Recreating the RNA World in the Laboratory

Gerald Joyce, Scripps Research Institute

Jan 22, 2007

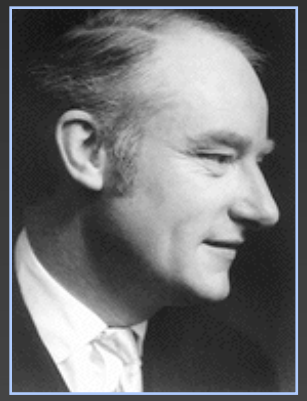
KITP Program: Evolution of Molecular Networks

DNA/protein-based life

3.6 billion years ago to present

genetic instructions

metabolic function

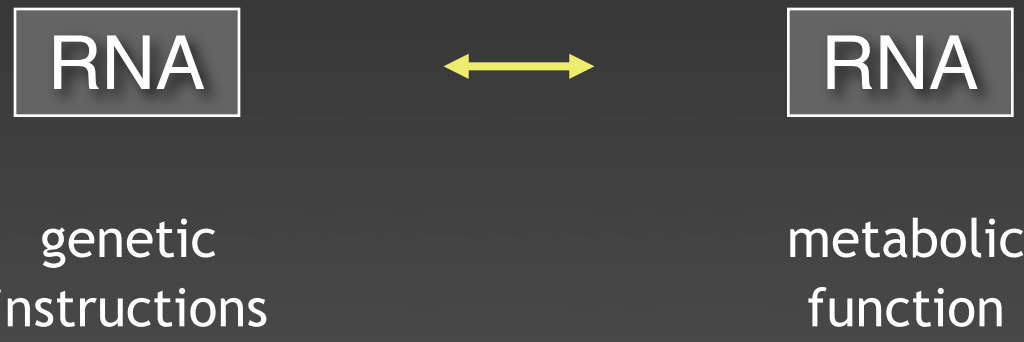


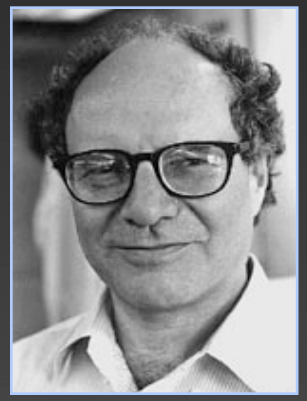
"Possibly the first 'enzyme' was an RNA molecule with RNA replicase properties."

Crick, F.H.C., *J. Mol. Biol.*, 1968

RNA-based life

circa 3.9 billion years ago

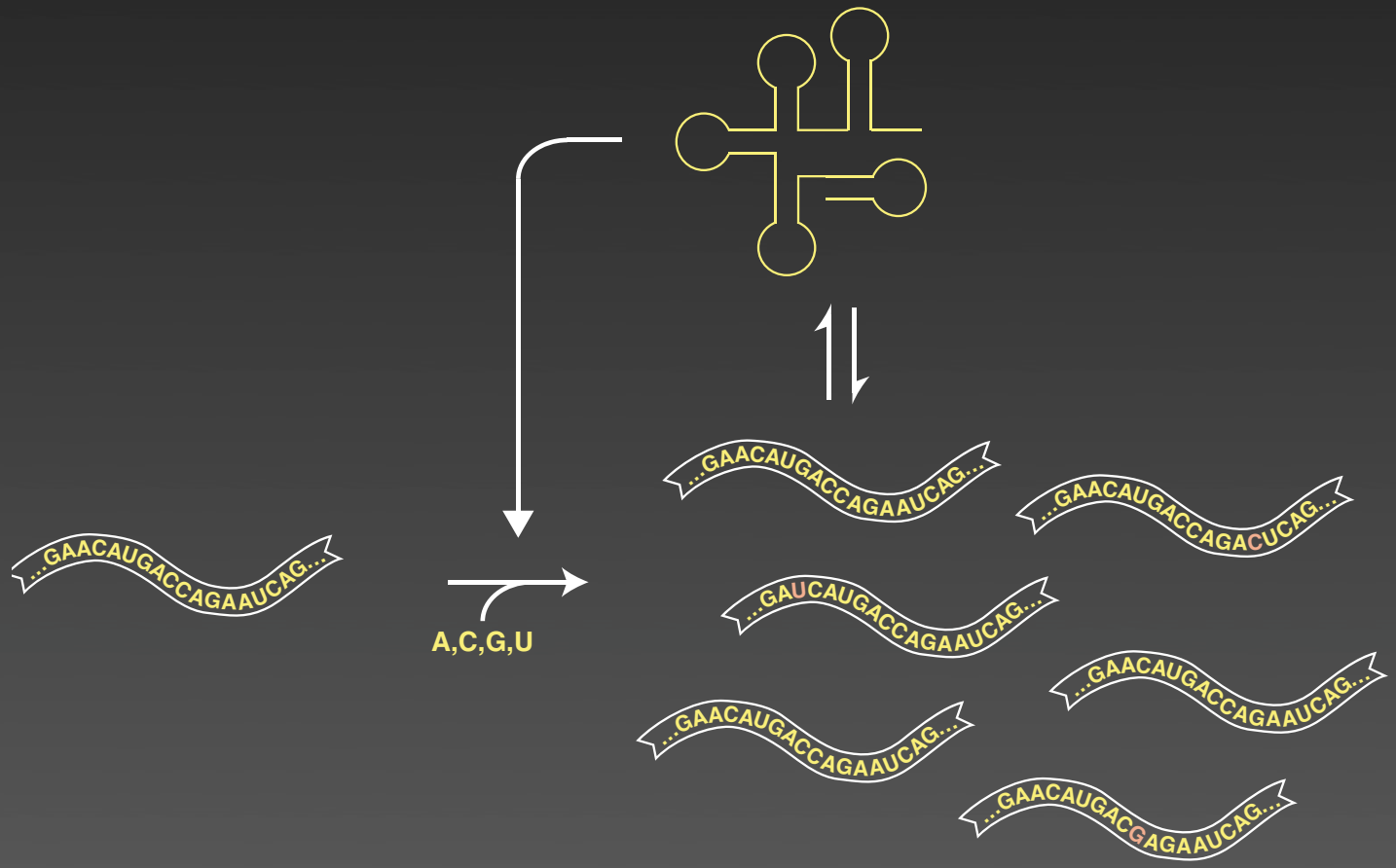




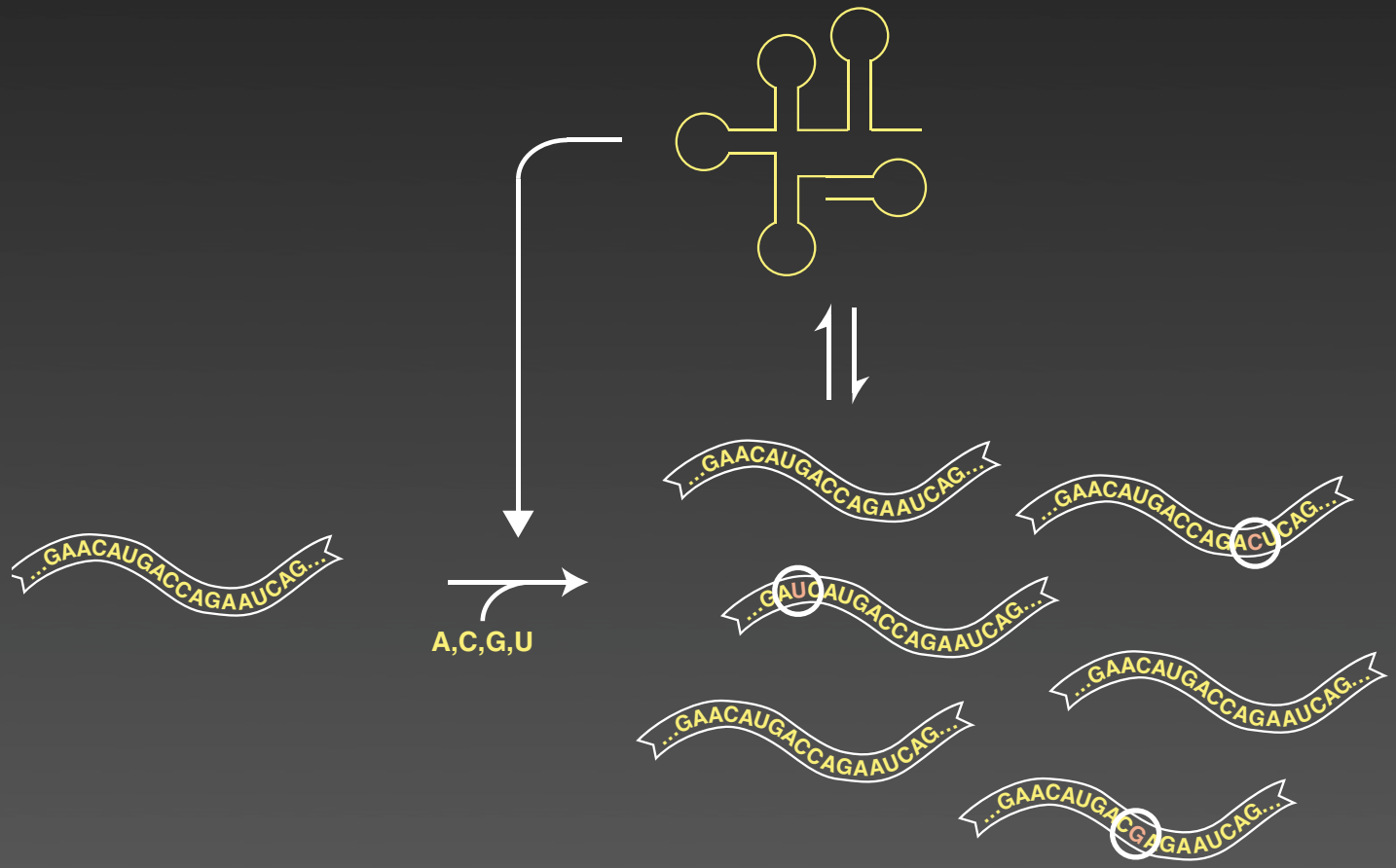
"One can contemplate an 'RNA world', containing only RNA molecules that serve to catalyze the synthesis of themselves."

Gilbert, W., *Nature*, 1986

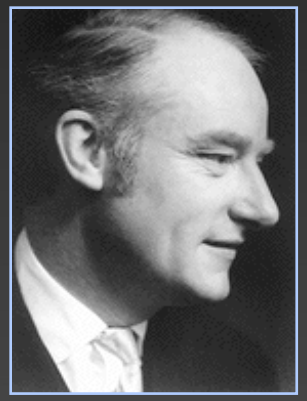
RNA-catalyzed RNA replication



RNA-catalyzed RNA replication

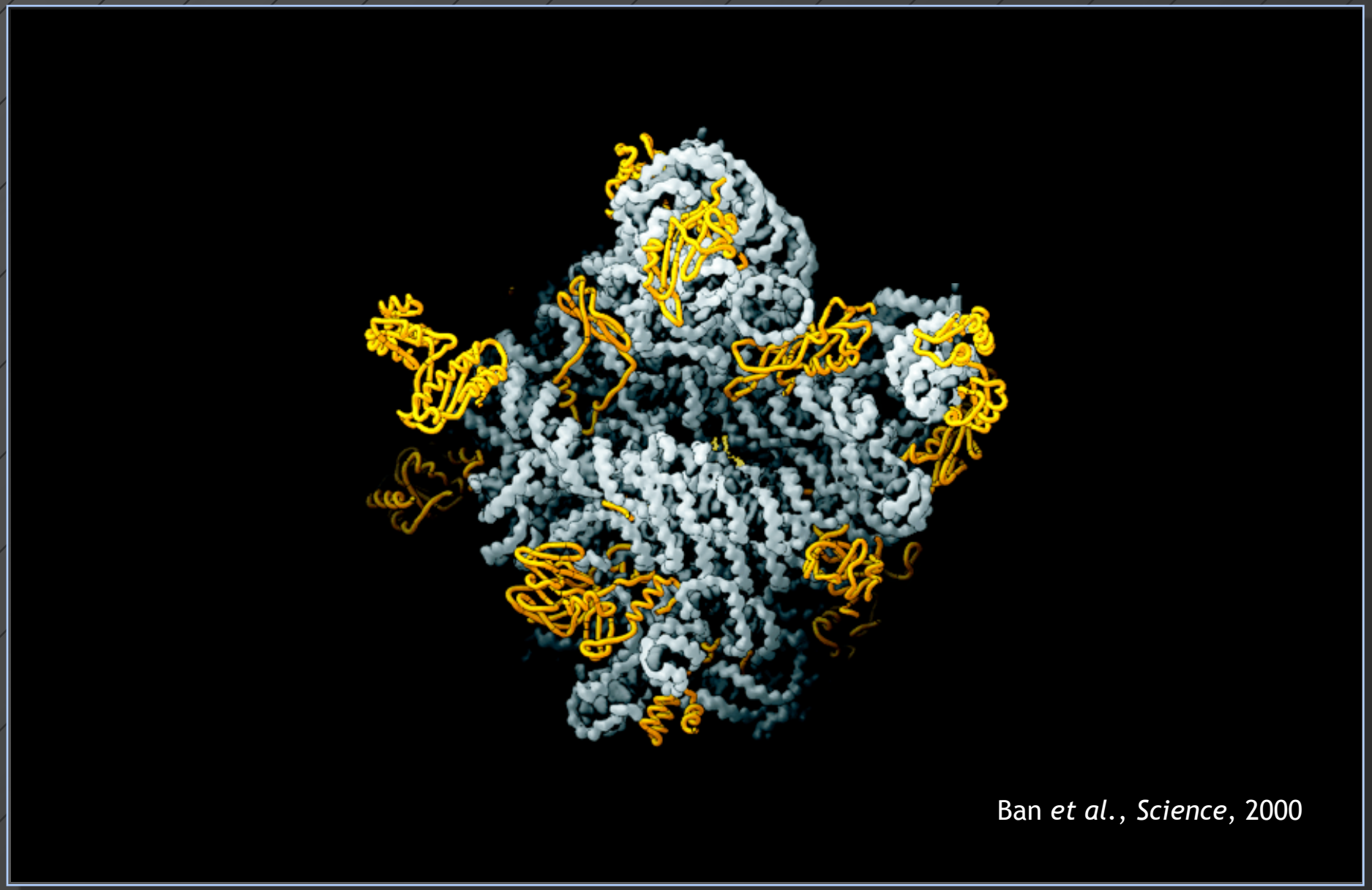


Did the RNA world really exist?



"It is tempting to wonder if the primitive ribosome could have been made *entirely* of RNA."

Crick, F.H.C., *J. Mol. Biol.*, 1968



Ban *et al.*, *Science*, 2000

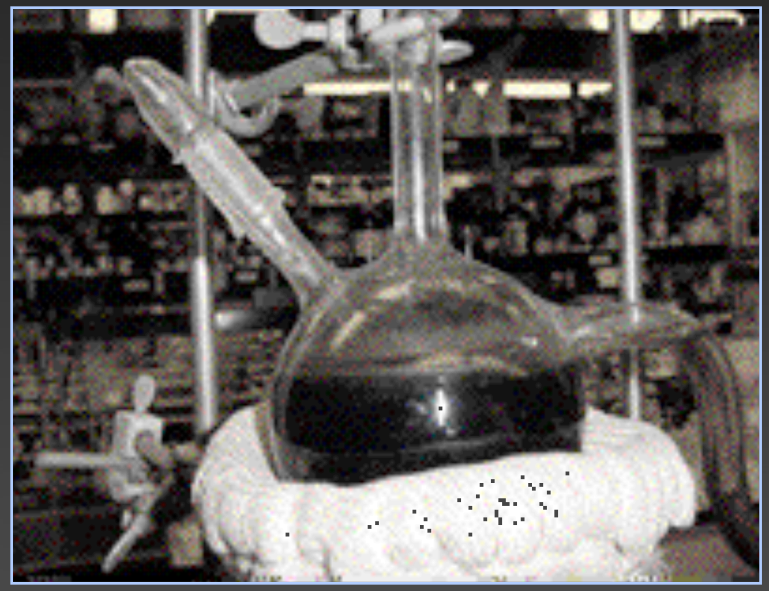
Can the RNA world be created in the laboratory?

Stanley Miller

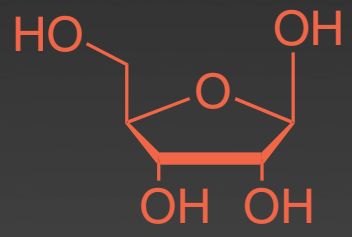
University of Chicago, 1953

Stanley Miller

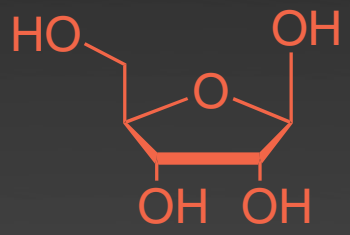
University of Chicago, 1953



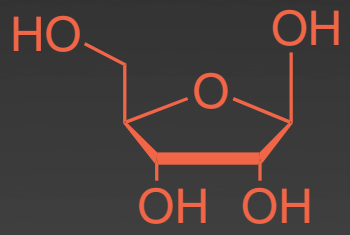
“prebiotic soup”


HO
OH
O
OH
OH

β D ribo furanose



β	D	ribo	furanose
α	L	lyxo	pyranose
		xylo	
		arabino	
		(tetroses)	
		(hexoses)	
		(branched sugars)	



β	D	ribo	furanose
α	L	lyxo	pyranose
		xylo	
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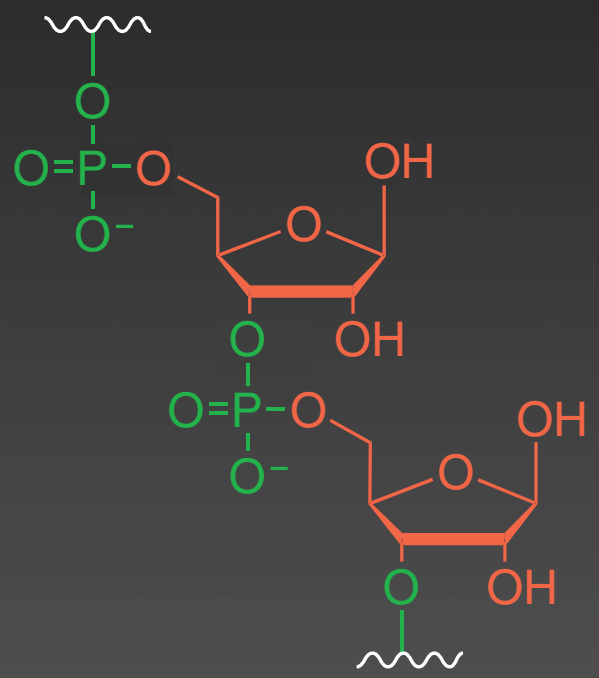
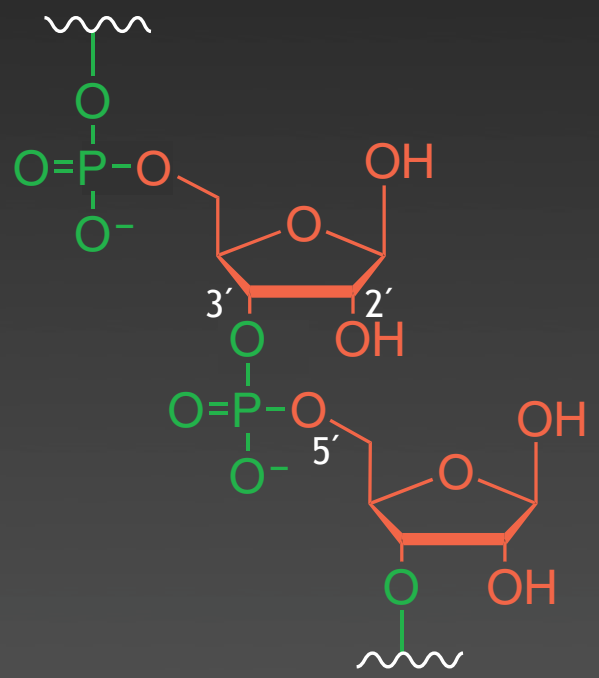


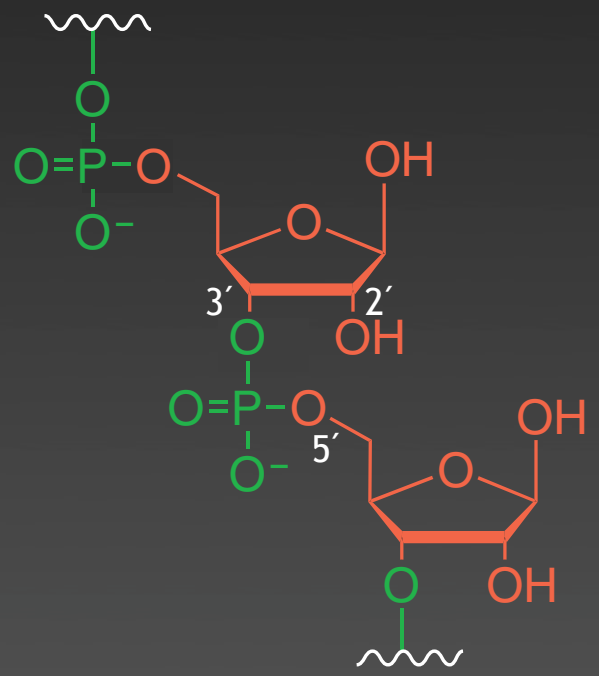
3′,5′ phosphate



β	D	ribo	furanose
α	L	lyxo	pyranose
		xylo	
		arabino	
		(tetroses)	
		(hexoses)	
		(branched sugars)	

3′,5′	phosphate
2′,5′	pyrophosphate
2′,2′	polyphosphate
3′,3′	alkylphosphate
5′,5′	

β	D	ribo	furanose
α	L	lyxo	pyranose
		xylo	
		arabino	
		(tetroses)	
		(hexoses)	
		(branched sugars)	



3′,5′ phosphate

2′,5′ pyrophosphate

2′,2′ polyphosphate

3′,3′ alkylphosphate

5′,5′

β D ribo furanose

α L lyxo pyranose

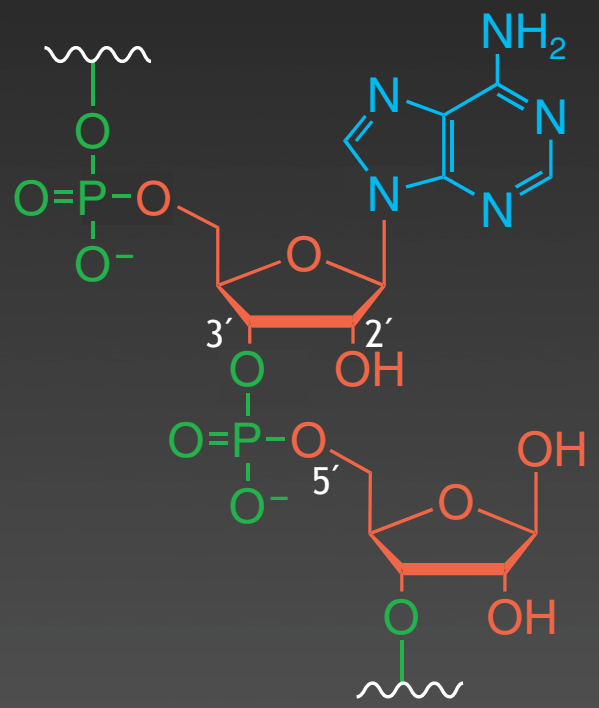
xylo

arabino

(tetroses)

(hexoses)

(branched sugars)



adenine, guanine

3′,5′ phosphate

2′,5′ pyrophosphate

2′,2′ polyphosphate

3′,3′ alkylphosphate

5′,5′

β D ribo furanose

α L lyxo pyranose

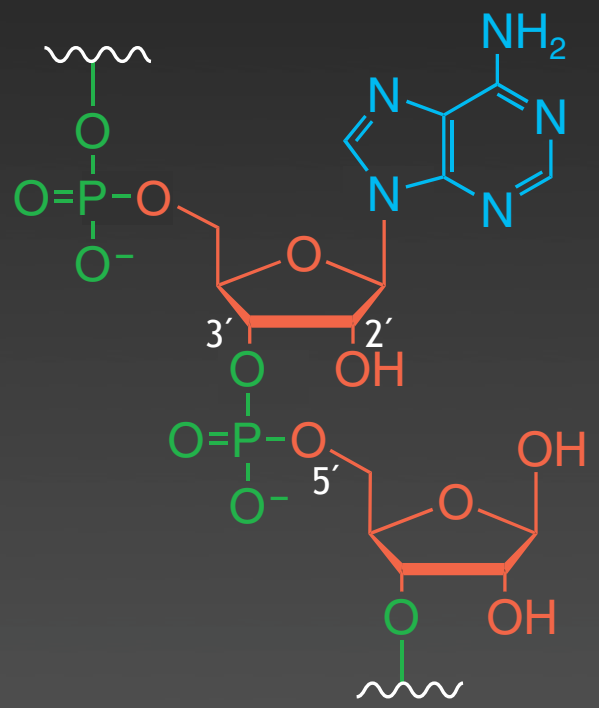
xylo

arabino

(tetroses)

(hexoses)

(branched sugars)



adenine, guanine

diaminopurine

hypoxanthine

xanthine

isoguanine

N6-substituted

C8-substituted

3′,5′ phosphate

2′,5′ pyrophosphate

2′,2′ polyphosphate

3′,3′ alkylphosphate

5′,5′

β D ribo furanose

α L lyxo pyranose

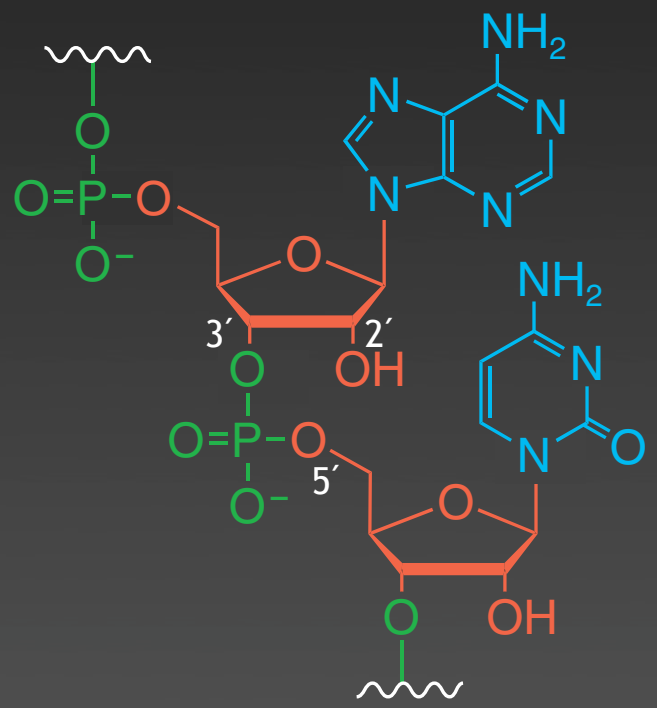
xylo

arabino

(tetroses)

