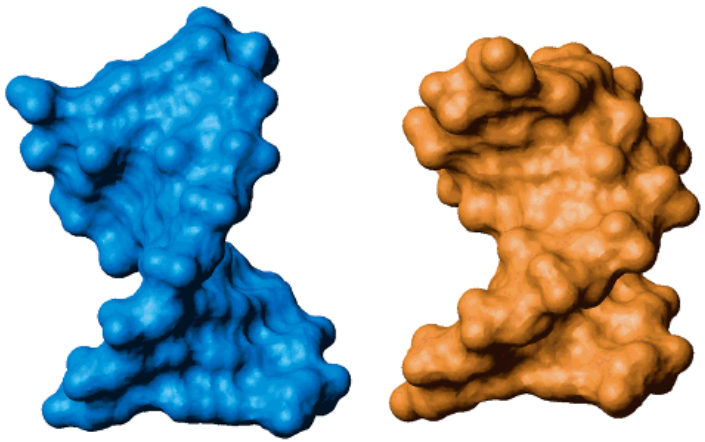
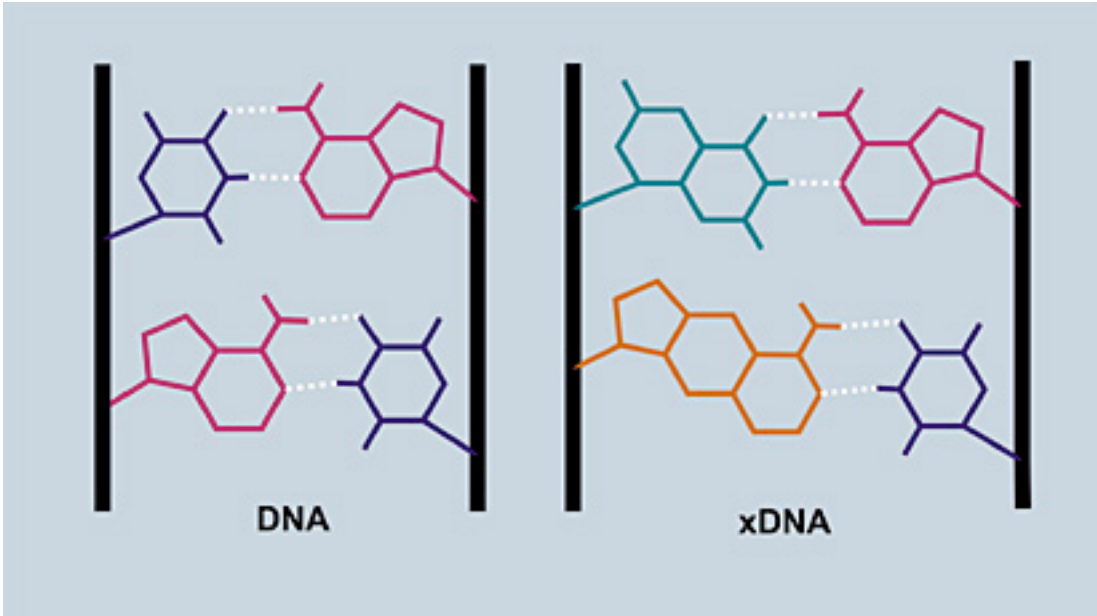
xDNA: A New Genetic System?





Toward a Designed, Functioning Genetic System with Expanded-Size Base Pairs: Solution Structure of the Eight Base xDNA Double Helix

