ZIERLER: Okay, it is March 30th, 2020. It is my great pleasure -- David Zierler, Oral Historian with the American Institute of Physics. It's my great pleasure to be here with Dr. Robert Best of the NIH. Dr. Best, can you tell us your affiliation at the NIH, and your title?

BEST: Yeah, hi David. I'm affiliated with the Laboratory of Chemical Physics in the National Institute of Diabetes and Digestive and Kidney Diseases. I'm afraid it's a bit of a mouthful. My title is a Senior Investigator.

ZIERLER: I'm curious from your perspective - why is chemical physics in NIDDK? Of all of the institutes, why NIDDK?

BEST: I think it's a little bit of a historical accident. The lab is actually very old. I think it dates back to '40s, even. Why it's in NIDDK, I'm not sure, to be honest.

ZIERLER: So, there isn't any substantive reason why -- I mean, with kidneys and diabetes, it doesn't intrinsically make sense, as far as you can tell?

BEST: No, it doesn't. There are other labs in other institutes that you could say have some overlap with us in terms of using physical approaches to look at things. NHLBI, for example. Especially with the older institutes, the correlation with the institute name and the research is not that tight, and there's a lot of fundamental biology research, and biophysics research that goes on at NIH in addition to the clinical side of things.

ZIERLER: Okay, alright. So, let's start right at the beginning. Tell us about your early childhood in Cape Town.

BEST: Okay, well, I guess --

ZIERLER: I'll make it easier for you. Tell us about your parents. Where are they from, and what did they do?

BEST: Okay. My mother is South African, and by father is British. He was a scientist also, a marine biologist, actually. He studied whales. So, there's probably inspiration that comes from that to get into science.

ZIERLER: Where did your parents meet?

BEST: In Cape Town.

ZIERLER: Is that where your father was doing research?

BEST: Exactly. The whales that he worked on were southern right whales. One of the places they have a high population around the western cape in South Africa. Their population was reduced to, I don't know, 7%, or maybe even less of its original level, and it's managed to recover remarkably well. Unlike in the Northern Hemisphere, the right whales are kind of really struggling. The gene pool doesn't seem to be, maybe, large enough to sustain a population.

ZIERLER: And your mom? Did she work outside the house?

BEST: Yeah, she was a teacher -- elementary school teacher.

ZIERLER: Now, your father, was he affiliated with the university? Who was his employer?

BEST: He was affiliated with the University of Pretoria, which is kind of odd, because it's landlocked in the middle of the country. So, he actually worked in Cape Town, but he had a research unit. I think once a year he went to Pretoria to give a lecture, or something, but the rest of the time he was based in Cape Town.

ZIERLER: But he grew up in England?

BEST: Exactly. He was really into biology, I think, from an early age. I think his interest in science probably rubbed off on me to some extent, although I was not attracted to the biological end in the beginning.

ZIERLER: Right. You clearly went the chemistry route. Now, was his work mostly on boats?

BEST: Yeah, I guess some of it was on larger research vessels, and on small boats on the coast of South Africa. He did also helicopter work to do aerial surveys to survey the population, basically, every year.

ZIERLER: Did you ever accompany him on any of these trips?

BEST: That's a funny thing. My main experience of accompanying him was to see dead whales, stranded on the beach. So, maybe that's why I didn't want to get into biology. They were very smelly, but I guess it was a great opportunity for him to collect specimen, and so on. So, actually, the first time I actually went out in the boat with him was, I think, when I was in university, or something. So, only quite a bit later.

ZIERLER: Your primary education, you went to public school, or private school?

BEST: I went to private school, yeah. So, I was lucky that I had very good science teachers there.

ZIERLER: What about for high school?

BEST: Yeah, for high school, the same school.

ZIERLER: Was this a secular school, or a religious school?

BEST: It was a religious school, an Anglican school.

ZIERLER: So, not particularly religious?

BEST: No, no, the school was Diocesan College, and we had to go to chapel every day. I mean, I was not particularly religious, but --

ZIERLER: And the science teachers taught evolution? There was no apparent conflict between --

BEST: I'm pretty sure the science teachers were all atheists.

ZIERLER: Okay. And the decision to go to the city of Cape Town, was that sort of -- I guess, the first question is when did you develop a real interest in math and chemistry? Was this in high school, or did this develop in college?

BEST: I think I got more into chemistry when I was at university. I guess I liked math when I was in school.

ZIERLER: And Cape Town -- was it the premier school in South Africa? Was that the main drive, or was it more that it was close to home?

BEST: Kind of a combination of the two. I got a scholarship to go there, so that kind of made the decision easier. It also was close to home, and it's, I think, one of the two or three best universities in the country.

ZIERLER: Was the school integrated at that point, when you were in college, or no?

