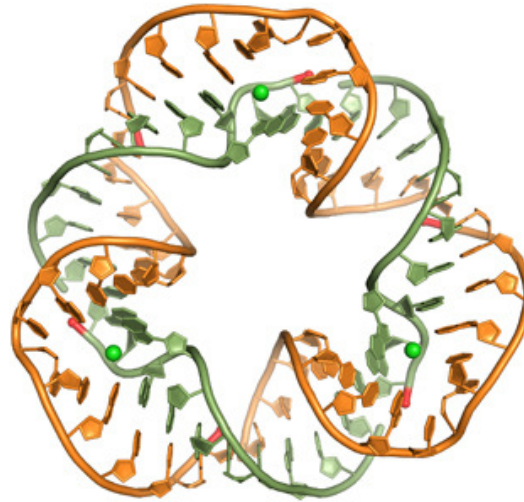


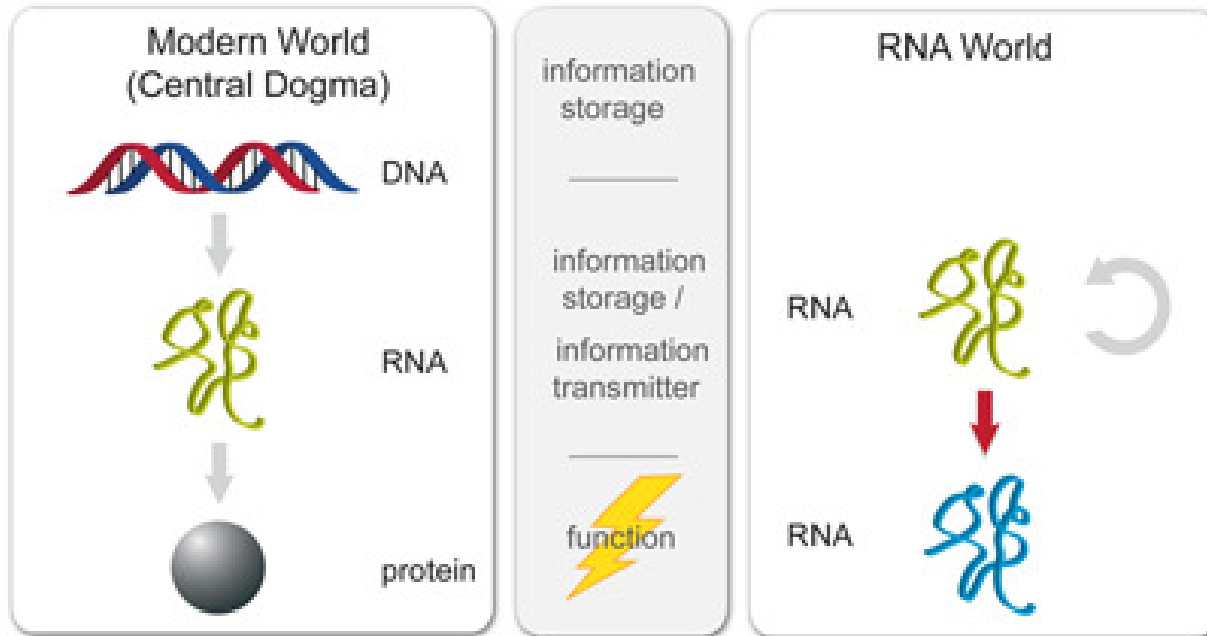
CHAPTER 1



OLIGONUCLEOTIDES

Part 2 – noncanonical nucleobases

The RNA world



During earliest stages of life emergence, RNA (or its structurally simplified analogue) may have carried out most of the information processing and metabolic transformations needed for biology to emerge from chemistry

Ribozymes

Riboswitches

Viroids

Co-enzymes derived from nucleosides

Ribosome is a ribozyme

Non-enzymatic replication of RNA

Autocatalytic short RNA networks

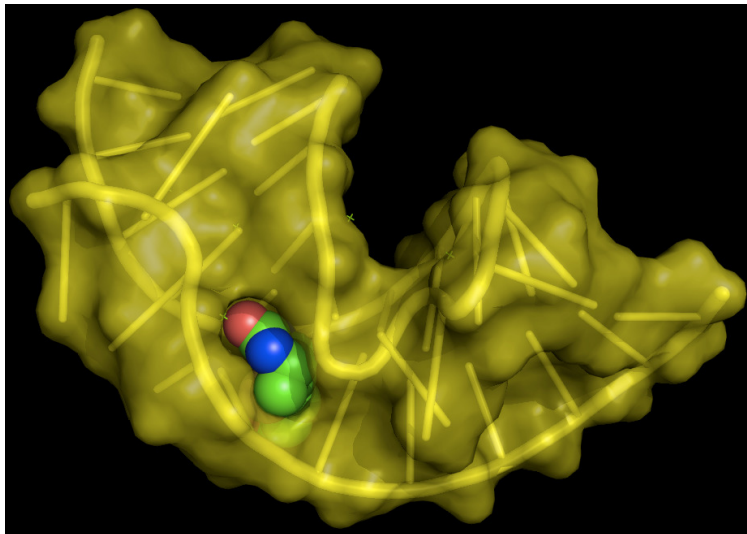
Can we create a xenonucleic acid world?

Aptamers

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

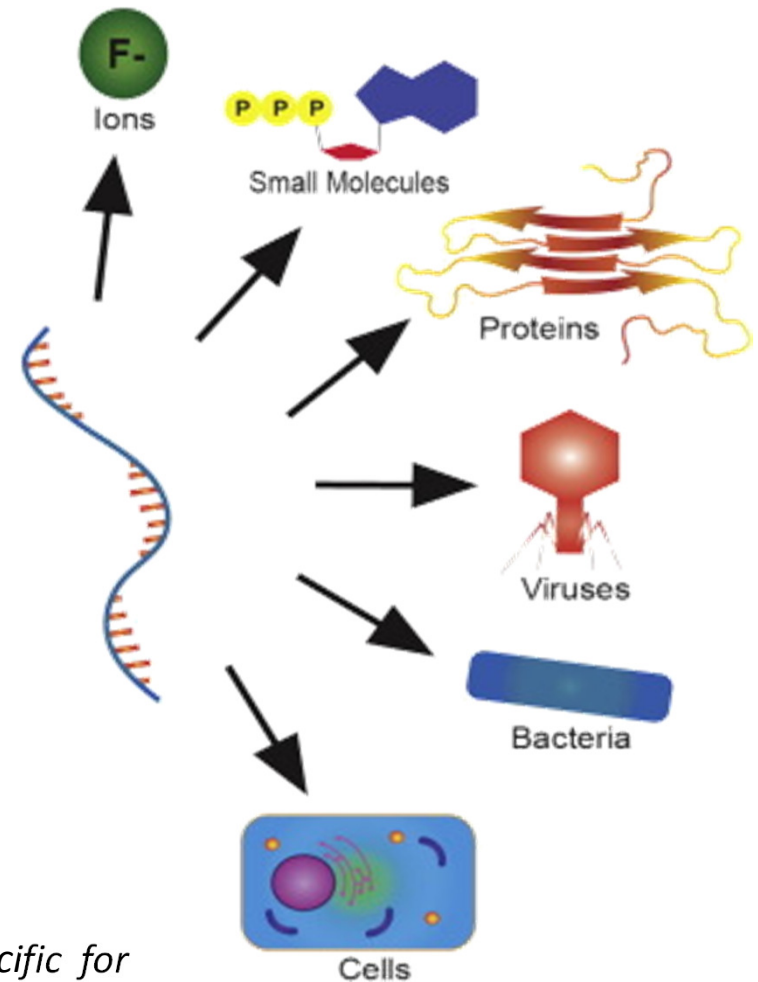
Aptamers are usually created by selecting them from a large random sequence pool, but natural aptamers also exist in riboswitches.

- **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

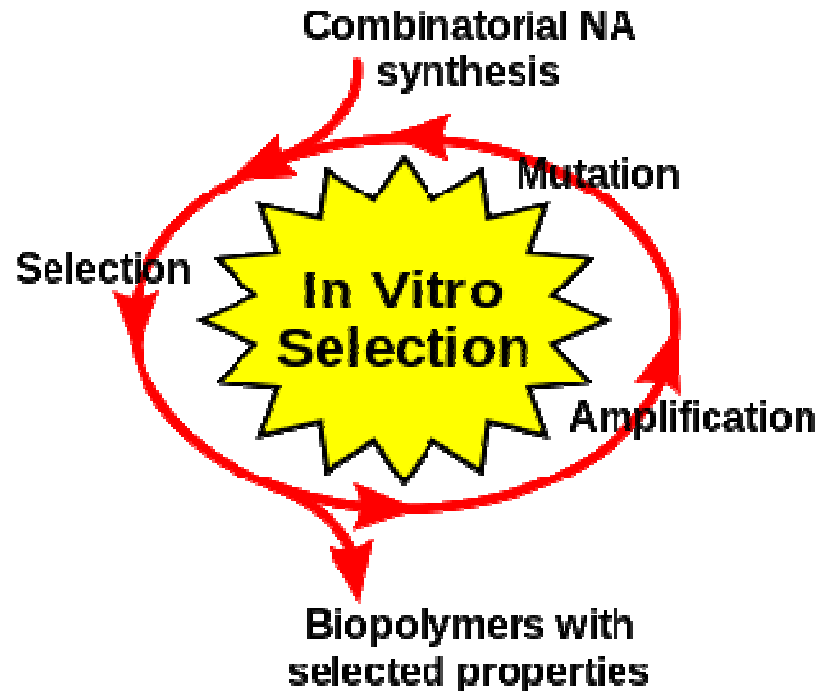


Variety of target molecules

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



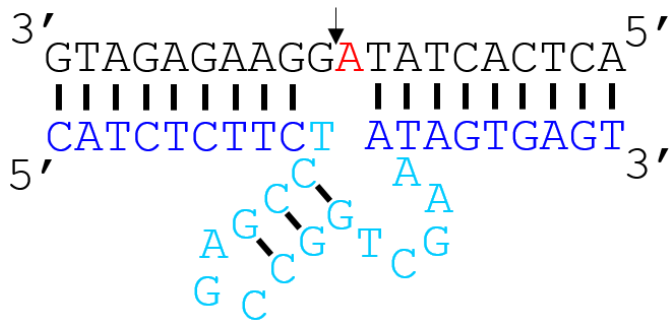
A general overview of in vitro selection protocol. NA stands for Nucleic Acids (DNA, RNA) which start as a random pool, and are enriched through the selection process

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.

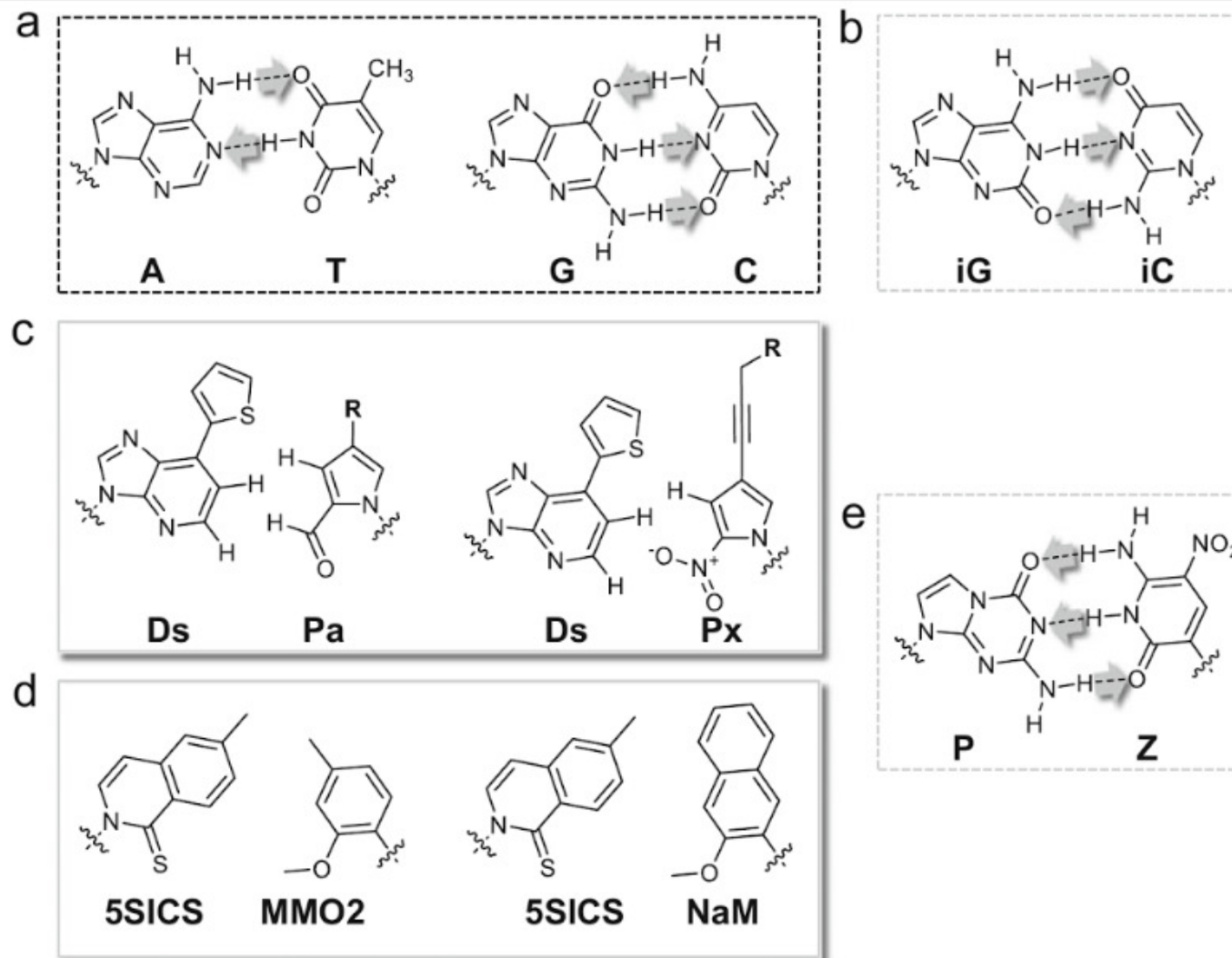
1994 – the first DNAzyme (a ribonuclease) – R. Breaker, G. Joyce – Pb²⁺ GR-5

Currently known:

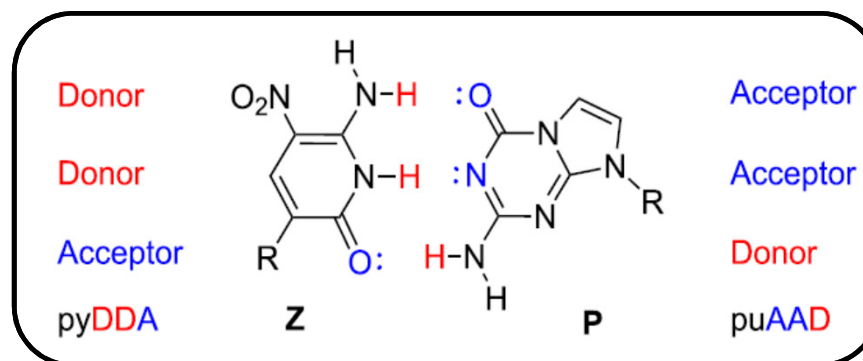
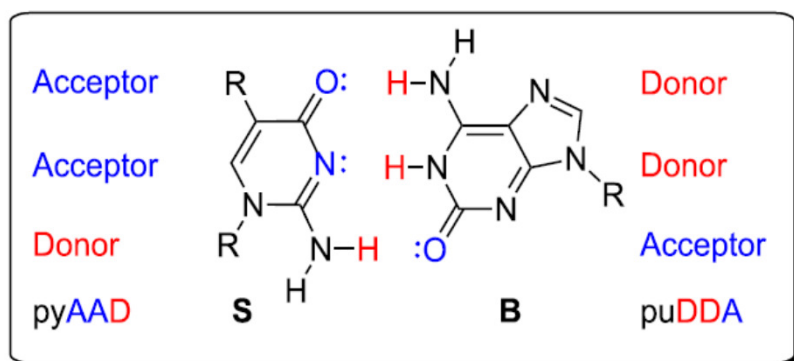
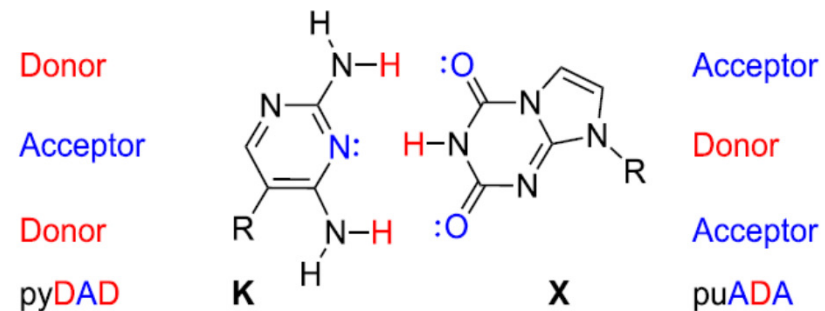
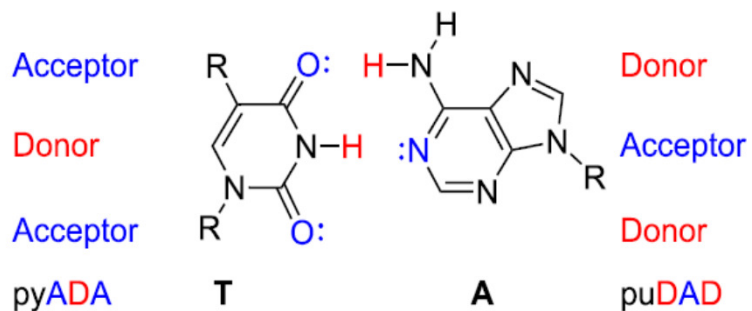
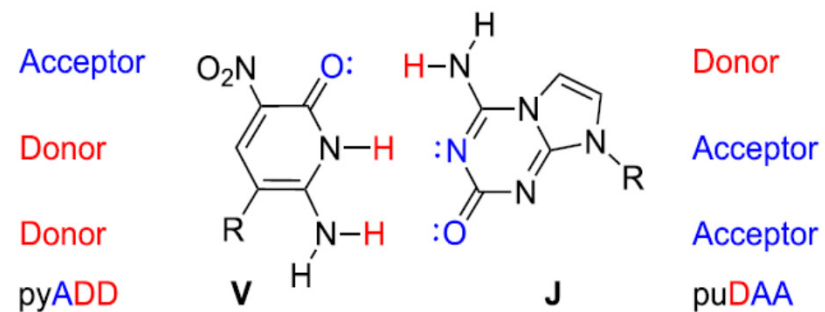
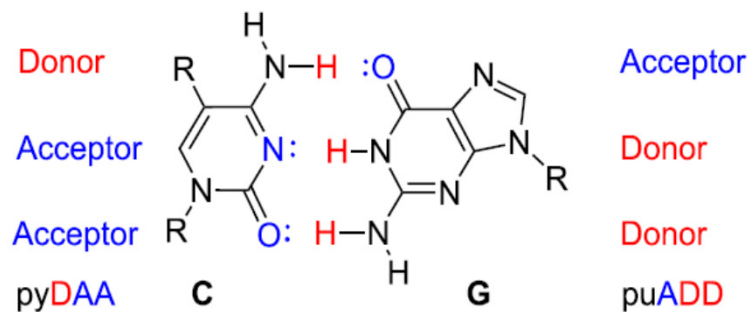
- Ribonucleases
- RNA ligases
- DNA phosphorylation, adenylation, deglycosylation
- DNA cleavage

