On behalf of my Co leader, Barbara Burtness and I, I'm pleased to introduce Bill Jorgensen, one of our developmental therapeutic program members and also my long term collaborator. Bill is a graduate of Princeton and Harvard, spent 15 years on the faculty at Purdue, and in 1990 he moved to Yale, where he's currently Sterling Professor in the Chemistry department. Bill is internationally recognized as one of the world leaders in computational chemistry and drug design.
His research has been recognized by many honors, and among those include the American Chemical Society Cope Scholar Award, the ACS Award for Computers, the ACS Hildebrand Award, the ISTQB P award in computational biology, the Sato International Award from the Pharmaceutical Society of Japan. He’s been elected to membership in the International Academy of Quantum Molecular Science, the American Academy of Arts and Sciences, and the US National Academy of Sciences.
2020 includes one of the 16.

Researchers selected for a Nobel Laureate Citation for individuals considered doing Nobel Class Research that has been cited over 2000 times.

Today he's going to tell you a little bit about some of his work on SARS COVID 2.

So without further ado,

So the our work I'll tell you about today is totally a collaboration between my
So I’ll talk a little bit in general about computer aided drug discovery and then specifically about our work and finding very potent protease inhibitors for SARS. Move two. So a key element of drug design is the fact of trying to make inhibitors that bind to an enzyme typically. And we’ll be talking about again a small molecule binding to SARS Cove 2 protease. And so this is governed by an equilibrium where you have the protein and water, the inhibitor and water, there’s a free energy of binding and then the complex.
So the free energy of binding the G because we’re working in the constant pressure, constant temperature world is for Gibbs. So it’s a Gibbs free energy and I put the stamp of our former colleague Jay Willard. Gibbs here is the father of Thermodynamics. So the free energy binding just to introduce the concept of a nanomolar inhibitor. So the free energy of binding is given by minusRTL and the dissociation constant if you have a dissociation constant of 10 to the minus nine molar. That would correspond to A1 nanomolar. Inhibitor or an inhibitor that has that KD,
one that has a KD of 10 to the
minus six would be a micromolar
inhibitor and our binder.
And the reason I bring this up
is that most drugs turn out to be
typically one to let’s say 20 or
so nanomolar in a binding assay.
And this all ultimately has to do with
the farm human pharmacology and just
how big a pill one is willing to take.
So this obsession with nanomolar
inhibitors just to, you know,
reflects this fact,
so.
Ultimately here we’re going to have to
do simulations on computer simulations
00:04:03.936 --> 00:04:07.450 of proteins binding to ligands in water.

00:04:07.450 --> 00:04:09.088 And so how did this arise?

00:04:09.090 --> 00:04:11.729 When did it with such things happen?

00:04:11.730 --> 00:04:13.406 And the answer is,

00:04:13.406 --> 00:04:15.501 there really wasn’t any significant

00:04:15.501 --> 00:04:18.297 work on doing computer simulations of

00:04:18.297 --> 00:04:21.052 molecular fluids before the late 1970s.

00:04:21.052 --> 00:04:25.030 And then of course it grew slowly after that.

00:04:25.030 --> 00:04:27.145 The problem is you have a lot of particles,

00:04:27.150 --> 00:04:29.182 you’re using classical force.

00:04:29.182 --> 00:04:31.214 Those describe the interactions,

00:04:31.220 --> 00:04:33.754 but there’s still a lot of particles

00:04:33.754 --> 00:04:35.140 and you have to.

00:04:35.140 --> 00:04:37.750 Observe the system over a significant

00:04:37.750 --> 00:04:38.620 time period.
So if you’re doing molecular dynamics, we might want to run the molecular dynamics for picosecond hundreds of picoseconds, nanoseconds and this just we didn’t have the computer resources to do that. And then making it more complicated by putting a protein into it and describing the energetics of the protein and the water. That really didn’t happen until mid. 1980s and my colleague here, Julian Torrado Rivas and I published one of the first calculations for a protein in water published one of the first where we did molecular dynamics for 100 picoseconds and that was in 1988.
So doing the type of calculations we're talking about today is relatively recent phenomenon. This is a picture we'll talk about HIV reverse transcriptase and just to get the sense, I usually give this to less. Sophisticated audiences to point out the yellow, little yellow pieces inhibitor and that's enough to shut down this enzyme and this is an example of one of the compounds that developed through Karens and in our work that is a inhibitor of
HIV RT that little molecule.

So here’s the way we do it.

We normally start with an X-ray structure and the first phase of this we’re looking for micromolar hit compounds that then we have to do a lot of hard work on to bring them to the low nanomolar level.

So we normally start with an X-ray structure and this can be from somebody else’s work and we remove the ligand that might be in that X-ray structure and we try to design our new own inhibitors and that.

Started out we do a virtual
screening which is docking.
And I'll tell you a little bit more about that,
where we literally fly molecules into the protein structure and see which ones look the best.
Or are we do denovo design where we use a growing program that I wrote a while back that starts with the little seed core of a molecule of, say benzene you place in the binding site.
And then the program will build libraries of compounds starting from that core, growing them out in the binding site. And then you have to.
A score of them, evaluate them in the same manner that this invariably gives us these micromolar hit compounds. But we’ve never been fortunate enough to do this initial part of the work and end up with nanomolar inhibitors. We’re close, you know, single digit micromolar. So then the hard part is the lead optimization because we’re going to have to refine the micromolar hits by making small changes that we decide. What to do? By a lot of structure building and energy minimizations.
So this bond program of mining can rapidly build protein ligand complexes. We can energy minimize them. That’s just a fast calculation compared to adding the water doing the molecular anamax. And so we do a lot of the structure building, energy minimization and then for select cases we will do, excuse me, the free energy calculations that are sort of our hallmark. We call them FEP, free energy perturbation calculations. Virtually all pharmaceutical companies today are,
you know,

Everybody's doing FP calculations for a drug design.

