Mechanochemistry

The Amazing Viral DNA Packaging Molecular Motor

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Nano-sized molecular motors, which consume chemicals and do mechanical work are ubiquitous in nature. One of the most powerful such motors is the viral packaging motor, which consumes ATP and packages the viral DNA into the procapsid (the protein shell) of the virus. A pulling force applied to the loose end of the DNA can slow down the rate of packaging, thus showing that a mechanical force can slow down a chemical reaction. In this article we describe this packaging process and what is known about the mechanochemistry of the motor.

1. Introduction

Richard Feynman, in a visionary lecture [1] at the American Physical Society meeting in 1959, posed two challenges to the audience. The first was to build an electric motor that would fit within a cube of side 1/64 of an inch (~ 0.4mm). The second was to write the information contained on the page of a book to an area 1/25,000 smaller and read it, using an electron microscope. He offered a prize of $1,000 to the first person who achieved each one of these tasks. Though Feynman thought that both would take a long time to achieve, the prize for the first was claimed by an engineer in 1960, who used conventional techniques and constructed a very small motor. The prize for the second was given to a Stanford University graduate student in 1985, who used electron beam lithography to write a part of Charles Dickens' novel *A Tale of Two Cities*, on a page of length 1/160 mm.

Feynman's visions on nanotechnology are being realized and it has become a very important area of research. Today we know that nature is abundant with very small, nano-sized molecular motors. Millions of them are active inside the human body and in

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other living organisms. Even a virus has molecular motors doing useful work for it, and this article is about one such molecular motor. For an interesting introduction to molecular motors see the article by Chowdhury [2] .

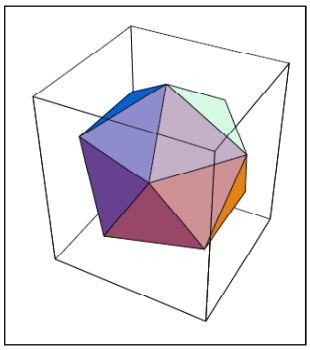

Figure 1. The icosahedron. It has 12 vertices and 20 faces. Passing through two diagonally opposite verticles is a 5-fold rotational axis. There are 6 such axes. (Figure drawn using MATHEMATICA.)

2. The Structure and Life Cycle of a Bacteriophage

Everyone knows about viruses that infect human beings and cause serious diseases such as hepatitis, AIDS, etc. But there are viruses that infect a bacterium. These are known as bacteriophages. The word bacteriophage originates from 'bacterium' and Greek word *phagein* which means 'to eat'– so bacteriophages are bacterium-eaters. Usually one uses the shorter word phage, to talk about them. Typically, a phage has an outer protein hull within which the genetic material is enclosed. The genetic material can be single/double stranded RNA or DNA. The phages have sizes varying between 20 and 200 nm and there is a large diversity of such phages. They are found in all places where there are bacteria, like the soil, the intestine of animals or sea water.

The bacteriophage, $\phi 29$ has a head that is icosahedral in shape. The icosahedron is a highly symmetric platonic solid having 20 faces and six C_5 (five fold rotational) axes and is shown in *Figure* 1. The head is connected to a tail, which has leg like parts that are referred to as tail fibers. The part that connects the head to the tail is referred to as the connector, whose structure has been determined recently [3].

The life cycle of the phage is shown in *Figures* 2–6 [4]. The virus can attach to specific receptors on the surface of a bacterium (*Figure* 2). After the contact has been made, the tail fibers bring the virus closer to the surface of the cell. The bacterial surface is then punctured and the DNA is injected into the bacterium (*Figure* 3). After the injection, the viral DNA takes over the cellular machinery of the bacterium and makes several copies of itself (*Figure* 4). It also instructs the protein factory of the bacterium viz., the ribosome, to produce the proteins that it needs. These proteins self-assemble into a precursor capsid (the outer

