It’s been an unprecedented year for protein research, as the pandemic closed most of our lab operations for several months in spring. We froze down proteins, stayed connected with journal clubs and worked from home on creative projects.

By June 1, we cautiously reopened to join the larger COVID-19 research effort. Doing our part, we launched three SARS-CoV-2 related projects. Two were eventually published in *Science*, and the last is still making headway.

At the same time, our co-founder Timothy Springer soared to media fame because of his founding investment in Moderna. In 2010, he had decided to put up $5M of his funds to back his colleague’s idea to develop modified RNA as therapeutics. A decade later, Springer became a billionaire and Moderna’s COVID-19 vaccine was racing through development toward worldwide impact.

While reporters from *Forbes, the Wall Street Journal* and *Fox News* came calling, it was surreal to witness the unimaginable day-to-day case counts, death and suffering. We witnessed the tremendous power of science rising from the calamity and …history. Long vilified, biotech rose as “saviors,” Springer told reporters, and the 21st century emerged as the beginning of the era of biotech.  

At IPI, there was a mix of fear and exhilaration, isolation and camaraderie. We rolled up our sleeves to “get it done,” and are delighted to now be back full force with a growing and evolving team.

We restarted our Antibody Pipeline and successfully characterized antibodies to 182 targets. We entered into a record number of partnerships and collaborations—and our first licensing agreements—to test and use IPI antibodies in other researchers’ assays. We brought in a new director of the Antibody Initiative (see page 4) to ready us for sales and distribution of IPI antibodies. While the news portrayed the tumult of race relations in the US, we continued to diversify with a wealth of new voices (see page 16) from many backgrounds and places. Now that we have the resources, vision and richness of scientific and administrative talent, we are on our way toward achieving the impact promised at IPI’s launch.

As we look back on such a historical year, we are thrilled and awed to be working so close to the cutting edge.

The Executive Leadership Team
While 2020 was dominated by the pandemic and low-profile quarantining, IPI’s founder Tim Springer experienced a surge of high-profile attention.

Stories appeared in Forbes, the Boston Globe, the Wall Street Journal and elsewhere. These outlets applauded his early stake in Moderna; Springer had been a founding investor in 2010, after channeling $5M of his personal funds to launch the biotechnology company. It soared to fame as one of the first manufacturers of a SARS-Cov-2 mRNA vaccine, causing Springer’s early investment to yield $2.2B.

While financial reporters focus on his “very high batting average” in starting Moderna and seven other thriving biotech companies, Springer saw the success merely as a validation of his science. “I’m a scientist at heart,” he says. “I love discovering things.”

“I never rest on my laurels,” he says. “As a scientist, I’m just too busy working on the next discovery.”

He also loves creating companies that help advance science and therapeutics. That is why he donated $10M of his fortune in 2017 to establish the Institute for Protein Innovation. As a nonprofit, it is not only unique in his portfolio, but also best positioned to build a new model of antibody discovery and distribution without the investor constraints that hinder the impact of for-profit companies.

“My motivation behind [the Institute] is not only to help develop new reliable antibodies that scientists around the world can use for biological discovery,” he says, “but also, develop new technology that will just allow many more discoveries to be made.”

When asked to think of a moment when Moderna’s impact really hit home, Springer was almost at a loss. “I never rest on my laurels,” he says. “As a scientist, I’m just too busy working on the next discovery.”
Joe Bertelsen’s foray into the reagent antibody world began with a chance meeting at a networking event. It launched a two-decade career, leading teams that developed and implemented commercial and research and development strategies. With stints in leadership at Abcam, Covance and Signet Laboratories, Bertelsen has cultivated a deep understanding of what it takes to succeed in the antibody business. He talks about his work and IPI’s unique ability as a nonprofit to disrupt the antibody market.

Q&A WITH JOE BERTELSEN, NEW DIRECTOR OF IPI’S ANTIBODY INITIATIVE

Can you describe what led to your 20 plus year career in the antibody field?

Berelsen In 1999, I met the owner of an antibody start-up, Signet Laboratories, at a biotech networking event. He had just acquired antibodies to neurobiology targets related to Alzheimer’s and Parkinson’s diseases. He asked me if I would head up the product line, and having degrees in chemical engineering and pharmaceutical medicine and an interest in neuroscience, I decided I would try it for six months. Ten years later, I was still there as the company was sold to Covance.
WHAT WERE THE KEY FACTORS INFLUENCING YOUR DECISION TO JOIN IPI?

