

What Can Nanotechnology Do for Biology?

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What I want to do in the next half hour or so is talk about nanotechnology for biology from my own perspective and that's a perspective that sees nanoscience and nanotechnology providing us tools. Enabling tools for the kinds of research projects that a wide variety of us are interested in. And eventually the kinds of applications that many of you are interested in. If you think about tools, it's kind of inspiring to think about how the introduction of a few simple, now simple tools, have changed the way we do our work. And you think about PCR. Think about various fluorescent proteins, microarrays, monoclonals. And imagine doing what you do without those tools. And that, to me, really captures the essence of what physical sciences can bring to the health sciences. Nano provides a whole set of tools and it cuts across much more than just the health sciences. As you heard from Richard in the introduction where he sketched out how the various federal agencies are involved in nano, you see that a lot of this work has been driven by electronics and trying to get to faster, smaller, cheaper, etc. computation. That's all still some ways off in the future. This is still very much at an early research stage. Nano provides materials having new properties. Properties we didn't have access to before, and there are a number of technologies and applications that are going to roll out from that. And some of that's happening and it's going to continue to happen. And then, in the applied biology side where I think we've seen the most important and the most rapid introduction of some of these technologies is in the area of sensors and other materials that are used for either scaffolds or for drug delivery. These are areas where things are happening today. The pace of this research and development is quite substantial and I would see over the next ten years a lot of new tools in the spirit of the previous slide, coming available for this, these broad fields. Okay. There we go. Just to give some sense for biosensors, I think what's driven the investment in biosensors and taking nanotechnologies in that direction is that they intersect many different activities. And this is put in the context of drug discovery, but certainly all of the basic research, which is done. One doesn't do an experiment without measuring something and better sensors and sensors capable of multi-analyte recognition, these are the sorts of things where the need remains high. And one understands that assays which can be applied in high throughput formats, performing thousands of assays a day are going to be, and remain, important in drug discovery, diagnostics, and then managing disease treatment are areas where sensing strategies are still needed. And then a whole associated area of consumer and environmental monitoring. What I'd like to do is give a little bit of context again before I go through some specific technical examples and I want to discuss "Why nano?" What's the deal? And why now? And to answer the "Why nano?" I think. The thing that's got a lot of us in the sciences excited is, as Professor Ratner said "When you take a material and you break a piece of it off,

you still have a material with the same properties.” If you took that material down to its atom components, clearly the atom is going to have very different properties and there’s that in-between range when you move from the properties of a bulk material to the properties of the atomic constituents where you get a whole interesting set of properties which can be tuned by the sizes and the shapes of those chunks of material. And so new physics emerge. It’s not just smaller, but new physics. That gives us ways of creating new transduction strategies for implementing sensors. You don’t have to use a fluorescent molecule. There are other strategies and I’ll show you some of those. And there are associated benefits in terms of the practical side of developing and using sensors. And the second big reason here, “why nano?” and you again heard this in the introductory lecture, it that biology is nano. That’s the size scale of which our behaviors and our activities are governed and if you want to study biological problems, you’ve got to have in one respect a pair of tweezers that can go in and hold on to those problems and that’s in the size range of tens of nanometers and that’s what this technology is providing. New ways of isolating, of controlling, of grabbing on to, and studying biomolecular assemblies. Now in terms of the new physics, this slide just captures a few of the ideas that are coming out, and some of these are being implemented. But what are these new properties you get? Well, if you have nanoparticles, which have some electron affinity, these particles cannot be conductive unless you move them within some certain distance of one another and then you can create conductive paths that can move electrons around. And if you think about it, if you had those particles coming together by way of biomolecular interactions, an analyte molecule will cause a particle to line up next to another particle, you can see how you can create sensors that might use nanoparticles as a transduction strategy. Now one reason that I think isn’t fully appreciated and why that’s so interesting is that a nanoparticle has a small surface area, which means that if you want to move a nanoparticle around and govern where it’s attached, you can do that with a very small number of analytes. And some of the sensors, and I’ll show you one, can measure the presence of ten molecules of a protein. And that’s because you are working on very, very small sensing areas. And that’s very different from a microscope slide or even a microchip array where the areas in which you sense are many tens of nanometers. So that’s one thing to keep your eye on. If you want to look at small numbers of **analytes**, this is one driver for this technology. One can do this with light too and you heard a little bit about **plasmons**, but it’s kind of neat. You can put light energy into a nanoparticle. That energy can hop from one particle to another, ultimately scatter and give off a photon of light at the end and you can create nano light pipes or nano wave-guides and these again, can be adapted to sensing strategies. This is an interesting one. Can you use mechanical forces to sense and to detect? And it’s looking like we will be able to. If you take a **resonator**, a diving board, and you tap it, it will vibrate at some frequency. If you make that diving board small, it vibrates at a quicker, a faster frequency, higher. And if you take it down to size scales of tens of nanometers, it vibrates at an incredibly high frequency. And at frequencies high enough that if you mass load