(hexoses)

(branched sugars)



adenine, guanine

diaminopurine

hypoxanthine

xanthine

isoguanine

N6-substituted

C8-substituted

cytosine, uracil

3′,5′ phosphate

2′,5′ pyrophosphate

2′,2′ polyphosphate

3′,3′ alkylphosphate

5′,5′

β D ribo furanose

α L lyxo pyranose

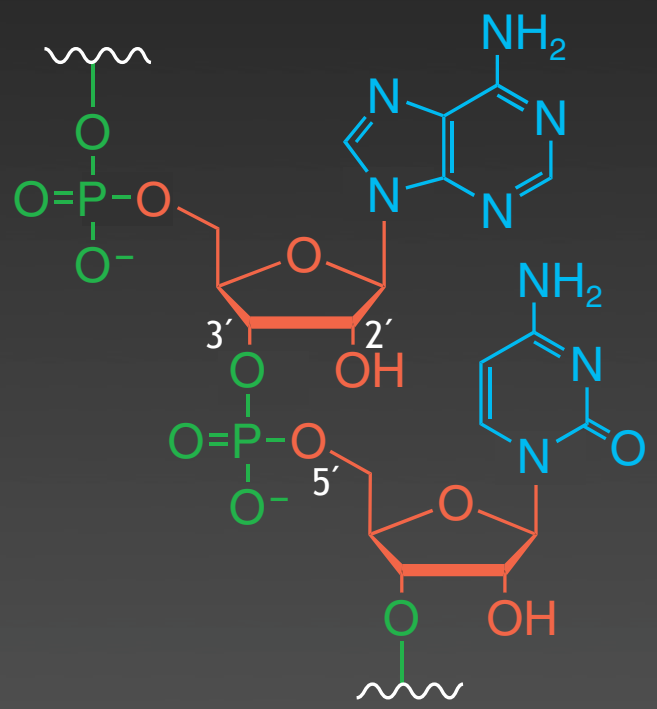
xylo

arabino

(tetroses)

(hexoses)

(branched sugars)



adenine, guanine

diaminopurine

hypoxanthine

xanthine

isoguanine

N6-substituted

C8-substituted

cytosine, uracil

diaminopyrimidine

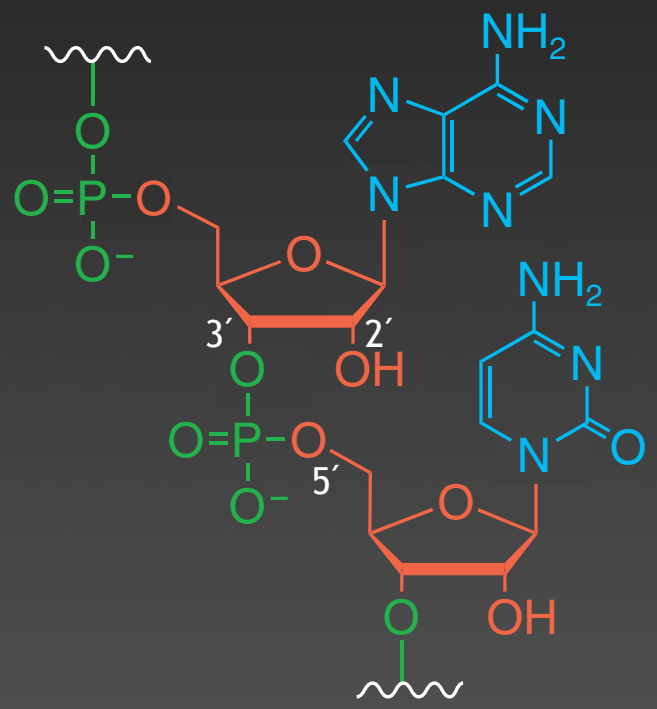
dihydrouracil

orotic acid

C5-substituted

3′,5′ phosphate

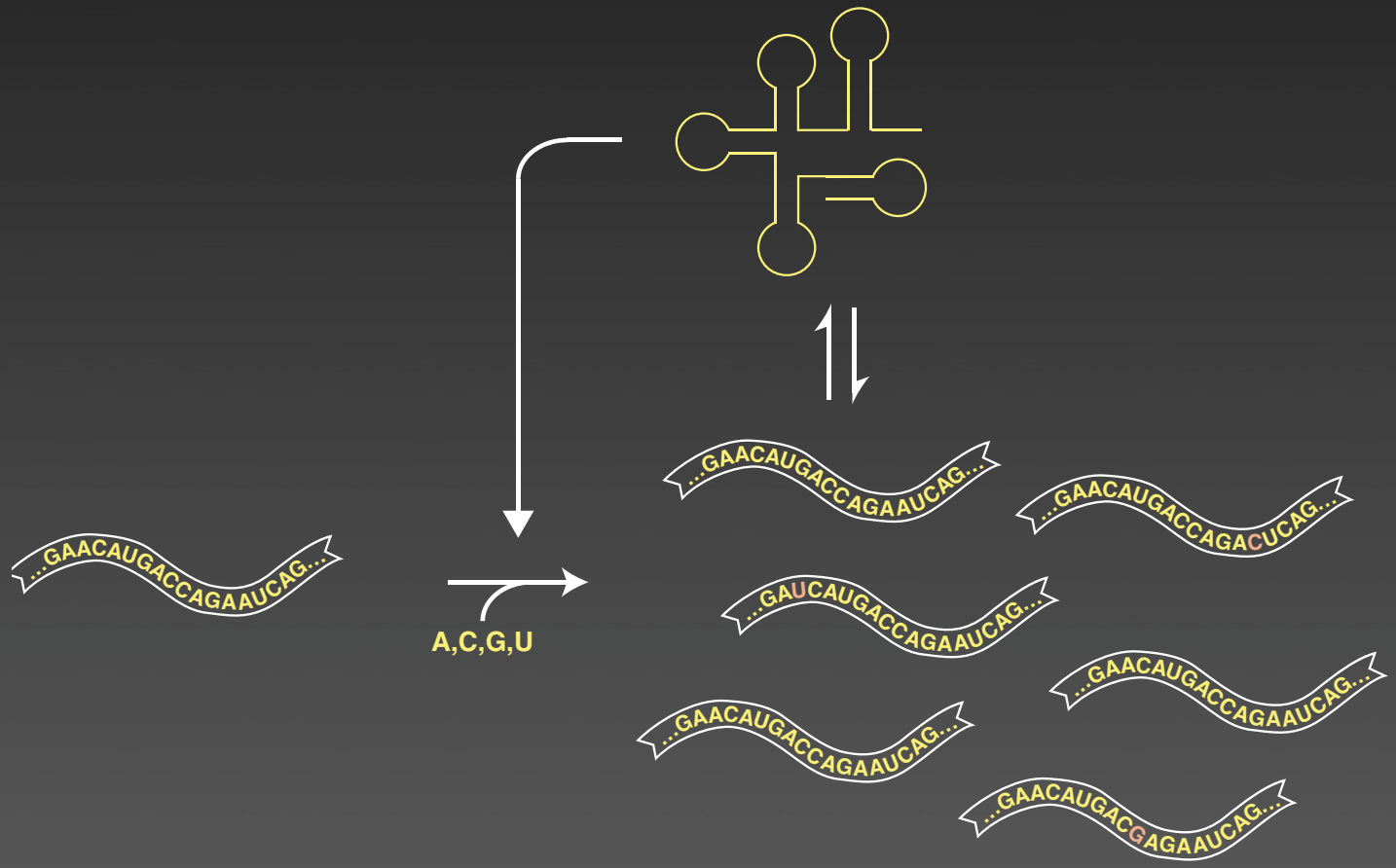
adenine, guanine



β D ribo furanose

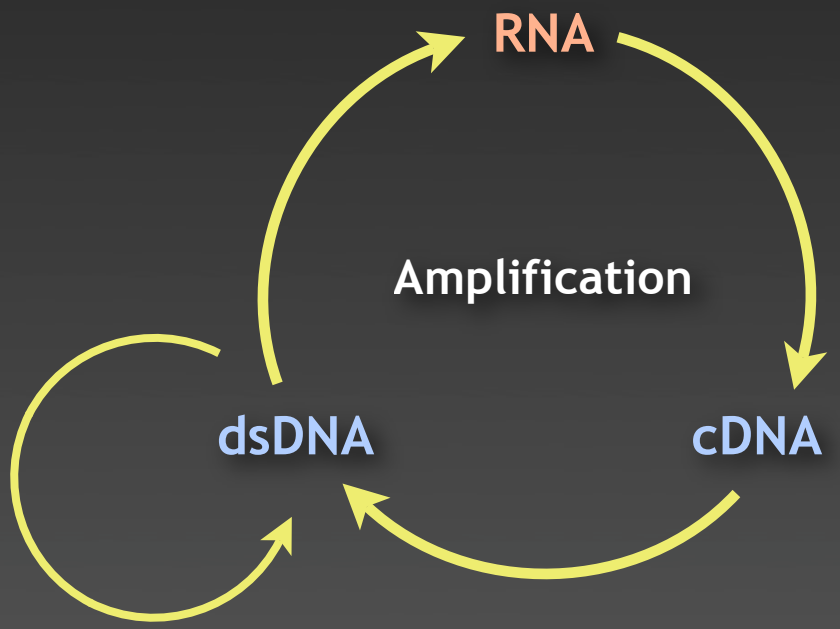
cytosine, uracil

RNA-catalyzed RNA replication

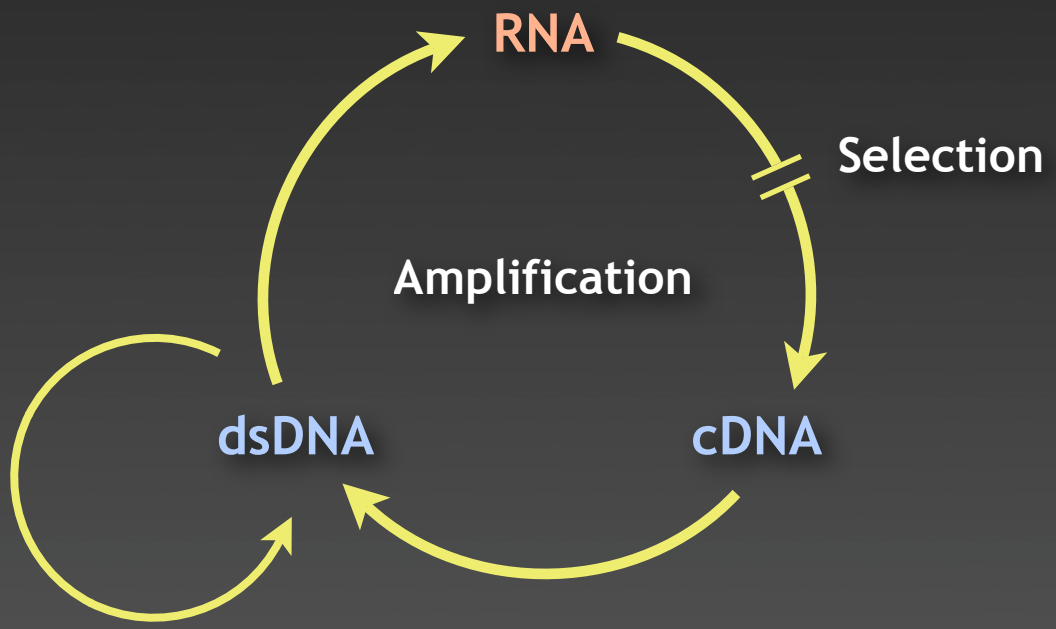


In vitro evolution of RNA

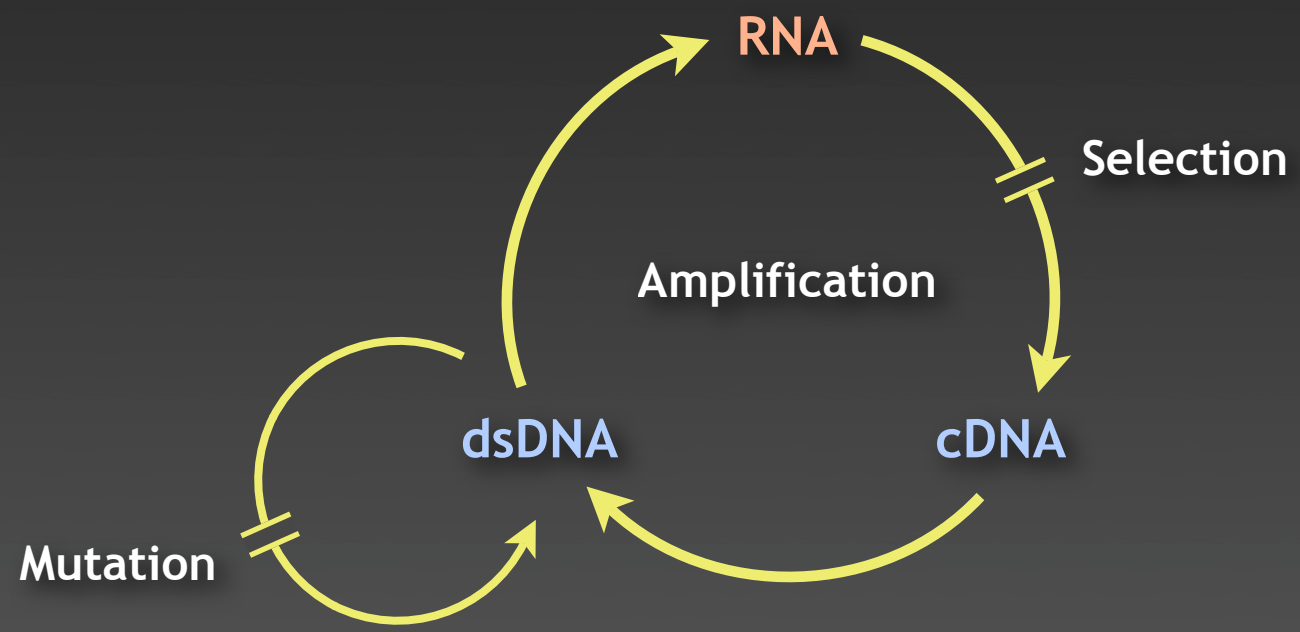
In vitro evolution of RNA



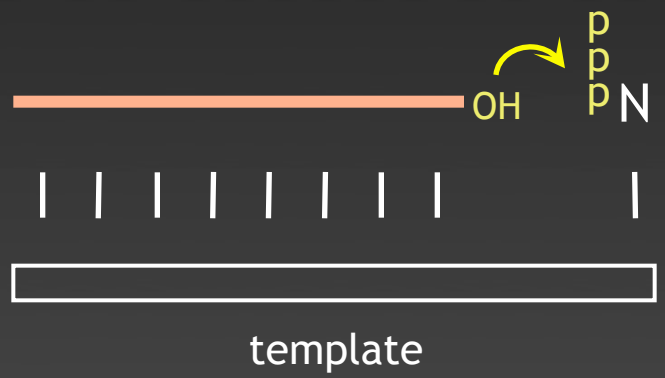
In vitro evolution of RNA



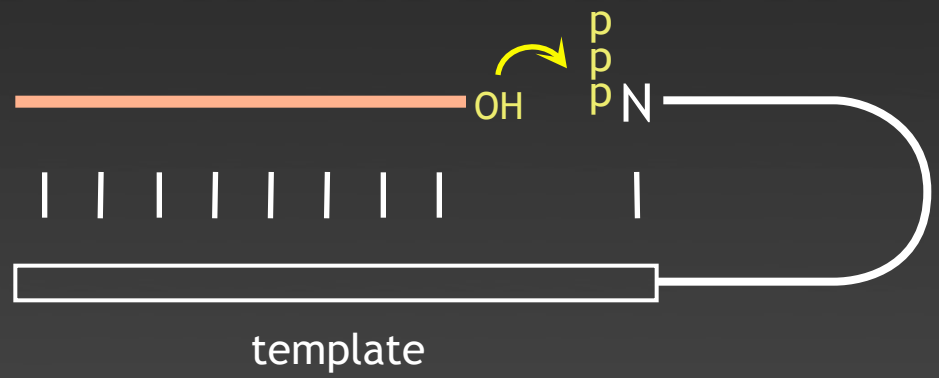
In vitro evolution of RNA



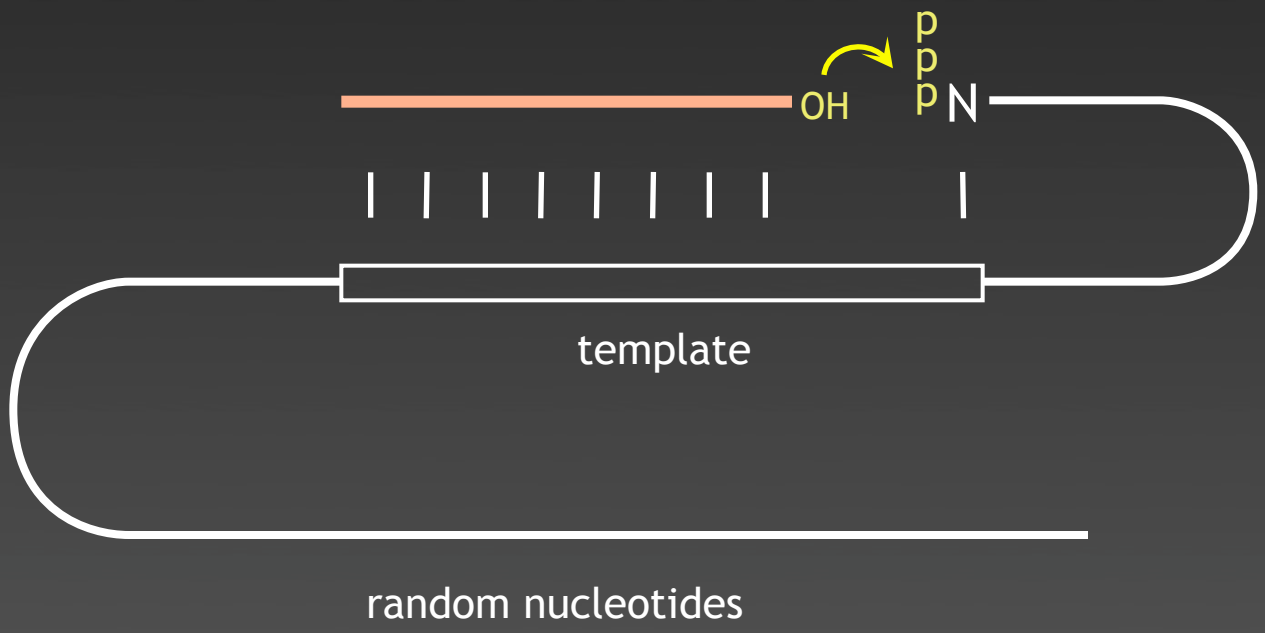
Select for RNA ligase activity



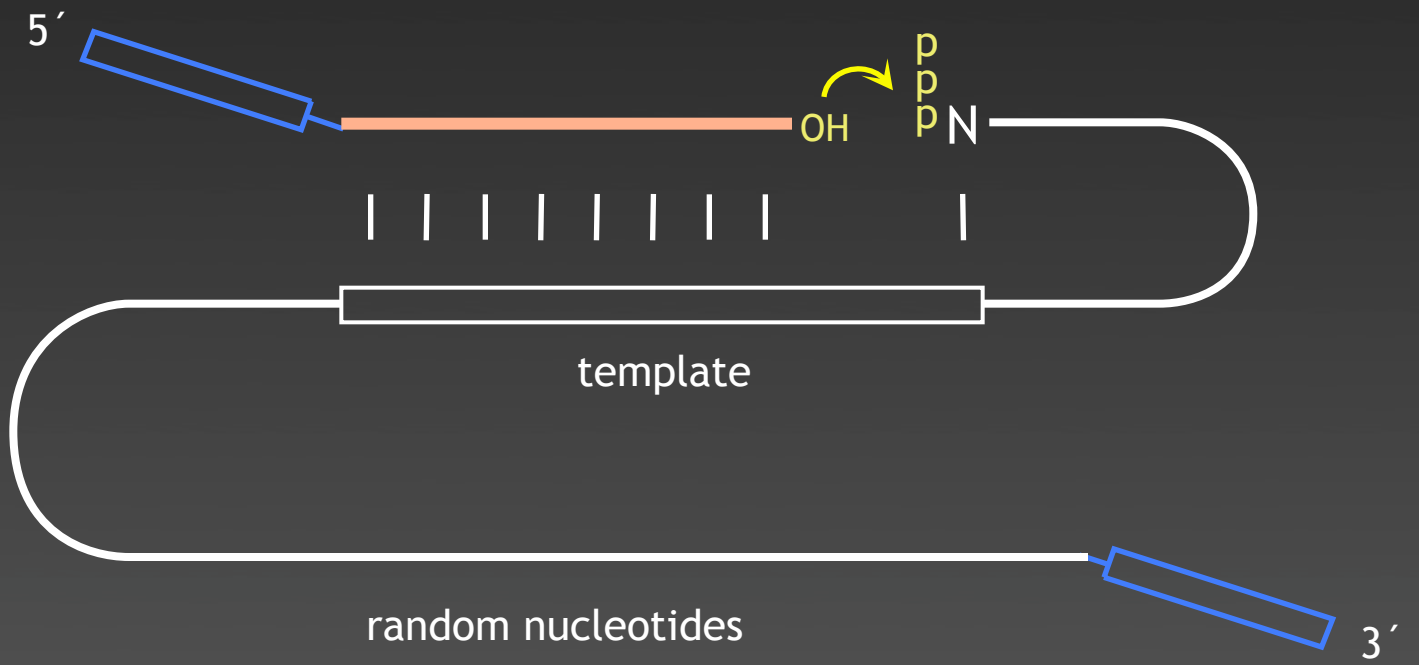
Select for RNA ligase activity



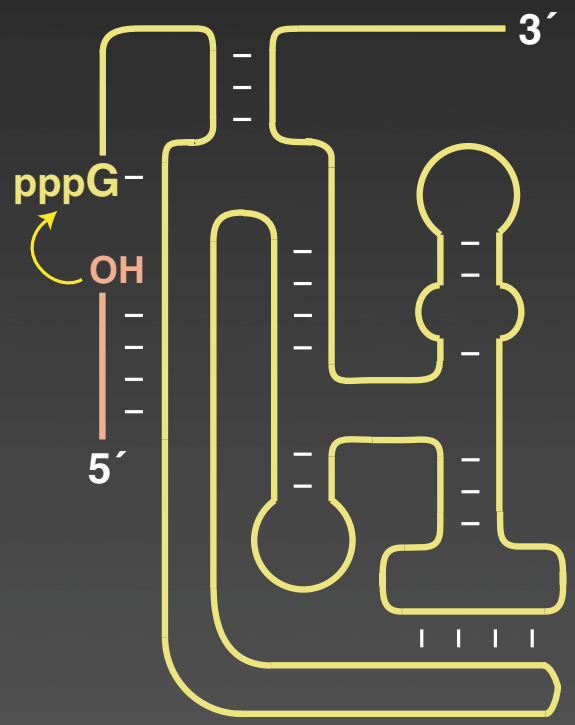
Select for RNA ligase activity



Select for RNA ligase activity



Class I RNA ligase



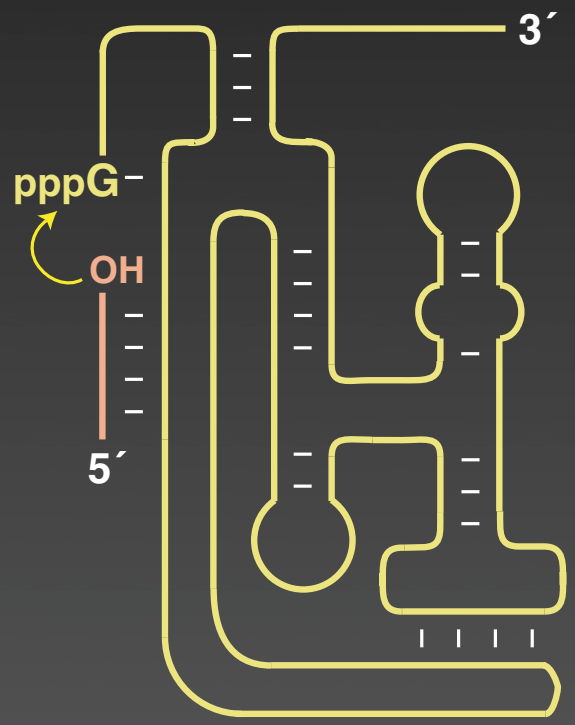
118 nucleotides

k_{cat} = 14 min^{-1}, K_m = 9 µM

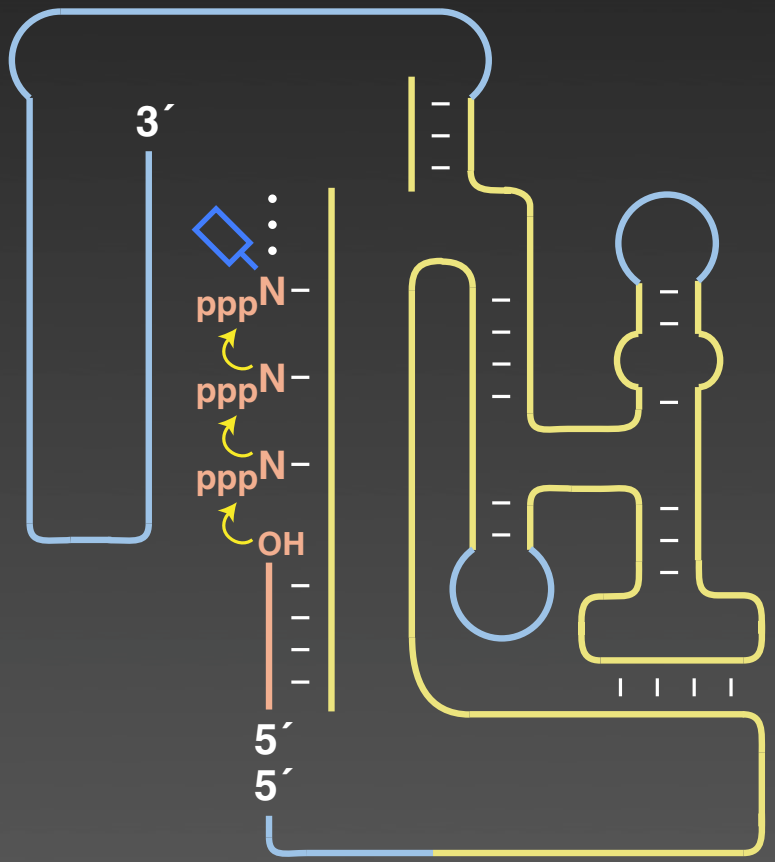
15 mM $MgCl_2$, pH 8.5, 37 °C

Bartel & Szostak, 1993

Class I RNA ligase

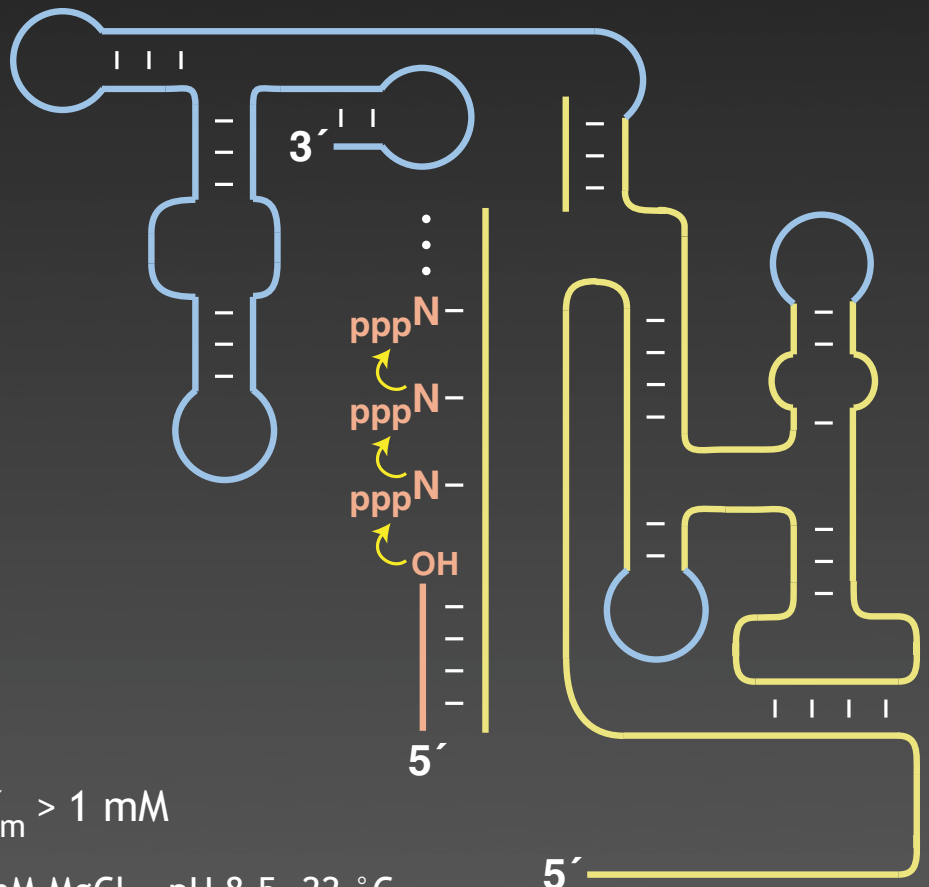


Ligase evolved to polymerase



Johnston *et al.*, 2001

Ligase evolved to polymerase



196 nucleotides

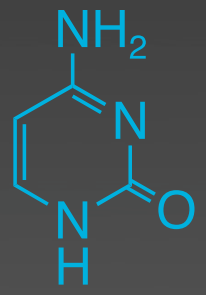
k_{cat} > 1 min^{-1}, K_m > 1 mM

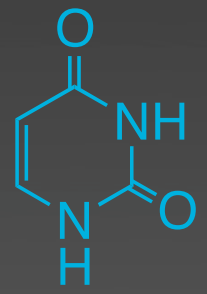
4 mM NTPs, 200 mM $MgCl_2$, pH 8.5, 22 °C

