

SUPERANTIBIOTICS TO COMBAT SUPERBACTERIA



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Novel types of drugs can very precisely target harmful proteins in our bodies.

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Modern medicine strives to use novel, highly-specialized drugs that work effectively in small doses with minimal adverse effects. Classic drugs typically fall into the category of inhibitors – small molecules that bind to specific proteins to block their activity. However, finding or designing such drugs is no easy task. Each human cell contains tens of thousands of diverse proteins that are responsible for most of the processes in the human body. Certain proteins form the building blocks of cellular structures, while others are responsible for recognizing or transporting metabolites. Many proteins are enzymes, or nano-scale machines that orchestrate chemical reactions resulting in complex compounds being synthesized, transformed, or broken down. In many diseases, defective proteins may disrupt the balance of cellular processes – for example by causing uncontrolled cell growth, as seen in cancer. Ideally, a drug should selectively deactivate the defective protein, while leaving the others unharmed and sparing the healthy cells. However, since all proteins are encoded using the very same set of 20 amino acids, it is challenging to distinguish one particular protein from the rest.

Thankfully, each protein chain, which may consist of thousands of amino acids, has a distinct sequence, allowing each chain to fold into its characteristic, preprogrammed three-dimensional structure. It contains pockets and surfaces responsible for interaction between proteins and other molecules (substrates, regulators, or other proteins). Proteins are seldom independent actors; they work as part of larger systems and cascades. At the molecular level, therefore, life resembles a continually changing 3D puzzle, with a major role being played by the coordination of various proteins. To effectively remove a defective component from the cell's molecular machinery, a drug molecule must fit snugly into a unique pocket within the target protein's structure, much like a key in a lock.

3D matchmaking

In order to design a drug that acts as a unique key for a specific protein, we need to know what that pro-

tein looks like in 3D. Proteins typically range in size from several to tens of nanometers, so they can be observed using the techniques of X-ray crystallography or cryogenic electron microscopy (the latest technology). Frozen protein molecules can be visualized using an electron microscope, and the resultant images are used to computer-generate a 3D model of the molecule. This process can also be applied to protein complexes, with a drug molecule bound to the protein's pocket. Through this method, we can refine the drug to perfectly fit the pocket of the protein responsible for enzymatic activity and outcompete its natural substrate, thus blocking the protein's function as an enzyme. Another crucial aspect of drug development involves ensuring that the drug in question does not block the activity of *other* essential proteins, resulting in its toxicity.

One well-known historical example is that of thalidomide, a drug that was given to pregnant women back in the late 1950s to ease anxiety and morning sickness. Unfortunately, it turned out that thalidomide in

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the human body exists as a racemate, or as a mixture of two mirror-image forms called the (*R*)-enantiomer and the (*S*)-enantiomer. While the (*R*)-enantiomer delivers the desired effect, the (*S*)-enantiomer binds to several key proteins in fetal development, thus acting as a teratogen. Worldwide, thalidomide administration led to an estimated 10,000 children being born with limb defects, and 40% of them did not survive at birth. The drug was taken off the market in 1961. Several years later, thalidomide and similar immunomodulatory imide drugs (IMiD) were found to inhibit angiogenesis (the growth of new blood vessels) and reduce inflammation. IMiDs have made a comeback in the therapy of certain cancers, skin disorders, and infections, but they are not used in patients who are trying to conceive.