Stephen R. Lynch, Haibo Liu, Jianmin Gao, and Eric T. Kool
Stanford University

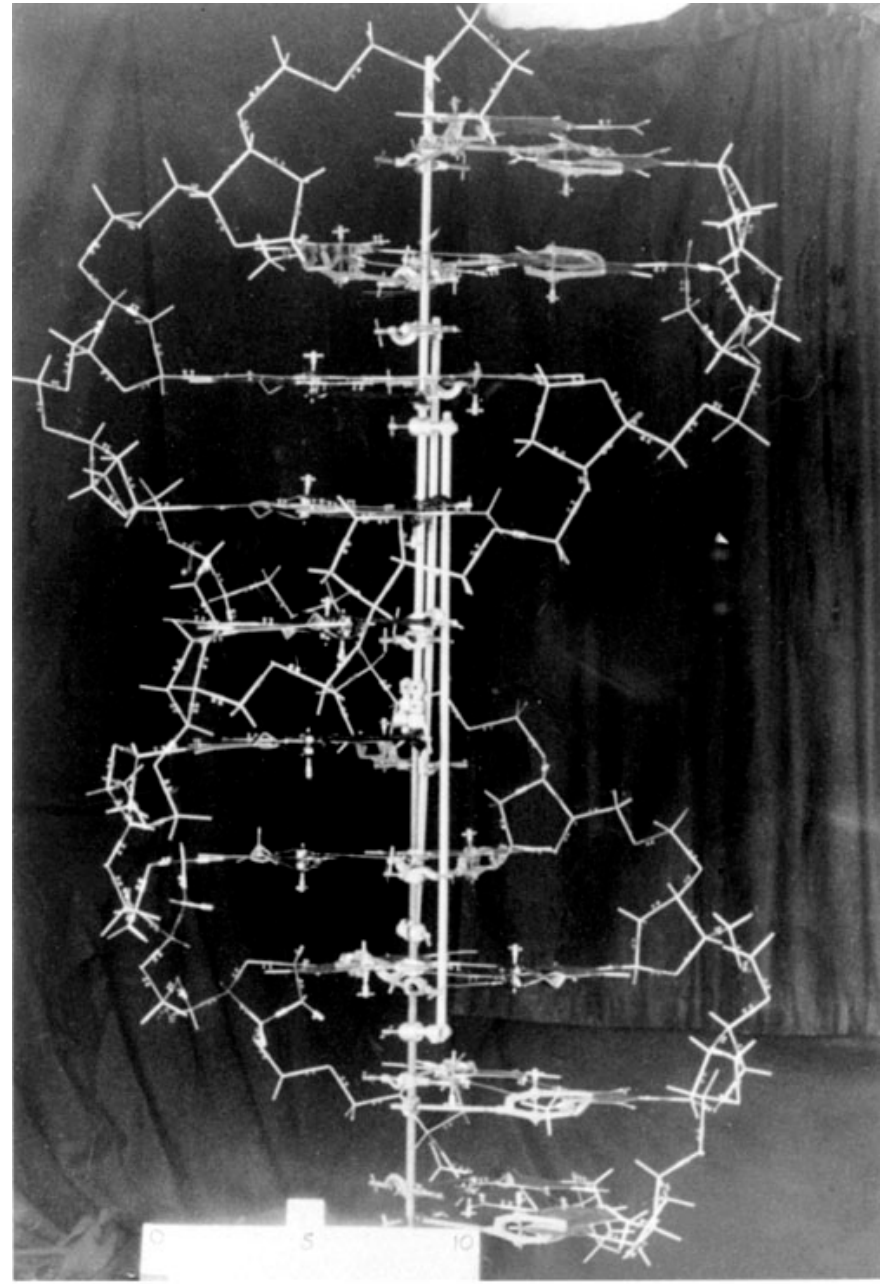
JACS, **2006**, ASAP

Toward A Designed Genetic System: Why?

The design of synthetic DNA sequences that can interact with natural DNA could provide interesting biological probes.

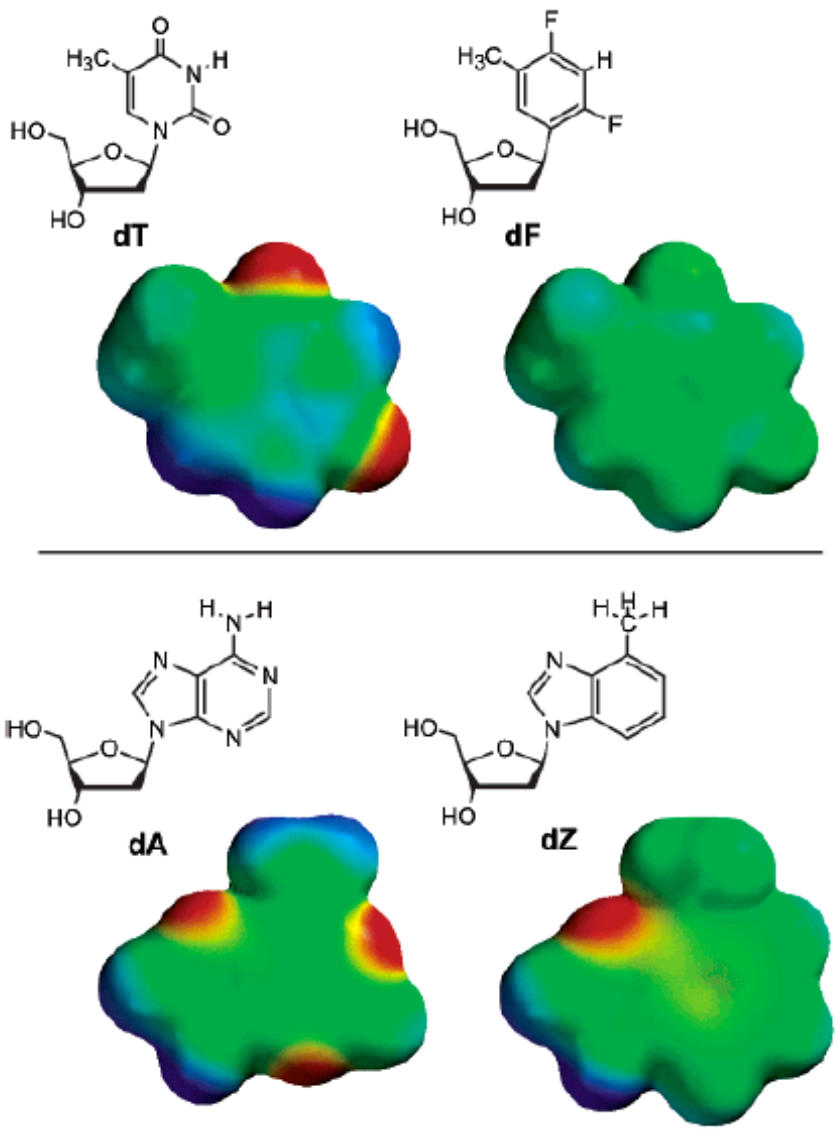
Synthesis and study of novel DNA strands can help scientists understand the function of natural DNA and possibly allow for the development of novel therapies.

