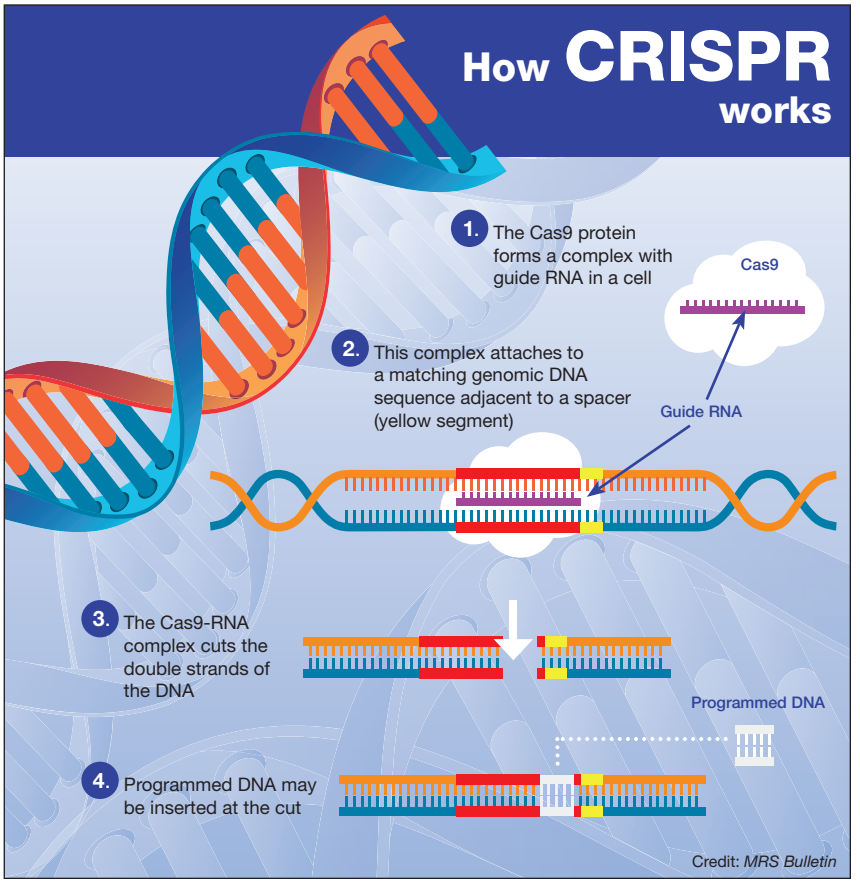
CRISPR: Implications for materials science

By Philip Ball

The ascent of CRISPR

The latest possibilities for editing DNA with pinpoint accuracy are transforming genetic science and may soon have biomedical implications for humans. It has been less remarked, though, that the new technology should have implications outside the life sciences, in particular for the design and synthesis of new materials. Not only might it streamline the modification of living organisms so that they can produce useful materials or their precursors, but it can also expand the possibilities for using DNA itself as a constructional material.



CRISPR-Cas9 is a method of genome editing that exploits a natural DNA-snipping enzyme in bacteria, called Cas9 (**C**RISPR-**as**sociated protein **9**) to target and edit particular genes. CRISPR stands for **C**lustered **r**egularly **i**nterspaced **s**hort **p**alindromic **r**epeats, which are segments of DNA of a particular structure found widely in bacteria and archaea (prokaryotes). In the wild, the CRISPR-Cas9 system is part of the prokaryotic immune system, which can snip out of the genome DNA acquired from foreign sources such as phages (bacterial viruses). The same molecular machinery is now being used to enable genetic material to be cut from and pasted into the genomes of other organisms, including eukaryotes such as humans. It might offer a tool for curing genetically based diseases.

DNA has become a versatile polymeric substrate for making nanotechnological structures and artificial molecular-scale machinery for computation, pattern formation, and nanoscale assembly. For several decades now, these efforts have drawn on methods developed in and for biotechnology, and similarly they are likely to find ways of exploiting the advantages of the new technique called CRISPR/Cas9 for manipulating DNA.

