LECTURE-39

DETECTION SYSTEM: DIFFRACTION-BASED BIOSENSORS

TRANSCRIPT

Welcome to the proteomics course. Today I have a guest with me Prof. Cynthia Goh. She is a professor in Department of Chemistry at the University of Toronto. She is also director of optical sciences in University of Toronto. We have been discussing about label-free methods to measure biomolecular interactions especially protein-protein interactions and we have discussed different type of methods which are currently being used including SPR-based optical sensors. Today with Dr. Cythia Goh we will discuss about diffraction based biosensors which her lab is actively working on and during discussion she will also show some examples of how these type of diffraction-based biosensors can be used for measurement of protein-protein interactions and it can also be applied for different type of diagnosis and point of care diagnostics. With that, I would like to welcome Prof. Cynthia Goh and we will talk on the how to measure protein interactions. Welcome Cynthia.

A- Prof. Sanjeeva Srivastava B- Prof. Cynthia Goh

B- Thank you. It's pleasure to be here. Biosensing is about the measurement of interaction between two biomolecules and I will be discussing an unusual approach which is actually surprising that it has not been used before but it was invented in my lab which is to examine to interactions between two sets of the molecules using the principles of diffraction. Let me just take you back to what you may remember from your basic physics course or perhaps you have met the diffraction in the context of X-ray diffraction on crystals. If you look at the right hand side where I have two slits if you have been watching waves of water passing through two slits, you see there is diffraction. There is little wavelets, that are formed and resolves interference pattern that has light and dark spots. So in the middle part of the slide we see a beam of light passing through diffraction grating and it shows the main beam in the lot of little beams

that are generated and the pattern of this diffraction or the image of the diffraction depends on the pattern of the grating. So let me show you how we can use the principle of diffraction to measure interaction between molecules.

So let me take a piece of glass slide here and put a coating, just one molecular layer thick of a coating that is a pattern so this is the grating lines made up of biomolecules that are spaced approximately a micron or micron and half and such that when light is shined through the grating which is very faint there is little bit diffraction, not much. You can barely see it in the cartoon drawing. However if binding were to take place so that this molecule has now a complementary binding partner so you can imagine the yellow one is protein-1 and the green one is protein-2, what you see is that the grating becomes more pronounced and so when light shines through the grating you are going to get much brighter spot so again let's just do this in different representation. I will take you to different slide where…

A- Cynthia, this means you are actually measuring how much material is there and to begin with small material 1 nm size and then we add more material to it and there is change in the diffraction, that is being measured. Right?

B- That is correct. So effectively we have a surface like piece of a glass, light will go through and when there is just imagine writing with your pencil lines on the piece of glass if you shine light, the light will begin to get diffracted except the lines which we were writing is one molecule thick which is one protein layer thick and so the diffraction is very faint. So let me illustrate that in this little cartoon so sensor surface is a piece of glass, it has lines as you can see and when I shine light through it there is going to be a very faint signal. Suppose I introduce molecules that bind right on the top of line the signal gets darker and its represented in the right hand side here by the intensity of the light so if I have a detector in one location you can see the signal increases with time as binding takes place. Now I was to introduce a second molecule that binds to the first one, again the signal gets darker so my detector has an intensity increase with time.

A- So good thing is that multiplexing is possible that way.

B- We can talk about multiplexing in different one. This one is basically about one molecule is binding to another molecule, you can also bind second one so you can imagine an antigen and an antibody and a secondary antibody. If you want to measure relative strength of interaction you can imagine whether you can displace the antibody with another thing so here is something coming in, another molecule and if the binding is stronger it may actually detach the previous one and that will be indicated by a change in signal in this case a decrease in signal.

A- So I think very similar to what we have talked in the previous class the SPR methods. I think same way we have a baseline here and we are measuring time versus intensity on X and Y axis and then we will have an ON rate and OFF rate depending upon the interaction, how it is weak or strong so one can compute the values for measuring the ON rate and OFF rate and kinetics of it.

