Journal of Cosmology, Vol. 25, No. 19, pp 13730-13776.

Life emerged on Earth by cometary panspermia, The sun is formed within a protoglobular starcluster clump of a trillion gassy planets that merge to form the star, bringing life along.

The standard model for Life's origin on Earth

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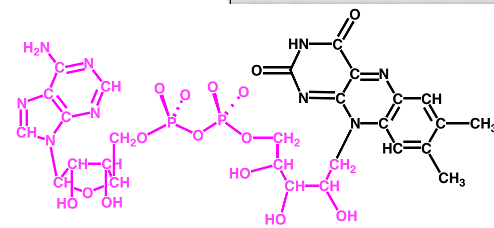
The Earth cooled; organic material formed

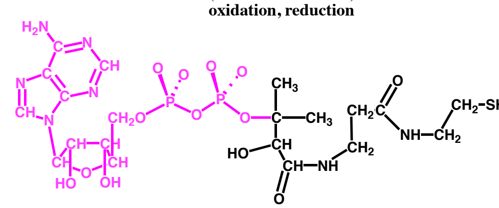
RNA molecules able to catalyze template-directed synthesis of RNA (with replicable errors) emerged.

Darwinian evolution began with **RNA** the sole genetically encoded part of biocatalysts; **RNA** cofactors emerged, now throughout terran life.

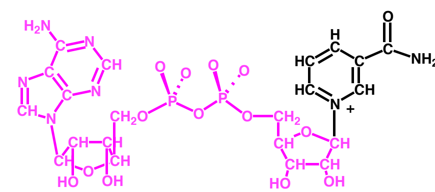
Rich, A. (1962). On the problems of evolution & biochemical information transfer. in *Horizons In Biochemistry* (eds. M. Kasha, B. Pullmann) 103-126. Academic Press, New York.



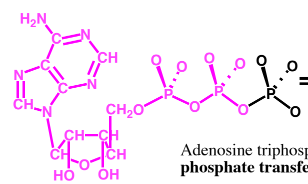
Flavin adenine dinucleotde
(vitamin = riboflavin)
oxidation, reduction



Coenzyme A
(vitamin = pantothenic acid)
carbon-carbon bond formation



Nicotinamide adenine dinucleotde
(vitamin = niacin)
oxidation, reduction

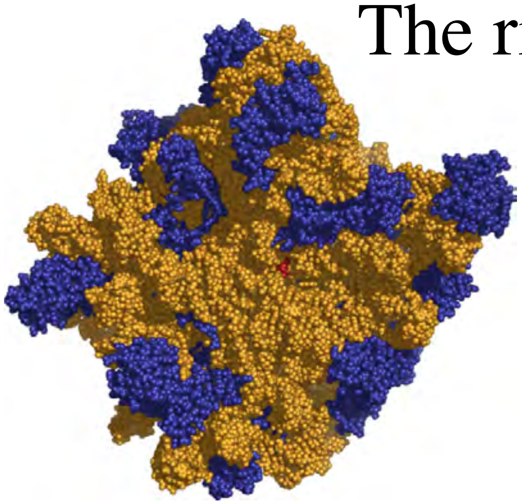


Adenosine triphosphate (ATP)
phosphate transferenergy transfer

DNA then emerged to "do" genetics; proteins to "do" catalysis.

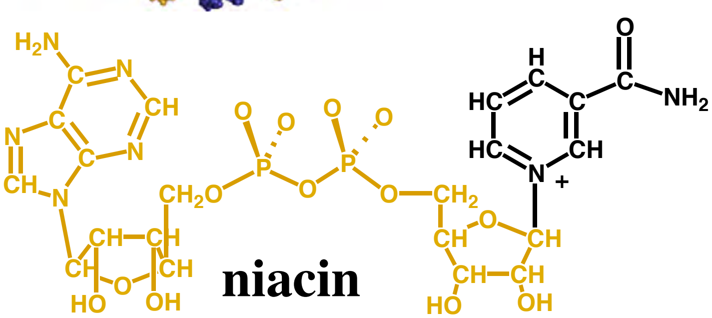
Standard model explains …

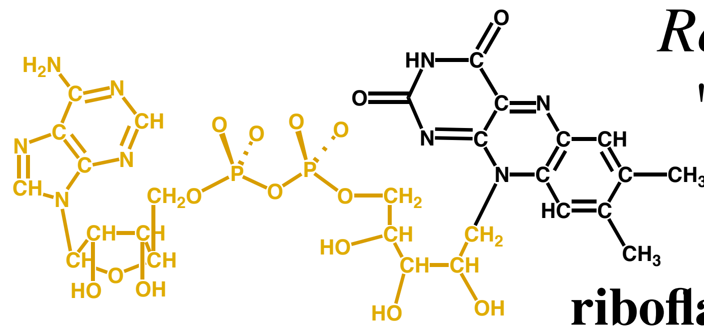
DNA ← → messenger RNA → proteins
and avoids the chicken-or-egg problem



The ribosome, the machine that *makes* **proteins**, is an **RNA** catalyst

The **RNA** pieces of cofactors used in metabolism make sense as handles arising at a time when RNA is all that one had.





Redox cofactors; "business ends" in black

Self-sustaining molecular system that can be replicated with errors, with the errors themselves replicable.

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For this model to make sense…

The RNA world must have had a complex metabolism, including redox metabolism (flavin, niacin), Claisen carbon-carbon bond forming (coenzyme A), one carbon transfer (S-adenosylmethionine), and phosphate metabolism (ATP).

It would have been ecologically diverse, for the same reason that the modern biosphere is.

It made efforts to expand the catalytic potential of RNA before it invented proteins (modified RNA bases).

It may be the most abundant kind of life in the cosmos today.

Harold White (1976); Cornelius Visser, Richard Kellogg (1978)

Benner et al. (1989) Modern metabolism as a palimpsest of the RNA world. *Proc. Nat. Acad. Sci.* **86**, 7054-7058

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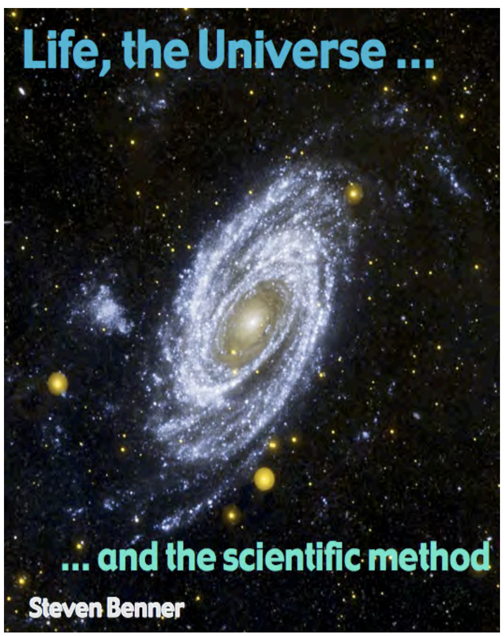
And then the troubles begin…

We should be able to construct in the modern laboratory components of the RNA world from "prebiotic precursors", *especially since we have "intelligent design" capability and biochemistry suppliers unavailable to the prebiotic world.*

Compared to the prebiotic Earth, modern molecular biologist have the power of gods.



50 years of failure, but instructive failure.

Follow the "paradoxes"

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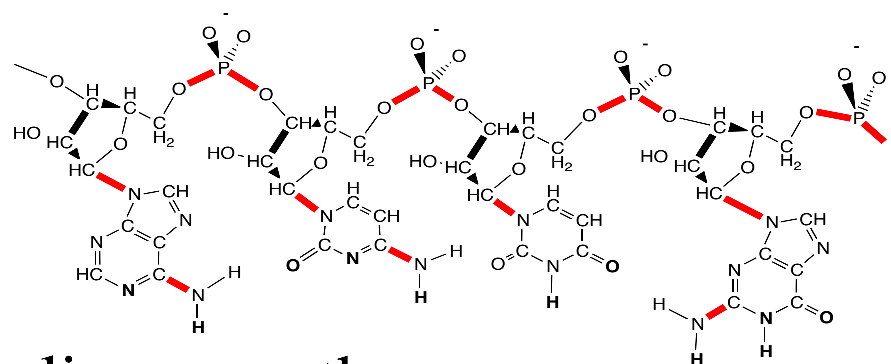
Some of the paradoxes in the RNA-first model for life's origins

- Tar paradox: Give energy to organic matter but no access to Darwinian evolution, one gets tar, not RNA building units. *Well validated, from the kitchen to the coal field*.
- Oligomer entropy paradox. Even if we get units, high concentrations needed to get oligomers.



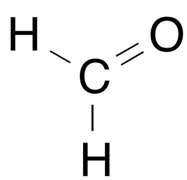
- Water paradox. Even if we get oligomers, they are hard to make in water and, if made, are destroyed by water.
- Single biopolymer paradox. Even if we get the oligomers, the needs of catalysis contradict the needs of genetics.
- Even if RNA is a good compromise for catalysis vs. genetics, destructive catalysts >> productive catalysts.

The tar paradox is especially well known with sugars

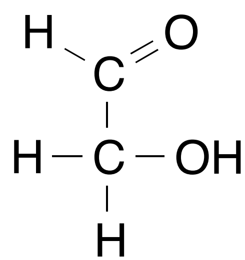
Sucrose:
"Rock candy"

And well known to *you*;
Heat it a bit,
get carmel
Heat more,
get more.

