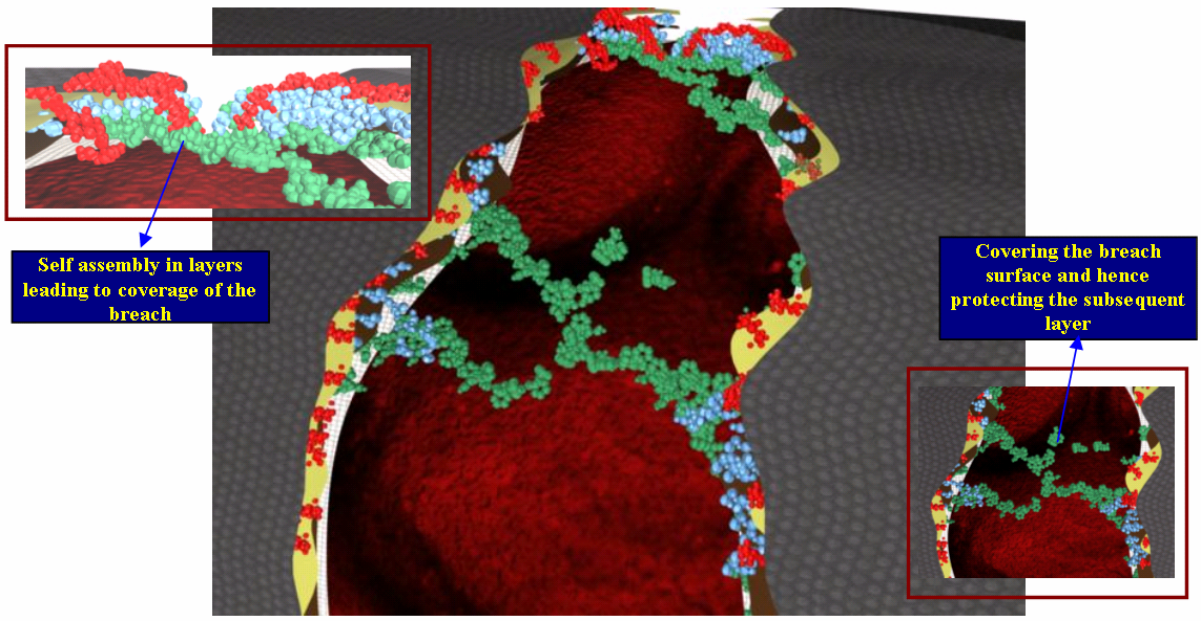


Figure 6-4: *Mechanism of curing a wound by the ATB gear. Shown are the bio-nano robots flowing through various layers and binding amongst themselves and forming a cover over the wound. Also shown are the drug molecules which will be injected into the infected area and thereby will be enhancing the overall rate of recovery for the astronaut.*

Also, the harmful radiation protection layer on the outside of the layer will help the astronauts from getting affected by harmful radiations. If the radiations somehow penetrate through the outer layer then the bio robots will trigger an event and will alert

the astronaut to seek a more protective place and simultaneously try to cover that breach through the movement of protective agents at that point. Further, in this chapter we discuss about the relevance of detecting space radiations and focus on designing ATB for such an event. There are many ways to detect radiations, but we offer a non-invasive way of detecting space radiations which can cause cancer or other long term problems in astronauts.

6.2 Space radiation & significance of its detection

For any human space mission, space radiation is one of the most important concerns. There are numerous health risks that can be caused from the radiations, when astronauts are exposed to various degrees of these radiations. The exact effects and how they propagate are not exactly known, though the intensity of these effects could be related to the type and amount of radiation exposure [http://srag-nt.jsc.nasa.gov/AboutSRAG/Why/Why.htm].

A human body can react to radiation exposure differently, but usually the short term and recoverable effects are nausea, and the long term and significant effects are damage to the central nervous system or even death. The other long term effects are the development of cataracts and chances of development of cancer in any part of the body [http://srag-nt.jsc.nasa.gov/AboutSRAG/Why/Why.htm, http://www.windows.ucar.edu/spaceweather/effects2.html].

This is one of the biggest concerns for human missions. There is an increasing need to determine:

i. How much radiation exposure if safe for humans?

ii. How to determine when the radiation exposure is getting into high risk zone?

iii. How to signal or caution astronauts when the radiations become dangerous?

iv. How we can autonomously detect as well as prevent certain level of radiations encountered by astronauts?

There are many experimental studies which links the exposure to space radiations to the long term biological damage (which could lead to cancerous conditions) [http://science.nasa.gov/headlines/y2005/09may_mysteriouscancer.htm,http://science.education.nih.gov/supplements/nih1/cancer/guide/understanding2.htm]. Detecting this range of exposure which would have a potential to cause long term damage in humans is one of the key objectives of ATB.

Long term biological damage is the principle concern for the human missions in space and this risk goes far in time, even when the mission is over. The high energy radiations to which astronauts are exposed can damage the cells by ionizing the molecules, such as water or other compounds. There is a certain degree to which cells can repair the damage caused to them by the radiations. But beyond that degree cells can be permanently affected and even die. The cells which are permanently affected are very dangerous as they may become cancerous.

Figure 6-5 & 6-6 shows a cartoon representation of how radiations interact with a DNA molecule. There are two major ways in which radiation can damage cells:

i. Water gets ionized to form highly reactive radicals and these free radicals reacts with the cell's DNA causing it to get oxidized or break its bonds.

ii. The high energy radiation collides with the DNA molecule directly and results in knocking off the electrons and breaking or completely annihilating molecules.

In either case, the DNA molecule breaks. A DNA molecule is composed of two strands of nitrogen-containing molecules which link together to form bonds with each other similar to the rungs in a ladder. These linked strands then twist around one another to form a helical structure that carries genetic information. Breaks can occur at either or both of the strands, but interactions that result in breaks to both strands are believed to be more biologically significant. With single-strand breaks, the cell can usually repair itself

and resume normal function. This repair process can happen because the double-stranded nature of the DNA molecule allows the undamaged strand to serve as a template for the repair of the broken one. With double-strand breaks, however, the repair is more difficult, and the cells may either be changed permanently or die [http://srag-nt.jsc.nasa.gov/AboutSRAG/Why/Why.htm, http://www.windows.ucar.edu/spaceweather/ effects2.html].

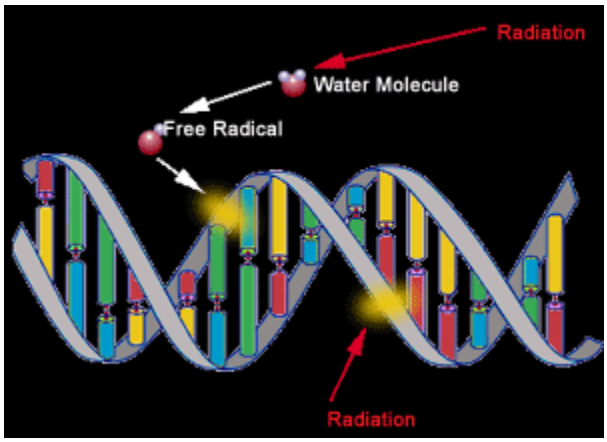


Figure 6-5: *Interaction of DNA with the radiation [http://srag-nt.jsc.nasa.gov/AboutSRAG/Why/Why.htm]*

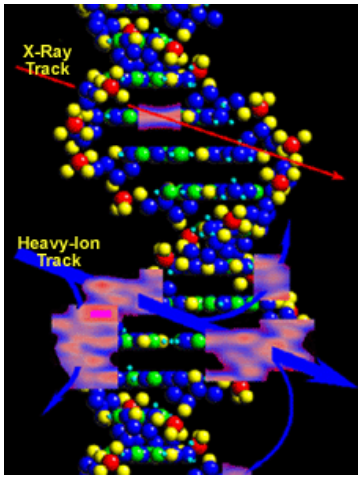


Figure 6-6: *Collision of the radiation with the DNA Molecule. [http://srag-nt.jsc.nasa.gov/AboutSRAG/Why/Why.htm,http://www.windows.ucar.edu/spaceweather/ effects2.html*

Monitoring of the radiation exposure for astronauts is a key requirement for the space missions. NASA has adopted the recommendations of the National Council on Radiation which sets the guidelines of the amount of radiation exposure for the astronauts [http://srag-

nt.jsc.nasa.gov/AboutSRAG/Why/Why.htm,http://www.windows.ucar.edu/spaceweather/effects2.html]. These are higher levels as compared to the terrestrial workers which are exposed to radiations.

6.3 Health hazards from the space radiations

The preliminary objective of the *"All Terrain Bio-nano* suit*, ATB"* is to detect the long term biological damages caused by the space radiations. Our hypothesis is to create a device which could interact with the space radiations and can simulate certain similar conditions as would be induced in human cells. We are focusing on utilizing bio-nano robots to interact with these radiations and signal us whether the intensity of the radiation is enough to cause cancerous effect in humans or not. These bio-nano robots have to be designed for the whole range of radiation effects. Based on the energy and kind of radiations encountered, we have to create a matrix of signals which corresponds to the biological effects. The hypothesis *(Figure 6-7)* used to design this device is described as following:

- Let **H[i]** → represents an affect 'i' caused by radiations in humans.
- Let **A[j]** → represents an affect 'j' caused by radiations in bio-nano robots
- Let **G[i]** → represents the energy of the radiations which causes this affect 'i'.
- Let the space radiation is represented by, its amplitude **I**, its magnetic field **B** and its electric field **E**.

The ability to encapsulate the biological like conditions and reaction with bio-nano robots renders them with a great potential and they evolve as the best suited concepts for this application. In our initial design thrust, we would be focusing on a particular space radiation effect, i.e., radiations which are capable of inducing cancer like conditions in human cells. While we can never ascertain the exact limit where this occurs, but with

experiments in laboratory, we can statistically ascertain a particular limit in terms of energy and kind of radiation where such conditions might occur. *Figure 6-8,* represents our initial design goal.

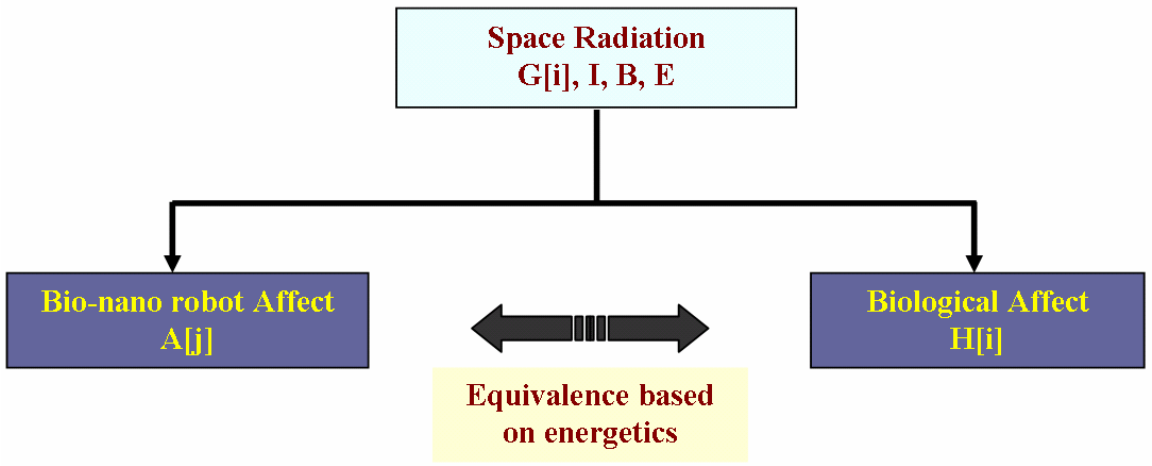


Figure 6-7: *The energy of the space radiation responsible for causing a particular biological affect H[i] in human cell is the same energy required to cause a particular bio-nano robot affect A[j].*

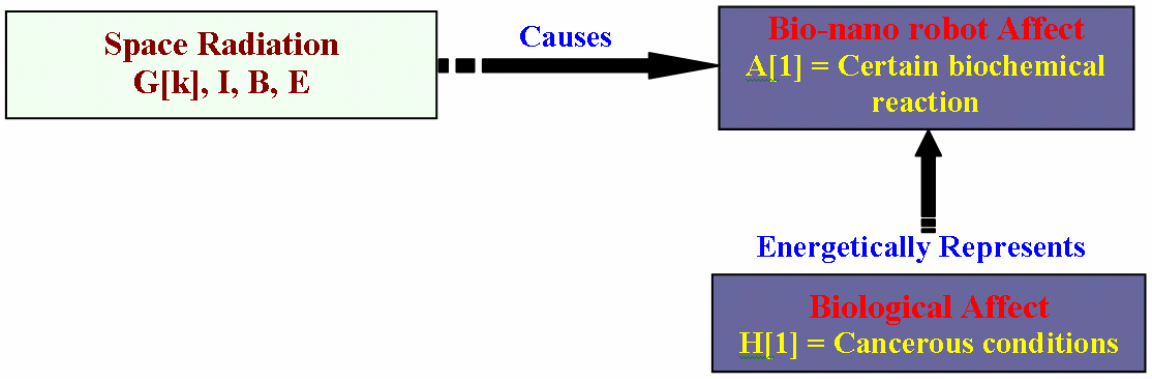


Figure 6-8: *The certain biochemical reaction A[i] represents energetically the cancerous condition H[1] in the human cell, both caused by the same space radiation, G[k]*

6.4 Design of All Terrain Bio-Nano suits: Architecture and Goals

This section details the preliminary design of ATB for astronauts for detection of space radiations. This design represents a *"non-invasive & approximate cancerous level*

radiation detection system". As described in the proposal, ATB gear would be made of various micro membranes and surface sheets. Each of these membranes and sheets would contain swarms of bio-nano robots capable of the required sensing and signaling capabilities. This proposed design consists of following layers:

a. *An intermediary space radiation detection layer –* ***'Layer A'***

b. *An intermediary space radiation prevention layer* ***–'Layer B'***

c. *An innermost medicated layer for wound and external surface injuries* ***– 'Layer C'***

d. *Mechanistic top layer for Astronaut suit mechanical fault detection* ***– 'Layer D'***

e. *Mechanistic top layer for self repairing of Astronaut suit mechanical faults* ***– 'Layer E'***

In this report we would only talk about Layer A and its design. This is an intermediary space detection layer. The following subsection will detail the design and architecture for this layer.

Figure 6-9, shows the placement of these layers in ATB. The two sides are defined the "space side" and the "astronaut side". The space radiation detection layer A is an intermediary layer as seen here.

6.4 The design of the "Layer – A"

This layer will contain the bio-nano robots which will demonstrate equivalence between the invasive and non invasive radiation effects as described in the initial section. Here we need to define the precise reaction chemistry within the bio-nano robots such that we can attain this equivalence.

The design has to be such that an 'equivalent' effect has to be induced and detected in a non-invasive manner. For this we employ bio-nano robots:

i. First we need to simulate the physiological conditions in the channels.

ii. Second, we need to select particular proteins and their combinations with nano-polymers to make architectures where the space radiations can interaction in a modular fashion and where the process of detection can initiate. This design requires following steps:

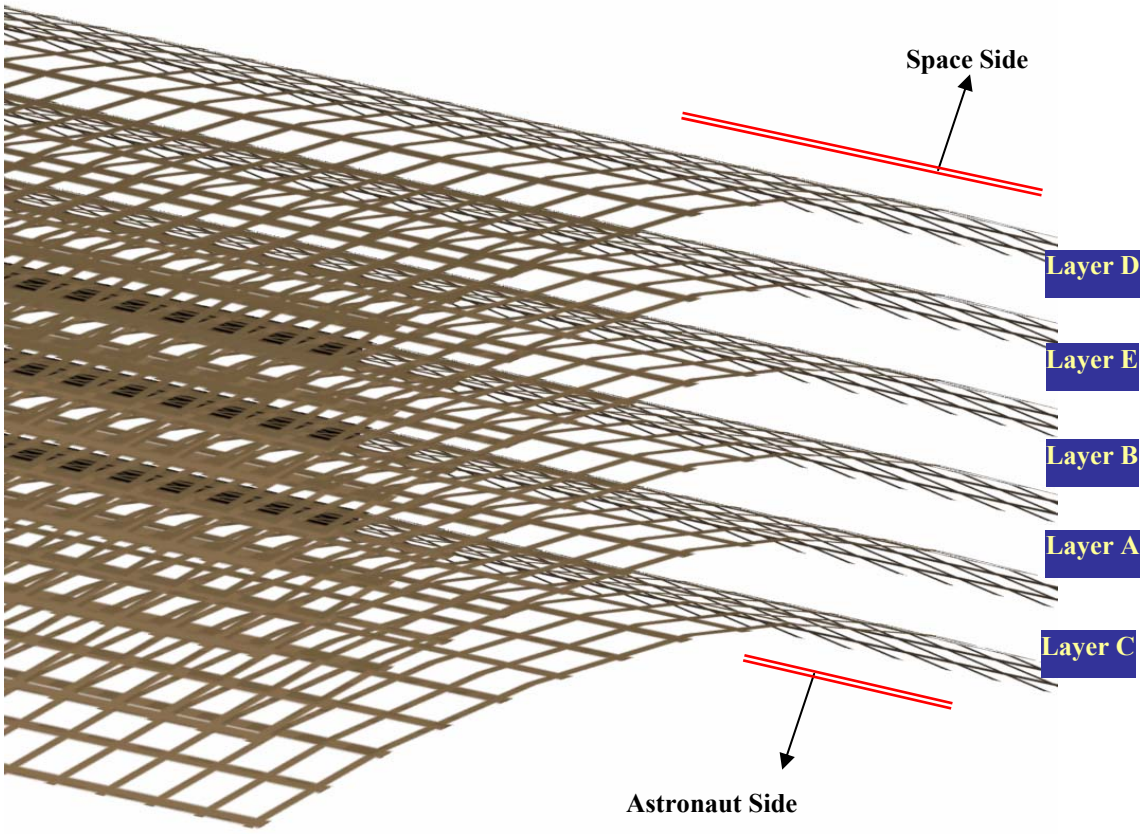


Figure 6-9: *Overall arrangement of the layers. The mesh represents the various placements of the layers with respect to the astronaut side*

a) Design of a reaction pathway. There are various possibilities here: these reaction pathways have to follow the sequence or consequence of the reactions which happen inside the human cell. Like the generation of toxic oxides and its reaction with DNA and collision of high energy field with the molecules. We need bio nano components, because we need to signal an instant when we detect these radiations damaging them. We require

this to establish the equivalence of the space radiation affects. This design must have various stages. One stage has to detect the initial range of radiations and signal, the other stage has to survive the radiations and has to detect more harsh levels of radiations, which are in the range of cancerous inducing levels in humans. For these stages we might use similar design architectures, but we need to have radiation resistance of the molecules.

b) The molecular components utilized to make these reaction pathways - the main radiation interacting molecular architecture has to be designed before we design the reaction pathways. Here we are employing a modular approach in which we will create autonomous *reaction centers (represented by spheres in the figure 6-10 & 6-11)*, which would be the centers of space radiation interaction initiations.

c) Survival of the molecular component - we cannot use the same exposed molecules without treating them. Therefore, we require a system where we can refill or refresh the system with these components. This calls for a very simple and fluidic based system where these components can be delivered. The fluid flow has to be such that the alignment of the components in the channels is exact for the detection to take place. Here the components have to be designed such that their interaction with the radiation is independent of their orientations. The best possible geometry for this is spherical (*Figure 6-10, 6-11, 6-12 & 6-13*). Self organizing ability of bio-nano robots would be useful to orient these in the channels.

d) Possible characteristics of the reaction pathways - destruction of a molecule trigger a reaction or breaking of a bond and its remaking triggers a reaction. This reversibility is hard to achieve, but by employing certain radiation resistant molecules we try to achieve this.

