

Novel Bionanostructures

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5.1 Definition

For the purpose of this report bionanostructures are defined as engineered nanoscale structures made from biological material or for biological purposes.

5.2 Short Description

Self assembling molecules which form novel nanostructures have already found applications in different areas ranging from drug delivery to cosmetics. Lipid-based nanostructures like liposomes and nanosomes have been used in the cosmetic industry for the last two decades. Proteins e.g. albumin have been modified to create nanoparticles for applications in cancer therapeutics. While the applications of self assembling molecules are slowly reaching the market, fundamental research on the self assembly of molecules to understand the complex cellular processes as well as next generation electric circuits are gaining pace. Synthetic cells which replicate the cellular functions enable to understand the biological processes taking place have implications in drug discovery. Liposomes, polymers and nanoemulsions have been used to create synthetic cells.

In addition, novel fabrication techniques along with nanomaterials have been used to create synthetic molecules by a bottom-up approach. To overcome the constraints in IC manufacturing as explained by Moore's law, the efforts are now on developing molecular switches. Chemically self assembling molecules which can be switched on and off have been proposed as molecular switches. Carbon nanotubes and nanowires have been used to link together different molecules in logic circuits. Complex molecules like catenanes and rotaxanes have been used to create molecular motors. DNA which can be programmed to self assemble, has been used to develop biosensors. RNA has also been used to produce devices which can be organised to detect molecules and perform drug delivery. They have also been used to build logic gates (AND, NOR, NAND, or OR gates) as well as signal filters.

DNA motors which can transport cargo (like natural molecular motors myosin and kinesin) have been developed. Different types of powering strategies have been developed for molecular motors. Models have been developed for DNA nanomotors powered by energy from DNA and RNA hydrolysis, ATP hydrolysis and DNA hybridisation. The report looks into the development of these novel bionanostructures and its manipulation for different applications.

5.3 State of R&D

5.3.1 Synthetic Cells

Cells are very complex structures. Understanding the complexity in cellular operations has been a challenge since the concept of cell was incorporated and still is the case. Significant advances have been made in this respect due to novel tools and techniques which have enabled scientists to look inside the cell with much more precision and to manipulate the cytoplasmic molecules to learn how one impacts the other. However explaining this is very complex as these molecules are heterogeneous in nature. Different factors affect the dynamic nature of molecules in the cells and understanding their behaviour to external stimuli as well as other factors will provide valuable information to prevent diseases as well as discover new drugs. Several proposals have been made to mimic the cellular structure and to create a model which is capable of carrying out normal cell operations including self replication. The concept of artificial cells or synthetic cells has received an additional momentum due to the tools and techniques provided by nanotechnology. It is essentially assembling synthetic materials to create a cell which can function similar to a normal cell. The design of such a structure benefits from the tools which are available due to nanotechnology. These tools will help the scientists to work at the biological level (nanoscale) and help them to create cells parts by parts using a bottom up methodology. However, the difficulty lies in the fact that cells are capable of carrying out thousands of functions at a time with high efficiency which may never be matched by an artificial cell.

Several approaches to create containers that can incorporate fluids and molecules as in a natural cell have been proposed. The advantage of such as small container is that small volumes help to understand the molecular reaction systems and self organization at the cellular scale with high efficiency¹. It also avoids the requirement for mixing and the small number of molecules in the artificial system enables the study of reactions involving single molecules². These containers have a small internal volume and may be used for single cell analysis, high throughput screening, protein synthesis or single molecule enzymology³.

5.3.1.1 Liposomes

Phospholipids have been used to create a variety of nanostructures called liposomes, niosomes, nanosomes, cubosomes, solid lipid nanoparticles, nanostructured lipid particles, etc. These structures have already found applications in drug delivery and some of them have been commercialised for use in the cosmetic industry to carry cosmetic ingredients. More information about lipid based nanobiostructures and their applications can be found in the Therapeutics and Cosmetics subsector reports.

Enzymes have been encapsulating inside liposomes⁴ and have subsequently been used in diagnostics, metabolising toxic reagents and as catalysts⁵. Successful reactions have been performed inside liposomes to produce proteins. Expression of green fluorescent protein (GFP) was used as an indicator in these reactions to verify that proteins have been produced⁶.