B- That is right. So it is very similar to surface Plasmon and in fact lot of the principles are similar it depends on the index of refraction, the main difference here is that in surface plasmon resonance you only looking at the main beam. In this case because we are putting things in the pattern then you are going to have a diffracted beam and we are looking specifically at the diffracted beam and there are advantages of doing that. So why would be actually want to measure this way instead of directly through the surface Plasmon?

Well, you can actually imagine in one area if you are doing surface Plasmon you can only put one molecule in that area. Is that correct?

A- Yes. Right. There are also some newer methods where people are trying to have four-plex or six-plex.

B- That is correct. Now you have different areas you can put things in different areas. So in the case of diffraction you notice that there is one to one correspondence between what your grating look like and what your diffraction image looks like. So even if I have a grating facing this way or another way I will have this grating will have dots in

this direction and this grating will have dots in this direction. You can identify whether molecules A is binding to this one or to this one. So you can multiplex very easily. That is one advantage. From the technical perspective we can actually choose that kind of pattern to enhance the signal therefore create a better sensor in many cases. But if you are trying to look into the diagnostic area in the future one of the advantages of getting diffraction pattern is that if molecules B is not binding but just drops somewhere accidently, it is called non-specific binding. In surface Plasmon you will measure that because it attaches to the surface. In a diffraction experiment, if you don't drop in a linear or a grating pattern then you would not get a signal.

A- So it means you are able to increase specificity here in much more controlled manner as compared to what one can do in SPR or other methods.

B- Yes. You can reduce what's called false positives where you get a signal that is not really meant to be a signal.

A- Sure. I think that is big advantage when you are talking about diagnostics and looking at very specific signal and getting a false signal may be problematic.

B- For diagnostics that is very important. But even for experiments in your lab you don't want a big error bars because some proteins just like falling out of your solution. So let me show you implementation wise how simple this can be. As I said this technique was invented in my lab and here is the example of substrate where we have patterned biomolecules, this protein, on the piece of glass. It's submonolayer coverage it's very sparse amount of protein in there and it's imperfect. I can see it's not even at all but it does not matter because diffraction is defect tolerant.

A- What do you mean by defect tolerant because if there is some defect, are we going to have different type of diffraction pattern or that can be compensated because of the nature of the diffraction.

B- Well the nature of the light is such that the light will pick out the repeats of the order of its wavelength or higher so if you look at this picture this is an atomic force

microscope image so little dots in there are proteins and you can see that there are area where are sparse coverage and the areas where there are more of them and you can see lot of clumps but as far as light is concerned it does not matter because these clumps are non-repeating if they are not periodic it does not show up as a signal and so it ignores all this, this is probably a protein or may be this is the piece of junk and it ignores that completely.

A- OK. So I think some error can be tolerated in that way.

B- That is right. From the point of view building a device or building an instrument it does not have to cost much because you don't have to make things perfect, making things perfect is very expensive.

A- Yeah still but at the end we can get the perfect signal and I think that is what matters.

B- So let me just show you different regions we have here a grating made up of individual protein molecules and then binding takes place on it, more binding takes place and what happens is here is the surface before binding and coverage is not very strong after binding the coverage is strong and you get much better intensity of the signal. So you can quantify that here is implementation how simple it can be in our lab. It has three components- light source which is in this case a laser pointer, it's a 3 mW red laser and the detector here is this is cmos detector it could be a webcam it could be a photodiode which is very inexpensive piece and here is the sample cell and let me enhance that.

A- It seems you have a prototype earlier to begin with.

B- Well this is how we build it in the lab. Right. Because you know you take pieces and put them together this is where actual interaction takes place. Let me just do that schematically at the bottom here I took pieces of glass slide separated by a double sided tape and that makes a channel about 50 microns that's the thickness of the tape. And on one of the glass slide you put down pattern of the protein here so that you can

flow your analyte, your medium with the analyte in between and on the other side we put the prism it helps to guide the light- that is called total internal reflection so the light does not go all the way through it just scans the surface. And naturally detects the binding on the upper substrate so it is very simple.

A- You have a prism then you a matching fluid for diffractive index correction then you have got the slides which contain the protein and then with the light beam you are initiating the diffraction.

