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3 UNITED STATES PATENT AND TRADEMARK OFFICE
4 _____
5 BEFORE THE PATENT TRIAL AND APPEAL BOARD
6 _____
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8
9 MERCK SHARP & DOHME LLC,
Petitioner
10
v.
11
HALOZYME INC.,
12 Patent Owner
13 _____
14
Case PGR2025-00003
15 U.S. Patent No. 11,952,600
16 _____
17
18 **Video Deposition of: James Naismith, Ph.D.**
19
Taken on: Friday, 6 February 2026
20
Held at: Randolph Hotel, Beaumont St, Oxford, UK
21
Time: 8.03 a.m. - 6.26 p.m.
22
23
24 Reporter: Amy Coley
25

Halozyme EX2301
Merck v. Halozyme
PGR2025-00009

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1
2 THE VIDEOGRAPHER: Good morning.
3 Here begins Media Number 1 in the deposition of
4 Dr. James Naismith in the matter of Merck Sharp &
5 Dohme LLC v Halozyme Inc in the United Sates
6 Patent and Trademark Office, case number
7 PGR2025-00003.
8 Today's date is February 6th 2026,
9 and the time is 8.03 a.m. This deposition is
10 taking place at the Randolph Hotel, Beaumont
11 Street, Oxford, United Kingdom. The Videographer
12 today is Wendy Viner.
13 Could I ask counsel to please
14 identify themselves and state whom they represent,
15 beginning with everybody in the room followed by
16 our Zoom participants.
17 MS. MARTIN: Lauren Martin from
18 Quinn Emanuel on behalf of Halozyme. With me
19 I have Eliot Choi from Quinn Emanuel and
20 Haley Ball from Sterne, Kessler.
21 MR. KUSHAN: Jeff Kushan from
22 Sidley Austin for Merck with Amy Mahan.
23 THE VIDEOGRAPHER: Thank you.
24 Would the Zoom participants please introduce
25 themselves for the record.

1 NAISMITH - MARTIN
2 MR. MACK: This is Josh Mack for
3 Halozyme.
4 MS. ELLISON: This is Eldora
5 Ellison from Sterne, Kessler, Goldstein & Fox on
6 behalf of Halozyme.
7 MR. MAJCHRZAK: Eric Majchrzak from
8 Merck Sharp & Dohme LLC.
9 THE VIDEOGRAPHER: Thank you. Our
10 Court Reporter today is Amy Coley. Could I ask
11 the Court Report to please swear in the witness
12 and we can proceed.
13 THE COURT REPORTER: Dr. Naismith,
14 do you swear that the testimony you shall give
15 shall be the truth, the whole truth and nothing
16 but the truth?
17 DR. NAISMITH: Yes, I do.
18 THE COURT REPORTER: Thank you.
19 Q. (BY MS. MARTIN): Good morning
20 Dr. Naismith or Professor Naismith. Am I saying
21 that ----
22 A. Doctor is fine. It is the
23 difference between the US and the UK. I'm fine
24 with "Dr."
25 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?

1 NAISMITH - MARTIN
2 A. I was testifying on behalf of
3 Amgen.
4 Q. Did you testify at trial in that
5 case?
6 A. Yes, I did.
7 Q. Any other depositions?
8 A. Apart from connected with that
9 case.
10 Q. Mmm-hmm.
11 A. No.
12 Q. Under the Patent Trial and Appeal
13 Board Standing Order in this matter I am obligated
14 to give you the following instruction. You must
15 ask me, the deposing counsel, rather than your own
16 counsel for any clarifications, definitions or
17 explanations of any words, questions or documents
18 presented during the cross-examination. Do you
19 understand that instruction?
20 A. Yes, I do.
21 Q. Your attorney may object to my
22 questions but unless he instructs you not to
23 answer you should still answer my question. You
24 understand that?
25 A. Yes, I understand that.

1 NAISMITH - MARTIN
2 Q. When were you contacted for this
3 case?
4 A. Probably around August of last
5 year.
6 Q. August 2025?
7 A. Yes.
8 Q. And by whom were you contacted?
9 A. Mr. Kushen.
10 Q. What did you do to prepare for this
11 deposition?
12 A. Prepare for the deposition or
13 prepare for the report?
14 Q. The deposition?
15 A. Just for the deposition:
16 I familiarised myself, sat and read through my
17 report a few times with the paper copy.
18 Q. Did you meet with counsel?
19 A. Yes, did.
20 Q. For how long?
21 A. Probably about five or six hours.
22 Q. Was that yesterday?
23 A. Yesterday and we caught up just in
24 the evening of the day before.
25 Q. Okay. At any time in your work on

1 NAISMITH - MARTIN
2 this matter have you -- strike that. Let me start
3 over again. So before your work in this matter,
4 had you heard of Dr. Hecht?
5 A. No, I had never met or heard of
6 Dr. Hecht.
7 Q. What about Dr. Park?
8 A. I have never heard of or met
9 Dr. Park.
10 Q. So as part of your work in this
11 case, did you speak with Dr. Hecht?
12 A. No, I have never spoken with
13 Dr. Hecht.
14 Q. And what about ----
15 A. I should correct that, I don't
16 believe we've ever met or spoken to one another,
17 certainly not in this case, but I have given
18 lectures at Princeton so it is possible I've
19 bumped into him.
20 Q. Okay.
21 A. I don't recall doing so.
22 Q. Okay, what about Dr. Park?
23 A. I'm fairly certain I've never met
24 Dr. Park and I certainly didn't -- to be clear
25 I didn't speak to him or meet him as part of this

1 NAISMITH - MARTIN
2 case. It could be possibly when he was a student
3 at some meeting I was at or something, you know?
4 I can't exclude those but I've never knowingly met
5 him or spoken to him.
6 Q. Okay. Did you ask to speak with
7 Dr. Hecht as part of your work in this case?
8 A. No.
9 Q. What about Dr. Park?
10 A. No.
11 Q. So you read Dr. Hecht's expert
12 declarations; correct?
13 A. I read his declaration, yes.
14 Q. His declaration?
15 A. I know they come in flavours but
16 ----
17 Q. Yes, okay.
18 A. ---- yes, I've read them.
19 Q. You read one of them, you didn't
20 read all of, you know, 15? Is that what you're
21 saying?
22 A. I read -- I saw the differences
23 between the other ones ----
24 Q. Okay.
25 A. ---- but I read his declarations.

1 NAISMITH - MARTIN
2 Q. Okay. And what about Dr. Park's
3 declaration?
4 A. Yes, I've seen his declaration too,
5 his analysis, yes.
6 Q. Mmm-hmm, okay. Did you see the
7 spreadsheet that Dr. Park prepared?
8 A. Are we talking about the exhibit
9 that he was offered? He offered an exhibit with a
10 spreadsheet.
11 Q. Yes.
12 A. Is that one the one we are
13 discussing?
14 Q. Yes, Dr. Park's spreadsheet. There
15 are, like, multiple spreadsheets in this case. Is
16 that what ----
17 A. Yes.
18 Q. ---- the one that Dr. Park
19 prepared?
20 A. The one that he prepared and it's
21 marked as an exhibit ----
22 Q. Yes.
23 A. ---- that's referenced in my
24 contents; that's the one I've seen.
25 Q. Okay, got it. You understand that

1 NAISMITH - MARTIN
2 Dr. Hecht and Dr. Park were deposed in this
3 matter?
4 A. Yes, I did.
5 Q. Have you read their deposition
6 transcripts?
7 A. I have looked through them. They
8 are rather long, but yes.
9 Q. When did you start -- strike that.
10 So you submitted declarations in this proceeding;
11 correct?
12 A. Yes, I did.
13 Q. Did you write those declarations?
14 A. The declarations are my opinion, my
15 opinions. The final versions are with the
16 assistance of counsel.
17 Q. And how long approximately did you
18 spend writing those declarations?
19 A. Over 20 days.
20 Q. Did you yourself spend over 20 days
21 preparing the declarations?
22 A. Yes, in formulating my opinion
23 reading papers yes.
24 THE COURT REPORTER: I am sorry,
25 "Before"?

1 NAISMITH - MARTIN
2 A. I don't think I said "before".
3 Q. (BY MS. MARTIN): Can you just
4 restate your answer? I don't think that she heard
5 it.
6 A. Could you give me the question,
7 again?
8 Q. Did you yourself spend 20 days
9 preparing your declarations?
10 A. Yes, including reading papers,
11 formulating my opinions, it would be over 20 days
12 of work.
13 Q. Okay. Do you know approximately
14 how many hours you spent?
15 A. The university measures my time by
16 eight hours, so eight times 20.
17 Q. Okay. Is there a cap on the amount
18 of time you can consult as part of your work with
19 the university?
20 A. I need permission to do any
21 consultancy, which I obtained before I worked on
22 this.
23 Q. Okay.
24 A. There is in the university
25 regulations a 30-day cap that I have to get

1 NAISMITH - MARTIN
2 additional permission if I go beyond 30 days in
3 what the university judges as the academic year.
4 Q. Oh, okay. So have you had to get
5 special approval to go beyond 30 days for this
6 case?
7 A. I haven't gone beyond 30 days in
8 this case.
9 Q. So how many days have you spent on
10 this case?
11 A. Just over 20 days.
12 Q. Have you ever consulted with Merck
13 besides your work in this case?
14 A. No, I've never consulted with
15 Merck. I've given a lecture at one of their sites
16 probably a couple of times over the years.
17 Sometimes they gave me an honorarium, take you out
18 for dinner, but I never consulted for them.
19 Q. Okay. Have they ever funded any
20 research in your lab?
21 A. Not to my knowledge but companies
22 in this field change quite often, so I can't swear
23 that they haven't bought some company in years
24 ago. The companies I've ever worked with are
25 listed in my CV.

1 NAISMITH - MARTIN
2 Q. Have you ever heard of a company
3 called Altergen?
4 A. Only in this connection, with this
5 case.
6 Q. Okay, but not outside of this case?
7 A. No.
8 Q. Have you ever done any consulting
9 work for Halozyme?
10 A. No, not that I'm aware of.
11 Q. Have you heard of Halozyme outside
12 of this case?
13 A. No.
14 Q. What's the subject of your
15 research?
16 A. So my research is broadly defined
17 structure function relationships in proteins
18 particularly interested in enzyme mechanism or the
19 interaction between two proteins. I'm
20 experimentalist so particularly around structural
21 techniques, so protein crystallography;
22 electromicroscopy; electron tomography;
23 competitional approaches to try to understand
24 mechanism; little bits of chemical synthesis
25 around substrates, analogs; assay development and

1 NAISMITH - MARTIN
2 attempts to commercialise some of those ideas.
3 I range most into bacterial proteins but I've also
4 worked in human proteins and actually from plants
5 and such.
6 Q. Sorry, what was the last thing you
7 said, "human proteins and actually from plints"?
8 A. Plants.
9 Q. Plant proteins?
10 A. Plant proteins.
11 Q. Okay.
12 A. They are a kind of class and
13 unusually -- they have some unusual properties.
14 Q. Okay. Would you say that you spend
15 most of your time working with bacterial enzymes?
16 A. I do not know "most" of my time.
17 It's a significant portion of my work working on
18 enzymes, bacterial enzymes. If I integrate over
19 my career, they were much easier in the 1980s to
20 work with, but increasingly we shifted to more
21 human complex systems. So it's a balance over my
22 career. More recently it would be more focused on
23 human proteins.
24 Q. What types of human proteins do you
25 work with?

1 NAISMITH - MARTIN
2 A. So we actually have been working on
3 trying to determine the structure of nucleosomes
4 by cryo-electron tomography. So nucleosomes are
5 inside cells, so we've been quite interested in
6 that. We've also been looking at immunity viral
7 proteins and trying to understand how they form
8 aggregates and spindles inside cells.
9 Q. What's an "immunity viral" protein?
10 A. So, it's kind of -- that's related
11 to Cas-KRISPR. So bacteria themselves are immune
12 to some viruses. Bacteria get virus, out of
13 interest. It's one of these weird things, but
14 they have an unusual defence mechanism and so we
15 are quite interested in the proteins that are
16 involved in that defence mechanism.
17 Q. And you said you are trying to
18 understand how they form something and spindles
19 inside cells?
20 A. Yes. So, they form these really
21 long like helical filaments.
22 Q. Filaments? Oh, "filaments" is the
23 word?
24 A. My apologies. As you hear, I have
25 a Scottish accent.

1 NAISMITH - MARTIN
2 Q. No, I am trying to make sure that
3 the record is clear and that I understand. Okay.
4 So they form filaments inside cells. Is a
5 filament -- would you call that like an aggregate?
6 A. Actually it's an order of aggregate
7 but it is an aggregate, yes. It is ill-defined in
8 its length we think. So this is very much our
9 current experimentation.
10 Q. It is similar to a Fibrel?
11 A. Yes, there are similarities but
12 also some differences that make it particularly
13 interesting.
14 Q. Okay, you mentioned I think you
15 said cryo-TM?
16 A. Yes, cryo-EM single particle
17 cryo-EM and I also work on cryo-electron
18 tomography.
19 Q. Okay, yes. So what is the
20 difference between cryo-electron microscopy and
21 cryo-electron tomography?
22 A. So in cryo-electron microscopy, we
23 tend to work with a purified sample. It does not
24 have to be, we've learned to be able to tolerate
25 some impurities. That might be complex but it's

1 NAISMITH - MARTIN
2 defined. And so you expose it to an electron beam
3 and you average out the images, and from that you
4 reconstitute the structure of the protein. So it
5 is analogous, the end result is analogous to
6 protein crystallography or AlphaFold.
7 In cryo-electron tomography, you
8 are looking at a slab of a cell or a species live.
9 So the goal that we have been engaged in is to be
10 able to take human tissue and examine the
11 structure of the proteins in the human tissue
12 directly without any purification. So that's
13 cryo-electron tomography.
14 The challenge is that electron
15 beams can only go through about 200 nanometers of
16 ice. So these are frozen samples but natively
17 frozen. So you need to thin the sample. So you
18 need some way to find the bit that what we call
19 the ROI inside the human tissue and then you
20 examine it. So this is very much a technological
21 frontier of structural biology.
22 Q. Okay, sorry, there was a word that
23 we didn't -- you want "to find the bit that is
24 inside the human tissue to examine", we did not
25 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.

1 NAISMITH - MARTIN
2 Q. Apart from your work on this case,
3 have you ever analyzed any structure of
4 hyaluronidase?
5 A. Apart from my work with this case,
6 I have never examined hyaluronidase.
7 Q. Have you ever performed an assay to
8 measure hyaluronidase activity?
9 A. I have never performed an assay nor
10 has my lab to analyse hyaluronidase activity. We
11 have assay polysaccharide degradation but not
12 hyaluronic acid.
13 Q. Also not hyaluronic acid and also
14 not hyaluronic acid degradation by hyaluronidase?
15 A. That is correct.
16 Q. Have you ever performed a
17 microtiter plate assay with a hyaluronidase?
18 A. I have performed microtiter plate
19 assays but not with hyaluronidase.
20 Q. Have you ever performed a
21 microtiter plate assay with biotinylated HA?
22 A. I perform, use biotinylated but not
23 biotinylated HA.
24 Q. Okay. Have you ever been a referee
25 on a paper for hyaluronidase?

1 NAISMITH - MARTIN
2 A. I honestly can't tell you. I have
3 refereed thousands of papers in my career.
4 I can't recall thousands of papers, I'm sorry.
5 I just can't give you an honest answer. It's
6 certainly possible but I can't remember. I've
7 been a scientist for a long time.
8 Q. But none come to mind?
9 A. None come to mind but lots of
10 things at my age, when I talk about, when I look
11 back over thousands papers, it won't come to mind.
12 Q. Okay.
13 A. I've probably refereed several
14 hundreds of papers, maybe a hundred papers a year.
15 I have been an editor of journals. I honestly
16 can't tell you.
17 Q. Outside of your work in this case,
18 are you familiar with Dr. Greg Petsko's work?
19 A. Yes, I am familiar with Dr. Petsko.
20 I've met Dr. Petsko on several occasions and
21 I know some of his work. He publishes a lot of
22 work, but I know some of his work, yes.
23 Q. You said that you met him before?
24 A. Yes, I have.
25 Q. In what context?

1 NAISMITH - MARTIN
2 A. So I've bumped into him at
3 conferences. He came to give a lecture, a series
4 of prestigious lecture series in St. Andrews and
5 then Dundee which is where I used to work. So he
6 was, kind of, travelling the country giving
7 lectures and I met him as part of the faculty day
8 when we talked to the invited speaker. So I met
9 him there and I've met him at conferences.
10 Q. Are you familiar with his research?
11 A. I'm familiar with some of his
12 research. I don't have it -- yes.
13 Q. Sorry, you said you are familiar
14 with some of his research and then what was the
15 second thing you said?
16 A. That is what I said I think. Some
17 of his research.
18 Q. Okay.
19 A. I said I wasn't -- I couldn't
20 answer questions on the entire breath of his
21 research, sorry, yes.
22 Q. Okay. Would you consider Dr.
23 Petsko to be a well-respected scientist?
24 A. Yes, I would.
25 Q. Would you consider him to be well

1 NAISMITH - MARTIN
2 known in the field?
3 A. What field?
4 Q. Structural biology?
5 A. Yes, I would.
6 Q. Have you heard of Dr. Melanie
7 Simpson outside your work on this case?
8 A. Outside the work in my case, no, I
9 have not.
10 Q. You mentioned in your report that
11 you were familiar with Dr. Petsko's textbook?
12 A. Yes, I may -- That is correct, I've
13 read his textbook or looked through it.
14 Q. Have you used his textbook in your
15 courses?
16 A. I think it would be in the library
17 but I didn't teach from his textbook.
18 Q. Okay, but you have seen it in the
19 library?
20 A. I think it is in the St. Andrews
21 library. It is certainly in the Oxford library.
22 Q. Okay. Are you aware that
23 Dr. Simpson is the President of the International
24 Society for Hyaluronan Sciences?
25 A. I think in her expert report - and

1 NAISMITH - MARTIN
2 I would like to go to it - I think she mentions it
3 in there. She certainly mentions some of the
4 things she's involved in in her expert report.
5 Q. I am going to mark the first
6 exhibit, which is your Declaration of James
7 Naismith PhD in PGR matter 2025-03 and then I'm
8 also going to mark Declaration of Dr. Naismith
9 from matter PGR 2025 matter 4, declaration of
10 James Naismith in PGR matter 2025 matter 6, and
11 declaration of James Naismith in matter PGR 2025
12 matter 9.
13 A. Ms. Martin?
14 Q. Mmm-hmm?
15 A. I have a correction to give you for
16 my report.
17 Q. Okay, we will get there. I'm going
18 to give you a stack of them. To be clear: matter
19 3; matter 4; matter 6 and matter 9.
20 MR. KUSHAN: These are all 1133?
21 MS. MARTIN: You know, it's not on
22 here, but I believe that that is right.
23 (**Exhibit 1133 marked for identification**)
24 MS. MARTIN: These will be exhibit number
25 -- you said it was what, sorry?

1 NAISMITH - MARTIN
2 MR. KUSHAN: 1133.
3 MS. MARTIN: Thank you.
4 Okay, so Dr. Naismith, can you
5 just confirm that those are the four declarations
6 that you submitted?
7 A. *(Pause)* Yes, these are the four.
8 Q. Okay. And you said that you had a
9 correction?
10 A. I do. I brought a sheet with a
11 correction for you, I brought copies.
12 Q. Okay.
13 A. I brought five copies.
14 Q. Let's mark this as an exhibit,
15 right. Did you ----
16 MR. KUSHAN: I've not got one.
17 A. Sorry. I printed them out this
18 morning.
19 MS. MARTIN: Oh, you have not seen
20 one?
21 MR. KUSHAN: Can I just have one?
22 MS. MARTIN: We're going to mark
23 this as an exhibit. I don't have a number, so I
24 don't know. Let's just say Exhibit 1 and then
25 we'll fix it later. Let's just say Exhibit 1 and

1 NAISMITH - MARTIN
2 we'll get the number later.
3 MR. KUSHAN: It's Exhibit 2446.
4 MS. MARTIN: I feel like fixing it
5 later will be better than getting it wrong now.
6 **(Exhibit 2300 marked for identification)**
7 Q. (BY MS. MARTIN): So Dr. Naismith
8 you prepared this errata?
9 A. Yes, I did.
10 Q. And so can you just explain what it
11 is that you want to change?
12 A. I want to correct a typo error that
13 I discovered when I was reading through it. There
14 is a stray sentence "and 17 total changes" that
15 doesn't belong there, my apologies.
16 Q. And so this is in paragraph 317 in
17 all four of your declarations?
18 A. It is.
19 Q. And there is a stray "and 17 total
20 changes" in the sentence that starts with, "Just
21 exploring"; is that right?
22 A. That's correct and I've underlined
23 the bit that should have been removed. My
24 apologies.
25 Q. Okay. Understood. All right. Is

1 NAISMITH - MARTIN
2 that the only change in you wanted to make?
3 A. It's the only error I discovered.
4 Q. All right. Are you aware that the
5 Patent Trial and Appeal Board instituted trial in
6 these matters?
7 A. I know there's some sort of Patent
8 Board process and there's a hearing I don't know
9 if it's called that.
10 Q. Okay.
11 A. I assume that's correct but I don't
12 always know the legal term.
13 Q. Have you read the Institution
14 decision?
15 A. No, I don't think so.
16 Q. And you listed the materials that
17 you considered in forming your opinions in this
18 case, in your Exhibit List; is that right?
19 A. Yes.
20 Q. And so I believe that that is
21 Appendix A which -- We are just for the ease of
22 everyone's ease here we will just use the Matter 3
23 declaration for purposes of this of our
24 discussion.
25 A. Is that the one here which is

1 NAISMITH - MARTIN
2 called 600?
3 Q. Yes, 600 BEN?
4 MR. KUSHAN: Let me just
5 double-check, sorry. Yes.
6 Q. (BY MS. MARTIN): So your Exhibit
7 List is Appendix A which is on page 269?
8 A. I have brought ringbound, if that
9 would make it easier for all of us.
10 Q. Yes, that would make it easier for
11 all of us. Except, is that marked at all?
12 A. It's pristine and looks exactly --
13 but this is just ringbound.
14 Q. Okay, perfect.
15 A. I have not touched these ones apart
16 from authoring them.
17 Q. Understood.
18 A. Can I just shove *these* out my road?
19 Q. Yes.
20 A. Is that okay?
21 Q. Yes.
22 A. I've got three just so we are all
23 on the same ----
24 Q. Got it thank you?
25 A. Okay.

1 NAISMITH - MARTIN
2 Q. Okay, and so your Exhibit List is
3 on page 269 hopefully?
4 A. Yes, it is.
5 Q. All right. So your Exhibit List,
6 that contains a list of all of the exhibits that
7 you have relied on in forming your opinions in
8 this declaration?
9 A. Yes, that and my knowledge of the
10 field which encompasses many other papers but
11 these are the ones I specifically cite and rely
12 on.
13 Q. Okay, and you signed your
14 declaration, your signature is on page 268; right?
15 A. That's correct.
16 Q. That is your signature?
17 A. Yes, it is.
18 Q. And you executed this particular
19 declaration on January 20th 2026?
20 A. Yes, I did.
21 Q. So earlier in your testimony we
22 talked a little bit about cryo-electron
23 microscopy; is that right?
24 A. We did.
25 Q. What would you consider to be good

1 NAISMITH - MARTIN
2 resolution for cryo-EM structure for a protein?
3 A. Oh, cryo-EM? These days you can
4 get below 2 angstrom resolution.
5 Q. Would you consider below angstrom 2
6 resolution to be a good resolution for cryo-EM?
7 A. Cryo-EM below 2 angstrom resolution
8 is really quite good.
9 Q. Okay. What about 3 angstrom: is
10 that good resolution for cryo-EM?
11 A. It depends on the region. So
12 cryo-EM doesn't have the -- So "resolution" is a
13 term that comes from protein crystallography where
14 it is mathematically defined. Cryo-EM uses what's
15 called an FSC curve so that is more of an average.
16 So parts of the structure are well ordered, other
17 parts might be less well defined.
18 Q. When you saw a cryo-EM structure
19 you are taking an average of the -- You are
20 generating a model based on the average of the
21 images that you have collected with the
22 microscope?
23 A. That is a reasonable summary of how
24 you work single particle averaging, yes.
25 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

1 NAISMITH - MARTIN
2 how two proteins interact for example, a
3 resolution of 3 and a half would be perfectly
4 fine. If I want to understand, as I have done in
5 the past, precise aspects of what's at the
6 catalytic core, catalysis movement around that
7 site, 1, you know, somewhere around 1, 1.2
8 angstrom resolution. If I wanted to have a broad
9 understanding of the chemical mechanism, anywhere
10 between 2 and 3 but it's a sliding scale. So the
11 closer you get to 2, the more information you
12 have.
13 Q. So let's say you have a 2 angstrom
14 crystal structure for a protein, what's the margin
15 of error in terms of atom positioning?
16 A. So typically around point 2
17 angstrom for any one atom at 2 angstrom resolution
18 is a reasonable guide.
19 Q. Okay.
20 A. But the atoms are coupled. It is
21 not simple, it's just they can all move that way.
22 Q. So what if you had a 3 and a half
23 angstrom structure, what's the margin of error?
24 A. Again, there is an element of
25 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

1 NAISMITH - MARTIN
2 structure has a plus or minus point 2 angstrom
3 error in it, can that error impact your ability to
4 see interactions between the atoms?
5 A. You are aware of it in your
6 analysis, but it's not that -- because its between
7 two atoms those are coupled so it's not just point
8 2 plus point 2. You have to -- it's a complicated
9 sum of squares. I'd have to go back and refresh
10 my memory. But the distance is not just --
11 certainly the distance is not simply point two
12 plus point 2, it is less than that.
13 Q. Okay. It is possible that the
14 error in the inherent or, like, the margin of
15 error in atom positioning could impact your
16 ability to determine if there's an interaction?
17 A. So, at the absolute, if you impose
18 an absolute rigid cut-off for hydrogen bonds, then
19 if you were at 3.55 you might be more careful in
20 your description to say it is at 3.55 in my model
21 which there is an element of uncertainty or 3.44
22 for example. So there, yes, but hydrogen bonds
23 have an additional feature which is helpful which
24 is there is a directionality about them. So there
25 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.

1 NAISMITH - MARTIN
2 Q. In your experience is AlphaFold 2.0
3 capable of generating a model that's comparable to
4 what you would get experimental results?
5 A. It depends on the resolution, but
6 it certainly is capable of producing models of a
7 quality from crystallography around 3, 2.5-3. I
8 would say and I haven't tested it rigorously since
9 I looked at 2, but it's pretty good.
10 Q. Okay. When you say "it depends on
11 the resolution" am I understanding correctly that
12 AlphaFold 2.0 may not be able to give you 1
13 angstrom accurate model but it can be give you a
14 3.2-3.5 angstrom accurate model?
15 A. Again overall, but where in the
16 core structure it will be as accurate as a 1.0
17 model. You know it's very accurate in core
18 structures but as you get to surface where things
19 are less well conserved, because that's kind of
20 how it works. You might see more uncertainty in
21 those regions. But it is hard to know. My
22 experience of AlphaFold is that it is pretty good,
23 you can -- there are some differences with
24 experimental structures in loops particularly.
25 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;

1 NAISMITH - MARTIN
2 absolutely. It's very good at that.
3 Q. Have you used SWISS-MODEL?
4 A. Years ago, yes.
5 Q. Did you -- so let's put out a
6 timeframe on this. In 2011/2012 timeframe were
7 you using the SWISS-MODEL?
8 A. I had been using it since it came
9 online, I think, which is the 90s, so I have used
10 it you think until really AlphaFold on and off.
11 Q. Okay.
12 A. Most of my lab was *de novo*
13 structure determination.
14 Q. What do you mean by "*de novo*
15 structure determination"?
16 A. Where we start from a structural
17 analysis based on an experimental structure.
18 Q. Okay, so you are trying to solve
19 the structure of a protein?
20 A. That's, kind of, what I built my
21 career on.
22 Q. Okay. All right, I am just trying
23 differentiate between, like, *de novo* protein
24 design and *de novo* structure determination?
25 A. Sorry, I mean from ----

1 NAISMITH - MARTIN
2 THE COURT REPORTER: I am sorry,
3 could you repeat your question, please?
4 MS. MARTIN: Sorry, I will go
5 slower.
6 THE COURT REPORTER: You will have
7 to go slower.
8 Q. (BY MS. MARTIN): I am trying to
9 differentiate between *de novo* protein design and
10 *de novo* structure determination.
11 A. Yes, my apologies. When I say "*de*
12 *novo* structure determination", I mean experimental
13 structure determination, and mostly in my lab we
14 were interested in the first structure of a
15 representative of a particular structure.
16 Q. So in what context would you use
17 SWISS-MODEL?
18 A. Usually if -- So most commonly in
19 my lab if we were in -- if we were able to solve a
20 structure from one organism and my collaborator
21 was interested if there was a particular single
22 site mutation in another, we might look at it in
23 that way, although in fact we tended to mostly
24 just use our own software which is a programme
25 called Coot, where you can model in single amino

1 NAISMITH - MARTIN
2 acid changes. But it wasn't in common use in my
3 lab. Collaborators often used it more until we
4 would provide an experimental structure.
5 Q. Do you know -- you said that
6 collaborators would use SWISS-MODEL until you were
7 provided experimental structure. Is that what you
8 said?
9 A. Yes.
10 Q. Do you know why your collaborators
11 were using the SWISS-MODEL, like, for what
12 purpose?
13 A. So, some information is better than
14 no information. So people maybe have been working
15 on a problem for many years, so the only
16 information they can get might be SWISS-MODEL
17 though when we would join the collaboration we
18 would come and give them a more accurate
19 structural model that was much more predictive in
20 understanding function.
21 Q. How would you generate that
22 structural model?
23 A. From X-ray crystallography or
24 single particle EM.
25 Q. Okay, so you would give them an

1 NAISMITH - MARTIN
2 experimentally determined structure as opposed to
3 the computer model?
4 A. Yes, and we would then work
5 together to try to understand the problem which we
6 were both interested in.
7 Q. And you mentioned -- you mentioned,
8 I thought, using SWISS-MODEL to model single amino
9 acid changes but you would also would use Coot?
10 A. Yes.
11 Q. So am I understanding correctly
12 that if you wanted to model what it looks like to
13 change a single amino acid, you could generate a
14 model using SWISS-MODEL or you could go into Coot
15 and just change the amino acid?
16 A. And then look at it yourself, yes.
17 Q. Did you ever use Modeller?
18 A. Not that I recall although I know
19 it was part of a software package. I'm aware of
20 Modeller but I don't think I've used it.
21 Q. Are you familiar with the phrase
22 homology modeling?
23 A. Yes, I'm familiar with that phrase.
24 Q. What is homology modeling?
25 A. So, where it's actually, it's what

1 NAISMITH - MARTIN
2 Dr. Park did. You have a structure of, in his
3 case, HYAL1. You have a Swiss-Prot to then make a
4 structure of PH20 and it uses the homology between
5 those two to then make a model of the PH20.
6 Q. In the 2011/2012 timeframe, would
7 you consider SWISS-MODEL to be a reasonably
8 accurate tool to use to generate a homology model?
9 A. It depends on the extent of
10 homology.
11 Q. In the case of PH20?
12 A. It's better than nothing; it's
13 reasonable.
14 Q. Okay. So do you think that
15 Dr. Park's homology model for PH20 is reasonably
16 accurate?
17 A. Depending on the purpose, you put
18 it to. The purpose he put it to was to look for
19 single amino acid changes and then assign them to
20 buckets. It's accurate enough for that.
21 Q. So it's accurate enough for the
22 purpose for which Dr. Park used it?
23 A. Yes, and he was careful to warn
24 that some regions were less well defined, I think
25 the CUE mean score, where he said, you know,

1 NAISMITH - MARTIN
2 "Well, it's a bit -- the model's not defined here
3 and the model at page 20 doesn't go all the way to
4 the end of the sequence 447".
5 Q. But the portions for which he used
6 the model to make his single amino acid
7 predictions do you think that it was reasonably
8 accurate?
9 A. It was reasonably accurate to
10 assign to it the buckets he did which was 1, 2 or
11 3 and he was careful not to say that he could
12 infer precise activity or action at a distance or
13 mutate effects elsewhere in the structure; he just
14 asked a single question which was, "How is the
15 local environment around this residue if I change
16 it, if I mutate it, one residue at a time, single
17 mutation?"
18 Q. But then he was actually drawing a
19 conclusion based on his local environment analysis
20 as to whether or not he would predict the protein
21 as a whole to retain enzymatic activity; right?
22 A. So he said he had signed it in
23 bucket 1 which he said was destabilizing. So
24 bucket 1 he said, "If it's this particular single
25 site mutation in bucket 1 in these non-conserved

1 NAISMITH - MARTIN
2 residues, it is likely to be destabilizing and
3 therefore I think it could adversely be
4 deleterious to the protein." When he assigned it
5 to bucket 2 he said, "Looking at the local
6 environment this single amino acid change probably
7 won't have an impact. It will be neutral" I think
8 his word was. And then in bucket 3 he said,
9 "Looking at that single site change in its local
10 environment I think that will be stabilizing."
11 So his expectation was that buckets
12 2 and 3 would be active, but he didn't draw a
13 conclusion that bucket 3 would be more or less
14 active than bucket 2 or any level of activity
15 within the buckets, just that it would be active.
16 Q. Okay. So he did in fact draw a
17 conclusion that for his bucket 2 and bucket 3 that
18 he would expect the protein to be active?
19 A. That was his prediction, yes, that
20 that single site mutation at this position, where
21 subject to his qualifications, yes, he would
22 expect those to be likely to be active.
23 Q. Okay, can you turn in your
24 declaration to paragraph 133.
25 A. Yes.