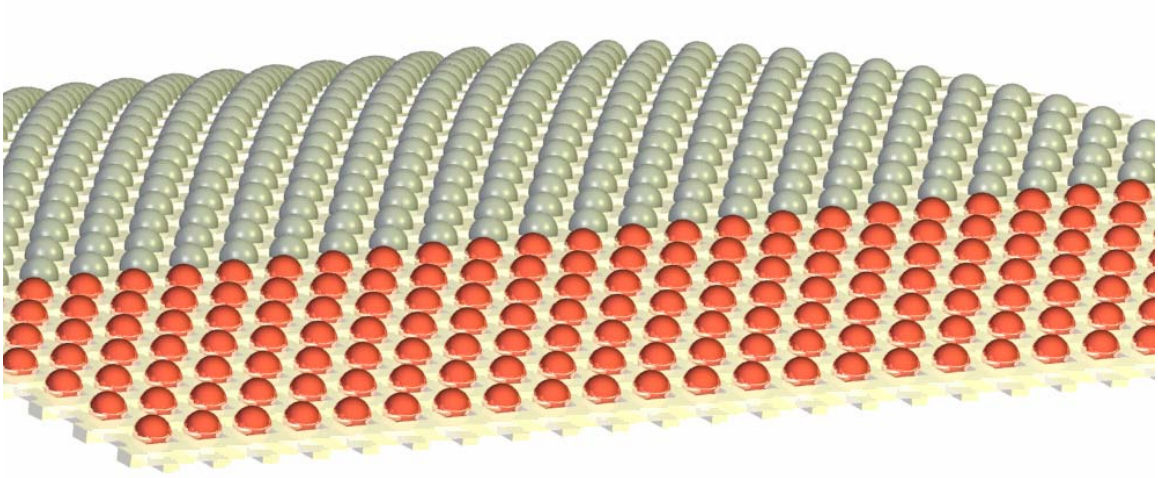


Figure 6-10: *A surface view of the radiation detection layer – the probabilistic reaction layer is represented by spheres. These spheres are embedded within the meshes created by radiation resistant nano-polymers.*

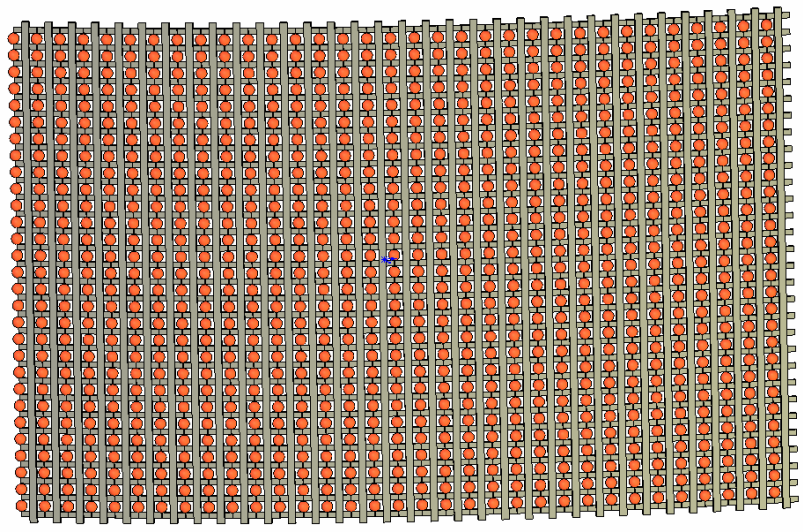


Figure 6-11: *The top view of the ATB radiation detection layer – this shows the overall arrangement of the spheres which makes the ATB layer.*

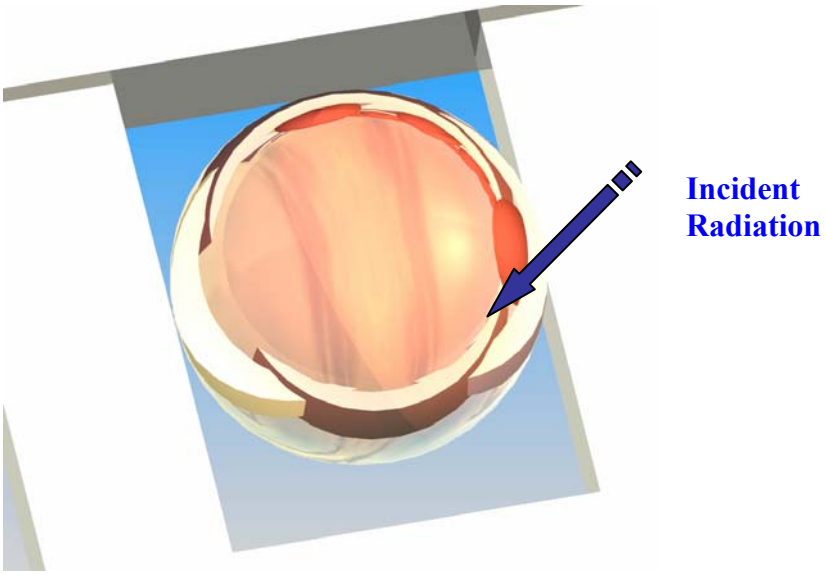


Figure 6-12: *Shows the sphere is detail – this represents the maximum probability regime for the reaction. This will contain all the machinery, bionano robots which will react with the radiation.*

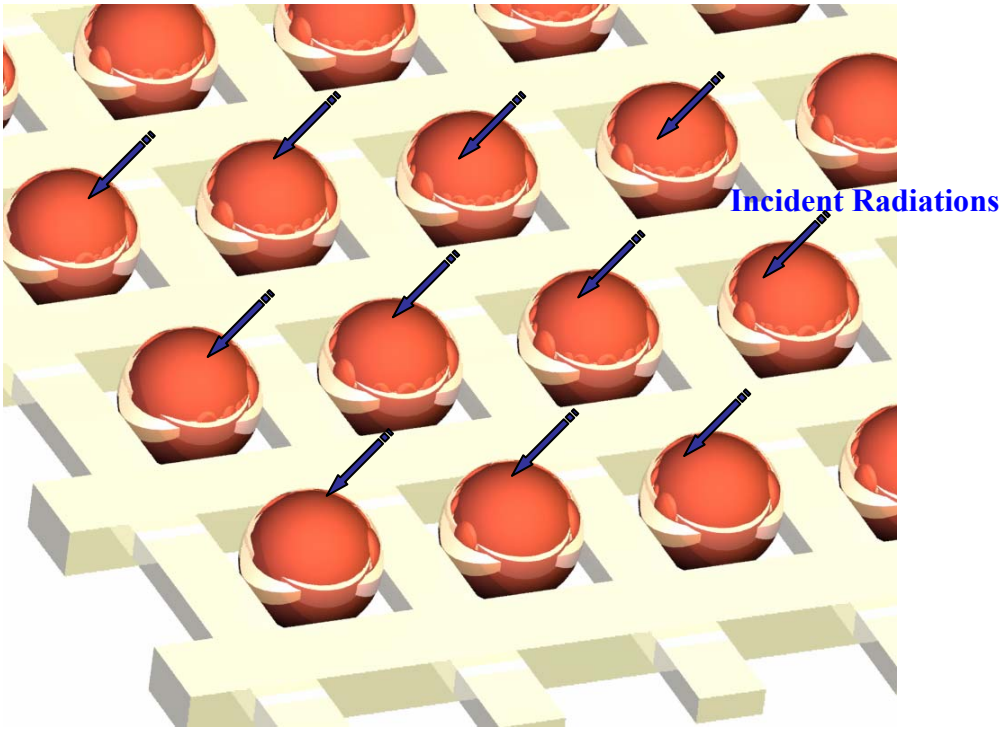


Figure 6-13: *Shows the close view of the sphere and its interaction with the Nano-polymer fibers.*

These modular reaction centers are the central to the design of ATB – Layer A. The next section describes these reaction centers in more details. These detection layers would be spread all along the space suit and astronaut's body. *Figure 6-14* gives a representation of how this would look like. These layers would be distributed radially and longitudinally and hence would probabilistically cover the space radiation exposure areas.

An insulation layer has to be provided between each layer so as to minimize cross-layer containments and affects. As all the layers perform a particular function this insulation would become very useful. This is especially important for the wound healing layer as that layer would involve would healing drugs and it would interact directly with the astronaut's skin. The following list provides the product plan and possible future releases of ATB:

- ATB v1 – Space radiation Detection
- ATB v1.5 – Radiation Detection and partial prevention
- ATB v2 – Added feature of wound healing layer
- ATB v3 – Mechanical Fault diagnosis and detection
- ATB v4 – Self repairing architecture implemented for the above layers.

6.5 Probabilistic molecular arrangement for optimized reaction initiation

This section describes more about the reaction centers. These reaction centers are represented by a sphere having an equal probability of reactivity in any orientation. Bio chemical reactions concerning as diverse and as large as bio components requires probabilistic design environment. And hence, this sphere creates optimized reaction initiation surroundings.

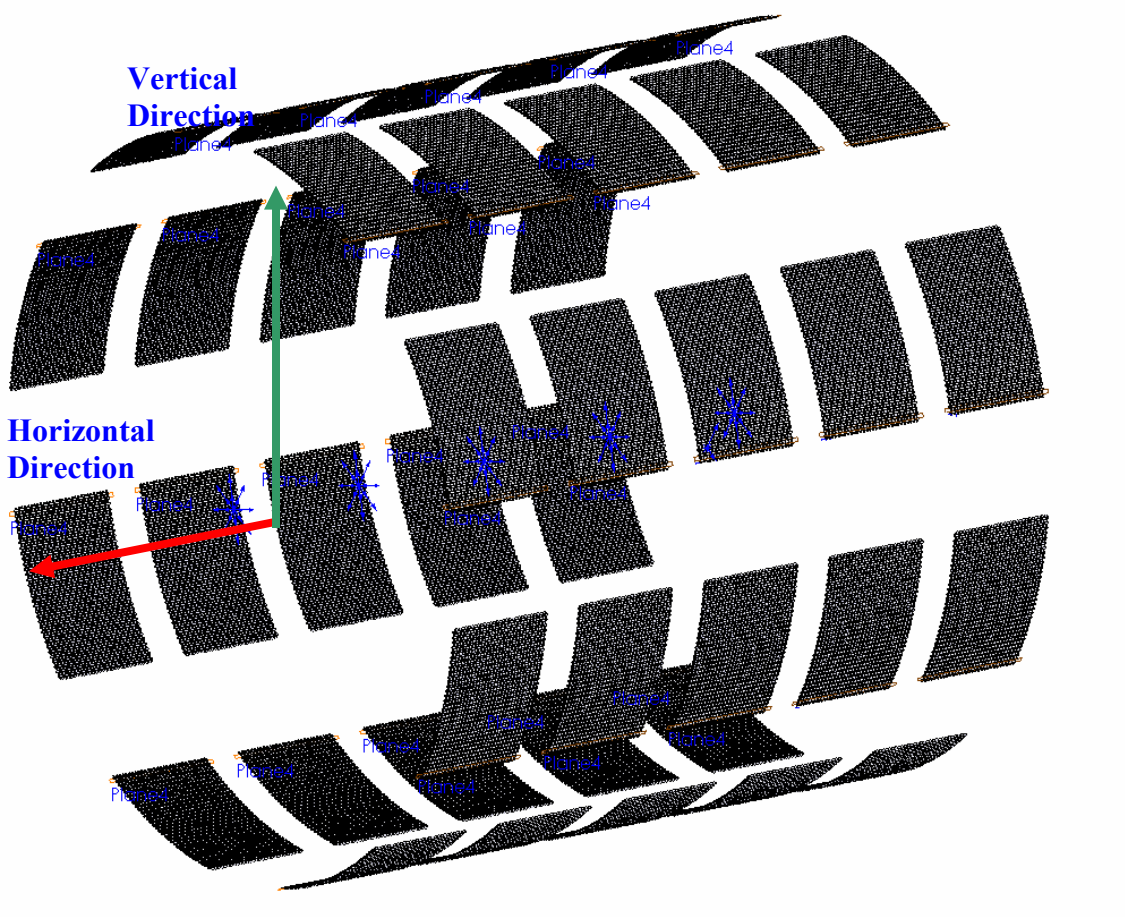


Figure 6-14: *The overall system design of the ATB radiation detection layer – this shows the arrangement of the ATB radiation detection layer along the whole space suit*

Many bacteria and in fact photosynthetic plants place their light sensitive proteins in a manner that it's most homologous to its subsequent reactants. Here we employ the similar design strategy to maximize the effects. This sphere represents the modular design strategy, where all the radiation related reactants and their signaling pathways are enclosed in the homologous manner. *Figure 6-15,* shows the concept of reaction centers. This design creates intensified reaction centers for carrying out the subsequent reactions. It is like creating a center which attracts the radiations towards itself, probabilistically. Because this setup is at a nano level, we have to create millions of centers and hence

carry out detection pathways throughout the astronaut's space suit. The centers are supported between radiation resistant polymers and nano fibers.

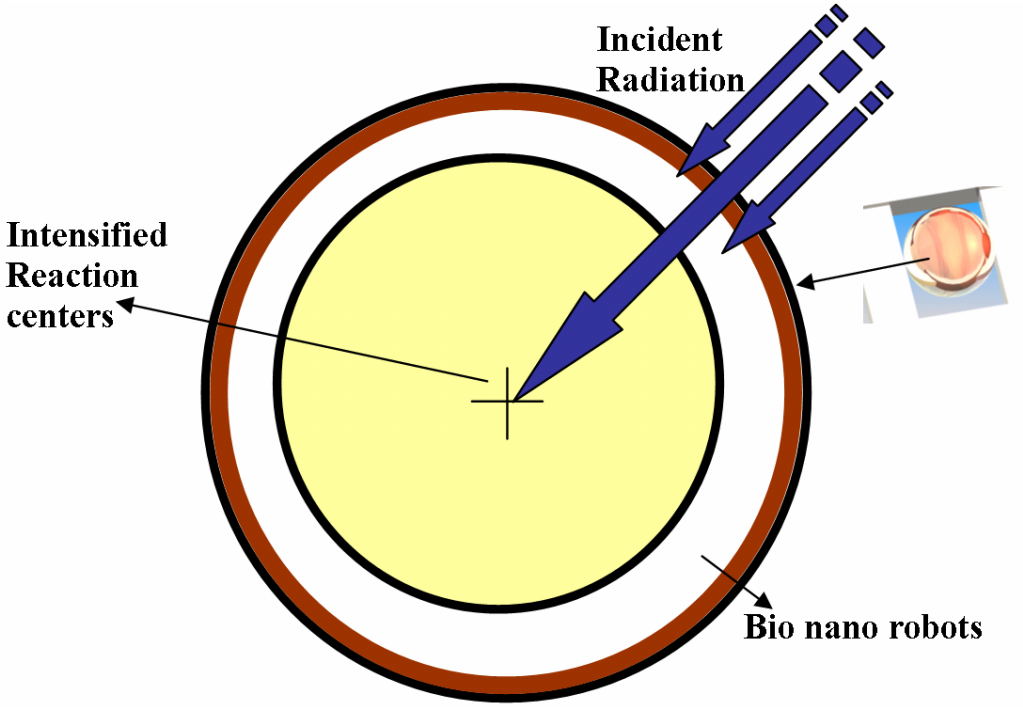


Figure 6-15: *Probabilistic molecular arrangement – creation of intensified reaction centers*

6.6 Inside reaction centers

In this section we detail the structure responsible for detecting space radiations within reaction centers as represented by the spheres. This radiation detection system would be termed as "*radiation-responsive molecular assembly*" (RMA).

There are numerous challenges for materials exposed to space radiations. Following summaries some of the main molecular level effects which are true for any material entity:

a) Damage to the molecular geometry: This implies that the incoming radiations damage the molecular geometry of the material structure. There is a direct

correspondence between the 3D structure of a bio-molecule and its functionality. Damage to the structure can totally or partially curb the functionality.

b) Destruction of the bonds between various atoms: Damage to the molecular structure implicitly implies that destruction of the molecular bonds takes place. This potentially means knocking off electrons from their bonding orbitals and creation of ions. These ionized atoms could themselves further attack other neutral species to generate further ionized species and hence totally destroy the molecular geometry. The free electrons have a potential to further collide with other atoms and thereby starting a cascaded reaction in the material.

c) Formation of harmful radicals: These radicals have potent destructive power to break or realign bonds not suitable for the material's molecular identity, thereby rendering the material of no use.

This list of effects is not extensive and only the beginning of the understanding of various harmful affects of space radiations. The challenges compound when we talk about these effects on a biological system (astronauts for instance). Based on the energy of the incoming space radiations, various affects could be caused. The exact details of these effects are not calculable and only specific instances can be studied at an instant and specified. Typically, atoms and molecules exist in a balanced state, i.e., number of electrons are same as number of protons. These orbiting electrons form bonds with other atoms and a molecule is formed (though the exact process is far more complex). The incoming radiations are in the form of high energy particles, which could be charged. Once these incoming particles interact with the atoms and molecules various effects come into picture. *Figure 6-16* shows these cascaded effects in more details and demonstrates

the fundamental difficulty in precise evaluations of these effects. Each atom in the molecular system is subjected to flux of incoming particles with very high energies. As these particles penetrate the molecular system, they interact with the atoms, exhibit scattering, nuclear decay all kinds of quantum – relativistic phenomena. *Figure 6-16* depicts a very simplistic view of the mentioned cascading effect. It is shown that how three atomic systems (namely a, b and c in the figure) interact under an incident radiation α and how the ionization takes place and creates other effects, such as, emissions of other particles (beta or gamma) and production of various ionized species including charged atoms, free electron and other harmful radicals.

Here, the Stage-1 is made up of outer most layer of the material or molecular system under study. The incident flux on this layer is the highest and so is the total energy of the incoming particles. As the cascading of ionization and other radiation effects takes place, some energy is absorbed and the subsequent stages (or layers) experience lesser and lesser effects. But the important thing to note here is that, in case of biological materials even the energies in the range of keV (kilo eV) are enough to create effects or demonstrate damage.

To summarize this qualitative argument, assume that the total effect on the molecular entity‘d’ as shown in figure 61 is given by:

$$[T.Effect]_d^{stage-2} \equiv \sum_{m_p=1}^{n_i} C_{m_p}^{n_i}$$

$$= \sum_{m_p} \frac{n_i!}{m_p!\left(n_i - m_p\right)!}$$

Where,

$\boldsymbol{n_i}$ = total number of ways of ionizing radiations that affects entity 'd'

In our case these ways are: $(1-\varphi)\alpha'_a$, $(1-\chi)\alpha'_b$, $(1-\psi)\alpha'_c$, I'_a, I'_b, I'_c and $\beta_{a,b,c}$, $\gamma_{a,b,c}$

$\boldsymbol{m_p}$ = possible ways of selecting the ionization radiations / particles / species

$(1, 2, 3, \ldots, n_i)$

Therefore, the total effect on 'd' could be evaluated in = $[T.Effect]^2_d$ number of ways

$$=1+\ldots+\frac{n_i!}{2!(n_i-2)!}+\frac{n_i!}{1!(n_i-1)!} \text{ number of ways}$$

6.6.1 Computational algorithm for evaluating these effects

As a first assumption in quantifying these effects, we need to select the most observable situation when a molecular system is subjected to ionizing radiations. Typically, *Monte Carlo simulations* [Cucinotta et. Al, 2003] are performed which involves selecting a particular probability distribution of the particles and their energies and tracking them through various layers and calculating their effects in terms of the pre defined parameters. We are most interested in calculating:

a) the net energy transfer or deposition [Cucinotta et. Al, 2003] through a certain layer

b) keeping track of the excited electrons

c) generating sequence of ionized particles with energy capable of carrying forward the cascaded effects

d) generation of other particles

This computational paradigm is indicative of the potential effects and the energy deposition in the molecular system. It is this molecular energy (and its gradients) which would give us pointers on how we signal this scenario and proceed towards space radiation detection sequence.

