INDIAN INSTITUTE OF TECHNOLOGY ROORKEE

NPTEL

NPTEL ONLINE CERTIFICATION COURSE

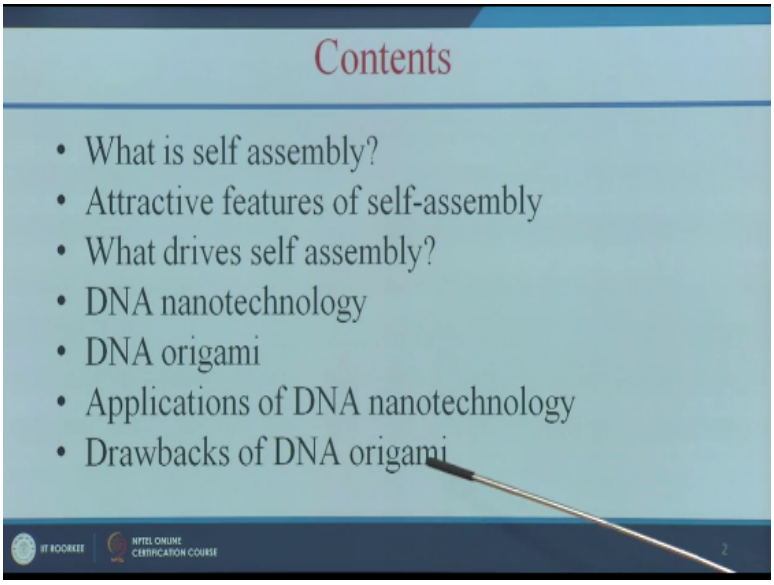
Biomedical Nanotechnology

Lec - 06
DNA Nanotechnology

Dr. Arup Kumar Das
Department of Mechanical and Industrial Engineering
Indian Institute of Technology Roorkee

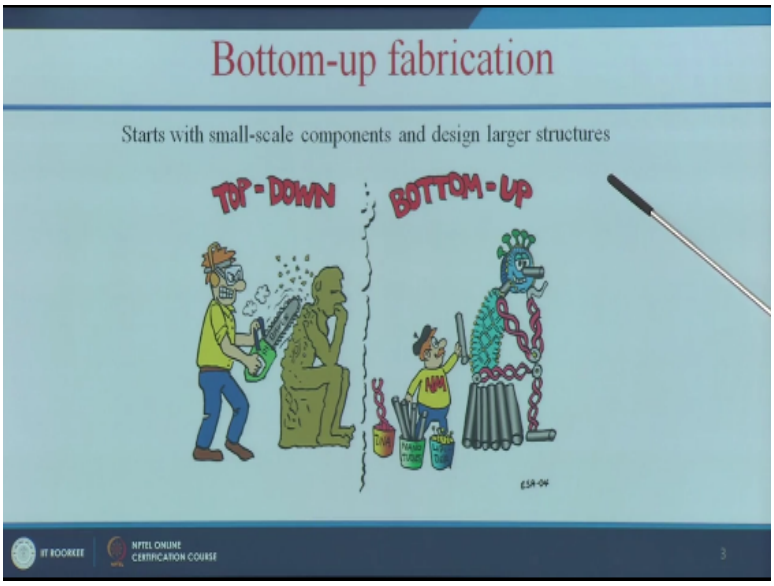
Hello everyone I welcome you all to the 6th lecture of this course that is DNA nanotechnology.

(Refer Slide Time: 00:29)



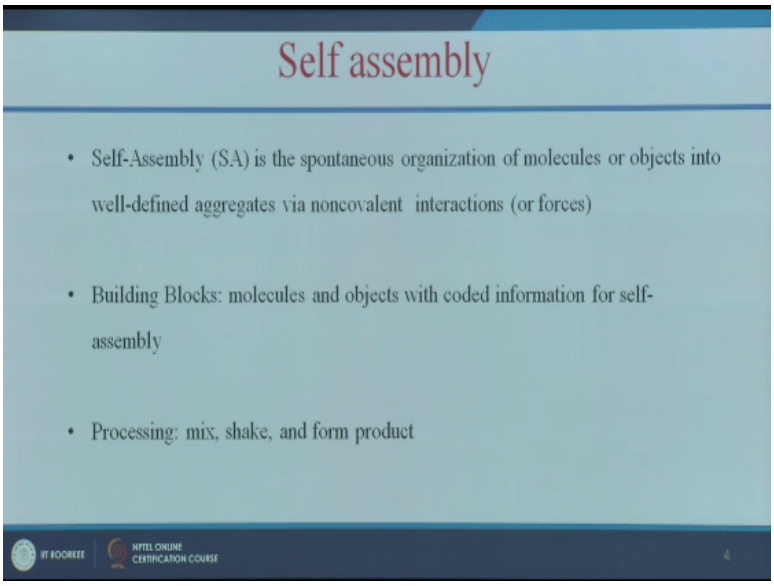
In this lecture we are going to learn what is self assembly and what are the attractive features of self assembly and what drives self assembly and we are also going to learn what is DNA nanotechnology and applications of nanotechnology and we are also going to learn what is DNA origami and what are the drawbacks of DNA origami.

(Refer Slide Time: 00:44)



So here the self assembly is a bottom up fabrication okay so in my first lecture I told you what is top down approach and bottom up approach top down means from bulk to nanostructures and bottom up approach means from atomic scale to nano scale okay so here we start with the small scale components and design the larger structures.

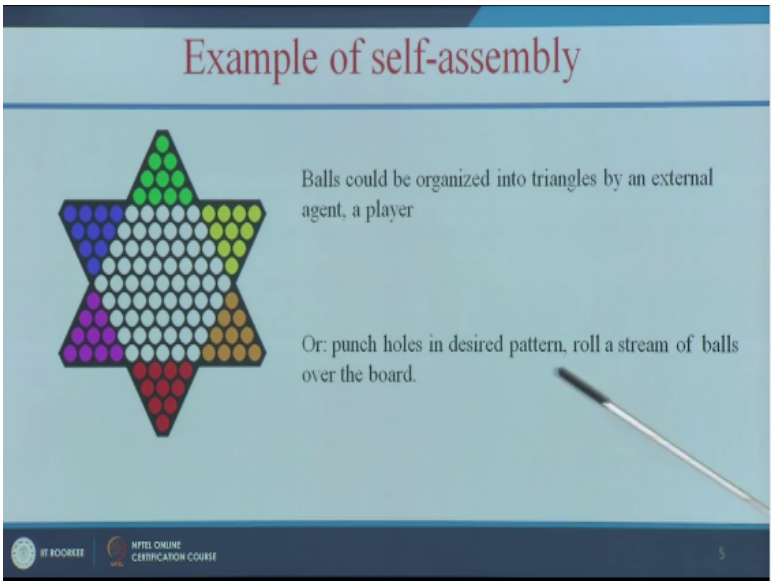
(Refer Slide Time: 01:04)



So what is self assembly self assembly is a spontaneous organization of molecules or objects into well defined aggregates via non covalent interactions or forces okay so here the building blocks is your molecules are any objects with the coded information for self assembly and here the process is mix and shake and it will form the product okay for example, here if you are losing the DNA molecule it have the coded information so the DNA as A it can form bond with T and G forms bond with C okay.

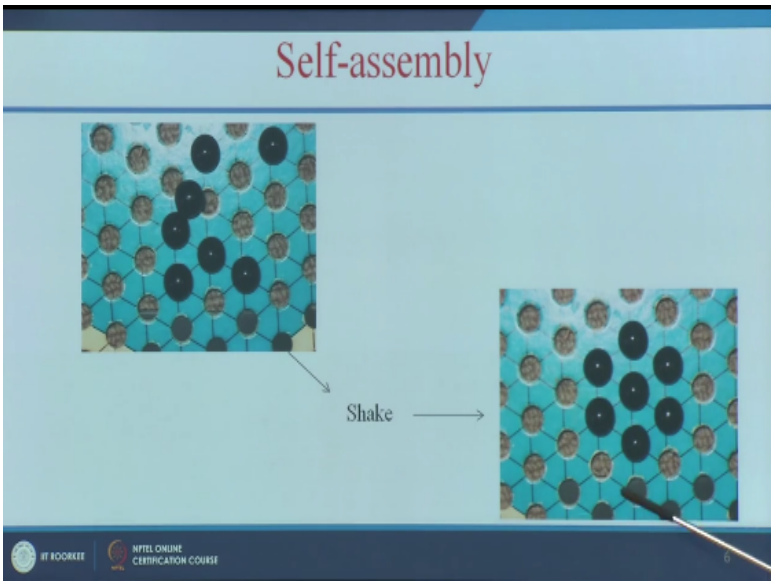
So that means the molecule is already having the coded information, so when you put in a solution so the DNA easily forms bonds okay so let us see a simple example to understand what is self assembly.

(Refer Slide Time: 01:58)



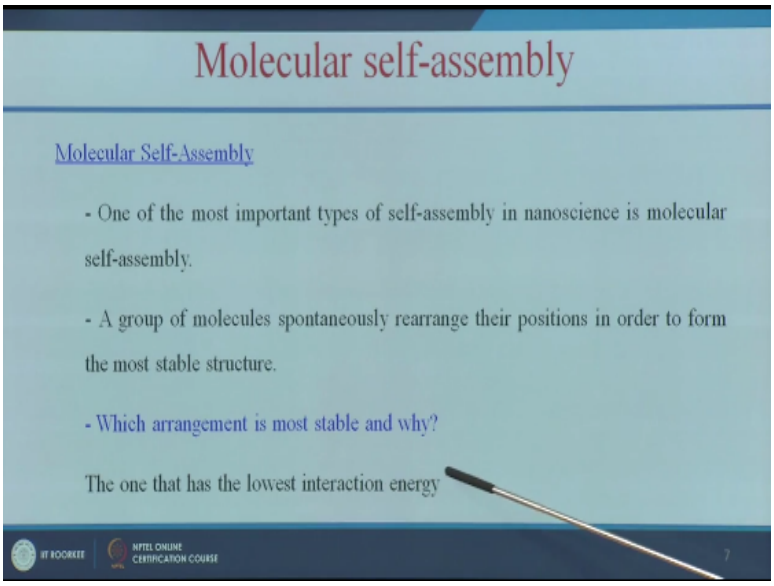
So this you might have played in your childhood okay so here the balls could be organized into triangles by an external agent or a player okay or what you can do is you can punch holes in the desired pattern and you can role a stream of balls over the board.

(Refer Slide Time: 02:13)



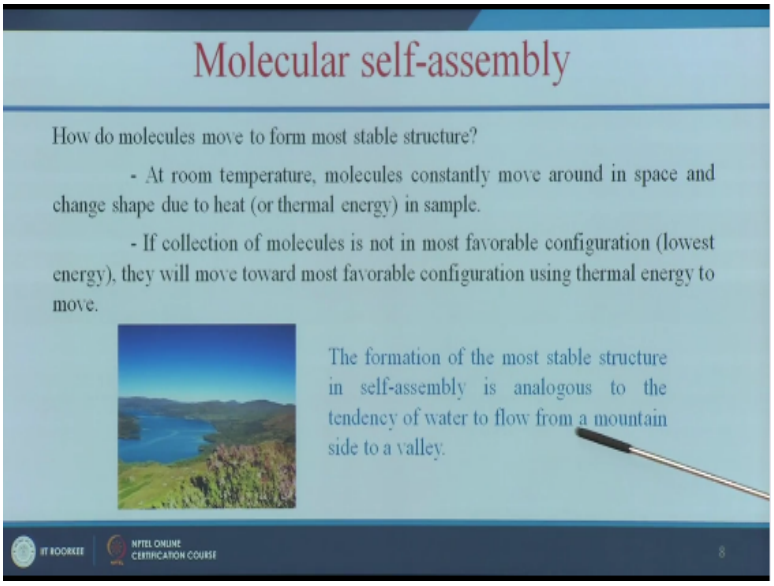
So here you can make a holes in this board okay and put the balls and shake it so it will automatically go and assemble so this is a simple example to understand what is self assembly.

(Refer Slide Time: 02:25)



So let us see what is molecular self assembly so one of the most important types of self assembly in nano science is molecular self assembly so here the group of molecules spontaneously rearrange their positions in order to form the most stable structure okay, so which arrangement is more stable and why, so here the one which has the lowest interaction energy is the more stable, so usually the nature favors which request low interaction energy and also high stable structure okay.

