SMB
Marius Clore
Remote Interview

by David Zierler
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DAVID ZIERLER: This is David Zierler, oral historian for the American Institute of Physics. It is my great pleasure to be here remotely with Dr. Marius Clore of the National Institutes of Health on March 23rd, 2020. Dr. Clore, thank you for being with me remotely today.

MARIUS CLORE: My pleasure.

ZIERLER: Would you please tell us your title and your affiliation at the NIH?

CLORE: NIH Distinguished Investigator, Chief of the Section of Protein NMR in the Lab of Chemical Physics, NIDDK—(National Institute for Diabetes, Digestive, and Kidney Diseases)—NIH.

ZIERLER: Wonderful. OK, so let's start right at the beginning. Tell us about your birthplace and your family background.

CLORE: There's not that much to tell. I was born, raised and educated in London.

ZIERLER: Where were your parents from?

CLORE: My father was from London. My mother was initially Dutch. Then she moved to Israel. She immigrated in 1936 with her family, and came to the U.K. in about 1952 or '53.

ZIERLER: And what was your father's profession?

CLORE: He was a film producer.

ZIERLER: A film producer! Of what kinds of film?

CLORE: Well, he did a lot of commercials, a lot of documentaries, and a number of major movies particularly in the '50s and '60s and some in the '70s. One that you might know is *French Lieutenant's Woman* in the 1970s that provided Meryl Streep with her break out role. Another is *Morgan a Suitable Case for Treatment* in the 1960s that provided Vanessa Redgrave and David Warner with their breakout roles.

ZIERLER: Do you have siblings?

CLORE: No.

ZIERLER: Only child?

CLORE: Yes.

ZIERLER: And your mother? Did she work outside of the house?

CLORE: Not while I was growing up. But she does have an interesting history. She was part of the Jewish Briage of the British Army stationed in Egypt in the second half of WWII, and was then part of the Hagannah prior to and during the 1948 Israeli war of independence. After that she served for about 2-3 years as David Ben Gurion's (Israel's first prime minister) chief of staff. She came to England in around 1951/1952 to learn documentary film making, with the aim of bringing this knowledge back to Israel, but she met my father, and the rest is history. She did return to Israel for a short while, but my father went after her, persuaded her to come back to England with him, and they got married in London in 1953.

ZIERLER: Tell us about your early schooling. What kind of a school did you go to?

CLORE: I went to the same school from K through 12, the French Lycee in South Kensington, London (now renamed the Lycee Charles de Gaulle) London. And the main reason I went there, and the same reason that many British kids in that area of London went there, is that the state schools in the Chelsea/Kensington area were not good at all. Although the French Lycée was private, it was extremely cheap as it was completely subsidized by teh French Government. The French kids who went there went for free (as they do in French Lycees all over the world, including in DC and New York) and the English kids only paid a nominal tuition fee (which at the time, in the 1960s/1970s, was about $80 per term). And so I went there all the way from K through 12.

ZIERLER: You took classes in French?

CLORE: Well, at the beginning yes. Basically total immersion up to and including 8th grade with the exception of three subjects: English, English math and English History. (English math was because at the time one had to learn to count in base 12 to deal with pounds, shillings and pence, plus the whole philosophy of teaching math in England and France is very different - in England the key was to get to a solution of a particular problem as quickly and simply as possible; in France they were stuck on theorems and rigorous proofs which doesn't exactly encourage creative thinking. So it was very good to have both English and French math classes. From 9th grade on, there was a major split with the French kids who were going to do the French exams (the baccalaureate), and the English kids who were going on to do O and A levels with the aim of attending British Universities. So obviously I was in the English section, and then the only thing subject taught in French was, you guessed it, French. One advantage is that they

forced us to do both O level and A level French, which was basically a freebie that required minimal study, since my friends and I were already fluent in French.

ZIERLER: Now, Americans sometimes have a hard time understanding the way class works in a place like London. So growing up, how did you see yourself in the class structure?

CLORE: Well class is a strange thing in the U.K. For sure, class was no longer what one would glean from 19th century novels such as Dickens. But the class structure was definitely there underneath and still is. What I think you're largely referring to is an impovorished working class. By the 60s that was largely gone in London, except perhaps in the East End of London. So I never came across any sort of rigid class hierarchy. But what there is in England is a lot of snobbery, and although I've been gone now for over 30 years, I suspect that hasn't changed much.

ZIERLER: So your family saw themselves as middle class or upper middle class, or you didn't even think like that?

CLORE: Well I wouldn't distinguish between middle and upper middle class. So middle class. i.e. not part of the aristocracy. But no doubt part of the highly educated elite, which is none too surprising given that my father was a film producer and therefore my parents friends were largely from the movie and theater business.

I should also say that while neither of my parents went to college (owing to WWII in my father's case, and the fight for the creation of the state of Israel in my mother's case), both were extremely well read. My mother especially in classical literature from all countries, and my

father in literature, in science, etc.... My mother was also exceptionally good at languages. She spoke 8 living languages completely fluently: in order of acquisition Dutch, German, Hebrew, English, French, Arabic, Italian and Russian; and she could read one dead language, Aramaic which she learnt to study the Deep sea scrolls in their original. Dutch was her mother tongue of course, although she refused to speak it except on very rare occasions, German, Hebrew, English, French and Arabic she learnt during her teens by necessity, and Italian and Russian later on in England out of interest. In fact remember she learnt to speak Italian fluently within the space of 2-3 weeks when we had an Italian au pair; and she learnt Russian equally fast because she wanted to read Tolstoy in the original. My father, on the other hand, was terrible at languages and he only spoke English. I'm more like my father. The only reason I speak French is because I went to the French Lycee and I haven't spoken it properly since high school - so I understand everything and my accent is perfect but my vocabulary is a bit rusty. When I went to work in Germany for 3 years at the Max Planck I learnt a bit of German but more often than not I would understand exactly the opposite of what people were actually saying. I took Russian in middle school for 3 years and it's the only subject I ever failed at!

ZIERLER: And when did you start to develop an interest in science? Early on?

CLORE: Very, very early on.

ZIERLER: Like what? What are some examples of your burgeoning interest?

CLORE: Maybe 5 years old. As early as I can remember. And I also wanted to be a medical doctor (which I became) as early as 5, and never wavered from that.

ZIERLER: How did you understand science or how did you see that you had an interest in science?

CLORE: So you've got to understand—in England, at least at that time, many, many, many boys from all walks of life were always interested in science, astronomy, electronics, cars, trains, planes, etc..... It is a different time from now, where everything is at school. Even in the 19th century, boys of all classes were very much into and reading about trains, and in the early 2th century about planes were reading about trains, and then in the early '20s, about planes and stuff. That was a common thing, so that wasn't exactly unusual. And it's not as if my parents guided me in that direction either.

ZIERLER: And in high school, did you sort of naturally gravitate towards physics or not yet, that was too early?

CLORE: So in England, things are much more specialized. In the A-level years, which is the bit that counts—so that's in 11th and 12th grades—one only studies 3-4 subject, so it's much more specialized than in the U.S. where they do everything. So at A level I did Chemistry, Physics, Math, Zoology and was forced to do French (but as I mentioned above that was a freebie requiring minimal if any studying). And I was exceptionally keen on Chemistry. We used to have Chemistry club on Friday evenings for those who were really keen where one would design one's own experiments and organic syntheses. And of course it was a great place to get one's hands on chemicals for home experimentation. The chemicals were in the back room, and one person would stand guard while the other one would be put chemicals in their pocket to take home. I once put copper nitrate in my pocket and the whole side of my leg was dehydrated. Took about 3 months to heal properly!

The other neat thing in England at the time is that one could buy all sorts of chemicals at the local pharmacy (knows as the chemist shop in England) - this included things like sulfuric acid, nitric acid, etc..... which would be unthinkable today. and I had my share of accidents - in one case I nearly sent off on fire a room in our house, but, fortunately I didn't panic, and was able to put this out. Fortunately my parents never discovered what had happened.

In addition, the husband of one my mother's best friends was a senior lecturer (equivalent to Associate Professor) in Chemistry at University College London, and he allowed me and friend to take all the glassware that we could carry from his lab home. He was actually quite a character - his name was Peter Pauling, the son of Linus Pauling. And his wife, Bud MacLennan was a published working at Weidenfeld and Nicholson, and she was responsible for the English publish of Watson's Double Helix.

ZIERLER: Much more specialized, OK. Can you tell us a little bit about your decision to enter University College London? What was the goal there?

CLORE: In England, one doesn't apply to a huge number of colleges as in the US. One is limited to a choice of 5. The other key to remember is that med school starts directly after school in the U.K., not after completing a separate undergraduate degree as in the U.S. Naturally, med school is therefore much longer than in the US: 6 years versus 4 years. So the first 2 years comprise preclinical studies (i.e. anatomy, physiology, biochemistry, immunology, neuroscience, psychology, etc....); in the third year (which was optional) one would do an intercalated BSc in which one joined the students majoring in the subject of one's choice (I did biochemistry, but one could do almost anything one wanted that was in any way medically related; friends of mine did neuroanatomy, neuroscience, physiology, anthropology, you name it; in fact for me it was a toss

up between biochemistry, immunology and neuroscience,as I was interested in all three subjects, but in the end I settled on biochemistry); and then the last 3 years constituted clinical studies. It's also worth noting that because of the specialized nature of the English system, one ends up being at a far more advanced stage at the end of one's third year (the intercalated BSc year) than one would at the end of 4 years of undergrad in the US. and likewise, one knows basically a year's worth more clinical medicine at the end of medical school in the UK than one would in the US (since the clinical component of med school in the US is 2 years, the first 2 years being preclinical, versus 3 years of clinical in the UK.).

Now I ended up going to University College London for preclinical and University College Hospital Medical School for clinical (the two institutions were linked and opposite the street from one another). UCL/UCH was actually my second choice initially. My first choice was Oxford but they decided to offer me a place to study chemistry or biochemistry rather than medicine with no guarantee that I could switch. They did so because they didn't think I'd ever become a clinician which ended up being true of course, but that proposition was of no interest to me so I turned them down.

ZIERLER: Undergraduate? I thought you went undergraduate.

CLORE: In England it works differently. You go to med school direct from school.

ZIERLER: So direct from high school, you go right to medical school.

CLORE: Exactly, just like I explained above. One tidbit that's worth noting is that the intercalated BSc year, those majoring specifically in a subject were not particularly keen on the

med students joining them. The reason was simple. In England, the only year that counts and determines what class of degree one is going to get (1st class honors, II.2, II.2 etc...) were the final exams at the end of the third year which were simply devoted to what was being learnt in the third year. These exams are not easy as one has to write long essay type answers under pressure to original questions that require a lot of thinking rather than pure regurgitation. Not only that, the level that is reached in the 3rd year is high because the course materials basically comprise the latest publications in the field. Grading is on a curve, and almost invariably the medical students would get 1st class honors, while the non-medical students would get 2nd class honors. This was perhaps not too surprising, because, in general in the UK the smartest kids tended to go to med school (where entrance is highly competitive) rather than read a specific science subject.

So I was qualified as an MD just before my 24th birthday. So just to give you an idea, my daughter started medical school when she was 25, and just became an MD at 29.

ZIERLER: Yeah, it's a very different model here. Right.

CLORE: For sure a different model, although some schools such as Brown have something similar

ZIERLER: In 1976 your degree is what?

CLORE: It's was BSc, so a bachelor's, in biochemistry with 1st class honors.

ZIERLER: I see. So if this is your focus, is there no core curriculum? Are you not taking classics and literature and that kind of thing? It's all science all the way through?

ZIERLER: Nobody does that in England. If you do chemistry, you're doing chemistry; if you're doing english literature that's what you're studying. Of course you will be doing relevant math or physics or maybe if you're interested, some biochemistry, but you're sure not doing English or philosophy or any other non-science subject. You're not messing around like you do here. In my view, what they do here, they might regard as good, but in some ways, it really isn't that good. It all depends on the person. Because in general—so what I found and I know this from the people who I went to school with all the way from K through 12, is that the—and this is very non-politically correct at this point in time, but I believe it's still true today— and I've seen this with my daughter and son —is that the girls, in general, are much better overall in school. So they're better at school, when you count everything - all subjects. But when you go into any individual subject, then they weren't better. For example, there was one girl who was at the top of the class, or close to the top of the class, all the way from K through 12, and while she was very good at everything, she wasn't brilliant at any one thing. She went and read chemistry at Cambridge initially, but didn't like Cambridge and moved to St. Mary's College in London after her first year where she did very well. But she wasn't the best at chemistry in high school by a long shot. So what happened was that the boys tended to be very good at those things that they were really interested in, and everything else, they didn't care about and didn't bother putting in any effort. And that was certainly true for me. So anything that I didn't care about, I just didn't bother. Not that I couldn't do it, but I just didn't care to. As an aside, there was one girl who joined us in 12th grade from Canada as she'd completed high school a year earlier in Canada and was too young to go to university - she was supposed to be a genius, but for sure at the time she was hopelessly behind our class in physics. Her name was Melissa Franklin. Her experience though must have had a big impact because she went on to study physics in Toronto, then went

to Fermi Lab, and after that Harvard and quickly became the first full Professor of Experimental Physics at Harvard.

ZIERLER: So this means that at 18 years old, you essentially know that you want to go into medicine. That's the idea?

CLORE: Yes.

ZIERLER: So any classical education you're getting, that essentially ends in high school for all intents and purposes?

CLORE: Actually any classical education ends at about tenth grade. But the point is—the fact is—it doesn't mean to say that one is badly read. I will say, for example, that most of the literature we studied in school was not exactly the stuff that interested me, but I still read plenty of classics, and plenty of English literature. This doesn't mean to say that people in the U.K. are poorly educated or not educated in the classics; it's just they don't do it at school.

ZIERLER: Sure, sure. Fair enough. So you enter college fully intending to become a medical doctor. That's the plan?

