

## **Disruptive: Rapid, Low Cost Detection of Zika & Future Pandemics**

Host Terrence McNally interviews James Collins and Keith Pardee. Podcast published on June 23, 2016.

McNally:

Hello, I'm Terrence McNally and you're listening to Disruptive, the podcast from Harvard's Wyss Institute for biologically inspired engineering. In early May, a low cost, rapid, paper-based diagnostic system for strain specific detection of the Zika virus was introduced by an international consortium of researchers led by synthetic biologist James Collins with the goal that it could soon be used in the field to screen blood, urine or saliva samples.

The core of the test kit is a piece of paper that changes color in the presence of Zika virus RNA and produces results in two to three hours. Much faster and cheaper than the PCR test used now according to Collins, a Wyss core faculty member - and it should cost less than a dollar per test adds University of Toronto biochemist Keith Pardee.

I'm going to speak with both of them about the real time story of a crisis inciting innovation. How a team from a number of different institutions came together, and in a matter of six weeks developed a new approach to detection and designed a system to deploy in the face of future pandemics. Collins says, in response to an emerging outbreak, a custom tailored diagnostic system could be ready for use within one week's time. We'll explore how they did it, what they've learned and what this might mean for the future.

The mission of the Wyss Institute is to transform healthcare industry and the environment by emulating the way nature builds. Our bodies and all living systems accomplish tasks far more sophisticated and dynamic than any entity yet designed by humans. By emulating nature's principles for self organizing and self regulating, Wyss researchers develop innovative engineering solutions for healthcare, energy, architecture, robotics and manufacturing.

In addition to leading the Living Cellular Devices and cellular devices platform at the Wyss, Jim Collins is the Termeer professor of medical engineering and science and professor of biological engineering at MIT. He's a member of the Harvard MIT health sciences and technology faculty and an institute member of the Broad Institute of MIT and Harvard.

Collins received a BA in physics from Holy Cross and a PHD in medical engineering from Oxford. A Rhodes Scholar, a MacArthur Fellow and a winner of the National Institutes of Health Directors Pioneer Award. Jim is one of the founders of synthetic biology as well as a pioneering researcher

in systems biology and his engineering ranges from the physical scale of wearable medical devices to that of molecules.

Collins says he found early inspiration both in the space program and much closer to home.

Collins: [02:38]

I was born and grew up initially in New York City. My dad was an electrical engineer who had worked in the aviation industry so he did work for NASA, he did work for the military, he did work for a number of companies building planes. My mom was a math teacher.

My dad was great. He would bring home a number of different devices and components that his group had built. Most notably his team was involved in building the altimeter for the lunar module for the Apollo project, so I remember as a four year old watching them land on the moon, and it was a very proud moment in our house. He could point to that unit landing on the moon and say hey we've got a piece of equipment in that system, but I was really struck by the fact that as a young kid I had both my grandfathers become disabled.

One grandfather went blind and the other had a series of strokes and he became a hemipalegic. Whilst I saw this amazing technology from the aviation side, I saw nothing being introduced or used to help these two guys who I loved very much restore function that they had lost when they still otherwise were relatively healthy.

That inspired me to think about how you could use technology to improve how the human body works, and this was starting in the mid to late '70s when bioengineering really did not yet exist as an academic discipline.

McNally: [03:58]

We're going to focus on your work with paper diagnostics, but briefly, what questions, if any, have consistently driven you over the years - besides the one you just laid out, which is how can we put our amazing technology to work to help individual human beings?

Collins:

I think that overall is really the dominant driving question and issue in my professional life. When I first started as an academic in 1990 as a professor, I was at Boston University, and for about the first decade my lab focused primarily on whole body dynamics. We studied how people walk, how people run, how people maintain balance, and our goal there was to use concepts from physics and engineering to better understand how the body does these amazing tasks that we take for granted on a daily basis. As well as, could we then harness those principles to come up with new technologies?

Probably most notably from the 90s - in work that we have since further developed and commercialized at the Wyss - we discovered that you could introduce small amounts of noise. Not noise in the sense of an unpleasant sound or even static on the radio, but noise in terms of fluctuating mechanical signal in the form of vibration could be introduced into sensory neurons in humans and improve their function.

We showed that you could take this very low level noise in the form of the vibrating insole, introduce it on the soles of subject's feet and have them balance better. That's most impressive ... We could take a 75-year old and have them balance as well as a 25-year old.

McNally: [05:24]  
What's going on there?

Collins:  
What's happening is that the noise, again in this example, in the form of either mechanical vibration, the type you might feel on a subway train, or in the form of electrical stimulation, a type of very low stimulation. It's basically tickling the neurons, and it's changing the detection threshold for the neurons.

All of our sensory neurons have some threshold. Well those thresholds go up as we age. They go up with certain diseases or injuries such as stroke. What the noise does is it tickles the neurons, effectively lowering the detection threshold, now making it easier for those neurons to send signals - either that they could never feel or to send signals that they could at one time feel, but then as a result of age or disease or injury now no longer can feel.

Going back to the early part of my life story that I shared, perhaps the most satisfying for me is that we showed that this noise in either the form of vibrating insoles or applied at other parts of the body could actually improve sensory and motor function in stroke patients. We showed we could significantly improve the balance abilities of stroke. Now this was developed decades too late to help my poor grandfather, but it's a technology that we think could have an impact on stroke patients, on diabetics, on generally older folks, on even young athletes and military personnel.