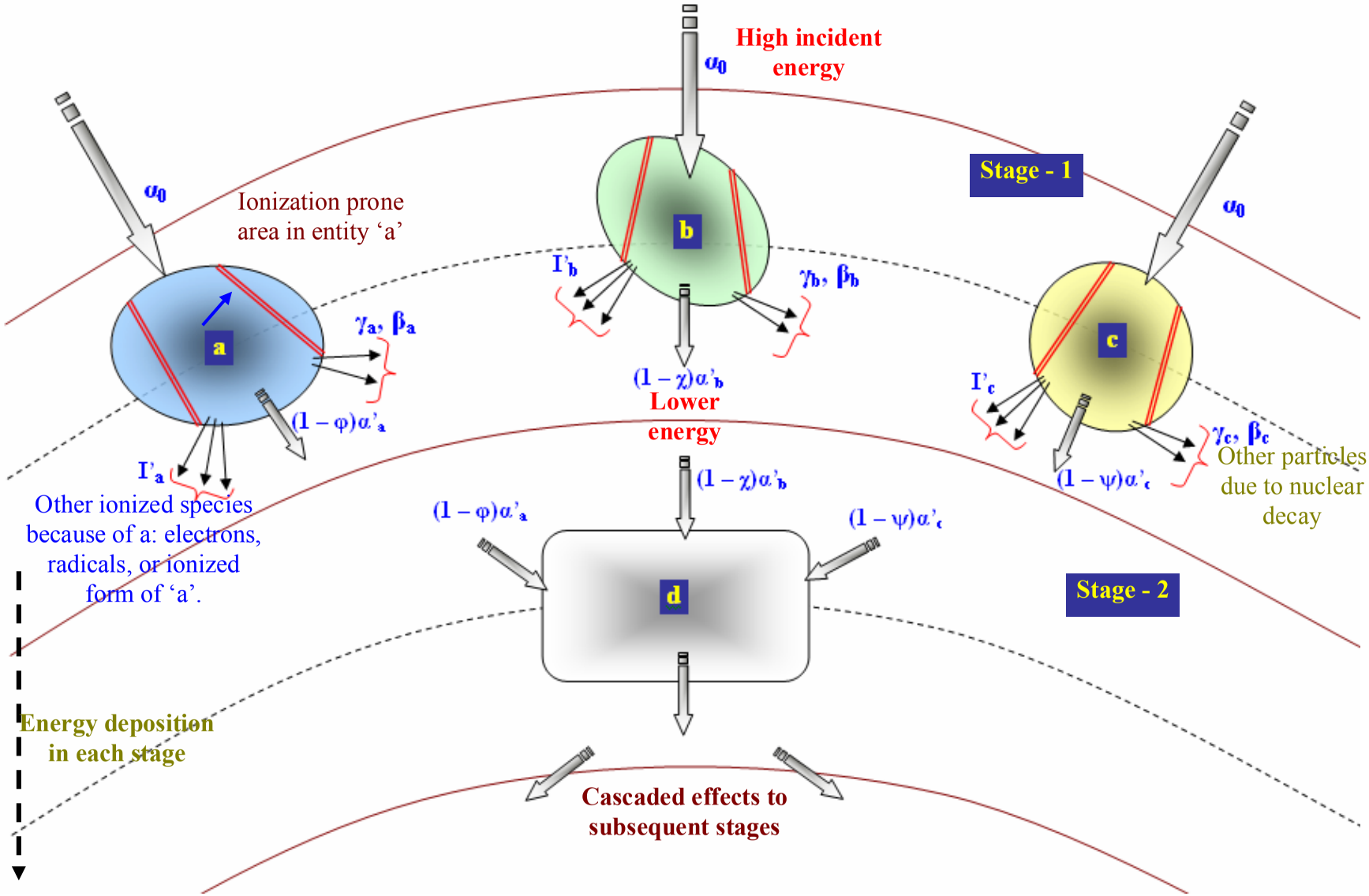


Figure 6-16: *Schematics showing the cascading mechanism induced in a molecular system subjected to ionizing radiations.*

6.6.2. Radiation fundamentals

Before getting into the framework for designing radiation responsive molecular assemblies, we would review some of the fundamentals of space radiations and how its effect is measured and its effectiveness evaluated. Some definitions:

Absorbed dose or dose is the term associated with the biological effects induced due to ionizing radiations. One can measure these doses through various instruments, which detects the average deposition of energy due to the radiations in a small volume of interest. Dose is measured in the units of *gray* (Gy) and it represents:

$$1\ \text{Gy} = \frac{\textit{Absorption of average of 1 joule energy}}{\textit{Kg of target material}}$$

Biological materials are made up proteins, DNAs, other cellular structures and have a varied degree of response to various different kinds of radiations. For instance, even if the energy deposition is similar, the effects induced can't be predicted. To compare these biological effects given the complexity of these materials, a concept called ***Relative Biological Effectiveness, RBE*** is introduced [http://www.nsbri.org/Radiation/].Its measurement is done in comparison to the effects induced by an equivalent dose of x-rays as shown in *figure 6-17* below.

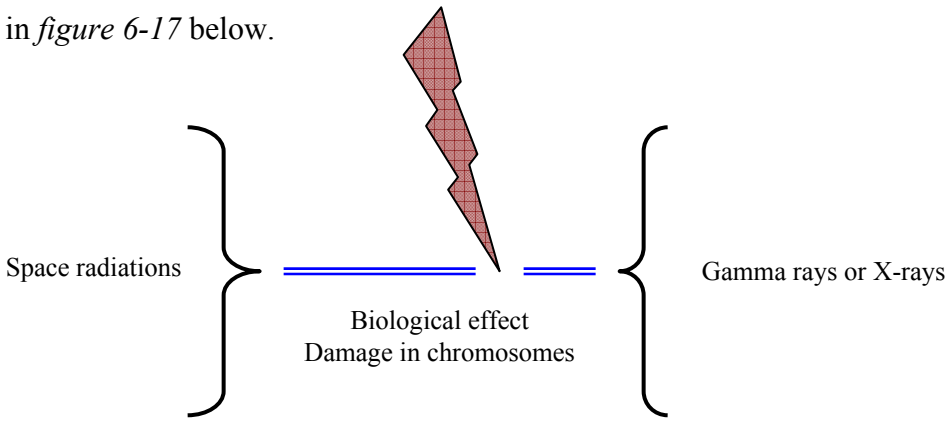


Figure 6-17: *Relative Biological Effectiveness measurement. Space radiations are measured against the equivalent of x-rays in terms of dose.*

Biologically equivalent dose (measured in the units of rem or Sieverts) can be calculated by simple product of the Absorbed dose (Gy or rad units) by the Relative Biological Effectiveness. For an instance, the RBE of alpha particles has been determined (by committee) to be 20. This means that 1 Gy of alphas is equivalent to 20 Gy of gammas/xrays [http://www.nsbri.org/Radiation/]. The absorbed dose is calculated as follows:

Biologically-equivalent dose (rem or Sievert) = Absorbed dose (rad or Gy) X (RBE)

The precise evaluation of the effects of ionizing radiations on astronauts is a very complicated process. But the overall classification of these effects can be done in terms of the time period within which potential macro-level damage could be detected. Usually such macro scale damages result from a considerable amount of molecular level damages. It is these molecular level damages which our *"radiation responsive molecular assembly"* proposes to detect. Once detected it can act as an early detection / warning system for the astronauts and would potentially assist the crew on a particular mission in their health management processes. The effects of a given dose of ionizing radiation on humans can be separated into two broad categories [http://www.nsbri.org/Radiation/, http://www.foe.arc.net.au/kohnlein/kohnlein.html/]:

a) Immediate or acute effects: Effects whose macro-level damages could be immediately observed are termed as immediate effects. These immediate effects might include skin-reddening, rashes, dehydration or nausea. Acute Radiation syndrome is another term used for immediate or acute effects.

b) Long term effects: Effects whose repercussions are very strong, but are not observed at a macro-scale immediately or in near short term could be termed as long term

effects. These effects are potentially very serious for the astronaut's health and at the very best should be avoided. But how the or when these effects arise is not certain. The complex cascading mechanism described in figure 1 shows how multitude of effects takes place simultaneously in the molecular structure. The long term effects could be caused by any of those ionizing elements. Typically the long term effects alter the DNA or destroy it. Bonds in the DNA breaks down which could lead to single strand breaking or the double strand and this causes many damaging problems in the organism. Usually cancer is associated with the mutation of DNAs exposed to ionizing radiations and is one of the most dangerous long term effects. Therefore, the other primary goals of the proposed "radiation responsive molecular assembly" is to evaluate these long term effects structurally and signal the various harmful effects. One way to associate these effects with the amount or intensity of ionizing radiations is to construct molecular bridges which are only susceptible to damage under long-term effect conditions. Hence, this is one of our design requirements.

6.6.3 Framework for the design of Radiation responsive molecular assembly (RMA)

Before introducing and exploring the concept of "radiation responsive molecular assembly", we will try to establish its design philosophy. The key advantage of such a design is its ability to interact with the radiation at the molecular scale; characterize its intensity based on energy deposition and relate it to the relative biological effectiveness based on the correspondence established through molecular structure and bond strength. Another feature of such a design is its integration with the current materials at the molecular scale and its widespread presence throughout the material structure. Other key advantages are mentioned in the ATB section. Some of the main design parameters are:

i) Selection of molecular structures with *simple and regularized geometries* for comparative ordering of cascading effects. More heterogeneous the molecular structure the more complex cascading effects.

ii) *Consistent usage of molecular entities*. This implies that number of different molecular entities to be utilized in the design should be minimized. This is again related to the cascading simplifications. This will render us with known values of ionization potentials of the electrons in the various structures and electron's interactions with the molecular entities could be pre-determined and used for more efficient design.

iii) *Electron de-accelerating molecular bridges.* Another parameter is the design of molecular bridges or homogenous structures which can absorb a high energy electron or react with it to produce a stable chemical species. This indicates presence of a solvent like material which is capable of de-accelerating or reducing the energy of free electrons or other ionized species.

iv) *Layered modules.* These layered modules would interact with various energy levels (and specifically within the acute effects inducing ionizing radiations). Because here we are dealing with the issue of radiation detection and not absorption or prevention, we have to lower down amount of incident radiations. Hence we need an inter layer medium which can potentially absorb certain magnitude of incident particles, while keeping the energies of the ones hitting unaltered. This is an approximation step, but is required so as to increase the life and usability of the molecular assembly. There is only a certain life and functionality associated with this design. Once reacted with the radiations, these may require replacement by other molecular assemblies, as discussed in the patch / modular design of space radiation detection layer in ATB.

v) *Signaling pathways.* Because we are interested in the characterization of various energy levels, we require a distinct pathway for various energy ranges so that when such a range is achieved, biological effectiveness information (in the form of electron flow or photo responsive reactions) could be transmitted.

6.6.4 Information flow in the molecular assembly concept

The key goal of this molecular design is to deduce information on the energy levels of the incident radiations, chemically relate it to biological effectiveness, and signal it through various pathways. Therefore, the net result of the integrated radiation responsive molecular assembly is to generate range of pre defined signals which could be correlated to the induced macro scale biological effects, such as, skin rashes, nausea etc.

The information flow and the correspondence between the targeted stages are shown in *figure 6-18*. The process initiates with the energy characterization which basically implies evaluating how much energy deposition per unit time and per unit mass is being done by the radiation. This information has to be evaluated structurally, through specific bond cleavages and structural modifications. Hence through this crucial step we have to determine the biological effectiveness of that particular energy range. These structurally evaluated effectiveness has to be corresponded with a signaling pathway which, is further related (or pre defined in this case) to the assumed induced acute effects. And hence a full loop is established between the ionizing radiations and the biological effect. Here, the described information flow loop has be to achieved in every energy range of the ionizing radiations.

6.6.5 Proposed design

Keeping in mind the above mentioned design parameters and the information flow targets, a multiple layered design for the radiation responsive molecular assembly is proposed.

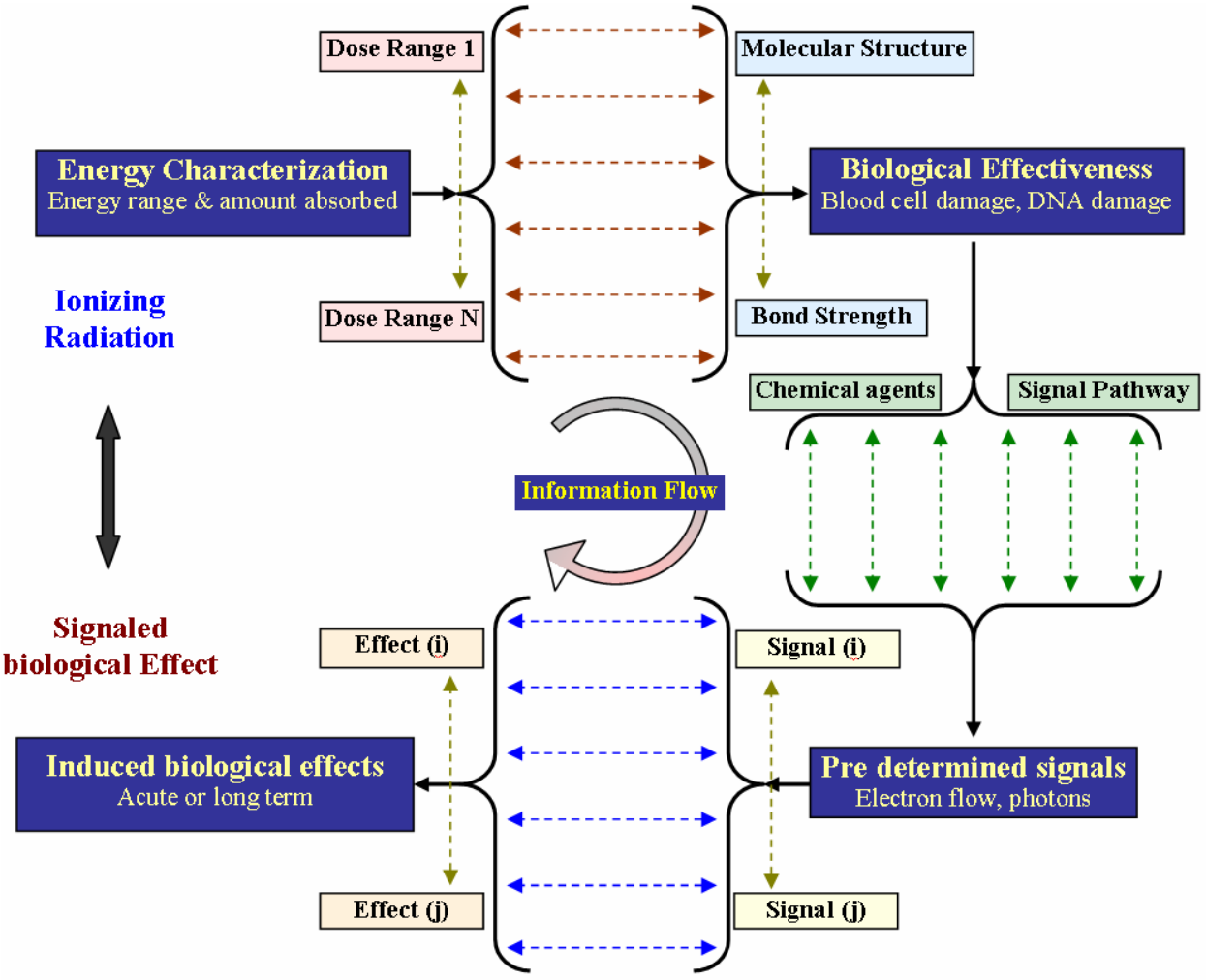


Figure 6-18: *Information flow in the each energy range characterized by the radiation responsive molecular assembly*

For this design we require following sub-modules (these are termed as sub modules, because in the paradigm of the radiation detection layers of ATB, the probabilistic reaction centers are termed modules. This molecular assembly is part of those probabilistic reaction centers):

a) Structural sub-module: This module is responsible for integrating the molecular assemblies within the probabilistic reaction centers. These structural elements could be made by nano – polymers which are 'normally' resistant to radiations. As these are passive elements they don't require an interaction capability with the ionizing radiations. The only other feature they require is to be able to transmit a coherent signal through the probabilistic reaction centers so that a unified signal is generated.

b) Detection sub-module: This is the energy range sensitive sub-module and is responsible for interacting with the radiation within the layer. Through this sub-module primary cascading effect originates and hence should be homogeneous as described in design parameters.

c) Relative effectiveness sub-module: This sub-module interacts with the first few generation of cascading effects and evaluates its relative effectiveness through its unique molecular structures and ordered layers of molecular bonds. The geometry of the structures and the strength of these bonds would potentially identify harmful effects of radiations and hence, would connect with the signaling sub-module to trigger a chemical pathway.

d) Signaling sub-module: As described above, these will be integrated with the relative effectiveness sub-module and would amplify the initial triggers and generate them into signals, such as, emission of light, flow of electrons, change of color or variations in the conformations. These pre determined range of signals would potentially correspond with a particular biological affect (acute or long term).

Each of these sub-modules is part of the individual layers which forms the core of the probabilistic reaction centers within the radiation detecting layer of the ATB. Hence in

each layer (which is based on the energy ranges) the whole information flow loop is covered and in the end we obtain a complex mixture of reactions and signaling pathways. Therefore, it is possible that more than one kind of biological effect is detected by an individual population of the probabilistic reaction centers. *Figure 6-19* details some of the keep design aspect of the radiation responsive molecular assemblies.

6.7 Radiation resistant bacteria – Selecting the bionano components for ATB

This section describes a radiation resistant bacteria **Deinococcus radiodurans** *(Figure 6-20)* which we would utilize to select bio nano components for ATB. Among the many characteristics of D. radiodurans, a few of the most noteworthy include:

- an extreme resistance to genotoxic chemicals
- resistance to oxidative damage
- resistance to high levels of ionizing and ultraviolet radiation
- resistance to dehydration

The ability to survive such extreme environments [Seipp 2002, http://www.microbe.org/microbes/Deinococcus.asp] is attributed to D. radioduran's ability to repair damaged chromosomes. It is known that heat, dehydration and radiation causes double-strand breaks in chromosomal DNA.

D. radiodurans usually repairs these chromosome fragments, within 12-24 hours, using a two-system process with the latter being the most crucial method.

i. D. radiodurans use a process called *single-strand annealing* to reconnect some chromosome fragments.

ii. D. radiodurans use a process known as *homologous recombination*, where a modified yet efficient RecA protein patches double-strand breaks.

