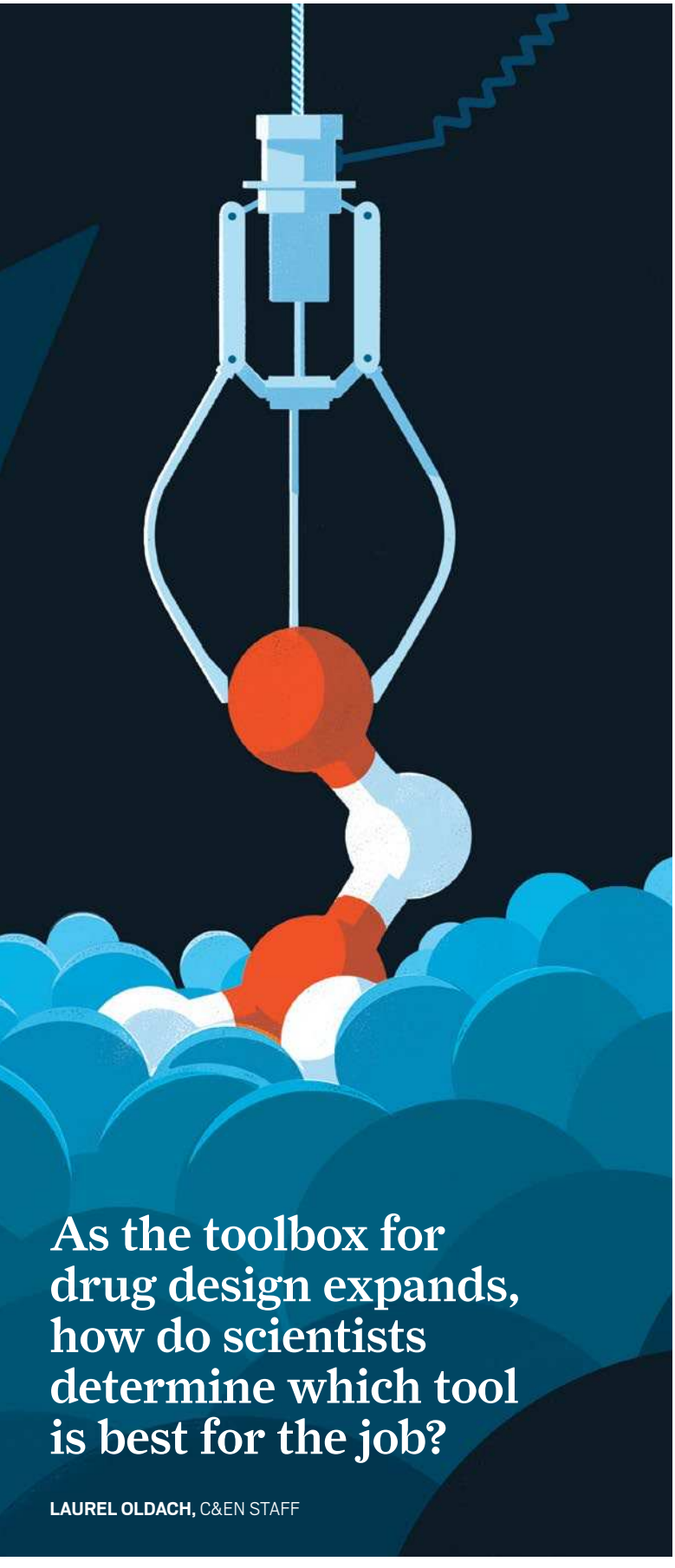
CHOOSE YOUR MODALITY



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As the toolbox for drug design expands, how do scientists determine which tool is best for the job?

LAUREL OLDACH, C&EN STAFF

Chemists and chemical biologists just keep dreaming up new ways to interfere with biomolecules. That research frequently leads to promising potential medicines, and there are more and more flavors of molecules at some stage of clinical or preclinical investigation. How do drug hunters choose between them?

