Interactomics: Protein Arrays and Label-Free Biosensors.
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Lecture-19.
Diffraction-based biosensors II

Welcome to MOOC Interatomic Course. In our last lecture we learnt about the basic of Diffraction based sensors from Cynthia Goh. Today we will continue with our discussion on this approach with her.

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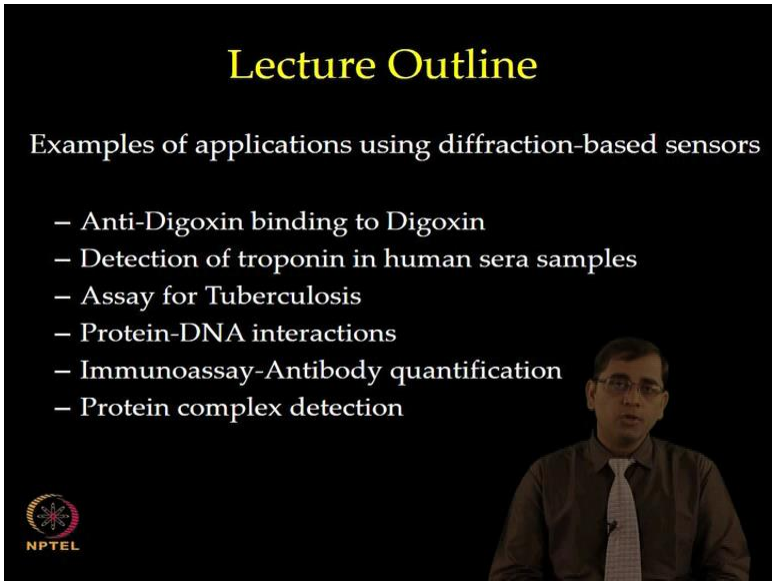

She will demonstrate various examples for measurement of molecular interactions using diffraction based biosensors. So let us start by viewing an example for direct and label free measurement of anti-digoxin binding to digoxin. We will talk more on the how to measure protein interactions. Welcome Ms. Cynthia.

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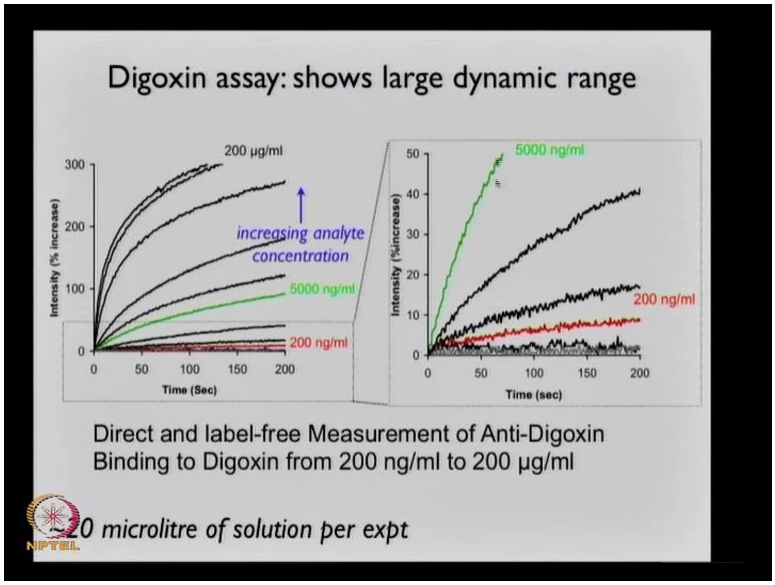

Cynthia: Thank You. I will just show you an example, there is an assay this resolves with this experiment that I showed you earlier this little proto type. so basically looking at the intensity of light as a function of time as we introduced anti-digoxin to the bind to digoxin which has been immobilized on the glass surface.

You can see when the anti-digoxin is 200 micro grams per mil you get the upper most curve. As it decrease the analyte concentration you get lower curves until down to 200 nanograms per mil. Here on the right hand side we blow it up, so that you can see 200 nanograms per mil. I can still believe signal above noise. So we can go from 200 micrograms per mil to 200 nanograms per mil label free in 20 microliter of solution in this experiment.

Sanjeeva Srivastava: I think that is quite good but probably we can tweak it around to (())(02:10).

Cynthia: This is still off course label free. This is also little in bread board set up which I showed you earlier, so not optimizing anywhere. in fact some of these experiments we have done earlier just on a web cam with manual capture of the intensity. So there are many ways to pushing it higher. One way of doing so is putting it in a label. I know this is label free course. You can see what label would do to you.

Sanjeeva Srivastava: I think broadly we are discussing about different types of (())(2:45) system, so I think it will fit.

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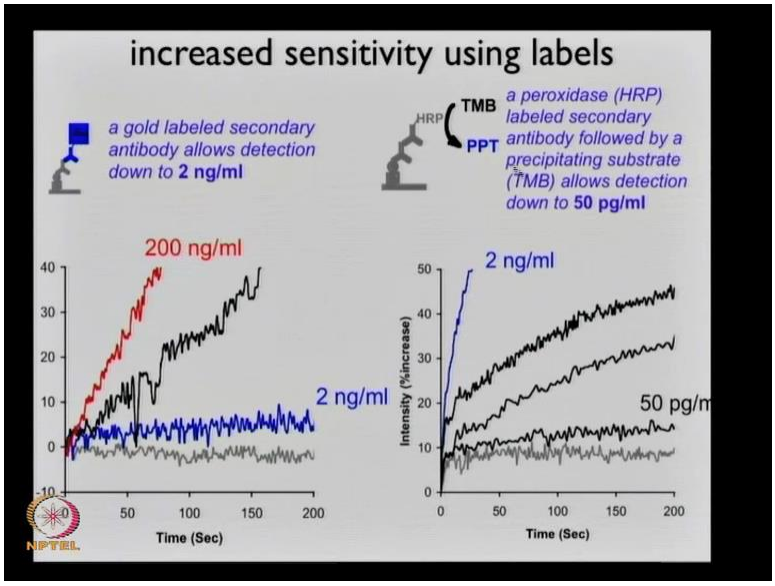

Cynthia: So you remember we had 200 nanograms per mil that was in the previous slide, but now I am going to introduce a secondary. Here is a secondary antibody and in this case labelled with a little piece of gold. Actually we found out later, we don't need that gold label. But anyway for this graph we have gold labelled in there.