BEST: There had only been a significant fraction of black students for a few years. So, I guess it was -- I started in '94, so that's like the same year -- sorry, no, I started in '95, so it was a year after the first democratic election. The university had, as far as I know, never had any racial selection. I mean, at least for a long time. For practical reasons, it was not possible for many black people to attend.

ZIERLER: You mean, simply because people wouldn't allow it, culturally?

BEST: No, I mean, in terms of affordability, and in terms of having the right schooling, and so on, to get in. I think some sort of combination of those things.

ZIERLER: And you declared as a double major in math and physics right away, or that was a sequential process?

BEST: No, I think I added the math later on because it turned out that I could get the extra major without too much extra work.

ZIERLER: Were you thinking, sort of, between the double major in math and chemistry, what were you thinking in terms of a career? Were you for sure going to go to graduate school? Did you consider going to industry? What were your goals as an undergraduate?

BEST: Well, when I started out, that's a good question.

ZIERLER: I mean, maybe you were just, like any undergraduate, not really thinking about a career at that point.

BEST: Yeah, exactly. I just wanted to -- I was just excited to learn about science. I guess, I had in the back of my mind, maybe a research career, but I guess I hadn't really thought it through that clearly at that point.

ZIERLER: But then you stayed on for the master's at the University of Cape Town, straight through?

BEST: Right. So, I guess I did a fourth year project with a guy, Kevin Naidoo, in my department. He was doing computational chemistry, and that was, I guess, my reason for staying on, for doing master's. I really enjoyed that project, so I decided to stay on with him.

ZIERLER: Was computational chemistry a new field at that point?

BEST: No, it's been around a long time. Probably, I would say, it was born in the late '70s, or something. The use of, basically, molecular dynamic simulation in chemistry probably dates from around about that time.

ZIERLER: And then what were your options after your master's degree? When you ultimately decided to go to Cambridge, were you thinking about leaving the country, or did yu have a specific opportunity at Cambridge that compelled you to leave?

BEST: Well, it was actually encouraged by my advisors to go overseas to study. So, I applied to Cambridge first, so I guess that's kind of how that unfolded.

ZIERLER: Was the idea that staying in South Africa you couldn't get the -- you would dead end faster there than had you gone abroad?

BEST: I think there's definitely opportunities to do interesting stuff in South Africa, but I think they felt like I would get more exposure -- I mean, it would be a good experience to basically be exposed to a big research university like Cambridge. In retrospect, I can understand why, because it's such a great academic environment.

ZIERLER: Had you spent any time in England before?

BEST: I had been there once before, just for a vacation.

ZIERLER: Your father had family still there at all?

BEST: Yeah, no, my dad didn't really have family there, but he had been to Cambridge himself, so I guess there was probably some bias there.

ZIERLER: Oh, he's an alumnus of Cambridge?

BEST: Yeah, exactly.

ZIERLER: Oh, he must have been quite pleased about that development, then.

BEST: Yeah, he was. He did his undergraduate degree there, and he did a PhD by submitting published work, so he worked very independently, basically, when he was in South Africa. Instead of doing a thesis, he just submitted a compilation of his papers, and they did an exam.

ZIERLER: And that worked.

BEST: And that was it, yeah.

ZIERLER: Now, when you got to Cambridge, was there coursework, or was it strictly lab work?

BEST: It was only really lab work, because the PhD there is three years, so you don't really have time to do formal coursework. So, I would say that's definitely a potential drawback of that system. They do have advanced level undergraduate courses that you could sit in on, but other than that it's kind of up to you to get up to speed in whatever you need to.

ZIERLER: And the lab work that you're doing, is it mostly what the professors are saying would be a good idea to work on, or is it more self-directed?

BEST: Well, I think when you first get in, usually your advisor is going to give you something to work on, because they can't let you wander aimlessly for a couple of years to find what your direction is going to be, but then usually you find a more independent direction moving on from that.

ZIERLER: And what was your process in identifying a dissertation advisor to work with?

BEST: Well, I was always interested in protein folding.

ZIERLER: Oh, even at that time you were?

BEST: Yes, I mean, I was interested in protein folding because I guess I had read some articles of some early lattice model studies of protein folding when I was doing my masters. So, I was interested in that, and then I also kind of felt at the time, in my naive way, that the genome projects were coming to an end, so the next thing would be to translate that genetic information into structural information, and that's one definition of the protein folding problem that's just how to find the structure given the sequence. That was my interest at the time, so I looked around at a couple of advisors -- Alan Fersht and Jane Clarke. I think Alan kind of steered me towards Jane, so I ended up working with her.

ZIERLER: Right. And what was your dissertation title, if I can jog your memory?