CLORE: Not necessarily. For sure I was going to become a medical doctor - there's no point starting and not finishing. What I didn't know was whether I was going to go into clinical medicine or into basic medical research. The point is that studying medicine gives one plenty of options and opportunities.

ZIERLER: And what kind of doctor did you think you were going to be, or what was your track?

CLORE: When you start med school there is no way for anybody to seriously know what will really interest them. You can't very well know until you've done it. Not only that, at least at the time I qualified as a doctor, you didn't decide right away what specialty you were going to pursue. So again, in England, it's a little bit different. Let's say you in the US you want to be a pediatrician, which is what my daughter is doing, you go into pediatrics right away after you finish med school. Well, in England, you don't. In England, you're first year of residency is six months medicine, six months surgery. Then at that point most people will either go into internal medicine or general surgery. You're not going to go into any specialized surgery, at that point of time. So if you want to be a neurosurgeon you first have to undertake general surgery training. So it's all a little different. So the truth is that in med school it's very hard to know what you're going to be interested in. I will tell you that in med school I was very good at internal medicine, and very keen on neurology. And in med school I hated surgery because surgery largely because there's not too much to it as a med student, and holding retractors for hours on end is extremely boring, particularly if you can't see anything.

ZIERLER: [laugh]

CLORE: But then when I was a resident in that first year, the first six months were in medicine at University College Hospital. There, you don't get to do that much because it's a teaching hospital. But surgery I did at St. Charles Hospital, which is a branch of St. Mary's teaching hospital. But because St. Charles is not the main teaching hospital it's a little bit freer, so I got to do a ton of surgery. So I operated on a lot of hernias, a lot of appendicectomies, and a lot of other relatively stuff totally on my own after a while. Had I continued—had I not gone into science (and I'll tell you why later) I would have probably become a surgeon. So even though I

hated surgery in med school, that's what I would have done in the end had I stayed in clinical medicine.

ZIERLER: Why do you think that would be? Why would you have gone on to be a surgeon?

CLORE: Because I enjoyed it. When you do it, it's fun. When you're holding a retractor, it's not fun.

ZIERLER: Right, OK. So the decision to transfer away from medicine—

CLORE: Any interesting story and a lucky break. So during that third year, the intercalated BSc year, what you do in addition to lectures is some research projects. And one of the research projects that I did was a project on cytochrome oxidase with Ted Chance, a senior lecturer equivalent to Associate Professor in US parlance. He was actually the nephew of Britton Chance, at Penn. He had written some programs to solve stiff differential equations and carry out non-linear optimization in collaboration with Alan Curtis at Harwell, the UK Atomic Energy Research Establishment. Alan and his colleagues were using this to simulate reactions in atomic bombs; Ted was using it to simulate kinetic reactions and fit complex transient kinetic data. It was some really neat stuff that was way ahead of its time, and I still use their program (known as FACIMSILE) today. I continued working with Ted on the side during my clinical years and I even went to the U.S. in the summer for a couple of weeks to collect data in Britton Chance's lab. So the net result was that by the time I finished med school—I mean, I can check exactly, but I published at least 15 or maybe about 15 to 20 papers, and a few even solo, and all first author. So I knew I was good at this stuff. What I did in December of 1978, at the suggestion of Michael Hollaway, another senior lecturer in the Biochemistry department at UCL, was to write to the then-director of the Medical Research Council (MRC) National Institute for Medical Research

(NIMR), Sir Arnold Burgen, and I described what I was interested in and what I wanted to work on, which largely entailed using low temperature kinetic methods in conjunction with optical and laser Raman spectroscopy to continue studying the reaction of cytochrome oxidase with oxygen in more detail and to pursue kinetic studies with various other metalloproteins. And so I presented him with a detailed research proposal, not by email of course because email didn't exist then. So by return of mail, he said, "Come on up and let's talk." NIMR was located at Mill Hill, on the outskirts of London, so not far to get to from central London by tube (i.e. metro or subway in the US). And we talked, and within ten minutes he offered me an MRC scientific staff position. So then I said, "OK, obviously I'm not coming right away. I'm not going to have done six years of medicine and not be able to prescribe anything even for myself." So I did the year (6 months medicine/6 month surgery) after med school—the first year of residency, which would have entitled me to actually practice should I want to later. So I did that; then joined Mill Hill in August 1980. So if I hadn't had that break, if you will, that lucky chance, I wouldn't have gone into research. So that's essential how I went on to a full time research career in basic medical science.

Now the thing was that at Mill Hill , nobody was doing what I had proposed to do and the equipment just wasn't there; but Sir Arnold knew that I was pretty smart and could probably do anything that I put my mind to. Sir Arnold had become interested in NMR spectroscopy. And at that time, the only two places in the U.K. doing any serious sort of biological NMR spectroscopy were in Oxford and at NIMR. The Mill Hill people in the Division of Molecular Pharamacology which was also headed by Sir Arnold, were working on dihydrofolate reductase which I thought was awfully boring as a research subject. Since Sir Arnold knew that my interests lay in physical biochemistry, he suggested nevertheless that I try my hand at NMR spectroscopy instead.

Fortunately for me NMR at that point in time wasn't too hard to learn and figure out. I read some books on it, figured out that the important experiments for biological NMR were a lot simpler than was made out, went to Mill Hill, and everything took off from there.

ZIERLER: Now, Mill Hill means what? That's a colloquial—?

CLORE: The MRC National Institute for Medical Research was in Mill Hill.

ZIERLER: Now is that the British equivalent of the NIH?

CLORE: Yes, sort of but on a much smaller scale. The intramural program at the NIH is much, much, much, much bigger. So NIMR was the largest MRC unit around, so it was bigger say than the size of NIDDK, for example, but not anywhere close to that of the whole NIH intramural program. As an example, NIMR was significantly larger than the MRC Lab of Molecular Biology in Cambridge (which I think is fair to say is probably the most successful research institution in the world, at least judging from the huge number of Nobel Prizes out of there, starting with Sanger, Perutz, Kendrew, Watson and Crick).

ZIERLER: All right. So just so that all of the readers will understand this, so the Medical Research Council is a branch within the National Institute for Medical Research, or what does that mean?

CLORE: No, no, no, no. The Medical Research Council itself is like the NIH, and they support research all over the U.K. They also have institutes of their own. Sometimes it's relatively small units within universities. Sometimes they're much bigger labs such as the MRC Laboratory of Molecular Biology, which is a standalone thing. And sometimes they're very big, relatively speaking, like the National Institute for Medical Research (NIMR).

ZIERLER: OK, So one part if you wouldn't mind repeating, the exact inflection point when you got involved in NMR spectroscopy, how did exactly that happen?

CLORE: Well, it happened between when I got hired in a faculty position by the MRC (and had I not had such an appointment, I wouldn't have comeand would have continued along a clinical career path), to work in the Division of Molecular Pharmacology, whose head was also Sir Arnold Burgen (he was both the director of the NIMR as well as head of one of the divisions within NIMR). So a division would be the equivalent of a department at a University or what is known as a Laboratory at the NIH. The stuff that I wanted to do or proposed to do, they weren't going to fund, but he said, "why don't you do NMR?" He'd already said that when I first met him 18 moths before actually coming to NIMR. So I did NMR. I had learned some NMR before coming, because I didn't want to know nothing, but there wasn't actually that much to know at the time, as it wasn't like now where there are hundreds and hundreds of experiments. And so I started doing NMR, and I realized immediately that NMR, unfortunately, is made a lot more complicated than it really is. It's actually really, really simple. But if you look at the books, I mean, it's like complete gobbledygook, unless you're into that sort of stuff. So I realized some very simple things. For example, you can understand the nuclear Overhauser effect (NOE) as you would a chemical reaction kinetic scheme, in terms of simple ordinary differential equations without knowing anything regarding the detailed physics of cross-relaxation (which is the basis of the NOE). So I knew exactly how to do that and solve such equations numerically from the work I'd done on kinetics, so I was able to figure out things extremely quickly.

ZIERLER: So in taking this appointment, had you already determined that you were not going to continue on with medicine? Was that the idea, or were you experimenting at this point?

CLORE: Well, in essence once you do that there's no turning back, at least not easily . If you go out of the clinical medicine track, it's hard getting back in - not impossible but hard. It's the same in research; if you go out, that's it. People may think they can do some administrative job for a year; but you're not coming back in. No. It's a break. Now I did think about keeping my hand in clinical medicine for a very short while, but then I really didn't bother.

ZIERLER: So you saw this as a true break. This was not really an opportunity to build on your medical degree. This was an entirely new experience.

CLORE: Well, I wouldn't say that. Sir Arnold Burgen was an MD, and a lot of the famous scientists at NIMR were MDs, so it wasn't like there weren't any MDs around. And it was not exactly unheard of (and in fact wasn't even that uncommon) for people with medical degrees to go into research. For sure, that was certainly true of many of the preclinical basic medical science departments at University College, especially in physiology, neurophysiology, neuroscience and immunology. It also wasn't that uncommon in the US. For example Harold Varmus is an MD.

ZIERLER: OK. And this is a theme that I want to return to, over the course of your career in NMR. When you first got involved with it relative to today, how far along was the field? How advanced was it? What was its value clinically or theoretically at the time?

CLORE: Clinically, nothing quite yet but just on the horizon. I can't remember quite when MRI was first developed—the first MRI work coming out Nottingham was done Mansfield and Andrews. I can't quite remember, OK? But it was probably 1980, 1981. Imaging at that time was primitive in terms of the type of resolution that would be required for clinical applications. At the time that I came to Mill Hill, all they were doing in terms of biological NMR was one-

dimensional NMR measurements. Most of the work at the time was focused on relatively simple things like titrating histidines. There really wasn't that much to do. But what I did figure out is that there were some simple yet interesting and very informative things that one could do with existing NMR technology. At the time the people at Mill Hill were doing transfer of saturation experiments to uncover the chemical shifts of bound ligands in the presence of a large (say 10 fold excess) of ligand over protein). The basis of the saturation transfer experiment is basically as follows. Let's say there are only two lines, two resonances, in two different states, and you saturate one resonance, you then look at the effect on the intensity of the other resonance. This would tell you that these two resonances were interconnected by exchange from state of the molecule to another. So what the people at Mill Hill were doing is they were saturating signals—they were scanning through the spectrum—for example, looking at complexes of dihydrofolate reductase with various drugs, with NAD, with NADPH, and they would see whether they could detect the positions of the bound resonances - when these are saturate and if the free and bound states are in exchange, a decrease in the corresponding free signals would be observed. Experimentally, one would scan through the spectrum, applying a weak saturation pulse at let's say 20-hertz intervals, and monitor the intensity of the resonances of the free ligand. And that way you could identify the positions of the resonances in the bound state. That's what they were doing, but they couldn't really figure out exactly what was going on. So one of the first things I did was figure out what was going on and what was involved. By that I mean to simulate the process in terms of two-site exchange, three-site exchange, and so on. And then I figured out by accident the one could observe a transferred nuclear Overhauser effect (TRNOE) to get the conformation of the bound by doing exactly the same thing experimentally; the difference is that the system had to be in fact exchange (so the observed shift is a population weighted average of

the free and bound states) rather than a slow-exchange system where the shifts are distinct different in the free and the bound state. Of course if you have ten- or twenty-fold excess of the free ligand, the observed shift is effectively at the free position; now the TRNOE will enable you to get information on the bound state conformation by transferring cross-relaxation information (the basis of the nuclear Overhauser effect) from the bound state to the observable free state. So the way that you would know it was from the bound ligand and not the free is that the free ligands were very small like ATP, cyclic AMP, this sort of thing. And their NOEs are positive in the free state. But when they're bound to a protein, the NOEs are negative (the NOE switches sign as the molecular weight and hence rotational correlation time increases). So if you see a negative effect, you know it's coming from the protein-bound state. So I figured all of that out, and that actually became a very widely used technique looking at conformations of bound ligands. The papers happen to be highly cited in terms of conformation of bound ligands and looking at drug binding, etc..... So that was one of the first things that I did at Mill Hill.

The other thing that I did was I realized very early on that one could use the NOE to actually get structural information. So at first in terms of getting confirmations of bound ligands, you could just model the transferred NOE data using some plastic models (with modeling kits for building molecules). You could figure out what the conformation of the ATP or the cyclic AMP was, whether it was in the syn or anti conformation, for example, very easily.

And then I could easily see that one could readily go ahead and do this on bigger things. Now at the time, when I first came to Mill Hill, the only spectrometer they had was a 270-megahertz spectrometer. So the resolution was very poor. Assigning a molecule as simple as NAD, which would take you all of let's say ten minutes today, was worth a thesis in those days. One of the people in the Division of Molecular Pharmacology actually was awarded her PhD two years

before on getting the assignments for NAD, something that I could literally do in like two minutes now.

ZIERLER: So it sounds like you saw that the field was primitive but that it was ripe for advancement.

CLORE: Exactly. It was totally primitive and I got in at such the right time when it was primed to explode. And since I know you've interviewed my colleague Ad Bax, who's in the same lab as me at the NIH, the same is true of him. The ground was fertile. The only reason we published so many papers, both of us, is that we got in at the ground floor, so everything is ripe for the taking, because if you're a little smarter than everybody else, you can do a lot of stuff very quickly. Now the 270 MHz NMR spectrometer was next to useless for tackling any system with significant chemical shift overlap, so we couldn't really do too much except these TRNOE experiments. In addition, the 270 MHz spectrometer couldn't run 2D experiments because the instrument and specifically the generated pulses weren't stable. So you can't have pulses that go from nine microseconds to ten microseconds to eight microseconds and monkey around. Plus the sensitivity was low. For example, it would take half an hour to an hour just to get a 1D spectrum of a 1 mM protein sample. Today, on a modern NMR spectrometer, whether it's a 500 or a 600 with a cryopepro be that could be done in a single scan; literally one second. So there's a huge improvement in technology.