5.3.1.2 Polymers

Polymers have found applications in drug delivery as polymer nanoparticles to carry drugs and to create conjugates to target certain molecules, and can also be used to create synthetic cells. The advantage of using polymers is that it is possible to change the characteristics and properties of these polymers to create vesicles of choice. In addition, polymers are easy to scale up, provide high biocompatibility and increase the stability of volatile materials that are encapsulated. Synthetic polymers provide much more stability to the structure compared to the amphiphilic molecules when used to create synthetic cells. The polymers can also be manipulated to self assemble to form novel structures. These polymers are also called polymersomes. Polymer-protein hybrid membranes that can act as a selective filter have been developed. Such hybrids can incorporate proteins even though the membrane is much thicker than lipid based membranes⁷.

Recently, energy-transducing proteins bacteriorhodopsins (BR) from *Halobacterium halobium* and cytochrome c oxidase (COX) from *Rhodobacter sphaeroides* have been used to create hybrid polymers⁸. Light-driven transmembrane pH gradients and pH gradient-induced electron release were observed in these polymers which created μA level currents without voltage. The technique has potential applications in high power density devices. More information about polymers and their applications are detailed in the Therapeutics and Regenerative Medicine subsector reports. Polymers have also been used to study the internal movement of molecules inside the cell. A polyethylene glycol (PEG) and dextran system was used to study how molecules behave inside the cell⁹. The polymers were mixed to form an aqueous two-phase polymer system (ATPS) inside a test tube coated with a dry lipid film. The lipid film got wet in the process and developed an elliptical two molecule-thick cell vesicle containing ATPS. When heat was applied the PEG and dextran separated to form two discrete phases. Dextran, which is heavier than PEG, sank to the bottom of the test tube while PEG went to the top. PEG collected on the inside perimeter of the vesicle wall and dextran collected at the centre forming a phase-separated microcompartment. Different responses can be studied by varying heat and osmotic pressure of the solution.

5.3.1.3 Nanoemulsions

Nanoemulsions are dispersions of nanoscale droplets of one liquid within another¹⁰. Nanoemulsions have a number of advantages over emulsions of larger particle size. Unlike microemulsions, nanoemulsions are kinetically stable due to their small droplet size¹¹.

The simple methods used in creating water-in-oil (W/O) emulsions can be used to create water droplets in the femto litre volume range. More sophisticated methods like ultrasonication have also been used to create droplets of smaller volumes. Droplets of the order of 10^{10} /ml can be created. Another advantage is the possibility of testing an entire DNA sequence or a single strand using nanoemulsions.

W/O emulsions have been used for high throughput screening of enzymes¹². The process, known as *in vitro* compartmentalisation, creates droplets which each contain a single gene. These droplets can act as single cells, facilitating the processes of transcription and translation. The oil phase is inert and prevents the diffusion of genes and proteins between different droplets that act as compartments. The advantage of such a system is that it enables the selection of enzymes based on the phenotype or the product due to the genotype activity. The high number of compartments created enables analysis of a larger number of gene libraries quickly and simultaneously.

Recently, a double emulsion IVC system was developed to sort single genes. Fluorescent markers were used and later sorted with FACS (fluorescence activated cell sorter). In this process w/o emulsions containing single genes were created with one gene per droplet. They were then transcribed and translated using compartmentalisation. The w/o emulsion is then converted into w/o/w emulsion, and the proteins with enzymatic activity change the non-fluorescent substrates into fluorescent products. FACS sorting isolated the genes that encoded active enzymes. These recovered genes can be used for further levels of selection process if required. Studies have shown that creating double emulsions does not disrupt the contents encapsulated inside the emulsions.

Current efforts are focussed on creating a system where the exchange of reagents between compartments becomes possible. Other applications of nanoemulsions are discussed in the Cosmetics and Therapeutics subsector reports.

5.3.1.4 Novel Fabrication techniques and Nanomaterials

The use of synthetic nanomaterials combined with novel fabrication technologies has been explored to create cell like structures. A variety of fabrication techniques have been successfully used to create synthetic membranes. These include surface micromachining, laser interference lithography, nanoimprint lithography, track etching, backside etching and phase separation. The advantage of these fabrication techniques is that by changing the parameters, it is possible to control the characteristics of the cells formed. By changing the thickness of the membrane, internal volume, catalyst used, crystallographic properties etc. it is possible to create a structure that closely resembles natural cells with membranes that can communicate each other. Another advantage is that these cells offer more robustness and rigidity compared to other types of synthetic cells. It can also store the contents for a long time in comparison with cells formed using phospholipids.

