

## Structural properties of Alien DNA, an alternative genetic system

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The simple elegance of the Watson-Crick DNA model reported in 1953[1] belies an underlying complexity that is central to all life. However, about thirty years then elapsed before the true complexity of DNA was revealed in high resolution crystals structures of oligonucleotides. In these structures, DNA was captured in three distinct helical forms, Z [2], B [2], and A [3], providing the first evidence for the remarkable ability of DNA to adopt different stable conformations influenced by nucleobase sequence. Since then, our understanding of the fundamental properties of DNA has been challenged further with efforts to expand the genetic code through the creation of unnatural nucleobases. These new entities include nucleobases that pair strictly through hydrophobic interactions [4, 5] and those that pair through hydrogen bonding interactions [6]. The latter nucleobases were created by Benner and coworkers and are referred to as the Artificially Expanded Genetic Information System (AEGIS) [7]. AEGIS takes advantage of alternative hydrogen bonding arrangements between Watson-Crick like pairs, a large purine-like nucleobase and a small pyrimidine-like nucleobase that exclusively pair to one another rather than natural nucleobases. This concept has produced an expanded genetic code, Hachimoji DNA [8] comprising 8 letters, 4 natural and 4 unnatural, and most recently Alien DNA, comprising 4 unnatural nucleobases. These systems including unnatural base pairs (UBPs) expand the structural landscape of DNA through the creation of duplexes that do not conform to known helical forms.

In previous work, we have reported structures including up to 6 UBPs within 16 bp duplex DNA structures [9]. Our most recent work on Alien DNA includes structures with 12 UBPs of 16 base pairs (almost Alien DNA) contained within the structure captured in B-like and A-like helical forms. The B-like structures were obtained through the use of our host-guest system, which is selective for DNA sequences that can adopt helical forms that are more similar to B than A-form DNA. In this system, the N-terminal fragment of Moloney murine leukemia virus reverse transcriptase serves as the host and a 16-mer DNA duplex as the guest [10]. Using this system, we have determined structures of numerous DNA sequences at relatively high resolution (1.6-1.8 Å) including now one with 12 UBPs. One of the almost Alien DNA sequences including 12 UBPs has crystallized in three different crystal forms, two that diffract to 1.2 Å, providing the first very detailed structural information for these UBPs including sugar conformations. These latest structures of almost Alien DNA will be presented here along with comparative analyses with natural and other less Alien DNA structures including UBPs.

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