Cellular Biophysics: A framework For Quantitative Biology
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Molecular Nature Of Water And Statistical Redundancy

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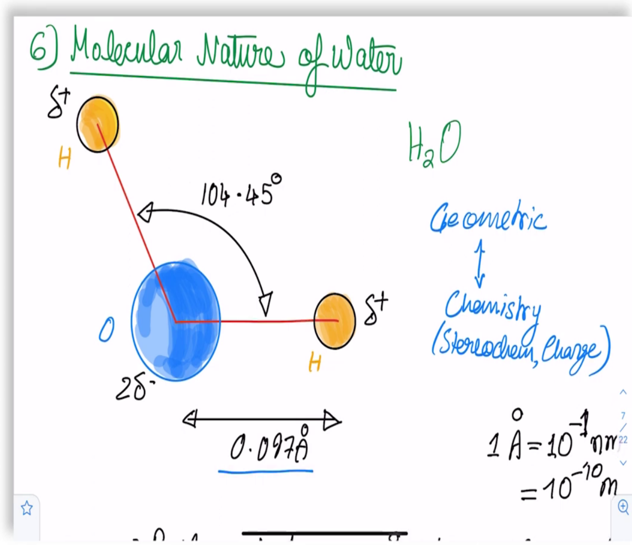

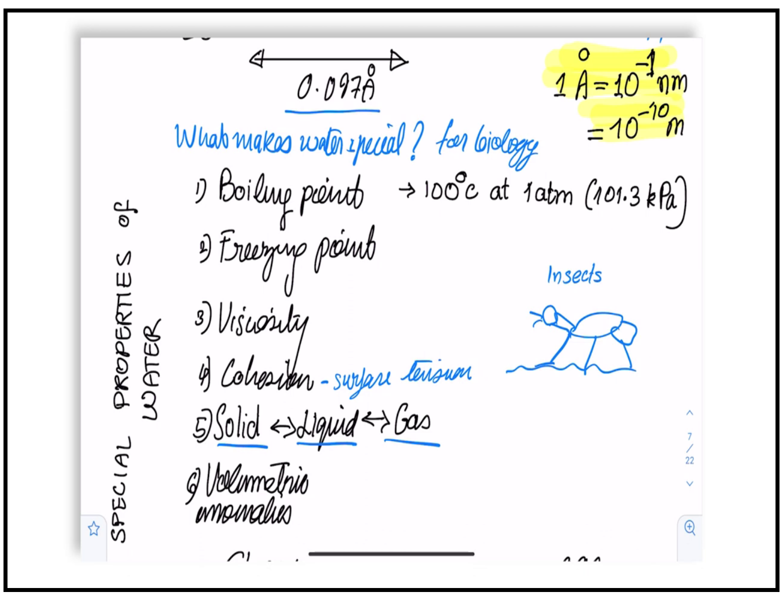

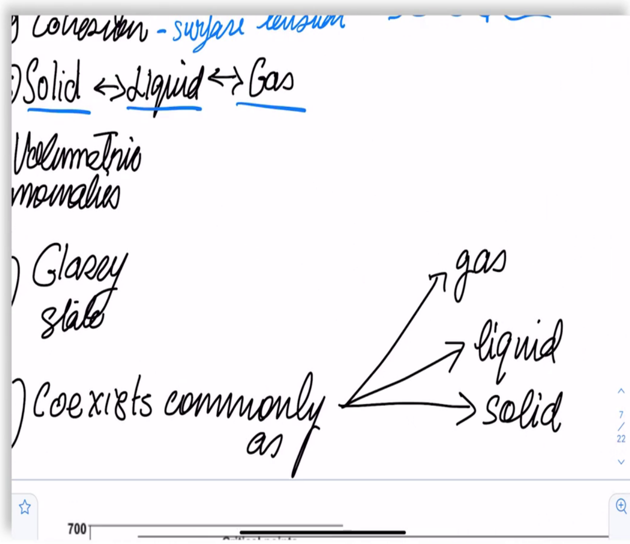

Hi, welcome back. We so for spoken about contrast between Newton's laws of elasticity and viscosity, the idea of viscous and elastic properties, Newtonian and non-Newtonian behavior. And now we would attention to a little bit more detailed view of water at the level of many molecules. So, this is the cause of the heading, which is the molecular nature of water.

What we are going to talk about now is to see if we can put what we know about the shape and structure of water into the context of its physical properties. And finally, do what I have been promising so far, which is related back to the biological relevance of this so, if we go back and look at our classic view of the molecule.

That is water it is, indeed, as everyone with a basic high school education knows H_2O where two hydrogen atoms bind to one oxygen atom the structure what is interesting, because as you see here, there is a central oxygen, which we say has a sort of charge, which is twice delta(δ) negative, and there is a perhaps balancing charge of small delta(δ) positive on the hydrogen.

And since there are two of them as delta minus delta plus plus and delta 2 delta minus the angle formed between these two in at the vertex of the oxygen is indeed the 104.45°. And the vertex of course, being defined by oxygen, the length of the bond OH is found to be 0.97 Angstroms. And this allows us to now think of water as a sort of geometric object. This is in contrast to what you traditionally read in the context of water in terms of the chemistry I mean, even add stereochemistry or charge.

