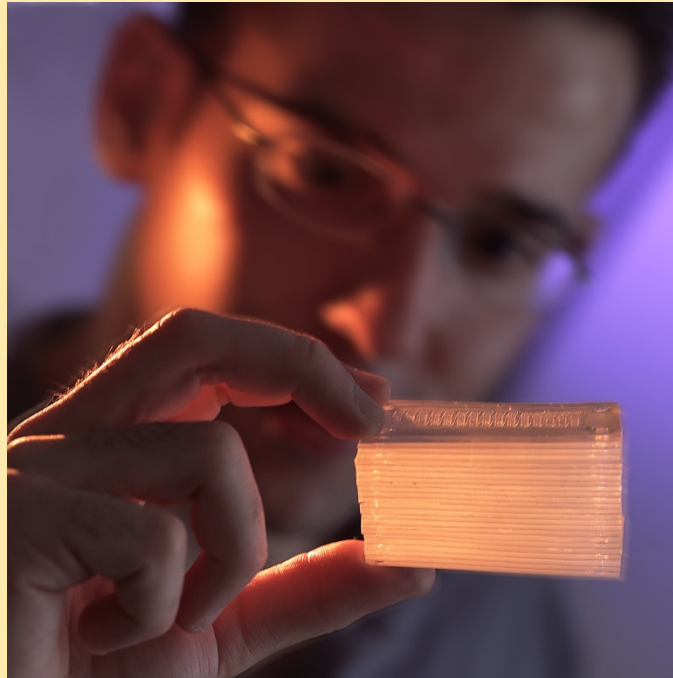


BIOMOLECULAR APPLICATIONS FOR BIOMEMS



A future artificial kidney
based on MEMS

Technology

*[Courtesy Center for Integration of Medicine and
Innovative Technology]*

Unit Overview

BioMEMS = Bio MicroElectroMechanical Systems

This unit discusses the characteristics and phenomena of biomolecules that make them attractive components for bioMEMS devices.

It provides information that help you understand how biological molecules can be used as working devices within bioMEMS.

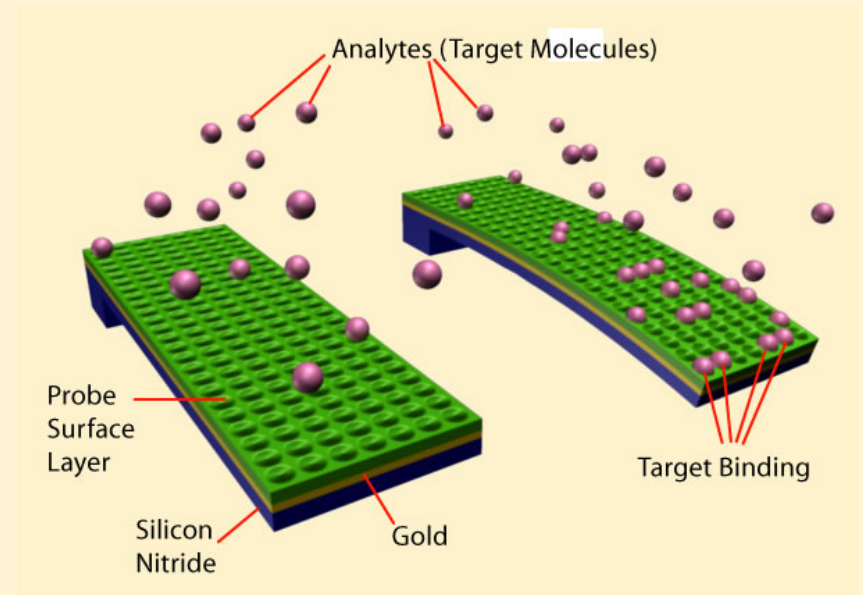
Objectives

- ❖ State the types of biological structures that are useful in bioMEMS.
- ❖ Describe at least three types of functions that biological structures can provide in bioMEMS design.
- ❖ State at least two size dimensions of biological structures used in bioMEMS.

Introduction

As MEMS devices become smaller, the use of biomolecules as a MEMS component becomes more attractive.

- ❖ Biomolecules self-assemble into predictable and precise structures in the nano range.
- ❖ Their functions in living organisms can be harnessed to perform the same functions in a bioMEMS device.



A MEMS cantilever coated with a monolayer of biological molecules can bind with and capture other molecules of complementary shapes

What You Will Learn

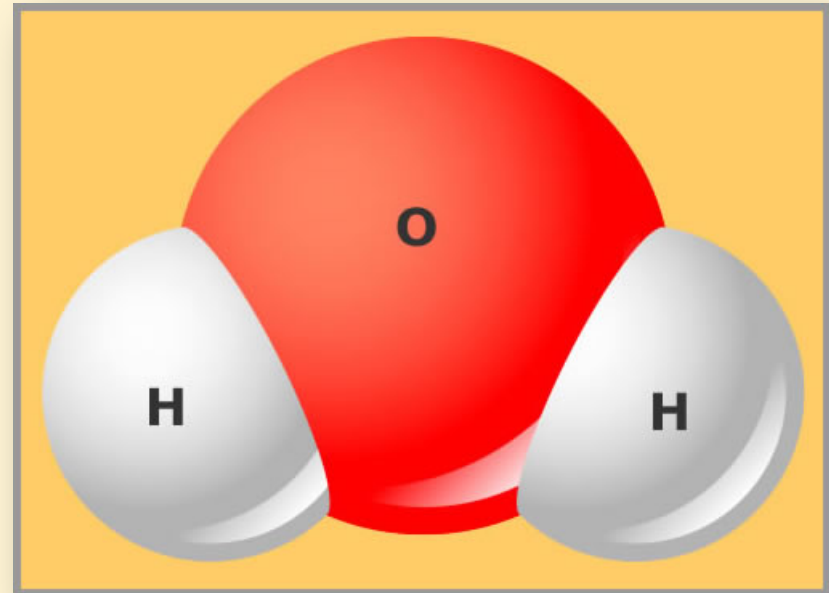
To understand how biomolecules can be used in bioMEMS, it is useful to learn about the types of biomolecules and how they associate with each other in functional units.

Following is an overview about the properties of biological molecules that make them functional in bioMEMS.

What is a Molecule?

- ❖ A molecule is the smallest form of a substance that contains all the properties of that substance.
- ❖ A molecule is made from atoms.

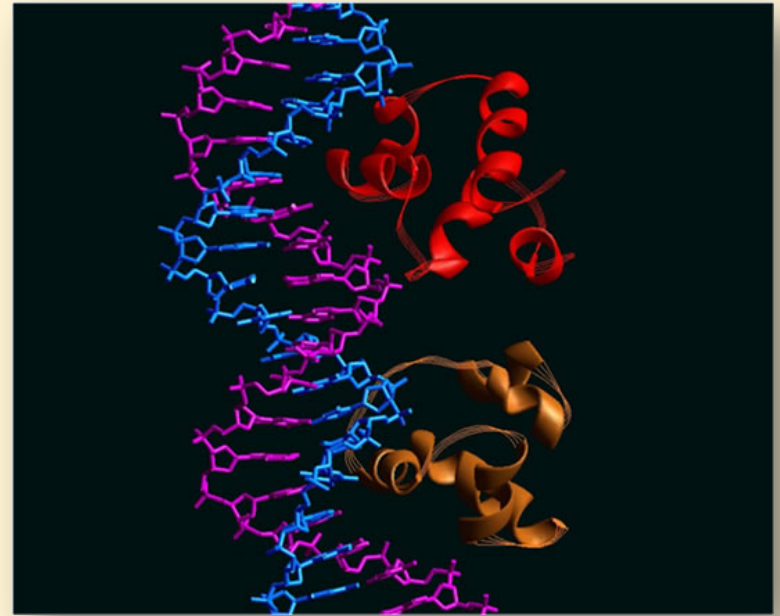
Example: An H_2O molecule is the smallest form of water. You break it up and you have two atoms of hydrogen and one atom of oxygen.



