Medicinal Chemistry
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Combinatorial and Parallel Synthesis

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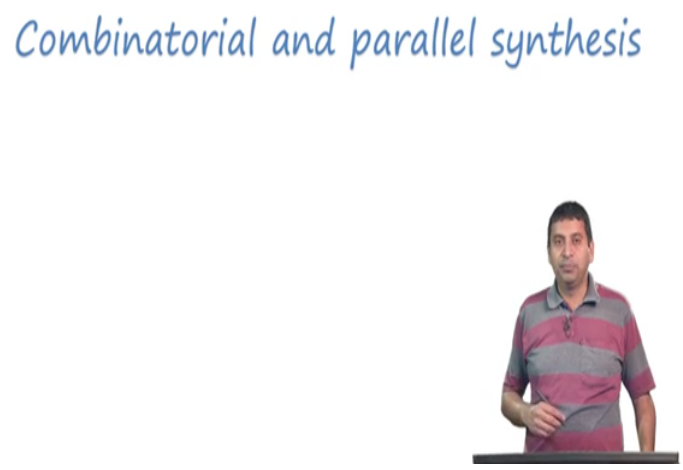

So in today's lecture we will be looking at combinatorial and parallel synthesis, before we get into the topic let us do a quick recap of what we have done in the past few weeks.

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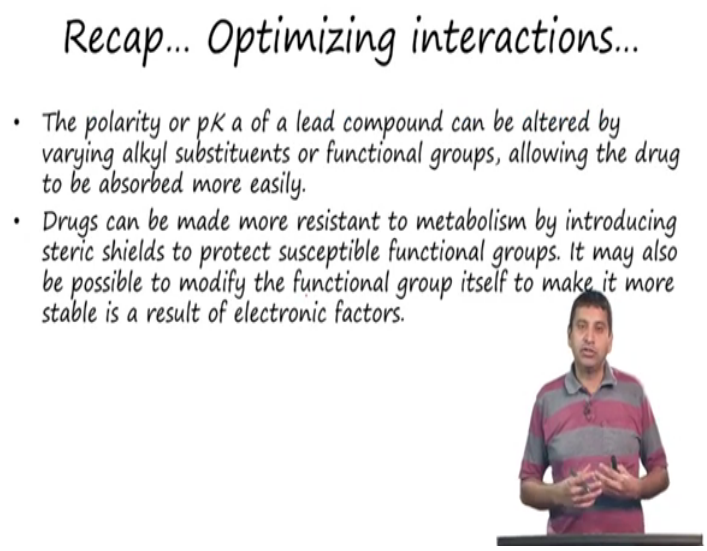

So what we have done so far is to figure out ways in which after we get a lead to optimize interactions between the target and the lead okay so here we have looked at a number of concepts that are going to be important. So for example the polarity or the p K a of the lead

compound, so here for example if you have a carboxylic acid in the lead compound then the p K a of the carboxylic acid or an amine the p K a of the amine whether it is able to get protonated or not all these things are going to be important and one can modify this by varying the alkyl substituent or functional groups, so this allows for the drug to be absorbed more easily.

Now another important concept in optimizing these interactions that is how to optimize interaction between you know to get to the target is to make it more resistant to metabolism. So we have spent a significant amount of time in trying to maximize the interaction between the target and the lead molecule, but if the lead molecule becomes very easy to get metabolized or if it is very easy to metabolize it and it if it breaks down then it would not reach the target of interest.

So therefore here we have looked at this important concept of steric shield, so steric shield helps in protecting susceptible functional groups. So we have looked at examples of how you have a hydroxyl group and hydroxyl group can be put next to a methyl for example can be put next to it and you can prevent the hydroxyl group from getting oxidized and so on. So by reducing the access of the important functional groups to metabolic enzymes one can create a situation where the molecule can actually reach its target intact, you can also modify a number of things to vary the electronic effects.

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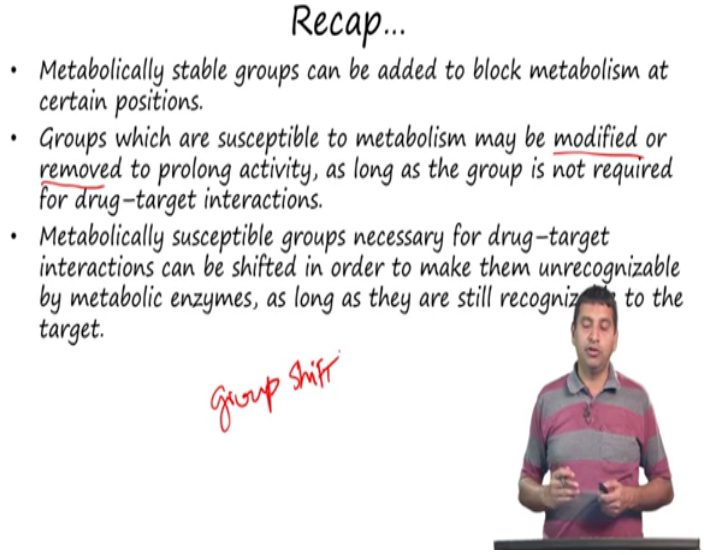

So the other so it took to continue along this (())(2:25) so metabolically stable groups can be added to block metabolism at certain positions and certain groups which are susceptible to

metabolism may be modified or removed altogether so that you can prolong the activity as long as the group is not involved in the major drug target interactions. So this removal or modification here we are talking about the groups which are not important for the drug target interactions.

Metabolically susceptible groups necessary for drug target inter interactions can actually be shifted, so we have looked at the example of how a phenol can be converted to a benzyl alcohol and in this process it becomes metabolically more stable, but it also continues to interact with the target. So this strategy is called the group shift strategy and becomes very important in certain kinds of optimizations, where we want to prevent metabolism from occurring.

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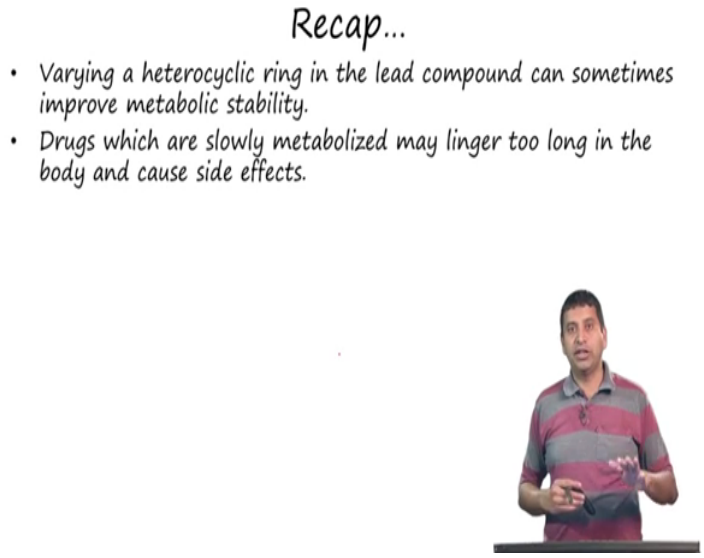

Now varying a hetero cycle ring is also something that we can do which will improve the metabolic stability, there are certain heterocyclic rings which are quite susceptible to metabolism and so we can remove them or we can replace them with alternate heterocyclic rings. And the one problem with drugs which are very slowly metabolized is that they may linger on too long in the body and cause side effects. So we do not want to make it so stable that it will remain in the system for too long as well.

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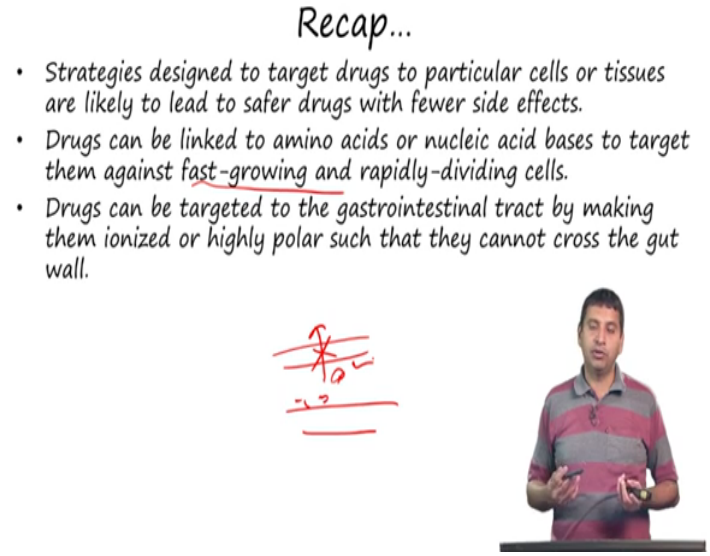

So strategies which are designed to target drugs to particular cells or tissues are likely to lead to safer drugs with fewer side-effects because this is because if they are targeted then they are not going to be distributed across or even if they are distributed across various other cells they are not going to be as active in those cells. So one can modify the or one can optimize for this kind of a preferential distribution or we can optimize for better targeting.

Drugs can also be linked to amino acids or (nucleic acids) nucleic acid bases to target them against fast growing or rapidly dividing cells. So here since fast growing cells such as cancers are going to actively take up number of these amino acids and nucleic acid bases or precursors to those then we can use this concept to deliver the drug to that place. Drugs can also be targeted to the GI tract that is a gastrointestinal tract by making them ionized.

So now what happens is that they are highly polar or ionized in the GI tract and so therefore they do not cross the gut membrane, so if they do not cross the gut membrane then they remain in the intestine and so this is useful for infections for example which are in the intestine.