Twenty years ago, monoclonal antibodies were just emerging as a promising type of therapy on the strength of their selective binding to highly specific molecular motifs. The class is now both a workhorse in the clinic and a molecular starting point for an expanding group of biological medicines that use antibodies' targeting power to home in on cell-surface targets.

Drugs aimed at targets within the cell have multiplied too. They have expanded beyond small molecules aimed at blocking enzyme activity by mimicking a substrate to encompass larger compounds that manipulate protein-protein interactions. Then there are new methods for targeting the DNA or RNA that encode problem molecules that cause disease.

Medicinal chemists refer to these different drug types—small and larger molecules, peptides, RNA, proteins, cell therapies, and hybrids like antibody-drug conjugates that remix and blur boundaries between classes—as modalities. Many researchers are excited about the new capabilities they can bring to bear with different types of molecules.

But during a panel discussion at a Cell Symposium in December called "Chemical Biology in Drugging the Undrugged," researcher Eric Fischer said that this profusion of modalities also presents a challenge for people in the business of drug discovery. For any given disease target, there may be 20 or 30 potential ways to go after it.

Fischer, whose lab at Dana-Farber Cancer Institute uses a variety of approaches to understand the molecular workings of protein degradation in cells, says testing how well every modality might work against a potential drug target would be prohibitive for most teams.

Because of this, Fischer and other experts say that the decision of which modality to use is usually ad hoc and involves balancing multiple factors. Those factors include what researchers know about the target they are aiming for, the

In brief

Many chemists are excited about the capabilities they can bring to bear with new drug modalities. But the profusion of new types of molecules also raises a tough question for early-stage drug discovery: Which modality would be the most effective way to drug a target of interest? Experts say there's no field-wide framework for making that decision. It involves weighing what you know about a drug target, what you need from a medicine, the strengths and weaknesses of each modality, and the expertise of the drug discovery team.

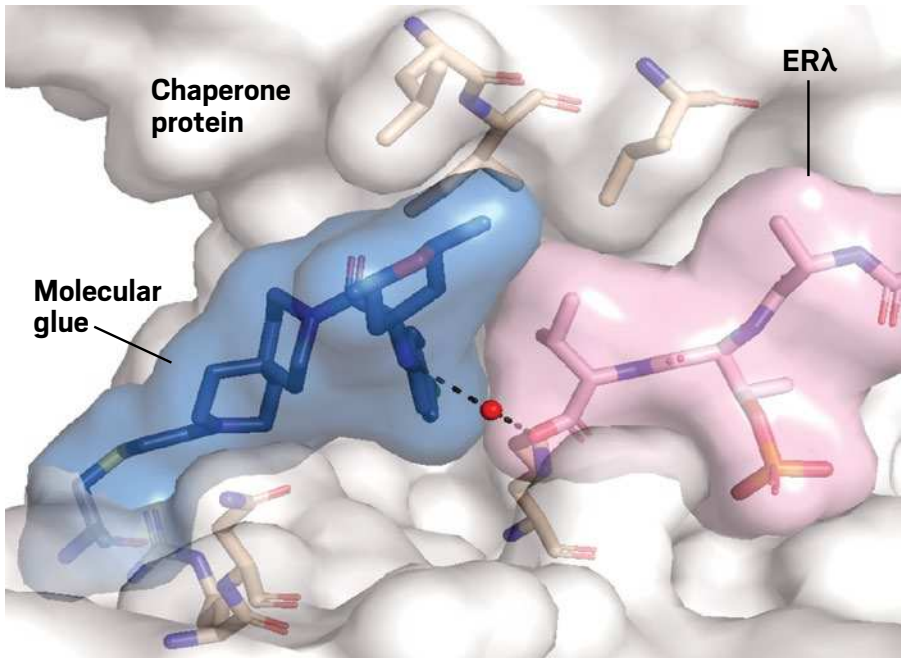
strengths and weaknesses inherent to each drug modality, and the resources and expertise available to the research and development team.