Before I left—a year before I left—so I think 1983 or so—Mill Hill got one of the first 500 MHz NMR spectrometers and I immediately starting doing 2D NMR experiments. I started on a small piece of DNA, a palindromic hexamer. And in fact for that project the resolution was sufficiently good at 500 MHz, that I could do all the NOE experiments using 1D NMR methods, saturating

individual resonances at a time. I took all these traces home at the weekend, and I figured out all the assignments within 30 minutes. So actually before others had done it, but unfortunately I and my colleague were a little too greedy, because we sent this to *Nature* and they didn't accept it—as usual, one referee said, "Oh, this is so fantastic" and the other said, "Oh, it's not of general interest." And then we tried somewhere else, and ended up publishing it in the *Biochemical Journal*. But of course, we weren't the only ones to think about doing this stuff, so other people published at roughly the same time. But it was quite obvious from that that one could get structures. Now I have to say, though, that, if you will, my mentor, Sir Arnold Burgen, didn't feel that way. He thought one could never do anything like that. So, you know, I decided to prove him wrong, which I did.

ZIERLER: [laugh] How did he receive that?

CLORE: Well, by that time, I had left. But if you ask him now—he's still alive at the ripe old age of 98. he's very pleased how things turned out.

ZIERLER: So now your PhD comes in 1982 in physical biochemistry.

CLORE: Yes.

ZIERLER: So when you entered in 1980, was it as part of a proper PhD program?

CLORE: Well, first, a proper PhD program, in the US sense, doesn't exit in the U.K. There's no coursework. So it's not like you spend a first year or two doing coursework, figuring out what lab you want to join. That's not how it works in England.

ZIERLER: Did you enter with the intention?

CLORE: Let me explain. So the thing was this — when I get to Mill Hill, I didn't have a PhD, and I couldn't use the work that I had already done—the 20 papers by that time — as the basis of a PhD—because I had already published those. One wasn't allowed to use work done before one registered for a PhD, as the basis for a PhD thesis. Also at the time, so not true today anymore—now they've changed—but when I was at med school, you couldn't register for two degrees at the same time. So I wasn't able, while doing my medical degree, to also register for a PhD and submit a PhD thesis based on my published work. But you'll realize that once you've published quite a large number of papers, you know what's what - i.e. how to write a paper, how to think critically and logically to present a piece of work in its best light, how to design experiments that will lead to rapid publication, etc..... So when I came to Mill Hill, I said, "Hey, I may as well register for a PhD. What's the big deal? Costs me nothing." I didn't need a PhD because I had an MD, but why not. And then there was an 18-month minimum time between registration for a PhD and the time to submit a thesis. You weren't allowed to do it in less time. And I could have done it much less time, because I had enough material within less than a year. So in the requisite18 months, I submitted a thesis. I think it was maybe ten papers' worth? So that's how that worked. So more than a little unusual. Not the regular path. It also only took me 3 weeks to write the thesis up, basically by doing a cut and paste on the papers I had published. Now this wasn't on a computer at the time, but on real paper using a real typewriter and with white out to correct typos etc... If this were today, I could easily have put together the thesis in a day.

ZIERLER: Right. And the PhD comes right in the middle of your tenure. The PhD is 1982, and you're there from '80 to '84.

CLORE: Yes.

ZIERLER: So what is your status there after the PhD? Does it become like a postdoc or your status really doesn't change as a result of the dissertation?

CLORE: Nothing changes. I mean, I was on the faculty before as a principal independent investigator. I was on the faculty after as a principal independent investigator Nothing changes.

ZIERLER: OK. So then—

CLORE: And the other thing is many of the MDs in basic research departments in the U.K. never bothered with PhDs. As they don't generally even in the U.S. Again Harold Varmus is an example, but there are many many others.

ZIERLER: Right. Now anything in terms of physics you're sort of learning on the fly at this point, right?

CLORE: Well, in terms of NMR, yes, but then so is everybody else. So that's not a big deal. But the bottom line—the thing was, I was very, very good at math, and I was also very good in high school at physics. And I kept my interest up in physics while in med school. So while I didn't study physics in med school obviously, I still was very interested in physics and taught myself things like special relativity, and then I taught myself general relativity from Dirac's book. An absolutely brilliant mathematical exposition of General Relativity that's only about 70 pages long—it literally only had something like ten lines of actual text in it and the rest was nothing but equations and very clear derivations that were easy to follow. I figured out how to do all of that stuff, and the various mathematical techniques such as covariant and contravariant differentiation associated with General Relativity from Dirac's book. So it wasn't

like I didn't know any physics or math. But in NMR, everybody was on a level playing field of "ignorance" if you will—at that stage, if you learned to do biological NMR, there really wasn't that much to know. So all that was required was street smarts and knowing a few things. For example, there was a book written by Abragam in 1960 which basically contains everything that one needs to know probably in NMR. But for practical purposes there is literally no need to know anything in that book. And for that matter I don't know anybody who has actually read and studied Abrgam's book. Maybe Ad has read it, but I've never managed to read it, except look up a few things. Because, at least as far as biological NMR is concerned, the key to being able to use and develop NMR effectively, the key is that one needs to know just enough to be able to do stuff, but not too much, so that you don't try and do things. Because if you know too much, you'll think everything is impossible. To be honest, that's the main reason I've been successful. In other words being able to see the forest for the trees, and getting rid of all the unecessary complexity. It's sort of a bit like Feynman and the Feynman diagrams - the underlying physics and math may be very complicated, but the Feynman diagrams enable anybody (with a little background) to understand very complex phenomena in the simplest of terms. (And I'm in no way equating myself with Feynman because he was many many orders of magnitude smarter than I am). But it's only when one can see complex phenomena is simple terms that one can have the necessary insights to make major advances. Otherwise, it's too easy to get bogged down into nitty gritty details.

ZIERLER: [laugh]

CLORE: If you're the type that needs to know everything before they can do anything, you're going to end up going nothing. So it's actually best not to know very much.

ZIERLER: So you were really perfectly positioned for this, it sounds like.

CLORE: Perfectly. Right.

ZIERLER: What did you see—once you were fully invested in terms of your career in NMR, what did you see as its big promise? For something where you're looking at decades of work in this area, what did you see as the main promise and what did you see as your main contributions at that time?

CLORE: I wasn't looking decades, OK, but once I realized that you could get structures, my initial main aim was to solve three dimensional structures of protein, DNA and their complexes in solution. And obviously that required the development of new methods to do that. But it was quite obvious that one could do it, because if you could get a sufficient number of distances by NOEs, it was clear that one should be able to translate that information into three-dimensional coordinates.. Now, going from distances between protons to atomic coordinates is still a complicated business, but that comes later, as we developed all the ideas of doing restrained molecular dynamics and simulated annealing to be able to get structures from sparse distance restraints later on. But nevertheless it was obvious that one could do it, at least in principle. A lot of people didn't think one could do it—and none of the crystallographers believed one could do anything? But it was obvious that a solution was possible. So once you knew that and bigger field NMR spectrometers were coming to the fore—two-dimensional NMR was developed, and then we started using that straightaway as well as developing new things—and it was not such a big deal to do that.

So I moved to the Max Planck Institute for Biochemistry in Martinsried (on the outskirts of Munich) in 1984 to jointly head an NMR group there with Angela Gronenborn, who I worked

with from 1980 until we split up in ’98. And actually, the reason we started working together is that we were put in an office in Mill Hill that was maybe like seven foot by seven foot; so we started working together, got married after a year or so, and continued working together. There were no big groups at Mill Hill, so it was just us two. And then in Martinsried, all of a sudden we had a big group. But I only interacted with a few people in the group (as most, believe it or not, could barely speak English). But once in Martinsried, we had a huge opportunity, because there we had a head start over all the people who were in England or for that matter in the U.S where multiple groups had to share NMR spectrometers. We had our own 500 MHz NMR spectrometer. So we could do plenty of stuff and try plenty of new things.

ZIERLER: So that was the big draw at Max Planck? It was your own equipment, essentially?

CLORE: That was the big draw, yeah.

ZIERLER: And how special was it to have your own equipment? Was it because it was so expensive?

CLORE: Yes.

ZIERLER: And what did Max Planck—what did they see in it, were they were willing to invest this kind of resources into NMR?

CLORE: Well, yes and no. They clearly decided to invest in NMR and recruit us. But Robert Huber who was there and is a very famous crystallographer, never thought it would amount to much. But he had the foresight tos ay, “Let’s give it a go. Let’s see what it can do.” And others thought the same, and they gave us a chance, so we did it.

ZIERLER: So if you can explain to a broader audience who's not familiar with these things—the excitement in NMR over being able to identify protein and DNA structures, what's the—

CLORE: Well, not identify; determine.

ZIERLER: Determine, right. What is so exciting about that? What is the value of that beyond—I mean, what is this for?

CLORE: Well, OK, so there are two aspects to it. The first aspect is that it's a challenge. Whether it's good for anything, it's still an incredible intellectual challenge to be able to do this—if you think about it, to have a liquid containing a protein in a small NMR tube that's let's say a centimeter high and five millimeters in diameter, you put it in a high field superconducting magnet, you put some radiofrequency pulses through it, and from that determine a three-dimensional structure—just to be able to do that is pretty amazing. In the early days of crystallography, being able to solve a structure, any structure, was an intellectual challenge. It didn't matter what the structure was or how exciting it was; it's was challenge. That's the first thing. The second thing is that the way you can look at molecular structure in relation to function is exactly analogous to looking at gross anatomy in relation to physiology. If you don't have the anatomy, you can do as much physiology as you like but you're not going to figure out how things actually work. So when Harvey figured out the circulation — that the right side of the heart was pumping out blood into the lungs and then back down into the left side of the heart, then through the body and back to the right side of the heart — then you can do a lot of physiology after that. If you don't have that basic information, you're nowhere. So in order to understand protein function, you need to know structure at atomic resolution. If you want to know how a protein recognizes a piece of DNA specifically, so it recognizes one DNA sequence

and not some other DNA sequence, you need to know what the structure of that protein-DNA complex is. Similarly, if you want to know how a protein works, or if you want to design drugs in a rational way as opposed to just screening—and that was a big thing at the time—structure is an essential prerequisite. Of course I think apart from HIV protease and maybe a few other example, rational drug design hasn't really contributed that much to drug development, largely because it's quicker to screen. But if you want to understand let's say how drugs work or you want to improve on drugs—once you've designed a drug, let's say even by screening, if you want to improve on it and you know the structure of the protein-drug complex, you can do so. It gives you hints about what to do. You're not flying blind. And that's why structural biology was—I would say was, not is anymore, because now it has become too easy, so I'm not doing structure stuff anymore—and you know, crystallographers are doing much bigger things now with cryoEM, because everybody and their brother can do small proteins by crystallography now. It's no longer a challenge. Whereas when Perutz and Kendrew were solving the structures of hemoglobin and myoglobin, respectively, in the 1950s that was a massive challenge. Now, anybody can do it. So in order to understand how any of these proteins work or how to inhibit them or how to manipulate them or how to understand let's say what factors stabilize a protein structure, you have to know what its three-dimensional structure is. So that's goal number two. And the idea at the time when people became interested in using NMR for doing structure was basically two-fold. One, the promise of NMR was that you could do this in solution and not in a crystal. And the idea was that things might be different in solution than in the crystal. Turns out usually they're not. And then that you could get dynamic information, which you can by NMR but not crystallography. And some of it is not so interesting, and some of the work that we and others are doing right now on excited, transient sparsely-populated states is super interesting,

especially since such states are invisible to conventional biophysical techniques (i.e. they are only accessible to NMR).

So that was the promise of NMR, and a lot of people were very interested in being able to do this, and we just happened to be a little bit faster off the starting block, and had a little bit more street smarts to be able to do it—not first; we solved the second proper protein structure. Kurt Wüthrich beat us to it, but he had been doing this for a long time. We hadn't. Which is why he never liked either me or Angela Gronenborn, because he felt we were upstarts. But it really wasn't that hard to do.

So when I came to the Max Planck, all the effort was channeled in developing NMR methods and associated computational techniques to solve three-dimensional structures. And I realized that for DNA, you can sort of model it as the different forms of DNA have regular helical structures (which is precisely why Watson & Crick were able to build the double helix based on a couple of spacing measurements from fiber diffraction in conjunction with Chargaff's rule that the amount A and T were the same, and likewise G and C, and hence they made the jump that A basepairs to T, and G basepairs to C. So you can figure out from the distances whether it was B DNA or A DNA or Z DNA, simply using plastic modeling kits. But for proteins, you can't model just based on distances, as the structure isn't regular. It's not simple like that. So you have to be able to determine a structure and the path was relatively clear. So there was a guy at Martinsried in another group who had worked with Martin Karplus, called Axel Brunger who then went back to work with Martin and subsequently on to a faculty position at to Yale; he also became a famous scientist and a member of the National Academy. Axel was working on molecular dynamics simulations, and he developed a program called CHARMMF, which was the Cray version of CHARMM program that had been developed in Martin Karplus' group at

Harvard, but it had its own syntax. So it really wasn't just a version of CHARMM for the CRAY but its own program, albeit derived from CHARMM. I got together with Axel and I said, “Hey, let’s incorporate NOE-derived distance restraints into the CHARMM empirical energy function to determine structures.” And I already knew that this was going to work because what I had already incorporated distance restraints like that into conventional X-ray refinement packages. In particular a package out of Birkbeck College in London called RESTRAIN, from Tom Blundell’s lab. RESTRAIN was fine for crystallography, because in crystallography, you model into an electron density map, you refine, you model, you refine. You only need to move atoms a little bit at a time. You could easily use such an approach for determining the structure of DNA by NMR: so let's say you had a piece of DNA that was clearly B DNA from a simple qualitative inspection of the NOE distances, you could construct a regular piece of B-DNA and then refine it, because RESTRAIN was basically a simple minimization routine. But what you couldn't do with RESTRAIN for example, was to start from A-DNA and go to B-DNA based on the NOE distance restraints, as one would get trapped in the closest local minimum. So I realized that one could do this in principle for a protein providing one used an algorithm that could get over local minima and reach the global minimum of the relevant target function. But in the case of a protein you were going to have to fold things from scratch either because you didn't know what was going on, or you didn't know what the fold was so you couldn't build an initial model based on some known, related protein structure—at that time, there weren’t thousands of protein structures—now, basically all the protein structures have been solved—but perhaps only a few dozen. So you would have to solve stuff from scratch. I realized that one could use molecular dynamics, not in the traditional sense of just carrying out a simulation at 300 Kelvin for many picoseconds, but one could just heat things up, cool things down, and in that way, get over

energy barriers to efficiently and reliably reach the global minimum. So I got Axel Brunger to quickly write a few lines of code to incorporate NOE-derived distances into his CHARMMF program, and then we started running this stuff on various test cases and on some peptides that we had done. And of course it worked. Sure there was a lot of tweaking and a lot of method development to obtain a robust protocol so that one obtained convergence 100% of the time as opposed to 1% of the time. But once we had done that, then everything depended basically on computer developments to be able to go faster. When I started, every structure—well, put it this way—what you could do on the Cray there overnight takes me two minutes today on a desktop. So that gives you an idea of the increase in speed. Now of course you can calculate hundreds and hundreds of structures and figure things out. In the mid to late 1980s, however, if you needed to calculate an ensemble of say 20 structures, it would take you a long time. So the path forward was clear, and it was obvious all of this was going to work. And it worked.

