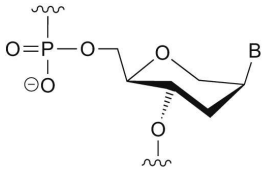
CHAPTER 1

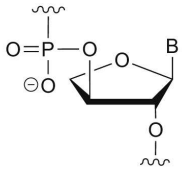
OLIGONUCLEOTIDES

Part 3 – noncanonical backbone – Xeno Nucleic Acids

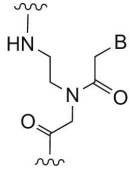
XNA – Xeno Nucleic Acids



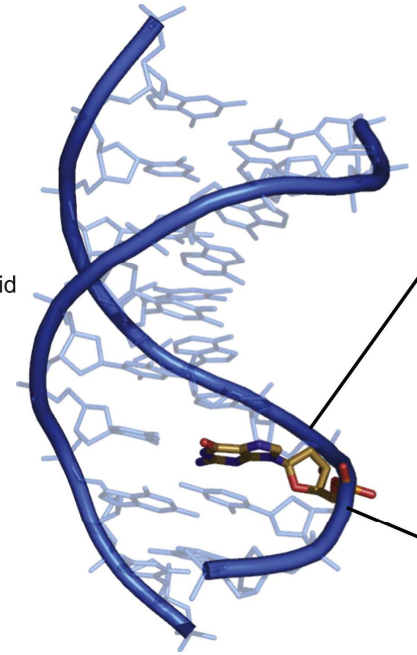
Hexitol Nucleic Acid
(HNA)

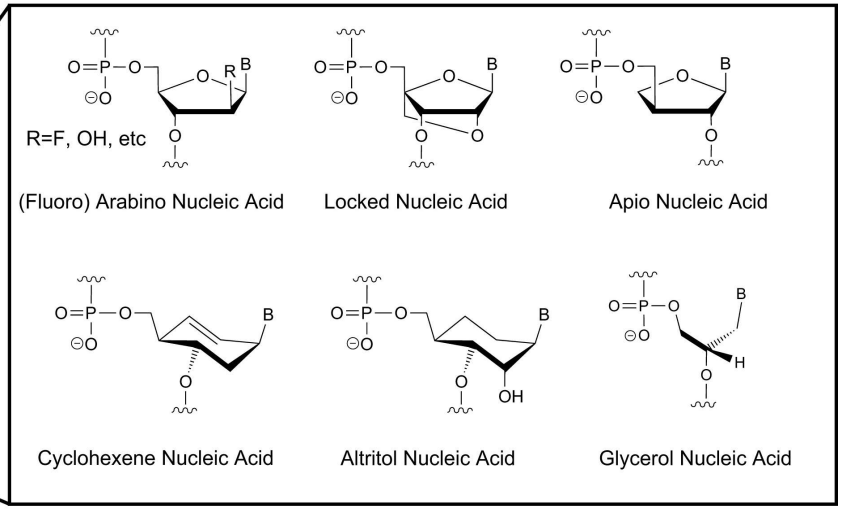


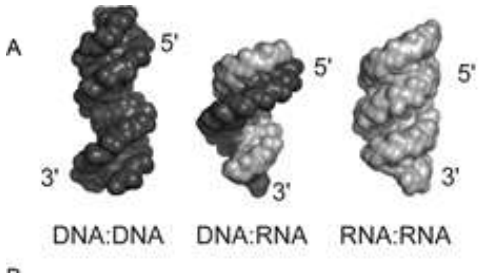
Threose Nucleic Acid
(TNA)

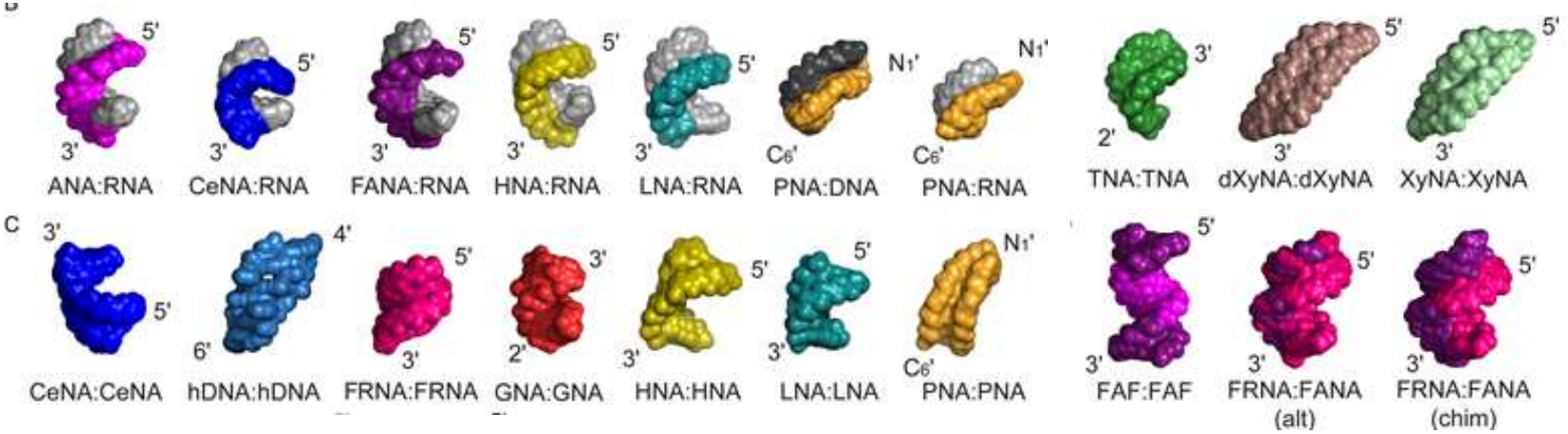


Peptide Nucleic Acid
(PNA)

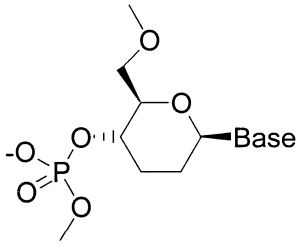




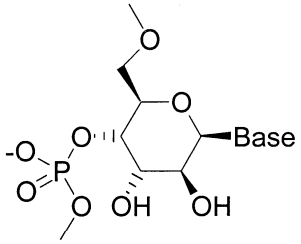




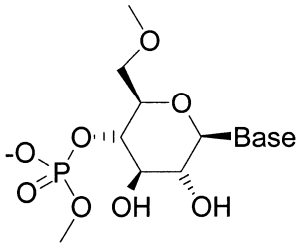
Overview of XNA



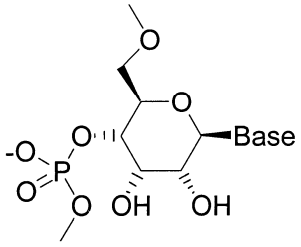
Homo-DNA
β-D, 4' → 6'



Altropyranosyl-NA
β-D, 4' → 6'

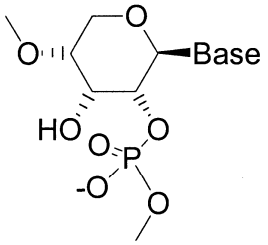


Glucopyranosyl-NA
β-D, 4' → 6'

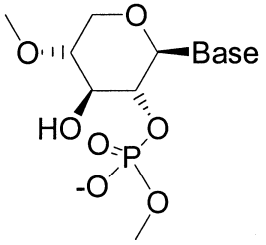


Allopyranosyl-NA
β-D, 4' → 6'

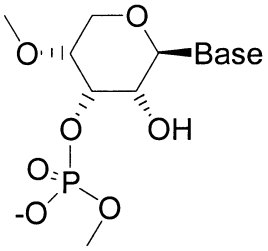
The 2'→4' linked pentopyranosyl family



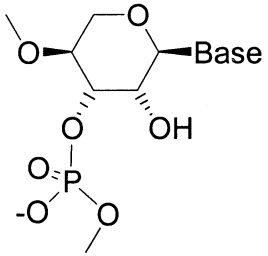
β-D-Ribopyranosyl-NA
(pRNA)



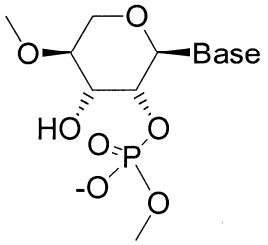
β-D-Xylopyranosyl-NA



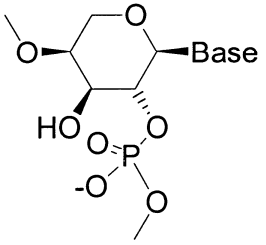
β-D-Ribopyranosyl-NA
3'→4'



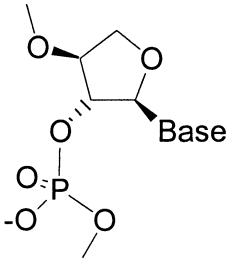
α-L-Lyxopyranosyl-NA
3'→4'



α-L-Lyxopyranosyl-NA



α-L-Arabinopyranosyl-NA



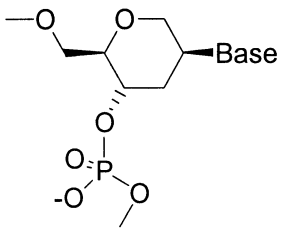
α-L-Threofuranosyl-NA
TNA

J. Hunziker, H. J. Roth, M. Bohringer, A. Giger, U. Diederichsen,M. Gobel, R. Krishnan, B. Jaun, C. Leumann and A. Eschenmoser, *Helv. Chim. Acta*, **1993**, *76*, 259–352

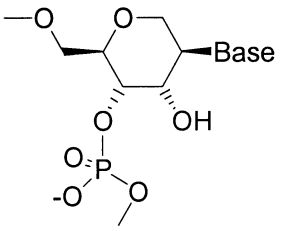
Review on the oligonucleotide modifications:
A. Eschenmoser *Angew. Chem.*, Int. Ed. **2011,** *50*, 12412-12472

C. J. Leumann, *Bioorg. Med. Chem.*, **2002,** *10*, 841–854

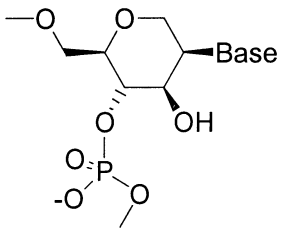
Overview of XNA



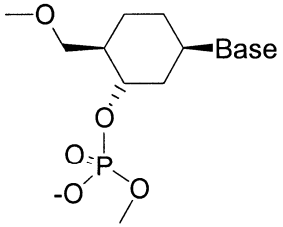
Hexitol NA
(HNA)



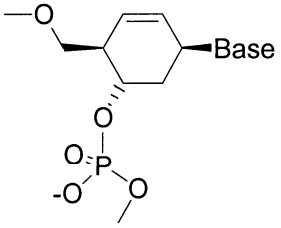
Altritol-NA
(ANA)



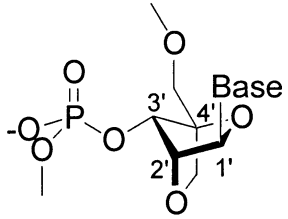
Mannitol-NA
(MNA)



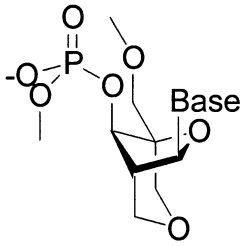
Cylohexanyl-NA
CNA



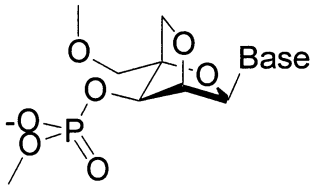
Cyclohexenyl-NA
CeNA



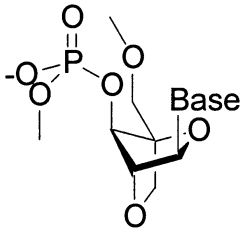
β-D-Ribo-LNA



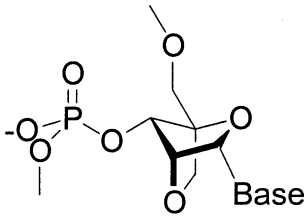
[3.2.1]-LNA



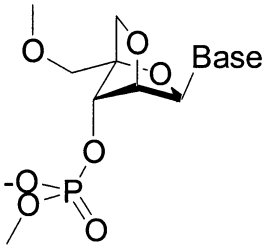
α-L-Ribo-LNA



β-D-Xylo-LNA



α-D-Ribo-LNA



α-L-Xylo-LNA

C. J. Leumann, *Bioorg. Med. Chem.*, **2002,** *10*, 841–854

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

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.