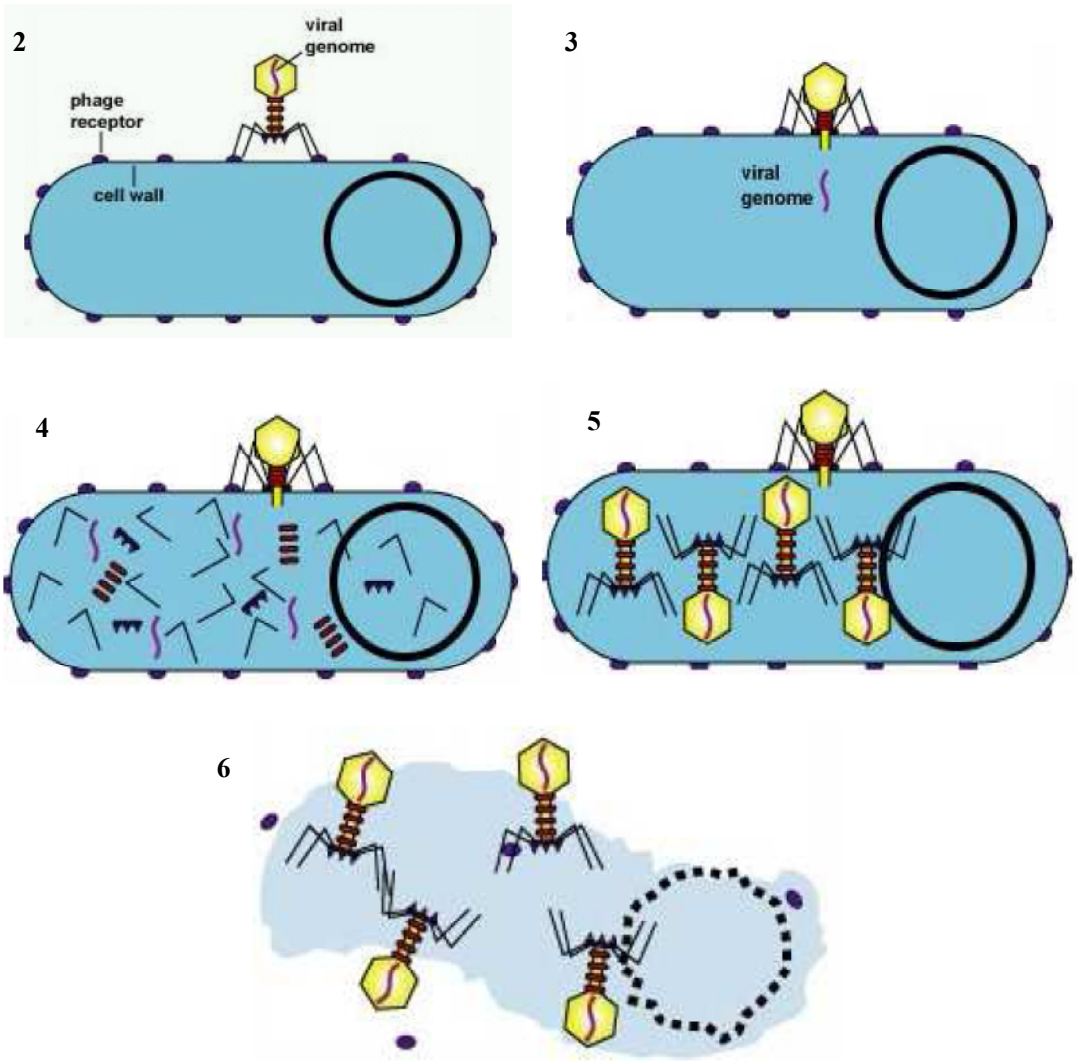

Figure 2. StepI: Adsorption: The phage attaches to receptors on the surface of the bacterium.

Figure 3. Step II Penetration: The phage punctures the bacterial surface and injects its DNA into the bacterium.

Figure 4 . Step III Replication: The bacterial DNA takes over the cell machinery. It replicates and makes the bacterial ribosome to produce the proteins that it needs.

Figure 5. Step IV Maturation: The proteins have assembled to form the procapsid and the viral DNA gets packaged inside it, using the molecular motor. The result is a new virus.

Figure 6. Step V Release: The bacterial cell wall is broken up and the mature viruses are released. About 20-30 new viruses are formed from each bacterium.

Figures 2–6 have been reproduced from the webpages of G Kaiser with permission [4].

cover of the virus, referred to as procapsid). Further, it makes proteins that form a molecular motor that sits at the mouth of the prohead and pushes the DNA into it, ultimately resulting in a new bacteriophage (*Figure* 5). In the final stage, the bacterial cell wall is destroyed and the several bacteriophages so formed are released (*Figure* 6) into the surroundings, ready to infect other bacteria.

