A proactive method to secure a PhD

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Hopefully some of you are here today because you are interested in doing a PhD. I'm going to talk to you about what I'm doing in my PhD, but I also want to raise your awareness about some really interesting science going on out there which you may not have heard about. I also want to give you some idea about how I went about getting my current position, because I think I had a bit of an unusual route to getting my PhD (but it might also work for you). I'm going to split the talk into two halves. First I'm going to talk about myself for a bit.

So I work at the MRC LMB in Cambridge, which is one of the best institutes in the world. I'm very luck to work there, it's absolutely brilliant. We've got a brand new building, there's lots of Nobel Prize winners everywhere, it's really nice. I work with a guy called Jason Chin and we work on a subject called Synthetic Biology. You may be familiar with it, but if not it's a relatively new field which has only existed in the last ten years or so. Feel free to google him if you want, because I do think what we do is quite cool. The textbook definition of Synthetic Biology is ''designing and construction of novel artificial biological pathways, organisms, devices and the redesigning existing biological systems" but I prefer to think of it as re-engineering life, because that's actually what I do; I go into the lab and think about how I can completely change how life works, which is pretty cool.

A quick skim through some of the relevant science now, which hopefully you'll find interesting. Presumably you're familiar with the genetic code and the amino acids that make up proteins. So, we have the standard 20 amino acids. Then there's Selenocysteine and Pyrrolysine, which are naturally occurring variants – important, but they're not standard. The question is, do we have to be locked into these twenty, or twenty two, or can we actually go beyond this and have other amino acids that we invent in the lab, that we synthesise and then put into proteins when and where we want?

The answer is that we can. So, what I do is bypass 4.7 billion years of evolution and put random amino acids with different functions into proteins exactly where I want, when I want. The slide from a 2010 review article shows a variety of different structures that can added in this way. Each introduces different properties, for example one has got a little group on the end where you can conjugate anything you want onto it. If you add a fluorescent molecule or drug or something like that you want to add to a protein of interest, you can add this amino acid to your protein via modified synthesis and then use this little chemical handle at the end to glue on your drug or your fluorophore, so it's quite a powerful technique. This is actually a relatively short list, at the moment we're up to about 170 unnatural amino acids that we can encode.

I'm going to explain a little bit more about this approach. Presumably you're all familiar with the ribosome and mRNA and how proteins are made. I'm not going to go into too much detail, but basically you have tRNA molecules and amino acids and you have tRNA synthetases which are specific for both the amino acid and their correct tRNA. These three work in combination. The synthetase is an enzyme, which takes the amino acid, which is the little hexagon in the diagram, and charges it to the tRNA. The synthetase then goes off and the tRNA goes to the ribosome and decodes the relevant codon, for example for Alanine, and the amino acid gets conjugated to the peptide chain, which is coming out of the ribosome. Then the tRNA goes off, everything shifts along and we have the peptide elongated by one amino acid. In all organisms you have matched pairs of these, so you have a set of a synthetase and tRNA for Alanine, another set for Lysine, a set for Serine, and so on. Each acts independently; the Serine synthetase, for example, doesn't interact

with Lysine, otherwise you wouldn't get the necessary specificity when it came to reading of the codon at the ribosome.

What I do in my research is to say "ok, instead of Lysine at a particular position, we want to put in one of those unnatural amino acids", for example the one I described to which we can conjugate a fluorophore. I'm giving this a little star symbol in the diagram, but I've kept it as green because it's still sort of similar to Lysine. So we've got our tRNA for Lysine, we've got this amino acid we're adding into the cell and we've got synthetase. We can actually do this with all sorts of organisms – bacteria, worms, human cells and so on – but for the example I'm going to use *E. coli*, because *E.coli* is very easy to work with. We have this unnatural amino acid we want to include in the protein, but it doesn't fit the shape specificity of the Synthetase. This is a problem because we're obviously trying to get that amino acid to stick there until it is loaded onto the tRNA. We can actually evolve that active site, using site-directed mutagenesis, basically engineering the active site to bind to what you want.

Hopefully you've spotted that there's a problem doing this. If you take the *E. coli* Lysine pair, the *E. coli* tRNA and the *E. coli* synthetase and you then put them on a plasmid and you engineer the active site, you're going to get cross reaction where this amino acid will be charged onto tRNAs inside the cell, and you're going to get the unnatural amino acid put into the newly forming protein chain at places other than where you specifically wanted it.

