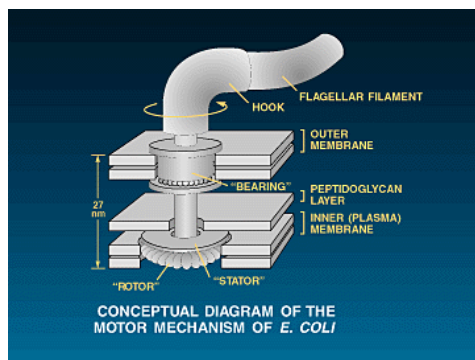


**Bio-Nanotechnology**  
**Molecular Machines & Molecular Self Assembly**  
Matthan Leo Easterlin, Siva Mandjiny, Ph.D.  
and Len Holmes, Ph.D.

**PART ONE – MOLECULAR MACHINES**



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Molecular self-assembly is a paradigm of nature. Self-assembly for this thesis is a “process in which pre-existing components aggregate in a reversible manner that is susceptible to control by design.” In this article we will review some of the main concepts of self-assembly and provide an introduction of supramolecular chemistry. First some of the best examples of molecular machines will be described and then in *Part II* we will explore the biophysical principles driving and controlling the spontaneous self-assembly of molecular machines. Specifically, the natural properties and results of (1) molecular size; (2) shape and (3) intermolecular forces will be related to spontaneous organization and assembly. Examples from laboratory studies and nature will be provided to illustrate the important principles.

### **Molecular Machines**

The cell can be imagined as a molecular-scale factory. It is jam-packed with small metabolites which are likened to raw materials and macromolecules which have functions of structure, enzymes, information storage or messengers. These molecular entities are organized, assembled and function in orchestrated ways to allow the cell to live, replicate and convert raw materials into cellular building blocks and useful energy. These macromolecules often serve as the cell’s machinery. Cells must also store and process information and generate motion.

Cellular factories face many of the same challenges that engineers in ordinary manufacturing facilities do. But there are distinct differences. Chemical, physical, optical and electronic properties at the scale of molecules are quite different from the observed properties of bulk matter. Thus, nature uses principles very different from the normal scale-down approach that an engineer might use to design and build smaller machines. For example, inertia, momentum and mass become less important at the molecular scale. Viscosity of fluids and frictional forces do not play count as much in the molecular world. Instead, at the nano-scale, it is electronic forces which predominate in holding an assembly together or in transducing (converting) the machine’s energy, from chemical to kinetic or perhaps electromagnetic to chemical energy.

It is also important to understand that compared to motion in the ordinary macroscopic world, motions at the molecular scale are incredibly rapid. For instance, a typical enzyme active site will randomly collide with hundred of thousands of substrate molecules each second. Due to thermal energy, soluble proteins spin at rates of a million times per second.

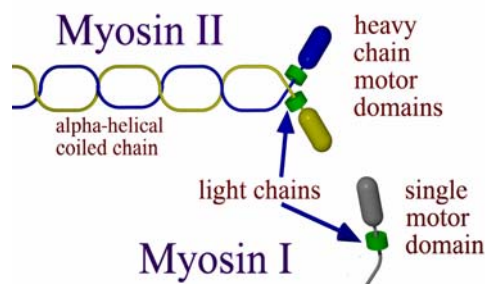
In nature there are three classes of molecular machines based upon their operational approach and the products they synthesize or physically move:

- a. Linear machines e.g. myosin
- b. Rotary machines e.g.  $F_0/F_1$  ATPase
- c. Translational machines e.g. DNA polymerase

### 1. Linear Machines: Myosin-actin:

Here we will examine a molecular motor involved in vertebrate striated muscle contraction. The basic mechanism of generating a mechanical force for the contractile process is the coupling of the hydrolysis of adenosine triphosphate (ATP) to the interaction of two proteins: myosin and actin. Multinucleated vertebrate muscle cells are bounded by a plasma membrane that is electrically excitable. This is important as well.

