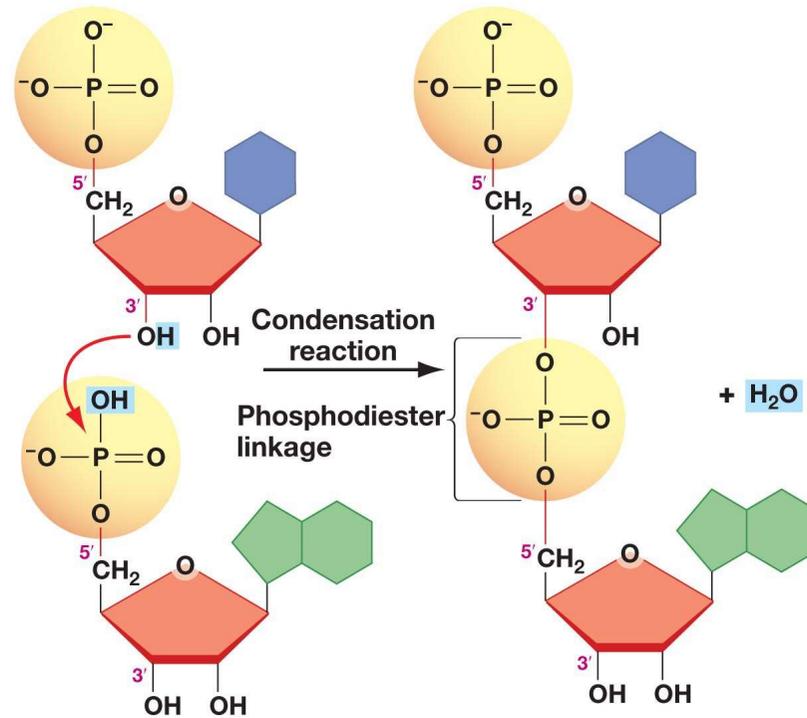
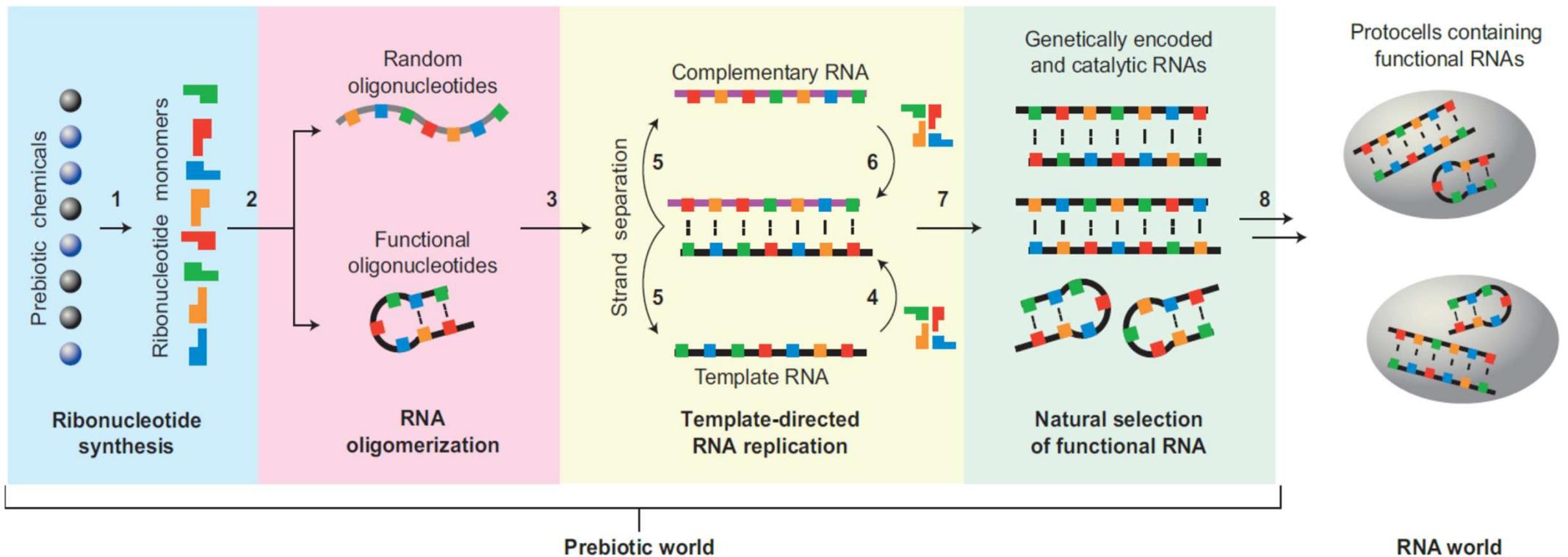


# Nucleotide polymerization

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

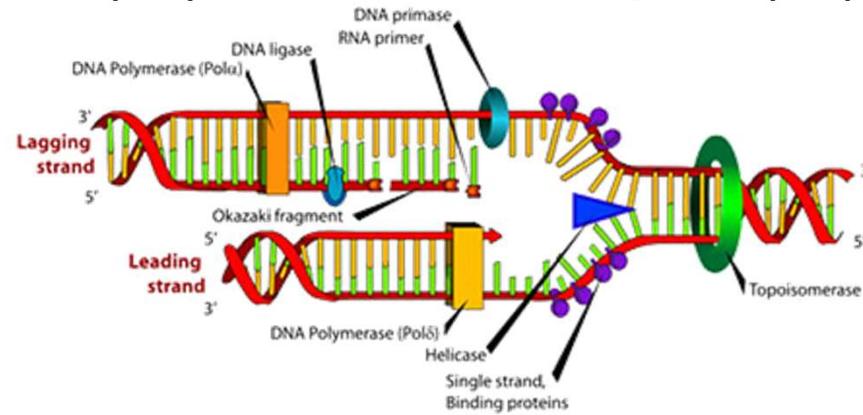


# Nucleotide polymerization – sequence control

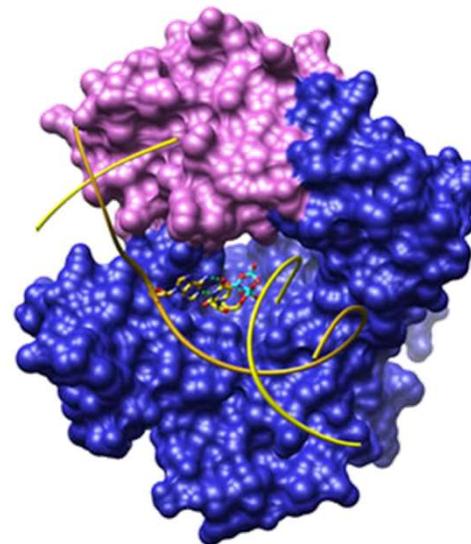
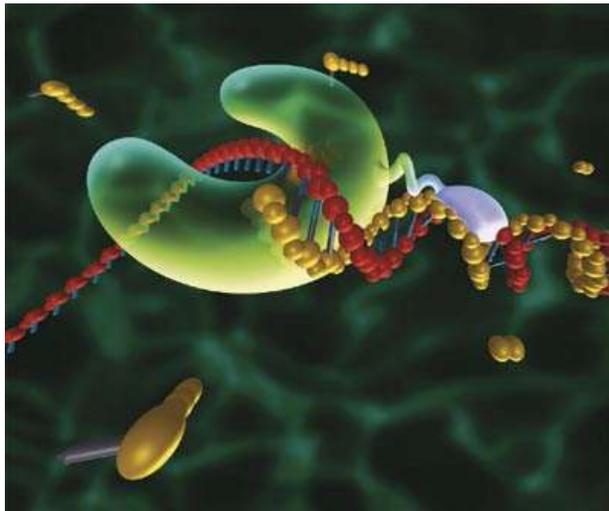


## Vital chemical reactions

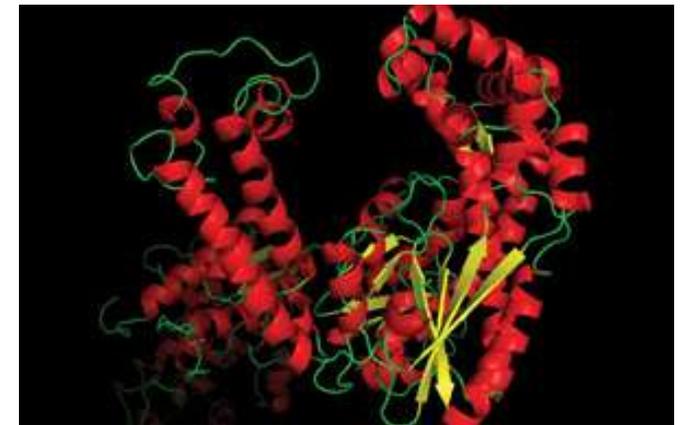
nucleotide polymerization → DNA/RNA polymerases



[dxline.info/img/new\\_ail/dna-polymerase\\_1.jpg](http://dxline.info/img/new_ail/dna-polymerase_1.jpg)



