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THE FATE OF FINGERS

Proteins with 'zinc fingers' designed to bind almost any DNA sequence will soon be available to any lab that wants them — from two very different sources. **Helen Pearson** reports on a revolution in designer biology.

"There is only one word that matters in biology," says pioneering molecular biologist Aaron Klug, "and that is specificity. The truth is in the details, not the broad sweeps." This neatly explains the importance of proteins equipped with structures called zinc fingers, on which Klug's team at the MRC Laboratory of Molecular Biology in Cambridge, UK, did pioneering work in the 1980s and 90s. The human body contains more than 700 different zinc-finger proteins, which bind to specific DNA sequences to switch genes on and off. For almost 20 years, would-be protein engineers have dreamed of taking the DNA-recognition ability of these zinc fingers and making it universal — of designing zinc-finger proteins targeted at any DNA sequence that catches their fancy. The world is about to reap the benefits of a decade of hard work realizing those dreams.

> **"How can we get this in the hands of every lab in the world that wants to use it?"**
> — Edward Lanphier

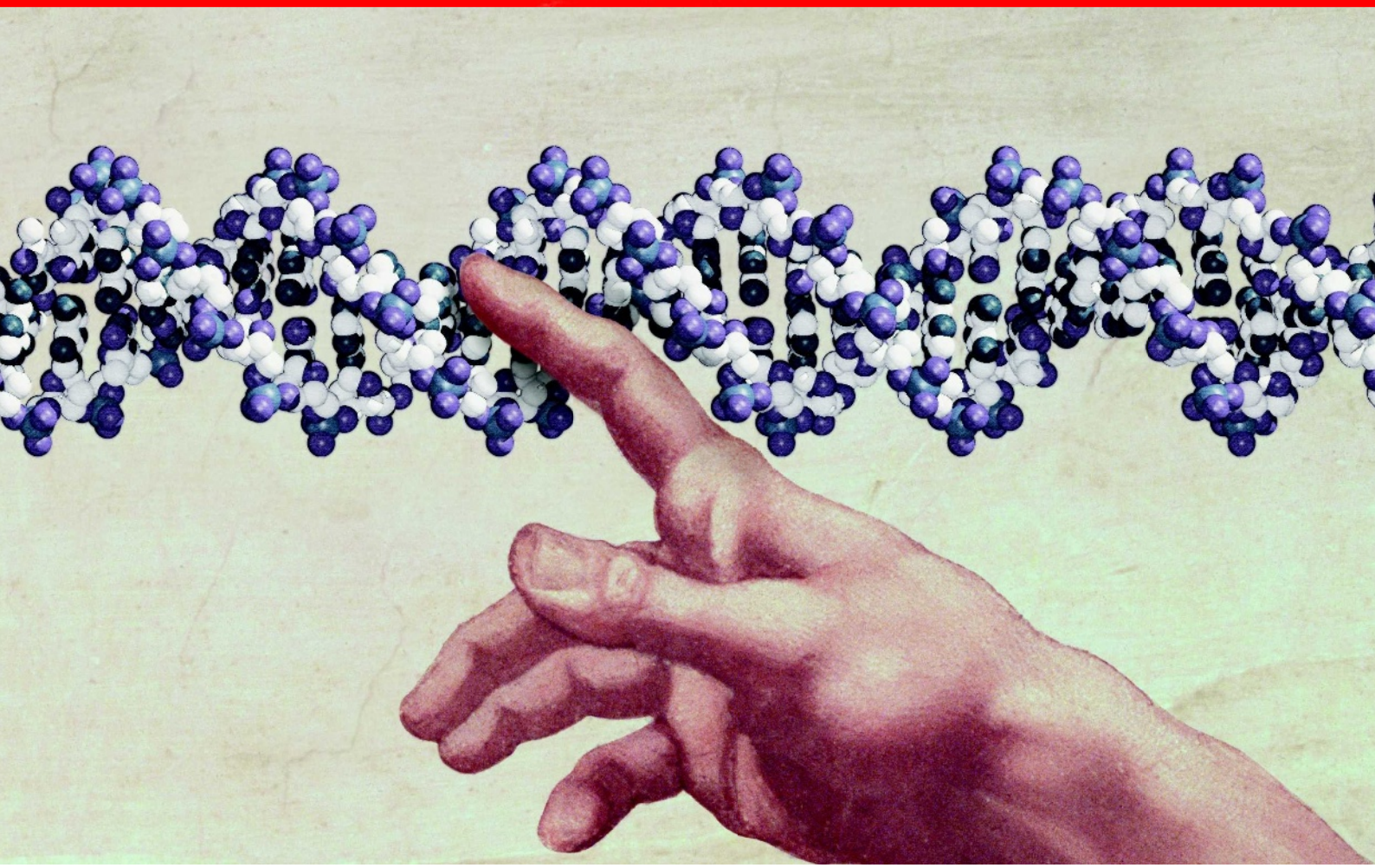
From the middle of September, designer zinc-finger proteins will be available to anyone with an idea, an Internet connection and US$25,000. The reagents company Sigma-Aldrich, based in St Louis, Missouri, has a splashy launch planned for CompoZr, a service that will provide its customers with zinc-finger proteins aimed at whatever DNA sequences they want. The service uses techniques developed by Sangamo Biosciences of Richmond, California. Zinc-finger technology has previously been the preserve of a select few, mostly working with Sangamo; with Sigma's cut-and-paste offering, Sangamo hopes to make zinc fingers far more widely used in commercial research and in academia.

