Life orthogonal

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It is surprisingly common for us to think that we are the apex of evolution. Similarly, it is easy to convince ourselves that after nearly 3bn years, life on Earth has tried everything: every possible chemical reaction, every possible solution and every possible arrangement. Fortunately, both statements are wrong. We now know that the DNA (and RNA) life on Earth is one solution, of many, on how chemistry can turn towards life. As we discover new ways to code genetic information, in what is an interdisciplinary basic science endeavour, we develop many of the technologies that will establish significant biotechnological and life-saving applications. Welcome to life orthogonal.

Uniquely suited but not unique

We know that life depends on our ability to copy our genetic information between generations and at every cell division. At the heart of the molecular mechanisms behind that process there is always an enzyme capable of 'reading' the old information and making a new copy. In organisms that use DNA as their genetic material, these enzymes are called DNA-dependent DNA polymerases.

We also know how easy it is to disrupt life. Errors in copying the DNA, if not corrected, can have a negative impact. Small changes in the DNA level can become the protein that functions a little differently, the interaction that shouldn't happen, the uncontrolled replication. We see them in the number of people we lose to cancer every day.

And not all threats are internal. Living agents, such as bacteria, and non-living ones, such as viruses, can also disrupt our lives. Their presence and uncontrolled replication (again requiring polymerases) in our bodies, whether *Mycobacterium tuberculosis*, HIV or COVID-19, can be deadly.

Naturally, polymerases have become targets for drug development. Understanding and comparing these enzymes has given us many insights into which compounds can be developed to interfere with their natural mechanisms and stop them from working. Medicinal chemists have come up with a plethora of compounds that are structurally very similar to the natural polymerase substrates, called nucleotides.

Nucleotides are small but complex molecules, harbouring three major chemical groups: a phosphate (which links the nucleotides into a polymer like DNA), a sugar (which in the case of DNA is deoxyribose) and a nucleobase (the variable group – our Gs, As, Ts and Cs; see Figure 1). Changing any one of those groups can affect the ability of polymerases to recognize, incorporate or continue extending after incorporating one of the resulting nucleotide analogues.

Some of those nucleotide analogues are now well-established therapies such as AZT and tenofovir against HIV, or cytarabine and gemcitabine against diverse types of cancer. Nonetheless, no drug development pipeline is perfect and many of those nucleotide analogues were found to interfere with the enzymes, but not sufficiently to be an effective drug. Some could be efficiently incorporated by polymerases becoming part of the genetic information.

If DNA components can be changed while maintaining their capacity to store genetic information, it raises a fundamental question over the centrality of DNA for the existence of life: must genetic information be stored only in DNA and RNA? And if life can depend on information stored in different molecules, what can we learn about life on our planet? Despite its great diversity, all life on Earth follows nearly universal principles: we are all different pages from a single (DNA) book. How can we dream about literature if all we have is a single book?

What is genetic information?

Intuitively, it seems obvious that the nucleobases, their order and the overall length of the resulting genetic polymer contain information. However, the concept of information is subtler, and its study was pioneered by telecommunication engineers in the mid-20th century. Information only exists during data transmission (or in the case of DNA, replication) and therefore biological access to information is the feature in DNA which makes it a genetic polymer. If a biological means of replication is not available, as is the case with proteins, the information encoded in the molecule is not accessible and therefore cannot be a genetic material.

That established a paradox. Chemists knew how to synthesize a wide variety of DNA-like molecules – later collectively called xenobiotic nucleic acids (XNAs) – some of which could bind DNA, RNA and themselves in a sequence-dependent manner but that was not sufficient to demonstrate that genetic information could be accessed; DNA and RNA remained safe in their uniqueness.

