WEBVTT

NOTE duration: ”01:05:05.268”

NOTE Confidence: 0.9498541

00:00:01.040 --> 00:00:02.500 You know, it's an absolute

NOTE Confidence: 0.92617553

00:00:02.960 --> 00:00:04.559 privilege and a pleasure for

NOTE Confidence: 0.92617553

00:00:04.559 --> 00:00:06.160 me to introduce Professor Lauren

NOTE Confidence: 0.92617553

00:00:06.160 --> 00:00:06.660 Wilenski

NOTE Confidence: 0.9294026

00:00:07.120 --> 00:00:08.580 for today's Grand Rounds.

NOTE Confidence: 0.97598875

00:00:09.519 --> 00:00:11.280 He graduated from Princeton as

NOTE Confidence: 0.97598875

00:00:11.280 --> 00:00:12.340 the class valedictorian

NOTE Confidence: 0.972646

00:00:13.119 --> 00:00:14.580 while majoring in chemistry.

NOTE Confidence: 0.95074266

00:00:15.275 --> 00:00:16.975 He completed his MD PhD

NOTE Confidence: 0.95074266

00:00:17.035 --> 00:00:18.954 at Johns Hopkins in seven

NOTE Confidence: 0.95074266

00:00:18.954 --> 00:00:19.454 years.

NOTE Confidence: 0.96536267

00:00:19.994 --> 00:00:21.275 He first came to Children's

NOTE Confidence: 0.96536267

00:00:21.275 --> 00:00:22.895 Hospital Boston as a pediatrics

NOTE Confidence: 0.96536267

00:00:23.114 --> 00:00:24.875 intern, then the Dana Farber

NOTE Confidence: 0.96536267

00:00:24.875 --> 00:00:26.335 Cancer Institute as a pediatric

NOTE Confidence: 0.96536267

00:00:26.395 --> 00:00:28.710 HemOnc fellow, and finally, Harvard

NOTE Confidence: 0.96536267

00:00:28.710 --> 00:00:29.990 is a faculty member where

NOTE Confidence: 0.96536267

00:00:29.990 --> 00:00:31.370 he's currently a full professor,

NOTE Confidence: 0.96536267

00:00:31.670 --> 00:00:32.330 the director

NOTE Confidence: 0.9448776

00:00:32.630 --> 00:00:34.870 of the, Lindy program in

NOTE Confidence: 0.9448776

00:00:34.870 --> 00:00:35.370 cancer,

NOTE Confidence: 0.9555676

00:00:35.830 --> 00:00:37.909 chemical biology, and the director

NOTE Confidence: 0.9555676

00:00:37.909 --> 00:00:39.830 of the Harvard MIT MD

NOTE Confidence: 0.9555676

00:00:39.830 --> 00:00:40.809 PhD program.

NOTE Confidence: 0.97453934

00:00:41.705 --> 00:00:43.385 His research focuses on using

NOTE Confidence: 0.97453934

00:00:43.385 --> 00:00:45.225 chemical biology to target cancer

NOTE Confidence: 0.97453934

00:00:45.225 --> 00:00:46.505 where he helped develop a

NOTE Confidence: 0.97453934

00:00:46.505 --> 00:00:48.185 new class of biologics called

NOTE Confidence: 0.97453934

00:00:48.185 --> 00:00:49.165 stapled peptides

NOTE Confidence: 0.9506878

00:00:50.025 --> 00:00:51.225 and then applied them to

NOTE Confidence: 0.9506878

00:00:51.225 --> 00:00:53.305 numerous pathways, including p fifty

NOTE Confidence: 0.9506878

00:00:53.305 --> 00:00:55.225 three, KRAS, as well as

NOTE Confidence: 0.9506878

00:00:55.225 --> 00:00:57.005 viral and bacterial targets.

NOTE Confidence: 0.9752593

00:00:57.650 --> 00:00:58.930 However, his most significant work

NOTE Confidence: 0.9752593

00:00:58.930 --> 00:01:01.110 has been elucidating the complicated

NOTE Confidence: 0.9752593

00:01:01.170 --> 00:01:02.530 functions of the BCL two

NOTE Confidence: 0.9752593

00:01:02.530 --> 00:01:04.610 family of proteins, both within

NOTE Confidence: 0.9752593

00:01:04.610 --> 00:01:06.310 and outside of apoptosis.

NOTE Confidence: 0.9755632

00:01:07.010 --> 00:01:08.370 And while he's made significant

NOTE Confidence: 0.9755632

00:01:08.370 --> 00:01:09.569 progress in our understanding of

NOTE Confidence: 0.9755632

00:01:09.569 --> 00:01:11.485 MCL one, today he's gonna

NOTE Confidence: 0.9755632

00:01:11.485 --> 00:01:13.085 focus on a little known

NOTE Confidence: 0.9755632

00:01:13.085 --> 00:01:14.385 protein called Bax,

NOTE Confidence: 0.9363349

00:01:14.685 --> 00:01:16.765 which you might recognize from

NOTE Confidence: 0.9363349

00:01:16.765 --> 00:01:17.805 the homologue of its more

NOTE Confidence: 0.9363349

00:01:17.805 --> 00:01:18.944 well known BAC

NOTE Confidence: 0.96900225

00:01:20.125 --> 00:01:20.625 protein.

NOTE Confidence: 0.9594086

00:01:21.725 --> 00:01:23.325 He served as the past

NOTE Confidence: 0.9594086

00:01:23.325 --> 00:01:24.945 chair of the Cancer Molecular

NOTE Confidence: 0.9594086

00:01:25.085 --> 00:01:25.585 Pathobiology

NOTE Confidence: 0.91712785

00:01:25.965 --> 00:01:27.920 Camp study section and has

NOTE Confidence: 0.91712785

00:01:27.920 --> 00:01:30.160 received numerous innovation awards, including

NOTE Confidence: 0.91712785

00:01:30.160 --> 00:01:30.900 an NCI

NOTE Confidence: 0.9592992

00:01:31.280 --> 00:01:33.200 outstanding investigator r thirty five

NOTE Confidence: 0.9592992

00:01:33.200 --> 00:01:34.400 award and one of the

NOTE Confidence: 0.9592992

00:01:34.400 --> 00:01:36.340 top twenty translational researchers

NOTE Confidence: 0.99820405

00:01:36.720 --> 00:01:37.700 of twenty nineteen.

NOTE Confidence: 0.98688114

00:01:38.480 --> 00:01:39.600 This shows in him being

NOTE Confidence: 0.98688114

00:01:39.600 --> 00:01:41.360 the scientific cofounder of five

NOTE Confidence: 0.98688114

00:01:41.360 --> 00:01:42.604 companies with with three lead

NOTE Confidence: 0.98688114

00:01:42.604 --> 00:01:44.384 compounds in clinical testing.

NOTE Confidence: 0.96055233

00:01:45.005 --> 00:01:46.924 However, arguably, the most meaningful

NOTE Confidence: 0.96055233

00:01:46.924 --> 00:01:48.445 awards, at least to me

NOTE Confidence: 0.96055233

00:01:48.445 --> 00:01:49.985 as a former lab member,

NOTE Confidence: 0.96055233

00:01:50.045 --> 00:01:51.244 is of Lauren being a

NOTE Confidence: 0.96055233

00:01:51.244 --> 00:01:53.244 superb mentor shown in his

NOTE Confidence: 0.96055233

00:01:53.244 --> 00:01:54.685 winning the Harvard Medical School

NOTE Confidence: 0.96055233

00:01:54.685 --> 00:01:56.045 Young Mentor Award in two

NOTE Confidence: 0.96055233

00:01:56.045 --> 00:01:57.024 thousand and seven,

NOTE Confidence: 0.98544633

00:01:57.380 --> 00:01:59.300 both the HST and BBS

NOTE Confidence: 0.98544633

00:01:59.300 --> 00:02:01.000 mentoring awards in twenty seventeen,

NOTE Confidence: 0.9893384

00:02:01.620 --> 00:02:03.300 and the MD PhD mentoring

NOTE Confidence: 0.9893384

00:02:03.300 --> 00:02:04.920 award in twenty twenty four.

NOTE Confidence: 0.9957421

00:02:05.780 --> 00:02:07.220 Lauren's ability to create a

NOTE Confidence: 0.9957421

00:02:07.220 --> 00:02:08.900 community and a family that

NOTE Confidence: 0.9957421

00:02:08.900 --> 00:02:09.880 is all inclusive

NOTE Confidence: 0.9982617

00:02:10.180 --> 00:02:11.240 is unsurpassed.

NOTE Confidence: 0.9962035

00:02:12.044 --> 00:02:13.245 As a postdoc in his

NOTE Confidence: 0.9962035

00:02:13.245 --> 00:02:14.944 laboratory with a young family,

NOTE Confidence: 0.9962035

00:02:15.165 --> 00:02:16.364 it was inspiring for me

NOTE Confidence: 0.9962035

00:02:16.364 --> 00:02:17.724 to see him excel with

NOTE Confidence: 0.9962035

00:02:17.724 --> 00:02:18.785 his three children,

NOTE Confidence: 0.9953251

00:02:19.245 --> 00:02:20.444 a wife as busy as

NOTE Confidence: 0.9953251

00:02:20.444 --> 00:02:20.944 himself,

NOTE Confidence: 0.99667966

00:02:21.485 --> 00:02:23.264 very sick clinical patients,

NOTE Confidence: 0.9946129

00:02:23.805 --> 00:02:25.905 multiple lab members with vastly

NOTE Confidence: 0.9946129

00:02:25.965 --> 00:02:26.785 different needs,

NOTE Confidence: 0.9798294

00:02:27.260 --> 00:02:28.459 and all the while advancing

NOTE Confidence: 0.9798294

00:02:28.459 --> 00:02:30.139 the most meticulous and beautiful

NOTE Confidence: 0.9798294

00:02:30.139 --> 00:02:31.359 scientific discoveries.

NOTE Confidence: 0.92819786

00:02:32.299 --> 00:02:33.579 I'm just truly grateful to

NOTE Confidence: 0.92819786

00:02:33.579 --> 00:02:34.780 be introducing my friend and

NOTE Confidence: 0.92819786

00:02:34.780 --> 00:02:36.560 mentor, professor Laura Glenske.

NOTE Confidence: 0.99641865

00:02:43.205 --> 00:02:44.725 Alright. Thanks so much. Thanks

NOTE Confidence: 0.99641865

00:02:44.725 --> 00:02:45.385 for coming.

NOTE Confidence: 0.9334847

00:02:46.325 --> 00:02:46.825 It's

NOTE Confidence: 0.97102726

00:02:47.205 --> 00:02:48.485 hard to top that, but

NOTE Confidence: 0.97102726

00:02:48.485 --> 00:02:49.044 I I,

NOTE Confidence: 0.9149888

00:02:49.764 --> 00:02:50.665 it's such

NOTE Confidence: 0.9570453

00:02:51.205 --> 00:02:52.245 so much fun to be

NOTE Confidence: 0.9570453

00:02:52.245 --> 00:02:52.745 here.

NOTE Confidence: 0.9985017

00:02:53.445 --> 00:02:55.250 I've had such great chats

NOTE Confidence: 0.9985017

00:02:55.250 --> 00:02:56.370 with Sam over the last

NOTE Confidence: 0.9985017

00:02:56.370 --> 00:02:57.269 twenty four hours.

NOTE Confidence: 0.9460636

00:02:58.290 --> 00:02:59.329 Those of you who have

NOTE Confidence: 0.9460636

00:02:59.329 --> 00:03:00.790 labs and have mentees,

NOTE Confidence: 0.93517524

00:03:02.930 --> 00:03:03.810 you should all be so

NOTE Confidence: 0.93517524

00:03:03.810 --> 00:03:04.310 lucky

NOTE Confidence: 0.90568244

00:03:04.689 --> 00:03:06.709 to have your first first

NOTE Confidence: 0.9711506

00:03:07.025 --> 00:03:08.705 group of postdocs have someone

NOTE Confidence: 0.9711506

00:03:08.705 --> 00:03:10.005 like Sam in the cohort.

NOTE Confidence: 0.9891018

00:03:10.865 --> 00:03:11.445 It's just

NOTE Confidence: 0.97279024

00:03:12.065 --> 00:03:13.185 the trust that folks have

NOTE Confidence: 0.97279024

00:03:13.185 --> 00:03:14.065 in you at the beginning

NOTE Confidence: 0.97279024

00:03:14.065 --> 00:03:14.645 of their,

NOTE Confidence: 0.93422383

00:03:15.105 --> 00:03:16.305 of your careers is like

NOTE Confidence: 0.93422383

00:03:16.305 --> 00:03:17.365 a very special

NOTE Confidence: 0.93563604

00:03:17.745 --> 00:03:19.585 thing. And so those always

NOTE Confidence: 0.93563604

00:03:19.585 --> 00:03:20.945 remain among the most special

NOTE Confidence: 0.93563604

00:03:20.945 --> 00:03:21.985 folks in your life because

NOTE Confidence: 0.93563604

00:03:21.985 --> 00:03:23.389 they kind of trusted you

NOTE Confidence: 0.93563604

00:03:23.389 --> 00:03:24.590 when you probably had no

NOTE Confidence: 0.93563604

00:03:24.590 --> 00:03:26.110 right to be trusted in

NOTE Confidence: 0.93563604

00:03:26.110 --> 00:03:27.889 terms of mentoring their careers.

NOTE Confidence: 0.990969

00:03:29.630 --> 00:03:30.770 So thanks for that.

NOTE Confidence: 0.9744835

00:03:32.910 --> 00:03:33.870 So what I was gonna

NOTE Confidence: 0.9744835

00:03:33.870 --> 00:03:35.310 do today is kind of

NOTE Confidence: 0.9744835

00:03:35.310 --> 00:03:36.190 a little bit of a

NOTE Confidence: 0.9744835

00:03:36.190 --> 00:03:37.710 nostalgia for me. I decided,

NOTE Confidence: 0.9744835

00:03:37.710 --> 00:03:38.864 you know, it's like twenty

NOTE Confidence: 0.9744835

00:03:38.864 --> 00:03:40.064 years since I started working

NOTE Confidence: 0.9744835

00:03:40.064 --> 00:03:40.965 on this protein,

NOTE Confidence: 0.990047

00:03:41.584 --> 00:03:42.944 and I thought it would

NOTE Confidence: 0.990047

00:03:42.944 --> 00:03:43.985 be a good opportunity to

NOTE Confidence: 0.990047

00:03:43.985 --> 00:03:45.665 look back to kind of

NOTE Confidence: 0.990047

00:03:45.665 --> 00:03:46.704 talk about all the things

NOTE Confidence: 0.990047

00:03:46.704 --> 00:03:48.405 that we've learned. And and

NOTE Confidence: 0.990047

00:03:48.465 --> 00:03:49.424 one of the beauties of

NOTE Confidence: 0.990047

00:03:49.424 --> 00:03:50.625 academic medicine is that you

NOTE Confidence: 0.990047

00:03:50.625 --> 00:03:51.584 could pick something that you

NOTE Confidence: 0.990047

00:03:51.584 --> 00:03:52.840 care about and really stay

NOTE Confidence: 0.990047

00:03:52.840 --> 00:03:54.120 focused on for many, many

NOTE Confidence: 0.990047

00:03:54.120 --> 00:03:55.880 years. In a world where

NOTE Confidence: 0.990047

00:03:55.880 --> 00:03:57.640 attention spans are always way

NOTE Confidence: 0.990047

00:03:57.640 --> 00:03:58.920 too short, we have the

NOTE Confidence: 0.990047

00:03:58.920 --> 00:03:59.880 luxury of being able to

NOTE Confidence: 0.990047

00:03:59.880 --> 00:04:01.160 focus on things that we

NOTE Confidence: 0.990047

00:04:01.160 --> 00:04:02.440 believe are very, very important

NOTE Confidence: 0.990047

00:04:02.440 --> 00:04:03.640 and stick to them because

NOTE Confidence: 0.990047

00:04:03.640 --> 00:04:05.100 we have that conviction.

NOTE Confidence: 0.96863496

00:04:06.185 --> 00:04:07.465 And I would say during

NOTE Confidence: 0.96863496

00:04:07.465 --> 00:04:09.065 these times, that is more

NOTE Confidence: 0.96863496

00:04:09.065 --> 00:04:10.125 important than ever.

NOTE Confidence: 0.9662817

00:04:10.585 --> 00:04:11.625 Stick to the things that

NOTE Confidence: 0.9662817

00:04:11.625 --> 00:04:12.905 you care about. Focus on

NOTE Confidence: 0.9662817

00:04:12.905 --> 00:04:14.445 them. Don't be distracted,

NOTE Confidence: 0.995565

00:04:14.825 --> 00:04:16.265 and and and really kind

NOTE Confidence: 0.995565

00:04:16.265 --> 00:04:17.785 of embrace and double down

NOTE Confidence: 0.995565

00:04:17.785 --> 00:04:19.305 on your scientific and medical

NOTE Confidence: 0.995565

00:04:19.305 --> 00:04:19.805 missions.

NOTE Confidence: 0.9920317

00:04:21.449 --> 00:04:22.669 So to start off,

NOTE Confidence: 0.9649553

00:04:24.250 --> 00:04:25.610 I got interested in this

NOTE Confidence: 0.9649553

00:04:25.610 --> 00:04:26.110 field,

NOTE Confidence: 0.98234814

00:04:27.449 --> 00:04:28.330 because when I was looking

NOTE Confidence: 0.98234814

00:04:28.330 --> 00:04:29.789 for a postdoctoral fellowship,

NOTE Confidence: 0.92313164

00:04:30.089 --> 00:04:30.910 Stan Korsmeyer

NOTE Confidence: 0.9785014

00:04:31.770 --> 00:04:33.050 basically started out with a

NOTE Confidence: 0.9785014

00:04:33.050 --> 00:04:34.669 verbal version of this slide.

NOTE Confidence: 0.9785014

00:04:34.889 --> 00:04:35.930 And he used to say,

NOTE Confidence: 0.9785014

00:04:35.930 --> 00:04:37.505 like, apoptosis is the best

NOTE Confidence: 0.9785014

00:04:37.505 --> 00:04:38.785 thing you could possibly study.

NOTE Confidence: 0.9785014

00:04:38.785 --> 00:04:40.005 Why? Because, like,

NOTE Confidence: 0.99371076

00:04:40.544 --> 00:04:41.044 every

NOTE Confidence: 0.98385984

00:04:41.985 --> 00:04:43.745 disease, and and overall health

NOTE Confidence: 0.98385984

00:04:43.745 --> 00:04:45.185 depends on a balance between,

NOTE Confidence: 0.98385984

00:04:45.185 --> 00:04:46.225 you know, new and dying

NOTE Confidence: 0.98385984

00:04:46.225 --> 00:04:48.165 cells. And he kind of

NOTE Confidence: 0.98385984

00:04:48.464 --> 00:04:49.824 convinced us that, like, okay.

NOTE Confidence: 0.98385984

00:04:49.824 --> 00:04:50.885 You could figure out

NOTE Confidence: 0.9604781

00:04:51.770 --> 00:04:52.810 this balance, and if you

NOTE Confidence: 0.9604781

00:04:52.810 --> 00:04:53.850 can modulate it in one

NOTE Confidence: 0.9604781

00:04:53.850 --> 00:04:54.810 direction or the other, you

NOTE Confidence: 0.9604781

00:04:54.810 --> 00:04:56.170 could tackle all the diseases

NOTE Confidence: 0.9604781

00:04:56.170 --> 00:04:57.130 where there's too much cell

NOTE Confidence: 0.9604781

00:04:57.130 --> 00:04:57.630 survival,

NOTE Confidence: 0.9707902

00:04:57.930 --> 00:04:59.870 like cancer and autoimmunity, inflammation.

NOTE Confidence: 0.9707902

00:05:00.170 --> 00:05:01.050 And then on the flip

NOTE Confidence: 0.9707902

00:05:01.050 --> 00:05:02.250 side, you know, you could

NOTE Confidence: 0.9707902

00:05:02.250 --> 00:05:03.610 tackle all the diseases where

NOTE Confidence: 0.9707902

00:05:03.610 --> 00:05:05.025 there's premature cell loss, like

NOTE Confidence: 0.9707902

00:05:05.185 --> 00:05:07.264 neurodegeneration and infertility and stroke,

NOTE Confidence: 0.9707902

00:05:07.264 --> 00:05:08.705 heart attack. And that was,

NOTE Confidence: 0.9707902

00:05:08.705 --> 00:05:09.205 like,

NOTE Confidence: 0.9813725

00:05:09.585 --> 00:05:11.104 very exciting concept that if

NOTE Confidence: 0.9813725

00:05:11.104 --> 00:05:12.705 you could understand this balance,

NOTE Confidence: 0.9813725

00:05:12.705 --> 00:05:14.384 you could potentially impact a

NOTE Confidence: 0.9813725

00:05:14.384 --> 00:05:15.745 whole host of diseases. So

NOTE Confidence: 0.9813725

00:05:15.745 --> 00:05:16.645 I was sold,

NOTE Confidence: 0.97616214

00:05:17.970 --> 00:05:19.089 and I started as a

NOTE Confidence: 0.97616214

00:05:19.089 --> 00:05:19.589 postdoc

NOTE Confidence: 0.9993797

00:05:19.890 --> 00:05:21.010 in an area where I

NOTE Confidence: 0.9993797

00:05:21.010 --> 00:05:21.910 knew absolutely

NOTE Confidence: 0.9991501

00:05:22.529 --> 00:05:23.029 nothing.

NOTE Confidence: 0.94578755

00:05:23.410 --> 00:05:24.610 I had never studied these

NOTE Confidence: 0.94578755

00:05:24.610 --> 00:05:26.130 proteins. I knew nothing about

NOTE Confidence: 0.94578755

00:05:26.130 --> 00:05:27.190 b c l two,

NOTE Confidence: 0.9804099

00:05:27.890 --> 00:05:29.190 and and in a way,

NOTE Confidence: 0.98507404

00:05:29.824 --> 00:05:30.885 I always try to encourage

NOTE Confidence: 0.98507404

00:05:30.945 --> 00:05:32.145 all the newbies in my

NOTE Confidence: 0.98507404

00:05:32.145 --> 00:05:33.824 lab that that having no

NOTE Confidence: 0.98507404

00:05:33.824 --> 00:05:35.104 background in an area of

NOTE Confidence: 0.98507404

00:05:35.104 --> 00:05:36.625 science is actually your biggest

NOTE Confidence: 0.98507404

00:05:36.625 --> 00:05:37.125 asset.

NOTE Confidence: 0.98122275

00:05:37.505 --> 00:05:38.705 Because, you know, I have

NOTE Confidence: 0.98122275

00:05:38.705 --> 00:05:40.145 to try to convince and

NOTE Confidence: 0.98122275

00:05:40.145 --> 00:05:41.845 remind myself over and over,

NOTE Confidence: 0.98122275

00:05:41.904 --> 00:05:43.270 like, the older you get

NOTE Confidence: 0.98122275

00:05:43.270 --> 00:05:44.150 in a field, the more

NOTE Confidence: 0.98122275

00:05:44.150 --> 00:05:45.270 you think you know things

NOTE Confidence: 0.98122275

00:05:45.270 --> 00:05:46.790 about it. And and that's

NOTE Confidence: 0.98122275

00:05:46.790 --> 00:05:48.710 actually a liability because then

NOTE Confidence: 0.98122275

00:05:48.710 --> 00:05:49.910 you start to turn off

NOTE Confidence: 0.98122275

00:05:49.910 --> 00:05:50.790 to things that may be

NOTE Confidence: 0.98122275

00:05:50.790 --> 00:05:52.150 completely new and unexpected that

NOTE Confidence: 0.98122275

00:05:52.150 --> 00:05:53.690 you hadn't appreciated before.

NOTE Confidence: 0.95462793

00:05:54.514 --> 00:05:56.035 So what I learned, was

NOTE Confidence: 0.95462793

00:05:56.035 --> 00:05:57.235 that there were two classes

NOTE Confidence: 0.95462793

00:05:57.235 --> 00:05:58.835 of survival proteins and death

NOTE Confidence: 0.95462793

00:05:58.835 --> 00:06:00.355 proteins, and the survival proteins

NOTE Confidence: 0.95462793

00:06:00.355 --> 00:06:01.794 in orange were like BCL

NOTE Confidence: 0.95462793

00:06:01.794 --> 00:06:02.294 two

NOTE Confidence: 0.91697437

00:06:02.595 --> 00:06:03.794 and, you know, its brothers

NOTE Confidence: 0.91697437

00:06:03.794 --> 00:06:05.794 and sisters, BCLXL and MCL

NOTE Confidence: 0.91697437

00:06:05.794 --> 00:06:07.014 one, which we'll talk about.

NOTE Confidence: 0.95472926

00:06:07.395 --> 00:06:08.355 And then there were death

NOTE Confidence: 0.95472926

00:06:08.355 --> 00:06:09.640 proteins, And at that time,

NOTE Confidence: 0.95472926

00:06:09.640 --> 00:06:10.680 it was BACs and BAC,

NOTE Confidence: 0.95472926

00:06:10.680 --> 00:06:11.880 and that one inhibited the

NOTE Confidence: 0.95472926

00:06:11.880 --> 00:06:12.380 other.

NOTE Confidence: 0.9762719

00:06:13.000 --> 00:06:14.200 And that most cells do

NOTE Confidence: 0.9762719

00:06:14.200 --> 00:06:15.720 fine because for the most

NOTE Confidence: 0.9762719

00:06:15.720 --> 00:06:17.000 part, the the system is

NOTE Confidence: 0.9762719

00:06:17.000 --> 00:06:18.120 rigged to keep your cells

NOTE Confidence: 0.9762719

00:06:18.120 --> 00:06:19.740 alive, keep tissues healthy.

NOTE Confidence: 0.9990561

00:06:20.120 --> 00:06:21.240 And then there's this third

NOTE Confidence: 0.9990561

00:06:21.240 --> 00:06:22.860 class of proteins that

NOTE Confidence: 0.994356

00:06:24.034 --> 00:06:25.714 are very diverse and that

NOTE Confidence: 0.994356

00:06:25.714 --> 00:06:27.094 have very heterogeneous

NOTE Confidence: 0.97821504

00:06:27.555 --> 00:06:29.074 structures and functions, but their

NOTE Confidence: 0.97821504

00:06:29.074 --> 00:06:30.275 job is like the antenna

NOTE Confidence: 0.97821504

00:06:30.275 --> 00:06:30.775 proteins

NOTE Confidence: 0.9954547

00:06:31.235 --> 00:06:32.675 that are situated all over

NOTE Confidence: 0.9954547

00:06:32.675 --> 00:06:34.594 the cell to sense different

NOTE Confidence: 0.9954547

00:06:34.594 --> 00:06:35.714 types of stress and then

NOTE Confidence: 0.9954547

00:06:35.714 --> 00:06:37.154 deliver that message to the

NOTE Confidence: 0.9954547

00:06:37.154 --> 00:06:38.630 kind of the big players,

NOTE Confidence: 0.9930669

00:06:39.089 --> 00:06:40.389 you know, at the mitochondria.

NOTE Confidence: 0.9793972

00:06:40.690 --> 00:06:41.490 And so these are the

NOTE Confidence: 0.9793972

00:06:41.490 --> 00:06:42.610 b h three only proteins,

NOTE Confidence: 0.9793972

00:06:42.610 --> 00:06:43.330 and I'll show you why

NOTE Confidence: 0.9793972

00:06:43.330 --> 00:06:44.130 they got their name in

NOTE Confidence: 0.9793972

00:06:44.130 --> 00:06:44.710 a minute.

NOTE Confidence: 0.99461806

00:06:45.330 --> 00:06:46.449 But the idea is is

NOTE Confidence: 0.99461806

00:06:46.449 --> 00:06:47.669 that when they get triggered,

NOTE Confidence: 0.9916988

00:06:48.370 --> 00:06:49.410 one of their ways to

NOTE Confidence: 0.9916988

00:06:49.410 --> 00:06:50.690 turn on death is to

NOTE Confidence: 0.9916988

00:06:50.690 --> 00:06:51.750 inhibit the inhibitor.

NOTE Confidence: 0.9816148

00:06:53.445 --> 00:06:54.404 And then the other way,

NOTE Confidence: 0.9816148

00:06:54.404 --> 00:06:55.845 which was much more controversial,

NOTE Confidence: 0.9816148

00:06:55.845 --> 00:06:57.125 was this idea that maybe

NOTE Confidence: 0.9816148

00:06:57.125 --> 00:06:58.805 they could also directly activate

NOTE Confidence: 0.9816148

00:06:58.805 --> 00:06:59.545 the activator.

NOTE Confidence: 0.9846944

00:07:00.085 --> 00:07:01.205 Okay. And most of the

NOTE Confidence: 0.9846944

00:07:01.205 --> 00:07:02.725 drug development, you know, in

NOTE Confidence: 0.9846944

00:07:02.725 --> 00:07:03.925 this area for the last

NOTE Confidence: 0.9846944

00:07:03.925 --> 00:07:05.525 twenty plus years has been

NOTE Confidence: 0.9846944

00:07:05.525 --> 00:07:07.305 the inhibit the inhibitor pathway.

NOTE Confidence: 0.89199686

00:07:08.710 --> 00:07:09.690 So the family

NOTE Confidence: 0.9598151

00:07:10.310 --> 00:07:11.909 grew and grew and grew

NOTE Confidence: 0.9598151

00:07:11.909 --> 00:07:13.430 based upon this homology map,

NOTE Confidence: 0.9598151

00:07:13.430 --> 00:07:15.430 and they're divided into multi

NOTE Confidence: 0.9598151

00:07:15.430 --> 00:07:17.030 domain proteins and then single

NOTE Confidence: 0.9598151

00:07:17.030 --> 00:07:18.389 domain proteins. So the multi

NOTE Confidence: 0.9598151

00:07:18.389 --> 00:07:20.229 domain homology is derived from

NOTE Confidence: 0.9598151

00:07:20.229 --> 00:07:21.985 what's called these BH or

NOTE Confidence: 0.9598151

00:07:21.985 --> 00:07:23.745 BCL two homology domains, one,

NOTE Confidence: 0.9598151

00:07:23.745 --> 00:07:25.185 two, three, and four. There's

NOTE Confidence: 0.9598151

00:07:25.185 --> 00:07:27.025 multidomain death proteins like BACs

NOTE Confidence: 0.9598151

00:07:27.025 --> 00:07:29.285 and BAC. But these eclectic

NOTE Confidence: 0.9598151

00:07:29.585 --> 00:07:31.505 antenna proteins are called BH

NOTE Confidence: 0.9598151

00:07:31.505 --> 00:07:33.505 three only because they only

NOTE Confidence: 0.9598151

00:07:33.505 --> 00:07:35.345 share the commonality of this

NOTE Confidence: 0.9598151

00:07:35.345 --> 00:07:36.785 one domain, and that domain,

NOTE Confidence: 0.9598151

00:07:36.785 --> 00:07:37.630 as I'll talk a lot

NOTE Confidence: 0.9598151

00:07:37.630 --> 00:07:39.230 about today, is a single

NOTE Confidence: 0.9598151

00:07:39.230 --> 00:07:40.050 alpha helix.

NOTE Confidence: 0.9912485

00:07:41.070 --> 00:07:42.190 And the idea was was

NOTE Confidence: 0.9912485

00:07:42.190 --> 00:07:43.550 that that alpha helix is

NOTE Confidence: 0.9912485

00:07:43.550 --> 00:07:45.010 the vehicle for communication

NOTE Confidence: 0.9964015

00:07:45.630 --> 00:07:46.830 between b h three only

NOTE Confidence: 0.9964015

00:07:46.830 --> 00:07:48.930 proteins and the downstream players.

NOTE Confidence: 0.9842645

00:07:49.865 --> 00:07:50.905 So to kind of put

NOTE Confidence: 0.9842645

00:07:50.905 --> 00:07:51.965 all this in motion,

NOTE Confidence: 0.9671252

00:07:52.745 --> 00:07:54.604 BAX lives in the cytoplasm

NOTE Confidence: 0.9671252

00:07:54.745 --> 00:07:55.865 for the most part as

NOTE Confidence: 0.9671252

00:07:55.865 --> 00:07:57.705 a latent inactive protein. And

NOTE Confidence: 0.9671252

00:07:57.705 --> 00:07:58.505 the way I kind of

NOTE Confidence: 0.9671252

00:07:58.505 --> 00:07:59.465 describe it is it's kind

NOTE Confidence: 0.9671252

00:07:59.465 --> 00:08:00.604 of like a hand grenade,

NOTE Confidence: 0.9700221

00:08:01.145 --> 00:08:02.104 and the pin is, like,

NOTE Confidence: 0.9700221

00:08:02.104 --> 00:08:03.759 firmly in place. And it's

NOTE Confidence: 0.9700221

00:08:03.759 --> 00:08:04.879 just kinda sitting there. And

NOTE Confidence: 0.9700221

00:08:04.879 --> 00:08:05.919 it's sitting there in cancer

NOTE Confidence: 0.9700221

00:08:05.919 --> 00:08:06.419 too,

NOTE Confidence: 0.9855052

00:08:07.120 --> 00:08:08.159 which is kind of the

NOTE Confidence: 0.9855052

00:08:08.159 --> 00:08:09.919 basis for my excitement about

NOTE Confidence: 0.9855052

00:08:09.919 --> 00:08:11.300 it. And then in response

NOTE Confidence: 0.9855052

00:08:11.360 --> 00:08:13.060 to stress, it undergoes this

NOTE Confidence: 0.9855052

00:08:13.120 --> 00:08:15.520 very extensive conformational change. It

NOTE Confidence: 0.9855052

00:08:15.520 --> 00:08:16.500 goes to the mitochondria.

NOTE Confidence: 0.97711104

00:08:16.879 --> 00:08:18.319 It self associates, and then

NOTE Confidence: 0.97711104

00:08:18.319 --> 00:08:19.995 it disrupts the outer mitochondrial

NOTE Confidence: 0.97711104

00:08:19.995 --> 00:08:21.914 membrane, and then second messenger

NOTE Confidence: 0.97711104

00:08:21.914 --> 00:08:23.195 signals come out and, you

NOTE Confidence: 0.97711104

00:08:23.195 --> 00:08:24.574 know, give you the irreversible

NOTE Confidence: 0.99499434

00:08:25.115 --> 00:08:26.175 cell death pathway.

NOTE Confidence: 0.9969911

00:08:26.555 --> 00:08:27.435 And so this is a

NOTE Confidence: 0.9969911

00:08:27.435 --> 00:08:29.195 very dynamic process. Right? You

NOTE Confidence: 0.9969911

00:08:29.195 --> 00:08:30.475 take something that starts in

NOTE Confidence: 0.9969911

00:08:30.475 --> 00:08:31.134 one compartment,

NOTE Confidence: 0.9842537

00:08:31.435 --> 00:08:33.490 completely changes its shape, shuttles

NOTE Confidence: 0.9842537

00:08:33.490 --> 00:08:35.250 to another compartment, then changes

NOTE Confidence: 0.9842537

00:08:35.250 --> 00:08:37.250 its behavior again, self associate.

NOTE Confidence: 0.9842537

00:08:37.250 --> 00:08:38.130 So there's a lot going

NOTE Confidence: 0.9842537

00:08:38.130 --> 00:08:39.650 on here. And and this

NOTE Confidence: 0.9842537

00:08:39.650 --> 00:08:41.010 slide is kind of, like,

NOTE Confidence: 0.9842537

00:08:41.010 --> 00:08:42.150 pretty much a systematic

NOTE Confidence: 0.9790804

00:08:43.090 --> 00:08:44.130 road map for what I've

NOTE Confidence: 0.9790804

00:08:44.130 --> 00:08:45.750 been dissecting for twenty years.

NOTE Confidence: 0.9758033

00:08:47.145 --> 00:08:47.885 And so

NOTE Confidence: 0.99395037

00:08:48.745 --> 00:08:50.445 when this system is blocked

NOTE Confidence: 0.9923275

00:08:50.905 --> 00:08:52.365 by cancer, for example,

NOTE Confidence: 0.9912783

00:08:52.985 --> 00:08:54.185 it's based upon this idea

NOTE Confidence: 0.9912783

00:08:54.185 --> 00:08:55.545 that a survival protein can

NOTE Confidence: 0.9912783

00:08:55.545 --> 00:08:56.985 go and latch on to

NOTE Confidence: 0.9912783

00:08:56.985 --> 00:08:58.745 that single alpha helix there

NOTE Confidence: 0.9912783

00:08:58.745 --> 00:08:59.785 and kind of arrest the

NOTE Confidence: 0.9912783

00:08:59.785 --> 00:09:01.145 whole process. And that single

NOTE Confidence: 0.9912783

00:09:01.145 --> 00:09:02.730 alpha helix that pops out,

NOTE Confidence: 0.9912783

00:09:02.809 --> 00:09:03.690 that's one of the b

NOTE Confidence: 0.9912783

00:09:03.690 --> 00:09:05.290 h three helices that I'll

NOTE Confidence: 0.9912783

00:09:05.290 --> 00:09:06.429 be talking a lot about.