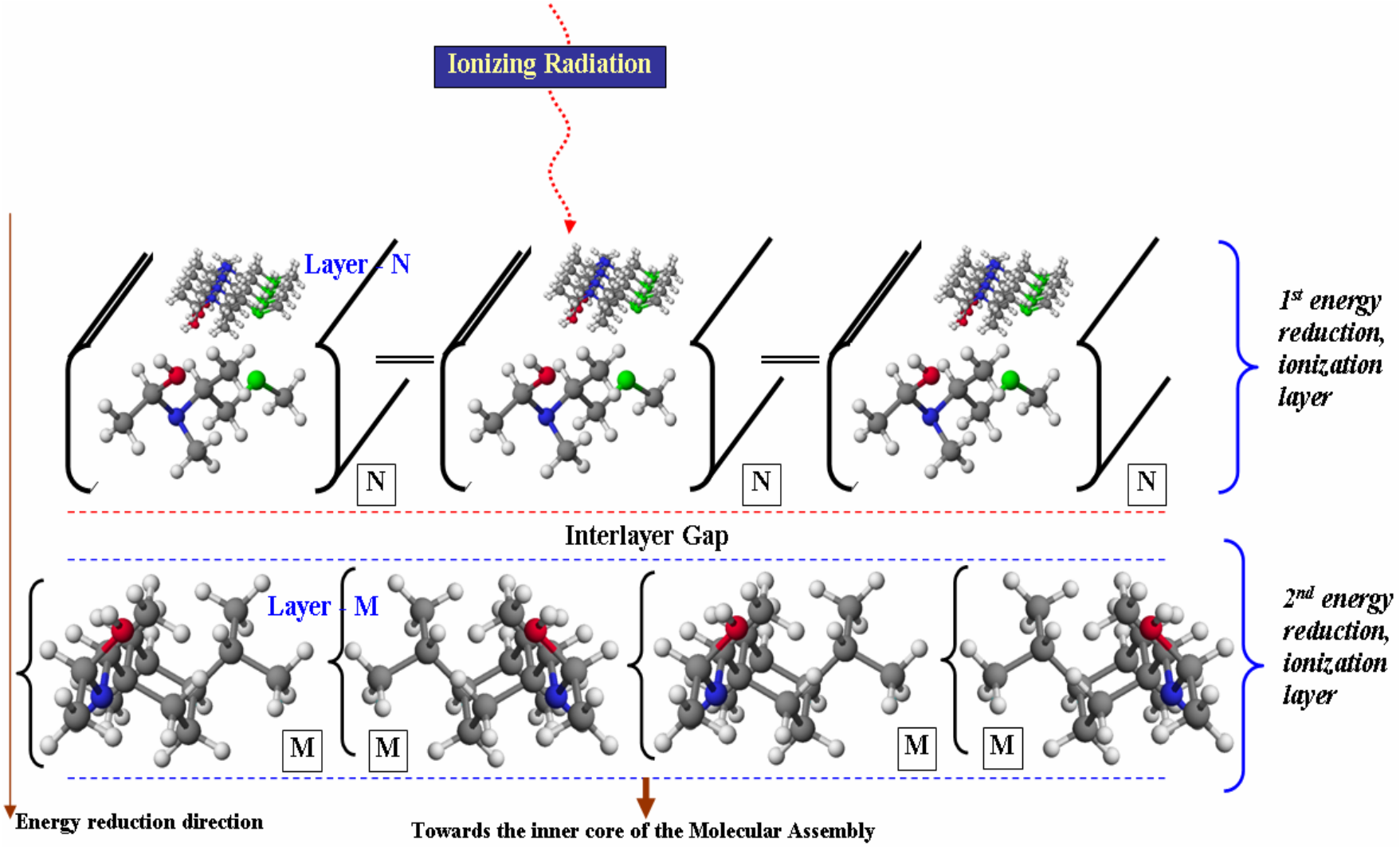


Figure 6-19: *The layered concept of radiation responsive molecular assembly. Within each assembly the information flow occurs and gives us an integrated signal based on the internally defined structure to 'affect' relationships.*

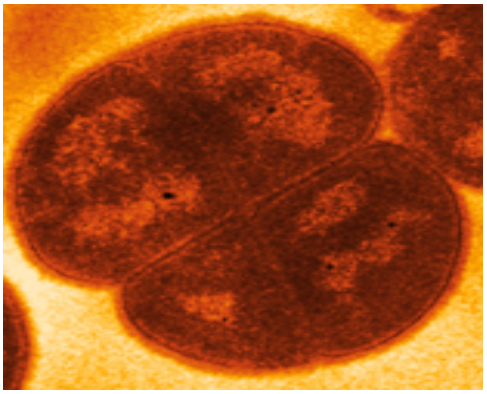


Figure 6-20: *Deinococcus radiodurans*

[http://www.microbe.org/microbes/Deinococcus.asp]

D. radiodurans usually repairs these chromosome fragments, within 12-24 hours, using a two-system process with the latter being the most crucial method.

i. D. radiodurans use a process called *single-strand annealing* to reconnect some chromosome fragments.

ii. D. radiodurans use a process known as *homologous recombination*, where a modified yet efficient RecA protein patches double-strand breaks.

RecA protein [Seipp 2002, http://www.microbe.org/microbes/Deinococcus.asp] works by cutting usable DNA from another molecule and then by inserting it into the damaged DNA strand. However, these repair methods alone are not unique to D. radiodurans, which therefore cannot account for its radiation resistance. There are still many proteins of this bacterium which have not been characterized and hence the exact radiation resistance mechanism is still unknown.

D. radiodurans also possess some other distinctive features [Seipp 2002, http://www.microbe.org/microbes/Deinococcus.asp, http://deinococcus.allbio.org/]:

- *Carotenoids*, which cause red pigmentation, are thought to be like free radical scavengers, which might increase resistance to DNA damage by hydroxyl radicals.

- *High levels of enzymes* such as superoxide dismutase and catalase both play a role in effective defense mechanisms against oxygen toxicity.
- *A cell wall forming three or more layers* with complex outer membrane lipids and a thick peptidoglycan layer containing the amino acid omithine also serves to protect D. radiodurans from lethal doses of radiation.

Currently the biochemical details of D. radiodurans's radiation resistance are not understood properly, but it certainly has proteins that are needed for cell survival which are synthesized in cultures exposed to ionizing radiation. Hence this bacterium might contain space resistant proteins and other mechanisms. RecA is one such protein which stitches the broken DNA and might have other radiation resistant proteins in this reaction pathway.

Further this bacterium is also known to survive in freezing or desiccating environments and hence could be extremely useful for constructing bio-nano robots for space applications. Hence, in future we have to study these bacteria and characterize its proteins and propose further designs.

Chapter 7: Experimental Activities

7.1 Introduction

The experimental efforts thus far have focused substantially on the mutagenesis, expression, purification, and characterization of *Loop 36,* a region of the hemagglutinin protein from *Haemophilus influenzae*. Loop 36 is centered at a hinge-like structural region of the hemagglutinin protein, and thought to play a role in inducing the dramatic conformational change of the hemagglutinin protein that occurs in response to exposure to endosomal pH (pH ~5). Previous studies have shown that Loop 36 undergoes a dramatic conformational change, from random coil to α-helix, in response to a change in pH from 7.0 to 4.0 [Carr, 1993]. We have cloned, mutagenized, purified and characterized Loop 36, and 10 genetically engineered mutants (Table 7-1) by circular dichroism (CD) spectroscopy. Our results raise interesting questions about the purported ability of wild-type Loop 36 to undergo a complete structural transition, while suggesting alternative approaches that substantially improve the pH-responsiveness of the wild-type peptide.

7.2 Description of Research Area & Project Objectives

Our objective is to characterize the suitability of Loop 36 as a bionanoactuator. In order to achieve this objective, we are studying the structural transitions induced in the Loop 36 peptide in response to changes in environmental conditions, such as pH. Our broad hypothesis is that the energy released during these structural transitions can be harnessed to perform useful work, on the nanoscale, in an ATP-independent manner.

Methods: *Mutagenesis Expression and purification.* Loop 36 mutants were constructed using the QuickChange mutagenesis kit (Stratagene). Wild-type Loop 36 and

mutants were expressed in *Escherichia coli* induced with isopropyl-β-D-thiogalactopyranoside (IPTG), at room temperature, and purified by affinity chromatography using a chitin-binding domain and chitin resin. The chitin-binding domain was removed via the self-cleaving intein protein. Further purification was accomplished by dialysis against 0.5 mM phosphate buffer (pH 7.2), using a 2 kDa molecular weight cut-off membrane, followed by a second dialysis step against a volatile carbonate buffer. This process yielded over 95% pure peptide, as determined by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and mass spectroscopy. The peptide was then concentrated by ultrafiltration.

Circular dichroism experiments: The peptide was lyophilized and re-dissolved in 250 μL of 10 mM phosphate buffer at pH 7.0 or 5.0, with or without sodium chloride (150 mM). Blanks consisted of phosphate buffer alone, at the respective pH and sodium chloride concentration. The final concentration for each sample was between 0.5 and 1.0 mg/mL. To compare our results with previously published data [Carr, 1993] CD spectra were obtained at 0 ^{o}C in the region of 200-260 nm using a 0.1 cm quartz cuvette. The secondary-structure content of the peptides was calculated using the CD spectra deconvolution software K2D.

Results: *Table 7-1* shows the mutants that were produced for this study. After an initial screen, for improvement in the random coil to α-helix transition, we focused our efforts on the characterization of the wild-type (*wt*), glycine-alanine mutation at position 22 (*G22A*), and histidine to glutamine mutation at position 11 (*H11Q*).

In contrast to the results of Carr and Kim, we did not observe a significant increase in the α-helical content of *wt* Loop 36 when changing the pH from 7 to 5. Carr and Kim

(1993) reported a high random coil content at pH 7 and a high α-helical content at pH 5 (more than 99% in each case). By contrast, our wild-type Loop 36 contained approximately 31% α-helical content at both pHs.

Position in Peptide	Native Amino Acid	Mutated Amino Acid
4	Glutamate (E)	Glutamine (Q)
8	Glutamate (E)	Glutamine (Q)
11	Histidine (H)	Glutamine (Q)
14	Glutamate (E)	Glutamine (Q)
16	Glutamate (E)	Glutamine (Q)
19	Glutamate (E)	Glutamine (Q)
21	Glutamate (E)	Glutamine (Q)
22	Glycine (G)	Alanine (A)
28	Glutamate (E)	Glutamine (Q)
32	Glutamate (E)	Glutamine (Q)

Table 7-1: *Mutations performed in the peptide*

There are several possible reasons that our results may not agree with those of Carr and Kim. The chemically synthesized peptide used by Carr and Kim was protected at both the C- and N-termini. It is known that C- and N- terminal substitution can influence the α-helix propensity of small peptides [Chakrabartty, 1990]. In addition, the purity of the chemically synthesized peptide in the paper by Carr and Kim was not specified. Recombinant Loop 36 is not protected, and we have shown that it is at least 95% pure by mass spectrometry analysis and SDS-PAGE. In addition, the sequence of the Loop 36 gene was confirmed by DNA sequence analysis and amino acid sequencing. CD spectroscopy requires highly pure protein to be correctly interpreted.

After characterizing the mutants listed in Table 7-1, we found two mutants with improved random coil to α-helix transitions, in comparison to the *wt*. Mutants *G22A* and

H11Q both exhibited an increase in α-helical content in response to a decrease in pH (*Figure 7-1*).

We have also generated melting curves for the *wt*, *G22A*, and *H11Q* constructs, to examine the changes in the secondary structures of the peptides in response to changes in temperature. As demonstrated by a decrease in the elipticity at 222 nm, our preliminary results indicate that all three constructs exhibit a tendency toward greater α-helical content as the temperature is raised from 0 to 60 ^{o}C, in the presence or absence of NaCl (*Figure 7-2*). Interestingly, the *G22A* mutant exhibits more complex behavior at pH 5, in the presence of salt (*Figure 7-2D*). We are currently focusing our efforts on characterizing the origins of this behavior, which we believe may arise from specific association between the peptides.

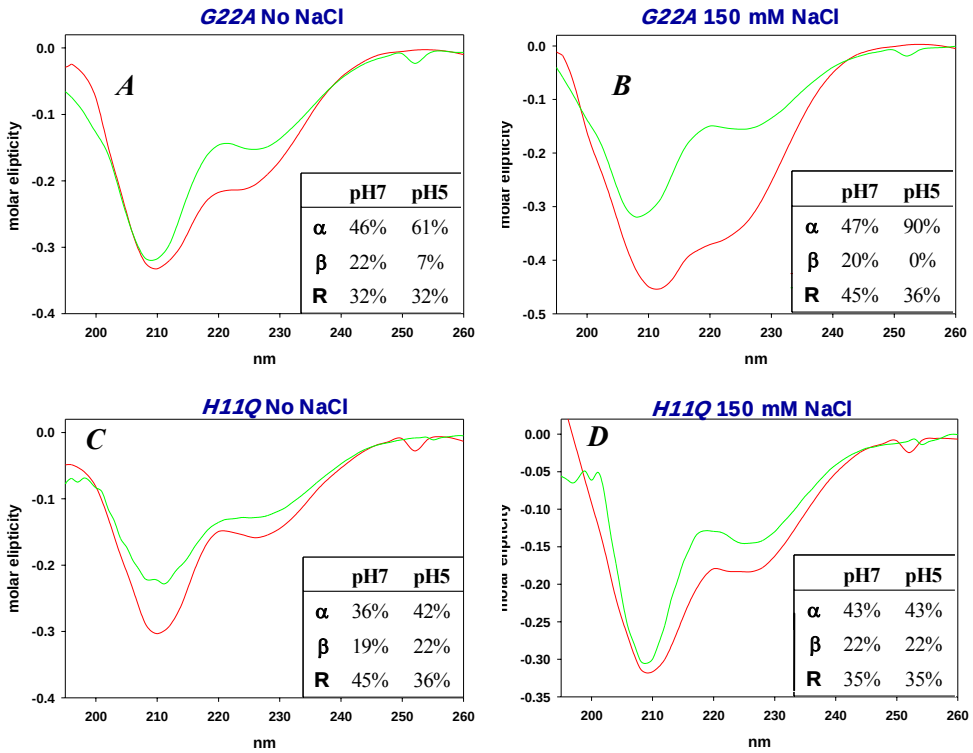


Figure 7-1: (A-B) *The G22A mutant exhibits an increased α-helical content at lower pH. This transition, which is more pronounced in the presence of 150 mM NaCl, comes at the expense of*

*β-sheet structure and random coils.**(B-C)**. The H11Q mutant exhibits a slight increase in α -helicity and β -sheet conformation, at lower pH, in the absence of NaCl, but not in its presence.*

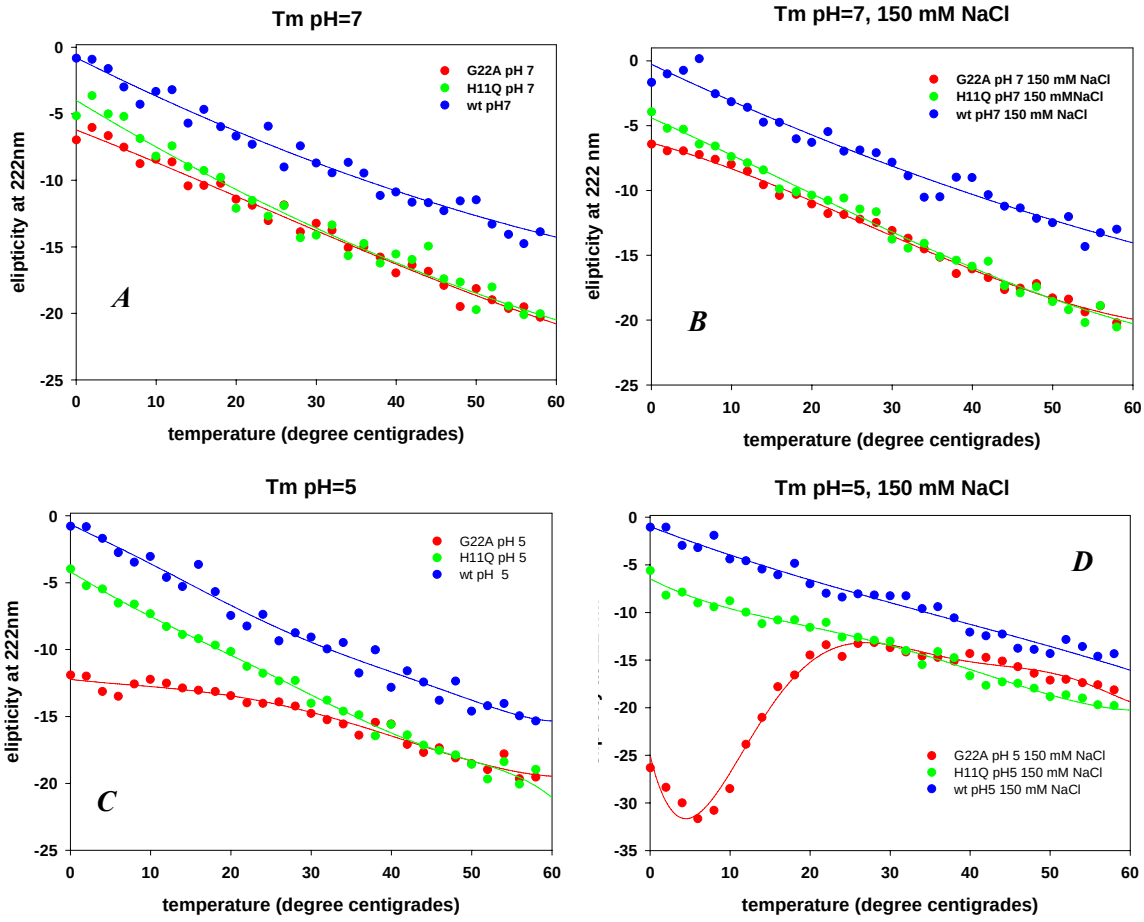


***Figure 7-2:** A-B. At pH 7, in the absence and presence of NaCl, all constructs exhibit a trend toward greater α-helicity at higher temperature. C-D. At pH 5, in the presence of NaCl, the G22A mutant exhibits complex behavior that may be characterized by helix formation, melting, and specific aggregation between helices.*

In addition to the work presented, we are collaborating with Matthew Lang, an Assistant Professor of Mechanical and Biological Engineering at the Massachusetts Institute of Technology to characterize the force produced during the single molecule phase transition of the elastin-like polypeptide $(GVGVP)_n$. Our future activities will

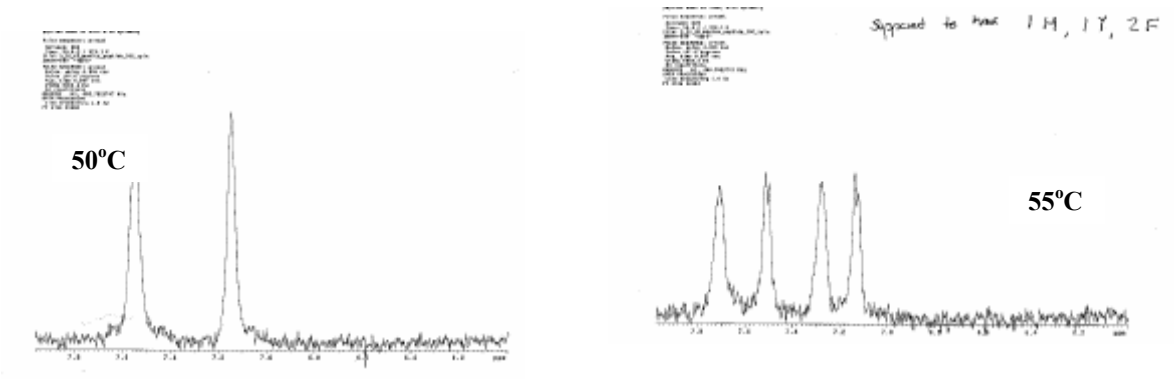
include a continuation of our characterization of Loop 36, including NMR experiments, and the characterization of the elastin-like polypeptide. In addition, we plan to combine these peptides to engineer materials that exhibit responsiveness to both pH and temperature.

