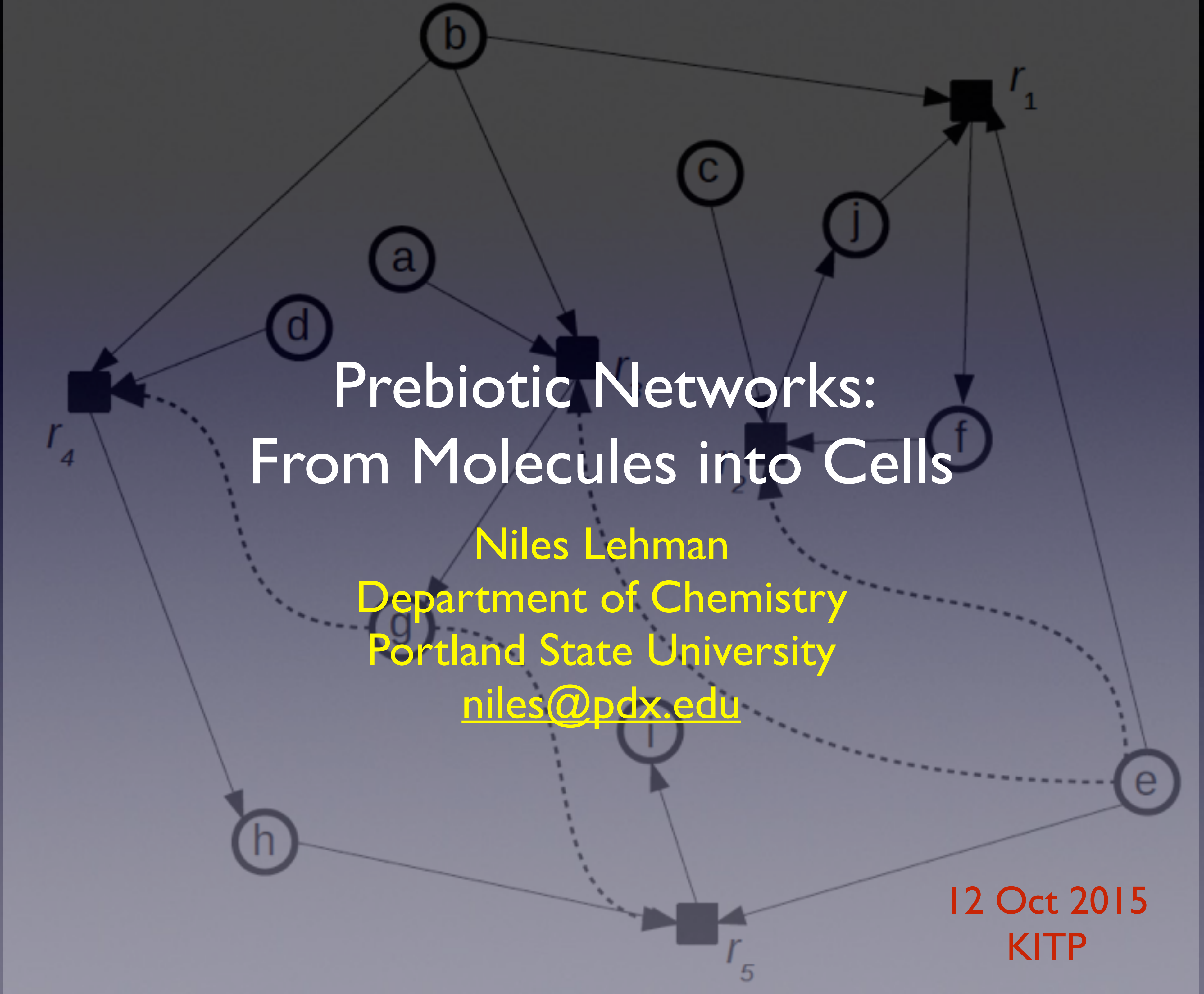

Prebiotic Networks: From Molecules into Cells

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12 Oct 2015
KITP

The Timeline of Life

OoL

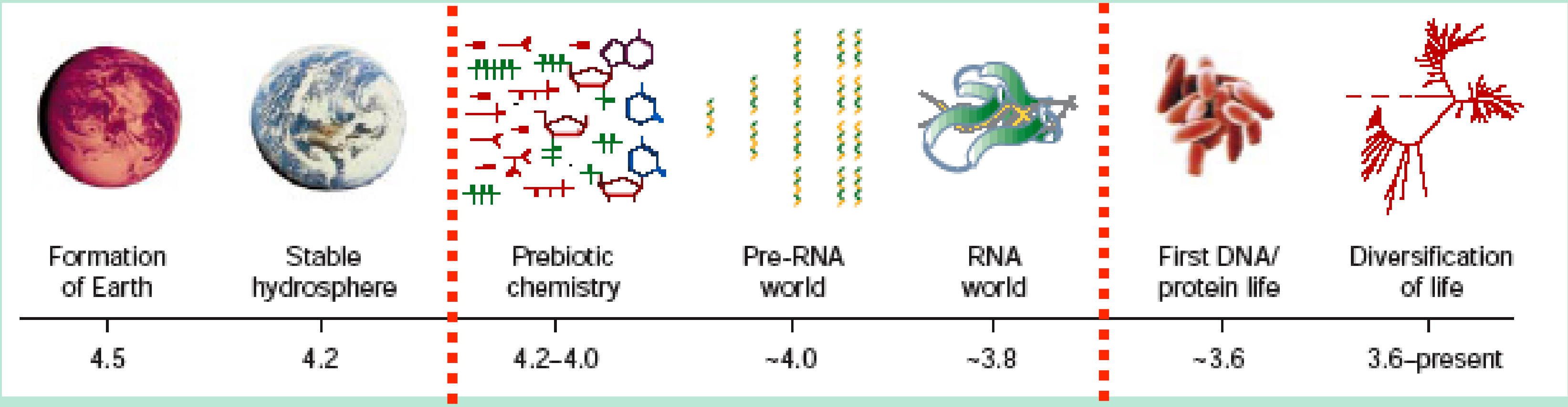
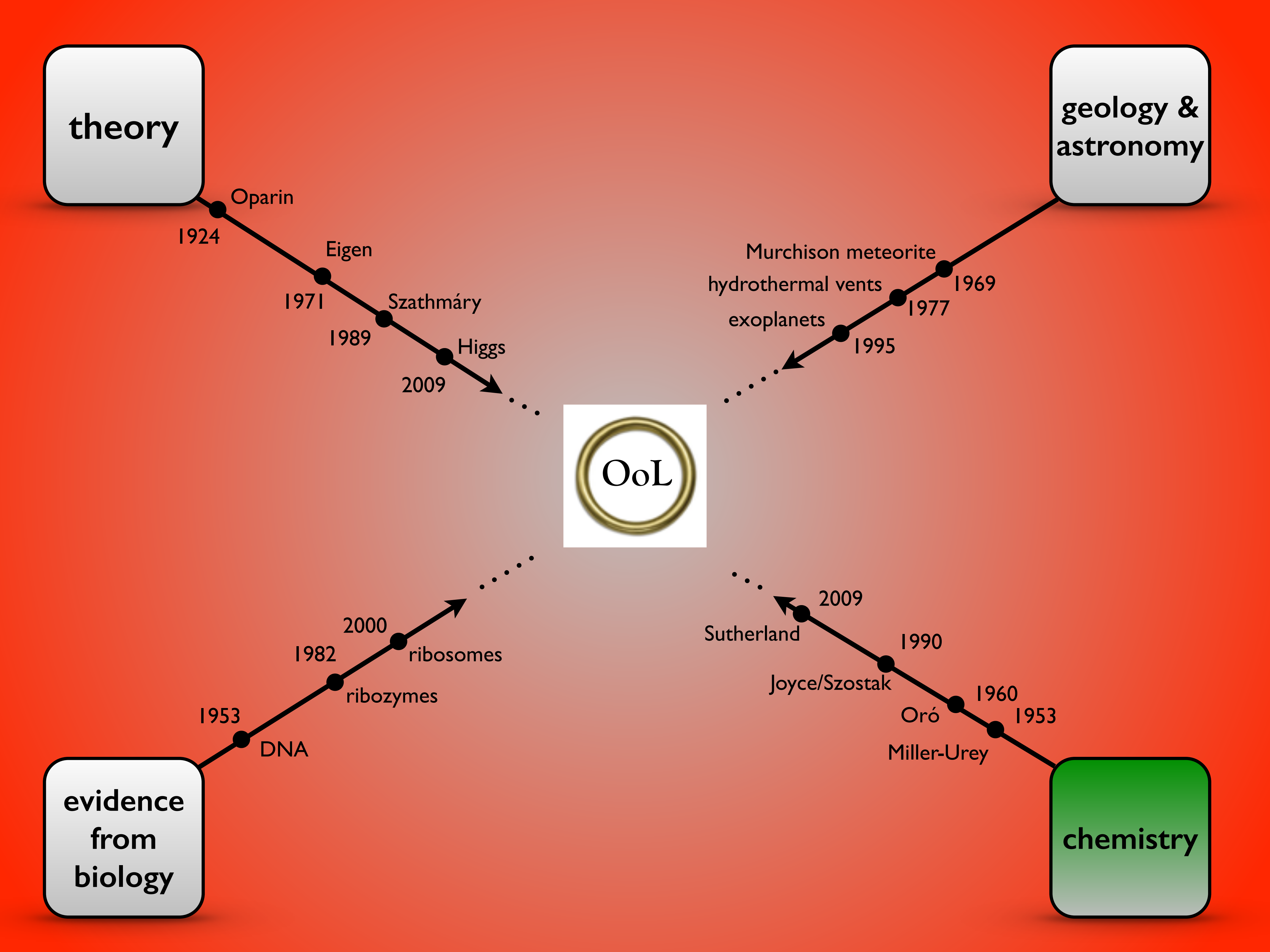


Figure 1 Timeline of events pertaining to the early history of life on Earth, with approximate dates in billions of years before the present.

Joyce (2002) *Nature* **418**, 214–221

LIFE = "a self-sustaining chemical system capable of darwinian evolution" (Joyce/NASA)

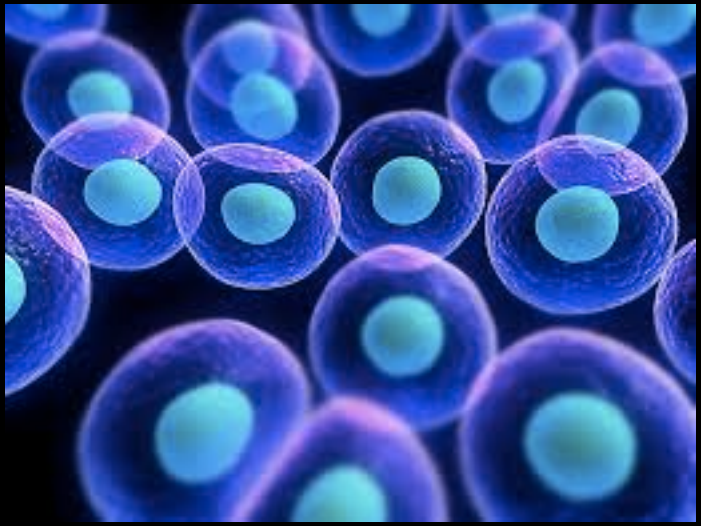

theory
Oparin
1924
Eigen
1971
Szathmáry
1989
Higgs
2009
geology &
astronomy
Murchison meteorite
1969
hydrothermal vents
1977
exoplanets
1995
OoL
evidence
from
biology
2000
1982
ribosomes
ribozymes
1953
DNA
chemistry
2009
Sutherland
1990
Joyce/Szostak
1960
Oró
1953
Miller-Urey

The Seven Challenges to a Prebiotic Chemist

1. The origin/source of the elements
2. The origin/source of small molecule precursors
3. The origin/source of monomers
4. The condensation problem
5. The (self)-replication problem***
6. The chirality problem
7. The compartmentalization problem*

the origin of cells

"linking genotype with phenotype"



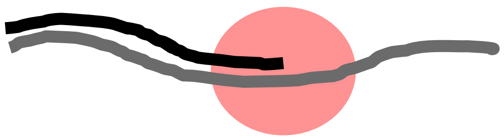
compartmentalization would offer life enormous advantages

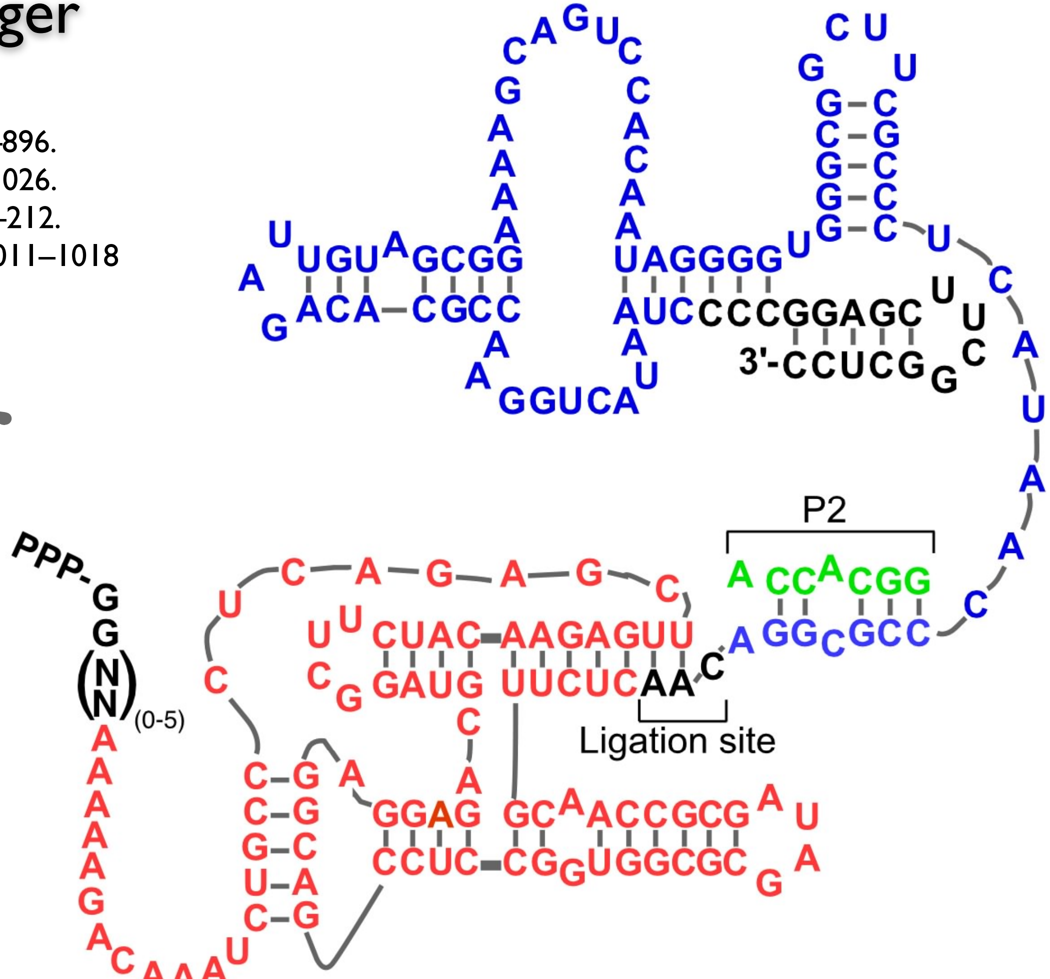
- keeping water concentrations low
- creating gradients
- allowing genotypes to harvest "the fruits of their labor"

the "holy grail" of the RNA World:
an RNA replicase ribozyme

the Bartel/Unrau/Holliger replicase ribozyme

Johnston *et al.* (2001) *Science* **292**, 883–896.
Zaher & Unrau (2007) *RNA* **13**, 1017–1026.
Wochner *et al.* (2011) *Science* **332**, 209–212.
Attwater *et al.* (2013) *Nature Chemistry* **5**, 1011–1018