The specificity zinc fingers offer could find a million uses in the lab, revolutionizing the techniques researchers use to work out what genes do. This summer two studies, one from Sangamo[1] and one from Scot Wolfe's lab[2] at the University of Massachusetts, Worcester, showed that bespoke zinc-finger proteins that can cut the DNA they recognize — zinc-finger nucleases — could, in principle, knock out any gene in zebrafish. This is something that researchers working with most model organisms have so far been unable to do with other methods. In an accompanying article[3], Ian Woods and Alexander Schier of Harvard University wrote that such tools might become "the major technology for genome manipulation". "It could have a monstrous impact," says Wolfe.

The technology could also prove valuable in the clinic. Monoclonal antibodies, a previous breakthrough in biological specificity, went through a phase of being just lab tools. Now they are the basis of a therapeutic market worth over $20 billion, and treat tens of thousands of patients annually. Sangamo hopes that therapies that use zinc fingers to turn genes on and off — or indeed to edit them — have similar potential. Earlier this year, in an illustration of what might be possible, the company built a zinc-finger protein that disables a protein by means of which HIV gains access to human cells. When applied in mice, the zinc-finger protein locked the virus out[4].

Exploiting that sort of capability, Sangamo plans first to open up and then dominate a whole new class of therapeutics. On the wall of his office looking towards San Francisco Bay, chief executive Edward Lanphier has framed copies of inspirational headlines from biotech history — such as the *San Francisco Examiner*'s 1980 "Genentech Jolts Wall St", commemorating the stock-market debut of the company on the other side of the bay that has done as much as any to capitalize on the specificity of antibodies. Asked whether he entertains such visions, though, Lanphier demurs: "My hubris is significant, but not that significant."

The CompoZr offering is intended in part to appease researchers who have expressed frustration at not being able to access Sangamo's proprietary technology. But following developments a couple of months ago, those academics may end up spoilt for choice. In July, a powerful new methodology for do-it-yourself zinc-finger



design was published in *Molecular Cell* by a consortium of scientists led by Keith Joung of the Massachusetts General Hospital in Boston[5]. Joung hopes the consortium's protocol — the culmination of ten year's work on his part — will allow any lab, anywhere, to make zinc-finger proteins at a fraction of Sigma's price.

Two ways forward

Joung says that he and the consortium are not seeking to undercut Sangamo, but rather to expand the zinc-finger universe. Still, some will inevitably see the two as rivals — or even as a new round in the struggle between academics devoted to open systems and companies built on the defence of intellectual property. "It's pretty unusual to have an academic research group as committed to an open platform in clear opposition to a firm," says Arti Rai, an expert in patent law and the biopharmaceutical industry at Duke University in Durham, North Carolina, who has studied Sangamo. Michael Eisen, at the Lawrence Berkeley National Laboratory in California, is blunter: "It's nice there's a reservoir of rebellion and people saying 'screw it, we're not going to be held back'."

Although the first zinc-finger protein was discovered in 1985 (ref. 6), the protein-engineering possibilities only really came to the fore in a May 1991 paper in *Science*[7] by Carl Pabo and Nikola Pavletich, then both at Johns Hopkins University in Baltimore, Maryland. This paper revealed the X-ray structure of three zinc-finger domains bound to a piece of DNA. The zinc fingers — each a chain of 30 amino acids folded back on itself and stabilized by a zinc ion — nestle in the major groove of the DNA molecule, touching three of the base pairs that provide the DNA with its sequence. The fingers lie one after the other in the groove, with three fingers recognizing a sequence of nine bases overall. "Everyone who saw that structure had the same thought," says Joung. "It might make a very nice scaffold for making designer DNA-binding proteins." Understand the relationship between the amino acids and the bases and you might design a protein that recognized any sequence of bases you chose, threading zinc fingers together as simply as beads on a string.