7.3 NMR analysis

(in collaboration with Dr. Mary Roberts, Boston College)

To gain a deeper and better defined understanding of the structural transitions that Loop 36 and its mutants undergo in response to changes in environmental conditions, we are using nuclear magnetic resonance (NMR) to study the structural features of the peptides. These results are still preliminary in nature, and we are continuing to refine the methods used to collect them. We anticipate having more detailed structural information sometime soon.

Preliminary spectra indicate a change in structure was observed 55-60°C. The following spectra (*figure 7-3*) show the aromatic region and a peak split when the temperature is raised from 50°C to 55°C.



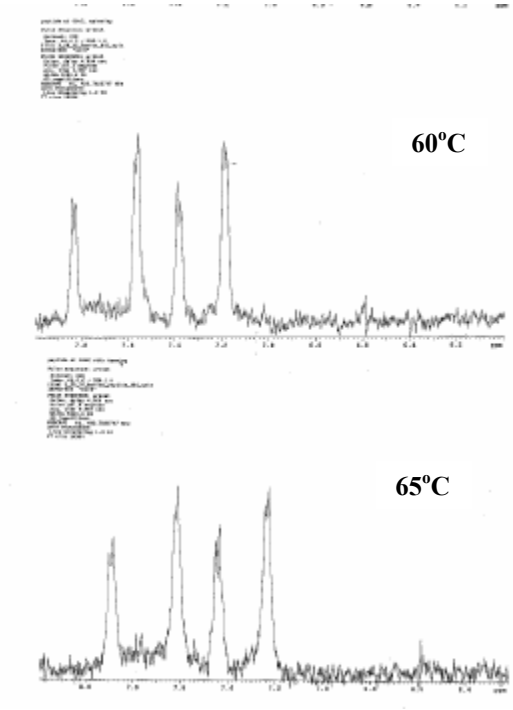


Figure 7-3: *The NMR spectra for Loop 36.*

Clearly, there is a larger chemical shift dispersion at higher temperature, indicating the adopting of structure. However, the intensity of the eight expected glutamic acids is less than expected, indicating possible impurities. We are currently focusing on producing large quantities of highly purified peptide for a second round of NMR analysis.

Chapter 8: Future Activities

In this chapter we list the future activities planned for the completion of NIAC phase II and beyond.

i) Design & Architecture

In this category, we plan to complete the designs of NTXp and ATB gears. This includes detailed component list and the integration with the electronic systems.

ATB gears for astronauts

- Design the reaction mechanism for radiation detection for ATB
- Design a detector layer complimentary to the Layer A
- Design the fluidic channels to transport the bio-nano robots to and from the reaction sites.
- Integrate the information obtained with the electronic systems for remote and local surveillance of the radiation damage.
- Following table gives the product release for ATB:

ATB v1	Space radiation Detection
ATB v1.5	Radiation Detection and partial prevention
ATB v2	Added feature of wound healing layer
ATB v3	Mechanical Fault diagnosis and detection
ATB v4	Self repairing architecture implemented for the above layers

NTXp

- Design the surface water / mineral detection network and reaction mechanism
- Design the multi channel pumping / actuating mechanism for transport of bio-nano robots in NTXp.
- Design the space condition tolerant outer skin for NTXp.

ii) Developing computational framework

- Work on the computational strategy detailed for designing bio-nano components and selecting their best mutants
- Integrate homology modeling of protein to expedite the process of design
- Develop algorithms for designing bio-nano assemblies using molecular docking techniques
- Computationally analyze the effect of radiation on bio-nano components
- Calculating the radiation effects in ATB and how the ion / electron transfer effects could be related to intensity of radiation damage.

iii) Experimental

- The work would be continued on the Loop 36 and other bio-nano components. Techniques from NMR would be used to exactly characterize the peptide structure when it changes its conformation.
- Explore the radiation resistant bacterium ***Deinococcus radiodurans*** for possible radiation resistant bio-mechanisms and proteins
- Develop other bio-nana components listed in the report
- Conduct experiments with carbon nano tube structures and bio-nano components and their integration.

iv) Integration

- Integrating the space devices with the MEMS.
- Amplifying the bio signals and integrating with the electronic controls.

v) Testing - Testing designed bio-nano components for radiations and temperature effects.

References

Ajayan et al. (2002), "Microfabrication technology: Organized assembly of carbon nanotubes", Nature 416, 495 - 496 .

Archives of Science. (2001). All about entropy, the laws of thermodynamics, and order from disorder. *http://www.entropylaw.com*, (c) Copyright 2001.

Amendola V, Fabbrizzi L, Mangano C, Pallavicini P. (2001). Molecular machines based on metal ion translocation. *Acc Chem Res*. 34: 488-93.

Bachand GD, Montemagno CD. (2000). Constructing Organic / Inorganic NEMS Devices Powered by Biomolecular Motors. *Biomedical Microdevices*. 2: 179-84.

Bala. P, Grochowski. P, Lesyng. B, McCammon. J.A (1996), Quantum-classical molecular dynamics: Models and applications, Quantum Mechanical Simulation Methods for Studying Biological Systems, Springer – Les Editions De Physique.

Balzani V, Lopez MG, Stoddart JF. (1998). Molecular Machines. *Acc Chem Res*. 31: 405-14.

Berg HC. (2000). Motile Behavior of Bacteria. *Physics Today*. 53: 24-9.

Berg HC. (1974). Dynamic properties of bacterial flagellar motors. *Nature*. 249: 77-9.

Berntson. G.M., (1997). "Topological scaling and plant root system architecture: developmental and functional hierarchies", New Phytol, 135, 621-634.

Block SM. (1998). Kinesin: what gives? *Cell*. 93: 5-8.

Block SM, Goldstein LS, Schnapp BJ. (1990). Bead movement by single kinesin molecules studied with optical tweezers. *Nature*. 348: 348-52.

Bohm KJ, Steinmetzer P, Daniel A, Baum M, Vater W, et al. (1997) Kinesin-driven microtubule motility in the presence of alkaline-earth metal ions: indication for a calcium ion-dependent motility. *Cell Motil Cytoskeleton*. 37: 226-31.

Boyer PD. Energy, Life and ATP", (Nobel Lecture). (1998). *Angewandte Chemie International Edition*. 37: 2296-307.

Braha, O. et al (1997), Designed protein pores as components for biosensors, Chem. Biol. 4, 497-505.

Carr, C.M. and Kim, P.S. (1993), A spring-loaded mechanism for the conformational change of influenza hemagglutinin. Cell. 73: 823-32.

Chakrabartty, A., Doig, A.J., and Baldwin, R.L. (1993), Helix capping propensities in peptides parallel those in proteins. Proc Natl Acad Sci U S A. 90: 11332-6.

Cucinotta F. A., Wu H., Shavers M., and George K. (2003), 'Radiation Dosimetry and biophysical models of space radiation effects'. Gravitational and Space Biology Bulletin 16 (2), June 2003.

Drexler Eric. K. (1992). Nanosystems: Molecular Machinery, Manufacturing and Computation: *John Wiley & Sons*.

Dubey A., Sharma G., Mavroidis C., Tomassone S. M., Nikitczuk K.P., Yarmush M.L. (2004), Computational Studies of Viral Pro-tein Nano-Actuators, Journal of Computational and Theoretical Nanoscience, Vol. 1, No. 1, pp. 18-28.

Eisenberg. B (1998), Ionic channels in biological membranes - Natural nanotubes, Accounts of Chemical Research, 31:117-125.

Farrell CM, Mackey AT, Klumpp LM, Gilbert SP. (2002) The role of ATP hydrolysis for kinesin processivity. *J Biol Chem*. 277: 17079-87.

Ferguson JA, Boles TC, Adams CP, Walt DR. (1996). A fiber-optic DNA biosensor microarray for the analysis of gene expression. *Nat Biotechnol*. 14: 1681-4.

Finer JT, Simmons RM, Spudich JA. (1994). Single myosin molecule mechanics: piconewton forces and nanometre steps. *Nature*. 368: 113-9.

Foresight Institute, (2000). "Molecular Nanotechnology Guidelines: Draft Version 3.7," 4 June 2000.

Frasch WD. (2000). Vanadyl as a Probe of the Function of the F1-ATPase-Mg2+ Cofactor. *Journal of Bioenergetics and Biomembranes*. 32: 2000.

Fukasaku. K, Takeda. K and Shiraishi. K (1997), Electronic Structures of Protein Nanotubes, J. Phy. Soc. Jpn. 66, 3387-3390.

Ghadiri. M et al (2001), Self-Assembling Organic Nanotubes, Angewandte Chemie International Edition, Volume 40, Issue 6 , Pages 988 – 1011.

Gyftopoulos. Elias P. et al. (2003), Quantum-theoretic Shapes of Constituents of Systems in various states, Journal of Energy Resources Technology.

Hackney DD. (1996). The kinetic cycles of myosin, kinesin, and dynein. *Annu Rev Physiol*. 58: 731-50.

Hamilton C.J. (2001), "Mars Introduction", http://www.solarviews.com/eng/mars.htm.

Harada A. (2001). Cyclodextrin-based molecular machines. *Acc Chem Res*. 34: 456-64, 2001.16.

Hellinga HW, Richards FM. (1991). Construction of new ligand binding sites in proteins of known structure. I. Computer-aided modeling of sites with pre-defined geometry. J Mol Biol. 222: 763-85.

Herbert R.A and Sharp R.J. (1992), "Molecular Biology And Biotechnology Of Extremophiles", Chapman and Hall.

Hess H, Vogel V. (2001). Molecular shuttles based on motor proteins: active transport in synthetic environments. *J Biotechnol*. 82: 67-85.

Horikoshi K and Grant W.D. (1998), "Extremophiles: Microbial Life In Extreme Environments", John Wiley & Sons.

Horikoshi K and Tsujii K. (1999), "Extremophiles In Deep-Sea Environment", Springer.

Howard J, Hudspeth AJ, Vale RD. (1989). Movement of microtubules by single kinesin molecules. *Nature*. 342: 154-8.

Hu J, Zhang Y, Gao H, Li M, Hartman U. (2002). Artificial DNA Patterns by Mechanical Nanomanipulation. *Nanoletters*. 2: 55-7.

Jet Propulsion Laboratory (JPL), California Institute of Technology. http://www.jpl.nasa.gov/.

Kasianowicz JJ, Bayley H, *http://www.cstl.nist.gov/biotech/biomat/Projects/metal.html*

Khan S, Zhao R, Reese TS. (1998). Architectural features of the Salmonella typhimurium flagellar motor switch revealed by disrupted C-rings. *J Struct Biol*. 122: 311-9.

Kinosita K. Jr, Yasuda R, Noji H, and Adachi K (2000). A rotary molecular motor that can work at near

100% efficiency. Phil.Trans. R. Soc. Lond. B 355, 473-489.

Kitamura K, Tokunaga M, Iwane AH, Yanagida T. (1999). A single myosin head moves along an actin filament with regular steps of 5.3 nanometres. *Nature*. 397: 129-34.

Koumura N, Zijlstra RW, van Delden RA, Harada N, Feringa BL. (1999). Light-driven monodirectional molecular rotor. *Nature*. 401: 152-5.

Levine I.N., (2000). Quantum Chemistry, 5th Edition, Prentice Hall.

Lewis Group 2004, http://webs.byu.edu/lewis/lewisgroup/research/peptidenanotubes.htm, Brigham Young University department of Physics and Astronomy.

Liu H, Schmidt JJ, Bachand GD, Rizk SS, Looger LL, *et al*. (2002). Control of a biomolecular motor-powered nanodevice with an engineered chemical switch. *Nat Mater*. 1: 173-7.

Mahadevan L, Matsudaira P. (2000). Motility powered by supramolecular springs and ratchets. *Science*. 288: 95-100.

Manning P, McNeil C. Microfabricated Multi-Analyte Amperometric Sensors. http://nanocentre.ncl.ac.uk/.

Mao C, Sun W, Shen Z, Seeman NC. (1999). A nanomechanical device based on the B-Z transition of DNA. *Nature*. 397: 144-6.

Mehta AD, Rock RS, Rief M, Spudich JA, Mooseker MS, *et al*. (1999). Myosin-V is a processive actin-based motor. *Nature*. 400: 590-3.

Messiah. A (1999), Quantum Mechanics, Dover Publications Inc.

MIT media laboratory Nanoscale Sensing, http://www.media.mit.edu/nanoscale/

Montemagno CD, Bachand GD. (1999). Constructing Nanomechanical Devices Powered by Biomolecular Motors. *Nanotechnology*. 10: 225-331.

Muller C., Gillotay D, Moreau D, Fonteyn D, "Exobiology: Martian environmental conditions", Space scientific research in belgium / volume 2 space sciences part 2.

Namba K, Vonderveczt F. (1997). Molecular Structure of Bacterial Flagellum. *1997*. 30: 1-65, 1997.

National Space Biomedical Research Institute, Copyright © 2000-2006. http://www.nsbri.org/Radiation/

Noji H, Yasuda R, Yoshida M, Kinosita K, Jr. (1997), Direct observation of the rotation of F1-ATPase. *Nature*. 386: 299-302.

Parr, R. G.; Yang, W., (1989). Density-Functional Theory of Atoms and Molecules, Oxford University Press.

Penrose LS, Penrose R, (1957). A self-reproducing analogue. Nature 1957; 179:1183.

Penrose LS, (1958). Mechanics of self-reproduction. Ann. Human Genetics 1958; 23:59-72.

Pieroni O, Fissi A, Angelini N, Lenci F. (2001). Photoresponsive polypeptides. *Acc Chem Res*. 34: 9-17.

Plant AL, Silin V. *http://www.cstl.nist.gov/biotech/biomat/Projects/warfare.html*.

Reysenbach A, Voytek M and Mancinelli R. (2001), "Thermophiles Bio diversity, Ecology, and Evolution", Kluwer Academic/Plenum Publishers.

Robert A. Freitas Jr. (1999). Nanomedicine, Volume I: Basic Capabilities, Landes Bioscience, Georgetown, TX, 1999.

Robert A. Freitas Jr. (2003). Nanomedicine, Volume IIA: Biocompatibility, Landes Bioscience, Georgetown, TX, 2003.

Robert A. Freitas Jr., Ralph C. Merkle, (2004). Kinematic Self-Replicating Machines, Landes Bioscience, Georgetown, TX, 2004; http://www.MolecularAssembler.com/KSRM.htm

Rohl CA, Strauss CE, Misura KM, Baker D, (2004). Protein structure prediction using Rosetta. Methods Enzymol 2004; 383:66-93.

Szabo. A, Ostlund. N.S., (1989). Modern Quantum Chemistry – Introduction to Advanced Electronic Structure, Mc-Graw Hill Publishing Company.

Schalley CA, Beizai K, Vogtle F. (2001). On the way to rotaxane-based molecular motors: studies in molecular mobility and topological chirality. *Acc Chem Res*. 34: 465-76.

Schnitzer MJ, Block SM. (1997). Kinesin hydrolyses one ATP per 8-nm step. *Nature*. 388: 386-90.

Seeman NC. (1998). DNA nanotechnology: novel DNA constructions. *Annu Rev Biophys Biomol Struct*. 27: 225-48.

Seipp. R (2002), Deinococcus radiodurans: Does this Bug Wear a Lead Vest or what?, Microbiology and Immunology, University of British Columbia.

Sharma G., Rege K., Mavroidis C. and Yarmush M., "Design and Modeling of a Peptide Based NanoGripper," Proceedings of the 2006 ASME Mechanisms and Robotics Conference, 2006 ASME Design Technical Conferences, September 10-13, 2006, Philadelphia, PA, USA. Paper DETC2006-99701.

Sigler. P et al (1998), Structure and function in GroEL-mediated protein folding, Annual Rev. Biochem, 67, 581-608.

Soong RK, Bachand GD, Neves HP, Olkhovets AG, Craighead HG, *et al*. (2000). Powering an inorganic nanodevice with a biomolecular motor. *Science*. 290: 1555-8.

Smith S.S (2001). United States Patent No. 6,200,782, 13 March 2001.

Tarek. M, Maigret. B and Chipot. C (2003), Molecular Dynamics Investigation of an Oriented Cyclic Peptide Nanotube in DMPC Bilayers, Biophysical Journal, Volume 85, October 2003 2287–2298.

Tobias I, Swigon D, Coleman BD. (2000). Elastic stability of DNA configurations. I. General theory. *Phys Rev E Stat Phys Plasmas Fluids Relat Interdiscip Topics*. 61: 747-58.

Ueno T, Oosawa K, Aizawa S. (1992). M ring, S ring and proximal rod of the flagellar basal body of Salmonella typhimurium are composed of subunits of a single protein, FliF. *J Mol Biol*. 227: 672-7.

Ueno T, Oosawa K, Aizawa S. (1994). Domain structures of the MS ring component protein (FliF) of the flagellar basal body of Salmonella typhimurium. *J Mol Biol*. 236: 546-55.

Vale RD, Milligan RA. (2000). The way things move: looking under the hood of molecular motor proteins. *Science*. 288: 88-95.

Wang MD, Schnitzer MJ, Yin H, Landick R, Gelles J, *et al*. (1998). Force and velocity measured for single molecules of RNA polymerase. *Science*. 282: 902-7.

Walker JE. (1998). ATP Synthesis by Rotary Catalysis (Nobel Lecture). *Angewandte Chemie Intternational Edition*. 37: 2308-19.

Walker. I, Hannan. M, The Elephant's Trunk Robotic Arm, http://www.ece.clemson.edu/crb/labs/biomimetic/elephant.htm, Manipulation & Biomimetics laboratory, Mechatronics, Clemson University.

Kohnlein. W, 1997. http://www.foe.arc.net.au/kohnlein/kohnlein.html/

Yan H, Zhang X, Shen Z, Seeman NC. (2002). A robust DNA mechanical device controlled by hybridization topology. *Nature*. 415: 62-5.

Yasuda R, Noji H, Kinosita K, Jr., Yoshida M. (1998). F1-ATPase is a highly efficient molecular motor that rotates with discrete 120 degree steps. *Cell*. 93: 1117-24.

Yuqiu J, Juang CB, Keller D, Bustamante C, Beach D, *et al*. (1992). Mechanical, Electrical, and Chemical Manipulation of Single DNA Molecules. *Nanotechnology*. 3: 16-20.

Yurke B, Turberfield AJ, Mills AP, Jr., Simmel FC, Neumann JL. (2000). A DNA-fuelled molecular machine made of DNA. *Nature*. 406: 605-8.