Water Molecule

What are Biomolecules?

- ❖ All known forms of life are comprised solely of biomolecules.
- ❖ Biomolecules are formed by the self-assembly of atoms (primarily carbon and hydrogen).
- ❖ Once formed, they continue to self-assemble into larger structures (larger molecules, compounds, organelles and cells)



Biomolecules - DNA and Protein

[Image source: Swiss Institute of
Bioinformatics (SIB) gallery]

Types of Biomolecules

There are four types of biomolecules that are abundant in all cells:

- ❖ Carbohydrates
- ❖ Nucleic acids
- ❖ Proteins
- ❖ Lipids

Biomolecule	Types and functions of biomolecules
Carbohydrates	<p>Have both structural and energy storage abilities.</p> <p>Monosaccharides (glucose)</p> <p>Disaccharides (sucrose)</p> <p>Polysaccharides (cellulose)</p>
Nucleic Acids	<p>DNA (hereditary characteristics)</p> <p>RNA (Triggers the manufacture of specific proteins. Involved in the transmission of DNA's genetic information.)</p>
Proteins	<p>Enzymes (Accelerates cellular reactions)</p> <p>Collagen, keratin, and elastin (Support proteins)</p> <p>Hemoglobin (Transport proteins)</p> <p>Casein, ovalbumin (Nutrient proteins)</p> <p>Antibodies (Necessary for immunity)</p> <p>Hormones (Regulate metabolism)</p> <p>Actin and myosin (Perform mechanical functions such as muscle contraction)</p>
Lipids (fats and oils)	<p>Triglycerides (A form of stored energy)</p> <p>Phospholipids (Major components of the biological cell membrane. Able to limit the passage of water and other water-soluble compounds through the membrane.)</p>

Biomolecules in MEMS

- ❖ Biomolecules come in all sizes and shapes within the nano-size range.
- ❖ Biomolecules assemble themselves from solutions into structures.
- ❖ Their designs have evolved to perform quite specific tasks; tasks which can be applied to a bioMEMS function.
- ❖ Following are examples of some of these tasks and functions.

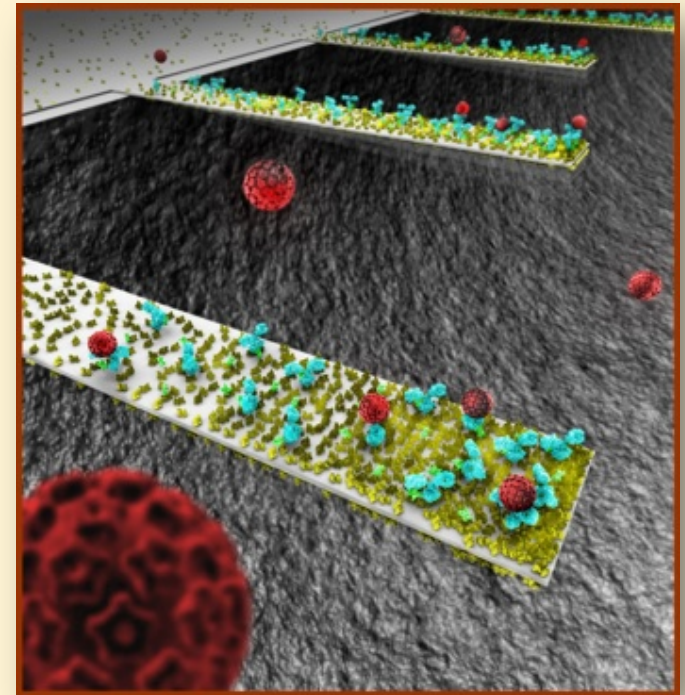
Tasks and Functions

Biomolecules can specifically recognize and bind to a particular molecule in a complex solution. (biosensor function).

Enzyme-catalyzed reactions (biosensor that detects the catalyzed reaction).

Enzyme-catalyzed reactions are used by living cells to generate energy from small molecules. (implantable devices to replace the need for batteries).

Proteins move particles within the cell, or move the cell itself (actuators).



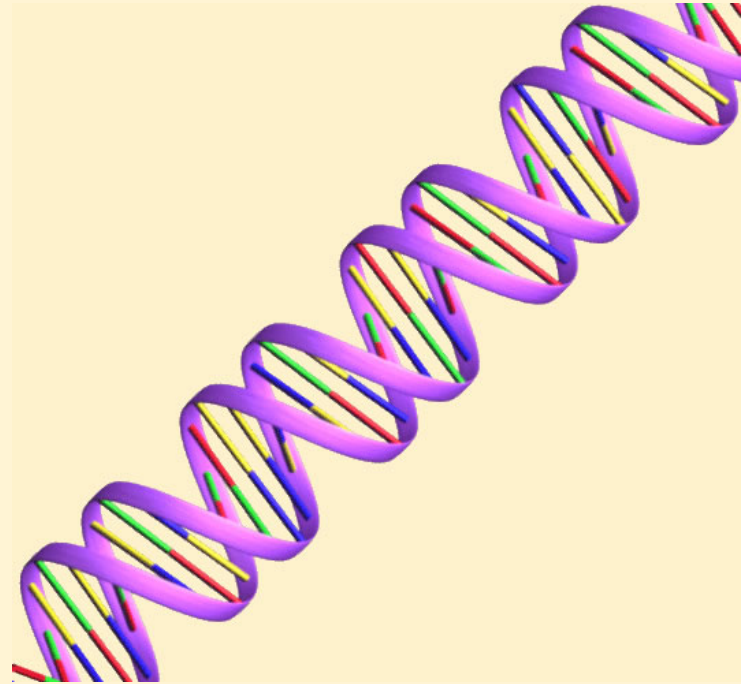
Nanocantilevers coated with antibodies that capture viruses (red spheres) [Image generated by and courtesy of Seyet, LLC]

Biomolecules for bioMEMS

Nucleic acids, such as DNA. (Used to carry genetic information).

Proteins, such as enzymes, fibers, molecular motors, channels and pores, vesicles. The "work horses" of the cell. They perform many of the jobs of cellular metabolism.

Lipids, such as phospholipid vesicles and membranes. Small molecules that self-assemble into very thin membranes in order to make separate compartments in the cell and provide a membrane barrier on the outside of all cells.



Double-stranded DNA molecule (a nucleic acid)

Let's Explore

- ❖ How nucleic acids, proteins, and lipids self-assemble into structures that have a functional application in bioMEMS devices.
- ❖ How some of the functions of biomolecules provide the specific recognition properties required in a biosensor-type application.
- ❖ Interesting applications of biomolecules based on their structure
- ❖ How some biological molecular motors move within the nanoscale.

Self-Assembly

A unique property of biological molecules is the ability to bind and assemble into a complex structure.

This self-assembly is like having a 3-dimensional jigsaw puzzle solves itself.

Self-assembly relies on the continuous motion of molecules in a solution and the resulting collisions. The molecules collide with each other until they encounter molecules to which they can bind.