> **"By a very aggressive, creative and smart licensing strategy Sangamo has swept up most of the intellectual property."**
> — Arti Rai

Lanphier, though, saw more than an engineering opportunity in the fingers. He saw a business break. Lanphier had spent his career in the business and strategic side of the pharmaceutical and biotechnology industries. In the 1990s he was head of commercial development at a California company called Somatix Therapy, which was developing the vectors that deliver genes into tissues for gene therapy. But although the company controlled intellectual property (IP) surrounding its vectors, others owned the genes that the company might want to put into them. "We were frustrated with the fact that we couldn't access proprietary genes," Lanphier says.

Zinc-finger proteins offered the opportunity to create those genes — newly written genes to describe newly imagined proteins. "If you have a motif that can be engineered, by definition those different proteins will be encoded by different genes," Lanphier says. "So you might have a technology platform here to generate an infinite number of genes." One of those designer genes, delivered to a cell, would make a zinc-finger protein that could control one of the cell's naturally occurring genes. With that in mind, Lanphier flew out to talk to some of the leading academic groups in the field, including Pabo's and Klug's.

Bolstered by those meetings and half a million dollars in venture capital, Lanphier started Sangamo in 1995. He hardly looks the part of a cut-throat monopolist as he pads around his office in hiking socks and Birkenstocks, but he went about developing the company with a fierce focus: he wanted an unassailable IP position. "By a very aggressive, creative and smart licensing strategy they've swept up most of the IP," Rai says. "It's unusual to have this vision."

Both inside and outside Sangamo, early

attempts to engineer zinc-finger proteins were based on two attractive and sometimes complementary ideas. One was the *a priori* approach: work out the rules specifying which amino acids direct a finger to which of DNA's 'letters' (the bases A, G, C and T) and rationally design new fingers. The other was the 'look-for-what-works' approach: design or try to identify zinc-finger domains that bind to each of the 64 possible three-letter sequences, and then mix and match them like Lego blocks to fit whichever base sequence is wanted.

If only the science of life were so simple. Researchers soon realized that there was no simple code, and that 'modular assembly' was not as easy as it looked. For one thing, the sequences bound by a series of zinc fingers turned out not to be completely distinct from each other but instead overlapped: the same DNA triplet can be touched by amino acids from more than one finger (see 'In the groove'). Almost everyone in the field came to believe that each finger had to be chosen in the context of the fingers next door. "You have to take into account the overlap or the failure rate is very high," says Mark Isalan of the EMBL/CRG Systems Biology Research Unit in Barcelona, Spain, who worked in Klug's lab and devised a way to design proteins that took context into account[8]. He took the technology forward at Gendaq, a company co-founded by Klug.

In April 2000 — amid a frenzy of anticipation over the human genome sequence — Sangamo took its stock public, raising $50 million in a day when the exuberant market valued the company at $375 million. In 2001, it bought Gendaq, and Isalan's approach has formed the basis of the company's protein building ever since. Flush with new money and science, Sangamo started to hit its stride.

Mix and match

Joung first got to grips with zinc fingers in 1998, when he joined Pabo's lab at the Massachusetts Institute of Technology (MIT) in Cambridge as a postdoc inspired by the idea of designing proteins *de novo*. Then — as now — he set about the task by a process of selection: "evolution in a tube", he says. Joung builds libraries of zinc-finger combinations, gets them mass produced in bacteria, and then identifies those that are best at binding a specific sequence. The strength of this approach is that if you start off with combinations of zinc fingers, rather than looking at them one at a time, the issue of context gets dealt with. The problem is that to cover all the possible combinations of key amino acids in a three-finger protein you would have to make 8×10^{24} different proteins. Joung calculates that working with such a library in bacteria would require an agar plate around 100 times the size of North America. By the end of his postdoc his library-building and selection method worked — but only for finding a single finger, not a set[9].