So, what we do, we actually take a synthetase, a Lysine synthetase, from an underwater organism that lives near one of these deep sea vents that you find in the middle of the Atlantic. We take this organism and we've sequenced their genome and we've identified the synthetases. However because these are so evolutionary diverse from *E. coli*, or from human cells or anything else, it won't cross react. We can engineer it in the same way I described and then we've got this specific tRNA which won't cross react with any other synthetase, or any other tRNA in the organism. We say that it's completely bio-orthogonal, which just means it is separate; it acts completely on its own.

But there's another problem with this. If we stick with the example of Lysine, this is added to a peptide chain when there is an AAA and AAG codon. If you're familiar with the genetic code though, there are about 20,000 instances of these two codons in the genome. So even though your unnatural amino acid is acting separately, it's still going to be put in everywhere across the proteome, into a variety of proteins and that's obviously not good when you were wanting to target one particular protein. It might even kill the cell, which is actually not that uncommon.

What we can do instead is to use a stop codon. A stop codon is basically where protein synthesis is supposed to terminate. There are only 321 copies of the UAG stop codon in *E. coli* and not many more in human cells either. So we can take the anticodon of the special tRNA and change it to a UAG. Now we've gone through a system which upwards of 20,000 instances of your amino acid being put in, down to literally 321. This means you can then take the protein that you're interested in, say a cancer protein, you can put it in your unnatural amino acid and you can then do whatever kind of chemistries you want to do and you've effectively expanded the genetic code. Even better, a paper came out a couple of years ago, in which a group of scientists completely re-engineered a strain of *E. coli* to have no UAG stop codons at all, only UAA. That's a pretty impressive feat. So using that strain you really can now put your special UAG codon in at one position at one time.

Just to go into a bit more detail about what I've been doing with this. The slide [number 11] shows Lysine with an extra molecule we call a photocage. When you shine light on this molecule it cleaves off, leaving your Lysine. This means that you can engineer your Lysine with a photocage into the active site of a

molecule, then shine light on it so the extra group comes off. The next slide shows the active site of a kinase enzyme, where they've put in an essential Lysine, which is photocaged and then you shine light on it and then ATP can bind. You're using light to activate a particular process within the cell at a particular time, completely under your control. That's the kind of thing I've been doing and gives you an idea about the sort of strategies that are appropriate in synthetic biology at the moment. If you want to know any more about this specifically, then by all means ask me about it, or look up our research.

Now I'm going to talk to you about how I got into this role, because there's a lot of research going on out there which is completely different to the things you've probably covered in your lectures so far. It was a big leap to me to go into something I've never heard of before.

I want to talk to you about what being a PhD student involves. You may be on the fence about whether or not you want to do one, so let me tell you the kind of things I have to deal with every day. As a PhD student you have your own research project to carry out. If you've done any lab research project over a summer vacation or if you're currently doing your third year, then you have a bit of an idea of what you have to do. You're in the lab, you're doing work, your handling data. Unlike final year projects and summer work, you are much more independent, your research is your own. Sometimes you will be working closely with another student or with a Postdoc, but I think most PhD students have their own projects that they're in charge of, that they pretty much drive, that they govern and move in a particular direction, with the assistance of their supervisor.

As a PhD student, you get to work in a cutting edge field, the research that goes on will not be in textbooks, it's brand new. Obviously that's very exciting and if you're interested in making new discoveries then a PhD is definitely for you. For the majority of bioscience PhDs it's going to involve lots of lab work, so if you don't like lab work don't do a PhD. I know people who've gone into a PhD because they love reading research papers, understanding new things and doing exams but then they realise that they actually hate doing the lab work. So, make sure you don't mind doing lab experiment, because otherwise you'll have made a terrible mistake! Doing a PhD also involves lots of data analysis and lots of reading. It is pretty interactive, you get to discuss your results, with your own team at lab meetings, and you may get to present what you're working on at conferences. It's quite cool being at the forefront of something, being involved in new discoveries. It's really exciting when you know your new results are something that no one's even seen before.

