Transcript of Dr. Sheldon Park

Date: August 7, 2025
Case: Merck Sharp & Dohme LLC -v- Halozyme Inc. (PTAB)

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Halozyme EX2077
Merck v. Halozyme
PGR2025-00030

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

MERCK SHARP & DOHME LLC,

Petitioner,

v.

HALOZYME INC.,

Patent Owner.

Case Nos. PGR2025-00003;

PGR2025-00004; PGR2025-00006;

PGR2025-00009

U.S. Patent No. 12,123,035

Videotaped Deposition of

DR. SHELDON PARK

Buffalo, New York

Thursday, August 7, 2025

8:45 a.m. EST

Job No.: 594918

Pages: 1 - 234

Reported By: Kadi A. Harmon

Deposition of DR. SHELDON PARK, held at the offices of:

Hyatt Regency Buffalo
Two Fountain Plaza
Buffalo, NY 14202

Pursuant to notice, before Kadi A. Harmon, Court Reporter and Notary Public in and for the State of New York.

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Raechel Meyerowich, Technician
Mark Stewart, Merck
Eric Majcherzak, Merck
Aubrey Hoddach, Halozyme
Joshua Mack, Halozyme
Dr. Michael Hecht

C O N T E N T S

E X H I B I T S

(Attached to the Transcript.)

PREVIOUSLY MARKED EXHIBITS

(Retained by Counsel.)

P R O C E E D I N G S

THE VIDEOGRAPHER: Here begins Media Number 1 in the videotaped deposition of Dr. Sheldon Park, in the matter of Merck Sharp & Dohme, LLC versus Halozyme Inc., in the United States Patent and Trademark Office, before the Patent Trial and Appeal Board; Case Numbers PGR2025-00003, PGR2025-00004, PGR2025-00006, and PGR2025-00009. 08:44:30 08:44:38 08:44:40 08:44:45 08:44:48 08:44:50 08:44:54 08:45:08

Today's date is Thursday, August 7th, 2025. The time on the video monitor is 8:45 a.m., Eastern time. 08:45:13 08:45:18 08:45:22

The videographer today is Charlotte Forrest representing Planet Depos. This video deposition is taking place at Two Fountain Plaza, Buffalo, New York, 14202. 08:45:22 08:45:25 08:45:25 08:45:31

Would Counsel please voice-identify themselves and state whom they represent? 08:45:33 08:45:34

ATTORNEY MARTIN: Lauren Martin from Quinn Emanuel on behalf of Patent Owner, and with me I have Elliot Choi, also from Quinn Emanuel, and Trey Powers, from Sterne Kessler. 08:45:36 08:45:39 08:45:42 08:45:50

ATTORNEY KUSHAN: Jeff Kushan from Sidney Austin on behalf of Petitioner, Merck. With me is Christine Engen. 08:45:50 08:45:55 08:45:55

Also, remotely we have attendees Kalen Sullivan and Chelsea Himes from Sidney Austin, and I believe Mark Stewart from Merck and Eric Majcherzak from Merck, and you'll have to get a spelling on the last one. 08:45:56 08:46:02 08:46:05 08:46:12 08:46:15

I think that's it for us. 08:46:17

Do you want to announce your remote participants? 08:46:18 08:46:22

ATTORNEY MARTIN: Yeah. Can someone announce the remote participants? 08:46:22 08:46:23

ATTORNEY ELLISON: I can do it. 08:46:32

This is Eldora Ellison from Sterne, Kessler, Goldstein & Fox on behalf of Halozyme. And it looks like we're joined by Zach Summers from Quinn Emanuel, Aubrey Hoddach from Halozyme, Josh -- Joshua Mack from Halozyme, and I think that's it. Thank you. 08:46:37 08:46:38 08:46:39 08:46:40 08:46:44 08:46:48

ATTORNEY MARTIN: Thank you. 08:46:50

THE VIDEOGRAPHER: All right. Thank you, 08:46:50

Counsel. 08:46:52

The Court Reporter today is Kadi Harmon 08:46:52

representing Planet Depos. The witness will now 08:46:55

be sworn. 08:46:58

THE COURT REPORTER: Okay. Would you

raise your right hand, please?

THE WITNESS: Yes.

THE COURT REPORTER: Do you swear or

affirm that the testimony you provide today will

be the truth, the whole truth and nothing but the

truth? 08:47:13

THE WITNESS: I do. 08:47:13

THE COURT REPORTER: Thank you. 08:47:16

E X A M I N A T I O N 08:47:17

BY ATTORNEY MARTIN:

Q Good morning, Dr. Park. 08:47:18

A Good morning. 08:47:19

Q Can you please state your full name and 08:47:19

address for the record? 08:47:22

A Sheldon Park, at 4 Fawn Meadows Court, 08:47:22

Amherst, New York, 14068. 08:47:26

Q Where are you employed, Dr. Park? 08:47:28

A I am employed at the University at Buffalo. 08:47:30 08:47:33

Q And how long have you been at the University of Buffalo? 08:47:33 08:47:36

A I have been there since year 2006. 08:47:37

Q And what's your position at University of Buffalo? 08:47:40 08:47:43

A I'm currently an associate professor of chemical and biological engineering. 08:47:44 08:47:49

Q Do you have any interest in the outcome of this litigation? 08:47:53 08:47:55

A No. I do not. 08:47:55

Q When were you first contacted about this case? 08:47:57 08:47:59

A I was first contacted back in July 2024. 08:48:03

Q And do you recall who contacted you? 08:48:06

A Mr. Jeff Kushan contacted me. 08:48:09

Q Have you ever been deposed before? 08:48:14

A No. This is the first time. 08:48:16

Q So Dr. Park, if at any time during the deposition you would like clarification on one of my questions, please ask me for clarification, and 08:48:18 08:48:26 08:48:30

not your counsel. 08:48:33
A I will. 08:48:34
Q Thank you. 08:48:35
Dr. Park, do you understand that you 08:48:36
cannot discuss the substance of this deposition 08:48:40
with anyone until the deposition is over, 08:48:42
including during breaks? 08:48:46
A I understand that. 08:48:47
Q Thank you. 08:48:48
Your counsel may object to some of my 08:48:49
questions during the course of the deposition, but 08:49:00
you have to answer every question unless you're 08:49:02
instructed not to answer by your counsel. 08:49:05
Do you understand? 08:49:08
A Yes. I understand. 08:49:08
Q And if at any time you need to take a 08:49:09
break, just let me know and we can take a break. 08:49:13
But I do ask that if a question is pending, you'll 08:49:16
answer the question and then we can take a break. 08:49:19
A I understand. 08:49:22
Q All right. Have you collaborated with 08:49:22
Merck before in your -- in your career? 08:49:32

A No. I have not. 08:49:34

Q Has Merck funded any of your research? 08:49:35

A No. 08:49:43

Q Have you ever had any -- any type of the relationship with Merck, other than this litigation? 08:49:44 08:49:55 08:49:57

A No. 08:49:58

Q Do you know Dr. Michael Hecht? 08:49:58

A I don't know him personally. I know of his name from his publication. I read his work. That is the extent of my familiarity with Dr. Hecht. 08:50:06 08:50:10 08:50:15 08:50:19

Q Have you ever collaborated with Dr. Hecht? 08:50:19

A He wrote a chapter for me in a book that I was editing. 08:50:23 08:50:30

(Reporter clarification.) 08:50:32

Q Any other collaboration? 08:50:32

A No. 08:50:35

Q Did you speak with him about his book chapter, or did you correspond with him about his book chapter? 08:50:36 08:50:39 08:50:43

A I emailed him, invited him to contribute a 08:50:44

chapter, and he was nice enough to do that. 08:50:47

Q Why did you ask him to contribute the chapter? 08:50:47 08:50:49

A Because he was an authority in the field. 08:50:50

Q Have you ever heard of the company Alteogen? 08:51:01 08:51:05

A What company? 08:51:06

Q Alteogen. 08:51:08

A No, never. 08:51:10

Q Do you have any experience with hyaluronidases? 08:51:11 08:51:27

A No. 08:51:28

Q Do you have experience with enzymes generally? 08:51:29 08:51:34

A No. 08:51:34

Q Do you know how many human hyaluronidases there are? 08:51:35 08:51:42

A Yes. I believe there are five versions of hyaluronidases. 08:51:45 08:51:49

Q Do you know what they're called? 08:51:50

A Hyaluronidase 1 through 4, and PH20. 08:51:52

Q Do you know which ones cleave hyaluronic 08:51:58

acid? 08:52:04

A HYAL1 and 2, and PH20 are believed to be hyaluronidases. HYAL4, I think is a -- got something related, the name of which just escapes me. And then Hyaluronidase 3, I don't think it has a known activity. 08:52:04 08:52:10 08:52:20 08:52:25 08:52:34

(Reporter clarification.) 08:52:38

A Known enzymatic activity. 08:52:38

Q Have you done any work with PH20? 08:52:45

A No. 08:52:49

Q What's the function of PH20? 08:52:50

ATTORNEY KUSHAN: Objection. Form. 08:52:57

A PH20, based on my reading, is an enzyme that cleaves hyaluronic acid. 08:53:01 08:53:06

(Reporter clarification.) 08:53:18

Q Do you know specifically how PH20 cleaves hyaluronic acid, what the mechanism of action is? 08:53:18 08:53:26

A I read papers on that, so I have some knowledge of it. 08:53:29 08:53:33

Q And did you read those papers in connection with your work on this case? 08:53:34 08:53:38

A I did. 08:53:39

Q So outside of your work on this case, do you have any -- strike that. 08:53:40 08:53:45

Before you started working on this case, had you ever had any experience with PH20? 08:53:50 08:53:56

A No. 08:53:58

Q Before you started working on this case, had you ever -- had you had any experience with hyaluronidases? 08:53:59 08:54:03 08:54:08

A No. 08:54:09

Q Did you know how many human hyaluronidases there were before you started working on this case? 08:54:10 08:54:14 08:54:16

A No. 08:54:17

Q Did you know which human hyaluronidases cleave hyaluronic acid before you started working on this case? 08:54:17 08:54:21 08:54:26

A No. 08:54:27

Q So is it fair to say that all of your hyaluronidase-related knowledge was learned in connection with this case? 08:54:27 08:54:31 08:54:36

ATTORNEY KUSHAN: Objection to form. 08:54:37

A Much of what I know about hyaluronidase 08:54:43

comes from the reading that I initiated in relation to this case. 08:54:46 08:54:52

Q Before you started working on this case, had you examined any structures of hyaluronidase proteins? 08:55:05 08:55:08 08:55:14

A No. 08:55:16

Q Before you started working on this case, had you performed any sequence assignment analysis for hyaluronidases? 08:55:16 08:55:19 08:55:26

A No. 08:55:26

Q Have you performed any structural analysis on any other carbohydrate active enzyme? 08:55:30 08:55:39

ATTORNEY KUSHAN: Objection to foundation. 08:55:46

A Any carbohydrate enzymes? 08:55:47

Q Uh-huh. 08:55:52

A Yes. I was working on a project, NIH-funded project. 08:56:02 08:55:58

Q And what enzyme was that? 08:55:59

A It was ST3GAL1. 08:56:14

(Reporter clarification.) 08:56:24

Q And what work did you do with ST3GAL1? 08:56:24

A It's an enzyme that transfers hyaluronic 08:56:26

acid to a glycan we were engineering. My -- my 08:56:30
collaborator and I were working on engineering 08:56:35
that enzyme into a glycan binding protein. 08:56:39
Q And did you succeed in transforming the 08:56:48
enzyme into a glycan binding protein? 08:56:54
A Yes. We did. 08:56:58
Q How did you do that? 08:56:59
A Combination of rational engineering and 08:57:01
directed evolution. 08:57:05
(Reporter clarification.) 08:57:15
Q And when -- when did you do that work? 08:57:15
A The work was funded around 2020. The 08:57:19
money might have started coming in 2021. I forget 08:57:27
the exact time. And for the last four or five 08:57:33
years, we've worked on it. 08:57:36
Q Have you done any work with any other -- 08:57:46
sorry. Strike that. 08:57:51
Have you done any work with glycoside 08:57:52
hydrolase enzymes? 08:57:58
ATTORNEY KUSHAN: Objection. Form. 08:58:01
A I have not worked with any other glycoside 08:58:03
hydrolase enzyme. 08:58:09

ATTORNEY MARTIN: I'm going to introduce the declaration -- your four declarations for the four PGRs that we're talking about. 08:58:11 08:58:13 08:58:17

These have been -- these are of record in the cases, so the first one is Exhibit 1004 to PGR2025-00003, regarding US Patent Number 11,952,600. 08:58:22 08:58:27 08:58:35 08:58:44

THE WITNESS: Okay.

(Previously marked Exhibit 1004 introduced and retained by counsel.) 08:58:54

ATTORNEY MARTIN: All right. The second one is Exhibit 1004 for PGR2025-00004, regarding US Patent Number 1,218,298. 08:58:54 08:58:55 08:59:03

THE WITNESS: Okay. 08:59:10

(Previously marked Exhibit 1004 introduced and retained by counsel.)

ATTORNEY MARTIN: The third is Exhibit 1004 for PGR2025-00006, regarding US Patent Number 12,152,262. 08:59:11 08:59:12 08:59:23

(Previously marked Exhibit 1004 introduced and retained by counsel.) 08:59:27

ATTORNEY MARTIN: And then the fourth is 08:59:27

Exhibit 1004 for PGR2025-00009, regarding US Patent Number 12,123,035. 08:59:29 08:59:39

(Previously marked Exhibit 1004 introduced and retained by counsel.)

BY ATTORNEY MARTIN:

Q Dr. Park, are these four the declarations that you submitted in support of each of the four PGRs? 08:59:45 08:59:48 08:59:52

A Yes. They are the four declarations I submitted. 09:01:16 09:01:19

Q And you signed each of those four declarations, correct? 09:01:20 09:01:23

A I did sign them. 09:01:24

Q So you have -- you have papers with you. Can you explain what that is? 09:01:26 09:01:33

A Oh, yeah. Absolutely. 09:01:34

I wanted to identify some minor errors -- 09:01:36

Q Okay.

A -- that came in. 09:01:39

Q Uh-huh.

A Should I give them to you first, or read them to you? They're minor. 09:01:44 09:01:47

First one, which appears in Paragraph 145(b) of the proceeding ending in 3, it reads, somewhere in there, 134 rows. It should be 95 rows. 09:01:50 09:01:57 09:02:08 09:02:15

The reason that this error came in is because initially the analysis was performed with the sequences that were available by the end of year 2012, December 2012. Afterwards, the sequences were revised to end in December of 2011, one year prior. As a result, the number of sequences that were included in the sample was reduced from 134 to 95. So that's what the first errata indicates. 09:02:17 09:02:21 09:02:26 09:02:28 09:02:39 09:02:49 09:02:54 09:02:58 09:03:08

Q Okay. So to be clear, if you're on Page 80 of the declaration for proceeding ending in 3 -- 09:03:08 09:03:11 09:03:17

A Uh-huh. 09:03:19

Q -- it says -- there's a sentence that says there were 134 rows, and you're saying that the number 134 should instead read 95? 09:03:19 09:03:27 09:03:32

A That is correct. 09:03:34

Q Thank you. Okay. 09:03:35

A And the corresponding errors in the other proceedings are, in the proceeding ending in 4, Paragraph 151(b); proceeding ending in 006, Paragraph 147(b); and the final proceeding ending in 9, Paragraph 127(b), the substance of the error is the same. 09:03:39 09:03:42 09:03:46 09:03:53 09:03:57 09:04:02

Q So in each of those cases, the -- there were 134 rows should read there were 95 rows? 09:04:02 09:04:05

A That is correct. 09:04:07

Q Okay. 09:04:08

A The next error is a typo, which is in paragraph -- they're all in the same paragraph of all four proceedings, Paragraph 97. 09:04:10 09:04:18 09:04:25

It reads, currently: Position 374 in PH20. 09:04:30 09:04:37

It should read: Position 372 in PH20. 09:04:38

That's a typographical error. 09:04:43

Paragraph 42 should read, instead of isoforms of PH20, the correct terminology should have been: Homologs of PH20. 09:04:47 09:04:53 09:05:00

(Reporter clarification.)

A Paragraph 12 -- 09:05:05

Q Sorry, what? Paragraph 42? 09:05:05

A 42. Yeah. 09:05:11

The last sentence of Paragraph 42 says: 09:05:23

At least four isoforms of PH20. 09:05:28

Q Oh, okay. Okay.

A Should read: Homologs of PH20. 09:05:31

Q Thank you. 09:05:36

A And Paragraph 12, that's the ending of 09:05:37

Paragraph 12. I believe just prior to the 09:05:43

parentheses should read, instead of structure, 09:05:48

structure relationship, should simply read: 09:05:53

Structure. 09:05:59

Q As well as with techniques and tools used 09:05:59

to analyze protein structure? 09:06:04

A That is correct.

Q Period.

A Period.

Q Or, actually, it's: Protein structure, 09:06:10

parentheses. 09:06:10

A That's correct. 09:06:11

Q Got it. 09:06:11

A There are errors unique to some of the 09:06:12

proceedings. 09:06:36

Proceeding ending in 03, Paragraph 109, it 09:06:16

lists a few amino acids in that paragraph. 09:06:23

109, yes. In the parentheses, it should 09:06:44

say: E31, P32 and D34, which is currently 09:06:51

missing. And L317, N321, E324. 09:06:57

Paragraph 100 in the same document also 09:07:06

lists several -- Paragraph 100, I think it should 09:07:20

have been in Paragraph 100. I don't see that. I 09:07:42

think the paragraph number is not correct. 09:07:49

Maybe I can -- I think that it should be 09:07:55

around -- let me see -- 109. So let me go there 09:08:00

and maybe I can find it. In fact, it's 09:08:04

Paragraph 110. 09:08:08

Q Oh, okay.

A Yeah. Not 100. 09:08:09

Q Okay. 09:08:11

A Yeah. In the -- in there, there's a 09:08:12

parentheses that ends in M323. M323 should not be 09:08:15

there. 09:08:24

Q Okay.

A So it ends in L319. 09:08:24

Okay. Appendix C in the same document, Row Number 4, Column J, says: K320 has 9 neighbors. 09:08:30 09:08:39 09:08:45

It should read: K320 has 8 neighbors. 09:08:45

Moving on to the next document, proceeding ending in 4, 004, Paragraph 134, typographical error of 34. 09:08:53 09:08:57 09:09:07

It says: Glutamate at position 34. 09:09:12

It should say: Glutamate at position 31. 09:09:17

Oh, yes. Yes. Halfway through glutamate at position 31. 09:09:32

(Reporter clarification.)

A Moving on to the next one. 09:09:53

Appendix C was cut off. It's a table of evaluation. And in an Excel spreadsheet, it should have been a wrap around, but the column got cut off, so some of the words got left out. So Appendix C -- yeah. 09:09:54 09:09:59 09:10:04 09:10:08 09:10:13

Okay. In this table, the column -- Row Number 4, Column 1 -- I'm sorry -- Column I, not Column 1, the comments column. 09:10:28 09:10:34 09:10:38

It should say: Hydrophobic contacts

to F64.

So that was left out. 09:10:45

Q Sorry. For which row? 09:10:45

A It says Row Number 4. 09:10:48

Q Number 4.

A Column I. The comments column, it says: 09:10:50

Hydrophobic contacts to --

(Reporter clarification.)

A Hydrophobic contacts to F64. 09:11:00

Appendix -- the same appendix, Row 09:11:05

Number 5 this time, the same column: Potential 09:11:08

charge repulsion. 09:11:13

Row Number 8: Hydrophobic contacts to 09:11:18

F64. 09:11:26

Row Number 10: Increased hydrophobic 09:11:26

contacts, hydrophobic exposed. 09:11:31

Row Number 11: Increased hydrophobic 09:11:36

contacts.

And finally, Row Number 13: Increased 09:11:39

hydrophobic contacts, Ncap. 09:11:45

So those are the minor errors I 09:11:49

identified. I wanted to bring them to your 09:11:54

attention. 09:11:56

Q Okay. Thank you. 09:11:57

Is that all the -- did we get all of them? 09:11:59

A Absolute yes.

Q Okay. Thank you. 09:12:01

ATTORNEY KUSHAN: Those are just copies. 09:12:01

Can I get one? 09:12:04

THE WITNESS: Okay. Absolutely. 09:12:05

ATTORNEY KUSHAN: Do you want to introduce 09:12:06

this as an exhibit? It's up to you. 09:12:08

ATTORNEY MARTIN: Yeah. Actually, that's 09:12:09

probably a good idea. 09:12:15

So Park Exhibit 1 will be the Errata. 09:12:17

ATTORNEY KUSHAN: Do you happen to know,

Trey, what the last exhibit... 09:12:23

ATTORNEY POWERS: 2037 [sic]. 09:12:23

ATTORNEY KUSHAN: So why don't we -- do 09:12:25

you want to do a --

ATTORNEY POWERS: Start at 2037. 09:12:29

ATTORNEY MARTIN: Okay. So 2037. 09:12:29

ATTORNEY KUSHAN: So this will be 2037. 09:12:30

ATTORNEY POWERS: Yeah.

(Park Deposition Exhibit 2068 marked for identification and attached to the transcript.)

ATTORNEY KUSHAN: Can you just --

THE WITNESS: Oh, okay. Yeah. 09:13:18

BY ATTORNEY MARTIN: 09:13:18

Q So Dr. Park, can you turn to the exhibit 09:13:20

list for -- 09:13:23

A Exhibit list. 09:13:23

Q Well, I guess let's use the --

A Sure. 09:13:23

Q -- the one ending in 3. 09:13:24

So each of your declarations has an 09:13:28

exhibit list, correct? 09:13:31

A I believe so. Should I check? 09:13:32

I believe that they are the same. I see 09:13:46

exhibit list here. There we go, assuming they are 09:13:49

the same. 09:14:02

Yes. I see an exhibit list there too. 09:14:02

Q Okay. So there's an exhibit list in each 09:14:05

of the four declarations, correct? 09:14:08

A That is correct. 09:14:09

Q All right. And just as an example, you 09:14:10

can look at the exhibit list for PGR 3, and that's on page -- starts on Page 99 of Exhibit 1004. 09:14:13 09:14:18

A Exhibit 1004?

Q Yes. 09:14:32

A 1004.

Q So that's the exhibit list? 09:14:34

A Yes. Yes. The declaration you mean. 09:14:35

Got it.

(Reporter clarification.) 09:14:40

Q So the exhibit list on -- you're at the exhibit list on Page 99 of the declaration on PGR 3? 09:14:40 09:14:44 09:14:46

A That is correct. I am. 09:14:47

Q All right. And Dr. Park, you listed all of the materials you considered forming your opinions in that declaration on your exhibit list, correct? 09:14:49 09:14:56 09:14:59 09:15:03

ATTORNEY KUSHAN: Objection. 09:15:04

A Yes. 09:15:07

ATTORNEY KUSHAN: Sorry. 09:15:08

Objection. Foundation. 09:15:11

Go ahead.

A Yes. I included the references that I read in preparation for this declaration. 09:15:12 09:15:13

Q Did you include all of the materials that you considered in forming your opinions on your exhibit list? 09:15:17 09:15:19 09:15:22

A I did. 09:15:27

Q Now your declaration in matter PGR 3, if you go to the cover page for that declaration, you see it says: Case Number PGR 2025-3, and then under that, there's a patent number, US Patent Number 11,952,600. 09:15:28 09:15:47 09:15:51 09:15:56 09:16:17

Do you see that? 09:16:05

A I see the patent number, yes. 09:16:05

Q Are you familiar with the '600 patent? 09:16:08

A No. I'm not familiar with this patent. 09:16:11

Q Have you ever seen the '600 patent? 09:16:13

A No. I have not seen this patent. 09:16:19

Q Did you consider the '600 patent in forming your opinions for this matter? 09:16:21 09:16:25

A No. I did not. 09:16:27

Q Did -- as part of your analysis in this case, did you go and look up the '600 patent? 09:16:33 09:16:35

A No. I did not look up the patent '600. 09:16:39

Q Have you ever read a patent? 09:16:43

A I read a patent that's listed in the 09:16:47

exhibit list for the first time. 09:16:51

Q And that patent is the '429 patent; is 09:16:54

that right? Exhibit 1005, US Patent Number 09:17:01

7,767,429? 09:17:09

A That's correct. '429 patent. 09:17:09

Q Is the '429 patent the first patent you 09:17:12

ever read? 09:17:15

A That is the first patent that I've ever 09:17:15

read. Yeah. 09:17:18

Q Okay. 09:17:19

A Except for my own patent, come to think of 09:17:31

it. 09:17:35

Q Uh-huh.

