Beyond Boundaries: The Future of Medical Therapeutics in Unraveling Undruggable Proteins

Interview with Professor Daniel Nomura

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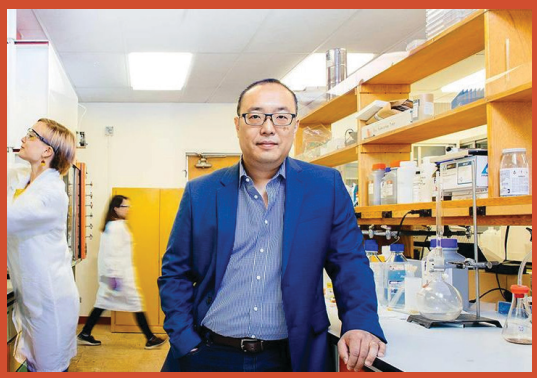

Dr. Daniel K. Nomura is a Professor of Chemical Biology within the College of Chemistry at the University of California, Berkeley. Dr. Nomura has a long history with Cal as he earned both his B.A. in Molecular and Cell Biology and Ph.D. in Molecular Toxicology from here. Currently, 90% of the human proteome is considered "undruggable." The Nomura Research Group is focused on using chemoproteomic platforms to transform how we approach therapeutic development to tackle this pressing issue. During our interview, we discussed what causes so many proteins to not be therapeutically accessible. Additionally, we gained insight into the different mechanisms that the Nomura Research Group are utilizing to increase druggability. Lastly, we examine the revolutionary impacts that Dr. Nomura's research can have on the future of pharmaceutical development.

BSJ: How did you first become interested in the expansion of molecular therapeutics and tackling the problem of undruggable proteins?

DN: One of the biggest bottlenecks that we face in modern drug discovery is that over 90% of human proteins are still considered undruggable. That means 90% of human proteins are unable to bind to conventional molecules because these proteins do not possess a well-defined binding pocket that a small molecule can bind to affect protein function. So, it is very likely that if you discover a new disease target, you are not going to be able to develop a drug against it using any current therapeutic approach. These current approaches include small molecules, antibodies, gene therapies, etc. This is a major problem because pharmaceutical and biotechnology companies have drained the top 10%, the low hanging fruit of drug targets. Currently, there are so many known critical drivers of disease that are part of the 90% that we just cannot therapeutically access. The Nomura Lab has been advancing an approach called "covalent chemoproteomics" to tackle this problem of the undruggable human proteome. Our premise is that if we can identify these binding pockets—what we call "ligandable hotspots"—across all proteins in the proteome, then we could begin developing small molecule binders against any protein desired. This would, in theory, allow us to target 100% of the human proteome.

BSJ: You mentioned that your lab is developing covalent chemoproteomics. Can you expand on this approach to tackling the undruggable protein crisis and the mechanisms of this method?

DN: Covalent chemoproteomics utilizes an electrophilic reactive probe that can covalently modify nucleophilic amino acids within various proteins. We can then couple these probes with proteomic approaches to be able to map proteome-wide reactivity and identify potential binding pockets across the entirety of the proteome. After using this kind of strategy for over 10 years, we have identified over 100,000 ligandable sites across nearly every human protein.

However, some proteins may not have functional pockets, like active sites in enzymes. What you end up finding might just be binders that do not do anything to the target protein's function; in that case, you need to couple other types of therapeutic modalities to the binder to force functionality and therapeutically exploit such proteins.

One major approach that was actually developed by a pioneer in the chemical biology field, Craig Cruz at Yale University, is known as "targeted protein degradation." This approach uses small dumbbell-shaped molecules called proteolysis-targeting chimeras (PROTACs). PROTACs have been used to essentially eliminate and destroy disease-causing proteins from cells.

The use of PROTACs has been a huge paradigm shift in drug discovery that has exploded in the last five years and is now being used across nearly every pharmaceutical company, resulting in hundreds of smaller biotechnology companies to utilize targeted protein degradation. Since then, we have used our technologies to really expand the scope of targeted protein degradation technologies by developing new recruiters against the over 600 E3 ligases that exist, enabling us to harness E3 ligases for targeted protein degradation applications. Now that we have the capabilities to develop ligands, we can develop small-molecule binders against any protein target—for example, deubiquitinase recruiters for targeted stabilization of