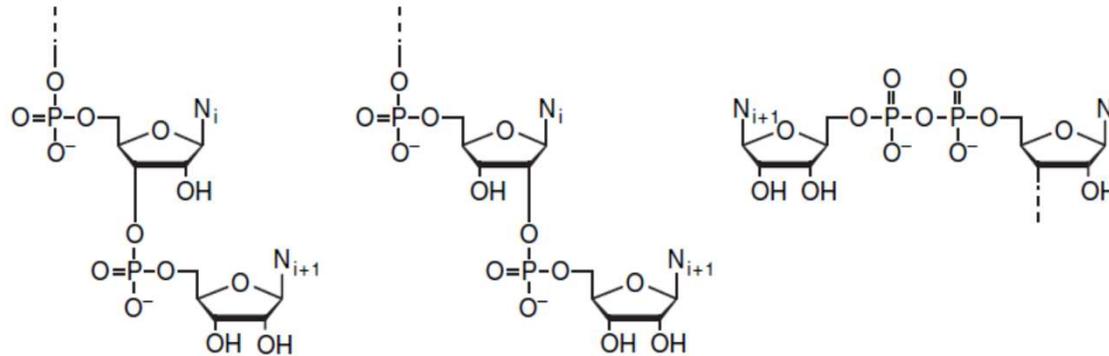
[niehs.nih.gov](http://niehs.nih.gov)



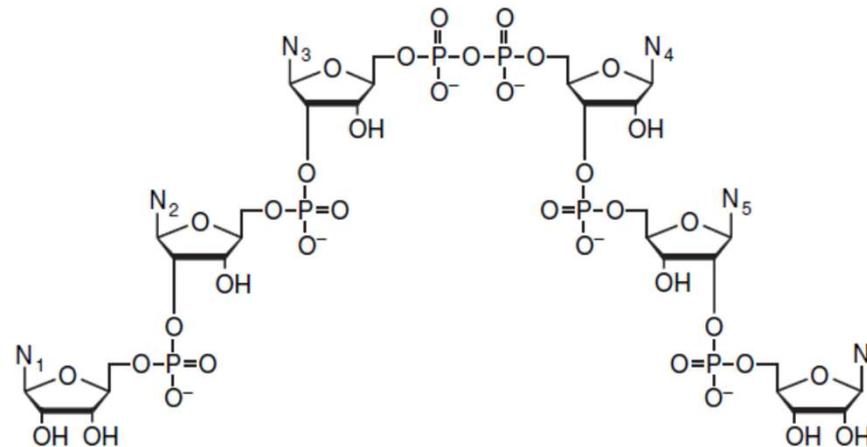
[www.neb.com](http://www.neb.com)

## Products of chemical condensation of nucleotides

**A**



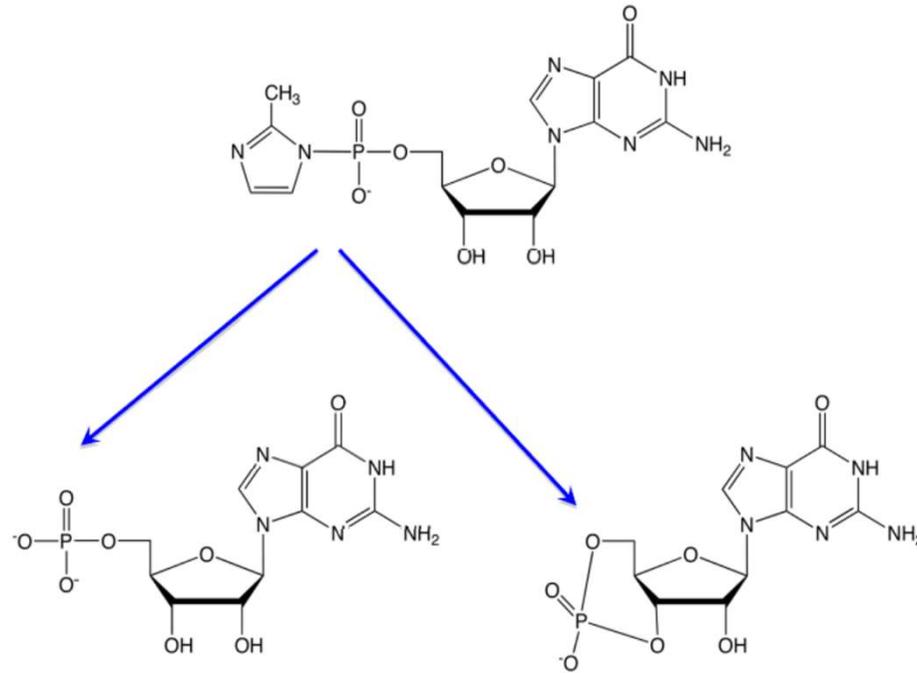
**B**



**(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

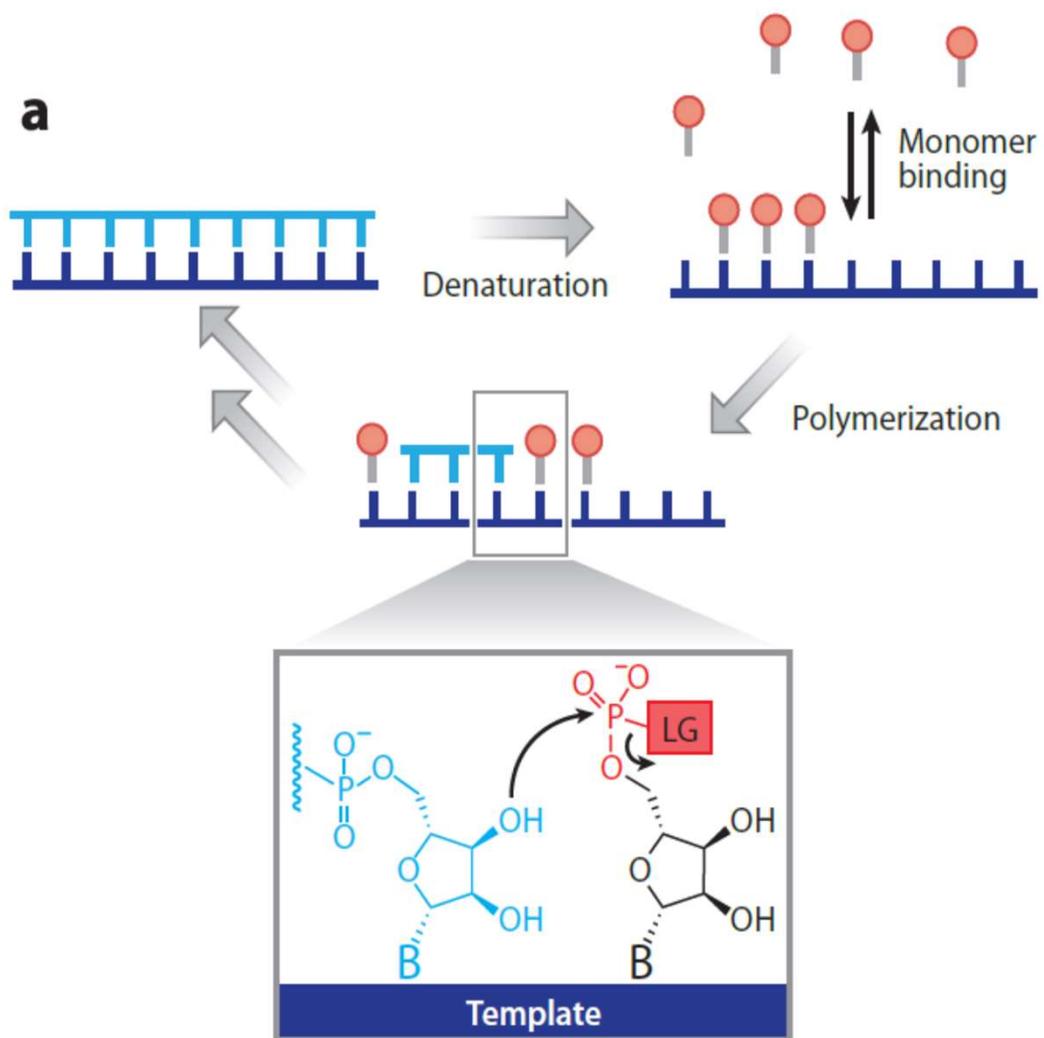
## *Degradation of activated nucleotides*



