UNITED STATES PATENT AND TRADEMARK OFFICE

_____

BEFORE THE PATENT TRIAL AND APPEAL BOARD

_____

MERCK SHARP & DOHME LLC,
Petitioner

v.

HALOZYME INC.,
Patent Owner

_____

Case PGR2025-00003
U.S. Patent No. 11,952,600

_____

**Video Deposition of: James Naismith, Ph.D.**

Taken on: Friday, 6 February 2026

Held at: Randolph Hotel, Beaumont St, Oxford, UK

Time: 8.03 a.m. - 6.26 p.m.

Reporter: Amy Coley



Halozyme EX2301
Merck v. Halozyme
PGR2025-00024

**APPEARANCES**

**FOR HALOZYME:**

QUINN EMANUEL URQUHART & SULLIVAN LLP

By Ms. Lauren Martin
Mr. Eliot Choi
11 Huntington Avenue
Suite 520
Boston MA 02199
laurenmartin@quinnemanuel.com
eliotchoi@quinnemanuel.com

and

STERNE, KESSLER, GOLDSTEIN & FOX PLLC
By Ms. Hayley Ball
1101 K Street NW
10th Floor
Washington, DC 20005
hball@sternekessler.com

**FOR MERCK:**

SIDLEY AUSTIN LLP
By Mr. Jeffrey P. Kushan
Dr. Amy L. Mahan
1501 K Street NW
Washington, DC 20005
jkushan@sidley.com
amahan@sidley.com

APPEARING VIA ZOOM:

Mr. Josh Mack
Executive Director, Patent Litigation Counsel at Halozyme, Inc.

Ms. Eldora L. Ellison
eellison@sternekessler.com
STERNE, KESSLER, GOLDSTEIN & FOX PLLC

Mr. Eric Majchrzak
MERCK SHARP & DOHME LLC

THE VIDEOGRAPHER:

Ms. Wendy Viner

THE COURT REPORTER:

Ms. Amy Coley

## EXAMINATION INDEX


| Deposition of: | | PAGE |
|---|---|---|
| DR. JAMES HENDERSON NAISMITH | | |
| | By Ms. Martin | 6 |
| WITNESS'S SIGNATURE | | 319 |
| REPORTER'S CERTIFICATION | | 320 |


## MARKED EXHIBIT INDEX


| NUMBER | DESCRIPTION | PAGE |
|---|---|---|
| 1133 | 4 Declarations of Dr. Naismith | 26 |
| 2300 | Errata sheet for Exhibit 1133 | 28 |


## PREVIOUSLY MARKED EXHIBIT INDEX


| | | |
|---|---|---|
| 1026 | US Patent Appn. No.13,694 | 169 |
| 2068 | Declaration of Dr. Simpson | 256 |
| 1131 | Deposition transcript of Dr. Petsko | 257 |
| 1026 | US Patent Appn. No.13,694 | 267 |

THE VIDEOGRAPHER: Good morning. Here begins Media Number 1 in the deposition of Dr. James Naismith in the matter of Merck Sharp & Dohme LLC v Halozyme Inc in the United Sates Patent and Trademark Office, case number PGR2025-00003.

Today's date is February 6th 2026, and the time is 8.03 a.m. This deposition is taking place at the Randolph Hotel, Beaumont Street, Oxford, United Kingdom. The Videographer today is Wendy Viner.

Could I ask counsel to please identify themselves and state whom they represent, beginning with everybody in the room followed by our Zoom participants.

MS. MARTIN: Lauren Martin from Quinn Emanuel on behalf of Halozyme. With me I have Eliot Choi from Quinn Emanuel and Haley Ball from Sterne, Kessler.

MR. KUSHAN: Jeff Kushan from Sidley Austin for Merck with Amy Mahan.

THE VIDEOGRAPHER: Thank you. Would the Zoom participants please introduce themselves for the record.

NAISMITH - MARTIN

MR. MACK: This is Josh Mack for Halozyme.

MS. ELLISON: This is Eldora Ellison from Sterne, Kessler, Goldstein & Fox on behalf of Halozyme.

MR. MAJCHRZAK: Eric Majchrzak from Merck Sharp & Dohme LLC.

THE VIDEOGRAPHER: Thank you. Our Court Reporter today is Amy Coley. Could I ask the Court Report to please swear in the witness and we can proceed.

THE COURT REPORTER: Dr. Naismith, do you swear that the testimony you shall give shall be the truth, the whole truth and nothing but the truth?

DR. NAISMITH: Yes, I do.

THE COURT REPORTER: Thank you.

Q. (BY MS. MARTIN): Good morning Dr. Naismith or Professor Naismith. Am I saying that ----

A. Doctor is fine. It is the difference between the US and the UK. I'm fine with "Dr."

Q. Okay. And Naismith is the correct

pronunciation?

A. Yes, that's right, like the basketball guy.

Q. Got it. Can you please state your full name and address for the record?

A. Yes, my full name is James Henderson Naismith and I live in Oxford at 12 Mount Street OX2 6DH.

Q. Have you been deposed before?

A. Yes, I have.

Q. When were you deposed before?

A. I think 2017.

Q. And was that in connection with a case in the UK?

A. No.

Q. In connection with a case in the United States?

A. Yes.

Q. What type of case was that?

A. It was the *Sandoz v Amgen* case, which was to do with Enbrel.

Q. Okay, were you an expert witness?

A. Yes, I was.

Q. On behalf of which side?

NAISMITH - MARTIN

A. I was testifying on behalf of Amgen.

Q. Did you testify at trial in that case?

A. Yes, I did.

Q. Any other depositions?

A. Apart from connected with that case.

Q. Mmm-hmm.

A. No.

Q. Under the Patent Trial and Appeal Board Standing Order in this matter I am obligated to give you the following instruction. You must ask me, the deposing counsel, rather than your own counsel for any clarifications, definitions or explanations of any words, questions or documents presented during the cross-examination. Do you understand that instruction?

A. Yes, I do.

Q. Your attorney may object to my questions but unless he instructs you not to answer you should still answer my question. You understand that?

A. Yes, I understand that.

NAISMITH - MARTIN

Q. When were you contacted for this case?

A. Probably around August of last year.

Q. August 2025?

A. Yes.

Q. And by whom were you contacted?

A. Mr. Kushen.

Q. What did you do to prepare for this deposition?

A. Prepare for the deposition or prepare for the report?

Q. The deposition?

A. Just for the deposition: I familiarised myself, sat and read through my report a few times with the paper copy.

Q. Did you meet with counsel?

A. Yes, did.

Q. For how long?

A. Probably about five or six hours.

Q. Was that yesterday?

A. Yesterday and we caught up just in the evening of the day before.

Q. Okay. At any time in your work on

this matter have you -- strike that. Let me start over again. So before your work in this matter, had you heard of Dr. Hecht?

A. No, I had never met or heard of Dr. Hecht.

Q. What about Dr. Park?

A. I have never heard of or met Dr. Park.

Q. So as part of your work in this case, did you speak with Dr. Hecht?

A. No, I have never spoken with Dr. Hecht.

Q. And what about ----

A. I should correct that, I don't believe we've ever met or spoken to one another, certainly not in this case, but I have given lectures at Princeton so it is possible I've bumped into him.

Q. Okay.

A. I don't recall doing so.

Q. Okay, what about Dr. Park?

A. I'm fairly certain I've never met Dr. Park and I certainly didn't -- to be clear I didn't speak to him or meet him as part of this

case. It could be possibly when he was a student at some meeting I was at or something, you know? I can't exclude those but I've never knowingly met him or spoken to him.

Q. Okay. Did you ask to speak with Dr. Hecht as part of your work in this case?

A. No.

Q. What about Dr. Park?

A. No.

Q. So you read Dr. Hecht's expert declarations; correct?

A. I read his declaration, yes.

Q. His declaration?

A. I know they come in flavours but ----

Q. Yes, okay.

A. ---- yes, I've read them.

Q. You read one of them, you didn't read all of, you know, 15? Is that what you're saying?

A. I read -- I saw the differences between the other ones ----

Q. Okay.

A. ---- but I read his declarations.

NAISMITH - MARTIN

Q. Okay. And what about Dr. Park's declaration?

A. Yes, I've seen his declaration too, his analysis, yes.

Q. Mmm-hmm, okay. Did you see the spreadsheet that Dr. Park prepared?

A. Are we talking about the exhibit that he was offered? He offered an exhibit with a spreadsheet.

Q. Yes.

A. Is that one the one we are discussing?

Q. Yes, Dr. Park's spreadsheet. There are, like, multiple spreadsheets in this case. Is that what ----

A. Yes.

Q. ---- the one that Dr. Park prepared?

A. The one that he prepared and it's marked as an exhibit ----

Q. Yes.

A. ---- that's referenced in my contents; that's the one I've seen.

Q. Okay, got it. You understand that

NAISMITH - MARTIN

Dr. Hecht and Dr. Park were deposed in this matter?

A. Yes, I did.

Q. Have you read their deposition transcripts?

A. I have looked through them. They are rather long, but yes.

Q. When did you start -- strike that. So you submitted declarations in this proceeding; correct?

A. Yes, I did.

Q. Did you write those declarations?

A. The declarations are my opinion, my opinions. The final versions are with the assistance of counsel.

Q. And how long approximately did you spend writing those declarations?

A. Over 20 days.

Q. Did you yourself spend over 20 days preparing the declarations?

A. Yes, in formulating my opinion reading papers yes.

THE COURT REPORTER: I am sorry, "Before"?

NAISMITH - MARTIN

A. I don't think I said "before".

Q. (BY MS. MARTIN): Can you just restate your answer? I don't think that she heard it.

A. Could you give me the question, again?

Q. Did you yourself spend 20 days preparing your declarations?

A. Yes, including reading papers, formulating my opinions, it would be over 20 days of work.

Q. Okay. Do you know approximately how many hours you spent?

A. The university measures my time by eight hours, so eight times 20.

Q. Okay. Is there a cap on the amount of time you can consult as part of your work with the university?

A. I need permission to do any consultancy, which I obtained before I worked on this.

Q. Okay.

A. There is in the university regulations a 30-day cap that I have to get

additional permission if I go beyond 30 days in what the university judges as the academic year.

Q. Oh, okay. So have you had to get special approval to go beyond 30 days for this case?

A. I haven't gone beyond 30 days in this case.

Q. So how many days have you spent on this case?

A. Just over 20 days.

Q. Have you ever consulted with Merck besides your work in this case?

A. No, I've never consulted with Merck. I've given a lecture at one of their sites probably a couple of times over the years. Sometimes they gave me an honorarium, take you out for dinner, but I never consulted for them.

Q. Okay. Have they ever funded any research in your lab?

A. Not to my knowledge but companies in this field change quite often, so I can't swear that they haven't bought some company in years ago. The companies I've ever worked with are listed in my CV.

NAISMITH - MARTIN

Q. Have you ever heard of a company called Altergen?

A. Only in this connection, with this case.

Q. Okay, but not outside of this case?

A. No.

Q. Have you ever done any consulting work for Halozyme?

A. No, not that I'm aware of.

Q. Have you heard of Halozyme outside of this case?

A. No.

Q. What's the subject of your research?

A. So my research is broadly defined structure function relationships in proteins particularly interested in enzyme mechanism or the interaction between two proteins. I'm experimentalist so particularly around structural techniques, so protein crystallography; electromicroscopy; electron tomography; competitional approaches to try to understand mechanism; little bits of chemical synthesis around substrates, analogs; assay development and

attempts to commercialise some of those ideas. I range most into bacterial proteins but I've also worked in human proteins and actually from plants and such.

Q. Sorry, what was the last thing you said, "human proteins and actually from plints"?

A. Plants.

Q. Plant proteins?

A. Plant proteins.

Q. Okay.

A. They are a kind of class and unusually -- they have some unusual properties.

Q. Okay. Would you say that you spend most of your time working with bacterial enzymes?

A. I do not know "most" of my time. It's a significant portion of my work working on enzymes, bacterial enzymes. If I integrate over my career, they were much easier in the 1980s to work with, but increasingly we shifted to more human complex systems. So it's a balance over my career. More recently it would be more focused on human proteins.

Q. What types of human proteins do you work with?

NAISMITH - MARTIN

A. So we actually have been working on trying to determine the structure of nucleosomes by cryo-electron tomography. So nucleosomes are inside cells, so we've been quite interested in that. We've also been looking at immunity viral proteins and trying to understand how they form aggregates and spindles inside cells.

Q. What's an "immunity viral" protein?

A. So, it's kind of -- that's related to Cas-KRISPR. So bacteria themselves are immune to some viruses. Bacteria get virus, out of interest. It's one of these weird things, but they have an unusual defence mechanism and so we are quite interested in the proteins that are involved in that defence mechanism.

Q. And you said you are trying to understand how they form something and spindles inside cells?

A. Yes. So, they form these really long like helical filaments.

Q. Filaments? Oh, "filaments" is the word?

A. My apologies. As you hear, I have a Scottish accent.

NAISMITH - MARTIN

Q. No, I am trying to make sure that the record is clear and that I understand. Okay. So they form filaments inside cells. Is a filament -- would you call that like an aggregate?

A. Actually it's an order of aggregate but it is an aggregate, yes. It is ill-defined in its length we think. So this is very much our current experimentation.

Q. It is similar to a Fibrel?

A. Yes, there are similarities but also some differences that make it particularly interesting.

Q. Okay, you mentioned I think you said cryo-TM?

A. Yes, cryo-EM single particle cryo-EM and I also work on cryo-electron tomography.

Q. Okay, yes. So what is the difference between cryo-electron microscopy and cryo-electron tomography?

A. So in cryo-electron microscopy, we tend to work with a purified sample. It does not have to be, we've learned to be able to tolerate some impurities. That might be complex but it's

defined. And so you expose it to an electron beam and you average out the images, and from that you reconstitute the structure of the protein. So it is analogous, the end result is analogous to protein crystallography or AlphaFold.

In cryo-electron tomography, you are looking at a slab of a cell or a species live. So the goal that we have been engaged in is to be able to take human tissue and examine the structure of the proteins in the human tissue directly without any purification. So that's cryo-electron tomography.

The challenge is that electron beams can only go through about 200 nanometers of ice. So these are frozen samples but natively frozen. So you need to thin the sample. So you need some way to find the bit that what we call the ROI inside the human tissue and then you examine it. So this is very much a technological frontier of structural biology.

Q. Okay, sorry, there was a word that we didn't -- you want "to find the bit that is inside the human tissue to examine", we did not get the word?

A. Region of Interest, was it?

Q. Region of Interest.

A. "ROI", we always abbreviate it to.

Q. Okay.

A. But the Region of Interest.

Q. Thank you. So the enzymes that you work with would you say they are typically enzymes that cleave a single molecule and produce a single product?

A. No. We have worked on -- Typically it's hard to define how. We work on enzymes that break things, make things, combine multiple things involved in multiple steps of process. So, one of the things we work on is assembly of carbohydrate polymers in bacteria. So those are makers. They take various things, add them together and grow a large polymer.

Q. Have you ever done any work with hyaluronidases in your lab?

A. I have never worked with hyaluronidase.

Q. So you have never worked with PH20?

A. I have never worked with PH20 nor has my laboratory.

NAISMITH - MARTIN

Q. Apart from your work on this case, have you ever analyzed any structure of hyaluronidase?

A. Apart from my work with this case, I have never examined hyaluronidase.

Q. Have you ever performed an assay to measure hyaluronidase activity?

A. I have never performed an assay nor has my lab to analyse hyaluronidase activity. We have assay polysaccharide degradation but not hyaluronic acid.

Q. Also not hyaluronic acid and also not hyaluronic acid degradation by hyaluronidase?

A. That is correct.

Q. Have you ever performed a microtiter plate assay with a hyaluronidase?

A. I have performed microtiter plate assays but not with hyaluronidase.

Q. Have you ever performed a microtiter plate assay with biotinylated HA?

A. I perform, use biotinylated but not biotinylated HA.

Q. Okay. Have you ever been a referee on a paper for hyaluronidase?

NAISMITH - MARTIN

A. I honestly can't tell you. I have refereed thousands of papers in my career. I can't recall thousands of papers, I'm sorry. I just can't give you an honest answer. It's certainly possible but I can't remember. I've been a scientist for a long time.

Q. But none come to mind?

A. None come to mind but lots of things at my age, when I talk about, when I look back over thousands papers, it won't come to mind.

Q. Okay.

A. I've probably refereed several hundreds of papers, maybe a hundred papers a year. I have been an editor of journals. I honestly can't tell you.

Q. Outside of your work in this case, are you familiar with Dr. Greg Petsko's work?

A. Yes, I am familiar with Dr. Petsko. I've met Dr. Petsko on several occasions and I know some of his work. He publishes a lot of work, but I know some of his work, yes.

Q. You said that you met him before?

A. Yes, I have.

Q. In what context?

NAISMITH - MARTIN

A. So I've bumped into him at conferences. He came to give a lecture, a series of prestigious lecture series in St. Andrews and then Dundee which is where I used to work. So he was, kind of, travelling the country giving lectures and I met him as part of the faculty day when we talked to the invited speaker. So I met him there and I've met him at conferences.

Q. Are you familiar with his research?

A. I'm familiar with some of his research. I don't have it -- yes.

Q. Sorry, you said you are familiar with some of his research and then what was the second thing you said?

A. That is what I said I think. Some of his research.

Q. Okay.

A. I said I wasn't -- I couldn't answer questions on the entire breath of his research, sorry, yes.

Q. Okay. Would you consider Dr. Petsko to be a well-respected scientist?

A. Yes, I would.

Q. Would you consider him to be well

known in the field?

A. What field?

Q. Structural biology?

A. Yes, I would.

Q. Have you heard of Dr. Melanie Simpson outside your work on this case?

A. Outside the work in my case, no, I have not.

Q. You mentioned in your report that you were familiar with Dr. Petsko's textbook?

A. Yes, I may -- That is correct, I've read his textbook or looked through it.

Q. Have you used his textbook in your courses?

A. I think it would be in the library but I didn't teach from his textbook.

Q. Okay, but you have seen it in the library?

A. I think it is in the St. Andrews library. It is certainly in the Oxford library.

Q. Okay. Are you aware that Dr. Simpson is the President of the International Society for Hyaluronan Sciences?

A. I think in her expert report - and

I would like to go to it - I think she mentions it in there. She certainly mentions some of the things she's involved in in her expert report.

Q. I am going to mark the first exhibit, which is your Declaration of James Naismith PhD in PGR matter 2025-03 and then I'm also going to mark Declaration of Dr. Naismith from matter PGR 2025 matter 4, declaration of James Naismith in PGR matter 2025 matter 6, and declaration of James Naismith in matter PGR 2025 matter 9.

A. Ms. Martin?

Q. Mmm-hmm?

A. I have a correction to give you for my report.

Q. Okay, we will get there. I'm going to give you a stack of them. To be clear: matter 3; matter 4; matter 6 and matter 9.

MR. KUSHAN: These are all 1133?

MS. MARTIN: You know, it's not on here, but I believe that that is right.

**(Exhibit 1133 marked for identification)**

MS. MARTIN: These will be exhibit number -- you said it was what, sorry?

NAISMITH - MARTIN

MR. KUSHAN: 1133.

MS. MARTIN: Thank you.

Okay, so Dr. Naismith, can you just confirm that those are the four declarations that you submitted?

A. *(Pause)* Yes, these are the four.

Q. Okay. And you said that you had a correction?

A. I do. I brought a sheet with a correction for you, I brought copies.

Q. Okay.

A. I brought five copies.

Q. Let's mark this as an exhibit, right. Did you ----

MR. KUSHAN: I've not got one.

A. Sorry. I printed them out this morning.

MS. MARTIN: Oh, you have not seen one?

MR. KUSHAN: Can I just have one?

MS. MARTIN: We're going to mark this as an exhibit. I don't have a number, so I don't know. Let's just say Exhibit 1 and then we'll fix it later. Let's just say Exhibit 1 and

we'll get the number later.

MR. KUSHAN: It's Exhibit 2446.

MS. MARTIN: I feel like fixing it later will be better than getting it wrong now.

**(Exhibit 2300 marked for identification)**

Q. (BY MS. MARTIN): So Dr. Naismith you prepared this errata?

A. Yes, I did.

Q. And so can you just explain what it is that you want to change?

A. I want to correct a typo error that I discovered when I was reading through it. There is a stray sentence "and 17 total changes" that doesn't belong there, my apologies.

Q. And so this is in paragraph 317 in all four of your declarations?

A. It is.

Q. And there is a stray "and 17 total changes" in the sentence that starts with, "Just exploring"; is that right?

A. That's correct and I've underlined the bit that should have been removed. My apologies.

Q. Okay. Understood. All right. Is

that the only change in you wanted to make?

A. It's the only error I discovered.

Q. All right. Are you aware that the Patent Trial and Appeal Board instituted trial in these matters?

A. I know there's some sort of Patent Board process and there's a hearing I don't know if it's called that.

Q. Okay.

A. I assume that's correct but I don't always know the legal term.

Q. Have you read the Institution decision?

A. No, I don't think so.

Q. And you listed the materials that you considered in forming your opinions in this case, in your Exhibit List; is that right?

A. Yes.

Q. And so I believe that that is Appendix A which -- We are just for the ease of everyone's ease here we will just use the Matter 3 declaration for purposes of this of our discussion.

A. Is that the one here which is

NAISMITH - MARTIN

called 600?

Q. Yes, 600 BEN?

MR. KUSHAN: Let me just double-check, sorry. Yes.

Q. (BY MS. MARTIN): So your Exhibit List is Appendix A which is on page 269?

A. I have brought ringbound, if that would make it easier for all of us.

Q. Yes, that would make it easier for all of us. Except, is that marked at all?

A. It's pristine and looks exactly -- but this is just ringbound.

Q. Okay, perfect.

A. I have not touched these ones apart from authoring them.

Q. Understood.

A. Can I just shove *these* out my road?

Q. Yes.

A. Is that okay?

Q. Yes.

A. I've got three just so we are all on the same ----

Q. Got it thank you?

A. Okay.

NAISMITH - MARTIN

Q. Okay, and so your Exhibit List is on page 269 hopefully?

A. Yes, it is.

Q. All right. So your Exhibit List, that contains a list of all of the exhibits that you have relied on in forming your opinions in this declaration?

A. Yes, that and my knowledge of the field which encompasses many other papers but these are the ones I specifically cite and rely on.

Q. Okay, and you signed your declaration, your signature is on page 268; right?

A. That's correct.

Q. That is your signature?

A. Yes, it is.

Q. And you executed this particular declaration on January 20th 2026?

A. Yes, I did.

Q. So earlier in your testimony we talked a little bit about cryo-electron microscopy; is that right?

A. We did.

Q. What would you consider to be good

resolution for cryo-EM structure for a protein?

A. Oh, cryo-EM? These days you can get below 2 angstrom resolution.

Q. Would you consider below angstrom 2 resolution to be a good resolution for cryo-EM?

A. Cryo-EM below 2 angstrom resolution is really quite good.

Q. Okay. What about 3 angstrom: is that good resolution for cryo-EM?

A. It depends on the region. So cryo-EM doesn't have the -- So "resolution" is a term that comes from protein crystallography where it is mathematically defined. Cryo-EM uses what's called an FSC curve so that is more of an average. So parts of the structure are well ordered, other parts might be less well defined.

Q. When you saw a cryo-EM structure you are taking an average of the -- You are generating a model based on the average of the images that you have collected with the microscope?

A. That is a reasonable summary of how you work single particle averaging, yes.

Q. Okay. Then in order to identify

positioning for the atoms in the protein do you use a template for cell structure?

A. Not always. You can *de novo* trace a cryo-EM structure but traditionally -- increasingly now we use AlfaFold 2 system because that is quite a good way to proceed now.

Q. So you use AlfaFold to predict the structure for the protein and then you use that model to -- You put the model into your cryo-EM. Would you call it electron density?

A. It's not quite electron density but it looks the same. It's a map. It's actually called a Coulombic Density map. But today that would be the more normal way to proceed. If there was an AlfaFold 2 model that might be the place you might start, but you can do *de novo* trace in the way we used to trace from electron density and protein crystallography maps.

Q. Okay.

A. It's much more difficult.

Q. And so you solve cryo-EM structures in your laboratory?

A. Yes, I have.

Q. If you have a two angstrom cryo-EM

structure what is the margin of error in terms of atom positioning?

A. That's interesting because it depends on the region as I said. So it's not as clear cut as it is in crystallography. There are Cruikshank errors which you can use in crystallography, but for EM it varies across the structure.

Q. So for a crystal structure you would have a B factor; is that right?

A. That's correct.

Q. Do you have a B factor for cryo-EM structure?

A. There is some sort of fudge factor that is used but it is not strictly a B factor. It's a different thing because cryo structural, cryo-EM, a single particle, is a real space technique. Protein crystallography is reciprocal space.

Q. Okay. So is there a statistic, if you will, that gives you a measure of the error at every position in cryo-EM?

A. You get an estimate of positions and, of course, it is coupled to the region

structure in the amino acids around it.

Q. What is that called?

A. I can't remember off the top of my head, it's like a precision index or whatever.

Q. Okay. So if I download a cryo-EM PDV from the PDV website is there a column in the PDV file that would give me that index?

A. Uncertainty.

Q. I am sorry?

A. Give you that uncertainties?

Q. Yes.

A. Not really. You can get there by looking at the structure in the map but usually the paper will call out if it thinks it's important, where the things are uncertain.

Q. Okay. Have you solved X-Ray crystal structures in your lab?

A. Yes, I have.

Q. What would you consider to be a good resolution for a protein crystal structure?

A. It depends on the purpose I wanted it for.

Q. Can you explain that?

A. Yes, so if I wanted to understand

how two proteins interact for example, a resolution of 3 and a half would be perfectly fine. If I want to understand, as I have done in the past, precise aspects of what's at the catalytic core, catalysis movement around that site, 1, you know, somewhere around 1, 1.2 angstrom resolution. If I wanted to have a broad understanding of the chemical mechanism, anywhere between 2 and 3 but it's a sliding scale. So the closer you get to 2, the more information you have.

Q. So let's say you have a 2 angstrom crystal structure for a protein, what's the margin of error in terms of atom positioning?

A. So typically around point 2 angstrom for any one atom at 2 angstrom resolution is a reasonable guide.

Q. Okay.

A. But the atoms are coupled. It is not simple, it's just they can all move that way.

Q. So what if you had a 3 and a half angstrom structure, what's the margin of error?

A. Again, there is an element of variation, because as you talked about the B

factor which you have for a crystal structure, the error can be over by an angstrom or more.

Q. Okay. And if you're determining interactions between atoms and a crystal structure, do you use a distance cut off to determine if there are potential interactions between atoms?

A. It depends on the interactions you are using. So there are different types of interactions. They have different thresholds.

Q. So like a hydrogen bond for example?

A. So a hydrogen bonds tends to be defined at up to 3.5 angstrom, sometimes defined at 3.2. It depends on the literature but 3.5 is a reasonable upper limit.

Q. And what about a Van der Waals?

A. Typically people put that cut off at 4.

Q. Okay. So if you have a point 2 angstrom error rate in your 2 angstrom crystal structure -- I'm sorry I'm sure that I just got that all garbled, so let me try again. If the atom positioning in your 2 angstrom crystal

structure has a plus or minus point 2 angstrom error in it, can that error impact your ability to see interactions between the atoms?

A. You are aware of it in your analysis, but it's not that -- because its between two atoms those are coupled so it's not just point 2 plus point 2. You have to -- it's a complicated sum of squares. I'd have to go back and refresh my memory. But the distance is not just -- certainly the distance is not simply point two plus point 2, it is less than that.

Q. Okay. It is possible that the error in the inherent or, like, the margin of error in atom positioning could impact your ability to determine if there's an interaction?

A. So, at the absolute, if you impose an absolute rigid cut-off for hydrogen bonds, then if you were at 3.55 you might be more careful in your description to say it is at 3.55 in my model which there is an element of uncertainty or 3.44 for example. So there, yes, but hydrogen bonds have an additional feature which is helpful which is there is a directionality about them. So there is also an angular component to how hydrogen bonds

interact. So there are other clues but you are correct that if I was imposing a cut-off and I was at the margin I would certainly comment on that.

Q. Okay. You mentioned AlphaFold 2.0?

A. Yes, I did.

Q. What about AlphaFold 3.0?

A. That is -- I think I can't remember if that's just come out. I know I've seen it, but I can't remember. It will be at 4.0 quite soon no doubt.

Q. Okay.

A. But AlphaFold 2.0 particularly because it is a seminal kind of moment in protein science.

Q. And when did AlphaFold 2.0 come out approximately?

A. 2021. I think Dr. Hecht has it in his report. I can go there and give you a precise date.

Q. Okay.

A. We were aware of it before it appeared in the literature.

Q. Have you used AlphaFold 2.0?

A. Yes, I have.

NAISMITH - MARTIN

Q. In your experience is AlphaFold 2.0 capable of generating a model that's comparable to what you would get experimental results?

A. It depends on the resolution, but it certainly is capable of producing models of a quality from crystallography around 3, 2.5-3. I would say and I haven't tested it rigorously since I looked at 2, but it's pretty good.

Q. Okay. When you say "it depends on the resolution" am I understanding correctly that AlphaFold 2.0 may not be able to give you 1 angstrom accurate model but it can be give you a 3.2-3.5 angstrom accurate model?

A. Again overall, but where in the core structure it will be as accurate as a 1.0 model. You know it's very accurate in core structures but as you get to surface where things are less well conserved, because that's kind of how it works. You might see more uncertainty in those regions. But it is hard to know. My experience of AlphaFold is that it is pretty good, you can -- there are some differences with experimental structures in loops particularly.

Q. Okay, and when you say "core

structure" what is a "core structure"?

A. The part in the middle.

Q. Okay. The part in the middle of the protein?

A. The hydrophobic core.

Q. Okay.

A. Yes, sorry, I should have defined that, my apologies. When I think about the core of structure, the sort of heart in the middle not the surface.

Q. Okay. And you said it has a harder time with loops. Is that because it is harder to predict the structure on a disordered loop?

A. No, I didn't say it had "a harder time", I said it would be less confident about its resolution in my experience, which is limited. I have not systematically tested AlphaFold 2.

Q. Okay. But is AlphaFold 2 then, does it have a -- Sorry, strike that, I'll be more precise here. Is AlphaFold 2 more confident in resolution with respect to alpha helices and beta sheets?