So then you have to make a decision on what molecules to synthesize for their assay.

So you need somebody like Karen to help out in the assaying and the crystallography, the crystallography isn’t.

But it sure is helpful if you know very much helps reinforce what the modeling is doing.

And also sometimes you’ll see that there’s a change in the protein structure from what you
00:08:55.304 --> 00:08:57.127 originally started with that you
00:08:57.127 --> 00:08:58.897 will see in the crystallography.
00:08:58.900 --> 00:09:00.188 You don’t necessarily see
00:09:00.188 --> 00:09:01.476 it in the computation.
00:09:01.480 --> 00:09:05.968 So the crystallography is really helpful.
00:09:07.690 --> 00:09:10.936 When the HIV area,
00:09:10.936 --> 00:09:13.050 Karen and I got along for quite a few
00:09:13.050 --> 00:09:14.655 but then once we certainly
00:09:14.655 --> 00:09:20.108 it certainly made life a lot more confident.
00:09:20.110 --> 00:09:22.936 So you repeat the cycle until
00:09:22.936 --> 00:09:25.790 you get the potency you want.
00:09:25.790 --> 00:09:29.078 All the while we are mindful of properties,
00:09:29.080 --> 00:09:33.013 so we want the compounds to be drug like.
And that requires a having things like reasonable solubility, reasonable cell permeability, no reactive functional groups. So we have software that checks that and then we also do some measurements of solubility and cell permeability, OK. So the FP calculations are done for where you do molecular dynamics or Monte Carlo simulations for protein ligand and a typically a ball of several 1000. Water molecules. And you do a calculation where you’re comparing the green ligand, green inhibitor with the blue.
So you do calculation. We have protein green legging to give complex protein blue ligand to give complex. And what we do on the computer, it turns out to be easier is to mutate the green leg into the blue unbound in water and then bound protein. And the difference in the two vertical numbers there then gives us the difference in predicted free energy binding. And so this type of calculation wasn’t done at all before 1985 or just the simple green to blue in water FP calculation.
It will take credit for doing the first calculation of that type again. Then there’s no software. You had to write all the software, you know the force fields we had to develop etcetera. So it was very different world in 1985, OK. So here are just a little bit on HIV. HIV is still a big problem. Some of the statistics are shown there for 2021. They’re, you know, close to 40 million people in the world that are infected with HIV and about 1 to 2,000,000 each year are becoming infected.
And they’re on the order of 650,000 deaths. So that’s down quite a bit from what it was. But still, you know, from a very serious problem and a Long story short.

We have worked on with Karen on the reverse transcriptase and the RNA virus and it has a reverse transcriptase which converts the RNA to DNA which is incorporated into the host genome by HIV integrase. So HIV reverse transcriptase has been the principal target for anti
HIV drugs and there are two classes, the nucleosides. And the non nuclear science, Karen has worked on both. So in our collaboration with Karen we’ve only worked on non nuclear sites, the NRT I and there are allosteric inhibitors. They bind in this little pocket that is about 10 angstroms or so from the polymerase active site. It’s one of the few examples of allosteric inhibitors that have become drugs. It’s very, very, very I’m, I’m, I’m. Have to think of it to find others. This is the principal example. The crystal structure.
Again a Yale connection.
The original crystal structure of HIV RT was done in the sites lab 1992.
This is a very big. You know,
This discovery at the time because the HIV crisis was so severe and it’s a big protein,
So Long story short,
we’ve tried to make better non nucleoside inhibitors.
The original ones have limitations.
The original ones have limitations.
They’re susceptible to mutations that arise quickly.
They also had some undesirable pharmacology.
So the way we proceed on
HIV is the same with the COVID and the trick in lead optimization is making systematic changes small changes in. Substituents on rings, the rings themselves and groups that link rings together, and if you know the right changes to make, they can have profound effects. So this is an early HIV compound of ours that we came about from a de Novo design and Karen’s lab. The assay they’re running is an infected T cell assay and this compound had an EC50. For inhibiting the reproduction of the HIV in the infected cells of 10 micromolar,
00:14:21.390 --> 00:14:22.668 so 10,000 nanomolar.

00:14:22.668 --> 00:14:25.224 So that’s a reasonable starting place,

00:14:25.230 --> 00:14:28.422 a small molecule, but we’ve got to

00:14:28.422 --> 00:14:31.370 increase the potency by a thousandfold.

00:14:31.370 --> 00:14:33.656 So I I point out here that if you

00:14:33.656 --> 00:14:35.758 happen to know to put a cyano group

00:14:35.758 --> 00:14:37.990 in the four position of this ring,

00:14:37.990 --> 00:14:40.186 you get a very big boost,

00:14:40.190 --> 00:14:44.390 50 fold boost to 200 nanomolar.

00:14:44.390 --> 00:14:44.850 OK.

00:14:44.850 --> 00:14:47.610 Then if you happen to change

00:14:47.610 --> 00:14:50.280 the thiazole into a pyrimidine,

00:14:50.280 --> 00:14:53.070 you get another tenfold boost and

00:14:53.070 --> 00:14:55.935 you’re at 17 nanul. So quite amazing.