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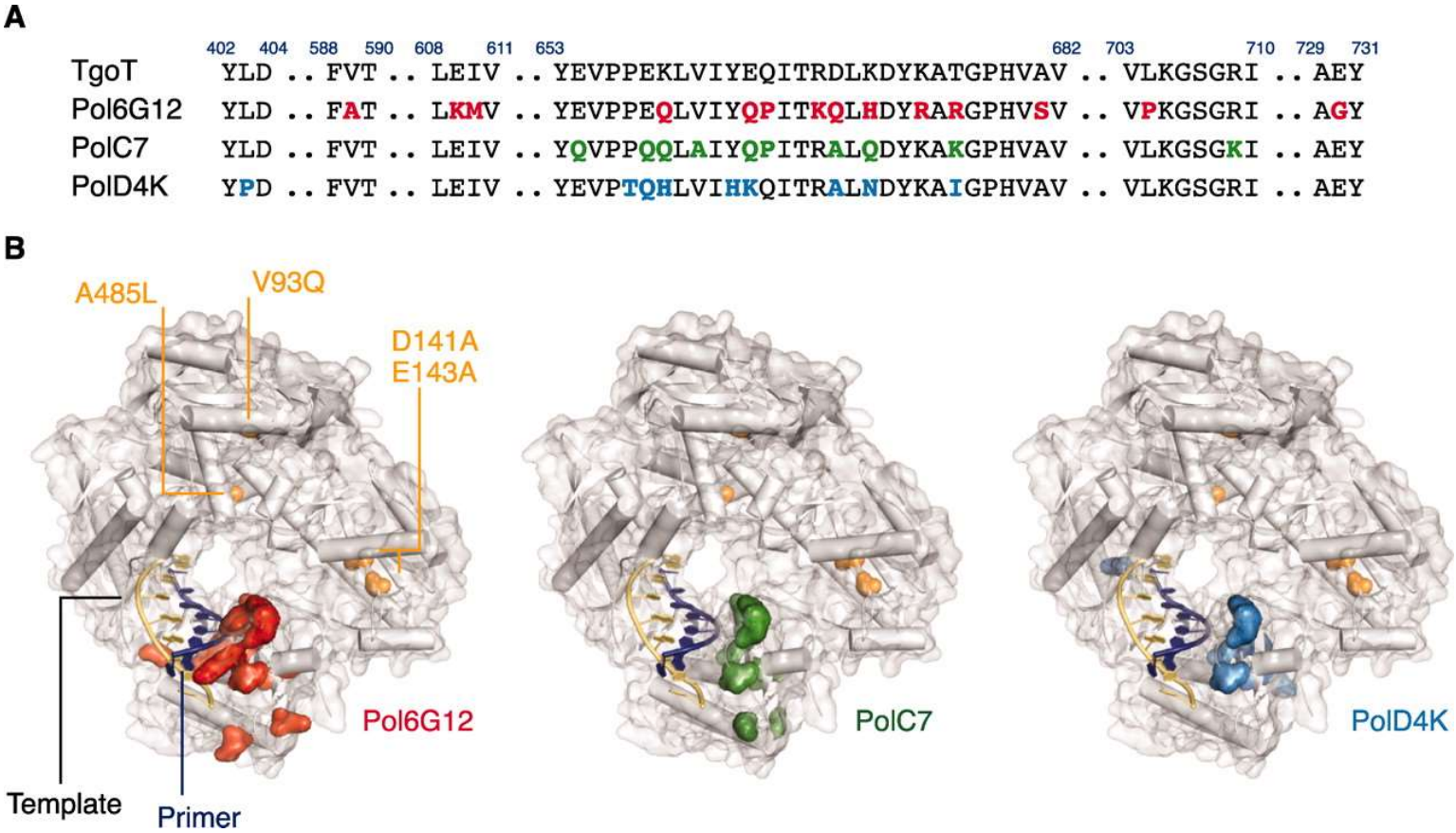
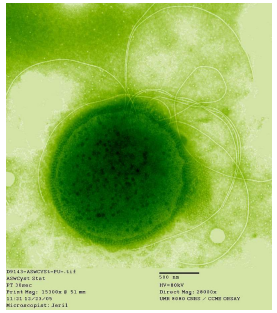
Engineering XNA polymerases

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

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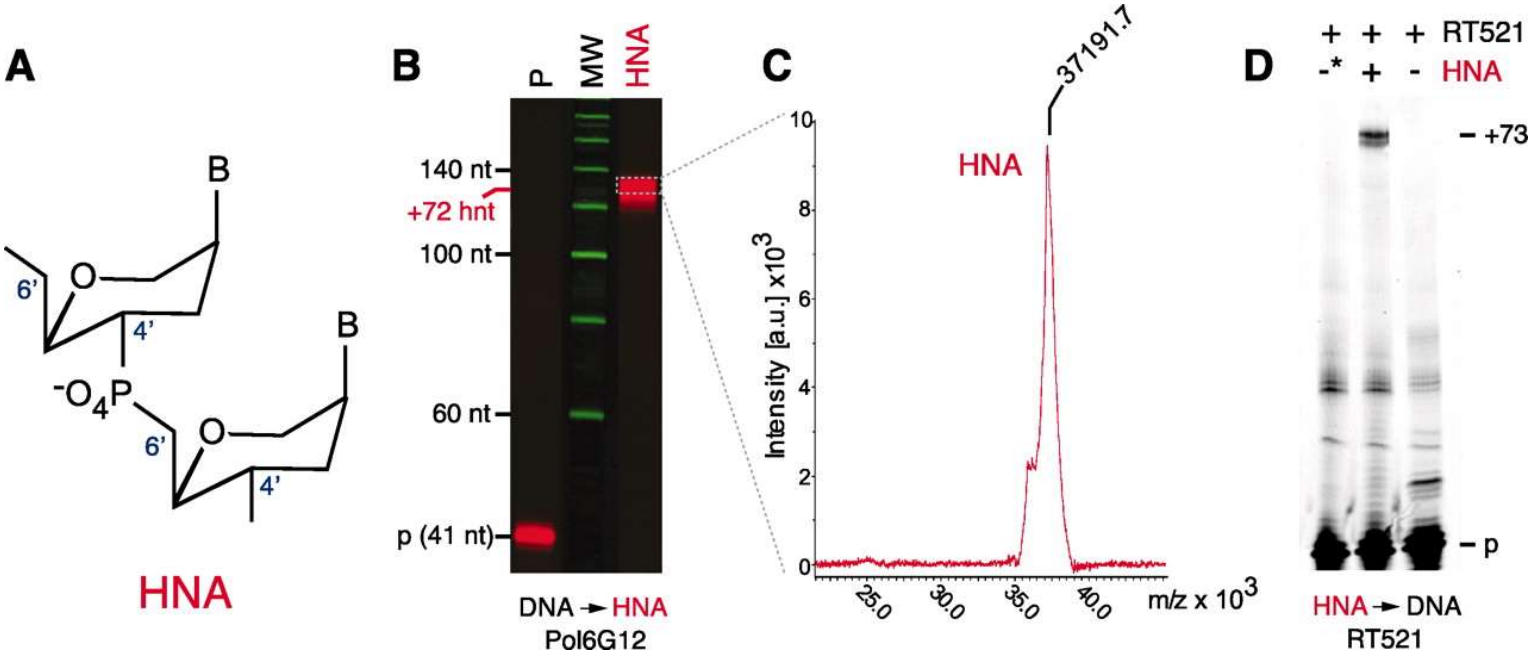
HNA synthesis



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

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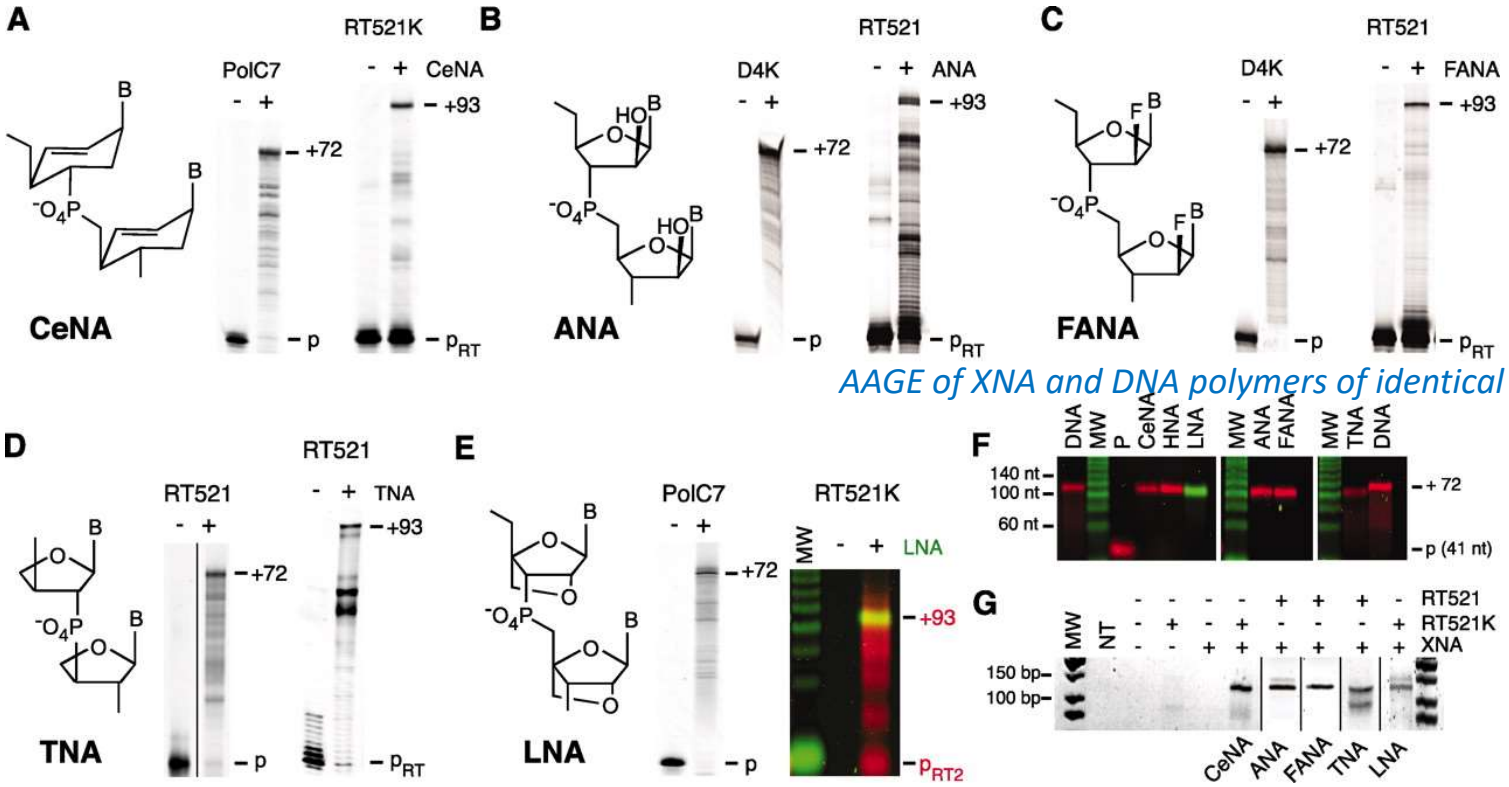
XNA genetic polymers.