Their unique shape and surface chemistry define how they assemble. This complex process is what takes place when you perform the simple task of blowing a bubble.

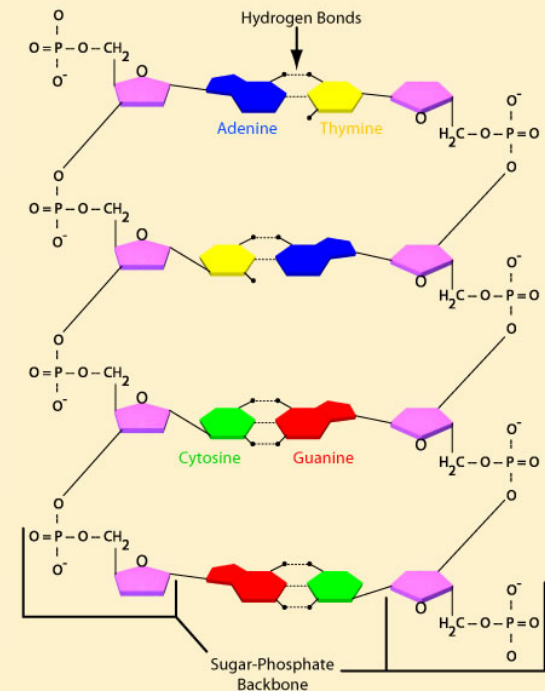
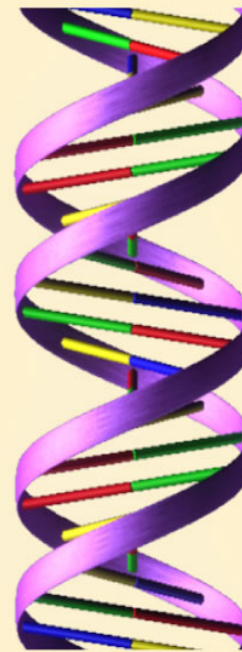


DNA Self-Assembly

DNA self-assembly is when two single strands of DNA assemble into a double helix.

This occurs if the nucleotide bases in each strand have complementary sequences where the bases pair off with the correct hydrogen bond between them.

The cumulative effect of large numbers of hydrogen bonds in a long DNA molecule stabilizes the double helical structure.



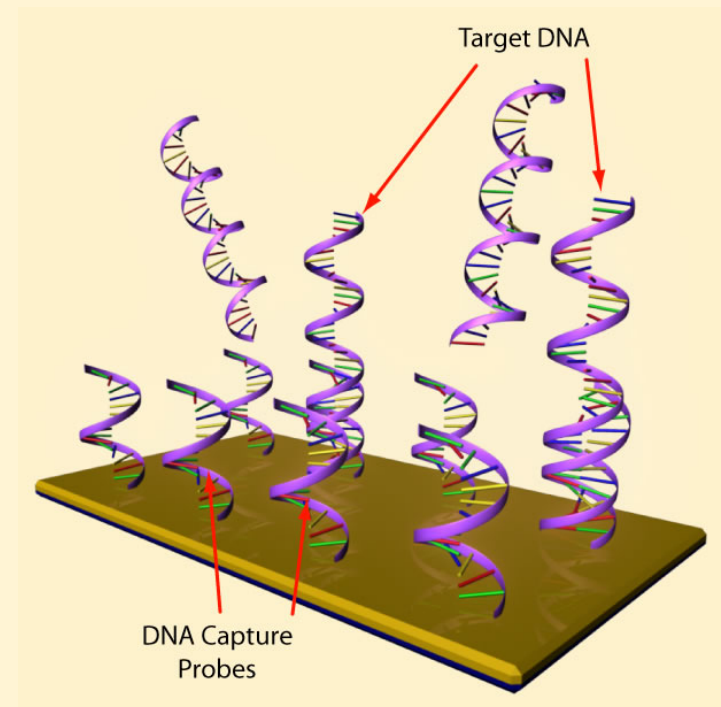
Double-stranded DNA

Application - Biosensor

In a biosensor that contains genetic information, a single strand of DNA can "capture" or detect its complementary strand of DNA (the "target"). This selective binding of a molecule is the basis of DNA microarrays, or gene chips.

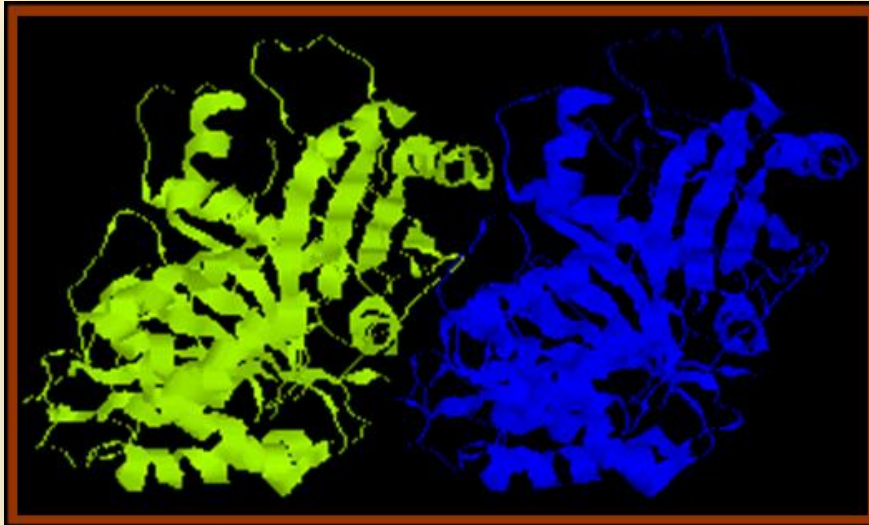
Applications of DNA microarrays in medicine include detection of

- ❖ infectious agents or genetic disease
- ❖ contamination in food and water.



A DNA molecule of a specific sequence ("target DNA") can be detected on a DNA microarray by binding to a single-stranded DNA strand ("probe") that has a sequence that is complementary to it.

Protein Self-Assembly



Quaternary Protein Structures

[Image Source: Research Collaboratory for Structural Bioinformatics Protein Data Bank]

Similar to DNA self-assembly, certain proteins will associate with other specific proteins that complement the shape and chemistry of their surfaces. This causes proteins to coordinate with each other in what is referred to as quaternary protein structures (see figure).

These protein structures assemble into superstructures using an instruction manual that has become internally "hardwired" through the evolution of cells and organisms.

Superstructures

Inside cell cytoplasm, newly assembled proteins are constantly moving, vibrating, rotating, and gliding in solution, bouncing off each other like dancers in a discotheque.

When a protein happens to bump into a "partner" protein with a complementary surface shape and chemistry, the two subunits "plug into" each other and continue their dancing motion as a functional pair. This continues until all the protein subunits have been found and assembled into the final functional structure.