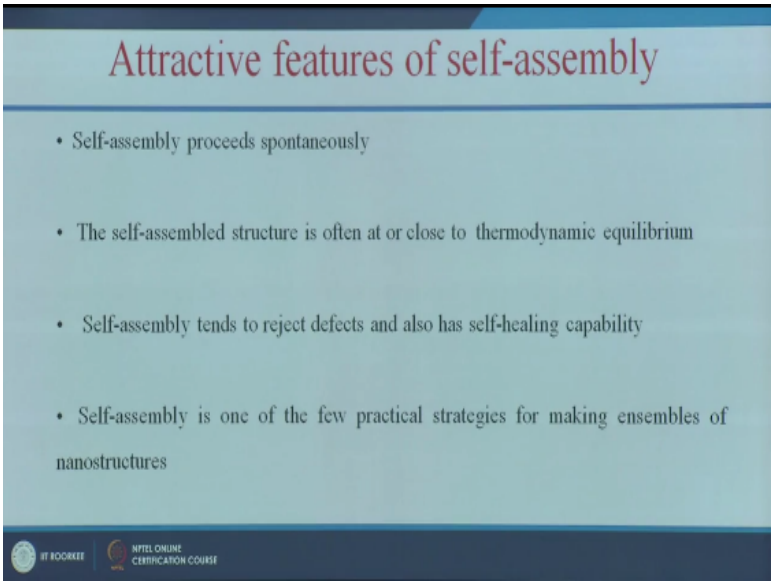
(Refer Slide Time: 02:56)



So how the molecules move to form most stable structure so at room temperatures the molecules constantly move around in space and change shapes due to heat or thermal energy in the sample okay so if a collection of molecules is not in the most favorable configuration that means not in the lowest energy form so they will move towards most favorable configuration using thermal energy to move to the highly stable structure.

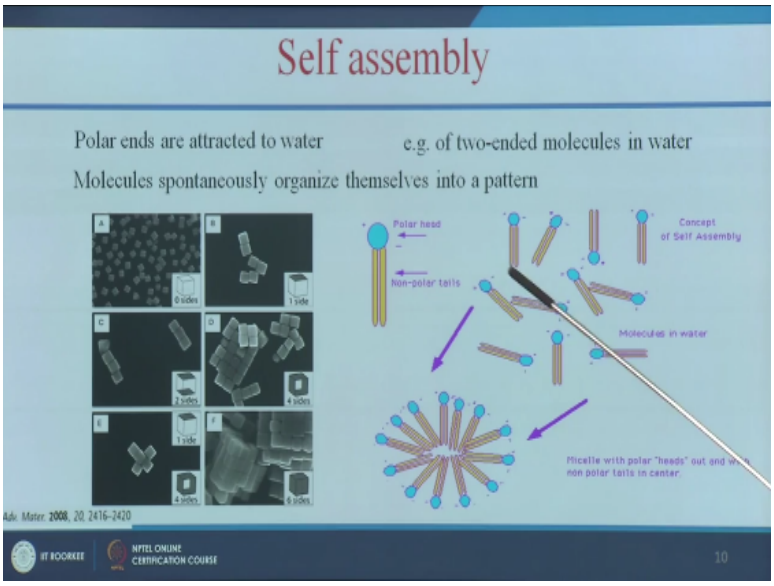
So the nature always as I told you earlier it also save as the which request low energy and highly stable structure okay so the simple example is the tendency of water to flow from mountain side to valley okay.

(Refer Slide Time: 03:41)



So what are the attractive features of self assembly? The self assembly proceeds spontaneously and it is often close to thermodynamic equilibrium okay and again it rejects the defects and also it has a self capability and self assembly is one of the few practical strategies for making the nano structures okay.

(Refer Slide Time: 04:05)

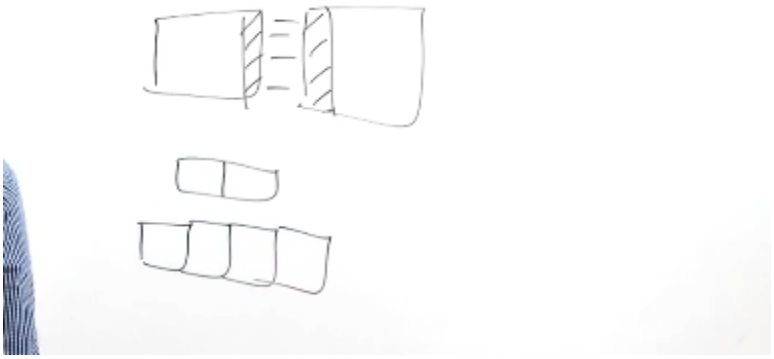


So let see some other examples suppose you are having a molecules two ended molecules in the water for example this kind of molecules which have the polar head that means water liking or hydrophilic molecule and it is having none polar riles that is hydrophobic so when you put this gram molecules in the water what happens although hydrophilic heads will be facing the water and all the hydrophobic tails will away from the water okay.

So you will get this kind of milieus structure a simple example is if you put a drop of oil in a water you will get a circular ring kind of structure right so the another example is we can make nano particle then this case they made a sliver nano cubes okay and they coded with a hydrophobic linger only one side of this cube so you will get this get kind of assembly and when you code on the both the sides you will get this kind of line kind of structure and when you cote it on the 4 sides all the 4 sides will get this kind of assembly and when you mix the 4 side coated nano cubes with the 1 side coated nano cubes I will get this kind of assembly.

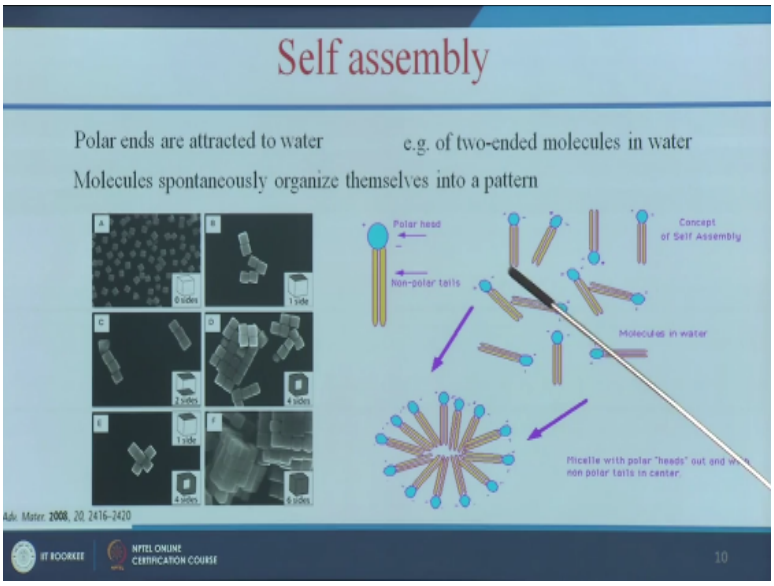
And when you place a like a 6 sided coated you will get this kind of assembly, so I will explain in detail like.

(Refer Slide Time: 05:25)



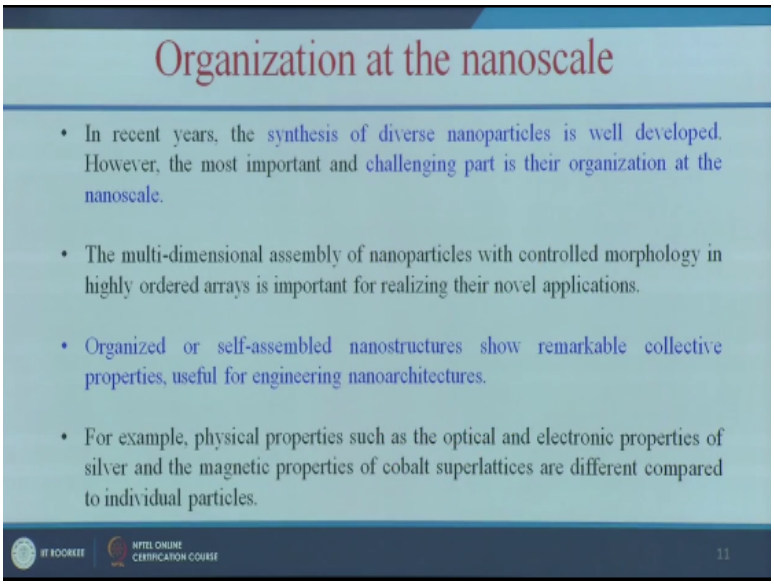
So this is your nano cubes and this coated with the hydrophobic ligand okay so it will join together it forms this kind of structure if your coating this nano cubes are only one side okay and if your coating this nano cubes on both the sides it will form this kind of assembly so you can understand from this picture.

(Refer Slide Time: 05:55)



So if you are coating it on only one side you will get this kind of assembly and if you are coating on both the sides you will get this kind of assembly.

(Refer Slide Time: 06:01)



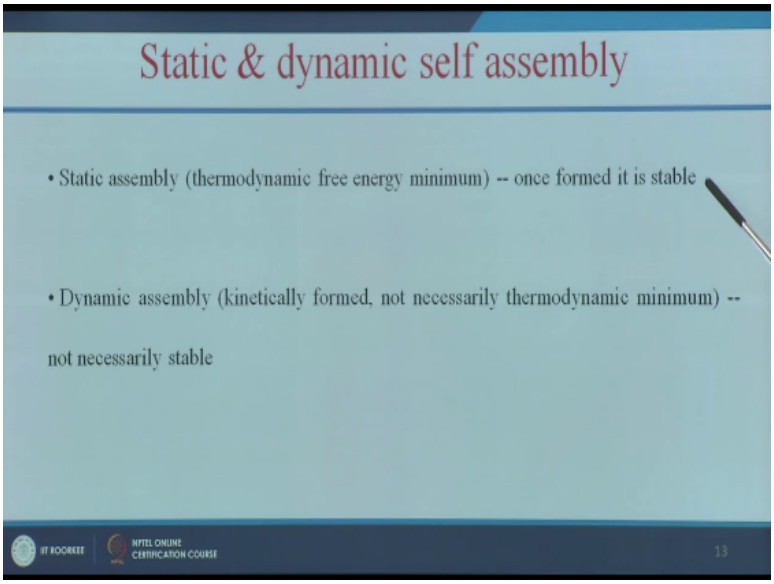
So why we need organization at the nano scale okay so the synthesis of dividers nano particle is well developed we are developed various methods to syntheses nano particles but the challenging task is how to arrange this nano particles okay challenging part is their organization at the nano scale okay so the multi dimensional assembly of nano particles with controlled morphology is very important for realizing their novel applications okay so the organized or self assembly nano structures it will show a remarkable collective properties which will be useful for engineering nano architectures.

A simple example is so if this all the alphabets are scatted you do not get any meaning so this alphabet should be arranged in a proper way to get the meaning for the word nano scale so similarly if the nano particles are disposed are scatted you do not get any meaning so you have to arrange in a proper structure okay so then only you can get various novel properties for example the physical properties and optical properties of the silver and magnetic materials is different okay when compare to the individual properties and another thing is it is a key to successful application of nano particles based devices and in this self assembly we wil get a defect free order nano structure okay.

So we can create periodic assemble nano structures to different and novel and innovative procedures so which has a various application by using self assembly we can arrange this nano particles and we can make it various nano structures which will have lot of application okay and how do we can do it we can do this nano organization by using top down approach like

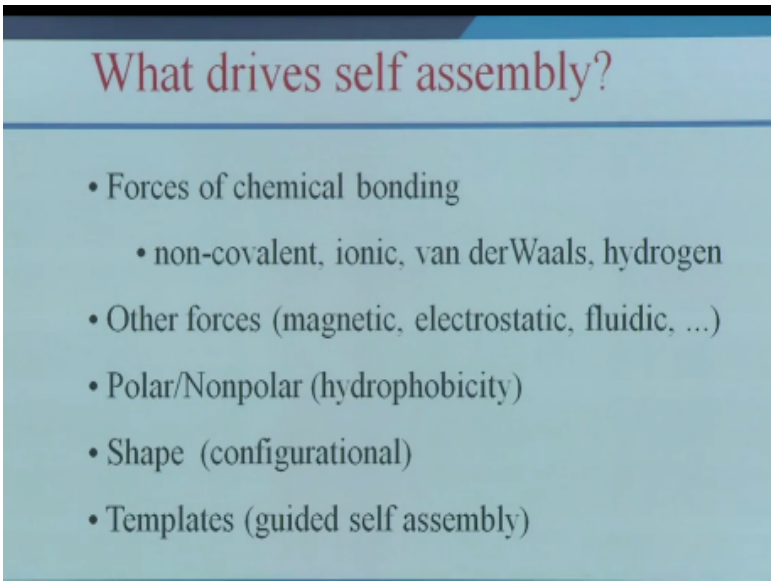
lithography but in this lecture we are going to learn how we can use this bottom up approach for self assembly.

