Cellular Biophysics
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Approximating Cellular and Molecular Size Scales

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Hi. So, with the goal of getting a feeling for numbers, in terms of times, energies and sizes, and concept of cellular organization.

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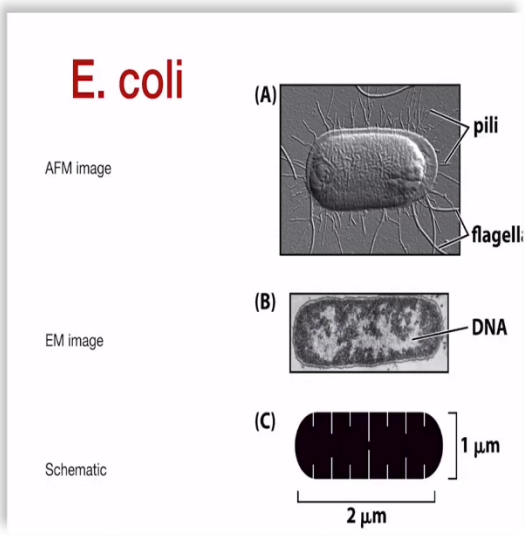

We are going to dive into the hydrogen atom of cells, namely, Escherichia coli. Take a look at images on the right. What you are looking at are atomic force microscopy, electron microscopy and schematic views of cell. The atomic force microscopy image, demonstrates cell which has membrane layer, something that looks like an inner membrane layer, some density in the middle something that is undulating, and then pili and thicker structures called flagella.

Now, some of you may be familiar with the microbiology of it, and know that the pili used for sexual reproduction and the flagella for motility or movement. An electron microscopy image shows a density map with less dense and more electron dense regions. Providing us a clue that the DNA or the chromosomal material is spread throughout the cell. This is an interesting idea. And we will come back to what it means for the chromosomal DNA to be spread through.

Finally, in the lowermost diagram, you see a schematic where the cell is represented as a sphero-cylinder or a scale bar you may say, length of 2 microns with a 1 micron. Now, this schematic with these artificial scales is meant to remind you that while we know that cell is more complex, it has other structures, it may not always be only 2 microns long, sometimes it will be 3 microns, when it is dividing, it is 4 microns, some individuals may be variable, etcetera, etcetera, that we can make a simplification. And that simplification will aid us in terms of making sense of what we are observing. So, let us see what we can do.

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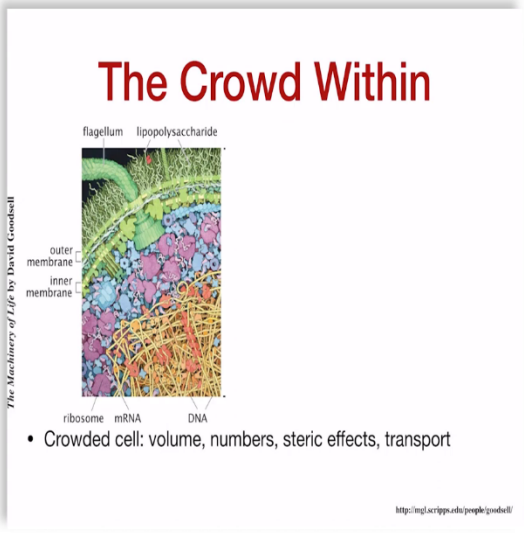

Now, it turns out that this cell is not just a small bag which contains molecules, but it has a crowded environment inside the intracellular region of even E. coli, a simple E. coli cell consists of ribosomes, mRNA, DNA, proteins that are diffusing of different sizes. And then at the membrane, there are inner membrane and outer membrane proteins. And what you are looking at is the flagellum itself, the flagellar motor as it is called.

On the outer side of the cell, as we go outwards is the lipopolysaccharide layer with a dense meshwork of fibers that are polysaccharides sugar molecules. Many of these are vitally important in disease and disease development. And this figure comes from David Goodsell, whose illustrations and biochemistry are very well known.

