Computational Systems Biology
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Lecture - 99
Introduction to Synthetic Biology

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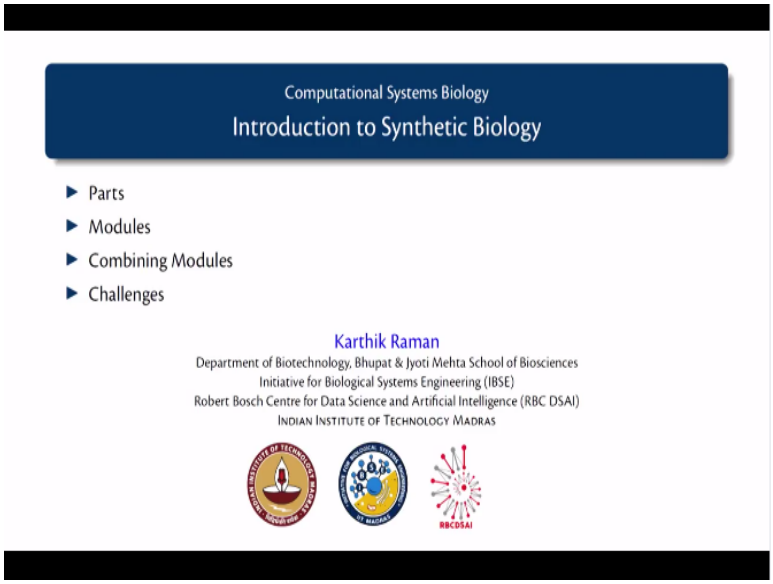

So in today's video, I will finally introduce you to synthetic biology which is a you know very interesting buzzword that is going around today and it is a very nice approach to engineering biological systems. So note that you know we are trying to systematically engineer biological systems. So you want to predictably engineer biological systems. So in today's lecture, I will overview some of the concepts.

What are the different synthetic biology parts that people have made? So these are akin to you know the logic gates or circuits that electrical engineers make. So can we make reliable parts, can we make modules building on these parts, can we combine modules together and what are all the challenges involved in all of these right. So these are some of the concepts I will try and overview today.

So what is synthetic biology? A reasonable description, it is a bottom of synthesis of a biological system. How is it different from genetic engineering? **"Professor - student conversation starts."** Well, you still stick in new components and so on in genetic engineering as well. So you can integrate a gene know, I can take a new gene and stick it into

a cell that is in a way synthetic biology in a way genetic engineering. **"Professor - student conversation ends."**

The key idea about synthetic biology is that you try to be a lot more rational here right, meaning you expect that this is what is going to happen and the key idea here is can you make it more like engineering right. So in engineering you understand that if I stick in this module into the circuit this is how this is going to work and it is an electrical engineering or something. So can you bring biology to that level of understanding right?

And people usually quote Feynman here who set something like what I cannot create I cannot understand right. So another very key aspect is in some sense understanding those design principles we were just talking about. So what are the design principles of biological systems or how do you design biological systems that can have the specific function right things like that.

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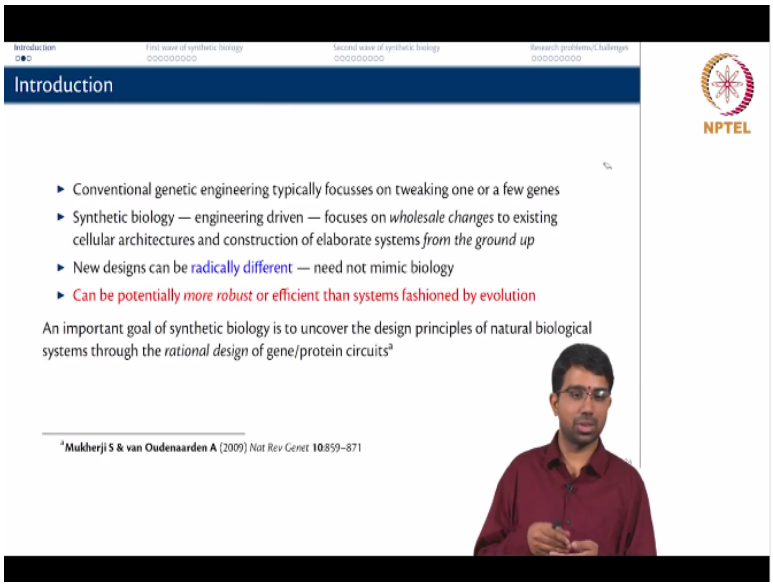

So conventional genetic engineering typically focuses on tweaking one or two genes right a few genes even but synthetic biology is engineering to when and focuses on wholesale changes to the existing cellular architecture and construction of elaborate systems in a bottoms of manner right and the idea is that new designs can be radically different, they do not need to mimic existing biology and they do not have to stay true to that and they can potentially be designed to be more robust than systems fashioned by evolution.

Because anything fashioned by evolution while undoubtedly very robust and so on, often has some artifacts because of its trajectory right. There is some history associated with the system right. One of the important goal of synthetic biology is to uncover the design principles of we were just discussing right. So can we rationally design circuits that are very robust that can exhibit a specific function and you know like oscillation or adaptation or switch like behaviour and so on in a reproducible reliable fashion.

And the important thing is there it has to work when you stick it into a system, so this is always a challenging part, you build something in-vitro it works right, what happens when you take it in-vivo, when you implement it within a cell does it work or not?

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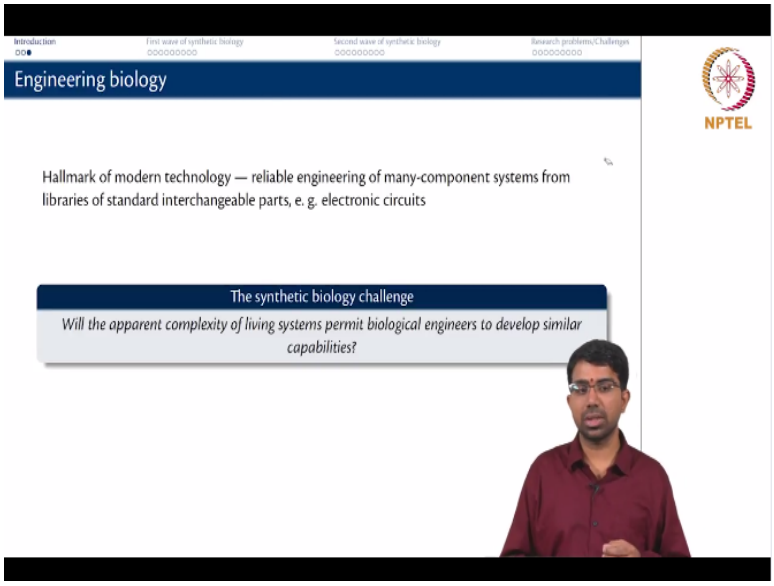

So the hallmark of modern technology is basically reliable engineering of many component systems right, so if you look at a system like this or a laptop or so on there are like thousands of small, small components but you know how they can be reliably composed to work together right. In biology, this composition is the toughest part. You cannot compose things when you take A and B and put together, you never see A+B.

You will see either you know A square+B square or A-B something you know not even linear right. So it is always going to be some really complex synergy or antagonism that happens when you put two things together because there are so many ways in which they can interact, there are so many molecules, so many proteins and so on.

And this synthetic biology challenge is will the apparent complexity of living systems permit biological engineers to develop such electrical engineering like capabilities right where I know take this module, take this oscillator and take this I can make a pump that so you take all these components you take this oscillatory, you take this module put it and maybe you can get a diabetes pump or something that secretes your insulin at specified intervals and so on.

So can you have this kind of a modular behaviour wherein I know if I put module X and module Y together I will get a particular behaviour right. So this predictability becomes a very important aspect and is difficult to achieve right.