Problems: product inhibition, often single-turnover

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

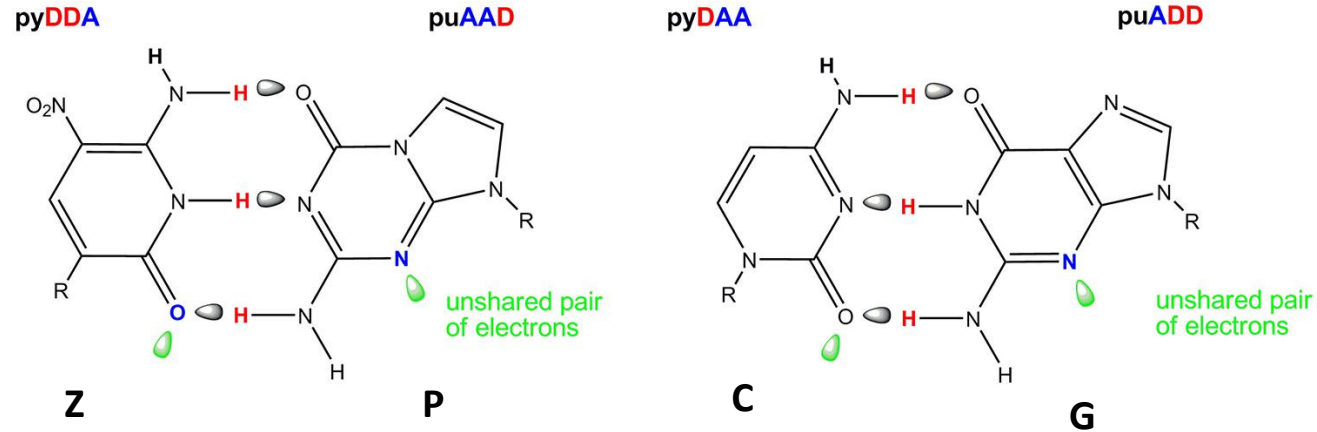
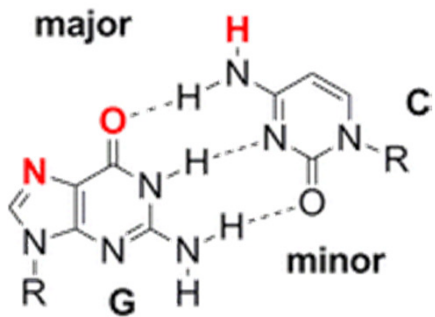
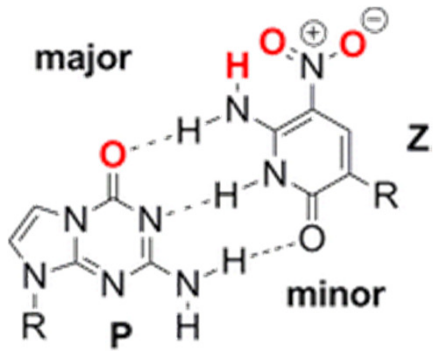


AEGIS – Artificially Expanded Genetic Information System



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

AEGIS – Artificially Expanded Genetic Information System



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

Multiple PCR amplification cycles achieved.

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

18-mers: 2+2 **Z:P** pairs → B-DNA

6 consecutive **Z:P** → A-DNA

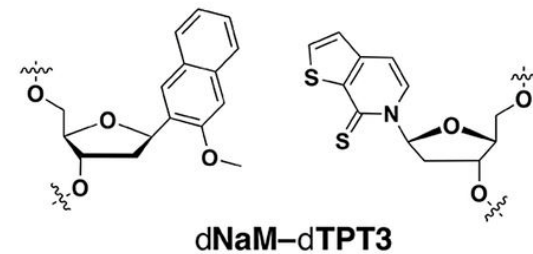
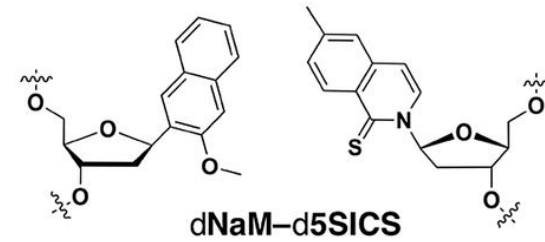
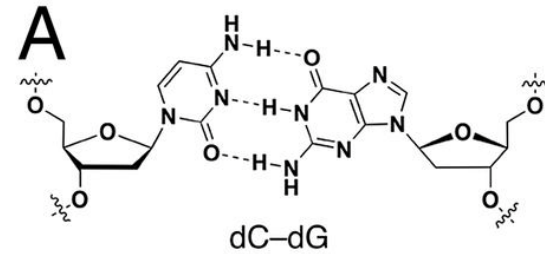
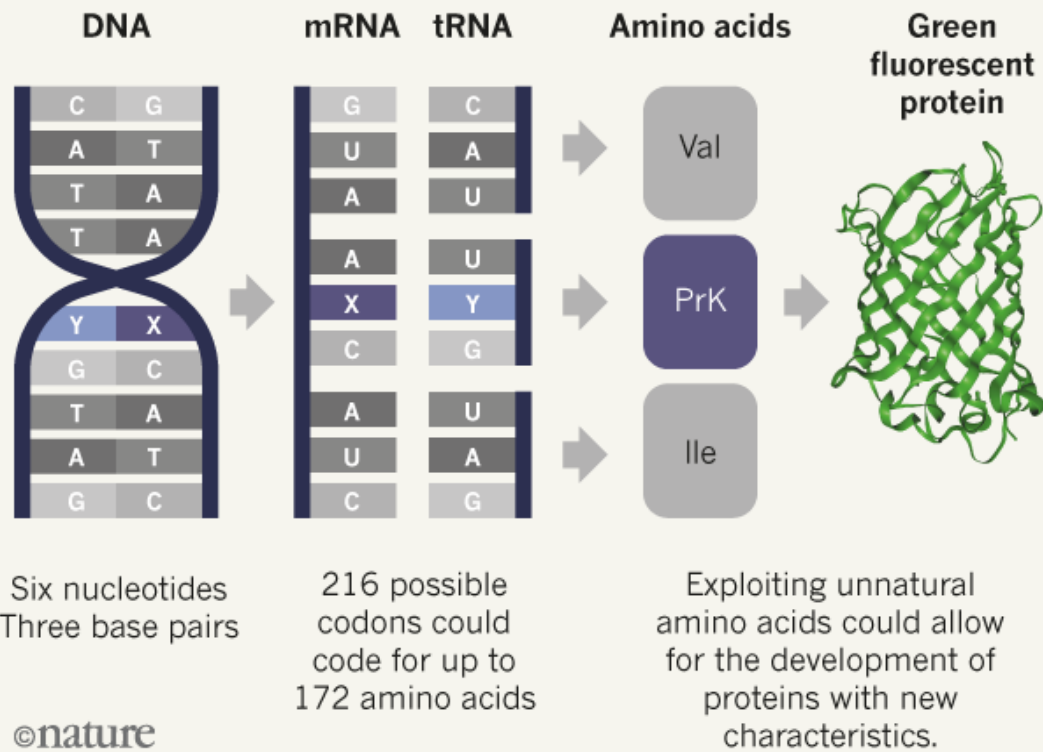
0,1 nm wider, but otherwise alike **G:C** pairs

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

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

A semi-synthetic organism with an expanded genetic alphabet

Researchers added a synthetic base pair (nicknamed X and Y; shown in blue) to DNA to encode new amino acids, which cells then incorporated into the fluorescent protein GFP.



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

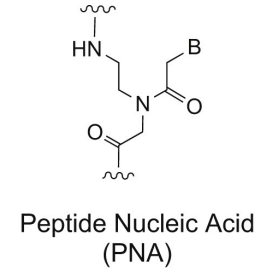
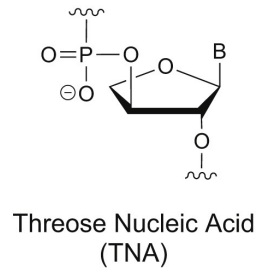
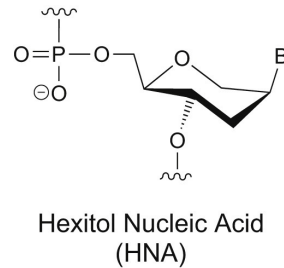
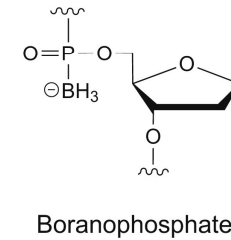
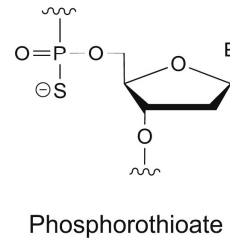
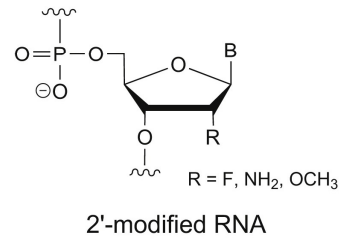
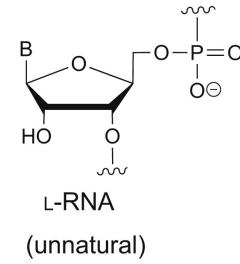
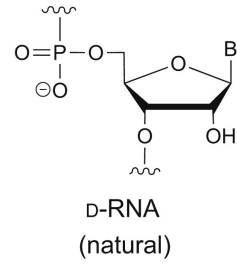
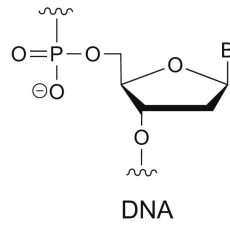
CHAPTER 1



OLIGONUCLEOTIDES

Part 3 – noncanonical backbone

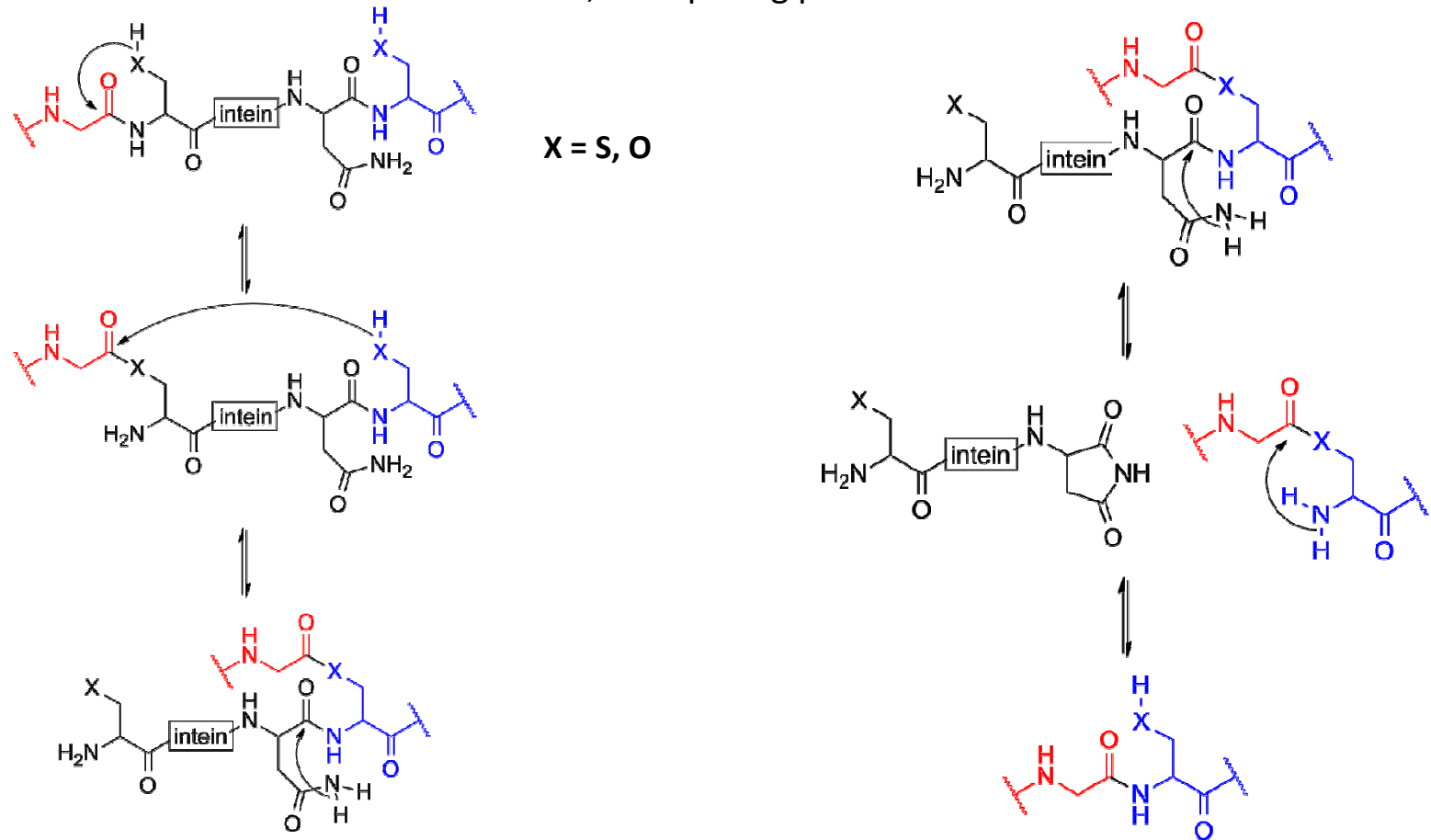
Artificial genetic polymers



Intein splicing

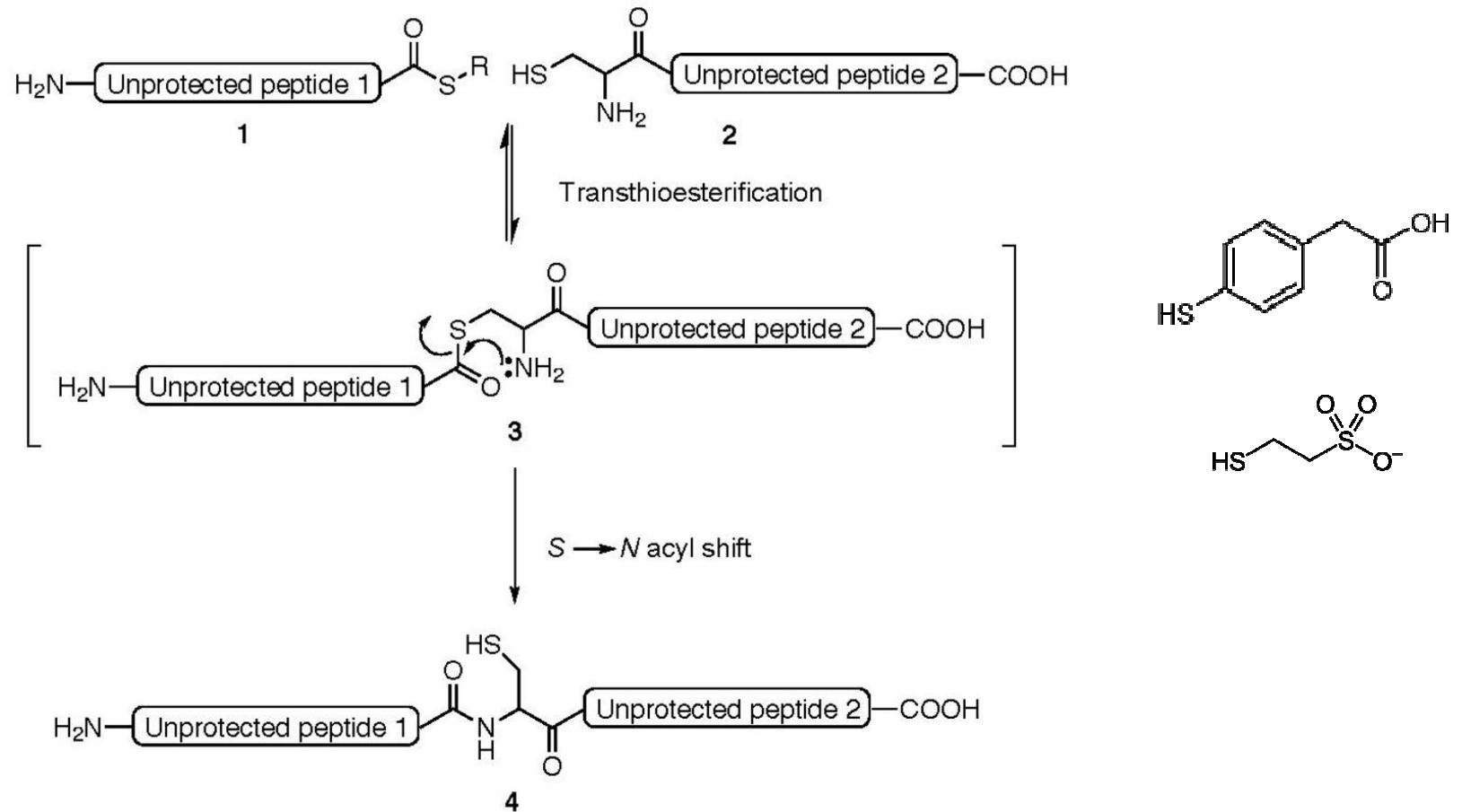
An **intein** is a segment of a protein that is able to excise itself and join the remaining portions (the **exteins**) with a peptide bond in a process termed protein splicing. Inteins have also been called "protein introns".