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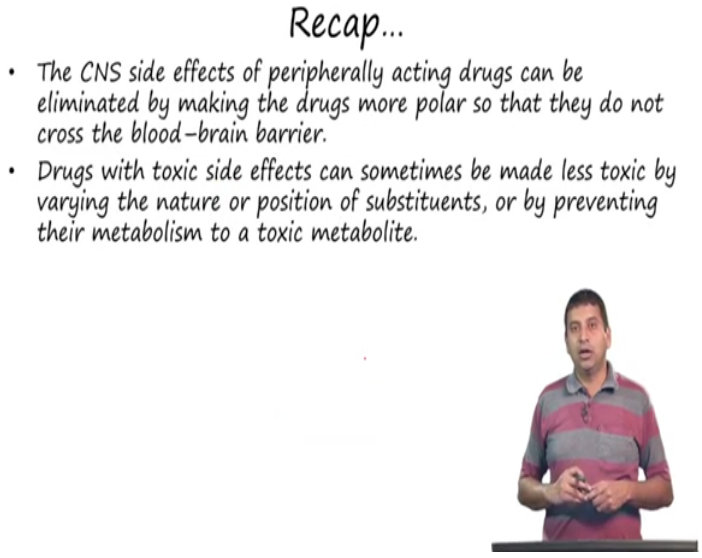

Now there is another important concept which we have looked at is the side effects associated with the central nervous system and so these side effects of these peripherally acting drugs can be eliminated by making the drugs more polar. So we already looked at how it is difficult to cross the blood-brain barrier by polar molecules unless there is a special mechanism by which they are taken up, but so by making the molecule more polar you can try to reduce the amount of CNS side effects.

Now drugs with certain toxic side effects can be made less toxic by varying the nature or position of the substituents or by preventing their metabolism to a toxic metabolite. So not just we want to optimize the drug to reach the target that is make it metabolically stable so that it reaches a target, but we also want to optimize for reduced toxicity. So you need to put in certain groups or remove certain groups so that you can prevent the formation of a toxic metabolite.

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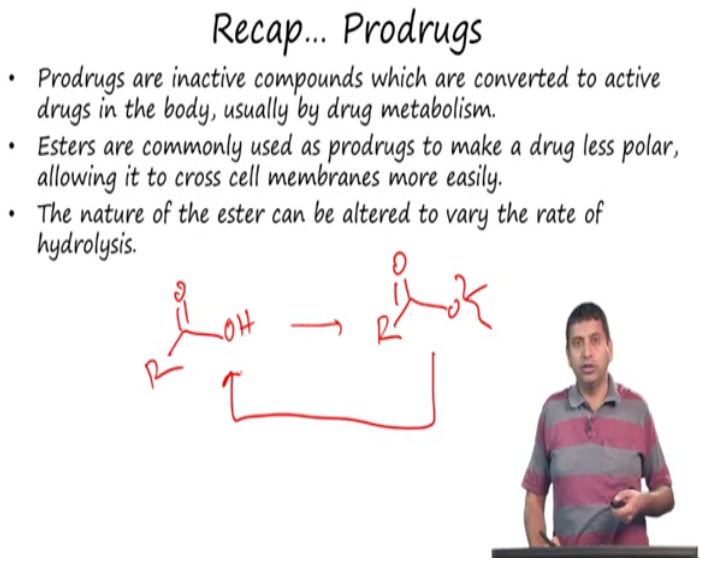

Here using this concepts of metabolism we introduced the concept of prodrugs, so prodrugs are by themselves inactive molecules but they are converted to active drugs in the body usually by drug metabolism. So here a knowledge of an intimate knowledge of how drugs are metabolized this becomes very useful, so most commonly used prodrugs are esters.

So here the carboxylic acids are themselves very poorly permeable, but once you convert them into an ester then they improve the permeability and (this esterase) esters are quite susceptible to hydrolysis and they can be converted to the active drug after they permeate cells. We have also looked at how to you know vary the nature of the ester so that you can alter the rate of hydrolysis.

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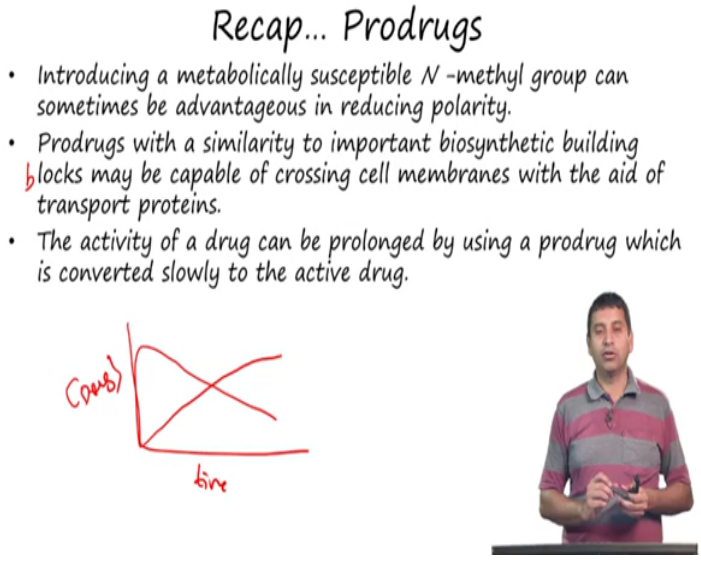

One can also think about making a prodrug of an amine by converting it to an N methyl group and these N methyl groups are actually metabolically susceptible and they can get converted back to the amine by demethylation. Prodrugs with a similarity to important by synthetic building blocks may be capable of crossing membrane with the aid of transport proteins.

So here we have seen that if you design the molecule such that they can be they can access these transport proteins then they may be able to get across. The activity of a drug can be prolonged by using a product which is then converted slowly to the active drug. So what we would do is that instead of you know sort of once you inject the drug or you administer the drug the concentration of the drug goes up and comes down like this, instead of that what we could do is by converting it to the prodrug we could slowly increase the concentration of the active drug.

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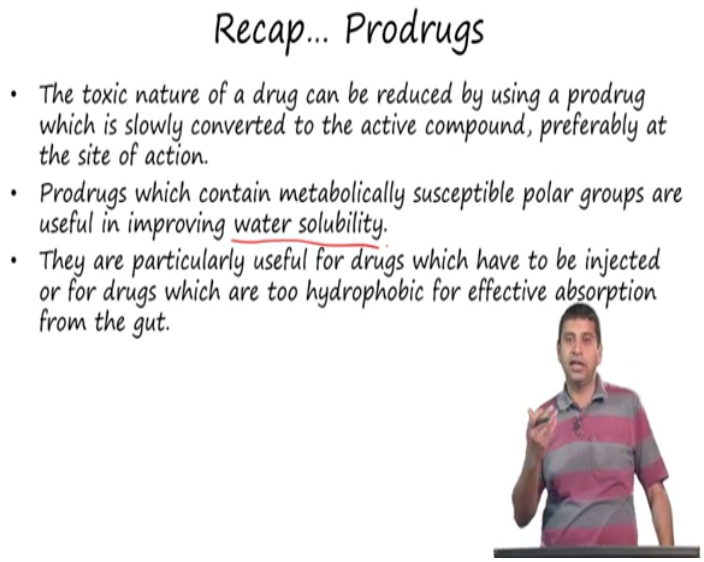

The toxic nature of a drug can also be reduced by using a prodrug which is then slowly converted to the active drug and so since we can now play with the therapeutic window by sampling the you know the toxic threshold at a later stage, so that we are able to modulate the level of toxicity. Prodrugs which contain metabolically susceptible polar groups are important in improving water solubility.

So if we have looked at cases where there are examples or there are situations where one needs to inject the drug and if the drug is extremely hydrophobic then it precipitates during the injection. So here what we would do is we would mask these groups with polar groups so

that they can improve the water solubility, you make the formulation, inject it and then once it gets into the bloodstream it will get cleaved off to produce the active drug.

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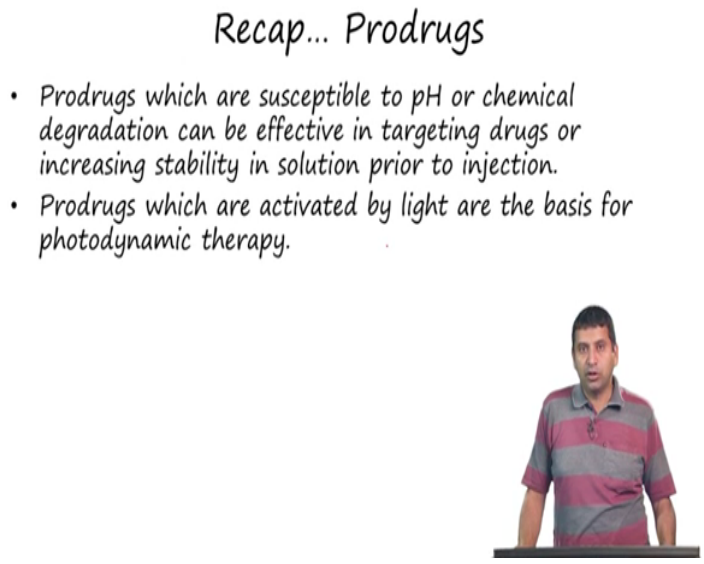

Prodrugs can also be made in such a way that they get cleaved by pH changes or by chemical degradation. So this can be effective in targeting drugs or increasing the stability of the solution prior to injection. So the example that we looked at was I know methenamine which is actually activated under low pH to produce the anti-bacterial formaldehyde and this preferentially happens in the infected urinary tract.

