Episode 57, "Two Protons Walk Into an H Bar"
Dramatis personae:
- Ben Tippett
- Brian Cross
- Nicole Prent
- Jacqueline Townsend

The Titanium Physicists Podcast
www.titaniumphysics.com

Ben: Never be afraid. There's nothing which is known which can't be understood. There's nothing which is understood which can't be explained. For over 50 episodes now, my team and I have brought you to the very frontier of knowledge in physics and astronomy. And still our mission goes on. To present you with your birthright: an understanding of the Universe. I've traveled the world seeking out a certain type of genius. Masters of not only their academic disciplines but also at explaining their research in understandable ways. And I've bestowed upon these women and men the title of Titanium Physicists. You're listening to the Titanium Physicists Podcast and I'm Ben Tippett. And now... *allez physique*!

01:12

[Intro song; *Tell Balgeary, Balgury Is Dead* by Ted Leo and the Pharmacists]

01:46

Ben: Only two women have won the Nobel Prize in physics. This shouldn't be taken as an indication that women haven't made outstanding discoveries in physics in XX century but rather of the sexism women in physics have often faced. For example, Madame Wu who was one of the greatest experimentalists of her day and designed and ran an experiment showing that our Universe is not symmetric under parity transformations. You remember, we covered this topic in episode 52 when we were talking about things going to the mirror world. Anyway, in 1957 Madame Wu was denied the Prize in favor of two of her colleagues who worked on the theoretical side of the problem. But like I said, only two women have been awarded this Prize in physics. You know the first one - it's Marie Curie but the second one was also brilliant. Her name was Maria Goeppert-Mayer and she shared the Prize in 1963 with J. Hans D. Jensen and Eugene Wigner for their work on the structure of atomic nuclei. Now, Maria Goeppert-Mayer's PhD thesis was kind of crazy - to my mind, at least - and crazy good. We've talked a lot on our show about quantum mechanics and atomic transitions. It takes a very specific wavelength photon energy to get an electron in one orbital to jump to a higher orbital. And upon dropping back down to the lower orbital, the electron will release a photon of the exact same wavelength. That atoms only interact with very specific colors of photons is the heart of the field we now call molecular spectroscopy which is recognizing which atoms or molecules are present in a cloud of gas based on which colors are absorbed or emitted. So you take a gas and you shine some light on it and then you look at the spectrum and the missing lines in the spectrum tell you what atoms are in it. Okay, but like I said - the atom will only absorb one specific energy of photon. It'll leave the rest untouched. Now, Maria Goeppert-Mayer's theory said that this wasn't exactly true. She said that if two photons with exactly half the required energy showed up at the same time, they'd get absorbed instead. It's like/ imagine if there was a parking meter that only accepted dimes. Maria Goeppert-Mayer proposed that putting two nickels in at the same time would also sometimes work. Or it's like two kids trying to sneak into a restricted movie by sitting on each other's shoulders and wearing an overcoat. It's a crazy argument but the effect works - these two little kid photons need to be really, really, really close to the atom at the same time to be absorbed.

Like, an attosecond apart. An attosecond, incidentally, is the amount of time that takes for light to travel the length of three hydrogen atoms. It's a really small distance. But, it works. So, today on the Titanium Physicists Podcast, we're gonna be talking about how this effect gets used to see inside things without perturbing them. Using lasers! It's called multiphoton microscopy and I'm so excited to be doing a show on biophysics. Now, the desire to see through a system without perturbing it is a lot like watching a play. The characters on the stage are meant to be as human as possible but somehow they're ignoring hundreds of faces that are staring at them and watching them and judging them. And somehow, in this most contrived of circumstance, the audience is meant to learn about the intimate workings of this human soul. So who better to invite on the show than a Broadway actor? Our guest today got a degree in Economics from Brown University and then moved to New York City, the Big Apple, to become an actor. In the last few years he's appeared in a variety of roles, both on and off Broadway. Welcome to the show, Brian Cross!

Brian: Hey!

Ben: So, Brian is currently featured on an off-Broadway play Desire, opening September 10th, at 59 East 59 Street Theater. Okay, so Brian.

Brian: Yes?

Ben: For you today, I've assembled two brand new Titanium Physicists. I call them my Biophysics Team. Arise, Doctor Nicole Prent!

Nicole: Choo choo! It's me!

Ben: Dr Nicole got her PhD from the illustrious University of Toronto in Biophysics, specializing in non-linear microscopy. She's currently teaching physics at Camosun College in a beautiful Victoria, British Columbia. Now arise, Doctor Jacqueline Townsend!

Jacqueline: Whoo-ooo-hooo!

Ben: Dr Jacqueline got her PhD from the University of Pittsburgh in Biophysics. She's currently faculty, teaching physics at Colorado State University! Alright everybody, let's start talking about light and cells and stuff. So, Brian, are there any questions to start off?

06:17

Brian: What is biophysics?

Jacqueline: Biophysics is basically a really broad field where we take a lot of sophisticated physics tools and principles of physics like thermodynamics and fluorescence and spectroscopy and all of these tools that come out of really high-end physics and turn those back around to look at life, to look at how proteins work, how enzymes catalyze reactions within the cells, how our muscles move. You know, all of the really physical and chemical processes that go about to enable life to be. You know, to look at those we have to go back and use really some sophisticated tools that have come out of the field of physics.

Brian: Hm. So in short/

Nicole: It's taking fundamental physics and putting it into a biological sample. You know, obviously makes it more complicated.

Brian: So, in a way it's a study of the way in which the physical, non-living phenomena on a very small scale affect and inform living phenomena?

Jacqueline: Yeah, that's a good way to put it.

Ben: So, today's topic involves the broader field of microscopy. Essentially, we wanna look inside stuff. And on the face of it, looking inside stuff should be fairly simple, right? Like, a microscope - what is it? We take some light and we shine it under a small sample of bacteria or whatever and the light's filtered through and then our eyes perceive it. And we go "oh, look, gross, I was drinking that". In some broader sense, though, if you want to go past that, we want to be able to discriminate between different things inside the system, right? I mean, one squiggly bit is going to look like another squiggly bit just in your microscope. So, essentially at this point in the game we want to move past that and we wanna talk about different techniques we've used to move past that to store or filter light in such a way that we can tell the difference between different things inside the cell.

08:20

Jacqueline: So I know a lot of the previous episodes on this show've been/ were about astrophysics and microscopy has followed sort of analogous paths to telescopes in terms of/ you know, when Galileo first invented the telescopes, you know, you could only see things very nearby, you know. And over last 150 years as the telescopes have gotten better, we can see further and further out into the Universe and into different galaxies and, you know, we have all those spectroscopic tools that let us see what types of elements are on different, you know, star systems and all that sort of thing. And with microscopy we've gone from - again, really, really basic - microscopes that just have lenses, you know, ground glass very similar in nature to the early telescopes, to developing tools that instead of letting us look further and further out now let us look further and further in. We are able to look at not just the cell itself but within cells. We're developing more and more sophisticated techniques that let us selectively look at different molecules within cells. And a lot of how we selectively look at different parts of cells has in the past mostly relied on staining things. And stains are molecules that, well, interact with specific other types of molecules in a very rough sort of way. You know, if you spill a glass of wine on your carpet, you get a stain on your carpet. If you dump a bunch of iodine in an onion cell - and a lot of you've probably done this in high school - you put iodine on an onion cell and then you look at it under a really basic microscope and you can see the outline of the cell. Because the molecule binds to specific parts of the cell and not others. And that early, early methods let us start developing a sense of what different parts of the cells were and there's a lot of limitations to those as stains only let us work at, you know, very very rough classes of molecules. Maybe they only let us look at lipids or only at proteins or only at cellulose. And so it's a very limited technique. It was best we had for a very long time but we're really moving a lot beyond that these days. So the types of stains that we've used historically have a lot of limitations to them. They only bind to very general classes of molecules and normally let us see very rough structures within the cell. And in recent decades we've been coming up with better and better ways of looking at finer and finer resolution images. Both in terms of the types of microscopy that we have available to us and also in terms of the ways that we actually visualize biological molecules. One of the other limitations of stains is that they kill the cells. So you can't look at something that's alive and also stained. You know, and dead cell are obviously not exhibiting a lot of very interesting biological behaviors because when we study biology, we wanna study things that are still alive. And so we're gonna kind of talk through some of the different types of imaging and get back around to how did we solve that problem of looking at very specific structures within living cells and the techniques that enable us to do that.