Carbon nanofibres (CNF) have been used to create synthetic cells that are able to transfer their contents between each other¹³. Vertically aligned carbon nanofibres (VACNF) grown using plasma-enhanced chemical vapour deposition (PECVD) were used in combination with silicon micromachining techniques to create synthetic membranes. Microfluidic channels were created using photolithography and etching. The catalyst was lifted off and CNF forests were grown on the surface of the substrate forming a complex interconnected cell mesh. The formed cells were then filled with molecules of interest and sealed with a transparent lid to create a synthetic cell completely isolated from the external environment. The nanosized pores created on the membrane supported the diffusion of fluids between cells thus enabling cell-to-cell communications. A similar approach was used by Fletcher *et al.*¹⁴.

However, controlling the pore size and membrane properties has been challenging. Coating the surface of VACNF with SiO₂ was found to be a better way of controlling the membrane characteristics.

Coating polymers on the surface of nanomaterials has also been explored to create active membranes and bionanostructures. Electrically conductive polymers such as polypyrrole (PPy) have been used to control the membrane properties of nanofibre based structures¹⁵. The polymer coatings were deposited on the sidewalls of nanofibres using electropolymerization. The ability to control the polymer characteristics helped to modify the properties of nanofibres and the structures. Carbon nanofibres have also been used as an interface in neural engineering and carbon nanotube fibres can promote neuronal cell growth¹⁶. The 'hair like' conductive wires incorporate the properties of electrodes, permeable microfluidic conduits and the porosity of the CNTs and were found to promote cell growth, migration and proliferation. CNFs have also been used for axonal regeneration by forming a novel nanofibre network which mimics the natural extracellular matrix to promote cell growth, adhesion and proliferation. The main advantage of these fibres are that they are immunologically inert thereby reducing the chances of rejection.

More information about the use of carbon nanofibres can be found in the subsector reports Regenerative Medicine and Implants, Surgery and Coatings.

5.3.2 Other Self Assembled Nanostructures

5.3.2.1 Albumin, chitosan, lecithin

5.3.2.2 Niosomes

Niosomes are non-ionic surfactant based vesicles that have a similar structure to that of phospholipid vesicles like liposomes and can be used to encapsulate aqueous solutes and act as drug carriers. They are formed by the self assembly of non-ionic amphiphiles in aqueous media. The application of heat or physical agitation helps the process to attain a closed bilayer structure¹⁷. The hydrophobic parts are shielded from the aqueous solvent while the hydrophilic head groups are in contact with it. They are used as anti-inflammatory agents¹⁸ as well as anti-infective agents¹⁹. They have also been used to enhance transdermal drug delivery. Recently, Paolino *et al.*²⁰ reported the development of a new type of niosome made of α,ω -hexadecyl-bis-(1-aza-18-crown-6) (Bola C16), Span 80® and cholesterol (2:5:2 molar ratio) called Bola-niosomes for percutaneous drug delivery applications. Studies have shown that these niosomes improves percutaneous passage of drugs through human stratum corneum and epidermis and are non toxic. This property has been exploited by the cosmetic industry. The first cosmetic products containing niosomes were developed and marketed by L'Oreal (www.loreal.com) in 1975. They hold the patent for the process of preparing compositions containing niosomes and a water-soluble polyamide for cosmetic and pharmaceutical applications²¹. The product also had its successors like 'Niosome Plus' anti-ageing cream by Lancome (www.lancome.com) which reached the market in the early 1990s.

5.3.2.3 Solid Lipid Nanoparticles

Solid lipid nanoparticles (SLNs) are particles of nanometre dimensions with a solid lipid matrix. They are oily droplets made from lipids which are solid at room temperature and stabilised by surfactants. Their production is a simple process where the liquid lipid (oil) in an emulsion is exchanged by solid lipids, i.e. lipids that are solid at body temperature²². The advantage of SLNs is that there is no need for organic solvents in the preparation, they provide protection from water and can be used for controlled drug release. Two different methods are generally used to prepare SLNs. First, the hot homogenization process in which melted lipids at high temperatures are dispersed in hot surfactant solutions. The second method is called cold homogenisation in which the lipid melt is cooled and dispersed in cold surfactant solution. When these suspensions are subjected to high-pressure, microparticles are broken down into SLNs²³. These nanoparticles have been used as colloidal carriers to deliver paclitaxel²⁴. Due to their occlusive properties, SLNs are considered as ideal for use in day creams. The compound coenzyme Q10 has been incorporated into an SLN for cosmetic applications²⁵.