However, there are some drawbacks. The hours are pretty long. That's certainly true for me, and I think most PhD students have a similar situation. My average day is at least 12 hours and I usually work most weekends as well. I'm actually quite lucky to have managed to get today off. I think I am a fairly extreme example, but long days are the norm.

You have to be able to cope with failures and setbacks. Most PhD students have experiments that fail. If you are doing science research you have to be prepared to have experiments that don't work, where you don't see the right result and you have to repeat it. It can be quite challenging to keep motivated when you're trying to do something that should work, where you should be seeing results, but it keeps failing repeatedly. I had quite a lot of this, so I can tell you from personal experience that it's quite demoralising. But if you're the sort of person that can battle through that and that you can see the end goal and you can work towards and motivate yourself, it's not actually that bad. There are some people who seem to get to work perfectly, so you might just be lucky.

You're likely to get quite a lot of independence. If you've done a final year project, or you're doing it at the moment, and you find you're the sort of person who knows what the next experiment should be without

talking to your supervisor, then that would be perfect. Supervisors are all different, some will be quite supportive day-to-day, but others basically leave you in the lab for 6 months without really talking through your results with you. If you have that experience it can be quite challenging!

Another thing that might be frustrating is limits on the funding you have available for the experiments. Fortunately that's not something I've had to cope with as the Chin lab is well resourced. But I do know a lot of labs, where maybe the next logical step in the research is going to cost £10,000 to do one experiment, it might not be that easy to justify. So you do have to think about where you will go and how well funded they are.

Your success is very dependent on your supervisor: how well they work with you and how well you work with them. It's obviously quite difficult to get an idea of this before you've even applied for a PhD, to know whether they're going to be good mentors or not. But, although it's hard it is something that you really do have to think about very carefully. When you are applying, and then when you meet them, you need to register that this is someone who will be your guidance for the next three or four years of your life. You have to be assured when you meet them and talk to them that this is someone you'll get on with and someone that you can work with.

Suppose you've decided that you do want to do a PhD, you want to carry on with science and you maybe want to be a group leader or just do some more experiments (there's lots of different reasons why you would want to do a PhD). One of the main things you need to do is find a topic that you're interested in. Don't just go and do a PhD because there's one on offer and you've got nothing else to do with your life, because that's going to be hard going. There are plenty of people who do that; they work on cancer because cancer is really cool, and then they realise that actually they hate what they do because their area of research involves staring down a microscope at cells for 8 hours a day. You have to really find something you love and this will probably require a lot of reading before you apply anywhere. By the time you're thinking about applying you should be used to reading primary research articles for your modules and project. So if you are thinking about doing a PhD you should definitely read papers on the areas that interest you.

If you've found a group, or found a topic that you're interested, then try to find out a bit more about the group. All groups have a reputation, unless the PI is brand new. So, if you say you want to work on a particular cancer kinase or say you're looking up synthetic biology, try and to see if there's anyone around Leicester who would be able to give you some advice. There's always someone who's heard of another group. I'm not saying that you should go and bother lecturers all the time because they're busy people but maybe a quick email or something to ask if they can offer any advice about this guy's research and they'll either say "yeah they're great" or they'll say "actually think of something else".

Have a look at their publication record. Most groups are quite well established, they've been around for a few years, so you should be able to find quite a few papers. You might get some idea from their university website, but often they're quite out of date. You need to dig into their research and find out what's going on, what kind of things they are working on at the moment. Generally groups might have a very long publication history, but their most recent publications, things in the last three years or so not stuff released in 2001, that's going to give you the best insight into the research they're doing. So find out what they've been doing in the last two years, three years, four years and see whether that applies to you because that's probably what you're going to be working on, at least something very similar.

Probably the most important thing if you want to do a PhD is to get a good grade in your degree. Things are very competitive now, for whatever you want to do not just if you don't want to do a PhD. You're paying so

much money on your fees; make sure you don't waste the opportunities you've got now. I can't stress that enough. It isn't necessary to do a Masters before a PhD; I'm working in a really amazing lab and I didn't do a Masters. If you're sure you want to do a PhD just go straight for it, there's no reason why you should hold yourself back.

