Synthetic life SL3-4

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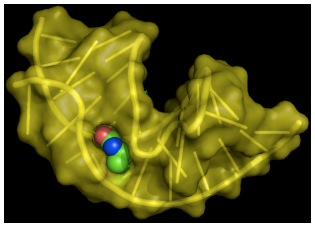
Aptamers

Aptamers (from the Latin *aptus* – fit, and Greek *meros* – part) are ***oligonucleotide*** or ***peptide*** molecules that bind to a specific target molecule.

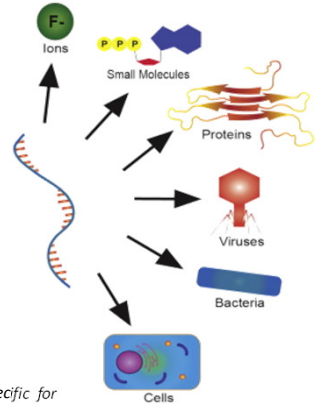
Aptamers are usually created by selecting them from a large random sequence pool, but natural aptamers also exist in riboswitches.

- ***DNA or RNA or XNA aptamers*** – oligonucleotide strands (usually short)
- ***Peptide aptamers*** - one (or more) short variable peptide domains, attached at both ends to a protein scaffold.



Fdardel

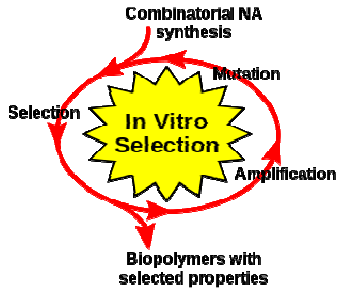
Structure of an RNA aptamer specific for biotin. The aptamer surface and backbone are shown in yellow. Biotin (spheres) fits snugly into a cavity of the RNA surface



Variety of target molecules

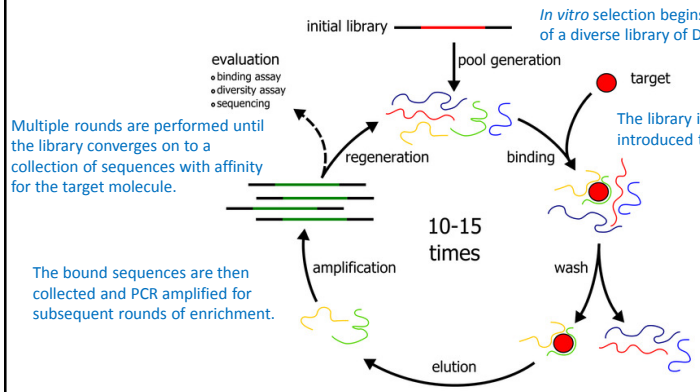
Systematic evolution of ligands by exponential enrichment - SELEX

1990 – Gold *et al.* – selection of RNA ligands against T4 DNA polymerase
1990 – J. Szostak *et al.* – selecting RNA ligands towards organic dyes



A general overview of in vitro selection protocol. NA stands for Nucleic Acids (DNA, RNA) which start as a random pool, and are enriched through the selection process

Systematic evolution of ligands by exponential enrichment - SELEX



In vitro selection begins with the generation of a diverse library of DNA or RNA molecules.

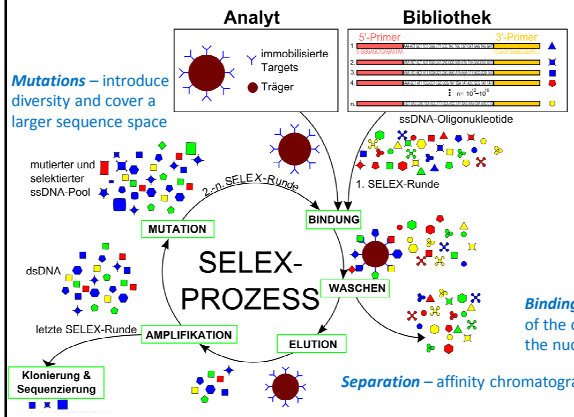
The library is then introduced to a target ligand

Multiple rounds are performed until the library converges on to a collection of sequences with affinity for the target molecule.

The bound sequences are then collected and PCR amplified for subsequent rounds of enrichment.

Sequences demonstrating affinity towards the target molecule are isolated from any unbound sequences.

Systematic evolution of ligands by exponential enrichment - SELEX



Mutations – introduce diversity and cover a larger sequence space

The initial library – usually contains sequences of 20-200 randomised nucleotides

The 5'- and 3'-termini are composed of constant primer sequences. Between them, the random sequences of *n* nucleotides result in 4^n combinations:

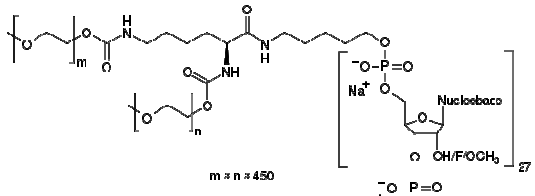
- n = 25 – a library of c.a. 10^{15} sequences,
- n = 50 – a library of c.a. 10^{30} sequences,
- n = 75 – a library of c.a. 10^{45} sequences,
- n = 100 – a library of c.a. 10^{60} sequences.

Binding strongly depends on the secondary structure of the oligonucleotides, which in turn depends on the nucleobase sequence

Separation – affinity chromatography

Amplification – reverse transcription RNA→DNA (only for RNA aptamers) + PCR (for RNA and DNA aptamers)

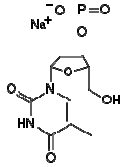
Systematic evolution of ligands by exponential enrichment - SELEX



Aptamers were evolved for a variety of target ligands:
- small molecules (ATP and adenosine)
- proteins: prions and vascular endothelial growth factor (VEGF),
- tumor cells.

Clinical uses are suggested by aptamers that bind tumor markers or GFP-related fluorophores.

A VEGF-binding aptamer trade-named ***Macugen*** has been approved by the FDA for treatment of macular degeneration.



Macugen

Additionally, SELEX has been utilized to obtain highly specific catalytic DNA or DNAzymes. Several metal-specific DNAzymes have been reported including the GR-5 DNAzyme (lead-specific), the CA1-3 DNAzymes (copper-specific), the 39E DNAzyme (uranyl-specific) and the NaA43 DNAzyme (sodium-specific).

Riboswitches

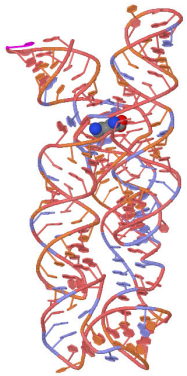
1990 - SELEX (Gold, Szostak)
2002 - the notion of aptamers in the natural world (Breaker and Nudler) – discovery of a nucleic acid-based genetic regulatory element – ***riboswitch*** - that possesses similar molecular recognition properties to the artificially made aptamers.

Riboswitches - naturally occurring regulatory segments of mRNA that bind small molecules specifically. The binding results in a change in production of the proteins encoded by the mRNA

Before discovery of ***riboswitches*** only ***proteins*** were supposed to do so in the biological context.

Most known ***riboswitches*** occur in bacteria, but functional riboswitches of one type (the TPP riboswitch) have been discovered in archaea, plants and certain fungi.

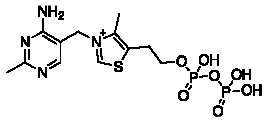
Riboswitches exist in all domains of life, and therefore are likely that they might represent ancient regulatory systems or fragments of ***RNA-world ribozymes*** whose binding domains remained conserved throughout the evolution



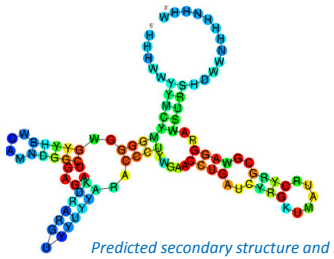
The lysine riboswitch

The TPP Riboswitch

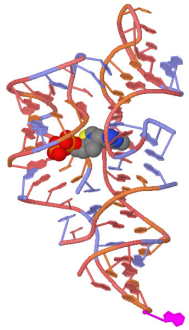
The **TPP riboswitch** (THI element and Thi-box riboswitch), is a highly conserved RNA secondary structure. It binds directly to thiamine pyrophosphate (TPP, a form of the vitamin B1, an essential coenzyme) to regulate gene expression through a variety of mechanisms in archaea, bacteria and eukaryotes.



Thiamine pyrophosphate TPP



Predicted secondary structure and sequence conservation of TPP riboswitch



The 3D structure of TPP riboswitch (by Benjamin Schuster-Böckler)

DNAzymes

Deoxyribozymes, also called ***DNA enzymes***, or catalytic DNA: DNA oligonucleotides that are capable of performing a specific chemical reaction, often but not always catalytic.

Although the working principle is similar to ***enzymes*** (and ***ribozymes***), there are no known naturally occurring ***deoxyribozymes***.

Deoxyribozymes should not be confused with ***DNA aptamers*** which are oligonucleotides that selectively bind a target ligand, but do not catalyze a subsequent chemical reaction.

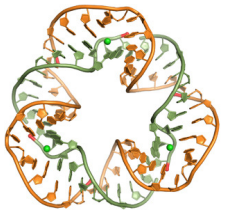
The trans-form (two separate strands) of the 17E DNAzyme. Most ***ribonuclease DNAzymes*** have a similar form, consisting of a separate enzyme strand (blue/cyan) and substrate strand (black: all-RNA or a DNA with one RNA nucleotide). Two arms of complementary bases flank the catalytic core (cyan) on the enzyme strand and the single ribonucleotide (red) on the substrate strand. The arrow shows the ribonucleotide cleavage site.

1994 – the first DNAzyme (a ribonuclease) – R. Breaker, G. Joyce – Pb^{2+} GR-5

Currently known:
- Ribonucleases
- RNA ligases
- DNA phosphorylation, adenylation, deglycosylation
- DNA cleavage

Problems: product inhibition, often single-turnover

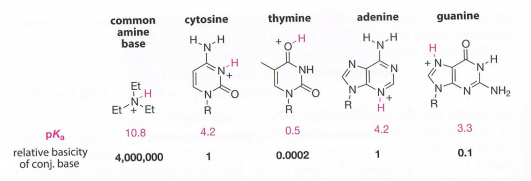
CHAPTER 1

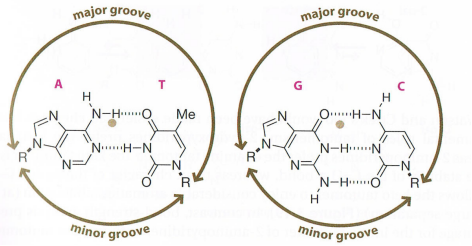


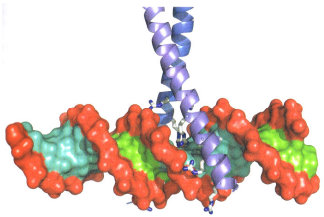
OLIGONUCLEOTIDES

Part 2 – noncanonical nucleobases

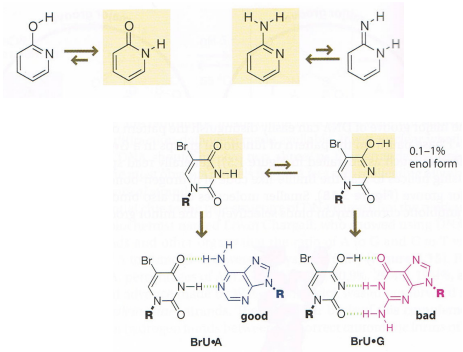
Canonical nucleobase pairing







Nucleobase modifications for biosynthetic



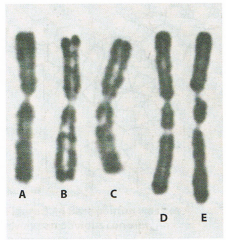
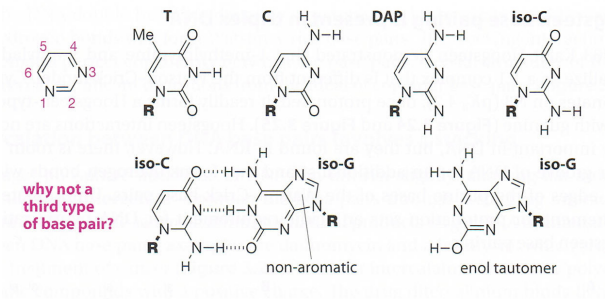


Figure 3.20 Chromosome 1 from hamster cells exposed to bromodeoxyuridine. (A) Normal chromosome. (B–E) Aberrant chromosomes. (From T.C. Hsu and C.E. Somers, *Proc. Natl. Acad. Sci. USA* 47: 396–403, 1961. With permission from the MD Anderson Cancer Center.)

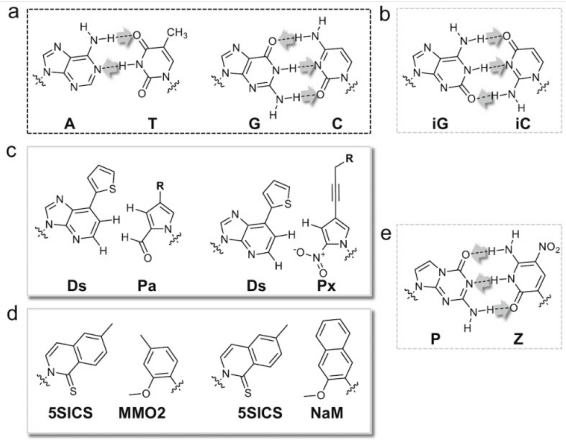
Alternative base pairs – synthetic biology



DAP – one tautomer forms a base pair with guanine

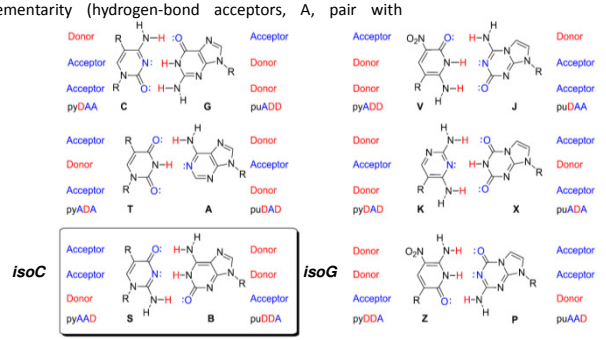
iso-C/iso-G
- specificity (the enol tautomer of iso-G, stabilized by aromatization, complementary to thymine)
- the 2-amino group of iso-C hydrolyses easily to uracil

Natural and non-natural base pairs that function in polymerase reactions



AEGIS – Artificially Expanded Genetic Information System

Watson–Crick pairing rules:
(a) size complementarity - large purines pair with small pyrimidines
(b) hydrogen-bonding complementarity (hydrogen-bond acceptors, A, pair with hydrogen-bond donors, D).