McNally:  
I like that sense of having a vision and muddling through this and that, and ending up actually succeeding to do something about it.

[06:49] Your current projects employ a range of approaches. You work with a number of the platforms at Wyss. Could you talk about how that works for you?

Collins:  
You know for me the Wyss is really a remarkable place. I've been with it since before it even began, so to speak, with Don Ingber and Dave Mooney. In

2005, as they began to explore this as a possibility and officially launched the institute in 2008, early 2009. It went from 0 to 60, Really I could say 0 to about 450 in record time.

It's fascinating to see how the teams are able to take innovative approaches but kind of going beyond the basic science to actually think about how do you now translate this into commercial and clinical space?

You know, you used the phrase "muddling through" earlier which I think nicely captures what happens in a lot of these projects. As nice as the stories will be and the anecdotes that I share with you today, it really, in most cases doesn't capture the failures that you face on a daily basis as you're trying to come up with a new technique, and the Wyss encourages one to take the failures. They actually like to celebrate the failures to their science advisory board and the board of directors. They encourage us to share and come forward with, "Okay, what didn't work?" That's very different from most settings and I consider myself really very fortunate to be part of this community.

We work on a whole range of efforts so we're still doing some wearable technologies, but much of our efforts now around this idea can you reprogram living organisms to serve as living diagnostics and living therapeutics? Can you program a bacterial cell or a human cell or a synthetic vesicle or synthetic membrane, endowing it with biological circuits, enabling it to sense something in its environment, and produce something in response to that that could improve or modify that environment?

McNally: [08:40]

Many of us envision scientists laser-focused on their current project. Could you talk a bit about how your personal response to news of the Zika outbreak evolved?

Collins:

Yeah so it was ... I think it will be an interesting case study in the history of the Wyss and certainly in the history of our lab and point to what one can do in academia. We tend to think of academics as very slow moving, laser-focused, wake up early, go to bed late but just drill down on that one experiment. What happened in this case is in late January, MIT leadership sent an email around asking who was working on Zika virus. We were not. I actually had not heard too much about it except for the couple of prior days several news headlines caught my attention that there was this growing outbreak.

I realized that a platform we had developed at the Wyss around paper-based technologies ... About a year and half earlier we had shown that you could take the inner machinery of a living cell, outside a cell, spot it on paper, freeze-dry it, store it indefinitely at room temperature, distribute it at room temperature, then sometime later rehydrate it, and whatever you had spotted would now function as if it was inside a living cell.

We had shown you could introduce biological sensors we had developed that could sense different RNA components onto this so that you could now have very cheap diagnostics. Back about a year-and-a-half ago we were initially working on it as a test for antibiotic resistance which is a big focus in my lab and is a big focus of the Wyss.

At that time in August of 2014, we were in the midst of the Ebola crisis, and our team responded very rapidly to that and showed you could in fact develop cheap and rapid effective Ebola sensors. At the time we came out with that which was late October 2014 the crisis was already past its peak point, and so we weren't able to contribute to that, but nonetheless we were advancing that platform. So now in late January when I received that note, I became intrigued. Could we do what we had done for Ebola but now for Zika and in time to actually impact the crisis?

[10:49] I reached out to Keith Pardee, who had been a post-doc of our group at the Wyss but now a professor at the University of Toronto, and I reached out to Alex Green, who also had been a post-doc in our group at the Wyss along with Peng Yin, who had been part of the Ebola effort as was Keith, but now professor at Arizona State University. We organized a call for later that day and we all agreed that this was a marvelous opportunity to step up.

Keith mobilized folks on his end, Alex mobilized folks on our end, and I began to put together a team focused at the Wyss, MIT and the Broad of post-docs and grad students. To a one, my team - which to capture your earlier point, each were focused in a laser-like way on their graduate project or post-doc project - dropped what they were doing and turned to see what we could do with Zika.

I was amazed, impressed, and really proud of what our team was able to do. These folks put in very long hours against some pretty daunting challenges, in part because the Zika virus when present in a patient is in very, very low concentrations, about one to three parts per quadrillion which is about a million times more diluted than what we could detect at the time.

McNally: [12:03]  
A million?

Collins:  
Over a million. Probably ten million times more dilute. Our team, led by Melissa Takahashi and Donna Braff, a post-doc and grad student in our group, and Melina Fan, who was the founder of Addgene and was on a brief sabbatical with our group at the time ...introduced a scheme where they could amplify a sample, enabling us to detect one-to-three parts per quadrillion, even lower than that, in about ninety minutes to two hours. We really lucked out for both who was present at the time and who was willing to step up and help us out.

McNally: [12:41]

How much could you see right away? What did you think you might be able to do and how fast did you think you might do it? You know that kind of...what you could envision and then how it plays out.

Collins:

Following my talk with Keith and Alex, I actually thought we could produce a reasonable output in two-to-three weeks. It took us in the end five-to-six weeks, and part of that was that the Zika virus was at a much lower concentration than I had anticipated. In blood it's one to three-parts-per-quadrillion. In semen, which is another sample that I think our test works well in, it's about a hundred-parts-per-quadrillion, which would be a little easier, but still on the order of a hundred-thousand times more dilute than we could do at the time.

