

Paradoxes of molecular biosignatures

Molecular systems that cannot possibly arise *without* life

The paradox: Such a life form could never arise.

For example, amino acids are bad biosignatures, as they can arise abiologically

Even enantiomerically enriched amino acids seem to arise without life (Sandra Pizzarello)

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The paradox is naively formulated

The (in)organic molecules are formed universally

We then look for Darwinian capability within them

A biopolymer requires homochiral building blocks to be evolvable. Further, it must be build from a controlled library.

What does Darwinism need by way of a genetic molecule?

- (a) A one-dimensional biopolymer*, that...
- (b) can change its structure to change its information, but ...
- (c) any changes in the structure of the biopolymer *cannot* change its physical chemical behavior substantially, e.g.:
its solubility, its molecular recognition, reactivity

* We have tried to assemble a two dimensional Cairns-Smith life form; so far no luck.

Data show that such systems are scarce ...

Proteins, polysaccharides, most every other class of molecules, including abiological polymers, physical behavior and reactivity change dramatically even with small sequence changes.

Sickle cell hemoglobin. Change one lousy amino acid in 576, and the protein precipitates.



**If DNA/RNA precipitated every
time a nucleotide changed,
evolution would grind to a halt**

**A genetic biopolymer must have fairly constant properties
if it is to support Darwinism, where it is replicated with
imperfections, with the imperfections themselves replicable**

Pretty much true with DNA and RNA

Pretty much every DNA/RNA sequence dissolves in water

Pretty much every DNA/RNA sequence binds its complement

Pretty much every DNA/RNA sequence precipitates in ethanol

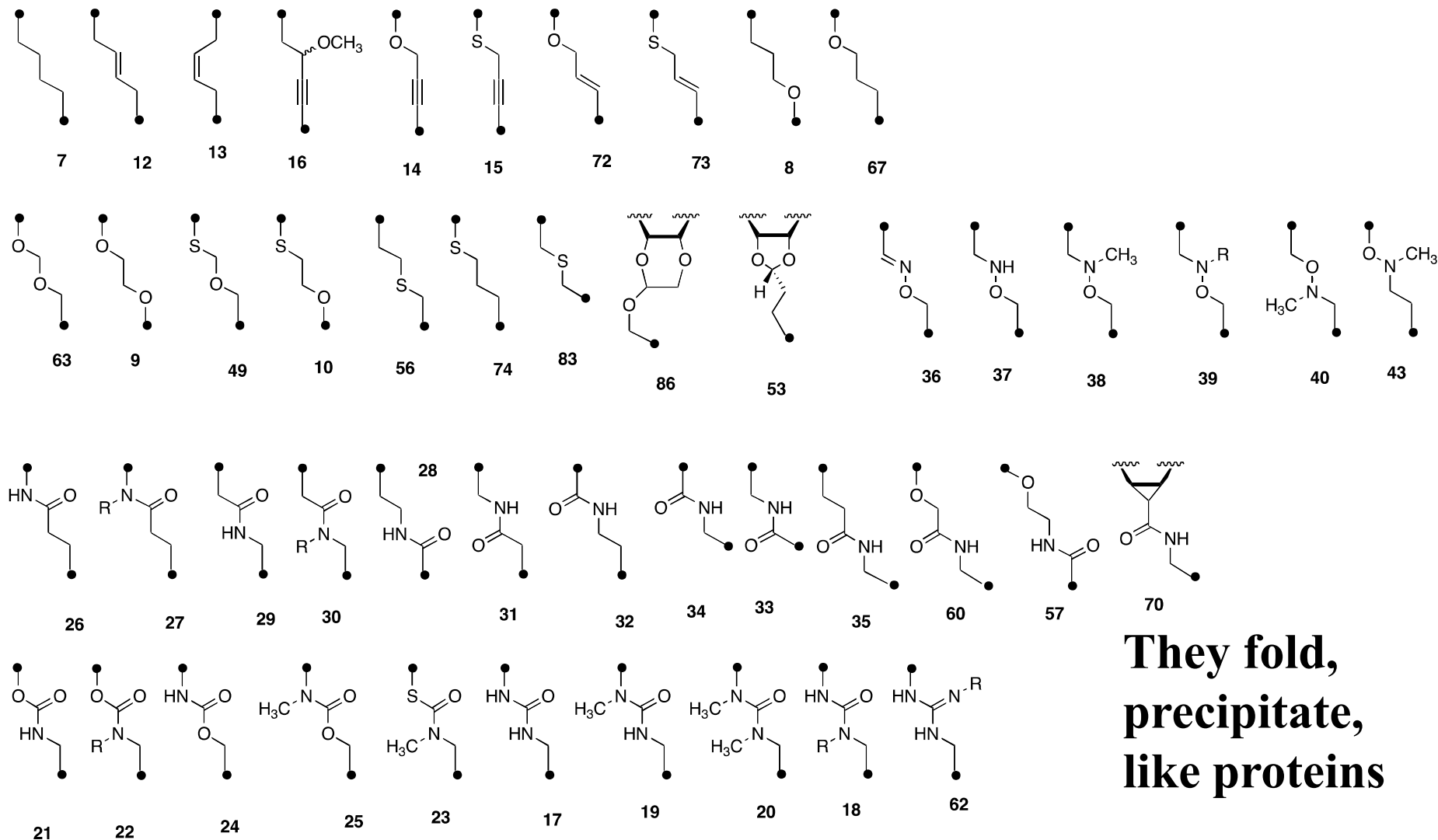
Pretty much every DNA/RNA sequence templates polymerases

What is it about the structure of DNA/RNA ?

Proteins have a repeating backbone dipole;

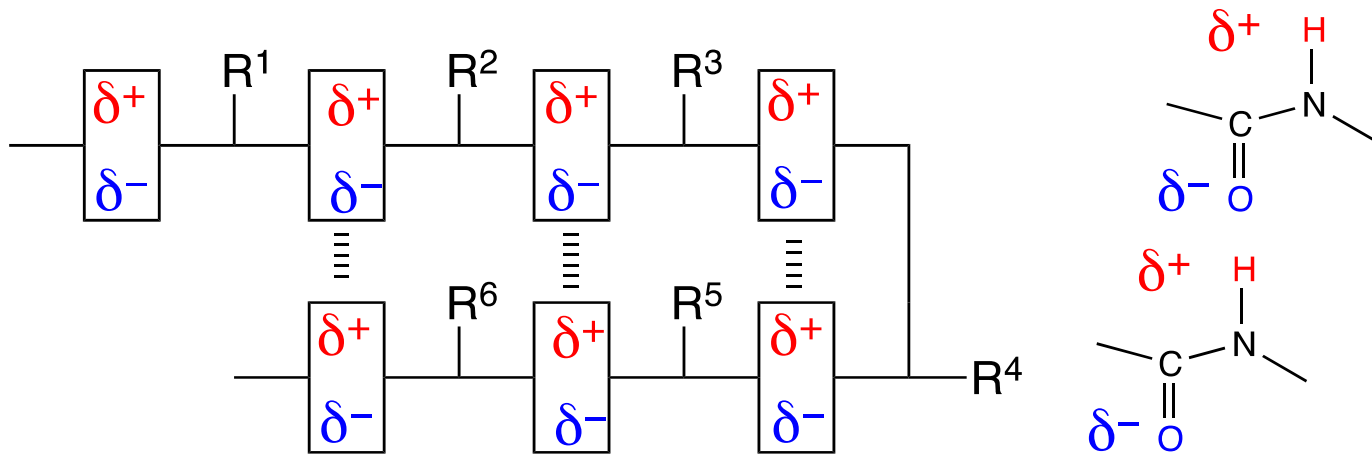
DNA has a repeating backbone monopole.

Many DNA analogs without the backbone charge have been synthesized



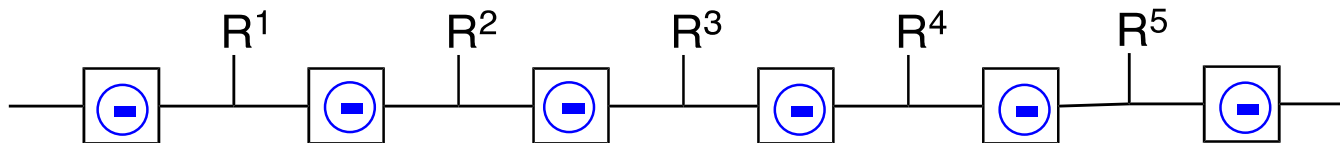
**They fold,
precipitate,
like proteins**

Compare peptides, with repeating dipole, to DNA/RNA, with a repeating monopole



**Think of
magnets
on a
string**

(above) A backbone with a repeating dipole easily folds
(below) A backbone with a repeating charge extends to template



Polyelectrolyte backbone prevents folding, allows templating.

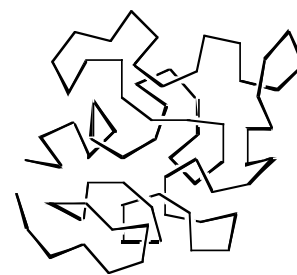
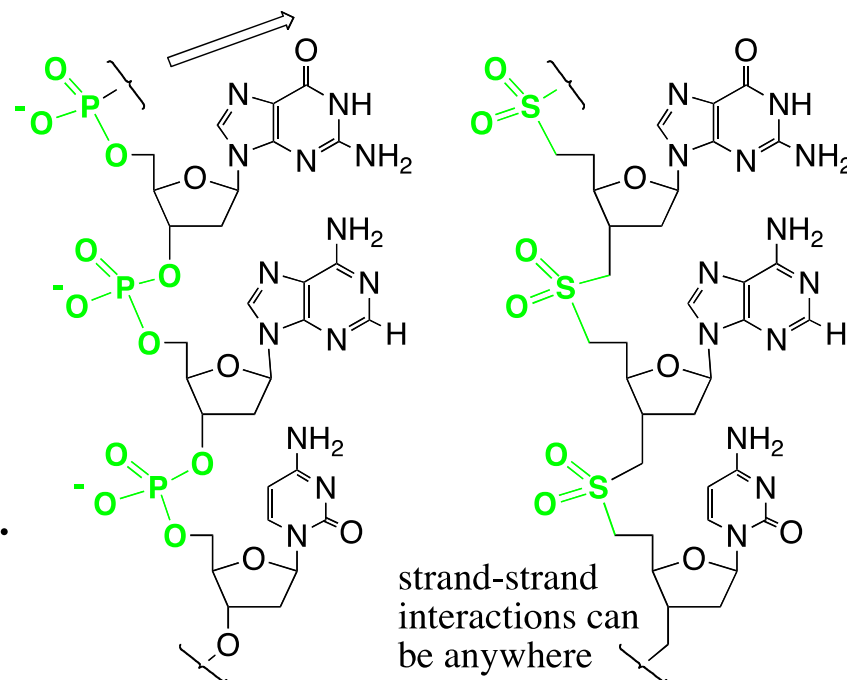
= polyelectrolytes are soluble in water.