We spent a good amount of time on this concept of photodynamic therapy. So here we sort of discussed about how you can use light to localize the generation of the off singlet oxygen and the singlet oxygen is going to you know produce highly reactive toxic species which can kill the cancer cell.

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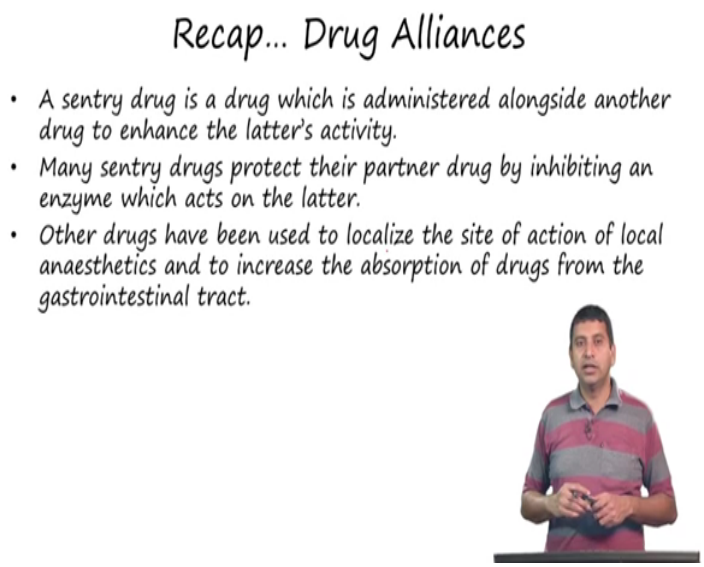

We also introduced the concept of a drug alliance, so here drug alliance is basically the making use of meaningful relationships between two different drugs. So what we could do is we could send a drug along with another drug to enhance the one of the drugs activity. So these are called a sentry drugs and these are typically used to protect the main drug and the way they protect it is they inhibit an enzyme which acts on the main drug, so we have looked at several examples of this.

Now other drugs have also been used to localize the site of action. So for example you know if you administer a local anaesthetic sometimes the adrenaline is given along with it so that the local anaesthetic does not spread across into various places and they also help with increasing the absorption from the GI tract.

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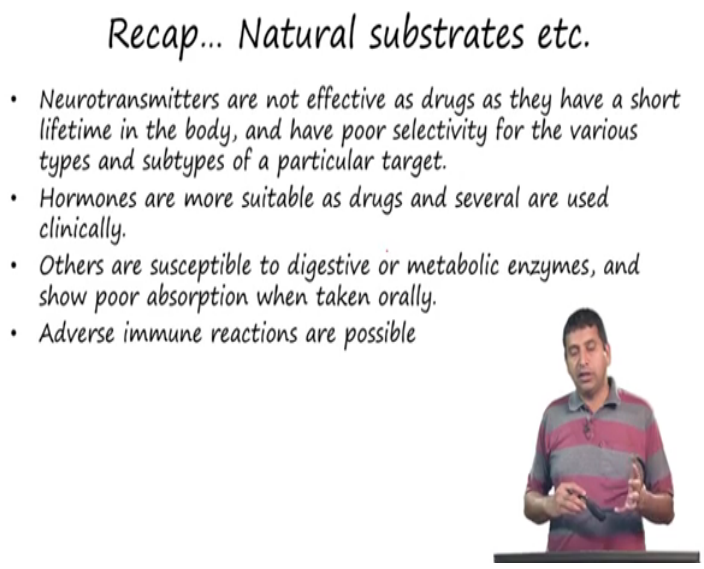

We spent time on discussing the pros and cons of using natural substrates as drugs. So here the natural substrates we divided them as neurotransmitters, hormones, you know peptides and nucleic acids. So neurotransmitters are not very useful because they have a very short half-life in the body they are metabolically quite susceptible and because they have poor selectivity among the various subtypes you know they will sort of have number of side effects which are not desirable.

Hormones on the other hand are actually metabolically more stable and so one could use this as a drug in certain cases and they are in fact used clinically. Now there are others type of hormones which are actually susceptible to digestive or metabolic enzymes and they show very poor absorption when taken orally. The other problem is that there are adverse immune reactions that are possible with these situations.

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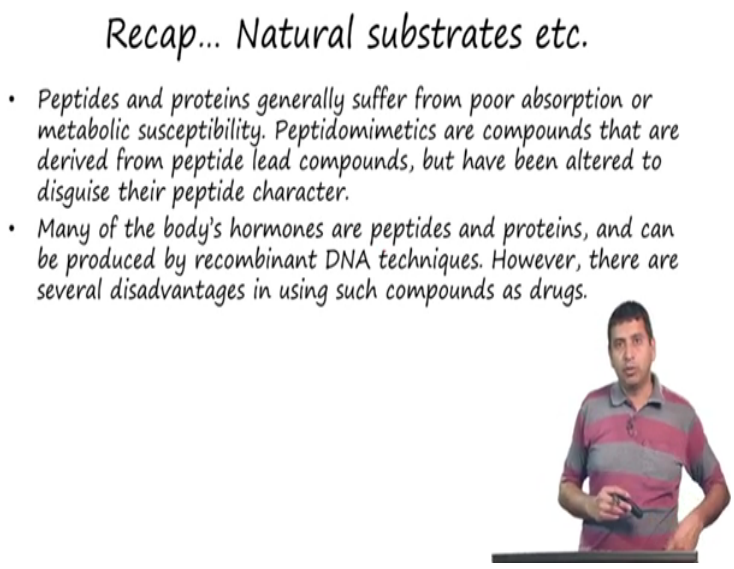

Peptides and proteins are also important drug candidates, but they are associated with poor absorption and extreme susceptibility to metabolism. So we described this concept of peptidomimetics which are you know sort of derived from peptide lead compounds they look like peptides but they are not actually peptides because or the natural peptides that we work with. And since many of the body's hormones are peptides or proteins, they can be produced by recombinant DNA techniques because you cannot possibly isolate large quantities of these from the bloodstream and of course because we are using recombinant DNA techniques there are several disadvantages because if they can trigger you know immune response.

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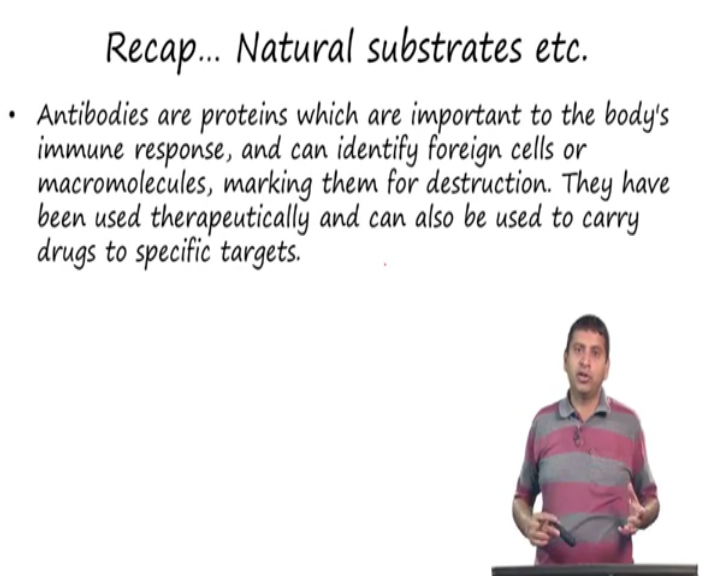

Antibodies are another very important naturally occurring macromolecule and these are again very important drugs, there are a number of drugs based on antibodies that have been introduced in the past decade and these are by enlarge the most exciting among the most exciting new therapies that are used and the biggest advantage to this is that they are quite they have very good specificity.

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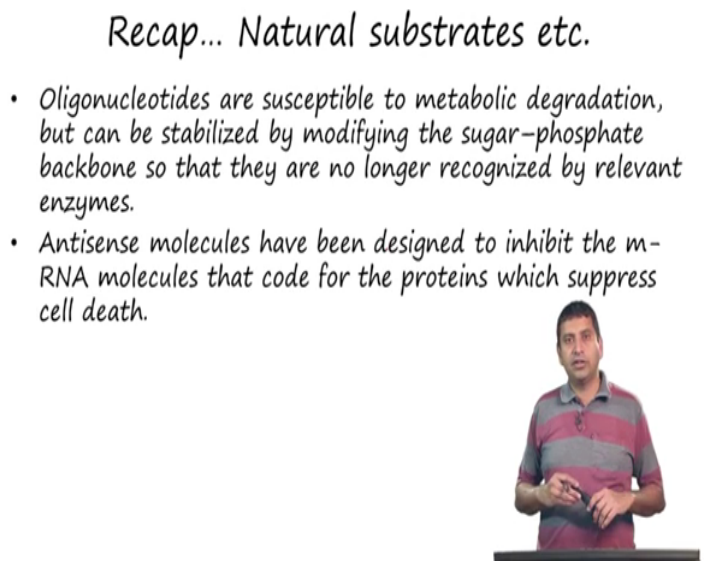

Oligonucleotides are again there are examples of this which can be used which are used in clinic and these have problems because again they are susceptible to metabolic degradation and therefore there are strategies that are used to modify the sugar phosphate backbone so that they do not get degraded. Antisense molecules have been designed to inhibit m-RNA molecules that code for proteins and suppress cell death, so again these are been used.