We had several other steps and new advances that we introduced as, in part, we really wanted to make this more fieldable, and that is something that could be distributed or shared with folks. Our Ebola test really was a lab demo that initially was in the nanomolar range demonstration which was about a thousand times off from what it needed to be when we initially published that piece in *Cell*. We got that down to close to what you needed shortly thereafter, but that still was way off from what we needed from Zika.

McNally: [13:52]

What we have going on here is not only the speed with which you want to accomplish it but that you don't want a proof of concept alone. You don't want to say this can be done. You want to say here it is, run and use it.

Collins:

What's interesting is we reached out to *Cell* which is one of the leading life science journals in the world that primarily focuses on basic science discoveries. When we spoke with our editor, Mirna Kvaajo, who handled our Ebola [diagnostic], she said, "Jim, this sounds very exciting to us, but we want to make sure that what you present is pragmatic, practical and fieldable."

It was fascinating, right... Here's *Cell*, open again to a cell-free technology - their name itself would be counter to that - and a journal that is primarily focusing on basic science work is pushing us, the engineering group, to be as pragmatic and practical as possible. It had a huge impact on us. It set us back a couple weeks to do even more to see what we could do to have something that would be cheap, that would be rapid, but that could be relatively easy to use in the field. By field I want to be clear. It's not something you would use yet at home or from a door-to-door, but something that you could nonetheless use in low resource areas in a doctor's office or a diagnostic center or at a national lab.

McNally: [15:00]

If there's anything that stands out to you that you learned in your effort in 2014 with Ebola that really set you ahead or a mistake we're not going to make, or that sort of thing, when you approach Zika here in 2016...

Collins:

You know that's a very interesting question. I don't think there was a mistake on the Ebola that motivated us or concerned us here. I think there we saw that we could rapidly use synthetic biology and bioengineering approaches to design in a multiplex way dozens of sensors as needed to go against the RNA virus. I think here we probably were motivated more on looking to see what were the challenges out there, and for me as a lab director, I think perhaps the strongest kind of reflection I have now on it is you just see how an external goal that really can help a lot of people can serve as a remarkable motivating factor.

We academics tend to operate on a slower scale than most of us would like, and yet these folks were hyper-caffeinated and hyper-motivated to move, and that's something I'd love to see reproduced. I think the pressure's now off the teams in some ways, and I think they've deserved a slight break, and I'm now looking to see in what way we can recapture that spirit on a more regular basis.

McNally: [16:20]

Oh very good. That does sound exciting. Where did you get your Zika samples?

Collins:

That's a great question. That actually speaks to another challenge. I think a broad challenge on diagnostics in general and diagnostics for new outbreaks and that is we did not have access to Zika samples. So what we did was what we also did in Ebola, which was create synthetic samples. We were able to synthesize small segments of the RNA, so in the Zika case it's an RNA genome, so the whole code for the virus is in an RNA form. We were able to synthesize small segments to then spike that into human blood serum samples firstly, to see, could we detect it with our sensors, and we could use that as a nice proof of cause of demonstration.

We then spiked in the synthetic samples into a lentivirus. This is a virus that's commonly used in labs around the world as a delivery mechanism for gene and cell therapies, and broadly a delivery mechanism just for experiments of mammalian cells. Very safe to use. And we showed that you could, when spiked in, now process it as if it was a virus sample in human serum and detect it at this one-in-three-parts-per-quadrillion.

We were very excited about it and did similar with dengue virus to show that our test could distinguish between dengue and Zika quite nicely. We submitted this to *Cell* and got a very constructive set of reviews - how to make it more pragmatic, practical, and one reviewer was really quite strong saying, "Boy, you know, your impacts are not going to be as big as you like

because these are synthetic Zika samples. It'd be great if you could get after real samples.”

McNally:  
Sure.

Collins: [17:54]

The challenge for the US community is, we as academics don't have access or limited access to patient samples. The editor at *Cell* was very excited about the paper and said, “Look, Jim, we're very excited, we want to move ahead with your paper, but we really would love to see what could you do to go after real samples.”

Now my lab is not a Zika lab. We did not have safety approval to work on Zika. Our lab is set up to have the right safety protocols but, and I shared that with her and said, “We'll do what we can,” and she said, “Well look, it's not a deal breaker but see what you can do.”

It turns out that a lab just above my lab at MIT, Lee Gehrke is a global health expert who had done work on Ebola, which I was aware of, and he himself had a paper-based beautiful test for Ebola. I reached out to him and he had been working on Zika, and he had live Zika virus. He had dengue virus as well, and within minutes, he said, “Jim we want to work... we're willing to team up.” And we teamed up with his group and ran a bunch of tests in his lab on live Zika to show that we could detect it in these low parts per quadrillion which was brilliant.

Simultaneously Keith Pardee had been in interactions with David O'Connor's group at University of Wisconsin, and David had been working on infected monkeys, monkeys infected with Zika. Keith reached out to David and within minutes David said, “We want to collaborate with you guys,” and we were able to obtain samples from David. Remarkably, they had samples at two-parts-per-quadrillion out of these monkeys, and we could brilliantly detect these quickly and readily with our samples.