Why genetic systems in water must have a polyelectrolyte backbone

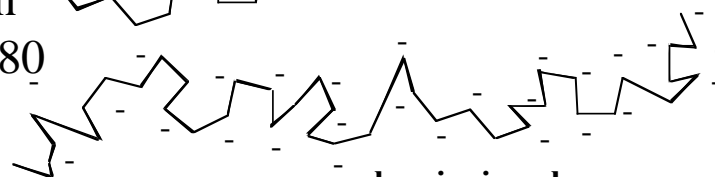
1. Keeps DNA soluble in water.
2. Backbone-backbone coulombic interactions force strand-strand contacts to Watson-Crick edges of the nucleobases (= rules).
3. Repeating charges discourages folding; “excluded volume” effect.
4. Repeating charge dominates the molecule’s properties, allowing mutation to occur without changing the bulk properties of the molecule

Benner, S. A., Hutter, D. (2002) Phosphates, DNA, and the search for nonterrean life. Second generation model for genetic molecules. *Bioorg. Chem.* **30**, 62-80

Polyelectrolyte Theory of the Gene
True for all life in water

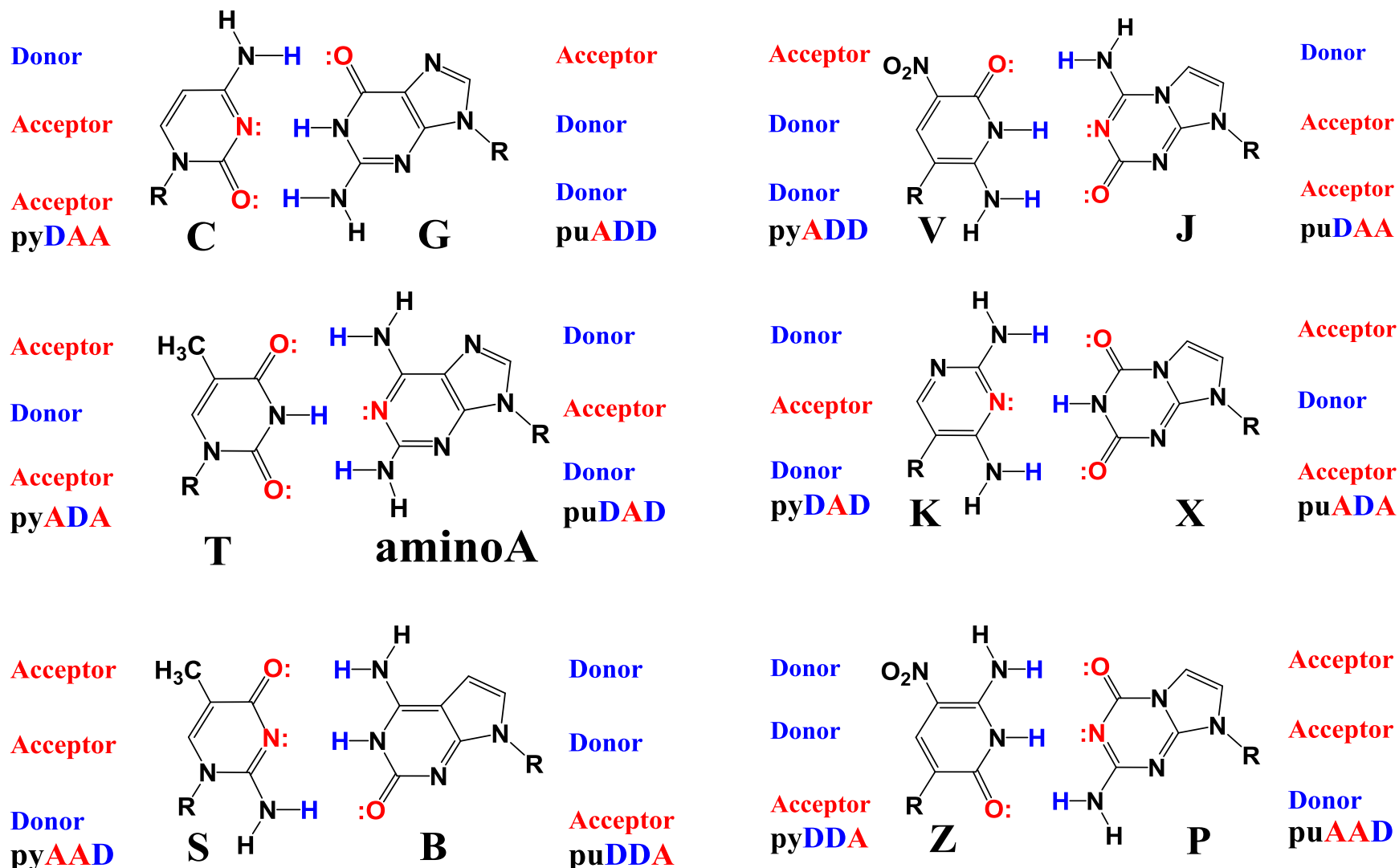


neutral polymer
Radius = length^(1/2)



polyanionic polymer
Radius = length^{>>(1/2)}

We can change the bases and retain Darwinism ...



But we cannot change the backbone charges. A universal.

Polyelectrolytes make excellent universal biosignatures

1. **Universal genetic biopolymers in water have repeating charges**
 2. This allows them to be easily concentrated from plumes, bulk water
 3. Darwinian biopolymers having this features are easily distinguished
- We can today detect extant life in H₂O *in situ*, terran & weird,**
4. we still lack a clear path to get RNA as the first terran Darwinian biopolymer, but it seems that we *will* find those paths.
 5. These paths are defeated by dilution into a global ocean
 6. Defeat is easily avoided in deserts with occasional water (e.g. Mars)

And non-aqueous life? An analogous universal biosignature?

7. Efforts to get life in cryosolvents defeated by solubility issues
8. Warm Titans, exotic polar solvents, still possible

Disequilibrium and the impotence of Darwinism

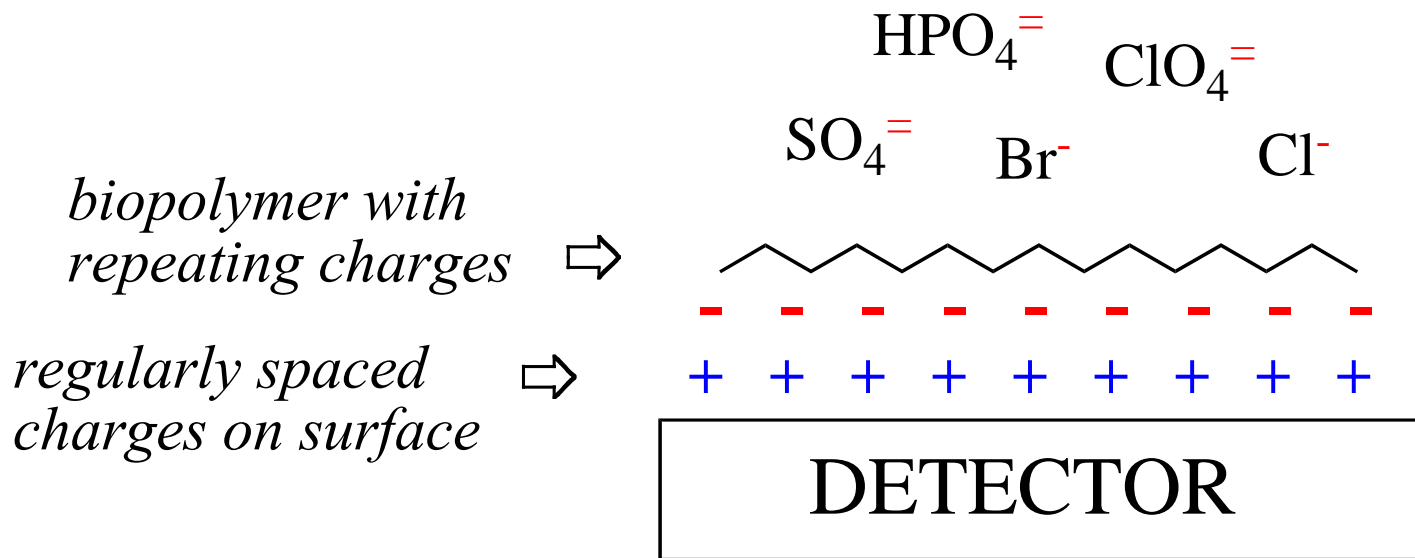
9. Smart life will escape Darwinism to gain Lamarckianism



Polyanions are easily captured dilute water on polycation supports

This is for a *universal* genetic biopolymer

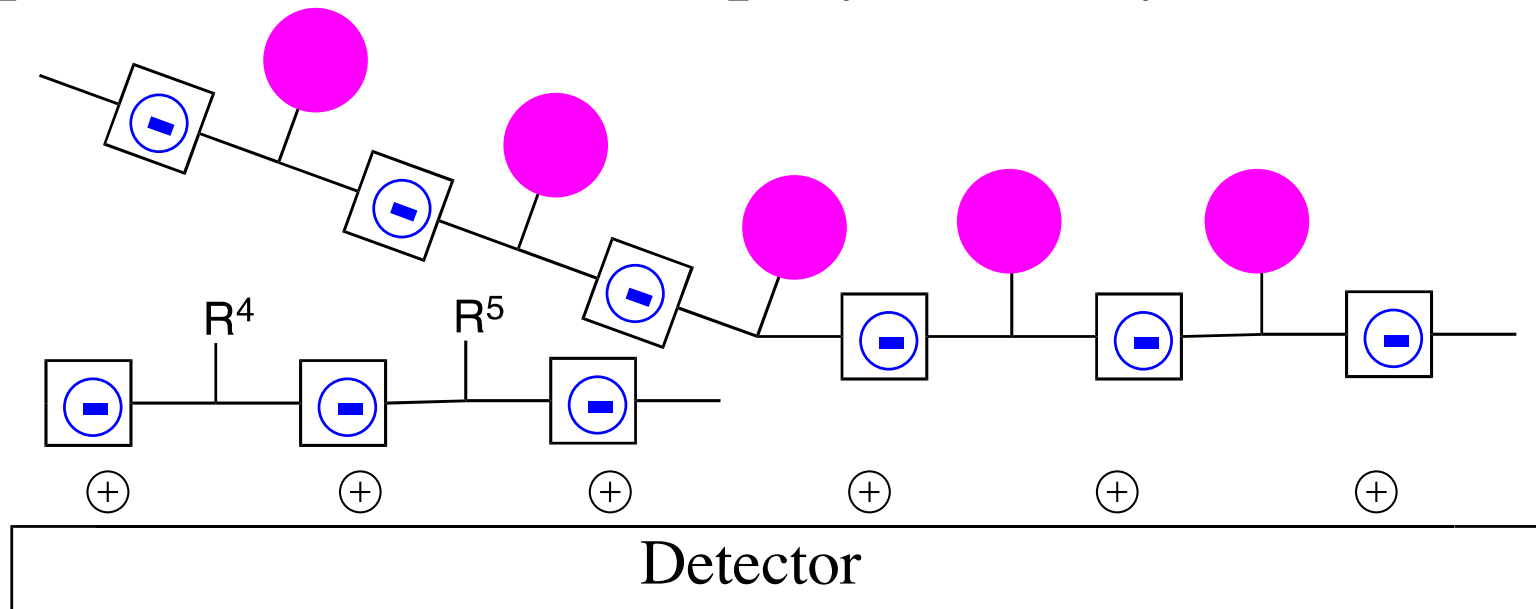
Polyelectrolytes bind in competition with simple ions



The sample can be indefinitely large and indefinitely dilute; no matter what the specifics of the molecule that support alien genetics, the detector will capture it, and present it to a “detection zone”.

The polyanion is easily detected as part of this capture

Displacement of labeled polyelectrolyte



Sensitivity depends on tags on displaced polyelectrolyte.
Can be radiolabels, fluorescent, UV active, chemically reactive, electrochemically reactive.

Once captured, polyanion easily analyzed to prove that it is Darwinian

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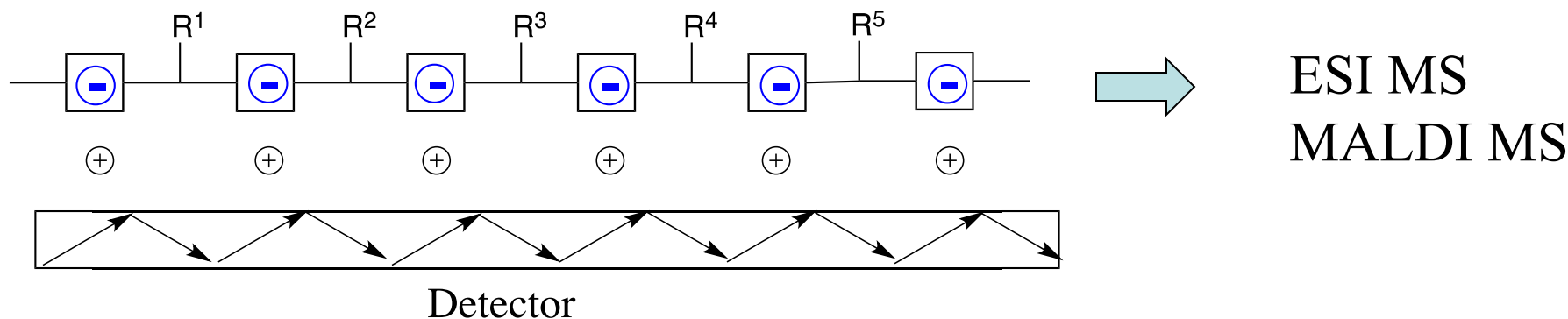
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Darwinian polyelectrolytes are built from a “controlled vocabulary”, a small set of building blocks.