A It was so long ago I forgot about it. The 09:17:36

patent was prepared by the lawyers, and I think I 09:17:39

read -- I wouldn't say I read the final patent, 09:17:44

but whatever was prepared at some point along the 09:17:50

process, I was involved in drafting that patent, 09:17:55

so you could say that might count as a patent. 09:18:04

Q Do you know if it issued as a patent? 09:18:10
A Say it again? 09:18:15
Q Do you know if your patent issued? 09:18:16
A Oh. It was issued, yes. 09:18:18
Q All right. But you don't know if you've seen the issued patent? 09:18:19
A I have seen it, but I haven't read it. 09:18:20
Q Okay.
A It was good enough to know that I was granted a patent. 09:18:23
Q Understood. 09:18:26
All right. So is it fair to say that the only issued patent that you've ever read is the '429 patent? 09:18:27
A That is correct, yes. 09:18:32
Q Did you -- do you know what's claimed in the '600 patent? 09:18:35
A I understand it's about a mutation in a protein, which I assume is PH20. But that's about it. 09:18:37
Q Have you ever seen the patent claims for the '600 patent? 09:18:50
09:18:57
09:19:03
09:19:09
09:19:15
09:19:15
09:19:19

A No. I have not. 09:19:20

Q Do you know what's disclosed in the 09:19:21

specification for the '600 patent? 09:19:25

A No. 09:19:27

Q Do you know -- do you have any idea of 09:19:28

anything that's disclosed in the '600 patent? 09:19:31

A No. 09:19:35

Q When you read the '429 patent, did you 09:19:35

read the claims? 09:19:46

A Can I get a copy of the '429 patent to see 09:19:50

where the claim is listed? 09:19:54

Q Sure. 09:19:57

So while we wait for the '429 patent, you 09:20:36

cited everything that you considered in forming 09:21:03

your opinions in your declaration, correct? 09:21:05

A I cited all the references that I felt 09:21:10

relevant to the declaration, yes. 09:21:15

Q And if something was, in your opinion, 09:21:16

relevant to your analysis, then you would have 09:21:19

cited -- cited it in the declaration, correct? 09:21:22

A I would have cited any relevant 09:21:25

references. 09:21:30

Q So if there's a reference that's not cited in your declaration, is it fair to assume that that reference is not relevant to your analysis? 09:21:30 09:21:33 09:21:36

ATTORNEY KUSHAN: Objection. Form. 09:21:38

A I would have felt that that reference was not directly relevant to my declaration. 09:21:40 09:21:44

Q All right. And so just quickly looking at the other -- your other three declarations, so the declaration for matter 4, do you see on the cover page, it references US Patent Number 12,018,298? 09:21:50 09:21:53 09:21:59 09:22:05

So do you understand that your declaration in PGR matter 4 was submitted with respect to the proceeding concerning the '298 patent? 09:22:11 09:22:16 09:22:20

A That seems reasonable. 09:22:24

Q Have you ever seen the '298 patent? 09:22:26

A No. I have not. 09:22:31

Q Did you consider the '298 patent in forming your opinions in PGR matter 4? 09:22:32 09:22:40

A No. I have not. 09:22:42

Q Do you know what's claimed in the '298 patent? 09:22:43 09:22:48

A I believe it's a mutation on a protein, 09:22:48

again probably PH20. Other than that, I'm not familiar with the content of that patent. 09:22:55 09:22:58

Q Do you know what's disclosed in the '298 patent? 09:23:01 09:23:05

A No. I don't. 09:23:05

Q And then PGR -- your declaration in PGR matter 6, on the cover page, lists US Patent Number 12,152,262. 09:23:12 09:23:18 09:23:24

So do you understand that your declaration in PGR matter 6 was submitted proceeding concerning the '262 patent? 09:23:27 09:23:30 09:23:35

A It appears that way. 09:23:38

Q Have you ever seen the '262 patent? 09:23:39

A I have not seen that patent, '262. 09:23:44

Q Do you know what's claimed in the '262 patent? 09:23:47 09:23:50

A Again, I'm assuming it's a mutation on a protein, PH20 perhaps. That's the extent of my assumption. 09:23:54 09:23:58 09:24:03

Q So you don't know for sure. You're just -- you're assuming that there's a mutation on PH20 that's claimed? 09:24:04 09:24:07 09:24:11

A I do not know that for sure. I've never seen the patent, so I don't know what's claimed in it. 09:24:12 09:24:17 09:24:21

Q Did you ask for the patent when you were performing your analysis? 09:24:21 09:24:24

A No. I did not. 09:24:28

Q Did you think that the patent was relevant to your analysis? 09:24:29 09:24:31

A No. I didn't think so. 09:24:34

Q So you don't know what's disclosed in the '262 patent? 09:24:38 09:24:41

A I don't know what's disclosed in that patent. 09:24:42 09:24:44

Q All right. And then finally, your declaration in PGR matter 9 lists US Patent Number 12,123,035. 09:24:50 09:24:52 09:25:02

So do you understand that your declaration in PGR matter 9 was submitted with respect to the '035 patent? 09:25:05 09:25:07 09:25:12

A That's my reading of it. 09:25:17

Q Have you ever seen the '035 patent? 09:25:18

A No. I have not seen the patent '035. 09:25:23

Q Do you know what's claimed in the '035 patent? 09:25:27 09:25:30

A Perhaps a mutation on PH20. 09:25:31

Q But you don't know for sure? 09:25:35

A I don't know it for sure. 09:25:37

Q And do you know what's disclosed in the '035 patent? 09:25:38 09:25:42

A No. I don't know what's disclosed in that patent. 09:25:42 09:25:46

Q All right. Dr. Park, do you know what a patent claim is? 09:25:47 09:26:13

A Only in a lay term. 09:26:15

Q And what's your lay understanding of what a patent claim is? 09:26:18 09:26:20

A You make a discovery, and you take ownership of that discovery, and the government gives you some authority, claims, for that discovery over other people. 09:26:22 09:26:26 09:26:31 09:26:37

Q Do you know what a -- what the terms -- strike that. 09:26:42 09:26:46

Have you ever interpreted a patent claim? 09:26:47

A No. I have not. 09:27:01

Q Okay. Do you understand that trial has been instituted in the four PGRs that we're talking about today? 09:27:02 09:27:14 09:27:17

A No. 09:27:20

Q Okay. Have you seen the institution decision for any of those -- any of these PGRs? 09:27:21 09:27:24

A What do you mean by institution decision? 09:27:28

Q It's a decision that was issued by the Patent -- by the PTAB? 09:27:31 09:27:35

A By the patent? 09:27:38

Q By the Patent Office? 09:27:40

A By the Patent Office. 09:27:42

What did they issue? 09:27:44

Q It's called an institution decision. 09:27:46

A And what does the -- what does the decision say? 09:27:49 09:27:51

Q I'm just asking if you've seen it. 09:27:51

A Oh, no. I've -- I have not seen it. 09:27:54

Q Okay. All right. 09:27:56

Okay. So Dr. Park, in your work, have you attempted to predict the impact of substitutions on protein function? 09:28:22 09:28:26 09:28:32

ATTORNEY KUSHAN: Objection. Form. 09:28:34

A In what work are you referring to? 09:28:35

Q Your work outside of this case. Just your -- in your career. 09:28:37 09:28:40

A In my career? 09:28:42

Q Uh-huh. 09:28:44

A Yes. 09:28:44

Q Can you estimate how many -- for how many proteins you've done this type of analysis, where you're trying to impact -- predict the impact of substitutions on protein function? 09:28:47 09:28:55 09:29:01 09:29:05

ATTORNEY KUSHAN: Objection. Form. 09:29:07

A The total number of proteins that I have ever worked on? Is that what you're asking? 09:29:11 09:29:14

Q Yes. 09:29:17

A In my career? 09:29:18

Q Yes. 09:29:24

A I would say 10. 09:29:27

Q And what types of proteins were those proteins that you worked on? 09:29:32 09:29:42

What was the function? 09:29:45

ATTORNEY KUSHAN: Objection. Form. 09:29:47

A They vary. They include binders, small molecule binders, antibody, small proteins that bind other proteins. 09:29:49 09:29:57 09:30:05

Q Anything else? 09:30:10

A Protein that binds DNA. Protein that binds carbohydrate. I think that's it. 09:30:13 09:30:19

Q And what kind of work have you done on antibodies? 09:30:35 09:30:38

A I use directed evolution to engineer an antibody that binds an antigen in a pH dependant manner. 09:30:43 09:30:49 09:30:57

Q When you say you used directed evolution, do you mean that you screened a library of antibodies to identify antibodies that had the desired pH dependant binding profile? 09:31:02 09:31:07 09:31:12 09:31:15

ATTORNEY KUSHAN: Objection. Foundation. 09:31:17

A That is exactly what I did. 09:31:20

Q Okay. How did you generate -- or, sorry. Strike that. 09:31:45 09:31:49

Did you generate the library of antibodies that you screened? 09:31:49 09:31:51

A How did I generate the library? 09:31:51

Q Well, did you generate the library? 09:31:53

ATTORNEY KUSHAN: Objection. Foundation. 09:31:56

A We constructed the library ourselves. 09:32:00

Q And how -- 09:32:03

A My students and I. 09:32:05

Q And how did you construct the library? 09:32:07

What -- I'll ask a better question. 09:32:14

What -- strike that. 09:32:15

Did you introduce specific mutations into 09:32:25
sequences when you generated the library, or was 09:32:43
it just a random library of antibodies? 09:32:46

ATTORNEY KUSHAN: Objection. Form. 09:32:51

Objection to foundation. 09:32:52

A It was a combination of both. 09:32:53

Q Okay. In the analysis that you performed 09:33:34
in these matters, you used several different 09:33:37
computational tools, correct? 09:33:43

A I used tools that involved computation in 09:33:46
the back -- background, yes. 09:33:52

Q And you -- specifically, you used PyMol, 09:33:54
SWISS-MODEL, Clustal Omega and BLAST; is that 09:33:58
right? 09:34:05

A Those were the four major tools and web services I used. 09:34:05 09:34:12

Q Are there any other computational tools that you used in your analysis for these PGRs? 09:34:13 09:34:17

A No. 09:34:20

Q And were all four of these tools known in 2011? 09:34:21 09:34:25

A They were well-known, and widely used. 09:34:29

Q In your work in the 2011 time frame, were you using programs like PyMol, SWISS-MODEL, Clustal Omega and BLAST? 09:34:33 09:34:49 09:35:02

A I was using them actively, yes. 09:35:03

Q Were there other computational tools that were in use in the 2011 time frame that are similar to PyMol, SWISS-MODEL, Clustal Omega and BLAST? 09:35:08 09:35:15 09:35:20 09:35:25

ATTORNEY KUSHAN: Objection. Form. 09:35:26

And objection. Foundation. 09:35:28

A Are there alternative tools? 09:35:31

Q Yes. 09:35:34

A For some of them, there were alternative tools. 09:35:35 09:35:44

Q And what are some examples of alternative tools that were available in 2011? 09:35:45 09:35:48

A For instance, PyMol, which is a molecular viewer. There was software, and even today there is that software, that was written out of use in San Francisco, Chimera, which does very similar work as PyMol. 09:35:55 09:36:00 09:36:05 09:36:10 09:36:17

Q What about Coot? Was that available? 09:36:19

A Coot is a much more advanced tool used in molecular modeling, determining X-ray crystal structure. It's a more niche application compared to PyMol or Chimera. 09:36:24 09:36:31 09:36:40 09:36:43

Q But in 2011, Chimera, PyMol and Coot were all available? 09:36:43 09:36:56

A Yes. 09:36:56

Q And SWISS-MODEL is a -- strike that. 09:36:56

Were there alternatives to SWISS-MODEL that were available in 2011? 09:37:04 09:37:09

A Yes. There were alternatives. 09:37:13

Q What were those? 09:37:16

A MODELLER, for instance. Perhaps Rosetta. 09:37:19

(Reporter clarification.) 09:37:33

Q Any others? 09:37:33
A There might have been. I don't remember. 09:37:35
Q In your work in 2011, were you using 09:37:38
structure prediction programs like SWISS-MODEL? 09:37:44
ATTORNEY KUSHAN: Objection. Form. 09:37:50
A Prior to 2011, I had used SWISS-MODEL. 09:37:55
Not in the year of 2011. 09:38:01
Q Okay. In that time frame, had you used 09:38:03
MODELLER? 09:38:10
A No. I did not use MODELLER. 09:38:12
Q And what about Rosetta? Did you use 09:38:15
Rosetta in that time frame? 09:38:18
A No. 09:38:21
Q How does SWISS-MODEL compare with MODELLER 09:38:23
and Rosetta? 09:38:30
ATTORNEY KUSHAN: Objection. Form. 09:38:31
A SWISS-MODEL is a web service, as opposed 09:38:33
to Rosetta and MODELLER that you need to run at 09:38:38
the command line. So one would have to be much 09:38:42
more sophisticated to be able to use them. 09:38:45
And they also required licensing 09:38:48
sometimes. Even if it's an academic licensing, 09:38:50

you would have to sign a form. So there was a lot more involved in launching those programs, and not many people had the -- the resources or the capability to do it. 09:38:54 09:38:57 09:39:01 09:39:03

Q So is that why you used SWISS-MODEL as opposed to MODELLER or Rosetta? 09:39:05 09:39:16

A That is correct. 09:39:17

Q And in the 2011 time frame, in your work were you using PyMol? 09:39:18 09:39:22

A Yes. I was. 09:39:25

Q Did you use Chimera or Coot in that time frame? 09:39:26 09:39:39

A I used Chimera sometimes, but in the end we decided to go with PyMol because it was easier to work with. 09:39:39 09:39:46 09:39:51

Q And do you have any experience with Coot? 09:39:59

A I have never used Coot. 09:40:03

Q Have you ever solved a protein crystal structure? 09:40:13 09:40:17

A I solved a crystal structure in collaboration with a crystallographer. 09:40:21 09:40:27

Q So -- 09:40:33

A Two structures. 09:40:33

Q Two structures?

A To be precise. 09:40:35

Q So and what was your involvement in 09:40:36

solving those structures? 09:40:40

A I made the protein, purify the protein, 09:40:41

and provide the protein to the crystallographer so 09:40:47

that they could crystalize and determine the 09:40:54

structure. And at the end, I interpreted the 09:40:58

structure and wrote the paper. 09:41:01

Q But you didn't actually -- you interpreted 09:41:03

the -- you interpreted the model once the 09:41:10

structure was solved; is that right? 09:41:12

ATTORNEY KUSHAN: Objection. Foundation. 09:41:14

A Once the structure has been determined by 09:41:16

the crystallographer, I interpreted it, compared 09:41:21

with the prediction, and ultimately wrote the 09:41:27

paper, which got published, as me as the leading 09:41:34

author. 09:41:43

(Reporter clarification.) 09:41:45

Q And what proteins were those when you were 09:41:45

involved in solving the structure? 09:41:49

A One is FK-506 binding protein. The second is monomeric streptavidin. 09:41:52 09:42:00

Q And the papers that you wrote in connection with those -- sorry. Strike that. 09:42:08 09:42:11

The papers that were published in connection with that work, are those on your CV? 09:42:14 09:42:18

A Yes. They are. 09:42:22

ATTORNEY MARTIN: So we've probably been going for an hour. Do you want to take a short break? 09:42:27 09:42:30 09:42:34

THE WITNESS: Sounds great. 09:42:35

THE VIDEOGRAPHER: We are going off the record. The time is 9:42 a.m. 09:42:38 09:42:40

(Recess from 9:42 a.m. until 9:54 a.m.) 09:42:44

THE VIDEOGRAPHER: We are back on the record. The time is 9:54 a.m. 09:54:23 09:54:24

BY ATTORNEY MARTIN: 09:54:28

Q Welcome back, Dr. Park. 09:54:29

So in the 2011 time frame, had you used the SWISS-MODEL program to predict protein structure? 09:54:31 09:54:36 09:54:41

ATTORNEY KUSHAN: Objection. Form. 09:54:41

A Predict a protein structure? I used SWISS-MODEL to model a protein. Predict might be a little over -- an overstatement. 09:54:45 09:54:50 09:54:57

Q Okay. Let me ask a more clear question then. 09:54:59 09:55:03

So in the 2011 time frame, had you used the SWISS-MODEL program to model a protein structure? 09:55:04 09:55:08 09:55:12

A Yes. I have. 09:55:12

Q Okay. So can you turn in your declaration for the matter 3 case? 09:55:13 09:55:30

A Which one? 09:55:32

Q The one that you have opened right there. 09:55:33

A Yes. 09:55:37

Q So Paragraph 12, which is on Page 3. 09:55:37

A Yes. 09:55:53

Q And you said: Counsel for Merck provided the following description of a person of ordinary skill of the art in the 2011 time frame for you to evaluate; is that right? 09:55:54 09:55:58 09:56:04 09:56:10

A Yes. 09:56:12

Q And your -- the definition that you 09:56:12

provided: A person of ordinary skill in the art in the 2011 time frame would have had an undergraduate degree, a Ph.D., and postdoctoral experience in scientific fields relevant to the study of protein structure and function, e.g., chemistry, biochemistry, biology, biophysics. From training and experience, the person would have been familiar with factors influencing protein structure, folding inactivity, production of modified proteins, using recombinant DNA techniques, and use of biological assays to characterize protein function, as well as techniques and tools used to analyze protein structure, i.e., sequence searching and alignments, protein modeling software, et cetera; is that right? 09:56:14 09:56:17 09:56:20 09:56:24 09:56:27 09:56:28 09:56:33 09:56:34 09:56:36 09:56:41 09:56:44 09:56:49 09:56:49 09:56:52 09:56:55 09:56:59

A That's correct. That was the description provided by counsel. 09:57:00 09:57:03

Q Okay. And so throughout this deposition, I'm going to use the term POSA to refer to person of ordinary skill in the art. 09:57:05 09:57:08 09:57:15

A Okay. 09:57:17

Q Okay. In the 2011 time frame, were you a person of ordinary skill in the art? 09:57:17 09:57:22

Did you meet this definition? 09:57:26

A In the year 2011, I was a POSA, yes. 09:57:29

Q And so in the 2011 time frame, a POSA could have used the SWISS-MODEL program to model a protein structure; is that right? 09:57:33 09:57:42 09:57:46

A That is correct. 09:57:47

Q And in the 2011 time frame, a POSA also could have used MODELLER and Rosetta to model a protein structure; is that right? 09:57:47 09:57:50 09:57:57

A If we say a POSA is a person of ordinary skill, I do not think it would be reasonable to expect a POSA to be able to use other software that I mentioned earlier, including MODELLER or Rosetta. 09:57:59 09:58:05 09:58:10 09:58:14 09:58:18

Q And is that because you would have to get a license to use those programs, and the POSA wouldn't necessarily have a license? 09:58:19 09:58:21 09:58:26

A No. The learning curve would have been very steep. 09:58:28 09:58:32

Q Oh. Okay. They're just more 09:58:32

technically -- 09:58:34

A Highly more -- 09:58:36

Q Okay.

A -- advanced. 09:58:38

Q Okay. So in your view, the POSA could 09:58:38

have used SWISS-MODEL, but not necessarily 09:58:41

MODELLER or Rosetta? 09:58:45

A That is correct. 09:58:48

Q Okay. Thanks. 09:58:49

All right. And then in the 2011 time 09:58:51

frame, had you used Clustal Omega for sequence 09:59:12

alignments? 09:59:17

A I did. 09:59:17

Q And the skilled person, or POSA, in 2011 09:59:17

could have used Clustal Omega to perform a 09:59:25

sequence alignment, correct? 09:59:28

A That's correct. 09:59:28

Q Were there other programs that the POSA 09:59:29

could have used in the 2011 time frame to run 09:59:34

sequence alignments? 09:59:38

A There were other sequence alignment 09:59:41

programs out there, but most of them were esoteric 09:59:46

in a way, not well-known. A person who specializes in bioinformatics might know the advantages of using different programs. But a POSA would not be in a position to compare and contrast different programs to see if there is a benefit in using other program. And as such, they would have used something like Clustal Omega as opposed to other algorithms. 09:59:55 10:00:00 10:00:05 10:00:10 10:00:14 10:00:18 10:00:24 10:00:28

Q Okay. And would the skilled person have been able to use BLAST to identify homologous sequences in the 2011 time frame? 10:00:30 10:00:41 10:00:46

A A POSA would have been able to use BLAST to find homologous sequences, yes. 10:00:50 10:00:55

Q Were there any other tools that a POSA could have used in the 2011 time frame to identify homologous sequences for alignment besides BLAST? 10:01:00 10:01:05 10:01:10

A I haven't used any other program besides BLAST personally. I would think that there might have been, but I'm not aware of any particular one that would be equally versatile as BLAST, and an ordinary person, a person of ordinary skill, would have chosen BLAST as a search tool as opposed to 10:01:14 10:01:19 10:01:23 10:01:26 10:01:36 10:01:40

any other tools out there. 10:01:46

Q How many times in your career would you say you've used SWISS-MODEL to generate a homology model of a protein? 10:01:49 10:02:14 10:02:22

A How many times? That's a very difficult estimate. 10:02:24 10:02:30

Q Okay. 10:02:30

A It's almost like asking how many times have you gone to the bathroom in your lifetime. I would say many. I don't know exactly how many. 10:02:33 10:02:39 10:02:43

Q Okay. So you used -- you've used SWISS-MODEL frequently in your work to generate homology models; is that right? 10:02:45 10:02:51 10:02:54

ATTORNEY KUSHAN: Objection. Form. 10:02:54

A At times, yes. 10:02:55

Q Okay. What's the purpose of generating -- well, actually strike that. 10:02:57 10:03:03

Like me make sure I'm using the right terminology here. 10:03:05 10:03:09

So the model that you generate using SWISS-MODEL, would you refer to that as a model, or a homology model, or is there some other term? 10:03:10 10:03:14 10:03:15

A You can use either one. 10:03:24

Q Uh-huh.

A Unless the structure of the protein is 10:03:26
known, any time you have a model, it's going to be 10:03:28
a constructed model as opposed to an experimental 10:03:32
structure. So in this particular case, we're 10:03:38
talking about homology model. 10:03:41

Can one generate a model any other way? I 10:03:44
don't know. So homology model/model, I think 10:03:50
either one would work here. 10:03:54

Q Okay. 10:03:56

A And when you say model, I would understand 10:03:57
that to mean homology model going forward. 10:04:01

Q Okay. Thank you. 10:04:04

So what's the purpose of generating a 10:04:05
homology model for a protein structure? 10:04:08

A It allows you to look at the molecule, as 10:04:18
opposed to imagining it in your head. 10:04:22

Q And it's useful to be able to look at the 10:04:27
molecule, right? 10:04:31

A Absolutely. 10:04:32

Q And you can use homology modeling to 10:04:38

predict the impact of substitutions in a protein? 10:04:42

ATTORNEY KUSHAN: Objection to foundation. 10:04:46

Also to form. 10:04:48

A A homology model can help you to evaluate 10:04:49

the impact of a substitution. Yes. 10:04:54

Q So can you turn in your declaration for 10:05:03

the -- the one that you have open for matter 3 -- 10:05:08

turn to Paragraph 20? 10:05:12

A Okay. 10:05:26

Q And in the first sentence, you say that: 10:05:27

Proteins often can tolerate a single amino acid 10:05:32

substitution in non-essential regions of the 10:05:35

protein structure; is that right? 10:05:38

A That's the sentence, yes. 10:05:42

Q What is -- what do you mean when you say 10:05:44

non-essential regions of the protein structure? 10:05:50

A A non-essential region of the protein 10:05:58

structure is the part of the protein that is not 10:06:04

critical to the folding of the protein. 10:06:09

(Reporter clarification.) 10:06:34

Q How can you tell whether a region of a 10:06:34

protein is essential to the folding of the 10:06:42

protein? 10:06:45

A You can tell by looking at a bunch of sequences that are related, homologous that is, to one protein, and -- and have high sequence similarity so that they all fold to the same structure, and by comparing those sequences, you can identify the amino acids that are important for folding and the amino acids that are less important for folding. 10:06:46 10:06:50 10:06:58 10:07:02 10:07:07 10:07:12 10:07:16 10:07:19

Q So wait. I'm going to unpack that. 10:07:22

So first -- and again, just to make sure that we're aligned on terminology -- so you used the word homologous. Can you explain what you mean when you say homologous? 10:07:28 10:07:31 10:07:37 10:07:42

A Absolutely. Homologous or homology refers to two or more genes in proteins that have a common ancestor. Two proteins that have originated from a common ancestral gene would have homology, and therefore be homologous. 10:07:43 10:07:51 10:07:55 10:08:01 10:08:08

Q Can you use the term homology to refer to -- strike that. 10:08:12 10:08:30

Does the term homology, as you're using 10:08:31

it, does that only refer to naturally occurring proteins? 10:08:35 10:08:42

A Yes. That's how the word should be used, and is used in practice. 10:08:42 10:08:46

Q Okay. And then you also talked about a high sequence similarity. So what do you consider to be a high sequence similarity? 10:08:50 10:09:00 10:09:04

A It's a spectrum. Similarity is something that can be measured based on the sequence. You can count the number of amino acids that are identical between two sequences, plus the pairs of amino acids, matching pairs, that are supposed to be similar in some chemical aspects. That definition can vary. Then you can count the number of those positions and divide by the whole length of the protein. That will give you a number, which would be the similarity of that protein, of those two proteins. 10:09:13 10:09:17 10:09:23 10:09:26 10:09:32 10:09:38 10:09:43 10:09:46 10:09:49 10:09:51 10:09:54

And now, getting back to high sequence similarity. It's not a black and white thing. It's more of a convention, perhaps used by practitioners in the field to indicate that two 10:09:57 10:10:00 10:10:07 10:10:11

proteins have sequences that are fully similar, and therefore have a similarity percentage above a certain number. 10:10:15 10:10:21 10:10:27

That number is not set in stone, unfortunately. However, practitioners would feel comfortable saying that, say, 50 percent would be a reasonable number to define high similarity. 10:10:31 10:10:34 10:10:38 10:10:42

Q Okay. And so if two sequences have, let's say, 60 percent sequence similarity, and before -- strike that. 10:10:48 10:10:52 10:10:57

Before we get there, I want to distinguish between similarity and identity, right? 10:10:57 10:11:01

So when you say high sequence similarity, you mean not necessarily identical, not necessarily identity, but that allows for some conservative substitutions or conservative changes; is that right? 10:11:04 10:11:08 10:11:11 10:11:14 10:11:18

ATTORNEY KUSHAN: Objection. Form. 10:11:19

Also objection to foundation. 10:11:20

A Identity is a subset of similarity, yes. And to go from identity to similarity, you do allow some slight changes. What's debatable is 10:11:24 10:11:28 10:11:32

exactly what should be counted as an allowed substitution. 10:11:38 10:11:42

Q Uh-huh.

A That -- I don't know the exact definition of that. But accounting for those substitutions, you would have a slightly higher number for similarity, as opposed to identity. 10:11:43 10:11:46 10:11:51 10:11:54

Both terms are used. One doesn't stick to one or the other religiously, but use the term with the expectation that the reader or the listener would understand what you mean by that. 10:11:57 10:12:04 10:12:11 10:12:15

Q Okay. So if a protein, or two proteins have sequence -- strike that. Let me start over. 10:12:19 10:12:25

You have two sequences that are, let's say, 60 percent similar. Would you consider those two sequences to be -- to have high sequence similarity? 10:12:26 10:12:36 10:12:42 10:12:46

ATTORNEY KUSHAN: Objection to foundation. 10:12:47

Objection as to form. 10:12:48

A Assuming certain conditions are met, I would say that they are similar. 10:12:53 10:12:58

Q What conditions? 10:13:00

A The similarity has to extend over a reasonable extent of the protein. In other words, you can't have similarity within the first 50 amino acids out of 500 amino acids and call that similar. Because the similarity would -- so you have to factor that in, how much of -- of the protein -- 10:13:03 10:13:08 10:13:12 10:13:17 10:13:23 10:13:29 10:13:34

Q So --

A -- would match. 10:13:37

Q Okay. So if the -- the sequence alignment for the entire protein had, let's say, 60 percent similarity, would you consider those two sequences to be highly similar? 10:13:37 10:13:42 10:13:46 10:13:51

ATTORNEY KUSHAN: Objection. Foundation. Objection as to form. 10:13:54 10:13:55

A If you're comparing just the sequence and limit your discussion and interpretation to the similarity alone? Yes. I would think that they are similar. 10:13:56 10:14:01 10:14:04 10:14:09

Q Okay. So you mentioned that if you have a bunch of sequences, a bunch of homologous sequences that have a high sequence similarity so 10:14:16 10:14:58 10:15:05

that they all fold to the same structure, you can compare those sequences and identify the amino acids that are important for folding. 10:15:09 10:15:12 10:15:15

How -- and my question is: If you have a bunch of sequences that are homologous, and have high sequence similarity, how do you know that they all fold to the same structure? 10:15:18 10:15:22 10:15:26 10:15:30

A That's the standard that the industry goes by. If you ask a practitioner in the field: I have two proteins that are homologous and have high sequence similarity, would I expect them to fold to the same structure, of course I would say yes. 10:15:36 10:15:40 10:15:50 10:15:53 10:15:58 10:16:01

Q And why is that? 10:16:01

A It's a matter of protein evolution. Protein structures, if one compares the folding structure to an entire space of -- conformational space that can be adopted by the polypeptide, it's a very small fraction. 10:16:15 10:16:19 10:16:28 10:16:34 10:16:41

Now, if you have homologous proteins, meaning they came from the same ancestor, and they have maintained similar sequence similarity, they 10:16:43 10:16:46 10:16:51

would be obligated to explore that very small sliver of the conformational space. They would not be able to go and randomly find another island of stability in that conformational space that can be occupied by that particular sequence. So that restricts what the protein can do. 10:16:56 10:17:03 10:17:08 10:17:13 10:17:17 10:17:19

So this is the foundation behind the assumption that homologous highly similar sequences would adopt the same conformational three-dimensional structure. 10:17:23 10:17:28 10:17:33 10:17:38

Q So you said at the beginning of your answer: If one compares folding structure to the entire space of conformational space that can be adopted by the polypeptides, it's a very small fraction. 10:17:46 10:17:49 10:17:53 10:18:06 10:18:00

Can you explain what you mean by that, and why that's the case? 10:18:01 10:18:03

A Conformational space, you can think of it as the ensemble of all the shapes that a given polypeptide can assume. It could be a folding structure. It could be DNA structure, some random structures. 10:18:08 10:18:12 10:18:18 10:18:23 10:18:28

The folding structure that one sees in a crystal structure or model structure, that's just a small fraction compared to whatever else, whatever other shapes this -- this particular polypeptide may assume. 10:18:29 10:18:34 10:18:38 10:18:42 10:18:46

Why is it a small fraction? Because folding structure will be defined by a combination of dihedral angles. And in a folded protein, you would have pretty well-defined values of dihedral angles, both main chain and side chains. The entire conformational space would encompass all the available values of all the dihedral angles. Therefore, the folding structure would correspond to a very small fraction of that entire ensemble. 10:18:48 10:18:52 10:18:55 10:19:03 10:19:04 10:19:09 10:19:15 10:19:20

(Reporter clarification.) 10:19:38

Q Okay. So the actual folded structure for a given sequence corresponds to a very small fraction of the potential conformational space that that -- that could be occupied by -- that could be occupied by folded protein structures? 10:19:45 10:19:59 10:20:10 10:20:14 10:20:17

ATTORNEY KUSHAN: Objection. Form. 10:20:17

Objection. Foundation. 10:21:54

A Could you repeat that question, please? 10:20:20

Q The actual folded structure for a given 10:20:23

sequence corresponds to a very small fraction of 10:21:46

the conformational space that -- that could be 10:20:28

occupied by that sequence? 10:20:35

ATTORNEY KUSHAN: Objection. Form. 10:20:37

And objection to foundation. 10:20:39

A Not by that sequence. 10:20:41

Q Uh-huh.

