An RNA World Scenario for the Origins of Life: Montmorillonite Clay-Catalyzed Formation of RNA Oligomers

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RNA WORLD

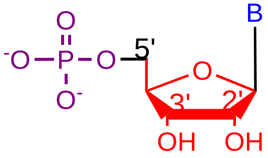
In the RNA world scenario of the origin of life RNA was the central biopolymer. It stores the genetic information and catalyzed reactions of RNA. The DNA/protein world evolved from the RNA world.

Evidence for the RNA world includes:

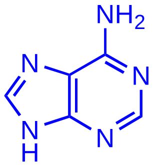
1. No proteins are formed proximate to RNA.
2. Ribozymes catalyze to the formation of RNA.

MONOMERS AND OLIGOMERS OF RNA

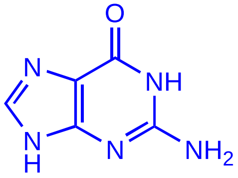
(a) RNA Monomer



(b) Bases B =

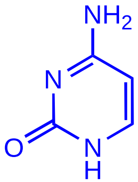


adenine

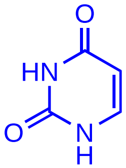


guanine

Purines



cytosine

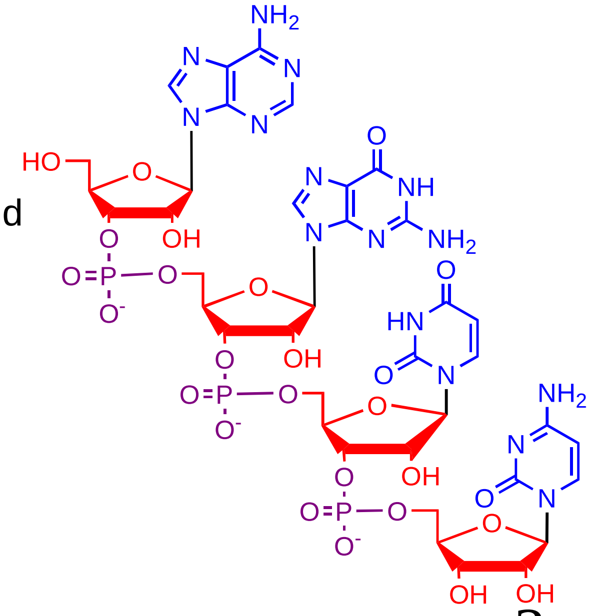


uracil

Pyrimidines

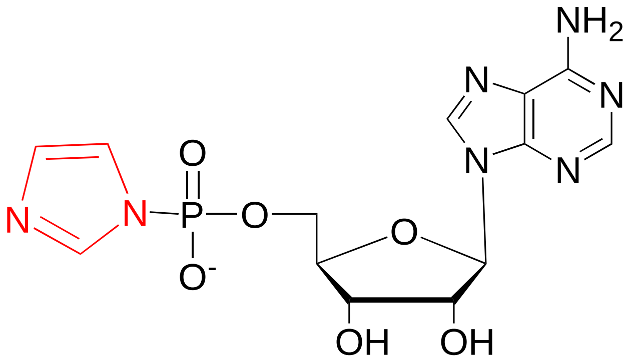
(c) RNA Oligomer

5'-end

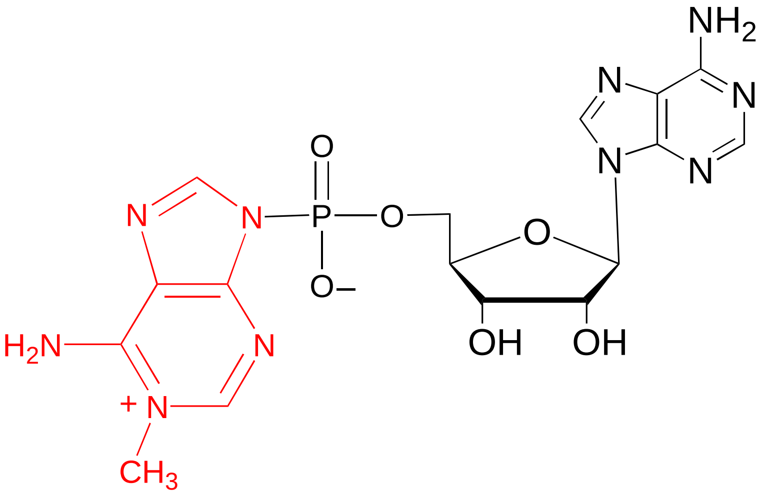


3'-end

ACTIVATED MONOMERS



5'-phosphorimidazolide of adenosine (ImpA)



1a. 1-Methyladenine-pA (1-MeadpA)

STATUS of the PREBIOTIC SYNTHESIS of RNA MONOMERS

Purines and Pyrimidines.

a. Purines present in small amounts in meteorites.
b. Purine and pyridine bases formed in low amounts from HCN.
c. Pyrimidines formed from cyanoacetylene.