Johnston *et al.*, 2001


O
N
NH
N
H
N
NH2

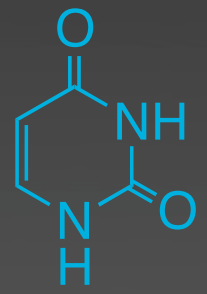

NH2
N
N
N
H
N


NH2
N
N
H
O

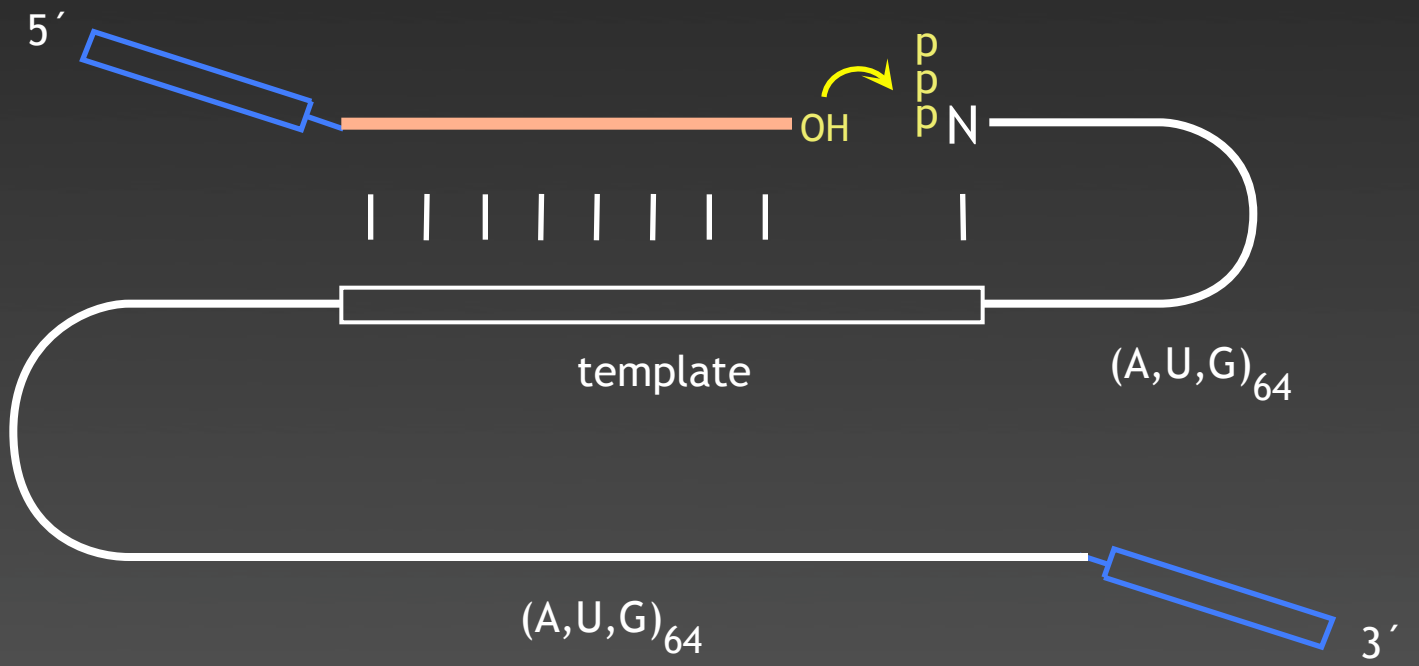

O
NH
N
H
O

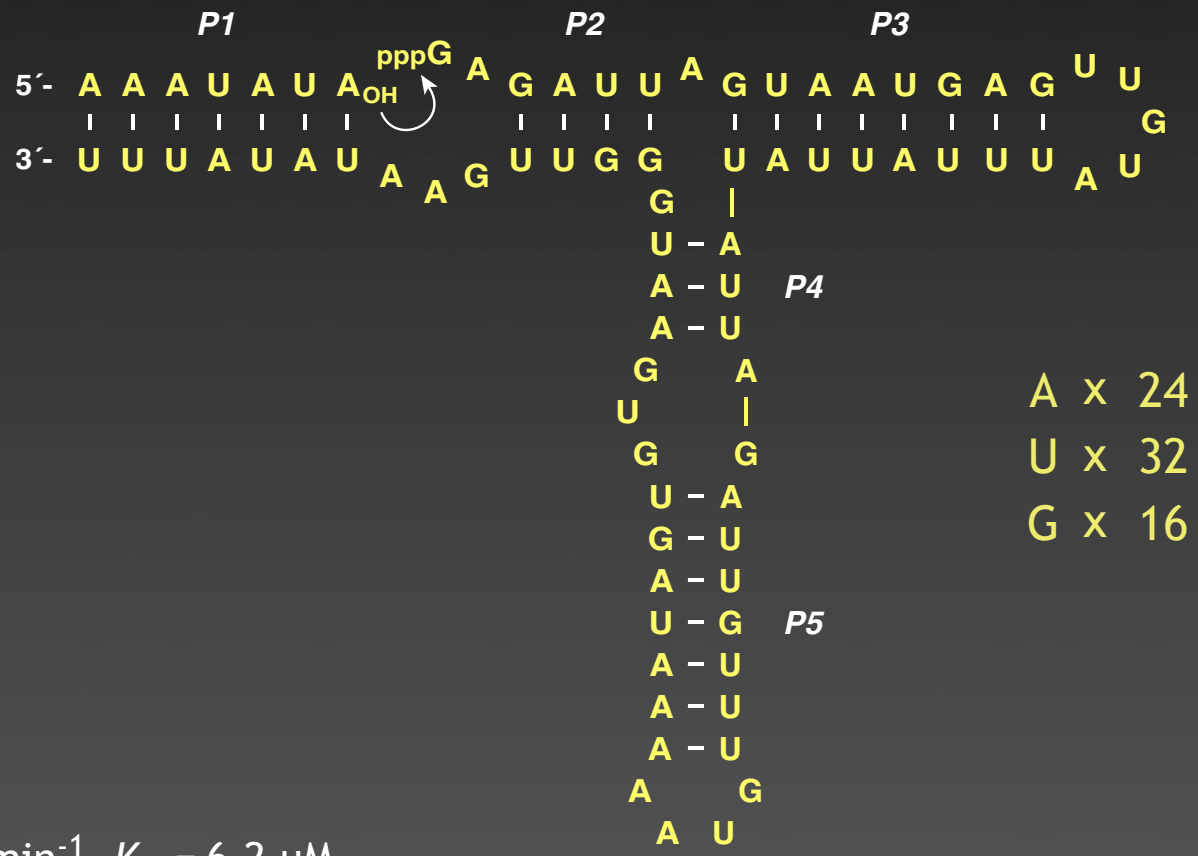

O
N
NH
N
H
N
NH2


NH2
N
N
N
H
N


O
NH
N
H
O

Evolution of a C-free ligase



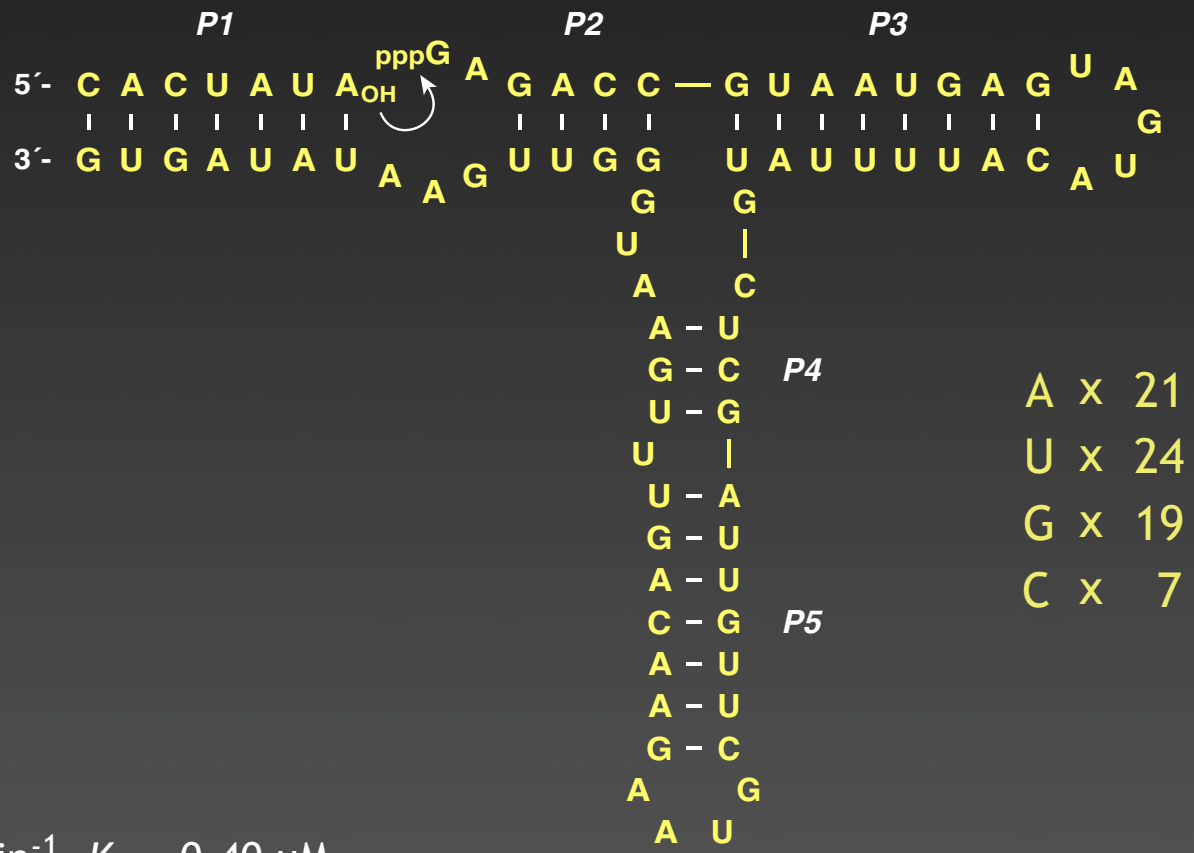


A x 24
U x 32
G x 16

k_{cat} = 0.013 min^{-1}, K_m = 6.2 μM

25 mM $MgCl_2$, pH 8.5, 23 °C

Rogers & Joyce, 2001

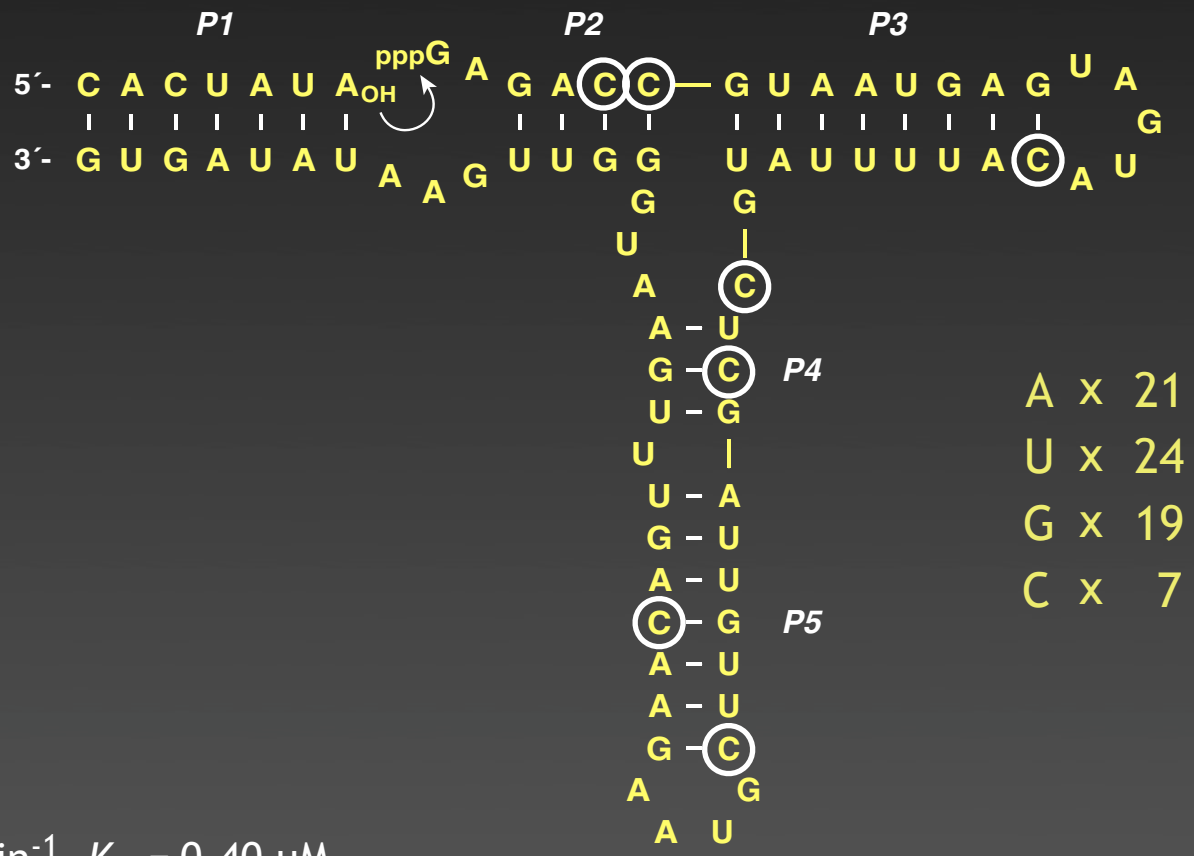


A x 21
U x 24
G x 19
C x 7

k_{cat} = 0.32 min^{-1}, K_m = 0.40 μM

25 mM $MgCl_2$, pH 8.5, 23 °C

Rogers & Joyce, 2001



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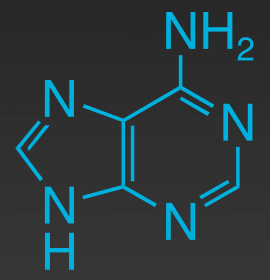
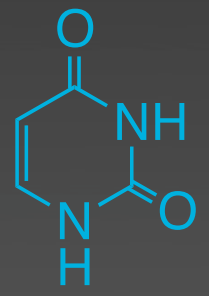
U x 24
G x 19
C x 7