We can bring it down from 200 nanograms per mil to 2 nanograms per mil. Now this this is now, this noise is now the noise of the detector. So in order to go better than we have better to do little bit more of us signal averaging. and we can do different type of labelling which is, this is precipitation assay, so here is initial antigen, and then you put in the antibody digoxin, then you put in um the anti-secondary which is Horseradish Peroxidase in it.

The Horseradish Peroxidase then acts on sub straight TMB to form a precipitate. If you do that we can actually go down to 50 picograms per mil. 2 nanograms per mil here, and other 2 nanograms per mil is huge, and we go down to 50 picograms per mil. I think one actually even tweak it further but you starting (())(04:07) cannot takes because if you very low now, this binding is taking very long period, because it takes some time for them to find each other.

Sanjeeva Srivastava: But this could be useful I think when we are talking about very weak protein interaction, different type of analyte which is very very low in abundance.

Cynthia: That is right. Yes, so that becomes an issue about assay development. What we have here is a tool that allow you to measure the signal. Now you can configure your assay so that you can (introduce). In this case we were introducing them leniently, but in some cases you can actually premix the cocktail and let it bind together, and that sometimes work better.

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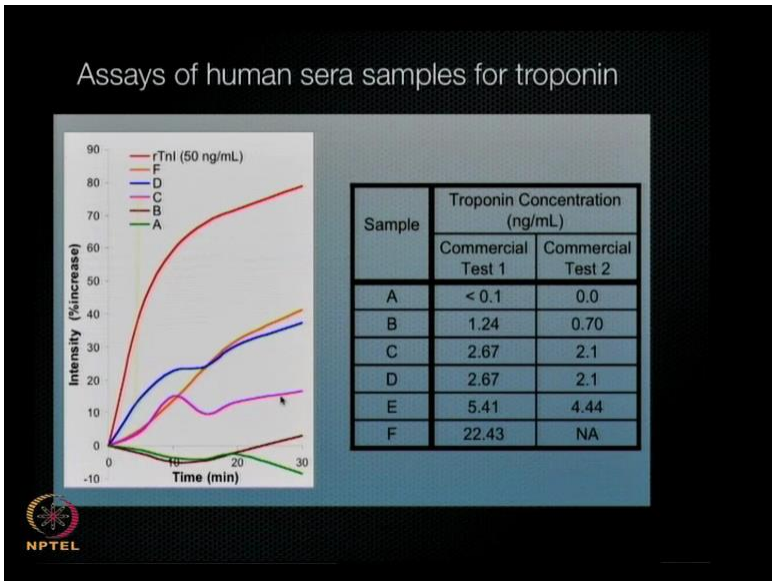

So it is not actually protein that we can it actually look for some data on troponin. This is protein antibody assay. the clinical relevant level is around here above this line, above 2, and you can see this is the signal. this is the indicative of the noise of the system that is why this is wiggles in that signal, but the interesting thing here is the point of view of clinical diagnostic is 10 minutes here. So in less than 5 minutes you already have difference between highest level clinically relevant level and clinically absence of troponin.

Sanjeeva Srivastava: I think in a very short time you are able to measure the high intensity.

Cynthia: That is right. So imagine this is the markers for cardiovascular of disease, markers for stroke, and you can know within few minutes that it is there or not.

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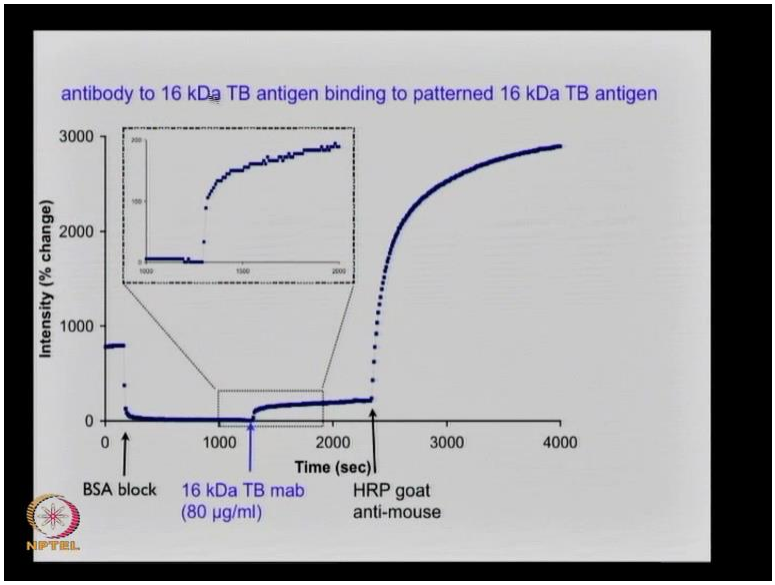

Here is an example for how you can actually assay for couple of antibodies for TB. Here looking at the 16kDa, we are just going to show you in this case example of two markers, two antibodies, two antigen binding for TB. Again at looking at two different spots simultaneously happening, and introduction of antibody to the 16 kilodalton TB. Antigen, again you start with an experiment here (and).

Sanjeeva Srivastava: How do you correct for the base line in the beginning, as I can see it is beginning from 800 or something.

Cynthia: Ops sorry about that. There is a base line here because there is a diffraction pattern, we put down 16 kilodalton antigen on the substrate, and so when you shine light on it this is going to be the base line.

Sanjeeva Srivastava: Right.

Cynthia: so we do nonspecific binding we put in BSA block, and so basically here. So that your medium will not have all the proteins sticking to the lines. So you introduce BSA block.

Sanjeeva Srivastava: You do the blocking surface and (then).

