

Scanning Life's Matrix: Genes, Proteins, and Small Molecules (2002)
Lecture Two—Probing Genes and Genomes
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1. Start of Lecture Two (00:15)

From the Howard Hughes Medical Institute, the 2002 Holiday Lectures on Science. This year's lectures-- "Scanning Life's Matrix: "Genes, Proteins, and Small Molecules"-- will be given by Dr. Stuart Schreiber, Howard Hughes Medical Institute investigator at Harvard University... and Dr. Eric Lander, Director of the Whitehead Institute/MIT Center for Genome Research. The second lecture is titled "Probing Genes and Genomes." And now, to introduce our program, the president of the Howard Hughes Medical Institute, Dr. Thomas Cech.

2. Introduction by HHMI president Dr. Thomas Cech (01:03)

Welcome back. In just a moment, Stuart Schreiber will discuss the proteins that are encoded by the human genome. He uses chemical genetics-- sort of a way to make small chemicals that interact specifically with these proteins-- sort of like molecular monkey wrenches to perturb their function and thereby learn what their roles are inside of living cells. Now, how did Stuart end up getting interested in this area of biological chemistry? He grew up not far from here in semirural Virginia, and when he was your age, he was really more interested in cars and girls and sports than he was in science. It was really only in college that his interest in chemistry caught fire, but even when he was much younger, some of the traits that he exemplified-- his huge curiosity and his drive to understand how natural systems work-- were really starting to become evident, and these are traits that we see in a lot of the nation's great scientists. Dr. Schreiber's talk is entitled "Probing Genes and Genomes." Let's introduce our speaker with a brief video.

3. Introductory interview with Dr. Stuart Schreiber (02:18)

When I was a high-school student, I was not very academically inclined. I really knew nothing about science. I had no understanding whatsoever of chemistry. In my first year as a freshman at the University of Virginia, I attended my first chemistry lecture, and that was really-- that was a life-transforming event. When I first started thinking about chemistry, these small molecules were, honestly, at the beginning just sort of beautiful art objects. I learned by studying organic chemistry that I could control their shape and build them sort of like an architect building a new building. But the key was to understand how this small molecule interacted with some other macromolecule. The use of small molecules allows you not just to modulate the function of a protein, but you actually have a chemical substance that can modulate function, then, of the protein to which it binds even within a human. The ability to manipulate the function with a small molecule makes the bridge between medicine and biology more direct. Those same probes can be the starting points for new medicines in the future. I love being able to wake up every morning and to ask myself, "What's the most interesting thing I could possibly do?" and go in and do it. That's what being a scientist is about. Trying to convert Nicky into a chemist from a biologist. But you know what? Science is very much a social phenomenon as well. The social network of science is the process that generates the most overall excitement and satisfaction. I work with a group of students who are in the very exciting phase of becoming young adults. Working with them keeps you young forever. My central goal for participating in the Holiday Science lectures is to leave students with the understanding that integrating disciplines today is the key to unlocking the mysteries of life.

4. If you want to understand life's processes, perturb them (04:48)

Good morning. It's a real pleasure for me to participate with my friend Eric Lander in the 2002 Holiday Lectures on Science. Although Eric and I get to stand before you and share with you some of our views on science, much of our lectures were made possible by the creative efforts of a dedicated and talented group of Institute colleagues to whom we are very grateful. Now... the simple theme that underlies almost everything I'm going to tell you today and tomorrow is shown on the screen. If you want to understand life's mysteries-- what I'll call life processes-- mess with them or perturb them and see what happens. Sometimes the perturbing part just happens, and we saw an illustration of that in the earlier lecture-- the natural and rare mutation in the cystic fibrosis gene that gives rise to this disease. We've certainly learned a great deal about the process that gives rise to the disease by identifying that mutation, but today, with increasing frequency, powerful tools of chemistry, biology, and genomics are providing for effective and specific means both to perturb life processes and to observe the consequences. I believe that these new tools are going to be key to understanding life's matrix as well as to create the new medicines that will ensure a high quality of life worldwide. So my goal today is to share with you some of these new tools that are being used to probe the genes and genomes to which Eric referred.

5. Proteins serve as the key mechanical components of life's processes (06:44)

Now, these life processes can be as simple as that membrane transporter that we saw in the earlier lecture or as complicated as the process by which a fertilized egg gives rise to a child. Understanding these life processes, of course, satisfies our sense of mystery and desire to solve mysteries, but I think it's also key to understanding what goes wrong when they lead to disease-- for example, the process by which the healthy cell on the left is converted into the cancerous cell on the right. We learned in the previous lecture that the period 1950 through 1975 provided the basic outline of life processes. We saw that genes are converted and read into proteins through the intermediary messenger RNA and that proteins serve as a key mechanical function in mediating these life processes.