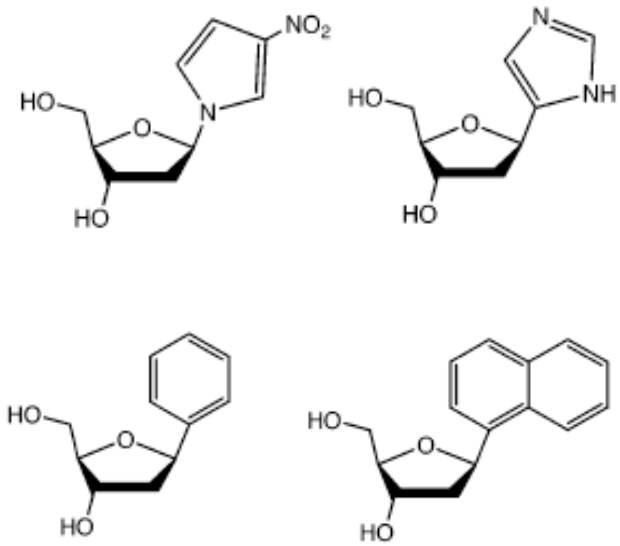
A more complex DNA system could lend itself useful for the storage of high-density information.



Original DNA demonstration model (scale gives distance in Angstroms) Cold Spring Harbor Laboratoy Archives

Molecular Replacements for DNA Bases



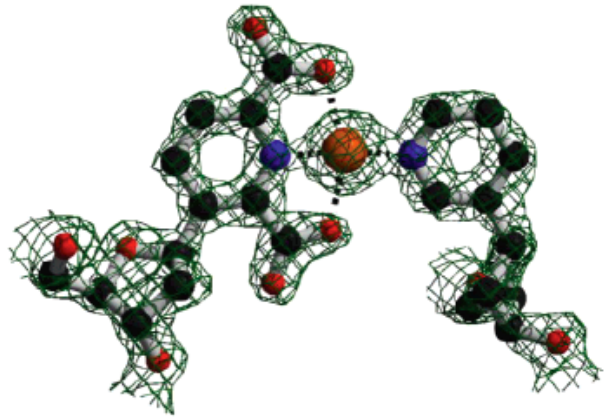


Much work has focused on the modification of DNA bases. Base alteration to study DNA function, improve base stacking (stabilize DNA) or create fluorescent bases for analytical study have been accomplished.

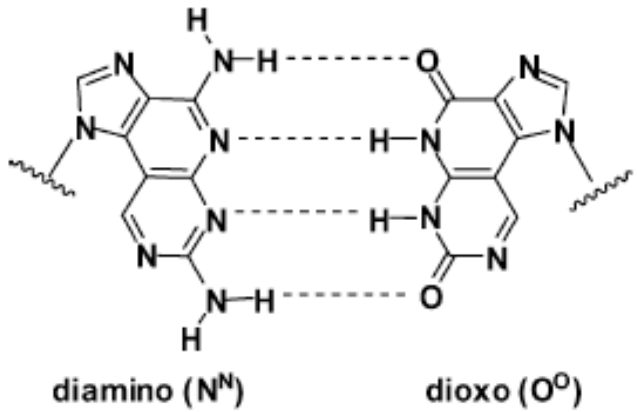
Additionally, modification of the DNA backbone has been accomplished and thoroughly studied.

Acc. Chem. Res. **2002**, *35*, 936.