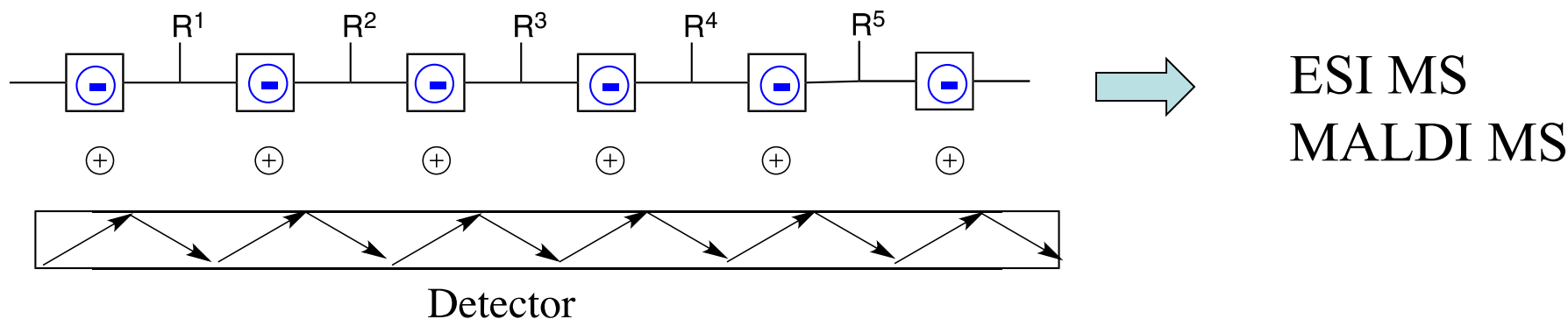
Displace adsorbed polyelectrolyte → Mass spec → fragments



Of course, these must be homochiral to support Darwinism

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Displace adsorbed polyelectrolyte → Mass spec → fragments



Of course, these must be homochiral to support Darwinism

This assay is available today for *in situ* biodetection.

It is universal for any life form in water, anywhere.

Assumes only the universality of Darwinism and the polyelectrolyte theory of the gene.

Which is why I raised my hand to say “yes”. We are ready to go.



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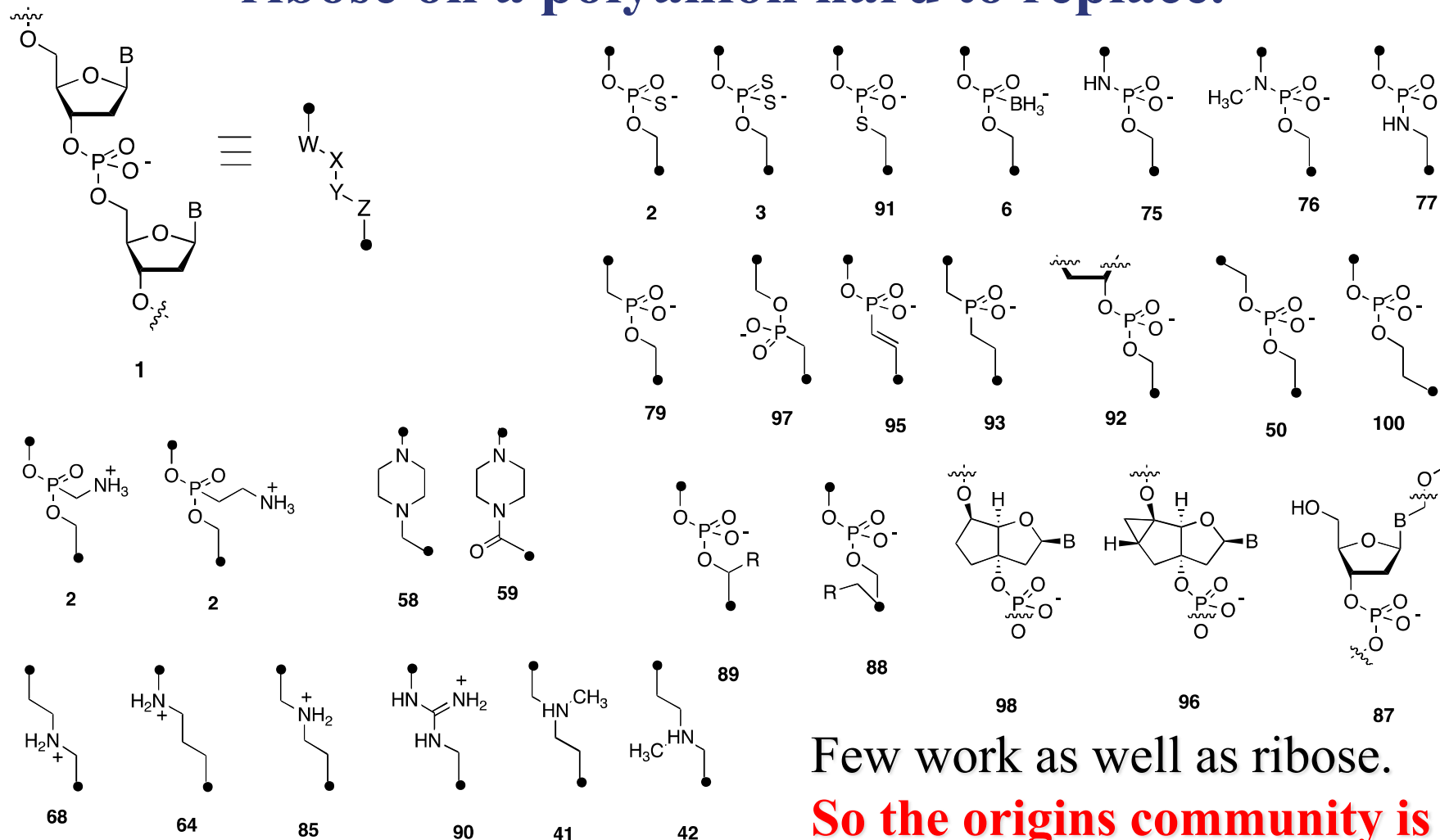
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The rest of genetic biopolymer can be anything. But again, synthesis teaches: ribose on a polyanion hard to replace.



Few work as well as ribose.

So the origins community is still focused on ribose RNA

We are learning more about how prebiotic RNA might be made



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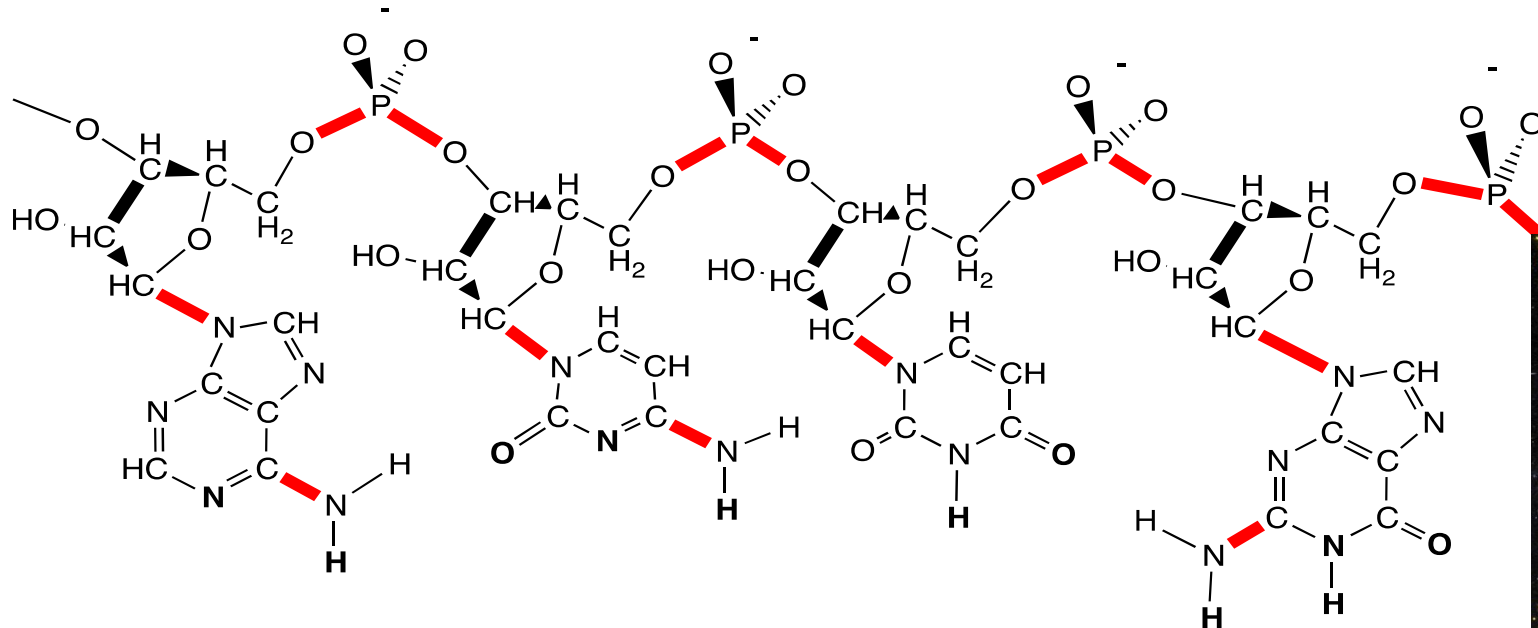
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I am optimistic because the main problems see solution

- Tar : 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.
- Phosphate. **Hard** to get high **phosphate** in **RNA**
- **Water**. Even if we get **RNA**, it is **unstable** in **water**.
Water essential but corrosive

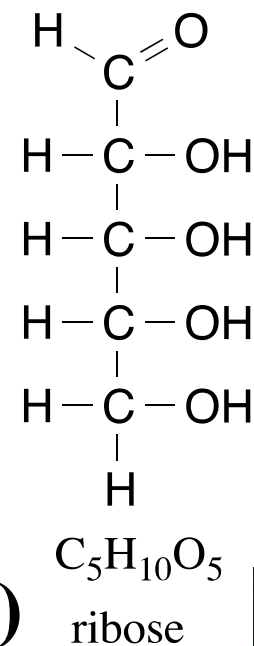
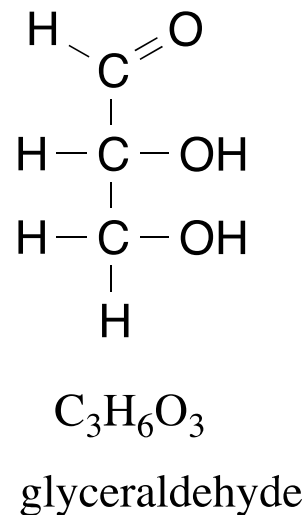
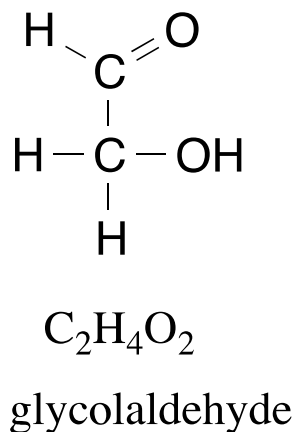
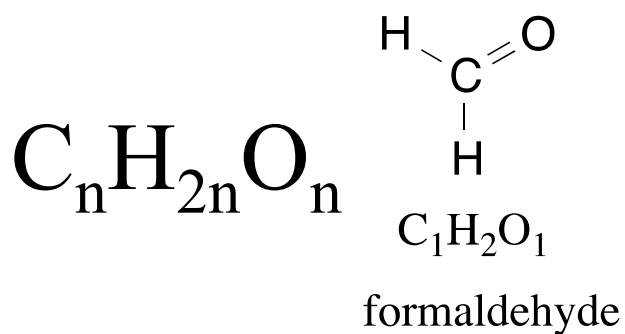