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)

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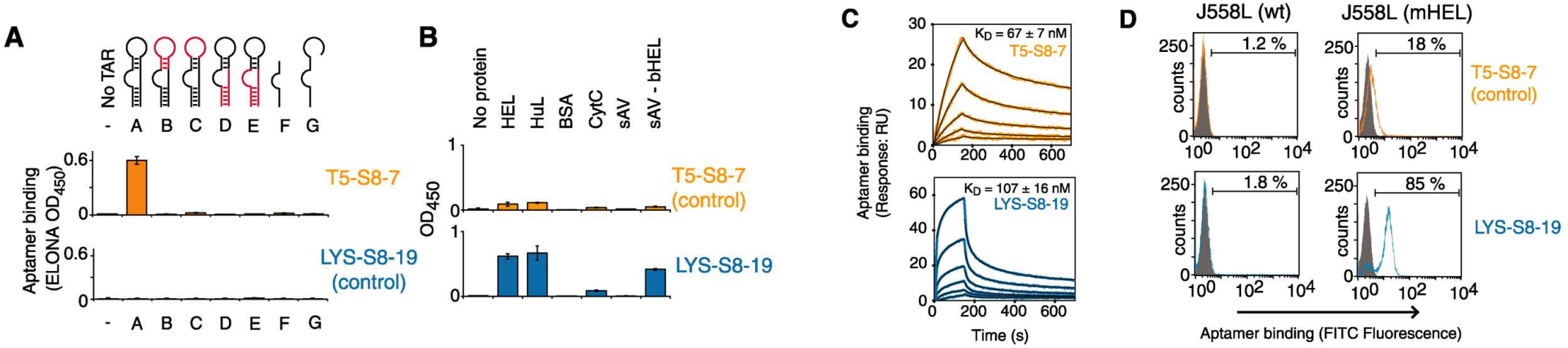
HNA aptamers



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.

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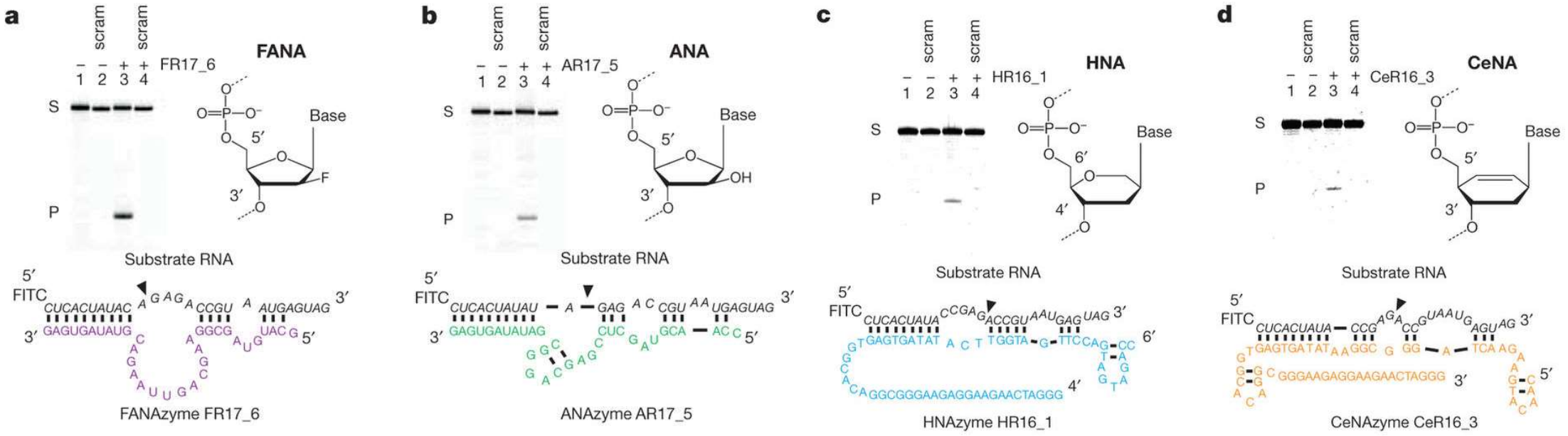
XNA – Xeno Nucleic Acids

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.



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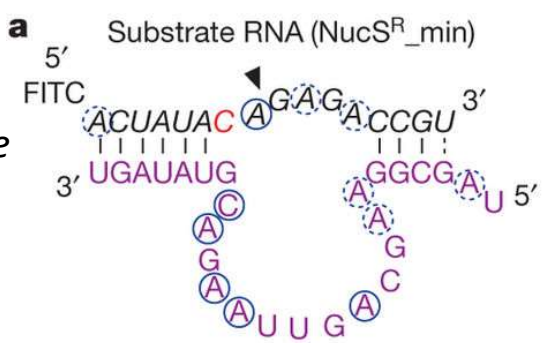
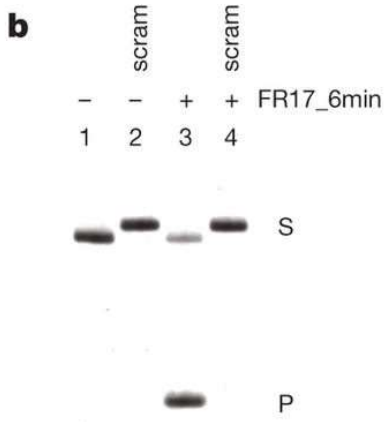
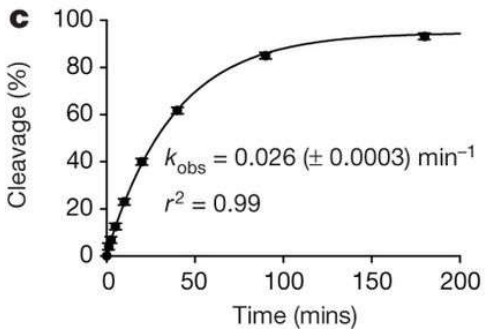
Chemical synthesis yields an active RNA endonuclease XNAzyme

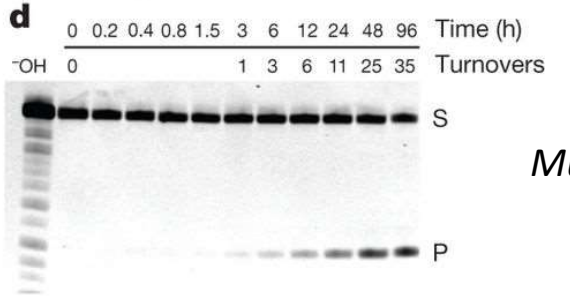
Secondary FANAzyme structure





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





Multiple turnovers

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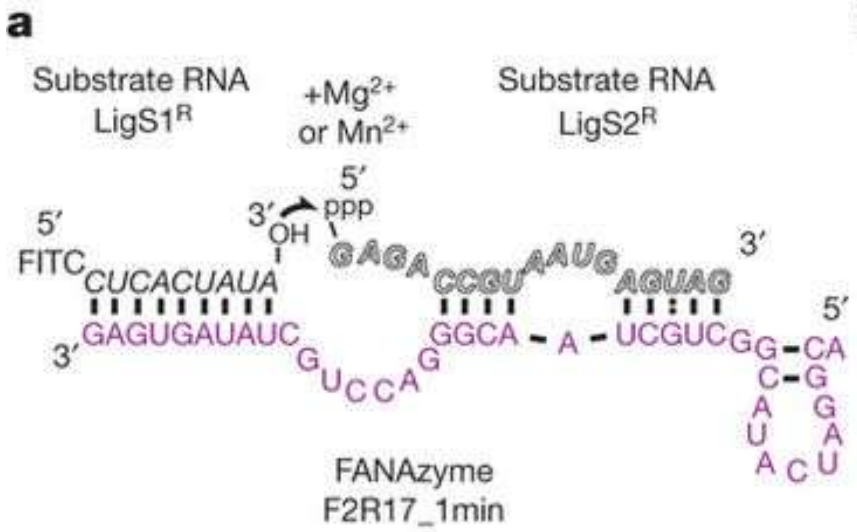
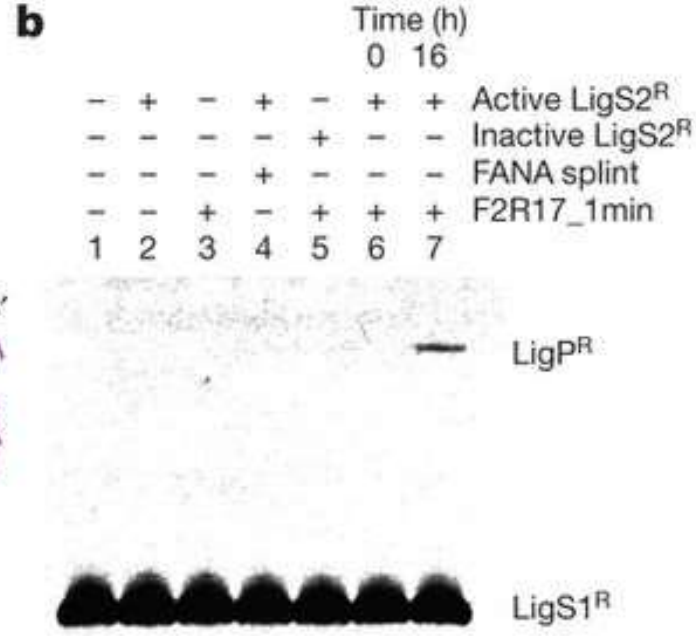
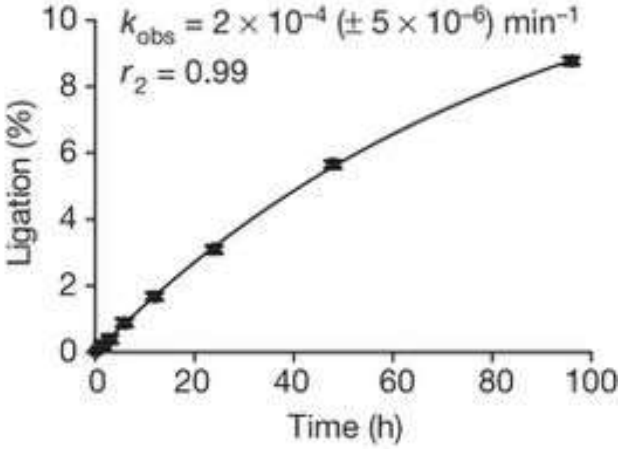
An RNA ligase XNAzyme (FANA)

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.).