A. Yes. It's pretty good at identifying alpha helices and beta sheets;

NAISMITH - MARTIN

absolutely. It's very good at that.

Q. Have you used SWISS-MODEL?

A. Years ago, yes.

Q. Did you -- so let's put out a timeframe on this. In 2011/2012 timeframe were you using the SWISS-MODEL?

A. I had been using it since it came online, I think, which is the 90s, so I have used it you think until really AlphaFold on and off.

Q. Okay.

A. Most of my lab was *de novo* structure determination.

Q. What do you mean by "*de novo* structure determination"?

A. Where we start from a structural analysis based on an experimental structure.

Q. Okay, so you are trying to solve the structure of a protein?

A. That's, kind of, what I built my career on.

Q. Okay. All right, I am just trying differentiate between, like, *de novo* protein design and *de novo* structure determination?

A. Sorry, I mean from ----

NAISMITH - MARTIN

THE COURT REPORTER: I am sorry, could you repeat your question, please?

MS. MARTIN: Sorry, I will go slower.

THE COURT REPORTER: You will have to go slower.

Q. (BY MS. MARTIN): I am trying to differentiate between *de novo* protein design and *de novo* structure determination.

A. Yes, my apologies. When I say "*de novo* structure determination", I mean experimental structure determination, and mostly in my lab we were interested in the first structure of a representative of a particular structure.

Q. So in what context would you use SWISS-MODEL?

A. Usually if -- So most commonly in my lab if we were in -- if we were able to solve a structure from one organism and my collaborator was interested if there was a particular single site mutation in another, we might look at it in that way, although in fact we tended to mostly just use our own software which is a programme called Coot, where you can model in single amino

acid changes. But it wasn't in common use in my lab. Collaborators often used it more until we would provide an experimental structure.

Q. Do you know -- you said that collaborators would use SWISS-MODEL until you were provided experimental structure. Is that what you said?

A. Yes.

Q. Do you know why your collaborators were using the SWISS-MODEL, like, for what purpose?

A. So, some information is better than no information. So people maybe have been working on a problem for many years, so the only information they can get might be SWISS-MODEL though when we would join the collaboration we would come and give them a more accurate structural model that was much more predictive in understanding function.

Q. How would you generate that structural model?

A. From X-ray crystallography or single particle EM.

Q. Okay, so you would give them an

experimentally determined structure as opposed to the computer model?

A. Yes, and we would then work together to try to understand the problem which we were both interested in.

Q. And you mentioned -- you mentioned, I thought, using SWISS-MODEL to model single amino acid changes but you would also would use Coot?

A. Yes.

Q. So am I understanding correctly that if you wanted to model what it looks like to change a single amino acid, you could generate a model using SWISS-MODEL or you could go into Coot and just change the amino acid?

A. And then look at it yourself, yes.

Q. Did you ever use Modeller?

A. Not that I recall although I know it was part of a software package. I'm aware of Modeller but I don't think I've used it.

Q. Are you familiar with the phrase homology modeling?

A. Yes, I'm familiar with that phrase.

Q. What is homology modeling?

A. So, where it's actually, it's what

Dr. Park did. You have a structure of, in his case, HYAL1. You have a Swiss-Prot to then make a structure of PH20 and it uses the homology between those two to then make a model of the PH20.

Q. In the 2011/2012 timeframe, would you consider SWISS-MODEL to be a reasonably accurate tool to use to generate a homology model?

A. It depends on the extent of homology.

Q. In the case of PH20?

A. It's better than nothing; it's reasonable.

Q. Okay. So do you think that Dr. Park's homology model for PH20 is reasonably accurate?

A. Depending on the purpose, you put it to. The purpose he put it to was to look for single amino acid changes and then assign them to buckets. It's accurate enough for that.

Q. So it's accurate enough for the purpose for which Dr. Park used it?

A. Yes, and he was careful to warn that some regions were less well defined, I think the CUE mean score, where he said, you know,

NAISMITH - MARTIN

"Well, it's a bit -- the model's not defined here and the model at page 20 doesn't go all the way to the end of the sequence 447".

Q. But the portions for which he used the model to make his single amino acid predictions do you think that it was reasonably accurate?

A. It was reasonably accurate to assign to it the buckets he did which was 1, 2 or 3 and he was careful not to say that he could infer precise activity or action at a distance or mutate effects elsewhere in the structure; he just asked a single question which was, "How is the local environment around this residue if I change it, if I mutate it, one residue at a time, single mutation?"

Q. But then he was actually drawing a conclusion based on his local environment analysis as to whether or not he would predict the protein as a whole to retain enzymatic activity; right?

A. So he said he had signed it in bucket 1 which he said was destabilizing. So bucket 1 he said, "If it's this particular single site mutation in bucket 1 in these non-conserved

residues, it is likely to be destabilizing and therefore I think it could adversely be deleterious to the protein." When he assigned it to bucket 2 he said, "Looking at the local environment this single amino acid change probably won't have an impact. It will be neutral" I think his word was. And then in bucket 3 he said, "Looking at that single site change in its local environment I think that will be stabilizing."

So his expectation was that buckets 2 and 3 would be active, but he didn't draw a conclusion that bucket 3 would be more or less active than bucket 2 or any level of activity within the buckets, just that it would be active.

Q. Okay. So he did in fact draw a conclusion that for his bucket 2 and bucket 3 that he would expect the protein to be active?

A. That was his prediction, yes, that that single site mutation at this position, where subject to his qualifications, yes, he would expect those to be likely to be active.

Q. Okay, can you turn in your declaration to paragraph 133.

A. Yes.

NAISMITH - MARTIN

Q. And I believe that you mentioned earlier you were looking for a date from Dr. Hecht's declaration regarding AlphaFold 2. Is that date 2019?

A. Yes, it would be 2019, 2020, when the publication started to appear.

Q. Okay. And so you say in paragraph 133, that the computational tools and in particular AlphaFold 2, "... did not reach predictive accuracy levels comparable to experimental determination of structure until around 2019 ...". Is that right?

A. Yes.

Q. Okay. So as of around 2019 AlphaFold 2 was generating predictive accuracy levels comparable to experimental?

A. I qualify that by the resolution, but, yes, for the overall fold of a protein by 2019, AlphaFold 2 was good.

Q. Okay. And there is a figure at the top of page 95, do you see that?

A. I do.

Q. Can you explain the figure?

A. So what that says is the mean C

alpha root mean square deviation. So what it shows is that if you look in the -- CASP is a competition. So people would submit sequences which were not known in the art. So there was no structure known for that sequence and a bunch of people would go along and try to predict using different software structure and then you would compare how well they did. And so this is, kind of, a measure of that. It is a root mean square deviation. What it shows is that AlphaFold boom certainly was more better than any of the competing programmes and you see here the other programmes similar sort of error ranges.

Q. You are pointing -- You said "similar sort of error ranges". What are you pointing to?

A. Oh, for the other programmes, the RMSDs and other approaches.

Q. And you are pointing to the figure, like the graph on the right of the top of page 95 in the error bars?

A. Yes.

Q. Okay and in that the X-axis has a bunch of like G numbers, and then AlphaFold. What

are the G numbers?

A. So those are the -- I believe those are -- I should actually like to probably go back to the original paper and check, but I think those are the different groups that were competing.

Q. Oh okay. So the other groups were competing with AlphaFold and you can see the median root mean square deviation for AlphaFold is around 1 whereas for the other groups that's like 3-4; is that right?

A. Yes.

Q. Okay, okay, got it. Okay, and in the graph on the left it says: "A score above 90 is considered roughly equivalent to an experimentally determined structure". And in 2020 the AlphaFold 2 score is approaching 90; is that right?

A. Yes.

Q. Did you use AlphaFold 2 to generate a model of PH20 in connection with your work in this case?

A. No, I did not.

Q. Did you use any software package to generate a model of PH20?

NAISMITH - MARTIN

A. I used the model that was provided to me and my counsel. It came from Dr. Park and Dr. Petsko. I think I used their PH20 model.

Q. Did you use Dr. Park's model or Dr. Petsko's model?

A. I would have to check. I think I used Dr. Petsko's PH20 model.

Q. Were you provided with Dr. Park's model?

A. That I can't recall. I was given one PDB coordinate set and I believe it is Dr. Petsko's, but I think I also had another PDB set which was Dr. Park's and they looked superimposable, but I did my analysis using the PH20 from Dr. Petsko's.

Sorry, do you want me to go again?

THE COURT REPORTER: I was right there but then I lost you. "... but I think I also had another PDB set which was Dr. Park's ----"

THE WITNESS: I think but I used all my analysis on Dr. Petsko's PH20.

Q. (BY MS. MARTIN): And I believe you said that you compared them and they were

superimposable?

A. They look superimposable to me but my analysis proceeded with Dr. Petsko's.

Q. Okay.

A. I think they both generated them the same way from Swiss-Prot so you would expect them to be identical.

Q. Okay.

A. So I didn't really probe whether there was any difference, but I used Dr. Petsko's one.

Q. Okay. Did you attempt to generate a model of PH20 with any mutations using AlphaFold 2?

A. No, I did not.

Q. You could have; right?

A. It wasn't relevant to the test I was given which was 2011/2012, this lens, so I did not do that.

Q. Okay. All right. We have been going for almost an hour, do you want to take a break?

A. If that's good with you, it's fine with me.

NAISMITH - MARTIN

MS. MARTIN: Okay, we will take a quick break.

THE VIDEOGRAPHER: We are going off the record. The time is 9.02 a.m.

**(A short recess from 9.02 a.m. to 9.11 a.m.)**

THE VIDEOGRAPHER: We are back on the record. The time is 9.11 a.m.

(BY MS. MARTIN): Welcome back, Dr. Naismith. Do you agree with the statement that: "Common structural features either side of those physical attribute that are common to the members of the genus of properly folded active PH20s?"

A. I think that's taken from my report. Could I just turn that page? I think there's ----

Q. I don't know if it's in your report or not, I'm reading from the submission, from Mark's submission.

A. Okay, so I was taught legal tests, so I want to get them in front of me and then I'll answer your question, if that's okay?

Q. Oh okay. Well actually let me -- I can get rid of the legal part of it. I am just

wondering if you agree that common structural features are a set of physical attributes?

A. So, "Common structural features are a set of ...". Yes, that's seems a reasonable statement to me, although I was told to be -- I was taught some legal principles too.

Q. I understand. I'm going to try to get rid of the legal principles because I am trying to understand the relationship between the structural features and the physical attributes; all right?

A. I understand.

Q. I want to focus on that, not the legal part?

A. Okay.

Q. Do you agree that structural features are physical attributes?

MR. KUSHAN: Objection foundation.

A. So a structure is a physical thing, and so, it is a physical thing that describes a protein three-dimensional folded structure or it could be describe a small molecule. So it is a three-dimensional visualization of a substance.

Q. (BY MS. MARTIN): Okay, let's go to

paragraph 85 of your declaration. In paragraph 85, you say: "A POSA would consider 'structural features' of a protein to be the physical characteristics of the protein molecule"; right?

A. Yes, that's what it says, "features", yes.

Q. Okay. So what do you mean by "physical characteristics" in that statement?

A. So the reality of what the molecule is, that's the physical reality characteristics. So "structural features" are a way of describing the physical -- some of the physical characteristics. It's this size, it's this amino acids arrangement in three-dimensional space.

I give an example in here if there is: "... a particular amino acid located at a particular ... sequence ..." then that would be a specific feature of the protein. I distinguish between folded proteins and non-folded proteins.

Q. When you say in the second sentence in paragraph 85, that: "... a particular amino acid located at a particular position in the amino acid sequence ... is a structural feature", are you referring to the three-dimensional positioning

of the atoms in the amino acid?

A. Not in this part because I actually differentiate between structure of a folded protein specifically. But here if you say, "I have an amino acid at position 320", which is this, then that is a physical characteristic of the protein, it is a primary structure definition.

Q. Okay, so do you agree that having a particular amino acid at, for example, position 320 is a common structural feature?

MR. KUSHAN: Objection form.

A. I say it's a physical characteristic and I differentiate between structure from the three-dimensional form of the protein as opposed to the primary structure which is the composition. But both are physical characteristics.

Q. (BY MS. MARTIN): So you agree that the amino acid sequence of a protein is a physical characteristic of the protein?

A. The amino acid sequence is a physical characteristic of a protein but it is different than the structure of a folded protein which is another physical feature, physical

characteristic.

Q. And the position of a particular amino acid in the sequence, for example, at position 320, that is a physical characteristic of the protein?

A. If -- Yes, if you have a specific residue, you have called out a specific amino acid at this position, then that is a physical characteristic of the protein.

Q. So, for example, let's say you have D320 K PH20, that means that the PH20 sequence has a K at position 320; right?

A. So when you talk about PH20, we are talking about a three-dimensional structure properly folded structure of the protein. There will be -- then from -- So that is when we use a the word "PH20" but you're correct that at position 320 you've defined a physical characteristic which is you got a lysine at position 320.

Q. Okay, so I am going to try to -- I am going to try to cut through some of this here. So SEQ.ID NO:3, if I talk about SEQ.ID NO:3, that is an amino acid sequence that has

amino acids from 1-447; right?

A. That's correct. It's a linear sequence, the primary structure.

Q. Okay. So I understand the issue with the PH20 and so again just trying to cut through that, so I'm going to try, this a different way and talk about SEQ.ID NO:3, so we're on the same page?

A. Understand.

Q. So the SEQ.ID NO:3 primary amino acid sequence let'S say it is D320 K, meaning that there is a lysine at position 320; right?

A. That is what D320 K; yes.

Q. Okay. So in that amino acid sequence, which is D320 K SEQ.ID NO:3, would you agree that having a lysine at position 320 is a physical characteristic of that amino acid sequence?

A. Yes, I would agree that D320 K is a physical because you defined it as definitely a physical characteristic of that sequence.

Q. Okay. In your view is having a lysine at position 320 in that SEQ.ID NO:3 amino acid sequence, is that a structural feature of

that amino acid sequence?

A. It is a primary structural feature. I'm going to distinguish between primary structure and structure of a folding protein. But, yes, it is a primary structure feature.

Q. Okay. So you said that PH20 is a folded protein; right?

A. As it's used in the common disclosure, it is a folded active protein.

Q. Okay. So outside of the context of the common disclosure, when you hear the word "PH20", do you think of a folded protein?

A. When I hear the word -- Yes, because I know it's in the surface of sperm cells it's a neutral active hyaluronidase. So I would assume it's the full length protein on the surface of the sperm cell. Outside the common disclosure it's defined slightly differently than common disclosure because we're got Sequence ID NO:3 here. But, yes.

Q. Okay. Had you heard of PH20 before your work on this case?

A. No.

Q. If I say "SEQ.ID NO:3", do you

think of a fully folded protein or do you think of a linear amino acid sequence?

A. So when you tell me SEQ.ID NO:3 I see that as a definition of a primary sequence.

Q. Okay. If you took PH20 and put it in a solution of saturating urea would you still think of it as a folded 3D protein?

MR. KUSHAN: Objection foundation.

A. So I haven't done that test but I would assume, based on what a POSA would expect, that PH20 and saturate urea would be an unfolded potentially aggregated protein. But I don't know that because I don't recall any data for PH20.

Q. (BY MS. MARTIN): But that's what POSA would expect?

A. That would be what a POSA would expect. An 8 Molar urea is what I'm guessing you mean by "saturated", 8M.

Q. So in your solution of unfolded potentially aggregated protein, is it still a PH20?

MR. KUSHAN: Objection foundation.

A. It still has the sequence of PH20 but it's no longer a folded active protein, which

I think we both agreed was how I defined and we define PH20.

Q. (BY MS. MARTIN): So if you take the amino acid sequence of PH20 and put it in a solution of saturating urea such that it unfolds, is it no longer PH20?

A. It is a sequence of PH20 but it would be -- unfolded PH20 would distinguish between that and normal PH20.

Q. So what would you call it if it's not PH20?

A. Inactive unfolded PH20, I would prefix it.

Q. Okay, so if you took -- Strike that. So let's say you have a D320 K SEQ.ID NO:3 PH20. If I use that phrase, do you think that that is a -- Do you consider that to be a fully folded protein?

MR. KUSHAN: Objection foundation.

A. So where it's used in the common disclosure then that is what I -- because that is what the claims speak to. Yes, I would consider that properly folded as does Dr. Petsko.

Q. (BY MS. MARTIN): Okay so SEQ.ID

NAISMITH - MARTIN

NO:3, 1-447 with a lysine at position 320 you consider that to be a folded PH20 protein?

MR. KUSHAN: Objection foundation.

A. What I said was as it appears in the declaration, I consider that to be -- in the common disclosure, I consider that to be an active protein. Now, you have discussed putting things into urea so I would be, given that you've already told me that, I would be more careful in answering your question. As a scientist I'd want to know, I'd ask you, "Is it still in urea?"

Q. (BY MS. MARTIN): Mmm-hmm, that was going to be my next question. So, yes. Without specifying whether or not it's -- Without specifying the conditions for your SEQ.ID NO:3, D320 K PH20, would you expect to it be folded?

A. So, and I mean no disrespect, if it was another scientist we are talking at PH20, that would be my assumption. You've asked -- you've talked about urea. So in your case specifically I would want to ask because you put into the notion that you might unfold it. But as it's discussed here in the common disclosure I take it to be the full deducted protein.

NAISMITH - MARTIN

Q. Okay.

A. I don't mean to disrespect lawyers, it's just scientists will use shorthand.

Q. Yes, I understand. It is good to be precise. Okay, so now let's do the example of the urea. If I tell you specifically that it's a SEQ.ID NO:3, D320 K PH20 that I've put in saturated urea, would you still consider it to be PH20?

A. I would consider it to have the same sequence that you've just defined but I wouldn't use it without qualification that either it's unfolded or here's some PH20 in 8 Molar urea. I would distinguish it from PH20. I would definitely add the qualifications either "unfolded" or "urea" when describing that molecule.

Q. Why is that?

A. Because when I use just PH20 that conveys to me, as it does in common disclosure, that is a folded active form of the protein but when it is in 8 Molar urea, that is an unfolded inactive form of the protein and that would mean a different thing to a scientist or, I assume,

anybody using it who wanted the material.

Q. Okay. So PH20 as it is found in nature has a GPI anchor; right?

A. Yes.

Q. So if I use the term "PH20", do you think that it has a GPI anchor?

A. So if we are talking as scientists outside the common disclosure ----

Q. Mmm-hmm.

A. ---- you are talking about the full length protein in the sperm cell I would assume. When we are talking disclosure we are directed to Sequence ID NO:3 and others. So there is a modification of that word that we're directed to in the common disclosure.

Q. Okay, so it sounds like you would need more information as to whether PH20 has a GPI anchor; right? You would need to know what sequence if we are talking about a C-terminally truncated sequence?

A. No.

Q. Okay.

A. I didn't say that. I said that as one scientist to another if we were talking about

NAISMITH - MARTIN

PH20 in general terms I would assume you are referring to the PH20 in the sperm cell. But I was careful to say that in the common disclosure PH20 is discussed as a soluble form with these various truncations and we are told that. So it has -- you know, we know that where PH20 comes from, but the one that is in the common disclosure is a truncated form, which lacks the GPI anchor.

Q. Let us go back to paragraph 85 in your declaration.

A. Yes.

Q. In the last sentence you say: "The phrase 'structure of a protein' would, unless prefixed by 'primary' or 'secondary', be taken by a POSA to refer only to the three-dimensional structure of a protein." Is that right?

A. Yes.

Q. If you were trying to define the three-dimensional structure of a protein for example, if you wanted to put it in a patent claim, how would you go about doing that?

MR. KUSHAN: Objection foundation, also objection as to form.

A. So I don't know how to write

patents, so I would have to ask a lawyer to help write a patent.

Q. (BY MS. MARTIN): Is it possible to describe the three-dimensional structure of a protein in words?

A. Scientists do describe and there is really important paper here from HYAL1. They describe the structure but they also deposit coordinates, three-dimensional coordinates. So for every atom you have an X, a Y and a Z coordinate. From that you can put it into a computer program and visualize it and you can write your description based on that. But scientists tend to do both. So we in structural biology we do both. We deposit the coordinates with something called the RCSB. It used to be called the PDB. Anyone can pull the coordinates out of that when we do an experimental structure.

Q. It is possible to describe the three-dimensional structure of a protein without physical -- without the coordinates?

A. It depends on the level of precision you want to apply. So you can describe

a structure but it depends on the precision you wish to use in your description.

Q. Can you explain what you mean by "precision"?

A. So if I wanted -- I could say, for example, bee venom hyaluronidase and human HYAL1 have an alpha-beta barrel motif. That is description but it is quite imprecise because there are important structural differences that we are drawing attention to between the bee venom non-and the human hyaluronidase. So if you were being precise then you would not just use alpha-beta barrel motif. That's is a very broad term that is not really helpful in getting to the precise defence situation you might want.

Q. So instead of using alpha-beta barrel, you would use -- Instead of using alpha-beta barrel motif you would use the structural coordinates?

A. Sorry, if I was trying to tell who, sorry, another scientist?

Q. Yes.

A. So depending on what they wanted from it, I could describe it as, "Here are some

detailed three-dimensional figures and a diagram where I outline a certain stop of helices and beta strands." But often we might just give at least some coordinates and say, you know, "Start from here".

Q. So you would need three-dimensional coordinates to describe the structure of a protein?

MR. KUSHAN: Objection foundation.

A. That's not quite what I said. I said depending on the level of precision you wanted I could describe for example as the people do in those two papers we discussed where the organisation of the beta sheets, the alpha helices and in the human HYAL1, the differences in that motif. So I could write that in words and describe. It clearly points me to those differences. If I wanted a very precise, ultimate precise definition, for example where exactly this residue is in the three-dimensional space, coordinates would probably be quite helpful but I could zoom in and make a figure of it.

Q. (BY MS. MARTIN): When your talking about the "papers" they are describing aspects of

NAISMITH - MARTIN

the structure, they not describing the entire structure; right?

MR. KUSHAN: Objection foundation.

A. I would say that both papers really do describe the structures that they are facing so the bee venom which has quite interesting differences from the human hyaluronidase 1. So both papers describe those structures. They don't describe every atom in the structure but they describe all the structural features, assign them and talk about the differences between the two.

Q. (BY MS. MARTIN): When you say they describe the structure, they describe the pattern of alpha helices and beta sheets and loops?

MR. KUSHAN: Objection form.

A. So they describe those. They produce figures. Figures are often one way scientists communicate with one another. So you do have visual information, particularly helpful for three-dimensional structure and they then highlight, for example, "Look at beta 2 in HYAL1 compared to bee venom".

Q. (BY MS. MARTIN): I appreciate that pictures are helpful, but I'm trying to understand

how you would use words to describe the structural aspect of a protein?

MR. KUSHAN: Objection foundation.

A. So if I was trying to talk to another scientist that is what my papers do. So my papers are ways to describing structural features. They are pages long. We put figures in. We all put figures in and we discuss structural features that we think are particularly interesting but we always give an overall structure description of the protein. We talk about the strands, helices, etc., loops.

Q. (BY MS. MARTIN): Do you expect that all natural full-length proteins are folded?

A. Do I expect it? If they are made in biology?

Q. Yes.

A. For all proteins? Is your question for all proteins?

Q. Yes.

A. No.

Q. Are there some naturally occurring proteins that are not folded?

A. Yes.

NAISMITH - MARTIN

Q. Like what?

A. They are called naturally unfolded proteins. That is actually a name.

Q. Okay.

A. And some proteins exist as proportions of both. So some proteins exist both in a folded form or an unfolded form. Some exist and it is in my declaration, Dr. Dobson's work of the various things. Now those often exist in equilibrium but there are natively unfolded proteins.

Q. You said that those various things often exist at equilibrium but there are natively unfolded proteins?

A. There are natively unfolded proteins that are natively unfolded. That's the phrase. So those are not folded. And there are proteins which, I would not like to speculate today without going back to literature, to speculate what percentage. But there are proteins that Dr. Dobson particularly is distinguished in which as they start to fold some of them get diverted off into aggregates, some of them get -- just remain as molten globules. Now there is an

equilibrium between those. But as a practical matter the aggregates tend not to be reversible. You can have all those things at the same time as a folded form of the protein. Many proteins, but not all, are, as you say, in nature, natural sequence folded and active.

Q. So you said that there is a natively unfolded protein. For those natively unfolded proteins are those molten globules?

A. That is not how people would normally tend to describe. Molten globule is on the way to folding. Natively unfolded is just what it says. It says that when it's made it remains unfolded. Sometimes you will see the phrase "unstructured", "natively unstructured". It's an important area of research.

Q. Do those proteins have a function?

A. Yes.

Q. What's the function?

A. They have lots of different functions.

Q. Okay.

A. So they are involved in lots of biological processes. It is a big area of

science. It's not one I've worked in myself but its natively unstructured proteins is an important area of science.

Q. So does a natively unstructured protein have a structure?

A. It has a primary sequence. It has a primary structure and sequence but it doesn't have a defined -- its by definition it says "unstructured". That is what people are trying to work out, that is what a whole research program is about.

Q. But it is a physical thing; right?

A. It is a physical sequence, yes.

Q. So it does actually exist in three-dimensional space?

A. Yes, but it is likely -- Yes, it does exist in three-dimensional space, but what we don't understand is how it exists in three-dimensional space.

Q. So we don't know what it looks like?

MR. KUSHAN: Objection foundation.

A. We have -- so people pursue it because they have some idea what is doing it, and

NAISMITH - MARTIN

what it might look like. But it's natively unstructured. That is the phrase that is used.

Q. (BY MS. MARTIN): So would you consider a natively unstructured protein to have structural features?

A. I'm not an expert on natively unstructured proteins. I would have to go and pull out some papers and look where the current state of the art is.

Q. So you do not know whether or not a natively unstructured protein has a structural feature?

MR. KUSHAN: Objection form.

A. So what I would know is there would be some structural features. There is a sequence which is the primary structure. They may exist as part of a structured assembly where there are portions of it that that are natively unstructured. It may be entirely natively unstructured. But those are known and as a specific category.

Q. (BY MS. MARTIN): So when you say "primary structure", when you are referring to the sequence, what does the term "primary" signify?

NAISMITH - MARTIN

A. It tells me it is the sequence. So primary structure is the amino acid sequence arranged in order.

Q. And the amino acid sequence arranged in order, is a structure or feature; right?

A. It is a structural characteristic, yes. But it is as I say, there is all, when we talk about proteins, this slight confusion or overlap by some people. Structure, when we talk about proteins we refer to the folded form, but the primary sequence or primary structure is the sequence.

Q. If we talk about polypeptides as opposed to proteins, is there is a distinction between whether a polypeptide is folded form?

MR. KUSHAN: Objection foundation.

A. So the experts have previously used those interchangeably in the reports so I am going to just say that proteins -- polypeptides mean the same thing. As regards the claims then we are talking about a folded form of the protein.

Q. (BY MS. MARTIN): In science, not in connection with patent claims, do you think of

NAISMITH - MARTIN

a polypeptide as a string of amino acids or as a folded protein?

MR. KUSHAN: Objection form.

A. So for here in this business I was very careful to use the terms that others have used. You know, polypeptide, it, kind of, means the same thing but sometimes some people draw differences but not here. I think Dr. Petsko picked up on this and said, "Yes, it's a bit sloppy in common usage" or something.

Q. Do you use the term "polypeptide" in your work as a scientist?

A. I would have to go and word search my publications. It is possible I've used it.

Q. But, like, as a common matter do you typically use that word?

A. When referring to a folded form of a protein?

Q. Just in general. I am just asking if you use the word "polypeptide" in your day-to-day life?

A. You know, I am struggling to think. I probably have used it interchangeably. I'm probably not as precise, as Dr. Petsko said, "We

are probably all a bit sloppy sometimes". I might have used it; I can't say I haven't.

Q. Okay.

A. Because it is possible you've searched my papers and found me saying "polypeptide"; I don't know.

Q. No, I am actually just trying to get an understanding as to, like, if you want to -- We are trying to be very precise with our language. "Polypeptide" is just a string of peptides; right? Many peptides?

MR. KUSHAN: Objection form and to foundation.

A. So to be precise, it is used interchangeably by POSAs as you saw from Dr. Petsko and Dr. Hecht. So I am going to use protein and polypeptide to be synonymous here. There's no hard and fast literature definition that would distinguish between them.

Q. (BY MS. MARTIN): Okay. Can we turn to paragraph 50?

A. I'm on paragraph 50.

Q. Okay, so do you see the sentence that starts: "Each additional permitted

substitution"?

A. Yes, I see that.

Q. And so in that sentence you say: "Each additional permitted substitution increases the size and structural diversity of the sequence space exponentially." Is that right?

A. Yes, I see that.

Q. What do you mean by "structural diversity" in that sentence?

A. So when we think about proteins and structure function relationship, they are introducing multiple mutations into a native sequence in all these permitted positions. You would begin to expect to see differences in the three-dimensional structure of the protein as well as, of course, you actually made primary structural differences, so .....

Q. What do you mean by a "structure function relationship"?

A. I am going to use it in the same way Dr. Petsko talked about, that a properly folded, he said, "'Activity' is a synonym for properly folded" I think he said. What I mean is the structure as a whole has a function. So that

is what I mean by the relationship between structure and function; in this case the hydrolysis of hyaluronic acid.

Q. So if you are talking about -- when you are talking about the structure function relationship am I understanding correctly that you are talking about the relationship between the three-dimensional structure of the folded protein and the function of hydrolyzing hyaluronic acid?

A. That is the -- Yes, for hyaluronic -- for the enzyme PH20 here that is the structure function relationship that is important in considering the common disclosure in the claims.

Q. What is the relationship between the three-dimensional structure of PH20 and the function of hydrolyzing hyaluronic acid?

A. So, the structure has to bring together the amino acids in such a configuration that allow it to bind the substrate, recognise it and then cleave the particular bond. So that is a three-dimensional, quite precise relationship between atoms.

What is interesting about hyaluronidase is that we know from the bee venom

structure, which is structurally very distinct to the mutant HYAL1, that it is possible to achieve a catalytically competent substrate binding form of the protein despite -- well, not "despite", but from two different structural arrangements.

So it is possible to contemplate as you introduce additional site directed mutagenesis, that you can start to change the structural arrangement of PH20. One of the questions is whether that preserves the structure function relationship in that it is still catalytically competent and active or whether it leads to unfolding or a lack of secretion or instability or preventing the protein being made. Because the parameter claim space is so vast you begin to expect to see all of those different things.