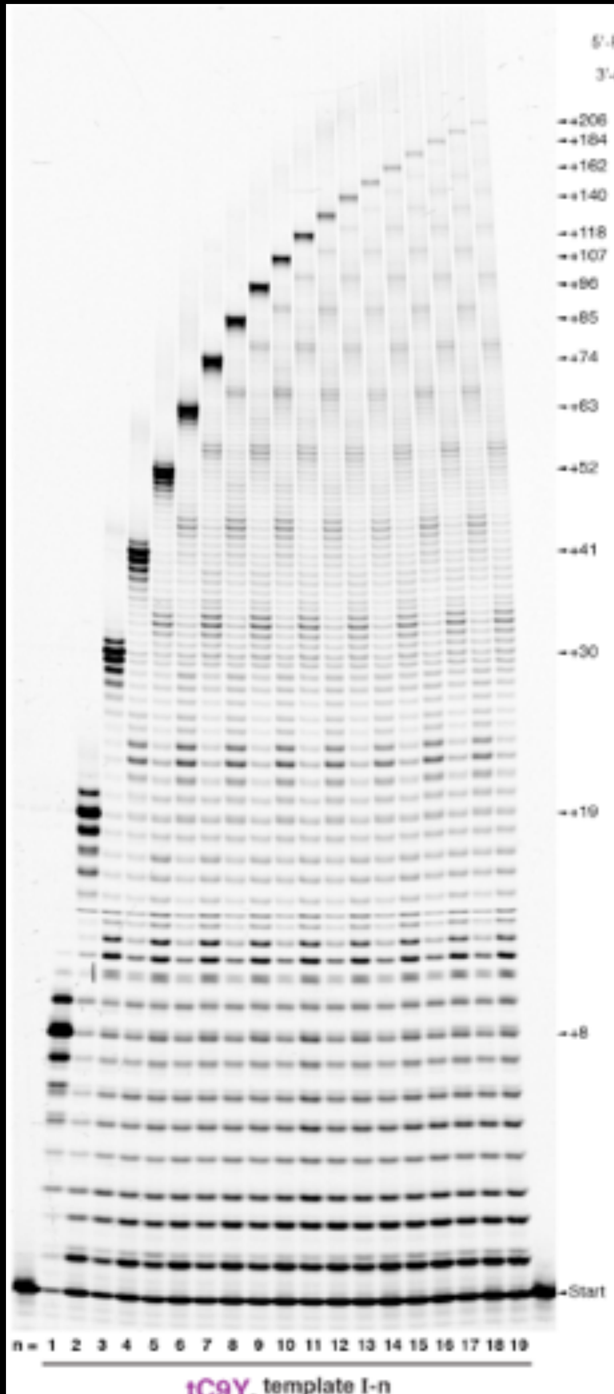




a 190-nt ribozyme that can polymerize a portion of itself

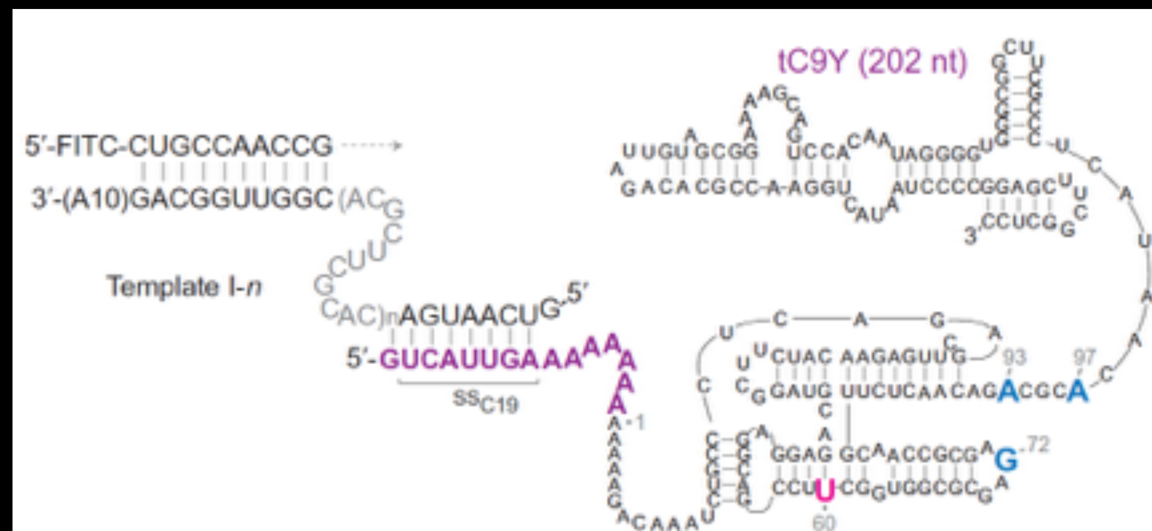
molecular <u>self</u>-replication

the world's record



206 nt

alterations of cold (–7˚C) and normal (17˚C) temperatures used to select this RNA



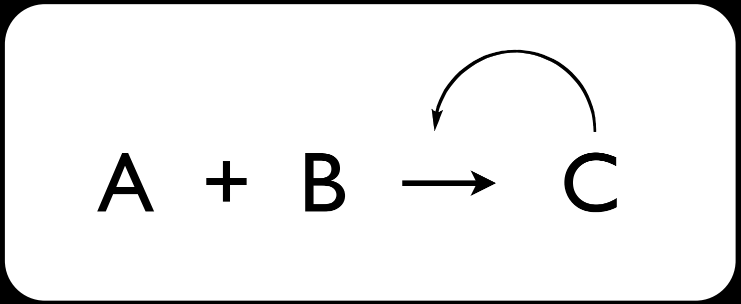
the tC9Y ribozyme can perform template-directed replication to elongate RNA to greater than its own length

(but it can't replicate itself)

Attwater *et al.* (2013) *Nature Chemistry* **5, 1011–1018**

autocatalysis

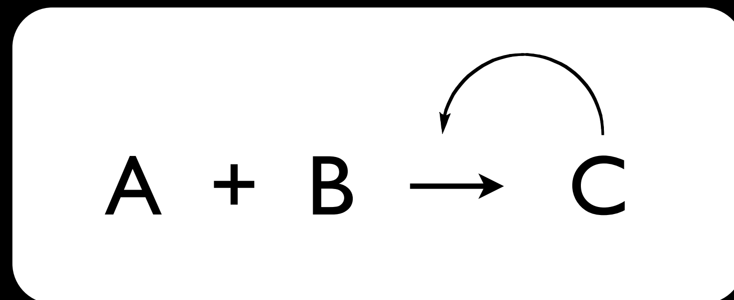
the chemical requirement for *self*-replication



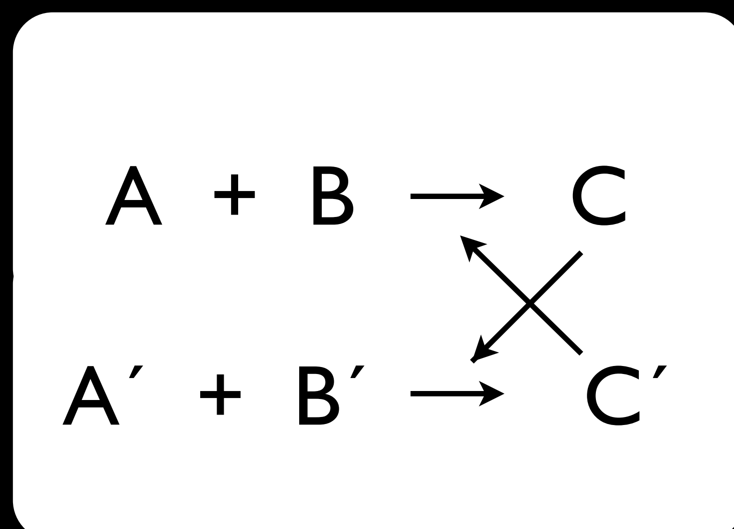
the product of a reaction catalyzes its own formation

from selfishness to cooperation...

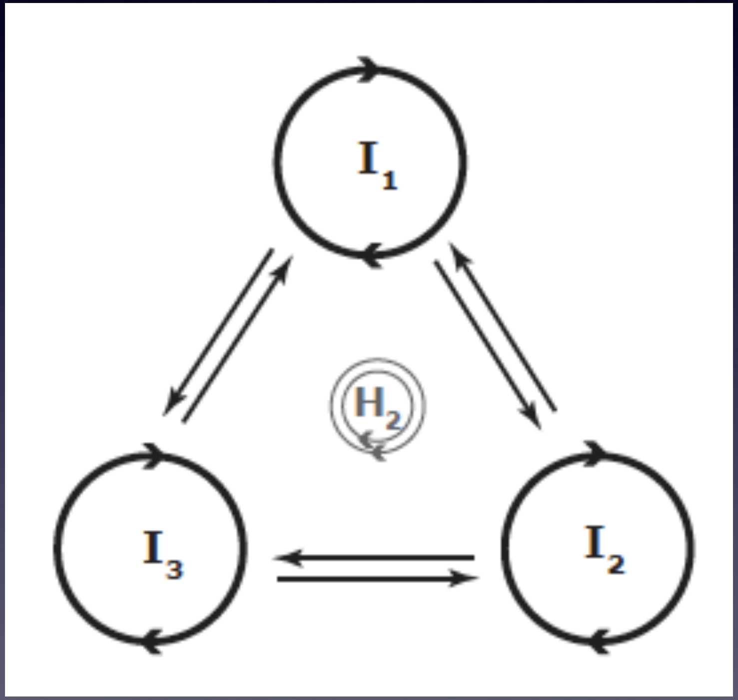
"selfish"



"cooperative"



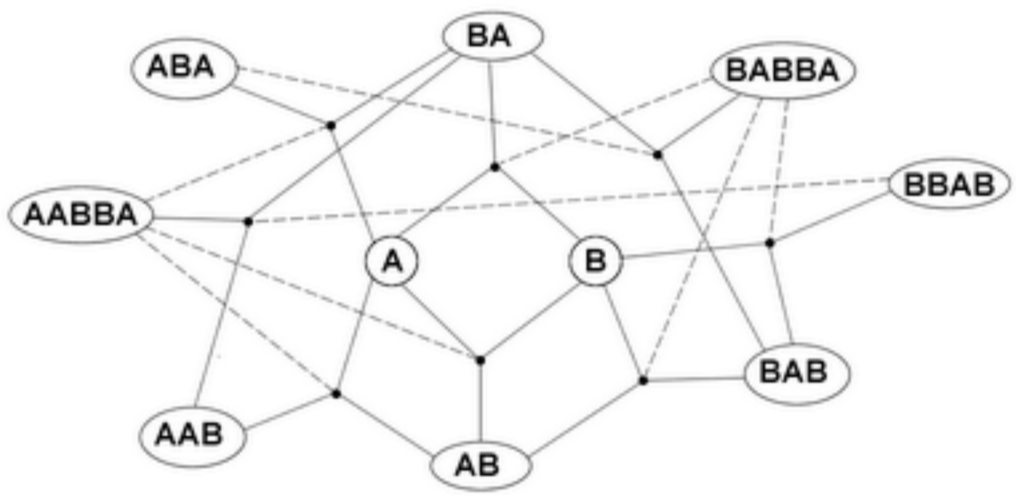
...extending cooperation to >2 “selves”...



Eigen & Schuster, 1977; 1978

... and from simple cycles to networks

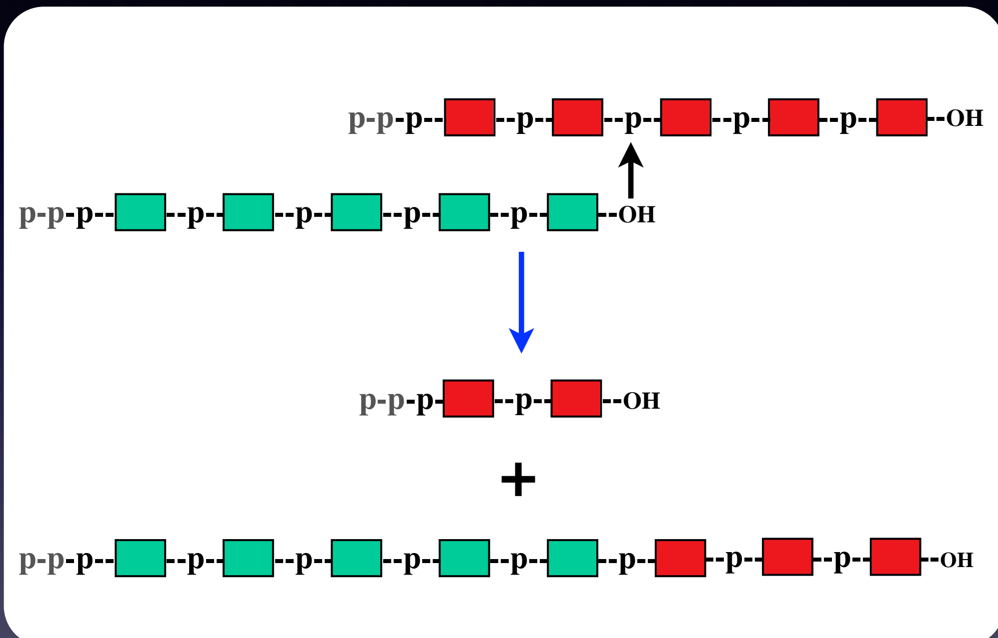
an autocatalytic set

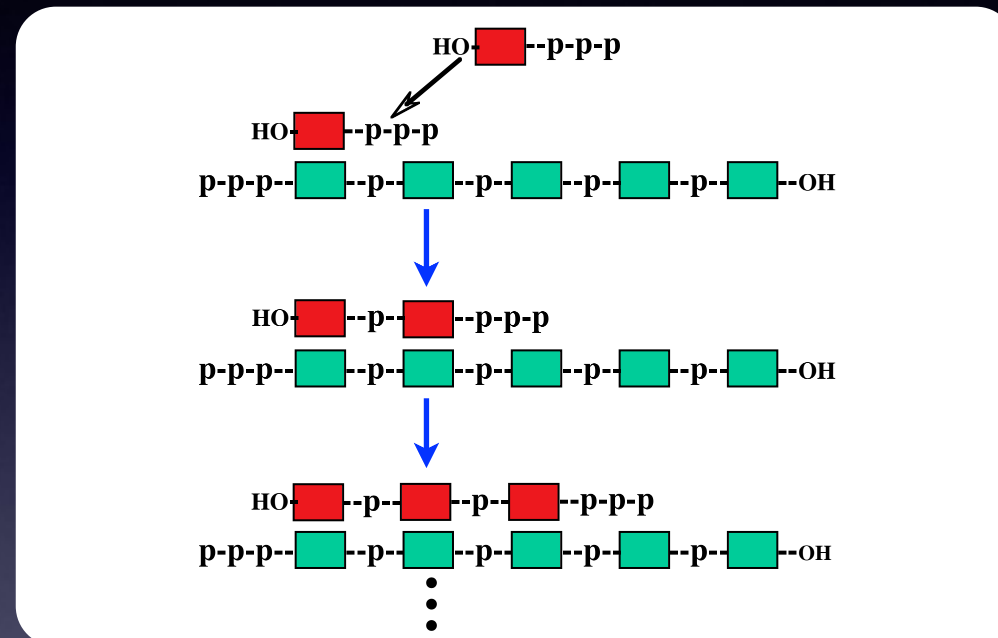


Kauffman (1993)

recombination

recombination, at the molecular level, is the breaking and re-formation of (phosphoester) bonds resulting in the swapping of ≥1 monomer units between two (nucleic-acid) strands





recombination

"easy" chemistry

polymerization

"hard" chemistry

Lehman (2003) *J. Mol. Evol.* ***56***, *770–777.*

Lehman (2008) *Chem. Biodiver.* **5,** 1707–1717.