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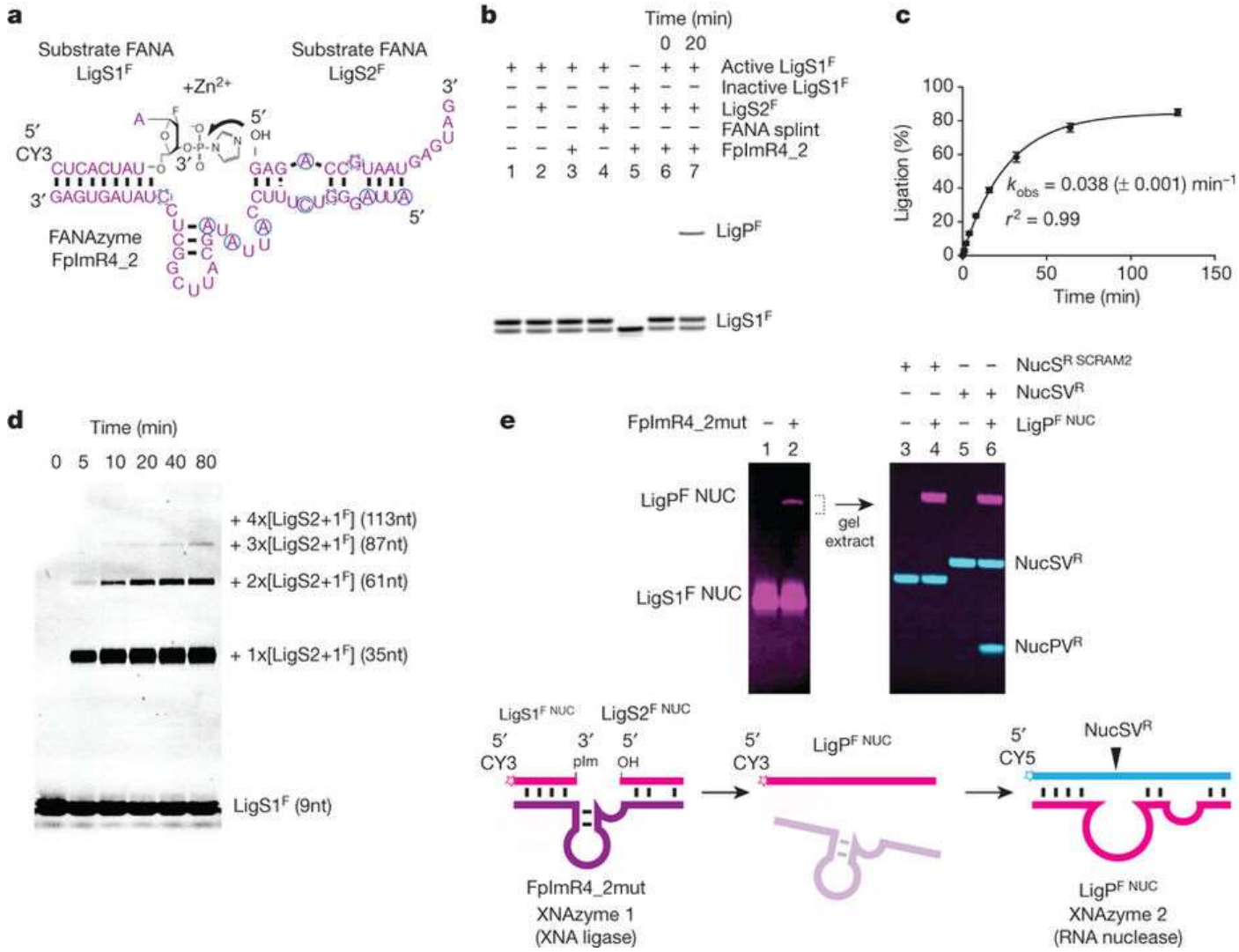
XNA–XNA ligase XNAzyme (FANA): catalysis without natural nucleic acids



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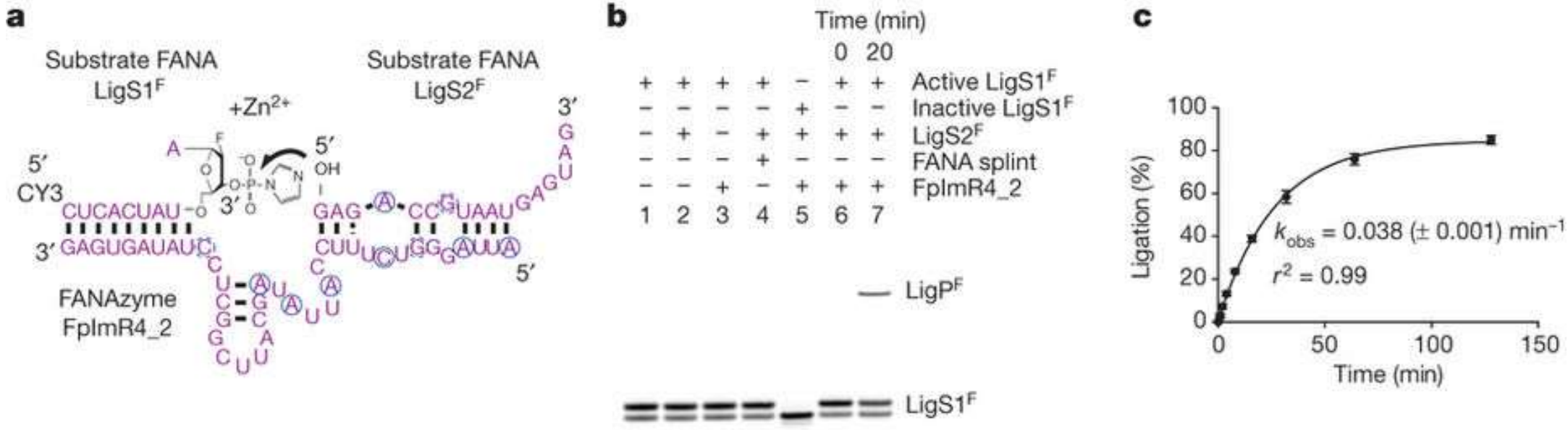
XNA–XNA ligase XNAzyme (FANA): catalysis without natural nucleic acids



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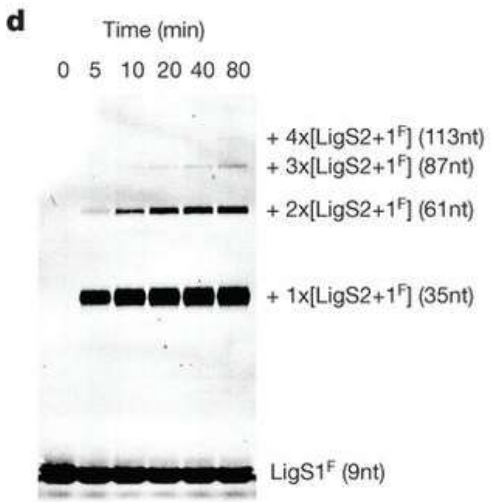
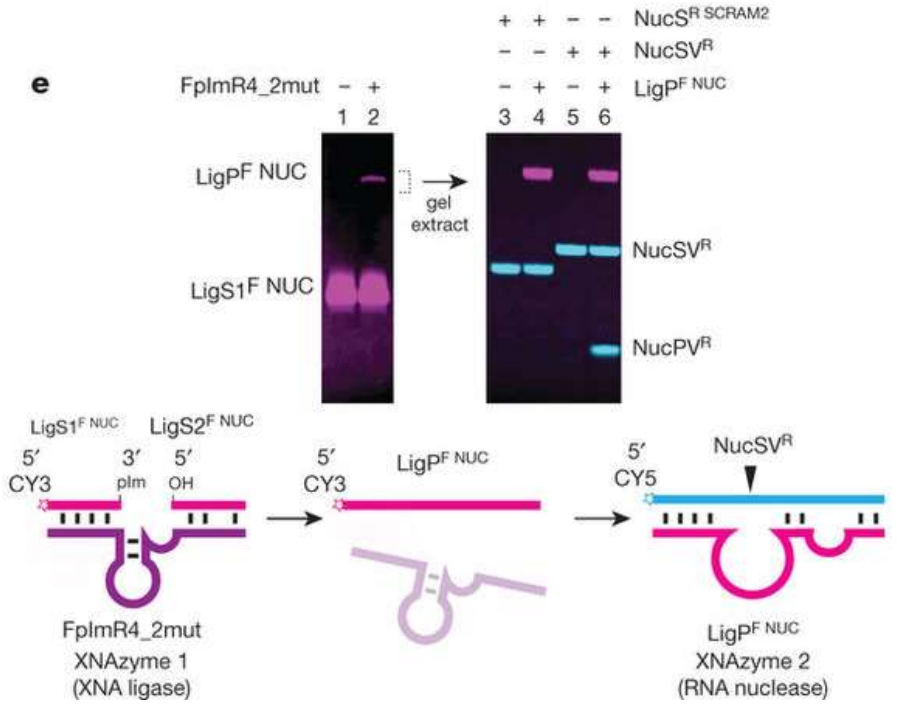
XNA–XNA ligase XNAzyme (FANA): catalysis without natural nucleic acids



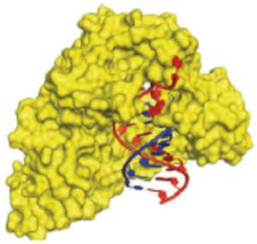
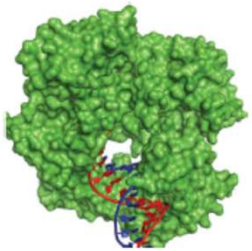
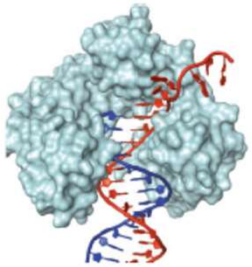
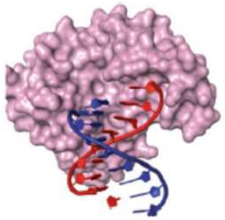
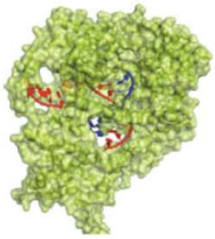
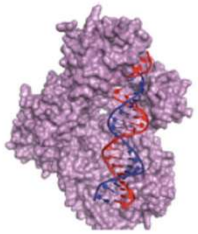
FpImR4_2-catalysed oligomerization of XNA (FANA) substrates



XNAzyme-catalysed assembly of an active XNAzyme. A variant XNA ligase (FpImR4_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

Table 1 Polymerase-mediated synthesis of XNAs

Pol Family		Polymerase	Novel Activity
Pol A		Taq Tth Pol θ	2′F RNA 2′OME RNA 2′-azido RNA
Pol B		Tgo KOD 9°N Pfu phi29	CeNA LNA phNA HNA FANA CyDNA 2′F RNA ANA TNA 2′azido RNA tPhoNA
Pol Y		(D-aa) Dpo4	L-DNA
Pol X		(D-aa) ASFV pol	L-DNA L-RNA
RNAP		T7 RNAP Syn5	2′F RNA 2′OMe RNA Ds-Pa UBP
RT		HIV-RT	pyDAD-puADA UBP