Starting with a hypothesis

Any drug discovery program depends on a therapeutic hypothesis—the idea that an intervention aimed at a particular target will improve disease outcomes. A therapeutic hypothesis can also be refined to include the way that someone will pursue that target. Each proposed mechanism is its own subservient hypothesis, even though they all aim at the same goal of ameliorating disease.

Michelle Arkin, co-organizer of last year's chemical biology symposium, offers an illustration using transcription factors, the proteins that bind to specific DNA sequences in a cell and direct gene expression. "You might well say, 'I want to inhibit the expression of the transcription factor; speed the degradation of this transcription factor; block the transcription factor binding to certain proteins it interacts with; stop its binding to DNA; stop the transcription of some of its downstream targets that I think are bad,' " Arkin says. "These are all hypotheses, and they'll all give you different results."

Arkin has an interest in transcription factors. Researchers in her lab at the University of California, San Francisco, focus on using small molecules to modulate protein-protein interactions. One of the therapeutic hypotheses they are exploring is that removing transcription factors from the nucleus, where the proteins can act on DNA, could help treat some cancers. To do this, the research team is experimenting with glue molecules that boost the interaction between a transcription factor and its chaperone protein.



A molecular glue designed in Michelle Arkin's lab at the University of California, San Francisco, stabilizes the interaction between the transcription factor estrogen receptor α(ERα) and a chaperone protein called 14-3-3. Because 14-3-3 binds to multiple transcription factors, researchers optimized the molecule for greater selectivity for ERα.

COURTESY OF MICHELLE ARKIN

This approach of using induced proximity between two disordered proteins to change the cellular location of one of them is a new twist for molecular glues, which are more often used to drive degradation, Arkin says. That demonstrates how drug designers are expanding not just the number of modalities but how they are used. "We're on the upslope of applying new technologies to our biggest problems," she says.

Formulating a therapeutic hypothesis is easier the more researchers know about the link between a target biomolecule and disease. But this type of understanding usually takes decades of experimentation, and many research perspectives, to uncover.

"There are a lot of layers of information, and they all have different layers of confidence," says David Ochoa, a scientist at the Open Targets initiative, a public-private partnership that generates

Modalities

Here are some of the modalities that medicinal chemists can choose among when they set out to interrupt the function of a disease-related protein or other biomolecule.

Small molecules

- **Competitive inhibitors:** Mimic a substrate and occupy the active site of an enzyme
- **Allosteric modulators:** Bind somewhere other than the active site to influence an enzyme's action
- **Covalent inhibitors:** Covalently bind to a target protein, and change its conformation to block activity
- **PROTACs and other bifunctional molecules:** Bind to two different proteins to drive outcomes such as protein degradation
- **Molecular glues:** Stabilize the interface between two proteins—often aiming to degrade one of the two
- **Splice modifiers:** Change the alternative splicing of an RNA molecule, affecting how the related protein is made

Biologics

- **Monoclonal antibodies:** Bind to targets on the cell surface with high specificity
- **Bispecific antibodies:** Modified to bind to two different targets
- **Antibody-drug conjugates:** Deliver drugs (usually chemotherapies) to cells specified by an antibody
- **Antibody fragments and light-chain antibodies:** Bind to specific epitopes, but are smaller than a monoclonal antibody
- **Peptides:** Usually bind to receptors, mimicking hormones
- **Cyclic peptides:** Act as linear peptides do, but with smaller 3D structure, offering large-molecule specificity but lower degradation
- **CRISPR and related technologies:** Alter DNA encoding disease-related proteins
- **Interfering RNA and antisense oligonucleotides:** Block production of proteins from RNA
- **RNA aptamers:** Bind to target molecules, sometimes used to block or activate signaling
- **mRNA:** Introduces desired proteins to be made
- **RNA editors:** Alter a target RNA by one or a few bases to change protein sequence
- **Cell therapies:** Bind to and kill specific cells, usually recognizing cancer epitopes

Ranking targets

This simplified version of a data visualization system used by Open Targets shows how scientists rank and prioritize the data supporting a list of potential drug targets. In this case, gene 1 would be the strongest target for disease X because strong evidence in several categories supports its druggability.