3. The Packaging Motor

Figure 8 shows the detailed structure of the motor that does the work of pushing the DNA into the capsid (see also *Figures* 14 and 15). It is to be noted that the capsid, shown in *Figure* 7 has dimensions of 42nm × 54nm, while the DNA that is packaged inside is very long ~ 6600nm in length. As one base in the DNA corresponds to a length of 3.4Å , the DNA is about 19,500 bases long. After the packaging, the density inside the capsid is very high, approaching that of the crystalline state. It is clear that a double stranded DNA would be much happier entropically if it is left outside, in the solution (i.e. outside its capsid), because the possible conformations available to it then would be very large. Thus packaging is an entropically unfavorable process. Further, inside the capsid, there would be electrostatic interactions between different regions of the packaged DNA, which would make packaging an unfavorable process energetically too. It has been

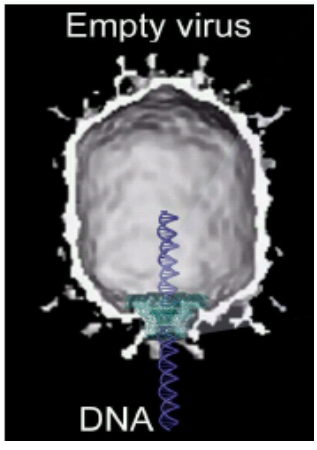

Figure 7(let). The empty procapsid, with the DNA and packaging machinery sitting at the lower end of the prohead.

Figure 8 (right). The structure of the packaging motor. The DNA and the dodecameric assembly of protein gp10 is shown. The DNA is shown in white and parts of each one of the proteins is marked with the same color.

Figures 7 and 8 reproduced from webpage of M Rossmann [5,6] with permission.

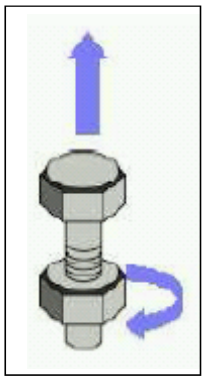

Figure 9. The rotation of the nut in the direction shown causes movement of the bolt in the direction indicated by the straight arrow.

estimated that the free energy difference ΔG = G(packaged state) – G(free state) = 20,500 kcal/mole! This makes it roughly 1 kcal/base pair. Therefore it is obvious that there has to be some driving force that pushes the DNA into the capsid – this is done by a nano-sized molecular motor, which works with the energy derived from a chemical reaction, viz., the hydrolysis of Adenosine triphosphate (ATP). The chemical energy causes a nut (see *Figure* 9) to rotate, which in turn causes the movement of the bolt. The nut in this case is made of proteins and is shown in *Figure* 8 and the bolt is just the double stranded DNA!

It has to be stressed that all aspects of the working of this wonderful machine is not known to us today and that this is a very active area of research. Interestingly, the overall efficiency of the motor has been estimated to be 30%. In the following, we summarize what is known about the workings of the motor.

4. The Symmetry Mismatch and Design of Nano-Sized Rotating Machinery

In our macroscopic world, construction of two objects one of which has to rotate smoothly in a hole within the other is easy – for example, the ball-bearing of *Figure* 10. Here, the two objects are both circular rings and to make them rotate smoothly, one has the spheres in between them. Suppose one wanted to make such a ball bearing, but having a size of a few nanometers.

Figure 10. In the macroscopic world, designing parts of rotating machinery, like the ball-bearing shown here is easy.

For this one needs two circular rings of diameter of a few nanometers. This however is impossible to have, because the only available building blocks are molecules, which themselves are discrete, mostly rod-like objects, of roughly a nanometer in size. Any assembly of rod like objects can at the most give a polygon, not a circle. Even if one had made two such circles, what would be the spheres, suitable to put in between the two, to facilitate rotation? One can think of a rare gas atom like Xe or perhaps an almost spherical molecule like CH_4 or perhaps C_{60}. However, these are not available within the living organism. Then, how does one construct a rotating machinery? Nature has found an