Cynthia: Yes, that is right. So here signal drops to 0 because of the blocking because BSA sticks in between the lines. Then at this point we introduce 16 kilodalton antibody for TB that will bind

to the antigen. You can see there is a little blip in here. Now let me expand this area and see the expansion here, that blip is actually real because the signal to the noise is good enough, but in case you basically wants something that is much more obvious or something that you can see, because this point when you have the precipitation you can actually see this signal ready, okay. Let me introduce HRP goal anti mouse TNB sub straight here, and you can see the enhancement and signal. This is actually very big intensity change. you can look at this, this is 3000, so 3000 percent intensity change.

Sanjeeva Srivastava: That is a clear sought of yes or no answer.

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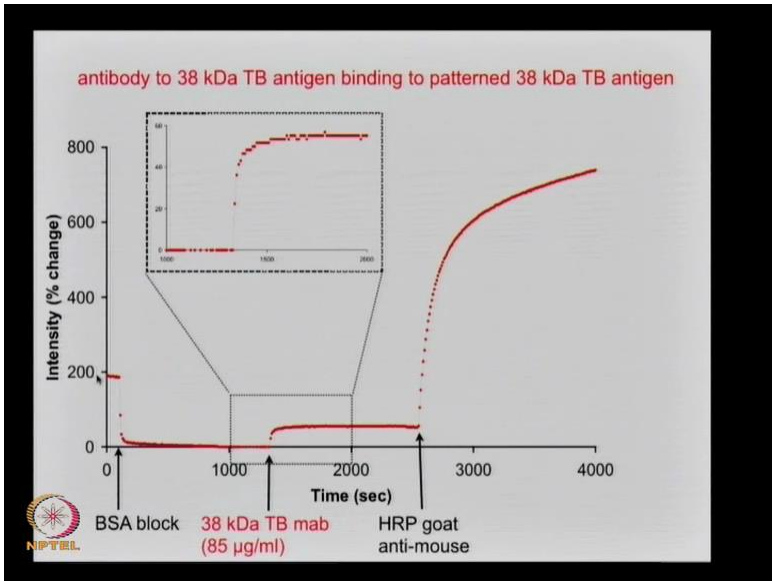

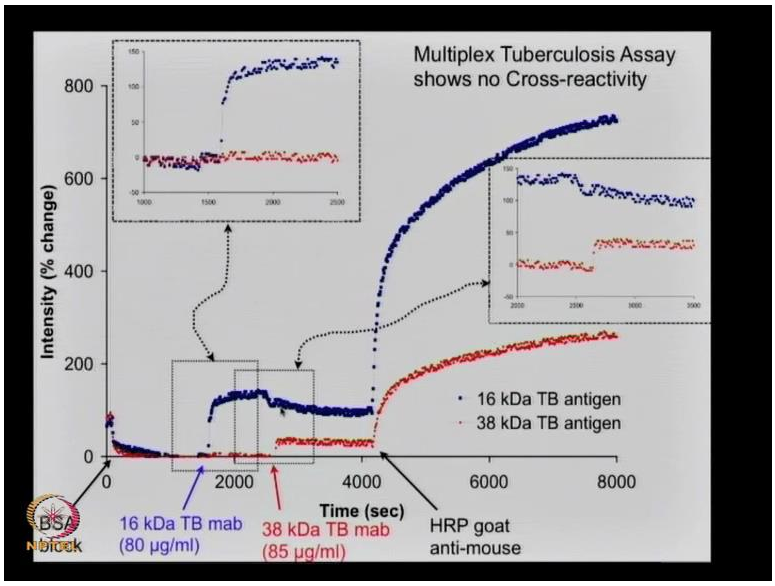

Cynthia: Yes, that is right. If you are trying to create a diagnosis yes or no, well here I can see yes or no. Yes, so that is one of the spot. If you look at the second spot which is filled with 38 kilodalton TB antigen, you can do the same experiment and then we can just put them together. These are the two parts together. at this point we introduced 16kilodalton TB antibody and one of its part get more intense, the other one did not. And then this point we introduced 38 kilodalton, and then you get the other part more intense. We amplify them both parts.

Sanjeeva Srivastava: Blue one is 16kDa, and 38kDa is red one.

Cynthia: That is right.

Sanjeeva Srivastava: It is able to measure simultaneously both.

Cynthia: That is right.

Sanjeeva Srivastava: And if we see the signal of both, then we know actually the signal is positive.

Cynthia: TB is a very high thing to detect, but at least you know two signs that it is there, and you can imagine array because there are other types of potential markers for TB that we can add through that, so you can get more and more sure that actually something is happening.

Sanjeeva Srivastava: It is a set of concept which you are showing, but I think it can be applicable depending on the context. Anyone can make the assay more robust with the introduction of right type of proteins, and more markers.

Cynthia: Yes, now other way to do is that you can imagine making the second part be a blank. Right. And that way you can make sure that the signal is in reference to a blank that you know you do not have (())(09:33) in there.

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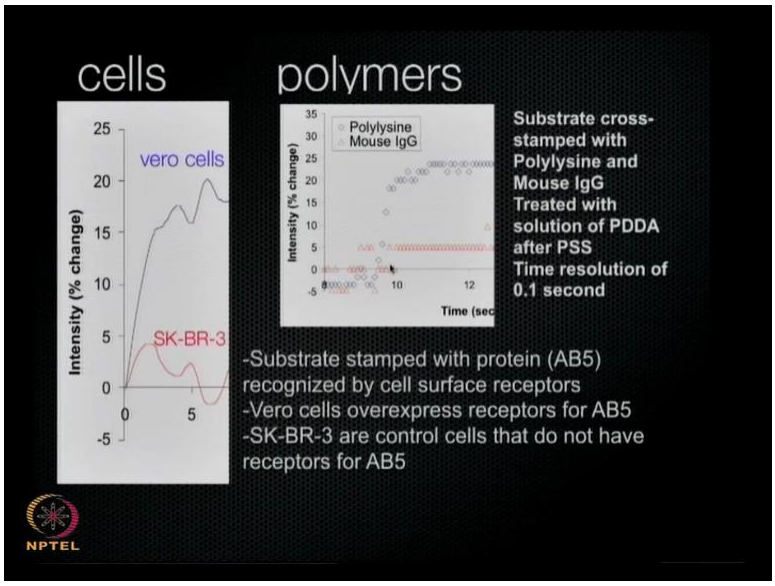

Okay, we can skip the other proteins, but just to show you that you can measure cells, you can look at polymers because actually the measurement of interaction is general. You can measure the interaction between two types of molecules.