Ribose Synthesis

Plausible prebiotic synthesis of ribose.

Nucleoside Synthesis

A major problem! The reaction of ribose with purine bases gives low yields of a mixture of nucleosides.

ASTEROIDS WERE A MAJOR SOURCE OF ORGANICS ON THE PRIMITIVE EARTH

Asteroids collide and propel dust and meteorites out of their orbits

The following are conservative are estimates of amounts of asteroidal material reaching the Earth:

1. 2×10^{21} kg, an amount equivalent to a layer 2.5 km thick on the Earth's surface.

2. About 1-2 % reduced carbon in this dust and rocks.

David Vokrouhlicky and Farinella, Nature 2000

M. Gaffey unpublished

CARBON IN THE MURCHISON METEORITE

Interstellar Grains	~ 2%
Amino acids	++[1]
Carboxylic acids	+++
Dicarboxylic acids	++
Hydroxy acids	++
Phosphonic acids	+
Sulfonic acids	++++
Basic N-heterocycles	+
Purines & Pyrimidines	+
Pyridine carboxylic acids	+
Amides	++
Amines	++
Alcohols	++
Aldehydes & Ketones	++
Aliphatic Hydrocarbons	++
Aromatic " "	++
Polar " "	+++
Sugar alcohols & acids	+

[1] ++++ > 1000 ppm, +++ > 100 ppm, ++ >10ppm, + > 1ppm

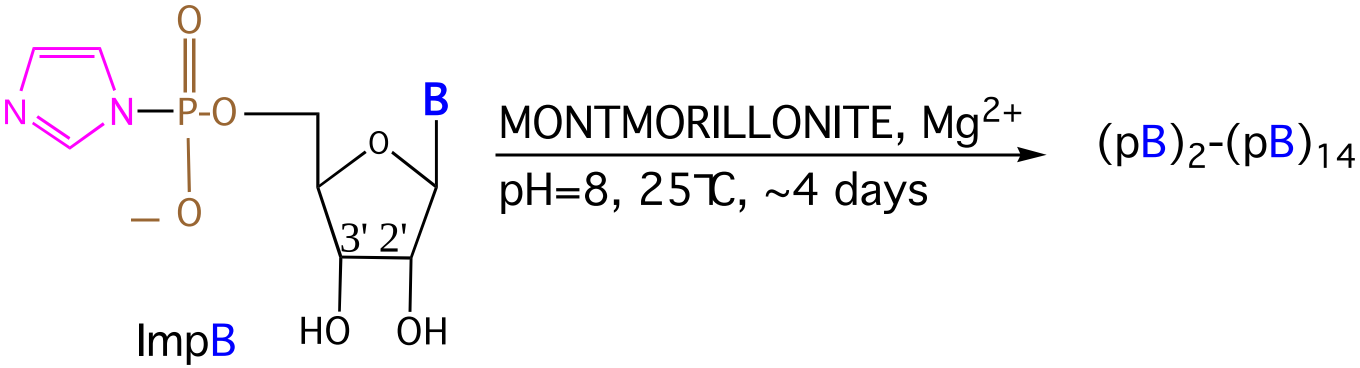
Relative Abundances of Volatile Organic Compounds in Comets (Mole %)

Molecule	*Hyakutake*	*Hale-Bopp*
H_2O	100	100
H_2O_2	<0.04	-
CO	6-30	20
CO_2	<7	6
CH_4	0.7	0.6
C_2H_2	0.5	0.1
C_2H_6	0.4	0.1
CH_3C_2H	-	<0.045
CH_3OH	2	2.4
H_2CO	0.2 – 1	1.1
$HCOOH$	-	0.08
$HCOOCH_3$	-	0.08
CH_3CHO	-	0.02
H_2CCO	-	<0.032
C_2H_5OH	-	<0.05
CH_3OCH_3	-	<0.45

Relative Abundances of Volatile Organic Compounds in Comets (Mole %)

Molecule	*Hyakutake*	*Hale-Bopp*
NH_3	0.5	0.7
HCN	0.1	0.25
HCNO	0.07	0.06
HNC	0.01	0.04
CH_3CN	0.01	0.02
HC_3N	-	0.02
$HCONH_2$	-	0.01
Glycine	-	<0.5
CH_2NH	-	<0.032
HC_5N	-	<0.003
H_2S	0.8	1.5
COS	0.1	0.3
SO	0.1	0.2 - 0.8
CS_2	0.1	0.2
SO_2	-	0.1
H_2CS	-	0.02