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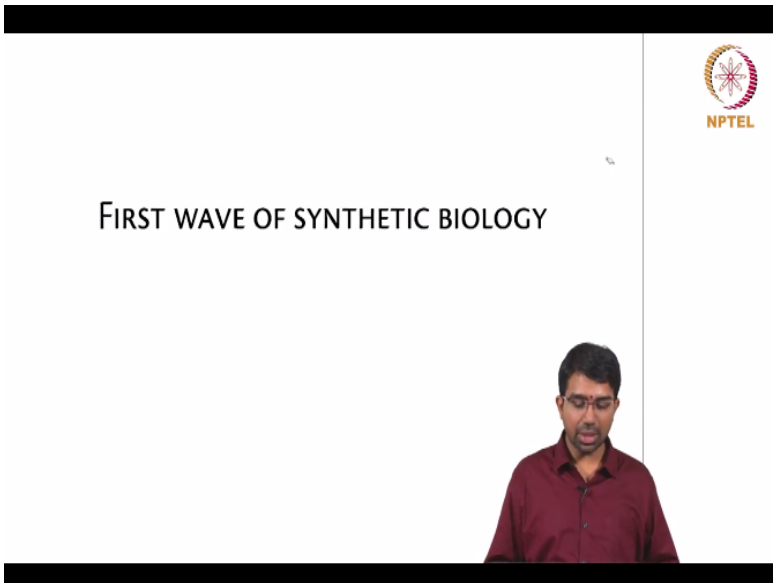

So let us look at what was the first wave of synthetic biology.

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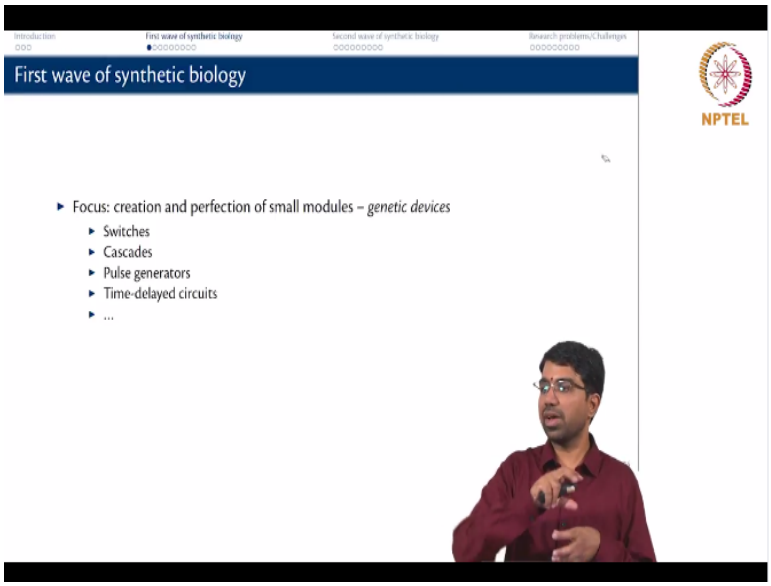

So the idea was very nice and lots of toys so what were the toys, people created small genetic devices like switches, cascades, pulse generators, time-delayed circuits and so on right. So all these modules or all these parts becomes central to creating more complex systems, so you stick all these so you want to build a more complex system, you need a you know a pulse generator or an oscillator or a switch and so on and you stick them with in tandem with some other components and finally you will get an interesting behaviour.

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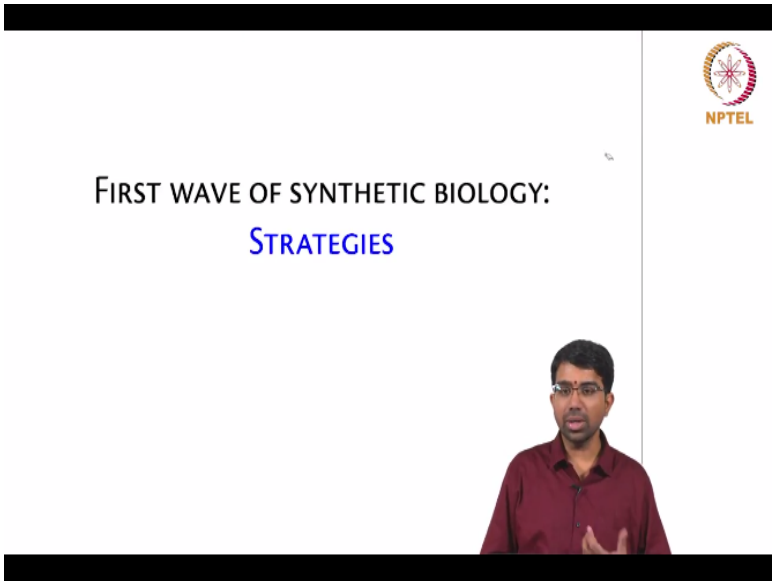

So what kind of strategies did people use?

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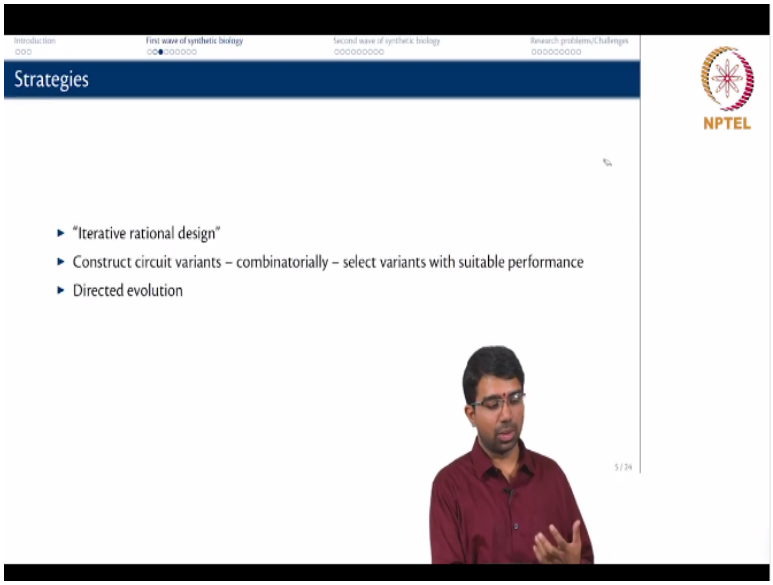

So it is what they call iterative rational design, so you construct circuit variance combinatorially and then filter out to those that have very good performance right. You can do this using laboratory evolution and so on right. You put a certain selection pressure, you

eliminate those that do not show the desired behaviour, there are directed evolution strategies so on and so forth.

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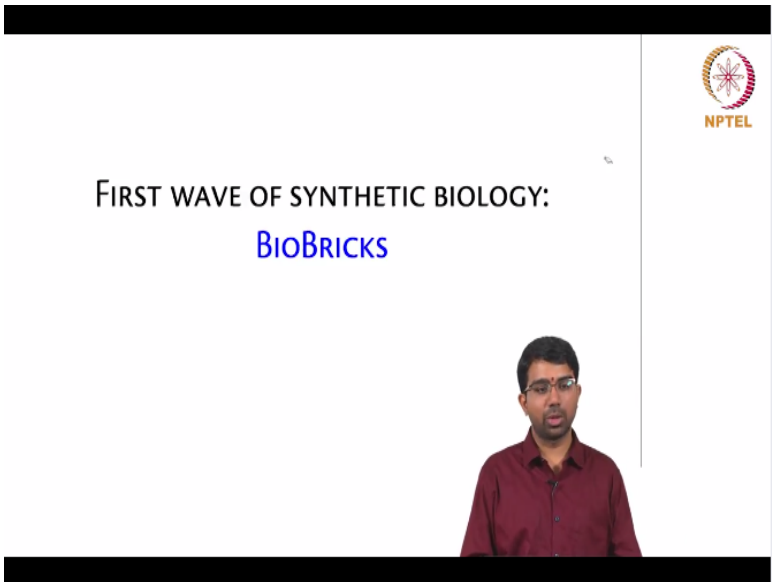

So this was one set of strategies that people used and you may have heard about BioBricks and iGEM competition and so on.

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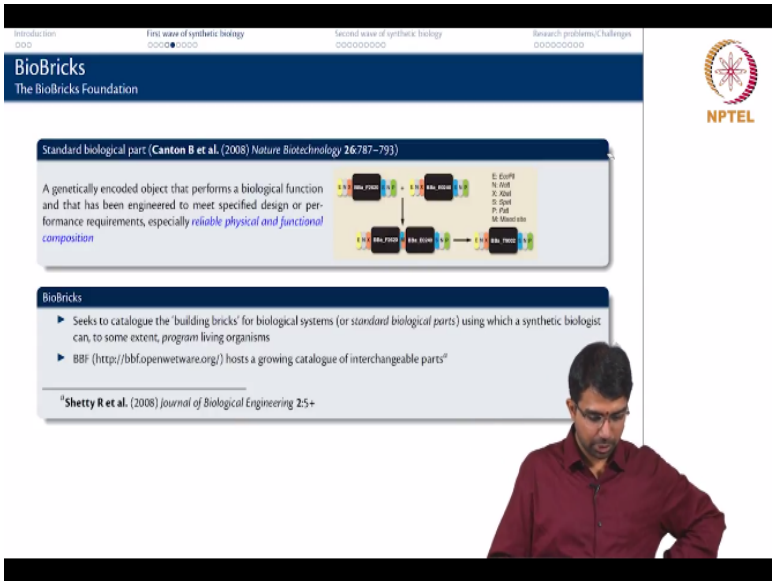

So what is the standard biological part? This is a nice definition, so it is a genetically encoded object that performs a biological function and that has been engineered to meet specified design or performance requirements right for the tough part especially reliable, physical and functional composition right. It means that you design a system, you put these whatever proteins or whatever together right actually DNA right.