Rearranging donor and acceptor groups on the nucleobases, while not changing the geometry of the Watson–Crick pair, creates an artificially expanded genetic information system (AEGIS). AEGIS components add information density to DNA strands built from them.

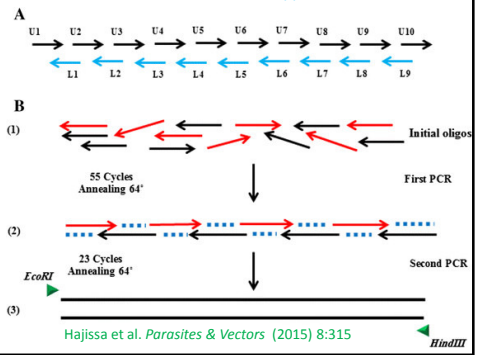
S. Benner *et al., Beilstein J. Org. Chem.* **2014,** *10,* 2348–2360. doi:10.3762/bjoc.10.245

Artificial Gene Synthesis

Artificial gene synthesis (DNA printing) - method in synthetic biology to create artificial genes in the laboratory:

- *currently based on solid-phase DNA synthesis,*
- *the user does not have to begin with preexisting DNA sequences.*
- *Therefore, it is possible to make a completely synthetic double-stranded DNA molecule with no apparent limits on either nucleotide sequence or size.*

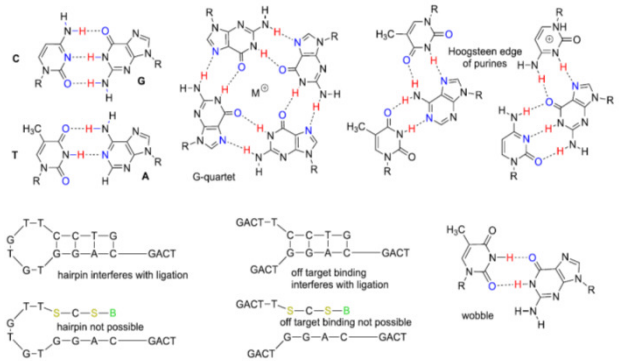
Applications:
- recombinant DNA technology including heterologous gene expression, vaccine development, gene therapy and molecular engineering.
- The synthesis of nucleic acid sequences can be more economical than classical cloning and mutagenesis procedures
- the ability to safely obtain genes for vaccine research without the need to grow the full pathogens.
- to optimize protein expression in a particular host, or to remove non-functional DNA segments
- For DNA digital data storage and computing
- For synthetic biological circuits



Hajissa et al. *Parasites & Vectors* (2015) 8:315

Self-assembly of whole genes and DNA nanostructures

Limitations of DNA puzzle assembly: unequal A:T vs. G:C strength, insufficient ACGT information density, higher-order structures

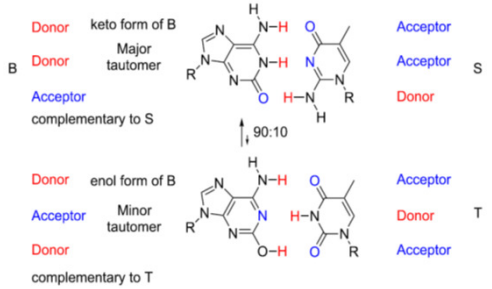


S. Benner *et al., Beilstein J. Org. Chem.* **2014,** *10,* 2348–2360. doi:10.3762/bjoc.10.245

Self-assembly of whole genes and DNA nanostructures

Solution: an orthogonal pair from the AEGIS system, that can be removed from the product, yielding native DNA structures

Conversion occurs when polymerases are forced to mismatch a standard nucleotide opposite an AEGIS nucleotide by
(a) not being provided the complementary AEGIS triphosphate and
(b) exploiting a chemical feature of the AEGIS nucleotide that directs a specific mismatch.



B in its major tautomeric form pairs with **S**; in its minor tautomeric form, **B** pairs with standard **T**. Asembly of the target gene/DNA nanostructure is followed by conversion of the **S:B** pairs to **T:A** pairs after two cycles of PCR:
B → **A** via an intermediate **B:T** mispairing, **S** → **T** (intermediate **S:B** followed by a second **B:T** mispairing).

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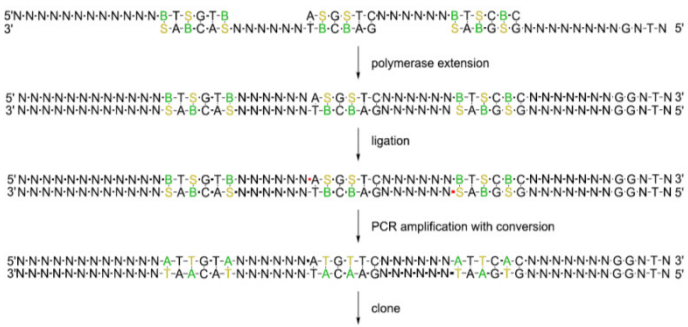
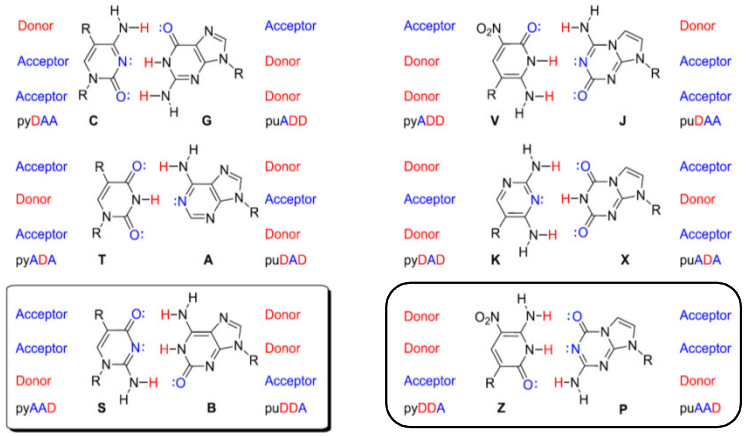
Self-assembly of whole genes and DNA nanostructures



The technology tested by assembly of the kanamycin-resistance gene and growing the bacteria in the environment containing kanamycin after assembly and conversion of that gene.

S. Benner *et al., Beilstein J. Org. Chem.* **2014,** *10,* 2348–2360. doi:10.3762/bjoc.10.245

AEGIS – Artificially Expanded Genetic Information System



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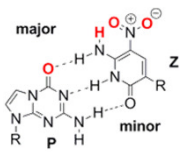
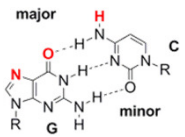
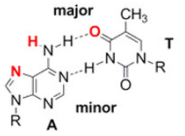
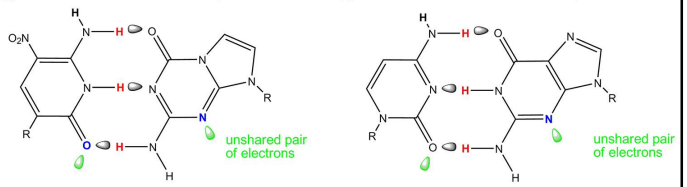
AEGIS – Artificially Expanded Genetic Information System







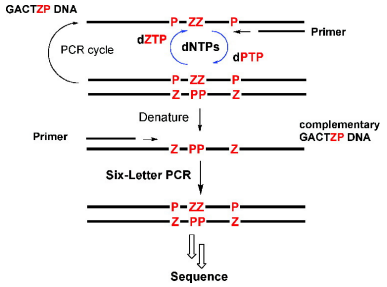
pyDDA puAAD pyDAA puADD



Z P C G

S. Benner *et al., J. Am. Chem. Soc.*, **2011**, *133 (38),* pp 15105–15112

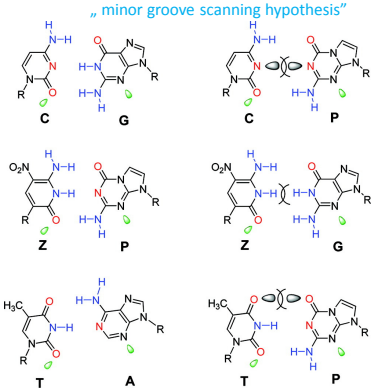
AEGIS – Permanent orthogonal nucleobases surviving PCR



Error rate 0,2% per a PCR cycle – both removal and incorporation of **Z** and **P** → the artificial genetic system capable to evolve.

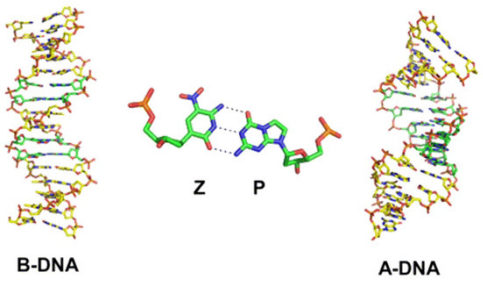
Pol: Deep Vent – 2 Z/P, Taq/Phu – 3-4 Z/P
dZTP (*deprotonated*) at higher pH pairs slightly with G
→ loss of some **Z**, but also gain of some new **Z** mutants.

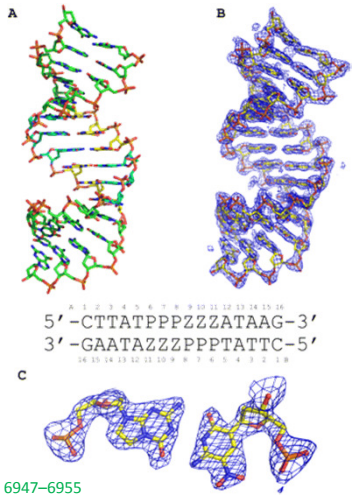
Electron density presented to the minor groove
→ recognition site by polymerases
„ minor groove scanning hypothesis"



S. Benner *et al., J. Am. Chem. Soc.*, **2011**, *133 (38),* pp 15105–15112

ACGTZP-DNA crystal structures

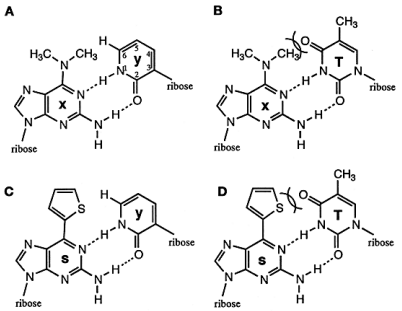




*18-mers: 2+2 **Z:P** pairs → B-DNA*
*6 consecutive **Z:P** → A-DNA*
*0,1 nm wider, but otherwise alike **G:C** pairs*

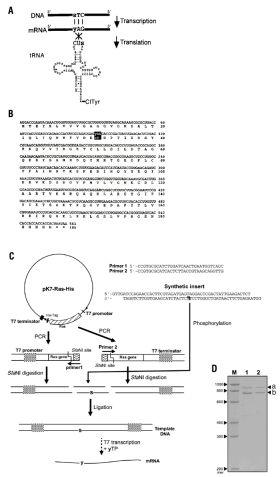
S. Benner *et al., J. Am. Chem. Soc.*, **2015**, *137,* pp 6947–6955

Unnatural aminoacid incorporation using a noncanonical base pair



Non-template strand 5′-d(ATAATACGACTCACTATAGGG)
Template strand 3′-d(TATTATGCTGAGTGATATCCCTTGCGCTCNTCACGCC)
N = **s**, **x**, A, or G
+1 +12 +19
↓ T7 RNA polymerase
↓ 1 mM N′TPs, [α-^{32}P]ATP, GMP
Full-length product (19-mer)
pGpGpGp*Ap*ApCpGpCpGp*ApGpN′p*ApGpTpGpCpGpG
↓ RNase T_2 digestion
↓ 2D-TLC
^{32}P-labeled nucleotides
Gp* x 2, Ap* x 1, **N′p*** x 1

I. Hirao *et al. Nature Biotechnology* **20**, 177–182 (2002)



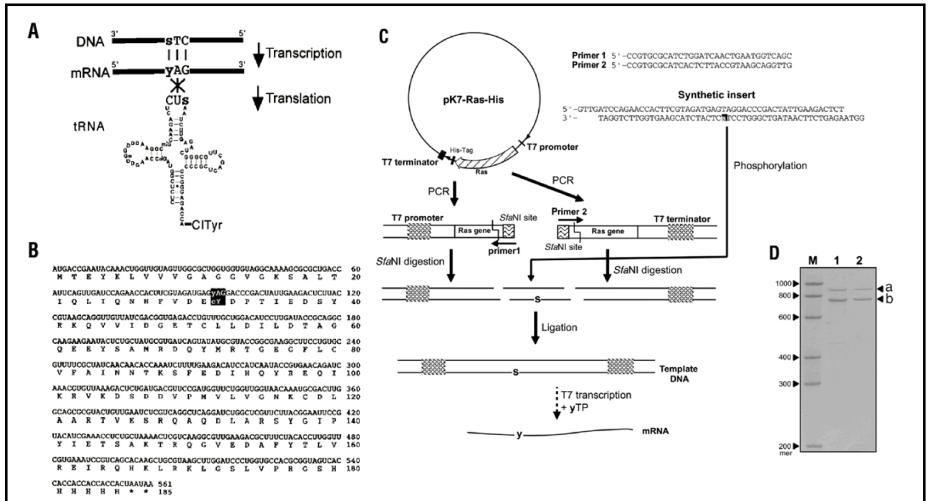
(A) The coupled transcription–translation system using the nonstandard codon–anticodon interaction for the site-specific incorporation of 3-chlorotyrosine into the Ras protein.

(B) The mRNA and amino acid sequences of human c-Ha-Ras replaced with the **y**AG codon for 3-chlorotyrosine at position 32.