11:49

Brian: So staining is kind of foreign process to me. So just to get an understanding of exactly what that is/ Um, when you stain a cell, so you take the onion cell example. You look at it under a microscope and you're looking at the stained version of what is a dead onion cell, right?

Jacqueline: Right.

Brian: Are you seeing the cell or are you seeing it the way you'd see the negative of a photo? You're seeing, like, parts of the cell that are highlighted because of the staining but not the cell itself? Does that question make sense?

Jacqueline: Yeah, that's not quite right but that's not a terrible analogy. So when you're adding the iodine to an onion cell, you're highlighting certain structures and not others.

Brian: Okay.

Ben: The stain molecules will bind to some of the different types of molecules in it. They'll bind preferably to, like, a lipid or something. That way they'll, you know, glow red.

12:40

Jacqueline: So, iodine binds to starch molecules really well. But there's a lot more in that cell than just starch.

Brian: So the limit of staining, as a technique of seeing cells, is one - it only works on certain kinds of cells and two - even on the cells it works on, you're only highlighting a limited number of things within that cell.

Jacqueline: Different stains will only work on specific types of cells and on those cells it only highlights certain types of molecules. Plus, it only highlights the very general class of molecules. So, say, if you're using a stain that binds to protein, you know, there's thousands of different types of protein molecules within a cell but it will just show you all of them. So it's not very good at differentiating things - it's very limited, you know. Plus, with the fact that it kills the cells. And a lot of times we wanna look at live cells.

Brian: If only there were a better way.

13:38

Nicole: If only there were a better way. [laughs]

Jacqueline: It was a big advantage. Because if you look at that onion cell with no staining it's practically invisible.

Nicole: So, it was definitely a big step over "no stain".

Ben: There's a general principle behind how staining works that's consistent in all the other techniques we'll see today, which is that it makes part of the cell that you're interested in more visible. Discriminates between that part of the cell and anywhere else. And so you can tell where the parts that are starchy are or where the proteins are, right? That alone is helpful information but if you want to understand how, say, cells work in more detail, you need an ability to discriminate between very, say, specific proteins. You wanna know where some very specific proteins are or where those proteins go. And so the common element in all the things we're gonna be talking about

today is that we are doing things that make certain parts glow, essentially. Should we talk about fluorescence, a little bit more? Um, what is it? Nicole, what's fluorescence?

14:46

Nicole: Yeah, so fluorescence. Basically when we're looking at fluorescence is we're looking at an emitted photon that are caused because one photon has been absorbed. And it's absorbed by exciting an electron into an excited or higher energy level. This electron, when it's in the higher energy level, doesn't like to be upstairs, so eventually it will relax. And when it relaxes, it emits this photon which we call a fluorescence photon. And what's happening is because some energy is dissipated during this relaxation process, the color has a slightly lower energy than the energy of the photon that we put into the sample. So we can look for this specific color. And the goal is to put in a molecule or what we like to call a chromophore that will go to a specific location in the cell and then we can look for this color, because we know what color it should be given, and then we can tell where in the cell this is being located. So it's very similar to this staining only now we can start to have this molecule or chromophore be much more specific in where it gets located. And that brings us to a really big next development in fluorescent proteins.

Brian: You're saying it'll absorb a photon at one energy and release that photon at a slightly lower energy than it absorbed it at?

16:16

Nicole: Yes.

Jacqueline: Yep, that's right.

Brian: So, what happens to that removed energy?

Nicole: Oh, well, yeah. It gets dissipated into the system so, you know - you have some thermal energy and there are few other processes that can go on as well, too. I'm using usually highly connected systems so you could have some intramolecular energy transfer.

Brian: Hm.

Jacqueline: So like when you turn on a lightbulb some of the energy gets dissipated as heat, um, if you're exciting a photon that is going to produce a form of luminescence, there's also some energy dissipation that happens.

Ben: One thing we've focused on whenever we've been talking about quantum mechanics and absorption and emission of light is that the emitted frequency, the emitted color, is always gonna be the same as the absorbed color. I mean, that's the basic idea. You have a hydrogen gas and you shine white light through it and the hydrogen gas will get excited and grab out one very specific frequency of light and then if we turn off the backlight it will then emit that frequency. What we're talking about here is cases which are a little bit more sophisticated. In cases where you have big complicated molecules, the difference between energy levels can depend on a variety of things including the shape of the molecule and how the different parts of the molecules are vibrating amongst themselves.

Jacqueline: Proximity of other atoms.

17:40

Ben: Yeah, it's a lot hairier in molecular physics than it is in simple gases inside of stars and stuff. Um, so what you can get is, you can get systems that absorb one frequency - take, like black lights, you know? They're a type of lightbulb that emit ultraviolet, right? And ultraviolet is invisible to our eyes, so we don't see any lights. It looks like the room is dark. But there are pigments in our white T-shirt that absorb that high frequency ultraviolet light, some of the energy turns into heat, some of it turns into bouncing of the molecules around with each other and some of it turns into an emitted photon, which is at a specific color. And so this way you don't end up with the white T-shirt emitting ultraviolet light, it's emitting colors in the spectrum that we can see. So at this level it's a little bit more complicated than a simple rule where it's always going to reemit at the same frequency as it absorbed because there's all these other details involved as these complicated pigments jiggle around. And so what you can get in these cases is a system where we shine one color of light on it and then the pigments that we've attached to proteins and stuff will reemit a different frequency light that we can then detect. So, we'll shine blue light on it, say, and it/ some of them will emit green light or something. And then we can say "Okay, so those green lights are the pigments that we attached to various things". So we can use that fact that a different color is being emitted than we're shining on it to figure out where the things are.

19:19

Jacqueline: Yeah, and that's a really useful property that you can take advantage of because it's not like: you shine a light on it and then you turn the light off and then your energy comes back to you, right? This is all happening at the same time. So, if you're getting back light at a slightly different frequency than the light you're shining on the system, it's a property you can exploit by saying "I'm gonna setup my emitters at a certain frequency and my detectors at a different frequency" that lets you filter out the background noise of the light that you're putting into the system.

Brian: So it's a little bit like the molecule that makes up that protein; because it emits a certain color frequency, that's the equivalent of it being stained.

Jacqueline: Mhm. Yep.

Brian: Okay, I see.

20:07

Jacqueline: Yeah, it's a parallel to it being stained. So, that was a good question, 'cause that's a great lead-in to talking about the fluorescent proteins. So, not every protein naturally has these fluorescent properties in the types of spectra we're used to being able to collect. So, there's a protein that's called, very inventively, Green Fluorescent Protein which is a protein that fluoresces green. Now, this is originally a protein that came from jellyfish. That's how certain jellyfish can emit green light. And scientists were able to isolate the gene for the protein that creates this green fluorescence. And what we can do is we can create what's called a fusion protein construct were through molecular cloning we can take the gene for green fluorescent protein and put what's called a linker sequence, which is basically just a bunch of really flexible amino acids and then fuse that to an existing protein that we wanna study. So, if we wanna study a very specific protein we create this fusion construct and then we can put it back into a living system and then by using fluorescence we can detect where that protein - and only that one very specific type of protein - is normally located within a cell.

Ben: So, it's/ I live in Canada, right? And it snows up here and it gets really snowy and grimy. At a certain time of winter maybe around February everything'd be brown and white. We had this little

dog and she was always running away and she was also brown and white and so what we did was we got her a red collar with bells on it. And that way everywhere she went, she was still the same color as the snow but you could look out for this little tag of red.

22:08

Jacqueline: Little tag, yeah.

Ben: And hear the ringing. And so, this fluorescence is the same thing. There's some specific protein that you wanna keep track of in a cell and you're like "where does it go? Does it go and fight the nucleus or what's up?" And then so you tie a little bit of fluorescing molecule to it and then wherever it goes it's gonna have this little fluorescent molecule attached to it and so you shine light on the cell and then it will glow by green. And you can be like "Alright, it's a/

Jacqueline: It's here. It's in the nucleus.

Ben: It's in a Golgi bodies. That's interesting."

Jacqueline: Yeah. Yeah. The little dog's a good analogy because, you know, people think of pictures of cells it's like, okay well, it's a blob and there's a dot in it and the dot is the nucleus but that's not what cells are like at all because all the proteins are constantly moving around, flipping around, going different places, doing different things. It's really busy in there, so things are literally moving all over the place. It's not static. Which is why, going back to the limitation of staining, you know, when the cell is dead all those things stop. But all of those things are what makes life happen. And you know, that's what we wanna study so we wanna be able to follow the protein as it's moving around, localizing different places, doing different things.