I think it was another example of how well this international community can work together and respond, and I was impressed with my colleagues and really appreciative of their willingness to drop what they were doing and it wasn't without some commitment on their part to rejigger some aspect of their lab and work with us in this remarkably timely fashion. So again within a matter of weeks we had beautiful clinical data.

McNally: [19:51]

Why would this method be better than standard antibody-based detection methods?

Collins:

I should say it's different is probably the better play and it's got complimenting features on a few accounts. One is it's cheaper, so it's both

cheaper to produce but it's also cheaper to develop. It's faster to produce, so whereas you may take many, many weeks for an antibody test typically, we think we now have a platform that in about five days you could develop appropriate diagnostic sensors for the next outbreak. In terms of speed, it's probably on par with speed now on output. In fact, I think our platform is amenable, that as antibody tests are developed, we think we can freeze-dry them and have them as part of our tests.

McNally:

Oh so work with it.

Collins:

Yeah work with it absolutely. Then I think also cost. So the antibody test will typically be on the order of several hundred dollars and many, many thousands to develop, ours costs less than a dollar to produce once made and required only about \$20 worth of DNA material plus the cost of supporting and feeding graduate students to make these things come to fruition.

McNally: [21:00]

What about the fact that antibodies for Zika and dengue, for instance, are very similar? Does the fact that this is sequence-based and not antibody-based help in that regard?

Collins:

It's different and it helps go after different aspects. So an important distinction here as well is that the Zika virus needs to be present for the current test that we have to work, and that is the virus itself has to be there for us to pick it up. In the case of infections, I think it's three-to-seven days still present in the blood after infection and about two weeks in urine, so if you've been infected a long time ago, the present test likely wouldn't work. Whereas you'd want to then use an antibody test where the antibodies will be present for a very long time subsequent to the infection.

McNally: [21:47]

You developed the whole workflow process for patient samples. How did you approach that?

Collins:

We internally wanted to think through and... the workflow we presented right now is really the development of the platform versus serious workflow on what you would use in the diagnostic center itself. The workflow we presented was, if you have a new outbreak, what would you need to do in order to develop the diagnostic? And there is really our sweet spot and specialty because it's coming up with a new technology platform and or a new version of that platform, and we stepped back and said okay if you had to set this up what would this look like? Now we envision that this is a platform that could be set up in a national lab or maybe as part of a company that is developing flu tests on a seasonal basis or lyme tests but is

also ready to respond to the next outbreak.

As for a workflow that could be fieldable, out into diagnostic centers around the world, we're in active discussion at the Wyss pulling teams together as to what does this really need to look like and what do the devices need to look like so that somebody without a lot of training could readily use that? We're in discussions now with many groups around the world that are eager to have our test in a pilot fashion put out into the field.

McNally:

When you are developing this workflow, as you say, just for how would you go from outbreak to diagnostic, right?

Collins:

Right.

McNally: [23:11]

What were some of the challenges and how you overcame them?

Collins:

Well there, the challenges are a few things. One is how quickly could you go from sequence to identifying parts of that RNA, be it an mRNA or the genome itself or some intermediate, to identify and come up with a sensor. Can you automate now the software that can look at these long strings of nucleotides to tell you where you should best target? That was challenge one.

Challenge two was, okay, now that you've come up with your design, how quickly can you synthesize these RNA-based sensors that are going to be the key part of your actual development test?

Next now it's, how do you best process the patient samples? And here's where I think we still have a number of challenges. As you think about blood, as you think about urine, as you think about sputum or even semen, how do you get out what you need to detect - or if it's there at all - in a timely fashion?

There was a new space for us and we're exploring other technologies that are being developed at the Wyss that I think could be relevant for this broad platform. Now once you pull that out, how do you amplify it, right? Again we're talking about something that is at 10 to the minus 15, so in the quadrillionth, how are ... maybe a little denser for some other conditions. How do you actually pull these very small numbers of molecules out of your sample and then couple it to our output?

McNally: [24:38]

With Zika appearing in such minute concentrations, amplification is crucial, and Jim points out that the team had a bit of luck in this regard.

Collins:

We were trying to initially take a synthetic biology approach to that, that is, could we design circuits themselves that were made out of nucleic acids that would be inserted into the sample that could themselves amplify it in a clever way? And I still think there may be possibilities there. We benefited from Melina Fan's very timely sabbatical with our group. Melina came over from Addgene, and this was a topic she was keen on, which was, could we develop an easy-to-use RNA-amplification scheme, DNA-amplification scheme, that could be potentially freeze-dried and not stored at room temperature... if you had to transport it around in an inexpensive manner? And she was developing that broadly for the platform and we just lucked out that she had made really nice advances in collaboration with Melissa Takahashi and Donna Braff as part of our lab, and we harnessed those advances quite quickly, and the team pushed it further during that five-to-six week development cycle.

McNally: [25:42]

Is this amplification process something that a non-scientist could understand? What are you doing when you do that?

Collins:

I guess the way general readers would think of it is it's equivalent to the old shampoo commercial. You know you tell two friends and they tell two friends and they tell two friends and you're making, you're doubling in multiple rounds what you have as you go, and or increasing the copies in an exponential fashion. Our advantage is that you can do this at a constant temperature.

PCR is a very common technique that's used, and it's the basis of the most popular and most commonly used tests right now for diagnosing Zika and other viruses. You have to do thermal cycling. You have to change the temperature in a quite dramatic way multiple times to get your sample suitably amplified and that requires a fair amount of input and requires decently expensive equipment. The technique that Melina pioneered in our group doesn't require such steps.