NOTE Confidence: 0.9840003

00:09:06.889 --> 00:09:08.170 And so if you were

NOTE Confidence: 0.9840003

00:09:08.170 --> 00:09:09.769 thinking about drug making, you

NOTE Confidence: 0.9840003

00:09:09.769 --> 00:09:11.070 would kind of wanna simulate,

NOTE Confidence: 0.99947125

00:09:11.449 --> 00:09:12.589 you know, this

NOTE Confidence: 0.9768218

00:09:12.970 --> 00:09:14.730 to disarm this inhibition in

NOTE Confidence: 0.9768218

00:09:14.730 --> 00:09:16.089 cancer. And you could imagine

NOTE Confidence: 0.9768218

00:09:16.089 --> 00:09:17.769 that, like, the mitochondria has

NOTE Confidence: 0.9768218

00:09:17.769 --> 00:09:20.155 these orange survival proteins literally

NOTE Confidence: 0.9768218

00:09:20.155 --> 00:09:21.595 sitting on it and as,

NOTE Confidence: 0.9768218

00:09:21.595 --> 00:09:22.955 like, a force field, like,

NOTE Confidence: 0.9768218

00:09:22.955 --> 00:09:24.395 to prevent the incomings, right,

NOTE Confidence: 0.9768218

00:09:24.395 --> 00:09:25.915 and protect the mitochondria. And

NOTE Confidence: 0.9768218

00:09:25.915 --> 00:09:26.955 that's a huge part of

NOTE Confidence: 0.9768218

00:09:26.955 --> 00:09:28.395 cancer. Right? Because cancer need

NOTE Confidence: 0.9768218

00:09:28.554 --> 00:09:30.090 you look at leukemia cells

NOTE Confidence: 0.9768218

00:09:30.250 --> 00:09:31.530 and do a mitochondrial stain,

NOTE Confidence: 0.9768218

00:09:31.530 --> 00:09:32.650 it is the scary thing

NOTE Confidence: 0.9768218

00:09:32.650 --> 00:09:33.610 to look at. Right? These

NOTE Confidence: 0.9768218

00:09:33.610 --> 00:09:35.390 cells, these little, like, lymphocytes

NOTE Confidence: 0.9768218

00:09:35.450 --> 00:09:36.890 that are now lymphoblasts are

NOTE Confidence: 0.9768218

00:09:36.890 --> 00:09:38.010 so small. They have all

NOTE Confidence: 0.9768218

00:09:38.010 --> 00:09:39.370 DNA, and then that thin

NOTE Confidence: 0.9768218

00:09:39.370 --> 00:09:41.230 rim of cytoplasm is choked

NOTE Confidence: 0.9768218

00:09:41.370 --> 00:09:43.370 with mitochondria after mitochondria. And

NOTE Confidence: 0.9768218

00:09:43.370 --> 00:09:44.990 they they need that protected

NOTE Confidence: 0.9768218

00:09:45.285 --> 00:09:46.165 so that they can keep

NOTE Confidence: 0.9768218

00:09:46.165 --> 00:09:47.225 cranking out energy.

NOTE Confidence: 0.98868144

00:09:47.684 --> 00:09:49.285 And so these antenna proteins,

NOTE Confidence: 0.98868144

00:09:49.285 --> 00:09:50.745 again, can inhibit the inhibitor,

NOTE Confidence: 0.99604785

00:09:51.444 --> 00:09:53.204 or they could potentially activate

NOTE Confidence: 0.99604785

00:09:53.204 --> 00:09:53.865 the activator.

NOTE Confidence: 0.98776037

00:09:54.644 --> 00:09:56.004 And so this field really

NOTE Confidence: 0.98776037

00:09:56.004 --> 00:09:56.745 kicked in,

NOTE Confidence: 0.982471

00:09:57.444 --> 00:09:58.504 once the structure

NOTE Confidence: 0.9448437

00:09:58.804 --> 00:10:00.324 of the first survival protein

NOTE Confidence: 0.9448437

00:10:00.324 --> 00:10:00.809 was

NOTE Confidence: 0.85638326

00:10:02.490 --> 00:10:04.410 collaboration between Craig Thompson and

NOTE Confidence: 0.85638326

00:10:04.410 --> 00:10:05.929 Steve Fesic. And and Steve

NOTE Confidence: 0.85638326

00:10:05.929 --> 00:10:07.210 Fesic was at Abbott Labs

NOTE Confidence: 0.85638326

00:10:07.210 --> 00:10:08.029 at the time.

NOTE Confidence: 0.9019821

00:10:08.890 --> 00:10:10.890 And so here's the structure.

NOTE Confidence: 0.9019821

00:10:10.890 --> 00:10:12.170 And what was discovered in

NOTE Confidence: 0.9019821

00:10:12.170 --> 00:10:13.850 the subsequent paper was that

NOTE Confidence: 0.9019821

00:10:13.850 --> 00:10:15.550 there's a surface groove

NOTE Confidence: 0.9894654

00:10:16.025 --> 00:10:17.465 shown in green, and that

NOTE Confidence: 0.9894654

00:10:17.465 --> 00:10:18.825 is the acceptor for these

NOTE Confidence: 0.9894654

00:10:18.825 --> 00:10:20.025 b h three helices. And

NOTE Confidence: 0.9894654

00:10:20.025 --> 00:10:20.825 this is kind of the

NOTE Confidence: 0.9894654

00:10:20.825 --> 00:10:22.184 basis for the protein protein

NOTE Confidence: 0.9894654

00:10:22.184 --> 00:10:23.545 interaction. This b h three

NOTE Confidence: 0.9894654

00:10:23.545 --> 00:10:25.325 helix fits into the groove,

NOTE Confidence: 0.9894654

00:10:25.625 --> 00:10:26.985 and that's the interaction. That's

NOTE Confidence: 0.9894654

00:10:26.985 --> 00:10:28.425 the wrestling match between life

NOTE Confidence: 0.9894654

00:10:28.425 --> 00:10:29.070 and death.

NOTE Confidence: 0.98349553

00:10:29.630 --> 00:10:30.750 And that kind of gave

NOTE Confidence: 0.98349553

00:10:30.750 --> 00:10:31.710 the road map for drug

NOTE Confidence: 0.98349553

00:10:31.710 --> 00:10:33.309 development in this field starting

NOTE Confidence: 0.98349553

00:10:33.309 --> 00:10:34.370 in the late nineties.

NOTE Confidence: 0.9960032

00:10:34.990 --> 00:10:36.590 And so Abbott went on

NOTE Confidence: 0.9960032

00:10:36.590 --> 00:10:37.090 to

NOTE Confidence: 0.96817815

00:10:37.470 --> 00:10:38.610 basically simulate

NOTE Confidence: 0.945733

00:10:39.070 --> 00:10:40.750 a helix that bound to

NOTE Confidence: 0.945733

00:10:40.750 --> 00:10:41.710 b c l two, b

NOTE Confidence: 0.945733

00:10:41.710 --> 00:10:43.070 c l x l, and,

NOTE Confidence: 0.945733

00:10:43.070 --> 00:10:43.809 you know,

NOTE Confidence: 0.9827829

00:10:44.165 --> 00:10:45.285 turned it into a drug

NOTE Confidence: 0.9827829

00:10:45.285 --> 00:10:46.725 eventually. It took probably, like,

NOTE Confidence: 0.9827829

00:10:46.725 --> 00:10:47.925 fifteen to twenty years to

NOTE Confidence: 0.9827829

00:10:47.925 --> 00:10:48.585 get there.

NOTE Confidence: 0.96876603

00:10:49.045 --> 00:10:50.405 And then now we have

NOTE Confidence: 0.96876603

00:10:50.405 --> 00:10:52.025 the selected BCL two inhibitor,

NOTE Confidence: 0.96876603

00:10:52.085 --> 00:10:53.605 venetoclax. And how we went

NOTE Confidence: 0.96876603

00:10:53.605 --> 00:10:55.045 from and how Abbott went

NOTE Confidence: 0.96876603

00:10:55.045 --> 00:10:56.245 from, you know, left to

NOTE Confidence: 0.96876603

00:10:56.245 --> 00:10:58.000 right there was just an

NOTE Confidence: 0.96876603

00:10:58.000 --> 00:10:59.360 amazing story that we won't

NOTE Confidence: 0.96876603

00:10:59.360 --> 00:11:00.579 talk about today, but just

NOTE Confidence: 0.96876603

00:11:00.800 --> 00:11:02.579 amazing fortitude, unbelievable

NOTE Confidence: 0.8991907

00:11:02.880 --> 00:11:03.380 persistence,

NOTE Confidence: 0.97503275

00:11:03.920 --> 00:11:06.079 people that were incredibly able

NOTE Confidence: 0.97503275

00:11:06.079 --> 00:11:07.600 to convince folks to keep

NOTE Confidence: 0.97503275

00:11:07.600 --> 00:11:08.720 on keeping on even when

NOTE Confidence: 0.97503275

00:11:08.720 --> 00:11:09.855 something seemed like it was

NOTE Confidence: 0.97503275

00:11:09.855 --> 00:11:11.375 never going to work, and

NOTE Confidence: 0.97503275

00:11:11.375 --> 00:11:12.255 even what happened in the

NOTE Confidence: 0.97503275

00:11:12.255 --> 00:11:13.934 early clinical trials and have

NOTE Confidence: 0.97503275

00:11:13.934 --> 00:11:15.054 that made folks have to

NOTE Confidence: 0.97503275

00:11:15.054 --> 00:11:15.934 go right back to the

NOTE Confidence: 0.97503275

00:11:15.934 --> 00:11:17.795 drawing board and start anew.

NOTE Confidence: 0.9703637

00:11:18.175 --> 00:11:19.774 It's a miracle that this

NOTE Confidence: 0.9703637

00:11:19.774 --> 00:11:20.895 drug actually made it to

NOTE Confidence: 0.9703637

00:11:20.895 --> 00:11:21.630 prime time.

NOTE Confidence: 0.9549466

00:11:22.110 --> 00:11:23.150 And now when you see

NOTE Confidence: 0.9549466

00:11:23.150 --> 00:11:24.510 as a clinician the amount

NOTE Confidence: 0.9549466

00:11:24.510 --> 00:11:25.550 of good that it's doing,

NOTE Confidence: 0.9549466

00:11:25.550 --> 00:11:27.309 it's really a remarkable story

NOTE Confidence: 0.9549466

00:11:27.309 --> 00:11:28.190 that I hope someone will

NOTE Confidence: 0.9549466

00:11:28.190 --> 00:11:29.410 write a book about someday.

NOTE Confidence: 0.94807017

00:11:30.910 --> 00:11:32.030 Now the challenge is is

NOTE Confidence: 0.94807017

00:11:32.030 --> 00:11:33.630 that there's other anti apoptotic

NOTE Confidence: 0.94807017

00:11:33.630 --> 00:11:34.910 proteins that this drug does

NOTE Confidence: 0.94807017

00:11:34.910 --> 00:11:35.985 not bind to because, again,

NOTE Confidence: 0.94807017

00:11:36.065 --> 00:11:36.945 it was engineered to be

NOTE Confidence: 0.94807017

00:11:36.945 --> 00:11:38.485 a selective BCL two inhibitor

NOTE Confidence: 0.94807017

00:11:38.545 --> 00:11:39.125 to avoid

NOTE Confidence: 0.9807667

00:11:39.505 --> 00:11:41.265 toxicities and avoid being too

NOTE Confidence: 0.9807667

00:11:41.265 --> 00:11:42.545 kind of panactive. And so

NOTE Confidence: 0.9807667

00:11:42.545 --> 00:11:44.885 MCL one and BFL one,

NOTE Confidence: 0.9818683

00:11:45.505 --> 00:11:47.285 are very commonly overexpressed,

NOTE Confidence: 0.99477774

00:11:47.825 --> 00:11:49.345 you know, to cause resistance

NOTE Confidence: 0.99477774

00:11:49.345 --> 00:11:50.005 to venetoclax.

NOTE Confidence: 0.96354955

00:11:50.790 --> 00:11:52.550 And then, in recent years,

NOTE Confidence: 0.96354955

00:11:52.550 --> 00:11:53.750 as more and more patients

NOTE Confidence: 0.96354955

00:11:53.750 --> 00:11:54.790 get the drug, it's become

NOTE Confidence: 0.96354955

00:11:54.790 --> 00:11:55.990 clear that b c l

NOTE Confidence: 0.96354955

00:11:55.990 --> 00:11:57.050 two very cleverly,

NOTE Confidence: 0.89549494

00:11:57.670 --> 00:11:58.149 can,

NOTE Confidence: 0.9843015

00:11:58.630 --> 00:12:00.149 basically mutate. So it doesn't

NOTE Confidence: 0.9843015

00:12:00.149 --> 00:12:01.029 bind the drug, but it

NOTE Confidence: 0.9843015

00:12:01.029 --> 00:12:01.990 still binds the b h

NOTE Confidence: 0.9843015

00:12:01.990 --> 00:12:03.345 three helix, which is quite

NOTE Confidence: 0.9843015

00:12:03.584 --> 00:12:04.245 a magnificent,

NOTE Confidence: 0.9625188

00:12:05.184 --> 00:12:06.944 structural biology trick that this

NOTE Confidence: 0.9625188

00:12:06.944 --> 00:12:08.625 protein figured out. Right? Because

NOTE Confidence: 0.9625188

00:12:08.625 --> 00:12:10.384 theoretically, the molecule on the

NOTE Confidence: 0.9625188

00:12:10.384 --> 00:12:11.665 the natural effect, they're all

NOTE Confidence: 0.9625188

00:12:11.665 --> 00:12:12.964 going at the same pocket,

NOTE Confidence: 0.9625188

00:12:13.024 --> 00:12:14.545 but this protein figured out

NOTE Confidence: 0.9625188

00:12:14.545 --> 00:12:15.665 how to mutate to not

NOTE Confidence: 0.9625188

00:12:15.665 --> 00:12:17.320 bind the drug but still

NOTE Confidence: 0.9625188

00:12:17.320 --> 00:12:18.679 bind the the natural target.

NOTE Confidence: 0.9625188

00:12:18.679 --> 00:12:19.420 It's amazing.

NOTE Confidence: 0.99612224

00:12:20.519 --> 00:12:21.820 And so I kind of

NOTE Confidence: 0.99612224

00:12:21.959 --> 00:12:23.500 consider this whole area

NOTE Confidence: 0.9813659

00:12:24.120 --> 00:12:25.800 of cancer biology and drug

NOTE Confidence: 0.9813659

00:12:25.800 --> 00:12:27.320 development on the anti apoptotic

NOTE Confidence: 0.9813659

00:12:27.320 --> 00:12:28.200 side as the whack a

NOTE Confidence: 0.9813659

00:12:28.200 --> 00:12:30.515 mole situation where drug companies

NOTE Confidence: 0.9813659

00:12:30.515 --> 00:12:32.195 really want selective inhibitors so

NOTE Confidence: 0.9813659

00:12:32.195 --> 00:12:32.915 that they don't have a

NOTE Confidence: 0.9813659

00:12:32.915 --> 00:12:33.735 lot of toxicities.

NOTE Confidence: 0.98054814

00:12:34.515 --> 00:12:35.475 But when you have selective

NOTE Confidence: 0.98054814

00:12:35.475 --> 00:12:36.595 inhibitors and then you have

NOTE Confidence: 0.98054814

00:12:36.595 --> 00:12:37.635 all of these brothers and

NOTE Confidence: 0.98054814

00:12:37.635 --> 00:12:39.415 sisters that have homologous functions,

NOTE Confidence: 0.98054814

00:12:39.475 --> 00:12:40.595 they're, of course, gonna pop

NOTE Confidence: 0.98054814

00:12:40.595 --> 00:12:41.475 up and take over, and

NOTE Confidence: 0.98054814

00:12:41.475 --> 00:12:43.190 that's exactly what's happened. And

NOTE Confidence: 0.98054814

00:12:43.190 --> 00:12:44.470 so, you know, the industry

NOTE Confidence: 0.98054814

00:12:44.470 --> 00:12:45.830 and, you know, academia and

NOTE Confidence: 0.98054814

00:12:45.830 --> 00:12:47.110 pharma alike have been going

NOTE Confidence: 0.98054814

00:12:47.110 --> 00:12:48.710 after, you know, different MCL

NOTE Confidence: 0.98054814

00:12:48.710 --> 00:12:49.830 one and BFL one and

NOTE Confidence: 0.98054814

00:12:49.830 --> 00:12:50.710 and to try to target

NOTE Confidence: 0.98054814

00:12:50.710 --> 00:12:51.589 them. And as some of

NOTE Confidence: 0.98054814

00:12:51.589 --> 00:12:52.390 you, I'm sure, have heard

NOTE Confidence: 0.98054814

00:12:52.390 --> 00:12:53.830 that these MCL one inhibitors

NOTE Confidence: 0.98054814

00:12:53.830 --> 00:12:56.309 have had toxicities, mostly cardiac

NOTE Confidence: 0.98054814

00:12:56.309 --> 00:12:57.345 in the clinic, and that's

NOTE Confidence: 0.98054814

00:12:57.584 --> 00:12:58.944 there's a biological reason for

NOTE Confidence: 0.98054814

00:12:58.944 --> 00:13:00.225 that because MCL one is

NOTE Confidence: 0.98054814

00:13:00.225 --> 00:13:01.444 very important in,

NOTE Confidence: 0.98707986

00:13:01.745 --> 00:13:03.285 what we've discovered in regulating

NOTE Confidence: 0.98707986

00:13:03.345 --> 00:13:04.964 fatty acid oxidation. And,

NOTE Confidence: 0.94159275

00:13:05.904 --> 00:13:06.785 I'm not gonna say more

NOTE Confidence: 0.94159275

00:13:06.785 --> 00:13:07.605 about antipoptopics

NOTE Confidence: 0.9692337

00:13:07.985 --> 00:13:09.345 side because I decided today

NOTE Confidence: 0.9692337

00:13:09.345 --> 00:13:10.429 to talk about BACs, but

NOTE Confidence: 0.9692337

00:13:10.429 --> 00:13:12.050 there's just still some amazing

NOTE Confidence: 0.9692337

00:13:12.110 --> 00:13:12.610 biology,

NOTE Confidence: 0.9752649

00:13:13.230 --> 00:13:14.589 being discovered on the anti

NOTE Confidence: 0.9752649

00:13:14.589 --> 00:13:16.029 apoptotic side about what these

NOTE Confidence: 0.9752649

00:13:16.029 --> 00:13:17.890 proteins do outside of doing

NOTE Confidence: 0.9752649

00:13:18.190 --> 00:13:19.230 latching on to b h

NOTE Confidence: 0.9752649

00:13:19.230 --> 00:13:20.589 three helices and shutting down

NOTE Confidence: 0.9752649

00:13:20.589 --> 00:13:21.089 apoptosis.

NOTE Confidence: 0.99562097

00:13:23.525 --> 00:13:24.585 But the other question

NOTE Confidence: 0.98007256

00:13:25.125 --> 00:13:26.825 aside from inhibiting the inhibitors

NOTE Confidence: 0.98007256

00:13:26.885 --> 00:13:28.085 was this idea that, you

NOTE Confidence: 0.98007256

00:13:28.085 --> 00:13:29.605 know, Stan Korsmeyer had and

NOTE Confidence: 0.98007256

00:13:29.605 --> 00:13:30.725 wrote about it in the

NOTE Confidence: 0.98007256

00:13:30.725 --> 00:13:32.745 earliest papers about discovering

NOTE Confidence: 0.94118506

00:13:33.125 --> 00:13:35.205 bid interacting with backs. What

NOTE Confidence: 0.94118506

00:13:35.205 --> 00:13:36.565 does that mean? You know,

NOTE Confidence: 0.94118506

00:13:36.565 --> 00:13:37.845 what could the activation look

NOTE Confidence: 0.94118506

00:13:37.845 --> 00:13:39.740 like? And the idea was

NOTE Confidence: 0.94118506

00:13:39.960 --> 00:13:42.120 that this interaction, instead of

NOTE Confidence: 0.94118506

00:13:42.120 --> 00:13:44.040 being a static interaction between

NOTE Confidence: 0.94118506

00:13:44.040 --> 00:13:45.000 b h three and groove

NOTE Confidence: 0.94118506

00:13:45.000 --> 00:13:45.880 that, you you know, sits

NOTE Confidence: 0.94118506

00:13:45.880 --> 00:13:46.940 there, you could immunoprecipitate

NOTE Confidence: 0.9778762

00:13:47.400 --> 00:13:48.440 it, you could solve a

NOTE Confidence: 0.9778762

00:13:48.440 --> 00:13:49.720 crystal structure of it, that

NOTE Confidence: 0.9778762

00:13:49.720 --> 00:13:51.225 this was somehow different, that

NOTE Confidence: 0.9778762

00:13:51.225 --> 00:13:52.425 this was gonna be a

NOTE Confidence: 0.9778762

00:13:52.425 --> 00:13:54.024 triggering interaction. It was gonna

NOTE Confidence: 0.9778762

00:13:54.024 --> 00:13:54.684 be dynamic,

NOTE Confidence: 0.9596257

00:13:55.065 --> 00:13:56.265 and they called it in

NOTE Confidence: 0.9596257

00:13:56.265 --> 00:13:57.625 the earliest paper a hit

NOTE Confidence: 0.9596257

00:13:57.625 --> 00:13:58.204 and run,

NOTE Confidence: 0.9996164

00:13:58.904 --> 00:13:59.404 mechanism.

NOTE Confidence: 0.99966264

00:14:00.505 --> 00:14:01.005 Now

NOTE Confidence: 0.9949962

00:14:01.464 --> 00:14:02.925 a lot of this idea

NOTE Confidence: 0.9995918

00:14:03.385 --> 00:14:04.365 was debunked

NOTE Confidence: 0.9519499

00:14:04.825 --> 00:14:05.325 because

NOTE Confidence: 0.9967362

00:14:06.530 --> 00:14:07.429 you couldn't immunoprecipitate

NOTE Confidence: 0.96915716

00:14:08.290 --> 00:14:09.650 b h three proteins with

NOTE Confidence: 0.96915716

00:14:09.650 --> 00:14:10.870 BAX. You couldn't

NOTE Confidence: 0.9979403

00:14:11.330 --> 00:14:13.090 solve the structure or even

NOTE Confidence: 0.9979403

00:14:13.090 --> 00:14:14.230 measure an interaction

NOTE Confidence: 0.9943657

00:14:14.770 --> 00:14:16.210 between a b h three

NOTE Confidence: 0.9943657

00:14:16.210 --> 00:14:18.390 helix and BAX. And so

NOTE Confidence: 0.9943657

00:14:18.610 --> 00:14:19.650 when I kind of started

NOTE Confidence: 0.9943657

00:14:19.650 --> 00:14:20.415 in at this,

NOTE Confidence: 0.92720985

00:14:20.894 --> 00:14:22.334 and was working on bid

NOTE Confidence: 0.92720985

00:14:22.334 --> 00:14:23.615 BAX, you know, b h

NOTE Confidence: 0.92720985

00:14:23.615 --> 00:14:24.595 three BAX interactions,

NOTE Confidence: 0.9785797

00:14:25.134 --> 00:14:26.975 you know, imagine writing your

NOTE Confidence: 0.9785797

00:14:26.975 --> 00:14:28.435 first r o one grant,

NOTE Confidence: 0.9785797

00:14:28.735 --> 00:14:30.735 okay, about how b h

NOTE Confidence: 0.9785797

00:14:30.735 --> 00:14:31.855 threes might be able to

NOTE Confidence: 0.9785797

00:14:31.855 --> 00:14:33.714 directly bind and activate BAX.

NOTE Confidence: 0.9785797

00:14:33.774 --> 00:14:34.894 But then this paper comes

NOTE Confidence: 0.9785797

00:14:34.894 --> 00:14:36.270 out in science and says

NOTE Confidence: 0.94876635

00:14:36.750 --> 00:14:38.510 aptosis is activated, you know,

NOTE Confidence: 0.94876635

00:14:38.510 --> 00:14:39.890 when you inhibit the inhibitors,

NOTE Confidence: 0.955369

00:14:40.270 --> 00:14:41.630 and that's it, comma, not

NOTE Confidence: 0.955369

00:14:41.630 --> 00:14:42.610 BAX or BAX.

NOTE Confidence: 0.9676998

00:14:43.310 --> 00:14:44.270 So that you know, that's

NOTE Confidence: 0.9676998

00:14:44.270 --> 00:14:45.790 pretty tough, you know, when

NOTE Confidence: 0.9676998

00:14:45.790 --> 00:14:47.070 your grant is, when you're

NOTE Confidence: 0.9676998

00:14:47.070 --> 00:14:48.110 asking someone to give you

NOTE Confidence: 0.9676998

00:14:48.110 --> 00:14:49.070 a million dollars, you know,

NOTE Confidence: 0.9676998

00:14:49.070 --> 00:14:50.610 to study direct BAX activation,

NOTE Confidence: 0.9676998

00:14:50.775 --> 00:14:52.596 and you have a science

NOTE Confidence: 0.9676998

00:14:52.596 --> 00:14:54.418 paper that says no. Great

NOTE Confidence: 0.9676998

00:14:54.418 --> 00:14:56.240 great hypothesis, but that doesn't

NOTE Confidence: 0.9676998

00:14:56.240 --> 00:14:58.426 occur. Now at that time,

NOTE Confidence: 0.9676998

00:14:58.426 --> 00:15:00.612 I had already known, that,

NOTE Confidence: 0.9676998

00:15:00.612 --> 00:15:02.433 like, they showed that these

NOTE Confidence: 0.9676998

00:15:02.433 --> 00:15:04.255 peptides did not bind to

NOTE Confidence: 0.9676998

00:15:04.255 --> 00:15:05.500 VAX very well. You couldn't

NOTE Confidence: 0.9676998

00:15:05.500 --> 00:15:06.460 really detect it. So we

NOTE Confidence: 0.9676998

00:15:06.460 --> 00:15:07.420 have the same results, and

NOTE Confidence: 0.9676998

00:15:07.420 --> 00:15:08.620 that's the flat red line

NOTE Confidence: 0.9676998

00:15:08.620 --> 00:15:09.900 here. You take a BID

NOTE Confidence: 0.9676998

00:15:09.900 --> 00:15:11.020 b h three peptide, a

NOTE Confidence: 0.9676998

00:15:11.020 --> 00:15:12.060 b h three only peptide.

NOTE Confidence: 0.9676998

00:15:12.060 --> 00:15:12.720 It's unstructured.

NOTE Confidence: 0.9604176

00:15:13.020 --> 00:15:13.820 You put it on to

NOTE Confidence: 0.9604176

00:15:13.820 --> 00:15:15.180 BAX, and you don't see

NOTE Confidence: 0.9604176

00:15:15.180 --> 00:15:16.220 binding. But if you do

NOTE Confidence: 0.9604176

00:15:16.220 --> 00:15:17.440 this to an anti apoptotic

NOTE Confidence: 0.9604176

00:15:17.500 --> 00:15:19.020 protein, you get beautiful binding.

NOTE Confidence: 0.9604176

00:15:19.020 --> 00:15:20.535 No problem. Right? So there's

NOTE Confidence: 0.9604176

00:15:20.535 --> 00:15:22.075 your yes and no, right,

NOTE Confidence: 0.9604176

00:15:22.135 --> 00:15:23.675 that underlies that paper.

NOTE Confidence: 0.9996114

00:15:24.935 --> 00:15:26.875 But I had been working

NOTE Confidence: 0.9996114

00:15:27.015 --> 00:15:27.515 on

NOTE Confidence: 0.95866257

00:15:28.535 --> 00:15:30.075 this problem for my postdoc,

NOTE Confidence: 0.95866257

00:15:30.215 --> 00:15:31.035 which was,

NOTE Confidence: 0.99928206

00:15:31.495 --> 00:15:32.535 you know, wouldn't it be

NOTE Confidence: 0.99928206

00:15:32.535 --> 00:15:33.035 great

NOTE Confidence: 0.9995402

00:15:33.950 --> 00:15:34.690 if bioactive

NOTE Confidence: 0.97171104

00:15:36.030 --> 00:15:37.630 motifs and proteins like alpha

NOTE Confidence: 0.97171104

00:15:37.630 --> 00:15:39.790 helices could actually be drugs?

NOTE Confidence: 0.97171104

00:15:39.790 --> 00:15:41.470 Because most drugs are modeled

NOTE Confidence: 0.97171104

00:15:41.470 --> 00:15:43.390 after bioactive motifs, and then

NOTE Confidence: 0.97171104

00:15:43.390 --> 00:15:44.670 you spend two billion dollars

NOTE Confidence: 0.97171104

00:15:44.670 --> 00:15:45.310 to come up with a

NOTE Confidence: 0.97171104

00:15:45.310 --> 00:15:46.965 small molecule that mimics that.

NOTE Confidence: 0.97171104

00:15:46.965 --> 00:15:47.685 But what if you could

NOTE Confidence: 0.97171104

00:15:47.685 --> 00:15:48.805 bypass that whole thing and

NOTE Confidence: 0.97171104

00:15:48.805 --> 00:15:49.925 then just take the peptide

NOTE Confidence: 0.97171104

00:15:49.925 --> 00:15:51.465 that nature already gave you

NOTE Confidence: 0.97171104

00:15:51.765 --> 00:15:52.885 and, you know, solve the

NOTE Confidence: 0.97171104

00:15:52.885 --> 00:15:54.565 problems of peptide therapeutics, which

NOTE Confidence: 0.97171104

00:15:54.565 --> 00:15:56.025 they're unstable, they get proteolyzed,

NOTE Confidence: 0.97171104

00:15:56.325 --> 00:15:57.285 but, you know, get rid

NOTE Confidence: 0.97171104

00:15:57.285 --> 00:15:58.725 of the unfolding problem, and

NOTE Confidence: 0.97171104

00:15:58.725 --> 00:15:59.685 then maybe you can make

NOTE Confidence: 0.97171104

00:15:59.685 --> 00:16:01.045 drugs much faster. That was

NOTE Confidence: 0.97171104

00:16:01.045 --> 00:16:02.560 the idea. And so when

NOTE Confidence: 0.97171104

00:16:02.560 --> 00:16:04.000 you look at, you know,

NOTE Confidence: 0.97171104

00:16:04.000 --> 00:16:05.440 helical motifs like a b

NOTE Confidence: 0.97171104

00:16:05.440 --> 00:16:06.560 h three in the context

NOTE Confidence: 0.97171104

00:16:06.560 --> 00:16:07.839 of a protein, yeah, you

NOTE Confidence: 0.97171104

00:16:07.839 --> 00:16:09.120 synthesize it, you know, in

NOTE Confidence: 0.97171104

00:16:09.120 --> 00:16:10.480 your lab and you do

NOTE Confidence: 0.97171104

00:16:10.480 --> 00:16:12.000 analyses and this is just

NOTE Confidence: 0.97171104

00:16:12.000 --> 00:16:12.660 a simulation.

NOTE Confidence: 0.9936688

00:16:13.040 --> 00:16:14.240 But, like, that thing's never

NOTE Confidence: 0.9936688

00:16:14.240 --> 00:16:15.120 going back to an alpha

NOTE Confidence: 0.9936688

00:16:15.120 --> 00:16:16.399 helix, okay, when you make

NOTE Confidence: 0.9936688

00:16:16.399 --> 00:16:16.899 it.

NOTE Confidence: 0.9571687

00:16:17.825 --> 00:16:19.445 And so the idea was,

NOTE Confidence: 0.9992004

00:16:19.905 --> 00:16:20.965 what if you installed

NOTE Confidence: 0.9218191

00:16:21.345 --> 00:16:22.705 non natural amino acids that

NOTE Confidence: 0.9218191

00:16:22.705 --> 00:16:23.585 you could kind of form

NOTE Confidence: 0.9218191

00:16:23.585 --> 00:16:24.805 a cross link with

NOTE Confidence: 0.97681224

00:16:25.265 --> 00:16:26.885 and try to reestablish

NOTE Confidence: 0.99714404

00:16:27.665 --> 00:16:28.165 structure

NOTE Confidence: 0.99129647

00:16:28.625 --> 00:16:30.145 to this peptide sequence that

NOTE Confidence: 0.99129647

00:16:30.145 --> 00:16:31.425 actually is meant to be

NOTE Confidence: 0.99129647

00:16:31.425 --> 00:16:32.310 an alpha helix?

NOTE Confidence: 0.9769681

00:16:32.870 --> 00:16:34.390 And so I I grew

NOTE Confidence: 0.9769681

00:16:34.390 --> 00:16:35.830 up as a, you know,

NOTE Confidence: 0.9769681

00:16:35.830 --> 00:16:37.190 undergrad that got bitten by

NOTE Confidence: 0.9769681

00:16:37.190 --> 00:16:38.550 the organic chemistry bug. So

NOTE Confidence: 0.9769681

00:16:38.550 --> 00:16:39.670 I started out with synthetic

NOTE Confidence: 0.9769681

00:16:39.670 --> 00:16:41.670 organic chemistry and always wanted

NOTE Confidence: 0.9769681

00:16:41.670 --> 00:16:42.310 to go back to it

NOTE Confidence: 0.9769681

00:16:42.310 --> 00:16:43.430 during my post doc even

NOTE Confidence: 0.9769681

00:16:43.430 --> 00:16:44.390 though my PhD was in

NOTE Confidence: 0.9769681

00:16:44.390 --> 00:16:45.750 molecular biology and and did

NOTE Confidence: 0.9769681

00:16:45.750 --> 00:16:46.870 a lot of biochemistry and

NOTE Confidence: 0.9769681

00:16:46.870 --> 00:16:47.445 animal work.

NOTE Confidence: 0.94232166

00:16:48.085 --> 00:16:49.925 And so I actually knew

NOTE Confidence: 0.94232166

00:16:49.925 --> 00:16:50.885 how to make these things.

NOTE Confidence: 0.94232166

00:16:50.885 --> 00:16:52.085 Like, these non natural amino

NOTE Confidence: 0.94232166

00:16:52.085 --> 00:16:53.445 acids were ten step chiral

NOTE Confidence: 0.94232166

00:16:53.445 --> 00:16:53.945 synthesis.

NOTE Confidence: 0.9827916

00:16:54.725 --> 00:16:56.085 Total nightmare, like, three and

NOTE Confidence: 0.9827916

00:16:56.085 --> 00:16:56.805 a half months of your

NOTE Confidence: 0.9827916

00:16:56.805 --> 00:16:57.765 life to be able to

NOTE Confidence: 0.9827916

00:16:57.765 --> 00:16:59.125 generate these non natural amino

NOTE Confidence: 0.9827916

00:16:59.125 --> 00:17:00.485 acids just to install them

NOTE Confidence: 0.9827916

00:17:00.485 --> 00:17:01.889 by hand, you know, into

NOTE Confidence: 0.9827916

00:17:01.889 --> 00:17:03.490 manual peptide synthesis back in

NOTE Confidence: 0.9827916

00:17:03.490 --> 00:17:04.070 the day.

NOTE Confidence: 0.9620887

00:17:04.690 --> 00:17:06.049 You know, while your beeper

NOTE Confidence: 0.9620887

00:17:06.049 --> 00:17:06.929 is going off and your

NOTE Confidence: 0.9620887

00:17:06.929 --> 00:17:08.049 patients are paging you as

NOTE Confidence: 0.9620887

00:17:08.049 --> 00:17:09.010 a first year fellow, and

NOTE Confidence: 0.9620887

00:17:09.010 --> 00:17:09.730 then you go back to

NOTE Confidence: 0.9620887

00:17:09.730 --> 00:17:10.609 the hood, you're like, now

NOTE Confidence: 0.9620887

00:17:10.609 --> 00:17:11.490 what step was I on

NOTE Confidence: 0.9620887

00:17:11.490 --> 00:17:13.250 of this manual peptide synthesis?