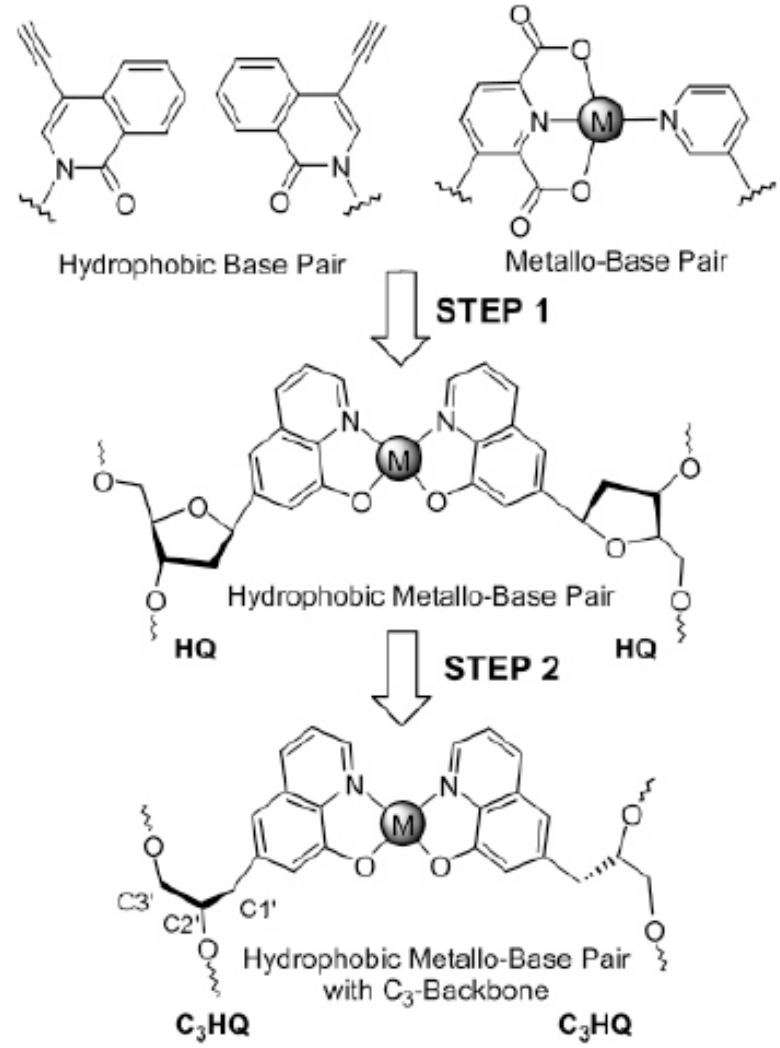
Strategies For Novel Base Pairing



JACS **2001**, *123*, 12364.

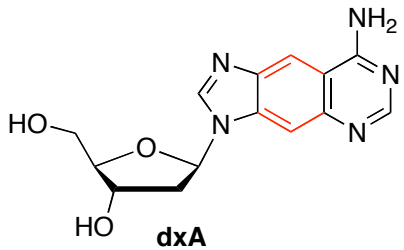


JACS **2003**, *125*, 9970.



JACS **2005**, *127*, 74.

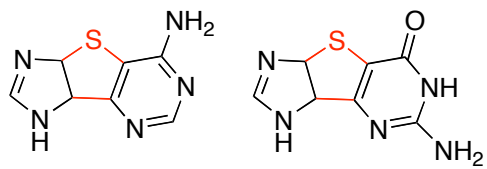
Origins of xDNA



1976-1984:

Leonard synthesized a "stretched out" form of adenosine in which a benzene ring was inserted between the two existing rings.

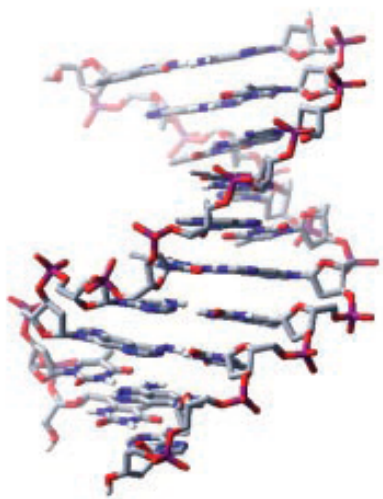
JACS, **1976**, *98*, 3987.
Biochemistry, **1984**, *23*, 3868



2000:

Seley synthesized thieno-separated purines and evaluated them as DNA probes and antitumor agents.

J. Med. Chem. **2000**, *43*, 4877.

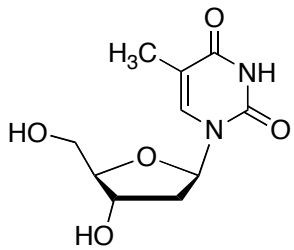


2003:

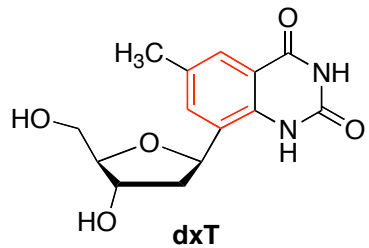
Kool proposed a new genetic system (xDNA) that is composed of both natural DNA bases and xDNA bases that showed preliminary evidence to form a natural looking helix that had increased size and stability.

Science, **2003**, *302*, 868.

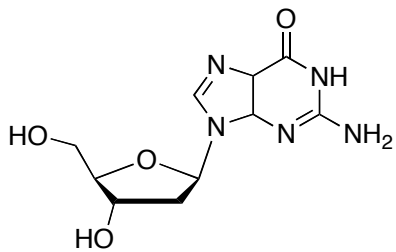
The Expanded Building Blocks: A Full Set