McNally: [26:40]

Here we turn to Keith Pardee, formerly a post-doctoral fellow at the Wyss and one of the Zika study's co-first authors. Pardee, now an assistant professor in the Leslie Dan Faculty of Pharmacy at University of Toronto, earned a B.S. in Biological Sciences from the University of Alberta, a Master of Science in Natural Products Chemistry from the University of British Columbia, and a PhD in Molecular Genetics from the University of Toronto. He shares a bit about his background.

Pardee:

I grew up in western Canada, and where I think the two main influences: one was spending a lot of time in nature with friends and family, hiking in the river valleys, exploring the creeks and stuff around where I lived, and the other was basically

tinkering. Building, taking things apart, and experimenting as a child. Bringing those together, I guess in hindsight, not too surprising that I have an affinity for synthetic biology, where we're building with components from nature.

My academic path has been pretty broad. At the University of Alberta I studied botany, and became very interested in how plants defended themselves. Plants, because they can't run away, need to use chemicals to basically deter herbivores. I got really fascinated with how these compounds could also be used for medicine, so that's how I ended up at the University of British Columbia studying natural product chemistry, where I was grinding up marine organisms looking for anti-viral compounds.

This led to me being really curious about what was on the other side of those compounds. What were the molecular switches that were being regulated by these compounds, and serving as therapeutics? That's how I ended up in Toronto for my PhD, where I was studying these molecular switches on the almost atomic level and bio-physical level.

But then after doing this basic research, I got really interested in being able to apply this basic science to global challenges. That's what drew me to Jim Collins. His work with synthetic biology was intriguing and fascinating. It was through Jim and his mentorship that I ended up at the Wyss, and did my post-doc in the wonderful collaborative environment that it is.

McNally: [28:50]  
So how did he get involved in the Zika project?

Pardee:  
In January, when Jim called me in Toronto and Alex Green in Arizona and said, "We've got this technology platform that I think it could really make a contribution to the effort on the ground with the Zika virus," we jumped on board. The team from our group and other groups assembled around that, and within a few days we had a strong group and we were up and at it. Like you said, within six weeks, we were submitting the paper for publication.

McNally:  
What happened right after the call on your part?

Pardee:  
Alex and I talked to a group of post-docs and students that are in Jim Collins's lab, and we basically split up the work, and everyone took the lead in the area that they were experts in. Alex Green, his strength is the design of the toehold switch, which serves as the sensor for the Zika virus diagnostic.

My end was more on the paper-based platform, and some of the technologies that were up front of that, like the sample prep. Figuring out, how can we amplify the RNA signal to the point that the RNA sensors can be detected?

McNally: [30:00]

Alex is in Arizona, you're in Toronto, Jim Collin's is at the Wyss in Boston, Lee Gehrke's at MIT, David who you just mentioned is at University of Wisconsin. How often is it that a team all over the place like this chooses to work together, and is able to get something done?

Pardee:

Exactly, I think that's a really interesting part of this project. I think we basically all recognize the importance of the work, and it catalyzed I think probably a year's worth of work into that six week period, where we all worked together. We were on the phone, Skyping, emails daily, all day, coordinating efforts, getting samples shipped from one place to another.

McNally: [30:43]

Keith points out some of the advantages of their diagnostic approach.

Pardee:

A lot of clinical diagnostics that look at the nucleic acid sequence of a pathogen use PCR, and this requires specialized equipment and specialized people. This is not a portable endeavor, and so right now when you're looking to do diagnostics on a Zika virus, samples are collected in the field and brought into larger urban centers where these facilities exist. Our hope is with the test that we've developed, that these molecular diagnostics can be done on the spot for under a dollar.

McNally:

Pardee had been involved with the earlier paper-based Ebola diagnostic in 2014, but Zika presented new challenges.

Pardee: [31:17]

I think our main gap with the original work was sensitivity. We were able to detect things down into the low nanomolar range. Viral concentrations for the Zika virus in patients are in the low femtomolar range, which is six to seven orders of magnitude difference. We needed to figure out how we could get that, our low RNA signal that's present in patient samples up to a level that the toehold switch could detect.

We employed preexisting isothermal amplification method, and what that means is amplifying the target RNA using enzymes at a single temperature. Using 41 degrees Celsius incubation, and primers directed to the regions of the genome that the toehold switches target, we were able to amplify the RNA.

McNally: [32:12]

What does that mean to amplify the RNA?

Pardee:

Basically what you're doing is you're making copies of the RNA. The RNA is a series of nucleic acids, and, borrowing the machinery from nature that exists out there in all of our cells and in life, bioengineers have figured out how to harness that and do it in a test tube. Basically it's like a photocopier of the RNA pumping out copies

of that region that we're interested in.

McNally: [32:41]

You've got the sample, you've developed the sensors on the paper-based diagnostic. When you drop a bit of the amplified sample on the paper, what's been done to the paper?

Pardee:

The paper's been, first of all it's been treated so that it's a better host for these molecular reactions. We coat it with a protein called bovine serum albumin, and then we freeze-dry it along with the enzymes of transcription and translation, and the toehold switches that were programmed to be sensitive to the Zika virus genome.