And we basically took it from there. The first thing we did—the first protein structure we published was purothionin, which was related to crambin, a protein whose crystal structure was known and where we had done model calculations to see whether you could get structures from these sorts of NMR distances and whether the structures were good enough, for example, to solve an x-ray structure by molecular replacement, and they were. So we knew everything was going to work. So we solved several small structures, and at one time, I think we published more structures in the Protein Data Bank than all other groups combined, for whatever that's worth. But none of these protein structures were of any particular great interest. I mean, any small protein that one could lay one's hands on, one would, but it was pretty obvious that the techniques at the time, the two-dimensional proton-proton techniques, were very limited. So they were good for proteins up to say 75 residues.

ZIERLER: Oh, no.

CLORE: Well not Oh no, but Oh yes. The first thing we tried to push the range of NMR further when I was still in Munich was a homonuclear three-dimensional NMR experiment which we published in Nature. The design principle for 3D NMR is really simple. Any 2D NMR experiment comprises a preparation pulse(s), an evolution period during which time spins are labeled according to their chemical shifts in an indirect dimension, a mixing period during which spins are correlated to one another (whether through scalar couplings or NOE), and finally a detection period in which spins are labeled in a second dimension. The construction of a 3D NMR pulse sequence is simply done by concatenating two 2D experiments leaving out the detection period of the first 2D pulse sequence and the preparation pulse of the second 2D pulse sequence. Sounds simple enough but the key to be useful is to design the right sort of experiments with signal-to-noise that is sufficiently high to make it useful. Homonuclear 3D NMR was not that useful in practice but it did demonstrate that 3D NMR could be done, and that is was we pushed forward when I came to the NIH but in a much more useful and powerful form.

So we moved to the NIH in 1988, largely because of Ad Bax, because I knew him. And he said, "Hey, why don't you come and work in the U.S.?" I didn't want to stay in Germany, because I really disliked living in Germany. For an Englishman, going to Germany is a very foreign country. Going to the U.S. is actually no different from being in London. Everything is the same. The way people buy houses, the banks, legal system, the way people think—there isn't that much difference between England and the U.S. But there's a huge difference between England and Germany. So I thought that this was great. And at that time, Ad had persuaded Bill Eaton, who was the department chair for Lab of Chemical Physics, that NMR was really a good thing to do to solve the structures of AIDS proteins. And Ad persuaded Bill that I and Angela Gronenborn

were the guys to be able to do this. And they had a lot of money. Or rather the Office of AIDS Research had a lot of money to do this, and we were able to get a 600-megahertz spectrometer from that, plus many, many other spectrometers thereon afterwards, year by year. And things really bloomed.

So the idea then was to use heteronuclear NMR and specifically three-dimensional heteronuclear NMR to expand the range and complexity of protein systems that one could usefully study by NMR. Now, I already knew that heteronuclear NMR was going to work, because even when I was at Munich, I had already done a three-dimensional heteronuclear NMR experiment just to see, on a sample at natural isotopic abundance with N15. So the sensitivity was very low, but we could see some cross-peaks. Once you isotopically label the protein with N15, you know that the sensitivity is going to be high (given that transfer between 15N atoms and their directly binded protons is very efficient owing to the large scalar coupling between these two atoms. And once you know how to go from two dimensions to three dimensions, you also immediately know how to extend three dimensions into four dimensions, and everything follows from there. Because the basic principles are all really, really simple. So to extend to four dimensions, you do exactly the same thing as from going from two to three-dimensions, basically combing a three-dimensional experiment with a two dimensional one, leaving out the detection period of the three dimensional experiment and the preparation pulse of the two dimensional one, and et voilas you have 4D . So we knew that this was going to work as well. At the time, of course, nobody believed it was going to work, because I think they couldn't see further than their noses. So had I not been at the NIH and had I been at a regular university, there's no way that the NIH would have given us funding to do any of this stuff, because the reviewers would have said, "Oh, it's not possible to do this. The signal to noise isn't good enough. You'll never get anywhere. It's completely

useless." In the same way that they used to say you couldn't get three-dimensional structures of proteins. Well, you could get three-dimensional structures of proteins. So it was very fortunate, in that regard, being at the NIH. And the thing is, it wasn't only that we knew it was going to work, but within the space of two years, not only had all the basic three-dimensional experiments that are still in use today been developed—I mean, they've been improved a little bit obviously, in whatever it is, 20 years—no, almost 30 years—yeah, 30—it's a long time—but all the basic experiments were there, and they're not that much different today. Sure there are some tweaks here and there, but basically all the basics had been done by 1991. Not only had we done three-dimensional NMR, we'd gone to four-dimensional NMR both with N15 and with C13. We had solved the structure of interleukin-1 which was an important protein. It's an important protein in the immune response, and at 153 residues was 50% larger than anything anybody else had previously done. Interestingly, it took about three years before anybody came close, because a lot of the stuff on the NMR instruments had to be built. Specifically, various timing devices not available on the commercial spectrometers at the time, and ways to be able to record the stuff so that one could actually do it with the existing spectrometers. Now of course everything is totally routine, because you can do anything you want on the current generation of spectrometers. They're sophisticated enough. So by that time—so by 1991, we had already solved a protein that was 153 residues in length. And just to put it in perspective if you look, even today, there are plenty of structures maybe up to 20 kDa, so maybe up to 200 residues— but there's very little beyond that. Because beyond that, it gets actually a lot more difficult, and we have to use some other approaches—you can't brute force it anymore. So it all started from there.

ZIERLER: I want to ask—going back to this idea of looking at NMR primarily or at least initially as an intellectual challenge, when you got to the NIH, and I think you said it was Bill

Eaton that said, “Focus on AIDS research,” was this the first time that you started to think about—I don’t know if this is the right phraseology, but more practical applications of NMR research? How it could be used to advance a given mystery in health sciences?

CLORE: Bill didn't say focus on AIDS research. The money was for AIDS research. But at that time—so the idea—he had sold it to the powers that be that NMR had this great potential to do all this structural stuff. But at that time, it hadn’t been demonstrated, right? Now, AIDS protein—I mean, of course, we’d love to do a lot of work on AIDS proteins, but AIDS proteins are notoriously badly behaved. I mean, we tried some things and we did some things, but they're notoriously badly behaved. But at that time, anything that was of any interest could be sold as AIDS-related. For example, take our work on interleukin-1: anything part of the immune system is AIDS-related in one way or another. You know, immunomodulators, this, that, and the other. So, looking at interesting systems is a no-brainer—once you developed all these techniques, you don’t want to look at some boring protein that nobody cares about. You want to look at some interesting things. But you can still look at interesting things and select what you want to look at, and what works, and still be able to push the technology to greater heights. So at that time, when we wrote our AIDS proposals every two years, we would write—it was like a two-page writeup to ask for $2 million worth of stuff (what a luxury!), we would just justify everything in terms of, “Oh, we're looking at a transcription factor interacting with DNA. This is involved in controlling transcription of immune modulators—involved in some way or another in the immune system, so it’s of interest to AIDS.” So it wasn’t like, “Oh, let’s focus specifically on this protein or that protein from HIV.” In fact, we didn't really look at that many HIV proteins. We looked at nucleocapsid, which was a two zinc knuckle thing, and that was—well, that was very easy. We tried to look at the RNAse H domain of reverse transcriptase, and we never really got anywhere

very far because the constructs that we had just weren't behaving very well; we were able to get the secondary structure and some backbone dynamics from it, but it turned out that the RNase H from the HIV strain we were looking at, even with some mutational tweeks, was badly behaved for both NMR and for that matter crystallography (and David Davies, a crystallographer in NIDDK in the Lab of Molecular Biology, upstairs from the Lab of Chemical Physics, had tried with no luck. But it turned out that a drug company called Agouron in San Diego worked on RNase H from a different HIV strain which behaved extremely well. In fact when we got a sample from Agouron it gave a fantastic NMR spectrum as we could have solved the structure in weeks. So that's how those things go. So it wasn't that we were focused specifically on HIV proteins.

ZIERLER: Did you ever find that you had to defend—in terms of securing funding for projects, that you had to demonstrate a particular clinical value of NMR spectroscopy? Was that ever an issue?

CLORE: Absolutely not. So the way that NIDDK works is that, in contrast to many of the other institutes at the NIH, is that part of NIDDK's written mission is to support basic research. And apart from the diabetes, kidney and liver branches, nobody else is doing much on any of those clinical things. So no. The way that we got judged—in terms of the NIDDK intramural program—was based on the quality and impact of the work we publsihed, irrespective of its relevance to any type of disease. We also got extra money from the Office of AIDS Research, or at least indirectly from the Office of AIDS Research, through the Office of the Director of the NIH, in what was known as the Intramural AIDS Targeted Research Program. So that was bonus money where we gotlarge amounts of capital equipment (including NMR spectrometers, high powered computers, etc...), that NIDDK couldn't afford. But as for the rest—we were judged, as

everybody is, in our institute, or for that matter at NIH as a whole, on what we've done, not what we propose to do. And providing you publish a lot of good papers what is anybody going to say? So you're not expected to do any clinical anything, or anything that's clinically relevant or even medically relevant.

ZIERLER: What is it about NIDDK that it does support that basic research component that makes it unique from the other institutes?

CLORE: The other institutes support basic research as well to a greater or lesser degree. There are some small institutes such as the Deafness institute where they only have ten PIs (so basically the size of the Lab of Chemical Physics!); they really do have to concentrate on deafness. NIDDK is a large institute; it's got about100 PIs. Well in that case you can obviously do a lot more. And basic research was also in the initial remit of NIDDK. Heart, Lung and Blood also supports all sorts of basic research that has no relation whatever to anything to do with heart, lung, or blood diseases. For NCI, on the other hand, there's always a cancer related theme. So NCI doesn't want people working on completely non-cancer related stuff. But there's still an awful lot of basic research going on. at NCI. And there was also a lot of HIV research going on in NCI, which only indirectly cancer-related because some HIV patients are susceptible to certain cancers such as Kaposi's sarcoma. So some institutes are much more specific than others. NIAID, so the infectious diseases and allergy institute, are really focused on immunology and infectious diseases, but that also entails a lot of basic immunology. So NIAID doesn't have any basic biophysics there, for example. NIDDK does. It just so happens that it does.

ZIERLER: What exactly does four-dimensional NMR spectroscopy mean? We live in a three-dimensional world, so what's the fourth dimension? What does that mean?

CLORE: I wish I could show you a picture of this, but I'll explain—it's really very easy simple. So let's say you've got a two-dimensional spectrum. So let's say the 4D spectrum that we're going to look at is going to be proton-attached-to -nitrogen, nitrogen, carbon, carbon-attached-to-proton, experiment. And what we want to do is look at NOE, so short interproton distances, between a proton attached to a nitrogen and a proton attached to a carbon. Now let's say I do a two-dimensional experiment to obtain a correlation between amide protons and the directly attached nitrogens. Now, I want to do a three-dimensional experiment in which the NOE effect originates on a particular proton attached to a nitrogen, and I want to know what other protons are close by. So those other protons are in the third dimension, so that's now a three-dimensional experiment. So the way you would look at that three-dimensional experiment is you would look at proton-proton planes within a cube—in effect let's say you were doing a proton-proton NOE experiment, two-dimensional, you would now take that single plane and you would divide it into n planes, usually 64, by the nitrogen chemical shift. OK? So that's your three-dimensional experiment. But now the problem is that the protons close by to the originating nitrogen-attached proton, unless they happen to be attached to nitrogen as well, are only identified by their proton chemical shift. But there are many, many more protons attached to carbons than there are protons attached to nitrogen. If you have a 200-residue protein there are 200 nitrogens, so there are 200 protons attached to backbone nitrogen atoms, give or take since the NH3 group at the N-terminus is not visible and the nitrogen of proline is not bonded to a proton. But give or take 200, OK? But there may be a thousands of carbon-attached protons—every side chain might have, I don't know, five, six protons—well, let's say—take a leucine; it will have an alpha proton, beta proton, gamma proton, and two methyl groups, so that's five sets of protons effectively. I mean, counting each methyl group as one prpton as opposed to three as

their degenerate. So a lot of protons, a lot of chemical shift overlap. So now, how do you get around that problem? Well you can do one of two things. You can either go to higher and higher magnetic field. Well, that depends upon NMR instrument manufacturing and technology related to magnet design. That's one way to go, a very expensive way to go. Plus let's say a 600 was routine—let's say in 1988, we got the first 600 in the U.S.; 600s are a dime a dozen now. Now the highest field spectrometer is 1.2 gigahertz. It has only improved by a factor of two, so it's not that big a gain in resolution, really. I mean, it's more than two, because it's actually eight-fold in 3 dimensions. It depends upon the number of dimensions as to the increase. Even if you get a two-fold increase in resolution one dimension, then in two dimensions you get fourfold, and in three dimensions, eightfold. But irrespective of that, the other way is to go to four dimensions. So in the four-dimensional spectrum, each plane in the three-dimensional spectrum now constitutes a cube separated by the carbon chemical shift of the carbons that are directly attached to the protons close by to the nitrogen-attached proton. So in other words, now—so you go from a two-dimensional plane from a 2D experiment to a cube edited by the nitrogen chemical shift(represented by a series of planes), to a four-dimensional experiment where every plane is then further edited by the carbon chemical shift. So now the NOE that you're looking at between the two protons is identified by the chemical shift of the two protons, and the chemical shift of the two heavy atoms, in this case nitrogen and carbon, to which they're attached. So there's a huge increase in resolution.