A A polypeptide of that length. 10:20:43

Q Oh, okay. 10:20:47

A Or if you do not constrain the protein to 10:20:48

adopt a structure and do something in nature, and 10:20:58

you consider all theoretical possibilities that 10:21:02

can be adopted -- it doesn't mean that it will 10:21:07

be -- it can be adopted by a polypeptide of that 10:21:12

length, that space would be much, much bigger 10:21:14

compared to the folded space, folded conformation. 10:21:19

Q Okay. All right. 10:21:26

And then going back to your previous 10:21:57

answer where you said that you compare the 10:22:00

sequences and identify the amino acids that are 10:22:03

important for folding, you're talking about amino acids that are important for folding are the ones that are conserved in the alignment, right? 10:22:06 10:22:10 10:22:13

A Yeah. Amino acids that are conserved in the alignment are the residues that are important for folding. Yes. 10:22:18 10:22:22 10:22:26

Q Okay. How many sequences do you need to have in your alignment to know that you've identified residues that are important for folding? 10:22:28 10:22:40 10:22:34

ATTORNEY KUSHAN: Objection. Foundation. 10:22:34

A The more sequences, the better. Since you're trying to get a statistical sense of what amino acids are conserved, a larger sample would be better. 10:22:44 10:22:48 10:22:56

There's no cut-off. You can do it with smaller set. And the difference is with a smaller set, it may look as if more residues are conserved just because you are starting with a smaller set, and you see less variation. 10:22:59 10:23:02 10:23:10 10:23:15 10:23:20

As you increase the sample size, you may observe some of those positions that begin to 10:23:22 10:23:28

vary. But the sample size can be small I suppose. There's no hard cut-off. 10:23:27 10:23:36

Q Okay. So we started this discussion by looking at Paragraph 20, and I asked about what are -- what you meant by non-essential regions of the protein structure, and am I understanding correctly that the non-essential regions of the protein structure are identified based on a sequence alignment, and specifically they are the residues in the sequence alignment that are not conserved? 10:23:57 10:24:01 10:24:07 10:24:13 10:24:17 10:24:20 10:24:23 10:24:25 10:24:30

A The positions, the residues, in a multiple sequence alignment that are not conserved are labeled as non-essential regions in this declaration, and they may vary. And the fact that they do vary among homologous high sequence similarity sequences suggests that they may be non-essential, although it's a quote/unquote non-essential. 10:24:33 10:24:39 10:24:48 10:24:54 10:25:06 10:25:14 10:25:18 10:25:24

Q Uh-huh. So what do you mean when you say quote/unquote non-essential? 10:25:24 10:25:26

A Meaning that it doesn't mean that 10:25:28

anything -- they're not anything goes residues. 10:25:32
You can't just randomly choose mutations at those 10:25:36
positions. They are still important, but they may 10:25:39
be qualitatively different than the residues that 10:25:43
are strictly conserved, or conserved to a very 10:25:46
high degree. 10:25:52
(Reporter clarification.) 10:25:54
Q So when you use the term non-essential, 10:25:54
non-essential region in -- in this declaration, 10:25:59
are you referring to positions in the sequence 10:26:01
that can potentially tolerate substitution, as 10:26:03
opposed to those that cannot tolerate 10:26:08
substitution? 10:26:11
A It -- it starts -- it starts with the -- 10:26:16
with the definition of what non-essential residues 10:26:25
are. So these are definitions, meaning the 10:26:30
positions in the multiple sequence alignment where 10:26:34
we see variation, we label them as non-essential. 10:26:37
Whether they tolerate substitutions or not comes 10:26:42
later. 10:26:44
Q Okay. But why did you label them 10:26:45
non-essential? 10:26:48

A We label them as non-essential in the sense that they are varied among homologous sequences that bode the same structure, which means those residues may allow variation. So in that sense, they're not critical. The identity of those amino acids are not critical to adopt this common fold shared by all homologous proteins in that group. 10:26:51 10:26:54 10:27:01 10:27:07 10:27:12 10:27:20 10:27:21 10:27:29

Q Did you use the term non-essential in your work in 2011 to identify non-conserved residues in sequence alignments? 10:27:30 10:27:35 10:27:41

ATTORNEY KUSHAN: Objection. Foundation. 10:27:42

A No. I did not use the term non-essential when I used it for my purpose. 10:27:47 10:27:51

Q Have you ever used the phrase non-essential region of a protein structure, outside of your work in this case? 10:27:53 10:27:57 10:28:01

A No. 10:28:05

Q So why did you use the term non-essential region to refer to non-conserved positions in sequence alignment for this case? 10:28:07 10:28:10 10:28:21

A The task that I was given was to identify 10:28:25

non-essential positions in PH20 and identify substitutions at those positions. So my task came down to two things: One to identify what those non-essential positions may be; and second, evaluate various substitutions at those positions to see if there are substitutions at non-essential positions that would be tolerated. 10:28:30 10:28:39 10:28:43 10:28:48 10:28:50 10:28:57 10:29:04

And that is motivated by patent '427, which I believe is quoted in Paragraph 32. We use the term non-essential because that's what is in the patent, and I was trying to see -- I was tasked to identify the substitutions at non-essential positions as described in the patent to see where they are and what they may be. 10:29:06 10:29:16 10:29:26 10:29:31 10:29:41 10:29:48 10:29:50

Q Okay. 10:29:54

A That's why I went about defining non-essential positions, whereas previously I did not do that in my other studies. 10:29:54 10:29:58 10:30:01

Q So in your opinion, would a POSA who sees the term non-essential position, would they understand that to mean a position in the sequence alignment that is not conserved? 10:30:05 10:30:18 10:30:21 10:30:25

A The other way around. If they see positions in a sequence alignment that is not conserved, they would think it's non-essential. 10:30:32 10:30:35 10:30:40

Q Oh.

A They wouldn't go, start with non-essential and say: Oh, aha. It's sort of backwards. 10:30:43 10:30:45

If they were to identify -- if they were tasked to identify non-essential positions, that is how they would go about doing that. 10:30:48 10:30:54 10:30:57

Q Okay. So if the POSA was tasked with identifying non-essential regions in a protein sequence, they would perform an alignment and identify non-conserved -- non-conserved residues? 10:31:00 10:31:04 10:31:07 10:31:09

A Yes. Since non-essential means opposite of essential; therefore, if you identify essential, you would have identified non-essential. 10:31:16 10:31:20 10:31:25 10:31:27

Q Okay. And the essential residues are those that are conserved in the alignment, right? 10:31:28 10:31:32

A That would be a reasonable interpretation. 10:31:35

Q Okay. So let me make sure I got this right. So a POSA tasked with identifying 10:31:37 10:31:41

essential residues in a protein sequence would perform an alignment of homologous sequences and identify those that are conserved, and that way identify the essential residues. And then those that are left, which are not conserved, are the non-essential residues; is that right? 10:31:44 10:31:48 10:31:51 10:31:55 10:31:56 10:32:00

A That would be the correct logic, yes. 10:32:04

Q Thank you. 10:32:06

And I believe you mentioned the '427 patent, but it's '429 patent, right? 10:32:07 10:32:11

A Oh. Oh, I'm sorry. '429 patent, yes. 10:32:13

Q Okay. Thank you. 10:32:17

So let's turn to Paragraph 32 -- are we already there -- in your declaration in matter 3? 10:33:01 10:33:05

And so the -- you have a block quote in Paragraph 32 from the '429 patent, right? 10:33:09 10:33:13

A Yes. 10:33:17

Q And the second sentence of that block quote says: Those of skill in this art recognize that in general, single amino acid substitutions in non-essential regions of a polypeptide do not substantially alter biological activity; is that 10:33:17 10:33:32 10:33:35 10:33:37 10:33:41

right? 10:33:46

A That's correct. 10:33:46

Q So in the 2011 time frame, a POSA would understand that if they performed a sequence alignment of homologous sequences and identified the non-conserved residues thereby giving them the non-essential regions, that substitutions in those non-essential positions would not substantially alter biological activity; is that right? 10:33:50 10:33:57 10:34:00 10:34:04 10:34:14 10:34:18 10:34:20

ATTORNEY KUSHAN: Objection. Form. 10:34:24

A POSA in 2011 would have done that multiple sequence alignment to identify non-essential regions, and reasonably expect that the amino acids found at those positions may be introduced without substantially altering biological activity, yes. 10:34:26 10:34:32 10:34:35 10:34:42 10:34:48 10:34:54

Q And how would -- what would the POSA understand the phrase "substantially alter biological activity" to mean? 10:34:54 10:34:57 10:34:59

A POSA would expect that sentence to mean without loss of structure or function. 10:35:10 10:35:14

Q So loss of function, that means no -- that 10:35:18

means that -- sorry. Strike that. 10:35:21

When you say loss of function, do you mean 10:35:22

that you've eliminated the function? 10:35:26

ATTORNEY KUSHAN: Objection. Foundation. 10:35:29

Also objection as to form. 10:35:31

A It says: Substantially alter biological 10:35:34

activity. 10:36:09

A substantial drop in the enzyme activity 10:35:41

would be considered a substantial alteration. 10:35:46

Therefore, those need to be guarded against. 10:35:50

Q Uh-huh.

A However, the way the sentence is written, 10:35:53

single amino acid substitutions may be introduced 10:35:57

without altering the biological activity 10:36:00

substantially. 10:36:07

Q If two highly similar polypeptides fold in 10:36:31

the same way, could a POSA predict that they will 10:36:39

function in the same way? 10:36:48

ATTORNEY KUSHAN: Objection. Form. 10:36:49

Also objection as to foundation. 10:36:50

A If two polypeptides, proteins, fold the 10:36:56

same way, can I -- can a person, POSA, expect them 10:37:00

to have the same activity? No. 10:37:04

Q Okay. Why is that? 10:37:07

A Because enzyme relating activity is a very subtle thing. Things can happen in the active site, which doesn't really alter the shape of the molecule, but can change the -- the function of the enzyme somewhat. 10:37:13 10:37:18 10:37:21 10:37:27 10:37:30

For instance, hyaluronidase 4 -- I remember hyaluronidase, yes. It hydrolyzes it from carbohydrate then PH20, which hydrolyzes hyaluronic acid. And maybe because of differences in the active site, for instance. 10:37:32 10:37:40 10:37:44 10:37:44 10:37:49

Q Uh-huh.

A So they may look the same, but they may act on different substrates, therefore, different activities. So a POSA would understand that kind of thing happens all the time. 10:37:52 10:37:56 10:38:00 10:38:02

Q Okay. Okay. So when you did your homology sequence alignment for -- for PH20, did you understand that all of the -- all of the hyaluronidases that you included in your alignment have -- I'm sorry. Strike that. 10:38:06 10:38:13 10:38:24 10:38:28 10:38:33

Your sequence alignment for PH20 includes, for example, HYAL4, right? 10:38:34 10:38:39

A Yes. 10:38:48

Q And HYAL4 does not cleave HA, right? 10:38:49

A Not very well. 10:38:49

Q So why did you include HYAL4 in your alignment for PH20 if they have different functions? 10:38:50 10:39:00 10:39:02

A Because the question that we were addressing was the -- the sequence structure relationship in this family of proteins, and you can learn by looking at all the sequences that fold to the same structure, which include all homologous sequences of PH20, regardless of how they're labeled, or what their specific enzyme activities may be, irrespective of their subtle differences in substrate specificity or the activity, enzymatic activity, doesn't change the fact that yeah, they fold to the same structure; therefore, the sequence -- all the sequences have they share, common sequence in amino acid restrictions and freedom, which can be beneficial 10:39:02 10:39:05 10:39:09 10:39:15 10:39:20 10:39:26 10:39:30 10:39:36 10:39:44 10:39:46 10:39:51 10:39:54 10:39:57 10:40:03

in our study. Therefore, it's important that we include all the sequences, not just a subset of highly similar sequences, for instance, by limiting the analysis to PH20. 10:40:09 10:40:13 10:40:18 10:40:23

Q Okay. So you included hyaluronidases in your alignment because you expect the homologous hyaluronidases to all adopt the same fold, to all fold in the same way, and as a result, that way you're able to identify which residues are essential for folding, as opposed to those which are not; is that right? 10:40:27 10:40:32 10:40:41 10:40:44 10:40:47 10:40:53 10:40:54

A That is correct. 10:40:55

Q Okay.

A That is a fundamental assumption -- 10:40:56

Q Okay. 10:41:01

A -- in this discipline.

Q And so why would you expect all of the hyaluronidases to fold the same way? 10:41:02 10:41:03

ATTORNEY KUSHAN: Objection. Foundation. 10:41:07

A In part for the reasons that I described earlier. They are related sequences, and the conformational space that can be explored by 10:41:15 10:41:19 10:41:23

related sequences will be clustered together into a very small fraction of the old conformational space that may be theoretically available to a polypeptide of that size. 10:41:28 10:41:33 10:41:38 10:41:44

Now, if they do not fold, two possibilities: One, they may fold to a completely different structure, which is not possible, not possible because they all came from the same ancestral gene. That means they started in some space, there would be no mechanism for them to go to the next island, which may be light years away, say. 10:41:46 10:41:48 10:41:52 10:41:55 10:41:57 10:42:02 10:42:08

Q Uh-huh.

A The other is they may not fold at all. If that were the case, you wouldn't have those concerned residues. Mutation would happen over time that would eliminate those conserved residues. 10:42:12 10:42:16 10:42:20 10:42:24 10:42:30

Evolution introduces mutations constantly at some rate. And over time, once a protein loses its benefit to the organism because it stopped folding, then those sequences would very quickly 10:42:30 10:42:37 10:42:42 10:42:47

mutate away, drift away in the sequence space. 10:42:53
They would look very, very different than what 10:42:58
they started with. 10:43:01
So the fact that they do have conserved 10:43:03
residues guarantee that they're doing something, 10:43:06
even when you don't know what they are doing, 10:43:10
which I believe is the case with HYAL3, which has 10:43:18
unknown function. But they may do something. 10:43:18
They must be doing something. Otherwise, would 10:43:20
have gotten rid of over time. So this is the 10:43:22
reason that they would fold to the same structure. 10:43:29
Q Okay. So when you -- when you did your 10:43:33
alignment for PH20, did you include all of the 10:43:36
hyaluronidase sequences that were available in 10:43:40
2011, or did you use some other cut-off? 10:43:45
A Yes. I used the sequences that were 10:43:52
available as of the end of year 2011. 10:43:55
Q Okay. Can you explain how exactly you ran 10:44:01
your -- strike that. 10:44:06
You used BLAST to create the -- or, to 10:44:09
identify the sequences that would be in the 10:44:10
alignment; is that right? 10:44:14

A I used BLAST to identify sequences that are similar to the seed sequence, or the target sequence, PH20 human. 10:44:15 10:44:19 10:44:25

(Reporter clarification.) 10:44:38

Q And how exactly did you use BLAST? 10:44:38

Did you set any parameters? 10:44:42

Can you just walk me through what you did in BLAST? 10:44:44 10:44:47

A BLAST is a web service. You enter the target sequence, in this case, human PH20, a protein sequence, and you hit -- run a button. 10:44:47 10:44:54 10:44:59

In my case, I did one thing that changed the default setting from the number return sequences expected was changed from 100, which is the default value, to the maximum that was allowed through the web service, which is 5,000. Because I wanted to see all the sequences that were available, not just the top 100. 10:45:10 10:45:14 10:45:20 10:45:27 10:45:30 10:45:35 10:45:38

Q Okay. Okay. And so then you then whittled that down by -- by removing the sequences that were only available after 2011 at the end? 10:45:41 10:45:51 10:45:56

A Only the sequences -- 10:45:59

ATTORNEY KUSHAN: Sorry. 10:46:02

Objection. Foundation. 10:46:02

Go ahead. 10:46:03

A Only the sequences that were available up to the end of year 2011; that is correct. 10:46:04 10:46:07

Q Okay. And then you removed -- sorry. 10:46:45

So you obtained the 95 sequences after you filtered out those available after December 29th, 2011, and then you removed 7 isoforms, leaving you with 88 sequences; is that right? 10:46:49 10:46:54 10:46:55 10:46:57

A That is correct. 10:46:59

Q Can you explain what 7 isoforms are, and why you removed them? 10:47:00 10:47:05

A Isoforms are different manifestations of the same gene, resulting from alternative splicing. They come from the same gene, but after MRNA synthesize, they undergo splicing event. And depending on where the splicing occurs, you get slightly different forms. 10:47:09 10:47:14 10:47:20 10:47:26 10:47:28 10:47:33

Since they essentially come from the same gene, having both of them represented in the final set would introduce a bias. I wanted to eliminate 10:47:35 10:47:37 10:47:40

those redundancies by identifying isoforms and ensuring that only one is represented in the final set. 10:47:45 10:47:49 10:47:53

ATTORNEY MARTIN: Okay. So let's introduce Exhibit 1054 and Exhibit 1057. 10:48:06 10:48:07

ATTORNEY KUSHAN: Lauren, do you want to take a break? 10:48:17 10:48:19

We've been going for an hour and twenty, or something. It's up to you. 10:48:20 10:48:23

ATTORNEY MARTIN: Yeah. That's fine. 10:48:26

THE WITNESS: Okay. Yeah. That would be great. Yes. Yes. 10:48:27 10:48:29

THE VIDEOGRAPHER: We are going off the record. The time is 10:48 a.m. 10:48:30 10:48:32

(Recess from 10:48 a.m. until 11:04 a.m.) 10:48:36

THE VIDEOGRAPHER: We are back on the record. The time is 11:04 a.m. 11:04:07 11:04:11

ATTORNEY KUSHAN: We -- Brian Goldberg, can you just announce yourself? 11:04:15 11:04:20

ATTORNEY GOLDBERG: Yes. Hi. This is Brian Goldberg from Dechert LLP, and with me is Dr. Michael Hecht, just listening in. 11:04:21 11:04:23 11:04:26

ATTORNEY MARTIN: All right. So welcome back, Dr. Park. 11:04:48 11:04:49

Right before we left, we were introducing Exhibits 1054 and 1057. 11:04:51 11:04:55

(Previously marked Exhibit 1054 introduced and retained by counsel.) 11:05:35

(Previously marked Exhibit 1057 introduced and retained by counsel.) 11:05:35

BY ATTORNEY MARTIN:

Q Did you prepare -- 11:05:35

A Yes.

Q -- 1054 and 1057? 11:05:37

A I did prepare both documents, yes. 11:05:38

Q Can you explain what Exhibit 1054 is? 11:05:41

A This is the header portion of the BLAST result that has been filtered based on the publication date of the individual sequences to correspond to pre-2011 set. 11:06:06 11:06:12 11:06:19 11:06:24

Q Okay. And so Exhibit 1054 includes the 88 sequences that you used in your sequence alignment; is that right? 11:06:25 11:06:29 11:06:33

A 1054 is a -- is a set of 95 sequences. 11:06:36

Q Oh. It's the 95? Okay. 11:06:41

A That's correct. 11:06:46

Q Okay. So Exhibit 1054 is the set of 95 sequences that you obtained from BLAST, and then you removed this 7 isoforms to arrive at the 88 sequences in your alignment; is that right? 11:06:51 11:06:57 11:07:01 11:07:02

A That is correct. 11:07:05

Q Okay. And so in Exhibit 1054, the first sequence listed is: Hyaluronidase PH20 isoform 2 Homo sapiens; is that right? 11:07:18 11:07:29 11:07:36

A That is correct. 11:07:41

Q Is that the sequence that you entered into BLAST? 11:07:45 11:07:49

A That is the sequence that was entered to do a BLAST search. Yes. 11:07:52 11:07:57

Q And so Exhibit 1054 identifies the protein in question, hyaluronidase PH20 versus -- actually, strike that. 11:08:06 11:08:19 11:08:28

So in Exhibit 1054, you can see which all of the proteins that are included in the alignment, and they include hyaluronidase PH20; sperm adhesion molecule 1 precursor; 11:08:29 11:08:33 11:08:36 11:08:43

hyaluronidase-5-like; sperm adhesion molecule 1; 11:08:47
hyaluronidase-4; LOW-QUALITY PROTEIN 11:08:52
hyaluronidase-4; hyaluronoglucosaminidase -- I 11:09:03
really butchered that -- 6. 11:09:03
This one is just hyaluronidase; 11:09:08
hyaluronidase-2; hyaluronidase PH20-like; 11:09:12
hyaluronidase-1; hyaluronidase PH20 precursor. 11:09:19
Let's see. Hyaluronidase-2 precursor; 11:09:27
hyaluronoglucosaminidase 6 precursor; 11:09:44
hyaluronidase-like; hyaluronidase-4-like; 11:09:53
hyaluronidase-3 precursor. 11:09:59
I think I've got them; is that right? 11:10:01
ATTORNEY KUSHAN: Objection. Foundation. 11:10:04
A I wasn't sure what you were doing. Are 11:10:06
you reading the entire table, or are you selecting 11:10:10
some of them for some reasons? 11:10:14
Q I'm trying to filter as I go through and 11:10:16
just read out -- 11:10:20
A Filter based on what? 11:10:21
Q The first row or the first column. 11:10:23
A Yes. But what are you filtering for? 11:10:27
Q So I'm not reading hyaluronidase-4 eight 11:10:31

times. I'm just saying hyaluronidase -- 11:10:35
A Oh. I see what you're saying. Okay. 11:10:35
Q Yeah. 11:10:36
A So you're just saying that there are different hyaluronidases here?
Q Yes. 11:10:36
A Okay. Yes. 11:10:37
Q Okay.
A I agree with that. 11:10:38
Q Okay. And then several columns over, is there -- is that a percent identity or a percent similarity that's given? 11:10:41 11:10:46 11:10:49
ATTORNEY KUSHAN: Objection. Foundation. 11:11:01
A I have to look back to the original file, but my recollection says identity. 11:11:04 11:11:07
Q Okay. And so that field would give the percent identity to the sequence that you entered, which is the -- the first sequence, the hyaluronidase PH20 isoform 2? 11:11:10 11:11:14 11:11:19 11:11:21
ATTORNEY KUSHAN: I'm sorry, Counsel. Which -- which column are you referring to in the exhibit? Maybe go from the far right, heading off 11:11:23 11:11:28 11:11:31

from the far right? 11:11:35

ATTORNEY MARTIN: Oh. From the far right. 11:11:35

Five. Right, so -- 11:11:38

THE WITNESS: In fact, yes. I do 11:11:39

apologize. There seems to be different numbers. 11:11:41

I do get confused. One of them does have a 11:11:47

percentage sign, but I'm not sure what the heading 11:11:51

of that is.

So perhaps I take back whatever I said 11:11:56

earlier, whether it was identity. I'll -- I'll 11:11:58

have to look at the original file with the 11:12:00

explanation of what each column means. 11:12:04

BY ATTORNEY MARTIN: 11:12:05

Q Okay. So you don't know if that's 11:12:05

identity or similarity? 11:12:07

A That I don't know right at this moment. 11:12:09

Yes. 11:12:12

Q Do you think it's either identity or 11:12:12

similarity, or is there another option? 11:12:15

A It makes me think that the one that says 11:12:17

percentage is not the identity or similarity. The 11:12:21

reason I say that is because I know the set of 88 11:12:24

or 95 sequences have sequences with similarity down to about 40 percent. Land that column doesn't seem to go down to 40 percent, so that makes me think it's a coverage. Coverage. 11:12:29 11:12:34 11:12:37 11:12:43

Q Oh. I see. 11:12:46

A The identity I think may be the third from the last column. 11:12:47 11:14:32

Q Okay. Okay. I got it. 11:12:51

Okay. So the column with the percentage sign is potentially coverage? 11:12:52

A Coverage. 11:12:52

Q Meaning what portion of the -- what portion of the -- is it the starting sequence is... 11:12:53 11:12:59 11:13:03

A I believe so. 11:13:04

Q What percentage of the starting sequence is being aligned with the test sequence? 11:13:05 11:13:08

A That's right. 11:13:12

Q Okay. Okay. And then -- and then the column that's three columns in from the far right, that number is potentially percent identity or similarity? 11:13:13 11:13:14 11:13:20 11:13:24

A Identity or similarity. I want to say identity. 11:13:24 11:13:27

Q Okay. 11:13:27

A Because I believe the similarities are -- most of them were 50 percent and higher, and identity here goes down to around 40 percent or just below. I think the lowest percentage that I remember was about 36 percent. So that seems to match that value. That column seems to be the identity. 11:13:28 11:13:32 11:13:36 11:13:41 11:13:45 11:13:49 11:13:53

Q Okay. And do you consider all of the sequences in this BLAST result to be -- to have a high sequence similarity, high degree of sequence similarity? 11:13:54 11:13:59 11:14:05 11:14:11

A Yes. I do. 11:14:12

Q And do you consider that all of the 95 sequences in this BLAST result to be homologous sequences? 11:14:14 11:14:17 11:14:20

A Yes. From the label of the individual entries, they do suggest that they're homologous proteins. 11:14:33 11:14:36 11:14:46

(Reporter clarification.) 11:14:49

THE VIDEOGRAPHER: Dr. Park, can you move your microphone up just a bit? 11:14:49 11:14:52

THE WITNESS: Sure. 11:14:57

THE VIDEOGRAPHER: Thank you. 11:14:58

BY ATTORNEY MARTIN: 11:15:09

Q When you say the label of the individual entry, what are you referring to? 11:15:21 11:15:23

A I mean the names that were given to the individual sequences. The first column that you were reading. 11:15:29 11:15:34 11:15:37

Q In other words, the hyaluronidases? 11:15:37

A That is correct. 11:15:41

Q Okay. The fact that they are all hyaluronidases suggests that they are homologous proteins? 11:15:42 11:15:44 11:15:49

ATTORNEY KUSHAN: Objection. Foundation. 11:15:50

A No. 11:15:51

Q Okay. 11:15:53

A If it's just a -- if I'm just focusing on the name, I would find other hyaluronidase enzymes out there that are not homologous to PH20. But in this set, the combination of the sequence identity 11:15:53 11:15:57 11:16:01 11:16:07

and the names do indicate that I'm looking at homologous proteins. 11:16:11 11:16:14

Q Okay. Okay. And when you say the combination of the sequence identity, so is it the sequence identity that is between 30 -- 36, 37 and 100, that combination, plus the name hyaluronidase indicates that they're homologous? 11:16:16 11:16:18 11:16:23 11:16:32 11:17:45

ATTORNEY KUSHAN: Objection. Form. 11:16:42

Also objection to foundation. 11:16:43

A Yes. That would be the case. 11:16:44

BLAST doesn't know what it's returning. It will return any sequence that looks similar to the target sequence, and it will give you the identity percentage. 11:16:47 11:16:50 11:16:54 11:16:57

So theoretically, one shouldn't be able to tell whether they're homologous or not. But combining that sequence identity with the names of the enzymes would convince you that they are homologous proteins. And the coverage. Identity plus coverage and the names. 11:16:59 11:17:03 11:17:06 11:17:10 11:17:14 11:17:17

Q Okay. Sequence identity plus sequence coverage plus the name indicates that they're 11:17:19 11:17:25

homologous? 11:17:29

A Yes. 11:17:31

Q Is there a range for sequence coverage that you look for to determine whether two proteins are homologous? 11:17:32 11:17:35 11:17:38

A No. Homologous proteins can have a wide range of sequence identity. There's high sequence similarity identity. There is a slightly lower range of percentage, sometimes referred to as the twilight zone, or you can have even lower percentage, which may be detected using some very sophisticated algorithms to establish some sort of relationship, but at a very, very low percentage. This is outside the realm of BLAST. BLAST would not return those sequences. 11:17:47 11:17:52 11:17:57 11:18:01 11:18:07 11:18:12 11:18:20 11:18:24 11:18:27 11:18:32

Q Okay. What do you mean when you said the twilight zone? 11:18:33 11:18:37

A Twilight zone refers to a range of sequence identity, somewhere between 20 to 35 percent, which is sort of a grey area in a way, in terms of interpreting sequence comparison. 11:18:39 11:18:44 11:18:51 11:18:54

Some sequences are very easy to interpret 11:18:58

because they are similar. Once a sequence identity drops below a certain number, it gets to be difficult. And even making the assumption that they would fold to the same structure can be questionable, and that would correspond to the twilight zone that I mentioned, or the midnight zone, that's even lower. So these are terms that people use to refer to different levels of sequence activity. 11:19:01 11:19:09 11:19:12 11:19:16 11:23:54 11:19:53 11:19:29 11:19:34 11:19:38

Q Do you know why they -- why it's called twilight zone? 11:19:39 11:19:42

A Because you can't really see very well. 11:19:44

Q Okay. Got it. 11:19:49

And then midnight zone because you can't really see? 11:19:58 11:20:01

A Too dark. Yeah. Pretty dark down there. 11:20:01

Q Okay. Got it. 11:20:02

So for the 95 sequences that are in Exhibit 1054, you expect them to all fold the same way? 11:20:05 11:20:09 11:20:14

A I do. 11:20:15

Q Would you expect that you could generate a 11:20:16

model of any of the sequences in Exhibit 1054 using the SWISS-MODEL? 11:20:29 11:20:33

