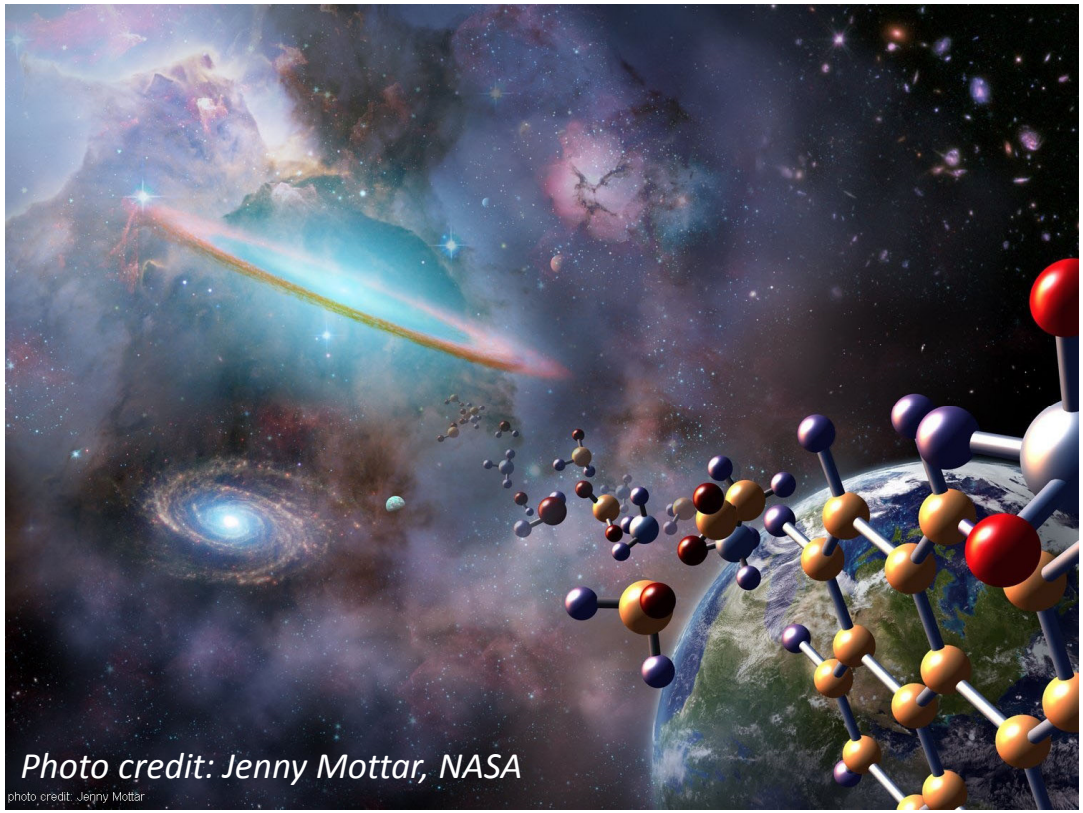
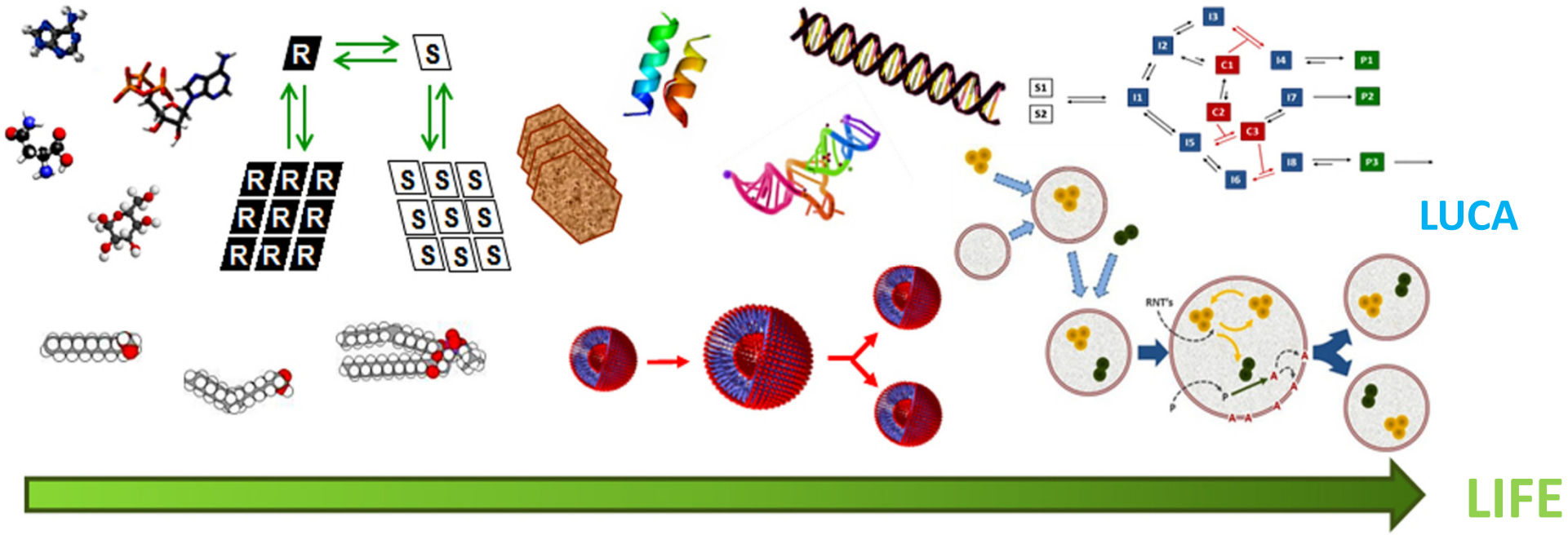
The molecular origins of life



Lecture 6, SoSe 2018 HD

Zbigniew Pianowski

Self-organization of molecules and chemical reactions



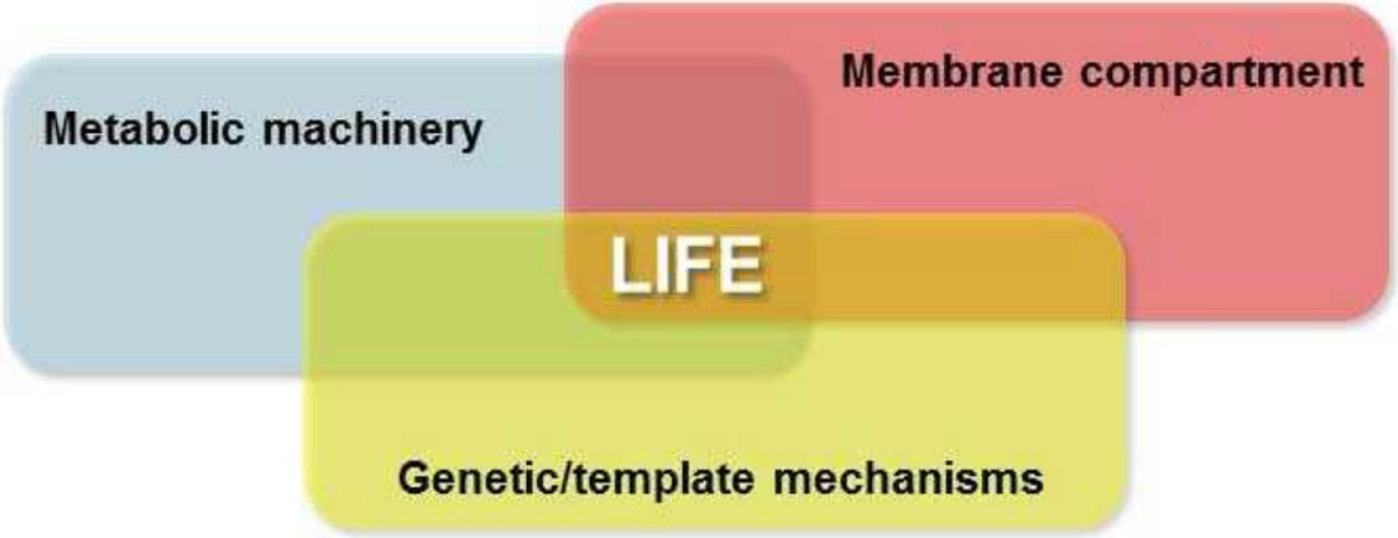
Increasing complexity from molecules to systems

Origin of the Universe – stars, planets, elements

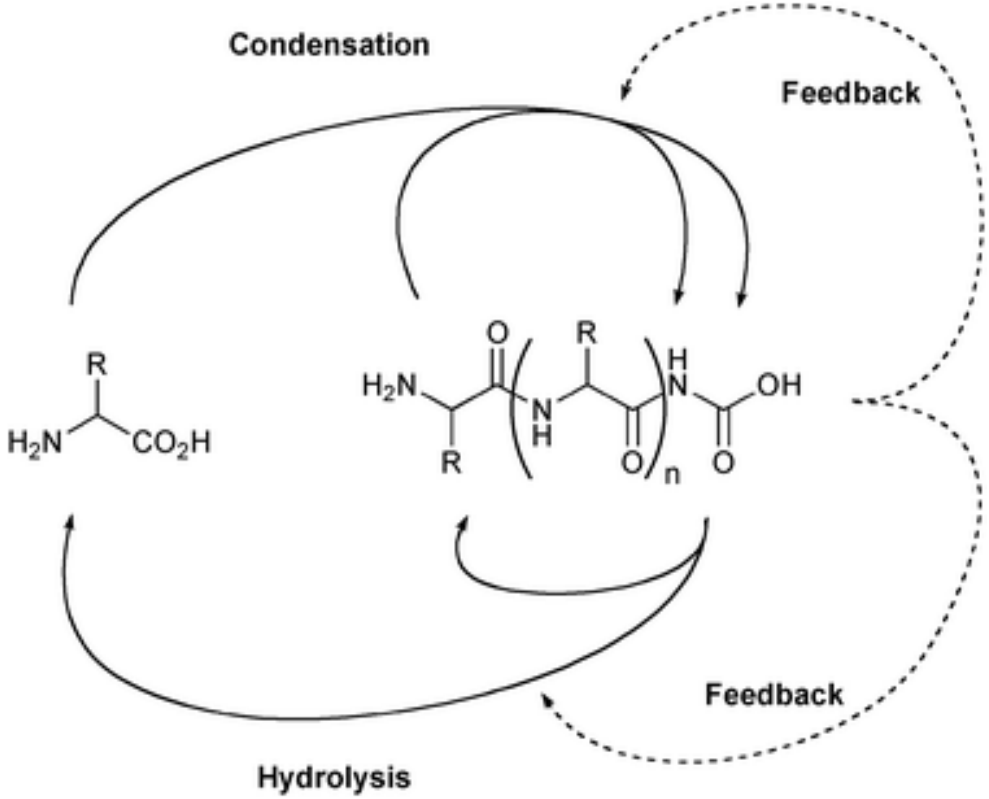
Origin of biorelevant monomers – primordial soup

Complex chemical processes on the way to living systems

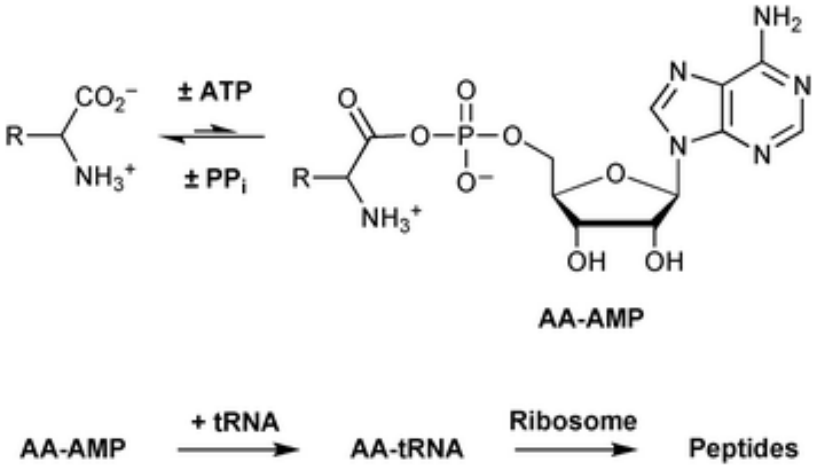
Protocells and LUCA

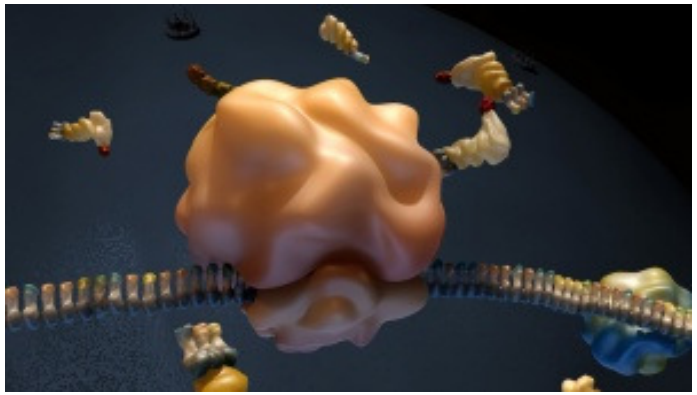

Membrane compartment
Metabolic machinery
LIFE
Genetic/template mechanisms

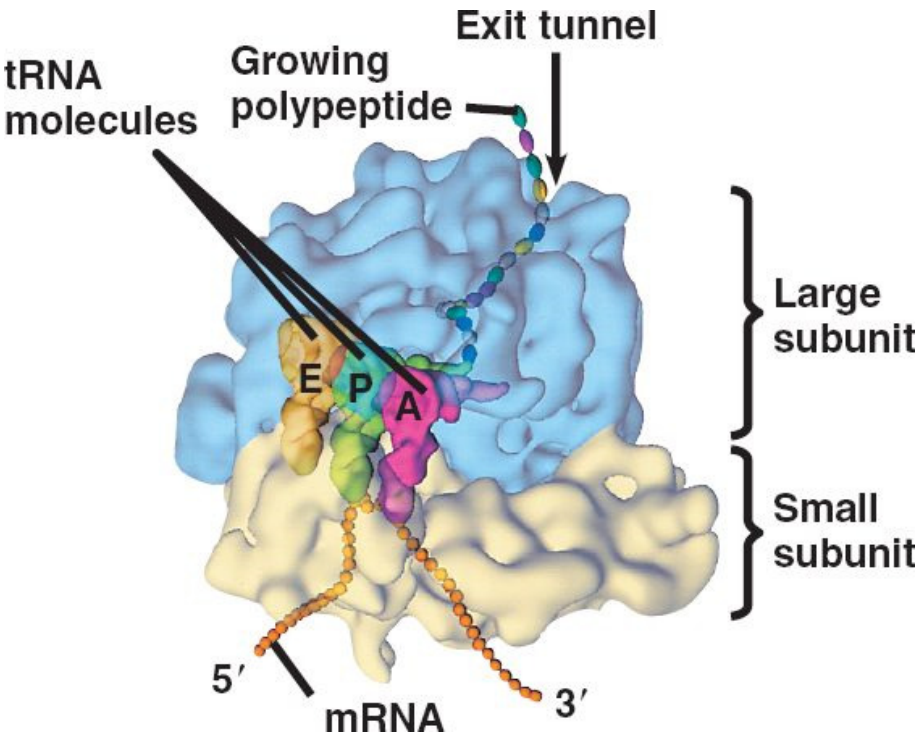
Condensation of aminoacids into peptides



Biochemical condensation of aminoacids into peptides

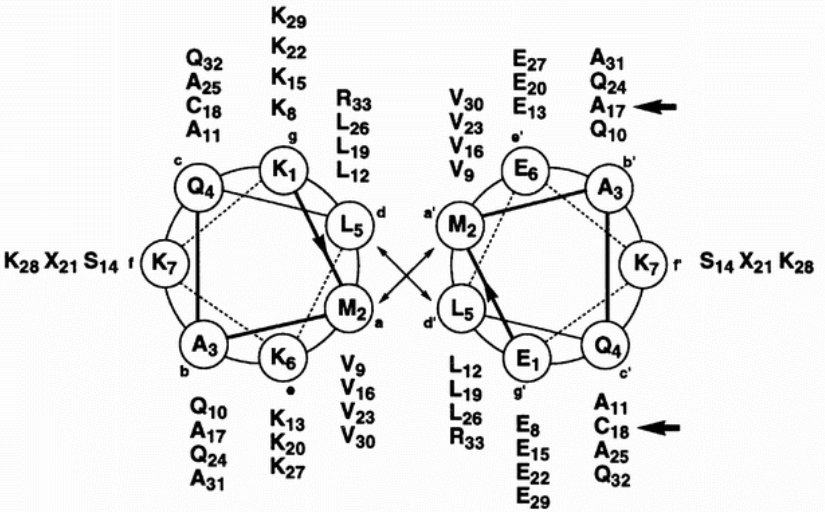




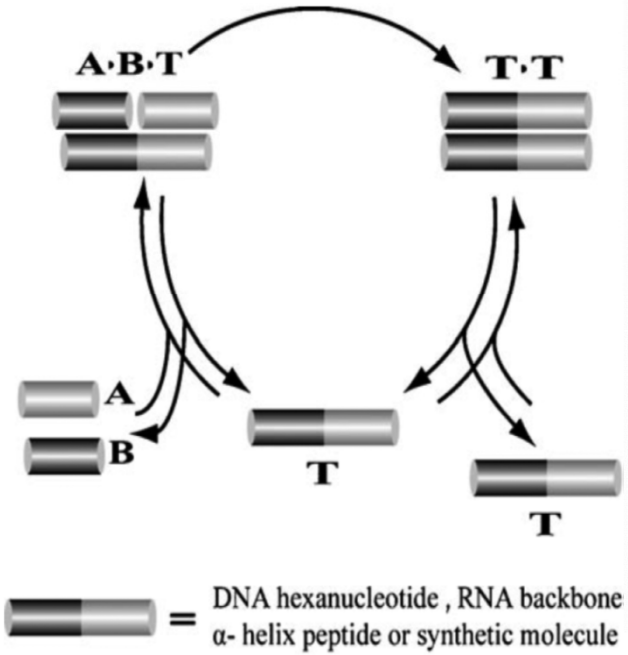


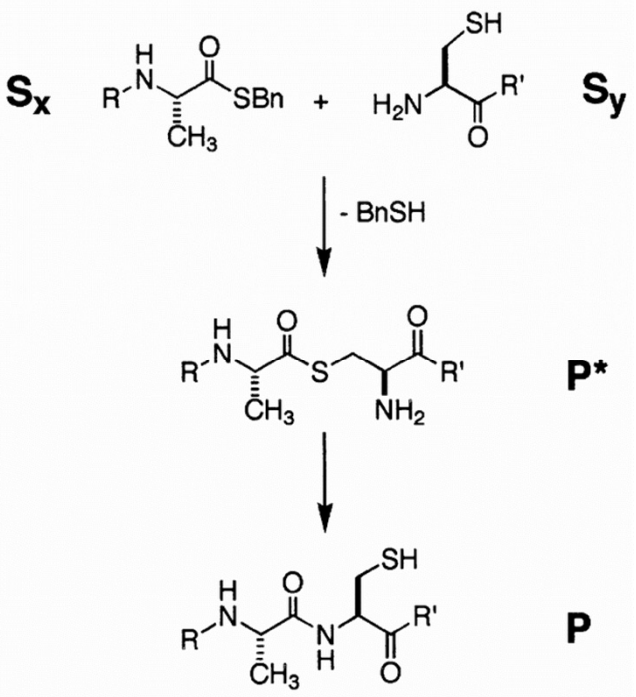
Nature Publishing Group, www.nature.com/nrg/multimedia

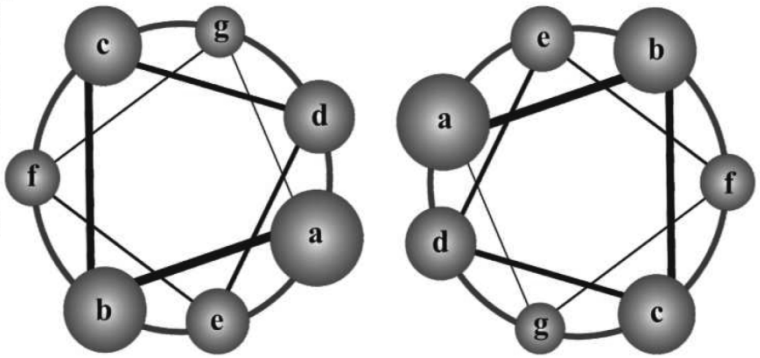
Peptide self-replication



E: Ar-**K**MAQL**KKK**VQAL**KSK**VA-CL**KXK**VQAL**KKK**VAQR-CONH$_2$
E*: Ar-**K**MAQL**KKK**VQAL**KSK**VA-SL**KXK**VQAL**KKK**VAQR-CONH$_2$
S$_1$: Ar-**E**MAQL**E**K**E**VQAL**E**S**E**VA-COSBn
S$_2$: Ar-**E**MAQL**E**K**E**VQAL**E**S**E**VA-COS(CH$_2$)$_2$CONH$_2$
S$_3$: Ar-**E**MAQL**E**K**E**VQAL**E**S**E**VA-CONHOH
S$_4$: Ar-**R**MKQLEE**K**VYELLS**K**VA-COSBn
S$_5$: Ar-**K**MAQL**KKK**VQAL**KSK**VA-COSBn
S$_6$: H$_2$N-CL**E**X**E**VQAL**E**K**E**VAQR-CONH$_2$
S$_7$: H$_2$N-CL**E**X**E**VARL**KK**LVGE-CONH$_2$
S$_8$: H$_2$N-(*d*-C)L**E**X**E**VQAL**E**K**E**VAQR-CONH$_2$
S$_9$: H$_2$N-GL**E**X**E**VARL**KK**LVGE-CONH$_2$



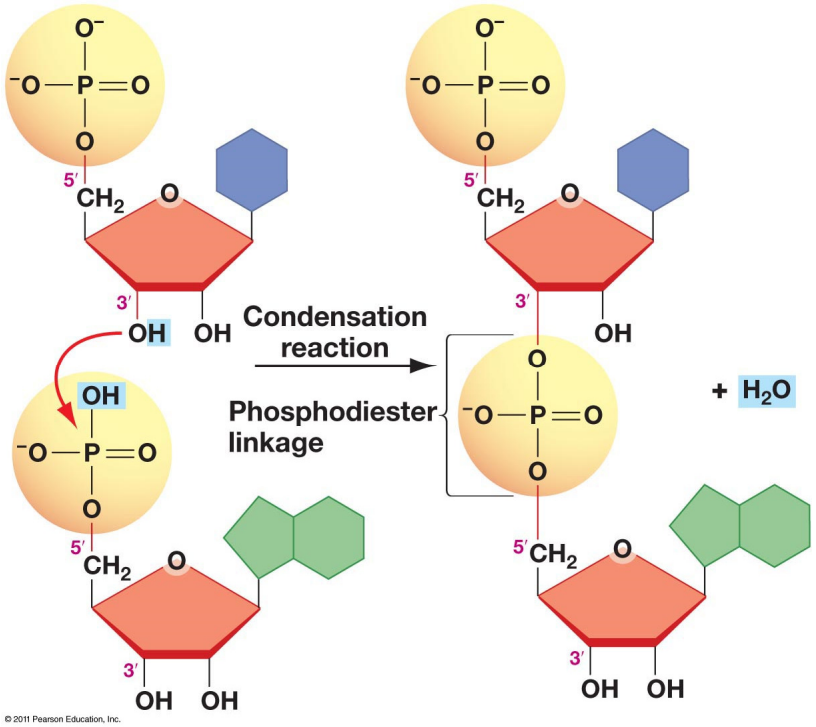




K. Severin, D. H. Lee, A. J. Kennan and M. Reza Ghadiri *Nature* **1997**, *389*, 706-709

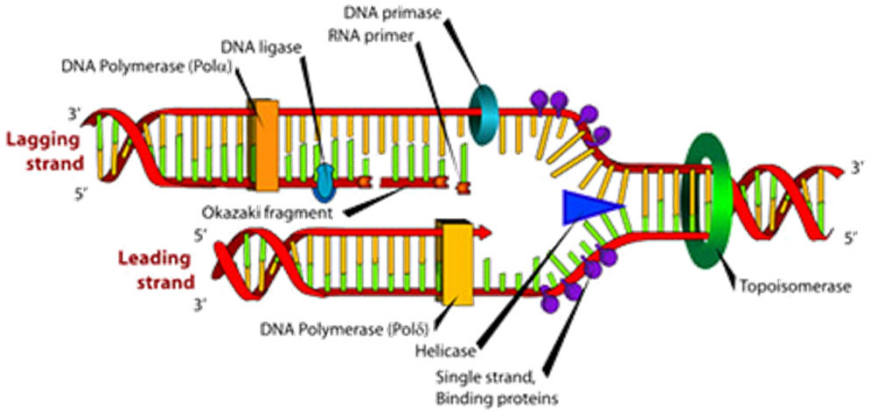
Nucleotide polymerization

Regioselective formation of 3’-5’ phosphodiester bonds between nucleotides

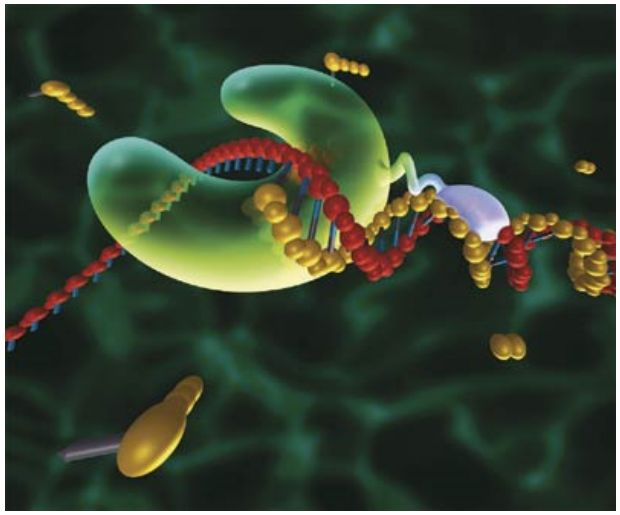


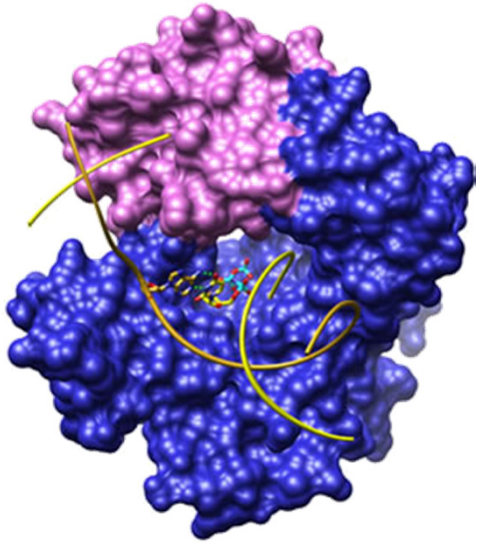
Vital chemical reactions

nucleotide polymerization → DNA/RNA polymerases

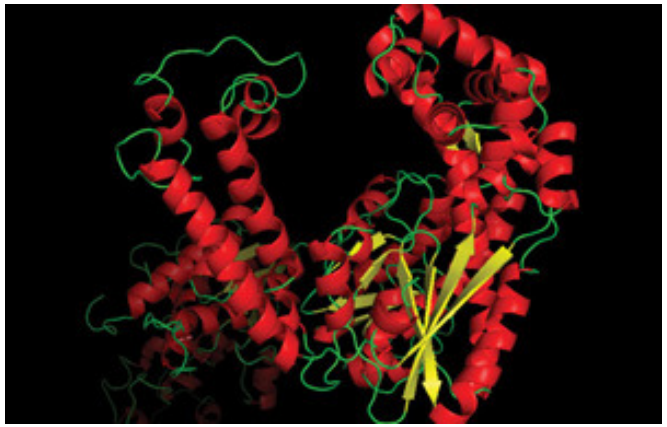


dxline.info/img/new_ail/dna-polymerase_1.jpg



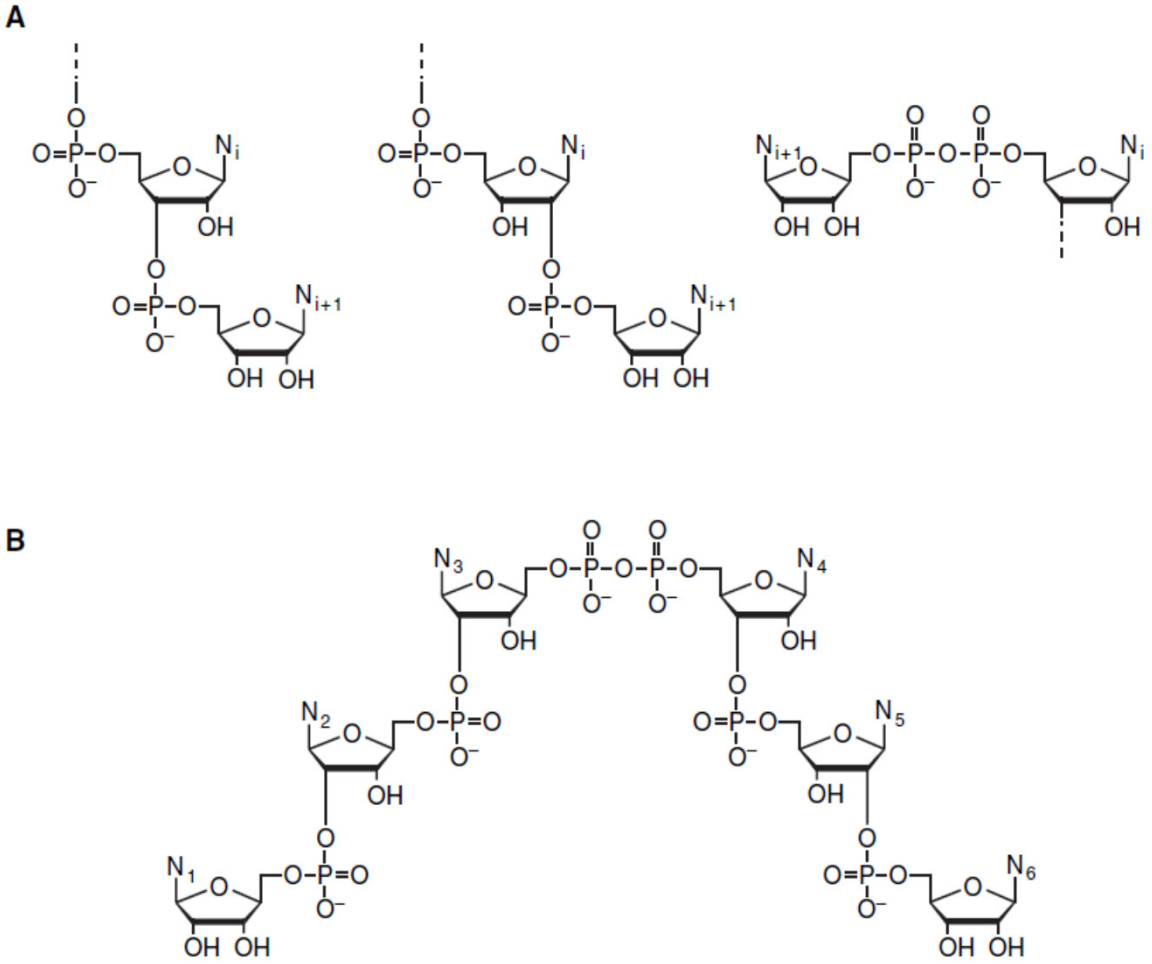


niehs.nih.gov



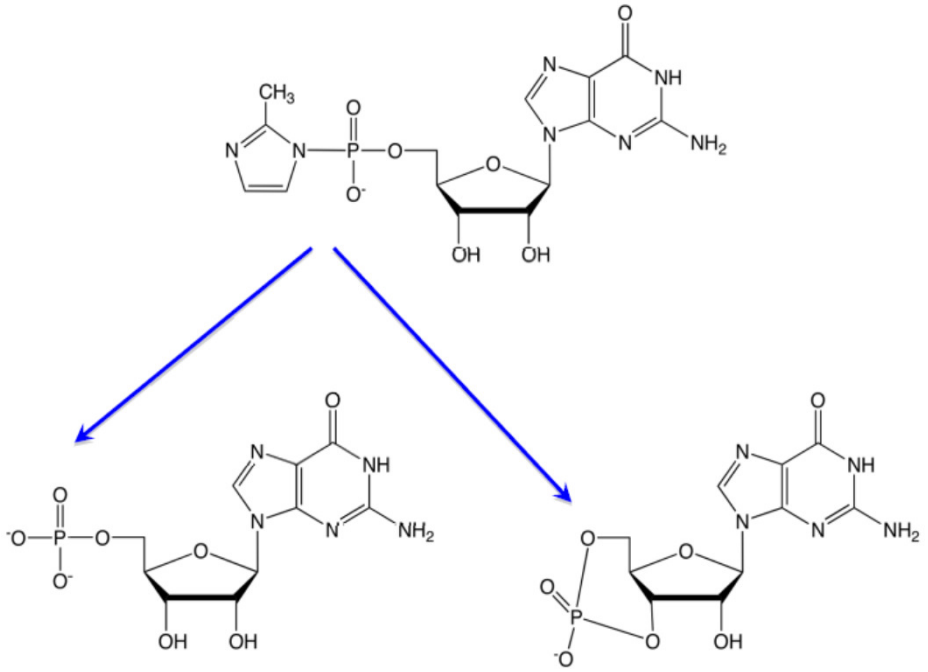
www.neb.com

Products of chemical condensation of nucleotides



(**A**) Reaction of an activated mononucleotide (N_{i+1}) with an oligonucleotide (N_1–N_i) to form a 3',5'-phosphodiester (left), 2',5'-phosphodiester (middle), or 5',5'-pyrophosphate linkage (right).
(**B**) Typical oligomeric product resulting from chemical condensation of activated mononucleotides