Appendix – 1- RMSD of the individual residues inside the peptide

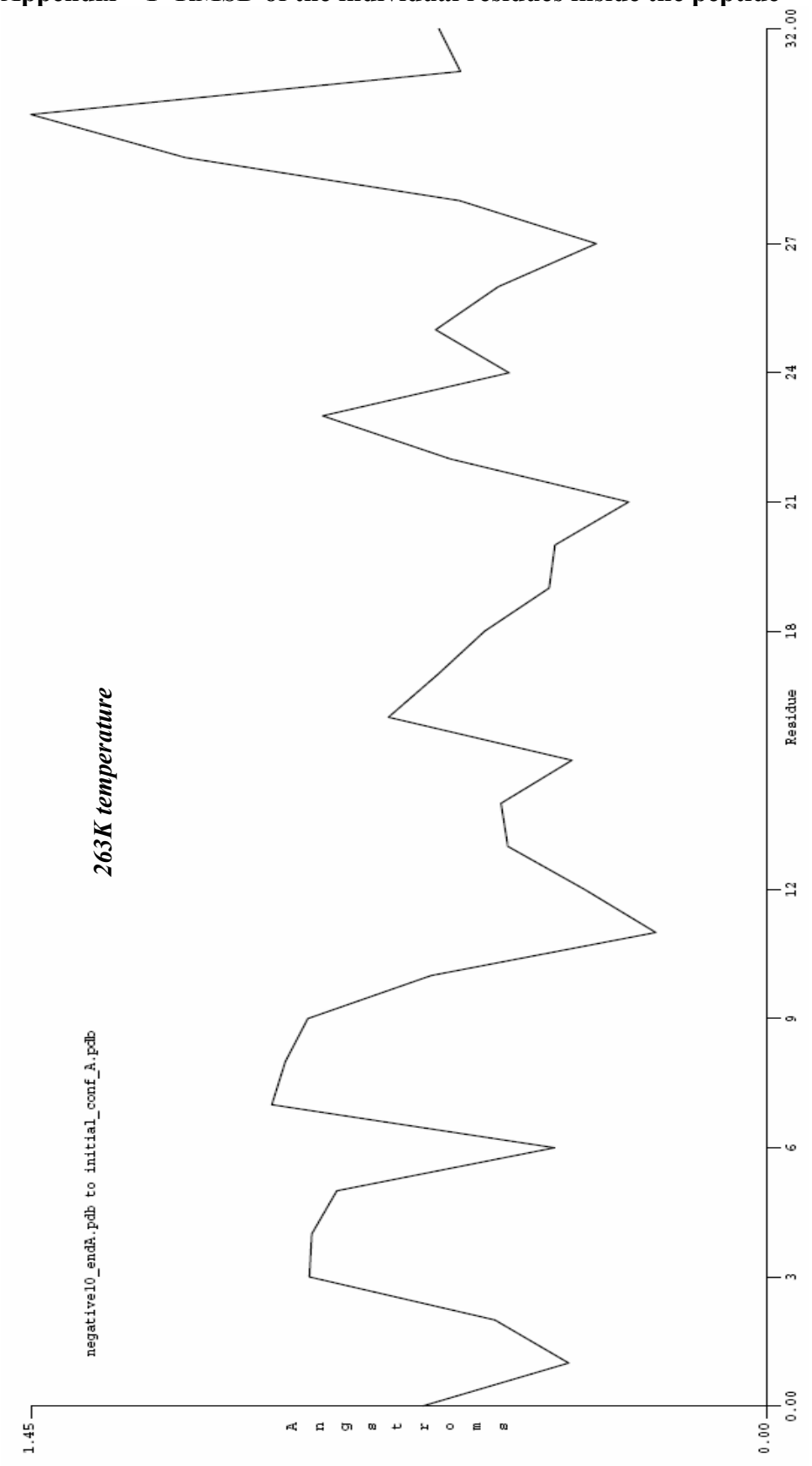


Figure 3-6: *The maximum rmsd was for residue 30 of 1.45 angstroms.*

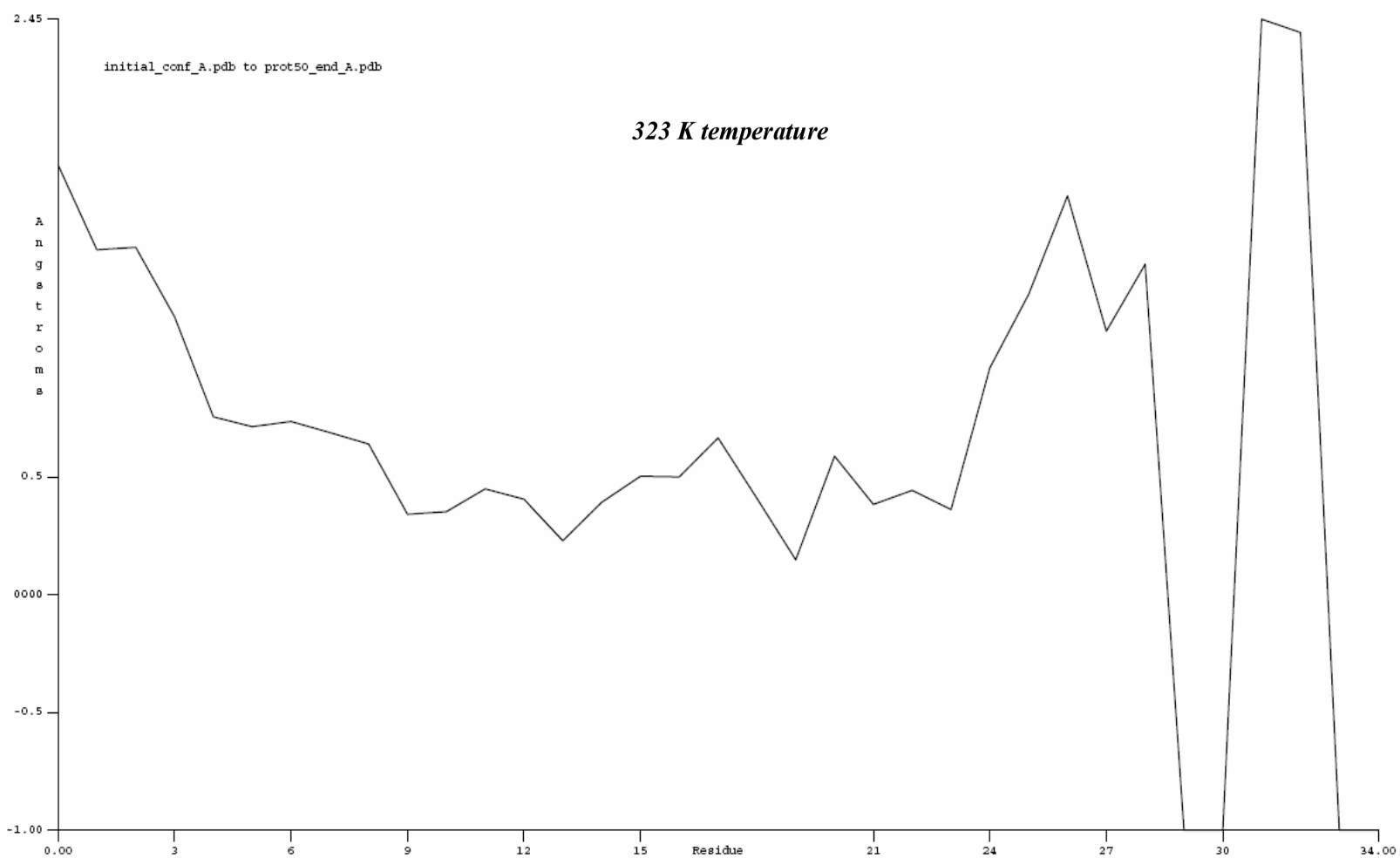


Figure 3-7: *The maximum rmsd was for residue 31 of 2.45 angstroms. We also observe negative rmsd values.*

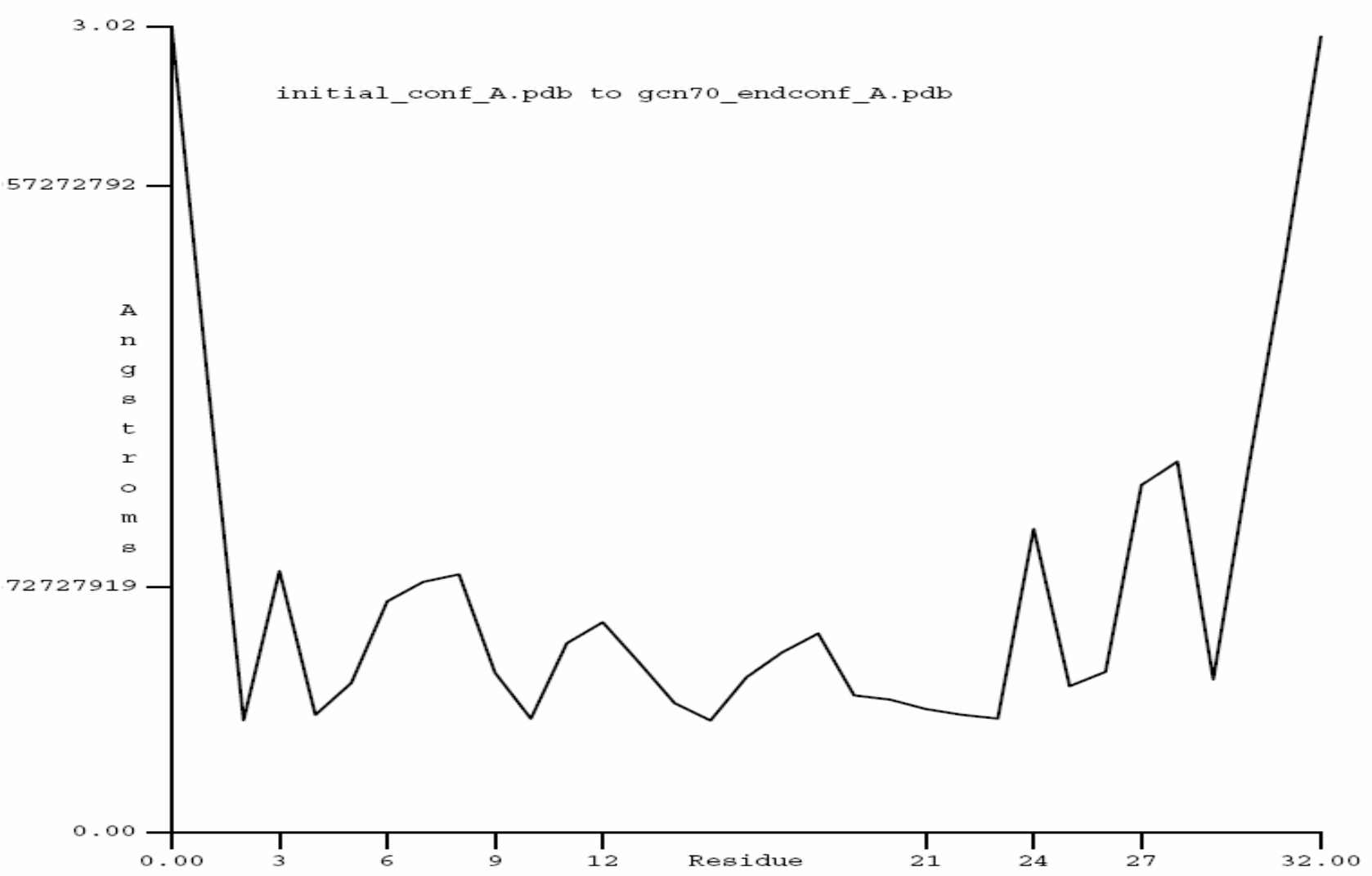


Figure 3-8: *The maximum rmsd was for residue 32 of 3.02 angstroms. We dont observe negative rmsd values in this case.*

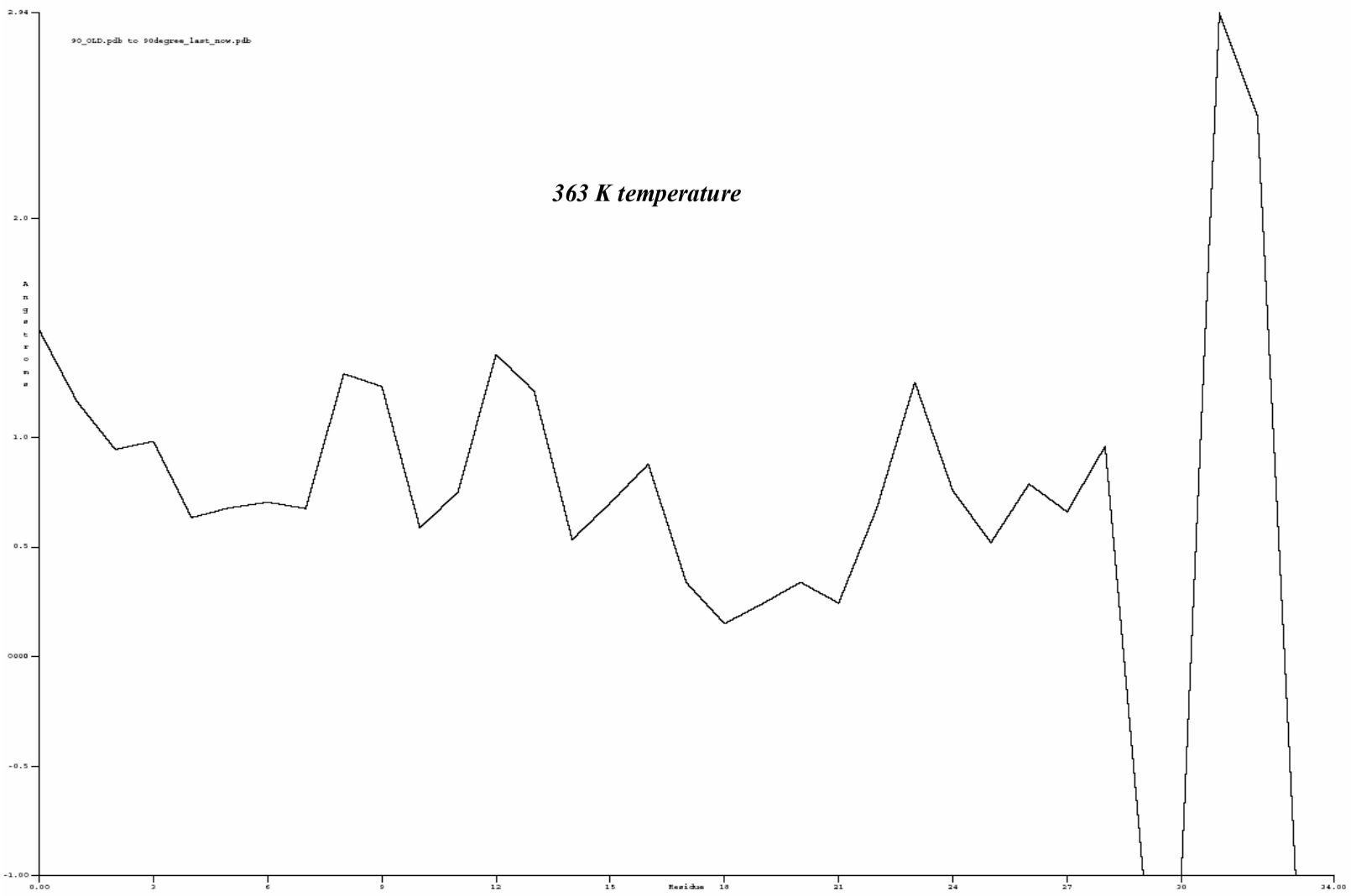


Figure 3-9: *The maximum rmsd was for residue 31 of 2.94 angstroms. Also we here observe the rmsd value of aroud 1.5 angstorms at residue 1 which is quire remarkable. We also observe negative rmsd values.*

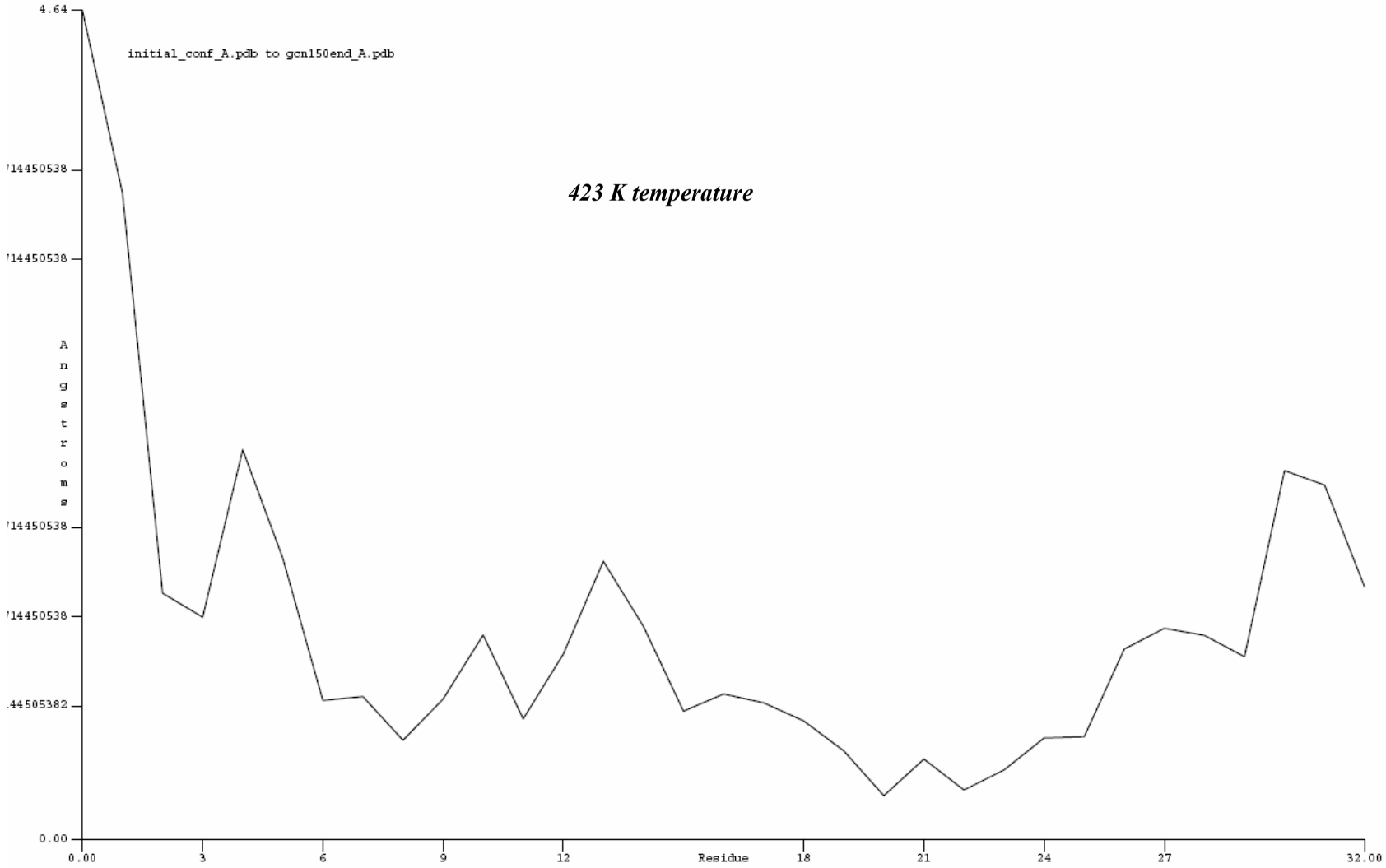
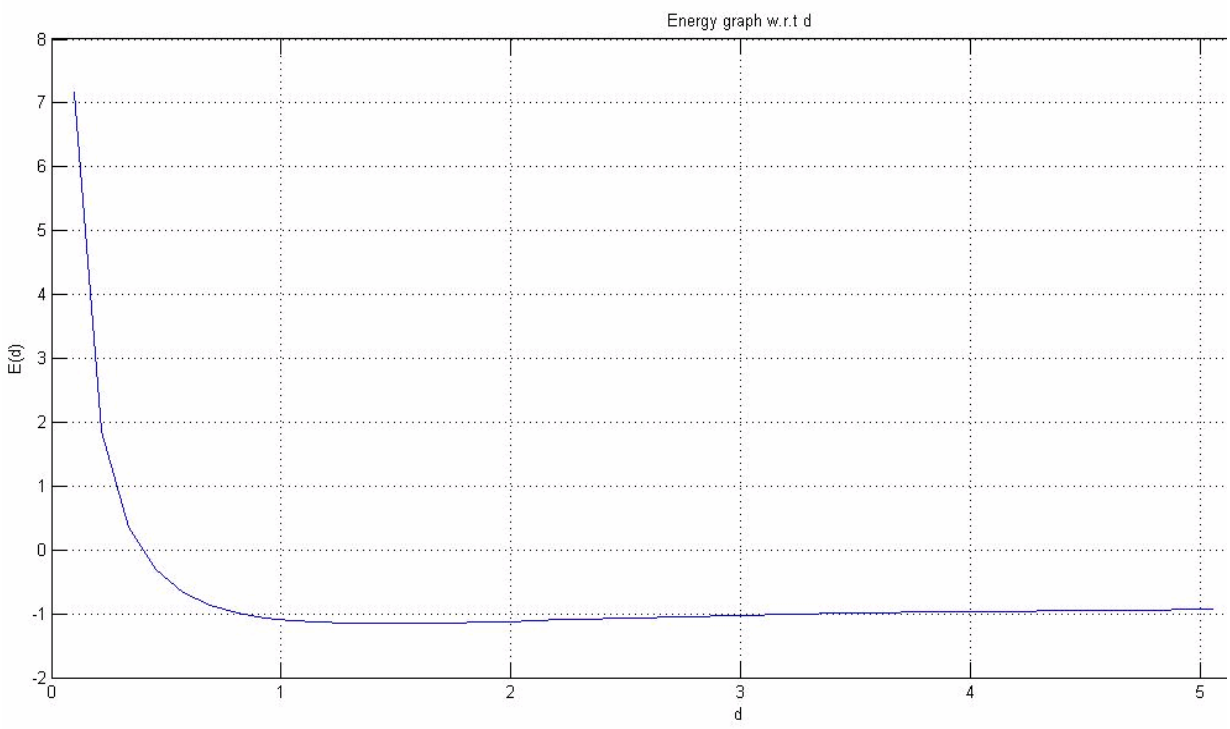


Figure 3-10: *The maximum rmsd was for residue 0 of 4.64 angstroms. We don't observe negative rmsd values here and this is quite remarkable.*

Appendix – 2 - Quantum Monte Carlo implementation for hydrogen molecule

In this code potential energy of the Hydrogen molecule is calculated through the Monte Carlo method. Following figure shows the 'energy ***E(d)*** versus distance ***d***' graph between two hydrogen atoms for this problem.