It is a stretch of DNA which will finally (()) (07:04) proteins whatever and so on but if you stick this DNA into a system, it is expected to perform an oscillator function right. So it meets specific design requirements and if you stick it into a system it behaves as expected right and the idea of BioBricks was to catalogue building bricks for biological systems are standard biological parts like all your electrical ICs and things like that using which a synthetic biologist can program living organisms right.

So you put an oscillator and put this then it is going to behave in a particular fashion. So the BioBricks Foundation holds lot of interchangeable parts and so on.

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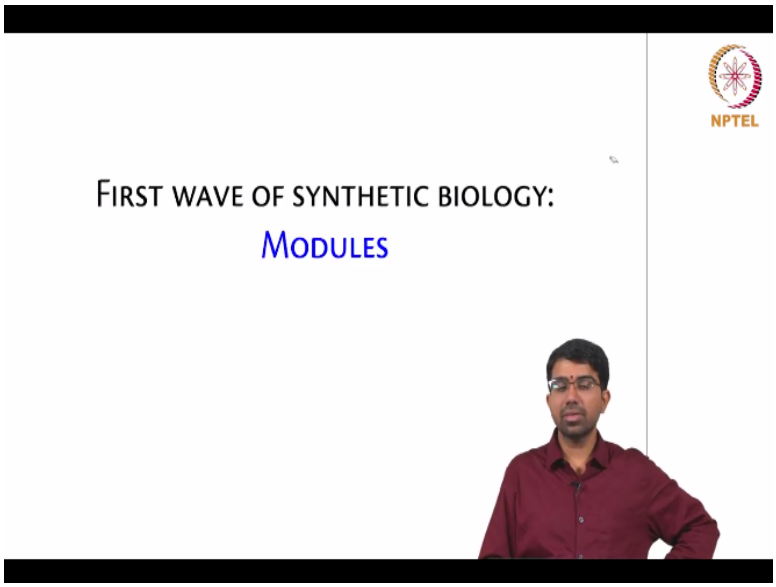

So then there were a lot of modules that were built.

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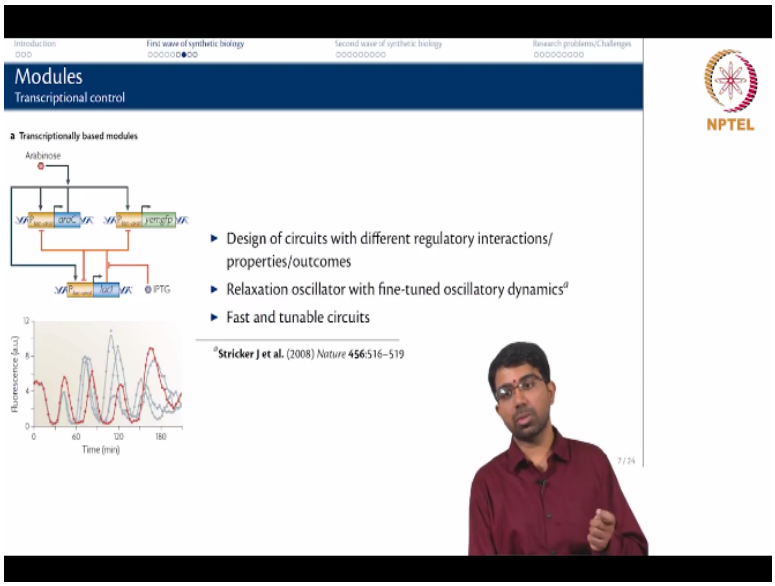

So there were some transcriptionally based modules, for example this module is dependent on Arabinose and so on. So if Arabinose is present, it will behave in a particular way, there will be a GFP that is triggered and there is some it also interacts with the lac i gene and so on so forth right and they found some oscillation in florescence. So this particular transcriptionally controlled system exhibits oscillations as you see in the bottom right.

So there are people who have built a relaxation oscillator with fine-tuned oscillatory dynamics and it is not sufficient if you build oscillators, are they fast and are they tunable right. By tuning, we mean that you can change the amplitude of frequency especially right. Can you change the frequency of the oscillations or even the amplitude? You want to tune the circuit to respond in a particular fashion right.

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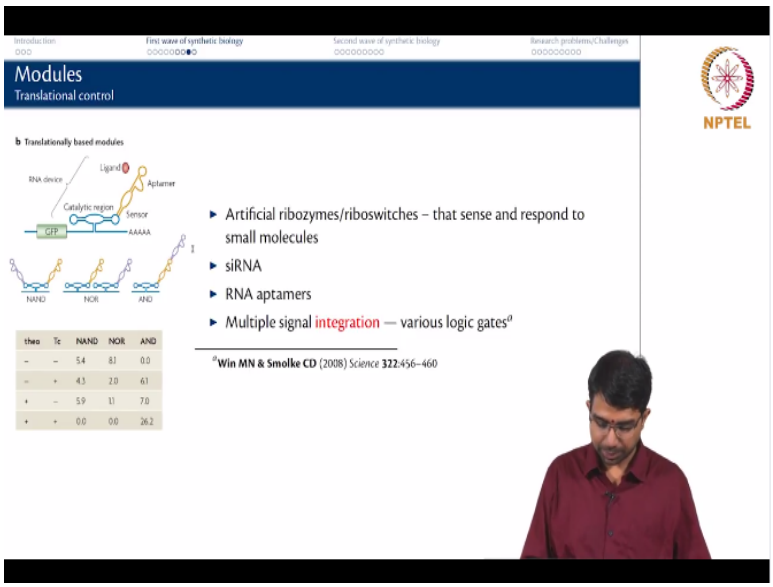

There are translational modules where you know there are you know ribozymes or riboswitches. So ribozymes are basically RNA molecules, it can act as enzymes. So RNA can fold into nice loops and so on and there are regions of RNA that can actually bind certain ligands. So you see there is a ligand you know there is a theobromine binding domain in one of these RNA and so on.

And they also built circuits that they could act as a NAND gate, NOR gate and AND gate and so on right. Only if both RNA bind when a particular response is produced or so a NAND gate kind behaviour and a NOR gate type of behaviour and so on. So you can see what is the output right. If both are not present, there is no output. If both are present, you get some output.

Even in these you need to get zero output but this is obviously not a digital gate, it is a little analogue. So these are low values which are essentially the equivalent of off and this is a high value the equivalent of on right and this uses various biology concepts such as siRNA and RNA aptamers and you can also integrate multiple signals right. So the NAND gate integrates two signals and then knocks them, NOR gate adds two signals and then knocks them, AND gate just multiplies two signals in some sense.

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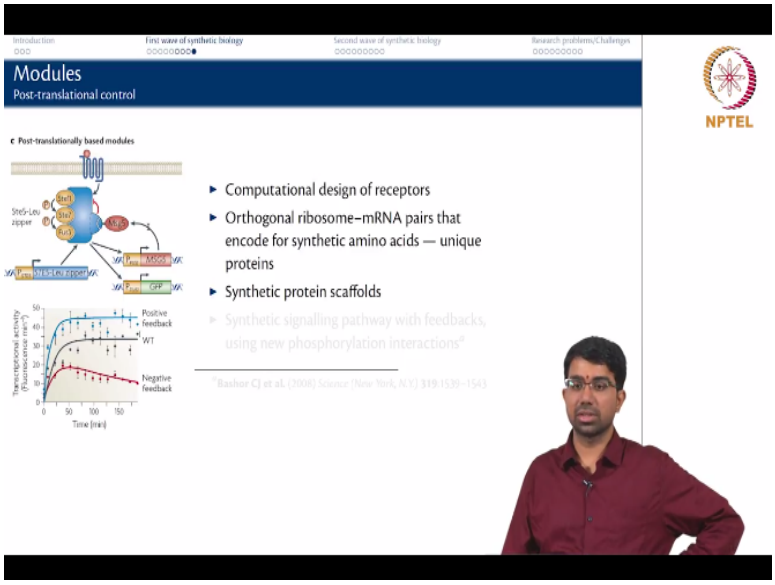

And there are also post-translational modules so if you go back to the very beginning of the course where we discussed the flow of information as transcription, translation followed by PTMs or post-translational modifications like phosphorylation and so on and things like phosphorylation give you very good fast switching behaviour right. You can quickly switch something on, switch something off and so on and so they are very useful.