NOTE Confidence: 0.9620887

00:17:13.250 --> 00:17:14.130 And then you didn't remember,

NOTE Confidence: 0.9620887

00:17:14.130 --> 00:17:14.850 so you had to throw

NOTE Confidence: 0.9620887

00:17:14.850 --> 00:17:15.809 it all out and start

NOTE Confidence: 0.9620887

00:17:15.809 --> 00:17:16.309 over.

NOTE Confidence: 0.9814086

00:17:17.965 --> 00:17:19.165 But it looked like this

NOTE Confidence: 0.9814086

00:17:19.165 --> 00:17:20.925 could do the job. And

NOTE Confidence: 0.9814086

00:17:20.925 --> 00:17:21.965 so, you know, these are

NOTE Confidence: 0.9814086

00:17:21.965 --> 00:17:22.845 some of the first data

NOTE Confidence: 0.9814086

00:17:22.845 --> 00:17:23.565 that I got as a

NOTE Confidence: 0.9814086

00:17:23.565 --> 00:17:25.005 postdoc. So here's a b

NOTE Confidence: 0.9814086

00:17:25.005 --> 00:17:26.705 h three peptide of BID.

NOTE Confidence: 0.98607755

00:17:27.484 --> 00:17:29.165 Circular dichroism gives you the

NOTE Confidence: 0.98607755

00:17:29.165 --> 00:17:30.045 sense of the shape and

NOTE Confidence: 0.98607755

00:17:30.045 --> 00:17:31.930 solution. This contour is a

NOTE Confidence: 0.98607755

00:17:31.930 --> 00:17:32.750 piece of spaghetti.

NOTE Confidence: 0.9038974

00:17:33.130 --> 00:17:33.850 And then you put the

NOTE Confidence: 0.9038974

00:17:33.850 --> 00:17:34.810 staple in and all of

NOTE Confidence: 0.9038974

00:17:34.810 --> 00:17:35.609 a sudden you could see

NOTE Confidence: 0.9038974

00:17:35.609 --> 00:17:37.150 the shape change, double hump

NOTE Confidence: 0.9387324

00:17:37.450 --> 00:17:38.970 pattern consistent with a with

NOTE Confidence: 0.9387324

00:17:38.970 --> 00:17:40.109 a pure alpha helix.

NOTE Confidence: 0.96257925

00:17:40.410 --> 00:17:41.450 You take these two things

NOTE Confidence: 0.96257925

00:17:41.450 --> 00:17:42.650 and you throw trypsin at

NOTE Confidence: 0.96257925

00:17:42.650 --> 00:17:44.030 them from, you know, sigma,

NOTE Confidence: 0.96257925

00:17:44.090 --> 00:17:44.810 like, right out of the

NOTE Confidence: 0.96257925

00:17:44.810 --> 00:17:46.730 vial, and the unstapled one

NOTE Confidence: 0.96257925

00:17:46.730 --> 00:17:48.665 is just destroyed. And the

NOTE Confidence: 0.96257925

00:17:48.665 --> 00:17:50.345 stable one magically seems to

NOTE Confidence: 0.96257925

00:17:50.345 --> 00:17:51.785 withstand this, and that's because

NOTE Confidence: 0.96257925

00:17:51.785 --> 00:17:53.165 amide bonds that are twisted

NOTE Confidence: 0.96257925

00:17:53.305 --> 00:17:54.585 are not great substrates for

NOTE Confidence: 0.96257925

00:17:54.585 --> 00:17:55.085 proteases.

NOTE Confidence: 0.99600166

00:17:55.545 --> 00:17:56.505 And this ends up being

NOTE Confidence: 0.99600166

00:17:56.505 --> 00:17:57.865 a really important aspect for

NOTE Confidence: 0.99600166

00:17:57.865 --> 00:17:59.165 the drug translation.

NOTE Confidence: 0.99663264

00:18:00.025 --> 00:18:01.225 And then the surprise was

NOTE Confidence: 0.99663264

00:18:01.225 --> 00:18:02.425 that some of them actually

NOTE Confidence: 0.99663264

00:18:02.425 --> 00:18:03.210 got into cells.

NOTE Confidence: 0.9463705

00:18:03.929 --> 00:18:04.809 Right? And so on the

NOTE Confidence: 0.9463705

00:18:04.809 --> 00:18:05.950 left, I have a fluorescently

NOTE Confidence: 0.9463705

00:18:06.010 --> 00:18:07.609 labeled unstable peptide, and then

NOTE Confidence: 0.9463705

00:18:07.609 --> 00:18:08.590 on the right, a fluorescently

NOTE Confidence: 0.9463705

00:18:08.649 --> 00:18:09.869 labeled staple peptide.

NOTE Confidence: 0.93580043

00:18:10.649 --> 00:18:11.850 And all of a sudden,

NOTE Confidence: 0.93580043

00:18:11.850 --> 00:18:12.730 you know, these jerk out

NOTE Confidence: 0.93580043

00:18:12.730 --> 00:18:14.490 leukemia cells had fluorescent peptide

NOTE Confidence: 0.93580043

00:18:14.490 --> 00:18:16.029 in their little scan cytoplasm,

NOTE Confidence: 0.9756962

00:18:16.330 --> 00:18:17.950 and that was pretty surprising.

NOTE Confidence: 0.9742233

00:18:18.715 --> 00:18:19.674 But that really opened the

NOTE Confidence: 0.9742233

00:18:19.674 --> 00:18:20.475 door to be able to

NOTE Confidence: 0.9742233

00:18:20.475 --> 00:18:21.994 do cell biology studies and

NOTE Confidence: 0.9742233

00:18:21.994 --> 00:18:23.195 to use them as potential

NOTE Confidence: 0.9742233

00:18:23.195 --> 00:18:24.634 therapeutics and treat tumors in

NOTE Confidence: 0.9742233

00:18:24.634 --> 00:18:25.754 mice, and it really kinda

NOTE Confidence: 0.9742233

00:18:25.754 --> 00:18:27.054 opened the door. And so

NOTE Confidence: 0.98825294

00:18:27.595 --> 00:18:28.975 I had been doing this

NOTE Confidence: 0.96838367

00:18:29.835 --> 00:18:31.034 and trying to figure out

NOTE Confidence: 0.96838367

00:18:31.034 --> 00:18:31.994 if there were any b

NOTE Confidence: 0.96838367

00:18:31.994 --> 00:18:33.034 h threes that bound to

NOTE Confidence: 0.96838367

00:18:33.034 --> 00:18:34.290 bacs, but I had

NOTE Confidence: 0.9666955

00:18:35.250 --> 00:18:36.850 stapled b h threes that

NOTE Confidence: 0.9666955

00:18:36.850 --> 00:18:38.210 were actually folded in the

NOTE Confidence: 0.9666955

00:18:38.210 --> 00:18:39.350 way that they were naturally.

NOTE Confidence: 0.9666955

00:18:39.570 --> 00:18:40.530 And when I did these

NOTE Confidence: 0.9666955

00:18:40.530 --> 00:18:42.770 same fluorescence polarization assays, in

NOTE Confidence: 0.9666955

00:18:42.770 --> 00:18:44.609 my hands, a subset of

NOTE Confidence: 0.9666955

00:18:44.609 --> 00:18:46.290 the stapled versions bound to

NOTE Confidence: 0.9666955

00:18:46.290 --> 00:18:47.250 backs just as if it

NOTE Confidence: 0.9666955

00:18:47.250 --> 00:18:48.950 was an anti apoptotic protein.

NOTE Confidence: 0.9826894

00:18:49.414 --> 00:18:50.794 So I got very excited.

NOTE Confidence: 0.99439067

00:18:51.255 --> 00:18:51.755 And

NOTE Confidence: 0.97628695

00:18:53.414 --> 00:18:54.455 so, at that point, the

NOTE Confidence: 0.97628695

00:18:54.455 --> 00:18:55.414 question was, well, now that

NOTE Confidence: 0.97628695

00:18:55.414 --> 00:18:56.534 you have a binding interaction

NOTE Confidence: 0.97628695

00:18:56.534 --> 00:18:57.734 and you can measure it,

NOTE Confidence: 0.97628695

00:18:57.734 --> 00:18:58.855 that was the door that

NOTE Confidence: 0.97628695

00:18:58.855 --> 00:19:00.135 was open to doing structural

NOTE Confidence: 0.97628695

00:19:00.135 --> 00:19:01.195 biology research.

NOTE Confidence: 0.9474043

00:19:01.575 --> 00:19:03.015 And so I, at that

NOTE Confidence: 0.9474043

00:19:03.015 --> 00:19:03.515 point,

NOTE Confidence: 0.9985402

00:19:04.320 --> 00:19:05.679 was given advice by my

NOTE Confidence: 0.9985402

00:19:05.679 --> 00:19:06.179 mentor

NOTE Confidence: 0.99900055

00:19:06.480 --> 00:19:07.220 to find

NOTE Confidence: 0.98311585

00:19:07.600 --> 00:19:08.820 the world's expert,

NOTE Confidence: 0.9650395

00:19:09.760 --> 00:19:10.260 in,

NOTE Confidence: 0.91505724

00:19:11.039 --> 00:19:12.480 back structural biology. And that

NOTE Confidence: 0.91505724

00:19:12.480 --> 00:19:14.080 was actually easy because the

NOTE Confidence: 0.91505724

00:19:14.080 --> 00:19:15.280 person that had done the

NOTE Confidence: 0.91505724

00:19:15.280 --> 00:19:16.905 first NMR structure of backs

NOTE Confidence: 0.91505724

00:19:16.984 --> 00:19:18.265 and published it in Cell

NOTE Confidence: 0.91505724

00:19:18.265 --> 00:19:19.965 was Niko Chandra, NHLBI.

NOTE Confidence: 0.94679475

00:19:20.984 --> 00:19:21.945 And I told this story

NOTE Confidence: 0.94679475

00:19:21.945 --> 00:19:23.085 at dinner last night,

NOTE Confidence: 0.9987872

00:19:24.025 --> 00:19:25.465 that when I called Niko

NOTE Confidence: 0.9987872

00:19:25.465 --> 00:19:26.285 the first time,

NOTE Confidence: 0.9779754

00:19:27.545 --> 00:19:28.665 you know, he disclosed to

NOTE Confidence: 0.9779754

00:19:28.665 --> 00:19:29.545 me you know, as a

NOTE Confidence: 0.9779754

00:19:29.545 --> 00:19:31.005 young person entering the field,

NOTE Confidence: 0.9779754

00:19:31.050 --> 00:19:32.090 I viewed him as, like,

NOTE Confidence: 0.9779754

00:19:32.090 --> 00:19:32.850 one of the kings of

NOTE Confidence: 0.9779754

00:19:32.850 --> 00:19:33.690 the field. He solved the

NOTE Confidence: 0.9779754

00:19:33.690 --> 00:19:34.750 structure of BACS.

NOTE Confidence: 0.9285963

00:19:35.210 --> 00:19:36.410 And I remember him saying,

NOTE Confidence: 0.9285963

00:19:36.410 --> 00:19:37.130 well, you know, I actually

NOTE Confidence: 0.9285963

00:19:37.130 --> 00:19:38.330 saw the structure of BACS

NOTE Confidence: 0.9285963

00:19:38.330 --> 00:19:39.690 to honor my mentor, Ad

NOTE Confidence: 0.9285963

00:19:39.690 --> 00:19:40.190 BACS.

NOTE Confidence: 0.9364477

00:19:41.130 --> 00:19:41.850 And that was why I

NOTE Confidence: 0.9364477

00:19:41.850 --> 00:19:43.290 did that. And so I

NOTE Confidence: 0.9364477

00:19:43.290 --> 00:19:44.570 was like, oh gosh. And

NOTE Confidence: 0.9364477

00:19:44.570 --> 00:19:45.130 while I said, I have

NOTE Confidence: 0.9364477

00:19:45.130 --> 00:19:46.330 a ligand, you know, that

NOTE Confidence: 0.9364477

00:19:46.330 --> 00:19:47.365 that that binds bacs. And

NOTE Confidence: 0.9364477

00:19:47.365 --> 00:19:48.484 he, you know, said, well,

NOTE Confidence: 0.9364477

00:19:48.484 --> 00:19:49.125 so do a lot of

NOTE Confidence: 0.9364477

00:19:49.125 --> 00:19:49.625 people.

NOTE Confidence: 0.9841671

00:19:50.085 --> 00:19:50.725 He said, oh, and by

NOTE Confidence: 0.9841671

00:19:50.725 --> 00:19:52.005 the way, your ligand binds

NOTE Confidence: 0.9841671

00:19:52.005 --> 00:19:53.684 and activates bacs. You can't

NOTE Confidence: 0.9841671

00:19:53.684 --> 00:19:54.804 do NMR on a moving

NOTE Confidence: 0.9841671

00:19:54.804 --> 00:19:55.765 target where, you know, the

NOTE Confidence: 0.9841671

00:19:55.765 --> 00:19:56.645 whole thing goes from a

NOTE Confidence: 0.9841671

00:19:56.645 --> 00:19:58.165 twenty kilodome protein to an

NOTE Confidence: 0.9841671

00:19:58.165 --> 00:20:00.244 oligomer in five minutes. So,

NOTE Confidence: 0.9841671

00:20:00.244 --> 00:20:01.619 you know, no chance of

NOTE Confidence: 0.9841671

00:20:01.619 --> 00:20:02.500 this working. And if you

NOTE Confidence: 0.9841671

00:20:02.500 --> 00:20:03.619 wanna do anything, just go

NOTE Confidence: 0.9841671

00:20:03.619 --> 00:20:04.580 make it weak enough so

NOTE Confidence: 0.9841671

00:20:04.580 --> 00:20:05.460 that it could bind but

NOTE Confidence: 0.9841671

00:20:05.460 --> 00:20:06.200 not activate.

NOTE Confidence: 0.99023044

00:20:06.900 --> 00:20:07.940 So as a young person,

NOTE Confidence: 0.99023044

00:20:07.940 --> 00:20:08.820 I was like, well, that

NOTE Confidence: 0.99023044

00:20:08.820 --> 00:20:10.340 sounded like really good advice.

NOTE Confidence: 0.99023044

00:20:10.340 --> 00:20:11.080 So I,

NOTE Confidence: 0.97371197

00:20:11.780 --> 00:20:12.500 I don't know if he

NOTE Confidence: 0.97371197

00:20:12.500 --> 00:20:13.300 thought I was going to

NOTE Confidence: 0.97371197

00:20:13.300 --> 00:20:14.500 follow-up his advice, but I

NOTE Confidence: 0.97371197

00:20:14.500 --> 00:20:15.220 went back and,

NOTE Confidence: 0.9823643

00:20:15.994 --> 00:20:16.795 a year and a half

NOTE Confidence: 0.9823643

00:20:16.795 --> 00:20:18.234 later, I found I was

NOTE Confidence: 0.9823643

00:20:18.234 --> 00:20:19.355 able to iterate this to

NOTE Confidence: 0.9823643

00:20:19.355 --> 00:20:20.635 make the peptide weak enough

NOTE Confidence: 0.9823643

00:20:20.635 --> 00:20:22.075 to bind but not activate

NOTE Confidence: 0.9823643

00:20:22.075 --> 00:20:23.035 over, like, a two hour

NOTE Confidence: 0.9823643

00:20:23.035 --> 00:20:23.535 period.

NOTE Confidence: 0.93220764

00:20:24.155 --> 00:20:25.775 And so I went back

NOTE Confidence: 0.93220764

00:20:25.915 --> 00:20:27.755 and, we collaborated with,

NOTE Confidence: 0.93021876

00:20:29.020 --> 00:20:30.220 one of his staff scientists

NOTE Confidence: 0.93021876

00:20:30.220 --> 00:20:31.340 who was the post doc,

NOTE Confidence: 0.93021876

00:20:31.340 --> 00:20:32.380 who's the first author on

NOTE Confidence: 0.93021876

00:20:32.380 --> 00:20:33.980 that BACS NMR paper, Matoshi

NOTE Confidence: 0.93021876

00:20:33.980 --> 00:20:34.480 Suzuki.

NOTE Confidence: 0.95444894

00:20:35.100 --> 00:20:37.840 And we added this peptide

NOTE Confidence: 0.95444894

00:20:38.059 --> 00:20:39.740 to Matoshi's n fifteen label

NOTE Confidence: 0.95444894

00:20:39.740 --> 00:20:41.420 BACS. And there were very

NOTE Confidence: 0.95444894

00:20:41.420 --> 00:20:42.240 subtle changes,

NOTE Confidence: 0.99942756

00:20:42.940 --> 00:20:44.000 but they were very

NOTE Confidence: 0.97404563

00:20:44.674 --> 00:20:46.595 specifically localized. And so when

NOTE Confidence: 0.97404563

00:20:46.595 --> 00:20:48.275 we mapped those residues or

NOTE Confidence: 0.97404563

00:20:48.275 --> 00:20:49.715 those chemical shift changes onto

NOTE Confidence: 0.97404563

00:20:49.715 --> 00:20:51.475 the protein, they all lined

NOTE Confidence: 0.97404563

00:20:51.475 --> 00:20:52.434 up on one side of

NOTE Confidence: 0.97404563

00:20:52.434 --> 00:20:53.095 the protein.

NOTE Confidence: 0.996198

00:20:53.715 --> 00:20:55.255 And what was interesting was

NOTE Confidence: 0.996198

00:20:55.395 --> 00:20:57.155 that that traditional groove that

NOTE Confidence: 0.996198

00:20:57.155 --> 00:20:58.195 I started out telling you

NOTE Confidence: 0.996198

00:20:58.195 --> 00:20:59.655 all about is over here.

NOTE Confidence: 0.996198

00:20:59.940 --> 00:21:00.440 So

NOTE Confidence: 0.97496945

00:21:01.059 --> 00:21:03.000 this chemical shift change perturbations

NOTE Confidence: 0.97496945

00:21:03.059 --> 00:21:04.100 that aligned on one face

NOTE Confidence: 0.97496945

00:21:04.100 --> 00:21:05.220 of the protein was not

NOTE Confidence: 0.97496945

00:21:05.220 --> 00:21:06.580 the famous part of the

NOTE Confidence: 0.97496945

00:21:06.580 --> 00:21:07.859 protein. It was not what

NOTE Confidence: 0.97496945

00:21:07.859 --> 00:21:09.480 we call the canonical groove

NOTE Confidence: 0.97496945

00:21:09.619 --> 00:21:10.820 that accepts b h threes.

NOTE Confidence: 0.97496945

00:21:10.820 --> 00:21:12.039 This was something different.

NOTE Confidence: 0.98231673

00:21:12.340 --> 00:21:13.460 And so what I did

NOTE Confidence: 0.98231673

00:21:13.460 --> 00:21:15.345 was I took that site

NOTE Confidence: 0.98231673

00:21:15.345 --> 00:21:16.304 and then I started, you

NOTE Confidence: 0.98231673

00:21:16.304 --> 00:21:17.505 know, painting it with the

NOTE Confidence: 0.98231673

00:21:17.505 --> 00:21:18.545 colors that,

NOTE Confidence: 0.94881594

00:21:18.865 --> 00:21:20.385 Steve Fesick and Craig Thompson

NOTE Confidence: 0.94881594

00:21:20.385 --> 00:21:23.025 painted the BCLXL binding pocket

NOTE Confidence: 0.94881594

00:21:23.025 --> 00:21:24.325 in their initial paper.

NOTE Confidence: 0.99815595

00:21:24.625 --> 00:21:25.845 And when I did that,

NOTE Confidence: 0.98502266

00:21:27.049 --> 00:21:28.169 I remember I mean, I

NOTE Confidence: 0.98502266

00:21:28.169 --> 00:21:29.470 remember the day

NOTE Confidence: 0.9690876

00:21:29.929 --> 00:21:31.210 that I did this in

NOTE Confidence: 0.9690876

00:21:31.210 --> 00:21:33.130 my, like, brand new office

NOTE Confidence: 0.9690876

00:21:33.130 --> 00:21:34.010 trying to figure out what

NOTE Confidence: 0.9690876

00:21:34.010 --> 00:21:35.049 the hell was going on.

NOTE Confidence: 0.9690876

00:21:35.049 --> 00:21:35.850 And I looked at this

NOTE Confidence: 0.9690876

00:21:35.850 --> 00:21:36.490 and I was like, well,

NOTE Confidence: 0.9690876

00:21:36.490 --> 00:21:37.470 there's that hydrophobic

NOTE Confidence: 0.96454746

00:21:37.770 --> 00:21:39.049 groove. That's nice. But what

NOTE Confidence: 0.96454746

00:21:39.049 --> 00:21:40.409 really blew my mind was,

NOTE Confidence: 0.96454746

00:21:40.409 --> 00:21:41.470 oh, look at the orientation

NOTE Confidence: 0.9355157

00:21:42.034 --> 00:21:43.234 of the positive charge and

NOTE Confidence: 0.9355157

00:21:43.234 --> 00:21:44.195 the negative charge in the

NOTE Confidence: 0.9355157

00:21:44.195 --> 00:21:45.475 hydrophilic. This looks like a

NOTE Confidence: 0.9355157

00:21:45.475 --> 00:21:46.755 very similar thing. Like from

NOTE Confidence: 0.9355157

00:21:46.755 --> 00:21:47.875 the upper left, blue, green,

NOTE Confidence: 0.9355157

00:21:47.875 --> 00:21:49.234 blue, red. That's the same

NOTE Confidence: 0.9355157

00:21:49.234 --> 00:21:49.955 thing going on on the

NOTE Confidence: 0.9355157

00:21:49.955 --> 00:21:50.755 other side. And then I

NOTE Confidence: 0.9355157

00:21:50.755 --> 00:21:52.115 got really excited. Like maybe

NOTE Confidence: 0.9355157

00:21:52.115 --> 00:21:53.635 this is an alternative BH3

NOTE Confidence: 0.9355157

00:21:53.635 --> 00:21:54.534 binding site.

NOTE Confidence: 0.9547226

00:21:55.369 --> 00:21:56.010 But then we had a

NOTE Confidence: 0.9547226

00:21:56.010 --> 00:21:57.290 problem because you can't solve

NOTE Confidence: 0.9547226

00:21:57.290 --> 00:21:58.750 a structure in two hours,

NOTE Confidence: 0.9667595

00:21:59.130 --> 00:22:00.330 by NMR. And so we

NOTE Confidence: 0.9667595

00:22:00.330 --> 00:22:01.050 had to come up with

NOTE Confidence: 0.9667595

00:22:01.050 --> 00:22:02.890 some alternative approach. And at

NOTE Confidence: 0.9667595

00:22:02.890 --> 00:22:03.550 that point,

NOTE Confidence: 0.79395354

00:22:04.010 --> 00:22:04.990 Everest Gavathiotis,

NOTE Confidence: 0.98710185

00:22:05.290 --> 00:22:06.250 you know, was giving a

NOTE Confidence: 0.98710185

00:22:06.250 --> 00:22:07.369 talk about how he had

NOTE Confidence: 0.98710185

00:22:07.369 --> 00:22:08.905 done some work on solving

NOTE Confidence: 0.98710185

00:22:08.905 --> 00:22:10.345 structures of weak protein protein

NOTE Confidence: 0.98710185

00:22:10.345 --> 00:22:11.565 interactions by NMR.

NOTE Confidence: 0.961976

00:22:12.025 --> 00:22:12.984 And I said, you gotta

NOTE Confidence: 0.961976

00:22:12.984 --> 00:22:13.785 come and help me with

NOTE Confidence: 0.961976

00:22:13.785 --> 00:22:14.905 this and join the lab

NOTE Confidence: 0.961976

00:22:14.905 --> 00:22:15.865 and be be a post

NOTE Confidence: 0.961976

00:22:15.865 --> 00:22:16.365 doc,

NOTE Confidence: 0.9292378

00:22:16.984 --> 00:22:18.505 again, around the time that,

NOTE Confidence: 0.9292378

00:22:18.744 --> 00:22:19.244 Sam,

NOTE Confidence: 0.9493155

00:22:19.545 --> 00:22:21.299 also joined. And and Everest

NOTE Confidence: 0.9493155

00:22:21.299 --> 00:22:22.139 was like, well, we could

NOTE Confidence: 0.9493155

00:22:22.139 --> 00:22:23.179 do p r e NMR

NOTE Confidence: 0.9493155

00:22:23.179 --> 00:22:24.320 where you stick a paramagnetic

NOTE Confidence: 0.9493155

00:22:24.539 --> 00:22:25.340 label at the end of

NOTE Confidence: 0.9493155

00:22:25.340 --> 00:22:26.000 the peptide,

NOTE Confidence: 0.99607944

00:22:26.539 --> 00:22:28.320 and that should disrupt the

NOTE Confidence: 0.98240685

00:22:28.619 --> 00:22:30.639 pattern of chemical shift perturbation.

NOTE Confidence: 0.98240685

00:22:30.779 --> 00:22:31.659 And then you'll see at

NOTE Confidence: 0.98240685

00:22:31.659 --> 00:22:33.794 least orientation wise, like, where

NOTE Confidence: 0.98240685

00:22:33.794 --> 00:22:34.914 this thing is binding on

NOTE Confidence: 0.98240685

00:22:34.914 --> 00:22:36.054 one side of the protein.

NOTE Confidence: 0.98240685

00:22:36.274 --> 00:22:37.394 And then you could stick

NOTE Confidence: 0.98240685

00:22:37.394 --> 00:22:38.355 the label on the other

NOTE Confidence: 0.98240685

00:22:38.355 --> 00:22:39.634 side and, you know, figure

NOTE Confidence: 0.98240685

00:22:39.634 --> 00:22:40.595 out where it's binding on

NOTE Confidence: 0.98240685

00:22:40.595 --> 00:22:41.634 the other side. And then

NOTE Confidence: 0.98240685

00:22:41.634 --> 00:22:42.994 things that don't change are

NOTE Confidence: 0.98240685

00:22:42.994 --> 00:22:43.955 usually the ones that are

NOTE Confidence: 0.98240685

00:22:43.955 --> 00:22:44.914 kinda, like, right there in

NOTE Confidence: 0.98240685

00:22:44.914 --> 00:22:46.375 the middle. And with these

NOTE Confidence: 0.99236417

00:22:47.540 --> 00:22:49.059 distance constraints, you could calculate

NOTE Confidence: 0.99236417

00:22:49.059 --> 00:22:49.960 a model structure.

NOTE Confidence: 0.9675325

00:22:50.260 --> 00:22:51.140 And so that's what we

NOTE Confidence: 0.9675325

00:22:51.140 --> 00:22:51.940 did, and we got this.

NOTE Confidence: 0.9675325

00:22:51.940 --> 00:22:53.640 And what was super satisfying

NOTE Confidence: 0.9675325

00:22:53.700 --> 00:22:55.059 about this is that that,

NOTE Confidence: 0.9675325

00:22:55.059 --> 00:22:56.359 you know, all the electrostatic

NOTE Confidence: 0.9675325

00:22:56.580 --> 00:22:57.080 pairings

NOTE Confidence: 0.9459207

00:22:57.460 --> 00:22:58.820 were perfectly lined up, the

NOTE Confidence: 0.9459207

00:22:58.820 --> 00:23:00.980 hydrophilic pairings perfectly lined up,

NOTE Confidence: 0.9459207

00:23:00.980 --> 00:23:02.440 and it looked really beautiful.

NOTE Confidence: 0.9459207

00:23:02.565 --> 00:23:03.445 You know, that you had

NOTE Confidence: 0.9459207

00:23:03.445 --> 00:23:05.205 this very tight binding interaction

NOTE Confidence: 0.9459207

00:23:05.205 --> 00:23:06.404 from the hydrophobic face, and

NOTE Confidence: 0.9459207

00:23:06.404 --> 00:23:07.684 then it was reinforced by

NOTE Confidence: 0.9459207

00:23:07.684 --> 00:23:09.865 this complimentary charge charge hydrophilic

NOTE Confidence: 0.9459207

00:23:09.924 --> 00:23:10.424 network.

NOTE Confidence: 0.924241

00:23:10.804 --> 00:23:12.004 And and that looked very

NOTE Confidence: 0.924241

00:23:12.004 --> 00:23:13.365 much like the traditional b

NOTE Confidence: 0.924241

00:23:13.365 --> 00:23:14.325 h three and group that

NOTE Confidence: 0.924241

00:23:14.325 --> 00:23:15.384 was solved for BCLXL,

NOTE Confidence: 0.99697053

00:23:16.085 --> 00:23:17.205 except this site was a

NOTE Confidence: 0.99697053

00:23:17.205 --> 00:23:18.105 bit more shallow.

NOTE Confidence: 0.95593536

00:23:19.100 --> 00:23:20.060 So we had, of course,

NOTE Confidence: 0.95593536

00:23:20.060 --> 00:23:21.100 prove this. So we started

NOTE Confidence: 0.95593536

00:23:21.100 --> 00:23:22.060 out with two assays, an

NOTE Confidence: 0.95593536

00:23:22.060 --> 00:23:22.560 oligomerization

NOTE Confidence: 0.9402629

00:23:22.940 --> 00:23:24.300 assay, where you actually look

NOTE Confidence: 0.9402629

00:23:24.300 --> 00:23:25.420 to see whether your ligand

NOTE Confidence: 0.9402629

00:23:25.420 --> 00:23:26.460 could make BACS go from

NOTE Confidence: 0.9402629

00:23:26.460 --> 00:23:27.420 a monomer to a higher

NOTE Confidence: 0.9402629

00:23:27.420 --> 00:23:29.180 order species. And then also

NOTE Confidence: 0.9402629

00:23:29.180 --> 00:23:30.885 like a more, physiologic one

NOTE Confidence: 0.9402629

00:23:30.965 --> 00:23:31.865 where you could take mitochondria

NOTE Confidence: 0.97822976

00:23:32.645 --> 00:23:33.765 and do the experiment on

NOTE Confidence: 0.97822976

00:23:33.765 --> 00:23:35.205 a mitochondria and see if

NOTE Confidence: 0.97822976

00:23:35.205 --> 00:23:37.065 they start releasing cytochrome c.

NOTE Confidence: 0.98045295

00:23:37.445 --> 00:23:38.325 And so we had our

NOTE Confidence: 0.98045295

00:23:38.325 --> 00:23:39.845 positive control, you know, that

NOTE Confidence: 0.98045295

00:23:39.845 --> 00:23:40.965 we had started with with

NOTE Confidence: 0.98045295

00:23:40.965 --> 00:23:42.484 our peptide and our protein,

NOTE Confidence: 0.98045295

00:23:42.484 --> 00:23:43.865 and then we started mutating,

NOTE Confidence: 0.98045295

00:23:43.925 --> 00:23:45.525 like, key residues, you know,

NOTE Confidence: 0.98045295

00:23:45.525 --> 00:23:47.480 in this interaction network. And

NOTE Confidence: 0.98045295

00:23:47.480 --> 00:23:48.679 every time we hit one

NOTE Confidence: 0.98045295

00:23:48.679 --> 00:23:50.119 key interaction, now all of

NOTE Confidence: 0.98045295

00:23:50.119 --> 00:23:51.240 a sudden these assays did

NOTE Confidence: 0.98045295

00:23:51.240 --> 00:23:51.720 not work.

NOTE Confidence: 0.9571413

00:23:53.080 --> 00:23:53.960 And so that got us

NOTE Confidence: 0.9571413

00:23:53.960 --> 00:23:55.080 very excited. And then the

NOTE Confidence: 0.9571413

00:23:55.080 --> 00:23:56.919 other thing we noticed was

NOTE Confidence: 0.9571413

00:23:56.919 --> 00:23:58.294 that every time we calculated

NOTE Confidence: 0.9571413

00:23:58.294 --> 00:23:59.734 a model structure based upon

NOTE Confidence: 0.9571413

00:23:59.734 --> 00:24:01.095 our data and we looked

NOTE Confidence: 0.9571413

00:24:01.095 --> 00:24:02.775 at Niko Chandra's structure where

NOTE Confidence: 0.9571413

00:24:02.775 --> 00:24:04.294 you have this protein and

NOTE Confidence: 0.9571413

00:24:04.294 --> 00:24:05.975 in in in magenta, I'm

NOTE Confidence: 0.9571413

00:24:05.975 --> 00:24:07.095 showing you the binding site,

NOTE Confidence: 0.9571413

00:24:07.095 --> 00:24:08.934 and then this green colored

NOTE Confidence: 0.9571413

00:24:08.934 --> 00:24:10.054 area is a loop, the

NOTE Confidence: 0.9571413

00:24:10.054 --> 00:24:11.430 alpha one alpha two loop

NOTE Confidence: 0.9571413

00:24:11.670 --> 00:24:12.790 that sits between the two

NOTE Confidence: 0.9571413

00:24:12.790 --> 00:24:14.250 alpha one alpha two helices.

NOTE Confidence: 0.9571413

00:24:14.470 --> 00:24:15.430 That was always in this

NOTE Confidence: 0.9571413

00:24:15.430 --> 00:24:16.390 what we ended up calling

NOTE Confidence: 0.9571413

00:24:16.390 --> 00:24:17.530 the closed confirmation.

NOTE Confidence: 0.99895054

00:24:18.310 --> 00:24:19.510 But when our peptide was

NOTE Confidence: 0.99895054

00:24:19.510 --> 00:24:20.170 in there,

NOTE Confidence: 0.97990346

00:24:20.470 --> 00:24:21.510 it was always in what

NOTE Confidence: 0.97990346

00:24:21.510 --> 00:24:23.050 we call the open confirmation.

NOTE Confidence: 0.97990346

00:24:23.190 --> 00:24:24.390 In other words, this loop

NOTE Confidence: 0.97990346

00:24:24.390 --> 00:24:25.369 was kicked out.

NOTE Confidence: 0.98467135

00:24:26.165 --> 00:24:26.965 And I showed you at

NOTE Confidence: 0.98467135

00:24:26.965 --> 00:24:27.924 the beginning that when this,

NOTE Confidence: 0.98467135

00:24:27.924 --> 00:24:28.804 you know, when the pin

NOTE Confidence: 0.98467135

00:24:28.804 --> 00:24:29.845 is pulled on this grenade,

NOTE Confidence: 0.98467135

00:24:29.845 --> 00:24:31.445 like, this protein totally changes

NOTE Confidence: 0.98467135

00:24:31.445 --> 00:24:32.184 its shape.

NOTE Confidence: 0.99149436

00:24:32.484 --> 00:24:33.525 And so we started to

NOTE Confidence: 0.99149436

00:24:33.525 --> 00:24:34.345 think maybe

NOTE Confidence: 0.98128974

00:24:34.885 --> 00:24:37.544 the the catalytic conformational change

NOTE Confidence: 0.98128974

00:24:37.605 --> 00:24:39.125 is this displacement of the

NOTE Confidence: 0.98128974

00:24:39.125 --> 00:24:40.565 loop from a closed to

NOTE Confidence: 0.98128974

00:24:40.565 --> 00:24:41.544 an open position.

NOTE Confidence: 0.9772209

00:24:42.140 --> 00:24:43.419 Now the one thing that

NOTE Confidence: 0.9772209

00:24:43.419 --> 00:24:44.619 got me even more excited

NOTE Confidence: 0.9772209

00:24:44.619 --> 00:24:45.820 about this was that at

NOTE Confidence: 0.9772209

00:24:45.820 --> 00:24:47.179 the time, if you were

NOTE Confidence: 0.9772209

00:24:47.179 --> 00:24:48.700 studying BAX, there was an

NOTE Confidence: 0.9772209

00:24:48.700 --> 00:24:49.200 antibody

NOTE Confidence: 0.95043606

00:24:49.580 --> 00:24:50.880 that everyone would buy,

NOTE Confidence: 0.944881

00:24:51.659 --> 00:24:53.260 came from Richard Ewell's lab,

NOTE Confidence: 0.944881

00:24:53.260 --> 00:24:54.619 also at NIH, and it

NOTE Confidence: 0.944881

00:24:54.619 --> 00:24:55.440 was the

NOTE Confidence: 0.9896816

00:24:56.014 --> 00:24:57.154 confirmation specific

NOTE Confidence: 0.9700732

00:24:57.534 --> 00:24:59.294 activated form of BAX. Right?