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).

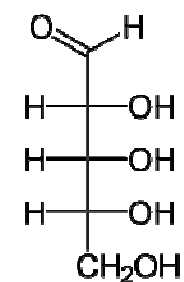
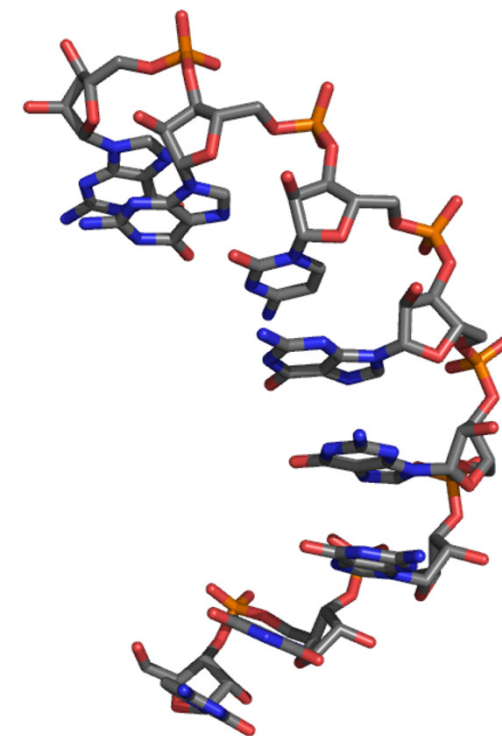


Spiegelmers: L-RNA

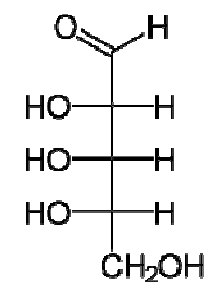
Aptamers (from the Latin aptus – fit, and Greek meros – part) are oligonucleotide or peptide molecules that **bind to a specific target molecule**. Aptamers are usually created by selecting them from a large random sequence pool, but natural aptamers also exist in riboswitches.

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.

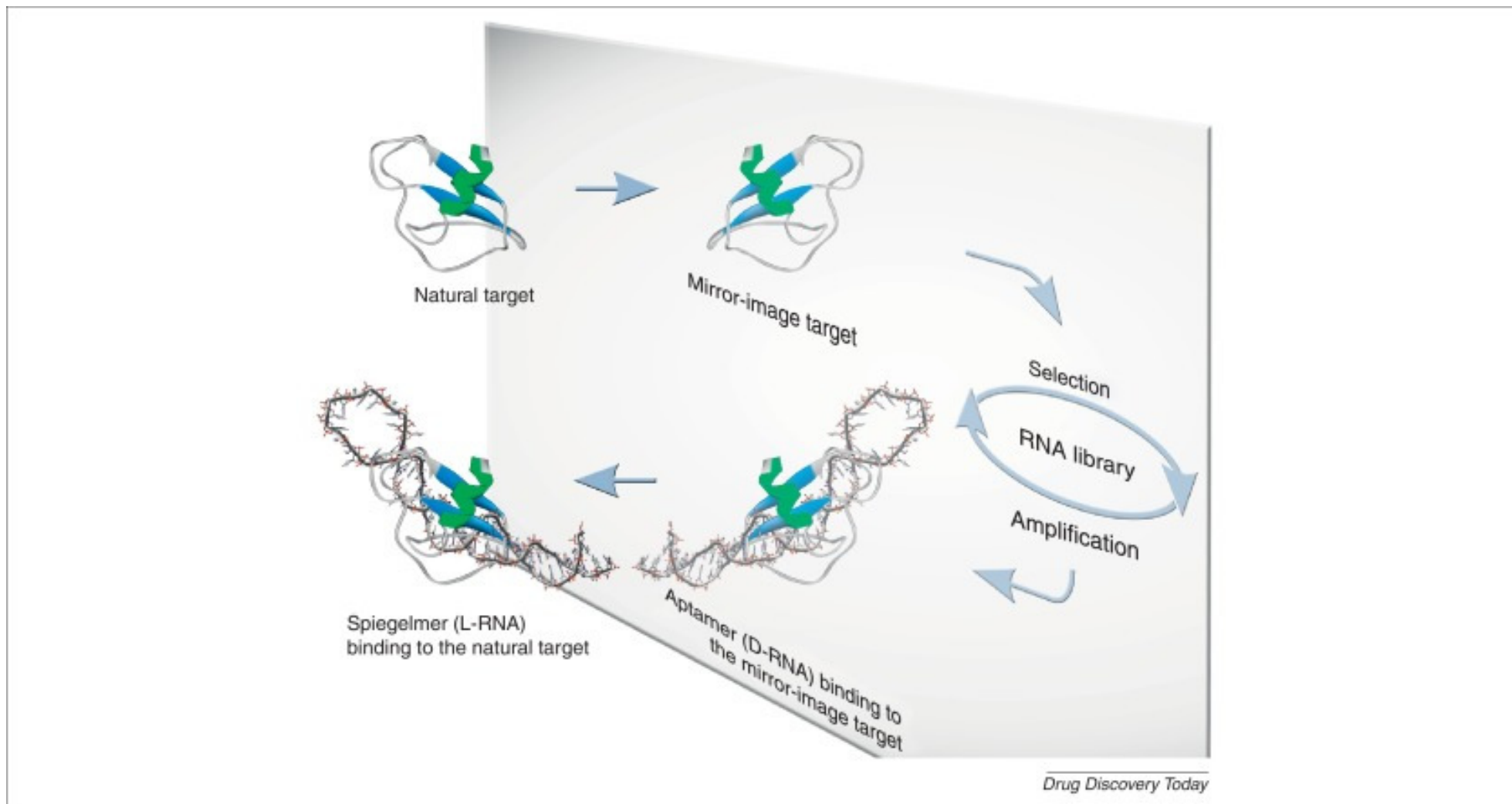
L-RNA aptamers are a form of aptamers. Due to their L-nucleotides, they are highly resistant to degradation by nucleases. **Spiegelmers** are considered potential drugs and are currently being tested in clinical trials.



D-Ribose



L-Ribose



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

Mirror-image RNA that binds D-Adenosine

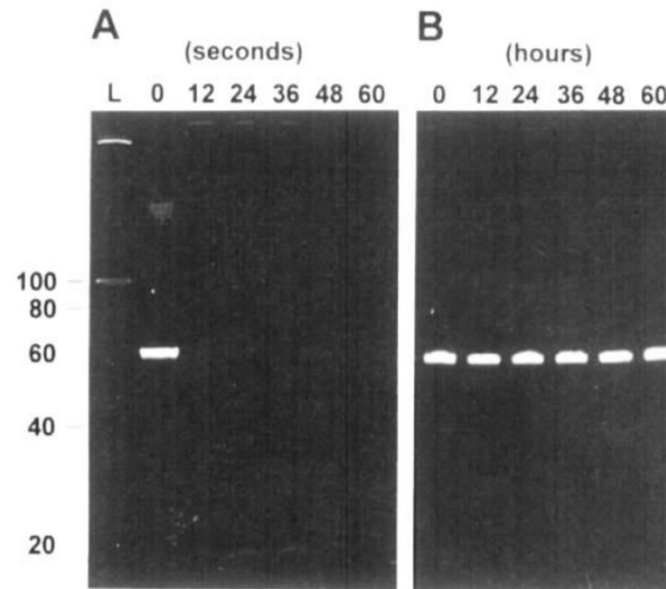
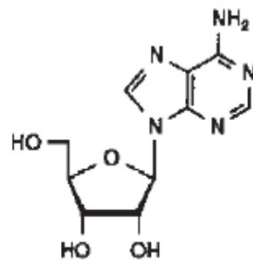
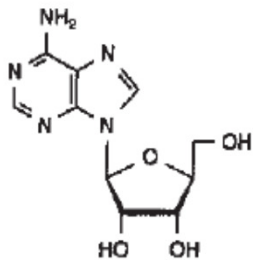
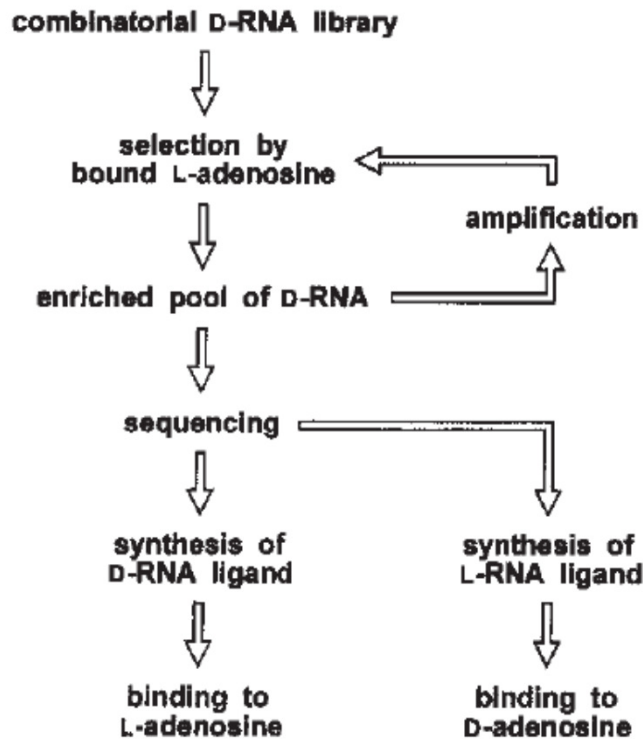
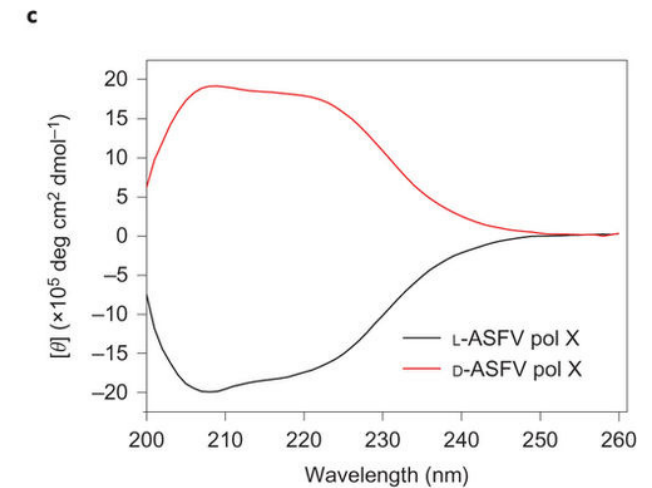
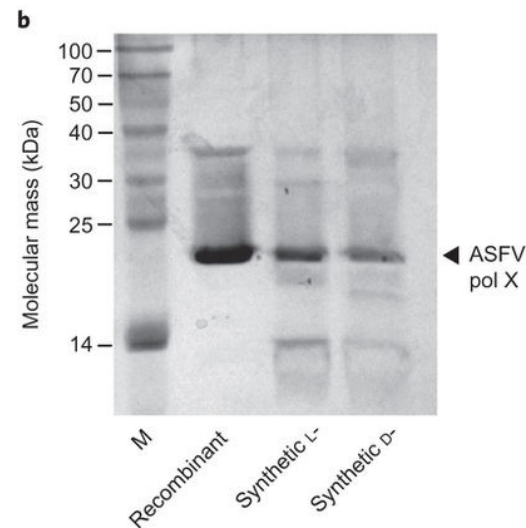
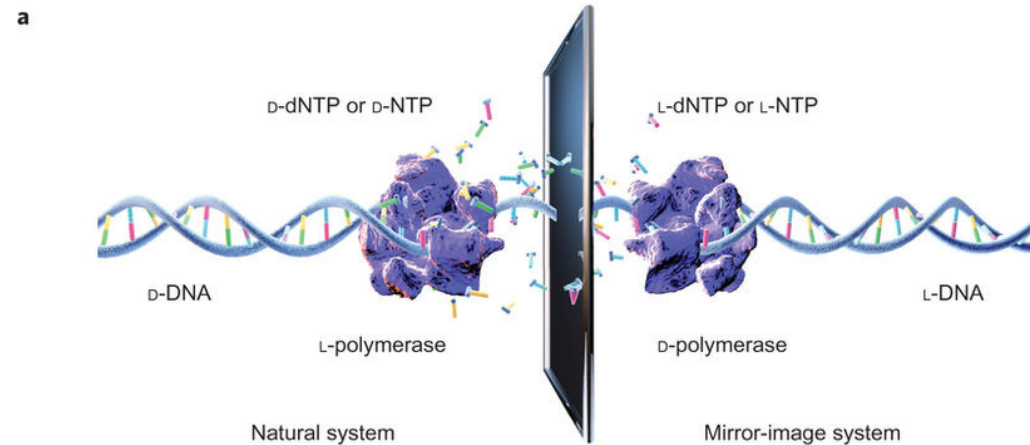


Figure 5. Stability of the 58-mer RNA ligands in human serum. (A) Aptamer D-A42d and (B) spiegelmer L-A42d. Aliquots were taken at the indicated times. L marks the size standard (10 bp DNA ladder). The results were reproduced in an independent experiment.

Spiegelmers: *L*-DNA polymerase

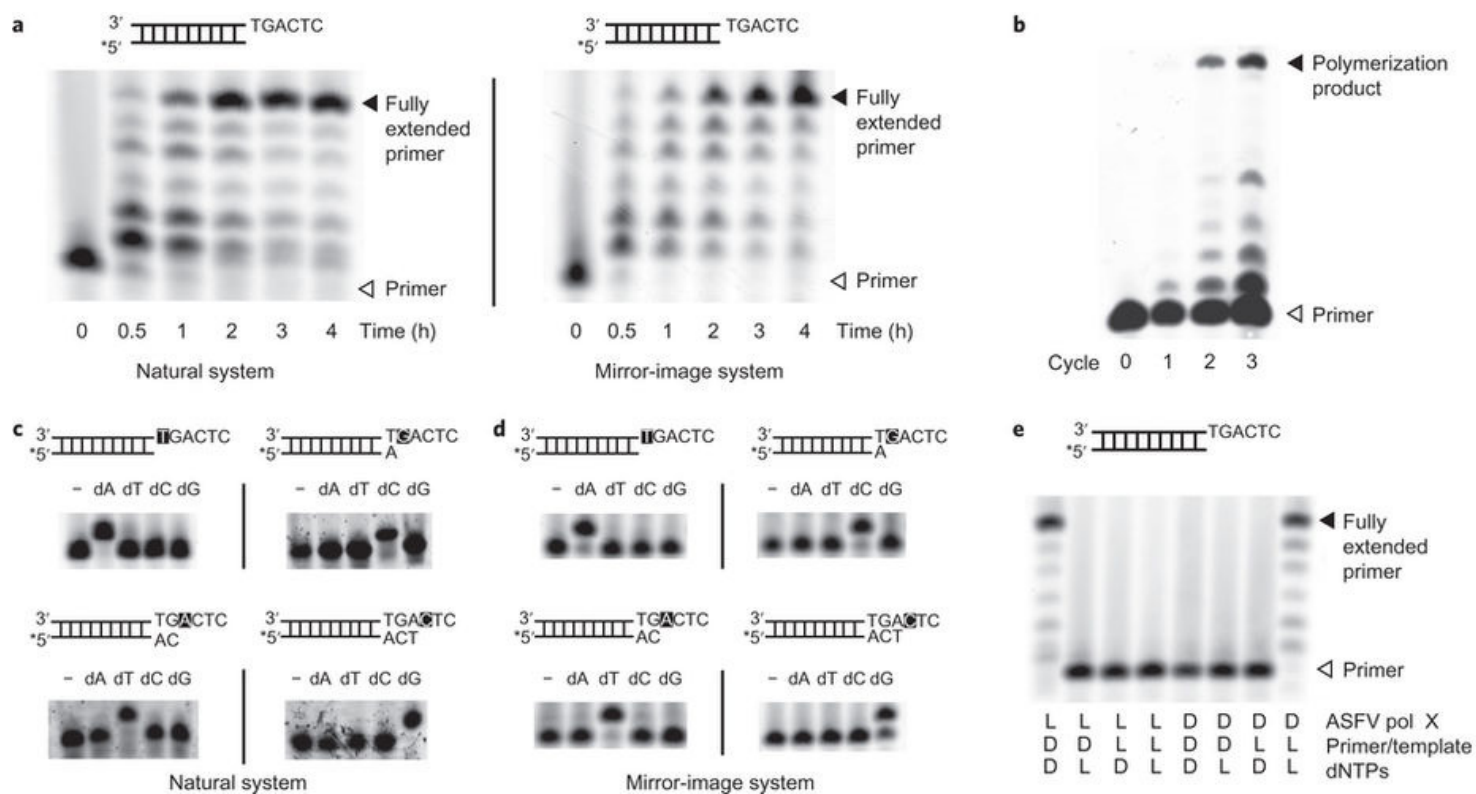
The mirror image configuration of polymerase X from African swine fever virus, the shortest known polymerase (174 amino acids), has recently been demonstrated to elongate an *L*-DNA primer with *L*-dNTPs; and a functional 56-mer *L*-DNAzyme was made within 36 hours.