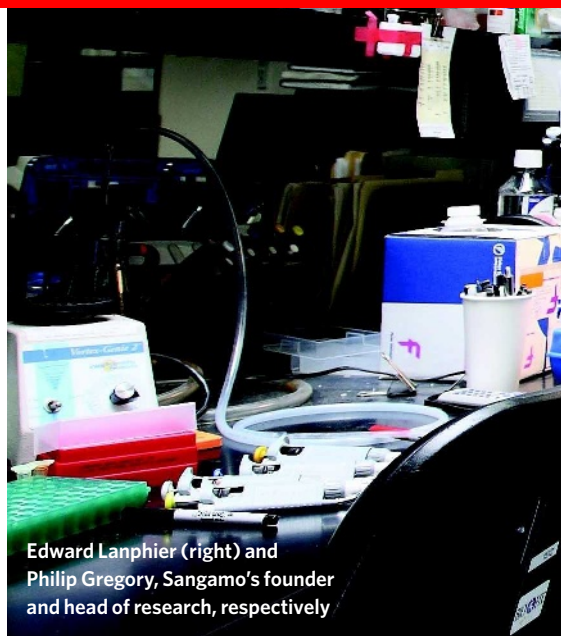
In September 2001, Pabo told his lab that he was moving to California to work for Sangamo. Joung says there was some discussion of him moving to Sangamo, too — the company had assessed his technology and licensed it, although it didn't end up using it — but he wanted an academic position, and one became available at Massachusetts General Hospital. Isalan, as it happens, made a similar decision: "[Sangamo] offered me a job but I'm really a scientist, and by that stage it was a production line," he says.

In the development of that production line, the company has built an extensive library of mainly two-finger proteins, designed so as to take into account interactions between neighbours. When the company's scientists want to design a protein for a new target gene, they feed the gene sequence into a computer program, which tells them various ways to piece together two-finger modules into a four-finger protein that might serve, and predicts how well each option will work. A robot then assembles the DNA for the new zinc fingers, picking out genetic fragments encoding the various modules from a standardized set of 384-well plates. "We've spent a lot of time and energy developing this kit of pieces, information about how these pieces function and then the software that knows best how to use them," says Jeff Miller, a one-time lab-mate of Joung's who made the transition from MIT to Sangamo with Pabo.

And the amount of time and energy Miller and his colleagues spent on honing their approach, not to mention the $230 million the company spent on this and other R&D, is far beyond what an academic group could hope to repeat. Another company might, but although no one can really know the strength of an IP position until it is tested in court, Sangamo's seems to have been strong enough to scare off most competition.

It's possible that this is good for the company but bad for the field. Richard Jefferson, founder of CAMBIA, a non-profit organization based in Canberra, Australia, that supports open access to life-sciences technology, claims that if a company had pursued monoclonal antibody or DNA sequencing IP as diligently as Sangamo has pursued zinc-finger IP "it would have set [those fields] back a long way". Lanphier rejects the idea: "On the contrary, I would argue that our patents have allowed us to access the funding that we have used to advance zinc-finger technology."

Sangamo has not just been amassing IP and developing its design processes: it has also started showing what zinc fingers might do. Much of the recent excitement in the field has focused on the zinc-finger nucleases, originally discovered in 1996 (ref. 10). In 2005, working with Matthew Porteous of



Edward Lanphier (right) and Philip Gregory, Sangamo's founder and head of research, respectively

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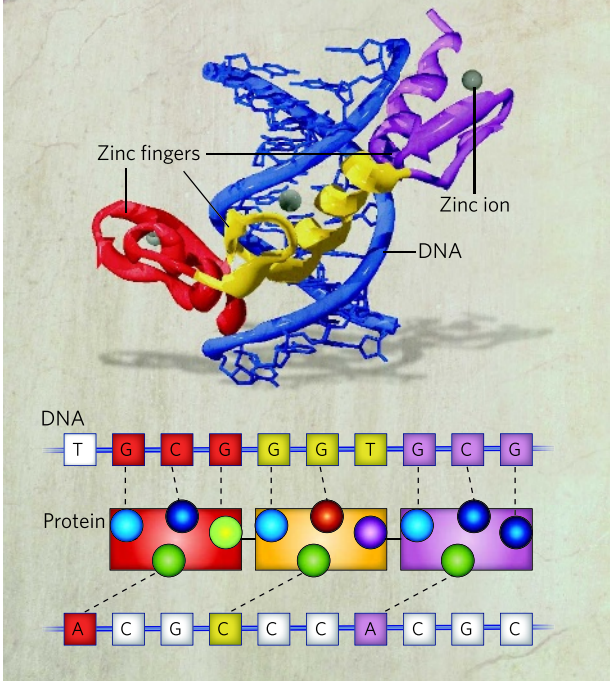
IN THE GROOVE

Three zinc fingers of the protein Zif268 nestle alongside the bases in their target stretch of DNA (top). Amino acids in each finger associate with specific bases in the DNA (bottom).