Sanjeeva Srivastava: Okay, I think now you got a prototype , and probably you can discuss that how you took the prototype, and how you put it at the commercial scale because ultimately you need an application to develop.

Cynthia: But you know I showed you what it looked like in my lab. I can teach you how to make one of those but unless you want to be an expert in optics then you really don't want to build one every time you do a measurement. so I could have just keep cracking out data but that is not the point. We want to be able to create an instrument that will be useful for other people and if you go for medical diagnostic it is important that the instrument is functional and useful for others.

And this is the role of commercialization, so at that point I actually around generate in 2002 or so.

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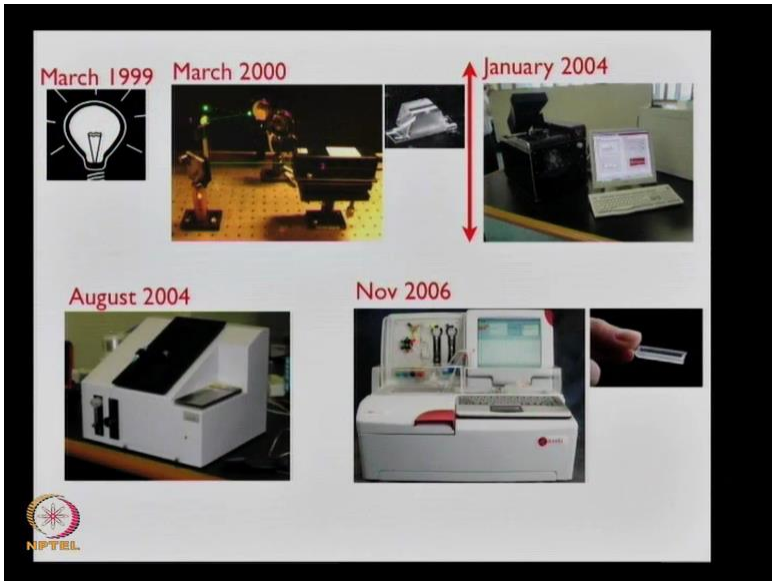

Basically I decided that we have to commercialize the device in order to get it to be useful by others and this is important thing that I believe in that science will be able to benefit the society. It has to be turned into the product that others can actually use.

This is how it was in my lab, and up to now this instrument is in my lab. We still work on the bench top. but what we did is we translate it into series of tabs, so that the current device rent out in the market late 2006, early 2007 is an instrument other people can use. In that little piece of double sided sticky tape, no it is not really gonna be good for you to just make that to yourself. It is now little piece of plastic that is actually a lot cheaper took a lot together, but it is now much more efficient and cheaper.

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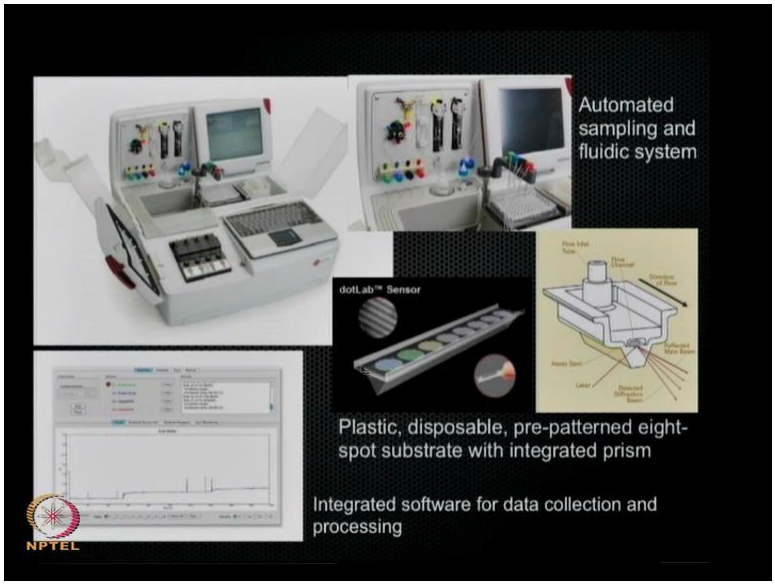

Okay, so here is the instrument , it is so computerised pumping system software , and it has little sample cell that has 9 parts that you can put down different proteins or different snippets of DNA whatever biomolecule you are trying to assay.

Sanjeeva Srivastava: You are trying to provide them multiplexing capability.

Cynthia: Yes, it has multiplexing capability, and instead of all together it is now in 8 different parts, and effectively in the instrument being runs through back and forth into this. And there is a channel there , there is an insertion port, the injection port where you inject the analytes and it just goes to the glow channel which is about 20 microliters or less. Okay, but it has a pumping system so you can actually control the fluid and so on which becomes important when you are doing kinetic studies.