6. Perturbing life's processes: The genetic approach (07:53)

This outline also gives us the blueprint for us to systematically perturb and therefore understand these life processes. During the past century, probably the most general and powerful way of perturbing a life system has been through the use of the tools and principles of genetics. We first then start with a mutation, and it can be naturally occurring as we saw. It can be randomly induced and now even targeted. These mutations give rise to a faulty protein, and the faulty proteins can either be inactive or overly active. A very good illustration of this is the study of cancer in recent years, where we've identified mutations in tumor suppressors that would render them inactive and oncogenes that render them overly active, and again, these mutations have given us a bright light-- shine a bright light on the process of cancer.

7. Perturbing life's processes: The chemical genetic approach (09:05)

Now, what I'm going to be discussing is the second approach. It is an approach that is grounded in the same principles of genetics but is made possible by advances in chemistry, and so we call this chemical genetics, and here we're going to bypass the gene. We're going to start with the normal protein, and we're going to use small molecules-- small molecules that can either, similarly, inactivate or, in cases, overactivate the proteins to which they bind. So let's

8. What are small molecules? (09:43)

start with the first question. What are these small molecules? Small molecules are difficult to define. I can define the macromolecules that we've been hearing about-- genes, proteins, the repeating elements

of nucleotides and amino acids. Small molecules are much more complex. Small molecules comprise the atoms, typically, carbon, hydrogen, oxygen, nitrogen. They're bonded together-- I give you one illustration on the slide-- in various ways in making different kinds of rings and substituents. If we can roll the first animation, I'll show you that, in fact, however, these structures are a bewildering array of different three-dimensional shapes, and it's these different shapes that's so key to allow them to interact with the many different proteins encoded by the genome. You're looking at some molecules. You're seeing the different kinds of shapes and even atoms-- the different atoms that I mentioned-- boron, sulfur, chlorine. The first molecule I'm going to stop with here is called rapamycin. As you'll see when I discuss this, it's a naturally occurring small molecule, and it's very useful in terms of teaching us about biology. The other molecules that you see now flashing before you in this collage of small molecules, showing you the many different shapes that they can acquire, are molecules that were synthesized in the laboratory. They're synthetic compounds, and I'll be discussing how we go about synthesizing such compounds. The small molecule that we're going to end with today is a molecule named furrowstatin. I have a molecular model of furrowstatin for you here.

9. Demonstration: Use of molecular models in chemistry (11:39)

We have some other molecular models of some of the small molecules flashing before you on the table. These molecular models are very useful to chemists to understand the various three-dimensional shapes that these molecules acquire, and it is their shapes that are so key for their function, and I think that the students in the audience received a little model-building kit. You can have some experience in putting these different kinds of shapes together maybe after the lecture.

10. Animation: How does a small molecule modulate a protein? (12:08)

So having defined or looked at some of these small molecules, now we're going to ask, "How do they modulate the proteins "to which they bind?" The first answer, of course, is that they do associate with the protein. They bind to the protein. The answer to my question, fundamentally, is going to be--the message that I want you to receive is that upon binding, these small molecules create a complex with the protein, and we should think about this complex as a completely new entity-- a new entity that can be either inactive or overactive. Can we roll the next animation? What you're seeing here is the cellular protein calmodulin in blue. It plays a key role in a number of cellular functions. It does so by using a cleft that you can see. The cleft has now been occupied by the gold small molecule, and so, in the process, we have a small molecule protein complex. You notice it has a very different shape. It is, in fact, in this case, inactive because that cleft is no longer available for its normal cellular function.

11. Animation: How does a small molecule activate a protein? (13:20)

What about the case of a small molecule binding to proteins that activate function? That one, perhaps, is a little bit more mysterious. It seems more intuitive how a small molecule could bind to a protein and inactivate it. On the next animation, if we could roll that one, please, you're going to see the blue protein is now a human protein called FKBP12. The purple protein is called FRAP. These two proteins normally do not interact with each other. You'll hear more about them later when I talk about nutrient sensing. The small molecule you've seen before is rapamycin. It has high affinity for FKBP. It binds tightly to it, and in so doing, it creates a composite surface-- a new surface that activates the blue protein to bind the purple one. So, previously, FKBP was unable to bind the purple one. By rapamycin binding to it, it creates this composite surface that now interdigitates with the purple FRAP protein-- an illustration of how small molecules can activate function.