Disease X	Drug candidate for target in the clinic?	Genetic links to the disease?	Chemical probe available?	Animal model evidence?	Essential gene, or other safety concerns?
Gene 1	●	■	●	▲	●
Gene 2	▲	●	▲	■	■
Gene 3	▲	▲	■	●	●

● Strong evidence ■ Weak evidence ▲ No evidence

therapeutic hypotheses from a variety of sources.

Bioinformatics researchers at Open Targets curate information from human genetics, perturbation studies like CRISPR screens, proteomics and transcriptomics data, and insights mined from the literature to identify and then prioritize potential drug targets. Though insights from data on human genetics can be translated the most easily, other lines of evidence are key to understanding a target's role in disease and whether it can be drugged, Ochoa says.

"The whole drug discovery process is mitigating risk," Ochoa says, citing the 90% failure rate of potential new drugs. "The more you understand about the problem, the less risks you have."

As researchers assess whether a target is druggable, they may also start thinking about which chemistries that biomolecule could be susceptible to. They have more options for hitting a protein on the cell surface than one within the cell, for example, and it's easier to get a drug to the liver than to the brain. Beyond these factors, researchers look to a biomolecule's 3D structures, scan for binding pockets, and examine its chemical reactivity.

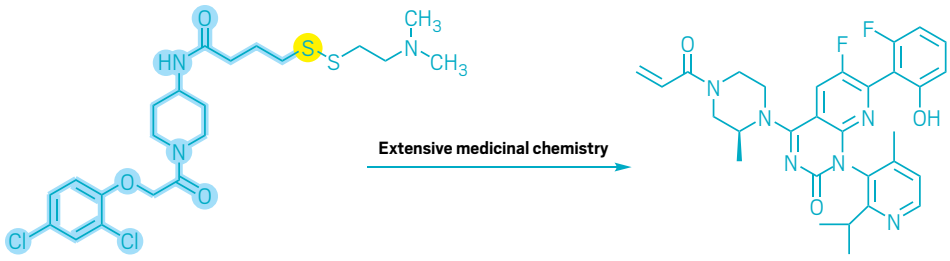
If a researcher is lucky, they may find that someone else has already sketched out some of the molecular vulnerabilities of a target they want to go after. For example, a previous research team might have reacted chemical probes with all the proteins found in the human body to detect the cysteine residues that can be bound by small molecules. If the drug designer's protein of interest appears in that catalog of ligandable cysteines, that indicates it might be a good candidate for a covalent cysteine-binding ligand.

Fischer says that even if the molecules used in these screens are not remotely drug-like, "going from a hit in one of these databases to a chemical probe is much faster than starting from scratch." If someone else has bound to, degraded, or inhibited a given protein, it's a hint that the target is tractable. A hit shows that there is "at least in principle, a molecule out there in the universe that does what I want it to," he says. "At least it de-risks the concept."

According to Arkin, drugs that target mutant KRas to treat cancer are an example of medicines inspired by a screen. KRas and other GTPases are often mutated and overactive in cancer, but they bind so well to their substrate, guanosine triphosphate, that researchers struggled to design an inhibitor that could compete. They found a solution when a screen using molecular fragments that react with cysteines uncovered a cryptic binding site in a cancer-related mutant KRas that has a glycine replaced by a cysteine. A cryptic pocket is one that does not appear in crystal structures but opens occasionally and can accommodate a small molecule. Covalent inhibitor medicines such as sotorasib and adagrasib have since exploited that G12C mutation, thanks to early tethering data that pointed the way to a successful choice of modality.