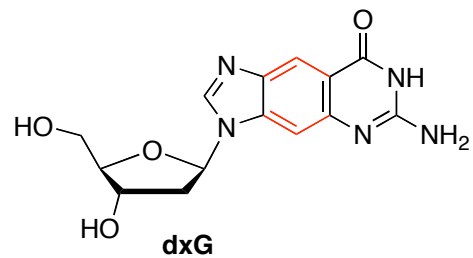
dT



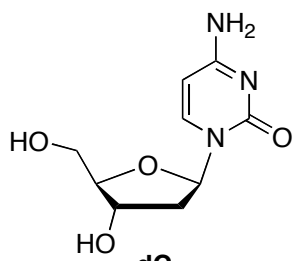
dxT



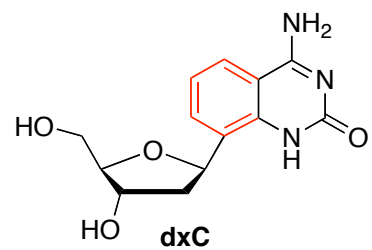
dG



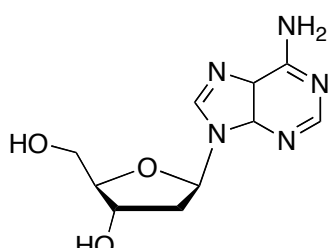
dxG



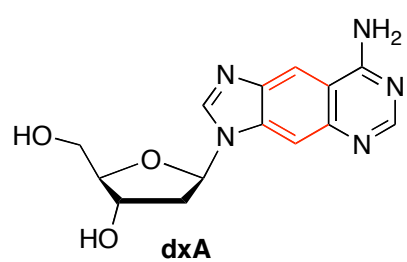
dC



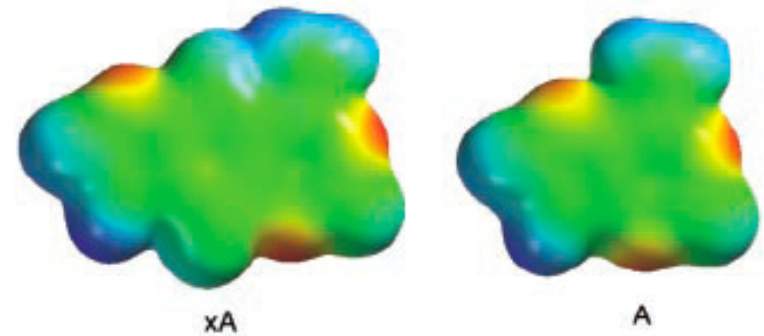
dxC

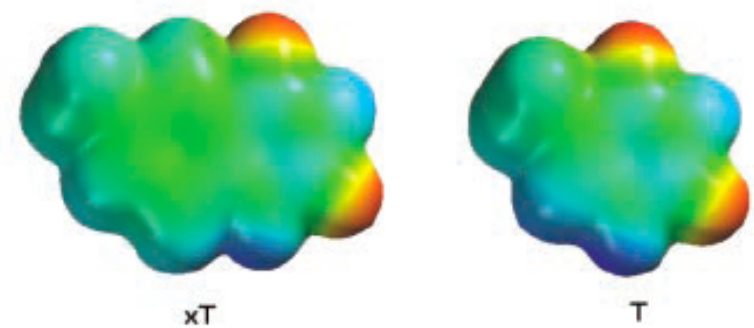


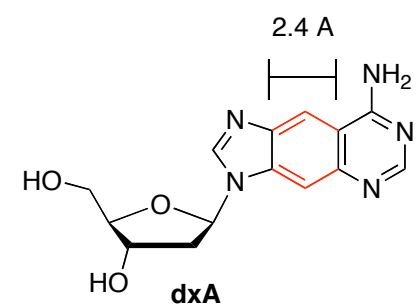
dA



dxA



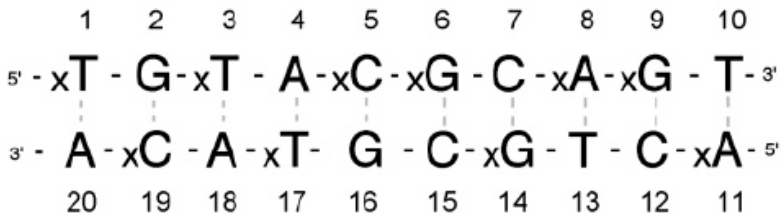




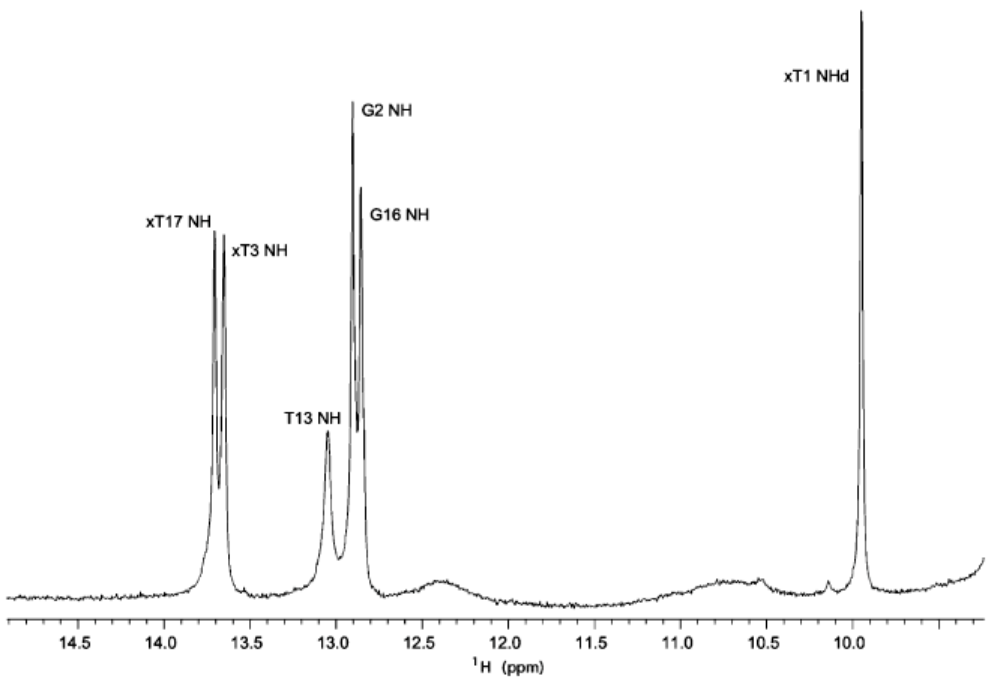
dxA

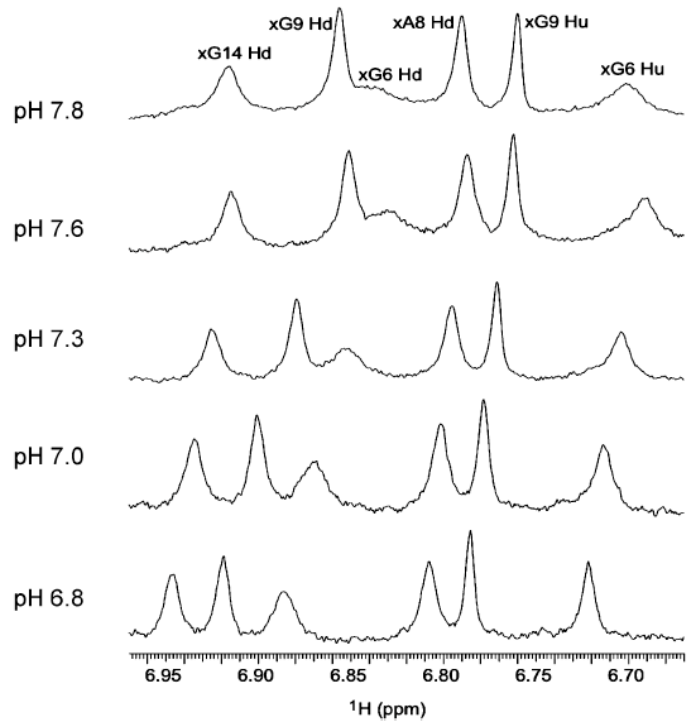
All building blocks are 2.4 Å wider than natural base pairs, yet interact with one another in analogous ways (Watson-Crick base pairing)