Lehman *et al.* (2011) *Entropy* **13,** 17–37.

Vaidya *et al.* (2012) *Nature* **490,** 72–77.

my claim...

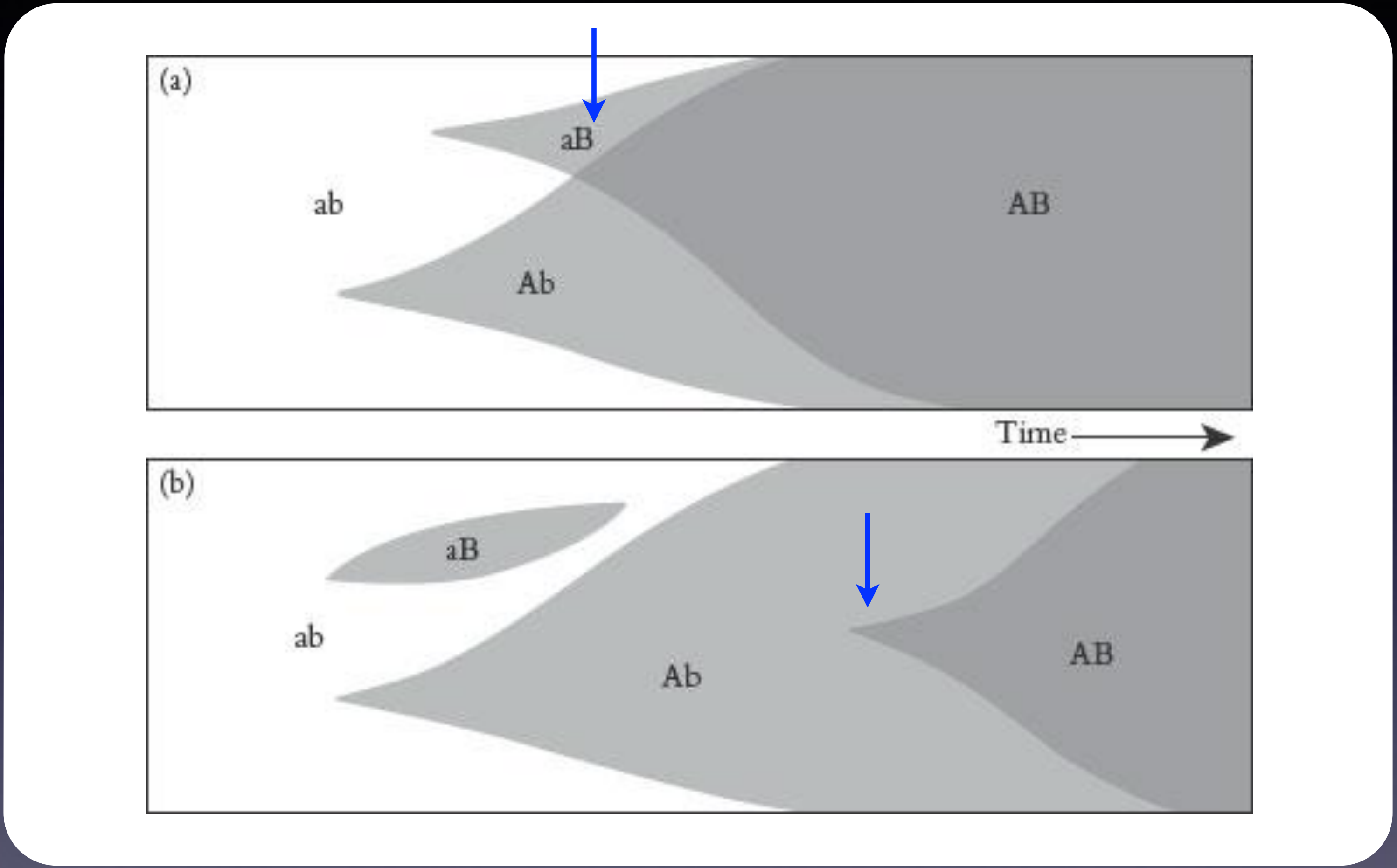
recombination can provide a mechanism for the initial build-up of complex catalytic RNAs

2-mer + 2-mer → 3-mer + 1-mer
3-mer + 3-mer → 5-mer + 1-mer
5-mer + 5-mer → 9-mer + 1-mer
9-mer + 9-mer → 17-mer + 1-mer
etc.

our goal: devise an all-RNA system that can exploit recombination to build up genetic information into a network of self-replication

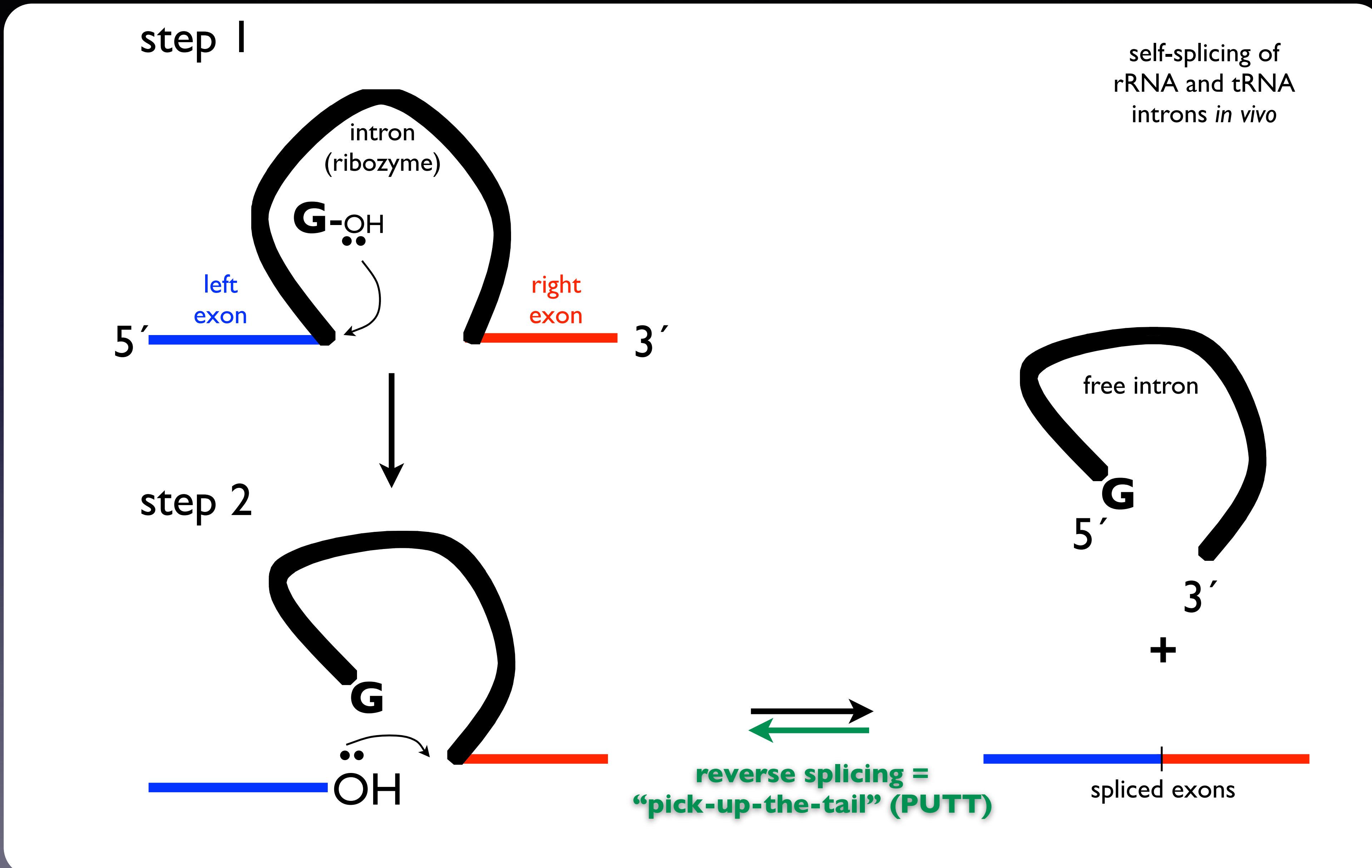
Lehman (2003)
J. Mol. Evol. **56,** 770–777.

analogy to “sexual” reproduction

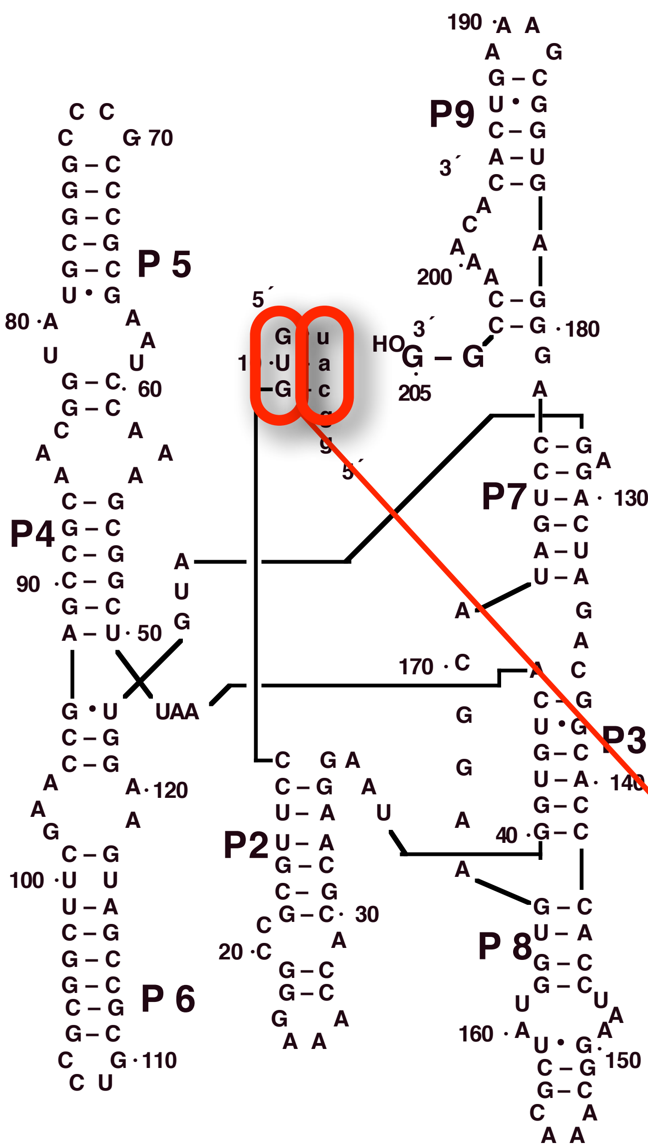


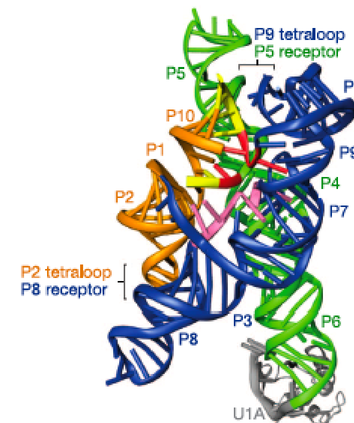
By analogy to the Fisher-Muller argument, recombination can hasten the appearance of multiple beneficial “traits” in the same “genome”

getting RNAs to recombine RNAs: group I introns do this in Nature



the *Azoarcus* ribozyme as a recombinase





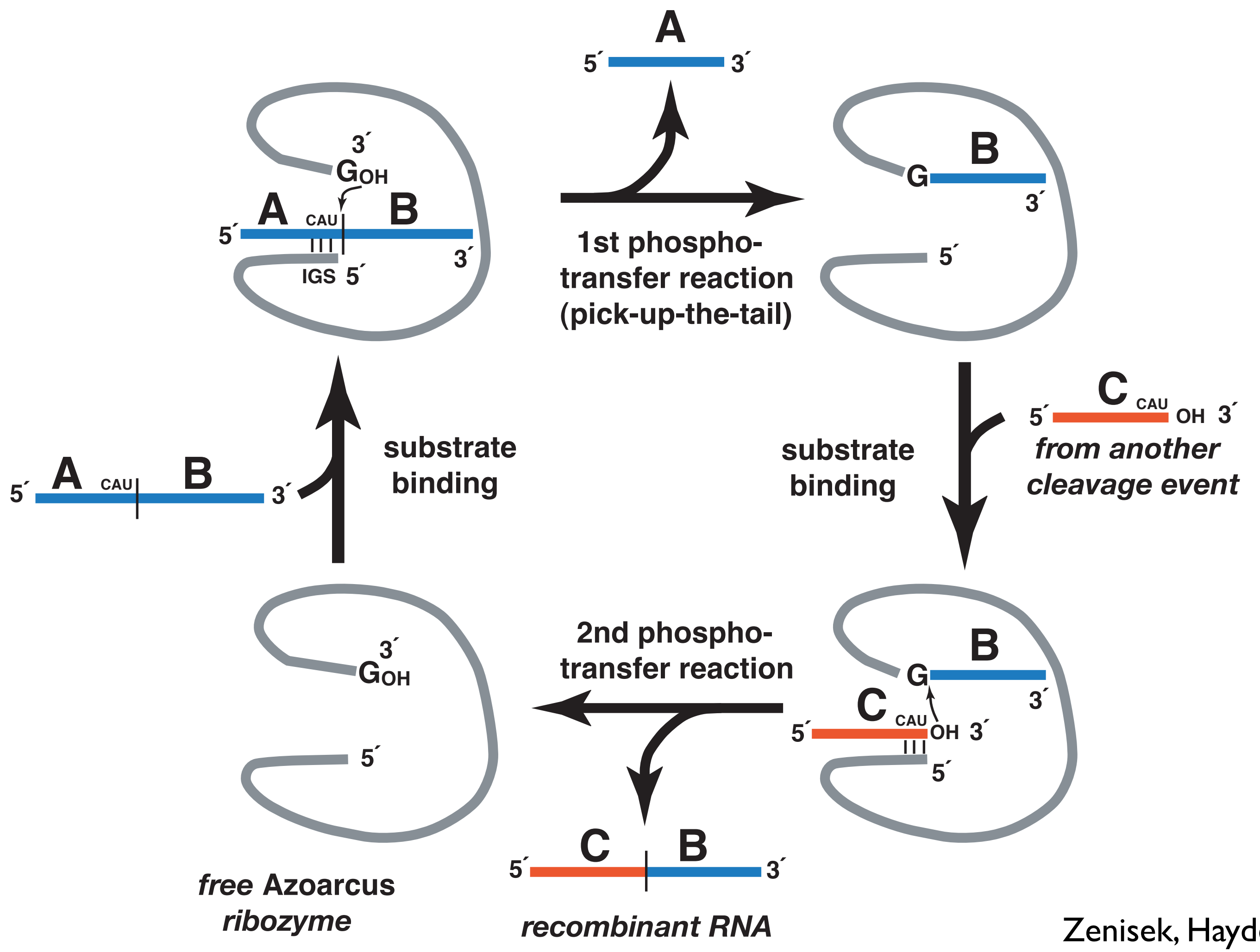
self-splicing intron from the isoleucine tRNA of the purple bacterium *Azoarcus*