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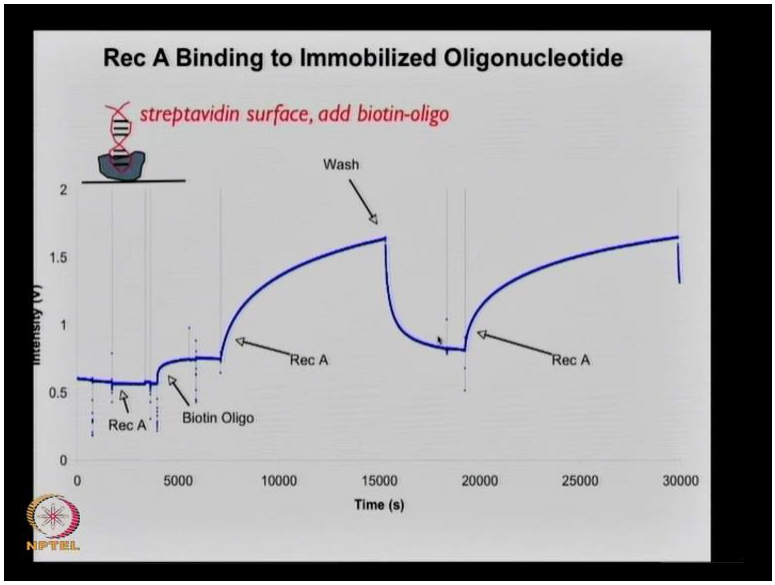

So here is an example of study of protein binding on DNA. So in this case this is the substrate you put down streptavidin in a pattern, and we basically take that. That is substrate, and that is the initial signal from that substrate.

Sanjeeva Srivastava: What is the Y axis here?

Cynthia: This is the time, intensity, and this is just intensity in arbitrary units at this point. So Rec A is a protein bind to the to the DNA, and what we wanted to see here is kinetics of binding and unbinding.

Sanjeeva Srivastava: Right.

Cynthia: so we start with the substrate that has only streptavidin, and when you introduce Rec A to that sample cell, nothing happens. Rec A does not bind. So at this point here we introduce a biotinylated Oligo.

Sanjeeva Srivastava: Right.

Cynthia: And so that binds to streptavidin, and you get a little blip on signal. When you introduce Rec A at this point you get big (())(13:45) signal, and then we want to unbind Rec A so we introduce just the buffer to your flush out the Rec A, and the signal goes down, that is

because interaction between Rec A and DNA is a lot weaker than antibody, antigen drug. And then you can repeat the experiment.

So you can actually analyse these curves to measure the binding and unbinding kinetics of Rec A and DNA.

Sanjeeva Srivastava: So this is similar to what we were talking about in SPR, like in terms of on rate and off rate.

Cynthia: That is right. So this is an on rate and off rate of Rec A on to DNA. Now both in here and in SPR, we put on the correct model in order to extract the correct kinetic.

Sanjeeva Srivastava: I think software play a role over there, how you best fit the model, and extract the (data).

Cynthia: Yes, I know SPR instruments come with associated software. now if we are actually trying to study specific system it may or may not be the right model for your system and in that case you can actually write down the equations and do your own fitting of the data. So that is the real extract of kinetic quantitative.

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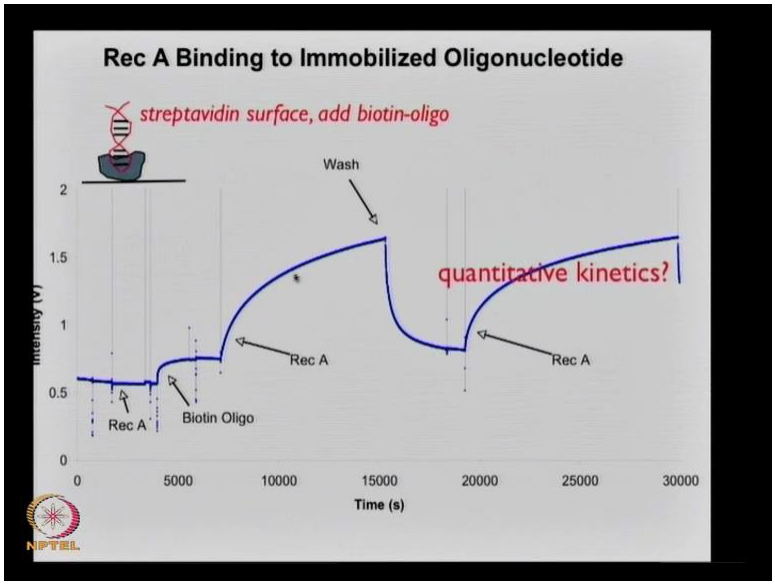

So in this case as long as reasonably at the certain range of concentration, the intensity is linear with concentration, and therefore you can actually model the kinetics nicely.

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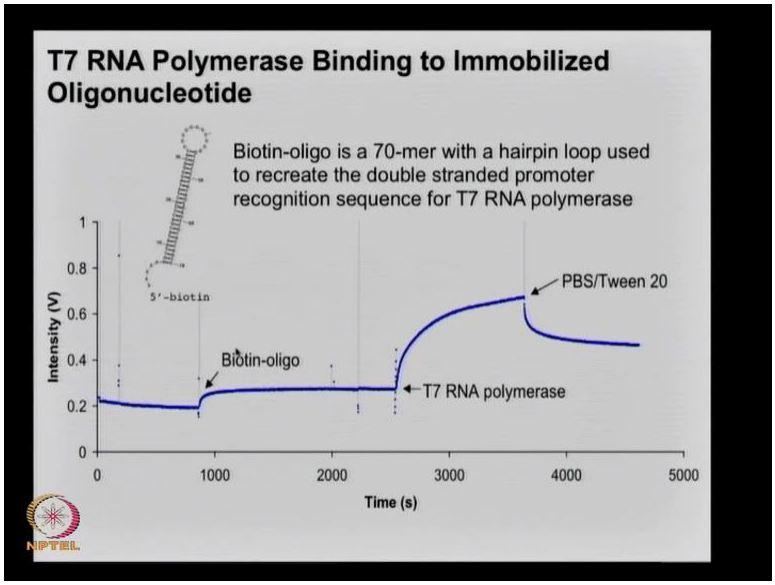

and this is similar experiment, and this is on the binding of Polymerase RNA Polymerase to Immobilized Oligo.

