Cellular Biophysics
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Energy Scale

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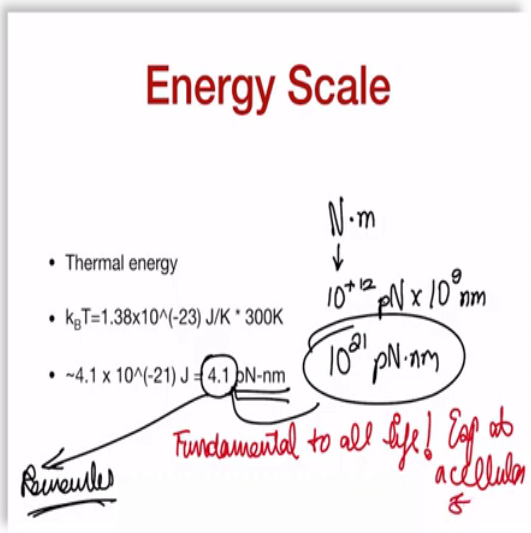

Energy scale is critical for understanding the dynamics of biological processes. In particular we are going to propose and explain in the coming sections how energy scale decides the time scale. But what is this energy scale, how much energy are we talking about? Certainly not like the atom bomb that we spoke about in the earlier sections.

We are talking in biological systems of a very tiny energy scale, these are small systems, these are molecular systems remember. We are talking about the product of Boltzmann's constant k_B times the temperature T which is in SI units 1.38 x 10^{-23} Joules per kelvin as the k_BT value and 300 kelvin which is 23 degrees Celsius, 27 sorry.

This gives us a number which is 4.1 x 10^{-21} Joules, this is the energy scale but this is a very difficult number to remember it is also annoying number to write down 10^{-21}, 4.1. So, we convert it Joules is nothing but newton meter. So, we can write this in terms of Pico newton that is 10^{12} Pico newton and nanometer into 10^{9} nanometer, which becomes 10^{21} Pico newton nanometer. As a result when we now convert this to Pico newton nanometer we are left with only 4.1. So, it is a number to remember and we will come back to it again. Because in some senses this is fundamental to all life! Especially at a cellular scale. Let me see how.

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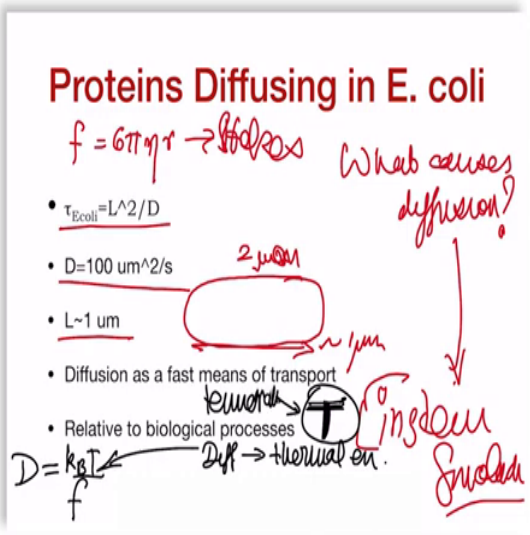

So, for example the diffusion of proteins in E. coli can be defined in terms of time scale which is set by L^2/D, D that is the diffusion coefficient of a typical protein is about 100 micron square per second, L the size of the cell is something in the range of a micron we know it is 2 micrometers in length.

Diffusion is a fast medium of transport. But what causes diffusion? To answer this question we needed a genius. Einstein and Smoluchowski and you may remember that we defined the drag coefficient f as $6\pi\eta r$ from stokes law. And therefore we get diffusion, the diffusion coefficient is equal to k_BT by f. This means that diffusion is related to thermal energy, thermal because T, T is nothing but temperature.