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

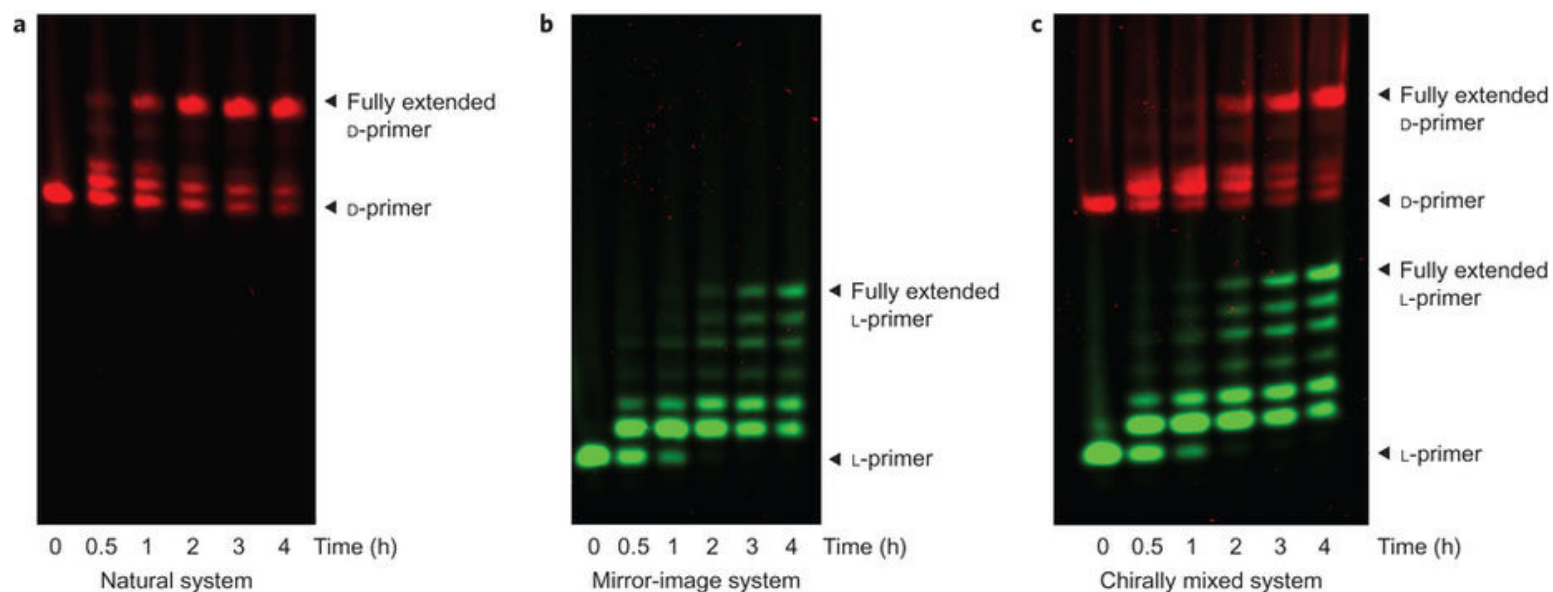
Spiegelmers: L-DNA polymerase



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

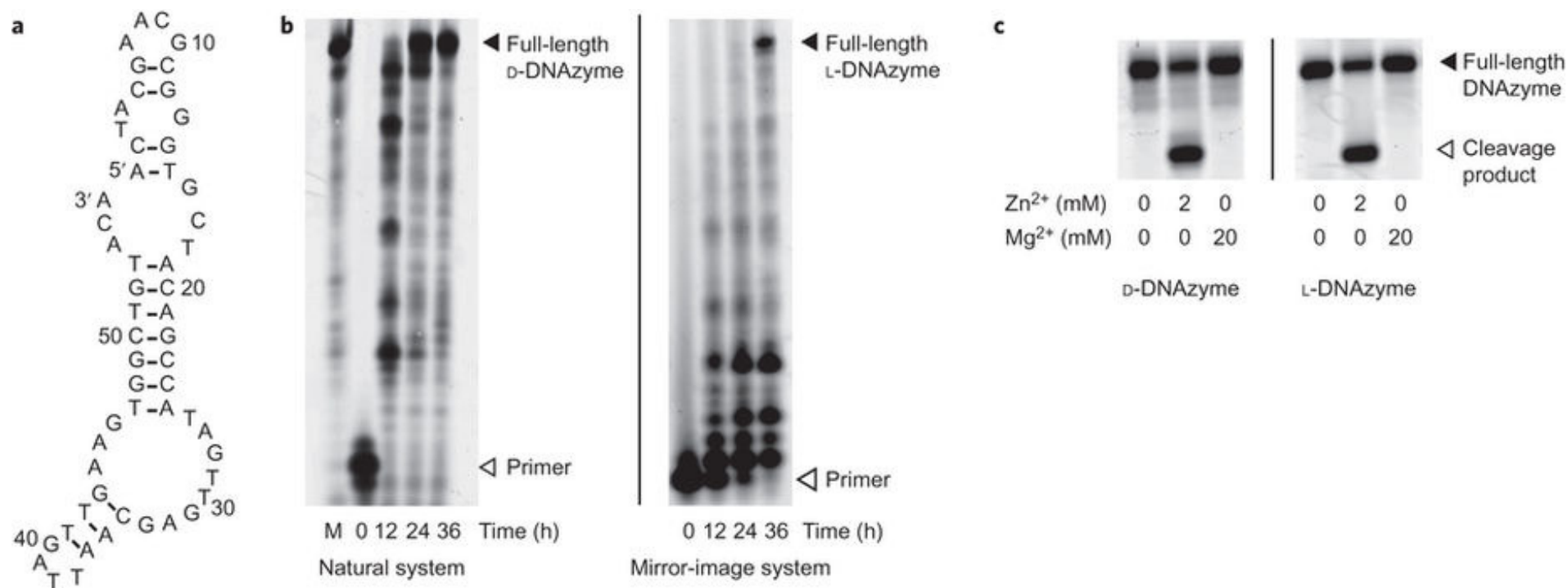
Spiegelmers: L-DNA polymerase



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

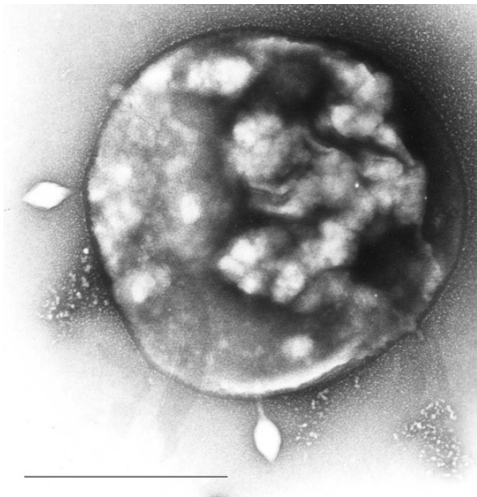


a, Sequence and predicted secondary structure of the previously reported Zn²⁺-dependent self-cleaving DNAzyme. **b**, Primer extension on a 66 nt template to produce the Zn²⁺-dependent self-cleaving DNAzyme. **c**, Self-cleavage of the enzymatically polymerized Zn²⁺-dependent D- and L-DNAzymes.

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

Spiegelmers: A thermostable D-polymerase

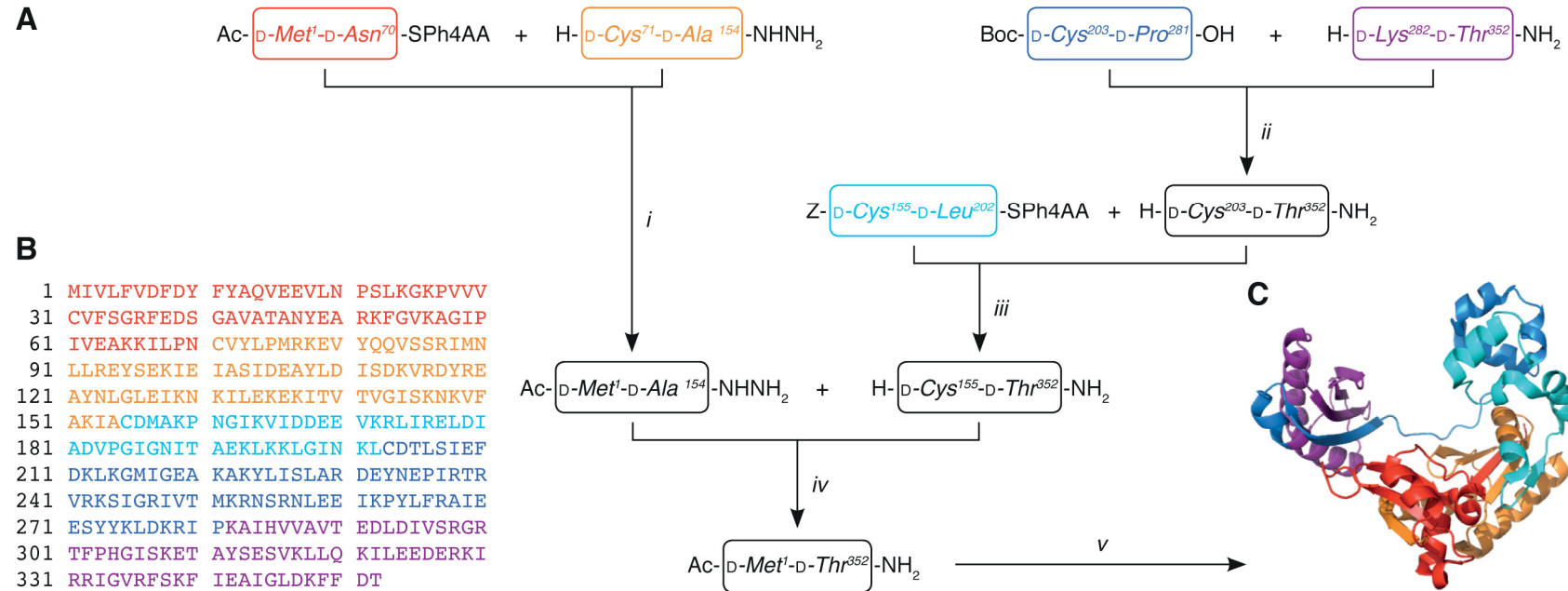
A thermostable mirror-image polymerase **D-Dpo4-3C** has been produced, that is able to amplify L-DNA in a classical PCR reaction and can even be used to assemble an L-DNA gene from L-DNA oligonucleotides. This artificial enzyme is a mutant of DNA polymerase IV from *Sulfolobus solfataricus*, a Y-family 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.

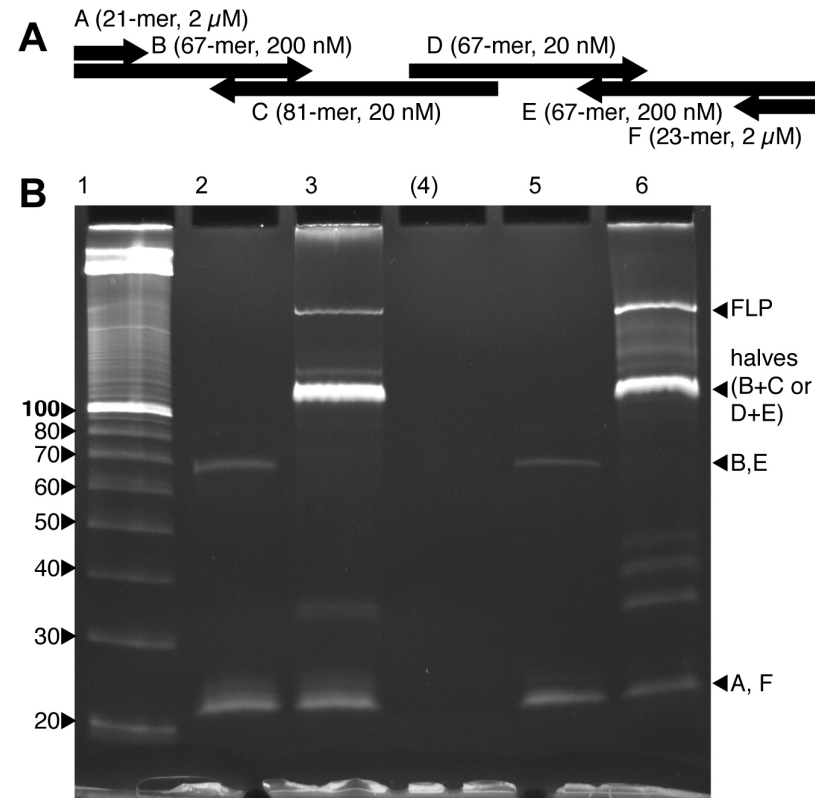
Furthermore, with an additional single point mutation (Tyr12Ala or Tyr12Ser), this DNA polymerase can be tuned to accept also ribonucleotides as substrates with reasonable efficiency. Thus, this enzyme may be hijacked to act as a DNA-dependent RNA polymerase to prepare longer stretches of L-RNA

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 \approx 18%. (ii) Segment condensation of fully protected fragments 4 and 5 followed by deprotection. Isolated yield \approx 15%. (iii) NCL of fragments 3 and 4•5 followed by Z-deprotection. Isolated yield \approx 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)).

Spiegelmers: A thermostable D-polymerase

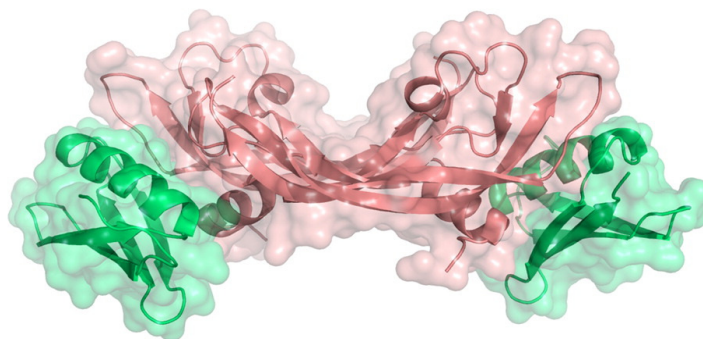


Assembly of a mirror-image gene. **(A)** schematic of the oligonucleotide setup. **(B)** lane 1, 3 μl of 10 bp DNA ladder. Lane 2, mirror-image no-enzyme control. Lane 3, mirror-image gene assembly. Lane 4, empty. Lane 5, natural handedness no enzyme control. Lane 6, natural handedness gene assembly.

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.

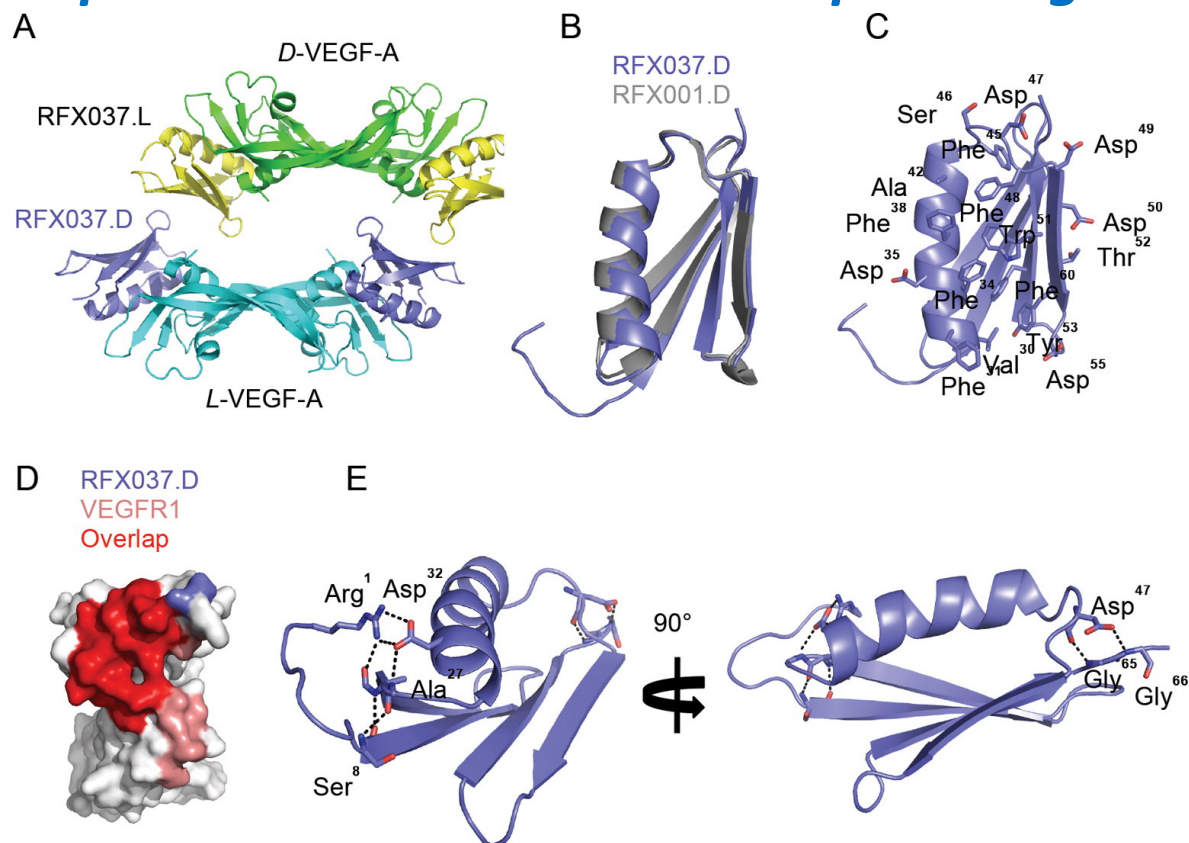


RFX037.D is a *D*-protein antagonist of natural vascular endothelial growth factor A (VEGF-A) that inhibited binding to its receptor, with extreme thermal stability ($T_m > 95\text{ }^\circ\text{C}$) and high affinity for VEGF-A ($K_d = 6\text{ nM}$).