Brian: Is the fluorescent tag otherwise benign to the system?

Jacqueline: Oh, that's a great question.

Nicole: Yes.

23:27

Jacqueline: So, yes, but it can potentially affect the behavior of the protein. What you would do when you're creating one of these constructs is, you know, test it to make sure that your system seems to be behaving the same way when you put it in as before. But if you're looking at something very sophisticated it might change the behavior. So, using the little dog analogy, maybe if it's got a big red bell, you know, it scares off the birds. And the dog would normally chase the birds but the birds were scared off by the bell so it doesn't do that anymore.

Brian: Like it drags on the snow.

Ben: Yeah, it's too heavy, so it can't run anymore.

Jacqueline: Yeah, so it's a much better tool than brute-force staining everything and it has a lot of advantages. One of the other advantages is that we've moved beyond just having green fluorescent proteins. There's ways that you can modify the protein molecule that, you know, some companies have, I think twelve different colors that you can get now. So, say, you have a little dachshund dog that you wanna see if it goes and hangs out and visits the neighbors' collie. So you put a red tag on your one little dog and a green tag on the other little dog and maybe they both go to the same place

in the cell. You know, they go to the same place in the neighborhood and they're hanging out together. Maybe they don't like each other, maybe they avoid each other. And so by tagging different color fluorescent proteins we can look at the behavior of a bunch of different proteins on the cell at the same time. But there is the caveat of it might be subtly modifying the behavior of those proteins in the ways that we're unaware of.

Brian: How does the binding happen?

25:12

Jacqueline: So the binding for a fusion protein construct is actually engineered. Which is one of the other major limitations of that system. So, you can only do that with something that you can genetically modify. So, if you're going to google and do a google search for GMO green fluorescent protein organisms, you'll have, you know, green glowing corn and green glowing kittens and green glowing, like, all different things. And these are all genetically modified organisms because to get these fusion constructs what you have to do is literally take the gene that makes green fluorescent protein and, like I said, add that linker sequence that's kind of maybe like a chain, like a ball and chain kinda looking thing. So, you take the gene for the green fluorescent protein, a DNA sequence that codes for the linker sequence, so the loose bit in between and the gene for the protein you wanna study and connect those all as a single coding sequence. So, you have to put that back into the organism to have it be expressed and functional with those cells. So you can only do this process for genes that you know what the sequence is, you have to be able to create the fusion construct, you have to be able to get it back into the organism, you know. So, this is something that is really useful for worms and fruit flies and plants and things like that but if you then wanna say "well, I wanna study where this protein goes in people", you run into some issues because we're obviously not gonna be able to do that.

Nicole: [laughs]

Jacqueline: So, that's the other really big limitation of this/ is you have to be able to create these fusion genes.

27:02

Ben: So, you're telling the cell's nucleus to make the fluorescent/

Jacqueline: The cell has a schematic that tells it "here's how I make"/ like, most proteins are tools. Like, here's a schematic for how I build a chainsaw. And then you take away its schematic that says "make a chainsaw" and you give it schematic that says "make a chainsaw with a little glowing ball attached to it". And it says "well, I need a chainsaw" but because you took the schematic for a normal chainsaw away, it makes a chainsaw with a little glowing ball attached to it 'cause that's the schematic it has to work from now.

Ben: I see. And that's why we can't do it to human cells 'cause that would involve mucking around with human DNA.

Jacqueline: Yeah.

Nicole: Yes. So, you know, you want to be able to see what's going on without really having to add anything in. So what we say is we're looking for something that's inherent in the system already. And not really lot of stuff likes to fluoresce naturally in cell. It's actually quite not exciting in that sense. There are a few things but we generally want avoid it because it ends up damaging the cell. However,

what we can do is we can look at non-linear interactions. So, what happens if rather than having just one photon show up, what happens if we have a whole busload of photons show up? And this is where we get into, you know, using the two nickels rather than the one dime. So if we have a whole bunch there, then there's a big probability that two of them might end up being within a certain distance in order to excite this electron. In this case, we begin by starting with fluorescence into its level and then we will see the emitted photon and this will be very close to half of the original wavelength. Now, I say very close because we still see this energy loss or energy deposition into the sample. Again, as I said, not a lot of stuff naturally likes to fluoresce, or emit this photon. It'll absorb but won't always emit a photon and we need to detect something so we're looking for something to see, to visualize. And so one thing we can do is we can look at the effect that is called harmonic generation. And basically it's frequency doubling or tripling the incident light. So, you send in your photon with one energy, you'll get the photon with double the energy. And depending on what you see, it's linked to the type of material.

Brian: Can you clarify that a little bit? So, is the issue that certain frequencies simply won't be absorbed at all or that there won't be any emission as a result of certain frequencies? What sort of system uses the half-wavelength that Benjamin introduced in the beginning?

30:00

Nicole: Yeah, so it's more that most don't give off anything that we can detect.

Brain: Okay.

Nicole: So, we could for example go back to the staining process and we can do this multiphoton fluorescence. And it does has some advantages because what we can do is we can localize the excitations to small area. For example, we can build a three-dimensional image of our cell. And in order for us to do this, to use this multiple photons, we have to get into really cool lasers. What we do with these lasers is we pulse them, so that instead of having a continuous amount of energy, we send in pulses of lots of energy. And this lots of energy comes in, excites the electrons and then you get a rare event where you might have two photons simultaneously absorbed and then you might get for example a fluorescence photon that you can detect.

Brian: Aaah.

Nicole: Yeah. [laughs]

Ben: Okay, let's back the track up. 'Cause we're going gangbusters and that's great.

Brian: [laughs]

31:05

Ben: At the very intro to this show, I talked about this photon doubling thing where your two photons come in and then they sit on each other's shoulders and then the atom goes "Oh yeah, you're an adult" and then it spontaneously absorbs and emits almost. It changes it into one photon. And the neat thing about this is that it's a completely different color from the two incidental photons. It's one that's like much more energetic, it's much more bluer than the other one.

Brian: Hmmm.

Jacqueline: Two photons enter, one photon leaves.

Ben: Yeah

Jacqueline: And it's totally different photon!

Brian: [laughs]

Ben: Yeah, so it's/ you bathe it in one color light, and then you get these emissions of another frequency and so you can see where this is happening but the downside to this/ the thing is, it only happens if the two little kid photons, those two nickel photons are really, really, really, really close together. And so, what Nicole's saying is, the trick here is, we're gonna make it happen in certain places/ you know how you take a, like a magnifying glass and you focus the rays of the sun, right? So, the sunrays come in, they're pretty much all parallel and then they pass through the magnifying glass and then they end up in a cone and they get smaller/ closer and closer together until they all pass through a point, right?

Brian: That point sometimes being an ant.

Ben: Yeah. Sure.

Nicole: Which would then light on fire.

Ben: Which would then light on fire. So at that point the density of photons is really, really high, right? So prior to that, on the other side, on any other point, the photons aren't all that dense but at that focal point, they're really, really, really super dense. So what you can do is: you can use a lensing system to take this laser and focus it down to some point. You can focus on a point inside the cell and at that point there'll be so many of these low energy photons that some of them will get changed into a high energy photons through this process.

33:01

Brian: Surely probabilistically, right?

Nicole: Yeah.

Brian: Like, there's just so many of them that the odds of two falling into orbital length, the distance you said, an attometer?

Ben: Yeah, the width of three hydrogen atoms, I think.

Brian: So the odds of that happening drastically increase when you increase the density of the photons.

Ben: That's right and you can do that by lensing the system.

Brian: Okay.

Ben: And so/

Nicole: Lensing and pulsing.

Ben: Yeah, yeah, so let's

Jacqueline: So the ant doesn't set on fire.

Brian, **Nicole:** [laugh]

Brian: I've never actually done that. I hope that you don't judge me. Anyway, go on, please.

Ben: So, in the system what'll happen is: you'll see this fluorescing only from the part where the light is focused. Only the atoms in that little region where it's dense enough will have this fluorescing effect. You'll only see photons coming from that little bit. And so you can select where in the cell you want to probe because only in that one place will these low energy photons be dense enough to cause one of these fluorescences. But here's the kicker - it's that you know how the ant melts?

Jacqueline: None of us have ever actually done that.

Nicole: [laughs]

Ben: Melting an ant? I've never/

Brian: I've never done that.