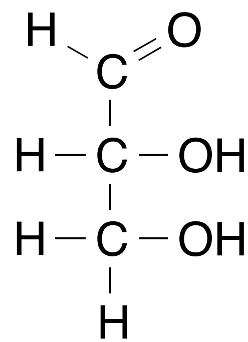
$C_nH_{2n}O_n$



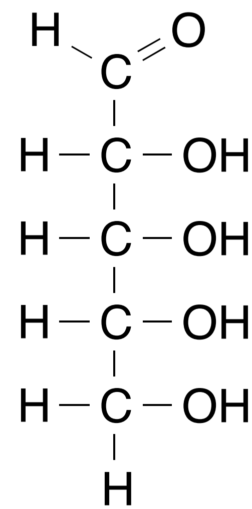
$C_1H_2O_1$
formaldehyde



$C_2H_4O_2$
glycolaldehyde



$C_3H_6O_3$
glyceraldehyde

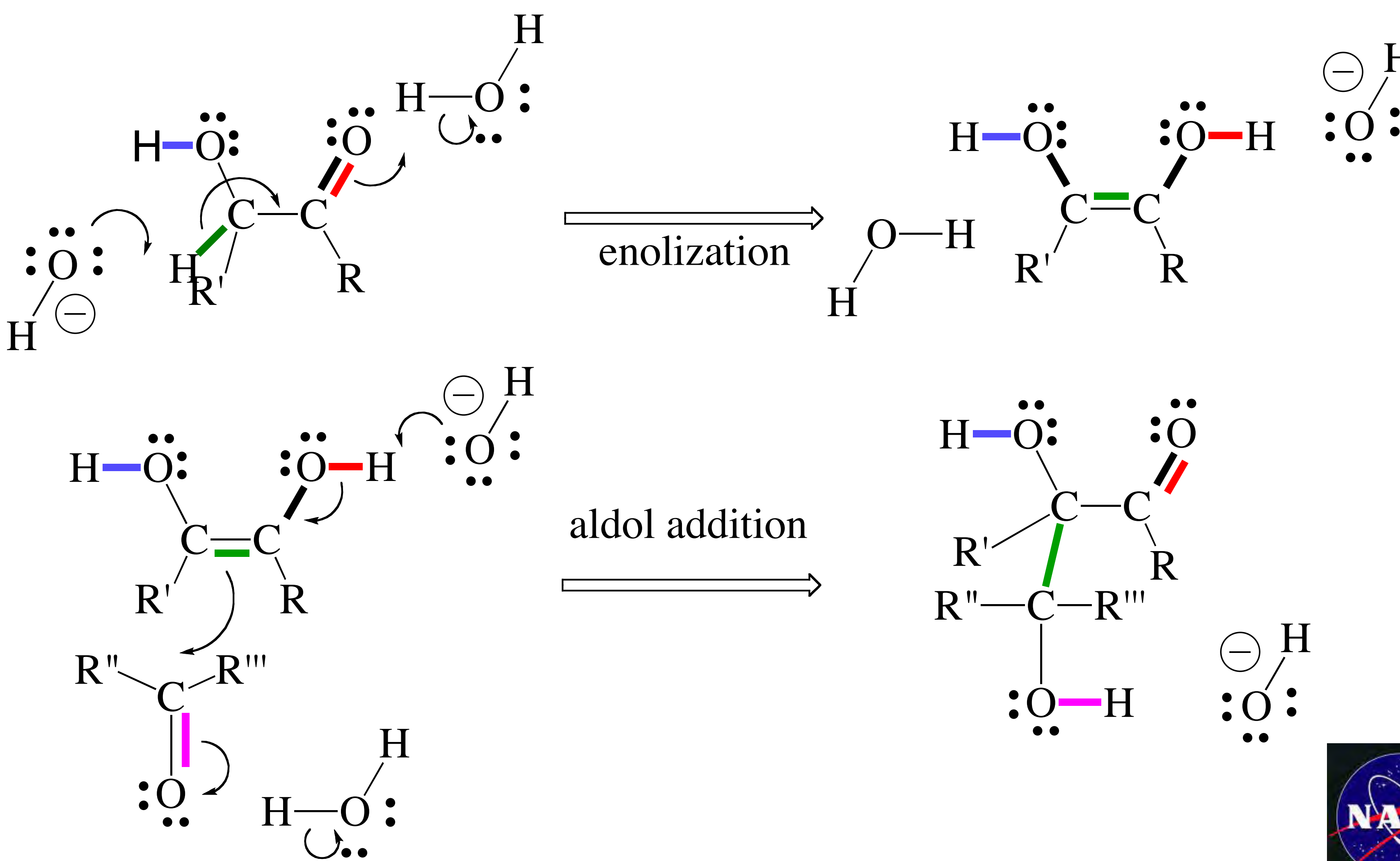


$C_5H_{10}O_5$
ribose

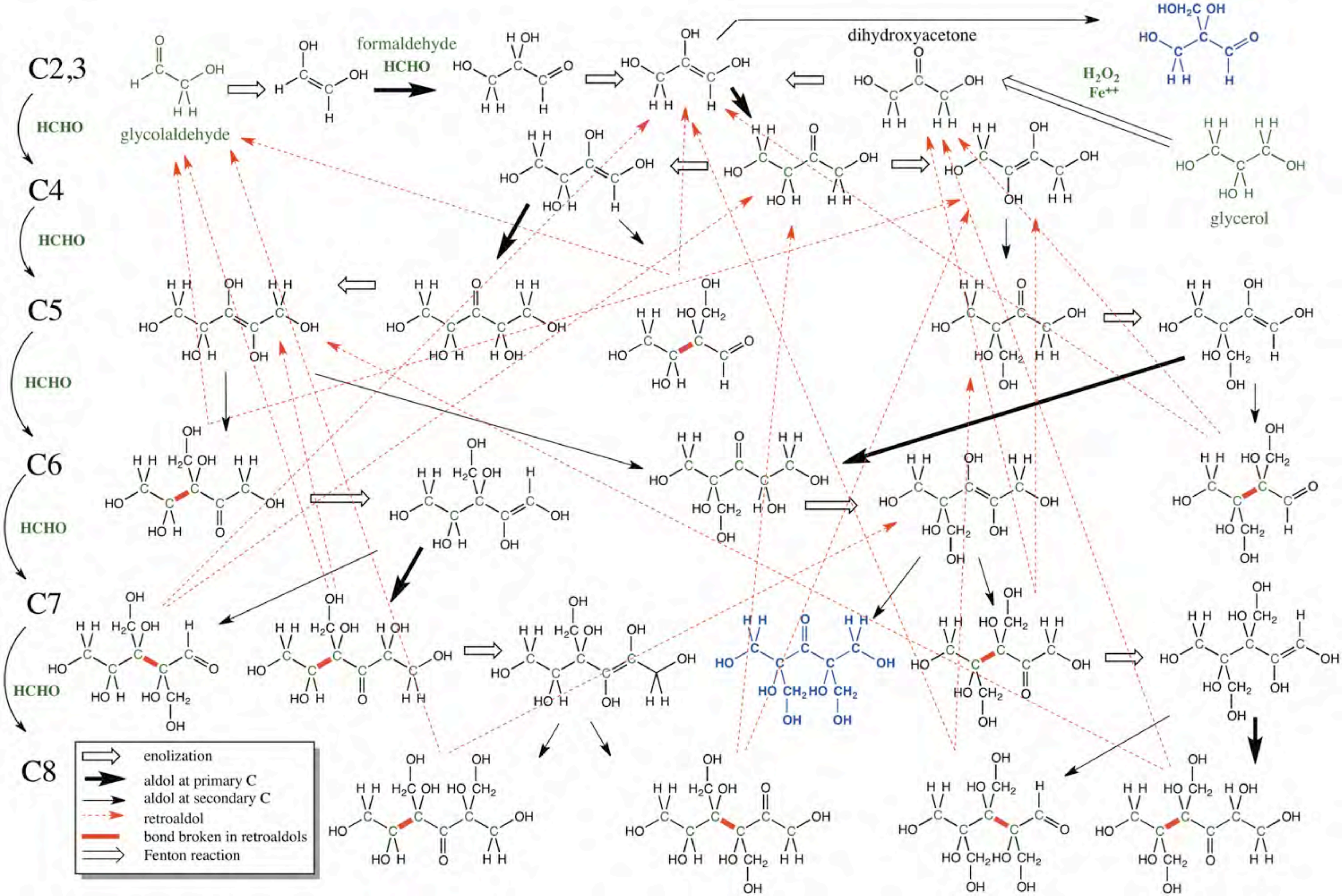
Unfortunately, RNA has a sugar component (R)

Why tar? Sugars can do repeated enolization/aldol addition

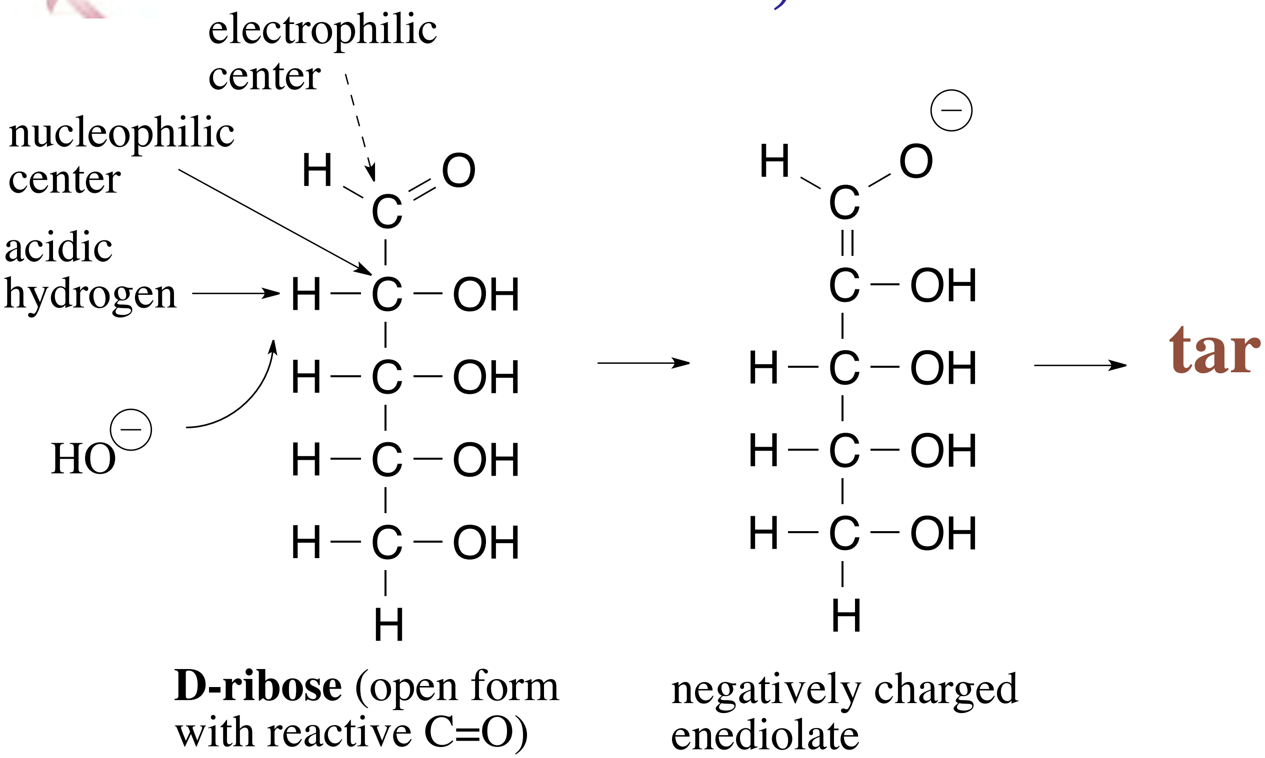
Note the C=O carbonyl group



The complexity is horrible



Ribose can also enolize, react with itself, and form tar



Stanley Miller measured the rate at which ribose formed tar; it is fast (~75 years at pH 7 at 50 °C, not millions of years

Larralde, Robertson, and Miller (1995) Rates of decomposition of ribose and other sugars. Implications for chemical evolution. *Proc. Natl. Acad. Sci. USA* 92, 8158

"stability considerations preclude the use of ribose and other sugars as prebiotic reagents …. It follows that ribose and other sugars were not components of the first genetic material…"

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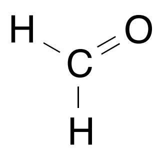
But origins occurred in a mineral environment

Sugars have adjacent hydroxyl (-OH) groups

Borate binds adjacent hydroxyl groups

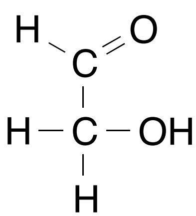
Borate binds ribose; prevent its tar-ization

Borate guides reaction of carbohydrates



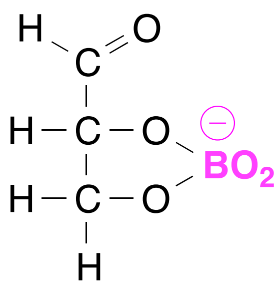
$C_1H_2O_1$

formaldehyde



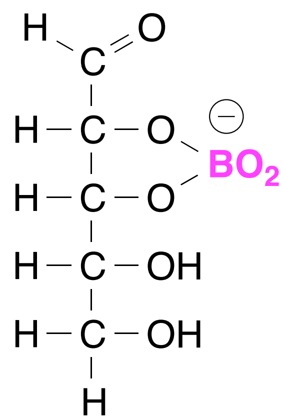
$C_2H_4O_2$

glycolaldehyde



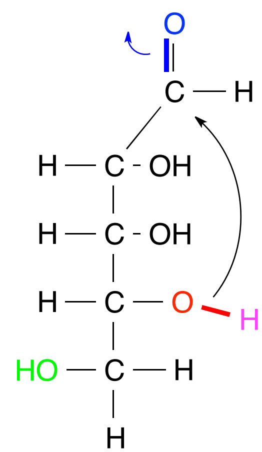
$C_3H_6O_3$

glyceraldehyde

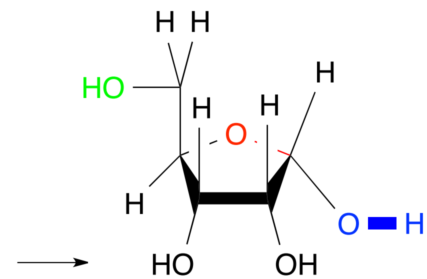


$C_5H_{10}O_5$

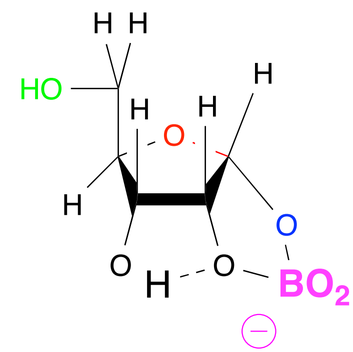
ribose



ribose (open form)



ribose (cyclic form)



Ricardo, A., Carrigan, M.A., Olcott, A., Benner, S.A. (2004) Borate minerals stabilize ribose. *Science* **303**, 196

Kim, et al. (2011) Synthesis of carbohydrates in mineral-guided prebiotic cycles. *J. Am. Chem. Soc.* **133**, 9457-9468

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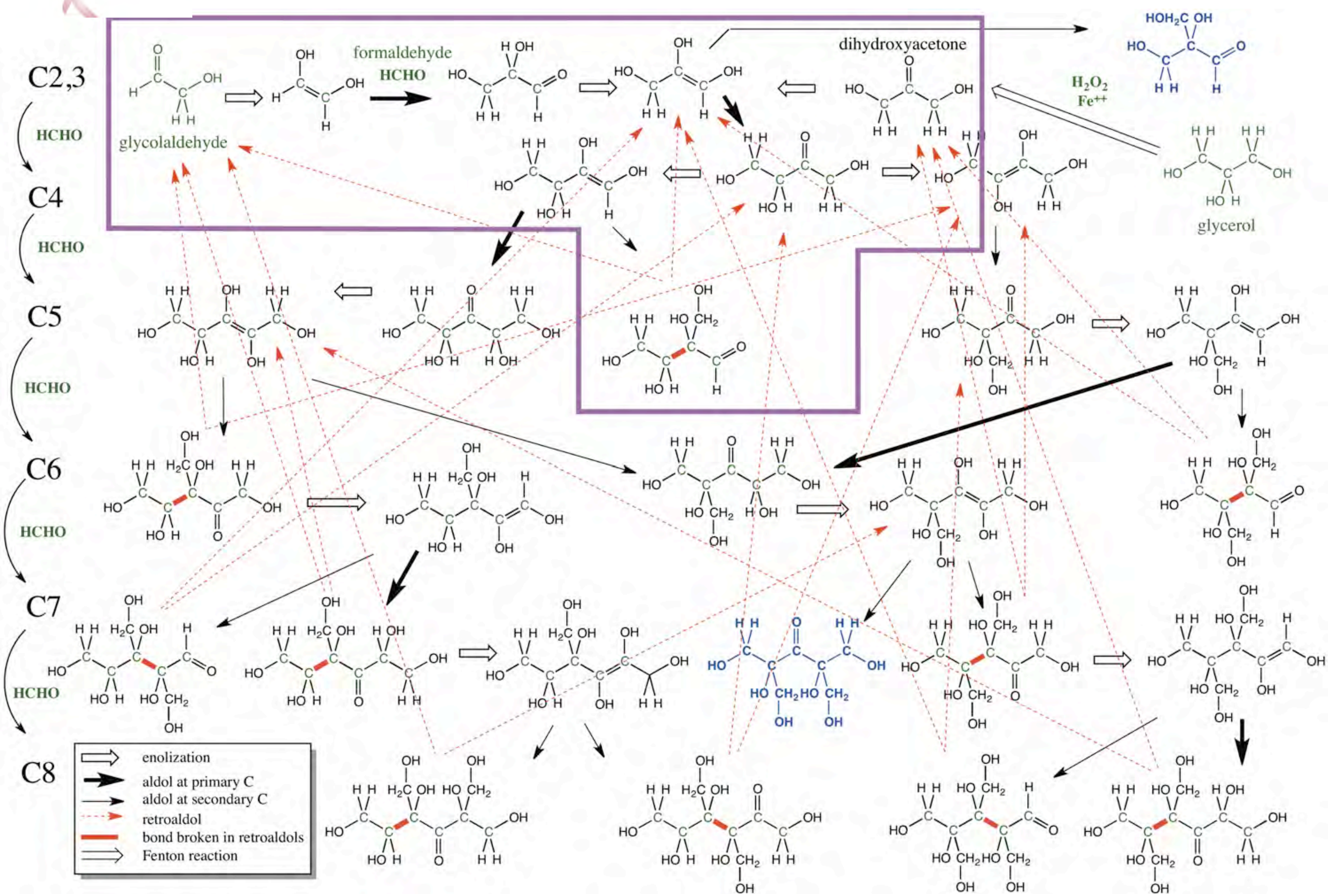
Borate constrains reactivity