The tar paradox is especially well known with sugars

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

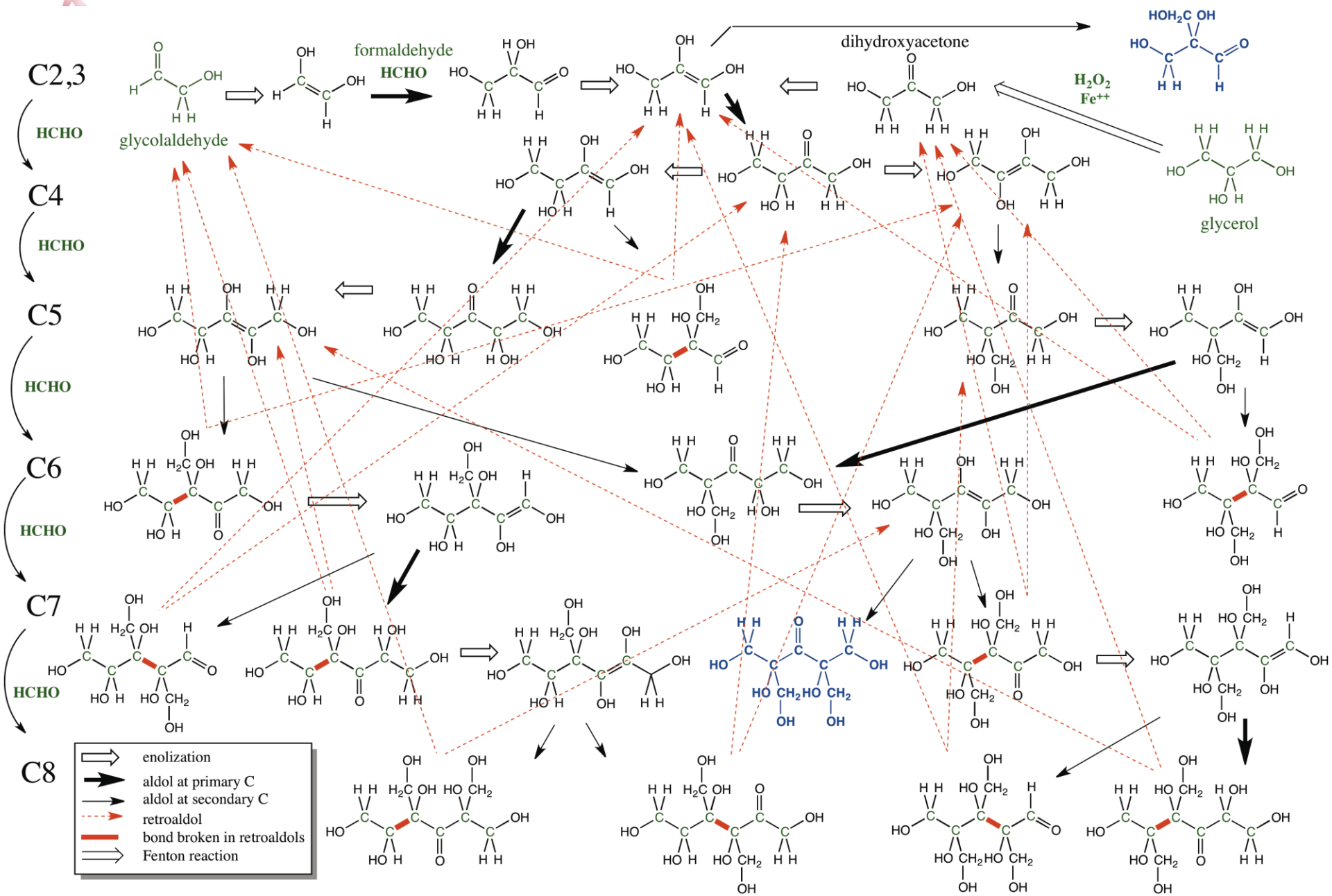


Sucrose:
"Rock candy"

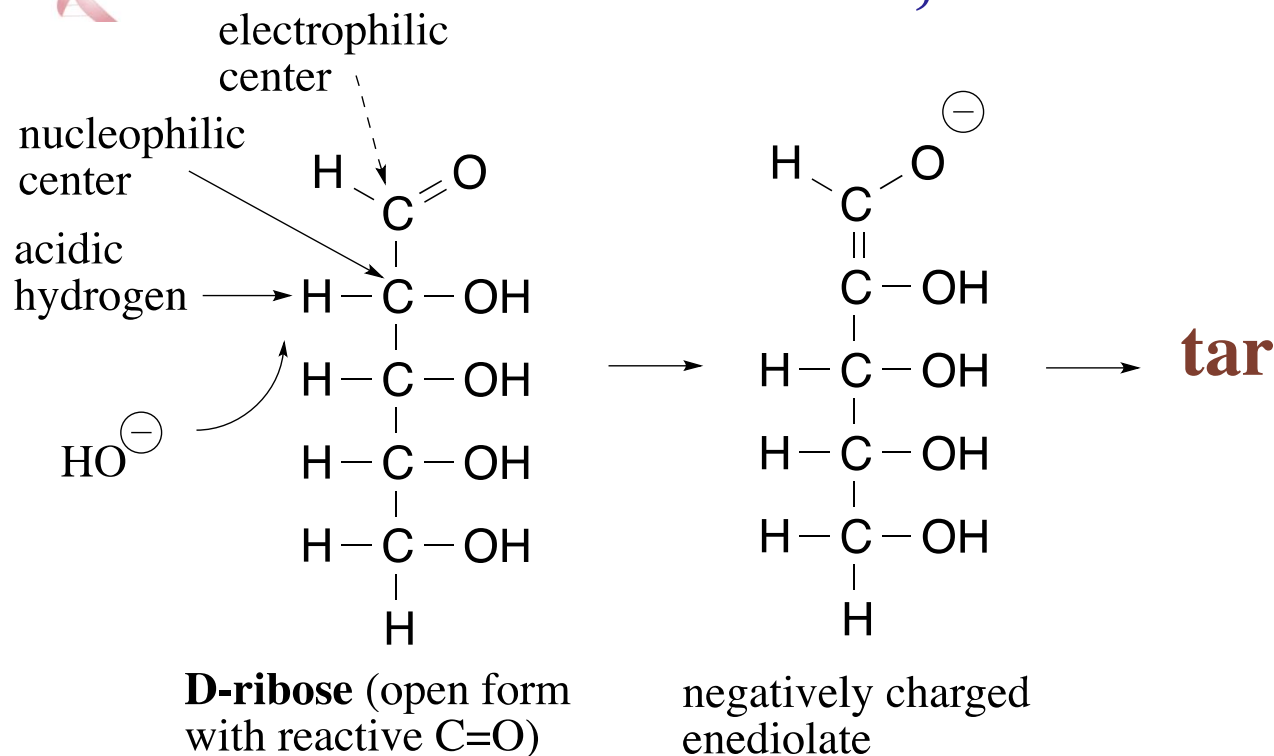


Unfortunately, **RNA** has a sugar component (**R**)

Complexity from C=O is horrible



Ribose has C=O, enolizes, reacts with itself, and forms tar

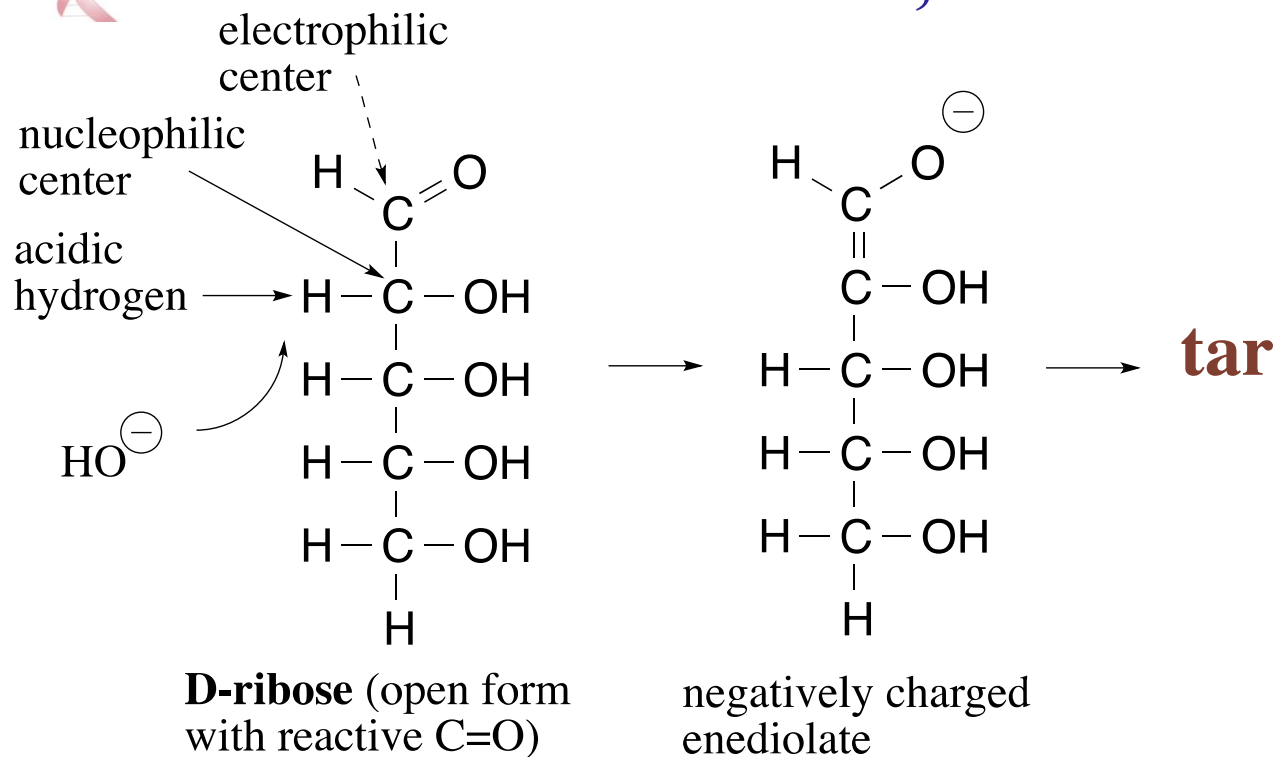


Stanley Miller measured the rate at which ribose formed tar; it is fast (~75 years at pH 7 at 50 °C, not millions of years)

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

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No R = no RNA

We turned mineralogy

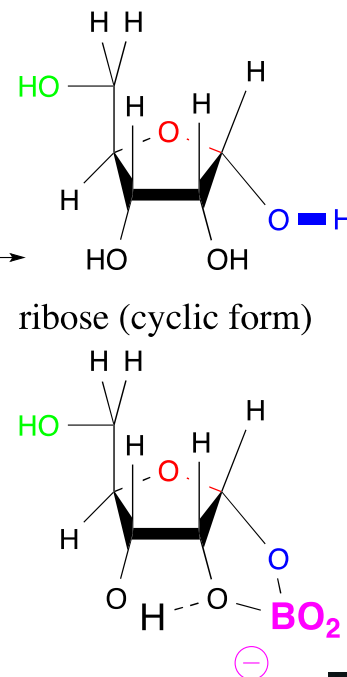
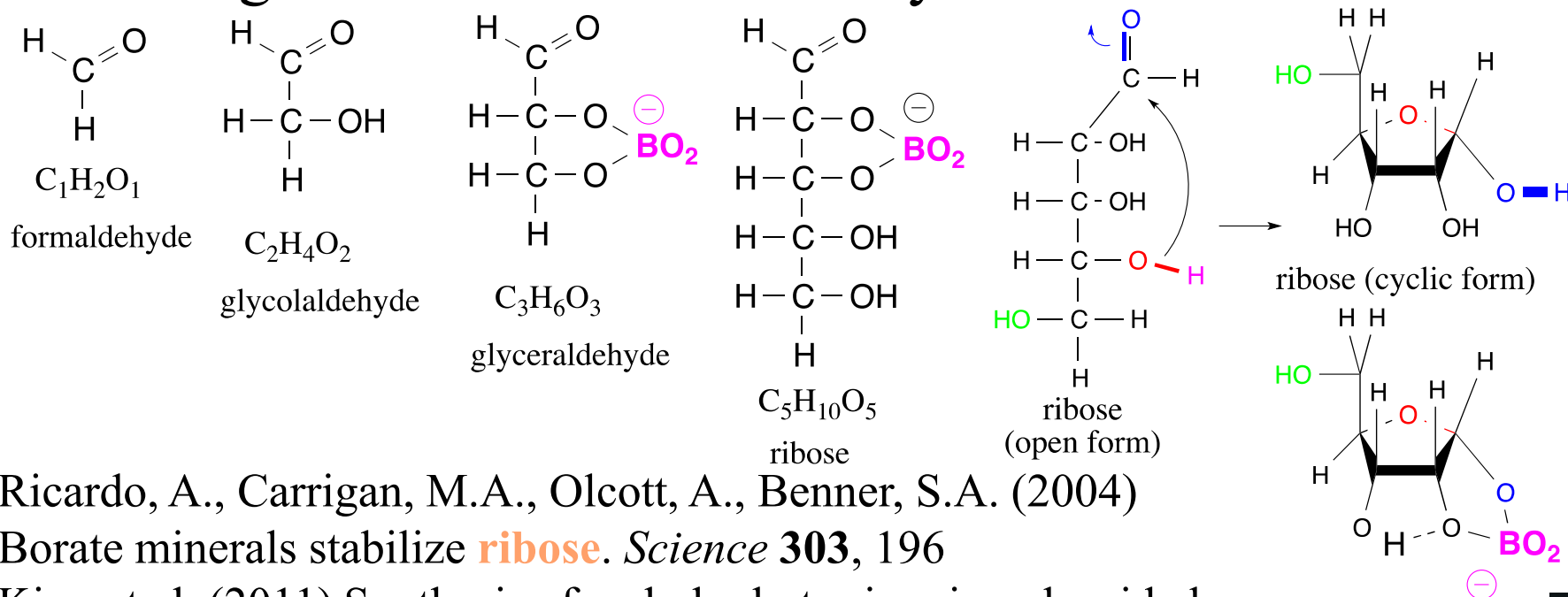
Life did not begin in a Pyrex baking pan