00:14:55.935 --> 00:14:58.698 And then if you happen to know to

00:14:58.698 --> 00:15:01.250
Put a methoxy group and the three position of the pyrimidine ring here 2 nanomolar. So you have more potency than you need for a drug. So this is all fine and this is what we use the FEP calculations to help us with because these changes are in a sea of possible changes. So we do however scans where we have a compound like this, have we have a compound like this, we’ll scan in chlorine atoms at each open. To see if we can add a little beef to it and that might have, if we did that it would show that this four position is good for chlorine,
well if it’s good for chlorine and may also be good or even better for cyano because they’re both somewhat electronic drawing. So then we would try siana. But we do these initial scans, we also do heterocycle scans of five and six membered rings because they are obviously affect hydrogen bonding patterns and hopefully that would have picked up that the pyrimidine was the way to go. And then finally we do another substituent scan on the pyrimidine of methyls and chlorines,
we would see that substitution and the three position is a good thing and before long we would come to the methoxy.

So that’s the way it’s done.

And that’s attuned animal or very potent compound we did in collaboration with Eddie Arnold got a crystal structure of that there’s quite a bit later and that was the only crystal structure we had until Karen’s group started getting some around 2012.

OK, so here is just some of the work with Karen.

These are all publications on different. And an RTI’s and you might say well Gee and from 2006 you have these two
national or compound aren't you done, why are you keeping doing this and the answer is that that number is against the wild type virus. But the virus as you know have mutates just like COVID is mutating and there's a whole panel of mutants with the HIV and you need to have efficacy against all of the common mutants with one compound. So it’s tough. So that initial compound like initial.

Compounds in this class, such as nevirapine, was the first approved drug in
00:17:25.259 --> 00:17:26.966 this class, like nevirapine.
NOTE Confidence: 0.825470216666667
00:17:26.966 --> 00:17:29.331 It was good against wild
NOTE Confidence: 0.825470216666667
00:17:29.331 --> 00:17:32.069 type but not not much else.
NOTE Confidence: 0.825470216666667
00:17:32.070 --> 00:17:33.985 So these other compounds that
NOTE Confidence: 0.825470216666667
00:17:33.985 --> 00:17:36.270 I’ll just skip to this one,
NOTE Confidence: 0.825470216666667
00:17:36.270 --> 00:17:37.700 one of our better compounds,
NOTE Confidence: 0.825470216666667
00:17:37.700 --> 00:17:39.376 we’ve increased the potency,
NOTE Confidence: 0.825470216666667
00:17:39.376 --> 00:17:41.890 but we very much increased the
NOTE Confidence: 0.825470216666667
00:17:41.890 --> 00:17:43.678 performance again mutant panels,
NOTE Confidence: 0.825470216666667
00:17:43.678 --> 00:17:45.518 here is A10 animal or EC50,
NOTE Confidence: 0.825470216666667
00:17:50.050 --> 00:17:52.314 Y181C and this compound
NOTE Confidence: 0.825470216666667
00:17:52.314 --> 00:17:55.518 here is A10 animal or EC50,
NOTE Confidence: 0.825470216666667
00:17:55.518 --> 00:17:58.094 which is you know good and great
NOTE Confidence: 0.825470216666667
00:17:58.094 --> 00:18:00.179 against that whereas the original
NOTE Confidence: 0.825470216666667
00:18:00.179 --> 00:18:02.304 compounds here would have had
no efficacy against that mutant. And we’ve gone on, we even see something that looks like a covalent inhibitor which it is. We with cooperation with Karen, we have covalent inhibitors for HIV RT Wild. Type and also the Y181C mutant. But I will go on now to what we did with COVID. So fortunately, because of our work on HIV. We’re pretty well positioned just to try to do something when COVID rolled around at the beginning of 2020. So this is the IT again an RNA genome. And it some of the proteins that
it encodes are indicated here, and not as many as with the HIV, but you do have. The There's a proteases here that are sort of papain like protease and then the main protease and what we've worked on is the main protease. There's also, you've probably heard of the RNA dependent RNA polymerase. This is just to reproduce the RNA genome. That's another possible target, and some of the structural proteins are over here. There's the spike and the famous spike that is mutating and causing
a lot of problems for the vaccines.
So the cycle, the life cycle involves the COVID virus binding to the ACE 2 receptors on the cells endocytosis. The RNA genome is unprocessed by a host of ribosomes to give you these two polyproteins. You similar situation with HIV, generating polyproteins that have to be cleaved by HIV. Areas. So here’s where if we can stop this proteolysis step, the rest of the reproduction cycle stops and it’s, I could say there aren’t as many targets here as with the HIV. There’s no integrase,
no reverse transcriptase.
And So what we picked in the beginning that we would work on the protease almost because there's hardly anything else to work on and there was a crystal structure. Reported so the first thing we did. So this came about as as you recall, things got serious in late January and then in March 2020 is one thing shut down. So we were. Sent out of the lab. You know, we could work from home. If you had special permission, you could work in the lab. But we didn’t pursue that.
But we decided for working at home that what we could do is we would do docking because we have the crystal structure,

And the typical way docking works is you have the crystal structure and you have a library of compounds and these are typically commercially available compounds.

