Click chemistry revolutionised our ability to create custom proteins, so could it do the same for DNA? Professor Tom Brown at the University of Oxford and Professor Afaf El-Sagheer at the University of Oxford and Suez University are performing truly groundbreaking research into the application of ‘click chemistry’ bioconjugation techniques to DNA. Their technique allows the creation of DNA and RNA constructs larger than any other chemical synthetic route and is more flexible than the use of enzymes. This is made possible by a novel chemical linkage which is bio compatible with DNA-manipulating cellular machinery in E. coli and human cells.

Every so often an observation, experiment or discovery changes more than just scientific understanding. Darwin, Copernicus, and Newton’s breakthroughs, while scientific, also had significance for philosophy, religion and metaphysics.

Perhaps Friedrich Wöhler’s 1828 finding – that urea can be made from potassium cyanate and ammonium chloride – should also be included in this list. Urea, in his own words an ‘animal substance’, was the first to be created without any biological starting materials, signalling the end for the doctrine of vitalism.

In the decades since Wöhler, the list of biochemicals created using purely chemical means has grown longer, and the molecules themselves have grown larger and more complex. Notable early advancements include glucose, vitamin B12 and insulin; more recently larger molecules have become possible, including chemotherapies, antibiotics, and even proteins. But despite our progress, there is still no effective chemical synthesis of probably the most famous biomolecule of them all: Long DNA.

The modern technique for constructing DNA sequences involves using biological enzymes to ligate (join together) short synthetic sections of DNA, called ‘oligonucleotides’ or ‘oligos’. Multiple oligos can be assembled into genes and plasmids.

Oligos are almost always produced using the solid phase phosphoramidite method. The error rate in this method is reasonably low – enough to create oligos typically around 100 bases long. However, at around the 150 bases mark, errors start to become a problem. As a result, long segments of DNA require large numbers of oligos, requiring complex biochemical techniques.

El-Sagheer and Brown’s triazole linkage is a unique example of a bio compatible artificial DNA linkage formed using chemical synthesis.

Since 2007, Afaf El-Sagheer and Tom Brown have been pioneering an alternative approach to the chemical synthesis of large DNA and RNA strands. Their premise is that longer oligos could be created from shorter oligos using chemical ligation rather than enzymatic ligation.

But this is no mean feat: it’s not possible for the chemical ‘links’ to be exactly the same as the natural phosphoester linkage found in natural DNA. Instead, the chemical links in these DNA replicas need to be ‘biocompatible’ – indistinguishable to normal enzymes from the real thing.

Finding a chemical reaction which produces a biocompatible chemical linkage is a major milestone in El-Sagheer and Brown’s work. The researchers outlined in their 2009 research how they use ‘click chemistry’, a set of chemical reactions known to efficiently form bonds between biomolecules and other molecules, a process known as bioconjugation.

Click chemistry reaction” by Sharpless, a pioneer of click chemistry techniques. CuAAC creates a triazole linkage – a five-membered ring of atoms containing two carbon and three nitrogen atoms. Initial experiments showed that it was possible to combine various oligomers using a version of CuAAC which joined two ‘T’ nucleotides with a triazole linkage.

These oligomers were then ‘amplified’ using PCR – the biochemical process used to replicate a DNA sequence. In PCR, the oligomer molecules containing the triazole linkage need to be ‘read’ by DNA polymerase, the key enzyme in the replication process. If the triazole linkage is biocompatible, then the PCR process should produce copies of the oligomer sequence. These resulting copies would not contain the triazole linkage.

DEVELOPING BETTER LINKAGES

With the promise of biocompatibility, El-Sagheer and Brown began redesigning similar linkages, applying their technique to both DNA and RNA.

In 2010 work, they demonstrated that a redesigned triazole linkage could be used to synthesise ribozymes, which are a type of cellular machinery formed from RNA. RNA is a similar molecule to DNA in many ways – it’s a polymer composed of nucleic acids – but it does have some key differences which make long RNA...
Behind the Research

Professors Afaf El-Sagheer and Brown investigate the chemical synthesis of long DNA and RNA strands.

Research Objectives

Mimicking DNA: using click chemistry to create DNA with a unique triazole linkage.

MEETING THE DEMANDS OF CRISPR

El-Sagheer and Brown's triazole linkage is a unique example of a biocompatible artificial DNA linkage formed using chemical synthesis. Almost 200 years on from Wöhler's synthesis of urea, the enzyme-free synthesis of DNA—or at least a functional mimic of it—is now possible, using click chemistry.

Details

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Afaf El-Sagheer and Brown were able to cross-link separate strands of oligomers together to form two RNA constructs, both almost 100 nucleotides in length. Both the constructs—a hairpin ribozyme with cross-links, and a hammerhead ribozyme with the improved linkage at the active site—functioned like their natural equivalents.

Repeating the test on E. coli cells deficient in DNA repair enzymes gave a similar survival rate, demonstrating that the success wasn't down to the linkage being 'corrected' by E. coli. The link is truly biocompatible with DNA-reading enzymes.

The resulting sgRNA allowed gene editing in cells with no unexpected off-target effects. Perhaps CRISPR will be where the triazole linkage leaves its mark on DNA chemistry.

References


In their 2019 research published in Nature Communications, the group detailed how the CuAAC reaction can be used to produce a library of sgRNAs in a single tube—by ligation of the two parts of sgRNA oligomers using click chemistry. The advantage of using this technique is that the Cas9-binding oligomer could be produced at a large scale, meaning only the 20-base oligomer needs to be tailored, and the two halves can be ligated.

The resulting sgRNA allowed gene editing in cells with no unexpected off-target effects. Perhaps CRISPR will be where the triazole linkage leaves its mark on DNA chemistry.

200 years on from Wöhler's synthesis of urea, the enzyme-free synthesis of long DNA—or at least a functional mimic of it—is now possible.

Personal Response

What would it take for this procedure to become a commercially available product?

The basic research has been carried out and patents have been filed. The next step is for an industrial partner to work on applications that could benefit from the approach. This could be for example in CRISPR/Cas but there are many other potential areas where click-ligation could offer advantages, such as modified genes, especially with epigenetic modification which can play a vital role in gene therapy.