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Protein Structure Determination

In this issue, we're reviewing the entire paradigm, organizing the industry into its key components, identifying the important players, and adding a new private company to our list: Triad Therapeutics.

Back in 1995, when Ernesto Bertarelli took the reins at the Swiss biotech firm Serono, S.A. [SRA] from his ailing father, Fabio, few people thought he would make the grade as a biotech entrepreneur. But seven years later, Serono is easily Europe's largest biotechnology company. Market capitalization over the period jumped ninefold to $12.2 billion.

But how can Serono keep up such impressive growth rates? The core of its business, fertility treatments, still relies on collecting 30,000 liters of female urine a day—much of it from menopausal nuns—and the nuns aren't getting any more productive. So Serono's betting its future on rational (a.k.a. structure-based) drug design.

Rational drug design is the heart of the new biodigital paradigm set forth in the first and subsequent issues of the *Gilder Biotech Report. (See GBR*, September 2001 and December 2001). Until recently, companies used a brute force approach. Chemists pumped out enough chemicals, figuring one would work eventually—a technique called combinatorial chemistry. It has turned out to be a less than efficient method, and with the glut of post-human genome map information, it's not even an option. It would take years to randomly screen millions of compounds against all the thousands of emerging new drug targets.

With rational drug design, rather than screening compounds randomly in wet labs, scientists design molecules digitally, based on their knowledge of molecular disease processes. Serono believes structure-based design increases drug discovery efficiency by at least 20-fold—last year alone it put seven new molecules into clinical trials.

In this issue, I review the key players and key technologies in the emerging industry, the first step to rational drug design: protein structure determination.

Dr. Scott Gottlieb

"Triad Therapeutics is at the vanguard of a group of talented new companies, turning data into biology."

INSIDE:

Unfolding Protein Structures

The key to structure-based design is uncovering the structure and function of proteins. Genes are merely blueprints for making proteins, the versatile molecules that trigger nearly every vital function in our bodies. Drugs work by binding to proteins to either directly block a protein's active site or to change its three-dimensional shape (upon which a protein's function depends). Often, the binding of drugs (known as "ligands") to the active site (also known as the "drug target", "combining site" or "receptor") of a protein results in the activation or discontinuation of a key cellular signaling mechanism. Understanding how a protein folds into a three-dimensional shape from a linear chain of amino acids is the key to identifying areas on the protein that can be targeted by new drugs, or to increasing the potency and reducing the side effects of existing drugs.

Rational drug design allowed Vertex to come up with Agenerase in less than one-third the time it normally takes to bring a drug to clinical trials

Big Pharma first looked at structure-based design about 10 years ago when it was equated with de novo design, or building a drug from the ground up. Big Pharma viewed the active site of a protein as merely a physical space to be filled with a molecule that complemented it in terms of shape, charge, and other binding components. Some of the elementary principles of structure-based drug design were first applied by Bristol-Myers Squibb Company with the popular blood-pressure medication Capoten. But Big Pharma tended to turn to structure-based tools only when its conventional chemistry wasn't working—too late in the drug discovery process to exploit dramatically accelerating increases in biodigital power. Moreover, the shape turns out to be only one important variable in linking drug compounds to protein sites.

So the molecules Big Pharma designed often didn't work as expected. The initial expectation of structure-based drug design—that companies could design molecules that worked right out of the box—was unrealistic. Companies didn't understand the thermodynamics of drug-binding well enough and so the molecules they made did not have all the properties of drugs. Companies and investors became disenchanted and moved on; many never returned.

This early experience left some on Wall Street skeptical, wondering where the payoff was in rational drug design. Some think companies like Serono are betting its fortunes on the wrong horse. But the skeptics are wrong. Over the last 10 years, new advances in computing power, combined with more detailed genomic knowledge, have made rational drug design techniques immensely powerful. Biotech is at an inflection point where companies that adopt tools which harness the computational power of microprocessors will generate more promising drug leads and bring new compounds to market faster than drug companies stuck in the era of test tubes and beakers.