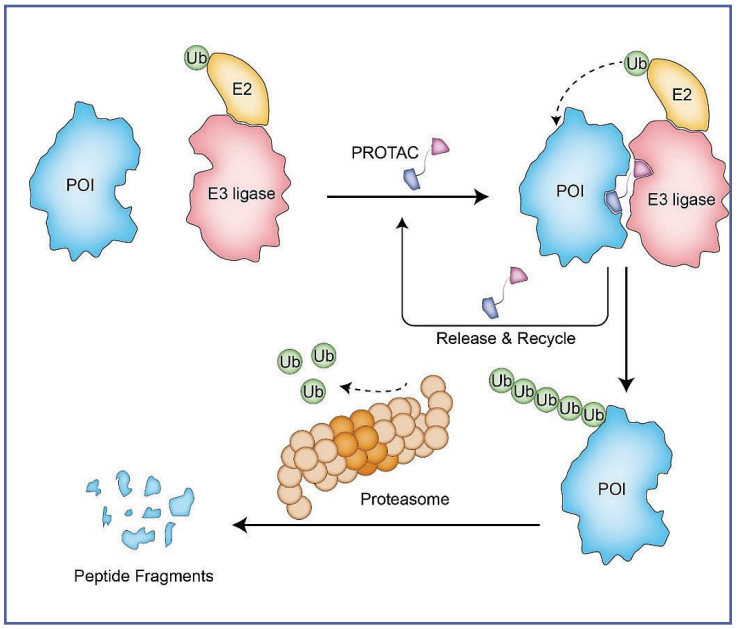

Figure 1: Degradation via PROTACS. This diagram illustrates the mechanism of degradation of a POI (Protein of Interest) by a Proteolysis-targeting chimera (PROTAC). In this scenario, the PROTAC promotes interaction between the POI and E3 ligase, which subsequently ubiquitinates the POI. The ubiquitinated POI is then degraded by the proteasome.

proteins, deacetylase recruiters for targeted deacetylation of proteins, and so on. This technology really adds to the collection of tools capable of manipulating protein function with surgical precision, which has immense potential for therapeutic benefit.

BSJ: You mentioned that the current goal of your lab is to increase druggability of the current 90% of human proteins that remain undruggable. How were you first introduced to the issue of the undruggable proteome, and how has your lab's objective evolved since it was founded in 2011?

DN: I remember learning about the problem of undruggable proteins in 1999 when I was pursuing my undergraduate degree in Molecular and Cell Biology here at UC Berkeley. The issue was not as heavily pursued back then, but I could tell that the problem would be a huge bottleneck in the future. Even as a freshman in college, I was reading about all these disease-causing proteins in cancer and neurodegenerative diseases that we have known for decades are major drivers of those diseases, yet we could not therapeutically manipulate them. If we could simply drug those proteins, either with a small molecule, antibody, or any other therapeutic, then we could potentially develop revolutionary cures to currently uncured diseases. But at the time we could not because these proteins were deemed undruggable. That notion seemed extremely limiting to me.

I remember being inspired by research conducted by Benjamin Cravatt at the Scripps Research Institute, who ended up being my postdoc mentor. His laboratory developed chemical proteomic approaches to assess the activities of large numbers of enzymes that were still relatively uncharacterized. He used these technologies to develop small molecule inhibitors against those enzymes. Since then, this approach for tackling the undruggable proteome has become incredibly popular within the pharmaceutical industry. Also, we have had major, long-standing collaborations with companies like Novartis to leverage chemoproteomic technologies to tackle the undruggable proteome. I have also begun start-up companies like Frontier Medicines in the Bay Area and also recently, Vicinitas Therapeutics.

BSJ: What is functionally different between proteins that can be inhibited through the use of drugs and those that are undruggable?

DN: Druggable proteins are proteins that oftentimes havewell-defined binding pockets. These include proteins like enzymes, which catalyze a reaction of a substrate into a product through very well-defined active sites. For enzymes, you can develop a small molecule that fits into that active site, like a key fitting into a lock, to inhibit the function of that enzyme.

However, the majority of proteins do not have obvious active sites. For example, things like protein complexes are simply proteins meant to bring together various other proteins to coordinate downstream biological action. It is really difficult to understand where the binding pocket may be within these more shallow, smooth interfaces between protein-protein interactions.

Another huge class of undruggable proteins are intrinsically disordered proteins; these are not made up of your typical alpha helix or beta sheets, but are instead more like giant spaghetti-like and poorly folded messes. These include important transcription factors that drive the majority of human cancer, like MYC, which is a massively important oncogenic transcription factor amplified across over 80% of human cancers; it is thought to be a major driver of these cancers. MYC does this by binding to a particular DNA consensus sequence across hundreds of cell-proliferation genes to jack up the proliferation of cancer cells. Other kinds of undruggable protein targets include tumor suppressors such as p53 that are oftentimes inactivated by mutation across a large proportion of human cancers to prevent cancer cell apoptosis. These mutations often cause the destabilization and unfolding of these gateway tumor suppressors so they can no longer function. Imagine trying to develop a drug where your molecule has to be able to restabilize, refold, and reactivate this now unfolded and inactivated tumor suppressor. That is a really hard thing to do, and that is the type of challenge my laboratory is trying to tackle.