And you will see that this approach has some advantages. Just to remind you, 1 Angstrom is nothing but 10^{-1} nanometers. It is important because in biology, we often use angstroms, because it is a convenient measure at a molecular scale or atomic scale. But SI units mean that it is meters, meters, is 10^{-10} meters for 1 angstrom. Or in other words, 1 nanometers, is equal to 10^{-9} meters.

So, why is water so special? What makes water special? We are going to first talk about what this is in terms of the physical chemistry of it, the physico chemical properties and then we will try to see if that allows us to answer the question. For biology, what makes it special? So, we are aware of the boiling point is 100° at 1 atmosphere, that is to say 101.3 kilo Pascal.

The freezing point is a triple point. The viscosity has unusual properties that arise out of a hydrogen bonding network there is cohesion, which leads to things like surface tension allowing, if you remember, on a static body of water, certain kinds of insects to walk on it water striding insects and we will not have time to discuss it, but these are all due to surface tension.

The triple point of water that I mentioned earlier allows us to observe that if we play around with temperature, pressure conditions, that water can coexist at the same point in P-V-T space that is pressure, volume, temperature space as a solid or a liquid and a gas. This makes it very unusual. I do not know if you realize this.

But think of any other liquid which coexists as a solid liquid and gas at the same time, think about ethanol, this is what is in the drinks that some people drink or think about petrol, or think about what are the fluid you want to think about ammonia the gas or you think about oxygen does it exist as a solid liquid and vapor at the same time, I mean you do not find this. And there are interesting volumetric anomalies, there is a glassy state and this coexistence makes all of these make water to be an unusual fluid.

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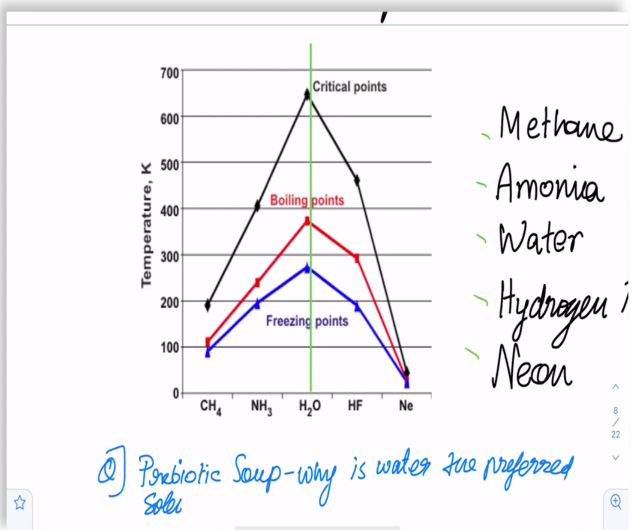

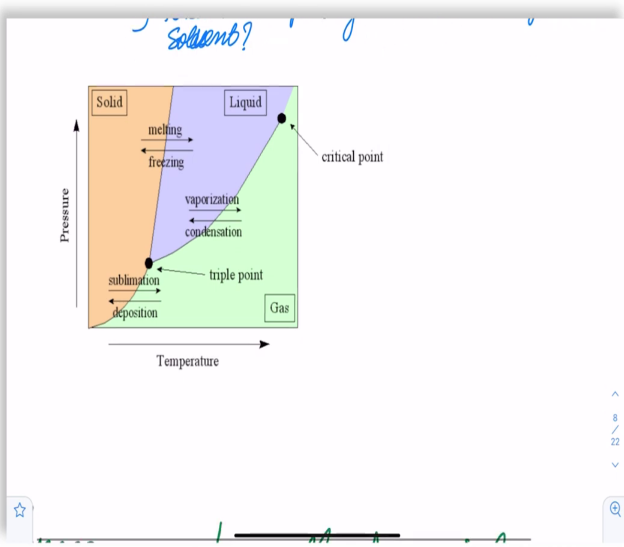

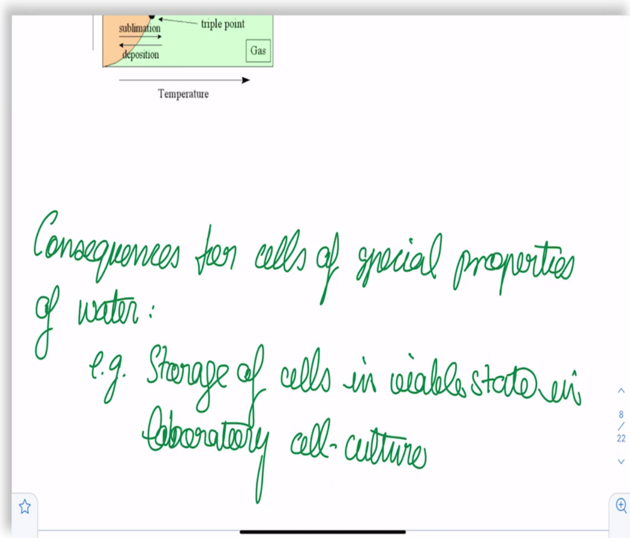

Indeed, anything we say about water in terms of being special is with relation to other fluids. So, here is an example of the temperature dependent state changes in the freezing and boiling points meaning to say the temperature below which that fluid will become a solid. And above which it will become a vapor or gas what you should notice is that our favorite fluid namely water has a freezing point higher than all others.

