Medicinal Chemistry
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Anti-Viral Agents Part – 2

So welcome to today's lecture in this lecture we will cover various aspects of anti-viral agents as we have discussed quite a bit about, how the HIV and related viruses can infect mammalian cell and how they are able to use the host machinery to produce new virions which then can go ahead and infect newer host sell and so on, so in today's lecture we will discuss at some strategies to counter this virus.

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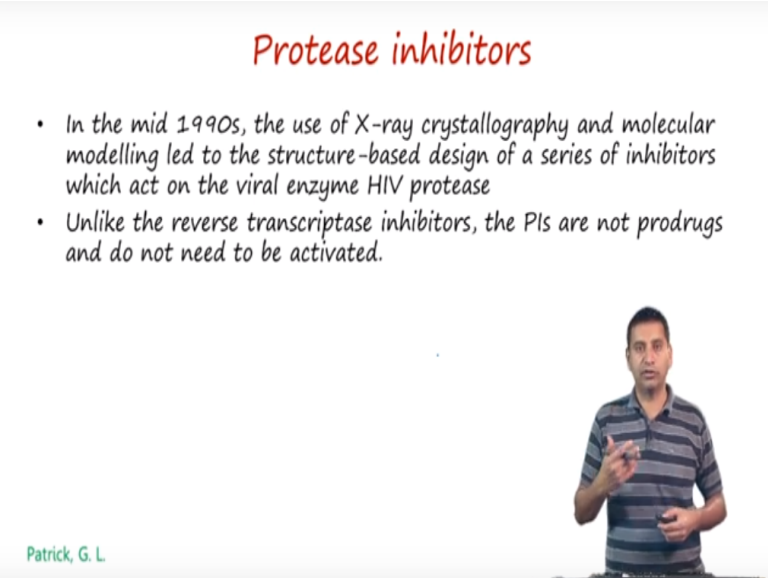

So the first major class of inhibitors of we want to look at today are this protease inhibitors, so somewhere in the 90's X-ray crystallography become quite prevalent in drug discovery and so you could crystalize proteins along with legant and therefore you could understand and how the major interaction occur and also parallelly molecular modeling had measure to some extend as a field and so it was being used to design new structure, so using the synergy of X-ray crystallography and molecular modeling a lot of structure design of inhibitors was possible.

So these enzyme called as viral enzyme HIV protease was important enzyme to inhibit, so we have already previously looked at the reverse transcriptase inhibitors and since reverse transcriptase is an enzyme that is present a primarily in a viruses is not a humans this was

something that we could exploit, so but unless unlike those reverse transcriptase inhibitor which need a phosphor relation to be activated these protease inhibitor is not pro-drug and so they need not be activated they can they are active in their actual administered form.

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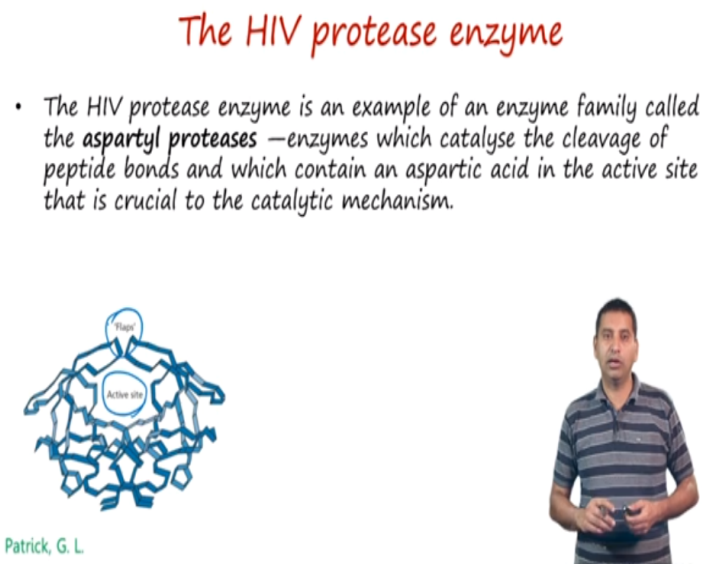

So look at the HIV protease enzyme, so this is an enzyme which is belong to the family of aspartyl proteases and we have already looked at this previously several lectures back or several weeks back actually about how aspartyl proteases is work will look at it briefly once again but these enzyme catalyze the cleavage of peptide bonds and they contain aspartic acid in the active site and that is crucial for the catalytic mechanism, so here is the active site of this enzyme and there is something interesting called as flaps which we are look at later.