Q. So HYAL1 and bee venom hyaluronidase have the same mechanism of action in that they both cleave hyaluronic acid through the same substrate mediated catalysis; is that right?

A. Yes, that is right. They are both able to do this piece of chemistry.

Q. And it is the same piece of

chemistry?

A. Yes, the cleavage of hyaluronic acid.

Q. It is the cleavage of hyaluronic acid but it is in the same way; right? They are cleaving the same bond, using the same residues?

MR. KUSHAN: Objection foundation.

A. So the chemistry of the bond cleavage is conserved between the two despite the fact that there are structural differences between them, which tells us that the structure function relationship obviously operates for both of these two despite the structural differences.

Q. (BY MS. MARTIN): Okay. When you say that "there are structural differences between them" are you talking about the lack of EGF motif or EGF-like motif in the bee venom structure?

MR. KUSHAN: Objection foundation.

A. Could we go to that bit in my report? I can find it for you.

Q. (BY MS. MARTIN): I don't know what bit it is. If you know where it is, that would be great.

A. It may take me a couple of minutes

to find it for you.

Q. That's okay then, we can come back to it.

But you mention there was a difference in structure and so I was trying to understand what you were talking about?

A. Again it is in my report. I extract part of the paper in HYAL1 and they call it the specific structural differences within the alpha-beta barrel motif.

Q. Oh, okay.

A. You also correctly reminded me and I should have said which was the EGF domain is, of course, missing in the bee venom.

Q. Mmm-hmm.

A. That is one of these interesting things about diversity is that it is possible to preserve a structure function relationship despite having quite different structures.

Q. Are there structural differences between HYAL1 and PH20?

MR. KUSHAN: Objection foundation also to form.

A. Dr. Petsko produced a nice super

position of PH20 and HYAL1. It is in my deposition (sic), I can turn to it, if that is okay?

Q. (BY MS. MARTIN): Yes. I think it is on maybe page 112?

A. That is the one, yes.

Q. Is that what you were thinking of?

A. Yes, that's right. He did that super position and said that they have -- I don't think, I don't use the word "alpha-beta barrel fold", I would say "motif" and the EGF domain is outside of that motif.

Q. Okay. While we are talking about terminology, PH20 is a single domain protein; is that right?

A. It is a single folded protein. So it is a single thing. So again there is a, we sometimes will say the "EGF domain" because that appears in the sequence. But the people who described HYAL1 were clear to say that the protein is a single thing. The domain is not separate. There are cases where the domain is attached essentially by a tether to another domain, but that is not one of them.

NAISMITH - MARTIN

Q. So it is not that -- it does not have a -- PH20 does not have a quaternary structure; right?

A. A quaternary structure is where you get multiple monomers together. I would have to go and check. I can't remember if PH20 exists as a dimer or sometimes they do.

Q. But you agree that the EGF portion is part of the PH20 protein, it is not a separate thing?

A. Yes, the PH20 is a single folded unit, yes.

Q. Okay.

A. As we discuss here with the 447. I don't know how the GPI anchor is arranged. I just, you know, I don't know, so my limit is the truncations.

Q. Okay, so I think the question that prompted us to find the figure on page 112 was whether there are structural differences between HYAL1 and PH20?

A. There will be small differences and, interestingly, you don't see any in these figures. So, you know, at that level there is no

difference, they both, as Dr. Petsko said, they adopt the same fold although I would refer to the whole protein as the fold not just alpha-beta barrel motif. So those are the same structures.

Q. When you say "at that level there is no difference" you mean the level of the fold, like a back -- that is formed by the backbone of the amino acid sequence?

MR. KUSHAN: Objection to form.

A. As it is shown here, you can see that the structures overlap completely. If you look carefully, maybe there is a little shift up here. But what is really interesting with that is despite the fact that they have the same fold, they actually have some differences in function, which shows you it is quite difficult to detect.

Q. (BY MS. MARTIN): And the difference in function is that one of them is active only at acidic pH and the other is active at neutral pH?

A. I set out somewhere in my report (I can turn to it) there is a table with the various ones. But there is are differences where they are active and also the substrate preference. One of

them prefers high weight hyaluronic acids.

Q. "High weight hyaluronic acids"? Is that what you said?

A. Yes.

Q. Okay. But they both cleave hyaluronic acid; right?

A. Yes, that is the amazing thing; right?

Q. Okay. They are only around 40% sequence identical; is that right?

A. That is correct. Now in reality we might expect those structures to be a little more different if we experimentally determined them. But using Swiss-Prot you will see here that, you know, you have got very closely aligned. I do note that if you experimentally determined them you may see bigger differences.

Q. Did you look at the experimentally determined PH20 structure?

A. I don't believe there is experimentally. There certainly wasn't one at 2011/2012.

Q. Okay. So you do not know if there is an experimentally determined PH20 structure?

NAISMITH - MARTIN

MR. KUSHAN: Objection to form.

A. There is not an experimentally determined structure for PH20 that I'm aware of and there certainly wasn't one in 2011/2012.

Q. (BY MS. MARTIN): Would you consider there to be structural diversity between HYAL1 and PH20?

MR. KUSHAN: Objection form.

A. I would expect -- Again, if I wanted to be precise I would define it. They had the same, based on what we would assume it to have the same arrangement of secondary structure elements based on the SWISS-MODEL. There will be local differences and you can see in Dr. Petsko's figures some little local differences. So you are beginning to introduce some structural diversity. The important thing is both of these enzymes are shaped by evolution. You have not introduced a bunch of sequence changes between them. Nature has selected them.

Q. (BY MS. MARTIN): There are a bunch of -- To be clear, there are a bunch of sequence changes between them because they are only around 40% identical. It is just that those changes have

been selected by nature; right?

A. That is correct in evolution.

Q. And you -- okay. When you talk about "local differences" are you talking about the slight difference in what looks like the loop positioning in the picture on page 112 or are you talking about something else?

A. No that's one of them. There's --- Yes, that's the one you can see at that level, yes.

Q. I'm just trying to understand what you mean by "local"?

A. Just that as Dr. Petsko qualifies with "essentially the same", he does not say "identical". So I'm happy to use the word Dr. Petsko uses which is "essentially the same". I am sure he used that word because he too can see there is a subtle difference here.

Q. Okay. Can you turn to paragraph 17?

A. I'm on paragraph 17.

Q. So you say in paragraph 17 that: "The scientific literature more frequently uses the term 'native state' or 'native fold' to refer

to the tertiary structure that exists in the folded form of a naturally occurring protein." Is that right?

A. Yes, but I see that I use them interchangeably here.

Q. So if we are talking about the structure, the 3D structure of a folded PH20 protein, would you consider, like, 3D structure and native fold to mean the same thing?

A. So, just to be clear, there are deletions so that does alter the structure. So if we -- if you say is Sequence ID NO:3 is exactly the same as the native protein that's not true because you are missing residues.

Q. Okay, so what does "native fold" mean to you?

A. "Native fold" means the form that is found on the sperm cell ----

Q. Okay.

A. ----- on the surface, that if I'm just talking about PH20 without giving any information about truncations that is what I would assume.

Q. Okay. So does SEQ.ID NO:3 which is

NAISMITH - MARTIN

C-terminally truncated, does that have a native fold since it is not a naturally occurring protein?

A. The parts that are folded, because they are active, are assumed to have the same fold as in the native protein. So those parts are natively folded, properly folded in Dr. Petsko's description. But there are additional regions that are not seen and so you might distinguish that between a modified PH20 and just PH20 because you've modified it with the truncation.

Q. And so did you also use the terminology "native state", and I'm wondering is there is a distinction between the word "state" and the word "fold"?

A. Not as I use it here, I use them interchangeably.

Q. In the literature is there is a distinction between "state" and "fold"?

A. I'm not aware of a precise definition between them. I've seen them used differently. I have seen them used, sorry, interchangeably in the literature.

Q. Okay. If you are talking about an

unfolded protein, would you say that an unfolded protein, even if it does not have a fold, it has a state?

MR. KUSHAN: Objection foundation and objection to form.

A. I would not use it when we are talking about unfolded protein.

Q. (BY MS. MARTIN): So you would not use, like, confirmational state to refer to an unfolded protein?

A. "Confirmational state" implies a definable arrangement of elements. You may have multiple confirmational states and you probably do, but that confirmational state does imply a description of something. Unfolded proteins tend to be thought of as just a collection of huge numbers of possible confirmational states.

Q. Okay. Is there any limit to the structure, the three-dimensional structure that an unfolded protein could take?

A. Dr. Dobson provides some mathematical formulations and we could turn to those. As a practical matter I've never considered it. It's huge numbers.

NAISMITH - MARTIN

Q. Okay.

A. I can't give you a number but it is exponentially large if you consider 15 mutations, for example. For unfolded proteins I would expect them to be very large.

Q. Is the confirmational space for an unfolded protein, is it limited by the dihedral angles of the sequence?

A. So for truly unfolded proteins there are very few limits. There are some limits and you are talking about the Ramachandran plot, I am guessing.

Q. Yes.

A. So there are energetic constraints but that doesn't mean you do not get forbidden angles too or so-called forbidden angles. So that is quite fuzzy but there are some regions you might not expect a particular confirmative dot. Dr. Dobson shows a formulation for you how you might calculate the, sort of, confirmational space that you can explore.

Q. So, for example, if you have a PH20 with a D320 K mutation in SEQ.ID NO:3 ----

A. Mmm-hmm.

NAISMITH - MARTIN

Q. ---- even though that protein has a mutation in it, does it still have a native fold?

A. As it is described in the patent, produced that way, yes, it does, not 8 Molar urea.

Q. Yes, understood. In -- yes, I mean, is there a shorthand for, you know, not in 8 Molar urea?

A. If we are both agree ----

Q. We can assume it is not in urea unless I tell you otherwise.

A. I know. Sorry, I am just trying to be precise.

Q. No, I understand. It is good to be precise. Okay. So, okay, but the question was if you have D320 K SEQ.ID NO:3 PH20 does it have a native fold even though it has a mutation in it?

A. So it obviously has differences to the full length native PH20 but the part that is folded because it is active we are going to assume it is properly un-natively folded.

Q. Okay, so I am just trying to make sure that I am understanding the terminology that the word "native" does not have a connotation, you know, of no mutations?

NAISMITH - MARTIN

A. For folding native when you talk about the sequence has that connotation, no mutations which is ----

Q. Yes, but for folding it doesn't?

A. For folding you can adopt a native fold, despite having sequence differences and that would be properly folded as it is used here.

Q. Okay. So is it fair to say that if you have an active PH20 you expect that that active PH20 has adopted the native fold?

A. You would assume that if it is properly folded, but there could be structural differences. But the fold is quite a broad term. It is relatively a distance high level term. But you would assume that it preserves a structure function relationship and we talked about the bee venom and the human HYAL1. So there are structural differences. So those are different structures ----

Q. Mmm-hmm.

A. ---- and that was noted in the publication. So you can envisage proteins having a proper fold which is, and we have used this term where it is active it is natively folded. But

structurally there still could be differences and structural diversity when you start to introduce mutations.

Q. Okay. You so I want to be trying to get an understanding of what you mean when you are using the terms that are in your declaration, or what you are thinking when you are using the terms in your declaration. So I understand that if you have, you know, a mutation for example at position 320 and you compare that to what to SEQ.ID NO:3, I don't want to say "wild type" because I understand there is the stuff at the end and I am trying to not deal with that.

A. No, I understand.

Q. Okay. So D320 K SEQ.ID NO:3 versus SEQ.ID NO:3, right, you have a lysine at position 320, so you have somewhat of a difference in the, you know, three-dimensional surface topology, if you will, on your PH20 but the fold is still the same?

A. I would use Dr. Petsko's logic, "essentially the same", yes. The fold and the arrangement for that one mutation, D320 K, the fold would be essentially the same. And based on

Dr. Petsko and Dr. Park's figures they also do not see any local pertubation; they see a stabilizing interaction.

Q. Hmm-mmm, okay. Now if you introduce mutations into PH20 and you obtain an active protein, do you expect that it still has the native fold or do you think it is possible that it has a totally different fold, like maybe it looks like a cat?

A. It certainly would not look like a cat.

Q. Okay. Would you expect it that has the native fold?

A. So I would expect at this same level of imprecision that we used the bee venom and human HYAL1 to say they are roughly the same structure but with important differences ----

Q. Yes.

A. ---- then I would expect that any active form of the PH20 within that level of imprecision would have the same fold.

Q. Okay. I am trying to understand that level of -- using that level of imprecision if you will. Like, it still is going to look,

NAISMITH - MARTIN

like, the same alpha-beta barrel-ish motif as opposed to, you know, an octagon or ----

MR. KUSHAN: Objection. Sorry.

Q. (BY MS. MARTIN): ---- hemoglobin or something?

MR. KUSHAN: Objection form, objection to foundation.

A. So fold is, the problem is fold is relatively imprecise.

Q. (BY MS. MARTIN): Yes.

A. The structure of the HYAL1 and the bee venom are different so therefore I would expect, based on what I knew at 2011/12, if I begin to introduce molten mutations and I found an active form of PH20 I would expect it to have the same fold as here, properly folded, but I could expect quite a lot of structural diversity, how the structure function relationship which is essential for activity was achieved.

Q. Okay, so now let's talk about what you mean by "structural diversity" in the answer that you just gave. So what do you mean by "structural diversity"?

A. For example the difference between

the bee venom and the human HYAL1, that is a good example of structural diversity. The one has a beta strand the other has a loop and the other one is missing an alpha helix.

Q. Okay. So there could be differences, for example, in secondary structure?

A. That is what the literature kind of shows us and also the patent. Dr. Simpson considers putting insertions and deletions in so those indeed could perturb some secondary structural elements.

Q. Okay. So if you have a difference in secondary structural elements would you still then have the same tertiary structure or is it a different tertiary structure?

A. I would say you would have broadly defined the same fold.

Q. Okay.

A. Tertiary structure begins to imply a bit more precision.

Q. Okay.

A. So that is why the people drew attention to difference between the bee venom and human HYAL1.

NAISMITH - MARTIN

Q. Okay. So if you introduced a change into PH20 that disrupted an alpha fold for example ----

A. An "alpha fold", sorry?

Q. I am sorry, an alpha helix. Let me start again. Strike that. If you introduce a change into PH20 that disrupted an alpha helix you would say that that is a change in secondary structure; right?

A. I would not know whether it did disrupt so I don't know how you know that it disrupted alpha helix.

Q. Oh okay.

A. I mean there are mutations that potentially and predictably can disrupt an alpha helix, but, you know, are you telling me that you know it disrupted an alpha helix or are you asking me to predict?

Q. Oh okay, I understand.

A. Sorry.

Q. Thank you. All right, so I am asking you to assume, just because I am trying to understand your opinion, that you introduce the change that's disrupted an alpha helix. So

assuming that that's what happened, would you consider that modified alpha helix PH20 to have a difference in secondary structure?

A. Is the protein active?

Q. Yes.

A. So I would say it is the same native fold. There are some details and difference in the structure however, exactly as you described alpha helix as somehow broken.

Q. Okay, so if you have an active PH20 you expect that it has the same native fold even though there could be differences in the secondary structure?

A. Yes, because we saw that with the bee venom; there is a clear difference between the secondary structure of the bee venom right within alpha-beta barrel motif between bee venom and human hyaluronidase. So we know that it is possible.

Q. Okay.

A. The biggest difference, of course, is bee venom does not require and does not have the EGF domain, which is essential for PH20.

Q. So it is possible to achieve the

NAISMITH - MARTIN

native fold of PH20 even if there are variations in secondary structure?

A. It depends how big those variations get. I mean, at some point you might begin to dispute whether it is the same, but it is certainly possible to imagine describable visible differences in secondary structure for proteins that shared the same fold and are active.

Q. Okay. Would you consider two PH20s to be precise so a modified PH20 that is active and SEQ.ID NO:3 PH20 to have common structural features if, let's assume, that there is a change in secondary structure like you have disrupted an alpha helix?

MR. KUSHAN: Objection form, objection foundation.

A. So I would expect if you were using that you would define the particular ones that are common between the two examples.

Q. (BY MS. MARTIN): What do you mean by that, like, what -- how would you define the particular ones that are common?

A. Well, I would say we have these secondary structure, so we are talking about them,

NAISMITH - MARTIN

---

Q. Mmm-hmm.

A. So you would say these secondary structure elements are the same, this secondary structure element is different, in the way that we did with the bee venom and the hyaluronidase.

Q. Okay, okay. So for HYAL1 and bee venom you could say that they have common structural features and that some of the alpha helices are in the same position?

A. They have -- Yes, the super position of HYAL1 and bee venom shows there are common structural features but there are really important structural differences. So if you restricted your discussion to the alpha-beta barrel motif you might say they have got a similar alpha-beta barrel motif but structurally they are different.

Q. Okay.

A. That is what the authors say in the paper.

Q. Okay, so they do have common structural features but they also have differences?

NAISMITH - MARTIN

A. That's correct but we do not know which one of those ones in common are relevant to the structure function relationship.

Q. Okay. So we have been going for a little over an hour, do you want to take another break?

A. If that's okay with you.

THE VIDEOGRAPHER: We are going off the record. The time is 10.18 a.m.

**(A short recess from 10.18 a.m. to 10.42 a.m.)**

THE VIDEOGRAPHER: We are back on the record. The time is 10.42 a.m.

Q. (BY MS. MARTIN): So does PH20 have more than one native fold?

A. PH20 protein has one native fold. You can modify PH20 and introduce different folds, but PH20 has one native fold that I'm aware of.

Q. Can you turn to paragraph 43 in your declaration?

A. Yes.

Q. In sub-item (iii) paragraph 43, you talk about: "... the same tertiary structure found in the native state of the wild-type PH20 protein ...".

NAISMITH - MARTIN

A. Yes, I do.

Q. And I just want to make sure I'm understanding that when you use "native state" there, are you talking about native, the native fold?

A. Yes.

Q. Okay.

A. I did say I would use them interchangeably, yes.

Q. Mmm-hmm. And similarly to the line of questioning I just did, is there more than one native state for PH20?

A. No, there's one properly folded native fold on PH20.

Q. Okay. And I have seen the phrase like "confirmational state" in the literature. Is that ----

A. "Confirmational state in the literature", I don't recall it for PH20. But in general confirmational state might imply there is -- that implies maybe local structural changes that are somehow important. There is in fact, if I remember correctly, in the bee venom structure there is some discussion of changes when it is

NAISMITH - MARTIN

bound to the substrate.

Q. Okay.

A. So you might say there are some confirmational changes in the loops.

Q. Okay. You used the term "epistasis" in your declaration; right?

A. I talked about the concept of epistasis and I use the word epistasis.

Q. What is the concept of epistasis and I think paragraph 253 potentially would help.

A. Yes, we begin at 253. So "epistasis" is just a word, a shorthand, if you like, for non-additivity of single site mutations.

Q. Okay, when you say "non-additivity", what does that mean?

A. So this is something all of the experts talk about, which is as you make an increasing number of single site mutations, so you go from 1 to 2 to 3 to 4 to 5, that you can't assume that the mutations do not interact with one another in unpredictable ways. So they start to like, to give you a concrete example I've given here, for on page (sic) 254. You can imagine I have two single site mutations. When they are

NAISMITH - MARTIN

made as single site mutations I want to combine them together to make a double mutant; right? So we will start simple. Now, let's say we assume they are additive, so they both individually actively -- they are both individually active on their own. If they are additive, we combine them, we would expect the protein to be slightly more active. So that is additive; okay? But we can get magnitude epistasis which means wow, the protein becomes way more active when I combine them as individuals. I can get sign, reciprocal sign epistasis when individually the two mutations are active. But when they go into the protein they actually combine to make the protein much less active. So that another one.

And then we can get just sign epistasis where individually this mutant is beneficial, if you like, active more active and this one is more active. But when I combine them I get one of them becomes deactivating deleterious. So those are the three broad categories of epistasis. This is, Dr. Hecht talks about this. This is why it is so difficult to predict as you combine multiple mutations based on

a single site dataset.

Q. I don't think Dr. Hecht used the word "epistasis"?

A. Dr. Hecht talked about the concept in both his deposition and his expert report. He did not use the word "epistasis" but he chose to explain the concept. I think he said "frequently non-additive" or "non-additive mutations" in his dep.

Q. Okay.

A. Which means the same thing and Dr. Petsko was careful about independent mutations which means mutations not subject to epistatic interactions.

Q. Okay. Is there another -- I don't think anyone used the word "epistasis" until your declaration. Is there, like, another word?

A. There is a phrase I think is probably, non -- The effect is people will use their own words to describe the phenomenon. I chose epistasis because I specifically cite to some of the foundational paper in that field which has the phrase epistasis and you will see it does crop up in other publications that I cited. So,

the concept is what people will describe. I just used a shorthand and the concept is although you may have single site mutations which, on their own, have some effect on the protein, when you start to combine them they become unpredictable and that is because you get these epistatic effects.

Q. So I think when Dr. Park in his deposition he talked about if you make changes in two different parts of the protein whether you could consider them to be independent. Is that, kind of, in the same general area of what you are talking about?

A. So I would like to turn to exactly what he used it as but, as a general point, if he is saying they are not independent, he is saying that there is a risk of epistasis. Dr. Park, in his declaration, was very clear that his approach only worked for single site mutations.

Q. Yes, no, I understand that. I am just trying to understand if the concept of two mutations being independent versus not independent is what you are talking about when you are talking about epistasis?

NAISMITH - MARTIN

A. So, that would be one way to express it. If you are saying -- if you say, "Mutation over here is independent from mutation over there", you are, by that word, saying there are no epistatic effects.

Q. Okay.

A. What I am trying to say is that the data in the patent show that single site mutations are perturbing the active site. Most of them perturb the structure function relationship at the active site. So we know that they are having some, I think Dr. Petsko's phrase was, "action at a distance".

Q. Mmm-hmm.

A. So finding mutations which are independent of one another is very, very challenging and that becomes exponentially almost impossible as you move to 5.

Q. Okay. So you saying that finding mutations that are independent of one another is very, very challenging because every single site mutation is resulting in a long range interaction?

A. I didn't say "every single site mutation". I said that -- and we can turn the

table in the patent, sorry, the table in my report where I show that when you look at the activity reported for active proteins they range from, I think one is 27 fold more active to ones that are, like, 25% active. That tells me that that mutation has done something to the protein; right? It has gained or it has been deleterious, so therefore adding another mutation. So that to change the activity you have in some way perturbed the active site. The protein is still active or vastly more active but it has had an effect.

So once when, let's say in my mutation over here, I have all 27 fold more active; right? The active site, something has changed, I don't know what. This other mutation over here which is two fold more active, something has changed over here. But putting both together is unpredictable since I can't figure out how their combined changes will influence the active site.

Q. Why are you as assuming that something changed in the active site?

A. Because the chemistry is 27 fold more active.

NAISMITH - MARTIN

Q. Are there other explanations for that besides something changing on the active site?

A. You could be 27 fold more expressed in the way the assay is conducted but that would be a feature of how the protein is folded and made, so you are still -- that is an assembly thing that is obviously interfering, also altering in some way the protein.

Q. What if the mutation improved the affinity of binding to HA but did not change the active site?

A. So you would need to do that by kinetic analysis to work out. So you are talking about KM versus Kcat?

Q. Mmm-hmm.

A. There is no data for me to assess that.

Q. That is a possible explanation; right?

A. For 27 fold more activity?

Q. Mmm-hmm?

A. Possibly. I gave you another one which was that it was made 27 times more readily

or a higher yield. I don't know because I can't use it but I have assumed because the patent uses the phrase much more active than native that it implies 27 fold more activity.

Q. But you assumed that the difference, the -- I am sorry, strike that. You assumed that the difference in activity is caused by a change in the active site?

A. No, I did not assume that, I used that as an explanation, but I am happy to take other explanations. But based on the common disclosure I am offered no other experimental evidence, other than it is 27 times more active than native.

Q. So you said that the explanation -- I am sorry, strike that. You said the reason why you can't identify independent mutations or independent positions is because every single change is impacting the active site and that, in itself, is premised on the assumption that a change in activity is the result of a change in the active site?

A. No, that is not what I said. So, what I said was that based on what is in the

patent information I have got is that I've got an increase in activity. I have given you possible reasons for increase in activity. But whether it affects the binding constant, the expression level, the secretion level, these are all whole protein complexes which would suggest a mutant is having an effect in some way upon the protein as a whole. So where I'm affecting the protein as a whole then I would have a concern to add another mutation which is affecting the protein as a whole. Now it is possible for mutations to be independent of one another but not, you would not know that without testing.

Q. So I don't understand why the changes are having an effect -- strike that. Let me ask a different question. What do you mean when you say that the change is having an impact on the protein as a whole?

A. So, if I talk about protein production levels for why it is 27 times made more, that is obviously something quite subtle about the whole protein.

Q. Okay. HA, hyaluronic acid, is the substrate; right?

NAISMITH - MARTIN

A. Yes.

Q. HA is big; right?

A. Yes.

Q. Very large, polymer?

A. Mmm-hmm.

Q. So I know in the bee venom structure, right, there is and I don't remember if it is an tetramer, but some portion of HA is shown in the bee venom structure; right?

A. Yes.

Q. Okay. That is not the whole HA?

A. No, it's a fragment, it's a product fragment.

Q. Yes. And PH20 binds to HA; right?

A. Yes.

Q. So PH20 is binding to this large polymer?

A. Not in the assay, in the patent though. The patent -- it's used in biotinylated versions. It's not quite the same as in nature.

Q. Okay, well let's be very precise about what we are talking about. In nature, actually in nature PH20 binds to HA; right?

A. It binds and cleaves HA, yes.

NAISMITH - MARTIN

Q. And HA is a large polymer?

A. Yes.

Q. So would you expect that PH20 is contacting HA at other positions outside of just the specific residues that are being cleaved?

MR. KUSHAN: Objection form.

A. So, I want to clarify your question. Are you talking about based -- do I think that the complete set of residues that interact with HA can be described solely from the bee venom complex?

Q. (BY MS. MARTIN): Yes.

A. The ones in contact with the bee venom or the carbohydrate are outlined and they do predict, they extrapolate based on the structure the other regions that are, sort of, just continue along that region. So there is a, sort of, a groove that they say they think but don't know that those are important for binding.

Q. Okay. Do you think that PH20 only binds for example like the tetramer of HA that is shown in the bee venom structure or do you think that PH20 binds more of the HA polymer?

MR. KUSHAN: Objection form.

A. So when you say "more", I think we know the substrate is longer than the product complex in the bee venom, and the bee venom does explain. And I would have thought, based on the bee venom structure, that I would expect that that groove they predict would be important in PH20 for binding polymer. But I don't know and I don't know any experimental data in 2012 that really tackled that question for PH20.

Q. (BY MS. MARTIN): You are talking about the "groove". What do you mean when you say the "groove", in PH20?

A. Yes, can we -- I will get to that structure, it is probably easier.

Q. That would be great.

A. *(Pause)* We are so used to electronic versions of documents.

Q. Maybe page 160 there is a picture?

A. Thank you. I knew it was in here somewhere.

Q. Okay.

A. Thank you very much.

Q. So we are on page 160. Does that show the groove that you are talking about?

NAISMITH - MARTIN

A. So the groove that they talked about in the paper can be located in this figure.

Q. Okay, and can you just explain what that is?

A. So the diagram is a primal image secondary structure. Do you want me to define it really clearly for the record or you would be happy to just say "this bit"?

Q. If you just "this bit" that's fine.

A. Okay. Sorry, I wasn't sure what you wanted. So you have a tetrasaccharide at the top. Then what we know, because that is cleaved product form, there must be some extension of the polysaccharide beyond that. In the paper as I describe they talk about what they think the residues that might be in contact are with that part of the substrate. So it is a continuous groove that runs, kind of, *here,* formed by these loops between the secondary structure elements. But there was no firm experimental evidence precisely what residues were in contact by the time you get to PH20.

Q. Okay. And so earlier in your testimony when you were talking about mutations

having an impact on the active site, when you are talking about the "active site" are you talking about the groove or are you talking about the specific residues that are involved in the substrate mediated catalysis?

A. So I am talking about the -- when you think about catalysis, it is a precise positioning of residues important to that, it's not just one residue of course. So positioning them with the correct electrostatic effect is really critical, and binding the substrate in the correct orientation for catalysis is also critical. So when you think about what you are altering in the active site, if I was to do it experimentally, I would be interested in all of it to try to understand that, but the patent only gives me the information about activity as a whole. So somehow it is altering the activity of the protein and I have given possible examples.

Q. Is it possible that some of the changes that impact activity are changes to residues that themselves contact HA even if they are not involved in catalysis?

A. Yes, it's possible. I mean, I have

no way to know.

Q. Did you account for that when you rendered your opinions regarding potential epistatic effects?

A. So that is the whole point about epistatic effects is that it is the unpredictability of combining mutations that is the problem. So anything which is deleterious to the binding of the protein or the correct binding of the substrate or deleterious to the correct positioning of the catalytic machinery, that would cause the protein to be, it could cause the protein to be inactive. Or you could also imagine that combinations which on their own are active result in protein that becomes unstable, because in some way they've altered the packing of the core of the protein. And that would also give rise to apparent inactivity although you could distinguish between those two forms of inactivity by experimentation.

So when I rendered my opinion I was guided solely by the common disclosure which reports activity measurements and those significantly differ from one for a large number

of mutations. And so I know something is happening and the patent doesn't tell me what. But some of them are more active, some of them are less active. And so combining those will be challenging in any event. Even if I -- The patent gives me that additional information. Even without that information I would ask still been worried about epistatic effects or non-deletative mutations. That was well known in the literature and I cite I think it is Fowler et al. where they talk about this, that by the time they got to three or four mutations then it became really difficult, you know, the thing became unpredictable. In part the unpredictability is what underpinned the work around directed evolution, because it is those complex interactions at a distance that are subtly altering the structural activity relationship that have bedevilled protein engineering to do rational combinations of single site mutations.

Q. And so I am trying to get an understanding of the potential independence of different mutations and the action at a distance as you put it. And I am just trying to understand

because you testified earlier that it would be very difficult to identify mutations that are independent on PH20 because all of the mutations are impacting the active site. And so I'm trying to understand if you took into account when you formed that opinion the possibility that what is happening is that the amino acid you are changing is binding to HA even though it is not in the active site?

MR. KUSHAN: Objection form, objection to foundation.

A. So there is a lot in that question. Could you break it up into smaller questions? Sorry, there is a lot. I heard a lot of words at the start and there is a lot of words at the end, I'm sorry.