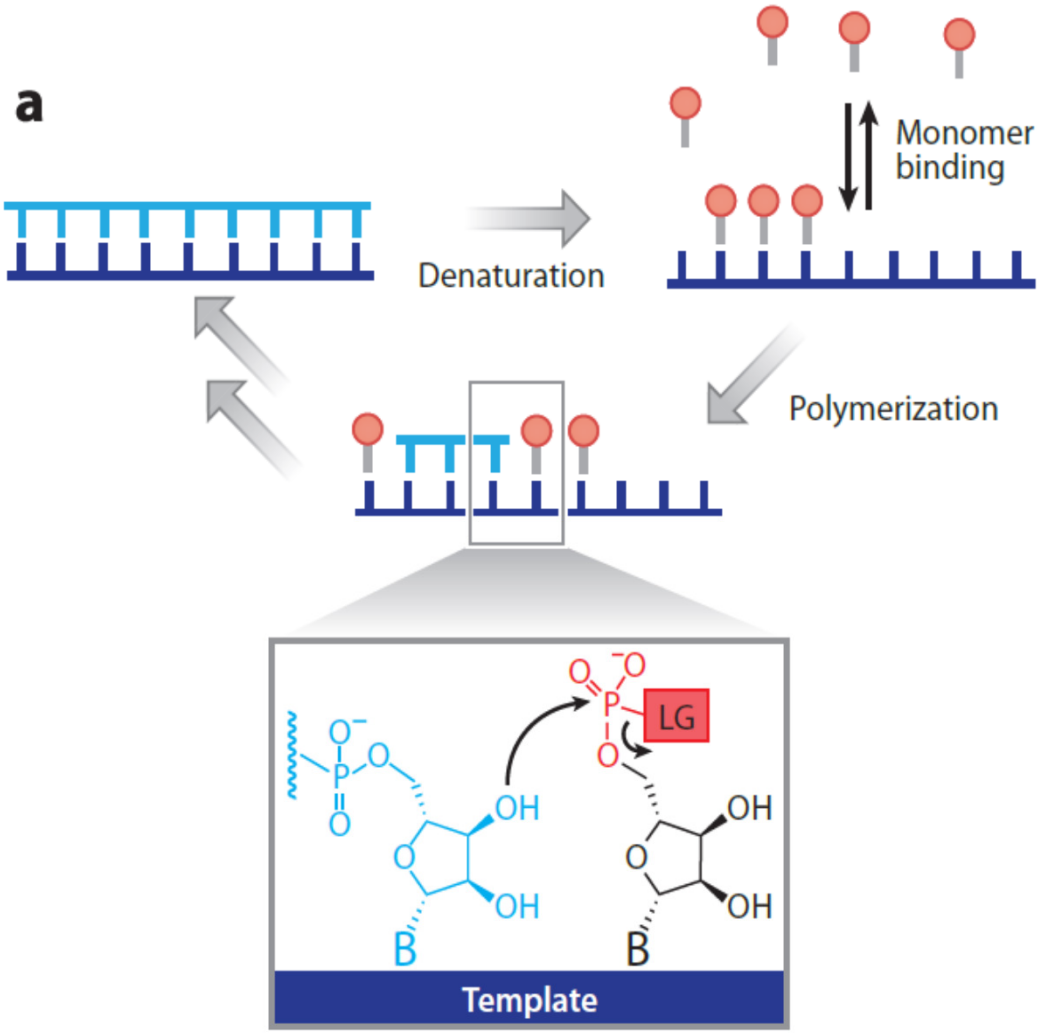
Degradation of activated nucleotides



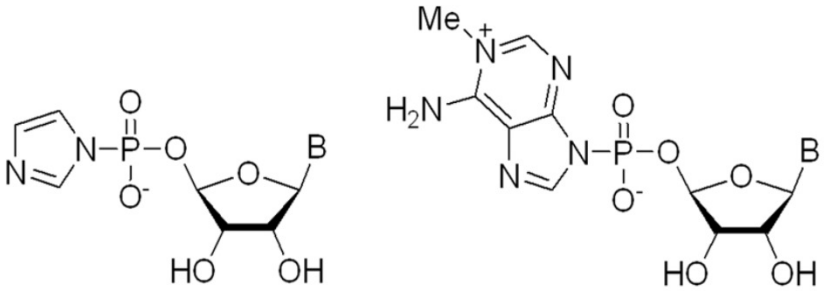
hydrolysis

3’,5’-cyclization

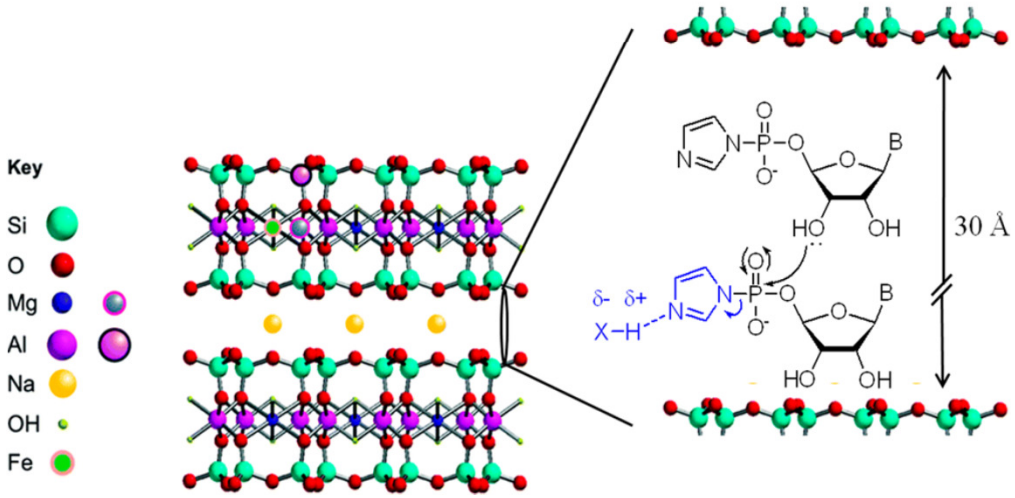
Template-directed synthesis



Montmorillonite



B = adenine, guanine, cytosine or uracil

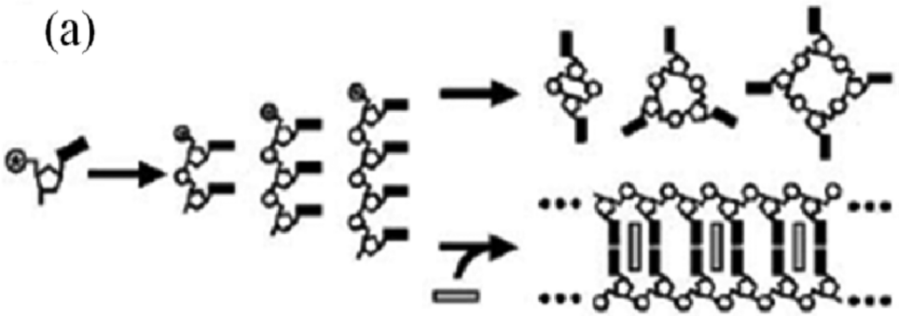


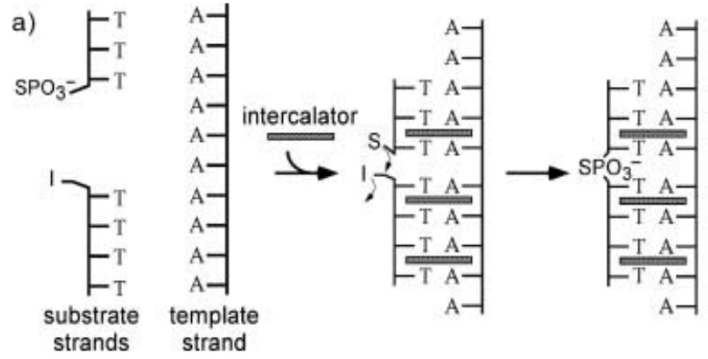
30-50 units successfully oligomerized

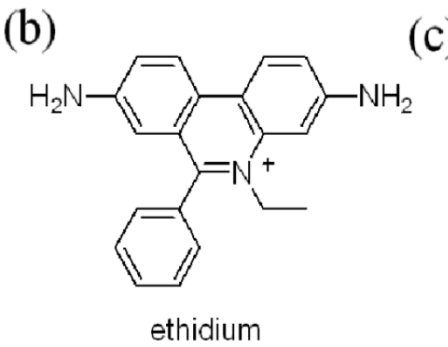
(Top) Structure of ribonucleotide 5′-phosphoimidazolides (left) and ribonucleotide 5′-phosphoro-1-methyladeninium (right). (Bottom) Unit cell of montmorillonite and phosphodiester bond formation within the clay interlayers, as proposed by Ferris and coworkers (right). XH, depicted in blue in the cartoon, is any undifferentiated protic species inside the clay galleries.

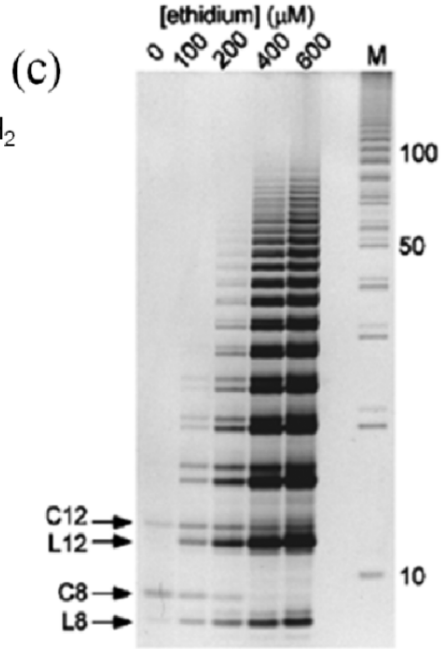
Joshi, P. C.; Aldersley, M. F.; Delano, J. W.; Ferris, J. P. *J. Am. Chem. Soc.* **2009**, *131*, 13369

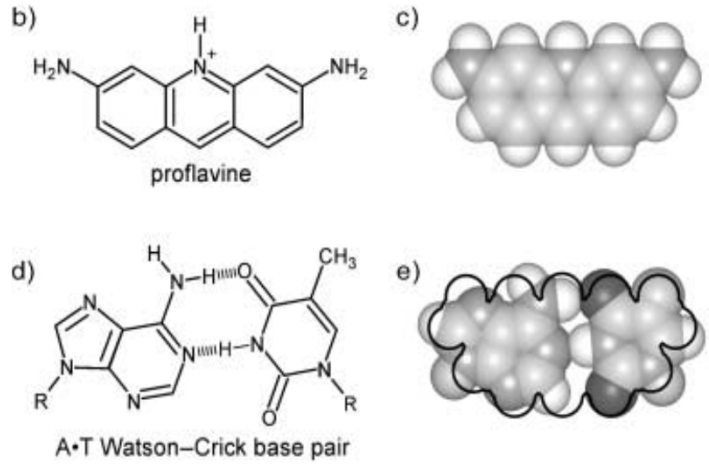
Intercalating agents









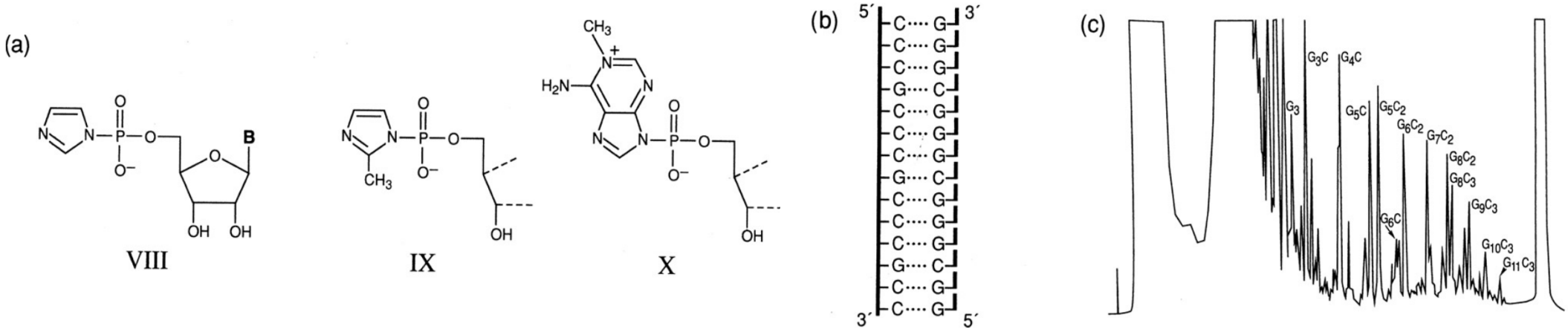


Rate increase by three orders of magnitude vs. ligation without proflavine

N. V. Hud *et al. Angew. Chem. Int. Ed.* **2004**, *43*, 2004 –2008

Template-directed synthesis

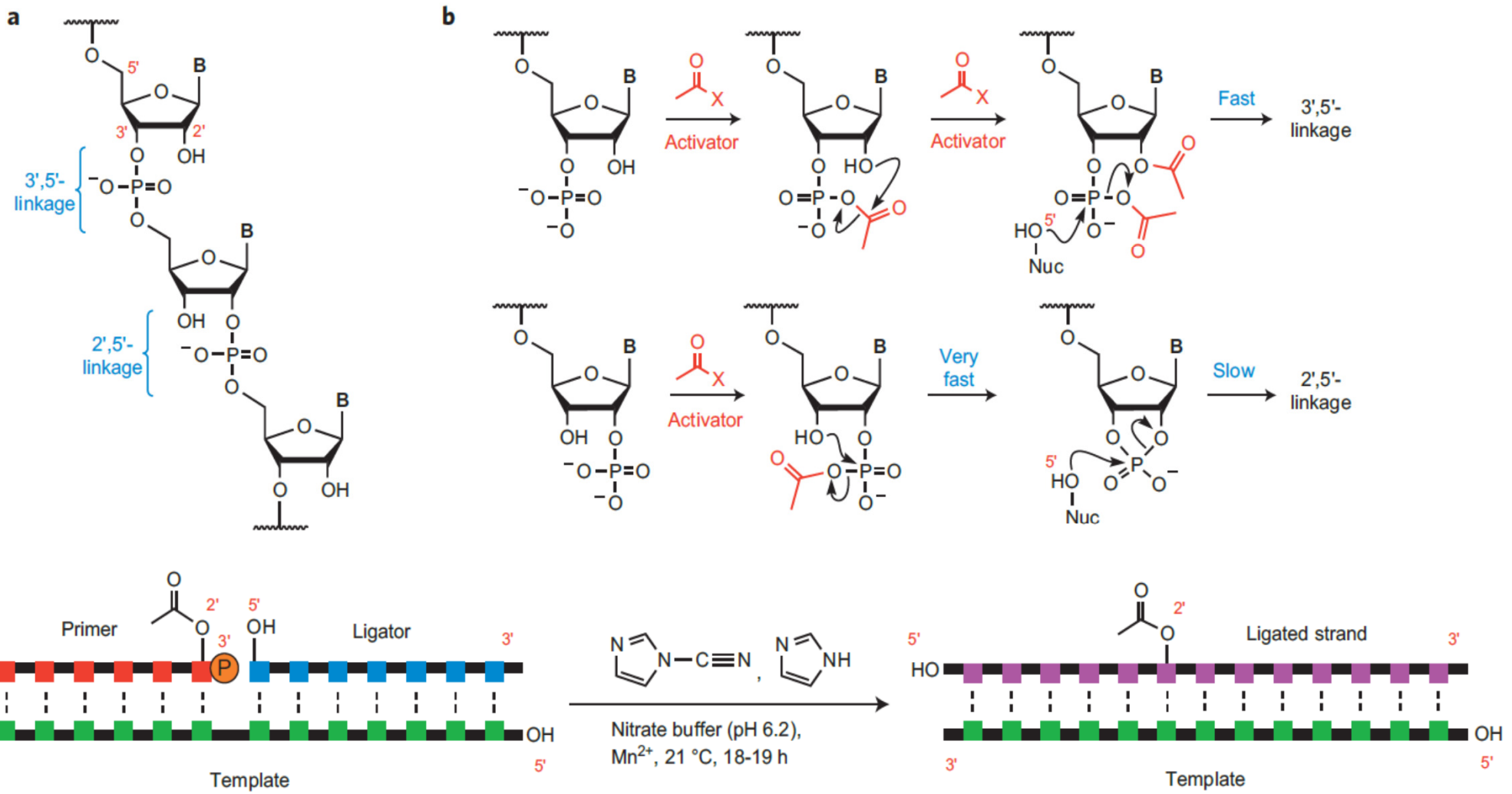
no example demonstrated yet, where single activated nucleotides would form a complementary strand on an RNA (or DNA) template without enzymatic support



Current experiments focus on ‚primer extention' or ‚filling abasic sites'– sequence-selective complementary nucleobase addition to a pre-existing strand (or between two pre-existing strands) already hybridized on a template. Here, pre-organization provided by the existing base-pairing network supports selection of the correct nucleoside to be joined.

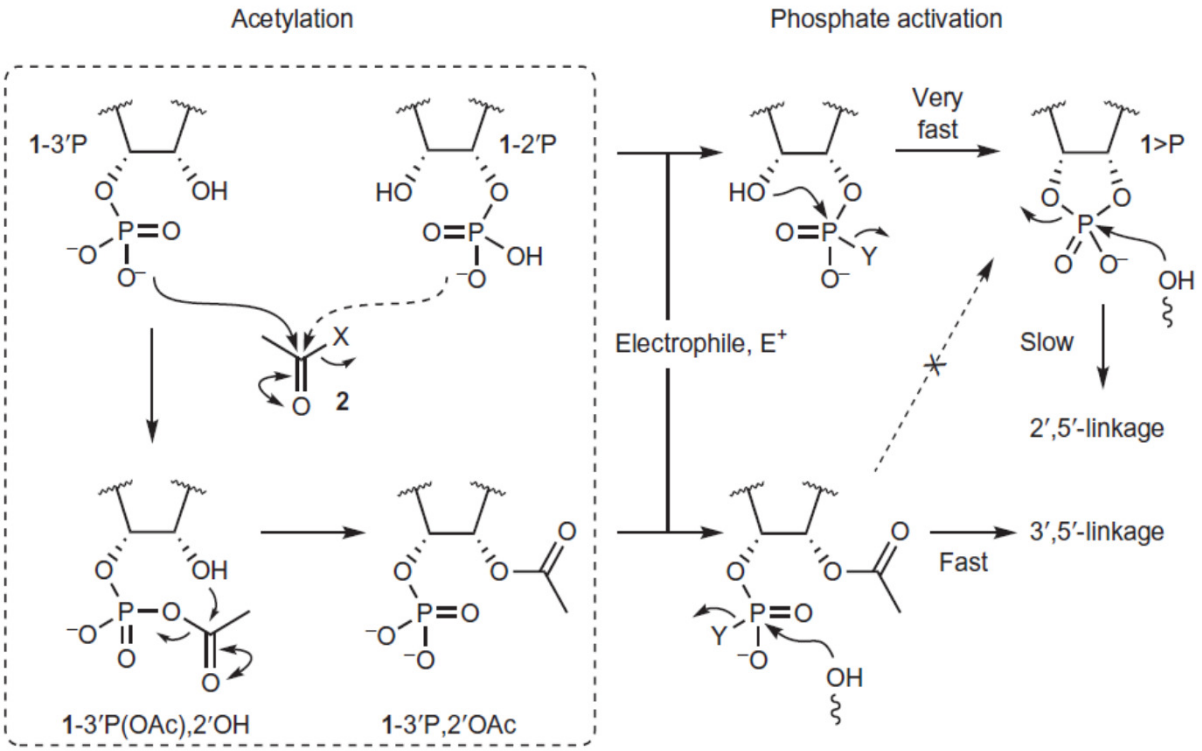
Complementary approaches are regioselective ligation reactions of short oligonucleotides on templates, or dynamic covalent chemistry, where nucleobase-containing components would be added sequence-specifically to a pre-existing ‚empty' backbone on a template

Regioselective ribonucleotide ligation



J. Sutherland *et al. Nature Chem.* **2013,** 383-389

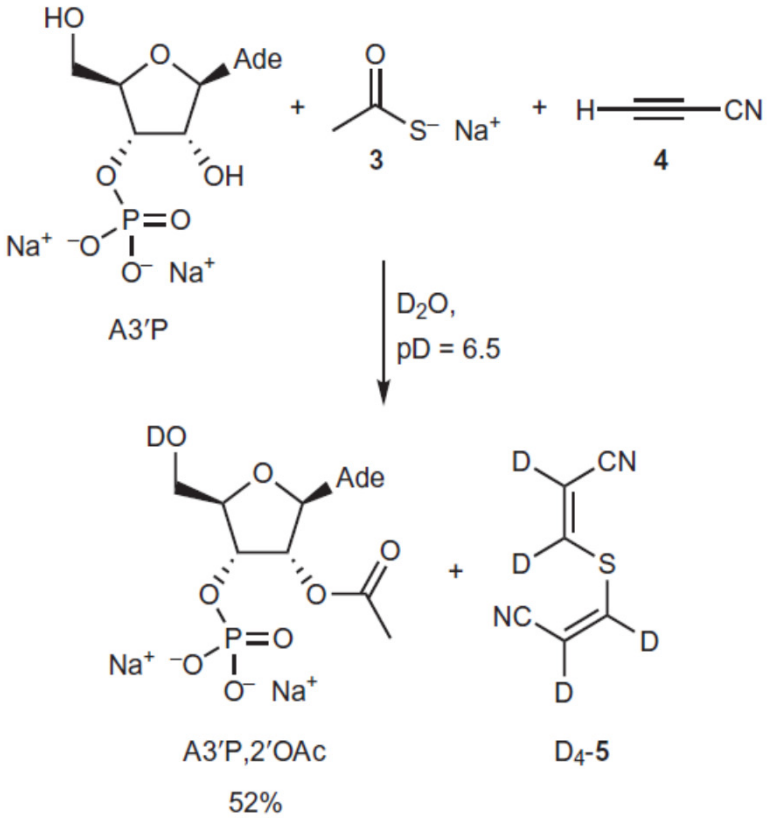
Regioselective ribonucleotide ligation



Protection of the 2'-OH group of **1**-3'P facilitates rapid template-directed 3',5'-ligation after electrophilic phosphate activation. The 3'-OH group of **1**-2'P is protected to a lesser extent, such that **1**>P is the major product of phosphate activation and slow template-directed 2',5' -ligation follows.
X = leaving group, Y = leaving group generated by electrophilic activation of phosphate oxygen with or without a subsequent nucleophilic displacement

J. Sutherland *et al. Nature Chem.* **2013,** 383-389

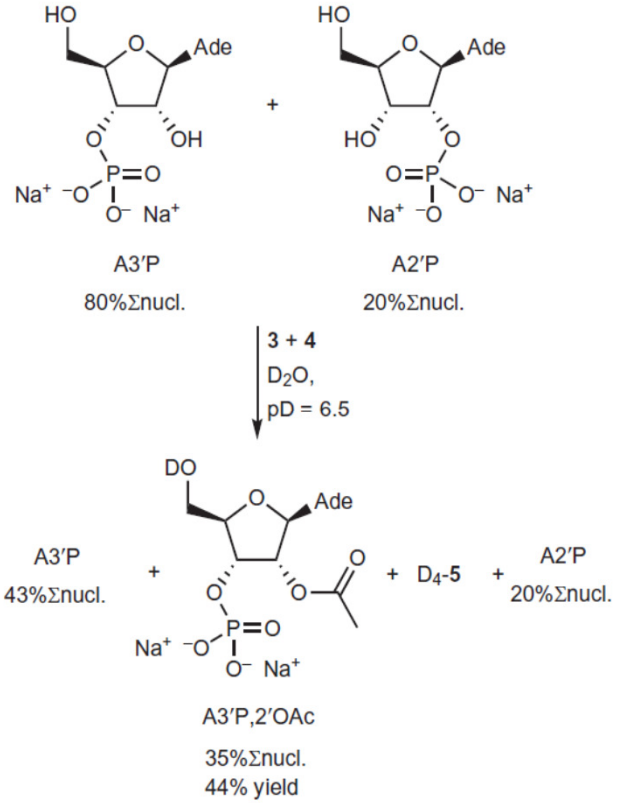
Regioselective ribonucleotide ligation

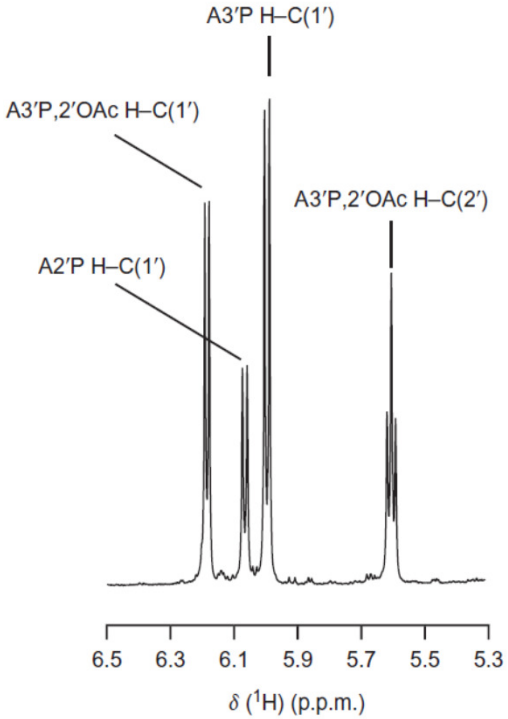


Treatment of adenosine-3'phosphate (A3'P) (100 mM) with sodium thioacetate 3 (100 mM) and cyanoacetylene 4 (200 mM) in D_2O at neutral pD for 24 hours results in selective acetylation of the 2-OH group.

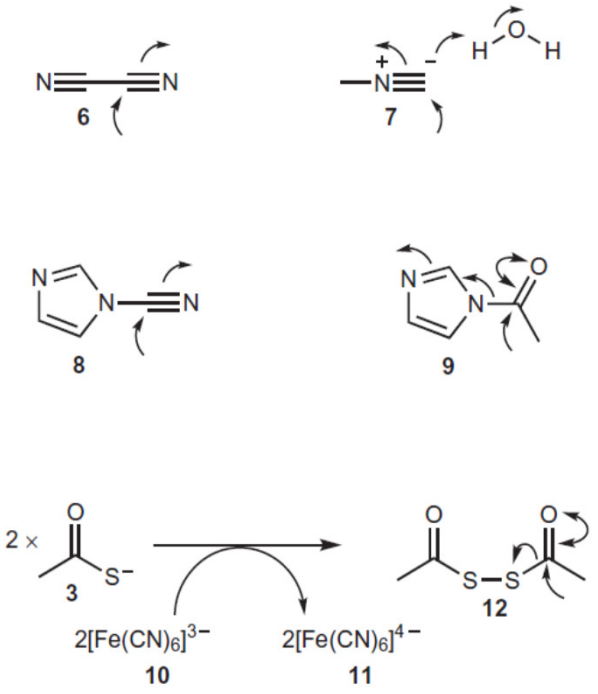
J. Sutherland *et al. Nature Chem.* **2013,** 383-389

Regioselective ribonucleotide ligation





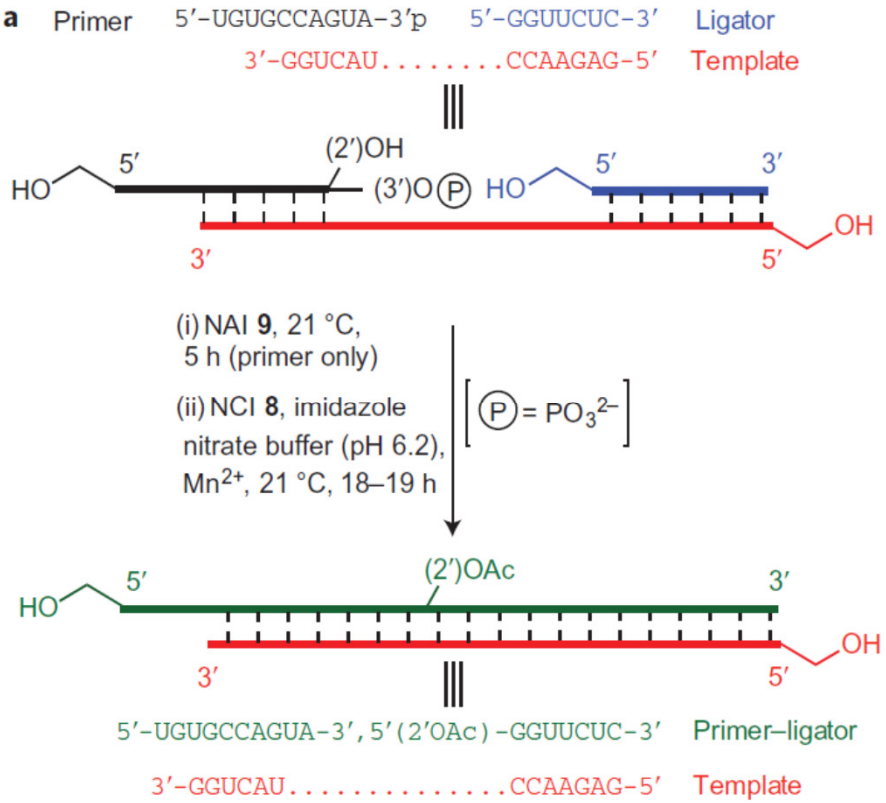
Treatment of A3'P (80 mM) and A2'P (20 mM) as given before results in the exclusive 2′-acetylation of the former nucleotide. Partial ^{1}H NMR spectrum of the reaction products.