Synthesis and Study of 10-Base Pair Strand



All eight components of xDNA are present and scattered on 2 strands; NMR data supports organized 3-D structure and most protons were assigned by 2-D NMR techniques.



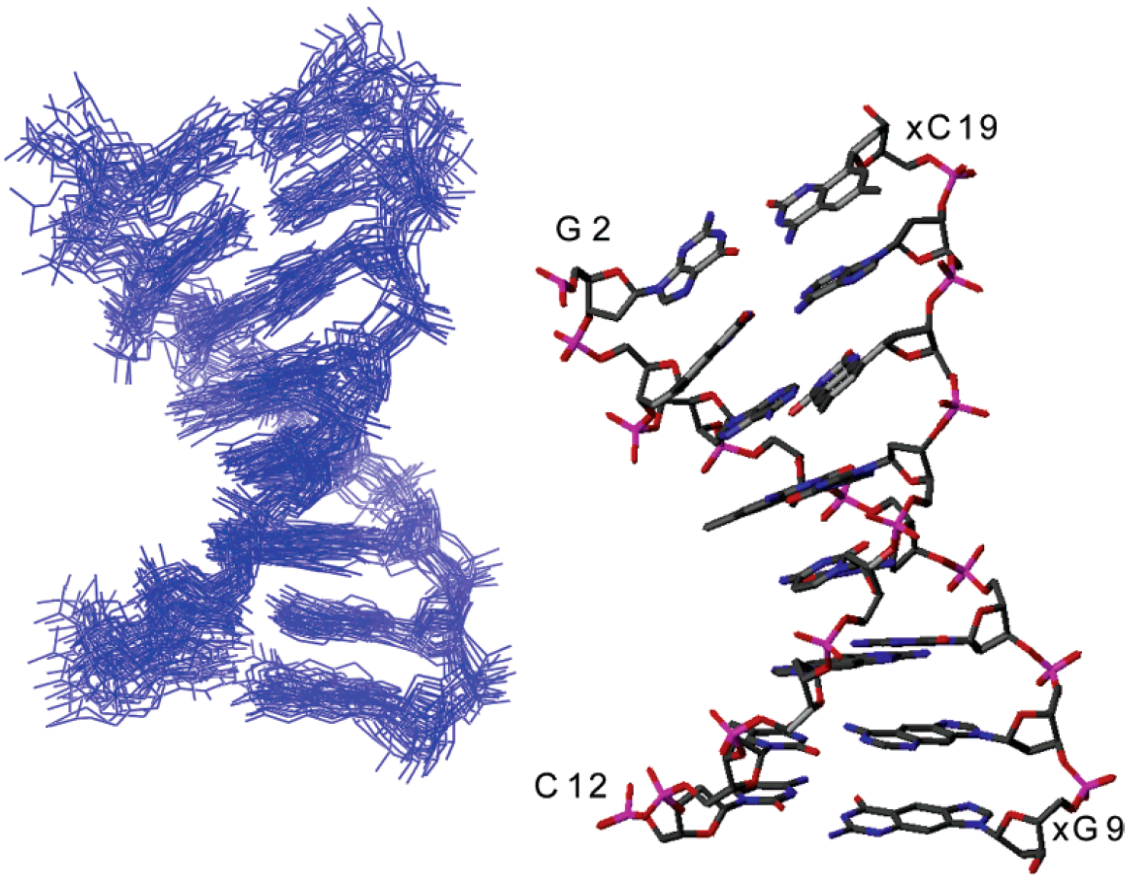


A pKa of 7.3 was found for dxG, more than 2 full units more acidic than dG (9.2). This caused difficulty in identifying the dxG imino protons (above, left); pH studies allowed for the identification of all imino protons except for those on terminal residues (above, right).