NOTE Confidence: 0.9700732

00:24:59.294 --> 00:25:00.095 So if you had BAX

NOTE Confidence: 0.9700732

00:25:00.095 --> 00:25:01.455 in a cell or BAX

NOTE Confidence: 0.9700732

00:25:01.455 --> 00:25:02.494 in a test tube and

NOTE Confidence: 0.9700732

00:25:02.494 --> 00:25:03.855 it was inactive, this antibody

NOTE Confidence: 0.9700732

00:25:03.855 --> 00:25:05.134 did not recognize it, couldn't

NOTE Confidence: 0.9700732

00:25:05.134 --> 00:25:05.634 immunoprecipitate

NOTE Confidence: 0.9331052

00:25:06.174 --> 00:25:07.715 anything. But if you activated

NOTE Confidence: 0.9331052

00:25:07.855 --> 00:25:08.975 BAX in a cell or

NOTE Confidence: 0.9331052

00:25:08.975 --> 00:25:10.480 in solution, and then you

NOTE Confidence: 0.9331052

00:25:10.480 --> 00:25:11.679 put this antibody and now

NOTE Confidence: 0.9331052

00:25:11.679 --> 00:25:13.040 magically it it binds to

NOTE Confidence: 0.9331052

00:25:13.040 --> 00:25:14.100 activated bacs,

NOTE Confidence: 0.99783576

00:25:14.880 --> 00:25:15.700 the epitope

NOTE Confidence: 0.9927562

00:25:16.160 --> 00:25:18.000 that that bound to was

NOTE Confidence: 0.9927562

00:25:18.000 --> 00:25:18.500 underneath

NOTE Confidence: 0.9983417

00:25:19.040 --> 00:25:20.820 the loop and became exposed

NOTE Confidence: 0.9978268

00:25:21.440 --> 00:25:22.580 when the loop opened.

NOTE Confidence: 0.9788801

00:25:23.105 --> 00:25:24.465 So we're like, wow. Okay.

NOTE Confidence: 0.9788801

00:25:24.465 --> 00:25:26.065 Maybe this is actually we're

NOTE Confidence: 0.9788801

00:25:26.065 --> 00:25:27.265 sniffing something out here that

NOTE Confidence: 0.9788801

00:25:27.265 --> 00:25:28.705 makes sense. That the six

NOTE Confidence: 0.9788801

00:25:28.705 --> 00:25:30.725 a seven epitope that defined

NOTE Confidence: 0.9788801

00:25:30.785 --> 00:25:32.945 activated backs was covered by

NOTE Confidence: 0.9788801

00:25:32.945 --> 00:25:34.244 the loop in the inactive

NOTE Confidence: 0.9788801

00:25:34.305 --> 00:25:36.085 form and was completely exposed

NOTE Confidence: 0.98977625

00:25:36.465 --> 00:25:37.765 when the loop was opened.

NOTE Confidence: 0.9996971

00:25:38.609 --> 00:25:39.730 So we wanted to test

NOTE Confidence: 0.9996971

00:25:39.730 --> 00:25:41.330 this. So what we did

NOTE Confidence: 0.9996971

00:25:41.330 --> 00:25:41.830 was

NOTE Confidence: 0.9802792

00:25:42.130 --> 00:25:42.630 we

NOTE Confidence: 0.9942313

00:25:43.970 --> 00:25:46.050 installed two cysteines and made

NOTE Confidence: 0.9942313

00:25:46.050 --> 00:25:46.869 a disulfide

NOTE Confidence: 0.9999558

00:25:47.250 --> 00:25:48.230 tethered version

NOTE Confidence: 0.92549783

00:25:48.690 --> 00:25:50.450 of backs where the loop

NOTE Confidence: 0.92549783

00:25:50.450 --> 00:25:51.430 could not open

NOTE Confidence: 0.9982013

00:25:51.875 --> 00:25:52.994 because now the loop is

NOTE Confidence: 0.9982013

00:25:52.994 --> 00:25:53.975 literally covalently

NOTE Confidence: 0.991486

00:25:54.515 --> 00:25:56.375 stuck into the binding site.

NOTE Confidence: 0.991486

00:25:56.595 --> 00:25:57.875 And we did, like, very

NOTE Confidence: 0.991486

00:25:57.875 --> 00:25:59.635 simple experiments. So we we

NOTE Confidence: 0.991486

00:25:59.635 --> 00:26:01.734 we basically made this protein.

NOTE Confidence: 0.991486

00:26:01.875 --> 00:26:03.955 We oxidized it. We added

NOTE Confidence: 0.991486

00:26:03.955 --> 00:26:05.395 the ligand, and we didn't

NOTE Confidence: 0.991486

00:26:05.395 --> 00:26:06.135 see anything.

NOTE Confidence: 0.9771528

00:26:06.460 --> 00:26:07.340 And we needed to prove

NOTE Confidence: 0.9771528

00:26:07.340 --> 00:26:08.800 that it wasn't just oxidizing

NOTE Confidence: 0.9771528

00:26:08.940 --> 00:26:10.060 VAX was the problem. So

NOTE Confidence: 0.9771528

00:26:10.060 --> 00:26:10.960 then we oxidized

NOTE Confidence: 0.9703632

00:26:11.340 --> 00:26:13.260 normal VAX, not mutated VAX,

NOTE Confidence: 0.9703632

00:26:13.260 --> 00:26:14.860 and it worked fine. So

NOTE Confidence: 0.9703632

00:26:14.860 --> 00:26:15.900 that was that was lucky

NOTE Confidence: 0.9703632

00:26:15.900 --> 00:26:17.100 for us that the oxidation

NOTE Confidence: 0.9703632

00:26:17.100 --> 00:26:18.300 of wild type VAX did

NOTE Confidence: 0.9703632

00:26:18.300 --> 00:26:19.744 not disrupt it. So then

NOTE Confidence: 0.9703632

00:26:19.744 --> 00:26:20.705 we did the experiment that

NOTE Confidence: 0.9703632

00:26:20.705 --> 00:26:21.904 we were really wanting to

NOTE Confidence: 0.9703632

00:26:21.904 --> 00:26:22.945 do is we took this

NOTE Confidence: 0.9703632

00:26:22.945 --> 00:26:23.445 disulfide,

NOTE Confidence: 0.9590231

00:26:24.304 --> 00:26:25.664 you know, closed version, and

NOTE Confidence: 0.9590231

00:26:25.664 --> 00:26:26.865 we just threw in reducing

NOTE Confidence: 0.9590231

00:26:26.865 --> 00:26:27.905 agent, and then all of

NOTE Confidence: 0.9590231

00:26:27.905 --> 00:26:29.125 a sudden it's back.

NOTE Confidence: 0.97116417

00:26:29.664 --> 00:26:30.705 Right? And then we had

NOTE Confidence: 0.97116417

00:26:30.705 --> 00:26:31.424 to show that if you

NOTE Confidence: 0.97116417

00:26:31.424 --> 00:26:32.880 just reduce wild type bacs,

NOTE Confidence: 0.97116417

00:26:32.960 --> 00:26:33.920 it doesn't just, like, auto

NOTE Confidence: 0.97116417

00:26:33.920 --> 00:26:35.140 activate it, and it doesn't.

NOTE Confidence: 0.97116417

00:26:35.359 --> 00:26:36.720 Okay. So clearly, when you

NOTE Confidence: 0.97116417

00:26:36.720 --> 00:26:38.000 allow that loop to open,

NOTE Confidence: 0.97116417

00:26:38.000 --> 00:26:38.960 it works. And when you

NOTE Confidence: 0.97116417

00:26:38.960 --> 00:26:40.160 don't allow it to open,

NOTE Confidence: 0.97116417

00:26:40.160 --> 00:26:41.619 now the protein is dead.

NOTE Confidence: 0.99773407

00:26:42.880 --> 00:26:44.240 So now we really started

NOTE Confidence: 0.99773407

00:26:44.240 --> 00:26:44.740 to

NOTE Confidence: 0.97335553

00:26:45.525 --> 00:26:46.805 think, okay, we're gonna start

NOTE Confidence: 0.97335553

00:26:46.805 --> 00:26:48.244 marching down that picture that

NOTE Confidence: 0.97335553

00:26:48.244 --> 00:26:49.125 I showed you of, like,

NOTE Confidence: 0.97335553

00:26:49.125 --> 00:26:49.925 what in the world is

NOTE Confidence: 0.97335553

00:26:49.925 --> 00:26:51.045 going on with this protein

NOTE Confidence: 0.97335553

00:26:51.045 --> 00:26:52.165 from start to finish? And

NOTE Confidence: 0.97335553

00:26:52.165 --> 00:26:52.965 that was kind of a

NOTE Confidence: 0.97335553

00:26:53.045 --> 00:26:54.085 that has been a project

NOTE Confidence: 0.97335553

00:26:54.085 --> 00:26:55.605 in my lab since I

NOTE Confidence: 0.97335553

00:26:55.605 --> 00:26:56.885 opened the lab. Like, step

NOTE Confidence: 0.97335553

00:26:56.885 --> 00:26:57.925 by step by step, what

NOTE Confidence: 0.97335553

00:26:57.925 --> 00:26:58.885 is going on with this

NOTE Confidence: 0.97335553

00:26:58.885 --> 00:27:00.105 protein? And so

NOTE Confidence: 0.9674205

00:27:00.950 --> 00:27:02.630 now we have the complex

NOTE Confidence: 0.9674205

00:27:02.630 --> 00:27:03.590 between the b h three

NOTE Confidence: 0.9674205

00:27:03.590 --> 00:27:04.730 and the and the protein.

NOTE Confidence: 0.9674205

00:27:04.950 --> 00:27:06.470 Then we had this loop

NOTE Confidence: 0.9674205

00:27:06.470 --> 00:27:07.290 thing happening,

NOTE Confidence: 0.98461205

00:27:07.750 --> 00:27:09.030 and then we thought, okay.

NOTE Confidence: 0.98461205

00:27:09.030 --> 00:27:10.630 But this protein goes from

NOTE Confidence: 0.98461205

00:27:10.630 --> 00:27:11.290 the cytoplasm

NOTE Confidence: 0.9991049

00:27:12.070 --> 00:27:12.890 to the mitochondria.

NOTE Confidence: 0.9827193

00:27:14.325 --> 00:27:15.845 How does that happen? And

NOTE Confidence: 0.9827193

00:27:15.845 --> 00:27:16.885 so we did some more

NOTE Confidence: 0.9827193

00:27:16.885 --> 00:27:18.265 short term NMR experiments,

NOTE Confidence: 0.99962395

00:27:18.725 --> 00:27:19.625 and we did

NOTE Confidence: 0.9828518

00:27:20.005 --> 00:27:21.044 a little bit longer and

NOTE Confidence: 0.9828518

00:27:21.044 --> 00:27:22.325 longer time courses up to

NOTE Confidence: 0.9828518

00:27:22.325 --> 00:27:23.684 two hours, higher and higher

NOTE Confidence: 0.9828518

00:27:23.684 --> 00:27:25.044 doses of our ligand. And

NOTE Confidence: 0.9828518

00:27:25.044 --> 00:27:26.405 what we saw was that

NOTE Confidence: 0.9828518

00:27:26.405 --> 00:27:27.765 there were chemical shift changes

NOTE Confidence: 0.9828518

00:27:27.765 --> 00:27:28.265 now

NOTE Confidence: 0.9836487

00:27:28.909 --> 00:27:30.510 in the c terminal helix

NOTE Confidence: 0.9836487

00:27:30.510 --> 00:27:31.169 of the

NOTE Confidence: 0.97484803

00:27:31.950 --> 00:27:32.990 protein. And the c terminal

NOTE Confidence: 0.97484803

00:27:32.990 --> 00:27:34.690 helix is the membrane insertion

NOTE Confidence: 0.97484803

00:27:34.750 --> 00:27:35.890 helix for BACS.

NOTE Confidence: 0.9968288

00:27:36.350 --> 00:27:37.470 So what we started to

NOTE Confidence: 0.9968288

00:27:37.470 --> 00:27:38.590 figure out here was that

NOTE Confidence: 0.9968288

00:27:38.590 --> 00:27:39.890 if you hit this thing,

NOTE Confidence: 0.9881813

00:27:40.269 --> 00:27:41.390 you know, on one side

NOTE Confidence: 0.9881813

00:27:41.390 --> 00:27:42.429 of the face, now all

NOTE Confidence: 0.9881813

00:27:42.429 --> 00:27:43.734 of a sudden the c

NOTE Confidence: 0.9881813

00:27:43.734 --> 00:27:45.355 terminal helix is allosterically

NOTE Confidence: 0.99965775

00:27:45.734 --> 00:27:46.554 popping out

NOTE Confidence: 0.9958546

00:27:46.855 --> 00:27:48.455 and probably responsible for it

NOTE Confidence: 0.9958546

00:27:48.455 --> 00:27:49.755 now going to the mitochondrial

NOTE Confidence: 0.9958546

00:27:49.815 --> 00:27:50.315 membrane.

NOTE Confidence: 0.9899999

00:27:50.695 --> 00:27:51.815 And so, again, that was

NOTE Confidence: 0.9899999

00:27:51.815 --> 00:27:53.255 a hypothesis based upon the

NOTE Confidence: 0.9899999

00:27:53.255 --> 00:27:54.615 NMR, and you could see

NOTE Confidence: 0.9899999

00:27:54.615 --> 00:27:56.715 these beautiful dose responsive changes

NOTE Confidence: 0.9899999

00:27:56.934 --> 00:27:57.960 in the shifts of all

NOTE Confidence: 0.9899999

00:27:57.960 --> 00:27:59.080 those residues in the c

NOTE Confidence: 0.9899999

00:27:59.080 --> 00:27:59.900 terminal helix.

NOTE Confidence: 0.9689477

00:28:00.440 --> 00:28:01.900 So, you know, our disulfide

NOTE Confidence: 0.9689477

00:28:01.960 --> 00:28:03.880 trick, you know, hit the

NOTE Confidence: 0.9689477

00:28:03.880 --> 00:28:05.000 payload the first time, so

NOTE Confidence: 0.9689477

00:28:05.000 --> 00:28:05.799 we thought let's just do

NOTE Confidence: 0.9689477

00:28:05.799 --> 00:28:07.320 it again. So we now

NOTE Confidence: 0.9689477

00:28:07.320 --> 00:28:09.179 installed the the the disulfide

NOTE Confidence: 0.9689477

00:28:09.400 --> 00:28:10.760 bond between the c terminal

NOTE Confidence: 0.9689477

00:28:10.760 --> 00:28:12.619 helix and the traditional groove.

NOTE Confidence: 0.96145976

00:28:13.275 --> 00:28:14.555 And we said, okay. Now

NOTE Confidence: 0.96145976

00:28:14.555 --> 00:28:16.155 is BACS unable to go

NOTE Confidence: 0.96145976

00:28:16.155 --> 00:28:16.975 to the mitochondria?

NOTE Confidence: 0.99937814

00:28:17.915 --> 00:28:19.035 So we did this very

NOTE Confidence: 0.99937814

00:28:19.035 --> 00:28:20.655 simple mitochondrial translocation

NOTE Confidence: 0.9583659

00:28:20.955 --> 00:28:22.555 assay where we took the

NOTE Confidence: 0.9583659

00:28:22.555 --> 00:28:24.635 the protein, again, oxidized condition,

NOTE Confidence: 0.9583659

00:28:24.635 --> 00:28:25.535 reduced conditions,

NOTE Confidence: 0.98702604

00:28:25.950 --> 00:28:26.990 see if it goes from

NOTE Confidence: 0.98702604

00:28:26.990 --> 00:28:28.850 the supernatant to the mitochondria.

NOTE Confidence: 0.97678864

00:28:29.470 --> 00:28:30.590 And the only time we

NOTE Confidence: 0.97678864

00:28:30.590 --> 00:28:31.389 saw it going from the

NOTE Confidence: 0.97678864

00:28:31.389 --> 00:28:32.830 supernatant to the mitochondria was

NOTE Confidence: 0.97678864

00:28:32.830 --> 00:28:34.210 when we reduced the protein.

NOTE Confidence: 0.998043

00:28:34.669 --> 00:28:35.950 And then, of course, once

NOTE Confidence: 0.998043

00:28:35.950 --> 00:28:36.909 you allow it to go

NOTE Confidence: 0.998043

00:28:36.909 --> 00:28:38.590 there, the cytochrome c now

NOTE Confidence: 0.998043

00:28:38.590 --> 00:28:40.190 leaves from the mitochondria and

NOTE Confidence: 0.998043

00:28:40.190 --> 00:28:41.250 goes into the supernatant.

NOTE Confidence: 0.9939064

00:28:41.715 --> 00:28:43.174 So it seemed that this

NOTE Confidence: 0.98579127

00:28:43.955 --> 00:28:45.635 direct displacement of alpha one

NOTE Confidence: 0.98579127

00:28:45.635 --> 00:28:47.075 alpha two loop and binding

NOTE Confidence: 0.98579127

00:28:47.075 --> 00:28:48.275 at that site was now

NOTE Confidence: 0.98579127

00:28:48.275 --> 00:28:49.875 being transmitted to the c

NOTE Confidence: 0.98579127

00:28:49.875 --> 00:28:51.075 terminal face of the protein

NOTE Confidence: 0.98579127

00:28:51.075 --> 00:28:52.674 and dislodging the c terminus.

NOTE Confidence: 0.98579127

00:28:52.674 --> 00:28:54.115 So now that pops out,

NOTE Confidence: 0.98579127

00:28:54.115 --> 00:28:55.315 sends your protein to the

NOTE Confidence: 0.98579127

00:28:55.315 --> 00:28:55.815 mitochondria.

NOTE Confidence: 0.95323443

00:28:58.440 --> 00:29:00.040 And we wanted to prove

NOTE Confidence: 0.95323443

00:29:00.040 --> 00:29:00.940 this, of course.

NOTE Confidence: 0.99683475

00:29:01.480 --> 00:29:02.520 And one of the things

NOTE Confidence: 0.99683475

00:29:02.520 --> 00:29:03.900 that was also a major

NOTE Confidence: 0.99683475

00:29:04.040 --> 00:29:05.800 aspect of the mechanism was

NOTE Confidence: 0.99683475

00:29:05.800 --> 00:29:06.300 that

NOTE Confidence: 0.9569088

00:29:06.680 --> 00:29:07.960 the b h three helix

NOTE Confidence: 0.9569088

00:29:07.960 --> 00:29:09.240 of BAX, I showed you

NOTE Confidence: 0.9569088

00:29:09.240 --> 00:29:10.200 that in the initial slide

NOTE Confidence: 0.9569088

00:29:10.200 --> 00:29:11.820 with the anti apoptotic protein,

NOTE Confidence: 0.975534

00:29:12.895 --> 00:29:14.175 BAX is blocked by the

NOTE Confidence: 0.975534

00:29:14.175 --> 00:29:15.695 anti apoptotic coming in and

NOTE Confidence: 0.975534

00:29:15.695 --> 00:29:16.895 grabbing onto the b h

NOTE Confidence: 0.975534

00:29:16.895 --> 00:29:18.815 three. So that means that

NOTE Confidence: 0.975534

00:29:18.815 --> 00:29:20.175 that b h three must

NOTE Confidence: 0.975534

00:29:20.175 --> 00:29:22.015 be exposed at some point

NOTE Confidence: 0.975534

00:29:22.015 --> 00:29:23.535 in this mechanism, or else

NOTE Confidence: 0.975534

00:29:23.535 --> 00:29:24.415 you would not be able

NOTE Confidence: 0.975534

00:29:24.415 --> 00:29:25.315 to block BAX.

NOTE Confidence: 0.9780525

00:29:25.980 --> 00:29:27.260 Okay? So we wanted to

NOTE Confidence: 0.9780525

00:29:27.260 --> 00:29:28.380 see in our system if

NOTE Confidence: 0.9780525

00:29:28.380 --> 00:29:29.500 we could detect for the

NOTE Confidence: 0.9780525

00:29:29.500 --> 00:29:30.240 first time

NOTE Confidence: 0.96403545

00:29:30.540 --> 00:29:32.300 this confirmational exposure of the

NOTE Confidence: 0.96403545

00:29:32.300 --> 00:29:33.120 b h three.

NOTE Confidence: 0.9804217

00:29:34.220 --> 00:29:36.080 So we used antibodies initially.

NOTE Confidence: 0.9804217

00:29:36.300 --> 00:29:37.580 And so first, we did

NOTE Confidence: 0.9804217

00:29:37.580 --> 00:29:39.360 this experiment with normal bacs,

NOTE Confidence: 0.9804217

00:29:39.420 --> 00:29:40.700 and what we found was

NOTE Confidence: 0.9804217

00:29:40.700 --> 00:29:42.184 that we could detect

NOTE Confidence: 0.9933992

00:29:42.485 --> 00:29:43.924 the six a seven epitope,

NOTE Confidence: 0.9933992

00:29:43.924 --> 00:29:44.885 but we could not detect

NOTE Confidence: 0.9933992

00:29:44.885 --> 00:29:45.865 the b h three.

NOTE Confidence: 0.9947312

00:29:46.644 --> 00:29:47.845 And so we thought, well,

NOTE Confidence: 0.9947312

00:29:47.845 --> 00:29:48.904 maybe that's because

NOTE Confidence: 0.98908687

00:29:49.205 --> 00:29:50.485 it pops out and goes

NOTE Confidence: 0.98908687

00:29:50.485 --> 00:29:51.765 to the mitochondria, and it's

NOTE Confidence: 0.98908687

00:29:51.765 --> 00:29:54.005 just so incredibly fast that

NOTE Confidence: 0.98908687

00:29:54.005 --> 00:29:55.524 we can't capture it. So

NOTE Confidence: 0.98908687

00:29:55.524 --> 00:29:56.325 we thought, okay. Well, we

NOTE Confidence: 0.98908687

00:29:56.325 --> 00:29:57.180 have a way of stopping

NOTE Confidence: 0.98908687

00:29:57.420 --> 00:29:58.940 mitochondrial translocation. We have our

NOTE Confidence: 0.98908687

00:29:58.940 --> 00:30:00.320 disulfide linked version,

NOTE Confidence: 0.98563457

00:30:01.100 --> 00:30:02.300 which doesn't allow the tail

NOTE Confidence: 0.98563457

00:30:02.300 --> 00:30:03.260 to come out. So maybe

NOTE Confidence: 0.98563457

00:30:03.260 --> 00:30:04.220 we'll trap this thing in

NOTE Confidence: 0.98563457

00:30:04.220 --> 00:30:06.220 some intermediate form. And so

NOTE Confidence: 0.98563457

00:30:06.220 --> 00:30:07.740 we tried it again, and

NOTE Confidence: 0.98563457

00:30:07.740 --> 00:30:09.180 there's your, you know, dose

NOTE Confidence: 0.98563457

00:30:09.180 --> 00:30:10.860 responsive induction of the six

NOTE Confidence: 0.98563457

00:30:10.860 --> 00:30:12.000 a seven recognition.

NOTE Confidence: 0.9719074

00:30:12.615 --> 00:30:13.255 And now all of a

NOTE Confidence: 0.9719074

00:30:13.255 --> 00:30:14.055 sudden for the first time,

NOTE Confidence: 0.9719074

00:30:14.055 --> 00:30:15.675 we got dose responsive recognition

NOTE Confidence: 0.9719074

00:30:15.735 --> 00:30:16.935 of b h three. We

NOTE Confidence: 0.9719074

00:30:16.935 --> 00:30:18.395 had never seen that before.

NOTE Confidence: 0.9951918

00:30:19.895 --> 00:30:21.495 And so now you kind

NOTE Confidence: 0.9951918

00:30:21.495 --> 00:30:22.795 of start to imagine

NOTE Confidence: 0.98826927

00:30:23.575 --> 00:30:25.095 these three very important parts

NOTE Confidence: 0.98826927

00:30:25.095 --> 00:30:26.455 of the protein undergoing a

NOTE Confidence: 0.98826927

00:30:26.455 --> 00:30:28.559 conformational change that's allowing it

NOTE Confidence: 0.98826927

00:30:28.559 --> 00:30:29.540 to go to the mitochondria

NOTE Confidence: 0.98826927

00:30:29.840 --> 00:30:30.720 and and go to the

NOTE Confidence: 0.98826927

00:30:30.720 --> 00:30:31.840 next step, which is, like,

NOTE Confidence: 0.98826927

00:30:31.840 --> 00:30:33.780 self association and membrane disruption.

NOTE Confidence: 0.999336

00:30:35.280 --> 00:30:36.100 But there was

NOTE Confidence: 0.9288978

00:30:36.559 --> 00:30:37.220 a weird,

NOTE Confidence: 0.8763703

00:30:38.080 --> 00:30:38.980 missing link,

NOTE Confidence: 0.99893653

00:30:39.840 --> 00:30:41.120 and that was that this

NOTE Confidence: 0.99893653

00:30:41.120 --> 00:30:42.340 process was catalytic

NOTE Confidence: 0.9706618

00:30:43.105 --> 00:30:44.065 so that you could put

NOTE Confidence: 0.9706618

00:30:44.065 --> 00:30:46.065 in the tiniest amount of

NOTE Confidence: 0.9706618

00:30:46.065 --> 00:30:47.825 a staple BIM peptide or

NOTE Confidence: 0.9706618

00:30:47.825 --> 00:30:49.424 the tiniest amount of the

NOTE Confidence: 0.9706618

00:30:49.424 --> 00:30:50.725 native bid protein,

NOTE Confidence: 0.95585215

00:30:52.384 --> 00:30:53.125 not stoichiometric,

NOTE Confidence: 0.99635714

00:30:54.065 --> 00:30:55.505 and this thing would just

NOTE Confidence: 0.99635714

00:30:55.505 --> 00:30:56.005 fire.

NOTE Confidence: 0.9995896

00:30:56.730 --> 00:30:58.270 And it was not clear

NOTE Confidence: 0.9251192

00:30:58.809 --> 00:30:59.950 how that happens.

NOTE Confidence: 0.9991658

00:31:00.890 --> 00:31:01.390 But

NOTE Confidence: 0.9508348

00:31:02.410 --> 00:31:03.929 we were staring, you know,

NOTE Confidence: 0.9508348

00:31:03.929 --> 00:31:05.130 at these sequences, and we

NOTE Confidence: 0.9508348

00:31:05.130 --> 00:31:06.650 realized that the backs b

NOTE Confidence: 0.9508348

00:31:06.650 --> 00:31:07.710 h three sequence

NOTE Confidence: 0.98498833

00:31:08.090 --> 00:31:10.350 in the core homology domain

NOTE Confidence: 0.98498833

00:31:10.645 --> 00:31:12.005 area that defines what a

NOTE Confidence: 0.98498833

00:31:12.005 --> 00:31:13.205 b h three is was

NOTE Confidence: 0.98498833

00:31:13.205 --> 00:31:15.385 essentially identical to the triggering

NOTE Confidence: 0.9916294

00:31:15.924 --> 00:31:17.145 b h three sequence.

NOTE Confidence: 0.99781185

00:31:18.565 --> 00:31:19.684 You could see that here.

NOTE Confidence: 0.99781185

00:31:19.684 --> 00:31:20.645 And so we thought, you

NOTE Confidence: 0.99781185

00:31:20.645 --> 00:31:22.565 know, maybe the mechanism is

NOTE Confidence: 0.99781185

00:31:22.565 --> 00:31:24.470 that BIM comes in, literally

NOTE Confidence: 0.99781185

00:31:24.470 --> 00:31:25.750 lights the match, goes to

NOTE Confidence: 0.99781185

00:31:25.750 --> 00:31:26.650 that new site,

NOTE Confidence: 0.9978175

00:31:27.670 --> 00:31:28.810 activates the protein,

NOTE Confidence: 0.9440513

00:31:29.190 --> 00:31:30.310 backs his b h three

NOTE Confidence: 0.9440513

00:31:30.310 --> 00:31:31.670 pops out. And now backs

NOTE Confidence: 0.9440513

00:31:31.670 --> 00:31:32.630 his b h three can

NOTE Confidence: 0.9440513

00:31:32.630 --> 00:31:34.390 function like the initiating BIM

NOTE Confidence: 0.9440513

00:31:34.390 --> 00:31:35.350 b h three, and then

NOTE Confidence: 0.9440513

00:31:35.350 --> 00:31:36.870 it just activates itself from

NOTE Confidence: 0.9440513

00:31:36.870 --> 00:31:37.610 there on.

NOTE Confidence: 0.9876502

00:31:38.164 --> 00:31:39.365 That was the idea. And

NOTE Confidence: 0.9876502

00:31:39.365 --> 00:31:40.164 then we're trying to figure

NOTE Confidence: 0.9876502

00:31:40.164 --> 00:31:40.885 out how in the world

NOTE Confidence: 0.9876502

00:31:40.885 --> 00:31:41.684 we were going to prove

NOTE Confidence: 0.9876502

00:31:41.684 --> 00:31:42.184 this.

NOTE Confidence: 0.94629127

00:31:42.485 --> 00:31:43.605 And so what we decided

NOTE Confidence: 0.94629127

00:31:43.605 --> 00:31:44.645 to do was, like, a

NOTE Confidence: 0.94629127

00:31:44.645 --> 00:31:45.544 reverse complementary

NOTE Confidence: 0.929121

00:31:45.845 --> 00:31:46.345 mutagenesis

NOTE Confidence: 0.9968623

00:31:46.645 --> 00:31:47.145 experiment

NOTE Confidence: 0.92461807

00:31:47.605 --> 00:31:49.044 because we knew from the

NOTE Confidence: 0.92461807

00:31:49.044 --> 00:31:50.389 BIM b h three BACS

NOTE Confidence: 0.92461807

00:31:50.389 --> 00:31:51.429 interaction that there was this

NOTE Confidence: 0.92461807

00:31:51.429 --> 00:31:52.870 very important k twenty one

NOTE Confidence: 0.92461807

00:31:52.870 --> 00:31:54.250 e sixty nine interaction.

NOTE Confidence: 0.9641772

00:31:54.950 --> 00:31:56.549 And we thought, well, if

NOTE Confidence: 0.9641772

00:31:56.549 --> 00:31:57.909 we could activate BACS in

NOTE Confidence: 0.9641772

00:31:57.909 --> 00:31:59.070 another way that didn't involve

NOTE Confidence: 0.9641772

00:31:59.070 --> 00:31:59.769 a ligand,

NOTE Confidence: 0.9967958

00:32:00.389 --> 00:32:01.830 right, we could see if

NOTE Confidence: 0.9967958

00:32:01.830 --> 00:32:03.029 maybe these two things would

NOTE Confidence: 0.9967958

00:32:03.029 --> 00:32:04.309 touch each other between two

NOTE Confidence: 0.9967958

00:32:04.309 --> 00:32:05.685 activated forms of BACS.

NOTE Confidence: 0.9385809

00:32:06.245 --> 00:32:07.525 And Doug Green at Saint

NOTE Confidence: 0.9385809

00:32:07.525 --> 00:32:08.645 Jude had published this great

NOTE Confidence: 0.9385809

00:32:08.645 --> 00:32:09.945 paper saying that, you know,

NOTE Confidence: 0.9385809

00:32:10.005 --> 00:32:12.005 heat activates backs, which makes

NOTE Confidence: 0.9385809

00:32:12.005 --> 00:32:13.385 sense because it's a confirmationally

NOTE Confidence: 0.9606963

00:32:14.245 --> 00:32:16.245 kind of labile protein. So

NOTE Confidence: 0.9606963

00:32:16.245 --> 00:32:16.965 you heat it up and

NOTE Confidence: 0.9606963

00:32:16.965 --> 00:32:17.925 you start allowing it to

NOTE Confidence: 0.9606963

00:32:17.925 --> 00:32:18.805 breathe, and then, you know,

NOTE Confidence: 0.9606963

00:32:18.805 --> 00:32:20.085 it would activate itself. So

NOTE Confidence: 0.9606963

00:32:20.085 --> 00:32:21.205 we thought, okay. That's gonna

NOTE Confidence: 0.9606963

00:32:21.205 --> 00:32:22.480 be the system we'll try.

NOTE Confidence: 0.9340002

00:32:23.020 --> 00:32:23.980 So we tried it and

NOTE Confidence: 0.9340002

00:32:23.980 --> 00:32:25.180 we just gave plain old

NOTE Confidence: 0.9340002

00:32:25.180 --> 00:32:26.460 wild type backs. We heated

NOTE Confidence: 0.9340002

00:32:26.460 --> 00:32:27.740 it up and over time

NOTE Confidence: 0.9340002

00:32:27.740 --> 00:32:29.180 it started to aligmarize, which

NOTE Confidence: 0.9340002

00:32:29.180 --> 00:32:30.380 is exactly what Doug had

NOTE Confidence: 0.9340002

00:32:30.380 --> 00:32:31.120 had published.

NOTE Confidence: 0.97988015

00:32:31.660 --> 00:32:33.180 And then we mutated one

NOTE Confidence: 0.97988015

00:32:33.180 --> 00:32:34.460 of these residues at a

NOTE Confidence: 0.97988015

00:32:34.460 --> 00:32:35.680 time. And if you mutated

NOTE Confidence: 0.97988015

00:32:35.820 --> 00:32:37.635 one of these electrostatic pairs,

NOTE Confidence: 0.9978303

00:32:38.515 --> 00:32:39.015 nothing.

NOTE Confidence: 0.93180573

00:32:40.035 --> 00:32:41.575 If you swap the position

NOTE Confidence: 0.93180573

00:32:41.635 --> 00:32:43.015 and did a reverse complementary

NOTE Confidence: 0.93180573

00:32:43.155 --> 00:32:44.435 mutagenesis, you were back to

NOTE Confidence: 0.93180573

00:32:44.435 --> 00:32:45.095 the beginning.

NOTE Confidence: 0.96431893

00:32:46.275 --> 00:32:47.475 And again, this was such

NOTE Confidence: 0.96431893

00:32:47.475 --> 00:32:48.515 a simple experiment. It was

NOTE Confidence: 0.96431893

00:32:48.515 --> 00:32:49.815 one of my favorite experiments

NOTE Confidence: 0.96431893

00:32:49.875 --> 00:32:51.095 from this paper because,

NOTE Confidence: 0.92343587

00:32:51.799 --> 00:32:52.679 you know, all you did

NOTE Confidence: 0.92343587

00:32:52.679 --> 00:32:53.480 was move the e to

NOTE Confidence: 0.92343587

00:32:53.480 --> 00:32:54.200 the other guy and move

NOTE Confidence: 0.92343587

00:32:54.200 --> 00:32:54.840 the k to the other

NOTE Confidence: 0.92343587

00:32:54.840 --> 00:32:55.480 guy, and then all of

NOTE Confidence: 0.92343587

00:32:55.480 --> 00:32:56.600 a sudden you restored wild

NOTE Confidence: 0.92343587

00:32:56.600 --> 00:32:57.880 type activity. So clearly, they

NOTE Confidence: 0.92343587

00:32:57.880 --> 00:32:58.919 were touching each other and

NOTE Confidence: 0.92343587

00:32:58.919 --> 00:33:00.679 clearly they could activate BAX

NOTE Confidence: 0.92343587

00:33:00.679 --> 00:33:03.020 could autoactivate itself and explained

NOTE Confidence: 0.92343587

00:33:03.159 --> 00:33:04.440 a really elegant paper done

NOTE Confidence: 0.92343587

00:33:04.440 --> 00:33:06.280 by David Andrews' group where

NOTE Confidence: 0.92343587

00:33:06.280 --> 00:33:07.725 they actually would take the

NOTE Confidence: 0.92343587

00:33:07.725 --> 00:33:09.325 BIDDH three, they put it

NOTE Confidence: 0.92343587

00:33:09.325 --> 00:33:10.684 on to BAX, you know,

NOTE Confidence: 0.92343587

00:33:10.684 --> 00:33:11.965 on a liposome, and then

NOTE Confidence: 0.92343587

00:33:11.965 --> 00:33:12.545 they would

NOTE Confidence: 0.9872994

00:33:12.924 --> 00:33:14.304 freeze stop the experiment,

NOTE Confidence: 0.98505604

00:33:14.765 --> 00:33:16.205 take away the supernatant. Right?

NOTE Confidence: 0.98505604

00:33:16.205 --> 00:33:17.325 They would do an immediate

NOTE Confidence: 0.98505604

00:33:17.325 --> 00:33:18.125 spin down, get rid of

NOTE Confidence: 0.98505604

00:33:18.125 --> 00:33:19.005 all the ligand, and they

NOTE Confidence: 0.98505604

00:33:19.005 --> 00:33:20.205 would have their pellet. And

NOTE Confidence: 0.98505604

00:33:20.205 --> 00:33:20.924 then they would take the

NOTE Confidence: 0.98505604

00:33:20.924 --> 00:33:22.045 pellet and just add more

NOTE Confidence: 0.98505604

00:33:22.045 --> 00:33:23.820 inactive BAX to it, and

NOTE Confidence: 0.98505604

00:33:23.820 --> 00:33:24.880 it always worked.