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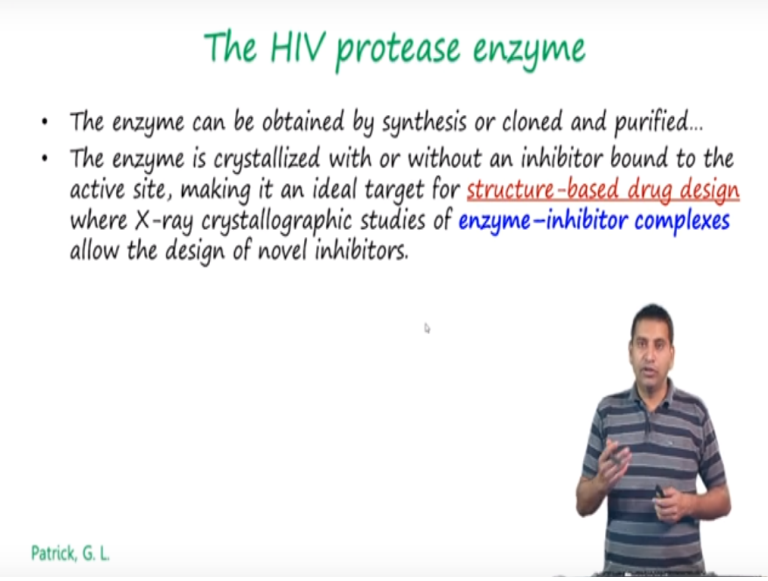

So this enzyme can be obtain by synthesis or it can be cloned and purified and so we can have access to large amount enzyme pretty much, so then it is crystalized with or without an inhibitor bound to be active site, so what happens is that when you crystalized it and it is may to form then you get a structure which probably resembles the form in which it is going to bind inhibitor and once the inhibitor in bound that would represent the induce fir that happens.

So now based on this we can have a good idea about what happens during the binding process and after it is bound so this kind of information provides an ideal mechanism or ideal vehicle for us to understand or to be able to carry out structure based design so using this we would be able design now inhibitors.

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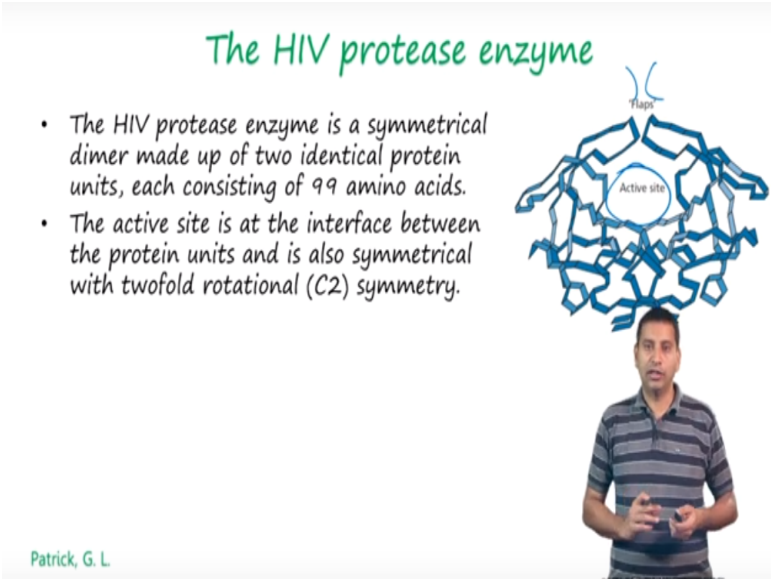

So the HIV protease is a symmetrical dimer made up of two identical units, so it is shown here this is one units and this is second unit and it contains about 99 amino acids the active site is at the interface between the protein units and is also symmetrical with twofold rotational symmetry, so here is the active site which is also has C2 symmetry in it.

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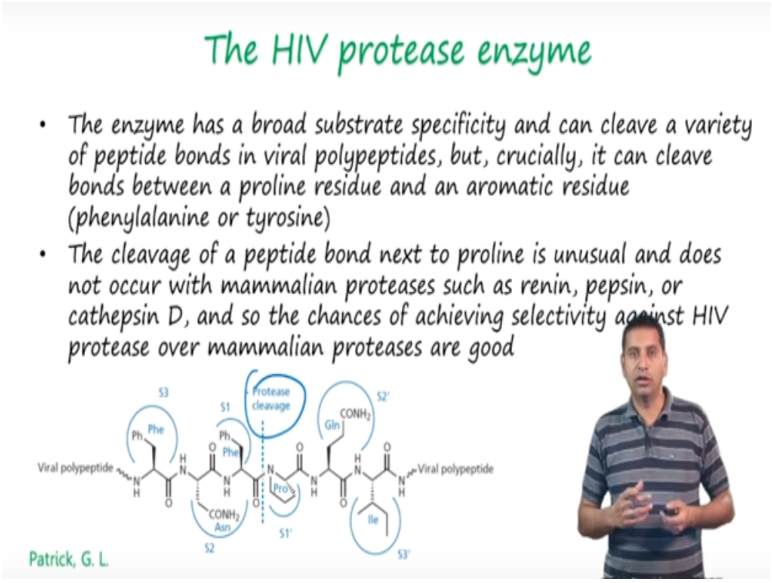

So the enzyme actually has broad substrate specificity and it can cleave a variety of peptide bond in viral polypeptides but crucially it can cleave the bond between a pro-line residue and I shown here pro-line residue and an aromatic amino acid residue such as phenylalanine or tyrosine, so

the cleavage of the peptide bond next to the pro-line is unusual and does not occur in mammalian proteases which we are already familiar with such renin, pepsin or cathepsin D, so the charges of achieving selectivity against HIV protease is actually quite good, so this is another aspects of drug design that we have discuss previously.