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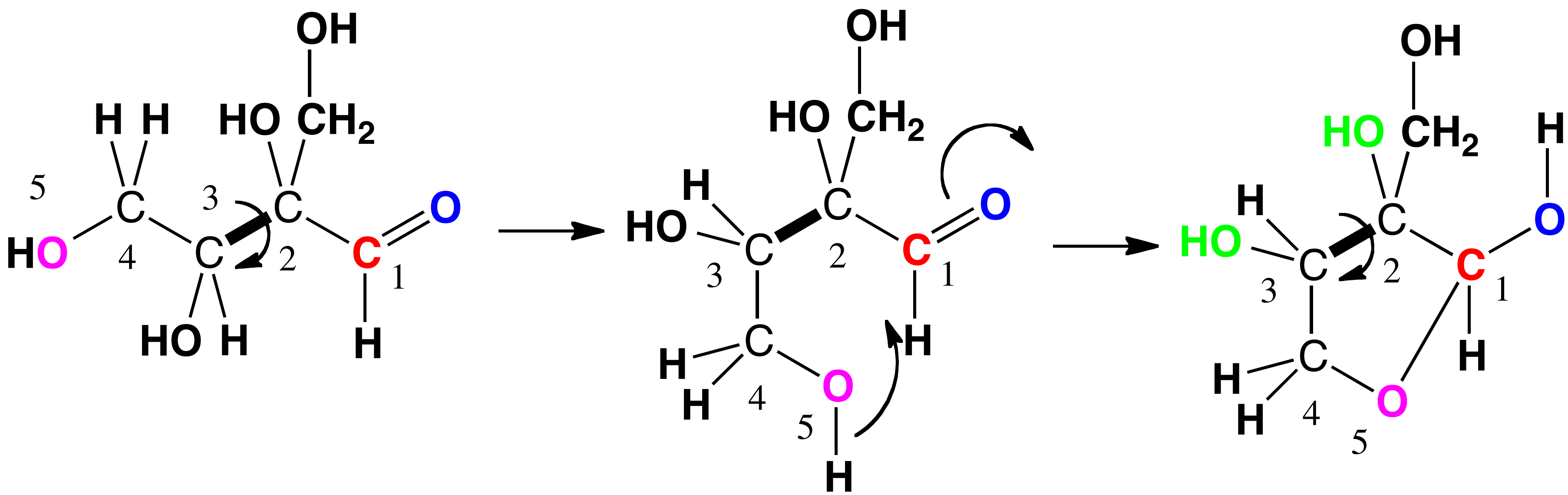
Forming a ring removes C=O, stabilizing the carbohydrate



A 5-membered ring, or a 6-membered ring is stable; thus, an oxygen (O) must be 5 or 6 (count ‘em) atoms in the chain from the C=O carbon atom

Borate can bind the adjacent OH groups

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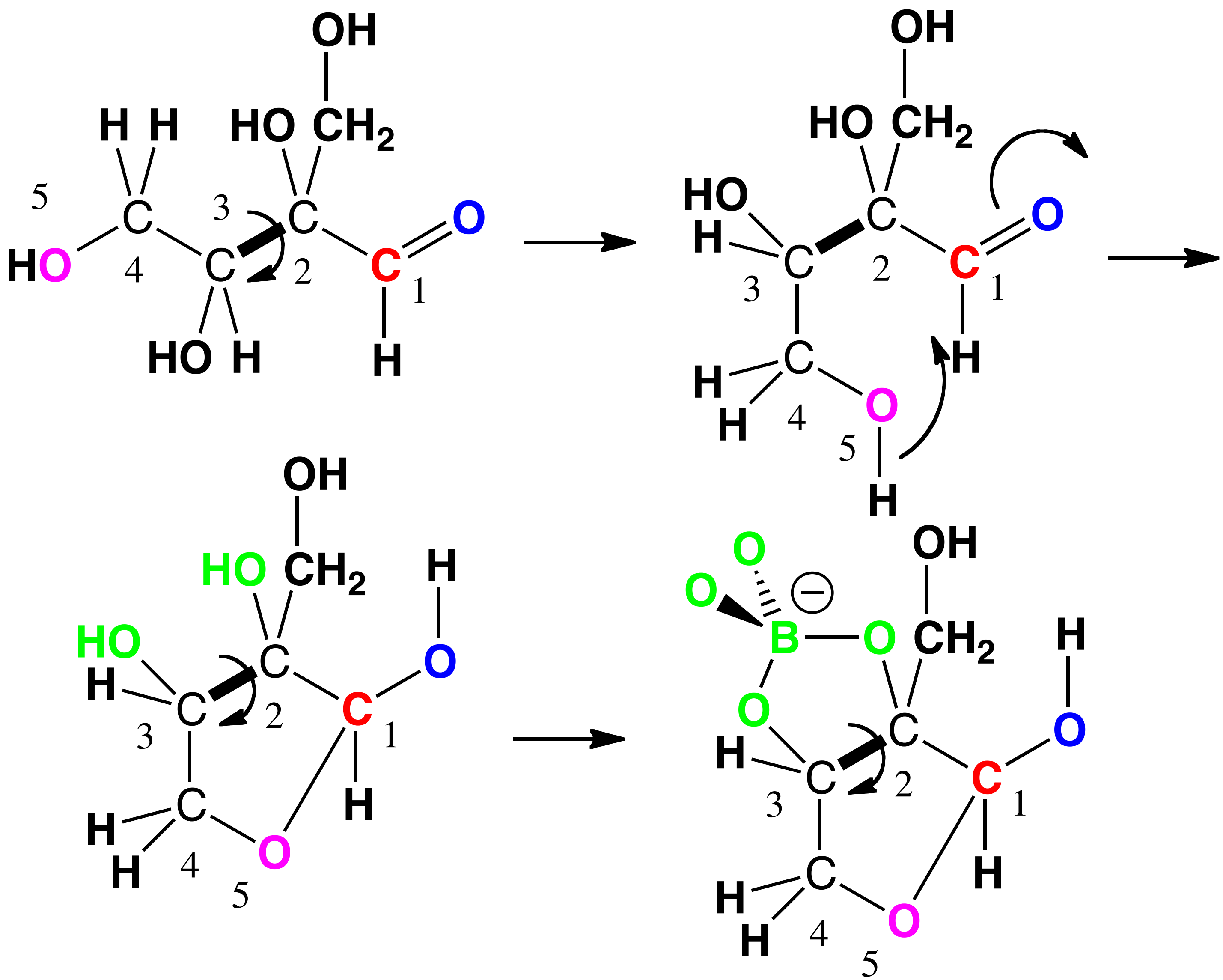
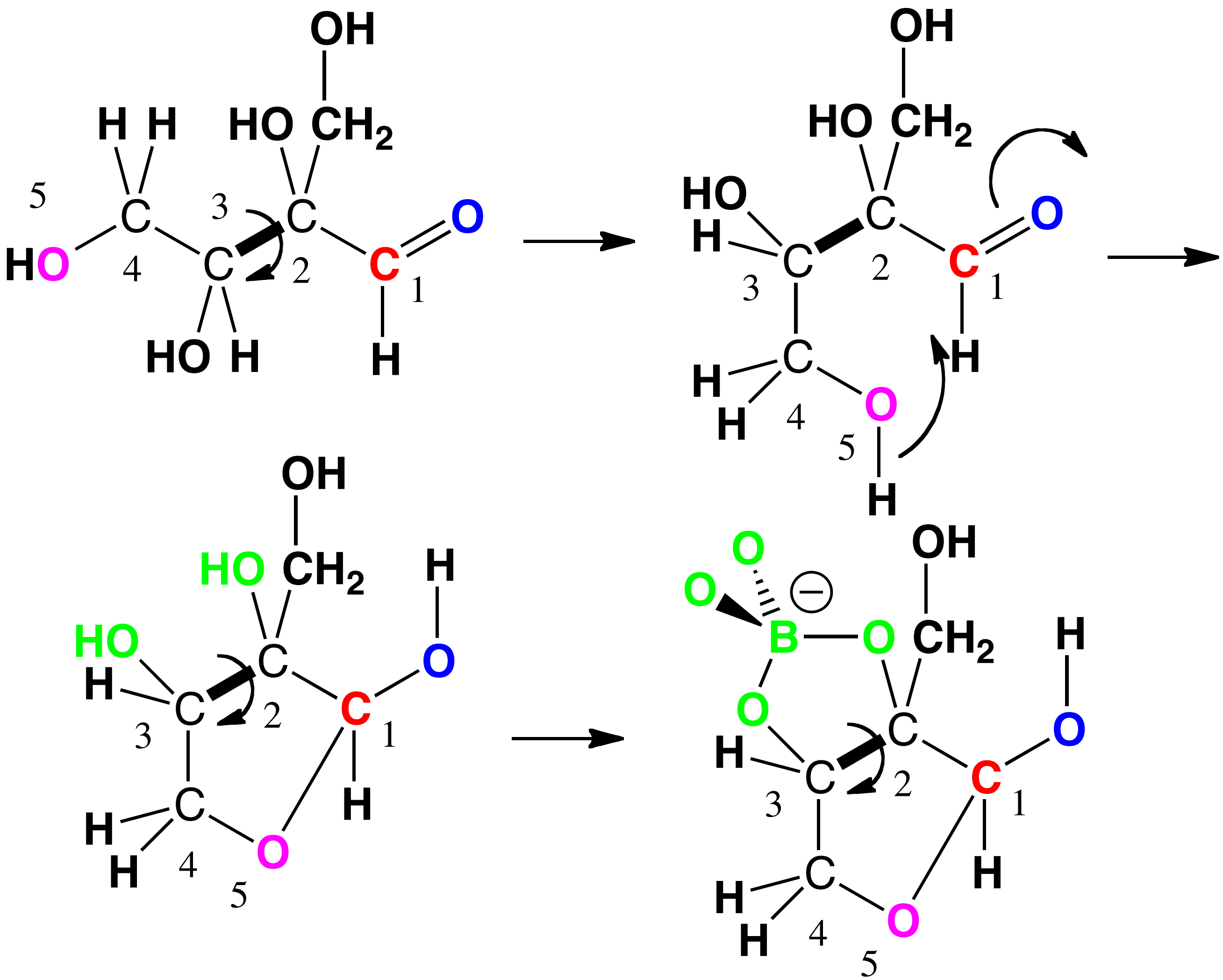
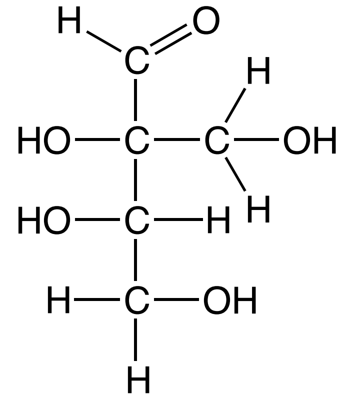
Borate binds the adjacent OH groups of the $C_5H_{10}O_5$

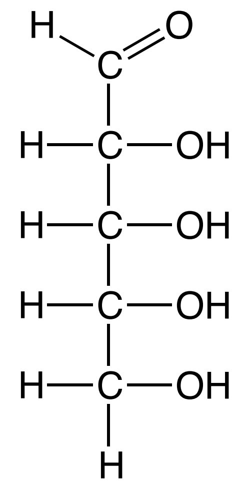


Problem: It is the wrong $C_5H_{10}O_5$





Borate gives you this branched sugar



This is ribose

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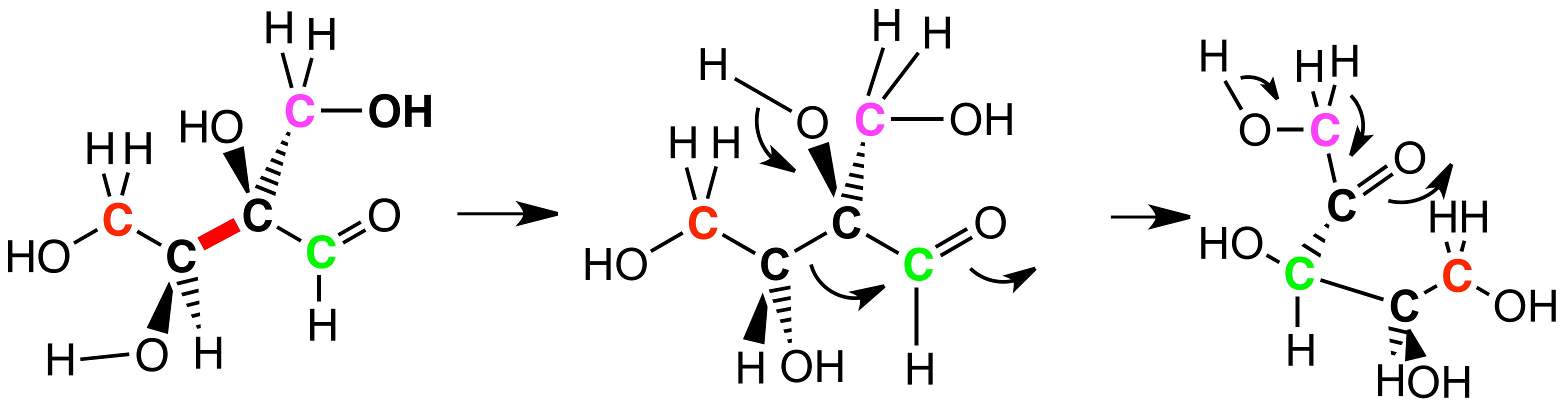
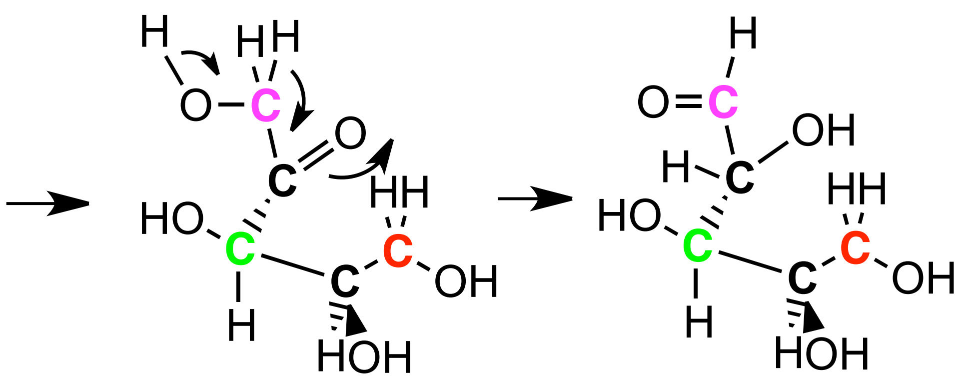
Molybdenum (+6) minerals convert the branched 5-carbon sugar into ribose



from borate-moderated formose process (Kim et al., 2011)

Bilik reaction catalyxed by Mo(6+)



ribulose

ribose

pH 6-7, minutes, *no tar*.
This kind of result surprises carbohydrate chemists.