NOTE Confidence: 0.9724411

00:33:25.260 --> 00:33:26.539 Right? And the reason why

NOTE Confidence: 0.9724411

00:33:26.539 --> 00:33:28.059 it worked was because there

NOTE Confidence: 0.9724411

00:33:28.059 --> 00:33:29.100 was active backs in the

NOTE Confidence: 0.9724411

00:33:29.100 --> 00:33:30.220 membrane already, and then you

NOTE Confidence: 0.9724411

00:33:30.220 --> 00:33:31.500 added inactive backs and the

NOTE Confidence: 0.9724411

00:33:31.500 --> 00:33:33.500 backs activated itself. So it

NOTE Confidence: 0.9724411

00:33:33.500 --> 00:33:34.400 explained that.

NOTE Confidence: 0.983859

00:33:35.659 --> 00:33:36.700 So here's kind of where

NOTE Confidence: 0.983859

00:33:36.700 --> 00:33:37.279 we were.

NOTE Confidence: 0.92150927

00:33:37.815 --> 00:33:38.554 Hand grenade,

NOTE Confidence: 0.97778904

00:33:38.934 --> 00:33:40.934 totally quiescent, nothing going on.

NOTE Confidence: 0.97778904

00:33:40.934 --> 00:33:42.375 You have a ligand in

NOTE Confidence: 0.97778904

00:33:42.375 --> 00:33:43.514 response to stress.

NOTE Confidence: 0.9629285

00:33:43.815 --> 00:33:45.335 It pops the loop. It

NOTE Confidence: 0.9629285

00:33:45.335 --> 00:33:47.174 changes the confirmation. Alpha nine

NOTE Confidence: 0.9629285

00:33:47.174 --> 00:33:48.534 pops out. Alpha three pops

NOTE Confidence: 0.9629285

00:33:48.534 --> 00:33:49.815 out. B h three pops

NOTE Confidence: 0.9629285

00:33:49.815 --> 00:33:51.340 out, goes to the mitochondria,

NOTE Confidence: 0.9629285

00:33:51.560 --> 00:33:52.680 and now it can start

NOTE Confidence: 0.9629285

00:33:52.680 --> 00:33:53.800 and activate more and more

NOTE Confidence: 0.9629285

00:33:53.800 --> 00:33:54.680 and more and trigger this

NOTE Confidence: 0.9629285

00:33:54.680 --> 00:33:55.420 chain reaction

NOTE Confidence: 0.9538191

00:33:55.800 --> 00:33:56.520 to the point where you

NOTE Confidence: 0.9538191

00:33:56.520 --> 00:33:58.120 have a critical mass of

NOTE Confidence: 0.9538191

00:33:58.120 --> 00:33:59.240 backs at the outer membrane

NOTE Confidence: 0.9538191

00:33:59.240 --> 00:34:00.140 and you get permeabilization.

NOTE Confidence: 0.99423474

00:34:01.320 --> 00:34:02.540 So that's where we were.

NOTE Confidence: 0.99423474

00:34:02.760 --> 00:34:03.980 Canonical pocket

NOTE Confidence: 0.9996634

00:34:04.385 --> 00:34:05.285 was not accessible

NOTE Confidence: 0.9720061

00:34:05.905 --> 00:34:07.265 to b h threes because

NOTE Confidence: 0.9720061

00:34:07.265 --> 00:34:08.545 the alpha nine stuffed in

NOTE Confidence: 0.9720061

00:34:08.545 --> 00:34:09.825 it. So that made a

NOTE Confidence: 0.9720061

00:34:09.825 --> 00:34:11.265 lot of sense. And so

NOTE Confidence: 0.9720061

00:34:11.265 --> 00:34:13.025 the actual trigger site was

NOTE Confidence: 0.9720061

00:34:13.025 --> 00:34:14.165 on the opposite side.

NOTE Confidence: 0.96475637

00:34:14.625 --> 00:34:15.665 Now here's a very kind

NOTE Confidence: 0.96475637

00:34:15.665 --> 00:34:16.705 of in the weeds subtle

NOTE Confidence: 0.96475637

00:34:16.705 --> 00:34:17.205 point.

NOTE Confidence: 0.952111

00:34:18.320 --> 00:34:19.440 If the b h three

NOTE Confidence: 0.952111

00:34:19.440 --> 00:34:20.739 is bound to anti apoptotic

NOTE Confidence: 0.952111

00:34:20.800 --> 00:34:21.300 proteins,

NOTE Confidence: 0.99392617

00:34:22.960 --> 00:34:24.160 why wouldn't they bind to

NOTE Confidence: 0.99392617

00:34:24.160 --> 00:34:24.960 this other site? I mean,

NOTE Confidence: 0.99392617

00:34:24.960 --> 00:34:25.840 it's sitting there. I drew

NOTE Confidence: 0.99392617

00:34:25.840 --> 00:34:26.900 it for you in orange.

NOTE Confidence: 0.9851671

00:34:27.440 --> 00:34:28.320 And it turns out the

NOTE Confidence: 0.9851671

00:34:28.320 --> 00:34:29.520 difference between the two sites,

NOTE Confidence: 0.9851671

00:34:29.520 --> 00:34:30.320 and you can see this

NOTE Confidence: 0.9851671

00:34:30.320 --> 00:34:31.119 with your own eyes right

NOTE Confidence: 0.9851671

00:34:31.119 --> 00:34:32.480 here, the red site is

NOTE Confidence: 0.9851671

00:34:32.480 --> 00:34:33.300 very deep.

NOTE Confidence: 0.9645587

00:34:33.924 --> 00:34:35.545 The orange site is shallow.

NOTE Confidence: 0.9739231

00:34:36.005 --> 00:34:37.684 And it turned out that

NOTE Confidence: 0.9739231

00:34:37.684 --> 00:34:39.144 a prefolded helix

NOTE Confidence: 0.98319834

00:34:39.525 --> 00:34:40.565 was needed to bind to

NOTE Confidence: 0.98319834

00:34:40.565 --> 00:34:42.085 the trigger site, but you

NOTE Confidence: 0.98319834

00:34:42.085 --> 00:34:43.525 didn't need a prefolded helix

NOTE Confidence: 0.98319834

00:34:43.525 --> 00:34:44.724 to bind to the canonical

NOTE Confidence: 0.98319834

00:34:44.724 --> 00:34:46.005 one because it was deeper

NOTE Confidence: 0.98319834

00:34:46.005 --> 00:34:47.525 and the, you know, induced

NOTE Confidence: 0.98319834

00:34:47.525 --> 00:34:49.305 folding was much more powerful.

NOTE Confidence: 0.98473245

00:34:49.690 --> 00:34:50.570 And so it just so

NOTE Confidence: 0.98473245

00:34:50.570 --> 00:34:51.950 happened that having a prefolded

NOTE Confidence: 0.98473245

00:34:52.090 --> 00:34:52.590 helix

NOTE Confidence: 0.9881125

00:34:52.890 --> 00:34:54.090 was what you needed to

NOTE Confidence: 0.9881125

00:34:54.090 --> 00:34:55.610 detect that other site because

NOTE Confidence: 0.9881125

00:34:55.610 --> 00:34:56.670 there wasn't enough

NOTE Confidence: 0.98977554

00:34:56.969 --> 00:34:58.730 interaction to cause induced folding

NOTE Confidence: 0.98977554

00:34:58.730 --> 00:34:59.469 at that site.

NOTE Confidence: 0.9546031

00:35:00.010 --> 00:35:01.710 But t bid, the ligand,

NOTE Confidence: 0.9546031

00:35:01.930 --> 00:35:03.450 is a highly structured protein.

NOTE Confidence: 0.9546031

00:35:03.450 --> 00:35:04.250 The b h three is

NOTE Confidence: 0.9546031

00:35:04.250 --> 00:35:05.150 already structured.

NOTE Confidence: 0.9402785

00:35:06.545 --> 00:35:07.424 So then at the same

NOTE Confidence: 0.9402785

00:35:07.424 --> 00:35:08.545 time, we're like, well, this

NOTE Confidence: 0.9402785

00:35:08.545 --> 00:35:10.065 could be a druggable binding

NOTE Confidence: 0.9402785

00:35:10.065 --> 00:35:11.344 site for turning on backs

NOTE Confidence: 0.9402785

00:35:11.344 --> 00:35:12.085 in cancer.

NOTE Confidence: 0.96732384

00:35:12.545 --> 00:35:13.424 But, again, we had a

NOTE Confidence: 0.96732384

00:35:13.424 --> 00:35:14.704 lot of limitations. And the

NOTE Confidence: 0.96732384

00:35:14.704 --> 00:35:16.625 limitation the biggest limitation was

NOTE Confidence: 0.96732384

00:35:16.625 --> 00:35:17.505 you couldn't make a ton

NOTE Confidence: 0.96732384

00:35:17.505 --> 00:35:18.690 of backs. And like I've

NOTE Confidence: 0.96732384

00:35:18.690 --> 00:35:19.489 been saying, it's not the

NOTE Confidence: 0.96732384

00:35:19.489 --> 00:35:20.609 most stable thing. So if

NOTE Confidence: 0.96732384

00:35:20.609 --> 00:35:21.570 you're going to do a

NOTE Confidence: 0.96732384

00:35:21.570 --> 00:35:23.010 drug screen, you know, with

NOTE Confidence: 0.96732384

00:35:23.090 --> 00:35:23.730 and you need a ton

NOTE Confidence: 0.96732384

00:35:23.730 --> 00:35:24.690 of ton of backs, it's,

NOTE Confidence: 0.96732384

00:35:24.690 --> 00:35:26.130 like, very difficult because it's,

NOTE Confidence: 0.96732384

00:35:26.290 --> 00:35:27.090 now you see it, now

NOTE Confidence: 0.96732384

00:35:27.090 --> 00:35:28.550 you don't. And so

NOTE Confidence: 0.96520555

00:35:28.930 --> 00:35:30.369 we later conquered that problem.

NOTE Confidence: 0.96520555

00:35:30.369 --> 00:35:31.815 But but at the beginning

NOTE Confidence: 0.96520555

00:35:31.815 --> 00:35:32.855 days, we're like, well, we're

NOTE Confidence: 0.96520555

00:35:32.855 --> 00:35:33.494 gonna have to do in

NOTE Confidence: 0.96520555

00:35:33.494 --> 00:35:34.614 silico screening. Now look at

NOTE Confidence: 0.96520555

00:35:34.614 --> 00:35:36.215 the date. So the until

NOTE Confidence: 0.96520555

00:35:36.295 --> 00:35:37.094 you know, the paper was

NOTE Confidence: 0.96520555

00:35:37.094 --> 00:35:38.135 twenty twelve, which means, you

NOTE Confidence: 0.96520555

00:35:38.135 --> 00:35:39.335 know, we probably started this

NOTE Confidence: 0.96520555

00:35:39.735 --> 00:35:40.935 and we started this pretty

NOTE Confidence: 0.96520555

00:35:40.935 --> 00:35:41.975 much right when we had

NOTE Confidence: 0.96520555

00:35:41.975 --> 00:35:43.094 solved the complex, I would

NOTE Confidence: 0.96520555

00:35:43.094 --> 00:35:44.455 say, two thousand and nine,

NOTE Confidence: 0.96520555

00:35:44.455 --> 00:35:44.955 maybe.

NOTE Confidence: 0.9835997

00:35:45.400 --> 00:35:46.760 We started doing in silico

NOTE Confidence: 0.9835997

00:35:46.760 --> 00:35:47.560 screening to try to figure

NOTE Confidence: 0.9835997

00:35:47.560 --> 00:35:48.440 out if we could find

NOTE Confidence: 0.9835997

00:35:48.440 --> 00:35:49.640 something. And and the tools

NOTE Confidence: 0.9835997

00:35:49.640 --> 00:35:50.440 back then were not like

NOTE Confidence: 0.9835997

00:35:50.440 --> 00:35:51.560 they are today. That's for

NOTE Confidence: 0.9835997

00:35:51.560 --> 00:35:52.300 sure. But,

NOTE Confidence: 0.9367711

00:35:52.680 --> 00:35:53.560 I think we got very

NOTE Confidence: 0.9367711

00:35:53.560 --> 00:35:54.940 lucky. And and and Everest,

NOTE Confidence: 0.9367711

00:35:55.000 --> 00:35:55.960 who's the post doc doing

NOTE Confidence: 0.9367711

00:35:55.960 --> 00:35:57.239 this work, had actually spent

NOTE Confidence: 0.9367711

00:35:57.239 --> 00:35:58.200 time at a company in

NOTE Confidence: 0.9367711

00:35:58.200 --> 00:35:58.700 England,

NOTE Confidence: 0.96421844

00:35:59.239 --> 00:36:00.280 before he did his post

NOTE Confidence: 0.96421844

00:36:00.280 --> 00:36:01.045 doc doing,

NOTE Confidence: 0.9866574

00:36:01.445 --> 00:36:03.285 in silico screening using novel

NOTE Confidence: 0.9866574

00:36:03.285 --> 00:36:04.325 methods. And so that was

NOTE Confidence: 0.9866574

00:36:04.325 --> 00:36:05.925 a very useful skill that

NOTE Confidence: 0.9866574

00:36:05.925 --> 00:36:06.885 he brought to the table

NOTE Confidence: 0.9866574

00:36:06.885 --> 00:36:08.245 here. And so he did

NOTE Confidence: 0.9866574

00:36:08.245 --> 00:36:09.545 that in silico screen,

NOTE Confidence: 0.9780008

00:36:09.844 --> 00:36:10.885 and we found a bunch

NOTE Confidence: 0.9780008

00:36:10.885 --> 00:36:12.005 of compounds. And we just

NOTE Confidence: 0.9780008

00:36:12.005 --> 00:36:13.364 did a competitive binding assay

NOTE Confidence: 0.9780008

00:36:13.364 --> 00:36:14.965 against our fluorescent b h

NOTE Confidence: 0.9780008

00:36:14.965 --> 00:36:16.710 three peptide to see which,

NOTE Confidence: 0.9780008

00:36:16.710 --> 00:36:17.590 if any, of them could

NOTE Confidence: 0.9780008

00:36:17.590 --> 00:36:18.489 actually displace.

NOTE Confidence: 0.9860904

00:36:19.110 --> 00:36:20.489 And it turned out that,

NOTE Confidence: 0.9860904

00:36:20.710 --> 00:36:21.590 you know, most of them

NOTE Confidence: 0.9860904

00:36:21.590 --> 00:36:22.550 didn't work, and a few

NOTE Confidence: 0.9860904

00:36:22.550 --> 00:36:23.369 of them did.

NOTE Confidence: 0.9740328

00:36:24.230 --> 00:36:25.350 And, you know, we then

NOTE Confidence: 0.9740328

00:36:25.350 --> 00:36:26.469 went ahead and tried a

NOTE Confidence: 0.9740328

00:36:26.469 --> 00:36:28.150 dose responsive experiment. And most

NOTE Confidence: 0.9740328

00:36:28.150 --> 00:36:29.430 of them that did actually

NOTE Confidence: 0.9740328

00:36:29.430 --> 00:36:31.350 compete were quite weak except

NOTE Confidence: 0.9740328

00:36:31.350 --> 00:36:31.875 for one.

NOTE Confidence: 0.9847399

00:36:32.995 --> 00:36:34.035 And, you know, we had

NOTE Confidence: 0.9847399

00:36:34.035 --> 00:36:35.395 gotten this from a library

NOTE Confidence: 0.9847399

00:36:35.395 --> 00:36:36.435 that came with, you know,

NOTE Confidence: 0.9847399

00:36:36.435 --> 00:36:37.555 tons and tons of compounds.

NOTE Confidence: 0.9847399

00:36:37.555 --> 00:36:38.675 So to make sure about

NOTE Confidence: 0.9847399

00:36:38.675 --> 00:36:39.635 the integrity of this thing,

NOTE Confidence: 0.9847399

00:36:39.635 --> 00:36:41.475 we resynthesized it ourselves, repeated

NOTE Confidence: 0.9847399

00:36:41.475 --> 00:36:42.135 the experiment,

NOTE Confidence: 0.98806524

00:36:42.435 --> 00:36:44.055 and the compound was legit.

NOTE Confidence: 0.89633507

00:36:45.150 --> 00:36:46.030 And that's what it looked

NOTE Confidence: 0.89633507

00:36:46.030 --> 00:36:47.650 like. Pretty greasy compound.

NOTE Confidence: 0.96501905

00:36:48.030 --> 00:36:49.069 And we went ahead and

NOTE Confidence: 0.96501905

00:36:49.069 --> 00:36:50.349 did the NMR. We repeated

NOTE Confidence: 0.96501905

00:36:50.349 --> 00:36:51.390 all the experiments that we

NOTE Confidence: 0.96501905

00:36:51.390 --> 00:36:52.030 had done with the b

NOTE Confidence: 0.96501905

00:36:52.030 --> 00:36:52.829 h three, but now with

NOTE Confidence: 0.96501905

00:36:52.829 --> 00:36:53.950 this molecule, and most of

NOTE Confidence: 0.96501905

00:36:53.950 --> 00:36:55.150 the results were identical. So

NOTE Confidence: 0.96501905

00:36:55.150 --> 00:36:56.609 here's, you know, the

NOTE Confidence: 0.93225235

00:36:56.910 --> 00:36:58.495 the chemical shift perturbations that

NOTE Confidence: 0.93225235

00:36:58.495 --> 00:37:00.015 occur. Again, alpha one, alpha

NOTE Confidence: 0.93225235

00:37:00.015 --> 00:37:00.515 six.

NOTE Confidence: 0.9816302

00:37:01.215 --> 00:37:02.415 You see where the the

NOTE Confidence: 0.9816302

00:37:02.415 --> 00:37:03.935 docking suggested that it was

NOTE Confidence: 0.9816302

00:37:03.935 --> 00:37:05.295 disposed, that k twenty one

NOTE Confidence: 0.9816302

00:37:05.295 --> 00:37:06.495 residue, which we found was

NOTE Confidence: 0.9816302

00:37:06.495 --> 00:37:07.875 very important with the natural

NOTE Confidence: 0.9816302

00:37:08.015 --> 00:37:09.695 interaction, again, was very much

NOTE Confidence: 0.9816302

00:37:09.695 --> 00:37:10.915 part of how the molecule

NOTE Confidence: 0.9816302

00:37:10.975 --> 00:37:11.475 interacted,

NOTE Confidence: 0.9855115

00:37:12.015 --> 00:37:12.810 at this site.

NOTE Confidence: 0.9341225

00:37:13.290 --> 00:37:13.930 And then we did a

NOTE Confidence: 0.9341225

00:37:13.930 --> 00:37:15.930 oligomerization assays and showed that

NOTE Confidence: 0.9341225

00:37:15.930 --> 00:37:16.810 you could get, you know,

NOTE Confidence: 0.9341225

00:37:16.810 --> 00:37:17.550 dose responsive

NOTE Confidence: 0.8459063

00:37:17.850 --> 00:37:18.890 triggering of BAX in a

NOTE Confidence: 0.8459063

00:37:18.890 --> 00:37:20.350 liposomal release assay.

NOTE Confidence: 0.99080765

00:37:20.810 --> 00:37:21.530 But if you had a

NOTE Confidence: 0.99080765

00:37:21.530 --> 00:37:22.670 ligand that,

NOTE Confidence: 0.9560959

00:37:23.130 --> 00:37:24.090 looked a lot like the

NOTE Confidence: 0.9560959

00:37:24.090 --> 00:37:25.690 triggering one but did not

NOTE Confidence: 0.9560959

00:37:25.690 --> 00:37:26.810 do anything in this assay,

NOTE Confidence: 0.9560959

00:37:26.810 --> 00:37:27.975 it didn't do anything either.

NOTE Confidence: 0.9720226

00:37:28.695 --> 00:37:29.815 And then if you mutated

NOTE Confidence: 0.9720226

00:37:29.815 --> 00:37:31.095 that lysine twenty one on

NOTE Confidence: 0.9720226

00:37:31.095 --> 00:37:32.695 VAX, which doesn't have any

NOTE Confidence: 0.9720226

00:37:32.695 --> 00:37:34.535 endogenous activity, you also saw

NOTE Confidence: 0.9720226

00:37:34.535 --> 00:37:35.515 much, much blunted

NOTE Confidence: 0.95532364

00:37:36.135 --> 00:37:37.755 activation by the small molecule.

NOTE Confidence: 0.95532364

00:37:37.895 --> 00:37:38.775 So it looked like it

NOTE Confidence: 0.95532364

00:37:38.775 --> 00:37:40.055 was obeying a lot of

NOTE Confidence: 0.95532364

00:37:40.055 --> 00:37:40.955 the same criteria,

NOTE Confidence: 0.99792624

00:37:41.735 --> 00:37:42.935 that we had established for

NOTE Confidence: 0.99792624

00:37:42.935 --> 00:37:43.595 the peptide.

NOTE Confidence: 0.9660899

00:37:44.190 --> 00:37:44.989 And then, you know, among

NOTE Confidence: 0.9660899

00:37:44.989 --> 00:37:46.349 the more exciting results is

NOTE Confidence: 0.9660899

00:37:46.349 --> 00:37:47.390 that if you took cells

NOTE Confidence: 0.9660899

00:37:47.390 --> 00:37:48.829 that had BAX in it

NOTE Confidence: 0.9660899

00:37:48.829 --> 00:37:50.750 but no BAX, you saw,

NOTE Confidence: 0.9660899

00:37:50.989 --> 00:37:51.810 dose responsive,

NOTE Confidence: 0.97998005

00:37:52.349 --> 00:37:54.190 induction of cell death. But

NOTE Confidence: 0.97998005

00:37:54.190 --> 00:37:55.230 if it didn't have BAX

NOTE Confidence: 0.97998005

00:37:55.230 --> 00:37:56.109 in it, then the cell

NOTE Confidence: 0.97998005

00:37:56.109 --> 00:37:57.310 didn't respond. And if it

NOTE Confidence: 0.97998005

00:37:57.310 --> 00:37:58.545 didn't have BAX or BAX,

NOTE Confidence: 0.97998005

00:37:58.545 --> 00:37:59.505 of course, it then didn't

NOTE Confidence: 0.97998005

00:37:59.505 --> 00:38:00.944 respond either as the ultimate

NOTE Confidence: 0.97998005

00:38:00.944 --> 00:38:01.844 negative control.

NOTE Confidence: 0.97012824

00:38:02.305 --> 00:38:03.344 And, again, you could look

NOTE Confidence: 0.97012824

00:38:03.344 --> 00:38:04.645 at this under the microscope,

NOTE Confidence: 0.91112155

00:38:05.505 --> 00:38:07.665 and so satisfying to actually

NOTE Confidence: 0.91112155

00:38:07.665 --> 00:38:08.405 just like

NOTE Confidence: 0.90605855

00:38:08.944 --> 00:38:09.744 I I have to say,

NOTE Confidence: 0.90605855

00:38:09.744 --> 00:38:11.105 by the way, histology and

NOTE Confidence: 0.90605855

00:38:11.105 --> 00:38:12.030 I'm not saying this because

NOTE Confidence: 0.90605855

00:38:12.030 --> 00:38:12.830 of the crowd because you

NOTE Confidence: 0.90605855

00:38:12.830 --> 00:38:14.110 can valid you could verify

NOTE Confidence: 0.90605855

00:38:14.110 --> 00:38:15.570 my comments here. Histology

NOTE Confidence: 0.96205515

00:38:15.870 --> 00:38:17.310 was always my favorite subject

NOTE Confidence: 0.96205515

00:38:17.310 --> 00:38:18.430 in med school. I TA

NOTE Confidence: 0.96205515

00:38:18.430 --> 00:38:19.730 histology and histopathology

NOTE Confidence: 0.9685967

00:38:20.030 --> 00:38:21.390 at Hopkins for years all

NOTE Confidence: 0.9685967

00:38:21.390 --> 00:38:22.530 throughout grad school.

NOTE Confidence: 0.9714921

00:38:22.830 --> 00:38:23.969 And a lot of times

NOTE Confidence: 0.9714921

00:38:24.190 --> 00:38:25.214 and and so everyone in

NOTE Confidence: 0.9714921

00:38:25.214 --> 00:38:26.654 this room really enjoys seeing

NOTE Confidence: 0.9714921

00:38:26.654 --> 00:38:27.614 things with their own eyes.

NOTE Confidence: 0.9714921

00:38:27.614 --> 00:38:28.494 But a lot of times,

NOTE Confidence: 0.9714921

00:38:28.494 --> 00:38:30.094 people don't look, you know,

NOTE Confidence: 0.9714921

00:38:30.094 --> 00:38:31.454 at their experiment. Like, you

NOTE Confidence: 0.9714921

00:38:31.454 --> 00:38:32.255 know, they'll do a ninety

NOTE Confidence: 0.9714921

00:38:32.255 --> 00:38:33.295 six well plate or something.

NOTE Confidence: 0.9714921

00:38:33.295 --> 00:38:34.015 It's like, did you look

NOTE Confidence: 0.9714921

00:38:34.015 --> 00:38:34.815 at the cells? What do

NOTE Confidence: 0.9714921

00:38:34.815 --> 00:38:35.454 they look like? Oh, I

NOTE Confidence: 0.9714921

00:38:35.454 --> 00:38:36.255 didn't no. I just put

NOTE Confidence: 0.9714921

00:38:36.255 --> 00:38:36.989 it in the plate reader.

NOTE Confidence: 0.97355366

00:38:37.710 --> 00:38:38.510 And when this was happening,

NOTE Confidence: 0.97355366

00:38:38.510 --> 00:38:39.070 I was like, you know,

NOTE Confidence: 0.97355366

00:38:39.070 --> 00:38:40.030 it'd be kinda nice to

NOTE Confidence: 0.97355366

00:38:40.030 --> 00:38:41.070 look at this and see

NOTE Confidence: 0.97355366

00:38:41.070 --> 00:38:42.210 if it looks like apoptosis

NOTE Confidence: 0.97355366

00:38:42.430 --> 00:38:43.469 as opposed to God knows

NOTE Confidence: 0.97355366

00:38:43.469 --> 00:38:44.130 what else.

NOTE Confidence: 0.97223705

00:38:44.510 --> 00:38:45.710 And so for me, I

NOTE Confidence: 0.97223705

00:38:45.710 --> 00:38:46.830 love this experiment because I

NOTE Confidence: 0.97223705

00:38:46.830 --> 00:38:47.950 just went to the, you

NOTE Confidence: 0.97223705

00:38:47.950 --> 00:38:49.469 know, microscope and, you know,

NOTE Confidence: 0.97223705

00:38:49.469 --> 00:38:51.070 face contrast microscope and started

NOTE Confidence: 0.97223705

00:38:51.070 --> 00:38:52.085 taking pictures. I was like,

NOTE Confidence: 0.97223705

00:38:52.085 --> 00:38:53.285 that looks like really nice

NOTE Confidence: 0.97223705

00:38:53.285 --> 00:38:54.404 apoptosis, like, right out of

NOTE Confidence: 0.97223705

00:38:54.404 --> 00:38:55.924 a textbook, which was very

NOTE Confidence: 0.97223705

00:38:55.924 --> 00:38:56.424 reassuring.

NOTE Confidence: 0.96500015

00:38:56.805 --> 00:38:57.605 So, you know, I don't

NOTE Confidence: 0.96500015

00:38:57.605 --> 00:38:58.404 have to convince you a

NOTE Confidence: 0.96500015

00:38:58.404 --> 00:38:59.684 picture's worth a thousand words,

NOTE Confidence: 0.96500015

00:38:59.684 --> 00:39:00.484 you know. Yeah. I mean,

NOTE Confidence: 0.96500015

00:39:00.484 --> 00:39:01.864 that that it really is.

NOTE Confidence: 0.96729803

00:39:03.860 --> 00:39:05.380 So, you know, my message

NOTE Confidence: 0.96729803

00:39:05.380 --> 00:39:06.100 for this part of the

NOTE Confidence: 0.96729803

00:39:06.100 --> 00:39:07.460 talk, you know, what is

NOTE Confidence: 0.96729803

00:39:07.460 --> 00:39:07.960 that,

NOTE Confidence: 0.96847373

00:39:08.500 --> 00:39:09.300 you know, a lot of

NOTE Confidence: 0.96847373

00:39:09.300 --> 00:39:10.820 basic science, you know, went

NOTE Confidence: 0.96847373

00:39:10.820 --> 00:39:12.500 into figuring, you know, these

NOTE Confidence: 0.96847373

00:39:12.500 --> 00:39:14.100 steps out. And, you know,

NOTE Confidence: 0.96847373

00:39:14.100 --> 00:39:16.035 we kinda walked along from

NOTE Confidence: 0.96847373

00:39:16.194 --> 00:39:17.555 discovering the trigger site and

NOTE Confidence: 0.96847373

00:39:17.555 --> 00:39:18.674 then figuring out what all

NOTE Confidence: 0.96847373

00:39:18.674 --> 00:39:20.515 the downstream steps were and

NOTE Confidence: 0.96847373

00:39:20.515 --> 00:39:21.635 then figuring out if that

NOTE Confidence: 0.96847373

00:39:21.635 --> 00:39:23.075 site could be drugged. And

NOTE Confidence: 0.96847373

00:39:23.075 --> 00:39:24.594 then my postdoctoral fellow is

NOTE Confidence: 0.96847373

00:39:24.594 --> 00:39:25.795 now full professor of biochem

NOTE Confidence: 0.96847373

00:39:25.795 --> 00:39:26.994 at Albert Einstein, went on

NOTE Confidence: 0.96847373

00:39:26.994 --> 00:39:27.875 to work on this more

NOTE Confidence: 0.96847373

00:39:27.875 --> 00:39:28.994 and took it into animals

NOTE Confidence: 0.96847373

00:39:28.994 --> 00:39:29.635 and worked a lot on

NOTE Confidence: 0.96847373

00:39:29.635 --> 00:39:31.174 the pharmacology and the optimization.

NOTE Confidence: 0.9814439

00:39:33.210 --> 00:39:34.650 And that gives you an

NOTE Confidence: 0.9814439

00:39:34.650 --> 00:39:35.150 entire

NOTE Confidence: 0.9921724

00:39:35.690 --> 00:39:36.890 other arm of the cell

NOTE Confidence: 0.9921724

00:39:36.890 --> 00:39:37.630 death pathway

NOTE Confidence: 0.96976167

00:39:38.090 --> 00:39:39.790 to think about drugging. Right?

NOTE Confidence: 0.96976167

00:39:39.850 --> 00:39:40.350 There's

NOTE Confidence: 0.95208704

00:39:40.890 --> 00:39:42.090 billions and billions of dollars

NOTE Confidence: 0.95208704

00:39:42.090 --> 00:39:43.450 focused on the anti apoptotic

NOTE Confidence: 0.95208704

00:39:43.450 --> 00:39:44.570 side, but there's a whole

NOTE Confidence: 0.95208704

00:39:44.570 --> 00:39:46.145 another side to this pathway,

NOTE Confidence: 0.9828257

00:39:46.605 --> 00:39:47.905 and that's the pro apoptotic

NOTE Confidence: 0.9828257

00:39:47.965 --> 00:39:49.245 side. And, you know, we

NOTE Confidence: 0.9828257

00:39:49.245 --> 00:39:50.285 talked about this at dinner

NOTE Confidence: 0.9828257

00:39:50.285 --> 00:39:51.165 last night as well, but,

NOTE Confidence: 0.9828257

00:39:51.165 --> 00:39:51.665 like,

NOTE Confidence: 0.99773127

00:39:52.045 --> 00:39:53.085 you might have something that

NOTE Confidence: 0.99773127

00:39:53.085 --> 00:39:54.205 you're very excited about, but

NOTE Confidence: 0.99773127

00:39:54.205 --> 00:39:55.245 that doesn't mean that anyone

NOTE Confidence: 0.99773127

00:39:55.245 --> 00:39:56.125 else is going to get

NOTE Confidence: 0.99773127

00:39:56.125 --> 00:39:57.505 excited about it. Right?

NOTE Confidence: 0.97355586

00:39:57.940 --> 00:39:59.060 Youth may think you have,

NOTE Confidence: 0.97355586

00:39:59.060 --> 00:40:00.739 you know, great mechanism, great

NOTE Confidence: 0.97355586

00:40:00.739 --> 00:40:02.360 target, great prototype therapeutic,

NOTE Confidence: 0.955993

00:40:02.820 --> 00:40:04.100 but then the the the

NOTE Confidence: 0.955993

00:40:04.100 --> 00:40:06.040 bigger job and and sometimes

NOTE Confidence: 0.955993

00:40:06.260 --> 00:40:07.860 the job that never pays

NOTE Confidence: 0.955993

00:40:07.860 --> 00:40:09.860 off, not not literally, but

NOTE Confidence: 0.955993

00:40:09.860 --> 00:40:10.360 figuratively,

NOTE Confidence: 0.98205984

00:40:11.300 --> 00:40:12.660 is, like, getting someone to

NOTE Confidence: 0.98205984

00:40:12.660 --> 00:40:13.695 give it a shot because

NOTE Confidence: 0.98205984

00:40:13.695 --> 00:40:14.735 giving it a shot is

NOTE Confidence: 0.98205984

00:40:14.735 --> 00:40:16.575 incredibly expensive. And so, you

NOTE Confidence: 0.98205984

00:40:16.575 --> 00:40:17.455 know, I felt like my

NOTE Confidence: 0.98205984

00:40:17.455 --> 00:40:18.335 part was to kind of

NOTE Confidence: 0.98205984

00:40:18.335 --> 00:40:19.295 write a review and say,

NOTE Confidence: 0.98205984

00:40:19.295 --> 00:40:20.335 hey. VAX is a great

NOTE Confidence: 0.98205984

00:40:20.335 --> 00:40:21.395 target. You know?

NOTE Confidence: 0.97581166

00:40:22.895 --> 00:40:23.695 And and so I think,

NOTE Confidence: 0.97581166

00:40:23.695 --> 00:40:24.415 you know, there's a lot

NOTE Confidence: 0.97581166

00:40:24.415 --> 00:40:25.635 of, you know, sociology

NOTE Confidence: 0.9825915

00:40:25.935 --> 00:40:26.975 around how do you go

NOTE Confidence: 0.9825915

00:40:26.975 --> 00:40:29.210 from new discovery to new

NOTE Confidence: 0.9825915

00:40:29.210 --> 00:40:30.170 drug, and it may or

NOTE Confidence: 0.9825915

00:40:30.170 --> 00:40:31.930 may not happen. But, you

NOTE Confidence: 0.9825915

00:40:31.930 --> 00:40:32.969 know, I'm I'm one who

NOTE Confidence: 0.9825915

00:40:32.969 --> 00:40:34.170 believes that you just gotta

NOTE Confidence: 0.9825915

00:40:34.170 --> 00:40:35.369 keep trying. Right? If you

NOTE Confidence: 0.9825915

00:40:35.369 --> 00:40:36.410 just keep trying, you just

NOTE Confidence: 0.9825915

00:40:36.410 --> 00:40:37.530 keep trying. You know, you're

NOTE Confidence: 0.9825915

00:40:37.530 --> 00:40:38.890 destined to fail so many

NOTE Confidence: 0.9825915

00:40:38.890 --> 00:40:40.325 times, but if you give

NOTE Confidence: 0.9825915

00:40:40.325 --> 00:40:41.364 up with your first, second,

NOTE Confidence: 0.9825915

00:40:41.364 --> 00:40:42.484 or third failure, like, you

NOTE Confidence: 0.9825915

00:40:42.484 --> 00:40:43.444 know what the numbers are.

NOTE Confidence: 0.9825915

00:40:43.444 --> 00:40:44.325 You know it's a ninety

NOTE Confidence: 0.9825915

00:40:44.325 --> 00:40:45.605 nine percent failure rate. But

NOTE Confidence: 0.9825915

00:40:45.605 --> 00:40:46.664 there's a lot of blockbuster

NOTE Confidence: 0.9825915

00:40:46.805 --> 00:40:48.244 drugs. And, like, we started

NOTE Confidence: 0.9825915

00:40:48.244 --> 00:40:49.924 out talking about venetoclax. Like,

NOTE Confidence: 0.9825915

00:40:49.924 --> 00:40:51.844 that could so have easily

NOTE Confidence: 0.9825915

00:40:51.844 --> 00:40:53.364 been on the cutting room

NOTE Confidence: 0.9825915

00:40:53.364 --> 00:40:53.864 floor

NOTE Confidence: 0.92654574

00:40:55.000 --> 00:40:55.820 years ago,

NOTE Confidence: 0.96207666

00:40:56.120 --> 00:40:57.320 but it was or human

NOTE Confidence: 0.96207666

00:40:57.320 --> 00:40:59.239 beings that that kind of

NOTE Confidence: 0.96207666

00:40:59.239 --> 00:41:00.600 forced that to keep going.