Confirmation of Helical Structure

The overall structure of xDNA is a right handed helix that is remarkably similar to natural DNA. The most obvious differences are the width and depth of the 2 grooves and in the number of residues per turn (12 vs 10).

The stability of the helix is also of interest - it is up to 2x more thermally stable than natural helices due to improved base pair stacking interactions.

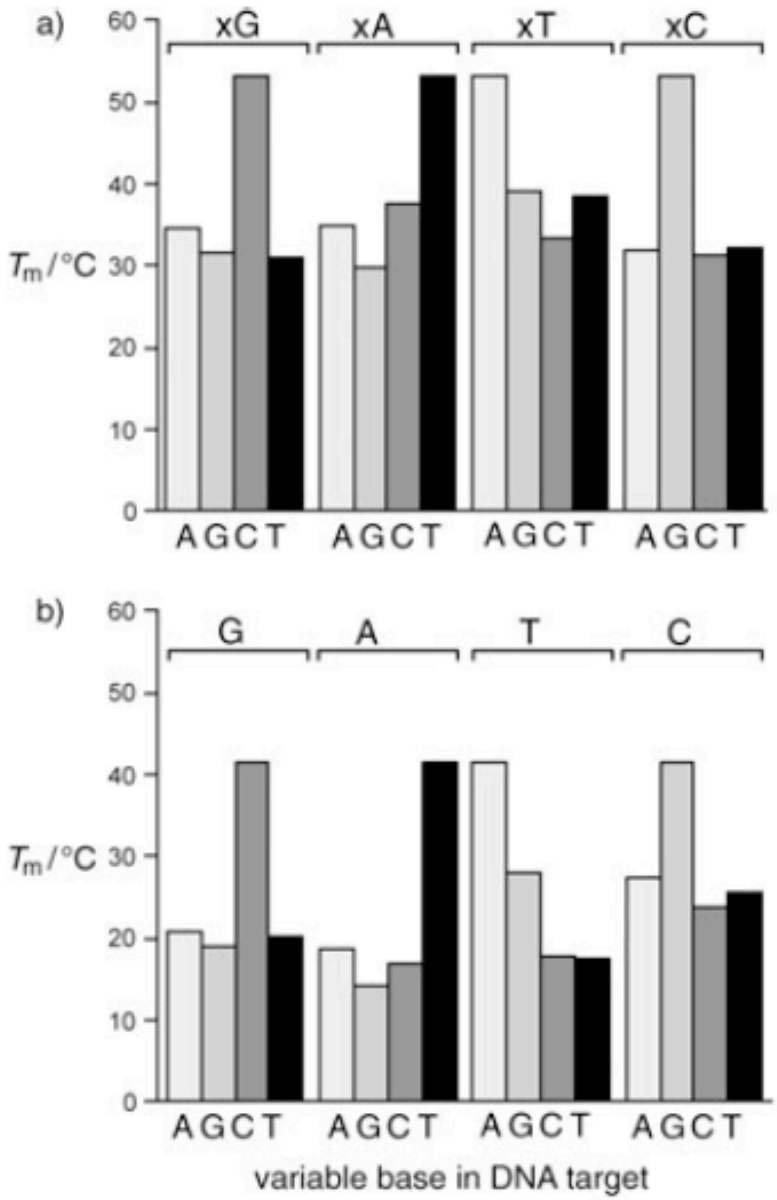


Average Structural Parameters for xDNA and B-DNA

	xDNA	B-DNA
helix handedness	right	right
no. of bp's per repeating unit	1	1
no. of bp's per turn	12	10
helix twist (deg)	30	36
rise per bp (deg)	4.0	3.4
helix pitch (deg)	46	34
base pair inclination (deg)	24	2.4
diameter (Å)	20.5	18.4
X displacement from bp to helix axis (Å)	−1.8	−0.2
glycosidic bond orientation	*anti*	*anti*
sugar conformation	2-*endo*	2-*endo*
major groove depth (Å)	2.5	4.1
major groove width (Å)	12.4	11.4
minor groove depth (Å)	2.6	5.5
minor groove width (Å)	13.1	5.9

Calculated structures of xDNA helix based on NMR data.