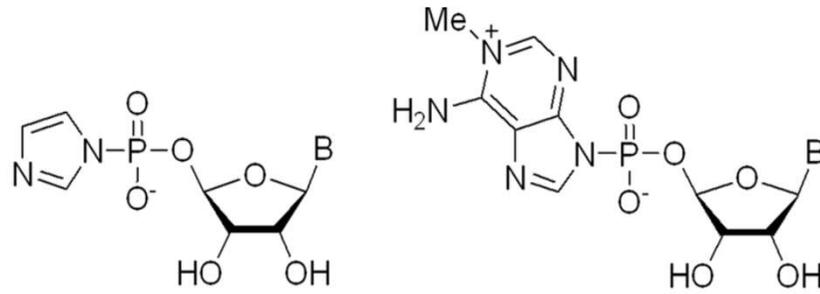
hydrolysis

3',5'-cyclization

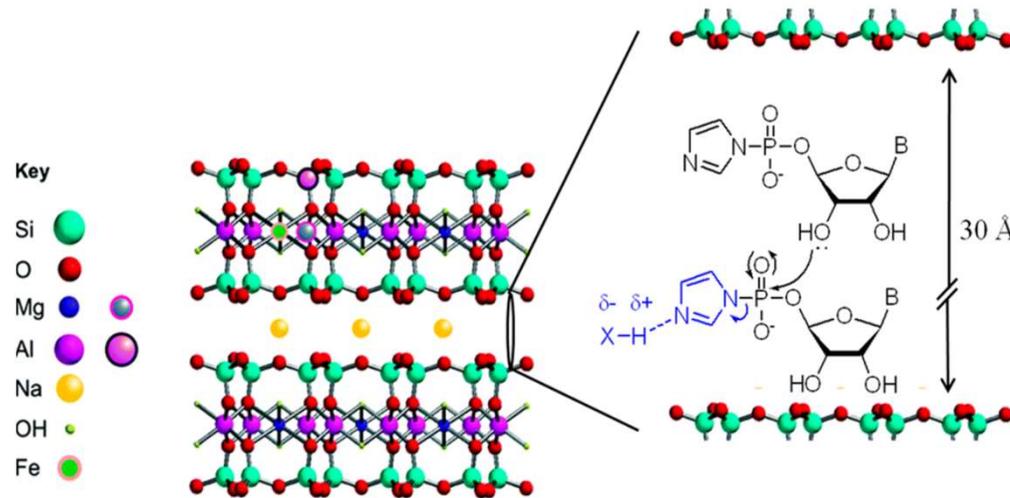
## Template-directed synthesis



## Montmorillonite



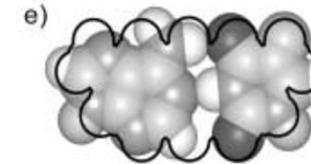
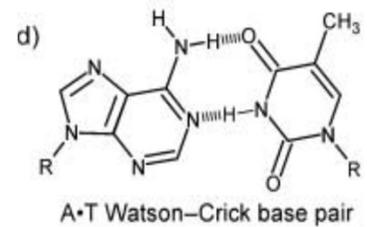
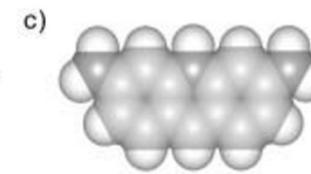
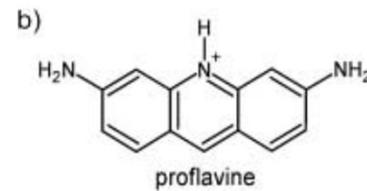
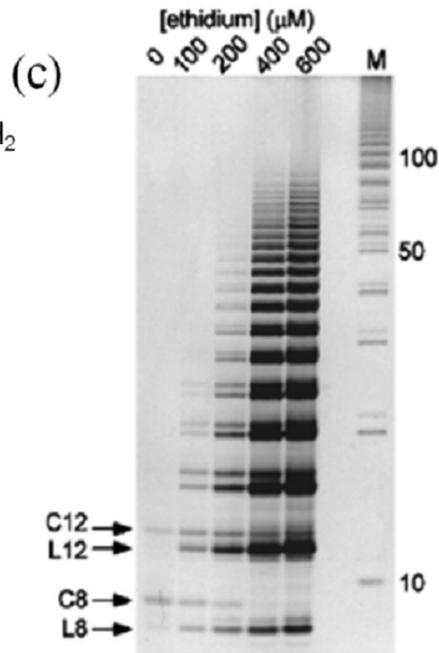
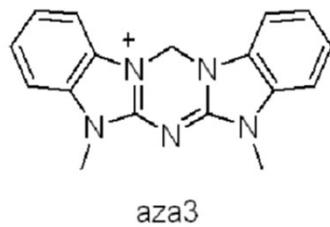
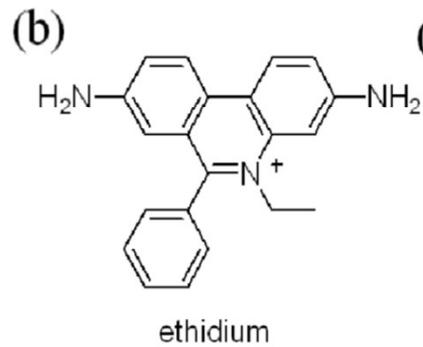
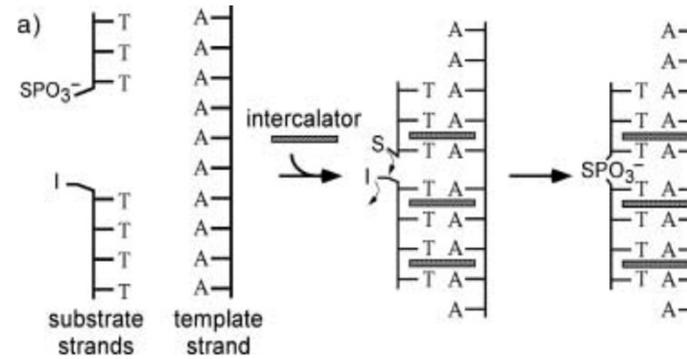
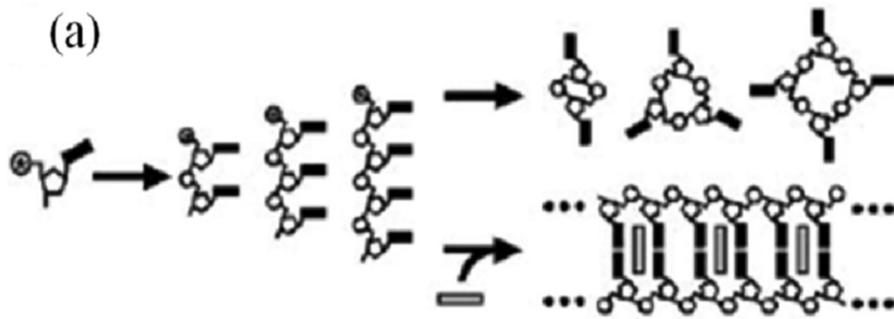
B = adenine, guanine, cytosine or uracil



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

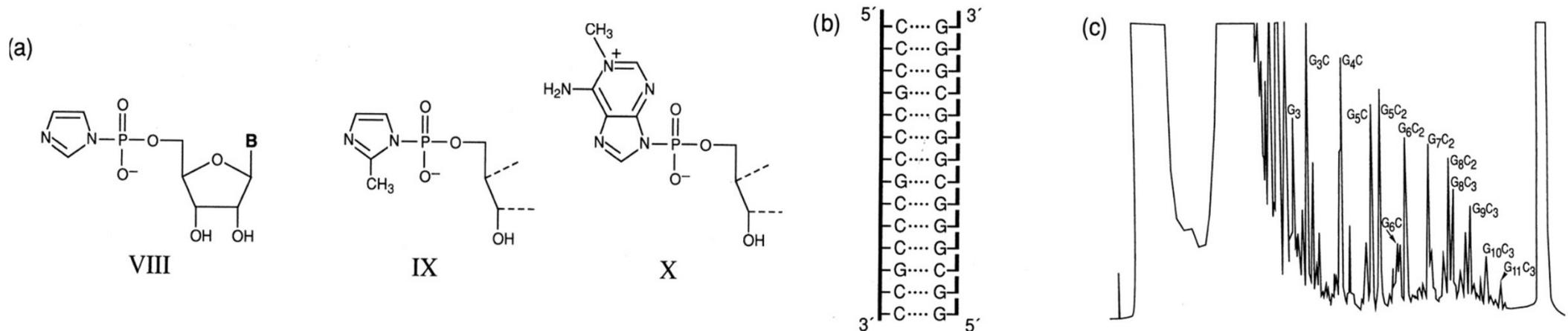


*Rate increase by three orders of magnitude vs. ligation without proflavine*

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

## Template-directed synthesis

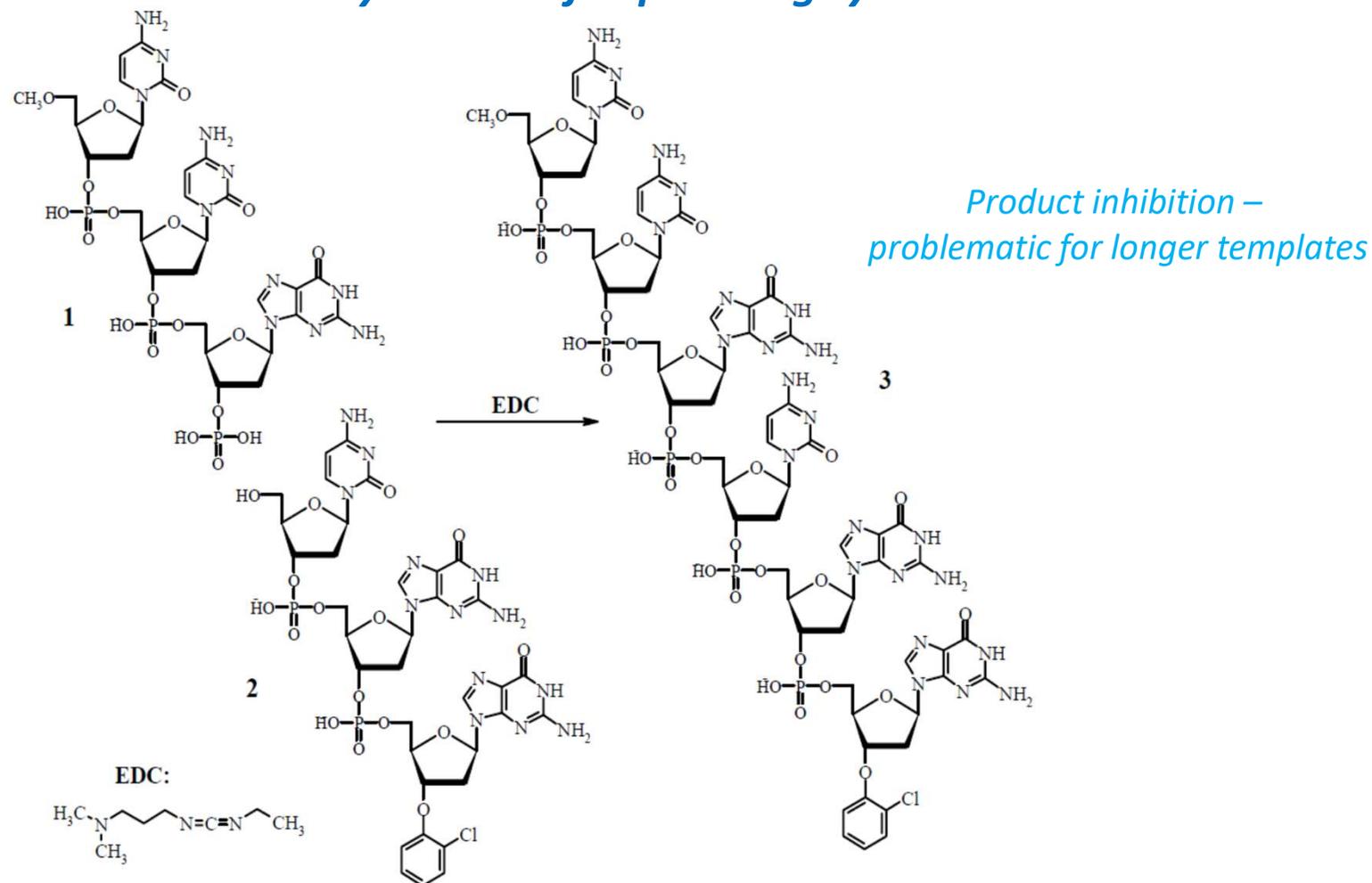
*no example demonstrated yet, where single activated nucleotides would form a complementary strand on an RNA (or DNA) template without enzymatic support*