Devised in 2012, CRISPR/Cas9 exploits a natural DNA-snipping enzyme in bacteria, Cas9, to target and edit particular genes. The target sequence of the DNA is recognized by a matching sequence on a "guide RNA" molecule carried alongside Cas9. This enables, for example, modified forms of the respective genes to be pasted into a genome. The method, and related approaches using other enzymes of the Cas family, could potentially supply a powerful way to cure diseases caused by mutations of one or a few specific genes, such as muscular dystrophy and thalassemia. A US clinical trial to assess the safety of CRISPR/Cas9 in a form of cancer therapy that enlists the body's immune response to fight tumors has already received approval. The discovery of the technique, for which the key contributions are generally attributed to biochemists Emmanuelle Charpentier, Jennifer Doudna, and Feng Zhang, is now widely tipped for a Nobel prize.

DNA nanotechnology and materials

Such a tool for targeting and editing DNA with high precision could be a fantastic addition to the toolbox of the many researchers seeking to repurpose this programmable molecule for making artificial, self-assembling constructs and materials.

That vision began with the pioneering work of Nadrian Seeman of New York University, who showed in 1991 that DNA strands could be encoded with the information they need to assemble spontaneously into cube-shaped molecules. Since then, the field of DNA nanotechnology has expanded in many directions. It has been used for complicated "molecular origami," such as boxes with lids that can be opened with a molecular trigger, and extended two-dimensional patterned arrays.

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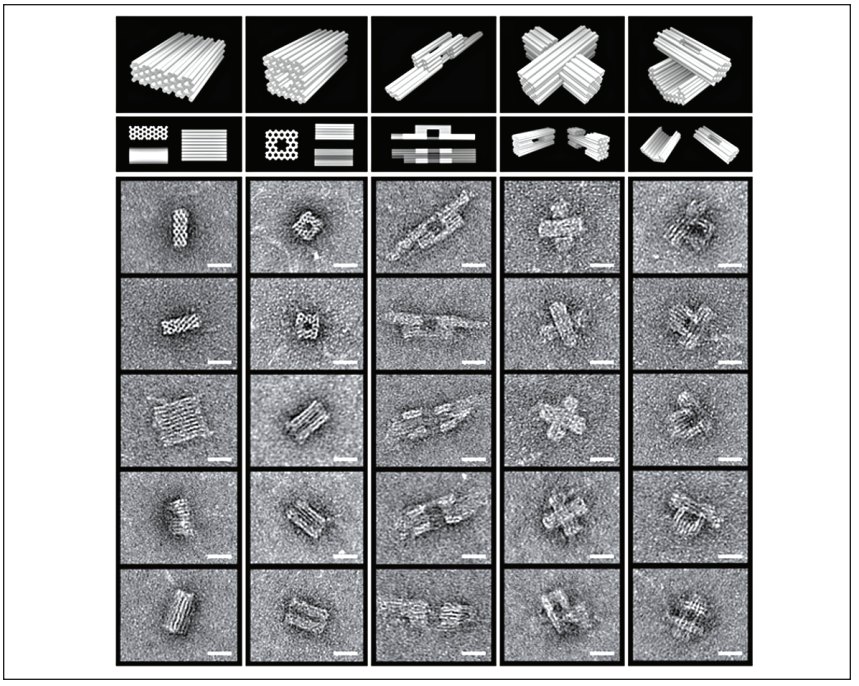
Erik Winfree and co-workers at the California Institute of Technology (Caltech) have developed a form of DNA computation in which tile-like structures are programmed to assemble like cellular automata. Several researchers have made DNA molecular machines that can change shape in a controllable and perhaps cyclic fashion. In 2006, Paul W.K. Rothemund, also at Caltech, showed how to program DNA sequences systematically so that they fold up into any arbitrary structure—an approach that can now yield intricate three-dimensional (3D) architectures. DNA has proved useful for boosting the performance of photonic devices such as organic light-emitting diodes, assembling nanoparticles, and storing data.