Boiling point or higher than all others and critical point higher than all others. And by others, we mean Methane, Ammonia, Water, Hydrogen Fluoride, and Neon. And there is a lot of work on the prebiotic life that has asked the question whether in the prebiotic soup why is water the preferred solvent?

And in order to answer some these questions, and we are not going to answer them, you actually go back need to go back and look at the geochemistry of prebiotic earth. And this is a topic unto itself. I think for those who are interested in this you should actually go and listen to lectures on the origin of life on earth and so called chemical evolution of life and the biological chemistry of it.

Suffice to say that, this is sort of the triple point that I was mentioning in terms of temperature pressure, phase diagram of water, where a transition between gaseous solid and liquid phases exists with these critical points marked that lead to transitions between the three states in the lowercase at the triple point and a critical point of transition from gas lower into liquid.

And these terms sublimation, deposition, freezing, melting, also tell you that you can go in the case of water from gas to solid directly. So, basically go from water vapor into ice or snow and I am sorry, that is deposition and from ice or snow to go straight into water vapor that is sublimation, and melting, freezing, you are familiar with vaporization and condensation, you are also familiar with.

So, this is sort of a very nice reminder that water has amazing physical properties and the chemistry that is related to it helps us understand it, but we want to know what the consequences of the special properties of water are for cells. One example is what happens when we do cell culture and those of you who have ever grown bacteria or fungi or mammalian cells.

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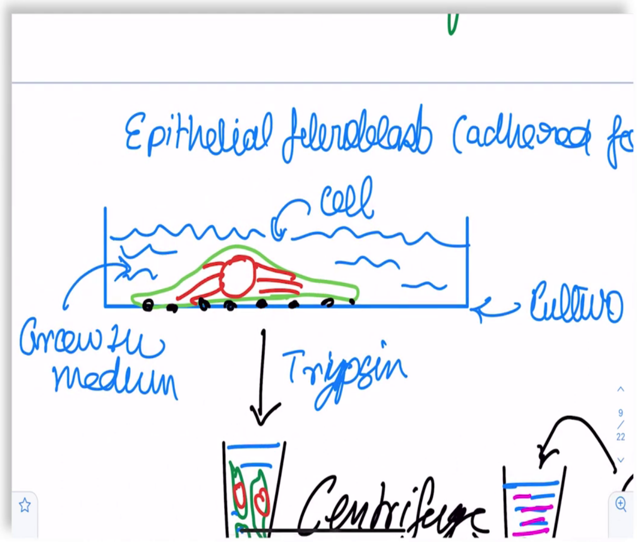
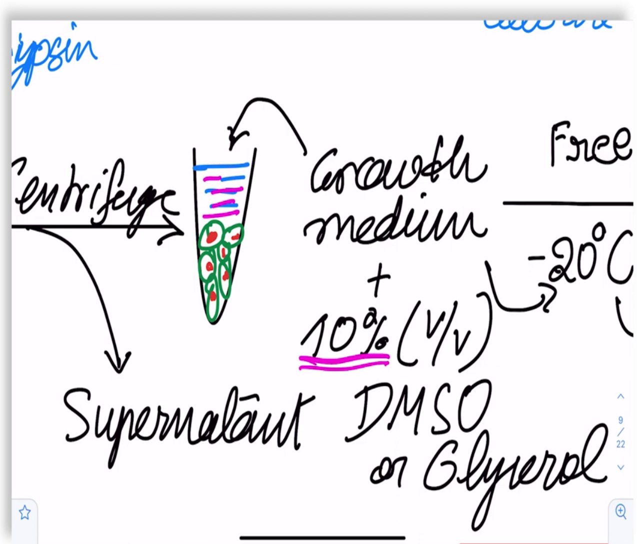
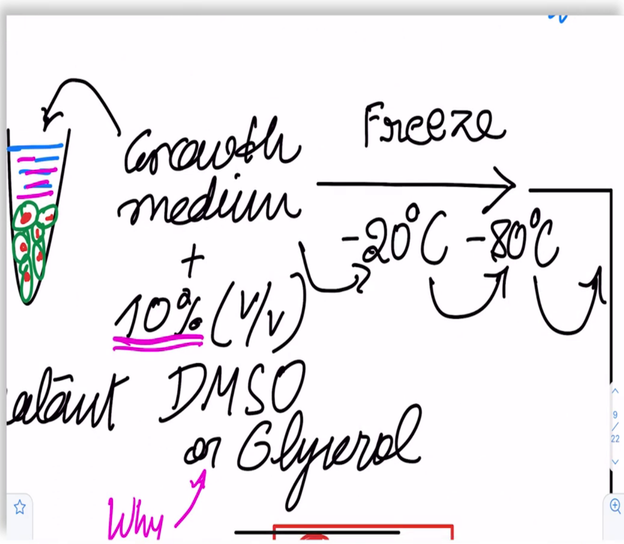
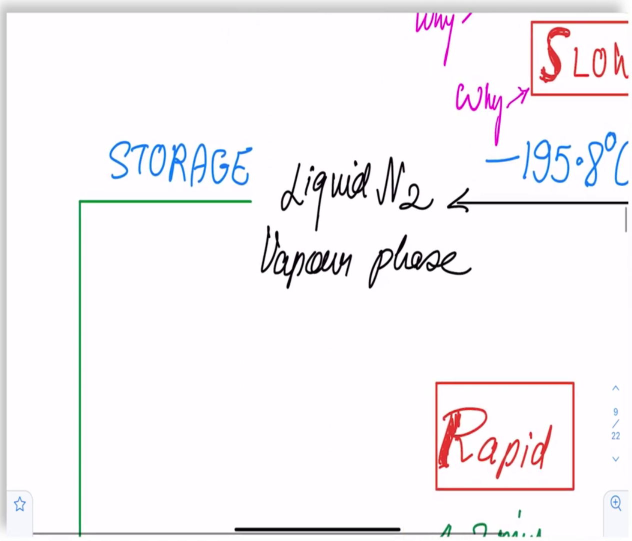
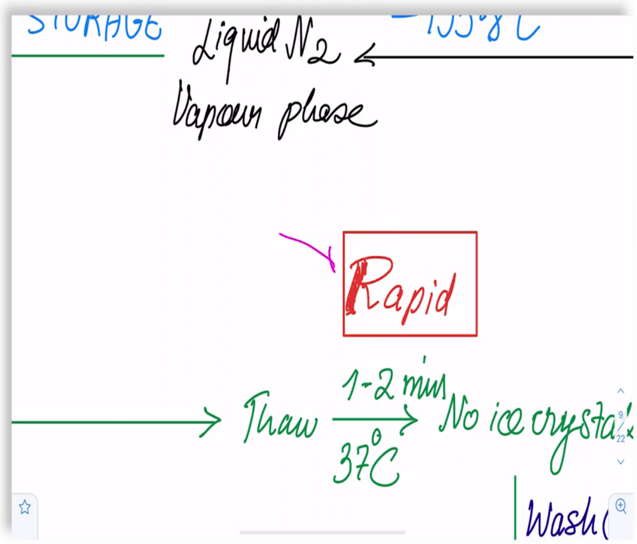
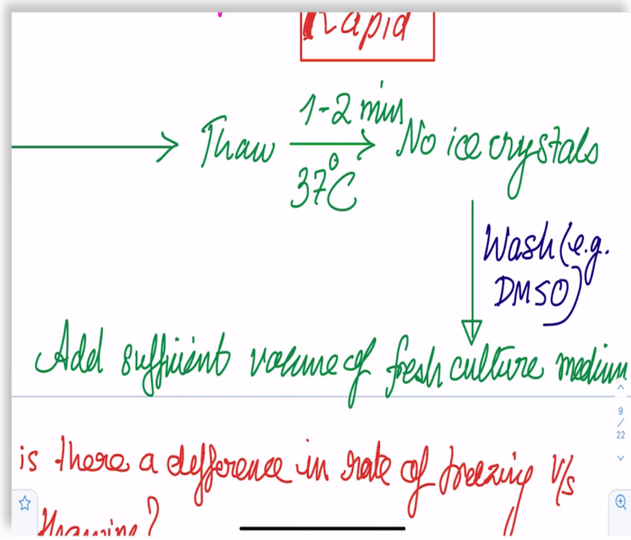