Current experiments focus on *primer extension* 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

## First non-enzymatic self-replicating system

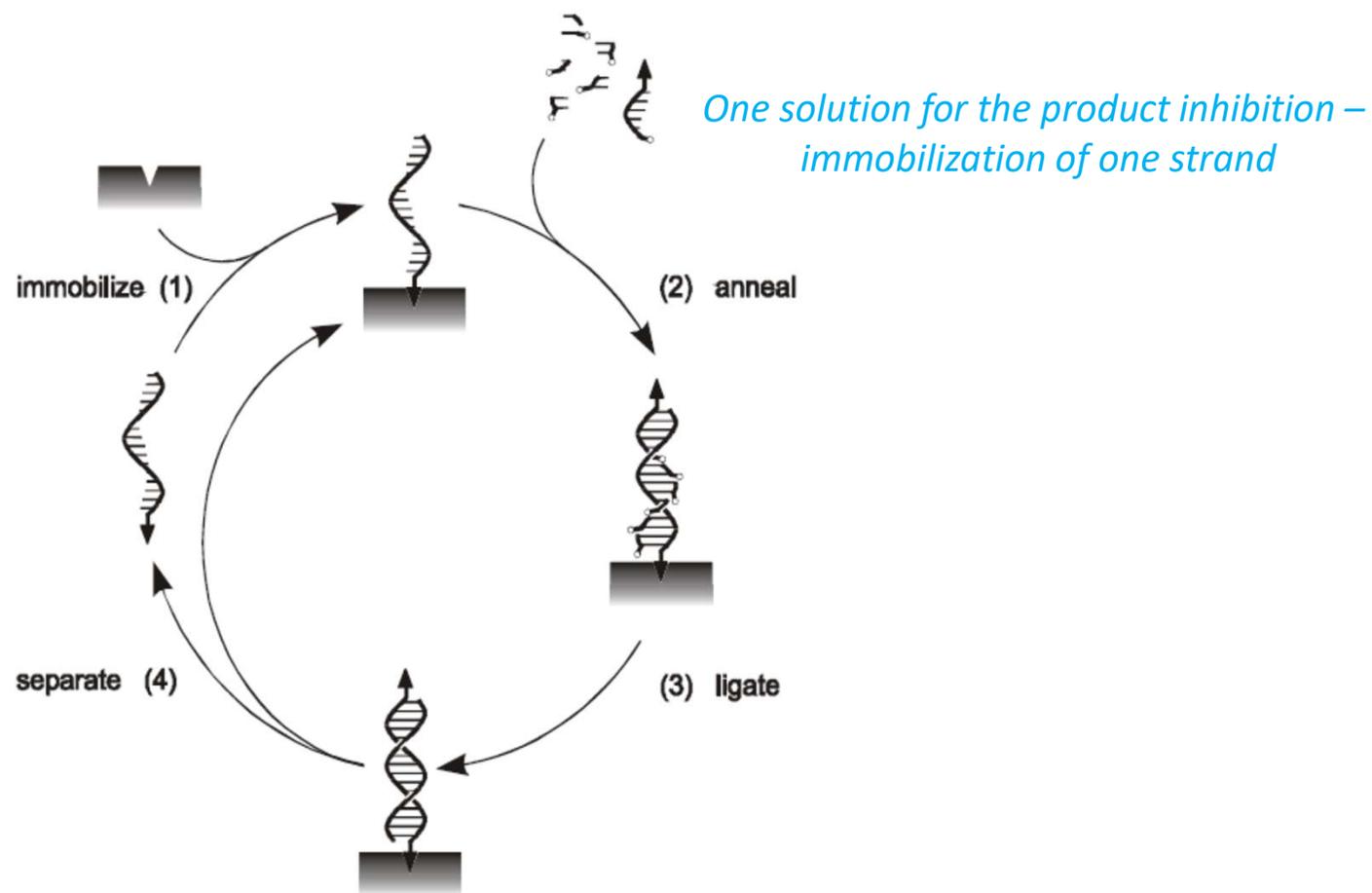


V. Patzke, G. von Kiedrowski *ARKIVOC* **2007** 293-310

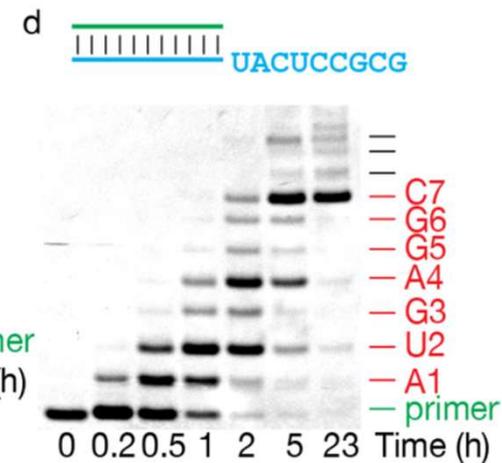
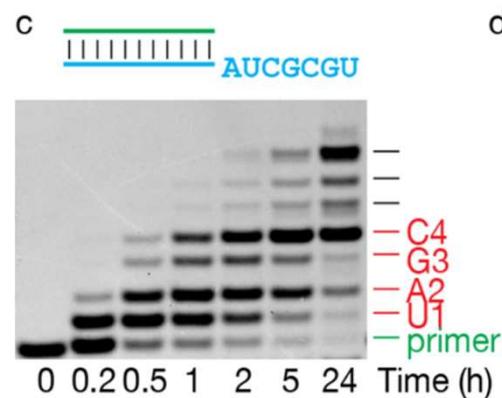
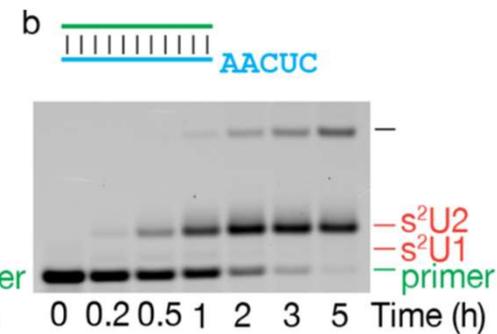
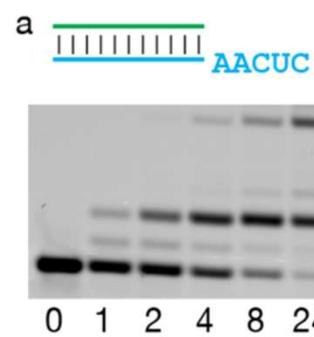
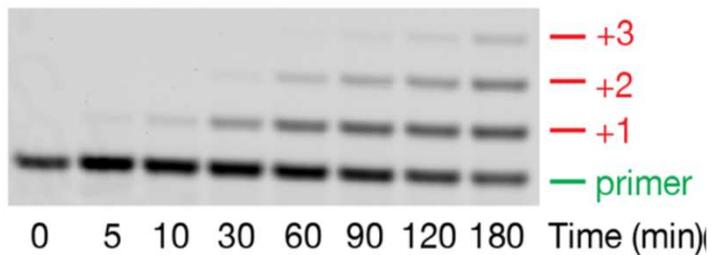
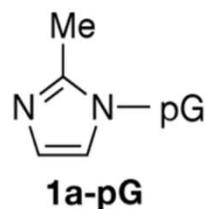
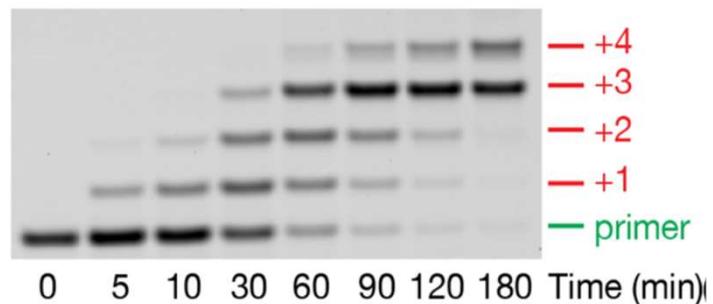
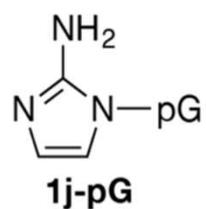
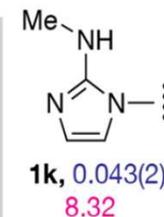
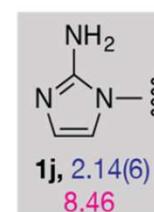
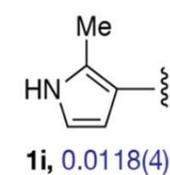
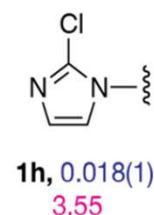
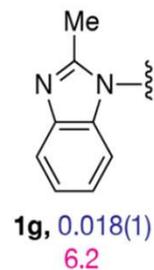
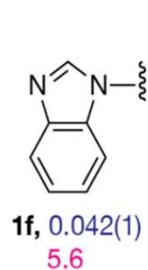
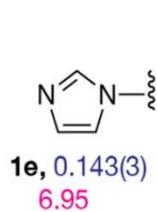
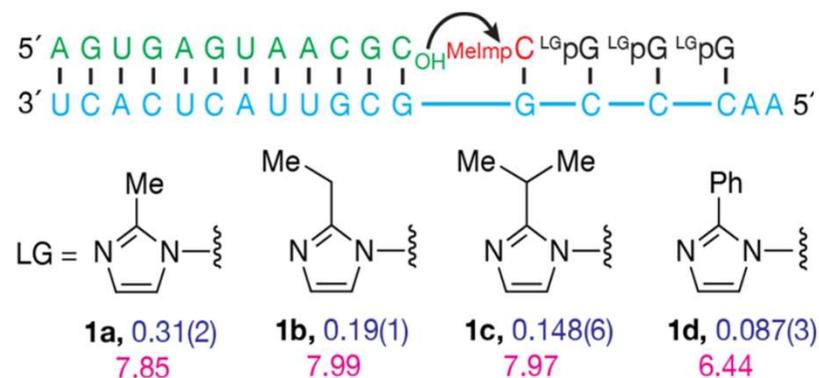
D. Sievers, G. von Kiedrowski *Nature* **1994** 369(6477), 221-224