12. Video: Using small molecules to study the cleavage furrow of cell division (14:32)

So having defined some of the key terms and, hopefully, some of the key concepts, I'm gonna start with an example that shows you how we use small molecules to interrogate a biological process. I'm not gonna tell you how we come upon these small molecules, how we identify the interesting ones. That will come a little later. The example that I've selected is a process in cell division all cells go through when they divide. During cell division, they create a cleavage furrow. I'm gonna talk a little bit about this cleavage furrow. So you know that when cells are dividing, the first thing they do is replicate their chromosomes. They align them in the middle of the cell. The microtubule network in green tugs them apart and segregates them to opposite poles of the cell. At this stage, a cleavage furrow forms that comprises the actin cytoskeleton. What we don't know is how the cell knows when to form the cleavage furrow, where to position the cleavage furrow, and finally, how to pull the purse string on the cleavage furrow so as to contract it and pinch the cell into the mother and daughter. The next animation I'm going to show you gives you an illustration of this process, and the main point that I'd like to make in this illustration is that this is an example of a very dynamic process. The chromosomes you see are aligned. They're segregating, and the cleavage furrow is forming, and the mother and daughter cell has been created.

13. Video: Furrowstatin can freeze the dynamic process of cell division (16:12)

Very dynamic processes, of which there are many in life, are very difficult to study through the use of mutations because a mutation is inherited in a cell. It's always there, and to study dynamic processes like cleavage furrow formation, you'd like to be able to turn the perturbation on and turn it off precisely with fine temporal control. This is where small molecules are so valuable, and the example that I'm gonna show you is this molecule furrowstatin I showed you before. This molecule was discovered by Tim Mitchison at Harvard University and was used to explore the cleavage furrow. The reason furrowstatin is of such interest to us is that it prevents the cleavage furrow from functioning. The cleavage furrow can form but doesn't function in the presence of furrowstatin. Furrowstatin has no effect on chromosome replication, chromosome segregation, all other processes in the cell. It's just targeting this cleavage furrow, and one of the beauties of the molecule, as well as other small molecules, is this temporal control. You can add cleavage furrowstatin prior to cleavage furrow formation, and you'll stall it right in the beginning, or you can allow the cleavage furrow to form, add the molecule, and essentially, instantaneously freeze out the cleavage furrow. On the next animation that I'm going to show you, you're going to see two cells dividing. They have both replicated their chromosomes. This cell, in the upper right-- it's a little bit advanced relative to this cell, and that's very nice for this experiment. When you see the blue splash, that symbolizes-- that's precisely when we've added a drop of the furrowstatin molecule. You're going to see that we're going to add it right after the cleavage furrow forms in the upper right-hand cell. Can we roll the film now? So here's the chromosomes. They have segregated. We add the furrowstatin. The cleavage furrow is frozen. These cells continue to segregate their chromosomes, but there's no cleavage furrow, so we can arrest the furrow, generating a cell either with no furrow or with an interrupted furrow.

14. Video: Furrowstatin perturbation is reversible (18:39)

Another nice feature of the small molecule perturbation to explore biology in this sort is that these processes are usually reversible, so you can simply wash the compound out and allow the process to proceed. Roll the next one, please. So here you see a cell treated with furrowstatin. The chromosomes can segregate, no problem, but no cleavage furrow. Wash it away. The cleavage furrow forms just where it's supposed to, pulls the purse string, mother and daughter cell are formed.

15. Animation: Furrowstatin works by binding to nonmuscle myosin II (19:13)

Now it turns out that we've learned about how furrowstatin works. How we did that is a subject for lecture number four-- the final lecture. Furrowstatin interacts with a key protein that is part of this cleavage furrow. It's not the actin polymer itself, but it's a protein that communicates with the actin protein-- a protein called non-muscle myosin II. You can roll this one. This is an illustration of the myosin protein shown here, which is a motor. It gives the cell the mechanical force, and it's actually motoring along across the actin fiber itself of the cleavage furrow. When furrowstatin is added, it binds to the non-muscle myosin II protein, inhibits--in this case, inactivates its function, and freezes the mechanical actions of the cleavage furrow.

16. Using the arrested furrow to study cell division (20:11)

Now, the ability to take dynamic life processes-- in this particular case, the cleavage furrow, but many others-- and freeze it temporarily is very valuable as an experimental tool. In this particular case, the cell has been stained with two different pairs of reagents. It's the same cell. You've seen an image of it, and we're going to use these stains to interrogate where various proteins and macromolecules in the cell are at the stage of stalled cleavage furrow right at the beginning. You can see that here, these cells--the cell treated with furrowstatin had the chromosomes segregating to opposite poles. There's the microtubules that were tugging them apart. First we're gonna stain for the target of furrowstatin-- myosin II. Well, happily, that protein is right where the cleavage furrow should be, so that's interesting and satisfying that it's targeting a protein that's right where the cleavage furrow is. But this technique allows you to look at other proteins that are less well-defined in terms of their function. One example is the protein anillin. If we looked at anillin in various stages of the cell, it would be distributed throughout the cell. When the cell is frozen with furrowstatin-- we stain for anillin-- you see that anillin protein is right where the cleavage furrow is forming. It's right where myosin is. Now, this doesn't prove anything. This is not a case of guilt by association, but it's provocative and it generates a hypothesis-- the hypothesis being that maybe anillin is somehow part of this process as well. Now, it turns out the story is even nicer than this. Chemical genetics is-- through the use of different disciplines and integrating different disciplines of chemistry and biology and engineering and computer science-- is yielding more and more of these probes, these small molecules like furrowstatin. For example, I raised the question, "How did the cleavage furrow know where to go "and when to form?" Well, probably, there's got to be some regulatory mechanism. There's gonna be some signaling molecules talking to myosin and anillin. There are some candidate signaling molecules already for which we have small molecule probes. We can manipulate their function and ask, "Does myosin go to the right place? "Does anillin go "to the right place?" If it perturbs one of their localizations but not the other, it tells you that the signaling is distinct for the two. If it affects both of them, it might suggest that they're on the same regulatory circuit.