BERTELSEN I am excited by IPI’s mission and its potential to create a disruptive entity. Having worked for more than 20 years in the antibody field, I am cognizant that changes need to be made to ensure scientists get quality tools to perform their critical research.

FROM YOUR EXPERIENCE, WHAT MAKES A COMPANY OR INSTITUTION SUCCESSFUL IN THE REAGENT ANTIBODY INDUSTRY?

BERTELSEN Success first and foremost is based on delivering a high-quality solution that addresses a critical need. At the same time, significant effort needs to be made upstream and downstream from the creation of the product in order to impact a market. Developing strong relationships with key opinion leaders in a field is essential.

DO YOU SEE THESE CHARACTERISTICS AT IPI?

BERTELSEN Yes. The Institute is not just connected with key opinion leaders in the antibody and protein field, it is composed of key opinion leaders in the antibody and protein field.

YOUR PROFESSIONAL POSITIONS TO THIS POINT HAVE BEEN WITH FOR-PROFIT ENTITIES. HOW WILL THESE EXPERIENCES HELP YOU AT IPI?

BERTELSEN It is critical to have an intimate understanding of the needs of the scientific community, regardless of the business model.

WHAT EXCITES YOU ABOUT BEING WITH A NON-PROFIT?

BERTELSEN The freedom to focus on technologies that are best suited to scientific advancement and improving human health, as opposed to being constrained by fiscal results.

IN YOUR VIEW, WHAT IS IPI’S FUTURE?

BERTELSEN IPI will bring scientists closer to the development and characterization of the antibodies they need, as opposed to choosing them after they are developed and on the market.
IPI’s Antibody Platform

We’ve constructed the IPI Antibody Platform around sophisticated yeast display technology. We deploy a robust high-throughput strategy that involves both positive and negative selections. Aligned with our commitment to open science, we aim to share our scientific data publicly and distribute our antibodies both commercially and through collaborations and partnerships.

### Antigen Expression

- Design and synthesize target DNA.

### Antibody Selection

- Yeast Library - $10^{11}$
- Target binding fragment (Fab)

#### Our Antibody Screen

**Basis of Screen**
Each of 15 billion yeast carries a different human antibody fragment (Fab) that may bind the target (antigen).

**Positive Magnetic Activated Cell Sorting (MACS)**
Antigen on magnetic beads binds to specific Fabs. Nonbinders are eliminated. (Repeated 2x)

**Negative Magnetic Activated Cell Sorting (MACS)**
Empty magnetic beads bind to nonspecific Fabs. Binders are eliminated.

**Positive Fluorescence Activated Cell Sorting (FACS)**
Antigen binds to specific Fabs. Fluorescent chemical detects binders (Repeated 3x)

**Negative Fluorescence Activated Cell Sorting (FACS)**
Insect cell extracts binds nonspecific Fabs. Binders are eliminated (Repeated 2x)

**Antibody Candidates**
Strongest, specific binders remain.

8 Rounds of Selection

Fab sent for next generation sequencing
Antibody Production

- Analyze DNA sequences of best candidates and choose finalists.
- Insert DNA sequences of finalist into vector.
- Express recombinant antibodies from vector using mammalian cells.
- Purify recombinant antibodies.
- Analyze DNA sequences of best candidates and choose finalists.

Antibody Characterization

- Capture ELISA: 384-well plate coated with target. Measures antibody binding at a range of concentrations.
- Cell Display: Target on surface used to profile antibody specificity and affinity.
DESPITE THE LAB SHUTDOWN DUE TO THE PANDEMIC, WE MANAGED TO KEEP OUR PLATFORMS UP, RUNNING AND OPTIMIZING. HERE ARE SOME HIGHLIGHTS OF THE YEAR:

- We’re proud to announce that our first antibody campaign, based on the immunoglobulin superfamily (IGSF), was a success! We made hundreds of antibodies, and in some cases, the first of their kind against challenging targets.
- In our first “workshop,” we shared our synthetic IgG antibodies with researchers in the neuroscience community for testing in their assays. Based on their feedback, we are further optimizing our process to enhance the quality and quantity of our antibodies.
- With an eye toward antibody commercialization in 2022, we are using feedback from collaborators in particular scientific domains to shape a new and potentially disruptive model of antibody production/distribution based on market needs.
- We have launched campaigns for two new families of target receptors important in cancer and diabetes.
- Two efforts related to SARS-CoV-2 have yielded promising success and two publications in the journal *Science*.
- On the protein design front, we are thrilled to report that Chris Bahl, PhD, Head of Protein Design, has been awarded a $750,000 grant from the Massachusetts Life Sciences Center. As a result, he will launch a new project to generate large-scale datasets of proteins, testing their properties using laboratory experiments combined with machine learning.
- Using three different approaches for engineering mini protein affinity reagents, the Protein Design group has identified promising potential “binders” for sponsored research collaborators.
The Antibody Initiative in Full Swing

IPI’s Antibody Initiative is our flagship effort designed to make well-characterized, recombinant antibodies to all proteins in the extracellular and secreted proteome; validate our antibodies internally and with collaborators and distribute those proven to work in specific assays to the research community through an open science model.

This year, streamlining workflows and increasing cross-collaboration, we restructured the IPI pipeline into one Antibody Platform, now headed by Director Rob Meijers, PhD. Also joining the leadership team is Joe Bertelsen, Director of the Antibody Initiative (introduced page 4), brought in to steer the Platform around a clear business strategy and position it uniquely in the reagent antibody space.

Wnt and TGFβ Campaigns

Moving beyond IgSF, we are now gearing up for upcoming campaigns targeting Wnt—a group of signal transduction molecules involved in cancer and type II diabetes—and transforming growth factor beta (TGFβ)—a family of multifunctional cell signaling molecules important in cancer, auto-immune and infectious diseases. We have produced 70 single antigens at scale and systematically investigated how to create receptor/ligand complexes, an unprecedented feat.

Variant-Resistant Antibodies to Fight COVID19

Pitching in with SARS-CoV-2 efforts with a novel approach, IPI developed antibodies to a portion of the virus’s spike protein, S2. This region contains the fusion machinery necessary for the virus to enter the host cell and therefore does not evolve as fast as other portions of the spike protein.

Employing our Antibody Platform, we discovered several candidates that bind to a shortened version of S2 displayed on Chinese hamster ovary cells. These potential antibodies do not attach to the spike regions involved with receptor binding. In collaboration with the lab of John Briggs at the Medical Research Council in Cambridge, UK, we are exploring whether these antibodies influence viral fusion. We also plan to conduct electron microscopy studies.

In related work, Wei Yang, PhD, former Director of Target Discovery, used the antibody production and characterization capabilities at the IPI to contribute to two structural studies, both published in the prestigious journal Science by Bing Chen, PhD, at Harvard Medical School. The researchers showed that a slight change in the protein’s amino acid sequence could alter its structure, rendering it more virulent.
Mini Protein Affinity Reagents

In a sponsored research collaboration, Chris Bahl’s team successfully designed novel disulfide-rich mini proteins de novo. The group generated a diverse library (~10⁹ diversity) and obtained binders to four antigens of interest. The team also used this library to create binders for TNF-α, c-Kit, the spike protein of SARS-CoV-2 and six different antibodies, including clinically relevant auto-antibodies and FDA-approved antibodies such as adalimumab. The Protein Design team is now further “maturing” these mini protein hits via site-saturation mutagenesis for detailed characterization.

The Protein Design group used another approach to rationally design a separate library of 10⁶ additional disulfide-rich mini proteins. Each bears an entirely different tertiary structure and the library exhibits high shape and chemical diversity. The team has successfully identified binders for three targets of interest to our sponsor and is subjecting them to affinity maturation.

Finally, the group developed novel design algorithms to craft mini protein structures around a binding site, providing optimal shape complementarity and binding energy. The result was mini protein binders to three targets: parathyroid hormone one receptor, a G protein-coupled receptor that recognizes peptide hormones; LapG protein, part of a highly conserved system that regulates biofilm formation in many Gram-negative bacteria; and the collagen-binding site of the integrin α 2 I domain, as part of another sponsored research project.
Synthetic Antibodies for Neurobiology Validation Campaign

No antibody is a good antibody if not valuable for scientists. Thus, we engaged scientists in the axon guidance receptor community to help validate our first IgSF antibodies. Specifically, we asked them to join our pilot validation “workshop” based on the roundabout (ROBO) receptor family, including human ROBO1, ROBO2 and ROBO3. We selected eight antibodies to each receptor and produced endotoxin-free antibodies for distribution to five laboratories.

