BIOCENTURY
Innovations
FROM IDEA TO IND

REPRINT FROM NOVEMBER 3, 2016

TOOLS & TECHNIQUES

ROCKET SCIENCE GUIDANCE

By Selina Koch, Staff Writer

Headed by former SpaceX engineers, Synthego Corp. has put its engineering prowess to work by creating an automated manufacturing process that can pump out nearly pure batches of the first fully synthetic guide RNAs on an industrial scale.

Guide RNAs are a key component of the CRISPR gene editing machinery, normally produced by biological methods, whose role is to target the nuclease, usually Cas9, to the genomic site of interest.

While other companies are tinkering with the composition of the molecules in the CRISPR machinery to increase editing efficiency or accuracy, Synthego thinks using synthetic rather than biologically generated guide RNAs holds the key to both of those things and more — including increasing the consistency of results between experiments, and offering greater ease of use and faster production to shorten experiment times.

CEO Paul Dabrowski told BioCentury Synthego didn't invent new chemistry to create its molecules, but rather increased the precision and throughput of decades-old chemistry by designing automated liquid handling procedures, instrumentation and software that take humans out of the workflow.

"When we saw what was going on in biotech, how people did lab work, we were kind of awe-struck that researchers were still in the lab pipetting, having to do all this manual work and potentially introducing errors. We thought as engineers we could help cut down on the wasted time, the mundane work and the mistakes," he said.

Dabrowski told BioCentury the company decided to focus first on CRISPR because of its potential to create curative therapies and "fundamentally change the way synthetic biology is done."

He added that despite the rapid evolution of CRISPR technology, "it's actually still kind of difficult to do a CRISPR experiment," because conventional methods of creating guide RNAs are costly and time-consuming and frequently result in impurities that sabotage experiments.

Most strategies involve altering the design of guide RNAs to optimize editing by modifying length and sequence structure. But Dabrowski argued that the key to precision and efficiency is to change the way guide RNAs are produced from noisy biological methods, which generate multiple RNA species, to more precise chemical synthesis routes (see "Seeking Purity").

"Our process yields extremely high quality and purity, which are very important if you want to develop therapeutics," said Dabrowski.

> "This is really cheap chemistry from the '80s that you can buy for five or six times cheaper than all the new chemistries. The thing is no one else has had the engineering or automation to be able to make it work."
>
> Paul Dabrowski, Synthego

It's unclear how many competitors Synthego is up against, since companies are continuing to announce interest in developing CRISPR tools as reagents, but heavy hitters range from large, established companies such as the MilliporeSigma unit of Merck KGaA and Thermo Fisher Scientific Inc., to 2013 start-up Twist Bioscience Corp.

Nevertheless, Dabrowski said, "we expect to become one of the largest sellers of CRISPR kits worldwide by early next year."

The company emerged from stealth mode in August to launch the kits, but Dabrowski said Synthego is already selling them in 20 different countries to academic institutions, synthetic biology companies, biotechs and pharmas.

For the last three years, the company has been running on an $8.5 million series A round from Founders Fund and Menlo Ventures, as well as the Chinese firm WI Harper and "a couple of undisclosed rounds," said Dabrowski.