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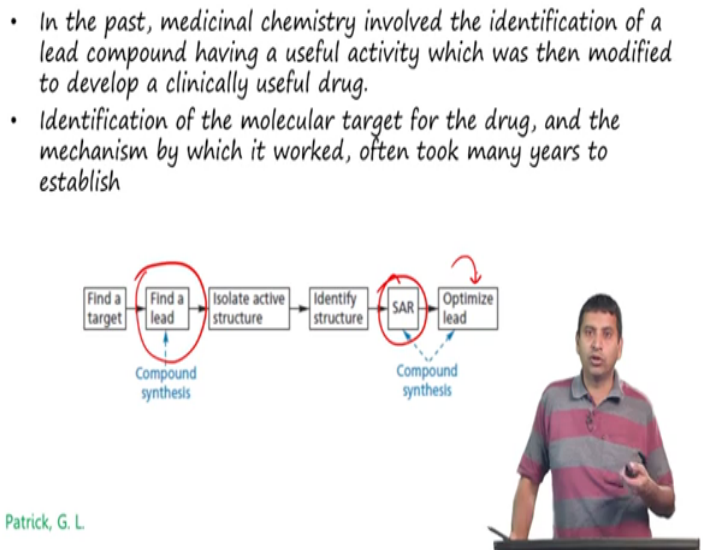

So now coming to the main topic of combinatorial and parallel synthesis what we sort of need to look at is a global view of medicinal chemistry. So in the past medicinal chemistry involved the identification of a lead compound. So this is how the drug discovery pipeline was working in the previous generations and then what we do is that once you find a lead then you isolate the active structure that is you find what is the pharmacophore and then you walk around this pharmacophore and try to make optimize for binding, optimize for metabolism and so on and this would be your structure activity relationship. And then you sort of go to the final optimized lead which is taken forward for preclinical evaluation.

But in this whole process we do not really spend a lot of time in identifying what the target is, often finding the target takes many years and it requires a large number of complementary experiments to actually do it. So this is not a very desirable approach in certain situations because you want to have you want to be aware of what the target is when you start out the drug discovery process.

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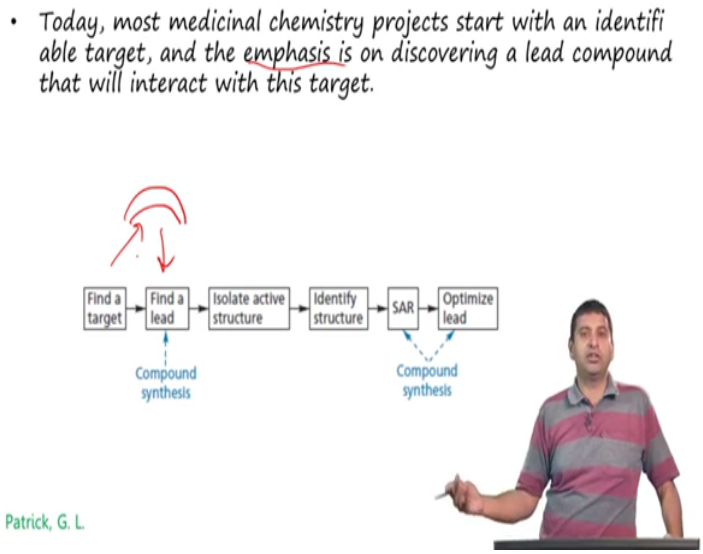

So today in recent times most medicinal chemistry projects start with an identifiable target okay and therefore the emphasis is not on you know finding a lead which does not have a target but using the target to discover a lead compound. So here you would find a lead based on a particular target. So let us say you have an enzyme as a target or a receptor as a target does this molecule go and bind to it? Does this molecule go and inhibit it? This is the first question that we ask, rather than after you optimize the lead you ask the question what the target is.

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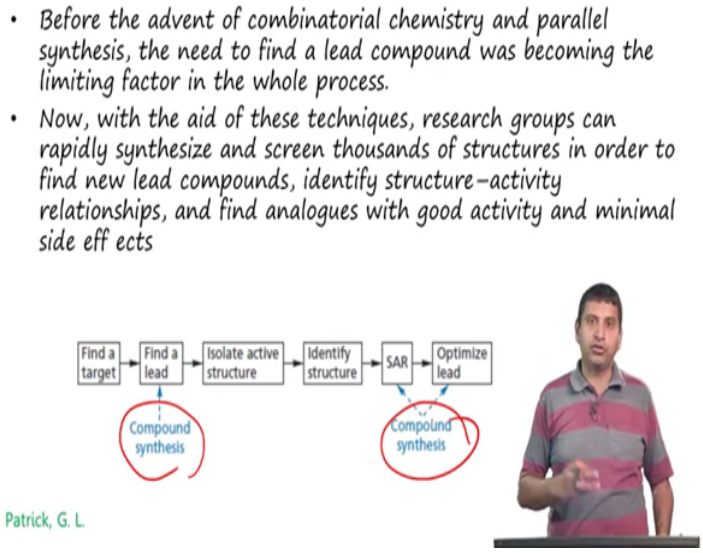

So before the advent of combinatorial chemistry and parallel synthesis, the need to find a lead was becoming the limiting factor in the whole process. Now with the aid of these techniques

which we shall look at in this lecture, research groups can rapidly synthesize and screen thousands of structures in order to find new lead compounds, also identification of structure activity relationship becomes quite easy and finding analogues with good activity and minimal side effects have become more (())(15:47).

So if you look at this diagram again there are a couple of places where you would need to do extensive compound synthesis. In some of the cases that we looked at you need to go back and synthesize an analogue which is going to be important for us to optimize. So these are some challenges that medicinal chemists faced and one of the ways out is to use a combinatorial chemistry and parallel synthesis.

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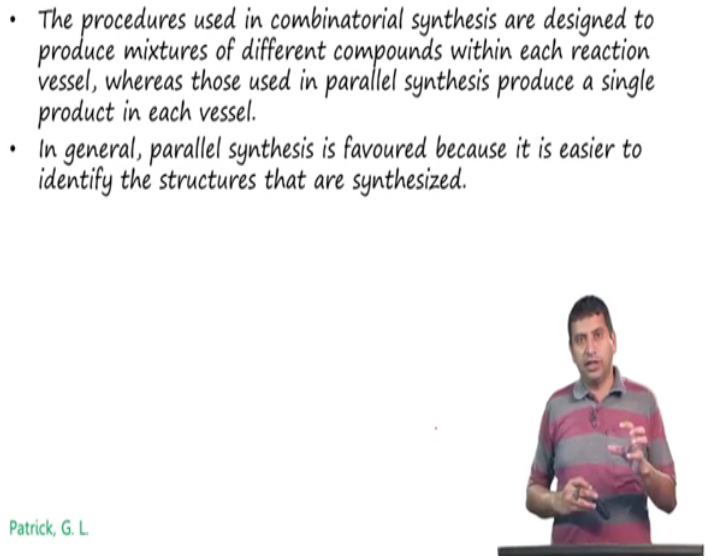

So these techniques are designed to produce mixtures of different substances and within each reaction vessel there would be for example in parallel synthesis you would produce a single product in each vessel, whereas in combinatorial synthesis they produce mixtures of different compounds. In general between the two approaches parallel synthesis is favoured because it is easier to identify the structure that are synthesized.

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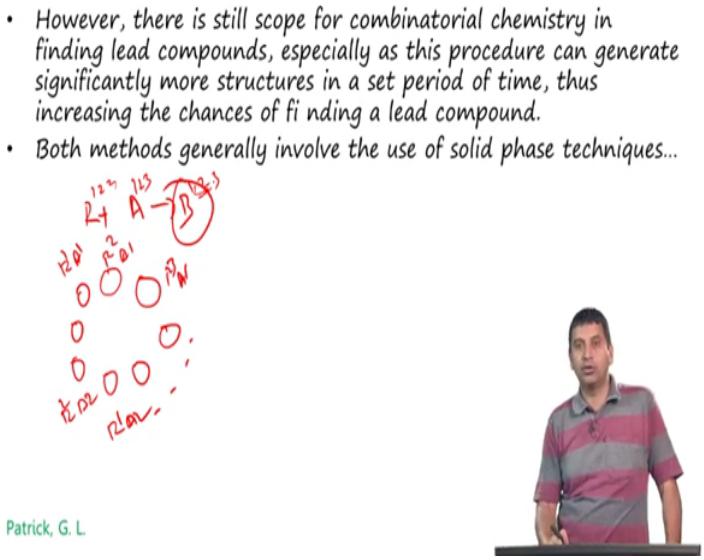

In order to understand this better we will look at both of these techniques in a little bit of detail. However, there is still scope for combinatorial chemistry in finding lead compounds especially if this procedure can generate significantly more structures in a set period of time. Thus, the chances of increasing the chances of finding a lead compound go up. So both these methods involve solid phase techniques, before we go into solid phase techniques let us look at this concept of parallel synthesis again.

So here you will carry out various reactions, so you have A going to B, what you would do is you would (sorry or A plus) R plus A goes to B and B is your product. So now you would vary R 1, R 2, R 3 and so on A 1, A 2, A 3 and so on you will get B 1, B 2, B 3 the only thing is that you will have R 1 here you will react it with A 1, R 2 here react it with A 1, R 3 here react it with A 1 and so on, you can have R 1 reacting with A 2, R 2 reacting with A 2 and so on.

So if you follow this approach then in each reaction vessel you would get a single compound which is B 1, B 2, B 3 and so on. So you may have to add another number here to increase to reflect accurately what the products are.