17. Chemical genetics lets us study dynamic processes (01:22:56:04) I'm gonna leave you with the analogy here before I take some questions from the audience of high-speed photography. I want to leave you with the impression that the life processes encoded within the human genome that we heard about are very dynamic, and, therefore, it makes them difficult to study, but having these kinds of tools now with increasing frequency that can freeze them out and allow them to progress is like looking at individual snapshots. If I were to show you a film of a bullet piercing the queen of hearts, you probably could tell very little about that process. You probably, if you didn't listen to it, couldn't even tell the direction from which the bullet was derived, but with high-speed photography, we can see all the details of the rotating bullet. We can see the cleavage furrow in the queen of hearts. So with that analogy, I'm gonna stop here and see if I can get some questions from the audience.

18. Q&A: Does furrowstatin inhibit furrow formation or furrow function? (23:52)

Yes? Does the furrowstatin actually inhibit the cleavage furrow ever taking place, or does it only stall it? Like, does it eventually-- It all depends on when you add the furrowstatin. The cleavage furrow looks

like it's actually forming in the presence of furrowstatin. It's just not functioning, and I think the reason for that is that the functioning of the furrow that yields that dynamic process involves the mechanical motor-like actions of the myosin protein. It has to ratchet along the actin and drag its cargo with it, and somehow, that's leading to a constriction of the cell. So the furrow forms in the presence of furrowstatin, but it doesn't function. If we add it a little later, the furrow forms-- begins to function, and we stop it right in the middle of it. So it doesn't complete division? It doesn't complete division as long as the furrowstatin is present. Wash it away, the cells proceed through division.

19. Q&A: Are small molecules useful for treating disease? (24:54)

Yes? With these small molecules, what you've shown here is mainly used for determining the properties of proteins and what they do-- their functions. Is it also used at all to treat any diseases? That's a very good question. What I'm focusing on here is the use of small molecules to perturb biological systems and biological processes. Perturbing them is a first step towards using them as therapeutic agents. It turns out it's a lot easier to make small molecules that serve as these probes than to take them to the next step and make them safe therapeutic agents, but it is the first step, so it's another dividend of probing biology-- this route by looking at the proteins and small molecules that interfere with them rather than through the genes themselves because you at least do have a starting point on intervening in a therapeutic manner. Now, I'm not sure that non-muscle myosin II is going to be a very good therapeutic target because, probably, many cells will require the same protein in that cleavage furrow process and during cell division. What would be very exciting is to find components of this cleavage furrow or other steps of the cell division-- for example, that we find cancer cells have a particular dependence on-- so this, again, requires the dissecting of the functions of these proteins in healthy and in disease states.

20. Q&A: How do you figure out which small molecule to use? (26:36)

Yes? I was wondering, how do you determine-- I mean, how do you figure out what small molecules to use? Do you use a program, or do you just--hmm. Or do you just figure out how they bond and just do, like, a guess-and-check method? Another terrific question. Yep. You know, this field has evolved over the past several decades. There have been various times-- I'll take you way back. Decades ago. That answer is you searched randomly and hoped that you had the good luck that Eric referred to, OK? Then there was a period of time where we thought we were, in fact, a little smarter than we really were. We thought, "Ah, we have so much new information-- "structures of proteins. "We understand how these molecules-- "the shapes that they adopt-- "by looking at their models. "We'll start just designing them, "getting them to fit together properly." There's been some success in that, but the truth is, it's mostly been a success at the stage of fine-tuning the molecules, giving them the slight alterations that just tweak the specificity a little bit more. What's happened, in fact, and these, again, are such great questions. You keep taking us to future topics. What's happened is I would say we've gone back to the future. We've gone back to the old idea that we should allow an element of chance in the whole process. It's just that we've gotten really good-- through the same process that you heard about in the first lecture-- of increasing the throughput of experimentation. By high throughput means, we can now perturb much more broadly with many different possible solutions, and we can observe the consequences much more precisely and pick the winner to find the needle in the haystack. So it's back to the future. It's a lot of chance involved, but we're just getting so good at the process that we can pick out the winner with very high frequency. And I'm--before I get too far behind here, we have a-- we don't call this the Holiday Lectures for anything. This is the gift that goes along with the holiday. Had one good question here. We had one good question over here, and thank you very much.