ATTORNEY KUSHAN: Objection. Foundation. 11:20:41

A Generate a homology model of the sequences in this list? 11:20:42 11:20:46

Q Yes. 11:20:47

A Using SWISS-MODEL? 11:20:47

Q Yes. 11:20:50

A That depends on the sequence identity between the individual sequences and the model, which is from HYAL1. In the case of PH20, the identity is sufficiently high. 41 percent, 45 percent, somewhere around there. 11:20:55 11:21:01 11:21:05 11:21:15 11:21:17

For other sequences, once you do the comparison with HYAL1, I don't know what that sequence percentage is. The percentage that's listed here is an identity to PH20 doesn't guarantee that they would similarly have high percent -- percentage identity with respect to HYAL1, and that would determine whether a reliable homology model can be generated for these entries. 11:21:19 11:21:24 11:21:30 11:21:30 11:21:33 11:21:37 11:21:40 11:21:47

Q Okay. I understand. 11:21:53

So you said 45-ish percent sequence identity for PH -- between PH20 and HYAL1, that's sufficiently high to use SWISS-MODEL to generate a homology model; is that right? 11:21:54 11:22:00 11:22:01 11:22:07

ATTORNEY KUSHAN: Objection. Foundation. 11:22:09

A That will give you a structure model that is overall reliable and can be used for subsequent studies, yes. 11:22:11 11:22:19 11:22:25

Q Is there a minimum amount of sequence identity that you need to satisfy in order to use SWISS-MODEL to generate a homology model? 11:22:30 11:22:33 11:22:37

ATTORNEY KUSHAN: Objection. Foundation. 11:22:44

A This is another one of those questions where one doesn't have a solid number, except a number that's commonly used and understood. A 30 percent identity between two proteins that are homologous would be assumed to fold the same structure. 11:22:46 11:22:50 11:22:54 11:23:00 11:23:07 11:23:10

Q Okay. 11:23:11

A So if it's 31 percent, I don't know. But if it's 45 percent, that seems like a pretty safe place to be. 11:23:12 11:23:16 11:23:21

Q Okay. Got it. 11:23:22

And so the 30 percent identity between two 11:23:26
proteins that are homologous, they would be 11:23:31
assumed to fold the same structure? Is that 30 11:23:35
percent number, is that well-known in the field? 11:23:38

ATTORNEY KUSHAN: Objection. Foundation. 11:23:42

A If you ask a POSA to put a number on that, 11:23:43
I think they would name 30 percent. 11:23:48

Q Okay. And was that the case in 2011? 11:23:51

A The understanding of 30 percent being sort 11:23:57
of the cut-off has been around for a long, long 11:24:01
time, including 2011, yes. 11:24:05

Q Okay. Would you consider proteins that 11:24:08
have more than 40 percent sequence identity to be 11:24:33
highly homologous? 11:24:37

ATTORNEY KUSHAN: Objection. Form. 11:24:39

A No. Those are unrelated issues. 11:24:43
Similarity is a similarity. Homology -- 11:24:47
homologous -- homologous proteins. 11:24:52

Q Oh, okay. 11:24:54

A You know, homology and homologous proteins 11:24:55
are -- that's a separate issue. They tend to go 11:25:00

hand in hand, but there's no direct correlation between the two. 11:25:04 11:25:08

Q Okay. I understand. 11:25:10

Because you could have proteins that are more similar in sequence that are completely evolutionarily unrelated, so those would not be homologous proteins; is that what you're saying? 11:25:11 11:25:15 11:25:17

A Yes. Although that might be difficult to -- to -- I don't know. It may be difficult to imagine. I don't know. 11:25:20 11:25:24 11:25:32

Q Okay. 11:25:33

A But again, those two are separate issues. They should not be mixed. 11:25:34 11:25:38

Q Okay. Understood. 11:25:40

Okay. All right. So then looking at 10 -- Exhibit 1057. 11:25:47 11:25:48

A 57. Yes. 11:25:57

Q So Exhibit 1057 is the alignment that you generated using Clustal Omega, right? 11:25:57 11:26:03

A This is the alignment that was produced by Clustal Omega, yes. 11:26:04 11:26:09

Q And you generated the alignment -- you 11:26:11

used Clustal Omega to generate the alignment, right? 11:26:12 11:26:15

A That's how I generated the sequence alignment, yes. 11:26:16 11:26:20

Q And I believe I asked earlier about other programs that could have been used for sequence alignments, and I believe you said that a POSA in 2011 would have used Clustal Omega. 11:26:24 11:26:28 11:26:31 11:26:35

Is that the case just for multiple sequence alignments? 11:26:38 11:26:41

A Clustal Omega is -- it's an algorithm that's designed to align multiple sequences. 11:26:44 11:26:50

Q If a POSA wanted to do a pairwise alignment, what would they have used in 2011? 11:26:54 11:26:58

A They would have used BLAST. 11:27:02

Q Okay. 11:27:04

A BLAST does have a feature that allows you to submit two sequences and do pairwise alignment. 11:27:05 11:27:10

Q Okay. And you can match the sequences that are in Exhibit 1057 with the sequences that are in Exhibit 1054 based on the designation? 11:27:16 11:27:30 11:27:35

I'm not sure what the right term for that 11:27:42

is, but in the left-hand most column on 1057; is that right? 11:27:46 11:27:50

A The accession numbers are -- 11:27:50

Q Accession numbers. Thank you. 11:27:54

A Yes.

Q Okay. So yeah. You use the accession number -- you can use the accession numbers in Exhibit 1057, which are in the left-hand most column to match the sequences in Exhibit 1057 with the sequences in 1054; is that right? 11:27:56 11:27:57 11:28:02 11:28:05 11:28:09

A Yes. 11:28:09

Q So the first -- the sequence at the very top in Exhibit 1057 has accession number NP003108.2, and that sequence in Exhibit 1054, that's the third sequence on the list. 11:28:15 11:28:19 11:28:29 11:28:33

It's PH20 isoform number 1; is that right? 11:28:39

A That is correct. Yes. 11:28:42

Q Do you know why you used the isoform number 1 as your lead sequence in the alignment in 1057, as opposed to the isoform 2 that you entered into BLAST in Exhibit 1054? 11:28:43 11:28:49 11:28:52 11:28:57

A Yeah. That's a byproduct of the 11:29:04

processing steps that I took in order to reduce the set of 95 sequences to 88 by illuminating redundancy. 11:29:11 11:29:15 11:29:22

At the time I assumed that the longest sequence might be what we want. Given that two -- given two sequences that are from the same gene, I decided to go with the longest isoform. That was the logic in the script that I developed in order to identify redundancy and filter it down to 88. 11:29:25 11:29:29 11:29:35 11:29:42 11:29:46 11:29:49

It turned out that isoform 1 has 511 amino acids; whereas isoform 2 has 9 -- 509 amino acids. So the program picked isoform 1 as opposed to isoform 2 as the sequence to keep in the final 88. 11:29:57 11:30:04 11:30:10 11:30:15

They are different in the final 14 amino acids of isoform 2. They are identical up to 495, but afterwards they deviate. And for that reason, isoform 1 ended up being included in the final set. And because I didn't -- yeah. So that was that. 11:30:20 11:30:25 11:30:32 11:30:43 11:30:49 11:30:50

Q Okay. Okay. So you selected the longest isoform, which is isoform 1, which has the 511 amino acids as the lead sequence in your 11:30:50 11:30:56 11:31:01

alignment? 11:31:02

A That's correct. Yes. 11:31:02

Q Okay. All right.

A 511, minus the ones that didn't match to 11:31:05

isoform 2. In other words, the sequences that 11:31:09

were used in the multiple sequence alignment are 11:31:13

the portions of the sequences that were matched by 11:31:17

BLAST. 11:31:22

Q Okay. 11:31:24

A So comparing isoform 2 and 1, that 11:31:25

alignment went up to 495. So only the first 495 11:31:29

amino acids of isoform 1 was used in the final 11:31:36

multiple sequence alignment, which incidentally is 11:31:42

exactly the same as isoform 2. 11:31:46

Q Okay. I understand. 11:31:49

A So despite the fact that it has a 11:31:49

different accession number, the results would have 11:31:52

been identical. But I used... 11:31:57

Q Okay. Okay. I see. Okay. I understand. 11:31:58

All right. So let's turn in your 11:32:39

declaration to Paragraph 30. 11:32:40

A Okay. 11:33:07

Q All right. So in Paragraph 30, you say: Using the alignment identified 68 largely invariant residues that a skilled artisan would have deemed essential residues in PH20 based on the sequence alignment, and then you have the table, right? 11:33:10 11:33:13 11:33:17 11:33:23 11:33:29 11:33:28

A Yes. 11:33:29

Q Can you explain what you mean by the term invariant in that sentence? 11:33:31 11:33:36

A By invariant, I mean that once the sequences have been aligned by Clustal Omega, there are certain positions where the identity of the amino acid does not change at all, or it changes only ever so slightly. 11:33:45 11:33:49 11:33:55 11:33:58 11:34:02

Q And the cut-off that you used to identify invariant residues is 95 percent; is that right? 11:34:09 11:34:12

A I used 95 percent as the cut-off, yes. 11:34:21

Q And how did you arrive at the 95 percent number? 11:34:25 11:34:29

A Well, there are two ways to think about it. I wanted to ensure that the preference of the wild-type amino acid at that position is 11:34:37 11:34:43 11:34:50

statistically significant compared to other amino acids that may occur at that position. And 95 percent seemed pretty convincing. 11:34:55 11:35:01 11:35:02

(Reporter clarification.) 11:35:11

A That would be a simple explanation. 11:35:11

Q Had you used a 95 percent cut-off in your work before to identify invariant residues? 11:35:17 11:35:21

ATTORNEY KUSHAN: Objection. Form. 11:35:27

A Never had the occasion to having to identify invariant residues before. So no, I have not used 95 percent per se. 11:35:31 11:35:36 11:35:44

Q So in your work, when you perform sequence alignments, you never identified invariant residues in those alignments? 11:35:46 11:35:50 11:35:56

ATTORNEY KUSHAN: Objection. Foundation. 11:36:01

A I do identify. However, the focus of my past studies have not been on invariant residues. Therefore, having to put a number on where the cut-off needs to be has never occurred. 11:36:02 11:36:07 11:36:20 11:36:24

Q So are you saying that in your work, when you would perform a sequence alignment like this, you would identify residues that were conserved, 11:36:28 11:36:32 11:36:35

but you never had to go through the exercise of categorizing residues, as invariant versus not invariant, and so that's why you haven't -- you didn't use a cut-off? 11:36:40 11:36:43 11:36:50 11:36:52

ATTORNEY KUSHAN: Objection. Form. 11:36:53

A Right. My goals were different and did not require having to identify invariant residues and having to put a number on the conservation. 11:36:54 11:36:58 11:37:05

Q So in your work in 2011, did you perform BLAST searches and then form sequence alignments of homologous proteins, like you did here? 11:37:10 11:37:15 11:37:19

ATTORNEY KUSHAN: Objection. Foundation. 11:37:22

A Are you referring to my independent work? 11:37:23

Q Yes. 11:37:27

A That might have happened in 2011? 11:37:28

Q Yes. 11:37:31

A Which, by the way, I haven't done any in 2011, I don't think. 11:37:31 11:37:35

But prior to that, perhaps? 11:37:36

Q Uh-huh. Yeah. In your independent work. 11:37:39

A Yeah. What about it? 11:37:40

Q Had you used BLAST to identify homologous 11:37:42

sequences and then perform the multiple sequence alignment? 11:37:46 11:37:50

A That I have, yes. 11:37:53

Q Okay. And why did you do that? What was the goal? 11:37:54 11:37:57

A The goal was to identify what other amino acids we might try at various positions in the protein that we're working with. 11:37:58 11:38:02 11:38:06

Q So you were -- you performed this alignment to identify potential candidates for substitution in the protein that you were working with? 11:38:10 11:38:13 11:38:17 11:38:21

A That is correct. 11:38:21

Q Okay. Did you already -- did you know what residues were essential for function in that protein? 11:38:22 11:38:30 11:38:34

ATTORNEY KUSHAN: Objection. Foundation. 11:38:35

A Essential residues weren't of interest to us. It was actually a non-essential residue, a residue that actually varied quite a bit from one protein to another that we were interested in. We wanted to see whether we could introduce a 11:38:39 11:38:43 11:38:47 11:38:52 11:38:56

mutation at that variable position in order to improve the function of that protein that we were working with. 11:39:02 11:39:05 11:39:06

Q And why were you focussed on that one specific position? 11:39:06 11:39:10

A There have been a lot of studies on this protein. This is a well-known protein. And the amino acids important for function were also well known, so we paid our attention to those positions more than amino acids. 11:39:12 11:39:15 11:39:22 11:39:27 11:39:32

Q Okay. What protein? 11:39:37

A Streptavidin. 11:39:39

Q So you were trying to improve the function in streptavidin by introducing mutations at a particular position; is that right? 11:39:58 11:40:02 11:40:08

A That is correct. 11:40:08

Q And how did you arrive at the particular position that you wanted to change in streptavidin? 11:40:09 11:40:13 11:40:17

A That position was known to be important. 11:40:17

Q Was it known to be important through studies in the literature? 11:40:24 11:40:29

A Yes. 11:40:28

Q When you say important, what do you mean? 11:40:39

A The residue at that position seems to be involved in ligand binding. 11:40:45 11:40:52

(Reporter clarification.) 11:41:03

Q And were you able to improve streptavidin activity by introducing mutations at that position? 11:41:03 11:41:13 11:41:12

A Yes. It did improve. 11:41:14

Q Did you do the work in your lab that identified that position as important in ligand binding? 11:41:23 11:41:25 11:41:32

A Did we do the work in the lab? 11:41:33

Repeat that question, please. 11:41:37

Q Let me try to ask a different question. 11:41:38

How do you know that that position was -- that that position was important in ligand binding? 11:41:42 11:41:47 11:41:49

A As I said, previous studies by other labs have identified that position to be involved in ligand binding. 11:41:50 11:41:56 11:42:02

Q Was that position that you were trying to 11:42:32

change in streptavidin, was that position essential to the protein folding? 11:42:36 11:42:39

A No. I don't think so. 11:42:46

Q Okay. In your -- in your work, in terms of introducing substitutions into proteins, have you ever tried introducing substitutions into a position that is essential for protein folding? 11:42:51 11:42:59 11:43:04 11:43:06

ATTORNEY KUSHAN: Objection. Form. 11:43:10

A No. I have never tried to introduce a mutation at a position that is known to be important for folding. 11:43:14 11:43:17 11:43:21

Q Would you expect that if you tried to introduce a mutation into a position known to be important for folding, that the -- that the protein would not be able to fold correctly? 11:43:22 11:43:25 11:43:28 11:43:29

A I would expect that; therefore, I would not try that. 11:43:36 11:43:39

Q Is it fair to say that a person skilled in the art would expect that if you introduced positions into residues that are important for folding, the resulting protein will not be properly folded? 11:43:49 11:43:54 11:43:58 11:44:02 11:44:06

ATTORNEY KUSHAN: Objection. Foundation. 11:44:08

A I think you may have garbled up words 11:44:12
there. 11:44:17

Q That's possible. Let me try to ask that 11:44:17
better. 11:44:20

A Okay. 11:44:20

Q So okay. So would persons skilled in the 11:44:20
art understand that if you try to introduce a 11:44:28
substitution into a position known to be important 11:44:32
for folding, the protein would not be able to fold 11:44:36
correctly? 11:44:39

ATTORNEY KUSHAN: Objection. Foundation. 11:44:40

Objection as to form. 11:44:41

A That really depends on the nature of the 11:44:43
conservation. Some amino acids and residues are 11:44:49
important for reasons other than folding itself. 11:44:55
If it's an enzyme, for instance, the residue may 11:45:00
be important for catalysis.

(Reporter clarification.) 11:45:15

A And mutating those residues would abrogate 11:45:15
enzyme function; however, it would have minimal 11:45:23
impact on the folding of the protein. 11:45:27

So knowing what the conservation is for would be relevant to calibrate one's expectation. 11:45:27 11:45:32

Q Okay. So along those lines, the skilled person would expect that if you make a substitution in a residue that's essential for catalysis, the skilled person would expect that you're going to inactivate the protein; is that right? 11:45:37 11:45:43 11:45:46 11:45:51 11:45:53 11:45:57

ATTORNEY KUSHAN: Objection. Foundation. Objection as to form. 11:45:57 11:45:58

A Introducing a mutation at a position that is catalytically important often abrogates the enzyme function. That would be the expectation. 11:46:01 11:46:06 11:46:14

Q So let's turn to Appendix D-1. 11:46:26

A D-1?

Q D-1, yeah. Which starts on Page 126. Okay. You have it. 11:46:41 11:46:44

A I'm on that. 11:46:44

Q Can you explain what you have -- what you've set forth in exhibit -- or, Appendix D-1, Appendix D, Part 1, rather? 11:46:46 11:47:11 11:46:57

A D, Part 1. Yes. 11:46:59

This is the output of the analysis of the multiple sequence alignment from Clustal Omega that we saw earlier in Exhibit 1057. I developed a script that took this as an input, and for each amino acid in PH20 -- that's the top line -- I would give it a residue number -- that's wild-type 1, wild-type 2 -- corresponding to the first amino acid, second amino acid, of PH20. And for that position, from the alignment, I counted the appearance of various amino acids and computed the actual number, as well as the percentage, which is divided by -- which is obtained by dividing that number by 88. 11:47:06 11:47:14 11:47:18 11:47:27 11:47:33 11:47:46 11:47:51 11:47:54 11:48:00 11:48:05 11:48:10 11:48:16 11:48:20

So for the first position, you have methionine, M, wild-type position number 1, methionine. If you look at the alignment, you see some sequences that have methionine at the beginning. But then there are a lot of sequences that don't have any matching residue. The absence of a matching residue is indicated by dashes. 11:48:24 11:48:33 11:48:34 11:48:38 11:48:41 11:48:45 11:48:48

So there are a lot of dashes. So the dash appears 72 times. Methionine appears 16 times, 11:48:51 11:48:53

divided by 88, will give you 18.18. So the top line says: Methionine appears 18.18 percentage, but the most frequent residue, in this case a dash, appears 81.81 percentage. 11:48:59 11:49:00 11:49:05 11:49:15

Then you will do that for every position of PH20, all the way down to the last amino acid, which I believe is 495 -- yeah, 495 -- for the reasons that I explained earlier. 11:49:18 11:49:21 11:49:28 11:49:35

Q Okay. And the sequence that you used includes the 35 amino acid signal peptide, right? 11:49:37 11:49:40

A It does include the 35 signal sequence at the beginning, yes. 11:49:45 11:49:50

Q Okay. So we start with position -- let's see. Actually, let's just do -- so you refer to position 320, or in the full length sequence it's position 355, right? 11:49:51 11:49:57 11:50:05 11:50:09

A The full length sequence number would be 355. That is correct. 11:50:11 11:50:15

Q So in Appendix D-1, the residue in question is residue 355? 11:50:16 11:50:22

A That is correct. 11:50:24

Q And that's on Page 146? 11:50:26

A Okay. 11:50:31

Q Okay. So it says: Wild-type 355 is D, 11:50:34

right? 11:50:40

Did you get it? 11:50:42

A I thought I did. 11:50:43

Q Wild-type residue position 355 is D, 11:50:45

right? 11:50:50

A Yes. 11:50:51

Q And if I'm understanding this correctly, 11:50:51

then D appears 10.22 percent of the time? 11:50:54

A Yes. 10.22 percent of the time, out of 11:50:59

the 88 sequences. 11:51:02

Q Okay. And the most common residue at that 11:51:03

position is K, right? 11:51:07

A That is correct. 11:51:09

Q And K appears 57.95 percent of the time? 11:51:10

A That's right. It appears 51 times out of 11:51:15

88, corresponding to 57.95 percent. Yes. 11:51:20

Q Okay. So why does it say, like, res394? 11:51:24

A Okay. One, you can ignore that number. 11:51:29

Q Okay.

A It's just a bookkeeping number. 11:51:32

Q Okay. 11:51:36

A Including all the dashes in the alignment. 11:51:37

Yeah. So I didn't know what I was using this 11:51:41

output for, ultimately when I was developing the 11:51:44

algorithm. So I just kept track of all the 11:51:48

positions. Because of the dashes, the wild-type 11:51:52

number actually corresponds to the amino acid 11:51:58

number in PH20. 11:52:02

Q Uh-huh.

A But res394, that's just a position in this 11:52:03

particular format. 11:52:09

Q Oh, okay. Okay. Got it. Thanks. 11:52:10

Okay. So in -- at the position 355, in 11:52:14

your alignment then all of the residues that 11:52:21

appear there, which are K, D, H, R, N, Q, S, G, E, 11:52:30

those are all of the amino acids that appear at 11:52:38

position 355 in the alignment; is that right? 11:53:10

A Yeah. The position corresponding to 11:52:44

wild-type residue in PH20, 355, yes. 11:52:50

Q Yes. Okay. 11:53:04

All right. So can you turn to 11:54:06

Paragraph 31? 11:54:07

A Okay. 11:54:18

Q So -- oh, actually. Sorry. 11:54:19

So we've talked about Paragraph 30, which 11:54:29

is where you have the 95 percent cut-off, right, 11:54:32

the essential residues? 11:54:38

A Yes. 11:54:40

Q Okay. And then you have a chart on 11:54:40

Page 14, and that gives all of the essential 11:54:42

residues; is that right? 11:54:47

A Page 14 lists essential residues, yes. 11:54:50

Q Okay. And so you have the residue number, 11:54:53

which is the -- the position in the wild-type 11:54:58

sequence that includes the signal sequence, right? 11:55:03

A Yes. Residue number -- yes. 11:55:11

Q Okay. And then the mature residue number 11:55:15

is the position in the sequence that does not 11:55:18

include the signal sequence? In other words, you 11:55:21

just subtract 35 from the residue number? 11:55:23

A That's correct. 11:55:27

Q Okay. And then the PH20 residue, and then 11:55:28

you have residue percent. So is the residue 11:55:34

percent the frequency with which the PH20 residue 11:55:40

occurs in the alignment of that position? 11:55:44

A That is correct. The residue percentage 11:55:47

corresponds to the wile type residue of PH20. 11:55:50

Q Okay. So for example, the first entry in 11:55:57

your chart is mature residue position 14, and it's 11:56:00

PH20 residue F, and the residue appears 95.5 11:56:09

percent of the time. So is it the case then 11:56:16

that -- am I understanding this correctly, that in 11:56:18

the alignment at mature residue position 14, 95.5 11:56:20

percent of the time there is an F? 11:56:26

A That is correct. 11:56:27

Q Okay. So then -- and there are 68 11:56:37

residues that you identified as essential, right? 11:56:44

A There are 68 residues in this table 11:56:49

labeled essential, yes. 11:56:53

Q And those 68 residues are the residues 11:56:56

that the skilled person would understand to be 11:57:01

essential to protein folding? 11:57:05

ATTORNEY KUSHAN: Objection. Foundation. 11:57:06

A Essential, not necessarily for folding. 11:57:11

Again, some residues may be conserved for other 11:57:16

reasons besides folding a cell. For this class of 11:57:22

proteins, that really comes down to the catalatic residue. So catalatically important residues also be conserved, and therefore included in this list. 11:57:26 11:57:32

Q So -- okay. So is the 68 residue list then, does that include residues that are essential for folding and/or catalytic activity? 11:57:35 11:57:40 11:57:41

ATTORNEY KUSHAN: Objection. Foundation. 11:57:47

A One would think that. 11:57:48

Q Okay. All right. 11:57:50

So now let's go to Paragraph 31 on the next page. 11:57:53 11:57:56

So the second and third -- I'm looking at the second and third sentence in Paragraph 31. 11:58:07 11:58:13

A I did get it. 11:58:17

Q Nice. Okay. 11:58:18

The second and third sentence in Paragraph 31: The existence of evolutionary segregation at these positions indicates that the homologous proteins have tolerated different amino acids at those positions. 11:58:21 11:58:24 11:58:30 11:58:32 11:58:34

And so am I understanding correctly that because the positions that you've labeled as 11:58:35 11:58:46

non-essential, because there's variation in the amino acid at that position in the sequence, for homologous proteins, that would indicate to a POSA that the POSA could potentially introduce substitutions at those positions? 11:58:48 11:58:52 11:58:56 11:59:00 11:59:05

A Because there is variation among naturally occurring homologous proteins, a POSA would be justified in thinking that a mutation may be made at those positions without damaging the enzyme. 11:59:13 11:59:17 11:59:22 11:59:28

Q Okay. And then you say: Amino acids at these positions in the different hyaluronidase proteins would be considered non-essential residues because the proteins presumably still exhibit hyaluronidase activity when they are mutated; is that right? 11:59:33 11:59:36 11:59:38 11:59:41 11:59:43 11:59:51

A They are considered non-essential, given that there is variation. In other words, the wild-type residue identity is not strictly required, and that makes it non-essential from that sense. 11:59:53 11:59:57 12:00:01 12:00:06 12:00:09

Again, it doesn't mean that it's anything goes. One has to be careful. But from that 12:00:12 12:00:15

definition, yes. 12:00:19

Q But there are -- there are proteins in the alignment that don't exhibit hyaluronidase activity, right? 12:00:24 12:00:28 12:00:32

A That is correct. Yes. HYAL3, as I mentioned earlier, doesn't seem to have known activity. It must still be folded, and must be doing something. Otherwise, there's no reason to keep those amino acids where they are. It's so easy to mutate them away. 12:00:33 12:00:38 12:00:41 12:00:44 12:00:48 12:00:49

In fact, hyaluronidase 6 in human is supposed to be a pseudo gene, but it has diversed so much you can't even find it, actually. It's not easy to find. 12:00:51 12:00:53 12:01:00

The same fate would have happened to hyaluronidase 3. The fact that it's around and has all those conserved residues suggests that it must be doing something. And in order to do be doing something for a protein, it has to be folded. 12:01:05 12:01:07 12:01:11 12:01:14 12:01:18

So one can deduce based on those observations that -- yeah. So they are doing 12:01:18 12:01:23

something. They might not have strictly the hyaluronidase activity. However, what's important is that they do have a structure that is sufficient to support activity. 12:01:24 12:01:28 12:01:31 12:01:35

That's what one would take away from this multiple sequence alignment. Not a strict reading that these enzymes exist because in the group, they exhibit hyaluronidase activity, which we know to be not true, with 3 and 4, for instance. 12:01:40 12:01:45 12:01:47 12:01:50 12:01:54

Q Okay. So the person of ordinary skill in the art would understand that amino acids at those non-essential positions and the different hyaluronidase proteins -- actually, strike that. It's going to be garbled. 12:02:09 12:02:12 12:02:30 12:02:23 12:02:24

I mean, the skilled person would understand that the non-essential residues are potentially amenable to change because different amino acids are allowed at those positions and the resulting proteins are folded and exhibit whatever activity it is that they do? 12:02:24 12:02:27 12:02:35 12:02:40 12:02:45 12:02:48

A That was a long sentence. If you could repeat some part of that sentence? 12:02:55 12:03:00

Q Uh-huh. 12:03:02

So the amino acids at the -- at the 12:03:06

positions that you termed non-essential would be 12:03:09

considered non-essential because the protein -- 12:03:13

the resulting protein is still folded and exhibits 12:03:15

some activity? 12:03:21

A They must have activity. Some activity, a 12:03:27

lot of activity, that's hard to say. But they 12:03:30

must have activity, and you still see a variation 12:03:35

in those individual sequences. Coupling those two 12:03:41

things was -- would lead one to believe that you 12:03:47

can have variation at these positions without 12:03:53

compromising the activity of the protein, which in 12:03:57

turn assumes that the protein would be folded. 12:04:02

ATTORNEY MARTIN: So it's 12:05. Is now a 12:04:13

good time to break for lunch? 12:04:17

ATTORNEY KUSHAN: Sure. 12:04:22

THE WITNESS: Okay. 12:04:23

THE VIDEOGRAPHER: We are going off the 12:04:23

record. The time is 12:04 p.m. 12:04:27

(Recess from 12:04 p.m. until 1:06 p.m.) 12:04:32

THE VIDEOGRAPHER: We are back on the 13:06:31

record. The time is 1:06 p.m. 13:06:35

BY ATTORNEY MARTIN: 13:06:38

Q Welcome back, Dr. Park. 13:06:40

Did you discuss the substance of your deposition with anyone during the break? 13:06:42 13:06:44

A No. I did not. 13:06:47

ATTORNEY MARTIN: Okay. So can I ask, is Dr. Hecht still on the Zoom? 13:06:48 13:06:51