BSJ: Can you briefly explain the process of designing these glue degraders? What methodology does the Nomura Research Group implement to study the effectiveness of your synthesized degraders?

DN: There are currently two major ways to degrade proteins: PROTACs and molecular glue degraders. PROTACs are bifunctional compounds. They consist of a small molecule, which binds to the target you want to degrade, linked via a linker to another small molecule, which recruits an E3 ubiquitin ligase. These PROTACs can induce the proximity of the protein you want to degrade with an E3 ubiquitin ligase, which tags the protein with ubiquitin chains. This then signals the cell to destroy the protein using the cellular trashcan called the proteasome. You can potentially apply this strategy to any intracellular protein by linking a small molecule that binds to the

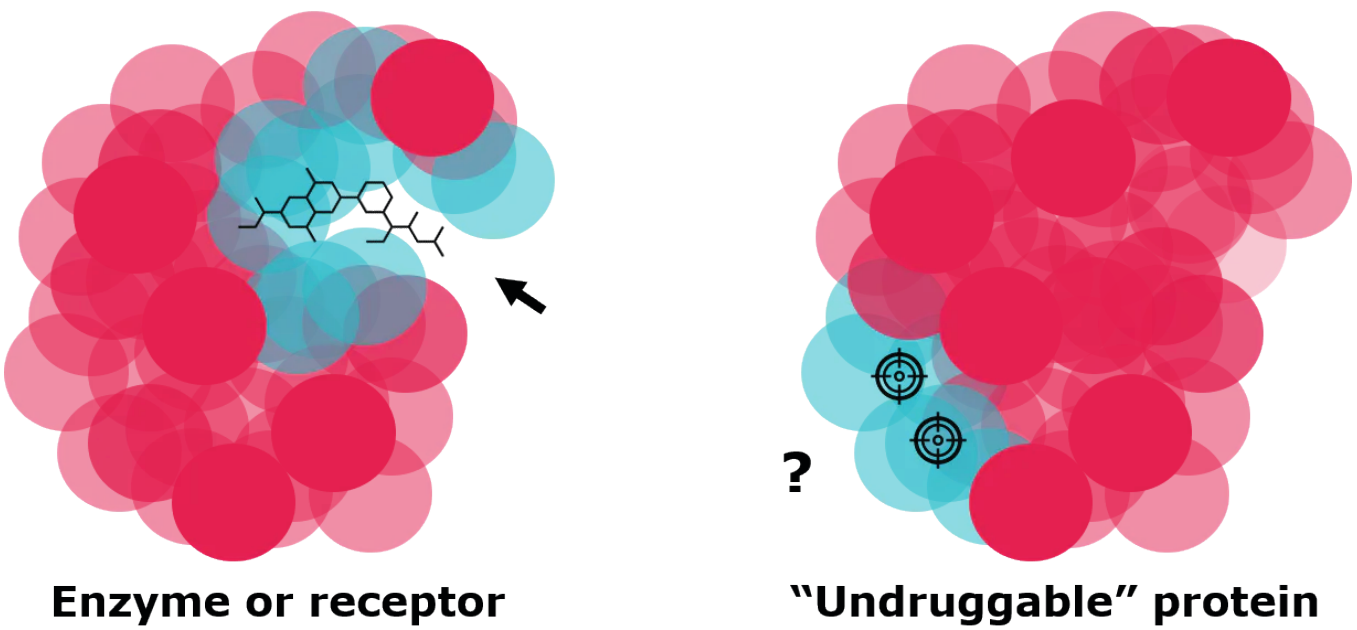

Figure 2: "Undruggable" Protein Site. The enzyme/receptor on the left represents a "druggable" protein due to its well-defined binding site, while the right represents a "undruggable" protein due to its indiscernible targeting points.

protein of interest to an E3 ligase recruiter. Thus, PROTACs are more modular in their design.