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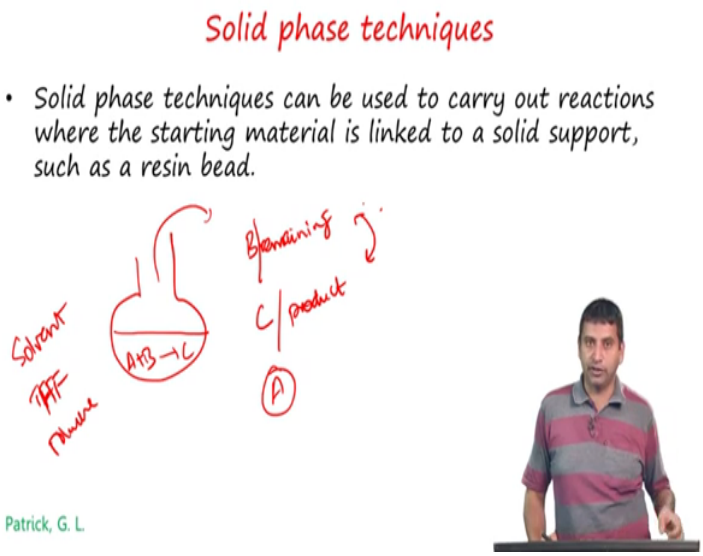

Okay, so solid phase techniques are something that many of you may not be aware of all the you know reactions that we normally carry out are in liquid phase. So here in this liquid phase what happens is that you have a solvent let us say a Tetrahydrofuran or Toluene and then here you add your reactants are A plus B let us say and now you will get C. So let us say you have at the end of the reaction you have B remaining, you have C that is being produced and A is completely consumed. So here what you would need to do is to take out this and then separate B from C.

Now the separation process can involve either column chromatography or you can do a distillation or you can do you know recrystallization and so on. Now if you imagine that this entire reaction can be done on a solid support that is I will take one of these molecules and immobilize it to a solid support such as resin bead or something. Now at least separating out this product from the reactant is not that difficult.

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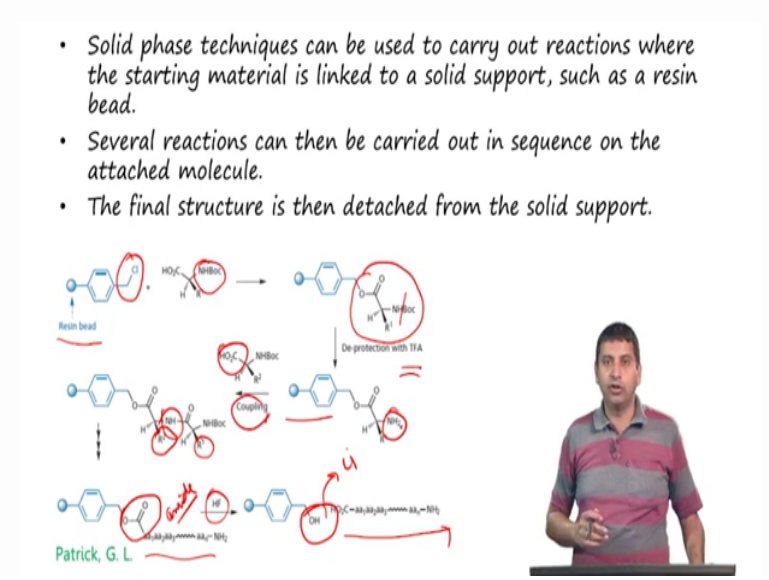

So here is how the technique works so solid phase technique has been used to carry out reactions where the starting material is linked to a solid support such as resin or a bead. Now several reactions can be carried out in sequence on the attached molecule and the final structure is then detached from the solid support. So here is the sequence of reactions that we can look at. So here is the resin bead which has a chlorobenzene functional group on it.

Now if you react this with a carboxylic acid, now you get this molecule which is an ester, so this is a nucleophilic substitution reaction and you protected the amine with a BOC functional group, then what do you do is you de-protect this BOC functional group using trifluoroacetic acid. So this BOC is cleaved off and you get a free amine. So notice that all of this is done in the solid phase.

Next you add another amino acid so the examples that we are going to look at is a peptide synthesis but it can be extended to other similar molecules. So here you add the next amino acid and here this coupling reaction that can occur between this NH 2 and this carboxylic acid will give you the new amide bond. So here notice that the first substrate that we started with is different from the second substrate. So in combination you can make R 2, R 1, R 3, R 4, R 5 and so on and you can extend this chain length for as much as you desire.

In the end all these bonds are amide bonds, whereas this is an ester bond. So the bond between your (solid support) solid phase and the growing peptide chain or the grown peptide change is actually an ester. So now ester can be cleaved off much easier compared to an amide and so here you add HF and you cleave it and you get back the alcohol. So through

some techniques you can reconvert this to the chloride and reuse the solid support. So this is the way in which you can make a oligopeptide or a polypeptide using solid phase synthesis.

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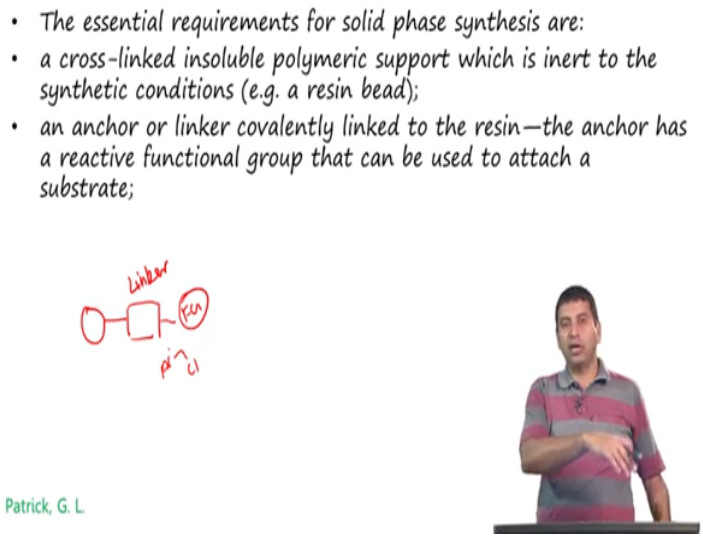

So the essential requirements for solid phase synthesis are you need a cross-linked insoluble polymeric support. So this is the support that you need and a resin bead and you need an anchor or linker that is covalently linked to the resin, so each of these resin beads must have a linker or anchor. So this linker should have a reactive functional group such as the chloride. So if you have that (reaction) reactive functional group this can be used to attach the substrate.

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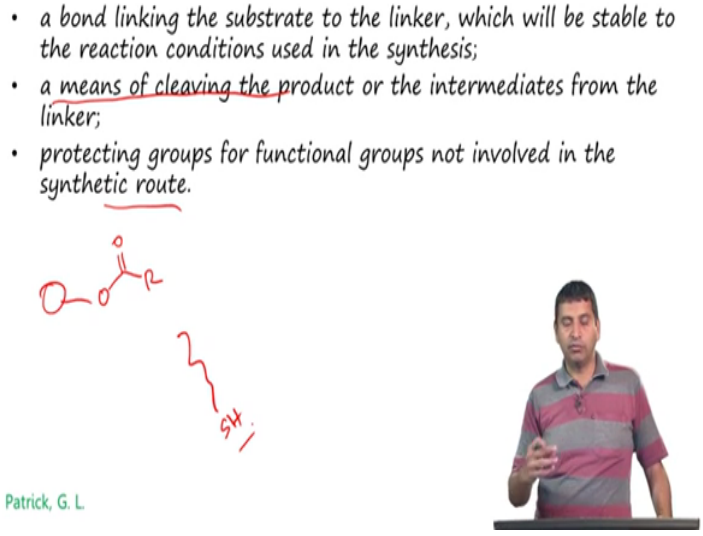

Then once the substrate is attached there is a bond that is formed. So for example we form a ester bond, this bond is very important because it should be stable under the conditions of the reaction that we are going to conduct after this point, but it should be cleavable under certain other reaction conditions so that you can recover the product. So we should have a clear means of cleaving the product or the intermediates from the linker.

So therefore identifying the right protective group is very important, the protecting groups of functional group not involved in the synthetic route are also very important because we need to be able to protect like say there is a side chain with a thiol on it, so this thiol may actually react with one of your other reactants and so therefore you may want to protect the thiol in some form so that you can de-protect it later.

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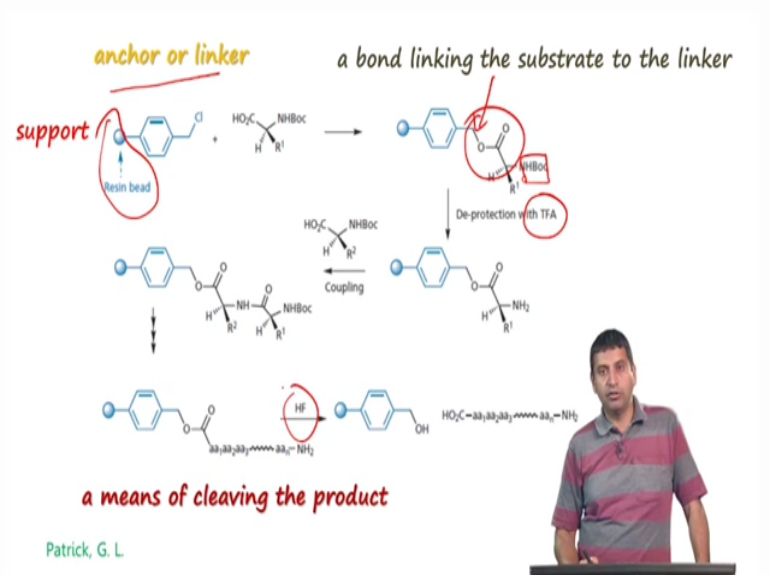

So going back to our synthetic scheme, so you see here that this is the resin bead which is the support, here is the anchor or linker, here is the bond you know the ester bond binding the linker to the substrate and you have a de-protection regime which is fairly favourable. So here if you are TFA you can de-protect this molecule and carry out the reaction and in the end you have a means of cleaving the product which is by using HF.