Additional electrophiles 6–8 shown to drive the acetylation of ribonucleotides with thioacetate 3. Direct acetylation with 9 is also possible, as is oxidative activation of 3 with ferricyanide 10 to afford ferrocyanide 11 and a dimeric acetylating agent 12. Curly arrows indicate electrophilic activation/acetylation steps.

Regioselective ribonucleotide ligation

Chemoselective acetylation of 3'P-oligoribonucleotides expedites templated ligation



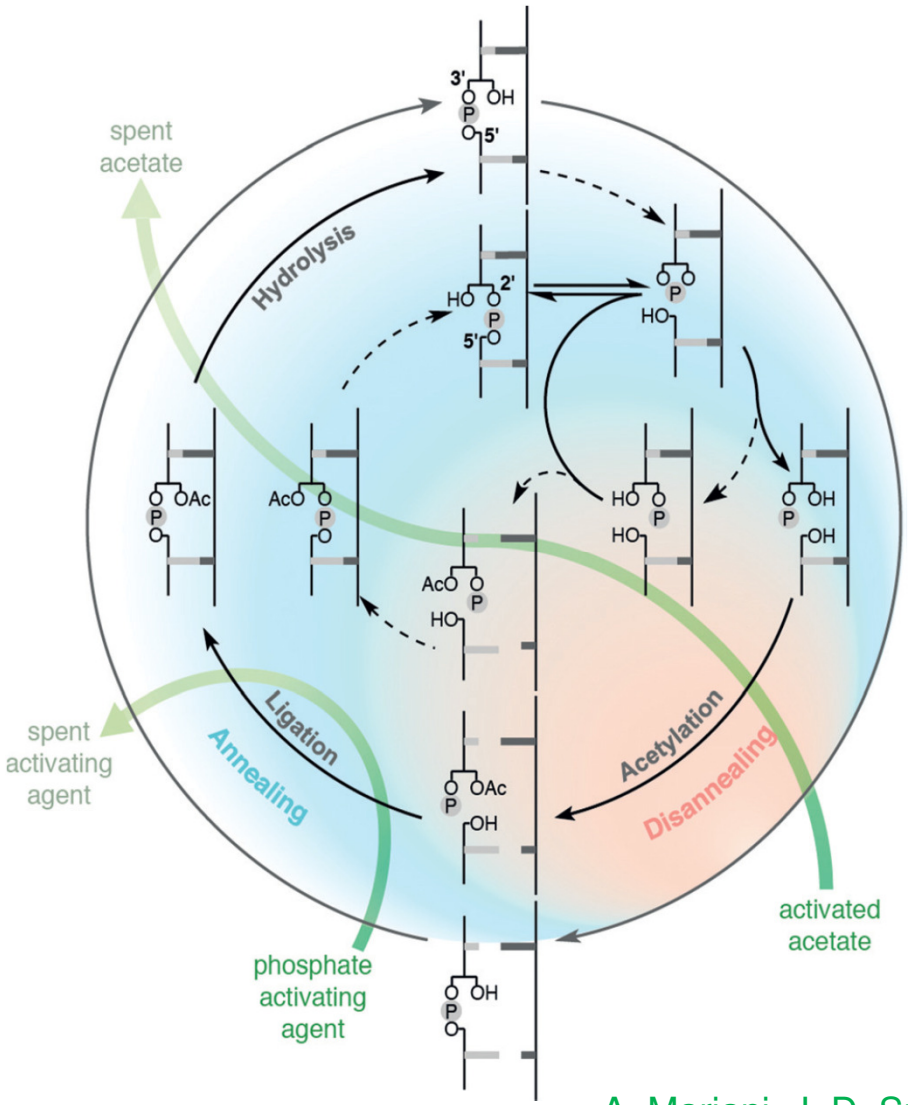
NAI – *N*-acetylimidazole

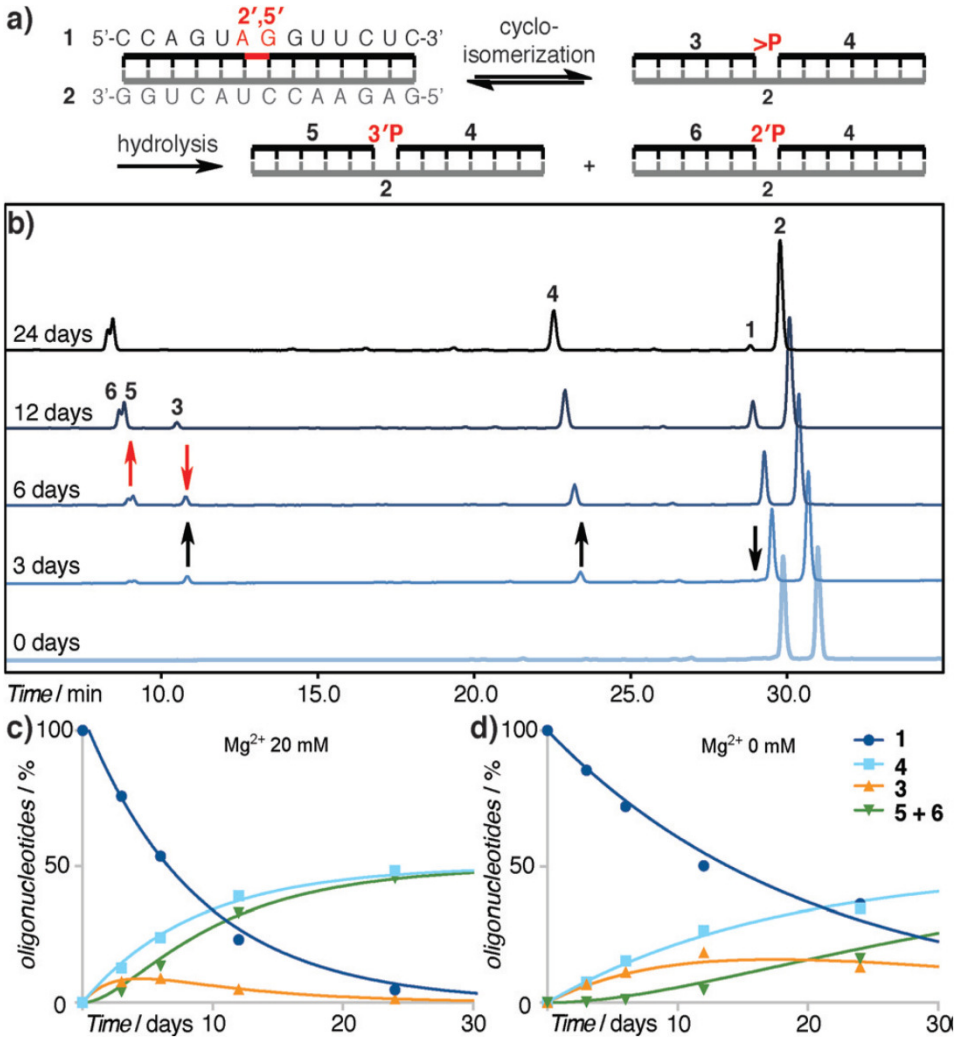
NCI – *N*-cyanoimidazole

Sequences and reaction conditions employed for acetylation (i) and subsequent templated ligation (ii). The acetylation mixture contained 80 mM primer and 50 mM NAI; the ligation mixture contained 4 mM primer from the acetylation reaction, 25 mM template, 30 mM ligator, 200 mM imidazole nitrate buffer (pH 6.2), 10 mM $MnCl_2$ and 100 mM NCI.

J. Sutherland *et al. Nature Chem.* **2013,** 383-389

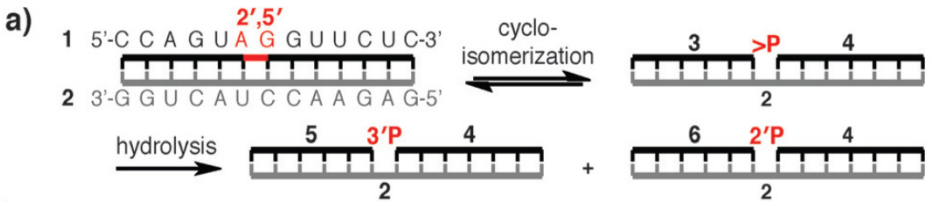
Correction mechanism 2'-5' → 3',5'



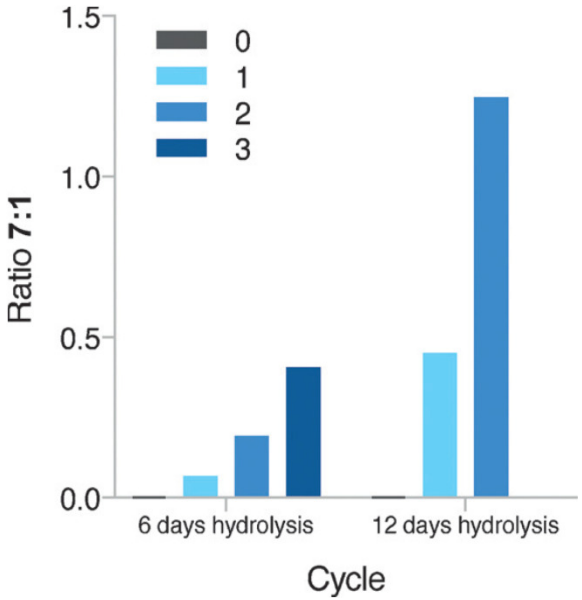


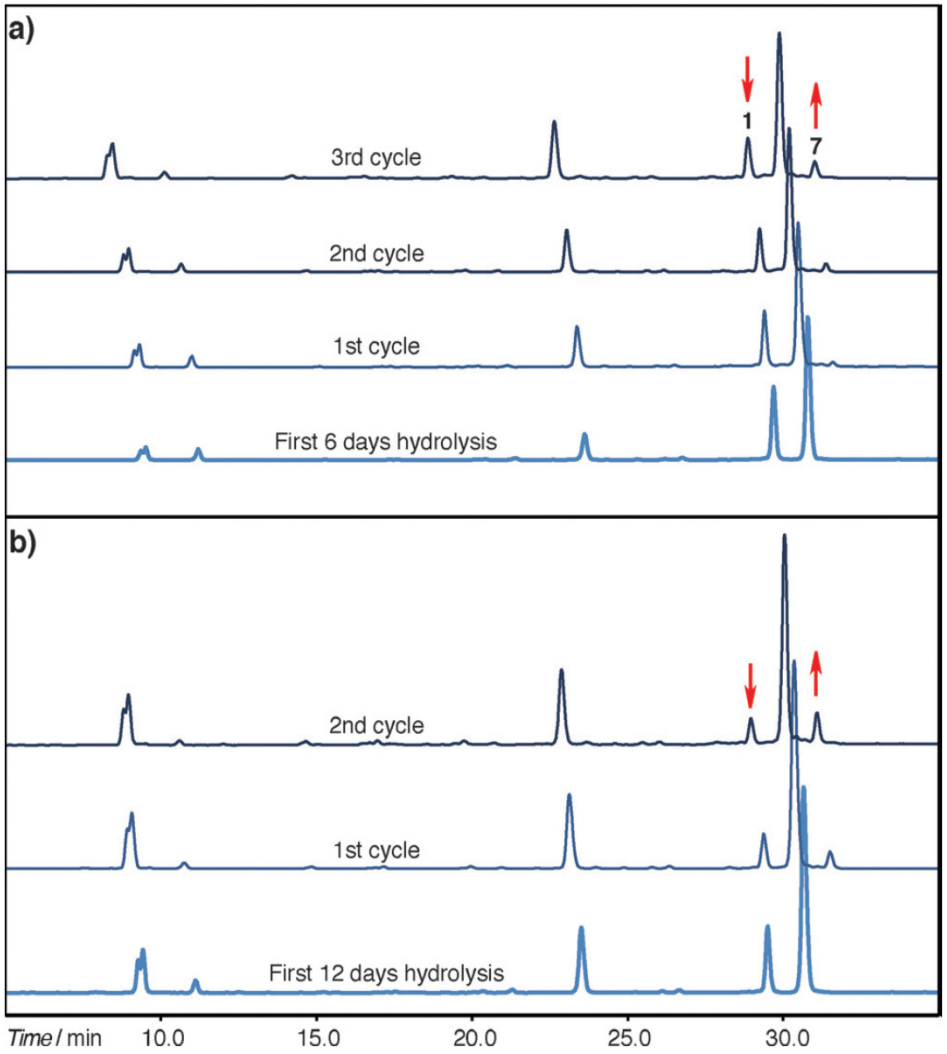
A. Mariani, J. D. Sutherland *Angew. Chem. Int. Ed.* **2017**, *56*, 6563-6566

Correction mechanism 2'-5' → 3',5'



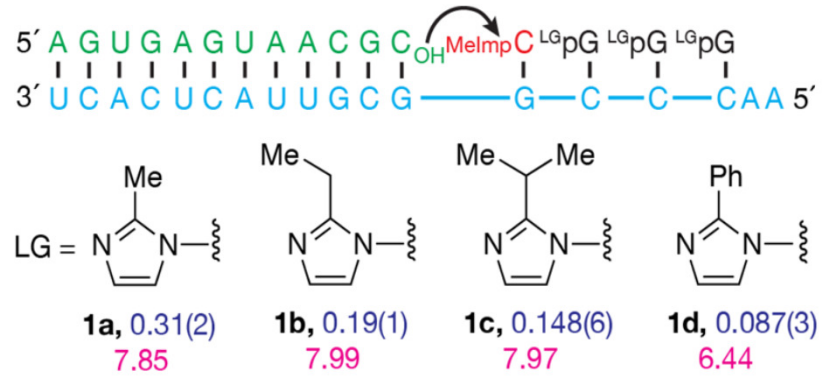
1: full 2',5' link
7: full 3'5' link

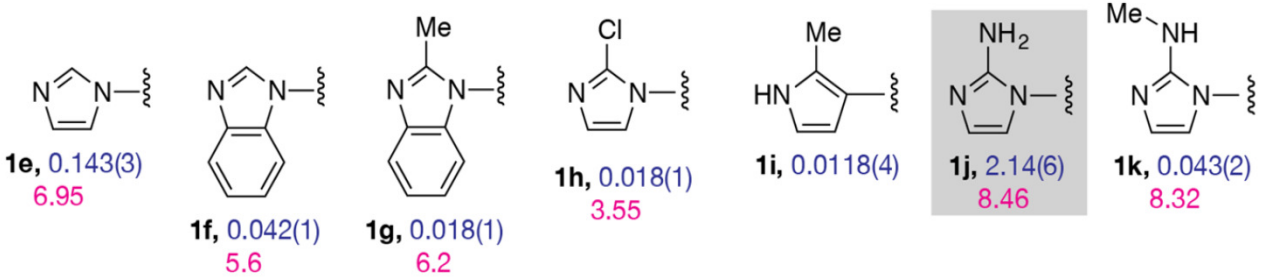


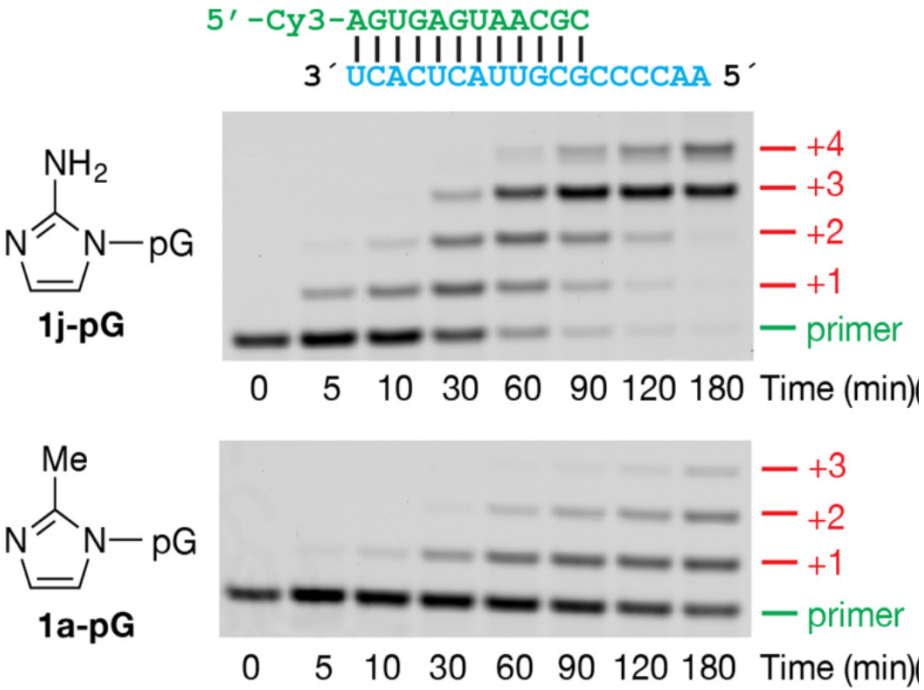


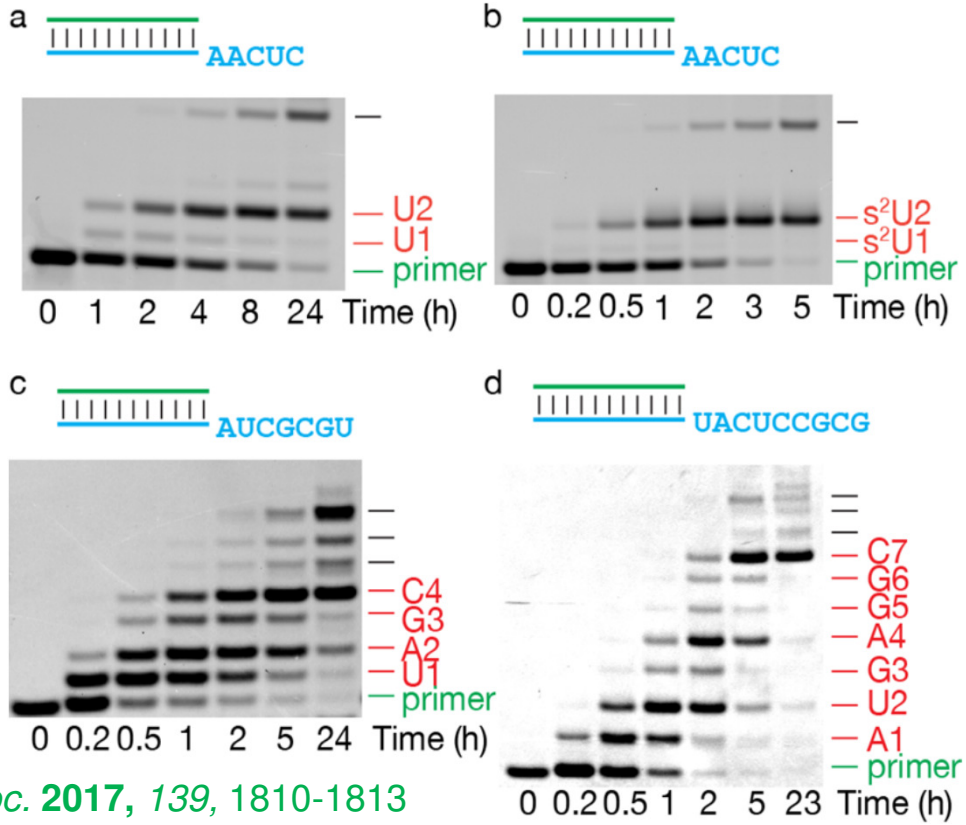
A. Mariani, J. D. Sutherland *Angew. Chem. Int. Ed.* **2017**, *56*, 6563-6566

Nonenzymatic primer extension



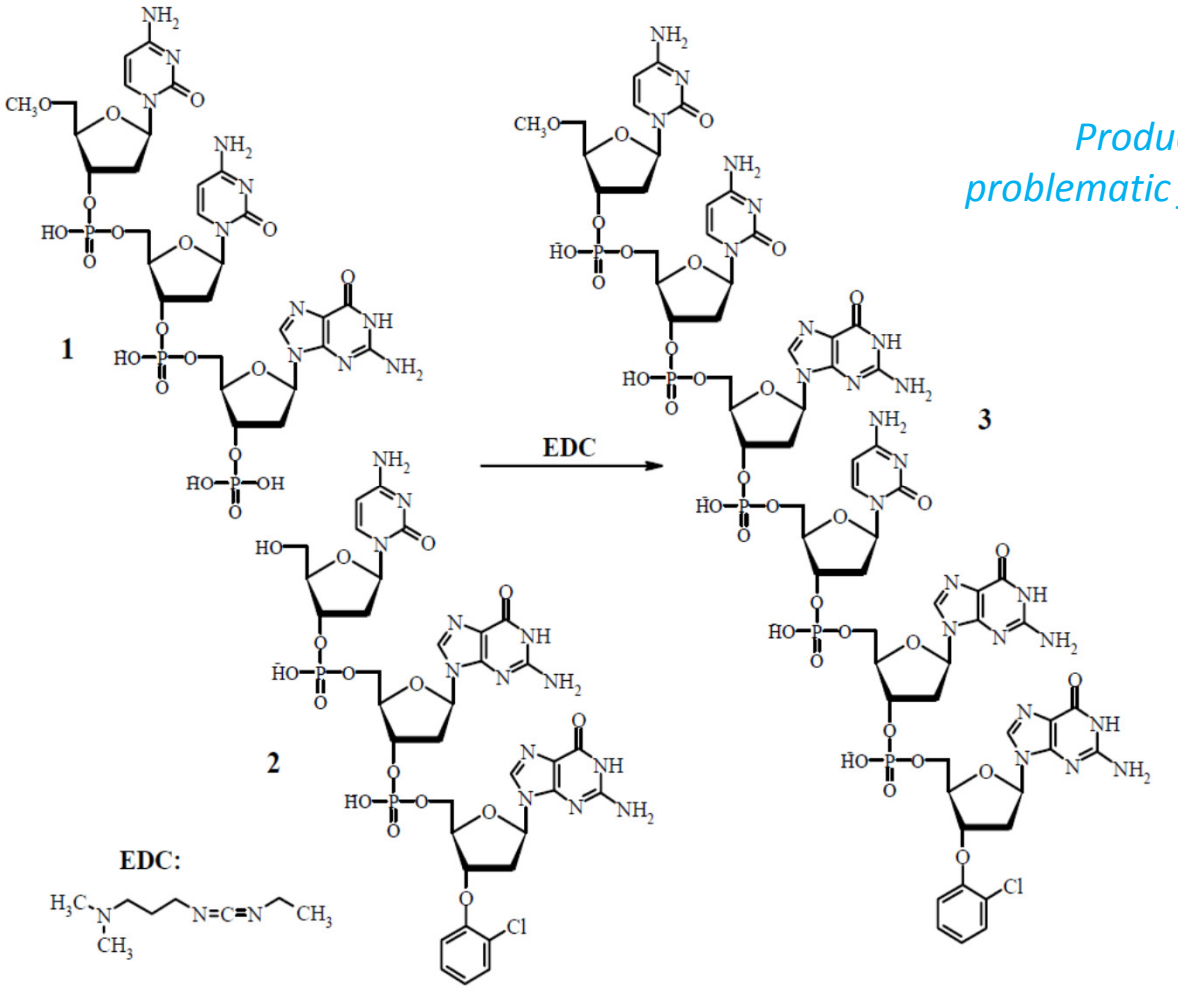






J. Szostak *et al. J. Am. Chem. Soc.* **2017,** *139,* 1810-1813

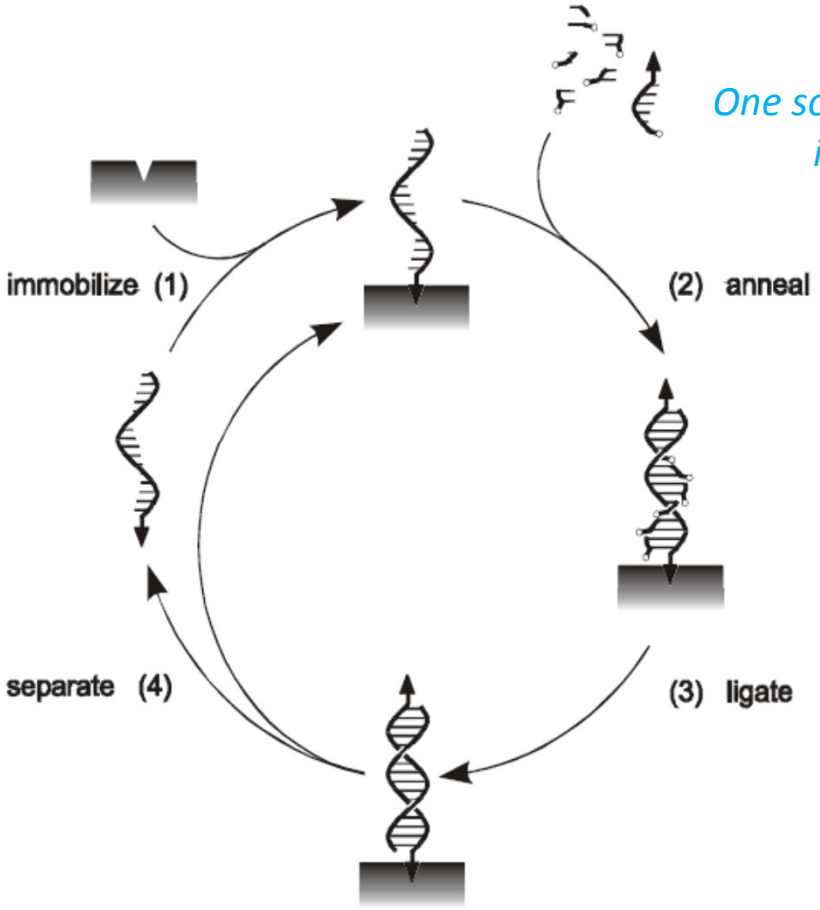
First non-enzymatic self-replicating system



Product inhibition –
problematic for longer templates

V. Patzke, G. von Kiedrowski *ARKIVOC* **2007** 293-310

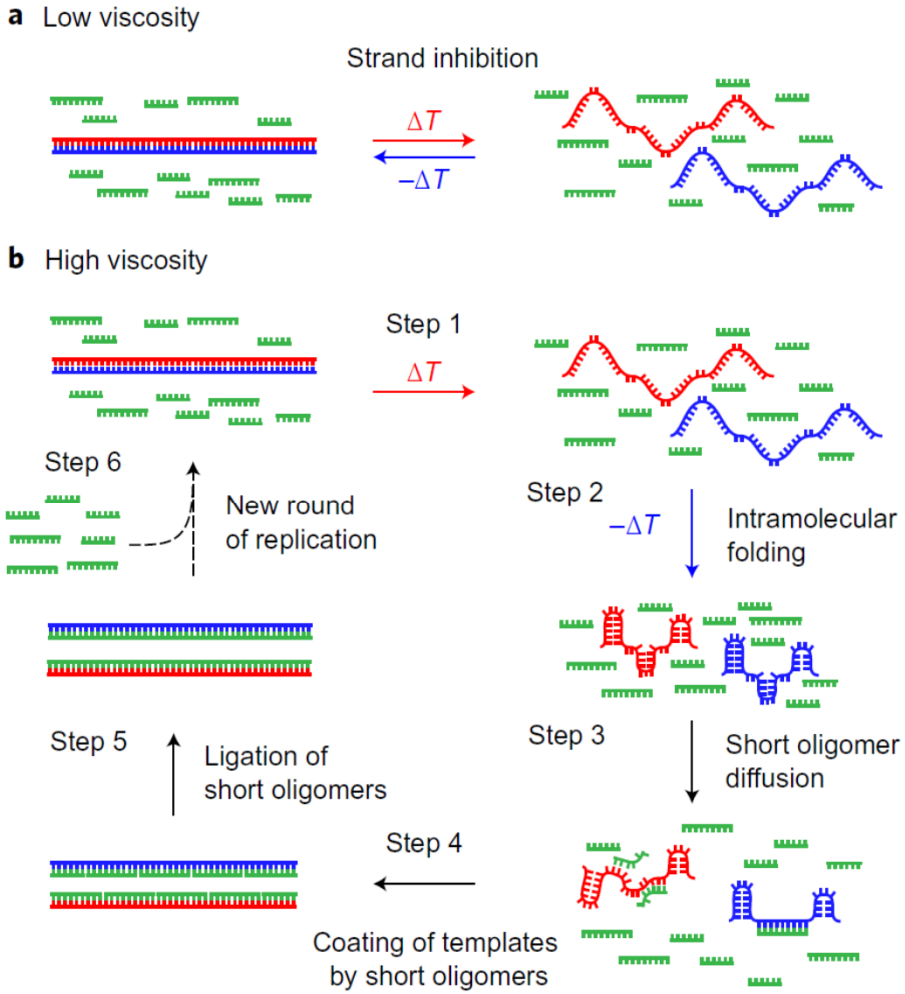
SPREAD – Surface-Promoted Replication and Exponential Amplification of DNA Analogues