Ben: I think that it's true. An older kid once told me to do it but then I don't think he could get it to work. But, you know...

Brian: Crisis averted.

Ben: That's right.

Nicole: Crisis averted

Jacqueline: Maybe grass I've tried to set on fire

Nicole: Yeah, I've/

Brian: Or leaves.

Jacqueline: I've done the leaves thing.

Ben: So why does the leaf heat up and catch on fire? It's a good question. And the answer is: it has to do with temperature, right? It has to do with energy density, if it's hot enough, something will catch on fire, right? Fire isn't some magical liquid, it's just a criterion. If there's enough temperature and there are oxygen atoms around and things for the oxygen atoms to bind to, you'll get fire happening. So, in the leaf what'll happen is: you'll put the leaf in the focal point and the energy is building up in that little concentrated region faster than it can leave leaf.

Brian: [laughs]

Jacqueline: Leave the leaf.

Ben: Faster than it can dissipate. And then so the temperature increases. And you run the same risk inside of the cell. So you shine a laser through the lens and it focuses down to a point and if you're not careful, you can melt everything in that point, right? You can mess up/

Brian: Hmmm.

Ben: Trying to study a protein or something, you can totally mess that protein up with too much. So what do they do? They use femtosecond lasers.

Nicole: Yes, I've actually unfortunately blown up a few yeast in my lab.

Ben: Really?

Nicole: Yeah. [laughs]

Brian: [laughs] What does it look like?

Nicole: I was just trying to see them. Well, it looks like an explosion really on your microscope screen. It starts as a little ball and it gets bigger and it's basically something that's happening at such a timescale that normally wouldn't be biological. Yeah. So what we do is we take that energy and we put it into a femtosecond pulse - a very short amount of time. So we get that energy density but we give the system a long break. So we kind of give it a shot and we let it wait. Then we give it a shot and we let it relax. And this prevents us from damaging the cell so we can do this non-invasive imaging. Um, so fluorescence is great but there are some disadvantages. Because you are, you know, exciting the system and it's relaxing and the energy is being put in. So, there's something else we can do, and that's where we can look at harmonics. So, you know, if you're familiar with music, you know about overtones, you have a fundamental frequency and you can get multiples of that frequency. So what ends up happening when you drive the system with a lot of intensity, these harmonics have a greater probability of being able to see them. And they don't depend on the frequency you put in. So yeah, so you end up with exactly double the frequency or double the energy or half the wavelength. So it's a very narrow wavelength that you can look for so it's very, very specific. So for example where we can see this/ you know, obviously crystals is one of the first places where the second harmonic was observed because of this high-ordered structure of these crystals. And there are certain biological structures like collagen which is located in your tendons as well as in your muscle and they are ordered enough that you can efficiently generate the second harmonic. And so you don't need to add anything to the cell. It's just purely based on the structure of the cell. And of course having enough intensity to go in there and probe the system.

Brian: Can I sum it up in a sentence to see if I get it and then ask a follow-up question?

Ben: Uh-huh.

Nicole: Yes.

Brian: To be clear: this mechanism of trying to locate molecules using lasers, you can use a high density/ Is a laser just a series of photons in high density?

Nicole: Photons are waves, yeah. [laughs]

38:19

Brian: Okay, so you shoot a lot of photons in high density at a point that you want to explore and the molecule will, under the right circumstances, absorb two photons and emit one photon back at a much higher energy, correct?

Nicole: Yes. Exactly double. Yep.

Brian: At exactly double. And when you can detect that photon, you can deduce things about where it came from i.e. whatever that protein or molecule was you were trying to explore, right?

Nicole: Where it is, what kind of structure. So in general most biological media is isotropic or random, so you would actually not see the second harmonic for most tissue. But when you do see it, you know that there's something very special about that structure what you're looking on there.

Brian: Can you explain to me why you can't just shoot photons at double the energy at them in a first place? Have that one to one thing?

Nicole: You can. This process doesn't actually involve having to be excited. So here we've taken away that dependence on matching the energies of your incident photon and the excitation energy of your electron. Yeah, so other things for example if you go to a longer wavelength, you can actually propagate further into tissue. So there are few other, like, little advantages that we talk about. Like, we call this penetration depth. There's less scatter, which is, you know, important when you're trying to get a nice, clear image. You get a slightly better resolution because the light's confined to this focal region, so you know that it has to be located within that focal spot area.

40:00

Brian: So there's the benefit to using lower energy photons in terms of the information you get back?

Nicole: Yeah, of course it has its limitations in the sense that only very specific structures will be able to do this. Turns out that these structures are very interesting. Collagen, for example, for cancer research; muscles for, you know, you can go into robotics, you know. There's all sorts of places where you can go once you sort of understand the fundamental physics of the biological process that's going on.

Ben: So what you're saying is, you do this laser thing where you're focusing the laser to a point and then you'll see a different amount of frequency doubled and tripled harmonic photons reemitted or scattered, depending on the type of material it is. And so you can use this to tell what type of material is at the focal point of the laser at that point in time.

Nicole: Yes. Yes.

Jacqueline: Mhm. It lets you be more selective.

Ben: Right. So there's a couple of benefits to this that I can see. And one of them is/ you asked why we don't just shine really intense light on it. We want to perturb the system as little as possible, right? So, the drawback to using staining is that it kills everything. And the drawback to using the green fluorescent protein is that it adds like, you have to change the DNA and it adds a little ball and chain to the protein and you're not sure if that changes things, right?

41:31

Nicole: Mhm

Brian: Uh-huh.

Ben: Similarly, if you use really high frequency light, you end up, you know, denaturing proteins or shooting of electrons or who knows. The lower frequency light you use, the less it's gonna muck up the system. And so we use/

Brian: Ooooh.

Ben: really low, gentle light that penetrates deep in the system, that doesn't muck anything up and it ends up emitting a different color that's really easy to spot.

Brian: So it's like you're avoiding giving the system a sunburn.

Nicole: Yeah. Or, you know, like an X-Ray. You know that X-Ray is bad because it's ionizing radiation. Because it, you know, basically can ionize the electrons 'cause that's the higher energy. So, it/ obviously the imaging process is a little bit different because they're really just looking for attenuation rather than emission but still, like, you basically wanna avoid being X-Rayed because it has potentially damaging side effects.

42:28

Ben: Another reason it seems to me why you wouldn't just shine a whole bunch of really intense light/ I mean, you could. You could use an optical microscope just like you use a regular microscope and shine a bunch of intense light up through the bottom and then look at the shadow of different things. And you could do that. It seems to me that the benefit of this particular method is when you're doing it that way, you just see the shadow of everything. You can't see, say the vertical structure of the system is. In this way, it's kind of like being in a car, driving your car during like a fog storm or like a snow storm. So you're trying not to hit the car in front of you so you're looking out for the brake lights of the car in front of you on the highway, right? If you wanna see them, there's light being emitted from those brake lights and you can see them but if you turn on your own headlights, brighter, so you turn on your high beams, the reflected light from all the stuff between you and the car in front of you would wash out the image that you're looking for. So it might be more helpful if you're looking for particular structures to have those structures emit light themselves than to try to get them to reflect or absorb light that you're shining on them.

Brian: Interesting.

Jacqueline: Very good.

Nicole: Mhm.

43:40

Ben: Oh yeah, so one interesting thing that we're emphasizing is that you don't actually need to tune the frequency. So in a regular laser when you're trying to get emission, you have to choose a really specific frequency. In this case do you not have to? You can use any frequency you want, essentially to get this frequency doubling or?

Nicole: Yeah, there are some things like, for example, you go too low in the infrared, like 800 nanometers, you can actually excite some natural biological molecules like NADH. You know, so you might wanna avoid that. And if you go too high into the infrared, the water starts to absorb and of course/

Jacqueline: You start microwaving the cells and it's bad.

Nicole: Yeah. [laughs] So yeah, this little/

Jacqueline: That's how microwaves work.

Nicole: [laughs]

Ben: So there's a band that you need to live inside. But you can choose any frequency, any convenient frequency in that band and you'll see this frequency doubling.

Nicole: Mhm. Yeah, you can see some like resonance effects. So if you're actually close to an absorption, you might see an enhancement of the effect but it really depends on, you know, what you're looking for when you ultimately design your system. You want to avoid all fluorescence, you know, to protect the system. Or do you want to have fluorescence with your harmonic generations so that you get more information. And we can learn more.