OK, so you've found a lab, you're interested in what they want to do, but now you've got to actually apply. What do you do next? You want to see if they're offering a PhD and there are a few places you can find this. There's a website called findaphd.com and places are also advertised on the main university websites or departmental pages. Many group leaders have their own website. See if they're actively offering a PhD to start off with. But even if they're not, don't necessarily let that hold you back. I'll say more about that in a second.

If you know they're offering something then that's a clear indication that there's an opportunity for you. Also, see if the position is funded. I think most positions that are advertised are, but obviously you don't want to be in a position where you have to use your savings, or your parent's savings to get yourself through a PhD because it's not really necessary. There are positons out there that are funded and they're very good positions too, so make sure you just keep an eye on that.

Also, keep track of the application dates. When I applied Oxbridge was in September, so if you're looking for a PhD somewhere like that you're probably already too late to apply for a start this autumn. Most universities are later, but they are getting earlier, so give yourself plenty of time and make sure you're ahead of the game with regards to dates.

Now I'm going to tell you a bit about the approach I specifically took in my application because I don't think a lot of people do this, but it worked out really well for me. I picked out four group leaders I was interested in working for and I wrote directly to each of them. I aimed pretty high and I didn't really have any back up plans if it had all gone wrong. I sat down and I designed a potential project I could do with them, which was pretty challenging to do. I went through pages and pages of research that they had done and thought "ok, where do I think they are going next?" A group leader isn't going to tell everyone what they're doing. They don't want their competitors to know what their next project or the next five years' worth of projects is going to be. But if you start reading through their publications, you will get an idea of where they're going and you should be able to generate some ideas about where you could fit into that, where your experience or skills would fit, or even to come up with completely fresh ideas that you've just come up with.

This works out really well if you come up with a good idea and its well received, because it show a lot of initiative and it shows that you're interested. When you fill out the standard online application forms where you just type in your name and your lab experience those go initially to some person in HR who doesn't understand any of the details in the things you're talking about. So, this alternative approach is bypassing all of that because you're talking directly to the person you want to be employed by. It is risky because you don't want to be writing just some old rubbish to the person whom you're really trying to impress. You want to make sure the project that you've proposed or even anything that you write them is well thought out. It could potentially be poorly received. I mean you're just some student right? Who are you to tell a group leader what they should be doing? So, there are risks involved, but at the same time I would say that if you're a group leader, you're sitting down at your desk and you've got an email from someone who says, not only have I seen then project your advertising or the work that you've done, but I can see where you might be going in the future. You're more likely to pay attention to that than some CV that comes onto your desk.

If you do think that you've got some good idea you want to email someone, see if your tutor or a PI here that your know is willing to give you a few minutes to discuss what you're talking about, they can vet it and tell you whether you're talking about something that's completely ridiculous, or whether you've actually got a good idea. That's what I did. I had the opportunity to speak to some people in the Chemistry department about what I was proposing and it was very useful for developing my ideas and putting them forward.

Also, meet personally with the supervisor if you can. By all means email them and ask if you meet for a coffee, or can I give you a call on Skype or something, to talk about me and talk about my research and talk about you and where I can fit in. The most important message here is if you choose to do this it can pay off, but make sure you do your reading because they can see right through you if you do not actually know what you're talking about. Believe me, I've had people come into my lab and the students come round and say 'yeah I'm really interested in things like Biology' and you say 'ok what do we do?' and they don't have a clue because they haven't bothered reading it, they just know that my group leader has a big name. You can't be that person; you have to actually know what you're talking about.

I'll give you an example of the sort of thing I wrote. "Dear whoever [the group leader you're interested in], I'm a third year undergraduate student at Leicester. I'm interested in your group's research. I've been following your publications [make sure that you have been following the publications, because otherwise that's a complete lie] and I think there's potential to investigate a particular subject". For me it was to investigate genetic code expansion, to investigate novel proteins with novel chemistries using a particular technique. For me it was evolving the active site of a synthetase to recognise a different molecule. Then I went into a bit more detail and I said "ok I think the experiment we could do is to synthesise this photocaged lysine, which I described earlier, and then we could evolve the active site using site-directed mutagenesis and we could add it to the cell and see what happens and that would give us insight into how a kinase work, or insight into whether we can work with particular amino acid". Then it's quite important, to say something like "would you be free to have a chat about this?" Because group leaders do have time to chill out and discuss science with young minds, so make sure you're open to doing that and it will make a difference. I actually got a chance to speak to my current group leader, when he came to Leicester to give a presentation. I jumped on that as an opportunity to show him that I know what I'm on about.