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

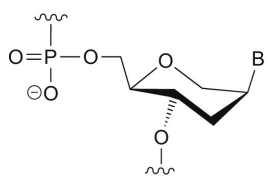
D-proteins: almost ideal therapeutic agents



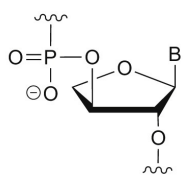
X-ray crystal structure of RFX037:VEGF-A heterochiral protein complex. (A) Two RFX037.L molecules (yellow) bound to one d-VEGF-A homodimer (green) and two RFX037.D molecules (blue) bound to one l-VEGF-A homodimer (cyan). (B) Superposition of RFX037.D (blue) and RFX001.D (gray, rcsb accession 4GLS). (C) 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

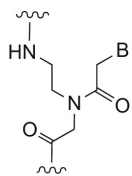
XNA – Xeno Nucleic Acids



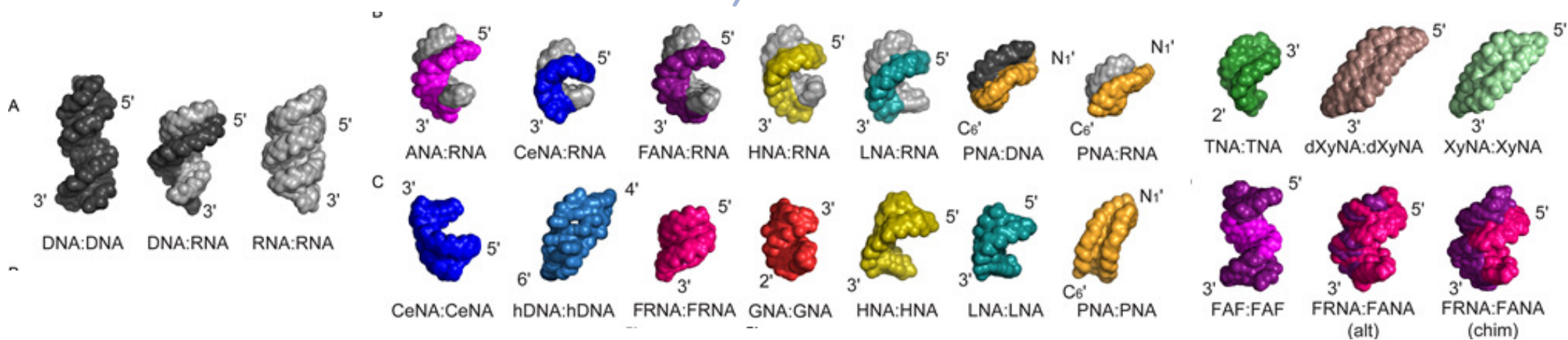
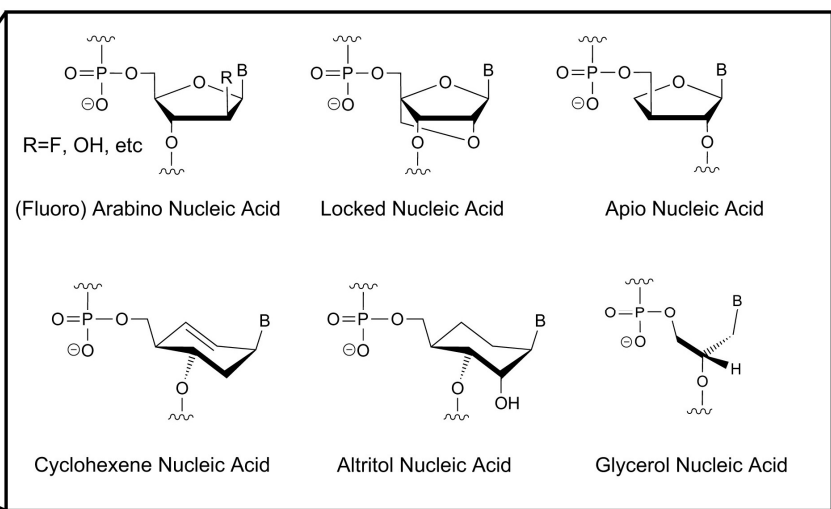
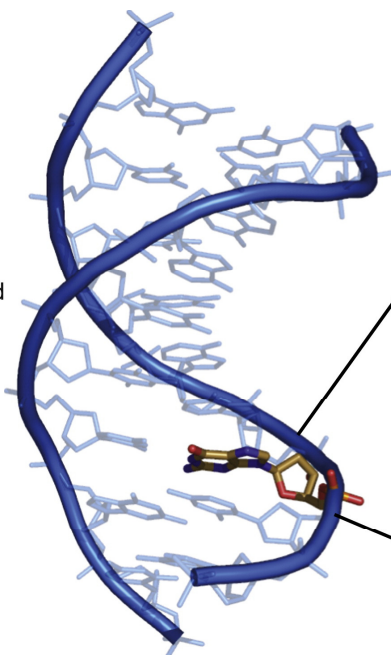
Hexitol Nucleic Acid (HNA)



Threose Nucleic Acid (TNA)



Peptide Nucleic Acid (PNA)



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**: *Primordial 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

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*.

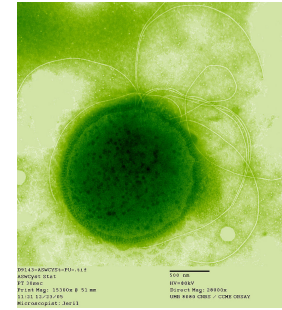
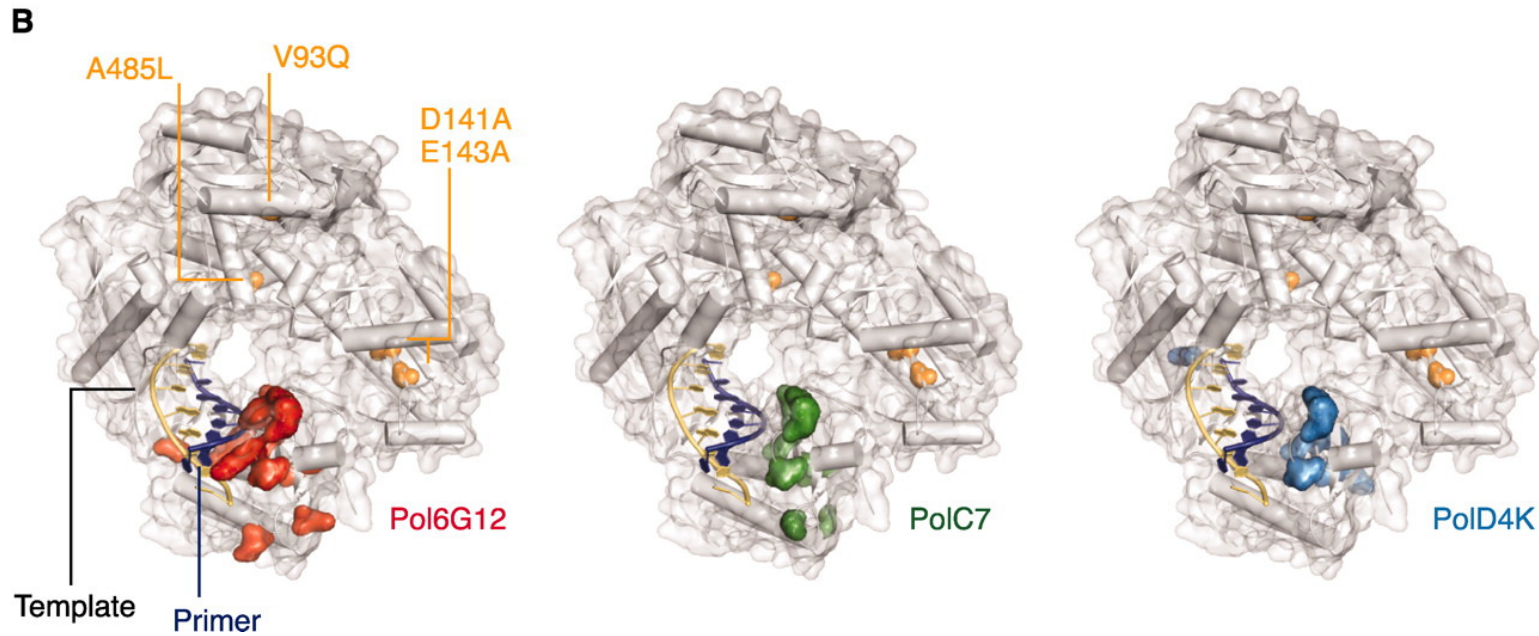
Thus, heredity and evolution, two hallmarks of life, are not limited to DNA and RNA but are likely to be emergent properties of polymers capable of information storage.

Engineering XNA polymerases

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

A

	402	404	588	590	608	611	653		682	703		710	729	731
TgoT	YLD	..	FVT	..	LEIV	..	YEV	PPEKLV	IEQITRDLKDYKATGPHVAV	..	VLK	GSGRI	..	AEY
Pol6G12	YLD	..	F A T	..	L K MV	..	YEV	PPE Q LVIY Q PIT K Q L H D Y R A R G P H V S V	..	V P K	GSGRI	..	A G Y	
PolC7	YLD	..	FVT	..	LEIV	..	Y Q V	P P Q Q L A IY Q PIT R A L Q D Y K A K G P H V A V	..	V L K	G S G K I	..	AEY	
PolD4K	Y P D	..	FVT	..	LEIV	..	YEV	P T Q H L V I H K Q IT R A L N D Y K A I G P H V A V	..	V L K	GSGRI	..	AEY	



Thermococcus gorgonarius
(Angels Tapias)

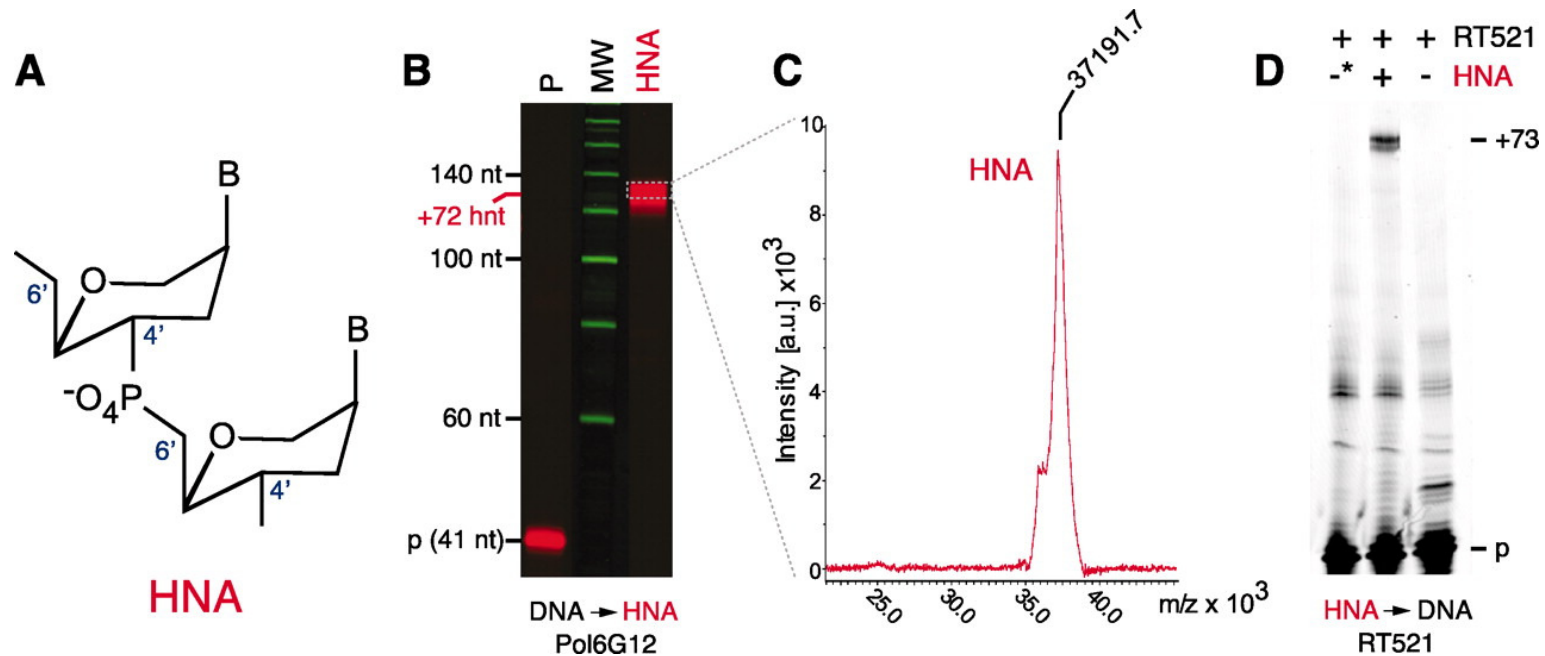
(A) Sequence alignments showing mutations from wtTgo in polymerases Pol6G12 (red), PolC7 (green), and PolD4K (blue).

(B) Mutations are mapped on the structure of Pfu (PDB: 4AIL).

Yellow - template; dark blue - primer; orange - mutations present in the parent polymerase TgoT

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HNA synthesis

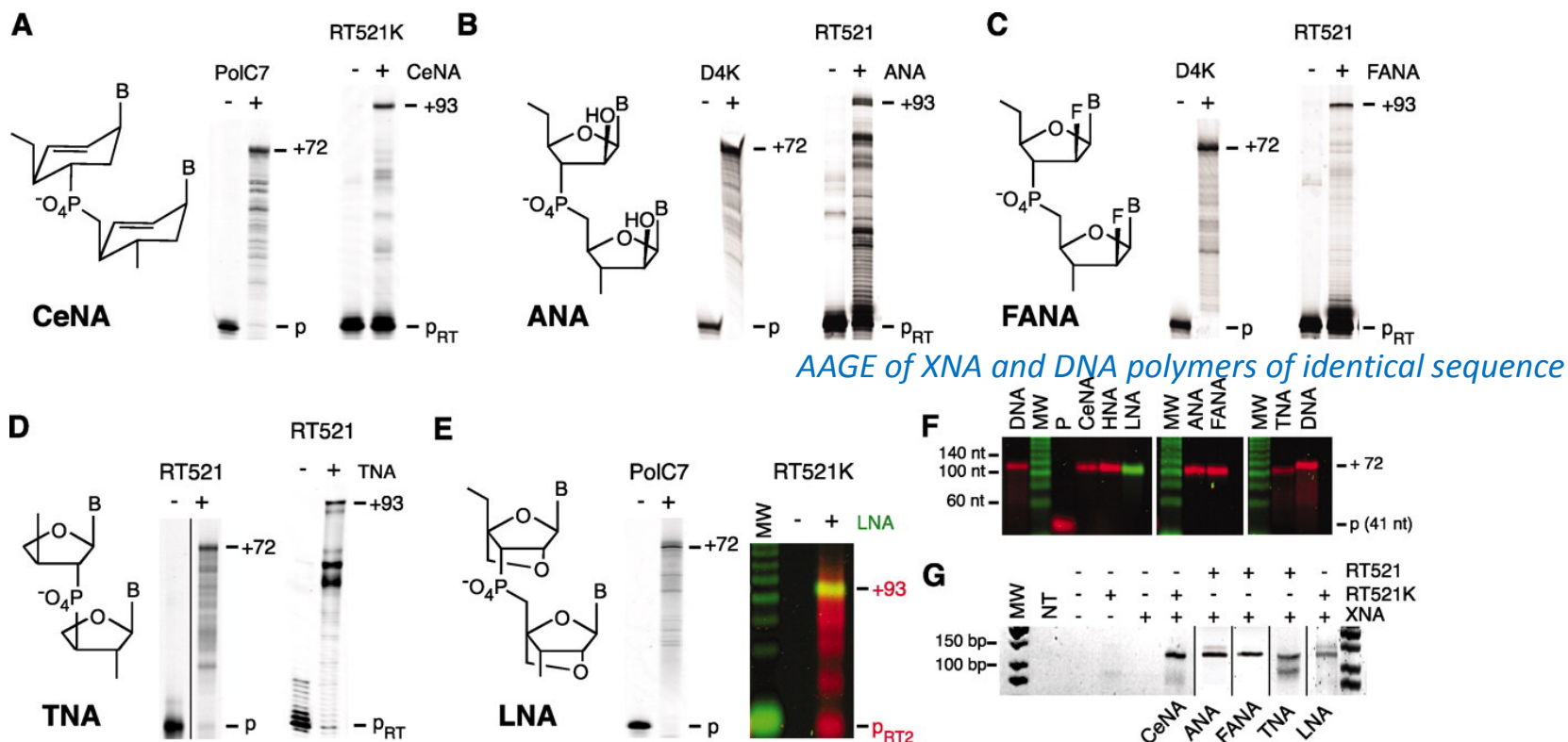


Pol6G12 extends the primer (p) incorporating 72 hNTPs against template T1 to generate a full-length hybrid molecule with a 37,215-dalton expected molecular mass.

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

XNA genetic polymers.

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

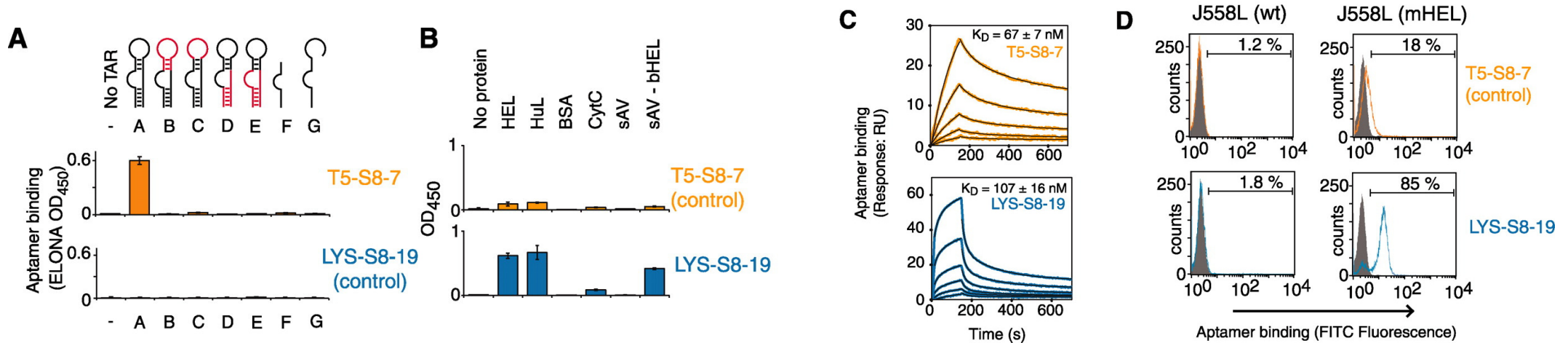


(E) PAGE of LNA synthesis [primer (41 nt) + 72 Int] and LNA-RT (red). LNA synthesis (green) migrates at its expected size (113 nt) and comigrates with reverse transcribed DNA (red) synthesized from primer PRT2 (20 nt).

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)

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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.