BEST: Yeah, it was an easy title to remember, because I think it was something generic like Studies of Protein Folding. I did a number of different projects during my PhD, so that was kind of the way that they could be fitted together. So, I guess I started out using -- this was, I guess -- the project that Jane Clarke put me on at the beginning was using atomic force microscopy to unfold proteins. So, that was kind of a new thing at that time, and the idea was to combine the mutagenesis approach to studying protein folding, where you make a bunch of single point mutations, and see how they change the folding rates, and the stability of the protein so you can infer something about the folding mechanism from that. So, the idea was to translate that to this mechanical unfolding approach. There are some proteins which are subjected to force in the cell, so it's kind of more of interest to know what the effect of the force is on the folding energy landscape.

ZIERLER: Now, at this point, did you think of yourself as working in the field of health science research? Were you making that connection, or was this more, sort of, pure chemistry, and you weren't really focusing on its application or clinical value?

BEST: Those projects are definitely fundamental research interest mainly, but I got into protein folding with the thought that it might be eventually useful for health research.

ZIERLER: What were some of the -- early on, what were some of the ways you think this research might have been helpful? At the time, what were you thinking it might be useful for?

BEST: I guess, at the time I had a lot more optimism about that. I still am, obviously, optimistic, but I guess I had some naive optimism about how accurate the models we have are for folding. It could be useful, as I said, in the first place, to predict protein structure, and that would then be immediately relevant to biomedicine, because of their sort of structure function hypothesis. If you know the structure of the protein, then you can figure out what its function should be. It's kind of amusing now that I'm working on intrinsically disordered proteins, but anyway...

ZIERLER: So, the concept -- and we'll return to this, but I'm fascinated by the concept of "naive optimism". So, you had these big ideas as a graduate student about where this research could be applied, and obviously, that suggests that the applications, as you've learned more about the issues, are probably, what? What would be fair to say? That they're less applicable than you would have hoped? Or, what are essentially the limitations that give you pause on the optimism that you felt at the time?

BEST: I now know that it's much more challenging to devise predictive models for protein folding, although we've really come a long way in doing that. I guess I've also come to appreciate where protein folding is -- where it's important to understand protein folding in biomedicine. It definitely is important in and of itself. It's not just like translating sequence to structure, but the process by which you get there can actually be important. If it doesn't go quite correctly, then you can get misfolding, and that can cause serious misfolding diseases. So, it turns out that it actually is relevant to understand these basic processes of protein folding.

ZIERLER: Okay, so you defend the dissertation, and then did you have the post-doc at NIH already set up, or did you defend and you were sort of weighing your options at that point?

BEST: Yeah, at that point I hadn't decided what to do, so I actually ended up doing a six month post-doc in Cambridge with Michele Vendruscolo, who was I guess, at that stage, just kind of starting out as a new faculty member there. So, that was actually a great experience. In that time, I got back to doing much more computational work, and working with some very smart people in Michele's group. So, that was a very interesting time.

ZIERLER: So, what were the circumstances that connected you with Bill Eaton and Gerhard Hummer?

BEST: I guess, the fact that I was working on protein folding, and they were one of the premier labs studying protein folding. Then, also, I think that Gerhard Hummer was interested in working with me because I had done these folding experiments, and he had been working on the theory for those experiments. So, I think he was interested in getting closer to the experimental side. It didn't eventually work out that way, but that was the original thinking.

ZIERLER: Now, at what point are you aware of the field of biophysics and thinking of yourself as working in that field? Does that happen before you get to NIH, or afterwards? Many different people have many opinions about what biophysics is, where it originated from, is it a real discipline, is interdisciplinary? So, I'm curious about your entrance into that world, and where you saw yourself.

BEST: Yeah, I would have said that I considered myself to be doing biophysics during my PhD already. So, biophysics is like a real umbrella term. I mean, if you go to a meeting of the Biophysical Society, you'll see people there who range from people doing molecular biology to people doing much more physical kind of experiments. So, yeah, it is very diverse and multidisciplinary.

ZIERLER: And you're drawing mostly on your physics education as an undergraduate?

BEST: Well, partly that, and partly what I learned in my graduate work in Cape Town. Also, the other training I had in physics was during my post-doc. Gerhard Hummer was a very good advisor to learn from.

ZIERLER: Okay. So, when you get to NIH, what is the project? This is something that Bill and Gerhard are telling you to work on, or you're going there with your own research agenda?

BEST: So, it was essentially something that -- I was working with Bill Eaton to begin with, and the idea was to develop a single molecule fluorescence that he had just started using a few years before that for studying protein folding. So, I was working with a couple of other post-docs -- Kusai Merchant and Sara Vaiana -- on setting up that instrument. And then I started a project on the side with Gerhard Hummer using molecular simulations to try and interpret the mechanical

unfolding experiments that I previously had been doing. So, I guess it's sort of -- again, those things were projects that my advisors were interested.