Famous library called Zinc that has up to 100 million compounds and then the computers software.
00:21:27.708 --> 00:21:30.519 combines them and it makes the
NOTE Confidence: 0.90919661
00:21:30.519 --> 00:21:33.416 complexes and then it has to score
NOTE Confidence: 0.90919661
00:21:33.416 --> 00:21:36.678 the complexes which is the weak spot.
NOTE Confidence: 0.90919661
00:21:36.680 --> 00:21:38.570 Often the scoring isn’t very
NOTE Confidence: 0.90919661
00:21:38.570 --> 00:21:40.897 accurate but you can then test
NOTE Confidence: 0.90919661
00:21:40.897 --> 00:21:42.423 the high scoring molecules.
NOTE Confidence: 0.90919661
00:21:42.423 --> 00:21:46.230 Well that’s a lot of compounds to deal with
NOTE Confidence: 0.90919661
00:21:46.309 --> 00:21:49.594 so I thought well we would do 1st instead.
NOTE Confidence: 0.90919661
00:21:49.600 --> 00:21:52.060 Is to dock known drugs,
NOTE Confidence: 0.90919661
00:21:52.060 --> 00:21:53.252 approved FDA approved drugs.
NOTE Confidence: 0.90919661
00:21:53.252 --> 00:21:55.873 So I happen to keep a library of
NOTE Confidence: 0.90919661
00:21:55.873 --> 00:21:58.547 these in the computer and there are
NOTE Confidence: 0.90919661
00:21:58.547 --> 00:22:00.427 three-dimensional structures of the drugs,
NOTE Confidence: 0.90919661
00:22:00.430 --> 00:22:02.866 which is this is all three-dimensional.
NOTE Confidence: 0.90919661
00:22:02.870 --> 00:22:07.049 And so I asked Muhammad and Julian.
NOTE Confidence: 0.90919661
00:22:07.050 --> 00:22:11.784 To dock the 2000 known drugs to see if
we could see get some reasonable hits

The docking was done in a consensus fashion,

meaning they used four different
docking protocols,

3 different programs and four ways
doing the docking because any
one program we don’t fully trust.

So we’re hoping that there will be a
consensus where you score well in all four.

Protocols. And so we got the list.

Excuse me. I don’t have code and I’ve tested.

But.

We got the list of the top compounds and then, very importantly,
we visualize the predicted poses,

And based on that visualization,

And I also was very concerned about

making analogs of these compounds

because I didn’t expect to have

again come up with a 10 nanomolar

compound we never have in the past.

So we purchased 17 compounds and.

Gave them to Karen’s lab,

and Karen had meanwhile obtained

the protein, expressed it,

and she also had implemented the A fret
00:23:45.423 --> 00:23:48.267 assay that was from the literature.
00:23:48.270 --> 00:23:50.406 So she was ready to go.
00:23:50.410 --> 00:23:53.890 And the 17 compounds arrived.
00:23:53.890 --> 00:23:57.526 And to our surprise, in Karen’s lab,
00:24:01.626 --> 00:24:05.182 14 of them showed some inhibition of the
00:24:05.190 --> 00:24:06.210 protease activity of Massar Scope 2 Proteus.
00:24:06.210 --> 00:24:06.550 So that was.
00:24:06.550 --> 00:24:10.002 And so we were did very well on the
00:24:10.002 --> 00:24:12.222 compound selection and the most
00:24:12.222 --> 00:24:14.370 potent compounds are listed here.
00:24:14.370 --> 00:24:19.120 They were single digit micromolar.
00:24:19.120 --> 00:24:22.160 And but we had a bunch that were
00:24:22.160 --> 00:24:24.220 under about 50 micromolar.
00:24:24.220 --> 00:24:28.054 So that this we published and this is a
00:24:28.054 --> 00:24:31.289 picture of one of the dock structures.
NOTE Confidence: 0.902261927142857
00:24:31.290 --> 00:24:34.097 The binding site is you know is
NOTE Confidence: 0.902261927142857
00:24:34.097 --> 00:24:36.131 meant to accommodate a peptide
NOTE Confidence: 0.902261927142857
00:24:36.131 --> 00:24:38.770 that’s going to get cleaved and we
NOTE Confidence: 0.902261927142857
00:24:38.770 --> 00:24:41.435 have site sub sites we call S1S1,
NOTE Confidence: 0.902261927142857
00:24:41.435 --> 00:24:44.901 Prime S2 and then this channel S3S4S5.
NOTE Confidence: 0.902261927142857
00:24:44.901 --> 00:24:47.918 So here’s just a picture of a
NOTE Confidence: 0.902261927142857
00:24:47.918 --> 00:24:49.910 compound in that binding site.
NOTE Confidence: 0.902261927142857
00:24:49.910 --> 00:24:52.754 So we published that but of course
NOTE Confidence: 0.902261927142857
00:24:52.754 --> 00:24:55.280 we were looking very much now.
NOTE Confidence: 0.902261927142857
00:24:55.280 --> 00:24:57.945 And one of these compounds we’re
NOTE Confidence: 0.902261927142857
00:24:57.945 --> 00:25:00.238 going to take and try to optimize it.
NOTE Confidence: 0.902261927142857
00:25:00.240 --> 00:25:02.180 And the compound we picked,
NOTE Confidence: 0.902261927142857
00:25:02.180 --> 00:25:04.068 we were we didn’t say what it was
NOTE Confidence: 0.902261927142857
00:25:04.068 --> 00:25:06.099 going to be in this paper and it was
NOTE Confidence: 0.902261927142857
00:25:06.099 --> 00:25:07.977 not one of the most potent ones.
In fact, it was this one param panel. Which is only 100 to 250 micromolar, so a relatively weak hit. But the fact was I liked the way it looked. And this was the dock structure. I’m orienting them all in the same way as 1S Primus 2 and I felt that the dock structure looked reasonable. Sometimes they have features, they just say this doesn’t feel right. But this looked reasonable. But I could also see that it had features that were not optimal. So looking at it over here, so the yellows are carbons.
00:25:51.620 --> 00:25:54.878 Reds are oxygens, Blues or nitrogens.
NOTE Confidence: 0.902261927142857
00:25:54.880 --> 00:25:57.448 I could see features that were not optimal.
NOTE Confidence: 0.902261927142857
00:25:57.450 --> 00:26:00.355 There’s a histidine here and it could,
NOTE Confidence: 0.902261927142857
00:26:00.360 --> 00:26:02.504 it would be nice if it could form
NOTE Confidence: 0.902261927142857
00:26:02.504 --> 00:26:04.318 a hydrogen bond with this ring.
NOTE Confidence: 0.902261927142857
00:26:04.320 --> 00:26:06.464 So you probably want to put a nitrogen
NOTE Confidence: 0.902261927142857
00:26:06.464 --> 00:26:08.480 in here, this nitrogen of the purity,
NOTE Confidence: 0.902261927142857
00:26:08.480 --> 00:26:10.346 and that’s not doing any good.
NOTE Confidence: 0.902261927142857
00:26:10.350 --> 00:26:12.639 So we can get rid of that.
NOTE Confidence: 0.902261927142857
00:26:12.640 --> 00:26:15.076 It’s just spacing out into solvent.
NOTE Confidence: 0.902261927142857
00:26:15.080 --> 00:26:17.588 There’s an NH over here that’s.
NOTE Confidence: 0.902261927142857
00:26:17.590 --> 00:26:19.498 I would like to be in a hydrogen bond,
NOTE Confidence: 0.902261927142857
00:26:19.500 --> 00:26:20.853 but it isn’t.
NOTE Confidence: 0.902261927142857
00:26:20.853 --> 00:26:21.304 Meanwhile,
NOTE Confidence: 0.902261927142857
00:26:21.304 --> 00:26:23.559 this carbonyl is just interacting
NOTE Confidence: 0.902261927142857
00:26:23.559 --> 00:26:24.660 with solvent,
00:26:24.660 --> 00:26:27.383 so maybe I could flip that from moving over left to there.

