So welcome to handout four and what we're looking at in this handout is enzymes. So the kind of logic here is that we've talked about how proteins fold, and how they get into a particular shape, but now we want to focus on a particular family of proteins that many of you are familiar with, and that's enzymes. So here I wanted to initially, I suppose, reflect a little bit on the key features, so we did a lot of exercises in class around writing down key points and you know, going back over things that we might be aware of generally about enzymes and then adding other layers to that. So we talked about specificity, we talked about active sites, we talked about enzymes not getting used up, we discussed the idea of negative feedback, we discussed the idea of sequential, kind of stepwise, patterns and pathways that involve enzymes and how the product of one reaction is actually the substrate for another enzyme, and so on. And we were really looking at the idea of the catalytic power of enzymes, and what they can do so a good example was the sugar on the shelf versus the sugar that we intake into our bodies and what happens in the presence and absence of enzymes. We then started talking a little bit about enzymes, how they need to have the right temperature, and they need to have the right pH. That came all the way back to the enzymes having the right shape, active site, because we really wanted to make sure we understood the idea that a substrate had to kind of fit into the active site for it to be converted to product or multiple substrates. But it's that idea again, back to proteins being folded and then maintained in their native forms, supported by the right temperature and the right pH. Soon, you will be doing experiments in the lab where temperature and pH will be controlled so as to maximize the enzyme’s functionality. Once we knew a little bit about enzymes, we reminded ourselves about how you knew about their key characteristics and what they do, and how they catalyze reactions. We were talking about naming them and we looked at how a lot of them end in -ase. The name often tells you what goes on, like we talked about Urease which catalyzes the hydrolysis of urea, DNA polymerase helps generate chains of DNA, but then there were another few enzymes that had different names. The one we focused on here was Trypsin, and we were talking about that a little bit because we will also be going to get you to think about proteins being ingested and then how we deal with them, so we chop them up into smaller bits when we want to harness their energy and recycle components that we might use again. So looking at that trypsin idea, we said trypsin actually cuts on the carboxyl side of arginine and lysine residues, so we had some examples about how the active site must be able to find the right place, the right amino acids, to dock onto and then the enzyme knows where to cut the protein chain and break the peptide bond. Hence, we were really trying to consider the idea that the active site actually must have something that's going to bind to those targets, so the example we had was trypsin and it actually has a negatively charged aspartate inside in the active site, and that why it's able to interact with the lysine or the arginine on the substrate which we want to cleave. So trypsin was a good example that we talked about, but our main focus jumped then to how enzymes actually work, so we had covered the idea of catalysis and what catalysis meant as in increasing the rate of the reaction and we talked a lot about the sugar and so on, but we began to look at activation energies and Gibbs’ free energy, graphs. Our analogy really was that an enzyme reaction needs an input of energy, so our analogy was a high jumper and in the high jump in the Olympics, you have the bar increased to a certain height and at the end of the day only one athlete is able to jump over a particular height, so that athlete has to put in so much energy to get over the bar, so it's very infrequent that lots of athletes get over the bar. The question I asked you was well, how would we get every athlete to get over the bar? Your answer to me was well let's just lower the bar, and when we look at these Gibbs energy profiles for enzyme reactions that take place, they often have these peaks in the middle. At the top of that peak is the transition state, the amount of energy that you need, that the substrate needs to receive or have input into the reaction to get to this transition state. This is essentially the athlete jumping over the bar, with the transition state being the height of the bar. Essentially, in our analogy, and what enzymes do is they effectively lower the bar, so you'll look at the diagrams in the handout and you'll see that when enzymes are there the bar, the amount of energy that's needed, is lowered and that means that we can get to product much easier - Just like you can get more athletes to get their medal if they can all just hop over a very low bar, instead of having a big high jump. So we were talking about what happens if reactions want to go the other way also, from product to substrate, because we often hear about reversible reactions but we were trying to look at the energy that's needed for the product to go back to the substrate. Looking back at some of the diagrams will remind you of our conversation. The catalysed components which lower the energy of activation really do speed up the rate. We were looking at what kind of speeds, so we were reflecting on some of those rates of enhancement. But one thing I really want you to consider in that section was that we have these, let's call them, barriers