(Refer Slide Time: 07:52)



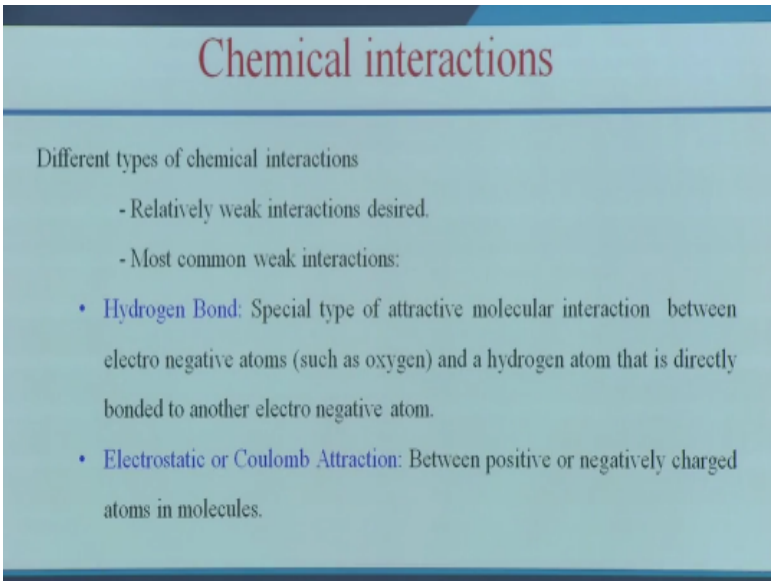
Okay again this self assembly is divide into two types the static assembly and dynamic assembly so the static assembly is thermodynamic free energy minimum okay. So once it is formed it is stable and dynamic assembly it is kinetically formed it is not necessarily thermodynamic minimum and not necessarily stable, okay.

(Refer Slide Time: 08:14)



So what are the forces driving this self assembly, so we can see that these are the forces of chemical bonding for example non-covalent interactions, ionic, Van Der Waals and hydrogen and other forces such as magnetic as well as hydrofluoric or hydrophobic and these are some of the forces which play a major role in driving this self assembly.

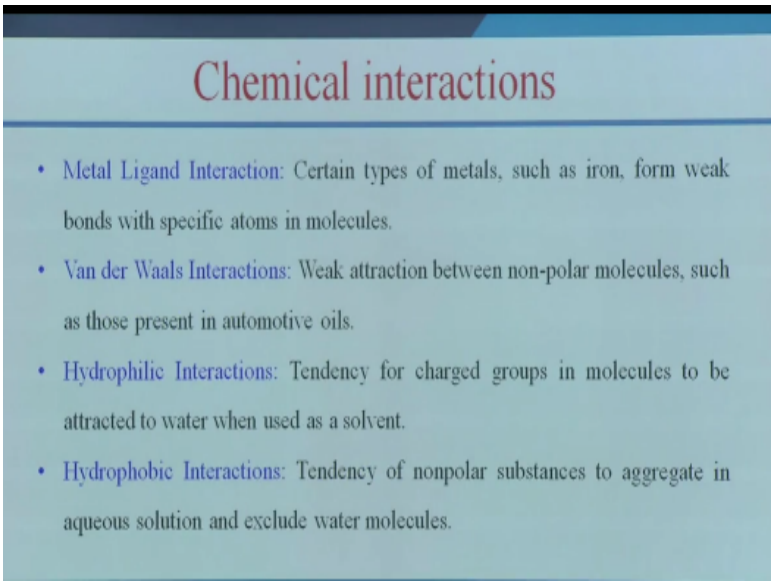
(Refer Slide Time: 08:34)



And let us say what are the chemical interactions play a role in this self assembly so mainly the weak interactions play a major role in this self assembly, okay. So let us say example hydrogen bond, it is a special type of attractive molecular interaction between electro negative atom such as oxygen and hydrogen atom that is directly bonded to another electro negative atom and you know what is electrostatic or Coulomb attraction.

That means so it is forming between the positively or negatively charged atoms in a molecule that is called as electrostatic attraction.

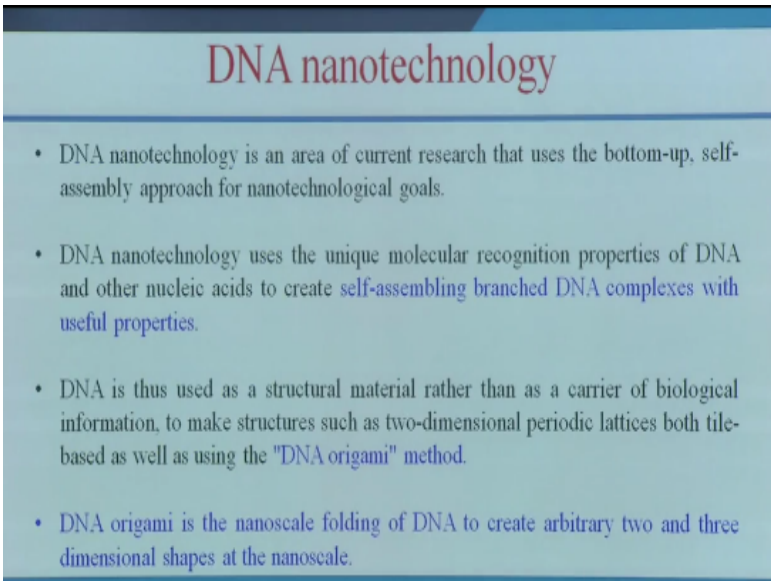
(Refer Slide Time: 09:09)



The third one is, metal ligand interaction so here the certain types of metals such as iron form a big bonds with the specific atom in the molecules and Van der Waals interactions so here the weak attraction between the non polar molecules such as those present in the automatic oils and hydrophilic interaction that means the tendency for charged groups in molecules to be attracted to water when use as a solvent.

The last one is the hydrophobic interactions that means the tendency of non polar substance to aggregate in aqueous solution and exclude the water molecules, okay.

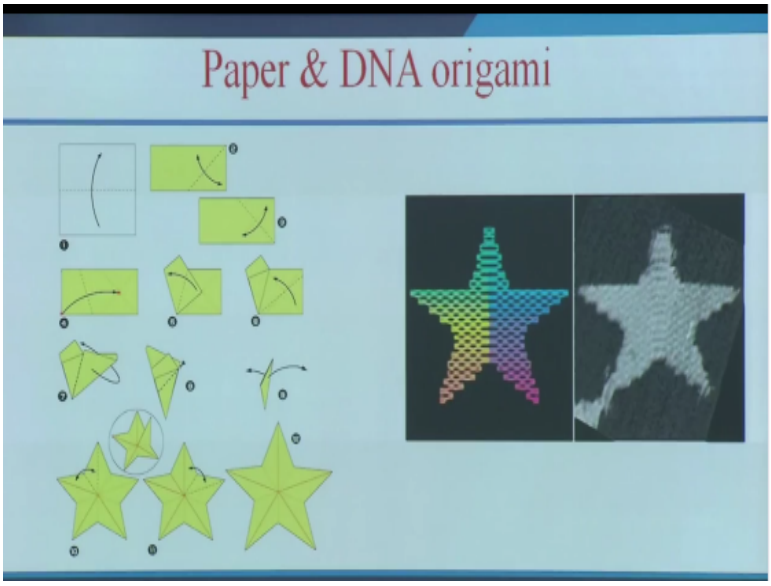
(Refer Slide Time: 09:44)



So let us see what is DNA nanotechnology, so DNA nanotechnology uses the bottom of that is self assembly approach for making DNA base nano structures, so here the DNA nano technology uses unique molecular recognition properties of DNA and it create the self assembly branched DNA complex with useful properties, okay. So here DNA is used as a structural material so usually the DNA are genetic material which carries a biological information.

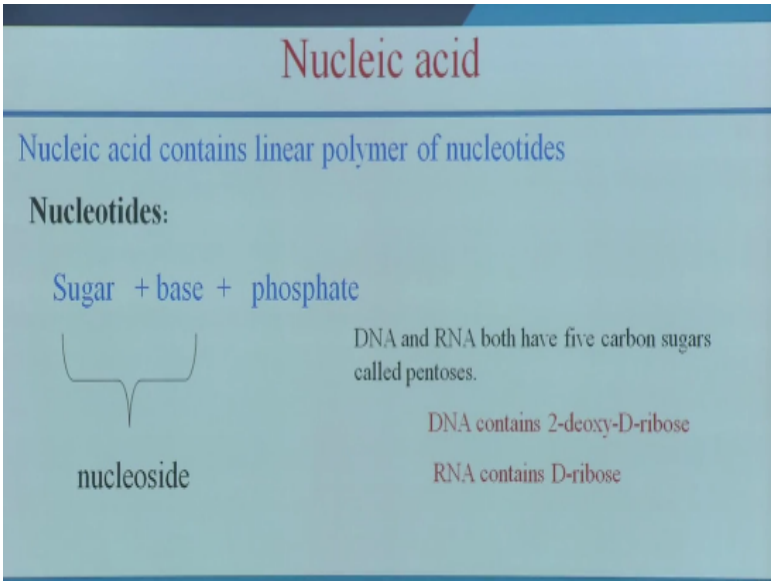
But here the DNA will be used as a structural material for making different nano structure, so that is called as DNA origami method, so the DNA origami is that nano scale folding up DNA to create arbitrary two or three dimensional shapes at the nano scale.

(Refer Slide Time: 10:33)



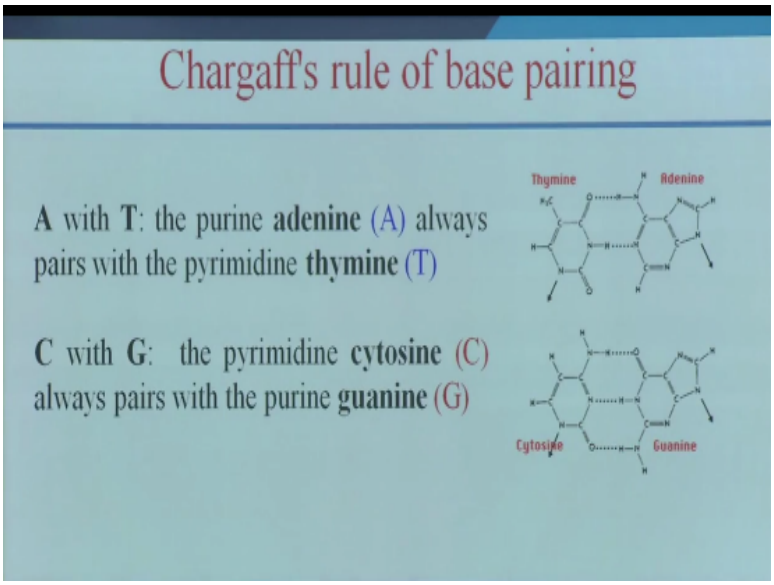
So you know what is paper origami so using a paper we can make a different kind of structures, okay. So similarly we can use the DNA to make different kind of structures, so this is the paper origami you can use the paper and we can make different kind of shapes or structures and if you are trying to mimic the similar kind of things you thing DNA that is called as DNA origami.

(Refer Slide Time: 10:55)



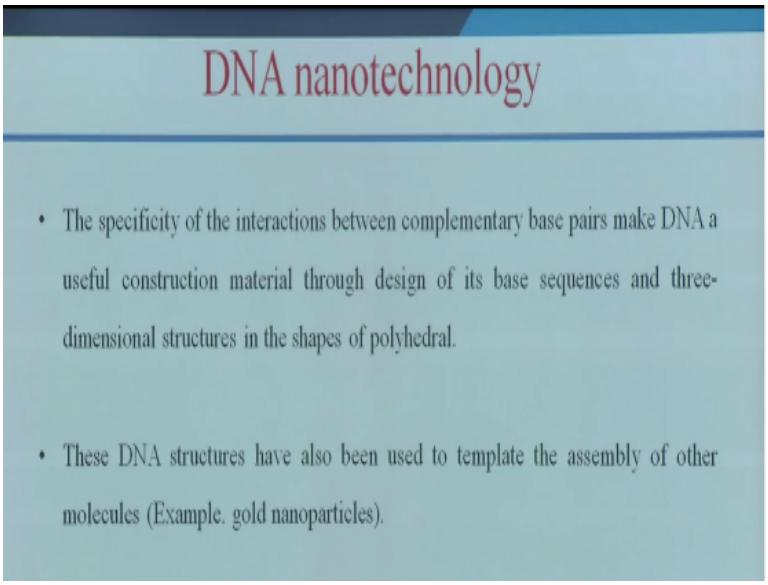
So let us see some basics okay, so the nucleic acid contains linear polymer of nucleotides, so what is nucleotide, what is nucleoside? So the DNA contains sugar, base and phosphate so the sugar + base is nucleoside and the combination of sugar base and phosphate is nucleotide in case of DNA will have 2-deoxy –D-ribose sugar in case of RNA it contains D-ribose sugar okay. So here the base is in simple you can 80 GC that is your adenine thymine, guanine and cytosine okay.