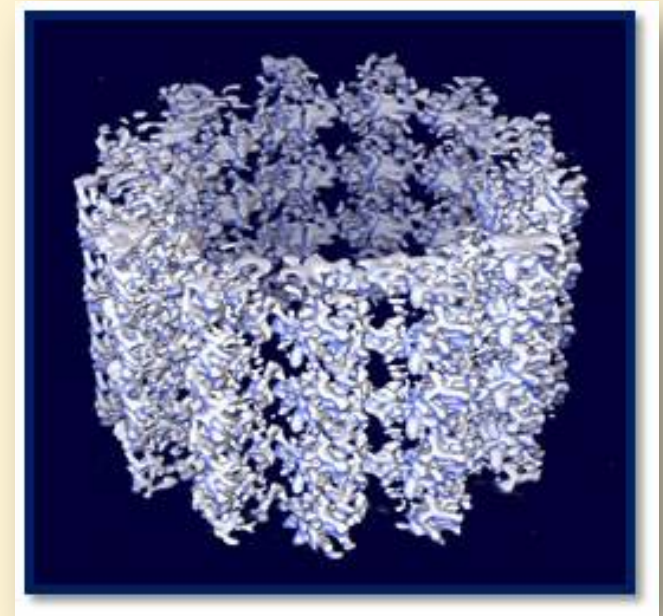
At that point, the work of self-assembly is finished.

Example of Protein Self-Assembly

This image shows a microtubule, a principal component of the cell's skeleton. This microtubule is formed by the self-assembly of tubulin dimers (a type of protein molecule). Each tubulin dimer binds to other tubulin dimers in order to "grow" this large spiral microtubule.

Thousands of tubulin dimers in a long microtubule is an example of a supramolecular structure.

This microtubule is a nanostructure, 20 - 30 nanometers in diameter.



Microtubule
[Image courtesy of Lawrence
Berkeley National Laboratory]

Applications of Self-Assembly

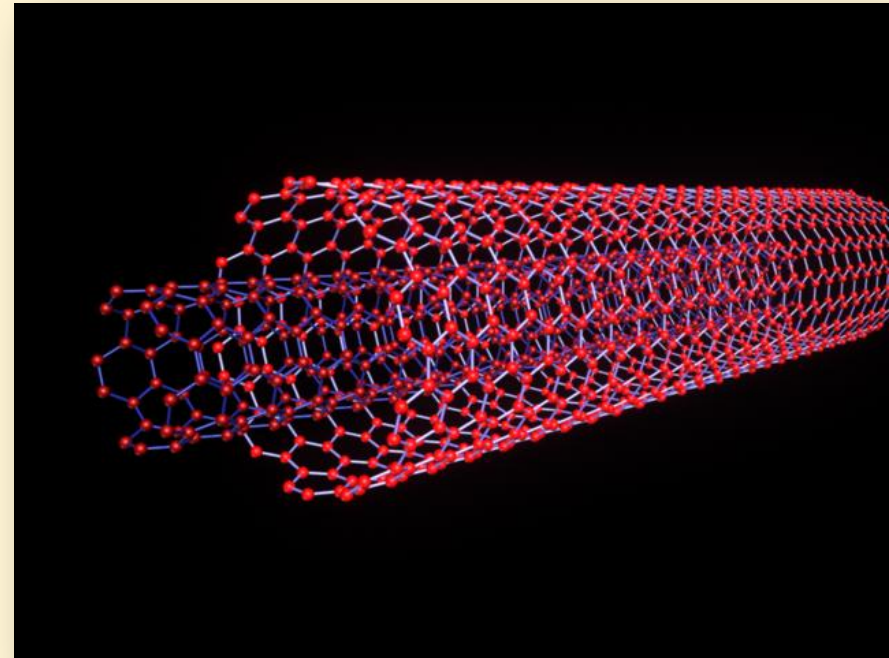
Because of their size, biomolecules provide a convenient means to further miniaturize MEMS devices.

The highly predictable and unique nanoscale structures created by biomolecules range in size from sub-nanometer to micrometer, and in some cases millimeter, depending on the type of biomolecule.

Applications of Self-Assembly

The ability of biomolecules to self-assemble provides a faster, cheaper, and more reliable means of generating a 2-D pattern or a 3-D architecture on a bioMEMS surface.

Structures formed through self-assembly can be used as templates or scaffolding for deposition of other organic or inorganic molecules. Such structures form useful nanoscale particles or fibers such as nanowires or nanotubes.



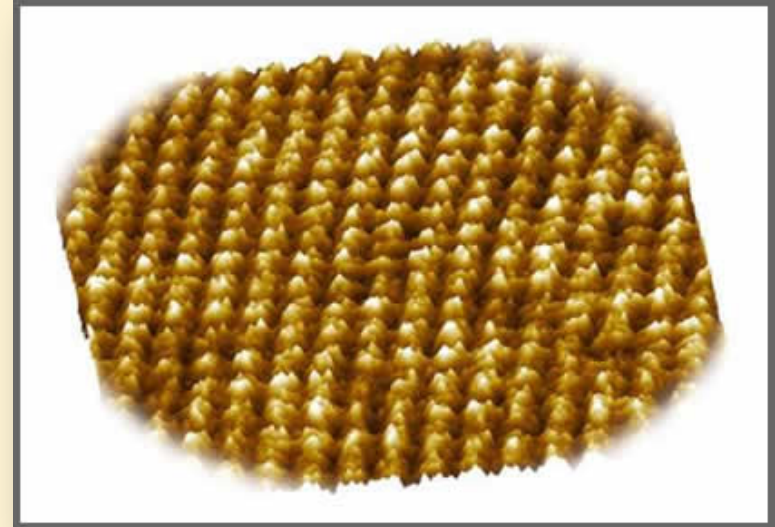
Multi-wall Nanotube [Graphic by Junifer Nez, SCME]

Biomolecular Filter

Crystalline bacterial cell surface layer (S-layer) proteins (see figure) are building blocks of one of the simplest self-assembly systems.

S-layer proteins form the outer most cell envelope of bacteria by building a monolayer lattice 5 – 10 nm thick. .

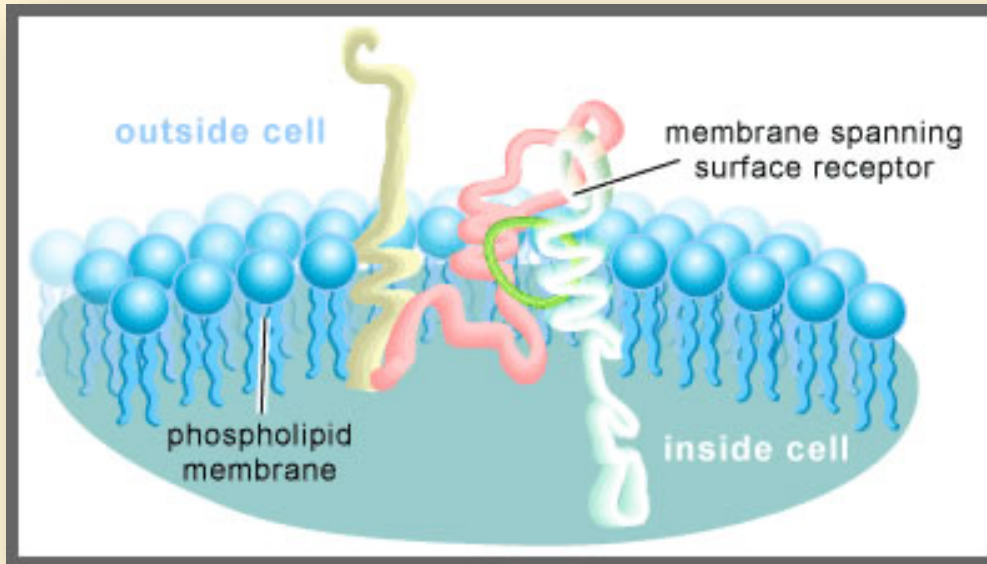
These have the ability to separate and identify molecules, acting as a filter. Such nanofilters offer new approaches to water purification and environmental cleanup.