One solution for the product inhibition – immobilization of one strand

V. Patzke, G. von Kiedrowski *ARKIVOC* **2007** 293-310

Prebiotic replication in a viscous solvent

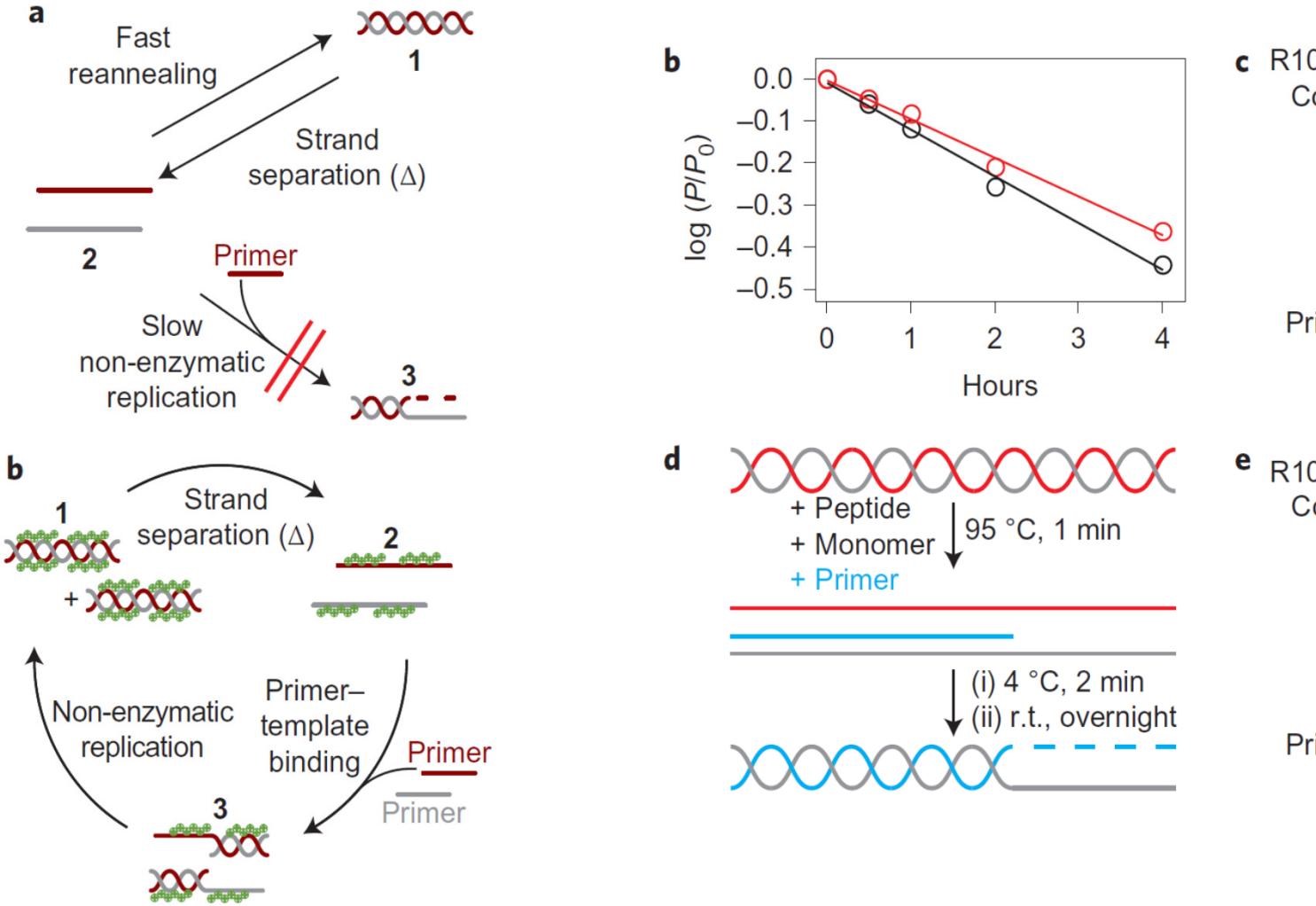


At low viscosity: warming causes duplex dissociation cooling – direct hybridization

At high viscosity: warming causes duplex dissociation cooling – intramolecular folding which prevents back-hybridization
Then slowly short oligomers diffuse into the folds and coat the templates preventing their re-annealing.
The following templated ligation of the short oligomers provides new generation of templates without product inhibition

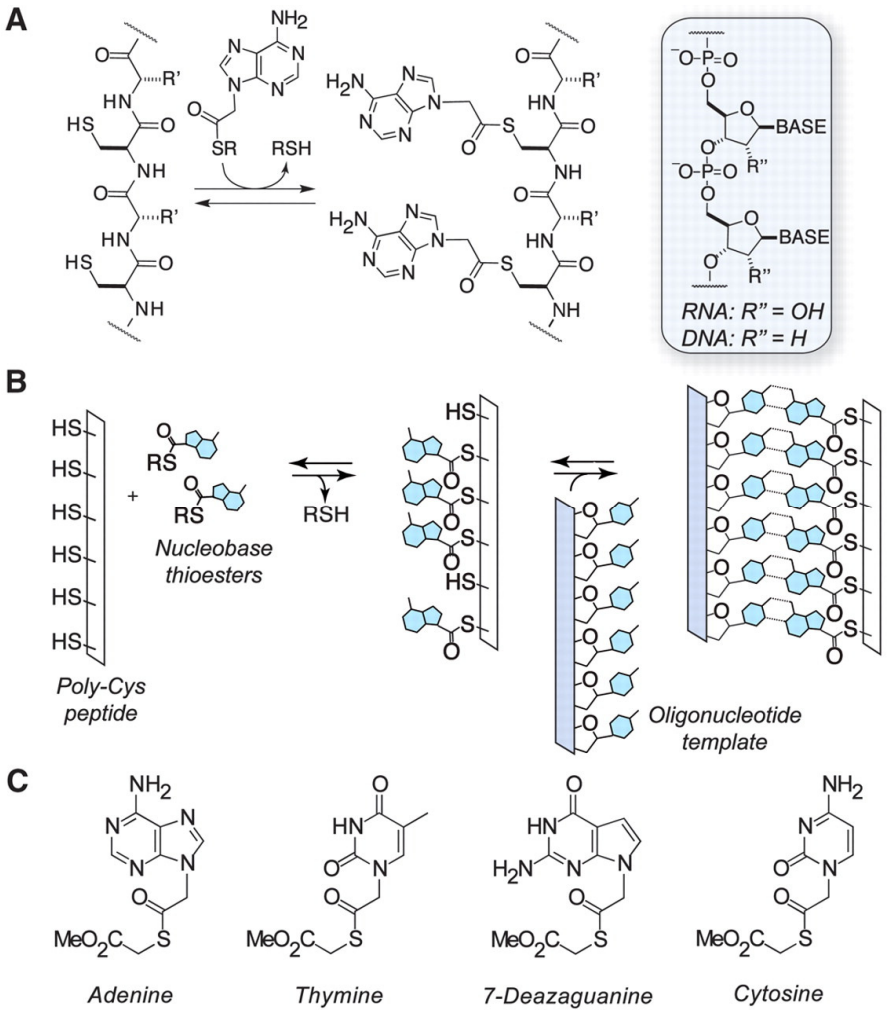
N. V. Hud *et al. Nature Chem.* **2017**, *9*, 318-324

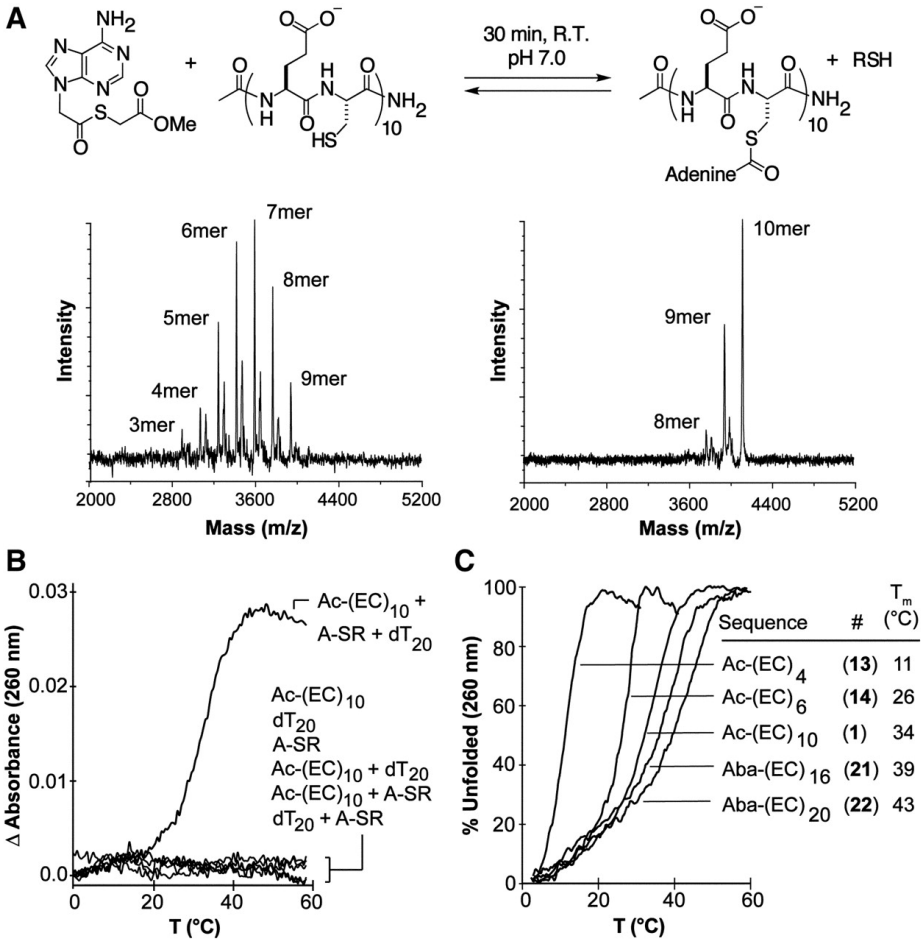
Nonenzymatic primer extension in presence of oligoarginine peptides



J. Szostak *et al. Nature Chem.* **2016,** *8,* 915-921

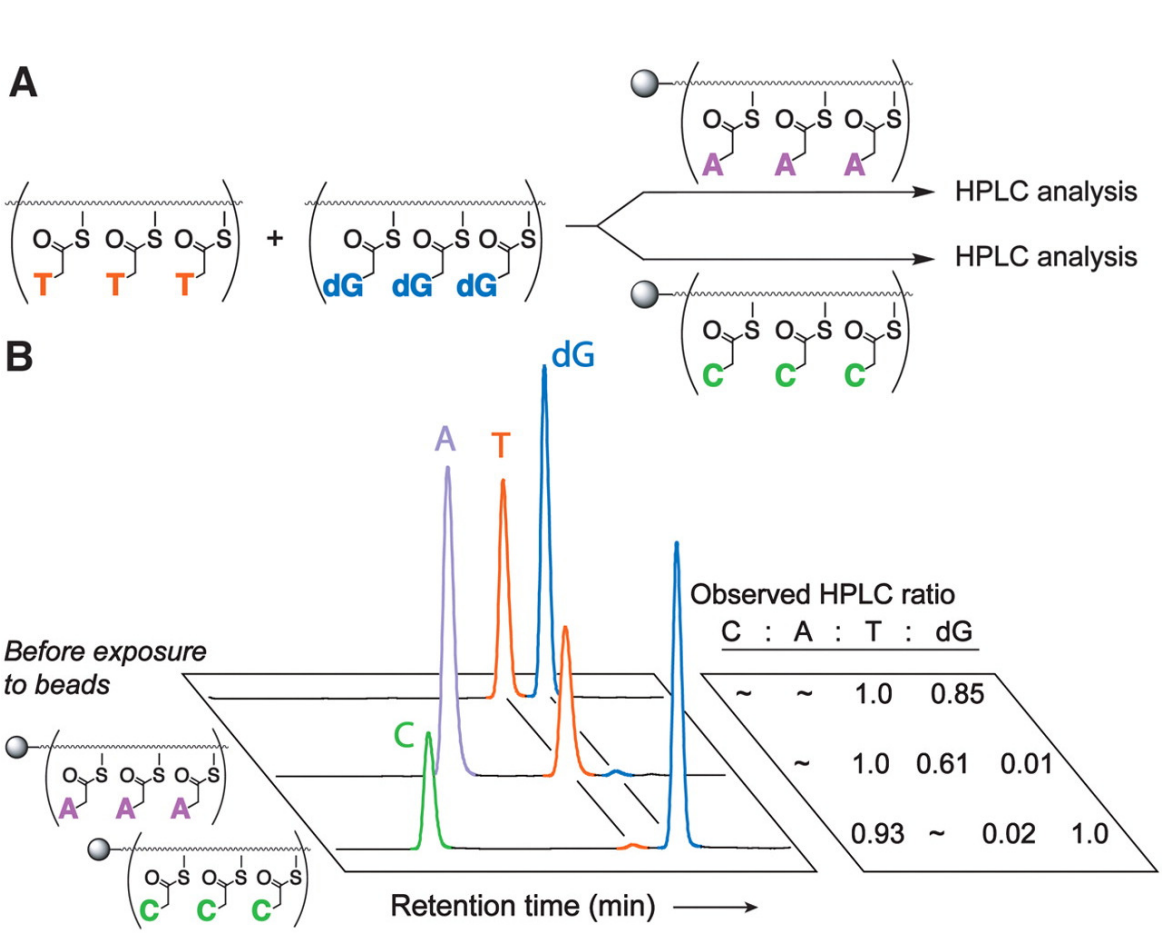
Dynamic oligonucleotide analogue sequence-specific assembly

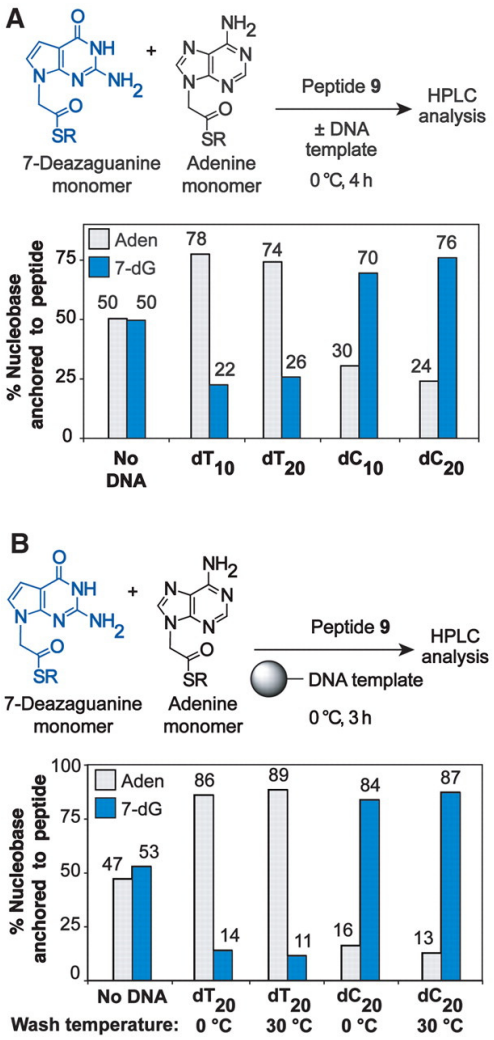


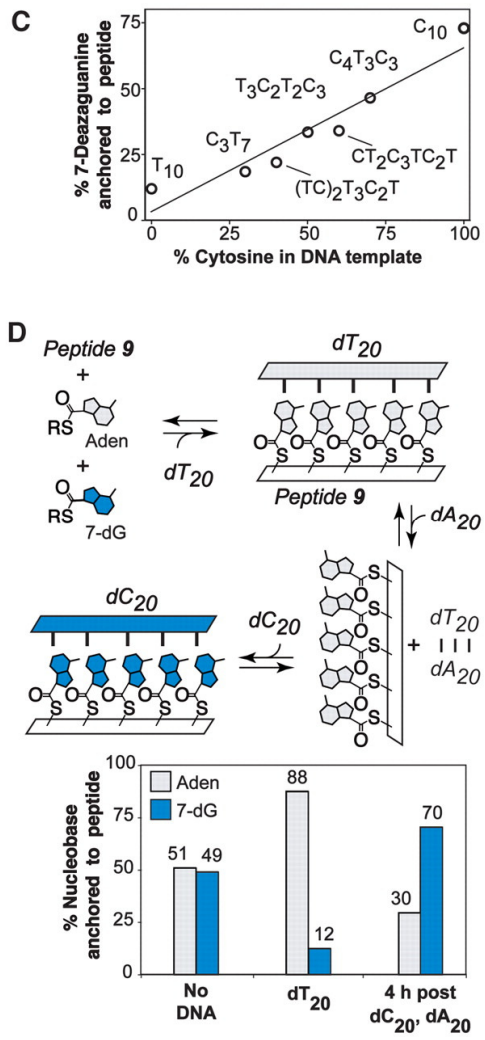


M. R. Ghadiri *et al. Science* **2009,** *325*, 73-77

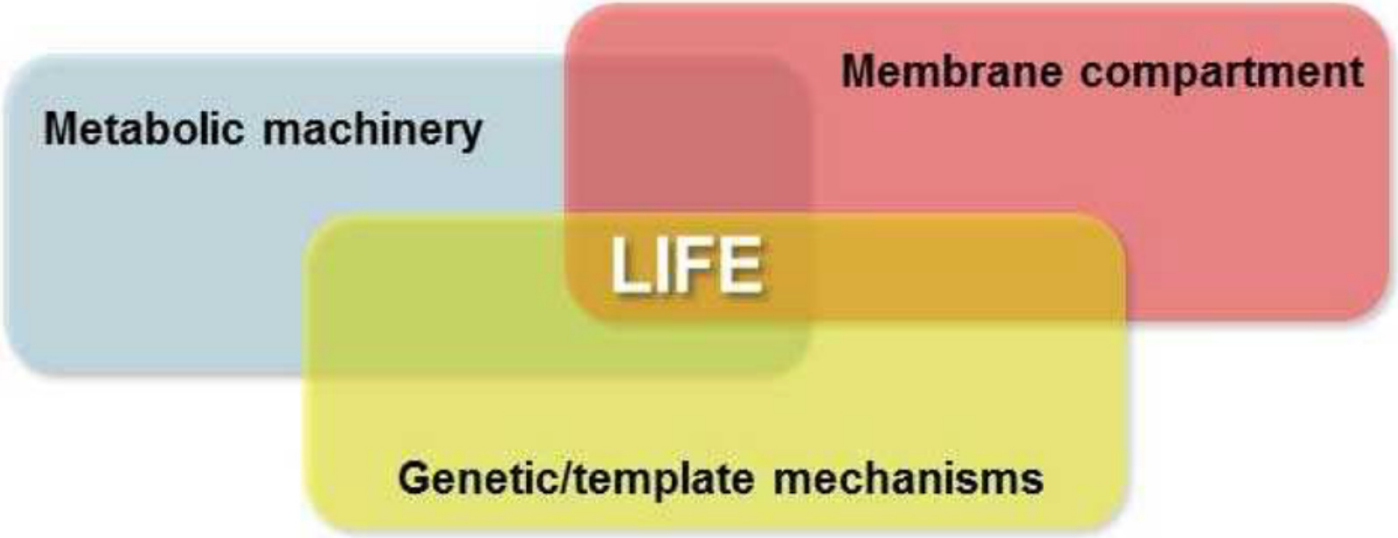
Dynamic oligonucleotide analogue sequence-specific assembly



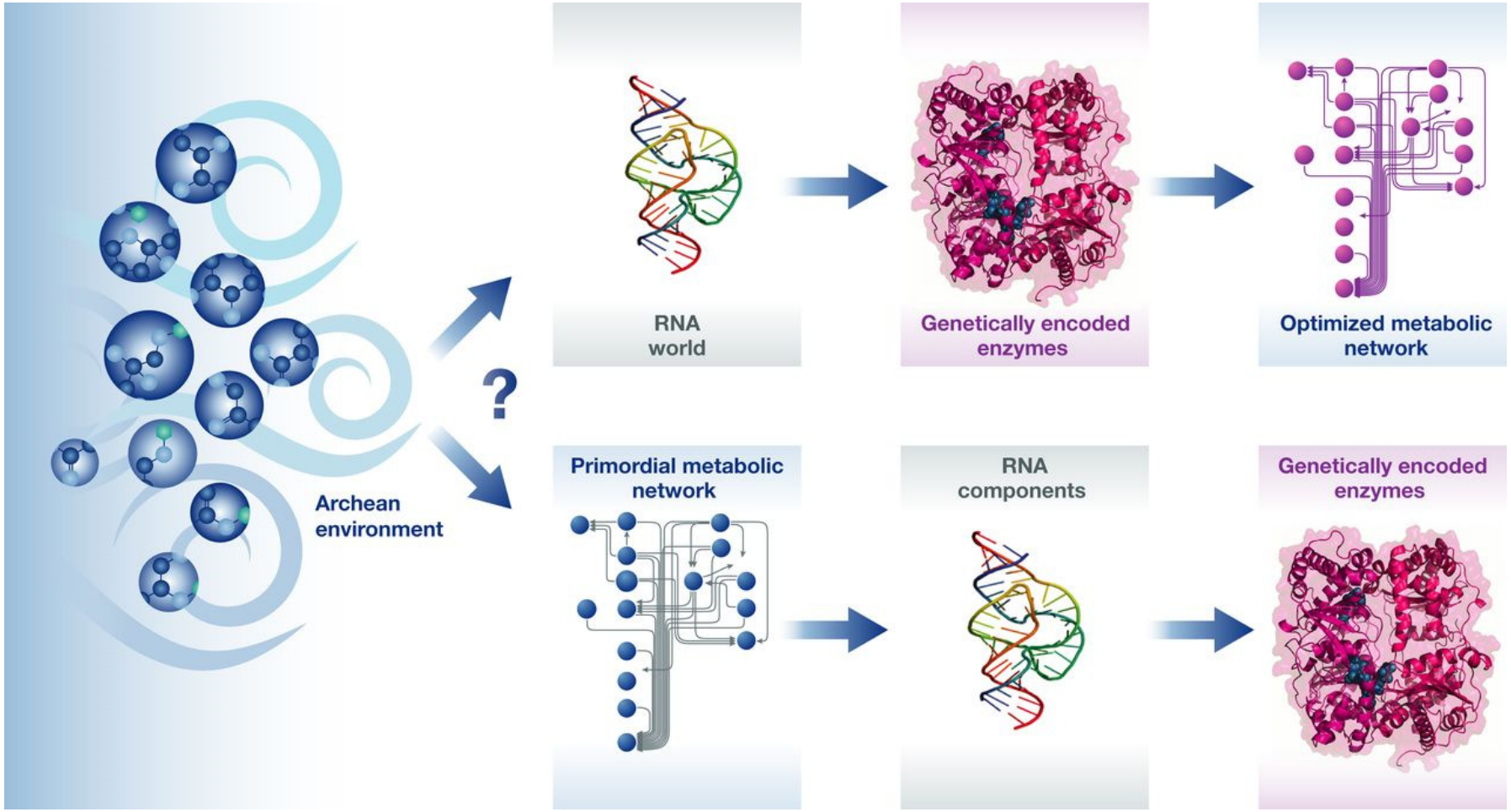




M. R. Ghadiri *et al. Science* **2009,** *325*, 73-77


Membrane compartment
Metabolic machinery
LIFE
Genetic/template mechanisms

Route to life by chemical networks



P. L. Luisi *Mol Syst Biol.* **2014,** *10,* 729

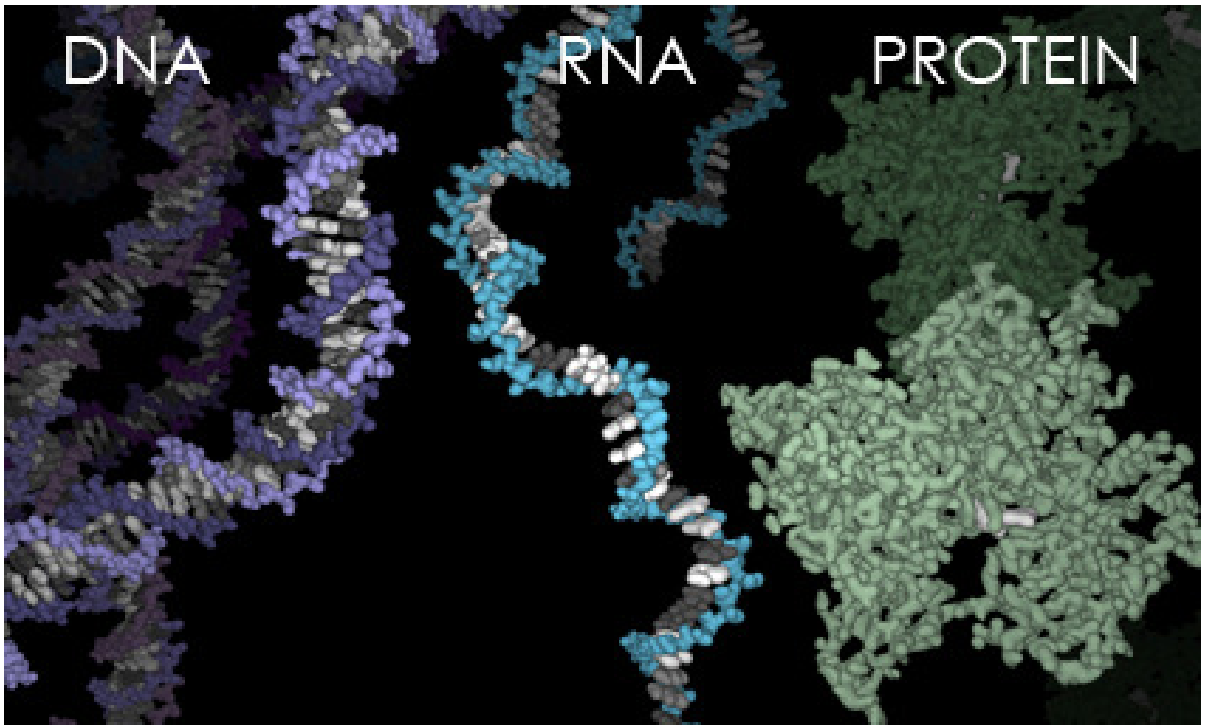
Metabolism-first vs. Genes-first

Genetics/replication-first: an information-carrying polymer capable of replication (RNA or something simpler) spontaneously arose from available prebiotic molecules available on early Earth. Metabolism incorporated later as a mean to receive energy from the surroundings in a controlled manner.

Metabolism-first: primitive metabolic cycles spontaneously assembled from simple prebiotic organic molecules or inorganic carbon sources as CO_2. And the cycles produced a set or more or less complex molecules needed for the replication process and construction of the genetic apparatus.

The supposed *proto-metabolism* would differ from the currently known one, because the chemical reactions were not catalysed by efficient enzymes, nor were aminoacid and peptide sequences determined by DNA.
The involved reactions were either spontaneous, or catalysed by inorganic catalysts or peptides.
Inorganic catalysts would be molecules, or ions, in solutions or on surfaces of solids such as clays or pyrites.
Peptides (or peptoids) formed either by random oligomerization or mutual catalysis.

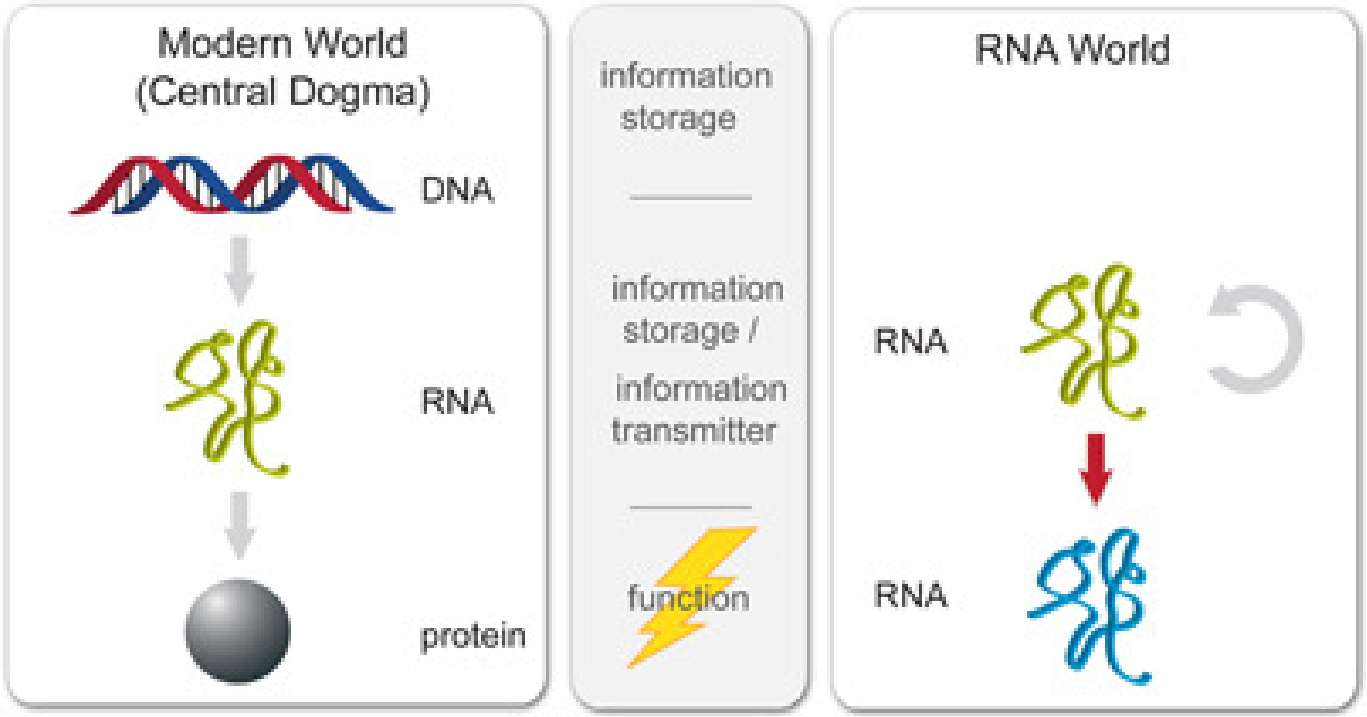
„Genes-first”



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

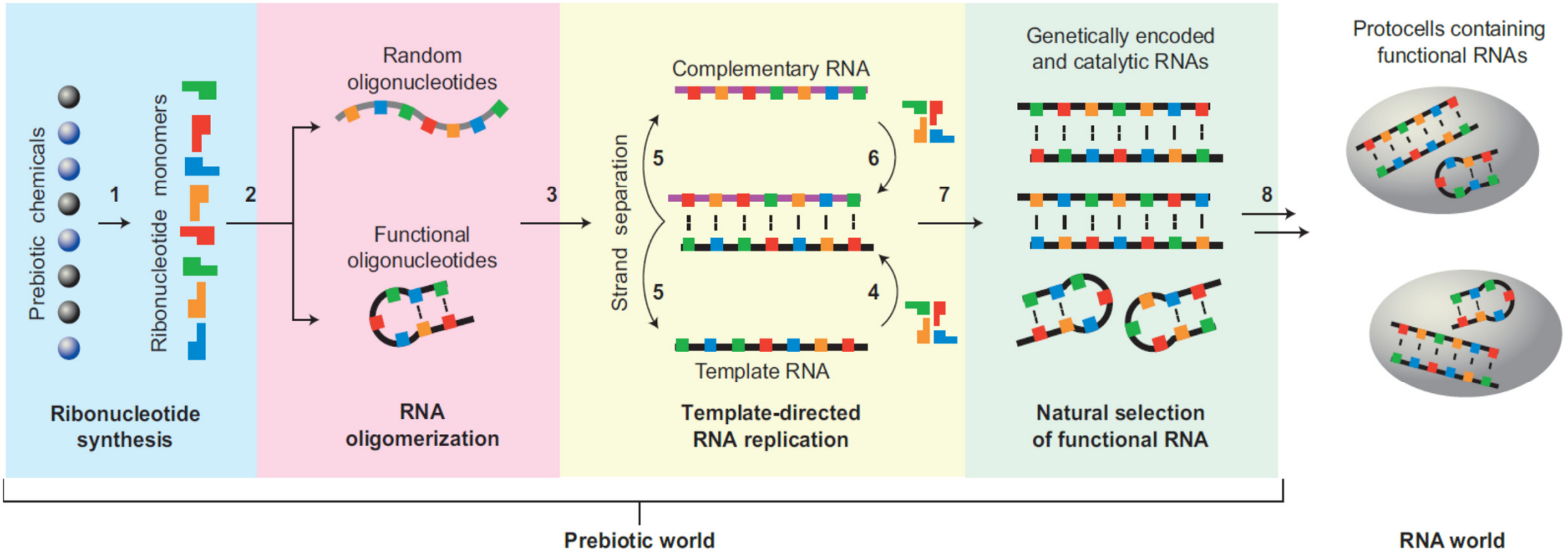
RNA folding is mediated by base-pairing interactions along different regions of a single-stranded RNA.