21. Q&A: Can you define again what small molecules are? (29:04)

Uh, let's see--in the back. Yes. Can you redefine what small molecules is? Ah. Redefine the small molecules. That's--this is the toughest question because I didn't really-- you know, I said I was gonna try, and I said I was gonna have a hard time because small molecules are smaller than macromolecules, OK? They are formed by bonding of-- generally speaking-- generally speaking, four or five atoms. Carbon, hydrogen, oxygen, nitrogen are the standard ones, but then more rarely, phosphorus, sulfur, chlorine, bromine. The bonding themselves follows the basic rules of chemical bonding. Chlorine forms two bonds, carbon forms four. Now, beyond that, if you consider the number of permutations that are conceivable for small molecules to satisfy those requirements, it's literally ten to the sixty of molecules having less than five hundred molecular weight, or more molecules than exist atomic particles in the universe. So, they are very difficult to define, as you can hear. They--you know what? The best thing I can say is that if you-- the more you see them, the more you recognize them. You start to put them into a bucket and say, "That looks like a small molecule." OK. I think that's our last question. Thanks for one more great question. Good catch. And one I'm missing over here. OK. Let's go on with the second part now of my presentation.

22. Where do these small molecules come from? (30:47)

I want to come back to some basic questions. Where did these small molecules come from? And the answer is, two basic sources--one from nature, one from the laboratory. Molecules from nature we call natural products. You are familiar with these, actually. They're all around you. The color in the leaves, the change throughout the season--those are natural products. The sweet taste of a fresh orange--that's a natural product.

23. Rapamycin, a small molecule from nature (31:22)

The natural product that I'm gonna be showcasing here, named rapamycin, is typical of many natural products. It actually comes from the soil. It comes from a microorganism. This particular microorganism was discovered on Easter Island. Some of you may recognize the icon. Literally, from a sample of soil, microbiologists cultured organisms within that soil sample--microorganisms, bacteria in this case, a streptomyces strain, and then searched for small molecule natural products made by the microorganism that may have some valuable property. The small molecule that resulted I showed you earlier, rapamycin, has had very valuable properties, not only as a biological probe--as I'm gonna be focusing on in even greater detail in my final lecture--but as a medicine. Rapamycin turns out to be, now, a lifesaving medicine. It's one of the most recently approved clinical drugs. In this case, it's thus far been used in organ transplantation. Rapamycin has the ability to tame the immune system down so that a patient receiving a foreign heart during heart transplantation no longer will attack that foreign heart through its immune system in the presence of rapamycin. Rapamycin is also being shown more recently to have very promising, exciting properties as an anticancer agent. And, again, some of this, I hope, will become a little more clear at the end of my lecture tomorrow.

24. Small molecules from laboratory synthesis (32:59)

I want to focus, however, on small molecules from laboratory synthesis because there are some really major advances in laboratory synthesis that are allowing greater access to a more--a broader range of small molecule structures. Now, small molecule synthesis in the laboratory came about first from target-oriented synthesis. This is a venerable field. It's been around for 60, 70, 80 years now, and as its name implies, it's a kind of synthesis that targets a particular small molecule. I'm gonna be focusing mostly on a new type of synthesis called diversity-oriented synthesis, and as its name implies, it aims to synthesize highly diverse small molecule structures.

25. Target-oriented synthesis and its limitations (33:50)

Target-oriented synthesis comes about from our understanding today of hundreds, if not thousands, of chemical reactions--chemical transformations. Now, that may sound daunting to you to think about studying chemistry. You have to learn about all these reactions, but the good news is, these reactions follow some very simple basic principles, and once you learn those principles, you don't actually have to know all the reactions. The reactions become much more reasonable and easy, even, to predict. Now, synthesis requires learning how to juggle those reactions, usually in a linear sequence, many steps in a row, to take very simple compounds into very complex ones. And the example that I'm gonna show you here is again my favorite natural product, rapamycin. My lab, fourteen years ago when we started our studies with rapamycin, decided that we needed to synthesize it. We thought an alternative source, not just from the soil from Easter Island, might be the laboratory. We succeeded in that synthesis. It took us about five years. It took about 5 student trainees during that period of time to work their way through what turned out to be about fifty consecutive reactions. When we were done, we had one very precious new compound, small molecule, rapamycin, but only one, and it was a long and arduous task to get there.