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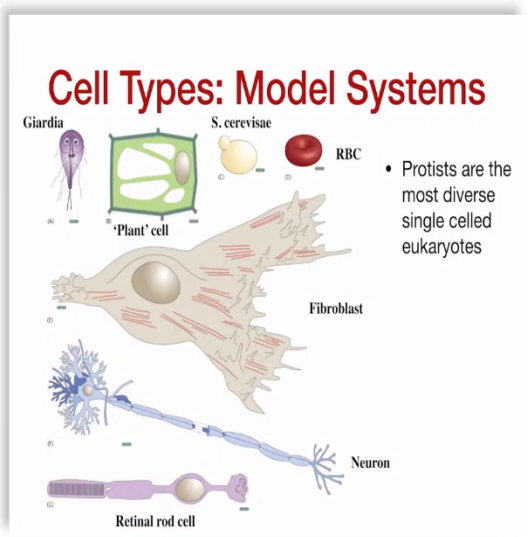

But when we look at other cell types, we find suddenly a huge diversity. What you should notice that the scale bar is still there. So, in other words, giardia, which is drawn, which is depicted here, is in length, roughly 1, 2, 3, 4, about 5 times as long as an E. coli cell and about twice as wide as the length. In other words, it is about four microns in this and about 10 microns in this dimension.

A plant cell is enormous comparatively, you can say 1, 2, 3, 4, 5, 6, 7, 8, 9, 9 to 10. E. coli cells will fit in along the length and about 5 in the width. A cerevisiae cell is closer with about 1, 2, 3, 4 maybe, so 6 microns across, about 4 or 5 microns across in the short axis. And this little structure here is the bud.

Saccharomyces cerevisiae as some of you may remember from biology lessons is a very important yeast, one it is important because it has been used and harnessed for making bread and wine and beer. And through human history, people have been doing this. But second, it is also an amazingly versatile tool for genetic research. In fact, for cell cycle research, and cell cycle research as some of you may know, is important for cancer research.

So, studying a very strange, a simple, humble yeast in basic science applies to make better sense of cancer, which is an applied problem or very, very difficult and uncurable disease with huge diversity across people. What you are looking at here is about 7 micron across red blood cell with the doughnut shape. And this enormous pizza slice like shaped cells are fibroblasts, it is what is available in our skin, our skin cells look like this.

Finally, we look lower below, in F at our neuron, which has the classic Soma, dendrites and along the axon, the axon of some neurons can extend up to 1 meter, these are spinal cord neurons in human beings. We also have retinal rod cells, which are consist of a stack which is photosensitive and a neuro active transmitting part at the base.

So, on top of all this, we have chosen a bacterium E. coli because it is very well studied, hydrogen atom as its said, but there are many more types of cells. And this diversity is what makes cell biology both fascinating and difficult. And we try to make some measure progress to our approach of quantification and numbers.

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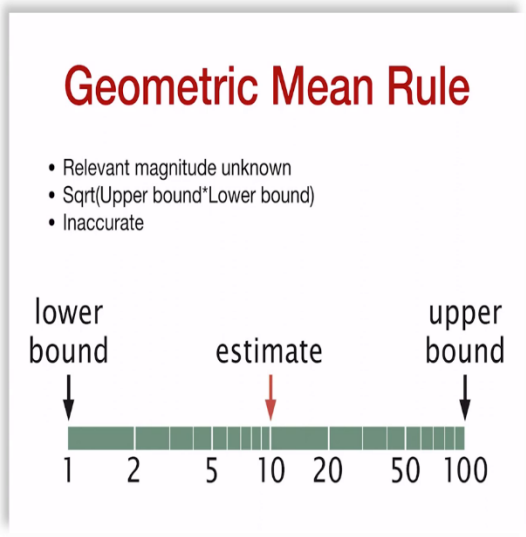

One of the rules I want to introduce to you is a geometric mean rule. So, when I want to find out what is the estimate of a certain value of something, let us say I want to know the size of a typical red blood cell, I know that the lower bound should be 1 micron, it cannot be smaller than an E. coil cell and E. coli and other pathogenic organisms, other pathogenic organisms like plasmodium, in fact, this must be bigger and lower bound is 1 micron. Upper bound it cannot be bigger than 100 microns because 100 microns is the size of a fibroblast or even bigger, so it has to reach that.