Growth
medium
+
10% (v/v)
alant DMSO
or Glyerol
Why
Freeze
-20°C -80°C
9
22


Why
SLO
Why
-195·8°C
STORAGE
Liquid N2
Vapour phase
Rapid
9
22





And this diagram summarizes how for example, Epithelial fibroblast cells in an adhered form can be stored for long term use storage in a frozen state. So, the idea is that they are going in a petri dish in a culture dish, the cells are submerged in growth medium, and you need to detach them typically this is done using Trypsin, which breaks the bonds of the cells to the substrate.

Very suspended in a tube the tube centrifuged to get rid of the supermatant add fresh growth medium along with the cryoprotect and this sort of depends, but typically, you will find protocols or reproducible methods recommend to you 10 percent volume by volume of DMSO, or glycerol. My first question to you is why? Why do we use DMSO or glycerol?

Then, the next step is cooling down the culture by gently freezing it in a stepwise manner with -20, -80 and followed by minus 195.8° Celsius in liquid nitrogen vapor phase. So, the next question I have is why, why do we do this process so, slowly. And then we store it, the second step, which is when we actually would use it, we revive and that is rapid.

Where we thaw it for 1 to 2 minutes, 37° Celsius for example, with these epithelial cells and observed in the point that there are ice crystals then wash meaning to say, centrifuge, discard the supernatant replaced with growth medium and fresh culture, medium and growth.

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Wash (e.g. DMSO)

Add sufficient volume of fresh culture

Why is there a difference in rate of freezing and rate of thawing?

Why do we add Glycerol or DMSO?