In prior issues of the *GBR*, we identified three outstanding companies that use structural techniques to accelerate their drug discovery efforts: **Gilead Sciences** [GILD], **Quorex**, and **Vertex**

Table 1. Drugs Developed with Structure Based Technology

Company	Protein Structure	Drug
Agouron (Pfizer)	HIV Protease	Viracept
Biota/Glaxo	Neuraminidase	Relenza
Gilead/Roche	Neuraminidase	Tamiflu
Novartis	c-Abl Kinase	Gleevac
Vertex	HIV Protease	Agenerase

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Pharmaceuticals [VRTX]. Vertex Pharmaceuticals has already turned industry skepticism into its own success story, using rational drug design to develop two HIV protease inhibitors. One, Agenerase, is already on the market and the other is likely to be approved this year. Rational drug design allowed Vertex to come up with Agenerase in two years, less than one-third the time it normally takes to bring a drug to clinical trials.

Wet lab and digital tools are not necessarily competing technologies, but complementary capacities. The best companies are those which, like Vertex, couple outstanding wet-lab capacities with advanced computational tools for determining protein structures. Computational tools grow smarter as researchers collect more experimental data, eventually leading to the development of entirely in silico platforms.

What will this new industry look like? In this issue, we're reviewing the entire paradigm, organizing the industry into its key components, identifying the important players, and adding a new private company to our list: **Triad Therapeutics**, of San Diego, California.

Experimental Structure Determination

The advent of new high-throughput technologies and computational advances for structural genomics accelerate the industry-wide move to structure-guided drug design.

Right now, data on protein structures is obtained by two basic approaches: experimentally or computationally (more on the latter below). Experimental structure determination is very important. Before researchers can create effective digital models, they need good data, i.e. information on the relationship between DNA, amino acid sequence, protein shape, and function. And after running digital experiments, companies still need wet-lab capacities to confirm the results of their model.

The two main experimental technologies for protein mapping are *nuclear magnetic resonance* (NMR), and *x-ray crystallography*. (Other less commonly used methods discussed below include *mass spectrometry* and *Multiple Coupling Spectroscopy* [MCS].)

Ideally, NMR and x-ray crystallography complement one another, but most experimental structure determination companies use only one method of structure determination, at least initially. Only a handful of structure-based companies (including Vertex and Ariad Pharmaceuticals [ARIA]) are equally adept at both.

Structural GenomiX and Syrrx are clearly the leaders in this space...

One of the driving forces behind structure-based drug design is the increasing need for *lead optimization*. The influx of genomic information has rapidly expanded potential drug targets, from about 500 to 10,000, or more. Which molecules and drug targets should a company invest time and resources in pursuing? Knowing the structure of a protein is key to making better decisions early in the process. Ten years ago, experimental structure determination was too slow and expensive a process to be much use. But thanks to technological advances and high-throughput, the time and cost of identifying a protein structure experimentally has dropped from about two years and $200,000 to a week and $20,000.

Table 2. Experimental Structure Modeling Companies

Company	Technology
Astex Technology	x-ray crystallography
Integrative Proteomics	NMR, x-ray crystallography
MediChem Life Sciences	x-ray crystallography
Structure Function Genomics	NMR
Structural GenomiX	x-ray crystallography
Syrrx	x-ray crystallography
Triad Therapeutics	NMR, x-ray crystallography

High-Throughput X-Ray Crystallography

Pioneering one form of high-speed, protein-structure determination are two privately traded companies: San Diego companies **Syrrx** and **Structural GenomiX.** Both specialize in high throughput x-ray crystallography.

How does it work? Proteins are coaxed into forming crystals and then bombarded with intense x-rays. X-rays bounce off a protein crystal, reflecting into a particular diffraction pattern that scientists can translate into a three-dimensional digital image, suitable for further computational manipulations to produce drug leads.

Structural GenomiX and Syrrx are clearly the leaders in this space, turning x-ray crystallization into

Update: Gilead Sciences – "The Stock Has Room to Run"

Despite the biotech slump, Gilead's stock has been a strong performer in recent weeks, largely due to encouraging news about its new HIV-drug Viread, released last November.

Gilead's senior management reported good news at the recent Bear Sterns Healthcare Conference in London. The European Union gave Viread the green light in February of this year. Sales are off to a strong, early start. About 15,000 patients were treated with the drug in January, and company data indicate that average prescriptions have been rising each week.

Viread is a novel, once-daily oral reverse transcriptase inhibitor for the treatment of AIDS. The FDA approved Viread for use in combination with other anti-retroviral agents to treat adult HIV-infected patients experiencing early failure with standard drug regimens, about 200,000 patients in the United States alone. But early indications are that doctors are already prescribing Viread more widely, sometimes as the first-line of defense against HIV.