The estimate is a geometric mean, that is to say multiply the two numbers and take the square root of it and find 10 microns and the estimate, that is very close to the diameter of about 7 micron of a red bold cell. So, when the relative magnitudes are unknown, we put an upper and lower bound, multiply them take the square root and get a number, it is inaccurate, as you saw 7 microns was 10 but it is a good idea to get ahead. So, let us use this on something that we are not necessarily familiar with because we will use the simplicity to get some clarity.

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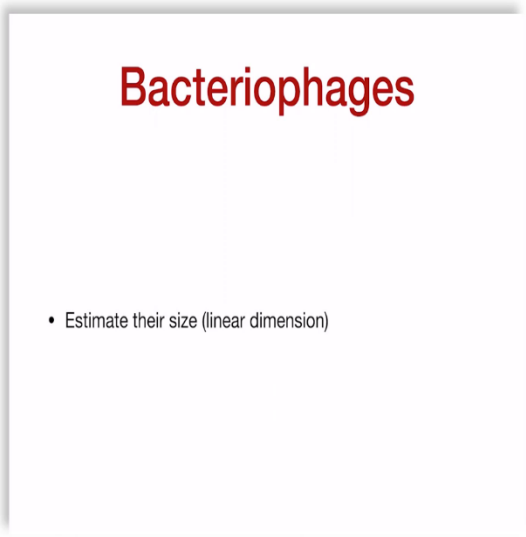

Bacteriophage, how can you estimate their size and linear dimensions?

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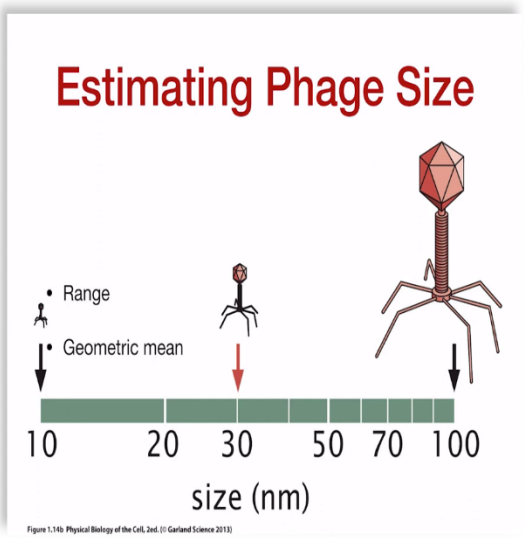

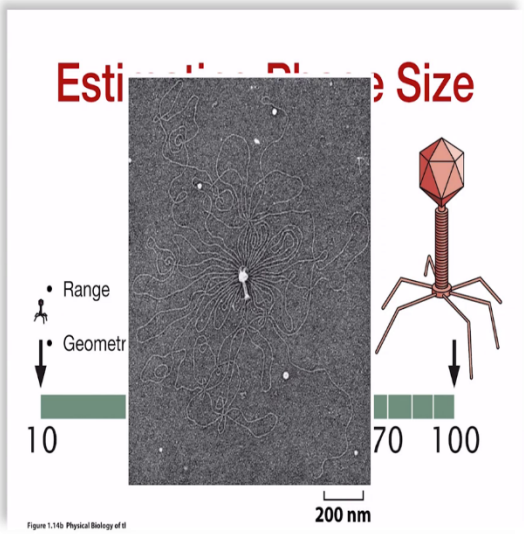

So, we can say that a bacteriophage must be smaller than an E. coli cell because it has to infect it, it has to go inside it maybe about a 10th of the E. coli cell. So, 1 micron is a cell size of E. coli. So, 100 nanometers must be 0.1 microns must be the size, upper limit of bacteriophage. But it cannot consist of just 1 or 2 atoms, so it must be about 100s of atoms, 1 atom is 0.1 nanometers, 100s of atoms is 10 nanometers, so we can consider about 10 nanometers as the lower bound, multiply 100 by 10, we get 1000, take the square root, we end up at 30 nanometers.

