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Innovations

Astex, Structural Genomix, and Syrrx

I Can See Clearly Now:

Structural Biology and Drug Discovery

In 1999, after a decade that had spawned a glut of companies immersed in high-throughput screening techniques for drug hunting, three pioneering biotechnology ventures emerged with a fresh approach. What set Syrrx, Structural Genomix, and Astex apart from the crowd was their idea to harness the powerful technology of structural biology in a whole new way to change the rules drug discovery. Since 1840, when the first protein crystal structure was documented by F.L. Hunfield, who undertook the extraordinary feat of uncovering the structure of earthworm hemoglobin, the field of protein crystallography had progressed to a point where it was available for use by researchers worldwide. However, in general it remained confined to the realm of an academic community of structural biologists who used this then cumbersome and time-consuming method to create beautiful detailed images of proteins to gain an understanding, from looking at the structure itself, of how proteins form and how form relates to function. Syrrx, Structural Genomix, and Astex have revolutionized structural biology into a sleek tool and are now neck-and-neck in competing to put the first structure-based designed drug in clinical trials.

Structure-Based Drug Design: Driven by Knowledge

San Diego-based enterprises Structural Genomix and Syrrx both began with a plan to automate the structural biology process to rapidly crystallize proteins on a massive scale, but soon realized that this alone was not a marketable tool, and expanded their horizons to utilize the crystal structures that they were creating for drug discovery. Ned David, who cofounded Syrrx with Peter Schultz and Raymond Stevens, both currently at Scripps, explains, "Over the years, we have figured out that the business we should be in is getting unique access to [protein] structures which are targets that the entire [pharmaceutical] industry values and leveraging those structures as starting points for structure-based design programs. The idea is that when we have unique access to structure, we are going to be more efficient than the rest of the industry on those targets." "What has happened is that people with more pharmaceutical experience were added to the management team and [they] have been changing the company and focusing on the downstream transformation of the structural information into drug leads," continues Keith Wilson, Syrrx's VP of Discovery Technologies and Structural Chemistry. Structural Genomix had a similar wake-up call after beginning with a plan to establish a database of the structures of medically relevant proteins and to sell access to that information. "That model changed in May 2001 when Structural Genomix purchased Prospect Genomics, an in silico drug discovery company of which I was one of the cofounders," says Stephen Burley, CSO and Senior VP of Research. "By combining that with the high-throughput structure determination platform and medicinal chemistry program that we have built up over the last six months, we have become a drug discovery company."

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In contrast, Astex, based in Cambridge, United Kingdom, distinguishes itself from Syrrx and Structural Genomix because it was never part of the structural genomics initiative. Harren Jhoti, cofounder and CSO of Astex, emphasizes this fact, "From day 1 we were focused on chemistry. We never had a business model that said that we would solve crystal structures of lots of protein which we would then commercialize to pharmaceutical companies." Jhoti had accumulated 10 years of experience in structural biology as head of structural biology and bioinformatics with Glaxo Wellcome when he set up Astex with Tom Blundell and Chris Abell from the University of Cambridge, UK. "The idea and key driving force behind the company was to think about X-ray crystallography in a different way and specifically to develop high-throughput X-ray crystallography so that we could use structure-based design for screening technology as well as for lead [drug] optimization. The initial idea therefore hasn't changed significantly over the last three years." In the nineties, the trend among pharmaceutical companies was to identify potential drug leads or "hits" by brute force: if a chemist could make enough compounds and screen enough targets, it was likely that they would find some desirable activity. But Jhoti states that this is not an effective method for drug discovery, "We are anticipating most drug discovery to be structure-driven in the next five to ten years. Clearly, [stucture-based] knowledge-driven approaches are coming back into favor now because the lack of knowledge-driven approaches have not delivered."

Protein Crystals by Robotics

All three companies have adapted X-ray crystallography for the fast-paced biotech world in similar ways, with some unique differences. In this trio's race to market the first struc-

ture-based rationally designed drug, it is these differences which may prove the cornerstone to success and subsequent longevity. Syrrx prides itself on its gene-to-structure approach and has highly automated high-throughput crystallography. Using a parallel fermentation system and the Sonic Hedgehog robot, Syrrx can make and purify 100 proteins in 5 hr. The purified protein is then moved to the Agincourt crystallization robot, which was reengineered from an automobile factory by Bob Downs from the Genomics Institute of the Novartis Research Foundation. Agincourt can accurately generate 100 nl crystallization droplets, enabling the structural information to be obtained from very small amounts of protein. In fact, Keith Wilson notes that this is a notable triumph for Syrrx, and patent filings are in process for the enabling robotics: "It depends on the protein, but the small volumes are crucial. No one can explain this, but the crystals grow faster in the small droplets, sometimes in a few hours or a day instead of a few days to a week." Remarkably, the integrity of the crystals that develop in the small droplets is also higher than for crystals developed with regular methodology, and they diffract X-rays better too, allowing for high-quality imaging. With a crystal structure in hand, medicinal chemists can be more efficient and can use this information to make a hundred specific compounds rather than thousands. At this stage, screens for drug leads using rational drug design are carried out both in vitro and in silico.