(Refer Slide Time: 11:33)



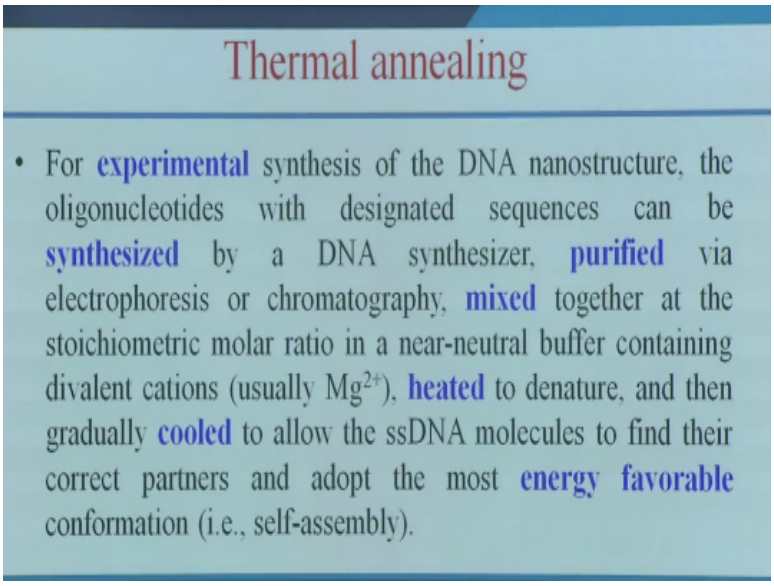
And we have to understand what is Chargaff rules that is very important in this DNA nano technology, okay. So this A always form bond with T, okay you can see here this adenine always form bond with thymine using two hydrogen bonds and other thing is the cytosine bonds with guanine with 3 hydrogen bonds, okay. So this is the Chargaff rules and where A always bonds with T and G always pair with the C.

(Refer Slide Time: 12:04)



So this specific interaction between the complementary base pairs that means like A how it forms bond with T and G forms with C so that makes the DNA as attractive material attractive construction material, so using the property we can make different kind of nano structures, okay.

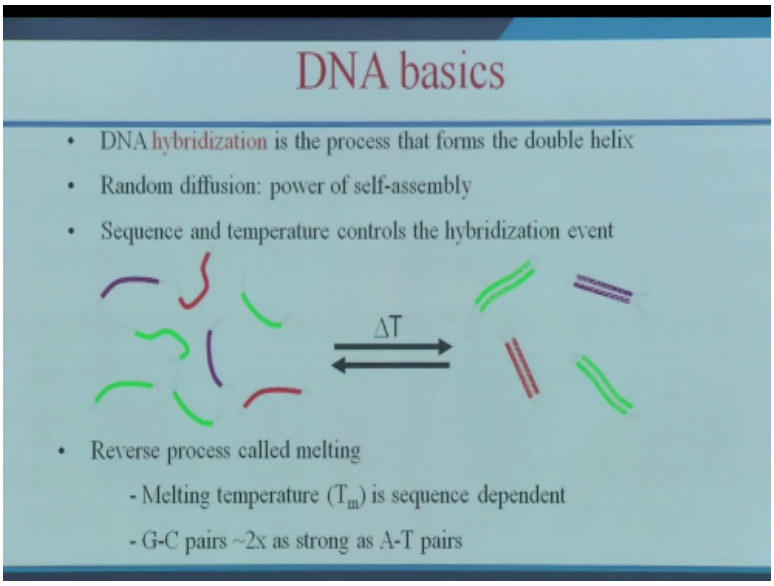
(Refer Slide Time: 12:22)



So for experimental synthesis of DNA nano structure we need more amount of DNA so how we can synthesize with DNA, so this oligonucleotides can be synthesized by DNA synthesizer and it can be purified by techniques like electrophoresis or chromatography and it can be mixed together at the stoichiometric molar ratio in a near neutral buffer containing divalent cations, okay.

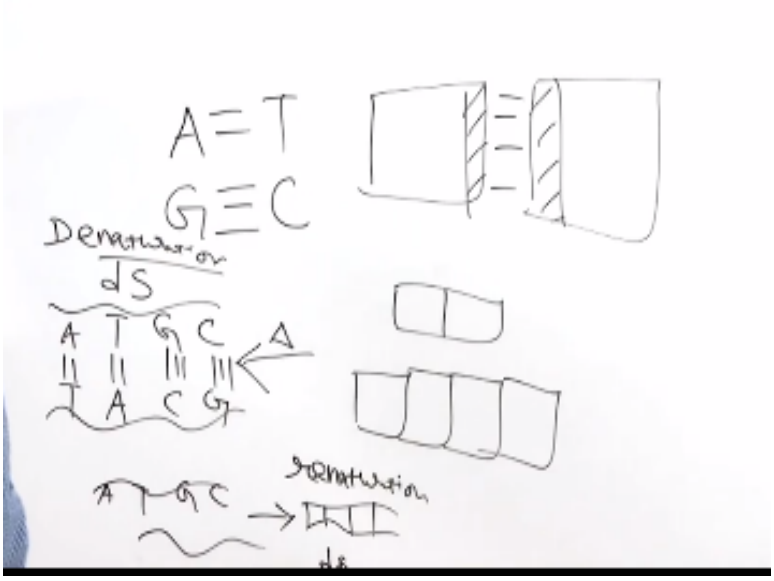
And if you want to denature the DNA it can heat the DNA that will break the hydrogen bond and it will denature the DNA and if you want to make it re-nature it can be cooled so that the single cyanide DNA combine and forms a double standard DNA, okay. And these are the very good example for the self assembly.

(Refer Slide Time: 13:09)



So let us see what is DNA hybridization.

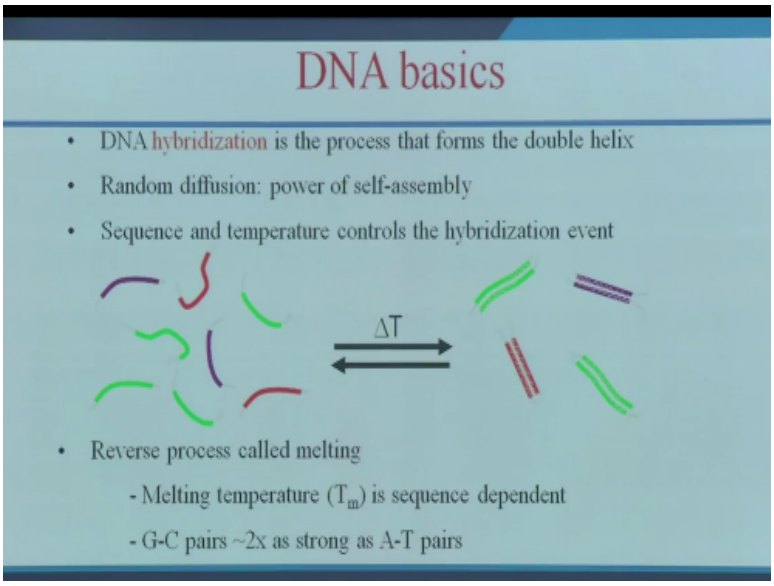
(Refer Slide Time: 13:13)



So as I told you this A always form bonds with T and G always form bond with C, okay. So when you have the DNA like this and this is your complementary based on the other stand and it forms the hydrogen bond, so when you apply this heat what happen this double standard DNA became a single standard DNA and when you cool it again it forms the double standard DNA, so when you apply heat it will breaks the hydrogen bonds.

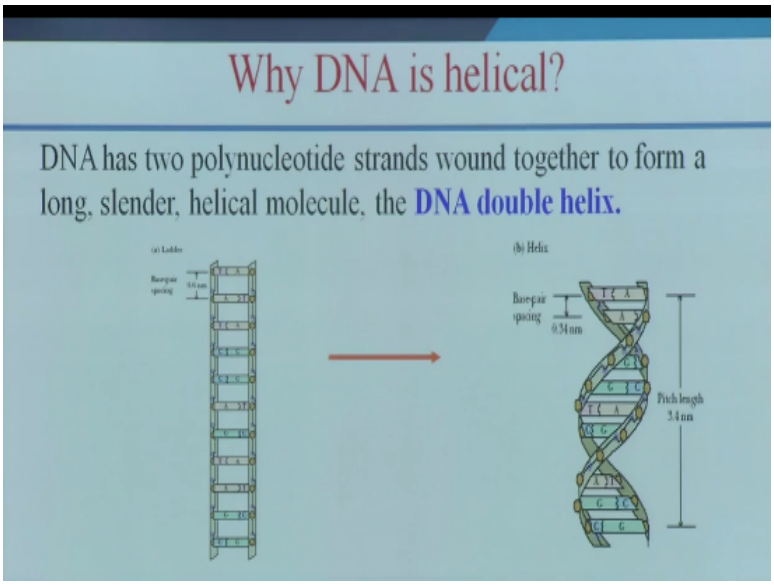
And it will become a single standard DNA and when you reduce the temperature and allow it to cool it will form a again double standard DNA that is called as renaturation, so this is your denaturation and this is called renaturation.

(Refer Slide Time: 14:28)



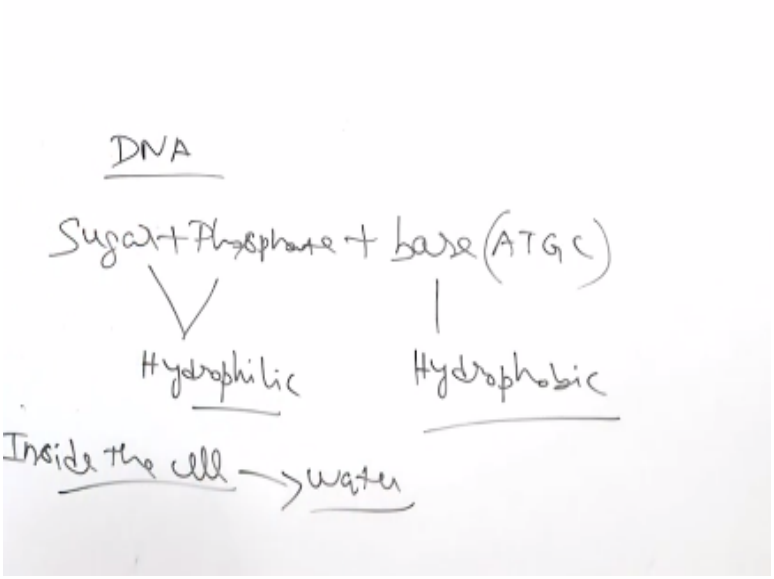
So that is called as DNA hybridization so here you can here simple example so you can use that color code so you can see here this green color DNA bind to green color DNA and red color is binding to red color, so it is like a this sequence are complemented to the other sequence so that is why it is forming a double standard DNA, so when you apply the heat it can be become single standard and when you remove the temperature it can be like a double standard DNA. So using this we are going to make different kind of nano structures.

(Refer Slide Time: 14:59)



So next thing is, why the DNA is helical in nature, okay. So usually it can be like a ladder like structure so why it is forming this kind of helical structure.

(Refer Slide Time: 15:25)

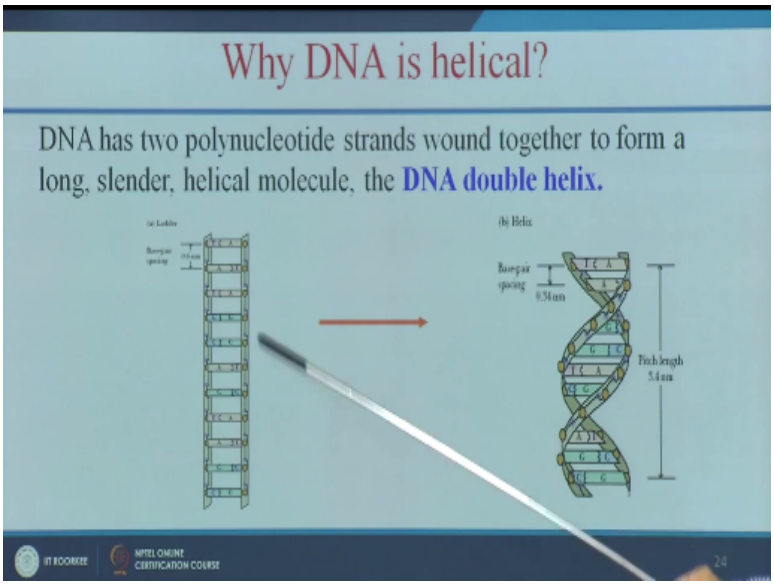


As I told you the DNA has 3 main components sugar okay phosphate and base that is your nitrogen base ATGC okay so in this, these two are hydrophilic and this is hydrophobic and inside the cell, cell is full of water right. So what happens is in this if we have a wave length like this like a ladder structure the water molecules can disturbed the structure and this ways has to be escape from the water because this is hydrophobic.