k_{cat} = 0.32 min^{-1}, K_m = 0.40 μM

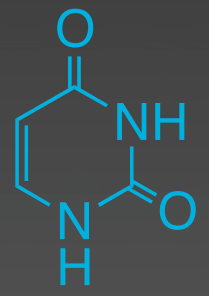
25 mM $MgCl_2$, pH 8.5, 23 °C

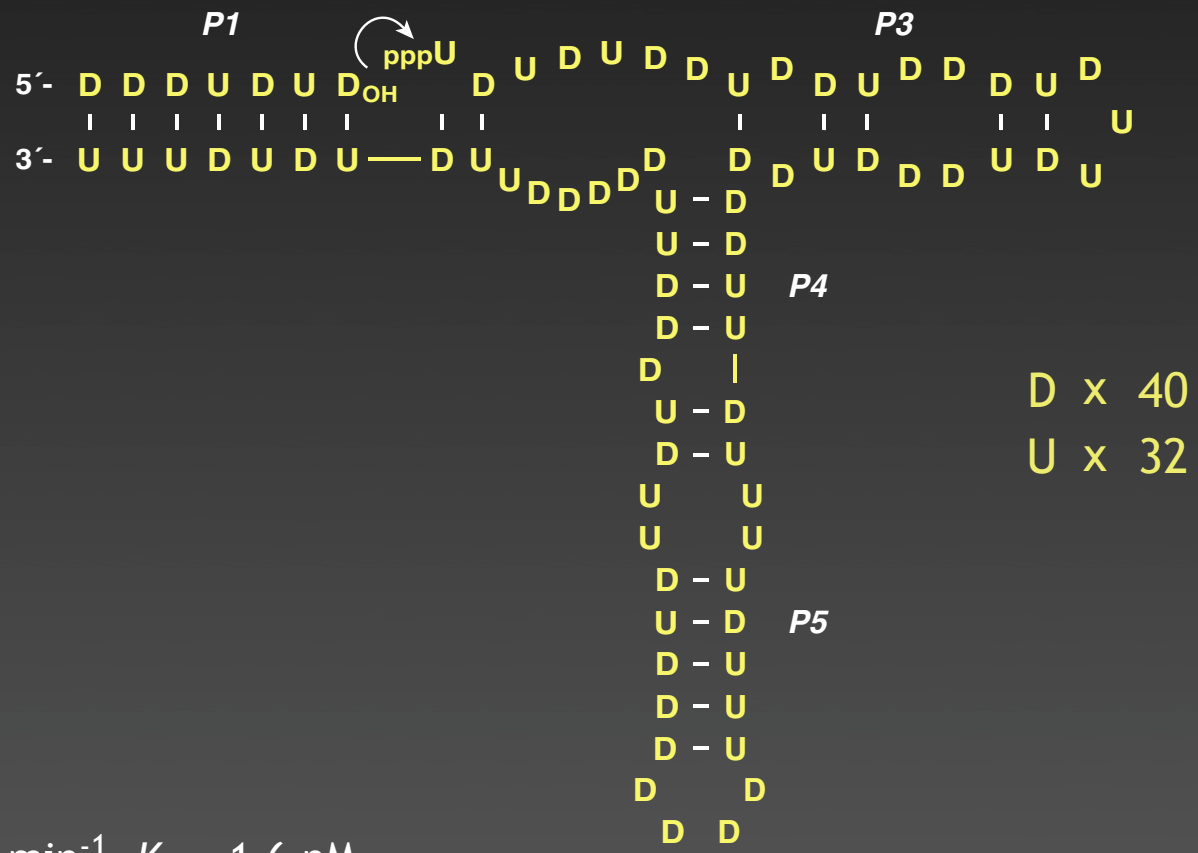
Rogers & Joyce, 2001


NH2
N
N
N
N
H


O
NH
O
N
H


NH2
N
N
N
NH2
H

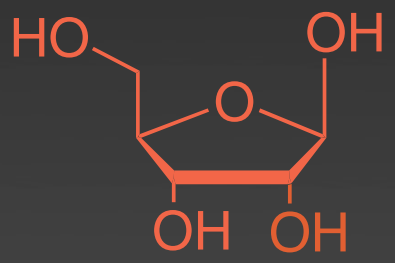

O
NH
N
O
H

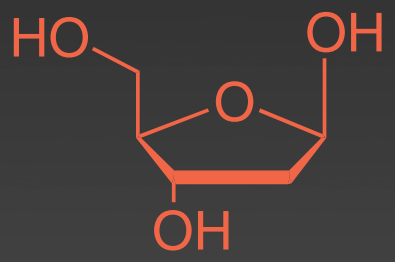


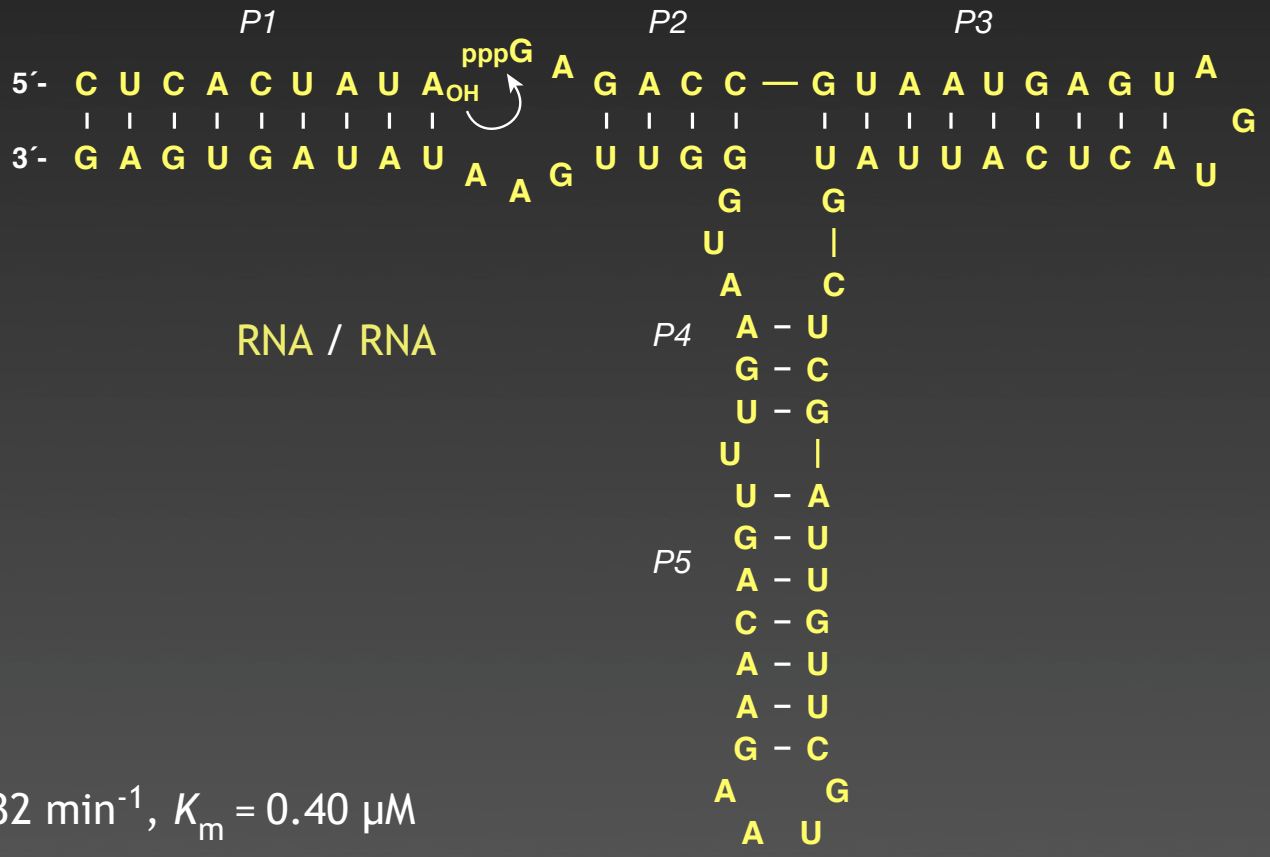
k_{cat} = 0.0011 min^{-1}, K_m = 1.6 nM

25 mM $MgCl_2$, pH 8.5, 23 °C

Reader & Joyce, 2002


HO
OH
O
OH
OH


HO
OH
O
OH

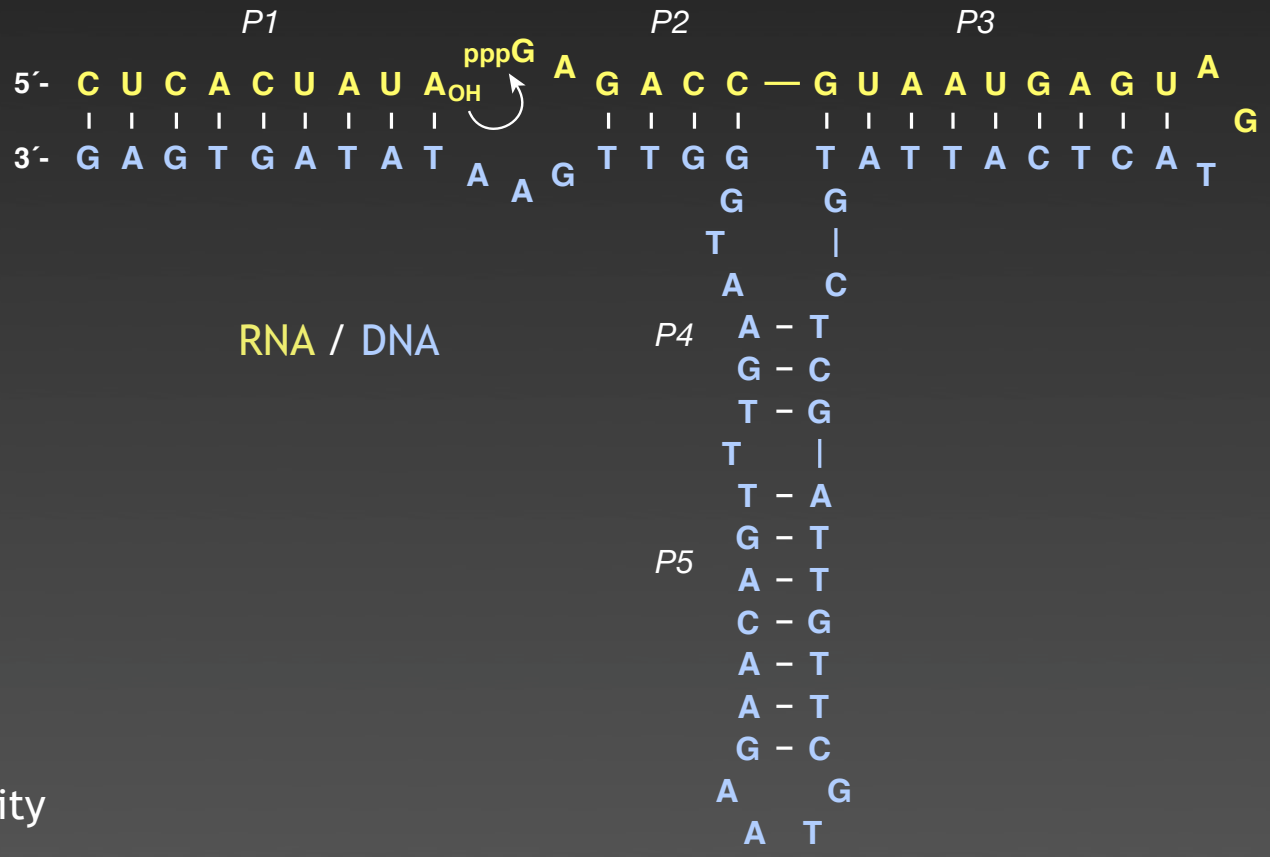


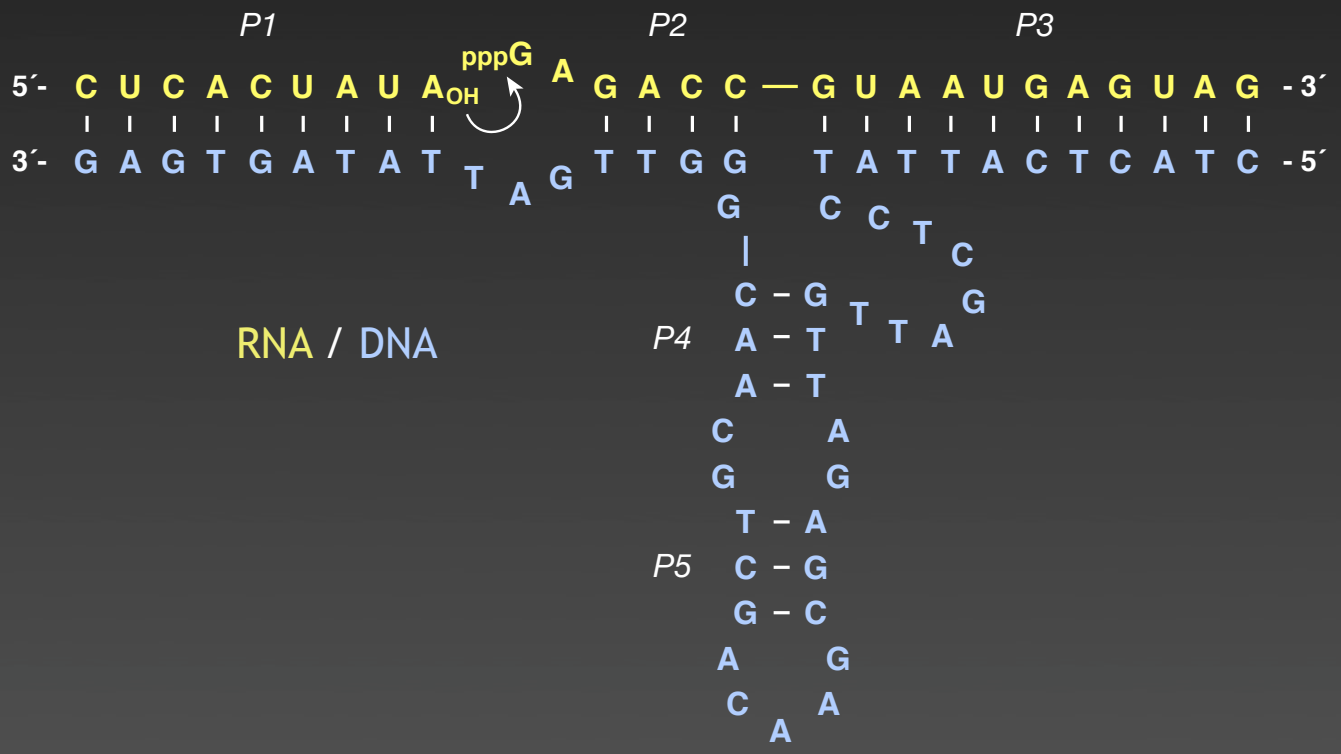
RNA / RNA

k_{cat} = 0.32 min^{-1}, K_m = 0.40 μM

25 mM $MgCl_2$, pH 8.5, 23 °C

Rogers & Joyce, 2001

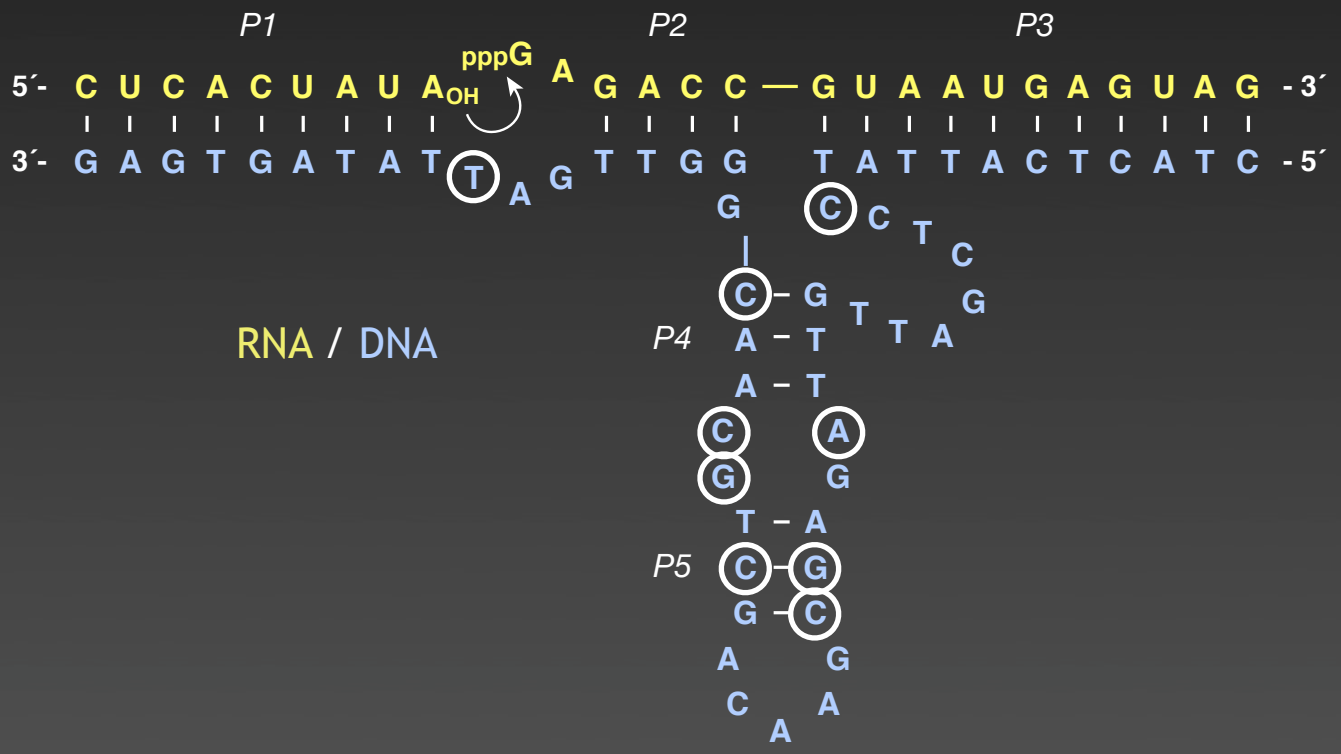

P1
P2
P3
5´- C U C A C U A U A OH pppG A G A C C — G U A A U G A G U A G
3´- G A G T G A T A T A A G T T G G T A T T A C T C A T
G G
T |
A C
P4
A – T
G – C
T – G
T |
T – A
P5
G – T
A – T
C – G
A – T
A – T
G – C
A G
A T
RNA / DNA
no activity



k_{cat} = 0.052 min^{-1}, K_m = 0.45 μM

25 mM $MgCl_2$, pH 8.5, 23 °C

Paul *et al.*, 2006

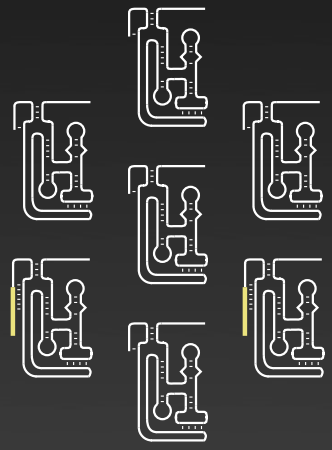


k_{cat} = 0.052 min^{-1}, K_m = 0.45 μM

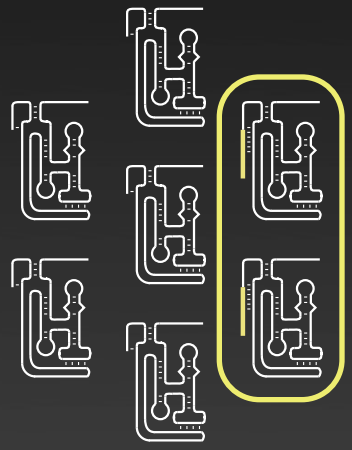
25 mM $MgCl_2$, pH 8.5, 23 °C

Paul *et al.*, 2006

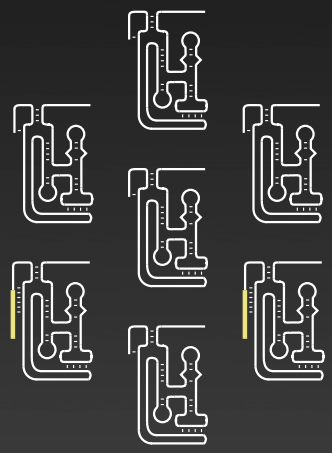
react



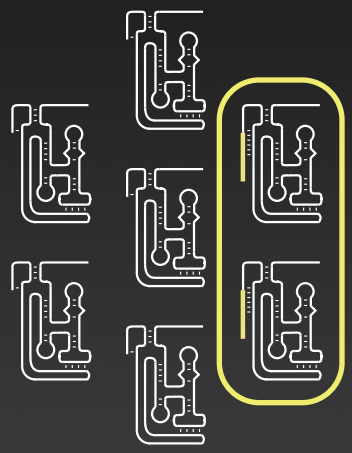
react



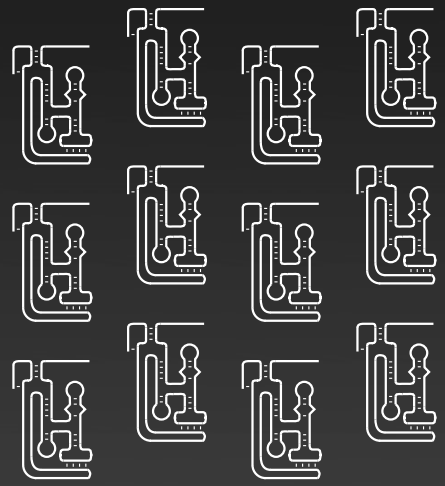
select



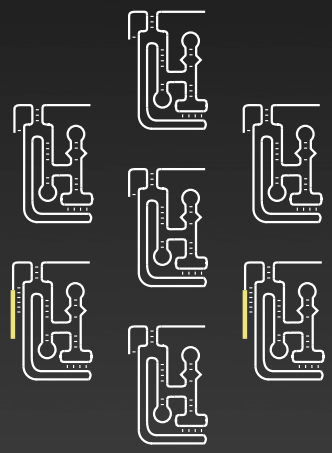
react



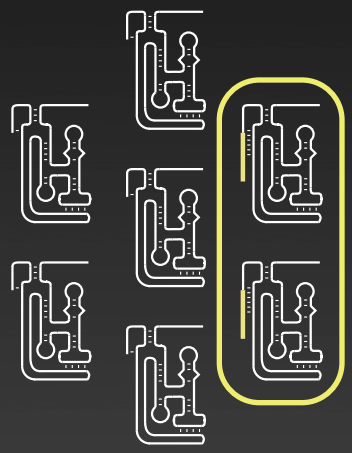
select



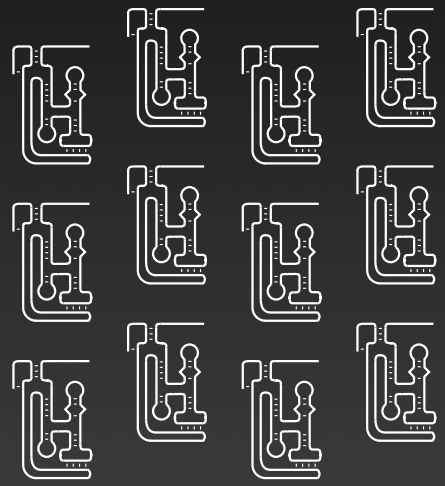
amplify (mutate)



react

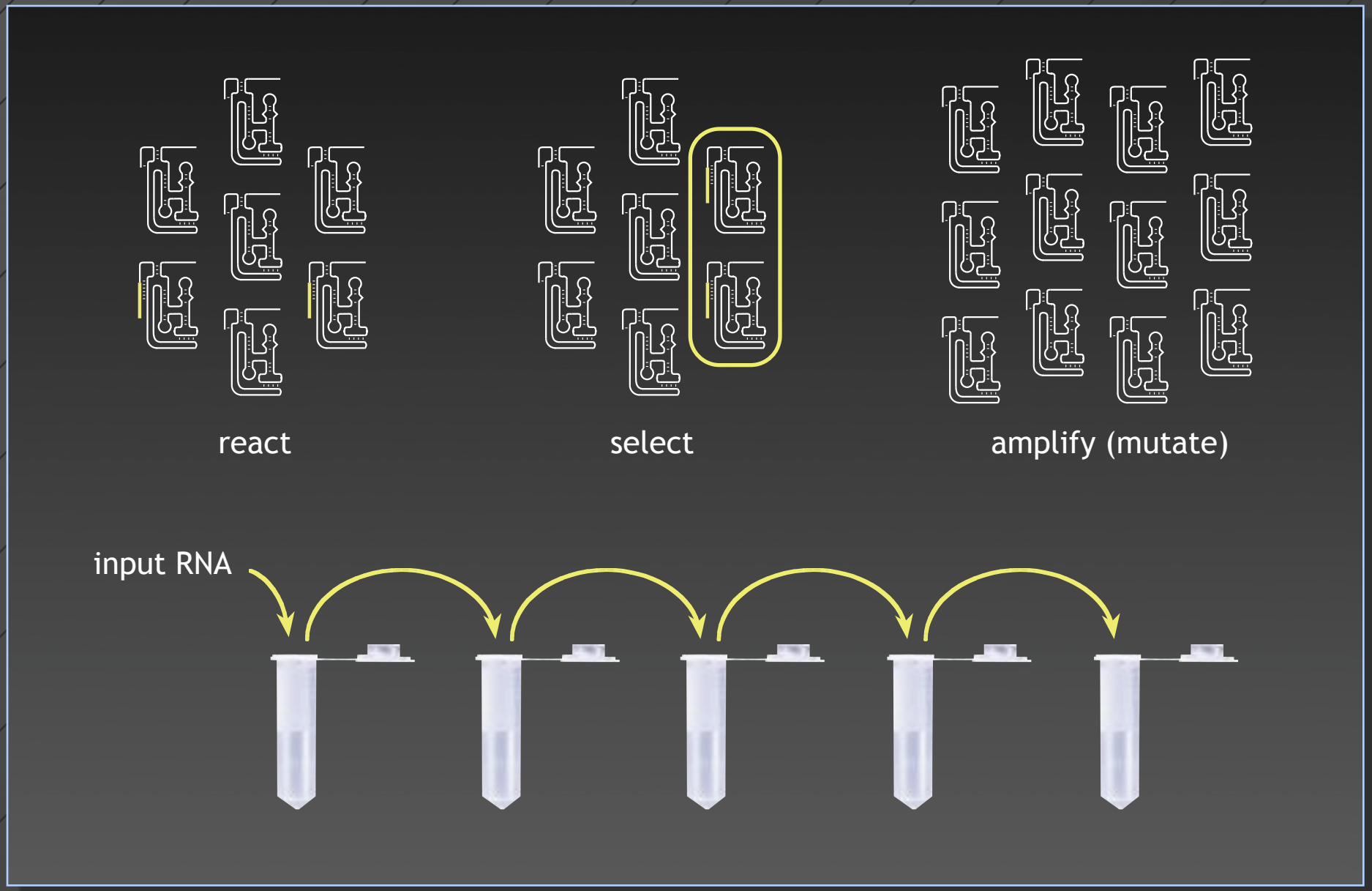


select



amplify (mutate)