(C) Construction of the DNA template containing the **s**TC sequence.

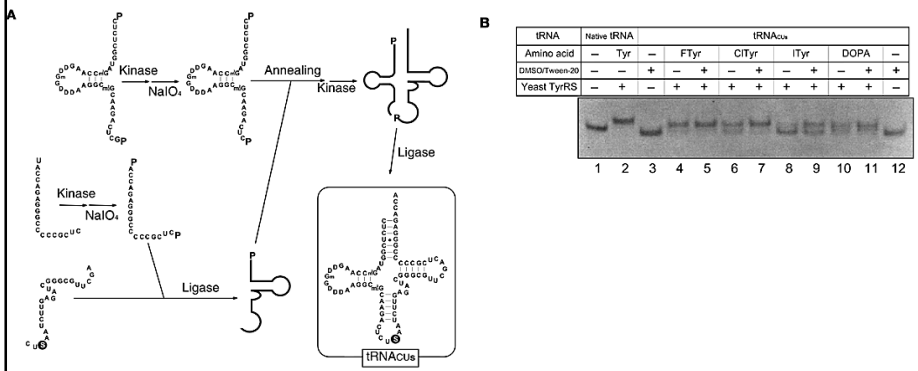
(D) Gel electrophoresis of the T7 transcripts. Lane 1 shows the transcripts from the DNA template containing the **s**TC sequence, and lane 2 shows those from the DNA template without **s** for the wild-type Ras protein. Bands a and b show the transcript terminated at the end of the template DNA (863-mer) and at the T7 terminator (747-mer), respectively.

I. Hirao *et al. Nature Biotechnology* **20**, 177–182 (2002)



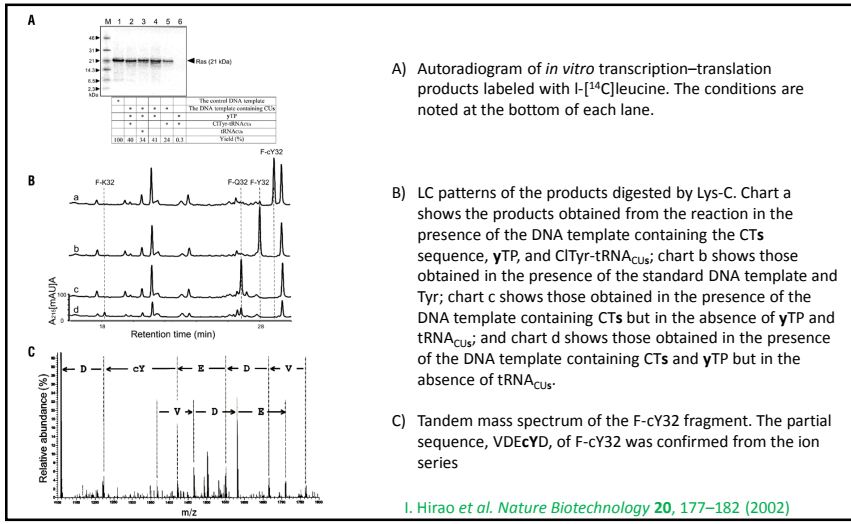
I. Hirao *et al. Nature Biotechnology* **20**, 177–182 (2002)

Unnatural aminoacid incorporation using a noncanonical base pair



(A) Construction of $tRNA_{CUs}$. (B) Acidic-gel electrophoresis of the products after aminoacylation of *S. cerevisiae* tRNA and $tRNA_{CUs}$ with tyrosine and the 3′-substituted analogs, in the absence or presence of 20% DMSO and 0.25% Tween-20. The upper bands are the aminoacylated tRNAs, and the lower bands are the noncharged tRNAs.

I. Hirao *et al. Nature Biotechnology* **20**, 177–182 (2002)



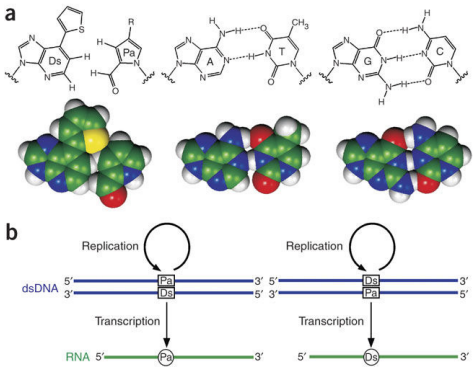
A) Autoradiogram of *in vitro* transcription–translation products labeled with l-[^{14}C]leucine. The conditions are noted at the bottom of each lane.

B) LC patterns of the products digested by Lys-C. Chart a shows the products obtained from the reaction in the presence of the DNA template containing the CT**s** sequence, **y**TP, and ClTyr-$tRNA_{CUs}$; chart b shows those obtained in the presence of the standard DNA template and Tyr; chart c shows those obtained in the presence of the DNA template containing CT**s** but in the absence of **y**TP and $tRNA_{CUs}$; and chart d shows those obtained in the presence of the DNA template containing CT**s** and **y**TP but in the absence of $tRNA_{CUs}$.

C) Tandem mass spectrum of the F-cY32 fragment. The partial sequence, VDE**cY**D, of F-cY32 was confirmed from the ion series

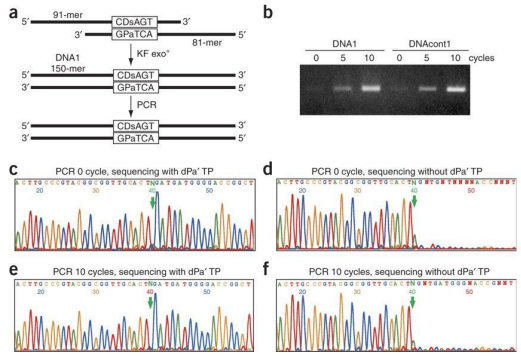
I. Hirao *et al. Nature Biotechnology* **20**, 177–182 (2002)

Unnatural aminoacid incorporation using a noncanonical base pair



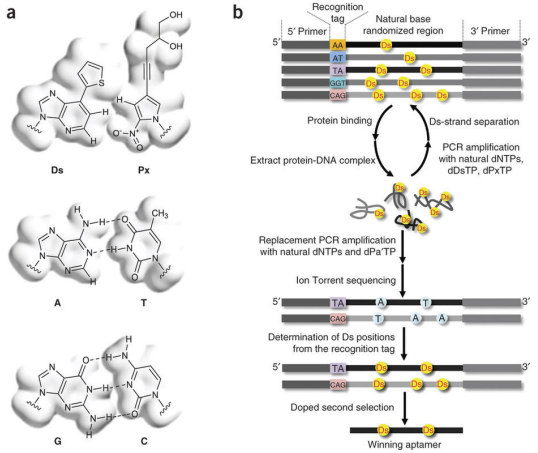
(**a**) The unnatural Ds-Pa and natural base pairs (Pa: R = H, Pa': R = C≡C–CH_3). (**b**) The unnatural base pair system that functions in PCR amplification, primer extension, DNA sequencing and T7 transcription. Original DNA templates were prepared by chemical synthesis and ligation, and were amplified by PCR with unmodified dNTPs (dPaTP, dGTP, dCTP and dTTP) and modified $dNTP_N$s (γ-amidotriphosphates, $dDsTP_N$ and $dATP_N$). RNA molecules containing Pa or modified Pa bases at specific positions were transcribed from DNA templates containing Ds in the template strands, by T7 RNA polymerase with PaTP (or modified PaTP) and the natural NTPs (left). RNA molecules containing Ds were transcribed from DNA templates containing Pa in the template strands, with DsTP and the natural NTPs (right).

I. Hirao *et al. Nature Methods* **2006**, *3*, 729-735



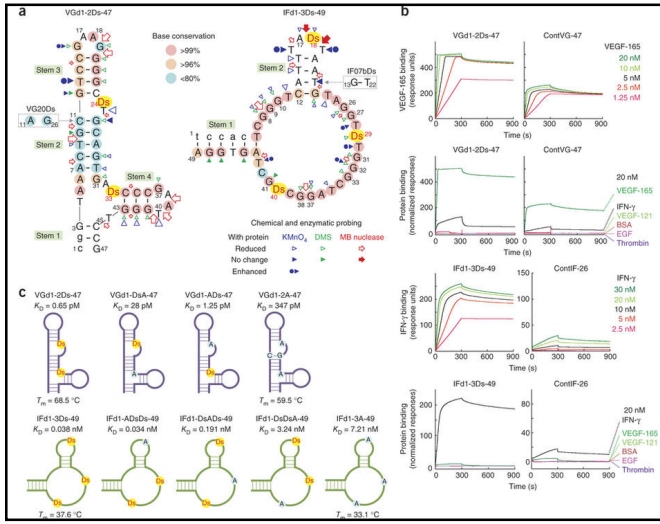
(**a**) The double stranded DNA fragment (150-mer, DNA1) was prepared by primer extension using chemically synthesized DNA fragments (91-mer and 81-mer) containing Ds and Pa. (**b**) Agarose-gel analysis of original DNA fragments (0 cycle) and PCR products after 5 and 10 cycles of amplification. For DNA1, PCR was performed with 0.04 unit/μl Vent DNA polymerase and the following cycle conditions: 0.5 min at 94 °C, 0.5 min at 45 °C and 4 min at 65 °C. For DNAcont1 consisting only of the natural bases, PCR was performed with 0.01 unit/μl Vent DNA polymerase with the following cycle conditions: 0.5 min at 94 °C, 0.5 min at 45 °C and 1 min at 72 °C. (**c**–**f**) DNA sequencing, in the presence (**c**,**e**) or absence (**d**,**f**) of dPa'TP, of the original DNA1 (**c**,**d**) and PCR-amplified DNA1 after 10 cycles using the unnatural base pair system (**e**,**f**).

Ds-Px noncanonical base pair – High-affinity DNA aptamers



Targets:
VEGF-165
and IFN-γ

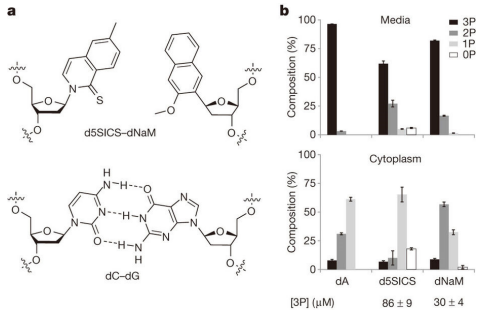
I. Hirao *et al. Nature Biotechnology* **2012**, *31*, 453-458



Characterizations and binding affinities of anti–VEGF-165 aptamer (VGd1-2Ds-47) and anti–IFN-γ aptamer (IFd1-3Ds-49)

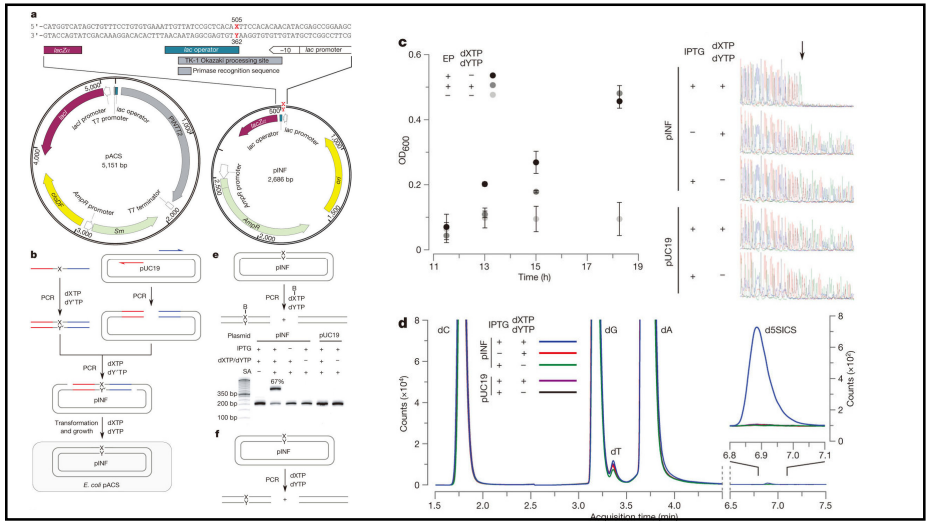
I. Hirao *et al. Nature Biotechnology* **2012**, *31*, 453-458

A semi-synthetic organism with an expanded genetic alphabet

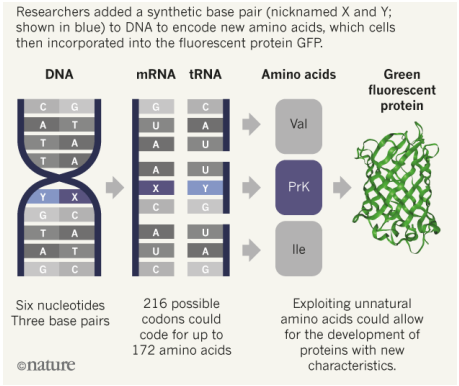


a, Chemical structure of the d5SICS–dNaM UBP compared to the natural dG–dC base pair. **b**, Composition analysis of d5SICS and dNaM in the media (top) and cytoplasmic (bottom) fractions of cells expressing *Pt*NTT2 after 30 min incubation; dA shown for comparison. 3P, 2P, 1P and 0P correspond to triphosphate, diphosphate, monophosphate and nucleoside, respectively; [3P] is the intracellular concentration of triphosphate.