We were thrilled by the enthusiastic response of our collaborators. For example, Alex Jaworsky showed that the IPI antibodies worked at high receptor density. While they failed to stain mouse brain tissue, his results led us to further analysis. As a result, we found that antibodies were indeed specific but limited in their applicability. Using that feedback, we launched a second-generation discovery campaign for ROBO receptors to obtain better-behaved antibodies.

In a second collaboration, Lisa Goodrich at Harvard Medical School tested eight IPI antibodies for the LINGO2 receptor and achieved even greater success. Two showed specific staining of mouse brain tissue, coinciding with mRNA expression for Lingo2, as observed in the Allen Brain Atlas.

A special thanks to our collaborators: Alexander Jaworsky, PhD, at Brown University; Avihu Klar, PhD, at Jerusalem Hebrew University; Frederic Charron, PhD, at Montreal University; Greg Bashaw, PhD at the University of Pennsylvania, Marc Tessier-Lavigne, PhD, at Stanford University, and Lisa Goodrich, PhD, at Harvard.
A Diamond in the Lab

Affecting only about 1 in 80,000 newborns, Shwachman-Diamond syndrome is inherited and marked by a failure to thrive, pancreatic deficits and bone marrow failure. Its symptoms, like poor growth and fevers, typically appear in infants by four to six months of age. With treatment and regular monitoring, children with SDS can lead fulfilling lives. However, five percent can develop leukemia, with the risk rising to 30 percent of patients by 30 years of age.

“It’s horrible to watch these children develop cancer,” says pediatric oncologist Alyssa Kennedy, MD, PhD, at Dana-Farber/Boston Children’s Cancer and Blood Disorders Center. She joined the laboratory of pediatric hematologist Akiko Shimamura, MD, PhD, “to figure out what can help predict who goes on to develop leukemia and why.”

Shimamura had spent 20 years painstakingly building a registry of blood and bone marrow samples from SDS patients of all ages. “I realized that there are certain mechanistic questions you can ask in the laboratory,” Shimamura says. “But if you really want to understand the disease, you have to partner with the patients.”

Joining forces with Coleman Lindsley, MD, PhD, an expert in genomic analysis at Dana-Farber Cancer Institute, Kennedy and Shimamura began to probe the genetic mutations that drove specific stem cells to develop into cancer-prone cells.
**Mutation after Mutation**

Most SDS patients carry mutations in their SBDS genes, which normally provide cells with the instructions for producing a critical protein that assembles cell structures called ribosomes. Made up of two parts, or subunits, ribosomes carry out the vital function of making proteins. The SBDS protein preps the ribosome’s large subunit by removing another protein, called EIF6, that caps the subunit and prevents its interaction with the small subunit.

“SBDS is almost like a little bottle opener that kicks off the bottle top (EIF6) to get what you want,” says Kennedy, “which is the mature ribosome.”

When the gene is mutated, as in Shwachman-Diamond syndrome, the ribosomal subunits remain “capped,” immature and unable to make proteins. The result is a body-wide defect in stem cells that shows up in patients as bone marrow failure, among other symptoms.

What the Boston Children’s team learned, and perhaps the most fascinating aspect of this biochemical story, is that stem cells are resilient. To survive SDS, they acquire more mutations, one set of which can reverse the defect. These rescue mutations arise early in an infant with SDS and most commonly in the capping gene, EIF6. In 110 patients studied, the investigators identified 265 EIF6 mutations that arose after their primary SBDS mutations.

These mutations came in two flavors. The first stopped the production of EIF6 before the cell could finish making it, leading to a shortened stub of a capping protein. Lacking the cap on the large subunit, it could once again join its smaller partner and make a functional ribosome. In other words, the second mutation (EIF6) reversed the damage of the first (SBDS).