So this base will be inside okay, and your sugar and phosphate will be on the outside, where these are water allowing okay and these base are hydrophobic that is water re plant or. So after that also it can be like this structure even the base can be inside, so then also it can be like a DNA ladder like structure, so why it has to attend like this kind of helical structure.

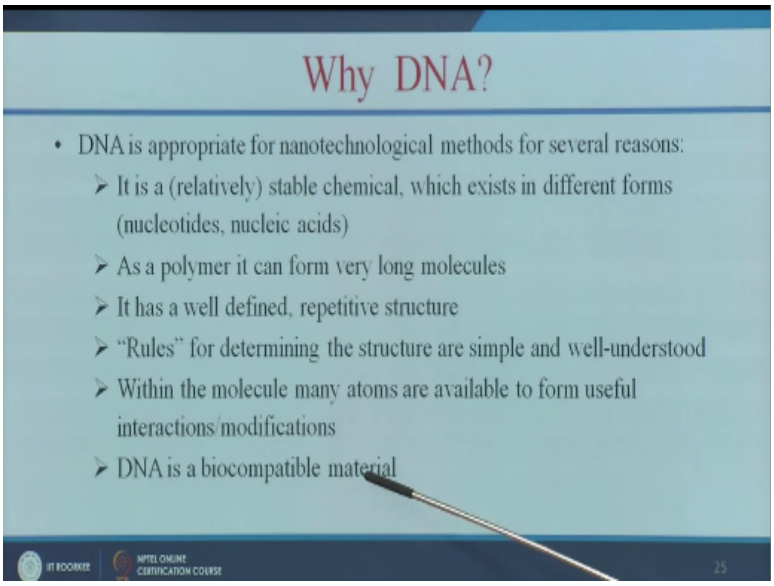
Yes if we have this kind of space gap again the water molecule can enter inside and it can disturb this structure so that is why it has to attend this kind of helical thinner structure to our the entry of water molecule okay, and another reason is the bonding angle between this A and D and G and C also place measure role in giving this helical structure another simple analogy to understand why it is helical is suppose you are cloth is wet, so what you do you twist your cloth to remove the water right.
The same principle here also, so the base is hydrophobic so it has to be produced so it is forming a helical like structure.

(Refer Slide Time: 17:45)



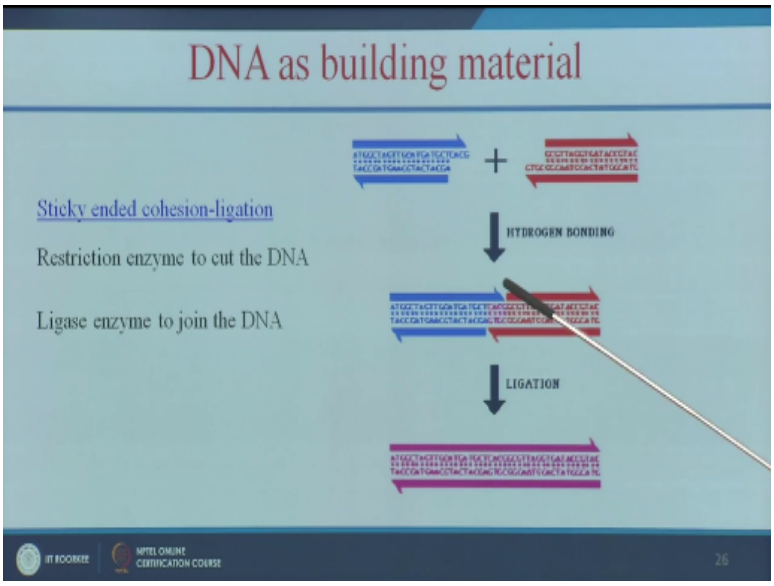
So it is tightly coiled and not align the water to enter and disturb the structure, so that is why the DNA is helical like structure not a ladder like structure.

(Refer Slide Time: 17:55)



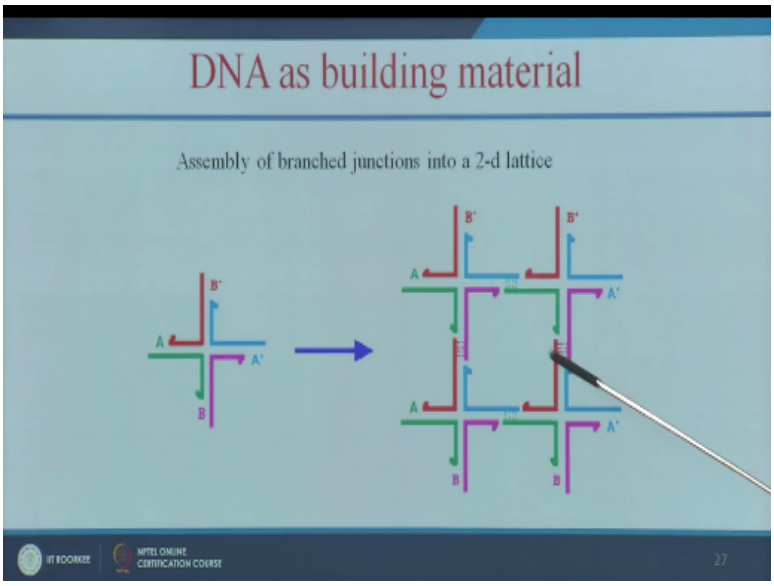
And why we have to select DNA for making this nano structures okay, so where the DNA is a appropriate nanotechnological method for several reasons, so first one is this relatively stable chemical and which exists in different forms okay and again as a polymer it can form a very long molecules and it has a well defined and repetitive structure, and again the rules for determine the structure or simple and well understood okay and within the molecule many atoms are available to form useful interaction and modification and by in the DNA is a again biocompatible material okay, so that is why we are using DNA as a structural material for constructing various nano structures.

(Refer Slide Time: 18:39)



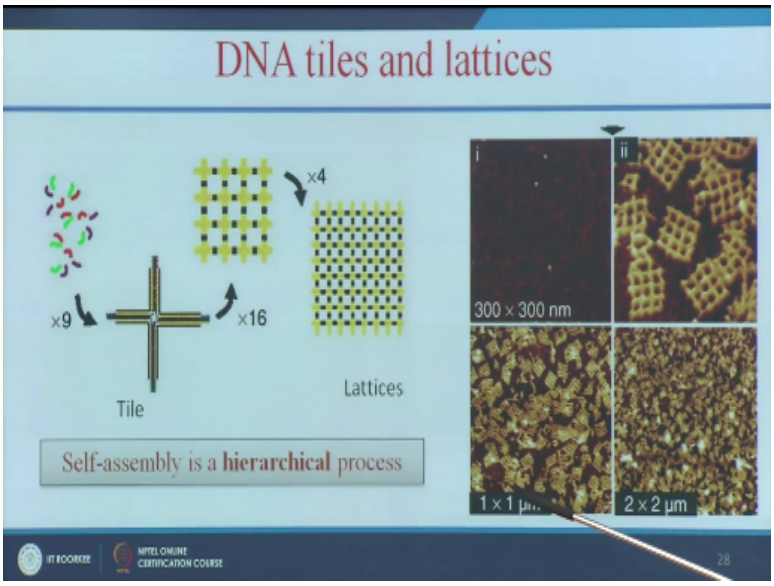
So we can use the DNA as a building material so here two important enzyme play a major role the first one is restriction enzyme okay, so this enzyme is also called as molecular scissor so it can cut the DNA at specific site okay when another enzyme is ligase enzyme so it can join the two DNS fragments.

(Refer Slide Time: 19:01)



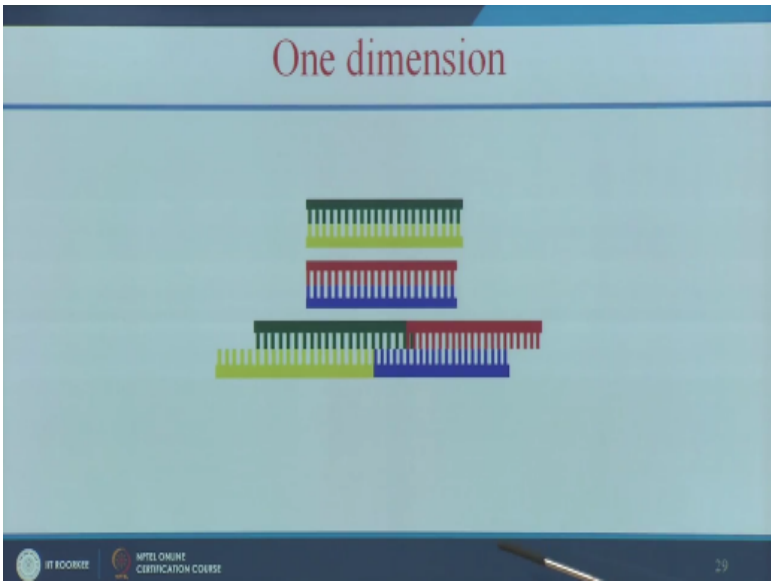
So using this self assembly principle we can make a different kind of nano structures and we can reach even two dimensional lattice.

(Refer Slide Time: 19:10)



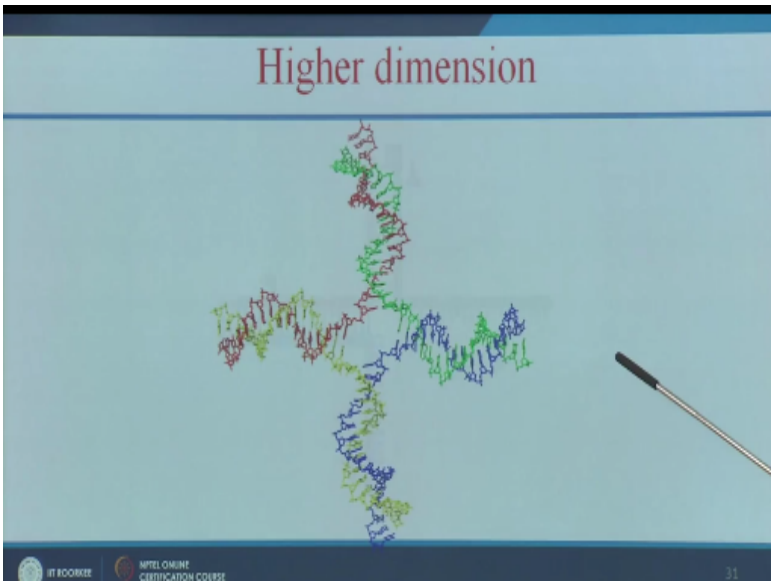
Here you can see here this is a DNA style okay, and the time will be arranged and formed the lattice like structure these are called a DNA tiles and DNS lattice.

(Refer Slide Time: 19:23)



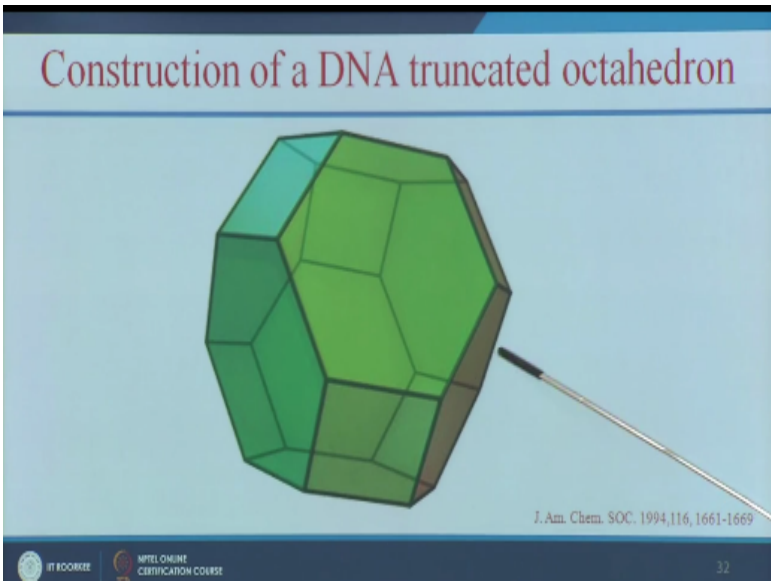
So using this self assembly and the complementary base sparing.