1 NAISMITH - MARTIN
2 Q. And I believe that you mentioned
3 earlier you were looking for a date from
4 Dr. Hecht's declaration regarding AlphaFold 2. Is
5 that date 2019?
6 A. Yes, it would be 2019, 2020, when
7 the publication started to appear.
8 Q. Okay. And so you say in paragraph
9 133, that the computational tools and in
10 particular AlphaFold 2, "... did not reach
11 predictive accuracy levels comparable to
12 experimental determination of structure until
13 around 2019 ...". Is that right?
14 A. Yes.
15 Q. Okay. So as of around 2019
16 AlphaFold 2 was generating predictive accuracy
17 levels comparable to experimental?
18 A. I qualify that by the resolution,
19 but, yes, for the overall fold of a protein by
20 2019, AlphaFold 2 was good.
21 Q. Okay. And there is a figure at the
22 top of page 95, do you see that?
23 A. I do.
24 Q. Can you explain the figure?
25 A. So what that says is the mean C

1 NAISMITH - MARTIN
2 alpha root mean square deviation. So what it
3 shows is that if you look in the -- CASP is a
4 competition. So people would submit sequences
5 which were not known in the art. So there was no
6 structure known for that sequence and a bunch of
7 people would go along and try to predict using
8 different software structure and then you would
9 compare how well they did. And so this is, kind
10 of, a measure of that. It is a root mean square
11 deviation. What it shows is that AlphaFold boom
12 certainly was more better than any of the
13 competing programmes and you see here the other
14 programmes similar sort of error ranges.
15 Q. You are pointing -- You said
16 "similar sort of error ranges". What are you
17 pointing to?
18 A. Oh, for the other programmes, the
19 RMSDs and other approaches.
20 Q. And you are pointing to the figure,
21 like the graph on the right of the top of page 95
22 in the error bars?
23 A. Yes.
24 Q. Okay and in that the X-axis has a
25 bunch of like G numbers, and then AlphaFold. What

1 NAISMITH - MARTIN
2 are the G numbers?
3 A. So those are the -- I believe those
4 are -- I should actually like to probably go back
5 to the original paper and check, but I think those
6 are the different groups that were competing.
7 Q. Oh okay. So the other groups were
8 competing with AlphaFold and you can see the
9 median root mean square deviation for AlphaFold is
10 around 1 whereas for the other groups that's like
11 3-4; is that right?
12 A. Yes.
13 Q. Okay, okay, got it. Okay, and in
14 the graph on the left it says: "A score above 90
15 is considered roughly equivalent to an
16 experimentally determined structure". And in 2020
17 the AlphaFold 2 score is approaching 90; is that
18 right?
19 A. Yes.
20 Q. Did you use AlphaFold 2 to generate
21 a model of PH20 in connection with your work in
22 this case?
23 A. No, I did not.
24 Q. Did you use any software package to
25 generate a model of PH20?

1 NAISMITH - MARTIN
2 A. I used the model that was provided
3 to me and my counsel. It came from Dr. Park and
4 Dr. Petsko. I think I used their PH20 model.
5 Q. Did you use Dr. Park's model or Dr.
6 Petsko's model?
7 A. I would have to check. I think I
8 used Dr. Petsko's PH20 model.
9 Q. Were you provided with Dr. Park's
10 model?
11 A. That I can't recall. I was given
12 one PDB coordinate set and I believe it is Dr.
13 Petsko's, but I think I also had another PDB set
14 which was Dr. Park's and they looked
15 superimposable, but I did my analysis using the
16 PH20 from Dr. Petsko's.
17 Sorry, do you want me to go again?
18 THE COURT REPORTER: I was right
19 there but then I lost you. "... but I think I
20 also had another PDB set which was Dr. Park's
21 ----"
22 THE WITNESS: I think but I used
23 all my analysis on Dr. Petsko's PH20.
24 Q. (BY MS. MARTIN): And I believe you
25 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.

1 NAISMITH - MARTIN
2 MS. MARTIN: Okay, we will take a
3 quick break.
4 THE VIDEOGRAPHER: We are going off
5 the record. The time is 9.02 a.m.
6 **(A short recess from 9.02 a.m. to 9.11 a.m.)**
7 THE VIDEOGRAPHER: We are back on
8 the record. The time is 9.11 a.m.
9 (BY MS. MARTIN): Welcome back,
10 Dr. Naismith. Do you agree with the statement
11 that: "Common structural features either side of
12 those physical attribute that are common to the
13 members of the genus of properly folded active
14 PH20s?"
15 A. I think that's taken from my
16 report. Could I just turn that page? I think
17 there's ----
18 Q. I don't know if it's in your
19 report or not, I'm reading from the submission,
20 from Mark's submission.
21 A. Okay, so I was taught legal tests,
22 so I want to get them in front of me and then I'll
23 answer your question, if that's okay?
24 Q. Oh okay. Well actually let me --
25 I can get rid of the legal part of it. I am just

1 NAISMITH - MARTIN
2 wondering if you agree that common structural
3 features are a set of physical attributes?
4 A. So, "Common structural features are
5 a set of ...". Yes, that's seems a reasonable
6 statement to me, although I was told to be --
7 I was taught some legal principles too.
8 Q. I understand. I'm going to try to
9 get rid of the legal principles because I am
10 trying to understand the relationship between the
11 structural features and the physical attributes;
12 all right?
13 A. I understand.
14 Q. I want to focus on that, not the
15 legal part?
16 A. Okay.
17 Q. Do you agree that structural
18 features are physical attributes?
19 MR. KUSHAN: Objection foundation.
20 A. So a structure is a physical thing,
21 and so, it is a physical thing that describes a
22 protein three-dimensional folded structure or it
23 could be describe a small molecule. So it is a
24 three-dimensional visualization of a substance.
25 Q. (BY MS. MARTIN): Okay, let's go to

1 NAISMITH - MARTIN
2 paragraph 85 of your declaration. In paragraph
3 85, you say: "A POSA would consider 'structural
4 features' of a protein to be the physical
5 characteristics of the protein molecule"; right?
6 A. Yes, that's what it says,
7 "features", yes.
8 Q. Okay. So what do you mean by
9 "physical characteristics" in that statement?
10 A. So the reality of what the molecule
11 is, that's the physical reality characteristics.
12 So "structural features" are a way of describing
13 the physical -- some of the physical
14 characteristics. It's this size, it's this amino
15 acids arrangement in three-dimensional space.
16 I give an example in here if there
17 is: "... a particular amino acid located at a
18 particular ... sequence ..." then that would be a
19 specific feature of the protein. I distinguish
20 between folded proteins and non-folded proteins.
21 Q. When you say in the second sentence
22 in paragraph 85, that: "... a particular amino
23 acid located at a particular position in the amino
24 acid sequence ... is a structural feature", are
25 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

1 characteristic.
2 Q. And the position of a particular
3 amino acid in the sequence, for example, at
4 position 320, that is a physical characteristic of
5 the protein?
6 A. If -- Yes, if you have a specific
7 residue, you have called out a specific amino acid
8 at this position, then that is a physical
9 characteristic of the protein.
10 Q. So, for example, let's say you have
11 D320 K PH20, that means that the PH20 sequence has
12 a K at position 320; right?
13 A. So when you talk about PH20, we are
14 talking about a three-dimensional structure
15 properly folded structure of the protein. There
16 will be -- then from -- So that is when we use a
17 the word "PH20" but you're correct that at
18 position 320 you've defined a physical
19 characteristic which is you got a lysine at
20 position 320.
21 Q. Okay, so I am going to try to --
22 I am going to try to cut through some of this
23 here. So SEQ.ID NO:3, if I talk about SEQ.ID
24 NO:3, that is an amino acid sequence that has
25

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

1 NAISMITH - MARTIN
2 think of a fully folded protein or do you think of
3 a linear amino acid sequence?
4 A. So when you tell me SEQ.ID NO:3
5 I see that as a definition of a primary sequence.
6 Q. Okay. If you took PH20 and put it
7 in a solution of saturating urea would you still
8 think of it as a folded 3D protein?
9 MR. KUSHAN: Objection foundation.
10 A. So I haven't done that test but
11 I would assume, based on what a POSA would expect,
12 that PH20 and saturate urea would be an unfolded
13 potentially aggregated protein. But I don't know
14 that because I don't recall any data for PH20.
15 Q. (BY MS. MARTIN): But that's what
16 POSA would expect?
17 A. That would be what a POSA would
18 expect. An 8 Molar urea is what I'm guessing you
19 mean by "saturated", 8M.
20 Q. So in your solution of unfolded
21 potentially aggregated protein, is it still a
22 PH20?
23 MR. KUSHAN: Objection foundation.
24 A. It still has the sequence of PH20
25 but it's no longer a folded active protein, which

1 NAISMITH - MARTIN
2 I think we both agreed was how I defined and we
3 define PH20.
4 Q. (BY MS. MARTIN): So if you take
5 the amino acid sequence of PH20 and put it in a
6 solution of saturating urea such that it unfolds,
7 is it no longer PH20?
8 A. It is a sequence of PH20 but it
9 would be -- unfolded PH20 would distinguish
10 between that and normal PH20.
11 Q. So what would you call it if it's
12 not PH20?
13 A. Inactive unfolded PH20, I would
14 prefix it.
15 Q. Okay, so if you took -- Strike
16 that. So let's say you have a D320 K SEQ.ID NO:3
17 PH20. If I use that phrase, do you think that
18 that is a -- Do you consider that to be a fully
19 folded protein?
20 MR. KUSHAN: Objection foundation.
21 A. So where it's used in the common
22 disclosure then that is what I -- because that is
23 what the claims speak to. Yes, I would consider
24 that properly folded as does Dr. Petsko.
25 Q. (BY MS. MARTIN): Okay so SEQ.ID

1 NAISMITH - MARTIN
2 NO:3, 1-447 with a lysine at position 320 you
3 consider that to be a folded PH20 protein?
4 MR. KUSHAN: Objection foundation.
5 A. What I said was as it appears in
6 the declaration, I consider that to be -- in the
7 common disclosure, I consider that to be an active
8 protein. Now, you have discussed putting things
9 into urea so I would be, given that you've already
10 told me that, I would be more careful in answering
11 your question. As a scientist I'd want to know,
12 I'd ask you, "Is it still in urea?"
13 Q. (BY MS. MARTIN): Mmm-hmm, that was
14 going to be my next question. So, yes. Without
15 specifying whether or not it's -- Without
16 specifying the conditions for your SEQ.ID NO:3,
17 D320 K PH20, would you expect to it be folded?
18 A. So, and I mean no disrespect, if it
19 was another scientist we are talking at PH20, that
20 would be my assumption. You've asked -- you've
21 talked about urea. So in your case specifically
22 I would want to ask because you put into the
23 notion that you might unfold it. But as it's
24 discussed here in the common disclosure I take it
25 to be the full deducted protein.

1 NAISMITH - MARTIN
2 Q. Okay.
3 A. I don't mean to disrespect lawyers,
4 it's just scientists will use shorthand.
5 Q. Yes, I understand. It is good to
6 be precise. Okay, so now let's do the example of
7 the urea. If I tell you specifically that it's a
8 SEQ.ID NO:3, D320 K PH20 that I've put in
9 saturated urea, would you still consider it to be
10 PH20?
11 A. I would consider it to have the
12 same sequence that you've just defined but
13 I wouldn't use it without qualification that
14 either it's unfolded or here's some PH20 in 8
15 Molar urea. I would distinguish it from PH20.
16 I would definitely add the qualifications either
17 "unfolded" or "urea" when describing that
18 molecule.
19 Q. Why is that?
20 A. Because when I use just PH20 that
21 conveys to me, as it does in common disclosure,
22 that is a folded active form of the protein but
23 when it is in 8 Molar urea, that is an unfolded
24 inactive form of the protein and that would mean a
25 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

1 NAISMITH - MARTIN
2 PH20 in general terms I would assume you are
3 referring to the PH20 in the sperm cell. But I
4 was careful to say that in the common disclosure
5 PH20 is discussed as a soluble form with these
6 various truncations and we are told that. So it
7 has -- you know, we know that where PH20 comes
8 from, but the one that is in the common disclosure
9 is a truncated form, which lacks the GPI anchor.
10 Q. Let us go back to paragraph 85 in
11 your declaration.
12 A. Yes.
13 Q. In the last sentence you say: "The
14 phrase 'structure of a protein' would, unless
15 prefixed by 'primary' or 'secondary', be taken by
16 a POSA to refer only to the three-dimensional
17 structure of a protein." Is that right?
18 A. Yes.
19 Q. If you were trying to define the
20 three-dimensional structure of a protein for
21 example, if you wanted to put it in a patent
22 claim, how would you go about doing that?
23 MR. KUSHAN: Objection foundation,
24 also objection as to form.
25 A. So I don't know how to write

1 patents, so I would have to ask a lawyer to help
2 write a patent.
3 Q. (BY MS. MARTIN): Is it possible to
4 describe the three-dimensional structure of a
5 protein in words?
6 A. Scientists do describe and there is
7 really important paper here from HYAL1. They
8 describe the structure but they also deposit
9 coordinates, three-dimensional coordinates. So
10 for every atom you have an X, a Y and a Z
11 coordinate. From that you can put it into a
12 computer program and visualize it and you can
13 write your description based on that. But
14 scientists tend to do both. So we in structural
15 biology we do both. We deposit the coordinates
16 with something called the RCSB. It used to be
17 called the PDB. Anyone can pull the
18 coordinates out of that when we do an experimental
19 structure.
20 Q. It is possible to describe the
21 three-dimensional structure of a protein without
22 physical -- without the coordinates?
23 A. It depends on the level of
24 precision you want to apply. So you can describe
25

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2 a structure but it depends on the precision you
3 wish to use in your description.
4 Q. Can you explain what you mean by
5 "precision"?
6 A. So if I wanted -- I could say, for
7 example, bee venom hyaluronidase and human HYAL1
8 have an alpha-beta barrel motif. That is
9 description but it is quite imprecise because
10 there are important structural differences that we
11 are drawing attention to between the bee venom
12 non-and the human hyaluronidase. So if you were
13 being precise then you would not just use
14 alpha-beta barrel motif. That's is a very broad
15 term that is not really helpful in getting to the
16 precise defence situation you might want.
17 Q. So instead of using alpha-beta
18 barrel, you would use -- Instead of using
19 alpha-beta barrel motif you would use the
20 structural coordinates?
21 A. Sorry, if I was trying to tell who,
22 sorry, another scientist?
23 Q. Yes.
24 A. So depending on what they wanted
25 from it, I could describe it as, "Here are some

1 NAISMITH - MARTIN
2 detailed three-dimensional figures and a diagram
3 where I outline a certain stop of helices and beta
4 strands." But often we might just give at least
5 some coordinates and say, you know, "Start from
6 here".
7 Q. So you would need three-dimensional
8 coordinates to describe the structure of a
9 protein?
10 MR. KUSHAN: Objection foundation.
11 A. That's not quite what I said.
12 I said depending on the level of precision you
13 wanted I could describe for example as the people
14 do in those two papers we discussed where the
15 organisation of the beta sheets, the alpha helices
16 and in the human HYAL1, the differences in that
17 motif. So I could write that in words and
18 describe. It clearly points me to those
19 differences. If I wanted a very precise, ultimate
20 precise definition, for example where exactly this
21 residue is in the three-dimensional space,
22 coordinates would probably be quite helpful but
23 I could zoom in and make a figure of it.
24 Q. (BY MS. MARTIN): When your talking
25 about the "papers" they are describing aspects of

1 NAISMITH - MARTIN
2 the structure, they not describing the entire
3 structure; right?
4 MR. KUSHAN: Objection foundation.
5 A. I would say that both papers really
6 do describe the structures that they are facing so
7 the bee venom which has quite interesting
8 differences from the human hyaluronidase 1. So
9 both papers describe those structures. They don't
10 describe every atom in the structure but they
11 describe all the structural features, assign them
12 and talk about the differences between the two.
13 Q. (BY MS. MARTIN): When you say they
14 describe the structure, they describe the pattern
15 of alpha helices and beta sheets and loops?
16 MR. KUSHAN: Objection form.
17 A. So they describe those. They
18 produce figures. Figures are often one way
19 scientists communicate with one another. So you
20 do have visual information, particularly helpful
21 for three-dimensional structure and they then
22 highlight, for example, "Look at beta 2 in HYAL1
23 compared to bee venom".
24 Q. (BY MS. MARTIN): I appreciate that
25 pictures are helpful, but I'm trying to understand

1 NAISMITH - MARTIN
2 how you would use words to describe the structural
3 aspect of a protein?
4 MR. KUSHAN: Objection foundation.
5 A. So if I was trying to talk to
6 another scientist that is what my papers do. So
7 my papers are ways to describing structural
8 features. They are pages long. We put figures
9 in. We all put figures in and we discuss
10 structural features that we think are particularly
11 interesting but we always give an overall
12 structure description of the protein. We talk
13 about the strands, helices, etc., loops.
14 Q. (BY MS. MARTIN): Do you expect
15 that all natural full-length proteins are folded?
16 A. Do I expect it? If they are made
17 in biology?
18 Q. Yes.
19 A. For all proteins? Is your question
20 for all proteins?
21 Q. Yes.
22 A. No.
23 Q. Are there some naturally occurring
24 proteins that are not folded?
25 A. Yes.

1 NAISMITH - MARTIN
2 Q. Like what?
3 A. They are called naturally unfolded
4 proteins. That is actually a name.
5 Q. Okay.
6 A. And some proteins exist as
7 proportions of both. So some proteins exist both
8 in a folded form or an unfolded form. Some exist
9 and it is in my declaration, Dr. Dobson's work of
10 the various things. Now those often exist in
11 equilibrium but there are natively unfolded
12 proteins.
13 Q. You said that those various things
14 often exist at equilibrium but there are natively
15 unfolded proteins?
16 A. There are natively unfolded
17 proteins that are natively unfolded. That's the
18 phrase. So those are not folded. And there are
19 proteins which, I would not like to speculate
20 today without going back to literature, to
21 speculate what percentage. But there are proteins
22 that Dr. Dobson particularly is distinguished in
23 which as they start to fold some of them get
24 diverted off into aggregates, some of them get --
25 just remain as molten globules. Now there is an

1 equilibrium between those. But as a practical
2 matter the aggregates tend not to be reversible.
3 You can have all those things at the same time as
4 a folded form of the protein. Many proteins, but
5 not all, are, as you say, in nature, natural
6 sequence folded and active.
7 Q. So you said that there is a
8 natively unfolded protein. For those natively
9 unfolded proteins are those molten globules?
10 A. That is not how people would
11 normally tend to describe. Molten globule is on
12 the way to folding. Natively unfolded is just
13 what it says. It says that when it's made it
14 remains unfolded. Sometimes you will see the
15 phrase "unstructured", "natively unstructured".
16 It's an important area of research.
17 Q. Do those proteins have a function?
18 A. Yes.
19 Q. What's the function?
20 A. They have lots of different
21 functions.
22 Q. Okay.
23 A. So they are involved in lots of
24 biological processes. It is a big area of
25

1 NAISMITH - MARTIN
2 science. It's not one I've worked in myself but
3 its natively unstructured proteins is an important
4 area of science.
5 Q. So does a natively unstructured
6 protein have a structure?
7 A. It has a primary sequence. It has
8 a primary structure and sequence but it doesn't
9 have a defined -- its by definition it says
10 "unstructured". That is what people are trying to
11 work out, that is what a whole research program is
12 about.
13 Q. But it is a physical thing; right?
14 A. It is a physical sequence, yes.
15 Q. So it does actually exist in
16 three-dimensional space?
17 A. Yes, but it is likely -- Yes, it
18 does exist in three-dimensional space, but what we
19 don't understand is how it exists in
20 three-dimensional space.
21 Q. So we don't know what it looks
22 like?
23 MR. KUSHAN: Objection foundation.
24 A. We have -- so people pursue it
25 because they have some idea what is doing it, and

1 NAISMITH - MARTIN
2 what it might look like. But it's natively
3 unstructured. That is the phrase that is used.
4 Q. (BY MS. MARTIN): So would you
5 consider a natively unstructured protein to have
6 structural features?
7 A. I'm not an expert on natively
8 unstructured proteins. I would have to go and
9 pull out some papers and look where the current
10 state of the art is.
11 Q. So you do not know whether or not a
12 natively unstructured protein has a structural
13 feature?
14 MR. KUSHAN: Objection form.
15 A. So what I would know is there would
16 be some structural features. There is a sequence
17 which is the primary structure. They may exist as
18 part of a structured assembly where there are
19 portions of it that that are natively
20 unstructured. It may be entirely natively
21 unstructured. But those are known and as a
22 specific category.
23 Q. (BY MS. MARTIN): So when you say
24 "primary structure", when you are referring to the
25 sequence, what does the term "primary" signify?

1 NAISMITH - MARTIN
2 A. It tells me it is the sequence. So
3 primary structure is the amino acid sequence
4 arranged in order.
5 Q. And the amino acid sequence
6 arranged in order, is a structure or feature;
7 right?
8 A. It is a structural characteristic,
9 yes. But it is as I say, there is all, when we
10 talk about proteins, this slight confusion or
11 overlap by some people. Structure, when we talk
12 about proteins we refer to the folded form, but
13 the primary sequence or primary structure is the
14 sequence.
15 Q. If we talk about polypeptides as
16 opposed to proteins, is there is a distinction
17 between whether a polypeptide is folded form?
18 MR. KUSHAN: Objection foundation.
19 A. So the experts have previously used
20 those interchangeably in the reports so I am going
21 to just say that proteins -- polypeptides mean the
22 same thing. As regards the claims then we are
23 talking about a folded form of the protein.
24 Q. (BY MS. MARTIN): In science, not
25 in connection with patent claims, do you think of

1 NAISMITH - MARTIN
2 a polypeptide as a string of amino acids or as a
3 folded protein?
4 MR. KUSHAN: Objection form.
5 A. So for here in this business I was
6 very careful to use the terms that others have
7 used. You know, polypeptide, it, kind of, means
8 the same thing but sometimes some people draw
9 differences but not here. I think Dr. Petsko
10 picked up on this and said, "Yes, it's a bit
11 sloppy in common usage" or something.
12 Q. Do you use the term "polypeptide"
13 in your work as a scientist?
14 A. I would have to go and word search
15 my publications. It is possible I've used it.
16 Q. But, like, as a common matter do
17 you typically use that word?
18 A. When referring to a folded form of
19 a protein?
20 Q. Just in general. I am just asking
21 if you use the word "polypeptide" in your
22 day-to-day life?
23 A. You know, I am struggling to think.
24 I probably have used it interchangeably. I'm
25 probably not as precise, as Dr. Petsko said, "We

1 NAISMITH - MARTIN
2 are probably all a bit sloppy sometimes". I might
3 have used it; I can't say I haven't.
4 Q. Okay.
5 A. Because it is possible you've
6 searched my papers and found me saying
7 "polypeptide"; I don't know.
8 Q. No, I am actually just trying to
9 get an understanding as to, like, if you want to
10 -- We are trying to be very precise with our
11 language. "Polypeptide" is just a string of
12 peptides; right? Many peptides?
13 MR. KUSHAN: Objection form and to
14 foundation.
15 A. So to be precise, it is used
16 interchangeably by POSAs as you saw from Dr.
17 Petsko and Dr. Hecht. So I am going to use
18 protein and polypeptide to be synonymous here.
19 There's no hard and fast literature definition
20 that would distinguish between them.
21 Q. (BY MS. MARTIN): Okay. Can we
22 turn to paragraph 50?
23 A. I'm on paragraph 50.
24 Q. Okay, so do you see the sentence
25 that starts: "Each additional permitted

1 substitution"?
2 A. Yes, I see that.
3 Q. And so in that sentence you say:
4 "Each additional permitted substitution increases
5 the size and structural diversity of the sequence
6 space exponentially." Is that right?
7 A. Yes, I see that.
8 Q. What do you mean by "structural
9 diversity" in that sentence?
10 A. So when we think about proteins and
11 structure function relationship, they are
12 introducing multiple mutations into a native
13 sequence in all these permitted positions. You
14 would begin to expect to see differences in the
15 three-dimensional structure of the protein as well
16 as, of course, you actually made primary
17 structural differences, so .....
18 Q. What do you mean by a "structure
19 function relationship"?
20 A. I am going to use it in the same
21 way Dr. Petsko talked about, that a properly
22 folded, he said, "'Activity' is a synonym for
23 properly folded" I think he said. What I mean is
24 the structure as a whole has a function. So that
25

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

1 NAISMITH - MARTIN
2 structure, which is structurally very distinct to
3 the mutant HYAL1, that it is possible to achieve a
4 catalytically competent substrate binding form of
5 the protein despite -- well, not "despite", but
6 from two different structural arrangements.
7 So it is possible to contemplate as
8 you introduce additional site directed
9 mutagenesis, that you can start to change the
10 structural arrangement of PH20. One of the
11 questions is whether that preserves the structure
12 function relationship in that it is still
13 catalytically competent and active or whether it
14 leads to unfolding or a lack of secretion or
15 instability or preventing the protein being made.
16 Because the parameter claim space is so vast you
17 begin to expect to see all of those different
18 things.
19 Q. So HYAL1 and bee venom
20 hyaluronidase have the same mechanism of action in
21 that they both cleave hyaluronic acid through the
22 same substrate mediated catalysis; is that right?
23 A. Yes, that is right. They are both
24 able to do this piece of chemistry.
25 Q. And it is the same piece of

1 NAISMITH - MARTIN
2 chemistry?
3 A. Yes, the cleavage of hyaluronic
4 acid.
5 Q. It is the cleavage of hyaluronic
6 acid but it is in the same way; right? They are
7 cleaving the same bond, using the same residues?
8 MR. KUSHAN: Objection foundation.
9 A. So the chemistry of the bond
10 cleavage is conserved between the two despite the
11 fact that there are structural differences between
12 them, which tells us that the structure function
13 relationship obviously operates for both of these
14 two despite the structural differences.
15 Q. (BY MS. MARTIN): Okay. When you
16 say that "there are structural differences between
17 them" are you talking about the lack of EGF motif
18 or EGF-like motif in the bee venom structure?
19 MR. KUSHAN: Objection foundation.
20 A. Could we go to that bit in my
21 report? I can find it for you.
22 Q. (BY MS. MARTIN): I don't know what
23 bit it is. If you know where it is, that would be
24 great.
25 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

1 NAISMITH - MARTIN
2 position of PH20 and HYAL1. It is in my
3 deposition (sic), I can turn to it, if that is
4 okay?
5 Q. (BY MS. MARTIN): Yes. I think it
6 is on maybe page 112?
7 A. That is the one, yes.
8 Q. Is that what you were thinking of?
9 A. Yes, that's right. He did that
10 super position and said that they have -- I don't
11 think, I don't use the word "alpha-beta barrel
12 fold", I would say "motif" and the EGF domain is
13 outside of that motif.
14 Q. Okay. While we are talking about
15 terminology, PH20 is a single domain protein; is
16 that right?
17 A. It is a single folded protein. So
18 it is a single thing. So again there is a, we
19 sometimes will say the "EGF domain" because that
20 appears in the sequence. But the people who
21 described HYAL1 were clear to say that the protein
22 is a single thing. The domain is not separate.
23 There are cases where the domain is attached
24 essentially by a tether to another domain, but
25 that is not one of them.

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2 Q. So it is not that -- it does not
3 have a -- PH20 does not have a quaternary
4 structure; right?
5 A. A quaternary structure is where you
6 get multiple monomers together. I would have to
7 go and check. I can't remember if PH20 exists as
8 a dimer or sometimes they do.
9 Q. But you agree that the EGF portion
10 is part of the PH20 protein, it is not a separate
11 thing?
12 A. Yes, the PH20 is a single folded
13 unit, yes.
14 Q. Okay.
15 A. As we discuss here with the 447.
16 I don't know how the GPI anchor is arranged.
17 I just, you know, I don't know, so my limit is the
18 truncations.
19 Q. Okay, so I think the question that
20 prompted us to find the figure on page 112 was
21 whether there are structural differences between
22 HYAL1 and PH20?
23 A. There will be small differences
24 and, interestingly, you don't see any in these
25 figures. So, you know, at that level there is no

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2 difference, they both, as Dr. Petsko said, they
3 adopt the same fold although I would refer to the
4 whole protein as the fold not just alpha-beta
5 barrel motif. So those are the same structures.
6 Q. When you say "at that level there
7 is no difference" you mean the level of the fold,
8 like a back -- that is formed by the backbone of
9 the amino acid sequence?
10 MR. KUSHAN: Objection to form.
11 A. As it is shown here, you can see
12 that the structures overlap completely. If you
13 look carefully, maybe there is a little shift up
14 here. But what is really interesting with that is
15 despite the fact that they have the same fold,
16 they actually have some differences in function,
17 which shows you it is quite difficult to detect.
18 Q. (BY MS. MARTIN): And the
19 difference in function is that one of them is
20 active only at acidic pH and the other is active
21 at neutral pH?
22 A. I set out somewhere in my report (I
23 can turn to it) there is a table with the various
24 ones. But there is are differences where they are
25 active and also the substrate preference. One of

1 NAISMITH - MARTIN
2 them prefers high weight hyaluronic acids.
3 Q. "High weight hyaluronic acids"? Is
4 that what you said?
5 A. Yes.
6 Q. Okay. But they both cleave
7 hyaluronic acid; right?
8 A. Yes, that is the amazing thing;
9 right?
10 Q. Okay. They are only around 40%
11 sequence identical; is that right?
12 A. That is correct. Now in reality we
13 might expect those structures to be a little more
14 different if we experimentally determined them.
15 But using Swiss-Prot you will see here that, you
16 know, you have got very closely aligned. I do
17 note that if you experimentally determined them
18 you may see bigger differences.
19 Q. Did you look at the experimentally
20 determined PH20 structure?
21 A. I don't believe there is
22 experimentally. There certainly wasn't one at
23 2011/2012.
24 Q. Okay. So you do not know if there
25 is an experimentally determined PH20 structure?