Malyshev, Denis A.; Romesberg, Floyd E. *et al.* *Nature* **2014**, *509,* 385–388



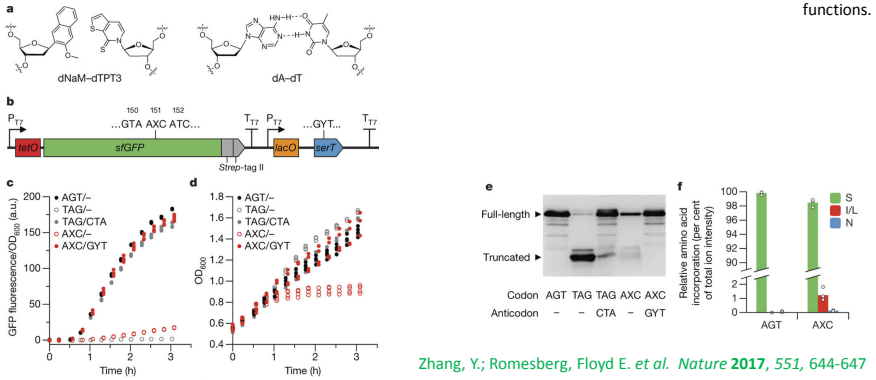
A semi-synthetic organism with an expanded genetic alphabet



Zhang, Y.; Romesberg, Floyd E. *et al.* *Nature* **2017**, *551,* 644-647

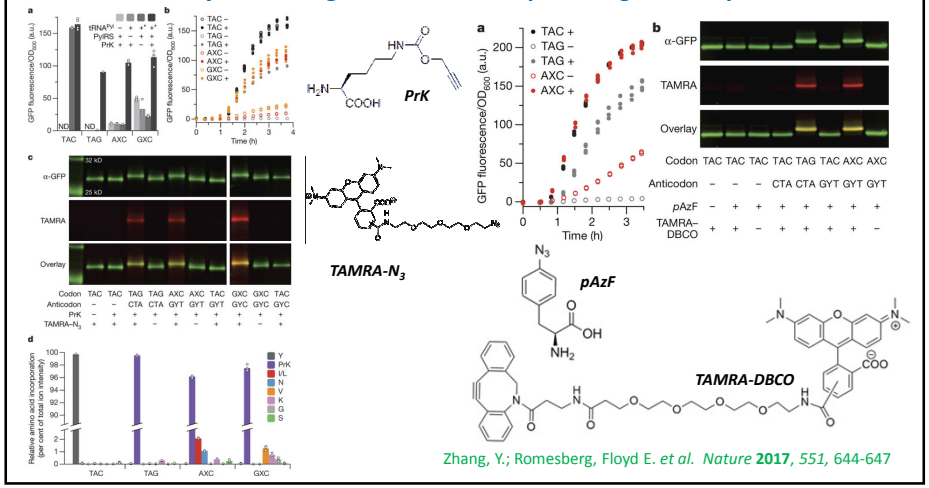
A semi-synthetic organism with an expanded genetic alphabet

The *in vivo* transcription of DNA containing dNaM and dTPT3 into mRNAs with two different unnatural codons and tRNAs with cognate unnatural anticodons, and their efficient decoding at the ribosome to direct the site-specific incorporation of natural or non-canonical amino acids into superfolder green fluorescent protein. The resulting semi-synthetic organism both encodes and retrieves increased information and should serve as a platform for the creation of new life forms and functions.



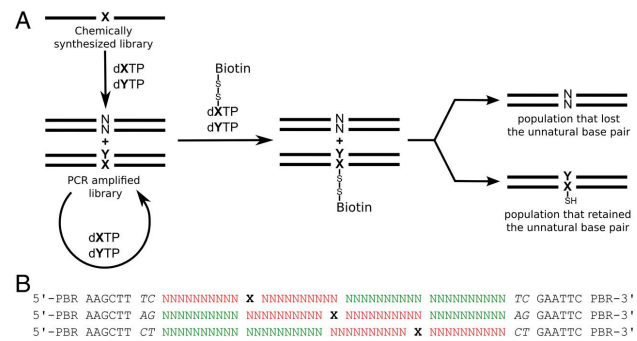
Zhang, Y.; Romesberg, Floyd E. *et al.* *Nature* **2017**, *551,* 644-647

A semi-synthetic organism with an expanded genetic alphabet



Zhang, Y.; Romesberg, Floyd E. *et al. Nature* **2017**, *551,* 644-647

A semi-synthetic organism with an expanded genetic alphabet



(A) PCR selection scheme. X = NaM (or when biotinylated, its analog MMO2; see Fig. S5) and Y = 5SICS. (B) Library design. The regions proximal to the unnatural base pair that were analyzed for biases are shown in red, and the distal regions used as a control are shown in green. Sublibrary-specific two-nucleotide barcodes that indicate the position of the unnatural base pair flank the randomized regions and are shown in italics. Primer binding regions are denoted as PBR

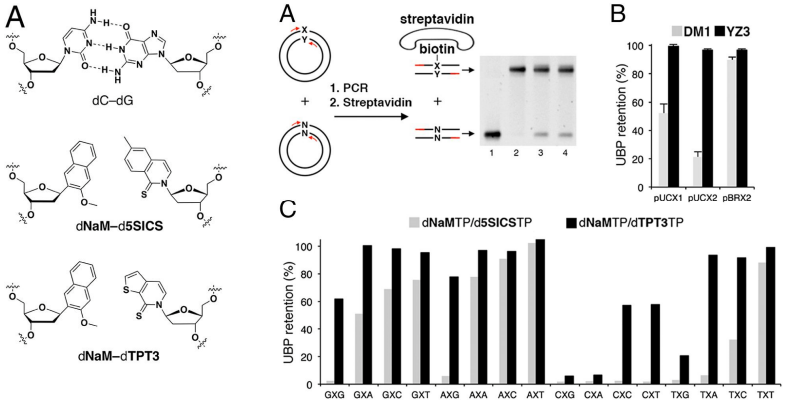
Malyshev, Denis A.; Romesberg, Floyd E. *et al. PNAS* **2012**, *109 (30),* 12005-12010

A semi-synthetic organism with an expanded genetic alphabet

- An unnatural base pair (UBP) would increase the information storage potential of DNA
- and semisynthetic organisms (SSOs) that stably harbor this expanded alphabet would thereby have the potential to store and retrieve increased information,
- *Escherichia coli* grown in the presence of the unnatural nucleoside triphosphates dNaMTP and d5SICSTP, and provided with the means to import them via expression of a plasmid-borne nucleoside triphosphate transporter, replicates DNA containing a single dNaM-d5SICS UBP,
- to fortify and vivify the nascent SSO, a more chemically optimized UBP dTPT3 was used, and the power of the bacterial immune response was harnessed by using Cas9 to eliminate DNA that had lost the UBP.
- The optimized SSO grows robustly, constitutively imports the unnatural triphosphates, and is able to indefinitely retain multiple UBPs in virtually any sequence context. This SSO is thus a form of life that can stably store genetic information using a six-letter, three-base-pair alphabet

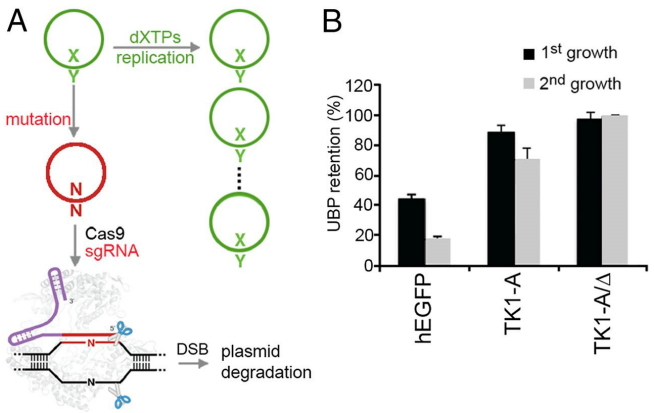
Malyshev, Denis A.; Romesberg, Floyd E. *et al. PNAS* **2017**, *114,* 1317-1322

A semi-synthetic organism with an expanded genetic alphabet



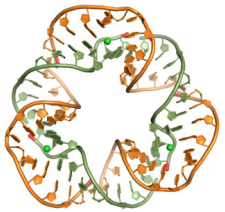
Malyshev, Denis A.; Romesberg, Floyd E. *et al. PNAS* **2017**, *114,* 1317-1322

A semi-synthetic organism with an expanded genetic alphabet



Malyshev, Denis A.; Romesberg, Floyd E. *et al.* *PNAS* **2017**, *114*, 1317-1322

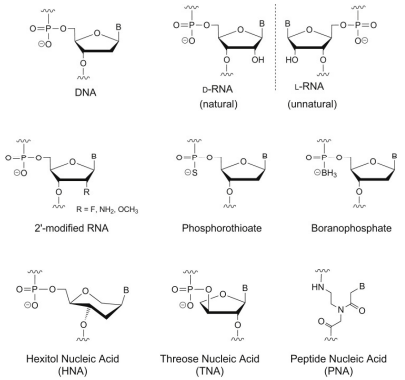
CHAPTER 1



OLIGONUCLEOTIDES

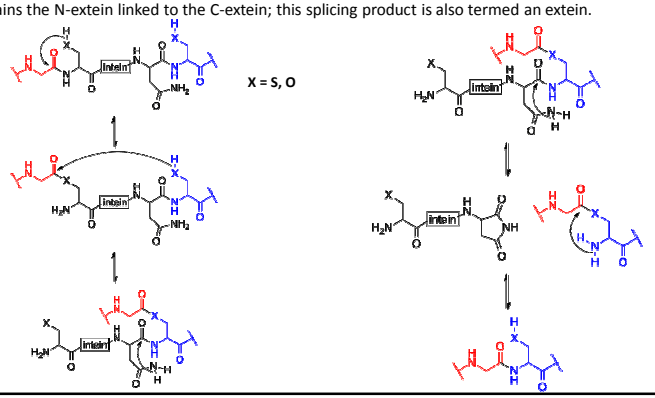
Part 3 – noncanonical backbone

Artificial genetic polymers



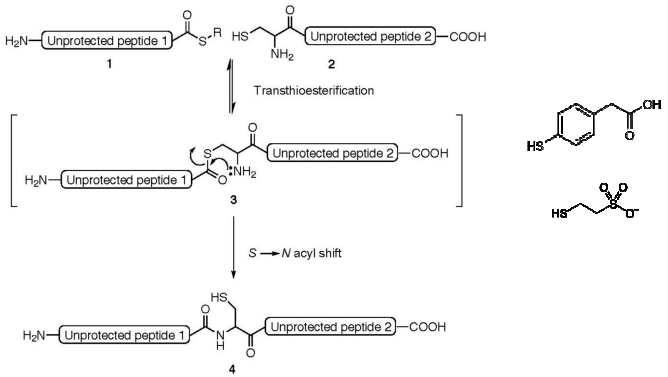
Intein splicing

An **intein** is a segment of a protein that is able to excise itself and join the remaining portions (the **exteins**) with a peptide bond in a process termed protein splicing. Inteins have also been called "protein introns".
Intein-mediated protein splicing occurs after the intein-containing mRNA has been translated into a protein. This precursor protein contains three segments—an **N-extein** followed by the intein followed by a **C-extein**. After splicing has taken place, the resulting protein contains the N-extein linked to the C-extein; this splicing product is also termed an extein.



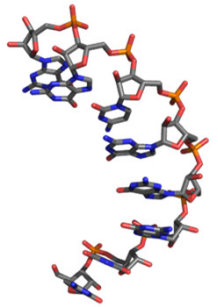
Native chemical ligation

Native chemical ligation or ***NCL*** is an important extension of the chemical ligation field, a concept for constructing a large polypeptide formed by the assembling of two or more unprotected peptides segments. Especially, NCL is the most powerful ligation method for synthesizing proteins (native or modified) of moderate size (i.e., small proteins< 200 AA).



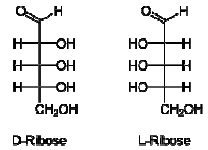
Spiegelmers: L-RNA

Aptamers (from the Latin aptus – fit, and Greek meros – part) are oligonucleotide or peptide molecules that ***bind to a specific target molecule***. Aptamers are usually created by selecting them from a large random sequence pool, but natural aptamers also exist in riboswitches.

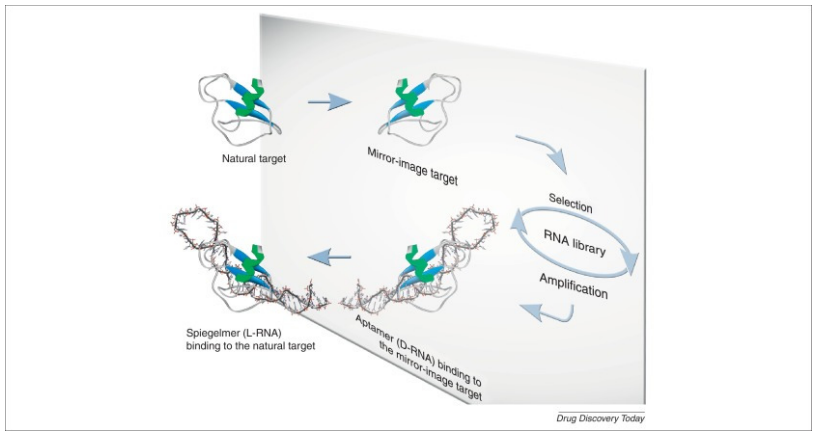


An ***L-ribonucleic acid aptamer*** (L-RNA aptamer, trade name ***Spiegelmer*** – from German Spiegel "mirror" – by Noxxon Pharma) is an RNA-like molecule built from L-ribose units.
It is an artificial oligonucleotide named for being a mirror image of natural oligonucleotides.

L-RNA aptamers are a form of aptamers. Due to their L-nucleotides, they are highly resistant to degradation by nucleases. ***Spiegelmers*** are considered potential drugs and are currently being tested in clinical trials.

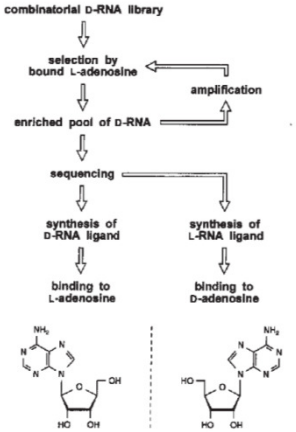


A. Vater, S. Klussmann, *Drug Discovery Today* **2015**, *20,* 147-155



A. Vater, S. Klussmann, *Drug Discovery Today* **2015**, *20,* 147-155

Mirror-image RNA that binds D-Adenosine



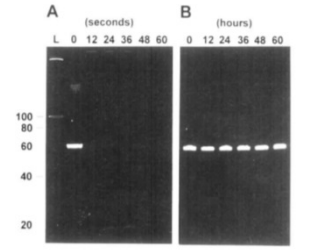


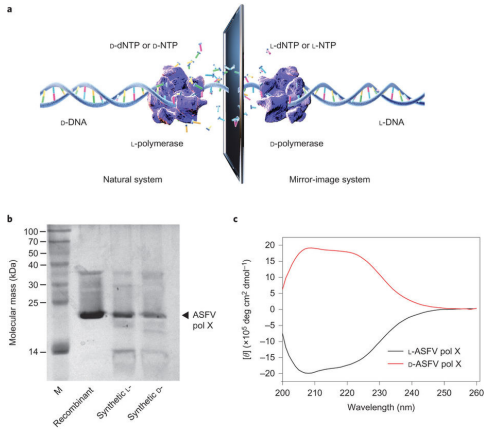
Figure 5. Stability of the 58-mer RNA ligands in human serum. (A) Aptamer D-A42d and (B) spiegelmer L-A42d. Aliquots were taken at the indicated times. L marks the size standard (10 bp DNA ladder). The results were reproduced in an independent experiment.