45:00

Brian: What kind of scale are we talking about here? In terms of the information you collect. How small are we talking?

Nicole: We're looking at cells. You know, a typical image might be, you know, as small as 10 by 10 micron image to 100 by 100 micron. You know, of course, every system is a little bit different depending on how fast you wanna scan the image. If they're looking for dynamics or high resolution. Yeah, there's a lot of development and, you know, do you wanna build an endoscope? So, you know, for example they're designing these things that can go on the end of an endoscope so they can go inside your body for example to detect cancer. You know, in situ rather than having to take sample and stain it and go through all that process.

Ben: Tell us about the videos you make and how you make them of muscle cells flipping out and stuff. 'Cause those are really awesome.

Nicole: Yeah, so. Muscle as you know, its main function is to move things, right? So, you know, someone throws a ball at your head. Hopefully, you're able to react and have your arm catch the ball before it hits your head. So, it wants to move. So how can it move? So you have what we call myosin nanomotors that undergo conformational changes that allow what we call filaments to slide, which allows the muscle to contract, to elongate.

46:19

Ben: So inside the muscle cell there are these fibers and there are these things, myosin nanomotors, that slide the fibers around, that causes/

Nicole: Yeah, picks it up and pulls it/

Jacqueline: Think like, the train tracks, you know, and then there's this little cart things, you know, they show in the old timey movies and the people with the carts with the handles they push up and down that go along

Ben, Nicole: [laugh]

Jacqueline: And it's a lot like that, how they work.

Nicole: So yeah, the way muscle is made: you have two main flavors of muscle. Smooth muscle which we'll kind of ignore and we have striated muscles. This is your heart muscle cells, your regular skeletal muscle cells. They're striated or striped. And so part of the stripes are what we call anisotropic and they're what produces the second harmonics. So it will frequency double the incident light, provided that you have enough intensity. And then right beside it is an isotropic band, which does not do this frequency doubling. So you get a very clear image of bright bands and dim bands. And what can happen is when you image this live, right? You can see the striations moving back and forth. And one of the things that was really interesting is if you look at your first-year biology textbook, muscle's not really doing very much until it's been activated and it contracts and it goes to not doing very much. But what we see is that it's actually more of a dynamic system. It's kind of moving around a little bit, even though it's not undergoing any net big changes. So it's sort of like it's ready to contract. It's kind of at that/ Like a scale - if you just tip it over a little bit, and you can have that really fast response. If you really think about the chain of events that happen between you seeing the ball and your hand catching it, there's a lot of things going on and it happens so quickly, all based on essentially gradients, chemical gradients, electric or potential gradients across membranes.

48:18

Ben: Oh man, I don't think Brian's quite getting the enormity of what you're saying.

Nicole, Jacqueline: [laugh]

Ben: So, what she does is, she takes one of these little muscle cells or bunch of them and she hooks up her laser whose focal length can be moved throughout the cell and then they essentially shine up a cross-section of the shell to glow in some places. And so, so the myosin is going to/ it's gonna do this frequency doubling and it'll emit these bluer colored photons and so you can see them. And so she'll take one photo after another of it as it evolves in about a video photograph lengths of time. Like, you know, sixteenths of a second amount of time. She'll do it over and over and over and over and what you end up with is a video through the middle of a cell and see what it's doing/ how it's jiggling around. And there's a video of this thing.

Brian: That's really cool.

Ben: Amazing, absolutely amazing.

49:11

Nicole: And one of the things to remember is that we've haven't changed anything other than the fact that we dissect it a little bit. But we haven't added anything to the system. So it's behaving hopefully as much as it naturally would in the organism itself. I believe that the one we did was actually just a (...) fruit fly maggot. We basically just kinda cut them in half [chuckles] and imaged them like he was alive and maybe not super happy but/

Brian: [laughs]

Nicole: His muscles were contracting and frequency doubling light and showing a great movie of a muscle contracting in action.

Brian: So, this technique, just to be clear, it allows you to see like, relatively speaking, very high definition photos/videos of what before was more of a grainy picture of these cells?

Nicole: Yeah, you could use, like, a white light colorization microscope and you can see the striations of muscle.

Jacqueline: You don't really get the movement.

Nicole: Yeah.

Brian: Uh.

Jacqueline: 'Cause again you're staining cells and they're dead. That's why we always had/ we had the pictures in the textbook of like, "oh, here's a contracted muscle and here's a relaxed muscle" and, you know, it's this way or that way and nothing in between. But, like she was just saying, it's a lot twitchier if you look at it live.

Brian: I see.

50:32

Ben: Yeah, she can watch a live cross-section of the cell jiggle around as opposed to essentially dying the parts that you want to, killing the cell and then projecting light through it and looking at it.

Nicole: When you project light through it, 'cause you're looking at the part of thickness or all on one projection as well, so remember we have that focus in there so we're just looking in a small piece of that muscle. Oh and the other thing too is, going even further from there is, can we actually rather than just looking at the contractions, can we learn something? So for example maybe if we add other parameters like looking into polarization of the light might tell us information on the structure of the myosin as it's undergoing the contractions and that's basically, like, the goal is we start with these tools and we try to answer important questions, we're trying to learn important things, based on what we know about physics.

51:32

Brian: So, not everything does this frequency doubling?

Nicole: No, no. It's actually most things don't.

Brian: But if it did, it would be like/ it sounds like it'd be a useless tool in this sense. Because if everything did it then you wouldn't be able to detect anything individually. But by the virtue of the fact that some things do and some things don't, you can see the juxtaposition, right?

Nicole: Exactly.

Jacqueline: Right, 'cause if everything did it, again you'd just see everything and wouldn't be able to tell one thing from another. So this is another tool in our toolbox

Brian: Does everything that exhibits frequency doubling, like Benjamin was saying, it can occur at any wavelength?

Nicole: Yeah, theoretically, yeah. Like, if the sample happens to absorb the color that you shine in, you know, there might be other nitty-gritty details you might need to watch out for. But if you just talk about the theory, theoretically whatever you put in, you get double out.

Brian: So the things that don't do it, don't do it at any wavelength but the things that do it, can do it at any wavelength?

Nicole: Yeah, you're getting there. It has to do with like, for example the symmetry of the system.

Brian: Hmm.. Why do some things do it and some things not do it?

52:44

Nicole: Yeah. So for example with the second harmonic, it needs a non-centrosymmetric system. So one way to sort of think about it is you have a guitar string. If you pluck it in the middle, you're basically forcing there always to be an antinode or a vibration in the middle, so you're only gonna be able to see like odd harmonics from that type of system. But if you induce an anisotropy across it, or for example don't make it symmetric, than you can for example see the other harmonic. And with second harmonic, basically what we're looking for, what it probes, is highly ordered structures that aren't what we call inversion symmetric. So if you rotate it, it looks different. So it's not the same.

Brian: Hmm...

Nicole: If you go forwards, if you go backwards. And then, for example, if you go to frequency tripling, we end up/ In a microscope, we end up seeing that happen at interfaces. So for example, you might see a membrane interface where we have a change in what we call the index of refraction of the media. So you get different/

Brian: Oh, wows.

Nicole: Yeah, you get different effects depending on, you know, what you're sort of looking for in it. You know, you get into, you know, lot of material properties.

Brian: So, things can frequency double or frequency triple, you're saying? Or go to the n-th degree?

54:08

Nicole: Yep. Just less and less probable. 'Cause you need more/ Now you need three photons.

Brian: Yes.

Nicole: Yep.

Brian: But every now and then, not sure how precise or obviously I don't know the specifics of how these experiments are conducted but if you throw a lot of photons in high density at a place and you're looking for the frequency doubled, every now and then can you also detect a triple?

Nicole: Yeah. It all happens at the same time. So you put in that intense light, you're gonna get, for example, if it's non-centrosymmetric and there's an interface, you can get, you know, both signals out from that same spot.

Brian: And you're saying that a frequency triple can convey to you different information than frequency doubling?

Nicole: Yes. Yes.

Brian: That's very cool. [laughs] That's so cool.

Nicole: So, for example with the muscle. Like, with the muscles I can see this myosin, like this striation, these stripes in the muscle cell, but then I can see the sarcolemma on top of the muscle fiber which is this membrane on top of it with the third, the frequency tripling on top. You know, another idea is to get into looking at mitochondria, which have a multi-layered structure and using looking at the third harmonic to look at the, you know, the energy or the powerhouse of the cell and then we have their second harmonic to see the response of the muscle. Sort of like this idea, combining different modalities to try and learn as much as we can. With the same system, you could put in chromophores, you could maybe stain, you know, membrane of your neuron. You know, you might be able to look at the fluorescence of your neuron and look at all three different things interacting at the same time.