So if you see there is negative feedback which works in a particular way, there is positive feedback which gives you an amplified response and so on. So they design very nice receptors that like this which can give you interesting behaviour. So they had an orthogonal ribosome mRNA pair that could encode for even synthetic amino acids right, not just the 20 amino acids, there are even very interesting studies that have been done which use a different translational machinery.

So no longer three-base codons or four-base codons right which can potentially be an orthogonal translational machinery in an organism right and another very interesting concept

is that of synthetic protein scaffolds right. So this is especially useful in say polymerization reactions and so on.

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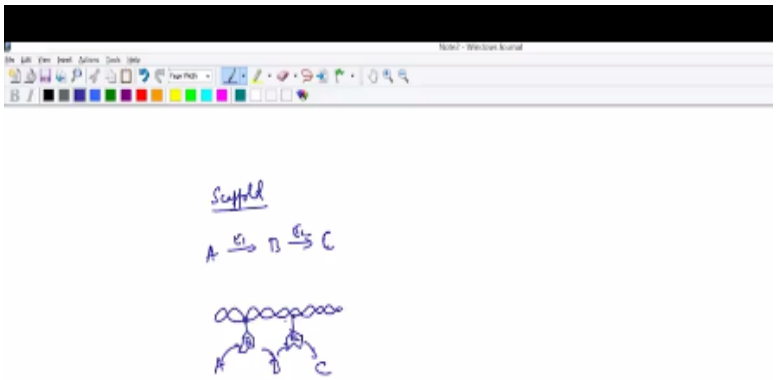

Or in any pathway wherein you have let us say you have A e1 makes it B e2 makes it C right. So the idea here is you take a DNA and you use it as a scaffold and you put some nice protein molecules on it. So this could be your e1 and this could be your e2. So this will facilitate better reactions right. So now A goes in, comes out as B, goes in this spatial colocation comes out as C.

So these enzymes are stuck onto a DNA scaffold to bring them closer and to improve reactivity and so on.

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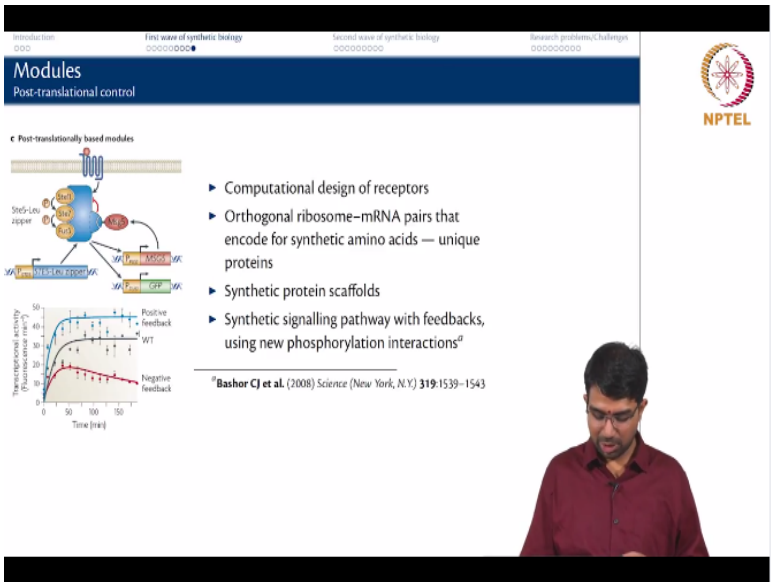

So there are very interesting studies which have used the concept such as these and they have also studied synthetic signaling pathways with different kinds of interactions. If you see the signal binding domains and proteins are very modular and so there are people who actually swap, mix and match those domains to create artificial signaling pathways which work very differently and so on.

But basically there are modules that are built on transcription, translation as well as post-translational modifications. I am just giving you an overview here. You can always go back and read a lot more and some nice paper such as these right.

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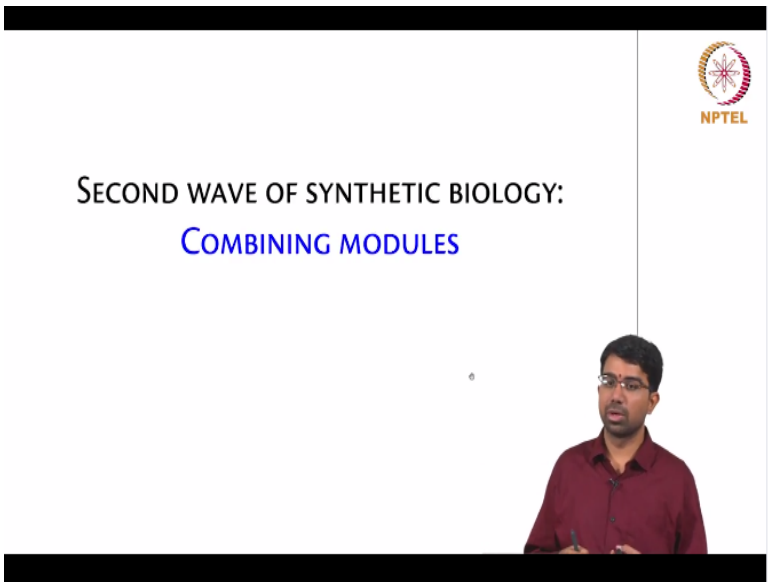

The next wave of synthetic biology is combining these modules. How do you put these modules together to build more complex systems, more complex functions?

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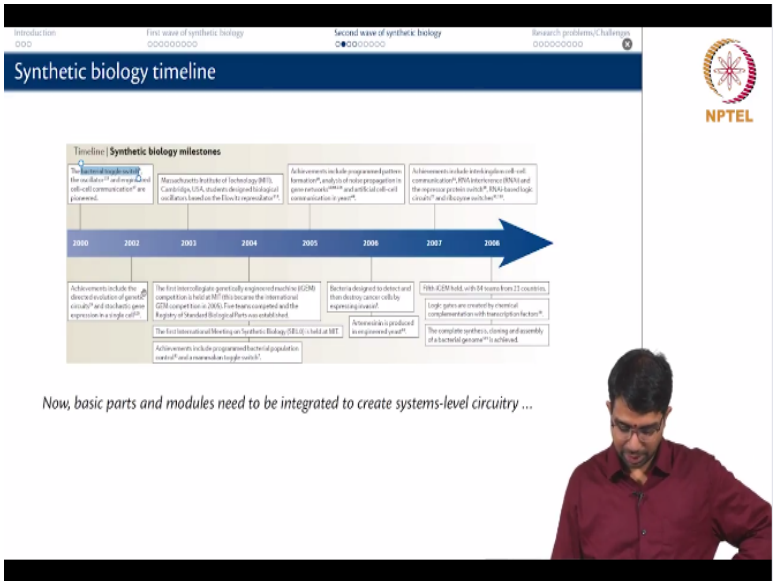

And this is a nice slide telling you the milestones of synthetic biology. So the first bacterial toggle switch and then you know the iGEM competition. The first iGEM competition was in 2004 and is a nice strategy wherein bacteria were designed to and this was called a pathogen find and destroy right. It could search for pathogens and then destroy cancer cells. This is cancer cells so they could sense a hypoxic environment.

There are also studies which try to detect a pathogenic organism and then secrete certain because things like cancer or even an infection, the difficulty is in selective destruction of the disease cells right. So how do you do that? So in this case they try to selectively detect cancer cells by searching for hypoxic environments because in cancer there is lot of hypoxia right and then this artemisinin we will try to study that.

This is a little old and still talks about the fifth iGEM. We regularly have teams from all over the country even India participating in iGEM so last year I think IIT won some very good medals. This year IISC won the gold medal and so on. These are the local chapter gold medals and there is also the final contest in Boston which was far more competitive. So now we are slowly going towards combining basic parts and modules to create systems level circuitry right.