5.3.2.4 Nanostructured Lipid Carriers

In order to overcome the drug loading inefficiency associated with SLNs, a second generation of lipid particles have been developed by mixing solid lipids with liquid lipids. They are called nanostructured lipid carriers (NLC). Different methods have been proposed for creating NLCs. The first is by mixing solid lipids with small amounts of oil (liquid lipids) to improve the drug loading capacity. These are called imperfect type NLCs. When mixed with large amount of oil, the lipid was found to solubilise certain drugs which were not soluble otherwise. They are called multiple type NLCs. A third type called amorphous NLC was created to reduce drug expulsion by mixing special lipids like hydroxyoctacosanylhydroxystearate and isopropylmyristate. Compared to SLNs, NLCs have a distorted structure which makes the matrix structure imperfect creating spaces to accommodate active compounds. The high loading capacity and long term stability offered by the NLCs makes it a superior structure in many of the cosmetic applications. The first product utilising the lipid nanoparticle characteristics reached the market in 2005. The products called NanoRepair Q 10 Cream and NanoRepair Q 10 Serum are marketed by the German company Dr. Rimpler (<http://www.rimpler.de/intro/>).

5.3.2.5 Cubosomes

Cubosomes are discrete, sub-micron, nanostructured particles of bicontinuous cubic liquid crystalline phase²⁶. Bicontinuous cubic liquid crystalline phase is an optically clear, very viscous material that has a unique structure at the nanometre scale²⁷. They are formed by the self assembly of liquid crystalline particles of certain surfactants when mixed with water and a microstructure in a certain ratio. What makes cubosomes unique compared to the parent surfactants is that they offer a large surface area and lower viscosity. The relative insolubility of cubic phase-forming lipid in water allows them to exist at almost any dilution level making them an attractive structure to incorporate into many formulations. Produced by high-energy dispersion of bulk cubic phase, they have high heat stability and are capable of loading hydrophilic and hydrophobic molecules²⁸. Combined with the low cost of raw materials required, they are an attractive choice for cosmetic applications. The technology has been widely investigated for applications in the cosmetic industry. For more information see the Cosmetics subsector report.

5.3.2.6 DNA Nanocages

DNA nanocages are novel structures formed by self assembly of DNA molecules under certain conditions. The structures are hollow inside and can be used to encapsulate nanoparticles and other protein drugs which can be released at a target site using external stimuli. Typical cages sizes range from 2 nm to 200 nm. Although it is possible to change the size depending on the type of applications required, it is preferred to keep the diameter in the range of 1 to 50 nm and the length from 100 nm to 10µm to keep the nanolevel advantages. Kazunori *et al.*²⁹ have developed a process to produce DNA nanocages in a single step. The cages are formed by the self assembly of three types of two dimensional oligonucleotides by hybridisation. The advantage is that the process doesn't consume energy and the structure, which has tridirectionally branched DNAs with self-complementary chains, can be obtained by the simple process of mixing oligonucleotides. Highly symmetrical cages (spherical) are obtained if the lengths of the nucleotides are same. However, different shapes (tube, egg etc.) can be obtained by changing parameters such as length of the assembling components, concentration of the DNA etc.

DNA nanocages offer potential as drug carriers for pharmaceutical applications. The advisable size of the nanoparticle for incorporation into the hollow space of the nanocages is between 2 nm to 200 nm. There is a high probability of particle leakage if the particles are smaller than 2 nm. Different types of particles and molecules can be incorporated into the nanocages, including metal nanoparticles, semiconductive nanoparticles, photocatalytic nanoparticles, magnetic nanoparticles, biological molecules like viruses, proteins, peptides, polysaccharides and other DNA molecules. The desirability of the cages in delivery applications is increased by their ability to bind cell-targeting molecules onto the surface in addition to the slow release capability. The drug release can be controlled by making it to respond to changes in conditions like temperature. It also offers potential in DNA molecular recognition.