SOURCE: JAMIESON, A. C. *ET AL. NATURE REV. DRUG DISC.* **2**, 361–368 (2003)

the University of Texas Southwestern Medical Center in Dallas, scientists at Sangamo showed that a zinc-finger nuclease aimed at a mutation in a gene called *IL2Rγ* erased the mutation in over 18% of cells, converting the gene into one that worked properly[11]. The mutation in question causes X-linked severe combined immune deficiency (SCID), a fatal genetic disease; the results looked all the more promising because gene-therapy trials for SCID a few years previously — in which a working copy of the gene was introduced into cells — had been successful in compensating for the defect but resulted in several children developing leukaemia. "People were very excited," Porteous says. The efficiency was good enough that if it could be made to work *in vivo* it might actually cure people.

Spreading the message

Sangamo has been racking up further scientific and commercial testaments to the potential applications of mutation-correcting zinc-finger nucleases. Plant biologists are drooling with delight over the enzymes, because they should enable precise genetic modifications to be made in plants to genes that have been impossible to target precisely with conventional techniques — opening up new possibilities for adding to, knocking out or overwriting genes associated with yield, nutritional content, pest resistance and other useful traits.

Zinc-finger believer: Keith Joung.

The company has an agreement with Dow Agrosciences, based in Indianapolis, Indiana, to develop the technology for plants. "It's revolutionizing plant breeding — that's not too strong a word for it," says Klug, who serves on Sangamo's scientific advisory board. Together with drug giant Pfizer and Genentech, Sangamo is also engineering cell lines to improve their protein productivity. And the company has an agreement with Genentech's corporate parent Roche for the creation of transgenic cells and animals.

But as Sangamo went from strength to strength, progress in the field as a whole was not so inspiring. Only a handful of groups, such as Joung's and Wolfe's, were able to make proteins using their own methods. "Many people have got excited by the technology and try it and it doesn't work and they give up," Isalan says. "It's been a real problem." Sangamo thus became a target of envy. "I think there has been some frustration," says Porteous, who no longer collaborates with the company. "They publish and present at conferences as if they've invented sliced bread, but they are very closed about helping anyone else do it."

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> — Mark Isalan

Joung — who, were he not devoted to the lab, would make a first-rate diplomat — is less critical: "I think Sangamo has done a really amazing job of moving the field forward." The problem he saw was not that Sangamo had too much technical ability — it was that the rest of the world had too little. "For many years I'd felt academics needed to develop a technology of their own," he says. "Sangamo was putting so much time and resources into this, there was no way that one lab was going to be able to keep up with them, so if we were to stay relevant to the field we had to band together."

In the summer of 2005, Joung got in touch with another zinc-finger veteran, Dan Voytas, then at Iowa State University. In 2000, Voytas had started a small company called Phytodyne to develop zinc-finger nucleases for plants. Voytas says that the company folded in 2004 after failing to come to a deal with Sangamo over access to the company's proteins. "We were bumped out of business. Their pockets had more cash in them than ours." (Sangamo says it would have loved to collaborate, but Phytodyne's lack of funding made it too unstable to partner with.) The zinc-finger consortium that grew out of a dinner Voytas and Joung had later that year now has 14 member labs, including many big names in the field, but most of its protein engineering has been done in Joung's lab.

Joung's current technique uses a battery of small libraries, or what he calls 'pools', that each contains just less than 100 proteins selected to bind a target sequence. One pool, for example, is full of protein domains that bind well to the sequence GAA, but only when they are the first finger in a three-finger protein. Another pool contains domains that bind to GAA when they are the second finger, and so on. To create a three-finger protein that binds at a specific sequence, three pools are combined and subjected to a second round of selection, from which the best-binding proteins are pulled out. "This is an advanced form of mix and match," Joung says. Sixty-eight of 192 possible pools now sit behind the ice-caked door of a −80 °C freezer off his lab.