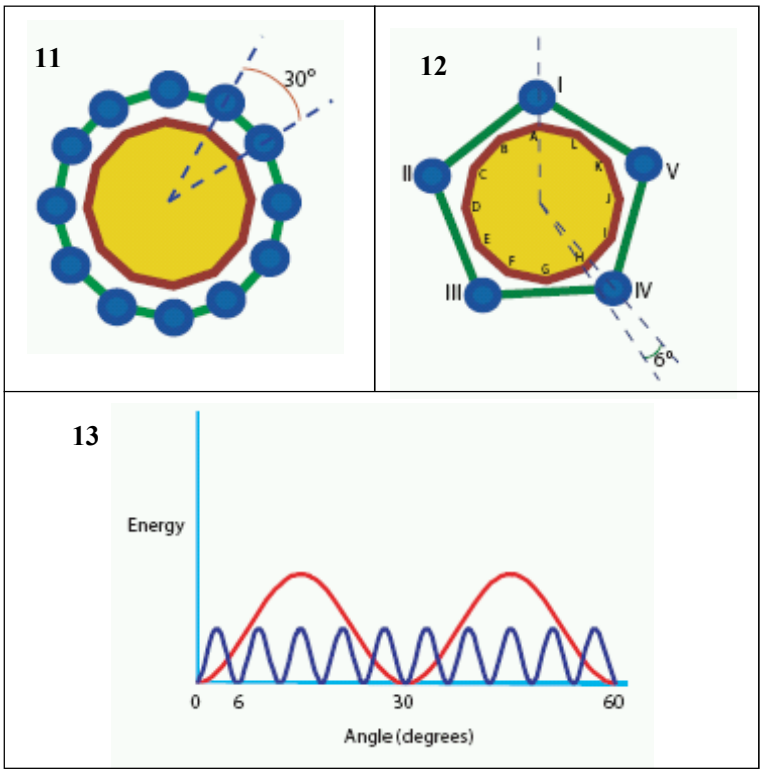

Figure 11. The outer ring as well as the inner ring have 12 fold axes. The result is a periodic variation of energy with a period of 30°.

Figure 12. The inner ring has 12 fold symmetry while the outer one has only 5 fold. Now if the inner one rotates with respect to the outer one, the energy changes in such a fashion that it is periodic with a period of 12 degrees.

Figure 13. The variation of energy as one rotates the inner part relative to the outwer part in Figures 11 and 12. The red curve has periodicity of 30 degrees and corresponds to Figure 11 while the blue curve has a periodicity of only 6 degrees and corresponds to Figure 12. Obviously rotation is easier in the second case.

elegant solution, as pointed out long ago by Hendrix [7]. The idea is to have a symmetry mismatch between the two rotating objects. To understand this, consider two nanosized rings having the shape of a regular dodecagon (a polygon of 12 equal sides and angles), shown in *Figure* 11. The separation between the two rings is of nanometer size, as a result of which there will be molecular interactions between the outer and inner rings that would keep the inner ring in place. If one ring rotates with respect to the other, the energy of interaction will change. In the case where both of them are regular dodecagons, the variation in the energy will be periodic, with a period of 30°, as shown by the red curve in *Figure* 13. On the other hand, if the two rings were both pentagons, then the periodicity would be 72°. But now suppose, the outer ring has a 12-fold axis while the inner one has only a 5-fold axis as in *Figure* 12. Then, what would be the periodicity with which the energy will vary? A moment's thought should convince everyone that it is only 12°. Therefore the variation of

the energy would be much smoother than if both had 12-fold axes or 5-fold axes, as shown by the blue curve in *Figure* 13. Nature has used this idea very efficiently to design rather smoothly rotating machinery of nanodimensions as we see in the next section.

5. Parts of the Motor

The procapsid has an opening at one of the vertices of the icosahedron. This is the portal for DNA entry. The DNA that is to be packaged has to have the protein gp3 attached to its end. The packaging takes place in about three minutes. As noted earlier, this vertex has a 5-fold axis of symmetry. The connector, a dodecamer of a protein referred to as gene product 10 (gp10) occupies this vertex (see *Figure* 7). The connector is conically shaped. At the narrow end, the external radius is 33 Å while at the wider end, it is 69 Å. The internal radii are 18 Å and 30 Å. It has a height of 75 Å. The narrow end protrudes from the portal vertex and is associated with a pentamer of pRNA-ATPase complex sitting at the lower end of the dodecameric assembly of gp10 (see *Figure* 7). A side view of the motor, which has the DNA that is being packaged in it is shown in *Figure* 14. A view of the same from below, is shown in *Figure* 15. Note that the labels A, B, C, ... L denote gp10 protein molecules while the labels I, II ... V