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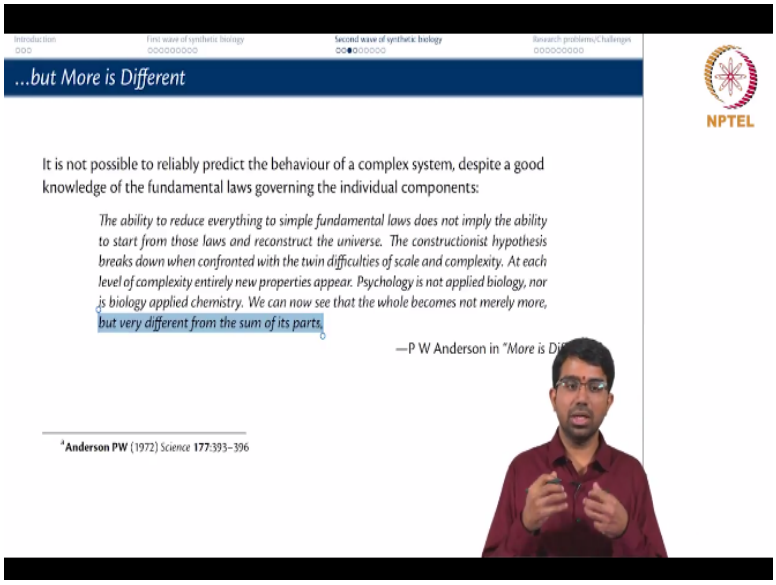

But the problem here is this is something I probably showed you in the very first lecture of the course, more is different. So this is Anderson in 1972 Nobel Laureate who saw things way before others did. So he said psychology is not applied biology nor is biology is just applied chemistry right. See biology is nothing but a combination of various biochemicals.

But there are so many emergent properties that occur and the whole becomes not merely more but very different from some of the parts and this is the challenge that one needs to tackle when you look at even synthetic biology or of course systems biology itself right. You have a very complex system. It behaves very differently from the basic modules that make it up right.

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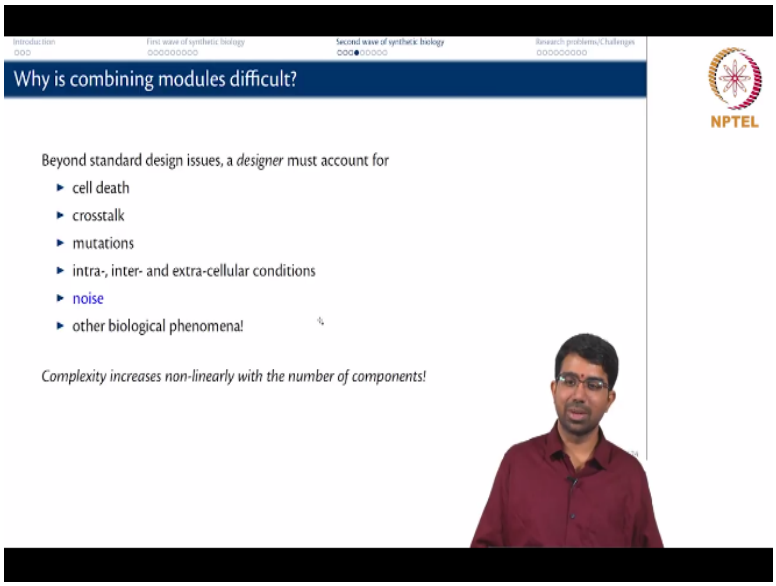

So beyond the regular standard design issues, you must worry about things like cell death, crosstalk between different modules right. There are some molecules, some chemicals, some signaling molecules present in different parts of the cell and they could all be integrating with your module and other modules. Mutations that can happen spontaneously, the cell may just try to kick out your module in the first place right.

Intra, inter and extracellular conditions, noise which becomes a major challenge right. There is so much of heterogeneity in cellular gene expression or in any of the other components within a cell and so on and various other biological phenomena not in the least evolution right. The cell can just you know mutate, change, evolve, behave very differently after a few generations right and complexity increases really nonlinearly with the number of components right.

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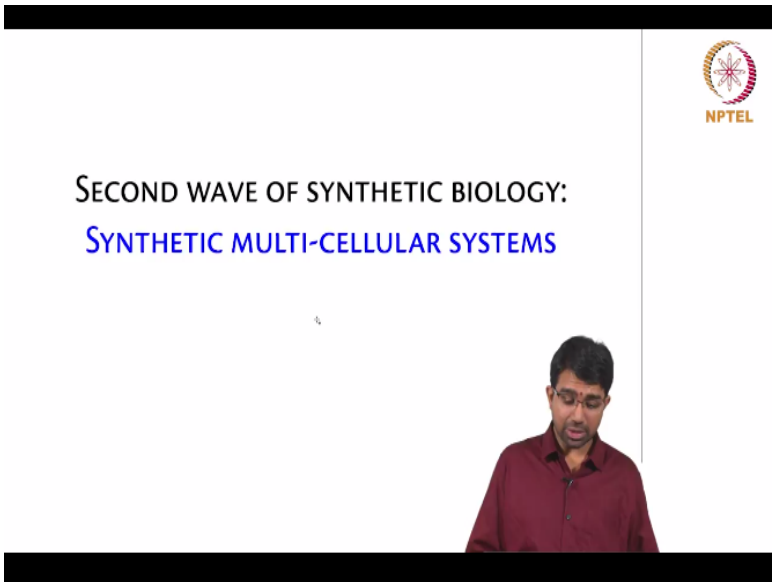

So this is also very interesting studies that have come up which talk about synthetic multicellular systems.

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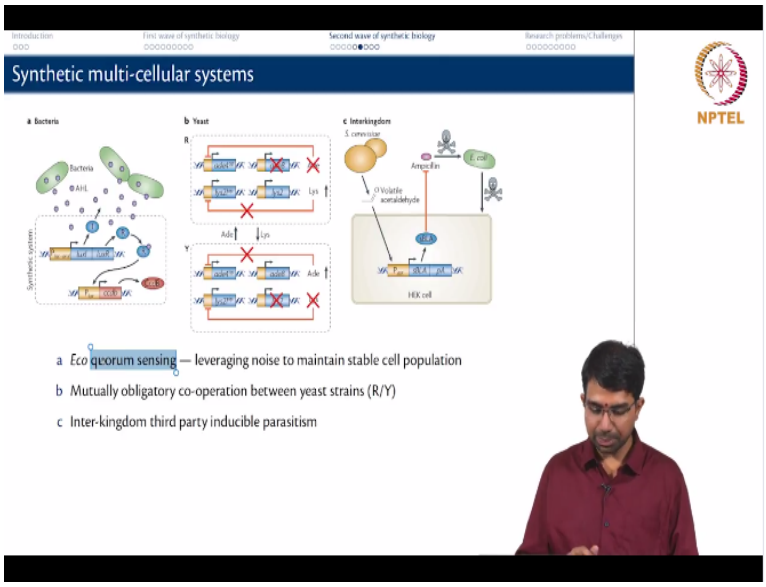

Some of them are still toys like for example there was this system wherein you know an adenine utilizing enzyme was destroyed in one and a lysine gene was removed in the other. So this cannot produce adenine, this cannot produce lysine and they were made to obligately cooperate with one another right. They are obligated to cooperate because they cannot survive otherwise right.

So this is a very interesting study and then there are many quorum sensing studies which leverage noise in the levels of various proteins to maintain a stable population and so on and very interestingly they also tried some inter-kingdom interactions right. So there is E. coli and yeast right. So this was just two yeast cells, this is just all bacterial cells but this E. coli and yeast cells which try to find a particular kind of cell and you know they induce parasitism and so on.

Like you can then destroy a particular cell and things like that, so many interesting studies, lot of them based on quorum sensing because any cellular communication involves a lot of quorum sensing right.

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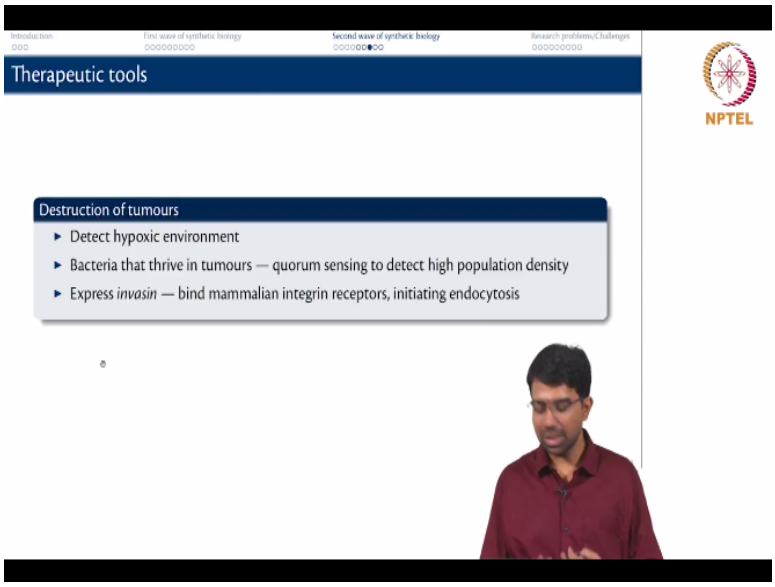

And then there are some nice therapeutic tools that have been built so which for example destroy tumours by detecting a hypoxic environment in the first place and there are bacteria that thrive in tumours using quorum sensing to detect high population density and then they express invasin which bind mammalian receptors and initiating cell destruction endocytosis right.