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2 MR. KUSHAN: Objection to form.
3 A. There is not an experimentally
4 determined structure for PH20 that I'm aware of
5 and there certainly wasn't one in 2011/2012.
6 Q. (BY MS. MARTIN): Would you
7 consider there to be structural diversity between
8 HYAL1 and PH20?
9 MR. KUSHAN: Objection form.
10 A. I would expect -- Again, if I
11 wanted to be precise I would define it. They had
12 the same, based on what we would assume it to have
13 the same arrangement of secondary structure
14 elements based on the SWISS-MODEL. There will be
15 local differences and you can see in Dr. Petsko's
16 figures some little local differences. So you are
17 beginning to introduce some structural diversity.
18 The important thing is both of these enzymes are
19 shaped by evolution. You have not introduced a
20 bunch of sequence changes between them. Nature
21 has selected them.
22 Q. (BY MS. MARTIN): There are a bunch
23 of -- To be clear, there are a bunch of sequence
24 changes between them because they are only around
25 40% identical. It is just that those changes have

1 NAISMITH - MARTIN
2 been selected by nature; right?
3 A. That is correct in evolution.
4 Q. And you -- okay. When you talk
5 about "local differences" are you talking about
6 the slight difference in what looks like the loop
7 positioning in the picture on page 112 or are you
8 talking about something else?
9 A. No that's one of them. There's ---
10 Yes, that's the one you can see at that level,
11 yes.
12 Q. I'm just trying to understand what
13 you mean by "local"?
14 A. Just that as Dr. Petsko qualifies
15 with "essentially the same", he does not say
16 "identical". So I'm happy to use the word Dr.
17 Petsko uses which is "essentially the same". I am
18 sure he used that word because he too can see
19 there is a subtle difference here.
20 Q. Okay. Can you turn to paragraph
21 17?
22 A. I'm on paragraph 17.
23 Q. So you say in paragraph 17 that:
24 "The scientific literature more frequently uses
25 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

1 NAISMITH - MARTIN
2 C-terminally truncated, does that have a native
3 fold since it is not a naturally occurring
4 protein?
5 A. The parts that are folded, because
6 they are active, are assumed to have the same fold
7 as in the native protein. So those parts are
8 natively folded, properly folded in Dr. Petsko's
9 description. But there are additional regions
10 that are not seen and so you might distinguish
11 that between a modified PH20 and just PH20 because
12 you've modified it with the truncation.
13 Q. And so did you also use the
14 terminology "native state", and I'm wondering is
15 there is a distinction between the word "state"
16 and the word "fold"?
17 A. Not as I use it here, I use them
18 interchangeably.
19 Q. In the literature is there is a
20 distinction between "state" and "fold"?
21 A. I'm not aware of a precise
22 definition between them. I've seen them used
23 differently. I have seen them used, sorry,
24 interchangeably in the literature.
25 Q. Okay. If you are talking about an

1 NAISMITH - MARTIN
2 unfolded protein, would you say that an unfolded
3 protein, even if it does not have a fold, it has a
4 state?
5 MR. KUSHAN: Objection foundation
6 and objection to form.
7 A. I would not use it when we are
8 talking about unfolded protein.
9 Q. (BY MS. MARTIN): So you would not
10 use, like, confirmational state to refer to an
11 unfolded protein?
12 A. "Confirmational state" implies a
13 definable arrangement of elements. You may have
14 multiple confirmational states and you probably
15 do, but that confirmational state does imply a
16 description of something. Unfolded proteins tend
17 to be thought of as just a collection of huge
18 numbers of possible confirmational states.
19 Q. Okay. Is there any limit to the
20 structure, the three-dimensional structure that an
21 unfolded protein could take?
22 A. Dr. Dobson provides some
23 mathematical formulations and we could turn to
24 those. As a practical matter I've never
25 considered it. It's huge numbers.

1 NAISMITH - MARTIN
2 Q. Okay.
3 A. I can't give you a number but it is
4 exponentially large if you consider 15 mutations,
5 for example. For unfolded proteins I would expect
6 them to be very large.
7 Q. Is the confirmational space for an
8 unfolded protein, is it limited by the dihedral
9 angles of the sequence?
10 A. So for truly unfolded proteins
11 there are very few limits. There are some limits
12 and you are talking about the Ramachandran plot,
13 I am guessing.
14 Q. Yes.
15 A. So there are energetic constraints
16 but that doesn't mean you do not get forbidden
17 angles too or so-called forbidden angles. So that
18 is quite fuzzy but there are some regions you
19 might not expect a particular confirmative dot.
20 Dr. Dobson shows a formulation for you how you
21 might calculate the, sort of, confirmational space
22 that you can explore.
23 Q. So, for example, if you have a PH20
24 with a D320 K mutation in SEQ.ID NO:3 ----
25 A. Mmm-hmm.

1 NAISMITH - MARTIN
2 Q. ---- even though that protein has a
3 mutation in it, does it still have a native fold?
4 A. As it is described in the patent,
5 produced that way, yes, it does, not 8 Molar urea.
6 Q. Yes, understood. In -- yes,
7 I mean, is there a shorthand for, you know, not in
8 8 Molar urea?
9 A. If we are both agree ----
10 Q. We can assume it is not in urea
11 unless I tell you otherwise.
12 A. I know. Sorry, I am just trying to
13 be precise.
14 Q. No, I understand. It is good to be
15 precise. Okay. So, okay, but the question was if
16 you have D320 K SEQ.ID NO:3 PH20 does it have a
17 native fold even though it has a mutation in it?
18 A. So it obviously has differences to
19 the full length native PH20 but the part that is
20 folded because it is active we are going to assume
21 it is properly un-natively folded.
22 Q. Okay, so I am just trying to make
23 sure that I am understanding the terminology that
24 the word "native" does not have a connotation, you
25 know, of no mutations?

1 NAISMITH - MARTIN
2 A. For folding native when you talk
3 about the sequence has that connotation, no
4 mutations which is ----
5 Q. Yes, but for folding it doesn't?
6 A. For folding you can adopt a native
7 fold, despite having sequence differences and that
8 would be properly folded as it is used here.
9 Q. Okay. So is it fair to say that if
10 you have an active PH20 you expect that that
11 active PH20 has adopted the native fold?
12 A. You would assume that if it is
13 properly folded, but there could be structural
14 differences. But the fold is quite a broad term.
15 It is relatively a distance high level term. But
16 you would assume that it preserves a structure
17 function relationship and we talked about the bee
18 venom and the human HYAL1. So there are
19 structural differences. So those are different
20 structures ----
21 Q. Mmm-hmm.
22 A. ---- and that was noted in the
23 publication. So you can envisage proteins having
24 a proper fold which is, and we have used this term
25 where it is active it is natively folded. But

1 NAISMITH - MARTIN
2 structurally there still could be differences and
3 structural diversity when you start to introduce
4 mutations.
5 Q. Okay. You so I want to be trying
6 to get an understanding of what you mean when you
7 are using the terms that are in your declaration,
8 or what you are thinking when you are using the
9 terms in your declaration. So I understand that
10 if you have, you know, a mutation for example at
11 position 320 and you compare that to what to
12 SEQ.ID NO:3, I don't want to say "wild type"
13 because I understand there is the stuff at the end
14 and I am trying to not deal with that.
15 A. No, I understand.
16 Q. Okay. So D320 K SEQ.ID NO:3 versus
17 SEQ.ID NO:3, right, you have a lysine at position
18 320, so you have somewhat of a difference in the,
19 you know, three-dimensional surface topology, if
20 you will, on your PH20 but the fold is still the
21 same?
22 A. I would use Dr. Petsko's logic,
23 "essentially the same", yes. The fold and the
24 arrangement for that one mutation, D320 K, the
25 fold would be essentially the same. And based on

1 NAISMITH - MARTIN
2 Dr. Petsko and Dr. Park's figures they also do not
3 see any local pertubation; they see a stabilizing
4 interaction.
5 Q. Hmm-mmm, okay. Now if you
6 introduce mutations into PH20 and you obtain an
7 active protein, do you expect that it still has
8 the native fold or do you think it is possible
9 that it has a totally different fold, like maybe
10 it looks like a cat?
11 A. It certainly would not look like a
12 cat.
13 Q. Okay. Would you expect it that has
14 the native fold?
15 A. So I would expect at this same
16 level of imprecision that we used the bee venom
17 and human HYAL1 to say they are roughly the same
18 structure but with important differences ----
19 Q. Yes.
20 A. ---- then I would expect that any
21 active form of the PH20 within that level of
22 imprecision would have the same fold.
23 Q. Okay. I am trying to understand
24 that level of -- using that level of imprecision
25 if you will. Like, it still is going to look,

1 NAISMITH - MARTIN
2 like, the same alpha-beta barrel-ish motif
3 as opposed to, you know, an octagon or ----
4 MR. KUSHAN: Objection. Sorry.
5 Q. (BY MS. MARTIN): ---- hemoglobin
6 or something?
7 MR. KUSHAN: Objection form,
8 objection to foundation.
9 A. So fold is, the problem is fold is
10 relatively imprecise.
11 Q. (BY MS. MARTIN): Yes.
12 A. The structure of the HYAL1 and the
13 bee venom are different so therefore I would
14 expect, based on what I knew at 2011/12, if I
15 begin to introduce molten mutations and I found an
16 active form of PH20 I would expect it to have the
17 same fold as here, properly folded, but I could
18 expect quite a lot of structural diversity, how
19 the structure function relationship which is
20 essential for activity was achieved.
21 Q. Okay, so now let's talk about what
22 you mean by "structural diversity" in the answer
23 that you just gave. So what do you mean by
24 "structural diversity"?
25 A. For example the difference between

1 the bee venom and the human HYAL1, that is a good
2 example of structural diversity. The one has a
3 beta strand the other has a loop and the other one
4 is missing an alpha helix.
5 Q. Okay. So there could be
6 differences, for example, in secondary structure?
7 A. That is what the literature kind of
8 shows us and also the patent. Dr. Simpson
9 considers putting insertions and deletions in so
10 those indeed could perturb some secondary
11 structural elements.
12 Q. Okay. So if you have a difference
13 in secondary structural elements would you still
14 then have the same tertiary structure or is it a
15 different tertiary structure?
16 A. I would say you would have broadly
17 defined the same fold.
18 Q. Okay.
19 A. Tertiary structure begins to imply
20 a bit more precision.
21 Q. Okay.
22 A. So that is why the people drew
23 attention to difference between the bee venom and
24 human HYAL1.
25

1 NAISMITH - MARTIN
2 Q. Okay. So if you introduced a
3 change into PH20 that disrupted an alpha fold for
4 example ----
5 A. An "alpha fold", sorry?
6 Q. I am sorry, an alpha helix. Let me
7 start again. Strike that. If you introduce a
8 change into PH20 that disrupted an alpha helix you
9 would say that that is a change in secondary
10 structure; right?
11 A. I would not know whether it did
12 disrupt so I don't know how you know that it
13 disrupted alpha helix.
14 Q. Oh okay.
15 A. I mean there are mutations that
16 potentially and predictably can disrupt an alpha
17 helix, but, you know, are you telling me that you
18 know it disrupted an alpha helix or are you asking
19 me to predict?
20 Q. Oh okay, I understand.
21 A. Sorry.
22 Q. Thank you. All right, so I am
23 asking you to assume, just because I am trying to
24 understand your opinion, that you introduce the
25 change that's disrupted an alpha helix. So

1 NAISMITH - MARTIN
2 assuming that that's what happened, would you
3 consider that modified alpha helix PH20 to have a
4 difference in secondary structure?
5 A. Is the protein active?
6 Q. Yes.
7 A. So I would say it is the same
8 native fold. There are some details and
9 difference in the structure however, exactly as
10 you described alpha helix as somehow broken.
11 Q. Okay, so if you have an active PH20
12 you expect that it has the same native fold even
13 though there could be differences in the secondary
14 structure?
15 A. Yes, because we saw that with the
16 bee venom; there is a clear difference between the
17 secondary structure of the bee venom right within
18 alpha-beta barrel motif between bee venom and
19 human hyaluronidase. So we know that it is
20 possible.
21 Q. Okay.
22 A. The biggest difference, of course,
23 is bee venom does not require and does not have
24 the EGF domain, which is essential for PH20.
25 Q. So it is possible to achieve the

1 NAISMITH - MARTIN
2 native fold of PH20 even if there are variations
3 in secondary structure?
4 A. It depends how big those variations
5 get. I mean, at some point you might begin to
6 dispute whether it is the same, but it is
7 certainly possible to imagine describable visible
8 differences in secondary structure for proteins
9 that shared the same fold and are active.
10 Q. Okay. Would you consider two PH20s
11 to be precise so a modified PH20 that is active
12 and SEQ.ID NO:3 PH20 to have common structural
13 features if, let's assume, that there is a change
14 in secondary structure like you have disrupted an
15 alpha helix?
16 MR. KUSHAN: Objection form,
17 objection foundation.
18 A. So I would expect if you were using
19 that you would define the particular ones that are
20 common between the two examples.
21 Q. (BY MS. MARTIN): What do you mean
22 by that, like, what -- how would you define the
23 particular ones that are common?
24 A. Well, I would say we have these
25 secondary structure, so we are talking about them,

1 NAISMITH - MARTIN
2 ----
3 Q. Mmm-hmm.
4 A. So you would say these secondary
5 structure elements are the same, this secondary
6 structure element is different, in the way that we
7 did with the bee venom and the hyaluronidase.
8 Q. Okay, okay. So for HYAL1 and bee
9 venom you could say that they have common
10 structural features and that some of the alpha
11 helices are in the same position?
12 A. They have -- Yes, the super
13 position of HYAL1 and bee venom shows there are
14 common structural features but there are really
15 important structural differences. So if you
16 restricted your discussion to the alpha-beta
17 barrel motif you might say they have got a similar
18 alpha-beta barrel motif but structurally they are
19 different.
20 Q. Okay.
21 A. That is what the authors say in the
22 paper.
23 Q. Okay, so they do have common
24 structural features but they also have
25 differences?

1 NAISMITH - MARTIN
2 A. That's correct but we do not know
3 which one of those ones in common are relevant to
4 the structure function relationship.
5 Q. Okay. So we have been going for a
6 little over an hour, do you want to take another
7 break?
8 A. If that's okay with you.
9 THE VIDEOGRAPHER: We are going off
10 the record. The time is 10.18 a.m.
11 **(A short recess from 10.18 a.m. to 10.42 a.m.)**
12 THE VIDEOGRAPHER: We are back on
13 the record. The time is 10.42 a.m.
14 Q. (BY MS. MARTIN): So does PH20 have
15 more than one native fold?
16 A. PH20 protein has one native fold.
17 You can modify PH20 and introduce different folds,
18 but PH20 has one native fold that I'm aware of.
19 Q. Can you turn to paragraph 43 in
20 your declaration?
21 A. Yes.
22 Q. In sub-item (iii) paragraph 43, you
23 talk about: "... the same tertiary structure
24 found in the native state of the wild-type PH20
25 protein ...".

1 NAISMITH - MARTIN
2 A. Yes, I do.
3 Q. And I just want to make sure I'm
4 understanding that when you use "native state"
5 there, are you talking about native, the native
6 fold?
7 A. Yes.
8 Q. Okay.
9 A. I did say I would use them
10 interchangeably, yes.
11 Q. Mmm-hmm. And similarly to the line
12 of questioning I just did, is there more than one
13 native state for PH20?
14 A. No, there's one properly folded
15 native fold on PH20.
16 Q. Okay. And I have seen the phrase
17 like "confirmational state" in the literature. Is
18 that ----
19 A. "Confirmational state in the
20 literature", I don't recall it for PH20. But in
21 general confirmational state might imply there is
22 -- that implies maybe local structural changes
23 that are somehow important. There is in fact, if
24 I remember correctly, in the bee venom structure
25 there is some discussion of changes when it is

1 NAISMITH - MARTIN
2 bound to the substrate.
3 Q. Okay.
4 A. So you might say there are some
5 confirmational changes in the loops.
6 Q. Okay. You used the term
7 "epistasis" in your declaration; right?
8 A. I talked about the concept of
9 epistasis and I use the word epistasis.
10 Q. What is the concept of epistasis
11 and I think paragraph 253 potentially would help.
12 A. Yes, we begin at 253. So
13 "epistasis" is just a word, a shorthand, if you
14 like, for non-additivity of single site mutations.
15 Q. Okay, when you say
16 "non-additivity", what does that mean?
17 A. So this is something all of the
18 experts talk about, which is as you make an
19 increasing number of single site mutations, so you
20 go from 1 to 2 to 3 to 4 to 5, that you can't
21 assume that the mutations do not interact with one
22 another in unpredictable ways. So they start to
23 like, to give you a concrete example I've given
24 here, for on page (sic) 254. You can imagine I
25 have two single site mutations. When they are

1 NAISMITH - MARTIN
2 made as single site mutations I want to combine
3 them together to make a double mutant; right? So
4 we will start simple. Now, let's say we assume
5 they are additive, so they both individually
6 actively -- they are both individually active on
7 their own. If they are additive, we combine them,
8 we would expect the protein to be slightly more
9 active. So that is additive; okay? But we can
10 get magnitude epistasis which means wow, the
11 protein becomes way more active when I combine
12 them as individuals. I can get sign, reciprocal
13 sign epistasis when individually the two mutations
14 are active. But when they go into the protein
15 they actually combine to make the protein much
16 less active. So that another one.
17 And then we can get just sign
18 epistasis where individually this mutant is
19 beneficial, if you like, active more active and
20 this one is more active. But when I combine them
21 I get one of them becomes deactivating
22 deleterious. So those are the three broad
23 categories of epistasis. This is, Dr. Hecht talks
24 about this. This is why it is so difficult to
25 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,

1 the concept is what people will describe. I just
2 used a shorthand and the concept is although you
3 may have single site mutations which, on their
4 own, have some effect on the protein, when you
5 start to combine them they become unpredictable
6 and that is because you get these epistatic
7 effects.
8 Q. So I think when Dr. Park in his
9 deposition he talked about if you make changes in
10 two different parts of the protein whether you
11 could consider them to be independent. Is that,
12 kind of, in the same general area of what you are
13 talking about?
14 A. So I would like to turn to exactly
15 what he used it as but, as a general point, if he
16 is saying they are not independent, he is saying
17 that there is a risk of epistasis. Dr. Park, in
18 his declaration, was very clear that his approach
19 only worked for single site mutations.
20 Q. Yes, no, I understand that. I am
21 just trying to understand if the concept of two
22 mutations being independent versus not independent
23 is what you are talking about when you are talking
24 about epistasis?
25

1 NAISMITH - MARTIN
2 A. So, that would be one way to
3 express it. If you are saying -- if you say,
4 "Mutation over here is independent from mutation
5 over there", you are, by that word, saying there
6 are no epistatic effects.
7 Q. Okay.
8 A. What I am trying to say is that the
9 data in the patent show that single site mutations
10 are perturbing the active site. Most of them
11 perturb the structure function relationship at the
12 active site. So we know that they are having
13 some, I think Dr. Petsko's phrase was, "action at
14 a distance".
15 Q. Mmm-hmm.
16 A. So finding mutations which are
17 independent of one another is very, very
18 challenging and that becomes exponentially almost
19 impossible as you move to 5.
20 Q. Okay. So you saying that finding
21 mutations that are independent of one another is
22 very, very challenging because every single site
23 mutation is resulting in a long range interaction?
24 A. I didn't say "every single site
25 mutation". I said that -- and we can turn the

1 NAISMITH - MARTIN
2 table in the patent, sorry, the table in my report
3 where I show that when you look at the activity
4 reported for active proteins they range from, I
5 think one is 27 fold more active to ones that are,
6 like, 25% active. That tells me that that
7 mutation has done something to the protein; right?
8 It has gained or it has been deleterious, so
9 therefore adding another mutation. So that to
10 change the activity you have in some way perturbed
11 the active site. The protein is still active or
12 vastly more active but it has had an effect.
13 So once when, let's say in my
14 mutation over here, I have all 27 fold more
15 active; right? The active site, something has
16 changed, I don't know what. This other mutation
17 over here which is two fold more active, something
18 has changed over here. But putting both together
19 is unpredictable since I can't figure out how
20 their combined changes will influence the active
21 site.
22 Q. Why are you as assuming that
23 something changed in the active site?
24 A. Because the chemistry is 27 fold
25 more active.

1 NAISMITH - MARTIN
2 Q. Are there other explanations for
3 that besides something changing on the active
4 site?
5 A. You could be 27 fold more expressed
6 in the way the assay is conducted but that would
7 be a feature of how the protein is folded and
8 made, so you are still -- that is an assembly
9 thing that is obviously interfering, also altering
10 in some way the protein.
11 Q. What if the mutation improved the
12 affinity of binding to HA but did not change the
13 active site?
14 A. So you would need to do that by
15 kinetic analysis to work out. So you are talking
16 about KM versus Kcat?
17 Q. Mmm-hmm.
18 A. There is no data for me to assess
19 that.
20 Q. That is a possible explanation;
21 right?
22 A. For 27 fold more activity?
23 Q. Mmm-hmm?
24 A. Possibly. I gave you another one
25 which was that it was made 27 times more readily

1 NAISMITH - MARTIN
2 or a higher yield. I don't know because I can't
3 use it but I have assumed because the patent uses
4 the phrase much more active than native that it
5 implies 27 fold more activity.
6 Q. But you assumed that the
7 difference, the -- I am sorry, strike that. You
8 assumed that the difference in activity is caused
9 by a change in the active site?
10 A. No, I did not assume that, I used
11 that as an explanation, but I am happy to take
12 other explanations. But based on the common
13 disclosure I am offered no other experimental
14 evidence, other than it is 27 times more active
15 than native.
16 Q. So you said that the explanation --
17 I am sorry, strike that. You said the reason why
18 you can't identify independent mutations or
19 independent positions is because every single
20 change is impacting the active site and that, in
21 itself, is premised on the assumption that a
22 change in activity is the result of a change in
23 the active site?
24 A. No, that is not what I said. So,
25 what I said was that based on what is in the

1 NAISMITH - MARTIN
2 patent information I have got is that I've got an
3 increase in activity. I have given you possible
4 reasons for increase in activity. But whether it
5 affects the binding constant, the expression
6 level, the secretion level, these are all whole
7 protein complexes which would suggest a mutant is
8 having an effect in some way upon the protein as a
9 whole. So where I'm affecting the protein as a
10 whole then I would have a concern to add another
11 mutation which is affecting the protein as a
12 whole. Now it is possible for mutations to be
13 independent of one another but not, you would not
14 know that without testing.
15 Q. So I don't understand why the
16 changes are having an effect -- strike that. Let
17 me ask a different question. What do you mean
18 when you say that the change is having an impact
19 on the protein as a whole?
20 A. So, if I talk about protein
21 production levels for why it is 27 times made
22 more, that is obviously something quite subtle
23 about the whole protein.
24 Q. Okay. HA, hyaluronic acid, is the
25 substrate; right?

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.

1 NAISMITH - MARTIN
2 Q. And HA is a large polymer?
3 A. Yes.
4 Q. So would you expect that PH20 is
5 contacting HA at other positions outside of just
6 the specific residues that are being cleaved?
7 MR. KUSHAN: Objection form.
8 A. So, I want to clarify your
9 question. Are you talking about based -- do
10 I think that the complete set of residues that
11 interact with HA can be described solely from the
12 bee venom complex?
13 Q. (BY MS. MARTIN): Yes.
14 A. The ones in contact with the bee
15 venom or the carbohydrate are outlined and they do
16 predict, they extrapolate based on the structure
17 the other regions that are, sort of, just continue
18 along that region. So there is a, sort of, a
19 groove that they say they think but don't know
20 that those are important for binding.
21 Q. Okay. Do you think that PH20 only
22 binds for example like the tetramer of HA that is
23 shown in the bee venom structure or do you think
24 that PH20 binds more of the HA polymer?
25 MR. KUSHAN: Objection form.

1 NAISMITH - MARTIN
2 A. So when you say "more", I think we
3 know the substrate is longer than the product
4 complex in the bee venom, and the bee venom does
5 explain. And I would have thought, based on the
6 bee venom structure, that I would expect that that
7 groove they predict would be important in PH20 for
8 binding polymer. But I don't know and I don't
9 know any experimental data in 2012 that really
10 tackled that question for PH20.
11 Q. (BY MS. MARTIN): You are talking
12 about the "groove". What do you mean when you say
13 the "groove", in PH20?
14 A. Yes, can we -- I will get to that
15 structure, it is probably easier.
16 Q. That would be great.
17 A. *(Pause)* We are so used to
18 electronic versions of documents.
19 Q. Maybe page 160 there is a picture?
20 A. Thank you. I knew it was in here
21 somewhere.
22 Q. Okay.
23 A. Thank you very much.
24 Q. So we are on page 160. Does that
25 show the groove that you are talking about?

1 NAISMITH - MARTIN
2 A. So the groove that they talked
3 about in the paper can be located in this figure.
4 Q. Okay, and can you just explain what
5 that is?
6 A. So the diagram is a primal image
7 secondary structure. Do you want me to define it
8 really clearly for the record or you would be
9 happy to just say "this bit"?
10 Q. If you just "this bit" that's fine.
11 A. Okay. Sorry, I wasn't sure what
12 you wanted. So you have a tetrasaccharide at the
13 top. Then what we know, because that is cleaved
14 product form, there must be some extension of the
15 polysaccharide beyond that. In the paper as
16 I describe they talk about what they think the
17 residues that might be in contact are with that
18 part of the substrate. So it is a continuous
19 groove that runs, kind of, *here,* formed by these
20 loops between the secondary structure elements.
21 But there was no firm experimental evidence
22 precisely what residues were in contact by the
23 time you get to PH20.
24 Q. Okay. And so earlier in your
25 testimony when you were talking about mutations

1 NAISMITH - MARTIN
2 having an impact on the active site, when you are
3 talking about the "active site" are you talking
4 about the groove or are you talking about the
5 specific residues that are involved in the
6 substrate mediated catalysis?
7 A. So I am talking about the -- when
8 you think about catalysis, it is a precise
9 positioning of residues important to that, it's
10 not just one residue of course. So positioning
11 them with the correct electrostatic effect is
12 really critical, and binding the substrate in the
13 correct orientation for catalysis is also
14 critical. So when you think about what you are
15 altering in the active site, if I was to do it
16 experimentally, I would be interested in all of it
17 to try to understand that, but the patent only
18 gives me the information about activity as a
19 whole. So somehow it is altering the activity of
20 the protein and I have given possible examples.
21 Q. Is it possible that some of the
22 changes that impact activity are changes to
23 residues that themselves contact HA even if they
24 are not involved in catalysis?
25 A. Yes, it's possible. I mean, I have

1 no way to know.
2 Q. Did you account for that when you
3 rendered your opinions regarding potential
4 epistatic effects?
5 A. So that is the whole point about
6 epistatic effects is that it is the
7 unpredictability of combining mutations that is
8 the problem. So anything which is deleterious to
9 the binding of the protein or the correct binding
10 of the substrate or deleterious to the correct
11 positioning of the catalytic machinery, that would
12 cause the protein to be, it could cause the
13 protein to be inactive. Or you could also imagine
14 that combinations which on their own are active
15 result in protein that becomes unstable, because
16 in some way they've altered the packing of the
17 core of the protein. And that would also give
18 rise to apparent inactivity although you could
19 distinguish between those two forms of inactivity
20 by experimentation.
21 So when I rendered my opinion I was
22 guided solely by the common disclosure which
23 reports activity measurements and those
24 significantly differ from one for a large number
25

1 NAISMITH - MARTIN
2 of mutations. And so I know something is
3 happening and the patent doesn't tell me what.
4 But some of them are more active, some of them are
5 less active. And so combining those will be
6 challenging in any event. Even if I -- The patent
7 gives me that additional information. Even
8 without that information I would ask still been
9 worried about epistatic effects or non-deletative
10 mutations. That was well known in the literature
11 and I cite I think it is Fowler et al. where they
12 talk about this, that by the time they got to
13 three or four mutations then it became really
14 difficult, you know, the thing became
15 unpredictable. In part the unpredictability is
16 what underpinned the work around directed
17 evolution, because it is those complex
18 interactions at a distance that are subtly
19 altering the structural activity relationship that
20 have bedevilled protein engineering to do rational
21 combinations of single site mutations.
22 Q. And so I am trying to get an
23 understanding of the potential independence of
24 different mutations and the action at a distance
25 as you put it. And I am just trying to understand

1 NAISMITH - MARTIN
2 because you testified earlier that it would be
3 very difficult to identify mutations that are
4 independent on PH20 because all of the mutations
5 are impacting the active site. And so I'm trying
6 to understand if you took into account when you
7 formed that opinion the possibility that what is
8 happening is that the amino acid you are changing
9 is binding to HA even though it is not in the
10 active site?
11 MR. KUSHAN: Objection form,
12 objection to foundation.
13 A. So there is a lot in that question.
14 Could you break it up into smaller questions?
15 Sorry, there is a lot. I heard a lot of words at
16 the start and there is a lot of words at the end,
17 I'm sorry.
18 Q. (BY MS. MARTIN): Did I understand
19 your testimony correctly that you believe it would
20 be difficult to identify positions to mutate on
21 PH20 that are independent?
22 A. Okay, so you can experimentally
23 identify those. My point was it would be hard, *a*
24 *priori*, to predict them. That's my point, is that
25 *a priori* you can't, you would not easily know for

1 NAISMITH - MARTIN
2 any protein whether sites were independent, even
3 at two mutations. Now you might assume you can
4 get away with it which is why in my opinion
5 Dr. Hecht and actually Dr. Petsko all said up to 5
6 you can probably manage but beyond that it does
7 become unpredictable and the literature even at 3,
8 so that is point. Now, when you say they are
9 independent can I identify them? I can. I can do
10 the experiment. I can make the combined mutation
11 and measure the output. And if the output is an
12 additive combination then I know they are
13 independent because that is the definition of
14 independence.
15 Q. I am going to break that down. So,
16 first, I want to understand why the cut off that
17 you are using is 5? So why is it that you can
18 probably manage 5 but if you tried 6 they would
19 become unpredictable?
20 A. So I said in my report that POSAs
21 would be confident at 1 and less confident by the
22 time you get to 3 and different POSAs would draw
23 the line differently. I've said for this report
24 I will draw it at 5. But another POSA might say
25 4. 5 is the outer limit, I think, that any POSA

1 NAISMITH - MARTIN
2 would draw.
3 Q. Okay. So a POSA could potentially
4 think that they could manage 5 mutations but 6
5 would be too many?
6 A. It is decreasing confidence in your
7 predictability. At 5 I think you would have very
8 little confidence in your predictability. By the
9 time you get to 6 you would have no confidence in
10 your predictability.
11 Q. And you think that all POSAs would
12 agree with that assessment?
13 A. In 2011 and 12 timeframe, yes,
14 I would because the literature was there at that
15 time to warn you about that, it was well known.
16 And Dr. Hecht and Dr. Petsko both touch on this
17 issue quite clearly. Dr. Hecht says, you know,
18 the same, kind of, number round about 5, up to 5.
19 So I would say, yes, that is where POSAs would
20 draw. I don't know any analysis of anyone who can
21 predict beyond 5 that I'm aware of in the 2011/12
22 timeframe.
23 Q. Okay, do you consider Dr. Petsko to
24 be a POSA?
25 A. Yes.

1 NAISMITH - MARTIN
2 Q. And he said that you could make
3 more than five changes with reasonable
4 predictability; right?
5 MR. KUSHAN: Objection foundation.
6 A. Can I have his report, because
7 I think he qualifies things so I'd like to see his
8 report where he says this.
9 Q. (BY MS. MARTIN): Okay so you don't
10 agree that -- I want to make sure that I'm
11 understanding because you said that no POSA would
12 be comfortable making predictions with more than
13 five changes and you said that Dr. Petsko is a
14 POSA. So are you saying that Dr. Petsko took the
15 position that you couldn't make any predictions
16 above five changes?
17 A. So Dr. Petsko when he talked -- he
18 used other evolutionary species when he gave
19 examples of more than five changes. But I don't
20 recall in his deposition where he said he could
21 confidently predict five or more single site
22 mutations that he could combine to retain
23 activity.
24 Q. So you don't think that he took
25 that position?

1 NAISMITH - MARTIN
2 MR. KUSHAN: Objection foundation.
3 A. So Dr. Petsko's opinion was based
4 on the tolerance table. He thought a POSA could
5 somehow use that but in his deposition he didn't
6 give any example of how to do that and I don't
7 think in my opinion that's wrong. I don't think
8 it is possible so I would disagree with his. He
9 never said in his opinion, "Here is a way to
10 combine five single site mutations or more that I
11 am confident will work."
12 Q. (BY MS. MARTIN): Okay. And to the
13 extent that he did in fact say that it was
14 possible to combine more than five single site
15 mutations in the way that would work, you would
16 disagree with him?
17 A. So, Dr. Petsko may have imagined
18 some very narrow definition of five, as an example
19 of which it might be possible. But as a general
20 rule, my opinion is that a POSA could not pick
21 five single site mutations and combine them with
22 any confidence in the prediction of the outcome.
23 Q. Okay. Do you think that
24 Dr. Simpson was a POSA?
25 A. Yes, I do.