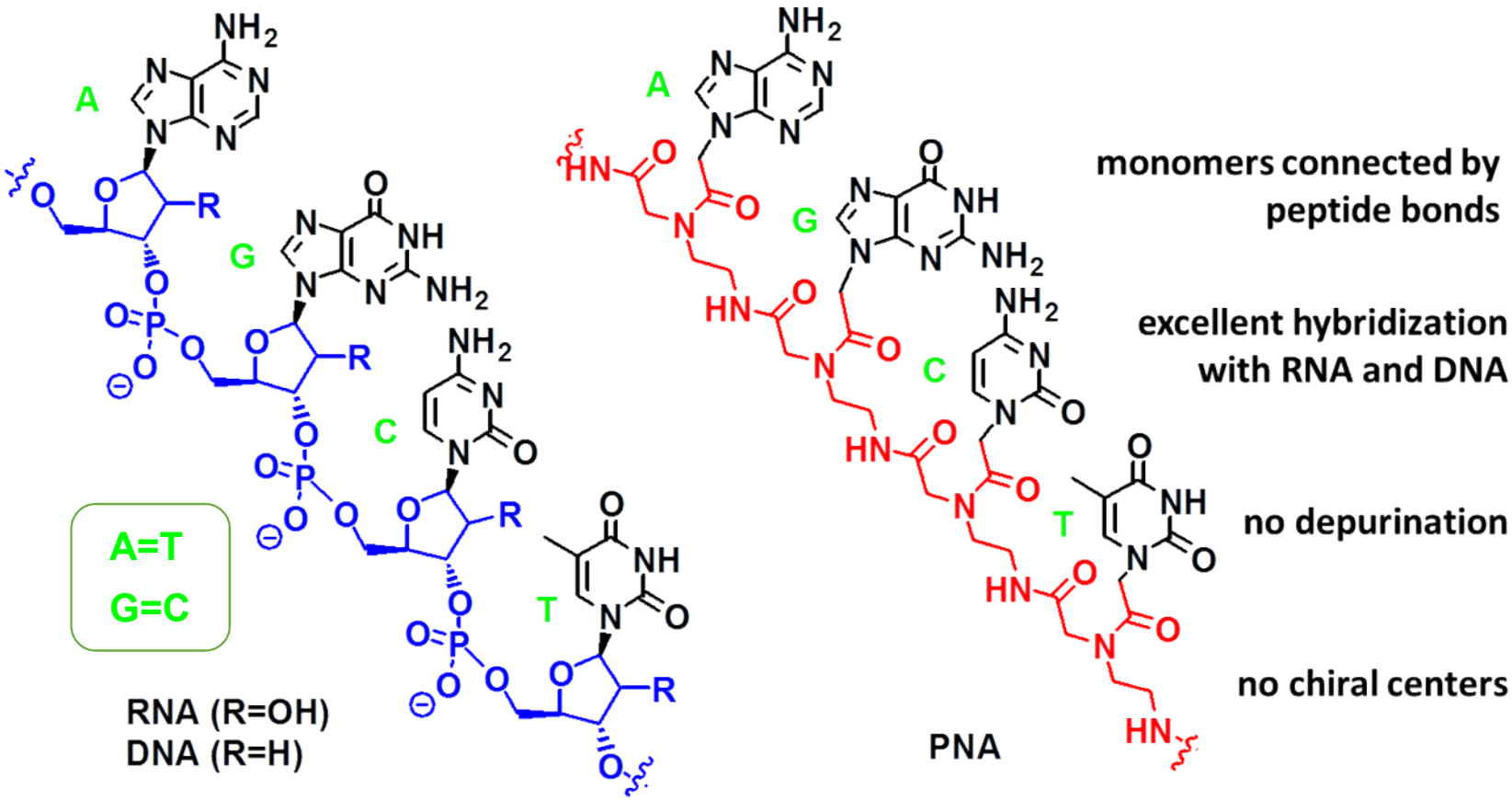
K. Duffy, S. Arangundy-Franklin, P. Holliger *BMC Biology*, **2020**, *18*, Art.# 112

Table 2 FDA-approved nucleic acid therapeutics as of February 2020

Drug name (trade name)	Target	Modifications	Mechanism	Indication	Approval
Fomivirsen (Vitravene)	mRNA of the CMV immediate-early (IE)-2 protein	PS	ASO (translation blocking)	Cytomegalovirus retinitis (CMV)	FDA (1998) and EMA (1999) approved. FDA (2001) and EMA (2002) withdrawn
Pegaptanib (Macugen)	Vascular endothelial growth factor (VEGF165)	2′F, 2′OMe, PEG conjugate	Aptamer	Neovascular (wet) age-related macular degeneration	FDA approved (2004)
Mipomersen (Kynamro)	Apolipoprotein B-100 mRNA	2′MOE, PS, 5mC	ASO (RNase H)	Homozygous familial hypercholesterolemia	FDA approved (2013)
Eteplirsen (Exondys 51)	Exon 51 in dystrophin mRNA	PMO	ASO (splicing modulation)	Duchenne muscular dystrophy	FDA approved (2016)
Nusinersen (Spinraza)	Survival of motor neuron 2 (SMN2) pre-mRNA	2′MOE, PS, 5mC	ASO (splicing modulation)	Spinal muscular atrophy	FDA (2016) and EMA (2017) approved
Patisiran (Onpattro)	Transthyretin (TTR) mRNA	2′OMe	siRNA	Hereditary transthyretin-mediated amyloidosis	FDA and EMA approved (2018)
Inotersen (Tegsedi)	Transthyretin (TTR) mRNA	2′MOE, PS, 5mC	ASO (RNase H)	Hereditary transthyretin-mediated amyloidosis	FDA and EMA approved (2018)
Volanesorsen (Waylivra)	Apolipoprotein C_3 (apo-CIII) mRNA	2′MOE, PS, 5mC	ASO (RNase H)	Familial chylomicronemia syndrome	EMA approved (2019)
Givosiran (Givlaari)	Aminolevulinate synthase 1 (ALAS1) mRNA	PS, 2′F, 2′OMe, GalNAc conjugate	siRNA	Acute hepatic porphyria	FDA approved (2019)
Golodirsen (Vyondys 53)	Exon 53 in dystrophin mRNA	PMO	ASO (splicing modulation)	Duchenne muscular dystrophy	FDA approved (2019)

K. Duffy, S. Arangundy-Franklin, P. Holliger *BMC Biology*, **2020**, *18*, Art.# 112

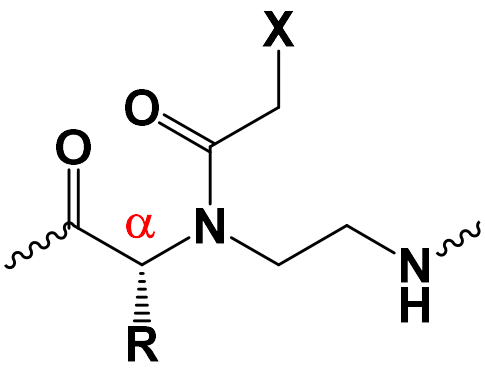
Peptidonucleic acids – functional DNA analogues



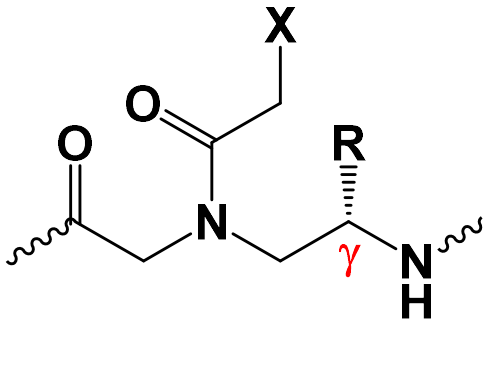
PNA – stable *ex vivo*, the backbone detected in cyanobacteria

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

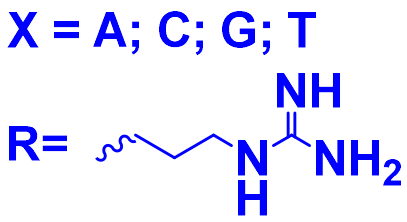
Structural modifications of the PNA - αGPNA, γGPNA



αGPNA

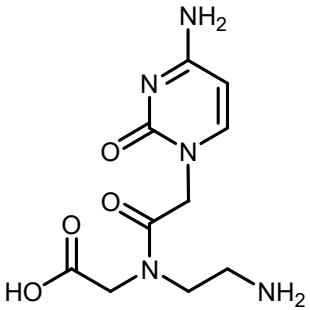


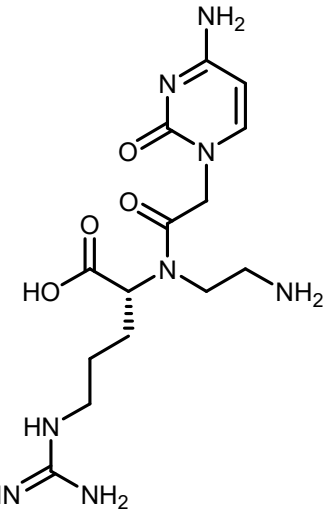
γGPNA

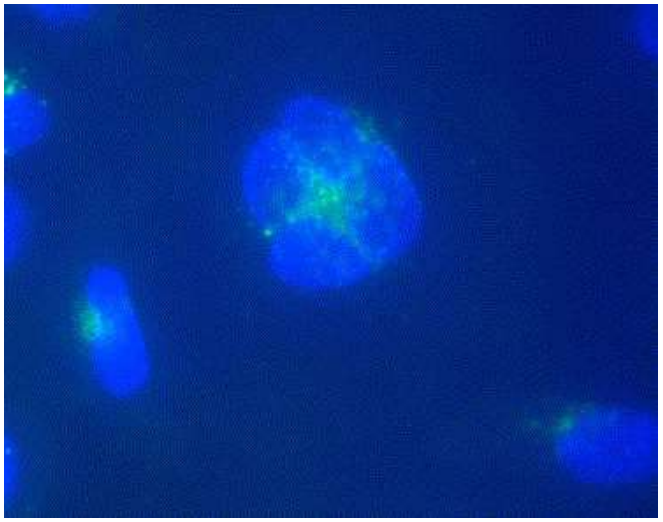


- GPNA: Alkylguanidinium residues (Arg side chains)
- enhanced water solubility
- cell permeability (analogous to oligoarginine CPPs)
- α position ← *D*-arginine
- γ position ← *L*-arginine