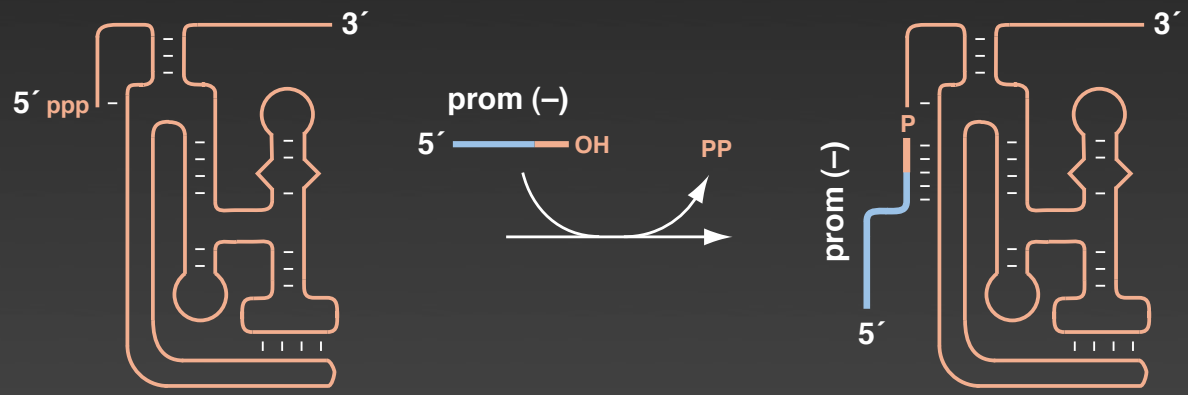
input RNA


react
select
amplify (mutate)
input RNA

Continuous *in vitro* evolution

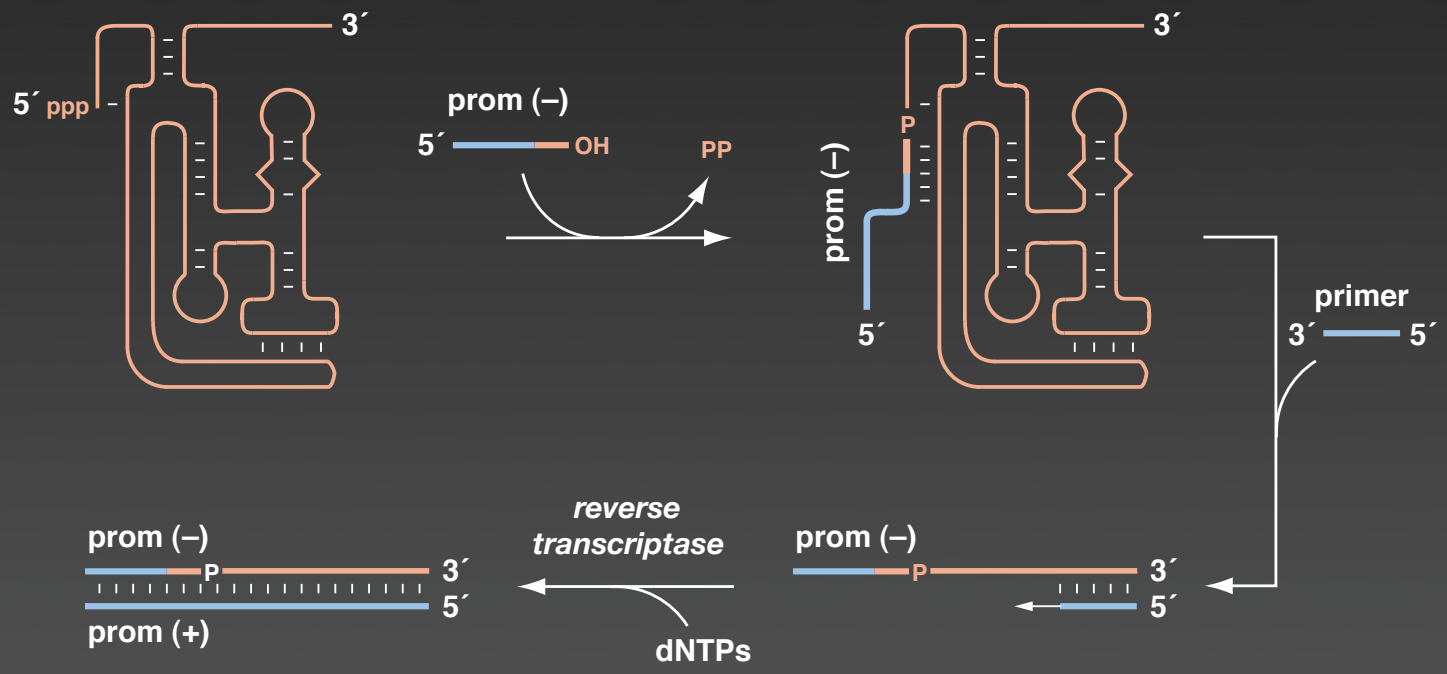
Wright & Joyce, 1997

Continuous *in vitro* evolution



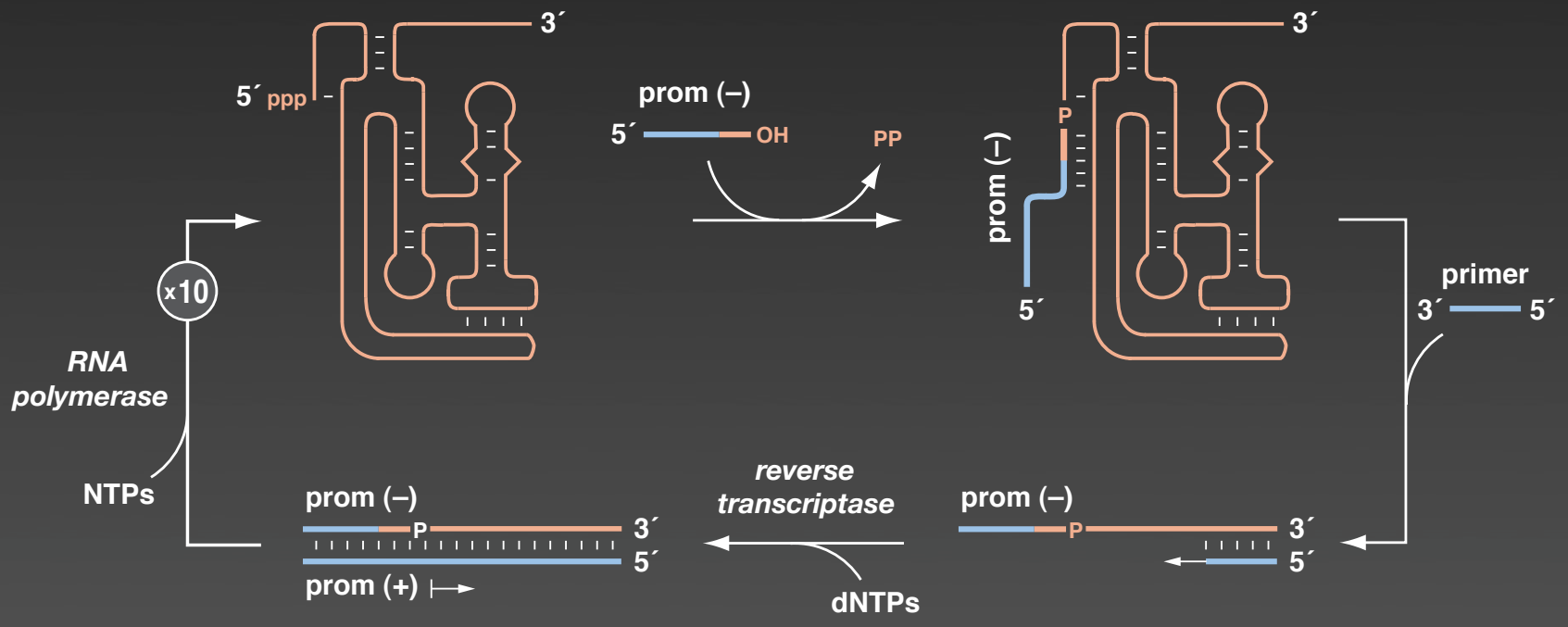
Wright & Joyce, 1997

Continuous *in vitro* evolution



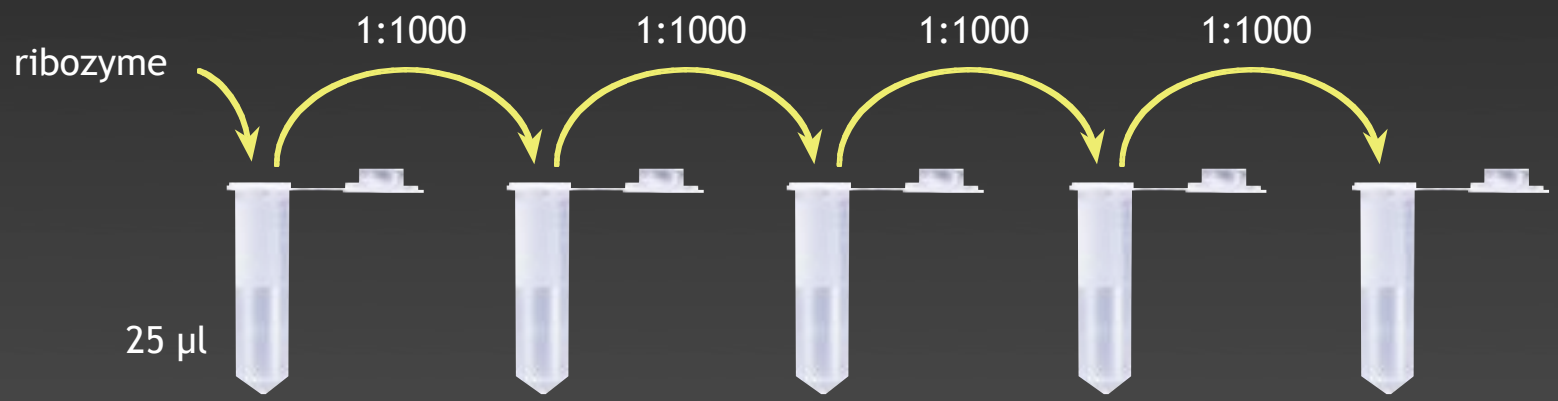
Wright & Joyce, 1997

Continuous *in vitro* evolution

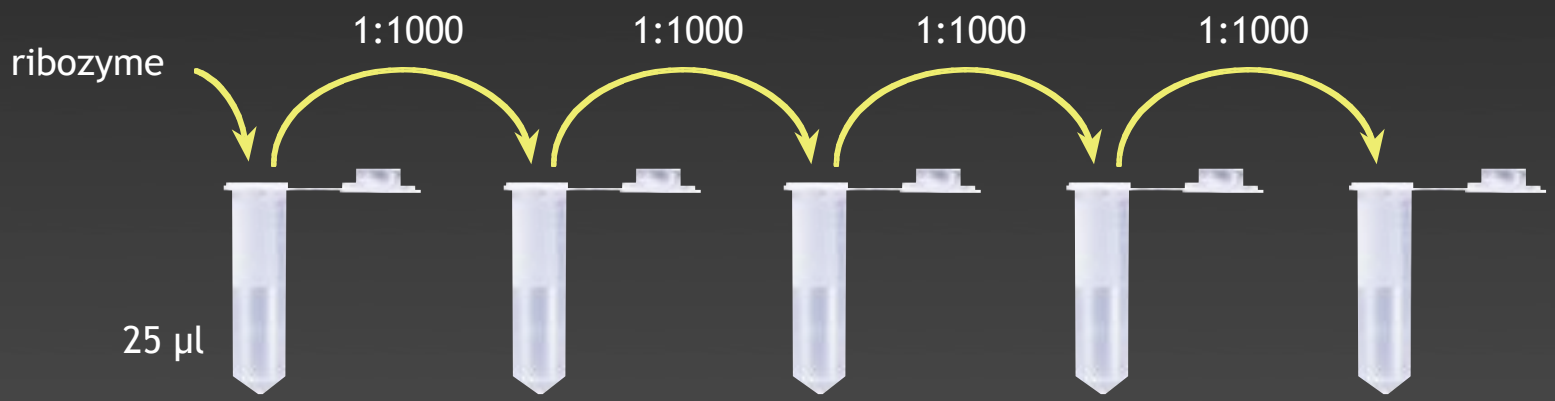


Wright & Joyce, 1997

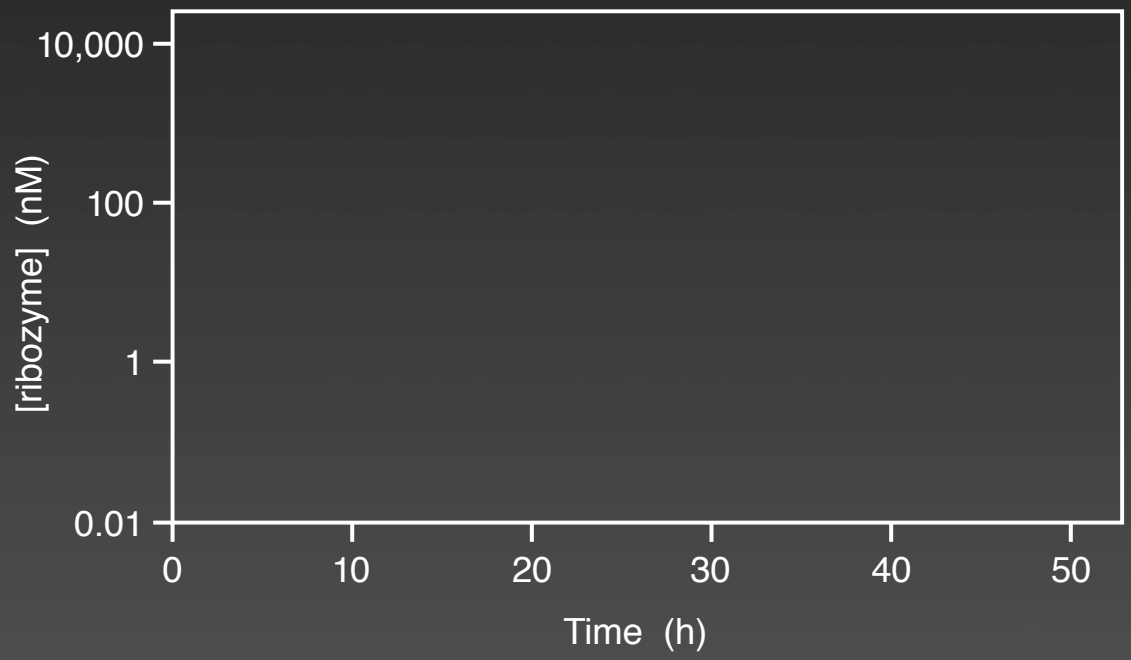
Continuous *in vitro* evolution



Continuous *in vitro* evolution

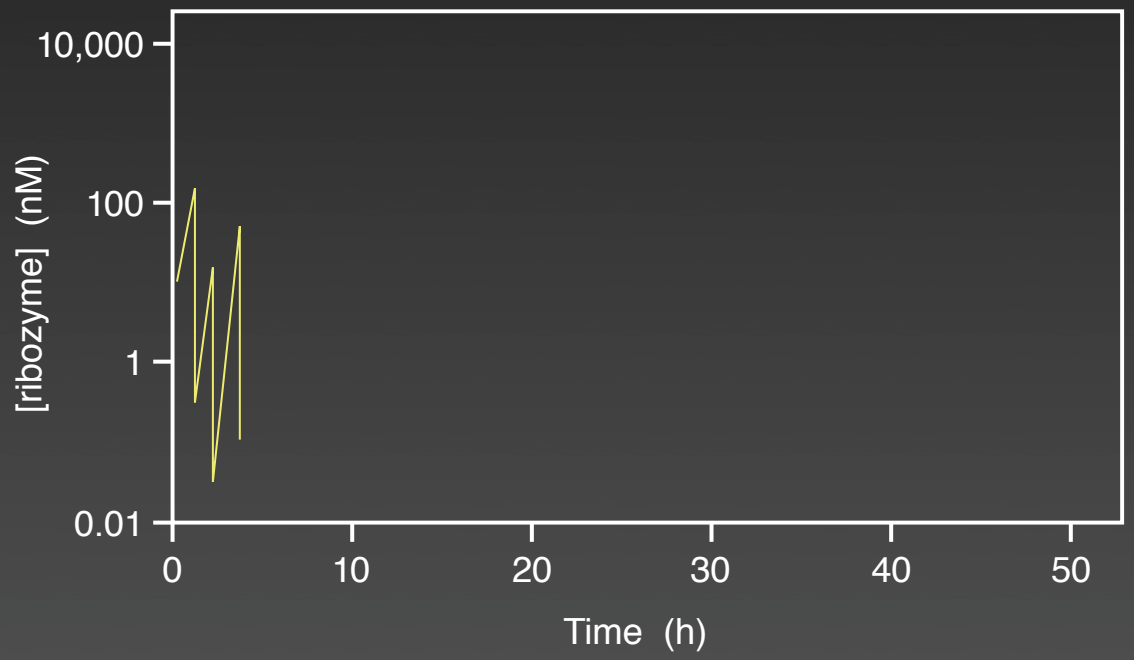


5 µM substrate, 2 µM RT primer, 2 mM NTPs, 0.2 mM dNTPs,
8 U/µl reverse transcriptase, 4 U/µl T7 RNA pol, 5 mM DTT,
25 mM $MgCl_2$, 50 mM KCl, 2 mM spermidine, pH 8.5, 37 °C



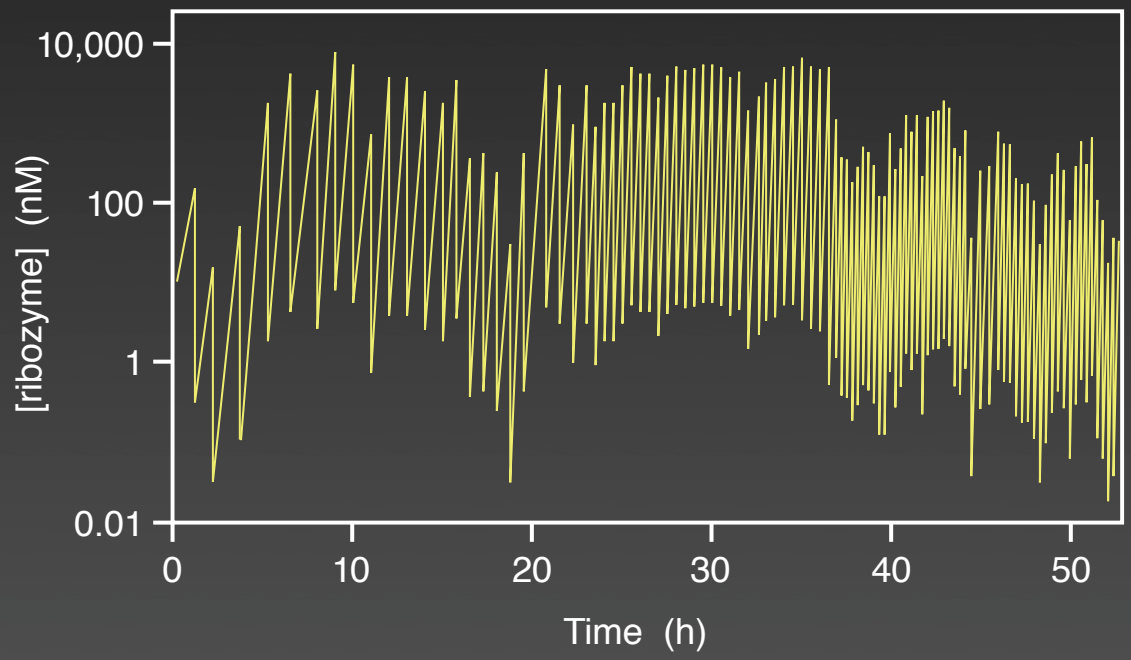
25 mM $MgCl_2$, pH 8.5, 37 °C

Wright & Joyce, 1997



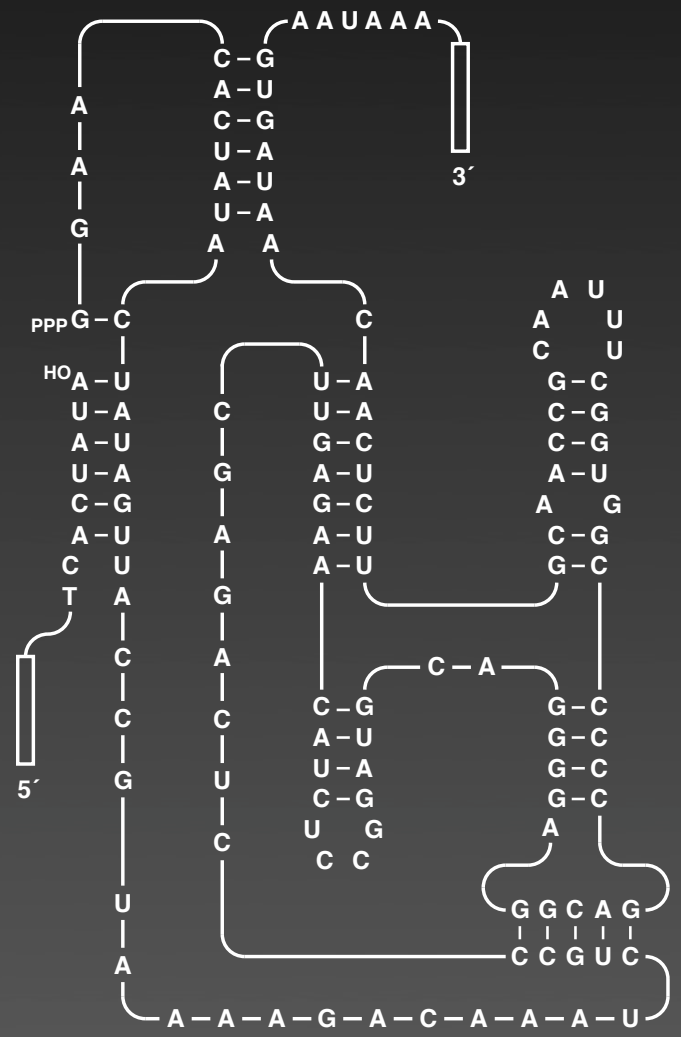
25 mM $MgCl_2$, pH 8.5, 37 °C

Wright & Joyce, 1997



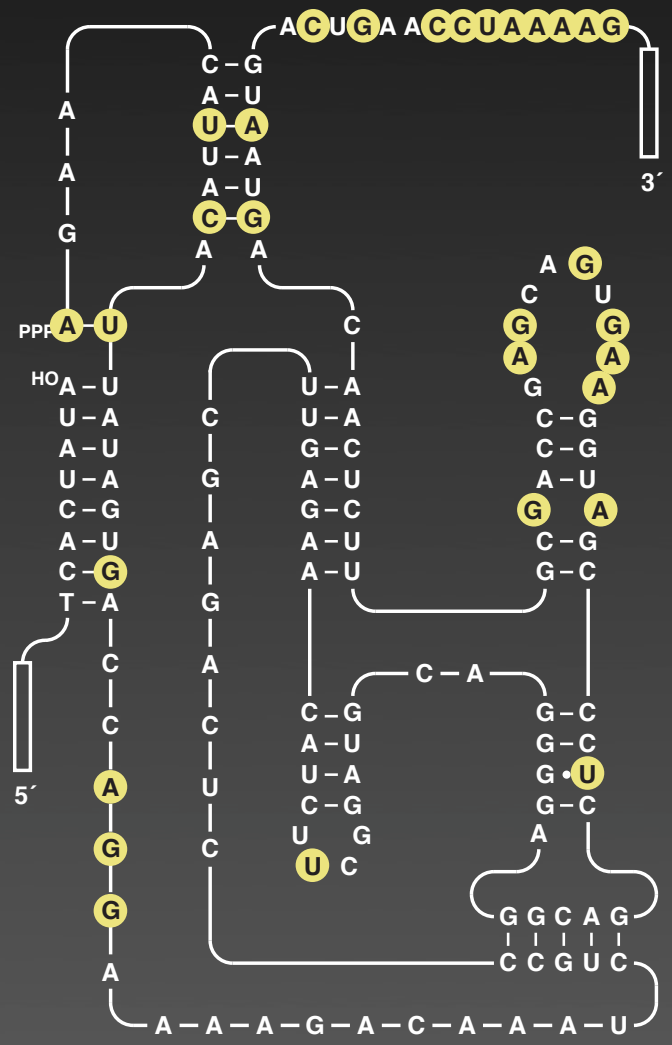
25 mM $MgCl_2$, pH 8.5, 37 °C

Wright & Joyce, 1997



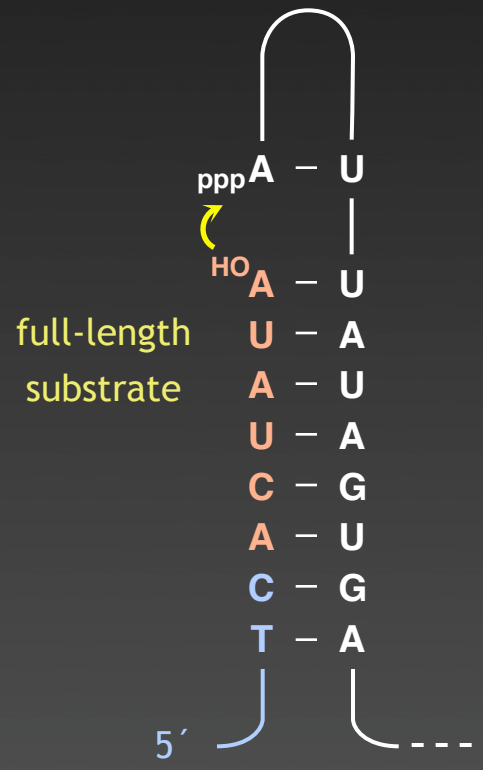
starting ligase

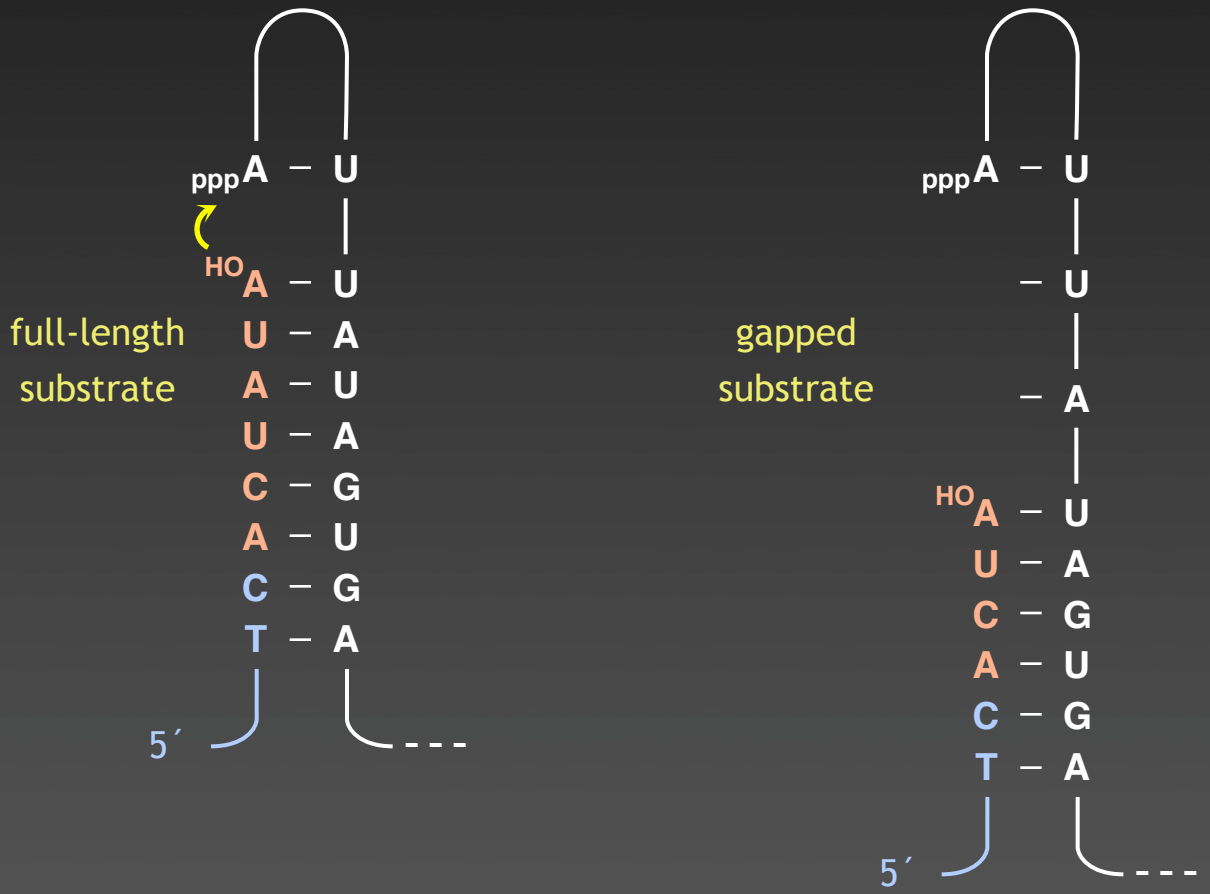
Ekland *et al.*, 1995

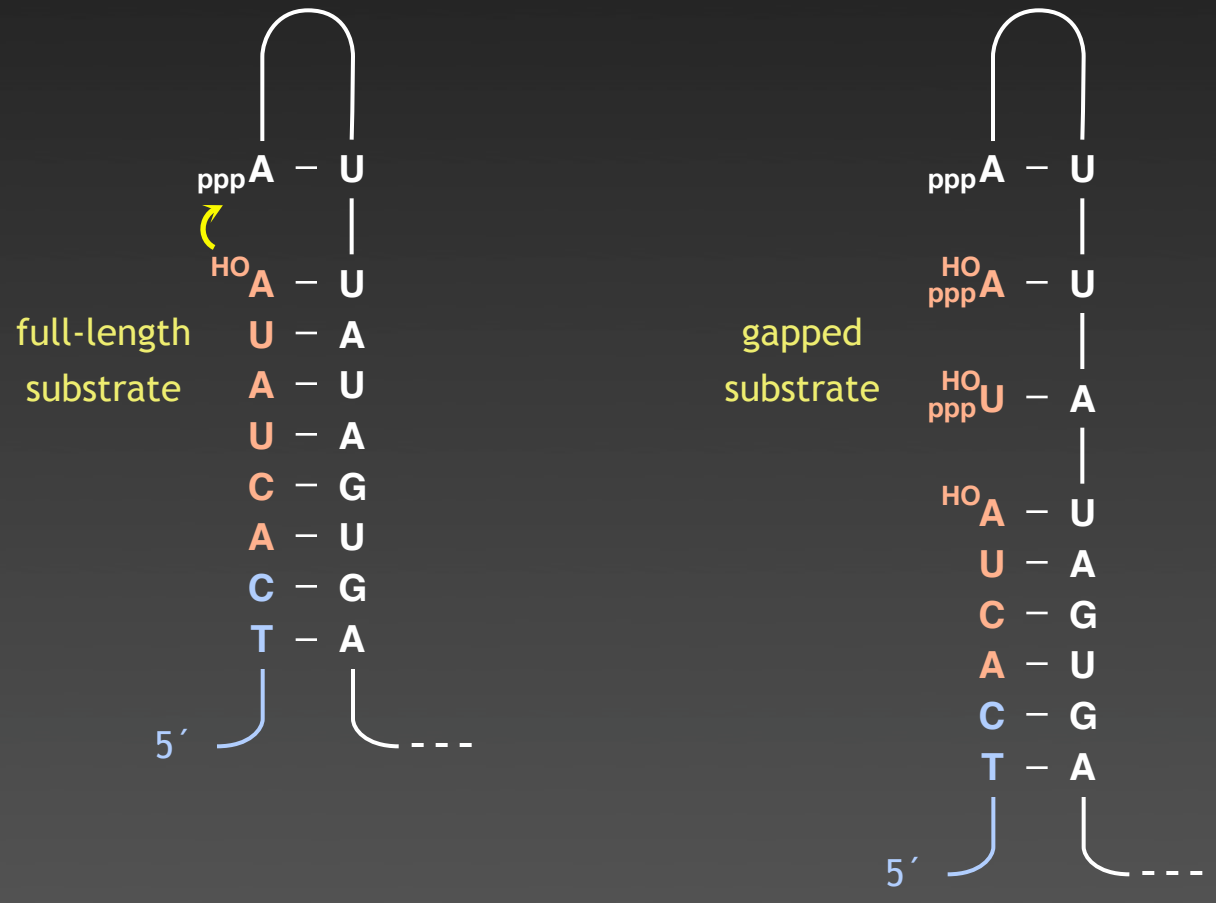


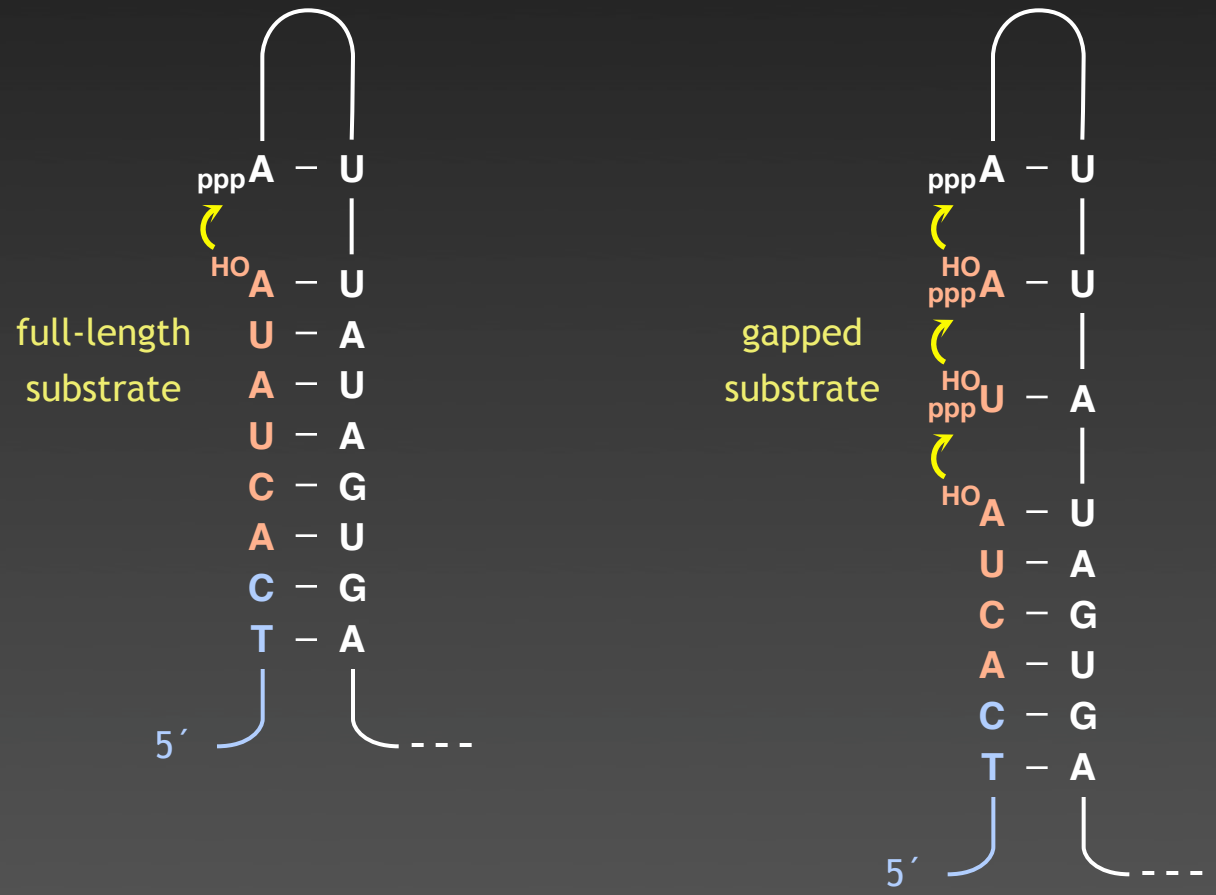
evolved ligase

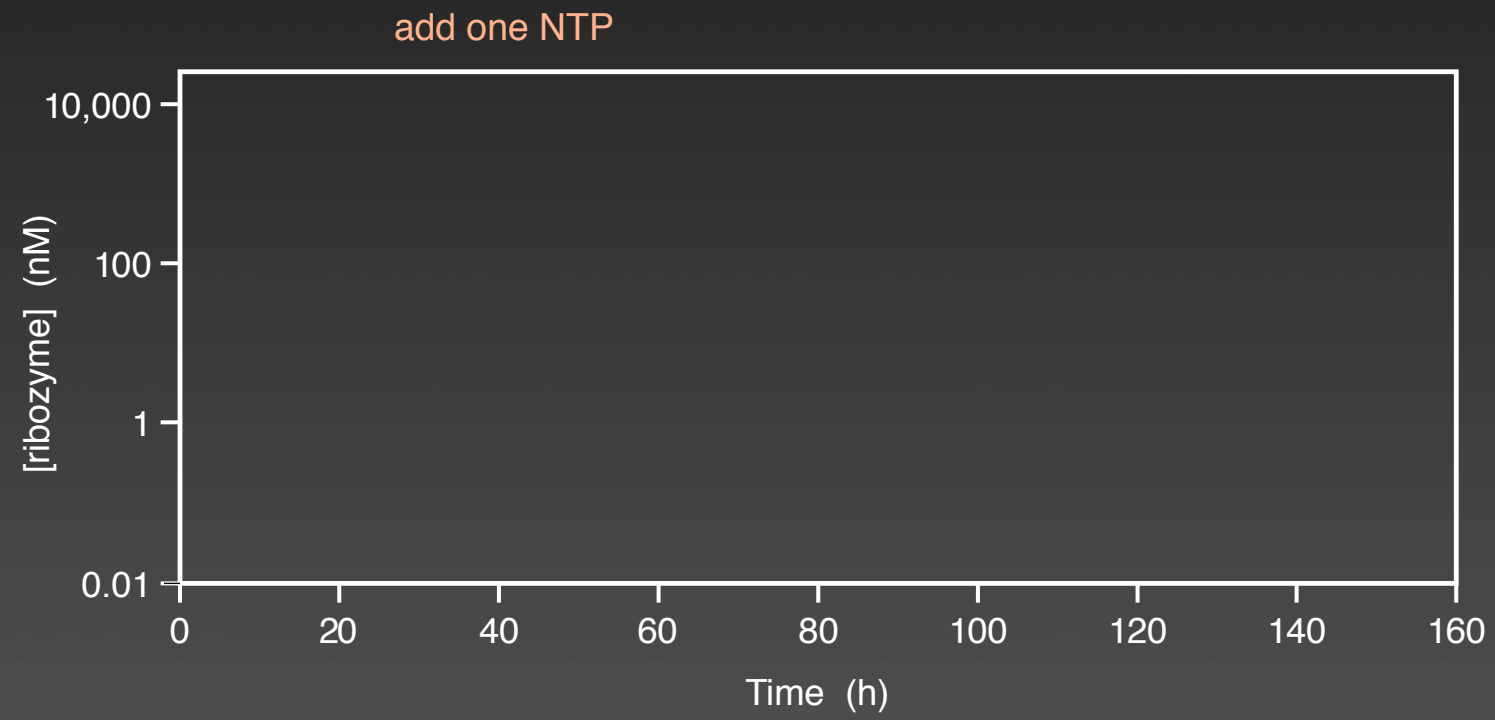
Wright & Joyce, 1997


pppA – U
HO
A – U
full-length
substrate
U – A
A – U
U – A
C – G
A – U
C – G
T – A
5´


full-length
substrate
pppA – U
HO A – U
U – A
A – U
U – A
C – G
A – U
C – G
T – A
5′
gapped
substrate
pppA – U
– U
– A
HO A – U
U – A
C – G
A – U
C – G
T – A
5′

