

Practical NanoBioTechnology

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The question

Can the "rule-based" molecular recognition displayed by DNA be exploited to practical ends?

The ultimate measure of "practical success": A commercial product

The answer is "yes", but it remains an open question whether nano-visualization is an essential part of this success.

The chemistry

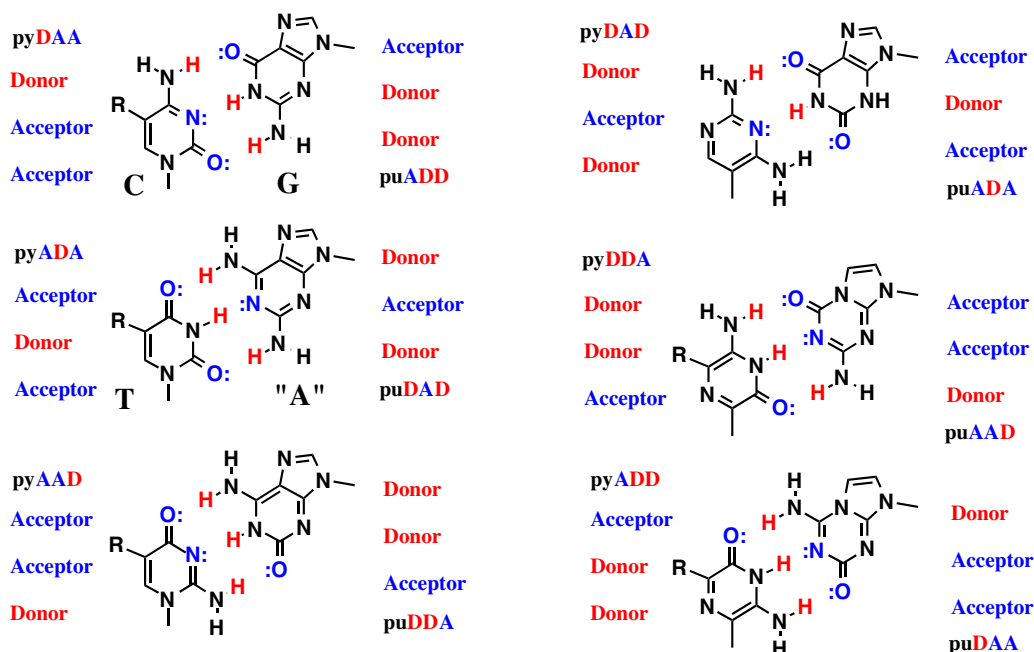
There is no doubt that the chemistry is essential for practical success.

Rule-based molecular recognition

- Rule-based molecular recognition is virtually unknown in organic chemistry (this is why designing drugs is difficult).
- Rule-based molecular recognition is, however, displayed by DNA.
- DNA is "Capable Of Suffering Mutation Independent of Concern over Loss Of Properties Essential for Replication" (COSMIC-LOPER), a property that permits it to support Darwinian evolution.
- The remarkable discovery of the last decade is that the focus of COSMIC-LOPER properties is not the nucleobase pair (the focus of "first generation" Watson-Crick theory), but rather the backbone.
- This leaves open the possibility of changing the nucleobases, perhaps even expanding the genetic alphabet, to get an artificial genetic system.

An Expanded Genetic Information System (Aegis)

- Underlying base pairing are two complementarity rules
Large pairs with small (purine vs. pyrimidine)
Hydrogen bond donors (N-H) pair with acceptors (O: or N:)
Generalizing these rules give an expanded genetic alphabet with 12 letters

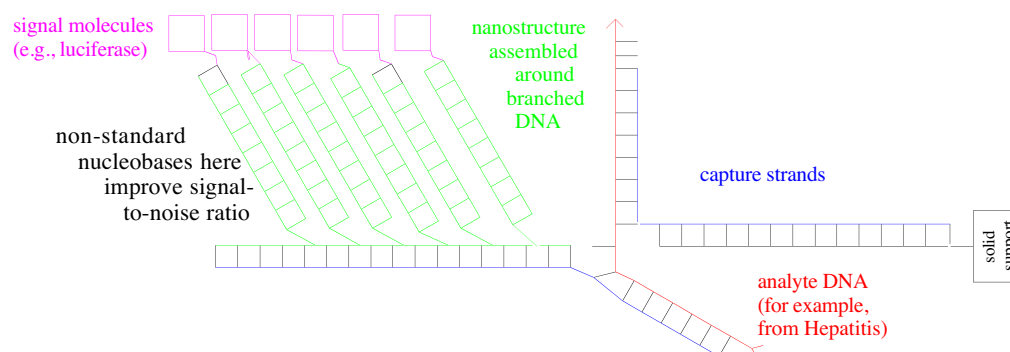


- Each of these has been synthesized in the Benner group.
- They display "orthogonality"; each match is favored over all other mismatches.
- DNA built from Aegis components does not bind to natural DNA.
- Higher information density allows more rapid assembly of nanostructures.

Real technology

The "branched DNA" DNA-based diagnostics system

- Chiron attempted to detect DNA by building a nanostructure based on DNA
- The nanostructure was essentially a DNA-based dendrimer, and served as an amplification system.