that sensor, attach a protein to it, there is a frequency shift and so one can actually measure small numbers of molecules binding to a mechanical element. Think about sensors that would operate on that principle. Okay. The second question is "Why now?" I mean, I'm humbled by Feynman's speech in 1960, which laid out some of this vision. Now it's 2005, 45 years later and we're talking about nanoscience and technology as if it's the new field. And my perspective on "Why now?" is that we've only recently, last 15 years or so, gotten our hands on tools that can be used to build nanostructures. So we can actually make these things, measure their properties and start to make them for application. And that's, in fact, then a big, big driver in this field and one of the areas that the NSF has had an important role in. Developing methods for manufacture, for assembling and fabricating and characterizing those structures. And these are various things, some of these I'll come back to. Now this is a slide that you have seen earlier and this is a slide that Dr. Ratner used to show that the properties of materials can be very dependent on, on the sizes, the shapes, the compositions. Why am I showing this? I don't know, Mark, Chad pays me 50 dollars each time I show his slide. I don't know what you get, but, no, I show this because it is a really striking, a very clean example of how you can tune property from the same kind of material. And you've heard that already. Okay. Let me take this concept and show you some exciting work that Rick Van Dyne has done at Northwestern University. Many of you, I think, are familiar with the **Biacore** instrument. This is an instrument that is based on surface plasmon resonance spectroscopy and lets one measure protein-protein association by tethering one protein to a gold film and measuring the association in a soluble protein with that. Turns out that you can do a type of SPR on a nanoparticle and these are particles, which are of order 20 nanometers in size. The physics is a little bit different, but it's related. And what Rick has developed are these nanoparticle sensors that have surface chemistries on them to create selective binding sites. And on binding of a protein, the optical properties surrounding that particle are changed and you can actually optically measure a shift in the resonance properties of the particle and see binding of something. Now I'm going to come back to the earlier point. Rick has shown that you can do this and monitor a single particle and you can measure the binding of order a dozen proteins to that particle. These are measurements that can be done on an open bench top with a device that costs ten thousand dollars at most. And that's really revolutionary. Compare that to a high-resolution transmission electron microscope, which would be the other way you would measure the presence of 12 proteins. It turns out that with nano, again, you get new properties. With conventional SPR or Biacore, one is looking at the binding of proteins that happen within about a hundred nanometer distance of the surface and that's because the fields, the k over these length scales. With the nanoparticle, one is measuring the binding of particles within five nanometers of a surface. So you get both a better sensitivity, but you also can get information on the orientation of bound proteins and the states of denaturation of bound