55:45

Jacqueline: Yeah, like you could put a GFP tag on a signaling molecule and see what the response time of, you know, the signaling molecule triggering of muscle contraction or something like that.

Ben: GFP, by the way, is Green Fluorescent Protein, you should say.

Brian: Yep, thank you.

Ben: That's the thing we were talking about earlier.

Brian: Yep.

Nicole: And what's great is this all happens simultaneous. If we're only using one color, you could look at three different colors that are being outputted and they're all giving different information. And then you could go to your biochemistry and maybe have some single molecule experiments and you might want to learn about how protein's shaped. And, you know, you talk with that person, you research everything and altogether you learn about the system, 'cause it's so complicated. There's no one golden imaging device.

Brian: But it seems like staining is to a painting as this technique is to a sculpture. Is that accurate? You're getting multiple layers.

56:45

Jacqueline: It's more like the difference/ so, if staining is a painting, then this is a Michael Bay movie with, like, explosions and cool video and lots of stuff happening. Maybe not Michael Bay but, you know, like you're getting the 3D and the things happening in real time and/

Brian: Oh, I see.

Jacqueline: A lot/ that you can see a lot more of the dynamics of the system.

Ben: You know, before we only had paintings of trucks to look at whereas now we can tell that trucks stand up and walk around and talk to people.

Jacqueline: Yeah [laughs]

Ben: Since Michael Bay came along our understanding of trucks is greatly improved.

Jacqueline, Brian: [laugh]

57:33

Ben: Well, that was fantastic. Thank you, Nicole. Thank you, Jacqueline. You've pleased me. Your efforts have borne fruit and that fruit is sweet. Here's some fruit. Nicole, you get a nectarine.

Nicole: Om nom nom.

Ben: Jacqueline, you get a bowl full of cherries.

Jacqueline: Om nom nom!

Ben: Fantastic. I'd like to thank my guest, Brian Cross. Hey Brian, I hope you had fun.

Brian: It was really fun. Thanks for having me.

Ben: Break a leg on that stage! Alright, that's it.

Brian: Thank you.

Jacqueline: Thanks, Brian!

Nicole: Thanks, Brian!

Brian: Thanks, guys! Thanks for teaching me!

58:04

Ben: Well, that was fun. That episode was pretty good and if you listen past the announcements and the end song, you'll hear another half hour of conversation where we talk even more about various other things. It's pretty fun. It's a pretty fun show. This was fun. I had fun. Okay, so, it's announcement time. First, I hope you're enjoying the Question Barn episodes - we haven't made one in a little while, we'll get back on the horse. We're still taking your questions and we like answering them. So, I've got a bunch but if you send me your questions, I'll add them to the pile and we'll get to them eventually, hopefully. I'm optimistic we will, at least. So, if you'd like us to answer your questions, send your questees to tiphyter@titaniumphysics.com. Second, we're looking for a person or people to help transcribe our episodes. We can pay a fee per episode for your work and we have enough saved to transcribe a few dozen episodes. So email me if you're interested and we'll talk about the details. Now second second, on that note, I'd like to remind you that we're now accepting donations. We'd be grateful to receive your support. You can make a one-time donation using PayPal through our website or you can set up automatic donations every time I finish an episode through our Patreon website. This particular episode of the Titanium Physicists has been sponsored by a collection of very generous people. I'd like to thank the generosity of Hannah Walls, Tony and Sam Bougue. I'd like to also thank miss Melissa Burk, Yaseem Owarzazeem, Spider Rougue, Insanity Orbits, Robin Johnson, madam Sandra Johnson, Mr. Jacob Wick, Mr. John Keese, a Mr. Victor C., Ryan Close, Peter Clipsham, Mr. Robert Halpen, Elizabetha Theresa, and Paul Carr. A Mr. Ryan Noule, Mr. Adam K, Thomas Sharay and Mr. Jacob S. A gentleman named Brett Evans also supported us, a lady named Jill, a gentleman named Greg, thanks Steve, Mr. James Clawson, Mr. Devin North, a gentleman named Scott, Ed Lowlington, Kelly Wienersmith, Jocelyn Read, a Mr. S. Hatcher, Mr. Rob Aberzado and Robert Stietka. So, that's it for Titanium Physicists this time. Remember, if you like to

listen to scientists talk about science in their own words, there are lots of lovely other shows on the Brachiolope Media Network. So, follow 'em from our webpage. The intro song to our show is by Ted Leo and the Pharmacists and the end song is by John Vanderslice. Good day, my friends. And until next time, remember to keep science in your hearts.

60:50

[Outro song; *Angela* by John Vanderslice]

61:44

Brian: Can I ask more questions?

Ben: Sure, go ahead.

Brian: So, you describe some of the images that your work has produced. What are some of the other things that biophysicists have learned using this technique about cells?

Nicole: Okay, so one thing that my group is/ my old group, I guess they're doing a lot of work on is, I guess, like early cancer detection type thing. Focusing on collagen. Collagen is another frequency doubler. You see collagen, it's in your tendons. Actually my dog recently has ruptured her cruciate ligament and the vet gave me her ligament and collagen is really beautiful. Sort of iridescent. If you look at it in the microscope it has beautiful patterns and zig-zags. But the idea is with cancer is you get a little bit more collagen buildup. So as sort of a precursor or an indicator that cancer might be there. And the idea is trying to move to something that's more in vivo versus sort of, you know, if you go and get a biopsy, they take it out and they stain it and usually you can't tell until it's pretty far along.

Jacqueline: 'Cause again, the staining kills the cell and modifies a lot of things and so you can get earlier detection if you're using this type of live cell imaging.

63:14

Nicole: Yeah, and I don't know, Jacqueline, if you also want to talk about (...), cause we kind of skipped over FRET. It's kinda cool.

Jacqueline: That's like a whole other lots of stuff.

Nicole: Yeah. It's kinda cool. I don't know. As far as, like, learning information and how this is being applied. Like I know, I've been to a couple conferences and they have these mice with basically a microscope slide installed overhead and they were using a lot of these fluorescent proteins and to watch how, you know, the neurons change and grow over time. So it was really interesting stuff. Like there's all sorts of places we can go.

Jacqueline: With different imaging systems, yeah. So, Nicole mentioned FRET, so that's fluorescent/ now I'm gonna screw it up. We said it like 50 times (...)

Ben: Förster...

Jacqueline: Förster Resonance Energy Transfer. I said it 50 times this afternoon and I can't say it anymore. But that's another one of these fluorescence energy transfer techniques where instead of, you know, shooting a bunch of photons at, you know, at a live cell, and looking for these stochastic

doubling effects, what you can do with FRET is looking at the way/ Basically the way that energy can transfer between very close molecules. So, this is another system where we have to modify things so we can't do FRET on just whatever. But we can get away with much smaller modifications. So instead of, you know, a ball and a chain that we're tying to our dog, you know, to go back to that metaphor, it's maybe like a little jingle bell instead. So it's gonna affect the system a lot less. So that marker would be called/ that's a light-sensitive molecule called a chromophore. So we're just putting a really small one on a protein. And so where the energy transfer comes in is that if there's two chromophores in close proximity, one of them can through a resonance process transfer energy to the other one. Getting back to some of the metaphors and analogies with soundwaves... So, you know, if you start/ if you have a singer singing at a certain frequency and she's really close to that crystal wine glass and it starts resonating in response to the frequency of the energy that the soundwaves that the singer's creating. You can see a similar sort of thing with electromagnetic energy. So this type of fluorescence we're talking to. So, you excite one chromophore and get it emitting. So that's referred to as the donor chromophore. And if there's another chromophore, that's referred to as an acceptor chromophore, in very close proximity, it can respond to the energy that the donor chromophore is generating. So, why this is useful is because it lets you do things like look at the proximity between things that are very close together within cells, such as/ So, instead of looking at this like big/ these large scale changes in muscle contractions, we can look at things like within one single molecule/ how is one single molecule interacting with itself. Or interacting with another protein that it might be binding to very closely. And you can look at things like - you know - under certain conditions is it getting closer, is it getting further away, how tight are those associations. And it's really useful to be able to look at something at very, very small scale distance changes because you can look at conformational changes within a single molecule of protein. And that's important because, again, most of these biological processes are coming about as different protein molecules, different enzymes within the cell are undergoing these conformational changes like, you know. Like the Transformers, right? You know? Trucks aren't just trucks sitting on a parking lot somewhere. They're turning into giant robots and making ATP and making our muscles move and doing all these different cool things and it's hard to see within a single molecule what's happening but it's so important to know how these things work because these are the processes that make life happen, you know. This is how we learn how to understand disease. This is how we learned all these different things, you know. What makes them break and how we can fix them and understand all of the biochemical reactions that create life. We need to know the enzymes that are creating the energy, the enzymes that are, you know, modulating these reactions and enabling them to happen by looking at these types of protein conformational changes. Like Nicole was saying, we can also do things like sometimes look within an organism to see, you know, are different proteins associating with each other under different types of circumstances? Like, what's triggering different reactions, what causes them to happen.