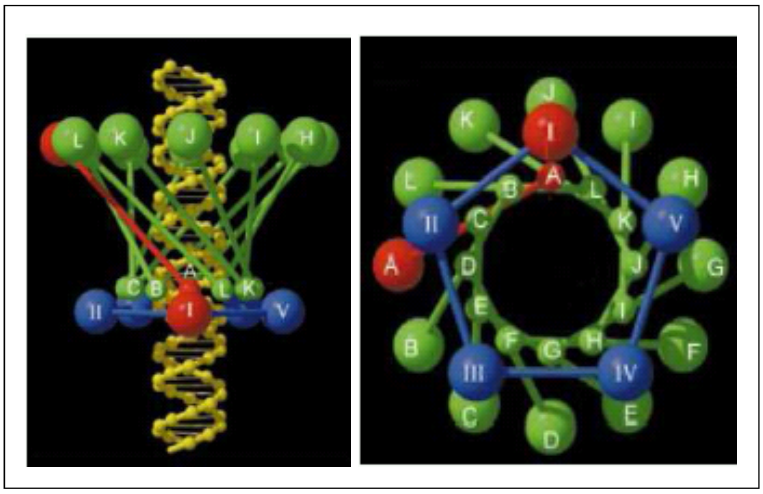

Figure 14 (left). The dodecameric proteins as well as penameric pRNA-ATPase complex are shown here.

Figure 15 (right). The dodecameric proteins as well as penameric pRNA-ATPase complex (view from below). The DNA shown in Figure 14 is not shown here. Reproduced with permission from [3].

denote pRNA-ATPase complexes, which can hydrolyze ATP. In the figures, each protein has two parts, a lower part, close to the pRNA, and another upper part. The two parts of the same protein are given the same color and labeled with the same letter. What is most important is to notice that the lower part of the connector, having 12-fold symmetry, is able to rotate inside the pentamer, which has 5-fold symmetry. Note further that in *Figures* 14 and 15 some parts are colored red. This is to make the reader notice that in this arrangement, the lower part of gp10, labeled by A is in close proximity to the pRNA marked I. None of the other proteins are this close to any other pRNA. It is believed that in this configuration, the ATPase of I is able to catalyse the hydrolysis of ATP. The hydrolysis causes the dodecamer to rotate in the anticlockwise direction, relative to the pentameric pRNA. This rotation drives the double stranded DNA which is sitting inside the dodecamer, much like the movement of a bolt inside a rotating nut. Obviously, the grooves of the double stranded DNA are very useful for this! Further, it is believed that the consumption of one ATP causes a rotation by 12^o, and this results in the movement of 6.8 Å (two base lengths) of the DNA into the capsid. The rotation by 12^o brings the pRNA-ATPase marked as C into close proximity with the protein labeled as II. This is then able to catalyze the hydrolysis of another molecule of ATP, causing a further rotation by 12^o and packaging of two more base pairs. This continues until the whole DNA has been packaged.

6. Can one Pull the DNA and Prevent it from Going into the Capsid?

The answer to the above question is definitely yes! However, this would not be easy, as one has to take hold of the DNA at the loose end and pull it. It is possible to do this using what are known as optical tweezers. The trick is to attach the loose end of the DNA to polystyrene microspheres of diameter of 2μm (10^{-6}m). These microspheres are coated with molecules of streptavidin. The loose end of the DNA has the molecule biotin attached to it. Biotin has great affinity for streptavidin and the loose end would