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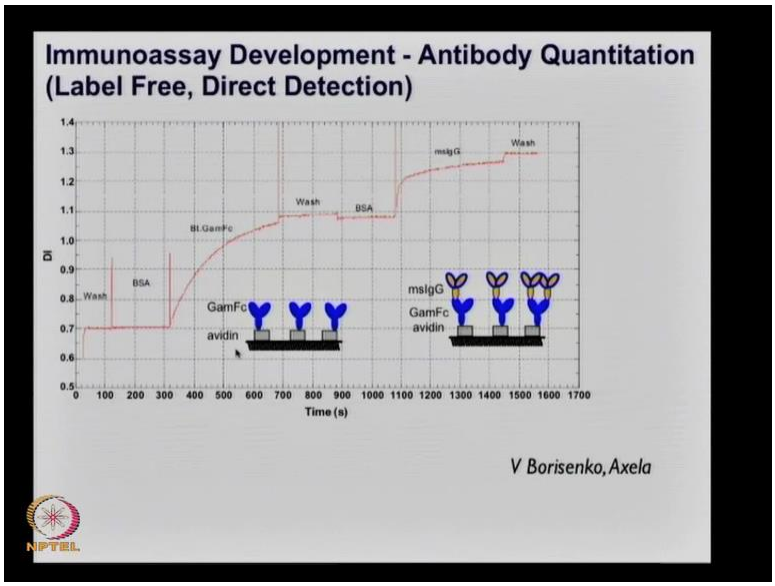

Sanjeeva Srivastava: Shown the different kind of level of applications, not only strong antibody, and protein, but also protein protein or DNA protein or polymers or cells.

Cynthia: Yes. Cells are actually very easy because cells are big, right. Yes. In fact challenge with cells is that we have to redesign the lines because lines are originally about 1 point 5 microns but you know cells are bigger than that, and so we redo the whole thing much bigger line. But it is the same principle, and it is actually very simple to detect cells.

Cynthia: So this example is actually antibody amino acid, so you can place them on that. What you have on this. Yes, this is just antibody quantitation, and showing us slightly different surfaces evident with Gamfc. It is not different from the other one.

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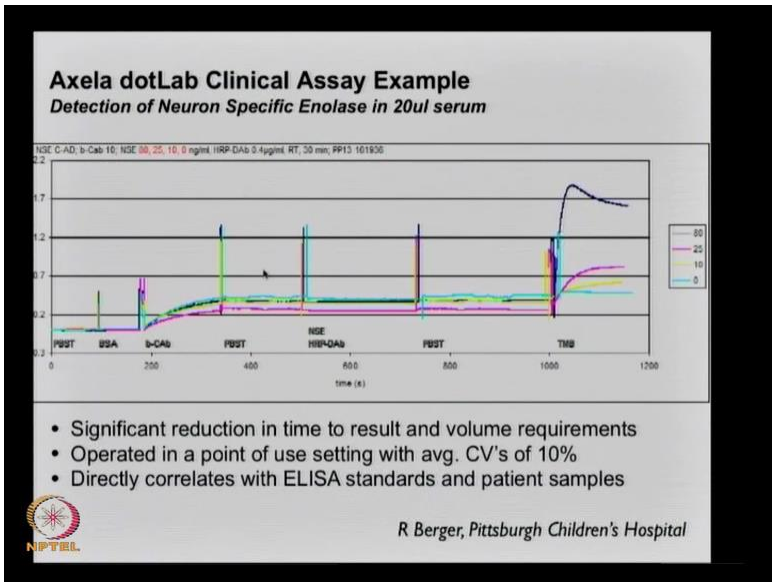

Here maybe some more interesting example, and this one is the early clinical example using now the instrument. Now the fact that this is the instrument that somebody else can use, that means people can actually configure their Assay and play around with how you are going to get to work best for measuring whatever you want to get measure.

So this is the detection of Neuron Specific Enolase which is basically associated with traumatic brain injury. And in this (case)...

Sanjeeva Srivastava: Is this any kind of marker which indicates the traumatic injury.

Cynthia: That is correct, yes. I am not an expert on the subject but Dr. Berger was using this primarily to look at markers in babies. Babies have shaken baby syndrome. They actually have

issues whether babies have shaken, and in this case being able to use only 20 microliters is very important because you cannot get too much blood from babies.

Sanjeeva Srivastava: Right.

Cynthia: Here are some examples where they it is effectively the same as we have been discussing before. We put down protein that identify another protein, and should (())(17:35) works. As a physical (())(17:36) if it works for one, it has work for another. It is a question of how strong the signal is which is dependent on how big the sample is.

Sanjeeva Srivastava: But the challenge is in terms of the level of the (protein).

Cynthia: Yes, what is so significant left? In designing bio diagnostic instruments one has to always ask if I am getting for detect disease X then what is the relevant concentration to detect.

Sanjeeva Srivastava: Whether I am able to capture the dynamic range.

Cynthia: That is right or whether it is even important to have dynamic range. Okay, so if you are actually looking for a marker that you know is present in micromolar quantity, that is not a challenge, you can use anything that you have. But also then there is no sense building an instrument that can go down to Nanomolar if all you are detecting is micromolar.

Now for many if you are primarily interested in terms of kinetics then you really cannot run kinetics at low concentration, and so you don’t have to do an instrument that cannot do nanomolar if you are trying to actually do kinetic but usually you run that at micromolar.

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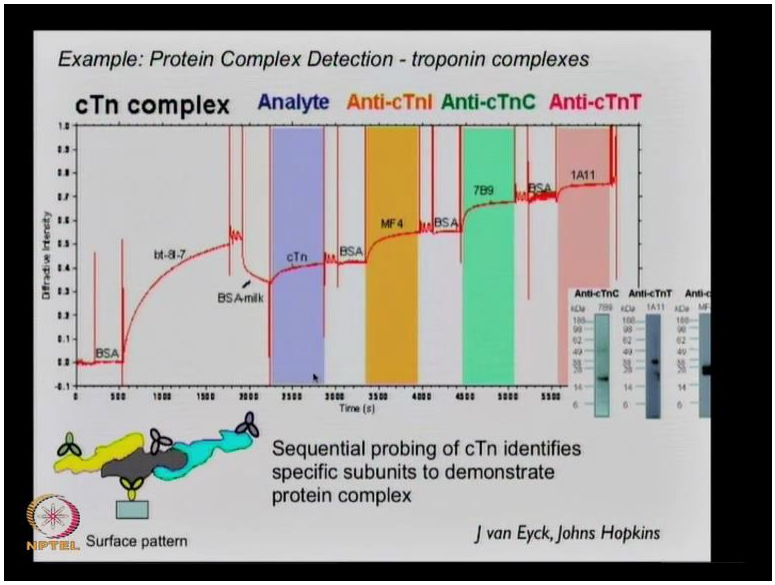

