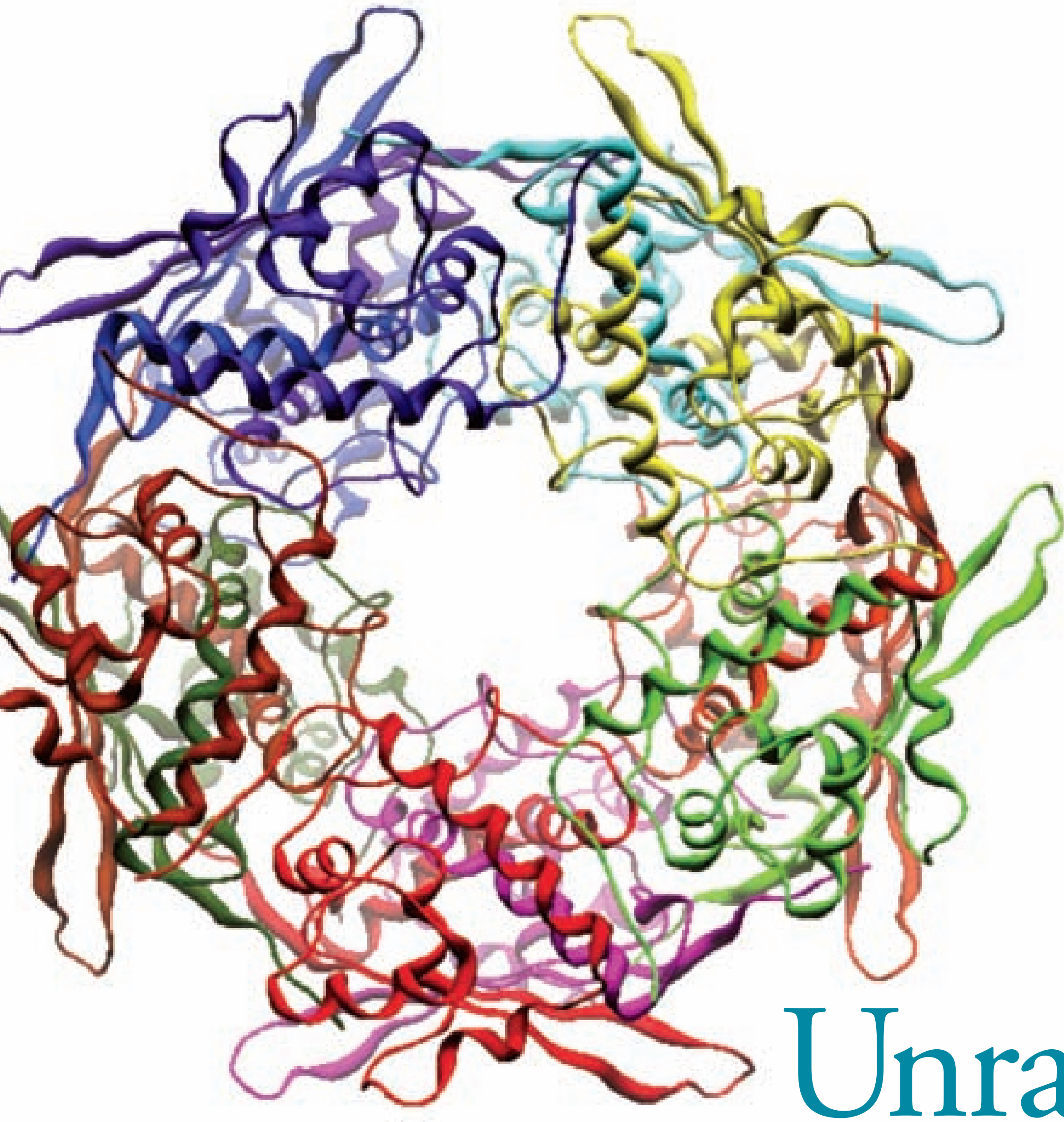


Unraveling

Two Rensselaer researchers team up to conduct groundbreaking biotechnology research—and to attract more young people to their fields.