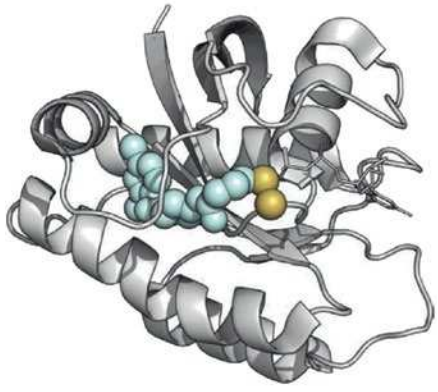
Playing to a modality's strengths

In addition to considering the specific vulnerabilities of a target, researchers may look at the strengths and the drawbacks of any modality they consider matching to it. Because modalities that have never been proved in a clinical context are riskier bets, R&D teams often gravitate toward tested methods, like small-molecule inhibitors and receptor antagonists.



Tethering fragment — Sotorasib

Sotorasib, sold by Amgen under the brand name Lumakras, inhibits a cancer-related mutant KRas protein. The drug was inspired by a fragment screen.



A fragment screen identified a compound (blue and yellow) that binds to a version of the protein KRas that drives cancer.

"If I can develop a straight-up inhibitor—[if] I have high conviction I can achieve a chemical structure that's selective, that has the desired target occupancy, efficacy, and potency," Fischer says, then "I would really question if someone wanted to make a PROTAC." Proteolysis-targeting chimeras (PROTACs) are molecules that are opening up new targets by driving their degradation, but regulators have not yet approved any PROTACs as drugs. Fischer points out that in addition to being newer, PROTACs are large and complex, which can make development more challenging.

ROB BRYSON/C&EN/ADAPTED FROM *NUCLEIC ACIDS RES.* 2025, DOI: 10.1093/NAR/GKAE1128 (TABLE); OSTROM ET AL., *NAT. CHEM. BIOL.* 2024 (COMPOUND)

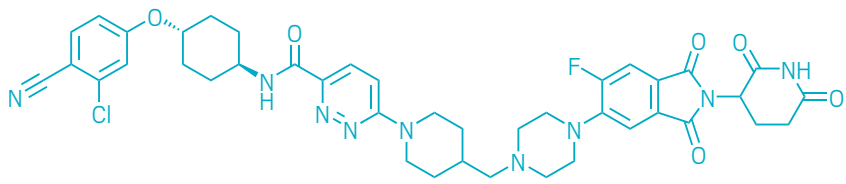
Ochoa says it's not unusual to hear at conferences these days that any protein could be a drug target. "But the level of investment you require to develop different types of modalities is very different." He also sees differences in investors' willingness to take risks in treating various diseases. Newer modalities tend to see their first applications in severe rare diseases, he says, because what constitutes an acceptable risk for patients changes—and the possible price point for a curative therapy may outweigh the risk of failure for a company. While the first choice might be the classic small-molecule inhibitor, researchers opt for an emerging modality if the classics have fallen short or for some reason can't address the target of interest.

According to Arkin, knowledge about the downsides of a modality can be a helpful guide in such cases but may also hold back innovation. After all, the expanding toolkit of options for drug hunters exists because some chemists are willing to make molecules that break rules.

Arkin says it can be worthwhile to make "a molecule that's a thousand Daltons, or has some peptide character, or whatever it is that was [once] verboten," then assess its problems and then try to solve them if needed. She considers that strategy a better use of time than "using a doctrine to keep me from ever making it."

Strengths inherent to your team

Another reason for choosing a particular modality is that it is a team's specialty. Strategic choices can be less about determining which modality best fits a potential target than about choosing a target that matches a company's favorite modality.



ARV-110

ARV-110, a proteolysis-targeting chimera (PROTAC) being developed by Arvinas, targets the androgen receptor protein.

Take, for example, Arvinas, which specializes in protein degradation. Angela Cacace, the firm's chief scientific officer, says there are certain "tenets of PROTACs" that she and her colleagues use to choose likely targets: "Is the target mutated? Can we actually target specifically that mutation in a selective manner? Is the target overexpressed in a certain setting?"