Q. (BY MS. MARTIN): Did I understand your testimony correctly that you believe it would be difficult to identify positions to mutate on PH20 that are independent?

A. Okay, so you can experimentally identify those. My point was it would be hard, *a priori*, to predict them. That's my point, is that *a priori* you can't, you would not easily know for

any protein whether sites were independent, even at two mutations. Now you might assume you can get away with it which is why in my opinion Dr. Hecht and actually Dr. Petsko all said up to 5 you can probably manage but beyond that it does become unpredictable and the literature even at 3, so that is point. Now, when you say they are independent can I identify them? I can. I can do the experiment. I can make the combined mutation and measure the output. And if the output is an additive combination then I know they are independent because that is the definition of independence.

Q. I am going to break that down. So, first, I want to understand why the cut off that you are using is 5? So why is it that you can probably manage 5 but if you tried 6 they would become unpredictable?

A. So I said in my report that POSAs would be confident at 1 and less confident by the time you get to 3 and different POSAs would draw the line differently. I've said for this report I will draw it at 5. But another POSA might say 4. 5 is the outer limit, I think, that any POSA

would draw.

Q. Okay. So a POSA could potentially think that they could manage 5 mutations but 6 would be too many?

A. It is decreasing confidence in your predictability. At 5 I think you would have very little confidence in your predictability. By the time you get to 6 you would have no confidence in your predictability.

Q. And you think that all POSAs would agree with that assessment?

A. In 2011 and 12 timeframe, yes, I would because the literature was there at that time to warn you about that, it was well known. And Dr. Hecht and Dr. Petsko both touch on this issue quite clearly. Dr. Hecht says, you know, the same, kind of, number round about 5, up to 5. So I would say, yes, that is where POSAs would draw. I don't know any analysis of anyone who can predict beyond 5 that I'm aware of in the 2011/12 timeframe.

Q. Okay, do you consider Dr. Petsko to be a POSA?

A. Yes.

NAISMITH - MARTIN

Q. And he said that you could make more than five changes with reasonable predictability; right?

MR. KUSHAN: Objection foundation.

A. Can I have his report, because I think he qualifies things so I'd like to see his report where he says this.

Q. (BY MS. MARTIN): Okay so you don't agree that -- I want to make sure that I'm understanding because you said that no POSA would be comfortable making predictions with more than five changes and you said that Dr. Petsko is a POSA. So are you saying that Dr. Petsko took the position that you couldn't make any predictions above five changes?

A. So Dr. Petsko when he talked -- he used other evolutionary species when he gave examples of more than five changes. But I don't recall in his deposition where he said he could confidently predict five or more single site mutations that he could combine to retain activity.

Q. So you don't think that he took that position?

NAISMITH - MARTIN

MR. KUSHAN: Objection foundation.

A. So Dr. Petsko's opinion was based on the tolerance table. He thought a POSA could somehow use that but in his deposition he didn't give any example of how to do that and I don't think in my opinion that's wrong. I don't think it is possible so I would disagree with his. He never said in his opinion, "Here is a way to combine five single site mutations or more that I am confident will work."

Q. (BY MS. MARTIN): Okay. And to the extent that he did in fact say that it was possible to combine more than five single site mutations in the way that would work, you would disagree with him?

A. So, Dr. Petsko may have imagined some very narrow definition of five, as an example of which it might be possible. But as a general rule, my opinion is that a POSA could not pick five single site mutations and combine them with any confidence in the prediction of the outcome.

Q. Okay. Do you think that Dr. Simpson was a POSA?

A. Yes, I do.

NAISMITH - MARTIN

Q. And Dr. Simpson also opined that you could make more than -- you could predictably make more than five single site mutations; right?

A. Could I turn to her declaration of what she actually said, if that's okay?

Q. We can get there. I am trying to have a general understanding again as to -- Because again you said that no POSA would take the position that you could predictably make more than five changes. So is it your understanding that Dr. Simpson also imposed a five change maximum?

MR. KUSHAN: Objection foundation.

A. Dr. Simpson did not discuss epistasis at all and did not shed any light of what was a well-known literature phenomenon that Dr. Petsko was clearly aware of. He uses the phrase "independent". So Dr. Petsko is using end result which is, they are independent, so you can combine them. But that you would only know by experimentation. Dr. Simpson does not express, that I recall, any method or literatures for support for her position that you could use Dr. Petsko's tolerance table and combine mutations. My argument -- opinion is based on the fact that

NAISMITH - MARTIN

the literature shows that not to be true.

Q. (BY MS. MARTIN): So there is literature on PH20 that shows that you can't use what you did in air quotes, Dr. Petsko's air quotes tolerance table to make predictions. There is literature on that?

MR. KUSHAN: Objection foundation, objection to form.

A. The literature shows quite clearly that the problem of combining in any protein single site mutations once you start to combine them the literature shows that they are unpredictable and I cite to Fowler where this is discussed at some length.

Q. (BY MS. MARTIN): But Fowler wasn't talking about PH20, was it?

A. He was not talking about PH20 but the principles around protein structure that Dr. Hecht and Dr. Petsko both opine in their introductory matters, these phenomenon will apply across all protein structures.

Q. All right, so you also said of your answer a little while ago that the definition, something like the definition of independent

NAISMITH - MARTIN

changes would be if they are additive. Is that about right?

MR. KUSHAN: Objection foundation.

A. So Dr. Petsko did not define what he meant by independent.

Q. (BY MS. MARTIN): I thought that that used the word definition in your answer?

A. I gave you independent of epistatic effect which is that they are simply additive.

Q. Okay.

A. Now Dr. Petsko could have had the other, which is they are not even additive, but that is actually -- I did, you know, he just said "independent" which means he is avoiding the epistatic effect.

Q. Okay. Is it your testimony -- is it your testimony that in order for two mutations to be independent their effects have to be additive?

MR. KUSHAN: Objection form.

A. So for them not to have epistasis, for there to be no epistasis the definition is the effects are additive. That is the definition of epistasis.

NAISMITH - MARTIN

Q. (BY MS. MARTIN): And so am I understanding "additive" correctly in that if you have one mutation that gives you 100% activity and another mutation that gives you 100% activity, when you combine them you would still have 100% activity?

A. If they were independent of one another, yes.

Q. Okay, and ----

A. Because there is no epistatic effect.

Q. I am trying to understand the additive part?

A. Okay. So it is based on the thermodynamic but then it translates to multiplying the activity measurement; so one times one is one.

Q. Okay. So, can you explain what you just said about thermodynamic?

A. So, the epistasis is thinking about what is happening in the structure so effects are in some way added. But if the protein has an activity of one and the second independent mutation has an activity of point 5, you multiply

the two activities to get the resulting activity which would be point 5 in the absence of epistatic effects.

Q. Okay, so if one had -- I'm trying to understand how you would actually add them. If one of them had an activity of point 5 and the other one had an active of point 75, then would you multiply them or would you add them?

A. If they are independent, you multiply them.

Q. Okay. So point 75 times point 5, that would give you the ----

A. Point 35.

Q. Point 35, okay.

A. Yes.

Q. But in that example point 35 is worse than point 5 and point 75?

A. That is correct because you are combining two deleterious mutants.

Q. Okay, oh, I understand. So the direction is the directionality?

A. Correct. And what would be an example of epistatic if I combined them and I got 1.5 activity, that is an example of ----

NAISMITH - MARTIN

Q. Okay?

A. ---- where you would see epistatic effects.

Q. So you are combining two deleterious mutants one of which halves activity and one of which drops it be a quarter. So you are expecting that when you combine them you are going to similarly decrease activity?

MR. KUSHAN: Objection form.

A. That's not quite what I said. I said if you assume them to be independent ----

Q. (BY MS. MARTIN): Yes, if you assume them to be independent.

A. ---- then you multiply the two activities.

Q. Okay. And so in this example when you are using the term "activity" and you are multiplying the values, what are you measuring? What is the activity?

A. I am using the definition that is given in the patent, which is the native protein is 1 and the other ones are fractions, decimal places of that in the tables in the common disclosure.

NAISMITH - MARTIN

Q. Is there anything in the patent that talks about multiplying activities to determine whether or not two changes are independent?

MR. KUSHAN: Objection form, objection foundation.

A. The patent doesn't give any teaching about combining multi-mutations at all. I drew it from, it was responding to Dr. Petsko's opinions.

Q. (BY MS. MARTIN): Do you consider the values that are given in the tables in the patent to be, like, absolute activity measurements?

A. These are relative to the native protein, that is how they are measured. So they are relative to the native protein, that is why they are expressed as 1.0 or 1.3 or whatever.

Q. Okay. Can you go to paragraph 191?

A. That's on page 136? I just want to check, you said 191. I did not quite catch it, sorry?

Q. Yes, 191?

A. I was pouring my drink, sorry.

NAISMITH - MARTIN

Q. 191.

A. On page 136?

Q. Yes, on page 136. You say in paragraph 191 that: "Chao et al. ... defined the HYAL-EGF fold as Ser353 to Trp 435 ..."; is that right?

A. That is, yes, that is what I have said.

Q. And then: "Using this definition, the EGF fold in PH20 comprises Leu336 to Asp416." Is that right?

A. Yes.

Q. But you used the term "fold" to refer to the EGF domain in this paragraph; right?

A. Because it refers to the whole fold of the protein. So the HYAL-EGF fold is the -- we are talking about specific motif within the EGF. But it is the HYAL-EGF fold of the protein which has the EGF motif within it.

Q. I don't understand what you mean when you say "the HYAL-EGF fold of the protein"?

A. What I am saying is that the fold of the HYAL-EGF protein has the EGF motif in it and that motif is defined between these residues.

NAISMITH - MARTIN

Q. But the HYAL1 protein -- you are talking about the HYAL1 protein; right? You are not talking about the HYAL-EGF protein?

A. No, I'm talking about the HYAL1 protein, yes.

Q. Okay. And the HYAL1 protein has more to it than just the EGF; right?

A. It does. It has the alpha-beta barrel motif.

Q. Okay. If the word "fold" refers to the entire three-dimensional structure of the protein, why did you use it in this paragraph to refer to just the EGF motif?

A. So that is an imprecision in my language, it should have said -- to be more consistent I should have said as I defined earlier the fold to be whole protein and I have used the phrase "EGF motif" or domain.

Q. Okay.

A. But you are correct and I apologise. There is an imprecision in the language there.

Q. But when you are talking about the EGF fold here you mean the EGF motif?

NAISMITH - MARTIN

A. Yes, and I should have said so, my apologies.

Q. Can you turn to paragraph 59?

A. 69?

Q. 59.

A. On page 30, yes?

Q. Yes. So in paragraph 59 you say: "While enzymatic activity confirms that an enzyme has folded, there would need to be additional evidence to justify a conclusion that the folded enzyme is sufficiently stable to retain its folded state." Is that right?

A. Yes, that is the start of paragraph 59.

Q. You then go on to say: "... a POSA would not believe an enzyme had 'properly folded' if it did not maintain activity over a meaningful period or if it formed aggregates within a short period"; right?

A. Yes, that what I said.

Q. Okay. So if you have an active PH20 that gives you enzymatic activity let's say in a microtiter plate assay, would you expect that the PH20 has the PH20 native fold?

NAISMITH - MARTIN

A. So, for the methodology described in the patent where you have these longer incubation times, if you have active protein then I would assume those are active folded natively folded protein.

Q. Okay. So how can you tell if the folded enzyme is sufficiently stable to retain its folded state?

A. You would look at different time points. A protein which -- in the assay they talk about overnight for example. That could have been one of the things you could do to see if the protein was stable overnight or for a few hours.

Q. Okay. I think in the Example 3 activity assay they were using an incubation time of 90 minutes?

A. Yes.

Q. Is that what you would consider?

A. Yes, but they had isolated the protein too. So there is more to it than just, you know, they purified it. Sorry, they took the extract I should say, not "purified it". They took the extract. So protein has a bit of time, not just 90 minutes.

NAISMITH - MARTIN

Q. Oh okay, yes. Because they are testing supernate, right, they are not testing purified protein?

A. Yes.

Q. Okay. I am trying to get an understanding of what "a meaningful period of time" is, as you use that phrase in paragraph 59. So, like, would 90 minutes in the assay in the patent, is that a sufficiently meaningful period for it to have been considered properly folded?

MR. KUSHAN: Objection foundation and objection to form.

A. There is, it is obviously a spectrum. To understand it you would take different time points, and two reasonably close together. You would see if there is was a slope between them for example. For the protein to be useful and I would anchor my expectations around what the patent said which was for the inactive mutants they ran they overnight ----

THE COURT REPORTER: Sorry, "My expectations around what the patent said which was for"?

THE WITNESS: They did overnight

incubations for some of the testing they did. So, that would seem a reasonable place to start around sort of, 8-10 hours.

Q. (BY MS. MARTIN): Okay, so do you think that the activity assay in Example 3 that used the 1.5 hour incubation time, that that was not sufficient for a POSA to determine whether or not the enzyme had properly folded?

A. In the assay at the time, the protein was properly folded. The assay does not disclose if the protein then subsequently lost activity, which is perfectly possible if it was unstable.

Q. Do you have a sense of the timeframe over which a PH20 needs to be sufficiently stable to be clinically useful?

A. So the patent does talk about pharmaceuticals. And one of the things Dr. Petsko has pointed out is that aggregated forms, unfolded forms of protein, are not good. So I would say that you would, based on the envisaged use in pharmaceuticals, you would not want any aggregates or misfold the proteins over, sort of, a 12-hour period till you could formulate your medicine. So

NAISMITH - MARTIN

that is a, kind of, minimum timeframe.

Q. So you would consider 12 hours to be the minimum timeframe over which the protein needs to be stable in order for it to properly fold?

MR. KUSHAN: Objection foundation.

A. No. What I said is it can be properly folded -- So you would have the fold at the time of the assay if it's active but you could already have a proportion of inactive protein because it could be unfolding during the assay. So just it is -- and the patent does talk about uses of the protein. It talks about an assay over 12 hours. So I think it is not a hard and fast rule like eleven 59 minutes is not any good, 12 minutes one is good. It is somewhat of a spectrum. But the point I am trying to make is that a protein which was active at 90 minutes and was completely inactive at three hours would not meet the test of a properly folded protein that was stable, which is what a POSA would want.

Q. (BY MS. MARTIN): So you can't tell from the results in Example 3 whether any of those PH20 proteins are a properly folded protein

NAISMITH - MARTIN

because the test didn't involve a 12-hour incubation?

MR. KUSHAN: Objection foundation.

A. I can have a reasonable expectation since there were single site mutations. I can have a reasonable expectation that most of the ones that were active were properly folded but I cannot exclude the possibility that some of them subsequently fell apart, but not in the 90 minute -- not meaningfully in the 90-minute assay period plus the time for preparation. The significance that I wanted to draw out was that the problem with the classification of inactive; those proteins could have been active when they were first in the supernative but the time you get them into assay they could have unfolded. So that is what I am trying to draw out in my distinction here.

Q. (BY MS. MARTIN): Did the conditions at which you are incubating the protein or keeping the protein impact whether or not it is going to stay folded?

A. Yes, we -- you and I talked about put thing it in 8 Molar urea for example would

NAISMITH - MARTIN

unfold the protein. The protein may be stable in some buffer, but if you add it to 8 Molar urea it is going to unfold, likely unfold and be unstable.

Q. So could you impact whether or not the -- could you change the stability of a protein over time by changing the buffer in which the protein is?

MR. KUSHAN: Objection foundation.

A. So a protein can have different -- So a stable protein, as we have shown with 8 Molar urea, is no longer able to adopt a stable fold. It is possible, and the patent does not discuss this, hypothetically to think of a mutation that is stable in one buffer but starts to become unstable in another. The patent does address a particular form of instability, which is the addition of compounds that are denaturing. And one of the things they look for is whether the activity is preserved or not for the sum of the single site mutations in the presence of the unfolding agent.

Q. (BY MS. MARTIN): So how do you go about determining whether a PH20 is properly folded?

NAISMITH - MARTIN

MR. KUSHAN: Objection foundation.

A. So native PH20 or single site mutations or multisite mutation?

Q. (BY MS. MARTIN): Let's start with native PH20.

A. So I would assume it is stably folded and I would run an assay.

Q. Okay, like C-Giga 3 PH20?

A. Yes, I would assume it was stably folded and active.

Q. Why?

A. Because there had been literature where people had purified it all the way down, purified it and assayed it or HYAL1, I should say, had been purified and tested activity, PH20 also. I think Dr. Simpson had looked at purified PH20.

Q. If I hand you a tube of a solution and I tell you that it has SEQ.ID NO:3 PH20 in it would you assume it is properly folded?

MR. KUSHAN: Objection foundation.

A. You have PH20 in a tube?

Q. (BY MS. MARTIN): In solution.

A. In solution. Did you purify it?

Q. I don't know, I am just handing it

to you. Would you assume it is properly folded?

A. Does it have 8 Molar urea present?

Q. No, no urea.

A. Did it come from the supernatant in the assay.

Q. Yes.

A. Then I would assume it would be active and properly folded.

Q. Would you have to do any testing or would you just assume?

THE COURT REPORTER: Sorry, did you say "active" or "Then I would assume it would be"?

THE WITNESS: Active and properly folded.

Q. (BY MS. MARTIN): Okay, why?

A. Because we knew from the literature that SEQ.ID NO:3 was active and had been isolated and so I would have a reasonable expectation the sample made the same way and your supernatant would be natively folded.

Q. Is it possible for SEQ.ID NO:3 PH20 to unfold?

A. We discussed if you put it in 8 Molar urea it could unfold.

NAISMITH - MARTIN

Q. What if it is just still in the supernatant?

A. There is no discussion in the patent about that at all. There is no discussion in the common disclosure on whether people tested anything about unfolding of PH20 except in the example where they added this denaturing compound.

Q. But just by the virtue of the fact it is a protein would you expect that it is folding and unfolding itself?

A. Sorry, I understand the theme there to your question. So proteins are sort of metastable, and over time most, but not all, proteins start to aggregate and unfold over time. But that might be days or weeks.

Q. Okay. So if you were PH20 how can you -- Would you consider PH20 to be properly folded if it is in the process of unfolding and aggregating or degrading or whatever?

MR. KUSHAN: Objection form, objection foundation.

A. Sorry, so I now have a sample you have given me and you are telling me the protein is unfolding in this sample?

NAISMITH - MARTIN

Q. (BY MS. MARTIN): Well, I am saying I don't know, like, how do you know whether -- So to be clear, if it is the case that as a protein it is eventually going to unfold, how do you know that the PH20 in that vial has not started to unfold?

A. I see. So I would ask you how you prepared the vial and I would look at your conditions, buffers, times, how you purified it etc. or in this case it was supernatant, you didn't purify it, you just took it. So I would ask all those questions, what temperature you did at, for example.

Q. Okay. Do you know when PH20 would start to unfold?

A. In a timeframe, no, I've never, haven't looked at that data. I think I can assume with a reasonable certainty that it won't unfold over a 12-hour timeframe.

Q. What if it's been, you know, 48 hours?

MR. KUSHAN: Objection foundation.

A. I haven't worked enough with PH20 to answer your question, and I don't recall the

data to give you a clear and accurate answer to 48 hours but I would certainly agree that 24 overnight it would be fine.

Q. (BY MS. MARTIN): Okay.

A. It may be discussed in the publications of Dr. Simpson, I don't recall.

Q. So the testimony that you gave in terms of proteins being metastable and eventually subject to unfolding and aggregation, was that known in 2012?

A. So proteins -- "metastable" is an imprecise definition, but proteins are marginally stable. That was well known by 2011/2012.

Q. Okay.

A. As a result a POSA would expect if you put most, but not all, proteins in solution and left out on the bench for a couple of weeks the protein would -- there would be aggregates and misfolds and all sorts of stuff there.

Q. When you saying "aggregates" in that context, is that something that you could see?

A. You could test to find, yes, you could do analytical methods to find if there was

aggregates, yes.

Q. But I'm just saying, like, is it going to be aggregated enough that it is going to precipitate and you can just see it, like in the tube?

A. It depends on the concentration you have and whether you are using plastic tubes. You can certainly imagine conditions where you would see some turbidity.

Q. Okay. If you have a PH20 supernatant that has been left out on the bench for three days, you don't know whether that PH20 is properly folded or not, do you?

A. We are back to the native PH20 SEQ.ID?

Q. Yes SEQ.ID NO:3?

A. 3D, as I've said, I would struggle to have hard and fast data to know. I would have to go back and look in the literature to give an answer to that question. I don't -- it was active at three hours. If I could do an assay at three days I could tell you straightaway.

Q. Is that because if it's still active then you would conclude that it is properly

folded?

A. Correct and if it has the same, so whatever my number was, one, if at three days measured one then I would know that the protein has remained all of it fully folded and native.

Q. And what if it is, you know, only half -- only like point 5?

A. So, now we are into the realms of speculation. So in the supernatant you could have proteases present, it could have degraded the protein. So that could be a cause and there are ways to test that. It could have unfolded that we are talking about. It could have interacted with something in the plastic and the buffers and stuff. So you would want to do quite -- To answer your question precisely there needs to be other experiments done.

Q. Okay. So you don't know whether or not it is properly folded?

MR. KUSHAN: Objection foundation.

A. Sorry, so what is properly folded?

Q. (BY MS. MARTIN): The PH20 in your three-day old tube?

A. So if I have measured activity at

point 5?

Q. Mmm-hmm.

A. The narrow assumption is that half of it is properly folded.

Q. Oh, okay, I see. Okay. Now when you are testing supernatant the concentration of PH20 in the supernatant is going to vary from sample to sample; right?

A. Well, that is an assertion, I think. I don't know that there is -- you might, it is possible, yes.

Q. So you are expressing PH20 -- let's say you are expressing PH20 in different cell lines; okay?

A. Would I get different amounts of protein is your question?

Q. Yes.

A. So if I was expressing at different cell lines I could get different amounts of protein.

Q. So you can't just assume that all of the supernatants have the same concentration of PH20, right, if they came from different cell lines?

NAISMITH - MARTIN

A. If they came from different cell lines I could not assume without some form of normalization that they had the same concentration in them.

Q. Okay. What if I gave you a PH20 SEQ.ID NO:3 supernatant from a Chao cell and then a SEQ.ID NO:3 from a bacterial cell: would those have the same fold?

MR. KUSHAN: Objection foundation, objection to form.

A. So you have given me two separate tubes. Have you measured activity on them or they are just two tubes?

Q. (BY MS. MARTIN): They are just two tubes.

A. How did you -- Did you set up a secretion signal in both so that they should both secrete into the supernatant?

Q. Yes, let's assume that we know that there is actual PH20 in both of the tubes. But one, yes, do you know if they are the same fold?

A. You would expect that to be true, you would have a reasonable expectation that that would be true and they would both be natively

NAISMITH - MARTIN

folded and active.

Q. Okay. Does PH20 need to be glycosylated in order to be active?

A. Does PH20 ----

Q. Need to be glycosylated in order to be active?

A. There is some data in that where they've knocked out some of the glycosylations. So we could turn to it. This is single and double triple mutant, so they made an inactive form by I think a triple mutant but they retained activity with at least one of the single site ones if I remember. But it is in my report, we can go to it.

Q. Okay. If it were the case that you needed to have glycosylated -- PH20 needed to have glycosylation to be active, would you expect that the PH20 from a bacterial cell would be active?

A. So is your requirement that bacterial cell doesn't, so no glycosylation at all?

Q. Correct.

A. So your statement is that it is known that if you have no glycosylation it is

inactive?

Q. Correct.

A. In that case a bacterial cell would not give an active protein.

Q. Okay. So if have your unglycosylated inactive PH20 is it still properly folded?

MR. KUSHAN: Objection foundation.

A. So how did I get my unglycosylated inactive protein? It's in the bacteria?

Q. (BY MS. MARTIN): Yes, or secreted from the bacteria?

A. Bacteria don't secrete.

Q. Okay, then I don't know.

A. Sorry.

Q. It's from a bacteria.

A. So you wouldn't know is the answer ----

Q. Okay.

A. ---- because the machinery for folding the protein is very different in eukaryotes and prokaryotes.

Q. Oh okay.

A. One of the hindrances in structural

biology is that bacteria are great for making proteins but they are limited in their ability to fold complex human proteins.

Q. So before I started asking you about glycosylation, you said that a POSA would expect that PH20 from a bacterial cell would have the same fold and activity as PH20 from a Chao cell?

MR. KUSHAN: Objection foundation.

A. I don't remember saying that. I thought you said Chao. I don't recall saying that. We can go to the transcript if I made an error. I would have assumed we were talking about human or mammalian cell lines. My apologies, if I have. I don't remember saying that I could make it in a bacterial cell. I think you said cell lines.

Q. (BY MS. MARTIN): Yes, I said what if I gave you a PH20 SEQ.ID NO 3 supernatant from a Chao cell and a SEQ.ID NO 3 from a bacterial cell, would those have the same fold?

A. Okay, I misheard your question and I apologise because I said you can't secrete PH20 ----

NAISMITH - MARTIN

Q. Okay.

A. ---- from a bacterial cell. My apologies.

Q. So how do you get PH20 from a bacterial cell?

A. Well, you would have to burst open the bacterial cell and get the protein there, and you would want to assay it first and foremost.

Q. So you don't know whether or not it's properly folded if it came from a bacterial cell?

A. I would do an assay to work out whether it was properly folded or not.

Q. And how would you do that?

A. In the teaching of the patent I would take the supernatant from my burst cells and I would do an assay with it.

Q. Okay. And if it is active then you would assume it is properly folded?

MR. KUSHAN: Objection foundation.

A. So, if it is active, demonstrates activity, I would want to check my concentrations and do controls. But if the protein, I'd done all my controls, was active I would assume the active

NAISMITH - MARTIN

component of the protein was properly folded.

Q. You would assume the active component of the protein was properly folded?

A. Yes.

Q. What is the active component of the protein?

A. So you could have a situation in bacterial cells where a portion of the protein was folded and active and a portion of the protein that was unfolded and inactive.

Q. So part of the same protein? Are you talking about a singular structure and part of it is active and part of it is not active?

A. I am not talking about a single structure, I'm talking about a single sequence.

Q. Oh, okay.

A. A single sequence can be both folded and can be unfolded or aggregated.

Q. Okay.

All right, we've been going for over an hour. Do you want to take another break?

MR. KUSHAN: Sure.

MS. MARTIN: Do you want to break for lunch? Can we go off the record?

NAISMITH - MARTIN

THE VIDEOGRAPHER: We are going off the record. The time is 11.52 a.m.

**(A short recess from 11.52 a.m. to 1.37 p.m.)**

THE VIDEOGRAPHER: We are back on the record. The time is 1.37 p.m.

Q. (BY MS. MARTIN): Dr. Naismith, did you discuss your deposition testimony with counsel during the break?

A. No, I did not.

Q. Okay. So can we turn to paragraph 43 in your declaration?

A. This is on page 19 I think?

Q. Yes.

A. Yes.

Q. And do you see the sentence that starts: "Instead, I read the claims as a whole"?

A. Yes, I can see that sentence.

Q. So it says: "Instead, I read the claims as a whole as being directed to modified PH20 polypeptides that (i) contain an amino acid sequence that satisfies the sequence identity claim parameters, (ii) meet the position 320 substitution requirement, (iii) have folded to adopt the same tertiary structure found in the

native state of the wild-type PH20 protein and (iv) are enzymatically active."

A. Yes.

Q. Okay. So is it your interpretation of claim 1, which appears to be on page 18?

A. Yes.

Q. That the genus modified PH20 polypeptides covered by claim 1 is limited to the modified PH20 polypeptides that have the four characteristics that I just read off from your paragraph 43?

A. So I believe that is what the claims read as a whole actually direct you to. So that is what is covered. So there are tests that you have to meet in the claims that fall within the mathematical boundary of 95%. There is a modification of 320 set forth in SEQ.ID NO:3 and that modification has to be one of these four residues.

Q. And then you have two other requirements?

A. That is correct. They talk about activity and they have a stable tertiary structure that resembles the native fold.

NAISMITH - MARTIN

Q. Okay. And so it is your testimony that claim 1 requires the PH20 to have folded to adopt the same tertiary structure as the native PH20 and also are enzymatically active; is that right?

A. It is my testimony that when you read the claims as a whole in light of the common disclosure they are directed exactly to that, that the additional two things are that it is the same fold as the native protein and that it is enzymatically active.

Q. Okay. So what limitation in the claim tells you that the claim proteins have to have folded to adopt the same tertiary structure found in native state of the wild-type PH20 protein?

A. So the fold as Dr. Petsko talks is that the human PH20 is a protein from a stable tertiary structure under physiological conditions it is. And he, like me, for the active subset of the proteins if he covers all of them but I think it only covers active subset, we would believe those to have the same fold as the native protein, and be enzymatically active.

NAISMITH - MARTIN

Q. Is it your testimony that Dr. Petsko is interpreting the claims as limited to active folded PH20s?

MR. KUSHAN: Objection foundation.

A. So Dr. Petsko and Dr. Simpson appear to include all of the different diversity that could be envisaged under the mathematical boundaries but Dr. Petsko does say when I am talking about PH20 I am referring to the active PH20.

Q. (BY MS. MARTIN): So ----

A. It's as a folded protein. It says on page 113, POSAs think of PH20 as a folded proteins, as a folded protein.

Q. So when he said that, was he talking about interpreting the claim as limited to folded PH20s?

A. I would like to turn to his deposition, sorry, his expert report, but what I am showing is that Dr. Petsko certainly, in my opinion, associated PH20 as a folded protein.

Q. So I would like a "yes" or "no": did Dr. Petsko interpret the claims as limited to folded PH20?

NAISMITH - MARTIN

MR. KUSHAN: Objection form and objection foundation.

Q. (BY MS. MARTIN): Or I guess, "Yes", "No" or "I don't know", sorry?

MR. KUSHAN: Objection form, objection foundation.

A. Dr. Petsko in part said that he -- that it covered the mathematical fence but elsewhere in his report he did focus on the folded protein.

Q. (BY MS. MARTIN): Okay. Okay, so it sounds like you are agreeing with me that Dr. Petsko interpreted the claim as covering all of the sequences but then he also said somewhere that the PH20 refers to the folded protein. Am I getting that right?

MR. KUSHAN: Objection foundation, objection as to form.

A. So I think Dr. Petsko -- So my opinion is that it applies to an active folded from the protein. Dr. Petsko opined that initially that his opinion was it covered mathematical things but then later on in his deposition he does say: "POSAs would understand

that ... variants with ... a small number of substitutions" -- which is the 95% -- "[would] adopt the same general tertiary structure ...". So he is defining here what PH20 variants are.