DNA nanotechnology is now moving from the test tube to living cells, for example for imaging, drug delivery, and smart therapeutics. Some of this work overlaps with the uses of artificial DNA in synthetic biology, where for example some researchers imagine creating autonomous DNA machines to monitor health in the body and respond to the threat of disease. Carolyn Bertozzi and Zev Gartner of the University of California, Berkeley, have shown that DNA tags on the surfaces of living cells can be used to assemble them in a programmable way into controlled micro-tissues: "living" biomaterials, you might say.

Underpinning all of these efforts are advances in biotechnology and chemical synthesis that have made it possible to specify the precise base sequence of artificial DNA strands, so that the way they stick together through base-pairing into double helices and other structures can be predicted and directed. The CRISPR/Cas9 method makes this kind of control easier and more precise. But where exactly might that be useful for DNA nanoengineers?

One answer is that it might simply help to make the long strands needed for DNA origami. "At the moment, DNA origami relies on a natural long DNA from the M13 bacteriophage [a bacterial virus]," says biochemist Anthony Genot of LIMMS/CNR-IIS and The University of Tokyo, "because it is difficult to synthesize chemically strands longer than 100–200 nucleotides." CRISPR could be used to splice fragments into longer strands and police the accuracy of their sequences.



Three-dimensional DNA origami shapes. Scale bars: 20 nm. Credit: *Nature* **459**, 414 (2009).

CRISPR might also be used to introduce functionality into DNA origami, says Chase Biesel of North Carolina State University, who uses gene editing to engineer gut bacteria. "I can imagine CRISPR/Cas9 being used to actively modify such nucleic-acid constructs," he says—"either by cleaving existing structures to drive large structural changes or to release an entrapped chemical."

Functional DNA circuits

Increasingly, researchers are interested in finding ways of combining artificial DNA strands and machines to make functional circuits that can carry out specified tasks, such as the computational processes observed by Winfree and others. André Estevez-Torres of the Laboratory of Photonics and Nanostructures, near Paris in France, working with Yannick Rondelez at The University of Tokyo and colleagues, has made DNA constructs that interact to generate copies in an autocatalytic process, producing a so-called reaction-diffusion chemical system which can form macroscale tunable patterns. Such interplay of DNA strands can also be considered a form of computation.

"One of the challenges is to scale up these circuits," Rondelez's colleague Genot says, "and readout is one of the bottlenecks. We need better ways to monitor our DNA systems to debug and optimize them." So far, one way of keeping track of what specific DNA sequences are up to in a dynamic system is to tag them with fluorescent nanoparticles that are themselves labeled with a complementary DNA sequence. "But the number of fluorescent colors that can be read simultaneously is severely limited in practice to 5 or 6, whereas we're aiming for systems with hundreds to thousands of strands," Genot explains.

"How can we encode information about our DNA circuits in DNA strands to be read by the next generation of sequencing techniques?" he asks. "I think CRISPR/Cas9 could be handy for that." In this context, one might regard the Cas9 enzyme within the same paradigm as the artificial DNA constructs themselves: as a kind of programmable machine, instructed in this case by the guide RNA. Genot imagines repurposing the enzyme as a

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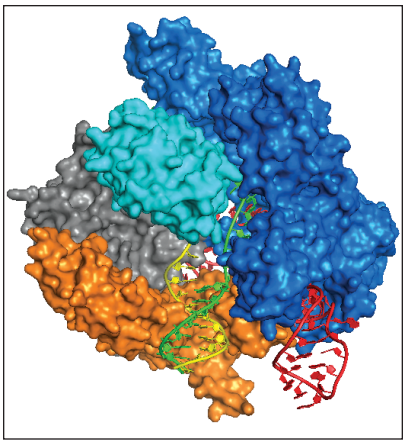

Crystal structure of Cas9 bound to DNA. Rendition was performed using UCSF's chimera software.

molecular recorder that detects the presence of certain DNA strands and encodes this information by editing a long DNA sequence at precise positions—a kind of molecular memory. "By reading which sequences were edited and by how much, we could reconstruct the history of the DNA systems," Genot says. "That could be essential for complex computing."