ZIERLER: Now, when you got to NIH -- you had earlier interests in applying to work in health sciences. Did you feel like, when you got to NIH, that you could more fully express those interests, just by virtue of being at the NIH with all of the things that they're involved with there? Or, as opposed to, did you still feel like it was more isolated from a clinical setting?

BEST: To be honest, in our lab, we are isolated from a clinical setting. There aren't any people doing clinical research in our building. That's not to say that we can't collaborate with people doing clinical work, and there are at least two groups in our lab that are -- Bill Eaton's being one of them -- that collaborate with clinical researchers. So, we weren't directly involved in clinical collaborations.

ZIERLER: Now, the post-doc -- were you thinking about converting the post-doc into a full-time position, or were you thinking about going back into a university setting? Basically, what were the circumstances that led you to go back to Cambridge after the post-doc?

BEST: I don't think that a full-time position was really an option. That's something that used to happen in national labs that people would be able to stay on after their post-doc, but that practice has sort of ended.

ZIERLER: So, when you came to NIH in 2004, it was on the assumption that it was a limited appointment? You went in on that basis?

BEST: Yeah. Exactly. Well, I mean, I went in for five years, and I could have stayed on after that, but I was looking for a more permanent position with more stable funding, and so on, than I had at that point.

ZIERLER: So, being a research fellow at the Royal Society at the University of Cambridge, was that a teaching position?

BEST: No. In fact, it doesn't require any teaching. It's essentially like a tenure track position, but not really. So, the Royal Society pays for you to essentially be an independent member of the

faculty. You're not required to do any teaching, but they recommend you do some teaching so that you can make yourself more appealing to eventually be hired later on.

ZIERLER: Uh huh. And then a year later, you become college lecturer. So, I assume you are teaching at that point?

BEST: Right, exactly. So, "college lecturer" just means that you are involved in doing supervisions for undergraduates. They have this system of small group teaching in Cambridge.

ZIERLER: And your research at Cambridge, is this a continuation of what you were doing at NIH, or are you pursuing new projects at this point?

BEST: I was pursuing new projects. So, at the time I got to Cambridge, I was doing only simulation work, and I wanted to get more into using physics based models rather than, say, coarse-grained models. Maybe coarse-grained models could also be considered physics models.

ZIERLER: Could you explain the difference? What's the difference between physics based and coarse-grained?

BEST: So, with coarse-grained models, you usually put in a lot of assumptions out of necessity. So, that can be very instructive, because it can show you what assumptions might lead you to the behaviors you're seeing, but at the same time, they're not predictive models. So, I was hoping to -- well, and I did get more into using atomistic simulations, or molecular dynamics forcefields to study protein folding, and such. So, that got me into finding out that there were some major deficiencies in those models. So, I spent several years improving that, and I think that's where I made my name as an independent researcher, making an impact and making those models more useful.

ZIERLER: And useful how? What does that mean? To whom?

BEST: Well, in the sense of being able to fold proteins. Previously, they would have, generally, a strong bias towards a particular kind of secondary structure. So, what I did was I used experimental data, particularly from nuclear magnetic resonance spectroscopy, to try and improve these energy functions. Basically, just [variationally] optimizing the energy function to

reproduce these experimental data with some well-chosen model systems. Then, it turned out that those improvements helped in order to fold entire proteins. So, we were able to show with the resources we had that we could fold 30 or 40 residue peptides. Others, namely David Shaw, in New York, had this special purpose supercomputer, just ordering molecular dynamics simulations. His group was able to fold a number of small proteins, up to 100 small residues, using these improved energy functions.

ZIERLER: So, you come back to NIH in 2012. Was that the plan? Did you love NIH so much that you were sort of just looking for a way back in, or was this sort of a unique opportunity, and it was one of a few that you were thinking about?

BEST: Well, there were certainly a few I was thinking about. The Royal Society fellowship is renewable after five years for another five years, and I'd just gone through that renewal process --

ZIERLER: Would the next five years be like a tenure situation at the end?

BEST: No, it was still not tenured. Cambridge doesn't usually tell you until you get to you 8th year whether they might actually offer you a position, so I wasn't really willing to wait around for that, so I applied to various places, and as it happened, this position in LCP came up at the same time. But that really is a unique opportunity. Those positions do not come up very often. The research environment in NIH has really major advantages over an academic environment, namely that you have access to stable funding. These days, most academics spend almost all their time applying for funding. Teaching can be enjoyable sometimes, too, but I think being able to focus entirely on research, there's definitely a major advantage of the way things work at NIH. So, I guess, I applied to several places, and got offers from several places, but I decided to come to NIH because the research environment, which, of course, I knew very well.