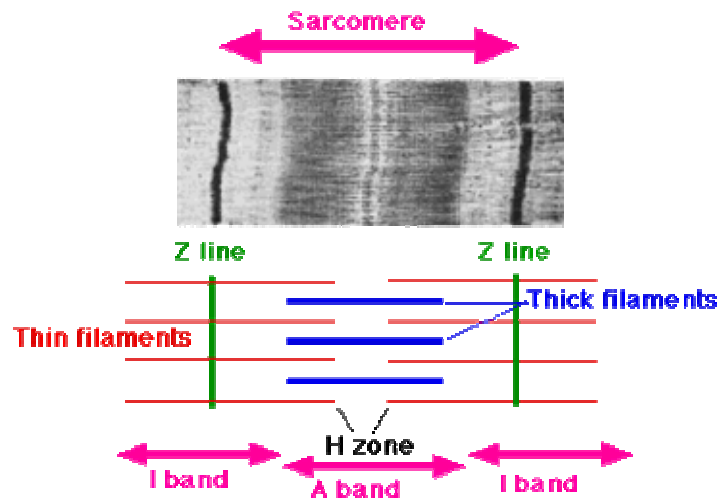
Skeletal muscles contain two kinds of filaments which are involved directly in the contractile process. “Thick filaments” are composed primarily of the protein myosin. There are several types of myosin, here we consider type II myosin. Myosin is a hexameric protein consisting of two trimers. Each trimer is composed of an identical heavy chain (220 kd) and two light chains (each 20 kd). Myosin hexamers bundle into thick filaments that are about 15 nm in diameter and 1.6  $\mu\text{m}$  in length.. The amino terminal of the heavy chains is a globular head that contains the binding sites for actin, two light chains and the site for binding and hydrolyzing ATP. The light chains binding near the amino end of the heavy chain have regulatory roles. The tail of myosin is a two-stranded alpha helical coiled coil of the heavy chains. The two chains have the same amino-to-carboxyl direction relative to each other. The absence of the amino acid proline in this long coiled coil (approx. 170 nm) is significant. Structurally, proline is a “helix breaker.”



**Diagram of myosin assembly.**

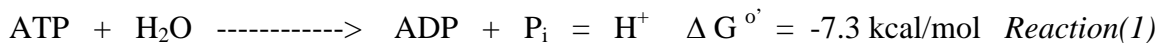
“Thin filaments” are composed of the proteins actin, tropomyosin and troponin complex. The thin filaments have diameters of about 9 nm and are 2.0  $\mu\text{m}$  in length. Interestingly, tropomyosin is also a coiled coil structure. Domains of myosin form cross-bridges that interact with the thin filaments. Both of these filament types run parallel to the muscle fiber, partially overlapping each other to form cylindrical-shaped columns called myofibrils. These myofibrils are about 1  $\mu\text{m}$  in diameter and immersed in the cytosol.

This overlapping thick filament/thin filament structure of the myofibril is seen as a striated appearance (sarcomere) under light microscope magnification:



The myosin/actin motor interaction produces a force. Striated muscle action is most simply described according to a "sliding-filament model." It is observed that muscle shortens as much as 33% during contraction. The filaments themselves do not shorten, but the length of the sarcomere does shorten. Sarcomere shortening occurs because the over-lap between the thick and thin filaments increases. Interaction of myosin and actin can only occur when calcium ions are bound to particular sites on troponin in actin.

Elegant experiments showed that myosin will spontaneously assemble into thick filaments. It binds to polymerized actin of the thin filaments and then it hydrolyzes ATP:



It is the free energy of ATP hydrolysis by myosin that drives the sliding filaments and the shortening of the sarcomere.

Actin, the major component of the thin chain is also an ATPase, but unlike the myosin activity, actin hydrolysis does not power muscle contraction. Actin organization and assembly with tropomyosin and troponin into thin filaments requires the free energy released by hydrolysis of ATP.