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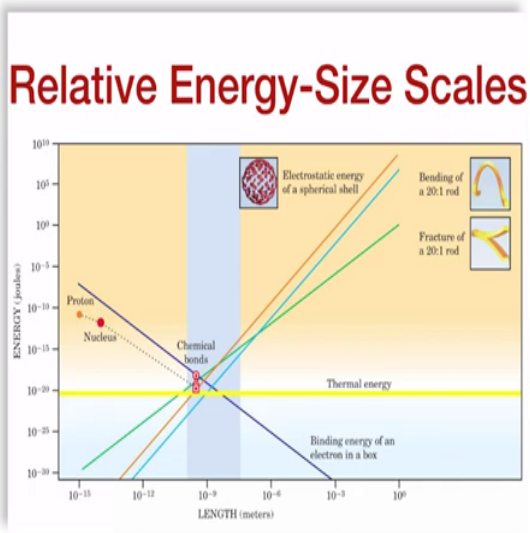

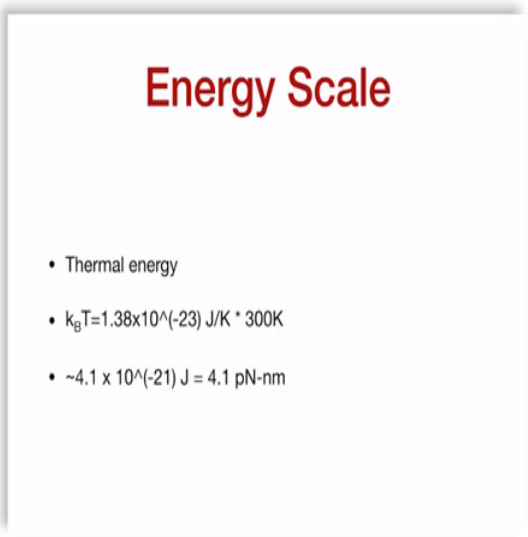

So, when we now consider this temperature thermal energy scale 4.1 Pico newton nanometer to remind ourselves or 10^{21} Joules is here, this is the horizontal line. You are looking at a graph of energy on the y-axis in Joules and the length in meters on the x-axis. This length goes from 10^{-15}, so 10^{0} meters is 1 meter, millimetre, micrometer, nanometer, Picometer, femtometer.

And the scale as we have discussed in the past of cells is over here which means that the thermal energy is greater than these lines. So, what are these lines? Now, if you consider any physical law, we always want to know how it scales. Meaning to say if you change some

independent variable or some variable what happens to the outcome there, final so-called outcome variable.

Here our outcome variable is energy, by a rule that we have not yet studied if you look at the energy of fracture or bending of a rod whose shape is defined by a factor 20 to 1 then you will find that as it goes to smaller and smaller energy scales, I am sorry length scales, so as you go down this way or down this way, somewhere the energy becomes comparable to thermal energy.

Now, for bending rods this is at nanometer scale, you could say roughly protein scale. Something above it already the energy is higher than thermal energy which would suggest that purely thermal energy will not bend a rod of ratio to 20 is to 1. At the same time or requires an energy which is higher than the thermal energy.

At the same time chemical bonds which exist in this length scale of 10^{-9} and 10^{-10} angstroms to nanometers transition. The energies required to form and break them are very comparable to thermal energy, this is suggesting to you, that thermal energy or k_BT has very important role to play when the size scale is small enough, namely here.

And when the size scale is bigger we need more energy input in order to perform this kind of activity. So, this scaling graph is telling us quite a few different things there is also a third one which I did not talk about that is electrostatic energy of a spherical shell. Again large spherical shell of meter scale versus micron scale versus nanometer scale, the energies change and we find that we come back to thermal energy scales at nanometer-sized objects.