NOTE Confidence: 0.96207666

00:41:00.600 --> 00:41:01.820 So, anyway,

NOTE Confidence: 0.97202843

00:41:02.360 --> 00:41:03.180 don't get discouraged.

NOTE Confidence: 0.9800915

00:41:05.400 --> 00:41:06.840 So then, you know, a

NOTE Confidence: 0.9800915

00:41:06.840 --> 00:41:07.880 big part of science also

NOTE Confidence: 0.9800915

00:41:07.880 --> 00:41:08.840 is, like, knowing what other

NOTE Confidence: 0.9800915

00:41:08.840 --> 00:41:09.820 people are doing.

NOTE Confidence: 0.9124233

00:41:10.335 --> 00:41:11.455 Right? You can't live in

NOTE Confidence: 0.9124233

00:41:11.455 --> 00:41:12.755 a like, I love facts,

NOTE Confidence: 0.97333384

00:41:13.455 --> 00:41:14.835 but, like, I can't just

NOTE Confidence: 0.97333384

00:41:14.974 --> 00:41:15.935 live in a vacuum. And

NOTE Confidence: 0.97333384

00:41:15.935 --> 00:41:16.815 so, you know, you read

NOTE Confidence: 0.97333384

00:41:16.815 --> 00:41:17.535 and you talk and you

NOTE Confidence: 0.97333384

00:41:17.535 --> 00:41:18.594 go to meetings. And,

NOTE Confidence: 0.9254388

00:41:19.215 --> 00:41:20.335 and we had come across

NOTE Confidence: 0.9254388

00:41:20.335 --> 00:41:21.855 this beautiful paper in cell

NOTE Confidence: 0.9254388

00:41:21.855 --> 00:41:23.715 by, Doug Green's group. Jerry

NOTE Confidence: 0.9254388

00:41:23.890 --> 00:41:25.010 was the first author. He's

NOTE Confidence: 0.9254388

00:41:25.010 --> 00:41:26.369 at Mount Sinai, a full

NOTE Confidence: 0.9254388

00:41:26.369 --> 00:41:26.869 professor.

NOTE Confidence: 0.9792655

00:41:27.570 --> 00:41:28.530 And they had found this

NOTE Confidence: 0.9792655

00:41:28.530 --> 00:41:29.810 interesting thing where, like, it

NOTE Confidence: 0.9792655

00:41:29.810 --> 00:41:31.250 seemed like certain aspects of

NOTE Confidence: 0.9792655

00:41:31.250 --> 00:41:32.550 sphingosine lipid metabolism,

NOTE Confidence: 0.8998764

00:41:33.650 --> 00:41:35.270 was relevant to BACS activation.

NOTE Confidence: 0.9639772

00:41:35.650 --> 00:41:36.930 And they did this study

NOTE Confidence: 0.9639772

00:41:36.930 --> 00:41:37.969 showing that, you know, he

NOTE Confidence: 0.9639772

00:41:37.969 --> 00:41:39.705 literally took every byproduct of

NOTE Confidence: 0.9639772

00:41:39.785 --> 00:41:41.385 singamyel and metabolism and showed

NOTE Confidence: 0.9639772

00:41:41.385 --> 00:41:42.905 that only one of the

NOTE Confidence: 0.9639772

00:41:42.905 --> 00:41:44.844 byproducts, this this lipid hexadec

NOTE Confidence: 0.9639772

00:41:45.065 --> 00:41:45.645 e now,

NOTE Confidence: 0.93051136

00:41:48.105 --> 00:41:50.185 like, sensitized release of, you

NOTE Confidence: 0.93051136

00:41:50.185 --> 00:41:51.465 know, cytochrome c when you

NOTE Confidence: 0.93051136

00:41:51.465 --> 00:41:52.344 threw Bax on there. None

NOTE Confidence: 0.93051136

00:41:52.344 --> 00:41:53.385 of the other lipids did

NOTE Confidence: 0.93051136

00:41:53.385 --> 00:41:53.700 that.

NOTE Confidence: 0.96406156

00:41:54.260 --> 00:41:55.300 And it was really interesting,

NOTE Confidence: 0.96406156

00:41:55.300 --> 00:41:56.260 and it suggests that there

NOTE Confidence: 0.96406156

00:41:56.260 --> 00:41:57.880 was crosstalk between the endoplasmic

NOTE Confidence: 0.96406156

00:41:58.020 --> 00:41:59.239 reticulum and the mitochondria

NOTE Confidence: 0.9392574

00:41:59.619 --> 00:42:00.900 and that, you know, lipids

NOTE Confidence: 0.9392574

00:42:00.900 --> 00:42:01.859 made in the ER were

NOTE Confidence: 0.9392574

00:42:01.859 --> 00:42:03.160 being shuttled to the mitochondria

NOTE Confidence: 0.9392574

00:42:03.300 --> 00:42:04.119 and it's sensitized.

NOTE Confidence: 0.9804901

00:42:04.500 --> 00:42:05.540 And he did beautiful work.

NOTE Confidence: 0.9804901

00:42:05.540 --> 00:42:06.260 You know, if you shut

NOTE Confidence: 0.9804901

00:42:06.260 --> 00:42:07.785 down this pathway, BAX doesn't

NOTE Confidence: 0.9804901

00:42:07.864 --> 00:42:08.984 activate as well in the

NOTE Confidence: 0.9804901

00:42:08.984 --> 00:42:09.484 mitochondria.

NOTE Confidence: 0.99701244

00:42:10.105 --> 00:42:10.905 And so I had a

NOTE Confidence: 0.99701244

00:42:10.905 --> 00:42:12.204 chemistry grad student,

NOTE Confidence: 0.9359283

00:42:12.664 --> 00:42:14.105 in the that joined the

NOTE Confidence: 0.9359283

00:42:14.105 --> 00:42:15.224 lab, and he read the

NOTE Confidence: 0.9359283

00:42:15.224 --> 00:42:16.825 paper, and he looked at

NOTE Confidence: 0.9359283

00:42:16.825 --> 00:42:18.424 the hexadec enal. And there

NOTE Confidence: 0.9359283

00:42:18.424 --> 00:42:19.325 was no structure,

NOTE Confidence: 0.9638982

00:42:20.140 --> 00:42:21.580 you know, and there were

NOTE Confidence: 0.9638982

00:42:21.580 --> 00:42:23.340 no there was no you

NOTE Confidence: 0.9638982

00:42:23.340 --> 00:42:23.980 know, you had to be

NOTE Confidence: 0.9638982

00:42:23.980 --> 00:42:25.100 like a chemistry person to

NOTE Confidence: 0.9638982

00:42:25.100 --> 00:42:26.060 kind of look at this.

NOTE Confidence: 0.9638982

00:42:26.060 --> 00:42:26.859 He looked at this, and

NOTE Confidence: 0.9638982

00:42:26.859 --> 00:42:27.900 in one second, he said,

NOTE Confidence: 0.9638982

00:42:27.900 --> 00:42:29.340 oh, that's a lipid electrophile

NOTE Confidence: 0.9638982

00:42:29.340 --> 00:42:31.020 off of beta unsaturated aldehyde.

NOTE Confidence: 0.9638982

00:42:31.020 --> 00:42:32.239 That is super reactive.

NOTE Confidence: 0.9736181

00:42:32.780 --> 00:42:33.820 And I was like, okay.

NOTE Confidence: 0.9736181

00:42:33.820 --> 00:42:34.555 He's like, oh, I bet

NOTE Confidence: 0.9736181

00:42:34.555 --> 00:42:35.835 you that, you know, any

NOTE Confidence: 0.9736181

00:42:35.835 --> 00:42:37.195 cysteine in BACs is gonna,

NOTE Confidence: 0.9736181

00:42:37.195 --> 00:42:39.455 like, fire and covalently modify.

NOTE Confidence: 0.9580638

00:42:40.235 --> 00:42:41.114 And so I was like,

NOTE Confidence: 0.9580638

00:42:41.114 --> 00:42:42.235 alright. Well, go check it

NOTE Confidence: 0.9580638

00:42:42.235 --> 00:42:43.114 out. So, you know, he

NOTE Confidence: 0.9580638

00:42:43.114 --> 00:42:44.155 comes back with this mass

NOTE Confidence: 0.9580638

00:42:44.155 --> 00:42:44.955 spec, and look at these

NOTE Confidence: 0.9580638

00:42:44.955 --> 00:42:45.915 doses. I mean, you need

NOTE Confidence: 0.9580638

00:42:45.915 --> 00:42:46.500 it a lot.

NOTE Confidence: 0.95115125

00:42:47.060 --> 00:42:47.940 But he was able to

NOTE Confidence: 0.95115125

00:42:47.940 --> 00:42:50.100 completely convert inactive backs in

NOTE Confidence: 0.95115125

00:42:50.100 --> 00:42:50.600 solution

NOTE Confidence: 0.9084047

00:42:51.060 --> 00:42:52.280 to fully,

NOTE Confidence: 0.9591334

00:42:52.660 --> 00:42:53.940 you know, what what I

NOTE Confidence: 0.9591334

00:42:53.940 --> 00:42:54.500 don't know what the right

NOTE Confidence: 0.9591334

00:42:54.500 --> 00:42:55.480 word is. Hexadec

NOTE Confidence: 0.81134427

00:42:55.780 --> 00:42:56.520 e analyzed,

NOTE Confidence: 0.9759929

00:42:56.820 --> 00:42:57.719 you know, derivatized

NOTE Confidence: 0.9680074

00:42:58.020 --> 00:42:58.520 backs.

NOTE Confidence: 0.9683237

00:42:59.234 --> 00:43:01.015 And then if you reduced

NOTE Confidence: 0.9859968

00:43:01.555 --> 00:43:03.154 that and delivered, you know,

NOTE Confidence: 0.9859968

00:43:03.154 --> 00:43:04.535 not alpha beta unsaturated

NOTE Confidence: 0.98844063

00:43:04.835 --> 00:43:06.194 aldehyde, then it it didn't

NOTE Confidence: 0.98844063

00:43:06.194 --> 00:43:07.015 work at all.

NOTE Confidence: 0.9640767

00:43:07.714 --> 00:43:08.835 So, clearly, this was a

NOTE Confidence: 0.9640767

00:43:08.835 --> 00:43:10.454 chemical reaction that was happening.

NOTE Confidence: 0.96255904

00:43:11.634 --> 00:43:12.835 And the question was, you

NOTE Confidence: 0.96255904

00:43:12.835 --> 00:43:13.954 know, was this actually what

NOTE Confidence: 0.96255904

00:43:13.954 --> 00:43:14.580 was happening,

NOTE Confidence: 0.97254366

00:43:15.060 --> 00:43:16.600 in in in in cells?

NOTE Confidence: 0.97254366

00:43:16.739 --> 00:43:17.700 And so, you know, we

NOTE Confidence: 0.97254366

00:43:17.700 --> 00:43:18.739 put it through the system

NOTE Confidence: 0.97254366

00:43:18.739 --> 00:43:19.700 and show that when you

NOTE Confidence: 0.97254366

00:43:19.700 --> 00:43:20.580 add more and more of

NOTE Confidence: 0.97254366

00:43:20.580 --> 00:43:22.340 this, hexadec e now, you

NOTE Confidence: 0.97254366

00:43:22.340 --> 00:43:23.560 get more and more oligomerization.

NOTE Confidence: 0.99630237

00:43:24.260 --> 00:43:25.300 If you use the reduced

NOTE Confidence: 0.99630237

00:43:25.300 --> 00:43:26.280 form, you don't.

NOTE Confidence: 0.93774927

00:43:27.140 --> 00:43:28.100 We then went ahead and

NOTE Confidence: 0.93774927

00:43:28.100 --> 00:43:28.760 did NMR,

NOTE Confidence: 0.9990114

00:43:29.295 --> 00:43:29.795 and

NOTE Confidence: 0.9949651

00:43:30.094 --> 00:43:31.375 completely different area of the

NOTE Confidence: 0.9949651

00:43:31.375 --> 00:43:33.295 protein was was modified as

NOTE Confidence: 0.9949651

00:43:33.295 --> 00:43:34.335 opposed to all the other

NOTE Confidence: 0.9949651

00:43:34.335 --> 00:43:35.375 NMR that I've showed you

NOTE Confidence: 0.9949651

00:43:35.375 --> 00:43:35.875 today.

NOTE Confidence: 0.9989667

00:43:36.655 --> 00:43:37.535 And a lot of the

NOTE Confidence: 0.9989667

00:43:37.535 --> 00:43:38.835 action was around,

NOTE Confidence: 0.9502365

00:43:39.535 --> 00:43:40.035 cysteine

NOTE Confidence: 0.9707112

00:43:40.335 --> 00:43:41.474 one twenty six.

NOTE Confidence: 0.99612945

00:43:42.519 --> 00:43:43.480 And you can see the

NOTE Confidence: 0.99612945

00:43:43.480 --> 00:43:45.000 residues that underwent chemical shift

NOTE Confidence: 0.99612945

00:43:45.000 --> 00:43:46.039 change here. They were all

NOTE Confidence: 0.99612945

00:43:46.039 --> 00:43:46.859 at the core,

NOTE Confidence: 0.9416813

00:43:47.719 --> 00:43:49.079 of the protein and and

NOTE Confidence: 0.9416813

00:43:49.079 --> 00:43:50.200 the in and the residues

NOTE Confidence: 0.9416813

00:43:50.200 --> 00:43:51.900 that it interacted with circumferentially.

NOTE Confidence: 0.9704655

00:43:53.480 --> 00:43:54.519 And, again, I kept I

NOTE Confidence: 0.9704655

00:43:54.519 --> 00:43:55.719 keep saying, like, grenade, but

NOTE Confidence: 0.9704655

00:43:55.719 --> 00:43:56.599 part of the analogy of

NOTE Confidence: 0.9704655

00:43:56.599 --> 00:43:57.480 the grenade is that, like,

NOTE Confidence: 0.9704655

00:43:57.480 --> 00:43:58.614 alpha five is kind of

NOTE Confidence: 0.9704655

00:43:58.614 --> 00:43:59.655 like the pin. Right? That's

NOTE Confidence: 0.9704655

00:43:59.655 --> 00:44:00.935 the helix that kind of

NOTE Confidence: 0.9704655

00:44:00.935 --> 00:44:02.375 traverses through the whole protein.

NOTE Confidence: 0.9704655

00:44:02.375 --> 00:44:03.415 It's the one helix that

NOTE Confidence: 0.9704655

00:44:03.415 --> 00:44:05.114 interacts with every other helix,

NOTE Confidence: 0.98515826

00:44:05.495 --> 00:44:06.375 in the structure of the

NOTE Confidence: 0.98515826

00:44:06.375 --> 00:44:07.655 protein. So we thought, well,

NOTE Confidence: 0.98515826

00:44:07.655 --> 00:44:08.795 that's kind of interesting.

NOTE Confidence: 0.8787741

00:44:09.175 --> 00:44:09.975 You know, how do we

NOTE Confidence: 0.8787741

00:44:09.975 --> 00:44:11.015 prove this? So we have

NOTE Confidence: 0.8787741

00:44:11.015 --> 00:44:12.420 this liposomal system

NOTE Confidence: 0.9522071

00:44:12.719 --> 00:44:14.260 that has fluorophore in it.

NOTE Confidence: 0.9522071

00:44:14.320 --> 00:44:15.440 You add the protein. You

NOTE Confidence: 0.9522071

00:44:15.440 --> 00:44:16.719 add the ligand. It triggers

NOTE Confidence: 0.9522071

00:44:16.719 --> 00:44:18.160 backs to translocate, and then

NOTE Confidence: 0.9522071

00:44:18.160 --> 00:44:19.460 you just measure the fluorophore.

NOTE Confidence: 0.9564485

00:44:20.480 --> 00:44:22.160 Sometimes the simplest assays are

NOTE Confidence: 0.9564485

00:44:22.160 --> 00:44:23.840 really the best assays because

NOTE Confidence: 0.9564485

00:44:23.840 --> 00:44:25.005 it's just like here, it's

NOTE Confidence: 0.9564485

00:44:25.005 --> 00:44:26.285 like, does the fluorophore get

NOTE Confidence: 0.9564485

00:44:26.285 --> 00:44:27.585 released or does it not?

NOTE Confidence: 0.9564485

00:44:27.645 --> 00:44:29.005 And so you can detect

NOTE Confidence: 0.9564485

00:44:29.005 --> 00:44:30.204 this, you know, and you

NOTE Confidence: 0.9564485

00:44:30.204 --> 00:44:31.265 can see that inactive,

NOTE Confidence: 0.94386756

00:44:32.204 --> 00:44:33.404 BACS, you know, has this

NOTE Confidence: 0.94386756

00:44:33.404 --> 00:44:34.924 little background release because, again,

NOTE Confidence: 0.94386756

00:44:34.924 --> 00:44:35.724 you know, because pure as

NOTE Confidence: 0.94386756

00:44:35.724 --> 00:44:36.605 you make it, there's always

NOTE Confidence: 0.94386756

00:44:36.605 --> 00:44:38.030 some activated in there, so

NOTE Confidence: 0.94386756

00:44:38.030 --> 00:44:38.750 you get a little tiny

NOTE Confidence: 0.94386756

00:44:38.750 --> 00:44:39.869 bit of release. But then

NOTE Confidence: 0.94386756

00:44:39.869 --> 00:44:40.830 you put in the staple

NOTE Confidence: 0.94386756

00:44:40.830 --> 00:44:41.869 peptide, and you get nice

NOTE Confidence: 0.94386756

00:44:41.869 --> 00:44:42.369 release.

NOTE Confidence: 0.9870529

00:44:43.550 --> 00:44:45.250 When you make the liposomes

NOTE Confidence: 0.9870529

00:44:45.390 --> 00:44:46.590 with these lipids, which was

NOTE Confidence: 0.9870529

00:44:46.590 --> 00:44:47.710 what we did, if you

NOTE Confidence: 0.9870529

00:44:47.710 --> 00:44:49.469 take the reduced version of

NOTE Confidence: 0.9870529

00:44:49.469 --> 00:44:50.430 the lipid, it was no

NOTE Confidence: 0.9870529

00:44:50.430 --> 00:44:51.469 different than not having a

NOTE Confidence: 0.9870529

00:44:51.469 --> 00:44:52.844 lipid added at all.

NOTE Confidence: 0.98629063

00:44:53.165 --> 00:44:54.285 But if you take the

NOTE Confidence: 0.98629063

00:44:54.285 --> 00:44:54.785 hexadecenal

NOTE Confidence: 0.9826606

00:44:55.325 --> 00:44:56.765 version in the liposomes, now

NOTE Confidence: 0.9826606

00:44:56.765 --> 00:44:58.045 all of a sudden, the

NOTE Confidence: 0.9826606

00:44:58.045 --> 00:44:59.984 BAX alone starts to activate.

NOTE Confidence: 0.9826606

00:45:00.205 --> 00:45:01.005 And then when you add

NOTE Confidence: 0.9826606

00:45:01.005 --> 00:45:01.965 the BIM in there to

NOTE Confidence: 0.9826606

00:45:01.965 --> 00:45:03.805 trigger BAX, it activates way

NOTE Confidence: 0.9826606

00:45:03.805 --> 00:45:04.305 better.

NOTE Confidence: 0.93652046

00:45:05.020 --> 00:45:06.020 Now this starts to sound

NOTE Confidence: 0.93652046

00:45:06.020 --> 00:45:06.860 a little bit like what

NOTE Confidence: 0.93652046

00:45:06.860 --> 00:45:07.980 Jerry Chipick was saying in

NOTE Confidence: 0.93652046

00:45:07.980 --> 00:45:10.080 his cell paper that this

NOTE Confidence: 0.93652046

00:45:10.300 --> 00:45:10.800 lipidation,

NOTE Confidence: 0.97544026

00:45:11.980 --> 00:45:13.739 right, this covalent lipidation that

NOTE Confidence: 0.97544026

00:45:13.739 --> 00:45:15.360 we discovered was somehow

NOTE Confidence: 0.9954052

00:45:16.220 --> 00:45:17.760 sensitizing BACCS activation.

NOTE Confidence: 0.99155194

00:45:19.015 --> 00:45:20.535 And so the next question

NOTE Confidence: 0.99155194

00:45:20.535 --> 00:45:21.835 was, you know, which cysteine?

NOTE Confidence: 0.99155194

00:45:22.055 --> 00:45:22.855 Right? So we kind of

NOTE Confidence: 0.99155194

00:45:22.855 --> 00:45:23.915 did the same experiment,

NOTE Confidence: 0.982642

00:45:24.375 --> 00:45:25.735 but we took out cysteine

NOTE Confidence: 0.982642

00:45:25.735 --> 00:45:27.175 one twenty six, on the

NOTE Confidence: 0.982642

00:45:27.175 --> 00:45:28.055 left, and we took out

NOTE Confidence: 0.982642

00:45:28.055 --> 00:45:29.415 cysteine sixty two on the

NOTE Confidence: 0.982642

00:45:29.415 --> 00:45:30.695 right. And you can clearly

NOTE Confidence: 0.982642

00:45:30.695 --> 00:45:32.695 see that cysteine one twenty

NOTE Confidence: 0.982642

00:45:32.695 --> 00:45:34.010 six is your cysteine. Right?

NOTE Confidence: 0.982642

00:45:34.010 --> 00:45:35.130 Because it looks just like

NOTE Confidence: 0.982642

00:45:35.130 --> 00:45:36.569 wild type, you know, with

NOTE Confidence: 0.982642

00:45:36.569 --> 00:45:38.589 no goosing from the lipid,

NOTE Confidence: 0.9860967

00:45:39.130 --> 00:45:40.170 on the left. But on

NOTE Confidence: 0.9860967

00:45:40.170 --> 00:45:40.969 the right hand side, when

NOTE Confidence: 0.9860967

00:45:40.969 --> 00:45:41.930 you get your cysteine one

NOTE Confidence: 0.9860967

00:45:41.930 --> 00:45:42.969 twenty six back, now it

NOTE Confidence: 0.9860967

00:45:42.969 --> 00:45:44.250 looks just like, you know,

NOTE Confidence: 0.9860967

00:45:44.250 --> 00:45:46.010 triggering and sensitizing with the

NOTE Confidence: 0.9860967

00:45:46.010 --> 00:45:47.125 lipid. And And so we

NOTE Confidence: 0.9860967

00:45:47.125 --> 00:45:48.565 did this in mitochondria. Again,

NOTE Confidence: 0.9860967

00:45:48.565 --> 00:45:49.785 we saw the same thing

NOTE Confidence: 0.9860967

00:45:49.925 --> 00:45:51.445 that we got sensitization just

NOTE Confidence: 0.9860967

00:45:51.445 --> 00:45:53.145 like wild type on mitochondria,

NOTE Confidence: 0.9860967

00:45:53.205 --> 00:45:54.185 which has native,

NOTE Confidence: 0.99730116

00:45:54.485 --> 00:45:55.045 you know,

NOTE Confidence: 0.9815356

00:45:55.445 --> 00:45:56.645 ligand in there. Here, we're

NOTE Confidence: 0.9815356

00:45:56.645 --> 00:45:57.925 just taking advantage of the

NOTE Confidence: 0.9815356

00:45:57.925 --> 00:45:58.905 native hexadecimal

NOTE Confidence: 0.98499125

00:45:59.285 --> 00:46:00.165 or whatever else is in

NOTE Confidence: 0.98499125

00:46:00.165 --> 00:46:01.610 the outer membrane. And when

NOTE Confidence: 0.98499125

00:46:01.610 --> 00:46:02.730 you mutate that c one

NOTE Confidence: 0.98499125

00:46:02.730 --> 00:46:04.410 twenty six, much, much, much

NOTE Confidence: 0.98499125

00:46:04.410 --> 00:46:04.910 blunted,

NOTE Confidence: 0.9615863

00:46:05.450 --> 00:46:07.370 mitochondrial cytochrome c release when

NOTE Confidence: 0.9615863

00:46:07.370 --> 00:46:08.410 you add the triggered back.

NOTE Confidence: 0.9615863

00:46:08.410 --> 00:46:09.390 So that was reassuring.

NOTE Confidence: 0.9980243

00:46:09.930 --> 00:46:10.810 And then we did some

NOTE Confidence: 0.9980243

00:46:10.810 --> 00:46:11.310 reconstitution

NOTE Confidence: 0.965168

00:46:11.610 --> 00:46:13.290 studies where we put into

NOTE Confidence: 0.965168

00:46:13.290 --> 00:46:14.190 double knockout

NOTE Confidence: 0.92408705

00:46:14.505 --> 00:46:15.704 cells wild type and then

NOTE Confidence: 0.92408705

00:46:15.704 --> 00:46:16.744 c one twenty six a

NOTE Confidence: 0.92408705

00:46:16.744 --> 00:46:18.105 mutant back and then treated

NOTE Confidence: 0.92408705

00:46:18.105 --> 00:46:19.644 with the pro apoptotic stimuli.

NOTE Confidence: 0.9654323

00:46:19.944 --> 00:46:21.305 The double knockouts are very

NOTE Confidence: 0.9654323

00:46:21.305 --> 00:46:23.065 resistant, not surprisingly, when you

NOTE Confidence: 0.9654323

00:46:23.065 --> 00:46:24.025 give them a combined,

NOTE Confidence: 0.94191194

00:46:24.744 --> 00:46:25.944 BCL two and MCL one

NOTE Confidence: 0.94191194

00:46:25.944 --> 00:46:26.444 inhibitor.

NOTE Confidence: 0.9857815

00:46:27.039 --> 00:46:28.559 When you reconstitute wild type,

NOTE Confidence: 0.9857815

00:46:28.559 --> 00:46:30.400 you restore killing. And our

NOTE Confidence: 0.9857815

00:46:30.400 --> 00:46:31.519 c one twenty six a

NOTE Confidence: 0.9857815

00:46:31.519 --> 00:46:32.579 mutant was

NOTE Confidence: 0.9979491

00:46:32.880 --> 00:46:34.480 somewhere in the middle. It

NOTE Confidence: 0.9979491

00:46:34.480 --> 00:46:35.539 was just not

NOTE Confidence: 0.95779246

00:46:36.079 --> 00:46:37.119 all of what wild type

NOTE Confidence: 0.95779246

00:46:37.119 --> 00:46:38.559 bacs was before when you

NOTE Confidence: 0.95779246

00:46:38.559 --> 00:46:39.539 took out that cysteine.

NOTE Confidence: 0.9766663

00:46:40.005 --> 00:46:41.204 And so what we discovered

NOTE Confidence: 0.9766663

00:46:41.204 --> 00:46:42.565 was that there's this non

NOTE Confidence: 0.9766663

00:46:42.565 --> 00:46:43.065 enzymatic

NOTE Confidence: 0.96615523

00:46:44.005 --> 00:46:46.085 lipidation that occurs when BAX

NOTE Confidence: 0.96615523

00:46:46.085 --> 00:46:46.964 ends up at the outer

NOTE Confidence: 0.96615523

00:46:46.964 --> 00:46:48.565 membrane where c one twenty

NOTE Confidence: 0.96615523

00:46:48.565 --> 00:46:49.844 six gets modified by this

NOTE Confidence: 0.96615523

00:46:49.844 --> 00:46:51.065 hexadec enal,

NOTE Confidence: 0.98317593

00:46:51.684 --> 00:46:53.545 lipid electrophile, and that sensitizes,

NOTE Confidence: 0.9947187

00:46:54.565 --> 00:46:55.464 BAX activation.

NOTE Confidence: 0.9648436

00:46:56.619 --> 00:46:58.000 So that led us to

NOTE Confidence: 0.9648436

00:46:58.060 --> 00:46:59.420 the next thing, which was,

NOTE Confidence: 0.9648436

00:46:59.420 --> 00:47:01.580 well, cysteine one twenty six,

NOTE Confidence: 0.9648436

00:47:01.580 --> 00:47:04.219 covalent inhibitors, covalent activators. That's

NOTE Confidence: 0.9648436

00:47:04.219 --> 00:47:06.380 in. Right? Like, ten years

NOTE Confidence: 0.9648436

00:47:06.380 --> 00:47:07.580 ago or whenever that was,

NOTE Confidence: 0.9648436

00:47:07.580 --> 00:47:08.694 if you mentioned a covalent

NOTE Confidence: 0.9648436

00:47:08.694 --> 00:47:09.734 inhibitor. I had a grad

NOTE Confidence: 0.9648436

00:47:09.734 --> 00:47:10.694 student in my lab that

NOTE Confidence: 0.9648436

00:47:10.694 --> 00:47:11.255 was gonna work on a

NOTE Confidence: 0.9648436

00:47:11.255 --> 00:47:13.174 covalent inhibitor. Her DAC said

NOTE Confidence: 0.9648436

00:47:13.174 --> 00:47:15.094 absolutely not. Only focus on

NOTE Confidence: 0.9648436

00:47:15.094 --> 00:47:17.015 noncovalent inhibitors. You know, covalent

NOTE Confidence: 0.9648436

00:47:17.015 --> 00:47:18.795 inhibitors and activators are passe.

NOTE Confidence: 0.9648436

00:47:18.934 --> 00:47:20.135 Now, of course, they're all

NOTE Confidence: 0.9648436

00:47:20.135 --> 00:47:21.480 the rage. So so when

NOTE Confidence: 0.9648436

00:47:21.480 --> 00:47:22.920 this happened, you know, people

NOTE Confidence: 0.9648436

00:47:22.920 --> 00:47:24.460 were getting excited about covalent

NOTE Confidence: 0.9648436

00:47:24.520 --> 00:47:25.719 modifiers again. So we're like,

NOTE Confidence: 0.9648436

00:47:25.719 --> 00:47:26.600 okay. Let's see if we

NOTE Confidence: 0.9648436

00:47:26.600 --> 00:47:28.540 can get a covalent modifier

NOTE Confidence: 0.9648436

00:47:28.600 --> 00:47:29.960 of the cysteine. And Jim

NOTE Confidence: 0.9648436

00:47:29.960 --> 00:47:31.480 Wells' group had published his

NOTE Confidence: 0.9648436

00:47:31.480 --> 00:47:33.560 disulfide tethering strategy, and they

NOTE Confidence: 0.9648436

00:47:33.560 --> 00:47:34.935 used that to develop, you

NOTE Confidence: 0.9648436

00:47:34.935 --> 00:47:36.155 know, KRAS inhibitor,

NOTE Confidence: 0.96946406

00:47:36.695 --> 00:47:38.055 with Kevan Shokat. And so

NOTE Confidence: 0.96946406

00:47:38.055 --> 00:47:38.855 we thought this would be

NOTE Confidence: 0.96946406

00:47:38.855 --> 00:47:40.135 a really cool technique to

NOTE Confidence: 0.96946406

00:47:40.135 --> 00:47:41.815 apply to back cysteine one

NOTE Confidence: 0.96946406

00:47:41.815 --> 00:47:43.015 twenty six. So we gave

NOTE Confidence: 0.96946406

00:47:43.015 --> 00:47:44.215 it a shot, and the

NOTE Confidence: 0.96946406

00:47:44.215 --> 00:47:45.975 grad student who discovered the

NOTE Confidence: 0.96946406

00:47:45.975 --> 00:47:47.895 alpha beta unsaturated lipid, you

NOTE Confidence: 0.96946406

00:47:47.895 --> 00:47:48.935 know, we shipped him to

NOTE Confidence: 0.96946406

00:47:48.935 --> 00:47:49.739 Jim's lab,

NOTE Confidence: 0.97736937

00:47:50.140 --> 00:47:51.020 for two months, and it

NOTE Confidence: 0.97736937

00:47:51.020 --> 00:47:52.060 was he loved that because

NOTE Confidence: 0.97736937

00:47:52.060 --> 00:47:53.280 he was from San Francisco,

NOTE Confidence: 0.97736937

00:47:53.340 --> 00:47:54.140 so he got to go

NOTE Confidence: 0.97736937

00:47:54.140 --> 00:47:55.420 home and work in Jim's

NOTE Confidence: 0.97736937

00:47:55.420 --> 00:47:56.560 lab to do the screen.

NOTE Confidence: 0.97736937

00:47:56.619 --> 00:47:57.580 And then he brought the

NOTE Confidence: 0.97736937

00:47:57.580 --> 00:47:58.560 compounds back,

NOTE Confidence: 0.9702082

00:47:59.020 --> 00:48:00.060 and it turned out that

NOTE Confidence: 0.9702082

00:48:00.060 --> 00:48:01.420 this screen was successful that

NOTE Confidence: 0.9702082

00:48:01.420 --> 00:48:03.180 we found, binders that were

NOTE Confidence: 0.9702082

00:48:03.180 --> 00:48:04.995 very effective at covalently modifying

NOTE Confidence: 0.9702082

00:48:04.995 --> 00:48:06.275 cysteine one twenty six even

NOTE Confidence: 0.9702082

00:48:06.275 --> 00:48:07.395 in the presence of, you

NOTE Confidence: 0.9702082

00:48:07.395 --> 00:48:08.615 know, lots of BME.

NOTE Confidence: 0.99013525

00:48:09.955 --> 00:48:11.555 And you could then go

NOTE Confidence: 0.99013525

00:48:11.555 --> 00:48:12.515 ahead and say, okay. I'm

NOTE Confidence: 0.99013525

00:48:12.515 --> 00:48:14.055 back to my liposomal experiment.

NOTE Confidence: 0.970331

00:48:14.835 --> 00:48:16.355 We call the compound covalent

NOTE Confidence: 0.970331

00:48:16.355 --> 00:48:17.980 BAX inhibitor one, And you

NOTE Confidence: 0.970331

00:48:17.980 --> 00:48:19.260 could see that, you know,

NOTE Confidence: 0.970331

00:48:19.260 --> 00:48:20.480 it really didn't affect,

NOTE Confidence: 0.9777903

00:48:21.100 --> 00:48:22.540 BACS alone other than actually

NOTE Confidence: 0.9777903

00:48:22.540 --> 00:48:23.660 get rid of your background

NOTE Confidence: 0.9777903

00:48:23.660 --> 00:48:25.020 BACS activation. So that was

NOTE Confidence: 0.9777903

00:48:25.020 --> 00:48:25.900 kind of like a nice

NOTE Confidence: 0.9777903

00:48:25.900 --> 00:48:27.420 thing. That little background BACS

NOTE Confidence: 0.9777903

00:48:27.420 --> 00:48:28.540 activation that you always see

NOTE Confidence: 0.9777903

00:48:28.540 --> 00:48:30.219 with pure BACS, it suppressed

NOTE Confidence: 0.9777903

00:48:30.219 --> 00:48:30.719 that.

NOTE Confidence: 0.9459273

00:48:31.260 --> 00:48:32.480 But then when you trigger

NOTE Confidence: 0.9459273

00:48:32.540 --> 00:48:34.505 BACS with the normal ligand,

NOTE Confidence: 0.9459273

00:48:34.505 --> 00:48:35.805 you got this big release

NOTE Confidence: 0.9459273

00:48:35.864 --> 00:48:37.145 and the compound seemed to

NOTE Confidence: 0.9459273

00:48:37.145 --> 00:48:38.344 be blocking that to some

NOTE Confidence: 0.9459273

00:48:38.344 --> 00:48:39.864 extent. And when we repeated

NOTE Confidence: 0.9459273

00:48:39.864 --> 00:48:41.145 this and did dose responsive

NOTE Confidence: 0.9459273

00:48:41.145 --> 00:48:42.105 work, I mean, it looked

NOTE Confidence: 0.9459273

00:48:42.105 --> 00:48:43.005 very real.

NOTE Confidence: 0.92752117

00:48:43.785 --> 00:48:44.825 So we went ahead and

NOTE Confidence: 0.92752117

00:48:44.825 --> 00:48:45.480 then had to go

NOTE Confidence: 0.9720851

00:48:46.040 --> 00:48:46.239 back and do the same

NOTE Confidence: 0.9720851

00:48:46.239 --> 00:48:47.320 thing, which cysteine. Let's make

NOTE Confidence: 0.9720851

00:48:47.320 --> 00:48:48.540 sure it's the right cysteine.