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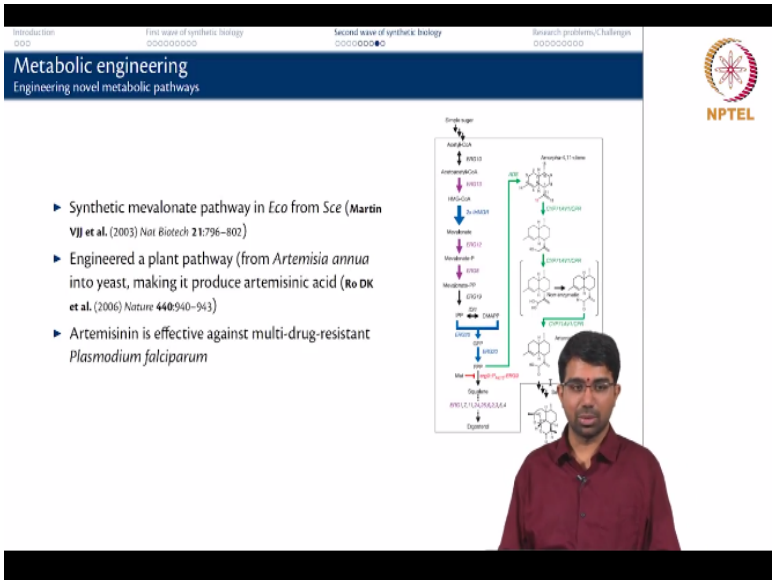

So there is some very interesting applications of synthetic biology. Another classic metabolic engineering application is how they stuck this pathway from artemisinin from Artemisia annua a particular tree to produce artemisinic acid. So if you see they produce artemisinic acid through metabolic engineering followed by semisynthesis, this is chemical synthesis. So they could not make artemisinin directly.

But they found a way to actually make artemisinic acid by integrating these genes from Artemisia annua into Saccharomyces cerevisiae followed by chemical synthesis of artemisinin and this is a very important anti-malarial drug. I have recently heard that this has not been very stable system right, although this engineering was successful in the beginning but there are some issues when you try to scale it and so on.

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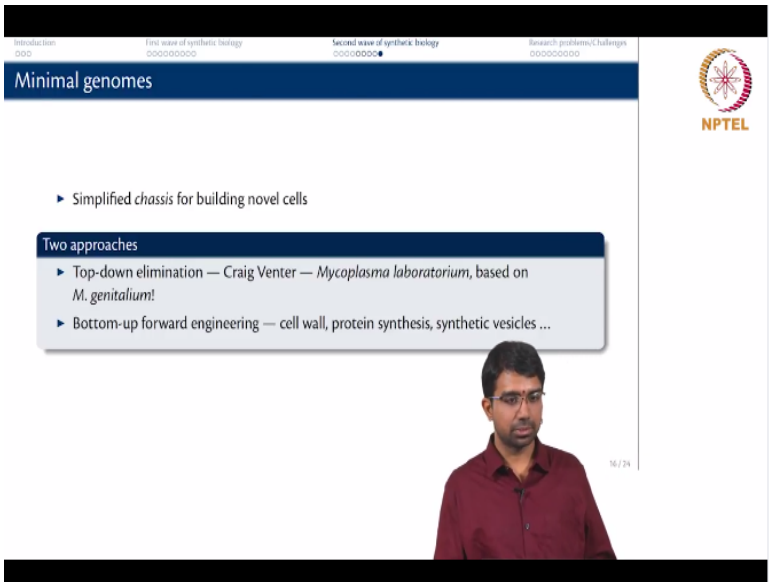

So there are always challenges that we need to worry about and the other very interesting aspect is how do you design minimal systems or minimal genomes right? This is one interesting direction for synthetic biology because if you want to engineer an E. coli or Saccharomyces cerevisiae cell you already have to live with all its existing complexity and an E. coli cell already has 4000 genes into which you are integrating your one new module.

And it has to interact with all those 4000 genes and still survive and so on right. So could you build a minimal genome like could be build a minimal genome which say 300 genes or 400 genes? It is still a large number but potentially less interaction, less complexity to worry about right and there are two interesting sets of studies that have been done. So one is Top-down elimination wherein there are groups which have been knocking out genes from different organisms.

And then there is also Craig Venter who has been I think some of this work is already published now which talks about say Mycoplasma laboratorium based on Mycoplasma genitalium. Mycoplasma genitalium is already a very fastidious organism, it is a very small genome and it is pathogenic and it can only grow in certain conditions and so on right which

means that it needs a lot of nutrients to survive and so on because it cannot make too much on its own.

And they made Mycoplasma genitalium encode the genome for a new organism and start producing this organism. There is a very interesting set of studies and there are two very nice books if you want to read about synthetic biology. There is Regenesis and that is by George Church and there is I think Life at the Speed of Light by Craig Venter himself. So Craig Venter is the genome sequence expert.

There are also groups that are trying to bottom-up forward engineering so how do you put up a minimal cell wall, how do you just integrate some basic protein synthesis machinery or can you have synthetic vesicles and so on and there is also the other synthetic biology is becoming an ocean now, so the courses on synthetic biology I cannot do much just as a synthetic biology in a lecture right.

But the other interesting concept is that of cell free synthetic biology. So can you have essentially in-vitro synthetic biology? You do not have the full cell but you have a circuit that just operates within a test tube literally. **"Professor - student conversation starts."** Robustness will go away but yeah robustness will go away. In fact, they also found that the ATP maintenance then which also went up and so on.

So the organism becomes must more fastidious, much more fragile and it needs more pampering to survive literally. **"Professor - student conversation ends."**

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And there are many, many interesting research problems and challenges as you would expect in a nascent and young field.

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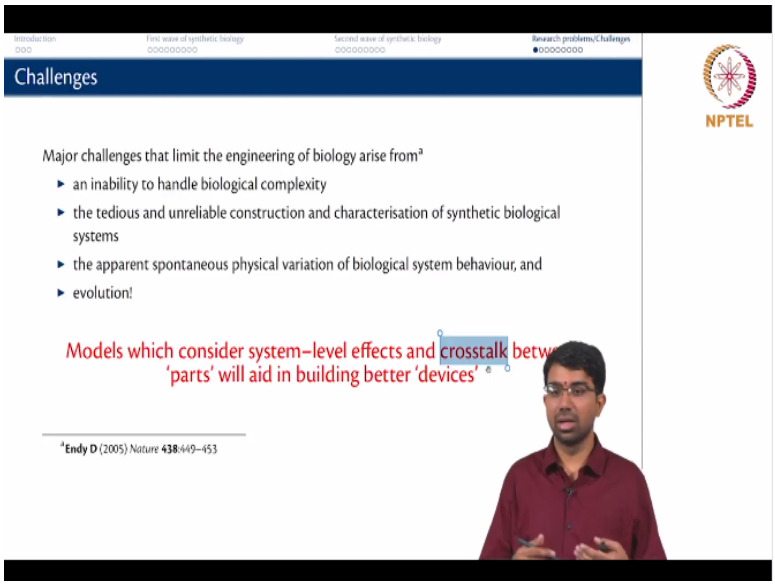

So the major challenges that limit engineering of biology arise from a complexity very difficult to handle and still the characterization of the systems is insufficient that has even synthetic systems and there is so much of inherent heterogeneity right. So if you try to stick a system in or even look at a single gene in a population that gene will have a curve, a distribution of expression not a just nice spike right.

So because of these complexities, it becomes very challenging to model such systems and of course evolution itself right. This is some which just evolve to a different state or reject the changes that you are putting in it so on right. So importantly models which consider system-

level effects and crosstalk between parts will aid in building better devices. So in fact this is something that some of the folks in my lab are working on like how can you consider what is shown as retroactivity right.