5.3.3 Molecular Switches and Molecular Motors

Self assembly of individual molecules to form complex structures is a common phenomenon that takes place in biological systems. The individual molecules self assemble under certain conditions to form higher order structures to carry out various biological operations. The technique has been adopted by researchers to create novel nanobiostructures to create models that mimic the living environment.

Similar principles have been adopted to create molecular switches and motors by altering the conditions and environment. Molecular switches that switch reversibly between two different positions and synthetic molecular motors which functions in a similar manner by utilising the energy from ATP have been developed. Though at the very early stage of development, these novel structures have significance in areas like electronics, drug delivery, drug discovery and drug design. Researchers are already aware that Moore's law won't be applicable after a certain period in the future and there won't be any room at the bottom to incorporate more transistors. In the current environment it is possible to produce integrated circuits with transistors of resolution in the range of 100 nm³⁰. The peak in the number of transistors implies that new methods are required to continue the process of miniaturisation. One of the possibilities lies in manipulating molecules to create novel devices and switches.

The method of 'bottom-up' manufacturing is considered as the best possible approach to create molecular devices from individual molecular components. These molecular components can be controlled and manipulated to create logic gates and circuits which are able to communicate to each other and with the external environment³¹. Unlike a switch which returns to its original state after the switching process, a molecular motor is a device which can do work repeatedly and progressively on a system³². So developing a molecular motor is a much more complex procedure. These devices should also be controllable, reversible and readable at the molecular level.

5.3.3.1 Self Assembling Molecular Switches

A molecular switch has been developed which has an electrically adjustable tunnel junction between the two connecting wires³³. The device operates using the oxidation and reduction of the molecules sandwiched between the wires. The redox reaction of the molecules affects the tunnelling height of the two wires and thereby controls the rate of charge flow through the junctions. The tunnelling resistance between the two wires depends on the chemical state of the molecular switches. Monolayers of molecular switches are connected by nanowires at each of the junctions of the grid. However, one of the major problems with this device was that the switch was irreversible and repeated logic operations were impossible. Another issue was that the wires used in the switches were made using conventional lithographic process, limiting the ability to shrink the devices.

To overcome this problem, CNTs and nanowires were used as links between different molecules in logic circuits. Nanowires of carbon or silicon can also chemically self-assemble like the molecules used in these switches, and a similar device was synthesised using these³⁴. Rotaxane was used as the molecular monolayer and by applying -2V the switch was closed and a current flow was observed. However, when the voltage was raised to +.7V the switch opened due to oxidation of the monolayer molecules. Repeated switching was observed in these molecular switches and the system was configured to create logic gates.

Molecular devices that can display hysteretic switching between two metastable states can also act as memory cells³⁵. As well as redox activity, light has been used as a stimulus to reverse the states of switches. Dithienylethene derivatives have been widely explored as photoswitches. The switching behaviour of self assembled molecules of dithienylethene on gold has been investigated using STM³⁶. Light of a certain wavelength was used to induce a change in the molecule that resulted in a lengthening of -0.1 nm. The two different lengths were observed by STM. The switching behaviour was found to depend on the nature, length and position of the spacer linking switching unit to the anchor group. These differences can be explored to create a unidirectional or bidirectional switching.

A detailed review of integrating molecular switches into electronic circuits is available by Mayor *et al.*³⁷.

A molecular switch that can change states without a change in geometry has been developed. The molecule used is naphthalocyanine, which has two opposing hydrogen atoms which can flip in opposite directions without a change in the geometry of the structure. When the voltage is low, the atoms won't switch and the state of the molecule can be read. By changing the applied voltage, flip of hydrogen atoms (tautomerisation) can be induced³⁸. This flip can be measured using STM as a change in conductivity of the molecules. It has also been demonstrated that a charge injection in one molecule induces tautomerization in an adjacent molecule. However, the system currently works only at very low temperatures (5K) and requires vacuum preventing its practical use.

Rotaxanes and catenanes are examples of interlocked molecules. They consist of two or more component molecules that are not covalently bonded, but are intrinsically linked, through a mechanical bond. Catenanes consist of two or more interlocked rings. Rotaxanes have a central rod and rings are trapped on this rod by bulky stoppers at the ends. Once complicated to synthesise, they are much more readily accessible through modern templating strategies.