ZIERLER: Bureaucratically, were you, sort of, picking up where you left off with your post-doc, or was this a new area, a new opportunity, a new place in the structure of the lab? Is this essentially a continuation, or this is more new coming into 2012?

BEST: Well, this is new. So, now I actually started my own research group, and I was working on different things.

ZIERLER: Right. And the Best Lab, you started that at the beginning? This was right in 2012?

BEST: Yeah, exactly. I was brought on as a Principle Investigator when I came in.

ZIERLER: So, this is a continuation of your research at Cambridge, or you're starting on new projects at this point?

BEST: Some things were continued. I still had several people who decided to stay in Cambridge for various family reasons, and so on, so I had a group in Cambridge that was continuing to work on the stuff we were doing there, which was a mixture of doing atomistic protein folding simulations, and studying binding of intrinsically disordered proteins at targets, as well as some work on protein misfolding in multi-domain proteins. So, the stuff that I started when I came to NIH, some of it was a continuation of that. For example, I continued working on the misfolding stuff in multi-domain misfolding, trying to develop those models for more different proteins. But I also started some new areas as well. For example, one of the things that we got into is trying to use coevolutionary models for protein sequences to do some interesting things.

ZIERLER: So, let's stop there. What is a coevolutionary model of a protein sequence?

BEST: Basically, it's a Potts type model that has essentially a propensity of each amino acid to be at a given position in the protein, and also, a coupling between amino acids at one position of the sequence to another position of the sequence. So, this is just a model that you can use to essentially model the fitness, if you like, of a given protein sequence for a particular protein structure. So, I guess, one piece of information that the listeners may not be aware of is that although there's a huge number of proteins in the PDB, the Protein Data Bank, the number of actual really different structures is much more limited. So, if you consider independently folding domains, there's maybe only a few thousand different ones. So, if you look at those -- of the sequence which fold to these structures, there are now databases of hundreds, or even thousands of sequences that fold to the same structures, which allows you to build a statistical model. That's what's modeled by this Potts model. So, one can fit those models to the available sequences, and then use it for different things. So, the thing that the models had been used for was to try and predict which residues were in contact in the native state. So, you expect that if you have residues that are in contact, then there should be some significant correlation between the residue

types that you put in those positions in the sequence, and that will show up in the Potts model coupling parameters. So, that was a pretty simple application. Our thought was to try, firstly, to try and predict new sequences that could fold to a given structure based on those models, and to basically test how general they were, and then also the idea was to look at so-called fold switching proteins, which are sequences that fold into two different structures. So, that sort of emerged as an area of a lot of interest lately. Our goal was essentially to model a sequence based model for one structure, and for the other structure, and use those to find sequences which would potentially fold to two structures. Sorry, I've been kind of rambling on for a while about this.

ZIERLER: That's fine, that's fine. Now, when we're talking about proteins, proteins of what? Where are these proteins to be found?

BEST: The proteins that we looked at were from Streptococcal bacteria. The reason we chose them was simply that they had been previously been well characterized. There were also a significant number of sequences available to look at.

ZIERLER: The advances in computational power over the course of your career, has this been something that has always allowed you do new things as computers have become more powerful?

BEST: Yeah, it has, I guess, to some extent. Every now and then, you realize that you can access, now, a timescale that allows you to do something really different. I don't think it's the driving factor. I think developing methods is at least as important as the advances in computational power.

ZIERLER: Now, when you get tenure in 2016, are you looking at this mostly as -- well, obviously, it's a relief I'm sure. But do you see it as your peers recognizing your contribution to the field? Is that primarily what it is? Is it allowing you to do things that you wouldn't otherwise be able to do? Does it free up concerns about funding? How are you interpreting whatever it is that being conferred tenure means? What does that mean for you?

BEST: I think it is a huge relief, because it sort of -- I mean, of course it's nice to have the recognition, but I think the main thing is that before you have tenure, there's this stress of --

ZIERLER: Like, being uncertain.

BEST: I guess, you feel like you're constrained to do things that are a bit, maybe, more conservative. Having tenure does give you a bit more freedom.

ZIERLER: Freedom, in the sense that you feel like you can pursue that might have less likelihood to succeed? Is that part of it?

BEST: Right, yeah. Exactly.

ZIERLER: So, how do you define in your lab setting, what does success and failure mean? When you're setting up a project, and you're defining the parameters of what success looks like, how do you define those things? Or can you point to areas in your research where it's been clear you've hit a wall, this is a dead end, this clearly isn't going anywhere, this is a failure, versus you feel like you've come upon a breakthrough, you've significantly advanced understanding in your field. Working at your level, how do you know when you've arrived at either place?