The RNA world

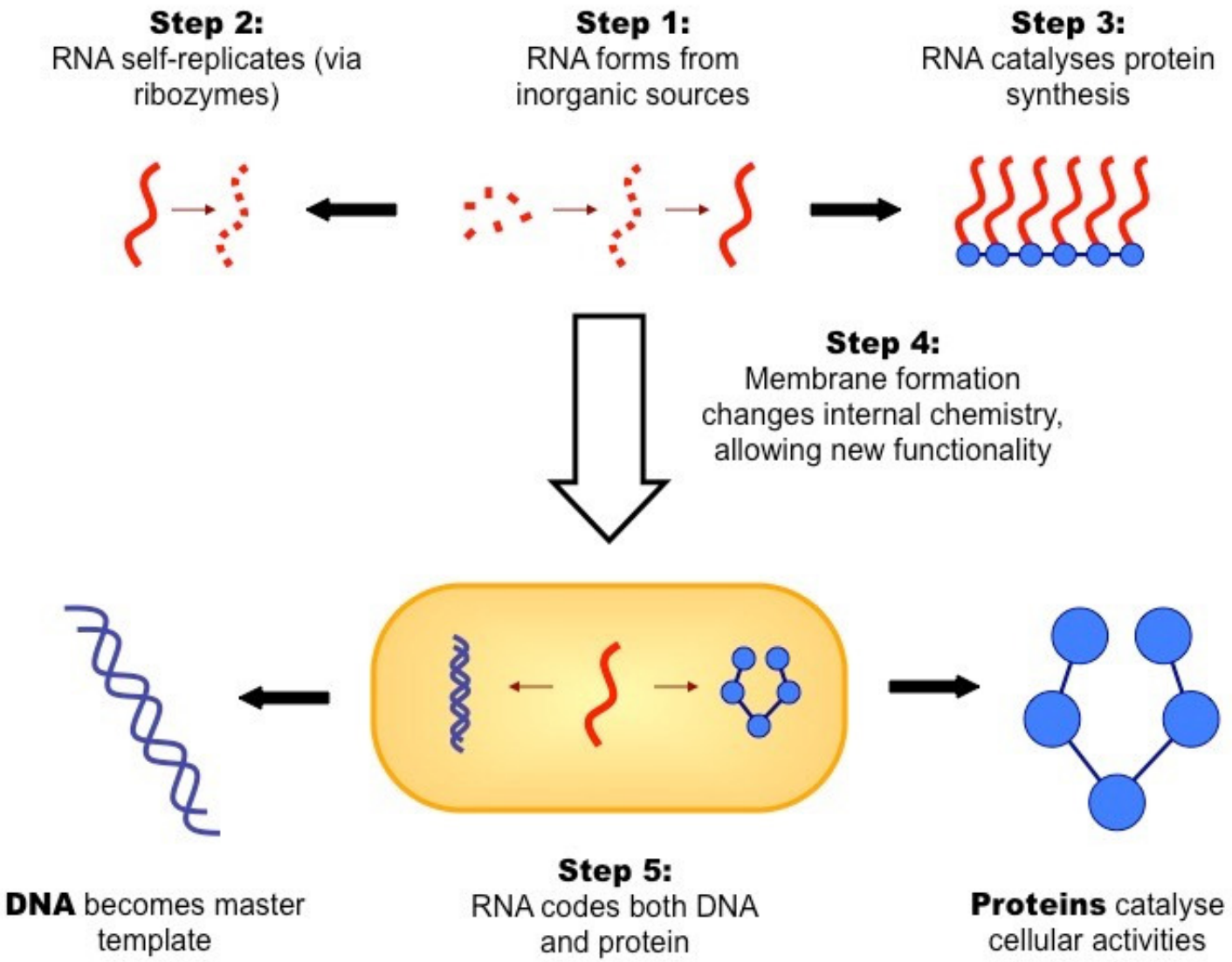


Conceptual idea that there was a period in the early history of life on Earth when RNA (or its structurally simplified analogue) carried out most of the information processing and metabolic transformations needed for biology to emerge from chemistry

The RNA world



The RNA world

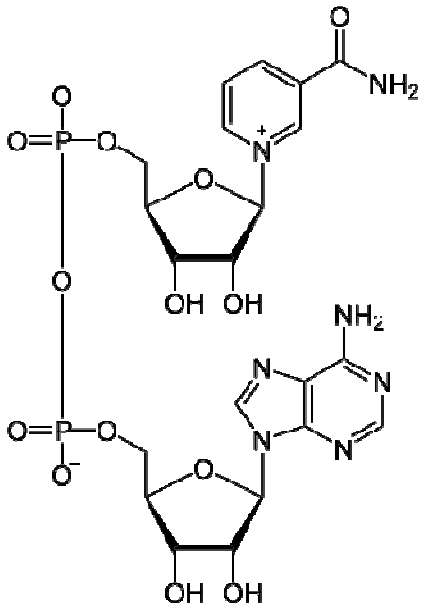


The RNA world

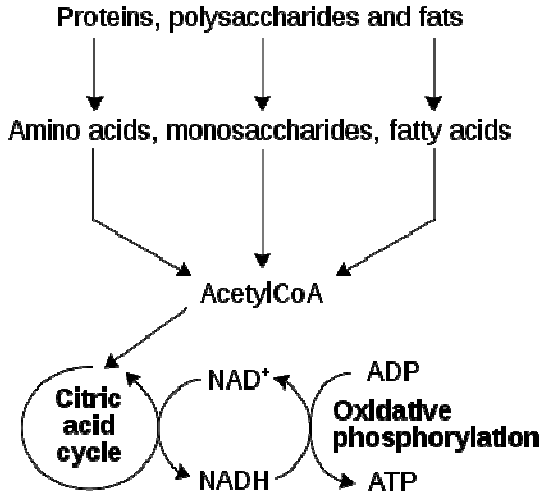
Crick, Orgel and Woese speculated in 1968 that, because RNA can form secondary structures, it has both a genotype and a phenotype and is a good candidate for the emergence of life

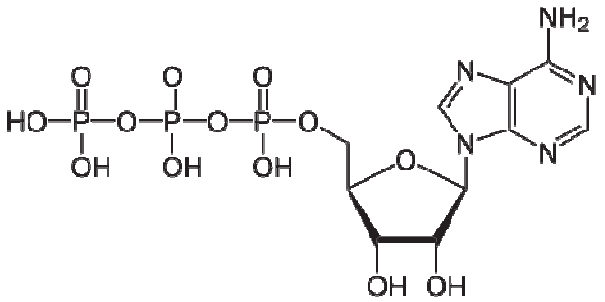
F. H. C. Crick *J. Mol. Biol.* **1968**, *38,* 367-379, L. E. Orgel *J. Mol. Biol.* **1968**, *38,* 381-393

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



Nicotinamide adenine dinucleotide (**NAD⁺**)



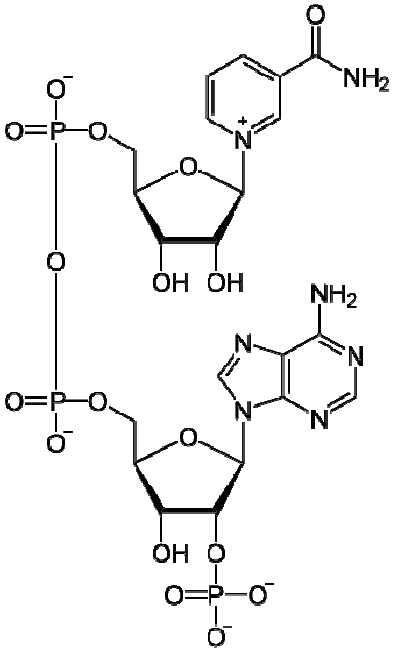


Adenosine triphosphate (**ATP**)

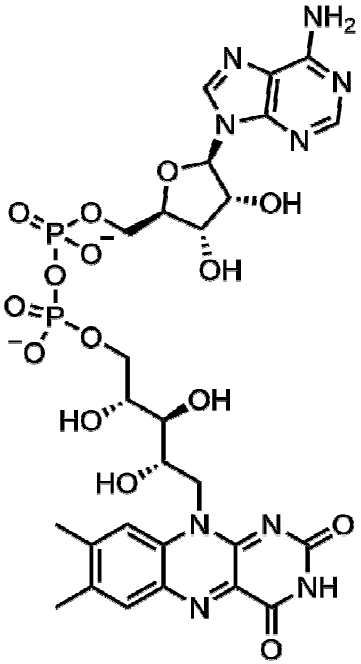
H. B. White III *J. Mol. Evol.* **1976**, *7,* 101-104

The RNA world

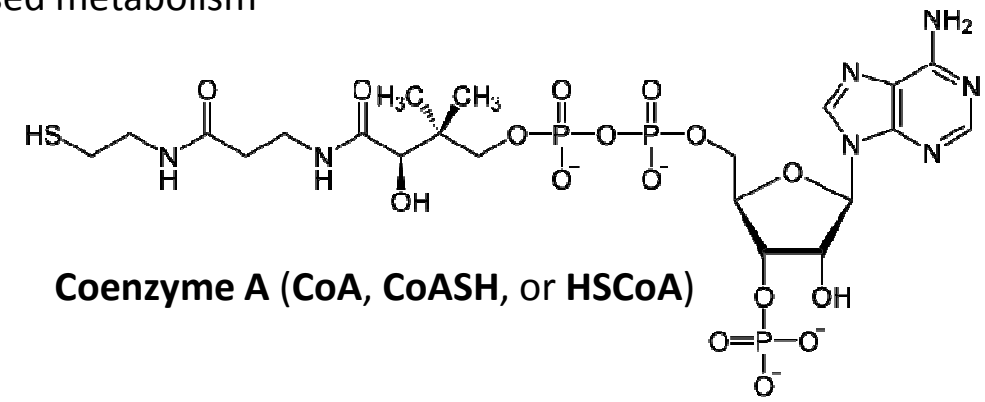
Ribonucleotide coenzymes now used by many proteins may be molecular „fossils” from the primoridal RNA-based metabolism



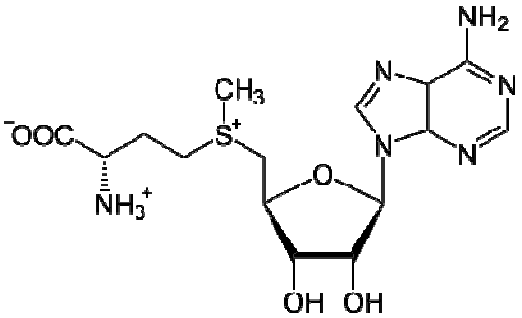
Nicotinamide adenine dinucleotide phosphate ($NADP^+$)



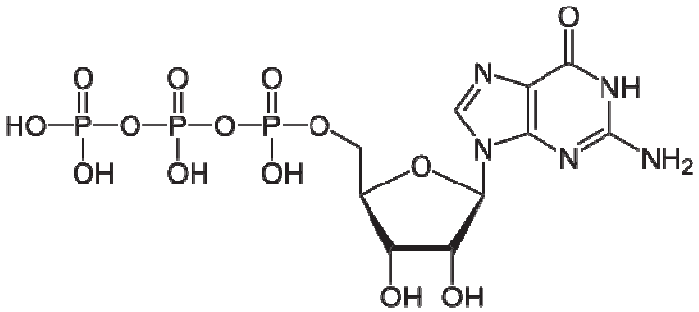
flavin adenine dinucleotide (FAD)



Coenzyme A (CoA, CoASH, or HSCoA)



***S*-Adenosyl methionine**

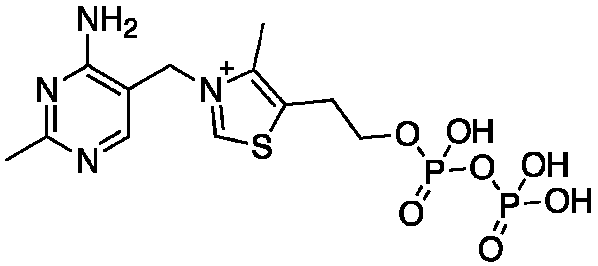


Guanosine-5'-triphosphate (GTP)

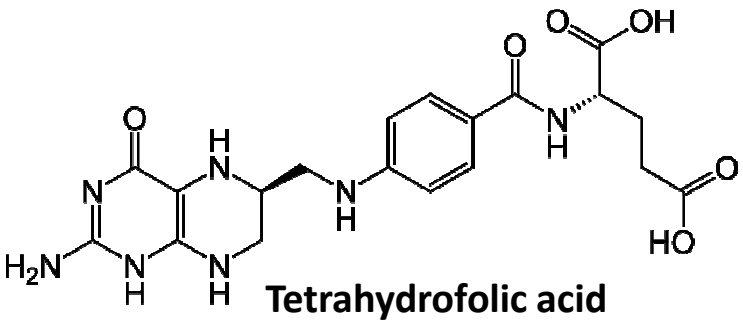
H. B. White III *J. Mol. Evol.* **1976**, *7*, 101-104

The RNA world

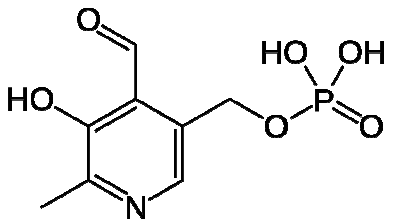
Other coenzymes contain cyclic nitrogen-containing bases that can also derive from nucleotides



Thiamine pyrophosphate (TPP or ThPP) – Vit. B_1



Tetrahydrofolic acid



Pyridoxal phosphate (PLP) – Vit. B_6

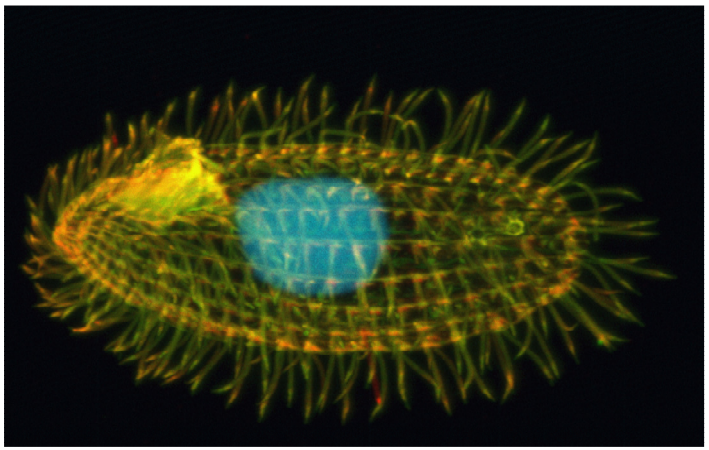
H. B. White III *J. Mol. Evol.* **1976**, *7*, 101-104

The RNA world

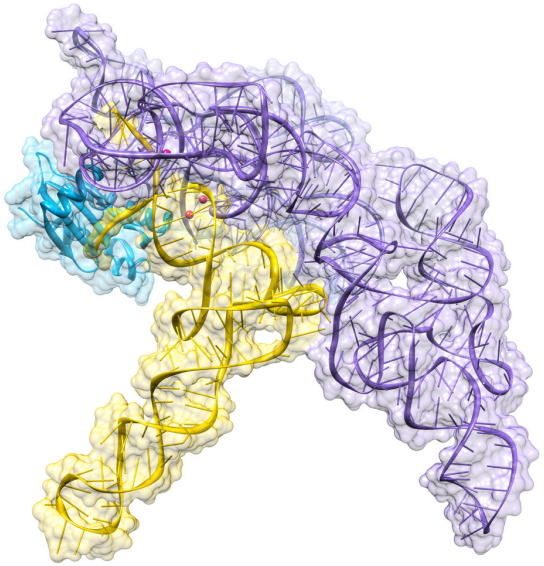
Ribozymes – Ribonucleic acid enzymes

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.



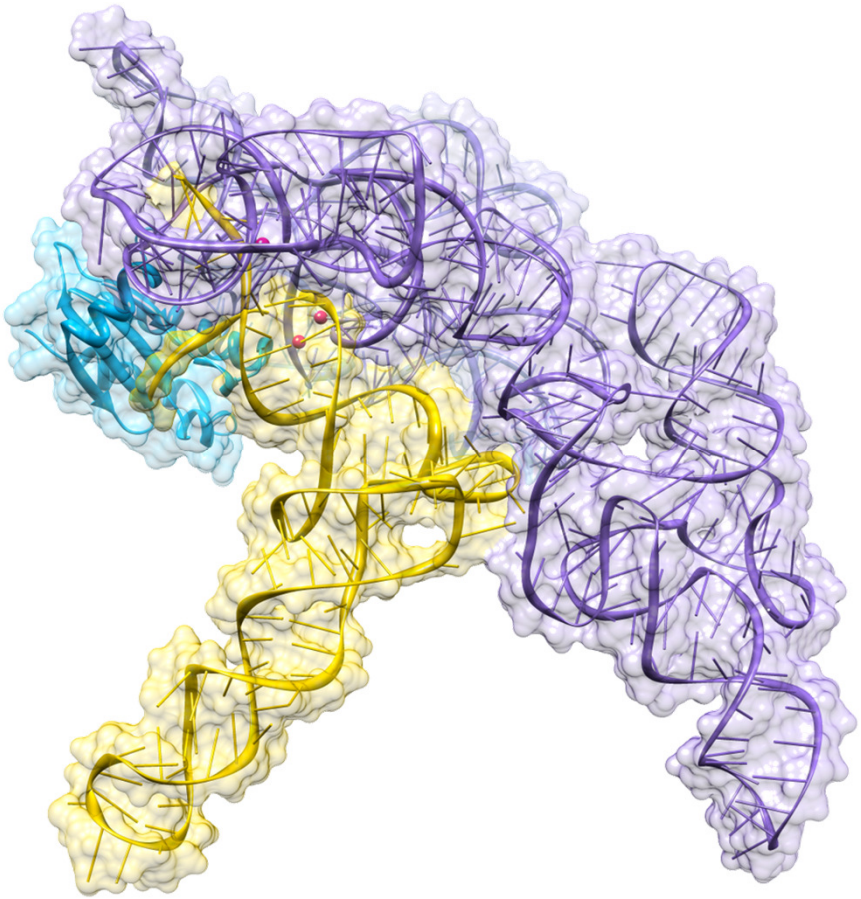
Tetrahymena thermophila



Bacterial RNAse P

The RNA world

Ribonuclease P

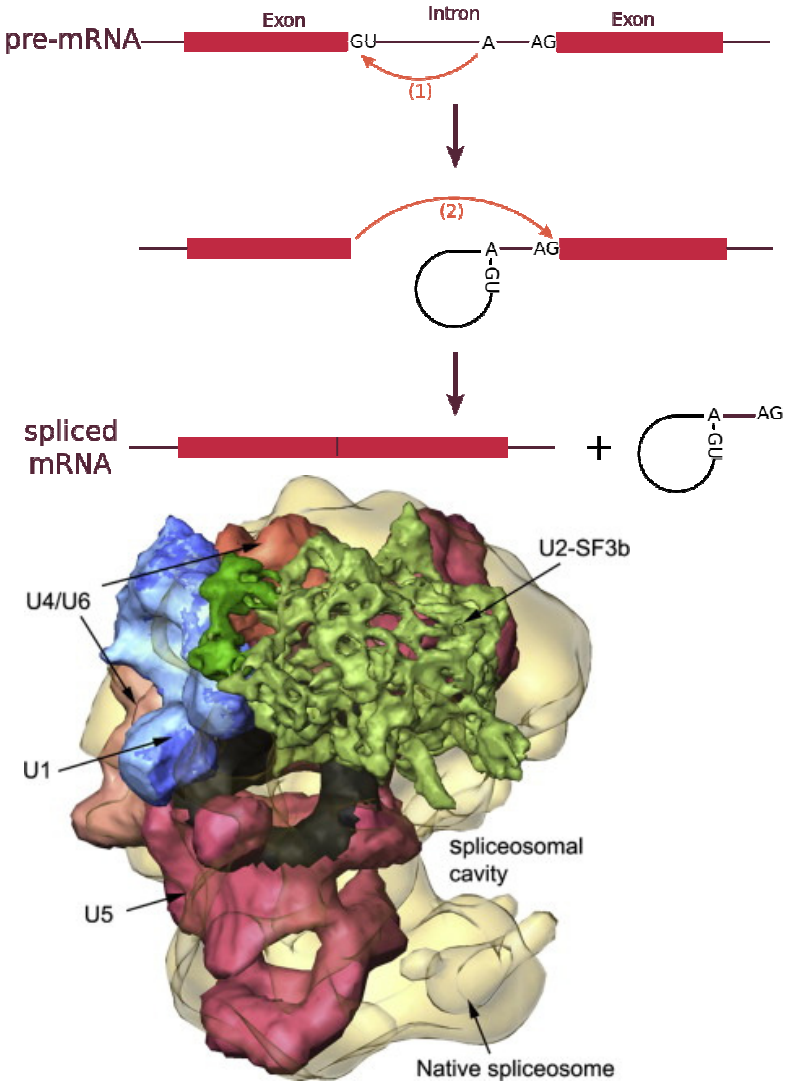


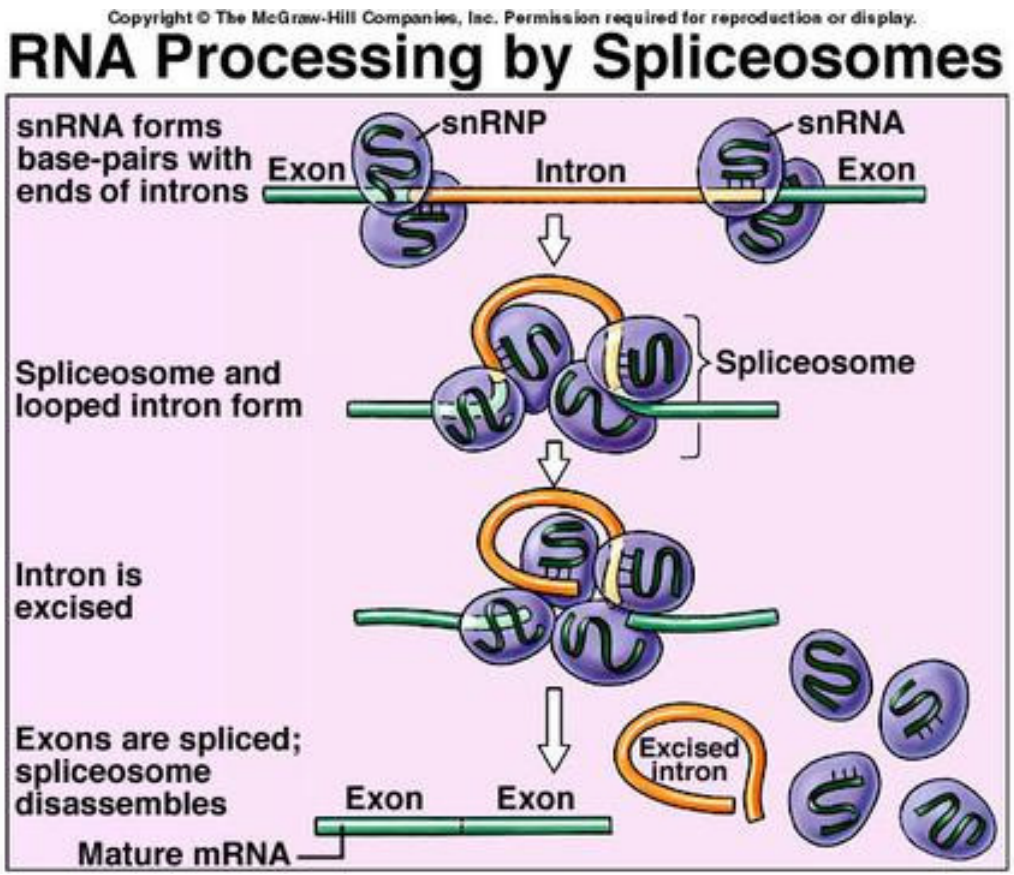
Ribonuclease P (RNase P) is a type of ribonuclease which cleaves RNA. RNase P is unique from other RNases in that it is a ribozyme – a ribonucleic acid that acts as a catalyst in the same way that a protein based enzyme would. Its function is to cleave off an extra, or precursor, sequence of RNA on tRNA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

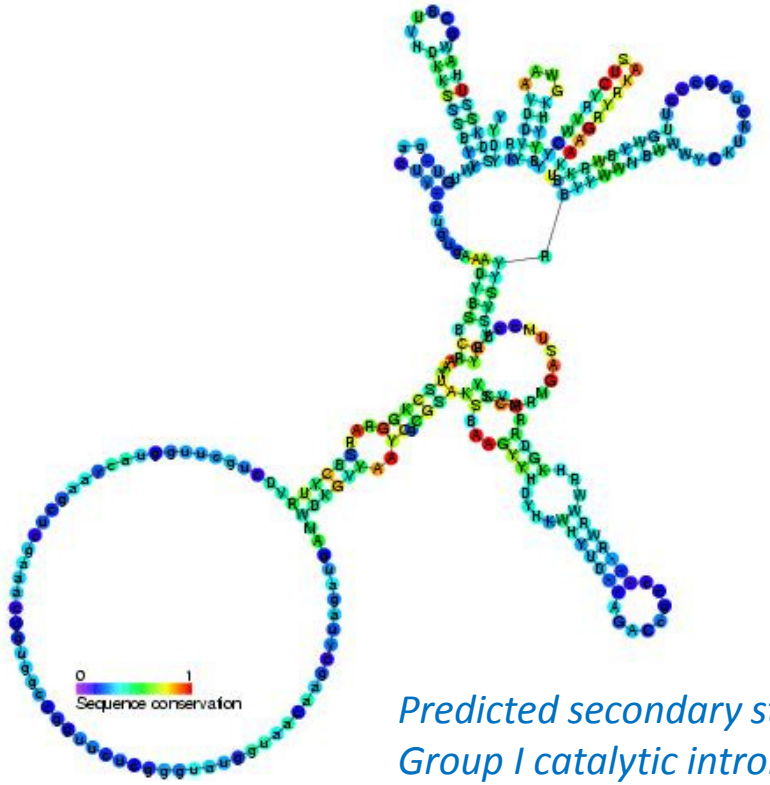
RNA splicing





Spliceosome – a complex of ribonucleoproteins

RNA splicing

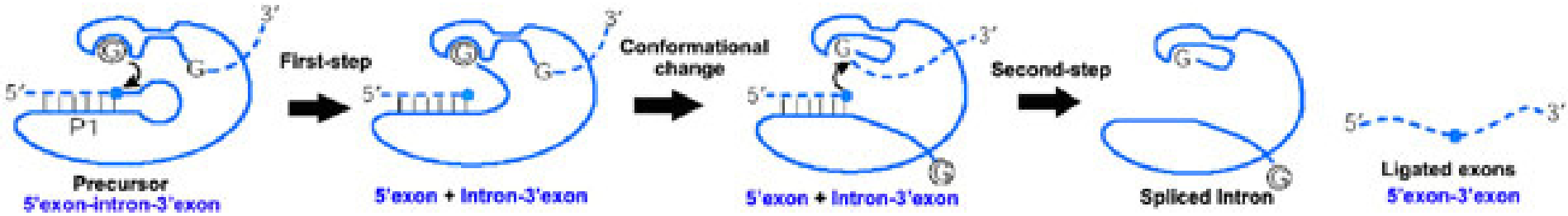


Predicted secondary structure and sequence conservation of Group I catalytic intron

Self-splicing RNA introns

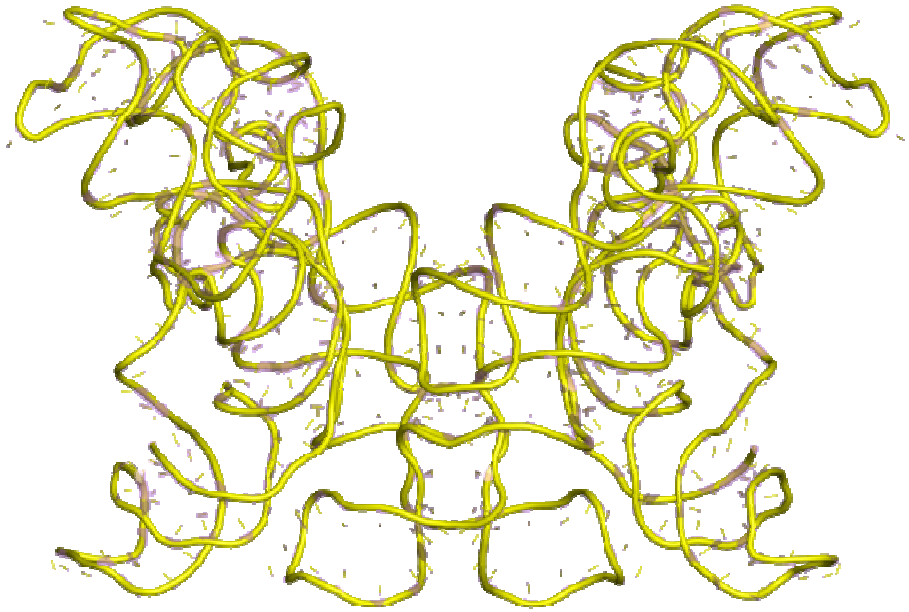
RNA splicing in *Tetrahymena* was taking place also in absence of the spliceosome - the ‚negative control' obtained after protease digestion also spliced.

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.