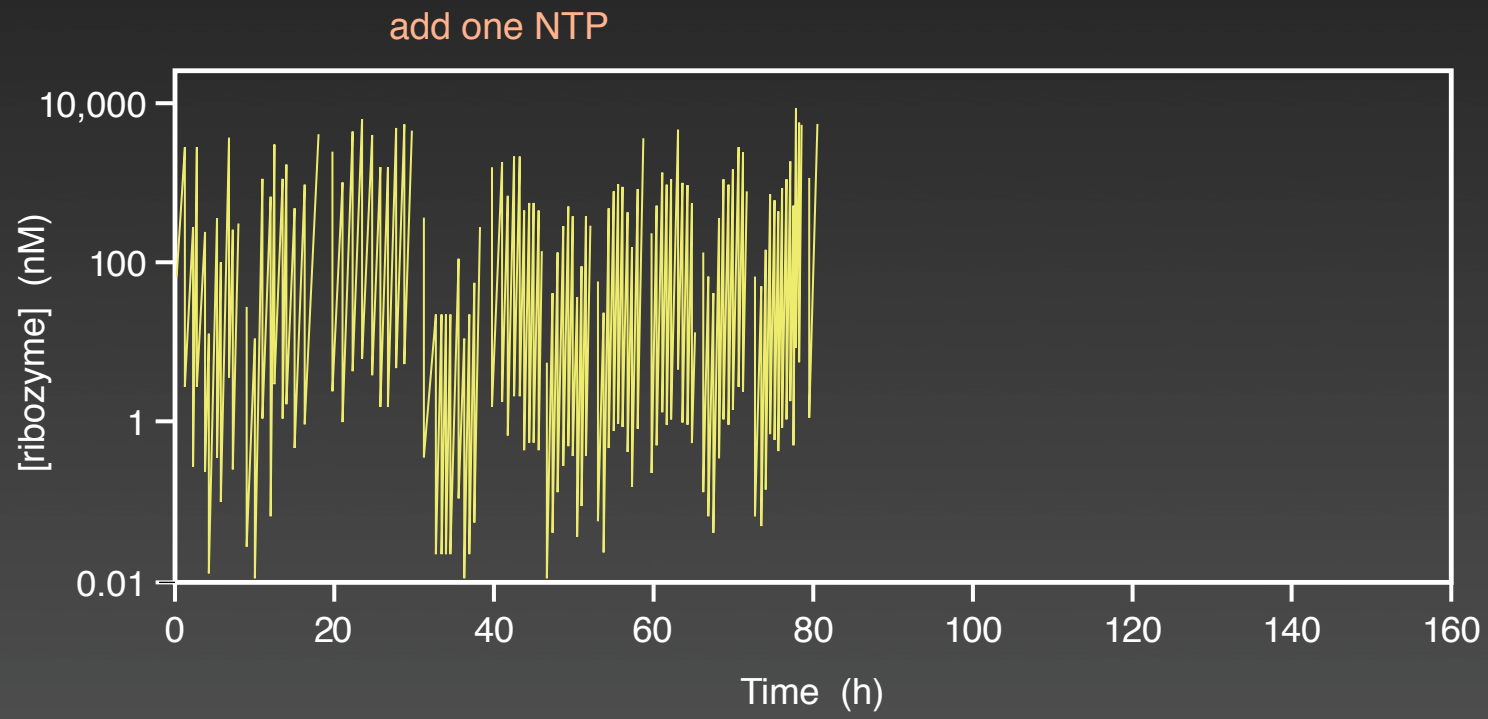

full-length
substrate
pppA – U
HO A – U
U – A
A – U
U – A
C – G
A – U
C – G
T – A
5′
gapped
substrate
pppA – U
HO
pppA – U
HO
pppU – A
HO A – U
U – A
C – G
A – U
C – G
T – A
5′


full-length
substrate
pppA – U
HO A – U
U – A
A – U
U – A
C – G
A – U
C – G
T – A
5′
gapped
substrate
pppA – U
HO
pppA – U
HO
pppU – A
HO A – U
U – A
C – G
A – U
C – G
T – A
5′