So you have a system, you integrate it into another system, there is retroactivity right. The fact that you integrate into the system has a pull on the other system which basically causes a different behaviour right not the desired behaviour. You have an oscillation initially but you stick into the system, the oscillation dies down but there are some systems that seem to be inherently resistant to this kind of retroactivity and so on right.

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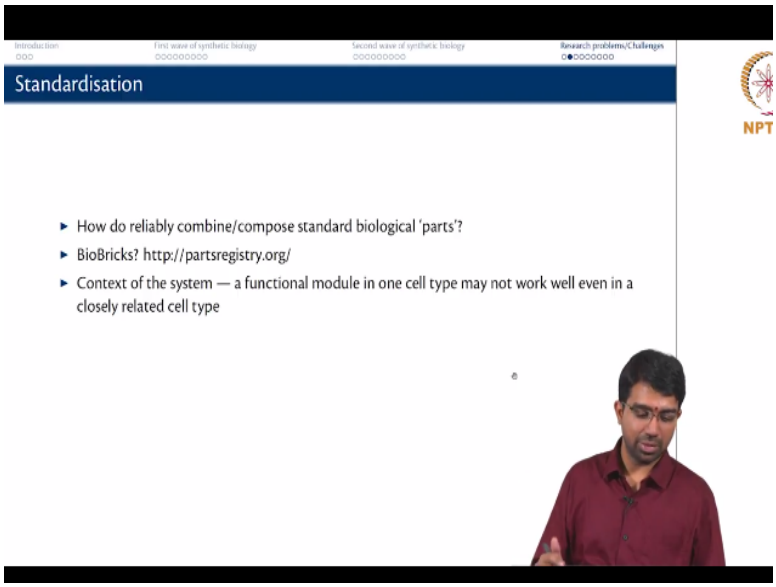

So how do you standardize? The goal is this right, in fact if you see any of the synthetic biology columns or magazines and popular sense articles, the idea is they will say that you just sit on a computer, you key in some genome sequence then order it then you get an organism that can do something cool right. So we are far from that but that is the eventual thing that we want to work towards.

But how do you reliably compose or combines the standard biological parts. So the BioBricks and so on and very importantly what kind of context can it work in right. So you build an oscillator, you stick it into one cell, it works beautifully, you stick into another cell it does not work at all right. So depending upon the context the behaviour might vary right.

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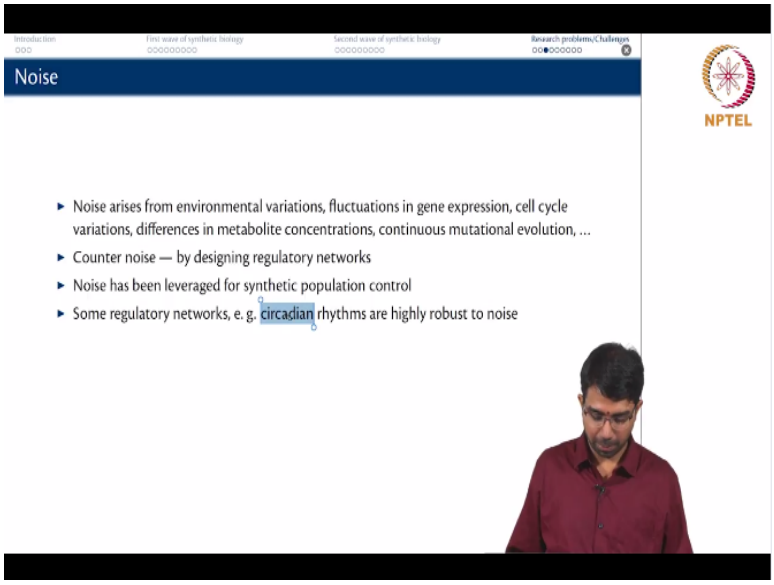

Then noise is another major factor right. Noise arises from several sources that could be environmental variations, generic fluctuation in gene expression, what stage of the cell cycle the cell is in, maybe there are some metabolite concentration which are massively influencing it and mutation evolution and so on and one can counter noise, so there are systems so there are places where noise is an advantage right.

For bacteria that are resistant noise is the great advantage right, save 100 bacteria each expressing different levels of a particular you know protein (()) (25:41) from antibiotics. Those which express a higher level of them will survive when you throw a drug right. Everything express the protein at the same level without noise. Then, you know all of them would have died right.

But the ability to fluctuate their gene expression levels gives them the power to survive right and there are interesting studies where noise has been leveraged for population control as well and there are some regulatory networks that are very robust to noise right and there is a lot that we can learn from these kinds of complex networks like circadian rhythms.

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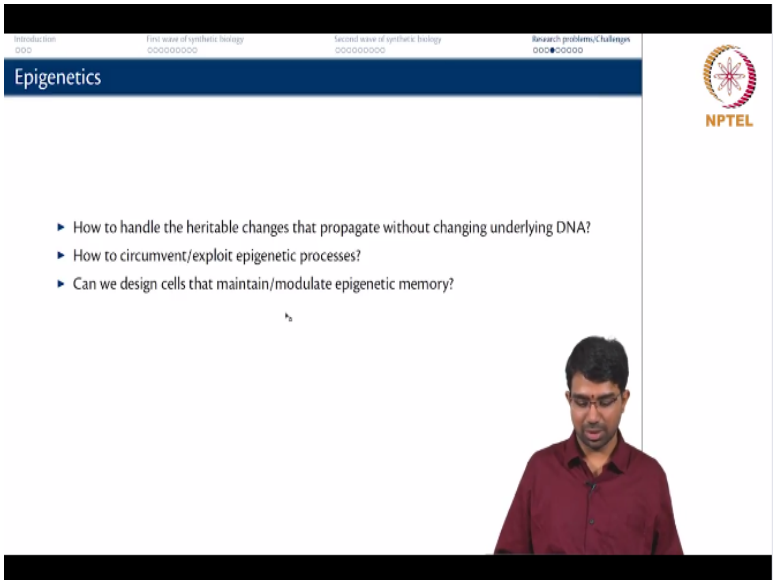

And then there are epigenetics and maybe we should not go into that too much because you have not done lot of biology in this course but there are many heritable changes that propagate without changing the underlying DNA right so maybe there is a methylation of DNA which passes on right. So can you circumvent or exploit these epigenetic processes? And can you design cells that will modulate the epigenetic memory and so on. Can you just remove it or preserve it right?

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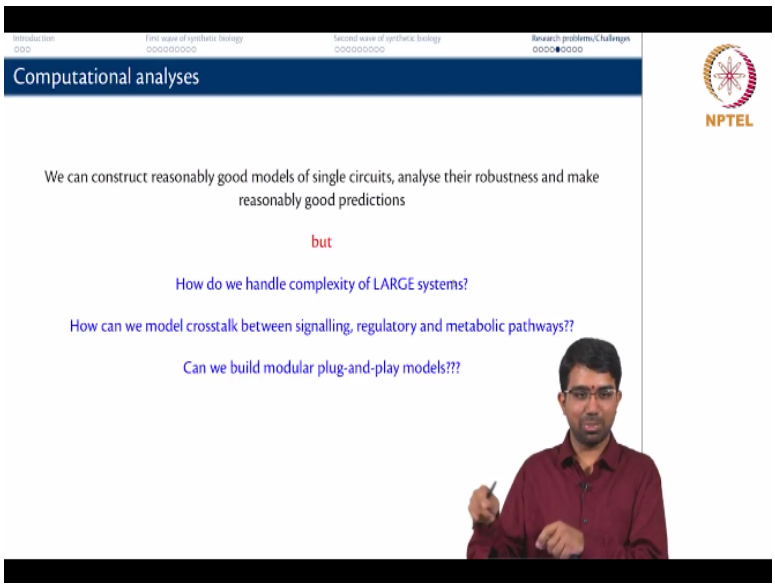

And computational analysis this is why we are talking about synthetic biology in this course right. A lot of background work for synthetic biology comes from computational and systems biology right. How do you model these systems? So like ensemble modelling or like sampling the parameter space and topology space to identify reliable circuits and so on. So we can still construct reasonably good models of single circuits.

You can also analyze the robustness and you can make reasonably good predictions but how do you handle very large systems right? If you wanted to make a 7 node oscillator, there is no way you can exhaustively sample. If you are talking about a 7-node oscillator. you are talking about something like 3 to the 49 circuits or something like that and that is astronomical number right and you can never sample that space.