Another type of degrader are molecular glue degraders. As opposed to PROTACs, which are bifunctional, dumbbell-shaped molecules, molecular glue degraders are monovalent molecules that physically glue together two protein interfaces between an E3 ligase and a target protein. This allows for the target protein to be tagged with ubiquitin and subsequently degraded. As you might imagine, this strategy is not as modular as PROTACs; almost every molecular glue degrader has been fortuitously discovered. In fact, a major class of molecular glue degraders known as Immunomodulatory Drugs or IMiDs was based on a drug that was developed in the 1950s called thalidomide. . Thalidomide was originally developed as a sedative but eventually used to combat morning sickness in pregnant women; however, it was later found to cause birth defects. Later, thalidomide analogs such as pomalidomide were developed as blockbuster cancer drugs. It was only decades after the discovery of thalidomide that it was found that thalidomide acted as a molecular glue degrader; it bound to an E3 ligase called cereblon to create a unique protein interface that could recruit "neo-substrate" proteins that cereblon does not otherwise interact. This interaction resulted in cereblon ubiquitinating these proteins, ultimately leading to their degradation. One of the proteins that was degraded by thalidomide was SALL4, whose degradation led to birth defects, and another was the cancer target Ikaros, which is also degraded by anti-cancer IMiDs. In fact, IMiDs are currently exploited as major blockbuster anti-cancer drugs in the clinic and are still giving rise to new cancer drugs. These discoveries of anti-cancer effects were all made decades after this sedative drug thalidomide was developed in the 1950s and found to cause birth defects. Subsequent new molecular glue degraders have also mostly been accidentally discovered or found through phenotypic screens. Thus far, there have not been rational design strategies for discovering molecular glue degraders.

Now, the advantage of molecular glue degraders in comparison to PROTACs is that they are much smaller in molecular weight and much more drug-like. The issue with the larger molecular weight of PROTACs is that it is more challenging to make these molecules into orally bioavailable drugs that can be taken as pills. Furthermore, many undruggable proteins do not necessarily have deep enough binding pockets to even bind to a synthesized ligand. A molecular glue is able to access the shallow interfaces between two proteins and cooperatively glue them together. This allows for therapeutic access into difficult targets, such as transcription factors.

BSJ: How has the Nomura Research Group attempted to develop novel molecular glue degraders?

DN: The approach that we took was to pick well-established drugs where we know exactly how they bind to their protein targets. We started with a CDK4 inhibitor for breast cancer called ribociclib. Then, we began our work appending various chemical handles onto ribociclib to look for chemical handles that would induce the degradation of CDK4 rather than just inhibiting it. My graduate student, Ethan Toriki, and a postdoc in our lab, James Papazimas, were able to find a covalent chemical handle that, when appended onto a variety of protein-targeting small molecules, converted these molecules into molecular glue degraders of their targets. We figured out that the mechanism through which this was working was through covalently targeting a cysteine on this E3 ligase, called RNF126. Our study turns out to be the one of the first kinds of rational chemical design strategies that can be used to convert any non-degradative protein-targeting ligand and convert it into a molecular glue degrader.

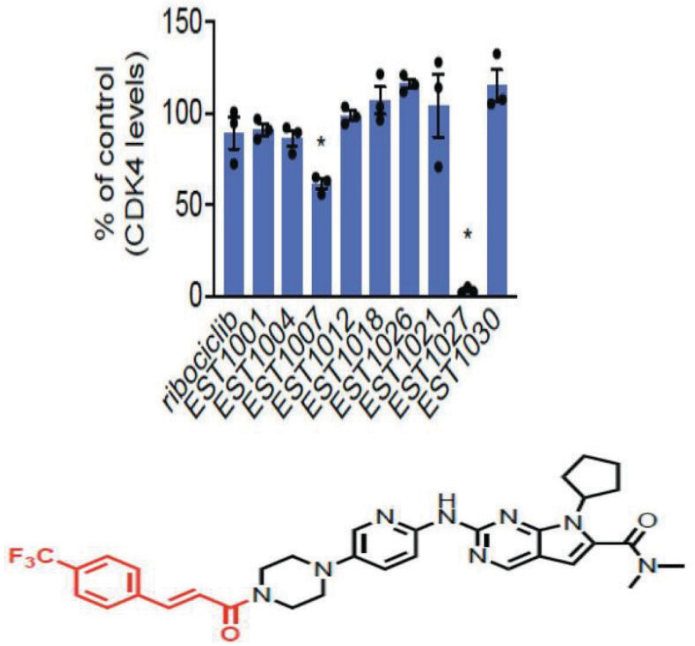

Figure 3: Efficacy of Molecular Glue Degraders. The table shows the analysis of multiple analogs to the common breast cancer drug, ribociclib, and their efficacy in degrading CDK4. The schematic illustrates the chemical structure of EST1027, the most successful analog which used proteasome-mediated degradation.

I see this as just the tip of the iceberg of other chemical handles that will likely be able to convert molecules into molecular glue degraders of their targets.