Sequence Specificity Remains Intact

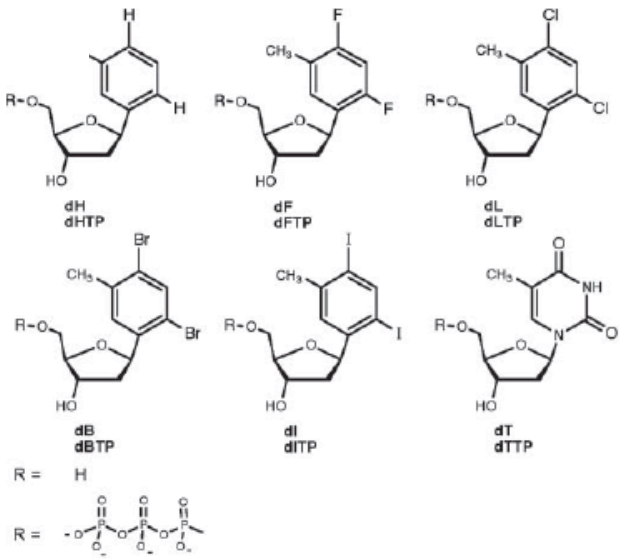


Sequence specificity was determined by comparing the melting temperatures for matched and all possible mismatched pairs.

xDNA and DNA showed an overall identical ability to discriminate against incorrect bases.

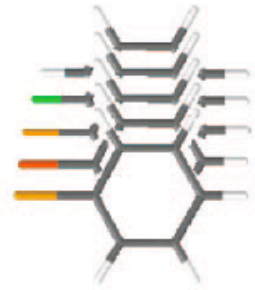
ACIE, **2005**, *44*, 3118.

The Next Step: Can xDNA be Replaced?



b

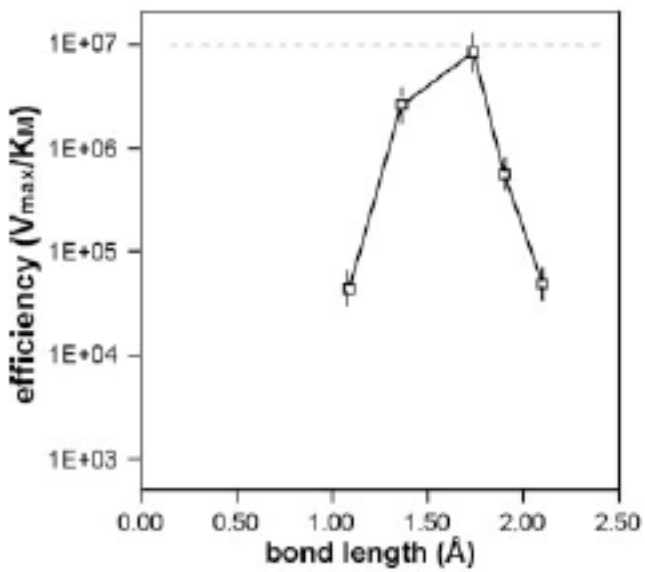
bond	length
Ar-H	1.08
Ar-F	1.36
Ar-Cl	1.74
Ar-Br	1.90
Ar-I	2.10
C=O (thymine)	1.22

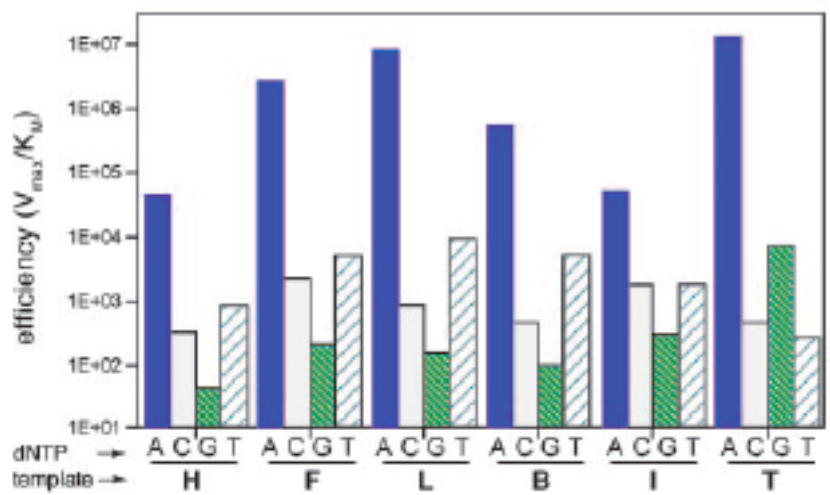


A series of gradually expanding thymine nucleobase analogues were studied for their DNA polymerase efficiency and fidelity.

It was found that even high-fidelity polymerase pockets tolerate bases of increased size, possibly to allow for evolutionarily advantageous mutation rates.

PNAS, **2005**, *102*, 15803.





Can a polymerase be found or developed that can accommodate the 20% larger xDNA?

Conclusions

"We've designed a genetic system that's completely new and unlike any living system on Earth," said Eric T. Kool, a professor of chemistry at Stanford and co-author of the Science study. "Unlike natural DNA, our expanded molecule is fluorescent and is considerably more stable when subjected to higher temperatures."

"That means each base pair could change color or intensity when it finds a complementary strand of natural DNA or RNA." This fluorescent property could prove useful for medical biopsies, he said, adding: "You need fast and accurate ways of genetically typing cells, and I think color is an interesting way of doing that. You'd put a thin slice of tissue on a slide, stain it with your molecule, look at it under a microscope and say, 'Ah! This tumor has this mutation in its DNA, so now we know what drugs to use to treat it.'"