L–8 ribozyme is 197 nt long, and has a 71% G+C content

active up to 70˚C

internal guide sequence is GUG, its complement (*i.e.*, "tag") is CAU

recombination scheme by group I ribozymes

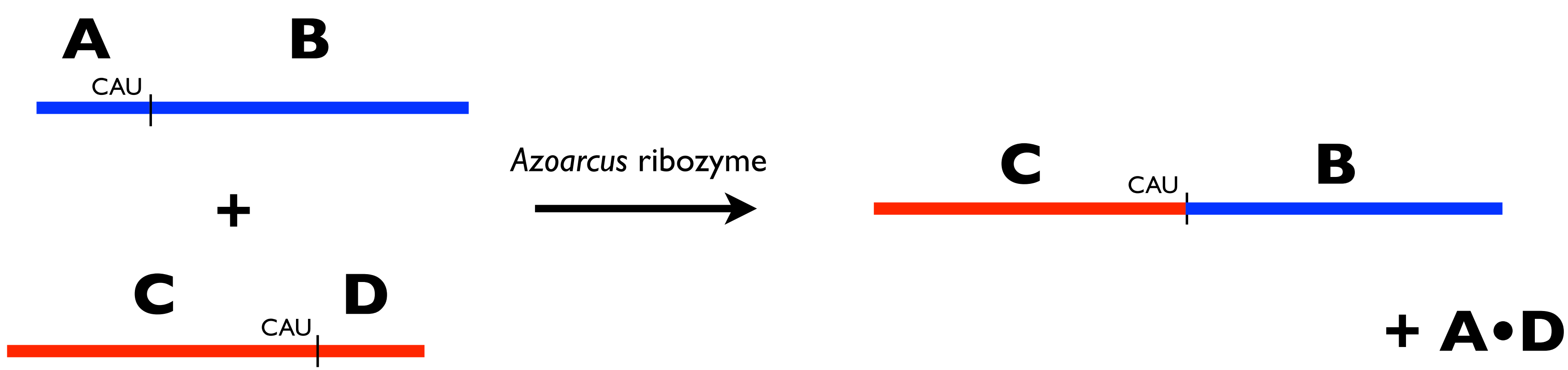


Zenisek, Hayden & Lehman (2007)
Artif. Life **13,** 279–289.

RNA-directed recombination of short oligomers

***Azoarcus* ribozyme: IGS = GUG; target = CAU**

SNL-1a	GGCAU	•AAAUAAAUAAAUAAAUA	22-mer
SNL-2a	GGAAAGGCAU	•AAAUA	15-mer
SNL-4a	GGCAU	•GGCCGAAACAGC	17-mer
SNL-5a	GGGAGUCUGAUGAGGCAU	•AAAUA	23-mer
	"head"	**• "tail"**	

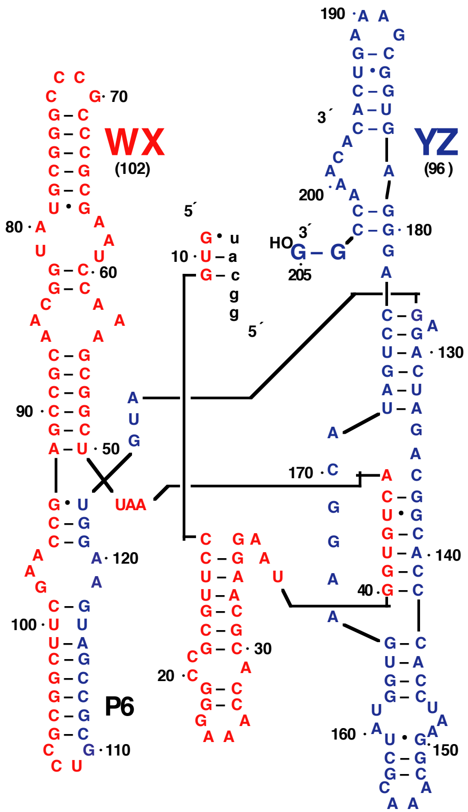


SNL-1a X SNL-2a: 22-mer + *15-mer → *27-mer + 10-mer

recombining the recombinase itself

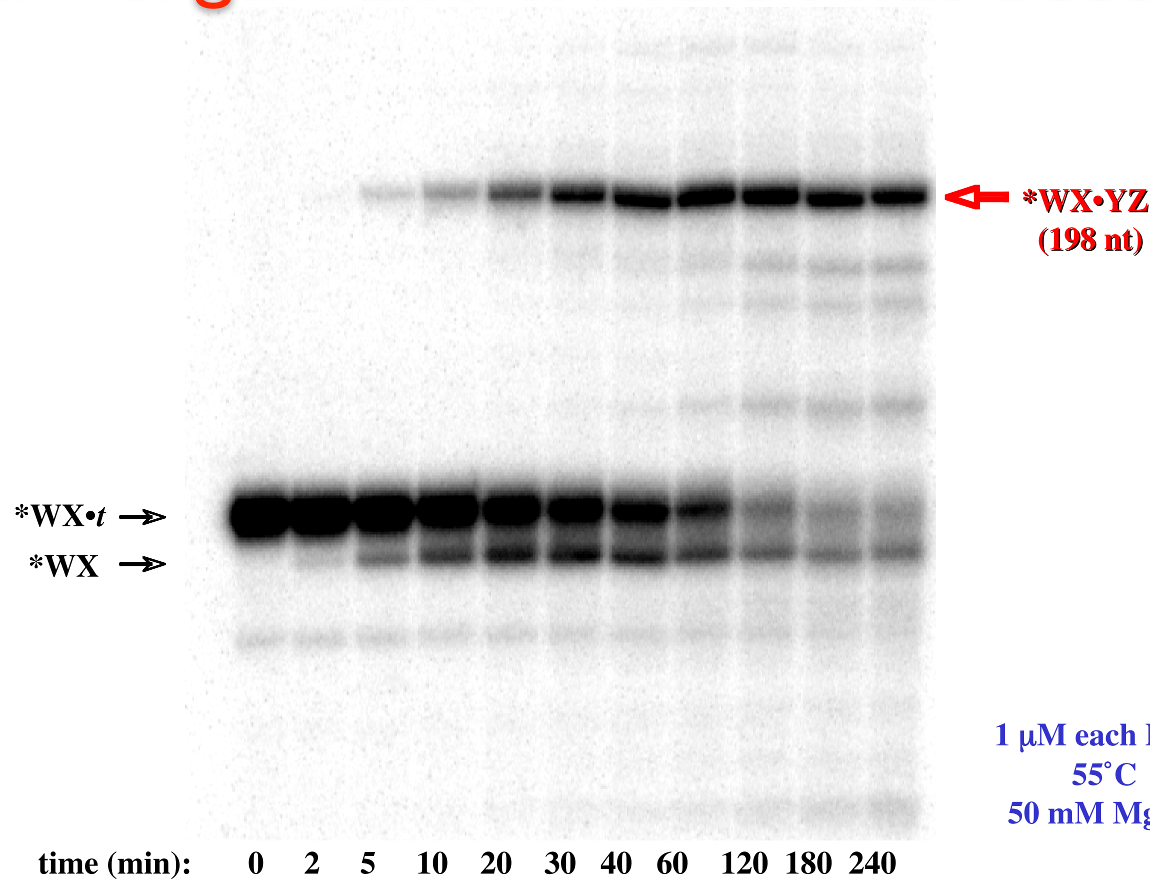
AZOARCUS RIBOZYME

198-nt *Azoarcus* ribozyme



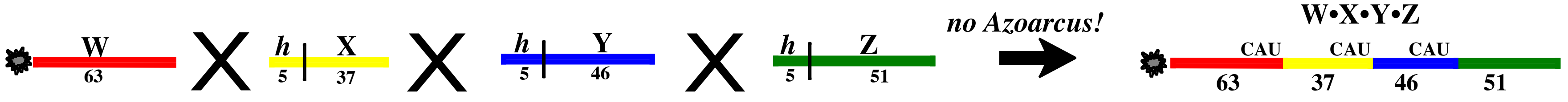
***Azoarcus* RCL6**
"binary"

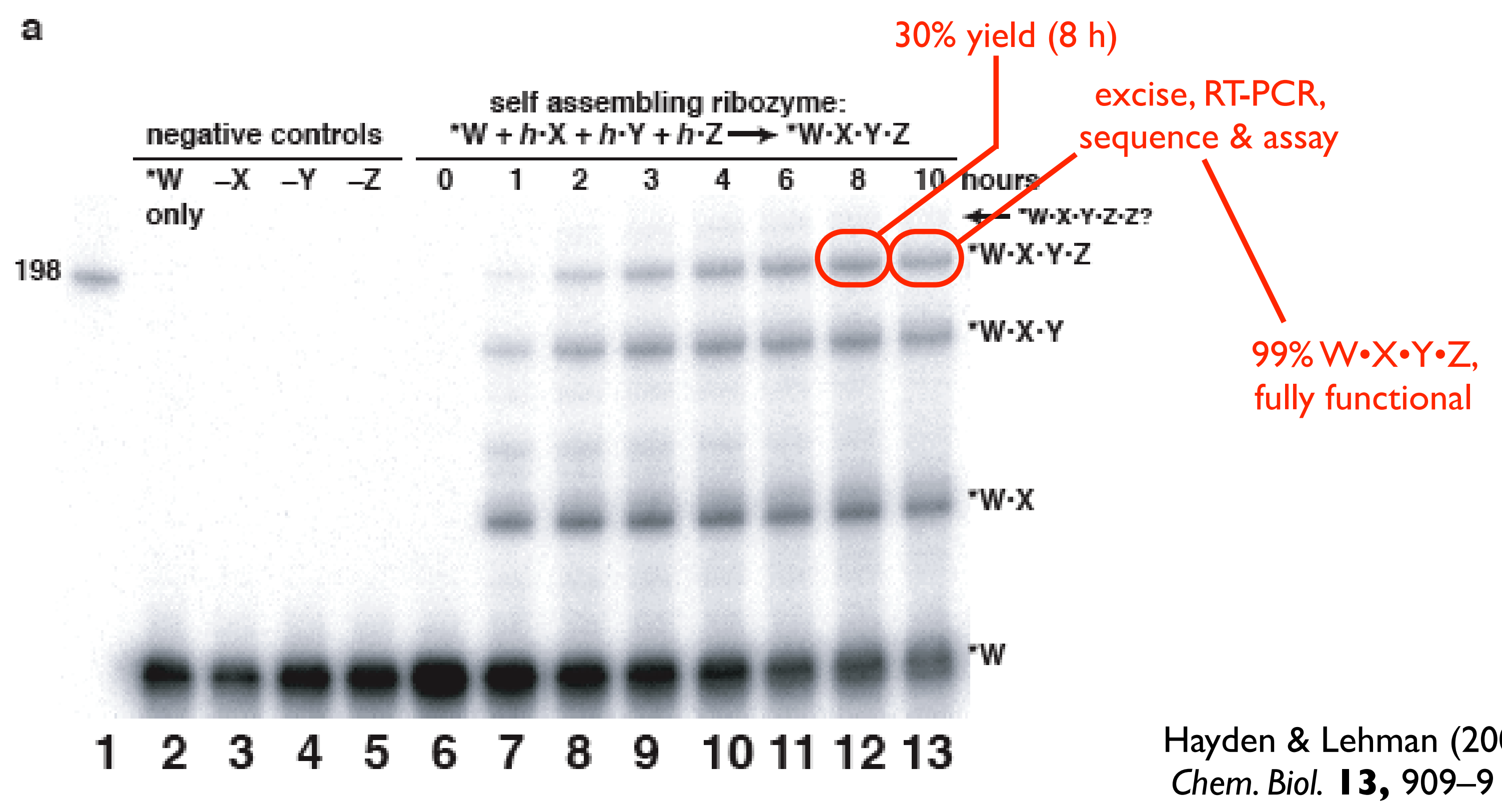
no full-length *Azoarcus* RNA was added!



