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Innovations

Affinium Pharmaceuticals, Inc.:
Structure-Guided Drug Discovery

The assessment of function inferred from structure is the name of the game in structural proteomics. There is an abundance of information to be acquired from genomic and proteomic studies, and there is a lot that is needed. Less than 30% of all predicted eukaryotic proteins have a known function, leaving an abundance of proteins uncharacterized. Affinium Pharmaceuticals Inc. is in the business to assess the three-dimensional structure of proteins that participate in bacterial or viral infection. The characterized structure of a protein provides insight into its function as well as a template for the synthesis of a chemical inhibitor.

The infected world of antibiotic resistance will most likely be alleviated by an antibiotic that was designed by structure-guided research. Continuing to serendipitously acquire new antibiotics, as has been done in previous decades, will only create short-term relief until resistance evolves. Structural biology allows for more control over the mechanism of bacterial inhibition.

"We can take an atomic walk around the molecule and make a list of targets based on druggability," explains John Mendlein, PhD and CEO of Affinium Pharmaceuticals. This approach involves the crystallization and three-dimensional analysis of bacterial and viral proteins. The specific structure information provided by this analysis helps determine if the protein has accessible and vulnerable sites, such that cognate chemical compounds can be synthesized with the hope that they will inhibit the bacterial or viral target. Subsequent cocrystallization of the chemical inhibitor bound to the protein can then reveal any remaining sites to be masked or modified for enhanced potency. "If we see the (inhibitor-target complex) wagging in solution, we may decide to put a carboxylic acid on to make it more water soluble," says Mendlein. "You want active chemical matter that you can visualize in the target, and then you can start to see where to make modifications." He adds, "Everybody will design drugs this way in the future."

The founding scientists of Affinium, Aled Edwards, PhD, Cheryl Arrowsmith, PhD, and Jack Greenblatt, PhD, have made progress in the field of structure biology and have shown that structural proteomics can contribute significantly to functional genomics. One considerable academic contribution was reported in *Nature Structural Biology* in 2000. This article showed the scientific muscle they put behind informative proteomics with their ability to characterize ~20% of 424 non-membrane proteins from *Methanobacterium thermoautotrophicum*, an archeon which shares many sequence and functional features with eukaryotic proteins but is smaller and more robust, and thus an excellent model system for complex processes. The goal of this large undertaking was to determine a sufficient number of three-dimensional structures necessary to define a "basic parts list" of protein folds [1].

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For Affinium, an ongoing goal beyond obtaining X-ray crystallographic data for various proteins is to find adequate methods for automating the process wherever possible. The laborious protocol for obtaining a protein structure involves purifying highly concentrated protein (>10 mg/ml), applying between 1,000–10,000 drops of the protein to obtain an accurate image, and, finally, inspecting and analyzing thousands of data sets corresponding to each drop of protein. It is important to recognize that data storage of thousands of images is rather daunting. If one image requires 2 MB and there are 10,000 drops (images), this could result in 20 GB of data on a daily basis.

But beyond the computer storage requirements, automated computers, in the way of robots, are also essential for sufficiently paced progress. Affinium has designed and developed its own automated high-throughput, large-scale purification system, called ProteoMax. Depending on expression levels, ProteoMax can purify between 20–150 mgs of protein in less than 24 hr. This custom-made apparatus is fully automated, and the protein product is >95% pure. For protein application, Affinium has developed the Gem crystallization system using an industrial Cartesian robot from Adept Technology. The Gem is customized for versatile protein crystallization, and was designed to automatically prepare the crystallization screens from the stock solutions as well as carry out preprogrammed crystal optimization screens. The more difficult automation task is determining the optimization strategy for each individual crystallization experiment, which can number in the thousands. And, while there have been advances in automated image capturing, there is no system in existence that can completely replace human inspection.

Affinium, located in Toronto, Canada, was founded in 2000 by Edwards, Arrowsmith, and Greenblatt as Integrative Proteomics, Inc (IPI). After two years of intensive work automating their setup and incorporation of new management, IPI changed its name to Affinium Pharmaceuticals, Inc., which they felt more accurately reflected their strategic focus of integrated, structure-guided drug discovery.

With the automation systems in hand, Affinium's primary focus has been in the area of anti-infectives. In the antiviral and antimicrobial research realms, it has been noted that the amount of structure data for viral proteins far exceeds that available for microbial proteins. Dr. Mendlein explains that this discrepancy is caused by two things: "The advent of AIDS in the early 1980s," which created a big push for viral research, and the fact that "cell-based assays are so easy in the antibacterial arena that there was not much drive on the structure side." Another contributing factor, according to Mendlein, was that in the 1970s the World Health Organization claimed that infectious disease had been cured and there was no need for new antibiotics. This claim about antibiotics was obviously incorrect, as resistance to current antibiotic drugs is a definite and threatening problem.

Using the approach whereby acquisition of a bacterial (or viral) protein structure is used as a template to design chemical inhibitors, Affinium has obtained intellectual property around their high resolution targets and novel chemical inhibitors against *Staphylococci*. Thus far, they have been able to show excellent antibacterial activity against methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-intermediate and vancomycin-resistant *Staphylococcus aureus* (VISA, VRSA), and coagulase-negative *Staphylococcus* (CoNS). Specifically, Affinium has novel chemical inhibitors against these four types of Staph infections that have never been found in the public domain, and are set to enter clinical trials at the end of 2004. These chemical inhibitors target the FabI protein involved in the essential fatty acid biosynthesis pathway in bacteria. In addition to chemical inhibitors of the FabI protein in *S. aureus*, Affinium has also synthesized molecules for the chemical inhibition of FabK in *Streptococcus pneumoniae* and FabI in *Helicobacter pylori*. Mendlein claims that these synthetic antibiotic molecules will be the first in the clinic from a bacterial genomics target.

Affinium is certainly not the only research company focusing on structure-guided anti-infective research. However, it is the only company with these particular Staph inhibitors. Their competitors (albeit friendly) include SGX (Structural Genomix), Syrrx, Astex, and Quorex. The need for many types of antibiotics is vast, and these companies coexist quite happily. As Mendlein explains, "Nature's a big place, and there are a lot of important therapeutic proteins. There are 900 kinases, and we aren't going to get them all and neither is one of our competitors."

In addition to their Staph inhibitors, Affinium has an antiviral program including structure studies on the hepatitis C viral protein. They also have programs focused on high-resolution target structure pertaining to pharmaceutically or biologically validated drug targets and a number of promising active small molecules. Furthermore, Affinium has over 200 internally generated proprietary targets from infectious organisms that are in the early stages of discovery, many of which have high-resolution structural information.

Mendlein claims that the ability to triage targets based on the volumes of acquired structural information is crucial for efficient pursuit of novel targets. He points out, "We are swimming in a sea of targets. We can screen millions of compounds virtually—that is a lot faster than building an assay and retesting your compounds, which literally could take months." In just three years, Affinium, with its 55 employees, has established the ability to determine protein structures in as little as 20–30 days and filed patents for over 400 targets and 16 small molecule inhibitors in anti-infectives and other disease categories. With humble enthusiasm, Mendlein claims, "On the ability to produce active targets and determine their structures, we are one of the top three companies in the world."

References

1. Christendat, D., Yee, A., Dharamsi, A., Kluger, Y., Saychenko, A., Cort, J.R., Booth, V., Mackereth, C.D., Saridakis, V., Ekiel, I., et al. (2000). Structural proteomics of an archaeon. Nat. Struct. Biol. *7*, 903–909.

***Chemistry & Biology* invites your comments on this topic. Please write to the editors at chembiol@cell.com.**

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