00:26:27.383 --> 00:26:29.359 Plus it looked like there was a little space in the meta position.

00:26:29.360 --> 00:26:31.817 So.

00:26:31.817 --> 00:26:34.772 What happened next was we did some FEP calculations to test those ideas and this is what those are.

00:26:34.772 --> 00:26:37.024 raw data looks like in an Excel sheet so that the the things I'm trying here are for the left ring.

00:26:37.030 --> 00:26:38.900 So ring scan where I did 234 pyridinyl 4 pyrimidine 2 triazine,
so a bunch of different rings there.

They also did a calc and that those calculations said that the three pyridine

the the negative number here is good.

This is the change in free energy

So this was saying go for the three pyrene.

Also I checked that ring flip of the

carbonyl and that was very good,

minus 4.7 and then over on the right

indeed the meta position when we did

a chlorine scan at each position,

the meta here shed very good.

Looks like we should put a chlorine there.
So combining those three ideas led to the three initial compounds that were synthesized. Now I'm aligning everything so you can see the changes from parent panel. The carbonyl's been flipped and we've added the chlorine and we've left the cyano phenyl from parent panel. I also from modeling with my bond program. Again, the slow part in all of this is synthesis. So we have plenty of time to do computer
00:28:25.219 --> 00:28:28.149 work while people are doing synthesis.
NOTE Confidence: 0.869961365
00:28:28.150 --> 00:28:31.594 So it’s a natural thing to, you know,
NOTE Confidence: 0.869961365
00:28:31.594 --> 00:28:33.346 look very hard at these structures.
NOTE Confidence: 0.869961365
00:28:33.350 --> 00:28:35.915 And I had looked hard at this and I
NOTE Confidence: 0.869961365
00:28:35.915 --> 00:28:37.943 recognized maybe I could do something
NOTE Confidence: 0.869961365
00:28:37.943 --> 00:28:40.315 over with this ring because there’s an
NOTE Confidence: 0.869961365
00:28:40.315 --> 00:28:42.527 edge that will show more clearly here
NOTE Confidence: 0.869961365
00:28:42.527 --> 00:28:46.554 of a loop that could use some hydrogen bonds.
NOTE Confidence: 0.869961365
00:28:46.560 --> 00:28:48.765 And I thought a uracil might work,
NOTE Confidence: 0.869961365
00:28:48.770 --> 00:28:49.730 so I’d modeled.
NOTE Confidence: 0.869961365
00:28:49.730 --> 00:28:51.330 Got with the program complex
NOTE Confidence: 0.869961365
00:28:51.330 --> 00:28:52.619 has looked very good.
NOTE Confidence: 0.869961365
00:28:52.620 --> 00:28:56.302 So we synthesized a uracil and also
NOTE Confidence: 0.869961365
00:28:56.302 --> 00:28:59.068 just this 35 dot clock compound.
NOTE Confidence: 0.869961365
00:28:59.068 --> 00:29:02.469 So this is a very happy day now.
NOTE Confidence: 0.869961365
00:29:02.470 --> 00:29:05.620 Because the potency of those original 3
00:29:05.620 --> 00:29:08.610 compounds was 10-6 and four micromolar.

00:29:08.610 --> 00:29:11.850 So here we’ve gotten a huge boost as expected from the FEP calculations.

00:29:11.850 --> 00:29:14.448 And this was the wonderful and I’ll tell you the timing more in a bit,

00:29:14.450 --> 00:29:16.676 but this is now June of 2020.

00:29:16.676 --> 00:29:19.100 So we didn’t get back into our lab until May.

00:29:19.100 --> 00:29:22.855 And now in June we have these,

00:29:22.855 --> 00:29:27.302 this 4 micromolar. Compound.

00:29:27.302 --> 00:29:31.446 We’ve only,

00:29:31.450 --> 00:29:33.634 this 4 micromolar. Compound.