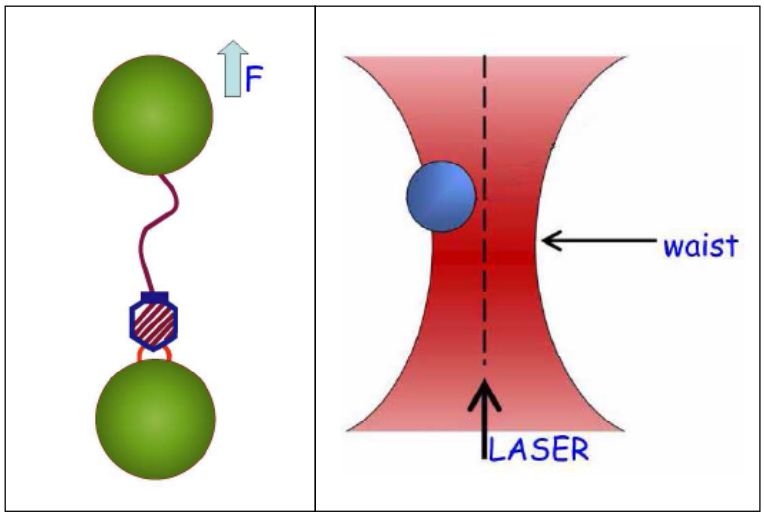

Figure 16 (left). The loose end of the DNA and the viral capsid are attached to two microspheres and one of the spheres is kept fixed, while a force F is applied to the DNA by moving the other sphere.

Figure 17 (right). The laser beam is focussed to the smallest possible spot, the size of which is of the order of its wavelength. The microsphere has lower energy at the point where the electric field is largest, and hence would be trapped at the centrer of the beam, at its waist.

therefore attach to the bead, thereby attaching the DNA to it. The binding, though not covalent, is quite strong. In a similar fashion, the capsid into which the DNA is going in, is attached to another microsphere (see *Figure* 16). Now we have to pull only the spheres apart, to pull the DNA out of the virus. This can be achieved using laser trapping of the beads. What is done is to focus a laser beam, to the smallest possible size and then have the bead sitting at the focus. Then, the electromagnetic field of the laser would induce a dipole in the bead, and this induced dipole would interact with the original electric field, to lower the energy of the system. Hence, if the bead is subjected to the inhomogeneous laser field, it would move into the region where the field has the largest value, and would remain trapped there. The electromagnetic field would have its largest value at the waist of the laser beam (see *Figure* 17), and therefore the lowering of the energy would have the largest value if the bead is there. If one moves the laser focus, the bead would move along with it. To exert a pulling force on the DNA that is being packaged, all that one needs now is to trap both the beads in two separate laser foci, and then move the two apart. This was done in beautiful experiments reported in the journal *Nature* [8]. They found that on an average, the packing can take place even for forces as large as 50–70 picoNewtons, making this one of the strongest known molecular motors. The initial packaging rate is about 110 base

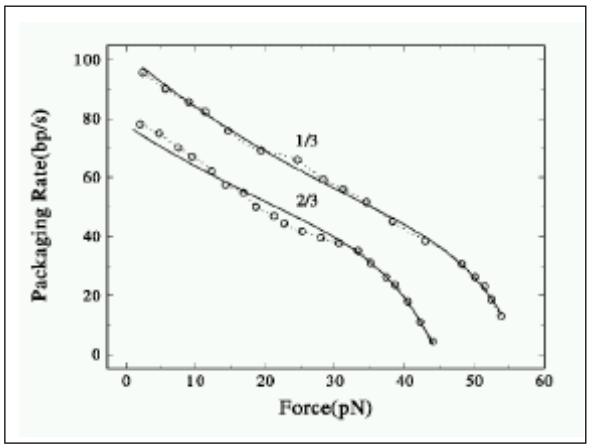

Figure 18. The circles show the rate of packaging as a function of force, for two fractions of packaging, viz., 1/3 and 2/3. The full lines are the results from the simple model [9].

pairs/second, but the rate decreases as the packaging proceeds because it is more and more difficult to push the DNA in. Experimentally measured packaging rates, as a function of the applied force, at two different fractions of packaging are shown in *Figure* 18.

7. Mechanochemistry: Influence of Mechanical Force on a Chemical Reaction.

How does one account for the observed force dependence of the rate of packaging? This is quite interesting, as a mechanical force is able to influence the rate of a chemical reaction. We briefly outline one of the models that has been suggested for this. It is to be noted that this is only one of the simplest possible models and that as more experimental information is obtained, the model may have to be refined.