Cell-penetrating αGPNA





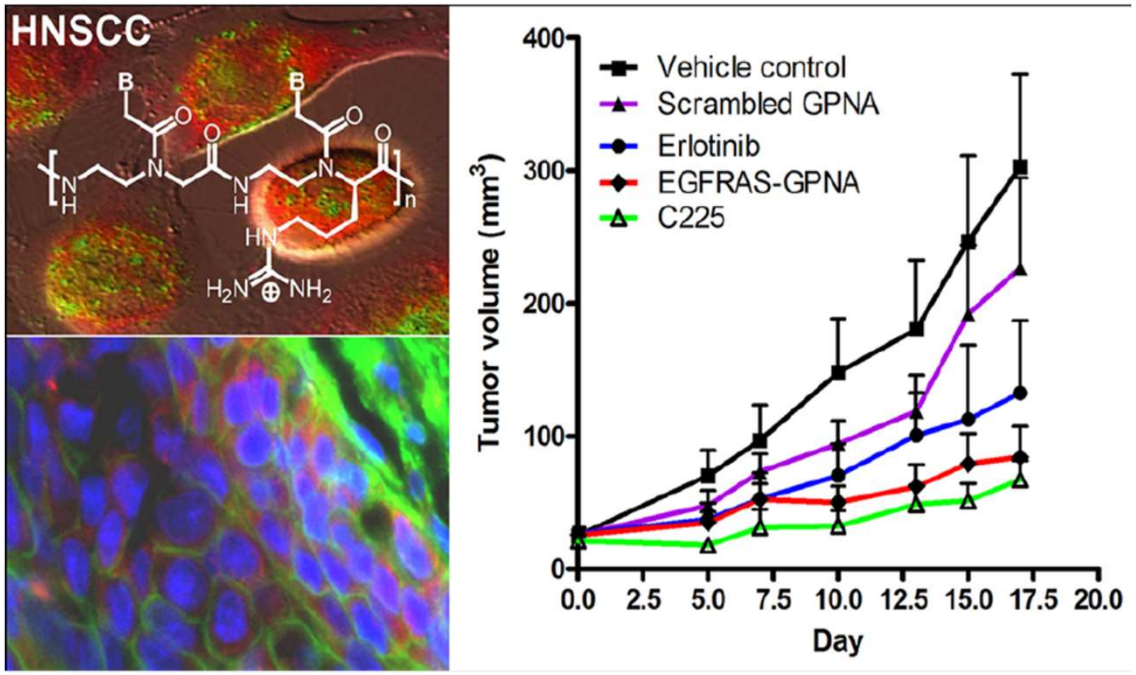


HeLa cells incubated with 1 μM GPNA (FITC-^{D}**C**^{D}**A**C^{D}**C**T^{D}**C**T^{D}**G**C^{D}**C**A^{D}**A**C^{D}**G**G^{D}**G**T-NH_2) for 16 h, Fixed, stained with DAPI. Nuclei (blue), GPNA (green).

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

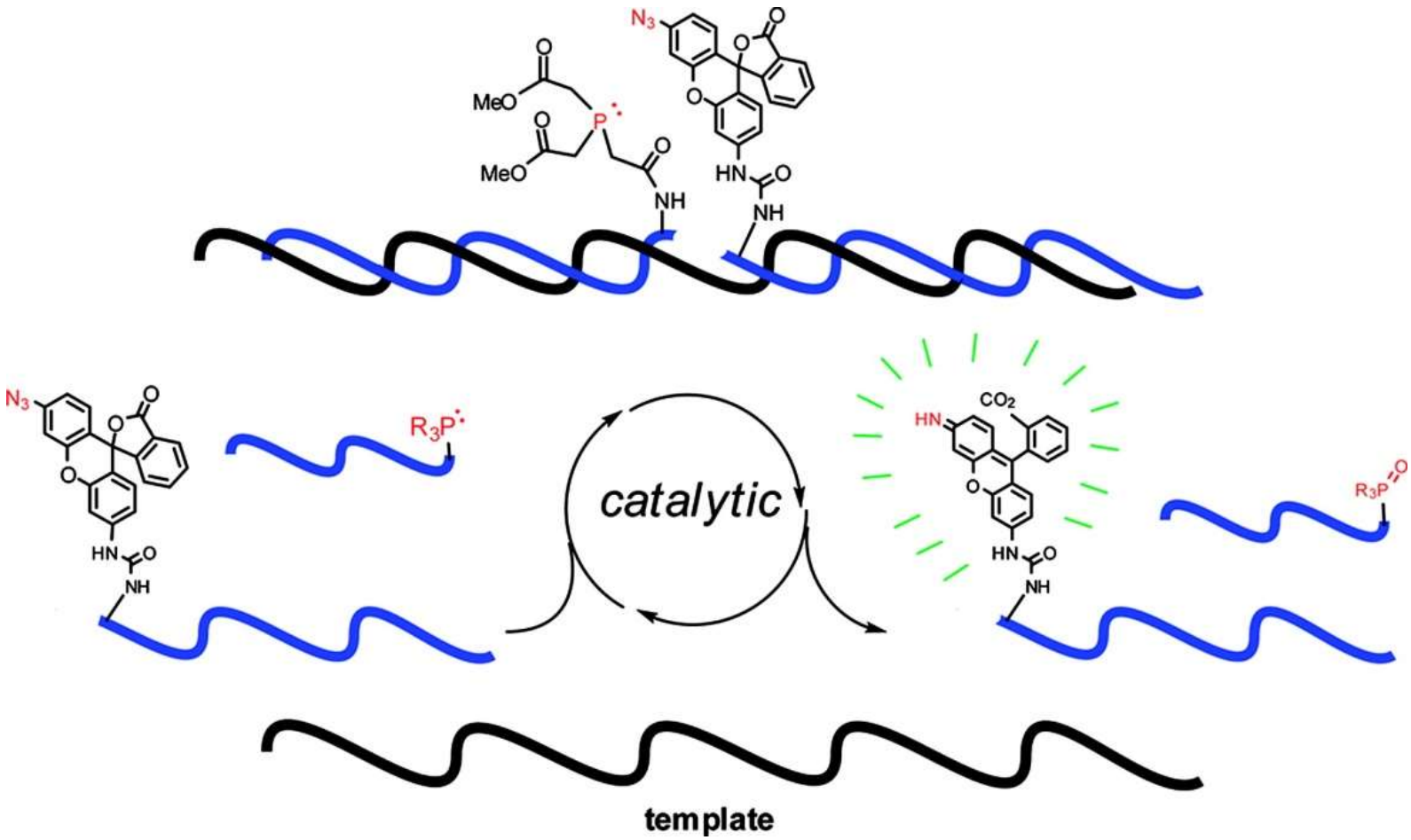
Antisense activity of αGPNA in vivo



- 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

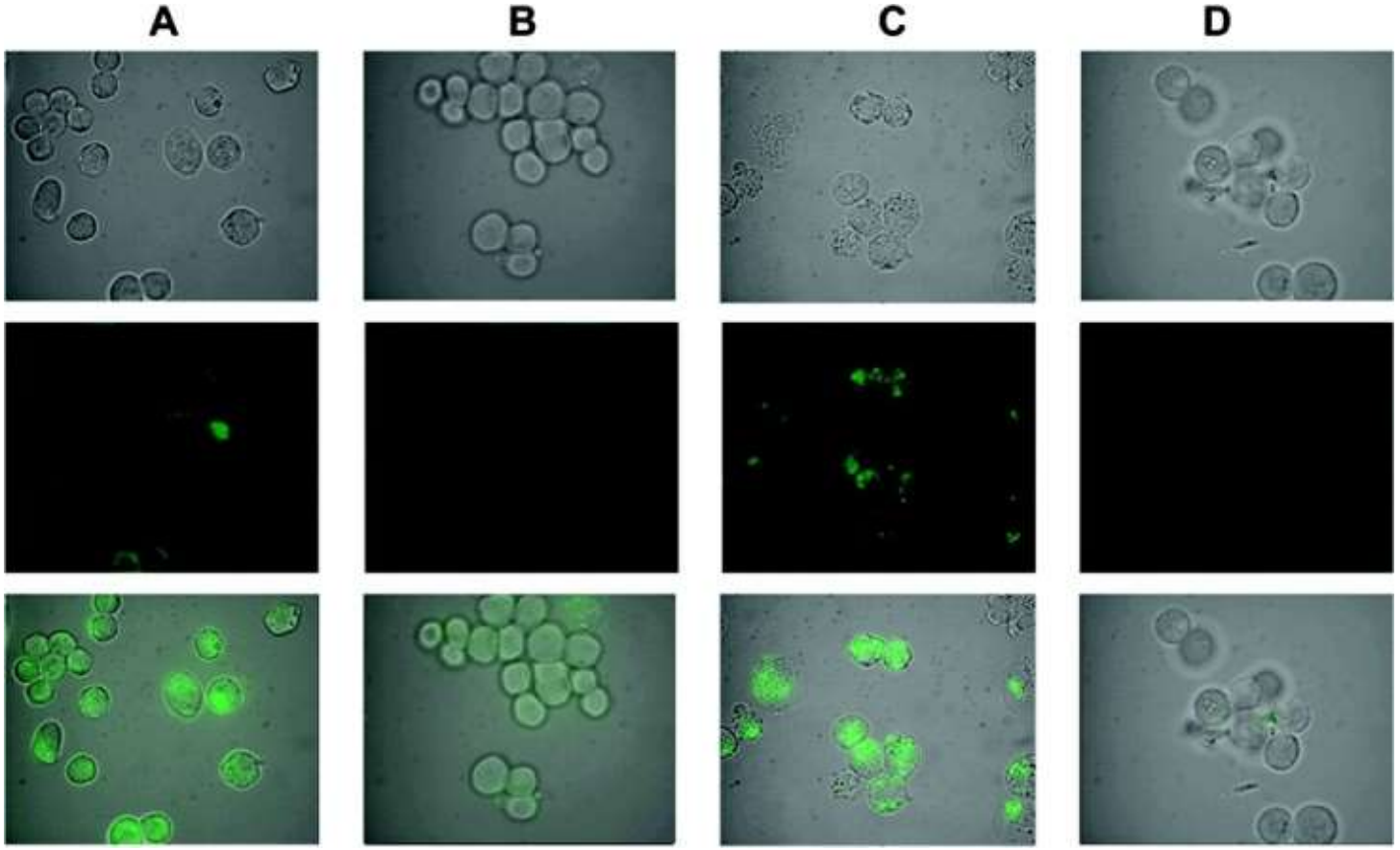
Cell-penetrating αGPNA for in vivo catalytic oligonucleotide sensing