*AFM image of bacterial cell S-layer proteins
(Scan size 250 nm x 250 nm)*

[Printed with permission from Agilent Technologies. Image Courtesy of T.Hopson]

Cell Surface Receptor



Cell Surface Receptor.

[Courtesy of The Science Creative Quarterly, scq.ubc.ca. Illustrator: Jane Wang]

A protein embedded in the plasma membrane of a cell is directly concerned with cell communications (getting information from outside the cell to the inside of the cell). The information transferred comes from signal molecules.

These receptors span the cell membrane and detect chemical signals on the outside of the cell and transmit this detection inside the cell.

Lock and Key Models

Cell surface receptors have another site for binding to a signal molecule.

- ❖ The signal molecule binding site has the exact shape and chemistry required for binding to the protein. This is sometimes referred to as a lock and key model, where the protein is the lock that fits one exact key.
- ❖ Only a signal molecule of the correct shape and position will bind and become "locked to" the protein.
- ❖ All other molecules are rejected as "imposters".

Function of Enzymes

Like receptor proteins, enzymes can also recognize specific molecules and bind to them. Like a cell surface receptor, an enzyme active site has a shape and surface chemistry that allows for recognition and binding of specific or complementary molecules.

The binding process is through multiple weak intermolecular interactions (such as hydrogen bonds).

Enzymes

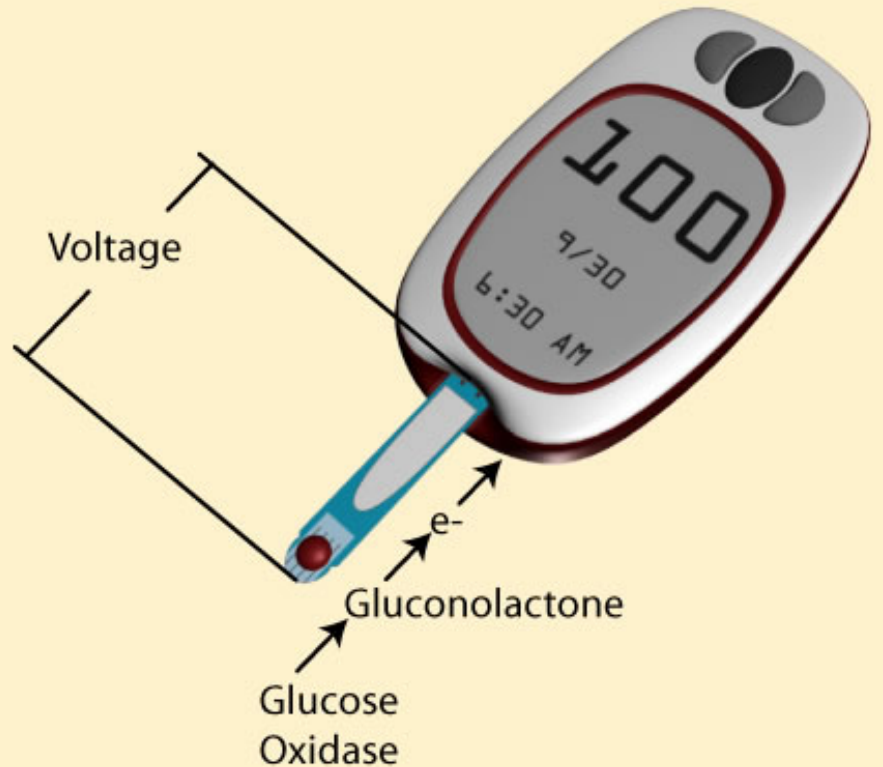
- ❖ Unlike cell surface receptors, once a target molecule binds to a capture molecule on the enzyme active site, the enzyme catalyzes a chemical reaction to form products that leave the enzyme.
- ❖ This leaves the active site open to catalyze reactions again and again between target and capture molecules.
- ❖ Enzyme active sites generate a "signal" as a result of the binding of molecules and the catalyzed reactions.
- ❖ This signal is monitored in enzyme biosensors.

Enzymes as Biosensors

An example of an enzyme biosensor is the glucose monitor (see figure). The glucose monitor is the most common diagnostic bioMEMS on the market.

It uses a MEMS transducer and the enzyme "glucose oxidase" in a blood sample (red dot) to recognize and transform glucose molecules.

Glucose molecules are oxidized to gluconolactone molecules, releasing electrons (e^-) that can be detected at an electrode as current.



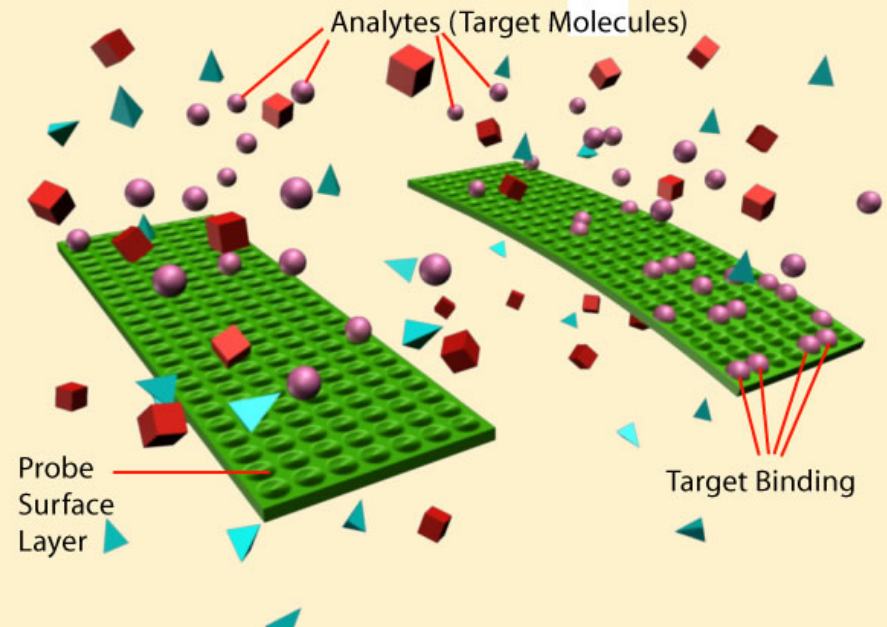
Glucose Monitor - Biosensor

MEMS Biosensor

This ability of cell receptors and enzymes to recognize an exact molecule in chemically complex body fluids can be simulated in a MEMS biosensor.

The graphic illustrates the surface of the microtransducers (cantilevers) coated with capture molecules (*a specific molecule that will detect one and only one type of analyte*).

A target molecule binds to a capture molecule creating a change in a property of the cantilever. The bioMEMS sensing circuitry detects the change, processes the signal and identifies the type and/or the amount of the analyte.

