Targeted protein degraders are redefining how small molecules look and act

The new modality promises to open up the human proteome to drug developers, but first they will need to work out the rules

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When the first human genome was sequenced, researchers finally got a glimpse of the true dimensions of our proteome—the tens of thousands of proteins responsible for sickness and health. Ever since, scientists have explored the morass for drivers of diseases, generating a long wish list of proteins they'd like to control.

IN BRIEF

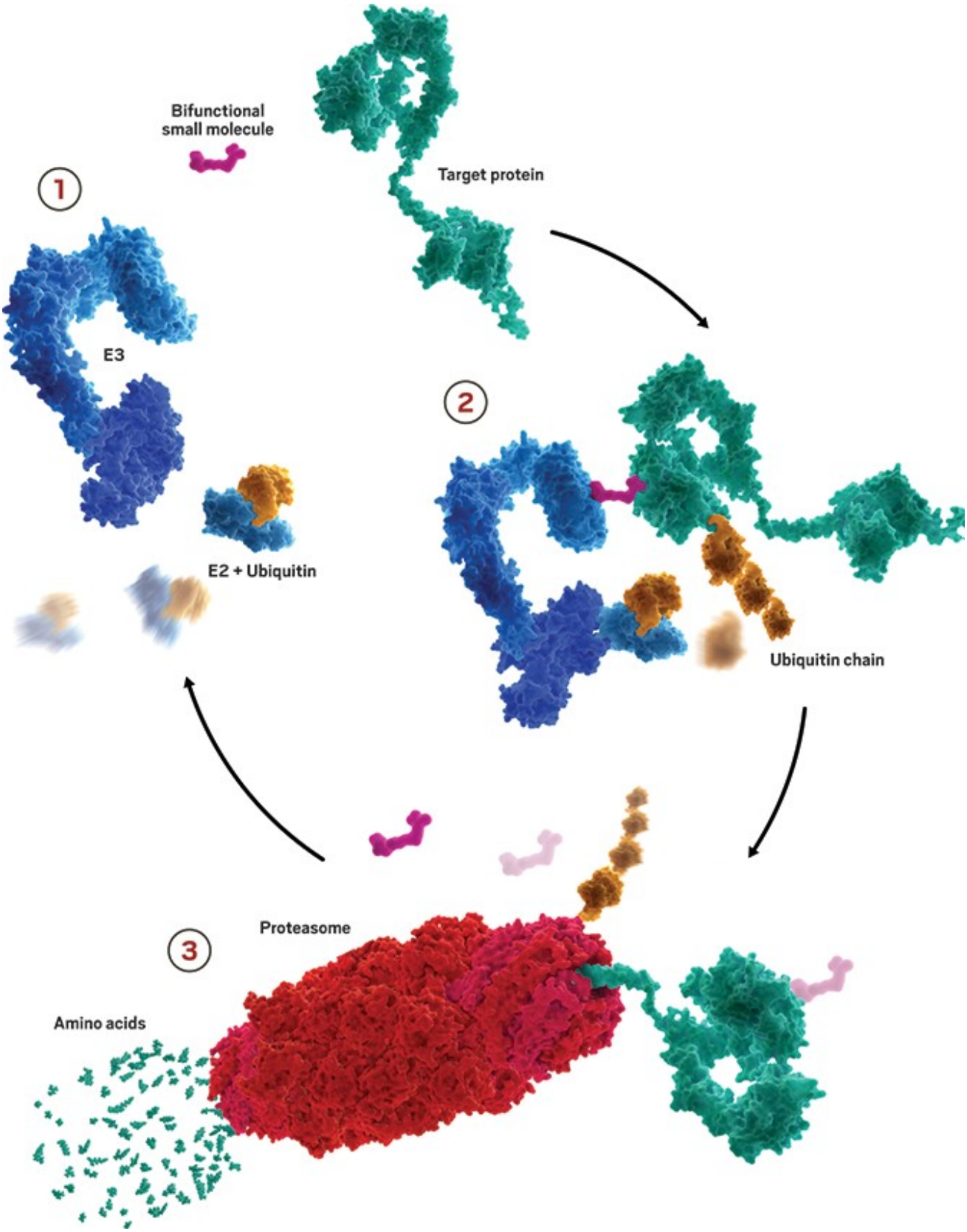
Targeted protein degradation, a way of sending bad-behaving proteins to the cellular trash compactor, is a hot new area in drug discovery. In just two years, multiple companies have bought into the idea that bifunctional small molecules can open up a broad swath of the human proteome. But much work remains to translate what was until recently an academic endeavor into a commercial opportunity. Read on to find out how biotech and big pharma firms are trying to take protein degraders from the lab to the marketplace.