RNA splicing

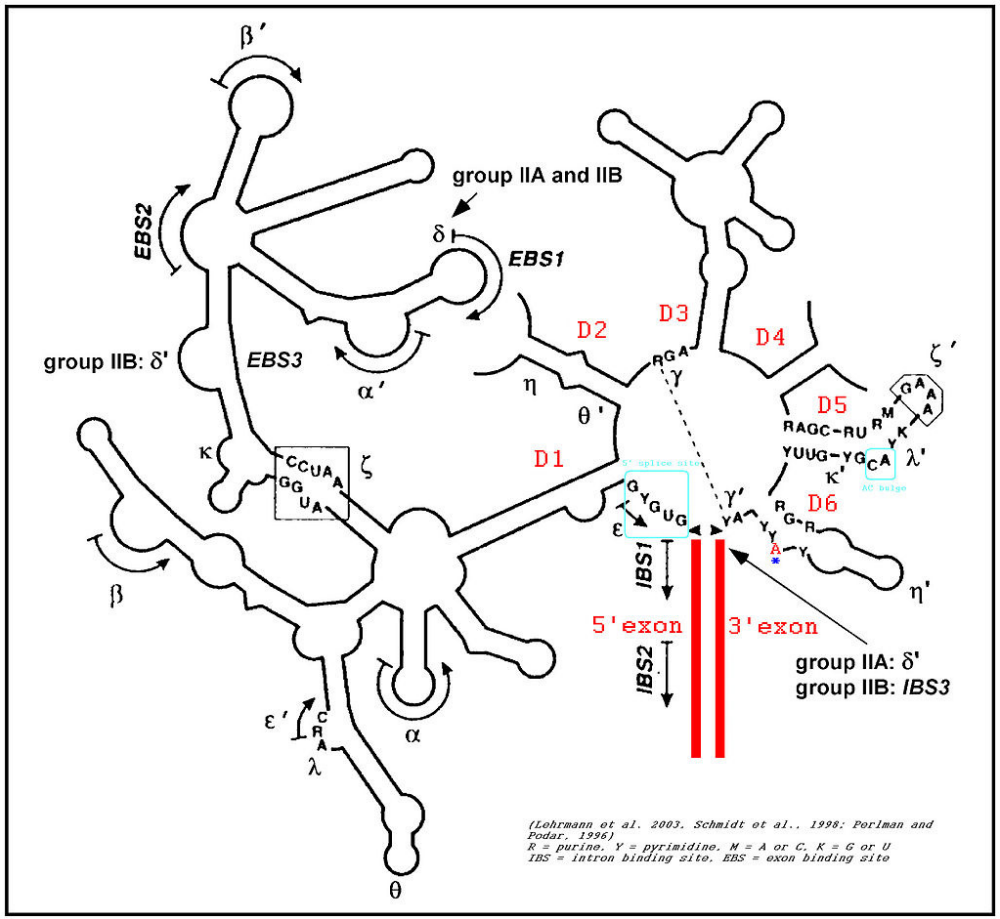
Group I catalytic introns



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

RNA splicing

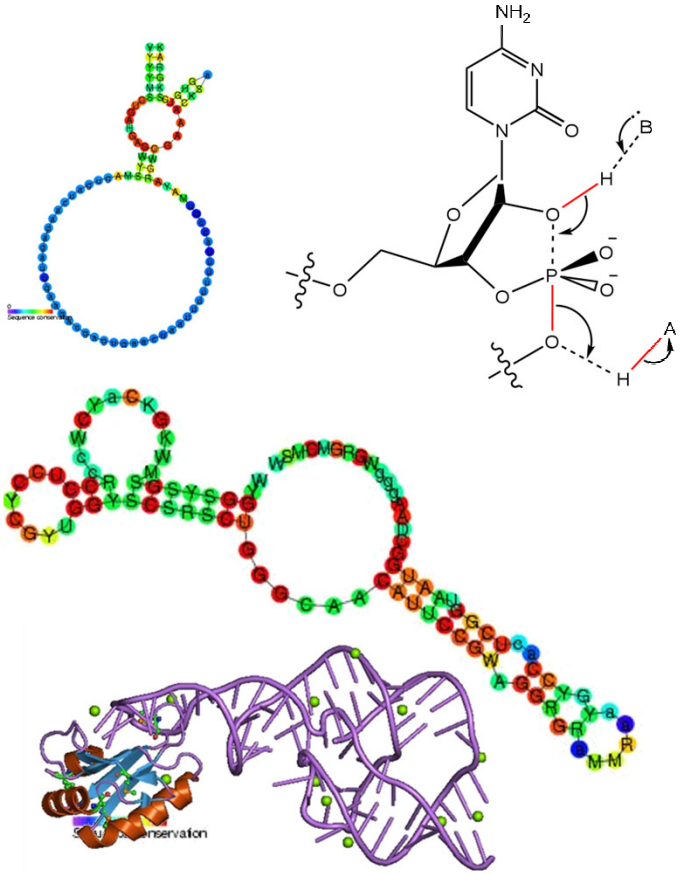
Group II catalytic introns



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

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 and riboswitches



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; targeted RNA cleavage experiments)

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.

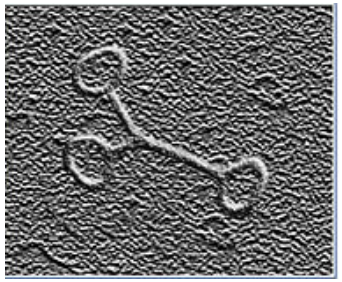
The ribozyme acts to process the RNA transcripts to unit lengths in a self-cleavage reaction. The ribozyme is found to be active in vivo in the absence of any protein factors and is the fastest known naturally occurring self-cleaving RNA.

Riboswitches

A riboswitch is a regulatory segment of a messenger RNA molecule that binds a small molecule, resulting in a change in production of the proteins encoded by the mRNA (bacteria, TPP riboswitch also in plants and funghi)

Viroids

Viroids ("subviral pathogens„) are mostly plant pathogens, which consist of short stretches of highly complementary, circular, single-stranded, and non-coding RNA without a protein coat. Viroids are extremely small - 246 to 467 nucleobases (genomes of smallest viruses start from 2,000 nucleobases). Viroids are plausible "living relics" of the RNA world.



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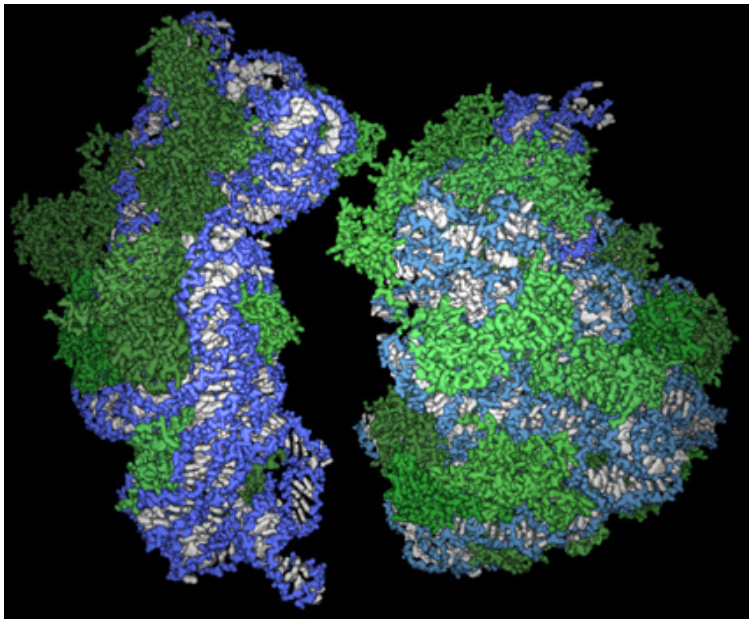
PSTVd-infected potatoes (right)

Viroid properties:

- small size (error-prone replication)
- high G-C content, (stability and replication fidelity)
- circular structure (complete replication without genomic tags)
- lack of protein-coding ability, consistent with a ribosome-free habitat; and replication mediated in some by ribozymes—the fingerprint of the RNA world.

Putative secondary structure of the PSTVd viroid

Ribosome – the ‚smoking gun'



Ribosome: green - proteins, blue and white - RNA

The ribosome is a 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.

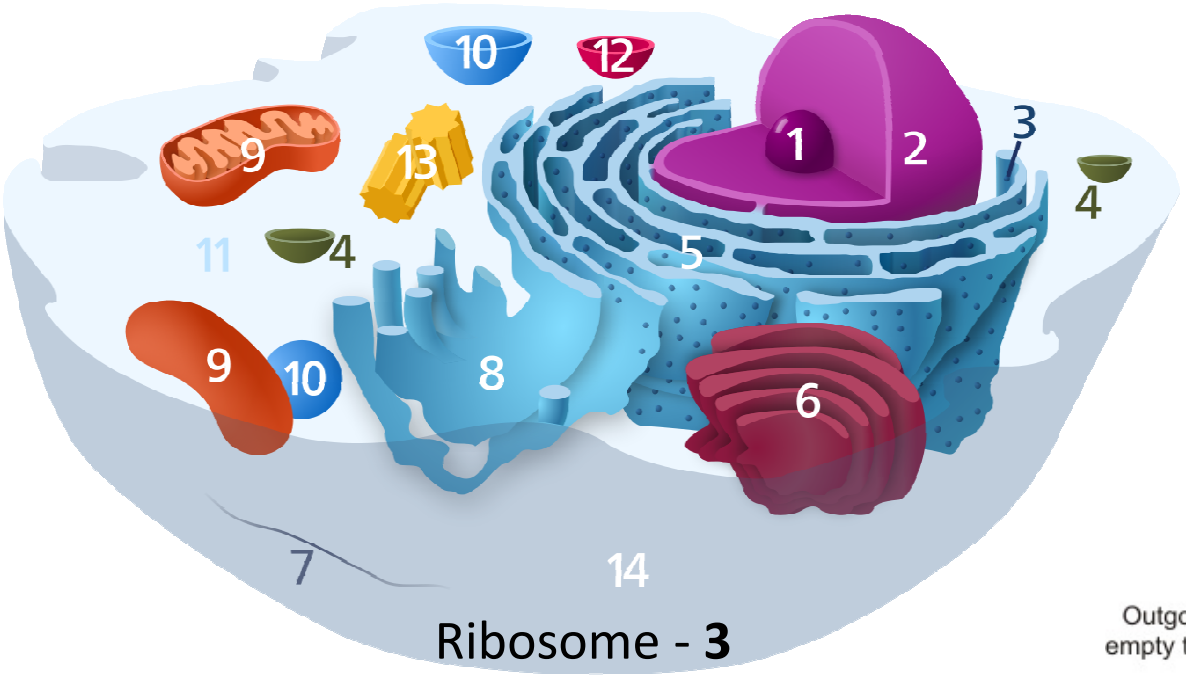
Ribosome is structurally highly conserved among all living species – most likely present in LUCA

Ribosomes:

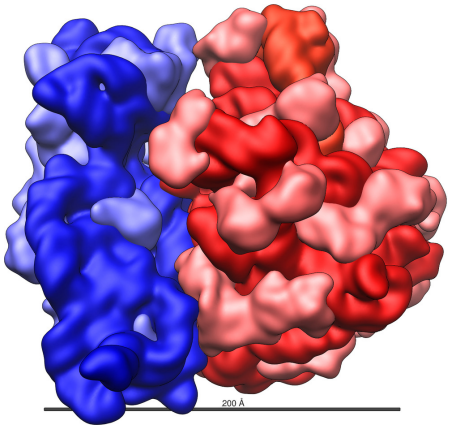
- the *small ribosomal subunit*, which reads the RNA
- the *large subunit*, which joins amino acids to form a polypeptide chain.

Each subunit is composed of one or more ribosomal RNA (rRNA) molecules and a variety of ribosomal proteins.

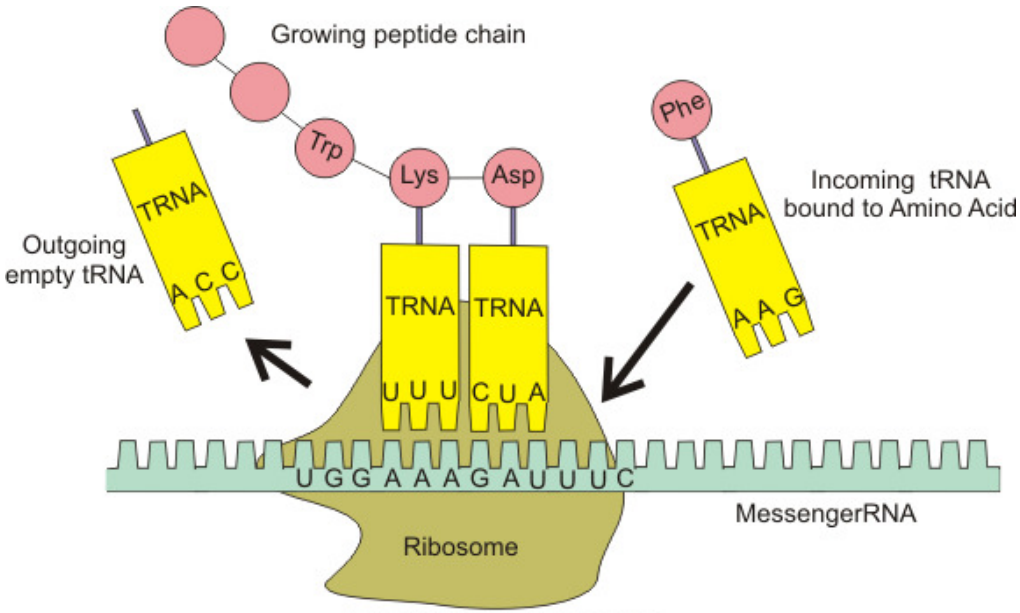
Ribosome – the ‚smoking gun'



Ribosome - **3**



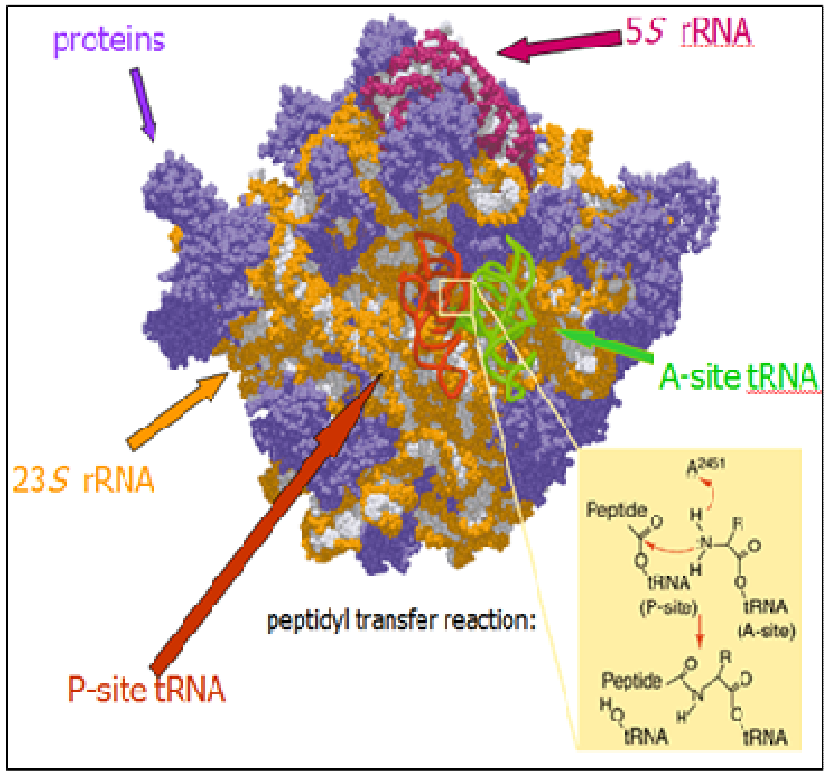
Large and small subunit



Peptide Synthesis

Ribosome – the ‚smoking gun'

Ribosome is a ribozyme!



No protein is present 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

The RNA world

RNA as catalyst

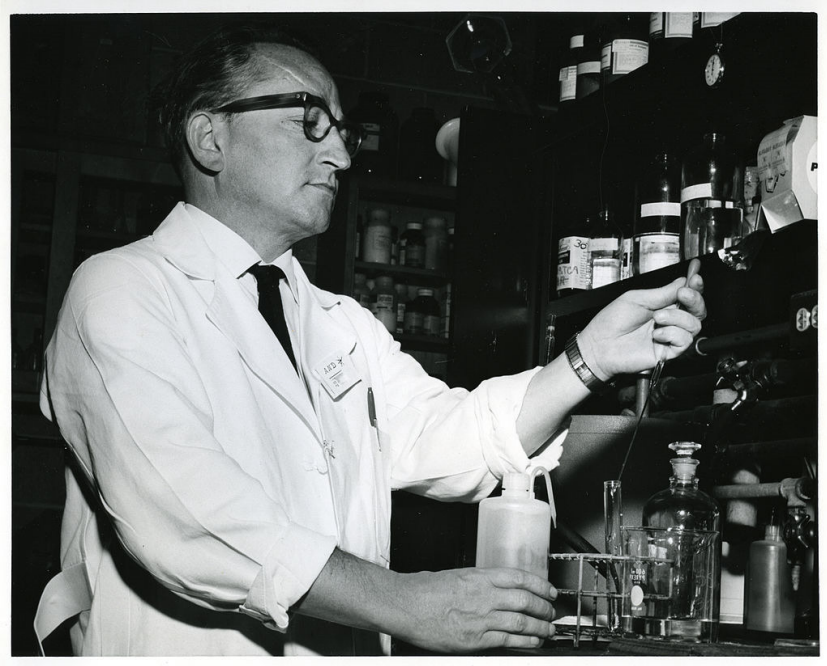
Currently known co-enzymes
Ribozymes
Ribosome

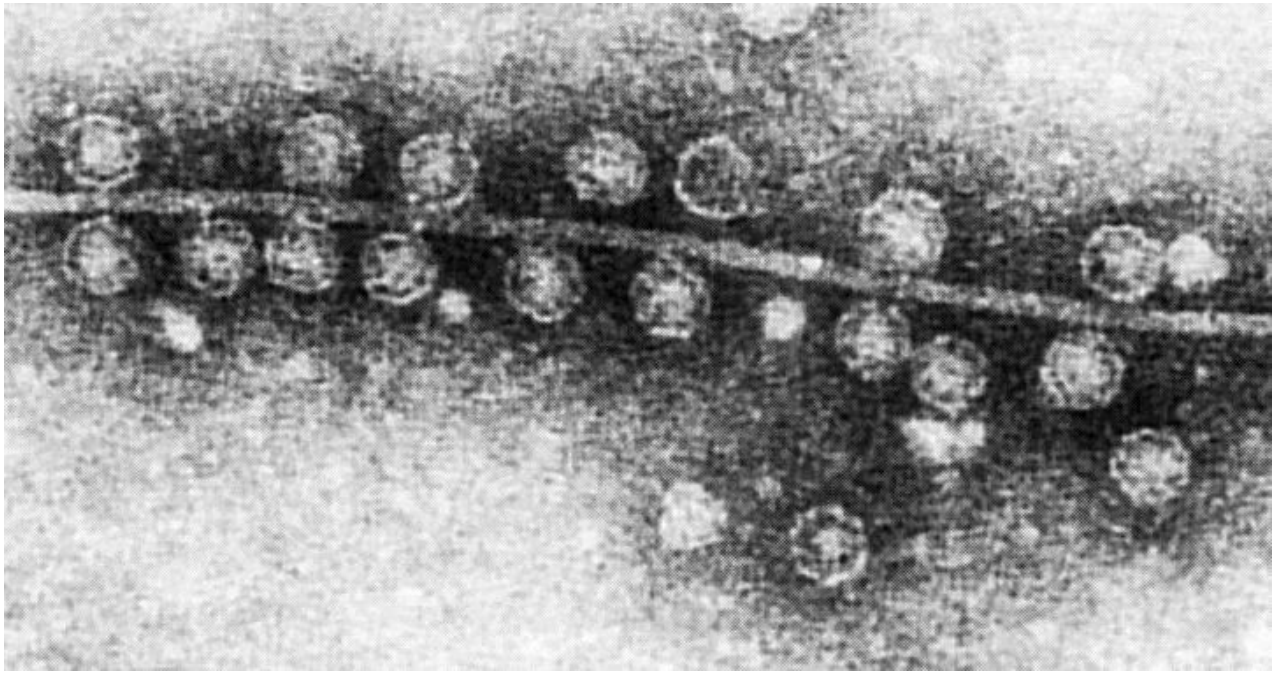
Can RNA evolve?

Can RNA replicate itself?

The RNA world

Can RNA evolve?



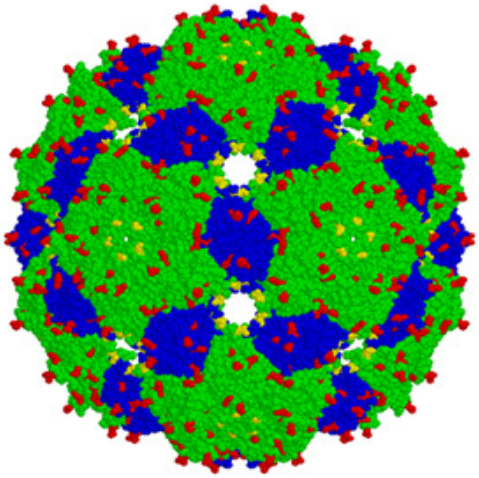


Spiegelman's monster

The RNA world

The bacteriophage Qβ – a virus containing RNA-dependent RNA polymerase (protein, enzymatic replicase)

Spiegelman's monster



Spiegelman mixed the Qβ RNA, the Qβ enzymatic replicase, mononucleotides and some salts (buffer). RNA replication begun.
An aliquot was transferred several times to a fresh solution without template.

Shorter RNA chains replicate faster. The selection in this system favors speed.
And no evolutionary pressure on pathogenicity was present anymore.
So the RNA became shorter and shorter due to random mutations during copying.

After 74 passages, the original 4500 nt RNA strand was reduced to 218 nt.
Such a short RNA chain replicated very quickly under these unnatural circumstances.
Of course, it lost all its genes and was unable to produce any useful proteins anymore.

First example of *in vitro* RNA evolution

Kacian D. L., Mills D. R., Kramer F. R., Spiegelman S. *PNAS* **1972**, *69*, 3038-3042.

Spiegelman's monster can be also formed by simple mixing of activated RNA monoers and the Qβ enzymatic replicase, in absence of any RNA template!

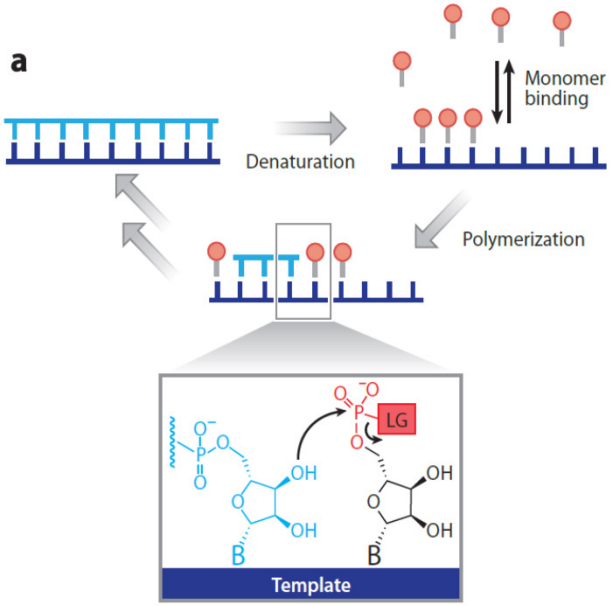
Sumper M., Luce R. *PNAS* **1975**, *72*, 162-166.

The RNA world

RNA self-replication

Nonenzymatic template-directed RNA polymerization

Maximally 30-50 nt extension, fidelity strongly sequence-dependent

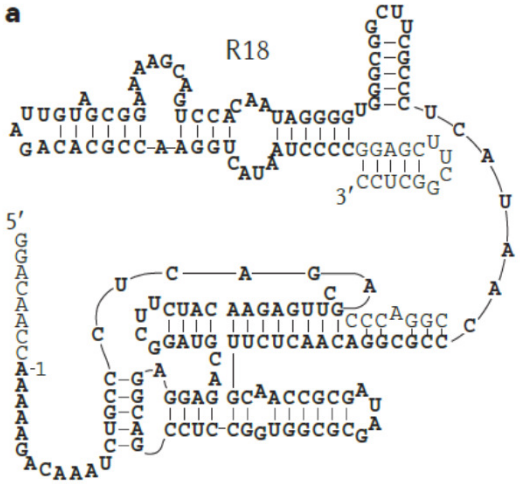


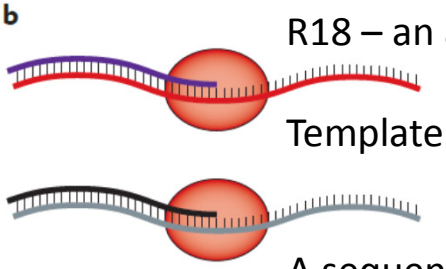
General RNA polymerase ribozyme (‚replicase')

Networks of RNA molecules that mutually catalyse their replication – autocatalytic replication of the whole network

The RNA world

RNA-dependent RNA polymerase ribozyme – Replicase - the ‚holy Grail' of the RNA world

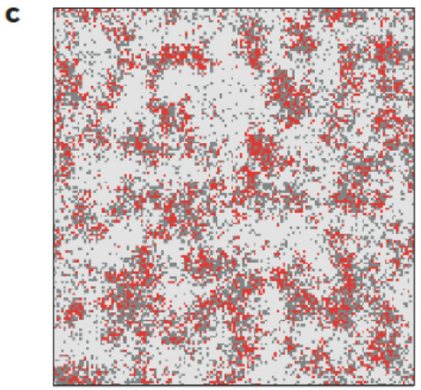


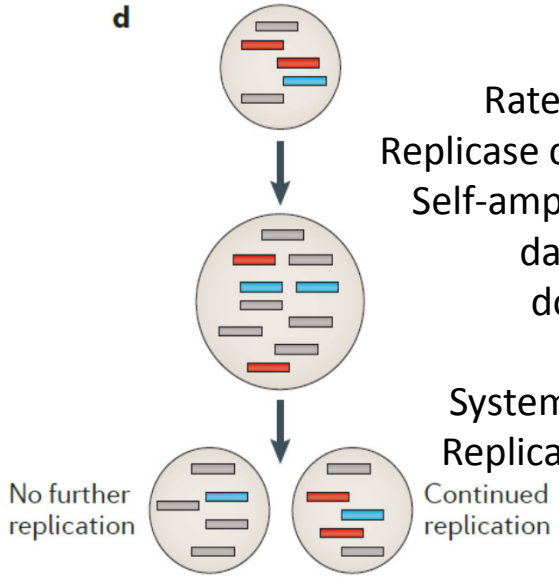


R18 – an artificial polymerase evolved from the class I ligase ribozyme.

Template: another copy of itself (red) or an unrelated sequence (grey).

A sequence of 206 nt was copied (fidelity 97.4%) at low temperatures by an engineered R18 mutant – first ribozyme capable to synthesize RNA oligomers longer than itself (though NO self-replication yet!)





Rate of replication not sensitive on the template's sequence.
Replicase could replicate other ribozymes (e.g. with metabolic functions).
Self-amplifying replicase needs a working complementary replicase – danger of paraistes (templates that copy themselves but do not contribute to the replication of the polymerase).

Systems of altruistic replicators are destroyed by parasites (grey).
Replicators (red) can survive e.g. by diffusion on 2D surfaces (**c**) or selection inside compartments (**d**)

Johnston, W. K., Unrau, P. J., Lawrence, M. S., Glasner, M. E. & Bartel, D. P. *Science* **2001**, *292*, 1319–1325.

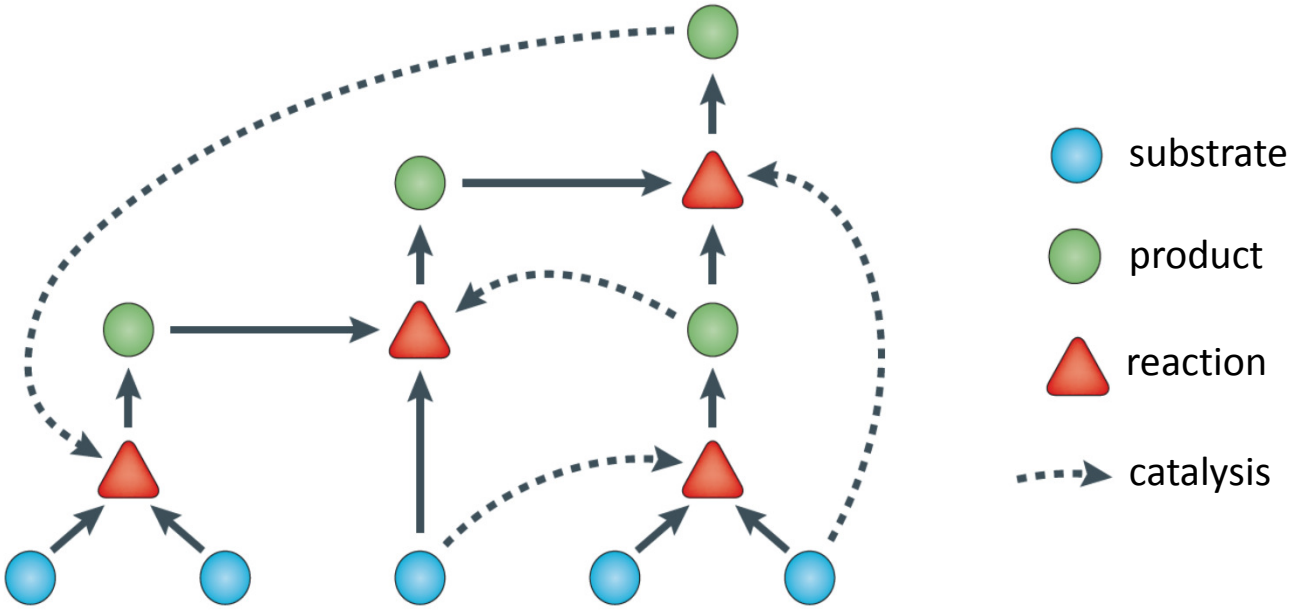
Attwater, J., Wochner, A. & Holliger, P. *Nature Chem.* **2013**, *5*, 1011–1018.

The RNA world

Replicase - problem

The replicase most likely needs to be long (> 200 nt) for the efficient replication –
How could such long fucntional RNA be spontaneously generated?