This is actually not a very, this is not a very bad estimate because as you see with this scale bar, about 60 to 100 nanometers is the size of the bacteriophage, t7 in this case. In fact, the same

image, this image is an electron micrograph classic one seen in many textbooks can be used to also estimate the genome length of a bacteriophage. Based on the counting of the strand of DNA that has come out when this is osmotically lysed and fixed an image. This is a very similar exercise to the one that Geoffrey Taylor performed for the atomic bomb. And I encourage you to try to make your own estimates and check. Do not cheat, try to do this yourself and work your way back.

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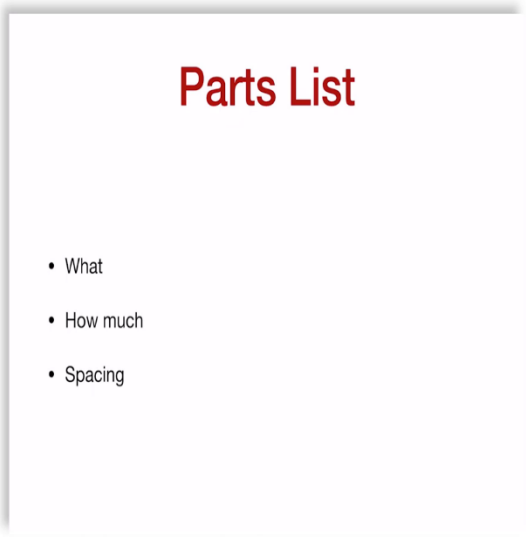

In order to get a good handle on the interactions between cells and their components, we need parts, we need to know what is there in the cell, we need to know how much of it is there, we need to know how much it is spaced.

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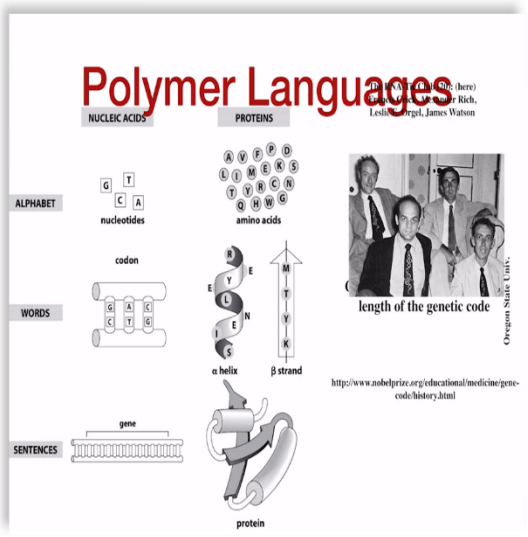

So, for that, we need to go back to biochemistry. And we know that there is a polymer language in cells, alphabet of nucleic acid is G, A T G C. The words are codon that is ATGC arranged in triplets and sentences are the genes. You will be surprised to know that after so many years, decades of working on genetics and genomics, we are still nowhere closer to a universal definition of a gene, what is a gene.

Those of you who are appearing for competitive exams, please look this up in a textbook. Because these answers are not as simple as this is, it is a very easy question to ask what is the gene, some of you will say the coding sequence, some of you will say the collection of triplets that makes sense, some of you will say it could be coding or non-coding, some of you will say that you have a promoter and some of them, this gets more complicated.

In a way the purpose of bioinformatics is to find how nucleotides form sentences, how the language of the genetic code is written. Proteins on the other end consists of amino acids which are more complex. You should go back and look how many amino acids that are naturally occurring. And you will find that some of the famous tables of amino acids, give a very nice illustration of the question I asked you.