69:21

Ben: Okay. Okay. Wait, wait, wait, hold on.

Jacqueline: [laughs] I'm just going and going and no one's asking questions about (...)

Ben: Okay, so in this thing you have a bunch of, they're called chromophores, right? And the chromophores are the - what's it called - fluorescing little bits. They're the little bells that

Jacqueline: Little bells.

Ben: That fluoresce, right?

Jacqueline: Yup, yup.

Ben: And you're saying that the idea is that if two of them are close enough, if one is fluorescing, the other will fluorescing.

Jacqueline: Yeah, in response at a different frequency.

Ben: What's causing/ at a different frequency/ what's causing the first one to fluoresce?

Jacqueline: Um, so you have to/ This is another one where you need to add energy to the system.

Ben: Right.

Jacqueline: So, you're shining some light, you know. And there's different ways of doing this, depending of what kind of/

Ben: You're shining/ So, if there's two chromophores

Jacqueline: Uh-huh.

Ben: You shine green light on it, one of them starts glowing blue and then the other one, if it's close enough, it'll start glowing orange. Is that correct?

Jacqueline: Yeah, like orange or yellow, or/

Ben: Some other color.

Jacqueline: Yeah.

Ben: And then so that tells you how close they are together, right?

Jacqueline: Yep.

Ben: And so the idea is that, you know, one of the things we're interested in, like you said, is protein folding.

Jacqueline: Mhm.

Ben: 'Cause it's not just the chemistry of a protein that determines what it does, it's also its overall shape.

Jacqueline: It's the structure and how it changes.

Ben: They flip and flop around like slinkies, doing whatever, right?

Jacqueline: Mhm. It's a lot more sophisticated than that.

Ben: Yeah, that's right.

Jacqueline: It is a lot like Transformers.

Ben: You were saying it's like Transformers, right? So, Optimus Prime has/ Like, he has a giant man mode. And so we attach one chromophore to his head and the chromophore to his hand and then

we say, "go into truck mode" and he does, and using/ Then, looking to see how the orange one and the blue one glow we can see how close they are together.

Jacqueline: Yeah.

Ben: And from that tell how the protein has changed shape.

Jacqueline: Yep.

Ben: Or alternatively, if there are two different proteins and we wanna see how they interact, we attach chromophores to each of them and then make one glow and see if the other one glows and that will tell you how they're attached and wiggling together.

71:44

Jacqueline: Yep.

Ben: Okay.

Brian: Is the second one glowing/ This could be wrong. And if it's wrong let me know. Is the second one glowing because if they're close to each other, it's absorbing the photons emitted by the first one? Why is the second one glowing at all?

Jacqueline: Yes. Yes. So what happens is you put energy into the system. So say you're shining, you know, UV light at a certain frequency into the system and so the first chromophore, the donor chromophore reacts to that. So, you know, the opera singer is singing and the wine glass starts resonating. Say there's a slightly different wine glass, like, right next to it. And it picks up on that energy as sort of a secondary effect.

Brian: Hmmm...

Jacqueline: So another way of thinking about it/ An analogy I use a lot is: so if you have kids on a swing set, right? Like, say you've got two different kids on a swing set right next to each other. And I don't know if you've ever done this or seen this but someone's pushing the first kid, you know. And they get going really good. And the kid's swinging and swinging and swinging and the energy kind of builds up and transfers along the top bar of that swing set and travels down and so that second kid will start swinging at slightly a different frequency. But you know, that energy transfers over.

Brian: Hmmm...

Jacqueline: To create an effect. This is kind of that on a quantum level. So, the energy coming into the system gets the donor chromophore glowing and making a certain kind of light and then a nearby acceptor chromophore is absorbing that light and emitting another light. So, how this works is because/ So we were talking about how with fluorescence you have energy coming in, exciting a photon and it's a little bit of the energy kind of gets lost and it emits at a slightly lower energy level, right?

Brian: Mhm

Jacqueline: So, this is kind of like a cascade effect. So, you're putting in energy at a certain level that gets your donor excited to a certain degree and then it's emitting photons that are a little bit relaxed, a little bit more chill, a little bit lower energy. And some of those hit the nearby acceptor which is

absorbing at that particular frequency. And then those relax and lose a little bit more energy as it emits in a different color. So you get this kind of downhill effect of the energy.

Brian: So in theory, you could have a chain of these.

Jacqueline: Yeah, you could theoretically have a chain of these.

Brian: Are the information you're gleaning through this technique is how close they are together?

Jacqueline: Yeah, 'cause how efficient the transfer of energy is depends very/ it's very sensitive to distant effects. It's non-linear. As they get closer together, the signal will exponentially be stronger or weaker as the distance is changed.

75:28

Ben: You're sure about "exponentially"?

Jacqueline: Um..

Ben: Isn't it like one over R squared or/

Jacqueline: Yeah, it's inverse square law. So, not exponentially.

Ben: [laughs]

Jacqueline: It follows the same inverse square law. It would be nice to fit it (...)

Ben: I have a "(...) up my ass" about people misusing the word "exponentially"

Jacqueline: No, no. That's a good/

Ben: At least physicists should use it correctly.

Jacqueline, Brian: [laugh]

Ben: So I guess she's saying that's exactly what you said. One guy emits/ You know, you shine blue light on it, one guy catches some of those blue photons, starts emitting green photons and then the other on will catch the green photons and emit a yellow photon. And if they're too far apart those/ the photons

Jacqueline: It will be too weak or it won't work out.

Ben: The closer they are together the more the second one will get hit by the ones emitted by the first one.

Jacqueline: Yeah.

76:26

Ben: Neat. Alright, what other questions have you got?

Brian: How close they are together - that information was previously difficult to glean and through this technique it's easier to determine?

Jacqueline: Oh yeah. It's really hard to measure things on scales that are that small and

Brian: You need a tiny ruler.

Jacqueline: Yeah, you need a teeny-tiny ruler and at that scale the ruler you need is, like, you know, light waves themselves, energy waves. So part of the thing is that, you know, so these things are so small. Like, you can't see individual protein molecules under normal type of/ You know, you can't just stick it under a microscope and look for it. You can do some things with scanning electron microscopes and stuff like that but/

Ben: It's like X-Ray/

Jacqueline: Yeah, it's /

Ben: They used to X-ray diffraction to see them, right?

Jacqueline: Okay, okay. So, we still use x-ray diffraction, we still do crystals for a lot of different proteins but there's a lot of caveats and drawbacks to that as well. So, x-ray crystallography is still very, very widely used. Which is how we get all those pretty ball and stick and ribbon diagrams of what certain different proteins look like that you see all the time. But that requires you to be able to crystallize the protein and a lot of proteins will not crystallize at all. And unfortunately a lot of the most interesting proteins are the ones harder to crystallize because the more anisotropy and structural irregularity and motion and conformational changes that the protein is capable of doing, the harder it is to crystallize. Because to crystallize something you want it to be as simple and still and stable as possible. Because, you know, by definition a crystal is a bunch of repeated, ordered structures. And so the more, you know, motions and changes and interesting things the protein does, the harder it is to crystallize. And if you don't have a crystal, you can't do x-ray diffraction. And the other thing is even a lot of the proteins that you can crystallize you can, there's kind of two additional caveats with that - one is that you might be stabilizing them in a conformation that is not necessarily very biologically relevant - so just because it stacks up and orders in that conformation, isn't necessarily the shape it's usually in in the cell and furthermore a lot of the different structural changes and conformational motions, you know, you might be able to crystallize one of them but never any of the others. So, again with the Transformer example - so it'd be like, you know, I can only ever get the firetruck shape and I would never see all these cool things that Optimus Prime can do if only ever use x-ray diffraction. I have to look at it with something like FRET to see what it does when it's moving around.