The second type of mutation, called a missense mutation, introduced an error in the EIF6 code. It allowed the full-length SBDS protein to form, albeit with an anomaly. What was the defect, and how did it rescue the SDBS mutation that marked the disease?

For answers, Shimamura approached IPI’s Chris Bahl, a protein structure and design expert whose laboratory was located, literally, down the street. Shimamura believed Bahl might help her by deploying a computational approach to ascertain what the mutant EIF6 proteins were doing to fix ribosome assembly.

**All in the Family**

On the day that Shimamura discussed her goals with Bahl, Bowman was working in the tissue culture room. Unbeknownst to Shimamura or Bahl, Bowman had a cousin, Annabel, who had been born with a “mystery illness that nobody could really figure out.” Annabel’s father (Bowman’s uncle and godfather) had Googled the girl’s symptoms, such as failure to thrive. He came up with a rare genetic disease: Shwachman-Diamond syndrome. It bore a striking overlap with Annabel’s symptoms.
Initially, the doctors were skeptical: “everybody Googles stuff and thinks they have things figured out,” Bowman concedes. But a subsequent genetic test confirmed that Annabel did indeed have SDS. Even more uncanny, Annabel, who lives in Seattle, became a patient of Shimamura, who later moved from Seattle Children’s to Boston Children’s Hospital.

And now, Shimamura was standing in Bahl’s lab looking for a computational protein engineer, which Bowman was, to help her unravel the mystery of the rescue EIF6 proteins. Bowman quickly realized that Shimamura’s registry contained his cousin’s tissue samples, albeit carefully de-identified. That meant by participating in this study, Bowman would directly impact Annabel’s life.

“It was just crazy how serendipitous and how small of a world Boston science is,” he says. “Without even searching, you get an opportunity like this.”

Under the guidance of Bahl, Bowman created a model of EIF6 protein using similar known structures from yeast, slime mold and archaeabacteria. Through computer modeling, he learned that the EIF6 missense mutations affected protein function in two ways. One destabilized the protein, making the “cap” too flimsy to prevent ribosomal subunit joining. The other impaired its binding to the large subunit, making it too loose to hold on.

The results, recently published in *Nature Communications*, illuminated a new direction for therapy, currently relegated to bone marrow transplant. If the team could find agents that mimic the genetic crippling of EIF6, making a patient’s caps too flimsy or loose, investigators could help treat the disease and prevent its other, more sobering consequence: cancer.

**Preventing Cancer**

When ribosomal joining is impaired, cells respond by activating a stress pathway that involves the tumor suppressor p53. In essence, p53 prevents damaged cells from reproducing or forces them to commit cell suicide. In SDS, EIF6 mutations rescue the ribosome assembly defect, spurring improved stem cell production. At the same time, EIF6 mutations are not associated with the conversion of SDS into cancer. Something else is the culprit.

Through genetic studies, Kennedy found that “something else” is the second most common crop of mutations that occur in SDS patients: those in the TP53 gene, which encodes the p53 protein that controls cell division and survival. Mutations in TP53, like EIF6, initially rescue cell growth and reproduction. Unlike EIF6, however, TP53 mutations fail to improve ribosome assembly and can be found in patients’ blood-producing stem cells when leukemia develops.

All humans have two copies of any gene, one maternal and the other paternal. Kennedy observed that patients had to acquire mutations in both copies of TP53 for blood-producing cells to become cancerous. She could detect that malignant transition several years before the development of leukemia. Thus, testing for these so-called “bi-allelic mutations” might identify patients at high risk of leukemia; they might benefit from early intervention with a bone marrow transplant.
Another key finding was that TP53 mutations were not observed in cells that had acquired EIF6 mutations. This suggests that correcting the SDS defect with EIF6 therapy might keep stem cells fitter for longer. Their risk of developing cancer-causing TP53 mutations would diminish because the SDS defect had already been rescued by EIF6 alteration.

Confused? Shimamura, citing a colleague, explains it this way: an SBDS mutation is like blowing the transmission on a car (the stem cells) that should be motoring down the highway (making proteins). Adding in a TP53 mutation revs the car back up but tragically drives it off the road into a cancer ditch. By contrast, EIF6 mutations keep the car going and on the highway. By promoting the good repair via EIF6 therapy, the cells bypass the bad one, TP53.