B- That's right and with this simple assembly you can actually measure down to nano grams/ml label-free. So these are the components in this setup are simple mirrors to make it more compact.

A- That is very neat concept and I think one can actually build various applications oon it.

B- That's right. As I said in our first implementation its diode laser or laser pointer and a webcam and these are the pictures captured from the webcam as you monitor the change in the intensity on one spot of the diffraction image upon addition of analyte so after few minutes it gets darker and it gets even darker there. The role of the prism is to make sure that light beam does not actually get captured by whatever is in solution so here is the picture of what diffraction spot would be if there was no prism if it's not total internal reflection. With total internal reflection we get it a lot cleaner that means you can actually use a fluid like blood or something that is equivalently murky.

A- I think many times the intension is to look for some biomarker or some diagnostics so measuring blood or serum becomes very important. Actually measuring that is very challenging because of the issues.

B- But even if you are doing an experiment like if you are using cell lysate for example and it has lot of particles in it to scatter light.

A- Yes. Actually we have lot of complex samples; it's not always the clean purified protein.

B- So in this case you don't have to purify your protein sample before you actually do the experiment. We talk earlier about multiple analytes so in this case it can have protein-1 in one direction and the protein-2 as the other grating and you end up with two grating patterns and here is the webcam image what would it look like this path here would be due to protein-1 whereas this path perpendicular will be due to protein-2 and so if you introduce your medium, if this path lights up you know there is binding to protein-1 and if this path lights up there is binding to protein-2. So you can examine multiple analytes that way. So to show specificity here is our example now, we have names of the analytes this is mouse IgG as one analyte, the other one is rabbit IgG so when we introduce anti-mouse IgG you can see increase in signal in one of the spots but not in the other spot, the red versus the blue. Then we introduce anti-rabbit IgG in which case second spot increase but the first one remains constant.

A- Actually one need to see the specificity of the assay and I think to test it out you have probably immobilized different type of proteins including one from rabbit and one from mouse and now you are looking at how specific the signals are then only anti-mouse is binding on the feature where we have the mouse IgG and the one where we have anti-rabbit IgG it's only binding with the rabbit IgG.

B- That's right. This is showing with two so now you can actually imagine and generalizing it with more and it is only a question of how many you want to pattern into the little substrate that you have.

A- Important point here is you are able to measure the signal simultaneously for all the features, even you can compare those visibly while experiment is going on so it just gives it a bit more room for even more arrays, one can try to change the concentration of antibodies and analytes and one can have different room. I think that is one of the other major advantages of having the label-free system where user can have the visible feel of the experiment as it is progressing.

B- So label-free detection how would you actually do it best if you are trying to measure kinetics for example because you have the actual signal, not adulterated by a secondary reaction but in this case having multiple analytes is actually very good in building controls because you can imagine one of your spot is always a control and in fact we do it routinely in my lab, when we are doing measurements.

A- I think controls are very important.

B- So to just to summarize features advantages- we actually talked about being able to detect more than one at a time simultaneously because the question is you know why would you want that and how many is a good number and that really depends on what project you are engaged in.

A- But usually based on your experience in the field for diagnostic purpose what do you feel like what will the good number be in terms of how many to measure simultaneously.

B- I think it's the question of cost now. The more things you put down the more expensive it becomes so in any disease that you identify how many markers do you want or if you want multiple diseases how many of them are likely to occur at same time. So I would say it should be less than 10 because chances are you are not going to be sick with 10 or more than 10 different things. In the complex illness like cardio-vascular diseases probably there are 4-5 relevant markers that one would like to detect.

A- So one has to actually take a call like what they are actually trying to measure and I think having as good marker as always good but having too many is also not good because controlling them and actually keeping them functional for long time again all the cost for the measurement and everything comes into the picture.

B- Yes so I would say somewhere between 4 and a dozen is probably a typical number.

A- Sure.

B- The approach is also quantitative, because of course the intensity of the signal is proportional to the amount of material that has to be spun down and you have to run calibration curves to get the standards going. As we have mentioned earlier, there are a little false positives because if things don't fall down on a grating then it just won't be measured, and the information is real-time, which is characteristic of all label free techniques, the measurement of the actual interaction is real-time and therefore you can extract from it kinetic information and binding information. In our case, the sample volume that is needed is really small. It is really all depends on how good the sample cell is. Using double-sided tape we can get it down to 20 µL; that is like a small droplet.