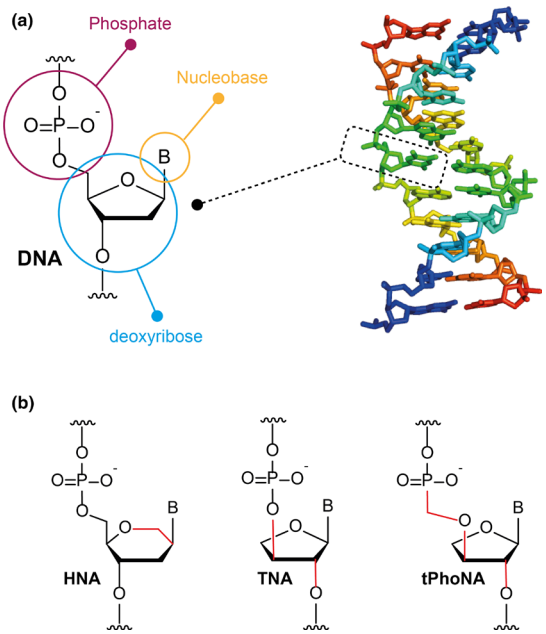

Figure 1. Modifying the chemistry of life. (a) DNA polymers are composed of nucleotide monomers. Each monomer has three chemical moieties that all contribute to DNA function – even if we normally only focus on the nucleobase, shown as B. (b) Examples of XNA developed in the last decade and with potential for both *in vivo* and *in vitro* applications. Chemical bonds changed with respect to DNA are shown in red.

To demonstrate their potential as genetic materials would require us to copy biology by using polymerases. If information could be transferred from DNA to an XNA and later transferred back to DNA, by XNA polymerases, it would demonstrate that a given XNA was a *bona fide* genetic material.

Nonetheless, no natural polymerase could be found capable of using those modified nucleotides to make an XNA. That raises the second complex question: how could it be possible to invent an XNA polymerase that never existed in biology?

Finding needles in a biological haystack

We don't know enough (yet) about proteins and their function to simply invent a protein. Using the 20 amino acids commonly used by biology to make proteins, we know that even for a small (100 amino acids) protein, the number of potential amino acid combinations (20^{100}) exceeds the number of atoms in the universe. The smallest polymerase is close to 200 amino acids and the ones used in biotechnological applications can be nearly 800 amino acids long, thus, it would be impossible to search randomly for an answer.

Here, science again copied biology, harnessing evolution to solve the apparently impossible. Evolution, under the moniker of directed evolution, can be implemented in the lab by linking a desired activity (e.g., XNA synthesis) to the genetic information encoding it (e.g., the gene encoding a polymerase capable of XNA synthesis). This is commonly described as the genotype–phenotype linkage, and it is central for the success of the approach (see Figure 2).

The power of directed evolution is easy to estimate. We have been practicing it from before we even gave it a name. It was directed evolution that allowed us to domesticate animals and improve our crops. Directed evolution has given us antibody therapeutics, improved enzymes of commercial importance (e.g., biological detergents) and the potential to address some of our current urgent needs (e.g., enzymes that can degrade plastics and other pollutants). It has already secured a Nobel Prize.

It was directed evolution that enabled the isolation of DNA polymerase variants that could also synthesize a few different XNAs. In the last decade, the number of possible genetic polymers grew from the natural 2 to over 10 different chemistries, clearly demonstrating that DNA and RNA are not the only answer for the chemical storage of information, but rather 'frozen accidents'. Prebiotic Earth created conditions where RNA and DNA could emerge: where precursors were sufficiently abundant and where sufficient information could be stored for long enough for the Darwinian ratchet of evolution to take hold. Once the RNA/DNA world got started, less efficient information systems or ones where precursors were not as abundant could not compete and were lost. Any new XNA systems that may have emerged after that would never be fit enough to compete with an established system.

Although significant, demonstration of XNAs as potential genetic materials is a first step along multiple paths: in the pursuit of a new biology based on XNA, as well as applications based on XNAs.

What will XNAs ever do for us?

On a conceptual level, life based on DNA was our first book and developing life to use XNA (and for that matter organisms using new DNA bases, or ones using expanded or recoded genetic codes) will give us deeper insights into how biology works, how it emerged and where it can go. As the new books are written, some may be able to coexist with natural biology – thus being orthogonal life. Far from building our welcomed overlords, these XNA platforms will enable an improved description of the complexity of life and the evolvability of each of life's components,