ATTORNEY KUSHAN: I don't know. 13:06:55

Brian, are you on the Zoom? 13:06:58

ATTORNEY GOLDBERG: Sorry. I was just unmuting. 13:07:02 13:07:05

Yes. Dr. Hecht and I are on the Zoom. 13:07:06

ATTORNEY MARTIN: Okay. So we object to Dr. Hecht listening in to this deposition because we believe it's improper witness coaching. 13:07:09 13:07:11 13:07:15

And so we're going to ask Dr. Hecht to stop listening to the deposition. 13:07:20 13:07:21

ATTORNEY KUSHAN: How is this witness coaching? 13:07:21 13:07:24

ATTORNEY MARTIN: Because he's listening to the deposition, presumably to prepare for his 13:07:26

own deposition.
ATTORNEY KUSHAN: He can review the deposition transcript, too.
ATTORNEY MARTIN: But it's different to listen in to the deposition of another expert in the same case and then --
ATTORNEY KUSHAN: How?
ATTORNEY MARTIN: -- see how depositions go so that you can understand, you know, what to do for your deposition.
ATTORNEY KUSHAN: Look, I've been in depositions where an expert who is not being deposed is attending, and never been the basis for objection.
ATTORNEY MARTIN: I have never had a deposition where another -- an expert for the same side is listening to -- who has not yet been deposed is listening to another expert be deposed before him. I have never had that, ever.
ATTORNEY KUSHAN: Well, you can object. I don't know how long he's going to be on. You know, he can remove himself. If you want to

consult with this -- with me on this, we can do that on the next break. 13:08:11 13:08:15

ATTORNEY MARTIN: Okay. So our objection is noted on the record, and understand that you're not willing to remove Dr. Hecht from the deposition. 13:08:15 13:08:17 13:08:20 13:08:23

ATTORNEY KUSHAN: Correct. 13:08:23

ATTORNEY MARTIN: All right. And to be clear, the objection is witness coaching under FRE-615. 13:08:24 13:08:29 13:08:37

BY ATTORNEY MARTIN: 13:08:49

Q Okay. So at the beginning -- at the very beginning of this deposition, Dr. Park, you gave -- you gave me a chart that had the corrections to your declarations, right? 13:08:50 13:08:54 13:08:58 13:09:01

A Yeah. 13:09:03

Q So when did -- when did those corrections first come to your attention? 13:09:04 13:09:06

A I noticed these errors while I was reading the declarations in preparation for today's deposition. 13:09:21 13:09:27 13:09:30

Q Okay. And when did that -- when did that 13:09:30

happen? 13:09:37

A The past three weeks, I would say. 13:09:41

Q Okay. And when was the chart prepared? 13:09:44

A This chart? 13:09:49

Q Yes. 13:09:50

A This was given to me this morning. 13:09:56

Q Got it. 13:09:59

Dr. Park, what did -- what did you do to prepare for this deposition? 13:10:15 13:10:17

A To prepare for this deposition, I read the declarations over. I read the articles over. I went over the data to make sure that I was familiar with the content of each file that was being submitted. 13:10:22 13:10:29 13:10:34 13:10:38 13:10:42

Q When you said the data, you mean the data in the appendices, or the exhibits to your declaration? 13:10:47 13:10:50 13:10:54

A That's correct. 13:10:54

Q Okay. Did --

A I wanted to know what was exactly in that file. I think I knew what it was, but wanted to go over that one more time. 13:10:55 13:10:59 13:11:02

Q Did you meet with anyone to prepare for this deposition? 13:11:03 13:11:06

A I met with counsel. 13:11:10

Q Was there anyone present who was not a lawyer? 13:11:12 13:11:15

A No. 13:11:20

Q Okay. Have you had any communications with Dr. Hecht regarding this case? 13:11:21 13:11:32

A We had Zoom meetings together. 13:11:40

Q About how many Zoom meetings? 13:11:43

A I want to say maybe four. I know it was less than seven for sure, because I elaborated on some of the positions, and there were seven of them. 13:11:51 13:11:59 13:12:06 13:12:12

At some of the meetings, I discussed more than one position, so it was definitely less than seven. I don't remember exactly how many I discussed at which days. My feeling is doubling up -- doubling up on some of the days. It might have been four meetings, say. 13:12:12 13:12:15 13:12:19 13:12:21 13:12:27 13:12:30

Q And approximately when did those meetings take place? 13:12:33 13:12:37

A I think it was toward the end of last year and beginning of this year. 13:12:44 13:12:48

Q Approximately how long were those meetings? 13:12:54 13:12:57

A Those Zoom meetings lasted an hour and a half to two hours each. 13:13:02 13:13:07

Q Did you -- did you ask to speak to Dr. Hecht, or did he ask to speak to you, do you know? 13:13:16 13:13:20 13:13:26

A During the Zoom meeting you mean? 13:13:26

Q Did you initiate -- in order to initiate the meeting, did you request to speak with Dr. Hecht? 13:13:29 13:13:31 13:13:37

A To initiate the meeting? 13:13:37

Q Yes. 13:13:40

A No. Those meetings were set up by Mr. Kushan. 13:13:40 13:13:47

Q Okay. Got it. 13:13:48

Was any -- was anyone else present -- strike that. 13:13:49 13:13:51

Was counsel present during the meetings with Dr. Hecht? 13:13:51 13:13:54

A Yes. 13:13:55

Q Okay. Did you communicate with Dr. Hecht about the substance of your declarations after you signed them? 13:13:57 13:14:23 13:14:30

A After I signed them? I'm afraid I can't tell which happened first, signing or the discussion. 13:14:28 13:14:39 13:14:42

Q Okay. So let's turn -- did you -- did you read Dr. Hecht's declarations? 13:14:43 13:15:43

A No. 13:15:45

Q Do you know what Dr. Hecht opined on in connection with these PGRs? 13:15:45 13:15:51

A Pardon? 13:15:53

Q Do you know what the substance of Dr. Hecht's testimony is? 13:15:54 13:15:56

A No. 13:15:58

Q Did you ask doctor -- or, sorry. Strike that. 13:15:58 13:16:00

So let's turn to Paragraph 85. 13:16:00

ATTORNEY KUSHAN: I'm sorry, Counsel. 13:16:35

Before you proceed, we're going to have Dr. Hecht leave this Zoom. We, of course, 13:16:36 13:16:39

disagree with your objection, but as of respect of your request, we're going to honor your request and have him leave. 13:16:43 13:16:47 13:16:51

ATTORNEY MARTIN: Thank you. 13:16:52

BY ATTORNEY MARTIN: 13:17:07

Q Okay. So in this section -- so, yeah. 13:17:09

Are you in Paragraph 85? 13:17:28

A I am in Paragraph 85. I wanted to see where Paragraph 85 comes in, in the overall scheme of things as I'm flipping through here. 13:17:30 13:17:34 13:17:39

Okay. I'll listen to your question first. 13:17:43

Q Okay. So the second sentence in Paragraph 85, you say: I balanced the type of impact of the substitutions based on the magnitude of each interaction may have on the protein structure. 13:17:48 13:17:52 13:17:55 13:18:00 13:18:03

Can you explain what you mean by the impact of the substitutions, like, what -- what you mean by impact, I guess? 13:18:04 13:18:06 13:18:09

A When a substitution is made, changes happen. Some are favorable changes. Others are unfavorable changes, and those changes are listed 13:19:00 13:19:04 13:19:09

in the paragraphs before this. There are many of them. 13:19:14 13:19:17

Q Uh-huh. 13:19:18

A I was just looking through them. Many of them are enumerated. I am factoring all of those positive and negative changes trying to come up with an estimate of where the net effects falls. 13:19:21 13:19:24 13:19:32 13:19:37

(Reporter clarification.) 13:19:44

Q Okay. So is it fair to say that you're analyzing all the potential effects of a substitution, and then trying to arrive at the overall net effect? 13:19:44 13:19:50 13:19:54 13:19:58

A That would be a correct summary, yes. 13:19:59

Q Okay. And when you reference the magnitude of each interaction, what do you mean -- what do you mean by magnitude? 13:20:04 13:20:06 13:20:10

A It's more of a perception. When there are two conflicting changes, I need to decide whether there are of equivalent magnitude, meaning is one as positive as the other one is negative, or is one factor more significant than the other. And if so, what would be the sum of all those number 13:20:19 13:20:25 13:20:30 13:20:36 13:20:41 13:20:44

of interactions.

So without putting a number on the magnitude, which obviously cannot be done, based on visual inspection, I try to apply my experience and my viewpoint as a POSA, to determine whether the net change would be in favor of the protein structure and stability and function, or the other way around, whether it would negatively impact the structure and function. 13:20:51 13:20:54 13:20:58 13:21:03 13:21:09 13:21:22 13:21:27 13:21:31 13:21:34

Q And so based on that assessment, you used a scoring system of -- using the numbers 1, 2 and 3, right? 13:21:45 13:21:54 13:21:58

A I did use a system of 1, 2 and 3. Yes. 13:21:58

Q Had you used that scoring system before in your work, outside of this case? 13:22:01 13:22:05

A Maybe implicitly, but not in those terms. 13:22:11

Q So is it fair to say that in your work outside of this case, you had perhaps looked at potential substitution and determine whether you would expect it to improve the stability or have a neutral effect or have a negative effect, but you wouldn't necessarily give it a 1, 2 or 3 rating? 13:22:17 13:22:22 13:22:25 13:22:29 13:22:33 13:22:37

ATTORNEY KUSHAN: Objection. Form. 13:22:41

A That is correct. I never had the need to put a number on what I see. 13:22:45 13:22:49

Q And why did you need to put a number on what you see in your analysis in this case? 13:22:55 13:22:58

A In order to put those substitutions in 2/3 bins so that I can say whether a mutation would be likely to be tolerated or not tolerated. I had to make that qualitative decision for the purpose of this declaration, and that required me to classify the substitutions in different bins. There could have been two, yes or no. But as I was looking through the substitutions, some stood out and I felt comfortable classifying some of them as a favorable mutation, not just acceptable mutual mutations. 13:23:08 13:23:12 13:23:20 13:23:27 13:23:34 13:23:37 13:23:42 13:23:45 13:23:48 13:23:55 13:24:22

Q So did you arrive at the 1, 2, 3 scoring system as you were doing your analysis in this case? 13:24:02 13:24:06 13:24:11

A Yes. I developed the system 1, 2 and 3 as I was looking at the substitutions. 13:24:11 13:24:16

Q Okay. So is it fair to say that a score 13:24:39

of 3 would be expected to be a favorable substitution; is that right? 13:24:42 13:24:47

A That's how the system is set up, yes. 13:24:50

Q Okay. 13:24:53

A By classification. System is set up, so that 3 indicates a favorable substitution. 13:24:53 13:24:58

Q And then is 2, is that a neutral substitution? 13:25:01 13:25:05

A Neutral plus/minus delta. In other words, I -- if there's a change in stability in either direction, I thought it would be minor change, not really worthy of separate classification, but something equivalent to wild-type. 13:25:06 13:25:14 13:25:18 13:25:22 13:25:30

Q Okay. And 1, is that a disfavored substitution, or unfavorable substitution? 13:25:35 13:25:43

A 1 corresponds to substitutions for which I had real reservation. Something about the substitution, say, worried me, then I would put that substitution in the category of 1. 13:25:47 13:25:51 13:25:56 13:25:59

Q And when you say it worried you, does that -- does that mean that you would expect it to potentially have a detrimental impact on protein 13:26:01 13:26:05 13:26:08

structure or function? 13:26:19

A Yes. That's what I mean. 13:26:19

Q Okay. And for a neutral change, would you expect that change to not have a meaningful effect on protein structure and function? 13:26:21 13:26:24 13:26:28

A That's correct. There may be a change, but I didn't think the change would be meaningful enough. 13:26:32 13:26:35 13:26:38

Q So for those residues where you scored the mutation as a 2, you would expect those changes not to meaningfully impact the protein structure and function? 13:26:39 13:26:43 13:26:48 13:26:54

A That would be the correct way to interpret a score of 2. 13:26:55 13:26:57

Q And for a score of 3, you would expect that the substitution potentially would improve function, or would have no effect on function? 13:26:59 13:27:02 13:27:08

ATTORNEY KUSHAN: Objection. Foundation. 13:27:11

A Score of 3 is a substitution that I can get excited about. Yes. So those are substitutions I had high hopes for. 13:27:15 13:27:20 13:27:26

Q And when you say you have high hopes for 13:27:31

them, what would you expect to see with those substitutions? 13:27:34 13:27:38

A I would expect those substitutions to improve the stability and perhaps function of the enzyme. 13:27:39 13:27:41 13:27:45

Q How could you tell if a mutation improves the stability of an enzyme? 13:27:46 13:27:51

A There are a number of things one needs to consider when entertaining a substitution. If the individual factors are favorable, then I would expect the net effect of the substitution would be overall favorable. And if I see several such effects, combined with an absence of any noticeable negative effect, I would think that that substitution would improve the protein, not necessarily the enzyme, but a protein in terms of stability. 13:27:55 13:27:59 13:28:06 13:28:11 13:28:15 13:28:21 13:28:27 13:28:32 13:28:37 13:28:40

Q Okay. So you mentioned that in your work outside of this case you would analyze potential substitution and determine whether it would be favorable, unfavorable or neutral. Was that the case in the 2011 time frame? 13:28:41 13:29:09 13:29:12 13:29:14 13:29:18

A Again, in 2011, I don't think I was doing that particularly. But if you're referring to time before then, I was looking at various substitutions. Most of the time, I wouldn't care for substitutions that are 2 or 1. Neutral substitutions are not that interesting from my perspective. 13:29:24 13:29:29 13:29:32 13:29:36 13:29:40 13:29:44

My ultimate goal as an academic person is to publish. You need to have an exciting discovery to publish. A neutral substitution does not constitute an exciting discovery that deserves a publication. As such, I wouldn't care much about substitutions that are 2, much less 1. It would have to be 3, and plus. 13:29:46 13:29:50 13:29:53 13:29:59 13:30:02 13:30:06 13:30:09

So those are the substitutions that I would care mostly about. There were many substitutions that would be in the groups of 2's and 1's. I wouldn't care much about them. 13:30:11 13:30:14 13:30:19 13:30:21

Q So if you were looking to make a substitution into a -- into a protein, you wouldn't be looking for a substitution that would have no effect. You would be looking for a 13:30:40 13:30:43 13:30:47 13:30:50

substitution that would have an improved effect, or at least some difference in effect; is that right? 13:30:55 13:30:56 13:30:58

ATTORNEY KUSHAN: Objection. Foundation. Also objection as to form. 13:30:58 13:31:00

A We're talking about a different situation, different scenario. My goal as an independent researcher would not necessarily be aligned with the goals of this particular declaration. My objective is quite different. 13:31:03 13:31:08 13:31:12 13:31:16 13:31:20

As you said, I would be interested in improving the enzyme, not necessarily finding a substitution that would be neutral and doesn't really change the protein per se. That is not really the mandate for this work. 13:31:23 13:31:25 13:31:30 13:31:34 13:31:40

As I understand it, I was instructed to go find substitutions that would be tolerated at non-essential positions in this enzyme, and that's what I did. 13:31:47 13:31:49 13:31:54 13:31:57

Q Okay. And when you say tolerated, you mean substitutions that were neutral or beneficial; is that right? 13:31:58 13:32:17 13:32:05

ATTORNEY KUSHAN: Objection. Foundation. 13:32:07

A I define the word tolerate in the 13:32:08

declaration. 13:32:14

Q Uh-huh.

A It's in the terminology used in the 13:32:19

declaration, Page 5 I believe. 13:32:22

Q Oh. It's actually in Paragraph 20. 13:32:33

A Oh, is it. Okay. The following, yes. 13:32:35

I use the word tolerate -- it should be in 13:32:40

quotation -- here to mean that the presence of a 13:32:44

single amino acid at a particular position of the 13:32:47

protein's amino acid sequence does not materially 13:32:50

alter the local structure around that position in 13:32:55

the protein, and thus does not meaningfully alter 13:32:55

the biological activity of the protein.

In terms of ranking, that would correspond 13:32:59

to 2 and 3 of the classification. 13:33:02

Q Okay. So going back to -- let's see. 13:33:08

So do you -- would you expect that 13:33:29

mutations that you've scored as 2 or 3, would you 13:33:42

expect those to impact protein function? 13:33:47

A Hard to say. I have not considered that 13:33:59

aspect much. I was mostly concerned with the compatibility of the substitution in terms of the structure, whether the substitution would be tolerated by the structure of the PH20. 13:34:03 13:34:09 13:34:18 13:34:20

If those substitutions are within the active site, which I can roughly guess based on the shape of the molecule. However, precisely what residues are involved in catalysis is not well known, so that would at best, an educated guess. If I see a position that is close to where I would expect the ligand or the substrate would be, then I would try to include that in the consideration. But I was mostly interested in the compatibility of the substitution with the structure of the enzyme more than anything else. 13:34:24 13:34:28 13:34:32 13:34:37 13:34:42 13:34:45 13:34:49 13:34:53 13:34:59 13:35:04 13:35:08

Q Okay. So did you -- did you evaluate whether you would expect the change to impact the function of the protein? 13:35:11 13:35:16 13:35:19

A I did not address that question directly. However, when it was apparent to me that the substrate would be near that particular position, I tried to model that as much as possible. 13:35:23 13:35:27 13:35:36 13:35:39

However, the substrate bound structure of the enzyme is not known. Therefore, those considerations may not be very accurate. 13:35:45 13:35:49 13:35:55

Fortunately, there weren't that many positions, because the active site residues are fewer than the rest of the protein. 13:36:00 13:36:04 13:36:14

In other words, there are more amino acids away from the active site than at the active site. So a fraction of the residues might be in the active site, and I would, if I can, fold that into the consideration, but not actively. If I get the -- the role of the enzymatic function incorrect, I do not worry about that aspect much. 13:36:16 13:36:19 13:36:25 13:36:28 13:36:33 13:36:38 13:36:43

Q So you say in Paragraph 20, in the context of your definition of tolerate, that the -- it doesn't materially alter the local structure, and thus does not meaningfully alter the biological activity of the protein, right? 13:37:04 13:37:38 13:37:25 13:37:28 13:37:35

ATTORNEY KUSHAN: Objection. Foundation. 13:37:39

A Does not meaningfully alter the biological activity in the way of structural preservations. 13:37:42 13:37:48

In other words, a substitution that doesn't 13:37:55

change the structure of a protein, it would not affect the activity of the enzyme unless, of course that residue happens to be involved in catalysis. 13:37:59 13:38:05 13:38:09 13:38:16

Some of them are known. We know from other publications that there are catalytic residues. If you mutate them, you would lose the activity 100 percent, and those positions are also unlikely to be structurally important. 13:38:18 13:38:22 13:38:26 13:38:31 13:38:34

Based on the structure, one can surmise that some of those substitutions are important for catalyst, but not necessarily for the folding of the protein. So here, what is meant is it doesn't affect the biological activity because the structure will be maintained upon substitution. 13:38:38 13:38:44 13:38:51 13:38:56 13:39:32 13:39:07

Q So you expect that after the substitution is made, that the -- the protein structure -- the protein will maintain the same structure, and so therefore it will also maintain the same function; is that right? 13:39:10 13:39:15 13:39:20 13:39:23 13:39:26

ATTORNEY KUSHAN: Objection. Foundation. 13:39:26

Also objection as to form. 13:39:28

A Given that the structure of the protein is critical for its activity, if you introduce a substitution that leaves a structure untouched, it would be reasonable to think that the biological activity would remain the same. 13:39:34 13:39:38 13:39:44 13:39:50 13:39:54

Q And so is it your opinion that a person of ordinary skill in the art would expect that if you leave a structure untouched that the biological activity would remain the same? 13:40:04 13:40:08 13:40:12 13:40:16

ATTORNEY KUSHAN: Objection. Foundation. Also objection as to form. 13:40:19 13:40:23

A To a degree that would be true, as long as, as I said earlier, the mutation does not affect known catalytically important residues. In this case, you can keep the structure the same and you would lose activity. So all bets are off in those cases. 13:40:26 13:40:37 13:40:45 13:40:51 13:40:55 13:40:58

Q Okay. When you look at the model, the structural model, are you able to identify residues that are important for protein folding? 13:40:59 13:41:04 13:41:09

A From the structure alone, no. That would be hard. 13:41:17 13:41:22

Q In connection with the -- are you able to use the structure in connection with the alignment to identify residues that important for protein folding? 13:41:28 13:41:31 13:41:34 13:41:37

A I think the alignment can help you greatly. It will help you identify a significant number of residues that are important for folding. 13:41:40 13:41:45 13:41:50

Q I'm just trying to understand what is it about a residue that makes it important for protein folding? 13:41:55 13:41:58 13:42:01

ATTORNEY KUSHAN: Objection to form. 13:42:03

A That's the essence of protein folding. If I knew the answer, it would be great. I don't think anybody does, unfortunately. 13:42:09 13:42:14 13:42:18

Q Okay. So when you designed the scoring system that you used in this declaration, you were approaching the issue from the perspective of a POSA; is that right? 13:42:20 13:42:56 13:43:01 13:43:05

A Yes. 13:43:07

Q Okay. 13:43:12

Okay. So can we turn to Appendix C? 13:43:35

A I am at Appendix C. 13:43:59

Q Okay. So Appendix C has your analysis for position 320; is that right? 13:44:02 13:44:12

A That is correct. 13:44:18

Q And is it correct that the alternate -- strike that. 13:44:21 13:44:29

So for position 320, the alternative residues that you considered are those alternative residues that appear at position 320 in the alignment; is that right? 13:44:29 13:44:36 13:44:40 13:44:43

ATTORNEY KUSHAN: Objection. 13:44:46

A Yes. 13:44:47

ATTORNEY KUSHAN: Objection to foundation. 13:44:48

Go ahead. 13:44:51

Q So the only residues that you consider as alternative residues are those that appear in the alignment at that position, right? 13:44:51 13:44:54 13:44:58

ATTORNEY KUSHAN: Objection. Foundation. 13:45:00

A I only consider the residues that appeared in the multiple sequence alignment for consideration at this position, yes. 13:45:04 13:45:07 13:45:10

Q Why did you only consider the residues that appear in the multiple sequence alignment at 13:45:12 13:45:15

that position? 13:45:18

A That goes back to the assumption that was 13:45:25

stated earlier in the declaration, that the 13:45:28

residues that appear among homologous sequences 13:45:31

have been tested by evolution and proven to be 13:45:40

compatible with the structure. Therefore, looking 13:45:44

at those possibilities would make sense. 13:45:48

And so that is in the Paragraph 31, I 13:46:07

think, which is the one that you referred to 13:46:09

earlier. Maybe... 13:46:11

The existence of evolutionary variation at 13:46:29

these positions indicates that the homologous 13:46:31

proteins have tolerated different amino acids at 13:46:38

those positions, including 320. Those proteins 13:46:42

presumably exhibit hyaluronidase activity when 13:46:44

they are mutated. 13:46:54

So in other words, the homologous 13:46:56

sequences that are part of the multiple sequence 13:46:57

alignment, they are -- they have explored other 13:46:59

possible substitutions at various non-essential 13:47:04

positions, including 320, and somehow they have 13:47:08

survived. They continue to exhibit activities, if 13:47:15

necessary, or at a minimum, managed to stay folded, which, as we discussed earlier, is supported by the fact that conserved residues are conserved, and they're conserved for a reason. Without a reason, there is no reason for them to be conserved. 13:47:20 13:47:26 13:47:30 13:47:37 13:47:42 13:47:46

So that is the evidence that they would fold to the same structure. So that's the reason for testing these residues, as opposed to other residues that are not listed in the -- 13:47:51 13:47:51 13:47:55

Q Okay. 13:48:00

A -- in the multiple sequence alignment. 13:48:01

Q A POSA would understand that they could also -- that they could, for example, at position 320, that the aspartic acid could be mutated to one of the other -- another amino acid that's not listed here; is that right? 13:48:07 13:48:10 13:48:15

(Reporter clarification.) 13:48:33

A Could you repeat the question? What a POSA might assume again? 13:48:33 13:48:36

Q Oh. A POSA would understand that if they were looking to substitution position 320 -- 13:48:36 13:48:39

A Yes. 13:48:42

Q -- they could change position 320 13:48:42

theoretically to any of the other 19 amino acids, 13:48:46

right? 13:48:49

A Not if they have any hope of finding an 13:48:53

acceptable mutant. If they're looking for an 13:48:59

acceptable substitution, a POSA who's familiar 13:49:03

with the literature wouldn't just try any amino 13:49:07

acid. There has to be a foundation for what they 13:49:10

try, and this would give them the foundation. The 13:49:14

other substitutions wouldn't make any sense, so 13:49:18

they wouldn't try. Yeah. That's not what a POSA 13:49:22

would do. 13:49:27

Q So a POSA wouldn't just make random 13:49:34

changes and kind of hope for the best? 13:49:37

ATTORNEY KUSHAN: Objection. Foundation. 13:49:39

Also objection as to form. 13:49:42

A Not a POSA. A person off the street, yes. 13:49:43

But a person who is supposed to be 13:49:48

educated in the field and understand the factors 13:49:51

that contribute to protein folding, stability, no. 13:49:55

They wouldn't randomly try a substitution. No. 13:50:01

They wouldn't. 13:50:07

Q So in exhibit -- I'm sorry -- Appendix C, 13:50:38
the far right of Appendix C in your chart, you 13:50:43
have factors, hydrophobicity, secondary structure 13:50:49
and interactions, and there's the Number 1, for 13:50:51
some of them, but not all of them. 13:50:55

Can you just explain how you -- what this 13:50:57
means -- what that means? 13:50:59

A First of all, 1 is meant to be a check. 13:51:03
So you can understand 1 as a substitute for a 13:51:10
check. 13:51:16

(Reporter clarification.) 13:51:16

A Checkmark. I was doing this in Excel 13:51:16
spreadsheet, and it's easier to enter 1, as 13:51:24
opposed to a checkmark. 13:51:27

The three columns, which have the labels 13:51:28
of: Hydrophobicity, secondary structure, and then 13:51:33
I forget what the third one says. 13:51:36

Q Interactions. 13:51:38

A Interactions -- are the three main 13:51:39
categories of interactions, attributes that I 13:51:41
should consider during a substitution, and they 13:51:46

correspond to the intrinsic properties of that amino acid, which is supposed to be the first column. 13:51:51 13:51:56 13:51:59

I labeled it as hydrophobicity, but it should really be hydrophobicity/hydrophilicity, lots of other things that one might think of, that's -- that belongs to that amino acid. 13:52:00 13:52:07 13:52:07 13:52:10

The second column is the secondary structure, meaning the compatibility of this amino acid with the underlying secondary structure of that protein. 13:52:13 13:52:17 13:52:21

And the third column is the interaction, meaning the interaction of the wild-type and mutant residue with its neighbors. 13:52:21 13:52:25 13:52:29

So these are the three aspects that I consider as a personal note, as I was scoring different substitutions. It is important to note that I considered all three for every substitution, but sometimes I'd make a checkmark as an -- as an indication to myself, oh, pay attention to that, what just sort of jumped out, that kind of a thing. 13:52:32 13:52:34 13:52:38 13:52:42 13:52:47 13:52:50 13:52:54 13:52:58

Q So does the check mean, for example, for secondary -- you have a check for R, for secondary structure, does that mean that the R gene at that position is compatible with the secondary structure; is that what that means? 13:52:59 13:53:03 13:53:07 13:53:13 13:53:17

A One shouldn't look at it as a compatibility, but rather secondary structure is an aspect that I considered and it jumped out as one of the factors that would affect the final decision. 13:53:18 13:53:23 13:53:27 13:53:32 13:53:37

Q Okay. I'm just trying to understand if the check is good -- a good thing or a bad thing, or just a note to yourself? 13:53:38 13:53:40 13:53:42

A Just a note. 13:53:44

Q Just a note. 13:53:45

A Yes.

Q Okay. Got it. Okay. 13:53:45

And so when you said the hydrophobicity should really be the properties of the amino acid, is that looking to see if the two amino acids are comparable in terms of, like, size and chemistry, or is that something else? 13:53:47 13:53:50 13:53:53 13:53:56 13:54:00

ATTORNEY KUSHAN: Objection. Foundation. 13:54:04

A All of them. 13:54:05

Q Okay. 13:54:06

A All of them. It could be a hydrophilic 13:54:06

amino acid being substituted with a hydrophilic 13:54:15

amino acid. Well, I took that into consideration. 13:54:18

Checkmark. 13:54:21

Q Okay. 13:54:22

A That's what it means. 13:54:23

Q Okay. Can you explain the FSASA? 13:54:24

A Oh. The FSASA. 13:54:48

(Reporter clarification.) 13:54:50

A I call it accessible surface area -- 13:54:50

Q Uh-huh. Okay.