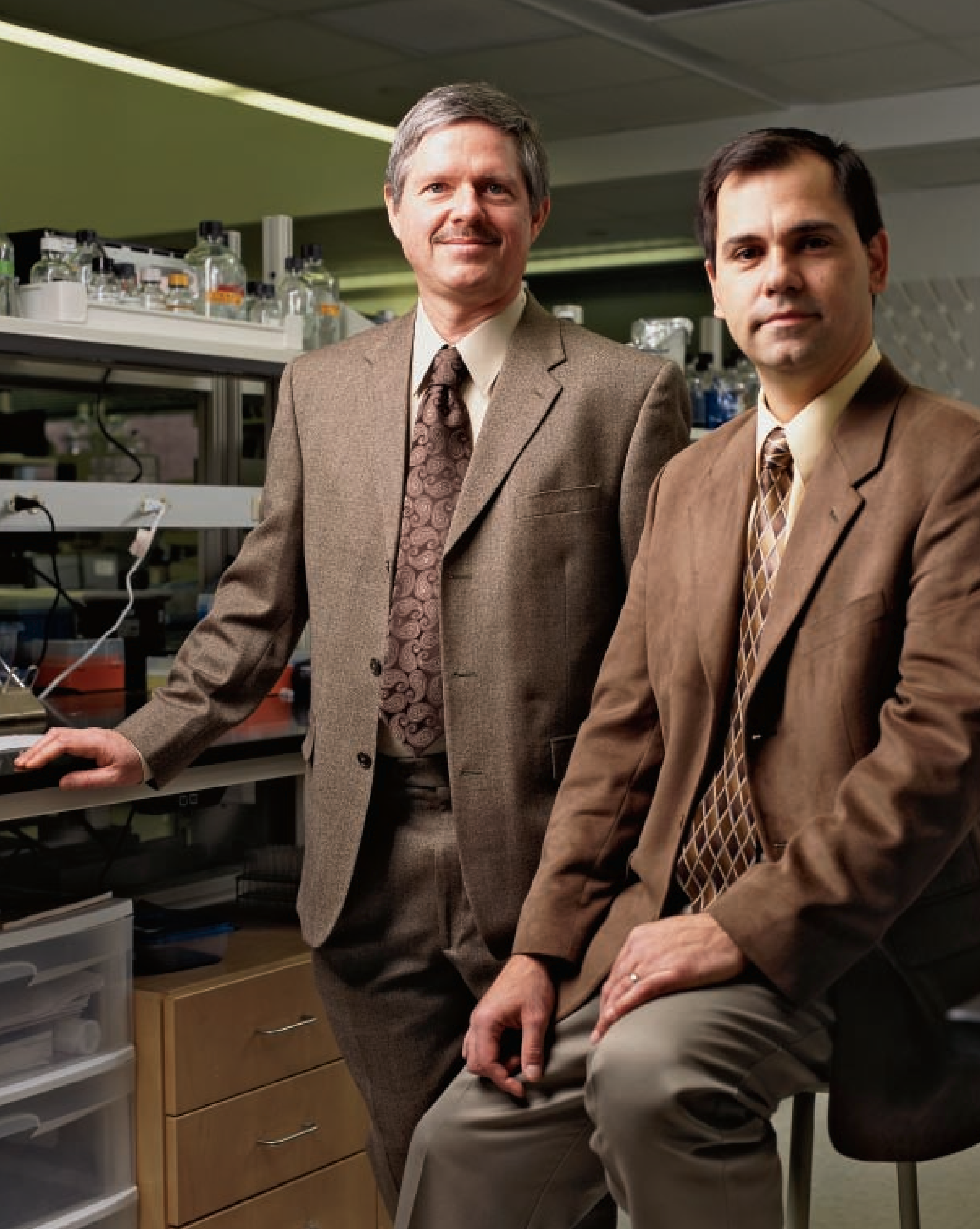
Wilfredo Colón and Christopher Bystroff make a formidable research team, bringing together their individual expertise in biochemical techniques and computer modeling to better understand why proteins sometimes become trapped in a specific structure—knowledge that could lead to early detection for diseases like amyotrophic lateral sclerosis (Lou Gehrig's disease) and Alzheimer's. Colón, associate professor of chemistry and chemical biology, and Bystroff, associate professor of biology, are prime examples of the multidisciplinary research teams supported by the Center for Biotechnology and Interdisciplinary Studies.