Joung doesn't have a robot — but two years ago he hired a young technician called Morgan Maeder and she has been spending near-robotic hours honing the technique. The first time she showed that the pools had generated a zinc-finger nuclease that corrected a gene in human cells "was the most exciting thing ever",

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A. BEKKER

Production factors: Morgan Maeder in Joung's lab (top); Jeff Miller and Sangamo's robot (right)

she says. "Even Keith was giddy; he said 'give me a high five' and we were jumping up and down, it was so exciting. For me it's two years' worth of work — for him it's a whole career." On 24 July, the consortium published Joung's methods for zinc-finger protein engineering[5], along with work from other members of the consortium showing that the proteins accurately cut genes in human and plant cells. They named the technique OPEN, for Oligomerized Pool Engineering. Joung is now arranging to make the pools available to other labs; he estimates the set will cost around $5,000. But the technique is hardly workaday. Maeder says her protocol runs to 20 pages.

Porteous says he would have liked to be a fly on the wall in Richmond when Lanphier's crew first saw the OPEN paper. Whatever such a fly might have heard, though, in public Sangamo has no problem. The company is much more focused on its first clinical results. Later this year, phase II trials will reveal whether a zinc-finger protein designed to activate a growth factor called VEGFA has promoted the recovery of damaged nerves in diabetics: the first chance for zinc fingers to show beneficial effects in humans. And in the next few months the company also plans to file two investigational new drug applications with the US Food and Drug Administration to test two zinc-finger nucleases — including one that disrupts the HIV receptor CCR5 — in humans for the first time.

Philip Gregory, Sangamo's vice-president of research, does not think that OPEN will be much competition for the click-and-go convenience of ordering from Sigma. "If you're a biologist and want to knock out a gene you might not want to set up as a zinc-finger lab to answer that one question. So with labs less fiscally constrained the advantage of working with a quicker product, and what's probably a better product anyway, will be convincing."

But convenience does not always win out. Eisen sees parallels with the stand-off in the 1990s between Affymetrix of San Jose, California, and academics, including Eisen, who thought the cost of the company's DNA chips was restricting research and published their own system for manufacturing microarrays that did the same thing. "Just the mindset — the transition from thinking it's inaccessible to something people could use — was important," he says. "It allowed arrays to be integrated into the fabric of research in a way that they wouldn't have if they had remained a commercial product only, and I can see exactly the same here."

An integrated, improvable, hands-on experience might give OPEN an edge in some academic settings. But that could suit Sangamo fine. Success for the consortium will increase the total amount of zinc-finger research, and that may matter more to Sangamo than what reagents are being used. Lanphier says that one of the original motivations for the CompoZr service came from Sangamo's management asking themselves why biotech companies focused on RNA interference were gaining value while theirs wasn't. They concluded that RNAi was a hot technology in part because it was widespread. "RNAi was ubiquitously available and easy to use, high-school kids were using it," recalls Lanphier. "So we said: 'how can we get this in the hands of every lab in the country or the world that wants to use it?'." The more zinc-finger research is done, in this analysis, the more exciting Sangamo looks.

> **"All the consortium is doing is loading up Sangamo's pipeline."**
> — Richard Jefferson

And the more commercial possibilities it might have. Sangamo is confident that when that research gets to the stage of generating products, its hard-bought IP position will win through. "Ultimately, from a commercialization perspective, you'd run into Sangamo's IP at some point," says Gregory. If Sangamo's patent position is as strong as the company claims, then "all the consortium is doing is loading up Sangamo's pipeline", says Richard Jefferson.

Since the OPEN paper came out, Joung says he has had some 15 requests for the pools. Now that researchers have the proteins in their hands, he says, they can start to tackle all kinds of other pressing questions, such as how to deliver them efficiently to cells, and whether they might make unwanted cuts in the genome. "Unless you were willing to use [Sangamo's] proteins there was nothing to work with — now we can ask those questions," says Joung.

But for all the possible applications, his personal fascination remains the motif itself. "I look at the sequences as a way to see that they worked, but I don't really care what they are," says Maeder. "But he loves it. Every time it works, it's like further proof that the past ten years of his career have been worthwhile." Joung and some of his colleagues still think it's possible that there is some kind of code — an algorithm for writing the best amino-acid sequence with which to target a piece of DNA from first principles. It's even conceivable that the tools with which to crack that code are sitting in the databases that are now filling up with successful designs, awaiting release through bioinformatic wizardry.

Still, complex though it is, there's something satisfying about the current process — at least to Joung. "You start with 200 million different things and you end up with this very small number that are very similar," he says. "It's exciting and gratifying to me that the system actually works and that you don't always get the same finger depending on which context it was selected in. I had the thought nearly ten years ago, so yes, it's pretty cool to see it come out this way." To come out in all its glorious specificity — which is what matters in biology. ■

Helen Pearson is *Nature*'s biology features editor.

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