A- Right, that is very important, right if you are talking about clinical samples and measuring things in a clinical setting, I think it is very important how low we can go.

B- But even in your experiments in the lab, proteins are very expensive, so the smaller it is, the more experiments you can do for cheap.

A- Right, it is better to do in the small volume, what is possible.

B- Now, the sensitivity, people ask me how sensitive can this get. Well, if you notice, its all about measuring the grating. So the more pronounced your grating, the bigger your signal. And if you want to work with low concentrations, it depends on how big your molecules are. The bigger the molecules, the better your signal will be. But also, the stronger the binding, the better your signal will be at low concentrations. So there is no direct answer to that, but in some sense it would be comparable to SPR, because it based on a similar principle, which is that there is an index of refraction change at that interphase. It is label-free right now, but if you want to get better sensitivity, you can also add labels, so you can start with protein 1 or an antigen, and you can put in an antibody and that should bind. If that signal is too weak, you can put in a secondary antibody.

A- That is very important, because if you cannot detect the signal at a very low level, then obviously, you have to have some mechanism to bring the signal up.

B- That is right, you, can amplify. Perhaps, the other way of doing it too is that the signal may be low, you need an instrument, a good photodye to that, but sometimes you want to amplify it so its visible to the eye and then you can add your secondary.

A- Right.

B- Let me just show you an example. Here is an assay with the results from the experiment, this setup I showed you earlier, that little prototype. So basically, looking at the intensity of light as a function of time, as we introduce anti-digoxin to bind to digoxin which has been immobilized on the glass surface. So you can see that when the analyte, the anti-digoxin is 200 ug/ml, you get the uppermost curve. As you decrease the analyte concentration, you get the lower and lower curves till you are down to 200 ng/ml and here on the right hand side we blow it up so you can see, 200 ng/ml, still pretty reasonable, I can believe that is signal above noise. So we can go from 200 ug/ml to 200 ng/ml label free in 20 ul of solution in this experiment.

A- Right, I think that is quite good, but we can tweak it around to increase the signal.

B- This is still label free. It is still in the breadboard setup I showed you earlier so not optimized in any way. In fact, some of these experiments we had done earlier just with a webcam with the manual capture of the intensity. So, now there are many ways of pushing it higher, but one way of doing so is by putting in a label. I know this is a label-free course, but you can see what a label would do to you.

A- Broadly we are discussing about different types of detection systems so it should fit in that.

B- Remember you have 200 ng/ml which was in the previous slide, but now I am going to introduce a secondary antibody, here, labeled with a little piece of gold. I found out later we did not need that gold label. But anyway, in this graph, it had a gold label in there and we can bring it down from 200 ng/ml to 2 ng/ml. This is now the noise of the detector. In order to go better than that we have to have a little bit more of signal averaging and we can do a different type of labeling, this is a precipitation assay so

here is the initial antigen and then you put in the antibody, digoxin and then you put in the secondary which has horse radish peroxidase(HRP) in it. The HRP then acts on a substrate, TMB to form a precipitate. If you do that, you can go down to 50 pg/ml.

A- So you achieve much higher sensitivity

B- So 2 ng/ml here, which is huge and then you go down to 50 pg/ml. I think one can even tweak it further but now you are starting to fight kinetics because if you are very low, of course this binding is taking a very long period because it takes a little while for them to find each other. In order to go better than that you have to do a little bit of signal averaging.

A- This could be useful when talking about very weak protein protein interactions for different types of analytes which are very low in abundance.

B- That becomes an issue of assay development. What you have here is a tool, which allows you to measure signal. You can configure your assay to introduce—in this case you are just introducing them linearly, but in some cases you can actually premix a cocktail and let it bind together and that may sometimes work better. So its not just protein we can actually look for. This are some data on troponin, this is still a protein antibody assay. The clinically relevant level is about here, above this line, above 2 and you can see this is the signal. This is indicative of the noise of the system that is why you can see wiggles in that signal, but the interesting thing here is that from the point of view of clinical diagnostics, that is 10 minutes here. So in less than 5 mins, you have a difference between higher levels, clinically relevant levels and clinically, absence of troponin.