Beta (*beta*) is the Variational parameter used here. For every d, an optimized beta is evaluated to calculate the true ground state energy. Through various iterations we therefore, obtained the above graph between the ground state energy and the distance between two protons.

```
implicit real*8(a-h,o-z)
common/laplacian/h

beta = 0.08d0 !a value for the variational parameter
h=0.0001d0
iseed = 1 !input to the srand func & generates particular rands
nstep = 1000000 !number of points generated
eps = 0.6d0 !dimension of the random cube for Metropolis
naccept = 0.d0 !number of transitions accepted, hence the trial walks
nconfig = 0.d0

dini = 0.1d0
dfin = 6.0d0
dh = (dfin - dini)/50.d0
```

```
      do k=1,50
      d = dini + dfloat(k-1)*dh
      tempi = 0.d0
c     a = 0.d0

      call whatsa(d,a)

      kc = 40
      betapo = 0.01d0
      betapf = 1.01d0
      betah = (betapf - betapo)/100.d0

      do j=1,100
        call srand(iseed)
        betap = betapo + dfloat(j-1)*betah

        x1o = 1.d0
        y1o = 1.d0
        z1o = 1.d0
        x2o = 0.5d0
        y2o = -0.5d0
        z2o = 0.5d0

        edensity = 0.d0
        enum = 0.d0
        eden = 0.d0
        esig = 0.d0

        do i=1,nstep
          x1n = x1o+eps*(2.d0*rand()-1.d0) !trial step electron 1: x dir
          y1n = y1o+eps*(2.d0*rand()-1.d0) !trial step electron 1: y dir
          z1n = z1o+eps*(2.d0*rand()-1.d0) !trial step electron 1: z dir
          x2n = x2o+eps*(2.d0*rand()-1.d0) !trial step electron 2: x dir
          y2n = y2o+eps*(2.d0*rand()-1.d0) !trial step electron 2: y dir
          z2n = z2o+eps*(2.d0*rand()-1.d0) !trial step electron 2: z dir

          psi_old=psi(x1o,y1o,z1o,x2o,y2o,z2o,d,a,beta) !old wave function
          psi_new=psi(x1n,y1n,z1n,x2n,y2n,z2n,d,a,beta) !new wave function
          r = (psi_new/psi_old)**2 ! the ratio of probabilities
          if (r.ge.rand()) then !accept the move
           naccept = naccept + 1 !updating number of acceptances
           if(mod(i,kc).eq.0) then
             psi_new=psi(x1n,y1n,z1n,x2n,y2n,z2n,d,a,beta)
             psi_newp = psi(x1n,y1n,z1n,x2n,y2n,z2n,d,a,betap)
             rp = (psi_newp/psi_new)**2
             ep = elocal(x1n,y1n,z1n,x2n,y2n,z2n,d,a,betap)
```

```
            enum = enum + rp*ep
            esig = esig + (ep**2)*rp
            eden = eden + rp
            nconfig = nconfig + 1
          endif
          x1o = x1n
          y1o = y1n
          z1o = z1n
          x2o = x2n
          y2o = y2n
          z2o = z2n
        endif
      enddo

      edensity = enum/eden

      if(j.eq.1) then
        tempi = edensity
      else
        if(edensity.lt.tempi) then
         tempi = edensity
c        write(6,*) tempi
        endif
      endif

c     write(2,*) betap, edensity
c     write(6,*) betap, edensity
    enddo
      write(6,*) d, tempi
    enddo

    end

! PSI = Electronic wave function of the H2 molecule
    function psi(x1,y1,z1,x2,y2,z2,d,a,betap)
    implicit real*8(a-h,o-z)
    r1r = dsqrt(x1**2 + y1**2+(z1-0.5d0*d)**2) !electron 1 from proton R
    r1l = dsqrt(x1**2 + y1**2 + (z1+0.5d0*d)**2) !electron 1 from proton L
    r2r = dsqrt(x2**2 + y2**2 + (z2-0.5d0*d)**2) !electron 2 from proton R
    r2l = dsqrt(x2**2 + y2**2 + (z2+0.5d0*d)**2) !electron 2 from proton L
    r12 = dsqrt((x1-x2)**2 + (y1-y2)**2 + (z1-z2)**2) !rel. distance 1&2

    psi_r1 = dexp(-r1l/a) + dexp(-r1r/a)
    psi_r2 = dexp(-r2l/a) + dexp(-r2r/a)

    psi = psi_r1*psi_r2*dexp((0.5d0*r12)/(1.d0+betap*r12))
```

```
      return
      end

! AVALUE = the coefficient in the wave function
      subroutine whatsa(d,a)
      implicit real*8(a-h,o-z)
      h3 = 0.01d0
      hstep = 1.0E-6
      to = 0.d0
      conv = 1.0E-10
      t1 = to + hstep
      call poly(t1,fto,d)
 100     continue
      tn = t1 + h3
      call poly(tn,ftn,d)
      if(fto*ftn.le.0.d0) then
        h3 = -h3/2.d0
      endif
      fto = ftn
      t1 = tn
      if(dabs(ftn).ge.conv) then
        goto 100
      endif
      a=tn

      return
      end

! POLY = calculate the polynomial in a
      subroutine poly(t,f,d)
      implicit real*8(a-h,o-z)
      b = dexp(-d/t)
      f = t - 1/(1.d0 + b)
      return
      end

! ELOACL = Electronic energy density of the H2 molecule
      function elocal(x1,y1,z1,x2,y2,z2,d,a,betap)
      implicit real*8(a-h,o-z)
      common/laplacian/h

      elocal=-12.d0*psi(x1,y1,z1,x2,y2,z2,d,a,betap)
      elocal=elocal+psi(x1+h,y1,z1,x2,y2,z2,d,a,betap)
      elocal=elocal+psi(x1-h,y1,z1,x2,y2,z2,d,a,betap)
      elocal=elocal+psi(x1,y1+h,z1,x2,y2,z2,d,a,betap)
```

```
      elocal=elocal+psi(x1,y1-h,z1,x2,y2,z2,d,a,betap)
      elocal=elocal+psi(x1,y1,z1+h,x2,y2,z2,d,a,betap)
      elocal=elocal+psi(x1,y1,z1-h,x2,y2,z2,d,a,betap)
      elocal=elocal+psi(x1,y1,z1,x2+h,y2,z2,d,a,betap)
      elocal=elocal+psi(x1,y1,z1,x2-h,y2,z2,d,a,betap)
      elocal=elocal+psi(x1,y1,z1,x2,y2+h,z2,d,a,betap)
      elocal=elocal+psi(x1,y1,z1,x2,y2-h,z2,d,a,betap)
      elocal=elocal+psi(x1,y1,z1,x2,y2,z2+h,d,a,betap)
      elocal=elocal+psi(x1,y1,z1,x2,y2,z2-h,d,a,betap)

      elocal=elocal/psi(x1,y1,z1,x2,y2,z2,d,a,betap)
!     write(6,*)elocal
      ! Calculating distances
      r1r = dsqrt(x1**2 + y1**2+(z1-0.5d0*d)**2) !electron 1 from proton R
      r1l = dsqrt(x1**2 + y1**2 + (z1+0.5d0*d)**2) !electron 1 from proton L
      r2r = dsqrt(x2**2 + y2**2 + (z2-0.5d0*d)**2) !electron 2 from proton R
      r2l = dsqrt(x2**2 + y2**2 + (z2+0.5d0*d)**2) !electron 2 from proton L
      r12 = dsqrt((x1-x2)**2 + (y1-y2)**2 + (z1-z2)**2) !rel. distance 1&2

      ! Calculating Potential terms
      epot1l = -1.d0/r1l
      epot1r = -1.d0/r1r
      epot2l = -1.d0/r2l
      epot2r = -1.d0/r2r
      epot12 = 1.d0/r12
      eproton = 1.d0/d

      epot = epot1l + epot1r + epot2l + epot2r + epot12 + eproton

      elocal = -0.5d0*elocal/h**2 + epot
c     write(1,*) epot, elocal,betap
      return
      end
```

Appendix – 3 - Solving Schrodinger wave equation for H2

In this code we have solved Schrödinger wave equation for the H2 molecule. Shooting method is employed to calculate the wave function for various states, ground and excited. Outputs are given to explain the working of the code and detail the basic results:

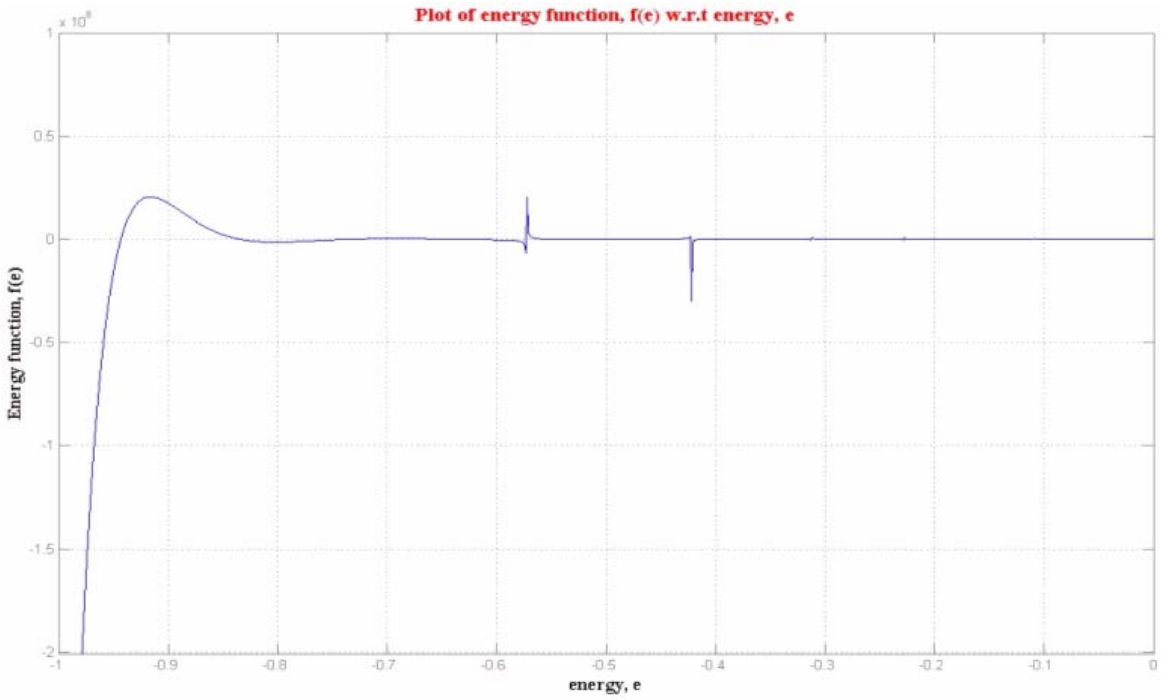
1) Ground State and excited state energies:

Energy Eigen value for (n= 0) is = -0.943278695
Energy Eigen value for (n= 1) is = -0.834828304
Energy Eigen value for (n= 2) is = -0.733137729
Energy Eigen value for (n= 3) is = -0.638162504
Energy Eigen value for (n= 4) is = -0.549572785
Energy Eigen value for (n= 5) is = -0.467657937

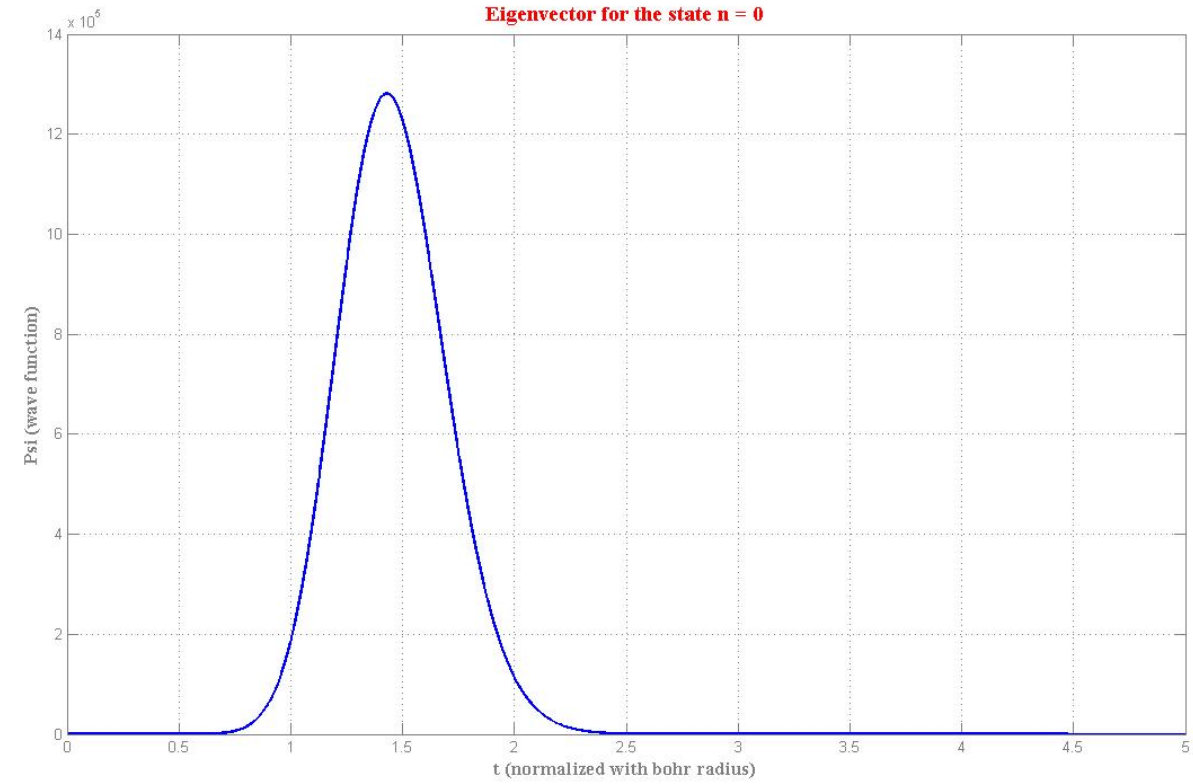
The above energies are normalized with the minimum morse potential value of 4.747 eV.

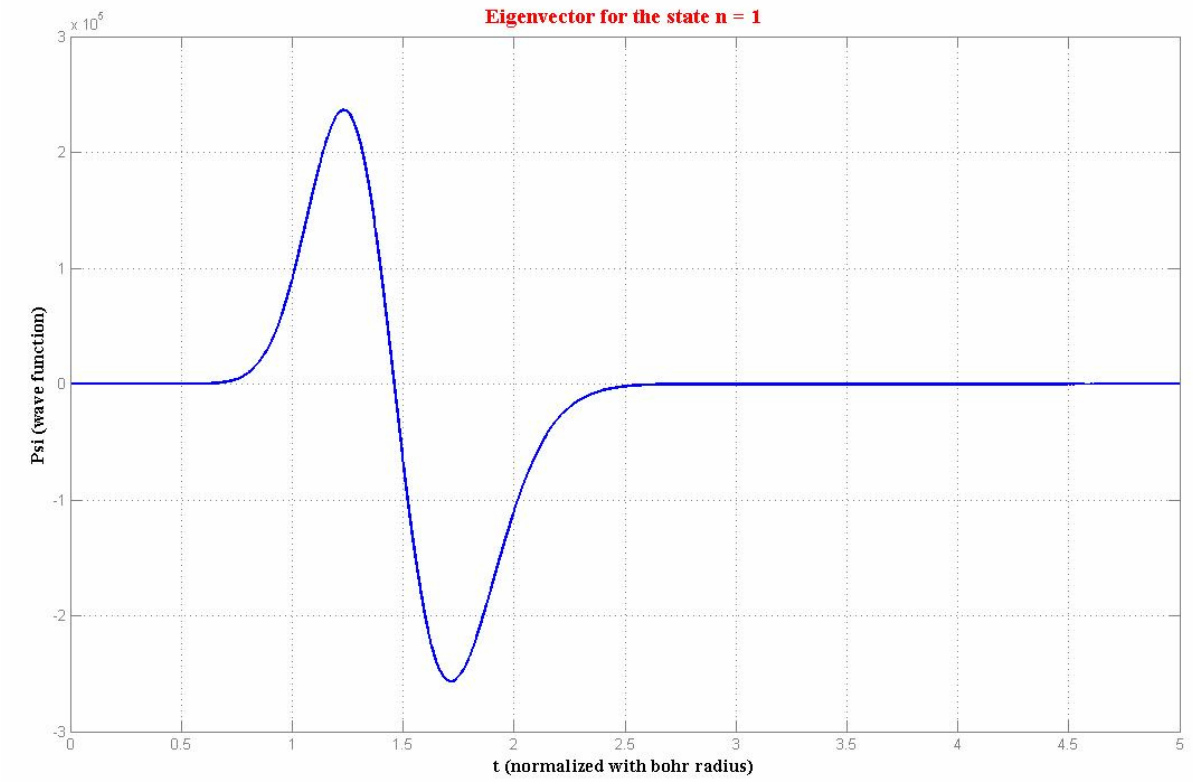
Calculated exact ground state energy = -4.47774396 eV

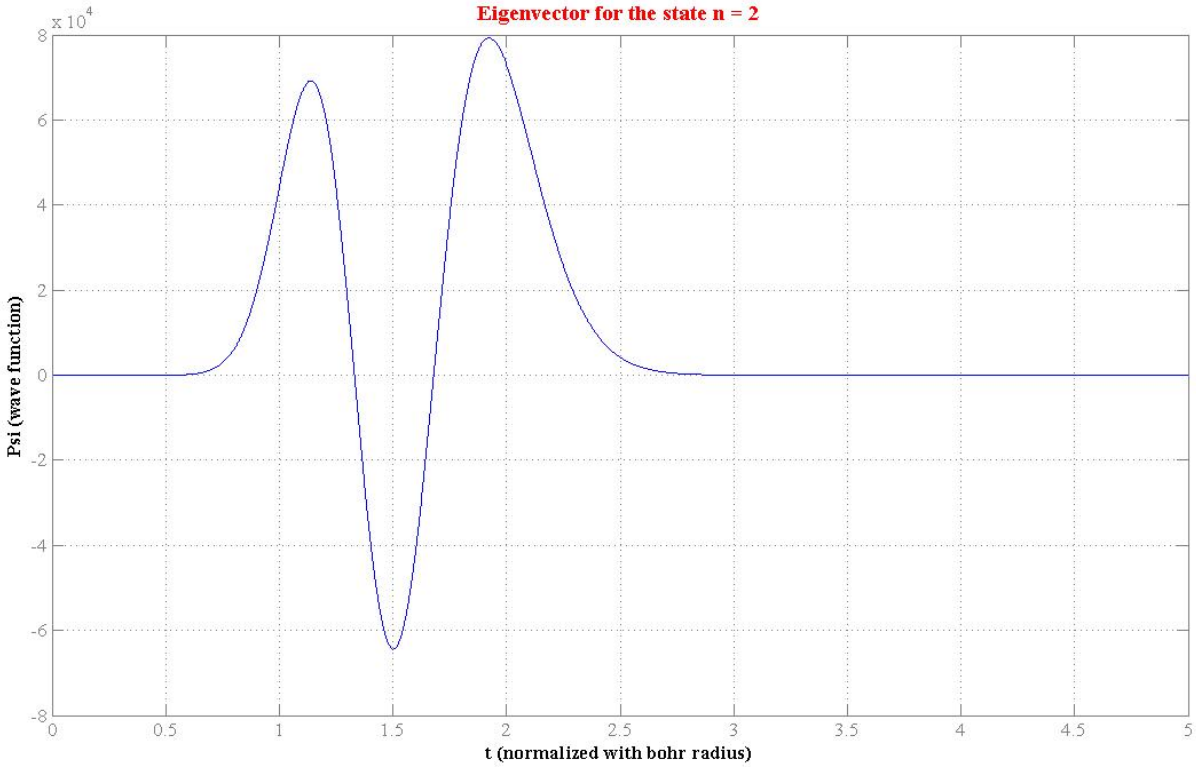
Following is the plot for the energy function. Roots of the energy function f(e) gives the value of the energy where the wavefunction for various states are optimized w.r.t the shooting method criterion.