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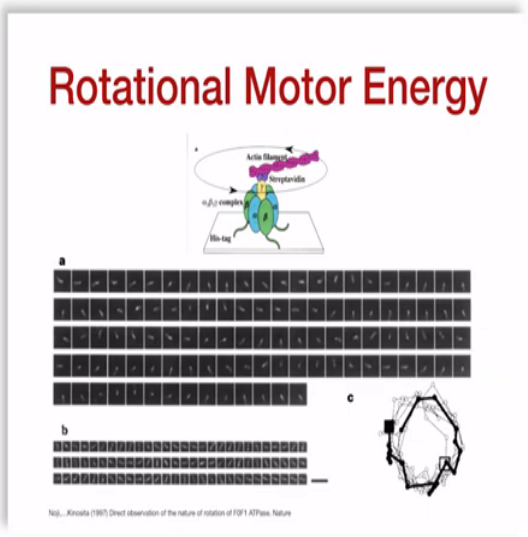

And an exhibition of how the thermal energy and chemical energy interact with each other is in molecular motors. The rotational energy of a motor, F0F1 ATPase driven by ATP activity was measured by Noji and Kinoshita where they attached a cytoskeletal filament as marker of rotational motion. Imagine that we are looking at rotational motion of nanometer sized objects, this is very hard to see, you cannot resolve it in fact.

So, to overcome the limitations Kinoshita and company attached an actin filament with this biotin streptavidin linkage to the rotor and then saw this beautiful rotational trajectory which they could map as start and endpoints demonstrating that the direct observation of rotation of F0F1 could be observed and that because of the presence of ATP this was able to perform despite thermal energy which was ubiquitous because it is performed at room temperature.

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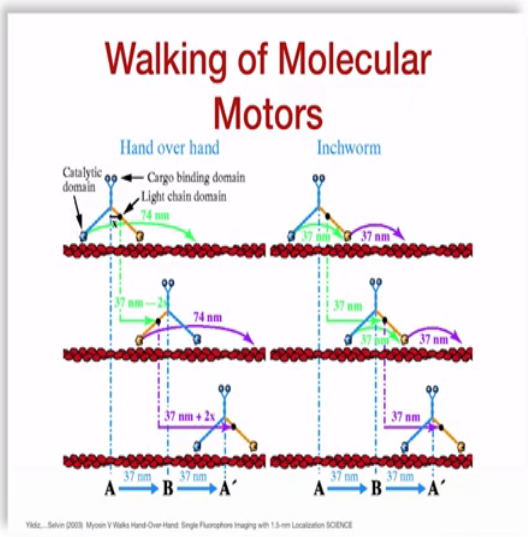

Another classic example is of walking molecular motors and the question about whether they work hand over hand over inch one which has trouble a lot of motor people for a while. What you are looking at here are schematics from Ahmet Yildiz and Paul Selvin's paper demonstrating this particular people's publishing science in 2003 that a tag on the light chain domain that binds to the leg of myosin would exhibit initially only a 37 nanometer displacement and then a 74 nanometer displacement if it was working as a handover and an inch worm would demonstrate 37 nanometer equal steps.

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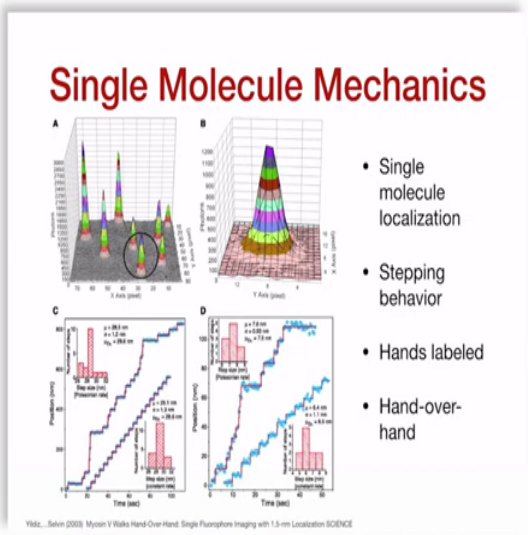

Given this they, when they examined the experimental data first they had to figure out how to detect single molecule and they used point spread function mapping to attain single molecule localization and they did a lot of tests to confirm that this was happening.