When the water from the amplified sample hits the paper, all these molecules come back into solution, transcription starts, which codes basically the DNA coating for the toehold switch is transcribed, the RNA hairpin which forms the toehold switch comes together. If the Zika virus sequence is present, that RNA hairpin unfolds, allowing the ribosomes to bind, which are also freeze-dried in the sample, and an enzyme, LagZ, is expressed, causing a color change that is visible by the naked eye.

McNally: [33:48]

You've mentioned several times this toehold switch. For someone who's not familiar with that, what is that, and how does it operate in this system?

Pardee:

The toehold switch sits on basically the starting point of an mRNA. mRNA's code for proteins, and ribosomes are responsible for running along these RNAs and translating them into proteins. But in order for the ribosome to bind, it needs to be able to interact with what's called a ribosomal binding site. The toehold switch basically occludes the ribosome from binding by steric strain.

This ribosomal binding site sits at the end of an RNA hairpin in a loop, and the ribosome can't bind. In the presence of the trigger, that RNA hairpin linearizes, and everything becomes conducive to ribosomal binding, and translation can go forward.

The switch is comprised of, at the three prime end, a toehold, which allows single-stranded RNA to interact, and basically is like the tab on the zipper. Once you have the correct sequence interacting with the toehold, that RNA hairpin starts to come undone just like a zipper, and the binding continues going up that RNA hairpin, driving the linearization process.

McNally:

What triggers that switch to go on or off, or yes or no?

Pardee:

Basically the RNA hairpin is comprised of two complimentary strands of RNA that

are connected together by a loop. They're attracted to each other by base-pairing, but the way these switches have been designed is that they prefer the target sequence. When the toehold interacts with the target sequence, it starts this unraveling, and you get this base pairing, now instead of the hairpin base pairing with itself, it starts to base pair with the target sequence.

McNally: [35:48]

I ask Jim Collins – what exactly makes the color change?

Collins:

On the paper now is you've got your inner machinery of your cells, which will be several dozen enzymes, there will be the RNA sensors themselves, and then molecular machines like ribosomes that can be used to interact with those RNA sensors and produce protein. If then in your sample that you've now amplified you have the triggers for those RNA sensors, when you drop those onto the sensor, they will turn on the RNA sensors and the RNA sensor's now, interacting with the cell-free extracts machinery will be turned on to produce a protein, and this protein is then set up that it will actually, if made, will change the color of the sensor on the paper from yellow to purple.

McNally:

Right, and, as I've read, it's as simple as when people read a color-changing pregnancy test.

Collins:

That's right.

McNally:

Available to the naked eye. You look at it and you know whether there's Zika there or not.

Collins:

That's right.

McNally: [36:54]

Okay. Once you put the sample on, the color doesn't change, it means there's no Zika in that sample as far as we know, right? If the color changes, what do you know at that point and what do you want to know next?

Collins:

At that point if you now have a positive detection event, you have a good sense and we would multiplex this so we likely would have a number of different spots sensing different parts of Zika. You definitely would want to have more than one be triggered to give you a true positive and not a false positive. You now might next be interested to know what strain of Zika do you have? Do you have the African strain, do you have the Asian strain, do you have an American strain?

McNally: [37:35]  
And you can do that?

Collins:

We can do that. In a brilliant innovation, Guillaume Lambert, who again we now benefited... He's a visiting scholar in our lab. He's been with us for almost a year. He finished a post-doc at the University of Chicago and he already has a new faculty position at Cornell University, and he came to spend a year with us before he launched his new lab as a junior faculty at Cornell. And Guillaume came up with a brilliant idea on using CRISPR/Cas9.

CRISPR/Cas9 is the latest rage in biotechnology, maybe the biggest rage ever in biotechnology. It's a system that enables you to do genome editing and genome engineering and specifically allows you in a sequence-specific way to target different aspects of different sequences of RNA or DNA.

Guillaume had the idea to see that you could use then CRISPR to target certain RNA sequences that would distinguish or discriminate between different strains of Zika, so that you could identify a large number of different sequence-specific differences between, for example, the African and the American strain. And he had the idea that you could then design triggers for our Toehold sensors or RNA sensors that were specific around these differences.

Now your next step would be, take your samples and introduce Guillaume's CRISPR-based modules that are specific to the African strain or the American strain. If say you take it from the African strain, if it finds those sequences, it's going to cut the RNA at those points, and now not trigger the RNA sensors. If you now do your second step, and for sample A, you get no color change... Well, it would indicate you actually have the African strain, whereas if you get a color change, it would indicate you have the American strain.

We are able to develop down to a single difference in nucleotide-base-pair discrimination abilities with this. And most impressive for the listeners who are familiar with CRISPR/Cas9, we showed you could freeze-dry the Cas9 components in the guide RNAs and, in many cases, in most cases, we actually increased their activity as a result of the freeze-drying.

McNally: [39:45]  
...and the freeze drying helps you because...?

Collins:

The key aspect of the freeze-drying is it really allows you to easily and readily deploy these biological sensors and materials. Typically, if you're going to work with biological material, you're going to have to keep it in a freezer, in a very large refrigerator. So you go into any biotech lab or biotech company, you'll have corridors or large rooms full of these very big freezers. and When you think about putting these out into the field, be it in low-resourced areas

or even decently resourced areas, suburbs and sorts, most places will not have the electricity or the interest in supporting a large refrigerator.