But even as they add to the list, drug hunters live with a maddening reality: So many of its entries are out of reach. Conventional small-molecule and antibody drugs can access only about 20% of the proteins we make.

So when a new technology comes along promising to tap into the other roughly 80%, everyone pays attention. Over the years, approaches such as RNA-based silencing and gene editing have raked in billions of dollars in financing for their potential to turn off the activity of previously unreachable proteins.

But new modalities like these come with growing pains—delivery problems, potency issues, safety questions—that make the arc from idea to marketed treatment excruciatingly long. What if instead you could reimagine an old workhorse—the small-molecule drug—into a new kind of medicine?

Researchers developing **targeted protein degraders <https://cen.acs.org/articles/94/i3/Chemists-Sending-Bad-Proteins-Cellular.html>** aim to do just that. Conventional small molecules block the activity of a protein. In contrast, these bifunctional small molecules eliminate the protein altogether by routing it to the proteasome, the cell's trash



Breakdown

Protein degraders tackle tough drug targets by hijacking a cell's natural system for disposing of spent or

E2 protein and tagging the protein for ubiquitination (2) and subsequent breakdown into amino acids in the proteasome (3). The molecules then move on to repeat that process.

Credit: Kymera Therapeutics

Our cells are always working to maintain just the right levels of proteins—making and disposing tens of thousands of them at any given time. A key player in managing that balance is a small protein called ubiquitin. When tacked onto exhausted proteins, it routes them to the proteasome for disposal.

Researchers developing protein degraders want to break into that ubiquitin-proteasome machinery to change the destiny of disease-causing proteins. To do that, they design small molecules with two active ends: one that binds to the protein of interest and the other that binds to a protein called E3 ubiquitin ligase. These bifunctional molecules force a handshake between the ubiquitin and the protein, sending it to the trash. The job done, they move on to repeat the process, quickly depleting levels of the unwanted protein.

It sounds devilishly simple. But protein degraders break all the rules about how a small molecule looks and behaves. To accommodate both functional parts, the molecules tend to be bulky on the ends and floppy in the middle. They also weigh more than is typically considered acceptable for a drug.

Unlike conventional drugs, these molecules act kind of like an enzyme or catalyst, with each one sending multiple target proteins to the trash. And for reasons still being explored, they upend conventional wisdom about drug potency and selectivity.