Like most other companies, Syrrx is striving to develop drugs that target proteases and kinases and, in fact, is specifically targeting proteins that already have inhibitors in clinical trials. "The Syrrx philosophy is to take this target protein and use the structural information generated in-house to produce compounds that can compete with the drug under trial as soon as possible," explains Keith Wilson. "The key to our success will be whether our inhibitors are different, at least as good, if not better, and that they are going to come fast, and this is facilitated by being able to see how everything fits together (protein and compound)." Syrrx's technology has attracted the attention of pharmaceutical groups including Hoffman-La Roche, Sanyko Co. LTD, Pharmacia, and Cubist Pharmaceuticals, all of whom have formed collaborative alliances with Syrrx. Two of Syrrx's most promising in-house projects involve dipeptidyl peptidase IV (DPP-IV) and histone deacetylases (HDACs). The group has solved novel structures for both human DPP-IV and a human HDAC and is currently screening for specific inhibitors of both enzymes. DPP-IV is an amino peptidase that cleaves the N terminus of incretins (peptide hormones that are released from the gut in response to nutrients that enhance glucose-stimulated insulin secretion). Inhibitors of DPP-IV activity would result in enhanced incretin activity and may represent a novel treatment for type II diabetes. Inhibition of HDAC activity has strong therapeutic potential to inhibit the onset and progression of cancer, since HDAC activity is associated with chromatin remodeling and decreased rates of transcription.

Like Syrrx, Structural Genomix has a considerable focus on targeting protein families, such as kinases, and is working on targets relevant to oncology, cardiovascular disease, and immune dysfunctions such as asthma and inflammation. Similarly, Structural Genomix has a broad base of corporate alliances, including technology partnerships with Aventis, Millennium, and Boehringer Ingelheim Pharmaceuticals and drug discovery partnerships with Asahi and Anadys. Interestingly, the company has established novel research partnerships with The Cystic Fibrosis Foundation to study the structure of CFTR (cystic fibrosis transmembrane conductance regulator), the Hereditary Disease Foundation, where they are working on the Huntington protein, and the NIH, with which they are collaborating with eight other research centers in the public Structural Genomics Research Consortium.

Structural Genomix has adopted a modular approach to their highly automated protein crystallization technology to enable the expression, purification, and crystallization conditions to be precisely controlled for each particular protein family of interest. As protein crystals emerge, they are sent to the company's proprietary third-generation beamline synchrotron at the Advanced Photon Source in Chicago. "This is arguably the best protein crystallography beamline in the world, and it has been tailored to our needs," Stephen Burley proudly explains. "In lay terms, the reason that this photon source is so effective is that the electron beam is extremely small, and that means that all of the X-ray photons end up in a very narrow beam, and you can work with small crystals." Crystals, frozen in liquid nitrogen, are shipped from San Diego to Chicago daily, and diffraction experiments are conducted at the beamline to quickly generate complete de novo structures using technology developed by Wayne Hendrickson (Columbia University), one of Structural Genomix's cofounders. This photon source also allows for precise cocrystal structure determination that is protein with inhibitor ligand bound, which is crucial for Structural Genomix's recently developed highly integrated medicinal chemistry, inhibitor activity screening, and computational chemistry process for drug development, which ultimately directly looks at how an inhibitor is bound in the active site of a protein. The process begins once the target protein structure is known, and researchers use a combination of real libraries (one of which is focused around particular pharmacophores; another contains drug-like fragments) and virtual compound libraries to look for compounds that bind to target proteins. Compounds that are found to bind to the target either in vitro or in silico are confirmed with actual enzyme assays, and then the crystal structure of the target protein with the successful inhibitor bound to its active site is obtained. By directly observing how compounds bind to their target and by using extensive computational analysis, particular inhibitors are then chosen as chemical scaffolds and will be repeatedly modified by medicinal chemists to generate a more potent drug. "To give you an idea of how rapidly we can proceed through this cycle, we have repeatedly documented turnaround times of less than one week from assay to cocrystallization data," comments Stephen Burley. He fur-

ther explains, "We are doing two things to ensure that what we are taking through lead optimization had the highest chance of success. We are examining selectivity by conducting cocrystallography with related targets to understand how these compounds are binding to the off-targets, while increasing the potency of binding to the target itself. The other issue is whether the compound that we are making is going to be useful as a drug, so we are doing cell-based assays and preliminary ADMETs." (ADMETs are adsorption, distribution, metabolism, excretion, and toxicology analyses.)