So when we are using or when we are identifying enzyme as a target we need to keep in mind that enzyme may or may not be present in the mammalian cell or the host cell as well as the is there anything unique about the one code explode, so that one code achieve selectivity so here this enzyme satisfies both the criteria that is it is present in mammalian cells or human but it is not going to cleave that particular bond and so therefore one could use if for drug discontent.

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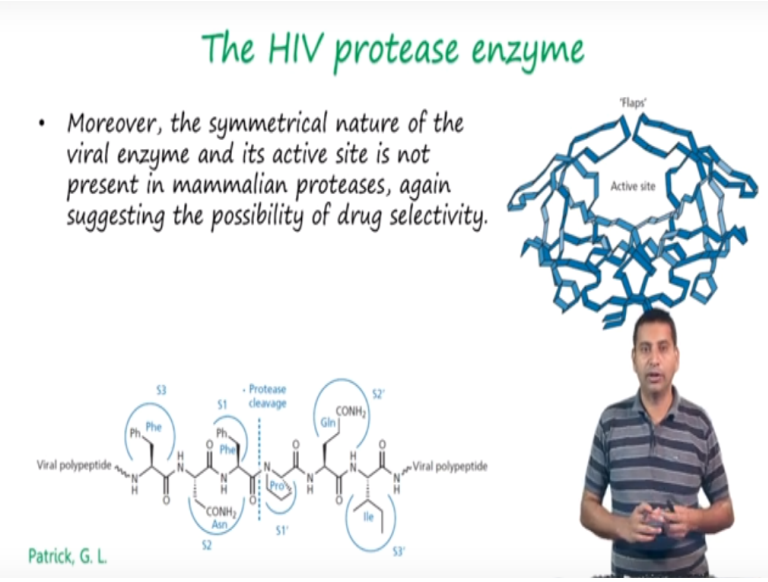

So the symmetrical nature of the viral enzyme and it is active site is not present in mammalian proteases, so there is a significant difference on the activity of the structure of the mammalian version and the viral version.

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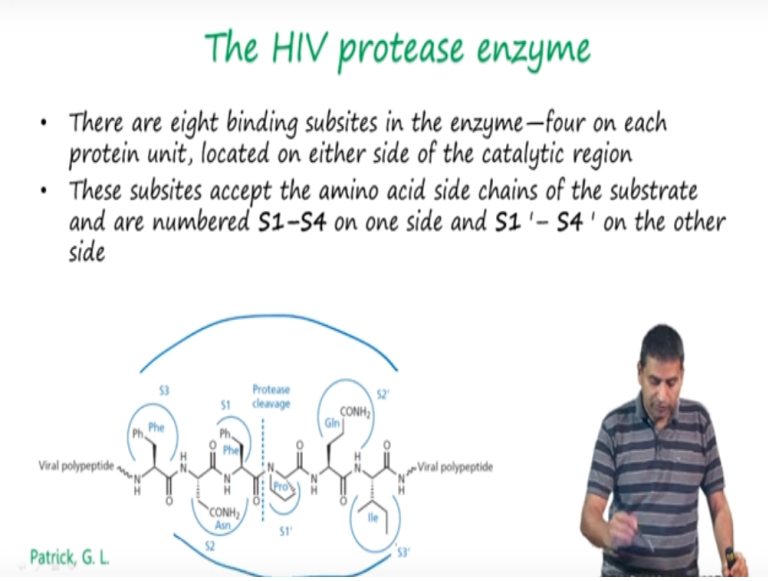

So in this enzyme there are binding sub-sites in the enzyme four on each of the protein unit and located on either side of the catalytic region and so these are numbered S and S4 and or S1 prime to S4 prime on the other side so you can see here this is these are the sub-units.