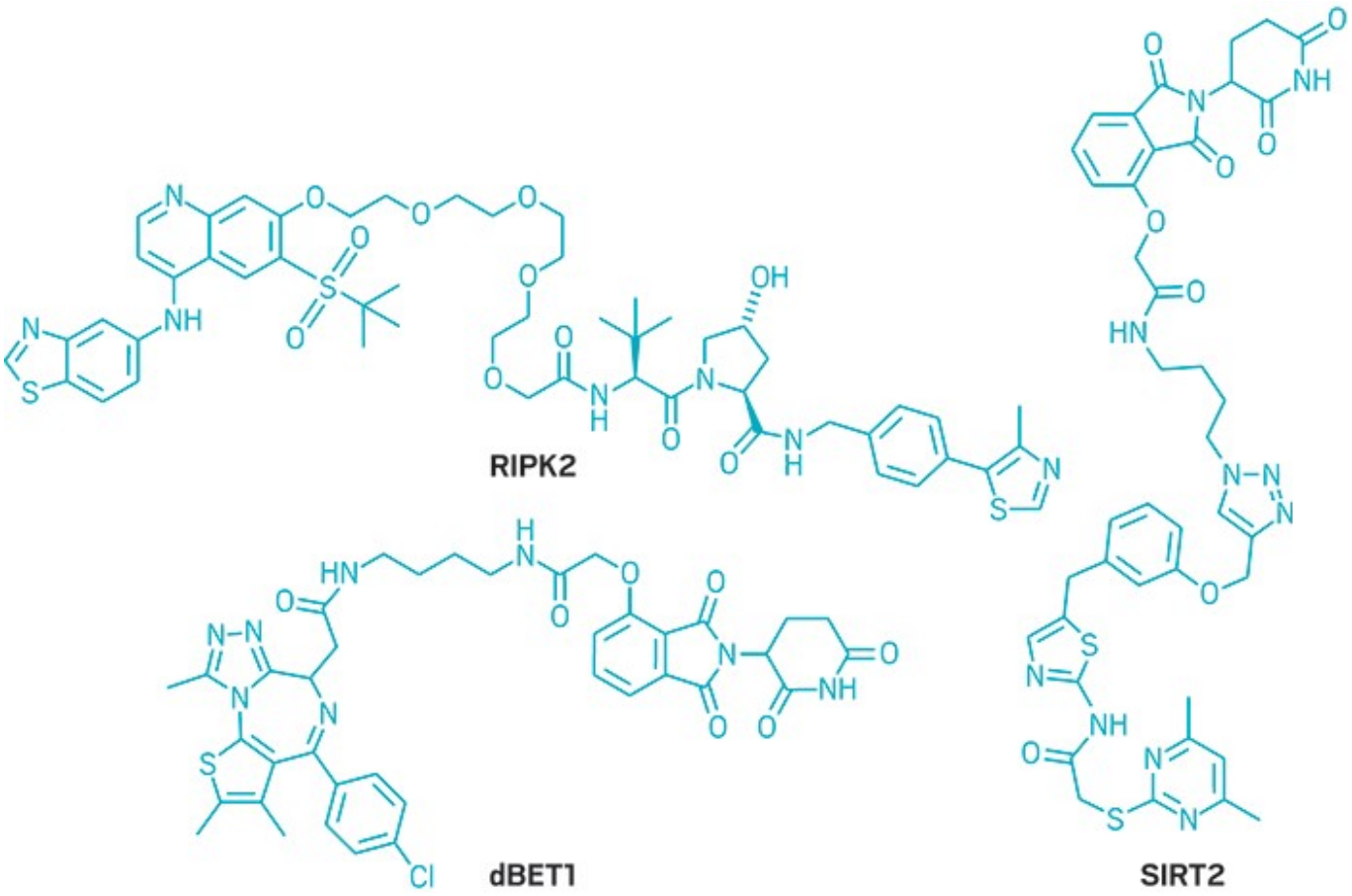
Because of those quirks, protein degraders might easily have been relegated to the annals of academic oddities. Indeed, not even five years ago, “people were queuing up to tell us the reasons why this wouldn’t work,” says Ian Churcher, vice president of drug discovery at BenevolentAI. Churcher previously led GlaxoSmithKline’s protein degradation unit, which was an early adopter when it launched in 2012. “To many medicinal chemists, these structures were truly strange.”

But thanks to a flurry of academic work proving, in cells at least, that targeted degradation is a powerful way to silence errant proteins, industry has **taken notice <https://cen.acs.org/articles/96/i2/Pfizer-inks-protein-degradation-deal.html>** . Since 2016, multiple protein-degradation-focused biotech firms have emerged with ample funding. Big pharma companies have launched internal efforts and forged partnerships to explore the modality.

Momentum is building, but drug company scientists still need to confront a few critical questions. The biggest—whether this new modality will be safe and effective in humans—could be answered soon. Arvinas, one of the first protein-degrader companies to emerge, plans to put two cancer drug candidates into clinical studies this year.

Researchers also need to push beyond the status quo. The molecules published so far were discovered empirically and act against well-established proteins. If companies want to access the other 80% of the proteome, their medicinal chemists will need to forge a new set of rules for how to rationally build drugs that consistently degrade proteins.

Research executives seem confident that the remaining challenges boil down to elbow grease. As Jay Bradner, president of the Novartis Institutes for BioMedical Research, puts it, “Our field is really good at



Wonderfully weird
Targeted protein degraders defy the conventional rules for small-molecule drugs. Shown here, the all-chemical bifunctional molecules developed by Crews (RIPK2) and Bradner (dBET1), and a recently discovered compound called SIRT2.

Curiosity to commercial opportunity

Although venture capital firms and big pharma companies are waking up to targeted protein degradation, the concept is far from new. In fact, a biotech company called Proteonix filed a patent in the late 1990s outlining how a bifunctional molecule could hijack the cellular ubiquitin system. One of the patent's authors, John Kenten, says he never could get his bosses excited about the concept. He eventually left the company.

About that time, academic scientists including Yale University chemical biologist Craig Crews and California Institute of Technology cell biologist Raymond Deshaies began publishing peptidelike

properties needed to be drugs, and industry largely overlooked the work.

“The initial observations sat in the literature for at least 10 years with not too many people paying attention,” BenevolentAI’s Churcher recalls.