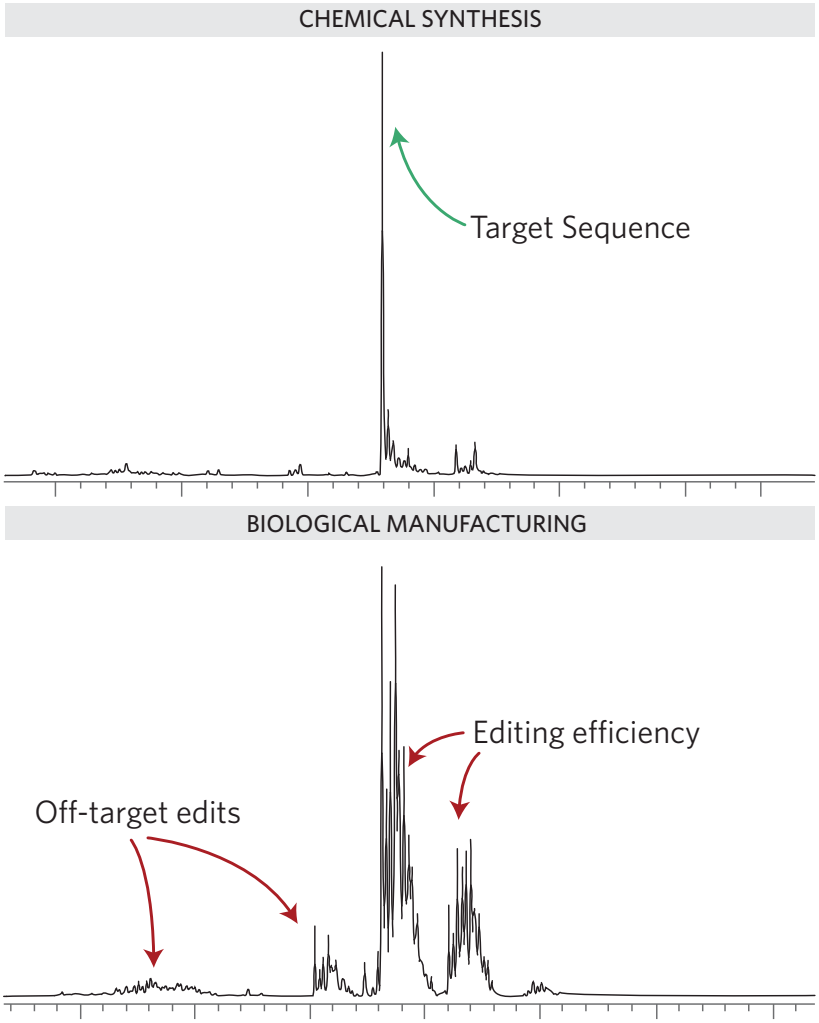
SEEKING PURITY

Synthego Corp.'s automated manufacturing process generates industrial-scale batches of fully synthetic guide RNAs containing far fewer impurities than those produced by conventional, small-batch biological methods. The company thinks the synthetic guide RNAs can increase the precision of CRISPR-based gene editing.

The diagram shows mass spectrometry analyses of the same guide RNA produced either by the company's **chemical synthesis** route or a standard **biological manufacturing** method involving transcription of DNA.

Synthego's product is nearly pure, containing a single peak representing the **target sequence**. However, the biologically produced guide RNA contains a number of additional sequences both shorter and longer than the target. Shorter sequences can increase **off-target edits** because they are more likely to complement multiple DNA regions, while longer sequences can decrease **editing efficiency** if the extra bases do not complement the target DNA site.

Source: Adapted with permission from Synthego.



Now, it's looking to put together a series B.

After that, the company wants to integrate additional services, which will include developing web services for hosting experimental data.

"Our long-term vision is to allow any scientist in the world to access biological experiments in the 'cloud', with quality, consistency, speed, and scalability advantages — much like what Amazon AWS has done for the world of IT," said Dabrowski. "Synthego's hardware and software platforms have been designed and built for this from the beginning."

> "With the old ways of doing CRISPR you might expect 10-20%, or maybe 30% of your cells to be modified correctly, that would be quite good. With our product we routinely see 70-80%."
>
> Paul Dabrowski, Synthego

DNA-FREE RNA

Dabrowski said that until now, almost all guide RNAs have been produced in one of two ways: by encoding them in plasmids and using cellular machinery to transcribe them; or through *in vitro* transcription, which, despite being cell-free, also relies on enzymes and DNA.