(Refer Slide Time: 19:27)



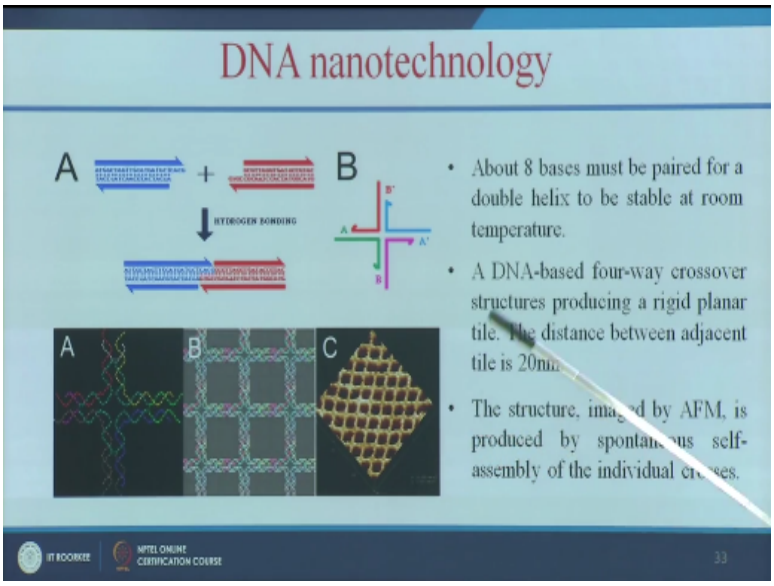
We can make one dimension and we can also achieve this kind of higher dimension structures.

(Refer Slide Time: 19:35)



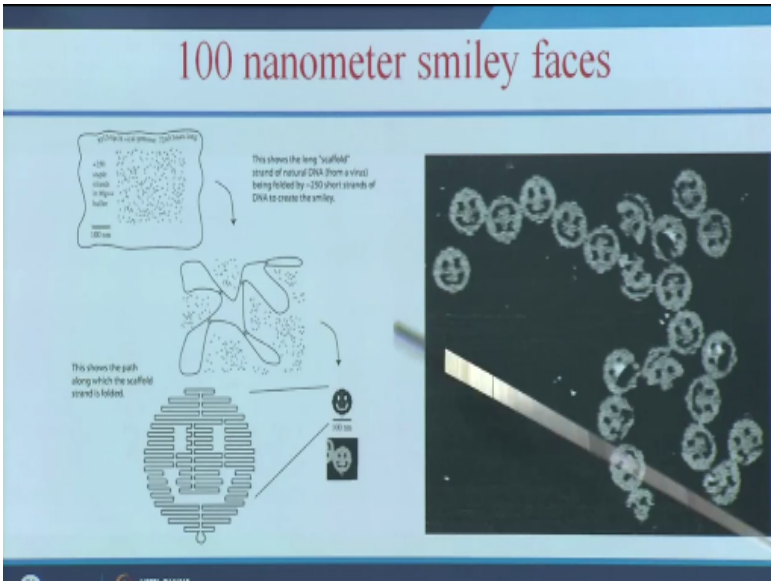
And we can also make a DNA truncated octahedron structures okay.

(Refer Slide Time: 19:40)



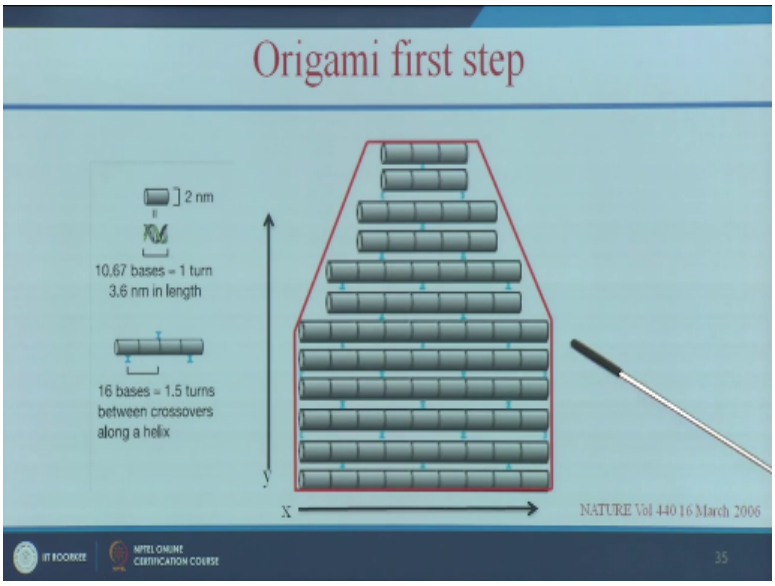
And here it need about a eight basis must be paired for a double helix to be stable at room temperature and this DNA based four way cross over structures producing a rigid plan a tile okay and the distance between the adjust and tile is 20 nano meter and as i told you in the previous lecture for a biological molecule like DNA are put in we have to use the enzyme first understanding the structure, so this is a from picture and we can see here, how it forms a beautiful lattice structure.

(Refer Slide Time: 20:13)



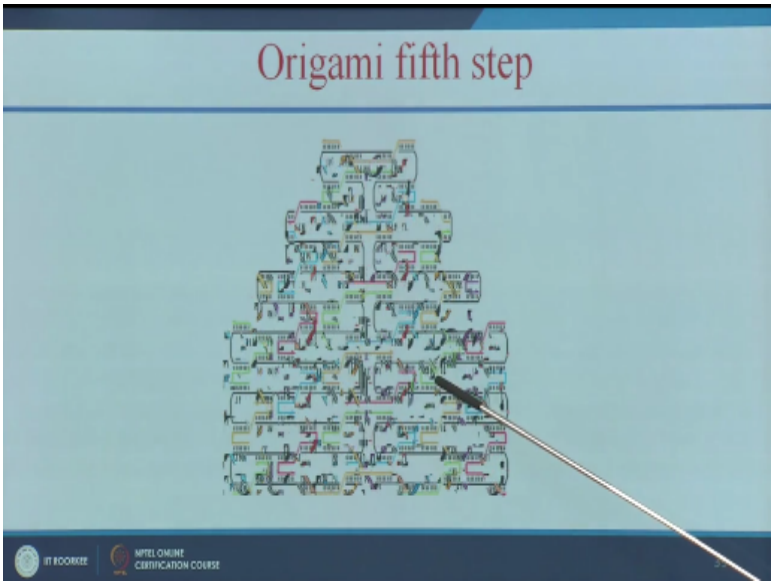
Let us see how to make 100 nanometers smile faces using this DNA okay, so here we can use a viral you know which is off 7000 to 49 basis long okay, and this venuesgance will be made into this kind of smile structures and using a small stands, that means where we going use like more than 250 short stands and we are going to make this kind of smile structure so this short stands are called as also called as staple stands, so for example if you want to staple the bunch of papers in this staple right. Similarly the DNA can be join to whether using this short fragments of DNA that is called as staple stand.

(Refer Slide Time: 20:55)



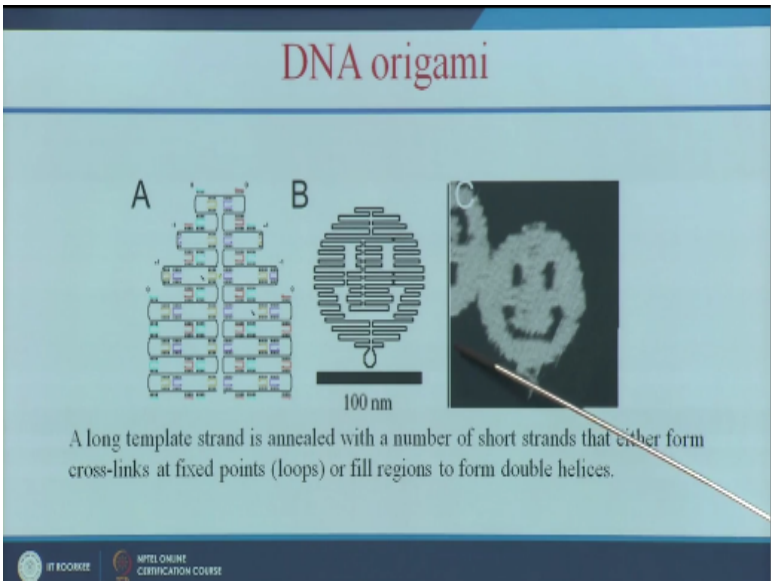
So if you want to make the smile capacity if face you should have a plan how to make this smile structure okay, so here you can see here the DNA is 2 nano meter diameter and the length is 3.6 nanometer for each helix. So this is a first step to understand how the staple stands you need to connect the 0 to make this smile shape.

(Refer Slide Time: 21:22)



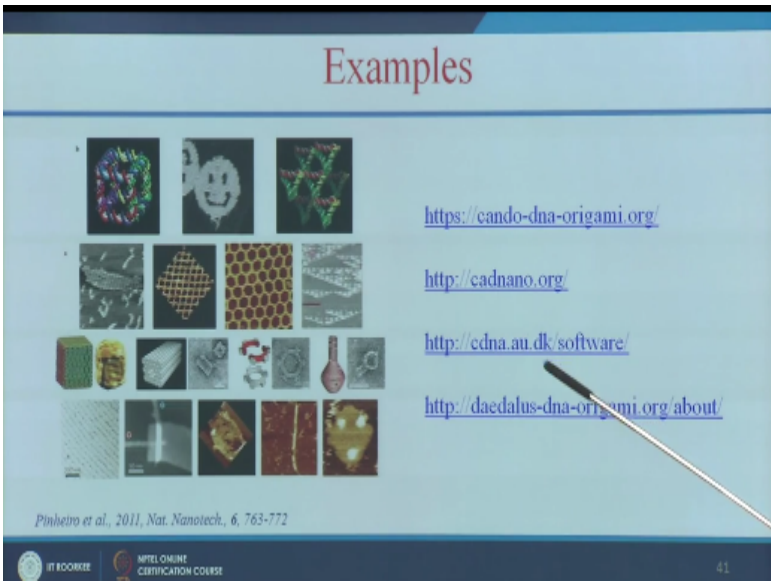
So once you made the plan so then second step is, so based on plan we have to draw the structure what are what can a smile you are going to make and then you will make the DNA sort fragments sequences so that will bind to this and we can add a some enzymes which will make that complete DNA sequence and finally you will get the complete structure.

(Refer Slide Time: 21:47)



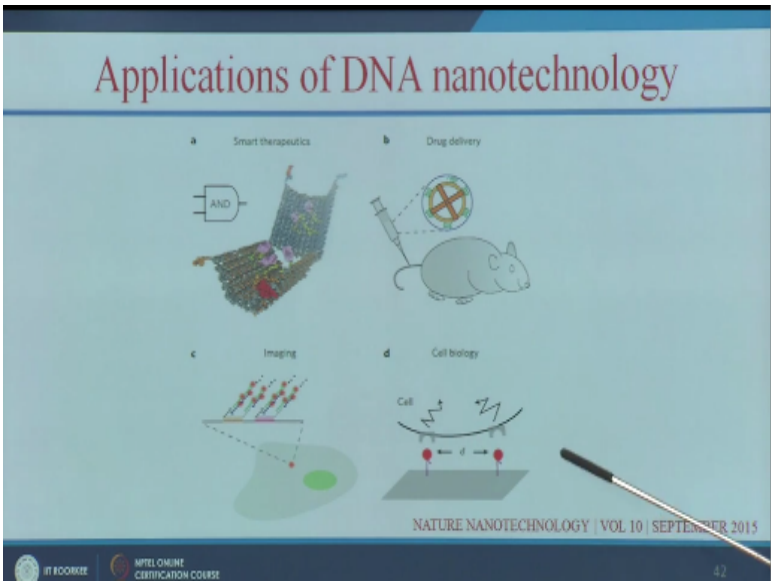
So here the long template strand is annealed with the number of short strands okay, so that either form at cross link at fixed points allows and to fill the regions to form the double helix so we will get the 100 nanometer smile face using this DNS.