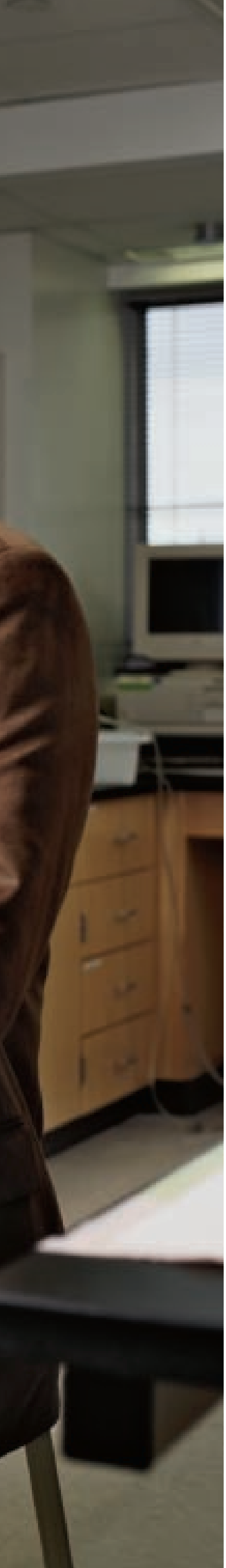
"This is exactly what the biotechnology center was intended to do," says Robert Linhardt, the Ann and John H. Broadbent Jr. '59 Senior Constellation Professor of Biocatalysis and Metabolic Engineering and acting director of the center. "To bring groups from different disciplines together."

Rensselaer's emerging biotechnology enterprise seeks to break down the traditional walls between researchers. "It's interesting how few biologists really talk to chemists," Linhardt says. "This way of working that we have now at Rensselaer basically cuts down those barriers."

Proteins

Colón and Bystroff are extending their collaboration to helping attract and mentor the next generation of researchers—especially young people from underrepresented groups in the sciences and academia. Students speak highly of the researchers as teachers and colleagues whose talent, energy, and commitment to leading-edge discovery is drawing promising undergraduates and graduate students to Rensselaer. BY JILL U. ADAMS





Mark McCarty

Learning the structure of proteins provides clues to their function. And yet, proteins are by no means static creatures. They fold and unfold, they glom on to one another and let go. Dancing, tumbling, and partnering with others, these are dynamic, interactive molecules. All the time, they obey the laws of physics.

THE PROTEIN DANCE Proteins are essential to life. They participate in every biological process in the body. They are carriers like hemoglobin delivering oxygen to every cell, enzymes like DNA polymerase aiding gene replication, structural elements like actin and myosin responsible for muscle contraction, signaling molecules like insulin and endorphins, and antibodies that target foreign substances in the body for destruction.

Learning the structure of proteins provides clues to their function. And yet, proteins are by no means static creatures. They fold and unfold, they glom on to one another and let go. Dancing, tumbling, and partnering with others, these are dynamic, interactive molecules. All the time, they obey the laws of physics. Despite the remarkable diversity in functions of which proteins are capable, they have a major liability—they are marginally stable. Stable proteins would be highly valued in a variety of industrial applications—think insulin with a long shelf life.

It's easier than ever to decipher the linear sequence of proteins—imagine a string of pearls, each bead an amino acid. The challenge for researchers is determining the three-dimensional shape that proteins will assume in the cell—picture that pearl necklace in a velvet pouch. Figuring out protein conformations—both good ones and bad ones—will answer so many important biological questions and improve the practice of medicine.

"I've been looking at proteins now for 20 years," says Bystroff. "I was always curious about how these things got into their convoluted state."

Both Colón and Bystroff have received National Science Foundation (NSF) CAREER Awards to support their protein folding research and educational activities.

Protein folding has been a major research field for some time, with tens of thousands of published articles in the scientific literature—hundreds this year alone—and numerous well-funded laboratories around the world. Bystroff has found his own niche by seeking out a less-studied problem—unfolding.

However, protein unfolding happens fast.

At left, Christopher Bystroff and Wilfredo Colón.