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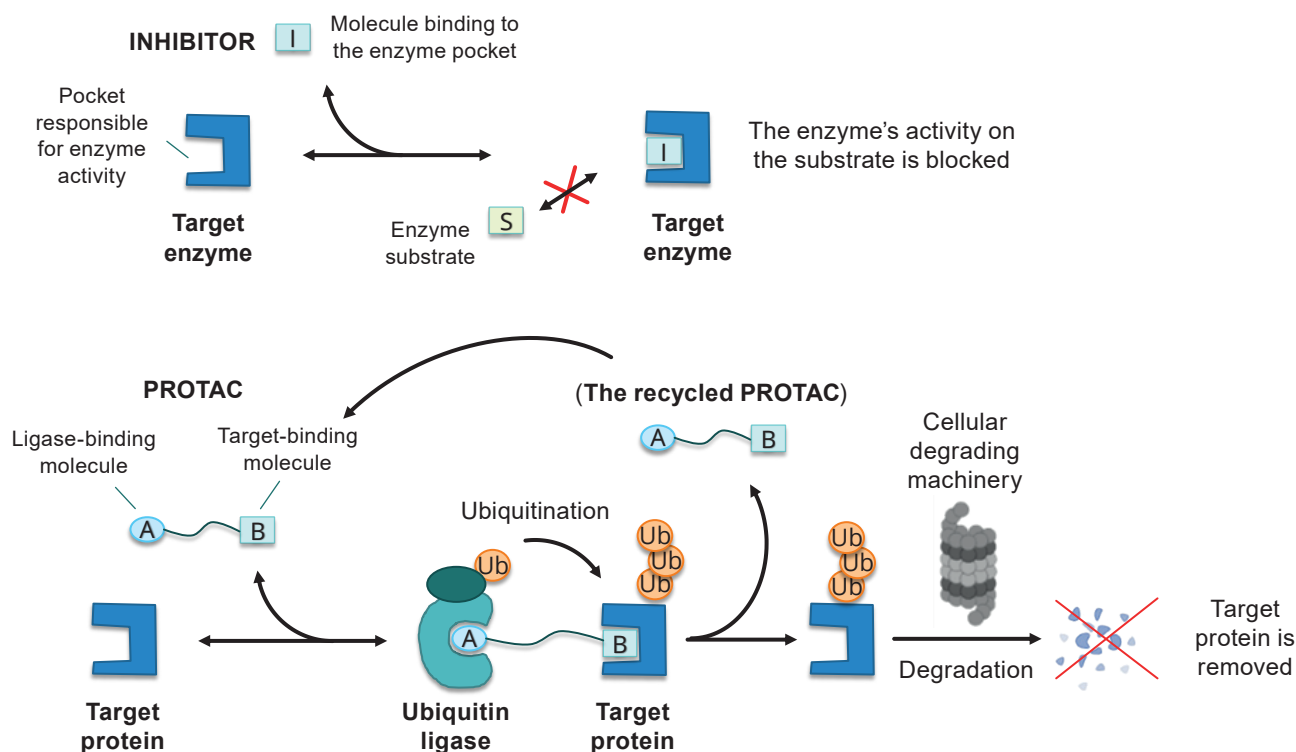
Degraders

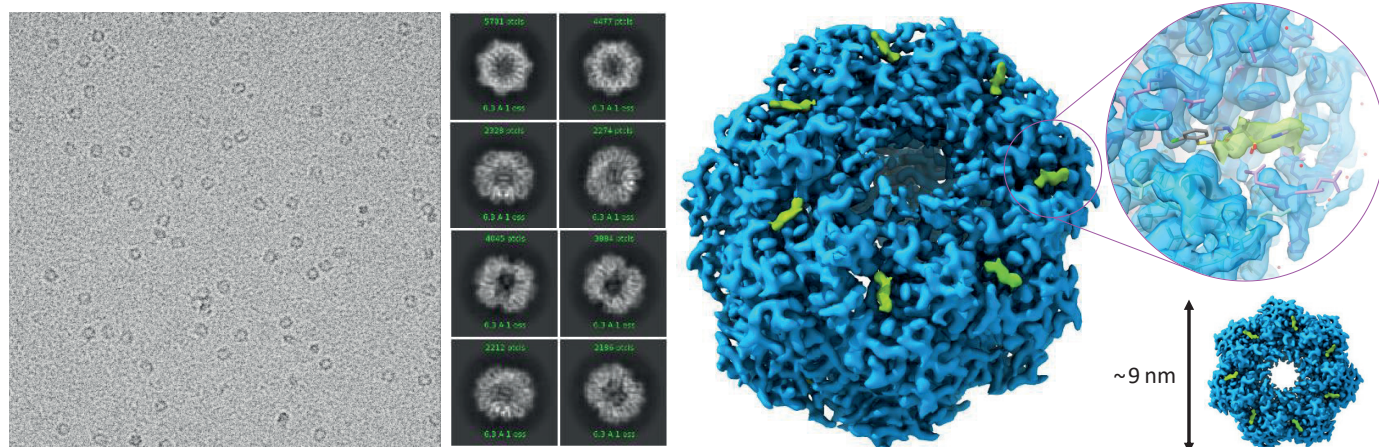
Adverse effects can also be minimized by administering a drug only where it is needed, either by delivering it to the affected tissue or by targeting a specific protein. That is why the majority of drugs currently in the developmental stage have a two-module structure: one module pinpoints the target, the other is responsible for the drug's activity. Examples of such novel drugs include bipartite molecules called proteolysis-targeting chimeras (PROTACs), which are capable of removing specific proteins inside cells. One part binds to the target protein, the other binds to an enzyme found in human cells called ubiquitin ligase. The ligase attaches molecules of ubiquitin to selected proteins, usually ones that are abnormal or no longer needed by the cell, thus tagging them for removal through cleavage (degradation). PROTACs are therefore part of a fascinating novel class of drugs known as degraders – molecules that cause the proteolytic degradation of target proteins.