The problem, he said, is that transcription is error-prone. "If you use biological methods they're actually very inaccurate and you'll end up making a bunch of sequences that are similar but not exactly right. That increases off-target effects and decreases the on-target effects."

People are also error-prone, said Dabrowski. Consequently, Synthego has forgone traditional RNA production methods in favor of a fully automated procedure that involves using *tert*-butyldimethylsilyl (TBDMS) amidite chemistry to string together nucleotides without enzymes or DNA, and with robotics for precise liquid handling.

"This is really cheap chemistry from the '80s that you can buy for five or six times cheaper than all the new chemistries. The thing is no one else has had the engineering or automation to be able to make it work," said Dabrowski.

In the chemical reaction, TBDMS serves as a protecting group that masks certain functional groups in the nucleotide-containing chemical where a reaction with the RNA strand is unwanted, ensuring binding occurs at the desired reaction site.

"The basic chemical synthesis iteratively builds up your strand of RNA one base at a time. Then you have to basically cut all that RNA off of the support, purify it in an industrial way and quality control it," he said.

He said the platform contains three core steps on which Synthego has done "considerable innovation": liquid handling, RNA purification and quality control.

Perhaps the most important innovation, said Dabrowski, was the development of "a fundamentally novel liquid handling architecture which allows for new types of scientific instrumentation." The ability to develop new instruments then allowed additional innovations in the other two core steps, he said.

Dabrowski said using robotic liquid handling virtually eliminates pipetting errors and variability. "If you look at biology now, everything is moving to liquids," he added.

The other key innovation, said Dabrowski, was the creation of software that can monitor all of the steps in the reaction to ensure quality. "Our software removes any human decision-making from the process, so there's less room for error. If anything's going wrong, or is even a little bit off, we find out really early" and can take the appropriate steps to correct it, he said.

PURE AND SIMPLE

Dabrowski told BioCentury that in addition to fast, low-cost production, which shortens experiment times and lowers barriers to access, the principal advantage of synthetic guide RNAs over standard ones is their purity.

He said Synthego's process more than doubles the number of guide RNAs in a batch with the correct sequence, which increases editing efficiency — the fraction of target cells with the desired genetic modification — and consistency from experiment to experiment, while decreasing unwanted edits.

"With the old ways of doing CRISPR you might expect 10-20%, or maybe 30% of your cells to be modified correctly,

that would be quite good. With our product we routinely see 70-80%," said Dabrowski.

And he thinks the company can push that up even higher. "Some of our clients are already seeing over 90% efficiency. Once you get to 100% editing that's a game changer for therapeutics, and that's a possibility we're very excited about."

He said the company has several collaborations with undisclosed biotechs and pharmas to develop guide RNAs for preclinical research. "In the future, some of those might transition into therapeutics," he said.

Dabrowski said the company has filed several patents covering the software and automation but declined to disclose how many. He noted that because software patents "can be hard to enforce internationally, there's a lot of trade secret component to this too."

COMPANIES AND INSTITUTIONS MENTIONED

Merck KGaA (Xetra:MRK), Darmstadt, Germany
Space Exploration Technologies Corp. (SpaceX), Hawthorne, Calif.
Synthego Corp., Redwood City, Calif.
Thermo Fisher Scientific Inc. (NYSE:TMO), Waltham, Mass.
Twist Bioscience Corp., San Francisco, Calif.

TARGETS AND COMPOUNDS

Cas9 - CRISPR-associated protein 9

REFERENCES

Martz, L. "Writing DNA with a twist." *BioCentury Innovations* **(2015)**

BIOCENTURY INC.

NEWSROOM
pressreleases@biocentury.com

SAN CARLOS, CA
+1 650-595-5333; Fax: +1 650-595-5589

CHICAGO
+1 312-755-0798; Fax: +1 650-595-5589

WASHINGTON, DC
+1 202-462-9582; Fax: +1 202-667-2922

UNITED KINGDOM
+44 (0)1865-512184; Fax: +1 650-595-5589