ZIERLER: And the value in increased resolution is what?

CLORE: The problem is let's say you run a NOE experiment where you want to look at short interproton distance contacts (less than five angstroms). That's the mainstay of structure determination by NMR. Now we can do other things, and we can measure much longer distances

using other approaches, which we'll come on to later, that are more exciting,. But the mainstay is still short interproton distances derived from NOEs. So you've got to be able to assign the cross-peaks in a multi-dimensional spectrum arising from the NOEs. If the two shifts of the two protons happen to be unique, do not overlap with anything else, you're cool. But if they overlap with the chemical shifts of other protons , not so cool, because you can't make a unique assignment a priori— you've got to use iterative structure refinement to figure out what they might belong to. But then you can have a situation where you've just got a big blob and you can't resolve anything at all. So in our first 4D paper, we've got this really neat picture where you see the two-dimensional plane, and it's just like a complete smear, just of a very small region between one and 2 ppm in the methyl region of the spectrum. And then you go to three dimensions, and after that four dimensions, and you see all the peaks comprising this blob in the two-dimensional spectrum fully resolved within individual planes of the 4D spectrum. So now, you don't even have to make any guesses. You just pick the peaks, you've got the NOE information assignment right off the bat, and you can use that to calculate the structure.

ZIERLER: OK! Can you explain how NMR helps to analyze and characterize protein dynamics?

~~**CLORE:** Yeah. So the thing is that – so long ago, so Atilazaba [?] who you contacted, actually – hold on a second.~~

~~**ZIERLER:** No worries.~~

CLORE: OK, so it turns out that NMR can also measure relaxation times: longitudinal relaxation, transverse relaxation, and something called the heteronuclear NOE. For example, say you are looking at backbone nitrogens. So you'll measure the N15 transverse relaxation, the N15 longitudinal relaxation, and the N15-1H heteronuclear NOE. So from that data what information can you get? You can get the overall correlation time of the molecule; that's the overall tumbling time. And then you can get order parameters along the backbone indicating how mobile the backbone nitrogens are. If the order parameter has a value of 1, it's rigid as a rock; if it's 0.9, it's not. The normal order parameter for a "rigid" backbone would be around 0.85 as there are always bond librations that reduce the order parameter for a value of 1. Now, let's say that there are highly mobile backbone nitrogens, they will have low order parameter values (say less than 0.6). This may seem a bit mundane, but there are plenty of other sorts of dynamic information that one can access by NMR which I'll talk about later, because first it's what we're doing now, and second it involves more chemically and biologically interesting processes, such as the interconversion between species and the detection of excited, sparsely-populated transient states. Nevertheless, at the time, in the early 90s, one could map out in very simple terms the mobility along the backbone.

ZIERLER: What does relaxation mean in the context of NMR?

CLORE: Well, oh god, that's—relaxation is a pain to explain and is sort of complicated.

ZIERLER: [laugh]

CLORE: I mean, it really is. But let's try and make some things simple. For example, what does transverse relaxation mean. Transverse relaxation is related basically to the line widths. So the line width at half height is one over pi times the transverse relaxation time. And basically the

reason signals are broad is dependent upon how rapidly the signal dephases in the xy plabe. There are other types of relaxation, for example longitudinal relaxation which relates to decay in the z direction as opposed to in the xy plane. And I'm not the best to explain this in a really simple way, but what can one obtain from this in a practical sense. First T1 is the longitudinal relaxation time; T2 is the transverse relaxation time. Those measurements give you information on rotational correlation times. So T2 gets shorter the bigger the molecule (and the broader the linewidths). T1 initially becomes shorter, and then it becomes longer again as the rotational correlation time increases (i.e. the bigger the molecuke). And by looking at the T1/T2 ratio one can get the rotational correlation time, and use expressions for T1, T2 and the heteronuclear NOE one can obtain atom specific order parameters. Basically that's what those things are about: transverse relaxation is how rapidly a signal dephases once it's in the xy plane; the longitudinal one is how—once a signal is in the xy plane, which is where signal detection occurs, how long does it take to get back into the z direction.

ZIERLER: OK. Let's talk about the XPLOR-NIH program. How did that get started and what was its relation to the original XPLOR program?

CLORE: Right. So the original XPLOR program came out Axel Brunger's CHARMMF variation of CHARMM. When he left Karplus, he renamed CHARMMF as XPLOR. Then there was a big kerfuffle with a company called Accelerys about rights. So Axel made a few trivial syntax changes to XPLOR and called it CNS. Of course by that time XPLOR had evolved considerably from the original CHARMMF to include all sorts of additional functionality related both to crystallography (developed in Axel's lab) and NMR developed in my lab. So CNS was basically the same as XPLOR, with some really minimal differences. All the NMR stuff in CNS came basically from us. And all the NMR stuff in XPLOR, the original came from us. And Axel

was mainly interested in the crystallography. And what happened with the crystallography was that I said—when he was still in Martinsried—just before he left to go back to the U.S., , “We should do what we did for NMR in terms of restrained molecular dynamics to crystallography. It’s trivial.” He didn't do that while in Martinsried; he did it unfortunately when he went back to Martin Karplus' lab, otherwise I’d have been involved with that, too. But be that as it may.

Now new functionality is being added all the time to XPLOR/CNS, every time we develop new NMR experiments or measure new structurally relevant NMR parameters. First, we just incorporated NOE-derived distance restraints. Then we put in three-bond scalar coupling constants which are related to dihedral angles. Then we put in other stuff like database potentials of mean force, restraints for residual dipolar couplings (which provide orientational information), etc.... So all of this got added into XPLOR and then into CNS. And then there were problems with CNS in relation to distribution as a consequence of the link between Accelerys and Harvard. So Axel continued developing the crystallography side in CNS, and eventually stopped further development of CNS and wrote a completely new program with Paul Adams. And we developed completely new NMR related stuff in a new program called XPLOR-NIH. What we did is we kept the legacy code so that labs could still run old scripts that are dated from 30 years ago if they wished to, and those scripts are perfectly fine, easy to understand and very powerful. But at the same time, we incorporated completely new code, now written in C++ as opposed to FORTRAN, and developed a Python interface. While Python is more complex than the original XPLOR scripting language it is vastly more powerful and flexible. Not only that it enables one to easily add additional potential terms and try out things in Python without having to code them up in C++ right away. And then if that proves to be useful one can always then code this up in C++ for increased computational speed. And of course all sorts of other functionalities and facilities

were added over time in Xplor-NIH. For example, we developed new torsion angle dynamics code which was much more effective and had a variable time stepintegrator; we also have code that allows one to combined rigid body dynamics with torsion angle dynamics and cartesian dynamics all in one: for example, some portion of a protein or protein domain can be treated as a rigid body (especially useful for protein-protein complexes), while regions at the protein-protein interface can be handled by torsion angle dynamics and if necessary can be dealt with in cartesian space, depending on the circumstances and the problem at hand.. It all originates from the same place.

ZIERLER: OK. Let's talk a little bit about your current research. What are some of the things that you're working on now?

CLORE: Right. Well, so let me tell you how I got into it. So this was back in maybe 2004 or so. We decided that NOEs were a bit limiting, because you could only get short distance information between protons separated by less than about 5-6 Å. But what about if you want to get long-range distance information? Now there's a way of getting long-range information that had been known since the late '50s, early '60s, and that's paramagnetic relaxation enhancement (PRE). So what that involves is you attach a paramagnetic center to the protein of interest, and then you look at broadening of signals. And that broadening of signals—so basically you measure transverse relaxation rates—and the difference in transverse relaxation rates with or without the paramagnetic center (which can be a paramagnetic metal or a nitroxide label) is the paramagnetic relaxation enhancement rate. So people have known about the PRE for a very long time, but it wasn't being used properly. Because the thing is that the paramagnetic labels move around a lot and sample a wide range of conformational space. So having a restraint that says something is 20 angstroms plus or minus ten angstroms is not very helpful in terms of structure

determination. If you say something is less than five angstroms when it could be 100 or 150 angstroms away, is obviously highly restraining. Saying 20 plus or minus ten angstroms, not so useful. So what we did—and the postdoc, Junji Iwahara, that I had at the time was absolutely brilliant—is he developed the necessary theory to take into account that the paramagnetic centers were mobile, and how to directly refine against the paramagnetic relaxation enhancement rates. So we did that. We developed all the relevant theory and necessary computational tools in XPLOR-NIH and went ahead and used it. We first validated the approach on a protein-DNA complex whose structure we had previously solved, and that was the SRY-DNA complex. So SRY is the male sex-determining factor. So this is a sort of an interesting system. So we knew it all worked. At the time I had already solved a fair number of binary protein-DNA complexes. So what I wanted to do was look at ternary protein-protein-DNA complexes, so two proteins and a DNA. And there's a lot of literature on this, about proteins interacting on the DNA, and a ton of literature, most of which turned out to be wrong, because people weren't purifying their proteins properly and they were using immunoprecipitation to claim interactions between the proteins on the DNA. So you've got two DNA binding proteins and you precipitate them out, then they're going to co-immuno-precipitate even if they bind non-specifically to the DNA and they don't need to interact at all on the DNA. But they were claiming they did. So in any case, at the time, there was a ton of literature on a related protein to SRY called HMGB which is a non-specific HMG box protein similar to SRY but doesn't bind DNA specifically, HMGB is an architectural DNA-binding protein that bends the DNA and it was claimed to interact with a transcription factor called HOX (a homeodomain protein), thereby enhancing transcription. So there was a ton of stuff in the literature claiming to show that the proteins interacted with one another on the DNA .Turns out the two proteins didn't interact with one another at all. But there was all sorts of

functional data suggesting that the presence of the HMG protein enhanced transcription by HOX, because HOX is a transcriptional activator. So we didn't realize at the time that all this data saying these two proteins interacted was problematic, so we were trying to look for such interactions using intermolecular PREs—so the paramagnetic label was covalently linked to the DNA and the PREs were specifically observed on amide protons of the protein by isotopically labeling the protein with N15—to figure out what was going on, and we could never figure out anything. By that I mean, it was clear they weren't interacting, and depending on how you did the experiment, you'd get different results. But then we were doing a control experiment where we first had looked at HOX in the presence of DNA at low salt, and the PRE data agreed perfectly with the crystal structure. And by perfect I mean there was excellent quantitative agreement. So the Q factor, which is a measure of agreement between observed and calculated PREs, sort of analogous to the crystallographic R-factor, was 20%. You can't get better than that. It's like perfect. Then you raise the salt—and this was the control experiment—we raised the salt, and I'm not sure why we did this control experiment, but it was a control experiment nonetheless, because we needed to do that to see whether HMGB was really interacting with HOX or not. So we raised the salt, and we're looking at this, just the binary complex, and we measure the PREs. The 1H-15N NMR correlation spectrum at high and low salt looks exactly the same, so the specific complex between protein and DNA is unchanged. Nothing has changed. But when we measured the intermolecular PREs at high salt, it looked as if binding was completely non-specific. This obviously couldn't be the case, because if you interact the HOX domain with a piece of DNA that doesn't contain the specific site, the 1H-15N correlation spectrum looks completely different. So it's not that it's all of a sudden binding non-specifically. What was going on is that we were actually detecting minor species present at the 1% level,

which we're observing on the visible species—the specific complex—and these invisible species basically comprised proteins sliding up and down the DNA or jumping from one DNA to another.

Now the way the PRE works, and the reason it works, is the following, is that the PRE, just like the NOE—and I didn't mention this before—is dependent upon the r^{-6} distance between the paramagnetic center and each proton being observed. So you can measure all the PRE effects to every proton in the protein if you want from a paramagnetic center. But the difference between the NOE and the PRE is that the NOE effect is tiny, tiny. So to see an even smaller effect on a small effect, you're not going to see it. But to see a small effect on an absolutely massive effect, you can see. So the way it works is the following—just us an example, let's say you have a major species populated at 99% (e.g. the specific protein-DNA complex) and the distance between the paramagnetic center that's labeled on the DNA and a proton on the protein is say around 30 angstrons; the PRE rate is going to be about two per second. If that distance is reduced to eight angstroms; the PRE rate is going to be around 5,000 per second. Now if the two species, the one at eight angstroms, the one at 30 angstroms, are exchanging slowly relative to that 5,000 per second, so very slowly, then you won't see any line broadening on the signal that you're looking at, which is the signal of the specific complex (the one populated at 99%). But if there's rapid exchange between a minor species, the 1% minor species and the 99% major species, then you will see it. So if there's rapid exchange, very fast exchange, then you'll just see a population-weighted average of 5,000 and 2 per second. So if there's 1% minor species, you'll see a line broadening effect, basically, equivalent to 50% increase in linewidth. So that's how it works. So what we did is we figured that this was going on. At first, Junji ouldn't figure out what was going on, but I immediately realized what this was—sliding of the protein up and down on the DNA

and hopping of the protein from one DNA to another directly without going into free solution. You can hop from one to one DNA to another directly because although a homeodomain is considered a one-domain protein, it really isn't, as it has a globular domain containing a helix-turn-helix motif that binds to the major groove of DNA, and a flexible N-terminal tail that interacts with the minor groove of the DNA. In the free protein, that N-terminal tail is all over the place. Now both the globular core and the N-terminal tail can bind on their own to DNA but very weakly. So let's say each binds to DNA with an equilibrium dissociation constant (K_D) of say 100 μM. Then combined, it's going to bind very tightly with a K_D of 10 nM. That's because if the N-terminal tail is off, the core is still bound, and vice versa. So in any case, the way HOX can hop from one DNA to another is that the tail can go on and off the DNA. And if there's enough DNA around, another piece of DNA can hook onto the N-terminal tail and then bring the globular core up for the ride onto the other DNA. Obviously, there's a probability of going either way, and we could demonstrate this. So that was the first thing that we did.