1) Statistical redundancy of water conformations

STORAGE

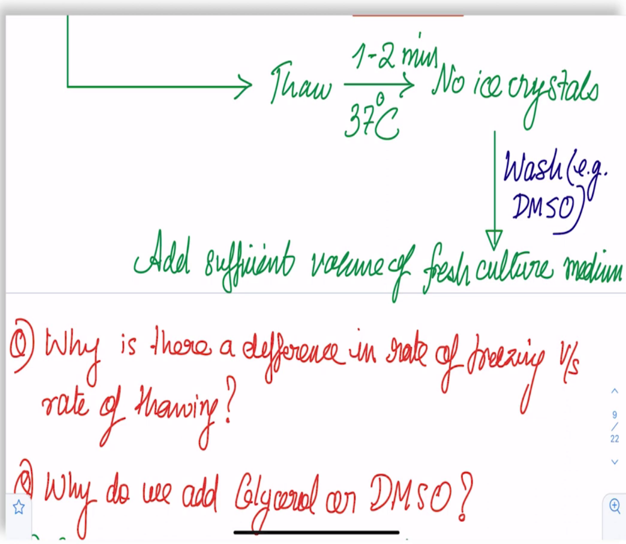
Liquid N_2 ← −195·8°C

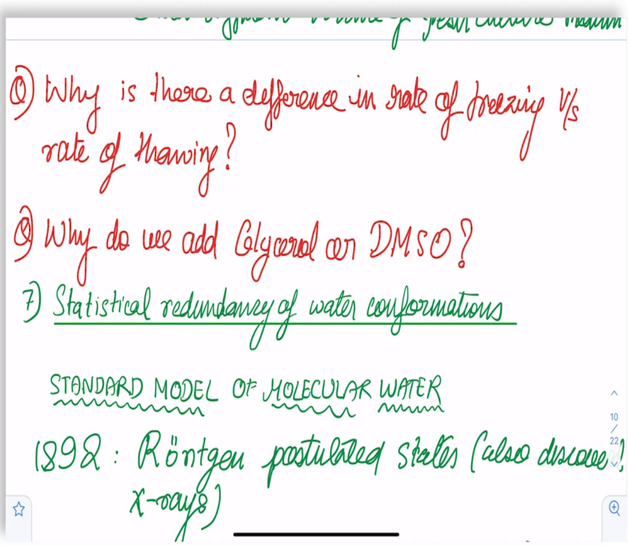
Vapour phase

Why? → **Rapid**

→ Thaw $\xrightarrow[37^\circ C]{1\text{-}2\ min}$ No ice crystals

↓ Wash (e.g. DMSO)





Why is there a difference in the rate of freezing rate point? And why do we add Glycerol or DMSO? Part of the answer to these questions lies in the fact of the structure of molecular water. And the fact that while 75 percent of the cell mass is indeed made up of water, when we are freezing it.

If you allow it to form the normal ice structure, then there is a very good chance you are going to disrupt the cell structure or break it in and this comes to viability. So, in other words, the freezing and thawing process are crucial to maintain the viability of cryopreservation of cellular material. Cryopreservation is referring to the fact that we preserve the cells at low temperature, cryo meaning low temperature cold.

So, what is the special structure of ice that requires such again? And that is what we are going to talk about next. And we are going to talk about this in the context of what I refer to as the statistical redundancy of configurations of molecular water.

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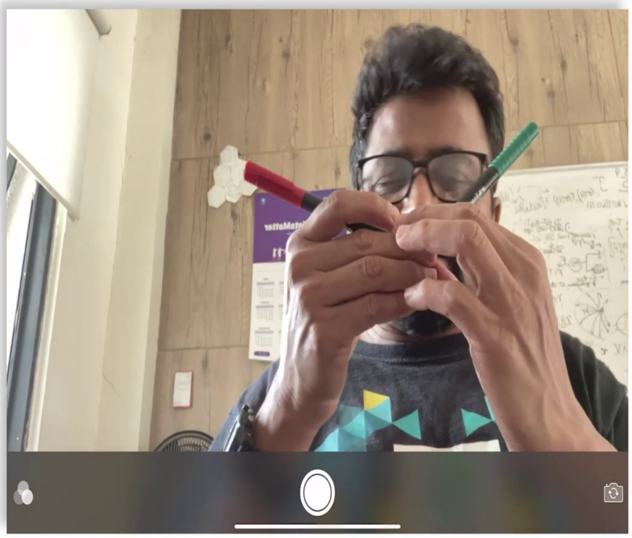

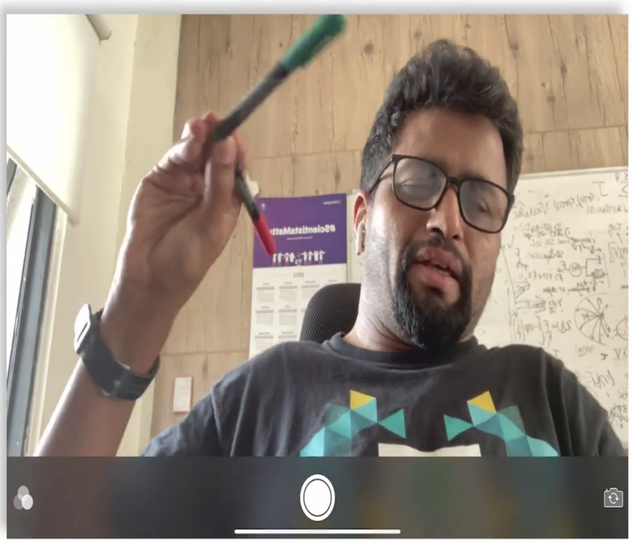

So, we said this earlier about the structure of water, which consists I have taken these two pens to represent the two hydrogen bonds O to H joint at the center by the oxygen and an angle approximately 105° between the four points. This picture is a static picture. You are aware that molecules are not always just stationary, especially not in a fluid. They are moving around and when they move around. They keep interacting with other molecules and this is just one water molecule, water in water pure water consists of multiple water molecules. So, let us see what that gives us.