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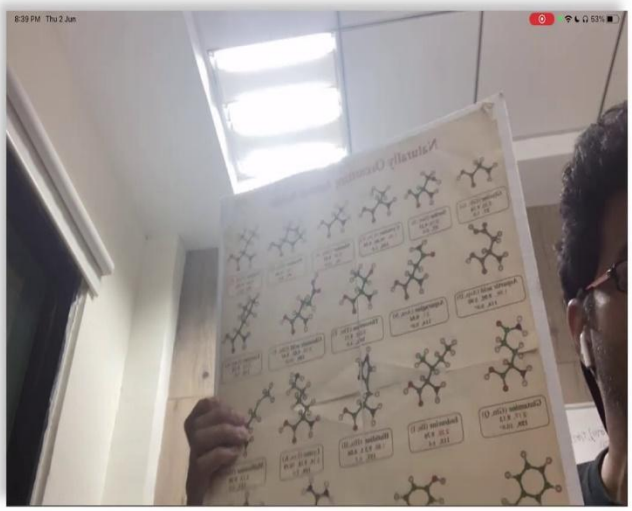

So, this table here is taken from the magazine resonance. And you can see the naturally occurring amino acids 1 2 3 4 5 6, 1 2 3 4, 24 in this particular case, and 21 is what I remember, and you should go back and look.

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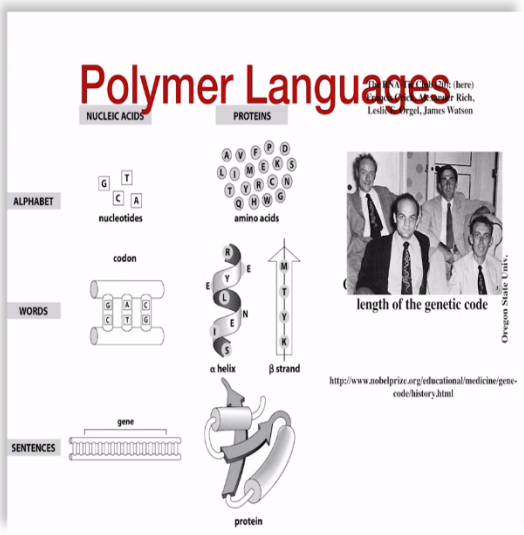

But the most important part is that those amino acids they form sentences, they form sentences out of words. And those words form sentences. And in some senses, the words are a little more complex in amino acids as compared to nucleotides, that is DNA or RNA. Because alpha helix and beta strand are the most easily identified structures. But forming a protein is not trivial.

And the universal solution to protein folding is still elusive. What you are looking at in the right-hand side is an image of the pioneers of genetic code cracking, which includes Watson and Crick, Lee Orgel. And it is part of the history of genetics, which all of you who are interested in the field of biology should be familiar with.

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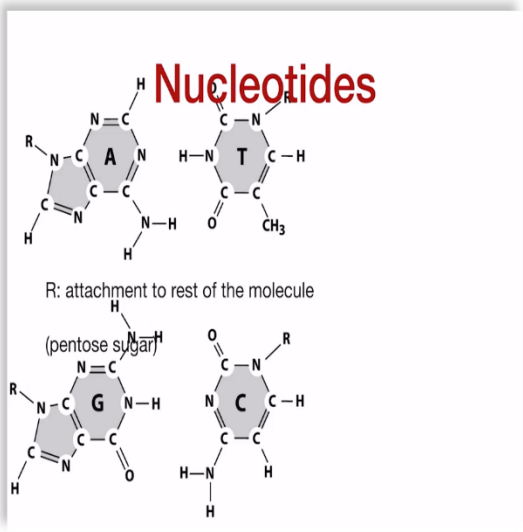

Nucleotides look like this with A, T, able to form a non-covalent bond through hydrogen bonding and G, C forming three not two non-covalent bonds.