Sugars have adjacent hydroxyl (-OH) groups

Borate binds adjacent hydroxyl groups

Borate binds **ribose**; removes C=O,
prevent tar-ization, allows **ribose** to form.

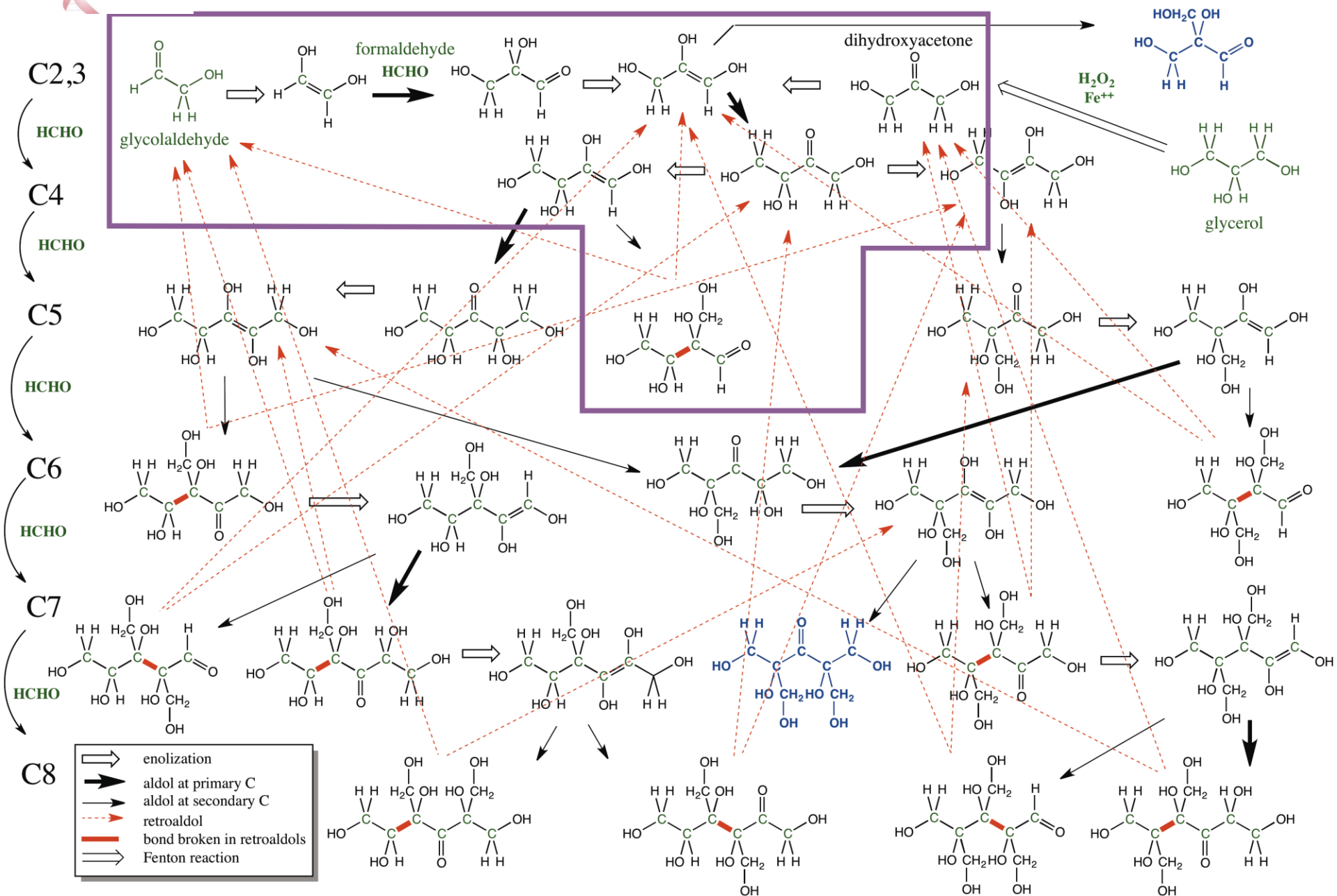
Borate guides reaction of carbohydrates



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



Desert environments where borate minerals accumulate also have low water activity.



Peridot in basalt
→
generates the base



Tourmaline (borate)

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)

Evaporite minerals saturated borate
Colemanite= boron, calcium, oxygen, hydrogen



Death Valley



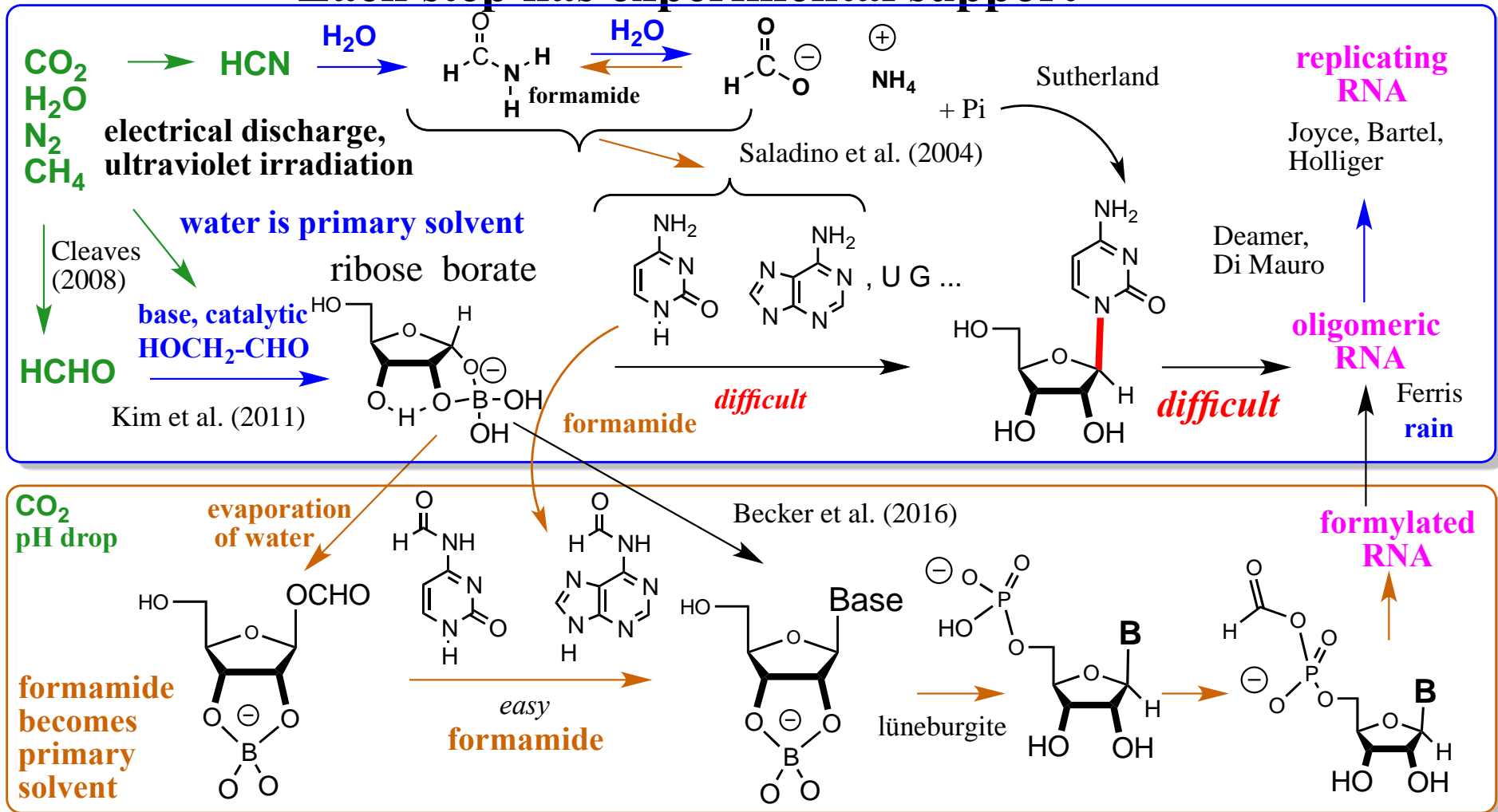
Origin of life jewelry



Discontinuous Model for RNA synthesis

I think the solution will look something like this.

Each step has experimental support



Key: in the atmosphere

in the aquifer;
Nucleophilic Context

in desert evaporite;
Electrophilic Context

Discontinuous RNA synthesis needs intermittant water

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Were these minerals and deserts present on early Earth?



wulfenite

**Early Earth borate? Hazen and Grew say “no”.
Early Earth evaporites? Kirschvink says “no”.**

*What can you give me by way
of minerals on early Earth?*

What do you need?

Molybdates are highly oxidized



tourmaline



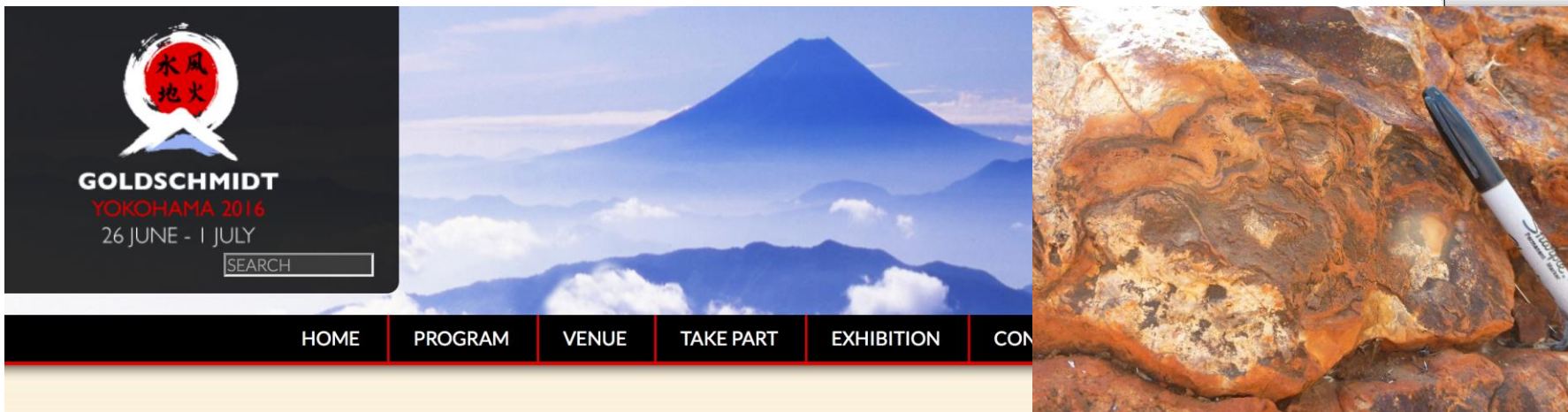
colemanite



Tourmalines, colemanite, and other borate minerals are formed only if scarce boron is enriched. In the lithosphere, only after some cycling time? In the hydrosphere, only in deserts.