This represents a completely new mechanism of action, where the cleaved protein is permanently eliminated from the cell, putting a conclusive stop to its activity. In contrast, a traditional inhibitor must stay bound to the pocket in the protein for its entire lifetime to block its activity. After ubiquitin is ligated to one molecule of the target protein, the PROTAC molecule can release it and swiftly move on to attacking the next one. Consequently, whereas an inhibitor needs to “keep one protein molecule imprisoned” at

all times, a PROTAC can keep handing down “death sentences” to successive molecules, making it significantly more effective even in small doses. Degraders work with such speed and effectiveness that, in certain instances, they can impede the emergence of mutations that lead to drug resistance in cancer cells or in the viral genome. Moreover, by eliminating the entire protein, degraders also get rid of its other non-enzymatic functions, such as interactions with other proteins, which frequently play a role in the progression of diseases.

While the PROTAC molecule still needs to fit spatially into a specific pocket of the target protein, this pocket does not necessarily have to be responsible for enzymatic activity. Therefore, the potential targets of these drugs are not limited to enzymes. In 2019, the first PROTAC drugs to undergo clinical trials were designed to target steroid hormone receptors – proteins which had been earlier considered “undruggable” due to the absence of enzymatic activity. However, PROTAC ARV-110 and PROTAC ARV-471 (targeting the androgen receptor in prostate cancer and the estrogen receptor in breast cancer, respectively) have already delivered promising outcomes and are now in the next stage of clinical trials. Interestingly, the ligase-binding module forming these and many other PROTACs is that of an IMiD. Studies of the proteins targeted by thalidomide revealed that it binds to a relatively commonly found ubiquitin ligase called cereblon. Consequently, the use of a well-characterized IMiD molecule to recruit cereblon to the target





protein has emerged as a crucial factor in the development of the next generation of pharmaceuticals. Thanks to the advantages offered by degraders, there has been a substantial surge in the development of drugs relying on targeted protein degradation (TPD) over the past decade. Most of the leading pharmaceutical companies are now involved in developing degraders, and new companies have been established with the same goal in mind, including in Poland. Currently, clinical trials are underway for more than 20 degraders, usually PROTACs or molecular glues. The latter, which are slightly smaller than PROTAC molecules, “glue” the target protein to the ubiquitin ligase. These drugs can target proteins associated not only with cancer, but also with autoimmune and neurodegenerative diseases. Soon, therefore, we will enjoy access to novel therapies harnessing highly specialized molecular “hit-men” capable of targeting specific proteins.

Drug-resistant infections

Research and development in the field of degraders also extends to the treatment of viral and bacterial infections. Considering their reduced potential for inducing drug resistance, degraders offer a promising approach to treat antibiotic-resistant infections. The continuous increase in antimicrobial resistance has resulted in a worldwide health crisis, as highlighted in the largest study of this kind published in November 2022 in *The Lancet*. Its findings show that bacterial infections were the second most common cause of death worldwide in 2019, accounting for one in every seven to eight deaths. The deadliest pathogens were the Gram-positive bacterium *Staphylococcus aureus*, which was the leading bacterial cause of death in 135 countries with over a million deaths in 2019, and the Gram-negative bacterium *Escherichia coli*, which was the leading bacterial cause of death in 37 countries, especially in Central and Eastern Europe and Central

Asia. Additionally, from 2019 onwards, the COVID-19 pandemic has likely contributed to a surge in the use of antibiotics and speeded up the spread of antibiotic resistance. According to a 2021 report by the World Health Organization (WHO), not one of the 43 antibiotics currently in development targeted multidrug-resistant Gram-negative bacteria, and only two out of the 11 antibiotics approved since 2017 belonged to a novel class of these drugs. These figures point to an urgent need for new antibiotics, especially those targeting Gram-negative pathogens and using a new mechanism of action. This is where PROTACs could come into play, given their groundbreaking impact on cancer drug development.

Unfortunately, however, substantial adjustments are needed to make this technology effective against bacteria, as such PROTACs must employ a different mechanism for targeting proteins for degradation – no ubiquitin ligases are found in bacteria. Recently, we witnessed the first and, to date, the only known example of a PROTAC molecule effectively combating *Mycobacterium tuberculosis*. The BacPROTAC prototype is based on cyclomarin, a molecule that can effectively recruit the bacterial protein-degradation machinery (just as IMiDs do with the cereblon ligase) – unfortunately, only the one present in mycobacteria. To be effective against other bacteria, future PROTACs would have to incorporate a different molecule, one that has yet to be developed. Bacterial degraders are being developed in several laboratories around the world, including the Structural Biology Group at the University of Warsaw. The research utilizes cryogenic electron microscopy to help find and design molecules that will bring the target bacterial protein straight to the protein-degrading machinery that is naturally found inside the bacterial cells. Hopefully, a few years from now, we will be testing the first degraders effective against the dangerous Gram-negative pathogens. As for new superantibiotics, it seems we will have to keep waiting a little longer. ■

Further reading:

Izert M.A., Klimecka M.M., Górna M.W., Applications of Bacterial Degrons and Degraders – Toward Targeted Protein Degradation in Bacteria, <https://pubs.frontiersin.org/articles/10.3389/fmolb.2021.669762/full>

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