So, it can be useful taking this more pro-active kind of approach, but as I said earlier be very careful; make sure if you send an email like this you're not just talking rubbish. You have to be able to back it up. Do your research thoroughly, take it seriously. If you've read a paper make sure you real understand it, not just the rough gist. But if you have, be confident about what you're saying. Also, make sure you mention your undergraduate project, or any work you've done over the summer, or anything like that is related, because it is useful to be able to say that you've got hands-on experience of some technique or area of research. Don't overdo it but show that you're not just someone who's read papers but you've also been in the lab a bit too.

Also, don't just copy the project description that they've put on their website. They know what they're advertising; the trick is to notice what they're not advertising, to spot what they're potentially doing next. If you just throw their own words back at them they'll see right through it! On the other hand, don't email them an essay either. These people are busy, you want to put your point across concisely and to show some initiative that's going to make you stand out from other applicants, in a good way. I stress again though, don't take this approach if you wouldn't feel able to sit down with the potential supervisor and have a sensible conversation with them about what you'd be interested in doing. These guys know what they're talking about, their top scientists in their field, they'll spot a bluffer a mile off.

Even if you make direct contact in a positive way you'll likely still have to apply formally through the University website. That's what happened to me; I still had to apply through the Uni and cough up 50 quid which was a right headache. You still run the risk of getting sifted out by some HR person, so be prepared to have a half decent CV as well. I'm fortunate because I have actually ended up working on the project I proposed, but that might not happen for you. At the end of the day you are not trying to tell your group leader what he should be writing in his grants proposals, you're just trying to get in.

So, that's how I did it. I made four applications, had four interviews and received four offers. I'm not trying to brag, I'm pointing out that the effort to research the labs and email people paid off. Without doing something to make yourself stand out you run the risk that your application to a top lab will get lost in a sea of paper, applications from other people from America, or from Europe. Do something to get your name out there. You can do this if you're confident, if you believe you can come up with a great idea, then it does work.

If you do get an interview be prepared to have a presentation ready about your undergraduate projects or any of the projects you have done and offer to present it. Don't wait for the offer; be like "I'd be very happy to talk about what I've been doing". Again, it's an initiative thing; not only have you turned up to the interview but you're ready to give a talk about what you've done, what it means and why it's meaningful.

Also if you do get an interview, talk to people in the lab. I said already that you can probably find out about the reputation of the group leader whilst you're here in Leicester, but getting a chance to talk to the PhD students in the lab, to the Postdocs in the group, that's invaluable. Find out about what they're doing, find out about the university or the town, what they think of your ideas. These are the people you are potentially going to be working alongside for the next three or more years. You don't want to end up somewhere that's not right for you. So do the reading, make the application and then be confident. At interview make it clear you're there to get the job; you're not just there to turn up and listen, you're there to show you're the right person for the job.

Good luck.

Questions

***Q**: If you're not confident about your ability to come up with your own extremely novel ideas, and your competing with someone who knows a whole lot more than you on a particular subject, would you say there's still value in contacting the scientist, to partially bypass HR and get their attention before you apply?*

A: Yes, I'd say that there's definitely value in contacting them, because the worst case scenario is that they say "I'm sorry". To be honest that's something you're definitely going to be facing, if someone's got a load of CVs on their desk some are going to get rejected at that stage. You don't necessarily have to come up with a brilliant new idea, but do at least take the opportunity to show you've read up on their work. Make it clear that you're well aware of what they are doing and why they are doing it and why it matters to you and why you are interested in it, as well as pointing out any relevant experience you already have.

Dr Chris Willmott: I would also say, a year from now you will of possibly had the opportunity to do a summer project between second year and third year. You will have done your first term including doing a lab project (certainly if you're intending to be applying directly to a PhD then you need to have been doing a lab project) and so you'll be much more familiar with a particular field than you are now. So although, that might seem impossible right now, in a year's time, that might well be more of a realistic possibility. If it's not, then there are the more traditional ways of getting into a PhD as well.