If early trends continue, Viread will be an even bigger seller than Wall Street estimates

Gilead reiterated expectations of company profitability in 2002, noting expected sales of $160 million for Viread, a slight boost from earlier estimates. Expect another report from Gilead on Viread sales mid-year. Most Wall Street analysts now expect $260 million in sales, versus earlier estimates of $230 million. On the heels of news about the increasing prevalence of HIV strains that are multi-drug resistant (and the positive response of doctors to Viread), sales should be even stronger.

Doctors have been switching aggressively to Viread because it's one of the few drugs capable of knocking down the drug-resistant HIV virus. Perhaps, most encouragingly, Gilead is seeing (primarily in certain U.S. West Coast treatment centers) some proactive switching of patients on first-line defense and early therapies to Viread-plus regimens, even before failure of other drugs becomes evident.

Doctors and patients are keen to avoid long term toxicity, especially from first-line HIV drugs such as AZT and d4T. And it is becoming clear that induced viral mutations caused by such drugs not only blunt these antivirals, but also result in a more pernicious virus resistant to later drugs. Some doctors believe that strategic, early use of Viread preserves the potential of these more established drugs for use later in the course of the disease. If this is true, up earnings estimates for Gilead considerably.

Also noteworthy: Gilead is about to begin human testing with GS 7340, a *prodrug* (or more active version) of Viread. Unlike Viread (which breaks down in the bloodstream), GS 7340 only releases the active drug once it's inside the cell. The result? Higher intracellular concentration of the active drug, i.e., more potency. GS 7340 should offer increased effectiveness, with Viread's low side effects and convenient once-a-day dosing. Human trials begin this month.

Doctors, not the FDA, ultimately decide how a drug will be used. If this kind of prescription writing continues, Viread will be an even bigger seller than Wall Street estimates. Gilead's stock has room to run. **B**

an almost fully automated process. Syrrx has focused on miniaturizing, parallelizing, and automating the crystallization process, and Syrrx is able to use smaller volumes—requiring mere milliliters rather than liters of cell culture—for protein production.

Like Syrrx, Structural GenomiX also invested in processes that automate structure determination. Initially, Structural GenomiX's main goal was to develop a comprehensive database of protein structures. Although the company still adds to the database, its focus has broadened. Like Syrrx, Structural GenomiX is now using its expertise in protein structure determination to develop intimate knowledge of particular protein classes, and then using that expertise to develop its own new drugs.

Syrrx has the best technology for experimental structure determination. Structural GenomiX's distinction is its earlier decision to branch out from its experimental methods into computational methods for structure determination. It acquired San Francisco-based Prospect Genomics, a computational genomics company more than a year ago. One area in which Structural GenomiX has focused its expertise is obtaining targets in bacterial genomes for the development of anti-infectives, solving dozens of promising crystal structures that are in the company's database.

X-ray crystallography isn't perfect. Crystallizing proteins for x-ray analysis can sometimes result in structural anomalies, masking their actual shapes in the body. And membrane-embedded proteins, which are important for regulating cellular signaling and involved in nearly every disease process, are not readily amenable to existing crystallization methods.That's where the competing experimental tool for protein structure determination, NMR, has an edge.

Nuclear Magnetic Resonance

NMR determines the position of atoms relative to one another, based on their interaction within a magnetic field. One of the main advantages of NMR over x-ray crystallography is that NMR can determine the structure of a protein without first going through the difficult and time-consuming process of purifying large amounts of a protein or growing very accurate crystal models of proteins.

Thus NMR provides an important alternative to crystallography for many finicky proteins that are difficult to purify or crystallize. Although x-ray crystallography has the advantage of defining ligand-binding sites with more certainty than NMR, NMR can measure proteins in their natural state. This is important since many proteins change form by the time they're coaxed into crystals. The downside? NMR is generally slower for obtaining structures and can't be used to obtain three-dimensional structures of large proteins (although new types of NMR are faster and somewhat better at resolving large proteins).

...Syrrx has the best technology for experimental structure determination

Table 3. Biotechnology Companies Using NMR as a Core Technology

GeneFormatics	RiboTargets
Integrative Proteomics	Triad Therapeutic
Metabometrix	Vertex Pharmaceuticals
Novaspin Biotech	

Another advantage of NMR is its ability to detect weak interactions, particularly between small molecules and a macromolecule. Looking for weak interactions might seem counterintuitive. However, weak binders can be modified to improve both their binding affinity and their drug-like characteristics. Weak binders are the compounds that big pharmaceuticals, seduced by potency, often overlook as potential drugs.

