Chemistry & Biology
Innovations

Order Up! The Custom Enzyme Shop Is Open for Business

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A new Seattle startup currently has just four employees, but big plans. Arzeda, a company spun out of the technology developed at the laboratory of University of Washington researcher David Baker, PhD, is making plans to move out of the UW launch center and into their own laboratories. Their aim is to design and create catalytic enzymes that are not found in nature but that have the ability to tackle a variety of big, real-world issues.

Popular at Meetings

"When we developed our methods for designing catalysts for, in principle, any arbitrary chemical reaction, it was clear there were lots of possible applications," says David Baker, UW Professor of Biochemistry, and Arzeda Scientific Advisory Board (SAB) member. "I went to an enzyme engineering meeting [that]…was largely attended by companies and I was approached by a lot of people each with a collaboration they wanted to do." But Baker's funding prohibits company-sponsored research. "So we thought we should start a company that could then design enzymes for the many different chemical reactions involved in so many areas of life. If we were going to apply these methods seriously to real-world problems, we would need to move outside the academic lab." The idea—and the opportunity—for Arzeda were conceived.

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Michael Martino, Arzeda CEO

Active Sites Decorated with Functional Groups

Designing any type of protein from scratch is an achievement. This methodology has only matured in the last 5–10 years. Baker's lab showed in 2003 that they could design new proteins from scratch that were unrelated to existing proteins (Kuhlman et al., 2003). But the protein generated, "Top7," was nonfunctional. "In the last 6 years, we have been focused on methods to design new protein functions, like enzymes," says Baker.

Baker's group starting cooking up novel enzymes by essentially working backward from the traditional way of protein engineering: "First, we started with a chemical reaction of interest and literally drew in the ideal positions of the catalytic groups—a hydrogen bond here, ring there, negative charge here—but with no enzyme yet," he says. "It is essentially a disembodied active site. Then you have to design a protein that has that active site."

The basic process starts with an understanding of a desired chemical reaction. From that understanding, the first step in the process is to design the active site: the minimal active site. What it contains is the transition state and the minimal functional groups around that transition state required to achieve a desired reaction. "Then, using computation, those active sites are mixed and matched with a virtual library of over 200 protein scaffolds, looking for the right fit within those scaffolds and the right geometric placement of that active site in the scaffold," explains Michael Martino, Arzeda CEO. "This is a 3D modeling exercise."

Landmark Publications

Fueling interest in Baker's work and the development of Arzeda was the 2008 publication of two papers highlighting the de novo synthesis of two enzymes.

"It has been very difficult to make catalysts for any different reaction because to design an enzyme means you have to control the position of many, many atomic groups," says Baker. "That is something people have not been able to do until recently." In a 2008 *Science* paper, Baker's group showed just that. "We designed retro-aldolases that use four different catalytic motifs to catalyze the breaking of a carbon-carbon bond in a nonnatural substrate," explains the study authors (Jiang et al., 2008). This work was quickly followed by another groundbreaking paper in *Nature* (Rothlisberger et al., 2008). Here the, team described the computational design of eight enzymes that use two different catalytic motifs to catalyze the Kemp elimination: a model reaction for proton transfer from carbon. "This involved extracting a proton away from a carbon, which is a very widespread feature of catalysis," explains Baker. "You take the hydrogen away from the carbon and it's activated to do all kinds of things."

But the problem easily acknowledged by Baker is that the designed enzymes, although they have function, do not operate with the efficiency of enzymes found in nature. He has described it as the proverbial tortoise running the race. However, pending publications will show that the Baker lab has since boosted the functionality and speed of new enzymes. "We have a considerably faster tortoise now," he says. "In at least one case, it isn't winning the race, but it's in the lead pack."

Rosetta

To handle the monumental computational requirements of the enzyme design program, Baker's lab designed the Rosetta computer program to do the prediction and design calculations. "With this work, the design problem is saying 'here is the ideal structure of an enzyme, now go find a sequence that will fold up to that,'" says Baker.

The Rosetta program is an open-source algorithm for predicting how a unique

string of amino acids will fold into a given protein's characteristic shape. Rosetta helps determine which folded shape will be most stable, meaning it rests in a lower energy state and can be more efficient.