1 μM each RNA
55°C
50 mM $MgCl_2$

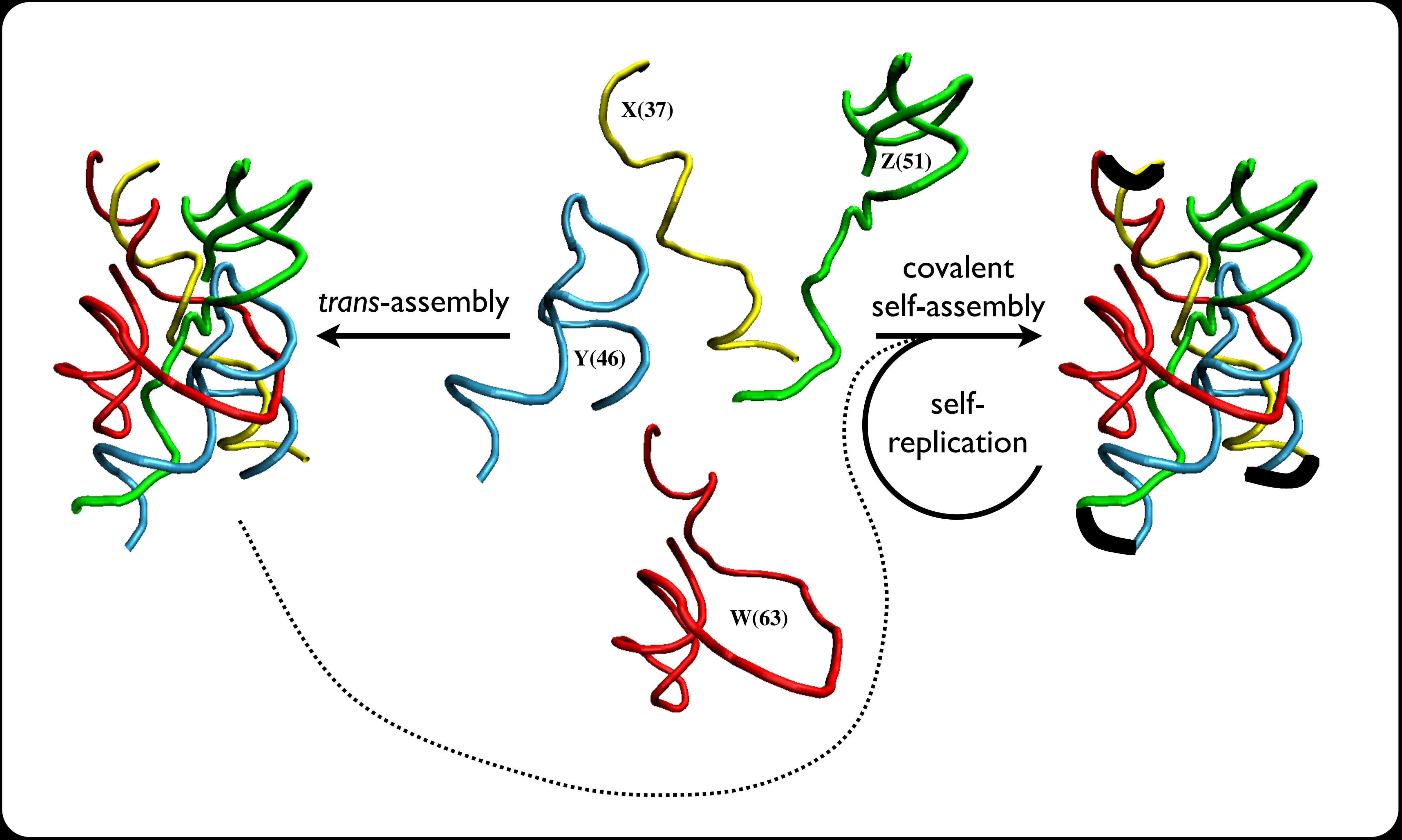
four-piece (quad) self-assembly



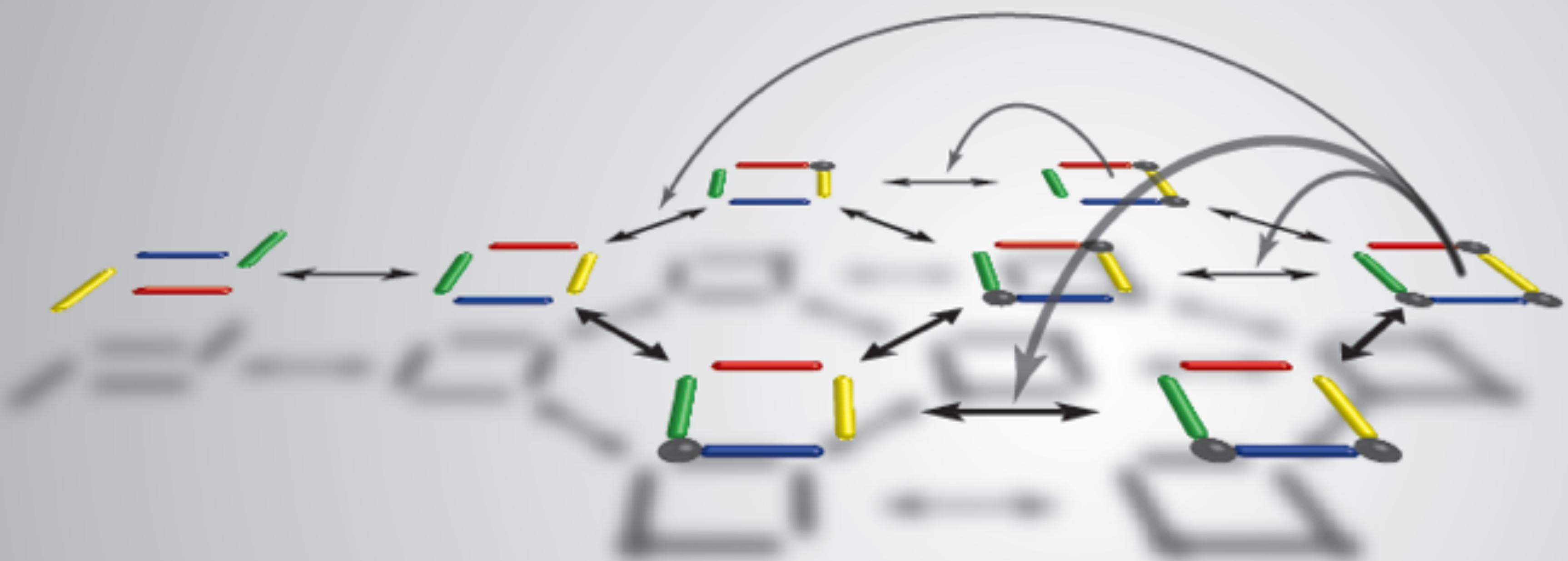


Hayden & Lehman (2006)
Chem. Biol. **13,** 909–918.

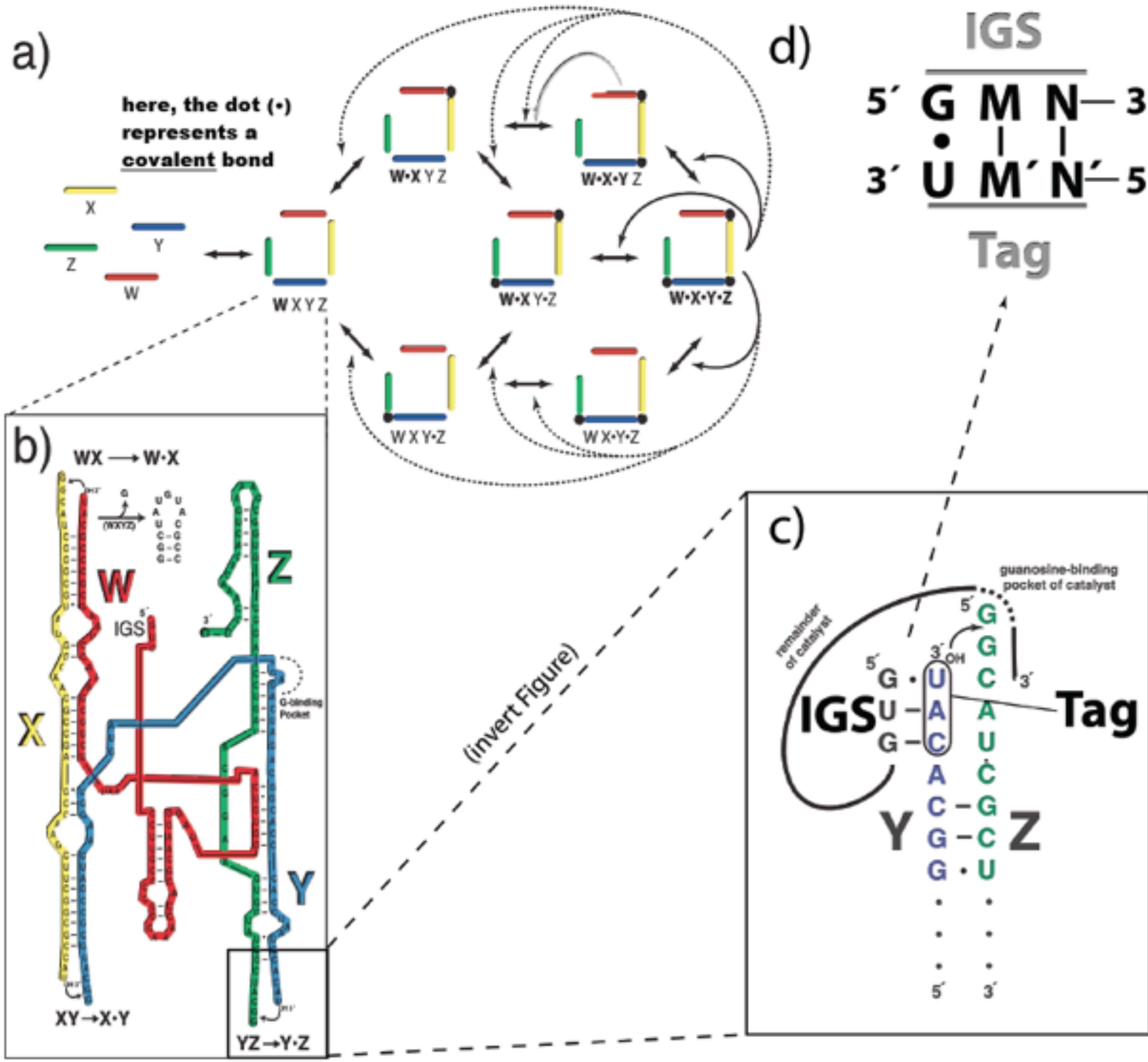
trans-catalysis first



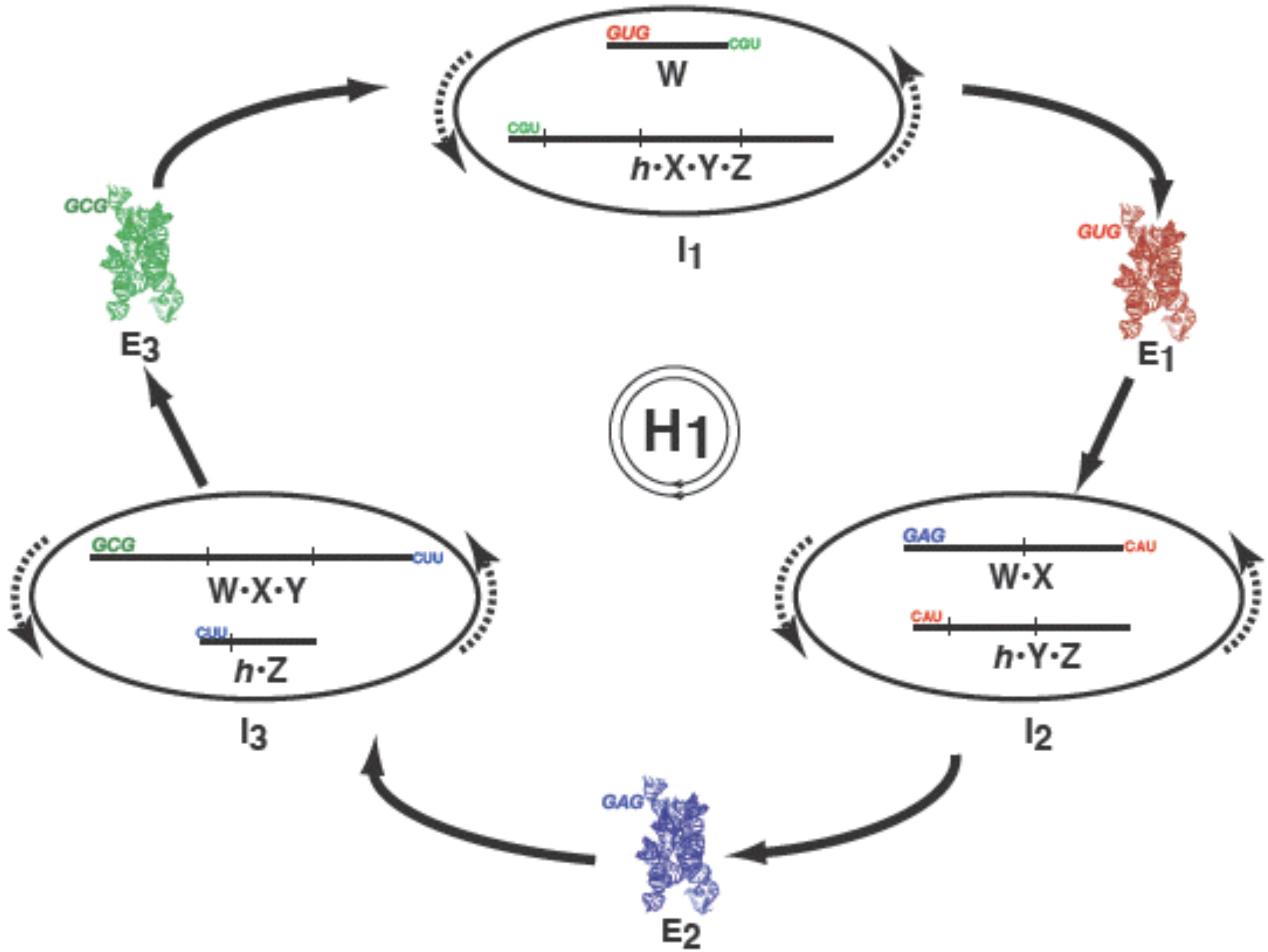
a small “selfish” autocatalytic network



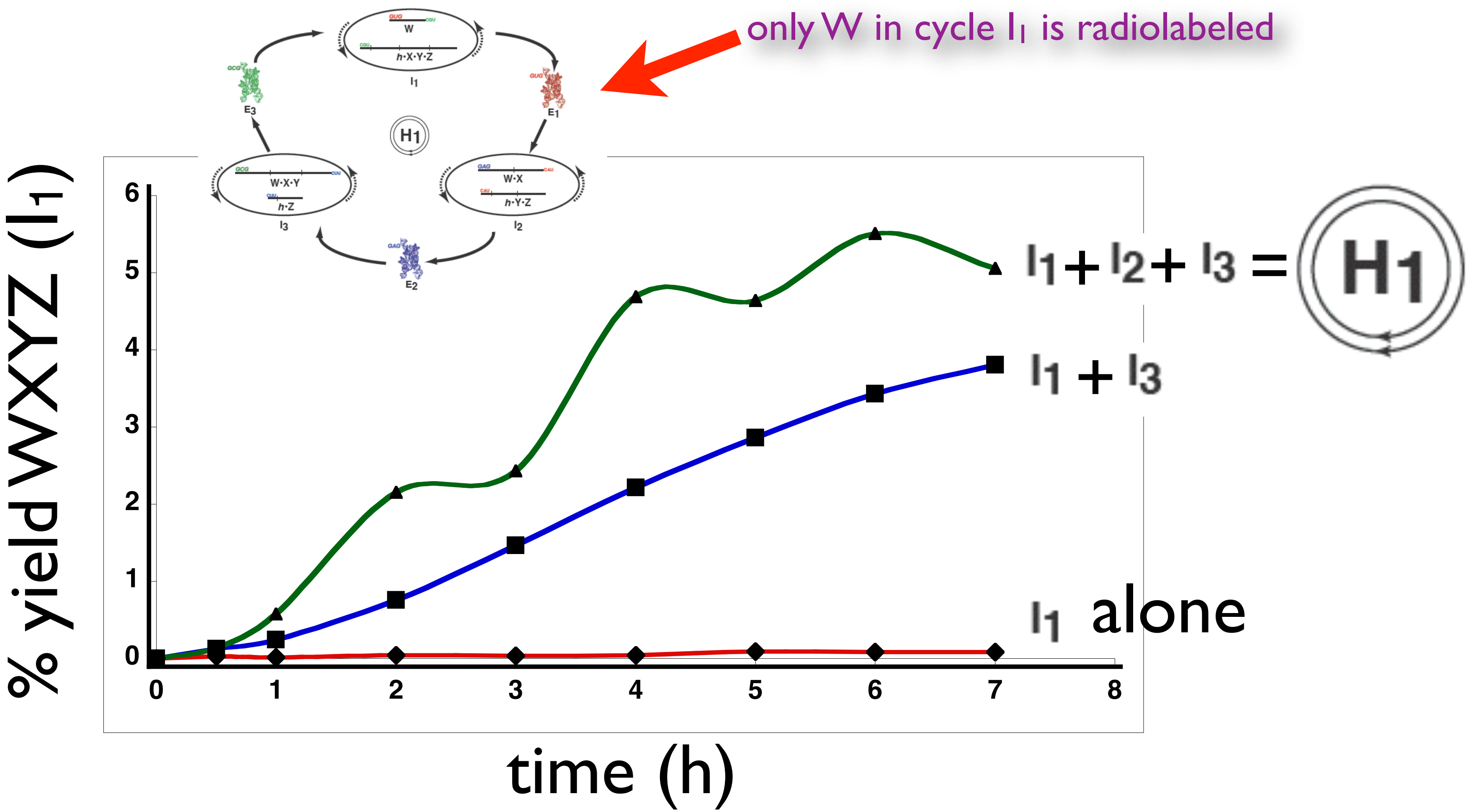
Hayden, von Kiedrowski, Lehman (2008)
Angew. Chem. Int. Ed. **47**, 8424–8428.