26. Introduction to diversity-oriented synthesis (DOS) (35:19)

I'm gonna contrast that with what I'll be discussing with you today. I'm gonna give you a little tutorial today about this new kind of synthesis, very exciting kind of synthesis--DOS, or diversity-oriented synthesis. We again start with a simple compound, but now what we do is, we run a very limited number of steps. Two reactions is what I'm gonna show you. Sometimes three or four, but usually no more. Two or three types of reactions. The big difference is, we're going to take our simple compound and run that one reaction but many slight variations of it to radiate outward like a starburst with different building blocks being used in this reaction. If we follow one product, we'll take that one and pick a second reaction, and we'll starburst out--not in a linear fashion now--but expand outwards with many subtle variations in the building blocks that we'll use with that single reaction, and maybe then, we'll even do it a third time. So I'll give you a specific example that was just recently completed about a year ago in my lab. This is an example of a simple small molecule. We selected it for some subtle features that it contains within it. There's a functionality off here that you'll see is really quite important in terms of the two reactions we're going to subject this compound to. Now, if I were performing a target-oriented synthesis, I would take my compound, I'd put it in a big flask, like you may see over there on the shelf, and I'd take all that compound through the first chemical transformation.

27. DOS process 1: Split to many tubes and attach to plastic beads (37:05)

In diversity-oriented synthesis, we take that single compound, and we split it. Shown here is about 25 different flasks. In reality, in this case, we took a little bit over 40 different flasks, OK? Now, boy, it's starting to sound more complicated, but, in fact, this process is not that complicated. A couple tricks we do in this process. The small molecule itself is actually not alone. It's attached to a little, polymeric plastic bead. I don't know if you're gonna be able to see this here up on the screen. So these little plastic beads are about the size of a fish egg--if you've ever had caviar. And we attach the simple small molecule to these beads. That's the first step. The second step is, we pour equal amounts of those beads into a reaction flask. It's now not a flask. It's a little tube, and we're gonna use over forty of these tubes, but, in fact, we're only subjecting them to one type of reaction. It's the same reaction. So we build a little contraption like this, which my students in the lab refer to as the cow for reasons that might be apparent to you. So there's forty different little tubes that have been attached, and we can filter them, and we can aspirate through a connection over here, but we can manipulate all forty in one single operation.

28. DOS process 2: Perform first reaction and split again (38:41)

OK. Let's take a look now at just one of those tubes. We're gonna focus on just one and see what's gonna happen to it. This is the tube. This is the small molecule. We've learned about these reactions and rules of chemical bonding, and we know that this orange compound is a partner for the blue compound. We know that there's functionality within this orange compound that will join together with functionality within the blue compound in the fashion that's shown. This is an example of a chemical transformation, one of those thousands. It has a particular name. It's called the Diels-Alder reaction. The name's not important, but it turns out to be a very useful, general kind of reaction process. So that yields the blue-orange compound. Now, the next step is, we take each one of these now, which has a slight variation of the blue-orange compound, and we take it, and we split it into forty new flasks. We take 1/40 of each one of these, reattach them to our cow.

29. DOS process 3: Perform second reaction and further reactions (39:51)

And I'm gonna just focus on the outcome of one of these. Let's look at just one to typify this process. There's our blue-orange compound. We look at this compound. Once again, we see functionality within it that's chemically compatible with, now, the red compound, the red building block. So we mix together the red compound with the blue-orange compound, and the outcome is yet another small molecule. This we'll call the orange-blue-red compound. The checkerboard symbolizing this one is shown. OK. So let's review what we've done. We took the simple building block, we split it into forty-some-odd different flasks, we focused just on one of them, and we saw that by using just two chemical reactions we could generate the blue-orange compound and the blue-orange-red compound, but remember, for each one of the original 40, we're generating variations of those compounds with different building blocks. Now let's just focus on the blue-orange compounds. In principle, we could look within those structures and find more chemical functionality that would allow us to run the third reaction. We've got that on the books, but we haven't done it yet. So let's just focus on what's been accomplished. Let's look at these 3 compounds, remind you of their structures, and now conceptually, I'm gonna ask you to think about what we've done just to make these three in the form of a matrix. You'll see why in a moment. Now we start with a sort of trivial 1x1x1 matrix that yields three new compounds. Now I'm gonna take you to what happens when we go to a 2x2x2 matrix, and then we're gonna expand it all the way as we actually did in the synthesis. Can we roll the next animation,

30. Animation: DOS matrix (41:48)

please? So here's our blue compound, and the white one is now a cousin. Here's our orange compound, and the white variation is a cousin of the orange, and there's our 2x2 matrix. We've already seen what happens when you mix the blue and the orange--you get the blue-orange compound. Now we're gonna get the white-orange combination and the blue-white combination and, of course, the white-and-white combination. That's just the 2x2 matrix. Now we're gonna consider a 2x2x2 matrix. Remember, we have the second reaction, the red building block. We've already seen that the red compound joins together with the blue-orange to generate the blue-orange-red compound, but, of course, now the other combinations will occur. The red's gonna join with the other compounds, generating four new compounds. We've got a total of eight, and we're gonna complete this with a cousin of the red to make a 2x2x2 matrix, four more compounds. That's now twelve. But now simply imagine what happens when you did this in the actual 40x40x40, plus or minus some, matrix. We generate 88,400 new compounds.