79:51

Ben: Right, let me/ Okay, so I said x-ray diffraction because I wanted to get. Okay, so x/ quickly, for the audience at home, x-ray diffraction is something that happens when we shine a wave at an object or a system of objects where the size of the structure is comparable to the wavelength that we're shining on it. Okay? And so, what happens is you get these weird interference patterns. And if you're shining your waves through a crystal, then you get really ordered interference patterns and you can deduce from those what the shape of the crystal is. So, with x-ray diffraction what we're saying is that the size and structure of one of these folded over proteins, if you can crystallize, like you said, is comparable to the width of x-rays. And the thing is, if you want to see what something looks like using light in sharp detail, you need to use wavelengths that are smaller.

Jacqueline: Yep.

Ben: In other words, you need to shine really, really energetic light. Really, really energetic light would be required to see the internal structure of a protein, if we wanted to look at it by shining light on it. And the problem with that is that really, really energetic light shakes the hell out of things. It would melt the protein if we shined, you know, gamma ray at it. It's not helpful for doing microscopy. Which is why we can't rely on light to do it - because they are too small. So we have to rely on these other things like putting little tags on it that glow and seeing where those tags go. Does that make sense?

81:28

Jacqueline: Yeah, like so for x-ray diffraction we're usually using really, really high energy x-rays to get those patterns clear enough. So what they usually do is they go to synchrotron beams and get kind of the off-shoots of the beam line of the really high energy particle accelerators to even get the x-rays for stuff like that. So, you know that's not something you can put in a living cell. And the crystals often will even melt, so they degrade over time as you're shining the radiation through so you can only try and image them so many times. You have to be careful balancing the energy that you're putting through 'em as you're going through that process.

Nicole: And there's similar thing with electron microscopy. Like, I'm sure you've seen beautiful images of, you know, small, little single cellular organisms. But they need to be essentially coated and dead in order to visualize the structures. So if you wanna again see this live, we're back to some sort of optical type/ to optical, visible regime, less energy.

Brian: Um, was it Nicole or Jacqueline talking about crystals in your long explanation?

Ben: Jacqueline, I think.

Jacqueline: Yep.

Brian: So, Jacqueline. What you were saying was: a lot of the proteins that are the most interesting for life are not great at this because they're disordered relative to well-ordered crystals?

Jacqueline: Yeah, so you can't get them to crystallize at all.

Brain: Is this too heavy a conclusion to draw that like/ It sounds what you're saying is that a lot of the coolest, most interesting building block-life proteins require or seem to have a pattern of some amount of disorder relative to crystals?

Jacqueline: Um, disorder starts/

Brian: Do any really ordered molecules make life? Oh, I'm sorry. What were you saying?

Jacqueline: Well, so okay. That's getting into a few different things in terms of thermodynamics. The disorder has very specific entropy/

Ben: It's kind of a philosophical question, isn't it?

Jacqueline: Yeah

Brian: It's too heavy, we can cut it.

83:53

Jacqueline: I will say that, um, you know, and I can talk about that. That's probably a whole different other show, where we talk about thermodynamics and order and disorder and all that kind of stuff. But, uh, so in a very general sense, and I'm talking very general and hoping that my PhD advisor doesn't listen to this, 'cause she

Ben, Brian: [laugh]

Jacqueline: She'd facepalm but in very, very general sense the proteins that are often easier to crystallize tend to be more solid and stable structures with fewer major conformational changes. In a very general sense. But we haven't even been able to look at enough proteins to even necessarily, you know, because that's the catch-22 in that we're only just now being able to look at a lot of these more, you know, these proteins that have more/ There's a difference between motion and disorder. Just because something can undergo a lot of different conformational changes does not mean that it's intrinsically more disordered. And, as I was saying, you can impose an artificial degree of order on a protein by crystallizing it if it's a simple one. So, that does get very philosophical

Ben: Let's try to answer it this way. So you think about, like, kitchen stuff, right? A bowl is nice, useful enough. You can't beat an egg with a bowl, you know?

Jacqueline: But you still need it, right? You know?

Ben: Right. So bowls crystallize really well. You can stack a set of the same bowl really easily, get a nice, even center, right? You can't stack egg-beaters, right? You just throw them in the drawer and they don't make/ You know what I'm saying?

Jacqueline: Yeah. But if you're like "what do you need for life?" Like, you need all of these things.

Ben: Yeah, that's right.

Jacqueline: Like, you need the boring structural, you know - I shouldn't say boring - but you need these simple structural proteins that hold everything together because otherwise you'd fall apart into soup.

86:21

Ben: Right.

Jacqueline: You know? Like/

Brian: Hm-m.

Jacqueline: Life is not, you know, all like/ These proteins aren't just like sitting around, twiddling their protein thumbs. They're all doing essential functions/

Ben: Yeah, proteins are tools. They do things inside the cell, right? And so you need some that do all sorts of crazy things and really specific things and why would we require something that complicated to be stackable, like a set of cups?

Jacqueline: Yes.

Ben: I mean, we need cups too. Like, you need them both for a kitchen. You can't do anything without a bowl but you can't do very specific things without big, complicated gizmos that you only use once and don't fit in the drawer, right?

Jacqueline: Yeah. I mean, like, the bricks that make up your house, you know, are still necessary to make your house even though they're simple. You know, the thermostat might be what/ or the furnace, you know? So saying like the mitochondria is the powerhouse of the cell and you know all of the enzymes in the mitochondria that are helping you generate ATP which you need to, you know, create heat and stay warm and be alive are essential but at the same time if you didn't have the bricks making up your house, all the energy would just dissipate into the world and you'd still freeze to death in winter. You know, you still need the complex things like your furnace as well as the bricks that are defining the space that is your house.

Brian: I see. So, it's almost - using the kitchen metaphor - if alien came down and needed to deduce what a human kitchen contained but it could only ask that question based on what it was able to stack and not stack, it would wrongfully, wrongly deduce that human kitchen has bowls, plates and spoons and cups and miss/

Jacqueline: And nothing else.

Brian: And nothing else.

Jacqueline: Right.

Brian: So by virtue of the coincidence of the fact that this particular mode of observation requires crystallization and order in that sense, we can look at the ones that crystallize easily but it doesn't mean that the ones that can't crystallize aren't equally important to life.

Jacqueline: Right. Right.

Brian: Mhm. Gotcha.

Ben: I think you could probably crystallize fridges. What do you think?

Jacqueline, Nicole: [laugh]

Jacqueline: So, if you want me to start going that way, technically you can't crystallize plates either because they have to be/ You know, there's certain/ we can talk about different types of symmetry. Plates are kind of/ they're radially symmetrical but not three dimensionally symmetrical in the type of way that you get good crystals from, in a three dimensional sense. [laughs]

Ben: Right.

88:59

Jacqueline: Like a lot of tessellations, so like if you look at M. C. Escher's work, you know, the tessellated lizards and stuff a lot of that type of symmetry, you know. It doesn't need to be radially symmetrical if they/

Ben: Yeah, well, I mean, yeah

Jacqueline: If it tessellates. So that gets into/ but you need it to tessellate in three dimensions.

Ben: So like what does tessellate well in 3 dimensions? Like fridges? Forks?

Jacqueline: Ugh..

Ben: I think

Jacqueline: Refrigerators, maybe.

Ben: I think stoves can tessellate.

Jacqueline: That's the thing is, like, not a lot of things. Not a lot of things tessellate in a way that makes nice, pretty crystals.

Ben: In the kitchen.

Jacqueline: Or in a cell, which is the thing. So, an interesting aside about Escher actually is that one of the faculty on my committee actually has one of his early drawings, like, saved away. Very specially is that when he was a poor starving artist on the street before he got, you know, more better. "More better"? I can't talk anymore. Before he got better known as an artist the people keeping Escher alive as a quasi-starving artist was mathematicians and crystallographers because those were the only people buying his work at first. Nobody else; they're like "Why are you drawing these patterns? They're really boring." And all these crystallographers were like "These are so cool. I'm gonna use these in my class".

Brian, Jacqueline: [laugh]

Ben: He did like hyperbolic tessellations. That's pretty impressive.

Jacqueline: Yeah, he did. Uh-huh.

Ben: Tessellation on hyperbolic space. That was pretty good.

Jacqueline: Yup.