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

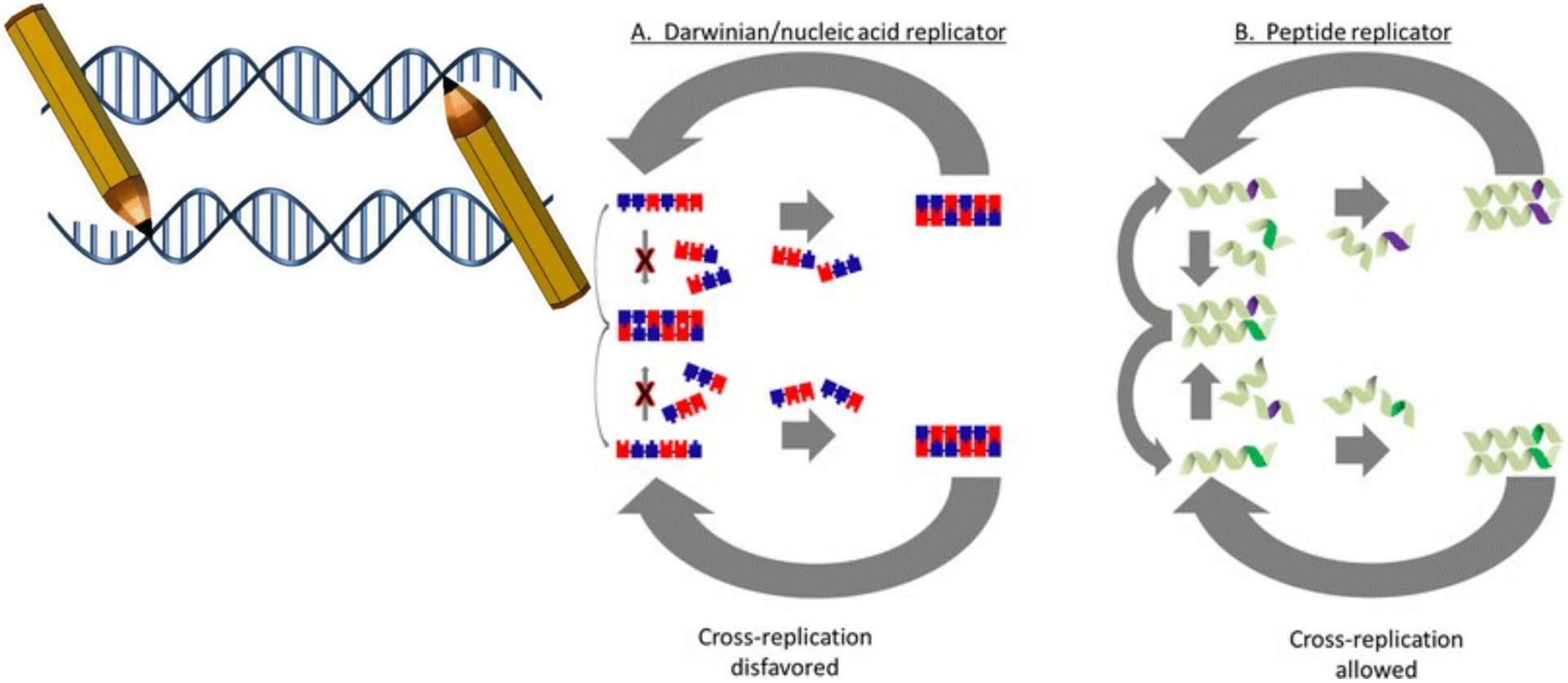
Inside living cells



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

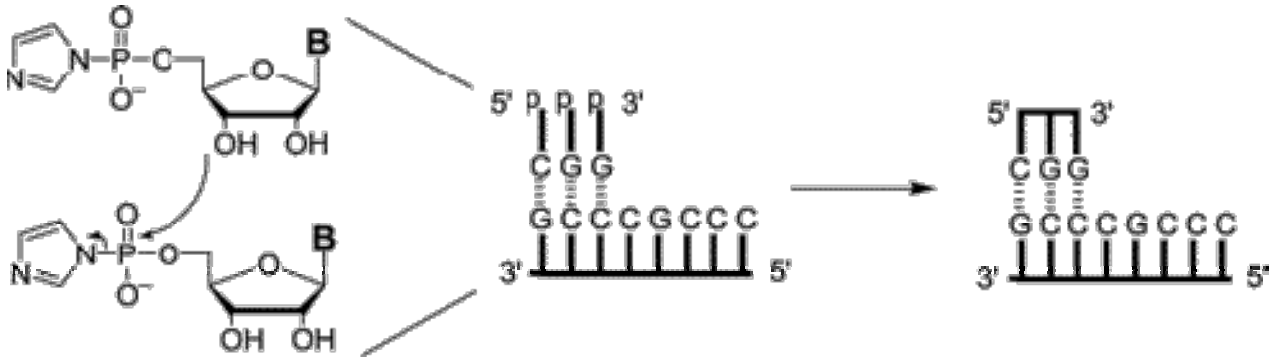
Abiotic self-replication



(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.

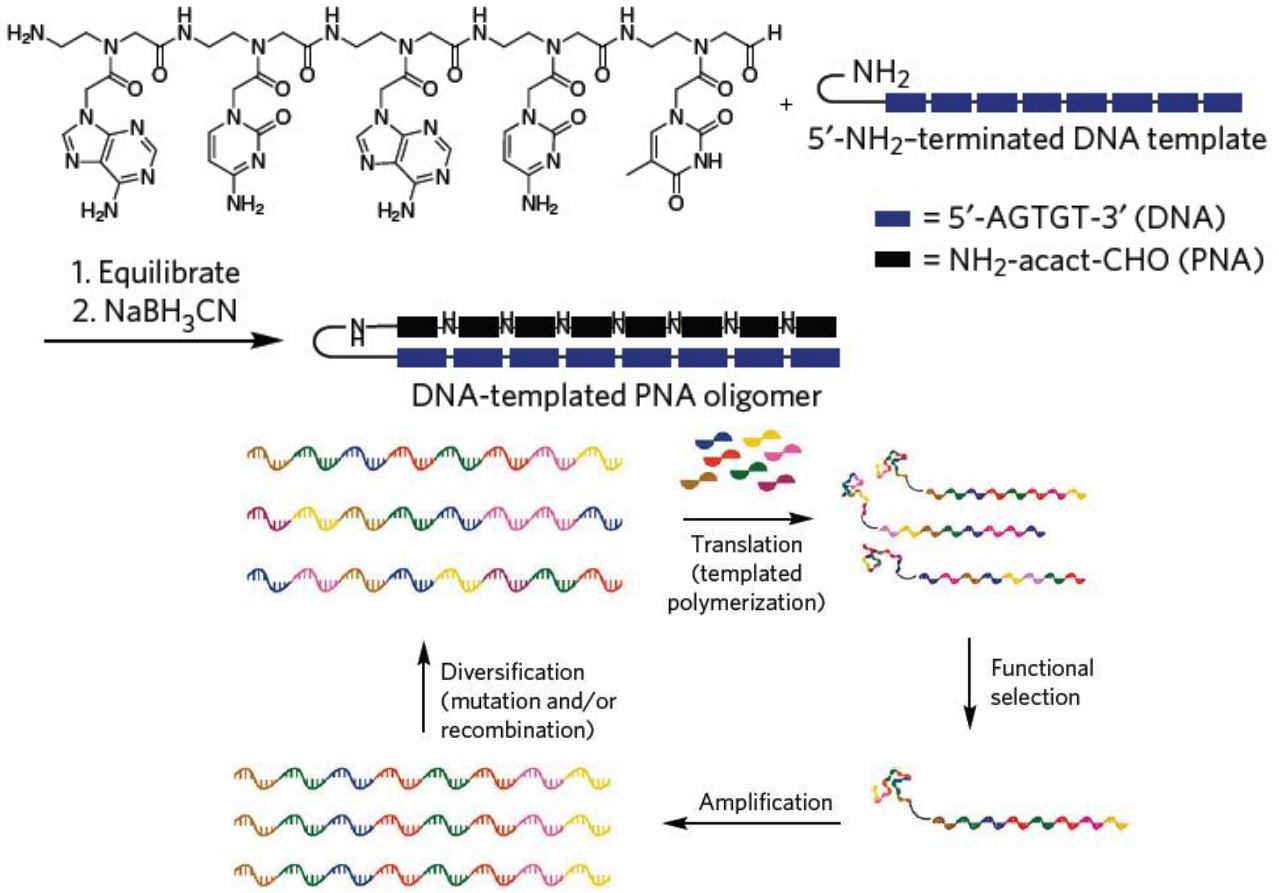
Nonenzymatic templated nucleic acid synthesis – monomer/short oligomer



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

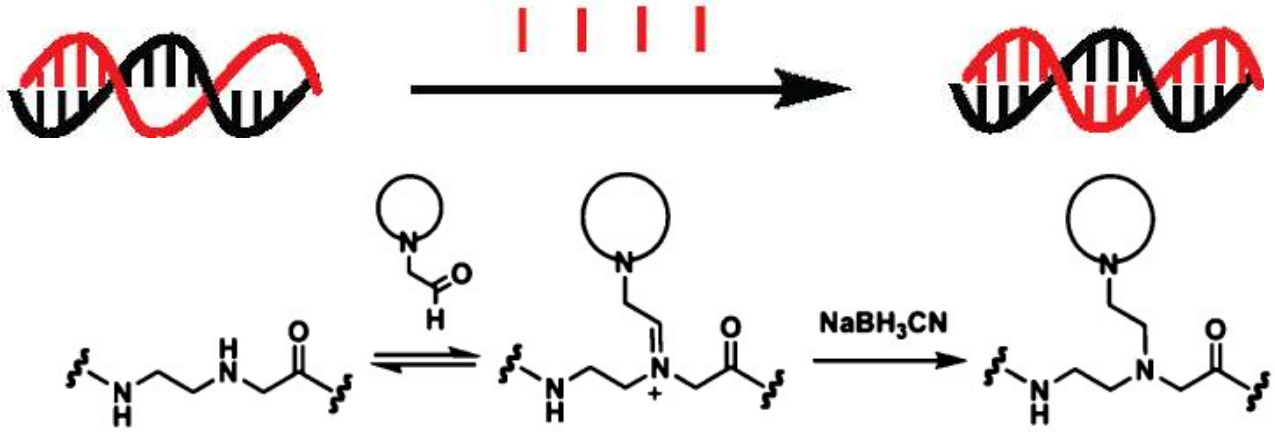


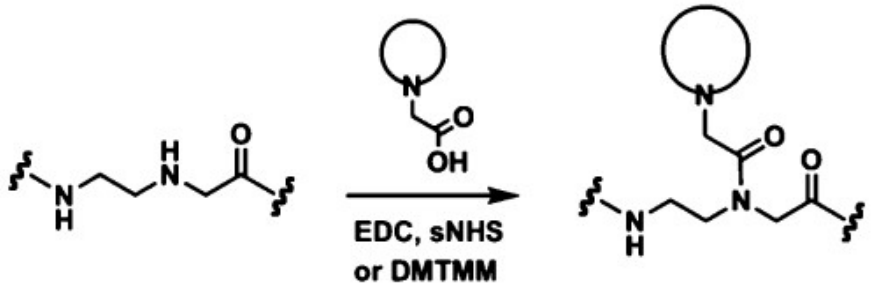
Limitations:

- 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

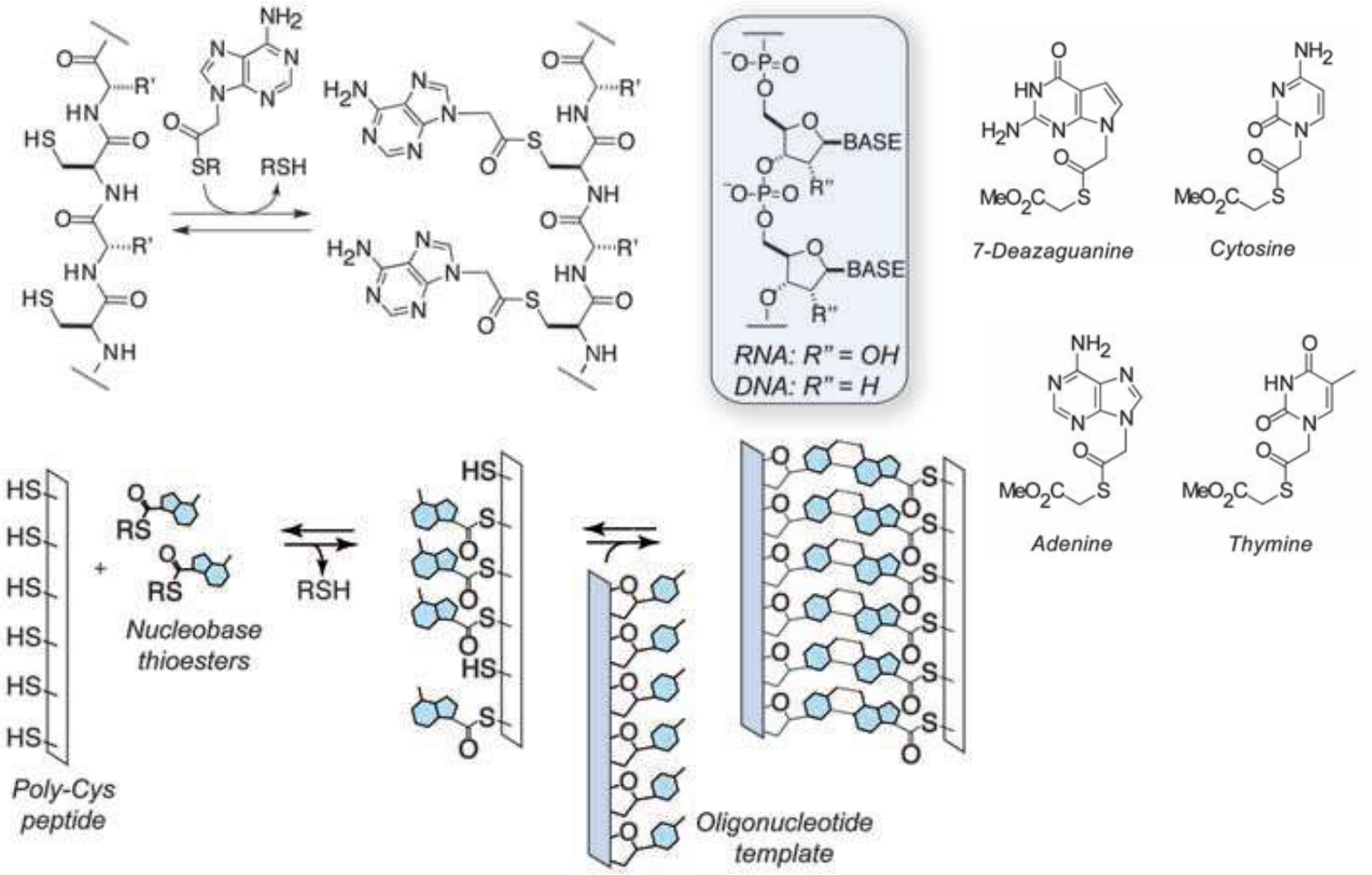
Limitations:

-single or double abasic sites (most efficient inside of the chain)

- Aldehydes give better yields and accuracy, but worse hybridization of the product

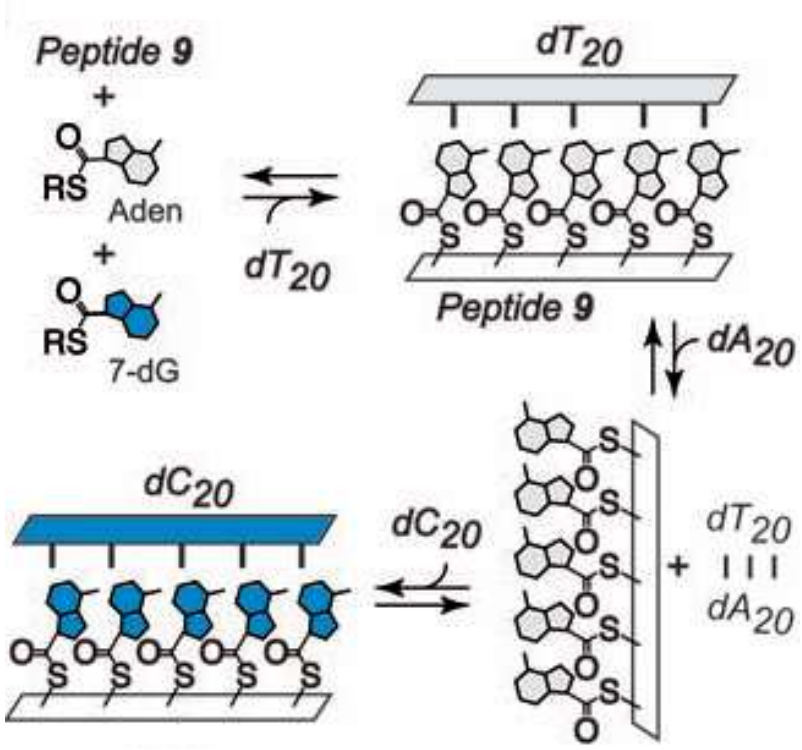
Heemstrra JM, Liu DR. *J. Am. Chem. Soc.* **2009**, *131,* 11347-11349.

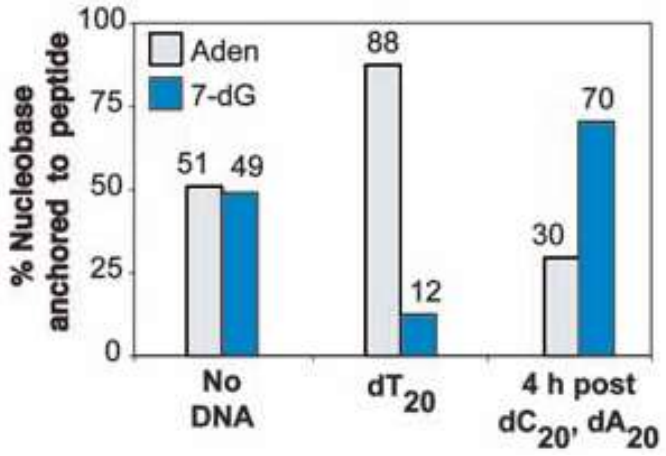
A polyamide responsive to selection pressure



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

A polyamide responsive to selection pressure

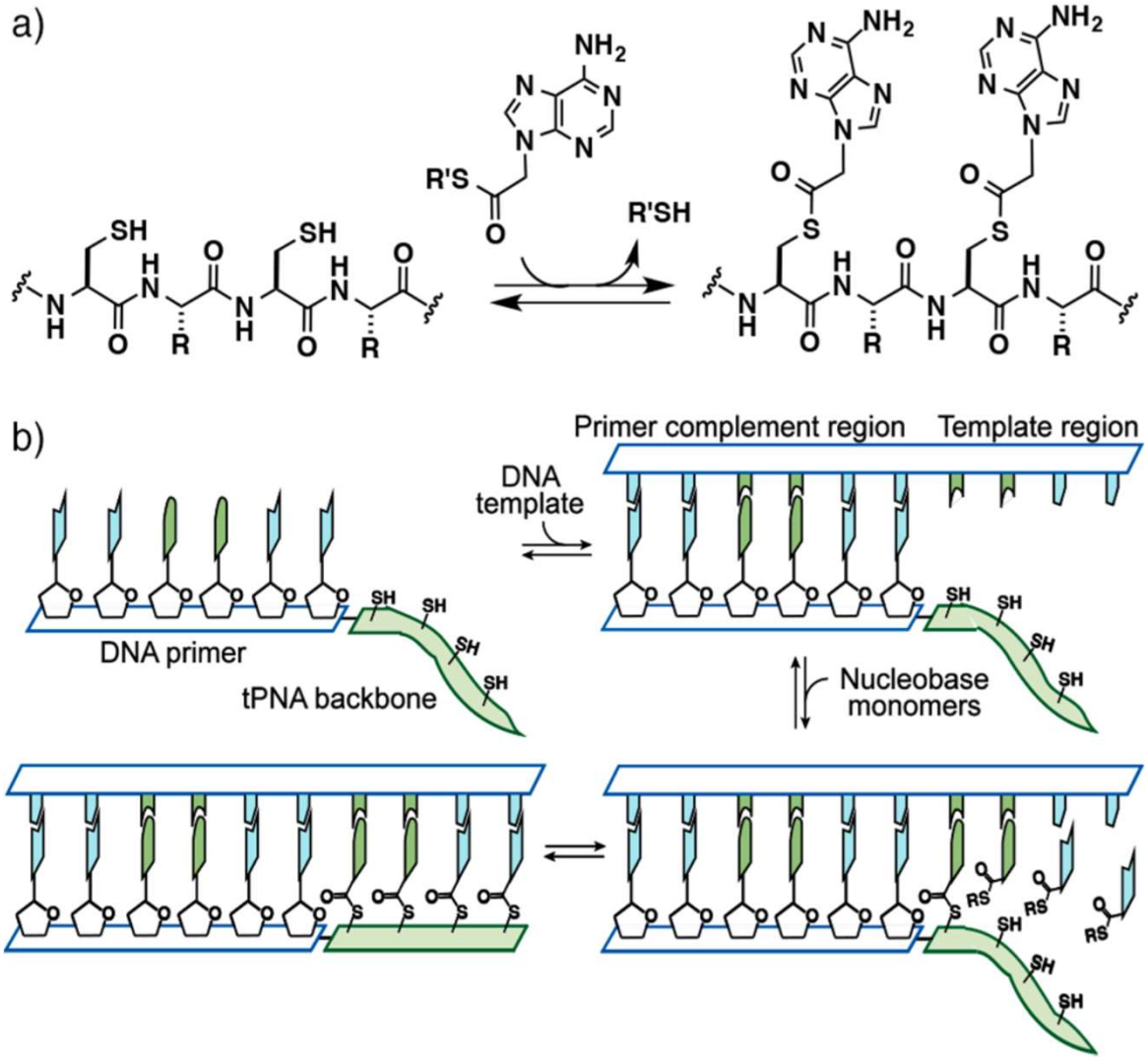




Dynamic polymer responsive to template changes with high fidelity

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

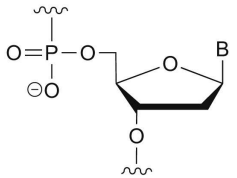
Templated Self-Assembly of Dynamic Peptide Nucleic Acids



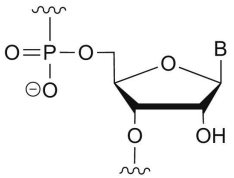
The DNA primer region affords a high level of control over the location and register of the tPNA backbone in relation to the template strand.

Beierle J, Ura Y, Ghadiri MR., Leman L *Biochemistry* **2018**, *57, 1,* 160–172

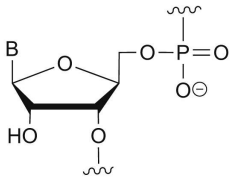
Artificial genetic polymers



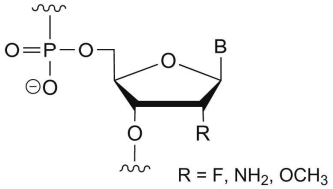
DNA



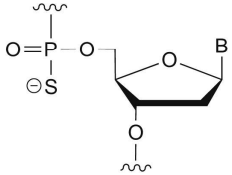
D-RNA
(natural)



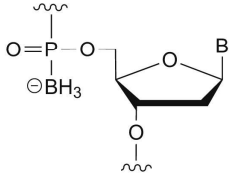
L-RNA
(unnatural)



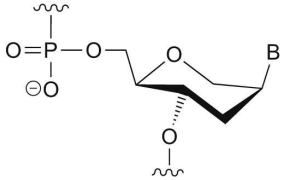
2'-modified RNA



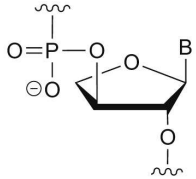
Phosphorothioate



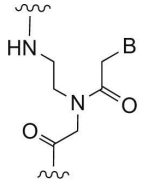
Boranophosphate



Hexitol Nucleic Acid
(HNA)



Threose Nucleic Acid
(TNA)



Peptide Nucleic Acid
(PNA)