A- In a very short time, you are able to measure the signal with high intensity.

B- That is right. Imagine this is a marker for cardiovascular disease, a marker for stroke, you can know in a few minutes whether it is there or not. Here is an example of how you can assay for a couple of antibodies for TB. Here is looking at the 16 kDa—in this case we are going to show you an example of looking at 2 antibodies- markers for

Ag-Ab binding for TB. Again it is looking at 2 different spots. Again, introduction of antibody to the 16 kDa TB Ag, again you start with the experiment here.

A- How do you correct for the baseline here because it is beginning from 800 or something..

B- There is a baseline here because there is a diffraction pattern. We've put down the 16 Kda Ag on the substrate, so when you shine light on it there is a baseline. And to actually reduce non-specific binding we put in a BSA block so basically, your medium will not have all the proteins sticking to the lines. So you introduce a BSA block.

A- So first you do the blocking of the surface..

B- That is right, so the signal drops to 0 because BSA sticks in between the lines. At this point we introduce the 16 kDa antibody for TB that binds to the antigen and as you can see, there is a little blip in here. Let me expand this area so you can see the expansion here. That blip is actually real because signal to noise is good enough. But in case you want something that is much more obvious or something that you can see, because this point when you have the precipitation you can see the signal already. We introduced the HRP-goat anti mouse and the TMB substrate here and you can see the enhancement in signal and it is a very big intensity change. You can look at it, this is a 3000 % intensity change.

A- That is, I think, a clear yes or no answer right?

B- That is right. If you are trying to create a diagnostic, just yes or no, then here I can see and say yes or no. That is one of the spots. Here if you can see the second spot filled with the 38 kDa TB antigen, you can do the same experiment and we will put them together..ie the two spots together. At this point we can introduce the 16 kDa TB antibody, and one of the spots get more intense, the other did not. At this point, we can introduce the 38 kDa and the other spot got more intense. At this point we can amplify both spots now.

A- So the blue is 16 kDa and the other one, 38 kDA is the red one and it is able to measure both simultaneously, and if we see the signal of both, we know that the person is actually positive for that disease.

B- TB is a very hard thing to detect and if you can see 2 signs that it is there, and you can imagine adding, there are other potential markers for TB and we can add that, so you can get more and more sure that something is happening.

A- It is more proof of concept what you are showing but it can be applicable depending on the context. One can make the assay more robust with the introduction of the right type of proteins and more markers.

B- Now the other way to do it you can imagine making the second spot be a blank, that way you can be sure that the signal is, you know, in reference to a blank, and you know you don't have false positive in there.

We can skip the other known proteins here, but you can look at cells, you can look at polymers, because the measurement of interactions is general, you measure the interactions between 2 types molecules. Left one is interesting because it is cells that are over-expressing receptors for some cancer and you can build in other kinds of stuff in there, so we can skip on that one.

A- Ok, so now you you've got a prototype, and you can discuss how you got that prototype and actually took it at commercial scale so you have applications to develop.

B- I can show you what I did in my lab, but unless you want to be an expert in optics, you don't want to have to build one every time you do a measurement. I could have just kept cranking out data. But that is not the point, right, we want to be able to create an instrument that will be useful to other people and if you want to go for medical diagnostics, it is important that the instrument is functional and useful to others. This is the point of commercialization. So this is the point that around Jan 2002, I decided that we had to commercialize the device to get it to be useful by others and this is important thing that I believe in that for science to be able to benefit society it has to be turned into

a product that others can actually use. And so this is the path, so this is how it was in my lab and up till now this is the instrument we have in my lab, we still work on the benchtop, but what we did is we translated it into a series of steps so the current device that went out in the market, late 2006, early 2007 is an instrument that other people can use. And that little piece of double sided sticky tape, it is not good for you to make that yourself, its now a little piece of plastic that is a lot cheaper, took a lot for it to get there but is now a lot more efficient and cheaper. So here is the instrument, it is all computerized, there is a pumping system inside, there is software and it has the little sample cell that has 8 spots in it that you can put down different proteins or different snippets of DNA or whatever bio-molecules you are trying to assay.