There have already been preliminary efforts to create such a "cellular recording device" for living cells (bacteria) using the CRISPR/Cas system. Genot sees potential for merging this sort of DNA-based information processing with cells to produce another kind of living biomaterial, for example by enabling artificial tissue growth in an adaptive manner. "A molecular computer could grow some biomaterials or tissue, remembering its past actions to determine the next chain of instructions," he says—that is the sort of responsiveness to circumstance exhibited by tissues in the body. But this, he admits, is still a distant and speculative prospect.

DNA is showing immense potential as an ultrahigh-density data storage system in its own right, because it can encode information at the molecular scale in a way that can be easily copied, amplified, and edited. Here, says Biesel, "CRISPR could be used to actively modify such a memory or affect which part of the memory is accessible." Already, computer scientist Olgica Milenkovic and co-workers at the University of Illinois at Urbana-Champaign have shown how CRISPR/Cas9 may be used to find and amplify or rewrite data stored in strings of DNA in a kind of biochemical random-access memory. They used the memory to encode and edit parts of the Wikipedia pages of six US universities.*

Can DNA nanotechnology enhance CRISPR?

DNA nanotechnology expert Hao Yan of Arizona State University thinks that the benefits of CRISPR/Cas9 could be two-way: DNA nanotechnology, he says, might be harnessed to provide molecular machinery that enhances the performance of CRISPR/Cas itself. "I envision that the marriage of DNA nanotechnology with CRISPR might overcome some shortcomings of the CRISPR/Cas9 technology, such as the high off-target rates"—the tendency of Cas9 to hit the wrong sequence, which seems to be a challenge for safe use in humans. "DNA technology-mediated delivery of CRISPR/Cas9 might also provide new ways to probe cellular pathways by allowing specific knockin or knockout of target genes or regulatory elements," Yan says.

Given that living organisms are currently being re-engineered in biotechnology and synthetic biology as "living factories" for making materials, there could also be indirect impacts of the new high-precision gene editing in materials technologies. Chemist Yamuna Krishnan of The University of Chicago sees a possibility to use CRISPR/Cas9 to "tailor organisms to metabolize inorganics and produce inorganic materials"—for example, retooling existing organisms that already process inorganics, such as magnetotactic bacteria and fungi, "to produce a panoply of inorganic nanoparticles in a truly 'green' way." Just as synthetic biologists are hoping to rewire microorganisms to make biofuels, hydrogen gas, and other useful chemical commodities, microorganisms might also be designed for constructing complex inorganic and composite functional materials. CRISPR/Cas9 seems sure to facilitate such efforts.

In short, it seems that the collaboration of CRISPR/Cas editing and artificial DNA-based nanosystems and materials will be limited only by our imaginations. As with any tool, the challenge is to figure out creative ways of using it.

*The universities were the University of Illinois at Urbana-Champaign; University of California, Berkeley; Harvard University; the Massachusetts Institute of Technology; Princeton University; and Stanford University.

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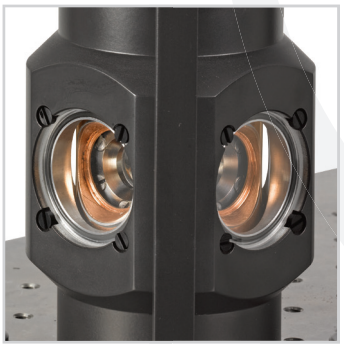

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