A -- as a way of quantifying how much a 13:54:54

given amino acid is exposed to the solvent. So 13:54:57

that pulls information regarding the -- the 13:55:05

neighbor, the environment where this residue 13:55:09

appears. If that residue is solvent exposed, then 13:55:11

this accessible surface area, fraction of 13:55:16

accessible surface area, would approach 1, because 13:55:20

the measured value at that position in this 13:55:23

protein is nearly as great as the maximum solvent accessible surface area for that amino acid. 13:55:27 13:55:35

Q So in order to calculate the fractional solvent accessible surface area, are you determining the solvent accessible surface area in the PyMol for that residue, and then dividing by the maximum solvent accessible surface area for that residue? 13:55:38 13:55:46 13:55:49 13:55:52 13:55:57 13:56:07

A That is correct, yes. 13:56:09

Q Okay. So the wild-type had -- the wild-type, you have a fractional solvent accessible surface area of .17. 13:56:29 13:56:33 13:56:39

Would you consider that to be varied? 13:56:42

ATTORNEY KUSHAN: Objection. Foundation. 13:56:45

A It is somewhat varied for that amino acid, aspartic acid. 13:56:49 13:56:54

The important quantity to bear in mind is not just the number that's listed here, but a comparison of that number to the typical value for that amino acid. For instance, the aspartic acid has a much higher median fractional accessible surface area, which I think is listed further down 13:56:58 13:57:01 13:57:04 13:57:10 13:57:19 13:57:22

in appendix -- which is page -- this is Appendix E. This is an output from PyMol. 13:57:25 13:57:41

As I consider wild-type on each substitution, I would look at the numbers that are reported back to me, and I get this information because it's written into the script that I developed. 13:57:47 13:57:49 13:57:56 13:58:00 13:58:05

And this one says, on Page 192: For aspartic acid at 355, position 355, the relative area, or relative solvent accessible surface area, 0.17, whereas the mean is 0.388. 13:58:05 13:58:13 13:58:20 13:58:23

So on average, this amino acid is much more solvent exposed, but at this position, it has much smaller solvent accessible surface area. So it gives you an idea of what kind of position we're looking at, because not every position is equal. 13:58:30 13:58:33 13:58:39 13:58:41 13:58:45 13:58:49

Q Can you turn to Page 178, Appendix D, Part 5, solvent accessible surface area? 13:58:57 13:59:04

A Page 178? 13:59:10

Q Yeah. 13:59:15

A Okay. Sure. 13:59:15

Q Yeah. The chart is on 179. 13:59:16

A Sure. Sure.

Q Can you just explain the chart that's on Page 179? 13:59:22 13:59:26

A Uh-huh. 13:59:27

So this is the normalizing factor, normalization factor, that you mentioned earlier. 13:59:28 13:59:31

You measure the surface area of that amino acid at position 320 in the structure, and divide that number by something else. And that something else is the maximum area possible for that amino acid, which has been published. 13:59:34 13:59:38 13:59:43 13:59:48 13:59:53

Q Oh, okay. So am I understanding right that the max solvent accessible surface area and the median solvent accessible surface area have -- are published values from the Lins paper, and then you've calculated the median solvent accessible surface area fraction based on those numbers, and that's in the third column? 13:59:55 13:59:58 14:00:05 14:00:09 14:00:16 14:00:18

A That is correct. Yes. 14:00:21

Q Okay. Got it. 14:00:23

Okay. So going back to Appendix C, 14:00:40

Page 125, so for N, you just have neutral in the comments. What does -- and also for E, you have neutral in the comment, and that's it. 14:00:53 14:01:11 14:01:17

Can you explain what that means? 14:01:20

A Yes. I consider that substitution to be so unremarkable that there was nothing to say about it. Oftentimes, D to N substitution is done because they are similar in size, both hydrophilic, and often no substitutions are made. 14:01:22 14:01:29 14:01:33 14:01:40 14:01:46

When I make that substitution in the structure, I don't see a great deal of change one way or another. Not -- nothing much. As a result, I just give it a score of 2 with very little comment. Nonetheless, I do consider all three factors listed in the last three columns, which includes intrinsic properties of the individual amino acids, compatibility with the secondary structure, and how that residue wild-type and mutant might interact with its neighbors. 14:01:50 14:01:53 14:01:57 14:02:01 14:02:08 14:02:14 14:02:18 14:02:22 14:02:25 14:02:31 14:02:34

So these are done, but in this case its so automatic and it's so quick, really nothing stood 14:02:35 14:02:39

out, so I didn't really felt the urge to mark it in anyway, other than noting that it's a very neutral substitution with a score of 2. 14:02:43 14:02:46 14:02:51

Q Okay. So you analyzed eight different potential alternative residues, right, for position 320? 14:02:55 14:03:06 14:03:11

A There are eight alternative potential substitutions at this position, yes. 14:03:12 14:03:18

Q And you conclude that all but one -- so seven of them -- would be tolerated, right? 14:03:19 14:03:24

A Yes. Seven substitutions are listed here with a score of 2 or 3, would be tolerated at this position of 320. 14:03:27 14:03:31 14:03:37

Q And you would expect that one glycine would be not tolerated, right? 14:03:38 14:03:43

A I had a strong feeling that it would not be tolerated. 14:03:45 14:03:49

Q So what do you expect would happen if you put glycine in position 320? 14:03:53 14:03:59

Do you think the protein wouldn't fold? 14:04:03

A It may fold. Its stability may be significantly lower than that of wild-type, thus 14:04:06 14:04:12

resulting in a substantial loss of activity. 14:04:19

Q Okay. So you would expect that if you put glycine in position 320, you would have a substantial loss in activity? 14:04:22 14:04:26 14:04:31

A That is my expectation, yes. 14:04:32

Q But for the other seven amino acids that you considered, you would expect the activity to be comparable or better than wild-type; is that right? 14:04:32 14:04:35 14:04:36 14:04:40

A That's correct. 14:04:40

ATTORNEY MARTIN: All right. We've been going for another hour. 14:04:45 14:04:48

Want to take another short break? 14:04:51

THE WITNESS: That would be great. 14:04:54

Thank you. 14:04:56

THE VIDEOGRAPHER: We are going off the record. The time is 2:04 p.m. 14:04:56 14:04:58

(Recess from 2:05 p.m. until 2:15 p.m.) 14:05:03

THE VIDEOGRAPHER: We are back on the record. The time is 2:15 p.m. 14:15:26 14:15:29

BY ATTORNEY MARTIN: 14:15:32

Q Welcome back, Dr. Park. 14:15:33

Did you discuss the substance of your deposition during the break with anyone? 14:15:36 14:15:39

A No. 14:15:41

Q So the principles that we've been talking about in terms of -- in terms of protein structure and function in your analysis, that all applies to the 2011 time frame, right? 14:15:42 14:15:46 14:15:52 14:16:03

ATTORNEY KUSHAN: Objection. Form. 14:16:06

A Obviously the principles are timeless, but the analysis was based on the sample that was gathered with the time frame of 2011 in mind. 14:16:08 14:16:12 14:16:16

Q Okay. And the analysis that you performed was done from the perspective of the skilled person in 2011, right? 14:16:20 14:16:23 14:16:31

(Reporter clarification.) 14:16:34

A Yes. It was. 14:16:34

Q Okay. All right. If you have -- if you had homologous proteins that were 90 percent identical in sequence, would you expect that those proteins share the same structure and function? 14:16:35 14:16:45 14:16:52 14:16:55

ATTORNEY KUSHAN: Objection. Foundation. 14:16:58

A Two proteins that are homologous and have 14:17:05

a sequence identity/similarity, 90 percent, do I expect them to adopt the same structure? If these are naturally occurring sequences, yes. 14:17:11 14:17:18 14:17:23

Q Would you expect them to have the same function? 14:17:32 14:17:35

A No. They may have subtly or dramatically different functions, depending on what's going on at the active site. 14:17:38 14:17:48 14:17:51

Q Okay. 14:17:53

A Although that may be less likely once you have 90 percent. Was that the percentage you mentioned? 14:17:53 14:17:57 14:17:59

Q Yes. 90 percent. 14:18:00

A 90 percent? Yes. Chances are they would have very similar functions. 14:18:02 14:18:05

Q Okay. And I believe you mentioned earlier this morning that you had done work with antibodies? 14:18:27 14:18:29 14:18:32

A Yes. 14:18:33

Q Do you know whether changes in the framework region of an antibody can impact binding? 14:18:33 14:18:35 14:18:40

ATTORNEY KUSHAN: Objection. Foundation. 14:18:41

A Yes. It can. 14:18:44

Q Is it possible to make changes in the framework region of an antibody that will eliminate the ability of the antibody to bind the antigen? 14:18:49 14:18:51 14:19:12 14:19:02

A It can, too. 14:19:03

Q Let's go to Paragraph 88 in your declaration. 14:19:08 14:19:20

All right. So Paragraph 88 is under the header that reads: Analysis of published results for mutations of hyaluronidase proteins; is that right? 14:19:37 14:19:40 14:19:45 14:19:49

A That is correct. 14:19:49

Q And in Paragraph 88, you say: In my evaluation of single amino acid substitutions, I considered biochemical and structural data reported in the scientific literature before December of 2011, particularly those reported in Chao, Zhang, Stern and Arming; is that right? 14:19:51 14:19:53 14:19:57 14:20:00 14:20:00 14:20:08

A That's correct. 14:20:15

Q Why did you consider biochemical and 14:20:16

structural data as part of your analysis? 14:20:21

A Structural data as reported in, say, Chao paper is very useful in terms of developing our own model for evaluation of individual substitutions. 14:20:42 14:20:59 14:20:52 14:20:55

Biochemical data reported in other papers, they're useful because they report the consequences of introducing the mutations at various places in the protein. Some conserved, some less conserved. So they are useful. 14:20:55 14:21:01 14:21:05 14:21:10 14:21:15

Q So why are -- why is it that biochemical data that reports the consequences of introducing substitutions into a protein, why is that useful? 14:21:20 14:21:24 14:21:31

ATTORNEY KUSHAN: Objection. Foundation. 14:21:34

Also objection as to form. 14:21:36

A They are useful because they help you identify what amino acids are important, and in some cases, it can even be used to deduce whether a protein would fold. 14:21:39 14:21:42 14:21:46 14:21:53

For instance, if you introduce a mutation and you get very little protein expressed in the same expression system, that may be because the 14:21:57 14:22:00 14:22:05

protein has unfolded, and as a result is no longer soluble. So this would be the standard way of interpreting mutational effects. 14:22:08 14:22:13 14:22:18

Q And are mutation data for both conservative and non-conservative changes useful? 14:22:25 14:22:31

ATTORNEY KUSHAN: Objection. Foundation. 14:22:36

A They are useful because they say different things. 14:22:45 14:22:48

Q Can you explain that? 14:22:48

A If you introduce mutation at a conserved position and you lose activity, function, expression, then it confirms that those conserved residues are important. 14:22:52 14:22:58 14:23:00 14:23:04

If you introduce a mutation at a non-conserved position and nothing happens, the protein maintains the same activity, then you know those mutations are interchangeable to a degree and they would tolerate substitutions. 14:23:06 14:23:09 14:23:12 14:23:17 14:23:21

So they hold different information, all of which can be useful, and some, in some ways support our findings. 14:23:24 14:23:27 14:23:32

Q Okay. How did you identify the Chao, 14:23:45

Zhang, Stern and Arming references? 14:23:50

A I did a public search with some keywords. 14:23:57

I don't remember exactly what. Hyaluronidase 14:24:04

would be a good starting point, or PH20. 14:24:11

Sometimes I would look up bibliography references 14:24:13

at the end of a paper and move backwards in time 14:24:21

and find earlier examples. 14:25:11

Q Okay. Can you turn to Paragraph 91? 14:25:11

A Yes. I'm at Paragraph 91. 14:25:20

Q So in Paragraph 91, you state: Chao 14:25:22

identified the residues in the active site of 14:25:24

HYAL1 that are involved in catalysis as Asp129, 14:25:29

Glu131, and Tyr202, which correspond to Asp146, 14:25:33

Glu148, and Tyrosine 219 in PH20. It also 14:25:41

identified a number of other residues in a cleft 14:25:47

where the ligand binds.

And I'm not going to read them out. 14:25:49

So the question is: Do you understand -- 14:25:55

sorry. Strike that. 14:26:03

Is it correct that Aspartic Acid 146, 14:26:04

Glutamate 148, and Tyrosine 219 in PH20 are 14:26:12

involved in catalysis based on the Chao paper? 14:26:18

ATTORNEY KUSHAN: Objection. Foundation. 14:26:21

A Yes. I would think so. 14:26:25

Q Okay. And do you know if the residues 14:26:29
that are listed -- listed in the cleft, those 14:26:36
are -- are those the HYAL1 residue numbers, or 14:26:40
you're not sure? 14:26:47

A I'm pretty sure there are -- those are 14:26:48
HYAL1 residues. 14:26:51

Q Okay. So you'd have to go and convert to 14:26:52
figure out what the residues are in PH20? 14:26:56

A Yes. There is a table at the end that -- 14:27:01

Q Oh, okay. 14:27:05

A -- in those amino acids to PH20 residues. 14:27:07

Q Okay.

A It's on paragraph -- Page 170 -- it goes 14:27:07
on for a while. 168. It's Appendix D, Part 4. 14:27:14

Q Appendix D, Part 4.

A Page 7 -- starting at Page 167. 14:27:20

Q Oh. I got it.

A It allows the mapping of PH20 to HYAL1. 14:27:23

Q Okay. Great. 14:27:28

Okay. And so would you expect that in 14:27:55

PH20 those residues -- the PH20 version of those residues are also in the cleft where the ligand binds? 14:27:59 14:28:00 14:28:04

A Yes. 14:28:05

Q Okay. And so if you turn to -- can you please turn to Paragraph 95? 14:28:10 14:28:37

A Yes. I'm at Paragraph 95. 14:28:54

Q So in Paragraph 95, you reference the testing in Zhang of single mutant HYAL1 proteins; is that right? 14:28:56 14:29:00 14:29:06

A These are mutational results from single point mutations, yes. 14:29:11 14:29:14

Q And in the -- the Zhang paper, am I understanding correctly that the mutations at the D129N, E131Q, Y202F, Y247F, and R265L mutations, have little or no activity? 14:29:18 14:29:23 14:29:30 14:29:47

A The figure shows that, yes. 14:29:54

Q Okay. 14:29:58

Okay. And so then the -- the results from Zhang, from the mutagenesis in Zhang, those are shown in a table in Paragraph 99 in your declaration, right? 14:30:16 14:30:20 14:30:26 14:30:30

A Paragraph 99? All right. Okay. 14:30:31

Q So just the -- in Paragraph 99, you've reproduced the chart from the Zhang paper that has the results of the mutagenesis testing? 14:30:43 14:30:49 14:30:52

A That is correct. 14:30:58

Q Do you know what the distinction is between catalytic mutants, substrate binding mutants, and punitive structural mutants? 14:31:03 14:31:05 14:31:10

ATTORNEY KUSHAN: Objection. Foundation. 14:31:17

A My reading of that table with those three classifications would be catalytic mutants are mutants where catalytic residues or residues that are directly involved in catalysis have been mutated. 14:31:27 14:31:31 14:31:37 14:31:39 14:31:42

Substrate binding mutants are the mutations where an amino acid involved in substrate recognition and binding has been mutated. 14:31:42 14:31:45 14:31:48 14:31:52

And punitive structural mutants would be mutations that affect the enzyme in some other ways. 14:31:52 14:32:01 14:32:03

Q Okay. And then can you turn to 14:32:03

Paragraph 101? 14:32:29

So and in Paragraph 101, you list the 14:32:38

results from Arming, where Arming identified five 14:32:43

single mutations that greatly reduced PH20 14:32:48

activity, right? 14:32:52

A Yes. 14:32:53

Q And then, as well as four conserved 14:32:53

cysteine residues that formed disulfide bonds, and 14:32:58

that PH20 truncated at position 341 exhibited no 14:33:02

activity, right? 14:33:07

A That's correct. 14:33:07

Q So how is the Arming data, how did you use 14:33:08

that in your analysis? 14:33:12

A I use these biochemical data to confirm 14:33:23

the finding from the multiple sequence alignment, 14:33:27

which suggested conservation of certain positions, 14:33:32

and they track the biochemical data, in that if 14:33:37

you mutate those residues, you lose activity. 14:33:40

Q So the biochemical data, consistent with 14:33:48

the multiple sequence alignment, and confirmed 14:33:53

that certain residues are essential for activity; 14:33:54

is that right? 14:33:58

A Yes. 14:33:58

ATTORNEY KUSHAN: Objection. Foundation. 14:33:58

And also objection as to form. 14:34:00

A Yes. That's how the biochemical data 14:34:02

would be useful to our analysis. 14:34:07

Q So would you expect based on the 14:34:16

biochemical data, for example in Arming, that if 14:34:18

you change position 113, that you're likely to 14:34:25

reduce PH20 activity? 14:34:33

ATTORNEY KUSHAN: Objection. Foundation. 14:34:41

Also objection as to form. 14:34:43

A These note that -- these note that 14:34:44

glutamine 113 is not wild-type. This is the 14:34:49

mutant -- 14:34:52

Q Oh, oh. Sorry. 14:34:53

A Yes. 14:34:55

Q Okay. So if you change position 113 to 14:34:55

glutamine -- 14:35:01

A That is correct. 14:35:01

Q -- then you will -- okay. 14:35:02

A That is correct. 14:35:03

Q Okay. Thank you. 14:35:04

A Yes. 14:35:05

Q Would you expect based on the Arming 14:35:06

results that if you change the four conserved 14:35:13

cysteine residues that you would reduce PH20 14:35:20

activity? 14:35:24

ATTORNEY KUSHAN: Objection. Foundation. 14:35:24

Also objection as to form. 14:35:26

(Reporter clarification.) 14:35:42

A Yes. I would totally expect mutating any 14:35:42

of the conserved cysteine residues involved in 14:35:46

that sulfuric bond would result in total loss of 14:35:51

protein activity. 14:35:56

Q All right. So let's turn to 14:36:09

Paragraph 102. 14:36:11

So in Paragraphs 102 and 103, you describe 14:36:28

your unbiased scoring system, right? 14:36:33

A That's correct. 14:36:44

Q And you said that it's necessary for you 14:36:45

to evaluate a lot of different types of 14:36:49

substitutions, and through that process you 14:36:52

developed a consistent methodology; is that right? 14:37:00

A That's correct. 14:37:05

Q So why was it necessary to evaluate a lot of different types of substitutions? 14:37:06 14:37:08

A It gives you a breadth in your analysis. It tells you how to deal with different types of substitutions. Some conserved, some not conserved, small to large, large to small, all kinds of differences. You want to be familiar with different types of substitutions that can happen so that you can develop a more consistent methodology that can be applied to all substitutions in your data set. 14:37:17 14:37:23 14:37:27 14:37:31 14:37:35 14:37:37 14:37:40 14:37:46 14:37:54

Q So when you say -- so did, as part of your analysis, did you evaluate a lot of different types of substitutions? 14:37:58 14:38:06 14:38:10

A I did. I mapped the types of substitutions by tabulating different combinations of from and to amino acids, and it was pretty well populated in that distribution. 14:38:14 14:38:18 14:38:25 14:38:33

Q How many different types of substitutions did you consider? 14:38:36 14:38:39

A I haven't counted, so out of the 20 by 20 minus 20, 380 types of substitutions that can 14:38:43 14:38:52

exist, a pretty significant fraction of them were found to exist in this PH20 data set. 14:38:59 14:39:05

(Reporter clarification.) 14:39:14

Q And what are you referring to when you talk about the PH20 data set? 14:39:14 14:39:18

A That's the multiple sequence on alignment -- 14:39:22 14:39:26

Q Okay. 14:39:26

A -- data set that I am speaking of. 14:39:27

Q Okay. Did you consider substitutions at every position in PH20? 14:39:30 14:39:35

A I considered as many substitutions as possible. There were approximately 3,000 substitutions suggested by the multiple sequence alignment. That was too many for me to consider. I considered about one quarter of them. 14:39:38 14:39:42 14:39:49 14:39:52 14:39:57

Q Okay. And have you provided -- when you say you considered them, can you explain what you did for each one of those? 14:40:01 14:40:05 14:40:09

A Absolutely. For each substitution that was suggested by the sequence alignment, starting from those substitutions involving amino acids 14:40:11 14:40:18 14:40:23

that appear with decent frequency, which I started with 10 percent, but ultimately went down to 5 percent. 14:40:27 14:40:35 14:40:39

So in the end, any substitutions to an amino acid that occur with a frequency 5 or greater percentage, I considered the wild-type residue in the context of the modeled PH20 structure, as well as the individual substitutions listed in the multiple sequence alignment. And for each substitution, I scored the substitution on a scale of 1 to 3, using the metric that was demonstrated in Appendix C earlier. 14:40:39 14:40:42 14:40:45 14:40:50 14:40:55 14:40:59 14:41:03 14:41:09 14:41:16

Q And did you record -- did you record the results of that analysis for every substitution that you analyzed? 14:41:20 14:41:24 14:41:27

A Yes. I created a spreadsheet and provided that to counsel. 14:41:29 14:41:35

Q You provided a spreadsheet with your analysis for every single residue that you considered to counsel? 14:41:36 14:41:40 14:41:44

A Yes. I did. 14:41:44

Q So you said that when you went through the 14:41:45

analysis, you would revisit the scores for each position multiple times to make sure that the way you assigned scores was consistent. 14:42:10 14:42:12 14:42:13

Can you explain what you mean by that? 14:42:17

A Yes. I would look at a substitution, give it a score, move on to the next position, consider substitutions at the next position, finish them, move on to the next position, consider substitutions. 14:42:20 14:42:28 14:42:31 14:42:36 14:42:38

I finished this exercise all the way to the end of the structure, and just to be consistent, I went back to the beginning, and repeated that exercise to either confirm what I had found the first time around, or I might have decided, perhaps I was a bit too harsh here, and I would update the score a little bit. This I did twice for the whole set. 14:42:39 14:42:42 14:42:46 14:42:49 14:42:54 14:42:58 14:43:05 14:43:09

Q So you looked at every single substitution twice? 14:43:10 14:43:15

A Every single substitution which amounted to roughly one quarter of the complete set of possible substitutions, which as I said was 14:43:16 14:43:21 14:43:26

about 3,000 substitutions. 14:43:29

Q Okay. And did your analysis in total for all of those positions, did you rely on that analysis in forming the opinions that you set forth in your declaration? 14:43:47 14:43:35 14:43:39 14:43:42

A That analysis was the backbone of the current declaration. 14:43:48 14:43:52

Q And was it important for you to consider every single position and substitution that you considered in order to validate your scoring system? 14:43:54 14:43:56 14:44:00 14:44:04

ATTORNEY KUSHAN: Objection to form. 14:44:05

A Was it necessary? Maybe not. I suppose I could have done less work. I wanted to be thorough about my analysis and be as rigorous as possible. If I had looked at only 10 substitutions, it's hard to know that I am doing it objectively. 14:44:09 14:44:17 14:44:22 14:44:31 14:44:34 14:44:37

It's only through repetition that I developed confidence in my scoring system and can vouch that I am being objective when evaluating the substitution. So for that reason I thought it 14:44:38 14:44:42 14:44:49 14:44:51

was important that I do a bit of a blind study in a way, look at the substitutions without paying attention to, you know, why they may be important. 14:44:52 14:44:54 14:44:59

Look at it as cleanly as possible and score it and see if I can come back at a later time and arrive at the same score. This was the objective. And for that I thought it was important that I start with a very large set, or set a goal for a larger set as what's practical. 14:45:03 14:45:07 14:45:11 14:45:17 14:45:21 14:45:24

Q Okay. And do you know, did you change the score when you went back and revisited some of the substitutions? 14:45:27 14:45:31 14:45:34

A Yes. I wasn't surprised that this happened. It's not unlike looking at students' exams. You start grading them, and once you get to the end of it and you realize that maybe I was a bit too harsh on this question. Let me go back and try to do a slightly different job. 14:45:35 14:45:39 14:45:43 14:45:48 14:45:53 14:45:56

I found that a fraction of the substitutions which I had previously scored could have been interpreted differently, and I updated them. In the process, I think it helped really to 14:46:01 14:46:03 14:46:07 14:46:13

refine my methodology, became more consistent, which then led to more confidence in how I was evaluating individual substitutions. 14:46:19 14:46:25 14:46:32

Q So did you rely on the -- the spreadsheet that you provided to counsel for your analysis for each of the declarations that you submitted? 14:47:08 14:47:10 14:47:13

ATTORNEY KUSHAN: Objection to form. 14:47:18

A The -- the complete set of analysis, which was recorded in a spreadsheet, formed the foundation for these four sets of declarations. 14:47:19 14:47:26 14:47:29

ATTORNEY MARTIN: Okay. So counsel, we would like to request the spreadsheet, since it was the foundation for all four declarations. 14:47:33 14:47:48 14:47:53

ATTORNEY KUSHAN: Okay. Do you want to have a conversation on the break about that? 14:47:57 14:47:58

ATTORNEY MARTIN: Sure. 14:48:00

BY ATTORNEY MARTIN: 14:48:07

Q So you've mentioned that when you went back and did your analysis again, the second analysis, you noticed that you were too harsh for some of the substitutions. 14:48:08 14:48:11 14:48:15 14:48:20

Can you just explain what you meant by 14:48:21

that? 14:48:24

A Being too harsh was an example. I 14:48:24

wouldn't say that the correction was always in one 14:48:30

direction. It might have gone both directions, 1 14:48:32

going to 2, 2 going into 3, vice-versa. So they 14:48:36

did move around. 14:48:40

So based on the follow-up examination, 14:48:42

where I either noticed other interactions which I 14:48:44

had missed earlier, or I have seen similar 14:48:49

interactions elsewhere, which then led me to one 14:48:55

score, which I then felt should be applied 14:49:02

similarly at a different position. 14:49:06

So that influenced my thinking, and hence 14:49:10

the final score. And the score got updated in 14:49:15

some of the cases, but it wasn't always 14:49:21

improvement. Sometimes it could have been the 14:49:27

other way around. 14:49:30

Q So in the 2011 time frame, if a POSA is 14:49:39

trying to determine the impact of a potential 14:49:42

substitution, would they have done the similar 14:49:45

analysis where they look at -- where they look at 14:49:49

the substitution and determine if it's potentially 14:49:54

going to favorable, neutral or unfavorable? 14:49:58

A In the year 2011, to consider a mutation, 14:50:02

it would have been very common and expected for a 14:50:08

POSA to generate a model and consider the 14:50:15

substitution in that structural context. 14:50:27

Q So you said that you looked at about -- I 14:50:41

think you said you looked at about a quarter of 14:50:44

the 3,000 and some; is that right? 14:50:51

A About a quarter, yes. 820 or 830. 14:50:51

(Reporter clarification.) 14:51:03

A I don't know exactly. Somewhere around 14:51:03

there, slightly over 800 substitutions. 14:51:05

Q How did you pick those 800-some -- how did 14:51:05

you pick the ones that you looked at? 14:51:12

A I started with substitutions that appeared 14:51:15

with high frequency. Ultimately I looked at all 14:51:19

substitutions that involved amino acids that 14:51:26

appeared with a frequency of 5 or greater 14:51:30

percentage. So I started at the top. After doing 14:51:59

10 percent, I think I had about 450. 14:51:37

I said: Okay. 450's not bad but I can do 14:51:40

better. So let's do additional 5 percent. That 14:51:44

added another 400. 14:51:50

Q Okay. Got it. 14:51:53

Okay. So can you turn to Paragraph 149? 14:52:32

A I'm at Paragraph 149. 14:52:57

Q So starting on Paragraph 149 is how you -- 14:52:58

is where you explain how you generated the 14:53:01

homology model using the SWISS-MODEL, right? 14:53:03

A That's correct. 14:53:08

Q So can you just explain kind of 14:53:08

step-by-step how you went about generating the 14:53:12

homology model using the SWISS-MODEL? 14:53:16

A Okay. SWISS-MODEL is a web service which 14:53:46

makes it very convenient for somebody who's not an 14:53:52

expert or a specialist in structural 14:53:55

bioinformatics to use the structure of a known 14:54:00

homologous protein as the starting point to 14:54:05

generate a model for a protein of interest, such 14:54:09

as PH20. 14:54:14

You visit the website, enter PH20 14:54:15

sequence. The website will take the sequence, 14:54:19

compare the sequence with the structures in their 14:54:22

database. And based on the matching, it will give 14:54:27

you a list of potential templates that you can use with a QME score -- that's quality of model estimate -- QME score. The higher the better. So it gives you idea of which template you might want to use. 14:54:31 14:54:36 14:54:43 14:54:45 14:54:48

At that point, you have a choice of choosing one or more template, and submit the job. And the job runs in the background, at the end of which you will be notified and then you'll be able to download the homology model of your protein. 14:54:49 14:54:51 14:54:55 14:55:00 14:55:02

That's it. 14:55:08

Q Okay. So you entered -- so you went to the SWISS-MODEL website and you entered the sequence for PH20, and then you asked the program to identify potential templates; is that right? 14:55:09 14:55:15 14:55:17 14:55:22

A That is correct. 14:55:27

ATTORNEY KUSHAN: Or, sorry. 14:55:28

Objection to form. 14:55:31

Q And so then it give you potential templates and then you selected the HYAL1 as the template; is that right? 14:55:32 14:55:35 14:55:37

A Yes. 14:55:38

Q Okay. So you selected HYAL1 as the template to use for the homology model? 14:55:43 14:55:48

ATTORNEY KUSHAN: Objection to form. 14:55:55

A I used the HYAL1 structure as the template for my homology model, yes. 14:55:56 14:56:01

Q Why did you use HYAL1 as the template? 14:56:03

A That was really the only template that was viable. When I read the service, only two structures came back as actually: HYAL1 structure, and another structure that had nothing to do with this protein. 14:56:07 14:56:11 14:56:15 14:56:20 14:56:25

Q Okay. 14:56:27

A Apparently there was some similarly over a span of, perhaps 50 amino acids, which is not even a protein. So it was pretty clear that I don't have a whole lot of options, other than HYAL1. So you pick that, and the rest is automated. 14:56:27 14:56:32 14:56:36 14:56:41 14:56:47

Q Okay. And so on Page 84, you have the SWISS-MODEL result; is that right? 14:56:50 14:57:10

A Yes. This is the result of homology modeling. Yes. 14:57:19 14:57:29

(Reporter clarification.) 14:57:30

Q And can you explain what the GMQE is? 14:57:30

A Yes. G -- GMQE stands for global model 14:57:39

quality estimate. It give you an idea of how good 14:57:44

the template is so that you can choose the right 14:57:48

template among several that are returned 14:57:56

initially, so that way you don't waste your time 14:58:00

modeling it against a template that's not even 14:58:05

appropriate. 14:58:10

So higher the better. Here it's 0.69. 14:58:15

The second template that came back I think had

a 0.1 or something.