a)
here, the dot (•) represents a covalent bond
X
Y
Z
W
W X Y Z
W•X Y Z
W•X•Y Z
W•X Y•Z
W•X•Y•Z
W X Y•Z
W X•Y•Z
b)
WX → W•X
XY → X•Y
YZ → Y•Z
W
X
Y
Z
IGS
G-binding Pocket
c)
guanosine-binding pocket of catalyst
remainder of catalyst
IGS
Tag
Y
Z
(invert Figure)
d)
IGS
5′ G M N — 3′
3′ U M′ N′ — 5′
Tag

a putative cooperative cycle



replicator yield is highest when all three components are present



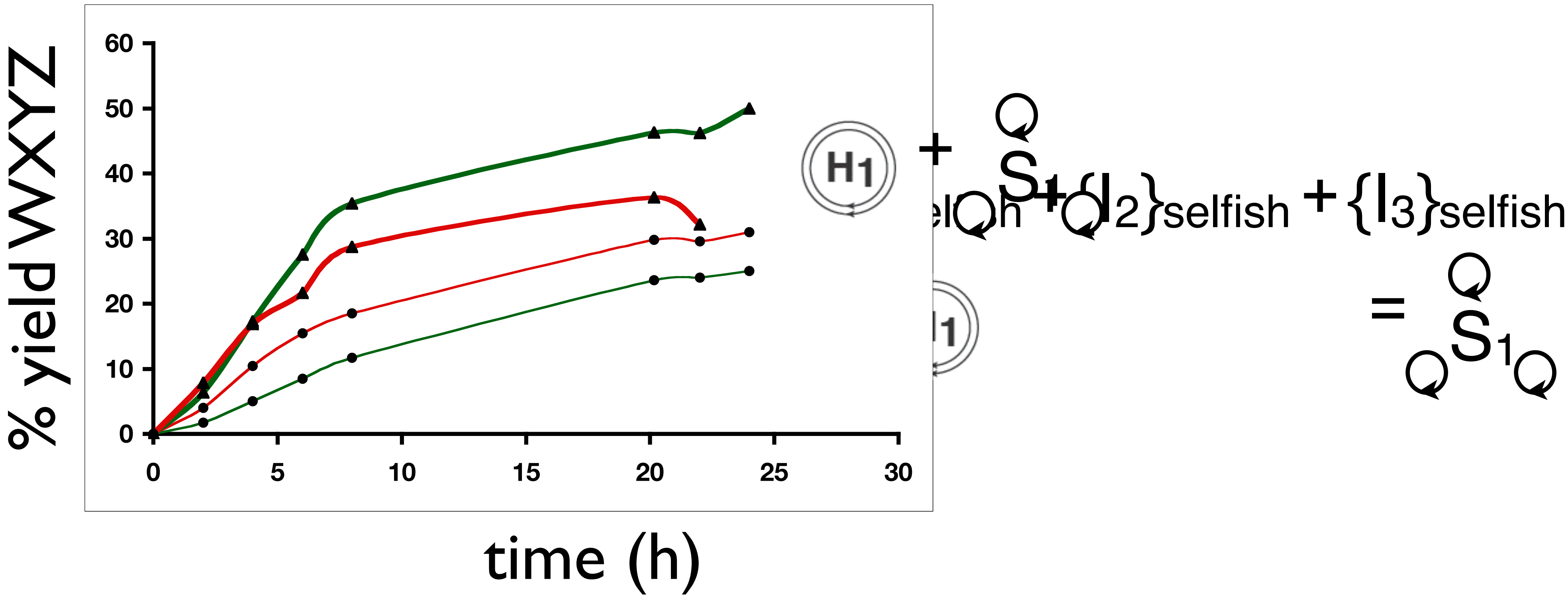
"closed" reaction

a competitive advantage to cooperation

the cooperative cycle out-competes the selfish replicators…

mismatched guides & tags

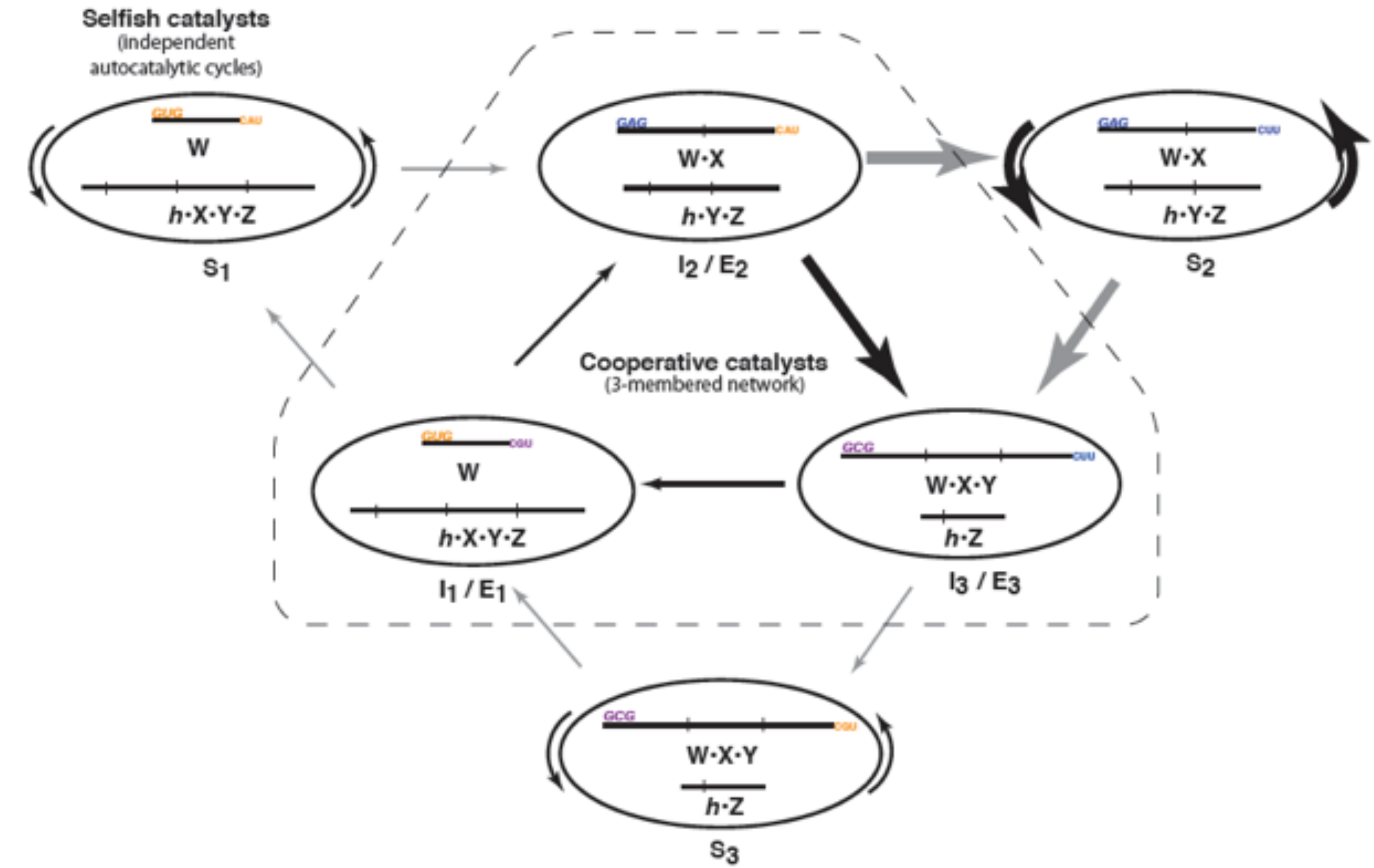
matched guides & tags



… but only when in mixed in the same population

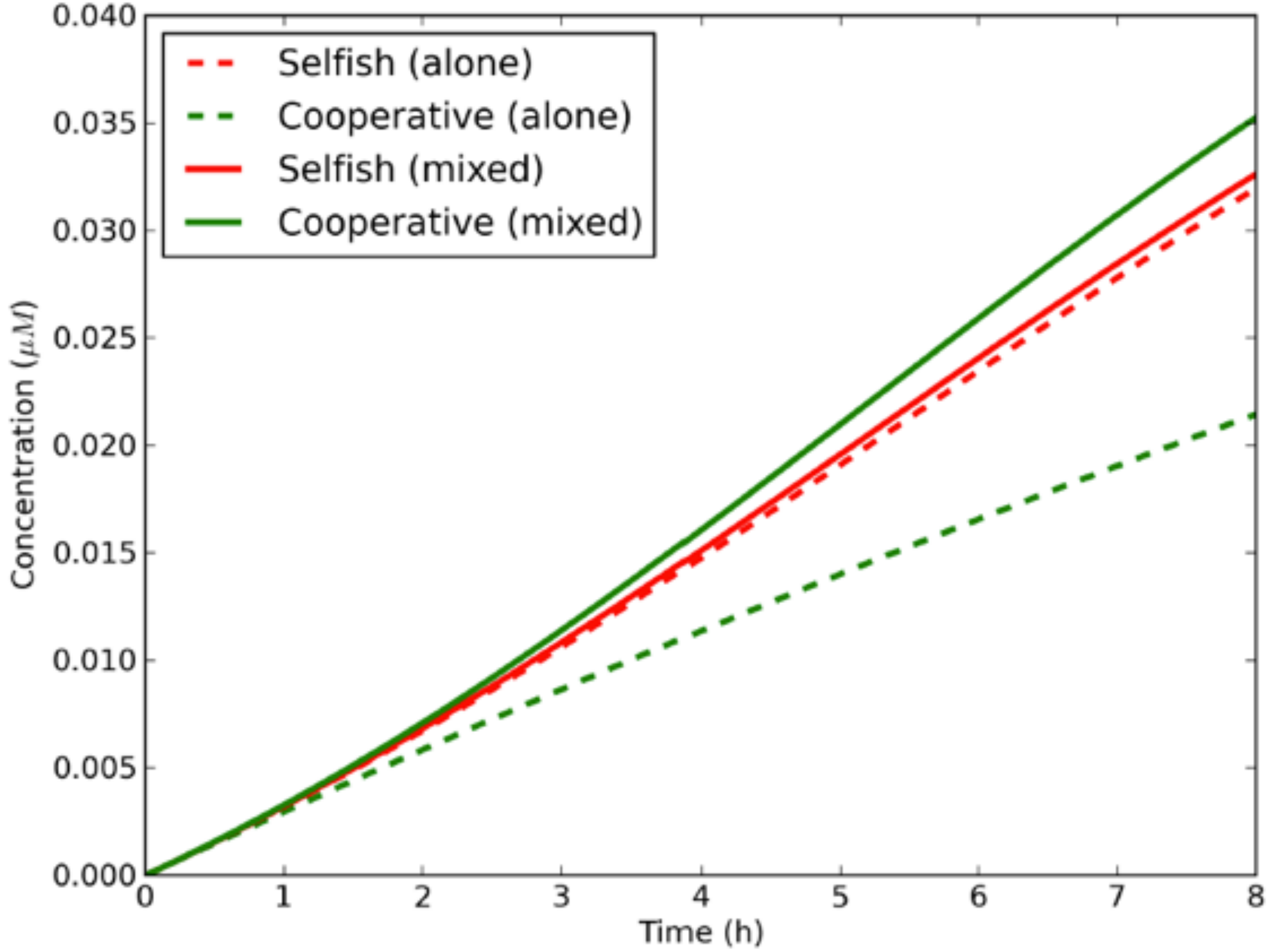
Vaidya, Manapat, Chen, Xulvi-Brunet, Hayden, Lehman (2012) *Nature* 490, 72–77.

a mechanism by which networks “assimilate” autocatalysts?

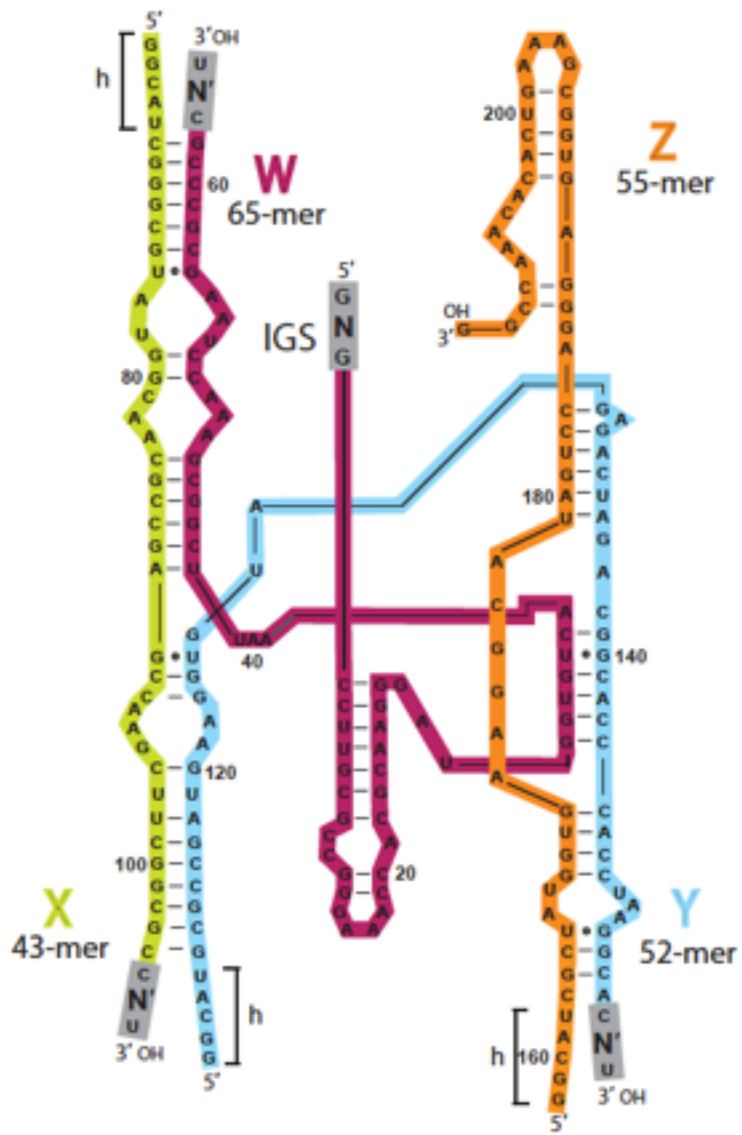


inequality in rate constants for the subsystems (arrow thickness) leads to time lags

mathematical modeling supports empirical data
(Michael Manapat / Irene Chen)