proteins. I won't go into all of what one does with that, but again the excitement is that by going small one does get cheaper, faster, etc., but one gets new capability. And that I think is what ultimately will drive a lot of additional work in this area. Okay, that's a bit of an introduction. Now, Richard my time has been completely disoriented since the start so don't be shy about letting me know when I'm 30 minutes past time, okay. Just kidding, Richard. Okay. So what I want to do is talk a little bit about an area that's been close to us and this has been learning how to develop model substrates, scaffolds that can be used to organize cells and study cells. And this is an area that has its roots in micron scale technologies and has quickly been moving to nanoscale technologies. And the notion here is that one can create model surfaces that are derivatized with proteins. We've got a particular interest in extracellular matrix proteins. One can create devices where these surface chemistries are present and then various kinds of plastics and molds can be applied on top to create wells, wells where assays can be done. And these have become very useful tools in cell biology, in drug discovery, in screening, and let me share a few examples of what can be done. This is a set of tools referred to as soft lithography and this has been developed in the Whiteside's Laboratory at Harvard. And what this really is alternate forms to lithography where one creates stamps. And those stamps have surface release on, relief on them. And they can be used to transfer material to a solid substrate, a planar substrate. And in this case, what you are seeing is that either proteins are transferred to a surface in a pattern or surface chemistries are transferred which are then subsequently immobilized with protein. And one can pattern here down to the submicron length scale, in special cases sub-hundred nanometer length scale. And the practical benefit is it's possible to organize cells and pattern cells out on a surface. Now that's proven quite useful. You can pull on cells in ways that they normally wouldn't experience. Here is a surface that was patterned into a square and it's stained for actin, so you see the cytoskeletal structures and you see the actin, in this case really concentrating around the perimeter. This is a co-culture, that was assembled using methods I won't fully get into, but using combinations of surface chemistries where one cell type is directed to attach to these circular features and a second cell type is then attached to the surrounding areas. These are exciting methods for creating co-cultures and learning how in the laboratory, and with scaffolds, to organize multiple cell types into models of tissue and then to understand the roles of cells in their more natural, in their in vivo environment when they are in fact organized with other unlike cell types. And there's been a lot of work that has gone on in the specific area of patterning neuronal cells. And a lot of these technologies can be applied in that vein as well. Okay. Here is my other 50 dollars I make for the day. This is, again, Chad Mirkin's dip pen nanolithography tool. And the point I want to make here is that this tool didn't exist ten years ago and now this tool is available as a commercial instrument. Not an inexpensive one, but certainly inexpensive by the alternate comparisons. That is, a lot cheaper than buying an electron microscope in order to do patterning at this scale. And, as Mark Ratner explained, here one uses a tip and uses that to apply an ink to a substrate. And

our work with Chad's group on this has developed inks so that we can pattern surfaces with chemistries that allow us to then go and decorate those chemistries with proteins and carbohydrates and other extracellular matrix components. And here are a couple of examples of pattern proteins. Here you see an array. The white features are in height, showing where the proteins have been immobilized. And this is an interesting example because we patterned out an array of fibronectin and the notion here is that when a cell attaches normally to a substrate and it assembles focal adhesions, these sites where the integrin receptors cluster and recruit the actin filaments and a number of cytoskeletal proteins, one can see those focal adhesions. Now, here is the point again about "bio is nano". How big is a focal adhesion? Well, we know that they're heterogeneous. Some are a couple of microns. Some are a couple of hundred nanometers. And to really understand how focal adhesions operate, we'd like to be able to control their size independently of how a cell attaches to a substrate. And with the nanopatterning tool we can now lay out patches of fibronectin and then allow a cell to attach to that patch and we can find situations where there is a critical island size that's necessary to recruit a stable focal adhesion. Now these here are kind of experiments that are interesting for discovery and again are fully enabled through these tools, these tools that come from the physical sciences. Let me take this a step further. As we think about creating scaffolds, and I am emphasizing scaffolds here because I know that in practice in the eye and in a lot of tissue, one worries a lot about cell adhesion, about cell extracellular matrix interaction, and about helping cells find their right locations. And so one recognizes when we pattern surfaces we are controlling where the extracellular matrix proteins are but we're not really controlling the properties of that extracellular matrix. It's still a complex goo. Proteins **adsorb** to surfaces and while those adsorbed proteins will mediate cell adhesion events and downstream activities, it can be difficult in practice to control that extracellular matrix because when you coat a surface with a protein it goes down in different orientations, it denatures, it gets remodeled by the cells. And that's led our group and others to develop surface chemistries that allow us to very clearly define the properties of an extracellular matrix to make sure we can control what peptides are present, what proteins are present, what carbohydrates are present. And this has offered quite an exciting opportunity. And before I show you one of those examples, here is the way we do this. These are surfaces that are made by letting molecules order themselves or self assemble on gold-coated glass slides. So if you take a gold-coated glass slide, and you put it in a solution of an **alkanethiol**, the sulfur atoms like the gold, so they attach. They attach at a high density so you wind up with a full monolayer of anchored molecules. They are standing straight up. They are packed almost like a cell membrane. And the charm of this is that one can control the chemistry of the surface by controlling the functional groups at the ends of those chains. What you see here is a monolayer that has the RGD peptide. That's an important peptide for cell adhesion. And indeed, these monolayers will support cell adhesion and cell spreading. And on these surfaces, you even find that cells, these are **3T3**