Astex's research program has a different flavor because of its consistently strong bias toward chemistry. The company's core focus is on fragment-based drug discovery, using 24/7 high-throughput X-ray cocrystallography to identify critical fragments with trademarked technologies known as Autosolve and Pyramid. "In the nineties, we all observed that there were fundamental problems with combinatorial chemistry type of approaches, where compounds were being synthesized and screened at tremendous rates," comments Harren Jhoti. "But if you actually looked at the size of the compounds, they were generally increasing in size, and so the actual molecular weight in a pharmaceutical company's collection was increasing as the years went on." Large compounds often become problematic at the later stages of development, because they are less likely to make it into cells and therefore have a lesser chance of being a successful drug. Consequently, Astex decided to take a Lego-like approach to drug design and start lead generation using very small compounds indeed. Jhoti elaborates, "If we could use fragments to start our chemistry, we would be much smarter because we would only add on to the initial fragment what was necessary." The problem Astex had to overcome was identifying the initial target binding fragments, because they are inherently very weakly binding entities with a size of less than 150 MW, and they often bind with very low potency (with high hundreds of a micromolar to a millimolar affinity). However, this binding interaction can be monitored by biophysical methods such as crystallography and NMR. "We felt that crystallography should give an advantage over NMR because it gives you much more accurate information about the binding of small molecules," says Jhoti. "In a nutshell, what Astex has been doing for the last three to four years is using crystallography in a high-throughput way to screen fragment libraries and look for binding of these fragments to a target. We have really pioneered this area and are quite different from the other guys as far as we can tell, in that they have not really been using their high-throughput crystallography in the same way."

Astex is validating its expertise by using well-characterized target proteins. Jhoti clarifies, "We never figured that there was a huge need for new target proteins; in fact the industry is target rich and lead poor, and there are plenty of good targets out there. The problem has been coming up with good lead compounds. So many of our targets are [also] on the [target] lists of many pharmaceutical companies, but we think that our technology should give us an advantage in coming up with lead drugs." Since both the structures and biological activities of these target proteins have been well defined, the researchers can make well-informed decisions about where they would like the fragments to bind to have a disruptive effect on the function of the protein. "We have potent lead compounds in the nanomolar range that are active in cells, so we have shown that we can take millimolar hits and grow them using structural knowledge into nanomolar lead compounds. We are also looking at proteins that are less characterized, and we may think about looking at fragment screening in a different way," but it is too early to discuss this at this time, continues Jhoti.

A project unique to Astex is their cytochrome P450 study, in which it collaborates with Astrazeneca, Aventis, and Mitsubishi Pharma. "A lot of drugs fail due to metabolic problems, or they get turned over by these cytochrome P450 enzymes, and that can produce toxic products that give you problems with side effects or drug-drug interactions," says Jhoti, explaining, "We have applied our structural biology capabilities to solve the crystal structure [of these enzymes], which was a very difficult task—many people had been trying to solve these structures for about 15 years. At the end of 2001, we announced that we had solved the first human P450 structure, and the second was solved in 2002. Last year we disclosed which isoforms they were as well (3A4 and 2C9). Those are two of the four key drug metabolizing proteins." Astex is now using this structural information to optimize drugs to ensure that metabolic turnover is reduced. However, Jhoti warns, "There is a balance here; you don't want to completely obliterate the interaction because you want there to be some turnover."

Despite the differences in their initial perspectives, scientific strategies, and technologies, Astex, Syrrx, and Structural Genomix all obviously share a common vision: that structure-based drug design will have a strong impact on the future of drug discovery. But does structure-based drug design hold the key to the future of drug discovery? Considering this prospect, Jhoti, of Astex, is exuberant. He exclaims, "Of course! I left a good position at Glaxo Wellcome to set up Astex, and we are all absolutely committed to this new approach. For example, several of our senior management team have come from big pharma companies, such as Robin Carr, VP of Drug Discovery, who was head of lead discovery and high-throughput screening for Glaxo Wellcome. This exemplifies their belief in the structure-based approach over conventional methods for drug discovery." When posed the same question, Burley, of Structural Genomix, is positive: "Structure-based approaches to drug discovery represent the newest technology to come into the arena. Five years ago, everyone was talking about the potential impact of high-throughput screening, and what we have seen is that high-throughput screening has been very effective in some contexts and not as effective in others, and that overall it did not represent the panacea that some people thought that it was going to be. By the same token, it would be unrealistic to expect that structure-based techniques and strategies are

going to solve all of the problems in drug discovery, but I don't think that there is any substitute for a direct look at how an inhibitor is binding to a target, and more importantly, how the inhibitor may interact with other potential targets in the body, some of which you may want to inhibit and others which would obviously be very deleterious for the patients." Wilson and David from Syrrx agree: "Other things have come and gone, and there has certainly been hype about structural information and its utility, but I don't think that structure-based drug design is the pinnacle," Wilson responds. "However, I do think that it will rise above lots of other technologies in terms of utility, and I think it may have already." If the world of drug discovery is likened to a chessboard, then David believes that structure-based drug design is analogous to the queen. "It's not the king because you don't need the king to play, but the queen allows you to move in any direction," he says. "You can keep playing the game without the queen, but you just play a lot worse."

The power of clear, precise visualization of protein targets with X-ray crystallography will undoubtedly mold and reshape the conventional strategies of the drug hunters of tomorrow. For Syrrx, Structural Genomix, and Astex, their impact on the global biotech community will be marked by the unveiling of the first successful drug designed with structure-based technologies. None of these young companies have compounds in clinical trials at this time, but the race to the finish line has begun.

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

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