1 NAISMITH - MARTIN
2 Q. And Dr. Simpson also opined that
3 you could make more than -- you could predictably
4 make more than five single site mutations; right?
5 A. Could I turn to her declaration of
6 what she actually said, if that's okay?
7 Q. We can get there. I am trying to
8 have a general understanding again as to --
9 Because again you said that no POSA would take the
10 position that you could predictably make more than
11 five changes. So is it your understanding that
12 Dr. Simpson also imposed a five change maximum?
13 MR. KUSHAN: Objection foundation.
14 A. Dr. Simpson did not discuss
15 epistasis at all and did not shed any light of
16 what was a well-known literature phenomenon that
17 Dr. Petsko was clearly aware of. He uses the
18 phrase "independent". So Dr. Petsko is using end
19 result which is, they are independent, so you can
20 combine them. But that you would only know by
21 experimentation. Dr. Simpson does not express,
22 that I recall, any method or literatures for
23 support for her position that you could use Dr.
24 Petsko's tolerance table and combine mutations.
25 My argument -- opinion is based on the fact that

1 NAISMITH - MARTIN
2 the literature shows that not to be true.
3 Q. (BY MS. MARTIN): So there is
4 literature on PH20 that shows that you can't use
5 what you did in air quotes, Dr. Petsko's air
6 quotes tolerance table to make predictions. There
7 is literature on that?
8 MR. KUSHAN: Objection foundation,
9 objection to form.
10 A. The literature shows quite clearly
11 that the problem of combining in any protein
12 single site mutations once you start to combine
13 them the literature shows that they are
14 unpredictable and I cite to Fowler where this is
15 discussed at some length.
16 Q. (BY MS. MARTIN): But Fowler wasn't
17 talking about PH20, was it?
18 A. He was not talking about PH20 but
19 the principles around protein structure that
20 Dr. Hecht and Dr. Petsko both opine in their
21 introductory matters, these phenomenon will apply
22 across all protein structures.
23 Q. All right, so you also said of your
24 answer a little while ago that the definition,
25 something like the definition of independent

1 NAISMITH - MARTIN
2 changes would be if they are additive. Is that
3 about right?
4 MR. KUSHAN: Objection foundation.
5 A. So Dr. Petsko did not define what
6 he meant by independent.
7 Q. (BY MS. MARTIN): I thought that
8 that used the word definition in your answer?
9 A. I gave you independent of epistatic
10 effect which is that they are simply additive.
11 Q. Okay.
12 A. Now Dr. Petsko could have had the
13 other, which is they are not even additive, but
14 that is actually -- I did, you know, he just said
15 "independent" which means he is avoiding the
16 epistatic effect.
17 Q. Okay. Is it your testimony -- is
18 it your testimony that in order for two mutations
19 to be independent their effects have to be
20 additive?
21 MR. KUSHAN: Objection form.
22 A. So for them not to have epistasis,
23 for there to be no epistasis the definition is the
24 effects are additive. That is the definition of
25 epistasis.

1 NAISMITH - MARTIN
2 Q. (BY MS. MARTIN): And so am I
3 understanding "additive" correctly in that if you
4 have one mutation that gives you 100% activity and
5 another mutation that gives you 100% activity,
6 when you combine them you would still have 100%
7 activity?
8 A. If they were independent of one
9 another, yes.
10 Q. Okay, and ----
11 A. Because there is no epistatic
12 effect.
13 Q. I am trying to understand the
14 additive part?
15 A. Okay. So it is based on the
16 thermodynamic but then it translates to
17 multiplying the activity measurement; so one times
18 one is one.
19 Q. Okay. So, can you explain what you
20 just said about thermodynamic?
21 A. So, the epistasis is thinking about
22 what is happening in the structure so effects are
23 in some way added. But if the protein has an
24 activity of one and the second independent
25 mutation has an activity of point 5, you multiply

1 NAISMITH - MARTIN
2 the two activities to get the resulting activity
3 which would be point 5 in the absence of epistatic
4 effects.
5 Q. Okay, so if one had -- I'm trying
6 to understand how you would actually add them. If
7 one of them had an activity of point 5 and the
8 other one had an active of point 75, then would
9 you multiply them or would you add them?
10 A. If they are independent, you
11 multiply them.
12 Q. Okay. So point 75 times point 5,
13 that would give you the ----
14 A. Point 35.
15 Q. Point 35, okay.
16 A. Yes.
17 Q. But in that example point 35 is
18 worse than point 5 and point 75?
19 A. That is correct because you are
20 combining two deleterious mutants.
21 Q. Okay, oh, I understand. So the
22 direction is the directionality?
23 A. Correct. And what would be an
24 example of epistatic if I combined them and I got
25 1.5 activity, that is an example of ----

1 NAISMITH - MARTIN
2 Q. Okay?
3 A. ---- where you would see epistatic
4 effects.
5 Q. So you are combining two
6 deleterious mutants one of which halves activity
7 and one of which drops it be a quarter. So you
8 are expecting that when you combine them you are
9 going to similarly decrease activity?
10 MR. KUSHAN: Objection form.
11 A. That's not quite what I said.
12 I said if you assume them to be independent ----
13 Q. (BY MS. MARTIN): Yes, if you
14 assume them to be independent.
15 A. ---- then you multiply the two
16 activities.
17 Q. Okay. And so in this example when
18 you are using the term "activity" and you are
19 multiplying the values, what are you measuring?
20 What is the activity?
21 A. I am using the definition that is
22 given in the patent, which is the native protein
23 is 1 and the other ones are fractions, decimal
24 places of that in the tables in the common
25 disclosure.

1 NAISMITH - MARTIN
2 Q. Is there anything in the patent
3 that talks about multiplying activities to
4 determine whether or not two changes are
5 independent?
6 MR. KUSHAN: Objection form,
7 objection foundation.
8 A. The patent doesn't give any
9 teaching about combining multi-mutations at all.
10 I drew it from, it was responding to Dr. Petsko's
11 opinions.
12 Q. (BY MS. MARTIN): Do you consider
13 the values that are given in the tables in the
14 patent to be, like, absolute activity
15 measurements?
16 A. These are relative to the native
17 protein, that is how they are measured. So they
18 are relative to the native protein, that is why
19 they are expressed as 1.0 or 1.3 or whatever.
20 Q. Okay. Can you go to paragraph 191?
21 A. That's on page 136? I just want to
22 check, you said 191. I did not quite catch it,
23 sorry?
24 Q. Yes, 191?
25 A. I was pouring my drink, sorry.

1 NAISMITH - MARTIN
2 Q. 191.
3 A. On page 136?
4 Q. Yes, on page 136. You say in
5 paragraph 191 that: "Chao et al. ... defined the
6 HYAL-EGF fold as Ser353 to Trp 435 ..."; is that
7 right?
8 A. That is, yes, that is what I have
9 said.
10 Q. And then: "Using this definition,
11 the EGF fold in PH20 comprises Leu336 to Asp416."
12 Is that right?
13 A. Yes.
14 Q. But you used the term "fold" to
15 refer to the EGF domain in this paragraph; right?
16 A. Because it refers to the whole fold
17 of the protein. So the HYAL-EGF fold is the -- we
18 are talking about specific motif within the EGF.
19 But it is the HYAL-EGF fold of the protein which
20 has the EGF motif within it.
21 Q. I don't understand what you mean
22 when you say "the HYAL-EGF fold of the protein"?
23 A. What I am saying is that the fold
24 of the HYAL-EGF protein has the EGF motif in it
25 and that motif is defined between these residues.

1 NAISMITH - MARTIN
2 Q. But the HYAL1 protein -- you are
3 talking about the HYAL1 protein; right? You are
4 not talking about the HYAL-EGF protein?
5 A. No, I'm talking about the HYAL1
6 protein, yes.
7 Q. Okay. And the HYAL1 protein has
8 more to it than just the EGF; right?
9 A. It does. It has the alpha-beta
10 barrel motif.
11 Q. Okay. If the word "fold" refers to
12 the entire three-dimensional structure of the
13 protein, why did you use it in this paragraph to
14 refer to just the EGF motif?
15 A. So that is an imprecision in my
16 language, it should have said -- to be more
17 consistent I should have said as I defined earlier
18 the fold to be whole protein and I have used the
19 phrase "EGF motif" or domain.
20 Q. Okay.
21 A. But you are correct and
22 I apologise. There is an imprecision in the
23 language there.
24 Q. But when you are talking about the
25 EGF fold here you mean the EGF motif?

1 NAISMITH - MARTIN
2 A. Yes, and I should have said so, my
3 apologies.
4 Q. Can you turn to paragraph 59?
5 A. 69?
6 Q. 59.
7 A. On page 30, yes?
8 Q. Yes. So in paragraph 59 you say:
9 "While enzymatic activity confirms that an enzyme
10 has folded, there would need to be additional
11 evidence to justify a conclusion that the folded
12 enzyme is sufficiently stable to retain its folded
13 state." Is that right?
14 A. Yes, that is the start of paragraph
15 59.
16 Q. You then go on to say: "... a POSA
17 would not believe an enzyme had 'properly folded'
18 if it did not maintain activity over a meaningful
19 period or if it formed aggregates within a short
20 period"; right?
21 A. Yes, that what I said.
22 Q. Okay. So if you have an active
23 PH20 that gives you enzymatic activity let's say
24 in a microtiter plate assay, would you expect that
25 the PH20 has the PH20 native fold?

1 NAISMITH - MARTIN
2 A. So, for the methodology described
3 in the patent where you have these longer
4 incubation times, if you have active protein then
5 I would assume those are active folded natively
6 folded protein.
7 Q. Okay. So how can you tell if the
8 folded enzyme is sufficiently stable to retain its
9 folded state?
10 A. You would look at different time
11 points. A protein which -- in the assay they talk
12 about overnight for example. That could have been
13 one of the things you could do to see if the
14 protein was stable overnight or for a few hours.
15 Q. Okay. I think in the Example 3
16 activity assay they were using an incubation time
17 of 90 minutes?
18 A. Yes.
19 Q. Is that what you would consider?
20 A. Yes, but they had isolated the
21 protein too. So there is more to it than just,
22 you know, they purified it. Sorry, they took the
23 extract I should say, not "purified it". They
24 took the extract. So protein has a bit of time,
25 not just 90 minutes.

1 NAISMITH - MARTIN
2 Q. Oh okay, yes. Because they are
3 testing supernate, right, they are not testing
4 purified protein?
5 A. Yes.
6 Q. Okay. I am trying to get an
7 understanding of what "a meaningful period of
8 time" is, as you use that phrase in paragraph 59.
9 So, like, would 90 minutes in the assay in the
10 patent, is that a sufficiently meaningful period
11 for it to have been considered properly folded?
12 MR. KUSHAN: Objection foundation
13 and objection to form.
14 A. There is, it is obviously a
15 spectrum. To understand it you would take
16 different time points, and two reasonably close
17 together. You would see if there is was a slope
18 between them for example. For the protein to be
19 useful and I would anchor my expectations around
20 what the patent said which was for the inactive
21 mutants they ran they overnight ----
22 THE COURT REPORTER: Sorry, "My
23 expectations around what the patent said which was
24 for"?
25 THE WITNESS: They did overnight

1 NAISMITH - MARTIN
2 incubations for some of the testing they did. So,
3 that would seem a reasonable place to start around
4 sort of, 8-10 hours.
5 Q. (BY MS. MARTIN): Okay, so do you
6 think that the activity assay in Example 3 that
7 used the 1.5 hour incubation time, that that was
8 not sufficient for a POSA to determine whether or
9 not the enzyme had properly folded?
10 A. In the assay at the time, the
11 protein was properly folded. The assay does not
12 disclose if the protein then subsequently lost
13 activity, which is perfectly possible if it was
14 unstable.
15 Q. Do you have a sense of the
16 timeframe over which a PH20 needs to be
17 sufficiently stable to be clinically useful?
18 A. So the patent does talk about
19 pharmaceuticals. And one of the things Dr. Petsko
20 has pointed out is that aggregated forms, unfolded
21 forms of protein, are not good. So I would say
22 that you would, based on the envisaged use in
23 pharmaceuticals, you would not want any aggregates
24 or misfold the proteins over, sort of, a 12-hour
25 period till you could formulate your medicine. So

1 NAISMITH - MARTIN
2 that is a, kind of, minimum timeframe.
3 Q. So you would consider 12 hours to
4 be the minimum timeframe over which the protein
5 needs to be stable in order for it to properly
6 fold?
7 MR. KUSHAN: Objection foundation.
8 A. No. What I said is it can be
9 properly folded -- So you would have the fold at
10 the time of the assay if it's active but you could
11 already have a proportion of inactive protein
12 because it could be unfolding during the assay.
13 So just it is -- and the patent does talk about
14 uses of the protein. It talks about an assay over
15 12 hours. So I think it is not a hard and fast
16 rule like eleven 59 minutes is not any good, 12
17 minutes one is good. It is somewhat of a
18 spectrum. But the point I am trying to make is
19 that a protein which was active at 90 minutes and
20 was completely inactive at three hours would not
21 meet the test of a properly folded protein that
22 was stable, which is what a POSA would want.
23 Q. (BY MS. MARTIN): So you can't tell
24 from the results in Example 3 whether any of those
25 PH20 proteins are a properly folded protein

1 NAISMITH - MARTIN
2 because the test didn't involve a 12-hour
3 incubation?
4 MR. KUSHAN: Objection foundation.
5 A. I can have a reasonable expectation
6 since there were single site mutations. I can
7 have a reasonable expectation that most of the
8 ones that were active were properly folded but
9 I cannot exclude the possibility that some of them
10 subsequently fell apart, but not in the 90 minute
11 -- not meaningfully in the 90-minute assay period
12 plus the time for preparation. The significance
13 that I wanted to draw out was that the problem
14 with the classification of inactive; those
15 proteins could have been active when they were
16 first in the supernative but the time you get them
17 into assay they could have unfolded. So that is
18 what I am trying to draw out in my distinction
19 here.
20 Q. (BY MS. MARTIN): Did the
21 conditions at which you are incubating the protein
22 or keeping the protein impact whether or not it is
23 going to stay folded?
24 A. Yes, we -- you and I talked about
25 put thing it in 8 Molar urea for example would

1 NAISMITH - MARTIN
2 unfold the protein. The protein may be stable in
3 some buffer, but if you add it to 8 Molar urea it
4 is going to unfold, likely unfold and be unstable.
5 Q. So could you impact whether or not
6 the -- could you change the stability of a protein
7 over time by changing the buffer in which the
8 protein is?
9 MR. KUSHAN: Objection foundation.
10 A. So a protein can have different --
11 So a stable protein, as we have shown with 8 Molar
12 urea, is no longer able to adopt a stable fold.
13 It is possible, and the patent does not discuss
14 this, hypothetically to think of a mutation that
15 is stable in one buffer but starts to become
16 unstable in another. The patent does address a
17 particular form of instability, which is the
18 addition of compounds that are denaturing. And
19 one of the things they look for is whether the
20 activity is preserved or not for the sum of the
21 single site mutations in the presence of the
22 unfolding agent.
23 Q. (BY MS. MARTIN): So how do you go
24 about determining whether a PH20 is properly
25 folded?

1 NAISMITH - MARTIN
2 MR. KUSHAN: Objection foundation.
3 A. So native PH20 or single site
4 mutations or multisite mutation?
5 Q. (BY MS. MARTIN): Let's start with
6 native PH20.
7 A. So I would assume it is stably
8 folded and I would run an assay.
9 Q. Okay, like C-Giga 3 PH20?
10 A. Yes, I would assume it was stably
11 folded and active.
12 Q. Why?
13 A. Because there had been literature
14 where people had purified it all the way down,
15 purified it and assayed it or HYAL1, I should say,
16 had been purified and tested activity, PH20 also.
17 I think Dr. Simpson had looked at purified PH20.
18 Q. If I hand you a tube of a solution
19 and I tell you that it has SEQ.ID NO:3 PH20 in it
20 would you assume it is properly folded?
21 MR. KUSHAN: Objection foundation.
22 A. You have PH20 in a tube?
23 Q. (BY MS. MARTIN): In solution.
24 A. In solution. Did you purify it?
25 Q. I don't know, I am just handing it

1 NAISMITH - MARTIN
2 to you. Would you assume it is properly folded?
3 A. Does it have 8 Molar urea present?
4 Q. No, no urea.
5 A. Did it come from the supernatant in
6 the assay.
7 Q. Yes.
8 A. Then I would assume it would be
9 active and properly folded.
10 Q. Would you have to do any testing or
11 would you just assume?
12 THE COURT REPORTER: Sorry, did you
13 say "active" or "Then I would assume it would be"?
14 THE WITNESS: Active and properly
15 folded.
16 Q. (BY MS. MARTIN): Okay, why?
17 A. Because we knew from the literature
18 that SEQ.ID NO:3 was active and had been isolated
19 and so I would have a reasonable expectation the
20 sample made the same way and your supernatant
21 would be natively folded.
22 Q. Is it possible for SEQ.ID NO:3 PH20
23 to unfold?
24 A. We discussed if you put it in 8
25 Molar urea it could unfold.

1 NAISMITH - MARTIN
2 Q. What if it is just still in the
3 supernatant?
4 A. There is no discussion in the
5 patent about that at all. There is no discussion
6 in the common disclosure on whether people tested
7 anything about unfolding of PH20 except in the
8 example where they added this denaturing compound.
9 Q. But just by the virtue of the fact
10 it is a protein would you expect that it is
11 folding and unfolding itself?
12 A. Sorry, I understand the theme there
13 to your question. So proteins are sort of
14 metastable, and over time most, but not all,
15 proteins start to aggregate and unfold over time.
16 But that might be days or weeks.
17 Q. Okay. So if you were PH20 how can
18 you -- Would you consider PH20 to be properly
19 folded if it is in the process of unfolding and
20 aggregating or degrading or whatever?
21 MR. KUSHAN: Objection form,
22 objection foundation.
23 A. Sorry, so I now have a sample you
24 have given me and you are telling me the protein
25 is unfolding in this sample?

1 NAISMITH - MARTIN
2 Q. (BY MS. MARTIN): Well, I am saying
3 I don't know, like, how do you know whether -- So
4 to be clear, if it is the case that as a protein
5 it is eventually going to unfold, how do you know
6 that the PH20 in that vial has not started to
7 unfold?
8 A. I see. So I would ask you how you
9 prepared the vial and I would look at your
10 conditions, buffers, times, how you purified it
11 etc. or in this case it was supernatant, you
12 didn't purify it, you just took it. So I would
13 ask all those questions, what temperature you did
14 at, for example.
15 Q. Okay. Do you know when PH20 would
16 start to unfold?
17 A. In a timeframe, no, I've never,
18 haven't looked at that data. I think I can assume
19 with a reasonable certainty that it won't unfold
20 over a 12-hour timeframe.
21 Q. What if it's been, you know, 48
22 hours?
23 MR. KUSHAN: Objection foundation.
24 A. I haven't worked enough with PH20
25 to answer your question, and I don't recall the

1 NAISMITH - MARTIN
2 data to give you a clear and accurate answer to 48
3 hours but I would certainly agree that 24
4 overnight it would be fine.
5 Q. (BY MS. MARTIN): Okay.
6 A. It may be discussed in the
7 publications of Dr. Simpson, I don't recall.
8 Q. So the testimony that you gave in
9 terms of proteins being metastable and eventually
10 subject to unfolding and aggregation, was that
11 known in 2012?
12 A. So proteins -- "metastable" is an
13 imprecise definition, but proteins are marginally
14 stable. That was well known by 2011/2012.
15 Q. Okay.
16 A. As a result a POSA would expect if
17 you put most, but not all, proteins in solution
18 and left out on the bench for a couple of weeks
19 the protein would -- there would be aggregates and
20 misfolds and all sorts of stuff there.
21 Q. When you saying "aggregates" in
22 that context, is that something that you could
23 see?
24 A. You could test to find, yes, you
25 could do analytical methods to find if there was

1 NAISMITH - MARTIN
2 aggregates, yes.
3 Q. But I'm just saying, like, is it
4 going to be aggregated enough that it is going to
5 precipitate and you can just see it, like in the
6 tube?
7 A. It depends on the concentration you
8 have and whether you are using plastic tubes. You
9 can certainly imagine conditions where you would
10 see some turbidity.
11 Q. Okay. If you have a PH20
12 supernatant that has been left out on the bench
13 for three days, you don't know whether that PH20
14 is properly folded or not, do you?
15 A. We are back to the native PH20
16 SEQ.ID?
17 Q. Yes SEQ.ID NO:3?
18 A. 3D, as I've said, I would struggle
19 to have hard and fast data to know. I would have
20 to go back and look in the literature to give an
21 answer to that question. I don't -- it was active
22 at three hours. If I could do an assay at three
23 days I could tell you straightaway.
24 Q. Is that because if it's still
25 active then you would conclude that it is properly

1 folded?
2 A. Correct and if it has the same, so
3 whatever my number was, one, if at three days
4 measured one then I would know that the protein
5 has remained all of it fully folded and native.
6 Q. And what if it is, you know, only
7 half -- only like point 5?
8 A. So, now we are into the realms of
9 speculation. So in the supernatant you could have
10 proteases present, it could have degraded the
11 protein. So that could be a cause and there are
12 ways to test that. It could have unfolded that we
13 are talking about. It could have interacted with
14 something in the plastic and the buffers and
15 stuff. So you would want to do quite -- To
16 answer your question precisely there needs to be
17 other experiments done.
18 Q. Okay. So you don't know whether or
19 not it is properly folded?
20 MR. KUSHAN: Objection foundation.
21 A. Sorry, so what is properly folded?
22 Q. (BY MS. MARTIN): The PH20 in your
23 three-day old tube?
24 A. So if I have measured activity at
25

1 point 5?
2 Q. Mmm-hmm.
3 A. The narrow assumption is that half
4 of it is properly folded.
5 Q. Oh, okay, I see. Okay. Now when
6 you are testing supernatant the concentration of
7 PH20 in the supernatant is going to vary from
8 sample to sample; right?
9 A. Well, that is an assertion,
10 I think. I don't know that there is -- you might,
11 it is possible, yes.
12 Q. So you are expressing PH20 -- let's
13 say you are expressing PH20 in different cell
14 lines; okay?
15 A. Would I get different amounts of
16 protein is your question?
17 Q. Yes.
18 A. So if I was expressing at different
19 cell lines I could get different amounts of
20 protein.
21 Q. So you can't just assume that all
22 of the supernatants have the same concentration of
23 PH20, right, if they came from different cell
24 lines?
25

1 NAISMITH - MARTIN
2 A. If they came from different cell
3 lines I could not assume without some form of
4 normalization that they had the same concentration
5 in them.
6 Q. Okay. What if I gave you a PH20
7 SEQ.ID NO:3 supernatant from a Chao cell and then
8 a SEQ.ID NO:3 from a bacterial cell: would those
9 have the same fold?
10 MR. KUSHAN: Objection foundation,
11 objection to form.
12 A. So you have given me two separate
13 tubes. Have you measured activity on them or they
14 are just two tubes?
15 Q. (BY MS. MARTIN): They are just two
16 tubes.
17 A. How did you -- Did you set up a
18 secretion signal in both so that they should both
19 secrete into the supernatant?
20 Q. Yes, let's assume that we know that
21 there is actual PH20 in both of the tubes. But
22 one, yes, do you know if they are the same fold?
23 A. You would expect that to be true,
24 you would have a reasonable expectation that that
25 would be true and they would both be natively

1 folded and active.
2 Q. Okay. Does PH20 need to be
3 glycosylated in order to be active?
4 A. Does PH20 ----
5 Q. Need to be glycosylated in order to
6 be active?
7 A. There is some data in that where
8 they've knocked out some of the glycosylations.
9 So we could turn to it. This is single and double
10 triple mutant, so they made an inactive form by
11 I think a triple mutant but they retained activity
12 with at least one of the single site ones if I
13 remember. But it is in my report, we can go to
14 it.
15 Q. Okay. If it were the case that you
16 needed to have glycosylated -- PH20 needed to have
17 glycosylation to be active, would you expect that
18 the PH20 from a bacterial cell would be active?
19 A. So is your requirement that
20 bacterial cell doesn't, so no glycosylation at
21 all?
22 Q. Correct.
23 A. So your statement is that it is
24 known that if you have no glycosylation it is
25

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

1 NAISMITH - MARTIN
2 biology is that bacteria are great for making
3 proteins but they are limited in their ability to
4 fold complex human proteins.
5 Q. So before I started asking you
6 about glycosylation, you said that a POSA would
7 expect that PH20 from a bacterial cell would have
8 the same fold and activity as PH20 from a Chao
9 cell?
10 MR. KUSHAN: Objection foundation.
11 A. I don't remember saying that. I
12 thought you said Chao. I don't recall saying
13 that. We can go to the transcript if I made an
14 error. I would have assumed we were talking about
15 human or mammalian cell lines. My apologies, if I
16 have. I don't remember saying that I could make
17 it in a bacterial cell. I think you said cell
18 lines.
19 Q. (BY MS. MARTIN): Yes, I said what
20 if I gave you a PH20 SEQ.ID NO 3 supernatant from
21 a Chao cell and a SEQ.ID NO 3 from a bacterial
22 cell, would those have the same fold?
23 A. Okay, I misheard your question and
24 I apologise because I said you can't secrete PH20
25 ----

1 NAISMITH - MARTIN
2 Q. Okay.
3 A. ---- from a bacterial cell. My
4 apologies.
5 Q. So how do you get PH20 from a
6 bacterial cell?
7 A. Well, you would have to burst open
8 the bacterial cell and get the protein there, and
9 you would want to assay it first and foremost.
10 Q. So you don't know whether or not
11 it's properly folded if it came from a bacterial
12 cell?
13 A. I would do an assay to work out
14 whether it was properly folded or not.
15 Q. And how would you do that?
16 A. In the teaching of the patent
17 I would take the supernatant from my burst cells
18 and I would do an assay with it.
19 Q. Okay. And if it is active then you
20 would assume it is properly folded?
21 MR. KUSHAN: Objection foundation.
22 A. So, if it is active, demonstrates
23 activity, I would want to check my concentrations
24 and do controls. But if the protein, I'd done all
25 my controls, was active I would assume the active

1 NAISMITH - MARTIN
2 component of the protein was properly folded.
3 Q. You would assume the active
4 component of the protein was properly folded?
5 A. Yes.
6 Q. What is the active component of the
7 protein?
8 A. So you could have a situation in
9 bacterial cells where a portion of the protein was
10 folded and active and a portion of the protein
11 that was unfolded and inactive.
12 Q. So part of the same protein? Are
13 you talking about a singular structure and part of
14 it is active and part of it is not active?
15 A. I am not talking about a single
16 structure, I'm talking about a single sequence.
17 Q. Oh, okay.
18 A. A single sequence can be both
19 folded and can be unfolded or aggregated.
20 Q. Okay.
21 All right, we've been going for
22 over an hour. Do you want to take another break?
23 MR. KUSHAN: Sure.
24 MS. MARTIN: Do you want to break
25 for lunch? Can we go off the record?

1 NAISMITH - MARTIN
2 THE VIDEOGRAPHER: We are going off
3 the record. The time is 11.52 a.m.
4 **(A short recess from 11.52 a.m. to 1.37 p.m.)**
5 THE VIDEOGRAPHER: We are back on
6 the record. The time is 1.37 p.m.
7 Q. (BY MS. MARTIN): Dr. Naismith, did
8 you discuss your deposition testimony with counsel
9 during the break?
10 A. No, I did not.
11 Q. Okay. So can we turn to paragraph
12 43 in your declaration?
13 A. This is on page 19 I think?
14 Q. Yes.
15 A. Yes.
16 Q. And do you see the sentence that
17 starts: "Instead, I read the claims as a whole"?
18 A. Yes, I can see that sentence.
19 Q. So it says: "Instead, I read the
20 claims as a whole as being directed to modified
21 PH20 polypeptides that (i) contain an amino acid
22 sequence that satisfies the sequence identity
23 claim parameters, (ii) meet the position 320
24 substitution requirement, (iii) have folded to
25 adopt the same tertiary structure found in the

1 native state of the wild-type PH20 protein and
2 (iv) are enzymatically active."
3 A. Yes.
4 Q. Okay. So is it your interpretation
5 of claim 1, which appears to be on page 18?
6 A. Yes.
7 Q. That the genus modified PH20
8 polypeptides covered by claim 1 is limited to the
9 modified PH20 polypeptides that have the four
10 characteristics that I just read off from your
11 paragraph 43?
12 A. So I believe that is what the
13 claims read as a whole actually direct you to. So
14 that is what is covered. So there are tests that
15 you have to meet in the claims that fall within
16 the mathematical boundary of 95%. There is a
17 modification of 320 set forth in SEQ.ID NO:3 and
18 that modification has to be one of these four
19 residues.
20 Q. And then you have two other
21 requirements?
22 A. That is correct. They talk about
23 activity and they have a stable tertiary structure
24 that resembles the native fold.
25

1 NAISMITH - MARTIN
2 Q. Okay. And so it is your testimony
3 that claim 1 requires the PH20 to have folded to
4 adopt the same tertiary structure as the native
5 PH20 and also are enzymatically active; is that
6 right?
7 A. It is my testimony that when you
8 read the claims as a whole in light of the common
9 disclosure they are directed exactly to that, that
10 the additional two things are that it is the same
11 fold as the native protein and that it is
12 enzymatically active.
13 Q. Okay. So what limitation in the
14 claim tells you that the claim proteins have to
15 have folded to adopt the same tertiary structure
16 found in native state of the wild-type PH20
17 protein?
18 A. So the fold as Dr. Petsko talks is
19 that the human PH20 is a protein from a stable
20 tertiary structure under physiological conditions
21 it is. And he, like me, for the active subset of
22 the proteins if he covers all of them but I think
23 it only covers active subset, we would believe
24 those to have the same fold as the native protein,
25 and be enzymatically active.

1 NAISMITH - MARTIN
2 Q. Is it your testimony that Dr.
3 Petsko is interpreting the claims as limited to
4 active folded PH20s?
5 MR. KUSHAN: Objection foundation.
6 A. So Dr. Petsko and Dr. Simpson
7 appear to include all of the different diversity
8 that could be envisaged under the mathematical
9 boundaries but Dr. Petsko does say when I am
10 talking about PH20 I am referring to the active
11 PH20.
12 Q. (BY MS. MARTIN): So ----
13 A. It's as a folded protein. It says
14 on page 113, POSAs think of PH20 as a folded
15 proteins, as a folded protein.
16 Q. So when he said that, was he
17 talking about interpreting the claim as limited to
18 folded PH20s?
19 A. I would like to turn to his
20 deposition, sorry, his expert report, but what
21 I am showing is that Dr. Petsko certainly, in my
22 opinion, associated PH20 as a folded protein.
23 Q. So I would like a "yes" or "no":
24 did Dr. Petsko interpret the claims as limited to
25 folded PH20?