G. von Kiedrowski *Angewandte Chemie* **1986** 98(10), 932-934

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



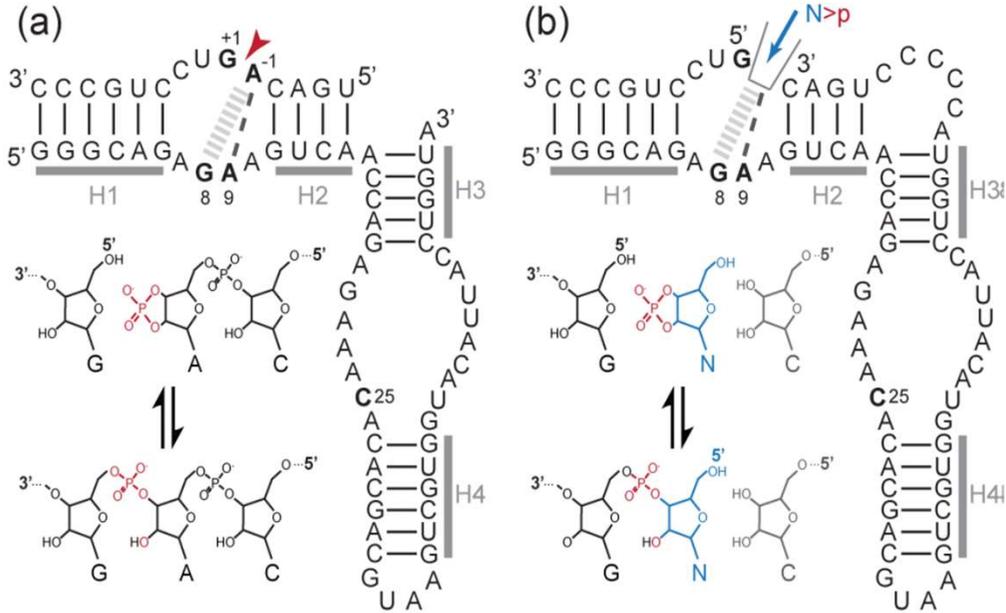
## Nonenzymatic primer extension



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

## Ribozyme-catalyzed primer extension

### Design of a 5'-nucleotidyl transferase for N>p's.

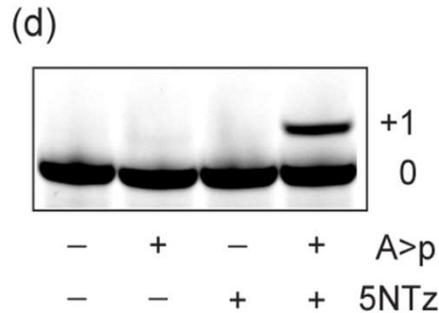
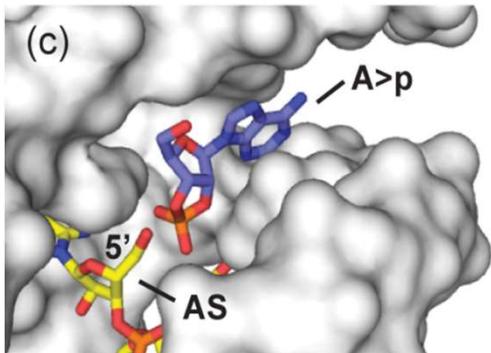


**(a)** Two-way junction HPz (small hairpin ribozyme), which catalyzes reversible RNA ligation using a 2',3'-cyclic phosphate.

**(b)** Redesign of HPz into 5NTz (nucleotidyl transferase).

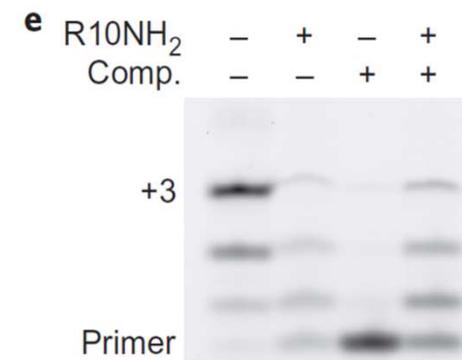
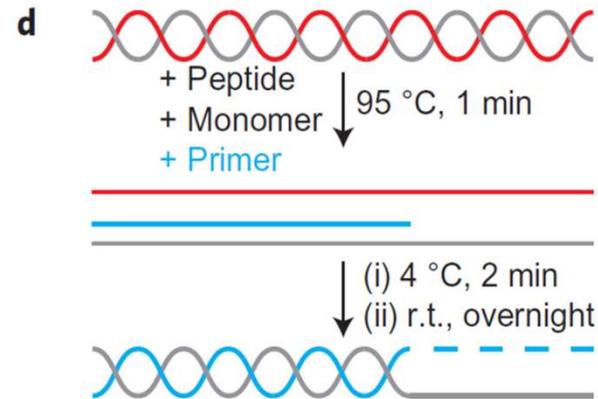
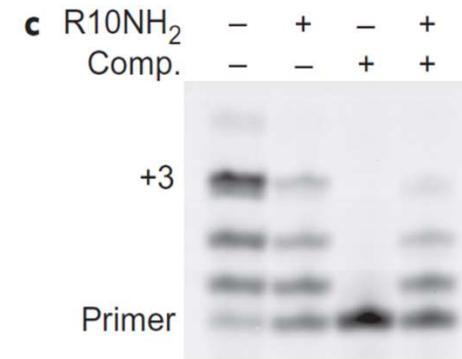
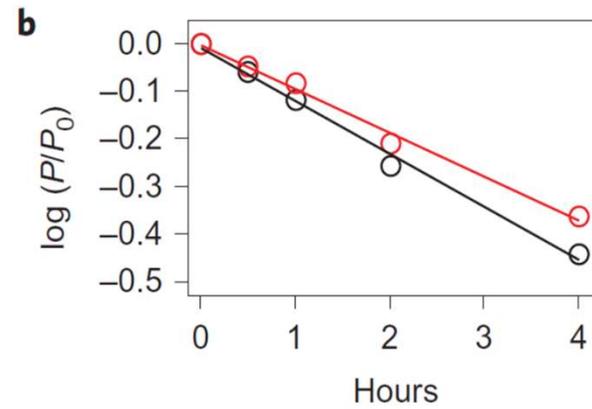
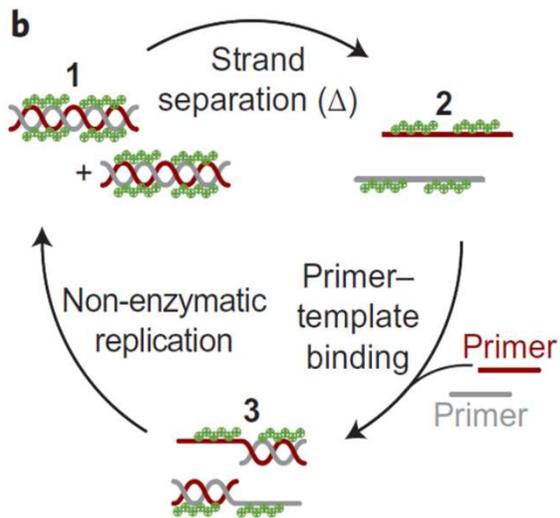
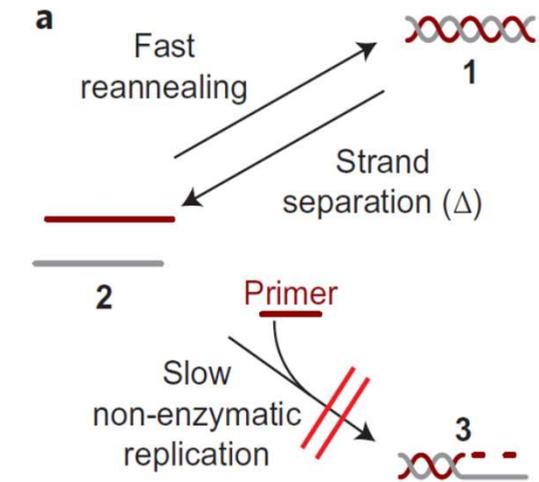
**(c)** Structural model of the substrate-binding pocket of 5NTz (based on PDB1M5V).