“This is really exciting,” Shimamura says. “We actually have a target that we can try to develop into a novel therapeutic for bone marrow failure that also prevents leukemia.

The impact would be large not only for Kennedy and Shimamura, who see SDS patients in the clinic but also for Bowman, who knows one so intimately.

“It just makes you realize that for every disease-relevant target, there’s somebody else with a story,” says Bowman. “And it matters a lot to them to have somebody who can do the science to improve their quality of life.”
There are the “typical” scientists who follow well-trodden career paths. But more often and interesting are the atypical, who find their passion for science through an odyssey of chance meetings, unexpected events and changes in personal circumstances.

Paula Garavito, a post-baccalaureate student at the Institute for Protein Innovation, tells one such story. In Bogota, Colombia, she was born to a 16-year old mother, and then, at the age of 15, brought to Houston, Texas, “to find a better life.” The transition brought significant challenges. Garavito, entering high school and speaking no English, immediately confronted stereotypes. “A lot of students didn’t know where Colombia was,” she explains. People thought, “I was just ‘from Mexico,’ and I wasn’t sure if that was a bad thing or a good thing. I felt like I didn’t have an identity anymore.”

But she overcame the hurdles of language, racism and American teen culture. She broke past financial obstacles by working as a waitress and dishwasher. She entertained dreams of becoming a sommelier and starting her own wine business.

“I believed that success was having stability, getting married,” she says. “I didn’t know that college was even an option.”

That all changed in 2017 when Garavito became a US citizen. Eligible for higher education funding, she won a full ride to Texas Tech University in Lubbock. Leaving a large gulf-coast city for a rural college 500 miles away, Garavito pursued a dream of getting into medical school. She volunteered at a hospital and a free clinic. She tried research, “because it’s the thing you need on your (med school) resume,” she admits.
Eventually, she joined a synthetic biology group, one of a very few in Texas. Mentored by graduate student Brandon Palomo, Garavito discovered that she liked research. She led a team that made their way into the annual international genetically engineered machine (iGEM) competition in Boston. At the event in 2019, Palomo coaxed her to introduce herself to IPI’s Chris Bahl. He had just given an inspirational talk about how researchers could bring engineering principles into science to impact society. She sought him out.

“I was immediately struck by Paula’s insightful questions and clear enthusiasm,” Bahl says. “She asked me about how she could learn computational protein design.”

As a member of RosettaCommons, the academic consortium which develops computational protein modeling and design software, Bahl was involved in a new post-baccalaureate program. It aimed to help underrepresented minority or disadvantaged students to succeed in PhD programs. Bahl and Garavito chatted about the Institute for Protein Innovation, where Bahl’s group focused on computational protein design. “It was a life-changing experience,” Garavito says. “I was like, IPI, that’s where I want to go.”

She applied to the Rosetta program. Bahl, who sat on the admissions committee recalls that her application was so strong, the only debate was whose lab she would join. The Institute for Protein Innovation won out. Garavito traveled to Boston to spend a year learning everything she could about protein design, boot camp style.

Somewhere in that year of intense training and mentorship, Garavito realized she had learned more than computational biology or protein engineering. “I found my passion,” she says.

Indeed. After a year, Garavito applied to graduate schools: Yale, Dartmouth, Johns Hopkins, Harvard. She got into each one, and in fact, was offered two fellowships to attend Harvard. Professors telephoned to recruit her, asking if she needed more money or what she wanted. “That is such a great feeling, right? Because three years ago, I was working at a restaurant, thinking I was gonna go into wine.”

In the end, she chose Harvard, doing her research in the Bahl lab. And now, she says, she feels proud: of her roots and her journey. Of being different. Of finally finding “her place.”

“Every time I said, I wanted to go to med school or Harvard,” she says. “Even my mentors were like, ‘oh, that’s cute…but you probably won’t make it.’ The main thing that I always kind of carry in the back of my head is that I can make it no matter what happened in my life.”
While many look back on this past year of fear and sadness with pessimism, we acknowledge the difficulty and also look forward with optimism. The coronavirus did take us down. But science helped bring us back up to where we could stand again. We believe in that science, especially when it is a force meant to do good. At IPI, we believe in our mission to advance protein science to accelerate research and improve human health. And we promise to work hard to help the scientific community take on even the most daunting of foes.