The freeze-drying will basically remove the fluid from these samples, and now what we discovered, allows you then to keep the stability of the biological components without the need for refrigeration. You eliminate the cold chain.

McNally:

You've created this diagnostic test. Now it can be sent out in paper form, temperature doesn't matter, and then activated on site.

Collins:

Exactly. I could put it in my pocket, get on a plane to LA, join you there in person, and we could run the test and it would function fine. We could store it in your desk, a year later you could take out and it would function the same. We could get on a plane, go around the world or stick it in a FedEx envelope or regular envelope, and easily distribute these and, perhaps more importantly, allow them to be stored at room temperature at sites around the world, which now makes it that much easier and that much cheaper to use.

McNally: [41:20]

Yeah that seems a phenomenal advantage. What are the limits to this system?

Collins:

You know I think you still have to think about amplification steps and then speed. I think right now we are focusing on both, so can we reduce the amplification steps? At present it's done as two separate tests. You amplify and then take the sample. Our team is aggressively looking to see can you do it in a one pot reaction, so to speak? That is, can you drop your sample on, having been amplified along with the sensors, and thus, once you hit the detection threshold of your sensor, you get the output? You don't have to wait through some prescribed scheme. I think that's a challenge and a limitation that we're now addressing.

The second is, how can you come up with sensors for other schemes that you might want to look at, whether it be external receptors on a pathogen or small molecules made by the pathogens? In present we are very good at detecting nucleic acids, so RNA or DNA of interest, but we are challenging ourselves, could you sense small molecules that are made by pathogens or cells that are bad? Could you detect other aspects of cells, be it metabolites that would be of interest - and that becomes a challenge. I wouldn't say it's a limitation but it's a challenge that we're taking on right now.

McNally: [42:38]

If you were to look back at this process - not just what goes on in the lab but what goes on in human motivation and teamwork, and so on, what are the

biggest lessons you've learned? What surprised you the most, those two?

Collins:

I think what surprised me the most was how willing and motivated folks were to a very specific goal. You know, in academia we tend to be a bit broad-based, big swath, and say, okay you know, it's a bit diffuse. Okay, here is generally what I want to do, but let's see what happens, and you explore and you hit a lot of bumps. Here we had a very clear goal and I was truly impressed and proud at how folks responded, and the time frame in which they did it was amazing. I similarly was impressed with some groups who we'd never met before - David O'Connor - on their willingness to step up and help us out and groups that, you know, they didn't know us from a hole in the wall. I think it speaks to very positive features of human nature and to academics. I think a lot of folks in the public think of academics in their ivory towers and-

McNally:

As even competitive...

Collins:

Well, look competition is good. I was a competitive athlete when I was much younger, and you know a lot of people hold up competition as bad. I think competition is good. It focuses the mind and it can drive, and I think here we were competitive more so against the virus because we really wanted to do something.

Certainly you have groups that can be competitive. They're all kind of racing, and I have to say we wanted to make sure we got ours out "before we were scooped." That would be a phrase that we used a lot. I think that can be very motivating but again it was interesting to see ... Lee Gehrke who was working on his own paper-based Zika virus diagnostic around antibodies. Here was a guy, he stepped up right away, and wanted to be part of the team, was willing to be part of the team. I again was very impressed with how he responded and David O'Connor and members of our team where they came together truly as a team where they had not been working as a team. It wasn't dysfunctional. They weren't together working and they came together and I think there will be interesting lessons that maybe the Harvard Business School should send a group over to look to see what happened there.

What was the secret sauce? I'm not sure what it was except I think people really felt they could help others quickly by doing this and getting involved.

McNally:

Yeah I think it's interesting what you point out there is that it's, competition is neutral. It's who are you competing against, and in this case you're competing against the virus, you're competing against time, you're competing against those sorts of things. Once you focus where the competition is, boom then you tap the power.

Collins:  
Yeah exactly.

McNally: [45:22]  
I ask both them about the future. First Keith Pardee –

Pardee:  
Our near term goals are to find the resources to take this project from the proof of concept stage through to product development, through the regulatory scheme, manufacturing.

Looking on the longer term, as you mentioned earlier, this is a platform that's programmable. With a turnaround time of about a week, we could make diagnostics for future outbreaks, and we could also make diagnostics for everyday things, like the cold that you have or things like that. Developing the broader platform for human health is our long-term goal.

McNally: [46:05]  
Where do you see your personal work evolving over the next year, next five to ten years?

Pardee:  
I'm fascinated with building these in vitro synthetic biology devices, and taking them further in applications and diagnostics, making the diagnostic platform more efficient, faster. Trying to figure out how to test more than one sample at a time, and applying this platform also to different domains. We focused a lot on diagnostics, but I think there's a lot of applications where we could use sensors, and other biological processes, for applications in the environment, and energy, and national security.

McNally:  
Can you throw out an example or two where someone would get a feel for what that might actually look like?

Pardee:  
Sure. You can imagine an ecologist who's in the field in a remote place, wanting to understand the microbial makeup of say, a water sample, or trying to figure out what the species are in an environment. Using this tool would basically allow you to do genotyping on the spot for low cost.

McNally:  
When you say this tool, this is still dealing in paper-based?