31. Recent advances allow one person to make 88,400 new compounds (43:09)

Now, once again, you might be daunted by this information. You might even be a little depressed about thinking about doing this kind of synthesis. I told you that five students struggled for five years to make rapamycin. What's going on with 88,400 new compounds? Well, one of the real messages, I think, Eric and I have to bring to you today is that modern science involves interdisciplinary research, bringing

different disciplines together. Synthesis alone would be still equally laborious, but there's all kinds of techniques, from computer science and engineering especially, that render this process rather simple. What do I mean by that? Well, the actual synthesis of these 88,400 compounds now was performed by one-- one person in the lab. This is Ohyun Kwon. Ohyun studied at Columbia learning about target-oriented synthesis, and she came to my lab a couple of years ago to learn about diversity-oriented synthesis. She spent about a year or so developing the basic chemistry that I've outlined for you, and it took her about four months to actually execute that synthesis of 88,400 compounds, and now Ohyun is a professor at UCLA teaching her own students in Los Angeles how to do diversity-oriented synthesis.

32. How do we discover which small molecules are useful? (44:29)

Now, one last introductory concept before we break for the next set of questions. All I've done thus far is given you a sense of-- using diversity-oriented synthesis-- how we can make these small molecules. I haven't told you how we can discover the furrowstatins among them or the furrowstatin orthologs that might target the other myosin family members. I want to leave you with a conceptual view of how we do that. Before, then, again in my last lecture, I'll take you through two specific examples in the context of the disease diabetes. I'll teach you about how we're learning about diabetes using these techniques and how conceptually, at least, we find the winning small molecule, the needle in the haystack. And the way I want you to envision this is that we developed a technique that allows those 88,400 compounds to flow as a stream, and we have a sensor that filters through each and every small molecule searching for a desirable process. Can we roll the final animation, please?

33. Animation: Screening small molecules with a protein (45:40)

So imagine these are Ohyun's 88,400 compounds flying by in this stream. Now, what is the filter? How do we screen for these small molecules? The filter's going to be a protein, a protein of interest. For example, here's that calmodulin protein. The vast majority of small molecules do not have the proper shape to be a magnet to the calmodulin protein, but the rare needle in the haystack does, and if you configure the experiment properly, you can pluck that small molecule out of the stream and find your winning probe molecule.

34. Two screening methods for identifying small-molecule function (46:18)

Finally, the last point before I take some questions. The--it turns out that's a good way to think about it conceptually, but there's two very important different-- distinct ways of doing this process. One of them says, "I think the myosin proteins "or the calmodulin proteins, "or the orthologs of the myosin proteins "are really important, "and I'm going to screen "my small molecules against them." In that case, you take a pure protein and screen your small molecules. There's a problem with this approach, however. It doesn't allow you to discover the chance, unusual, unexpected finding. And to do that, you need to screen the proteins while they're functioning in cells or in organisms. So I'm gonna introduce to you tomorrow the process of cellular and organismal screening, as well. We're gonna stream those small molecules while the cell-- while the protein is actually functioning inside of the cell. And with that, I think I'll stop and take a few questions.

35. Q&A: Do you accidentally make unexpected molecules? (47:24)

Question in the back. I was wondering, when you try to make a certain small molecule, do you ever end up, like, accidentally making other ones that are useful to you, or do you just screen out all those other ones? Uh, that's--that's a great question again. We like--we work so hard to develop the chemistry so that it's so precise that every theoretical compound that's made in that big 40x40x40 matrix is made and precisely only those compounds are made, and we're getting pretty good at it, pretty good at it. Maybe

we have, you know, at each step 95% success, but multiply 95% success by 95% success several times, and you start getting the unexpected surprises in there. They do exist, and we have had some experience with them. They've emerged-- unexpected structures that weren't supposed to be there, but they were actually the winners. So the downside of that is that it takes a little bit more time to do some detective work to figure out what the precise structure is as opposed to what it was supposed to be. I guess the upside is that you've created a little more chance, and this whole process is about taking advantage of chance observations.

36. Q&A: Wouldn't sorting through molecules of DOS take a long time? (48:50)

Question. I was wondering-- you said that in the beginning that target-oriented synthesis was often very long and laborious and going, and it just took a lot of effort on your behalf to do this. Would sifting through all these-- you know, you said-- what is it--88,400-- wouldn't that be almost equally laborious to-- to sort through them as opposed-- So I've given you a sense that we can now make them, but now what do you do with them? You got a big problem on hand. And once again, the reason we're able to sort through them is that chemistry connects with neighboring disciplines, and the tricks we learn from genomics, and the tricks we learn from biology and from engineering and computer science-- as some of the main ones-- have made extraordinarily powerful and effective ways to screen through all 88,000 in a very short period of time and with real precision. So there are some techniques now that are pretty widely available that would allow you to look at those 88,000 in maybe an hour, and with a single student with, in fact, six different microscope slides as I'll try to show you tomorrow in tomorrow's lecture. OK. Here, I get-- very good question here. And I think I owe someone in the back, all right? I hope I don't-- there we go. Good catch. Uh, let's see.