Cynthia: So here is an example of troponin detection. Interesting thing here is able to detect a complex system so in this (case), and this is the work of Professor John van Eyck, John Hopkins. Again you see the instrumental ready the commercial instrument. In the surface you put down the antibody, anti-troponin. in this case troponin has a complex that has three parts, and you can identify whether all three parts are present or mother of three parts are present. Putting now antibody for each of the parts of Troponin measuring its part. So it is almost multiplex assay except for it is all on the same protein.

And at each point here is the introduction of another thing. Introduction of Troponin or introduction of all antibodies to the different parts. The way they configured instrument is actually in order to introduce different solution. There is a little gas bubble, and so this gas bubble marks where you introduce it. It is a time 0, so if you are doing kinetic measurement, you need to know exactly where time 0 is when things are introduced.

Sanjeeva Srivastava: I think in this case they know the biology of it well, so probably measuring different components is easy, because they were able to generate Anti CTNL, Anti TNC, Anti TNT fragments, and then specific antibodies for it. I think you need measurement where you actually measure the complex protein but individual components for their specification.

Cynthia: Yes, and so you are being able to do, and that could configure assay that you do these placements, and take them away. Right, and this can be done in 5000 seconds, but it does not have to be even 5000 seconds. If you are not doing kinetics you can cut this thing shorter. It does not have to be that long. Comparable technique is to actually is western blood, and you know how long western blood stay.

Sanjeeva Srivastava: Whole day.

Cynthia: Lot of work, and lot of material. So that is actually very nice way you can experiment faster.

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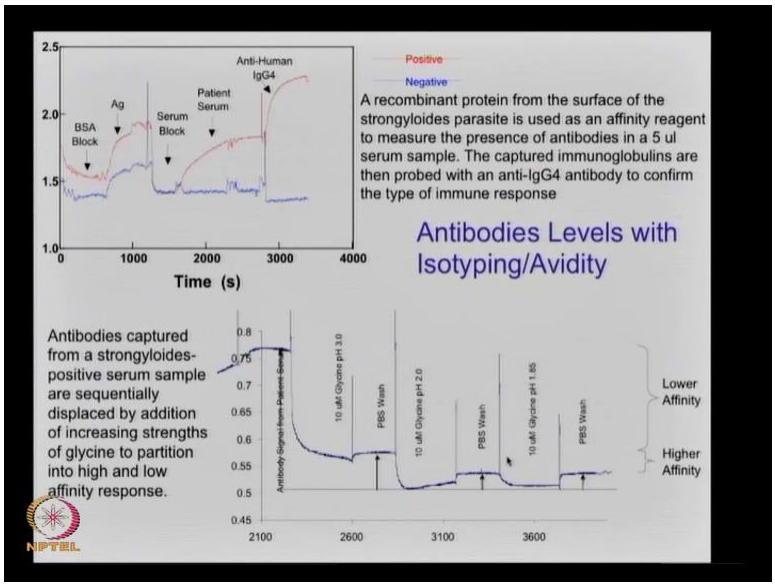

Cynthia: know there is various application that different groups of study, then this is about detection in PSA. Some interesting work was done on antibodies for Isotyping Avidity experiments. Once the instrument is commercial, I even do not know what these things are any more.

Sanjeeva Srivastava: These are the measuring interactions, different biomolecules, and one can use the same principle.

Cynthia: Yes, but you can use the same instrument, now you can configure your assay to get the number you want. Because for example, I told you that um we work best with large molecules.

Well how do you start doing small molecules. You have to be more clever to do displacement assay. It is label free but you still have to do displacement to compare the strengths of interaction. In this case the location of Isotyping Avidity, it is about displacement.

Sanjeeva Srivastava: This is seen for all technologies, right.

Cynthia: That is right, but you see I personally could not have done all this wonderful application. So again I go back to get this to benefit society by sharing your science. It is not just about publishing, but creating a device which that facilitates other studies.

Sanjeeva Srivastava: We can give rise to multiple applications.

Cynthia: That is right, and hopefully get it to be most useful quickly. If you have a diagnostics that are useful quickly that is great. Let me just show you how we actually did multiplex (())(22:28), it said there are 8 parts in there.

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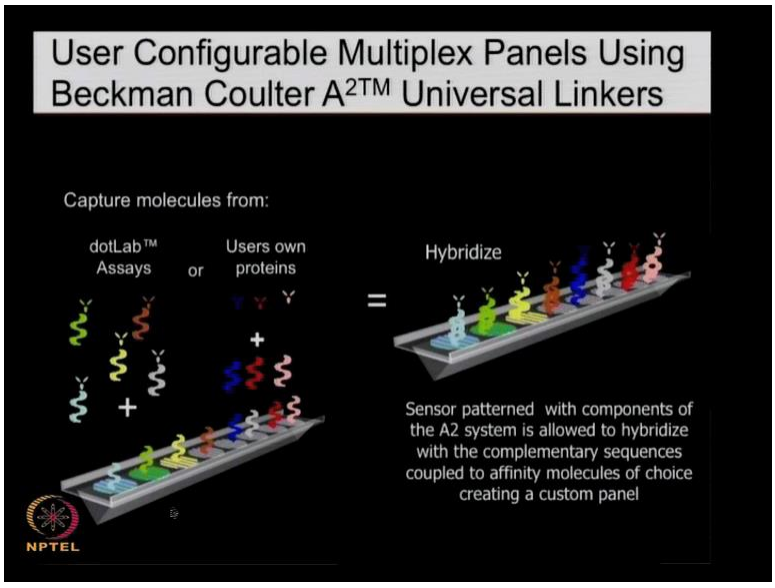

Cynthia: Put 8 different proteins on a piece of glass slide in a pattern is actually non trivial. And you know you can do it physically doing them one by one but that becomes very expensive prone to errors. So 1 approach which we have actually done in collaboration with (())(22:51) the company is to use what is called the Beckman Coulter, A squared linkers which are short

snippets of DNA that they actually have created in order to bind to proteins, but distant enough, so that each snippet is at one location.