These structures have been used for a number of applications, including molecular switches, molecular motors and nanovalves. The Stoddart group have advanced this field significantly since their first published rotaxane 'molecular shuttle' in 1991³⁹. They have used rotaxanes as molecular switches, moving a ring from one position ('ON') to another ('OFF') using a number of control methods, including pH, electrical and chemical control⁴⁰. Recently one of their rotaxanes has been used to construct a 160,000-bit molecular electronic memory circuit with a density of 10^{11} bits per square centimetre, using the rotaxane as the data storage element⁴¹. Rotaxanes attached to mesoporous silica nanoparticles to form nanovalves that can be opened with the appropriate stimulus have been reported⁴². They envisage trapping drug molecules within the pores of the silica and only allowing release under appropriate conditions, for controlled drug delivery.

Leigh and co-workers have built upon the use of catenanes and rotaxanes to create molecular machines⁴³. One of their rotaxanes works by attaching and removing silyl groups. The presence of silyl ether makes the systems unbalanced and the removal of the group makes the system balanced. The process, called linking, 'switches on' the exchange of the macrocycle between the destinations. The linking process moves the macrocycle into the destination. This removal restores the balance by a biased Brownian motion to a new equilibrium state. When the silyl group is attached again (unlinking), the system moves towards an unbalanced state again. The operational cycle is continued to move the macrocycles. Using this method, they have moved 56% of the macrocycle to its destination. The operation is irreversible and the state of machine does not determine the position of the substrate. They have also developed a catenane-based reversible synthetic rotary molecular motor that moves unidirectionally between four positions in a ring, controlled by hydrogen bonding⁴⁴.

5.3.3.2 DNA Switches

The simplicity of the structure and the interactions that are allowed by DNA has enabled researchers to control the assembly of these structures. Although RNA and proteins are more suitable to create molecular devices due to their structural versatility, the ease of controlling and manipulating DNA has made it an attractive choice to create synthetic devices. This control over the structure has been exploited to create self assembled molecular motors, molecular switches, electronic circuits and enzyme networks⁴⁵.

Some of the simplest devices created by manipulating DNA are molecular switches. Switching has been obtained by changing external conditions like pH, temperature, ionisation, by inducing chemical reactions, or through the binding of signalling molecules. DNA switches have been made using the i-motif, a three dimensional DNA structure formed by the folding of single strands with correctly spaced cytosine bases. This structure can be closed and opened, effectively turning on and off, by changing the pH⁴⁶.

Addition of DNA or RNA control strands can induce conformational changes in DNA for use as switches. This use of RNA is significant as it demonstrates that such molecular devices can be controlled by transcriptional circuits⁴⁷. Some DNA nanomachines have been placed into 2D crystalline DNA arrays while retaining full functionality⁴⁸.

Self-assembled DNA has been used to develop biosensors. Typically, a gene chip works by incorporating single stranded DNAs that search for its complementary fragments. The transduction process is performed by conventional electrochemical or optical methods.

However, a DNA biosensor which can also perform the function of a transducer has been reported⁴⁹. The principle is based on hybridization chain reactions where DNA molecules self assemble to form new structures when exposed to a target DNA fragment. When hybridised by a target, a DNA hairpin loop breaks to form a nicked double-helix. This change in conformation can be utilised for biosensing applications. An inverse relationship between the concentration of initiator and the average molecular weight of the resulting structures was seen and may allow for quantitative biosensing. Incorporation of aptamers into the DNA hairpins has been proposed to sense more complex biomolecules like protein, ATP or other smaller molecules.

Ribozymes, catalytic RNA molecules, have been manipulated by control strands to produce logic circuits for the detection of oligonucleotides⁵⁰. Changes can be configured into the system to create multilayered operational circuits.

An RNA device which can be used in drug delivery or diagnostic applications has been reported⁵¹. The programmable nature of RNA has been used to produce a device which can be manipulated to detect thousands of molecules simultaneously and perform cellular information processing operations. The three main components of the device - sensor, actuator, and transmitter - are made of RNA. When the input sensors detect a target, the transmitter is activated and triggers the actuator ribozyme molecules. RNA devices have been used to detect the drugs tetracycline and theophylline within yeast cells⁵².