A- So you are now providing a multiplexing capability..

B- Yes it has multiplexing. Instead of on being on top of each other it has 8 different spots. Effectively, in the instrument, the laser beam runs back and forth into this and there is a channel there- there is an insertion port, an injection port where you inject the analyte, and it goes through that little channel which is about 20 ul or less. But it has a pumping system so you can control the flow rate, which is important while doing kinetic studies. So here is an example on the study of binding of a protein to DNA. So in this case, this is the substrate, we have put down streptavidin in a pattern, basically, take that substrate and here is the initial signal from that substrate.

A- What is the Y axis?

B- This is time vs intensity (in arbitrary units). Rec A is a protein that binds to DNA. What we wanted to see here is the kinetics of binding and unbinding. So we start with a substrate that only has strepavadin and when you introduce Rec A to that sample cell, nothing happens, Rec A does not bind. At this point we introduce a biotinylated oligo and so that binds to streptavadin and you get a little blip. When you introduce Rec A at this point you get a big increase in signal. Then you want to unbind Rec A so you introduce the buffer to flush out the Rec A and the signal goes down. That is because the Rec A-DNA interaction is a lot weaker than Antigen-antibody interaction and you

can repeat the expt etc. You can analyze this curve to analyze the binding and unbinding of rec A to DNA.

A- This would be similar to what we were talking about in SPR, about on rate and off rate.

B- That is right, this is an on rate and off rate of Rec A on DNA. Now, a similar thing here and in SPR, one has to put together the correct model to extract the correct kinetics.

A- So I think the software play a role in how you best fit the model and analyze the data.

B- I know the SPR instruments come with associated software. Now, if you are trying to study a specific system, it may or may be the right model for your system. In this case, you can write down the equation and do your own fitting of the data. So that is in order to extract real quantitative kinetics. So in this case, as long as we are reasonably at a certain range of concentration, the intensity is linear with concentration and therefore you can model the kinetics nicely. This is a similar experiment and on the binding of RNA polymerase to the immobilized oligo.

A- It shows a kind of level of applications one can achieve. It is not only the strong antigen-antibody and protein-protein or DNA-protein or polymers or cells..

B- Cells, yeah. Cells are actually easy because cells are big. The challenge with cells is that we have to redesign the lines because, the lines originally we have are about 1.5 microns, but you know cells are bigger than that so we have to redo the whole thing with a much bigger lines.

A- So different settings have to be made.

B- But it is the same principle, but in fact it is very simple to detect cells. So this is just—this example is just antibody immunoassay so you can play games on that. So this

is just antibody quantitation showing just a slightly different surface, avidin with gamma Fc in there. Its not different from the other ones so I'll pass by it.

Here maybe is a more interesting example-- I think one of the early clinical examples using now the instrument. Now the fact that there is an instrument that somebody else can use, that means people can actually configure their assay and play around how you can get it to work best to measure whatever it is you want to measure. So this is the detection of neuron specific enolase, which is basically associated with traumatic brain injury.

A- So this is a marker that indicates the traumatic injury?

B- That is correct. I'm not an expert on the subject, but Dr. Berger was using this to look at markers in babies—ie in Shaken Baby Syndrome. There have been issues of whether the baby has been shaken and in this case, being able to use only 20 ul is very important because you cannot get too much blood from babies and so here is an example where they actually have—its effectively the same as we have been discussing before, we put down one protein that identifies another protein, and sure enough it works. As a physical chemist, I feel that if it works for one it should work for another. It is a question of how strong the signal is, which is dependent of how big the sample is.

A- But the challenge here will be in terms of the level of the protein in different patients?

B- That is correct, what is the significant level of the protein. In designing biodiagnostic instruments, one has to ask, if I am detecting for disease X, what is the relevant concentration to detect?