But our work has caused geologists to look again

If geologists *look* for Archaean borate ... They find it



Tourmaline-Bearing Crusts in the 3.48 Ga Dresser Formation: Derivation and Implications for the Origin of Life

Van Kranendonk M, Ota T, Nakamura E & Djokic T

Borate minerals unstable to metamorphism, give monazite, apatite, and tourmaline. These are in ~3.8 Ga metasedimentary rocks from the Isua supracrustal belt (Greenland) with biogenic (?) graphite.

Mishima, S., Ohtomo, Y., Kakegawa, T. (2016) Occurrence of tourmaline in metasedimentary rocks of the Isua supracrustal belt, Greenland. Implications for ribose stabilization in Hadean Marine Sediments. *Origin Life Evol. Biosphere* **46**, 247-271.

Grew, E. S. et al., (2015) Boron isotopes in tourmaline from the ca. 3.7-3.8 Ga Isua supracrustal belt, Greenland: Sources for boron in Eoarchean continental crust. and seawater. *Geochim. Cosmochim. Acta*. **163**, 156-177.

Are deserts available on early Earth?

Joe Kirschvink's objection to the Benner model. Models for planetary formation suggest that the inventory of water on early Earth did not leave *any* dry land before continental drift.

*No deserts, no borate evaporites, no **ribose**, no **R**, no **RNA**.*



Waterworld



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-ribose is there today.*

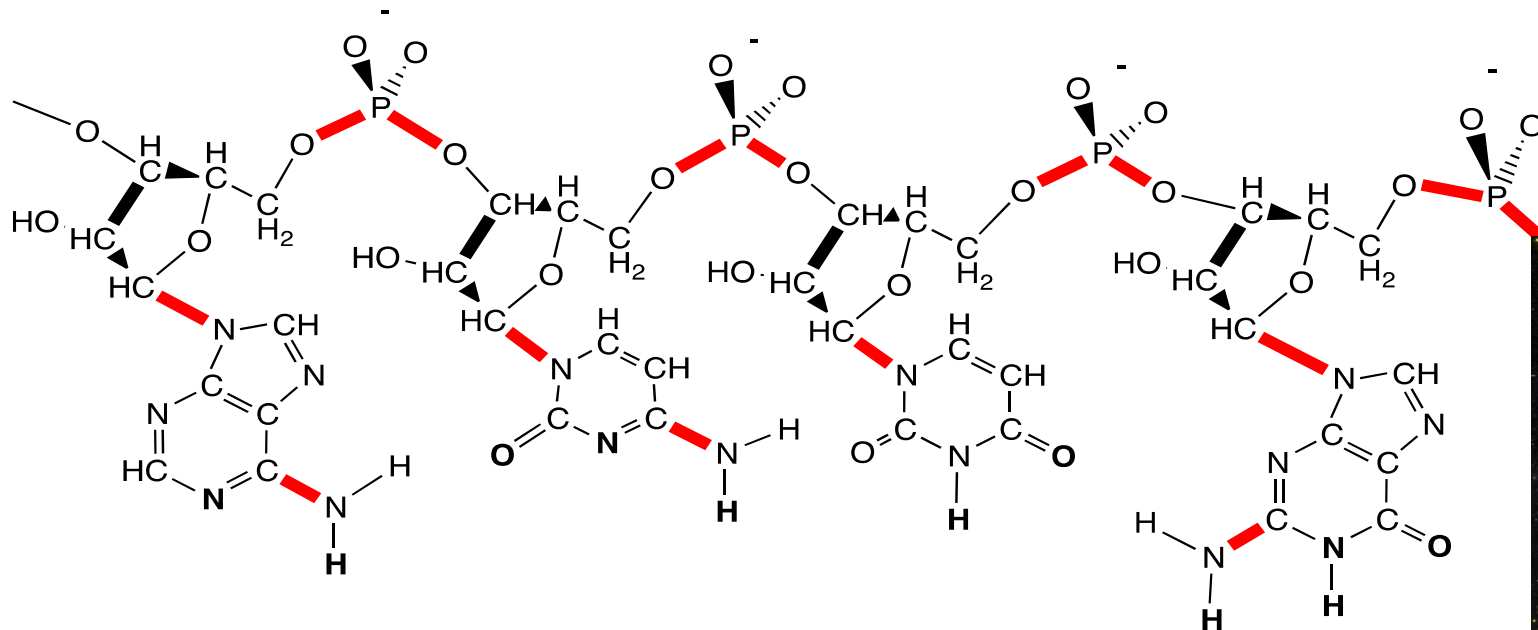


John Carter



I am optimistic because the main problems see solution

- Tar : 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.
- Phosphate. **Hard** to get high phosphate in **RNA**
- **Water**. Even if we get **RNA**, it is **unstable** in **water**.
Water essential but corrosive



Borate +phosphate = borophosphate minerals, not inaccessible apatite

27. LÜNEBURGITE $[\text{Mg}_3 (\text{PO}_4)_2 \text{B}_2\text{O} (\text{OH})_4 \times 6 \text{H}_2\text{O}]$ IN UPPER MIOCENE SEDIMENTS OF THE EASTERN MEDITERRANEAN SEA

Jens Müller and Frank Fabricius, Lehrstuhl für Geologie, Technische Universität, München, West Germany

ABSTRACT

Upper Miocene dolomitic marls drilled at Site 374 (Messina Abyssal Plain, Ionian Sea) contain small white mm-sized spherules which have been identified by X-ray, chemical, and IR-spectrometry techniques as lüneburgite $[\text{Mg}_3 (\text{PO}_4)_2 \text{B}_2\text{O} (\text{OH})_4 \times 6 \text{H}_2\text{O}]$.

We assume that the lüneburgite formed as a secondary product by upward migrating brines from the Miocene evaporites which contain highly soluble Mg- and B- salts. Enrichment of phosphorus may be the result of concentration in residual brines from the evaporites or to diagenetic decomposition of organic matter in a reducing environment.

chiefly of authigenic Ca-dolomite ($\text{Ca}_{55}\text{Mg}_{45}$) and the detrital components quartz, feldspar, smectite, illite, kaolinite, chlorite, and traces of (?) attapulgite. The fine-grained black sediment is slightly enriched in organic carbon (0.3-0.7%; Sigl, this volume) and when fresh, expells a bituminous odor. Secondary(?) gypsum occurs within the sequence containing lüneburgite. These homogeneous sediments were deposited as a transitional facies between the formation of evaporites and deposition of more open marine sediments (Site 374 Report, this volume).

Lüneburgite



Tribal jargon

Gypsum = $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$

Lüneburgite =

$\text{Mg}_3\text{B}_2(\text{PO}_4)_2(\text{OH})_6 \cdot 8\text{H}_2\text{O}$

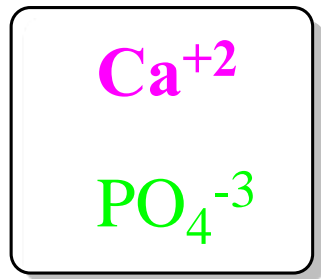
What does it mean if gypsum + lüneburgite occur together?

The phosphate problem in the “RNA first” model

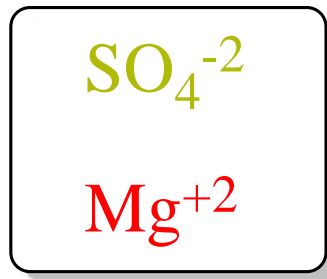
Clearly stoichiometric phosphate is necessary for RNA itself

Powner et al. (200) need it in quantity (1 M, 98 g/L)

But phosphate + calcium precipitate = apatite



apatite



epsomite



Apatite

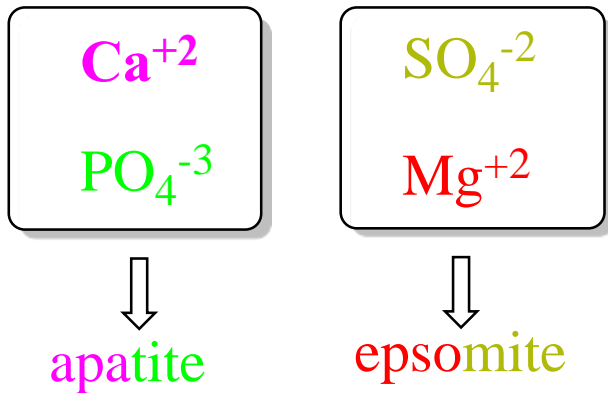
Benner collection of fine minerals

What happens with borate?

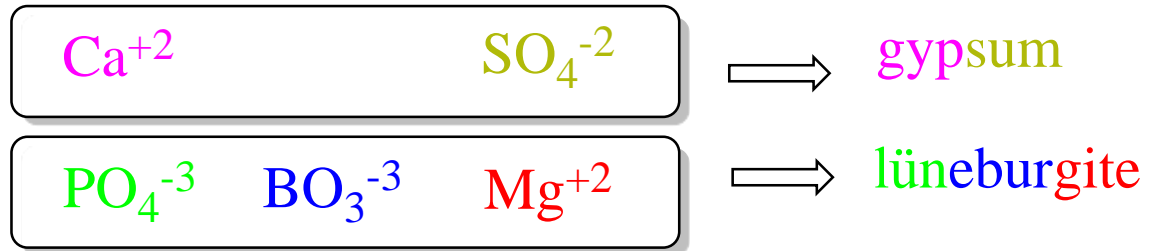
Borate keeps phosphate away from apatite

In natural environments, shown by observational geology

Partitioning of species
in absence of **borate**



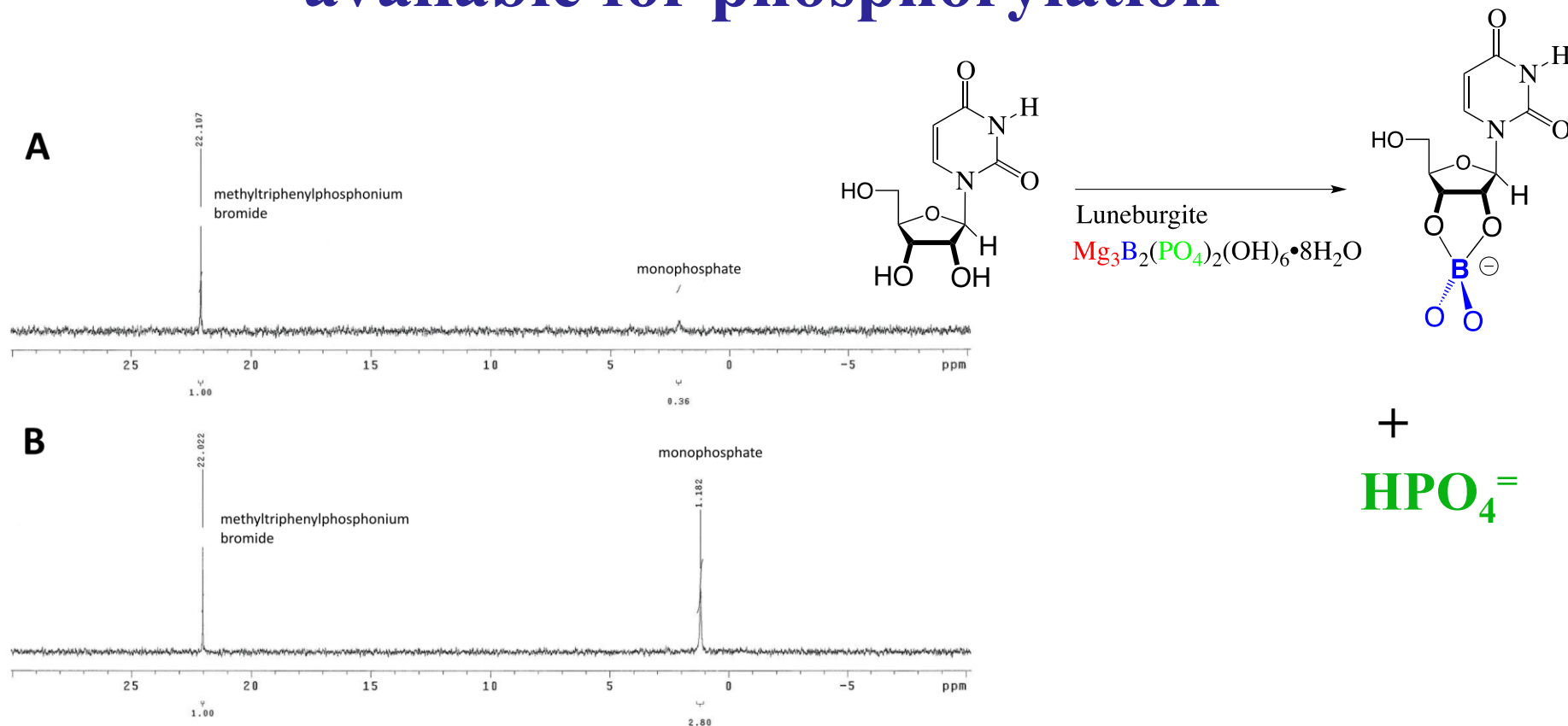
Partitioning of species
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This also happens in the laboratory if you mix MgO , CaO , H_3BO_3 , H_3PO_4 , and H_2SO_4 and evaporating.