(Refer Slide Time: 22:04)



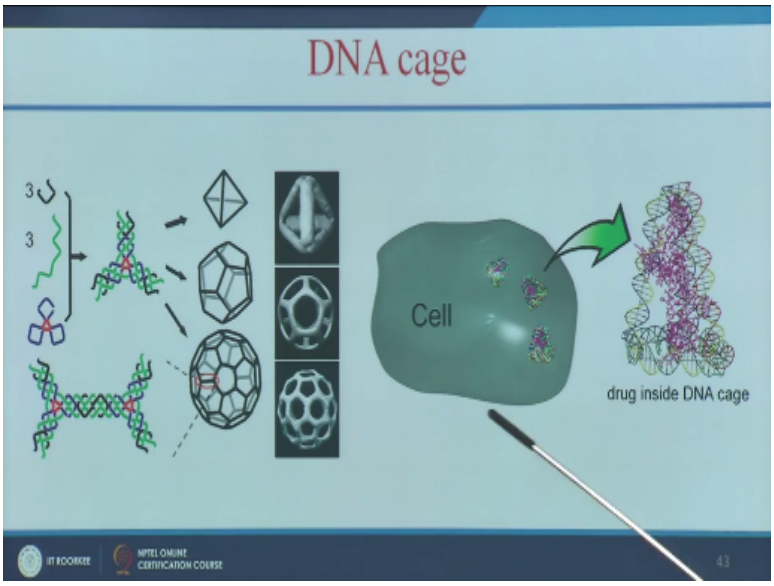
So similarly we can make a different kind of structures okay using this DNA as a construction material and these are the free software's available so design various nano structures using the DNA as a construction material.

(Refer Slide Time: 22:19)



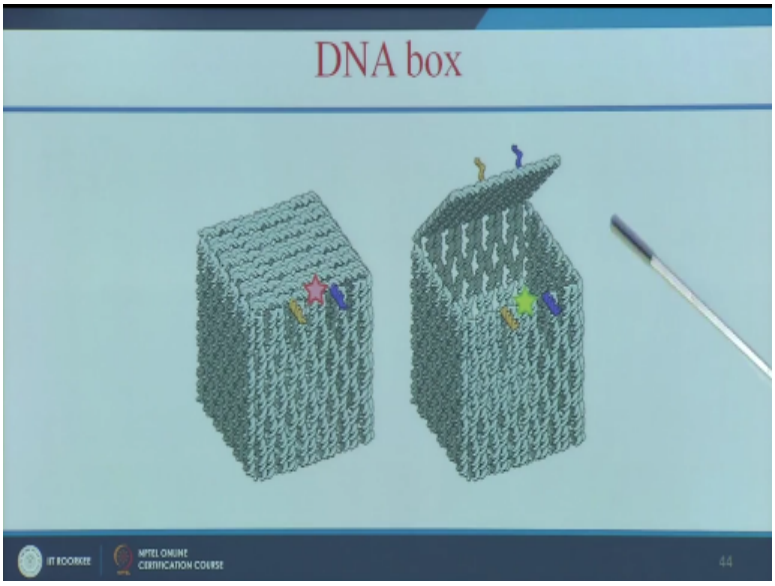
So let us see the applications of DNA nanotechnology so first one is smart therapeutics and the second one is drug delivery, so in the small therapeutics it will release the drug when there is particular signal is available, and we can also use this kind of nano carrier for drug delivery and we can use this DNA for imaging application for diagnosing various diseases and also we can use it for various cell barrage applications.

(Refer Slide Time: 22:50)



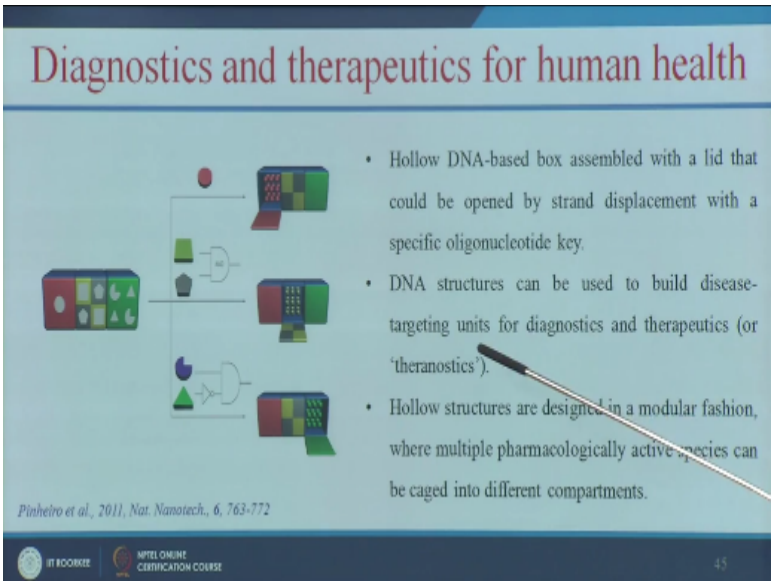
Let us see one by one the first one I told you like it can act like a DNA cage and it can carry any kind of drug, so we can load with any kind of anti drug and which can be transfer to the cell and it can kill the cells.

(Refer Slide Time: 23:04)



And we can also made a DNA based box okay, so this box can be loaded with anti drug or any therapeutic molecule and it has a lock this lock is also made by nucleic acid only so when we have the key of nuclei types is this open this box and it will release the drugs.

(Refer Slide Time: 23:27)

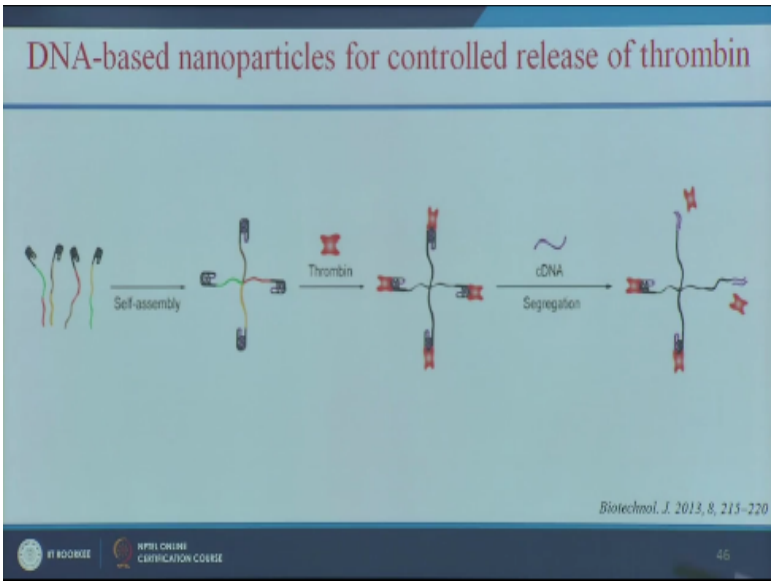


So we can adverse this DNA base box okay, so this hallow DNA box can be assembled with the late okay and it could be open by strand displacement with the specific oligonucleotide key, so this box can be opened with the oligonucleotide key so if we have a DNA sequence that will open this box and you can make this kind of box with the two different kinds of anti drugs or one box with the imaging agent.

So that is called as theranostics nano particle that means so the DNA nano structures which can be used to build disease targeting units for diagnostics as well as therapeutic purpose that means the same nano particle which can diagnosis as well as which can carry the therapeutic agent it is called as theranostic nano particle, okay and again this hollow structures can be designed where multiple pharmacologically active species can be caged into different compartments.

As I told you earlier in case of early stage of cancer if you want to release only this kind of drug, only this box can be open and the person who is having this advanced stage of cancer if you want to have two different kind of drugs and which can have the very good therapeutic effect in the cancer which is resistance to the drugs, okay.

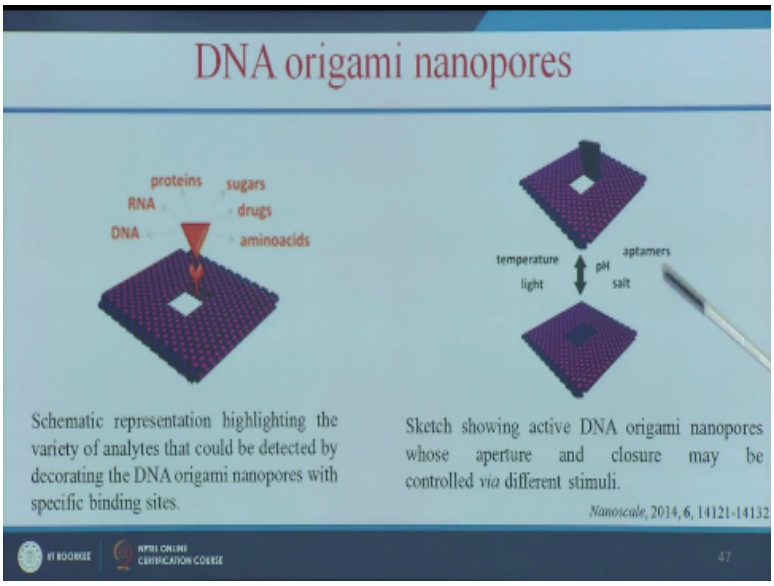
(Refer Slide Time: 24:44)



And also we can use this DNA based nano particles for controlled released of thrombin or any kind of molecules, so here you can see here we are going to use the small fragments have DNA pieces okay, and this DNA is specific for particular protein in this case it is specific for your Thrombin okay. So you can this self assembly process we can make X shape DNA tail and this thrombin will go and mind to this DNA and when you add the cDNA which is specific for this DNA.

What happens is the cDNA go and bind and it will release the thrombin so the thrombin can be released from this DNA. So that is why like we can use the DNA based nano particle for the control is of thrombin, if you have putting only one cDNA it will release only on molecule of thrombin and if you are adding like a four cDNA then it will release all the four thrombin. So similarly we can use that small fragment of DNA as a key to open the DNA based nano particles and it can release the drug according to the, our need.

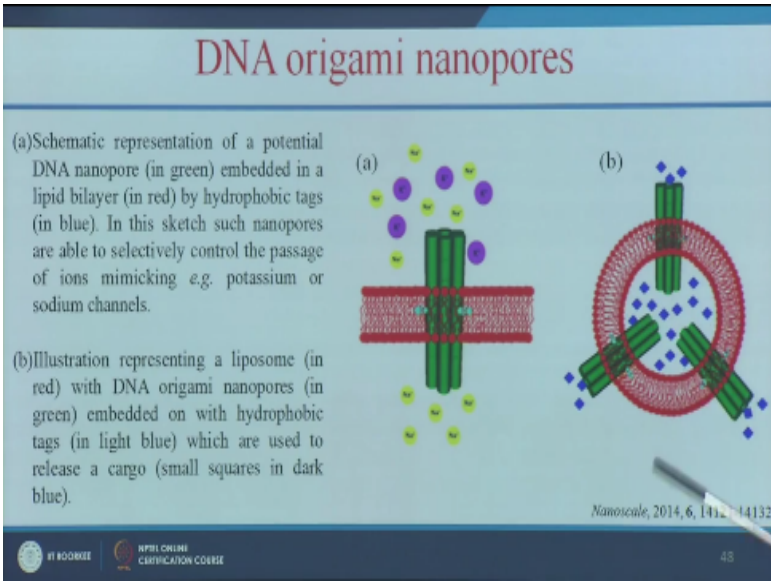
(Refer Slide Time: 25:47)



And we can also make DNA origami nanopores, so here you can see this example so here the it is a DNA origami nanopores okay, so that could be useful for detecting various compounds. For example, if you are having a receptor in this nanopores when you pass the protein or any passer a sugar or amino acids so it can easily detect and it can uses a kind of good sensor and again this DNA nanopores can be lock and open by using this kind of signals like temperature.

If I apply temperature it can be opened light or pH by using this kind of parameters we can open the pores or we can close the pores and this is also very important role in the control drug release.