And so we realized we could detect a very short-lived states with lifetimes say less than 250 microseconds to half a millisecond. And we could detect species at the half a percent to one percent level. So this is quite interesting, because people had talked about sliding up and down the DNA to do a one-dimensional search to increase to facilitate the search for a specific DNA site within a massive sea of non-specific sites, but nobody had actually demonstrated this phenomenon directly. So we were the first to demonstrate that directly.

So after that I immediately realized one could do something similar with protein-protein complexes. It so happened that at the time, we were interested in a series of protein-protein complexes from the bacterial phosphotransferase system. This is an interesting system with nine cytosolic complexes in this pathway, and I knew that for some of these, the intermolecular NOE

data was such that they weren't consistent with a single structure. But I knew that for chemical reasons that there has to be a single main structure, because you have to have phosphoryl transfer. which entails inline phosphorus transfer between two histidines. So there is a very specific geometry that has to occur. So I realized that something was going on. So we tried on one complex between enzyme I and HPr whose structure we had already solved. We measured the intermolecular PRE data. And of course we see PREs corresponding to the specific complex, the real complex, the complex where phosphoryl transfer can occur, but we saw other effects as well. And those other effects were the result of transient encounter complexes. People had talked about encounter complexes for years and looked at these computationally, but nobody had actually demonstrated their existence directly. So we did that.

Then we knew that there were other proteins where large domain movements were esential for function. So, for example, maltose-binding protein exists in two states involving very large-scale interdomain motions. It has two domains. It's basically like a Venus flytrap. In the absence of the sugar substrate maltose binding protein is in an open state; with sugar boun, the protein is in a closed state engulfing the sugar. And the question was, can you sample—in the absence of sugar, states that are close to the states sampled when sugar is bound? So a closed state. Lewis Kay had done a whole bunch of experiments with mutations where he showed that the probability of occupying this closed state, the real closed state, is very, very small, but that doesn't mean to say that you can't occupy other states that are still closed or partially closed that are close to but not identical to the holo closed state seen in the protein-sugar complex. So we could use the PREs to probe this phenomenon. So we put a paramagnetic label on one domain, and we looked at the PREs both within the domain and on the other domain. Within the domain they should agree with the crystal structure, which they do. And in the other domain, we see some other effects in

addition to the ones that you predict from the apo (without sugar) crystal structures. You then do the same experiments with the paramagnetic label attached to the second domain, and you can then use ensemble simulated annealing calculations with XPLOR-NIH based on these PREs and find that yes indeed there's about 5% of a minor species which is in a partially closed state, close to but not identical to the final closed state of the holo protein-sugar complex. So this minor state is partially open so a sugar could still go in, but it's a lot closer to the final state than the fully open state. So in this instance sugar binding can occur via a mixture of both induced fit and conformational selection.

We then did similar experiments on calmodulin, another two-domain protein separated by a central linker. In solution, the middle portion of that linker really doesn't make any contacts to the two domains, and it's very flexible, so you can sample a large range of conformational space. And people knew that these two domains tumble almost independently of each other. But we were able to demonstrate that you could actually sample conformations that were close to the closed state when calmodulin binds peptides. Because when calmodulin interacts with peptides, the two domains clasp onto the peptide ligand. In other words, we showed that one could sample states similar to but not identical to the closed ligand-bound state in the absence of ligand. So a variation on the theme we had observe with maltose binding protein.

The discovery of low population states that are very transient is exciting for a number of reasons. One, they're involved in a whole range of biological and chemical processes: for example macromolecular recognition, protein-DNA binding, assembly processes including amyloid fiber formation, etc..... So binding DNA non-specifically and sliding up and down the DNA basically reduces a three-dimensional search for the specific DNA target site to a one-dimensional search. Similar in protein-protein recognition, non-specific encounter complexes reduce the search for

the final specific configuration of the complex from three to two dimensions. So clearly these are intrinsically interesting phenomena. And then from a purely technical and experimental perspective, these phenomena are really challenging to study and really NMR is the only way to look at these. For example, let's consider cryo-electron microscopy it would be very hard to sort out a 20% minor species from an 80% major one, as one has to pick out all the images by hand and classify them. It's a mess and just wouldn't be possible in practical terms. In crystallography, you're only crystallizing one thing. You might be able to crystallize a minor conformer, but that's not by design, but purely by chance, and you wouldn't know whether what you had crystallized was the major or minor species, as everything depends upon which states crystallizes preferentially. And in single molecule fluorescence spectroscopy, one just doesn't have the signal-to-noise to detect species below say 10-15% occupancy. That was an exciting thing for NMR to be able to tackle.

So we pushed the PRE in a number of areas as obviously there's a lot of low-hanging fruit to be picked when one has basically created a brand new field. Now I had a postdoc come who thought we could use the PRE to look at the role oligomers or pre-aggregates/protofibrils play in amyloid fiber formation related to Alzheimer's. So the question was whether we could low populations of dimers, trimers, tetramers or higher order oligomers? So we carried out PRE experiments and could never see anything significant or reproducible. Initially it wasn't obvious what was going on. And then it occurred to us that another phenomenon was occurring by carrying out similar measurements as a function of concentration in the absence of a paramagnetic label: that phenomenon was something called lifetime line broadening. So what was happening was as follows: if we took a sample of say 300-micromolar amyloid β40 (Aβ40) and left it for a couple of weeks, at ten degrees, with no stirring or any other type of physical

perturbation, half the NMR signal would disappear. This state of the system would then remain stable for three, four weeks, and then only after that would it form fibrils. So in that pseudo-equilibrium, exchange phenomena were occurring . Now, if you just looked at the spectrum all you would see is the signal had dropped by half as large protofibrils are NMR invisible (since they tumble very slowly and are therefore broadened out beyond detection. But we could detect small difference in transverse relaxation rates between the concentrated sample where half the protein had become dark and a dilute sample (say 50 micromolar) where the sample was entirely monomeric and therefore 100% visible. These difference in transverse relaxation rates were small, and relatively uniform, but not quite. And the cause for this was due to exchange between monomers that are fully NMR visible, and very large species, protofibrils in this case, which are in the 2 to 8 megadalton range that are completely invisible or dark to NMR. When the monomer transiently binds to the protofibril, it loses magnetization very rapidly. So when it comes back into solution, there's line broadening. That effect is due to lifetime line broadening effect arising from exchange between a fast tumbling and very slow tumbling species, as opposed to the more usual cause of line broadening in exchanging systems that arises from differences in chemical shifts between the states. These experiments gave us information on the association rate constant, as the exchange regime we were in was such that lifetime line broadening was limited by the apparent associate rate constant. Then we realized that we can get information on the dark state that's completely invisible because while its resonances are so broadened out that they lie in the baseline they can still be saturated. So if you take a very dilute sample—and this goes back to experiments I was doing I was at Mill Hill, except we're doing these in two dimensions andand in a more sophisticated way,—and you apply a weak saturation pulse at say 100 hertz intervals you see decreases in intensity of the signals of the visible state even when the radiofrequency

pulse is 30 kilohertz off-resonance. On a very dilute sample where everything is a monomer, saturation is only achieved on-resonance. When you look at that profile of intensity of the resonances (cross-peaks since we're doing this in two dimensions) of the visible species as a function of the position of the weak saturation pulse, one obtains a profile that can be described by the Bloch-McConnell equations, and fitting then gives you information on the dissociation rate constant and the relaxation properties in the bound state. In other words it's possible to get information on really large dark states by looking at the effects of applying saturation pulses at different positions on the spectrum of the small NMR visible species, providing the NMR visible and the very large NMR invisible species exchange with one another in a certain time regime. That experiment was called dark state exchange saturation transfer or DEST for short.

In essence all the NMR experiments designed to look at transient sparsely-populated states operate in a similar manner to that of the TRNOE experiments that I described right at the beginning and that I had done at the MRC Mill Hill back in 1982. What in effect is being done is that one is transferring a magnetic property (whether relaxation, chemical shift, etc...) from an invisible state to a visible one through exchange. This is true of the TRNOE experiments where the ligand is in large excess over the protein, it's true of the PRE, lifetime line broadening, DEST, chemical exchange saturation transfer, relaxation dispersion and so on. Exchange itself can only be minimally manipulated (for example by temperature) but exchange has to be there to be able to detect and characterize low-populated transient states. If the two species don't exchange with one another or exchange too slowly there's nothing to observe. And depending on the particular type of experiment the exchange window (i.e. the scale of exchange) that is useful is different. So that’s how all these experiments work.

So those first experiments involving lifetime line broadening and DEST that we did with Aβ40 got us into looking at other visible proteins interacting with large macromolecular assemblies. So the next thing we looked at was the chaperonin GroEL which is close to 1 megadalton in size. GroEL accelerates protein folding. And a lot of the kinetic studies that have been done are complicated by the fact that the starting species that were being studied are really not very well characterized. The substrates were molten globule-like proteins , so from an NMR perspective that makes them very, very hard to look at. Basically impossible. So here we've got an eight hundred kilodalton system and we want to look at how GroEL affects protein folding using a protein system that we can actually observe well by NMR. We initially tried some of the systems that people had previously used but as I said they were poorly behaved and molten-globule like so that was going nowhere. But then I realized that Lewis Kay had looked at an SH3 domain that exchanged between a visible folded state, an intermediate state, and under some conditions, an unfolded state. So what we first did is we looked at the SH3 s under conditions where only two species are present: the major folded species, and a folding intermediated populated at around the 2% level. And so the goal was to to see whether we could detect any impact of the chaperonin on the exchange characteristics between the folded state and the intermediate state (the folding intermediate—and we're going to use not just the techniques that I've just described, but also some other techniques such as relaxation dispersion and exchange induced shifts (small population weighted effects arising from small differences in shifts between the free and bound states).So you do these experiments in the absence of GroEL, in the presence of GroEL, and you look at what’s going on by carrying out a global fit to all the experimental data which are described by some variant or other of the Bloch-McConnel equations. So that's a large optimization problem. And what it turns out is going on is the GroEL accelerates the exchange

between the folded state and the intermediate state about 500-fold and switches the equilibrium from 50-to-1 folded to intermediate for free SH3, to1-to-3 when bound to GroEL. I have to look the exact numbers. So GroEL switches the equilibrium in favor of the folding intermediate state. In other words GroEL biunds to SH3, stabilizes the folding intermediate state and accelerates the interconversion between folded and intermediate states. And what we were also able to show using some other constructs where you just had exchange betweenthe folding intermediate and a fully unfolded state is that GroEL not only stabilizes the folding intermediate relative to the folded state, but also stabilizes the intermediate relative to the unfolded state. In fact the unfolded state is actually destabilized within the large cavity of GroEL. So that's the sort of things we've been looking at.

Over the last couple of years we have tuned out attention to look at the earliest oligomerization events of the Huntington protein. The Huntingtin protein is responsible for Huntington's chorea, a fatal, autosomal, neurodegenerative condition—and there's a famous American who died of it, Woody Guthrie. Maybe before your time as well as mine, but Woody Guthrie had a huge influence on Bob Dylan.

ZIERLER: I know Woody Guthrie. [laugh]

CLORE: Yeah. Well, that's more than my postdocs, but they're not American. But in any case, soWoodie Guthrie died of Huntington's disease. It's an autosomal dominant condition (so you only need to inherit the bad gene from one parent), and all the bad stuff, specifically the formation of neuronal inclusion bodies composed of amorphous aggregates and fibrils of Huntingtin, involve the first portion of the protein encoded by exon 1. Huntingtin itself is around 3,000 residues, but all the bad stuff, namely the polyglutamine expansion that leads to

aggregation, is located within exon 1. Now the diseased state only occurs when there are too many glutamines inserted in this region (more than 35 glutamines in a row). Exon 1 comprises an N-terminal domain, a polyglutamine stretch, and a polyproline rich domain. So what we decided to do is see whether we could detect what was going on in the very, very earliest stages of oligomerization before you get fibril formation. In other words pre-nucleation events that eventually trigger fibril formation. And the way we did that is we titrated in how many glutamines you need to still maintain a stable NMR sample that doesn't just precipitate or form fibers right away, which would prevent one from doing any interesting NMR experiments, because NMR takes a long time. Every experiment of the type we're going to be doing may take 48 hours or so. But obviously you need a sample that's stable for a lot longer that, basically many weeks, because you don't want to make a new sample for every experiment- reproducibility would then be a big issue and it just gets too expensive and too time-consuming. So you want a sample that's stable but still forms fibrils eventually. So we ended up with something that had seven glutamine repeats. And again, using all these combined techniques of relaxation dispersion, which looks at chemical exchange line broadening and how you can suppress that, which is related to kinetic information and to chemical shifts between major species and minor species, exchange induced shifts, and other forms of relaxation, we were able to show that huntingtin oligomerizes via a branched pathway: there's an off-pathway, leading to a non-specific dimer that doesn't oligomerize any further, and then an on-pathway that leads first to a dimer, which we could show was an anti-parallel helical coiled-coil dimer with C2 symmetry, that tgets converted to a tetrameric coiled-coil with D2 symmetry. So we were able to resolve all the kinetics, resolve all the steps. We were also able to get the structures of the on-pathway species using paramagnetic relaxation enhancement, where we can detect what's going on in

something that's really populated at a very low level. So under the conditions of our experiment, the maximum population of these species is 2-3% at a concentration of around 1 mM. And that's the maximum concentration you can get under the conditions we're studying while keeping the sample fully soluble. If you increase the concentration of the sample further you reach a critical concentration and the protein comes out of solution. So we can detect really low populated species doing this sort of stuff. So that's what we're largely focusing for the moment.