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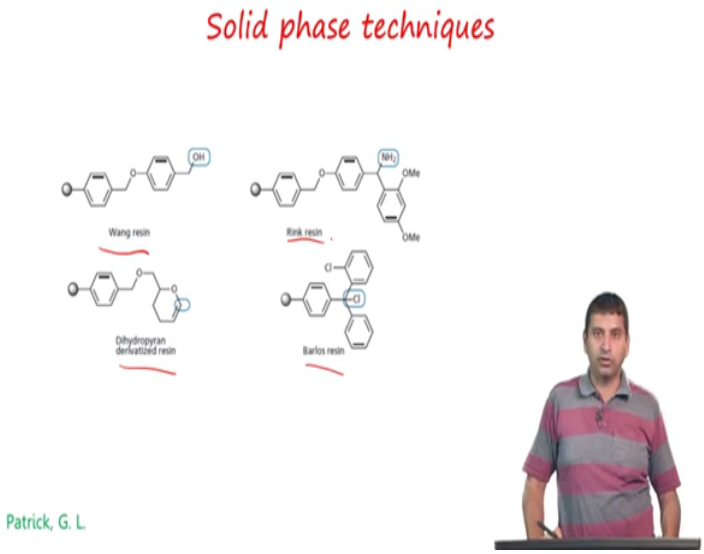

So here are some examples of many solid phase techniques that have been developed over the past 2, 3 decades and so you have a Wang resin you have a Dihydropyran, Barlos resin and Rink resin, we will not spend any time on this but these are available for using for solid phase synthesis.

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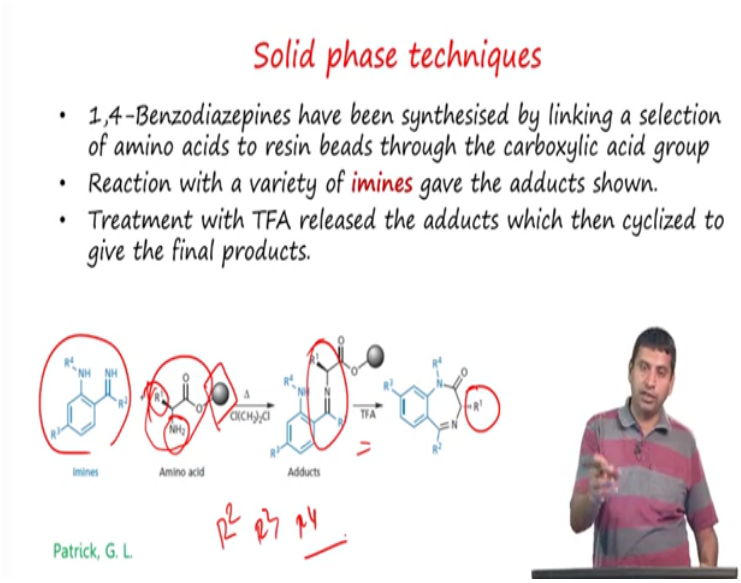

So here is an example of using this solid phase synthesis, so 1,4 benzodiazepines which we have looked at previously have been synthesized by linking a selection of amino acids to resin beads through carboxylic acid group. So here is your amino acid and you link it to the solid bead and now when you heat this with your amine what happens is that it undergoes a reaction which forms. For example if you have 50 of these different amino acids you know a

natural as well as unnatural amino acids then you can have each of these reactors which is attached to this amino acid in a bead and that bead is now going to react and form an amine.

So here is the amine and here it is going to displace the existing amine and form a new amine and then it is set up nicely to do cyclization if you add TFA. So R1 here is going to end up as R1 here, so now you can do R2, R3, R4 and so on and at the end of it you will get back your pure material because the solid phase is now going to be it can be washed off or the material can be washed off from the solid phase.

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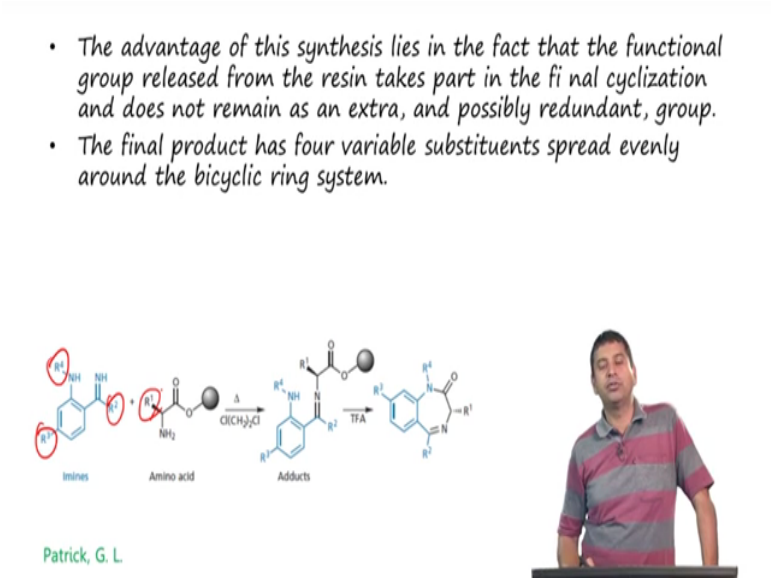

The advantage of the synthesis lies in the fact that the functional group release from the resin takes part in the final cyclization and does not remain as an extra and possibly redundant group. The final product has four variable substituent spread evenly around the bicyclic system. So if you notice here you have this R 3, R 4 and R 2, I was only talking about the amino acid previously, but now you can also change the amine part to get a large variety of different kinds of cyclized products.

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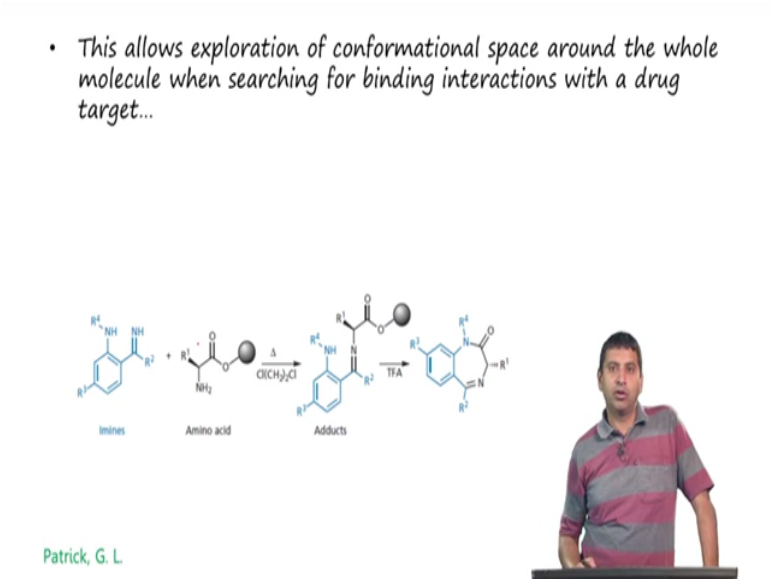

So this allows for conformational space exploration around the whole molecule when searching for binding interactions with a drug target.

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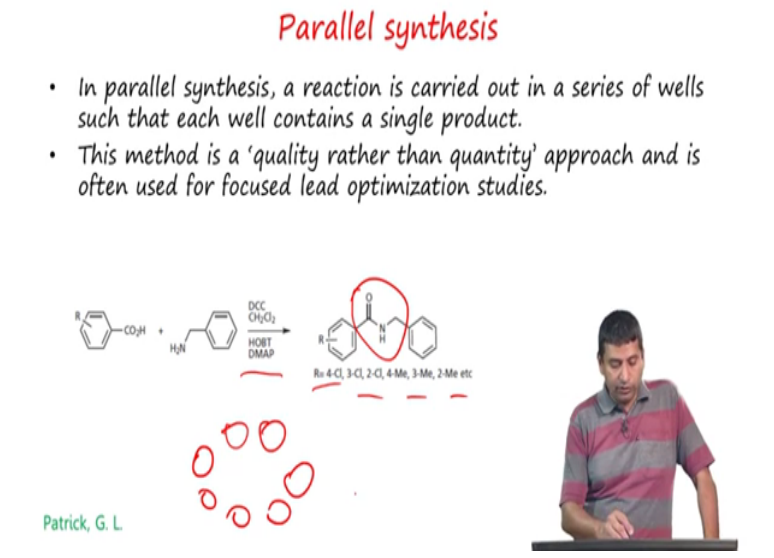

In parallel synthesis a reaction is carried out in a series of wells such that each well contains a single product. So the previous example that I gave you, you can do each of these things in a single well and you can generate a library of these molecules with single components in it. So this method ensures quality, so because it is limited by the number of wells that we have for example, but it compromises on quantity. So numbers of molecules that we can make is actually not very large but you can make good quality products.

So here is the example so you can do a DCC HOBT dmap coupling and form an amide and so here you can change each of these functional groups over here and each of these vessels will give you a single product.