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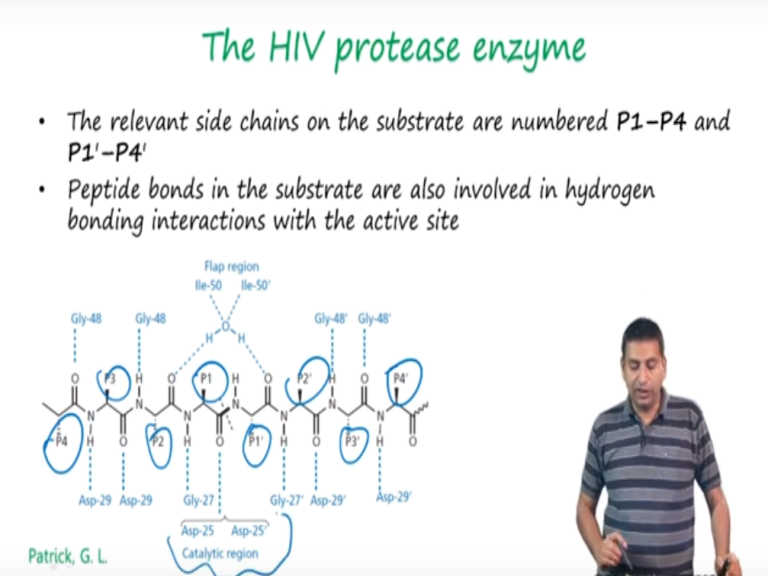

Now the relevant side chains on the substrate are numbered as P1 to P4 and P1 prime to P4 prime so here is P1 P2 P and P4 and the corresponding other sides so the petite bond in the substrate is also involved in hydrogen bonding interaction with the active site and here is the catholitic region.

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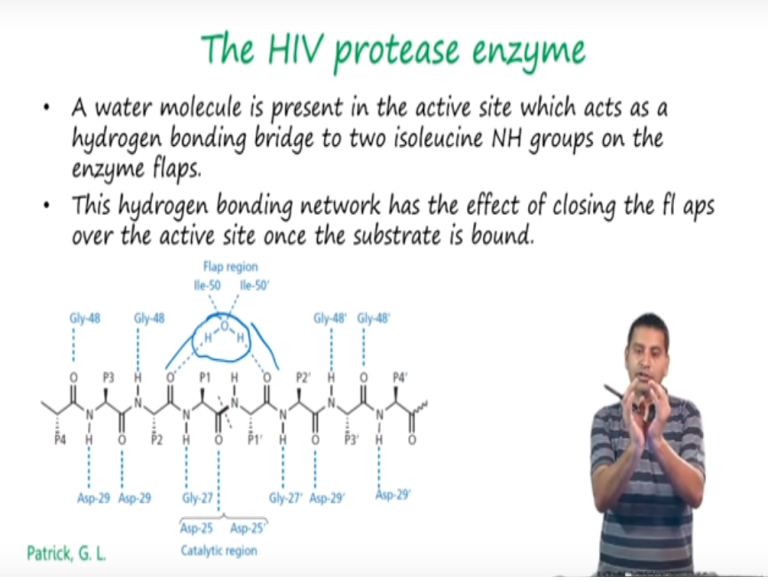

Now an interesting thing about this protease enzyme is that there is a water molecule present in the active site which acts as a hydrogen bond bridge, so here is the water molecule and this is the hydrogen bridge that we are talking about and what happens is that this bridges to isoleucine NH groups on the enzyme flaps, so we have already at those two flaps in the enzyme and so the hydrogen bonding network has the effect of closing the flaps over the active sites once the substrate is bound, so it is almost like how the flaps are open and after the substrate is bound the flaps closed.

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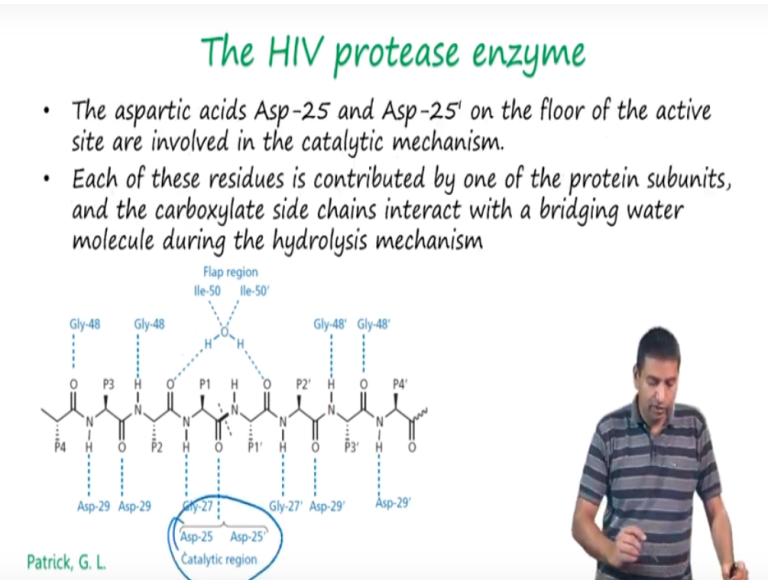

So the aspartic acids unit over shown here ASP-25 and 25 prime on the floor of the active site are involved in the catalytic mechanism, each of this residue is contributed by one of the protein subunits and the carboxylate side chains interact with a bridging water molecule during the hydrolysis mechanism this is again some thin that we looked at previously.