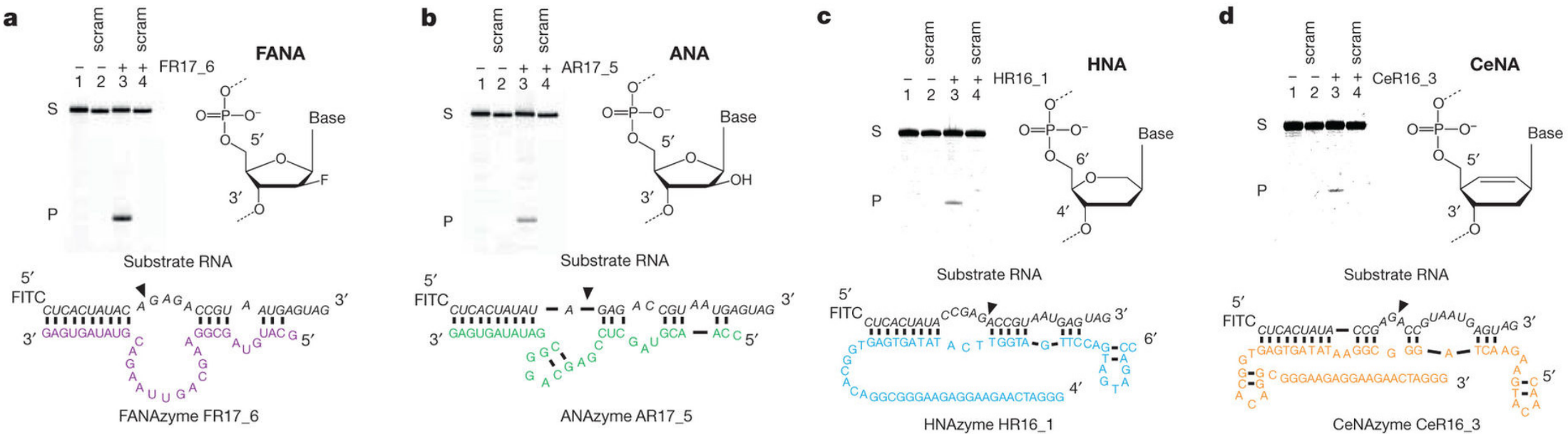
P. Herdewijn, P. Holliger, *et al.* *Science* **2012**, *336*, 341-344

XNA – Xeno Nucleic Acids

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

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

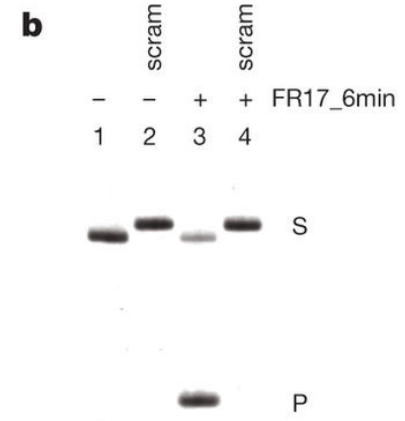
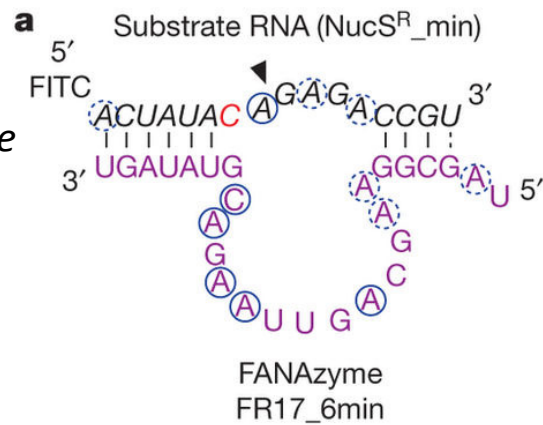
FANA XNAzymes can also ligate DNA, RNA and XNA substrates.



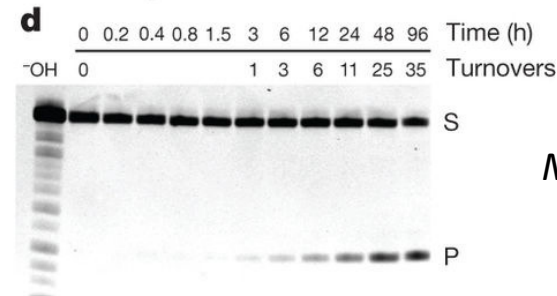
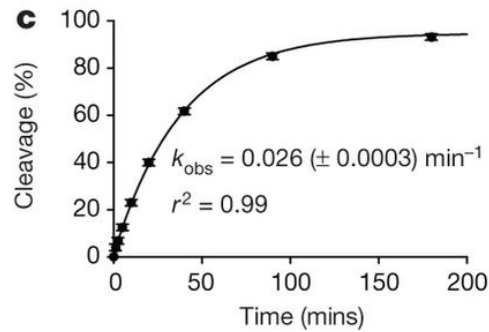
P. Herdewijn, P. Holliger, *et al.* *Nature* **2015**, *518*, 427-430

Chemical synthesis yields an active RNA endonuclease XNAzyme

Secondary FANAzyme structure



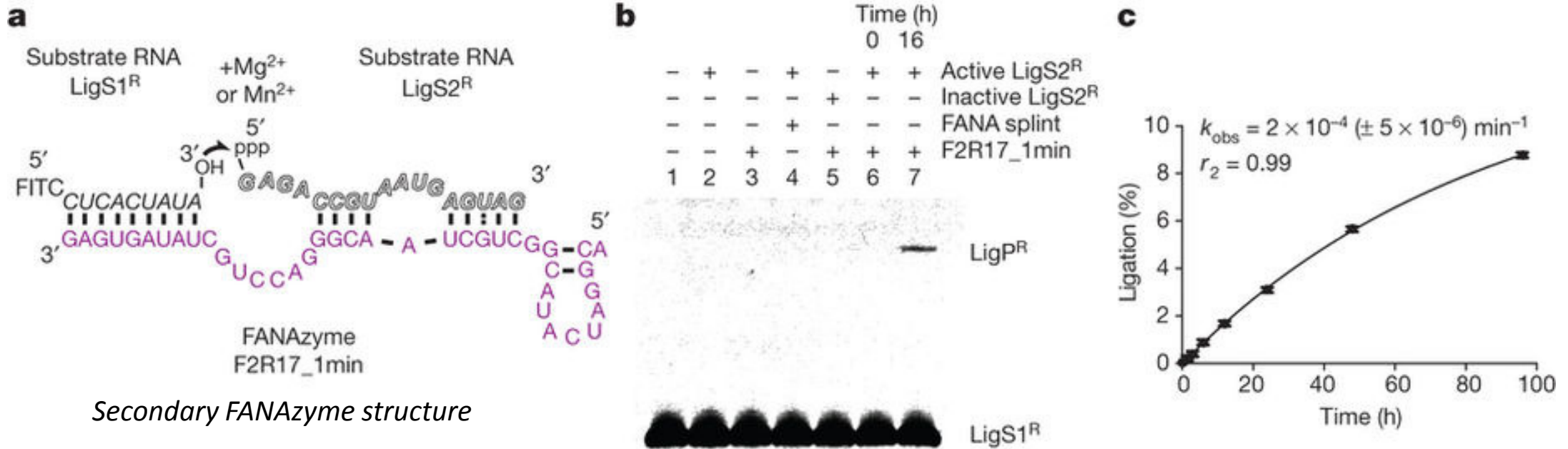
l. 1 and 3 - matching RNA
l. 2 and 4- scrambled RNA



Multiple turnovers

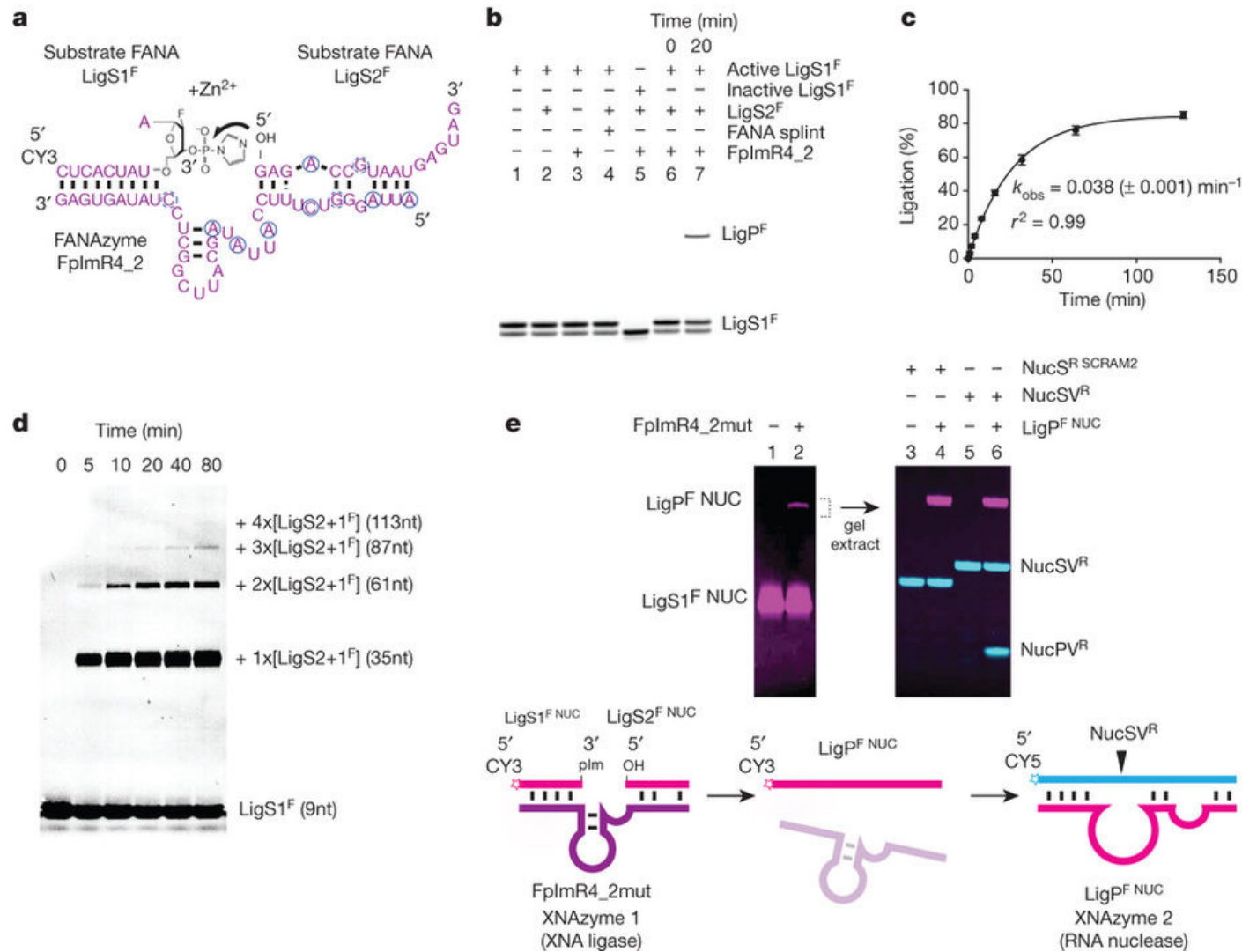
An RNA ligase XNAzyme (FANA)

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



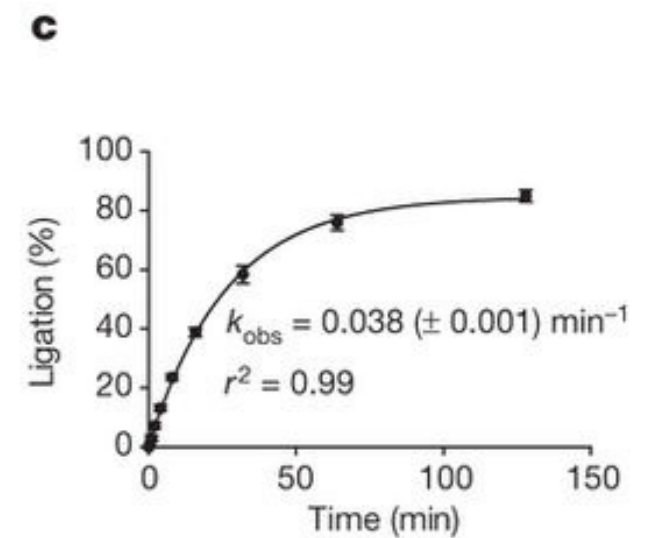
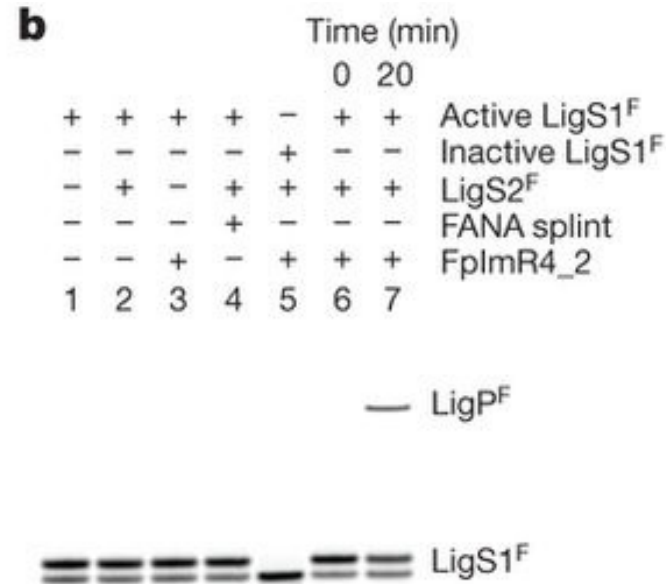
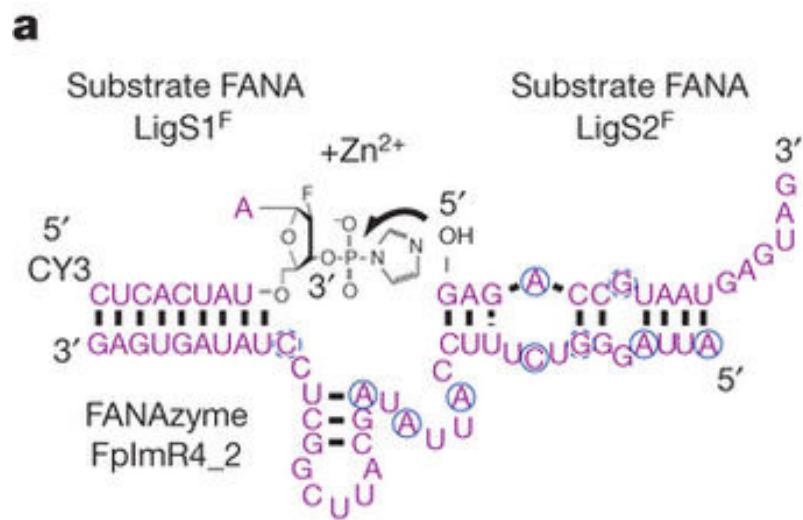
Pre-steady state trimolecular reaction rate (k_{obs}) at 25 °C ($n = 3$; error bars, s.d.).

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

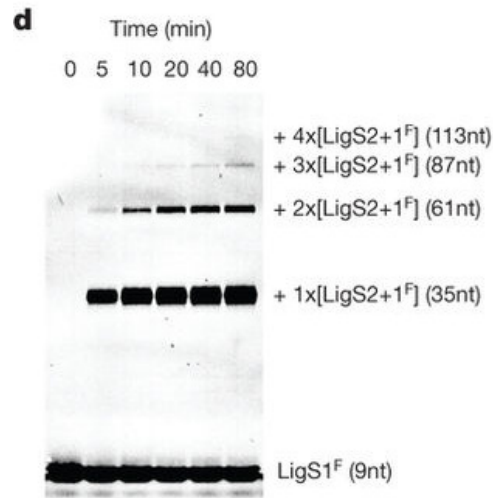


P. Herdewijn, P. Holliger, *et al.* *Nature* **2015**, *518*, 427-430

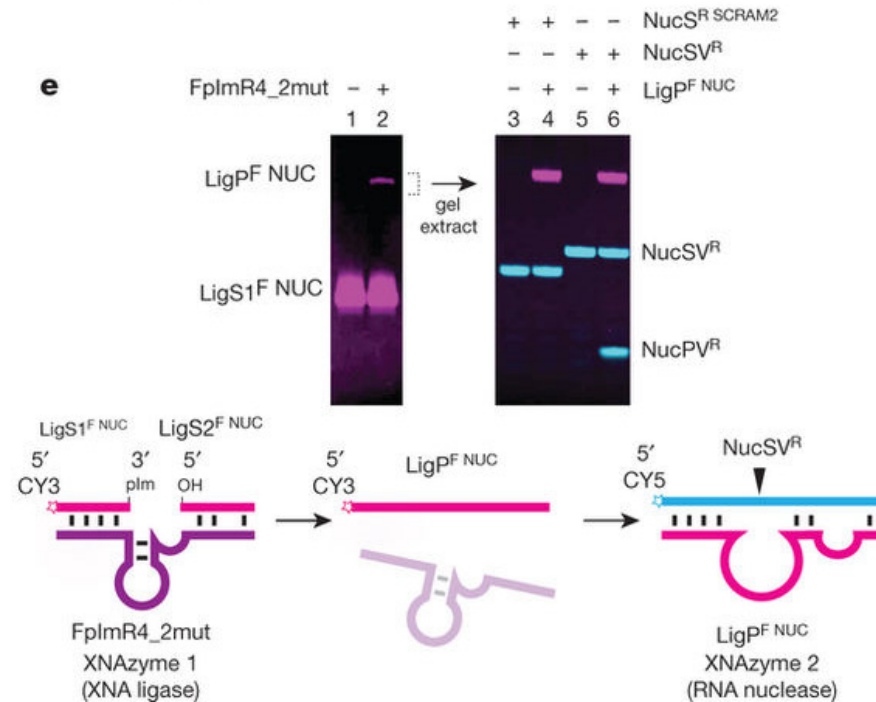
XNA–XNA ligase XNAzyme (FANA): catalysis without natural nucleic acids



