Designer peptoids – improving on Mother Nature

Interview

Ron Zuckermann, Facility Director of the Biological Nanostructures Facility at The Molecular Foundry, Lawrence Berkeley National Laboratory, http://foundry.lbl.gov, has long been fascinated by the way nature builds precise 3-dimensional nanostructures by the folding of linear polymer chains. He and his team are mimicking this to create novel and robust nano-scale bio-inspired polymers called 'peptoids'.

Peptoids bridge the gap between the intricate architectures of natural proteins and the robustness and chemical diversity of synthetic polymers. In short, they offer a way of improving on nature in specific application areas. There are, for example, lipid-like peptoids that mimic natural membranes and can self-assemble into a sheet that is thinner and more stable than a soap bubble. The sheet can selectively bind to biological targets with surface loops that resemble something like a sheet of molecular Velcro. They can withstand submersion in a range of liquids and in some cases, even repair themselves. Artificial membranes like these offer new ways of exploiting the properties of natural membranes, for example to create water filters, sensors, drug delivery devices or fuel cells.

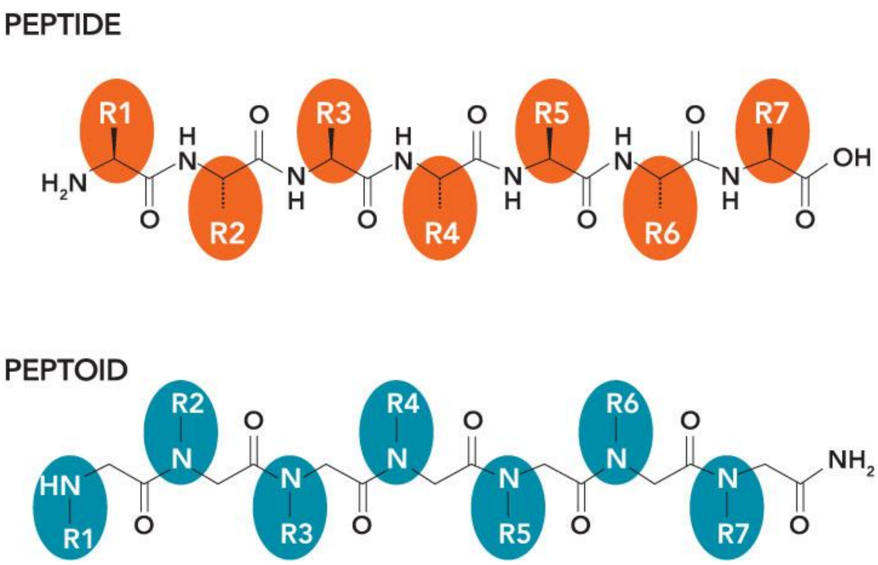

Figure 1. Peptoids are peptidomimetic polymers composed of repeating N-substituted glycine monomer units, where the side chain is appended to the nitrogen atom rather than the α-carbon. They can be readily assembled from cheap building blocks using automated synthesizers. (Image adapted from *http://www.neuromics.com/overview-of-peptoids-and-peptoid-libraries*)

The subtle structural modification in peptoids increases their resistance to proteolysis compared to peptides and enables a high degree of chemically diverse sequence information to be encoded into the chain. Peptoids can be synthesized with high efficiency by solid-phase methods using readily available reagents with complete control over chain length, side-chain chemistry, and monomer sequence. Peptoids adopt secondary structures and undergo supramolecular self-assembly that is mainly steered by side-chain−side-chain interactions.

Ron Zuckermann and his team have set up a peptoid synthesis core facility available to users of the Molecular Foundry, where several synthesizers from Gyros Protein Technologies play a central role. The team are in the process of developing vast combinatorial libraries of information-rich peptoid polymers that can be screened for a variety of functions. A key enabler in the research effort at the Molecular Foundry is Michael Connolly, Principal Scientific Engineering Associate, who is responsible for providing high efficiency synthesis support. We took the chance to ask Ron and Mike about their work, and why the Symphony® X peptide synthesizer has proved to play such a central role in their work.

Ron

Q: What is the promise of peptoid research?