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Alkaline borate is in igneous rocks

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Peridot in basalt

generates the base

Tourmaline (borate)

Death Valley

Evaporite minerals saturated borate

Colemanite= boron, calcium, oxygen, hydrogen

Borate excluded from silicates, comes to surface in igneous rocks, easily weathered, most salts water soluble, collect in dry basins. Peridot in basalt weathers to creates alkali.

--> Borate moderated formose

Wulfenite (molybdate)

NASA

We make (and sell) "origins of life" jewelry

Apatite (phosphate)
Tourmaline (borate)
Peridot (alkali)
Wulfenite (molybdate)
Citrine (chirality)
Black diamond (carbon)

Orders to:
Kellie Rucker
krucker@ffame.org

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Five paradoxes in the RNA-first model for life's origins

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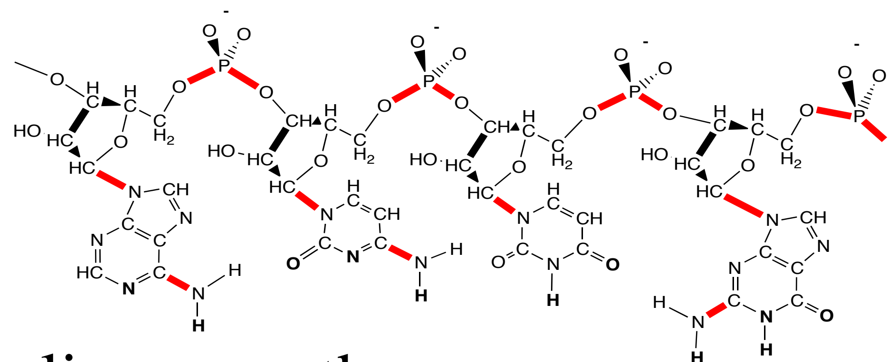
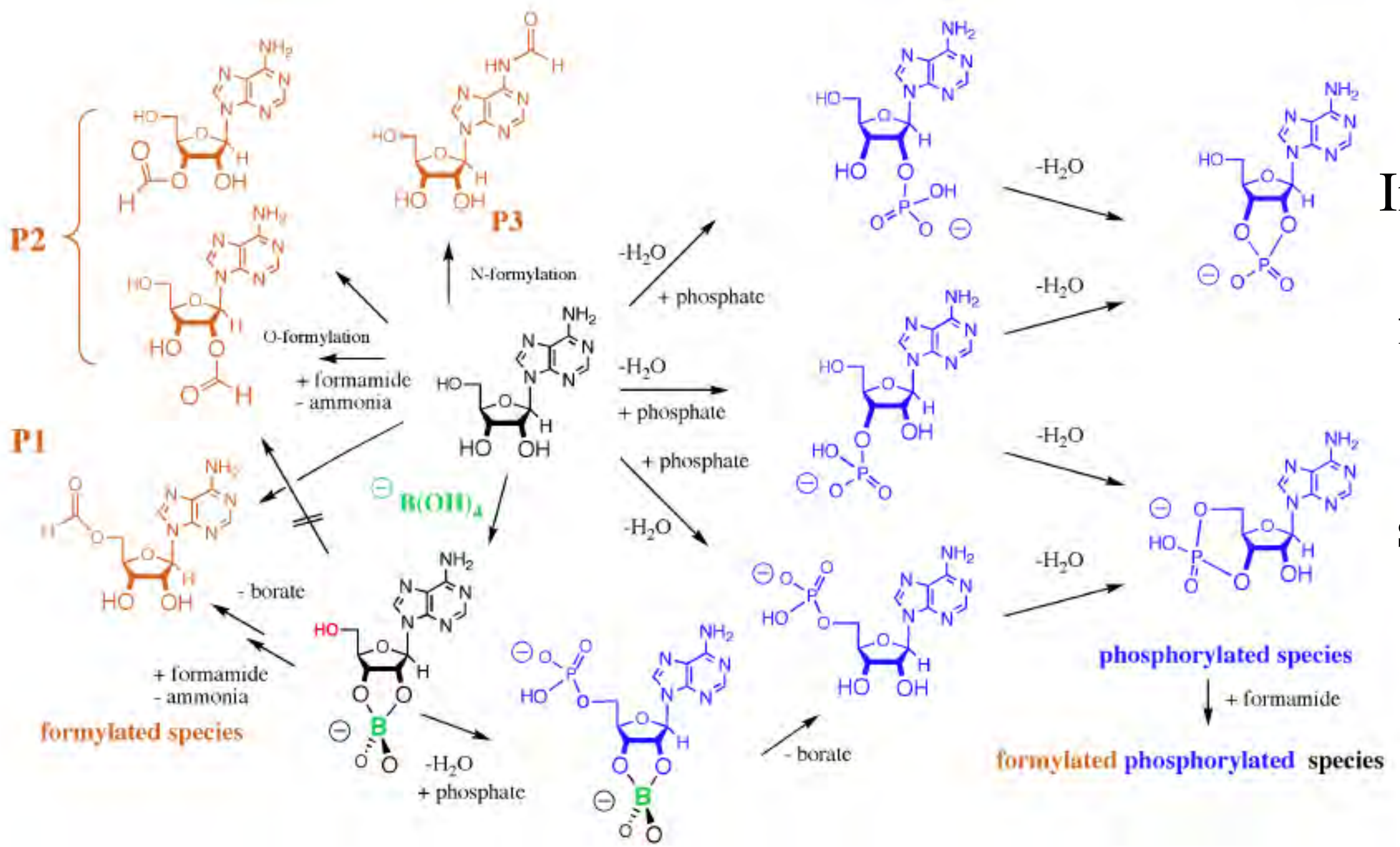
- Tar paradox: Give energy to organic matter but no access to Darwinian evolution, one gets tar, not RNA building units. *Mineral interactions can manage this problem.*

- Oligomer entropy paradox. Even if we get units, high concentrations needed to get oligomers.



- Water paradox. Even if we get oligomers, they are hard to make in water and, if made, are destroyed by water.
- Single biopolymer paradox. Even if we get the oligomers, the demands for catalysis contradict those for genetics.
- Even if RNA is a good compromise for catalysis and genetics, destructive catalysts >> productive catalysts.

The desert helps solve the water paradox



Intermittant water; formamide as an alternative solvent; bp >220 °C

Furukawa, Y., Kim, H.-J., Hutter, D., Benner, S. (2015) Regio-phosphorylation in borate-formamide. *Astrobiology* **15**, 259-267

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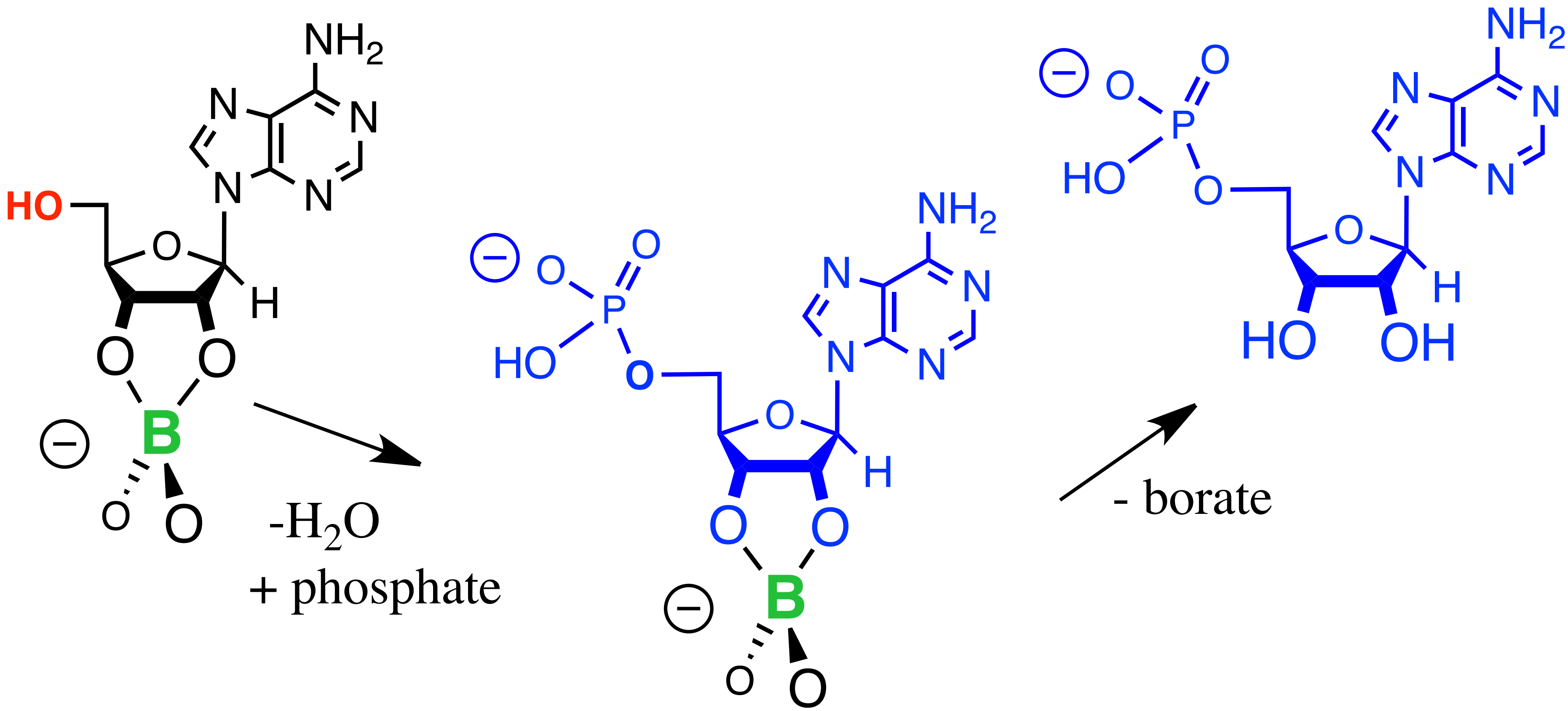
Solvent is formamide
Borate minerals control where phosphate goes



Journal of Cosmology, Vol. 25, No. 19, pp 13730-13776.

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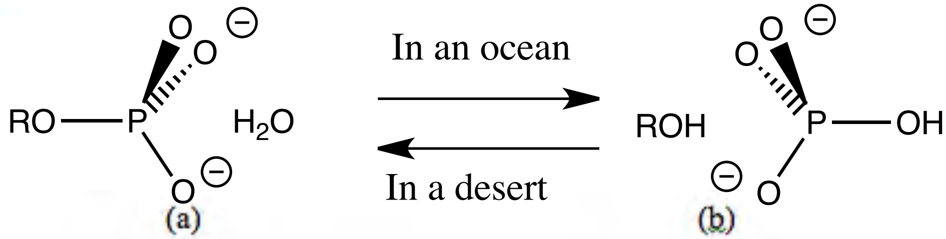
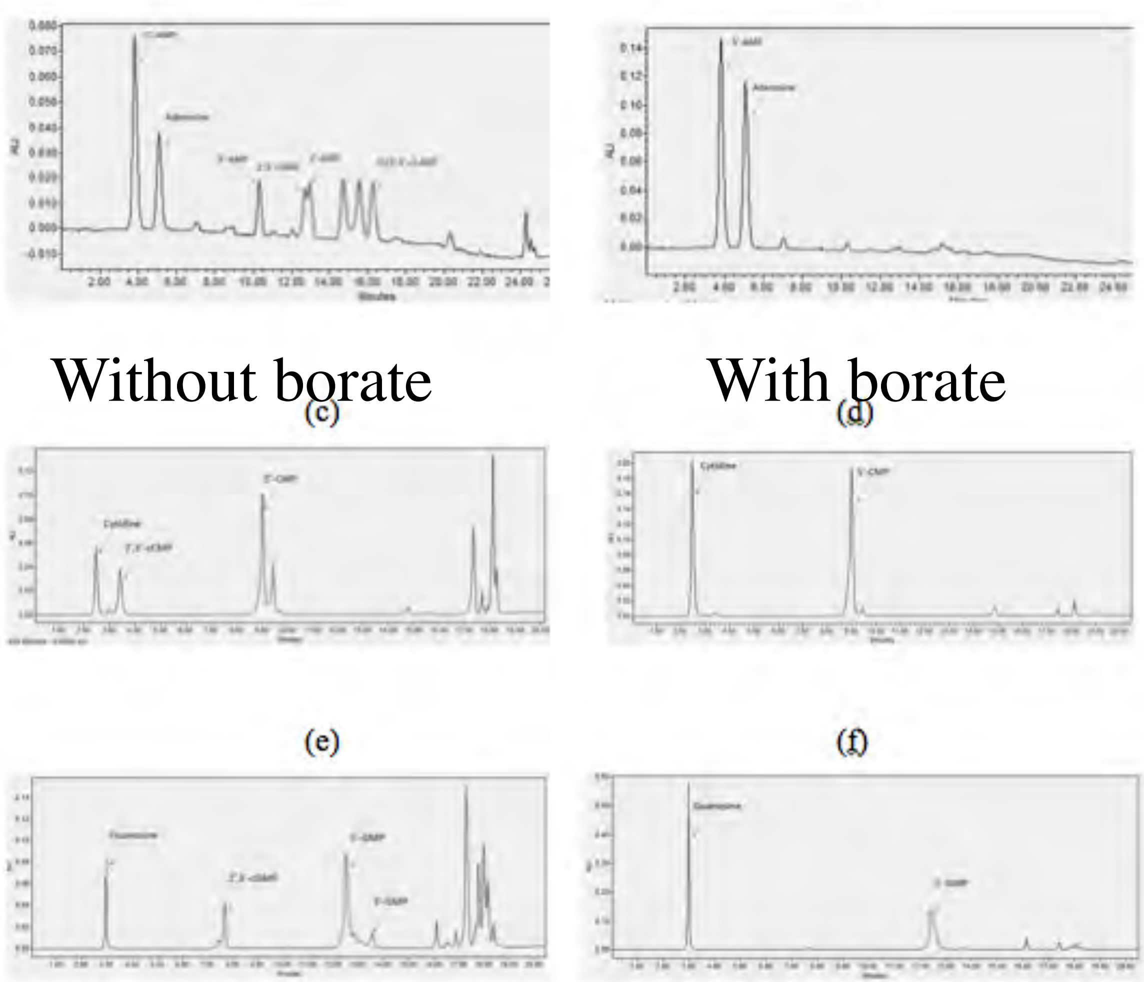
Make hydrolytically unstable phosphate esters in deserts





