BioMEMS and Microfluidics
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Lecture No - 40
Review Lecture of 23, 24 and 25

Hello and welcome to this review lecture on the lecture twenty third to twenty fifth of this NPTEL course BioMEMS and micro fluidics. In the last lectures, we had talked about the various processes associated with how proteins can get initiated through an mRNA which is nothing but a transcript of the DNA present in the nucleolus of a particular cell And then we covered the whole process of transcription an in the last lecture. In this particular session I would like to just review whatever lectures have been associated with the later on process or later on part of the process were proteins are finally, synthesized which is called translation.

So, in the translation process, as we know that there is a this protein warehouse of the cell which is called the ribosome which is nothing but essentially a two sub units like a jaw or a claw together of different proteins and RNA's. And what happens is that the mRNA in a sequence based manner comes and gets encoded by the transfer RNA the amino aisle transfer RNA which is otherwise present in the cell. And it tries to synthesize amino acid sequence finally, from the transfer RNA where the RNA part actually bonds covalently to the mRNA which is there in the lower rail of ribosome.

So, this is a very interesting process where finally, the amino acid chain is resulting from the sequence of the base pairs or bases which are there on the mRNA itself . So, we talked about the various sequences and you know we finally, found out that there about close to 20 different amino acids which are present. And they are coded by its something called codan on the mRNA which is nothing but a set of 3 basis on the mRNA. And as we all know that there are different basis which can occupy those three positions at least 4 different basis which is adenine, guanine, cytosine and uracil. Therefore, there is a possibility that these 4 different bases can occupy 3 positions in exactly 4 to the power of 3 sequences. So, therefore, 4 into 4 i.e. 16 times for 64 such sequences are present. So, therefore, there is defiantly a certain amount of degeneracy in coding the 20 different amino acids. Because on one hand we are saying there about 64 sequences which are which can code various different proteins on other hand we are saying that there are only 20 amino acids.

So, out of the 64 normally 3 such sequences are treated as a stop coders where the process of translation really undergoes you know no further it stops actually the process of translation actually stops on those 3 stop coders. So, remaining 61 are coding 20 amino acids and thereby we showed that what are those different sequences 61 sequences on the mRNA which is corresponding to again the DNA base triplets. And these 3 corresponding to what kind of amino acids. So, all the 20 different amino acids were shown to be coded in that manner. And a comparison was made of in a similar manner to what a computer does by having a floppy disk which actually records the information the hard copy of the information of whatever is stored in the electronic space of the computer. So, in a similar manner a chromosome is represented in a floppy disk in case of the information stored in a cell living cell. Similarly, there is a file in the floppy disk which can be mapped to a gene in a chromosome a particular gene in a chromosome.

Then, finally, the files are constituted out of these bytes which are 8 bit characters in the similar manner in genes there are codons and these codons have 3 different basis. So, it is something like a smiley between these two strategies and finally, this by it is again reconstituted in to a bit which can be either a 0 or 1 in case of a computer, because it is digital in nature. In this case; however, in case of a cell; however, the bit is mapped in to a base and there are mind you 4 basis. So, definitely the living system computer or the cell based computer is actually based on a quaternary system rather than a binary system evaporation. And finally, needless to say if you have a corrupted file the whole structure collapses. And there is no retrieval possible from the floppy disk in a similar manner if there is some kind of a mutation. The whole outcome of the mutation is so, significantly different that you may ultimately result in another kind of gene pull all together. And it is very high it is very unlikely that any of the characteristics of the previous gene pools are formulated in case there is even a single mutation of such nature. That is why DNA is very stable in nature and it does not allow a much of errors or much of changes during the process of its production.

We also talked about the different aspects of the bio links particularly the protein complexes. And in this slide we mentioned about the biotin, avidin, molecular lock which is considered to be one of the high affinity locks in biochemistry. We specifically talked about antigen antibody interactions, we also talked about the strategies of attachments on surfaces particularly substrate surfaces like silicon oxide or silicon so and so forth where we use either simple absorption or even covalent bonding. And sometimes ionic or physical bonding

between the various moieties to absorb some particular biological entity on to reference substrate or surface in relation to that. We started discussing about little bit about the enzyme kinetics, because as we know that everything including you know the polymerize enzyme or even the arti polymerized. They are all based on how an enzyme behaves and participates in the process of catalysis. So, a little bit of quantification of how the enzyme does the catalysis was evaluated by looking at so called the Michaelis-Menten equations where we talked about how a substrate binding to an enzyme would create an intermediary state.