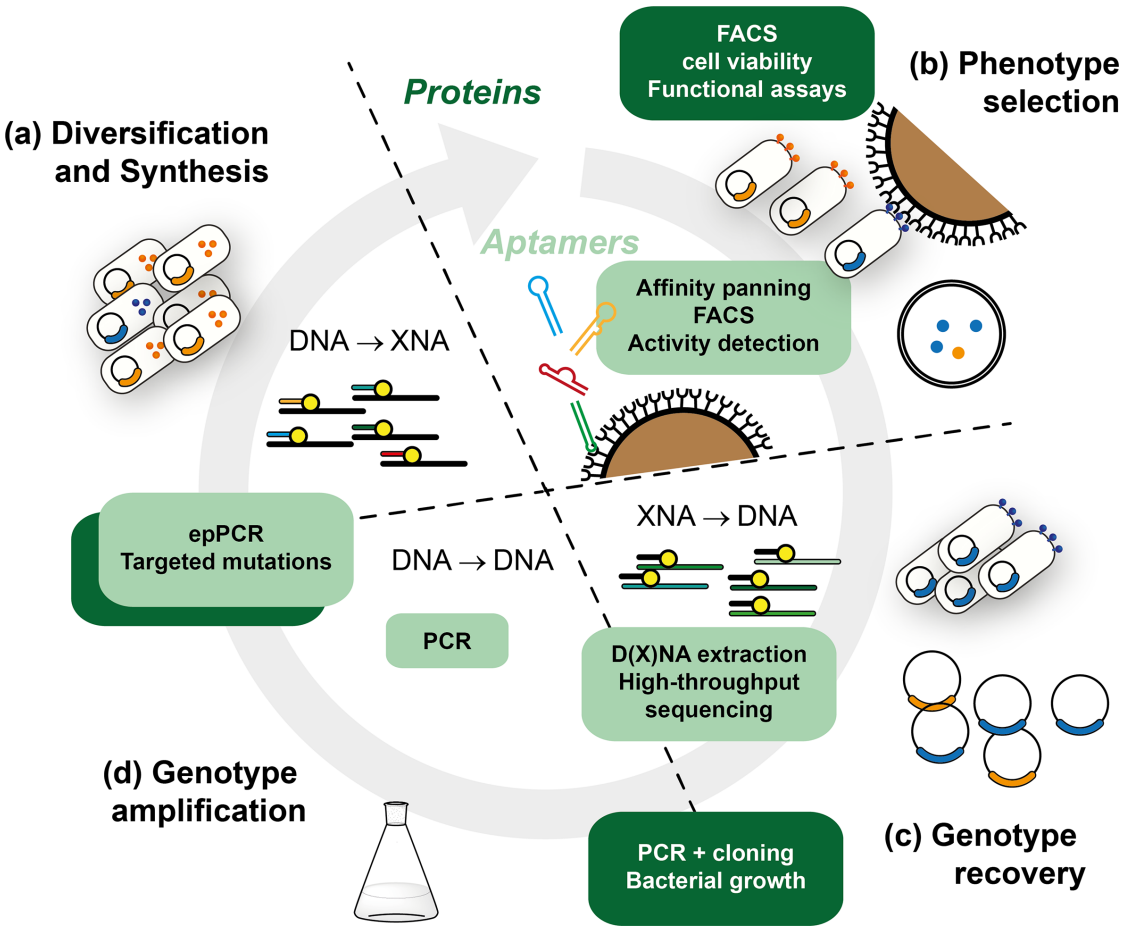

Figure 2. The directed evolution cycle. The systematic optimization of a protein or nucleic acid aptamer follows broadly the same approach. It's a cycle of creating variation (a) followed by the selection of active variants using a wide variety of screening or partitioning methodologies (b) – a small range is shown in green. The genetic information coding for the active molecules has to be recovered (c) and amplified (d), allowing the cycle to be restarted, with or without additional variation.

including a range of enzymes and processes that need to be rebuilt and assembled systematically.

The quest for an XNA life will also force us to mature many areas of our knowledge and methodology. We cannot reach XNA life with our current tools, but we can develop better ones helping the development not only of novel XNA enzymes, but also of other applications. For instance, improvements to all stages of directed evolution can be readily deployed against other targets, accelerating the development and improvement of greener chemistries, new biomaterials and new technologies.

XNA life can also provide an insulation layer for the exploitation of living organisms. Uncertainty over whether our biological tools can turn against us in unanticipated ways limits our exploitation of life as a tool. Creating an insulating layer that cannot communicate or exchange genetic material with existing life is a safety measure – one that can be easily monitored, one that can be legislated and less suitable for the development of biological weapons.