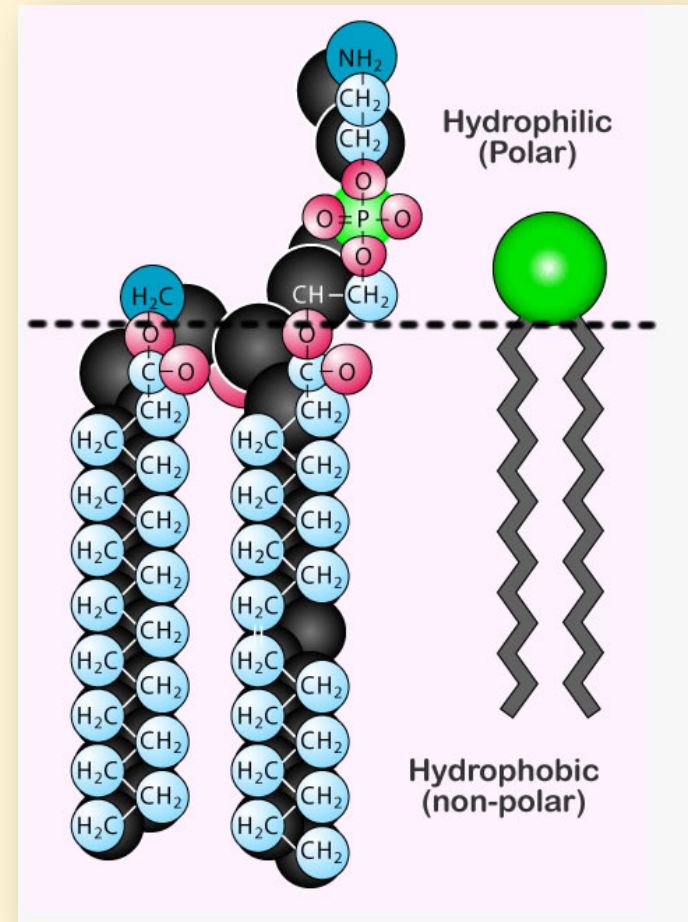


Biosensor using Cell Surface Receptors

Phospholipids

Phospholipids are very small lipid molecules that are a major component of all biological membranes.

Phospholipids self-assemble into membranes through hydrophobic and hydrophilic interactions. The graphic shows a phospholipid with its hydrophobic tail and hydrophilic head.

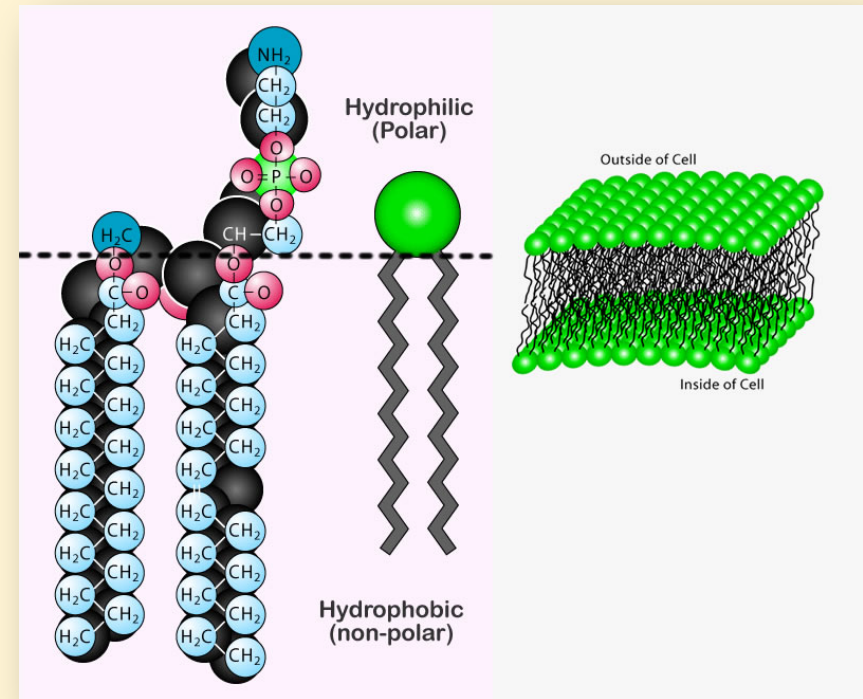


Phospholipid Bilayers

To form a bilayer membrane, the hydrophobic tails associate with each other and the hydrophilic heads line up together on either side of the membrane.

The graphic shows a bilayer membrane (the hydrophilic heads in green and the hydrophobic tails in black).

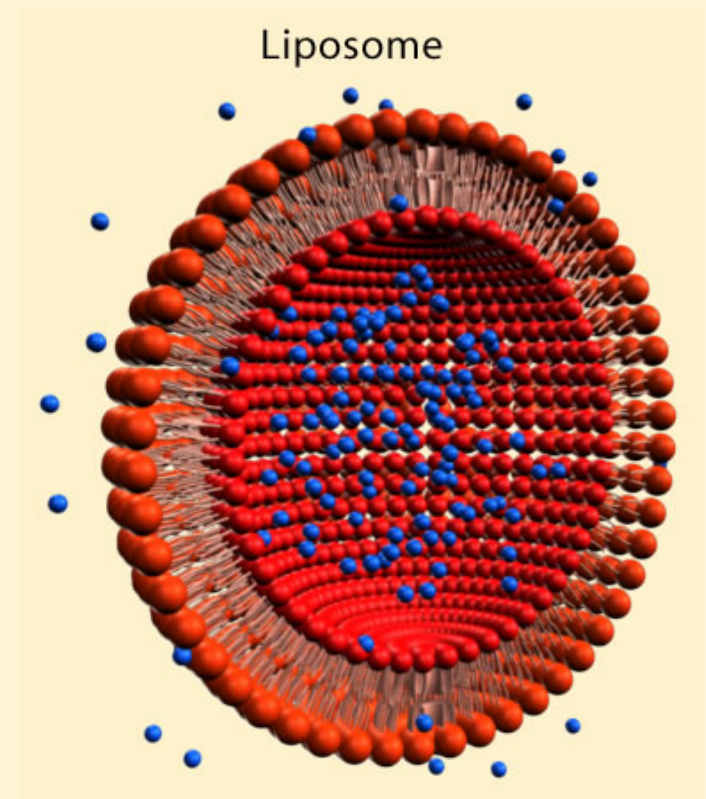
This membrane serves as a very thin, but very effective barrier.



Liposome Vesicle

This graphic shows a liposome (a spherical vesicle composed of a phospholipid bilayer membrane with an aqueous solution in the core).

Can you identify the phospholipid heads and tails in this graphic?



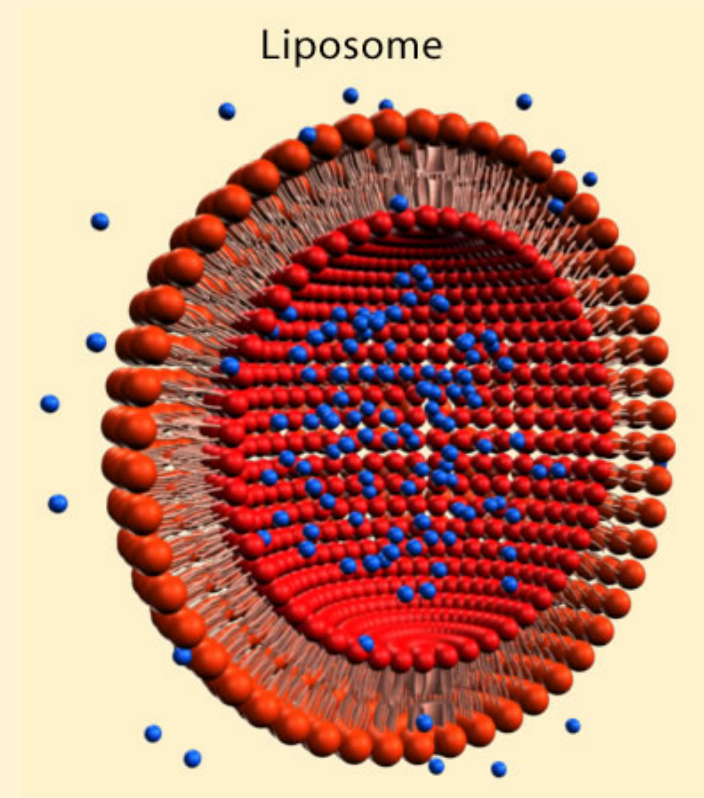
Liposome Drug Delivery System

In bioMEMS liposomes are being designed for drug delivery systems.