**(d)** 5NTz catalyzes 5'-adenylation in ice (2 mM A>p, 2  $\mu$ M 5NTz, 1  $\mu$ M 3'-FITC-labeled AS, 72 h in ice at  $-7^\circ\text{C}$ ).



An engineered hairpin ribozyme catalyzes addition of all four N>p's (2',3'-cyclic A-, G-, U-, and CMP) to the 5'-hydroxyl termini of RNA strands (eutectic ice phase formation at  $-7^\circ\text{C}$ ). 5' addition of 2',3'-cyclic phosphate-activated  $\beta$ -nicotinamide adenine dinucleotide (NAD>p), as well as ACA>p RNA trinucleotide, and multiple additions of GUCCA>p RNA pentamers was also observed.

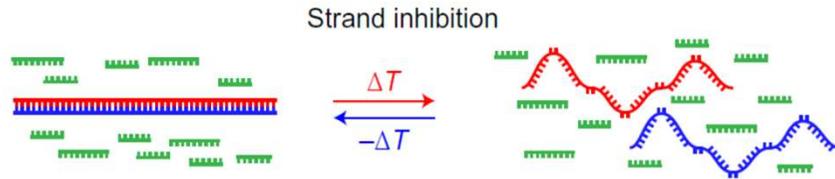
# Nonenzymatic primer extension in presence of oligoarginine peptides



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

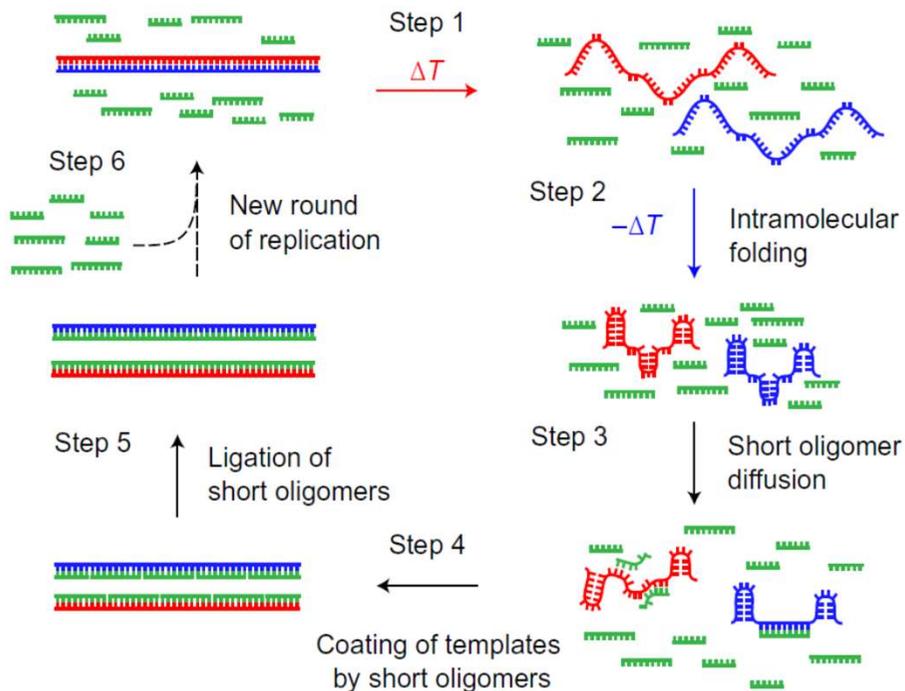
## Prebiotic replication in a viscous solvent

**a** Low viscosity



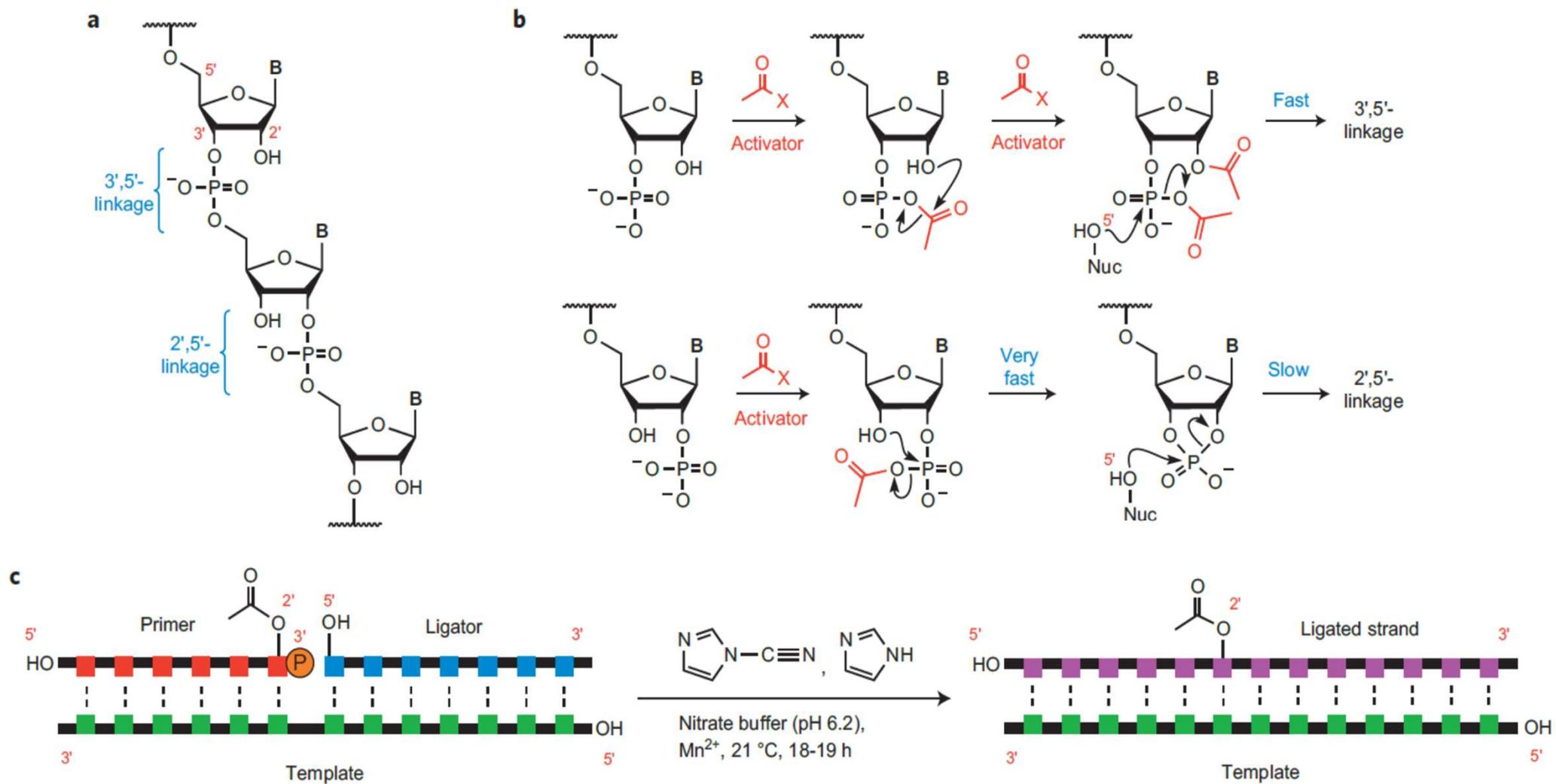
*At low viscosity: warming causes duplex dissociation  
cooling – direct hybridization*