Some of the XNAs developed have properties that make them relevant for potential therapeutic and diagnostic applications, such as increased chemical and biological stability, lack of immunogenicity or even simply stronger base-pairing potential. Several XNA-based therapeutics with phosphate modifications (e.g., Vitravene), sugar-phosphate modifications (e.g., Vyondys 53) and phosphate, sugar and nucleobase modifications (e.g., Spinraza) are already commercially available. Many therapeutic nucleic acid-based applications coming to the clinic today can be replaced by XNAs, whether already described or ones yet to be developed.

In our current crisis, the mRNA vaccines against COVID-19 took the limelight demonstrating their potential and DNA vaccines are not far behind. An XNA vaccine with enhanced nuclease resistance and compatibility with the human translation machinery can also be developed, delivering potentially stronger and more durable immune responses – since the stable XNA can keep being used by our cells to produce the vaccine

epitope. Gene-editing technology via CRISPR-Cas nucleoproteins rely on an RNA guide, which again can be readily replaced with XNAs to increase their effectiveness while reducing off-target effects.

Finally, XNAs are likely to have the biggest impact on the development of aptamers and aptazymes. Single-stranded oligonucleotides can fold upon themselves forming stable structures that can have catalytic activity (in the case of aptazymes) or can create binding surfaces comparable in binding affinity and specificity with antibodies (in the case of aptamers).

Aptamers have been held as a promising replacement for antibodies in the clinic, but that promise is yet to materialize since their original characterization 30 years ago. Aptamers can be readily isolated by directed evolution in RNA and DNA, but those chemistries are not suitable for therapy; they trigger our immune system as potential signatures of attacking viruses or bacteria and are rapidly destroyed. Initially, classical medicinal chemistry methods were used to improve DNA/RNA aptamers but even small chemical changes to the natural chemistries can affect the overall fold of the molecule decreasing its effectiveness.

Here, platforms that allow evolution in XNA can lead to aptamers being isolated already in chemistries compatible with therapy: by using XNA chemistries that are biologically stable and able to go unnoticed by our immune system. In principle, XNA aptamers isolated from directed evolution can be immediately tested as a clinical candidate, making selection and maturation of nucleic acid therapeutics faster than antibodies. Chemically synthesized, aptamers can also be made more cheaply and rapidly than other biotherapeutics. In a world of increasing pandemics and dwindling effective drugs, we need fast drug development pipelines and XNA aptamers could deliver effective clinical candidates after as little as 2 weeks from target identification.

In conclusion, whether we pursue immediate or long-term applications for the XNA technology, it has already shown us that there is more to life than DNA. As it grows, the XNA book will start as a copy of our biology but (mostly) unhindered by the evolutionary pressures that shaped DNA life. It will create new parallels while it traces its own orthogonal path. ■

Further reading

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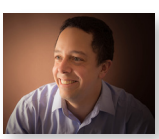
Paola Handal-Marquez obtained her bachelor's degree in molecular biology at University College London (UK) and later obtained her MPhil in structural and molecular biology at University College London (UK) focusing on sampling the functional sequence neighbourhood of Phi29 DNA polymerase for HNA synthesis under the supervision of Prof Vitor Pinheiro. Paola is currently pursuing a PhD in medicinal chemistry at the KU Leuven Rega Institute under the guidance of Prof Vitor Pinheiro particularly focusing on mapping the sequence and functional landscapes of DNA and XNA polymerases to get a better understanding of these complex enzymes and engineer more efficient XNA polymerases.



Graduated from University of Cambridge (UK) in Natural Sciences, Prof. Pinheiro pursued a PhD in bacterial pathogenesis at University of Cambridge looking at the molecular evolution of Yersinia pestis, guided by Prof. David Ellar. Interested by the behaviour of complex systems, Vitor pursued his post-doctoral research at the UK Medical Research Council where he pioneered the development of the first synthetic or xenobiotic genetic materials (XNAs), working with Dr. Phil Holliger. Vitor joined University College London in 2013 as an independent researcher focused on applying Directed Evolution to Synthetic Biology and on further developing the XNA world. Despite its acknowledged impact, Directed Evolution (DE) remains an artisanal practice and the Pinheiro group works to transform DE into an engineering tool to probe the limits of functional biopolymers. Since 2018, Vitor is an Associate Professor at KU Leuven as a member of the Medicinal Chemistry group at the Rega Institute. Email: v.pinheiro@kuleuven.be