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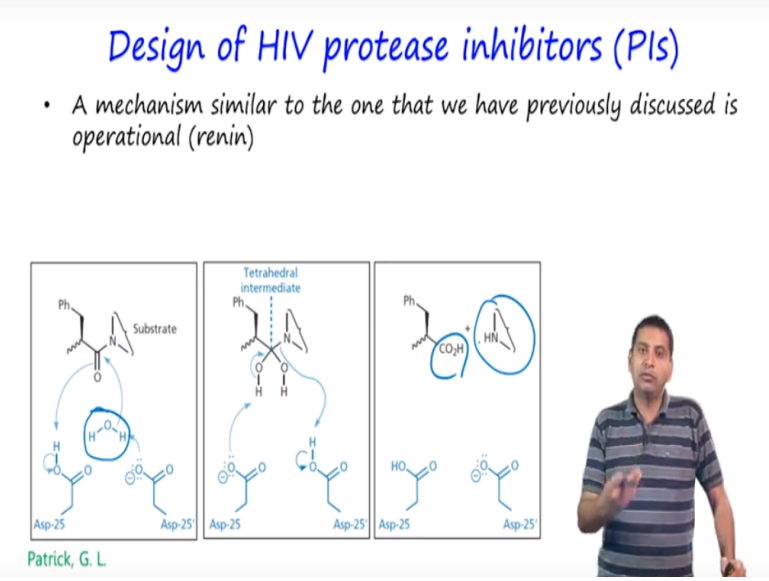

So here is the mechanism that we discuss previously for a renin so here is the bridging water molecule and this water molecule is actually activated by the aspartate residue here and by assistance from the aspartate these water molecule attacks the might bond and forms the tetrahedral intermediate and subsequently under goes in which to produce an are main and a carboxylic acid and therefore this mechanism is what is operating in renin a very similar mechanism is operational in HIV protease.

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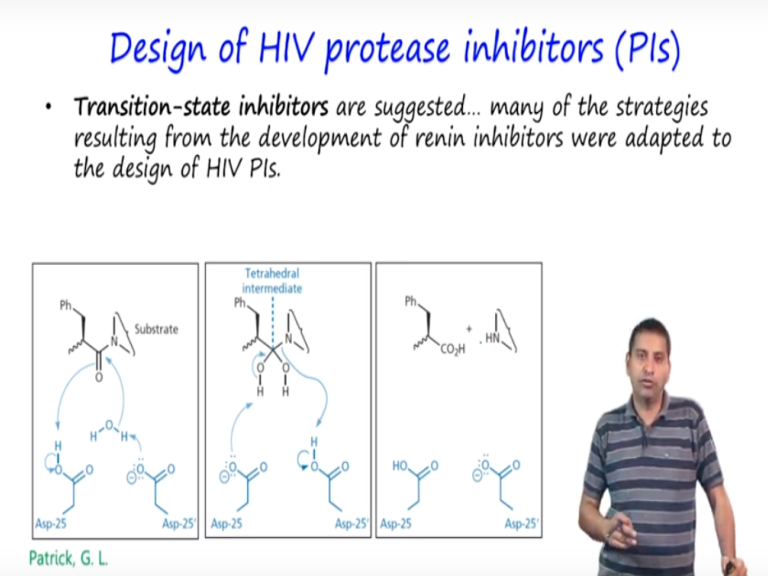

So based on this mechanism one could suggest that transition state inhibitors can be used and now there are many strategies there have been used which have previously used for inhibitors and this can be adapted for the design of HIV protease inhibitors.

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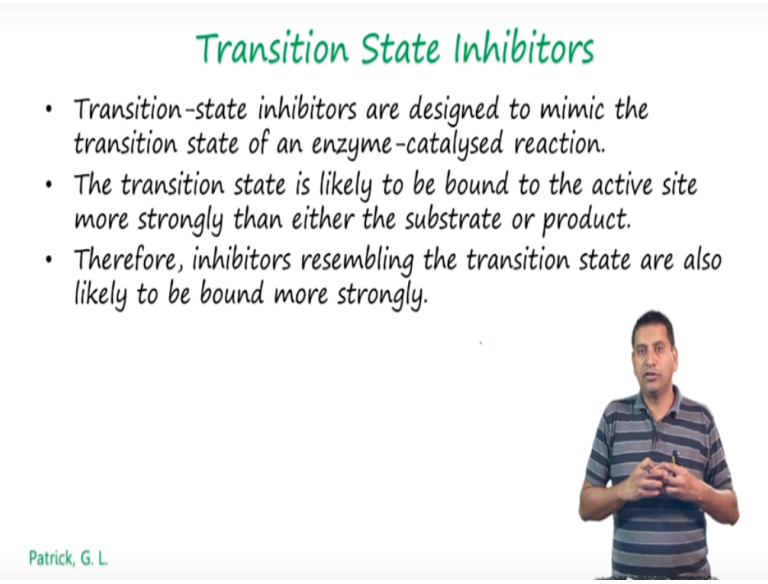