Q Okay.

A So pretty clear cut. 14:58:16

Q Is 0.69, is that a -- is that a good 14:58:26

value, in your experience? 14:58:24

A I don't know actually. I think 0.69 is a 14:58:27

reasonable score based on the publication. I 14:58:44

don't have a lot of experience working with a 14:58:36

template that has lower QM -- GMQE value, so I 14:58:39

can't quite say. 14:58:43

Q Okay. 14:58:53

A But what's comforting is that we know 14:58:53

HYAL1 and PH20 are pretty similar based on the sequence alignment. So if that high sequence identity translates into 0.69, I think it's reasonable to assume that that is an acceptable value. 14:58:58 14:59:03 14:59:04 14:59:09 14:59:12

Q And the sequence identity is given as 40.24 percent; is that right? 14:59:12 14:59:18

A Yes. That's what this website says. 14:59:20

Q And then there's also a QMEAN DisCo Global. Can you explain what that is? 14:59:26 14:59:35

A QMEAN DisCo Local stands for qualitative model energy analysis, distance correlation local, meaning individual analysis. 14:59:39 14:59:44 14:59:47

Q And the value is given as .75 plus or minus .05. 14:59:49 14:59:58

What does that mean? 14:59:59

ATTORNEY KUSHAN: Objection. Foundation. 15:00:02

A In this graph, 0.75 I think is the average or mean, and anything above that is colored blue. Anything below that is colored red. 15:00:04 15:00:09 15:00:16

The critical number is 0.6, according to publication. It says 0.6 and up, you would have 15:00:19 15:00:22

some confidence in that modeling structure at that residue position, and sometimes it falls below because the sequence identity might be low in that region. 15:00:30 15:00:34 15:00:38 15:00:49

(Reporter clarification.) 15:00:59

Q Okay. And so when you did your analysis, did you -- at the positions that you looked at, did you consider what the -- what the value was at that -- at that position for the QMEAN DisCo? 15:00:59 15:01:04 15:01:06 15:01:11

A Yes. 15:01:16

Q Okay.

A I checked it and it was about .6. I forget the exact value, but -- 15:01:17 15:01:29

Q Okay.

A -- above that threshold. 15:01:25

Q Okay. 15:01:27

Okay. And so then you have -- on Page 86, you have: The relative QMEAN assessment for the entire protein and the local QMEAN quality assessment; is that right? 15:01:39 15:01:52 15:02:03 15:02:06

A That is correct. 15:02:07

Q Can you explain the graph on the left on 15:02:08

Page 86?

A QMEAN 4, or quality model energy analysis, is a statistical energy comprised of four independent terms, including torsion energy, solvent interaction, secondary structure and atomic interaction. Those four terms are added up some way to give a total score of QMEAN 4. 15:02:14 15:02:19 15:02:26 15:02:32 15:02:38 15:02:41 15:02:47

And what's plotted on this graph is a Z score, which indicates the multiples of the standard deviation for our structure compared to the average energy expected for a protein of that size. 15:02:54 15:03:02 15:03:05 15:03:14 15:03:19

Q And so what does this graph tell you about the model that you generated? 15:03:37 15:03:40

A The graph tells you that the model is reasonable. 15:03:43 15:03:47

Q Okay. And what about the graph on the right? 15:03:53 15:03:57

A The graph on the right is the -- the local quality estimate, which is the expanded view of the earlier tiny graph where 0.6 is the number to watch out for. And the position where the local 15:04:00 15:04:06 15:04:11 15:04:19

quality estimate is about .6 is a place where one can trust the model and carry on with the analysis. 15:04:24 15:04:28 15:04:32

Q Okay. 15:04:32

(Reporter clarification.) 15:04:47

Q And you also, above those graphs at the very top of Page 86, you mention that the whole protein had an overall QMEAN score of minus 2.83, which is the -- which is below the threshold of minus 4, indicative of acceptable quality. 15:04:47 15:04:56 15:05:02 15:05:08

So what is the QMEAN score of minus 2.83? 15:05:17

A Right. So QMEAN stands for qualitative model energy analysis. That's the aggregate sum of the four terms that I mentioned earlier -- torsion energy, solvent exposure, blah, blah -- and minus 2.83 corresponds to the multiple of the standard deviation from the average energy expected for a protein of that size. So that is what's shown on the left of the figure. 15:05:21 15:05:25 15:05:30 15:05:33 15:05:35 15:05:44 15:05:46 15:05:51

The star is our model, and what this graph says is that that star is minus 2.83 standard deviation away from the median, or median or 15:05:53 15:05:57 15:06:06

average, of the energy at that protein size, for that protein size, which is the X axis. 15:06:11 15:06:17

Q Oh, okay. Okay. Got it. Okay. Thanks. 15:06:21

So in Paragraph 153, you refer to B factor scores? 15:06:49 15:06:58

(Reporter clarification.) 15:07:00

Q And you equate a local QMEAN reliability to a B factor score? 15:07:00 15:07:06

A Yes. 15:07:09

Q Is that right? 15:07:09

A Yes. 15:07:10

Q Okay. And so the graph on the right, in the local quality estimate graph on the right on Page 86, is that a graph of B factor scores? 15:07:12 15:07:19 15:07:23

A That is correct. 15:07:28

Q Okay.

A The values shown on the right side of that graph is recorded in the B factor column of the PDB file. You can extract that value. 15:07:30 15:07:34 15:07:45

(Reporter clarification.) 15:07:48

Q Okay. So the PDB file that you download from SWISS-MODEL, in the B factor column, it has 15:07:48 15:07:54

the numbers that are -- for each atom, right? 15:07:54

A Residue. 15:07:56

Q For each residue? 15:07:58

A Yes.

Q That provides the local QMEAN reliability? 15:08:00

A That's correct, yes. So you can extract 15:08:02
that information from that B factor column. 15:08:05

ATTORNEY MARTIN: Okay. Okay. Got it. 15:08:34

All right. We've probably been going for 15:08:34
another an hour. 15:08:39

Do you want to take another break? 15:08:40

THE WITNESS: Yes, yes, yes. 15:08:43

ATTORNEY KUSHAN: Why don't we stay on the 15:08:44
record for a minute, and let Dr. Park step out. 15:08:46

THE WITNESS: Okay.

ATTORNEY KUSHAN: So we can discuss an
issue you raised. 15:08:51

THE VIDEOGRAPHER: Do you want to stay on 15:08:51
the video record as well? 15:08:53

ATTORNEY KUSHAN: You can take the video 15:08:56
off. 15:08:57

THE VIDEOGRAPHER: We are going off the 15:09:00

video record. The time is 3:08 p.m. 15:09:02

ATTORNEY KUSHAN: So we're willing to entertain a request to produce the spreadsheet that he referred to. I want to make clear that, you know, you've had these declarations since November/December. 15:09:06 15:09:10 15:09:14 15:09:19 15:09:20

You filed Patent Owner response, preliminary responses. We filed replies. And what we pointed out in our replies was that you were misreading paragraphs of the declaration. 15:09:21 15:09:24 15:09:27 15:09:27

We pointed you to Paragraphs 102 and 103 in our replies to the Board, and what we said was your characterization that we had directed him to specific residues to consider only, was false. We made it very clear, and we also pointed out this additional explanation in the declaration, that he had -- he's telling you he evaluated lots of positions. That's what he just confirmed in his testimony. 15:09:30 15:09:34 15:09:38 15:09:41 15:09:44 15:09:48 15:09:51 15:09:54 15:09:59

So I want to make it clear, you had a chance to ask for this many months ago, and we don't expect that you should, you know, seek to 15:09:59 15:10:02 15:10:05

get an additional deposition of him or anything like that, claiming prejudice, because you've had the opportunity, you've had the knowledge, you've had our position about him doing more work than what was limited to each individual declaration as specific position. 15:10:08 15:10:11 15:10:14 15:10:14 15:10:15 15:10:18

So we're happy -- like I said, we can work to produce this spreadsheet. We can add it in as an exhibit if you want, and we can provide it to you. 15:10:19 15:10:23 15:10:28 15:10:30

ATTORNEY MARTIN: So a couple of things. 15:10:31

There's no way from his declaration that one could extract that he looked at 820 to 830 different substitutions, and gave you a spreadsheet with the data. It's not clear from this declaration at all how many positions he looked at, and then how many substitutions he looked at in each position. 15:10:32 15:10:35 15:10:41 15:10:44 15:10:47 15:10:50 15:10:53

He could have looked at 10, right? He could have looked at, you know, 10 different positions of like, a handful of different substitutions, maybe, like, 10 at each position. 15:10:55 15:10:58 15:11:01 15:11:03

That's very different from what he just said, which is 100 and -- 820 to 830. 15:11:04 15:11:07

There are like -- this is purely qualitative. There -- there is no way for us to know, based on the declaration, what he did. And so there's no -- there's no way -- there is no possible way that we could have predicted that he gave you a spreadsheet with 820 to 830 substitutions in it, and then asked for it and then considered it. We just did not have that level of information. 15:11:09 15:11:12 15:11:15 15:11:17 15:11:21 15:11:25 15:11:27 15:11:29 15:11:32

You guys are you ones who -- hold on. I listened to you go on for a very long time, so let me -- 15:11:33 15:11:35 15:11:39

ATTORNEY KUSHAN: Go ahead. 15:11:40

ATTORNEY MARTIN: -- say my piece and then, you know, you can respond. 15:11:42 15:11:44

You know, you guys had the opportunity to make clear what he did or didn't do. You had the opportunity to frame what he did and didn't do. And that's what you did in your declaration. We read it. Literally, we read it, and we understood 15:11:45 15:11:47 15:11:51 15:11:55 15:11:55

the best that we could. And if it's the case that he considered 800 and some positions, you should have told us that, right, as opposed to trying to put it on us to somehow psychically extrapolate out of this declaration what it was that he did, and put the onus on us to go and ask you. 15:11:57 15:12:00 15:12:04 15:12:06 15:12:09 15:12:11

And also, I mean, you guys have been serially filing PGRs over time, right, where you come up with new tables with new positions. Like when you filed the fist one, how could we possibly know what positions he looked at. We get no information or positions he looked at because you're filing more PGRs, but there was no way that we could have known that at the very beginning. It's just totally unreasonable. 15:12:15 15:12:16 15:12:20 15:12:25 15:12:30 15:12:34 15:12:35 15:12:36 15:12:39

So it's really -- like, it's really, really not clear from anything that you guys have -- maybe it's clear for you because you know that there's a spreadsheet. It was not clear from our perspective that there was this spreadsheet that we should have known to ask about. We just didn't know. 15:12:41 15:12:42 15:12:44 15:12:46 15:12:47 15:12:49 15:12:52

And so in terms of asking for another deposition, I haven't seen the spreadsheet, right? We need to see the spreadsheet, and then we can go from there. But as an initial matter, we would like the spreadsheet. 15:12:53 15:12:54 15:12:57 15:13:00 15:13:03

ATTORNEY KUSHAN: So I'll be very brief. 15:13:04

The point I made was that when you read Paragraphs 102 and 103, and you see him explain that he did a lot of different types of -- evaluated a lot of different types of substitutions. He conducted an analysis that did not focus on any particular position. 15:13:06 15:13:09 15:13:13 15:13:15 15:13:17 15:13:20

And the natural thing would have been to -- if you had a question or you thought this was relevant information, would be to drop an email to me saying: Hey, you appear to be relying on additional information that you haven't included in the record. 15:13:24 15:13:27 15:13:27 15:13:31 15:13:33

ATTORNEY POWERS: Jeff, that's why we have a deposition. 15:13:34 15:13:36

ATTORNEY KUSHAN: What? 15:13:37

ATTORNEY POWERS: That's why we have a 15:13:38

deposition, so we can ask him these questions. 15:13:39

ATTORNEY KUSHAN: No. You chose to tell a narrative where you said we, you know, you've invented -- you've basically ignored these passages, and misquoted and mischaracterized the scope of work he was asked to do. 15:13:40 15:13:41 15:13:43 15:13:47

And then you went to the Board, and you said: Deny these institutions because they directed him to review only position 320. So it was advancing that narrative you were going for. 15:13:52 15:13:55 15:13:56 15:13:58

I have done this in many, many cases, where if we have an expert do analytical work, the other side can read the explanation that work has been done, and if you feel there's an incomplete record or you need more information about what he did, we would have responded, and we would have, you know, we could have provided or explained what additional information existed. That's my point. 15:14:00 15:14:03 15:14:08 15:14:09 15:14:13 15:14:16 15:14:17 15:14:19

And the Board obviously wants us to cooperate and not bring things to them that are, you know, going to waste their time. So that's why in a normal setting, you can contact opposing 15:14:22 15:14:23 15:14:26 15:14:28

counsel and ask questions.

Is there more evidence? Is there more work product of this expert? And we would have then had this conversation three months ago, and then maybe you wouldn't have filed, you know, all those arguments. 15:14:32 15:14:37 15:14:40 15:14:41 15:14:41

But the point of it is, you could have gotten -- you could have explored -- 15:14:42 15:14:45

ATTORNEY POWERS: Well, you withheld them. 15:14:45

ATTORNEY KUSHAN: We didn't withhold the document. 15:14:48

ATTORNEY POWERS: Certainly seems like it. 15:14:49

ATTORNEY KUSHAN: We made clear -- you can read the -- look, we've told you he didn't limit his analysis. We've told you that evaluated lots of positions, and we would have, you know, responded if you had contacted us about this. 15:14:49 15:14:51 15:14:54 15:14:57 15:14:59

So bottom line, we are going to go back and we will work to get this over to you as rapidly as we can. I think the question that you guys should think about is, you know, why did -- why did you wait? 15:15:02 15:15:06 15:15:10 15:15:12 15:15:15

And that's my point. So I'm happy to -- 15:15:16

ATTORNEY MARTIN: Yeah. We -- you've said 15:15:20

your piece. I've said my piece. Trey has said 15:15:21

his piece. 15:15:24

We didn't wait, right? Like, you guys had 15:15:25

the burden under the rules to provide the 15:15:28

information that your expert is relying on that 15:15:29

forms the backbone of the declarations that he 15:15:32

submitted. So you should have given us the 15:15:34

spreadsheet. 15:15:36

You can give us the spreadsheet now. 15:15:37

You're going to give us the spreadsheet, so... 15:15:37

ATTORNEY KUSHAN: Do you want it, like, 15:15:39

right now? 15:15:41

ATTORNEY MARTIN: We can move forward. 15:15:42

As soon as possible would be great. 15:15:43

ATTORNEY KUSHAN: I don't think we can 15:15:46

create -- I don't think we can turn it into paper 15:15:52

now, so that's the point. 15:15:55

ATTORNEY MARTIN: I mean, I would hope 15:15:56

that you would give it in, like, an electronic 15:15:57

format. I mean, if you hand me like -- if you 15:15:59

hand me something that's this big, you know, a foot-tall stack of paper with 800 and some rows, I'm going to be a little unhappy, so please just give me a PDF, or... 15:16:01 15:16:05 15:16:09

ATTORNEY KUSHAN: You'll get -- yeah. You'll get an electronic version. 15:16:12 15:16:13

ATTORNEY MARTIN: Okay. Thank you.

ATTORNEY KUSHAN: All right. So we'll... 15:16:16

ATTORNEY MARTIN: Want to take a break? 15:16:18

ATTORNEY KUSHAN: Yeah. 15:16:20

ATTORNEY MARTIN: Okay. 15:16:21

ATTORNEY KUSHAN: Now we can go off the record. 15:16:22 15:16:24

(Recess from 3:16 p.m. until 3:34 p.m.) 15:16:27

THE VIDEOGRAPHER: We are back on the record. The time is 3:34 p.m. 15:34:51 15:35:03

BY ATTORNEY MARTIN: 15:35:10

Q All right. Okay. Can you turn -- Dr. Park, can you turn to Paragraph 161? 15:35:11 15:35:25

A I'm at Paragraph 161. 15:35:34

Q Okay. Oh, actually. Let's look at 160. 15:35:37

A 160. 15:35:47

Q So you said that protein structure models generated by SWISS-MODEL, similar tools in 2011 would have been reliable for evaluating single amino acid substitutions that are in regions of PH20 where there is a sufficiently reliable local QMEAN score, right? 15:35:50 15:35:55 15:36:01 15:36:04 15:36:04 15:36:06

A Yes. That is correct. 15:36:09

Q And then you say: This is possible because only one residue is being changed and the remainder of the protein is still based on the reliable experimentally determined structure, right? 15:36:10 15:36:17 15:36:20 15:36:24 15:36:28

A Correct. 15:36:28

Q So when you say only one residue is being changed, are you referring to the fact that you're using the mutagenesis feature in PyMol to change one amino acid in the protein? 15:36:29 15:36:33 15:36:41 15:36:41

ATTORNEY KUSHAN: Objection. Foundation. 15:36:45

A Introducing a mutation in PyMol is for convenience only. You can see it in realtime in a matter of seconds. I do it so I can see the mutation quickly. However, if I want to generate 15:36:46 15:36:51 15:36:55 15:37:00

a more reliable model, I would generate a mutant sequence of PH20, resubmit it to SWISS-MODEL, and generate PyMol's model again. So I do this in parallel. 15:37:04 15:37:10 15:37:15 15:37:17

Q Okay. And is that because PyMol does not -- does not do an energy minimization when it does the mutagenesis? 15:37:18 15:37:21 15:37:26

ATTORNEY KUSHAN: Objection. Foundation. 15:37:29

A It does not do minimization, energy minimization. That's part of the answer. But it's also because when you introduce a mutation using PyMol, you're using PH20 as the template for generating mutant structure. It would be more fair if that mutant structure were generated using HYAL1 structure as the template, just so that you can treat wild-type and mutant the same way. 15:37:31 15:37:34 15:37:39 15:37:43 15:37:50 15:37:54 15:37:58 15:38:03

Q Okay. So hypothetically, you could take the PH20 sequence and introduce two substitutions, and then put that sequence -- put the two substitutions into SWISS-MODEL and generate a homology model using HYAL1 as the template, right? 15:38:06 15:38:10 15:38:15 15:38:19 15:38:27

ATTORNEY KUSHAN: Objection. Form. 15:38:28

A Yes. If you submit a sequence containing two substitutions through SWISS-MODEL, it will generate a homology model corresponding to that mutant structure. That is true. 15:38:29 15:38:32 15:38:34 15:38:39

Unfortunately, that's not the end of the story. Because modeling is not just a matter of generating a PDB file. It also involves the -- the subsequent aspect, which involves an expert looking at the structure and making sense of it, and that's where the problem occurs. 15:38:45 15:38:47 15:38:53 15:38:58 15:39:02 15:39:05

Q And what's the problem? 15:39:08

A The problem is when you're looking at one substitution, there are only so many interactions that a single amino acid can make, and those are relatively straightforward to discern for an expert. They look at the substitution. They can estimate the three properties that I mentioned: The intrinsic property of the amino acid, secondary structure compatibility and interactions. Those are relatively straightforward, relatively speaking. 15:39:11 15:39:15 15:39:21 15:39:29 15:39:35 15:39:42 15:39:45 15:39:47 15:39:48 15:39:52

Once you start introducing the mutations, 15:39:55

more than one mutation, and if those mutations happen to be -- to say, one, if they're close, or if they're far away. 15:40:01 15:40:11 15:40:14

If they're far away, you can treat them -- treat them as independent mutations so you can evaluate them one at a time. That's fine. However, if those substitutions happen to be very close to each other, what happens is now suddenly you have to deal with many more possible changes in the interactions, because the individual substitutions would now alter their interactions with their neighbor, and there would be interactions between them as well. 15:40:15 15:40:19 15:40:23 15:40:25 15:40:29 15:40:33 15:40:36 15:40:40 15:40:45 15:40:47

So now that results in both positive and negative interactions, all of which need to be tracked and estimated. Unfortunately, a person viewing the structure would not be able to differentiate many of these subtle changes, especially if there are multiple mutations nearby. 15:40:50 15:40:53 15:40:56 15:41:02 15:41:05 15:41:11

Q Okay. So let's say you have your PH20 structure, and you introduce two substitutions that are far apart in the structure. 15:41:16 15:41:21 15:41:25

Am I understanding correctly that you could -- you could evaluate those -- you could evaluate those substitutions using the model, the homology model that you generate, because they're far away from each other and so you're not going to have this neighbor effect? 15:41:29 15:41:31 15:41:34 15:41:39 15:41:43 15:41:46

ATTORNEY KUSHAN: Objection. Foundation. Objection as to form. 15:41:48 15:41:49

A One can make an approximation and say because they are spatially far apart from each other, their interactions would be minimal. Therefore, they can be treated independently, yes. 15:41:50 15:41:54 15:42:01 15:42:01

(Reporter clarification.) 15:42:08

Q And so when you say they can be treated independently, you mean that you can go and look in the structure at -- at the effect of the substitution at each position independently and see how that substitution is impacting the local environment, and it would be two different analyses because they're far apart in the protein; is that right? 15:42:08 15:42:11 15:42:13 15:42:17 15:42:20 15:42:28 15:42:33 15:42:37

A Yes. That would be the case. 15:42:37

Q Okay. 15:42:39

A That would be an approximation, but I would think it would be a reasonable approximation. Distant residues are known to be coupled in some cases, but they're not very common. 15:42:39 15:42:43 15:42:47 15:42:49

So if you introduce a mutation that are far apart from each other, then each mutation can be treated independently and evaluated independently, and one may be able to estimate the combined effect of the two or more mutations, if necessary, and include whether the mutant would be better or worse than wild-type. 15:42:50 15:42:52 15:42:57 15:42:59 15:43:06 15:43:11 15:43:17

(Reporter clarification.) 15:43:26

Q Okay. So then if you have two substitutions that are close together, am I understanding correctly that if you make two substitutions that are close together, then they could interact with each other, and if you're going through and identifying and cataloging every single interaction that -- every single interaction at a particular position, when you 15:43:26 15:43:30 15:43:33 15:43:36 15:43:41 15:43:44 15:43:47 15:43:50

then make a change, let's say right next to that position, you then have to go through and catalog every single one of the interactions at the second substitution, and so then it becomes difficult to tease out the effects of the combination, as opposed to just making the single substitution? 15:43:54 15:43:58 15:44:01 15:44:02 15:44:06 15:44:11

ATTORNEY KUSHAN: Objection to form. 15:44:15

Also objection to foundation. 15:44:16

A It does come down to the number game. As you introduce more and more mutations, the number of possible favorable and unfavorable interactions increases exponentially, and can quickly overwhelm a person's ability to evaluate them. 15:44:20 15:44:25 15:44:28 15:44:35 15:44:43

And many of the combinations may be very nuanced. In other words, the differences between different conformations and different combinations of mutations would be very nuanced, to be able to -- for a person to be able to -- to say whether one would be better than another set of mutants, mutations. 15:44:47 15:44:53 15:44:58 15:45:02 15:45:08 15:45:10 15:45:16

Q Okay. So let's say you're mutating two residues that are right next to each other in the 15:45:17 15:45:23

protein. Okay? Just the two. I'm trying to understand why it is that -- why it is that that creates an issue when you're doing your comparison? 15:45:26 15:45:30 15:45:38 15:45:41

So you have your PH20 sequence and you mutate two residues right next to each other, and you compare that model with the wild-type PH20 model. So what is it about the fact that the residues are right next to each other that is making the analysis difficult? 15:45:44 15:45:48 15:45:53 15:45:58 15:46:01

ATTORNEY KUSHAN: Objection. Form. 15:46:03

A Yeah. Again, the problem is not the generation of the model, per se. But here I'm using the word modeling, not only to indicate the mechanics of going through the SWISS-MODEL portal downloading PBD, which can be done regardless of what you put in, but the subsequent step -- the human intervention portion of the modeling, which involves interpretation of the mutations, becomes very quickly intractable. 15:46:06 15:46:11 15:46:14 15:46:19 15:46:22 15:46:26 15:46:32 15:46:36 15:46:40

And the reason for that is because of the -- the very large number of changes that occur 15:46:45 15:46:49

following multiple simultaneous mutations. They can't be broken down. In other words, the number of interactions they need to keep track of does not grow linearly, as you would expect if those sites are independent, but they would grow exponentially. 15:46:54 15:47:02 15:47:05 15:47:05 15:47:12 15:47:15

Q Okay. So you agree that technically you can introduce multiple mutations into a sequence -- into a sequence, and then you could submit that sequence for PH20 into SWISS-MODEL, and you download a PBD file with the resulting homology model, right? 15:47:16 15:47:21 15:47:27 15:47:30 15:47:33 15:47:38

A Well, I don't -- 15:47:40

ATTORNEY KUSHAN: Sorry. 15:47:40

Objection. Foundation. 15:47:41

A I don't argue that you can do it. If you have many combinations, it might take a while. But you can generate a PBD file, files. Unfortunately, it doesn't really get you anywhere, because SWISS-MODEL doesn't put a number on -- it doesn't tell you anything about the energetics of those substitutions. All it does is -- it's going 15:47:45 15:47:53 15:47:56 15:48:00 15:48:04 15:48:10 15:48:14

to give you the best structure it can, given the sequence. 15:48:20 15:48:24

It may be a horrendous combination of mutations. SWISS-MODEL would not know the difference between that and the best sequence. It just gives you the best structure it can by going through the set of routines that it's designed to do, and you generate a PBD file. 15:48:24 15:48:30 15:48:32 15:48:35 15:48:38 15:48:44

And sure, you can download it. But what do you do with it? The problem is you can't do anything, and there's no methodology that will allow you to compare two structures containing substitutions that are near each other if it's a large number. There's no proven and accepted methodology that one can go through systemically to evaluate and compare those sets of structures and sequences, which I consider still part of the modeling. 15:48:49 15:48:51 15:48:55 15:49:01 15:49:06 15:49:10 15:49:14 15:49:17 15:49:23 15:49:27