**Borate keeps phosphate from
being lost by calcium capture.
Borophosphate formed instead.**

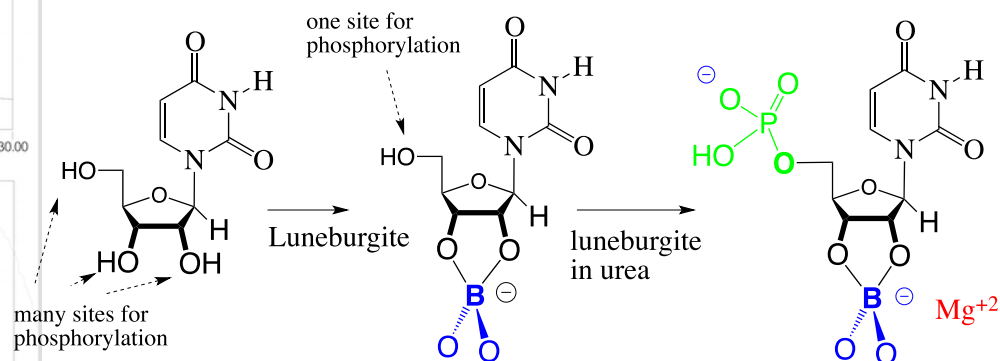
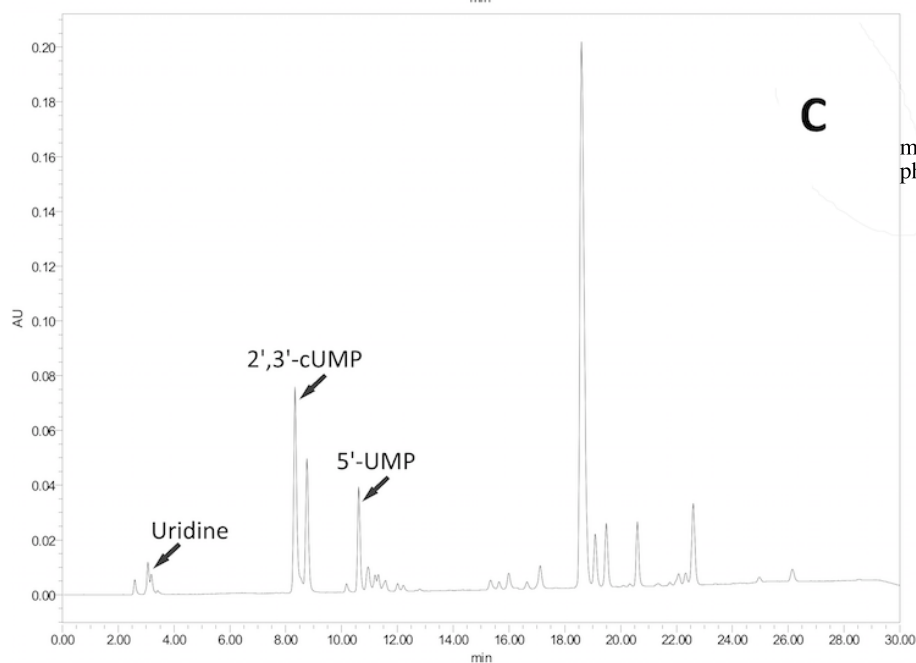
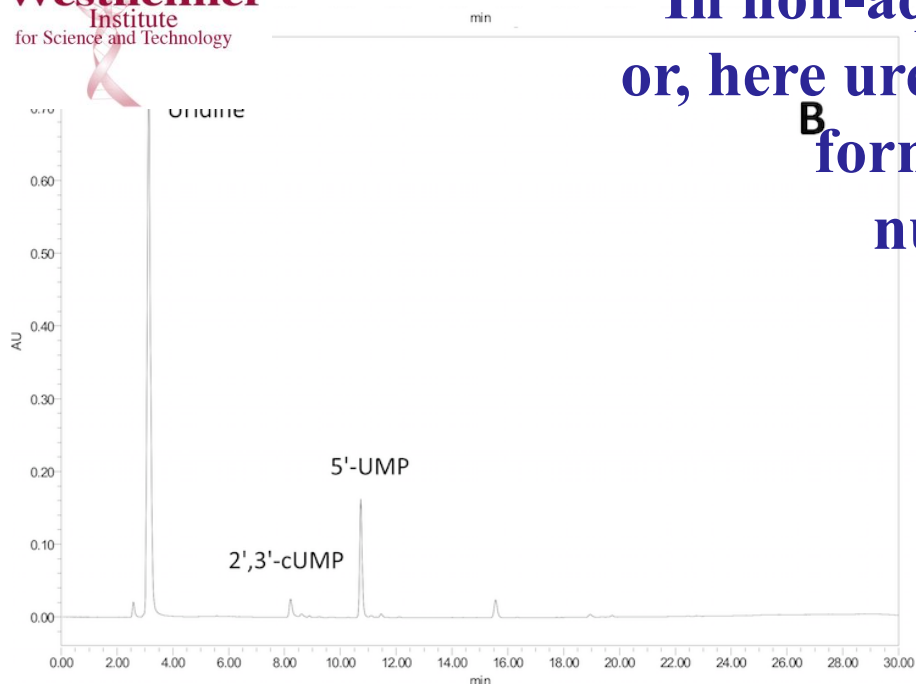
Ribose extracts borate out of lunenburgite, makes phosphate available for phosphorylation



Kim, H. J., Furukawa, Y., Kakegawa, T., Bitai, A., Scorei, R., Benner, S. A. (2016) Evaporite borate-containing mineral ensembles make phosphate available and regiospecifically phosphorylate ribonucleosides: Borate as a multifaceted problem solver in prebiotic chemistry. *Angew. Chem.* DOI: 10.1002/ange.201608001

In non-aqueous media (formamide or, here urea) where phosphate ester formation is thermodynamic, nucleosides regioselectively phosphorylated.

Absent borate, one gets a mixture.

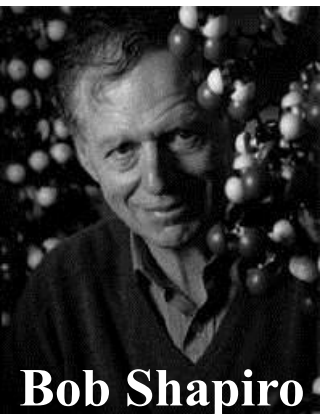
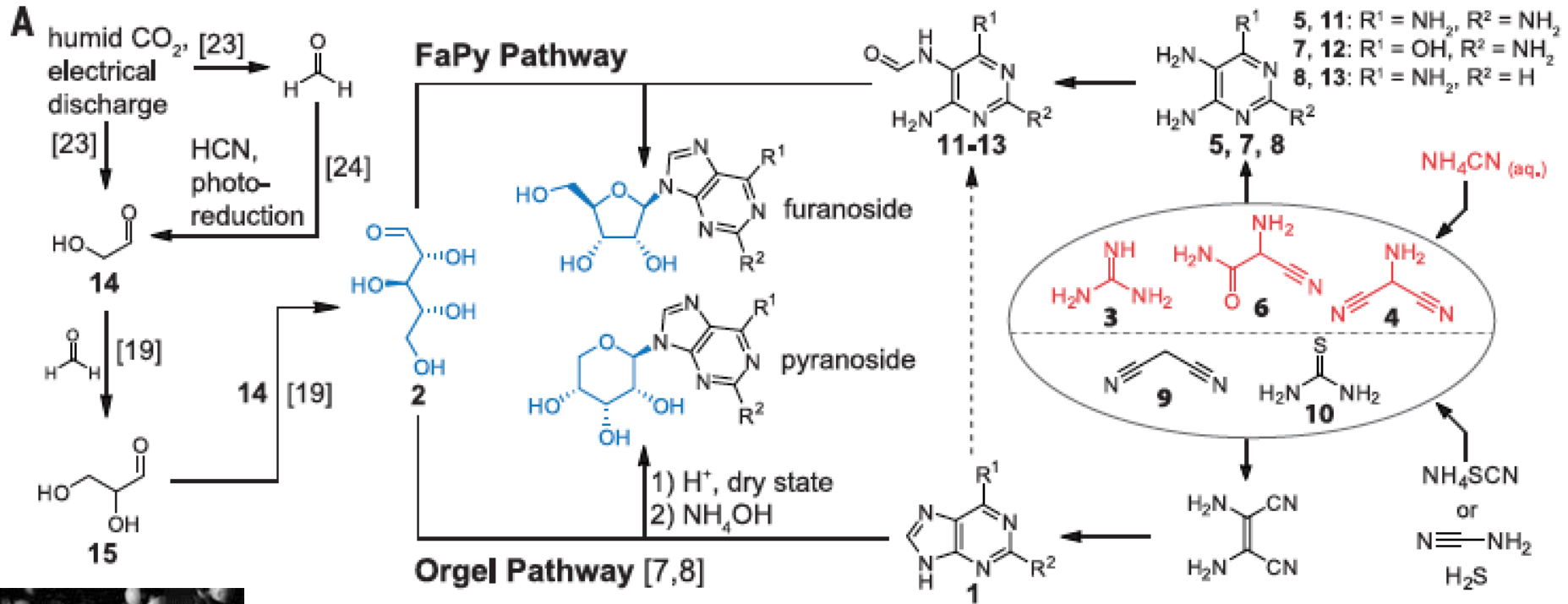


Two for the price of one.

Lüneburgite sequesters phosphate from calcium until nucleosides are present, then phosphorylates nucleosides regio-specifically after nucleosides decompose the mineral to release phosphate.

Powner and Sutherland have a route

Thomas Carell et al. have a better route

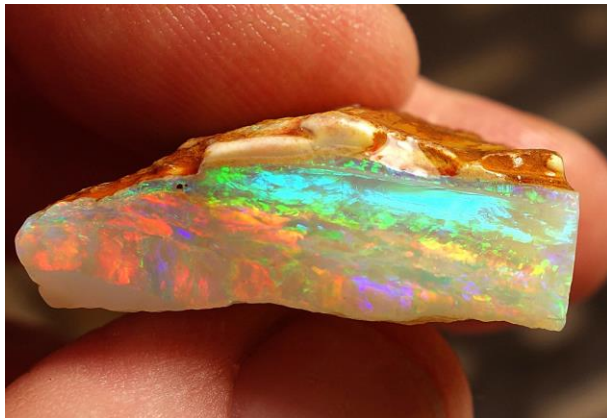


Carell route starts with ribose **borate**, *Unstable carbohydrates (glyceraldehyde) need not accumulate for Carell's route to work.*



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- **Water**. Even if we get **RNA**, it is **unstable** in **water**.
Once RNA formed, adsorbed onto CT-Opal



John Grotzinger, David Blake, **exactly like 3 Ga Mars**



A logic tree

RNA first?

No

Yes

A different set
of problems

Need to make RNA
prebiotically

**Templeton-FfAME
Origins Collaboration.**
Steve Mojzsis;
2 x California land.
Granite @ 4.2 Ga

Benner's stupid chemistry?