Using NMR, Vertex came up with some previously untried weak inhibitors, a promising new pre-clinical lead, and a potential Alzheimer's drug

Many really potent drug leads aren't useful anyway. They're strong binders because they're generally large-molecule drugs. The bigger the drug molecule, the more interactions it forms with its target. Large-molecule drugs bind well because they interact with the drug target site in many different ways. But many interactions also mean many potential

side effects. Big drugs also tend to be poorly absorbed in the stomach. So weak binders offer dramatic, untapped potential as drugs. But in ordinary wet-lab assays, these promising weak binders can be hard to detect. Not so with NMR.

The Astex platform is definitely fast when it comes to screening for drugs, one or two orders of magnitude faster than most other firms

Searching for weak binders sometimes results in a new class of compounds. Vertex, for example, used NMR to find inhibitors of JNK-3, a type of kinase involved in the development of Alzheimer's disease. Early high-throughput screens on tight binders led nowhere. Using NMR, Vertex came up with some previously untried weak inhibitors that were then optimized into powerful binders with a few simple modifications. Voila! A promising new pre-clinical lead. And a potential Alzheimer's drug [See *GBR*, March 2002].

Most importantly, NMR can tell researchers a lot about the chemical interactions that are taking place between a drug and a protein. This kind of information, dubbed the physics or thermodynamics of drug design, is essential for deciding which compounds will make good drugs and what changes can be made to a drug to make it an even better binder. It is generally the lack of this kind of information that limits the ability of computational tools to predict drug-protein binding and slows the evolution of truly in silico efforts.

With x-ray crystallography, companies get an excellent picture of how tightly a drug binds to a particular protein, but this technology does not reveal which parts of the drug are actually binding. For screening libraries of potential drugs against a protein in order to find some that stick (dubbed docking) and for optimizing the drugs that do stick, the key is understanding the dynamics of the drug-protein interaction. NMR can show in real time the different residues in contact with each other.

This is one important advantage of NMR over crystallography: NMR helps the algorithms decide not only if a drug has the right geometric shape, but also the right electronic binding properties to stick. To make smarter in silico docking programs, you need to know about a host of subtle changes that take place in the dynamic structures of proteins, data that static x-ray crystallography pictures fail to yield. NMR is slower thus far, but as better magnets and better ways of assigning resonances are developed, expect the speed gap between NMR and x-ray crystallography to narrow dramatically.

NMR and x-ray crystallography technologies thus offer advantages in different kinds of data. While both help determine the structure of proteins, they are complementary rather than competing technologies.

The privately held **Triad Therapeutics** uses NMR as the centerpiece of its drug discovery platform, which it calls *integrated object-oriented pharmacoengineering*, or IOPE (more on this company below). **Vertex** also uses a novel NMR method called SHAPES. [See *GBR* September 2001] Privately held **GeneFormatics**, a San Diego-based company is also adding NMR to its repertoire of experimental structure-determination capabilities.

Multiple Coupling Spectroscopy

Other alternative technologies for experimental structure determination worth considering include *mass spectrometry* (which we discuss in the January 2002 *GBR*) and **Signature Bioscience's** *Multiple Coupling Spectroscopy* (MCS).

Microwave spectroscopy is already extensively used in the computer chip industry (to ascertain the purity of semiconductor crystals), but Signature Bioscience has adapted the technology to protein structure determination. MCS probes proteins with microwaves. In less than one millisecond, structural changes that occur when molecules, proteins, and cells interact can be mapped. Like NMR, Multiple Coupling Spectroscopy can generate important information about the underlying "physics" of the binding process.

Like other companies, privately held **Astex Technology** uses x-ray crystallography to determine protein structure. But then Astex takes an unusual shortcut: instead of screening its entire library of digital compounds against the drug targets, the company uses a much smaller subset of compounds called

fragments. These partial pieces of drugs represent the chemical diversity contained within the entire drug library. Carving large drugs or drug-like molecules into digital fragments, the company takes a Lego-like approach to drug design, fitting the fragments together to build large molecules likely to work as finished drugs.