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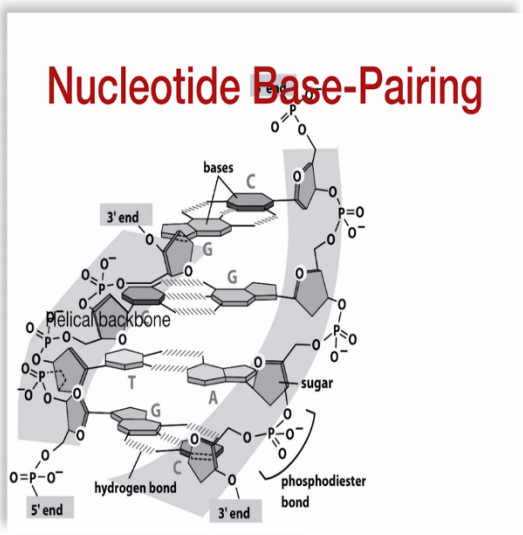

In a manner that gives rise to the base pairs that we are familiar with the helical backbone is made up of sugar molecules. The name DNA comes from deoxyribose sugar, one of the OH groups is lacking.

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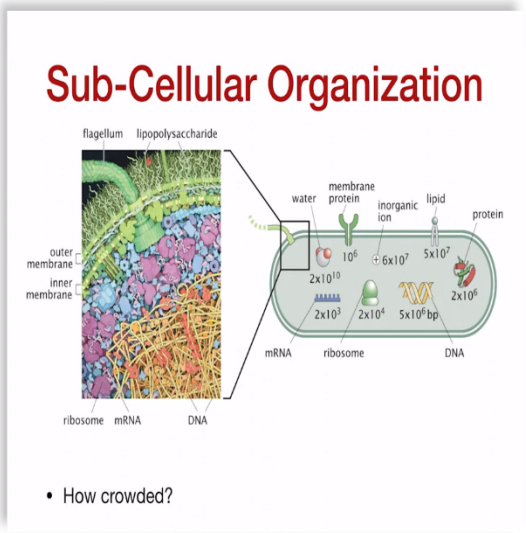

And then going zooming out again to the larger structure of a cell we know that the sub cellular organization just like the good cell cartoon had indicated, consists of water up to 10 to power 10 molecules, mRNA 10^3 molecules, 1000 roughly, 10,000 ribosomes, 60 million inorganic ions plus and minus, 50 million lipid molecules, 2 million proteins, and 5 million base pairs.

So, if you remember the genome length of E. coli is 4.8 into 10^6 base pairs. Now, for a cell to contain all this means that it might be very crowded, there is so many molecules in that. How crowded is it? It is my favorite question.

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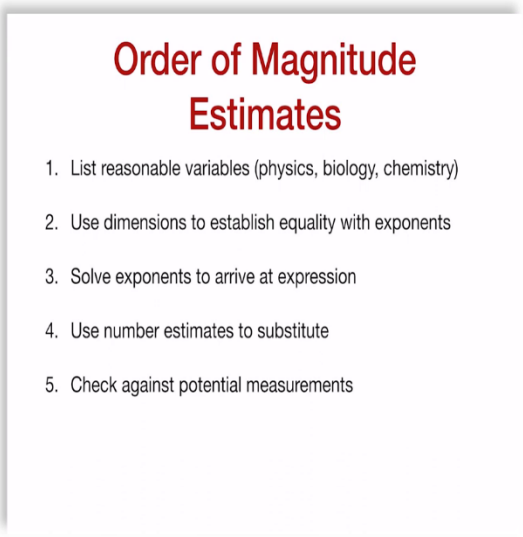

So, in order to do this, we need to make order of magnitude estimates. So, we need to list the reasonable variables, use dimensions and equality, establish equality with exponents, solve the exponents to arrive at expression and use the number estimates to substitute and check against potential measurements. You remember, this was exactly what G I Taylor did in order to estimate the yield of the bomb.