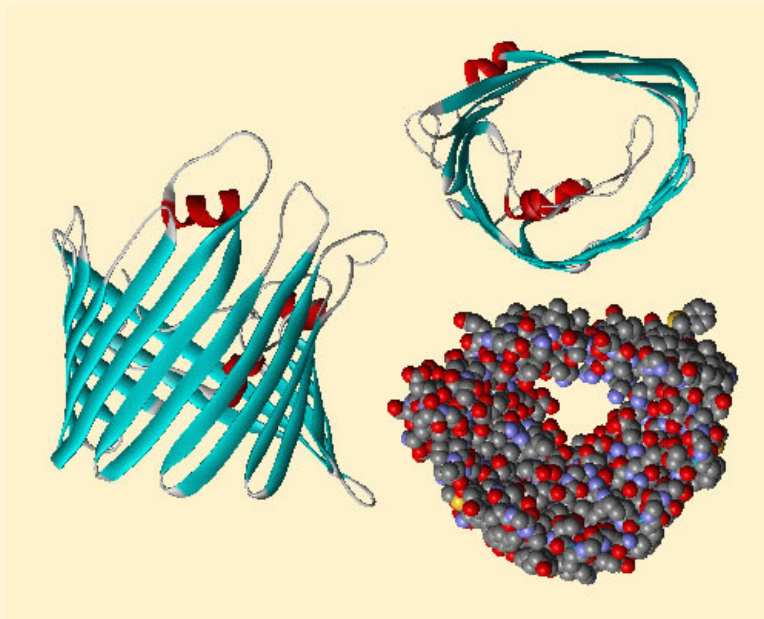
- ❖ The bilayer membrane sequester drugs within the liposome core (*drugs shown in blue*).
- ❖ The drug carrying liposomes are injected into the bloodstream and carried to the target area (such as a tumor).
- ❖ The liposome fuses to the membrane of the target molecule.
- ❖ The drug inside slowly travels through the membrane and into the cancerous cell (like a timed-release capsule).

(An animation of this process can be seen on the Tekmira Pharmaceuticals Corporation website -

http://www.inexpharm.com/Research/Inex_tcs.asp).



Porin Proteins



(Left) Ribbon diagram of a porin. (Right) Top view of the same porin molecule in a ribbon diagram (top) and atomic detail (bottom). Notice the pore through the middle of the structure.

[Graphic Source: Tulane University, BioChemistry/ RCSB Protein Databank]

Porin proteins are a class of outer membrane proteins found in bacteria. Porins are barrel proteins which cross a cell's membrane and act as "nanopores" through which molecules can diffuse. These nanopores act as channels which are specific to different types of molecules.

Porins in bioMEMS

Molecule separation - To separate molecules based on size, charge, and hydrophobicity

"Gating" channels— To control when a molecule is allowed to pass through the membrane.

Analyte detection – To selectively bind with specific analytes passing through the nanopore, thereby partially blocking the channel. By measuring the conductance of ions through channel, analyte concentration can be directly detected.

Molecular Machines / Motors

Strictly speaking, all cellular receptors and enzymes are molecular machines because they all have moving parts. Internal movement within protein and enzymes occurs when the capture molecules bind with the target molecules.

This binding process results in a change in shape or rearrangement of the atoms that make up the protein.

The range of motion of this rearrangement is generally very small and subtle, on the order of nanometers.

However, the movement is large enough to be detected with the right microsensor.

Motor Functions

In contrast, relatively large movements are produced by protein molecules or "motors" within cells. Protein motors move "cargo" around in the cell provide locomotion for the cell, or transport particles within the cell.

Some of these motors have a linear motion, while others have a rotary motion.

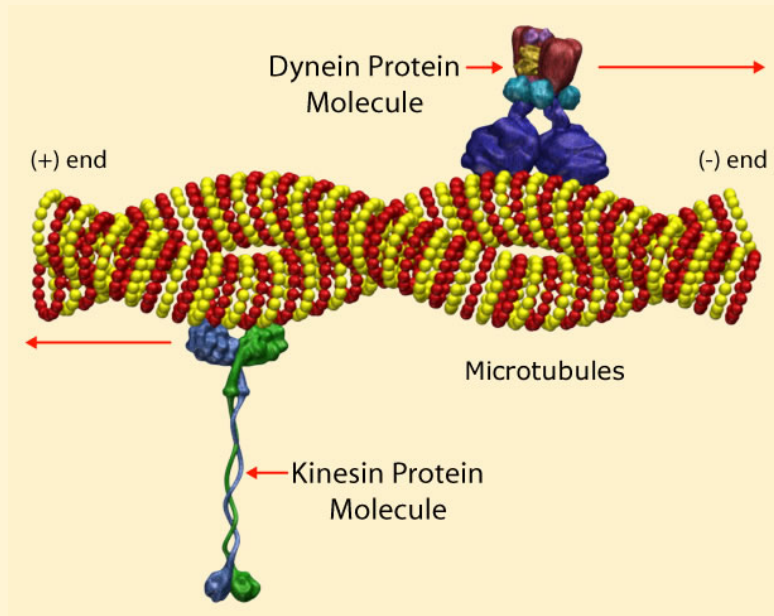
In bioMEMS devices, such motors can be used for similar functions as their macroequivalents.

Linear Motion Motors

Molecular motors that have linear motion move along microtubules or microfilaments within the cell.

Three such motors are the kinesin, dynein, and the myosin protein molecules. They all transport cargo that includes other proteins, membrane vesicles and organelles.

Kinesin and Dynein Proteins



Kinesin and Dynein Protein Molecules (Molecular linear motors)

The graphic shows that Kinesin travels toward the + end of the microtube while dynein travels toward the – end.

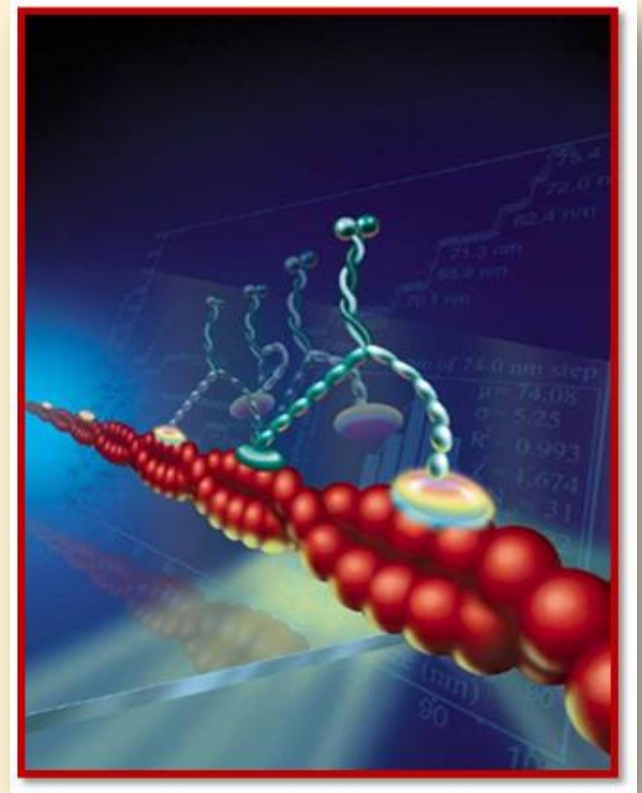
Kinesin and Dynein (shown in the graphic) are protein molecules that exist in the living cells of all plants and animals. These molecules can "walk" along tube-like material called microtubules. They crawl "hand-over-hand", using two "heads" like feet to move forward. This action allows these molecules to transport material within cells. Some viruses such as herpes and smallpox use kinesin molecules to move within an infected cell.