The force of striated muscle contraction is caused by the interaction of myosin, actin and ATP. The heavy chain myosin has two force-generating globular domains that serve as actin-binding sites and ATPase sites. The nervous system controls the action of the muscles. Nerves connect the spinal column to the muscle. The brain and spinal cord of vertebrates send signals in the form of action potentials conducted along the nerve fibers to the muscles, meeting at the "neuromuscular junction". When an electrical signal crosses this junction it is transmitted deep inside the muscle fibers and the muscle contracts. The nervous system controls the action of the muscles so that the force and movement are matched to the task to be performed. The brain and spinal cord accomplish this coordinating by sending signals in the form of action potentials that travel along the nerve fibers to the muscles. Nerves connect the spinal column to the muscle. The place

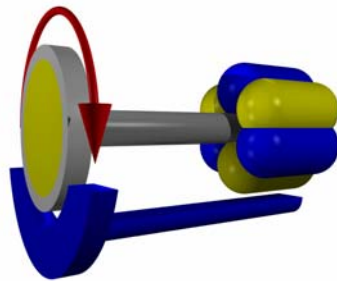
where the nerve and muscle meet is called the "neuromuscular junction". When an electrical signal crosses this junction it is transmitted deep inside the muscle fibers.

## 2. **Rotary motor: ATP Synthase (ATPase)**

The mitochondria is called “the power house” of the cell. ATP is the universal energy currency that is used to drive many cellular reactions and processes. ATP is manufactured by a protein assembly integrated into the inner mitochondrial membrane. Chloroplasts and bacteria cell also have similar ATP synthases. The phosphorylation of ADP is the opposite of reaction (1) above and is thermodynamically unfavorable ( $\Delta G^{\circ} > 0$ ).

These assemblies are composed of two types of protein subunits:  $F_0$  subunits function as a channel for the passage of proton ( $H^+$ ) down the concentration gradient from the intermembrane space into the mitochondrial matrix. The other type of subunit composing the ATPase is called  $F_1$ .  $F_1$  is a complex of five types of polypeptide chains and has a MW of 378 kd.  $F_1$  contains catalytic sites for phosphorylation of ADP into ATP. The  $F_0$  subunit is almost completely transmembrane.  $F_1$  is located on the matrix side of the inner mitochondrial membrane. The roles of the subunits was determined by sonication of intact mitochondria breaking them open. The inner membrane fragments then re-anneal to form inside-out submitochondrial vesicles (SMPs), with the  $F_1$  subunits now on the outside. If the  $F_1$  subunits are removed from the vesicles they catalyze ATP hydrolysis.

When  $F_1$  is dissociated from the ATPase assembly the SMPs becomes permeable to the movement of  $H^+$  down a concentration gradient into or out of the vesicles. Thus it was clearly established that  $F_0$  functions as a proton channel.



“Mechanical schematic” of ATPase motor showing 3 alpha and 3 beta subunits.

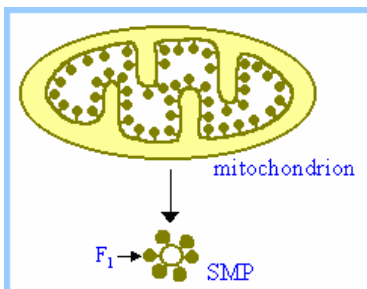
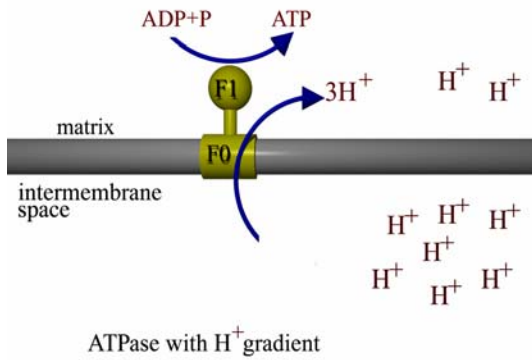


Diagram of the mitochondria indicating the  $F_1$  subunits in the matrix and the segregation of the matrix from the intermembrane space.