S. Klussmann, J. Furste, et al. *Nature Biotech.* **1996**, *14,* 1112-1115

Spiegelmers: L-DNA polymerase

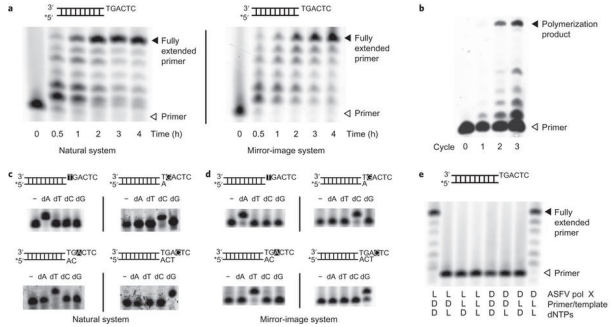
The mirror image configuration of polymerase X from African swine fever virus, the shortest known polymerase (174 amino acids), has recently been demonstrated to elongate an *L*-DNA primer with *L*-dNTPs; and a functional 56-mer *L*-DNAzyme was made within 36 hours.

This poses an important proof of concept, however, polymerase X is a thermo-labile repair enzyme and its catalytic activity does not meet the requirements for a standard PCR



Z. Wang, W. Xu, L. Liu, T. F. Zhu *Nature Chem.* **2016**, *8*, 698-704

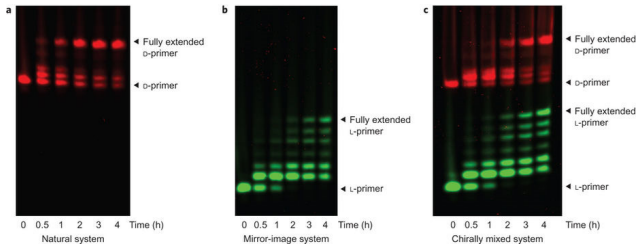
Spiegelmers: L-DNA polymerase



a, Template-directed primer extension by synthetic L-ASFV pol X (natural system) and D-ASFV pol X (mirror-image system) with the corresponding D- and L-DNA primers, templates and dNTPs. **b**, Repeated cycles of polymerization by D-ASFV pol X: **c**,**d**, The nucleotide substrate specificities of synthetic L- and D-ASFV pol X. **e**, Chiral specificity assay with different chiral combinations of polymerases, primer/template pairs and dNTPs.

Z. Wang, W. Xu, L. Liu, T. F. Zhu *Nature Chem.* **2016**, *8*, 698-704

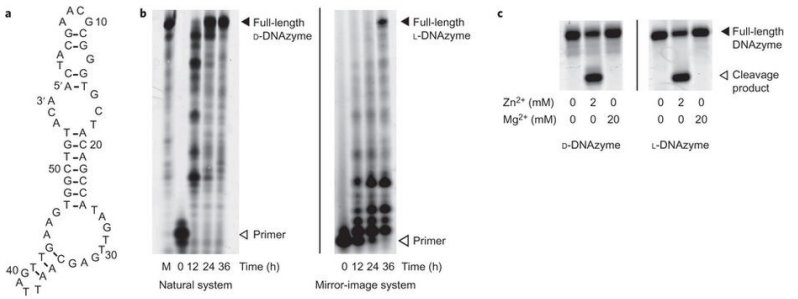
Spiegelmers: L-DNA polymerase



a,**b**, Primer extension by synthetic L- and D-ASFV pol X with the corresponding D-DNA primer (5′-Cy5 labelled) and L-DNA primer (5′-FAM labelled), templates and dNTPs. **c**, The above two polymerization reactions were carried out in a racemic mixture under the same conditions as described above, with the L- and D-ASFV pol X, D- and L-primers, D- and L-templates and D- and L-dNTPs added, incubated for up to 4 h at 37 °C.

Z. Wang, W. Xu, L. Liu, T. F. Zhu *Nature Chem.* **2016**, *8*, 698-704

Spiegelmers: L-DNAzyme

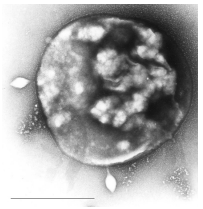


a, Sequence and predicted secondary structure of the previously reported Zn^{2+}-dependent self-cleaving DNAzyme. **b**, Primer extension on a 66 nt template to produce the Zn^{2+}-dependent self-cleaving DNAzyme. **c**, Self-cleavage of the enzymatically polymerized Zn^{2+}-dependent D- and L-DNAzymes.

Z. Wang, W. Xu, L. Liu, T. F. Zhu *Nature Chem.* **2016**, *8*, 698-704

Spiegelmers: A thermostable D-polymerase

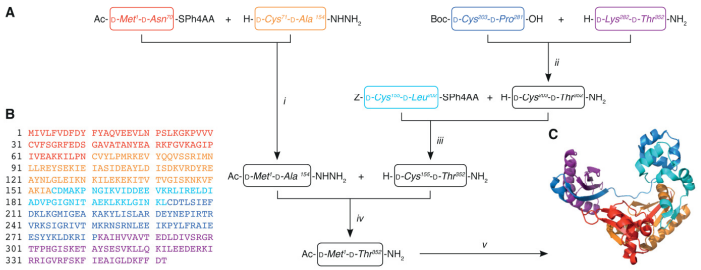
A thermostable mirror-image polymerase ***D*-Dpo4-3C** has been produced, that is able to amplify L-DNA in a classical PCR reaction and can even be used to assemble an L-DNA gene from L-DNA oligonucleotides. This artificial enzyme is a mutant of DNA polymerase IV from *Sulfolobus solfataricus*, a Y-family polymerase consisting of 352 amino acids, the longest protein made by chemical synthesis thus far.



Cell of *Sulfolobus* infected by virus STSV1 observed under microscopy. Two spindle-shaped viruses were being released from the host cell.

Furthermore, with an additional single point mutation (Tyr12Ala or Tyr12Ser), this DNA polymerase can be tuned to accept also ribonucleotides as substrates with reasonable efficiency. Thus, this enzyme may be hijacked to act as a DNA-dependent RNA polymerase to prepare longer stretches of *L*-RNA

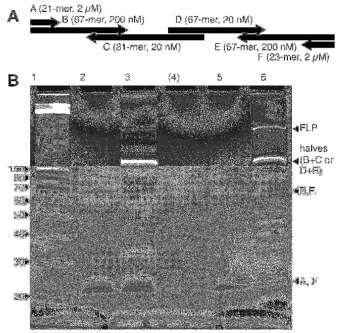
Spiegelmers: A thermostable D-polymerase



Synthesis strategy for d-Dpo4-3C. (**A**) five fragments were synthesized and assembled as follows: (i) native chemical ligation (NCL) of fragments 1 and 2. Isolated yield ≈ 18%. (ii) Segment condensation of fully protected fragments 4 and 5 followed by deprotection. Isolated yield ≈ 15%. (iii) NCL of fragments 3 and 4•5 followed by Z-deprotection. Isolated yield ≈ 25%. (iv) Thioester-conversion of fragment 1•2 and NCL with fragment 3•4•5. Isolated yield: 10%. (v) Folding. (**B**) sequence of d-Dpo4-3C; coloring as in panel A. (**C**) folded d-Dpo4-3C (artist impression based on PDB 3PR4 (31)).

S. Klussmann *Nucl. Acid Res.* **2017**, *45,* 3997-4005

Spiegelmers: A thermostable D-polymerase

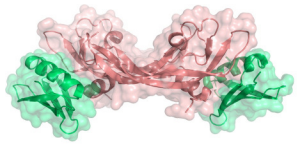


Assembly of a mirror-image gene. (**A**) schematic of the oligonucleotide setup. (**B**) lane 1, 3 µl of 10 bp DNA ladder. Lane 2, mirror-image no-enzyme control. Lane 3, mirror-image gene assembly. Lane 4, empty. Lane 5, natural handedness no enzyme control. Lane 6, natural handedness gene assembly.

S. Klussmann *Nucl. Acid Res.* **2017**, *45,* 3997-4005

D-proteins: almost ideal therapeutic agents

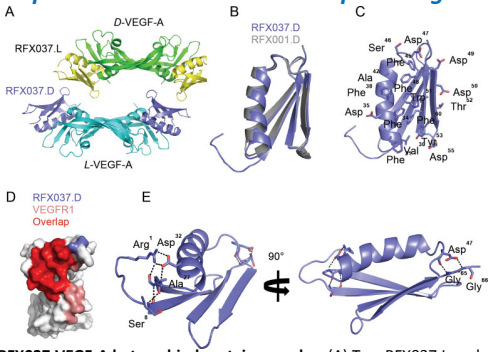
Polypeptides composed entirely of *D*-amino acids and the achiral amino acid glycine (*D* -proteins) inherently have *in vivo* properties that are proposed to be near-optimal for a large molecule therapeutic agent. Specifically, *D* -proteins are resistant to degradation by proteases and are anticipated to be nonimmunogenic. Furthermore, *D* -proteins are manufactured chemically and can be engineered to have other desirable properties, such as improved stability, affinity, and pharmacokinetics.



RFX037.D is a *D*-protein antagonist of natural vascular endothelial growth factor A (VEGF-A) that inhibited binding to its receptor, with extreme thermal stability (T_m > 95 °C) and high affinity for VEGF-A (K_d = 6 nM).
Comparison of the two enantiomeric forms of RFX037 revealed that the *D*-protein is more stable in mouse, monkey, and human plasma and has a longer half-life *in vivo* in mice. Significantly, RFX037.D was nonimmunogenic in mice, whereas the *L*-enantiomer generated a strong immune response. These results confirm the potential utility of synthetic *D*-proteins as alternatives to therapeutic antibodies.

S. Kent *et al.*, ACS Chem. Biol. **2016**, *11,* 1058-1065

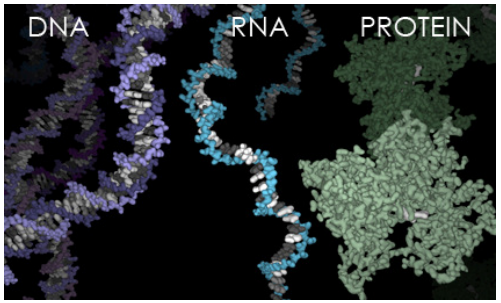
D-proteins: almost ideal therapeutic agents



X-ray crystal structure of RFX037:VEGF-A heterochiral protein complex. (A) Two RFX037.L molecules (yellow) bound to one d-VEGF-A homodimer (green) and two RFX037.D molecules (blue) bound to one l-VEGF-A homodimer (cyan). (B) Superposition of RFX037.D (blue) and RFX001.D (gray, rcsb accession 4GLS). (C) RFX037.D side chains (shown as sticks) that contact l-VEGF-A. (D) The contact surfaces of l-VEGF-A to RFX037.D (blue), VEGFR1 (salmon), or both (red). (E) Hydrogen bond networks formed by intramolecular polar contacts originated from additional N- and C-terminal residues in RFX037.D.

S. Kent *et al.*, ACS Chem. Biol. **2016**, *11*, 1058-1065

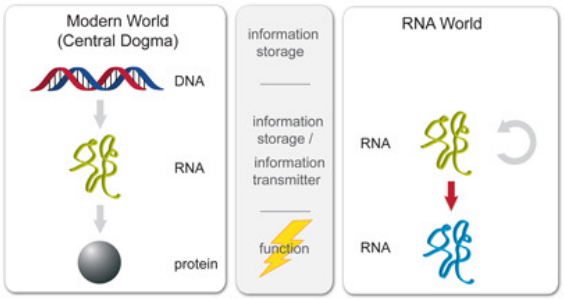
The RNA world



In modern cells, RNA (light blue, center) is made from a DNA template (purple, left) to create proteins (green, right).

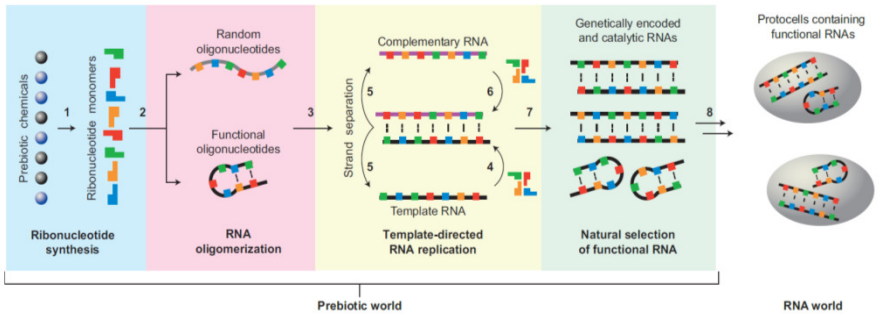
RNA folding is mediated by base-pairing interactions along different regions of a single-stranded RNA.