in the way, because we have to get over the barrier, over the bar, and in order to get to the ‘other side’, where the products are present (refer to diagrams). So we have that reaction path of having to go ‘up’ on the graph before we come ‘down’, just like the high jumper jumps over the bar and landing on the mat, but why do they exist? A lot of people think they’re to slow down the reactions, because you need an input of energy which is true, but they're there for a reason. The primary reason they're there is as barriers, where they're evolved I suppose, to make sure we carry out the reactions that we need when we need them, and we don't waste energy carrying out reactions that we don't need the products of any more. In fact, those energy barriers, that as in the height of the bar, the height of the transition state in those graphs is crucial for life, and they make sure that lots of reactions aren't happening spontaneously and that we're able to perform the reactions when we want them to take place. Hence, it’s worth refreshing your minds on those diagrams and graphs in the handout. We tried to distinguish a little bit between ‘equilibrium’ and ‘rate of reaction’. We highlighted how there was the sugar at home on the shelf, and the sugar in your body. Ultimately, we'll get to the same place; the sugar will be broken down to carbon dioxide and water and so on, and that's going to happen. But one takes years to get there and one takes seconds and in the presence of enzymes, we do that much quicker. So our analogy around this was kind of a car race. If there were two cars, say one that can accelerate much faster than the other. Well both of them might get to the target speed, let's say the speed is 120 kilometers an hour, both of them will get the target speed but one might accelerate much quicker and get there immediately within a few seconds, and with the other one it might take a few minutes for them to get up to that speed, but they'll both ultimately get to that speed in the end. So what we're talking about here is ultimately reaching the equilibrium. It's where the plateaus are in the graphs, essentially where the product kind of levels off over time, and that's essentially where the plateau is, is where both cars will reach 120 kph. However with the enzyme, i.e. with the faster car, you get to the equilibrium much quicker and that's to do with altering the rate of the reaction. Hence, the equilibrium is not altered in any way. We both get there. It's going to happen whether it's catalysed or not, just the time required is the difference. So enzymes alter the rate of the reaction but not the reaction equilibrium. Hence, it’s worth looking back at that graph in the handout on this topic, to remind yourselves of it. What I love about science is you read all these things, but they're all evidence based, and that's really key. The data is key to show, and to have validity and proof, and be able to emphasise/realise different points, so we started looking at how could we prove that enzymes and substrates actually interacted. Therefore, we talked a lot about enzyme substrate complexes, these ES complexes. We had a few examples we looked at that the speed, the initial velocity. So the speed of product being made at a specific limited amount of enzyme in every tube, even though if we flooded with substrate, it limits off, it plateaus, and we were asking why does it plateau? That came back to the idea that the active sites must be full. We had a nice analogy with car parking and cars, and flooding car parks with cars, and observing the speed of cars being able to park and so on. That section might be worth reflecting on a little bit, but it was nice to kind of see and understand about enzymes and substrates, and what happens when enzymes’ active sites become full when there's so many substrate molecules. We also looked at evidence from X-ray crystallography information. We looked at some spectroscopic characteristic changes that happened in experiments to give us evidence that enzymes interact with substrates, and then for the last section, we took a look at active sites. We looked at five key features of active sites. Firstly, what do they look like? They're little pockets or clefts. We saw how the amino acids far away from eachother, could be close together in the final folded protein as we mentioned in the last handout. They have very unique microenvironments, and we thought that there has to be some sort of weak attraction between substrate and enzyme because remember enzymes don't get used up, so have to release the product to have the enzyme available again, so you don't want to covalently bond the substrate and have it permanently wedged inside in the enzyme forever. We want the enzyme to be able to do its job, release the product(s) and then go and work again. That's much more efficient. Then we finished talking a little bit about how the enzyme and substrate fit together with models, lock and key that we often hear about but also the induced fit model whereby the enzyme is almost able to slightly move a little bit and make sure that it holds the substrate in the active site which is a very common way that a lot of enzymes were actually functioning. That handout was really just to kind of bring us all up to speed on enzymes and to go into a bit more depth about the rate of the reaction and get some of the language around enzymes in place, because our next handout is on enzyme kinetics and how enzymes become inhibited and that's something that's very important in the various industries that we discussed throughout our lecture series. So we'll continue with that in the next clip.