Without borate

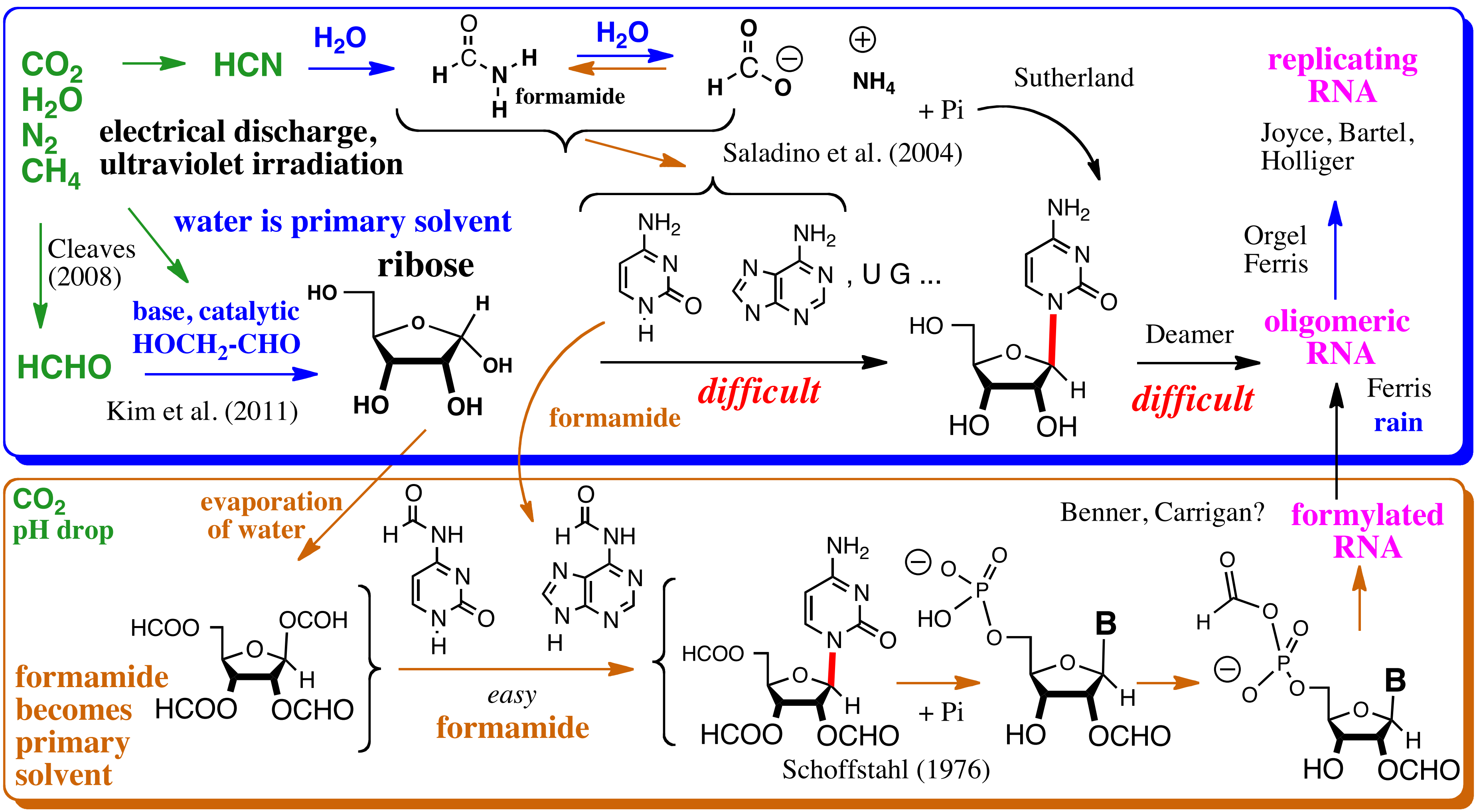
With borate

Hyo-Joong Kim

Need an alternative solvent; formamide or urea (from cyanide and cyanate)

Discontinuous Model for RNA synthesis

Every step has experimental support



Key: in the atmosphere — in the aquifer; Nucleophilic Context — in the desert; Electrophilic Context

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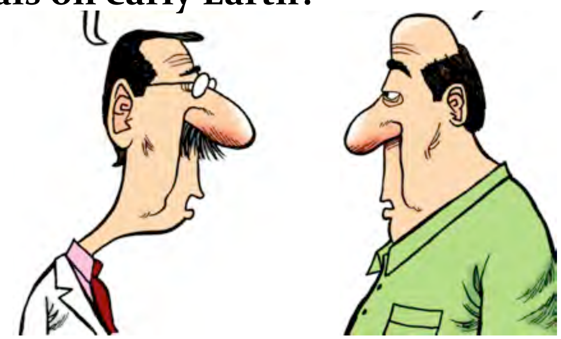
The chemistry is driving the geology

Hazen & Grew: Cannot have borates on early Earth; Too young for the lithosphere to have churned to concentrate scarce borate (2010).

Benner. Why not concentrate borate in the hydrosphere? (2012)

Van Kranendonk found tourmalines with borate in 3.5 Ga rocks (2014)



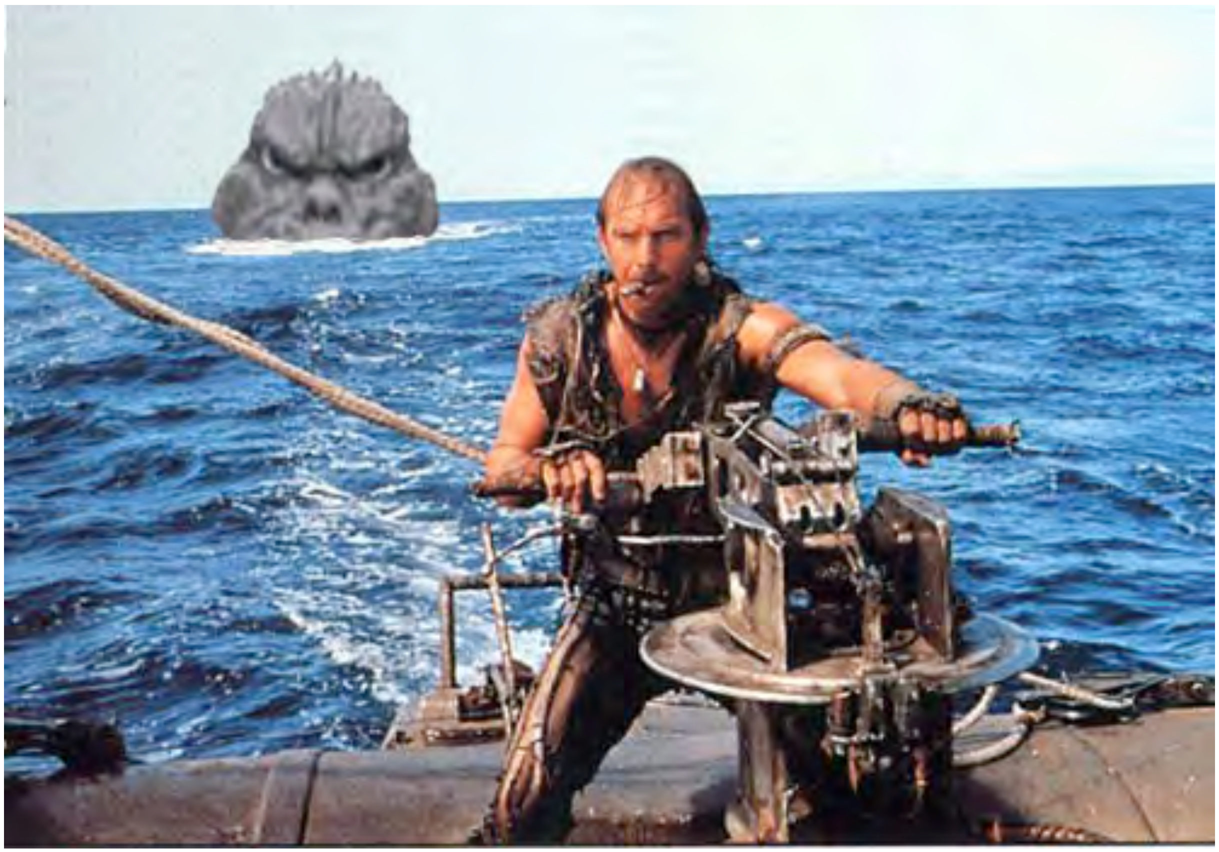
Tourmalines, colemanite, and other borate minerals are formed only if scarce boron is enriched

Molybdates are highly oxidized

But is Earth sufficiently oxidized to have molybdenum +6?

Kirschvink. The Discontinuous Synthesis of RNA is impossible on early Earth

Models for planetary formation suggest that the inventory of water on early Earth did not leave *any* dry land before continental drift.

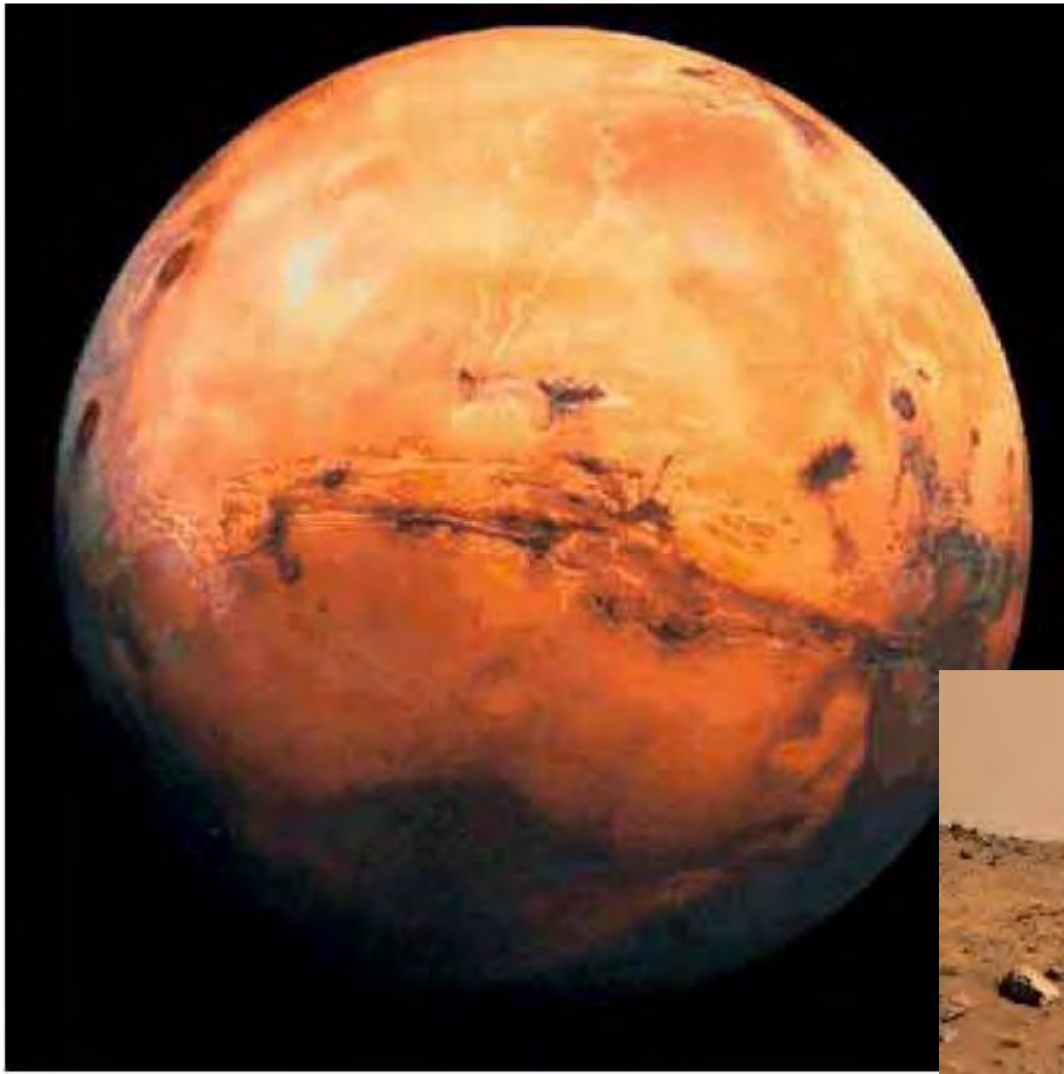


Waterworld

Scarce borate and molybdate diluted into a global ocean; huge tides inundate low lying land every four hours

But Mars has always had less water

Intermittant water.
More oxidized?
More molybdate?
Borate concentrated?



Life originated on Mars?

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The Kirschvink Modification of the Benner Model

Move it to Mars, where water was never as abundant and oxidation level has always been higher. *Perhaps borate-sugars there today*.

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A logic tree

RNA first?

No — Yes

No: A different set of problems

Yes: Need to make RNA prebiotically

Benner's stupid chemistry?

No — Yes

No: Get your own alternative

Yes: Need oxidized minerals, a desert

Can we have these on early Earth?

No — Yes

No: **We are all Martians**

Semi-continuous Synthesis Model

$$CO_2 + N_2 + H_2O$$

HCHO HCN H_2O Glycolaldehyde, formamide

A desert on Earth?
A valley on Mars?

Hyo-Joong Kim, Marc Neveu

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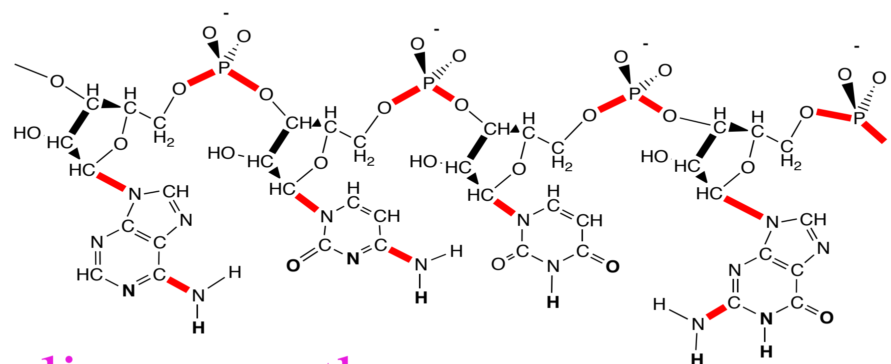

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- Single biopolymer paradox. Even if we get the oligomers, the demands for catalysis contradict those for genetics.
- Even if RNA is a good compromise for catalysis and genetics, destructive catalysts >> productive catalysts.