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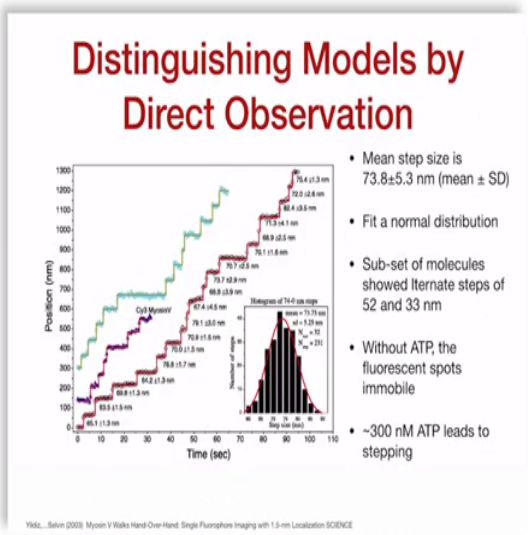

Followed by which they could then show that by direct observation a single ATP molecule led to motion which led to this frequency histogram of step size which had as its major peak 74 nanometers approximately 73-75. This means that ATP drives the motion, in fact, without ATP the fluorescent spots are immobile, not diffusing and the reason for that is because even without ATP the water can fight to the filaments.

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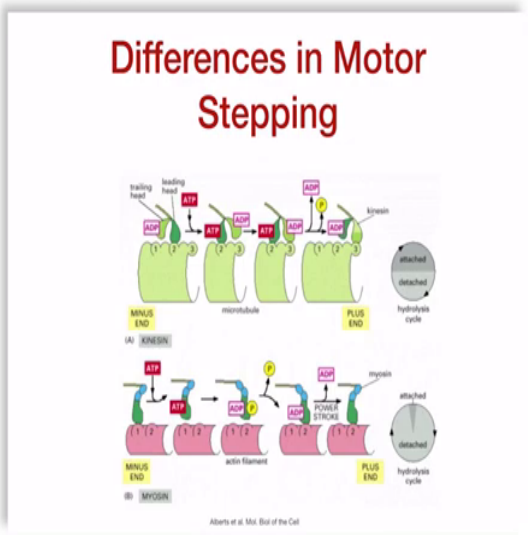

So, in summary the differences in motor stepping of course are based on attachment, detachment and hydrolysis cycles. And diffusion and thermal energy have a role to play when the motor is detached, but when it is attached ATP dominates.

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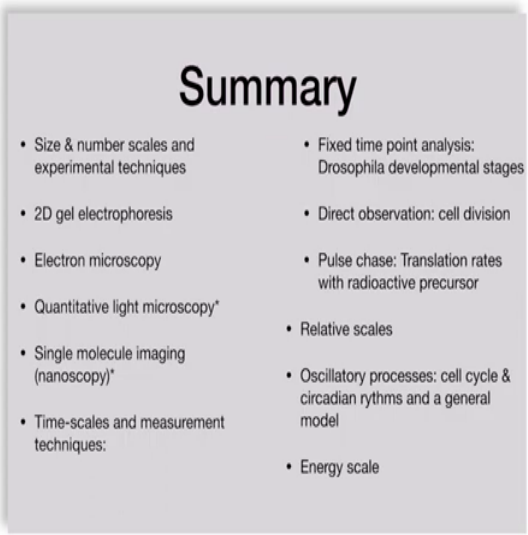

So, in summary we have talked about numbers and scales and experimental techniques to analyze them. We have talked about four different methods 2D gel electrophoresis, a brief introduction to electron microscopy, quantitative light microscopy and nanoscopy. And we have then discussed methods to measure time scales, fixed time point, direct observation and pulse chase.

Relative scales we have not discussed in great detail but we have gone into energetic relative scales. We also spoke about oscillatory processes cell cycle and circular rhythms and how at the end thermal energy plays a role in determining energy scales.