Protein degradation required a leap of faith for industry, says Andy Phillips, president and chief scientific officer of another new firm, C4 Therapeutics. After “120 years of pretty well-resolved knowledge around inhibition,” he says, it’s tough to rethink how small-molecule drugs look and act.

In 2008, Crews assembled a team of chemists and biophysicists to move beyond the early peptide compounds. Their goal: the first catalytic small-molecule drug, a modality he dubbed “PROTACs” for proteolysis-targeting chimeras.

By the end of the year, they had come up with a small molecule that recruited an E3 ligase using a ligand known as nutlin, but its activity was far too weak to turn it into a drug. Over the next few years, researchers chipped away at the problem, generating hundreds of molecules and solving many prote crystal structures. As Crews’s lab and ones run by researchers like University of Tokyo’s Yuichi Hashimoto published on bifunctional molecules, others started to take notice. Crews says he is convinced that without those years of work, “PROTACs would have died a very quiet death as a cute chemical biology curiosity.”

A key challenge for Crews’s team was finding an E3 ligase binder, the half of the bifunctional molecul that causes the ubiquitin disposal tag to be added. The problem is that harnessing an E3 ligase ofter means disrupting a multiprotein complex—a historically difficult feat.

In 2009, chemical biologist Alessio Ciulli spent a few months as a postdoctoral fellow in Crews’s lab t work on the problem. The collaboration continued when Ciulli started his group at the University of Dundee. In early 2012, they debuted a ligand for the well-characterized von Hippel-Lindau protein, giving the field its first small-molecule VHL binder to work with.

It was a critical step forward for the field. GlaxoSmithKline subsequently established its protein degradation group and partnered with Yale, providing Crews’s lab with key industrial medicinal chemistry expertise.

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Then in 2015, two sets of discoveries sparked the current frenzy for protein degradation. Bradner, at the time leading a chemical biology group at Dana-Farber Cancer Institute, published a small-molecule degrader featuring a new E3 ligase-binding component: a thalidomide group that binds to cereblon, a ubiquitin ligase (Science 2015, **DOI: 10.1126/science.aab1433 <http://dx.doi.org/10.1126/science.aab1433>**). Around that same time, **Crews’s team published**

degrader (ACS Chem. Biol. 2015, DOI: **10.1021/acschembio.5b00442 <http://cgi.cen.acs.org/cgi-bin/cen/trustedproxy.cgi?redirect=http://pubs.acs.org/doi/abs/10.1021/acschembio.5b00442?source=cen>**).

Investors took notice. “That combination of the field maturing with the opportunity to explore the uncharted territory of the remaining 85% of the human proteome with this technology was really impossible to pass on,” says Nello Mainolfi, founder and chief technology officer of **Kymera <https://cen.acs.org/articles/95/web/2017/10/Kymera-launches-30-million-tackle.html>** Therapeutics, a protein-degradation-focused company that Atlas Venture, a venture capital firm, began building in late 2015.

Crews had founded Arvinas two years earlier to commercialize PROTACs. In 2016, Bradner and other academic researchers formed **C4 <https://cen.acs.org/articles/94/i2/C4-Launches-Makes-De Roche.html>** , receiving $73 million in financing. Both firms quickly amassed partnerships with big pharma firms like Merck & Co., Pfizer, and Roche, all eager to test-drive the technology.

Meanwhile, other big drug companies, including Novartis and AstraZeneca, began investing in target protein degradation. Bradner, who joined Novartis in 2016 to head the firm’s research arm, personal leads the protein degrader unit. Some 200 chemists, biologists, and computer scientists contribute, says.

Rewriting the rules

As companies flood into the space, they will need to rethink small-molecule drug design. Constructing a protein degrader requires carefully considering each of its components: the protein-binding ligand, the E3 ligase binder, and the chemical chain linking the two.

Fully assembled, the molecules defy some of medicinal chemistry’s most sacred tenets. Most obviously, they break medicinal chemist Christopher A. Lipinski’s infamous rule of five, a set of parameters for predicting whether a molecule will behave like a good, orally available drug. By those standards, protein degraders weigh too much and feature too many hydrogen-bond donors and acceptors.

In addition to not looking like great drugs, protein degraders diverge from conventional concepts of drug binding, selectivity, and dosing. Historically, medicinal chemists have tried to craft a molecule that sticks to its target—and only its target—like glue. And they think in terms of “occupancy”—how much time the

"If I were to ask a pharmacologist would they rather have something that is a 95% inhibitor of a target or a 99% or 99.9%, most of the time they'll say, 'I'll take the 99.9% inhibitor, please,' " C4's Phillips says.