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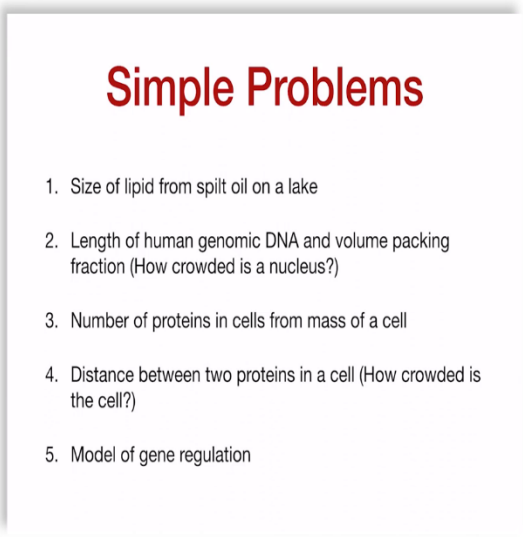

So, we are going to take a simple problem for this order of magnitude estimate problem. That is to say, we want to know what is the size of the lipid. But we need some data. So, it turns out

Benjamin Franklin, who is also one of the early presidents of the United States was also an experimentalist, he liked to do scientific experiments, he was an enlightened person, he read a lot and he tried things out there, some of his experiments were pointless some of his experiments are elegant, one of his experiments was to take oil, olive oil and spill it on a lake on a calm day when there no wind and he measured the size of the oil spill that he observed.

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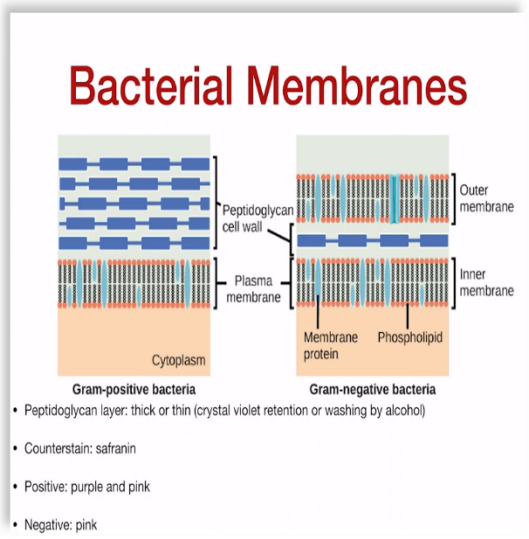

Now, we know that lipids form membranes, and in bacteria, gram positive and gram negative have slightly different structures, gram negative is like E. coli, gram positive you may say bacillus. The gram positives are a result of peptidoglycan layer which will retain crystal violet if it is thick or not retained if it is this thin when washed with alcohol. So, gram negative bacteria will turn pink because of the counter stain and will be purple and pink if they are positive, gram positive.

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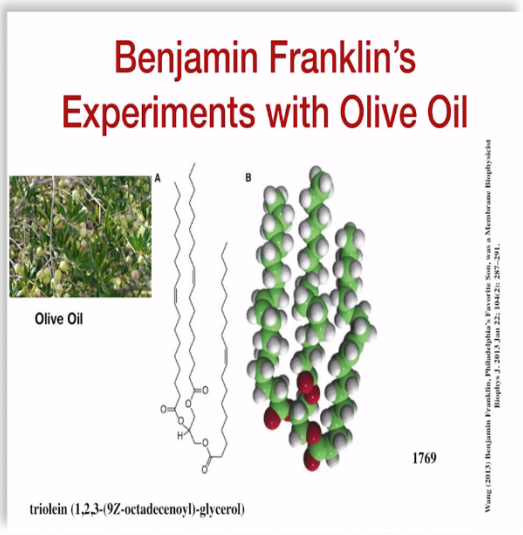

So, this Benjamin Franklin experiment, which involved lipids, the same lipids that are in the membrane consists of pouring olive oil which is mostly triolein 1, 2, 3, 9Z octyldodecanol glycerol. Experiment was done by the way in 1769. So, it is a classic experiment.