So just o sort of recaps transition state inhibitors are designed to mimic the transition state of an enzyme catalyzed reaction so here the logic is that if the inhibitor resembling the transition state then the enzyme binds to the transition state significantly stronger compared to the substrate or the pro-drug and, so once this happens then it results in a situations where it is compete the

natural substrate, so therefore inhibitors resembling the transition state are quite actively sort after.

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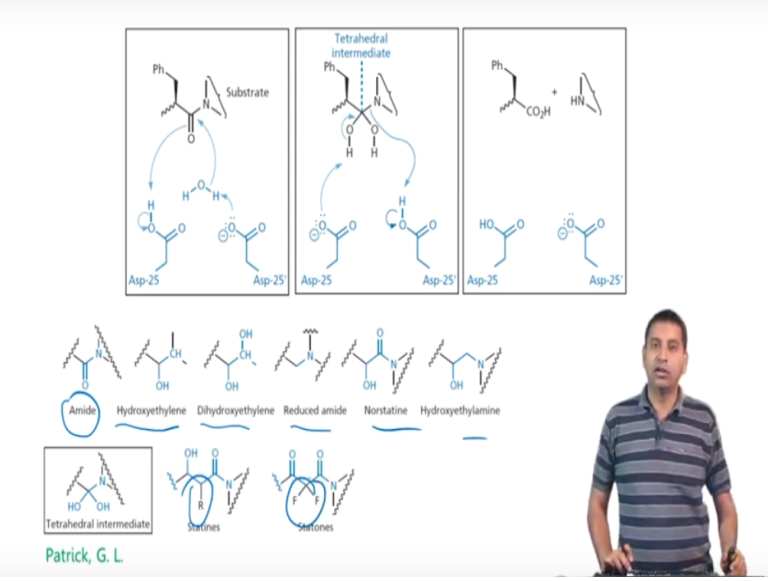

So based on the chemistry that had already been developed on from rinine inhibitors so there are number of sort of inhibitors that one could proposed which have all these various functional groups like dihydroxyethylene, hydroxyethylene, reduced amide, hydroxyethylamine and so on and so forth and of course we could also use a you know statines and statones as potential inhibitors.

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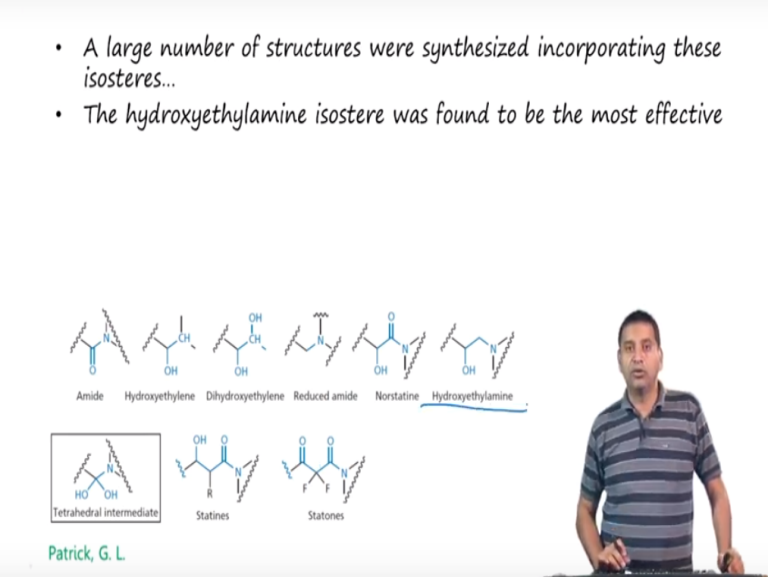

So large numbers of this structure were synthesis which incorporated these isosteares and what was found the hydroxyethylamine was found to be the most effective.