So how do you go about constructing such circuits? Many interesting challenges right and how do we model crosstalk between signaling regulatory and metabolic pathways. This becomes another major challenge, so we will talk about some of these when we wrap up the course. Can we build modular plug and play models? This is like the final aim of synthetic biology right.

I want to just have modules, have BioBricks, have DNA sequences, cut paste, cut paste, it all works beautifully right.

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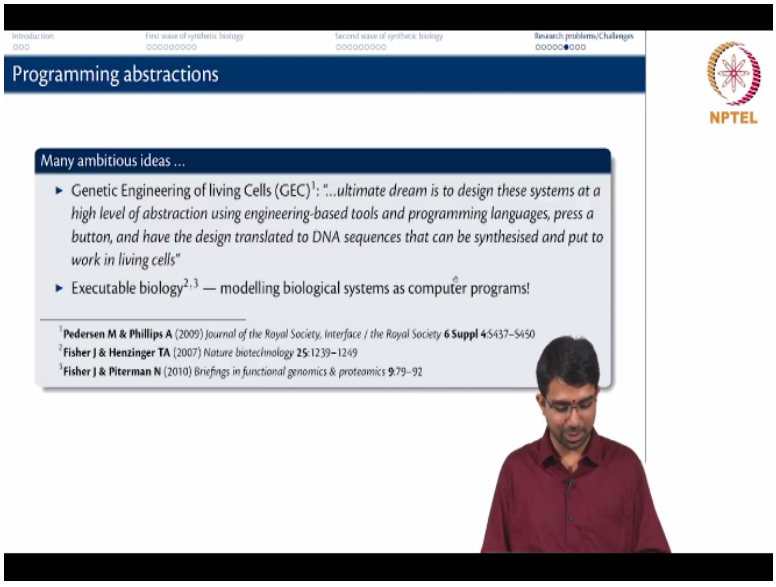

There are some very ambitious ideas such as genetic engineering of living cells and executable biology right wherein you basically use a high level engineering language like all the electrical engineers use right. There are many interesting design languages that electrical engineers use to design systems and so on right. Can you do something like that?

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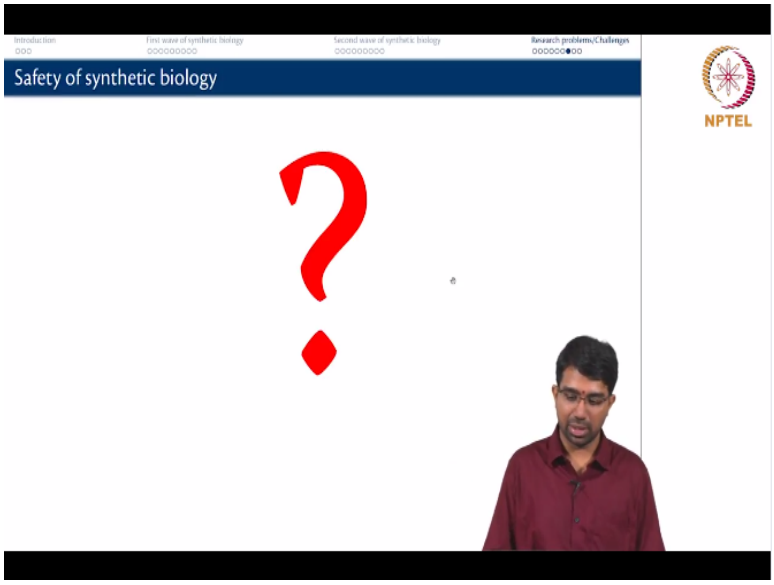

And safety remains a big question mark right. So there are valid concerns, there is paranoia but you know I think scientists are still working actively to resolve these. There are many challenges but there are many advances as well. So I am not going to these but there are very interesting things that people have been doing in the areas of safety.

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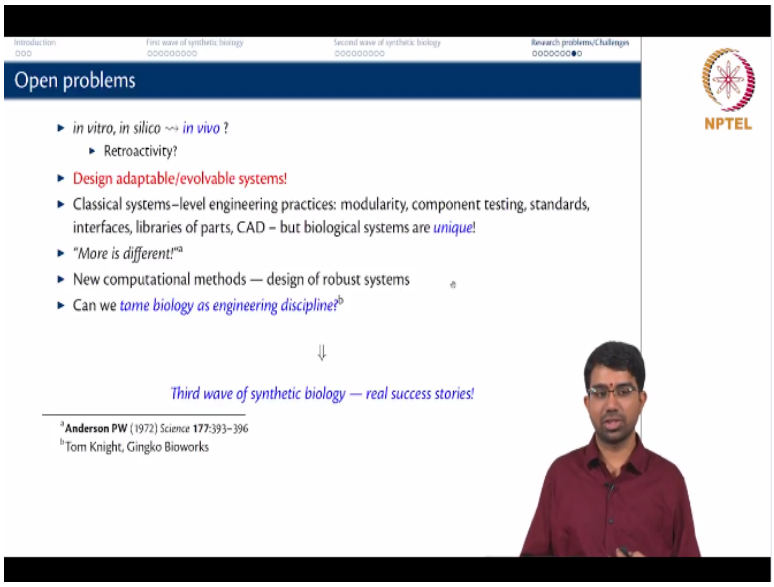

Lastly, some open problems, we have an in-vitro system or we have in silico system. What happens when you stick it in vivo, can you handle retroactivity right or can you design adaptable or evolvable systems right or so see classic systems in engineering practices can you apply them all in biology like modularity, component testing, standards, interfaces, parts library, computer-aided design right.

You want to apply all of them in biology but it is very challenging because biological systems are unique. Why unique? Highly nonlinear, lots of noise, lots of robustness, evolution and heterogeneity and so on and of course more is different right. You add two things together, you get something completely different but you know one needs to explore new computational methods to try and design robust systems in the first place right.

If you design a robust oscillator that is also a non-retroactive, it is going to likely be a very good synthetic biology module that you can reliably stick into a complex system right and as Tom Knight said the goal is to finally tame biology as an engineering discipline right. So biology is still not a truly an engineering discipline because there is so much you know how much of its art and how much of it is science right.

So any genetic engineering or synthetic biology experiment becomes quite an art rather than systematic, you integrate this with this, it just works right. So that is where it will become a science but that is where all the excitement is okay and hopefully you know that is where we will see the third wave of synthetic biology wherein we see mostly some interesting success stories which combine all these modules, complex cell communications, complex circuitry to solve real life engineering problems.

So this was like a quick introduction to synthetic biology.

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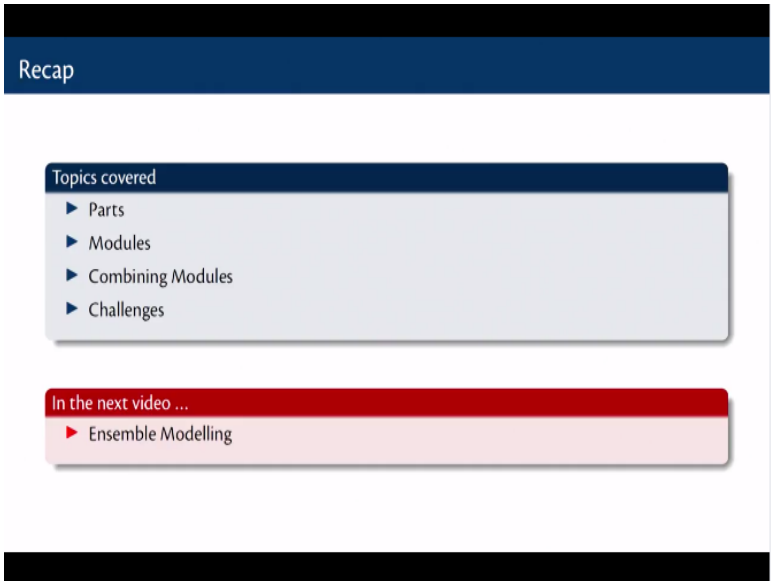

So this is very brief introduction to synthetic biology. I hope you have had a feel for what are the kind of parts that people have built so far and how you know some modules have also

been built and how one can try and combine these modules to build circuits that perform you know desirably. So the dream is to have a computer-aided design of biological systems wherein you know you decide that I want functionality X and I figure out what is the DNA corresponding to that functionality X.

And you know generate the DNA sequence, stick it into an organism and it works perfectly, although they are still a long way away from that. We have been making very interesting progress towards that and the very interesting papers that I showed you today, discussed many of these interesting studies. So from the next video onwards, we will look at some advanced topics beginning with this very interesting concept called ensemble modelling.