moving beyond this single example: randomization experiment



GNGWCN′U

GNGWXCN′U

GNGWXYCN′U

*h*XYZ

*h*YZ

*h*Z

51 species

randomization experiment

GNGWCN′U

GNGWXCN′U

GNGWXYCN′U

*h*XYZ

*h*YZ

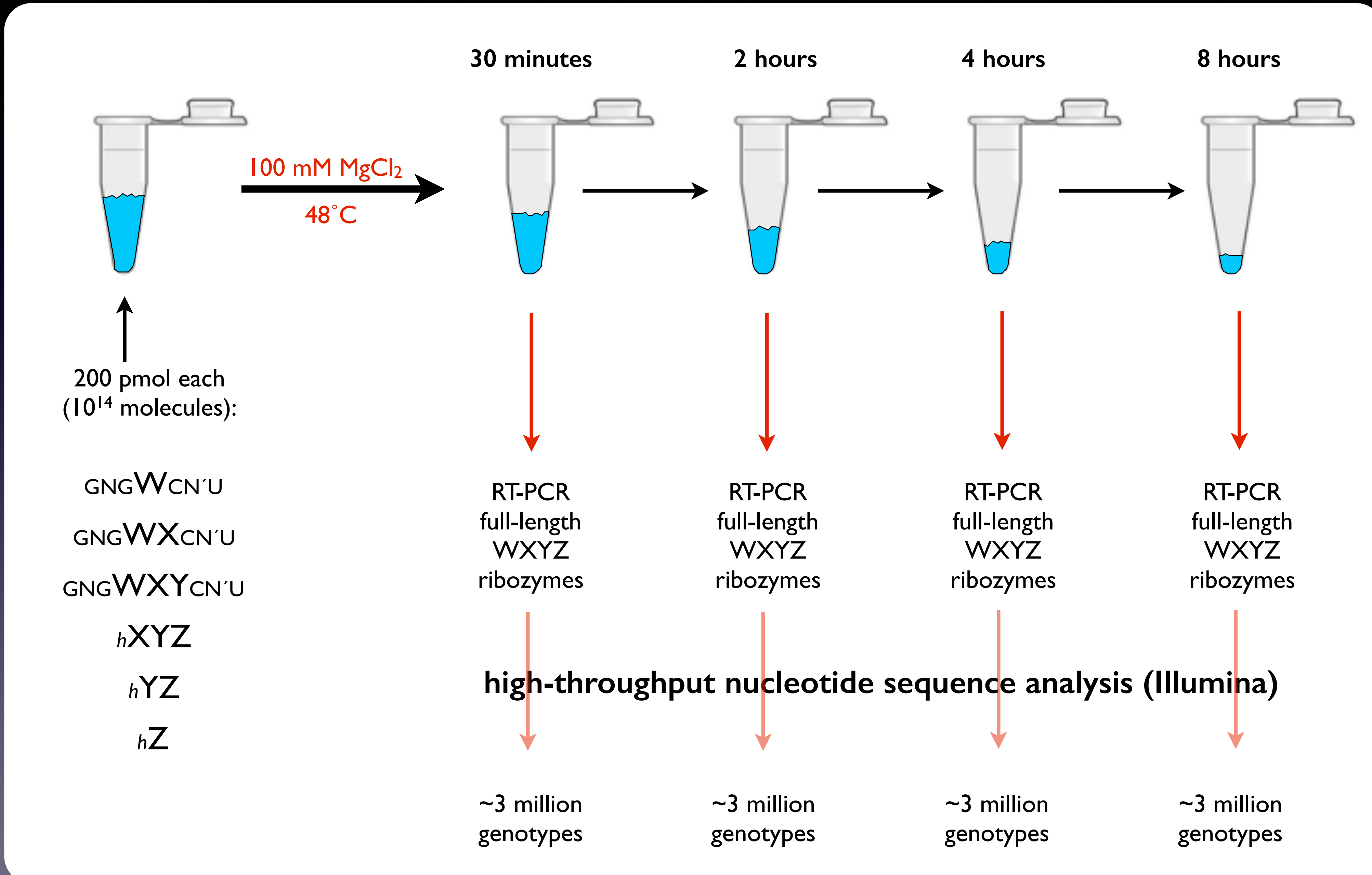
*h*Z

48 possible genotypes

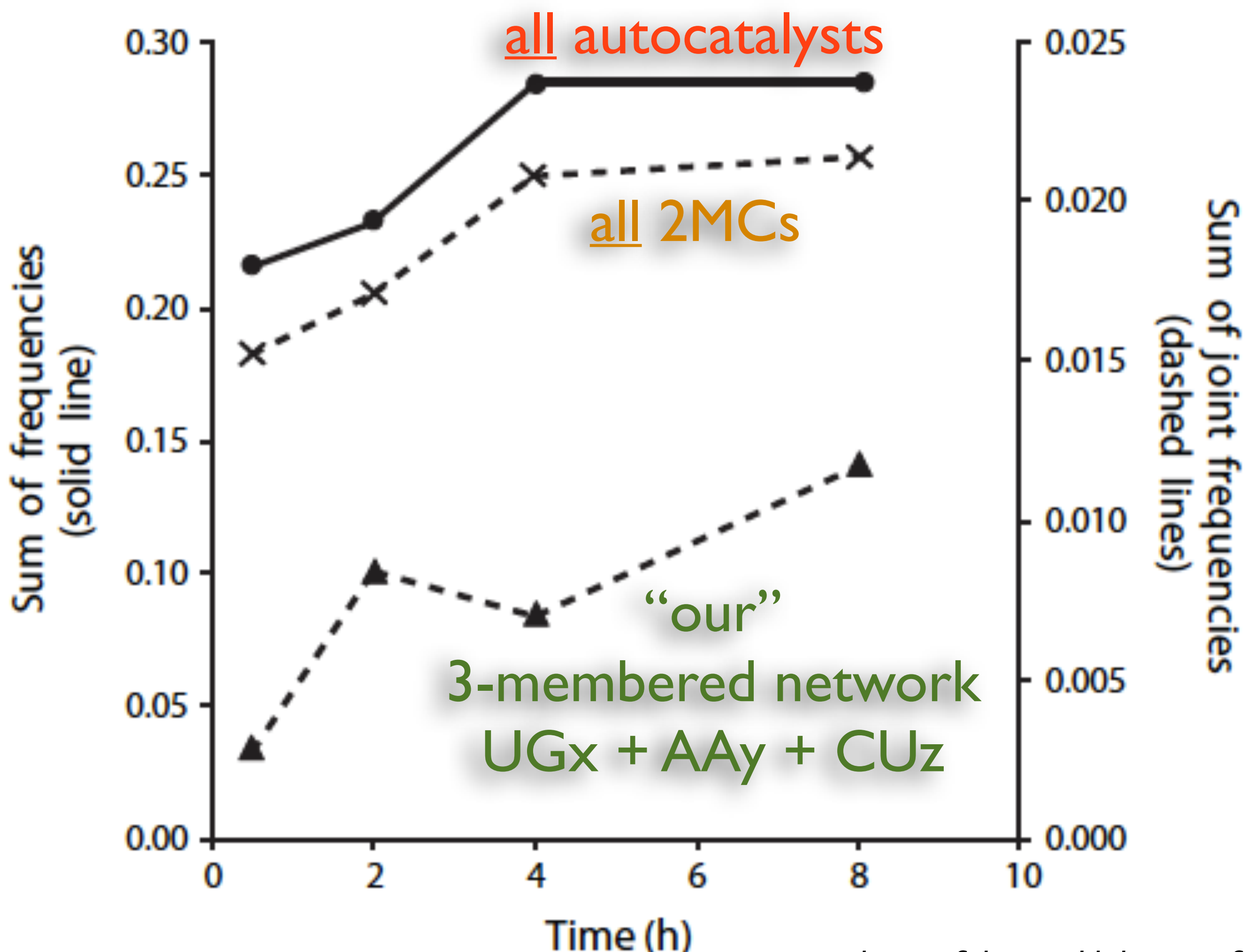
(4 IGS choices x 4 IGS tag choices x 3 junctions)

e.g., **C|U|x**

randomization experiment



summarized results



evidence of the establishment of cooperation over time

Vaidya, Manapat, Chen, Xulvi-Brunet, Hayden, Lehman (2012) *Nature* 490, 72–77.

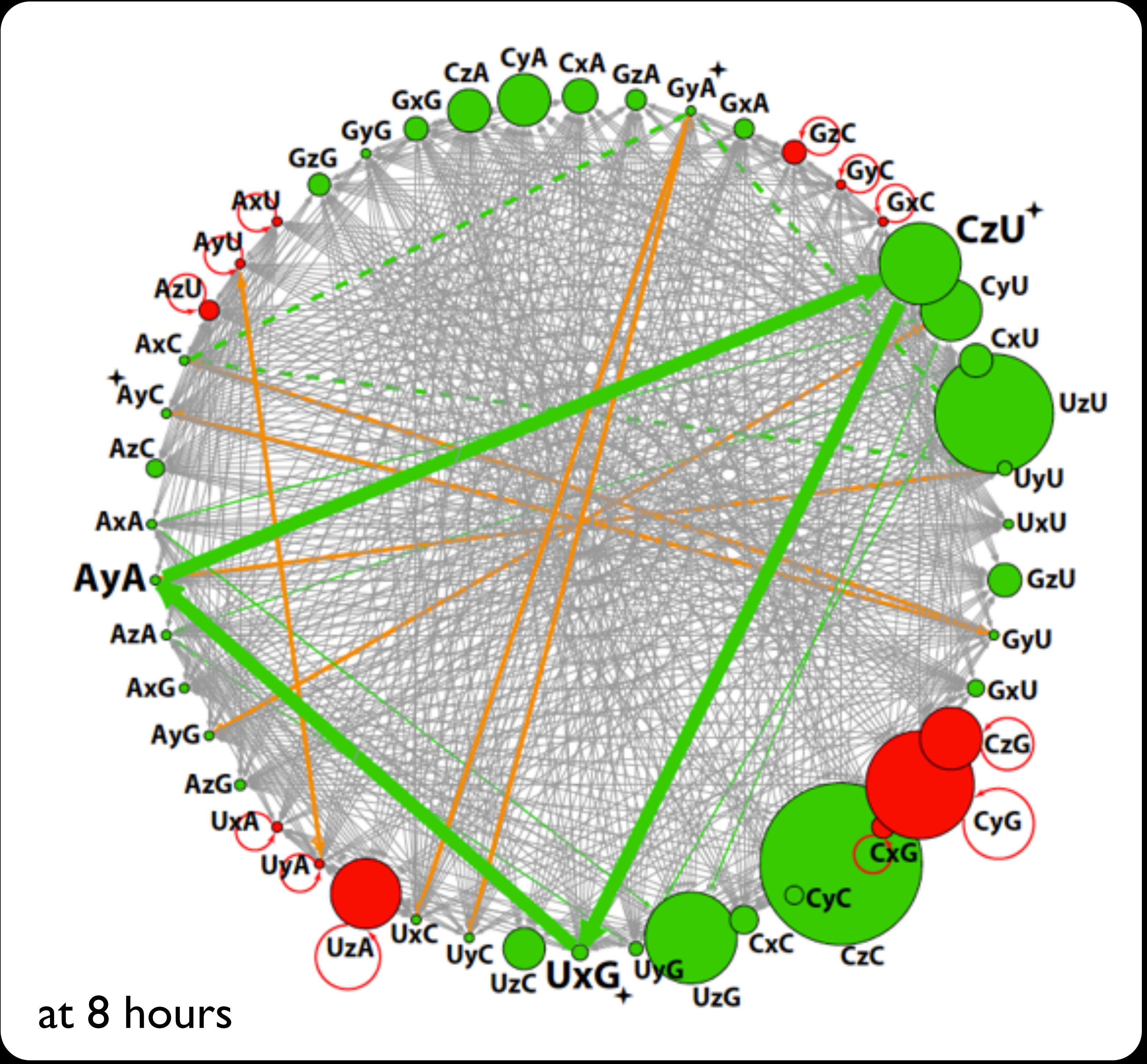
global visualization

red: autocatalysts

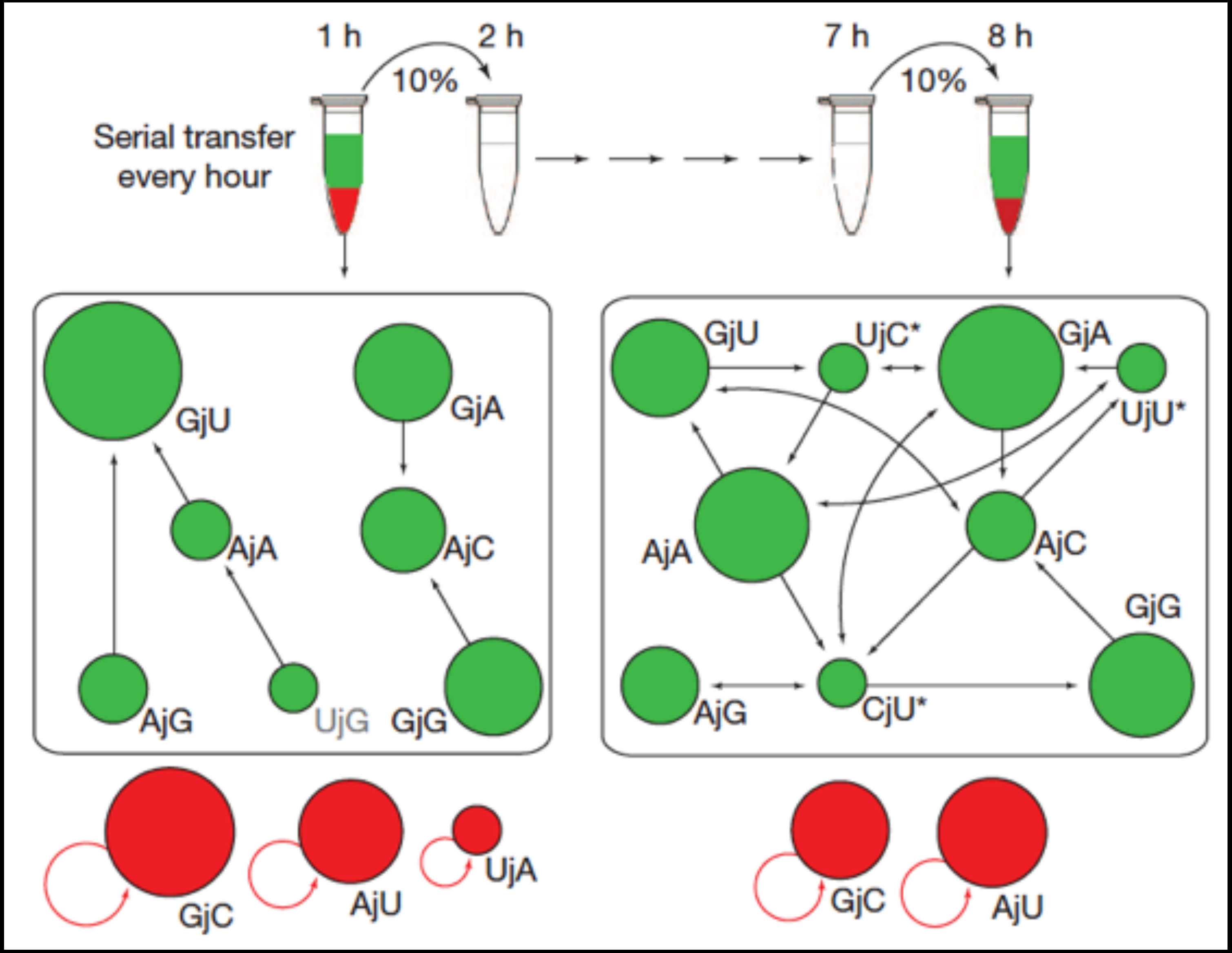
green: "cooperators"

orange: both members of 2MCs increasing over time

thick green: UGx + AAy + CUz

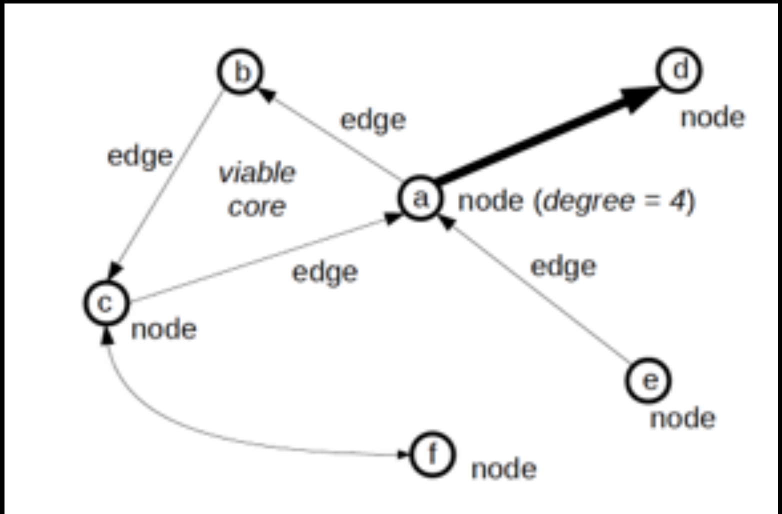


serial transfer experiments:
emulating a steady-state flow reactor



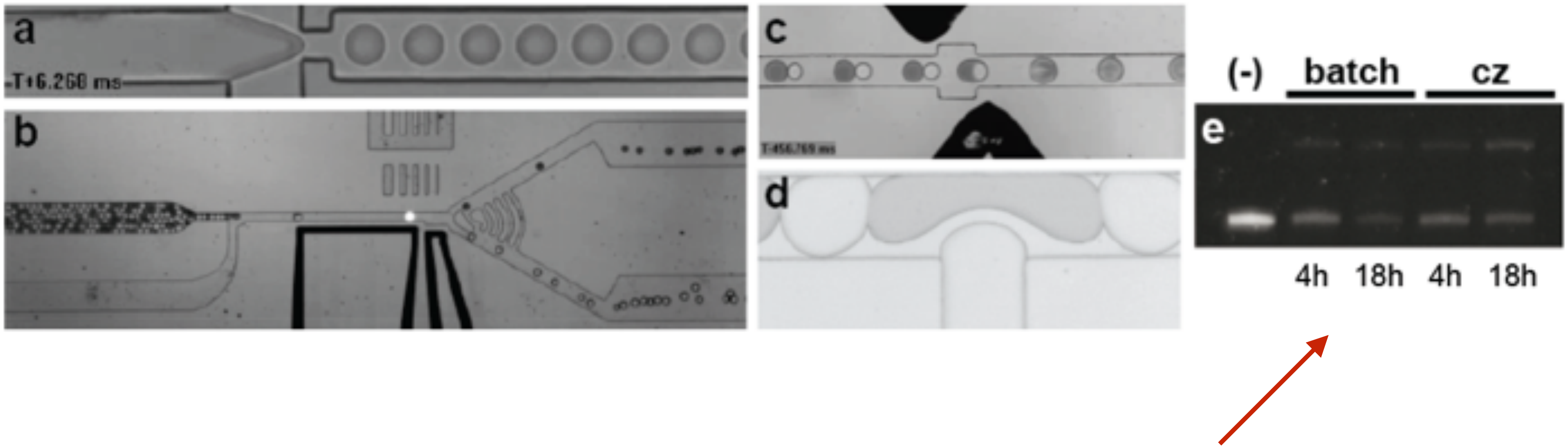
what matters to prebiotic networks?