BEST: I guess, sometimes you may make some progress, but you realize that it's -- I think success is when you can learn something new, and when you can -- I'm trying to think of an example of failure that -- hmm.

ZIERLER: Well, let's say like this: it's a success when you, say, learn something new. What does that mean? It means that this is something that was not known before, or it was theorized and now you are demonstrating it experimentally?

BEST: Well, I guess, demonstrating experimentally is something that we don't do directly, but we do with collaborators. A lot of what I do is trying to develop models that can explain what my collaborators are seeing in their experiments. So, in that sense, a success would be defined as coming up with a model that can explain what they see, and also can be used to make some sort of predictions that can be tested as well.

ZIERLER: So, what's a real-life example of a collaborator? What field is this collaborator coming from, for one? What are the kinds of experiments they're doing, and then what's the

model that you're building, and what's the value of that model to your collaborator? Could you just walk us through one example of that narrative?

BEST: Yeah, sure. One of my main collaborators is Ben Schuler at the University of Zurich. He does single molecule fluorescence experiments. One of the things we've been looking at is interactions of intrinsically disordered proteins with each other. Particular ones we've been looking at recently are highly charged proteins that interact with DNA, or with each other. So, these are challenging to study from an experimental point of view -- well, they're challenging experiments to interpret, because the proteins are disordered. So, trying to characterize what kind of complexes they might form, and what interactions are involved there, is something that's very difficult to do with limited experimental data. The number of possible structures is incredibly large. This is where we've used coarse-grained models to try and inform what's going on in the experiments, and have essentially been able to show that these proteins remain disordered when they're in a complex. They have this extremely high affinity for each other. More recently, we've been able to show that these proteins might play a role in acting as catalysts to get other proteins off of DNA. So, DNA binding proteins, like histones, are often extremely tightly bound to the DNA. There's this strong electrostatic interaction, and if you were to assume that the binding is two-state equilibrium with unbound and bound, and you can calculate what the on-rate is just from diffusion, then the off-rate would be many orders of magnitude too slow to be biologically useful. So, it turns out that, actually, there are other proteins that can help to facilitate a release of histones from DNA. So, I guess, that's an example of where we've been able to use simulations to show how that might actually happen, validated by experimental single molecule data.

ZIERLER: When you talk about proteins being disordered, what does disordered mean in this context? Disordered relative to what? I mean, the assumption is that you would expect them to be ordered, but they're not.

BEST: Yeah. I guess, it's maybe not the best term, but somehow the field decided that this would be the term they use. It means, essentially, that they have characteristics very similar to just an unstructured polymer. So, the name comes from -- as an opposite to the conventional view of protein folding to a specific structure. So, the idea is that those kind of proteins would only be disordered if they were unfolded before they fold into their eventual destination. But these

proteins are intrinsically disordered, because that is what their eventual destination is. So, they're basically just a disordered polymer, but that's a little bit misleading, because there usually is some degree of structure in there. Might be, sort of, local secondary structure, or long range interactions on different parts of the chain that's different from what you would expect from a polymer.

ZIERLER: Can you explain the importance of novel simulation methodology and theory for understanding protein function in evolution? What is the relationship of using this approach to understand protein function in evolution? I guess we could start first with definitions. What does "novel simulation methodology" mean?

BEST: Well, it could mean various things. On one level, it would mean devising new coarse-grained models. So, that comes down to putting in various ingredients into the model in order to capture the effect seen experimentally. Or, it could be an atomistic model, trying to do the sort of thing I did more in Cambridge, where you're trying to improve it systematically, to do more predictive types of simulations. Or, it could also be finding -- I mean, one of the major challenges that you have in running molecular dynamic simulations, which at the end of the day, are just large n-body, classical simulations, is that you have to use a very small time step, and they take a lot of resources than if you were just to run a brute force simulation. So, we're always trying to think of ways in which we could enhance the sampling without having to use a lot more computer power. So, I don't know. One example might be that we studied association of Alzheimer's beta peptide with the surface of an amyloid fibril. So, I guess one of the important proteins in Alzheimer's disease is this A beta peptide, which forms these long fibrils that may have a role in the disease. Of course, everything in the field is a little controversial. One of the interesting things, though, is this is kind of a nucleated process. You have to have some initial stable nucleus formed, and that forms the fibril. What's interesting is that the fibrils can nucleate the formation of additional fibrils, so that makes it much more sensitive to the concentration of the protein peptide. So, anyway, what we were trying to do is look at how this peptide associates with the fibril. We wanted to do it with an atomistic molecular dynamic simulation. If you just did that in a brute force way, you would have no hope of being able to sample all the ways that the peptide could bind to the fibril. So, we devised a way where we have this method based on replica exchange, which was originally a Monte Carlo method. In our method, we essentially are

dialing between a version of the energy function, which is kind of realistic, where the peptide sticks to the fibril, but that's difficult to sample. Then, one in which the peptide has a lower affinity for the fibril, so it's able to move around in solution. So, you're able to more easily look for different possible binding [pauses] that the peptide would have on the surface of the fibril. That's one example of a method that would enhance sampling.