ZIERLER: Let's talk a little bit about the future of NMR. Where do you see the field going? What are some of the frontiers that may be reached in your career or beyond?

CLORE: Well, NMR is one of those fields where it's very difficult to predict what the future will hold. And to be honest if I knew what could be done, I'd already be doing it, and I'd be on to the next thing. So I can think of plenty of things to keep us occupied for the next ten years, with no problem, because there are many, many interesting problems in biology to answer, and there are many challenging problems. And personally I'm most interested in tackling challenging problems that other people can't do. And just as an aside, one of the reasons I'm interested in that is at the NIH, you don't have large groups. So that's why I don't do structures anymore, because if somebody decides to do a structure and they've got a huge amount of resources behind them they are likely to be first, even if they do a sloppy job, and the current scientific environment is such that it no longer matters whether you get a more accurate structure if you're second. If they publish fast, they get the credit for it. So I want to do stuff that's really intellectually challenging as well as biologically interesting. In that sense it's very hard to actually predict exactly where NMR is going. There are a number of things that I can think of that Ad Bax has probably talked to you about. I'm thinking along the same lines as him. But it's hard to think of things right off the bat that I think that we could do, that I can see happening ten

years down the road, because if I could I would be doing it now. And that has been the history, I would say, of NMR, throughout. Not only that NMR has always advanced in quantum jumps. In the early days (1970s) a lot of people thought, "Oh, it's a boring" as all that one could do with proteins was histidine titrations. I mean, how much can you learn from histidine titrations? And even though it was realized very early on that NMR could in principle solve protein structures, nobody had a clue actually how to do it. There's a difference between thinking, "Oh, maybe it can be done," to really even writing down in practice how you might even think about doing it. Nobody had a clue. But then all of a sudden NMR advanced very rapidly largely due to the advent of higher field NMR spectrometers (500 megahertz and better electronics to provide appropriate control of the pulses). So in1985, Wüthrich published the first structure of a protein. In 1986, we published the second protein structure. But nobody in the mid-80s was thinking about heteronuclear NMR or three-dimensional NMR or four-dimensional NMR. But two dimensional homonuclear NMR is limited to around 70 residues. And how many proteins are there that are less than 70 residues? Not very many. You're going to run out fairly soon. And then all of a sudden, we think about it and say—"Oh, let's try three-dimensional NMR." But the three-dimensional NMR experiment that we first did was a demonstration of principle but completely useless in practice. But then Ad Bax had done some work with Dennis Torchia on heteronuclear NMR; and it was, immediately clear how to push things forward in a big way. Within two years—so basically from 1988, when I came to the NIH, to 1990, all the three-dimensional and four-dimensional experiments were developed, and have stood the test of time with only relatively minor tweaks resulting from improved spectrometer capabilities. And that carried over for the next, I don't know, 15 years until I stopped doing conventional NMR structure determination of proteins and protein complexes. There were more developments like

residual dipolar couplings, which were sort of found by chance. I think Ad went to a conference and got the idea to make liquid crystal lipid bicelles to induce very small degrees of alignment and thereby measure residual dipolar couplings that could provide long-range orientational information (that is orientation of bond vectors relative to an external alignment tensor) And that provided a lot of extra information which enabled us to tackle much higher molecular weight complexes because we could then use some alternative creativeapproaches—for example, if you knew the structure of individual domains or proteins and you want to put these things together, you don't need to do a full-blown structure determination anymore. You just needed to know the orientation of these domains from dipolar couplings and then combine this with small angle x-ray scattering to get the overall shape of the complex. Maybe you have one or two intermolecular NOE distances as well, and you've got the structure of these large complexes. So we've done something of 150 kilodaltons that way, just by piecing these things together in a very, very simple way, and in a way that's really fast. So that was another big quantum jump, that one wouldn't have thought that you could do initially. But to be honest, I don't really know what totally new stuff is going to be done.

ZIERLER: And you'd probably say the same from ten years ago to where you are now, it sounds like.

CLORE: Absolutely correct. And then the other thing to realize is that even if particular types of measurement are known it isn't necessarily obvious how to use them. For example, paramagnetic relaxation enhancement was alreadday known in the '60s. But the experiments that we were doing, that we ended up doing, there's no way you could have done them in the '60s because the spectrometer technology was simply not there. For that matter you couldn't have done these experiments in the '80s for the same reason. At that time we were working on

solutions that were five to ten millimolar in concentration. Now, we're working on samples at a concentration of 200 micromolar or so as PRE measurements have to be conducted on dilute samples to eliminate non-specific solvent PREs, arising basically from non-specific encounters due to excluded volume effects. So we couldn't have done those experiments without the massive increases in sensitivity that were obtained through the introduction of cryoprobe technology which affords a 3-5 fold increase in signal-to-noise over room temperature probes. In a cryoprobe, the sample is obviously at room temperature but all the electronics are at liquid helium temperatures, thereby reducing thermal noise. Right now, 1.2 gigahertz spectrometers are coming on board. Well, at first, one might say, “I don’t know what good this is for. I can’t see that it would be useful over existing 900 megahertz spectrometer” But I can see, let’s say, for carrying out the sort of work we've been doing on excited sparsely-populated states using relaxation dispersion, PREs, etc... that a 1.2 gigahertz spectrometer could be fantastically useful, not so much because of the increase in resolution, but because the increase in separation between the signals arising from major and minor species alters the exchange regime relative to the measurable that is being oberserved. And so the amount of information, for example, that you can get by recording relaxation dispersion data at 500 and 1.2 gigahertz is way greater than you could get for the same experiments recorded at 500 and 900 megahertz. There’s all sorts of stuff that will open, and once one does those experiments, one will immediately realize that there are other experiments that one can devise.

For example, our extension of the PREs to study sparsely-populated states was not planned but discovered by luck. It wasn’t like we thought, “Oh yeah, we can use this for looking at species present at 1%.” right off the bat. If we wouldn't have made that original observations on HOX-DNA where at low salt the PREs were fully consistent with the structure of the specific complex

while at higher salt (only 100-150 mM) the PREs were indicative of non-specific interactions despite the fact that the spectrum of the complex was unchanged by salt and therefore reflected the specific complex (the major species), we would never have cottoned on to using the PRE for detecting and characterizing transient, sparsely-poopulated states . Exactly the same sort of thing happened with DEST (dark state exchange saturation transfer). If we wouldn't have seen the lifeline line broadening effects—because we were really looking at something else involving PREs thatdidn't pan out—we wouldn't have gone on to be able to investigate the interaction of NMR visible molecules with very large macromolecular machines or assemblies that are completely dark to NMR. So it's not like I thought, "Oh, this is a great idea to do. Let's do it." It just—you see something, then you realize, "Aha! We can do this, this, and this."

ZIERLER: I want to ask some more generalized questions now, beyond the particularities of your field. All of the prestigious awards that you've gotten, and your membership in the greatest scientific societies and academies, do you see these things as merely recognition for the good work, or is this recognition actually useful for your research agenda? Does it open doors? Does it open funding opportunities? Does it establish connections that advance what you're doing? Or is it simply something where it's nice to be recognized?

CLORE: Well, there are two things. One, it's nice to be recognized.

ZIERLER: Of course.

CLORE: For what it's worth. I mean, I don't pay that much attention to those things, but it is very nice to be recognized in that way. And for example membership in the National Academy of Sciences is very useful at the NIH, because it not only gives me a numbered parking space (which is really helpful given how difficult parking is on campus) but even more importantly it

means I'm not going to get chucked out when I reach 80 or 85 or whatever. Now I must say I was particularly thrilled to get elected to the Royal Society this year for two reasons: first it really is an honor to be elected to the oldest and most prestegious scientific organization in the world dating back to 1660 and counting among its membership people like Newton, Maxwell, Darwin, Faraday, (and even Leibnitz who stole the calculus from Newton! Well I would say that wouldn't I as I'm originally British); second, it was really great that they remembered I was actually British despite having left the U.K. over 30 years ago; and third I only became eligible for election 2 years ago when they changed their rules—before that dual citizens not living in the U.K. or a Commonwealth country were considered as foreigners and it's next to impossible to get elected as a Foreign Member of the Royal society, and not only that who wants to be elected as foreigner when I still hold British citizenship (as well s US of course), and I was born, raised and educated in London, and never left England until I was 29.

ZIERLER: [laugh] Really? That's how it works?

CLORE: That's how it works.

ZIERLER: So you can have your office until you're 110 years old if you wanted.

CLORE: Well, there's a guy at the NIH called Herb Tabor, who was the editor of *JBC* for 50 years until he reached 98.

ZIERLER: [laugh]

CLORE: And he's 101 or 102 now, and he still comes in every day. So—

ZIERLER: I guess that's another question.

CLORE: I don't know if he does anything anymore, but he can come in.

ZIERLER: That's another question.

CLORE: I'm not going to work that long, but it does put one in a position of more security.

ZIERLER: Yeah. It sounds like now, just in terms of you recounting your career, you have as much curiosity and vigor today that you did at any other time in your career. Is that fair to say?

CLORE: Not quite. I mean, I've got the curiosity. I haven't quite got as much drive as I had when I was in my twenties and thirties. Put it this way—so when I was in my early twenties, I wanted to first get to 20 papers. Then I wanted to get to 50. Then I wanted to get to 100. By the time I interviewed at the NIH in January of 1987 (so 31 years old), I'd already reached a 100 publications. Then when I got to the NIH I'm competing with Ad Bax, albeit a friendly competition since we're close colleagues, but competition nevertheless. I then wanted to get to 500 papers. But once you get to 500, whether you publish 20 papers a year or ten papers a year or two papers a year doesn't make that much difference anymore. So I'm not driven—so I'm a bit slower than I was—in that regard, I'm probably much more careful and more thoughtful than I was when I was young. I'm certainly more mature than I was. That's for sure, and it's also for sure that it took a long time. But yeah, I'm not quite as driven. I used to be able to write papers like in a couple days. Now it takes a little bit longer, not that much longer but a little bit longer.

ZIERLER: I'm curious how you define yourself. You're operating in a field where you're moving very nimbly between physics and chemistry and biology. Do you see yourself primarily as one of those fields, operating in the others, or are these not very useful distinctions for you?

CLORE: Well, first, the field that I'm in is molecular biophysics, broadly speaking, and biophysics is not really physics at least in the generally understood way.

ZIERLER: I've heard a range of opinions about this very topic. What is your feeling about that? What is real physics as far as you're concerned?

CLORE: Well, let's say biophysics is not like theoretical physics or high energy physics or materials science. It's different—it's not in the same—I mean, it's very interesting, and it uses some tools from physics and for that matter chemistry as well, but it's not like you have to know that much to do what I'm doing. In fact knowing too much is a distinct disadvantage because it hinders one from doing new things because you'd always have an excuse as to why something wouldn't work; but of course if you don't know too much you don't think about something not working. That allows for a much more positive outlook. The other thing, of course, is that no matter how sophisticated some of the stuff that I and my colleagues are doing in the Laboratory of Chemical Physics, it's not like we're anywhere close to the physics greats. For example you don't need to be a like Feynman who had not only tremendous insight and clarity of thought, but tremendous depth of knowledge as well.. To be successful in biophysics, you need street smarts more than anything.

ZIERLER: Which is a term you used—street smarts—in the early 1980s. You said, "This is street smarts." Which means what?

CLORE: Street smarts means being able to see the forest from the trees easily. But the physics involved in biophysics is relatively simple, for example, compared to theoretical physics and astrophysics.

ZIERLER: The physics is relatively simple, so what's complex? The biology?

CLORE: Well, putting everything together might be complex, or knowing how to put it together might be complex. For example, NMR is intrinsically very complicated, but all that intrinsic detail was already figured out by 1960. Those guys who developed NMR in the first place—Purcell, Bloch, Rabi, all those people, were way smarter than any of us. But putting that information to practical use requires a different skill; less deep physical insight and more street smarts.

ZIERLER: I wonder if you can talk a little generally about your work process. Before you move on to another project, do you feel like you have to fully understand what you're currently working on? What's your feedback mechanism for defining success before moving on to something else? Or have you ever abandoned ship when you felt like you hit a dead end?

CLORE: Oh, I've abandoned ship when I've hit a dead end. That's for sure. I tell my postdocs this—one of the most important things to be able to do is to know when enough is enough. Because if you're hitting your head against a brick wall, maybe it's time to move on. And I've had that happen and then somebody else has come along and solved the problem two or three years later.

ZIERLER: What does it mean to hit your head against the wall? How do you know that you're doing that, that you're not just on the cusp of a discovery?