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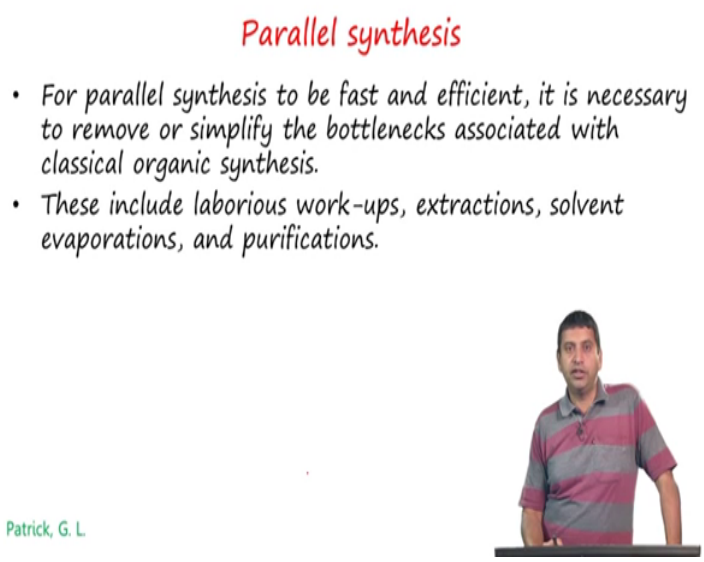

For parallel synthesis to be fast and efficient it is necessary to remove or simplify the bottlenecks associated with the classic organic synthesis. These include laborious work-ups, extractions, solvent evaporation and so on. So if we do this on a solid phase then you can sort of remove some of these aspects.

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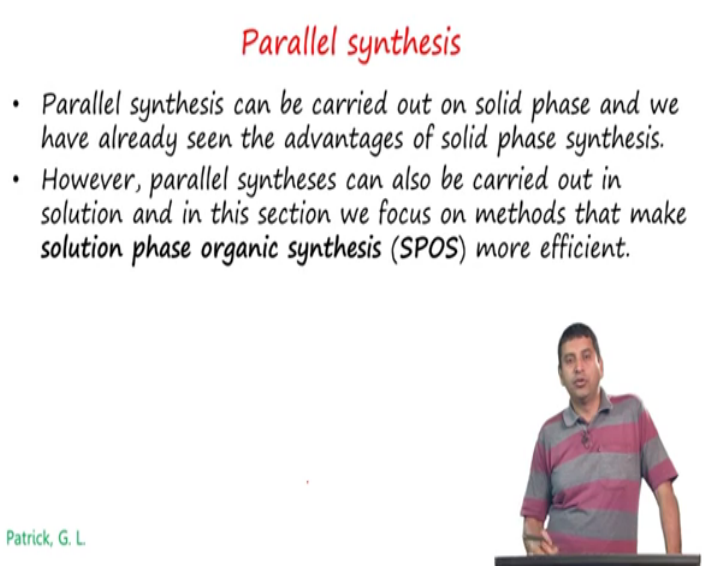

So instead of using solution phase synthesis, we would try and use as much as possible solid phase so that we can exploit the advantages of this method.

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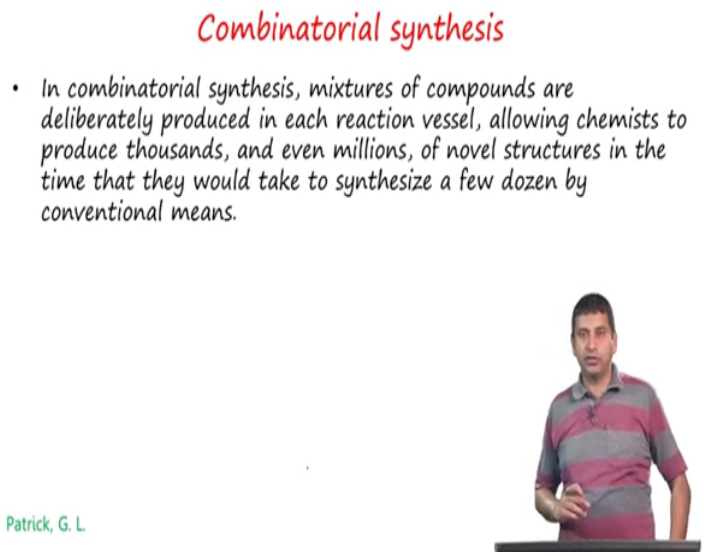

In combinatorial synthesis what we are aiming to get is actually mixtures of compounds. So these are deliberately produced in each reaction vessel which allows the chemists to produce thousands of not millions of molecules and it would take a long time to synthesize each of these molecules by conventional means.

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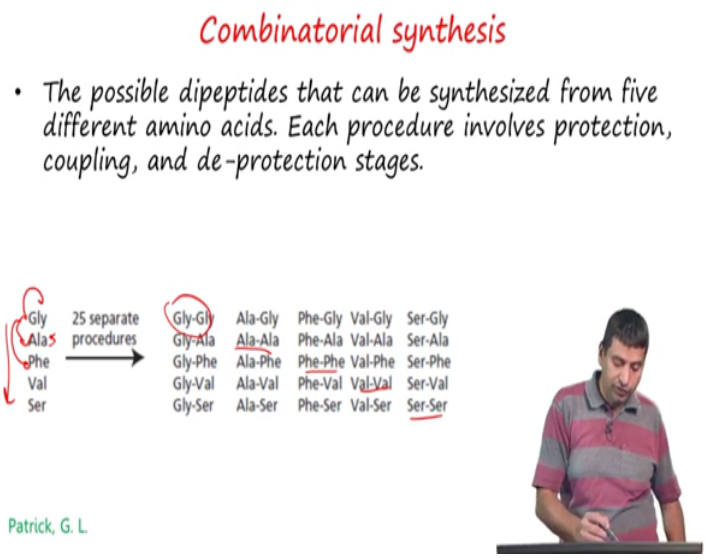

So let us look at this example, so if I have to go through a traditional synthesis and if I want to do a peptide synthesis and if I want to make only dipeptides, then I can take glycine couple

it with itself and get diglycine here but the objective is to make dipeptides. If I take alanine and couple it with itself then I get alanine dimer, phenylalanine dimer, valine dimer, serine dimer. So this I can do each of them by reacting it with itself, so each of this involves one protection and one de-protection strategy to get to the dimer.

But I can also react glycine with alanine, glycine with phenylalanine, glycine as valine and so on and I can get a library of these molecules.

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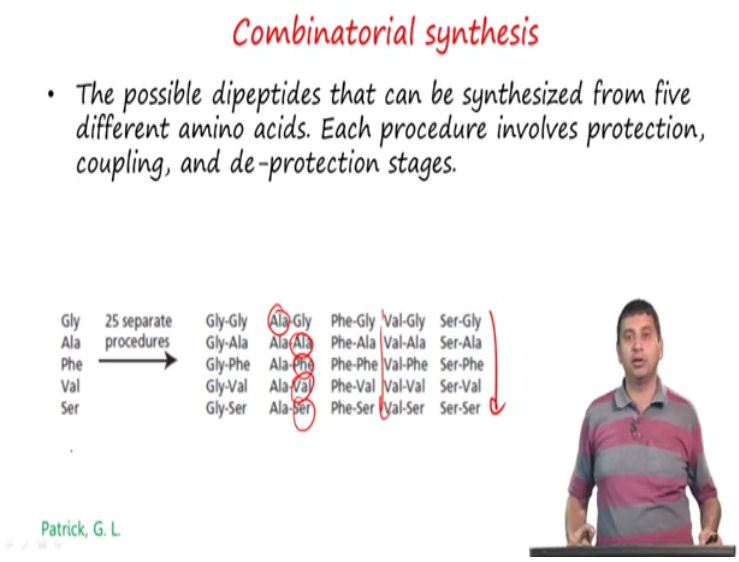

Similarly we can also make alanine, glycine alanine, phenylalanine alanine, alanine valine, alanine serine so these again can be made again I have to go through protection, de-protection strategies. And so if we follow this procedure I need to use 25 separate procedures to make 25 different dipeptides starting from 2, 3, 4, 5 of these amino acids.

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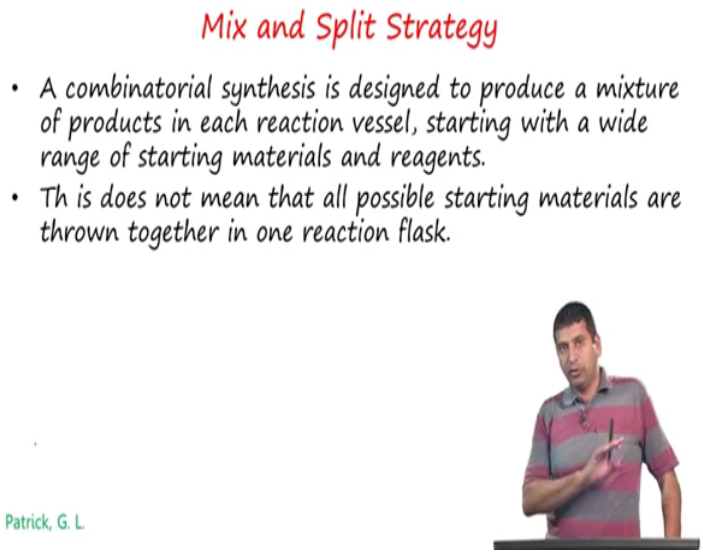

Now in combinatorial strategy we do not worry about these individual reactions, what we do is we design it in such a way that we get a mixture of products in each reaction vessel. So we aim to start with a wide range of starting materials and reagents so that we can get diversity in the library. So this does not mean that all possible starting materials are thrown together in one reaction flask. So there is a method that we follow and that is what we are going to explain now, because if you throw all of them in the flask then you will get a black tarry mask which will be useless to us.