"Too fast," says Bystroff. "And once it starts, it doesn't stop." It's extremely cumbersome to analyze using biochemical methods and even then, one only gets a blurry picture.

"Scientists have no clue of what the details are," says Bystroff. "And that's my justification for doing it computationally. We have a very detailed look at what's happening in the computer. And the trick is, to prove to people that what we're doing in the computer is the same thing that's happening in the cell."

Meanwhile, Colón is interested in proteins that misfold. He seeks to understand the fundamentals of protein folding and has zeroed in on the issue of stability.

Most proteins spend most of the time in their folded state, says Colón, but they're in equilibrium, going back and forth. "During that fraction of a second that they may be unfolded, that's where they could misfold," he says. If the misfolded state is more stable than the folded state, then that's going to be the preferred form—and likely a problem. Thermodynamics are one factor; kinetics (i.e. how fast?) also plays a role. "Is it faster to fold this way or that way?" he asks. Proteins that are prone to misfolding are often trapped in their native state so that they cannot unfold at all during their lifetime in the cell. This property devised by nature is known as kinetic stability.

Mistakes in protein folding are increasingly seen in neurological disorders, like Alzheimer's and Lou Gehrig's diseases. Misfit proteins are biological markers for these diseases at the very least, and many researchers are seeking to understand whether they have a causative role as well.

Colón compares a protein to a toddler to illustrate the difference between thermodynamic stability and kinetic stability. "If you have a toddler in a room with lots of toys and the door is open, chances are the toddler is going to stay, even though he may briefly leave the room," he says. "That's an example of a thermodynamically stable toddler. He's there—not because he can't get out—but because he wants to stay there."

Put a gate across the door to keep the toddler out of trouble and "then he's kinetically stable," Colón says. "It doesn't matter if he cries and wants to get out. He's kinetically trapped. Sometimes nature must put a 'gate' on proteins, an energy barrier to keep them out of trouble (i.e. misfolding). If one could figure out how nature does this, we may be able to use the same strategy to confer kinetic stability upon proteins."

The collaboration with Bystroff happened when Colón found a way to identify kinetically stable proteins and needed some way to characterize their common features. Bystroff, with his expertise in computer modeling and his drive to make his models relevant, was the perfect partner.

"It's a really great situation to have somebody doing experimental work in the same area that we're doing computational work," says Bystroff.

THE TWO-STEP With the wide variety of sizes, shapes, and charged characteristics of proteins, no one would think there's a simple way to categorize the molecules based on their dynamic properties. But that's exactly what Colón has developed.

Adapting an everyday method that is used time and again in biology labs, Colón has fashioned an assay, a regimented laboratory procedure that effectively separates kinetically stable proteins from all others. In a paper published two years ago with then graduate student Marta Manning '03, Ph.D. '06, Colón showed that proteins that don't easily unfold in nature were also resistant to a common laboratory treatment.

When proteins are unfolded or denatured, they can be sorted by running an electric current across a length of gel. Colón developed a simple assay on a gel that involves comparing the migration of heated and not heated protein samples containing a common chemical denaturing agent known as SDS. Most proteins migrated to the same location on the gel regardless of whether or not the sample was heated. In contrast, kinetically stable proteins exhibited a slower migration when the sample was not heated.

At first, Colón used purified proteins to make his case that kinetically stable proteins were resistant to unfolding with SDS treatment. Now, with one clever quarter turn of his gel, Colón has streamlined his procedure so that proteins, even messy mixtures of thousands of proteins, can be processed via a high-throughput assay, to identify those proteins that are kinetically stable.

With this method, he can screen the protein soup of an entire organism, as he has recently done with the bacterium *E. coli*, for kinetically stable proteins. Identifying the proteins that were sorted involved a series of biochemical methods and the help of Qishan Lin, director of the University at Albany's proteomics facility.

Ultimately, Colón wants to use this method with human plasma where it might be used to diagnose medical conditions. "For example," Colón says, "we hope to be able to identify whether any disease may be linked to the loss or gain of protein kinetic stability. The gel becomes almost like a fingerprint of kinetically stable proteins in human plasma."

"We have now what we didn't have before, what nobody had before: a list of kinetically stable proteins with known 3-D structures," says Colón. "Chris is analyzing the structure of these, looking for common features that may be responsible for kinetic stability."

