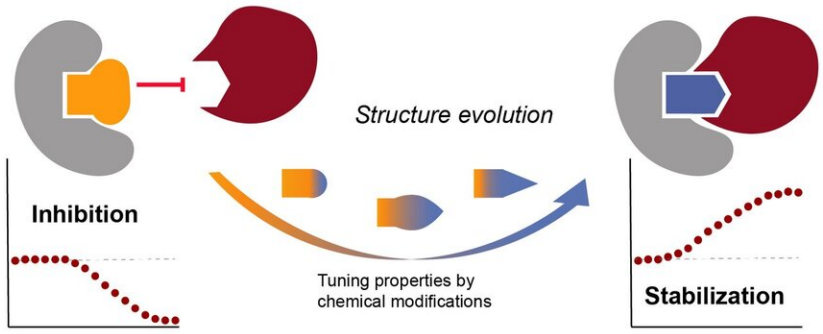
The search for molecular glue in targeted disease control

August 14 2020, by Hilde De Laat



On the left is the signal molecule that acts as an inhibitor, preventing other proteins from binding. On the right, the signal molecule that acts as a stabilizer, strengthening the binding of the protein complex. The researchers were able to convert the signal molecule in such a way that it changed from inhibitor to stabilizer. Credit: Visser and Sijbesma.

In cells, there are proteins that do the work and proteins that regulate them. The latter inhibit or enhance activity, depending on the need. However, in many diseases—for example cancer—there is so much overactivity in the cell that the regulator proteins can no longer keep up with it. Researchers at Eindhoven University of Technology therefore developed a kind of molecular "glue" in 2019 that helps the regulator to

inhibit faster. Now this technique has been further developed, and the researchers have found a completely unexpected way to look for new protein-gluing molecules. This offers prospects for the development of drugs for cancer, diabetes or cystic fibrosis, for example. They published their results last week in *Nature Communications*.

Overactive proteins are the cause of many diseases in our body. Doctors usually combat these directly by sending an inhibitory drug directed at the overactive protein. But that does not work for all diseases: the medicine sometimes inhibits not only the diseased proteins, but also the healthy ones. Researchers have therefore continued to look for other ways to inhibit overactive proteins, while the healthy proteins remain undisturbed.

Regulator proteins provide a logical route, because their natural function is to inhibit overactivity in the cell. If you can support those regulator proteins in their inhibitory power, like a volume knob that you turn up, then you have found a much more natural and effective way to suppress overactive proteins. TU/e-researchers Eline Sijbesma and Emira Visser of the Institute for Complex Molecular Systems have started working on this question.

Key and lock

The inhibition or amplification of activities in our cells takes place because a regulator protein binds to the process protein in the cell that needs regulation, together forming a complex. Sijbesma: "The shape of the two proteins and the place where they bind to each other creates a kind of cavity between the two proteins. It is precisely these cavities that are interesting for the targeted drug delivery. Such cavities are very specific; the binding sites available in a cavity are unique for each complex of two proteins. For us, these are the chemical handles we target with a new drug."

This is a familiar mechanism in the cell; cavities in protein complexes are bound by small signal molecules in the cell. They act as inhibitors, which ensure that no other protein can bind, or as stabilizers, which make the complex much more stable—this is what the researchers also want to do. Such a stabilizer acts as a kind of molecular glue that glues the two proteins together so that they can communicate better with each other, and as a result of which the regulator protein gets much more grip on inhibiting the process protein. An wayward disease protein can thus be powerfully corrected in a natural way.

Visser explains: "We want to make new stabilizers, but make them so unique that they only fit on one complex. So the crux is to find a particle that fits exactly in that specific cavity, like a key in a lock. Once you know how to do that, you can search for a suitable cavity per disease, and develop a very specific molecule for it."

Stabilizer versus inhibitor

In 2019 the researchers published a kind of glue molecule, which indeed fits exactly in the cavity of such a protein complex. As a result, the bond to the regulator protein actually became 40 times stronger than without the glue. Sijbesma: "Now that we had demonstrated that our hypothesis worked, we could look for new ways to find chemical starting points for glue molecules. We started that search with a set of virtual molecules, and then began tinkering to make one the exact fit for the complex we had in mind."

By coincidence, however, the researchers then discovered that one of the most promising molecules is a familiar inhibitor, which prevents the normal binding of proteins to the regulator protein. And that would mean that you can choose from a much larger pool of possible molecules. Visser: "We hadn't thought of that before, because you don't want the properties of the inhibitors, namely that no other protein is able to bind

anymore."

However, after many modifications to the inhibitor molecule, the researchers turned out to be able to convert those undesirable properties to desired ones. "We convert an inhibitor into a stabilizer, as it were," explains Sijbesma. Sijbesma and Visser feared for a while that this new molecule would not be specific enough, and would therefore have an effect on several protein complexes, but this turned out not to be the case after extensive experimental work. The researchers discovered thus an entirely new pool of molecules that can be used as a starting point for their molecular glue.

Diabetes, cystic fibrosis and cancer

The next step is to test the new molecules in the cell. Eventually, the researchers hope to be able to set up a platform on which they can apply the same trick they will soon have at their fingertips to many different diseases in the future. They are thinking, for example, of metabolic diseases such as diabetes, neurodegenerative diseases, cystic fibrosis and various types of cancer. Sijbesma concludes: "These are all diseases that are caused by wayward proteins, and which are also so complex that direct inhibition is often not selective enough."

These results were published on 7 August in the journal *Nature Communications*, titled "Structure-based evolution of a promiscuous inhibitor to a selective stabilizer of protein-protein interactions."

More information: Eline Sijbesma et al. Structure-based evolution of a promiscuous inhibitor to a selective stabilizer of protein–protein interactions, *Nature Communications* (2020). DOI: 10.1038/s41467-020-17741-0

Provided by Eindhoven University of Technology