1 NAISMITH - MARTIN
2 MR. KUSHAN: Objection form and
3 objection foundation.
4 Q. (BY MS. MARTIN): Or I guess,
5 "Yes", "No" or "I don't know", sorry?
6 MR. KUSHAN: Objection form,
7 objection foundation.
8 A. Dr. Petsko in part said that he --
9 that it covered the mathematical fence but
10 elsewhere in his report he did focus on the folded
11 protein.
12 Q. (BY MS. MARTIN): Okay. Okay, so
13 it sounds like you are agreeing with me that Dr.
14 Petsko interpreted the claim as covering all of
15 the sequences but then he also said somewhere that
16 the PH20 refers to the folded protein. Am
17 I getting that right?
18 MR. KUSHAN: Objection foundation,
19 objection as to form.
20 A. So I think Dr. Petsko -- So my
21 opinion is that it applies to an active folded
22 from the protein. Dr. Petsko opined that
23 initially that his opinion was it covered
24 mathematical things but then later on in his
25 deposition he does say: "POSAs would understand

1 NAISMITH - MARTIN
2 that ... variants with ... a small number of
3 substitutions" -- which is the 95% -- "[would]
4 adopt the same general tertiary structure ...".
5 So he is defining here what PH20 variants are.
6 Q. (BY MS. MARTIN): Can you tell me
7 where we you are reading from?
8 A. I am reading on page 59 paragraph
9 93.
10 Q. Okay, so it is your testimony that
11 Dr. Petsko initially found that the claim covers
12 "mathematical" things not proteins, but then later
13 in his deposition he admitted that he would
14 understand that the claims are limited to just the
15 folded proteins?
16 MR. KUSHAN: Objection foundation,
17 objection to form.
18 A. So it is my testimony that Dr.
19 Petsko talks about PH20 in the same way that I do
20 later in his declaration. I would have to go
21 through the declaration to see what -- in order it
22 was set. But Dr. Petsko does say that: "POSAs
23 think of PH20 as a folded protein ...".
24 Q. (BY MS. MARTIN): So are you basing
25 your interpretation of the word "PH20" in the

1 NAISMITH - MARTIN
2 claim on Dr. Petsko, "Yes" or "No"?
3 MR. KUSHAN: Objection foundation,
4 objection to form.
5 A. I am using the common disclosure to
6 define PH20.
7 Q. (BY MS. MARTIN): Not Dr. Petsko?
8 A. I have used the common disclosure
9 of Dr. Petsko as I said used it in the same way
10 I did but I am using my definition based on the
11 common disclosure.
12 Q. Are you trying to give Dr. -- I am
13 sorry, strike that. Let me start over. Are you
14 trying to interpret the claims on Dr. Petsko's
15 behalf?
16 A. No, I am not.
17 Q. Do you know as a matter of fact,
18 sitting here right now, what Dr. Petsko thinks the
19 claims cover?
20 A. I can read what Dr. Petsko produced
21 as his opinion and I have also pulled out a
22 paragraph where he talks about the word PH20
23 meaning a folded protein, and that is the
24 information I have.
25 Q. Yes, okay and that paragraph is not

1 NAISMITH - MARTIN
2 talking about what the claims cover; right?
3 A. I would have to see the context in
4 the deposition, but it is, he naturally defaulted
5 to -- he did say POSA's PH20 variants with a small
6 number of substitutions, PH20 is a folded protein
7 so he talks about a modified protein, modified
8 polypeptide.
9 Q. That is your interpretation of what
10 Dr. Petsko is construing?
11 A. I believe I am stating what
12 Dr. Petsko did. My interpretation is based on the
13 common disclosure.
14 Q. So is it your testimony that an
15 inactive PH20 variant does not fall within the
16 scope of claim 1 of the 600 patent?
17 MR. KUSHAN: Objection to form.
18 A. Does it have the M320 substitution
19 test?
20 Q. (BY MS. MARTIN): Yes.
21 A. Then it is my interpretation and
22 opinion that that would be outside of the scope of
23 the claims as I read them.
24 Q. If you made a D320K SEQ.ID NO:3
25 protein and let's say you also mutated position,

1 NAISMITH - MARTIN
2 one of the catalytic residues such that you killed
3 activity, would that protein fall within the scope
4 of claim 1, even though it meets the sequence
5 identity parameters?
6 MR. KUSHAN: Objection foundation,
7 objection as to form.
8 A. So I didn't catch the last bit of
9 your, could you just read that question, back,
10 sorry, I didn't catch something you said about the
11 claims.
12 Q. (BY MS. MARTIN): So SEQ.ID NO:3
13 with the D320 mutation and let's say you also
14 change one of the catalytic residues so you kill
15 activity, okay, would that protein, even though it
16 meets the sequence identity limitations, would
17 that protein fall within the scope of claim 1?
18 A. It is my opinion it would be
19 outside the scope of claim 1 because it's not
20 active.
21 Q. Okay. And that is because you
22 interpret modified PH20 polypeptide to require
23 activity; right?
24 MR. KUSHAN: Objection foundation,
25 objection as to form.

1 NAISMITH - MARTIN
2 A. So it is because taking the
3 disclosure as a whole, my opinion is the
4 disclosure as a whole and the POSA is supposed to
5 read the interpretation as a whole and then
6 interpret the claims in a way that is consistent
7 with the common disclosure in this case, I believe
8 that is where the activity flows from the
9 requirement.
10 Q. (BY MS. MARTIN): You are not a
11 lawyer; right?
12 A. I am not a lawyer.
13 Q. Do you understand that in order to
14 limit the scope of a claim, you have to be able to
15 point to some language in the claim in order to
16 limit the scope of the claim?
17 MR. KUSHAN: Objection form,
18 objection to foundation.
19 A. So my understanding of the legal
20 test that was explained to me was that I had to
21 approach this with a POSA's lens 2011/12, read the
22 common disclosure, read the claims and interpret
23 every word in the claims in light, through the
24 lens of what is communicated in the common
25 disclosure. And I set out in detail in my report

1 NAISMITH - MARTIN
2 the reasons why I believe that, a POSA would read
3 the claims having read the common disclosure and
4 believe them to be directed to the folded protein
5 and active protein.
6 Q. (BY MS. MARTIN): And the -- I am
7 sorry, go ahead, I did not mean to cut you off.
8 A. And active protein.
9 Q. The words in the claims that you
10 are relying on to support your interpretation of
11 the patent fully folded and active, the words
12 specifically is PH20; right?
13 MR. KUSHAN: Objection foundation,
14 objection to form.
15 A. So I read it, so we have the
16 requirement from modified. We have, in one of the
17 things that we know is the one modification that
18 is required are active mutations, so those
19 substitutions at 320 are all active. The field of
20 the invention talks about importance of activity.
21 When we read through the common disclosure we are
22 bucketing them as active or inactive mutations.
23 Then we move on to, additional mutations in the
24 patent talks about importance of, and this is set
25 out in my point 72: "[a] modified PH20 polypeptide

1 NAISMITH - MARTIN
2 ..." sorry, I use page 72: "[a] modified PH20
3 polypeptide can have up to 150 amino acid ..." so
4 a modified PH2 polypeptide: "... replacements, so
5 long as the resulting modified PH20 polypeptide
6 exhibits hyaluronidase activity." Dr. Hecht
7 offers essentially the same opinion. We both read
8 in light of the common disclosure that is claims
9 are directed to active form of the protein that is
10 properly folded.
11 Q. (BY MS. MARTIN): You agree that
12 the common disclosure uses the phrase modified
13 PH20 polypeptide to refer to inactives as well;
14 right?
15 MR. KUSHAN: Objection foundation.
16 A. Could you point me to that precise
17 phrase please? I think I just easier for me to
18 look at the data.
19 Q. (BY MS. MARTIN): Are you familiar
20 with that disclosure?
21 A. The common disclosure.
22 Q. The part of the common disclosure
23 where it uses modified PH20 polypeptide to refer
24 to inactives?
25 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

1 NAISMITH - MARTIN
2 vaccines."
3 Q. Yes.
4 A. That sentence.
5 Q. Yes, that sentence includes the
6 word PH20; right?
7 A. So my understanding is that in
8 forming my opinion I was to consider the
9 disclosure as a whole, and for my opinion, so that
10 this phrase which is "inactive" does appear in the
11 report, I note that in paragraph 109. But as I
12 understand the legal test my opinion should be
13 based not just on an isolated collection of words
14 but on the reading of the disclosure as a whole to
15 understand what the claims are directed to.
16 Q. Yes, that didn't answer my
17 question. The word PH20 appears in the sentence
18 that we just read, right, at line 21 on page 99,
19 it says: "... modified PH20 polypeptides that are
20 inactive ..."; right?
21 A. So, what is, the question is "PH20"
22 word appears here?
23 Q. Yes.
24 A. Yes, "PH20" word appears here.
25 Q. The phrase, specifically the phrase

1 NAISMITH - MARTIN
2 "modified PH20 polypeptides" appears in that
3 sentence; right?
4 A. It does.
5 Q. And then it says: "... that are
6 inactive ..."; right?
7 A. It does say that.
8 Q. So this sentence is using the
9 phrase: "modified PH20 polypeptides" to refer to
10 polypeptides that are in fact inactive?
11 MR. KUSHAN: Objection form,
12 objection to foundation.
13 A. So, I read the sentence and I said
14 that my test was not to read isolated sentences,
15 but as I read the sentence it is specifically
16 defining a "modified PH polypeptide" that is
17 inactive.
18 Q. (BY MS. MARTIN): So within the set
19 of all modified PH20 polypeptides, some of them
20 are active and some of them are inactive; right?
21 MR. KUSHAN: Objection foundation.
22 A. What set are we defining, sorry?
23 Q. (BY MS. MARTIN): I am just, like,
24 the phrase "modified PH20 polypeptide", it can be
25 active or it can be inactive?

1 NAISMITH - MARTIN
2 MR. KUSHAN: Objection to
3 foundation.
4 A. Sorry, you said a set, I am
5 interested to know what set? As I read the claim
6 the set of proteins that are covered are active,
7 and I, so what set are we discussing?
8 Q. (BY MS. MARTIN): Okay. Not in the
9 context -- okay, sorry, let me back up, let me try
10 this a different way.
11 The patent defines the phrase
12 "modified PH20 polypeptide", right, that is a
13 defined phrase?
14 A. It appears, yes.
15 Q. Okay, and as that phrase is used in
16 the specification, it can include active
17 polypeptides and inactive polypeptides; right?
18 A. So, the phrase is used in this
19 sentence coupled to the word "inactive".
20 Q. Yes, so there are some modified
21 PH20 polypeptides that are inactive; right?
22 A. One can make PH20 polypeptides that
23 are inactive and I said I don't believe they are
24 covered in my understanding of the test for the
25 claims.

1 NAISMITH - MARTIN
2 Q. Okay. The inventors are using the
3 phrase "modified PH20 polypeptide" in this
4 sentence to refer to inactive polypeptides; right?
5 A. I would say the inventors are
6 specifically defining, appearing in the patent are
7 PH polypeptides that are inactive and those appear
8 in the data table where you have classified
9 various single mutants as inactive.
10 Q. Okay, but they are still calling
11 them PH20 polypeptides, right, they are not
12 calling them something like a different type of
13 protein?
14 A. They use the word "inactive" when
15 they describe it to signal that they are talking
16 about inactive PH20 polypeptides.
17 Q. And they also use the word "active"
18 when they are talking about active PH20
19 polypeptides; right?
20 A. Not every time and in the claims
21 you talk about modified PH20 polypeptide and I set
22 out my reasoning for the various reasons but one
23 of the things is the specific mutations that are
24 later in the patent found as active mutations, at
25 320.

1 NAISMITH - MARTIN
2 Q. So it is your testimony that, yes,
3 the inventors use the phrase "modified PH20
4 polypeptide" to refer to inactives, but because
5 they use the word inactive when they are talking
6 about inactives, therefore "modified PH20
7 polypeptide" does not include inactives?
8 MR. KUSHAN: Objection to
9 foundation, objection to form.
10 A. So I don't think that is what
11 I said. So, if we -- what I said was that the
12 claim language is the one we are talking about and
13 it is my view in the claim language for the
14 reasons we went through and these are on
15 page 70-71 of my report, when you read the common
16 disclosure you conclude that the claims are
17 directed to active proteins of a particular
18 modification, they are stably folded. You are
19 asking sentences that appear and you asked me
20 about modified PH polypeptides that are inactive.
21 I agree. That is what they have said modified
22 PH20 polypeptides that are inactive. I don't
23 believe that phrase in isolation, I did not use
24 that phrase to render my reading of the claims.
25 Q. (BY MS. MARTIN): Okay. So you

1 NAISMITH - MARTIN
2 were not interpreting the "phrase modified PH20
3 polypeptide" in the claims, you were not relying
4 on that phrase to conclude that the claim requires
5 active proteins; is that right?
6 A. No, that is not what you are
7 supposed to do. The legal test is based on a
8 reading of the common disclosure, and through the
9 eyes of a POSA, then that is how you read the
10 claims. Every word has meaning in the claims.
11 Q. Okay. So you are interpreting the
12 claim as a whole based on your reading of the
13 common disclosure, you are not looking for
14 individual words in the claim to figure out which
15 words require activity?
16 MR. KUSHAN: Objection foundation,
17 objection to form.
18 A. No, again that is not what I did.
19 So, I read the common disclosure as a whole and
20 I point out that there are more than just words
21 that we specifically have these active
22 modifications at 320. We talk about classifying
23 these single site mutations as active or inactive.
24 We also talk about a threshold for activity that
25 is 40% and important. Then, particularly when we

1 NAISMITH - MARTIN
2 talk about modified polypeptides, as I set out in
3 72, can have up to 150. Now that is not an
4 exhaustive interpretation of all the things that I
5 read in the common disclosure. But these are the
6 things that I helped rely on to reach my opinion.
7 Q. (BY MS. MARTIN): Can you point to
8 a specific word in the claim and tell me that that
9 word is what you are anchoring your requirement
10 for a properly folded and active PH20; yes or no?
11 MR. KUSHAN: Objection foundation,
12 objection to form.
13 A. As Dr. Petsko said a PH20, a POSA
14 thinks of it as an active form of the protein.
15 Q. (BY MS. MARTIN): So, yes, and the
16 answer to the question is, yes, PH20 is the word
17 in the claim?
18 MR. KUSHAN: Objection foundation.
19 A. So my understanding is I have to
20 read the claims as a whole, one of the ways it
21 shapes my reading of the claims as a whole is to
22 look at all the words in the claim, there are,
23 PH20 is thought of, as Dr. Petsko said, a folded
24 active protein. But I did emphasise that the site
25 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

1 NAISMITH - MARTIN
2 -- so I read the common disclosure and then read
3 the claims.
4 Q. That is respectfully, that is not
5 actually the legal standard. So ----
6 MR. KUSHAN: Why don't you ask
7 questions and not make statements, okay?
8 MS. MARTIN: Let's make sure that
9 we are being really clear here.
10 Counsel, I would ask you not coach
11 the witness during the testimony.
12 You understand that you interpret
13 the claims first based on the language of the
14 claims, right, not by first reading the disclosure
15 and then figuring out what you think the inventors
16 wanted to claim.
17 A. I did not say that, I think, my
18 understanding is that you read the claims and make
19 sure that you understand them in light of the
20 common disclosure, so they have to be consistent
21 with the common disclosure.
22 Q. All right. Let's look at -- strike
23 that. Do you read claim 1 as being directed to a
24 modified PH20 protein?
25 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

1 NAISMITH - MARTIN
2 say, in the claims as they were rendered.
3 Q. Are you reading the claim to
4 require a stably-folded PH20?
5 A. Yes, I do say that, that you would
6 expect it to be stably folded.
7 Q. So if you made a SEQ.ID NO:3 PH20
8 that had a D320K mutation and you left it on the
9 bench for three days, would that protein satisfy
10 the claims?
11 MR. KUSHAN: Objection foundation,
12 also objection to form. Go ahead.
13 A. Is the protein still active?
14 Q. (BY MS. MARTIN): I don't know.
15 A. So I did, when we talked about how
16 long stability was I gave you an answer which is
17 about, you know, roughly 12 hours. So if it is
18 still active at 12 hours I would say it was
19 properly stable and folded.
20 Q. If it is still active after three
21 days on the bench, then you would say it meets the
22 claims?
23 A. So it is modified, it has the
24 mutation?
25 Q. Yes.

1 NAISMITH - MARTIN
2 A. And it is still active?
3 Q. Yes.
4 A. At three days; yes, that is within
5 the scope of the claims.
6 Q. Okay. What if it is no longer
7 active at three days; does it meet the claims?
8 A. I said the other test I would use
9 is around eight hours to 12 hours, so you said
10 three days, so it could well meet the test.
11 I don't know because I didn't have activity at the
12 time of eight to 12 hours. I said that would be
13 kind of the time frame I would think of.
14 Q. If I -- I am asking a slightly
15 different question, which is if I hand you a
16 sample of PH20 that has been sitting, that as the
17 D320K mutation, SEQ.ID NO:3 that has been sitting
18 on the bench for three days and is no longer
19 active, would that meet the claims?
20 A. So it is no longer active at three
21 days?
22 Q. Yes.
23 A. Was it -- I asked you, was it
24 active over eight hours which is where I, the
25 eight to 12 hours that I would say that that is

1 NAISMITH - MARTIN
2 the kind of time frame that I would, that I use
3 for my stably folded. So to be clear, if it was
4 active in that time frame, eight to 12 hours, it
5 would be stably folded. It could well be stable
6 for 12 hours and then subsequently unfold over
7 three days, two and a half days later.
8 Q. Does it meet the claims when it is
9 no longer active after three days?
10 A. It could have met the claims if it
11 was active, meets my definition of stable which
12 I told you was around eight to 12 hours, so if it
13 was still stably active, if it was still active at
14 eight to 12 hours then it is within the scope of
15 the claims.
16 Q. Okay, I am trying to understand if
17 it is no longer active, right, as it sits at three
18 days?
19 A. I don't have the information to
20 give that opinion because you didn't give me the
21 data eight to 12 hours.
22 Q. Why does the eight to 12 hours data
23 matter? I am asking you if after three days,
24 literally the sample I am handing you, if that
25 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

1 NAISMITH - MARTIN
2 that is sufficient for it to infringe?
3 MR. KUSHAN: Objection foundation,
4 objection relevance.
5 A. What do you mean, I don't know the
6 legal test infringement you are asking me.
7 Q. (BY MS. MARTIN): Let me ask it
8 without the word infringe. If I give you a D320K
9 sample from SEQ.ID NO:3 that is no longer active
10 because it sat out too long, you are saying that
11 it would still meet the claims if it was active
12 within -- if it showed activity within eight
13 hours?
14 MR. KUSHAN: Objection foundation,
15 objection to form.
16 A. So, if you -- so this sample we
17 have -- it is, when we say active eight to 12
18 hours, it behaves, you can measure activity, that
19 is the kind of thing that I have said that I would
20 interpret and POSAs as a stably-folded protein.
21 But I did say that, you know, we can stretch that
22 window either side, it is a little fuzzy. So that
23 would be active and within the scope of the
24 claims.
25 Q. (BY MS. MARTIN): That is the case

1 NAISMITH - MARTIN
2 even if it is no longer active at some point in
3 time, because it eventually degrades?
4 A. Yes, you can, proteins will all,
5 most of them anyway, as we said, would eventually
6 degrade at some point, it would be, it is not a
7 test that I imposed that they have to be stable
8 forever, I used because the patent talks about
9 this overnight, that seemed a reasonable place to
10 think of the stability.
11 Q. Okay, if the patent data is
12 provided in examples 3 and 4 based on a 90-minute
13 incubation time, so based on that test, you don't
14 know whether the protein is stable after eight
15 hours; right?
16 MR. KUSHAN: Objection foundation.
17 A. So it actually says there are
18 varying times and the samples were sometimes
19 frozen and then thawed, so you would have an
20 expectation that for the single mutations and I'm
21 careful to restrict here to the single mutations,
22 that if they were active in the assay they would
23 be active at eight to 12 hours. That would be, it
24 is not a foolproof, but you would have a
25 reasonable confidence that all of those active

1 NAISMITH - MARTIN
2 mutants in the assay, single mutant would be
3 active at eight to 12 hours.
4 Q. (BY MS. MARTIN): So let's say the
5 D320K mutant that is tested in the patent, if it
6 was no longer active after eight hours would it
7 meet the claims?
8 A. So what is a mutant of the D320?
9 Q. Just D320K, that is the mutant?
10 A. I would have a reasonable
11 expectation that it would be active, I would not
12 have any reason to not think it would be active
13 based on the common disclosure, so I would believe
14 it to be active. It is a single mutation of a
15 stably-folded protein which has activity. As a
16 single mutation I would confidently expect it to
17 be active at eight hours, but you are correct the
18 patent does not test stability in that way.
19 Q. If I am telling you, let's say just
20 hypothetically if it turns out that it is not
21 stable after eight hours, would it meet the
22 claims?
23 MR. KUSHAN: Objection foundation.
24 A. So, that would be like I don't
25 think that is, I would just be very surprised

1 NAISMITH - MARTIN
2 if that was true base on my understanding. It is
3 a hypothetical that I don't recognise.
4 Q. (BY MS. MARTIN): Okay. If I made
5 D320K SEQ.ID NO:3 and put it in the 8 molar urea,
6 such that it is no longer a stable folded protein,
7 does it meet the claims?
8 A. Yes, it meets the claims because
9 you told me you've made the D320K that we know to
10 be active. You subsequently inactivated it by
11 choice, but it would meet the claim.
12 Q. So it doesn't -- but the version of
13 it, the version of it that I'm talking about is
14 not active, so how could it meet the claims?
15 A. Because -- so is your thing, if I
16 handed me, and I've now put the PH20, D320K inside
17 the 8 molar urea and that is my new sample.
18 Q. Yes.
19 A. I would say that the protein was
20 made in the active form and you have inactivated
21 it and as a result you have changed what the
22 protein is by deliberately inactivating it.
23 Q. But does that now deliberately
24 inactivated protein meet the claims?
25 A. A question I haven't considered,

1 NAISMITH - MARTIN
2 that you are deliberately trying to prepare
3 samples in 8 molar urea to make them inactive to
4 test claim language; I don't recognise that
5 example in the patent or find anything in the
6 common disclosure or around that. So I'm -- that
7 is just not a hypothetical I considered.
8 Q. So you don't know whether or not
9 the unfolded D320K SEQ.ID NO:3 PH20 meets the
10 claims?
11 A. So unfolded?
12 Q. Let me be as precise as possible
13 here. You don't know if the sample of D320K
14 SEQ.ID NO:3 PH20, that is unfolded because it is
15 an 8 molar urea meets the claims?
16 A. I said I hadn't considered that
17 specific example. I'd like to think about it
18 before I give you an opinion. I think if you were
19 -- I think given that you have deliberately
20 modified that you would have believed that the
21 original protein met the claims, so that's the
22 physical thing, so then it does meet the claims.
23 Now you've done something to it to not meet -- I
24 mean, you could set it on fire or you could do
25 anything to it, but the protein itself met the

1 NAISMITH - MARTIN
2 claims, the modified PH polypeptide that you
3 describe did meet the claim, so I do think it
4 meets the claims.
5 Q. I am trying to be really precise
6 here about the tense you are using because you are
7 saying it met the claims now it sounds like it may
8 or may not no longer -- may or may not meet the
9 claims now it is unfolded in urea?
10 A. I don't think that is what I am
11 trying to say. I appreciate I had not considered
12 this hypothetical, so apologies if I'm not being
13 clear. The protein, the modified PH polypeptide,
14 in all the disclosure is talked about in a
15 secreted form of the protein. That is how you
16 imagine the protein and how it is discussed. So,
17 in our example that meets the claims, it is active
18 as it is produced. You are then saying, "Well, if
19 I deliberately had some way to destroy the
20 protein", in your case "with 8 molar urea, would
21 that still meet the claims?" My answer is the
22 physical modified PH20 polypeptide would meet the
23 claims, in that scenario.
24 Q. Even though it is unfolded and
25 inactive it would still meet the claims?

1 NAISMITH - MARTIN
2 A. It is specifically because you have
3 done something to the protein which was active and
4 you have specifically unfolded it, but the
5 modified PH20 polypeptide is still itself active
6 first, so I do believe it meets the claims.
7 Q. Sorry, you said the PH20 itself is
8 active, then what was the word?
9 A. When it is made and pure, in the
10 example we are talking about is stable and
11 therefore it meets the claims. You are then
12 saying if I choose to inactivate it would it still
13 meet the claims? My answer is, a modified PH20
14 polypeptide that is stably active for eight hours
15 meets the claims. You can think of many, many
16 ways to destroy the protein, but I don't think it
17 changes that the protein meets the claims as long
18 as it's active stably over the time period I gave
19 you, as a guide.
20 Q. So it sounds like, if I am
21 understanding correctly, if it is possible to
22 produce that sequence in a way that it will fold
23 into a stable active protein, then the sequence
24 meets the claims and whether -- is that right?
25 MR. KUSHAN: Objection foundation.

1 NAISMITH - MARTIN
2 A. So my contention is that my opinion
3 is that it is not possible to identify that
4 sequence within those sequences within the vast
5 array that could be mathematically described to
6 identify the particular combination of mutations,
7 it would give you that. There are single site
8 mutations that are clearly active and my belief
9 and I said is, that I would expect them to be
10 folded stably and active, because we measured
11 activity.
12 Q. (BY MS. MARTIN): Okay, but the
13 question again was as long as it is possible to
14 make a sequence specifically SEQ.ID NO:3 with
15 D320K to make that sequence such that it is
16 properly folded and active then that sequence
17 meets the claims; right?
18 MR. KUSHAN: Objection foundation,
19 objection as to form.
20 A. So the claim does talk about PH20
21 but a properly folded PH20 with a D320K mutation
22 that matches your mathematical description of 95%,
23 that would be within the scope of the claims.
24 Q. (BY MS. MARTIN): If you introduce
25 a second mutation ----

1 NAISMITH - MARTIN
2 A. Sorry, I would just like a glass of
3 water before you ask your question. I do not want
4 to be cheeky and interrupt you in the middle.
5 MR. KUSHAN: Can we take, like, a
6 two-minute break because this thing has been
7 buzzing in my pocket and I want to check it.
8 THE VIDEOGRAPHER: We are going off
9 the record. The time is 2.23 p.m.
10 **(Short recess from 2.23 p.m. to 2.24 p.m.)**
11 THE VIDEOGRAPHER: We are back on
12 the record. The time is 2.24 p.m.
13 Q. (BY MS. MARTIN): If you introduce
14 a second mutation into your D320K SEQ.ID NO:3
15 protein that causes the activity to fall below
16 40%, let's say it's at 38%, does that protein meet
17 the claims?
18 A. So how did I measure? Sorry,
19 I just want to be clear. The patent classifies
20 that as inactive, if I measured my activity by an
21 accurate method at 38%, is that the question? The
22 patent just says below 40% I don't know anything
23 else but why it could have been inactive,
24 unfolded, many different things, right, so we
25 don't know that. So I just want to be clear what

1 NAISMITH - MARTIN
2 you are asking. Are you saying you have made a
3 second site mutation, you have added one to D320
4 and you have measured reliably 38% activity.
5 Q. Yes.
6 A. Then that would fall within the
7 scope of the claims.
8 Q. So if it is 38% active it would
9 fall within the scope of the claims?
10 A. Yes.
11 Q. So you are not requiring the 40%
12 activity?
13 A. No. The patent uses 40%, and the
14 reason I pointed that out was because it seems
15 very focused on very -- that is part of my
16 understanding of reading the common disclosure is
17 it directed towards the active forms but, no, 38%
18 activity any, that is active protein; it is not as
19 active but it is active.
20 Q. Okay, what if it is 21% active,
21 reliably measured to be 21% active?
22 MR. KUSHAN: Objection foundation
23 objection to form.
24 A. So we are back to my D3 fits the
25 mathematical test, it has a D320.

1 NAISMITH - MARTIN
2 Q. (BY MS. MARTIN): Yes.
3 A. So, with those caveats you've now
4 measured it and it is 21%.
5 Q. Yes.
6 A. And it has one additional mutation.
7 Q. Yes.
8 A. Yes, that is active.
9 Q. Okay. So D320K and SEQ.ID NO:3
10 with one additional mutation and it is 19% active;
11 does that meet the claims?
12 A. Robustly measured 19%?
13 Q. Yes.
14 A. Yes, that is active.
15 Q. So what would you consider to be
16 inactive?
17 A. So, I would use, I think
18 Dr. Simpson, Dr. Petsko said below 10, I think
19 most POSAs would feel around 5% is roughly
20 knocking down by a factor of 10-20 would be
21 beginning to be inactive. Although in
22 Dr. Simpson's paper she is able to detect activity
23 lower than that, I think it was 3%, she called it
24 residual activity. But it was more activity than
25 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

1 NAISMITH - MARTIN
2 relationship between fold and percent, sorry for
3 my ignorance and stupidity with the math here, but
4 are you saying if it is more than 10 fold reduced
5 is that the same as, are you using that as the
6 same as 10%?
7 A. It is less than 10%, exactly, 100
8 divided by 10 is 10 fold. I'm really sorry,
9 scientists work this way. I'm sorry.
10 Q. Yes, I know. It is not you, it's
11 me. Okay, so, all right, so 10% active or 5%
12 active, that is, because I think that fold, for me
13 fold, I'm having difficulty with fold, let's do
14 percentages.
15 A. Okay, my apologies. I will try to
16 stick with percentages which is the order of
17 magnitude. So less than 10%, some people might
18 say it is inactive. Most people would agree by
19 the time you get to 5 it's inactive.
20 Q. Okay, got it. So is there, if you
21 introduce a second mutation into your D320K SEQ.ID
22 NO:3 protein and it's now 7% active, do you think
23 that that would meet the claim?
24 MR. KUSHAN: Objection foundation.
25 A. So, the common disclosure doesn't

1 NAISMITH - MARTIN
2 measure anything below that. Based if I had the
3 assay that I believe was a reliable 7% personally
4 I would tend to draw the line at 5%, but I'm,
5 other POSAs may draw it below 10. So there a bit
6 of, as I said, there is kind of a window there,
7 but personally, in my opinion, I draw it at 5 and
8 in my publications you will find that is usually
9 where I draw the line.
10 Q. (BY MS. MARTIN): Okay. Now, so
11 you would consider a 7% activity to be an active
12 protein; right?
13 MR. KUSHAN: Objection foundation.
14 A. Are we talking about PH20 here?
15 Q. (BY MS. MARTIN): Yes.
16 A. With the mutations.
17 Q. Yes.
18 A. In my view if you could accurately
19 measure 7% then it would, it would be within the
20 scope of the claims because I would consider that
21 active.
22 Q. Okay. Would you consider that
23 protein to have a stable fold if it has 7%
24 activity?
25 MR. KUSHAN: Objection foundation,

1 NAISMITH - MARTIN
2 objection to form.
3 A. It is just a single site mutation
4 of the -- so it has two mutations only?
5 Q. (BY MS. MARTIN): Yes.
6 A. And I expressed it in the
7 supernatant and done the same sort of experiment.
8 Q. Yes.
9 A. Although I used a more accurate
10 assay.
11 Q. You use whatever assay you want so
12 that you are happy with your 7% number?
13 MR. KUSHAN: Objection foundation.
14 A. So we are very much into the realm
15 of the hypothetical, but in that arena I would say
16 that would be classified as an active form, but
17 I'm -- of the enzyme because of the, we made it in
18 the supernatant, we purified it, it is only one
19 single mutation beyond the D320K and it was
20 reliably measured at 7% it would fit, in my view,
21 the test I gave you was more than 5%.
22 Q. Okay, so I understand that with
23 activity, but I am trying to understand if that
24 protein is stably folded?
25 A. I would have a reasonable

1 NAISMITH - MARTIN
2 expectation that it would be stable because we
3 have only made one additional mutation, and I did
4 say that I think the cut off for that is around 5,
5 so I would have a reasonable expectation. I did
6 say that people begin to get squirrely around 3,
7 but I said the outer limit is 5 for that type of
8 analysis.
9 Q. Again, I just want to make sure we
10 are on the same page with the terminology. You
11 said, we are talking about same example of the two
12 mutations, that is 7% active and you would have --
13 you said you would have a reasonable expectation
14 that it would be a stably-folded protein and I'm
15 wondering, would it have the native fold?
16 MR. KUSHAN: Objection foundation,
17 objection form.
18 A. So we have got D320K mutation, for
19 example, and we have introduced a second mutation,
20 the protein expresses in the supernatant we can
21 measure activity at 7%. So in that case I believe
22 that the protein would be properly folded and have
23 the same properly fold that we see in the active
24 form of PH20 because we've only made two
25 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?