Proteins that resist unfolding are a small minority in nature. "Proteins are in the stomach, in Yellowstone geysers, harsh environments like that," says Bystroff, describing environments so harsh, it's hard to imagine any dancing molecule surviving.

As an expert in protein unfolding, Bystroff is discover-

Last summer, 24 interns from the Howard Hughes Medical Institute (HHMI) Minority Undergraduate Research Program in Bioscience and Biotechnology did research in 18 labs on the Rensselaer campus. The program is funded through a $1.2 million, four-year grant in the Undergraduate Biological Sciences Education Program from HHMI.

ing how these proteins are kept folded. "I categorized things according to the topological features these proteins had," he says. Categories include "tucked in," where ends of the chain were buried like a tab, "latches," a long piece of chain that goes around the molecule like a belt, and "blocking."

"If you have a box that can only open one way and it's packed up against another box," like a hinge that can't move, "I call that blocking," Bystroff says.

"I'm interested in being able to design proteins," Bystroff says. "Proteins are incredibly flexible molecules," for which there are lots of industrial uses. To look at a protein and be able to make that protein kinetically stable, "it's sort of natural nanotechnology."

For all his modeling and computational expertise, Bystroff is not afraid to rely on his visual sense. "He does want to picture things," says Mohammed Zaki, an associate professor of computer science, who has worked with Bystroff on modeling protein unfolding pathways projects. "It's a very hard problem, a grand challenge, so one really needs all the ingenuity that we can get."

"The good thing about Chris is he has the ability and the skills to handle both the wet lab side of things as well as the computational side of things," says Zaki. "He's pretty well diversified in his interests. It allows him to collaborate with Freddie on the dynamic side and with me on the data-mining side."

INSPIRING FUTURE RESEARCHERS Colón and Bystroff are not only interested in creating scientific results, but also in nurturing the next generation of scientists.

Marta Manning began her career in Colón's lab as an undergraduate volunteer and then was welcomed to stay on as a graduate student. "He is an excellent person to work with," Manning says of her former mentor. "He is very friendly, great sense of humor, very open to students. He was an excellent mix of someone that let students work independently, but also offered support [when needed]." Manning is now doing postdoctoral work at Penn State.

Bystroff also inspires the next generation. "Chris always has a bunch of undergrads working for him," Zaki says. "They go on to do Ph.D.s or master's, either at RPI or elsewhere. From a training point of view, that's a really great thing."

A summer internship program seems a natural extension of this training mindset. Colón and Bystroff have taken the existing program—funded by an educational grant from the Howard Hughes Medical Institute and directed by Acting Provost Robert Palazzo—to the next level. Not only are they seeking undergraduates who are interested in research experience, they are intent on reaching students who might otherwise have little exposure to academic science at a research institution.

"I have enjoyed working with Freddy and Chris on launching an important effort to recruit exceptional women and minority students to Rensselaer for an undergraduate research experience," says Palazzo. "This program, funded by the Howard Hughes Medical Institute, provides support for a high-quality research experience for truly exceptional and gifted undergraduates from diverse backgrounds. Already some of these students have shown interest in applying to our own graduate programs and I am sure that many decided to attend graduate school somewhere after their experience at Rensselaer."

"We want to attract the best students we can," says Colón. Too often, he says, students have to make their decisions based on paper or what they see on the Internet. "The idea was to bring them here so they can experience what it is to be at Rensselaer," he says. "We all felt that would be a very effective recruiting strategy."

Their efforts have caused a ripple effect as fellow faculty in the biotechnology center take undergraduate interns into their laboratories—with the goal of identifying potential graduate students before the formal application process.

"This has been a wonderful thing that they've done," says Linhardt. "I had two students in my laboratory last summer. Both had very successful projects and I expect I will publish papers with both of them as co-authors."

For the students themselves—many from Puerto Rico or historically black colleges—a fascinating and challenging new world awaits, as the internships give them direct experience with the realities and the demands of experimental research—all with the goal of attracting them to careers in the sciences.

Exposing students to the life of a researcher in the sciences is the first step toward expanding the pool of young people who choose careers in these fields. "We hope that we tempt them to take research paths," Linhardt says.