ZIERLER: So, I'll ask a broader question, and you can contextualize this within the overall mission of NIH, or just in terms of your own research: why is it important -- again, this is a very broad questions -- why is it important to understand protein evolution and function?

BEST: Well, it's harder to make a case for why understanding evolution is important in the mission of NIH, but I think it's clear that understanding protein function is clearly important, because it also holds the key to understanding lack of function. So, I think that's clearly something that has relevance for the mission of the NIH, which is ultimately a healthcare mission.

ZIERLER: So, let's pause and go back, because you were hesitant to say -- so, what do we mean by "evolution" in this context? What does protein evolution mean?

BEST: Ultimately, it's the way that proteins have changed over time to end up with the versions that we have in the current species of animals.

ZIERLER: So, it's evolution in a Darwinian sense.

BEST: Yeah, exactly. I guess, it's molecular evolution. On a molecular scale, you find similar proteins doing -- sometimes similar, and sometimes slightly different things in different organisms. They have a clearly evolutionary relationship if you look at the similarity of the sequences.

ZIERLER: What would be an example to illustrate the point? You're talking about how two different organisms have evolved their proteins. That demonstrates --

BEST: I guess one example would be the proteins that occur in organisms that live in different temperatures. Mesophiles, or thermophiles, according to whether they like to live in cold

temperatures or hot temperatures. You find the same kinds of enzymes in these proteins -- well, enzymes doing the same thing, but they've been optimized to work at those kind of different temperatures.

ZIERLER: Which tells you what?

BEST: Well, at a basic level, it just tells you that they're optimized for their environment. The interesting thing is how that is actually achieved at a molecular level. How would you optimize an enzyme to work at a higher temperature, versus working at a lower temperature.

ZIERLER: Uh huh. Okay, and to go back to protein function, what are some examples -- again, I appreciate that your lab is more isolated from the clinical setting, but where do you see your work emanating beyond your lab? Not just NIH, but more broadly, what are the applications, or in what places is your work being cited that's advancing your research beyond the things that you're specifically doing?

BEST: Well, I guess, the one thing that's an obvious example is the methodology -- I mean, improving the energy functions or force fields for atomistic simulations, that's the most highly cited stuff that I've done. Those force fields are used by every computational person doing molecular simulations. So, if one can make an improvement there, indirectly, it could help a lot of people. That's really just a methodological improvement rather than a consequence of finding of my research. I guess something where our research has --

ZIERLER: Well, you could think back to your quadrennial -- your last four year review. What are the cases that you're making to set the budget for the next four years?

BEST: A field that our group has moved into in the last year or two is looking at these so-called liquid-liquid phase transitions in cells, which has become a bit of a hot area in the last few years. So, this is driven by the observation that there are a number of organelles in cells which appear to not be bounded by membranes. So, stress granules, and nucleoli, and so on. Some experimental groups have shown that these organelles are liquid-like in their properties, so they can flow, and coalesce, and so on. And they've also shown that you can reconstitute in-vitro, similar looking things using reduced number of components. Often even just single proteins.

Often these proteins are intrinsically disordered. So, that's obviously what kind of got our interest in this field, because we were already looking at intrinsically disordered proteins, so I guess the whole idea was, well, could we use some of the coarse-grained models that we'd developed to study the assembly of these liquid droplets. So, we developed a simple coarse-grained energy function. This was work that was done with a longtime collaborator of mine, Jeetain Mittal. So, we were able to show that we can develop a sequence based coarse-grained energy function that can be predictive of which sequences are able to undergo the space separation, and under which conditions. So, that's an area that we're working on quite a bit at the moment. What we did so far was kind of proof of principle, but really, the models need to be a bit more accurate, so we're working on improving that, and also incorporating interactions with DNA into them. The reason this is important for, let's say, health science, is there are a lot of diseases that are associated with these sort of droplets, one of which is ALS. It may be caused by the aggregation of this protein called fused in sarcoma, which may form -- well, these droplets may be somehow involved in the initial formation of aggregates of that protein. There are other examples of where these membranous organelles are involved in disease.

ZIERLER: So, your work is never focused on a particular malady. You're developing models that can be applied for experimenters who are working on their specific issues?

BEST: Yeah, exactly. So, I guess we're much more methodology driven than problem driven.