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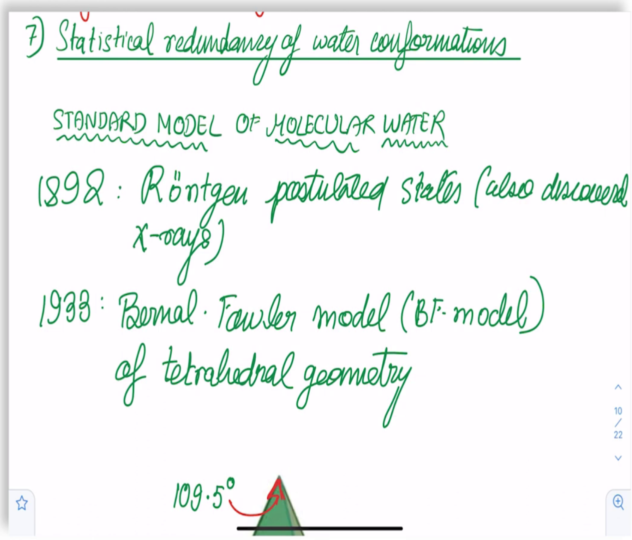
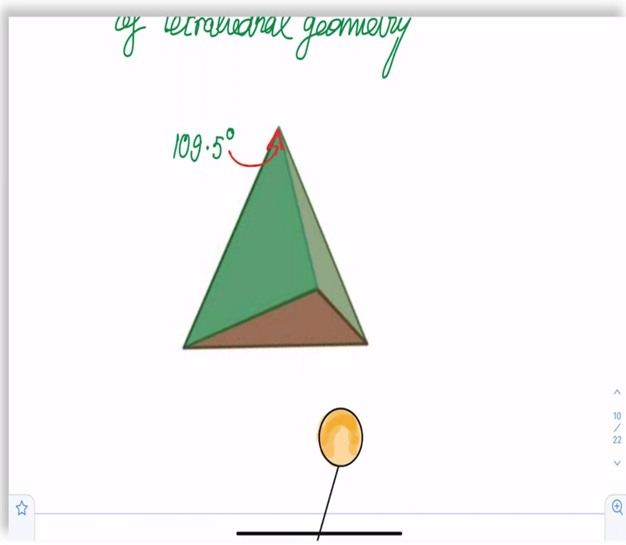
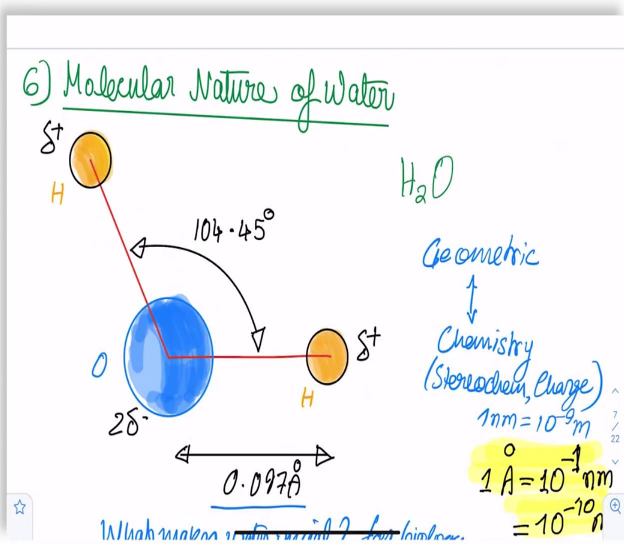
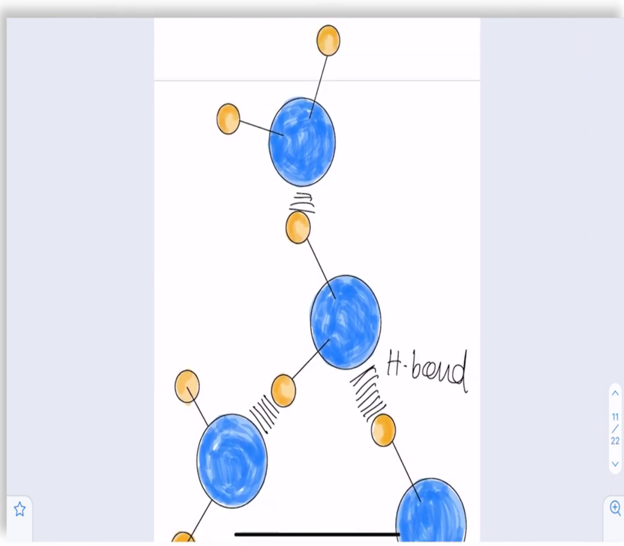
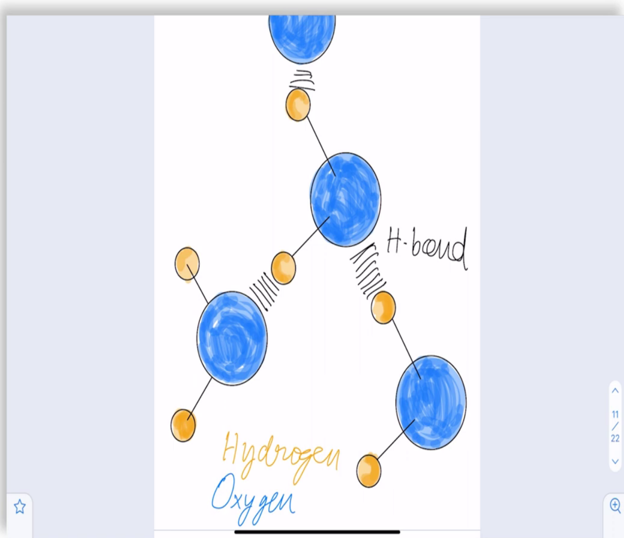