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

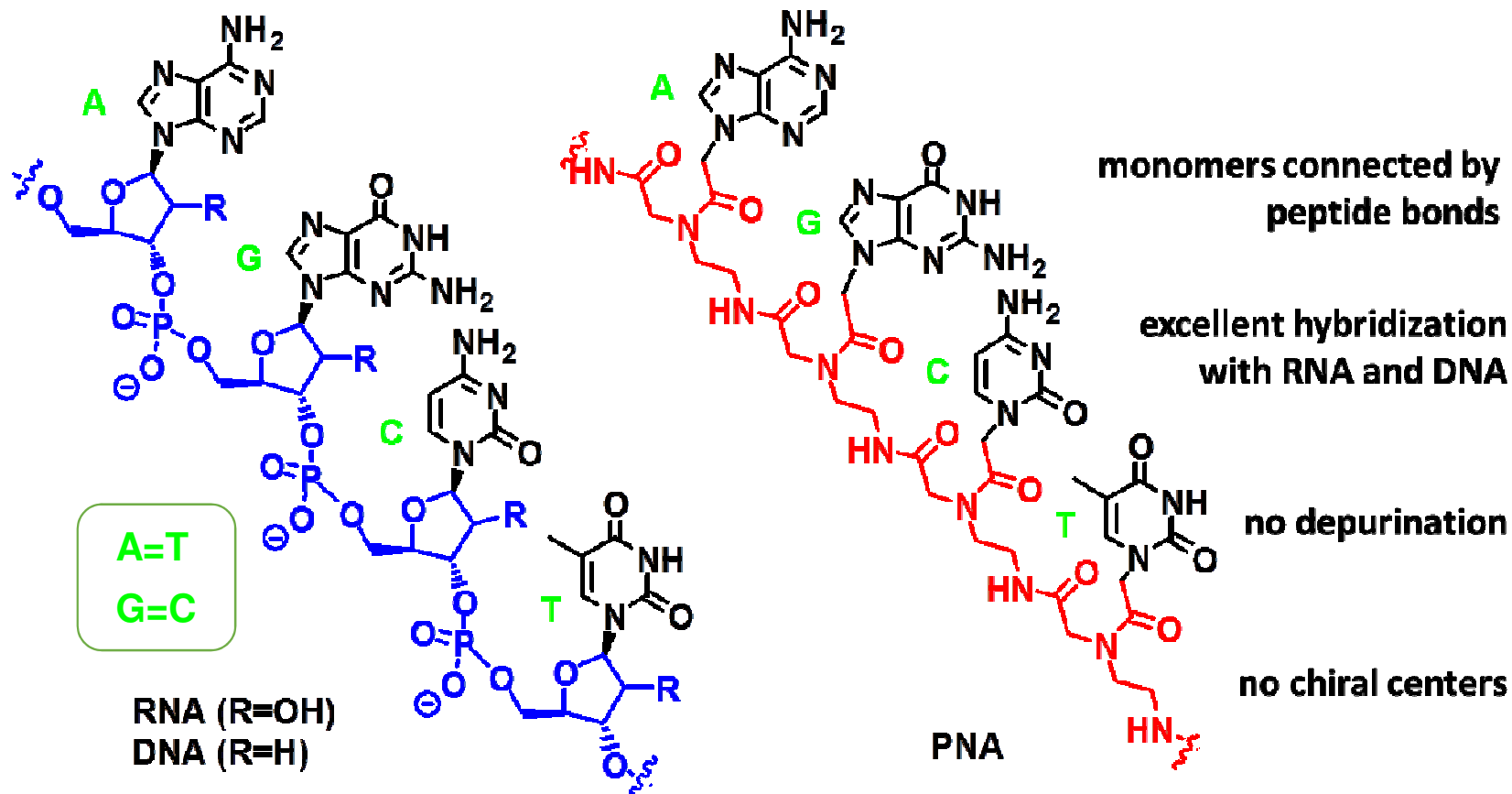


FplmR4_2-catalysed oligomerization of XNA (FANA) substrates



XNAzyme-catalysed assembly of an active XNAzyme. A variant XNA ligase (FplmR4_2mut) catalyses ligation (lane 2) of FANA substrates LigS1^F NUC and LigS2^F NUC. The product (LigPF NUC) is a variant of XNAzyme FR17_6 min (Fig. 2), which cleaves RNA substrate NucSV^R (lanes 5 and 6), but not scrambled RNA (NucS^R SCRAM2)(lanes 3 and 4).

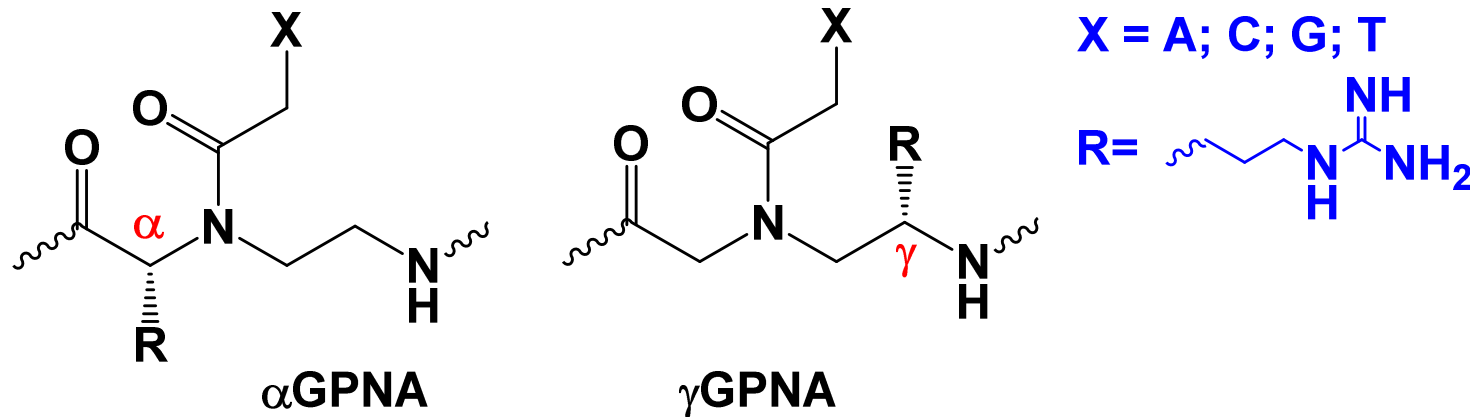
Peptidonucleic acids – functional DNA analogues



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

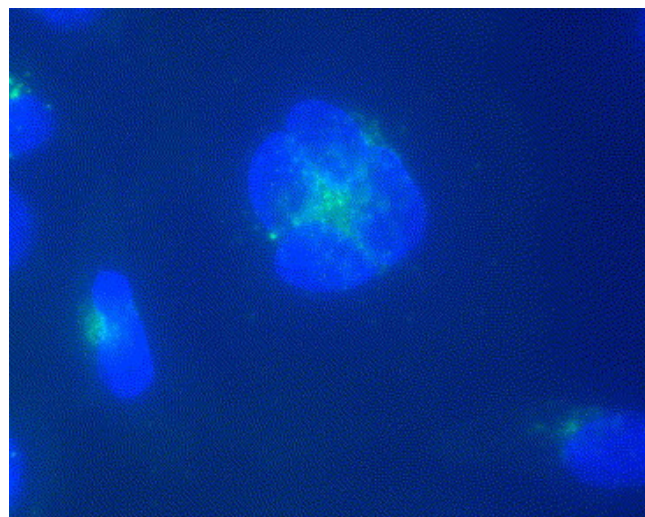
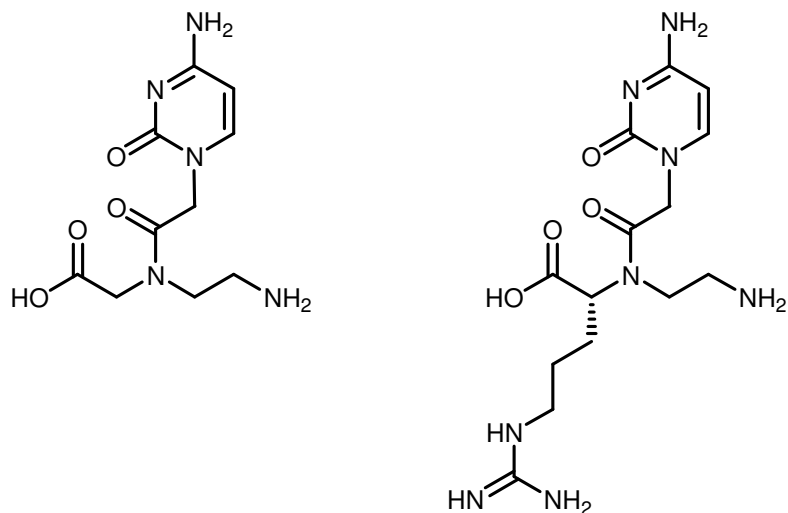
Applications: antigene, antisense agents; fluorescent DNA probes (FISH), anticancer, antiviral, antibacterial, antiparasitic agents; diagnostics, mol. biology

Structural modifications of the PNA - α GPNA, γ GPNA



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

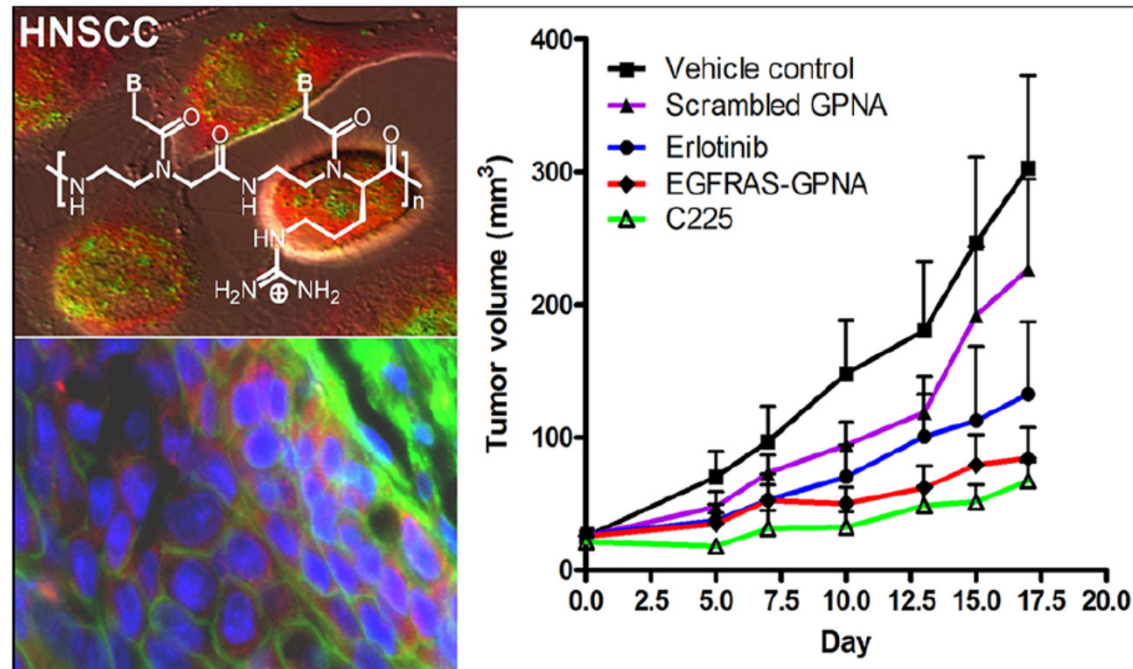
Cell-penetrating α GPNA



HeLa cells incubated with 1 μ M GPNA (FITC-^DCC^DAC^DCT^DCT^DGC^DCA^DAC^DGG^DGT-NH₂) 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

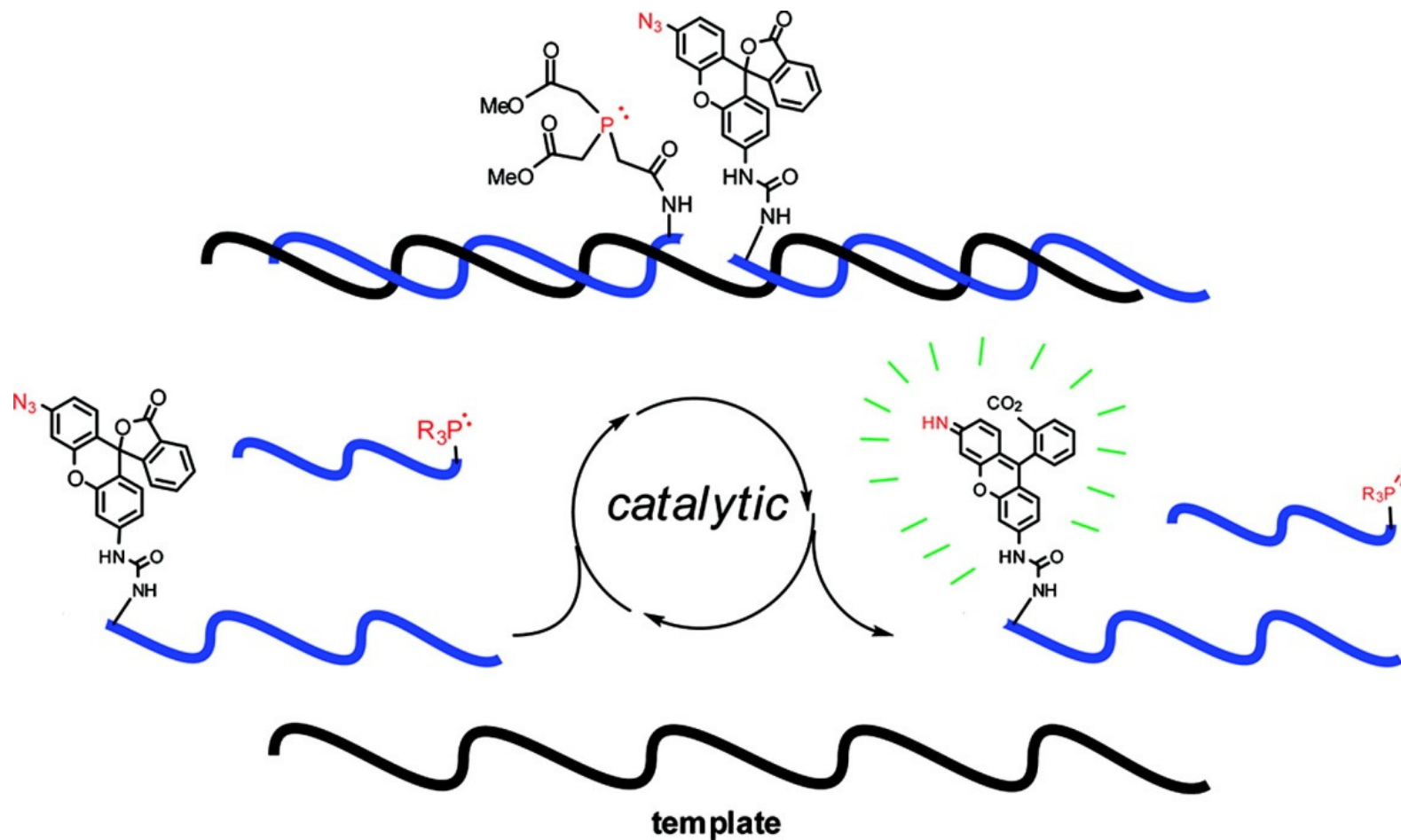
Antisense activity of α GPNA in vivo



- 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

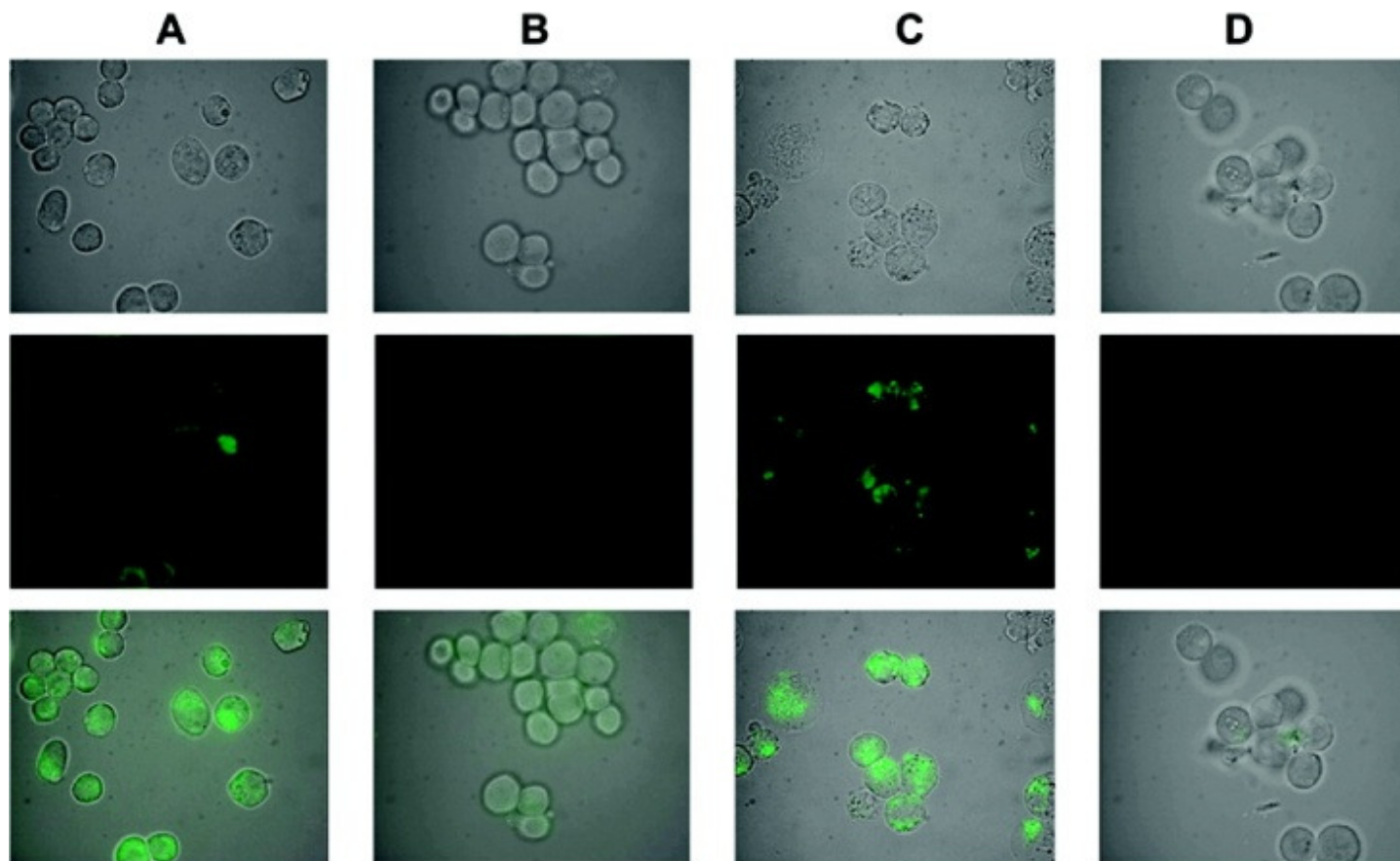
Cell-penetrating α GPNA for *in vivo* catalytic oligonucleotide sensing