ZIERLER: Uh huh. But you're methodology driven based on problems that are coming to you from your collaborators. Is that fair to say?

BEST: Exactly, yeah.

ZIERLER: So, if you could characterize, what are some of the major problems? Are there themes? Are there groupings of problems where your collaborators come to you? How do you categorize them? I guess the question is, is it really a unique, case-by-case basis where you have a collaborator who's working on something and you're just dealing with that particularly? Or are you taking a more systematic approach to all of the questions that are coming into your lab, and looking for models that answer these problems more globally?

BEST: So, the way we're maybe trying to look at things more globally is by developing models that are transferable to different problems. So, in the case of this liquid phase separation model, that's something which we ideally want to be applicable to any system which a collaborator from outside might come to us with. So, I guess that's the commonality there.

ZIERLER: Alright. So, I think we'll end with a few questions that are more introspective. Some are forward looking, and some are backwards looking. I want to return to your "naive optimism" as a graduate student. Is part of the optimism, or the naivety, however you want to look at that, is that sort of a function of how much more you know about this work now, meaning that the naivety is that you didn't appreciate the complexity of the issues as a graduate student? Is that part of it?

BEST: I think so, yeah. I think you learn from experience what are the really difficult things to do, and I think it's just a matter of experience, and understanding better what challenges are.

ZIERLER: What about your appreciation for the complexity of molecules? Is there a Socratic thing going on? You're obviously learning more and more every year, but in learning more, are you appreciation more about how much you don't know, or about how much the field doesn't know?

BEST: I think the more things that you solve, the more questions there are, usually, at the end of the day. Yeah, that's certainly true. You find, for example, in the case of the intrinsically disordered proteins that assemble in a disordered fashion, we're still uncovering things about them that we don't fully understand, and so we're trying to understand what's going on there.

ZIERLER: Are there certain mysteries that you might have perceived earlier in your career that are clearer now, that are less mysterious? You can never say you can fully understand any given thing, but have you made -- not in terms of your contributions to the field, but in terms of your own intellectual development -- are there certain things that you feel like you much more fundamentally understand now than ten-fifteen years ago?

BEST: I think I understand more about the interactions that are important for protein folding, for example.

ZIERLER: I could ask it like this: what do you see as -- so far. I mean, you're young. You have hopefully many decades ahead of you, right? What do you see as your primary contributions to the field, and what is the field as you define it?

BEST: I think as I said, my primary contributions are methodological, and the field as I define it is pretty broad. I started out working on protein folding, and essentially, I branched out into, essentially, a bunch of things that are related to protein folding. So, either misfolding, or aggregation of proteins, or association of -- well, intrinsically disordered proteins I got into originally because they often do fold. Some of them do fold into structures when they bind to other things. So, I was interested in that. So, essentially, I'm broadly interested in things that are related to folding. That's the field. It is kind of broad.

ZIERLER: So, for my last question, it's a looking forward question. What are the things that you hope to accomplish? Again, it's a very broad question, because some things you're not going to know are coming to you until a collaborator heads your way and presents you with an interesting problem. Not being able to predict the future in terms of the collaborators who are coming to you, what are the things that you're excited about for the future of your career? In terms of discovery, in terms of methodology, in terms of -- is there a breakthrough that you imagine you are a part of, and what might that be? The goals?

BEST: Well, I think, in terms of the goals, one of the most exciting things I've done is using coevolutionary models for modeling protein sequence propensity. That was something that really was driven by curiosity within our own group, and we only got on experimental collaborators to verify our predictions later on. I think that approach seems to be potentially very powerful for doing both understanding proteins which may fold into two different structures, as well as maybe designing new ones. That's kind of a biotechnology application, but designing new proteins that can fold into different structures, and maybe act as switchers, or something like that. That's something that I'd like to be part of in the future. I guess that's a possible breakthrough. We could really make that work. So far, we've only designed things that fold to one structure, and we're still working on the other part of it.

ZIERLER: And what would the breakthrough look like? How would you know when you've achieved that?

BEST: Well, I guess once we've gotten the experimental validation that that actually works. That would be kind of a breakthrough, I think. I don't think anybody has successfully done that before.

ZIERLER: And then, of course, you have to know, or you have to have an inkling, that just because it hasn't been done before, that it's possible and can be done.

BEST: Oh, yeah. It's possible because there are certain naturally occurring examples, but a limited number. So, I guess there is some interest in understanding how hard it is to find those sequences that fold into two different structures, and also whether you can design some new ones, which is the other half of that question.

ZIERLER: Okay. Alright, well, Dr. Best, thank you so much for your time. It's been a delight talking to you today.

BEST: Yeah, yeah. Thanks for your time. Okay, bye.