Astex's strategy is to look at diseases for which proteins can be crystallized, but where few good lead compounds currently exist. The company has developed software that actually identifies where the drugs are most likely to stick in a binding site, without the need for first using time-consuming x-ray crystallography to to find likely molecules. Only after the computer finishes its virtual screening, are a handful of candidate compounds then experimentally tested using x-ray crystallography. The company calls this process "structural screening." Unlike Structural GenomiX's and Syrrx's technology platforms, Astex can only generate crystal protein structures one-at-a-time. But the Astex platform is definitely fast when it comes to screening for drugs against the targets it generates. Astex can do hundreds of protein ligand experiments per week, a rate one or two orders of magnitude faster than most other firms.

Another company we like that has expertise in experimental protein structure determination is the privately held **Affinium Pharmaceuticals**, founded in Toronto in August 2000. Affinium uses three different tools—x-ray crystallography, mass spectrometry, and NMR—in a complementary fashion. If a protein is of the sort that crystallizes easily, it's sent to NMR. If it's a more difficult, generally larger protein, then it's sent for interrogation by x-ray crystallography. Mass spectrometry can be used to complement each of these technologies, providing lots of data about the physics of drug binding to complement the structural data generated by the other tools. Unlike its competitors, Affinium characterizes proteins from a biophysical point of view, looking at the physics interactions involved in binding rather than merely the shape.

Other companies with experimental structure determination expertise include: **Millennium Pharmaceuticals** [MLNM], which is investing heavily in NMR through its recent collaboration with Abbott. Both **BioCryst Pharmaceuticals** [BCRX] and **Ariad** have drugs in clinical trials that were developed using these techniques as well. **RiboTargets,** like Vertex, uses NMR and x-ray crystallography to build out its docking program, RiboDock. Two promising but early-stage academic spinouts are the privately held **Metabometrix**, and **Novaspin Biotech**. Neither anticipates a public offering for at least two years.

Another company we like that has expertise in experimental protein structure determination is the privately held Affinium Pharmaceuticals

Computational Structure Determination

Computational methods for structure determination are playing increasingly important roles in rational drug design. A great deal of information can be derived about protein structure and function using a purely in silico approach.Currently, the two main approaches to computational protein structure modeling are *homology-based* (or comparative) *modeling* and *ab initio protein prediction*.

How can a computer predict a protein's structure? Homology modeling is currently the most accurate of digital prediction techniques. Proteins run in families whose members share the same three-dimensional structure and have detectable similarities at the gene-sequence level. Homology-based protein modeling technologies use such previously determined three-dimensional structures as templates to infer the shape of proteins for which only the amino acid sequence is known. Homology modeling allows the prediction of the structure of all proteins in any given family, even if the structure of only one member is known.

Structural Bioinformics is a leader in homology modeling, achieving unusual accuracy in predicting the backbone structure of proteins

The privately held **Structural Bioinformatics** is a leader in homology modeling, achieving unusual accuracy in predicting the backbone structure of pro-

teins. Its ProMax database contains about 3,000 protein models. Although homology modeling is yielding quite accurate core structures, the prediction of surface structures (where biological activity of proteins resides) is so far less successful. Expect new computational methodologies and algorithms to continue to improve these models, however, as companies benefit from more experimental data. Structural Bioinformatics has one troubling drawback: it doesn't generate much of its own experimental data to feed into its algorithms, relying instead on other companies' databases. The most dramatic improvements in computational models will come from in silico companies with the core expertise to generate their own experimental data. Researchers need wet-lab experiments to validate digital models.

In computational structure determination, we're particularly impressed with GeneFormatics

The second method of computational structure determination is based on reading sequences of amino acids. DNA is a simple linear code of four chemical letters, while proteins are composed of 20 different amino acids that fold into complex, largely unpredictable arrangements of sheets and loops. The arrangement of the chemical letters in this sequence determines the protein's three-dimensional structure. Eventually, scientists will be able to use computers to predict protein structures directly from their DNA blueprints (called *ab initio structure determination*). But first they'll need to gain the breadth of information needed to teach software algorithms how proteins fold from their linear forms into three-dimensional structures. Given the speed with which this technology is advancing, expect this advance within five years.

Table 4. Computational Structure Modeling Companies

Company	Technology
DeNovo Pharmaceuticals	Homology modeling, docking
GeneFormatics	Ab initio, homology modeling
Inpharmatica	Homology modeling
Locus Discovery	Computational chemogenomics, docking
Structural Bioinformatics	Homology modeling, docking

The privately held company **Prospect Genomics** has a program called ROSETTA which is able to predict up to 70 percent of a protein's structure directly from amino acid sequence information. When **IBM** [IBM] completes its new supercomputer (dubbed "Blue Gene") in 2004, the machine will be left alone for an entire year to crunch the physical interactions between each atom of an average-sized, 150-amino-acid protein as it folds. Blue Gene will be capable of performing 1,000 trillion calculations per second, or 1,000 teraflops.