The RNA world

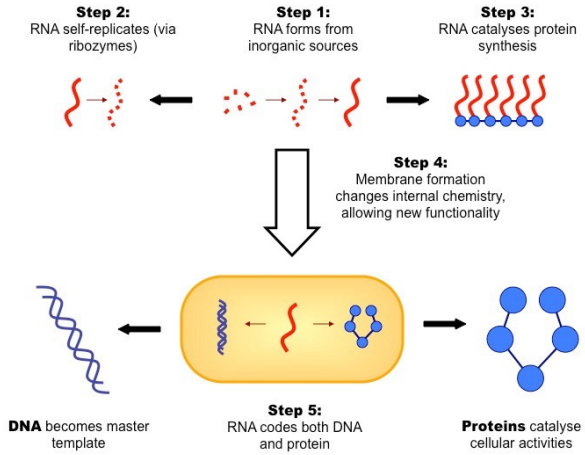


Conceptual idea that there was a period in the early history of life on Earth when RNA (or its structurally simplified analogue) carried out most of the information processing and metabolic transformations needed for biology to emerge from chemistry

The RNA world



The RNA world

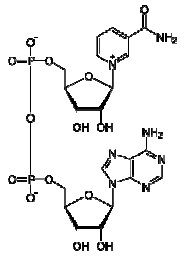


The RNA world

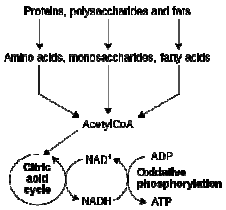
Crick, Orgel and Woese speculated in 1968 that, because RNA can form secondary structures, it has both a genotype and a phenotype and is a good candidate for the emergence of life

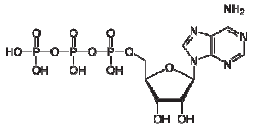
F. H. C. Crick *J. Mol. Biol.* **1968**, *38,* 367-379, L. E. Orgel *J. Mol. Biol.* **1968**, *38,* 381-393

Ribonucleotide coenzymes currently used by many proteins may be molecular „fossils" from the primoridal RNA-based metabolism



Nicotinamide adenine dinucleotide (NAD⁺)



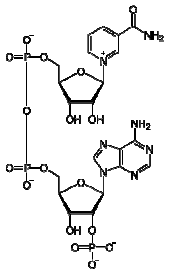


Adenosine triphosphate (ATP)

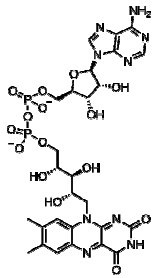
H. B. White III *J. Mol. Evol.* **1976**, *7,* 101-104

The RNA world

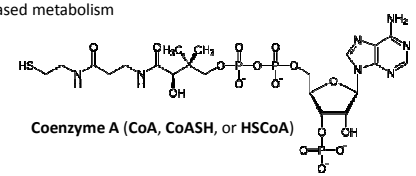
Ribonucleotide coenzymes now used by many proteins may be molecular „fossils" from the primoridal RNA-based metabolism



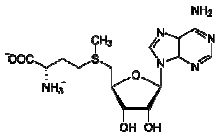
Nicotinamide adenine dinucleotide phosphate (NADP⁺)



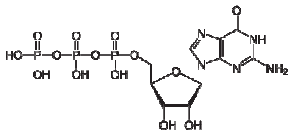
flavin adenine dinucleotide (FAD)



Coenzyme A (CoA, CoASH, or HSCoA)



***S*-Adenosyl methionine**

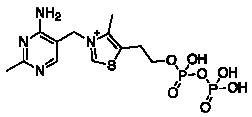


Guanosine-5'-triphosphate (GTP)

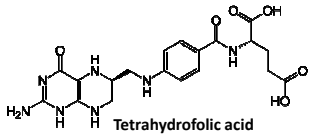
H. B. White III *J. Mol. Evol.* **1976**, *7,* 101-104

The RNA world

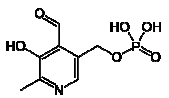
Other coenzymes contain cyclic nitrogen-containing bases that can also derive from nucleotides



Thiamine pyrophosphate (TPP or ThPP) – Vit. B_1



Tetrahydrofolic acid



Pyridoxal phosphate (PLP) – Vit. B_6

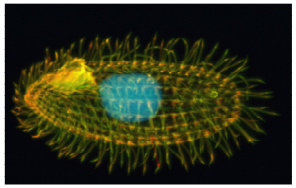
H. B. White III *J. Mol. Evol.* **1976**, *7,* 101-104

The RNA world

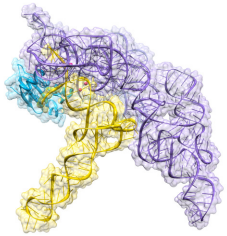
Ribozymes – Ribonucleic acid enzymes

1989 – Thomas Cech and Sidney Altman – Nobel Prize in chemistry for discovery of catalytic RNA

Thomas R. Cech was studying RNA splicing in the ciliated protozoan *Tetrahymena thermophila*
Sidney Altman and Norman Pace were studying the bacterial RNase P complex.



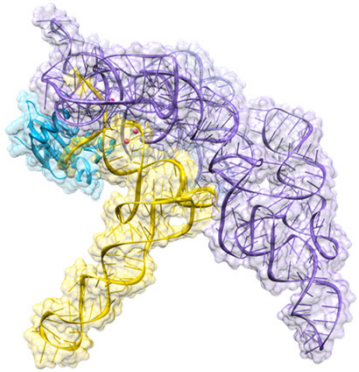
Tetrahymena thermophila



Bacterial RNAse P

The RNA world

Ribonuclease P

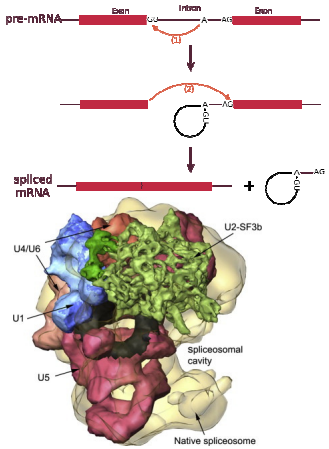


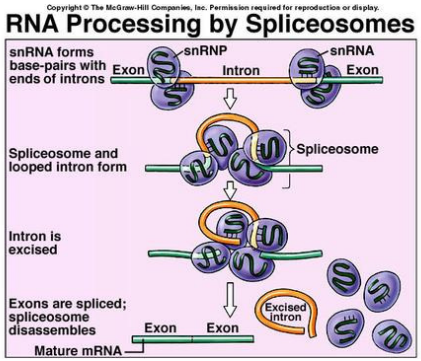
Ribonuclease P (RNase P) is a type of ribonuclease which cleaves RNA. RNase P is unique from other RNases in that it is a ribozyme – a ribonucleic acid that acts as a catalyst in the same way that a protein based enzyme would. Its function is to cleave off an extra, or precursor, sequence of RNA on tRNA molecules.

Bacterial RNase P has two components: an RNA chain, called M1 RNA, and a polypeptide chain, or protein, called C5 protein. *In vivo*, both components are necessary for the ribozyme to function properly, but *in vitro*, the M1 RNA can act alone as a catalyst. The primary role of the C5 protein is to enhance the substrate binding affinity and the catalytic rate of the M1 RNA enzyme probably by increasing the metal ion affinity in the active site.

Crystal structure of a bacterial ribonuclease P holoenzyme in complex with tRNA (yellow), showing metal ions involved in catalysis (pink)

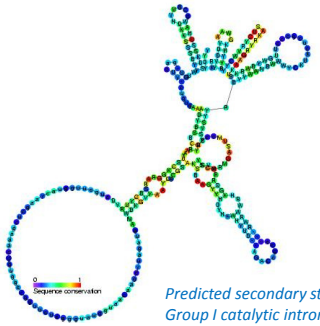
RNA splicing





Spliceosome – a complex of ribonucleoproteins

RNA splicing

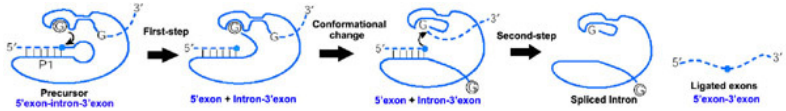


Self-splicing RNA introns

RNA splicing in *Tetrahymena* was taking place also in absence of the spliceosome - the ‚negative control' obtained after protease digestion also spliced.

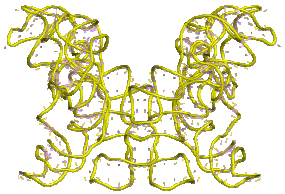
In contrary to the spliceosome, the ***catalytic*** motif ***does not*** contain protein part, ***only RNA***.
First known example of a ***ribozyme*** – ribonucleic acid-composed enzyme analogue.

Predicted secondary structure and sequence conservation of Group I catalytic intron



RNA splicing

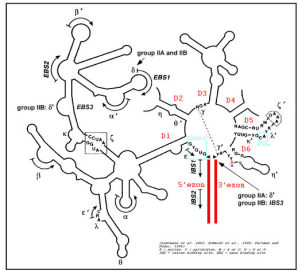
Group I catalytic introns



A 3D representation of the Group I catalytic intron. This view shows the active site in the crystal structure of the Tetrahymena ribozyme

Ribozyme activity (e.g., self-splicing) can occur under high-salt conditions in vitro. However, assistance from proteins is required for in vivo splicing

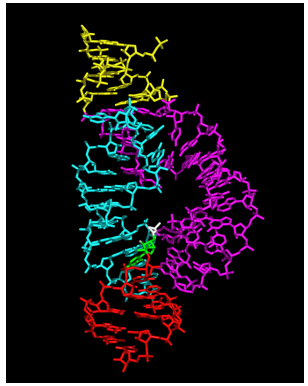
Group II catalytic introns



It is hypothesized that pre-mRNA splicing may have evolved from group II introns, due to the similar catalytic mechanism as well as the structural similarity of the Domain V substructure to the U6/U2 extended snRNA

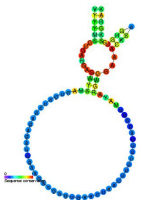
Ribozymes

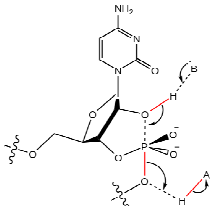
Hammerhead ribozyme



The hammerhead ribozyme is a RNA molecule motif that catalyzes reversible cleavage and joining reactions at a specific site within an RNA molecule.

- model system for research on the structure and properties of RNA,
- targeted RNA cleavage experiments,



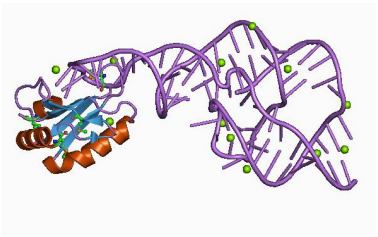


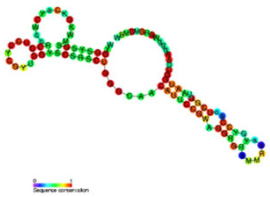
Ribozymes

HDV ribozyme

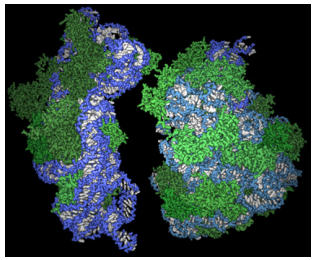
The hepatitis delta virus (HDV) ribozyme is a non-coding RNA found in the hepatitis delta virus that is necessary for viral replication and is thought to be the only catalytic RNA known to be required for viability of a human pathogen.

The ribozyme acts to process the RNA transcripts to unit lengths in a self-cleavage reaction. The ribozyme is found to be active in vivo in the absence of any protein factors and is the fastest known naturally occurring self-cleaving RNA.





Ribosome – the ‚smoking gun'



Ribosome: green - proteins, blue and white - RNA

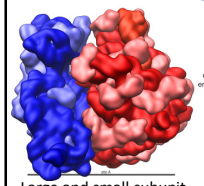
The ribosome is a simple molecular machine, found within all living cells, that serves as the site of biological protein synthesis (translation). Ribosomes link amino acids together in the order specified by messenger RNA (mRNA) molecules.

Ribosome is structurally highly conserved among all living species – most likely present in LUCA

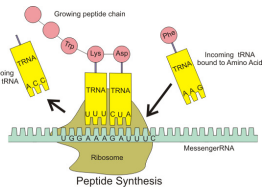
Ribosomes consist of two major components: the small ribosomal subunit, which reads the RNA, and the large subunit, which joins amino acids to form a polypeptide chain. Each subunit is composed of one or more ribosomal RNA (rRNA) molecules and a variety of ribosomal proteins.

Ribosome – the ‚smoking gun'

Ribosome - **3**

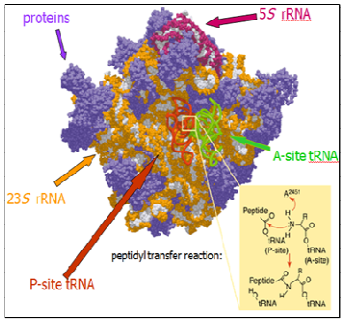


Large and small subunit



Peptide Synthesis

Ribosome is a ribozyme!



No protein within 18 Angstroms from the active site
→ proteins play a structural role, but
DO NOT CATALYZE THE ACYL TRANSFER PROCESS
T. Cech *Science.* **2000**, *289*, 878-879

Ribosome – the ‚smoking gun'

Ribosome is a ribozyme!

The ribosome may have first originated in an RNA world appearing as a self-replicating complex that only later evolved the ability to synthesize proteins when amino acids began to appear.

Studies suggest that ancient ribosomes constructed solely of rRNA could have developed the ability to synthesize peptide bonds.

In addition, evidence strongly points to ancient ribosomes as self-replicating complexes, where the rRNA in the ribosomes had informational, structural, and catalytic purposes because it could have coded for tRNAs and proteins needed for ribosomal self-replication.