NOTE Confidence: 0.95700234

00:48:48.840 --> 00:48:50.619 So we did, our mutagenesis

NOTE Confidence: 0.95700234

00:48:50.840 --> 00:48:51.800 work, and we showed that

NOTE Confidence: 0.95700234

00:48:51.800 --> 00:48:53.640 cysteine one twenty six. You

NOTE Confidence: 0.95700234

00:48:53.640 --> 00:48:54.680 you take that out, and

NOTE Confidence: 0.95700234

00:48:54.680 --> 00:48:56.140 there's now no longer derivatization.

NOTE Confidence: 0.97400063

00:48:56.440 --> 00:48:58.140 So it's very, very selective

NOTE Confidence: 0.9793407

00:48:58.505 --> 00:48:59.545 to the to the cysteine

NOTE Confidence: 0.9793407

00:48:59.545 --> 00:49:00.905 one twenty six. Didn't bother

NOTE Confidence: 0.9793407

00:49:00.905 --> 00:49:02.685 cysteine sixty two at all.

NOTE Confidence: 0.97415125

00:49:03.785 --> 00:49:04.585 So then we went to

NOTE Confidence: 0.97415125

00:49:04.585 --> 00:49:05.864 the NMR. Like, how does

NOTE Confidence: 0.97415125

00:49:05.864 --> 00:49:07.225 this look like libidation? And

NOTE Confidence: 0.97415125

00:49:07.225 --> 00:49:08.665 it turned out it kinda

NOTE Confidence: 0.97415125

00:49:08.665 --> 00:49:09.805 looked a lot like libidation.

NOTE Confidence: 0.97415125

00:49:09.864 --> 00:49:11.049 Again, it was, you know,

NOTE Confidence: 0.97415125

00:49:11.049 --> 00:49:12.510 causing chemical shift perturbations

NOTE Confidence: 0.94720876

00:49:12.890 --> 00:49:14.089 in and around cysteine one

NOTE Confidence: 0.94720876

00:49:14.089 --> 00:49:14.910 twenty six.

NOTE Confidence: 0.94266963

00:49:16.569 --> 00:49:17.630 But it's an inhibitor,

NOTE Confidence: 0.9988807

00:49:18.170 --> 00:49:19.210 and I've been telling you

NOTE Confidence: 0.9988807

00:49:19.210 --> 00:49:20.829 that libidation is an activator.

NOTE Confidence: 0.9965918

00:49:21.450 --> 00:49:22.190 And so

NOTE Confidence: 0.99109167

00:49:22.489 --> 00:49:23.630 what's going on?

NOTE Confidence: 0.9953981

00:49:24.075 --> 00:49:25.035 Right? And if you talk

NOTE Confidence: 0.9953981

00:49:25.035 --> 00:49:25.835 to Jim and you talk

NOTE Confidence: 0.9953981

00:49:25.835 --> 00:49:26.714 to the folks that do

NOTE Confidence: 0.9953981

00:49:26.714 --> 00:49:28.395 these studies with, you know,

NOTE Confidence: 0.9953981

00:49:28.395 --> 00:49:29.455 doing modification,

NOTE Confidence: 0.98558533

00:49:29.994 --> 00:49:30.875 they will tell you that

NOTE Confidence: 0.98558533

00:49:30.875 --> 00:49:31.994 they always get both sides

NOTE Confidence: 0.98558533

00:49:31.994 --> 00:49:33.114 of the coin in their

NOTE Confidence: 0.98558533

00:49:33.114 --> 00:49:34.635 compounds. They'll get activators and

NOTE Confidence: 0.98558533

00:49:34.635 --> 00:49:35.835 inhibitors. It kind of really

NOTE Confidence: 0.98558533

00:49:35.835 --> 00:49:36.875 depends on the protein and

NOTE Confidence: 0.98558533

00:49:36.875 --> 00:49:38.310 depends on the screen. And

NOTE Confidence: 0.98558533

00:49:38.310 --> 00:49:39.750 the difference between an activator

NOTE Confidence: 0.98558533

00:49:39.750 --> 00:49:41.210 and inhibitor is, like, mechanistically

NOTE Confidence: 0.98558533

00:49:41.430 --> 00:49:42.969 and structurally very interesting.

NOTE Confidence: 0.9459702

00:49:43.670 --> 00:49:44.790 But we were very intrigued

NOTE Confidence: 0.9459702

00:49:44.790 --> 00:49:45.750 that now we had ourselves

NOTE Confidence: 0.9459702

00:49:45.750 --> 00:49:46.790 what looked like an inhibitor

NOTE Confidence: 0.9459702

00:49:46.790 --> 00:49:47.750 even even though we were

NOTE Confidence: 0.9459702

00:49:47.750 --> 00:49:49.050 going after an an activator

NOTE Confidence: 0.9459702

00:49:49.190 --> 00:49:49.690 site.

NOTE Confidence: 0.9525327

00:49:50.150 --> 00:49:51.030 And at the same time,

NOTE Confidence: 0.9525327

00:49:51.030 --> 00:49:52.035 we had another grad student

NOTE Confidence: 0.9525327

00:49:52.194 --> 00:49:53.234 working on the kind of

NOTE Confidence: 0.9525327

00:49:53.234 --> 00:49:54.114 like the pin in the

NOTE Confidence: 0.9525327

00:49:54.114 --> 00:49:55.795 grenade and studying what residues

NOTE Confidence: 0.9525327

00:49:55.795 --> 00:49:56.694 were very important

NOTE Confidence: 0.97364724

00:49:56.994 --> 00:49:58.914 at keeping backs inactive, and

NOTE Confidence: 0.97364724

00:49:58.914 --> 00:49:59.954 it ends up being these

NOTE Confidence: 0.97364724

00:49:59.954 --> 00:50:01.714 four residues here. If you

NOTE Confidence: 0.97364724

00:50:01.714 --> 00:50:02.755 take them out one by

NOTE Confidence: 0.97364724

00:50:02.755 --> 00:50:04.619 one in combination, as, you

NOTE Confidence: 0.97364724

00:50:04.619 --> 00:50:05.900 know, two or three or

NOTE Confidence: 0.97364724

00:50:05.900 --> 00:50:07.579 four, you could completely create

NOTE Confidence: 0.97364724

00:50:07.579 --> 00:50:08.079 hyperactivated

NOTE Confidence: 0.9619893

00:50:08.380 --> 00:50:09.579 forms of bax just by

NOTE Confidence: 0.9619893

00:50:09.579 --> 00:50:10.700 playing around with those four

NOTE Confidence: 0.9619893

00:50:10.700 --> 00:50:12.079 residues in the core.

NOTE Confidence: 0.9778399

00:50:12.619 --> 00:50:13.660 So we thought, wow. This

NOTE Confidence: 0.9778399

00:50:13.660 --> 00:50:14.700 is a good assay for

NOTE Confidence: 0.9778399

00:50:14.700 --> 00:50:15.200 us.

NOTE Confidence: 0.99965644

00:50:15.500 --> 00:50:16.719 Let's take a hyperactive

NOTE Confidence: 0.8024485

00:50:17.694 --> 00:50:18.194 semiautoactivated

NOTE Confidence: 0.9471301

00:50:19.055 --> 00:50:20.174 form of bax and see

NOTE Confidence: 0.9471301

00:50:20.174 --> 00:50:20.994 if our molecule

NOTE Confidence: 0.9994048

00:50:21.375 --> 00:50:22.275 could restore

NOTE Confidence: 0.9977899

00:50:22.575 --> 00:50:23.875 the inactive state.

NOTE Confidence: 0.98358464

00:50:24.895 --> 00:50:25.855 And so we took the

NOTE Confidence: 0.98358464

00:50:25.855 --> 00:50:27.375 f one sixteen a single

NOTE Confidence: 0.98358464

00:50:27.375 --> 00:50:28.335 mutant because that was our

NOTE Confidence: 0.98358464

00:50:28.335 --> 00:50:30.094 most active single mutant. You

NOTE Confidence: 0.98358464

00:50:30.094 --> 00:50:31.135 could see I'm putting it

NOTE Confidence: 0.98358464

00:50:31.135 --> 00:50:31.635 on,

NOTE Confidence: 0.9942219

00:50:32.780 --> 00:50:34.219 the liposomes with no stimulant,

NOTE Confidence: 0.9942219

00:50:34.219 --> 00:50:35.339 and it just fires on

NOTE Confidence: 0.9942219

00:50:35.339 --> 00:50:36.000 its own.

NOTE Confidence: 0.9331895

00:50:36.380 --> 00:50:37.180 And then you put in

NOTE Confidence: 0.9331895

00:50:37.180 --> 00:50:38.219 the the small molecule, and

NOTE Confidence: 0.9331895

00:50:38.219 --> 00:50:39.359 we saw dose responsive

NOTE Confidence: 0.7608161

00:50:39.820 --> 00:50:40.320 inhibition.

NOTE Confidence: 0.98266906

00:50:41.099 --> 00:50:41.900 And we saw this in

NOTE Confidence: 0.98266906

00:50:41.900 --> 00:50:43.839 the mitochondrial experiment as well.

NOTE Confidence: 0.99671805

00:50:44.460 --> 00:50:45.660 And that was super exciting

NOTE Confidence: 0.99671805

00:50:45.660 --> 00:50:46.780 to us. And then we

NOTE Confidence: 0.99671805

00:50:46.780 --> 00:50:49.505 decided to, years ago, initiate

NOTE Confidence: 0.99671805

00:50:49.645 --> 00:50:50.925 the application of this really

NOTE Confidence: 0.99671805

00:50:50.925 --> 00:50:51.825 cool technique,

NOTE Confidence: 0.9678463

00:50:52.444 --> 00:50:53.244 to b c l two

NOTE Confidence: 0.9678463

00:50:53.244 --> 00:50:55.085 family structural analysis, which is

NOTE Confidence: 0.9678463

00:50:55.085 --> 00:50:57.184 hydrogen deuterium exchange mass spec,

NOTE Confidence: 0.99419034

00:50:57.964 --> 00:50:58.464 which,

NOTE Confidence: 0.9443445

00:50:59.405 --> 00:51:00.765 we started a collaboration with

NOTE Confidence: 0.9443445

00:51:00.765 --> 00:51:02.145 John Engen's group at Northeastern

NOTE Confidence: 0.9443445

00:51:02.285 --> 00:51:03.579 and Thomas Wells, and they

NOTE Confidence: 0.9443445

00:51:03.579 --> 00:51:04.940 were really world experts in

NOTE Confidence: 0.9443445

00:51:04.940 --> 00:51:06.140 this. And it's a really

NOTE Confidence: 0.9443445

00:51:06.140 --> 00:51:07.200 simple in principle,

NOTE Confidence: 0.95294106

00:51:07.500 --> 00:51:09.180 very technically difficult to do

NOTE Confidence: 0.95294106

00:51:09.180 --> 00:51:09.980 in terms of all the

NOTE Confidence: 0.95294106

00:51:09.980 --> 00:51:11.260 machinery and everything. But the

NOTE Confidence: 0.95294106

00:51:11.260 --> 00:51:12.619 concept is very simple, which

NOTE Confidence: 0.95294106

00:51:12.619 --> 00:51:13.819 is you have a protein

NOTE Confidence: 0.95294106

00:51:13.819 --> 00:51:15.280 that's got n h bonds.

NOTE Confidence: 0.95294106

00:51:15.500 --> 00:51:16.780 The h is exchange with

NOTE Confidence: 0.95294106

00:51:16.780 --> 00:51:17.904 water. If you put it

NOTE Confidence: 0.95294106

00:51:17.904 --> 00:51:19.344 in deuterated water, the h

NOTE Confidence: 0.95294106

00:51:19.344 --> 00:51:20.805 is will exchange with deuterium

NOTE Confidence: 0.8389295

00:51:21.825 --> 00:51:23.125 if those h is exposed.

NOTE Confidence: 0.9591487

00:51:24.144 --> 00:51:25.344 Right? So things on the

NOTE Confidence: 0.9591487

00:51:25.344 --> 00:51:26.805 surface, things that are flexible

NOTE Confidence: 0.9591487

00:51:26.944 --> 00:51:28.164 will exchange quickly.

NOTE Confidence: 0.9752194

00:51:28.625 --> 00:51:29.664 And then you allow that

NOTE Confidence: 0.9752194

00:51:29.664 --> 00:51:31.184 to happen over time if

NOTE Confidence: 0.9752194

00:51:31.184 --> 00:51:31.984 you'd like and do a

NOTE Confidence: 0.9752194

00:51:31.984 --> 00:51:34.070 time lapse photography style experiment.

NOTE Confidence: 0.9752194

00:51:34.290 --> 00:51:35.250 And then you could quench

NOTE Confidence: 0.9752194

00:51:35.250 --> 00:51:36.930 it all, digest everything, and

NOTE Confidence: 0.9752194

00:51:36.930 --> 00:51:37.730 throw it on the mass

NOTE Confidence: 0.9752194

00:51:37.730 --> 00:51:38.850 spec, and you're asking a

NOTE Confidence: 0.9752194

00:51:38.850 --> 00:51:40.370 pretty simple question. Which of

NOTE Confidence: 0.9752194

00:51:40.370 --> 00:51:42.070 my peptides gained weight?

NOTE Confidence: 0.9881525

00:51:42.690 --> 00:51:43.890 And you can watch certain

NOTE Confidence: 0.9881525

00:51:43.890 --> 00:51:45.330 peptides gain weight, and then

NOTE Confidence: 0.9881525

00:51:45.330 --> 00:51:46.550 you can watch certain peptides

NOTE Confidence: 0.9881525

00:51:46.610 --> 00:51:47.594 never gain weight if they're,

NOTE Confidence: 0.9881525

00:51:47.594 --> 00:51:48.234 like, in the core of

NOTE Confidence: 0.9881525

00:51:48.234 --> 00:51:49.275 the protein and never have

NOTE Confidence: 0.9881525

00:51:49.275 --> 00:51:50.315 access to the d two

NOTE Confidence: 0.9881525

00:51:50.315 --> 00:51:50.815 o.

NOTE Confidence: 0.96453977

00:51:51.275 --> 00:51:52.075 And then you can plot

NOTE Confidence: 0.96453977

00:51:52.075 --> 00:51:52.795 this out, and you could

NOTE Confidence: 0.96453977

00:51:52.795 --> 00:51:53.835 say, okay. Here's one that

NOTE Confidence: 0.96453977

00:51:53.835 --> 00:51:55.215 exchanges over time.

NOTE Confidence: 0.9266826

00:51:55.594 --> 00:51:56.475 And then you can look

NOTE Confidence: 0.9266826

00:51:56.475 --> 00:51:57.915 at all these pretty like

NOTE Confidence: 0.9266826

00:51:57.915 --> 00:51:59.215 fragments of your protein.

NOTE Confidence: 0.96467894

00:52:00.869 --> 00:52:02.630 I love this technique because

NOTE Confidence: 0.96467894

00:52:02.630 --> 00:52:04.069 it's not easy to do,

NOTE Confidence: 0.96467894

00:52:04.069 --> 00:52:05.530 you know, how to proteins

NOTE Confidence: 0.96467894

00:52:05.589 --> 00:52:06.710 change their structure in a

NOTE Confidence: 0.96467894

00:52:06.710 --> 00:52:08.410 membrane environment over time.

NOTE Confidence: 0.9706609

00:52:08.950 --> 00:52:10.549 And this gives you, like,

NOTE Confidence: 0.9706609

00:52:10.549 --> 00:52:11.690 essentially a movie

NOTE Confidence: 0.998299

00:52:11.989 --> 00:52:13.270 of what's happening over time

NOTE Confidence: 0.998299

00:52:13.270 --> 00:52:14.170 with your protein.

NOTE Confidence: 0.9896091

00:52:14.724 --> 00:52:15.844 And and so when we

NOTE Confidence: 0.9896091

00:52:15.844 --> 00:52:17.444 mutate the f one sixteen

NOTE Confidence: 0.9896091

00:52:17.444 --> 00:52:18.244 and I told you that

NOTE Confidence: 0.9896091

00:52:18.244 --> 00:52:19.765 it makes the protein more

NOTE Confidence: 0.9896091

00:52:19.765 --> 00:52:20.265 loose,

NOTE Confidence: 0.9714813

00:52:20.565 --> 00:52:21.444 you see now all of

NOTE Confidence: 0.9714813

00:52:21.444 --> 00:52:23.045 a sudden you're getting anything

NOTE Confidence: 0.9714813

00:52:23.045 --> 00:52:23.925 that's going up like a

NOTE Confidence: 0.9714813

00:52:23.925 --> 00:52:25.525 mountain is deep protection or

NOTE Confidence: 0.9714813

00:52:25.525 --> 00:52:26.265 or faster,

NOTE Confidence: 0.97574085

00:52:27.530 --> 00:52:29.290 conformational change and exposure than

NOTE Confidence: 0.97574085

00:52:29.290 --> 00:52:30.410 the normal one because everything

NOTE Confidence: 0.97574085

00:52:30.410 --> 00:52:32.010 is a subtraction here. So

NOTE Confidence: 0.97574085

00:52:32.010 --> 00:52:33.210 I'm showing you how does

NOTE Confidence: 0.97574085

00:52:33.210 --> 00:52:34.489 f one sixteen a move

NOTE Confidence: 0.97574085

00:52:34.489 --> 00:52:35.690 compared to wild type? Well,

NOTE Confidence: 0.97574085

00:52:35.690 --> 00:52:36.810 in those regions that are

NOTE Confidence: 0.97574085

00:52:36.810 --> 00:52:38.489 looking like mountains, those regions

NOTE Confidence: 0.97574085

00:52:38.489 --> 00:52:40.464 are magically moving more. And

NOTE Confidence: 0.97574085

00:52:40.464 --> 00:52:41.265 where are they? On the

NOTE Confidence: 0.97574085

00:52:41.265 --> 00:52:42.704 right hand side, oh, they're

NOTE Confidence: 0.97574085

00:52:42.704 --> 00:52:43.844 right where that phenylalanine

NOTE Confidence: 0.9656883

00:52:44.145 --> 00:52:45.585 used to be. So that

NOTE Confidence: 0.9656883

00:52:45.585 --> 00:52:47.045 aromatic and all those interactions

NOTE Confidence: 0.9656883

00:52:47.105 --> 00:52:48.065 around it are now gone

NOTE Confidence: 0.9656883

00:52:48.065 --> 00:52:49.425 because it's an alanine, and

NOTE Confidence: 0.9656883

00:52:49.425 --> 00:52:51.025 those areas are not being

NOTE Confidence: 0.9656883

00:52:51.025 --> 00:52:52.710 kind of tethered down anymore.

NOTE Confidence: 0.9656883

00:52:52.710 --> 00:52:54.150 And they're moving, and that's

NOTE Confidence: 0.9656883

00:52:54.150 --> 00:52:55.270 a problem because you get

NOTE Confidence: 0.9656883

00:52:55.270 --> 00:52:56.710 back starting to move whether

NOTE Confidence: 0.9656883

00:52:56.710 --> 00:52:57.590 by this or by heat,

NOTE Confidence: 0.9656883

00:52:57.590 --> 00:52:58.890 and it's gonna fire.

NOTE Confidence: 0.9788231

00:52:59.750 --> 00:53:00.630 When you add in the

NOTE Confidence: 0.9788231

00:53:00.630 --> 00:53:01.130 molecule

NOTE Confidence: 0.9509675

00:53:01.510 --> 00:53:02.630 to the f one sixteen

NOTE Confidence: 0.9509675

00:53:02.630 --> 00:53:03.830 a and say, what does

NOTE Confidence: 0.9509675

00:53:03.830 --> 00:53:04.950 it look like now when

NOTE Confidence: 0.9509675

00:53:04.950 --> 00:53:05.715 I add the molecule

NOTE Confidence: 0.9406224

00:53:07.075 --> 00:53:08.535 f one sixteen a hyperactive

NOTE Confidence: 0.9406224

00:53:08.675 --> 00:53:10.195 version of ax, it is

NOTE Confidence: 0.9406224

00:53:10.195 --> 00:53:11.255 a mirror image.

NOTE Confidence: 0.999089

00:53:12.114 --> 00:53:12.855 It completely

NOTE Confidence: 0.9995276

00:53:13.235 --> 00:53:13.735 eliminates

NOTE Confidence: 0.99767315

00:53:14.594 --> 00:53:15.575 all of the hyperactivity

NOTE Confidence: 0.91755986

00:53:16.675 --> 00:53:17.175 structurally

NOTE Confidence: 0.98880064

00:53:17.635 --> 00:53:19.094 of what you started with.

NOTE Confidence: 0.99831146

00:53:20.109 --> 00:53:20.910 And this is the type

NOTE Confidence: 0.99831146

00:53:20.910 --> 00:53:22.670 of experimental data that you

NOTE Confidence: 0.99831146

00:53:22.670 --> 00:53:23.810 can get from,

NOTE Confidence: 0.98439056

00:53:24.589 --> 00:53:25.550 HDX, and then you can

NOTE Confidence: 0.98439056

00:53:25.550 --> 00:53:26.430 go and map it onto

NOTE Confidence: 0.98439056

00:53:26.430 --> 00:53:27.550 your protein and kinda see

NOTE Confidence: 0.98439056

00:53:27.550 --> 00:53:28.589 where it is. And, again,

NOTE Confidence: 0.98439056

00:53:28.589 --> 00:53:29.869 it's so satisfying because, again,

NOTE Confidence: 0.98439056

00:53:29.869 --> 00:53:30.750 look where the c one

NOTE Confidence: 0.98439056

00:53:30.750 --> 00:53:31.630 twenty six is on that

NOTE Confidence: 0.98439056

00:53:31.630 --> 00:53:33.650 picture. It's that red cysteine,

NOTE Confidence: 0.98439056

00:53:33.869 --> 00:53:35.555 and look what's protected all

NOTE Confidence: 0.98439056

00:53:35.555 --> 00:53:36.214 of a sudden.

NOTE Confidence: 0.9993396

00:53:36.835 --> 00:53:37.575 The residues

NOTE Confidence: 0.99860334

00:53:37.875 --> 00:53:39.394 of those peptide fragments that

NOTE Confidence: 0.99860334

00:53:39.394 --> 00:53:41.255 are staring at the cysteine.

NOTE Confidence: 0.9981829

00:53:41.795 --> 00:53:43.634 Right? So that molecule attached

NOTE Confidence: 0.9981829

00:53:43.634 --> 00:53:44.454 to the cysteine

NOTE Confidence: 0.97264993

00:53:44.835 --> 00:53:46.835 is now replacing what the

NOTE Confidence: 0.97264993

00:53:46.835 --> 00:53:48.594 phenylalanine did and is interacting

NOTE Confidence: 0.97264993

00:53:48.594 --> 00:53:50.035 with those areas and sucking

NOTE Confidence: 0.97264993

00:53:50.035 --> 00:53:51.180 it in to the point

NOTE Confidence: 0.97264993

00:53:51.180 --> 00:53:52.540 that now BACS is inactive

NOTE Confidence: 0.97264993

00:53:52.540 --> 00:53:53.040 again.

NOTE Confidence: 0.9820099

00:53:53.900 --> 00:53:55.760 And even beyond wild type.

NOTE Confidence: 0.9820099

00:53:55.900 --> 00:53:57.020 So we looked at, okay,

NOTE Confidence: 0.9820099

00:53:57.020 --> 00:53:58.700 how does the pattern look

NOTE Confidence: 0.9820099

00:53:58.700 --> 00:54:00.060 of the molecule bound to

NOTE Confidence: 0.9820099

00:54:00.060 --> 00:54:01.820 our hyperactive BACS versus just

NOTE Confidence: 0.9820099

00:54:01.820 --> 00:54:02.960 plain old BACS?

NOTE Confidence: 0.99947387

00:54:03.260 --> 00:54:04.219 And you see that there's

NOTE Confidence: 0.99947387

00:54:04.219 --> 00:54:05.280 even more protection

NOTE Confidence: 0.9857606

00:54:05.835 --> 00:54:06.955 than what you started with.

NOTE Confidence: 0.9857606

00:54:06.955 --> 00:54:07.915 And where is that more

NOTE Confidence: 0.9857606

00:54:07.915 --> 00:54:08.415 protection?

NOTE Confidence: 0.9672673

00:54:09.675 --> 00:54:10.955 Right across the street from

NOTE Confidence: 0.9672673

00:54:10.955 --> 00:54:12.734 the molecule that just derivatized

NOTE Confidence: 0.9672673

00:54:12.795 --> 00:54:14.974 your cysteines. It's super intellectually

NOTE Confidence: 0.9672673

00:54:15.195 --> 00:54:15.695 satisfying.

NOTE Confidence: 0.9750087

00:54:16.875 --> 00:54:18.155 And then the other part

NOTE Confidence: 0.9750087

00:54:18.155 --> 00:54:19.035 of this, which was a

NOTE Confidence: 0.9750087

00:54:19.035 --> 00:54:20.635 little bonus prize, was that,

NOTE Confidence: 0.9081455

00:54:21.810 --> 00:54:23.110 not only did it confirmationally

NOTE Confidence: 0.9081455

00:54:23.330 --> 00:54:24.530 constrain and everything I just

NOTE Confidence: 0.9081455

00:54:24.530 --> 00:54:25.810 showed you was stuff in

NOTE Confidence: 0.9081455

00:54:25.810 --> 00:54:26.310 solution,

NOTE Confidence: 0.95731926

00:54:26.690 --> 00:54:27.489 but I told you that

NOTE Confidence: 0.95731926

00:54:27.489 --> 00:54:29.170 lipidation is sensitizing and that

NOTE Confidence: 0.95731926

00:54:29.170 --> 00:54:30.610 occurs at the mitochondria. Well,

NOTE Confidence: 0.95731926

00:54:30.610 --> 00:54:31.810 if I take that cysteine

NOTE Confidence: 0.95731926

00:54:31.810 --> 00:54:32.310 out

NOTE Confidence: 0.999393

00:54:32.690 --> 00:54:33.430 of the game

NOTE Confidence: 0.95822835

00:54:34.045 --> 00:54:35.405 and you're essentially, like, alkylating

NOTE Confidence: 0.95822835

00:54:35.405 --> 00:54:36.364 it. Right? Now all of

NOTE Confidence: 0.95822835

00:54:36.364 --> 00:54:37.485 a sudden, the lipid can't

NOTE Confidence: 0.95822835

00:54:37.485 --> 00:54:38.925 go there anymore, and it

NOTE Confidence: 0.95822835

00:54:38.925 --> 00:54:40.285 can't sensitize backs at the

NOTE Confidence: 0.95822835

00:54:40.285 --> 00:54:42.125 mitochondria anymore. So now you've

NOTE Confidence: 0.95822835

00:54:42.125 --> 00:54:43.905 done two things. You've confirmationally

NOTE Confidence: 0.95822835

00:54:44.045 --> 00:54:45.325 constrained the protein and you

NOTE Confidence: 0.95822835

00:54:45.325 --> 00:54:46.685 prevented it from interacting with

NOTE Confidence: 0.95822835

00:54:46.685 --> 00:54:47.805 the site that actually would

NOTE Confidence: 0.95822835

00:54:47.805 --> 00:54:49.610 stimulate its activation. And so

NOTE Confidence: 0.95822835

00:54:49.610 --> 00:54:50.190 we wanted to prove

NOTE Confidence: 0.9853372

00:54:50.489 --> 00:54:52.090 this, and so we took

NOTE Confidence: 0.9853372

00:54:52.090 --> 00:54:54.090 a reagent that reacted with

NOTE Confidence: 0.9853372

00:54:54.090 --> 00:54:54.830 free aldehydes.

NOTE Confidence: 0.97073185

00:54:55.210 --> 00:54:56.090 And when you form this

NOTE Confidence: 0.97073185

00:54:56.090 --> 00:54:57.370 covalent reaction, you still have

NOTE Confidence: 0.97073185

00:54:57.370 --> 00:54:58.810 your free aldehyde. It's a

NOTE Confidence: 0.97073185

00:54:58.810 --> 00:55:00.750 psi five labeled reagent.

NOTE Confidence: 0.9602058

00:55:01.050 --> 00:55:01.850 And you can see that

NOTE Confidence: 0.9602058

00:55:01.850 --> 00:55:02.989 when you add the vaccine

NOTE Confidence: 0.9602058

00:55:03.050 --> 00:55:03.610 with the,

NOTE Confidence: 0.98667383

00:55:04.635 --> 00:55:05.135 lipid,

NOTE Confidence: 0.9325323

00:55:06.155 --> 00:55:07.355 the lipid, you know, goes

NOTE Confidence: 0.9325323

00:55:07.355 --> 00:55:08.715 crazy and it modifies BAX,

NOTE Confidence: 0.9325323

00:55:08.715 --> 00:55:10.315 and then BAX becomes a,

NOTE Confidence: 0.9325323

00:55:10.315 --> 00:55:11.695 you know, heterogeneous

NOTE Confidence: 0.98364526

00:55:11.995 --> 00:55:14.255 oligomer and ladders like this.

NOTE Confidence: 0.9774125

00:55:14.875 --> 00:55:15.915 And when you add in

NOTE Confidence: 0.9774125

00:55:15.915 --> 00:55:17.114 the molecule, you get dose

NOTE Confidence: 0.9774125

00:55:17.114 --> 00:55:18.815 responsive inhibition of the laddering.

NOTE Confidence: 0.9774125

00:55:19.110 --> 00:55:19.910 And in fact, at the

NOTE Confidence: 0.9774125

00:55:19.910 --> 00:55:21.110 bottom right hand corner, you

NOTE Confidence: 0.9774125

00:55:21.110 --> 00:55:22.570 actually get your backs back.

NOTE Confidence: 0.98618805

00:55:23.110 --> 00:55:24.870 Right? Your your monomeric protein

NOTE Confidence: 0.98618805

00:55:24.870 --> 00:55:26.230 that, like, disappeared into this

NOTE Confidence: 0.98618805

00:55:26.230 --> 00:55:28.390 oligomer, you restored actually what

NOTE Confidence: 0.98618805

00:55:28.390 --> 00:55:29.450 you started with.

NOTE Confidence: 0.9990076

00:55:30.230 --> 00:55:31.270 So that kind of led

NOTE Confidence: 0.9990076

00:55:31.270 --> 00:55:32.650 us to this, you know,

NOTE Confidence: 0.97744507

00:55:33.350 --> 00:55:35.505 explanation that this cysteine one

NOTE Confidence: 0.97744507

00:55:35.505 --> 00:55:36.464 twenty six as a drug

NOTE Confidence: 0.97744507

00:55:36.464 --> 00:55:36.964 target

NOTE Confidence: 0.84056926

00:55:37.265 --> 00:55:38.005 can confirmationally

NOTE Confidence: 0.85400075

00:55:38.385 --> 00:55:39.444 constrain VACS,

NOTE Confidence: 0.9924223

00:55:39.905 --> 00:55:41.285 but could also competitively,

NOTE Confidence: 0.9961519

00:55:42.065 --> 00:55:43.045 inhibit VACS.

NOTE Confidence: 0.95668167

00:55:43.344 --> 00:55:44.625 And so that dual mechanism

NOTE Confidence: 0.95668167

00:55:44.625 --> 00:55:45.505 is kind of exciting to

NOTE Confidence: 0.95668167

00:55:45.505 --> 00:55:46.305 us. And if you remember

NOTE Confidence: 0.95668167

00:55:46.305 --> 00:55:47.184 at the beginning, I showed

NOTE Confidence: 0.95668167

00:55:47.184 --> 00:55:47.969 you this dial and you

NOTE Confidence: 0.95668167

00:55:47.969 --> 00:55:49.030 can block survival,

NOTE Confidence: 0.9670283

00:55:49.330 --> 00:55:50.210 you know, and do good

NOTE Confidence: 0.9670283

00:55:50.210 --> 00:55:51.250 in one area of diseases

NOTE Confidence: 0.9670283

00:55:51.250 --> 00:55:52.210 and you could block death

NOTE Confidence: 0.9670283

00:55:52.210 --> 00:55:53.330 in another area of diseases.

NOTE Confidence: 0.9670283

00:55:53.330 --> 00:55:54.530 And so we're really interested

NOTE Confidence: 0.9670283

00:55:54.530 --> 00:55:56.290 in this because, potentially, you

NOTE Confidence: 0.9670283

00:55:56.290 --> 00:55:57.650 could use this to, like,

NOTE Confidence: 0.9670283

00:55:57.650 --> 00:55:58.850 I don't know, infuse something

NOTE Confidence: 0.9670283

00:55:58.850 --> 00:55:59.744 that blocks back in the

NOTE Confidence: 0.9670283

00:55:59.744 --> 00:56:00.704 midst of a stroke or

NOTE Confidence: 0.9670283

00:56:00.704 --> 00:56:01.505 a heart attack or a

NOTE Confidence: 0.9670283

00:56:01.505 --> 00:56:03.285 nerve injury and temporarily

NOTE Confidence: 0.8515304

00:56:03.744 --> 00:56:04.244 arrest

NOTE Confidence: 0.95309246

00:56:04.625 --> 00:56:05.585 cell death. And we know

NOTE Confidence: 0.95309246

00:56:05.585 --> 00:56:07.344 that that's important because there's

NOTE Confidence: 0.95309246

00:56:07.344 --> 00:56:08.384 tons of mouse models out

NOTE Confidence: 0.95309246

00:56:08.384 --> 00:56:09.664 there and backs knockout mice

NOTE Confidence: 0.95309246

00:56:09.664 --> 00:56:10.944 and back knockout mice that

NOTE Confidence: 0.95309246

00:56:10.944 --> 00:56:12.005 you inhibit them

NOTE Confidence: 0.98504746

00:56:12.450 --> 00:56:13.650 genetically and do a, you

NOTE Confidence: 0.98504746

00:56:13.650 --> 00:56:15.330 know, induction of cardiac arrest

NOTE Confidence: 0.98504746

00:56:15.330 --> 00:56:17.010 or induction of stroke, and

NOTE Confidence: 0.98504746

00:56:17.010 --> 00:56:18.210 there's less death, you know,

NOTE Confidence: 0.98504746

00:56:18.210 --> 00:56:19.089 in the mice that don't

NOTE Confidence: 0.98504746

00:56:19.089 --> 00:56:19.969 have backs or backs. So,

NOTE Confidence: 0.98504746

00:56:19.969 --> 00:56:21.089 I mean, the the genetics

NOTE Confidence: 0.98504746

00:56:21.089 --> 00:56:22.050 part of this has already

NOTE Confidence: 0.98504746

00:56:22.050 --> 00:56:23.489 been proven, right, to be

NOTE Confidence: 0.98504746

00:56:23.489 --> 00:56:24.849 a potential benefit. Can we

NOTE Confidence: 0.98504746

00:56:24.849 --> 00:56:25.830 do something pharmacologic

NOTE Confidence: 0.903848

00:56:26.210 --> 00:56:27.750 now with with this site?

NOTE Confidence: 0.9904838

00:56:28.594 --> 00:56:29.555 Okay. So what I've told

NOTE Confidence: 0.9904838

00:56:29.555 --> 00:56:30.775 you today is,

NOTE Confidence: 0.995822

00:56:31.474 --> 00:56:32.835 we've spent a lot of

NOTE Confidence: 0.995822

00:56:32.835 --> 00:56:33.875 time looking for ways to

NOTE Confidence: 0.995822

00:56:33.875 --> 00:56:34.375 activate,

NOTE Confidence: 0.6698936

00:56:35.075 --> 00:56:35.555 BACs,

NOTE Confidence: 0.98065645

00:56:36.434 --> 00:56:37.795 and all of this I

NOTE Confidence: 0.98065645

00:56:37.795 --> 00:56:39.155 can't say it enough. All

NOTE Confidence: 0.98065645

00:56:39.234 --> 00:56:40.035 maybe I should say this

NOTE Confidence: 0.98065645

00:56:40.035 --> 00:56:41.635 at congress. All of this

NOTE Confidence: 0.98065645

00:56:41.635 --> 00:56:43.335 came from basic

NOTE Confidence: 0.9774201

00:56:44.650 --> 00:56:46.330 science, as basic as you

NOTE Confidence: 0.9774201

00:56:46.330 --> 00:56:47.610 possibly could be. Where does

NOTE Confidence: 0.9774201

00:56:47.610 --> 00:56:48.969 a peptide or a protein

NOTE Confidence: 0.9774201

00:56:48.969 --> 00:56:49.950 bind a protein

NOTE Confidence: 0.96412015

00:56:50.410 --> 00:56:52.010 target? Or where does oh,

NOTE Confidence: 0.96412015

00:56:52.010 --> 00:56:53.050 a lipid binds to a

NOTE Confidence: 0.96412015

00:56:53.050 --> 00:56:54.350 protein at the outer mitochondrial

NOTE Confidence: 0.96412015

00:56:54.410 --> 00:56:55.930 membrane. Oh, and that led

NOTE Confidence: 0.96412015

00:56:55.930 --> 00:56:57.050 to this whole idea about

NOTE Confidence: 0.96412015

00:56:57.050 --> 00:56:58.545 how to inhibit VAX. Like,

NOTE Confidence: 0.96412015

00:56:58.704 --> 00:57:00.224 connecting those dots. It all

NOTE Confidence: 0.96412015

00:57:00.224 --> 00:57:02.145 starts, you know, here. Right?