Docking Programs

The goal of computational structural determination is to couple these algorithms with docking programs that can search in silico for potent binders that might make good drugs. Once a researcher knows what a protein looks like, docking programs are used to test various compounds in silico to find one that binds well. Companies with expertise in structure determination are moving naturally into the docking program space in the process of developing their own pipelines. Syrrx, for example, licensed virtual ligand- screening technology (docking programs) from privately held informatics technology developer **MolSoft**, and then invested in a 500-processor Linux computing cluster that can digitally dock a million virtual compounds onto a drug target in a single day.

The privately held company **Locus Discovery** (spun out from the Sarnoff Corporation in 1999) has some fascinating, buzz-generating technology. Locus starts with a fragment-based approach to lead optimization. Starting with the crystal structure of a protein, Locus uses fragments of known drugs to identify drugs that might bind to a protein's active site. Software programs then move to find the best fit and to build the bound fragments back into drugs.

A palette of fragments of small molecules is combined with proteins, and the free energy expended in binding the fragments to the proteins is calculated. As its computational calculations about the "fit" of drugs into binding sites get better, Locus's goal is to get to the point where the molecules that come out of its process are practically clinical candidates, eliminating tedious, time consuming pre-clinical experimentation.

Locus has obtained proof-of-concept that its technology can generate compounds that are real drugs. How? By running its fragments against two known targets, the company accurately identified the targets' binding sites, predicted the structures of known interacting drugs, generated several novel classes of drug mimetics, and found novel binding sites for new active molecules with potentially improved efficacy and safety profiles.

The company recently started compiling a database of its fragments that relates them to known problems such as toxicity and absorption. The process works. For example, when the fragment library was run against HIV protease, the Merck drug Crixivan was one of the compounds generated. Locus is adding five new targets to its pipeline per quarter and hopes to design 20 targets per year. The company has ramped up its computational power, increasing the supercomputer clusters from approximately 200 parallel processors to 1,000.

Table 5. Publicly Traded Structure Based Drug Design Companies

Ariad Pharmaceuticals
Biocryst Pharmaceuticals
Gilead Sciences
Millenium Pharmaceuticals
Three-Dimensional Pharmaceuticals
Vertex Pharmaceuticals

Given the current state of the technology, structure-based extrapolation is preferable to purely sequence-based prediction. Similarities in structure are more recognizable than similarities in sequence. However, for proteins that have no similarity to known structures and which cannot be analyzed by NMR or crystallography, the only strategy for predicting the outcome of their structural folds is *ab initio modeling*.

Ab Initio Modeling

Historically, ab initio structure predictions have produced reliable models for only a few proteins. However, thanks to recent progress in model-building algorithms, it will soon be possible to generate low-resolution models for many more proteins in the genome, potentially producing a gold mine of truly novel target sites.

Right now, these tools are not very useful in structure-based drug discovery or optimization applications. The bottleneck is the experimental data needed to power the algorithms that predict protein structure directly from the amino acid sequence. In order for the algorithms to be smart enough to crunch these calculations, they have to be pre-programmed with thousands of amino acid sequences and protein structures. Essentially, it's an exercise in pattern matching. And the computers have to have seen enough patterns to know effectively how amino acid sequence turns into protein structure. The development of that critical mass of information from experimental data is still at least five years away.

GeneFormatics recently struck a deal with IBM to build out the processing power needed to run its simulation software

In computational structure determination, we're particularly impressed with **GeneFormatics**, a private San Diego-based company that applies a combination of ab initio and comparative modeling, focusing on the active sites of proteins. The company is developing a library of structural motifs called Fuzzy Functional Forms (FFF) which are essentially functional fragments of protein structures. They use these fragments like Lego pieces to construct entire proteins. By washing a library of Fuzzy Functional Forms over an amino acid sequence, computers can recognize certain stretches of the sequence and stick the right structure to the right stretch of amino acids. In this way, GeneFormatics is able to piece together proteins simply by looking at the amino acid sequence. GeneFormatics correlates each of their Fuzzy Forms with a particular function, which allows them to derive protein function directly from its models. The company recently struck a deal with IBM to build out the processing power needed to run its simulation software.