fibroblasts, you find that the cells assemble their focal adhesion structures and in, in fact, have a lot of the signaling that's associated with normal matrix. So these models where you really can define what **ligands** are present offer some important opportunities for deciphering the complexity of real ECM and that's the value. Now it turns out that when you combine the surface chemistry with the patterning, you can do some interesting things. And here's one example. Here's an example of a surface, which is actually dynamic, or some would call it smart. That depends who the surface is standing next to, I suppose. But what one has is a monolayer that's patterned into these 200 micron circular features. Those are cell adhesive. They present ligands that can mediate adhesion. And we can keep these cells on their pattern for days. But what we've done is to engineer this surface chemistry so that it's electrically active. These monolayers assemble on gold films. And so by applying electrical potentials to those gold films, we can trigger redox chemistry and in this case, when we apply a potential, we turn on an RGD ligand at these inert regions. And when you do that, you see that cells are now no longer patterned because they see ligand in the surrounding regions and they literally start to migrate out of their patterns. Now how do we do that? I'm actually not going to go through the chemistry. This talk reminds me of one I gave about a year ago. It was an evening talk. It was a clinical talk. I think I was the only non-clinician, if that's the word. I was the scientist who didn't do clinical work at the meeting and it was a dinner meeting. So, the two of us were speaking and the first gentleman spoke while I was half way through my dinner and he showed slides that kind of reminded me of Mark's slides with the eyeballs and slits and eyes pulled back. This isn't normally the kind of material I look at over dinner. And so I was a bit uncomfortable by all of that and heck, you know I didn't eat too well that night. So, it turns out I spoke next during dessert. I eventually got to this slide and I got the same reaction from the whole room because that's organic chemistry there. Anyhow, the chemistry audiences always find more humor in that joke. The way this works is that we attach molecules, which can be switched between two states, by applying potentials. And this is interesting because in one state, we have a reactive molecule that very selectively will immobilize the RGD peptide by way of a **Diels-Alder** reaction. So we can control when we turn this ligand on by controlling when we turn the reactivity of the surface on. And the chemistry is selective, so we introduce the ligand. And that notion, that notion of creating switchable surfaces, is one that we've generalized to quite many different activities and again, they provide tools where there have just been no alternative approaches available. How could one study how cells respond to a change in their extracellular matrix? An activity which is clearly important in a number of developmental and wound repair processes. But one which hasn't been accessible in the laboratory. These smart surfaces, which can switch their display of ligands, are providing those tools and I think that these are also going to be important in a number of more applied arenas where one wants to develop scaffolds to direct a cellular and a tissue response. And in this case, I'll go through this, we've been able to create assays which are alternatives for the common wounding assay and cell migration and to evaluate molecules that

have an effect on cell migration. Okay. I don't know. How is it looking Richard? Five? Okay. Let me go through now a much more biological example than the last example. And it's one that I know a number of people here work on and that's angiogenesis, the biological process for growing new blood vessels. And to give just a summary of some of the relevant biology for those of you who don't follow this, endothelial cells are recruited actively to form blood vessels. And there are a couple of proteins, which play important roles in this process. The first is a growth factor VEGF and the biology here is clear that VEGF will stimulate endothelial cells to organize themselves into tubes. These tubes, though, are not mature capillaries or vessels; they are, in fact, quite leaky. And it's only after that tissue is exposed to a protein known as **angiopoietin** that the, another cell layer is recruited and one gets to a functional, mature vessel. Now **Ang1**, discovered less than ten years ago, is known to act through a cell surface receptor known as **Tie2** and so in this respect, Ang1 had been regarded as a growth factor type molecule. It signals through a cell surface receptor and induces a transcriptional activity. If you look at genetic knockouts, what you find is that if you take a normal animal, you find that at the blood vessel wall there is a good cell-cell adhesion. In a knockout you find the mice are embryonically full, they die in their mid-stage embryonic development. And if you look, the phenotype is very leaky vessels, the poor adhesion of these cell types. Now we looked at that as physical scientists and with our collaborator, Alex Morla, somebody who knows a lot about this area, and we said, "To us it looks like you just have bad adhesion." And Alex responded by thinking about it, first telling us to go away and to go back to playing with surfaces, and then thinking about it and said "You know there is a lot of literature and some odd results that actually suggest that it does look like an adhesion defect when you lose Ang1". So we did some work and published this paper a few years ago and the essence was that we found if you coated dishes with Ang1, we found that HUVECS would attach and spread efficiently. That doesn't prove anything, but it's consistent with the notion that Ang1 can serve as an adhesion ligand, an extracellular matrix component. It turns that you didn't need Tie2 to mediate that adhesion. So it wasn't the case that a receptor which bound this protein would mediate the adhesion, but in a biologically non-relevant way. And we did a little bit more work and we came to the conclusion that Ang1 is a candidate extracellular matrix molecule. Well, that meant we had to identify within the protein what the active ligand was that mediated this adhesion. And here's a sequence of Ang1. You're not meant to see the sequence. I'm going to show you a piece of it in a moment. But we had reason to believe that somewhere between this point and this point there was an adhesion ligand. So how do you find it? Well, one way is to create a peptide array where you isolate overlapping peptides from that primary sequence and you immobilize them into an array. So this is a biochip type approach. And one then takes that chip, allows cells to adhere and we found that the HUVECS and 3T3s, this worked with many cell types, only adhere to a single peptide. And if we then took that peptide and we did truncations from both the C and N termini, we could narrow down based on a lack of adhesion or adhesion. We could identify a consensus sequence in Ang1 that mediated the