5.3.3.3 DNA Nanomotors

Biological molecular motors, like myosin and kinesin, use the energy from the hydrolysis of ATP to initiate mechanical movements. These motors are essential molecular machines for movement in living organisms. Synthetic molecular motors which derive their operating concept from natural motors have been designed in a similar way using ATP as the energy source. Additionally, studies have been conducted on motors which use energy from the hydrolysis of the DNA backbone and DNA hybridisation. Innovations in synthetic chemistry and genetic engineering enabled by nanotechnology are creating opportunities for scientists to create new motors which can complete a fully cycle of motion without external intervention. Although in the laboratory stage, some of the advances in the area are promising.

Two models of molecular motors powered by DNA and RNA hydrolysis have been developed^{53,54}. In one model the cargo strand is hybridised into an anchorage. The track for the movement of the motor is made of identical single stranded anchorages attached to the double stranded backbone. Once it is hybridised, the enzymes contained in the cargo cut the anchorage, releasing a small fragment. This allows the cargo to stick to the next anchorage. The cargo can be then transferred fully to the next anchorage by a branch migration reaction. The cycle is repeated to complete the operation. In a second system a similar track was used to carry out the movement of the device. However, instead of the '10-23' catalytic enzyme used in the first motor, a recognition site in the cargo-anchorage duplex for a restriction enzyme was present. Unidirectional motion was obtained by the destruction of the track once the motor has moved on. However, this reduces the potential use of these devices. The probability of the cargo moving out of the track can be reduced if the interaction between the cargo and anchorages are strong.

ATP hydrolysis has also been used to move DNA nanomotors and has also been developed to move cargo. Compared with other systems the probability of cargo moving out of the track is minimal as there is covalent anchorage rather than hybridisation. The track is made of four double stranded anchorages tied together by single strands with a double stranded DNA backbone. The cargo has two short fragments of DNA that are attached covalently to one set of anchorages. The second sets of anchorages have sticky ends which are complementary to that of the free ends of the cargo. The gap between the two sets of anchorages is closed by enzymatic ligation and the base pairs are matched by a restriction enzyme. Subsequently, the bonding is cleaved, the cargo is transferred to the second set of anchorages through a covalent attachment and the cycle continues. However, it has drawbacks. The simultaneous use of three enzymes makes the process complicated and backward motion is not possible.

DNA hybridisation has also been proposed as a suitable method to fuel molecular motors. The advantage is that the reaction can be controlled by manipulating the base pairs and concentrations of the control strands. Turberfield *et al.*⁵⁵ have developed a hairpin loop design to fuel DNA motors. The DNA hairpin has a single stranded loop with a double stranded neck which can hybridise with a complementary hairpin to release free energy. However, the process is hindered due to the neck-like structure of the hairpins. At least one neck needs to be opened to allow hybridisation to occur. For this purpose, a short strand is introduced as a catalyst which opens one of the loops. This opened loop can then hybridise with another loop releasing the introduced short strand catalyst. The reaction continues and a cascade of hairpin-hairpin reactions are followed⁵⁶. Although there are currently no motors that successfully use this as an energy source, a model of one has been proposed⁵⁷.

5.4 Additional Demand for Research

Although there have been significant advances in the development of synthetic cells and molecular motors, the field is still in its infancy. The current level of research deals with the development of fundamental units of molecular motors and switches. However, research into molecular motors is important as they are central to biological functions. Understanding how these motors function and the creation of controlled molecular motors will improve our knowledge of biological functions and aid in the design of functional materials accordingly. The following list provides an overview of some of the research requirements for this sector.

- DNA machines have shown promise in detecting biomolecules with potential applications in drug delivery. Further research is required to develop this potential for use in cells.
- Research on sources of energy for synthetic molecular machines to function is required, particularly natural molecular motors utilising energy from e.g. ATP.
- Additional research is required to make multidirectional movement molecular motors.
- Multidisciplinary research teams are required to design and assemble molecular devices for electronic applications.
- Molecular devices are currently proof of principle. Further research is required to go beyond this level to create integrated molecular devices which can function in the real world.
- Additional research is required to create suitable interfaces between molecules and electrodes in molecular electronic circuits.
- Another key issue is the development of suitable models for scale up and manufacturing of integrated molecular devices.
- Further study on nanomaterials in the design of synthetic membranes is required e.g. to control their self assembling properties and to enhance their integration with natural biomolecules.

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