1 NAISMITH - MARTIN
2 MR. KUSHAN: Objection foundation,
3 objection to form.
4 A. So what I'm saying is that because
5 you have a good native structure SEQ.ID NO:3 which
6 is stable and active, then if you make one or two
7 mutations, then if that protein is still active in
8 the way we've assayed it, then I have a reasonable
9 expectation that it would be stable so I don't
10 need to impose an additional test.
11 The issue comes where I start to
12 try to combine mutations, say six or more, then my
13 view is that there is, it begins to populate the
14 space where there will be aggregated unfolded or
15 unstable proteins.
16 Q. (BY MS. MARTIN): Okay, so let's
17 talk about that. Let's just have a kind of clean
18 slate for the hypothetical so that we are not
19 confusing things. So let's say you have SEQ.ID
20 NO:3 D320K and then you have introduced six more
21 mutations into that sequence, okay. So your
22 protein has a total of seven changes compared to
23 the wild type; right?
24 A. Correct, and to work out how many
25 possible protein there are we would have to raise

1 NAISMITH - MARTIN
2 19 to the power of 6.
3 Q. Okay. Let us take one of them in
4 our minds; right?
5 A. Okay. Can you tell me anything
6 about that one?
7 Q. No.
8 A. Okay.
9 Q. I will tell you that it is active,
10 okay, that it has like ----
11 A. You have told me it is active and
12 it has D320K and it has six changes?
13 Q. Yes.
14 A. So you have made it, you have
15 tested it and it is active.
16 Q. Yes, okay. Can you tell from the
17 fact that it is active that it has been stably
18 folded or do you need to do more testing to figure
19 out if it's been stably folded?
20 MR. KUSHAN: Objection to
21 foundation, objection to form.
22 A. In the assay you are using here
23 I would have an expectation that because there is
24 quite a bit of time here that it would be stably
25 folded in my test, so if you made your protein the

1 same way, supernate it and you measure the
2 activity, production, then my view is that you
3 would have a reasonable expectation that the
4 protein is stable over that 12 hours. But
5 reasonable expectation, I can imagine now that you
6 are at six that you could have a situation where
7 the protein might be unstable over that time
8 period.
9 Q. (BY MS. MARTIN): So would you have
10 to do another test to make sure that the protein
11 is stable after 12 hours?
12 A. So I think I'm saying that where
13 you start to introduce multiple mutations beyond
14 five, then you might want to do some
15 experimentation to be sure, because you are moving
16 quite far away from your knowledge base.
17 Q. Okay, so what would you have to do
18 in order to figure out if you were, you know,
19 seven mutated PH20 is stably folded?
20 A. You could just measure activity at
21 two time points.
22 Q. Okay. So if you just do the assay
23 that is in the patent where they measure it at 90
24 minutes, would you know whether that protein with
25

1 NAISMITH - MARTIN
2 the seven changes meets the claim?
3 MR. KUSHAN: Objection foundation.
4 A. So there is an issue we touched on
5 is the patent classifies things as inactive and
6 does not report activities below 20%, so I -- you
7 could even with a single mutation have a protein
8 that is, I think we discussed 70% active,
9 I believe that meets the test of the claim, so
10 that my test. The patent does a classification
11 thing that I think is separate.
12 Q. (BY MS. MARTIN): Let me ask that
13 again. So, if you have your seven mutation PH20
14 that you test, after 90 minutes you test it and it
15 is active, let us say it is 70% active at 90
16 minutes; does it meet the claim?
17 MR. KUSHAN: Objection foundation,
18 objection as to form.
19 A. So seven mutations and it is
20 active, under those conditions; I think I would
21 have a reasonable expectation that it would meet
22 the claims, to be certain I would like to,
23 because I'm now on seven mutations I would like to
24 do a second assay just 12 hours later or six hours
25 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?

1 NAISMITH - MARTIN
2 Q. (BY MS. MARTIN): Yes.
3 A. At 70% activity?
4 Q. Yes.
5 A. After an hour? I would think, in
6 the way that it does, that we do this I would
7 think it would fall within this claim, the claims,
8 without -- that would be my opinion, that that
9 would fall within the scope of the claims.
10 Q. You do not have to do another test
11 for a native fold?
12 MR. KUSHAN: Objection foundation,
13 objection as to form.
14 A. So the point I had raised is that
15 as you start to -- you are backwards solving, you
16 already told me it is active in the assay which
17 gives me some information about timing and the way
18 the protein was made, so that helps form my
19 expectation that it would fall within the scope of
20 the claims if it was active.
21 Q. (BY MS. MARTIN): Why are you
22 saying that you would expect it to fall within the
23 scope of the claims as opposed to saying that it
24 would fall within the scope of the claims?
25 A. You asked me could I be absolutely

1 NAISMITH - MARTIN
2 certain that it didn't degrade or fall apart so
3 fast that it maybe it was at 70% at 90 minutes and
4 5% at four hours? I think that is highly
5 unlikely, but certain is a difficult word.
6 Q. Okay, so if it were the case that
7 it is at 5% at four hours, then would it be your
8 testimony that that does not meet the scope of the
9 claims?
10 A. 5% is an inactive protein, I think
11 we agreed that is a structure, below 5% is
12 inactive.
13 Q. Okay. So if you have a PH20 with
14 seven changes in it, that is 5% active at four
15 hour then it would not meet the claims?
16 A. It would be outside the scope of
17 the claims because it is an active PH20, it does
18 have the other attributes I guess we agree.
19 Q. Yes, okay. But if that same
20 protein had 70% activity at 90 minutes, then would
21 it meet the claims after 90 minutes or would it
22 not meet the claims because it is only 5% active
23 after four hours?
24 MR. KUSHAN: Objection foundation,
25 objection as to form.

1 NAISMITH - MARTIN
2 A. I explained because of the way we
3 are making the protein and handling it, that I
4 would confidently expect that it would meet the
5 claims.
6 Q. (BY MS. MARTIN): But what if it is
7 the case that it is down to 5% after four hours,
8 then would it meet the claims?
9 A. It is a very unusual protein that I
10 haven't considered but it is -- in a truly
11 hypothetical protein, now, well, that is an
12 unusual protein because the time it takes for the
13 assay to get all the way down to 4 after four or
14 so hours, I can't really imagine that. But if you
15 had two time point measurements, such a
16 hypothetical, I can't imagine what that would
17 mean, but at four hours if it had no activity
18 I would not consider it to fall within the scope
19 of claims.
20 Q. (BY MS. MARTIN): Earlier you
21 agreed that a modified PH20 polypeptide with D320K
22 mutation in SEQ.ID NO:3 meets the claims; correct?
23 A. Yes. With these specific mutations
24 that are spelt out in the claims?
25 Q. Yes, just with D320K?

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?

1 NAISMITH - MARTIN
2 A. I would not -- I've never done that
3 experiment, we both agree it would seem unlike --
4 assuming it was inactive, then that would not meet
5 the scope of the claims for producing it in that
6 way, yes, because you would have a physical thing
7 that is, presumably my prediction but I don't know
8 is an unfolded aggregated mess, inclusion bodies
9 that commonly -- but when you try to express human
10 proteins and bacteria, so those would be outside
11 my reading of the statement of claims.
12 Q. It is not the case that for a
13 particular sequence it will always meet the
14 claims, it depends on how that sequence is
15 produced?
16 MR. KUSHAN: Objection foundation,
17 objection as to form.
18 A. Again, that is -- I don't think
19 what I'm saying, I'm saying that the test is just
20 that it is folded and active. I don't impose a
21 way to make it. You are specifically saying where
22 it is made inactive unfolded aggregate, I don't
23 believe that unfolded aggregate would meet the
24 scope of the claims because it is not properly
25 folded.

1 NAISMITH - MARTIN
2 Q. (BY MS. MARTIN): Okay. So let's
3 say you have your D320K SEQ.ID NO:3 protein that
4 is made in a CHO cell, so it is properly folded
5 and active?
6 A. Secreted.
7 Q. Secreted, if you heat the sample to
8 the point that it unfolds, does it meet the
9 claims?
10 A. Yes. We discussed this, that you
11 can -- it meets the claims, you can subsequently
12 destroy the protein but when it was -- it still
13 meets the claims as it was made and produced.
14 Q. Okay. If you put it in a solution
15 of m-cresol such that it no longer has activity,
16 would it meet the claims?
17 MR. KUSHAN: Objection foundation.
18 A. So we are back to my just the D320K
19 mutation.
20 Q. (BY MS. MARTIN): Yes?
21 A. I made it. I know it is stable.
22 You now render it unstable by adding cresol.
23 I still would think that meets the test of the
24 claims.
25 Q. Okay. Is there any way to know

1 NAISMITH - MARTIN
2 whether a protein has the native fold of PH20
3 decides solving an experimental structure for the
4 protein?
5 A. You could test activity of the
6 protein and if the protein is active we would
7 assume it has the native fold of the protein.
8 There may be structural differences but if it was
9 active we assume it is properly folded.
10 Q. Okay, I just want to try to
11 understand why -- you are using, you are saying
12 that it has the native fold, but then you are
13 saying that there could be structural differences,
14 so I am trying to understand how something could
15 have the native fold and have structural
16 differences?
17 A. Okay. So we, it is good to be
18 clear, and I apologize if I wasn't. We talked
19 about bee venom hyaluronidase and alpha-beta
20 barrel motif within, and alpha-beta barrel motif
21 within at HYAL1, those at a high level have the
22 same fold but there are important structural
23 differences. So for an active protein I would say
24 they have the same fold but there could be
25 important structural differences if the protein

1 NAISMITH - MARTIN
2 was modified in other ways. The difference would
3 be what a POSA would recognise as a different
4 structure and in bee venom to human enzyme HYAL1,
5 they clearly pull out what the structural
6 differences are between them. But it is within,
7 I think the phrase is loosely defined as
8 alpha-beta barrel motif in the same fold, so we
9 would use it in that sense.
10 Q. Is it the case that active modified
11 PH20 proteins share a common structure in terms of
12 the native fold of PH20?
13 MR. KUSHAN: Objection foundation,
14 objection as to form.
15 A. So they would have the same fold,
16 there could be differences as I described and we
17 know exist between the bee venom and the human
18 hyaluronidase, both are active enzymes.
19 Q. (BY MS. MARTIN): Is having the
20 same fold a common feature?
21 A. Of what?
22 MR. KUSHAN: Objection foundation.
23 Objection to form.
24 Q. (BY MS. MARTIN): If two proteins
25 have the same fold is that a feature that they

1 NAISMITH - MARTIN
2 share in common?
3 MR. KUSHAN: Objection foundation,
4 objection as to form.
5 A. Are we talking about active
6 proteins?
7 Q. (BY MS. MARTIN): Yes.
8 A. So it is an imprecise definition to
9 say they are the same fold. It can be a factual
10 statement but it is not a very precise one to say
11 they have the same fold. It is not a precise
12 structural definition.
13 Q. I am trying to understand because
14 in your testimony you use the phrase "same fold"?
15 A. Yes.
16 Q. So I am trying to understand if
17 that is a common feature or not?
18 MR. KUSHAN: Objection form.
19 A. The same fold is not -- is not an
20 accurate description of a common structural
21 feature and the reason I say it is because bee
22 venom and human HYAL1 have the same fold but there
23 are important structural differences between them
24 and they are both active.
25 Q. Okay, so I am trying to understand

1 NAISMITH - MARTIN
2 if having a common fold is itself a common feature
3 or if there has to be some deeper level of
4 identity in order to have a common feature?
5 MR. KUSHAN: Objection foundation,
6 objection as to form.
7 A. So my understanding that I was
8 taught in my legal principles was that the test
9 I applied for a written description is a precise
10 one and therefore, as I said, for the bee venom
11 and the human HYAL1 they have the same fold,
12 broadly defined for the alpha-beta barrel motif,
13 only that part, that motive is the same sort of
14 fold but there are important structural
15 differences. So it is not a precise description
16 to say those have common structural features
17 because there are important structural differences
18 between them.
19 Q. (BY MS. MARTIN): I think that we
20 can agree that neither bee venom nor HYAL1 meet
21 the claims because they are more than 95%
22 different from PH20; right?
23 A. Yes.
24 Q. So whether or not bee venom
25 hyaluronidase and HYAL1 share common structural

1 NAISMITH - MARTIN
2 features has nothing to do with whether or not
3 PH20 and 95% sequence identical PH20 share common
4 structural features; right?
5 MR. KUSHAN: Objection foundation.
6 A. No, I think it is useful to think
7 of that because what it shows you is that you
8 can't -- that it, if you had simply said, "Well,
9 the common structural feature is HYAL1", we know
10 that, just exactly that HYAL1, it turns out that
11 the alpha-beta barrel motif can tolerate
12 structural changes and remain active, it is the
13 difference between the two.
14 Therefore, when looking at the
15 scale of mutations envisaged in the parameter
16 claim space it is perfectly possible to imagine
17 structural differences between members of the
18 family that are still active, they will all have
19 the same fold as bee venom motif and the
20 alpha-beta barrel motif and the bee venom,
21 alpha-beta barrel motif in human HYAL1, but in the
22 same way that we have been taught by the
23 literature, you could imagine structural
24 differences between proteins that fall into the
25 set of active, stably folded PH20s that comply

1 NAISMITH - MARTIN
2 with the 95% test and they have the 320.
3 Q. (BY MS. MARTIN): I just wanted to
4 make sure that I am understanding this correctly.
5 You are saying that within the set of 95%
6 identical Ph20s, the sequence space, there are
7 proteins, there are proteins that have the same
8 fold but it is possible that there could be
9 differences in secondary structure, such that
10 there would be structural differences between
11 those protein; is that what you are saying?
12 MR. KUSHAN: Objection foundation,
13 objection as to form.
14 A. So on page 47, I say "the native
15 folding" and so what I'm saying is that within the
16 native fold there may be important structural
17 differences that a POSA would highlight, they
18 could be significant arrangements of loops or
19 secondary structural elements. The reason a POSA
20 would be alert to that is because they were aware
21 that the alpha-beta barrel motif between bee venom
22 and human hyaluronidase, HYAL1, had important
23 structural differences. So they share some common
24 structural features between them and you can
25 overlap the structure to define them. They are

1 NAISMITH - MARTIN
2 also important structural differences.
3 Q. (BY MS. MARTIN): So in the same
4 way that HYAL1 and bee venom share?
5 A. Common fold.
6 Q. Some common structural features you
7 would agree that PH20 and 95% identical PH20 share
8 some common structural features; right?
9 MR. KUSHAN: Objection foundation,
10 objection as to form.
11 A. Are the Ph20s active?
12 Q. (BY MS. MARTIN): Yes.
13 A. So we are on to the subset that are
14 active. So they will be folded in, as I predict,
15 properly folded adopting a native fold, but
16 I don't know what the common structural features
17 that would be shared amongst them would be,
18 because I had seen with bee venom and human HYAL1
19 that a core bit of secondary structure was
20 different. So it is possible that different
21 elements of the secondary structure, either in the
22 EGF domain motif or in site alpha-beta barrel
23 motif may be different in some other way than we
24 have seen between bee venom and human
25 hyaluronidase. We only had two structures and

1 NAISMITH - MARTIN
2 remarkably the secondary structure was different,
3 so defining what the common secondary structural
4 features are would be beyond a POSA in 2011/12 and
5 there is no description in the patent that would
6 direct me to some other way of doing, identifying
7 those common structural features.
8 Q. So you just agreed you just
9 testified -- strike that. You just testified that
10 bee venom and HYAL1 have common structural
11 features; right?
12 MR. KUSHAN: Objection foundation.
13 A. I said they have, and I was careful
14 to define that as the, "you can overlap the
15 structures and see what beta strands and alpha
16 helices overlap", but there are big differences
17 between the two.
18 Q. (BY MS. MARTIN): I understand that
19 there are differences; I am just trying to
20 establish that there are common structural
21 features?
22 A. Between those two proteins.
23 Q. Yes.
24 A. There are.
25 Q. Okay. In same way that there are

1 NAISMITH - MARTIN
2 some common structural feature between HYAL1 and
3 bee venom, there are also some common structural
4 features between PH20 and a PH20 that is 95%
5 identical assuming that both of them are active?
6 MR. KUSHAN: Objection foundation.
7 A. So they would have the same fold,
8 but I don't know anything about which of the
9 features there might be different between them so
10 I haven't got a definition for what common
11 structural features. The reason I can give you
12 them for bee venom and for HYAL1 is because I can
13 superimpose them and call it out.
14 Q. (BY MS. MARTIN): What is your
15 understanding of what -- strike that. What is
16 your understanding as to what is required to
17 qualify as a common structural feature?
18 MR. KUSHAN: Objection foundation.
19 MS. MARTIN: So the requirement is
20 so that I can visualize or recognise members of
21 the genus, and it requires a precise definition.
22 Q. (BY MS. MARTIN): So what is a
23 precise definition?
24 A. If, sorry, we are in hypothetical
25 because, of course, I didn't formulate a precise

1 description, that wasn't the test I was asked to
2 do. Had you described, for example, the common
3 structural feature is beta strand that starts
4 here, finishes there, beta strand there, alpha
5 helix here, arranged in this, that would be quite
6 a reasonable definition of a common structural
7 feature.
8 Q. (BY MS. MARTIN): So, okay a --
9 strike that. A description of the identity and
10 location of secondary structural motifs, that
11 would qualify as a precise definition of a common
12 structural feature?
13 A. No, in this case because you also
14 have the active site and loop, so you would also
15 want some description of the loop structure that
16 was critical for activity.
17 Q. How do you describe a loop
18 structure?
19 A. You can show figures or point to
20 coordinates that have been deposited, for example,
21 point to a model that you have created.
22 Q. So in order to satisfy the common
23 structural features test you would have to have a
24 precise description of the secondary structural
25

1 NAISMITH - MARTIN
2 characteristics and active site loop
3 characteristics that are shared by the claim genus
4 of proteins?
5 A. So my opinion --
6 MR. KUSHAN: Objection form.
7 A. Should you -- that would be one of
8 the ways that you would meet the test. If you had
9 a precise structural description of the protein,
10 calling out what you think are the structural
11 elements and how they interact together, that
12 would be a precise description.
13 Q. (BY MS. MARTIN): Now the amino
14 acid -- strike that. You acknowledge that the P10
15 may not adopt your claim construction; right?
16 A. Correct. That is in my report,
17 they are the trier of fact, I am only a scientist
18 offering an opinion, not a legal authority.
19 Q. So if it is the case that the
20 claims do not require activity but are in fact
21 defined by sequence, would you agree that a POSA
22 would be able to determine whether a particular
23 protein falls within the scope of the claims by
24 examining the sequence?
25 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

1 NAISMITH - MARTIN
2 ----
3 Q. Okay. So my question was if the,
4 as you say in paragraph 110, if the Patent Trial
5 and Appeal Board construes the claims such a that
6 they don't require activity, can you tell whether
7 a particular protein falls within the scope of the
8 claims or not just based on the sequence?
9 MR. KUSHAN: Objection to form,
10 objection to foundation.
11 A. So it rules out activity, I would
12 have to -- so there would be no written
13 description in that case, so if I did not have
14 activity then the protein could be anything, it
15 could be partially made, partially unfolded and
16 they are very different things. So the Patent
17 Board would -- so my view is that, based on what
18 I understand the legal principles are, you have to
19 describe in a precise way. So we have talked
20 about a description of an active protein, if you
21 have to describe aggregated protein you would need
22 to have some description of that if you were
23 considering within the written description of the
24 claims.
25 Q. (BY MS. MARTIN): So again that

1 NAISMITH - MARTIN
2 still isn't my question. The question is, can you
3 tell whether a particular protein meets the claims
4 based on the sequence if the claims don't require
5 activity?
6 MR. KUSHAN: Objection foundation,
7 objection relevance.
8 A. So I think that is different than
9 the question I asked myself. I asked as a legal
10 test if the Patent Board decided the claims were
11 not restricted to active proteins, but included
12 all these other forms, would that alter my
13 opinion? And the answer was, no, it would not
14 because the active form is not -- that you are
15 trying to aim for is not adequately described and
16 these other forms are not adequately described.
17 I'm sorry, I'm struggling with your hypothetical
18 of what the Patent Board are deciding, that is
19 what I am struggling with. I considered the
20 question, if they read the claims in a different
21 way.
22 Q. (BY MS. MARTIN): Have you
23 considered whether or not you could tell if a
24 particular protein meets the claims based on
25 sequence, if the claims don't require activity?

1 NAISMITH - MARTIN
2 MR. KUSHAN: Objection foundation,
3 objection as to form.
4 A. So, if the Patent Board say, "We
5 don't care about activity", then the claims would
6 then, and "don't care about fold or activity",
7 then a protein with a sequence would fall within
8 the scope of the claims, but it would not be
9 described because, as I said, you can imagine
10 there are, well, we know, there are will be
11 multiple different structural representations in
12 that population.
13 MR. KUSHAN: We have been going
14 about 90 minutes, I think it is a good time for a
15 break.
16 MS. MARTIN: We have been going for
17 ----
18 MR. KUSHAN: 89.5 minutes.
19 MS. MARTIN: No, we stopped at
20 2.23.
21 MR. KUSHAN: That was for a
22 90-second break. We have been going for about 90
23 minutes.
24 MS. MARTIN: Yeah, that's fine. We
25 can take a break.

1 NAISMITH - MARTIN
2 THE VIDEOGRAPHER: We are going off
3 the record. The time is 3.07 p.m.
4 **(Short recess from 3.07 p.m. to 3.24 p.m.)**
5 THE VIDEOGRAPHER: We are back on
6 the record. The time is 3.24 p.m.
7 Q. (BY MS. MARTIN): Dr. Naismith did
8 you discuss the contents of your testimony with
9 counsel during the break?
10 A. No, I did not.
11 Q. Okay. Earlier we were talking
12 about testing potential mutants for activity to
13 figure out whether they meet the claims?
14 A. We talked about how I read the
15 claims; I don't remember saying I had to test
16 mutants. If you made these very vast number of
17 mutants, you couldn't reliably predict their
18 activity so you would want to do tests. This is
19 just a very general statement, so I just want to
20 be clear what it is you are asking.
21 Q. Oh okay. I gave you a hypothetical
22 where I said you had D320K SEQ.ID NO:3 PH20 and
23 you made another mutation in it and it had
24 activity, and you determined that by testing;
25 right?

1 NAISMITH - MARTIN
2 MR. KUSHAN: Objection foundation.
3 A. I said that you could expect,
4 unless you had mutated, introduced that second
5 single site mutation at one of the inactivating
6 positions which were known, then you could
7 confidently expect that that second mutation would
8 be active.
9 Q. (BY MS. MARTIN): Okay, you could
10 predict that the -- it is your testimony -- strike
11 that, I will start over again. Is it your
12 testimony that you could reasonably predict
13 whether a dual mutant PH20 would be active without
14 having to test it?
15 MR. KUSHAN: Objection foundation,
16 objection to form.
17 A. So if dual PH20 data, you have that
18 and you introduce a second mutation and that
19 second mutation was one of the ones that was
20 tested as active from the patent and was active,
21 then you would have an expectation that the
22 combined would be active.
23 Q. (BY MS. MARTIN): Okay. So then if
24 you make a third mutation at a site that the
25 patent teaches retains activity, would you expect

1 NAISMITH - MARTIN
2 that triple mutant to be active?
3 MR. KUSHAN: Objection foundation,
4 objection as to form.
5 A. So you begin to get into the realm
6 of epistasis and also active, we talked about the
7 multiplicatively deleterious, so if you, for
8 example, 40, 40, 40% multiply that, for example,
9 then you get below, I would have to do my maths
10 but you can conceivably have ones which are
11 additive, but if you combine the multiplicatively
12 deleterious you get the lower level of activity
13 because you keep knocking away as you add
14 deleterious mutations. They are each individually
15 active, it is just a consequence of
16 multiplication.
17 Q. (BY MS. MARTIN): Okay. So if you
18 introduce two mutations that give you 40% activity
19 and one mutation that gives you 100% activity,
20 would you expect to have -- would you expect to
21 have a protein with reduced activity?
22 MR. KUSHAN: Objection foundation,
23 objection as to form.
24 A. I would expect it to have activity
25 if they were depending whether there was epistatic

1 NAISMITH - MARTIN
2 effects you begin to, as I said, worry about that,
3 but if you have no epistatic effect point 4 times
4 point 4 is point 16 and therefore by my 5%
5 threshold you would expect that to be active.
6 Q. (BY MS. MARTIN): So then, if you
7 are introducing five mutations at sites that the
8 patent teaches are active, would you expect that
9 protein to be active?
10 MR. KUSHAN: Objection foundation,
11 objection as to form.
12 A. So that is where I break down in my
13 ability to be confident of that prediction because
14 by the time I get to 5 I think, based on the
15 literature and what POSAs understood, that is at
16 the limit to where you really begin to expect to
17 have problems with predictability.
18 Q. (BY MS. MARTIN): Then you would
19 have to test for protein?
20 A. So as I said you would have a weak
21 prediction by the time you put 5 in. You, for the
22 protein you would need to know, for the protein to
23 be within the scope of the claims as I read them
24 it has to be folded active, by the time you put 5
25 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?

1 NAISMITH - MARTIN
2 A. I don't ----
3 MR. KUSHAN: Objection foundation.
4 Go ahead.
5 A. At that level I would have no
6 confidence in my prediction for active or
7 inactive, or folded or unfolded.
8 Q. (BY MS. MARTIN): So then you would
9 have to test it; right?
10 A. At six mutations to know if it was
11 within the scope of active folded proteins, based
12 on the common disclosure I would have no way to
13 know or reliably predict. So to be sure, to know
14 I would have to test.
15 Q. Okay, and could you use the test
16 that is described in the patent to determine
17 whether or not it is active?
18 MR. KUSHAN: Objection foundation,
19 objection as to form as well.
20 A. So the test in the patent is we
21 have already talked about the fact that some
22 mutations can have less than 20% activity that I
23 would classify as inactive, so the patents test,
24 as it is written in the common disclosure, could
25 classify a protein which is active as inactive.

1 NAISMITH - MARTIN
2 Q. (BY MS. MARTIN): Okay, if you
3 yourself were to follow the instructions in the
4 patent, could you perform that test and determine
5 whether or not your 6 mutant PH20 was active?
6 MR. KUSHAN: Objection foundation,
7 objection as to form.
8 A. So there are problems with the
9 assay that mean you would be uncertain. If it was
10 above 40% you would be confident that the protein
11 is active. The problem is that the patent doesn't
12 give any guidance to what is resulting in a
13 measure of activity in a sample of less than 20%
14 and I previously said that a protein which is 15%
15 active reliably measured would fall within the
16 scope of the claims.
17 Q. (BY MS. MARTIN): Okay, so that is
18 what I am trying to get at, is the reliably
19 measured part of what you just said. So, is it
20 your testimony that the assay in the patents is
21 not capable of reliably measuring whether or not a
22 PH20 is active?
23 A. "Reliably" identifies things that
24 are active above 40%, but it provides no insight
25 as to when you get an activity less than 20%, and

1 NAISMITH - MARTIN
2 as a result one can imagine proteins, modified
3 polypeptide proteins which have 18% activity which
4 are classified by the patents method, as inactive.
5 Q. So I'm trying to understand if
6 there is actually a problem with the assay or if
7 what you are talking about is the fact that the
8 patent doesn't tell you why you got you know value
9 below 20%. So that is why I was saying you
10 personally could run the assay, right, and you
11 would get a number and so would that number tell
12 you if the protein is active or not?
13 MR. KUSHAN: Objection foundation,
14 objection as to form.
15 A. So there are some specific flaws
16 with the assay that would meet. Those flaws, you
17 can deal with from measurements above 40%, but
18 there are, those flaws prevent you knowing whether
19 a protein is inactive that we have described at 5%
20 or active at 18% or misfolded. We don't know the
21 reasons for that, and the assay itself appears to
22 have quite large variations in it, so that you
23 can't reliably, at least there is no standard
24 curves or information that would allow you to
25 evaluate the robustness of the assay and the

1 NAISMITH - MARTIN
2 patent itself does not disclose any measurements
3 below 20.
4 Q. (BY MS. MARTIN): Do you believe
5 that if you yourself were to go and perform that
6 assay you could tell whether or not a protein was
7 active by running that assay?
8 MR. KUSHAN: Objection foundation.
9 A. By running that assay, I would tell
10 whether the protein had more than 40% activity
11 reliably.
12 Q. (BY MS. MARTIN): But you believe
13 that that particular assay will not tell you
14 whether or not a protein is inactive?
15 MR. KUSHAN: Objection foundation.
16 A. So you can't -- the problem is
17 the assay can't distinguish or doesn't
18 distinguish, nothing in the teaching on its own
19 distinguishing why you don't reach a threshold of
20 above 20%, there is nothing there that can help
21 you guide that in the patent.
22 Q. (BY MS. MARTIN): I don't
23 understand why that matters why is that important
24 in terms of figuring out whether a protein is
25 active or not?

1 NAISMITH - MARTIN
2 A. Because we have talked about that
3 for active protein we believe it is above 5%,
4 I have no information in the patent to know if as
5 performed in the assay, what accuracy the assay
6 has because I lack control experiments, and
7 I don't know what the noise level in the assay is
8 and I don't have standard curves.
9 Q. So if you were to personally
10 perform the assay, you would have that
11 information, right, you would have a standard
12 curve and control and noise level. I am asking
13 you is the assay itself capable of telling you
14 whether a protein is active or not?
15 MR. KUSHAN: Objection foundation.
16 A. Sorry, I tried to say that I have
17 considered the assay as it is described and
18 reported in the common disclosure, I believe if
19 the assay reported above 40% activity then that
20 would be an active protein.
21 Q. (BY MS. MARTIN): Okay, so it's
22 your testimony that the assay in the patent cannot
23 identify inactive proteins?
24 MR. KUSHAN: Objection foundation.
25 A. What I say is in the classification

1 NAISMITH - MARTIN
2 as inactive mutations, there are within them the
3 potential probability of proteins which have 18%
4 activity, proteins which are unfolded, proteins
5 which are not secreted, proteins which are
6 misfolded, proteins that are aggregated.
7 Q. (BY MS. MARTIN): And the patent
8 doesn't distinguish between those, right, just all
9 of them would fall within the bucket of inactives?
10 A. It is definition of
11 "classification", it puts them in that bucket as
12 inactive but it doesn't differentiate between
13 them.
14 Q. So using the assay in the patent
15 you could identify what the patent classifies as
16 inactive proteins?
17 MR. KUSHAN: Objection foundation.
18 A. So using the assays as described in
19 the patent following it, I would be able to
20 classify proteins in the same way the patent has
21 done as inactive or active, but you specifically
22 asked me about enzyme activity and therefore we
23 discussed it, there is a limit of about 5%.
24 Q. (BY MS. MARTIN): So you are
25 reading the claims to require an activity

1 NAISMITH - MARTIN
2 threshold of 5% enzyme activity?
3 MR. KUSHAN: Objection foundation,
4 objection as to form.
5 A. I am reading the claims to require
6 enzyme active and I said a POSA we, we might, we
7 would all, I think, draw a line at 5, some of us
8 might draw it below 10, but that is a definition
9 of an active protein.
10 Q. (BY MS. MARTIN): Okay. Now before
11 the break I was asking if the Patent Trial and
12 Appeal Board interpreted the claims to not require
13 activity. Could you tell whether a particular
14 protein meets the claims just based on the
15 sequence?
16 MR. KUSHAN: Objection foundation,
17 objection relevance.
18 A. So, I went, in the break, to
19 re-read my paragraph so that I am clear. So if
20 the claims define, the Judges define the claims
21 that it includes additional forms of the PH20
22 polypeptides, unfolded, misfolded, partially
23 folded, higher aggregates inactive forms, then
24 they would be within the scope of the claims, but
25 they would not be adequately described because an

1 NAISMITH - MARTIN
2 inactive protein shares no structural feature in
3 common with a folded protein. The only
4 commonality there is the primary sequence, not the
5 structure.
6 Q. I understand that that is your
7 testimony. What I am trying to get at is if you
8 could tell based on just the sequence alone,
9 whether a protein would meet the claims, if there
10 is no activity requirement, and it sounds like,
11 from what you just said, that the answer is, yes?
12 A. It is within the scope of the
13 claims because it includes those additional forms,
14 but those additional forms are not adequately
15 described.
16 Q. Okay. So you agreed that you could
17 differentiate proteins that meet the claims from
18 those that do not meet the claims based on the
19 amino acid sequence if the claim does not require
20 activity?
21 MR. KUSHAN: Objection foundation,
22 objection as to form.
23 A. So my testimony is if, if the
24 Patent Board conclude that all the universe of
25 possible claims are within the scope and that

1 would include these additional unformed, then
2 these would be covered by the claims.
3 Q. (BY MS. MARTIN): Okay, and so you
4 could use the amino acid sequence to distinguish
5 proteins that meet the claims from those that do
6 not meet the claims; right?
7 A. When you say meet within the scope,
8 but they would not be -- so they would be within
9 the scope of the claims, but you don't have a
10 written description to understand what structural
11 features are being described because there are
12 very different structural features between
13 unfolded proteins, folded proteins, aggregates
14 etc.
15 Q. It is your testimony that you would
16 need to have a description of structural features
17 of unfolded proteins in order to meet the written
18 description requirement?
19 A. My understanding of a written
20 description, and we are now into hypotheticals, is
21 that if you want to discuss unfolded proteins you
22 would have to describe what that meant, or
23 aggregates, you would have to give some
24 description of that.
25

1 NAISMITH - MARTIN
2 Q. Of what the structure of the
3 unfolded protein is?
4 A. The three dimensional structure not
5 necessarily, but you could define it in other
6 ways.
7 Q. Like how?
8 A. It forms a lump at the bottom, you
9 know, it is an aggregated mass. You could look at
10 other ways to try and describe aggregates but it
11 is difficult because they do have a very wide
12 range. You could, say, describe the aggregate
13 itself as a physical thing.
14 Q. So when the patent talks about
15 inactive PH20 polypeptide, your view is that that
16 is not a sufficient description of an inactive
17 PH20 polypeptide?
18 A. When the patent talks about
19 inactive polypeptides it is imposing a threshold
20 of 20% activity. I have said that there is a
21 whole range of reasons why you can have less than
22 20% activity. We talked about the example where
23 we had 18% activity, that would be classified as
24 inactive by the patent's classification, but
25 I would consider that protein to be active if

1 NAISMITH - MARTIN
2 reliably measured and, therefore, to fall within
3 the scope of the claims as I read them.
4 Q. Okay. In your view is it
5 required -- strike that. Is it your opinion that
6 in order to satisfy the written description
7 requirement you must be able to know the precise
8 number of embodiments that fall within the scope
9 of the claims?
10 A. That is not my opinion.
11 Q. Okay. So when you say that --
12 strike that?
13 A. Can I turn to that page, I am
14 flicking pages when I am trying to listen, my
15 apologies.
16 Q. So in order to satisfy the written
17 description requirement, is it your view that you
18 have to be able to approximate the number of
19 sequences that fold and are enzymatically active?
20 A. No, that is not what -- my opinion,
21 as I said, as legal principle you need a
22 representative number of species that fall within
23 the scope of the claims. Or, as we discussed
24 earlier, there are structural features common to
25 the members of the genus.