"I think making artificial proteins is the new frontier. If we can make protein-like nanostructures from non-natural building blocks then we can create catalysts and synthetic antibodies. And maybe they can do things as magical as biology and at the same time be much more stable. Structures like these would be very useful in applications that require molecular recognition, such as curing diseases, water purification, and detection of chemical and biological agents."

Q: How do you see peptoid research developing in the next five years?

"We are just at the edge of understanding of how chains fit together to produce higher order assemblies. It's a bit like discovering tiles and realizing that rectangular ones can be used to pave the bathroom floor, or hexagonal ones can be used to make a dome. Within the next five years we should be able to establish design rules for forming and assembling pre-defined peptoid nano-structures. We also hope to publish atomic resolution structures of peptoid assemblies in the next couple of years, just as we can today with X-ray crystal structures of proteins. This will help us to see how non-natural structures fit together and develop functionality, chemical stability and diversity that you cannot get in nature."

Mike

Q: Why is the Symphony X peptide synthesizer better than other systems you have in the lab?

"Having the ability to have specific sequence control over something between a peptide and a polymer gives us the flexibility we need to open new doors to materials scientists, chemists and physicists. Being able to use a whole catalog of primary amines as building blocks is really enabling. That diversity of functionality and sub-monomer control allows anyone to do the synthesis of materials relevant to their research area."

Q: How do you measure success?

"Success for me is a function of yield and purity, and also the speed in obtaining the product, together with the cost. This includes monetary cost and also how much chemical waste is being produced. The Symphony X and Prelude® X peptide synthesizers are great systems in maximizing our success rates."

Ron Zuckermann (left) and Mike Connolly in the lab.

Q: Are there experiments that were only made possible using these peptide synthesizers?

"The synthesizers from Gyros Protein Technologies have helped us to take on entire projects that we haven't been able to do before. This includes Ron's massive DARPA project, http://today.lbl.gov/mimicking-nature-for-homeland-security/, that aims to build the world's first library of 100 million peptoid-based nanosheets. The idea is to display chemically diverse loops, with varied size, charge, and hydrophilic or hydrophobic properties on the nanosheet surface. Each nanosheet variant will be tested for its effectiveness in detecting and neutralizing threat agents, such as anthrax. While we have other synthesizers, I would never attempt to run that project on those machines because they are so much slower or have septa that may degrade during the synthesis of longer sequences. On Symphony X, I can do 24 peptides at a time and we frequently synthesize 40-mers."

Q: Why do you think the Symphony X peptide synthesizer is faster?

Symphony X is much faster in delivering reagents than robotic type systems where the needle system can only add one reagent at a time, plus rinses. Pneumatic delivery speeds up the addition. And that can improve your chemistry and the precision in timing reactions. Extended delivery time on other systems is a problem if you are trying to run short incubations. Symphony X also supports independent parallel control of twelve reaction vessel pairs."

"The instrument helps us to develop peptoid synthesis protocols with very short sub-monomer method reaction times. The short synthesis cycles of Symphony X and its ability to handle up to 400 mL reactions for a large range of sub-monomer amines make this system ideal for rapid synthesis of a library of peptoids with sequences of up to 50 residues."

Q: How does software help you in optimizing your methods?

"Your software is more flexible than software from other vendors. This includes flexibility in the contents of the bottle and the way we name it. The high number of different sequences we can make in a simultaneous run helps us to support more of our users. I can put three or four projects on the machine at once, rather than one synthesis at a time like I have to do on some other instruments."

"Being able to run lots of syntheses in a shorter time is great. The Symphony X has *UV monitoring*, which is really useful if you were trying to figure out where a de-protection is causing trouble. We have research groups here that have trouble synthesizing their difficult peptides on other machines and the combination of UV and heating on Symphony X enables them to efficiently synthesize pure peptides with higher yields."

Find out more about how Molecular Foundry is pushing the frontiers in peptoids

Design, Synthesis, Assembly, and Engineering of Peptoid Nanosheets. Robertson EJ et al. Acc Chem Res. 2016 Mar 15;49(3):379-89. doi: 10.1021/acs.accounts.5b00439. Epub 2016 Jan 7.

https://www.ncbi.nlm.nih.gov/pubmed/26741294