A- Right, whether I am able to capture the dynamic range

B- That is right. It is important to have dynamic range., If you are looking for a marker present in micromolar quantities, that is not a challenge, you can use anything you have. But there is no sense in building an instrument that can go down to

nanomolar if all you are detecting is micromolar. Now, if your interest is primarily in kinetics, you don't run kinetics at low concentrations. So you don't have to use an instrument that can do nanomolar if you are primarily wanting to do kinetics, usually, you run that at micromolar.

So here is an example of troponin detection. The interesting thing here is to be able to detect a complex system. This is the work of Professor Jenny van Eyck at Johns Hopkins, again using the ready commercial instrument. In the surface, you put down the antibody, the anti-troponin. In this case, troponin is a complex that has 3 parts, and you can identify whether all 3 parts are present or in what amount are the three parts present, by putting now an Ab for each of the parts of troponin and measuring the response. It is almost a multiplex assay except for its all on the same protein. And again, at each point here, is the introduction of another thing, the introduction of troponin and the introduction of all the abs to the different parts. The way we configured the instrument is that is in order to introduce a different solution, there is a gas bubble, and so those gas bubble marks where you introduce it. So at time zero, if you are doing kinetic measurement, you need to know exactly what time zero is, when things were introduced.

A- In this case, you know the biology of it well, so probably, measuring different components is easy because they will be able to generate anti-CTNL and the anti-TNC and anti-TNT fragments and specific antibodies for it. So it is a pretty neat experiment where you can actually measure the complex proteins and also the individual components with great specificity.

B- So being able to do that and configuring your assay because you can do displacements and take them away. And this can be done in how long…this is 5000 seconds. But it does not have to be 5000 seconds-- if you are not doing kinetics you can cut this thing shorter. The comparable technique is to do a western blot and you know how long western blots take.

A- The whole day..

B- And a lot of work and a lot of material. So it is actually a very nice way you can do experiments faster.

A- Sure

B- You know, there are various applications that different groups have studied about detection in PSA and some interesting work has been done for antibodies with isotyping and avidity experiments. One the instrument is commercial, I don't even know what these things are anymore.

A- Yes, it is measuring interactions of different molecules and one can use the same principle.

B- But you can use the same instrument but now you can configure your assay to get the same number you want, because for example, if I told you that we work best with large molecules. How do you start doing small molecules? Well you have to more clever at it you may have to do a displacement assay. It will still be label-free but you have to do a displacement to compare the strengths of interactions. In this case, to look at isotyping and avidity, it is about displacement.

A- It is the same for all technologies right?

B- That is right, But you see, I personally couldn't have done all these wonderful applications, so again I go back to, you know, the desire to benefit society by sharing your science. It is not about publishing, but about creating a device that facilitates others.

A- Which can give rise to multiple applications, depending on the user.

B- And hopefully, get it to be more useful quickly. You know, if you have diagnostics that are useful more quickly then, that is great.

Let me just show quickly how we did multiplex. I kind of glossed over, there are 8 spots in there. But to put 8 different proteins in glass slide, in a pattern, is actually non-trivial.

You can do it by physically doing them one by one, but that becomes very expensive and prone to errors. So one approach which we have done actually in collaboration with Axela, the company, is to use the Beckman Coulter A2TM linkers, which are short snippets of DNA that they actually have created in order to bind to proteins, but are distinct enough so that each snippet is on one location so for example, when I bind this dark blue single stranded oligo to the protein, it will specifically bind to this site, so it will hybridize to a specific location. So it makes it a lot easier. You have to react your protein to this DNA, that chemistry is known and this you just inject them all and they goes to the right locations.

A- I think you can achieve more interactions simultaneously.

B- You can measure 8 different things at the same time. The instrument works and hopefully other people will start developing more. Right now the main people using it are in the diagnostics area, trying to develop ways of detecting certain illnesses. It can be used for research to look at binding kinetics.

A- Since we have developed this device, one has to think where next?

B- Yes. The dream is point-of-care diagnostics for many. Basically to be able to actually get yourself diagnosed without having to have, you know without having lots and lots of vials of blood extracted, sent to the clinic, sent to you know, laboratory. If you have diagnostics in the doctor's office you can know quickly. That is a dream and there is a lot of issues in it, and it is not scientific. It is really a question of business and policy and so on. So that is goal for the company.