Q Uh-huh. 15:49:28

A Because modeling is not just generating the PBD file, but also looking at it and making sense of it. And you will -- one will run into a 15:49:29 15:49:32 15:49:36

combinatorial, combinatorially intractable situation very quickly if mutations occur close to each other. 15:49:44 15:49:48 15:49:53

Q And the combinatorially intractability is because of all of the -- all of the new interactions that are forming, and you can't keep track of all of the new interactions that are forming when you're making multiple changes close together in the protein; is that right? 15:49:54 15:50:00 15:50:02 15:50:06 15:50:09 15:50:11

A That is true. Two parts: One, to share a number of interactions that one needs to keep track of increases very quickly, as I pointed out, exponentially, and that will expose the fundamental shortfalls, shortcomings of the energy field that is used to evaluate different substitutions. Those energy fields are incomplete. Incomplete. 15:50:14 15:50:20 15:50:22 15:50:29 15:50:36 15:50:39 15:50:43 15:50:47

And I think I got it. 15:50:51

They are unfortunately incomplete. There are many effects that are overlooked by the -- the force field, and those errors would compound as you begin to change more interactions, and now the 15:50:54 15:50:57 15:51:02 15:51:07

uncertainty that results from those shortcomings in the force -- force field would essentially prevent one to make a meaningful comparison between structures. 15:51:12 15:51:18 15:51:23 15:51:28

Q So is the problem that you're -- is the problem the comparison, that you're comparing the wild-type structure with the structure that has, let's say, two changes that are right next to each other, and you're trying to account for, let's take position 320 -- you're trying to account for all of the interactions at position 320, and then in the wild-type structure, and then if you make two changes -- well actually, wait. 15:51:30 15:51:31 15:51:35 15:51:37 15:51:40 15:51:43 15:51:46 15:51:53 15:51:59

Let me start over. Let me try this a different way. 15:52:04

All right. So you have your wild-type PH20 model. Let's say you make your position, your change at position 320, right? And you make your SWISS-MODEL model at position 320. 15:52:04 15:52:08 15:52:11 15:52:13

Could you then make a change at position 321, and then compare that model to the model at position in which you changed 15:52:15 15:52:18 15:52:22

position 320? 15:52:26

A Both mutations, or just one mutation? 15:52:28

Q So the -- wait. You have your wild-type, 15:52:32

and you have your mutant 320, and then you have 15:52:41

your mutant 320 and 321. 15:52:42

A Okay. Single point mutant versus double 15:52:43

mutant? 15:52:47

Q Yes. Could you do that -- could you do 15:52:47

that comparison, I guess three-way comparison -- 15:52:50

ATTORNEY KUSHAN: Objection. 15:52:54

Q -- to evaluate the -- to model the 15:52:55

mutation at positions 320 and 321? 15:52:59

ATTORNEY KUSHAN: Objection. Foundation. 15:53:02

Objection to form. 15:53:03

A It depends on the types of mutations that 15:53:05

you would introduce. Some combinations are more 15:53:08

straightforward than others. If one mutation is 15:53:12

outright bad, then no matter what you do at the 15:53:16

other position, you may just kill the protein. So 15:53:21

that -- that's an easy combination. 15:53:25

There may be scenarios where it's not 15:53:28

clear what happens. And now if you increase it to 15:53:31

three and four and five positions which are all simultaneously changing, and now all of a sudden, a lot of things are changing. 15:53:38 15:53:44 15:53:48

So you have to -- one has to compare the initial wild-type set of interactions with the interactions that you see in a single point mutant or a quintuple mutant, and a lot of things have changed, and unfortunately the energy function that is used and needed to evaluate these different changes and interactions are not very accurate. 15:53:50 15:53:54 15:53:58 15:54:04 15:54:11 15:54:16 15:54:19 15:54:23

And those errors would compound as you begin to introduce more and more, and there are other missing components to the force field which may not have been very important initially, for a single point mutant, will become very apparent as you -- it would become more significant because they individually introduce uncertainties, and errors so that it becomes nearly impossible to make any meaningful statement comparing one set of five mutants versus another set of five mutants, or a single mutant. That's the challenge. 15:54:25 15:54:28 15:54:33 15:54:38 15:54:42 15:54:48 15:54:53 15:54:58 15:55:03 15:55:08 15:55:15

Q So the challenge is that -- I'm trying to understand if the challenge is that you have to count all of the different interactions that have changed, and that's just a lot of interactions that have changed when you have, like, five mutations right next to each other on a protein, or if the problem is that you don't know if those -- let's say you're making five changes, if those replaced residues are in the correct position in the model, because there's an issue with the energetics that the computer program is using to build the homology model. 15:55:16 15:55:21 15:55:23 15:55:25 15:55:27 15:55:30 15:55:33 15:55:35 15:55:40 15:55:43 15:55:46 15:55:51

I'm trying to understand if which one of those two things it is that you're -- 15:55:54 15:55:56

A Oh, absolutely. 15:55:57

ATTORNEY KUSHAN: Objection. Foundation. 15:55:58

Objection to form. 15:56:00

A SWISS-MODEL does side chain modeling, meaning after the backbone structure has been determined, it would go through different rotamer states and identify the set of rotamers that the minimize the energy. 15:56:05 15:56:12 15:56:17 15:56:21 15:56:30

(Reporter clarification.) 15:56:32

A That it does pretty well, because it uses an external algorithm actually, SCWRL, I think that's called. 15:56:32 15:56:36

It's known to be pretty good at side chain modeling. And it will find the best combination of rotamers. So internally consistent. So comparing one rotamer set for that quintuple mutant to another rotamer set containing exactly the same mutations, in other words, if you change the confirmations of the five residues, SCWRL will know how to do it. 15:56:43 15:56:48 15:56:53 15:57:00 15:57:05 15:57:05 15:57:12

So it will give you the best rotamer. However, it doesn't give you a method of comparing that mutant with another mutant containing different amino acids at the same positions. That requires different analysis, post-model generation. You generate the model, and then comes the analysis. How does one analyze that? 15:57:13 15:57:17 15:57:20 15:57:26 15:57:29 15:57:33 15:57:38

For a single point mutant, visual inspection is often sufficient. That has been known to be very successful. And that's also 15:57:42 15:57:43 15:57:48

proven by publication. People have done that. 15:57:52

For multiple point mutations, it becomes 15:57:56

very difficult for a human to juggle many 15:58:00

different possible interactions. 15:58:07

But that's only the problem. As I said,

there are two parts to it: One is the sheer 15:58:09

number of conformations, or combinatorial 15:58:10

interactions that we -- we need to keep track of. 15:58:16

But even if we were to do that diligently 15:58:19

and somehow tabulate all 5,000 interactions that 15:58:25

have changed, it still doesn't give you the answer 15:58:26

because the force field, energy field, that is 15:58:31

needed to estimate the energies for those 15:58:34

individual interactions is not there. It's 15:58:38

incomplete. 15:58:43

And that is a struggle that the whole 15:58:43

community has to deal with, and was not a solved 15:58:47

problem back in 2011. I don't think it's a solved 15:58:59

problem as of today. 15:58:56

Q Okay. Okay. So I think that what I'm 15:58:58

struggling with is that PH20 is -- PH20 is 40 15:59:07

percent-ish identical to HYAL1, the template 15:59:17

structure, right? 15:59:26

A Yes. 15:59:26

Q Okay.

A It is. 15:59:27

Q Human PH20. Let's say Rhesus monkey PH20 15:59:27

is, I don't know, like 37 percent -- I'm just 15:59:37

making up a number -- 37 percent identical to 15:59:42

HYAL1, right? 15:59:46

Presumably there's more than one change, 15:59:47

one difference between Rhesus monkey PH20 and 15:59:52

human PH20 that presumably you could still 15:59:53

generate a Rhesus monkey model using SWISS-MODEL 15:59:54

for Rhesus monkey PH20, the same way you could for 15:59:58

human PH20. 16:00:01

And if based on what you're saying about 16:00:02

the multiple mutation issue, I don't understand 16:00:04

why it's okay to use SWISS-MODEL to generate a 16:00:09

human PH20 model, and that's okay, but somehow 16:00:13

there's no force field and you're going to get 16:00:20

gibberish if you try to generate a model for

Rhesus monkey PH20, despite the fact that the 16:00:24

Rhesus monkey PH20 and the human PH20 have 16:00:27

multiple differences in their sequences? 16:00:31
That's -- that's what I'm not 16:00:33
understanding. Because the template sequence that 16:00:35
you're using has more than one change, right, to 16:00:38
PH20? The HYAL1 sequence is more than one amino 16:00:42
acid off of PH20, and you're still able to use 16:00:47
that as a template, so -- 16:00:50
A Absolutely. No problem with the Rhesus 16:00:51
monkey. It can generate a model. 16:00:55
Q Okay. 16:00:56
A What you cannot do is compare the Rhesus 16:00:57
monkey PH20, compare that to human PH20. 16:01:00
Q Okay. Okay. It's the comparison that 16:01:01
we're talking about? 16:01:04
A It's the comparison that you cannot do. 16:01:05
Q Okay.
A That's what -- that's what comes down to 16:01:08
the mutant analysis. 16:01:10
Q Okay. 16:01:11
A If you introduce the mutation in PH20, the 16:01:12
model will not tell you how to compare the two 16:01:16
structures. 16:01:22

Q Okay. All right. I am now following. 16:01:18
Thank you for that. 16:01:24
A I'm sorry for that very long-winded and 16:01:24
somewhat confusing answer. 16:01:30
Q No. I'm now following. Thank you. 16:01:30
All right. So let's turn to 16:03:00
Paragraph 107. 16:03:02
A Okay. I'm at Paragraph 107. 16:03:22
Q So you say: I visualized position 320 -- 16:03:41
(Reporter clarification.)
Q Paragraph 107: I visualized position 320 16:03:52
within the PH20 model using PyMol. First, I 16:03:52
confirmed that position 320 was not near the 16:03:58
active site, right? 16:04:01
A That's correct. 16:04:02
Q How did you confirm that position 320 was 16:04:04
not near the active site? 16:04:07
A I built a model of PH20 with the substrate 16:04:15
in the binding pocket, and that substrate was
imported from the bee venom hyaluronidase. And 16:04:26
through superposition and transferring of the 16:04:27
glycan, I could see where the substrate would sit 16:04:31

in the binding pocket, and 320 was not near that binding pocket. 16:04:35 16:04:40

Q When you say not near, I'm just trying to understand what that means. Is that a quantitative or qualitative? 16:04:41 16:04:44 16:04:46

A Sure. I don't have an exact number in terms of the distance in angstroms, but it was sufficiently far away from where the bound substrate is, which led me to believe that it's unlikely that this residue 320, regardless of what that amino acids is, I didn't think that that would be in a position to interact with a substrate in any significant way. 16:04:49 16:04:52 16:04:56 16:05:00 16:05:05 16:05:10 16:05:14 16:05:16

Q And why is that evaluation as to whether position 320 is near the active site, why is that important in your analysis? 16:05:19 16:05:23 16:05:28

A When you start mutating residues in the binding pocket, now you have to worry about the impact of the substitution on catalysis directly, in addition to its potential impact on the structure. I wanted to isolate those two effects and consider only the structural aspect, not the 16:05:33 16:05:37 16:05:41 16:05:47 16:05:53 16:05:56

functional aspect. 16:06:02

Q Okay. 16:06:05

ATTORNEY KUSHAN: Can we take a one-minute break so I can let Christine in? 16:06:08 16:06:11

ATTORNEY MARTIN: Oh, yeah. 16:06:14

THE VIDEOGRAPHER: We are going off the record. The time is 4:06 p.m. 16:06:16 16:06:18

(Recess from 4:06 p.m. until 4:08 p.m.) 16:06:22

THE VIDEOGRAPHER: We are back on the record. The time is 4:08 p.m. 16:08:48 16:08:50

BY ATTORNEY MARTIN: 16:08:59

Q Okay. Dr. Park, can you please turn to Paragraph 52? 16:09:00 16:09:05

A Paragraph 52? I am at Paragraph 52. 16:09:26

Q So in Paragraph 52, you say that the Yue paper also pointed out that there was a better correlation between substitutions and destabilizing changes when the nature of an interaction was combined with this location. 16:09:39 16:09:44 16:09:47 16:09:50 16:09:54

Do you agree that the location of a residue is an important factor to consider when evaluating the impact of a substitution? 16:09:58 16:10:04 16:10:08

Actually, strike that. 16:10:30

If you turn to Paragraph 53 -- 16:10:33

A Okay.

Q -- in the last sentence, it looks like you address this.

You say: The location of the amino acid 16:10:36
being changed within the protein structure is an 16:10:37
important consideration in assessing whether a 16:10:39
single amino acid substitution will be tolerated 16:10:42
or not, right? 16:10:50

A That's correct. 16:10:50

Q So do you agree that the location of the 16:10:50
amino acid is an important consideration when 16:10:54
you're evaluating substitutions? 16:10:56

A Was the question: Is the location an 16:10:57
important consideration? 16:10:59

Q Yes. Yes. 16:10:59

A The location of the substitution is an 16:11:05
important consideration, yes. 16:11:08

Q So why -- why is the location of an amino 16:11:09
acid an important consideration? 16:11:11

A There are many factors that go in. It's 16:11:20

listed in that Paragraph 52 as well, where it says, for example: Proteins are more consistently destabilized by a single amino acid change that introduced a large side chain that caused a steric clash within a buried position of the protein. 16:11:24 16:11:30 16:11:37 16:11:41 16:11:43

(Reporter clarification.) 16:11:55

A That's because in a buried position, many structural constraints of what can go in, in terms of size, the shape, in addition to the hydrophobicity/hydrophilicity. 16:11:55 16:11:57 16:12:02 16:12:05

So the constraints are different whether a position is buried as opposed to, say, solvent exposed. In a solvent exposed position, there's a lot of freedom. You still need to satisfy some constraints, but having done that, there's a lot of freedom in terms of choosing an amino acid, just because there are fewer structural constraints. 16:12:09 16:12:16 16:12:23 16:12:26 16:12:32 16:12:38 16:12:43 16:12:48

Q What are -- what do you mean when you say structural constraints? 16:12:49 16:12:51

A Structural constraints that I refer to include the ones that I mentioned earlier, say, 16:12:53 16:12:58

the volume or the shape. The amino acids at a buried position will force the choice of amino acid to be introduced to be a certain way. It not only has to fit into that space, but it also needs to have the right shape. 16:13:01 16:13:11 16:13:17 16:13:22 16:13:27

Those constraints are not as prominent at a solvent exposed position. The amino acid will have to satisfy some constraint, for instance, based on hydrophilicity/hydrophobicity. 16:13:30 16:13:35 16:13:42 16:13:46

(Reporter clarification.) 16:13:57

A But beyond that, there is much liberty. 16:13:57

Q So as a -- is it correct that as a general matter, a buried hydrophobic residue is going to be less amenable to substitution than a solvent exposed hydrophilic residue? 16:14:05 16:14:09 16:14:16 16:14:21

A It's always dangerous to generalize, but in many cases, buried positions are harder to mutate properly, compared to a solvent exposed position. Yes. 16:14:26 16:14:30 16:14:37 16:14:41

Q Did you look -- did you identify where on the structure -- strike that. 16:14:46 16:14:50

If you were to introduce a single amino 16:15:23

acid change that introduced a large side chain, let's say a large charged side chain, into a buried hydrophobic pocket in the protein, would you expect that to cause problems for your protein? 16:15:26 16:15:29 16:15:33 16:15:38 16:15:43

ATTORNEY KUSHAN: Objection. Foundation. 16:15:44

A That's a very complicated scenario, introducing a large charged residue into a buried position, was it? 16:15:48 16:15:54 16:15:58

Q Uh-huh. 16:15:59

A It's hard to imagine a scenario where that would happen. The large side chain would have to be the right size to fit into it, first of all. And having a charge that's buried is a no-no when you're doing important engineering. This, I think, would be one of the worst combination of position versus amino acid choice. 16:16:00 16:16:05 16:16:09 16:16:15 16:16:22 16:16:26 16:16:31

Q Okay. 16:16:35

A Yes. So it would be a pretty bad mutation in general. Now, can one think of a scenario where this is not the case, well, anything is possible. But I think in general, that would be 16:16:35 16:16:41 16:16:45 16:16:47

challenging. 16:16:51

Q Okay. Would you expect -- would you 16:16:52

expect to get a misfolded protein, or an inactive 16:16:54

protein, if you did something like that? 16:17:01

A In all likelihood, and very high 16:17:06

probability, I think the -- that substitution 16:17:10

would be highly destabilizing. 16:17:13

(Reporter clarification.) 16:17:19

Q So if you -- let's say you change 16:17:19

multiple -- multiple buried hydrophobic residues 16:17:24

to large charged residues all at the same time, 16:17:33

would you expect to get a functional protein, or 16:17:36

would you expect to get not -- an inactive 16:17:40

protein? 16:17:43

ATTORNEY KUSHAN: Objection. Foundation. 16:17:44

Objection as to form. 16:17:46

A We're talking about a very highly 16:17:48

coordinated and orchestrated mutation, which I 16:17:58

think would be extremely difficult to pull off. 16:18:03

Q So you would expect that it would not -- 16:18:04

you would not get a functional protein out of 16:18:06

that, right? 16:18:10

ATTORNEY KUSHAN: Objection. Form. 16:18:10

A If I were a betting man, I would not bet 16:18:11

in favor of that mutation. 16:18:15

Q Okay. And you don't need a SWISS-MODEL 16:18:25

homology model to predict that that's not going to 16:18:21

work, right? 16:18:24

ATTORNEY KUSHAN: Objection. Foundation. 16:18:26

A SWISS-MODEL can generate a model that one 16:18:31

can expect to see whether such combination might 16:18:36

be possible, and being able to see the model with 16:18:41

your own eye might be very helpful. 16:18:47

What I am objecting earlier is that the 16:18:53

likelihood of finding a pair of charge mutations, 16:18:55

I think that was the scenario? 16:19:01

Q For multiple -- multiple charge. 16:19:03

A Multiple charge, no less. 16:19:04

Q Uh-huh.

A To fit into hydrophobic pocket and somehow 16:19:06

make everybody happy and keep the protein 16:19:15

functional, would be a very difficult task, with 16:19:19

or without SWISS-MODEL. 16:19:23

(Reporter clarification.) 16:19:29

ATTORNEY KUSHAN: Can we just -- whoever has joined, announce themselves? 16:19:29 16:19:33

THE VIDEOGRAPHER: It looks like Mr. Goldberg signed in again. 16:19:37 16:19:39

ATTORNEY KUSHAN: Brian, can you hear us? 16:19:42

ATTORNEY GOLDBERG: I can. 16:19:45

ATTORNEY KUSHAN: Are you by yourself? 16:19:47

ATTORNEY GOLDBERG: Yes. 16:19:49

ATTORNEY KUSHAN: Okay. Just wanted to make sure. 16:19:50 16:19:52

ATTORNEY MARTIN: Thank you. 16:19:53

ATTORNEY GOLDBERG: Yeah. 16:19:53

BY ATTORNEY MARTIN: 16:20:08

Q So you used a distance cut-off of 5 angstroms to identify neighbors in your analysis; is that right? 16:20:08 16:20:15 16:20:17

A I did. 16:20:17

Q Why did you use 5 angstroms as the cut-off? 16:20:18 16:20:22

A It's a number that's commonly used. 16:20:24

Q So is that a typical cut-off in the art to identify neighbors for a particular residue? 16:20:30 16:20:33

A I would say so. 16:20:39

Q And was that the case in 2011? 16:20:41

A Yes. The cut-off hasn't changed. 16:20:46

Q Okay. Let's turn to Paragraph 70. 16:20:51

A I'm at Paragraph 70. 16:22:02

Q And in Paragraph 70, you say that: The propensity of different amino acids to support helix or beta sheet formation was generally known; is that right? 16:22:04 16:22:10 16:22:12 16:22:13

A That's correct. 16:22:16

Q And was that generally known in 2011? 16:22:17

A Yes. 16:22:25

Q And you have a chart under Paragraph 70 that identifies good and poor alpha helix and beta sheet formers, right? 16:22:25 16:22:29 16:22:38

(Reporter clarification.) 16:22:41

A Yes. There's a chart. 16:22:41

Q Did you prepare this chart? 16:22:43

A Yes. I did. 16:22:49

Q And was this chart created based on what the skilled person would understand in 2011? 16:22:50 16:22:57

A Yes. 16:23:00

Q Okay. 16:23:05

A This would be regularly understood by a POSA. 16:23:06 16:23:09

Q So we had talked about the various interactions that you considered in your analysis to determine whether a substitution would be favorable or neutral or unfavorable, and you used two publications -- the Wang publication and the Yue publication -- for the -- to identify or list those interactions; is that right? 16:24:07 16:24:11 16:24:16 16:24:19 16:24:24 16:24:29 16:24:33

ATTORNEY KUSHAN: Objection. Foundation. 16:24:38

A Yes. I used those two publications as a demonstration that there were other precedents doing similar things. 16:24:40 16:24:46 16:24:55

Q And how did you identify those two papers? 16:24:56

A I don't remember the exact steps I took, but it must have been a combination of PubMed search and Google search. 16:24:59 16:25:09 16:25:20

(Reporter clarification.) 16:25:28

Q And those papers evaluate SNPs, right, single nucleotide polymorphisms; is that right? 16:25:28 16:25:38

A That's correct. 16:25:44

Q And in particular, they evaluate SNPs that are known to cause disease, right? 16:25:44 16:25:50

A That's right. 16:25:52

Q And they try to identify the molecular mechanism of action for why that particular SNP causes disease? 16:25:53 16:25:57 16:25:58

A That's correct. 16:26:00

Q So is it fair to say that those two papers, the Wang and the Yue paper, are evaluating the relationship between structure and function in terms of the impact on protein structure of a particular mutation, and the function being a destabilization and then the resulting disease? 16:26:01 16:26:04 16:26:07 16:26:11 16:26:18 16:26:23

ATTORNEY KUSHAN: Objection. Foundation. 16:26:27

A That's what they found in the paper. They reported that 90 percent of the time, SNPs cause instability in the protein, which is the -- the direct cause of the disease. 16:26:28 16:26:32 16:26:38 16:26:41

Q So are those papers evaluating structure function correlations for those various SNPs? 16:26:41 16:26:44

ATTORNEY KUSHAN: Objection. Foundation. 16:26:50

A Not structure function, but sequence 16:26:51

structure/stability. 16:26:53

ATTORNEY MARTIN: Okay. So we've been going for a long time. Do you want to take a quick break? 16:26:55 16:27:03 16:27:05

THE WITNESS: Sure. 16:27:09

ATTORNEY KUSHAN: Sure. 16:27:09

ATTORNEY MARTIN: See how much I have. 16:27:11

THE VIDEOGRAPHER: We are going off the record. The time is 4:27 p.m. 16:27:14 16:27:23

(Recess from 4:27 p.m. until 4:50 p.m.) 16:27:30

THE VIDEOGRAPHER: We are back on the record. The time is 4:50 p.m. 16:50:24 16:50:25

ATTORNEY MARTIN: All right. I just have one more question, and then some things to read on the record. 16:50:29 16:50:31 16:50:36

BY ATTORNEY MARTIN: 16:50:39

Q So -- so Dr. Park, you mentioned that you had several conversations with Dr. Hecht in connection with your analysis in this case. 16:50:40 16:50:41 16:50:45

So the question is: Did you rely on -- on anything that you learned from Dr. Hecht in -- in your analyses that are set forth in your 16:50:48 16:50:51 16:50:57

declarations? 16:51:02

A No. The conversation was more or less one 16:51:03

directional, me explaining to him what I had done, 16:51:11

and Dr. Hecht mostly agreeing with what I said. 16:51:14

ATTORNEY MARTIN: Okay. All right. So 16:51:24

let me just say what I'm going to say. 16:51:27

All right. So for the spreadsheet, we 16:51:30

object to the late production of the spreadsheet 16:51:34

under Rule 42.65. It's highly prejudicial that we 16:51:38

got the spreadsheet during the deposition, the 16:51:45

afternoon of the deposition. 16:51:49

And you know, it's very -- we appreciate 16:51:52

that you got us the spreadsheet, but at the same 16:51:55

time, right, I mean, it's a very small fonted, 16:52:00

large document with, like, 2,800 rows. 16:52:04

And it's in black and white, and there's a 16:52:08

figure that looks like it should be in color in 16:52:11

here, and we still don't have a color version of 16:52:15

it. 16:52:17

So it's obviously highly prejudicial that 16:52:18

we just got this now. We haven't been able to 16:52:21

talk to our experts about it. Obviously we can't 16:52:26

be expected to depose Dr. Park on it now, and so we reserve the right to request a further deposition and potentially other relief, so... 16:52:29 16:52:31 16:52:34

And we also object to the fact that Dr. Hecht attended a portion of this deposition, and we object to the potential -- we object to Dr. Hecht reading the transcript from this deposition because we think that it's witness coaching. So we're going to put that on the record as well. 16:52:45 16:52:51 16:52:53 16:52:58 16:53:02 16:53:05 16:53:08

And we object to, under Federal Rule of Evidence 615 specifically, that Dr. Hecht listened in on the deposition. We object to Dr. Hecht reviewing the transcript under Federal Rule of Evidence 615, and we object to the extent that Dr. Hecht relies on any of today's testimony. 16:53:11 16:53:17 16:53:22 16:53:25 16:53:26 16:53:30

Okay. All right. So I think I'm done, and pass the witness. 16:53:49 16:53:51

ATTORNEY KUSHAN: Can we have a five-minute break, and then we can proceed? 16:53:52 16:53:58

Before we do that, we, of course, disagree with the basis and propriety of the objections 16:54:01 16:54:04

you've raised about Dr. Hecht. 16:54:07

We also disagree with your arguments 16:54:09

regarding the late production -- or, supposedly 16:54:15

late production -- of this exhibit. And we are in 16:54:17

the process of emailing you the version of that 16:54:19

file with page numbers, which you should get 16:54:23

shortly. 16:54:27

But why don't we take our break? 16:54:28

THE VIDEOGRAPHER: We are going off the 16:54:32

record. The time is 4:54 p.m. 16:54:34

(Recess from 4:54 p.m. until 5:07 p.m.) 16:54:37

THE VIDEOGRAPHER: We are back on the 17:07:58

record. The time is 5:07 p.m. 17:08:01

ATTORNEY KUSHAN: Just before the break, 17:08:09

you made a number of comments, and my brief 17:08:12

response to that -- and we obviously can reserve 17:08:15

our ability to address other issues at a 17:08:18

subsequent point. 17:08:21

Promptly after you asked that Dr. Hecht 17:08:23

terminate his participation in this deposition, we 17:08:26

complied. And we don't think -- believe there's 17:08:26

any improper nature in his participation in the 17:08:30

deposition or, you know, watching it, and we don't think there's been any prejudice as a consequence of that. So we don't think there's any issue to address in the future. 17:08:34 17:08:39 17:08:41 17:08:45

And that's -- at this point, we have no questions for the witness, so we can end the deposition. 17:08:48 17:08:51 17:08:53

ATTORNEY MARTIN: Okay. Great. 17:08:54

THE VIDEOGRAPHER: This marks the end of the deposition of Dr. Sheldon Park. 17:08:55 17:08:59

We are going off the record at 5:08 p.m. 17:09:01

(The videotaped deposition of DR. SHELDON PARK was concluded at 5:09 p.m.)

CERTIFICATE OF SHORTHAND REPORTER - NOTARY PUBLIC

I, Kadi A. Harmon, Certified Reporter and Notary Public within and for the State of New York do hereby certify:

That Dr. Sheldon Park, the witness whose deposition is hereinbefore set forth, was duly sworn by me before the commencement of such deposition and that such deposition was taken before me and is a true record of the testimony given by such witness.

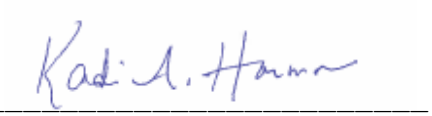
I further certify that the adverse party, Merck Sharp & Dohme, was represented by counsel at the deposition.

I further certify that the deposition of Dr. Sheldon Park, occurred at the offices of the Hyatt Regency Buffalo on Thursday, the 7th day of August, 2025, commencing at 8:45 a.m. to 5:09 p.m.

I further certify that I am not related to any of the parties to this action by blood or marriage, I am not employed by or an attorney to any of the parties to this action, and that I am in no way interested, financially or otherwise, in the outcome of this matter.

IN WITNESS WHEREOF, I have hereunto set my hand this 11th day of August, 2025.

My commission expires:
December 18, 2026



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