Schematic representation of ATPase function and the proton gradient driving force.

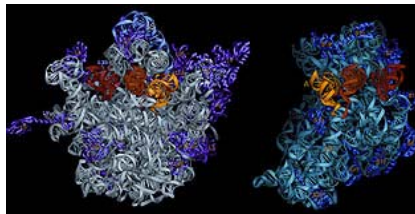
### **3. Translational Motor Ribosomes**

*Ribosomes are molecular machines that manufacture proteins.* Because protein synthesis is an extremely complex process, our understanding of it has lagged behind our understanding of the details of DNA replication and RNA transcription.

Cells must be able to manufacture copies of their machinery. The vast preponderance of molecular machinery is protein. But, elucidation of translation has given the molecular biologist a new appreciation for a broader function of RNA. Evidence indicates that in all stages of protein synthesis the rRNA has a primary functional role. The ribosomal protein appears to have the secondary role of facilitating rRNA function by inducing or stabilizing the conformational state of rRNA.

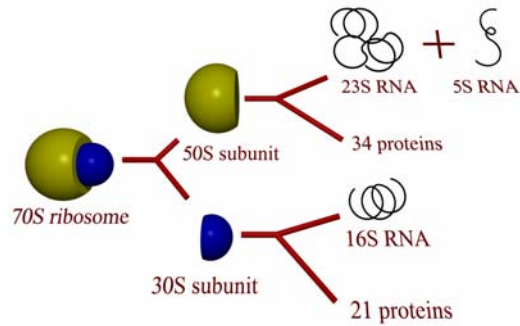
The ribosome is a complex molecular assembly built of more than 50 individual protein chains and three single-stranded RNA chains responsible for protein synthesis (translation). The RNA constitutes nearly 2/3 of the mass of ribosomes. It is composed of a large subunit (called the 50S) containing the active catalytic site for protein synthesis. The 50S subunit is constituted of 34 proteins and 2 rRNA molecules (5S and 23S). There is also a small subunit (30S) that controls the information used by the ribosome to manufacture the correct protein sequence. The small subunit contains some 21 unique proteins and a 16S rRNA molecule. In *E. coli*, the ribosome assembly has a mass of about 2700 kd and the 20,000 ribosomes in a bacterial cell account for 25% of the cell mass.

A definitive example of the functional importance of rRNA in the translational process is the base-pairing interactions between the purine-rich (Shine-Dalgarno) sequence upstream of the initiation codon on the mRNA and the complementary pyrimidine-rich sequence near the 3' end of the 16S rRNA.



**The large and small subunits of the ribosome.** (Adapted from *rib-x.com*)

23S (grey and the 5S (light blue) associate with their respective proteins (royal blue) to make up the large subunit of the ribosome (left), while the 16S RNA (cyan) associates with its proteins (dark blue) to make up the small subunit (right).

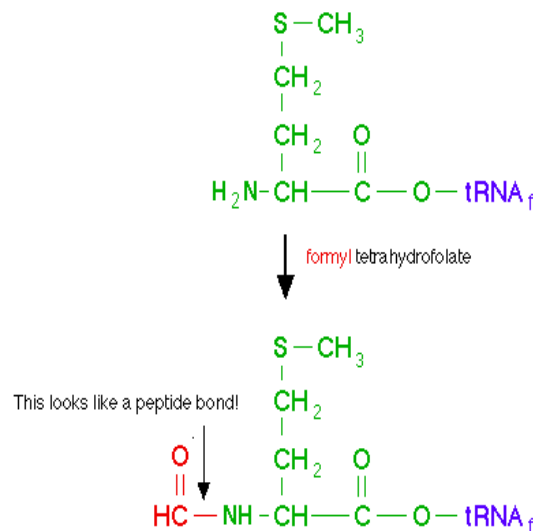


**A ribosome can be dissociated into its constituent protein and rRNA parts.**

**Over-all process:** The information encoded in mRNA is used by the ribosome in an integrated process that produces protein of the correct sequence. This process, called translation, takes place in the cytoplasm. The protein is synthesized in three stages in the amino-to-carboxyl direction. Protein synthesis requires several partners:

1. Ribosome
2. mRNA
3. Transfer RNA (tRNA)
4. energy source
5. amino acids
6. Protein release factors

**Stage One- Initiation:** This is a tRNA binding step. The ribosome facilitates binding of an “initiator tRNA” to the start signal on the mRNA. This initiator transfer RNA carries a specially modified amino acid, formylmethionine (f-Met). The first amino acid on the nascent protein is f-Met. The ribosome also binds the tRNA at a site called the Peptidyl (P) site.



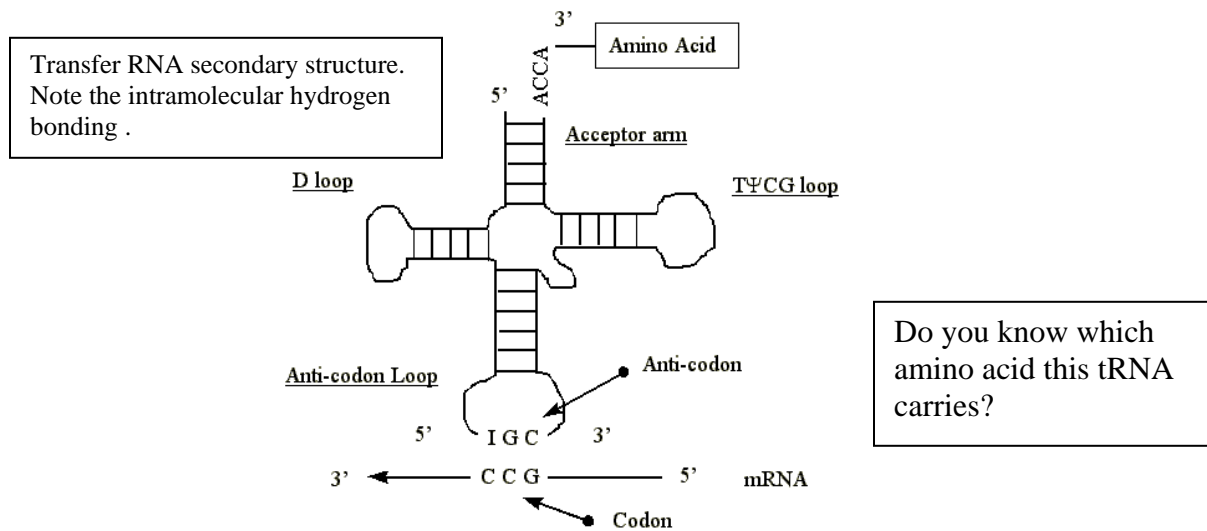
Formylation of methionine which is linked to f-met tRNA by formyl tetrahydrofolate. f-met is the first amino acid in the polypeptide chain.

**Stage Two- Elongation:** Another ribosome site (Aminoacyl site) binds the second tRNA which is charged with the amino acid corresponding to the second codon of the mRNA. A charged tRNA is called an aminoacyl tRNA. The ribosome then catalyzes the formation of a peptide bond between the carboxyl group of f-Met (on the P-site) and the amino group on the incoming (second) amino acid. This leaves the initiator tRNA without an amino acid (uncharged). It then transfers from the P-site to an Exit (E) on the

ribosome. The newly formed dipeptyl tRNA then shifts from the A-site to occupy the now empty P-site in preparation for the next amino acid specified by the next triplet (codon) on the message (mRNA). Then empty tRNA also dissociates from the E-site in preparation for the next peptide bond forming event. Research has shown that the rRNA also plays a critical elongation function by base pairing with mRNA and facilitating the retention of the correct reading frame.