37. Q&A: Where did you get the idea to use chemistry in biology? (50:19)

Question in the back. And you're next. I was wondering where you got the idea of using chemistry and biology to--and using small molecules to find out things about biological processes. Ah, that's great. You know, I, um--that's a-- I can give you my personal answer here. I--I, um--I didn't do it in any calculated way. I did it by a process of sort of waking up in the morning and just asking myself, you know, "What's the most fascinating thing out there?" And I think I first fell in love with these shapes. I didn't know anything about chemistry when I went to college. I had no clue what chemistry was about, and I attended a lecture, only at my sister's urging, three weeks into college, and to be perfectly honest, I was just about ready to quit college because I didn't find it to my liking, but I sat in on this lecture, and the professor drew up molecules of this sort, and I thought, "That's unbelievable. "I have no idea what the professor's drawing, "but it's beautiful." I thought it was an art class. So I fell in love with chemistry thinking I was taking art, and I learned about the rules of chemistry, and, uh, will carry that passion with me to my grave. But, you know, a number of years later, I started to realize what these molecules do, and I realized that they actually have a function out there in life and nature. They interact with proteins, and they modulate function and so forth, and that started-- life processes and life started to become just an amazingly interesting and fascinating challenge and exciting problem, so I thought I would start to study that problem, and I thought I was pretty smart. I thought, "Ah. I've figured out "a very special way of studying biology "with these small molecules-- "perturb them, see what happens." And then I was a century behind the scientist that Eric mentioned on January of 1900. I discovered a hundred years later that my idea was a very old one. It's called genetics, but that was OK. I thought, you know, "This is a slight variation of genetics," and this was my portal into biology. That certainly deserves-- I think we got two more. OK. Good catch. And let's see. I think I promised here in the middle. Please.

38. Q&A: Wouldn't rapamycin's immunosuppression cause other diseases? (52:52)

You had mentioned the rapamycin, and you said that it was used for organ transplants and it tames the immune system, but wouldn't that increase the chance of other illnesses with it down? Boy, you all are incredible. That's exactly correct. The most... paradoxically-- because I said it's actually being used for cancer. What happens is that patients that are on extended treatment of rapamycin will have a tendency to develop the earliest signs of cancer, presumably, it's been argued, because the immune system's been tamed and presumably because the immune system is constantly surveying the earliest signs of cancer. Now because there's some evidence of this, clinicians know of this, and they know how to regiment the use of the drug. So they guard against this happening. However, there was a totally unexpected finding with rapamycin that, again, I'm going to discuss in my next lecture because suppressing the immune system was not to have anything to do with diabetes, but that's a new and unexpected by-product of inappropriate use of rapamycin. Now, the good news is, again, through dosing and the fact that these drugs, like furrowstatin, are reversible, you can take a patient off rapamycin for a period of time, give them another drug, and let that deleterious side effect go away, because it's reversible, and then put them back. But this unexpected connection was one of the wonderful outcomes, in a sense, of this because it made a connection between fundamental processes of the immune system and fundamental processes leading to diabetes, which I think is gonna shine a new light on the process of acquiring type II diabetes.

39. Q&A: Could more than one small molecule interact with the protein of interest? (54:59)

I think I just got a sign we got one more-- time for one more question. With the small molecules that you create, and then you said you use a protein to filter them out, is it possible that two molecules or more could fit into the same protein, and then if that happened, how would you differentiate between which small molecule did what you wanted it to do and which ones don't, and how would you separate that one from the rest of them? That's a great question. In fact, it happens. It's rare because the single binding event itself is pretty rare, but the double binding event then might be, say, doubly rare but not so rare that it doesn't actually happen. So you got to be on the lookout for it, number one, and if you are on the lookout for it and you detect it, there's good news. If you find two molecules that bind to the same protein, you can, using chemistry, stitch them together and convert them into one, and you get a superstrong binder with very, very high selectivity. So you actually--it's rare, but you look for it because you hope it will happen because you can turn it to your advantage. So that's all the time we have for the questions. I have one--one, uh-- my favorite t-shirt left here, and I think I owe someone in the audience another one. We'll get that for you. Thank you.

40. Closing remarks by HHMI President Dr. Thomas Cech (56:23) Well, thank you, Stuart, for literally bringing chemistry to life for us, and thank you, students in the audience, for your great questions. This would be a good time to visit the Holiday Lectures web site, and please join us again tomorrow morning, where we're going to continue this fascinating journey through genomes and how to perturb genomes, as well as how to observe genomes. We'll see you again then.