One of the advantages of GeneFormatics is that it does not rely solely on computational models. Even the best computational tools are meaningless if they're not validated by meticulous experimental data. Structures derived from tools such as NMR and x-ray

crystallization are essential for validating the computational models. But many of the computational structure companies don't have experimental capabilities.

In effect, Triad begins its drug development process with a compound that's much closer to being an actual drug

Because these two components of structure determination—computational and experimental—greatly complement each other, I predict that many companies will follow the path of GeneFormatics and Structural GenomiX and seek to broaden their current technology platforms through consolidation with companies involved in complementary investigations.

Ultimately, however, none of these tools are useful unless they lead to better drugs. The greatest limitation of the in silico docking programs at the core of structural design is that scientists don't yet understand the physics of drug-protein interactions. Even when they have perfect protein structures and can model drugs that fit snugly into protein binding sites, scientists still can't predict all the different chemical interactions that will take place in the real world, or inside the human body, as drugs try to bind with proteins.

However, the more experimental data that companies generate, the smarter their computational models become. Like Vertex, **Triad Therapeutics** has a head start in this essential task of developing integrated digital- and wet-lab expertise.

Triad Therapeutics

Triad uses x-ray crystallography, but its specialty is NMR, which is used in a novel way: to speed the process of finding drug compounds by taking advantage of target class effects. Classes of targets, such as protein kinases, may contain many members, each with very different biological functions, but all the class members share certain structural features.

Triad bases its approach on its *integrated object-oriented pharmaco-engineering*, or IOPE technology. IOPE exploits information from NMR to map key structural elements computationally among the members of individual classes of enzymes and then uses the data to design libraries of compounds likely to bind to targets of that particular class.

Triad focuses on gene families with two-domain enzymes, particularly ones that require co-factors for their enzymatic activity. The process begins by creating compounds that bind to an enzyme co-factor site, a particular molecule needed to trigger a reaction. (Many vitamins are co-factors because they help the body with certain vital tasks.) These co-factor sites are common across all the targets in a specific class of genes. So if it is protein kinases at which Triad is aiming drugs, the library of co-factor binders it creates will stick to all the co-factor sites on every single protein kinase.

Triad then uses these co-factor binders as anchors and tries to aim a second drug at a different site on the protein kinase. Once Triad binds a drug to the second site, it then creates a linking molecule that binds the co-factor binder with the second binder.

Why take this approach? Each fragment by itself might bind weakly to the protein. But once the two drug fragments are linked, they create a stronger, more specific link, which in drug form would have more potency and fewer side effects. In fact, when the two fragments are linked together, they bind 100 to 1,000 times more tightly to the protein than either one would alone.

Screening programs from these carefully biased libraries should yield hits that bind tightly enough to their protein targets to be taken immediately into a medicinal chemistry optimization program, thereby leapfrogging most of the intermediate and often fruitless chemical revisions required to tweak a screening hit into a full-fledged lead candidate. In effect, Triad begins its drug development process with a compound that's much closer to being an actual drug.

Triad's a leader in using NMR to define the structure of some of the most difficult proteins. Its strategy of using experimental tools to power computational short-cuts is the best application of each of these powerful tools. The experimental tools make the computations smarter, putting the drug discovery platform on a pace that accelerates with increasing processing power. The type of drugs Triad aims to create (where two fragments that bind separate sites on the protein are linked together) are called *bi-ligands*. Two of the most successful drugs on the market are bi-ligands: one is Propecia, used for benign prostatic hypertrophy, and also for baldness. The others are the statins, used to treat high cholesterol.

Triad is private and closely held. Its current strategy is to focus on a common gene family called *oxidoreductase*, largely because this gene family is already well understood. Last year there were $20 billion in sales of drugs in this gene family. The lion's share was the statins, which accounted for $17 billion. But the other $3 billion's worth of other inhibitors included the blood-thinner Coumadin and the seizure medicine, valproic acid.

Last year, Triad raised $30 million in a second private round of financing led by CSFB Private Equity, the global private equity arm of Credit Suisse Group. Now they are out trolling for their first collaboration with a major pharmaceutical company. Triad is planning a series-C financing round this year, looking to raise an additional $25 million from private investors. It plans to take its first drug into the clinic by the first quarter of next year. Triad is waiting to hit a couple of milestones before going public, probably by 2003.

The integration of a wide array of technologies beyond structural genomics will be crucial to that success. The greatest advances will be made through the integration of computational methods with physical data obtained from x-ray crystallography, NMR, and other experimental results from a variety of disciplines in the functional genomics era. Companies like Triad are well positioned to combine these important tools.