Q. (BY MS. MARTIN): Can you tell me where we you are reading from?

A. I am reading on page 59 paragraph 93.

Q. Okay, so it is your testimony that Dr. Petsko initially found that the claim covers "mathematical" things not proteins, but then later in his deposition he admitted that he would understand that the claims are limited to just the folded proteins?

MR. KUSHAN: Objection foundation, objection to form.

A. So it is my testimony that Dr. Petsko talks about PH20 in the same way that I do later in his declaration. I would have to go through the declaration to see what -- in order it was set. But Dr. Petsko does say that: "POSAs think of PH20 as a folded protein ...".

Q. (BY MS. MARTIN): So are you basing your interpretation of the word "PH20" in the

NAISMITH - MARTIN

claim on Dr. Petsko, "Yes" or "No"?

MR. KUSHAN: Objection foundation, objection to form.

A. I am using the common disclosure to define PH20.

Q. (BY MS. MARTIN): Not Dr. Petsko?

A. I have used the common disclosure of Dr. Petsko as I said used it in the same way I did but I am using my definition based on the common disclosure.

Q. Are you trying to give Dr. -- I am sorry, strike that. Let me start over. Are you trying to interpret the claims on Dr. Petsko's behalf?

A. No, I am not.

Q. Do you know as a matter of fact, sitting here right now, what Dr. Petsko thinks the claims cover?

A. I can read what Dr. Petsko produced as his opinion and I have also pulled out a paragraph where he talks about the word PH20 meaning a folded protein, and that is the information I have.

Q. Yes, okay and that paragraph is not

talking about what the claims cover; right?

A. I would have to see the context in the deposition, but it is, he naturally defaulted to -- he did say POSA's PH20 variants with a small number of substitutions, PH20 is a folded protein so he talks about a modified protein, modified polypeptide.

Q. That is your interpretation of what Dr. Petsko is construing?

A. I believe I am stating what Dr. Petsko did. My interpretation is based on the common disclosure.

Q. So is it your testimony that an inactive PH20 variant does not fall within the scope of claim 1 of the 600 patent?

MR. KUSHAN: Objection to form.

A. Does it have the M320 substitution test?

Q. (BY MS. MARTIN): Yes.

A. Then it is my interpretation and opinion that that would be outside of the scope of the claims as I read them.

Q. If you made a D320K SEQ.ID NO:3 protein and let's say you also mutated position,

NAISMITH - MARTIN

one of the catalytic residues such that you killed activity, would that protein fall within the scope of claim 1, even though it meets the sequence identity parameters?

MR. KUSHAN: Objection foundation, objection as to form.

A. So I didn't catch the last bit of your, could you just read that question, back, sorry, I didn't catch something you said about the claims.

Q. (BY MS. MARTIN): So SEQ.ID NO:3 with the D320 mutation and let's say you also change one of the catalytic residues so you kill activity, okay, would that protein, even though it meets the sequence identity limitations, would that protein fall within the scope of claim 1?

A. It is my opinion it would be outside the scope of claim 1 because it's not active.

Q. Okay. And that is because you interpret modified PH20 polypeptide to require activity; right?

MR. KUSHAN: Objection foundation, objection as to form.

NAISMITH - MARTIN

A. So it is because taking the disclosure as a whole, my opinion is the disclosure as a whole and the POSA is supposed to read the interpretation as a whole and then interpret the claims in a way that is consistent with the common disclosure in this case, I believe that is where the activity flows from the requirement.

Q. (BY MS. MARTIN): You are not a lawyer; right?

A. I am not a lawyer.

Q. Do you understand that in order to limit the scope of a claim, you have to be able to point to some language in the claim in order to limit the scope of the claim?

MR. KUSHAN: Objection form, objection to foundation.

A. So my understanding of the legal test that was explained to me was that I had to approach this with a POSA's lens 2011/12, read the common disclosure, read the claims and interpret every word in the claims in light, through the lens of what is communicated in the common disclosure. And I set out in detail in my report

NAISMITH - MARTIN

the reasons why I believe that, a POSA would read the claims having read the common disclosure and believe them to be directed to the folded protein and active protein.

Q. (BY MS. MARTIN): And the -- I am sorry, go ahead, I did not mean to cut you off.

A. And active protein.

Q. The words in the claims that you are relying on to support your interpretation of the patent fully folded and active, the words specifically is PH20; right?

MR. KUSHAN: Objection foundation, objection to form.

A. So I read it, so we have the requirement from modified. We have, in one of the things that we know is the one modification that is required are active mutations, so those substitutions at 320 are all active. The field of the invention talks about importance of activity. When we read through the common disclosure we are bucketing them as active or inactive mutations. Then we move on to, additional mutations in the patent talks about importance of, and this is set out in my point 72: "[a] modified PH20 polypeptide

NAISMITH - MARTIN

..." sorry, I use page 72: "[a] modified PH20 polypeptide can have up to 150 amino acid ..." so a modified PH2 polypeptide: "... replacements, so long as the resulting modified PH20 polypeptide exhibits hyaluronidase activity." Dr. Hecht offers essentially the same opinion. We both read in light of the common disclosure that is claims are directed to active form of the protein that is properly folded.

Q. (BY MS. MARTIN): You agree that the common disclosure uses the phrase modified PH20 polypeptide to refer to inactives as well; right?

MR. KUSHAN: Objection foundation.

A. Could you point me to that precise phrase please? I think I just easier for me to look at the data.

Q. (BY MS. MARTIN): Are you familiar with that disclosure?

A. The common disclosure.

Q. The part of the common disclosure where it uses modified PH20 polypeptide to refer to inactives?

A. So I would like to just refresh my

memory because obviously this is an important test, an important part of the thing. I would like to go to the declaration, I believe there is certainly a discussion of inactive mutants of modified PH polypeptide and you are specifically saying inactive mutants.

Q. Okay.

**(Previously marked Exhibit 1026 was referred to)**

Q. So if you go to page 86?

A. Page 86 which I think, column 73.

Q. Page 86 line 20, page 86 of this 86?

A. Oh, the bold central 86.

Q. Yes. I guess Merck's page 99, so that the record is clear.

A. Column 86, yes, page 99.

Q. Yes, around line 20?

A. Yes, it begins: "In particular, provided herein are PH20 polypeptides that exhibit hyaluronidase activity ...".

Q. The next sentence?

A. "Also provided are modified PH20 polypeptides that are inactive, and that can be used, for example, as antigens in contraception

vaccines."

Q. Yes.

A. That sentence.

Q. Yes, that sentence includes the word PH20; right?

A. So my understanding is that in forming my opinion I was to consider the disclosure as a whole, and for my opinion, so that this phrase which is "inactive" does appear in the report, I note that in paragraph 109. But as I understand the legal test my opinion should be based not just on an isolated collection of words but on the reading of the disclosure as a whole to understand what the claims are directed to.

Q. Yes, that didn't answer my question. The word PH20 appears in the sentence that we just read, right, at line 21 on page 99, it says: "... modified PH20 polypeptides that are inactive ..."; right?

A. So, what is, the question is "PH20" word appears here?

Q. Yes.

A. Yes, "PH20" word appears here.

Q. The phrase, specifically the phrase

"modified PH20 polypeptides" appears in that sentence; right?

A. It does.

Q. And then it says: "... that are inactive ..."; right?

A. It does say that.

Q. So this sentence is using the phrase: "modified PH20 polypeptides" to refer to polypeptides that are in fact inactive?

MR. KUSHAN: Objection form, objection to foundation.

A. So, I read the sentence and I said that my test was not to read isolated sentences, but as I read the sentence it is specifically defining a "modified PH polypeptide" that is inactive.

Q. (BY MS. MARTIN): So within the set of all modified PH20 polypeptides, some of them are active and some of them are inactive; right?

MR. KUSHAN: Objection foundation.

A. What set are we defining, sorry?

Q. (BY MS. MARTIN): I am just, like, the phrase "modified PH20 polypeptide", it can be active or it can be inactive?

NAISMITH - MARTIN

MR. KUSHAN: Objection to foundation.

A. Sorry, you said a set, I am interested to know what set? As I read the claim the set of proteins that are covered are active, and I, so what set are we discussing?

Q. (BY MS. MARTIN): Okay. Not in the context -- okay, sorry, let me back up, let me try this a different way.

The patent defines the phrase "modified PH20 polypeptide", right, that is a defined phrase?

A. It appears, yes.

Q. Okay, and as that phrase is used in the specification, it can include active polypeptides and inactive polypeptides; right?

A. So, the phrase is used in this sentence coupled to the word "inactive".

Q. Yes, so there are some modified PH20 polypeptides that are inactive; right?

A. One can make PH20 polypeptides that are inactive and I said I don't believe they are covered in my understanding of the test for the claims.

NAISMITH - MARTIN

Q. Okay. The inventors are using the phrase "modified PH20 polypeptide" in this sentence to refer to inactive polypeptides; right?

A. I would say the inventors are specifically defining, appearing in the patent are PH polypeptides that are inactive and those appear in the data table where you have classified various single mutants as inactive.

Q. Okay, but they are still calling them PH20 polypeptides, right, they are not calling them something like a different type of protein?

A. They use the word "inactive" when they describe it to signal that they are talking about inactive PH20 polypeptides.

Q. And they also use the word "active" when they are talking about active PH20 polypeptides; right?

A. Not every time and in the claims you talk about modified PH20 polypeptide and I set out my reasoning for the various reasons but one of the things is the specific mutations that are later in the patent found as active mutations, at 320.

NAISMITH - MARTIN

Q. So it is your testimony that, yes, the inventors use the phrase "modified PH20 polypeptide" to refer to inactives, but because they use the word inactive when they are talking about inactives, therefore "modified PH20 polypeptide" does not include inactives?

MR. KUSHAN: Objection to foundation, objection to form.

A. So I don't think that is what I said. So, if we -- what I said was that the claim language is the one we are talking about and it is my view in the claim language for the reasons we went through and these are on page 70-71 of my report, when you read the common disclosure you conclude that the claims are directed to active proteins of a particular modification, they are stably folded. You are asking sentences that appear and you asked me about modified PH polypeptides that are inactive. I agree. That is what they have said modified PH20 polypeptides that are inactive. I don't believe that phrase in isolation, I did not use that phrase to render my reading of the claims.

Q. (BY MS. MARTIN): Okay. So you

were not interpreting the "phrase modified PH20 polypeptide" in the claims, you were not relying on that phrase to conclude that the claim requires active proteins; is that right?

A. No, that is not what you are supposed to do. The legal test is based on a reading of the common disclosure, and through the eyes of a POSA, then that is how you read the claims. Every word has meaning in the claims.

Q. Okay. So you are interpreting the claim as a whole based on your reading of the common disclosure, you are not looking for individual words in the claim to figure out which words require activity?

MR. KUSHAN: Objection foundation, objection to form.

A. No, again that is not what I did. So, I read the common disclosure as a whole and I point out that there are more than just words that we specifically have these active modifications at 320. We talk about classifying these single site mutations as active or inactive. We also talk about a threshold for activity that is 40% and important. Then, particularly when we

talk about modified polypeptides, as I set out in 72, can have up to 150. Now that is not an exhaustive interpretation of all the things that I read in the common disclosure. But these are the things that I helped rely on to reach my opinion.

Q. (BY MS. MARTIN): Can you point to a specific word in the claim and tell me that that word is what you are anchoring your requirement for a properly folded and active PH20; yes or no?

MR. KUSHAN: Objection foundation, objection to form.

A. As Dr. Petsko said a PH20, a POSA thinks of it as an active form of the protein.

Q. (BY MS. MARTIN): So, yes, and the answer to the question is, yes, PH20 is the word in the claim?

MR. KUSHAN: Objection foundation.

A. So my understanding is I have to read the claims as a whole, one of the ways it shapes my reading of the claims as a whole is to look at all the words in the claim, there are, PH20 is thought of, as Dr. Petsko said, a folded active protein. But I did emphasise that the site directed mutants that are reported also point me

towards an active form of the protein.

Q. (BY MS. MARTIN): Now, the protein can also include other mutations that would inactivate the protein; right?

MR. KUSHAN: Objection foundation.

A. But I don't believe the ones that are inactive are covered by the scope of the claims.

Q. (BY MS. MARTIN): Does the claim -- does the language in the claim specifically exclude mutations that inactivate the protein?

A. I believe the language of the claims is directed to the set of proteins which is active enzymatically and properly folded and has this additional mutation at D320.

Q. Okay, so the word PH20 and the specific mutational requirements at position 320 are the two portions of the claim that you are relying on to read in activity and proper folding; right?

A. No. I am relying on the common disclosure. You have to read the common disclosure as a whole to form this opinion. That is my understanding of the legal test that has to

NAISMITH - MARTIN

-- so I read the common disclosure and then read the claims.

Q. That is respectfully, that is not actually the legal standard. So ----

MR. KUSHAN: Why don't you ask questions and not make statements, okay?

MS. MARTIN: Let's make sure that we are being really clear here.

Counsel, I would ask you not coach the witness during the testimony.

You understand that you interpret the claims first based on the language of the claims, right, not by first reading the disclosure and then figuring out what you think the inventors wanted to claim.

A. I did not say that, I think, my understanding is that you read the claims and make sure that you understand them in light of the common disclosure, so they have to be consistent with the common disclosure.

Q. All right. Let's look at -- strike that. Do you read claim 1 as being directed to a modified PH20 protein?

A. Amongst other things I believe it

is directed to a modified PH20 protein, but there is a fence drawn around a mathematical sequence identity and then it will have a modification at 320 and it has one of these four active mutations.

Q. Does the word "protein" to you signify that it is a three-dimensional or a folded structure?

A. When we think about PH20 protein we think of it as a folded protein and we already discussed that people use polypeptide and protein interchangeably.

Q. So, do you think that what the inventors were trying to claim was a PH20 protein that is active?

A. So I believe that the claim covers PH20 proteins that are active with a mathematical fence around them that is defined in A and that they in addition have to have one of four known active mutations of aspartic acid 320.

Q. Do you think that the claim should have said PH20 protein?

A. I didn't form an opinion what the claim should or could have said. I was only asked to evaluate the, the common disclosure, I should

NAISMITH - MARTIN

say, in the claims as they were rendered.

Q. Are you reading the claim to require a stably-folded PH20?

A. Yes, I do say that, that you would expect it to be stably folded.

Q. So if you made a SEQ.ID NO:3 PH20 that had a D320K mutation and you left it on the bench for three days, would that protein satisfy the claims?

MR. KUSHAN: Objection foundation, also objection to form. Go ahead.

A. Is the protein still active?

Q. (BY MS. MARTIN): I don't know.

A. So I did, when we talked about how long stability was I gave you an answer which is about, you know, roughly 12 hours. So if it is still active at 12 hours I would say it was properly stable and folded.

Q. If it is still active after three days on the bench, then you would say it meets the claims?

A. So it is modified, it has the mutation?

Q. Yes.

NAISMITH - MARTIN

A. And it is still active?

Q. Yes.

A. At three days; yes, that is within the scope of the claims.

Q. Okay. What if it is no longer active at three days; does it meet the claims?

A. I said the other test I would use is around eight hours to 12 hours, so you said three days, so it could well meet the test. I don't know because I didn't have activity at the time of eight to 12 hours. I said that would be kind of the time frame I would think of.

Q. If I -- I am asking a slightly different question, which is if I hand you a sample of PH20 that has been sitting, that as the D320K mutation, SEQ.ID NO:3 that has been sitting on the bench for three days and is no longer active, would that meet the claims?

A. So it is no longer active at three days?

Q. Yes.

A. Was it -- I asked you, was it active over eight hours which is where I, the eight to 12 hours that I would say that that is

the kind of time frame that I would, that I use for my stably folded. So to be clear, if it was active in that time frame, eight to 12 hours, it would be stably folded. It could well be stable for 12 hours and then subsequently unfold over three days, two and a half days later.

Q. Does it meet the claims when it is no longer active after three days?

A. It could have met the claims if it was active, meets my definition of stable which I told you was around eight to 12 hours, so if it was still stably active, if it was still active at eight to 12 hours then it is within the scope of the claims.

Q. Okay, I am trying to understand if it is no longer active, right, as it sits at three days?

A. I don't have the information to give that opinion because you didn't give me the data eight to 12 hours.

Q. Why does the eight to 12 hours data matter? I am asking you if after three days, literally the sample I am handing you, if that literal sample in this hypothetical, if that

sample meets the claims?

A. And I have said that because of how we discussed what stability would be, that I don't have the information to give you an accurate answer. It could have been stable at eight hours and that would be within the scope of eight to 12 hours within what I perceive, my opinion is the scale of the claims. It could subsequently have unfolded after that, but it was within the scope of the claims.

Q. Okay, so understanding, as long as it was stably folded for eight to 12 hours and active then it meets the claims, even if it then unfolds and is no longer active?

MR. KUSHAN: Objection, foundation.

A. Not quite what I said. We talked about stability and I said looking in the patent they have that overnight. So, if it is within there overnight, eight to 12 hours, then if it is active then it is, falls within the scope of the claims.

Q. (BY MS. MARTIN): So it doesn't matter whether or not the sample is active after three days; if it was active within eight hours

that is sufficient for it to infringe?

MR. KUSHAN: Objection foundation, objection relevance.

A. What do you mean, I don't know the legal test infringement you are asking me.

Q. (BY MS. MARTIN): Let me ask it without the word infringe. If I give you a D320K sample from SEQ.ID NO:3 that is no longer active because it sat out too long, you are saying that it would still meet the claims if it was active within -- if it showed activity within eight hours?

MR. KUSHAN: Objection foundation, objection to form.

A. So, if you -- so this sample we have -- it is, when we say active eight to 12 hours, it behaves, you can measure activity, that is the kind of thing that I have said that I would interpret and POSAs as a stably-folded protein. But I did say that, you know, we can stretch that window either side, it is a little fuzzy. So that would be active and within the scope of the claims.

Q. (BY MS. MARTIN): That is the case

even if it is no longer active at some point in time, because it eventually degrades?

A. Yes, you can, proteins will all, most of them anyway, as we said, would eventually degrade at some point, it would be, it is not a test that I imposed that they have to be stable forever, I used because the patent talks about this overnight, that seemed a reasonable place to think of the stability.

Q. Okay, if the patent data is provided in examples 3 and 4 based on a 90-minute incubation time, so based on that test, you don't know whether the protein is stable after eight hours; right?

MR. KUSHAN: Objection foundation.

A. So it actually says there are varying times and the samples were sometimes frozen and then thawed, so you would have an expectation that for the single mutations and I'm careful to restrict here to the single mutations, that if they were active in the assay they would be active at eight to 12 hours. That would be, it is not a foolproof, but you would have a reasonable confidence that all of those active

NAISMITH - MARTIN

mutants in the assay, single mutant would be active at eight to 12 hours.

Q. (BY MS. MARTIN): So let's say the D320K mutant that is tested in the patent, if it was no longer active after eight hours would it meet the claims?

A. So what is a mutant of the D320?

Q. Just D320K, that is the mutant?

A. I would have a reasonable expectation that it would be active, I would not have any reason to not think it would be active based on the common disclosure, so I would believe it to be active. It is a single mutation of a stably-folded protein which has activity. As a single mutation I would confidently expect it to be active at eight hours, but you are correct the patent does not test stability in that way.

Q. If I am telling you, let's say just hypothetically if it turns out that it is not stable after eight hours, would it meet the claims?

MR. KUSHAN: Objection foundation.

A. So, that would be like I don't think that is, I would just be very surprised

if that was true base on my understanding. It is a hypothetical that I don't recognise.

Q. (BY MS. MARTIN): Okay. If I made D320K SEQ.ID NO:3 and put it in the 8 molar urea, such that it is no longer a stable folded protein, does it meet the claims?

A. Yes, it meets the claims because you told me you've made the D320K that we know to be active. You subsequently inactivated it by choice, but it would meet the claim.

Q. So it doesn't -- but the version of it, the version of it that I'm talking about is not active, so how could it meet the claims?

A. Because -- so is your thing, if I handed me, and I've now put the PH20, D320K inside the 8 molar urea and that is my new sample.

Q. Yes.

A. I would say that the protein was made in the active form and you have inactivated it and as a result you have changed what the protein is by deliberately inactivating it.

Q. But does that now deliberately inactivated protein meet the claims?

A. A question I haven't considered,

that you are deliberately trying to prepare samples in 8 molar urea to make them inactive to test claim language; I don't recognise that example in the patent or find anything in the common disclosure or around that. So I'm -- that is just not a hypothetical I considered.

Q. So you don't know whether or not the unfolded D320K SEQ.ID NO:3 PH20 meets the claims?

A. So unfolded?

Q. Let me be as precise as possible here. You don't know if the sample of D320K SEQ.ID NO:3 PH20, that is unfolded because it is an 8 molar urea meets the claims?

A. I said I hadn't considered that specific example. I'd like to think about it before I give you an opinion. I think if you were -- I think given that you have deliberately modified that you would have believed that the original protein met the claims, so that's the physical thing, so then it does meet the claims. Now you've done something to it to not meet -- I mean, you could set it on fire or you could do anything to it, but the protein itself met the

claims, the modified PH polypeptide that you describe did meet the claim, so I do think it meets the claims.

Q. I am trying to be really precise here about the tense you are using because you are saying it met the claims now it sounds like it may or may not no longer -- may or may not meet the claims now it is unfolded in urea?

A. I don't think that is what I am trying to say. I appreciate I had not considered this hypothetical, so apologies if I'm not being clear. The protein, the modified PH polypeptide, in all the disclosure is talked about in a secreted form of the protein. That is how you imagine the protein and how it is discussed. So, in our example that meets the claims, it is active as it is produced. You are then saying, "Well, if I deliberately had some way to destroy the protein", in your case "with 8 molar urea, would that still meet the claims?" My answer is the physical modified PH20 polypeptide would meet the claims, in that scenario.

Q. Even though it is unfolded and inactive it would still meet the claims?

NAISMITH - MARTIN

A. It is specifically because you have done something to the protein which was active and you have specifically unfolded it, but the modified PH20 polypeptide is still itself active first, so I do believe it meets the claims.

Q. Sorry, you said the PH20 itself is active, then what was the word?

A. When it is made and pure, in the example we are talking about is stable and therefore it meets the claims. You are then saying if I choose to inactivate it would it still meet the claims? My answer is, a modified PH20 polypeptide that is stably active for eight hours meets the claims. You can think of many, many ways to destroy the protein, but I don't think it changes that the protein meets the claims as long as it's active stably over the time period I gave you, as a guide.

Q. So it sounds like, if I am understanding correctly, if it is possible to produce that sequence in a way that it will fold into a stable active protein, then the sequence meets the claims and whether -- is that right?

MR. KUSHAN: Objection foundation.

NAISMITH - MARTIN

A. So my contention is that my opinion is that it is not possible to identify that sequence within those sequences within the vast array that could be mathematically described to identify the particular combination of mutations, it would give you that. There are single site mutations that are clearly active and my belief and I said is, that I would expect them to be folded stably and active, because we measured activity.

Q. (BY MS. MARTIN): Okay, but the question again was as long as it is possible to make a sequence specifically SEQ.ID NO:3 with D320K to make that sequence such that it is properly folded and active then that sequence meets the claims; right?

MR. KUSHAN: Objection foundation, objection as to form.

A. So the claim does talk about PH20 but a properly folded PH20 with a D320K mutation that matches your mathematical description of 95%, that would be within the scope of the claims.

Q. (BY MS. MARTIN): If you introduce a second mutation ----

NAISMITH - MARTIN

A. Sorry, I would just like a glass of water before you ask your question. I do not want to be cheeky and interrupt you in the middle.

MR. KUSHAN: Can we take, like, a two-minute break because this thing has been buzzing in my pocket and I want to check it.

THE VIDEOGRAPHER: We are going off the record. The time is 2.23 p.m.

**(Short recess from 2.23 p.m. to 2.24 p.m.)**

THE VIDEOGRAPHER: We are back on the record. The time is 2.24 p.m.

Q. (BY MS. MARTIN): If you introduce a second mutation into your D320K SEQ.ID NO:3 protein that causes the activity to fall below 40%, let's say it's at 38%, does that protein meet the claims?

A. So how did I measure? Sorry, I just want to be clear. The patent classifies that as inactive, if I measured my activity by an accurate method at 38%, is that the question? The patent just says below 40% I don't know anything else but why it could have been inactive, unfolded, many different things, right, so we don't know that. So I just want to be clear what

NAISMITH - MARTIN

you are asking. Are you saying you have made a second site mutation, you have added one to D320 and you have measured reliably 38% activity.

Q. Yes.

A. Then that would fall within the scope of the claims.

Q. So if it is 38% active it would fall within the scope of the claims?

A. Yes.

Q. So you are not requiring the 40% activity?

A. No. The patent uses 40%, and the reason I pointed that out was because it seems very focused on very -- that is part of my understanding of reading the common disclosure is it directed towards the active forms but, no, 38% activity any, that is active protein; it is not as active but it is active.

Q. Okay, what if it is 21% active, reliably measured to be 21% active?

MR. KUSHAN: Objection foundation objection to form.

A. So we are back to my D3 fits the mathematical test, it has a D320.

NAISMITH - MARTIN

Q. (BY MS. MARTIN): Yes.

A. So, with those caveats you've now measured it and it is 21%.

Q. Yes.

A. And it has one additional mutation.

Q. Yes.

A. Yes, that is active.

Q. Okay. So D320K and SEQ.ID NO:3 with one additional mutation and it is 19% active; does that meet the claims?

A. Robustly measured 19%?

Q. Yes.

A. Yes, that is active.

Q. So what would you consider to be inactive?

A. So, I would use, I think Dr. Simpson, Dr. Petsko said below 10, I think most POSAs would feel around 5% is roughly knocking down by a factor of 10-20 would be beginning to be inactive. Although in Dr. Simpson's paper she is able to detect activity lower than that, I think it was 3%, she called it residual activity. But it was more activity than truly dead, which I think was a thousand fold

less.

Q. So, sorry, there are a lot of numbers in that answer and I'm not sure what the right number is. So you said around 5% but then you also said by a factor of 10-20, and I don't know.

A. Fold.

Q. Okay, a 10-20 fold?

A. Yes. So depending, so you know, I would say there would be some perhaps -- typically for POSAs thinking about enzyme mechanism inactive protein is more than 10 fold reduced and Dr. Petsko sort of says that less than 10%, some people would draw the line at 5%.

Q. Sorry I am getting confused with the fold and the percent. I want to make sure that we are on the same page.

A. Apologies, because we always think of orders of magnitude. Sorry, my apologies.

Q. Sorry, yes ----

A. Fold is an over used word here.

Q. So if it is more than 10 fold reduced, are you saying that like, I just want to make sure that I'm understanding, like, the

NAISMITH - MARTIN

relationship between fold and percent, sorry for my ignorance and stupidity with the math here, but are you saying if it is more than 10 fold reduced is that the same as, are you using that as the same as 10%?

A. It is less than 10%, exactly, 100 divided by 10 is 10 fold. I'm really sorry, scientists work this way. I'm sorry.

Q. Yes, I know. It is not you, it's me. Okay, so, all right, so 10% active or 5% active, that is, because I think that fold, for me fold, I'm having difficulty with fold, let's do percentages.

A. Okay, my apologies. I will try to stick with percentages which is the order of magnitude. So less than 10%, some people might say it is inactive. Most people would agree by the time you get to 5 it's inactive.

Q. Okay, got it. So is there, if you introduce a second mutation into your D320K SEQ.ID NO:3 protein and it's now 7% active, do you think that that would meet the claim?

MR. KUSHAN: Objection foundation.

A. So, the common disclosure doesn't

measure anything below that. Based if I had the assay that I believe was a reliable 7% personally I would tend to draw the line at 5%, but I'm, other POSAs may draw it below 10. So there a bit of, as I said, there is kind of a window there, but personally, in my opinion, I draw it at 5 and in my publications you will find that is usually where I draw the line.

Q. (BY MS. MARTIN): Okay. Now, so you would consider a 7% activity to be an active protein; right?

MR. KUSHAN: Objection foundation.

A. Are we talking about PH20 here?

Q. (BY MS. MARTIN): Yes.

A. With the mutations.

Q. Yes.

A. In my view if you could accurately measure 7% then it would, it would be within the scope of the claims because I would consider that active.

Q. Okay. Would you consider that protein to have a stable fold if it has 7% activity?

MR. KUSHAN: Objection foundation,

NAISMITH - MARTIN

objection to form.

A. It is just a single site mutation of the -- so it has two mutations only?

Q. (BY MS. MARTIN): Yes.

A. And I expressed it in the supernatant and done the same sort of experiment.

Q. Yes.

A. Although I used a more accurate assay.

Q. You use whatever assay you want so that you are happy with your 7% number?

MR. KUSHAN: Objection foundation.

A. So we are very much into the realm of the hypothetical, but in that arena I would say that would be classified as an active form, but I'm -- of the enzyme because of the, we made it in the supernatant, we purified it, it is only one single mutation beyond the D320K and it was reliably measured at 7% it would fit, in my view, the test I gave you was more than 5%.

Q. Okay, so I understand that with activity, but I am trying to understand if that protein is stably folded?

A. I would have a reasonable

expectation that it would be stable because we have only made one additional mutation, and I did say that I think the cut off for that is around 5, so I would have a reasonable expectation. I did say that people begin to get squirrely around 3, but I said the outer limit is 5 for that type of analysis.

Q. Again, I just want to make sure we are on the same page with the terminology. You said, we are talking about same example of the two mutations, that is 7% active and you would have -- you said you would have a reasonable expectation that it would be a stably-folded protein and I'm wondering, would it have the native fold?

MR. KUSHAN: Objection foundation, objection form.

A. So we have got D320K mutation, for example, and we have introduced a second mutation, the protein expresses in the supernatant we can measure activity at 7%. So in that case I believe that the protein would be properly folded and have the same properly fold that we see in the active form of PH20 because we've only made two mutations. However, I can imagine you could

select by either bad luck or design, design is very hard, a mutation that does alter, for example, a deletion could involve perturbing the structure and we talked about the alpha helix where you gave me the specific example. So I could imagine the structure being perturbed but still properly folded and active.

Q. (BY MS. MARTIN): So even if the structure is perturbed, it could still be properly folded and active such that it would meet the claims?

A. To meet the claims it has to be active, and that means it has properly folded but there could be structural differences between that.