No

Yes

Get your own
alternative

Need oxidized
minerals, a desert

Can we have these on early Earth?

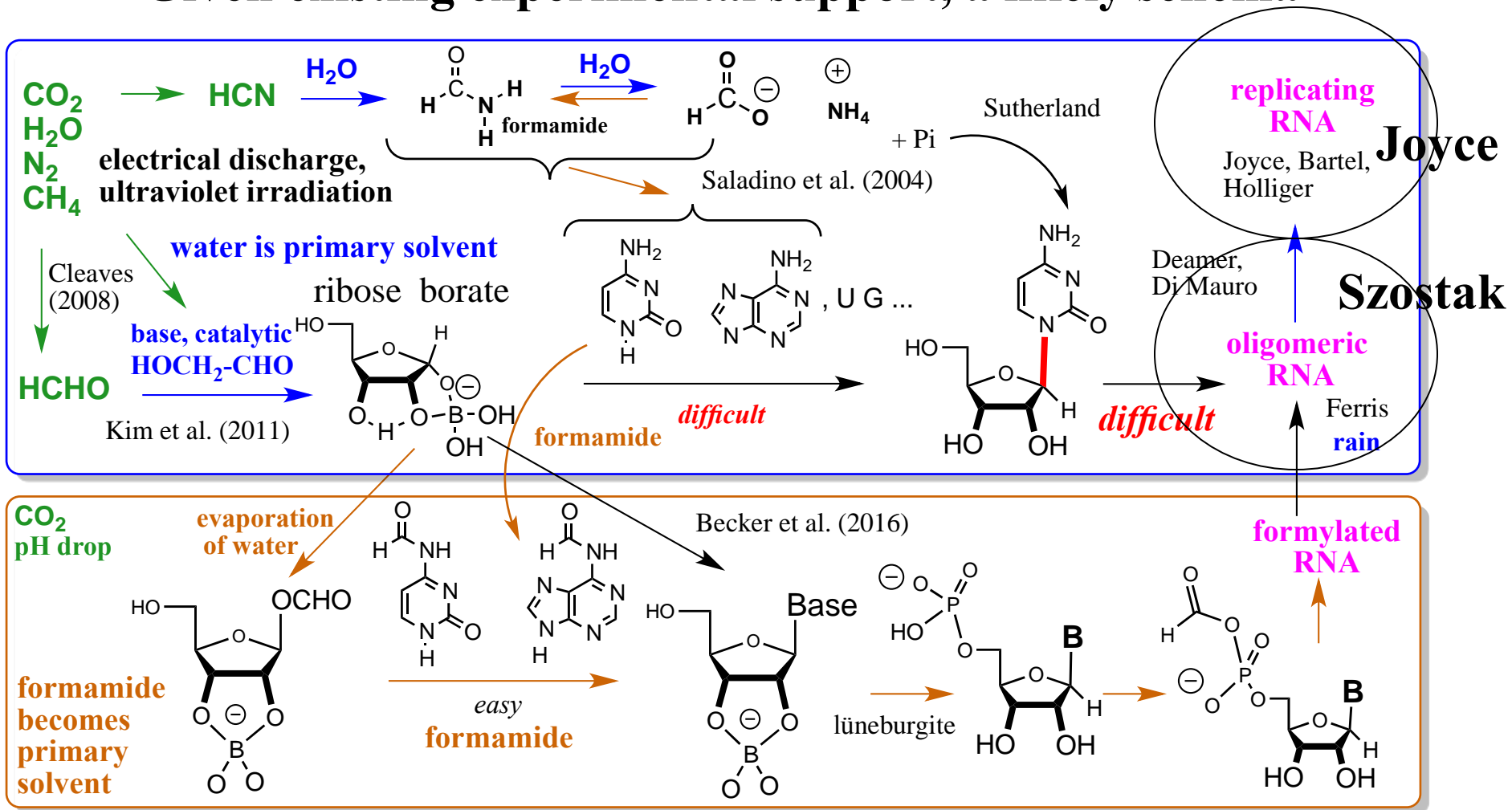
No

Yes

We are all Martians

Model for prebiotic synthesis of RNA

Given existing experimental support, a likely schema



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

There is hope, even though problems remain

Aqueous life needs dry land. Perpetual water worlds a problem.

1. Universal genetic biopolymers in water have repeating charges
 2. This allows them to be easily concentrated from plumes, bulk water
 3. Darwinian biopolymers having this features are easily distinguished
- We can today detect extant life in H₂O *in situ*, terran & weird,**
4. We still lack a clear path to get RNA as the first terran Darwinian biopolymer, but it seems that we *will* find those paths.
 5. These paths are defeated by dilution into a global ocean
 6. Defeat is easily avoided in deserts with occasional water (e.g. Mars)

And non-aqueous life? An analogous universal biosignature?

7. Efforts to get life in cryosolvents defeated by solubility issues
8. Warm Titans, exotic polar solvents, still possible

Disequilibrium and the impotence of Darwinism

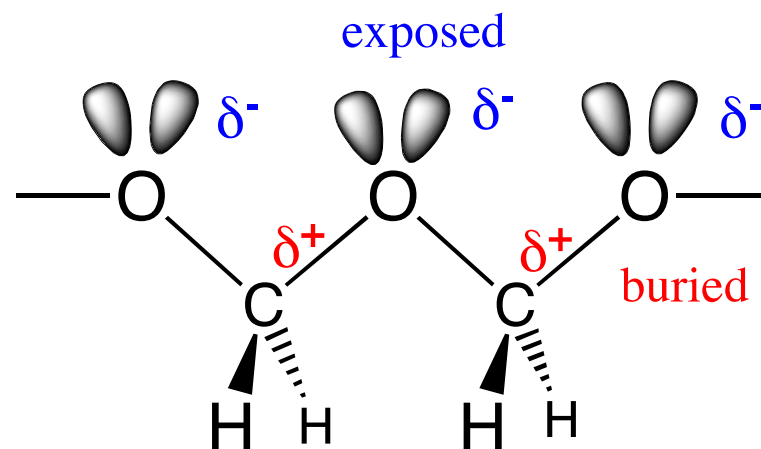
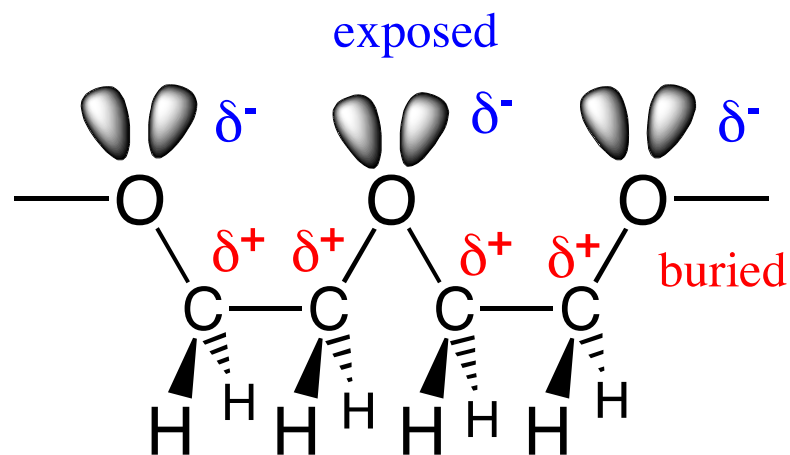
9. Smart life will escape Darwinism to gain Lamarckianism



Biopolymer with a repeating charge will not dissolve in hydrophobic solvents.

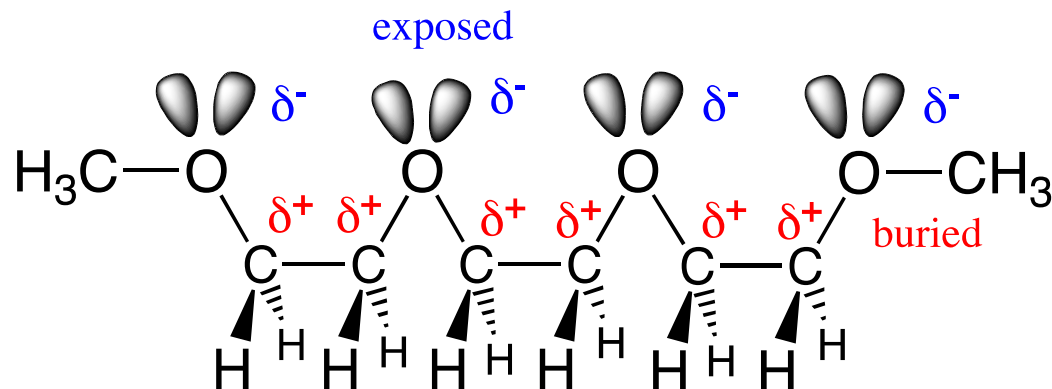
What about a biopolymer with a dipole, where the exposed part of the dipole is negatively charged?

By burying consistently one end of the dipole, and exposing the other, maybe the polymer cannot aggregate.



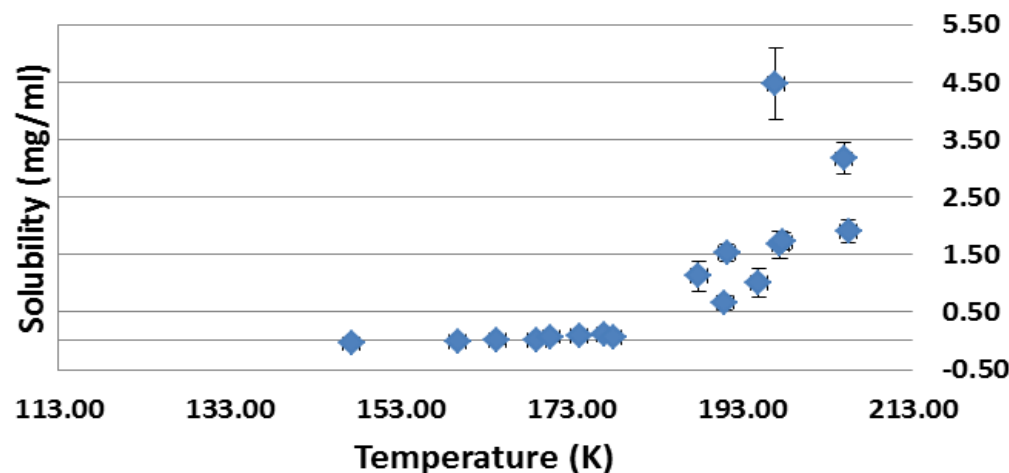
McLendon, C., Opalko, F. J., Illankoon, H. I., Benner, S. A. (2015) Solubility of polyethers in hydrocarbons at low temperatures. A model for potential genetic backbones on warm Titans. *Astrobiol.* **15**, 200-206.

They dissolve in warm hydrocarbons; aggregate (precipitate) in cold



Hydrocarbon	Freeze	Boil
Methane	91 K	112 K
Ethane	89 K	184 K
Propane	85 K	231 K
Butane	135 K	273 K

$C_8H_{18}O_4$ Tri-ethylene glycol dimethyl ether
(4 assays)



A "warm Titan" is OK.

*Water is a good solvent
because it is hot.*

William Bains

Cryosolvents are not good, if you think that biology needs dissolution

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Disequilibrium and the impotence of Darwinism



A disequilibrium that shows the impotence of Darwinism.
*It has not created a life form able to exploit the energy in
the tree-dioxygen combination. **The solution?***

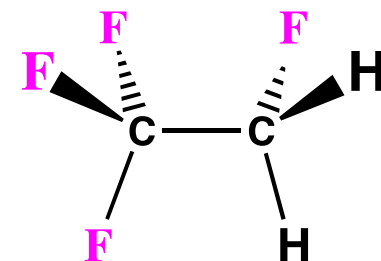


Homo
erectus
invented
Lamarckian
evolution

Any life form smart enough to talk to with us will soon get control of its biology to not need dying babies die to prevent sickle cell anemia. *Germ line gene therapy.*

Universal for life, regardless of core molecular biology.

What biosignatures would come from this life form?



Lamarckianism: universal end point for intelligent Darwinian systems



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**Search for Lamarckian biosignatures escapes conundrum
of remote detection of unknown molecular biology.**