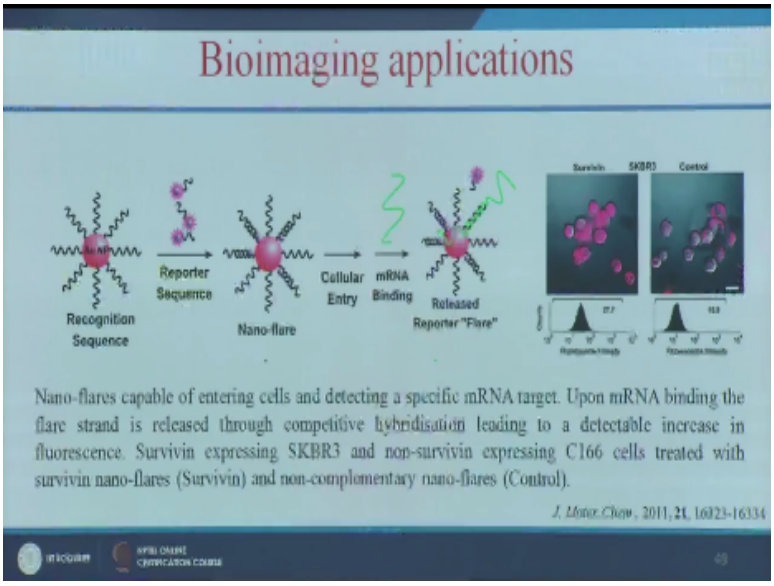
(Refer Slide Time: 26:34)



And next one is this DNA origami nanopores can also mimic like your potassium and sodium channels, so here you can see the example, so this DNA nano pore this is a green color one is a DNA nano pore and this red color is a liquid by a layer, okay and it is selectively acting like the potassium or sodium channels and here this DNA this green color is a DNA origami nanopores and it is emitted in this red color liquid by a layer.

And here also this blue color square is your anti cancer drug or any other therapeutic molecules, and this carrier can be useful for control delivery or control is of your therapeutical molecule.

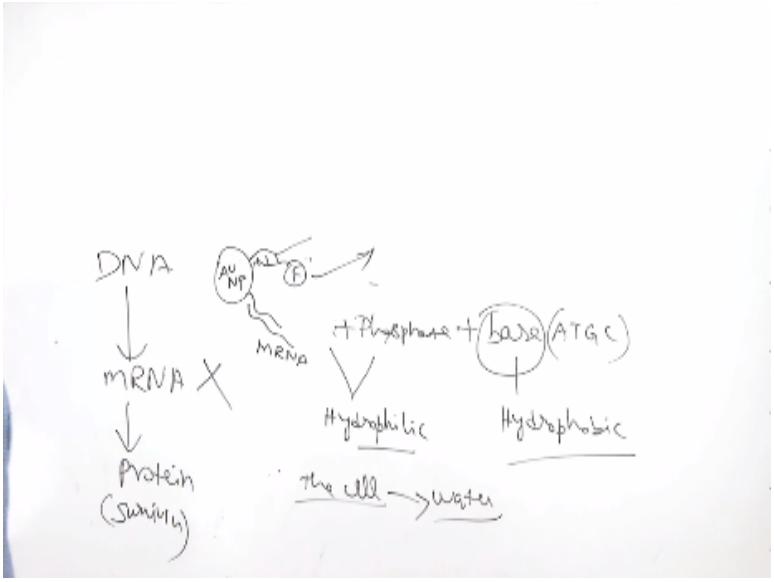
(Refer Slide Time: 27:14)



So the next application is we can used it for bioimaging application, here you can see here this grown nano particle it is tagged with a recognition sequence and we can add the reporter sequence, in the reporter sequence is tagged with some kind of f fluorine fluorescence molecule. So it is binding to this goal nano particle and form the nano flare once it enter the cells this sequence will target the mRNA, okay.

So when it is bind sources mRNA what happens is this fluorescence signal will be expressed okay, so it is releasing this reporter flare so it is giving a fluorescence signal. So in the example, so this is survivin is one of the anti upper protic that means this kind of protein mostly expressed in the cancer cells so you target this kind of proteins and it could be useful for cancer therapy, okay. So how do you stop this, we can stop at the protein level or we can stop at the mRNA level.

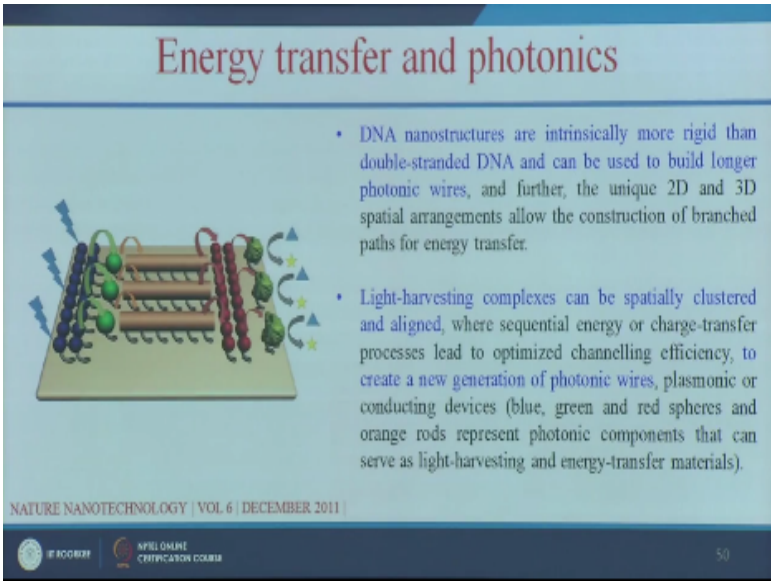
(Refer Slide Time: 28:17)



So from DNA we will get the mRNA and from here we will get the protein, so in this example so survivin you have to stop this survivin protein so we can stop at the mRNA level okay, so the sequence which is tagged to goal nano particle so this mRNA will bind to this okay, so what happens is when the mRNA binds the sequence with the fluorescence signal it will be released. So once it is released it will give the fluorescence signal.

So here you can see here the cells which have the more amount of surviving you can see here more amount of fluorescence signal when compared to the control cells where the survivin expression is less or no expression of survivin.

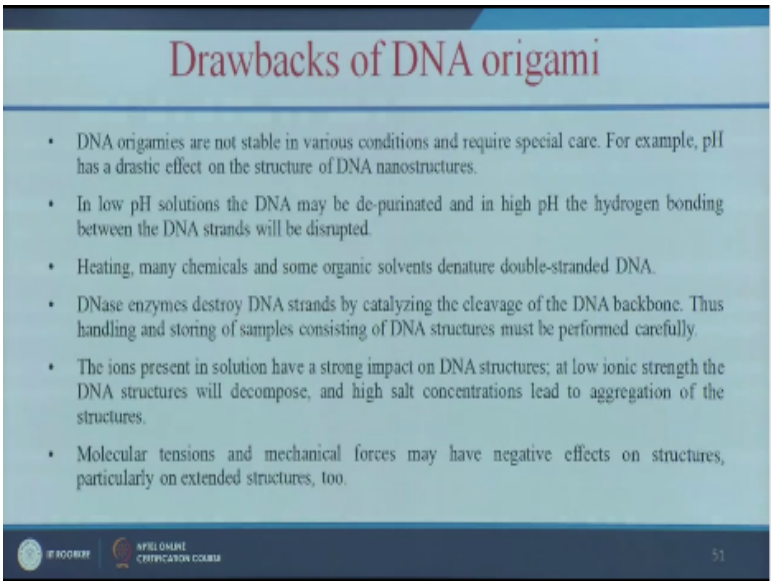
(Refer Slide Time: 29:12)



So we can use this DNA based nano flavor for various diagnostic as well as for therapeutic applications, and next application is we can also use this DNA based nano structures for energy transfer and photonics okay, so here the DNA nano-structures are intrinsically more rigid than the double standard DNA so it can be used to build a longer photonic wires, so using this we can harvest light it can act like a light harvesting complex okay, by especially clustered and aligned to create a new generation of photonic wires.

So using, we can use the DNA as a photonic wires and we can also use it for energy transfer as well as photonics.

(Refer Slide Time: 29:52)

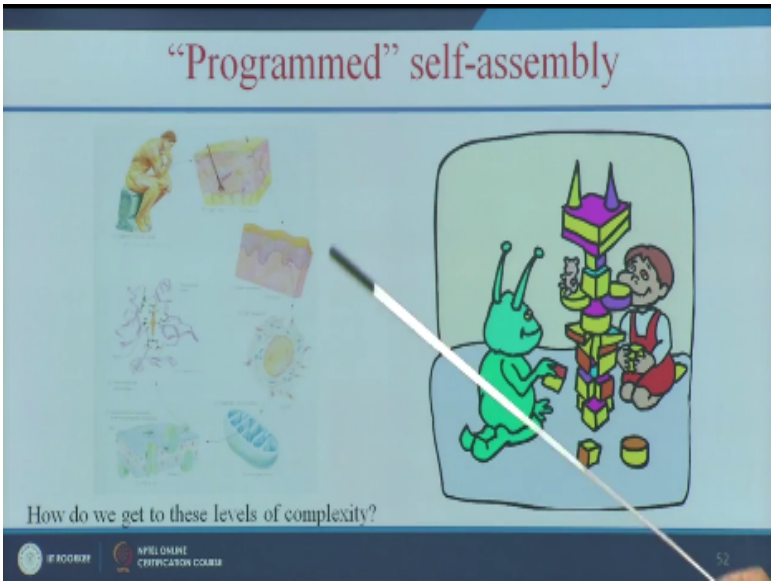


So let us see some of the drawbacks of DNA origami, so here the DNA origamies are not stable in various conditions. It required special care for example, pH has a drastic effect on the structure of DNA nano-structures. In low pH the DNA may be de-purinated and in high pH the hydrogen bonding between DNA stands will be disrupted, okay. And also the heating many chemicals and some organic solvents may denature the double standard DNA.

And again the enzymes like DNA enzymes which can destroy the DNA stands and it can also destroy the DNA back bone, okay. So thus, we have to store and handle this samples very carefully and again the ions present in the solutions have a strong impact on the DNA structures, okay. So at low ionic strength the DNA structures will decompose and at high salt concentration which lead to aggregation of the structures.

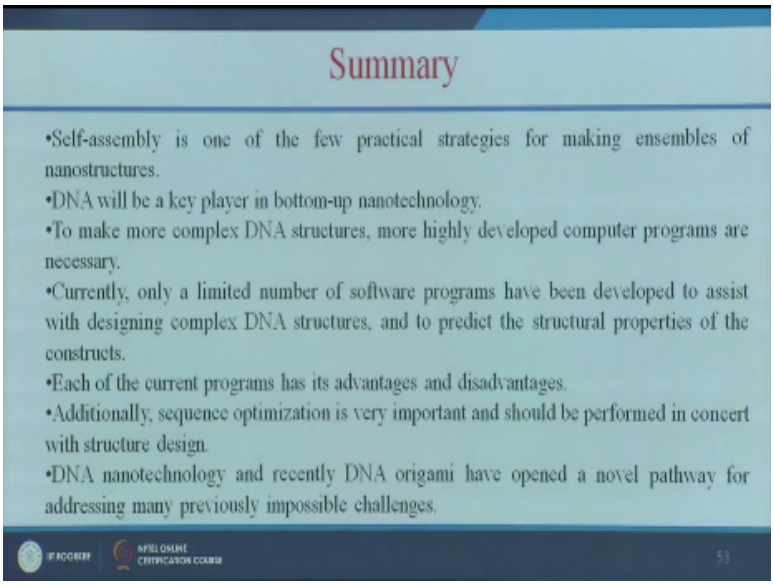
And also the molecular tensions are mechanical forces also have the negative effects on the structures.

(Refer Slide Time: 30:55)



So we are also example for the program self assembly, so if you see that we also assigned from a simple atoms the atom combines and found the cells and the cells combine and form the tissues and tissues and combine and form the organs and organs combine and form the complete body. So if you understand the self assembly we can also make such kind of complex structures and nano scale devices and everything. But our understanding is of self assembly is still in the elementary level.

(Refer Slide Time: 31:22)



And as a summary the self assembly is one of the few practical strategies for making enzymes nano structures and here the DNA will be the keep layer in bottom up nano technology okay, so to make more complex DNA structures you need more highly developed to compute a programs okay, and also we are limited with number of software programs to develop to understand the DNA structures are making the designing the complex DNA structures.

And each of the program has a own advantages and disadvantages and additionally we have to sequence optimization is very, very important okay, and again the DNA nano technology and the DNA origami it has opened a novel pathway for addressing many previously impossible challenges, okay. So I will end my lecture here thank you all for the thing in this lecture I will see you in another lecture.

For Further Details Contact
Coordinator, Educational Technology Cell
Indian Institute of Technology Roorkee
Roorkee – 247667
E Mail: etcell.iitrke@gmail.com. etcell@iitr.ernet.in
Website: www.iitr.ac.in/centers/ETC, www.nptel.ac.in

Production Team
Sarath Koovery
Mohan Raj. S
Jithin. K
Pankaj Saini

Graphics
Binoy. V. P
Camera
Arun. S

Online Editing
Arun. S

Video Editing
Arun. S

NPTEL Coordinator
Prof. B. K. Gandhi

An Educational Technology Cell
IIT Roorkee Production