Then finally, result in the enzyme coming of in the product and there would be several steps of a reversible processes involved in this substrate enzyme to substrate product enzyme product conversion. Then finally, we tried to look at how the equilibrium constants the rate constants can play a significant role in determining what is the enzyme substrate concentration for example, is a function of the initial enzyme concentration. And also the function of the substrate concentration things which we know and we formulated something called the Michaelis constant. And Michaelis constant is very important sort of you know intermediary which is needed to understand the reaction kinetics in terms of reaction velocity and substrate concentration. So, we had detailed plots of the same which we investigated in this matter. Then we started looking in to the whole theory of how anti bodies provide binding. And in this manner we considered the different heavy chain light chain sections of an antibody where there is the typically high affinity constants shown by the epitope sights on the light chain of the antibody with respect to the antigen which is present. And this affinity constant makes the antibody specifically bind to certain characteristics proteins or characteristic antigens and thus gives a response in this manner.

We also studied that anti-bodies are produced by a kind of white blood cell called b cell. And that there are several different types of anti-body heavy chain and several different kinds of antibodies which are grouped into different isotypes based on their heavy chain further. We also studied that there are 5 different antibody isotypes which are known particularly in mammalian species. And then of course, the high variability in the epitope side creating different binding chemistry with the varieties of antigens for those particular anti bodies. We also studied about different aspects of how to mobilize this antibodies like for example, physisorption or chemisorption of some of these species on a surface. And also had little bit of introduction in to the absorption kinetics where we did the Langmuir adsorption equations where we could find out the equilibrium constant rate constant by looking at the

concentration of the particles p. And the filled particle sights sp on a particular substrate and the empty surface sights s and so brought out an equilibrium constant of how the adsorption chemistry would work on a surface.

And finally, we studied about micro encapsulation technique where an inert membrane is used to trap the bio material on the surface of the sensor or the sensing surface. We also talked about various other immobilization strategies like entrapment within multiple layers of gels without really any physical connection between the sensing surface and the molecule. So, just sort of stabilizing the molecule between different layers of intermediary gelatinous kind of structures and in a way holding that the gelatinous structure to the sensor surface. We also studied about cross linking where the root we are direct covalent linkage between whatever groups are present on the sensory surface with respect to the antibody. And this could be generally done by a ligand where the ligand binds the antibody on one side and binds to itself to the substrate surface on another side. And we finally, did some introductory concepts about voltammetry, chronoamperometry. And some issues related to how you know the redox potentials can be looked at by sweeping the voltage in such an in such a plot which is commonly known as the voltammogram.

So, it is a v versus I plot where on one side you have the different oxidation and reduction potentials. And as soon as there is a reduction reaction on one side or oxidation reaction in another side there is a release or transfer on absorption of electrons which would result in a peaking of the current value. And this would actually indicate the extent up to which the particular reductant or oxidant is present by looking at the peak heights. And at the same time also indicate what is the exact reactant which is present because each of these oxidant species have different potentials so called reduction and oxidation potentials. So, looking at a comparative of that potential with respect to what species are present you can very well gauge about the concentration as well as the type of the species present in the sample. So, having said that we also can do this cyclically by having a you know reversible voltage sweep where we can increase the voltage per unit time and then again later on decrease it and make it negative. So that we can have both the processes in a single plot and sometimes this is also known as cyclic voltammetry a very important concept for electrochemical measurements of substrates or analytes of interest. And so basically we did some detail studies on these. And finally, we went into the fluidics domain where we talked about the continuum theory that how a fluid model can be envisaged as shifting layers with respect to each other.

And also how if you go to a particular point and keep on decreasing the volume to an extent where the volume starts interfering with the mean free path of the fluid particles. There is a sort of a non-continuum which results and thinks like density or volume velocity do not hold any constancy over time. And so therefore, there are highly variable parameters thermodynamic parameters off state at that particular time when the continuum is broken down. So, we also from our whatever applications we would be proposing in this course learned that we would by enlarge we referring to the continuum part of the modelling I am not going in to the discretized of the non-continuum domain. And so, basically in the microscopic scale whatever scales we are looking at typically in the 1 micron plus range with are still in the continuum domain. And there thereby we started all the continuum based analysis which would be helpful for us in studying of micro fluidics as in the later lectures.

Thank you.