Q. Okay. I just want to be very clear, what I am trying to understand is if there is a -- I understand the active part, right, what I'm trying to understand is if the stably folded portion of your opinion is a separate requirement such that you have to do a test to figure out if the protein is stably folded or has the native fold, is that a separate test you have to do besides the activity?

NAISMITH - MARTIN

MR. KUSHAN: Objection foundation, objection to form.

A. So what I'm saying is that because you have a good native structure SEQ.ID NO:3 which is stable and active, then if you make one or two mutations, then if that protein is still active in the way we've assayed it, then I have a reasonable expectation that it would be stable so I don't need to impose an additional test.

The issue comes where I start to try to combine mutations, say six or more, then my view is that there is, it begins to populate the space where there will be aggregated unfolded or unstable proteins.

Q. (BY MS. MARTIN): Okay, so let's talk about that. Let's just have a kind of clean slate for the hypothetical so that we are not confusing things. So let's say you have SEQ.ID NO:3 D320K and then you have introduced six more mutations into that sequence, okay. So your protein has a total of seven changes compared to the wild type; right?

A. Correct, and to work out how many possible protein there are we would have to raise

NAISMITH - MARTIN

19 to the power of 6.

Q. Okay. Let us take one of them in our minds; right?

A. Okay. Can you tell me anything about that one?

Q. No.

A. Okay.

Q. I will tell you that it is active, okay, that it has like ----

A. You have told me it is active and it has D320K and it has six changes?

Q. Yes.

A. So you have made it, you have tested it and it is active.

Q. Yes, okay. Can you tell from the fact that it is active that it has been stably folded or do you need to do more testing to figure out if it's been stably folded?

MR. KUSHAN: Objection to foundation, objection to form.

A. In the assay you are using here I would have an expectation that because there is quite a bit of time here that it would be stably folded in my test, so if you made your protein the

same way, supernate it and you measure the activity, production, then my view is that you would have a reasonable expectation that the protein is stable over that 12 hours. But reasonable expectation, I can imagine now that you are at six that you could have a situation where the protein might be unstable over that time period.

Q. (BY MS. MARTIN): So would you have to do another test to make sure that the protein is stable after 12 hours?

A. So I think I'm saying that where you start to introduce multiple mutations beyond five, then you might want to do some experimentation to be sure, because you are moving quite far away from your knowledge base.

Q. Okay, so what would you have to do in order to figure out if you were, you know, seven mutated PH20 is stably folded?

A. You could just measure activity at two time points.

Q. Okay. So if you just do the assay that is in the patent where they measure it at 90 minutes, would you know whether that protein with

NAISMITH - MARTIN

the seven changes meets the claim?

MR. KUSHAN: Objection foundation.

A. So there is an issue we touched on is the patent classifies things as inactive and does not report activities below 20%, so I -- you could even with a single mutation have a protein that is, I think we discussed 70% active, I believe that meets the test of the claim, so that my test. The patent does a classification thing that I think is separate.

Q. (BY MS. MARTIN): Let me ask that again. So, if you have your seven mutation PH20 that you test, after 90 minutes you test it and it is active, let us say it is 70% active at 90 minutes; does it meet the claim?

MR. KUSHAN: Objection foundation, objection as to form.

A. So seven mutations and it is active, under those conditions; I think I would have a reasonable expectation that it would meet the claims, to be certain I would like to, because I'm now on seven mutations I would like to do a second assay just 12 hours later or six hours later, it's not a lot of work but I would want to

do that.

Q. (BY MS. MARTIN): In order to be certain if it meets the claims you would have to do a separate assay to make sure that it is still active at 12 hours?

MR. KUSHAN: Objection foundation.

A. My position is that once you get into these multiple mutations there is not the teaching to allow you to make that prediction. You are sort of reverse solving and saying, "I've made one that I tell you is active" given that you've made it and its active, then I have a reasonable expectation that it will in fact meet the claims.

Q. (BY MS. MARTIN): Yes, this is really me trying to understand what meets the claims and does not meet the claims under your claim construction. You say you have a reasonable expectation that it meets the claims, but do you know for certain whether it meets the claims without doing the additional testing at 12 hours?

MR. KUSHAN: Objection foundation, objection as to form.

A. We are talking about a multiply mutated form, seven or more?

NAISMITH - MARTIN

Q. (BY MS. MARTIN): Yes.

A. At 70% activity?

Q. Yes.

A. After an hour? I would think, in the way that it does, that we do this I would think it would fall within this claim, the claims, without -- that would be my opinion, that that would fall within the scope of the claims.

Q. You do not have to do another test for a native fold?

MR. KUSHAN: Objection foundation, objection as to form.

A. So the point I had raised is that as you start to -- you are backwards solving, you already told me it is active in the assay which gives me some information about timing and the way the protein was made, so that helps form my expectation that it would fall within the scope of the claims if it was active.

Q. (BY MS. MARTIN): Why are you saying that you would expect it to fall within the scope of the claims as opposed to saying that it would fall within the scope of the claims?

A. You asked me could I be absolutely

certain that it didn't degrade or fall apart so fast that it maybe it was at 70% at 90 minutes and 5% at four hours? I think that is highly unlikely, but certain is a difficult word.

Q. Okay, so if it were the case that it is at 5% at four hours, then would it be your testimony that that does not meet the scope of the claims?

A. 5% is an inactive protein, I think we agreed that is a structure, below 5% is inactive.

Q. Okay. So if you have a PH20 with seven changes in it, that is 5% active at four hour then it would not meet the claims?

A. It would be outside the scope of the claims because it is an active PH20, it does have the other attributes I guess we agree.

Q. Yes, okay. But if that same protein had 70% activity at 90 minutes, then would it meet the claims after 90 minutes or would it not meet the claims because it is only 5% active after four hours?

MR. KUSHAN: Objection foundation, objection as to form.

NAISMITH - MARTIN

A. I explained because of the way we are making the protein and handling it, that I would confidently expect that it would meet the claims.

Q. (BY MS. MARTIN): But what if it is the case that it is down to 5% after four hours, then would it meet the claims?

A. It is a very unusual protein that I haven't considered but it is -- in a truly hypothetical protein, now, well, that is an unusual protein because the time it takes for the assay to get all the way down to 4 after four or so hours, I can't really imagine that. But if you had two time point measurements, such a hypothetical, I can't imagine what that would mean, but at four hours if it had no activity I would not consider it to fall within the scope of claims.

Q. (BY MS. MARTIN): Earlier you agreed that a modified PH20 polypeptide with D320K mutation in SEQ.ID NO:3 meets the claims; correct?

A. Yes. With these specific mutations that are spelt out in the claims?

Q. Yes, just with D320K?

NAISMITH - MARTIN

A. Yes, but there are others, H.

Q. Yes, okay, yes. So just to make sure that it is clean, you agreed that a modified PH20 polypeptide with a D320K mutation in SEQ.ID NO:3 meets the claims; right?

A. When its produced in the way we discuss rather than just made off a synthesizer or whatever, it is folded and active.

Q. Does it matter how it is made?

A. It matters that it is folded and active.

Q. Okay. So does it have to be made in a CHO cell?

A. No, but as we discussed earlier, a bacterial cell it might not fold in and one of the tests is that the protein is folded and active, so that isn't -- that is a test, it might have the sequence it conforms to the claim in the first tests, but when you think about it, as the claims require, it has to be folded and active.

Q. Okay. So if you took your D320K SEQ.ID NO:3 sequence and you expressed it in a bacterial cell then your resulting product would not meet the claims because it's not active?

NAISMITH - MARTIN

A. I would not -- I've never done that experiment, we both agree it would seem unlike -- assuming it was inactive, then that would not meet the scope of the claims for producing it in that way, yes, because you would have a physical thing that is, presumably my prediction but I don't know is an unfolded aggregated mess, inclusion bodies that commonly -- but when you try to express human proteins and bacteria, so those would be outside my reading of the statement of claims.

Q. It is not the case that for a particular sequence it will always meet the claims, it depends on how that sequence is produced?

MR. KUSHAN: Objection foundation, objection as to form.

A. Again, that is -- I don't think what I'm saying, I'm saying that the test is just that it is folded and active. I don't impose a way to make it. You are specifically saying where it is made inactive unfolded aggregate, I don't believe that unfolded aggregate would meet the scope of the claims because it is not properly folded.

NAISMITH - MARTIN

Q. (BY MS. MARTIN): Okay. So let's say you have your D320K SEQ.ID NO:3 protein that is made in a CHO cell, so it is properly folded and active?

A. Secreted.

Q. Secreted, if you heat the sample to the point that it unfolds, does it meet the claims?

A. Yes. We discussed this, that you can -- it meets the claims, you can subsequently destroy the protein but when it was -- it still meets the claims as it was made and produced.

Q. Okay. If you put it in a solution of m-cresol such that it no longer has activity, would it meet the claims?

MR. KUSHAN: Objection foundation.

A. So we are back to my just the D320K mutation.

Q. (BY MS. MARTIN): Yes?

A. I made it. I know it is stable. You now render it unstable by adding cresol. I still would think that meets the test of the claims.

Q. Okay. Is there any way to know

whether a protein has the native fold of PH20 decides solving an experimental structure for the protein?

A. You could test activity of the protein and if the protein is active we would assume it has the native fold of the protein. There may be structural differences but if it was active we assume it is properly folded.

Q. Okay, I just want to try to understand why -- you are using, you are saying that it has the native fold, but then you are saying that there could be structural differences, so I am trying to understand how something could have the native fold and have structural differences?

A. Okay. So we, it is good to be clear, and I apologize if I wasn't. We talked about bee venom hyaluronidase and alpha-beta barrel motif within, and alpha-beta barrel motif within at HYAL1, those at a high level have the same fold but there are important structural differences. So for an active protein I would say they have the same fold but there could be important structural differences if the protein

was modified in other ways. The difference would be what a POSA would recognise as a different structure and in bee venom to human enzyme HYAL1, they clearly pull out what the structural differences are between them. But it is within, I think the phrase is loosely defined as alpha-beta barrel motif in the same fold, so we would use it in that sense.

Q. Is it the case that active modified PH20 proteins share a common structure in terms of the native fold of PH20?

MR. KUSHAN: Objection foundation, objection as to form.

A. So they would have the same fold, there could be differences as I described and we know exist between the bee venom and the human hyaluronidase, both are active enzymes.

Q. (BY MS. MARTIN): Is having the same fold a common feature?

A. Of what?

MR. KUSHAN: Objection foundation. Objection to form.

Q. (BY MS. MARTIN): If two proteins have the same fold is that a feature that they

share in common?

MR. KUSHAN: Objection foundation, objection as to form.

A. Are we talking about active proteins?

Q. (BY MS. MARTIN): Yes.

A. So it is an imprecise definition to say they are the same fold. It can be a factual statement but it is not a very precise one to say they have the same fold. It is not a precise structural definition.

Q. I am trying to understand because in your testimony you use the phrase "same fold"?

A. Yes.

Q. So I am trying to understand if that is a common feature or not?

MR. KUSHAN: Objection form.

A. The same fold is not -- is not an accurate description of a common structural feature and the reason I say it is because bee venom and human HYAL1 have the same fold but there are important structural differences between them and they are both active.

Q. Okay, so I am trying to understand

NAISMITH - MARTIN

if having a common fold is itself a common feature or if there has to be some deeper level of identity in order to have a common feature?

MR. KUSHAN: Objection foundation, objection as to form.

A. So my understanding that I was taught in my legal principles was that the test I applied for a written description is a precise one and therefore, as I said, for the bee venom and the human HYAL1 they have the same fold, broadly defined for the alpha-beta barrel motif, only that part, that motive is the same sort of fold but there are important structural differences. So it is not a precise description to say those have common structural features because there are important structural differences between them.

Q. (BY MS. MARTIN): I think that we can agree that neither bee venom nor HYAL1 meet the claims because they are more than 95% different from PH20; right?

A. Yes.

Q. So whether or not bee venom hyaluronidase and HYAL1 share common structural

features has nothing to do with whether or not PH20 and 95% sequence identical PH20 share common structural features; right?

MR. KUSHAN: Objection foundation.

A. No, I think it is useful to think of that because what it shows you is that you can't -- that it, if you had simply said, "Well, the common structural feature is HYAL1", we know that, just exactly that HYAL1, it turns out that the alpha-beta barrel motif can tolerate structural changes and remain active, it is the difference between the two.

Therefore, when looking at the scale of mutations envisaged in the parameter claim space it is perfectly possible to imagine structural differences between members of the family that are still active, they will all have the same fold as bee venom motif and the alpha-beta barrel motif and the bee venom, alpha-beta barrel motif in human HYAL1, but in the same way that we have been taught by the literature, you could imagine structural differences between proteins that fall into the set of active, stably folded PH20s that comply

with the 95% test and they have the 320.

Q. (BY MS. MARTIN): I just wanted to make sure that I am understanding this correctly. You are saying that within the set of 95% identical Ph20s, the sequence space, there are proteins, there are proteins that have the same fold but it is possible that there could be differences in secondary structure, such that there would be structural differences between those protein; is that what you are saying?

MR. KUSHAN: Objection foundation, objection as to form.

A. So on page 47, I say "the native folding" and so what I'm saying is that within the native fold there may be important structural differences that a POSA would highlight, they could be significant arrangements of loops or secondary structural elements. The reason a POSA would be alert to that is because they were aware that the alpha-beta barrel motif between bee venom and human hyaluronidase, HYAL1, had important structural differences. So they share some common structural features between them and you can overlap the structure to define them. They are

also important structural differences.

Q. (BY MS. MARTIN): So in the same way that HYAL1 and bee venom share?

A. Common fold.

Q. Some common structural features you would agree that PH20 and 95% identical PH20 share some common structural features; right?

MR. KUSHAN: Objection foundation, objection as to form.

A. Are the Ph20s active?

Q. (BY MS. MARTIN): Yes.

A. So we are on to the subset that are active. So they will be folded in, as I predict, properly folded adopting a native fold, but I don't know what the common structural features that would be shared amongst them would be, because I had seen with bee venom and human HYAL1 that a core bit of secondary structure was different. So it is possible that different elements of the secondary structure, either in the EGF domain motif or in site alpha-beta barrel motif may be different in some other way than we have seen between bee venom and human hyaluronidase. We only had two structures and

remarkably the secondary structure was different, so defining what the common secondary structural features are would be beyond a POSA in 2011/12 and there is no description in the patent that would direct me to some other way of doing, identifying those common structural features.

Q. So you just agreed you just testified -- strike that. You just testified that bee venom and HYAL1 have common structural features; right?

MR. KUSHAN: Objection foundation.

A. I said they have, and I was careful to define that as the, "you can overlap the structures and see what beta strands and alpha helices overlap", but there are big differences between the two.

Q. (BY MS. MARTIN): I understand that there are differences; I am just trying to establish that there are common structural features?

A. Between those two proteins.

Q. Yes.

A. There are.

Q. Okay. In same way that there are

NAISMITH - MARTIN

some common structural feature between HYAL1 and bee venom, there are also some common structural features between PH20 and a PH20 that is 95% identical assuming that both of them are active?

MR. KUSHAN: Objection foundation.

A. So they would have the same fold, but I don't know anything about which of the features there might be different between them so I haven't got a definition for what common structural features. The reason I can give you them for bee venom and for HYAL1 is because I can superimpose them and call it out.

Q. (BY MS. MARTIN): What is your understanding of what -- strike that. What is your understanding as to what is required to qualify as a common structural feature?

MR. KUSHAN: Objection foundation.

MS. MARTIN: So the requirement is so that I can visualize or recognise members of the genus, and it requires a precise definition.

Q. (BY MS. MARTIN): So what is a precise definition?

A. If, sorry, we are in hypothetical because, of course, I didn't formulate a precise

description, that wasn't the test I was asked to do. Had you described, for example, the common structural feature is beta strand that starts here, finishes there, beta strand there, alpha helix here, arranged in this, that would be quite a reasonable definition of a common structural feature.

Q. (BY MS. MARTIN): So, okay a -- strike that. A description of the identity and location of secondary structural motifs, that would qualify as a precise definition of a common structural feature?

A. No, in this case because you also have the active site and loop, so you would also want some description of the loop structure that was critical for activity.

Q. How do you describe a loop structure?

A. You can show figures or point to coordinates that have been deposited, for example, point to a model that you have created.

Q. So in order to satisfy the common structural features test you would have to have a precise description of the secondary structural

NAISMITH - MARTIN

characteristics and active site loop characteristics that are shared by the claim genus of proteins?

A. So my opinion --

MR. KUSHAN: Objection form.

A. Should you -- that would be one of the ways that you would meet the test. If you had a precise structural description of the protein, calling out what you think are the structural elements and how they interact together, that would be a precise description.

Q. (BY MS. MARTIN): Now the amino acid -- strike that. You acknowledge that the P10 may not adopt your claim construction; right?

A. Correct. That is in my report, they are the trier of fact, I am only a scientist offering an opinion, not a legal authority.

Q. So if it is the case that the claims do not require activity but are in fact defined by sequence, would you agree that a POSA would be able to determine whether a particular protein falls within the scope of the claims by examining the sequence?

MR. KUSHAN: Objection foundation,

objection as to form.

A. I am going to go to the section in my report, if that is okay.

Q. (BY MS. MARTIN): Do you address this in your report?

A. I said I'm not a lawyer and talked about if the Patent Board read the finding, I just want to be sure I can refresh my memory. Page 73, paragraph 110. Can you repeat your question because my view is, I am talking about if the Patent Board say the claims cover all possible ten to the power of 60 variants for example. In that event, I would expect there to be a range, a very divers range of possible arrangements and structures that would be meaningfully different. So some would be folded, some would be unfolded, some would be aggregated, some might not be able to be produced. So I don't think there is any written description guidance to those other forms, there is not for the native that they folded, so there is no guidance to the others.

Q. That wasn't my question, my question was ----

A. Sorry, I'm just getting into the

NAISMITH - MARTIN

---

Q. Okay. So my question was if the, as you say in paragraph 110, if the Patent Trial and Appeal Board construes the claims such a that they don't require activity, can you tell whether a particular protein falls within the scope of the claims or not just based on the sequence?

MR. KUSHAN: Objection to form, objection to foundation.

A. So it rules out activity, I would have to -- so there would be no written description in that case, so if I did not have activity then the protein could be anything, it could be partially made, partially unfolded and they are very different things. So the Patent Board would -- so my view is that, based on what I understand the legal principles are, you have to describe in a precise way. So we have talked about a description of an active protein, if you have to describe aggregated protein you would need to have some description of that if you were considering within the written description of the claims.

Q. (BY MS. MARTIN): So again that

still isn't my question. The question is, can you tell whether a particular protein meets the claims based on the sequence if the claims don't require activity?

MR. KUSHAN: Objection foundation, objection relevance.

A. So I think that is different than the question I asked myself. I asked as a legal test if the Patent Board decided the claims were not restricted to active proteins, but included all these other forms, would that alter my opinion? And the answer was, no, it would not because the active form is not -- that you are trying to aim for is not adequately described and these other forms are not adequately described. I'm sorry, I'm struggling with your hypothetical of what the Patent Board are deciding, that is what I am struggling with. I considered the question, if they read the claims in a different way.

Q. (BY MS. MARTIN): Have you considered whether or not you could tell if a particular protein meets the claims based on sequence, if the claims don't require activity?

NAISMITH - MARTIN

MR. KUSHAN: Objection foundation, objection as to form.

A. So, if the Patent Board say, "We don't care about activity", then the claims would then, and "don't care about fold or activity", then a protein with a sequence would fall within the scope of the claims, but it would not be described because, as I said, you can imagine there are, well, we know, there are will be multiple different structural representations in that population.

MR. KUSHAN: We have been going about 90 minutes, I think it is a good time for a break.

MS. MARTIN: We have been going for ----

MR. KUSHAN: 89.5 minutes.

MS. MARTIN: No, we stopped at 2.23.

MR. KUSHAN: That was for a 90-second break. We have been going for about 90 minutes.

MS. MARTIN: Yeah, that's fine. We can take a break.

NAISMITH - MARTIN

THE VIDEOGRAPHER: We are going off the record. The time is 3.07 p.m.

**(Short recess from 3.07 p.m. to 3.24 p.m.)**

THE VIDEOGRAPHER: We are back on the record. The time is 3.24 p.m.

Q. (BY MS. MARTIN): Dr. Naismith did you discuss the contents of your testimony with counsel during the break?

A. No, I did not.

Q. Okay. Earlier we were talking about testing potential mutants for activity to figure out whether they meet the claims?

A. We talked about how I read the claims; I don't remember saying I had to test mutants. If you made these very vast number of mutants, you couldn't reliably predict their activity so you would want to do tests. This is just a very general statement, so I just want to be clear what it is you are asking.

Q. Oh okay. I gave you a hypothetical where I said you had D320K SEQ.ID NO:3 PH20 and you made another mutation in it and it had activity, and you determined that by testing; right?

NAISMITH - MARTIN

MR. KUSHAN: Objection foundation.

A. I said that you could expect, unless you had mutated, introduced that second single site mutation at one of the inactivating positions which were known, then you could confidently expect that that second mutation would be active.

Q. (BY MS. MARTIN): Okay, you could predict that the -- it is your testimony -- strike that, I will start over again. Is it your testimony that you could reasonably predict whether a dual mutant PH20 would be active without having to test it?

MR. KUSHAN: Objection foundation, objection to form.

A. So if dual PH20 data, you have that and you introduce a second mutation and that second mutation was one of the ones that was tested as active from the patent and was active, then you would have an expectation that the combined would be active.

Q. (BY MS. MARTIN): Okay. So then if you make a third mutation at a site that the patent teaches retains activity, would you expect

that triple mutant to be active?

MR. KUSHAN: Objection foundation, objection as to form.

A. So you begin to get into the realm of epistasis and also active, we talked about the multiplicatively deleterious, so if you, for example, 40, 40, 40% multiply that, for example, then you get below, I would have to do my maths but you can conceivably have ones which are additive, but if you combine the multiplicatively deleterious you get the lower level of activity because you keep knocking away as you add deleterious mutations. They are each individually active, it is just a consequence of multiplication.

Q. (BY MS. MARTIN): Okay. So if you introduce two mutations that give you 40% activity and one mutation that gives you 100% activity, would you expect to have -- would you expect to have a protein with reduced activity?

MR. KUSHAN: Objection foundation, objection as to form.

A. I would expect it to have activity if they were depending whether there was epistatic

effects you begin to, as I said, worry about that, but if you have no epistatic effect point 4 times point 4 is point 16 and therefore by my 5% threshold you would expect that to be active.

Q. (BY MS. MARTIN): So then, if you are introducing five mutations at sites that the patent teaches are active, would you expect that protein to be active?

MR. KUSHAN: Objection foundation, objection as to form.

A. So that is where I break down in my ability to be confident of that prediction because by the time I get to 5 I think, based on the literature and what POSAs understood, that is at the limit to where you really begin to expect to have problems with predictability.

Q. (BY MS. MARTIN): Then you would have to test for protein?

A. So as I said you would have a weak prediction by the time you put 5 in. You, for the protein you would need to know, for the protein to be within the scope of the claims as I read them it has to be folded active, by the time you put 5 mutations in, you are sampling quite a wide range

of possible structural diversity some of it, so you run the risk of unfolded aggregated protein even though the individual mutations would be active.

Q. But the question was, so you don't know whether or not it's go together be active and so you would have to test it?

A. At 5 I would have decreasing confidence, so because you've got 19 to the power of 5, that is a lot of variants, you begin, so that is -- you know, without some additional information, your confidence that it would be active is quite decreased.

Q. What additional information?

A. Some form of test of the ones that I have -- you know, if you made, for example, a quadruple mutant that was well behaved and active and tested it so you know you can combine these four and you introduce another one, that might help give you some confidence.

Q. Okay. So now, let's say you introduce six mutations that the patent says are active, would you expect that protein to be active?

NAISMITH - MARTIN

A. I don't ----

MR. KUSHAN: Objection foundation. Go ahead.

A. At that level I would have no confidence in my prediction for active or inactive, or folded or unfolded.

Q. (BY MS. MARTIN): So then you would have to test it; right?

A. At six mutations to know if it was within the scope of active folded proteins, based on the common disclosure I would have no way to know or reliably predict. So to be sure, to know I would have to test.

Q. Okay, and could you use the test that is described in the patent to determine whether or not it is active?

MR. KUSHAN: Objection foundation, objection as to form as well.

A. So the test in the patent is we have already talked about the fact that some mutations can have less than 20% activity that I would classify as inactive, so the patents test, as it is written in the common disclosure, could classify a protein which is active as inactive.

NAISMITH - MARTIN

Q. (BY MS. MARTIN): Okay, if you yourself were to follow the instructions in the patent, could you perform that test and determine whether or not your 6 mutant PH20 was active?

MR. KUSHAN: Objection foundation, objection as to form.

A. So there are problems with the assay that mean you would be uncertain. If it was above 40% you would be confident that the protein is active. The problem is that the patent doesn't give any guidance to what is resulting in a measure of activity in a sample of less than 20% and I previously said that a protein which is 15% active reliably measured would fall within the scope of the claims.

Q. (BY MS. MARTIN): Okay, so that is what I am trying to get at, is the reliably measured part of what you just said. So, is it your testimony that the assay in the patents is not capable of reliably measuring whether or not a PH20 is active?

A. "Reliably" identifies things that are active above 40%, but it provides no insight as to when you get an activity less than 20%, and

as a result one can imagine proteins, modified polypeptide proteins which have 18% activity which are classified by the patents method, as inactive.

Q. So I'm trying to understand if there is actually a problem with the assay or if what you are talking about is the fact that the patent doesn't tell you why you got you know value below 20%. So that is why I was saying you personally could run the assay, right, and you would get a number and so would that number tell you if the protein is active or not?

MR. KUSHAN: Objection foundation, objection as to form.

A. So there are some specific flaws with the assay that would meet. Those flaws, you can deal with from measurements above 40%, but there are, those flaws prevent you knowing whether a protein is inactive that we have described at 5% or active at 18% or misfolded. We don't know the reasons for that, and the assay itself appears to have quite large variations in it, so that you can't reliably, at least there is no standard curves or information that would allow you to evaluate the robustness of the assay and the

patent itself does not disclose any measurements below 20.

Q. (BY MS. MARTIN): Do you believe that if you yourself were to go and perform that assay you could tell whether or not a protein was active by running that assay?

MR. KUSHAN: Objection foundation.

A. By running that assay, I would tell whether the protein had more than 40% activity reliably.

Q. (BY MS. MARTIN): But you believe that that particular assay will not tell you whether or not a protein is inactive?

MR. KUSHAN: Objection foundation.

A. So you can't -- the problem is the assay can't distinguish or doesn't distinguish, nothing in the teaching on its own distinguishing why you don't reach a threshold of above 20%, there is nothing there that can help you guide that in the patent.

Q. (BY MS. MARTIN): I don't understand why that matters why is that important in terms of figuring out whether a protein is active or not?

NAISMITH - MARTIN

A. Because we have talked about that for active protein we believe it is above 5%, I have no information in the patent to know if as performed in the assay, what accuracy the assay has because I lack control experiments, and I don't know what the noise level in the assay is and I don't have standard curves.

Q. So if you were to personally perform the assay, you would have that information, right, you would have a standard curve and control and noise level. I am asking you is the assay itself capable of telling you whether a protein is active or not?

MR. KUSHAN: Objection foundation.

A. Sorry, I tried to say that I have considered the assay as it is described and reported in the common disclosure, I believe if the assay reported above 40% activity then that would be an active protein.

Q. (BY MS. MARTIN): Okay, so it's your testimony that the assay in the patent cannot identify inactive proteins?

MR. KUSHAN: Objection foundation.

A. What I say is in the classification

as inactive mutations, there are within them the potential probability of proteins which have 18% activity, proteins which are unfolded, proteins which are not secreted, proteins which are misfolded, proteins that are aggregated.

Q. (BY MS. MARTIN): And the patent doesn't distinguish between those, right, just all of them would fall within the bucket of inactives?

A. It is definition of "classification", it puts them in that bucket as inactive but it doesn't differentiate between them.

Q. So using the assay in the patent you could identify what the patent classifies as inactive proteins?

MR. KUSHAN: Objection foundation.

A. So using the assays as described in the patent following it, I would be able to classify proteins in the same way the patent has done as inactive or active, but you specifically asked me about enzyme activity and therefore we discussed it, there is a limit of about 5%.

Q. (BY MS. MARTIN): So you are reading the claims to require an activity

NAISMITH - MARTIN

threshold of 5% enzyme activity?

MR. KUSHAN: Objection foundation, objection as to form.

A. I am reading the claims to require enzyme active and I said a POSA we, we might, we would all, I think, draw a line at 5, some of us might draw it below 10, but that is a definition of an active protein.

Q. (BY MS. MARTIN): Okay. Now before the break I was asking if the Patent Trial and Appeal Board interpreted the claims to not require activity. Could you tell whether a particular protein meets the claims just based on the sequence?

MR. KUSHAN: Objection foundation, objection relevance.

A. So, I went, in the break, to re-read my paragraph so that I am clear. So if the claims define, the Judges define the claims that it includes additional forms of the PH20 polypeptides, unfolded, misfolded, partially folded, higher aggregates inactive forms, then they would be within the scope of the claims, but they would not be adequately described because an

inactive protein shares no structural feature in common with a folded protein. The only commonality there is the primary sequence, not the structure.

Q. I understand that that is your testimony. What I am trying to get at is if you could tell based on just the sequence alone, whether a protein would meet the claims, if there is no activity requirement, and it sounds like, from what you just said, that the answer is, yes?

A. It is within the scope of the claims because it includes those additional forms, but those additional forms are not adequately described.

Q. Okay. So you agreed that you could differentiate proteins that meet the claims from those that do not meet the claims based on the amino acid sequence if the claim does not require activity?

MR. KUSHAN: Objection foundation, objection as to form.

A. So my testimony is if, if the Patent Board conclude that all the universe of possible claims are within the scope and that

would include these additional unformed, then these would be covered by the claims.

Q. (BY MS. MARTIN): Okay, and so you could use the amino acid sequence to distinguish proteins that meet the claims from those that do not meet the claims; right?

A. When you say meet within the scope, but they would not be -- so they would be within the scope of the claims, but you don't have a written description to understand what structural features are being described because there are very different structural features between unfolded proteins, folded proteins, aggregates etc.

Q. It is your testimony that you would need to have a description of structural features of unfolded proteins in order to meet the written description requirement?