Right now Triad is focusing on collaborations with pharmaceutical companies. In a typical deal, a client company will bring Triad a protein to be screened. Triad turns it around in a few weeks. Clients will decide, based on in vitro data, whether or not it is worthwhile to take an exclusive license on the compound. But in addition, Triad is also pursuing its own leads to develop into clinical drug candidates.

Programs geared to 3D modeling of protein structure and in silico docking of potential drugs will improve, as more genuine structural information is gathered and used to facilitate connections between gene sequences, the physical conformation of proteins, and, ultimately, their function. The key players in the two segments of the industry—experimental and computational—realize that their tools are complementary and have been rapidly pairing together through collaborations and mergers. These are the sort of companies we like. Big Pharma, having given up on these technologies years ago, is scrambling to catch up, by striking collaborations and acquiring the talent and technologies to bring these tools in-house. They're way behind the curve.

Structural genomics will reduce the time spent turning hits into drug candidates and therefore the time it takes to bring drug candidates into human testing

Structural genomics will reduce the time spent turning hits into drug candidates (called lead optimization), and will therefore lessen the time it takes to bring drug candidates into human testing. Protein structure determination promises to be an excellent way of quickly determining how a drug candidate binds to the target of interest and how to use chemistry to modify the lead compound to increase its binding affinity and improve its properties.

As the number of drug targets expands exponentially, digital methods have the potential to compress the discovery and development cycle drastically. With 40,000 genes in the human genome, the proteome promises to hold many more targets for an industry that has built its business over the last 100 years on a mere 500 targets or so. Harnessing this information will require technology platforms that can rapidly derive meaning from large amounts of information.

Triad Therapeutics is at the vanguard of a group of talented new companies, turning information into biology, accelerating drug discovery at the pace of Moore's law

Triad Therapeutics is at the vanguard of a group of talented new companies, as well as older companies like Serono, that are turning information into biology, accelerating drug discovery at the pace of Moore's law.

Scott Gottlieb
April 9, 2002

BIOTECH COMPANIES

Company	Technology Leadership	Reference Date	Reference Price	4/8/02 Price	52-Week Range	Market Cap
Vertex (VRTX)	Rational Drug Design	9/17/01	28.60	26.85	15.50 - 52.25	2.02B
Human Genome Sciences (HGSI)	Cellular Signaling	10/26/01	43.97	17.58	19.02 - 77.00	2.24B
Nanogen (NGEN)	BioChips	10/2/01	4.95	4.32	3.00 - 10.60	94.0M
Gilead Sciences (GILD)	Rational Drug Design	12/05/01	33.88**	34.79	16.04 - 39.00	6.77B
Quorex (none*)	Rational Drug Design	12/05/01				
Sequenom (SQNM)	Pharmacogenomics	1/09/02	9.00	6.73	5.15 - 18.70	251.4M
MDS Proteomics (none*)	Proteomics	2/05/02				
CuraGen (CRGN)	Cellular Signalling	3/13/02	17.67	14.49	13.87 - 41.34	701.7M
Triad Therapeutics	Rational Drug Design	4/9/02				

* Pre-IPO startup companies.

** Split-adjusted price.

NOTE: This list of Gilder Biotech companies is not a model portfolio. It is a list of technologies in the Gilder biotech paradigm and of companies that lead in their applications. Companies appear on this list only for their technology leadership, without consideration of their current share price or the appropriate timing of an investment decision. The presence of a company on the list is not a recommendation to buy shares at the current price. Reference Price is the company's closing share price on the Reference Date, the day the company was added to the table, typically the last trading day of the month prior to publication. The author and other Gilder Publishing, LLC staff may hold positions in some or all of the companies listed or discussed in the issue.

References

Blundel, T. L., H. Jhoti, and C. Abell. 2002. High-throughput crystallography for lead discovery in drug design. *Nature Drug Discovery* 1:45-54.

Fejzo, J., et al. 1999. The SHAPES Strategy: An NMR-based approach for lead generation in drug discovery. *Chemical Biology* 6:755-769.

Pellecchia, Maurizio, Daniel Sem, and Kurt Wuthrich. 2002. NMR in drug discovery. *Nature Drug Discovery* 3:211-219.

Russell, R., and D. S. Eggleston. 2002. New roles for structure in biology and drug discovery. *Nature Structural Biology* 7:928-930.