"Suppose we want to predict the structure of a protein, with Rosetta we give it the sequence and the computers fold up the protein into many possible solutions," explains Baker. "With more than 100K participants volunteering their computer down-time to the Rosetta at Home effort, their computers fold up the protein and send us back the result so we get hundreds of thousands different possible solutions."

Adds Martino, "I think what made Rosetta particularly powerful and unique is that the library that it generated consisted mostly of folded stable proteins."

Ag-Bio First on Deck

Arzeda's three cofounders emigrated from the Baker lab where they were all pivotal players in the enzyme creation technology. Alexandre Zanghellini has a background in computational science; Daniela Grabs' is in protein and antibody design; Eric Althoff is chemist by training with special experience in moving catalytic antibody technology to proteins.

The 2008 *Science* and *Nature* papers drew a lot of attention from commercial players, including DuPont's Pioneer Hi-Bred Division, an ag/bio division in the genetically-modified seed business. "Arzeda will design enzymes to achieve specific things within seeds," says Martino. "Pioneer will then modify the genome of the seeds to express those enzymes." The overall objective is higher yields for crops including corn, soybeans, rice, cotton and canola.

"What is truly impressive about this technology is the sheer size of the libraries that are generated and the ability of the technology to filter that down to the top 100 candidates," adds Martino. Typically the group designs active motifs four at a time. In combining the 4 active motifs with over 200 protein scaffolds about 10^{50} possible enzyme candidates are created. "The beauty of this is we are doing it computationally in about a 4-6 week period of time," says Martino. "It would take years and years and years to get that number of candidates in the traditional directed evolution lab." From 10^{50}, the software filters it down in an intermediate step to 10^{6} candidates. The objective is to get it down to the top 100. "Literally what we have at that point are 100 enzyme candidates in the form of a sequence of amino acids," adds Martino. Arzeda then expresses them using standard expression technology and then assays them to determine both expression and activity.

Arzeda's ultimate business goal is to provide that fully validated enzyme.

Bioremediation and Drug Therapy

"We truly believe this is an enabling breakthrough technology," says Martino. Arzeda has its eye on two other areas where it hopes to leverage its ability to design active sites that don't exist in nature. "We look to the renewable chemical space along the lines of biomass conversion," says Martino. "We think we can provide tools to biorefineries and chemical refineries that give them more efficient and effective use of the biomass conversion they are doing." Baker is more to the point: "We want to see this technology used in bioremediation, to break down toxic compounds in the environment."

Beyond that, Arzeda looks to the pharmaceutical sector to make big contributions. Baker's lab is already making strides in both of those areas, with expectations that they will be commercially explored within Arzeda. "With therapeutics, we would like to see this technology used to enzymatically destroy toxic compounds in your body or for a new way to do gene therapy or for making novel vaccines," says Baker. The UW group currently has a large HIV vaccine program.

"While the technology can also be used to optimize existing enzymes, and in fact has been applied to that, we see the real opportunity in enabling reactions that can't be achieved today with the enzymes that exist in nature," summarizes Martino.

CalTech, Duke, Monsanto in the Game

The Baker lab has designed over 70 enzymes with good levels of starting activity, but they are not the only ones in the game. Stephen Mayo at the California Institute of Technology and Homme Hellinga at Duke University are also involved in the de novo enzyme and protein creation space. Baker, Mayo, and Hellinga run the three best-known academic groups in the country for computational protein design.

It is no surprise to see Mayo and Hellinga expanding into the commercial realm as well. What is interesting is that the two have done so together. As of June 2009, the two researchers joined commercial efforts to form their own company, Protabit LLC. "Steve and I formed the company to do computational protein design," says Hellinga. "We are developing a software suite to capture the best practice design approaches." Protabit's mission will be to develop new computational methods for improving protein design. Potential end goals include new proteins or "reengineered" proteins, according to Hellinga.

Seemingly walking in lockstep, Protabit's first partnership is with Monsanto, again focused on the biotech crop business. "Agricultural biotechnology is just one field of application," says Hellinga. "It just turns out that the two major players in that business think this is an important area to go into and they see a future in it."

So, academic competitors are now commercial competitors as well. Just as Baker, Mayo, and Hellinga strive for first in publication, now their commercial offspring in Arzeda and Protabit will strive for first to market. Place your order.

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