1 NAISMITH - MARTIN
2 Q. Okay. So, the fact that you don't
3 have a number, a precise number of those that fold
4 and are enzymatically active, that is not part of
5 the written description test?
6 MR. KUSHAN: Objection foundation,
7 objection to form.
8 A. So, the written description test
9 I use, is you have to have a representative number
10 of species. If we are talking about an active
11 protein parameter sequence space, we have a huge
12 number, ten to the power of 60, so you would want
13 to have representative number of species that
14 probe some of those structural divers -- sequence
15 I should say, diversity for active proteins
16 because you can imagine quite a lot of changes by
17 the time you get to 15. There is a vast array of
18 them, you would want some representation of that.
19 Q. (BY MS. MARTIN): Do you have to be
20 able to physically make all of the sequences that
21 fall within the sequence space in order to satisfy
22 the enablement requirement in the aggregate, to be
23 clear?
24 A. So you don't have to make every
25 single one. You must enable to, a person skilled

1 NAISMITH - MARTIN
2 in the art to make them use an entire class, so
3 you have to be directed how to make and use them,
4 you don't have to make every single one of ten to
5 the 60.
6 Q. Okay. So we agree that whether --
7 we agree that the fact that it is not physically
8 possible to make 10 to the 60th items is not, you
9 know, dispositive for 1/12 purposes here?
10 MR. KUSHAN: Objection foundation,
11 objection relevance, objection to form.
12 A. So what is dispositive is that in
13 that diversity you have wildly different types of
14 protein and there is no enablement to enable you
15 to choose the folded active protein. There is no
16 written description as to what those common
17 structural features would have to be. That is a
18 written description test so on the enablement
19 there is no direction to how to enable when you
20 get to 15 or more than 6, what, how you would get
21 there.
22 Q. (BY MS. MARTIN): So if I gave you
23 an amino acid sequence that had 16 changes in it
24 from wild-type PH20, is it your testimony that you
25 would not know how to make that protein?

1 NAISMITH - MARTIN
2 MR. KUSHAN: Objection foundation,
3 objection as to form.
4 A. So, I could make the protein but
5 I would have no way to know whether it was an
6 active form or whether it was inactive, I think I
7 could make it. So sometimes you can't make, you
8 get stalling on the ribosome, so if you gave me a
9 nucleotide sequence, I could make the nucleotide
10 sequence and do the experiment if that is your
11 question. But by the time you get to that large
12 number of mutations there are many reasons that
13 could lead to the protein appearing in a
14 different, entirely different region of structure,
15 unfolded, aggregated, whatever.
16 Q. (BY MS. MARTIN): Okay.
17 I understand that there is a question about what
18 it is going to look like at the end, but the
19 question is if I gave you the sequence, would you
20 know what to do with it, you know, if you wanted
21 to make it into a protein or would you have no
22 idea where to turn in terms of how to make that,
23 how to change that string of letters into a
24 physical item?
25 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.

1 NAISMITH - MARTIN
2 Q. It is predicting whether or not
3 your string of text is going to be turn into a
4 protein that has the native fold of PH20?
5 MR. KUSHAN: Objection form;
6 objection foundation.
7 A. So the issue for me is, as
8 I understand enablement I have to be able to probe
9 the full scope of the claims, right? So I have to
10 be able to make things out that touch, that enable
11 the full scope as defined by the claims and it is
12 my position that there is no teaching in the
13 patent that would enable me to practice the full
14 scope of the claims because, and, yes, the full
15 scope of the parameter claims base, that is my
16 opinion.
17 Q. Yes, I'm trying to understand why.
18 I mean, you are just giving me the legal test for
19 what is lack of enablement and I'm trying to
20 understand what the issue is, and it sounds like
21 the issue is, "I can't predict whether this string
22 of text is going to, you know, translate into a
23 protein with the native fold of PH20"?
24 MR. KUSHAN: Objection foundation.
25 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?

1 NAISMITH - MARTIN
2 A. That is the definition as I read
3 the claims, that they have adequate, they are
4 stable, native fold and they sit in that box.
5 Q. Okay, because, okay, so then above
6 that, right, there is a box that says: "Natively
7 folded modified PH20 polypeptide" and there are
8 arrows pointing to all of these boxes within the
9 heavy green outline?
10 A. So that points to the case where
11 there is a possibility, as I said, where the
12 protein is active for a very short period of time
13 and I would put that in this inadequate stability,
14 can't measure the enzyme activity reliably or
15 believe in it and, therefore, I don't think it
16 would fall within the scope of the patent claims.
17 Q. Okay, so the: "Inadequate
18 stability, enzymatically active" box, would not
19 fall within the scope of the claims?
20 A. As I read the claims that's my
21 definition because although it may have had
22 activity transitorily, once it becomes unstable,
23 unfolds, it no longer has activity. So,
24 therefore, it would not meet the scope of the
25 claims.

1 NAISMITH - MARTIN
2 Q. So for purposes of the test in the
3 patent, right, if you were to test such a protein
4 it would show up as inactive because it may have
5 had transient theoretical activity but by the time
6 you test it it doesn't have activity any more;
7 right?
8 A. So for the single mutations that
9 are recited in the patent, it is one of the
10 reasons why you might have assigned them or
11 classified them as inactive along with many other
12 reasons. As you say, it could have been secreted
13 and suddenly it just unfolded or it unfolds very
14 rapidly, by the time you get to the assay it is
15 less than 20%, you would have classified that as
16 inactive.
17 Q. I just want to understand, based on
18 the discussion that we had earlier about, you
19 know, you run the test at, after 90 minutes and
20 you show that it is active, the universe of
21 proteins that fall within that bucket, right, that
22 are active after 90 minutes, those are the ones
23 that are in the green shaded box?
24 MR. KUSHAN: Objection foundation.
25 A. So you are changing my definition

1 NAISMITH - MARTIN
2 of stability. That "stability", I am confident
3 for the single mutation, as I said confident that
4 those would be stable once I get to other, which
5 are not represented at all in the patent,
6 multimutations, then and I gave the answer about
7 eight hours. So, if the protein was completely
8 unfolded by eight hours and aggregated I would
9 think it would fall outside of the scope of the
10 claims as I read them. I think and I
11 differentiated between the single site mutations
12 that are reported in the patent and then these
13 multimutations.
14 Q. Yes, but I thought that we agreed
15 that even if you had multiple mutations, if you
16 did the test at 90 minutes and it was active, you
17 would expect that it was sufficiently stable to
18 meet the claims?
19 MR. KUSHAN: Objection foundation.
20 A. So with multimutations I was
21 careful to say I would like to know what the
22 activity was, and I would, because of
23 multimutations I might, I would want a set because
24 of my concern about stability, if it was a, you
25 know, very active protein, probably not. But

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2 I would begin to use some judgment here because as
3 I read the claims it has, the number given is
4 about eight hours, that is the kind of assay time
5 they want.
6 So I can imagine proteins which may
7 have some activity at 90 minutes which have none
8 at eight hours, but I said because of the way we
9 prepare it that you have some, if it's active at
10 90 minutes you have "some information" about
11 stability, you don't have "no information" about
12 stability at that point, because it's active at 90
13 minutes plus the handling.
14 So you would have an expectation
15 that it might be stable at eight hours, but once
16 you get to multiple mutations you begin to have
17 less confidence in that expectation because your
18 expectation is grounded in the original PH20 with
19 a single site mutation.
20 Q. (BY MS. MARTIN): So then you would
21 have to do an additional test at eight hours to
22 make sure that it is still active in order to tell
23 whether it meets the claims if it has multiple
24 mutations?
25 A. I don't think, I said depending on

1 NAISMITH - MARTIN
2 the purpose, if it was still active in the assay
3 you would have confidence, some confidence if it
4 was made and it was active in the assay, that it
5 would still be active at eight hours. But if it
6 was at the very edge of activity, on those, one of
7 those border lines, you might want to do a second
8 test at eight hours to see whether it retained its
9 activity.
10 Q. Do you read the claims as requiring
11 PH20 to be on the surface of the sperm?
12 A. No, I don't because I'm
13 specifically told in the claims and I've had
14 reference to SEQ.ID NO:3.
15 Q. SEQ.ID NO:3 is not a naturally
16 occurring sequence, is it?
17 A. It is a ----
18 MR. KUSHAN: Objection form.
19 A. As I read the claims, we are
20 directed to polypeptides and then we narrow it
21 down, so PH20 polypeptide would include the native
22 protein, but then we narrow it down and we say, we
23 specifically call it sequence 3 and 32-66, so we
24 are restricting here that universe.
25 Q. (BY MS. MARTIN): So the native

1 NAISMITH - MARTIN
2 PH20 which includes PH20 on the sperm is not part
3 of the claim because that would not satisfy the
4 requirements of the SEQ.ID NO:3 or 32-66; right?
5 MR. KUSHAN: Objection form.
6 A. I would want to check precisely on
7 whether the longer ones, the number of amino acids
8 in the longer one I have a table arming ----
9 Q. Oh?
10 A. Because if you get ----
11 Q. (BY MS. MARTIN): I will represent
12 to you that none of those have a GPI anchor.
13 A. Right, but it is the number of
14 changes relative, you know, deletions, because
15 I'm -- so you could imagine deletions that there
16 are so many other changes that are longer it falls
17 outside your 95%. I just want to check the number
18 of the full length of the sequence ----
19 Q. Okay.
20 A. ---- to answer your question. So
21 447 is the sequence I have, so I can't recall,
22 without going back, how many residues are in the
23 full length protein, I apologise. If there are
24 more than 18 residues longer than 447 then that
25 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

1 NAISMITH - MARTIN
2 Dr. Petsko which is Exhibit 1131.
3 **(Previously marked Exhibit 1131 was referred to)**
4 A. Before we start again could I go to
5 the bathroom? There is not a question pending so
6 I thought would now be okay?
7 MS. MARTIN: We will go off the
8 record.
9 THE VIDEOGRAPHER: We are going off
10 the record. The time is 4.05 p.m.
11 **(Short recess from 4.05 p.m. to 4.26 p.m.)**
12 THE VIDEOGRAPHER: We are back on
13 the record. The time is 4.26 p.m.
14 Q. (BY MS. MARTIN): All right.
15 Dr. Naismith, did you discuss the substance of
16 your testimony with counsel during the break?
17 A. No, not at all.
18 Q. Can you please turn in your report
19 to paragraph 340?
20 A. That is on page 248 I think.
21 Q. Yes.
22 A. Can I move these back out the road.
23 Q. Yes, you can move them aside for
24 now. We will come back to them. And so you say
25 in paragraph 340: "Dr. Park explained that he

1 investigated many individual substitutions at the
2 non-essential positions of PH20 within the portion
3 of the PH20 sequence that overlapped with the
4 Hyal-1 template used to create his PH20 homology
5 model, and for which the model had reliable local
6 QMEAN values." Right?
7 A. That is the sentence, yes.
8 Q. And so it is your testimony that
9 the positions that Dr. Park looked at all had
10 reliable local QMEAN values?
11 A. So he excluded some regions where
12 he felt the QMEAN score was not, and the model was
13 not good enough for him to use his local
14 environment test, so C-terminous being one.
15 Q. You have a colour coded chart on
16 page 249 of your declaration; right?
17 A. Yes.
18 Q. Did you prepare this chart?
19 A. With the assistance of counsel.
20 Q. And why did you colour code the
21 chart on page 249?
22 A. To help represent information and
23 there is a key below which say what is the colours
24 correspond to.
25

1 NAISMITH - MARTIN
2 Q. Were you trying to suggest through
3 your colour-coded chart that Dr. Park's analysis
4 provides a measure of tolerability of the
5 positions in your chart, in the positions in that
6 chart to substitution?
7 A. So I think I'm pretty clear as red
8 is where Dr. Park identified essential residues,
9 so he did not do anything there, there was no
10 probing by his methods. Then dark green is where
11 Dr. Park evaluated 60% of the total residues
12 identified in MSA. So he worked down a certain
13 list. Green, where he only probed between 30 and
14 66%, and light green where he didn't probe, he
15 probed less than 33% of alternatives suggested in
16 the MSA. The unshaded is the ones he didn't probe
17 at all.
18 Q. So the shading relates to the
19 extent to which, or the number of alternate
20 residues within the MSA that Dr. Park evaluated
21 for each position; right?
22 A. It represents the percentage of
23 those that were in his MSA if there was, say, 10,
24 if I coloured it dark green that means he tested
25 seven because it is more than 66%.

1 NAISMITH - MARTIN
2 Q. When you say tested, you mean he
3 looked at it in PIE model and evaluated it?
4 A. My apologies, he used modelling and
5 evaluated it and then he assigned individually
6 those mutations into his buckets of 1, 2, 3.
7 Q. You were not intending to represent
8 with your shading on the figure on page 249, you
9 weren't intending to represent any information
10 about the tolerability of those positions to
11 substitution, were you?
12 A. So the red ones would be ones that
13 he identified as essential, and so those would be
14 ones in his analysis that you would not start by
15 making single mutations and that is what he said.
16 But I don't have any -- the colours here don't
17 represent anything other than, as you say, the
18 numerical thresholds, but they are not -- there is
19 -- the green or dark green or light green don't
20 apply some tolerability.
21 Q. The numerical thresholds are the
22 number of substitutions that Dr. Park considered,
23 not the tolerability of that position to
24 substitution; right?
25 A. So, I don't -- tolerability is, I,

1 it is an odd expression, I wonder if you could
2 define that for me because it's defined
3 differently by different people. So tolerance
4 applies to a single site mutation is active or
5 inactive and Dr. Park uses it in that way. So he
6 said, you can tolerate, I've got a mutation,
7 whatever it is, if it falls into buckets 2 or 3 by
8 his analysis that is a tolerated mutation.
9 Q. Okay. So the colouring that is
10 shown in the graph on page 249 is not intended to
11 show the tolerability of each of those positions
12 using the definition that you just provided;
13 right?
14 A. What he said is, so the colour
15 scheme is designed to tell you how many of the
16 alternative residues were probed in the
17 multisequence alignment, that is what it does.
18 Q. Correct, not the tolerance of each
19 position to change?
20 A. You would need to go to his
21 spreadsheet to work out which amino acid
22 substitutions were tolerated.
23 Q. Which ones he predicted would be
24 tolerated?
25

1 NAISMITH - MARTIN
2 A. Correct, which ones, based on what
3 a POSA would do, that he assigned to buckets 2 or
4 3.
5 Q. You certainly weren't intending to
6 suggest that this chart would provide an
7 indication of the tolerability of each positioned
8 substitution; were you?
9 A. No, I would say that if you wanted
10 to look for mutations based on this alignment you
11 would go back to his main spreadsheet, that is a
12 definitive list of where you might find
13 substitutions that were tolerated buckets 2 or 3.
14 THE COURT REPORTER: Did you say
15 this alignment?
16 A. Multi-sequence alignment.
17 THE COURT REPORTER: You said "based
18 on this alignment".
19 MS. MARTIN: I think you said ----
20 A. Oh, this alignment, yes, the
21 multi-sequence alignment.
22 Q. (BY MS. MARTIN): In paragraph 343
23 the second to last sentence you say that Dr. Park:
24 "... did not assess alternatives at residues 4-7
25 and around 398-4033, because of the uncertainty in

1 NAISMITH - MARTIN
2 the structural model in those regions." Right?
3 A. That is correct.
4 Q. So were those the ones that he
5 excluded because of an unreliable QMEAN score?
6 A. I think it exceeded even the
7 template that he had to use, so he just excluded
8 them.
9 Q. Okay. Keep page 249 handy while
10 turning to paragraph 283, page 205?
11 A. You said keep something else open?
12 Q. 249. Actually let's go to page
13 206, the top of page 206?
14 A. 206, what was other page, sorry?
15 Q. 249?
16 A. So we are looking at the table.
17 Q. Yes. So you say at the last
18 sentence in paragraph 283 that: "In PH20,
19 positions 3-5, 27-35, 44-48, 104-105, 234-237,
20 256-262, 274-278, 357-358, 405-406 have unreliable
21 local QMEAN ..." scores: "... and modeling at
22 those positions would be unreliable." Correct?
23 A. They are less, yes, they would be
24 less reliable at those position.
25 Q. You don't say "less reliable"; you

1 NAISMITH - MARTIN
2 say "unreliable"?
3 A. "Unreliable", I do say
4 "unreliable", yes.
5 Q. Dr. Park in fact did model PH20
6 positions within those ranges, right, for example?
7 MR. KUSHAN: Objection.
8 Q. (BY MS. MARTIN): If you look at 27
9 through 35, you see that those are shaded green in
10 your chart on page 249, which means that Dr. Park
11 considered substitutions at those positions?
12 A. He did for the purposes of his
13 analysis, yes.
14 Q. Well, you agreed that his analysis
15 was reliable and appropriate; right?
16 A. He described how he did it, and
17 I think a POSA would follow his recipe and come up
18 with the same answers, so he clearly set out how
19 he did it.
20 Q. Now did you, when you were
21 eventuating Dr. Park's methodology, did you
22 consider the fact that he modeled positions that,
23 according to you, are unreliable?
24 A. So Dr. Park's analysis set out
25 illustration, he didn't exclude regions with lower

1 NAISMITH - MARTIN
2 QMEAN scores. So I noted that, but he did say
3 what the basis for his exclusions were. You know,
4 he told me not the templates and then I worked
5 through and looked at the MSA, so I understood how
6 Dr. Park had done his analysis.
7 Q. Okay, but his analysis included
8 estimations of whether or not particular mutations
9 would be tolerable at positions that you say, at
10 the positions for which you say modelling would be
11 unreliable; right?
12 A. I said insufficient reliability so
13 that would be, yes, if I was, you know, I do say
14 that that would be a caution you would use, that
15 QMEAN would be a caution in evaluating those
16 predictions.
17 Q. That is not what you said about
18 Dr. Petsko. You said that modelling at those
19 positions would be unreliable?
20 A. But I did not exclude them from my
21 -- the only test I impose -- it would say the same
22 thing to both Dr. Petsko and Dr. Park, is that
23 those would have insufficient reliability in some
24 way, but I didn't cut out those residues for
25 Dr. Petsko, when I went through Dr. Petsko's lists

1 NAISMITH - MARTIN
2 I did not impose a QMEAN cut off from Dr. Petsko.
3 Q. Okay, you are saying you didn't
4 ignore Dr. Petsko's analysis for those residues,
5 but you're just saying that it would have been,
6 that modelling at those positions was unreliable
7 for Dr. Petsko but you didn't take that position
8 for Dr. Park?
9 A. No. I said that in my view, where
10 the QMEAN score is less a POSA would be concerned
11 about quality of predictions there because it's
12 less reliable, unreliable I said. And that is
13 true for Dr. Petsko and Dr. Park's analysis,
14 Dr. Park himself talks about that the
15 unreliability in that region. But what I didn't
16 impose my judgment on that, on their analysis.
17 Q. You didn't say anything about
18 Dr. Park's analysis being unreliable at those
19 various positions, did you?
20 A. Well, I think I already, I didn't
21 repeat it, but I had talked about it in
22 paragraph 283.
23 Q. In paragraph 283 you are talking
24 about Dr. Petsko to be clear, you are criticizing
25 Dr. Petsko in 283; you don't criticize Dr. Park?

1 NAISMITH - MARTIN
2 A. I think am just talking in general,
3 portions of the PH20 in paragraph 283, I don't
4 think I specifically criticized Dr. Petsko in any
5 way in paragraph 283.
6 Q. Oh, yeah, okay, that is in 284.
7 You criticize Dr. Petsko in paragraph 284 for his
8 use, for his modelling at what you call to be,
9 what you determine to be unreliable positions?
10 A. Dr. Petsko's analysis did not
11 account for them, that is just a factual
12 statement.
13 Q. And neither did Dr. Park's; right?
14 A. Dr. Park didn't use a QMEAN cut
15 off, but he drew attention to it.
16 Q. Not for those positions he did not.
17 A. He didn't exclude them based on the
18 QMEAN score; that is correct.
19 **(Previously marked Exhibit 1026 was referred to)**
20 Q. Somewhere in there you have the 731
21 application, I think it is there.
22 A. Sorry?
23 Q. That one.
24 A. So I am looking for the last three
25 digits; is that correct?

1 NAISMITH - MARTIN
2 Q. Actually no, sorry. So the 731,
3 that one. The 731 application, so have that and
4 your declaration. Okay.
5 A. This is the common ----
6 Q. Common disclosure and your
7 declaration. Okay. So let's go to Example 5 in
8 the 371 application, which is on page 282?
9 A. Is that the common disclosure
10 numbering or the exhibit number?
11 Q. The common disclosure numbering.
12 It is exhibit page 295.
13 A. It has a table data then Example 5.
14 Q. Yes.
15 A. Indeed, I am now there.
16 Q. Example 5 is an assay for
17 hyaluronidase activity under temperature and
18 phenophilic conditions; right?
19 A. That is what it says. It is a test
20 of natants that were identified as active and then
21 tested under these conditions.
22 Q. And specifically for: "...
23 activity under temperature and phenophilic
24 conditions." Right? That is what it says in the
25 header, for Example 5?

1 NAISMITH - MARTIN
2 A. It defines 37°C, yes, higher than
3 room temperature, some people would say
4 thermophile is 100°C, but the patent is quite
5 clear the teaching is 37.
6 Q. So it says on exhibit page 295
7 under Example 5 that: "Supernatants from PH20
8 activity variants set forth in Table 9, as
9 identified in Example 4, were tested for stability
10 un[d]er thermophilic and/or phenophilic
11 conditions." Right?
12 A. That is what it says.
13 Q. So they were measured -- they were
14 testing supernatants, right, in Example 5?
15 A. Yes, it does say supernatants, that
16 is correct.
17 Q. So because they are testing
18 supernatants the concentration of protein from
19 each of the individual transfected cultures could
20 vary; is that right?
21 A. I would like to check. It is a
22 while since I read this. If they did any
23 normalization here between different ones,
24 I cannot recall if they actually controlled for
25 that in this particular example, I don't believe

1 NAISMITH - MARTIN
2 they did.
3 Q. Okay, we will look at that in a
4 second. As a theoretical matter though, when
5 you're testing supernatants from different
6 transfected cell cultures, the concentration of
7 protein that you are going to get out of those
8 various transfected cell cultures can vary; right?
9 MR. KUSHAN: Objection foundation.
10 A. As a hypothetical matter, that is
11 -- for different proteins, you know, if I changed
12 the protein in some way, correct; you would
13 expect, if I was making the same protein under the
14 same conditions I would get pretty much the same
15 result, or you would expect that to be true, same
16 amount protein, but if I was doing modified
17 proteins you could get different amounts.
18 Q. (BY MS. MARTIN): Let's say I take
19 my wild-type PH20 and I transfect it into a CHO
20 cell, so let us say I have five different
21 transfected cell lines of my wild-type PH20, are
22 you telling me that if I take out, let's say, you
23 know, 1 millilitre aliquot out of each one of
24 those five cell cultures, that every single one is
25 going to have the exact same concentration of

1 NAISMITH - MARTIN
2 PH20?
3 A. They would not have precisely
4 exactly to some measure the exact same, but the
5 variation you would expect if you had done the
6 experiments properly in a controlled manner, you
7 would expect by the particular hypothetical we are
8 exploring, for it to be very similar. You haven't
9 -- that is a test of reproducibility.
10 Q. That is a test of reproducibility
11 of protein concentration in a supernatant?
12 A. No, the test is how you approach
13 the experiment, if you had done it under identical
14 ways you should get pretty much the same answer.
15 Q. Okay, so when you say identical
16 ways, are you talking about identical cell culture
17 conditions?
18 A. Cell culture conditions, time,
19 buffer, if you've shaken the plate, all of how you
20 handle the cells for that experiment. If you've
21 kept them all the same then reproducibility, if
22 you have actually done that, would suggest you
23 would get very similar results, there may be an
24 inherent noise in your data that you can find out,
25 but if you've done it properly you would expect

1 NAISMITH - MARTIN
2 them to be very, very similar.
3 Q. Why is that?
4 A. It is the basis of the scientific
5 method, is it should be reproducible and one of
6 the ways with students, for example, doing
7 something completely different is they often
8 believe they have done everything exactly the
9 same, but get different results, wildly different
10 results, they are not doing everything the same in
11 that case.
12 Q. So is your testimony that if you
13 have two different cell lines, those two different
14 cell lines are going to secrete the exact same
15 amount of PH20 independently of one another?
16 A. No, because those are different
17 experiments; you have changed the cell line.
18 Q. So if you have the same cell line
19 in two, let us say, two different cultures, right
20 two different beakers, two different cultures, you
21 would expect that the cells would produce the
22 exact same amount of PH20?
23 MR. KUSHAN: Objection form.
24 A. I didn't say the exact same, I said
25 very similar, but you would be, characterise a

1 NAISMITH - MARTIN
2 level of noise in the data that you do get
3 statistical variation, but you would expect, if
4 you had done the experiment carefully and
5 properly, for that to be relatively small, but you
6 can measure it and people would measure it so they
7 would have confidence in that. Given that you
8 have done exactly the same thing, if you truly had
9 done exactly the same thing, then the difference
10 between them is a reflection of statistical noise
11 stochastic variation.
12 Q. (BY MS. MARTIN): Is not part of it
13 just a difference in the amount of PH20 secreted
14 by the cell? I mean, are you saying that all
15 cells are going to secrete the exact same amount
16 of PH20?
17 MR. KUSHAN: Objection foundation.
18 A. I keep saying that "exact" is a
19 demanding test, but given you have the same number
20 of cells, the same culture, exactly the same
21 conditions, that the variation you would see
22 should be small and what you would assign as
23 stochastic variation, if you had done everything
24 in the same way, that you hadn't obviously varied
25 the cell line as we talked about, if you hadn't

1 NAISMITH - MARTIN
2 come back tomorrow when the lab was 3° warmer, if
3 you hadn't used a different batch of media, all of
4 these things could lead to increase noise in the
5 data. But a basic premise of science, good
6 science, is that if you do everything the same
7 way, it doesn't matter, if you follow the recipe
8 you should get pretty much the same answer, and
9 pretty much, is not exact. Where you do not get
10 that, and you've excluded carefully all other
11 possibilities, then that is a stochastic noise of
12 the knowledge of the system.
13 Q. How do you know it is the same
14 number of cells and the different cultures?
15 A. So, you told me you started with
16 the same number of cells. I asked, you start
17 everything in the same ----
18 Q. Okay, then we are clearly not on
19 the same page here. I just said that you have two
20 different cell cultures of wild-type PH20, why
21 would you assume that they both have the same
22 number of cells in them?
23 A. Because I asked you if everything
24 was the same.
25 Q. Let's assume that it's not the

1 same.
2 A. Then I would expect to get
3 different results.
4 Q. Is it possible to control to make
5 sure that you have the exact same number of cells
6 in two different cultures?
7 A. Yes.
8 Q. Okay, and would you just expect
9 that to happen?
10 A. What do you mean expect what to
11 happen, sorry?
12 Q. If you transfect some CHO cells
13 with PH20 and grow them in a culture would you
14 expect that two different cultures, if you did
15 that, you know, in two different containers if you
16 will, that you are going to obtain, you know,
17 identical cell counts in your two different
18 containers?
19 A. If everything was done identically
20 then I would expect them to be pretty much the
21 same within some stochastic noise ratio, but if
22 you made differences in the volume or temperature
23 or other things then I would expect that to be
24 different.
25

1 NAISMITH - MARTIN
2 Q. Okay. Back to Example 5, so let's
3 go to the bottom of page exhibit 297, in the
4 common disclosure.
5 A. So this is Table 12?
6 Q. Sorry, it is exhibit page 297,
7 common disclosure page 284?
8 A. Okay, my apologies. So it is, yes,
9 284 starts the first words are: "1.5 hours at
10 37°C ..." at the top of the page.
11 Q. Yes, let's go to the last paragraph
12 on the page.
13 A. Yes.
14 Q. And it says in the second sentence:
15 "Absolute hyaluronidase activity (U/mL) was
16 generated from the standard curve." Right?
17 A. That's indeed what it says.
18 Q. And then it says: "In addition,
19 percent activity was determined as a ratio of
20 activity at 37°C/4°C, 37°C plus m-creso1/4°C, and
21 37°C plus m-cresol/37°C." Right?
22 A. Yes.
23 Q. And then: "The results are set
24 forth in Tables 11 and 12 below." Right?
25 A. Yes.