**b** High viscosity



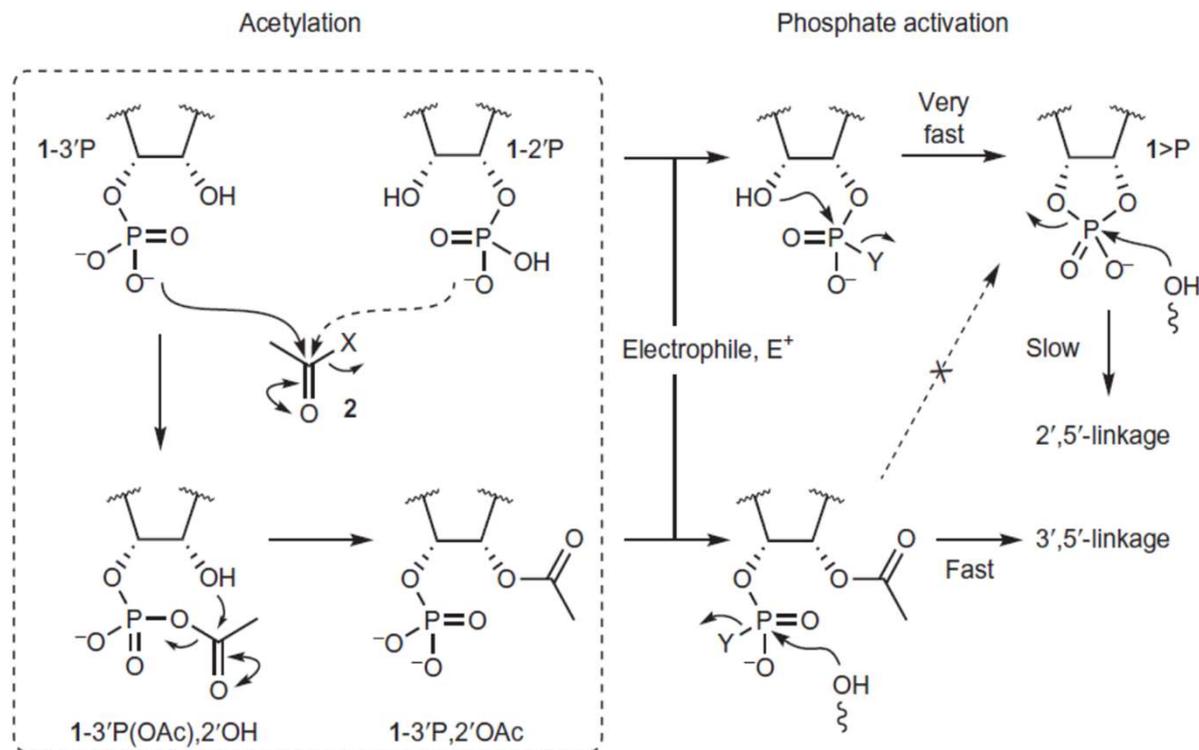
*At high viscosity: warming causes duplex dissociation  
cooling – intramolecular folding which prevents  
back-hybridization  
Then slowly short oligomers diffuse into the folds and  
coat the templates preventing their re-annealing.  
The following templated ligation of the short oligomers  
provides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

## Regioselective ribonucleotide ligation



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

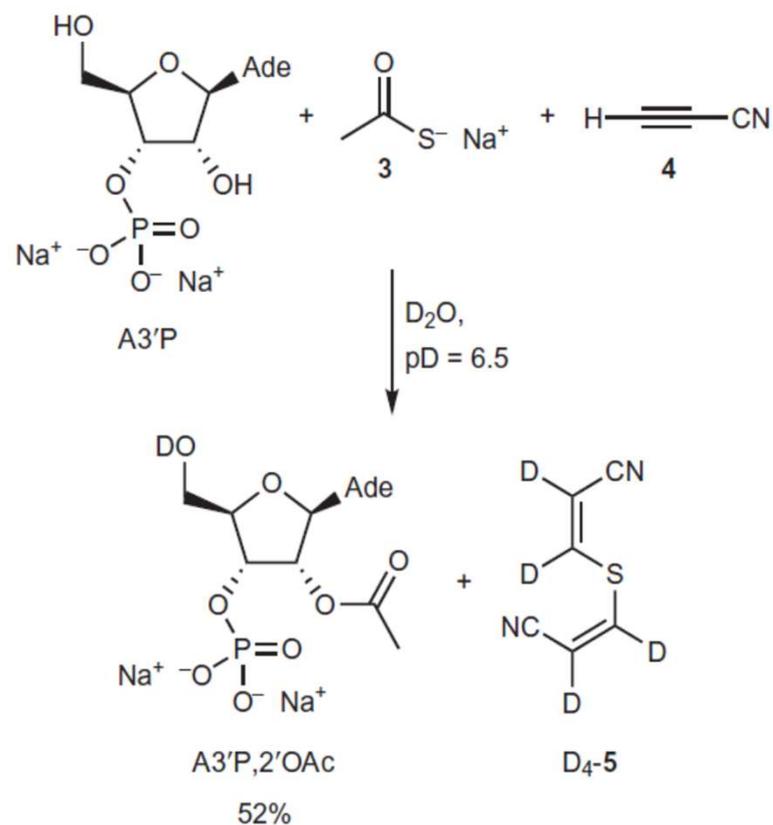
## Regioselective ribonucleotide ligation



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

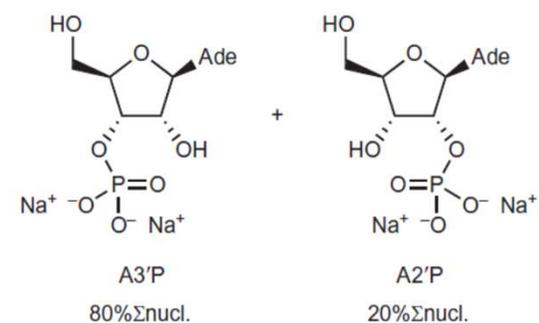
## Regioselective ribonucleotide ligation



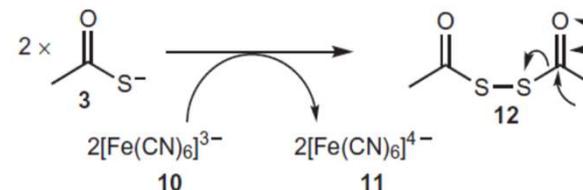
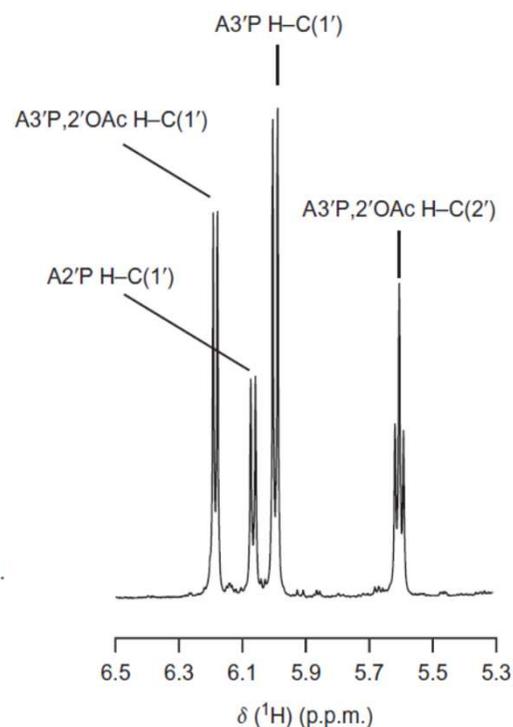
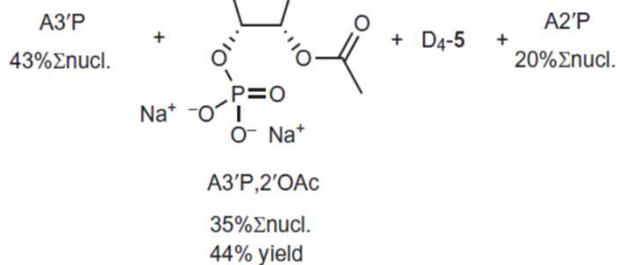
Treatment of adenosine-3'phosphate (A3'P) (100 mM) with sodium thioacetate **3** (100 mM) and cyanoacetylene **4** (200 mM) in D<sub>2</sub>O at neutral pD for 24 hours results in selective acetylation of the 2-OH group.

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

## Regioselective ribonucleotide ligation



3 + 4  
D<sub>2</sub>O,  
pD = 6.5



Additional electrophiles **6–8** shown to drive the acetylation of ribonucleotides with thioacetate **3**.

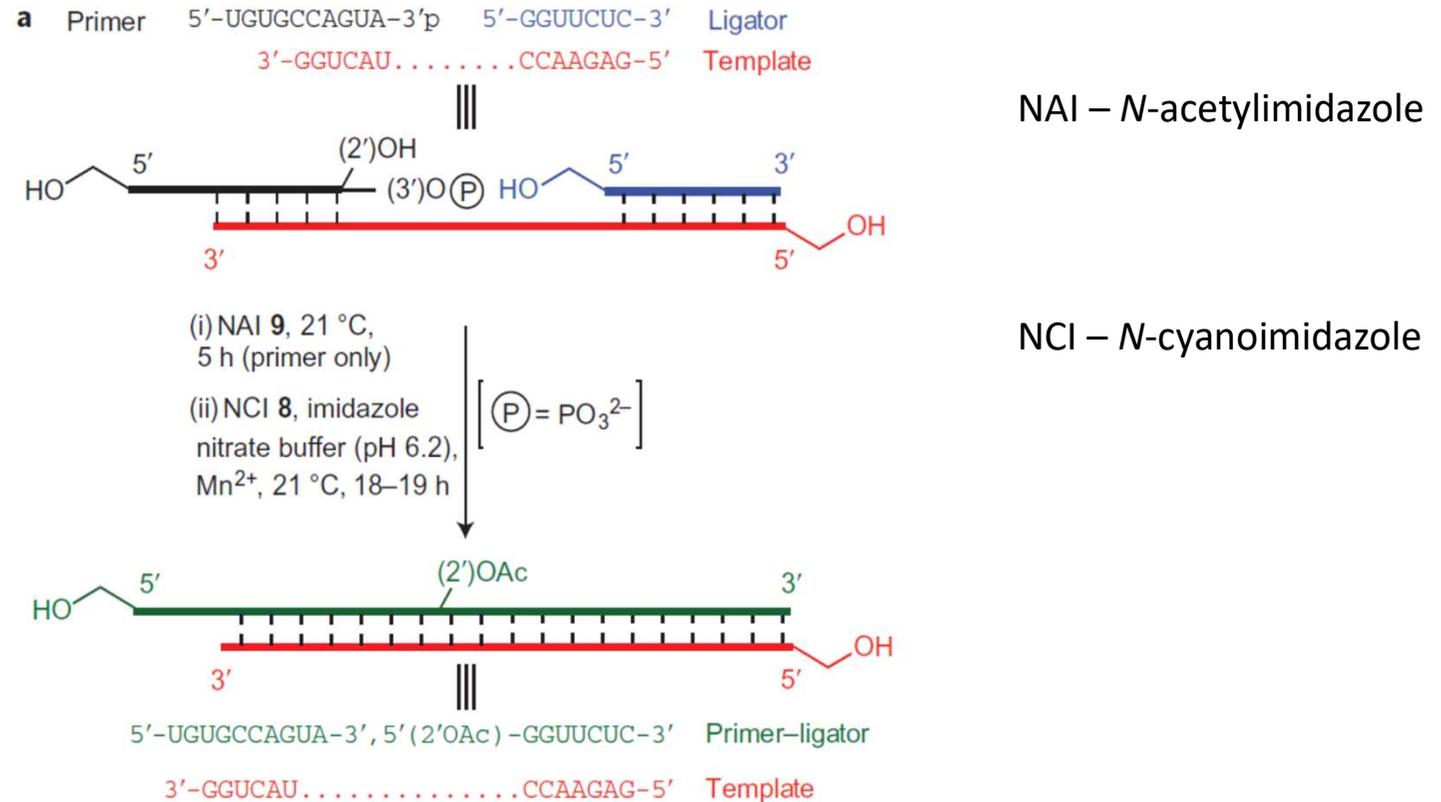
Direct acetylation with **9** is also possible, as is oxidative activation of **3** with ferricyanide **10** to afford ferrocyanide **11** and a dimeric acetylating agent **12**.