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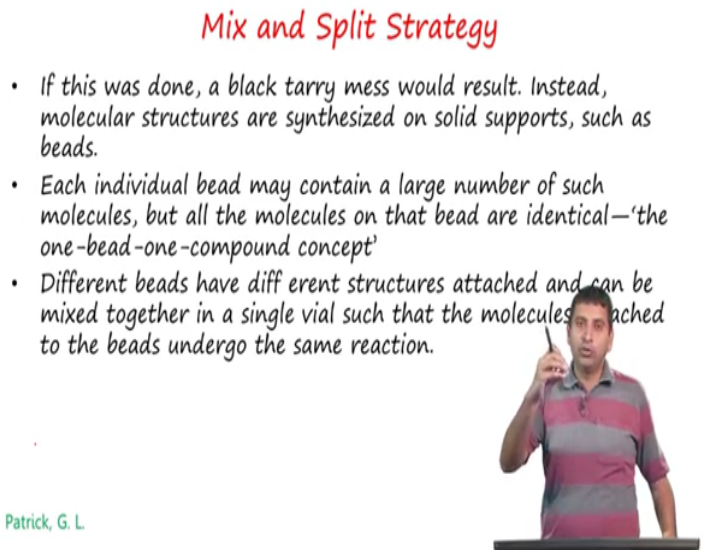

So what we do is you start with these solid phase beads at each individual bead may contain a large number of such molecules, but all the molecules on the bead are identical that means

that if you start with one bead you get one compound and then therefore different beads will have different structures attached and then can be mixed together in a single vial such that the molecules are attached to the beads undergo the same reaction.

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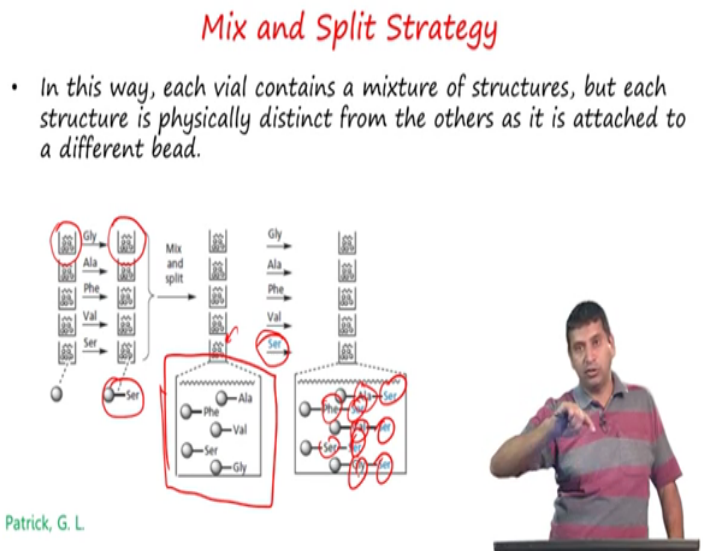

So we look at this a little bit more detail now. So let us say I have a set of beads where I couple it and produce the glycine bead, so each of these vessels contain for example this contains a serine bead, then what I do is I mix all of these okay so I mix the beads containing serine with the beads containing glycine with the ones that contain valine with the ones that contain phenylalanine and alanine and so on, so I have each of these vessels contains a mixture of these starting materials, to each of them individually now I add a different amino acid.

So in this final vial here for example I have added serine, once the coupling happens this will contain a mixture of alanine serine, phenylalanine serine, valine serine, serine serine, glycine serine they are all in present in the same reaction mixture, they are all coupled and they are all immobilized to a solid phase.

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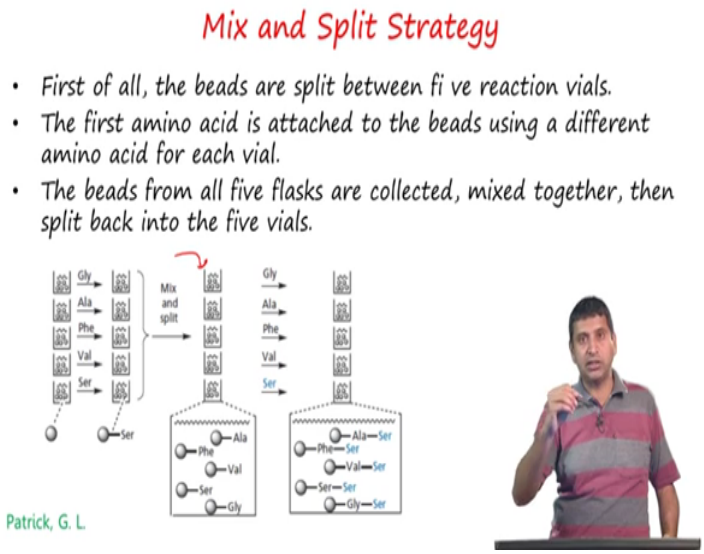

Now so to just to recap quickly the beads are split between the five reaction vials and then the first amino acid is attached to the beads using a different amino acid for each vial, then the beat from all the five flasks are collected, mixed together and split back into the five vials. So that is what we did in this process, so this is called the mix and split strategy.

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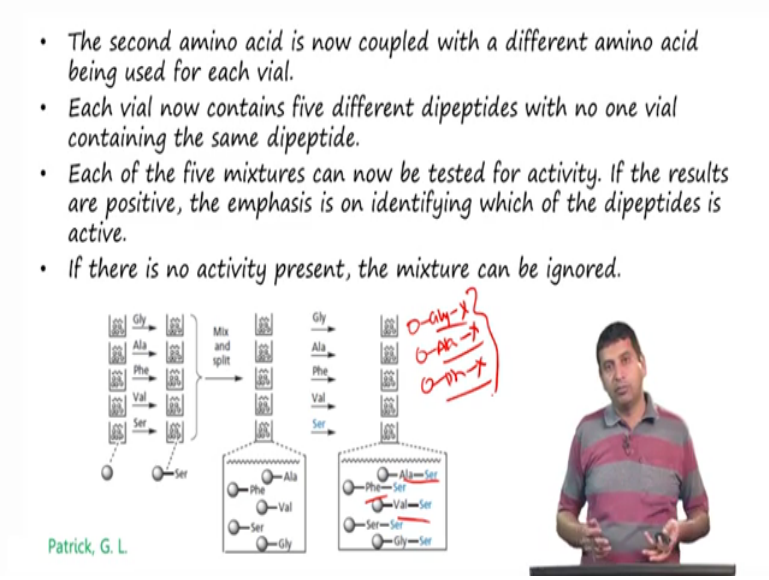

This means that each vial now has the same mixture, so at this point they all have the same mixture. The second amino acid is now coupled with a different amino acid being used for each vial so each vial now contains five different dipeptides with no vial containing the same dipeptide. So if you notice in this vial you have glycine with various amino acids, here you

have alanine with various amino acids, here you have phenylalanine with various amino acids. So these dipeptides are all different from these dipeptides.

So then what we do is that we take this vial and screen for activity. Let us say you are looking for a inhibitor of cancer growth then you do one (())(32:24) and find out whether they have any activity, if one of the mixtures has some activity or one or more of these mixtures have some activity then I can now go back and look at which of these dipeptides is actually active. But if no activity is present then that particular mixture vial can be ignored.

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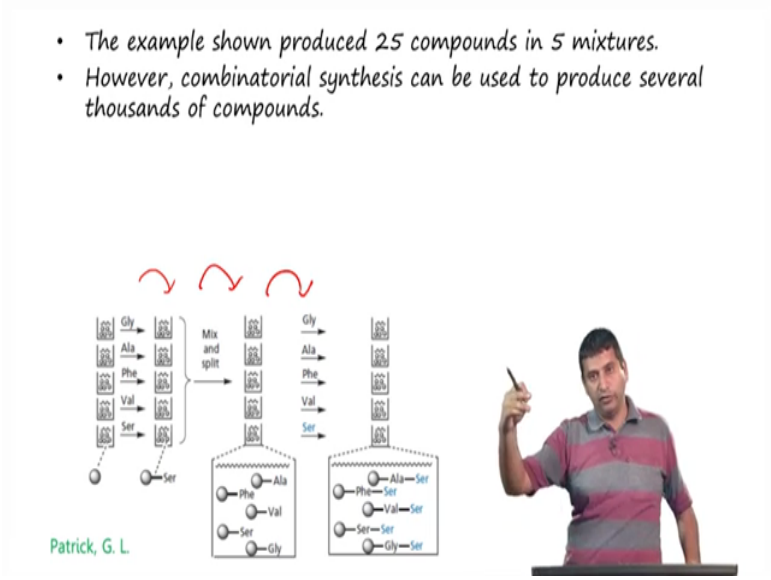

So here the example that we have shown is producing 25 component in 5 mixtures. But if you expand on this you can actually make several thousands of compounds with this kind of combination this is very widely used to come up with a large number of molecules whose structures we know but who are always evaluated as mixtures. So the principle that we use here is that the mixture if it is quite active it is at an elevated concentration, then it is likely that one of those ingredients is quite active.

So to summarize this lecture we need to come up with a large number of compounds and in parallel synthesis the number of compounds are fewer but the quality of the compounds are good that means you have a single component in each reaction vessel. In combinatorial chemistry what we do is we come up with large libraries of compounds that is each vial can have mixture of 10, 20, 30 different types of compounds, here we are trying to optimize for the numbers of diverse structures that we can make.

And the screen in both of these cases we screen for activity and if the compound turns out to be active or if the vial turns out to be active, then we go probe in and find out what the structure is, so in combinatorial chemistry if you keep track of this whole process you can actually exactly identify what are the various components of this mixture.