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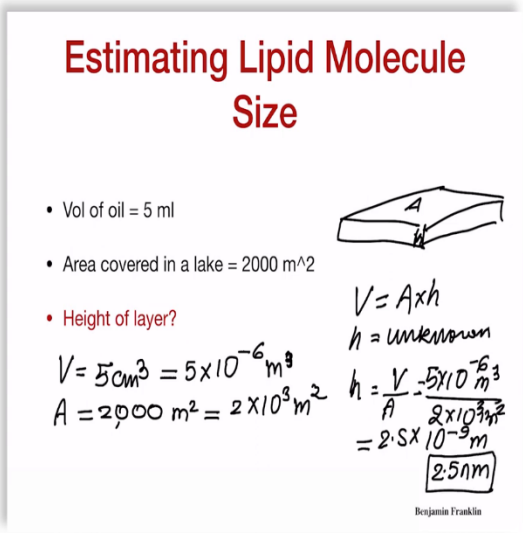

What he did is poor 5 milliliters of oil and covered an area of 2000 meter square, find the size of lipid layer, which is another way of saying what is the height of the layer. If we take our approximations seriously then we can say that 5-centimeter cube is the volume, area is 2,000 meters square.

So, 5-centimeter cube is 5 into centimeter to meters 10^{-2}, cubed is minus 6, meter cube, this is 2 into 10^{3} meter square. If we assume that the surface was covered uniformly then the volume is equal to area into height. But since h is the unknown, we can say h is equal to V by A which is 5 into 10^{-6}-meter cube upon 2 into 10^{3}-meter square. This leaves us with 2.5 into 10^{-9} meters. If you remember your units, this is 2.5 nanometers.

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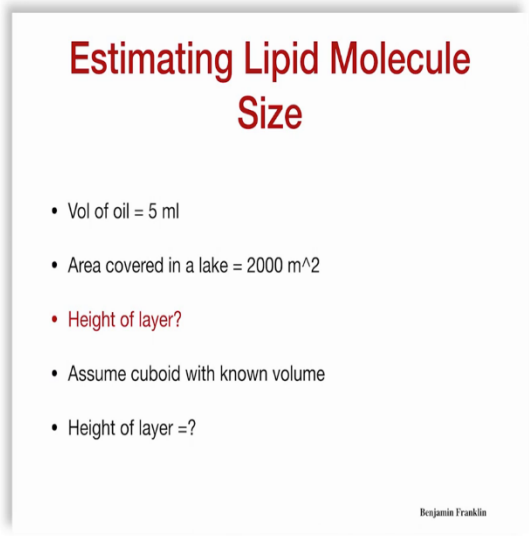

Surprisingly, this experiment performed so many years ago in 1769 give a fairly accurate estimate of the height of a lipid molecule, 2.5 nanometers. So, you see that assuming cuboid with known volume and some information, gives us very good estimate of molecular sizes.

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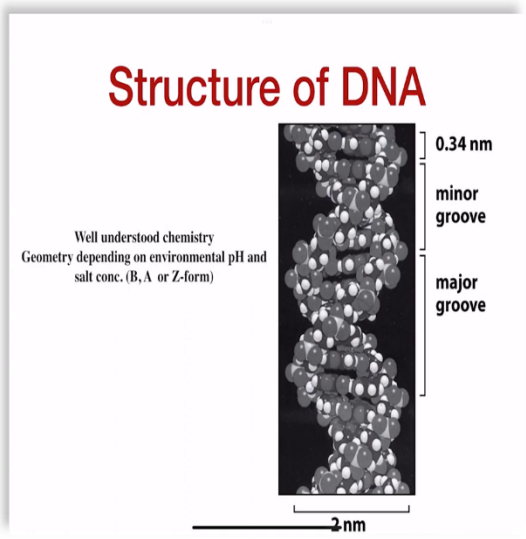

We are going to do similar ideas with the DNA