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We are not out of the woods

A catalytic biopolymer must *fold*, to surround a substrate with catalytically useful groups.
A genetic biopolymer must *not* fold, so that it can template the formation of its complement.

A catalytic biopolymer must have *many* building units, so as to have all of the functional groups needed for catalysis
A genetic biopolymer must have *few* building units, so as to have all of the functional groups needed for catalysis

A catalytic biopolymer must have *rich* catalytic power.
A genetic biopolymer must *not* have rich catalytic power, as the easiest reactions to catalyze are those that destroy the polymer.

People have done laboratory *in vitro* evolution to create RNA molecules that catalyze the destruction of RNA, and RNA molecules that catalyze the template-directed synthesis of RNA

~1 in 10^{14} increase rate of RNA *destruction* by 10^{4}.

~1 in 10^{33} perform the replicase function.

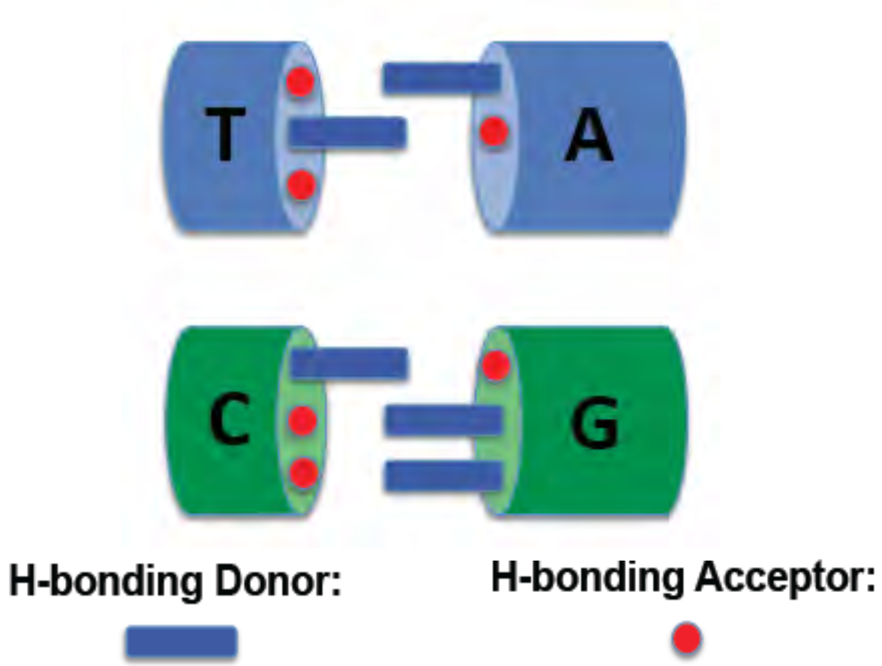
Destruction is 29 orders of magnitude more likely than construction.

All of our work to get RNA prebiotically a waste? But remember, this is *terran* RNA. *This need not be universal RNA.*

Landscape & One Biopolymer Paradoxes

Hard to get desirable function out of xNA.
One also gets undesirable function.

- **A quarter century of *in vitro* selection shows that *functional* xNA molecules are sparse in sequence space. This is not surprising.**
- ***xNA has only four building blocks; few binding/functional groups.***
- **Proteins have 20 building blocks and lots of functionality.**



What if xNA had more building blocks & more functionality? Could we get better xNAzyme catalysis with more control over undesirable catalysis (= "specificity")?

If you think of xNA like LEGO …

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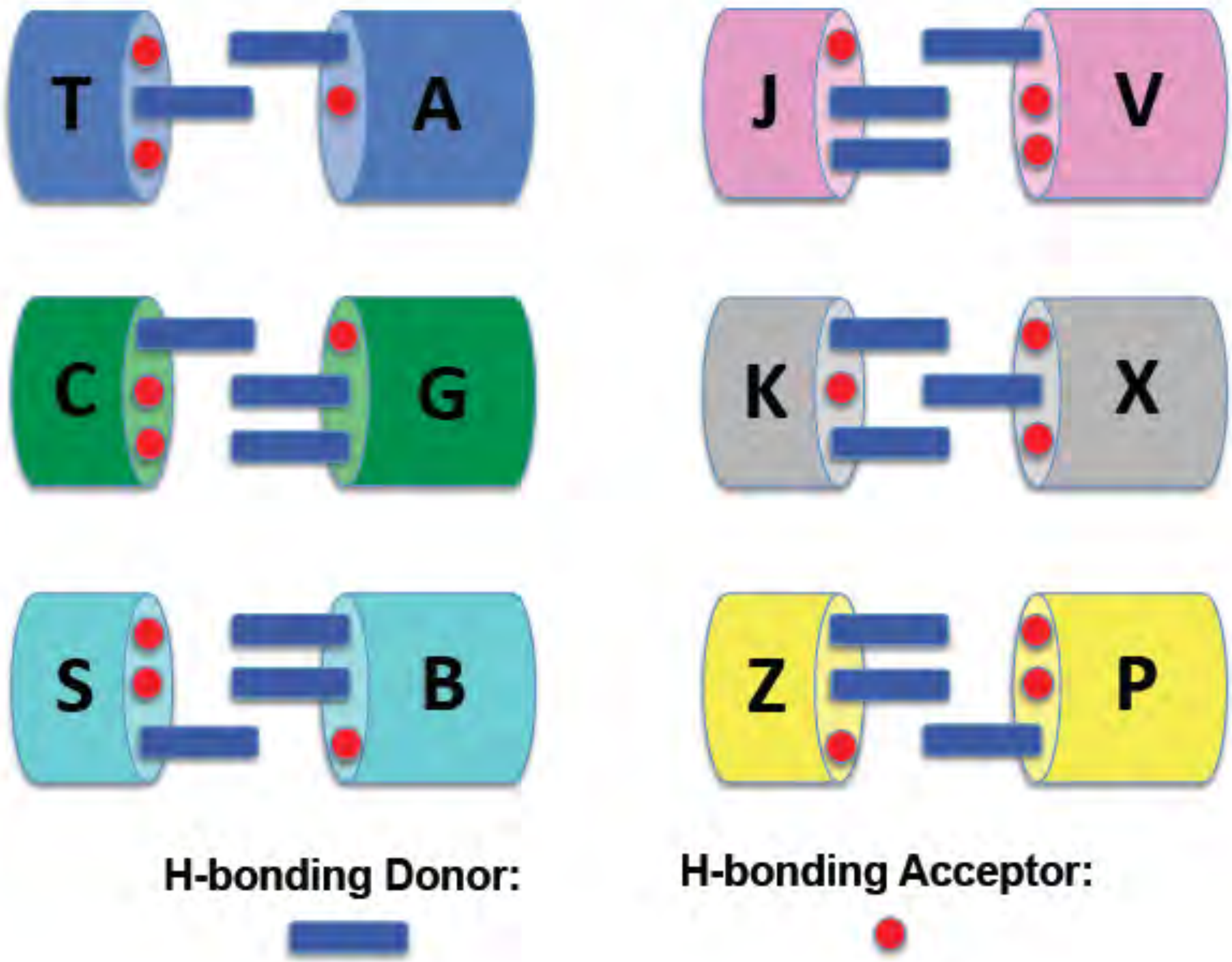
You can get 12 xNA building blocks by shuffling H-bonding units



Artificially Expanded Genetic Information System (AEGIS)

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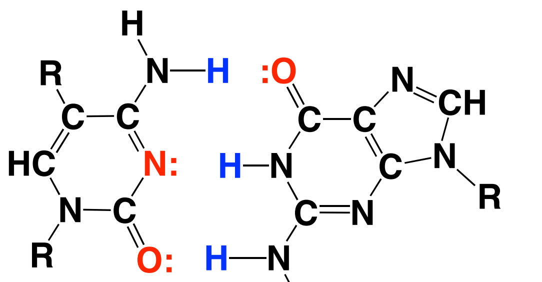
AEGIS in molecular form

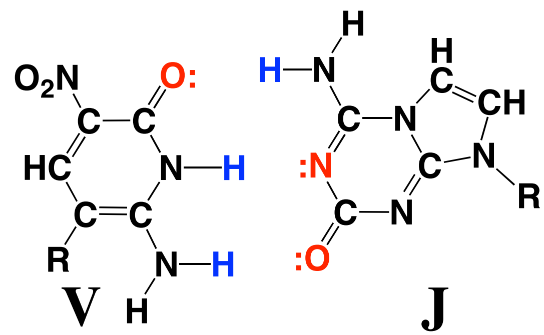
Donor
Acceptor
Acceptor
pyDAA



C G

Acceptor
Donor
Donor
puADD

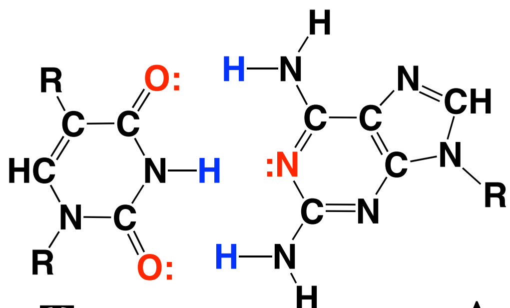
Acceptor
Donor
Donor
pyADD



V J

Donor
Acceptor
Acceptor
puDAA

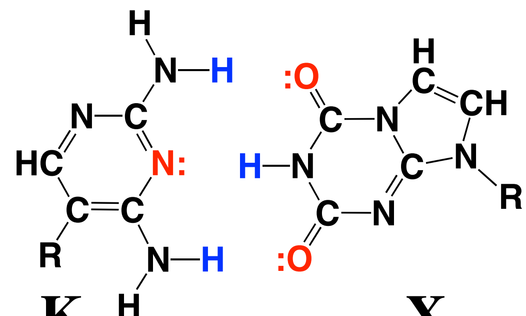
Acceptor
Donor
Acceptor
pyADA



T aminoA

Donor
Acceptor
Donor
puDAD

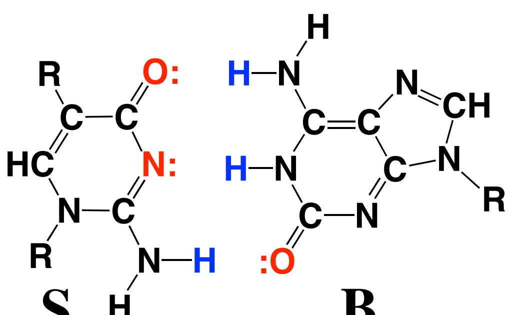
Donor
Acceptor
Donor
pyDAD



K X

Acceptor
Donor
Acceptor
puADA

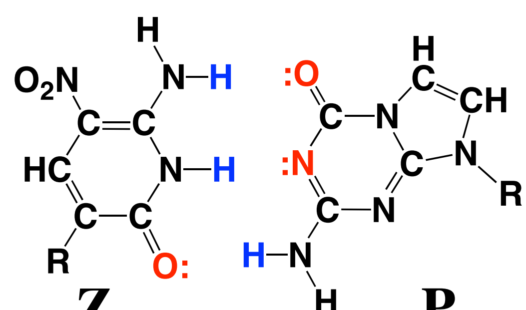
Acceptor
Acceptor
Donor
pyAAD



S B

Donor
Donor
Acceptor
puDDA

Donor
Donor
Acceptor
pyDDA



Z P

Acceptor
Acceptor
Donor
puAAD

Artificially Expanded Genetic Information System (AEGIS)

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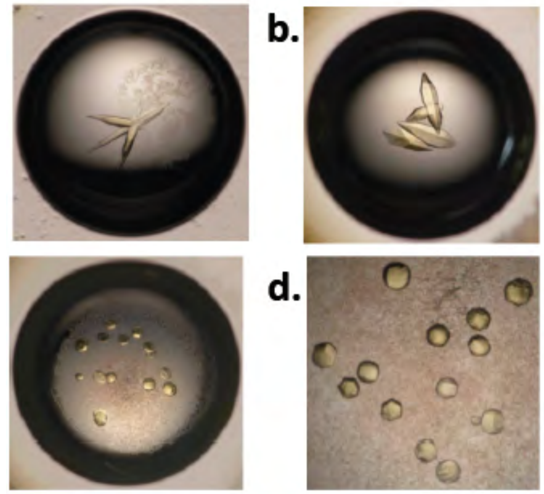
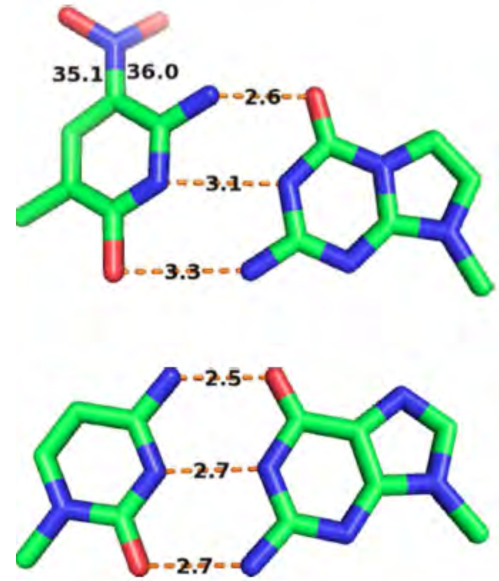
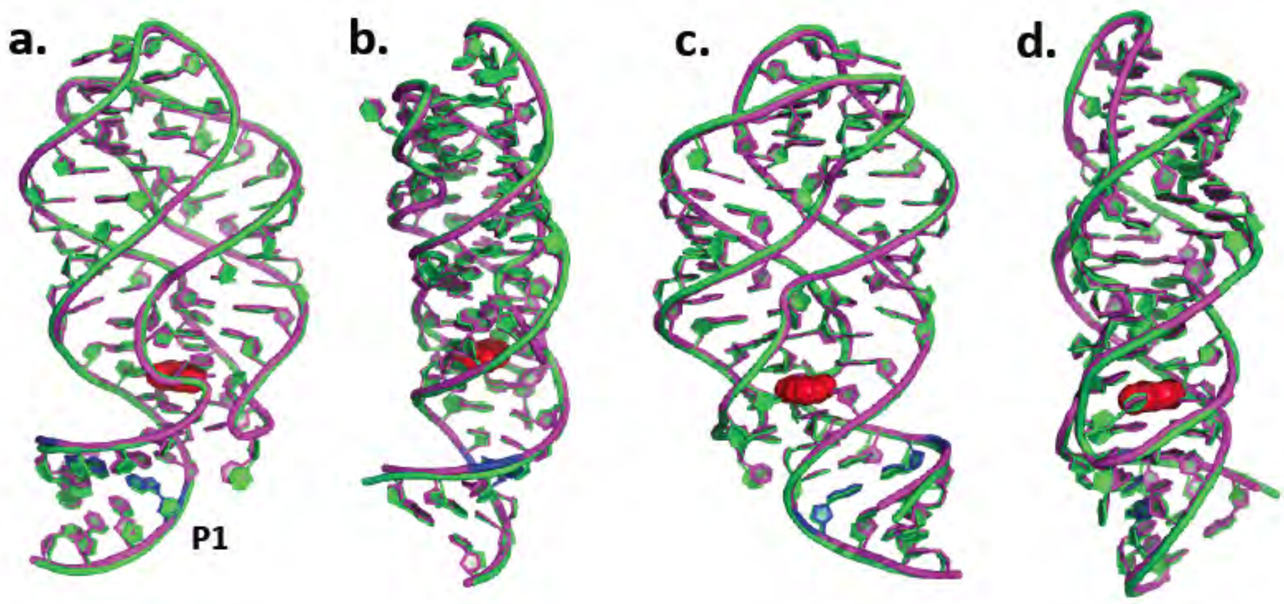
Crystals of AEGIS-pairs, here within the guanosine riboswitch

Joseph Piccirillli
Armando R. Hernandez
University of Chicago

Shuichi Hoshika
Hyo-Joong Kim
Myung-Jung Kim
Elisa Biondi







Green = native
Magenta = ZP
5 nM vs. 3.7 nM

Can AEGIS do Darwinism?