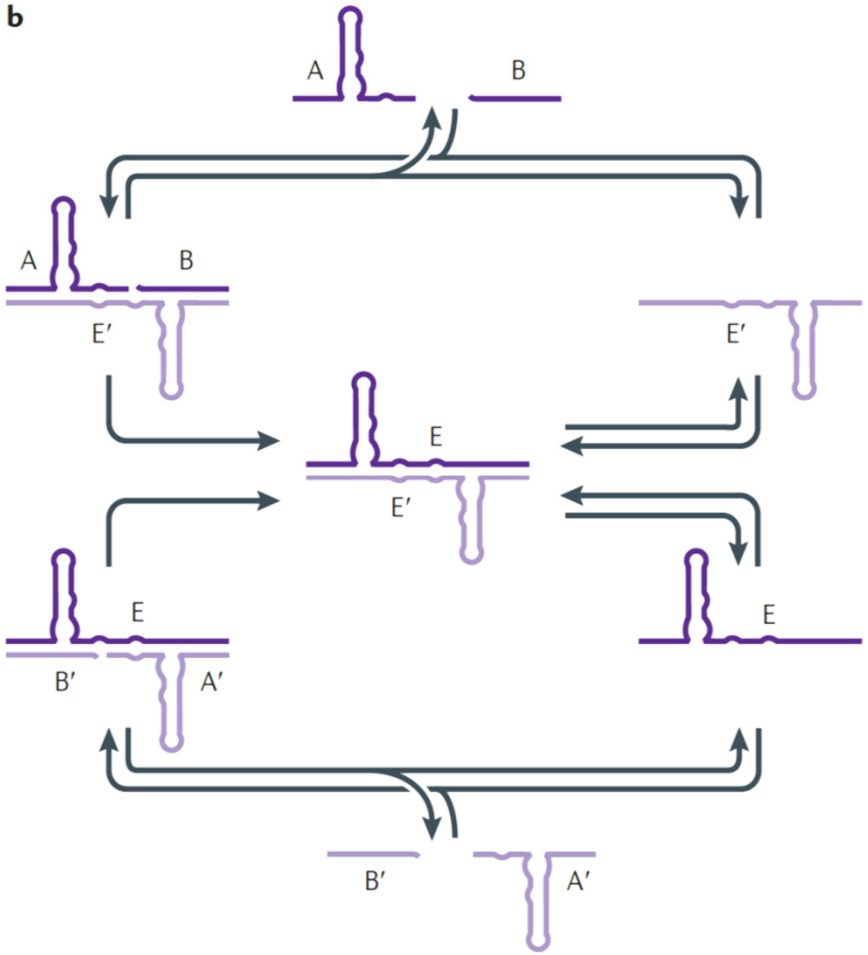
Possible solution – autocatalytic networks



No component can replicate without all the others

The RNA world

Mutually autocatalytic RNA networks

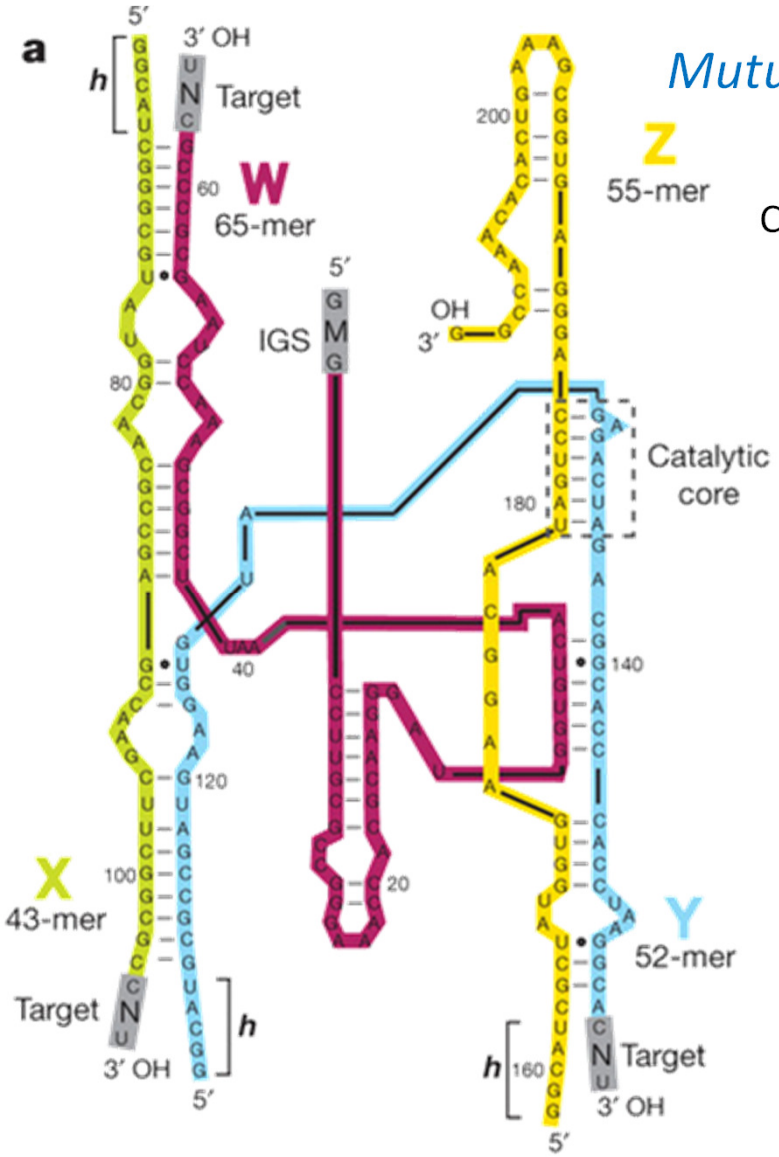


An autocatalytic set composed of two cross-catalytic ligases was demonstrated. RNA A and RNA B are ligated together by ribozyme E‘ to create ribozyme E, which can reciprocate and ligate RNA A‘ and RNA B’ to create ribozyme E‘.

Lincoln, T. A. & Joyce, G. F. *Science* **2009**, *323*, 1229–1232.

The RNA world

Mutually autocatalytic RNA networks

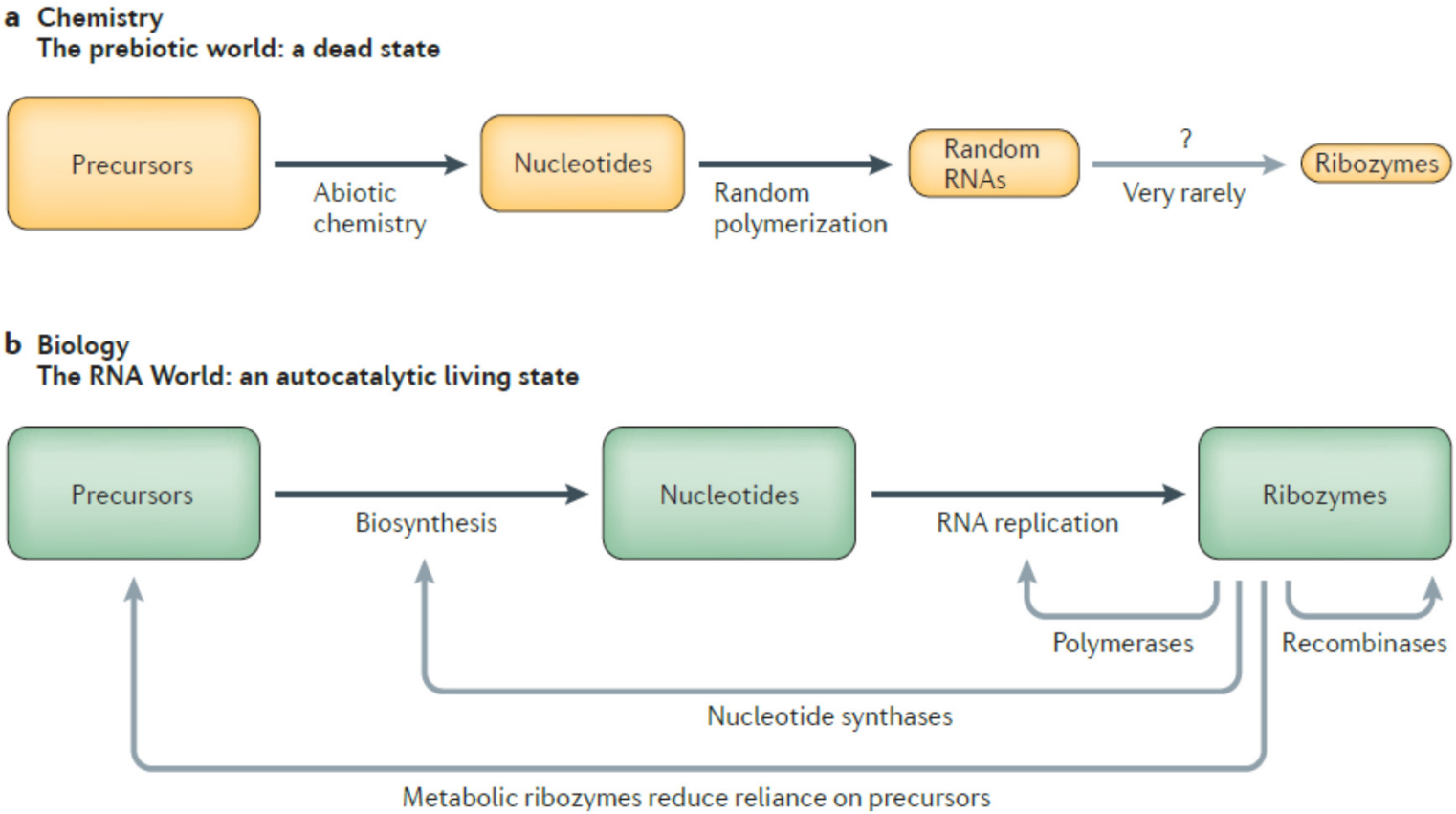


Cooperation between multiple strands that assemble to perform a single function.

Ribozymes, such as the *Azoarcus* recombinase, can be made from several short strands that assemble as a result of RNA secondary structure formation and information contained in internal guide sequences (IGSs) and complementary targets (grey).

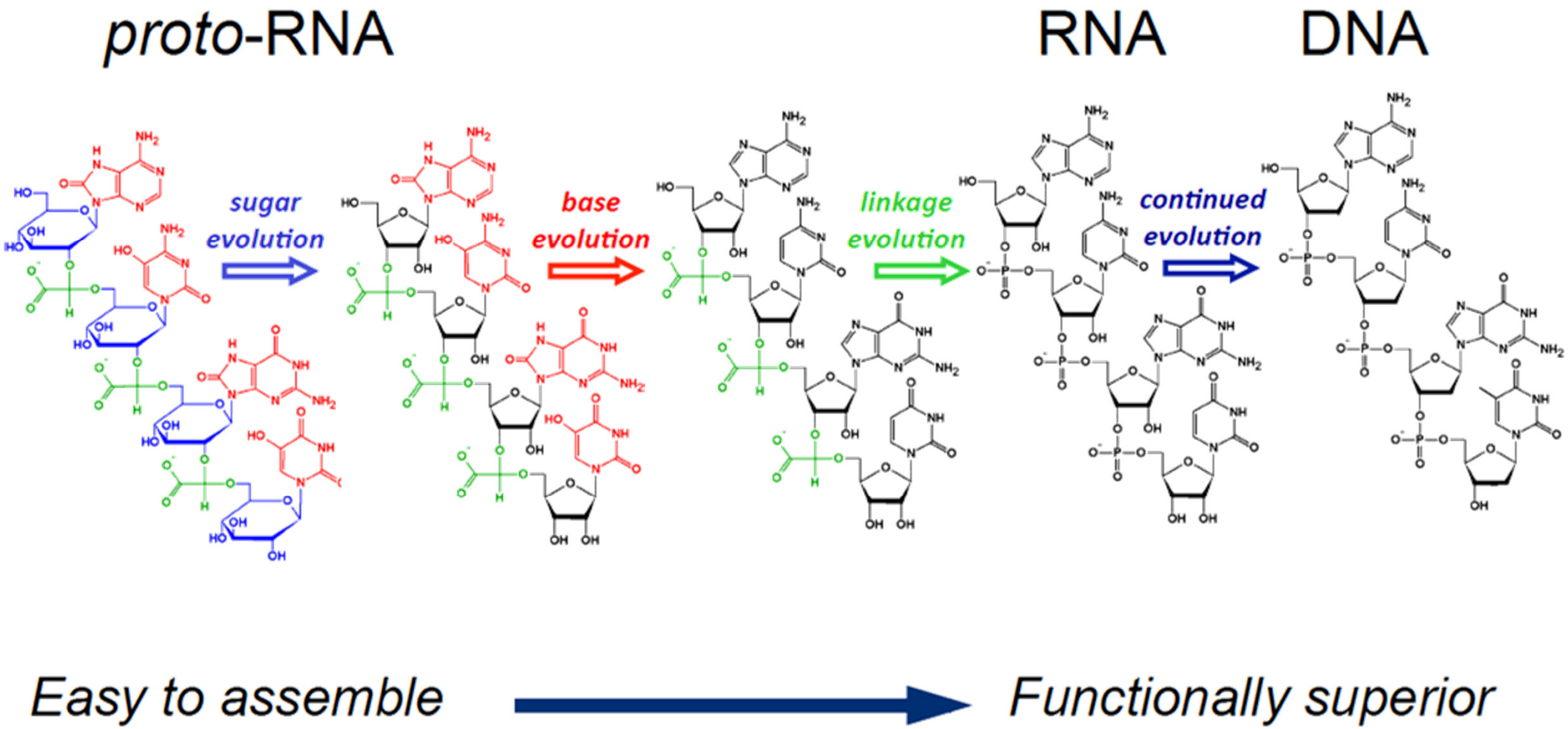
Vadia, N. *et al. Nature* **2012**, *491*, 72-77.

The RNA world



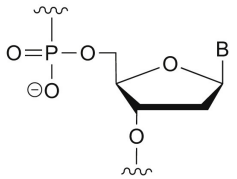
Transition from chemistry to biology involves autocatalytic feedbacks from ribozymes to all stages of the prebiotic chemistry

„RNA-second”

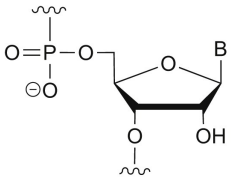


Proto-RNA evolution: According to the protoRNA theory, each of the components of RNA — sugar, base and phosphate backbone — may have originally taken different forms.

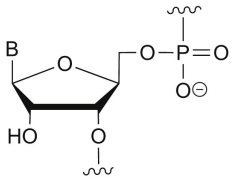
Artificial genetic polymers



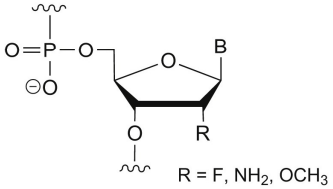
DNA



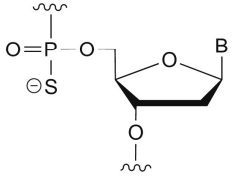
D-RNA
(natural)



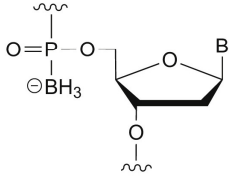
L-RNA
(unnatural)



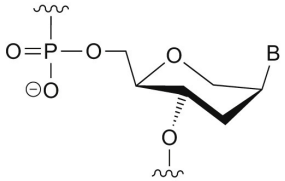
2'-modified RNA



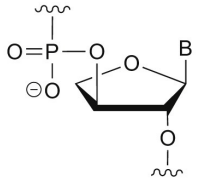
Phosphorothioate



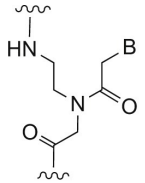
Boranophosphate



Hexitol Nucleic Acid
(HNA)

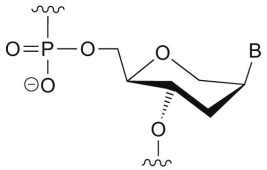


Threose Nucleic Acid
(TNA)

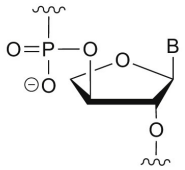


Peptide Nucleic Acid
(PNA)

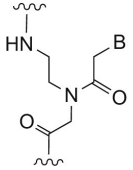
XNA – Xeno Nucleic Acids



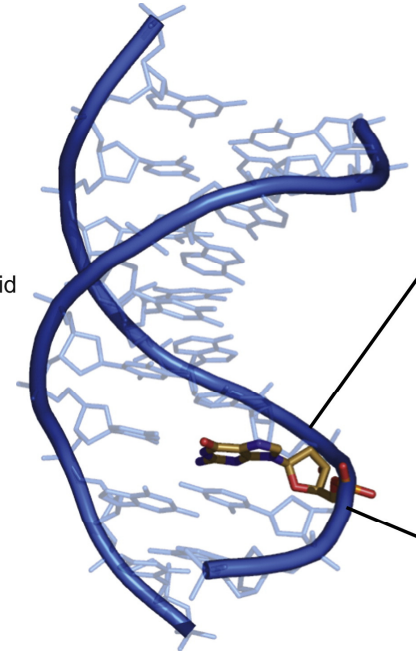
Hexitol Nucleic Acid
(HNA)

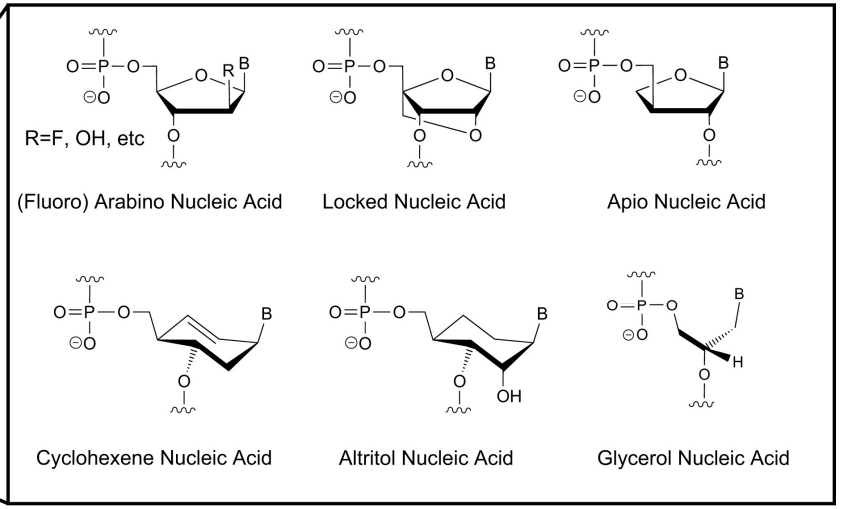


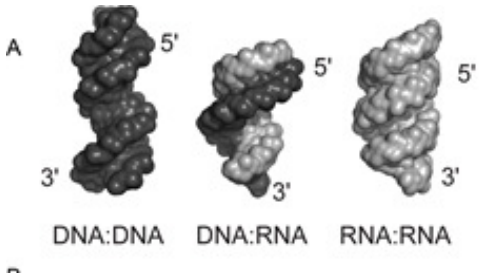
Threose Nucleic Acid
(TNA)

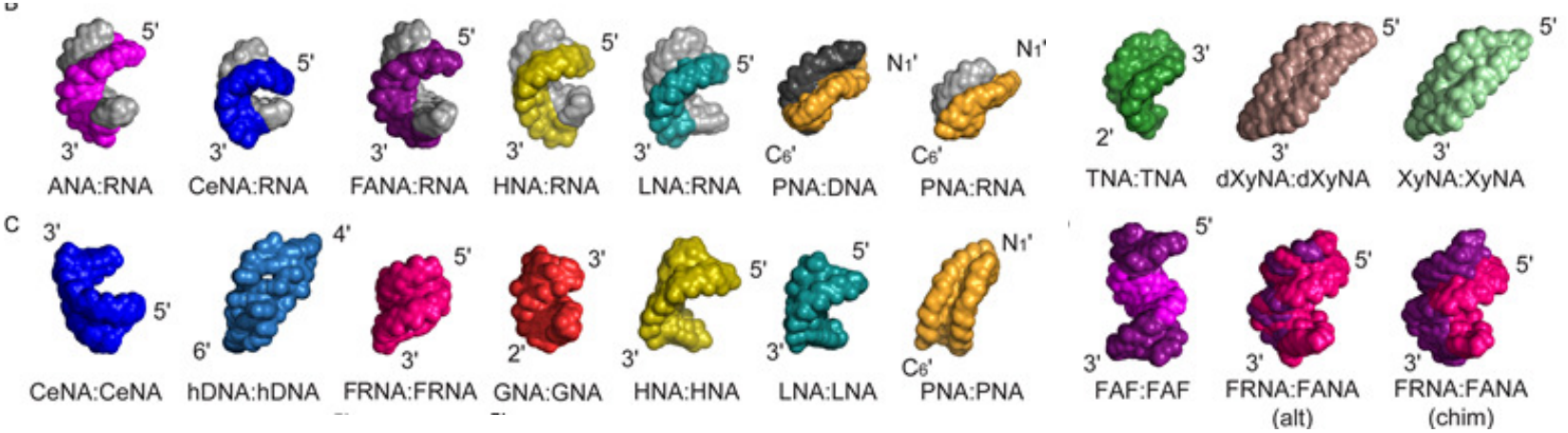


Peptide Nucleic Acid
(PNA)

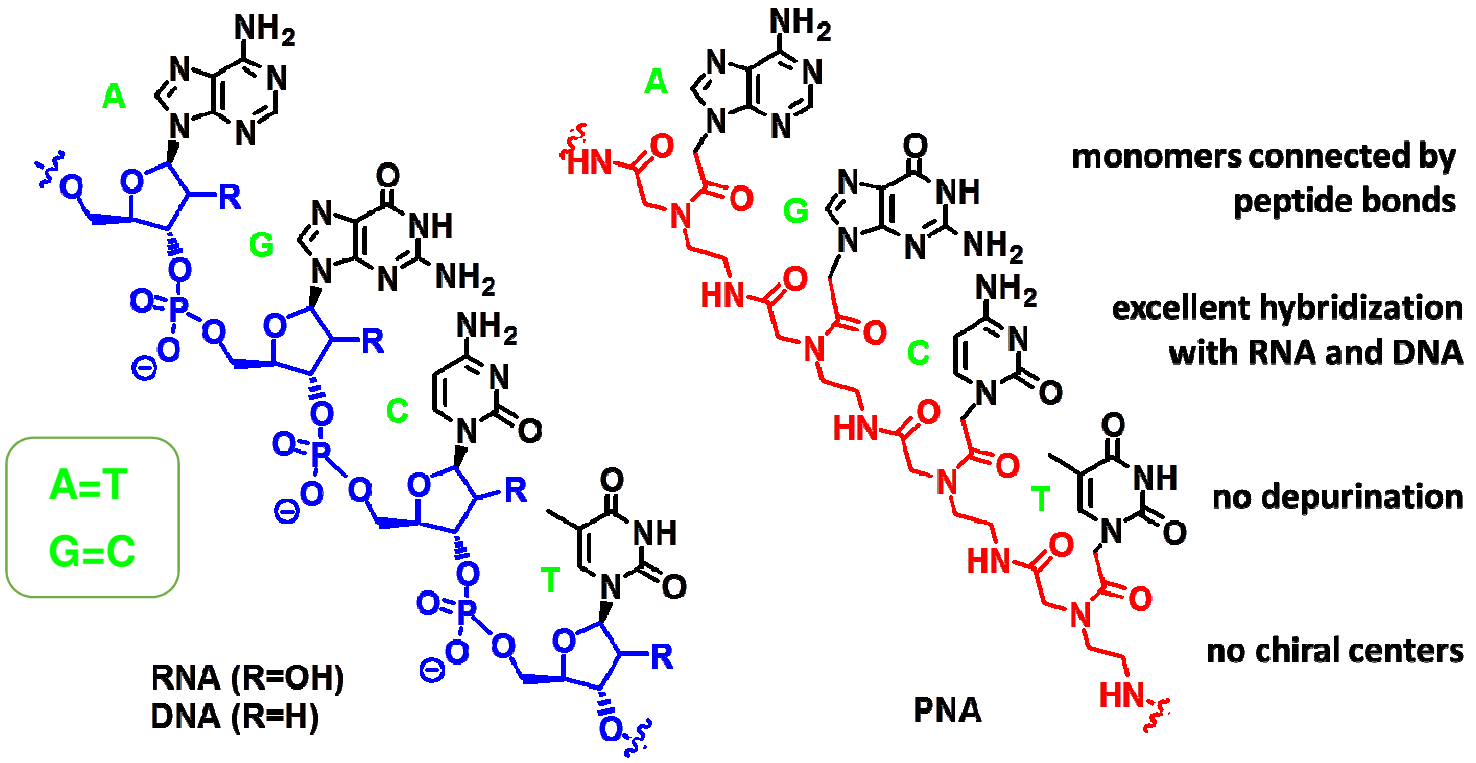








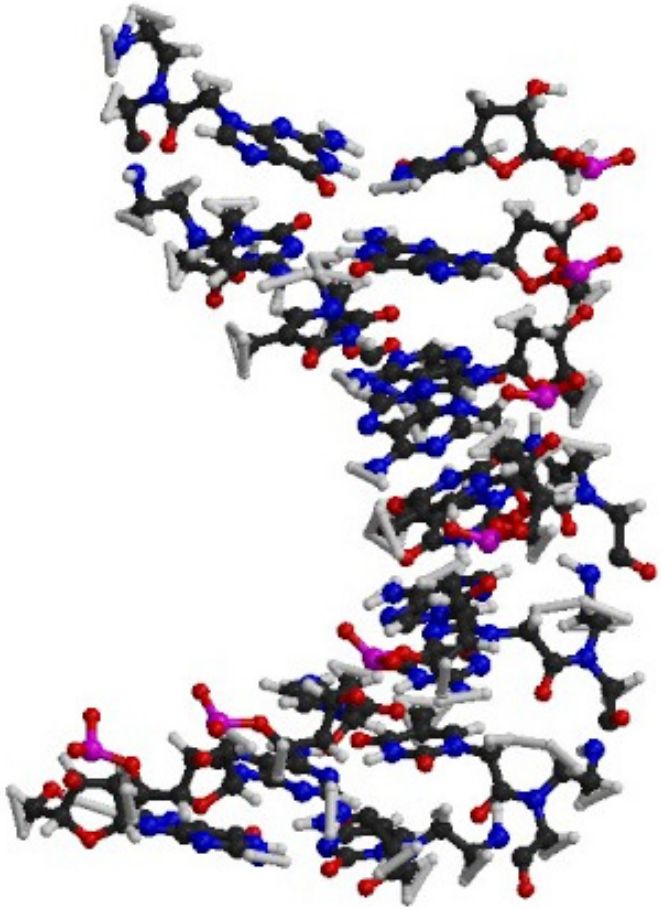
Peptidonucleic acids – functional DNA analogues



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

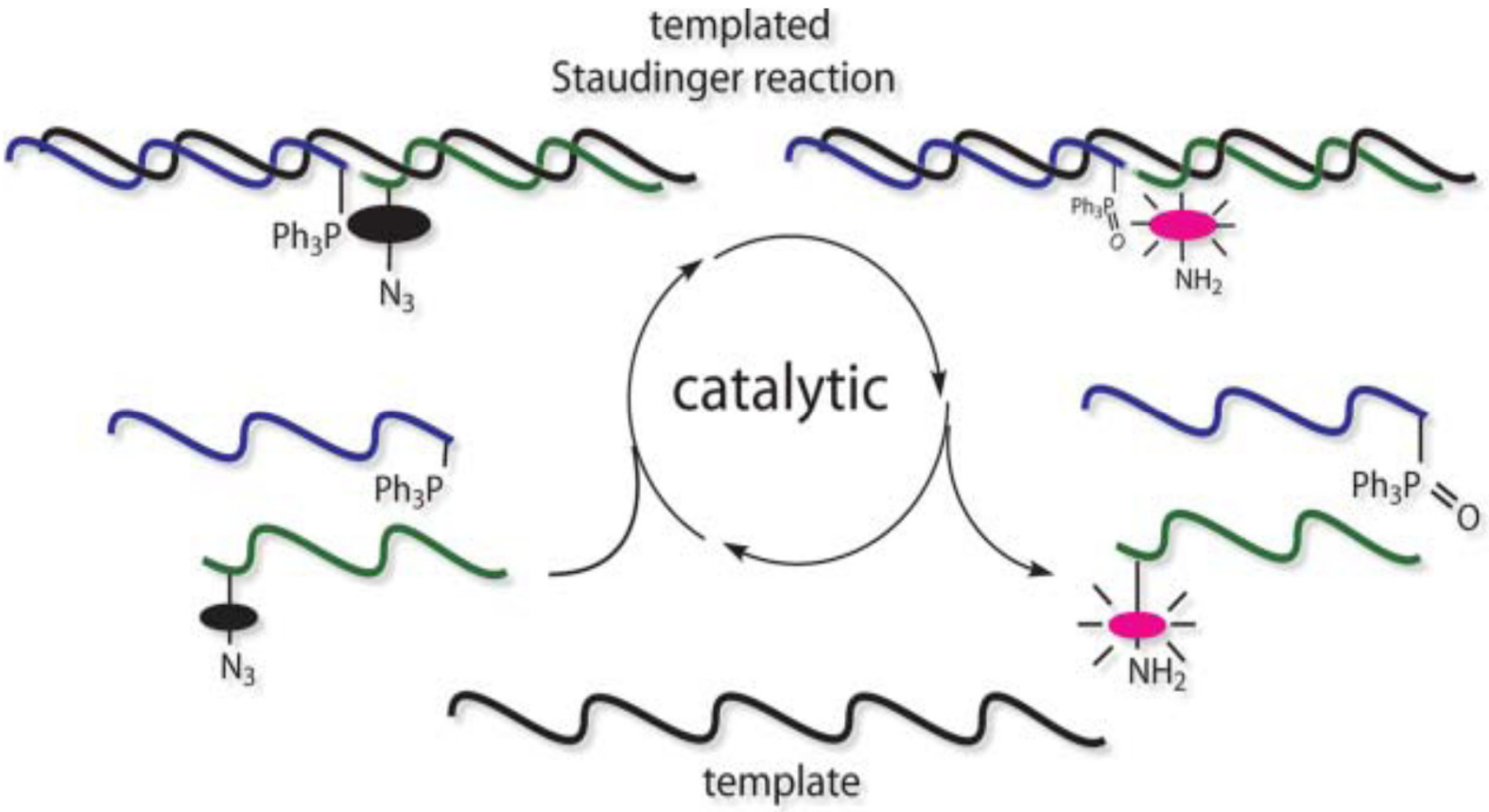
Peptidonucleic acids – functional DNA analogues



PNA-DNA duplex, NMR structure
PDB entry: 1PDT

PNA – dsDNA strand invasion due to lack of electrostatic repulsion

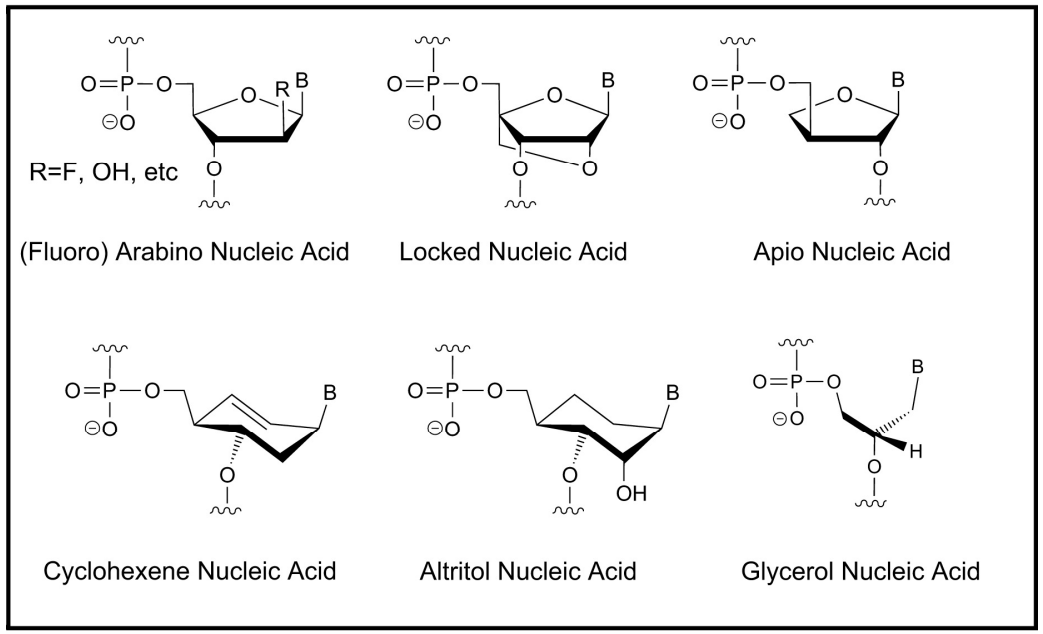
Bioorthogonal templated reaction for detection of oligonucleotides in vivo