adhesion. Now, why is this nano? Well, it's nano because it's a tool. And the self assembly monolayers are really among the early experimental systems that let people do nanoscience even though they're infinitely, they are infinite structures in the lateral dimension, in the z direction. They are only a few nanometers high and they let us build structures, which are otherwise inaccessible. So we have this peptide and I'm going to wrap up here because the details aren't so critical. We had this peptide and we wanted to identify what is the cell surface receptor. And so this is young Sam Lee, a student in my group. He kind of likes a combination of this and this, so I am not sure this belongs on the slide. But we did standard affinity purification and we identified a protein, which bound the peptide. And let me just come to the point. We actually found that on the cell surface the **ephrin** receptors are capable of mediating adhesion to angiopoietin and we kind of looked at that result and said, "That's interesting." And then we went to the library and you know the story here, a good four weeks in the laboratory can save you an hour in the library, so we did the experiment first. But then we went to the library and we had a little bit more work here. We, for example, showed that ephrins will co-localize with integrins in cells that are adhering to Ang1 giving more evidence that this ephrin receptor is functioning, in fact, as an adhesion receptor. We found that there was an activation of the F receptor and some down stream signaling events were still intact. So actually, the biology is starting to look encouraging here. But we went to the library, as I said, and there hadn't been anything clear that implicated ephrins in angiogenesis, but a lot of indirect evidence. And this is just one paper, one of the early ones, which show that mouse embryos lacking the ephrin receptors, or ligands, have defects in angiogenic remodeling that look like those that you see in an Ang1 or a Tie2 knockout. So that was kind of exciting that we have now come to a molecular model for the role of ephrin in angiogenesis which puts at least some hypotheses on the table that are now being actively investigated. And the point I want to make about that, the reason I discuss this, is that nobody would confuse, my group certainly is a group that's expert in angiogenesis, and there are a lot of groups that work in this field in doing a lot of good work. The thing that allowed us to make what might be a substantial contribution in the area is we had a good tool. And that, in fact, is what drives a lot of biomedical research. Developing tools that can be put into researchers' and clinicians' hands to solve problems. And this is an example that showed us humbly that with a good tool one can go into a system without knowing a lot of what one's doing and get to something that might be interesting. And that's kind of where I'm going to sum this up. That one view that you might keep in mind as you watch nanoscience and nanotechnology developing, and it's going to develop still for a long time, is that these are going to provide new experimental tools that are going to be useful. And you've seen some of those in this afternoon's lectures and I know in the following lectures you are going to see more. But the way to keep this in focus is nano gives us new properties. Small is small, which means cheap and multiplexible and all of those things. But also it means you can work with small numbers of molecules which means you can work with small numbers

of molecules you're trying to detect. And because it's small, it's less invasive. So one of the things you've seen is the notion of putting these sensors in cells and in tissue to do real time sensing and imaging. And then finally, this last point, which is "bio is nano". And, you know, we've studied biology with reagents, molecules, and proteins because that's the kind of relevant size scale we're trying to investigate. And nano has introduced now the ability to create tools or reagents which are structured at the tens of nanometer of scale and that lets us now ask questions about a number of biomolecular complexes in that size range, which was hard to address previously. So, I'm going to stop there. I'll thank Richard again for putting this thing together. Thank all of you for your kind attention. And carry on with the program. (Applause).