As amino acids gradually appeared in the RNA world under prebiotic conditions, their interactions with catalytic RNA would increase both the range and efficiency of function of catalytic RNA molecules. Thus, the driving force for the evolution of the ribosome from an ancient self-replicating machine into its current form as a translational machine may have been the selective pressure to incorporate proteins into the ribosome's self-replicating mechanisms, so as to increase its capacity for self-replication

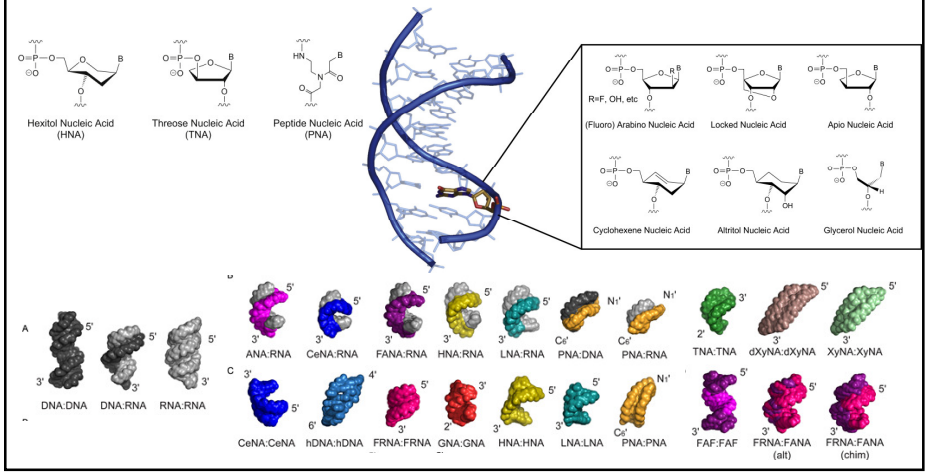
XNA – Xeno Nucleic Acids

XNA - synthetic alternative to DNA and RNA as information-storing biopolymers that differs in the sugar backbone.
- at least 6 XNAs can store and retrieve genetic information
- Ongoing research to create synthetic polymerases to transform XNA →

Xenobiology
- (XNA) as information carriers, expanded genetic code and, incorporation of non-proteinogenic amino acids into proteins
- the ***origin of life:*** *Primoridal soup → (XNA →) RNA → RNA(+DNA)+Proteins*
- development of industrial production systems with novel capabilities (pathogen resistance, biopolymer engineering)
- „genetic firewall" – excludes the risk of contaminating currently existing organisms (horizontal gene transfer)

The ***long-term goal*** - a cell that stores its genetic information on XNA, with different base pairs, using non-canonical amino acids and an altered genetic code.
So far cells have been constructed that incorporate only one or two of these features

XNA – Xeno Nucleic Acids



Synthetic genetic polymers capable of heredity and evolution

XNA are not recognized by natural polymerases.

One of the major challenges is to find or create novel types of polymerases that will be able to replicate these new-to-nature constructs. The method of polymerase evolution and design successfully led to the storage and recovery of genetic information (of less than 100bp length) from six alternative genetic polymers based on simple nucleic acid architectures not found in nature.

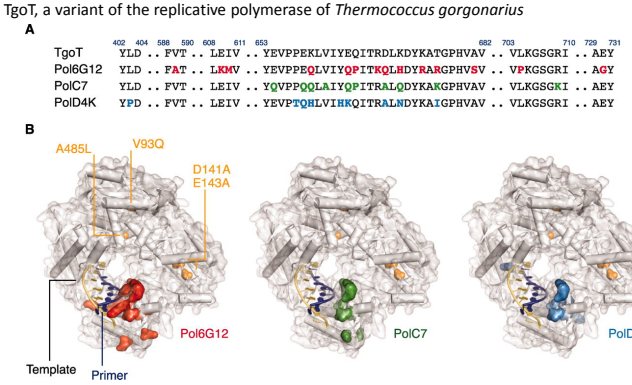
XNA aptamers, which bind their targets with high affinity and specificity, were also selected, demonstrating that beyond ***heredity***, specific XNAs have the capacity for ***Darwinian evolution*** and ***folding into defined structures***.

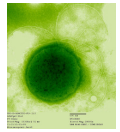
Thus, heredity and evolution, two hallmarks of life, are not limited to DNA and RNA but are likely to be emergent properties of polymers capable of information storage.

P. Herdewijn, P. Holliger, *et al. Science* **2012**, *336*, 341-344

Engineering XNA polymerases

TgoT, a variant of the replicative polymerase of *Thermococcus gorgonarius*



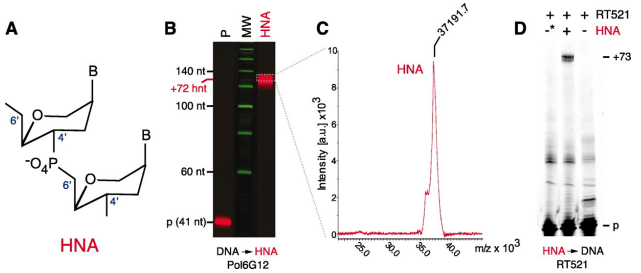


Thermococcus gorgonarius
(Angels Tapias)

(A) Sequence alignments showing mutations from wtTgo in polymerases Pol6G12 (red), PolC7 (green), and PolD4K (blue).
(B) Mutations are mapped on the structure of Pfu (PDB: 4AIL).
Yellow - template; dark blue - primer; orange - mutations present in the parent polymerase TgoT

P. Herdewijn, P. Holliger, *et al. Science* **2012**, *336*, 341-344

HNA synthesis



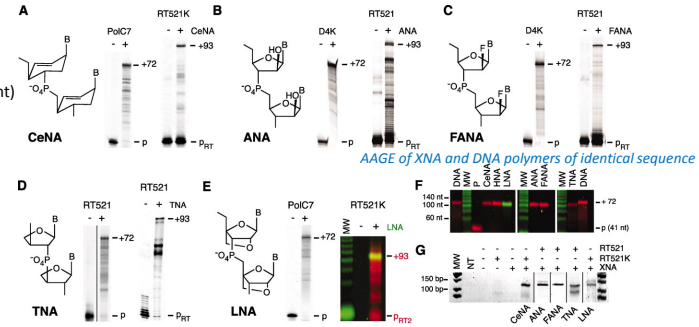
Pol6G12 extends the primer (p) incorporating 72 hNTPs against template T1 to generate a full-length hybrid molecule with a 37,215-dalton expected molecular mass.

HNA reverse transcription (DNA synthesis from an HNA template). Polymerase-synthesized HNA (from template YtHNA4) is used as template by RT521 for HNA-RT

P. Herdewijn, P. Holliger, *et al. Science* **2012**, *336*, 341-344

XNA genetic polymers.

Structures and PAGE of synthesis (+72 xnt), and reverse transcription (+93 nt)



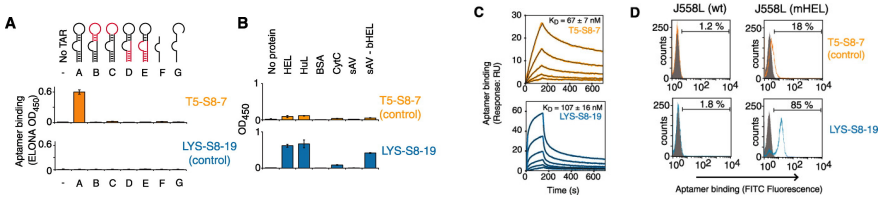
AAGE of XNA and DNA polymers of identical sequence

(E) PAGE of LNA synthesis [primer (41 nt) + 72 lnt] and LNA-RT (red). LNA synthesis (green) migrates at its expected size (113 nt) and comigrates with reverse transcribed DNA (red) synthesized from primer PRT2 (20 nt).

XNART–polymerase chain reaction. Amplification products of expected size (133 base pairs) are obtained only with both XNA forward synthesis and RT (RT521 or RT521K)

P. Herdewijn, P. Holliger, *et al. Science* **2012**, *336*, 341-344

HNA aptamers



Characterization of HNA aptamers. Anti-TAR aptamer T5-S8-7 and anti-HEL aptamer LYS-S8-19.

(A and B) Aptamer binding specificity against TAR variants (red, sequence randomized but with base-pairing patterns maintained) and different protein antigens (human lysozyme, HuL; cytochrome C, CytC; streptavidin, sAV; biotinylated-HEL bound to streptavidin, sAV-bHEL). OD, optical density.
(C) Affinity measurements of aptamer binding by SPR. RU, response units.
(D) FACS analysis of fluorescein isothiocyanate (FITC)–labeled aptamers binding to plasmacytoma line J558L with and without expression of membrane-bound HEL (mHEL). wt, wild type.

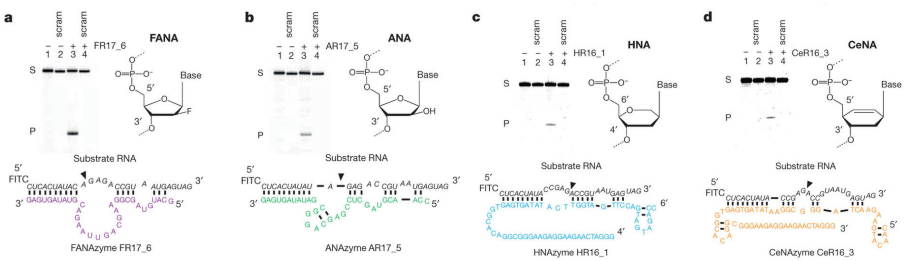
P. Herdewijn, P. Holliger, *et al. Science* **2012**, *336,* 341-344

XNA – Xeno Nucleic Acids

XNA – complementarity to DNA, also used as genetic catalysts.

FANA, HNA, CeNA and ANA - cleave RNA (***XNAzymes***).

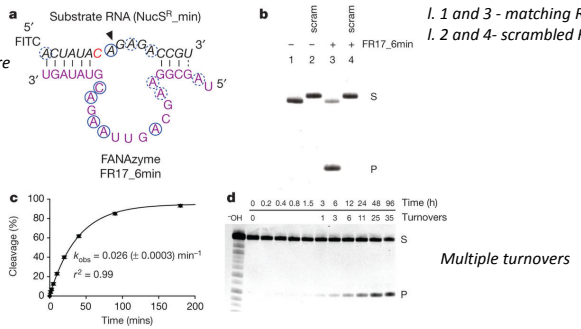
FANA XNAzymes can also ligate DNA, RNA and XNA substrates.



P. Herdewijn, P. Holliger, *et al. Nature* **2015**, *518,* 427-430

Chemical synthesis yields an active RNA endonuclease XNAzyme

Secondary FANAzyme structure



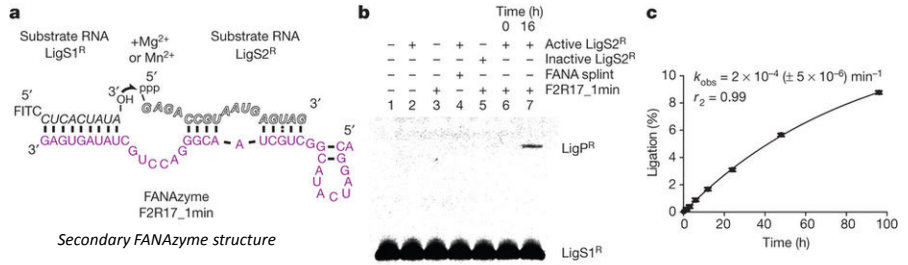
I. 1 and 3 - matching RNA
I. 2 and 4- scrambled RNA

Multiple turnovers

P. Herdewijn, P. Holliger, *et al. Nature* **2015**, *518,* 427-430

An RNA ligase XNAzyme (FANA)

FANA XNAzymes can also ligate DNA, RNA and XNA substrates.

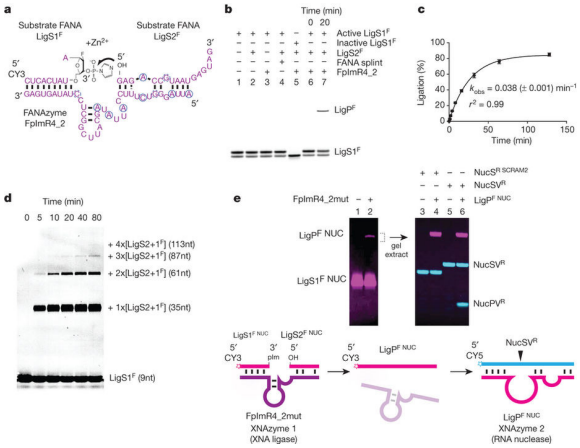


Secondary FANAzyme structure

Pre-steady state trimolecular reaction rate (k_{obs}) at 25 °C (n = 3; error bars, s.d.).

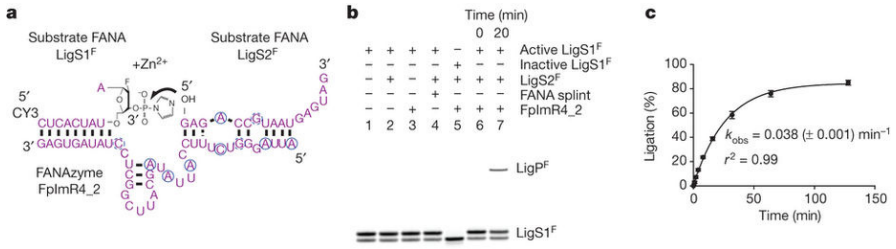
P. Herdewijn, P. Holliger, *et al. Nature* **2015**, *518,* 427-430

XNA–XNA ligase XNAzyme (FANA): catalysis without natural nucleic acids



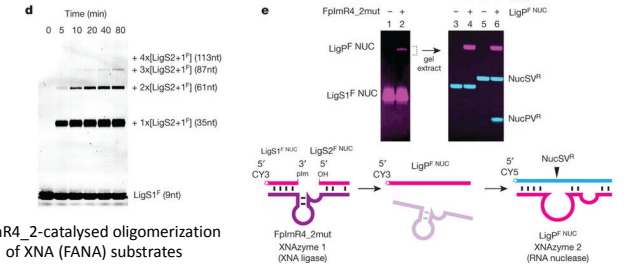
P. Herdewijn, P. Holliger, *et al. Nature* **2015**, *518*, 427-430

XNA–XNA ligase XNAzyme (FANA): catalysis without natural nucleic acids



P. Herdewijn, P. Holliger, *et al. Nature* **2015**, *518*, 427-430

XNA–XNA ligase XNAzyme (FANA): catalysis without natural nucleic acids

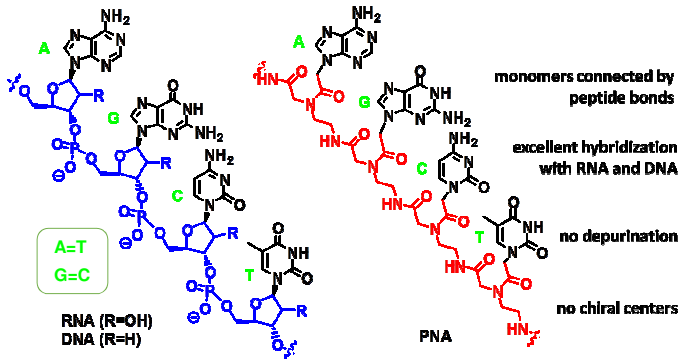


FplmR4_2-catalysed oligomerization of XNA (FANA) substrates

XNAzyme-catalysed assembly of an active XNAzyme. A variant XNA ligase (FplmR4_2mut) catalyses ligation (lane 2) of FANA substrates LigS1F NUC and LigS2F NUC. The product (LigPF NUC) is a variant of XNAzyme FR17_6 min (Fig. 2), which cleaves RNA substrate NucSVR (lanes 5 and 6), but not scrambled RNA (NucSR SCRAM2)(lanes 3 and 4).