6) Molecular Nature of Water
δ+
H
104.45°
O
2δ-
δ+
H
0.097Å
H2O
Geometric
Chemistry
(Stereochem, Charge)
1nm = 10^-9 m
1 Å = 10^-1 nm
= 10^-10 m


H-bond



So, it turns out that the Standard Model of molecular water was proposed in 1892 initially by some very simple ideas about multiple states by Rontgen was the German scientists who also discovered X-rays, I am sorry that in 1892. In 1933, Bernal, and Fowler came up with a model of a tetrahedral geometry so this is a tetrahedral object draw and geometry is tetrahedral.

That takes into account what we showed earlier about the molecular structure of water 104.45° between the H-O-H. Arranged in a tetrahedral how it is, of course, these two angles are not the same. So, what are we talking about? So, it turns out that water because of that small negative charge on the oxygen, delta(δ) two minus(-) if you recall, and the small positive charge delta(δ) plus(+) on each hydrogen atom is capable of now creating a hydrogen bonding network.

These dashed lines here indicate the fact that there is a weak interaction based on electrostatics between the hydrogen and the oxygen of the neighboring molecule. Now, this would suggest an interesting property, which means that you remember I said that the water molecules can move around because it is a fluid, but that certain positions are likely to be more favored than others energetically more favored than other. And this brings us to the question about the statistical redundancy of H_2O and its molecular components.

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1935: Linus Pauling - relation to RANDOMNESS

[Contribution from the Gates Chemical Laboratory, California Institute of Technology, No. 506]

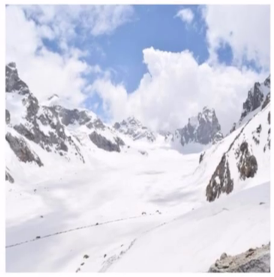
The Structure and Entropy of Ice and of Other Crystals with Some Randomness of Atomic Arrangement

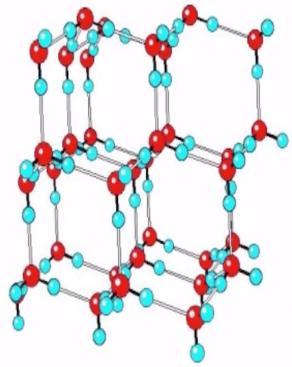
By Linus Pauling

Investigations of the entropy of substances at low temperatures have produced very important information regarding the structure of crystals, the work of Giauque and his collaborators being particularly noteworthy. For example, the observed entropy of crystalline hydrogen shows that even at very low temperatures the molecules of orthohydrogen in the crystal are rotating about as freely as in the gas;[1] subsequent to this discovery the phenomenon of rotation of molecules in crystals was found to be not uncommon. Also the entropy values of carbon monoxide[2] and nitrous oxide[3] show that in crystals of these substances the molecules are not uniquely oriented, but have instead a choice between two orienta-

covery of the hydrogen bond[4] that the unusual properties of water and ice (high melting and boiling points, low density, association, high dielectric constants, etc.) owe their existence to hydrogen bonds between water molecules. The arrangement of oxygen atoms (but not of hydrogen atoms) in crystals of ice is known from x-ray studies;[5] it is not a close-packed arrangement (as of sulfur atoms in the high-temperature form of hydrogen sulfide), but a very open one, like that of the silicon atoms in high-tridymite. Each oxygen atom in ice is tetrahedrally surrounded by four other oxygen atoms at the distance 2.76 Å., and it has been assumed that it is bonded to these