Myosin Motors

Another linear motor is myosin protein, which travels along a microfilament in muscle cells causing muscle contraction.

The figure shows a myosin (green) "walking" in nanosize steps along a microfilament (red).

As the myosin molecule walks it pulls on the microfilament causing the muscle cells to contract.



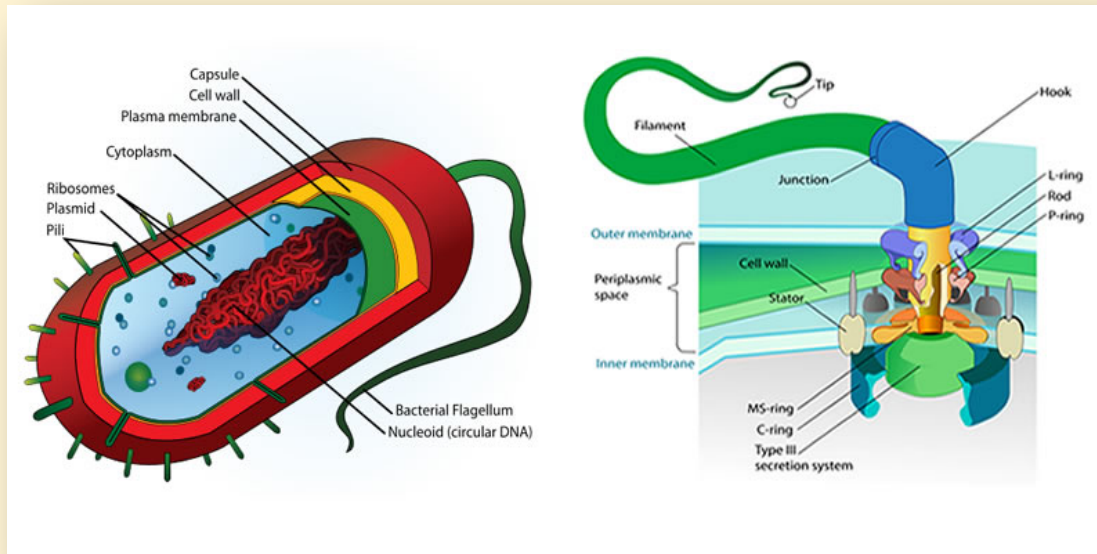
*Myosin Protein Molecule (A molecular linear motor)
 . [Printed with permission by the University of Illinois]*

Rotary Motors

Two types of molecular rotary motors are the

- ❖ bacterial flagellar motor and
- ❖ the enzyme ATP synthase.

Bacterial Flagellar Motor



**Bacterium Cell (left) and
Bacterial Flagellar Motor (right)**
[Graphics courtesy of Mariana Ruiz
Villarreal]

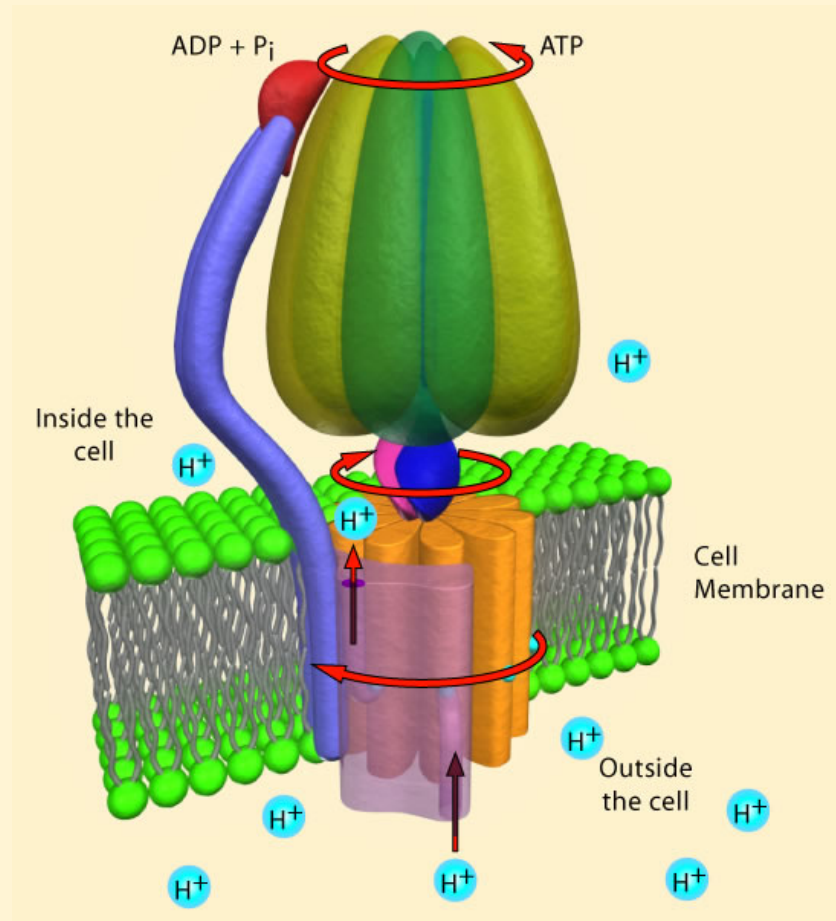
Motor responsible for the locomotion of bacterial cells.

A flagellum is a long, slender tail or tube that projects from a bacterium cell. The detail in the right figure illustrates the flagellum as an assemblage of rotor, stator and filament. The concentrated whip-like motion of the filament causes the rotor to turn. This propels the bacterium through its medium.

ATP Synthase Rotary Motor

This motor is an enzyme embedded in a cell membrane. It generates ATP (adenosine triphosphate), a high-energy molecule that is the fuel of cells. As the ATP synthase motor spins, ATP is created.

The ATP synthase motor is powered by incoming protons (H^+) causing a difference in electro-chemical potential to occur across its rotor membrane (F₀). The energy from this potential difference is transduced to mechanical energy which spins the motor. As the motor spins, ATP is generated by the rotor inside the cell.



Fueling Biomolecular Motors

Other molecular motors use this ATP as fuel. For example, each molecule of ATP that a linear motion kinesin motor encounters triggers precisely one, 8 nm step along its microtubule.

All of these molecular motors enable MEMS engineers to integrate the functions of macromotors into micro and nanoscale devices.

Let's Review

- ❖ *What types of biomolecules are being investigated for use in bioMEMS designs?*
- ❖ *How can the specific types of structures and functions of biomolecules be used to do the work in bioMEMS designs?*
- ❖ *On what scale are the sizes of the biomolecules being used in bioMEMS?*

Summary

The self-assembling structures is useful for biosensing and separations in bioMEMS design.

Complex 3-D structures provides a simpler, faster, and less expensive route to nanoscale structures than do most top-down types of fabrication.

Active site recognition of enzymes and other binding proteins is very useful in biosensor designs.

Microarrays can be used in a biosensor for analyzing genetic information.

Disclaimer

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