P. Herdewijn, P. Holliger, *et al. Nature* **2015**, *518*, 427-430

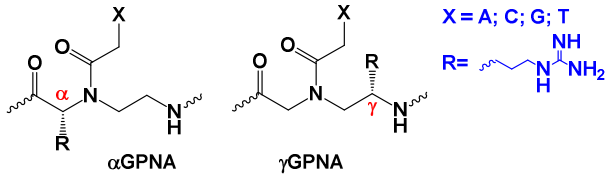
Peptidonucleic acids – functional DNA analogues



PNA – stable *ex vivo*, the backbone detected in cyanobacteria

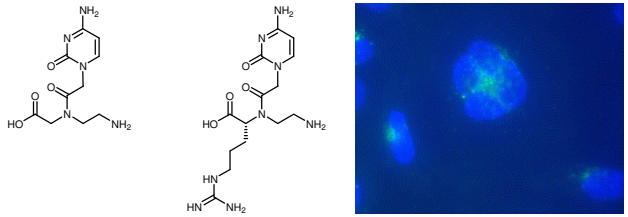
Applications: antigene, antisense agents; fluorescent DNA probes (FISH), anticancer, antiviral, antibacterial, antiparasitic agents; diagnostics, mol. biology

Structural modifications of the PNA - αGPNA, γGPNA



- GPNA: Alkylguanidinium residues (Arg side chains)
- enhanced water solubility
- cell permeability (analogous to oligoarginine CPPs)
- α position ← *D*-arginine
- γ position ← *L*-arginine

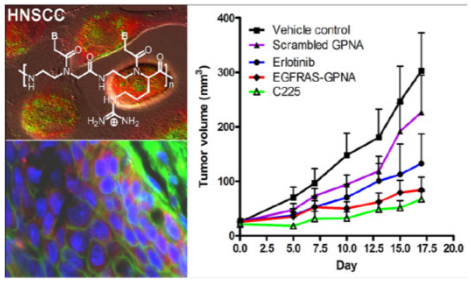
Cell-penetrating αGPNA



HeLa cells incubated with 1 μM GPNA (FITC-^{D}CC^{D}AC^{D}CT^{D}CT^{D}GC^{D}CA^{D}AC^{D}GG^{D}GT-NH_2) for 16 h, Fixed, stained with DAPI. Nuclei (blue), GPNA (green).

P. Zhou, A. Dragulescu-Andrasi, B. Bhattacharya, H. O'Keefe, P. Vatta, J. J. Hyldig-Nielsen and D. H. Ly *Bioorg. Med. Chem. Lett.* **2006**, *16*, 4931
A. Dragulescu-Andrasi, S. Rapireddy, G. He, B. Bhattacharya, J. J. Hyldig-Nielsen, B. G. Zon, and D. H. Ly *J. Am. Chem. Soc.* **2006**, *128*, 16104

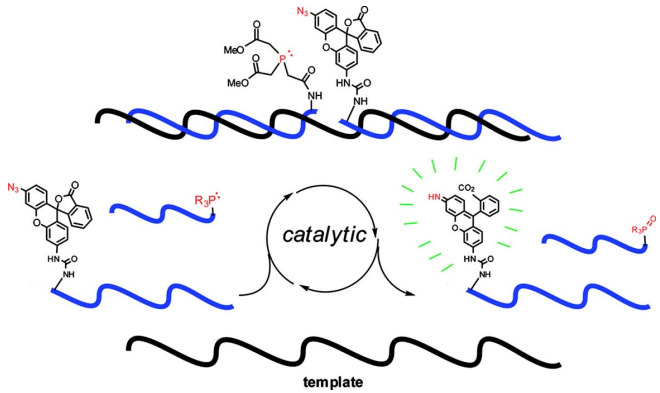
Antisense activity of αGPNA in vivo



- GPNA 16-mers targeting the epidermal growth factor receptor (EGFR) in preclinical models as therapeutic modality for head and neck squamous cell carcinoma (HNSCC) and nonsmall cell lung cancer (NSCLC)
- Elicited potent antisense effects in NSCLC and HNSCC preclinical models
- When administered intraperitoneally in mice, EGFRAS-GPNA was taken-up by several tissues including the xenograft tumor
- Systemic administration of EGFRAS-GPNA induced antitumor effects in HNSCC xenografts, with similar efficacies as the FDA-approved EGFR inhibitors: cetuximab and erlotinib.

D. Ly *et. al.* ACS *Chem. Biol.* **2013**, *8*, 345-352

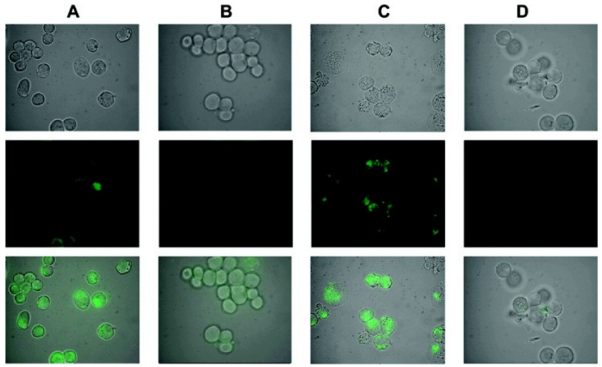
Cell-penetrating αGPNA for in vivo catalytic oligonucleotide sensing



Z. Pianowski, N. Winssinger *Chem. Comm.* **2007**, *37*, 3820-3822
Z. Pianowski *et al. J. Am. Chem. Soc.* **2009**, *131*, 6492-6497

Cell-penetrating αGPNA for in vivo catalytic oligonucleotide sensing

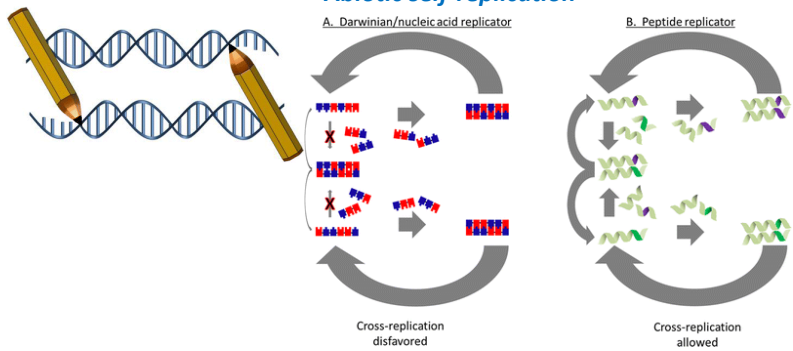
Inside living cells



A, B – controls (+/-) C – matching PNA D – mismatched PNA

Z.Pianowski, K. Górska, L. Oswald, C. Merten, N.Winssinger *J. Am. Chem. Soc.* **2009,** *131*, 6492-6497

Abiotic self-replication

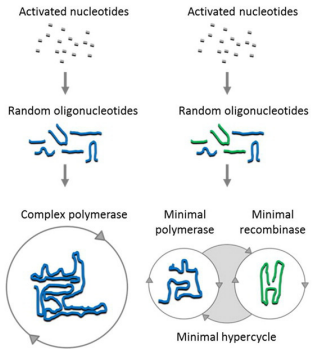


(A) For nucleic acids replicators, templating is based on base pairing, so the formation of a mutant template is rare. Once formed, the mutant replicator forms a competing replication cycle. (B) For a peptide replicator, templating is less exact, so the formation of a mutant template is common. The mutant template can catalyze formation of mutant progeny or parental progeny, and the two species form a mutualistic network.

Meyer AJ, Ellefson JW, Ellington AD. *Acc Chem Res.* **2012** *45(12)*:2097-2105.

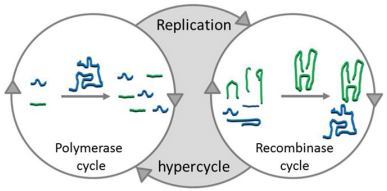
Emergence of a self-replication system through hypercycles

A. Complex emergence B. Cooperative emergence C. Cooperative-replication hypercycle



In the first scenario, a complex RNA-dependent RNA polymerase capable of full self-replication emerges from random oligonucleotides.

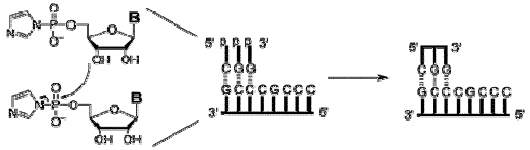
In the second scenario, a minimal polymerase and minimal recombinase emerge from random oligonucleotides. These ribozymes cooperate to perform replication.



The replication hypercycle: two intertwined polymerization and recombination cycles. In one cycle, polymerization of the short RNA fragments comprising the polymerase and recombinase occurs. In the other cycle, the reconstituted recombinase stitches the RNA fragments. Recombination is directed by internal guide sequences, forming longer, more complex ribozymes.

Meyer AJ, Ellefson JW, Ellington AD. *Acc Chem Res.* **2012** *45(12)*:2097-2105.

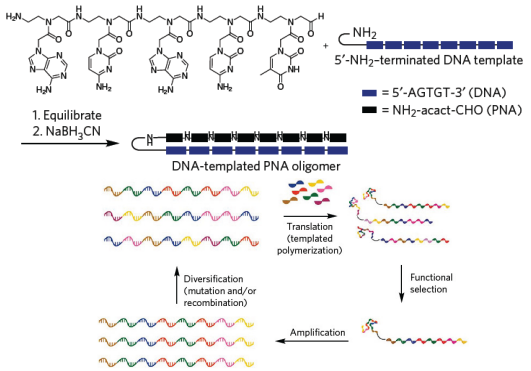
Nonenzymatic templated nucleic acid synthesis – monomer/short oligomer



Problems:
- very slow reactions
- limited range of templates (mostly C-rich)
- poor regiospecificity (2'-5' linkages, predominant in some cases)
- 3'-aminonucleotides perform better, but undergo intramolecular cyclizations as side reaction

Lohrmann, R.; Orgel, L. E. *Tetrahedron* **1978**, *34*, 853
A. Silverman, E. Kool *Chem. Rev.* **2006**, *106*, 3775

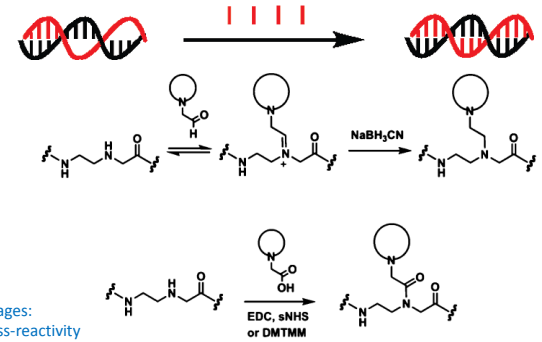
Templated nucleic acid synthesis – short oligomer coupling



Limitations:
- slightly distorted backbone (amine instead of amide backbone every 5 bases)
- only carefully designed pentamers work – limiting the diversity for functional selection

Brudno Y, Birnbaum ME, Kleiner RE, Liu DR. *Nature Chem. Biol.* **2010**, *6,* 148-155.

Templated nucleic acid synthesis – base filling

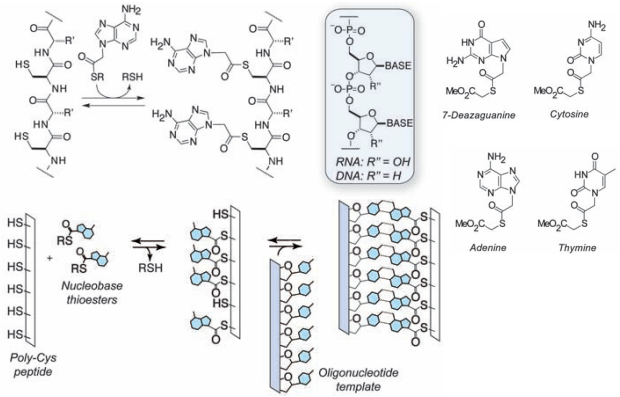


Advantages:
-no cross-reactivity
- selectivity increased by proximity of the reaction to the hybridization site

Limitations:
-single or double abasic sites (most efficient inside of the chain)
- Aldehydes give better yields and accuracy, but worse hybridization of the product

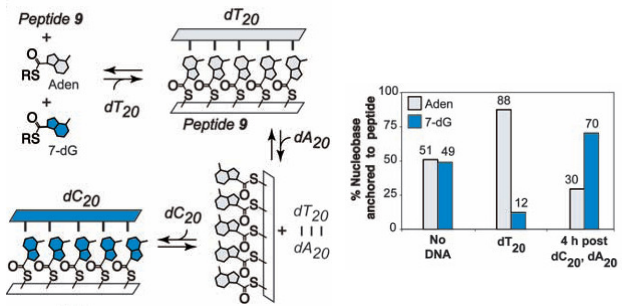
Heemstrra JM, Liu DR. *J. Am. Chem. Soc.* **2009**, *131,* 11347-11349.

A polyamide responsive to selection pressure



Ura Y, Beierle J, Leman L, Orgel LE, Ghadiri MR. *Science* **2009**, *325*, 73-77.

A polyamide responsive to selection pressure



Dynamic polymer responsive to template changes with high fidelity

Ura Y, Beierle J, Leman L, Orgel LE, Ghadiri MR. *Science* **2009**, *325*, 73-77.