Pardee:  
Absolutely. You can imagine a botanist, for instance, being able to go into a forest and determine what species the plants are on the ground by, instead of probing a viral genome, probing the plant genome and figuring out what's present.

McNally:

Again, it would be done through implanting diagnostic sensors within the paper?

Pardee:

That's right, yeah, toehold switches could probe really any biological sequence. While we've talked a lot about RNA-sensing today, this paper-based platform is also suitable for detecting heavy metals, contaminants, drugs. Anything that there's a transcription factors for that's responsive to these molecules, you can build gene switches that can detect these things.

McNally:

What I hear, and I guess I hadn't really thought about it before. I always thought about it in terms of, "Oh this paper-based system will work with Ebola." We assume, or we've seen some preliminary work that it might work with HIV, it might work, as you said with the common cold and so on. What you're saying is it's actually much broader than that. This paper-based system give you cheap, portable, user-friendly ways to do all sorts of things that were more expensive and more complicated in the past.

Pardee:

Exactly, and we see it as hopefully a tool that can replace expensive equipment that's not portable.

McNally: [48:35]

That's exciting. Where do you see yourself in five or ten years?

Pardee:

Hopefully building the next generation of these sensors, and finding new and interesting ways to apply them in research. I think it has excellent opportunities as a research tool, but also just in the space of energy and the environment, as well as health.

McNally:

Okay. Keith Pardee, thank you very much.

Pardee:

Thank you Terrence, I really enjoyed this.

TMN: [49:00]

I ask Jim Collins how he sees the big picture moving forward.

Collins:

I think there's two things. One is we have ambition to impact this outbreak and we're in discussions with groups in the Americas to see what kind of pilot testing we can do. I think more broadly we are quite excited with our teams at the Wyss, at MIT, at the Broad and our collaborators growing around the world, of having this as a broad diagnostic platform. We've done Zika, we've done Ebola, we've done antibiotic resistance. We have plans and efforts underway to extend this to HIV, to extend it to Lyme disease, to

extend it to leprosy, to extend it to antibiotic susceptibility testing, to extend it to the flu for having an at-home flu test.

We have interest in using it as a clinical and research tool for exploring the microbiome, an area of great excitement in biomedicine.

We have big plans now to extend it to cancer. Can we use it as a cheap rapid early diagnostic for a range of cancers? We think this is a transformative, disruptive platform that can impact many areas, and at the Wyss we are now aggressively exploring how we could best translate this commercially and clinically. Should we do this through a new co? Should we team up with an existing company and/or should we create a nonprofit that can address in particular the next outbreak for a newly emerging pathogen or a reemerging pathogen?

McNally: [50:20]

This is exciting. When you talk about this system, the key components of it that define it for you as this system are the paper base, the amplification, the use of CRISPR...

Collins:

Yeah I think it's broadly the workflow, that is, on the basis of sequence information, either DNA or RNA, we can rapidly and readily develop RNA sensors and other sensors, from syn-bio that could be utilized to detect some sample of interest. We then have this workflow that would allow us to couple this with cell-free extracts to freeze-dry these and to now readily and rapidly encode them in paper - and it's not limited to paper. In fact, this works on any porous media so we've shown it can work on plastic, quartz, cloth. By the system, I think it's the entire design and workflow leading now to a cheap, deployable system that doesn't need a cold chain.

To go back to the earlier point I made on the sensors, we've shown you can freeze dry other sensors, groups that have come up with ones that we didn't come up with...nano-based sensors, and in many cases, they've worked quite well, and so we think it's a system, a platform that is broadly applicable to different diagnostic challenges.

McNally: [51:35]

Where do you see this work or your work evolving in the near future or the five to ten years?

Collins:

I think that in this space we are really quite excited about how we can use synthetic biology, so the engineering of biology to really harness the power and diversity of biology for coming up with whole new classes of diagnostics and therapeutics. I think in this case of what we just talked about it's a great example of how you can harness the power of biology to come up with a whole new class of diagnostics.

I think we have potential to do something similar around therapeutics, so using this freeze drying to get after portable manufacturing of therapeutics that can be used on demand, on site. I think we are similarly excited about how you harness living cells themselves as diagnostics and therapeutics for either treating infection or a range of complex conditions, being inflammatory bowel disease or cancer. I think this interface of engineering and biology which is the centerpiece of the Wyss is going to introduce new classes into medicine that I think will really change the face of medicine in the coming decades.

McNally:  
Excellent, thank you Jim Collins.

Collins:  
Thanks Terrence.

McNally: [52:45]  
You've been listening to Disruptive: Rapid, low cost detection of Zika and future pandemics.

I'm Terrence McNally and my guests have been Jim Collins and Keith Pardee. You can learn about their exciting work with paper diagnostics for Zika and other viruses - as well as an exciting range of other projects at the Wyss website, [wyss.harvard.edu](http://wyss.harvard.edu). That's [w-y-s-s.harvard.edu](http://w-y-s-s.harvard.edu) where you'll find articles, videos, animations, additional podcasts - and to have podcasts delivered to you, you can sign up at the Wyss site or on iTunes or SoundCloud.com. My thanks to Seth Kroll and Mary Tolikas of the Wyss Institute and to JC Swiatek in Production and to you, our listeners. I look forward to being with you again soon. [53:30]