Ih form of I





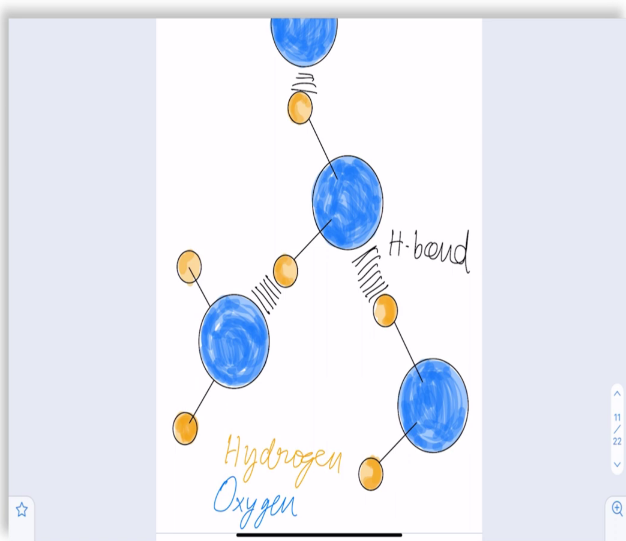
Chhota Sigri Glacier, H.P.
4,900 m ASL

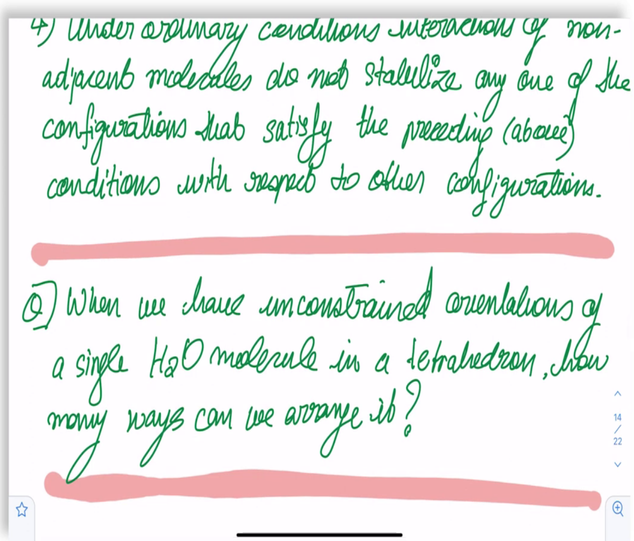
BF-Model adopted by Pauling

1) Ice has distance of 0.95 Å between O–H and H–O–H angle of 105°

2) Each H_2O molecule orients 2 Hydrogen atoms towards two O-atoms. Four (4) Oxygen atoms tetrahedrally surround a single O-atom.

3) Adjacent H_2O molecules orient approximately 1 H-atom along each O–O axis (O–H⋯O)





So, it brings us also forward to 1935. So, Bernal Fowler model of tetrahedral geometry was in 1933, this paper published by Linus Pauling Noble laureate in chemistry, and peace later describes the structure and entropy of ice and other crystals with some randomness of atomic arrangement. Now, this is interesting for 2-3 reasons. One is that it talks about the structure of ice.

Second is talks about entropy. So, far we have not talked about it. You are aware of entropy in other contexts. And he talks about randomness. And this is where my statistical variations business comes in. You can read this paper, it is available online. But for me, the most interesting part is that it describes one kind of water, one kind of water being ice, that ice that is formed in a freezer if you have one with ice cubes.

It is also found in the glaciers. This is the Chhota Sigri Glacier of Himachal Pradesh 4900 meters above sea level. That water has this structure. What you are looking at here, it is also called 1-h form. The Bernal Fowler model that was adopted by Pauling has the following properties. Ice has a distance of 0.95 Angstroms between the O and H.

And an angle of 105 degrees H-O-H, each H_2O molecule orients, 2 hydrogen atoms towards 2 oxygen atoms, which is sort of what I showed you in my very crude diagram here, we will get to it a little bit more in detail 4 oxygen atoms tetrahedrally surrounds single oxygen atom we will see how that works. Adjacent H_2O molecules orient approximately 1 hydrogen atom along each O-O axis that means you have O-H hydrogen bond O.

Under ordinary conditions, interactions of non adjacent molecules do not stabilize any one of the configuration that satisfy the conditions that we just discussed, meaning to say that normally the molecules that are not next to each other do not have a role to play in that direction. That is what the not stabilizing non-adjacent molecules means.

So, the question is if you have unconstrained orientation of a single hydrogen H_2O molecule in tetrahedron how many ways can we arrange it? By unconstrained I mean that there are, there is a tetrahedron, and no one position is favored over another, how many ways can we arrange it think about it for a while.

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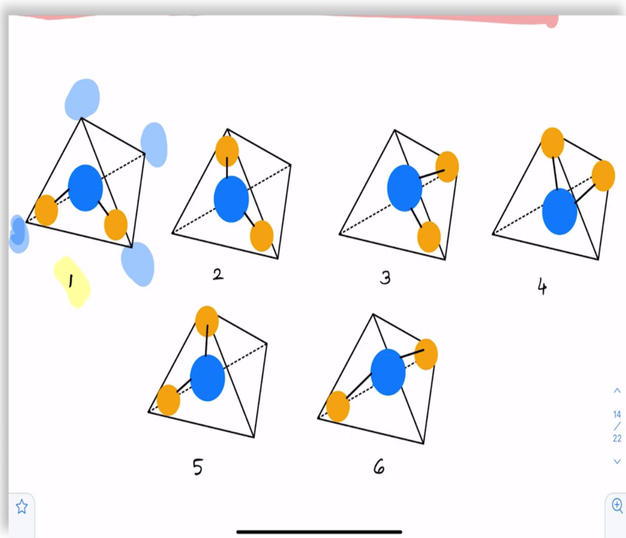

And I am going to show you this, which you may have also come across in a physical chemistry textbook. It is nothing but a tetrahedron. So, think of this as a 3-dimensional geometry, I have drawn the dashed line to indicate this in the third dimension through the plane below the plane of this screen that you are watching and the water molecule.

Then, which has oxygen at all 1-2-3-4 vertices, so each vertex is the location of a neighboring oxygen resulting in the fact that the hydrogens can be located at any of the vertices for the same charge issue. This is what the tetrahedral model looks like 1-H model of Pauling. It also however means that it does not have to be in state one which I have marked here. It could be in 2-3-4-5 or 6. In other words, what I have done is I have picked each pair of neighboring vertices and installed by H2O according to that, giving me 6 possibilities.