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

Cell-penetrating α GPNA for *in vivo* catalytic oligonucleotide sensing

Inside living cells

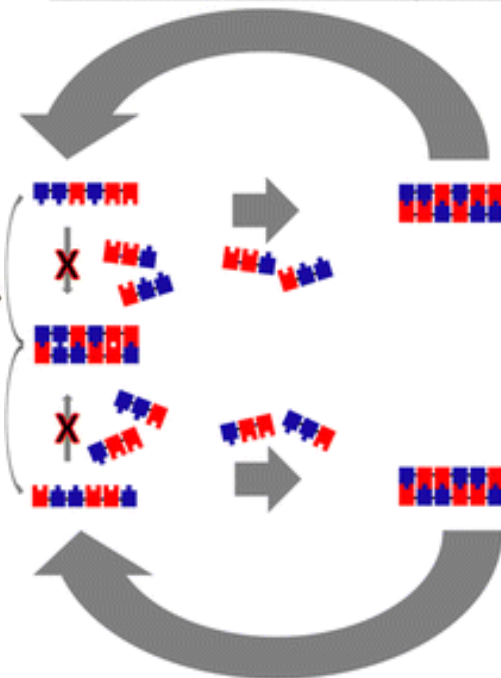


A, B – controls (+/-) **C** – matching PNA **D** – mismatched PNA

Abiotic self-replication

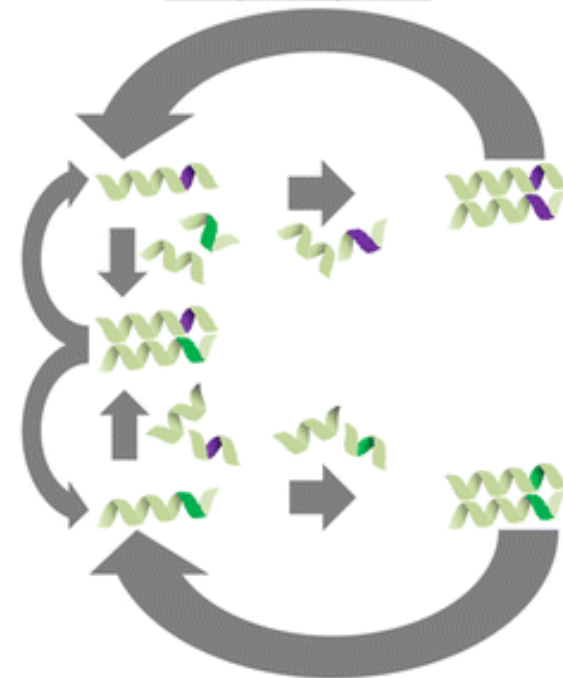


A. Darwinian/nucleic acid replicator



Cross-replication
disfavored

B. Peptide replicator

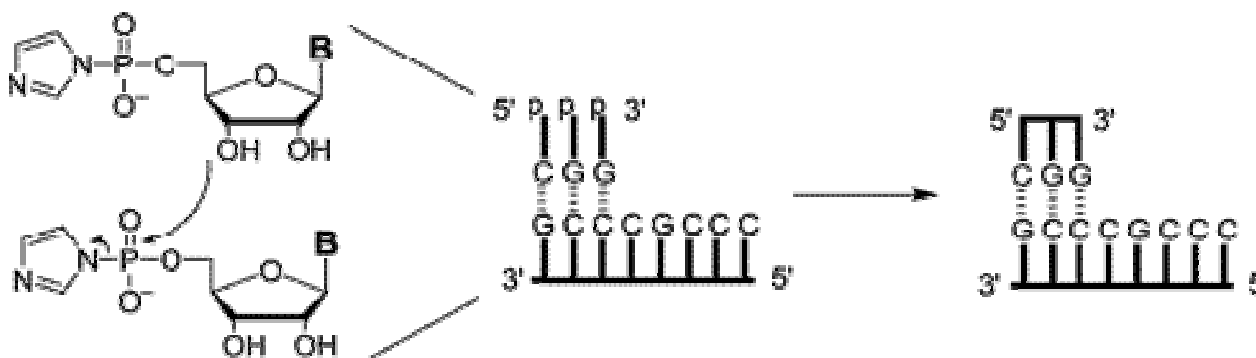
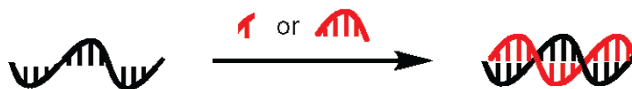


Cross-replication
allowed

(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.

Nonenzymatic templated nucleic acid synthesis – monomer/short oligomer



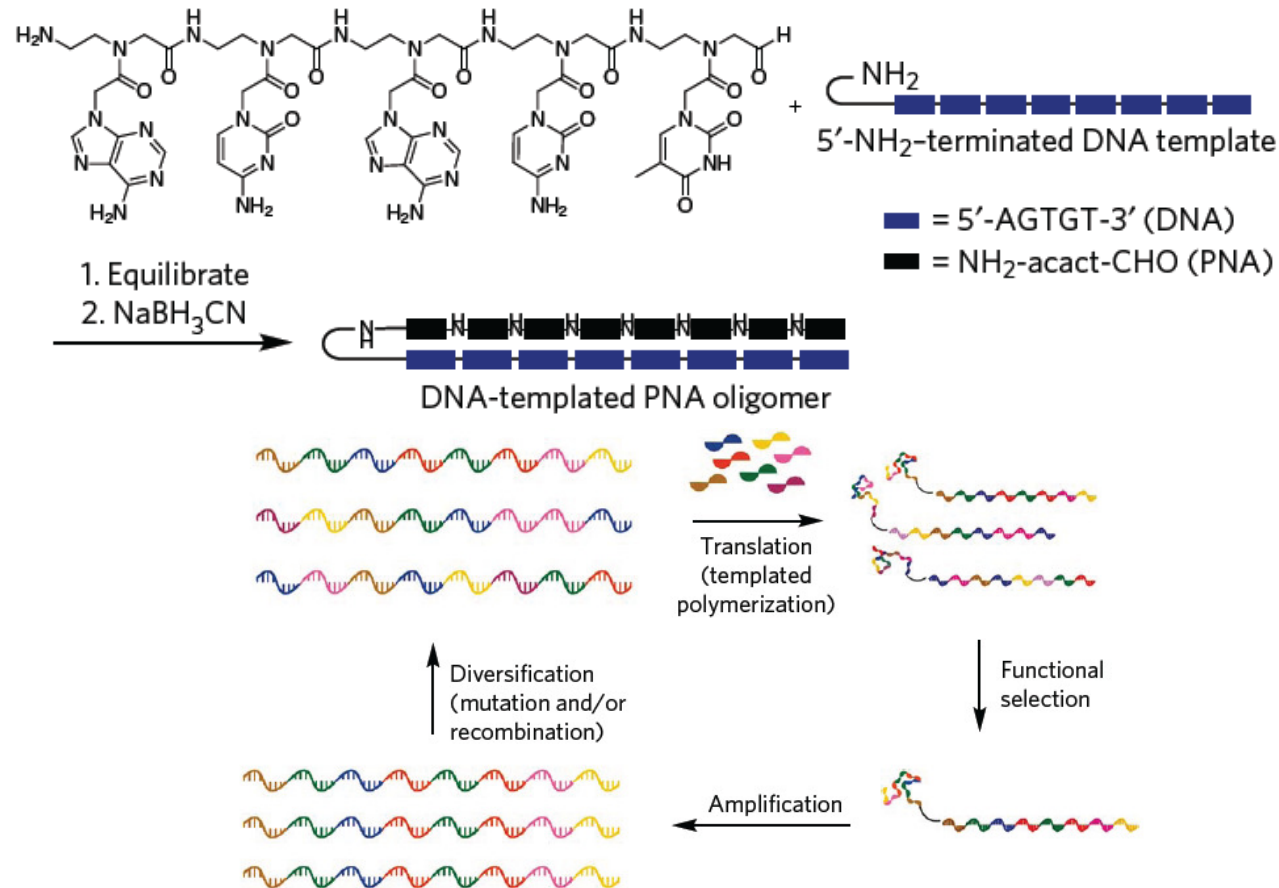
Problems:

- very slow reactions
- limited range of templates (mostly C-rich)
- poor regioselectivity (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

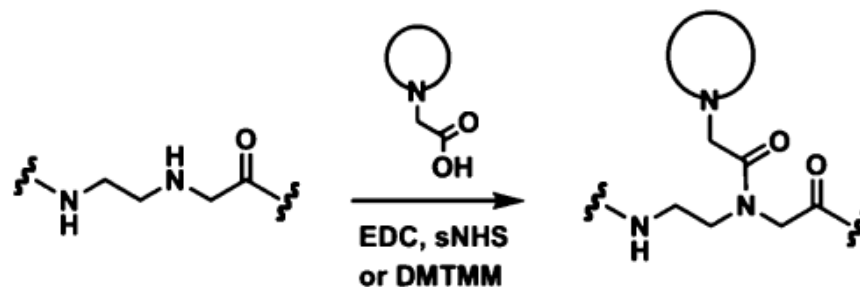
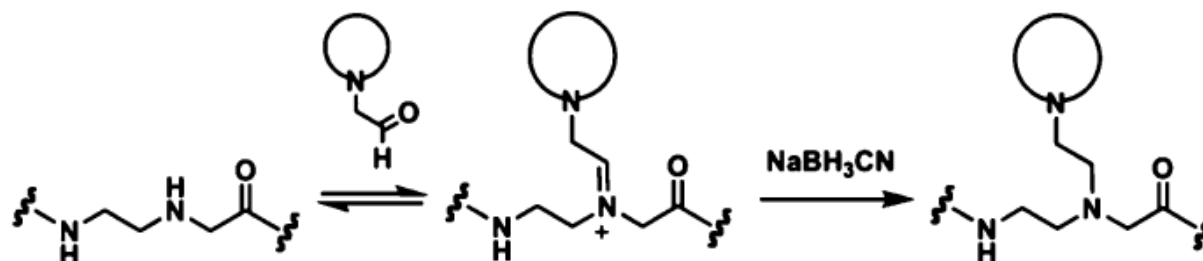


Limitations:

- 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



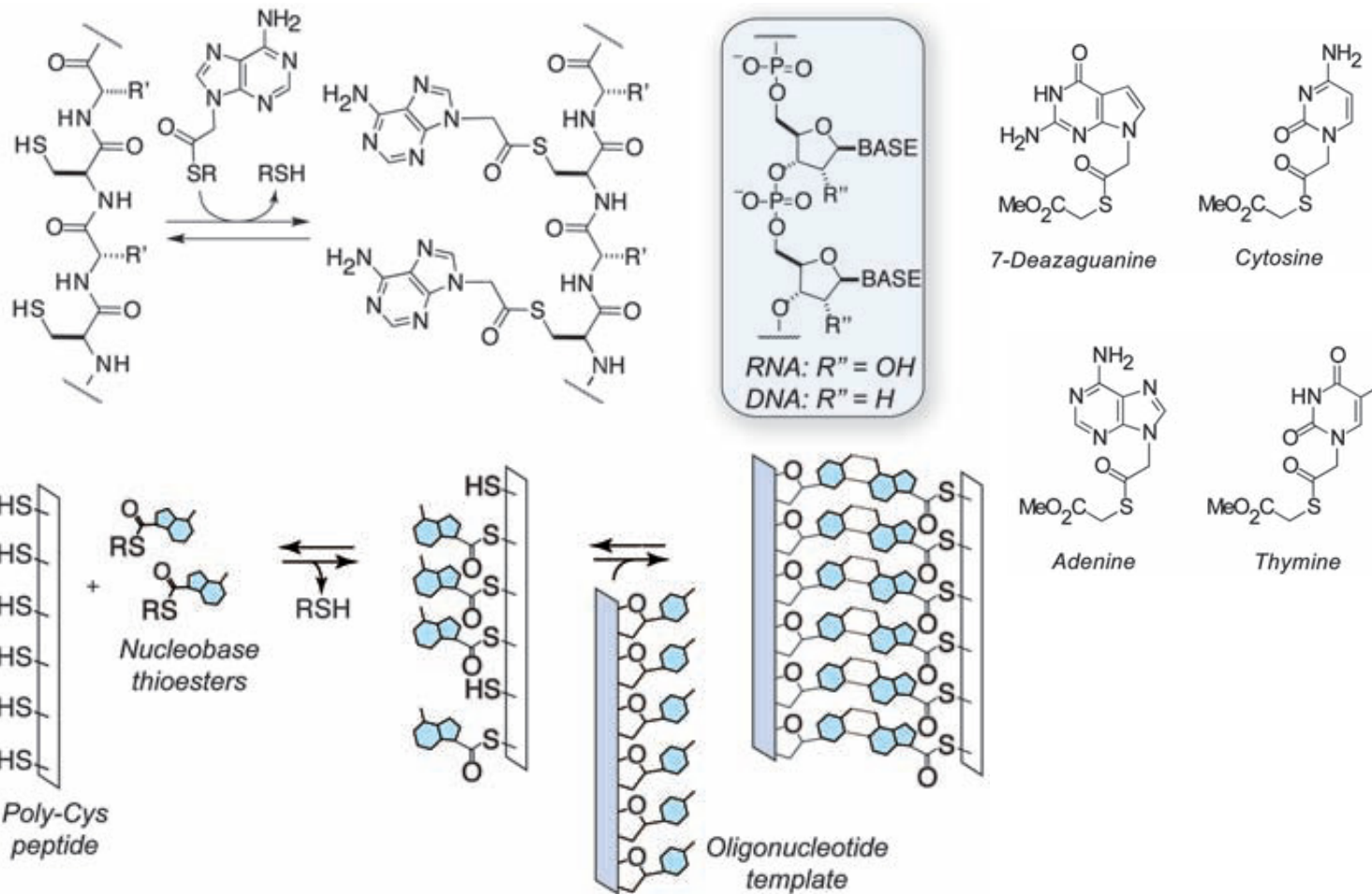
Advantages:

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

Limitations:

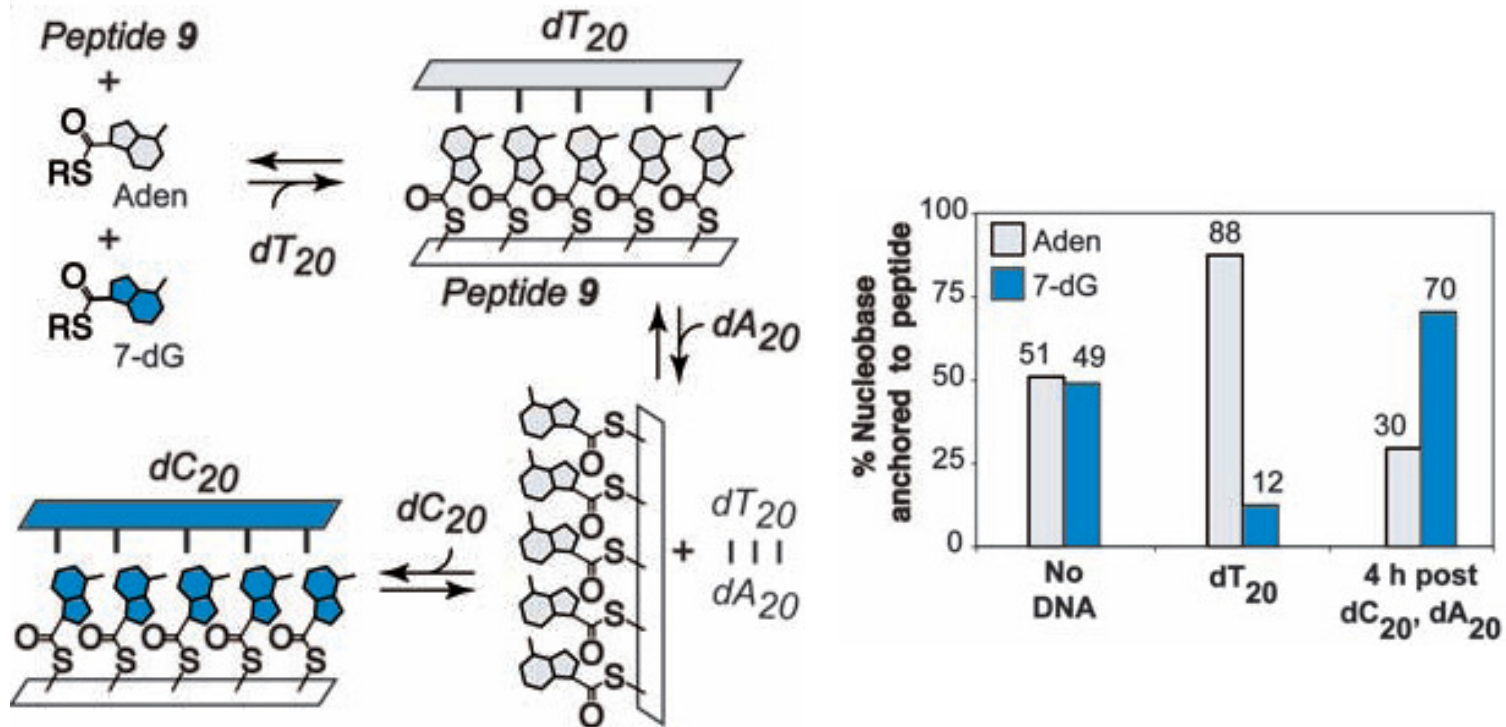
- single or double abasic sites (most efficient inside of the chain)
- Aldehydes give better yields and accuracy, but worse hybridization of the product

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