BSJ: Currently, the only FDA-approved glue degraders are thalidomide and its analogs, Revlimid and Pomalyst. In your opinion, when and in what capacity do you believe designed glue degraders will be available for broad public use?

DN: Currently, there are dozens of targeted protein degradation companies either developing PROTACS or developing glue degraders, with many companies already in clinical trials. As an example, Michael Rapé, who is now the head of UCB's Division of Molecular Therapeutics in the MCB department, is the founder of a company called Nurix. He is a world-class E3 ubiquitin ligase expert, and Nurix's lead program is on a PROTAC against a kinase called Bruton's Tyrosine Kinase (BTK). This PROTAC is able to take out BTK, an oncogenic kinase in cancer cells, and is already showing clinical efficacy in patients. Arvinas, which was started by the originator of PROTACs, Craig Crews, also has estrogen receptor and androgen receptor PROTACs that are in phase two clinical trials and have already shown efficacy in human patients.

There are also many molecular glue degraders that are in clinical development, including thalidomide analogues that lead to the degradation of proteins beyond the original targets of Revlimid degraders. Another interesting case of a molecular glue degrader is the breast cancer drug fulvestrant, sold by Genentech that was found to be a monovalent glue-type degrader of the estrogen receptor. With such examples, it turns out that these glue degraders are more common than we thought, and we just were not properly looking for them. However, now that we are looking for these molecular glue degraders, it is something that I think is going to pop up more and more. Our recent proof of concept study for rational design of molecular glue degraders, done in collaboration with Novartis, is still in its early stages, and we are further optimizing these initial strategies so they can be deployed in drug discovery applications.

BSJ: Over your time developing molecular glue degraders, what has been one of the most challenging hurdles that your research group has faced?

DN: I would say that our biggest challenge has been in opening up our minds to be as creative as possible toward imaging new therapeutic paradigms. Molecular glue degraders are just one type of therapeutic modality that we have been pursuing, but there are so many other types beyond degradation. As I mentioned, not every protein benefits from destroying it. One of the first new therapeutic modalities to come out of our lab is the deubiquitinase-targeting chimeras or the DUBTAC platform. DUBTACs, which are the opposite of PROTACs, are deubiquitinase recruiters that stabilize aberrantly ubiquitinated and degraded proteins to prevent disease. Using this DUBTAC approach, we were able to stabilize, increase the levels, and restore the function of mutant CFTR protein that drives cystic fibrosis because of its aberrant degradation and consequent loss. We were also able to apply this DUBTAC approach to stabilize and increase the levels of tumor suppressors in cancer that are otherwise aberrantly degraded and lost to fuel cancer cell proliferation.

Now that we have shown proof of concept of this targeted protein stabilization approach, we can potentially apply DUBTACs to the hundreds of genetic disorders caused by destabilization and degradation of a particular protein as well as to cancers fueled by the active elimination of tumor suppressors. These types of proteins were all previously inaccessible, but now we can potentially therapeutically access these targets with our new therapeutic modality. DUBTACs are just the tip of the iceberg of possible new therapeutic modalities. We are pushing very hard to show proof of concept of all these new therapeutic approaches, beyond degradation, that manipulate target protein function through forced proximity achieved by small molecules too. Once we demonstrate feasibility of these new therapeutic approaches, we hope to then translate these approaches into drugs through our pharmaceutical company collaborations or through spinning out new start-up companies.

BSJ: How do you envision further research into druggability using the chemoproteomic platforms you discussed to proceed? Specifically, how might these developments serve to help those experiencing illnesses and diseases that, in the past, have been seen as incurable?

DN: Over the past few decades, there has been a confluence and maturation of many different chemical biology approaches that are now all coming together to be applied for drug discovery applications. This includes advancements in chemoproteomic technologies that are coming together with PROTACs, targeted degradation, and molecular glues. We now have all the tools in place to be able to unmask and target the undruggable proteome and, in turn, develop an arsenal of therapeutic technologies, whether they be

PROTACs, glues, DUBTACs, or beyond, to really make drug discovery more modular. The goal of this modularity is to eventually be able to mix and match ligands and therapeutic modalities, along with different approaches, to get to drugs that effectively target any previously undruggable protein. These chemoproteomic platforms, coupled with the incredibly powerful therapeutic modalities arising from Jennifer Doudna's CRISPR/Cas9 discoveries (from the myriad of gene editing-based therapeutic platforms to immunooncology approaches to cell therapies), make me excited. I look forward to seeing what the next five to ten years will bring to transform the currently mostly undruggable proteome and genome to 100% druggable.

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