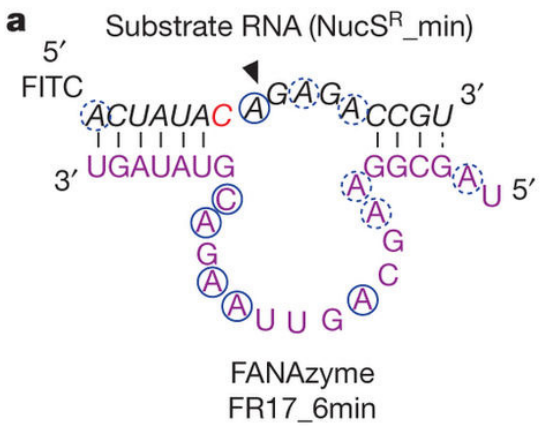


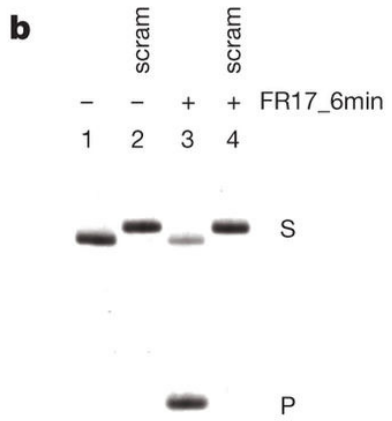
Z.Pianowski, N.Winssinger *Chem. Commun.* **2007**, *37*, 3820

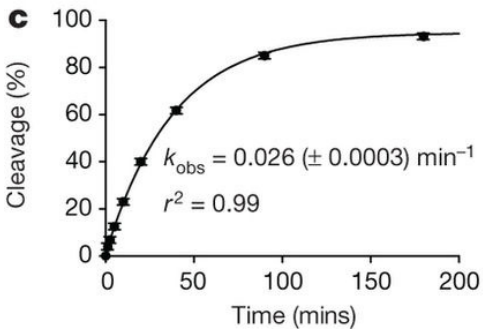
Z.Pianowski, K. Górska, L. Oswald, C. Merten, N.Winssinger *J. Am. Chem. Soc.* **2009,** *131,* 6492-6497

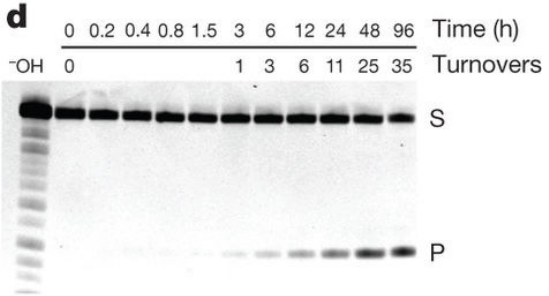
Chemical synthesis yields an active RNA endonuclease XNAzyme







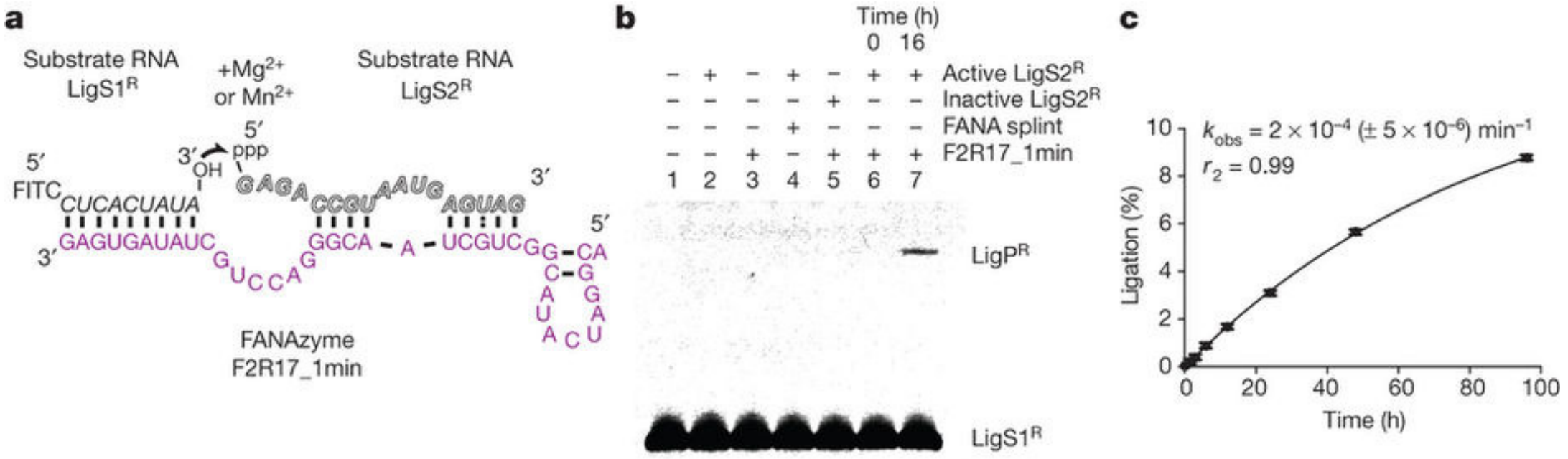




a, Secondary structure of truncated FANAzyme FR17_6 (FR17_6min, purple)
b, FR17_6min synthesized using FANA phosphoramidites cleaves cognate RNA substrate (NucSR_min; lanes 1 and 3), but not a scrambled RNA (NucSR SCRAM2; lanes 2 and 4), with...
c, essentially unchanged catalytic rate (k_{obs}) at 25 °C.
d, FR17_6min (10 nM) can perform multiple turnover cleavage of RNA NucSR_min (1 µM).

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

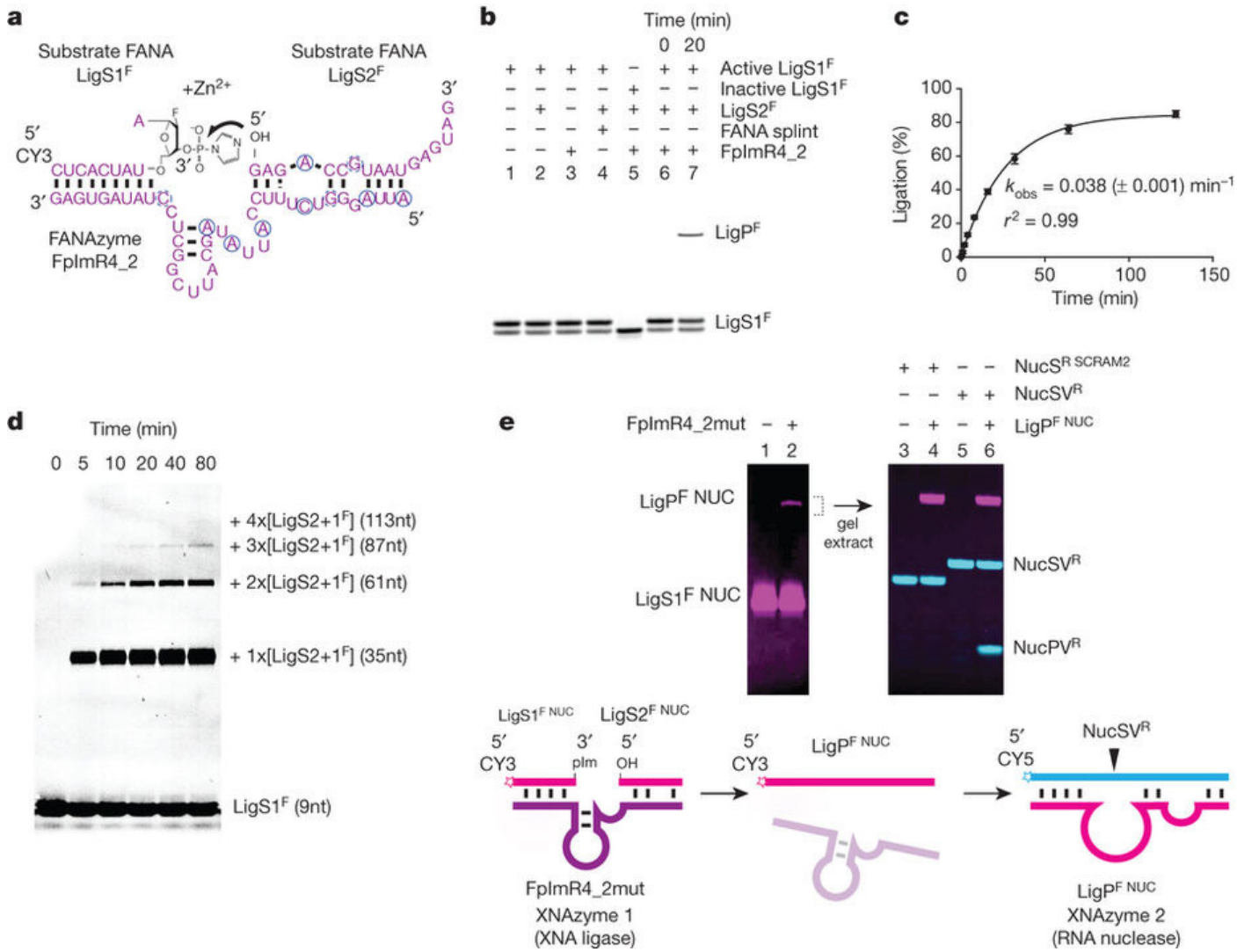
An RNA ligase XNAzyme (FANA)



a, Putative secondary structure of truncated chemically synthesized FANAzyme (F2R17_1min, purple) that ligates RNA substrate LigS1R to LigS2R, activated with 5′ triphosphate (ppp), in a trimolecular reaction in trans.
b, Urea–PAGE gel showing no significant product (LigPR) observed with: substrate LigS1R alone (lane 1), no XNAzyme (lane 2), no LigS2R (lane 3), complementary FANA splint (lane 4), or LigS2R lacking 5′ppp (lane 5); product formation is dependent on LigS1R, activated LigS2R and XNAzyme (lanes 6 and 7). No product was detectable with combinations of RNA, DNA or FANA versions of LigS1 and (5′ppp)LigS2, except DNA LigS1 and RNA LigS2, which showed ~1.5% ligation after 20 h (Extended Data Fig. 7g).
c, 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



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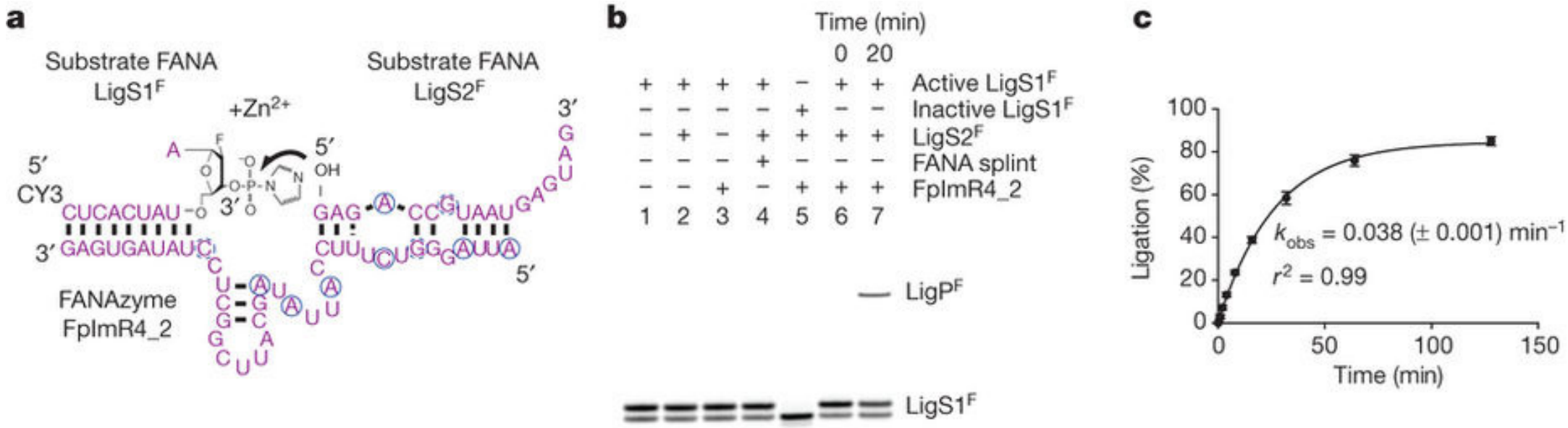

a, Secondary structure of chemically synthesized FANAzyme FpImR4_2, which ligates FANA LigS1F, activated with 3′ phosphorylimidazolide (pIm), to LigS2F in trans.

b, Urea–PAGE gel showing no product with: substrate LigS1F alone (lane 1), no XNAzyme (lane 2), no LigS2F (lane 3), splint (lane 4), or LigS1F lacking 3′pIm (lane 5); product formation is dependent on LigS2F, activated LigS1F and XNAzyme (lanes 6 and 7).

c, Pre-steady state trimolecular reaction rate (k_{obs}) at 35 °C (n = 3; error bars, s.d.).

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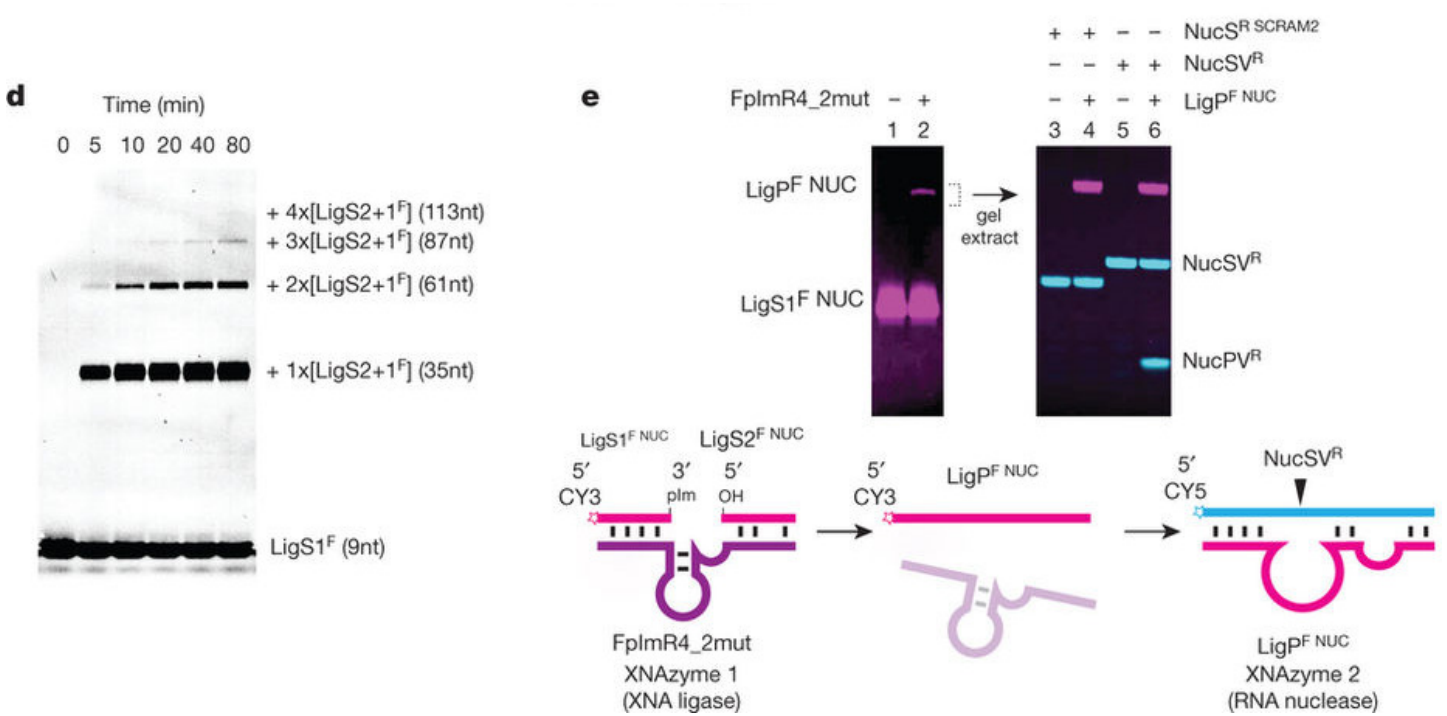

d, Urea–PAGE gel showing FpImR4_2-catalysed oligomerization of XNA (FANA) substrates. Substrate LigS2+1F is a 3′pIm-activated substrate containing the sequences of both LigS1F and LigS2F above.

e, Urea–PAGE gels and schematic diagram showing 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).

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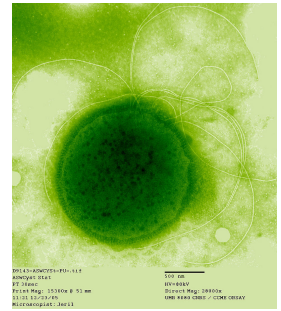
Engineering XNA polymerases

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

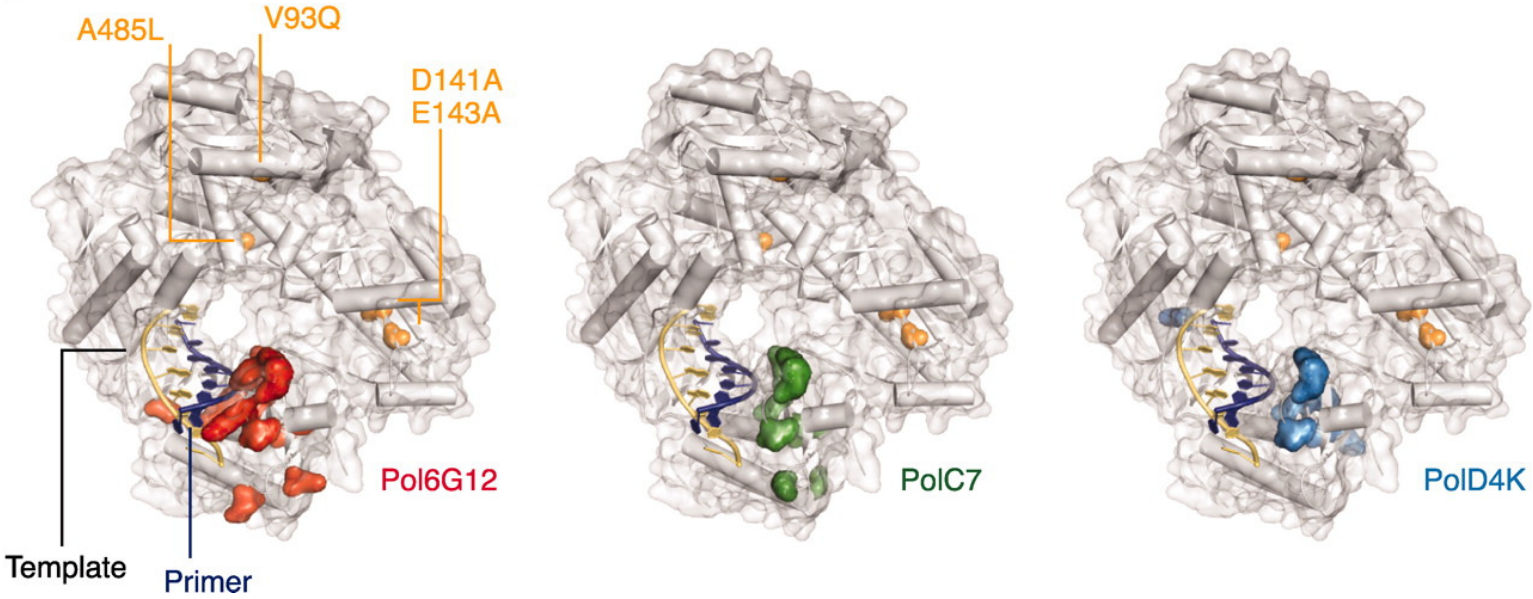


Thermococcus gorgonarius
(Angels Tapias)

A

	402	404	588	590	608	611	653	682	703	710	729	731
TgoT	YLD	..	FVT	..	LEIV	..	YEVPPEKLVIYEQITRDLKDYKATGPHVAV	..	VLKGSGRI	..	AEY	
Pol6G12	YLD	..	FAT	..	LKMV	..	YEVPPEQLVIYQPITKQLHDYRARGPHVSV	..	VPKGSGRI	..	AGY	
PolC7	YLD	..	FVT	..	LEIV	..	YQVPPQQLAIYQPITRALQDYKAKGPHVAV	..	VLKGSGKI	..	AEY	
PolD4K	YPD	..	FVT	..	LEIV	..	YEVPTQHLVIHKQITRALNDYKAIGPHVAV	..	VLKGSGRI	..	AEY	

B



(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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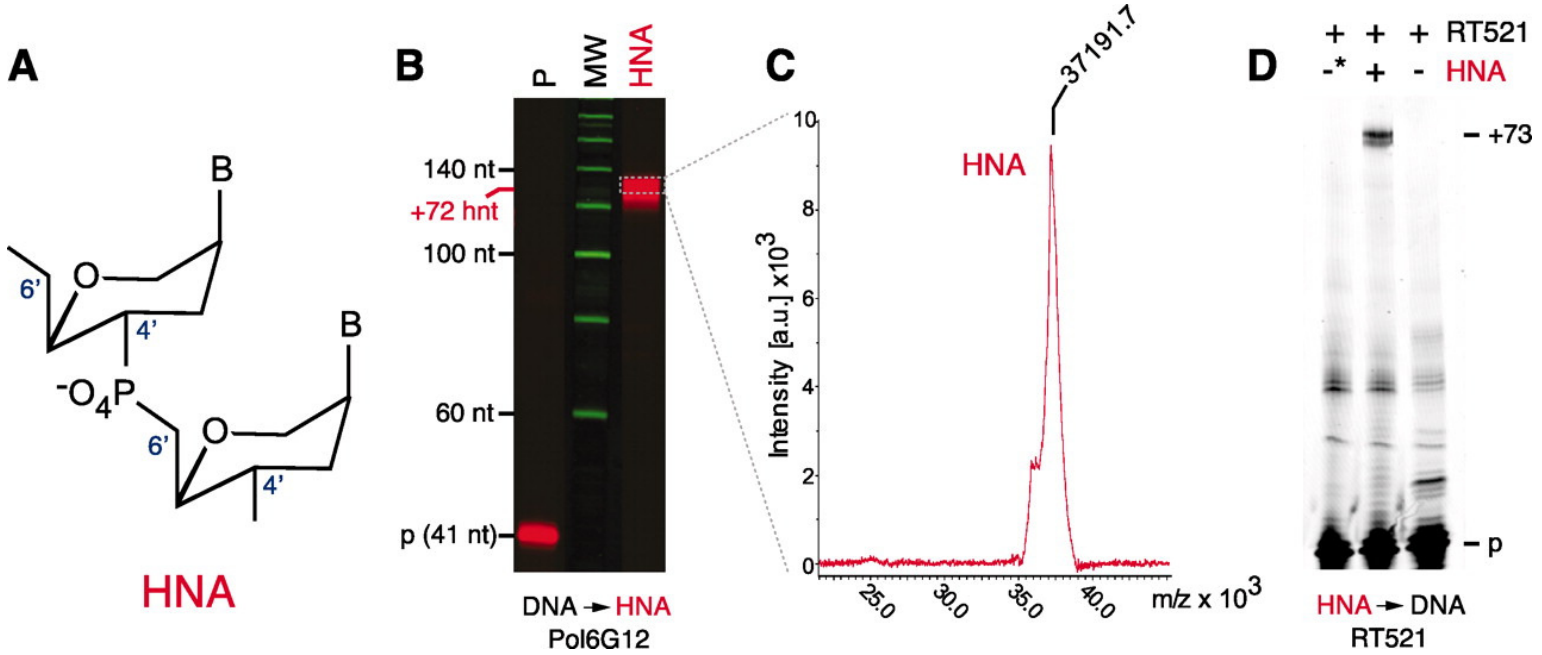
HNA synthesis



(A) Structure of 1,5-anhydrohexitol (HNA) nucleic acids (B, nucleobase).
(B) 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. (MW - ILS 600 molecular weight marker. P - primer-only reactions)
(C) MALDI-TOF spectrum of a full-length HNA molecule showing a measured HNAmass of 37,190 ± 15 daltons (n = 3 measurements). a.u., arbitrary units; m/z, mass-to-charge ratio.
(D) HNA reverse transcription (DNA synthesis from an HNA template). Polymerase-synthesized HNA (from template YtHNA4) is used as template by RT521 for HNA-RT (-* denotes a no HNA synthesis control to rule out template contamination).

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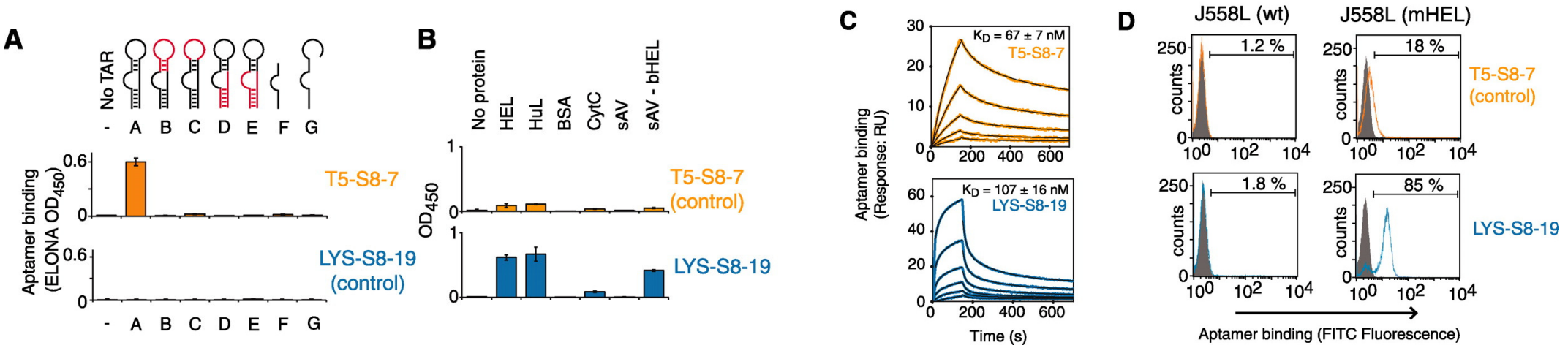
HNA aptamers



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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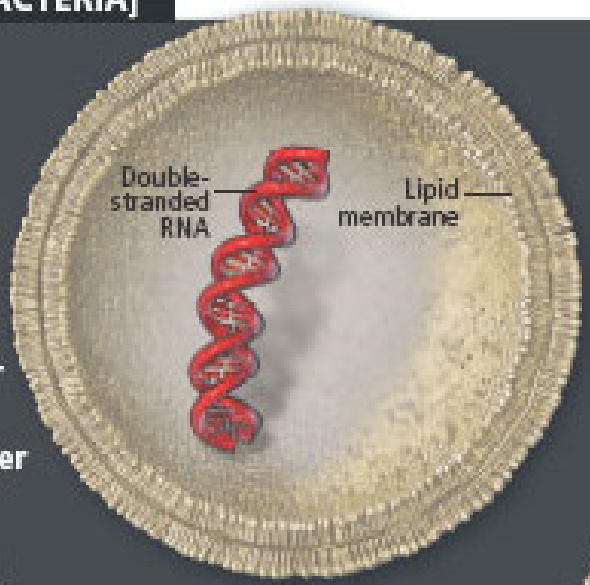
From RNA world to bacteria

[FROM RNA WORLD TO BACTERIA]

Journey to the Modern Cell

After life got started, competition among life-forms fueled the drive toward ever more complex organisms. We may never know the exact details of early evolution, but here is a plausible sequence of some of the major events that led from the first protocell to DNA-based cells such as bacteria.

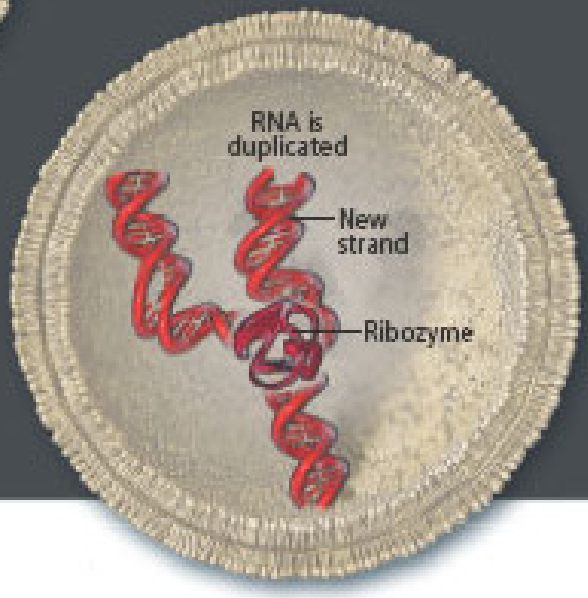


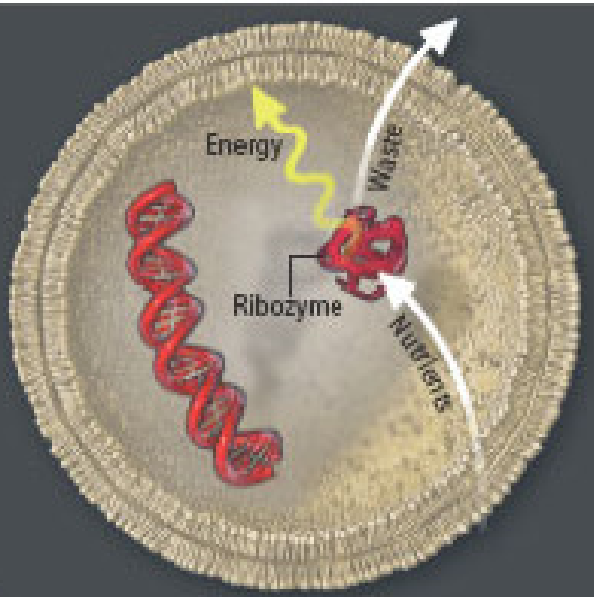
1 EVOLUTION STARTS ▲

The first protocell is just a sac of water and RNA and requires an external stimulus (such as cycles of heat and cold) to reproduce. But it will soon acquire new traits.

2 RNA CATALYSTS ▼

Ribozymes—folded RNA molecules analogous to protein-based enzymes—arise and take on such jobs as speeding up reproduction and strengthening the protocell's membrane. Consequently, protocells begin to reproduce on their own.





3 METABOLISM BEGINS ▲

Other ribozymes catalyze metabolism—chains of chemical reactions that enable protocells to tap into nutrients from the environment.

From RNA world to bacteria