00:29:33.634 --> 00:29:37.978 and then it came a little later was actually October and Karen’s group got a crystal structure for that dichloro compound and it’s basically identical to what we’ve predicted.
There's the carbonyl and hydrogen bond we wanted.

There's a hydrogen bond between the pyridine and the histidine.

We still have the nitrile hydrogen bonded in what he called the oxyanion sort of hole and the dichloro compound.

Is again looking very good.

Furthermore, we have this channel running N from the upper chlorine there and so we're ready to think about putting some of the something in that Channel.
interested in methyl particularly,

but just for grins, we did FP

calculations for methyl ethyl propyl,

O methyl ethyl propyl albuterol and

then some ones with a hydroxyl that

I figured probably wouldn’t be very

good problem with hydroxyl is it’s

Again, but you’re not going to gain much.

The way you gain is by having more

hydrophobic pieces that are binding into

hydrophobic part of the binding site.

So this told us.

Tried the O propyl compound
so we synthesize on.

There are two synthetic chemists are working on this so Lizzie and Chun way and so we they made.

The proxy compound in both the cyano phenyl and the urea series, and this turned out great, 140 nanomolar and 120 animal later on.

We had made the trifluoromethyl analogs. They’re more hydrophobic. They’re probably going to be better binders as they were showing this one even down at 25 an animal,

but generally I don’t like CF. Big groups and drug like molecules
because they really hurt the solubility of the compounds. So, but we're doing very well here. An animal or and I'll show you the timing on this, this I think is in August now and Karen’s group again got a crystal structure in October and it was exactly as expected including this bent. Hard at the at the end of the Propoxur group. And so it’s a Ghosh. We call it a gauche. You’ve all taken organic chemistry, I’m sure. So that’s the course you hated the most, but maybe, maybe not.
But there it is. There's this gosche OCC and we had figured that was the case. The modeling told us that because at that terminal methyl fits right in the S4 site of the channel. And so there's a lucine or problem, so hydrophobic site and so put it right in there also. Again, like I said, there's lots of time to do computing and so we considered benzel oxy groups. So you can imagine a benzene ring sitting here and potentially projecting. A substituent into that pocket.
NOTE Confidence: 0.784769928333333
00:33:18.185 --> 00:33:21.135 these benzyl oxy analogs and did a
NOTE Confidence: 0.784769928333333
00:33:21.135 --> 00:33:24.103 chlorine scan on the fennel which said
NOTE Confidence: 0.784769928333333
00:33:24.103 --> 00:33:27.343 in a methyl scan and both methyl and
NOTE Confidence: 0.784769928333333
00:33:27.343 --> 00:33:30.216 chlorine were predicted to be very good.
NOTE Confidence: 0.784769928333333
00:33:30.220 --> 00:33:34.077 And so those compounds were made and
NOTE Confidence: 0.784769928333333
00:33:34.080 --> 00:33:37.280 the parent compounds 120 micromolar,
NOTE Confidence: 0.784769928333333
00:33:37.280 --> 00:33:40.500 but the ortho chloro compound
NOTE Confidence: 0.784769928333333
00:33:40.500 --> 00:33:44.308 18 an animal compound and this
NOTE Confidence: 0.784769928333333
00:33:44.308 --> 00:33:47.743 we had in October of of 2020.
NOTE Confidence: 0.784769928333333
00:33:47.743 --> 00:33:51.481 And Karen’s group again got a
NOTE Confidence: 0.784769928333333
00:33:51.481 --> 00:33:55.367 crystal structure for the bend the
NOTE Confidence: 0.784769928333333
00:33:55.367 --> 00:33:58.537 parent Benz loxy components and
NOTE Confidence: 0.784769928333333
00:33:58.537 --> 00:34:01.669 is positioned as one expected.
NOTE Confidence: 0.784769928333333
00:34:01.670 --> 00:34:06.414 So this is just now a little video.
NOTE Confidence: 0.784769928333333
00:34:06.420 --> 00:34:10.380 To. Have those. Break this.
NOTE Confidence: 0.784769928333333
This is a dimer, so they’re two.

This is Karen’s crystal structure of the propoxur compound and just zeroing in on it.

There.

OK, so you can.

Run it again. So that little molecule is enough to shut down the enzymatic activity of that protein.

We’ve got to go into cells, infected cells and so that...

52
we published 28 compounds. Of course by the results we have lots of compounds here under the 50 nanomolar and you can see there are authors, lots of people involved and from the medical school, you know, fair and Isaacs and Brett Lindenbach, grouper and very important. Along with Karen in doing the cell assays that will describe in Miller in a minute and Scott Miller in chemistry had donated his graduate student Lizzie Stone.
to help us with the synthesis, along with my postdoc Chunwei Zang. So that was good. We published that in ACS Central science in February 2022. A little later we also replaced the benzyl Oxy with heterocycles. This is a standard, I’d say, medicinal chemistry. This isn’t, you know, genius stuff. Heterocycles often have some desirable properties over a substituted benzene. So we published some more compounds in the summer than of a 2021. Tested cell permeability with
a pampa assay in our lab and measured aqueous solubility.

So now we have uracil’s with the hydrogen or with a methyl.

So the ones with the methyl are going to have better cell permeability.

And so that is an issue because we want to show that we have efficacy and sell assay.

The BSL three facility was used.

Krassimir getting suited up because COVID, of course, is airborne.

He has to have a full breathing
apparatus and the assays that were done.

Karen certainly can describe these far better than I can, but there's one.

It's a plaque assay using infectious virus.

And so you have the live these are Vero cells infected with large live SARS Cove two.