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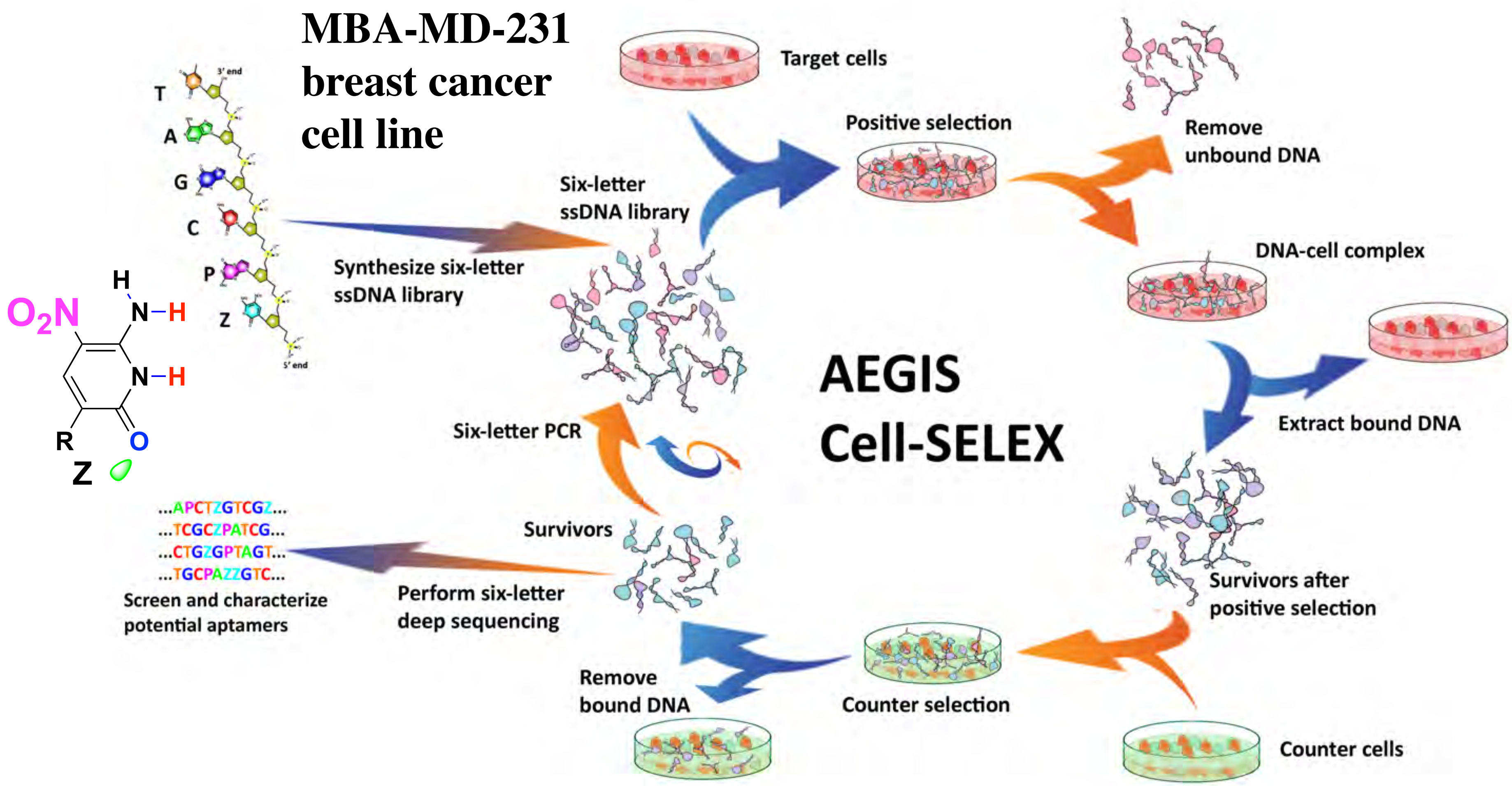
AEGIS Darwinism can give functional xNA (here, aptamers to bind cancer cells)



Sorry. Not relevant to the origin of life, but we don't have $$$ from the Simon Foundation or Harry Lonsdale. No bucks, no Buck Rogers.

Kwame Sefah, Zunyi Yang, Liqin Zhang, Weihong Tan

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xNA molecules that bind to liver cancer cells do indeed arise

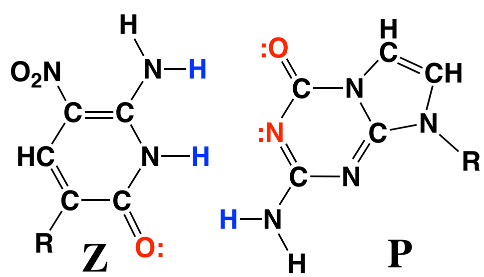
Dissociation constants range from 35 nM to 250 nM

Name	Sequence	K_{diss} (nM)	Error ±	% in pop
LZR1	~CGCCCACGGAAGAGTCTCTGCGGCC~	83	± 23	1.35
LZR2	~CCAACCTGCGACCCACAACCCTATG~	35	± 10	1.27
LZR3	~TTGCGCATGCCACCACCTACCAGGC~	52	± 12	1.25
LZR4	~GTGCGGCCACCATACCCTCCTGGGC~	53	± 6	0.91
LZR5	~TCCCTACATGCGAGTACCACCCCTG~	65	± 15	0.74
LZR6	~CCACCTAAGCTCTGGTTTCCCGTGG~	239	± 187	0.54

What about the rest of the population?

Where is the Z?

Where is the P?



DTRA

DEFENSE THREAT REDUCTION AGENCY · UNITED STATES OF AMERICA

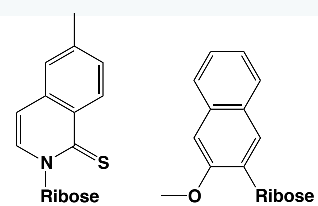
Rest of the population is in xNA aptamers containing AEGIS Z and P

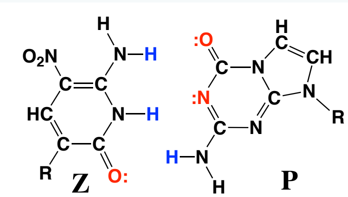
Over half have dissociation constants < 30 nM

Name	Sequence	K_{diss} nM	Error ±	% in pop
LZZP02	~CAATAATTCTPGCZGCGGTATTGGG~	8	± 1	23.0%
LZZP03	~CGACCZGACTTTTAGCPTCGAATAG~	6	± 1	7.84%
LZZP04	~TATCAGCCCGATTTAACTCPZATGG~	29	± 9	2.15%
LZZP05	~GCTACPTGGGCCCTGGTPTCTGTGC~	44	± 2	0.66%
LZZP06	~TATTAGTACGGCTTAACCCPCATGG~	23	± 8	0.65%
LZZP07	~GGATAAGTCTPACZGPGGTATCATG~	12	± 8	0.50%
LZZP08	~CCAATAAATCTPGCZGPGGTATCGG~	6	± 1	0.40%
LZZP09	~GGAAGTGACGGTAGCPTTTTGGAGG~	26	± 3	0.28%
LZZP10	~CGCCCGCZGAGCAGGPCCCCCCCCG-	283	± 16	0.27%
LZZP11	~CGGCTTGACAGACPGCATZGATCAG~	201	± 142	0.14%

Never see adjacency with Romesberg or Hirao bases

Their structures are too weird





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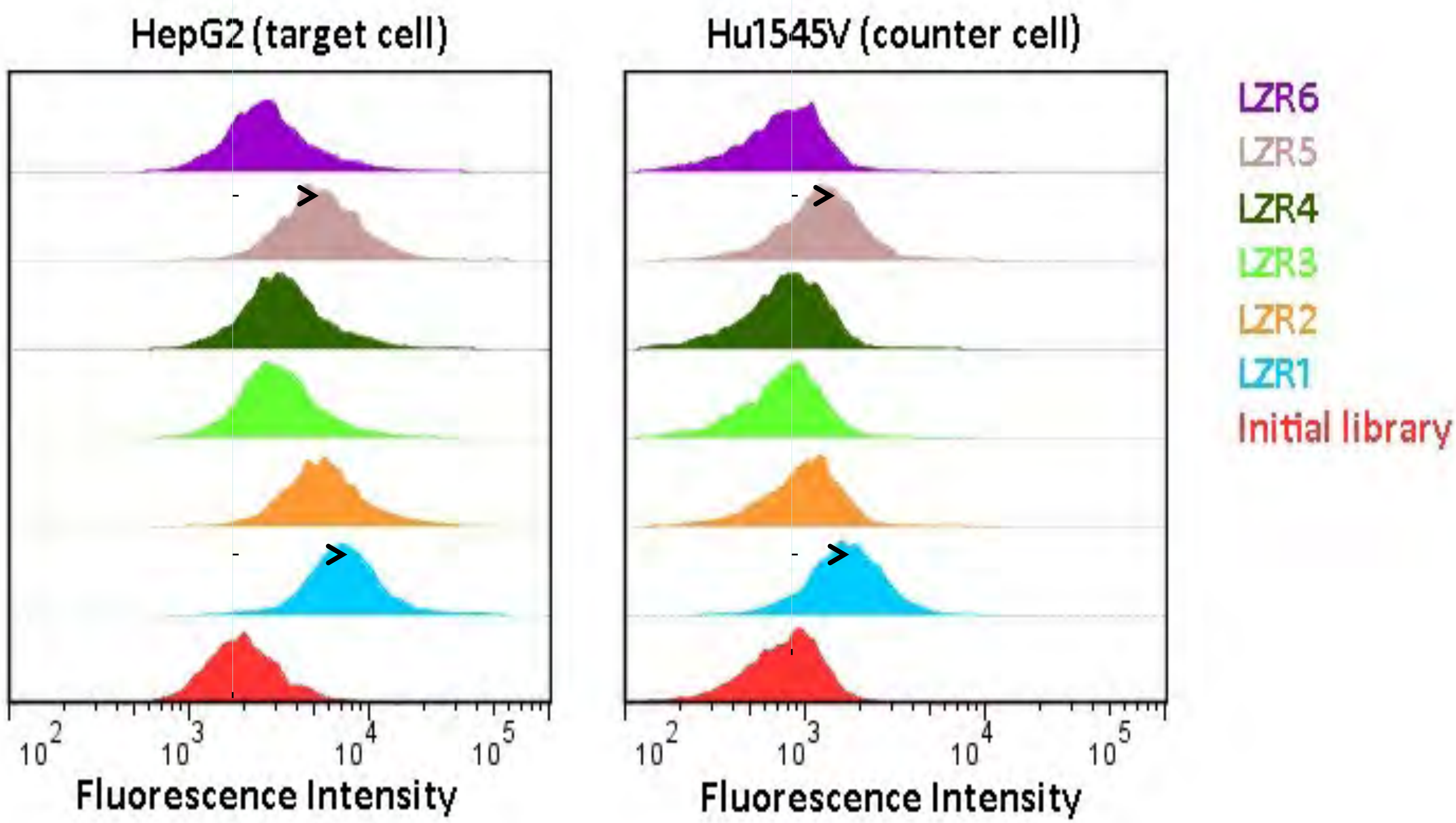
Selectivity

We counter-selected against normal liver cells
Cell sorting FACS shows some (imperfect) specificity