1 NAISMITH - MARTIN
2 Q. So Table 11 provides the absolute
3 hyaluronidase activity in units per millilitre as
4 generated from the standard curve; right?
5 A. The standard curve is not
6 disclosed, that is what the table reports.
7 Q. And there is no normalization or
8 concentration, is there?
9 A. I'd like to just read through to
10 check I read the method properly.
11 Q. Okay.
12 A. *(Pause for reading)* So as I read
13 it, I assume that they are trying to keep the same
14 volume. There is nothing that says they changed
15 the volume, they used the same CHO cells from the
16 same supplier. So I read it as they are trying to
17 make sure that they are using the same procedure
18 so that the results are comparable. And they are
19 only working on active proteins here, so they are
20 single site active mutations. So, while I might
21 expect for mutations that are classified as
22 inactive, for there to be very large deviations in
23 the amount of protein, at least here the single
24 site mutations I would assume that they are
25 probably about the same in general. But the

1 NAISMITH - MARTIN
2 stochastic noise, there is quite large variations
3 so it is possible they have different
4 concentrations.
5 Q. So by the fact that they are
6 testing the same volume and they use the same CHO
7 cells from the same supplier you assume that they
8 have cultured all of the variants and all of the
9 wild types in the exact same conditions, such that
10 there is no variation in temperature or shaking or
11 anything that could potentially result in a
12 difference in cell count in each of the cultures,
13 that would then result in a difference in
14 concentration?
15 MR. KUSHAN: Objection foundation.
16 A. So they are clear that they put
17 some controls in to try to control for that, so
18 there are controls mentioned clearly here in each
19 plate so that for each plate you are trying to
20 minimize. But plate-to-plate has variation but
21 that should be accounted for by the control
22 experiments.
23 Q. (BY MS. MARTIN): Okay, but the
24 issue is that in the data that are reported in
25 Table 11, as they are reported in Table 11 they

1 NAISMITH - MARTIN
2 are not normalized for protein concentration and
3 you cannot assume that every single well had the
4 exact same concentration of protein, because that
5 would be unreasonable; is that not true?
6 MR. KUSHAN: Objection foundation.
7 A. I can't assume that every single
8 well has an identical concentration but I would
9 be, as a POSA would normally do this experiment
10 one would seek to minimize any variation between
11 them, they explicitly put controls in to try to
12 control for that with their plates. So I think I
13 would therefore believe that what they have
14 reported reflects meaningful data rather than just
15 accidental you know there is one, they have gotten
16 times more of one than the other, because they are
17 trying to minimize variation within a plate and
18 they also control between plates.
19 Q. (BY MS. MARTIN): Okay. So in
20 Examples 3 and 4 they controlled for the
21 difference in concentration by normalizing based
22 on seep, but they didn't do that so that they
23 reported their data normalized for protein
24 concentration in Examples 3 and 4, but here they
25 didn't report the data normalized for protein

1 NAISMITH - MARTIN
2 concentration because they are not reporting it in
3 the same way. So it's not that they do care about
4 the difference in protein concentration, it's that
5 this particular table, with which you have decided
6 to complain about variability, does not actually
7 control for differences or does not actually
8 normalize for differences in concentration?
9 MR. KUSHAN: Objection foundation,
10 objection as to form.
11 Q. (BY MS. MARTIN): And Table 12,
12 right, provides percentages but the percentage
13 that is provided is not a comparison between the
14 different variants, such that there would be no
15 need to control or normalize for concentration,
16 for protein concentration between the variants?
17 MR. KUSHAN: Objection foundation,
18 objection form.
19 A. So here, so my criticism of the
20 assay is even within things that are in the same
21 plate and duplicates you see quite large
22 variations between them and that is a higher level
23 of variation than a POSA would believe for a
24 reliable assay.
25 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

1 NAISMITH - MARTIN
2 even with things that are in the same plate, you
3 see quite large variations?
4 A. You do, you can read down and see
5 them, that there are quite large variations
6 between duplicates.
7 Q. How do you know the duplicates are
8 on the same plate?
9 A. I'd have to check if they said
10 that, it would be my expectation they would do
11 that.
12 Q. Okay. So you are talking about the
13 ----
14 A. Let me just find it, sorry. So it
15 doesn't explicitly, I think, say that but it would
16 seem kind of crazy not to put them in the same
17 plate because you would introduce quite large
18 errors, you could introduce very large errors by
19 that and it would make your results even more
20 difficult to interpret.
21 Q. Ok. You expect that, if I am
22 understanding correctly, the wild-type data that
23 are on page 212 that you excerpted, right, that
24 the duplicates are on the same plate but each
25 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 ----

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

1 NAISMITH - MARTIN
2 much more similar results than you obtained.
3 Q. But assuming that you used the same
4 concentration of protein in each of the wells.
5 MR. KUSHAN: Objection, foundation.
6 A. I think it is rather assuming that
7 since I used the same media, the same cell line
8 for the native protein which should, and same
9 volumes, that I would expect that I would have,
10 within some measure, the same number of cells, a
11 transfect in a consistent way. If my assay was
12 reliable I would expect similar results between
13 plates. That the fact you don't see that, if it
14 was a student in my lab, I would want them to go
15 back and figure out what it is they are doing
16 differently.
17 Q. (BY MS. MARTIN): One of those
18 things could be purifying the protein and making
19 sure they are using the same concentration of
20 protein; right?
21 A. I think these are supernatants they
22 are using.
23 Q. Yes, they are supernatants.
24 A. So they could choose to use
25 different volumes of supernatants; is that what

1 NAISMITH - MARTIN
2 you are saying?
3 Q. They could purify the protein and
4 then just use a fixed amount of protein to make
5 sure that they are using the same concentration of
6 protein?
7 THE COURT REPORTER: Sorry, could
8 you repeat that please?
9 MS. MARTIN: They could purify the
10 protein and then use a fixed concentration to make
11 sure that they are using the same concentration of
12 protein?
13 A. So I think, you could have done an
14 assay where you probed to understand why you've
15 got that variation, I do not understand why you
16 have that variation, there's nothing in Example 5
17 that I could spot that says, "I know why they've
18 got such variation". So, to understand that, you
19 are correct, I would want to go back and try to
20 understand that and do more experiments. As a
21 normal rule in my lab we do triplicates and if a
22 student had produced that level of variation
23 between plates I would be concerned that they are
24 doing something that is not controlled for, they
25 are doing something different. I can't imagine

1 NAISMITH - MARTIN
2 that. It seems very odd. Okay, based on -- it
3 doesn't say in the description of the experiment,
4 "Some of the plates we put more in, some of them
5 we put less in". When people are comparing things
6 or analyzing things, it is kind of a rule of
7 science is, vary one thing at a time, try to keep
8 everything else constant.
9 Q. Couldn't they also have just
10 normalised for concentration?
11 A. They could have done a lot of
12 things but they didn't.
13 Q. You are criticizing, no, you are
14 not criticizing them for not normalizing because
15 you didn't take into account the fact that they
16 didn't normalize, right, you are criticizing them
17 for reporting the data like this without
18 normalizing?
19 MR. KUSHAN: Objection foundation,
20 objection as to form.
21 A. My criticism is that there is
22 nothing in the description that for the wild-type
23 protein that I can understand why they have such
24 large variation, that is my opinion. I can't
25 understand if they did the experiment properly why

1 NAISMITH - MARTIN
2 that is the case, that you've got such large
3 variation between what should be similar samples.
4 You are postulating that they had different
5 amounts of material, but that would not be normal
6 for a POSA to do, and they would certainly,
7 I would think, tell me that in the assay.
8 Q. (BY MS. MARTIN): So, okay, I just
9 want to make sure that the record is clear, that
10 when you said that you're testing protein
11 supernatants from different transfected cells that
12 you are assuming that the protein supernatants you
13 are testing all have an equivalent amount of
14 protein, the same concentration of protein across
15 the supernatants taken from the different cell
16 cultures?
17 MR. KUSHAN: Objection foundation,
18 objection as to form.
19 A. That's not what I said.
20 I restricted my answer to the wild type, which we
21 can agree, given it is the same cell line, grown
22 in the same way, there is nothing in the
23 description that I could see that tells me that
24 some plates were done differently. And as I said,
25 the default assumption of a POSA would be when you

1 NAISMITH - MARTIN
2 are measuring the wild type in different plates
3 you would try to keep the experiment or you would
4 aim to keep the experiment consistent, so you
5 would have the same volume, same cell line, same
6 temperature, same media.
7 What I am saying is that based on
8 that, when I look at the variation between the
9 plates, I can't understand why that variation is
10 so high. And there is nothing in the description
11 that says, "For some of the plates with these
12 variations we did things differently". That might
13 heap me understand, but they don't discuss at all
14 that ----
15 Q. (BY MS. MARTIN): So you just
16 assume that the data are unreliable for Examples 3
17 and 4 based on the wild-type variation in
18 Example 5?
19 MR. KUSHAN: Objection foundation,
20 objection as to form.
21 A. Unreliable for what test?
22 Q. (BY MS. MARTIN): For any test
23 because you said they are unreliable ----
24 A. I believe that the protein is
25 active in the assay, and the activity is decreased

1 NAISMITH - MARTIN
2 in the presence of cresol for the wild-type
3 protein at 37° but I am highlighting that the
4 large coefficient of variants would, and these
5 variations between what should be this, we would
6 expect as a POSA the same thing, would be alarming
7 and also there are quite large deviations between
8 duplicates, which are in some ways the absolute
9 definition of things you expect to be the same.
10 Q. (BY MS. MARTIN): Are you telling
11 me that you expect the duplicates to give exactly
12 the same number?
13 MR. KUSHAN: Objection foundation,
14 objection as to form.
15 A. I have never used the phrase "exact
16 number". What I'm saying is that where they are
17 different, if you have done everything exactly the
18 same, the difference arises from some stochastic
19 variation that you can quantify, and that is often
20 used as a reliability for the assay.
21 Q. (BY MS. MARTIN): I want to be
22 really clear about what you would consider to be
23 an acceptable stochastic variation. So, for
24 example, like the third line down has 3 and 2.725
25 and so you are saying that those two numbers are

1 NAISMITH - MARTIN
2 unacceptably different?
3 A. So you can't form an opinion what
4 the precise level of stochastic variation is until
5 you actually assess a large number of assays done
6 in the same way, it is not simple to just take two
7 separate numbers. It could arise from that. But
8 as a percentage, the numbers you gave 2.7 to 3,
9 that is 0.25 divided by 3.08, that is high but
10 Dr. Petsko's threshold, I think, was 10%.
11 Q. Is that a 10% difference?
12 A. Variation. But between duplicates
13 that is not nothing. If you go to the middle
14 column 37° with no cresol, you can see one of them
15 is 10.8 and the other one is 3.94, that is 1, 2,
16 3, 4, 5. Now, if I saw that in the student's
17 notebook I would be incredibly concerned about
18 what they are doing and probably get them to start
19 again, and watch them very carefully. I can see
20 other examples in the table where even within what
21 are supposed to be duplicates, I have these very
22 large differences.
23 Q. Okay, so let's turn to
24 paragraph 265 in your declaration. In paragraph
25 265 ----

1 NAISMITH - MARTIN
2 A. This is headed: "A POSA relying on
3 Dr. Petsko's ..."?
4 Q. Yes, and actually let's look at the
5 graph, the chart on page 193?
6 A. Okay, yes, I am looking at them.
7 Q. And so you have taken the relative
8 activity levels reported in the patent for
9 different mutations at, for example, position 74?
10 A. Yes.
11 Q. And you have done a statistical
12 analysis where you've calculated, among other
13 things, the standard deviation and the coefficient
14 of variation; right?
15 A. Yes, but these are not the same as
16 the coefficient of variation between assays.
17 Q. You are representing that this is,
18 you are representing these as error bars so you
19 are representing that, for example, T74 the
20 different mutations of T74, two different residues
21 are treated as, are being treat as replicates in
22 your analysis with a high error rate as opposed to
23 independent experiments.
24 A. No, that is not what I did at all.
25 Q. Well, that is what you did because

1 NAISMITH - MARTIN
2 I see error bars on your graph indicating that
3 there is a wide range of error for that particular
4 experiment.
5 A. No, that is not what it means. It
6 just shows you that what the table is plotting and
7 I apologize, it is not obviously clear, when you
8 look at the mutations at T74 as individual dots
9 you can point them. Then what these bars are is
10 just to give you a sense of what the variation of
11 those mutants are. Between the mutants it doesn't
12 speak to error of the measurement. Just the fact
13 that I can put one residue substitution, which has
14 a two point, roughly, seven, let's look at the top
15 blue number, so that makes the protein 2.7 fold
16 more active, that is the number reported in the
17 table. Another mutant has 0.5 mutant, so it is
18 reduced activity. So that is not an error
19 measurement, it is to give you a sense, the reason
20 the numbers are there was to just give a sense of
21 what the variation between single site active
22 mutations are at a position are.
23 Q. Okay.
24 A. And those are very large, and
25 because of that a POSA would look at that and

1 NAISMITH - MARTIN
2 realize that the difference between a mutation,
3 particular introduction, that makes the protein
4 2.5 times more active and one that makes a protein
5 half as active, roughly, right, I'm not going to
6 get into the precision here. That is a really
7 interesting issue about what is happening to the
8 protein active site or binding or whatever, that
9 is quite interesting. So it speaks to this
10 problem that this mutation is doing something to
11 the protein that we don't understand. We can see
12 their outcome. So therefore if you combine that
13 mutation which has done something to the active
14 site in some way, because we can see it in the
15 assay, with another mutation, that is where we
16 start to worry, really would worry about epistasis
17 because we know these are affecting at a distance
18 in some way the active site because you can see it
19 in the assay, and that is a big difference.
20 Q. When Dr. Park rated a particular
21 change as a "one", meaning that it would be
22 untolerated, is it your testimony that that
23 change, that would be untolerated according to
24 Dr. Park, would have an impact on the active site
25 of the protein, irrespective of where it is in the

1 NAISMITH - MARTIN
2 protein?
3 MR. KUSHAN: Objection foundation,
4 objection as to form.
5 A. So Dr. Park's analysis talks about
6 destabilizing of the local environment and,
7 therefore, those were single site mutations based
8 on his multiple sequence non-conserved regions,
9 that since they were destabilizing in their local
10 environment he would expect those to have a
11 negative consequence to the protein's activity.
12 He did not assign a particular level of activity
13 to his assays but he said those are the ones that
14 are likely to be inactive. But he didn't give a
15 number for that. He just said it would be
16 destabilizing, so I would not start here.
17 Q. Okay. So when Dr. Park determines
18 that a change will be destabilizing such that it
19 will inactivate the protein, it will inactivate
20 the protein because of a destabilization effect.
21 When the patent shows a reduction in activity you
22 assume that there is change in the active site
23 caused by some sort of magical epistasis effect;
24 is that right?
25 MR. KUSHAN: Objection foundation,

1 NAISMITH - MARTIN
2 objection as to form.
3 A. Epistasis is not a magical effect.
4 It is well-documented problem. Local instability
5 can affect global protein stability, affect at a
6 distance. So Dr. Park's analysis was confined to
7 single site mutations. What this is analysis is
8 doing is showing even for single site mutations at
9 one position, if I put one type of residue in,
10 I get a reduction of 50% activity. If I put
11 another in, I get 2.7. Now these mutations would
12 fall in and I haven't gone to check, but I think
13 these are non-conserved residues and I can go to
14 Dr. Park's spreadsheet, the ones that are found in
15 the MSA that are represented here, Dr. Park would
16 have assigned to the buckets 2 and 3 is my
17 prediction. I haven't checked every single one
18 but he selected his residues not universally, he
19 didn't test all of them because he only used MSA
20 to guide the choice.
21 Q. What if it's the case that he
22 assigned one of those residues to bucket 1, that
23 it would be untolerated? Based on your analysis,
24 right, when Dr. Park assumes that a mutation is
25 going to be not tolerated and inactivate the

1 NAISMITH - MARTIN
2 protein, it is because of a stability issue. But
3 when you look at the patent data and you find a
4 single point mutation inactivates the protein, it
5 is because that point mutation, wherever it is,
6 somehow impacts the active site, not because of a
7 stabilization issue, because it is actually
8 impacting the active site through some sort of
9 long-range situation?
10 MR. KUSHAN: Objection foundation,
11 objection as to form.
12 A. So there was a lot in there. So,
13 to go back, Dr. Park, his analysis is what a POSA
14 would reasonably predict and some, I think there
15 was one prediction in fact that Dr. Petsko
16 pointed out, didn't chime with the data. What you
17 are seeing -- so he assigns them to bucket 1, 2,
18 3. So he talks about destabilizing local
19 environment, but if you destabilize one bit of the
20 protein that will influence across the protein.
21 That is why he says you would not start here if
22 you were to try to make active single site
23 mutations. What I am talking about here, is
24 somebody has done all of these single site, well,
25 not all, but the ones I have plotted here, and

1 NAISMITH - MARTIN
2 I don't know, nor does the patent teach me, nor
3 can I model, why one mutation reduces the activity
4 by a factor of 2, and the other one increases the
5 activity by a factor of 2. But for activity
6 measurement that has to mean something is
7 happening at the catalytic site.
8 Q. Okay, so based on what you just
9 said, then when Dr. Park assumes that, or assigns
10 a particular mutation to bucket 1, then it must be
11 the case that something is happening at the active
12 site; right?
13 A. No, I didn't say that. Dr. Park's
14 analysis is based on he predicted that his
15 bucket 1 would be destabilizing for proteins, and
16 therefore likely to lead to inactive protein. He
17 did not pursue those further, he just said
18 destabilizing is likely to be a negative mutation
19 so not when a POSA would start for single site
20 mutations.
21 Q. And you would see that it would be
22 an inactive protein meaning that it was not
23 active?
24 MR. KUSHAN: Objection foundation,
25 and objection to the form of the question.

1 NAISMITH - MARTIN
2 MS. MARTIN: Let us take a break.
3 Let's go off the record.
4 THE VIDEOGRAPHER: We are going off
5 the record. The time is 5.22 p.m.
6 **(Short recess from 5.22 p.m. to 5.29 p.m.)**
7 THE VIDEOGRAPHER: We are back on
8 the record. The time is 5.29 p.m.
9 Q. (BY MS. MARTIN): Okay,
10 Dr. Naismith can you please turn to paragraph 124?
11 Are you at paragraph 124?
12 A. Yes, I am.
13 Q. Okay you say in paragraph 124 that:
14 "Dr. Petsko's practical answer that a POSA would
15 not have even tried to make modified PH20
16 polypeptides with more than about 5 modifications
17 reflects 2011-2012 timeframe for producing
18 multiply modified PH20 polypeptides." Is that
19 right?
20 A. Yes, that is what I said.
21 Q. Dr. Petsko didn't actually testify
22 that a POSA would not have even tried to make
23 modified PH20 polypeptide with more than about
24 five modifications, did he?
25 A. He did not specifically address

1 NAISMITH - MARTIN
2 five but he also said, you know, there is a lot of
3 stuff no POSA would go ahead making them all.
4 Q. Well, are you telling me that he
5 literally testified a POSA would not go about
6 making them all because there's a lot of stuff?
7 MR. KUSHAN: Objection foundation.
8 A. That's not what I said.
9 Dr. Petsko, when talking about the protein in his
10 deposition was asked, you know, of all the
11 variants and he said, "No POSA would go about a
12 rational design that way", that is what he said.
13 I took that to -- he did not offer any opinion on
14 how one might go about five or more mutations.
15 Dr. Hecht pointed out that that is where his view
16 a POSA draws the line, it's where a draw a line.
17 There's nothing in Dr. Petsko's book or any other,
18 I'm aware of, that suggest he has some particular
19 insight how you would get beyond 5. When he was
20 asked about how you explore large numbers he did
21 said, well, you wouldn't do that.
22 Q. Well, okay, let's be very precise
23 about what he did and did not say since you seem
24 to be inclined to give opinions on Dr. Petsko's
25 behalf. So he was asked in the testimony that is

1 NAISMITH - MARTIN
2 shown on page 84 in your declaration, if someone
3 were asked how to -- and they were asked to
4 determine the number of PH20 polypeptides in the
5 genus that were properly folded and enzymatically
6 active, how they would go about doing that. To be
7 clear he was asked to give a number, right, not --
8 he wasn't asked about making proteins with
9 multiple mutations. He was asked to give a number
10 of proteins; right?
11 MR. KUSHAN: Objection foundation,
12 objection as to form.
13 A. So, because he said the number he
14 was able to calculate mathematically and agreed
15 with the calculation that Dr. Park did, he is
16 being asked here, determine the genus, so what is
17 the molecules that were consistent with claim 1
18 and it is qualified in the question: "...
19 properly folded and enzymatically active, how
20 would they go about doing that?" So how might
21 they -- so in all these variations, of which we
22 agree five is included in that, he said you just
23 wouldn't do that. You: "... wouldn't even go
24 about doing that." He said.
25 Q. (BY MS. MARTIN): Sir, you are

1 NAISMITH - MARTIN
2 blatantly mischaracterizing is testimony.
3 MR. KUSHAN: Are you done with your
4 answer?
5 (To Ms. Martin) Don't interrupt
6 the witness in his middle of answer, please.
7 MS. MARTIN: Okay, please continue
8 to mischaracterize if you weren't done.
9 A. I am not trying to mischaracterize
10 his testimony.
11 Q. Okay, so, again because you just
12 **did**, right, you said: "... they were asked to
13 determine the **number** of PH20 polypeptides in that
14 genus of claim 1, that were properly folded and
15 enzymatically active, how would they go about
16 doing that?" And he said: "They wouldn't even go
17 about doing that." Meaning they would not go
18 about determining the **number** of PH20 polypeptides
19 in the genus of claim 1 that were properly folded
20 and enzymatically active; right?
21 MR. KUSHAN: Objection foundation,
22 objection to form.
23 A. How I read his testimony is that
24 presented with the challenge of what is covered by
25 the patent scope and it is qualified here as:

1 NAISMITH - MARTIN
2 "... properly folded and enzymatically active
3 ...", he was asked how would you know which ones
4 were active and properly folded? And he goes on
5 to say in the last answer apart: "... from
6 experimenting on the entire ensemble" he wouldn't
7 know.
8 Five mutations, for example, is
9 within the mathematical scope description, but
10 I believe Dr. Petsko would have been confident if
11 he want, he could have expressed a view and
12 I would have agreed with it, that up to five
13 mutations based on PH20 there is an element of
14 predictability but beyond that we all agree there
15 is not an element of predictability. In
16 Dr. Petsko's answer the point is that he is
17 talking about the genus, how would you know which
18 of the genus is active and folded? And in that
19 parameter space are five mutations and he says you
20 wouldn't go about doing it that way. Then in his
21 last answer: "Is it possible to experimentally
22 determine which ones are properly folded and
23 enzymatically active ..." and he said: "Not from
24 experimenting on the entire ensemble."
25 Q. Yes, it is not possible -- okay, we

1 NAISMITH - MARTIN
2 don't have to do that again. So now, let's see,
3 in fact in your declaration you admit that
4 Dr. Petsko describes an example of making 22
5 changes which would be more than five?
6 A. Could you refer me to that please?
7 Q. Page 92, you say: "In
8 paragraph 359 f his declaration (below), he
9 provides an example where the POSA inspects
10 positions that tolerated between 60% and 100% of
11 the tested changes using a homology model and
12 selects sets of 22 substitutions from those
13 positions." So, you recognized in your declaration
14 that in fact Dr. Petsko did not draw a line and
15 conclude that a POSA would not have even tried to
16 make modified PH20 polypeptides with more than
17 about five modifications because you acknowledge
18 that he provided examples of making 22 changes;
19 right?
20 MR. KUSHAN: Objection foundation,
21 objection as to form.
22 A. So Dr. Petsko, as I read it, he is
23 going beyond what is in the patent, and he is
24 saying that in his opinion that if -- and he
25 qualifies it later on by talking about

1 NAISMITH - MARTIN
2 independent, that he thinks that if I selected 23
3 residues that it would fold into an active, again
4 he uses active here, changes. And I disagree with
5 his opinion that you could in any way predict
6 which of the 23 is possible to make.
7 So, while I accept that I can
8 imagine a protein with 23 residues to be properly
9 folded and active just as Dr. Petsko talks about,
10 my point is that I had no way to predict or
11 reliably identify what that sequence would be for
12 the reasons that I have shown you in my deposition
13 -- sorry expert report, my apologies.
14 Q. (BY MS. MARTIN): Okay, so can we
15 turn quickly to Dr. Petsko's deposition?
16 A. Of course.
17 Q. At page 113?
18 A. Can I take some time to familiarize
19 myself with the document please?
20 Q. You want to read his deposition
21 transcript?
22 A. Just around the part here, help me
23 to set in context, if you don't want me to I will
24 try to answer the questions as best I can.
25 Q. I just want to point you to -- are

1 NAISMITH - MARTIN
2 you on page 113?
3 A. Page 113, yes.
4 Q. So there is a question and answer
5 starting on line 20 that says: "So using that
6 dataset, the person could predict which
7 combinations might yield an active form of a
8 mutated PH20 polypeptide let's say with 15 changes
9 to it?
10 "(A) You could, but as I said, you
11 would not tend to do that. A person of skill in
12 the art would tend to only be interested in a much
13 smaller number of mutations, because you can
14 almost certainly get where you want to go that
15 way." Right?
16 A. I can read that.
17 Q. All right. So we talked about
18 Example 5 in the common disclosure ----
19 A. Can I put away the deposition or do
20 you want me to keep it open?
21 Q. No, you can put that away.
22 A. Can you remind me what page it is
23 on, sorry? I know we talked about Example 5, just
24 to help me remember the page.
25 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

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.

1 NAISMITH - MARTIN
2 **(Short recess from 5.44 p.m. to 6.09 p.m.)**
3 THE VIDEOGRAPHER: We are back on
4 the record. The time is 6.09 p.m.
5 Q. (BY MS. MARTIN): Dr. Naismith,
6 what level of predictability are you requiring in
7 order to be confident that a particular mutant
8 will be active without having to test it?
9 MR. KUSHAN: Objection foundation,
10 also objection as to form.
11 A. So what particular mutant? Just
12 any random mutant?
13 Q. (BY MS. MARTIN): Yes, any random
14 mutant.
15 A. I would distinguish, I would use
16 some of what we already know to answer your
17 question, so is it in the non-conserved region
18 that Dr. Park analyzed?
19 Q. And if it is?
20 A. Then those particular mutants he
21 has assigned to buckets 1, 2, 3, and I have said
22 that I would have confidence that the ones that he
23 has assigned into buckets 2 and 3 you would have a
24 reasonable expectation that those single mutations
25 would be stable and active, natively folded and

1 active.
2 Q. And so, what does "reasonable
3 expectation" mean to you, how certain do you have
4 to be?
5 MR. KUSHAN: Objection foundation,
6 also objection as to form.
7 A. I don't need to -- a reasonable
8 expectation is that it is likely to be correct. I
9 don't think, the majority, I didn't think of a
10 single dividing line percentage if that is your
11 answer, your question.
12 Q. (BY MS. MARTIN): Would you say
13 more than 50% certain, more likely than not?
14 A. Yes, more likely than not that
15 would be, that would be one that I would be,
16 I would expect some surprises but I would expect
17 more likely than not about 50%, based on
18 Dr. Park's analysis and how he's gone about it,
19 that's for single sites, yes.
20 Q. And if you were to make, let's say,
21 a triple PH20 mutant at positions that are shown
22 to be tolerated or produce an active protein in
23 the patent, would you think it is more likely than
24 not that you would get an active protein?
25

1 NAISMITH - MARTIN
2 MR. KUSHAN: Objection foundation,
3 objection as to form.
4 A. So the triple mutation also has the
5 M320K?
6 Q. (BY MS. MARTIN): D320K.
7 A. I apologise, I am tired. So D3, so
8 that is one of my triple. So my two mutations
9 were both active in the single mutation?
10 Q. Yes.
11 A. And I've got a D320K and I have two
12 additional mutations both of them were acting
13 single, I would say more likely than not I would
14 expect those to be active. But I qualify only
15 that some mutations, some residues at that
16 position were either shown to be inactive, right.
17 So at some positions they were classified as
18 inactive when you made a particular change, so
19 I wouldn't have confidence that if I introduced
20 one which had been classified as inactive, but
21 that would give a predictable more likely than not
22 active.
23 Q. Do you agree that the cell culture
24 techniques that were described in the patent would
25 have been routine in 2012?

1 NAISMITH - MARTIN
2 A. Sorry, were what in 2012?
3 Q. The cell culture techniques
4 described in the patent?
5 A. Were what in 2012, sorry?
6 Q. Would have been routine in 2012?
7 A. They were within the skill of a
8 POSA 2012, yes.
9 Q. Do you agree that the patent
10 explains how to make modified PH20 polypeptide?
11 MR. KUSHAN: Objection foundation,
12 objection as to form.
13 A. So, the patent doesn't describe how
14 to make modified PH20 polypeptides beyond a
15 certain number of mutations that would retain
16 activity and properly folded.
17 Q. (BY MS. MARTIN): Did you consider
18 the fact that microtiter plates can vary in terms
19 of the amount of biotinylated HA, that is coded in
20 the well, informing your opinions regarding
21 variability of the assays in the patent?
22 A. So you are identifying something
23 that is not properly controlled and you would
24 expect to try to control for that, but nothing in
25 the disclosure tells me that they had a concern

1 they had some trouble with reproducibility.
2 Q. You can control for defects in a
3 plate?
4 A. That is a different question. So
5 you are now postulating that the reason for these
6 differences could be that there was a bad plate?
7 Q. Yes.
8 A. In that case I would have gone back
9 and done the experiment again if I got these
10 deviations.
11 Q. Okay. Did you consider variations
12 in the size of the HA polymer that is in the well
13 in rendering your opinions on variability?
14 A. So do you mean the variation HA
15 polymer between plates or the fact that you have a
16 population of species in a well.
17 Q. The difference in species between
18 wells?
19 A. So again the patent tells you how
20 they got to that, you are postulating that they
21 varied it in some uncontrolled way. I don't think
22 a POSA would normally do that. There could be
23 stochastic variation but you are told how these
24 source material, so again as a POSA, reading the
25

1 method, there is nothing to direct you that they
2 have wild variation in the species.
3 Q. You keep saying that there could be
4 stochastic variation, but is there not always
5 stochastic variation in life?
6 A. You are quite right and that is the
7 error threshold of the assay, but before a
8 scientist assigns difference to that, you want to
9 be sure you haven't eliminated obvious things
10 which you know could give rise to, a student uses
11 the wrong plate, you gave an example of a
12 defective plate or that two different people
13 prepared, one of them inexperienced prepared the
14 HA biotinylation or that somebody brought a
15 different batch of HA. All of those would give
16 rise to variation, but there is nothing in the
17 disclosure which would lead me to believe that
18 they set out to do that. As I say, it is not
19 normally how POSAs approach experiments, so I have
20 assumed that they would approach it as a normal
21 POSA would.
22 Q. You agree that a normal POSA would
23 expect there to be some stochastic variation, just
24 as matter of the fact that their experiments that
25

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.

1 NAISMITH - MARTIN
2 So normally it is one of the things
3 that I would have my students do, is make sure if
4 you are doing repeats or multiple plates you try
5 as hard as possible to have them done in exactly
6 the same way so that the variation you see is a
7 genuine reflection of stochastic variation rather
8 than error, poorly done assay. You can control
9 for temperature.
10 Q. What about humidity?
11 A. We actually measure humidity in a
12 lot of the labs. If you thought that the assay
13 was particularly sensitive to humidity you would
14 control for it.
15 When I used to worked in Dallas
16 some days were very humid or very hot so it can
17 affect an experiment and you would know that. So
18 you would try to replicate it under, as close as
19 possible, the same conditions. Labs are usually
20 air conditioned to try to minimize that variation.
21 Q. Even if you do try your hardest
22 there is still going to be some amount of
23 variability; right?
24 A. Yes, as Dr. Petsko said, well-run
25 10% or less, a well-run assay, he thought 10%

1 NAISMITH - MARTIN
2 roughly or less would be a well-run of this type
3 of ELISA assay and I would agree with him.
4 Q. Okay. Do you know if he was
5 thinking about testing supernatant that had not
6 controlled for differences in protein
7 concentration when he said 10%?
8 A. I think his opinion was that that
9 would be what he would imagine a well-run
10 experiment is. We talked about before, that based
11 on the way they approached the method, and
12 I talked about the wild-type protein only, then
13 you are trying to control for any variation by the
14 way you do the experiment in the same way. There
15 is nothing in the description that makes me or
16 guides me to them making a change to the
17 temperature, humidity and performing the assay.
18 MS. MARTIN: I don't have any
19 further questions.
20 MR. KUSHAN: Why don't we have a
21 short break and come back?
22 THE VIDEOGRAPHER: We are going off
23 the record. The time is 6.20 p.m.
24 **(Short recess from 6.20 p.m. to 6.25 p.m.)**
25 THE VIDEOGRAPHER: We are back on

1 NAISMITH - MARTIN
2 the record. The time is 6.25 p.m.
3 MR. KUSHAN: We have no further
4 questions.
5 THE COURT REPORTER: Could I ask
6 you to put your transcript orders on the record
7 before we go off, please?
8 MS. MARTIN: Whatever our normal --
9 wait its different. Can we do a rough and then
10 final?
11 THE COURT REPORTER: We will do it
12 off the record.
13 MS. MARTIN: Okay, that is easier.
14 THE VIDEOGRAPHER: This concludes
15 the deposition. We are going off the record. The
16 time is 6.26 p.m.
17 **(Deposition concluded at 6.26 p.m.)**
18
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1
2 **CERTIFICATE OF WITNESS**
3
4 I HEREBY CERTIFY that having been first
duly sworn to testify to the truth, I gave the
5 above testimony.
6
I FURTHER CERTIFY that the foregoing
7 transcript is a true and correct transcript of the
testimony given by me at the time and place
8 specified hereinbefore.
9
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18
DR. JAMES NAISMITH
19
20
Subscribed and sworn to before me
21
22
this_____day of_____20___
23
24
25

1
2 **CERTIFICATE OF COURT REPORTER**
3
4 I, AMY COLEY, Court Reporter, do
5 hereby certify that DR. JAMES NAISMITH the witness
6 whose deposition is hereinbefore set forth, was
7 duly sworn by me and that such deposition is a
8 true record of the testimony given by the
9 witness.
10 I further certify that I am not
11 related to any of the parties to this action by
12 blood or marriage, and that I am in no way
13 interested in the outcome of this matter.
14

18
19 .....
20 AMY COLEY
21
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2 **ERRATA SHEET**
3 DATE OF DEPOSITION: 2ND FEBRUARY, 2026
4 **WITNESS: DR. JAMES NAISMITH**
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