And there's also then the replicon.

So we're testing our compounds and we have as a reference compound remdesivir,

which is A1 micromolar EC50

and the assays that were done.

And long short, we have many
NOTE Confidence: 0.829809488333333
00:37:42.338 --> 00:37:44.520 compounds that are one micromolar.
NOTE Confidence: 0.829809488333333
00:37:44.520 --> 00:37:46.240 We also have some compounds.
NOTE Confidence: 0.829809488333333
00:37:46.240 --> 00:37:49.318 This one’s 38 nanomolar EC 50,
NOTE Confidence: 0.829809488333333
00:37:49.320 --> 00:37:51.628 that’s inhibition of the
NOTE Confidence: 0.829809488333333
00:37:51.628 --> 00:37:53.936 of the protease activity,
NOTE Confidence: 0.829809488333333
00:37:53.940 --> 00:38:00.078 but it’s not active in the replicant housing.
NOTE Confidence: 0.829809488333333
00:38:00.080 --> 00:38:01.420 And this simply because it
NOTE Confidence: 0.829809488333333
00:38:01.420 --> 00:38:02.760 doesn’t get into the virus.
NOTE Confidence: 0.829809488333333
00:38:02.760 --> 00:38:04.740 Cell permeability is too low,
NOTE Confidence: 0.829809488333333
00:38:04.740 --> 00:38:07.638 so the cell permeability is critical.
NOTE Confidence: 0.829809488333333
00:38:07.640 --> 00:38:11.735 The quite remarkable compound is number 19.
NOTE Confidence: 0.829809488333333
00:38:11.740 --> 00:38:14.200 So this.
NOTE Confidence: 0.829809488333333
00:38:14.200 --> 00:38:17.302 Benzyl oxy compound that has a
NOTE Confidence: 0.829809488333333
00:38:17.302 --> 00:38:20.865 methylated uracil and in the assay it
NOTE Confidence: 0.829809488333333
00:38:20.865 --> 00:38:24.375 was 80 nanomolar in the infectious
NOTE Confidence: 0.829809488333333
virus assay and 175 and the replicon assay.

So this became our lead compound for preclinical work.

Now unfortunately in our world we can’t, you know we’re not Pfizer, so we can’t take 10 compounds and put them all into preclinical studies but.

We did work on 19 and a pharmaceutical company was very interested in 19. They took 19 and did their own sell assay and they came back and their cell was 15 animals they can confirmed everything that we we had reported.

So that compound 19 is a very potent compound in infected cells and Karen’s group has been working on the PK, it has very good.
Basic PK bioavailability. And they have done with Pretty Kumar some initial mouse studies and this is with these humanized mouse mice, KTH 2 mice. And again Karen could describe the current status of this. But basically we were delighted a very low dose of the compounds that were using and if you don’t untreated mouse after six days as this is now fluorescent. Imaging of where the virus is. So initially the virus goes into the lungs, but it makes its way into the brain. And at day six,
the mouse is again horribly infected and dies. So we have tested we meaning Karen and pretty by both Ivy and oral. And the results have been very good. There's only one dose and you see protection for four days, you know, completely clean a mouse and even at 6 days. So with the oral, it's, really very clean. So if this was being dosed every day, the feeling is infection that wouldn't go on. So we have very, you know, concur raging data with this compound. There has been some. You know again external interest
in this compound, yeah, we think we if we had the resources we can come up with lots of other compounds, but we need support for this and are high level because these preclinical studies are are expensive. So just to compare what we’ve done. Versus others. So first of all our compound is a non covalent inhibitor by most of the other work in this area been covalent inhibitors. Up until recently covalent inhibitors were considered to be. Not desirable because you’re always worried about off target activity.
But here is how other people progress. Generally we don’t like peptidic inhibitors because they can be proteolysis by many. Proteolytic enzymes that exist in humans so but this is some of the compounds and EC 50 of 720. Remember we’re 50 or 80 nanomolar. This is the COVID moon shot that got quite a bit of publicity. They obtained an assay 2400 compounds and the best IC50 they obtained is basically 100 nanomolar. At 30, we had made no more than 30 compounds.
and we were at 18 nanomolar.

Another peptide peptide peptide, but this is a PAX lovin.

So Pax Lovid is this neurometrix alvir, but you have to include a SIP inhibitor ritonavir. So ritonavir is an HIV protease inhibitor. Not something you probably want to take for a long time and have their side effects of that. Of course you’re not going to take packs a little bit for a long time. So I guess it’s OK, but on the other hand having to have the SIP inhibitor to keep the.
Protease inhibitor from being chewed up. Metabolically. Is clearly not desirable because you don’t want to be, you know, can have drug, drug interactions. This is our compound. Again, by comparison, other things that you know. I’m obviously a little bit prejudiced here, but this to me is a tough molecule. All the stereo chemistry going to be tough to synthesize. You have high cost of goods. It’s peptic. You worry about that. It is a covalent inhibitor, covalently modifies the cyano.
but it’s probably reversible.
Are covalent.
There have been a synthesis
It’s also intrinsically not
as potent as our compound.
It’s a EC 50 or 740 whereas we’re at you
know 10 times more potent with there’s no,
we don’t we know from our preclinical
work on off target and SIP
activity that we don’t have any sip
problems with the compound either.
So the rest of the story.
So why isn’t our compound in clinical
trials and that’s a probably takes me
more than the last time I have here. But the packs of lovin and thermal, Trevor got into clinical trials very quickly because it was sitting on the shelf from the SARS Cove One project. They made a minor modification to make it have better solubility. So it was ready to go and so it’s off and running, I doubt seriously it’s the best. Drug possible, and there’s no way, and well, time will tell the problem for the pharmaceutical companies that they’re all in the business of making money. And so the before the end of last fall.
People are getting kind of cocky about, you know, covid’s under control, the vaccines are working. And then Omicron came along around December of last year and that’s changed things a bit. But we’ll see who has the, you know, stamina to advance additional protease inhibitors into the clinic because of the cost of the clinical trials. This is a timeline just showing the power I think of our approach. So June 15th all we had was parent panel. By August 3rd we had these six and four micromolar compounds.
September 2nd we had the proxy 140 nanomolar compound.