A. My understanding of a written description, and we are now into hypotheticals, is that if you want to discuss unfolded proteins you would have to describe what that meant, or aggregates, you would have to give some description of that.

NAISMITH - MARTIN

Q. Of what the structure of the unfolded protein is?

A. The three dimensional structure not necessarily, but you could define it in other ways.

Q. Like how?

A. It forms a lump at the bottom, you know, it is an aggregated mass. You could look at other ways to try and describe aggregates but it is difficult because they do have a very wide range. You could, say, describe the aggregate itself as a physical thing.

Q. So when the patent talks about inactive PH20 polypeptide, your view is that that is not a sufficient description of an inactive PH20 polypeptide?

A. When the patent talks about inactive polypeptides it is imposing a threshold of 20% activity. I have said that there is a whole range of reasons why you can have less than 20% activity. We talked about the example where we had 18% activity, that would be classified as inactive by the patent's classification, but I would consider that protein to be active if

NAISMITH - MARTIN

reliably measured and, therefore, to fall within the scope of the claims as I read them.

Q. Okay. In your view is it required -- strike that. Is it your opinion that in order to satisfy the written description requirement you must be able to know the precise number of embodiments that fall within the scope of the claims?

A. That is not my opinion.

Q. Okay. So when you say that -- strike that?

A. Can I turn to that page, I am flicking pages when I am trying to listen, my apologies.

Q. So in order to satisfy the written description requirement, is it your view that you have to be able to approximate the number of sequences that fold and are enzymatically active?

A. No, that is not what -- my opinion, as I said, as legal principle you need a representative number of species that fall within the scope of the claims. Or, as we discussed earlier, there are structural features common to the members of the genus.

NAISMITH - MARTIN

Q. Okay. So, the fact that you don't have a number, a precise number of those that fold and are enzymatically active, that is not part of the written description test?

MR. KUSHAN: Objection foundation, objection to form.

A. So, the written description test I use, is you have to have a representative number of species. If we are talking about an active protein parameter sequence space, we have a huge number, ten to the power of 60, so you would want to have representative number of species that probe some of those structural divers -- sequence I should say, diversity for active proteins because you can imagine quite a lot of changes by the time you get to 15. There is a vast array of them, you would want some representation of that.

Q. (BY MS. MARTIN): Do you have to be able to physically make all of the sequences that fall within the sequence space in order to satisfy the enablement requirement in the aggregate, to be clear?

A. So you don't have to make every single one. You must enable to, a person skilled

in the art to make them use an entire class, so you have to be directed how to make and use them, you don't have to make every single one of ten to the 60.

Q. Okay. So we agree that whether -- we agree that the fact that it is not physically possible to make 10 to the 60th items is not, you know, dispositive for 1/12 purposes here?

MR. KUSHAN: Objection foundation, objection relevance, objection to form.

A. So what is dispositive is that in that diversity you have wildly different types of protein and there is no enablement to enable you to choose the folded active protein. There is no written description as to what those common structural features would have to be. That is a written description test so on the enablement there is no direction to how to enable when you get to 15 or more than 6, what, how you would get there.

Q. (BY MS. MARTIN): So if I gave you an amino acid sequence that had 16 changes in it from wild-type PH20, is it your testimony that you would not know how to make that protein?

NAISMITH - MARTIN

MR. KUSHAN: Objection foundation, objection as to form.

A. So, I could make the protein but I would have no way to know whether it was an active form or whether it was inactive, I think I could make it. So sometimes you can't make, you get stalling on the ribosome, so if you gave me a nucleotide sequence, I could make the nucleotide sequence and do the experiment if that is your question. But by the time you get to that large number of mutations there are many reasons that could lead to the protein appearing in a different, entirely different region of structure, unfolded, aggregated, whatever.

Q. (BY MS. MARTIN): Okay. I understand that there is a question about what it is going to look like at the end, but the question is if I gave you the sequence, would you know what to do with it, you know, if you wanted to make it into a protein or would you have no idea where to turn in terms of how to make that, how to change that string of letters into a physical item?

MR. KUSHAN: Objection foundation,

objection as to form.

A. So you're using protein in the way I would which is, I assume, an active folded protein or active form polypeptide, so I would have no way to know how to make that into an active form of the protein or folded.

Q. (BY MS. MARTIN): If I say polypeptide, is that easier? I mean, if I give, is it honestly your testimony sitting here, honestly your testimony that if I give you an amino acid sequence you have no idea how to proceed in terms of how to actually turn that string of letters into a physical item?

A. That is not my testimony.

Q. Okay.

A. But "I would not know what that physical item would be" is my testimony. It could be aggregates misfolded in some cases; it might not actually get made and I would have no way to predict that.

Q. Okay, because the issue, I just want to be really clear on what the issue is for you, right?

A. Okay.

NAISMITH - MARTIN

Q. It is predicting whether or not your string of text is going to be turn into a protein that has the native fold of PH20?

MR. KUSHAN: Objection form; objection foundation.

A. So the issue for me is, as I understand enablement I have to be able to probe the full scope of the claims, right? So I have to be able to make things out that touch, that enable the full scope as defined by the claims and it is my position that there is no teaching in the patent that would enable me to practice the full scope of the claims because, and, yes, the full scope of the parameter claims base, that is my opinion.

Q. Yes, I'm trying to understand why. I mean, you are just giving me the legal test for what is lack of enablement and I'm trying to understand what the issue is, and it sounds like the issue is, "I can't predict whether this string of text is going to, you know, translate into a protein with the native fold of PH20"?

MR. KUSHAN: Objection foundation.

A. So the issue is that the written --

the description in the patent that I would use to enable me doesn't give me any guidance how to approach making any particular form of the protein. As I have said, I think it is active, so there is nothing in the patent to help me make your 16 mutant protein, so I don't think it is enabled.

Q. (BY MS. MARTIN): But you know how to make a -- you know how to take a string of text with 16 changes in it and, you know, make it into a vector and put it in a cell and express it. You know how to do that because that has been, you know, routinely in the art for 50 years?

A. But I would not know what I would get as a result. I could have a wildly varied product as a result so that is why I don't think it's enabled.

Q. Can we turn to paragraph 78 in your declaration. More specifically the figure on page 47 of your declaration?

A. You want me to go to page 47?

Q. Yes. Did you create the figure on page 47?

A. With the assistance of counsel

I did.

Q. Okay, can you explain to me what portion of the figure on page 47, in your view, meets the claims?

A. So some -- the, some subset of the green is within the claims, yes, so the green ones, yes, that is the bit that would meet the claims.

Q. To be clear, there is a -- okay, so we are looking at a box and then there is a dashed purple line, and then there is a heavy green square with two boxes in it, and then there is another heavy green square that has two more boxes in it. So, when you say green, what part of the green are you talking about?

A. Sorry, yes, so the -- as I read the claims, it is that inner square, that they form active, "adequate stability, enzymatically active" provided they have the D3HK20 reference, but there is a bigger green box, that is what I wanted to differentiate.

Q. Okay. So you are saying that the claims cover the proteins that fall within the green shaded box, the box that is shaded green?

NAISMITH - MARTIN

A. That is the definition as I read the claims, that they have adequate, they are stable, native fold and they sit in that box.

Q. Okay, because, okay, so then above that, right, there is a box that says: "Natively folded modified PH20 polypeptide" and there are arrows pointing to all of these boxes within the heavy green outline?

A. So that points to the case where there is a possibility, as I said, where the protein is active for a very short period of time and I would put that in this inadequate stability, can't measure the enzyme activity reliably or believe in it and, therefore, I don't think it would fall within the scope of the patent claims.

Q. Okay, so the: "Inadequate stability, enzymatically active" box, would not fall within the scope of the claims?

A. As I read the claims that's my definition because although it may have had activity transitorily, once it becomes unstable, unfolds, it no longer has activity. So, therefore, it would not meet the scope of the claims.

NAISMITH - MARTIN

Q. So for purposes of the test in the patent, right, if you were to test such a protein it would show up as inactive because it may have had transient theoretical activity but by the time you test it it doesn't have activity any more; right?

A. So for the single mutations that are recited in the patent, it is one of the reasons why you might have assigned them or classified them as inactive along with many other reasons. As you say, it could have been secreted and suddenly it just unfolded or it unfolds very rapidly, by the time you get to the assay it is less than 20%, you would have classified that as inactive.

Q. I just want to understand, based on the discussion that we had earlier about, you know, you run the test at, after 90 minutes and you show that it is active, the universe of proteins that fall within that bucket, right, that are active after 90 minutes, those are the ones that are in the green shaded box?

MR. KUSHAN: Objection foundation.

A. So you are changing my definition

of stability. That "stability", I am confident for the single mutation, as I said confident that those would be stable once I get to other, which are not represented at all in the patent, multimutations, then and I gave the answer about eight hours. So, if the protein was completely unfolded by eight hours and aggregated I would think it would fall outside of the scope of the claims as I read them. I think and I differentiated between the single site mutations that are reported in the patent and then these multimutations.

Q. Yes, but I thought that we agreed that even if you had multiple mutations, if you did the test at 90 minutes and it was active, you would expect that it was sufficiently stable to meet the claims?

MR. KUSHAN: Objection foundation.

A. So with multimutations I was careful to say I would like to know what the activity was, and I would, because of multimutations I might, I would want a set because of my concern about stability, if it was a, you know, very active protein, probably not. But

I would begin to use some judgment here because as I read the claims it has, the number given is about eight hours, that is the kind of assay time they want.

So I can imagine proteins which may have some activity at 90 minutes which have none at eight hours, but I said because of the way we prepare it that you have some, if it's active at 90 minutes you have "some information" about stability, you don't have "no information" about stability at that point, because it's active at 90 minutes plus the handling.

So you would have an expectation that it might be stable at eight hours, but once you get to multiple mutations you begin to have less confidence in that expectation because your expectation is grounded in the original PH20 with a single site mutation.

Q. (BY MS. MARTIN): So then you would have to do an additional test at eight hours to make sure that it is still active in order to tell whether it meets the claims if it has multiple mutations?

A. I don't think, I said depending on

the purpose, if it was still active in the assay you would have confidence, some confidence if it was made and it was active in the assay, that it would still be active at eight hours. But if it was at the very edge of activity, on those, one of those border lines, you might want to do a second test at eight hours to see whether it retained its activity.

Q. Do you read the claims as requiring PH20 to be on the surface of the sperm?

A. No, I don't because I'm specifically told in the claims and I've had reference to SEQ.ID NO:3.

Q. SEQ.ID NO:3 is not a naturally occurring sequence, is it?

A. It is a ----

MR. KUSHAN: Objection form.

A. As I read the claims, we are directed to polypeptides and then we narrow it down, so PH20 polypeptide would include the native protein, but then we narrow it down and we say, we specifically call it sequence 3 and 32-66, so we are restricting here that universe.

Q. (BY MS. MARTIN): So the native

NAISMITH - MARTIN

PH20 which includes PH20 on the sperm is not part of the claim because that would not satisfy the requirements of the SEQ.ID NO:3 or 32-66; right?

MR. KUSHAN: Objection form.

A. I would want to check precisely on whether the longer ones, the number of amino acids in the longer one I have a table arming ----

Q. Oh?

A. Because if you get ----

Q. (BY MS. MARTIN): I will represent to you that none of those have a GPI anchor.

A. Right, but it is the number of changes relative, you know, deletions, because I'm -- so you could imagine deletions that there are so many other changes that are longer it falls outside your 95%. I just want to check the number of the full length of the sequence ----

Q. Okay.

A. ---- to answer your question. So 447 is the sequence I have, so I can't recall, without going back, how many residues are in the full length protein, I apologise. If there are more than 18 residues longer than 447 then that would, in my view, be more than 17 changes or 5%.

Sorry 5% of 447 is 23 changes.

Q. Oh, okay, I see what you are saying.

A. And you would still have to have the DH320K mutation for it to fall within the scope of the claims. So my concern was just to be precise, you have to fall within the sequence identity claim parameter, so whether the full length one is within 95%, but if it was a native protein on its own it does not have the D320K, HRS so would be outside the scope.

Q. Let's turn -- let's mark Dr. Petsko's declaration which is Exhibit 2068.

**(Previously marked Exhibit 2068 was referred to)**

A. This is the declaration of Dr. Simpson?

Q. Okay, while we are at we will do Dr. Simpson's declaration. Dr. Simpson's declaration is 2068 and Dr. Petsko's declaration is 2070.

A. Which one do you want me to look at?

Q. While we are at it, let's just go ahead and also do the deposition transcript from

Dr. Petsko which is Exhibit 1131.

**(Previously marked Exhibit 1131 was referred to)**

A. Before we start again could I go to the bathroom? There is not a question pending so I thought would now be okay?

MS. MARTIN: We will go off the record.

THE VIDEOGRAPHER: We are going off the record. The time is 4.05 p.m.

**(Short recess from 4.05 p.m. to 4.26 p.m.)**

THE VIDEOGRAPHER: We are back on the record. The time is 4.26 p.m.

Q. (BY MS. MARTIN): All right. Dr. Naismith, did you discuss the substance of your testimony with counsel during the break?

A. No, not at all.

Q. Can you please turn in your report to paragraph 340?

A. That is on page 248 I think.

Q. Yes.

A. Can I move these back out the road.

Q. Yes, you can move them aside for now. We will come back to them. And so you say in paragraph 340: "Dr. Park explained that he

investigated many individual substitutions at the non-essential positions of PH20 within the portion of the PH20 sequence that overlapped with the Hyal-1 template used to create his PH20 homology model, and for which the model had reliable local QMEAN values." Right?

A. That is the sentence, yes.

Q. And so it is your testimony that the positions that Dr. Park looked at all had reliable local QMEAN values?

A. So he excluded some regions where he felt the QMEAN score was not, and the model was not good enough for him to use his local environment test, so C-terminous being one.

Q. You have a colour coded chart on page 249 of your declaration; right?

A. Yes.

Q. Did you prepare this chart?

A. With the assistance of counsel.

Q. And why did you colour code the chart on page 249?

A. To help represent information and there is a key below which say what is the colours correspond to.

NAISMITH - MARTIN

Q. Were you trying to suggest through your colour-coded chart that Dr. Park's analysis provides a measure of tolerability of the positions in your chart, in the positions in that chart to substitution?

A. So I think I'm pretty clear as red is where Dr. Park identified essential residues, so he did not do anything there, there was no probing by his methods. Then dark green is where Dr. Park evaluated 60% of the total residues identified in MSA. So he worked down a certain list. Green, where he only probed between 30 and 66%, and light green where he didn't probe, he probed less than 33% of alternatives suggested in the MSA. The unshaded is the ones he didn't probe at all.

Q. So the shading relates to the extent to which, or the number of alternate residues within the MSA that Dr. Park evaluated for each position; right?

A. It represents the percentage of those that were in his MSA if there was, say, 10, if I coloured it dark green that means he tested seven because it is more than 66%.

NAISMITH - MARTIN

Q. When you say tested, you mean he looked at it in PIE model and evaluated it?

A. My apologies, he used modelling and evaluated it and then he assigned individually those mutations into his buckets of 1, 2, 3.

Q. You were not intending to represent with your shading on the figure on page 249, you weren't intending to represent any information about the tolerability of those positions to substitution, were you?

A. So the red ones would be ones that he identified as essential, and so those would be ones in his analysis that you would not start by making single mutations and that is what he said. But I don't have any -- the colours here don't represent anything other than, as you say, the numerical thresholds, but they are not -- there is -- the green or dark green or light green don't apply some tolerability.

Q. The numerical thresholds are the number of substitutions that Dr. Park considered, not the tolerability of that position to substitution; right?

A. So, I don't -- tolerability is, I,

it is an odd expression, I wonder if you could define that for me because it's defined differently by different people. So tolerance applies to a single site mutation is active or inactive and Dr. Park uses it in that way. So he said, you can tolerate, I've got a mutation, whatever it is, if it falls into buckets 2 or 3 by his analysis that is a tolerated mutation.

Q. Okay. So the colouring that is shown in the graph on page 249 is not intended to show the tolerability of each of those positions using the definition that you just provided; right?

A. What he said is, so the colour scheme is designed to tell you how many of the alternative residues were probed in the multisequence alignment, that is what it does.

Q. Correct, not the tolerance of each position to change?

A. You would need to go to his spreadsheet to work out which amino acid substitutions were tolerated.

Q. Which ones he predicted would be tolerated?

NAISMITH - MARTIN

A. Correct, which ones, based on what a POSA would do, that he assigned to buckets 2 or 3.

Q. You certainly weren't intending to suggest that this chart would provide an indication of the tolerability of each positioned substitution; were you?

A. No, I would say that if you wanted to look for mutations based on this alignment you would go back to his main spreadsheet, that is a definitive list of where you might find substitutions that were tolerated buckets 2 or 3.

THE COURT REPORTER: Did you say this alignment?

A. Multi-sequence alignment.

THE COURT REPORTER: You said "based on this alignment".

MS. MARTIN: I think you said ----

A. Oh, this alignment, yes, the multi-sequence alignment.

Q. (BY MS. MARTIN): In paragraph 343 the second to last sentence you say that Dr. Park: "... did not assess alternatives at residues 4-7 and around 398-4033, because of the uncertainty in

the structural model in those regions." Right?

A. That is correct.

Q. So were those the ones that he excluded because of an unreliable QMEAN score?

A. I think it exceeded even the template that he had to use, so he just excluded them.

Q. Okay. Keep page 249 handy while turning to paragraph 283, page 205?

A. You said keep something else open?

Q. 249. Actually let's go to page 206, the top of page 206?

A. 206, what was other page, sorry?

Q. 249?

A. So we are looking at the table.

Q. Yes. So you say at the last sentence in paragraph 283 that: "In PH20, positions 3-5, 27-35, 44-48, 104-105, 234-237, 256-262, 274-278, 357-358, 405-406 have unreliable local QMEAN ..." scores: "... and modeling at those positions would be unreliable." Correct?

A. They are less, yes, they would be less reliable at those position.

Q. You don't say "less reliable"; you

NAISMITH - MARTIN

say "unreliable"?

A. "Unreliable", I do say "unreliable", yes.

Q. Dr. Park in fact did model PH20 positions within those ranges, right, for example?

MR. KUSHAN: Objection.

Q. (BY MS. MARTIN): If you look at 27 through 35, you see that those are shaded green in your chart on page 249, which means that Dr. Park considered substitutions at those positions?

A. He did for the purposes of his analysis, yes.

Q. Well, you agreed that his analysis was reliable and appropriate; right?

A. He described how he did it, and I think a POSA would follow his recipe and come up with the same answers, so he clearly set out how he did it.

Q. Now did you, when you were eventuating Dr. Park's methodology, did you consider the fact that he modeled positions that, according to you, are unreliable?

A. So Dr. Park's analysis set out illustration, he didn't exclude regions with lower

QMEAN scores. So I noted that, but he did say what the basis for his exclusions were. You know, he told me not the templates and then I worked through and looked at the MSA, so I understood how Dr. Park had done his analysis.

Q. Okay, but his analysis included estimations of whether or not particular mutations would be tolerable at positions that you say, at the positions for which you say modelling would be unreliable; right?

A. I said insufficient reliability so that would be, yes, if I was, you know, I do say that that would be a caution you would use, that QMEAN would be a caution in evaluating those predictions.

Q. That is not what you said about Dr. Petsko. You said that modelling at those positions would be unreliable?

A. But I did not exclude them from my -- the only test I impose -- it would say the same thing to both Dr. Petsko and Dr. Park, is that those would have insufficient reliability in some way, but I didn't cut out those residues for Dr. Petsko, when I went through Dr. Petsko's lists

I did not impose a QMEAN cut off from Dr. Petsko.

Q. Okay, you are saying you didn't ignore Dr. Petsko's analysis for those residues, but you're just saying that it would have been, that modelling at those positions was unreliable for Dr. Petsko but you didn't take that position for Dr. Park?

A. No. I said that in my view, where the QMEAN score is less a POSA would be concerned about quality of predictions there because it's less reliable, unreliable I said. And that is true for Dr. Petsko and Dr. Park's analysis, Dr. Park himself talks about that the unreliability in that region. But what I didn't impose my judgment on that, on their analysis.

Q. You didn't say anything about Dr. Park's analysis being unreliable at those various positions, did you?

A. Well, I think I already, I didn't repeat it, but I had talked about it in paragraph 283.

Q. In paragraph 283 you are talking about Dr. Petsko to be clear, you are criticizing Dr. Petsko in 283; you don't criticize Dr. Park?

A. I think am just talking in general, portions of the PH20 in paragraph 283, I don't think I specifically criticized Dr. Petsko in any way in paragraph 283.

Q. Oh, yeah, okay, that is in 284. You criticize Dr. Petsko in paragraph 284 for his use, for his modelling at what you call to be, what you determine to be unreliable positions?

A. Dr. Petsko's analysis did not account for them, that is just a factual statement.

Q. And neither did Dr. Park's; right?

A. Dr. Park didn't use a QMEAN cut off, but he drew attention to it.

Q. Not for those positions he did not.

A. He didn't exclude them based on the QMEAN score; that is correct.

**(Previously marked Exhibit 1026 was referred to)**

Q. Somewhere in there you have the 731 application, I think it is there.

A. Sorry?

Q. That one.

A. So I am looking for the last three digits; is that correct?

NAISMITH - MARTIN

Q. Actually no, sorry. So the 731, that one. The 731 application, so have that and your declaration. Okay.

A. This is the common ----

Q. Common disclosure and your declaration. Okay. So let's go to Example 5 in the 371 application, which is on page 282?

A. Is that the common disclosure numbering or the exhibit number?

Q. The common disclosure numbering. It is exhibit page 295.

A. It has a table data then Example 5.

Q. Yes.

A. Indeed, I am now there.

Q. Example 5 is an assay for hyaluronidase activity under temperature and phenophilic conditions; right?

A. That is what it says. It is a test of natants that were identified as active and then tested under these conditions.

Q. And specifically for: "... activity under temperature and phenophilic conditions." Right? That is what it says in the header, for Example 5?

NAISMITH - MARTIN

A. It defines 37°C, yes, higher than room temperature, some people would say thermophile is 100°C, but the patent is quite clear the teaching is 37.

Q. So it says on exhibit page 295 under Example 5 that: "Supernatants from PH20 activity variants set forth in Table 9, as identified in Example 4, were tested for stability un[d]er thermophilic and/or phenophilic conditions." Right?

A. That is what it says.

Q. So they were measured -- they were testing supernatants, right, in Example 5?

A. Yes, it does say supernatants, that is correct.

Q. So because they are testing supernatants the concentration of protein from each of the individual transfected cultures could vary; is that right?

A. I would like to check. It is a while since I read this. If they did any normalization here between different ones, I cannot recall if they actually controlled for that in this particular example, I don't believe

they did.

Q. Okay, we will look at that in a second. As a theoretical matter though, when you're testing supernatants from different transfected cell cultures, the concentration of protein that you are going to get out of those various transfected cell cultures can vary; right?

MR. KUSHAN: Objection foundation.

A. As a hypothetical matter, that is -- for different proteins, you know, if I changed the protein in some way, correct; you would expect, if I was making the same protein under the same conditions I would get pretty much the same result, or you would expect that to be true, same amount protein, but if I was doing modified proteins you could get different amounts.

Q. (BY MS. MARTIN): Let's say I take my wild-type PH20 and I transfect it into a CHO cell, so let us say I have five different transfected cell lines of my wild-type PH20, are you telling me that if I take out, let's say, you know, 1 millilitre aliquot out of each one of those five cell cultures, that every single one is going to have the exact same concentration of

PH20?

A. They would not have precisely exactly to some measure the exact same, but the variation you would expect if you had done the experiments properly in a controlled manner, you would expect by the particular hypothetical we are exploring, for it to be very similar. You haven't -- that is a test of reproducibility.

Q. That is a test of reproducibility of protein concentration in a supernatant?

A. No, the test is how you approach the experiment, if you had done it under identical ways you should get pretty much the same answer.

Q. Okay, so when you say identical ways, are you talking about identical cell culture conditions?

A. Cell culture conditions, time, buffer, if you've shaken the plate, all of how you handle the cells for that experiment. If you've kept them all the same then reproducibility, if you have actually done that, would suggest you would get very similar results, there may be an inherent noise in your data that you can find out, but if you've done it properly you would expect

NAISMITH - MARTIN

them to be very, very similar.

Q. Why is that?

A. It is the basis of the scientific method, is it should be reproducible and one of the ways with students, for example, doing something completely different is they often believe they have done everything exactly the same, but get different results, wildly different results, they are not doing everything the same in that case.

Q. So is your testimony that if you have two different cell lines, those two different cell lines are going to secrete the exact same amount of PH20 independently of one another?

A. No, because those are different experiments; you have changed the cell line.

Q. So if you have the same cell line in two, let us say, two different cultures, right two different beakers, two different cultures, you would expect that the cells would produce the exact same amount of PH20?

MR. KUSHAN: Objection form.

A. I didn't say the exact same, I said very similar, but you would be, characterise a

level of noise in the data that you do get statistical variation, but you would expect, if you had done the experiment carefully and properly, for that to be relatively small, but you can measure it and people would measure it so they would have confidence in that. Given that you have done exactly the same thing, if you truly had done exactly the same thing, then the difference between them is a reflection of statistical noise stochastic variation.

Q. (BY MS. MARTIN): Is not part of it just a difference in the amount of PH20 secreted by the cell? I mean, are you saying that all cells are going to secrete the exact same amount of PH20?

MR. KUSHAN: Objection foundation.

A. I keep saying that "exact" is a demanding test, but given you have the same number of cells, the same culture, exactly the same conditions, that the variation you would see should be small and what you would assign as stochastic variation, if you had done everything in the same way, that you hadn't obviously varied the cell line as we talked about, if you hadn't

come back tomorrow when the lab was 3° warmer, if you hadn't used a different batch of media, all of these things could lead to increase noise in the data. But a basic premise of science, good science, is that if you do everything the same way, it doesn't matter, if you follow the recipe you should get pretty much the same answer, and pretty much, is not exact. Where you do not get that, and you've excluded carefully all other possibilities, then that is a stochastic noise of the knowledge of the system.

Q. How do you know it is the same number of cells and the different cultures?

A. So, you told me you started with the same number of cells. I asked, you start everything in the same ----

Q. Okay, then we are clearly not on the same page here. I just said that you have two different cell cultures of wild-type PH20, why would you assume that they both have the same number of cells in them?

A. Because I asked you if everything was the same.

Q. Let's assume that it's not the

same.

A. Then I would expect to get different results.

Q. Is it possible to control to make sure that you have the exact same number of cells in two different cultures?

A. Yes.

Q. Okay, and would you just expect that to happen?

A. What do you mean expect what to happen, sorry?

Q. If you transfect some CHO cells with PH20 and grow them in a culture would you expect that two different cultures, if you did that, you know, in two different containers if you will, that you are going to obtain, you know, identical cell counts in your two different containers?

A. If everything was done identically then I would expect them to be pretty much the same within some stochastic noise ratio, but if you made differences in the volume or temperature or other things then I would expect that to be different.

NAISMITH - MARTIN

Q. Okay. Back to Example 5, so let's go to the bottom of page exhibit 297, in the common disclosure.

A. So this is Table 12?

Q. Sorry, it is exhibit page 297, common disclosure page 284?

A. Okay, my apologies. So it is, yes, 284 starts the first words are: "1.5 hours at 37°C ..." at the top of the page.

Q. Yes, let's go to the last paragraph on the page.

A. Yes.

Q. And it says in the second sentence: "Absolute hyaluronidase activity (U/mL) was generated from the standard curve." Right?

A. That's indeed what it says.

Q. And then it says: "In addition, percent activity was determined as a ratio of activity at 37°C/4°C, 37°C plus m-creso1/4°C, and 37°C plus m-cresol/37°C." Right?

A. Yes.

Q. And then: "The results are set forth in Tables 11 and 12 below." Right?

A. Yes.

NAISMITH - MARTIN

Q. So Table 11 provides the absolute hyaluronidase activity in units per millilitre as generated from the standard curve; right?

A. The standard curve is not disclosed, that is what the table reports.

Q. And there is no normalization or concentration, is there?

A. I'd like to just read through to check I read the method properly.

Q. Okay.

A. *(Pause for reading)* So as I read it, I assume that they are trying to keep the same volume. There is nothing that says they changed the volume, they used the same CHO cells from the same supplier. So I read it as they are trying to make sure that they are using the same procedure so that the results are comparable. And they are only working on active proteins here, so they are single site active mutations. So, while I might expect for mutations that are classified as inactive, for there to be very large deviations in the amount of protein, at least here the single site mutations I would assume that they are probably about the same in general. But the

stochastic noise, there is quite large variations so it is possible they have different concentrations.

Q. So by the fact that they are testing the same volume and they use the same CHO cells from the same supplier you assume that they have cultured all of the variants and all of the wild types in the exact same conditions, such that there is no variation in temperature or shaking or anything that could potentially result in a difference in cell count in each of the cultures, that would then result in a difference in concentration?

MR. KUSHAN: Objection foundation.

A. So they are clear that they put some controls in to try to control for that, so there are controls mentioned clearly here in each plate so that for each plate you are trying to minimize. But plate-to-plate has variation but that should be accounted for by the control experiments.

Q. (BY MS. MARTIN): Okay, but the issue is that in the data that are reported in Table 11, as they are reported in Table 11 they

are not normalized for protein concentration and you cannot assume that every single well had the exact same concentration of protein, because that would be unreasonable; is that not true?

MR. KUSHAN: Objection foundation.

A. I can't assume that every single well has an identical concentration but I would be, as a POSA would normally do this experiment one would seek to minimize any variation between them, they explicitly put controls in to try to control for that with their plates. So I think I would therefore believe that what they have reported reflects meaningful data rather than just accidental you know there is one, they have gotten times more of one than the other, because they are trying to minimize variation within a plate and they also control between plates.

Q. (BY MS. MARTIN): Okay. So in Examples 3 and 4 they controlled for the difference in concentration by normalizing based on seep, but they didn't do that so that they reported their data normalized for protein concentration in Examples 3 and 4, but here they didn't report the data normalized for protein

concentration because they are not reporting it in the same way. So it's not that they do care about the difference in protein concentration, it's that this particular table, with which you have decided to complain about variability, does not actually control for differences or does not actually normalize for differences in concentration?

MR. KUSHAN: Objection foundation, objection as to form.

Q. (BY MS. MARTIN): And Table 12, right, provides percentages but the percentage that is provided is not a comparison between the different variants, such that there would be no need to control or normalize for concentration, for protein concentration between the variants?