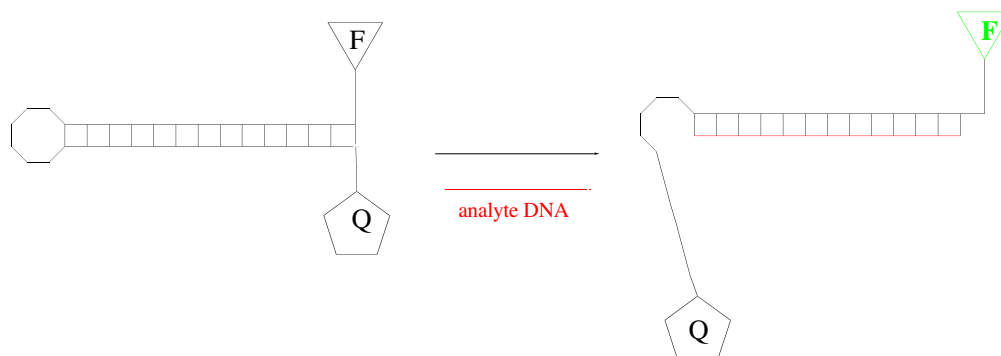
- A-T and G-C nucleobase pairing was used to assemble the nanostructure, as well as capture the analyte.
- Contaminant DNA in the biological specimen captured signaling molecules to the support, creating "noise".
- Signal not detectable above the noise unless analyte present >100,000 copies

Use Aegis components to assemble the nanostructure

- Retain standard A, T, G, and C in capture strands.
- Place Aegis components in nanostructure assembly units.
- Detection limit < 50 molecules.
- The *Quantiplex* system, marketed with a license to Aegis technology by Bayer, will have in 2001 (projected) sales of \$100 million.
- FDA approval is being sought

Alternative readouts

Molecular beacons from the Tan laboratory



Fang et al., *J. Am. Chem. Soc.* **121**, 2921-2922 (1999)

- Molecular beacons can be visualized at the single molecule level.
 - Can be used for studies inside of cells
- Perlette & Tan, *Proc. Nat. Acad. Sci.* (submitted) 2001.

In vitro selection

- Modified nucleotides can be incorporated into combinatorial tools to generate "receptors on demand"
- Battersby, T. R., Ang, D. N., Burgstaller, P., Jurczyk, S., Bowser, M. T., Buchanan, D. D., Kennedy, R. T., Benner, S. A. *In vitro* selection of an adenosine receptor from a library incorporating a cationic nucleotide analog. *J. Am. Chem. Soc.* **121**, 9781-9789 (1999)

The challenge: Single nucleotide polymorphism (SNP)

- Nanoprobng (e.g. AFM) can visualize DNA, especially DNA carrying appendages (see, for example, Woolley et al. *Nature Biotechnology* **18**, 760-763 (2000))
- In principle, this could enable single molecule multiplexed genotyping.
- Practical reality: binding strengths of a duplex pair with one mismatch is not sufficiently weaker than binding of an exact matched pair to create a robust platform for

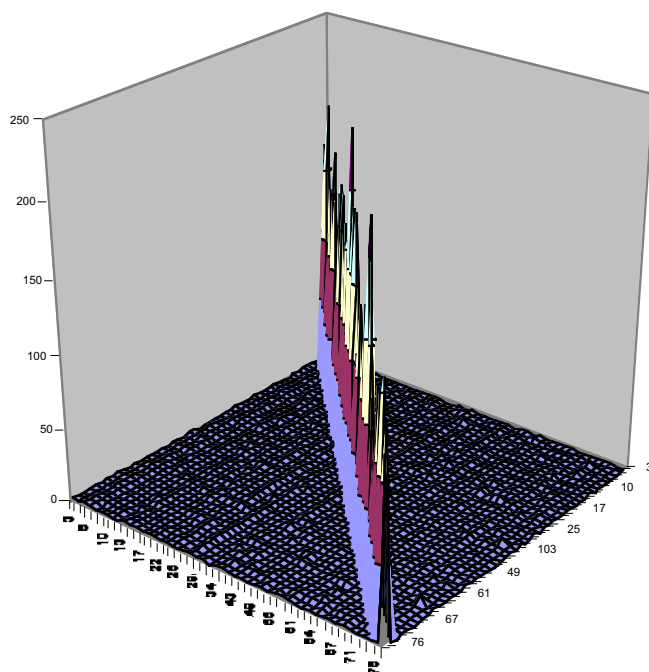
Enzymes that manipulate DNA also manipulate Aegis components

- Ribosomes take Aegis components better than standard nucleotides
- Kinases, ligases, and other standard tools of molecular biology also work well
- Polymerases handle Aegis components with a wide range of features
 - Evolutionary family is the determinative criterion
 - Some polymerases read through
 - Some terminate, depending on the Aegis component
- Nucleases likewise display a range of properties
- Exploiting these features generates a range of robust SNP detection strategies

EraGen Biosciences, based in Gainesville FL, and Madison WI, exploits these to develop a robust SNP platform.

Collaborative studies

- Combine Aegis components with carbon nanotube probes for single molecule genotyping.
- Highly multiplexed (> 100 assays per tube) genotyping based in the orthogonality of Aegis tags.
- Use of Luminex platform for rapid separation and tag identification.



Background reading:

For information behind the expanded genetic alphabet

Piccirilli, J. A., Krauch, T., Moroney, S. E., Benner, S. A. Extending the genetic alphabet. Enzymatic incorporation of a new base pair into DNA and RNA. *Nature* **343**, 33-37 (1990)

For early applications of the expanded genetic alphabet

Bain, J. D., Chamberlin, A. R., Switzer, C. Y., Benner, S. A. Ribosome-mediated incorporation of non-standard amino acids into a peptide through expansion of the genetic code. *Nature* **356**, 537-539 (1992)

To learn more about "second generation nucleic acid theory"

Benner, S. A. et al. Redesigning nucleic acids. *Pure Appl. Chem.* **70**, 263-266 (1998)

To learn more about the Quantiplex assay, see:

Collins, M. L. et al. (A branched DNA signal amplification assay for quantification of nucleic acid targets below 100 molecules/mL. *Nucl. Acids Res.* **25**, 2979-2984 1997).