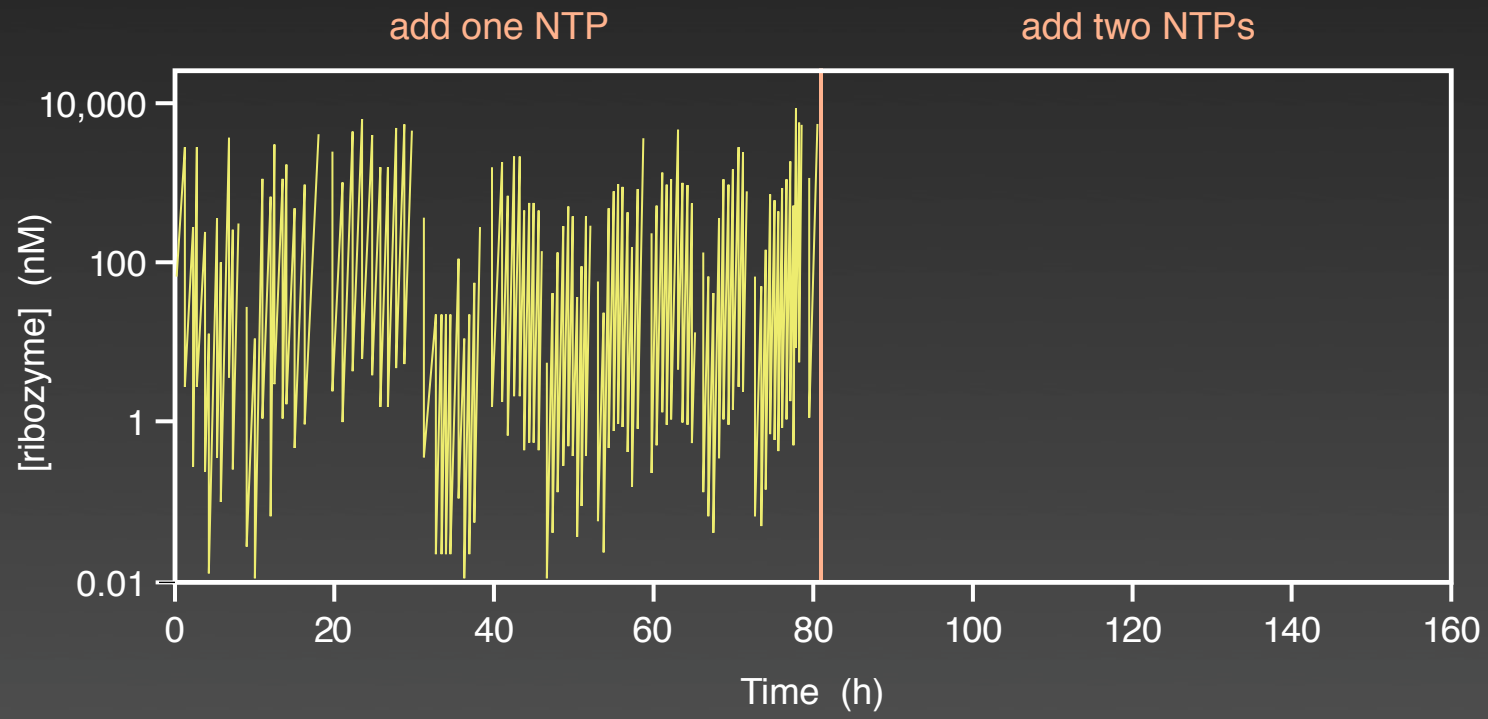
25 mM $MgCl_2$, pH 8.5, 37 °C

McGinness *et al.*, 2002



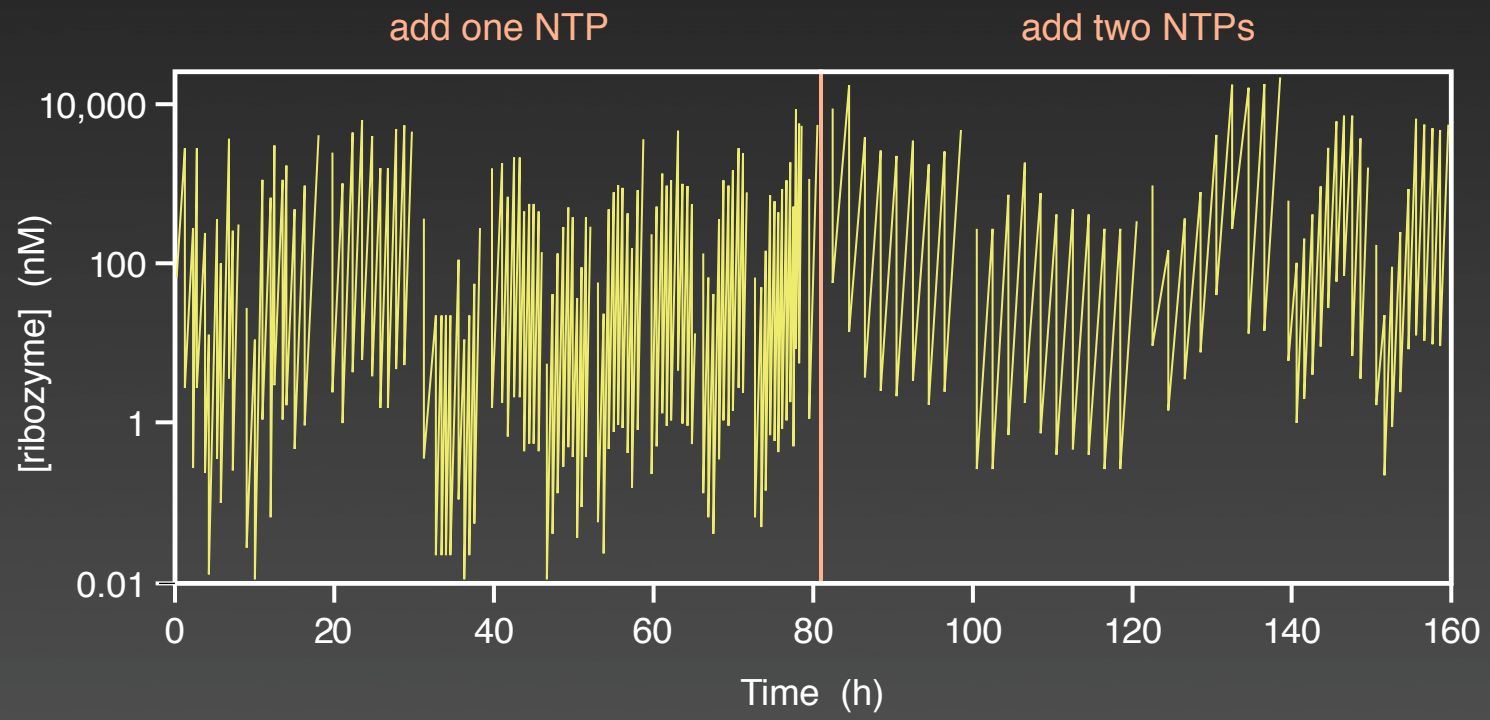
25 mM $MgCl_2$, pH 8.5, 37 °C

McGinness *et al.*, 2002



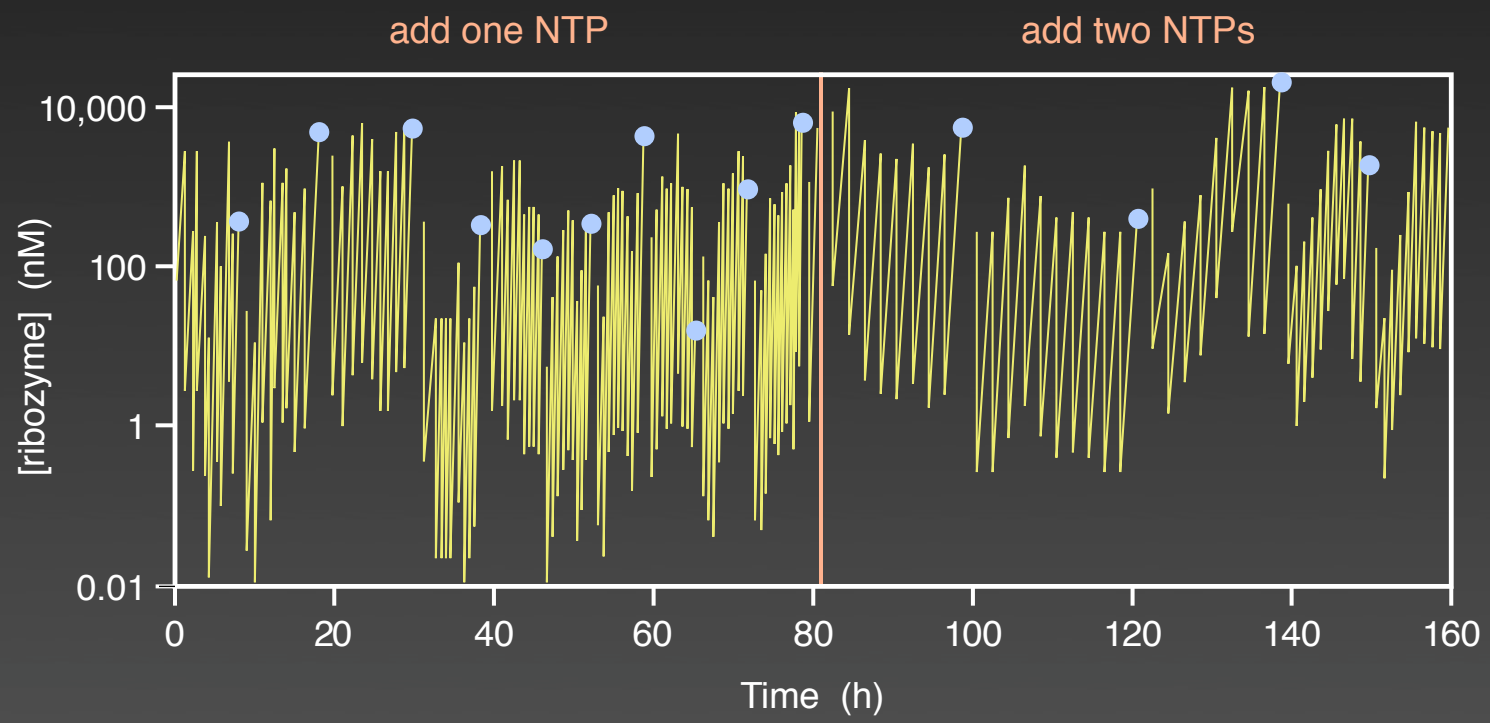
25 mM $MgCl_2$, pH 8.5, 37 °C

McGinness *et al.*, 2002



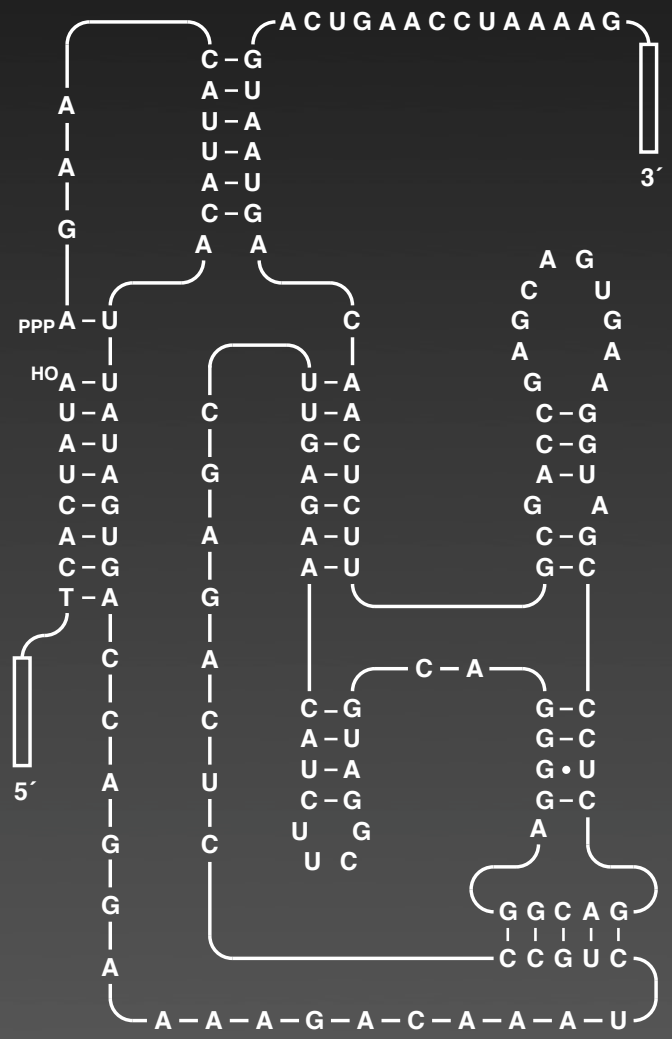
25 mM $MgCl_2$, pH 8.5, 37 °C

McGinness *et al.*, 2002



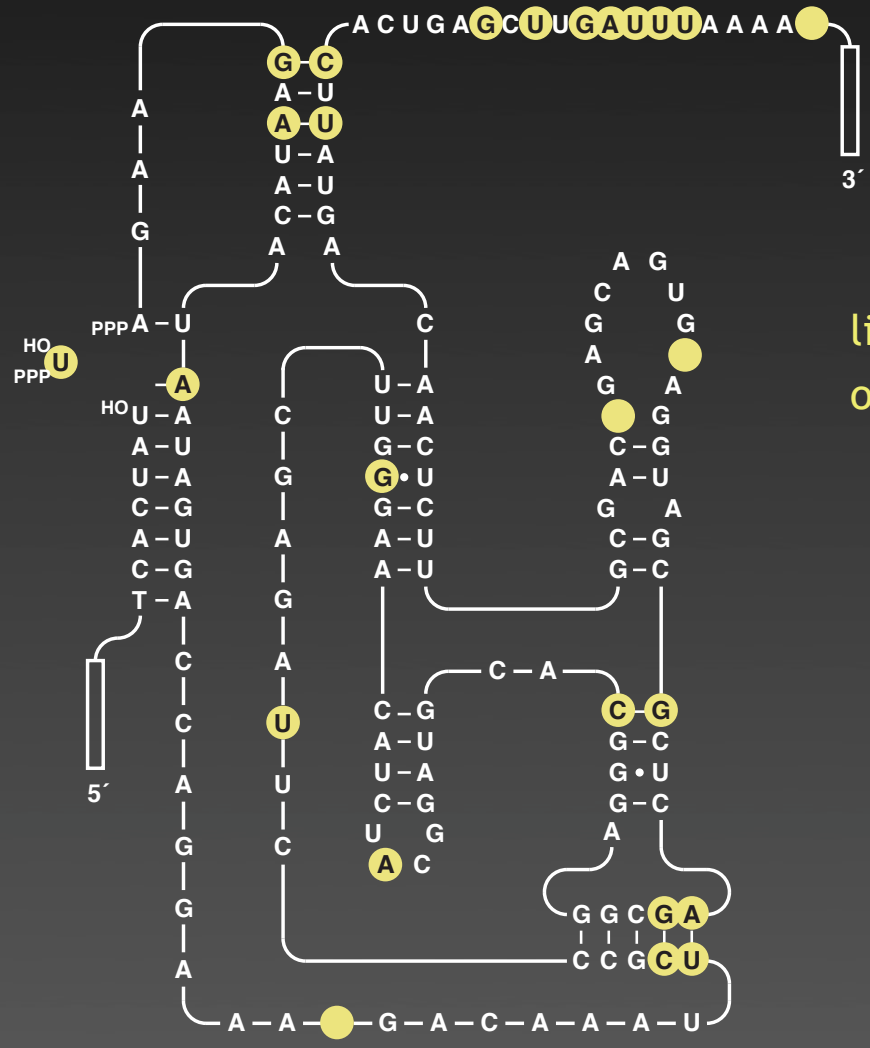
25 mM $MgCl_2$, pH 8.5, 37 °C

McGinness *et al.*, 2002



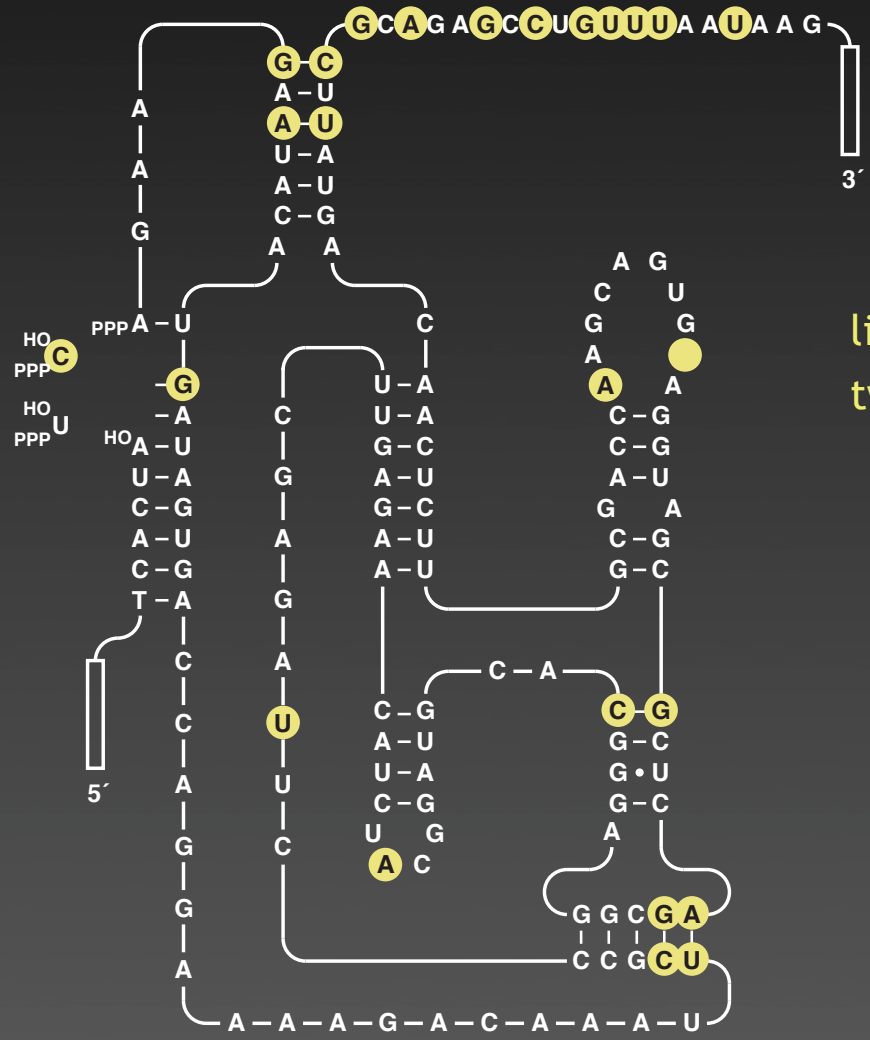
ligation only

Wright & Joyce, 1997



ligation plus
one NTP addition

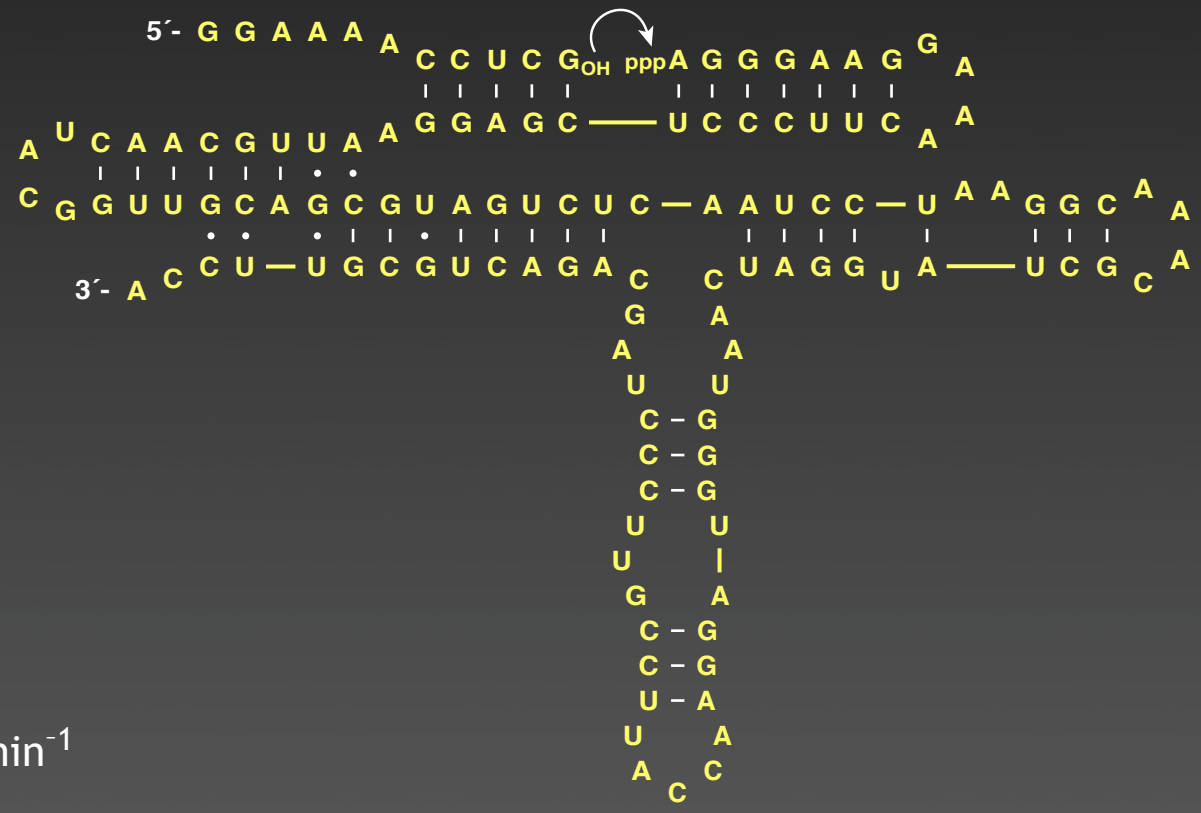
McGinness *et al.*, 2002



ligation plus
two NTP additions

McGinness *et al.*, 2002

DSL ligase ribozyme

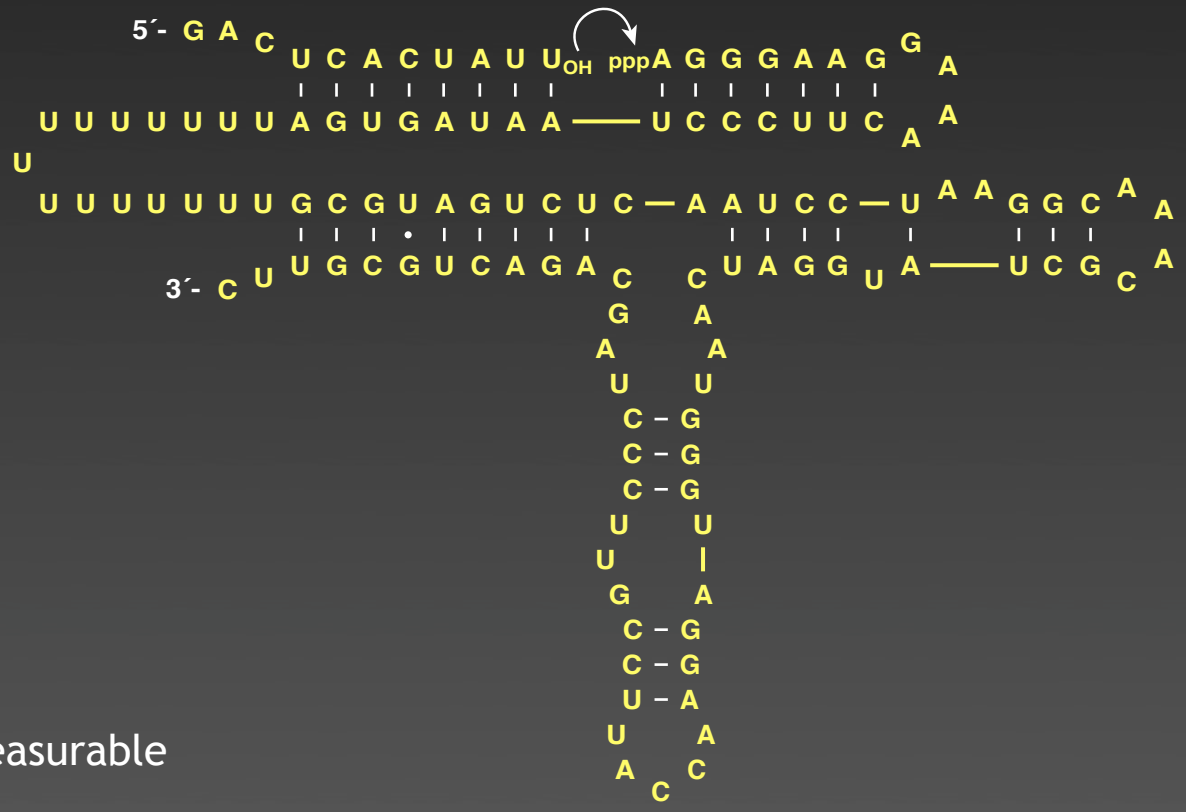


k_{cat} = 0.07 min^{-1}

50 mM $MgCl_2$, pH 7.5, 37 °C

Ikawa *et al.*, 2004

Add flexible tether

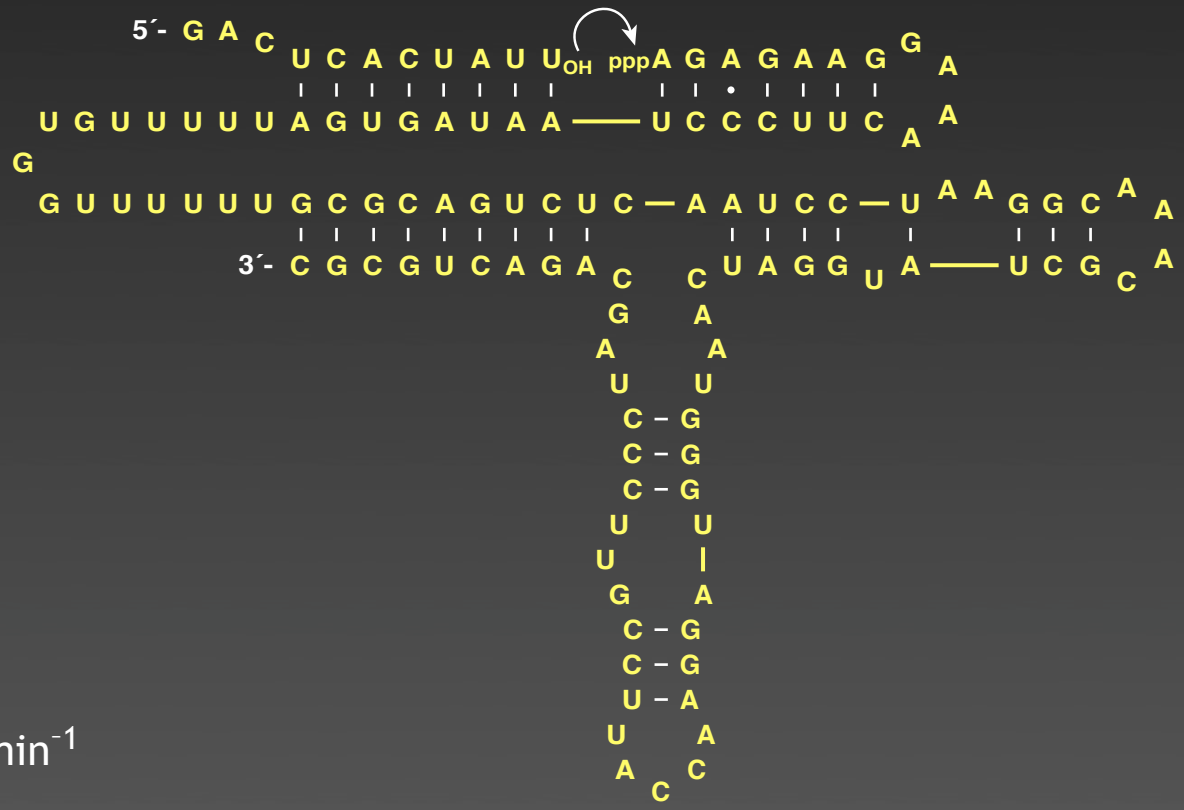


k_{cat} : not measurable

2 mM NTPs, 25 mM $MgCl_2$, pH 8.5, 37 °C

Voytek & Joyce, unpublished

Optimize by stepwise evolution

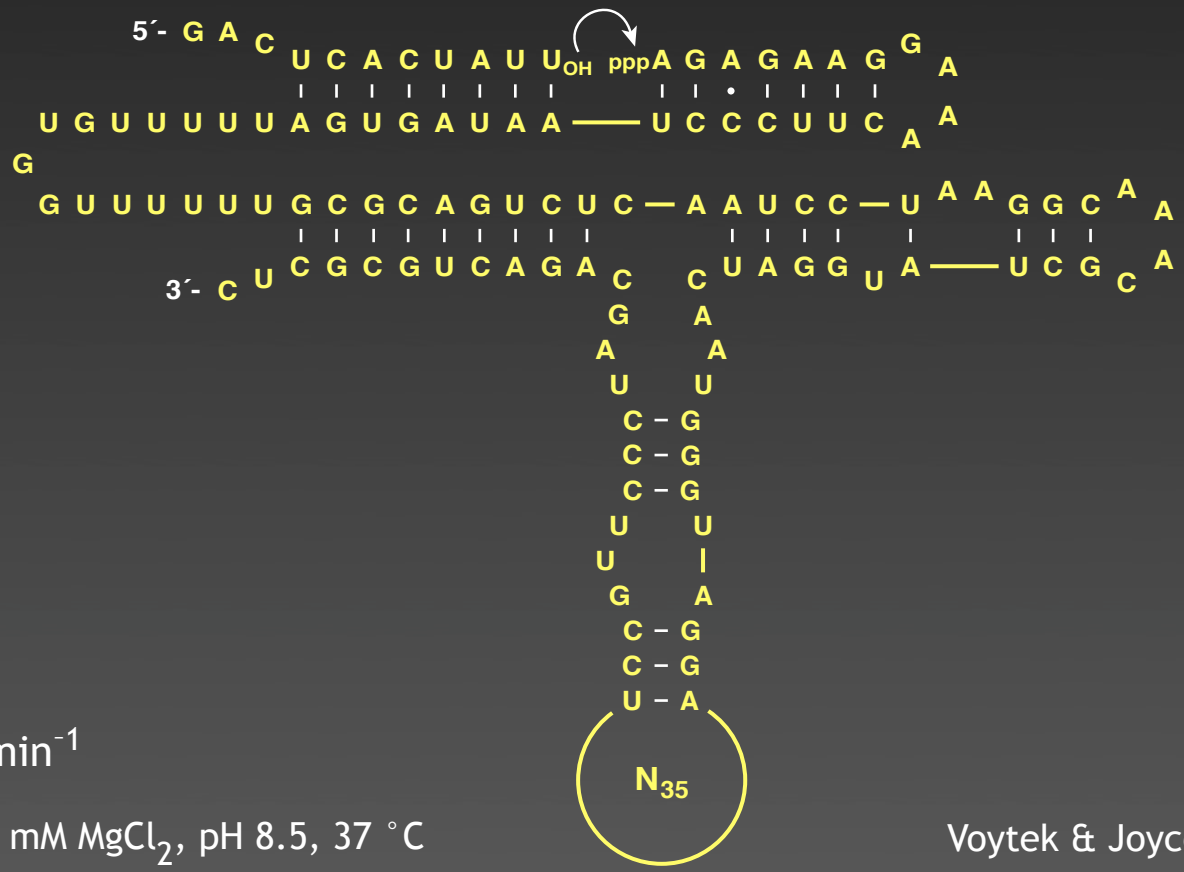


k_{cat} = 0.006 min^{-1}

2 mM NTPs, 25 mM $MgCl_2$, pH 8.5, 37 °C

Voytek & Joyce, unpublished

Add 35 random nucleotides

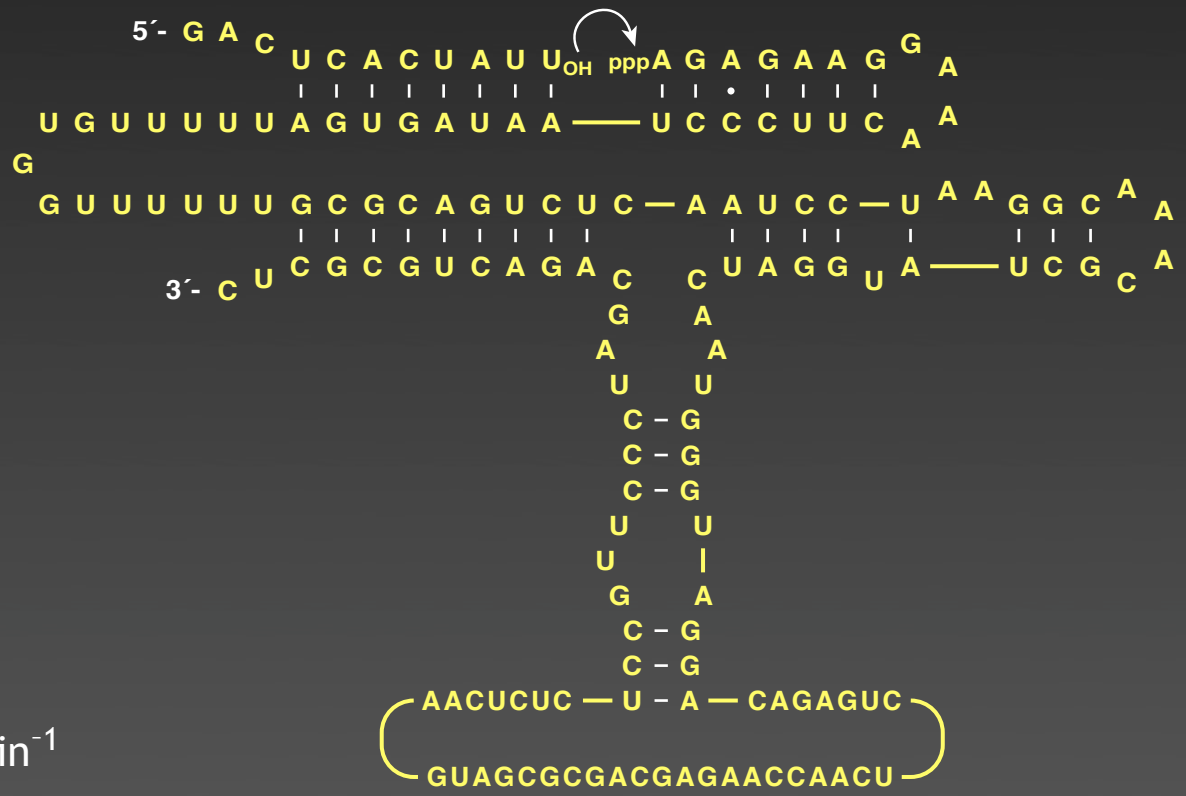


$k_{obs} \approx 0.006\ min^{-1}$

2 mM NTPs, 25 mM $MgCl_2$, pH 8.5, 37 °C

Voytek & Joyce, unpublished

Optimize by stepwise evolution

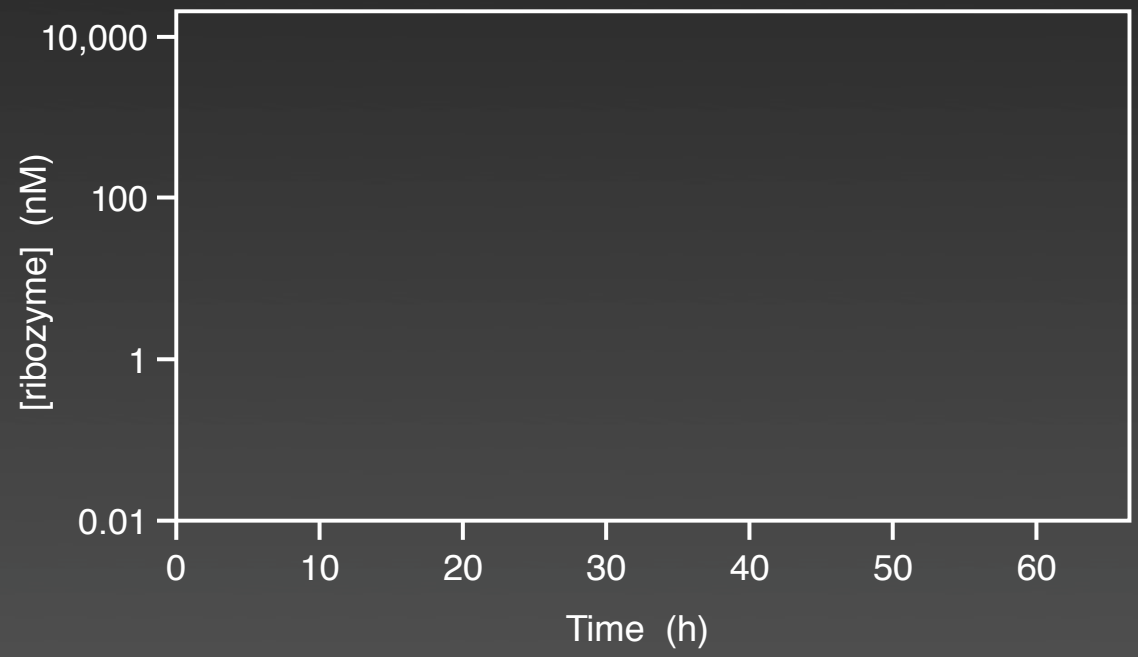


$k_{obs} \approx 0.35\ min^{-1}$

2 mM NTPs, 25 mM $MgCl_2$, pH 8.5, 37 °C

Voytek & Joyce, unpublished

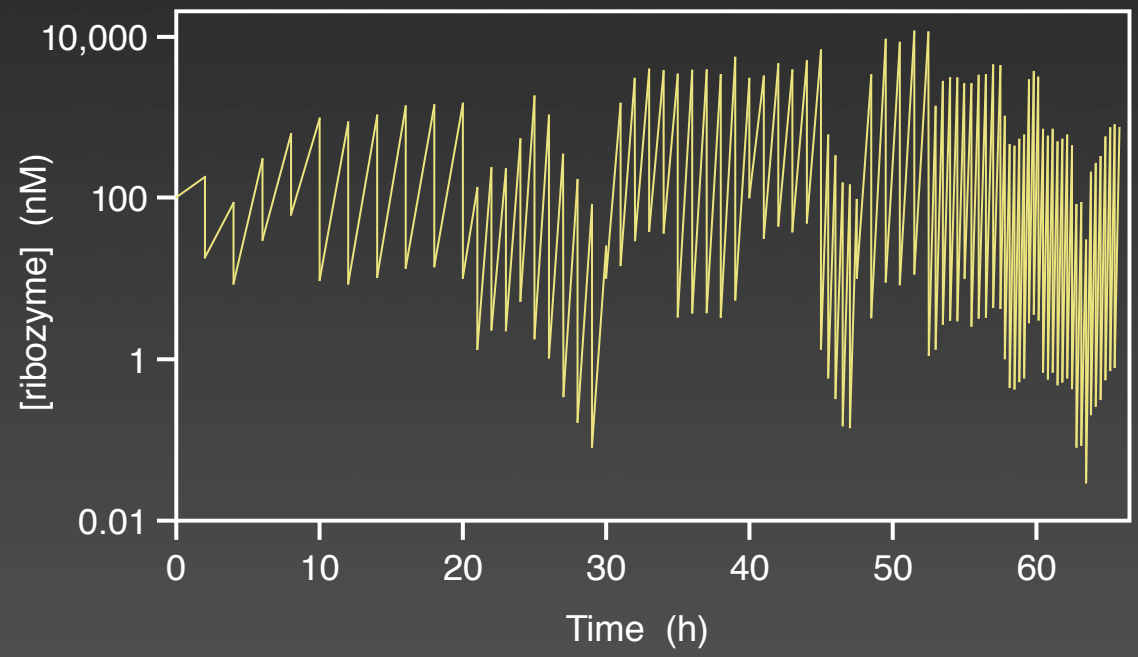
Initiate continuous evolution



2 mM NTPs, 25 mM $MgCl_2$, pH 8.5, 37 °C

Voytek & Joyce, unpublished

Initiate continuous evolution



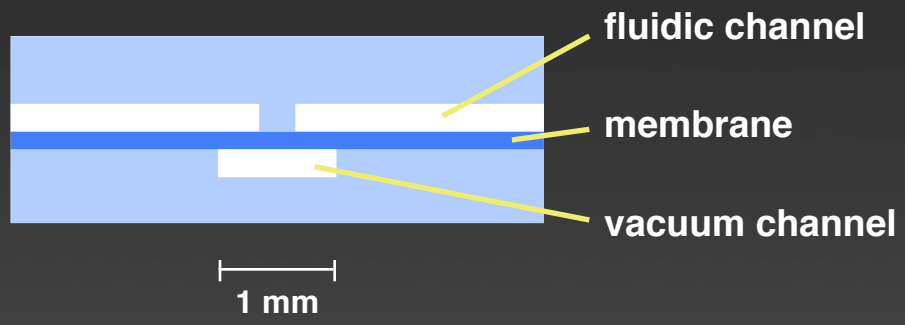
2 mM NTPs, 25 mM $MgCl_2$, pH 8.5, 37 °C