Certainly selection gave standard aptamers with some selectivity

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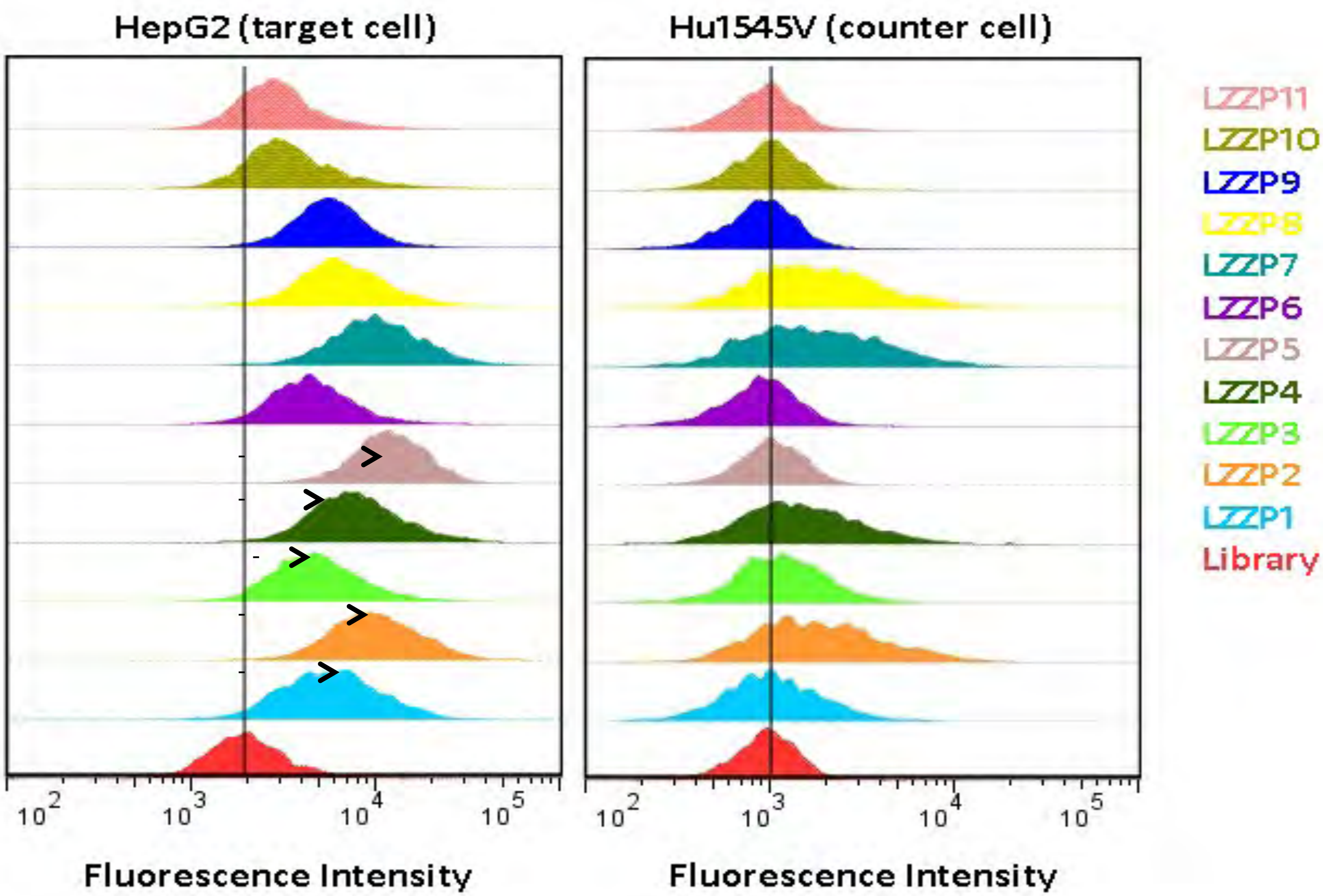
Selectivity is better with 6-letters

Aptamers with AEGIS bind better, are more selective



AEGIS: More of what you want; *less of what you do not want*

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AEGIS= More of what you want. Less of what you do not want (= specificity)

(e) Landscape Paradox. Even if we find a biopolymer (RNA?) that manages the contrasting needs of catalysis and genetics, data suggest that **xNA enzymes that *degrade* RNA** arise much more frequently than xNA enzymes that *make* RNA.

(d) One Biopolymer Paradox. Even if biopolymers did emerge prebiotically, it is not clear that one biopolymer can manage apparently contradictory demands of genetics and metabolism.

DTRA

My dinner with Jack, December 10, 1985

Jack Szostak

Andy Ellington

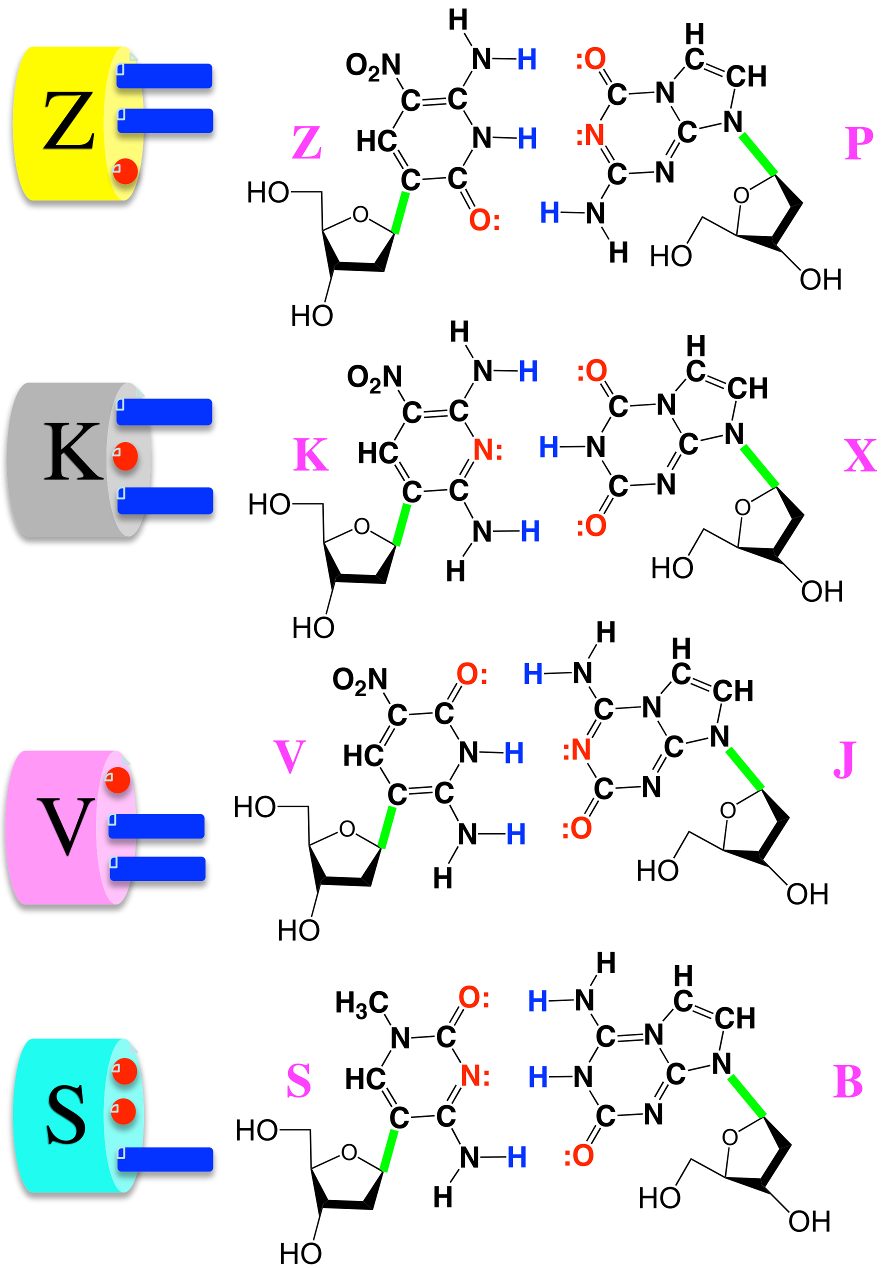
We both wanted catalysts, ligands, and receptors from nucleic acids.

Steve said: Selection with standard RNA/DNA will not yield a rich diversity of catalysis, as the four standard nucleotides do not have enough functional group diversity.

Jack said: You will take a decade to expand the genetic alphabet, and another decade to get polymerases to accept the extra nucleotides.

Prebiotic relevance??

Cannot make AEGIS components prebiotically??? They are C-glycosides



But (as John Sutherland emphasizes), we also cannot easily make N-glycosides.

Following some experiments from Nick Hud, Hyo-Joong Kim had an idea…

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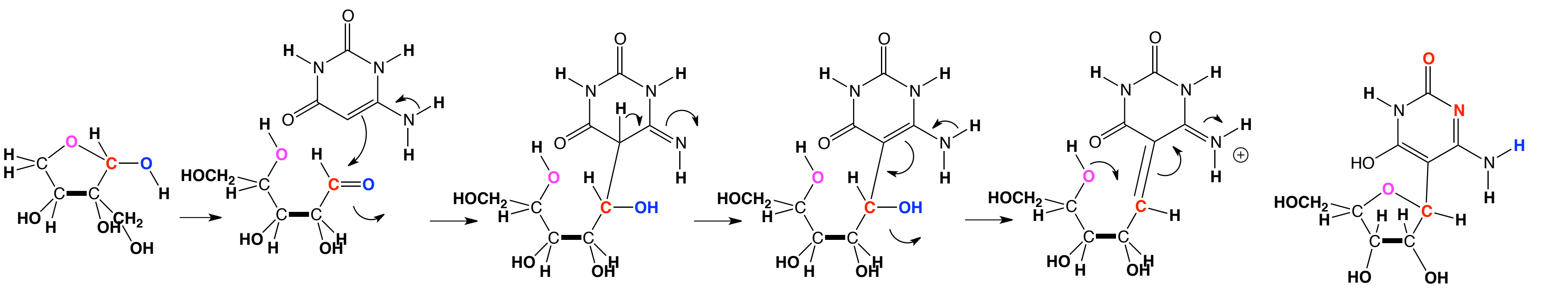
C-glycosides form in water if the base is nucleophilic enough

Hyo-Joong Kim's poster



(a) Asphalt Paradox. Organic molecules, absent biology, inherently devolve into “asphalts”, before they give Darwinian molecular systems.
(b) Water Paradox: Even if devolution is avoided, many bonds in Darwinian biopolymers are thermodynamically unstable in water.
This is not so with the C-glycoside bond.

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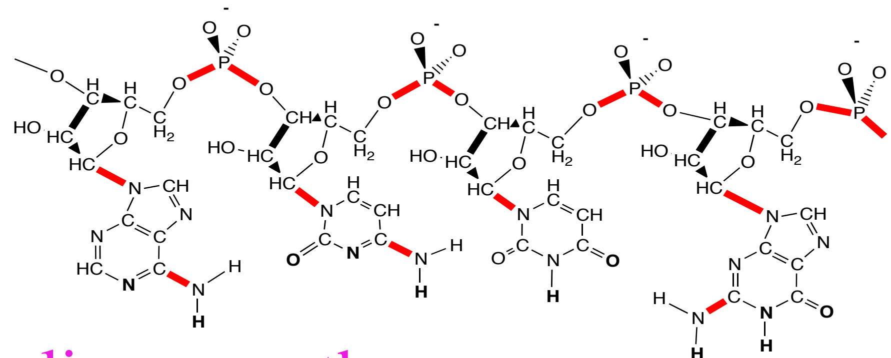
Five paradoxes in the RNA-first model for life's origins

- Tar paradox: Give energy to organic matter but no access to Darwinian evolution, one gets tar, not RNA building units. *Mineral interactions can manage this problem.*
- Oligomer entropy paradox. Even if we get units, high concentrations needed to get oligomers.



- Water paradox. Even if we get oligomers, they are hard to make in water and, if made, are destroyed by water.
- Single biopolymer paradox. Even if we get the oligomers, the demands for catalysis contradict those for genetics.
- Even if RNA is a good compromise for catalysis and genetics, destructive catalysts >> productive catalysts.

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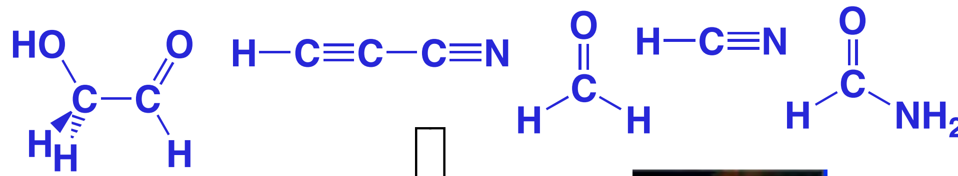
Work on Origins at the FfAME

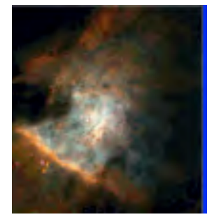
Prebiotic Chemistry



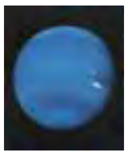
interstellar organics

Ricardo *et al*. (2004) *Science* **303**, 196

forward in time



Benner (2004) *Acc. Chem. Res.* **37**, 784-797



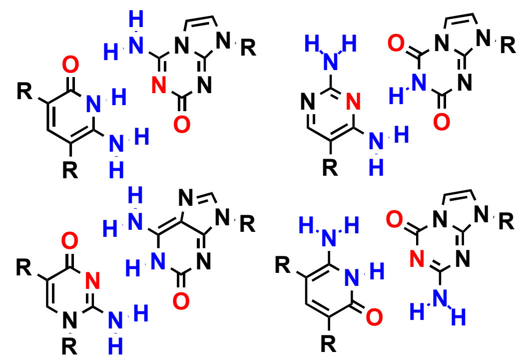
A path to the simplest first life

understand better the possibilities

an independent genesis?

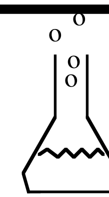
First system to support Darwin-ian evolution

Construct alternatives in the lab



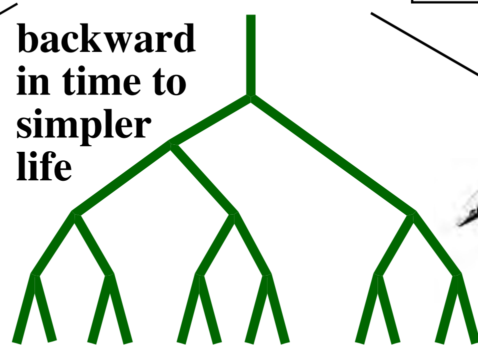
Explore Solar System

Synthetic biology



Baross, Benner, et al. (2007) *Limits of Organic Life in Planetary Systems*

backward in time to simpler life



infer ancestral life forms; resurrect for laboratory study

Eucarya Archaea Bacteria

Paleogenetics

Benner *et al*. (2007) *Adv. Enzymol. Mol. Biol. Protein Evol*. **75**, 1-132

The Benner organic chemistry is brilliant, and was probably quite relevant to the evolution of life in the organic soup of ~ 10^80 dark matter planets that formed the cosmic soup 2 Myr after the big bang; soon after the dark matter planets fragmented in Jeans mass clumps of a trillion at the plasma to gas transition at 10^13 seconds (300,000 years).