MR. KUSHAN: Objection foundation, objection form.

A. So here, so my criticism of the assay is even within things that are in the same plate and duplicates you see quite large variations between them and that is a higher level of variation than a POSA would believe for a reliable assay.

Q. (BY MS. MARTIN): Okay. I want to

make sure we are talking about the same thing. Within things that are in the same plate, so what things in the same plate are you talking about in that answer?

A. So each measurement is in duplicate.

Q. I am sorry, what are you talking ----

A. Sorry, apologies, I am on page 212 of my report, where I have extracted some of the data here.

Q. You said 212?

A. In my report, page 212 which is paragraph 293.

Q. Okay.

A. I have specifically focused on the wild-type controls to assess how reliable the assay is.

Q. You are assuming that the wild-type controls are on the same plate?

A. So they are in different plates, they are, "intra" is within the same and "inter" is between plates, I believe, I always get -- yes.

Q. I thought that you were saying that

even with things that are in the same plate, you see quite large variations?

A. You do, you can read down and see them, that there are quite large variations between duplicates.

Q. How do you know the duplicates are on the same plate?

A. I'd have to check if they said that, it would be my expectation they would do that.

Q. Okay. So you are talking about the ----

A. Let me just find it, sorry. So it doesn't explicitly, I think, say that but it would seem kind of crazy not to put them in the same plate because you would introduce quite large errors, you could introduce very large errors by that and it would make your results even more difficult to interpret.

Q. Ok. You expect that, if I am understanding correctly, the wild-type data that are on page 212 that you excerpted, right, that the duplicates are on the same plate but each individual wild type, so going down each

individual row, is on a different plate; is that right?

A. I don't assume that to be true, I think there will certainly be some of them on different plates, yes.

Q. I am sorry, you said some of them would be on different plates?

A. Well, the duplicate, I assume for each one of the duplicates it will be in the same plate.

Q. Okay?

A. And there are several duplicates here mentioned. I think they would all be in separate plates, the duplicate would be in the same plate.

Q. I understand, okay.

A. For the wild type.

Q. Yes, so I think there are 17 wild types here; right?

A. There are 17 wild-type measurements, yes.

Q. You are saying that the 17 are on definitely more than one plate, maybe not 17, but ----

NAISMITH - MARTIN

A. Yes.

Q. Okay. And so when you say that there is unacceptably high variation, you are talking about the variation between duplicates and not the variation between wild types?

A. No, I'm talking -- it is, both things are true, there is a large variation even between the duplicates, but there is extremely large variation between plates.

Q. And when you conclude that there is a very large difference between plates, you are not accounting for the fact that there may be different concentrations of wild type in the wells on those different plates?

A. You are correct that I am not accounting for it, but again if I, looking through the lens of a POSA, that based on the fact it is a wild-type protein which you should express in the same way that I have talked about here, it is a very high level of error between plates if you had done everything the same. They don't mention that they systematically did different things to the plates. So based on the description, if you replicated it I would have thought you would have

much more similar results than you obtained.

Q. But assuming that you used the same concentration of protein in each of the wells.

MR. KUSHAN: Objection, foundation.

A. I think it is rather assuming that since I used the same media, the same cell line for the native protein which should, and same volumes, that I would expect that I would have, within some measure, the same number of cells, a transfect in a consistent way. If my assay was reliable I would expect similar results between plates. That the fact you don't see that, if it was a student in my lab, I would want them to go back and figure out what it is they are doing differently.

Q. (BY MS. MARTIN): One of those things could be purifying the protein and making sure they are using the same concentration of protein; right?

A. I think these are supernatants they are using.

Q. Yes, they are supernatants.

A. So they could choose to use different volumes of supernatants; is that what

you are saying?

Q. They could purify the protein and then just use a fixed amount of protein to make sure that they are using the same concentration of protein?

THE COURT REPORTER: Sorry, could you repeat that please?

MS. MARTIN: They could purify the protein and then use a fixed concentration to make sure that they are using the same concentration of protein?

A. So I think, you could have done an assay where you probed to understand why you've got that variation, I do not understand why you have that variation, there's nothing in Example 5 that I could spot that says, "I know why they've got such variation". So, to understand that, you are correct, I would want to go back and try to understand that and do more experiments. As a normal rule in my lab we do triplicates and if a student had produced that level of variation between plates I would be concerned that they are doing something that is not controlled for, they are doing something different. I can't imagine

that. It seems very odd. Okay, based on -- it doesn't say in the description of the experiment, "Some of the plates we put more in, some of them we put less in". When people are comparing things or analyzing things, it is kind of a rule of science is, vary one thing at a time, try to keep everything else constant.

Q. Couldn't they also have just normalised for concentration?

A. They could have done a lot of things but they didn't.

Q. You are criticizing, no, you are not criticizing them for not normalizing because you didn't take into account the fact that they didn't normalize, right, you are criticizing them for reporting the data like this without normalizing?

MR. KUSHAN: Objection foundation, objection as to form.

A. My criticism is that there is nothing in the description that for the wild-type protein that I can understand why they have such large variation, that is my opinion. I can't understand if they did the experiment properly why

that is the case, that you've got such large variation between what should be similar samples. You are postulating that they had different amounts of material, but that would not be normal for a POSA to do, and they would certainly, I would think, tell me that in the assay.

Q. (BY MS. MARTIN): So, okay, I just want to make sure that the record is clear, that when you said that you're testing protein supernatants from different transfected cells that you are assuming that the protein supernatants you are testing all have an equivalent amount of protein, the same concentration of protein across the supernatants taken from the different cell cultures?

MR. KUSHAN: Objection foundation, objection as to form.

A. That's not what I said. I restricted my answer to the wild type, which we can agree, given it is the same cell line, grown in the same way, there is nothing in the description that I could see that tells me that some plates were done differently. And as I said, the default assumption of a POSA would be when you

are measuring the wild type in different plates you would try to keep the experiment or you would aim to keep the experiment consistent, so you would have the same volume, same cell line, same temperature, same media.

What I am saying is that based on that, when I look at the variation between the plates, I can't understand why that variation is so high. And there is nothing in the description that says, "For some of the plates with these variations we did things differently". That might heap me understand, but they don't discuss at all that ----

Q. (BY MS. MARTIN): So you just assume that the data are unreliable for Examples 3 and 4 based on the wild-type variation in Example 5?

MR. KUSHAN: Objection foundation, objection as to form.

A. Unreliable for what test?

Q. (BY MS. MARTIN): For any test because you said they are unreliable ----

A. I believe that the protein is active in the assay, and the activity is decreased

NAISMITH - MARTIN

in the presence of cresol for the wild-type protein at 37° but I am highlighting that the large coefficient of variants would, and these variations between what should be this, we would expect as a POSA the same thing, would be alarming and also there are quite large deviations between duplicates, which are in some ways the absolute definition of things you expect to be the same.

Q. (BY MS. MARTIN): Are you telling me that you expect the duplicates to give exactly the same number?

MR. KUSHAN: Objection foundation, objection as to form.

A. I have never used the phrase "exact number". What I'm saying is that where they are different, if you have done everything exactly the same, the difference arises from some stochastic variation that you can quantify, and that is often used as a reliability for the assay.

Q. (BY MS. MARTIN): I want to be really clear about what you would consider to be an acceptable stochastic variation. So, for example, like the third line down has 3 and 2.725 and so you are saying that those two numbers are

NAISMITH - MARTIN

unacceptably different?

A. So you can't form an opinion what the precise level of stochastic variation is until you actually assess a large number of assays done in the same way, it is not simple to just take two separate numbers. It could arise from that. But as a percentage, the numbers you gave 2.7 to 3, that is 0.25 divided by 3.08, that is high but Dr. Petsko's threshold, I think, was 10%.

Q. Is that a 10% difference?

A. Variation. But between duplicates that is not nothing. If you go to the middle column 37° with no cresol, you can see one of them is 10.8 and the other one is 3.94, that is 1, 2, 3, 4, 5. Now, if I saw that in the student's notebook I would be incredibly concerned about what they are doing and probably get them to start again, and watch them very carefully. I can see other examples in the table where even within what are supposed to be duplicates, I have these very large differences.

Q. Okay, so let's turn to paragraph 265 in your declaration. In paragraph 265 ----

NAISMITH - MARTIN

A. This is headed: "A POSA relying on Dr. Petsko's ..."?

Q. Yes, and actually let's look at the graph, the chart on page 193?

A. Okay, yes, I am looking at them.

Q. And so you have taken the relative activity levels reported in the patent for different mutations at, for example, position 74?

A. Yes.

Q. And you have done a statistical analysis where you've calculated, among other things, the standard deviation and the coefficient of variation; right?

A. Yes, but these are not the same as the coefficient of variation between assays.

Q. You are representing that this is, you are representing these as error bars so you are representing that, for example, T74 the different mutations of T74, two different residues are treated as, are being treat as replicates in your analysis with a high error rate as opposed to independent experiments.

A. No, that is not what I did at all.

Q. Well, that is what you did because

NAISMITH - MARTIN

I see error bars on your graph indicating that there is a wide range of error for that particular experiment.

A. No, that is not what it means. It just shows you that what the table is plotting and I apologize, it is not obviously clear, when you look at the mutations at T74 as individual dots you can point them. Then what these bars are is just to give you a sense of what the variation of those mutants are. Between the mutants it doesn't speak to error of the measurement. Just the fact that I can put one residue substitution, which has a two point, roughly, seven, let's look at the top blue number, so that makes the protein 2.7 fold more active, that is the number reported in the table. Another mutant has 0.5 mutant, so it is reduced activity. So that is not an error measurement, it is to give you a sense, the reason the numbers are there was to just give a sense of what the variation between single site active mutations are at a position are.

Q. Okay.

A. And those are very large, and because of that a POSA would look at that and

realize that the difference between a mutation, particular introduction, that makes the protein 2.5 times more active and one that makes a protein half as active, roughly, right, I'm not going to get into the precision here. That is a really interesting issue about what is happening to the protein active site or binding or whatever, that is quite interesting. So it speaks to this problem that this mutation is doing something to the protein that we don't understand. We can see their outcome. So therefore if you combine that mutation which has done something to the active site in some way, because we can see it in the assay, with another mutation, that is where we start to worry, really would worry about epistasis because we know these are affecting at a distance in some way the active site because you can see it in the assay, and that is a big difference.

Q. When Dr. Park rated a particular change as a "one", meaning that it would be untolerated, is it your testimony that that change, that would be untolerated according to Dr. Park, would have an impact on the active site of the protein, irrespective of where it is in the

protein?

MR. KUSHAN: Objection foundation, objection as to form.

A. So Dr. Park's analysis talks about destabilizing of the local environment and, therefore, those were single site mutations based on his multiple sequence non-conserved regions, that since they were destabilizing in their local environment he would expect those to have a negative consequence to the protein's activity. He did not assign a particular level of activity to his assays but he said those are the ones that are likely to be inactive. But he didn't give a number for that. He just said it would be destabilizing, so I would not start here.

Q. Okay. So when Dr. Park determines that a change will be destabilizing such that it will inactivate the protein, it will inactivate the protein because of a destabilization effect. When the patent shows a reduction in activity you assume that there is change in the active site caused by some sort of magical epistasis effect; is that right?

MR. KUSHAN: Objection foundation,

objection as to form.

A. Epistasis is not a magical effect. It is well-documented problem. Local instability can affect global protein stability, affect at a distance. So Dr. Park's analysis was confined to single site mutations. What this is analysis is doing is showing even for single site mutations at one position, if I put one type of residue in, I get a reduction of 50% activity. If I put another in, I get 2.7. Now these mutations would fall in and I haven't gone to check, but I think these are non-conserved residues and I can go to Dr. Park's spreadsheet, the ones that are found in the MSA that are represented here, Dr. Park would have assigned to the buckets 2 and 3 is my prediction. I haven't checked every single one but he selected his residues not universally, he didn't test all of them because he only used MSA to guide the choice.

Q. What if it's the case that he assigned one of those residues to bucket 1, that it would be untolerated? Based on your analysis, right, when Dr. Park assumes that a mutation is going to be not tolerated and inactivate the

NAISMITH - MARTIN

protein, it is because of a stability issue. But when you look at the patent data and you find a single point mutation inactivates the protein, it is because that point mutation, wherever it is, somehow impacts the active site, not because of a stabilization issue, because it is actually impacting the active site through some sort of long-range situation?

MR. KUSHAN: Objection foundation, objection as to form.

A. So there was a lot in there. So, to go back, Dr. Park, his analysis is what a POSA would reasonably predict and some, I think there was one prediction in fact that Dr. Petsko pointed out, didn't chime with the data. What you are seeing -- so he assigns them to bucket 1, 2, 3. So he talks about destabilizing local environment, but if you destabilize one bit of the protein that will influence across the protein. That is why he says you would not start here if you were to try to make active single site mutations. What I am talking about here, is somebody has done all of these single site, well, not all, but the ones I have plotted here, and

NAISMITH - MARTIN

I don't know, nor does the patent teach me, nor can I model, why one mutation reduces the activity by a factor of 2, and the other one increases the activity by a factor of 2. But for activity measurement that has to mean something is happening at the catalytic site.

Q. Okay, so based on what you just said, then when Dr. Park assumes that, or assigns a particular mutation to bucket 1, then it must be the case that something is happening at the active site; right?

A. No, I didn't say that. Dr. Park's analysis is based on he predicted that his bucket 1 would be destabilizing for proteins, and therefore likely to lead to inactive protein. He did not pursue those further, he just said destabilizing is likely to be a negative mutation so not when a POSA would start for single site mutations.

Q. And you would see that it would be an inactive protein meaning that it was not active?

MR. KUSHAN: Objection foundation, and objection to the form of the question.

NAISMITH - MARTIN

MS. MARTIN: Let us take a break. Let's go off the record.

THE VIDEOGRAPHER: We are going off the record. The time is 5.22 p.m.

**(Short recess from 5.22 p.m. to 5.29 p.m.)**

THE VIDEOGRAPHER: We are back on the record. The time is 5.29 p.m.

Q. (BY MS. MARTIN): Okay, Dr. Naismith can you please turn to paragraph 124? Are you at paragraph 124?

A. Yes, I am.

Q. Okay you say in paragraph 124 that: "Dr. Petsko's practical answer that a POSA would not have even tried to make modified PH20 polypeptides with more than about 5 modifications reflects 2011-2012 timeframe for producing multiply modified PH20 polypeptides." Is that right?

A. Yes, that is what I said.

Q. Dr. Petsko didn't actually testify that a POSA would not have even tried to make modified PH20 polypeptide with more than about five modifications, did he?

A. He did not specifically address

five but he also said, you know, there is a lot of stuff no POSA would go ahead making them all.

Q. Well, are you telling me that he literally testified a POSA would not go about making them all because there's a lot of stuff?

MR. KUSHAN: Objection foundation.

A. That's not what I said. Dr. Petsko, when talking about the protein in his deposition was asked, you know, of all the variants and he said, "No POSA would go about a rational design that way", that is what he said. I took that to -- he did not offer any opinion on how one might go about five or more mutations. Dr. Hecht pointed out that that is where his view a POSA draws the line, it's where a draw a line. There's nothing in Dr. Petsko's book or any other, I'm aware of, that suggest he has some particular insight how you would get beyond 5. When he was asked about how you explore large numbers he did said, well, you wouldn't do that.

Q. Well, okay, let's be very precise about what he did and did not say since you seem to be inclined to give opinions on Dr. Petsko's behalf. So he was asked in the testimony that is

shown on page 84 in your declaration, if someone were asked how to -- and they were asked to determine the number of PH20 polypeptides in the genus that were properly folded and enzymatically active, how they would go about doing that. To be clear he was asked to give a number, right, not -- he wasn't asked about making proteins with multiple mutations. He was asked to give a number of proteins; right?

MR. KUSHAN: Objection foundation, objection as to form.

A. So, because he said the number he was able to calculate mathematically and agreed with the calculation that Dr. Park did, he is being asked here, determine the genus, so what is the molecules that were consistent with claim 1 and it is qualified in the question: "... properly folded and enzymatically active, how would they go about doing that?" So how might they -- so in all these variations, of which we agree five is included in that, he said you just wouldn't do that. You: "... wouldn't even go about doing that." He said.

Q. (BY MS. MARTIN): Sir, you are

NAISMITH - MARTIN

blatantly mischaracterizing is testimony.

MR. KUSHAN: Are you done with your answer?

(To Ms. Martin) Don't interrupt the witness in his middle of answer, please.

MS. MARTIN: Okay, please continue to mischaracterize if you weren't done.

A. I am not trying to mischaracterize his testimony.

Q. Okay, so, again because you just **did**, right, you said: "... they were asked to determine the **number** of PH20 polypeptides in that genus of claim 1, that were properly folded and enzymatically active, how would they go about doing that?" And he said: "They wouldn't even go about doing that." Meaning they would not go about determining the **number** of PH20 polypeptides in the genus of claim 1 that were properly folded and enzymatically active; right?

MR. KUSHAN: Objection foundation, objection to form.

A. How I read his testimony is that presented with the challenge of what is covered by the patent scope and it is qualified here as:

NAISMITH - MARTIN

"... properly folded and enzymatically active ...", he was asked how would you know which ones were active and properly folded? And he goes on to say in the last answer apart: "... from experimenting on the entire ensemble" he wouldn't know.

Five mutations, for example, is within the mathematical scope description, but I believe Dr. Petsko would have been confident if he want, he could have expressed a view and I would have agreed with it, that up to five mutations based on PH20 there is an element of predictability but beyond that we all agree there is not an element of predictability. In Dr. Petsko's answer the point is that he is talking about the genus, how would you know which of the genus is active and folded? And in that parameter space are five mutations and he says you wouldn't go about doing it that way. Then in his last answer: "Is it possible to experimentally determine which ones are properly folded and enzymatically active ..." and he said: "Not from experimenting on the entire ensemble."

Q. Yes, it is not possible -- okay, we

NAISMITH - MARTIN

don't have to do that again. So now, let's see, in fact in your declaration you admit that Dr. Petsko describes an example of making 22 changes which would be more than five?

A. Could you refer me to that please?

Q. Page 92, you say: "In paragraph 359 f his declaration (below), he provides an example where the POSA inspects positions that tolerated between 60% and 100% of the tested changes using a homology model and selects sets of 22 substitutions from those positions." So, you recognized in your declaration that in fact Dr. Petsko did not draw a line and conclude that a POSA would not have even tried to make modified PH20 polypeptides with more than about five modifications because you acknowledge that he provided examples of making 22 changes; right?

MR. KUSHAN: Objection foundation, objection as to form.

A. So Dr. Petsko, as I read it, he is going beyond what is in the patent, and he is saying that in his opinion that if -- and he qualifies it later on by talking about

independent, that he thinks that if I selected 23 residues that it would fold into an active, again he uses active here, changes. And I disagree with his opinion that you could in any way predict which of the 23 is possible to make.

So, while I accept that I can imagine a protein with 23 residues to be properly folded and active just as Dr. Petsko talks about, my point is that I had no way to predict or reliably identify what that sequence would be for the reasons that I have shown you in my deposition -- sorry expert report, my apologies.

Q. (BY MS. MARTIN): Okay, so can we turn quickly to Dr. Petsko's deposition?

A. Of course.

Q. At page 113?

A. Can I take some time to familiarize myself with the document please?

Q. You want to read his deposition transcript?

A. Just around the part here, help me to set in context, if you don't want me to I will try to answer the questions as best I can.

Q. I just want to point you to -- are

NAISMITH - MARTIN

you on page 113?

A. Page 113, yes.

Q. So there is a question and answer starting on line 20 that says: "So using that dataset, the person could predict which combinations might yield an active form of a mutated PH20 polypeptide let's say with 15 changes to it?

"(A) You could, but as I said, you would not tend to do that. A person of skill in the art would tend to only be interested in a much smaller number of mutations, because you can almost certainly get where you want to go that way." Right?

A. I can read that.

Q. All right. So we talked about Example 5 in the common disclosure ----

A. Can I put away the deposition or do you want me to keep it open?

Q. No, you can put that away.

A. Can you remind me what page it is on, sorry? I know we talked about Example 5, just to help me remember the page.

Q. If you want to go to, so it starts

on page exhibit 295 and Table 11 is on page 298?

A. 295 is headed Example 5, I will just turn to that page in my report, if that is okay.

Q. Okay.

A. Thank you.

Q. So I just want to make sure that we are clear that data in Table 11 from Example 5 is the absolute activity, absolute hyaluronidase activity in units per millilitre and does not control for concentration, or is not -- sorry, strike that. The data in Table 11 in Example 5 provides absolute hyaluronidase activity in units per millilitre and was not normalized for concentration; right?

A. So it provides absolute values but reading the methodology, you think they are trying to keep it the same, so there is no, as I said before, there's no reason to assume or understand from the patent that they deliberately chose to vary things between experiments for the native protein, so didn't tell me that this plate was made in some different way. And my analysis is that therefore it is hard to understand why they

NAISMITH - MARTIN

get such variants between the native protein.

Q. Well, so it's a yes or no question, right, they didn't normalize for protein concentration for the data in Table 11 but they did normalize for protein concentration for the activity assays in Example 3 and 4?

MR. KUSHAN: Objection foundation, objection as to form.

A. What I'm saying is that I agree it is straight, yes, they used the absolute, but I'm saying by the method described you are trying to control for variation experimentally. What -- they did not apply a subsequent normalization based on concentration, that is -- there is no evidence that they did that. But my interpretation of the method is that they tried to control for differences by doing the same method with the same cell lines in the same way.

Q. (BY MS. MARTIN): Okay.

A. They appear to have been unsuccessful.

Q. All right. Can we take a break?

THE VIDEOGRAPHER: We are going off the record. The time is 5.44 p.m.

NAISMITH - MARTIN

**(Short recess from 5.44 p.m. to 6.09 p.m.)**

THE VIDEOGRAPHER: We are back on the record. The time is 6.09 p.m.

Q. (BY MS. MARTIN): Dr. Naismith, what level of predictability are you requiring in order to be confident that a particular mutant will be active without having to test it?

MR. KUSHAN: Objection foundation, also objection as to form.

A. So what particular mutant? Just any random mutant?

Q. (BY MS. MARTIN): Yes, any random mutant.

A. I would distinguish, I would use some of what we already know to answer your question, so is it in the non-conserved region that Dr. Park analyzed?

Q. And if it is?

A. Then those particular mutants he has assigned to buckets 1, 2, 3, and I have said that I would have confidence that the ones that he has assigned into buckets 2 and 3 you would have a reasonable expectation that those single mutations would be stable and active, natively folded and

active.

Q. And so, what does "reasonable expectation" mean to you, how certain do you have to be?

MR. KUSHAN: Objection foundation, also objection as to form.

A. I don't need to -- a reasonable expectation is that it is likely to be correct. I don't think, the majority, I didn't think of a single dividing line percentage if that is your answer, your question.

Q. (BY MS. MARTIN): Would you say more than 50% certain, more likely than not?

A. Yes, more likely than not that would be, that would be one that I would be, I would expect some surprises but I would expect more likely than not about 50%, based on Dr. Park's analysis and how he's gone about it, that's for single sites, yes.

Q. And if you were to make, let's say, a triple PH20 mutant at positions that are shown to be tolerated or produce an active protein in the patent, would you think it is more likely than not that you would get an active protein?

NAISMITH - MARTIN

MR. KUSHAN: Objection foundation, objection as to form.

A. So the triple mutation also has the M320K?

Q. (BY MS. MARTIN): D320K.

A. I apologise, I am tired. So D3, so that is one of my triple. So my two mutations were both active in the single mutation?

Q. Yes.

A. And I've got a D320K and I have two additional mutations both of them were acting single, I would say more likely than not I would expect those to be active. But I qualify only that some mutations, some residues at that position were either shown to be inactive, right. So at some positions they were classified as inactive when you made a particular change, so I wouldn't have confidence that if I introduced one which had been classified as inactive, but that would give a predictable more likely than not active.

Q. Do you agree that the cell culture techniques that were described in the patent would have been routine in 2012?

NAISMITH - MARTIN

A. Sorry, were what in 2012?

Q. The cell culture techniques described in the patent?

A. Were what in 2012, sorry?

Q. Would have been routine in 2012?

A. They were within the skill of a POSA 2012, yes.

Q. Do you agree that the patent explains how to make modified PH20 polypeptide?

MR. KUSHAN: Objection foundation, objection as to form.

A. So, the patent doesn't describe how to make modified PH20 polypeptides beyond a certain number of mutations that would retain activity and properly folded.

Q. (BY MS. MARTIN): Did you consider the fact that microtiter plates can vary in terms of the amount of biotinylated HA, that is coded in the well, informing your opinions regarding variability of the assays in the patent?

A. So you are identifying something that is not properly controlled and you would expect to try to control for that, but nothing in the disclosure tells me that they had a concern

they had some trouble with reproducibility.

Q. You can control for defects in a plate?

A. That is a different question. So you are now postulating that the reason for these differences could be that there was a bad plate?

Q. Yes.

A. In that case I would have gone back and done the experiment again if I got these deviations.

Q. Okay. Did you consider variations in the size of the HA polymer that is in the well in rendering your opinions on variability?

A. So do you mean the variation HA polymer between plates or the fact that you have a population of species in a well.

Q. The difference in species between wells?

A. So again the patent tells you how they got to that, you are postulating that they varied it in some uncontrolled way. I don't think a POSA would normally do that. There could be stochastic variation but you are told how these source material, so again as a POSA, reading the

method, there is nothing to direct you that they have wild variation in the species.

Q. You keep saying that there could be stochastic variation, but is there not always stochastic variation in life?

A. You are quite right and that is the error threshold of the assay, but before a scientist assigns difference to that, you want to be sure you haven't eliminated obvious things which you know could give rise to, a student uses the wrong plate, you gave an example of a defective plate or that two different people prepared, one of them inexperienced prepared the HA biotinylation or that somebody brought a different batch of HA. All of those would give rise to variation, but there is nothing in the disclosure which would lead me to believe that they set out to do that. As I say, it is not normally how POSAs approach experiments, so I have assumed that they would approach it as a normal POSA would.

Q. You agree that a normal POSA would expect there to be some stochastic variation, just as matter of the fact that their experiments that

are being performed, you know, in the world?

A. Yes, I would echo what Dr. Petsko said. You would expect a well-run assay in this type of plate to be less than 10%, that is what he would reckoned as a reasonable threshold. I'd be happy to agree with that.

Q. And he said less than 10%?

A. 10% or less, I think he said some numbers, you know, but I think -- I'd like, if you want me to be precise I will turn to exactly what he said, but I think he said 10%, that sort of thing.

Q. Do you agree that time, humidity, temperature and oxygen levels can all impact the number of cells in a culture and as a result the protein concentration in the supernatant from that culture?

A. So, again, that is, lab practice 101 is that when we describe experiments we describe the temperature they are run at precisely to control for that effect. Again there is nothing in this disclosure that told me we tried one day at one temperature for the native and one day at another.

NAISMITH - MARTIN

So normally it is one of the things that I would have my students do, is make sure if you are doing repeats or multiple plates you try as hard as possible to have them done in exactly the same way so that the variation you see is a genuine reflection of stochastic variation rather than error, poorly done assay. You can control for temperature.

Q. What about humidity?

A. We actually measure humidity in a lot of the labs. If you thought that the assay was particularly sensitive to humidity you would control for it.

When I used to worked in Dallas some days were very humid or very hot so it can affect an experiment and you would know that. So you would try to replicate it under, as close as possible, the same conditions. Labs are usually air conditioned to try to minimize that variation.

Q. Even if you do try your hardest there is still going to be some amount of variability; right?

A. Yes, as Dr. Petsko said, well-run 10% or less, a well-run assay, he thought 10%

roughly or less would be a well-run of this type of ELISA assay and I would agree with him.

Q. Okay. Do you know if he was thinking about testing supernatant that had not controlled for differences in protein concentration when he said 10%?

A. I think his opinion was that that would be what he would imagine a well-run experiment is. We talked about before, that based on the way they approached the method, and I talked about the wild-type protein only, then you are trying to control for any variation by the way you do the experiment in the same way. There is nothing in the description that makes me or guides me to them making a change to the temperature, humidity and performing the assay.

MS. MARTIN: I don't have any further questions.

MR. KUSHAN: Why don't we have a short break and come back?

THE VIDEOGRAPHER: We are going off the record. The time is 6.20 p.m.

**(Short recess from 6.20 p.m. to 6.25 p.m.)**

THE VIDEOGRAPHER: We are back on

NAISMITH - MARTIN

the record. The time is 6.25 p.m.

MR. KUSHAN: We have no further questions.

THE COURT REPORTER: Could I ask you to put your transcript orders on the record before we go off, please?

MS. MARTIN: Whatever our normal -- wait its different. Can we do a rough and then final?

THE COURT REPORTER: We will do it off the record.

MS. MARTIN: Okay, that is easier.

THE VIDEOGRAPHER: This concludes the deposition. We are going off the record. The time is 6.26 p.m.

**(Deposition concluded at 6.26 p.m.)**

**CERTIFICATE OF WITNESS**

I HEREBY CERTIFY that having been first duly sworn to testify to the truth, I gave the above testimony.

I FURTHER CERTIFY that the foregoing transcript is a true and correct transcript of the testimony given by me at the time and place specified hereinbefore.

.....

**DR. JAMES NAISMITH**

Subscribed and sworn to before me

this_____day of_____20___

**CERTIFICATE OF COURT REPORTER**

I, AMY COLEY, Court Reporter, do hereby certify that DR. JAMES NAISMITH the witness whose deposition is hereinbefore set forth, was duly sworn by me and that such deposition is a true record of the testimony given by the witness.

I further certify that I am not related to any of the parties to this action by blood or marriage, and that I am in no way interested in the outcome of this matter.



.....

AMY COLEY

**ERRATA SHEET**

DATE OF DEPOSITION: 2ND FEBRUARY, 2026

**WITNESS: DR. JAMES NAISMITH**

| PAGE | LINE | FROM | TO |
|---|---|---|---|
| | | | |
| | | | |
| | | | |
| | | | |
| | | | |
| | | | |
| | | | |
| | | | |
| | | | |
| | | | |
| | | | |
| | | | |

**MARTEN WALSH CHERER LTD.**
**LONDON, ENGLAND**
**Tel. 01144 (0) 20 7067 2900**
**(Out-of-hours tel. 01144 (0) 7889 198989)**
**info@martenwalshcherer.com**