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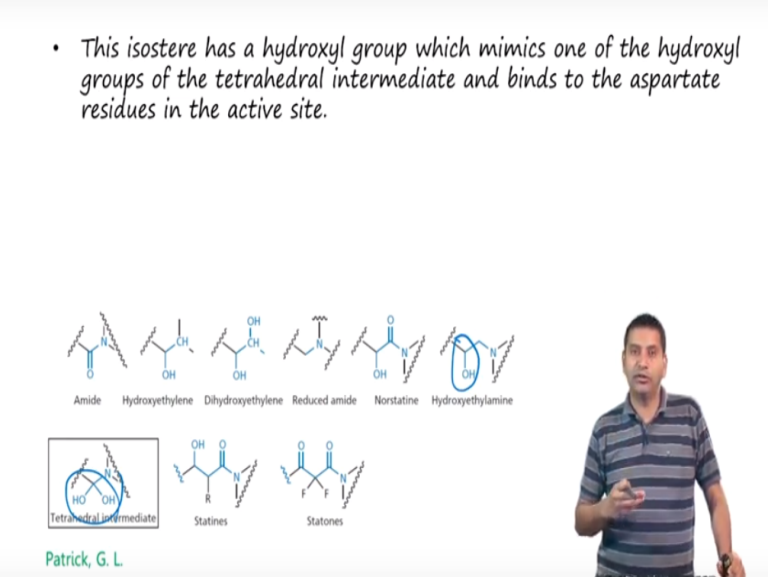

So this isosteare has a hydroxyl group which mimics the of the hydroxyl in the tetrahedral intermediate so here is the tetrahedral intermediate and here is the hydroxyl group and therefore it can bind to the aspartate residues in the active site.

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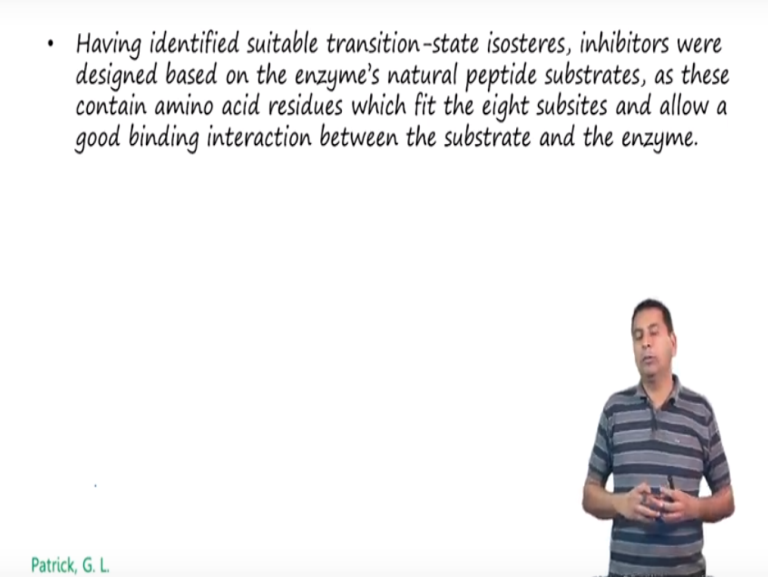

So having identified this suitable transition state isosteres, inhibitors were then designed based on the enzymes natural peptide substrates, so that it can go and bind to the HIV protease and because it is an inhibitor therefore does not undergo trend over it is there is no amide bond to cleave therefore it would bind to the enzyme and prevent for the substrate from acting on it.

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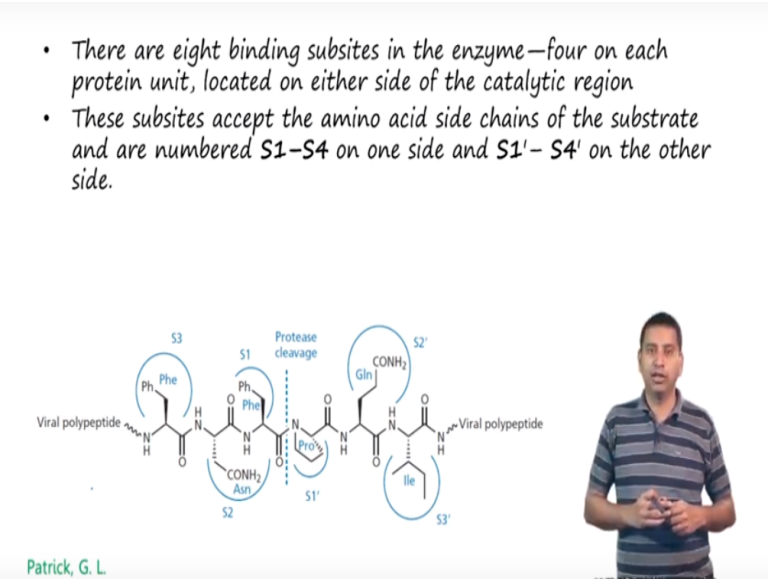

So there are eight binding sub-sites in the enzyme as we discuss previously and these are numbered are S1 to S4 on one side and S1 prime to S4 prime the other side.