But the measure of a protein degrader isn't how tightly it binds to a protein; it's how fast the bifunctional molecule can bring together all the right players to prompt the protein to be tagged for the trash.

That shift from binding thermodynamics to reaction kinetics is what gets everyone excited about protein degraders' ability to tackle previously out-of-reach drug targets.

The end of the molecule that seeks out the protein doesn't need to tuck into a pocket and turn it off; it just needs to mingle with its target long enough to get the job done. And while 75% of proteins lack th active site needed by a conventional small-molecule inhibitor, "if you look at the surface of them, the have nooks and crannies" to which a degrader can tether, Yale's Crews says.

Evidence is also mounting that the protein-binding half of the molecule doesn't need to have the selectivity typically required of small-molecule drugs.

Drug developers usually want molecules to latch on to only their intended target; a drug that binds to other proteins can cause toxic side effects. But academic scientists have repeatedly shown that promiscuous inhibitors can be turned into selective degraders. That selectivity has yet to be complete explained, but researchers point out that in order for a protein to be tagged with ubiquitin, degraders need to twist into the right shape at the right time across several steps.

For inhibiting molecules, "it's just all about binding," Novartis's Bradner notes. "But here, there are s many mechanistic steps involved—it's a catalytic cycle that you are delivering."

The selectivity of degraders makes perfect sense, Phillips agrees. "I'm not surprised by that, simply because of what Mother Nature has set up as background machinery." Proteins, he notes, are constantly being degraded using a "carefully orchestrated, three-dimensional relationship between the target protein and ubiquitin."

Into the unknown

But just because researchers don't need to adhere to the old medicinal chemistry rules when designing their drugs, it doesn't mean the human proteome is suddenly up for grabs.

"By definition, if it's an 'undruggable' protein target, you probably don't have a small molecule to target it," quips Daniel Nomura, a chemoproteomics expert at the University of California, Berkeley. So far, he points out, the bifunctional molecules that have been published feature high-affinity, protein-binding ligands that were already reported in the literature.

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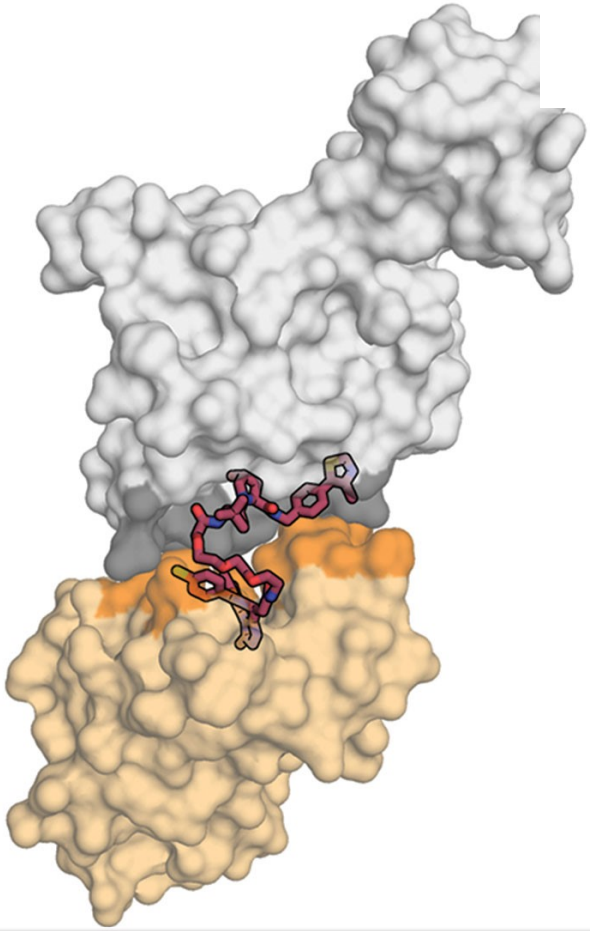
Indeed, when Arvinas moves its first two protein-degrading molecules into human studies later this year, they will be breaking down well-characterized proteins: the androgen receptor, which is a prostate cancer target, and the estrogen receptor, a breast cancer target. So far, no labs have publicly unveile potent molecules against undruggable targets, although companies and academic labs alike claim t have unpublished data proving it is possible.

Researchers are taking a range of approaches to identifying protein-binding ligands for tough targets Nomura, whose lab is part of a collaboration between Novartis and UC Berkeley that is focused on undruggable targets, has developed a way of using covalent small molecules to probe the surface of protein for hidden toeholds.