September 10th we had the corresponding benzyl oxy, uracil and then we started getting some crystal structures October 3rd.

We had the first crystal structure October 4th and also the Propoxur compound and

These sort of loading animal compounds again to get to 18 animal we had synthesized about 30 compounds and a few of them were things we probably or 10 of them were real or wild shots.

And this synthesis was done by a gun.

Postdoc Chunwei and graduate student Lizzie.
So that’s the story and I hope I’ve told you a little bit about what Karen and I do and the Hour of combining the computation with the, you know, reliable assaying and crystallography is such a different world than what we lived in 20 years ago. So just thanking people in my lab notably. And Julian is a long-term associate other so he’s a senior research scientist. Anna and Joe were both associate research scientist and other people listed here. Wonderful collaborator and
other Pi collaborators,

pretty yosi on our Jack projects and Brett,

Brett and Faron on the COVID project.

So pleasure to be here with you and thank you very much.

What a whirlwind journey.

Yeah, amazing.

Are there any questions here, Emily? Are you monitoring questions in the chat, Tommy?

Are there any questions here,

Yeah, these the COVID compounds are all binding to the active site of the Proteus.

So the cysteine, that’s a, there’s a cysteine protease,
00:47:46.380 --> 00:47:47.672 there’s a cysteine it.
00:47:47.672 --> 00:47:50.310 We’re sort of in the middle of all the structures I showed you and that’s the active site cysteine.
00:48:01.700 --> 00:48:02.330 Even fine.
00:48:07.620 --> 00:48:11.650 Yes, the Pfizer compound binds in that same site, and it covalently modifies that cysteine.
00:48:11.650 --> 00:48:15.150 And you? Does not covalent.
00:48:27.120 --> 00:48:27.450 Quite understand, he’s asking if in the crystal structure does it bind to the cleavage. Yes.
00:48:33.910 --> 00:48:34.290 And this is the.
The cysteine there cysts and this histidine over here. Are the catalytic residues, so our compound sitting right on top of them. The Pfizer compound covalently modifies that cysteine as do most of the other. There's a very few coat non covalent inhibitors have been reported for this. But we from the getco we wanted to pursue non covalent inhibitors just to avoid the potential issues of covalent inhibitors.

So you know, we’re extremely familiar with the hyper immutability of this virus in the spike protein to evade immunity.
I wonder if you’ve done sort of low dose exposure and if there’s a mutational response to a protease inhibitor like this?

Yeah, I haven’t maybe.

I mean, we haven’t to my knowledge, unless Karen’s been up to something I don’t know about,

the SARS Cove 1 protease and SARS Cove protease are extremely identical.

They’re the only differences are quite far from the protease active site.

So it’s it’s hoped that there won’t be.

A lot of mutations possible for the Proteus, however,
00:50:19.385 --> 00:50:21.360 it hasn’t been under pressure.
NOTE Confidence: 0.74597426
00:50:21.360 --> 00:50:24.472 So I think with the Pax lovid treatments
NOTE Confidence: 0.74597426
00:50:24.472 --> 00:50:27.686 we will probably begin to see some
NOTE Confidence: 0.74597426
00:50:27.686 --> 00:50:30.530 mutations closer to the binding site.
NOTE Confidence: 0.74597426
00:50:30.530 --> 00:50:33.218 And there there was a recent
NOTE Confidence: 0.74597426
00:50:33.218 --> 00:50:34.562 paper in science,
NOTE Confidence: 0.74597426
00:50:34.570 --> 00:50:39.708 I believe is it science indicating some
NOTE Confidence: 0.74597426
00:50:39.710 --> 00:50:43.406 mutations that might arise in this Proteus,
NOTE Confidence: 0.74597426
00:50:43.410 --> 00:50:46.066 so it was under some pressure that they.
NOTE Confidence: 0.74597426
00:50:46.070 --> 00:50:49.364 but we haven’t looked into that yet.
NOTE Confidence: 0.74597426
00:51:00.700 --> 00:51:03.472 So this is a this is a related question,
NOTE Confidence: 0.864771771111111
00:51:03.480 --> 00:51:05.853 but how different is the COVID 2
NOTE Confidence: 0.864771771111111
00:51:05.853 --> 00:51:07.700 protease active site from that
NOTE Confidence: 0.864771771111111
00:51:07.700 --> 00:51:09.615 of other common human proteases?
NOTE Confidence: 0.44774485
00:51:11.290 --> 00:51:16.818 Umm. Well, I would say it’s it’s the
COVID active site is quite unique, but it’s virtually identical to the SARS Cove one active site, but I don’t think there’s been a. I don’t think that these inhibitors are generally inhibiting other proteases. So I haven’t heard that, so I don’t expect it, but if they were, it would certainly be, I’d imagine it’d be assisting a protease would be the ones you’d be looking at. Well, we’re at the hour. I can’t thank you enough for this lucid explanation to a bunch of non chemists was really beautiful.
Also on behalf of healthcare workers who, you know, see people with COVID all the time. It’s wonderful work. Thank you very much. Thank you.