Curly arrows indicate electrophilic activation/acetylation steps.

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 <sup>1</sup>H NMR spectrum of the reaction products.

## Regioselective ribonucleotide ligation

Chemoselective acetylation of 3'P-oligoribonucleotides expedites templated ligation

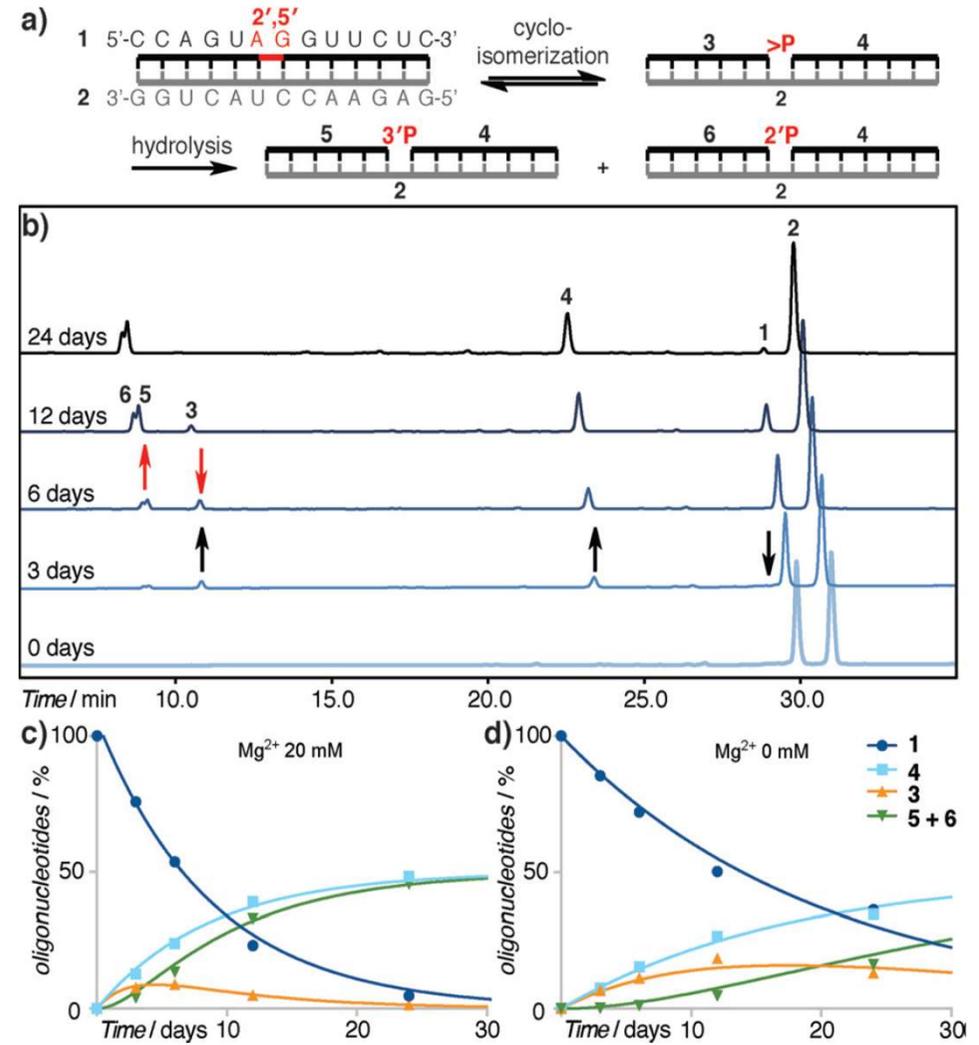
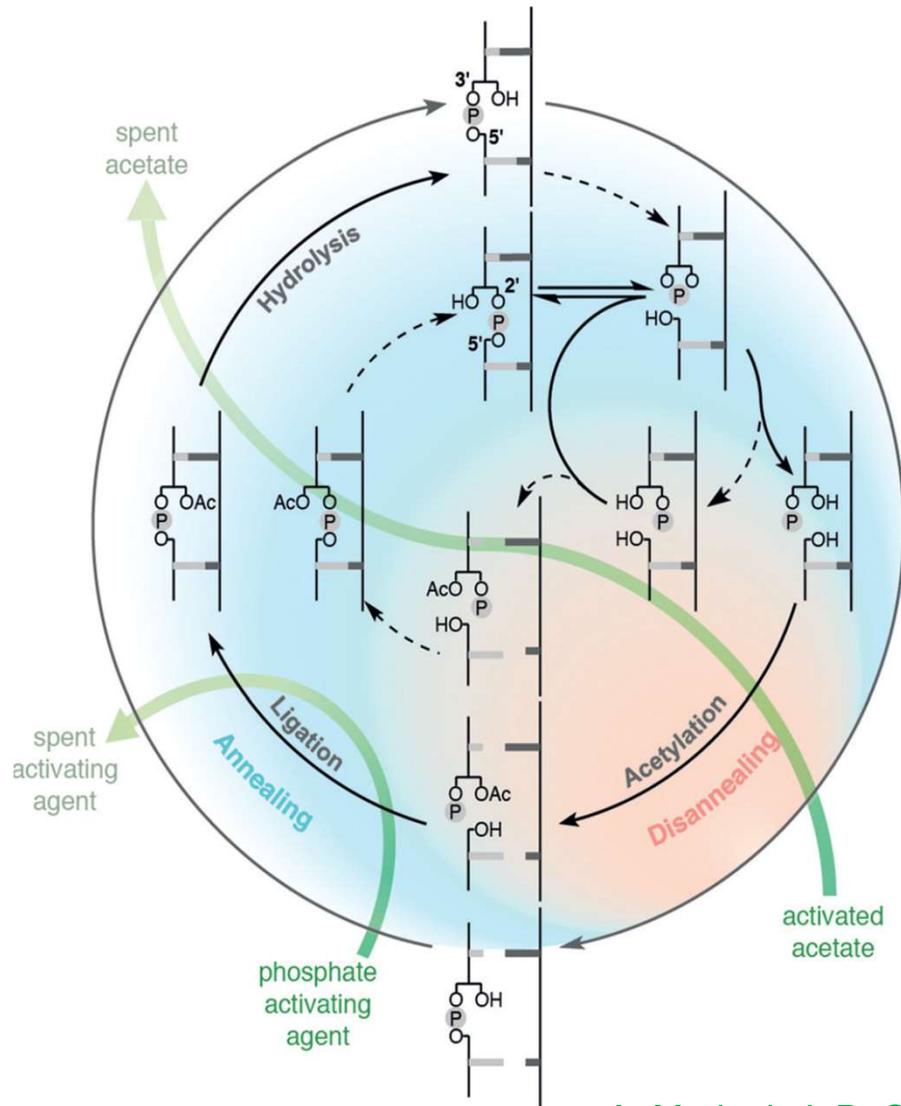


Sequences and reaction conditions employed for acetylation (i) and subsequent templated ligation (ii).

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<sub>2</sub> and 100 mM NCI.

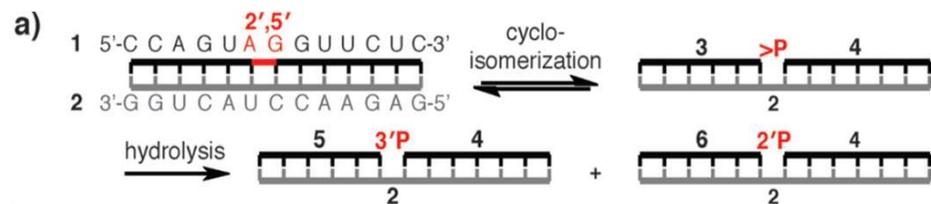
J. Sutherland *et al.* *Nature Chem.* **2013**, 383-389

## 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