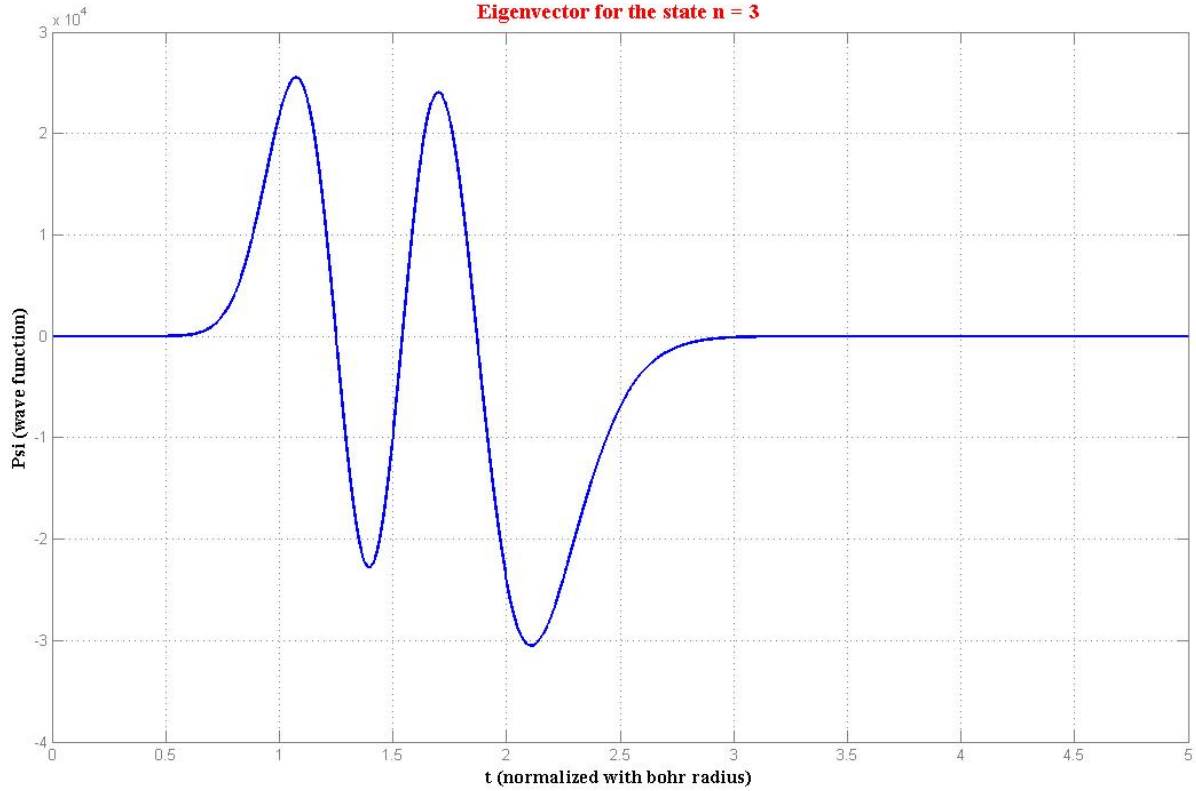


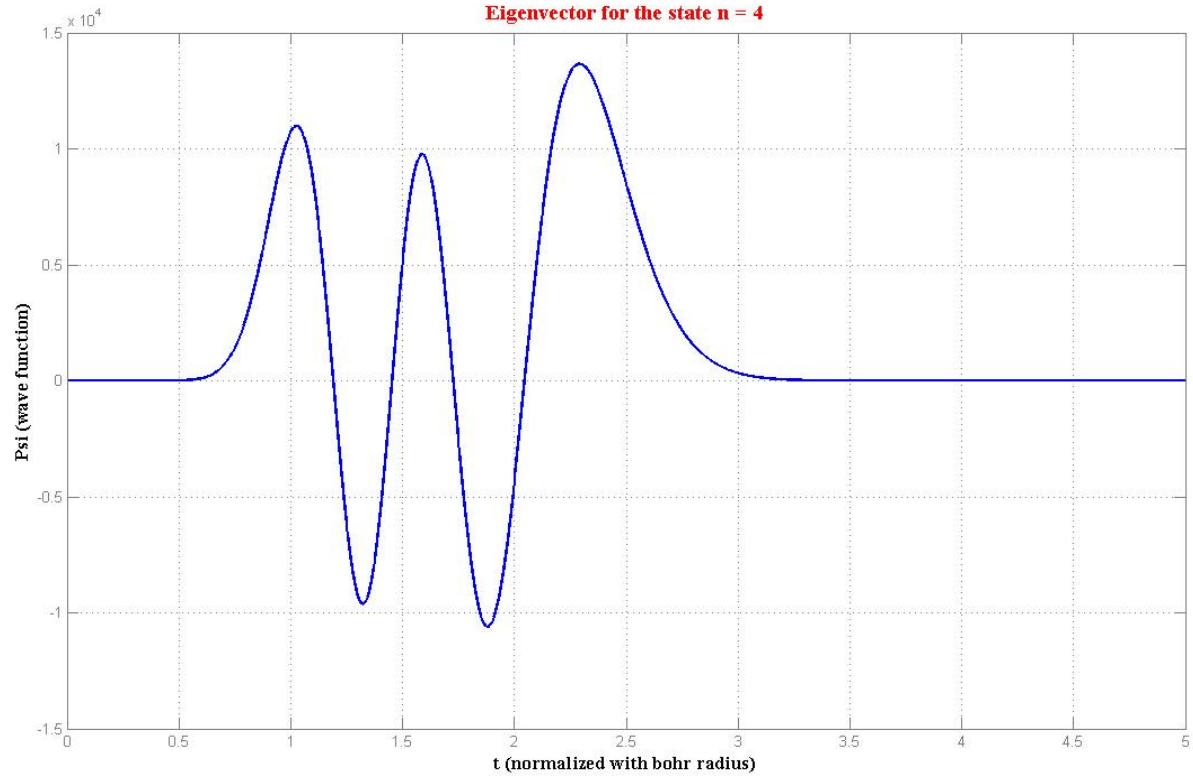
2) Plots for the wavefunction for various states (n = 0, 5)

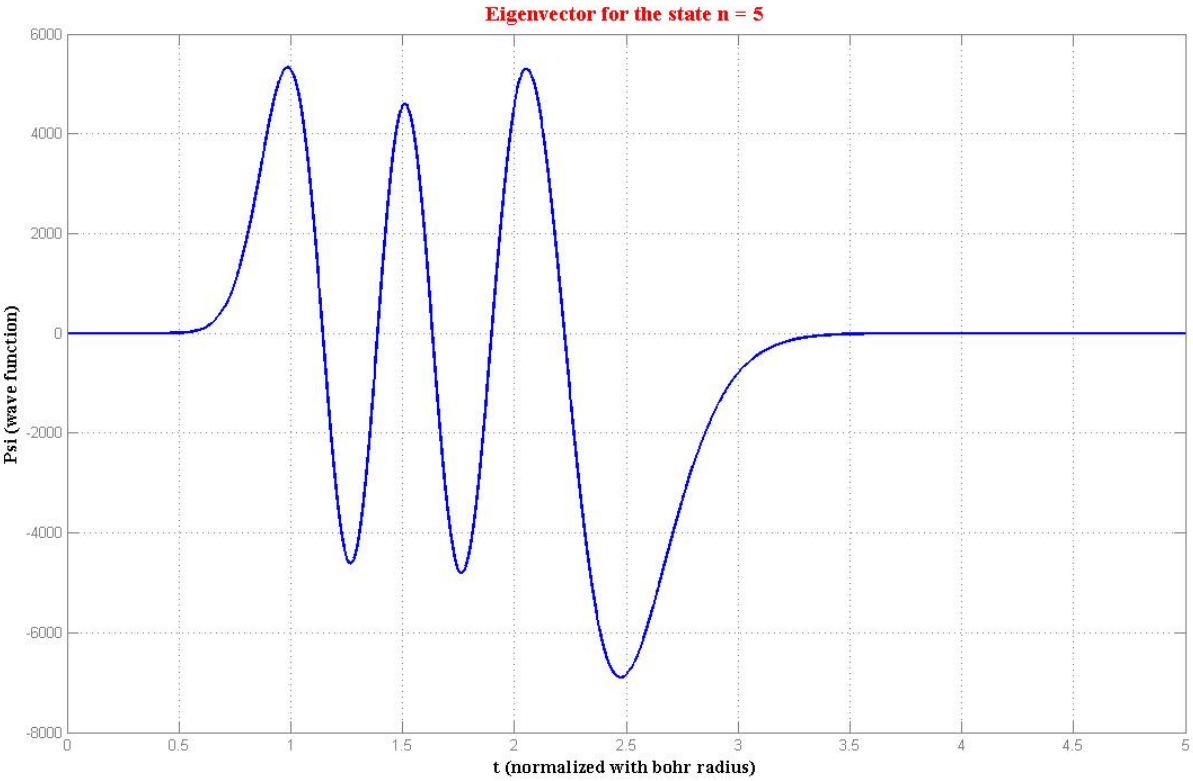





Eigenvector for the state n = 2
x 10^4
Psi (wave function)
t (normalized with bohr radius)


Eigenvector for the state n = 3
x 10^4
Psi (wave function)
t (normalized with bohr radius)


Eigenvector for the state n = 4
x 10^4
1.5
1
0.5
0
-0.5
-1
-1.5
Psi (wave function)
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
5
t (normalized with bohr radius)


Eigenvector for the state n = 5
6000
4000
2000
0
-2000
-4000
-6000
-8000
Psi (wave function)
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
5
t (normalized with bohr radius)

```
!    POTENTIAL USED: Morse Potential
!    OBJECTIVE: Eigenvalues E(n) and Eigenvectors psi(n)

     implicit real*8(a-h,o-z)
     real abohr,xmin,gamma2,gamma,vo,b
     common /coeff/ abohr,xmin,gamma2,gamma,vo,b
     dimension estate(6)

!    Parameters for the H2 molecule
     pi = 4.d0*datan(1.d0)
     hbar = 6.62608E-34/(2.d0*pi)
     ev = 1.602177E-19
     abohr = 5.291772E-11
     proton = 1.672623E-27
     hmass = proton
     redumass = hmass/2.d0

!    Parameters for the morse potential (in eV)
     vmin = -4.747d0
     vo = -vmin
     xmin = 0.74166E-10
     b = (1.03d0)/abohr !Still to be fine tuned
     gamma2 = ((2.d0*redumass*((abohr)**2)*vo*ev)/((hbar)**2)) !dimensionless
     gamma = sqrt(gamma2)
     write(6,*) 'Gamma squared = ',gamma2

!    Parameters for the wave function
     dt = 0.001d0
     t = 0.0d0
     write(6,*) 'Initial start point = ',t
     tf = 5.d0
     write(6,*) 'Final finish point = ',tf
     nstep = dint((tf-t)/dt)
     write(6,*)'No. of steps for Runge Kutta = ',nstep
     write(6,*) '=================================================='

!    Parameters for the initial conditions
     extn = t*abohr
     psi_nas = exp(-(gamma/(b*abohr))*exp(-b*(extn))) !asymptotic neg side psi
     phi_nas = psi_nas*(gamma*exp(-b*(extn))) !asymptotic neg side phi

     conv = 1.0E-5
     bound = 1.0E2
     h1 = 0.01d0
     estep = 0.01d0
     h2 = estep
```

```
      eo = -1.0d0
      do j=1,6
 400    continue
        e = eo + h1
        call funceval(e,t,tf,dt,psi_nas,phi_nas,nstep,fe)
        eigen1 = fe
 300    continue
        e = e + h2
        call funceval(e,t,tf,dt,psi_nas,phi_nas,nstep,fe)
        eigen2 = fe
        if(eigen1*eigen2.lt.0.d0) then
        h2 = -h2/2.d0
        endif
        eigen1 = eigen2
        if((dabs(e).gt.0.6d0).or.(dabs(e).lt.0.57d0)) then
          if(dabs(eigen2).ge.conv) then
            go to 300
          endif
        else
          eo = e
          h2 = estep
          go to 400
        endif
        estate(j)=e
        write(6,*)'Energy Eigen value for (n=',j-1,') is = ',estate(j)
        eo=e
        h2 = estep
      enddo                    !energy loop

      write(6,*) '=================================================='
      write(6,*) 'Value of beta = ',b,' ( = 1.03d0/bohr_radius)'
      write(6,*) 'Calculated ground state energy = ',estate(1)*4.747d0

!     Constructing Wave function for n=5 state.
!     This generates fort.1. Plots are included in the project submission
      k = 6
      econstruct = estate(k)
      write(6,*) '--------------------------------------------------'
      write(6,*) 'Constructing the eigenvector for n = ',(k-1)
      call construct(econstruct,t,tf,dt,psi_nas,phi_nas,nstep)

      end
c     ------------------------------------------------------------------------
c     ------------------------------------------------------------------------
```

```
!    Calculate the f(e)
     subroutine funceval(e,t,tf,dt,psi_nas,phi_nas,nstep,fe)
     implicit real*8 (a-h,o-z)
     dimension y(2),ymneg(2),ympos(2)
     real abohr,xmin,gamma2,gamma,vo,b
     common /coeff/ abohr,xmin,gamma2,gamma,vo,b

     psi_pas = exp(-(gamma)*sqrt(-e)*tf) !asymptotic pos side psi
     phi_pas = psi_pas*(-gamma*sqrt(-e)) !asymptotic pos side phi

     y(1)=psi_nas
     y(2)=phi_nas

     tneg = t !makes sure t remains constant for the energy do loop
     do i=1,2001
       call rgkuta(e,tneg,y,2,dt)
       tneg = tneg + dt
     enddo              !Shooting left

     ymneg(1)=y(1)
     ymneg(2)=y(2)

     y(1)= psi_pas
     y(2)= phi_pas

     tpos = tf !makes sure tf remains constant for the energy do loop
     do i=nstep,2000,-1
       call rgkuta(e,tpos,y,2,-dt)
       tpos = tpos - dt
     enddo              !Shooting right

     ympos(1)=y(1)
     ympos(2)=y(2)

     scaling = ymneg(1)/ympos(1)

     fe = ympos(2)*(scaling) - ymneg(2)
     return
     end

!    Construct Wavefunction
!    ----------------------------
     subroutine construct(e,t,tf,dt,psi_nas,phi_nas,nstep)
     implicit real*8 (a-h,o-z)
     dimension y(2),ymneg(2),ympos(2),yscale(5000),xt(5000)
     dimension y2scale(5000)
```

```
real abohr,xmin,gamma2,gamma,vo,b
common /coeff/ abohr,xmin,gamma2,gamma,vo,b

psi_pas = exp(-(gamma)*sqrt(-e)*tf) !asymptotic pos side psi
phi_pas = psi_pas*(-gamma*sqrt(-e)) !asymptotic pos side phi

y(1)=psi_nas
y(2)=phi_nas

tneg = t
do i=1,2001
  call rgkuta(e,tneg,y,2,dt)
  tneg = tneg + dt
  write(1,*) tneg,y(1)
enddo  !Shooting left

ymneg(1)=y(1)
ymneg(2)=y(2)

y(1)= psi_pas
y(2)= phi_pas

tpos = tf
do i=nstep,2000,-1
  call rgkuta(e,tpos,y,2,-dt)
  tpos = tpos - dt
  xt(i) = tpos
  yscale(i)=y(1)
  y2scale(i)=y(2)
enddo  !Shooting right

ympos(1)=y(1)
ympos(2)=y(2)

scaling = ymneg(1)/ympos(1)

do j=2000,nstep
  temp = scaling*yscale(j)
  yscale(j) = temp
  write(1,*) xt(j),yscale(j)
enddo

return
end
```

```
!    Here the external function is defined (for a given energy)
!-------------------------------------------------------------
      subroutine funcvec(e,t,y,f,n)
      implicit real*8(a-h,o-z)
      dimension y(n),f(n)
      real abohr,xmin,gamma2,gamma,vo,b
      common /coeff/ abohr,xmin,gamma2,gamma,vo,b

      gx = t*abohr-xmin
      vx = vo*((1-exp(-b*(gx)))**2-1.d0)
      v = vx/vo
      diff = e-v
      dkx2 = gamma2*diff

      f(1)= y(2)
      f(2)= -dkx2*y(1)

      return
      end

!    Here the ODE is calculated - Shooting for the given parameters
!-------------------------------------------------------------------
      subroutine rgkuta(e,to,y,n,h)
      implicit real*8(a-h,o-z)
      dimension ynew(100),y(100),f(100),g(100)

      call funcvec(e,to,y,f,n)

      do i=1,n
        g(i)=y(i)+0.5d0*h*f(i)
       ynew(i)=y(i)+h*f(i)/6.0d0
      end do

      th=to+0.5d0*h

      call funcvec(e,th,g,f,n)

      do i=1,n
       g(i)=y(i)+0.5d0*h*f(i)
       ynew(i)=ynew(i)+h*f(i)/3.0d0
      end do

      call funcvec(e,th,g,f,n)

      do i=1,n
       g(i)=y(i)+h*f(i)
```

```
 ynew(i)=ynew(i)+h*f(i)/3.0d0
end do

t1=to+h

call funcvec(e,t1,g,f,n)

do i=1,n
 y(i)=ynew(i)+h*f(i)/6.0d0
end do

return
end
```