One drug candidate under development at Arvinas is ARV-102, a PROTAC that targets a mitochondrial kinase linked to Parkinson's disease. Two other companies have molecules that target that same kinase, LRRK2. (Denali is working on a small-molecule LRRK2 inhibitor that is currently in Phase 3 trials; Ionis has an antisense oligonucleotide, although its development was discontinued in February.) But Cacace says Arvinas is still confident about its LRRK2 program because a PROTAC can change cellular chemistry in ways a kinase inhibitor can't.

"The advantage of going to a PROTAC modality is that you can also target the other functions of that protein," she explains. For LRRK2, those are its GTPase activity and the fact that it can act as a scaffold for signaling. It's worth the competitive gamble to continue to push this molecule forward, she says, because the different modality may give it a unique edge in the clinic. But Arvinas has terminated programs that did not offer such an edge, Cacace says. "What we do spend some time thinking about preclinically is, 'Is the PROTAC differentiated from other mechanisms?'"

Cacace has worked on many different modalities over her career and says that, for her, modality choice depends on being able to design a drug that suits a specific patient population. Besides its mechanism of action, a molecule's other desirable qualities could include pharmacological properties like half-life or delivery to target tissues.

Arvinas and other biotech companies exist to turn a specific technology into beneficial medicines. Fischer says that, in contrast, "most large pharma organizations can play the entire portfolio of modalities." They can test different approaches to the same molecule, choosing the most effective to advance—or licensing or buying up options developed at a smaller company.

Investors seeking to back promising drug candidates are similarly able to survey the field in search of the most promising opportunity. "We're relatively modality agnostic," says Sandy Madigan, a managing partner at Avalon BioVentures and a serial entrepreneur—although he expresses some concern about the complexity of cell therapies. "Whether it's a small molecule, large molecule, degrader, PROTAC, molecular glue . . . we'd want to see data. What was the data that supported that therapeutic modality for the therapeutic indication that's being supported by the entrepreneurs?"

Madigan says that even with a board of experts considering each potential therapeutic hypothesis, "I'm not sure there's ever a perfect answer when there's multiple modalities in play." Ultimately, the best strategy is to let data guide the choice. As with so much in life, he says, "the science has to dictate." ■

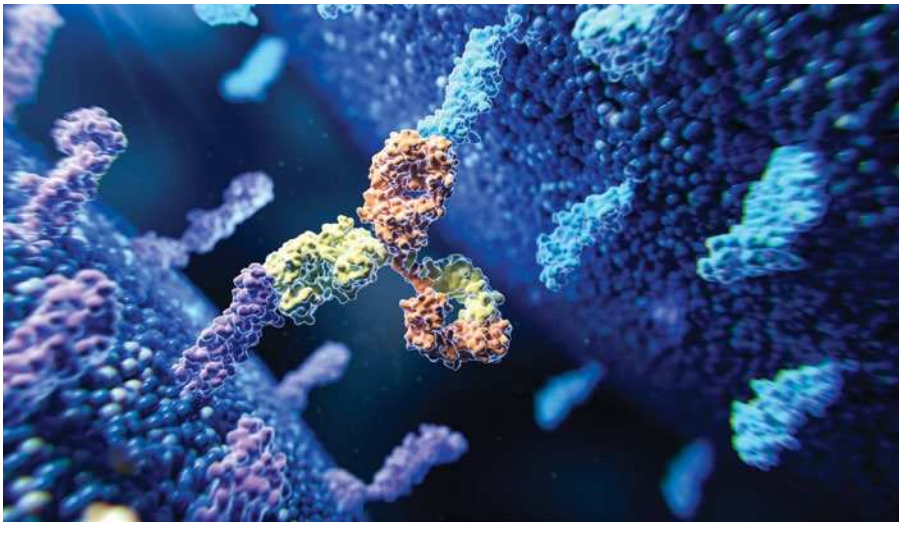


Illustration of a bispecific antibody (yellow and orange) binding to two different membrane proteins (purple and blue)

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