Voytek & Joyce, unpublished

Microfluidic-based evolution

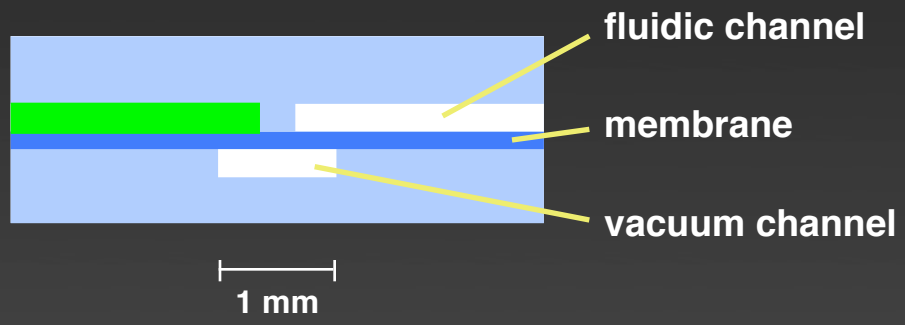
Grover *et al.*, 2003

Microfluidic-based evolution



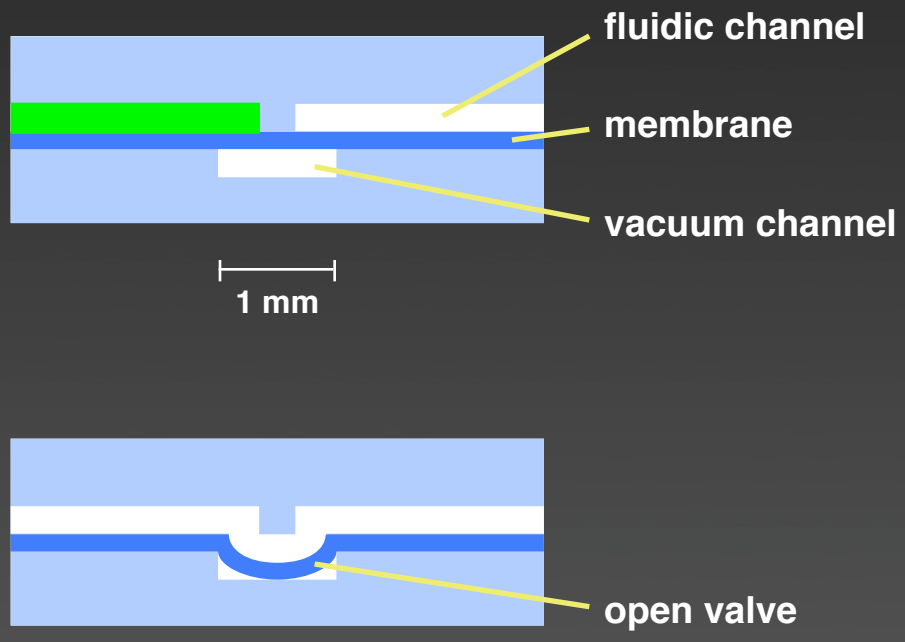
Grover *et al.*, 2003

Microfluidic-based evolution



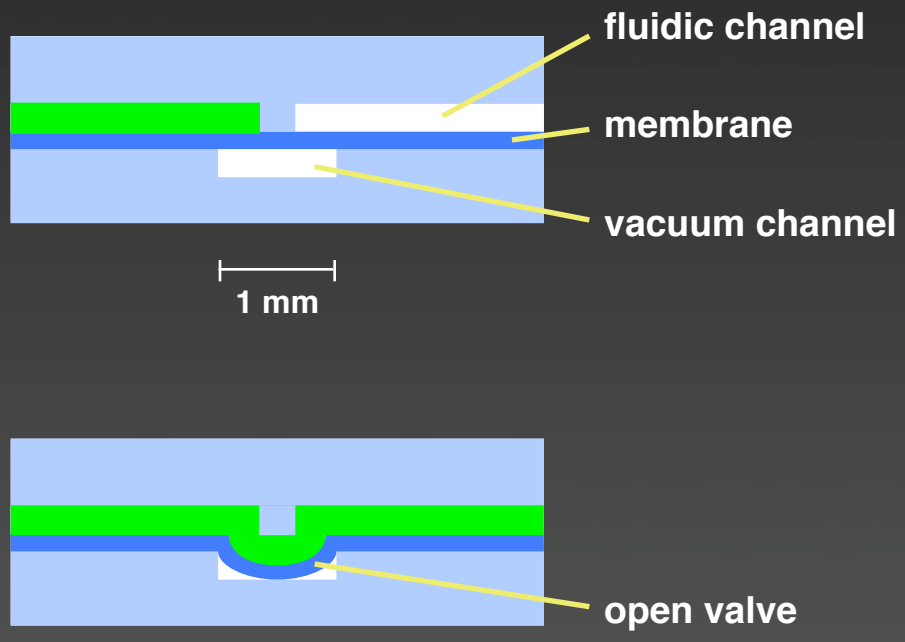
Grover *et al.*, 2003

Microfluidic-based evolution



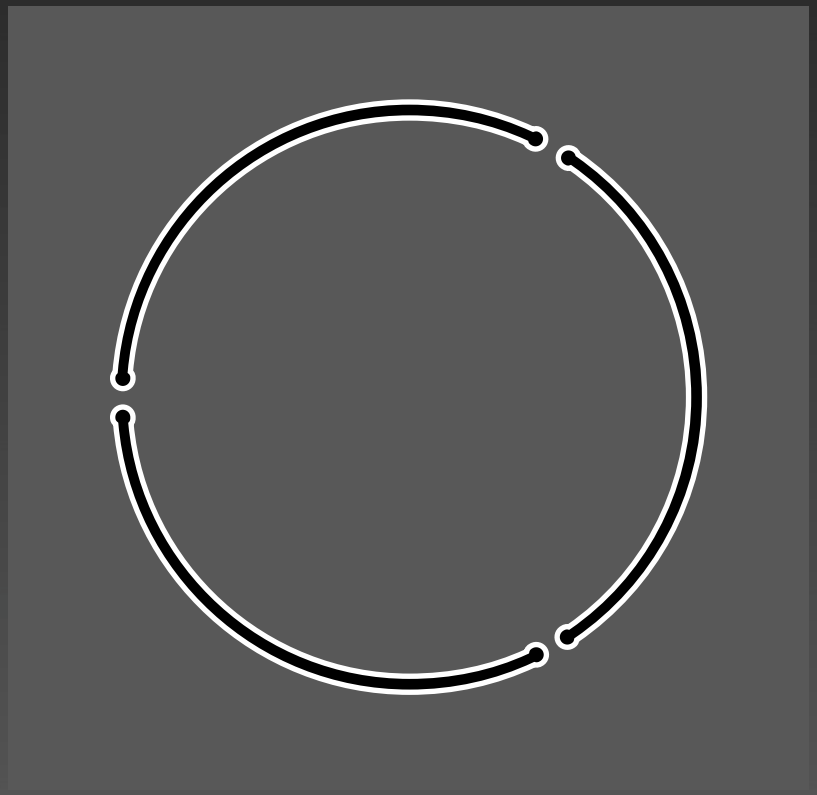
Grover *et al.*, 2003

Microfluidic-based evolution



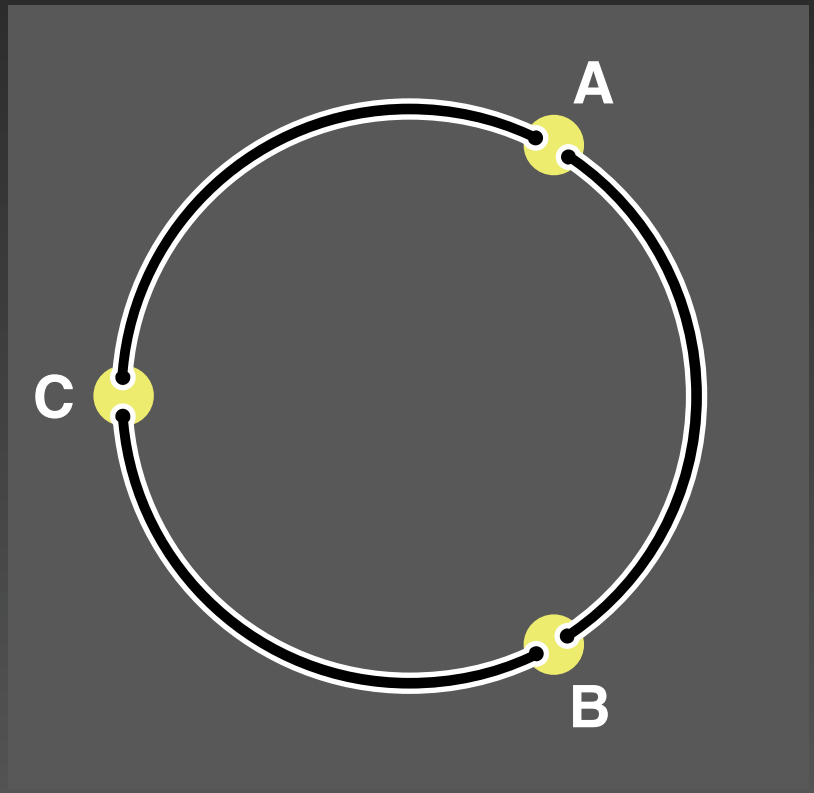
Grover *et al.*, 2003

Microfabricated mixing loop



Paegel *et al.*, 2006

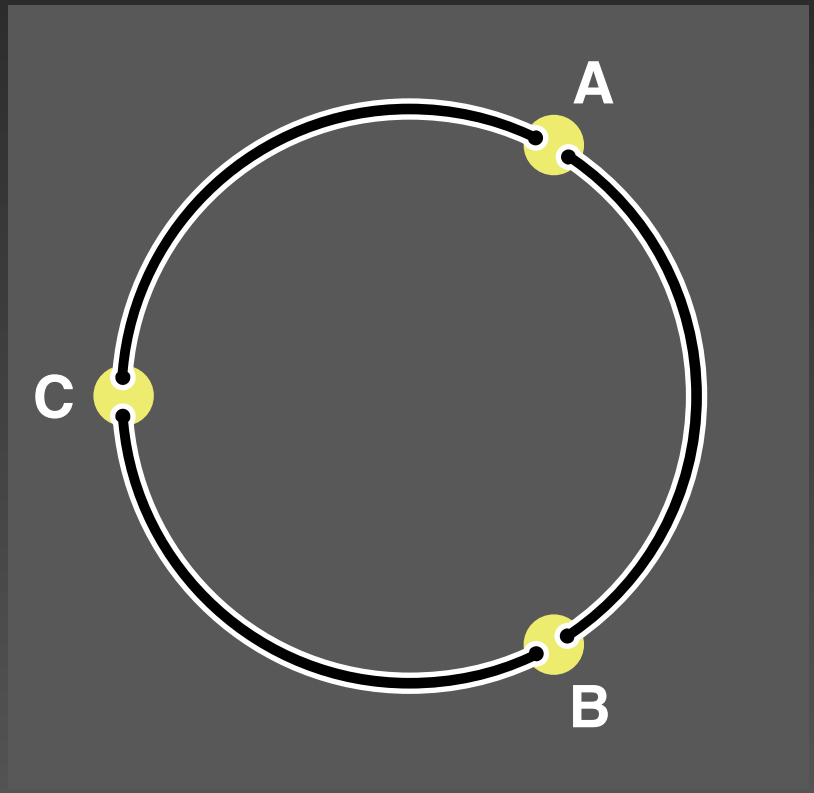
Microfabricated mixing loop



A, B, C **pumping valves**

Paegel *et al.*, 2006

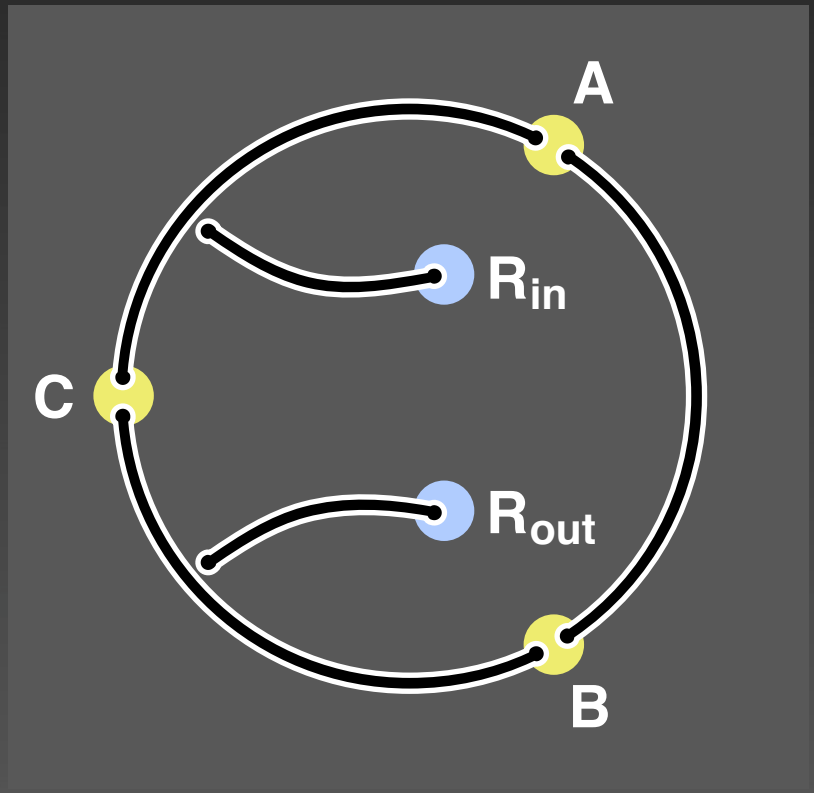
Microfabricated mixing loop



A, B, C **pumping valves**

Paegel *et al.*, 2006

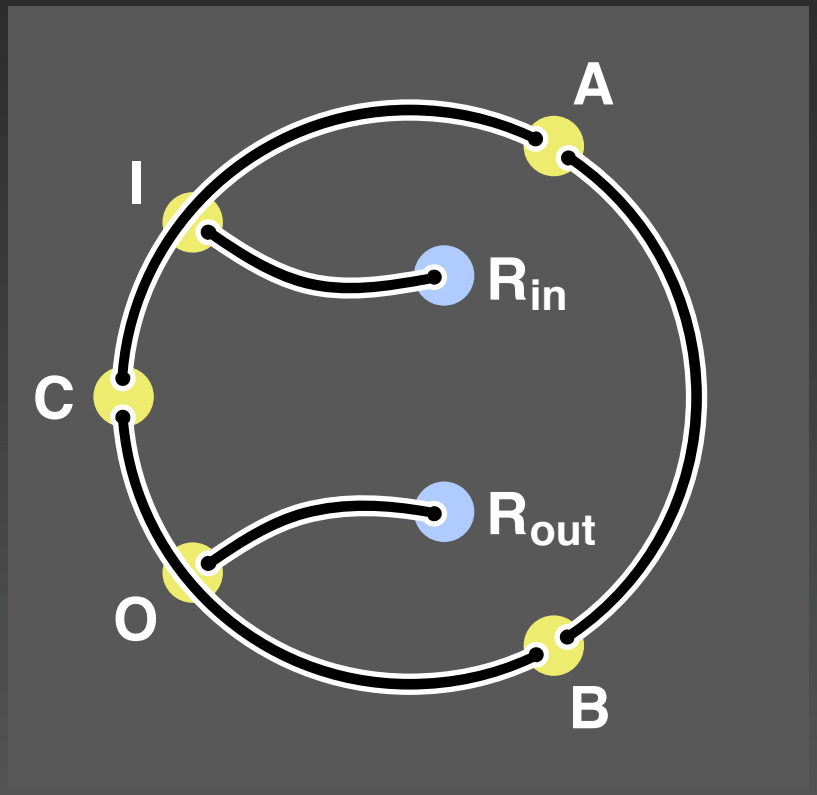
Microfabricated mixing loop



A, B, C	pumping valves
R_{in}	input reservoir
R_{out}	output reservoir

Paegel *et al.*, 2006

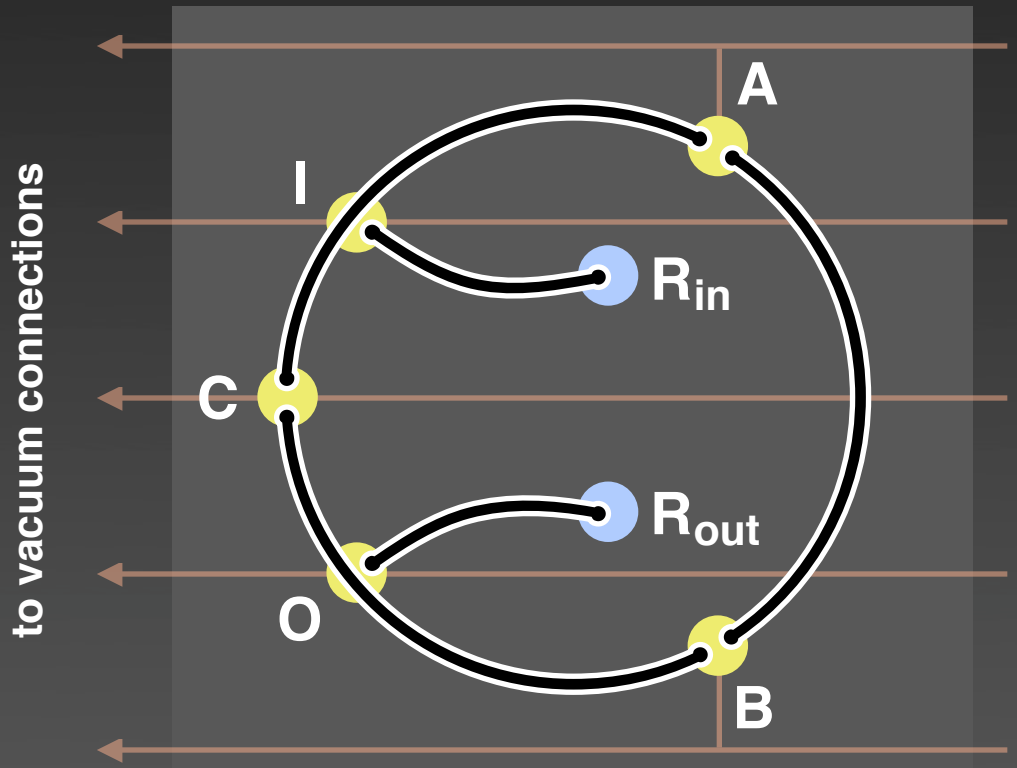
Microfabricated mixing loop



A, B, C	pumping valves
R_{in}	input reservoir
R_{out}	output reservoir
I	input valve
O	output valve

Paegel *et al.*, 2006

Microfabricated mixing loop



A, B, C	pumping valves
R_{in}	input reservoir
R_{out}	output reservoir
I	input valve
O	output valve

loop diameter: 1 cm

fluid channels: 300 x 50 μm

loop volume: 400 nL

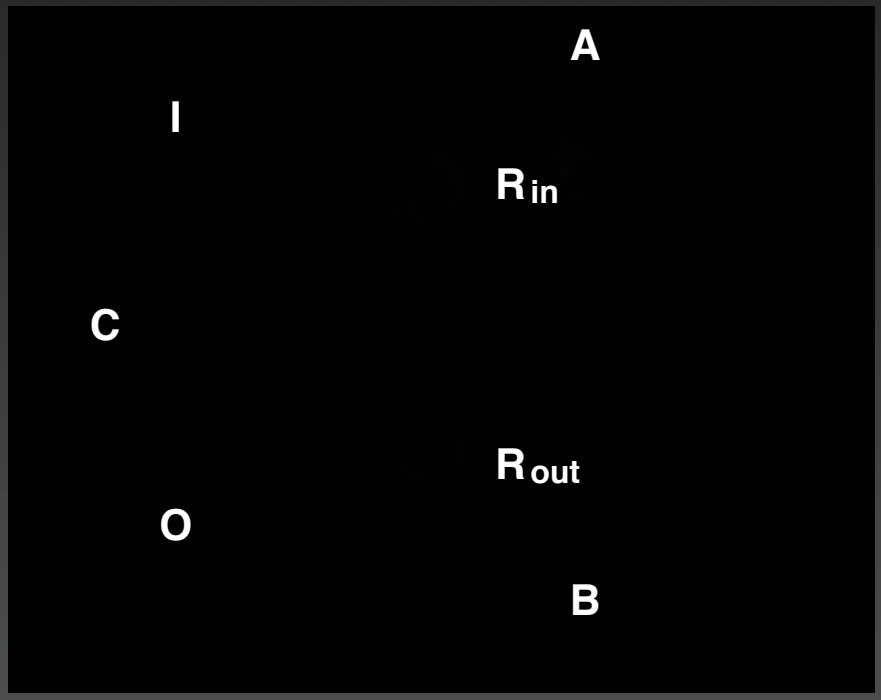
Paegel *et al.*, 2006

Isolate carryover fraction

Actuate I, A, B, and O with C closed

Paegel *et al.*, 2006

Isolate carryover fraction



Actuate I, A, B, and O with C closed

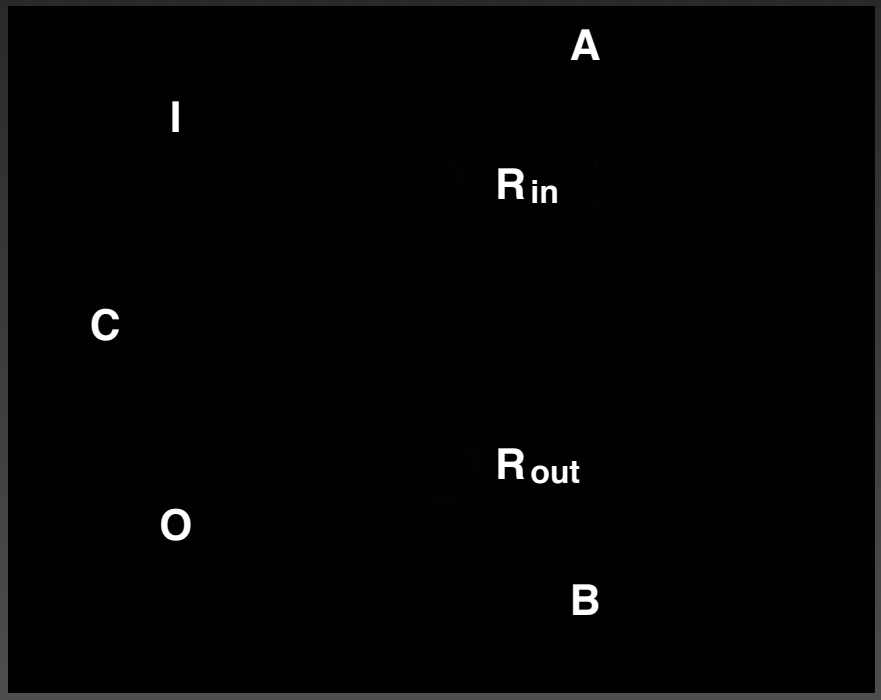
Paegel *et al.*, 2006

Dilute by cyclic mixing

Actuate A, B, and C with I and O closed

Paegel *et al.*, 2006

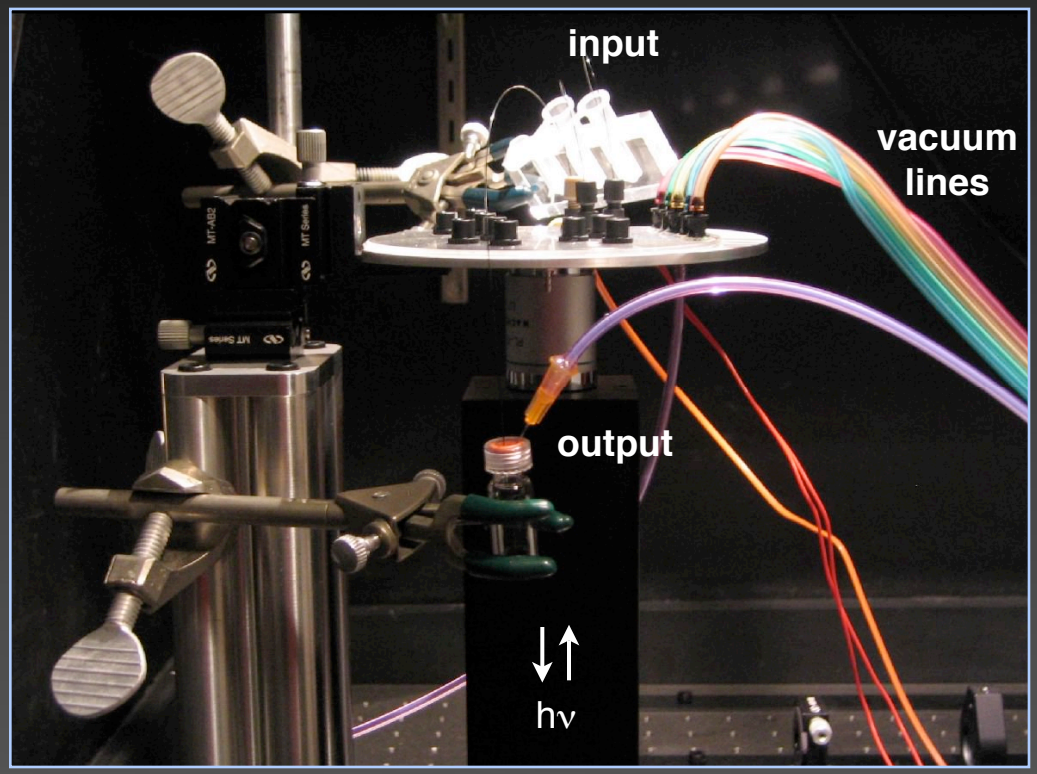
Dilute by cyclic mixing



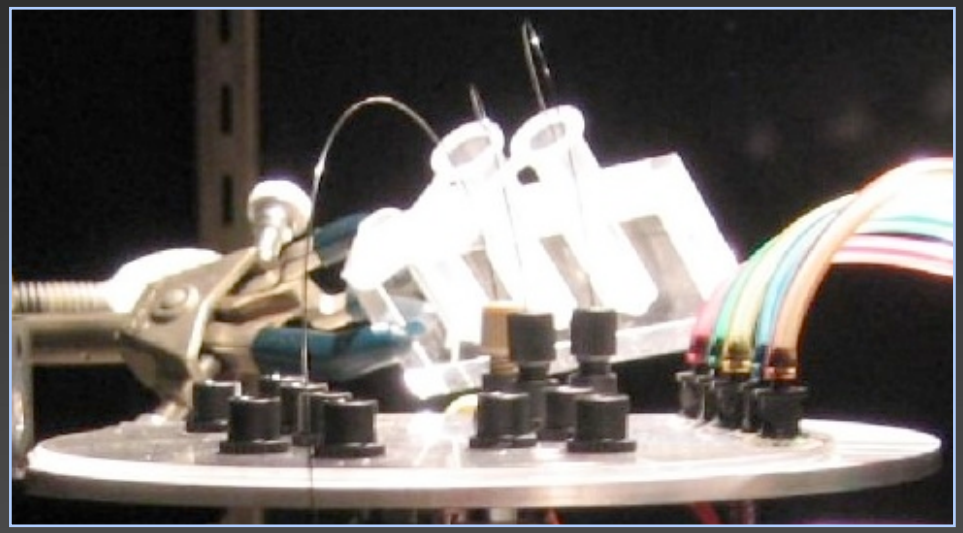
Actuate A, B, and C with I and O closed

Paegel *et al.*, 2006

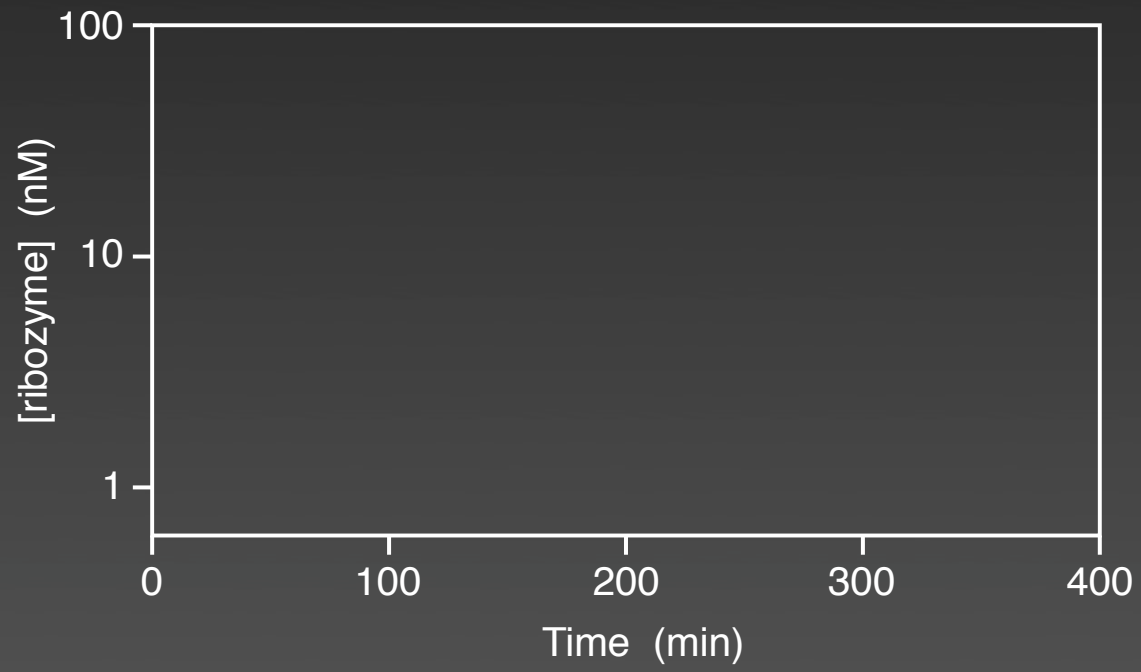
Monitor by confocal laser fluorescence microscopy



RNA evolution on a chip

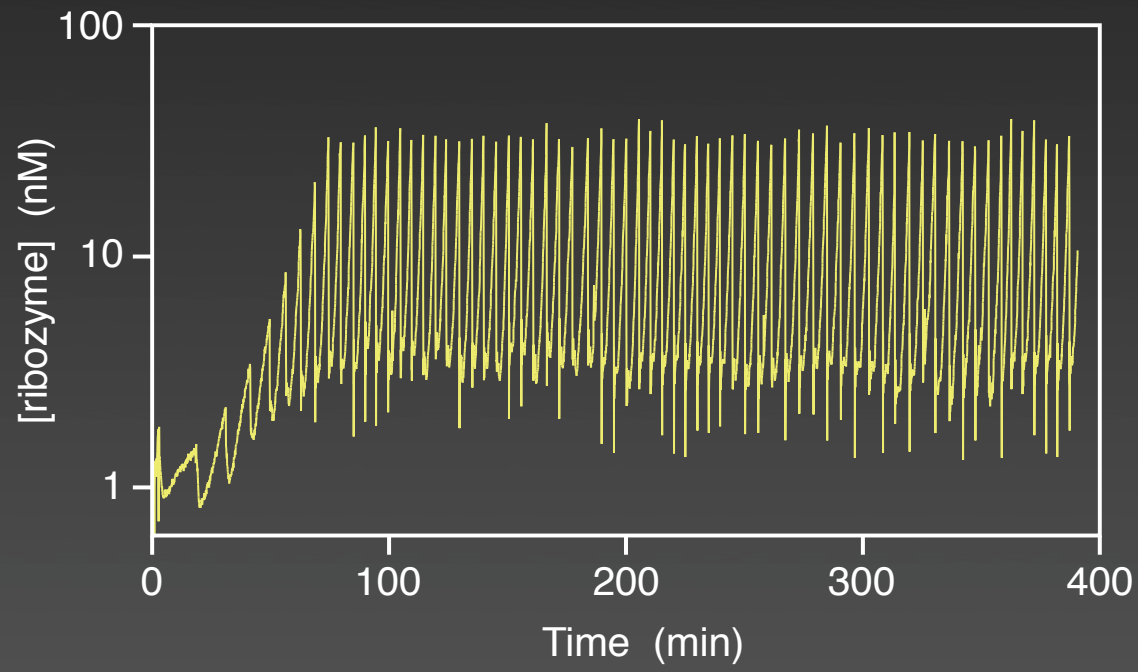


RNA evolution on a chip



25 mM $MgCl_2$, pH 8.5, 37 °C

RNA evolution on a chip



25 mM $MgCl_2$, pH 8.5, 37 °C

Ligase enzymes

Jeff Rogers
John Reader
Natasha Paul

Continuous evolution

Martin Wright
Kathleen McGinness
Sarah Voytek

Microfluidics

Brian Paegel

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