So for example, when I bind this dot Blue, single stranded Oligo to the protein, it will specifically bind to this side.

Sanjeeva Srivastava: Right.

Cynthia: So it will hybridise specific location. So it will make a life easier. You have to act your protein with this DNA, that chemistry is known. Then you just inject them all, and it goes to the right location.

Sanjeeva Srivastava: I think you can achieve more interactions simultaneously.

Cynthia: You can measure 8 different things at the same time that is the whole idea. So the instrument works hopefully other people will start developing more. Right now main people using it in the diagnostics area trying to actually develop ways of detecting certain illnesses but it can be used for research to actually look at the binding kinetics.

Sanjeeva Srivastava: Since you have developed this device, so where next.

Cynthia: Yeah, well of course the dream is point of care diagnostics basically to be able to get yourself diagnose without having lots and lots of blood extracted, sent to the some clinic, sent to some laboratory. (())(24:37) diagnostics into doctor's office you can know quickly, that is a dream. There are a lot of issues in it, it is not scientific. It is really a question of business and policy and so on. So that is a go for the Company.

Sanjeeva Srivastava: In terms of detection systems obviously you are looking at defection based system, or optical based system when different types of platforms are available. What will be your prospective, where the field is progressing and what do you think, is there any specific way where one can just select a platform, and move forward on it, or depending on our kind of application one has (to).

Cynthia: No, I do not think there is one answer to everything because different devices have different strengths. Okay for example, what is the weakness for something like defective optic. I remember I told you there has to be repeats, so therefore we cannot detect one single molecule.

Okay, so what is the best sensitivity you can achieve, I don't know the answer to that, but it certainly multiples of molecules. So if you are trying to go very very low numbers that is not a way to go.

so it works best, probably its main advantage is in detection of multiplexing particularly things that needs a wide dynamic range. So for example if we are looking for cardiac marker, some are present are Nanogram, some are present are Micrograms, it is very high to find that technique that will span a very wide dynamic range. but there are other advantages of different techniques, and one has to consider is also the ease of use, the cost, the time needed, etc.

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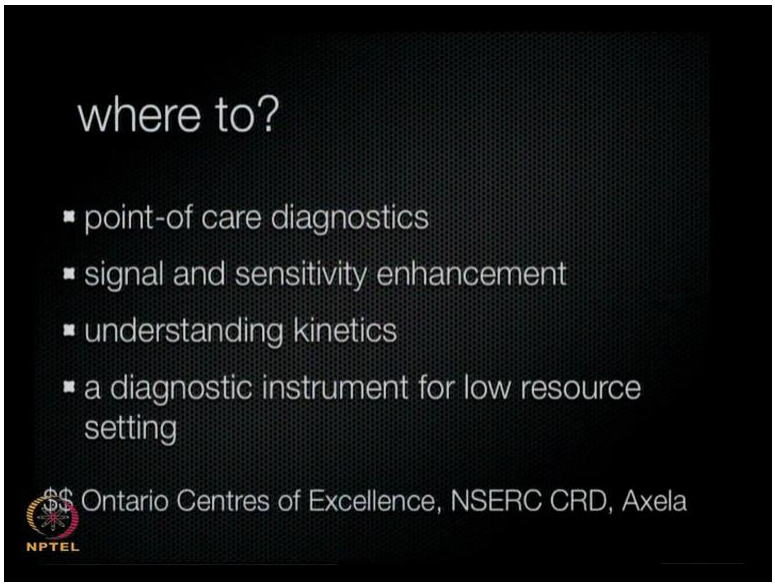

Cynthia: So sometimes we scientists tend to concentrate on signal and sensitivity. We want to have better signal, higher sensitivity, we want one more accurate diagnostic, but then again how accurate you just need it. Basically, usually when you have 90% chance of disease probably you get treatment for the disease. But from the basic science prospective we would like to understand kinetic better. You are modest software where you can press the button and out pops the answer, and sometimes you have to ask well is that what is really happening because once you start to deal with confine media very small volumes and you start having other issues especially if other things have solution you have to take into account.

Sanjeeva Srivastava: Do you foresee your diffraction based in terms of integrating it with more nano elements, nano material or with the Plasmons or integrating different components for having better applications.

Cynthia: Well that is one way of improving sensitivity. So right now we built this to be as cheap, as simple as possible. Three elements are light source, sample cell and a detector. Rest are the things that you can get removed, but it is not there nice for research. if you want to increase the signal you can put in Plasmon Surface.

Actually there are two approaches that I am working with colleagues. One is using plasmonics by having gold either on the surface or particles or on the lines, and other one is to actually do elements that are vertical to actually have confined what is called block surface waves. Again they will make the device more expensive because it is more complex, and probably because it is more complex it is less robust, but if you get two others more sensitivity then you have a chance of looking early stage cancer. So there are pluses and minuses.

Sanjeeva Srivastava: I think application is to look very very low abundant molecule at very early stage. Thank You Very Much Cynthia. I think as you have learnt from her lecture the science part of it how one has to think about simple physics principle and then build devices from it which can be applicable for various type of application. So I like to thank again Cynthia for sharing your work with us, and giving us a very good insight about different type of detection system including the diffraction basis, and how one can play with different type of molecules there, and have label free or label based detection for better sensitivity. Thank You Very Much.

Cynthia: Thank You.

Sanjeeva Srivastava: So in these two lectures from Cynthia Goh we learnt about the principle of diffractive optic technology, the feature, and advantages of this approach, and application of this approach in various types of diagnostics and other biological applications. Thank You.