1. viable cores (clusters)
2. connectivity kinetics (who's connected to whom)
3. information control (negative feedback)
4. scalability (scale-free networks)
5. resource availability (food supply)
6. compartmentalization (barriers to free flow)



Nghe, Hordijk, Kauffman, Walker, Schmidt, Kimble, Yeates, & Lehman (in press) *Molecular BioSystems*

testing predictions - artificial compartments

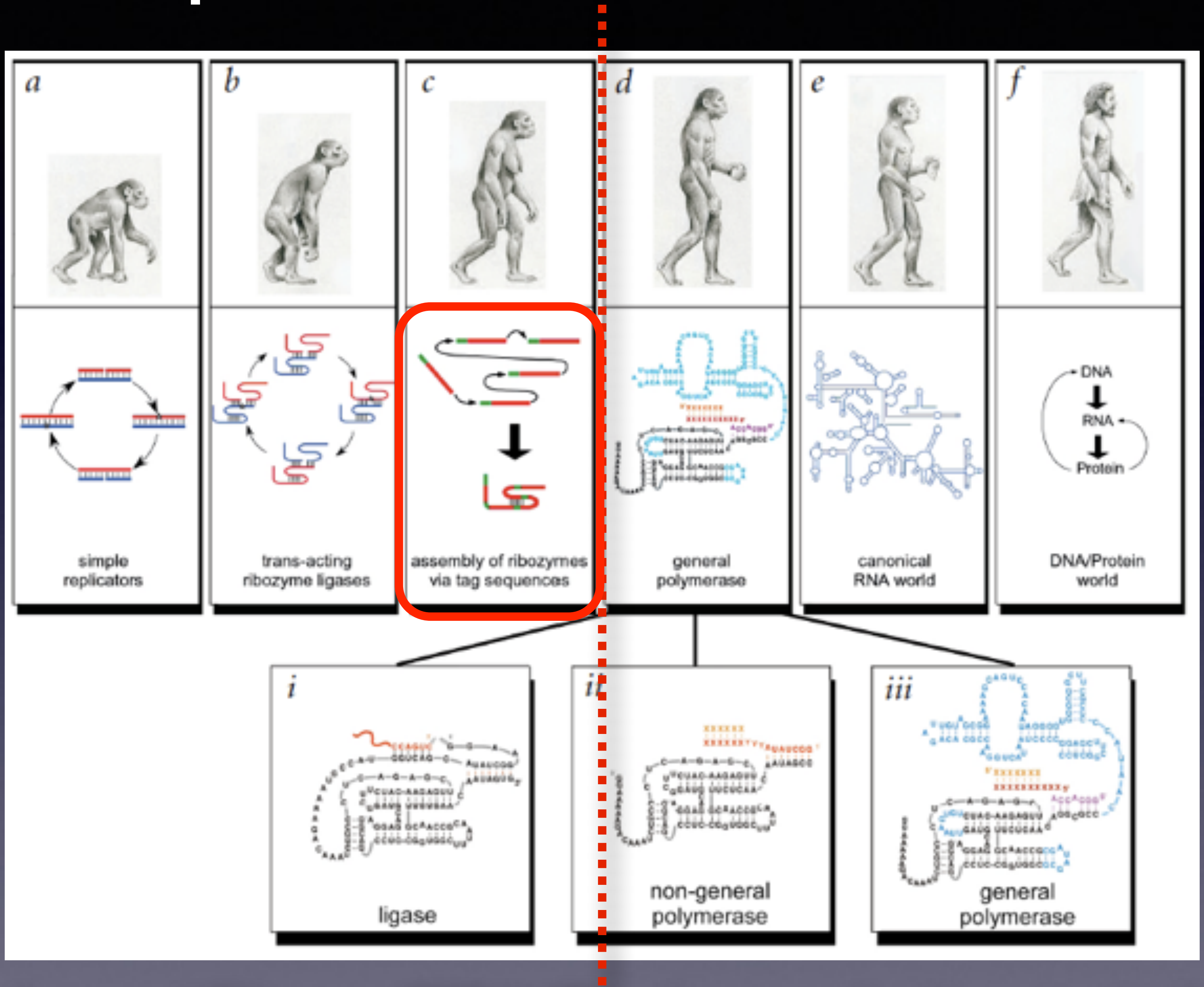


Yields of **WXYZ** RNA are 10–20% higher
in artificial water-in-oil (10 fL – 10 nL) droplets

we can test, for example, the Stochastic Corrector Model

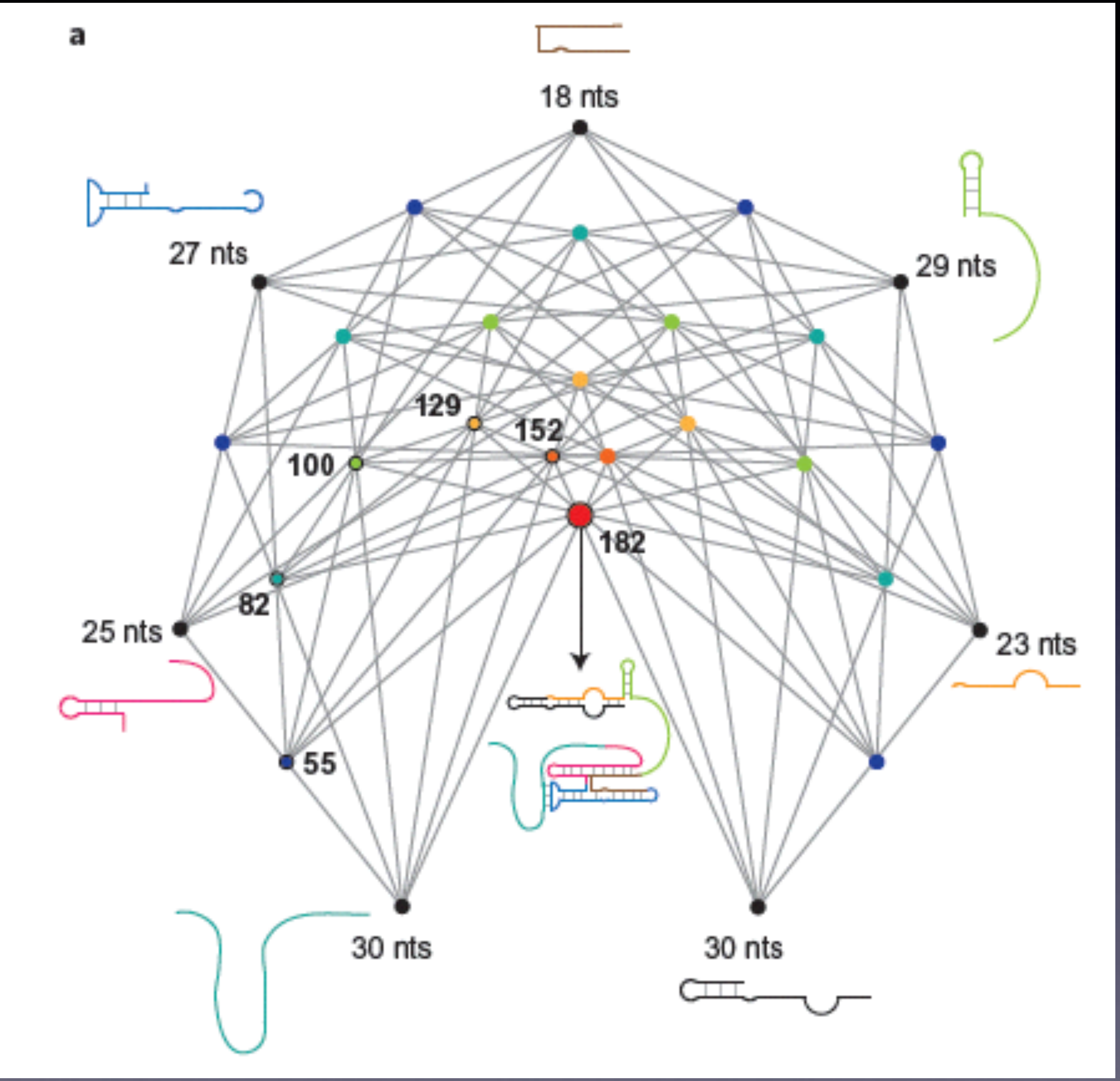
with Philippe Nghe & Andrew Griffiths, ESPCI ParisTech

cooperate ... then be selfish!



Levy and Ellington (2001) "The descent of polymerization"
Nat. Struct. Biol. **8**, 580–582.

cooperate … then be selfish!



Mutschler *et al.* (2015) Freeze-thaw cycles as drivers of complex ribozyme assembly. *Nature Chemistry* **7**, 502–508.

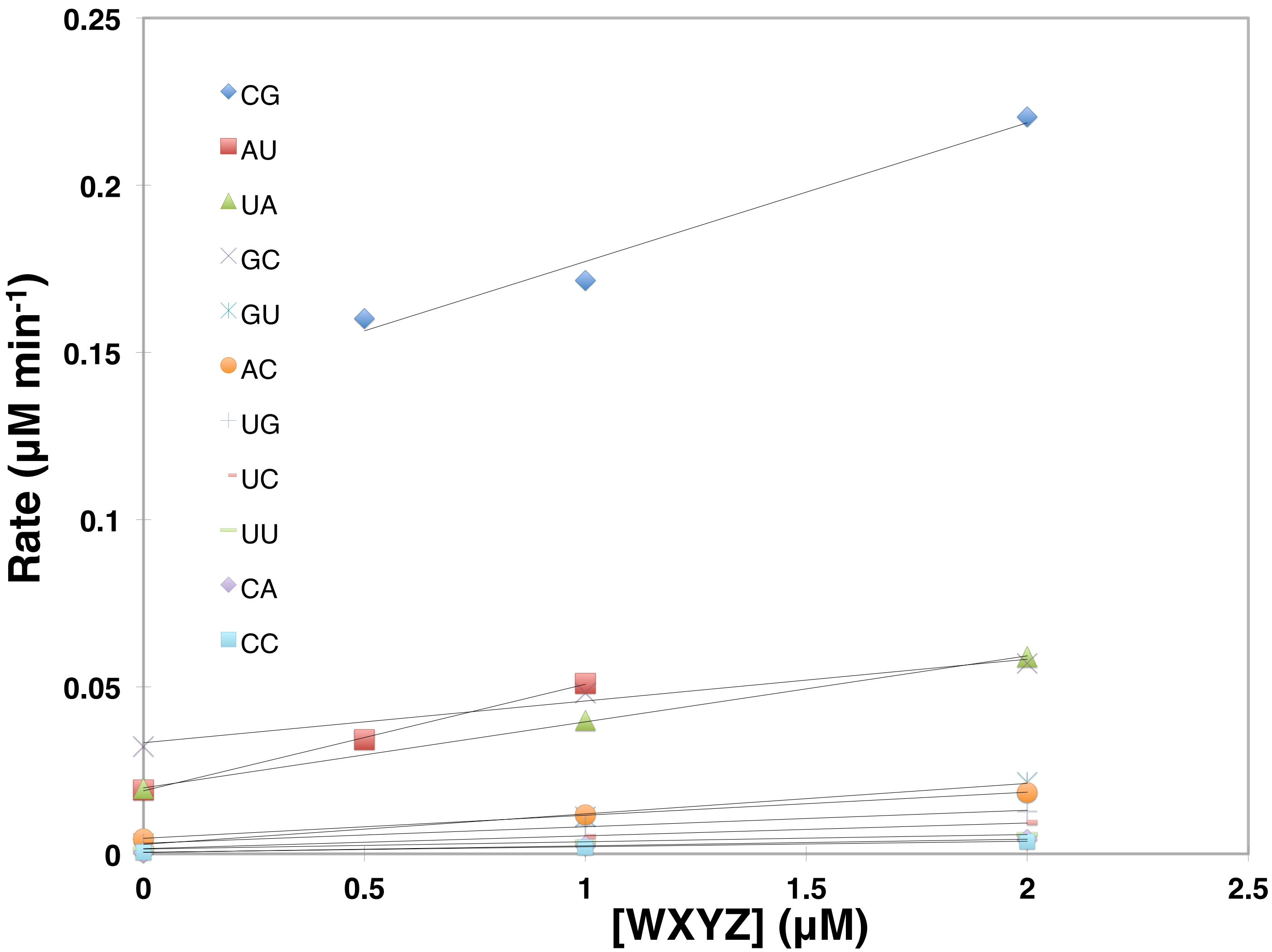
acknowledgements

Dr. **Nilesh Vaidya** (Portland State, now Princeton)
Dr. **Eric Hayden** (Portland State, now Boise State)
Dr. Prof. **Günter von Kiedrowski** (Bochum)
Dr. **Irene Chen** (Harvard, now UCSB)
Dr. **Michael Manapat** (Harvard, now Google)
Ms. Jessica Yeates (PSU Ph.D. student)

"NilesH"

NASA
National Aeronautics
and Space Administration

autocatalytic rate constants (k_a, min^{-1}) for the 16 **WXY** genotypes



Genotype	k_a (min^{-1})	Std. error	r^2
CG	0.0415	0.0066	0.98
AU	0.0319	0.0011	1.00
UA	0.0197	0.0004	1.00
GC	0.0125	0.0021	0.97
GU	0.0091	0.0007	0.99
AC	0.0069	0.0002	1.00
UG	0.0049	0.0004	0.99
UC	0.0038	0.0002	1.00
UU	0.0022	0.0001	1.00
CA	0.0020	0.0000	1.00
CC	0.0016	0.0001	1.00
GG	0.0006	0.0001	0.99
GA	0.0005	0.0001	0.98
AA	0.0004	0.0001	0.92
CU	0.0004	0.0000	1.00
AG	0.0001	0.0000	0.99