CLORE: Well, no, no. OK. So in the sort of biophysics that I'm doing—I'll give you an example. Let's say in the structural biology that I was doing when we were solving protein structures by NMR, one major bottleneck is very similar to that in crystallography. In

crystallography, you've got to get crystals. In NMR, you've got to get a sample that behaves well in the NMR tube. It can't aggregate. The lines should be reasonably sharp relative to its molecular weight. They shouldn't all be broadened out because there's all sorts of exchange processes going on and so on. So in the case of that RNAse H domain, for example, where we did what we could with the material at hand, I didn't realize at the time that there would be so much difference between HIV strains that one (the one used by Agouron) would give fantastic spectra and another (used by us) would give bad ones. We had some insights because thewild type RNase H spectrum was terrible, and I guessed that some of that had to with a tryptophan, where exchange between different conformations can result in a huge amount of line broadening as protons close to tryptophan are subject to massive ring current shifts. When wemutated that tryptophan to an alanine, the protein was still folded, and the spectrum was better but it still wasn't good enough to be able to do a full structure determination. Similarly, after we had determined the structure of interleukin-1, the next thing I wanted to do, which was the obvious thing, was to look at the interleukin-1 receptor, which is a soluble protein. And a postdoc worked on it for two years and got nowhere, and we said, "OK, let's move on to something else." After all postdocs have to get results and publications to be able to secure a faculty position. And then three or four years later, a crystallography group made some other constructs of the interleukin-1 receptor that happened to be that much better behaved, and they were able to crystallize it; and I have no doubt that those constructs would have looked good in the NMR tube, because it wasn't a size issue that was the question, but that everything was broad or aggregating and so on. So there's a limit to what one can do. And at that time, you had to make site-directed mutations oneself in the lab—now, you can just order the mutations, so it's really easy. You don't even have to do anything yourself. You just sit down there, design stuff, send it off, get the gene. So

that doesn't cost any of one's own time. When we were doing this in the early ‘90s, making mutants took a lot of effort, and there’s just so many you can make with a limited amount of resources. Manpower resources. I mean, if a drug company devotes 20 people to working on something and you've got one person, they're going to beat you to it, if they're just trying many different constructs and strains at random. And there’s a certain amount of luck—like with crystallization, you can use some intuition, but at the end of the day, it’s all luck, to get the sample. Once you've got a sample and it behaves well in the NMR tube, no matter how complicated the problem may be, if it gives a decent spectrum, you know you're going to be able to do something of one sort or another. So you have to get to a certain stage to know that you can actually do something. Then you know it might take you five years to do it, but you know that you can do it. For example, on the early oligomerization events involving huntingtin, the postdoc wanted to give up, but I knew it was all there. It was just a question of figuring out the correct model to fit the data and acquire the relevant data that could address the issue at hand. And yeah, it was complicated, but the data were good. So it was obvious this was a project that could be solved and was going to work. It’s just a question of being able to solve it. So that, you can do, because that’s a problem that requires brains as opposed to one that requires luck.

ZIERLER: So on that note, I wonder—a theme that has permeated your comments in our discussion is that so much of your motivation is the intellectual challenge, finding the solutions from an intellectual perspective, and you have kept sort of at arm’s length questions about the clinical value of your research. But clearly it’s there, but you've sort of like not dealt directly with that. So I wonder if I can sort of corner you a little bit to talk about, in broad terms, over your decades of working in this field, what has your work produced that has demonstrated clinical value?

CLORE: That's a challenging question, at least if one wants an honest answer rather than hype. I would say two things. One, even if had never done anything on any protein of any clinical interest, just developing the technology—and this comes back to the AIDS funding at the beginning— others can then use that technology to look at systems with direct clinical implications. So even if I'm not doing those clinical-related problems others can then go on and tackle them using the tools and methods and approaches that I've developed. So that's one thing. So in that broader sense, my work has had clinical impact, and that's the rationale behind supporting basic research. In terms of actually putting drugs into the clinic, that's a whole different ballgame. So the clinical implications might be a little bit overambitious, because how many people get to work on something that is actually going to result in a new drug. But in terms of knowledge, people have gained plenty of basic knowledge of biological problems using what we've developed. It has had broad lasting impact, irrespective of whether I've worked on some clinically-related problem or somebody else has done it. Of course, I want to look at interesting things that people are interested in. Work that is going to have high impact. And obviously I don't want to work on boring problems.. But I also want to look at things that are intellectually challenging from the point of view that very few people if any can tackle at the time. For example, let's say people might be looking at using our techniques to look at conformational exchange between two species. Well, that's a very easy problem to do. It doesn't require much intellect to be able to analyze the data and perhaps these people are making mutations here and there, and looking at their effects on exchange rates , and so on and so forth, but that's not intellectually challenging. You can do that sort of research as a black box. So I'm not interested in doing stuff that you can do as a black box. But other people can, and they can get very useful information out of it. So I like my research to be driven both by interesting problems but also by

doing stuff that's challenging, that requires some sort of new developments, and that's not easy or straightforward. But once that's done, then other people can use that methodology or approach, see how it's done and the different pieces put together to then apply it to biological problems that they're interested in. And what was challenging when I was first doing eventually becomes routine after the passage of time. So as an example, NMR structure determination of let's say proteins of around 200 residues, when I started, presented a massive challenge. Now, any postdoc without having any udnerstanding of NMR of simulated annealing calculations, etc. can do this—now, believe it or not there are plenty of postdocs around who don't know anything about NMR who are literally pressing the button. I mean, they've got no clue even how two-dimensional NMR data are even collected or what it involved. They can't even read and understand a simple pulse sequence. I had somebody the other day—she's a young and very smart tenure-track, and she was just using NMR on the side to verify some sructural predictions she had made, and she had absolutely no idea what a two-dimensional NMR experiment involved, or how it was collected. I was actually really shocked given that she was not only smart but had done physics as her major during undergrad. She was just pressing the buttons. Calibrate a pulse which is almost automatic, press go, put it into some program to Fourier transform the data, probably not even know how the Fourier transform is done, and out comes some spectrum that she's looking at. So now, 200 residue proteins, providing they behave well, anybody can do. The same is also true of crystallographers and that's why—the challenge in crystallography, for example, if you're a pure crystallographer, is not just to solve protein structures; it's to solve bigger and bigger things, whether it's the the ribosome or some other massive molecular machine. But just think about the ribosome. When the first three people solved the structure of the ribosome—that was a massive tour du force. In fact one of them, Ada

Yonath had worked on this problem for her entire career. And then the others got there in much shorter time because of the improvements in technology. But now look how many different ribosome structures have been solved with different drugs, etc.... Everybody can do it. It's not hard anymore, because all the hard work that went into solving the first structures has been done. So all the developments that are being done, other people can use them to advance their research which might be more targeted, let's say, towards clinical research or towards answering very specific biological problems that don't require, let's say, innovation from the point of view of the technology, but rather answering interesting questions. And let's face it, there are still many many interesting questions in biology.

ZIERLER: There's clearly a loss in the intellectual tradition where you have younger people who are just pressing a button. Is this concerning to you, that they don't have the background for the intellectual tradition that went into this advancement, or is that not a problem?

CLORE: Well, I'm going to answer your question in two ways. In some sense, it's not a problem. For example, you don't need to understand the physics of how gel electrophoresis works to be able to use it, and it's a completely routine tool in molecular biology or in any form of biology. You go to a biology talk; all they do is show gels. Totally routine. But you don't need to understand the physics behind it, and there is physics behind it. And the guys who first developed chromatography got a Nobel Prize for that, and look how that's so trivial these days. So there's that aspect of it. The other aspect is as far as NMR is concerned, it's a little disconcerting, because what it means is if they don't understand what's going on, they're not going to be able to push the frontiers of NMR. So the problem in NMR right now is that while there are plenty of labs who are doing NMR in a black box way, or even in a way where they sort of understand without pushing the frontiers - and the people coming out of those labs really don't

know much NMR so they can use it to solve interesting problems providing those problems fall within the range of what's already been developed; but there are very few labs few labs that are really pushing the frontiers of NMR. In fact, there are literally only a handful of labs in the world that are truly pushing the frontiers of NMR. So that means that advances are perhaps going to come a little bit slower, or maybe it means that NMR has reached maturity and it's not going to jump in leaps and bounds. For example, crystallography is not going to jump any further, if you discount Laue diffraction which is a second type of X-ray diffraction experiment and where you certainly do a lot of very new and cool stuff at picosecond time resolution, although nobody has really exploited Laue diffraction that much, yet. Conventional crystallography, on the other hand, is a pretty straightforward—it entails basically one experiment. In NMR, at least there are a hundred of different experiments. In conventional crystallography, there's only one experiment. There's nothing to it. So yes, it's challenging to go to bigger things for all sorts of other reasons, but the experiment itself has reached, in essence, the end of the road. That's why all the crystallographers are now going into cryoEM because they want to tackle bigger systems. But there again, cryoEM is almost becoming a black box—and once it becomes black boxn, you're not going to need the Richard Henderson's of this world to be able to tackle really interesting structural biological problems because it's all going to depend on instrument development, and most of the software is going to either be there or rely on a very few software developers in the field. Most people are going to be doing very interesting problems with cryoEM without really understanding it. So there are going to be very few people who are going to be able to say, push that field beyond what it is today. It's the same in NMR. There are very few people who are going to be pushing the field beyond what it is today, but there are going to be plenty of people who are going to be using it in a more and more and more routine sort of way. And the problems

that were really difficult, let's say ten years ago, are going to become easier and easier and easier.

ZIERLER: I think this will be my last question. You see yourself operating on the periphery of the clinical world, right? So obviously NIH is the best place for you to be. If it wasn't, you wouldn't be at the NIH. But I guess my question is because you're operating on the periphery of the clinical world, why are you not running a lab somewhere else? Why are you not at like a Harvard or a Stanford? My question really is, what is it about the NIH that has kept you here for all of these years?

CLORE: Well, everything has to do with serendipity. One of the issues in pursuing an academic career in science is lack of certainty. By that I mean the following by comparison with other professions. For example if you're a medical doctor, you can do good stuff and you can go wherever you want. You might not be a professor in an academic department, but you can still go into private practice and do extremely well. Same in law. You don't need to be at the biggest or more prestigious law firm. In science, you can't just decide where you'd like to end up; one can't just "Hey, I want to go to Harvard." You've got to be hired. It's like I tell my postdocs—where one ends up is very much a result of chance—my best postdoc ever ended up at UTMB. Another recent postdoc ended up at UC San Diego. Well, UC San Diego is better than UTMB even if that postdoc may not be quite as good as the postdoc who's at UTMB. So that's luck because it all depends where the openings happen to be, who has the encessary funds, what the different places are looking for at any point in time. But the other thing is this. So I've always worked at a research institution. So I was at the MRC, which is basically like the NIH. It's based largely on postdocs working under PI, and in fact when I came to the MRC at Mill Hill, it wasn't based on that as eEverybody was on their own basically, or in groups of two. I then went to the

Max Planck; it's a research institution but it's a little bit of a different construct because it's more hierarchical and the groups are bigger, which is why I didn't like it. I had a group of 20 people there; Ibut only spoke to two or three, because the rest were not very good, so I didn't bother. So I didn't find that a very productive environment. So the NIH was an ideal place to go. I jumped at it because first of all it was an environment that I was used to. I was used to working in small groups. I had always worked in small groups. So it wasn't a hindrance. Now, had I gone to Harvard, which I may have done, then I might have branched in quite different ways. Now, once you're at a place for a long time—one of the problems with NMR is it's not cheap. So if anybody wanted to hire me right now, they would have to fork out maybe $20 million for equipment. That's a lot of money in one go. If not more, right, because a 1.2-gigahertz instrument alone costs $15 million to start with. So why would I go if they didn't offer me more than what I've got now? But what I've got now I didn't get right away in one year. The facilities have been buolt up over 30 years. We started off with the 600. Then we wrote in and said, "Hey, we need a 500." We got a 500. Then we got another 600. Then we got a 700, 750, then an 800, then a 700, then a 900. So it goes on and on and on. But this has been built over 30 years. Well, it's still a lot of stuff, but the good thing about NMR is these magnets last forever. The consoles don't, so the electronics needs to updated as they improve and acquire new capabilities—so maybe a console a lifetime of let's say seven to ten years, although probably now the current NMR spectrometers are built in such a way and they're so modular that they can do basically anything that you can possibly dream of. I mean, any pulse sequence, anything you can think of, they can do now, as opposed to what it was in the past, where you could design pulse sequences that just couldn't be implemented on the existing spectrometers at the time. So now there may not be any need to improve the consoles other than replacing broken parts. So it's an expensive business which

makes it not so easy to move somewhere else. In addition, being at the NIH was great, because you have reliable funding. In fact we have excellent funding. And I've always generally gotten what I've asked for, within reason. On the other hand, if I wanted to branch out—and let's say I I decided, "OK, now I want to do single-molecule spectroscopy," it would be very difficult for me to do that at the NIH. And it would be very difficult to do for two reasons. First I would have to persuade the powers that be to give me the money. It's not an inconsiderable amount of money for the relevant equipment—not like NMR, but it's still expensive, particularly now in terms of constrained budgets. So first I'd have to justify the equipment which would be hard. And it's not like writing a grant. It's the scientific director who decides. So it's one person who decides. So it depends. They might say, "Hey, we can't afford it" and there would be little to no recourse. But the second important thing is at the NIH, we rely entirely on postdocs. Yes, there's a very small graduate program, but those graduate students are sort of very special and know exactly what they want to do. But at universities, if I were to decide to do something completely different, you've still got a whole pool of graduate students. They don't know anything to start with. They don't know whether I'm really well known in that particular area or not. Because most of them are very naïve at that stage. So you've got a pool of people that can come and work for you. At the NIH where you rely on postdocs, no postdoc who's any good is going to come and work for somebody, no matter how good they are, in a field that they're not known in. Why would any ambitious postdoc want do that except perhaps in exceptional circumstances? Why would ambitious postdoc planning a career in say single molecule fluorescence spectroscopy go and work with somebody who has done nothing yet in the field, when they could work with somebody very well known in that particular field. It wouldn't generally be a smart move. Unless that person was so good that they decided they want to work with somebody who has an interest

in this but who really isn't an expert in it as yet. And that of course can be a great opportunity for such a postdoc and they can say to themselves, "Aha, anything that I do in this, I'm going to get the credit for, because hey, nobody has heard of Marius in this field. He's an NMR guy." So then they can get the credit. But that's a very, very rare postdoc who does that most of the time. So there are pluses and minuses about being at a university or being at the NIH. At a university, if you write a grant and they say no, you can reapply. At the NIH if the scientific director says no, it's no. There's no other funder that you can go back to. So there are pluses and minuses about being there.

But here's the other thing. Not only does NIDDK pride itself on basic medical and biological research but I happen to be located in by far the most successful lab/department in the history of the NIH and that's an absolute privilege. Of the 8 PIs in the Lab of Chemical Physics currently, 5 are members of the National Academy, and that percentage is higher than the Chemistry Department at Harvard or for that matter anywhere else. So to have such great colleagues, and colleagues that one can easily interact with is really important, and really conducive to carrying out really original, ground breaking research.

ZIERLER: Dr. Clore, this has been a whirlwind of a discussion. I really appreciate your time. Thank you so much.

CLORE: Thank you. A pleasure and I really enjoyed this!

[End]