Now we discuss a simple model for the effect of exerting a mechanical force on the DNA on the rate of the reaction. The model is the simplest one that can be used to describe the process [8]. In chemical reactions, it is usual to think of what is referred to as the reaction co-ordinate, to measure the extend of the reaction. In this case, it is convenient to take this co-ordinate to be the length of the DNA that has been packaged in. A plot of the free energy as a function of the length of the DNA that has been

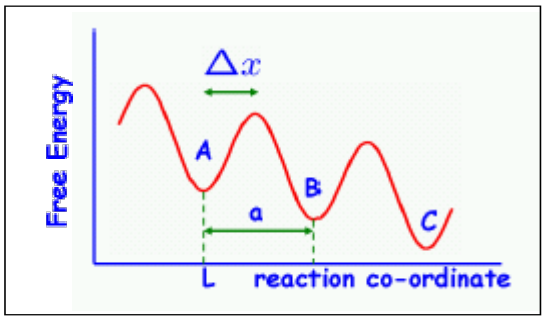

Figure 19. The free energy change as a function of the length of the DNA that has been packaged inside the prohead.

packaged in, is shown in *Figure* 19. At the point A in the figure, a length L of the DNA has already been packaged in. Then the consumption of another molecule of ATP will cause a further packaging of a length a (= 6.8 Å) of the DNA into the procapsid and the system moves from A to B. Even though the energy has been spent for the packaging, a molecule of ATP has been hydrolyzed, and thus the overall free energy has decreased and hence at B, the overall free energy of the system is lower. As this involves a chemical reaction, one naturally expects that there will be a barrier for this step. The barrier occurs at the point Δx away from A. According to the theory of rate processes, the rate of this in the absence of any pulling force would be given by the usual Arrhenius form $k_f^0 = \nu_f e^{-Ef/k_BT}$, where ν_f is an attempt frequency and E_f is the activation energy for the forward (going in) process. Similarly, it is also possible for the system at B to go back to A. The rate of this would be $k_b^0 = \nu_b e^{-Eb/k_BT}$. A pulling force F acting on the DNA implies that in order to reach the transition state, one has to do an additional mechanical work of $F\,\Delta x$, as a result of which the activation energy for the forward process is increased. *Note that the mechanical force changes the activation energy of the process – this is the key aspect of mechanochemistry.* The rate under the influence of the external force would then be $k_f = \nu_f e^{-(Ef + F\Delta x)/k_BT} = k_f^0 e^{-F\Delta x/k_BT}$. Similar arguments lead to the conclusion that the rate of backward reaction would be $k_b = k_b^0 e^{-F(a-\Delta x)/k_BT}$. Hence the net rate of the reaction is given by

$$\begin{aligned} \text{net rate} = k &= k_f - k_b \\ &= k_f^0 e^{-F\Delta x/k_BT} - k_b^0 e^{-F(a-\Delta x)/k_BT}. \end{aligned}$$

This equation can be used to fit the experimental data [8] and the full curve in *Figure* 18 is obtained using this equation. Obviously, this simple model reproduces the experimental data very well. It is interesting to note that this equation resembles the Butler–Volmer equation of electrochemistry [10]. In fact one gets the Butler–Volmer equation if one replaces k by the net current density i and the force F by the overpotential η.

8. Conclusion

Nature is abundant with nano-sized motors. At present there is very little understanding of the way in which they work. We have very briefly summarized what is known about the mechanochemistry of one such motor, viz., the viral DNA packaging motor. It is a fascinating example illustrating how a mechanical pulling force can influence the rate of a chemical reaction.

Suggested Reading

[1] **Feynman's whole lecutre is a must read for any student interested in nanotechnology, The full text is available at: http://www.zyvex.com/nanotech/feynman.html**

[2] **Debashish Chowdhury, Natural Nano-Machines, *Resonance*, Vol.12, Nos. 1 and 2, 2007.**

[3] **Alan A Simpson, Yizhi Tao, Petr G. Leiman, Mohammed O Badasso, Yongning He, Paul J Jardinek, Norman H Olson, Marc C Morais, Shelley Grimes, Dwight L Anderson, Timothy S Baker and Michael G Rossmann, *Nature*, Vol.408, p.745, 2000.**

[4] **The webpages of Prof G Kaiser contain beautiful illustrations of several biological processes. It may be found at: http://student.ccbcmd.edu/courses/bio141/lecguide/unit3/viruses/prodlc.html**

[5] **http://news.uns.purdue.edu/html4ever/001206.Rossmann.DNAmotor.html**

[6] **http://bilbo.bio.purdue.edu/~viruswww/Rossmann_home/index.shtml**

[7] **R W Hendrix, *Proceedings of the National Academy of Sciences*, Vol.75, p.2779, 1978.**

[8] **Douglas E Smith, Sander J Tans, Steven B Smith, Shelley Grimes, Dwight L Anderson and Carlos Bustamante, *Nature*, Vol.413, p.748, 2001.**

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