

The TOPAZ® System Enables Rapid Discovery at San Diego Biotech Company

If you visit Dr. Mark Hilgers' laboratory at Rx³ Pharmaceuticals (Rx³), you won't see a roomful of liquid-handling robots, storage racks filled with crystallization trays, or other hallmarks of high-throughput protein crystallization. Yet, Dr. Hilgers has been able to exhaustively screen a large number of target proteins and advance six of them to solved structures in just the past six months. His success is due to his lab's ability to generate a large number of variants, on a small scale, and to screen those variants in parallel using the TOPAZ® system. The TOPAZ system is uniquely suited to such a strategy because it delivers experimental throughput and reproducibility while requiring just 1 microliter of protein for 96 conditions.

San Diego-based Rx³ is an emerging drug discovery company whose focus is meeting the acute need for new and more effective antibiotics. "We realized back in the late 90s that knowledge of protein structure is the key to the antibacterial problem," said Rx³ President Dr. John Finn. "Just about every large pharmaceutical company was finding tons of hits using genomics and high-throughput compound screening. But, it's been nearly impossible to generate antibacterials that are optimized for spectrum and potency without knowledge of the target structure." For this reason, Rx³'s strategy capitalizes on structure-based (or "rational") drug design. Historically, an obstacle to this approach has been the high cost of protein crystallization, given both the expense and resource requirements to prepare enough sample for crystallization study.

MINIMAL PROTEIN

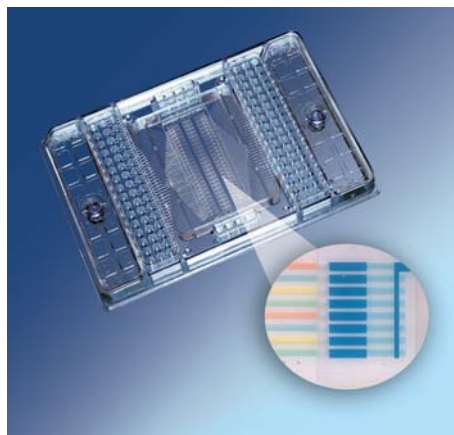
By using the TOPAZ system, Rx³ has managed to significantly reduce its protein crystallization costs. The TOPAZ system has an extraordinarily low sample burden compared to even the lowest-volume drop dispensing platforms because fluids are precisely metered into a highly uniform nanofluidic architecture. Fluidigm's latest crystallization screening chip,

the 8.96, runs 768 experiments in parallel at a sample consumption of less than 15 nanoliters per reaction.

In July of 2005, Fluidigm installed the TOPAZ system at Rx³. Dr. Hilgers first validated the system by running samples known to be difficult, but not impossible, to crystallize. In all cases the TOPAZ system gave results equivalent to those on other platforms. He also ran samples that previously had not been successfully crystallized on other platforms and, in a few cases, the TOPAZ system generated new crystals. Based upon this early success and on the minimal sample requirement, the company moved forward with the TOPAZ system as its front-line screening tool.

AN EFFICIENT APPROACH

The ultimate goal of Rx³ is to design drugs that are effective against multiple species of bacteria. Therefore, drug design at Rx³ benefits from structural knowledge obtained across orthologs. "Each enzyme target will be different, so compounds may bind effectively to one but not to another, which makes characterization so important," Hilgers explained.



The TOPAZ 8.96 Screening Chip is comprised of a network of channels and valves embedded in an integrated fluidic circuit or IFC (center), which is made of silicon. The IFC is encased in a SBS format carrier that accommodates eight distinct samples and 96 unique reagents.

Rx³ Pharmaceuticals at a glance:

Rx³ currently has a broad pipeline of antibacterial programs. Our focus is the design of small molecule drugs with novel modes of action. These drugs will be useful in a broad range of bacterial infections including those caused by resistant bacteria. Our targets have conserved active site molecular architecture throughout a range of pathogenic bacteria. We apply our leading structure-based drug design technology to design multiple safe and specific inhibitors for the targets. The best inhibitors can be optimized to deliver drugs with potent, broad-spectrum antibacterial activity, good drug properties and safety.

Learn more at www.rx3pharma.com.

Rx³ first produces small-scale volumes of several mutants or orthologs in parallel, and then uses TOPAZ chips to identify which among the variants show the most promise for producing crystals suitable for X-ray crystallography. Only then is an informed choice made as to which proteins should be scaled-up for structure determination. Hilgers reports that conditions producing hits in-chip can be reproduced using conventional crystallization methods. These larger, optimized crystals are then interrogated by X-ray analysis for structure determination. "After screening in-chip, I use the resulting conditions repeatedly, soaking the crystals or co-crystallizing the protein with different compounds," said Hilgers. The resulting co-crystal structures guide the design and optimization of compounds that are potent and exhibit broad spectrum antibacterial activity.