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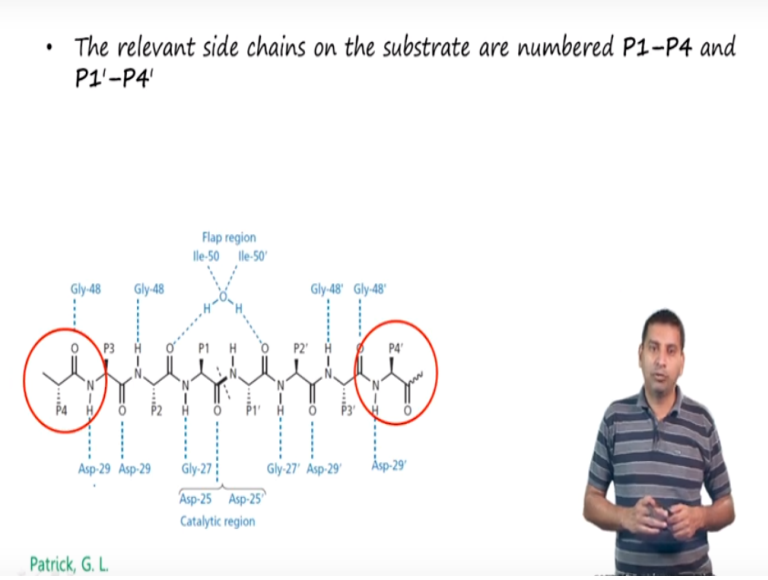

Now the relevant side chains on the substrate are again numbered labeled as P1 to P4 and P1 prime to P4 prime.

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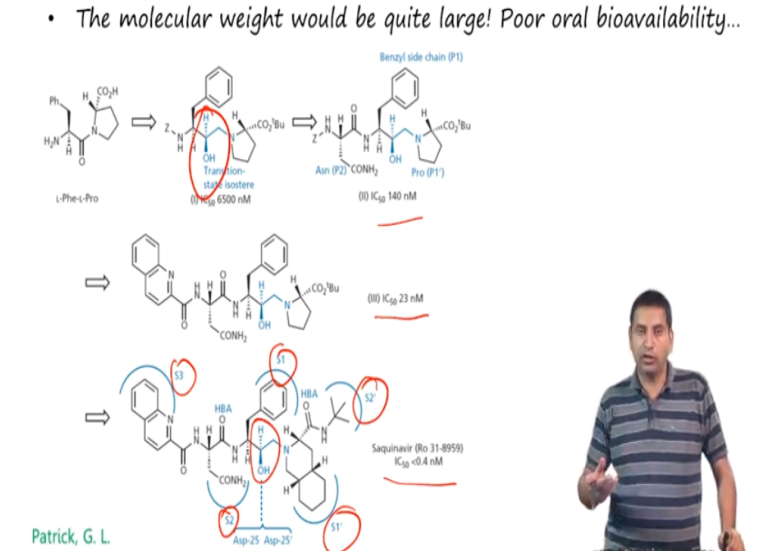

So using this concept there are been number of inhibitors that have been developed here is transition state isosteare which has a IC 50 of about 6500 Nano molar and subsequently this is been further optimized to reduce the CI 50 to 140 Nano molar and finally further modification relate to an improved IC 50 of 20 Nano molar, so we are getting really closed to where we want to get to and finally based on the interaction of the S3 S1 S2 sub-units and keeping in the

transition state in mind as well as S2 prime and S1 prime this molecule which is quinavir was identified this has an IC 50 of less than 0.4 Nano molar and that is fantastic for use as an HIV protease inhibitor.

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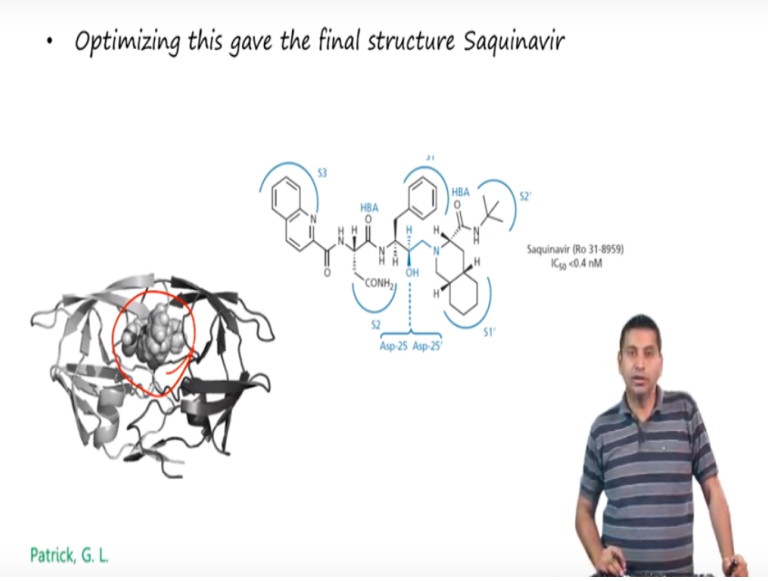

So finally this structure Saquinavir was developed which has the following structure and if you cocraslize this molecule in with HIV protease you find a rate fits very beautifully into the active sites with the following binding interactions.