A- In terms of detection systems, obviously you are looking at a diffraction-based system and you are aware there are optical-based systems and there are different types of other platforms available. How do you foresee the kind of, based on the user requirement, how the field will progress, one has to rely only on type of principle and device based on it and combination of it and depending on the type of samples one can select these types of things. So since you have been in this area for a long time, what is

your perspective, where the field is progressing and what do you think is, like the specific way one can select a platform and move forward on it or depending on our kind of application one has to…

B- I don't think there is one answer to everything because different devices have different strengths. For example, what is the weakness for something like diffractive optics? Remember I told you, there has to be repeats, so therefore we cannot detect one single molecule. What is the best sensitivity you can achieve, well, I don't know the answer to that but its certainly the multiples of molecules. So if you are trying to go very, very low numbers, well, that is not a way to go. So it works best, probably its main advantage is in detection of multiplexing—that needs multiplexing, particularly the thing that needs a wide dynamic range. For example, if you are looking at cardiac markers some are present in ng, some are present in ug, it is very hard to find a technique which will span a very wide range of dynamic range. But there are other advantages of different techniques and one has to consider the ease of use, the cost, the time needed etc. So if you are trying to do an early detection of cancer you are willing to pay premium because you know, its very, very important, whereas, if you are trying to do a surveillance of malaria, you are not willing to pay premium because after all what are you going to do? So there are a lot of considerations, not just technology. I think sometimes when people talk about diagnostics, they really don't think about path-to-market and in fact different countries of the world, in fact, different towns within a country may have different diagnostics that may suit them because of the local situation.

A- I think one has actually customize the devices based on the need for the local market.

B- And the regulations locally, and well as the kinds of illnesses that people may be having to address. So sometimes we scientists tend to concentrate on signal and sensitivity. We want to have better signal, higher sensitivity, we of course want more accurate diagnostics, but then again, how accurate do you need it? Basically, when you have 90% chance of having a disease, probably, you get a treatment for the disease.

But from the basic science perspective, we would like to understand kinetics a bit better. There are models, software, when you can press a button and out pops an answer and sometimes you have to ask, is that really what is happening? Once you start to deal with confined media, very small volumes and you start having other issues, especially if there are other things in solution that you have to take into consideration.

A- Do you foresee your diffraction-based sensors in terms of integrating it with more nano elements, more nano materials, more plasmons or integrating different components for having better applications?

B- That is one way of improving sensitivity. Right now we built this to be as cheap and simple as possible. There are 3 elements, a light source, a sample cell and a detector. The rest are things you can remove, but they are nice for research. If you want to increase the signal, you can put in plasmonic surfaces. Actually there are 2 approaches I'm working on with colleagues, one is using plasmonics by having gold either on the surface or as particles or one the lines. The other is to do elements that are vertical, to have confined 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 can get 2 orders more sensitivity, then you have a chance of looking at early stage cancer. So there are all these pluses and minuses.

A- So I think the application is to look for the very, very low abundant molecules at an early stage.

B- That is what everybody seems to be after. My main interest is to study it, to actually reengineer everything for a low resource setting. How cheap can we get it so we can actually do dengue surveillance? You don't want to spend too much, there is a breakout happening in a remote village. I need to be able to do very low cost, 1000s of experiments quickly in a device that is robust enough to probably be powered by the sun. That is what I'm after.

A- Thank you very much Cynthia. So as you learned from her lecture, the science part of it, how one has to think about even a physics principle and then build devices from it which can be applicable for various types of applications and also, she is being an entrepreneur, she always has an insight of making the devices of the low cost which can have better implications for various types of markets. That is where, I think, during our entire discussion, you have been hearing, like not only in terms of making the devices or its applications, but also how it can be applicable for the different type of consumer, different type of people, different type of point-of-care detection devices. So, like to thank you again, Cynthia for sharing your work with us and giving a very good insight of different types of detection system, including the diffraction-based sensors and how one can play with the different types of molecules over there and have either a label-based or label-free system for better sensitivity. Thank you very much!