"A lot of the sites we end up identifying are not obvious pockets if you look at the static crystal structure," he says.

Companies point to **DNA-encoded libraries <https://cen.acs.org/articles/95/i25/DNA-encoded-libraries-revolutionizing-drug.html>** as optimum starting points for finding protein degraders. Unlike a high-throughput screen, which captures compounds that modulate a protein's activity, DNA-encoded libraries are used to pan for molecules that merely bind to a protein. The technology allows companies to scan millions—even billions—of compounds tagged with DNA bar codes that can be sequenced to reveal the structure of any hits.



Ian Taylor, Arvinas's head of biology, points out that in addition to revealing protein binders, the DNA bar code is a natural starting point for constructing a protein degrader's linker—the chemical chain connecting the protein and ligase binders. Arvinas is about nine months into a collaboration with Macroceutics to use DNA-encoded libraries to explore previously inaccessible proteins. "We're going to be investing more into that this year," Taylor adds.

Another route to protein binders is to revive compounds that are known to latch onto their target but for other reasons weren't viable. They could include old drug

had off-target effects. In theory, those molecules could be reimagined as part of a protein degrader.

structure revealing how a degrader (purple) is bound to its targets, the E3 ligase VHL (gray) and the Brd4 bromodomain (orange).

Credit: Ciulli lab

Beyond finding ligands for previously unapproachable proteins, researchers have some honing to do on the other end of their bifunctional molecules, the E3 ligase binder. Although the human ubiquitin-proteasome machinery includes more than 600 E3 ligases, scientists have so far come up with ligands that bind to only a handful of them.

Companies say they will be able to degrade many proteins even with that limited tool kit. But researchers want to find other E3 ligase binders that could broaden their capabilities. For example, identifying ligands for E3 ligases that function only in specific tissues could allow developers to desig better drugs.

“Figuring out the master keys—or just any keys, frankly—to Mother Nature’s collection of E3 ligases is important medium-term goal for the field,” C4’s Phillips says. “We don’t necessarily need new E3 liga to meet unmet medical need, but if this field is going to truly capitalize on this groundswell, we’ll nee them.”

The next wave

To access more of the human proteome, researchers will need to industrialize the drug discovery process. In these early days of protein degrader research, molecules are primarily products of empiricism. The components have typically been assembled “in a nonguided manner,” University of Dundee’s Ciulli says. “There is a clear need to bring the field into the modern era of structure-based drug design.”

The influx of venture capital and interest from big pharma should help. “We’re doing our best to take away much of the empiricism associated with this technology,” says Laurent Audoly, CEO of Kymera. He notes that the company has built a drug discovery platform intended to design the best molecules, rather than “backing into a drug and really not being able to explain how you got there.”

Transitioning to rational drug design is important not just in speeding the process but also in understanding which targets to pursue. The reality is that chemists can’t predict which proteins can be degraded and whether their molecules—currently rather unwieldy—can get to hard-to-reach places such as the brain or consistently be turned into pills.

“People often confuse the fact that you don’t need a functional binder as meaning you basically can drug any kind of protein,” Kymera’s Mainolfi says. Kymera has spent a lot of time trying to define the parameters that turn a promiscuous protein binder into a selective protein degrader, Mainolfi says.

Churcher cautions. “That can be a good thing if pharmacology gives you efficacy and a bad thing if pharmacology also gives you toxicity.” BenevolentAI is using artificial intelligence analysis tools to figure out which targets have the best chance of treating a disease without dangerous side effects.

Even as researchers point out all the work that still needs to be done, they are also excited that protein degradation is moving into its next phase: human studies. Seeing whether these molecules can safely and catalytically degrade proteins in people will be “the first indication that, yes, these probably can be turned into drugs,” C4’s Phillips says.

Arvinas hopes to have its androgen receptor-targeted PROTAC ready for clinical trials by October. Its second drug candidate, which targets the estrogen receptor, could be in trials by the end of the year. Other biotech and big pharma firms are not far behind, with several molecules expected to be tested patients in the next two years.

As researchers wait for their first peek at how the drugs work in humans, optimism is high. “Since I joined Novartis, our scientists have degraded more than 30 proteins,” Bradner says. “It is proving ver facile.”

Some proteins remain elusive, but Bradner is not concerned. “With a little bit of luck and a lot of chemistry,” he says, “we can degrade almost anything.”