NOTE Confidence: 0.96412015

00:57:02.145 --> 00:57:03.525 It all starts in academia

NOTE Confidence: 0.96412015

00:57:03.665 --> 00:57:04.885 where you have the time

NOTE Confidence: 0.96412015

00:57:04.944 --> 00:57:06.224 over twenty years to flesh

NOTE Confidence: 0.96412015

00:57:06.224 --> 00:57:07.585 these things out so you

NOTE Confidence: 0.96412015

00:57:07.585 --> 00:57:08.385 can kind of figure out

NOTE Confidence: 0.96412015

00:57:08.385 --> 00:57:09.825 the biology, figure out what

NOTE Confidence: 0.96412015

00:57:09.825 --> 00:57:11.184 you could possibly do about

NOTE Confidence: 0.96412015

00:57:11.184 --> 00:57:12.645 it. Okay. In the last

NOTE Confidence: 0.96412015

00:57:12.704 --> 00:57:14.145 one minute, I'll leave you

NOTE Confidence: 0.96412015

00:57:14.145 --> 00:57:15.420 with, like, what's next.

NOTE Confidence: 0.9989548

00:57:16.440 --> 00:57:17.420 The last step.

NOTE Confidence: 0.9732726

00:57:18.440 --> 00:57:18.940 Okay?

NOTE Confidence: 0.9700985

00:57:19.720 --> 00:57:20.840 So, again, we've been kind

NOTE Confidence: 0.9700985

00:57:20.840 --> 00:57:22.440 of obsessively going through every

NOTE Confidence: 0.9700985

00:57:22.440 --> 00:57:23.400 step. The last step is

NOTE Confidence: 0.9700985

00:57:23.400 --> 00:57:24.120 how in the hell does

NOTE Confidence: 0.9700985

00:57:24.120 --> 00:57:25.960 this actually come together to

NOTE Confidence: 0.9700985

00:57:25.960 --> 00:57:27.655 dissociate, disrupt the membrane, and

NOTE Confidence: 0.9700985

00:57:27.655 --> 00:57:29.015 the answer is nobody knows,

NOTE Confidence: 0.9700985

00:57:29.015 --> 00:57:30.714 really. Okay? There's no structures.

NOTE Confidence: 0.99606454

00:57:31.734 --> 00:57:32.694 No one knows. So this

NOTE Confidence: 0.99606454

00:57:32.694 --> 00:57:33.815 is this has been called

NOTE Confidence: 0.99606454

00:57:33.815 --> 00:57:35.115 the holy grail of apoptosis

NOTE Confidence: 0.99606454

00:57:35.255 --> 00:57:35.755 research.

NOTE Confidence: 0.99470955

00:57:36.135 --> 00:57:37.015 Like, what does this look

NOTE Confidence: 0.99470955

00:57:37.015 --> 00:57:37.515 like?

NOTE Confidence: 0.9749731

00:57:38.775 --> 00:57:39.974 I showed you ladders of

NOTE Confidence: 0.9749731

00:57:39.974 --> 00:57:41.434 backs throughout this whole talk.

NOTE Confidence: 0.9455078

00:57:42.240 --> 00:57:43.200 I told you about how

NOTE Confidence: 0.9455078

00:57:43.200 --> 00:57:44.240 Niko Chandra said, how do

NOTE Confidence: 0.9455078

00:57:44.240 --> 00:57:45.840 you study moving target? That's

NOTE Confidence: 0.9455078

00:57:45.840 --> 00:57:46.420 the problem.

NOTE Confidence: 0.9512652

00:57:46.800 --> 00:57:47.680 And by the way, this

NOTE Confidence: 0.9512652

00:57:47.680 --> 00:57:49.200 isn't a membrane. And so

NOTE Confidence: 0.9512652

00:57:49.200 --> 00:57:50.160 we figured out that the

NOTE Confidence: 0.9512652

00:57:50.160 --> 00:57:50.980 only way

NOTE Confidence: 0.9400754

00:57:52.000 --> 00:57:53.119 to to think about this

NOTE Confidence: 0.9400754

00:57:53.119 --> 00:57:54.240 is to somehow create an

NOTE Confidence: 0.9400754

00:57:54.240 --> 00:57:56.020 oligomeric species that is homogeneous

NOTE Confidence: 0.9400754

00:57:56.240 --> 00:57:56.980 and stable,

NOTE Confidence: 0.999715

00:57:57.515 --> 00:57:58.335 which seems

NOTE Confidence: 0.9777222

00:57:58.635 --> 00:57:59.595 like god bless these two

NOTE Confidence: 0.9777222

00:57:59.595 --> 00:58:00.875 grad students, Hausman and Harvey.

NOTE Confidence: 0.9777222

00:58:00.875 --> 00:58:01.995 They took on this crazy

NOTE Confidence: 0.9777222

00:58:01.995 --> 00:58:03.275 project for their thesis. They

NOTE Confidence: 0.9777222

00:58:03.275 --> 00:58:04.415 actually did it together,

NOTE Confidence: 0.99906415

00:58:05.195 --> 00:58:06.075 to try to figure out

NOTE Confidence: 0.99906415

00:58:06.075 --> 00:58:06.955 how you take the most

NOTE Confidence: 0.99906415

00:58:06.955 --> 00:58:08.155 unstable thing and make it

NOTE Confidence: 0.99906415

00:58:08.155 --> 00:58:08.655 stable.

NOTE Confidence: 0.94625217

00:58:09.035 --> 00:58:09.995 And so they did a

NOTE Confidence: 0.94625217

00:58:09.995 --> 00:58:11.435 detergent screen, and they took

NOTE Confidence: 0.94625217

00:58:11.435 --> 00:58:12.870 inactive backs, and they took

NOTE Confidence: 0.94625217

00:58:13.030 --> 00:58:13.510 oligomer,

NOTE Confidence: 0.97725666

00:58:13.830 --> 00:58:15.110 different detergents and looked at

NOTE Confidence: 0.97725666

00:58:15.110 --> 00:58:16.230 what it did. And, you

NOTE Confidence: 0.97725666

00:58:16.230 --> 00:58:17.370 know, some of these detergents

NOTE Confidence: 0.97725666

00:58:17.430 --> 00:58:18.150 gave you a peak that

NOTE Confidence: 0.97725666

00:58:18.150 --> 00:58:19.770 looked fairly, you know, reliable,

NOTE Confidence: 0.97725666

00:58:19.830 --> 00:58:20.870 consistent. You run it out

NOTE Confidence: 0.97725666

00:58:20.870 --> 00:58:21.830 on a native page. It

NOTE Confidence: 0.97725666

00:58:21.830 --> 00:58:23.190 was like one band. That

NOTE Confidence: 0.97725666

00:58:23.190 --> 00:58:23.930 was exciting.

NOTE Confidence: 0.9851939

00:58:24.310 --> 00:58:25.930 Well, maybe it's this detergent

NOTE Confidence: 0.9851939

00:58:25.990 --> 00:58:27.530 doing something. Who knows? Repurify

NOTE Confidence: 0.9851939

00:58:27.750 --> 00:58:29.345 it without the detergent.

NOTE Confidence: 0.9631676

00:58:29.965 --> 00:58:31.485 And what was there that

NOTE Confidence: 0.9631676

00:58:31.485 --> 00:58:33.405 was induced actually survived after

NOTE Confidence: 0.9631676

00:58:33.405 --> 00:58:34.125 you got rid of the

NOTE Confidence: 0.9631676

00:58:34.125 --> 00:58:35.245 detergent, and there the band

NOTE Confidence: 0.9631676

00:58:35.245 --> 00:58:36.305 was still there again.

NOTE Confidence: 0.9834321

00:58:36.605 --> 00:58:37.645 So we said, okay. What

NOTE Confidence: 0.9834321

00:58:37.645 --> 00:58:38.925 what did we make? What

NOTE Confidence: 0.9834321

00:58:38.925 --> 00:58:39.585 is this?

NOTE Confidence: 0.9067016

00:58:39.965 --> 00:58:41.645 And so we showed that

NOTE Confidence: 0.9067016

00:58:41.645 --> 00:58:42.765 just like t bit triggered

NOTE Confidence: 0.9067016

00:58:42.765 --> 00:58:44.480 bacs that translocates to membranes,

NOTE Confidence: 0.9067016

00:58:44.480 --> 00:58:45.200 this thing you throw it

NOTE Confidence: 0.9067016

00:58:45.200 --> 00:58:45.920 on there, we call it

NOTE Confidence: 0.9067016

00:58:45.920 --> 00:58:47.220 Bax o for Bax oligomer,

NOTE Confidence: 0.986782

00:58:47.599 --> 00:58:48.980 automatically goes to the liposomes.

NOTE Confidence: 0.986782

00:58:49.119 --> 00:58:50.480 So it's behaving like the

NOTE Confidence: 0.986782

00:58:50.480 --> 00:58:51.780 ligand triggered Bax.

NOTE Confidence: 0.98889583

00:58:52.240 --> 00:58:53.460 We showed in our liposomal

NOTE Confidence: 0.98889583

00:58:53.599 --> 00:58:55.280 release assay that just like

NOTE Confidence: 0.98889583

00:58:55.280 --> 00:58:56.720 everything I've showed you multiple

NOTE Confidence: 0.98889583

00:58:56.720 --> 00:58:57.220 times,

NOTE Confidence: 0.9601326

00:58:57.915 --> 00:59:00.395 liposomes alone, VACS alone, ligand

NOTE Confidence: 0.9601326

00:59:00.395 --> 00:59:01.355 alone. You add the two.

NOTE Confidence: 0.9601326

00:59:01.355 --> 00:59:02.475 You get this nice release.

NOTE Confidence: 0.9601326

00:59:02.475 --> 00:59:03.915 You throw in oligomeric VACS.

NOTE Confidence: 0.9601326

00:59:03.915 --> 00:59:05.355 It looks just like TBID

NOTE Confidence: 0.9601326

00:59:05.355 --> 00:59:06.255 triggered VACS.

NOTE Confidence: 0.9592628

00:59:06.875 --> 00:59:07.515 Now you go to the

NOTE Confidence: 0.9592628

00:59:07.515 --> 00:59:09.035 electron microscope because we'd like

NOTE Confidence: 0.9592628

00:59:09.035 --> 00:59:09.995 to you know? Again, let's

NOTE Confidence: 0.9592628

00:59:09.995 --> 00:59:11.090 go look at this thing.

NOTE Confidence: 0.9592628

00:59:11.090 --> 00:59:12.610 Here are your liposomes. Here's

NOTE Confidence: 0.9592628

00:59:12.610 --> 00:59:14.310 your TBID triggered BACS liposomes

NOTE Confidence: 0.9592628

00:59:14.370 --> 00:59:15.330 with these nice holes in

NOTE Confidence: 0.9592628

00:59:15.330 --> 00:59:17.430 them. The BACS oligomeric form,

NOTE Confidence: 0.9592628

00:59:17.490 --> 00:59:18.850 nice holes. Looks just like

NOTE Confidence: 0.9592628

00:59:18.850 --> 00:59:19.990 the panel in the middle.

NOTE Confidence: 0.9925545

00:59:20.850 --> 00:59:21.990 Well, what about mitochondria?

NOTE Confidence: 0.9062933

00:59:22.530 --> 00:59:23.785 Same story. You got your

NOTE Confidence: 0.9062933

00:59:23.785 --> 00:59:25.145 TBID triggered VACS. You add

NOTE Confidence: 0.9062933

00:59:25.145 --> 00:59:26.105 in your dose response of

NOTE Confidence: 0.9062933

00:59:26.105 --> 00:59:26.925 VACS oligomeric

NOTE Confidence: 0.9242539

00:59:27.625 --> 00:59:29.305 species, gives beautiful dose response

NOTE Confidence: 0.9242539

00:59:29.305 --> 00:59:30.605 of cytochrome c release.

NOTE Confidence: 0.977694

00:59:30.985 --> 00:59:32.025 And then the last funny

NOTE Confidence: 0.977694

00:59:32.025 --> 00:59:32.825 thing that I will tell

NOTE Confidence: 0.977694

00:59:32.825 --> 00:59:34.025 you, which again is why

NOTE Confidence: 0.977694

00:59:34.025 --> 00:59:35.305 I really believe, like, you

NOTE Confidence: 0.977694

00:59:35.305 --> 00:59:36.205 have to just

NOTE Confidence: 0.9934982

00:59:36.840 --> 00:59:38.840 sometimes think do multiple things

NOTE Confidence: 0.9934982

00:59:38.840 --> 00:59:40.360 that seemingly have nothing to

NOTE Confidence: 0.9934982

00:59:40.360 --> 00:59:41.340 do with one another,

NOTE Confidence: 0.98838633

00:59:41.640 --> 00:59:42.680 and you may get an

NOTE Confidence: 0.98838633

00:59:42.680 --> 00:59:43.800 intersection that you never would

NOTE Confidence: 0.98838633

00:59:43.800 --> 00:59:45.480 have come up with. And

NOTE Confidence: 0.98838633

00:59:45.480 --> 00:59:46.520 this was a grad student

NOTE Confidence: 0.98838633

00:59:46.520 --> 00:59:47.560 coming to my lab and

NOTE Confidence: 0.98838633

00:59:47.560 --> 00:59:48.860 said, oh, antimicrobial

NOTE Confidence: 0.9749107

00:59:49.240 --> 00:59:50.775 peptides are very important in

NOTE Confidence: 0.9749107

00:59:50.775 --> 00:59:52.615 antibiotic resistance. And guess what?

NOTE Confidence: 0.9749107

00:59:52.615 --> 00:59:54.055 There's hundreds of alpha helical

NOTE Confidence: 0.9749107

00:59:54.055 --> 00:59:55.655 ones, and they're very, very

NOTE Confidence: 0.9749107

00:59:55.655 --> 00:59:57.335 toxic, you know, to membranes,

NOTE Confidence: 0.9749107

00:59:57.335 --> 00:59:58.375 and they're really good drugs

NOTE Confidence: 0.9749107

00:59:58.375 --> 01:00:00.454 against antibiotic resistant bacteria. Oh,

NOTE Confidence: 0.9749107

01:00:00.454 --> 01:00:01.415 but by the way, they

NOTE Confidence: 0.9749107

01:00:01.415 --> 01:00:02.635 they nuke every membrane.

NOTE Confidence: 0.9739791

01:00:03.339 --> 01:00:04.220 And I thought, well, that's

NOTE Confidence: 0.9739791

01:00:04.220 --> 01:00:05.579 great. Like, we're trying to,

NOTE Confidence: 0.9739791

01:00:05.579 --> 01:00:07.260 like, deliver staple peptides to

NOTE Confidence: 0.9739791

01:00:07.260 --> 01:00:08.780 cells, cancer cells, so they

NOTE Confidence: 0.9739791

01:00:08.780 --> 01:00:10.460 don't disrupt membranes. Maybe if

NOTE Confidence: 0.9739791

01:00:10.460 --> 01:00:11.660 we just study how they

NOTE Confidence: 0.9739791

01:00:11.660 --> 01:00:12.880 actually nuke membranes,

NOTE Confidence: 0.984144

01:00:13.260 --> 01:00:14.300 something will come of this.

NOTE Confidence: 0.984144

01:00:14.300 --> 01:00:15.260 Somewhere in the middle, what

NOTE Confidence: 0.984144

01:00:15.260 --> 01:00:16.619 we learn about nuking membranes

NOTE Confidence: 0.984144

01:00:16.619 --> 01:00:18.075 and preserving membranes will find

NOTE Confidence: 0.984144

01:00:18.075 --> 01:00:19.135 us something interesting.

NOTE Confidence: 0.97396827

01:00:19.755 --> 01:00:21.035 We had no idea we'd

NOTE Confidence: 0.97396827

01:00:21.035 --> 01:00:22.415 end up landing on backs.

NOTE Confidence: 0.97396827

01:00:22.714 --> 01:00:24.075 Okay? And so we published

NOTE Confidence: 0.97396827

01:00:24.075 --> 01:00:25.355 this paper about how to

NOTE Confidence: 0.97396827

01:00:25.355 --> 01:00:26.255 make antimicrobial

NOTE Confidence: 0.9995793

01:00:26.795 --> 01:00:27.295 peptides

NOTE Confidence: 0.96300054

01:00:27.675 --> 01:00:28.895 selective for bacteria

NOTE Confidence: 0.99507326

01:00:29.195 --> 01:00:30.255 and not disrupt

NOTE Confidence: 0.9612476

01:00:30.635 --> 01:00:32.075 mammalian membranes, a story for

NOTE Confidence: 0.9612476

01:00:32.075 --> 01:00:33.910 another day. But what what

NOTE Confidence: 0.9612476

01:00:33.910 --> 01:00:35.350 we've learned from that was

NOTE Confidence: 0.9612476

01:00:35.350 --> 01:00:36.970 that these were hydrophobic

NOTE Confidence: 0.96787274

01:00:37.830 --> 01:00:39.750 faced with cationic charges on

NOTE Confidence: 0.96787274

01:00:39.750 --> 01:00:41.030 the other side, and the

NOTE Confidence: 0.96787274

01:00:41.030 --> 01:00:42.630 mechanism how they work is

NOTE Confidence: 0.96787274

01:00:42.630 --> 01:00:43.290 they form

NOTE Confidence: 0.9246697

01:00:44.150 --> 01:00:46.230 toroidal pores. They cause negative

NOTE Confidence: 0.9246697

01:00:46.230 --> 01:00:47.850 Gaussian curvature to membranes.

NOTE Confidence: 0.96744317

01:00:48.470 --> 01:00:50.924 And when you scan bacs

NOTE Confidence: 0.96744317

01:00:50.924 --> 01:00:51.744 for hydrophobic

NOTE Confidence: 0.99956393

01:00:52.045 --> 01:00:52.865 and cationic

NOTE Confidence: 0.98549867

01:00:53.484 --> 01:00:53.984 sequences,

NOTE Confidence: 0.9739335

01:00:54.765 --> 01:00:55.505 guess what?

NOTE Confidence: 0.9533359

01:00:56.204 --> 01:00:57.644 Alpha six, if you didn't

NOTE Confidence: 0.9533359

01:00:57.644 --> 01:00:59.244 know anything about anything and

NOTE Confidence: 0.9533359

01:00:59.244 --> 01:01:00.144 you're an antimicrobial

NOTE Confidence: 0.99715304

01:01:00.525 --> 01:01:02.444 peptide professor, you would say,

NOTE Confidence: 0.99715304

01:01:02.444 --> 01:01:03.664 oh, that's an antimicrobial

NOTE Confidence: 0.9999708

01:01:04.045 --> 01:01:04.545 peptide

NOTE Confidence: 0.9657237

01:01:05.780 --> 01:01:07.320 as the alpha six helix

NOTE Confidence: 0.9657237

01:01:07.380 --> 01:01:08.040 of BACS.

NOTE Confidence: 0.9799489

01:01:08.500 --> 01:01:10.100 And there they are sitting

NOTE Confidence: 0.9799489

01:01:10.100 --> 01:01:11.480 on the surface of BACS,

NOTE Confidence: 0.96588874

01:01:11.859 --> 01:01:12.900 and you look at an

NOTE Confidence: 0.96588874

01:01:12.900 --> 01:01:14.420 anti apoptotic protein. Oh, they

NOTE Confidence: 0.96588874

01:01:14.420 --> 01:01:15.780 don't have those arginines on

NOTE Confidence: 0.96588874

01:01:15.780 --> 01:01:16.980 the surface of their alpha

NOTE Confidence: 0.96588874

01:01:16.980 --> 01:01:18.340 sixes. Oh, and I could

NOTE Confidence: 0.96588874

01:01:18.340 --> 01:01:19.460 put them on liposomes, and

NOTE Confidence: 0.96588874

01:01:19.460 --> 01:01:21.095 alpha six pops those liposomes

NOTE Confidence: 0.96588874

01:01:21.315 --> 01:01:22.615 all by itself in BAX,

NOTE Confidence: 0.96588874

01:01:22.675 --> 01:01:24.195 but the anti apoptotic one

NOTE Confidence: 0.96588874

01:01:24.195 --> 01:01:25.335 does not do that.

NOTE Confidence: 0.9599551

01:01:26.035 --> 01:01:27.255 And then I could start,

NOTE Confidence: 0.9599551

01:01:27.395 --> 01:01:29.235 you know, doing reverse polarity

NOTE Confidence: 0.9599551

01:01:29.235 --> 01:01:30.515 mutagenesis, and all of a

NOTE Confidence: 0.9599551

01:01:30.515 --> 01:01:31.795 sudden my BAX doesn't work

NOTE Confidence: 0.9599551

01:01:31.795 --> 01:01:33.155 in a liposomal assay as

NOTE Confidence: 0.9599551

01:01:33.155 --> 01:01:34.675 well, in the cytochrome c

NOTE Confidence: 0.9599551

01:01:34.675 --> 01:01:35.890 assay as well. If I

NOTE Confidence: 0.9599551

01:01:35.890 --> 01:01:37.430 do the double mutant experiments,

NOTE Confidence: 0.95490587

01:01:37.890 --> 01:01:38.710 even worse,

NOTE Confidence: 0.9591689

01:01:39.170 --> 01:01:39.990 less activity.

NOTE Confidence: 0.97035444

01:01:40.290 --> 01:01:41.410 And then the the most

NOTE Confidence: 0.97035444

01:01:41.410 --> 01:01:43.010 important experiment for the reviewers

NOTE Confidence: 0.97035444

01:01:43.010 --> 01:01:43.730 is show me that it

NOTE Confidence: 0.97035444

01:01:43.730 --> 01:01:45.010 works in cells, and you

NOTE Confidence: 0.97035444

01:01:45.010 --> 01:01:45.750 can reconstitute

NOTE Confidence: 0.9677169

01:01:46.130 --> 01:01:47.830 cells with wild type acts,

NOTE Confidence: 0.9815911

01:01:48.195 --> 01:01:49.875 in a DKO background and

NOTE Confidence: 0.9815911

01:01:49.875 --> 01:01:51.155 then take out just those

NOTE Confidence: 0.9815911

01:01:51.155 --> 01:01:52.995 two arginines, and bacs is

NOTE Confidence: 0.9815911

01:01:52.995 --> 01:01:54.595 now no longer working very

NOTE Confidence: 0.9815911

01:01:54.595 --> 01:01:55.495 well at all.

NOTE Confidence: 0.92827284

01:01:57.235 --> 01:01:58.675 So, you know, there's been

NOTE Confidence: 0.92827284

01:01:58.675 --> 01:01:59.475 a lot of work trying

NOTE Confidence: 0.92827284

01:01:59.475 --> 01:02:00.910 to figure out what BAX

NOTE Confidence: 0.92827284

01:02:00.910 --> 01:02:02.030 is doing and is it

NOTE Confidence: 0.92827284

01:02:02.030 --> 01:02:02.990 a poor and this and

NOTE Confidence: 0.92827284

01:02:02.990 --> 01:02:04.910 that. But, honestly, I,

NOTE Confidence: 0.9987777

01:02:05.470 --> 01:02:06.510 have come around to the

NOTE Confidence: 0.9987777

01:02:06.510 --> 01:02:07.010 idea

NOTE Confidence: 0.99562305

01:02:07.470 --> 01:02:09.090 that BAX is a membrane

NOTE Confidence: 0.99562305

01:02:09.310 --> 01:02:09.810 disruptive

NOTE Confidence: 0.9998863

01:02:10.190 --> 01:02:10.690 protein

NOTE Confidence: 0.9428219

01:02:10.990 --> 01:02:11.870 and that you get it

NOTE Confidence: 0.9428219

01:02:11.870 --> 01:02:12.990 on the mitochondria and it's

NOTE Confidence: 0.9428219

01:02:12.990 --> 01:02:14.350 self associating, and it's seeing

NOTE Confidence: 0.9428219

01:02:14.350 --> 01:02:15.895 those alpha six cationic

NOTE Confidence: 0.96952885

01:02:16.355 --> 01:02:16.855 hydrophobic

NOTE Confidence: 0.9877961

01:02:17.395 --> 01:02:19.155 amphipathic peptides there that look

NOTE Confidence: 0.9877961

01:02:19.155 --> 01:02:20.615 just like antimicrobial

NOTE Confidence: 0.98705107

01:02:21.075 --> 01:02:22.515 peptides, and it is lysing

NOTE Confidence: 0.98705107

01:02:22.515 --> 01:02:23.175 the membrane.

NOTE Confidence: 0.9726508

01:02:23.635 --> 01:02:24.755 And so, you know, of

NOTE Confidence: 0.9726508

01:02:24.755 --> 01:02:26.194 course, we're super interested in

NOTE Confidence: 0.9726508

01:02:26.194 --> 01:02:27.954 understanding what those structures are.

NOTE Confidence: 0.9726508

01:02:27.954 --> 01:02:28.650 We have sacks

NOTE Confidence: 0.9862324

01:02:29.210 --> 01:02:29.930 of our,

NOTE Confidence: 0.99472797

01:02:30.890 --> 01:02:31.390 stable,

NOTE Confidence: 0.93251705

01:02:32.410 --> 01:02:33.770 Bax o, and it looks

NOTE Confidence: 0.93251705

01:02:33.770 --> 01:02:34.970 like a finger. You know,

NOTE Confidence: 0.93251705

01:02:34.970 --> 01:02:36.089 you've got, like, what looks

NOTE Confidence: 0.93251705

01:02:36.089 --> 01:02:36.589 like

NOTE Confidence: 0.9640072

01:02:36.890 --> 01:02:38.490 three dimers there attached to

NOTE Confidence: 0.9640072

01:02:38.490 --> 01:02:39.609 one another, and you can

NOTE Confidence: 0.9640072

01:02:39.609 --> 01:02:40.569 kinda think about on the

NOTE Confidence: 0.9640072

01:02:40.569 --> 01:02:41.609 right hand side those holes

NOTE Confidence: 0.9640072

01:02:41.609 --> 01:02:42.410 that we showed in the

NOTE Confidence: 0.9640072

01:02:42.410 --> 01:02:43.450 liposomes. And you can kind

NOTE Confidence: 0.9640072

01:02:43.450 --> 01:02:45.494 of imagine these linear structures

NOTE Confidence: 0.9640072

01:02:45.494 --> 01:02:47.255 of these oligomeric dimers sitting

NOTE Confidence: 0.9640072

01:02:47.255 --> 01:02:48.635 around and just

NOTE Confidence: 0.9963936

01:02:49.255 --> 01:02:50.775 disrupting the membrane and causing

NOTE Confidence: 0.9963936

01:02:50.775 --> 01:02:52.055 deformation so that it leads

NOTE Confidence: 0.9963936

01:02:52.055 --> 01:02:52.714 to rupture.

NOTE Confidence: 0.97559196

01:02:53.255 --> 01:02:54.694 So lots more work to

NOTE Confidence: 0.97559196

01:02:54.694 --> 01:02:56.714 do on this part,

NOTE Confidence: 0.98790973

01:02:57.410 --> 01:02:58.290 but I feel like we're

NOTE Confidence: 0.98790973

01:02:58.290 --> 01:02:59.490 kind of inching closer to

NOTE Confidence: 0.98790973

01:02:59.490 --> 01:03:01.190 really trying to understand how

NOTE Confidence: 0.98790973

01:03:01.330 --> 01:03:02.710 backs and back really

NOTE Confidence: 0.9769445

01:03:03.250 --> 01:03:04.610 cause cytochrome c release by

NOTE Confidence: 0.9769445

01:03:04.610 --> 01:03:06.130 disrupting the outer membrane. So

NOTE Confidence: 0.9769445

01:03:06.130 --> 01:03:07.250 I know I'm right at

NOTE Confidence: 0.9769445

01:03:07.250 --> 01:03:08.130 the minute there, so I

NOTE Confidence: 0.9769445

01:03:08.130 --> 01:03:08.950 will stop.

NOTE Confidence: 0.99884605

01:03:09.410 --> 01:03:10.770 Thank everybody in the lab

NOTE Confidence: 0.99884605

01:03:10.770 --> 01:03:11.750 that has done it

NOTE Confidence: 0.9557369

01:03:13.224 --> 01:03:14.345 in recent years, that has

NOTE Confidence: 0.9557369

01:03:14.345 --> 01:03:15.305 done it like Sam in

NOTE Confidence: 0.9557369

01:03:15.305 --> 01:03:17.085 prior years, our amazing collaborators.

NOTE Confidence: 0.9557369

01:03:17.145 --> 01:03:17.885 And I just

NOTE Confidence: 0.9995141

01:03:18.345 --> 01:03:19.325 have to end

NOTE Confidence: 0.7615345

01:03:22.984 --> 01:03:23.484 with

NOTE Confidence: 0.97463924

01:03:24.185 --> 01:03:24.685 mentorship.

NOTE Confidence: 0.92905706

01:03:27.780 --> 01:03:29.480 You know, there is

NOTE Confidence: 0.99974734

01:03:31.220 --> 01:03:32.040 no chance

NOTE Confidence: 0.98398584

01:03:32.580 --> 01:03:33.700 that I would be standing

NOTE Confidence: 0.98398584

01:03:33.700 --> 01:03:34.200 here

NOTE Confidence: 0.98400384

01:03:34.580 --> 01:03:35.960 telling you any of this

NOTE Confidence: 0.98400384

01:03:36.180 --> 01:03:37.560 if it wasn't for him.

NOTE Confidence: 0.9716937

01:03:38.435 --> 01:03:40.115 Changed my life. And although

NOTE Confidence: 0.9716937

01:03:40.115 --> 01:03:40.995 many of you know that

NOTE Confidence: 0.9716937

01:03:40.995 --> 01:03:42.455 he died way too young,

NOTE Confidence: 0.9716937

01:03:42.515 --> 01:03:43.895 the age of fifty four,

NOTE Confidence: 0.96275395

01:03:44.595 --> 01:03:45.715 the impact that he has

NOTE Confidence: 0.96275395

01:03:45.715 --> 01:03:47.415 had on so many scientists

NOTE Confidence: 0.96275395

01:03:47.635 --> 01:03:48.995 and the reason why no

NOTE Confidence: 0.96275395

01:03:48.995 --> 01:03:49.815 matter how

NOTE Confidence: 0.9905238

01:03:50.270 --> 01:03:51.150 far away it is from

NOTE Confidence: 0.9905238

01:03:51.150 --> 01:03:51.950 the time of his death

NOTE Confidence: 0.9905238

01:03:51.950 --> 01:03:52.910 in two thousand and six,

NOTE Confidence: 0.9905238

01:03:52.910 --> 01:03:54.190 I will never be able

NOTE Confidence: 0.9905238

01:03:54.190 --> 01:03:55.410 to talk about him without,

NOTE Confidence: 0.9711254

01:03:56.270 --> 01:03:56.770 emotionality.

NOTE Confidence: 0.99851376

01:03:57.390 --> 01:03:57.890 But

NOTE Confidence: 0.9994149

01:03:58.190 --> 01:03:58.930 the impact

NOTE Confidence: 0.972042

01:03:59.470 --> 01:04:00.369 that your mentors,

NOTE Confidence: 0.98535514

01:04:00.829 --> 01:04:01.950 the young ones here today

NOTE Confidence: 0.98535514

01:04:01.950 --> 01:04:02.930 that will be mentors,

NOTE Confidence: 0.9961858

01:04:03.470 --> 01:04:04.770 you know, it is

NOTE Confidence: 0.95851743

01:04:05.385 --> 01:04:06.285 that relationship.

NOTE Confidence: 0.9859876

01:04:06.585 --> 01:04:07.965 It is that cultivation.

NOTE Confidence: 0.9580289

01:04:08.585 --> 01:04:09.865 You know, that's part of

NOTE Confidence: 0.9580289

01:04:09.865 --> 01:04:11.325 what we're trying to preserve

NOTE Confidence: 0.9580289

01:04:11.385 --> 01:04:13.165 literally today. That's at threat.

NOTE Confidence: 0.98001784

01:04:13.545 --> 01:04:15.385 Right? That continuity, that legacy,

NOTE Confidence: 0.98001784

01:04:15.385 --> 01:04:17.065 that chain reaction of the

NOTE Confidence: 0.98001784

01:04:17.065 --> 01:04:18.745 senior person inspiring the younger

NOTE Confidence: 0.98001784

01:04:18.745 --> 01:04:20.185 person who's then inspiring the

NOTE Confidence: 0.98001784

01:04:20.185 --> 01:04:21.910 younger person. And that is

NOTE Confidence: 0.98001784

01:04:21.910 --> 01:04:23.190 what our science is all

NOTE Confidence: 0.98001784

01:04:23.190 --> 01:04:24.150 about. And so we have

NOTE Confidence: 0.98001784

01:04:24.150 --> 01:04:25.510 to guard it and advocate

NOTE Confidence: 0.98001784

01:04:25.510 --> 01:04:26.950 for it and keep doing

NOTE Confidence: 0.98001784

01:04:26.950 --> 01:04:28.150 what we're doing, that we

NOTE Confidence: 0.98001784

01:04:28.150 --> 01:04:29.110 know that what we're doing

NOTE Confidence: 0.98001784

01:04:29.110 --> 01:04:30.150 is important. And at the

NOTE Confidence: 0.98001784

01:04:30.150 --> 01:04:32.070 same time, it's not only

NOTE Confidence: 0.98001784

01:04:32.070 --> 01:04:33.690 focusing and being persistent

NOTE Confidence: 0.9969429

01:04:34.405 --> 01:04:35.685 about our science, but we

NOTE Confidence: 0.9969429

01:04:35.685 --> 01:04:36.985 have to lead now too.

NOTE Confidence: 0.9969429

01:04:37.205 --> 01:04:38.425 We have to make sure

NOTE Confidence: 0.99485505

01:04:38.805 --> 01:04:40.165 that we get out there

NOTE Confidence: 0.99485505

01:04:40.165 --> 01:04:40.985 and somehow

NOTE Confidence: 0.9979267

01:04:41.285 --> 01:04:42.245 use what we think is

NOTE Confidence: 0.9979267

01:04:42.245 --> 01:04:43.845 so special about our institutions

NOTE Confidence: 0.9979267

01:04:43.845 --> 01:04:45.145 and what we're doing scientifically

NOTE Confidence: 0.9979267

01:04:45.205 --> 01:04:45.785 and medically

NOTE Confidence: 0.97149044

01:04:46.165 --> 01:04:47.605 and teach people that probably

NOTE Confidence: 0.97149044

01:04:47.605 --> 01:04:49.650 don't wanna listen and somehow

NOTE Confidence: 0.97149044

01:04:49.650 --> 01:04:51.410 convince, you know, elected folks

NOTE Confidence: 0.97149044

01:04:51.410 --> 01:04:53.190 that this is the lifeblood

NOTE Confidence: 0.97149044

01:04:53.410 --> 01:04:54.470 of health,

NOTE Confidence: 0.9888498

01:04:54.923 --> 01:04:56.523 you know, in America. Right?

NOTE Confidence: 0.9888498

01:04:56.523 --> 01:04:57.483 And so that's kind of

NOTE Confidence: 0.9888498

01:04:57.483 --> 01:04:59.083 a big new challenge that

NOTE Confidence: 0.9888498

01:04:59.083 --> 01:05:00.283 we've never ever had to

NOTE Confidence: 0.9888498

01:05:00.283 --> 01:05:01.343 think about before.

NOTE Confidence: 0.987748

01:05:01.723 --> 01:05:02.843 So, anyway, thank you so

NOTE Confidence: 0.987748

01:05:02.843 --> 01:05:03.723 much for staying. I know

NOTE Confidence: 0.987748

01:05:03.723 --> 01:05:04.943 I'm five minutes over.