**Stage Three- Termination:** The 16S rRNA recognizes the UGA stop codon. It was found that a deletion of a single 16S nucleotide allows the ribosome to read through the UGA termination codon. Protein synthesis halts when a special stop codon is recognized by translation release factors. Interaction with stop the codon facilitates the release of the fully translated protein from the ribosome complex. Release of the ribosome is followed by its dissociation into the 50S and 30S subunits. The subunits must then re-associate for the next round of protein synthesis.

So, in very brief summary: The mRNA molecules are information-carrying molecules that are decoded by the interaction of the ribosome machinery and adapter tRNA molecules. The nucleotide-coded information is read in a coordinated process by the ribosome and the tRNA in triplets called codons.



The tRNA molecules, transport the amino acids to the site of protein synthesis on the ribosome. They interact with both the small and large subunits of the ribosome and facilitate the decoding process. A portion of the tRNA associates with the large subunit of the ribosome and positions the amino acid corresponding to the triplet codon in a site called the peptidyl transferase center of the ribosome. Another portion of the tRNA (anticodon) base pairs with the codon, thus specifying which amino acid is added next in the sequence (amino-to-carboxyl).

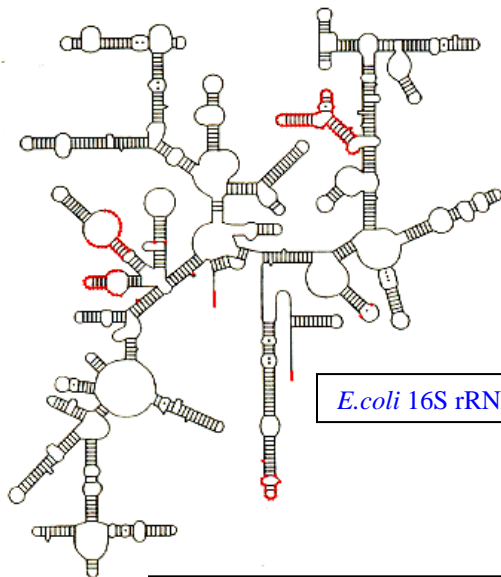
		2nd base in codon				
		U	C	A	G	
1st base in codon	U	Phe Phe Leu Leu	Ser Ser Ser Ser	Tyr Tyr STOP STOP	Cys Cys STOP Trp	U C A G
	C	Leu Leu Leu Leu	Pro Pro Pro Pro	His His Gln Gln	Arg Arg Arg Arg	U C A G
	A	Ile Ile Ile Met	Thr Thr Thr Thr	Asn Asn Lys Lys	Ser Ser Arg Arg	U C A G
	G	Val Val Val Val	Ala Ala Ala Ala	Asp Asp Glu Glu	Gly Gly Gly Gly	U C A G
						3rd base in codon

### The Standard Genetic Code

As stated above, it was initially believed that the ribosomal RNA served mostly a passive structural function, perhaps as a scaffold to assist the proteins of the assembly to bind together in a correct way so that they could catalyze protein synthesis. However, current knowledge suggests that the role of rRNA in synthesis is much more active. Experiments supporting this thesis have shown:

1. Ribosomes that have been stripped of nearly all the protein component still catalyze the formation of peptide bonds.
2. Protein synthesis inhibitors interact with rRNA rather than ribosomal protein.
3. The start sequence on the mRNA is recognized by 16S rRNA.

Biologists speculate that ribosomes once consisted entirely of RNA. Ribosomal protein later evolved to enhance the speed and accuracy of translation.



*E. coli* 16S rRNA (adapted from <http://prion.bchs.uh.edu>)

**More than structural importance-** 16S rRNA- (1542 bases) conformational changes in the 16S rRNA during ribosome subunit association are related to changes in the ribosome that occur during the movement of the tRNA during the elongation cycle. Interactions assist the mRNA to position itself on the ribosome during translation. There is also evidence that 16S rRNA is directly involved in the interactions between the large and small ribosomal subunits.

## **SOME USEFUL REFERENCES**

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