Researchers have long recognized the value of generating different orthologs and truncations at the molecular biology level. That's all well known, according to Hilgers. What's new is

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-Mark Hilgers
Rx³ Pharmaceuticals

that now we have the crystallization tools to rapidly sort through those constructs to find those most likely to yield structural data.

CROSS-SPECIES, MULTI-MUTANT PROJECTS

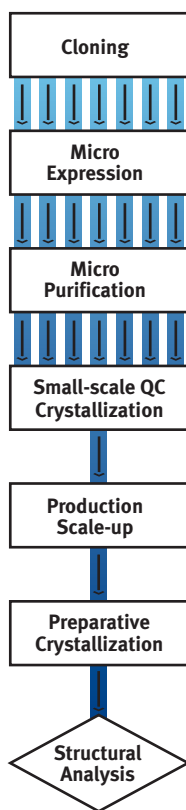
In a recent case, Rx³ pursued a cross-species screening effort to elucidate the structure of Gram-positive Methionyl-tRNA Synthetase (MetRS). MetRS is a gene essential to bacterial viability and an attractive drug target. Scientists at Rx³ approached this task by creating a two-dimensional grid: MetRS cloned from four species on one axis and orthologs of varying lengths or truncations on the other. Ultimately, over two dozen variants were prepared for crystallization screening. Results were analyzed on Fluidigm's AutoInSpeX® Workstation, which automatically assigns priority rankings. Although Hilgers admits that having structural data for multiple organisms remains the ultimate goal, he is encouraged by the company's initial success in solving the structure from *Staphylococcus aureus*.

Rx³ is now in the compound development phase of its MetRS project. If drug candidates bind well to the *S. aureus* enzyme, but less well to the enzyme from other pathogens, Rx³ will revisit the screening process. More granular grids will be developed to obtain the additional structural information that will guide the next iteration of compound design, and allow Rx³ to expand their antibacterial spectrum. On the other hand, if lead compounds show effectiveness for multiple Gram-positive organisms, this is a good indication of the predictive ability of *S. aureus* MetRS and its usefulness as a model. "That we are even in a position to be considering these options was certainly beyond our expectations when this project started six months ago," asserted Hilgers.

The TOPAZ system has also demonstrated its usefulness on other projects at Rx³. In one case, Rx³ had prepared three constructs, all of which had failed to crystallize using

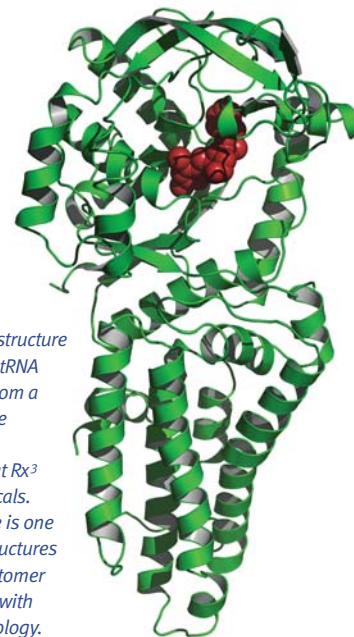
conventional methods. "We never got crystals," said Hilgers, "so we did surface mutagenesis, designing 29 different variants of the protein, each having between one and four point mutations." Those variants were subsequently screened for expression and solubility; seventeen were then advanced to crystallization screening.

Parallel Process with Many Micro Samples



The TOPAZ system provides an efficient means to use crystallization as a positive filter to identify targets that have the greatest potential for downstream success at a time when it is most cost effective.

In two days, Dr. Hilgers, working alone, was able to setup those 17 samples against 384 crystallization conditions, and, within a week, he had selected the most promising screening hits for scale-up. "I followed up these leads by preparing a limited number of hanging drop crystallizations on a macro scale," said Hilgers, "and it turned out that one of them gave me spectacular crystals." It was a matter of a couple of weeks before Rx³ had its prized structure.



The three-dimensional structure of Methionyl-tRNA Synthetase from a Gram-positive pathogen, determined at Rx³ Pharmaceuticals. This structure is one of over 30 structures solved in customer labs in 2005 with TOPAZ technology.

THE RIGHT MINDSET

John Finn and Mark Hilgers agree that achieving a high-throughput protein crystallization enterprise requires what, for some laboratories, may be a new mindset. "It's hard for people to switch from the idea of taking a single protein, purifying it, trying to crystallize it, and failing that, going back to the beginning and repeating the process," said Finn. "Investment in high-throughput robotics will certainly speed up your rate of crystallization screening, but if the process is flawed to begin with, you are apt to simply repeat your failures, albeit at a quicker pace." From Rx³'s perspective, the issue is not so much a question of speed, but of efficiency. Using the TOPAZ system, Rx³ is able to qualify many proteins for crystallization and invest only in those with proven potential, thereby freeing up precious drug discovery resources.

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