FORMATION OF RNA OLIGOMERS



ImpB

MONTMORILLONITE, Mg^{2+}

pH=8, 25°C, ~4 days

$(pB)_2$-$(pB)_{14}$

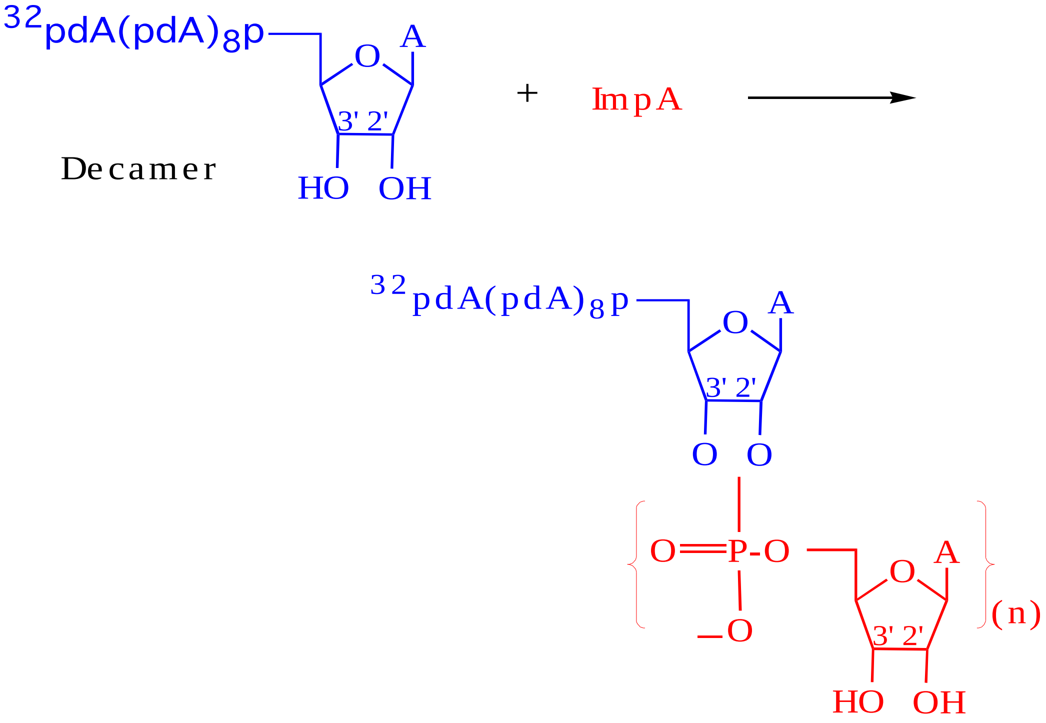
B= A, G, C, U, I

Ferris and Ertem, Science **257**, 1387 (1992)

Recent mass spectral data shows that oligomers of A longer than 30 mers are formed.

Zagorevski, Aldersley et al. J. Am. Soc. Mass Spectrum. 2006.

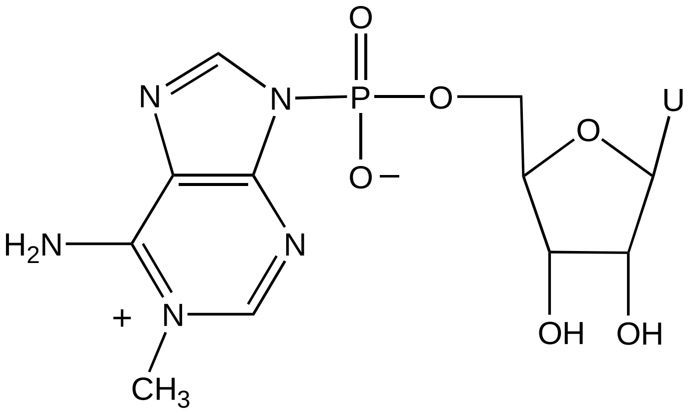
GENERATION OF LONGER RNA OLIGOMERS



Bind a ^{32}p-labelled decamer to Na$^+$-montmorillonite and add ImpA to it each day.   At the end of 14 days oligomers with a median chain length of 30 mers and as long as 50 mers were formed.

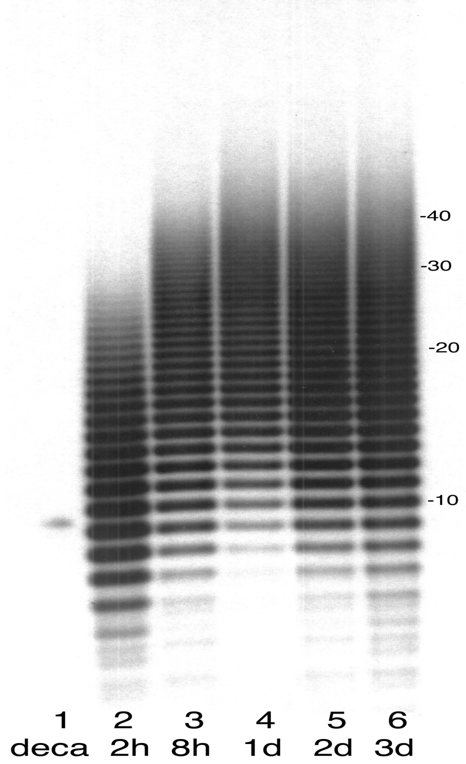
Ferris et al. Nature **35**1, 59 (1996)

ONE - STEP SYNTHESIS OF 35-40 MERS
REACTION of 1-MeadpU



1-Methyladenine-pU

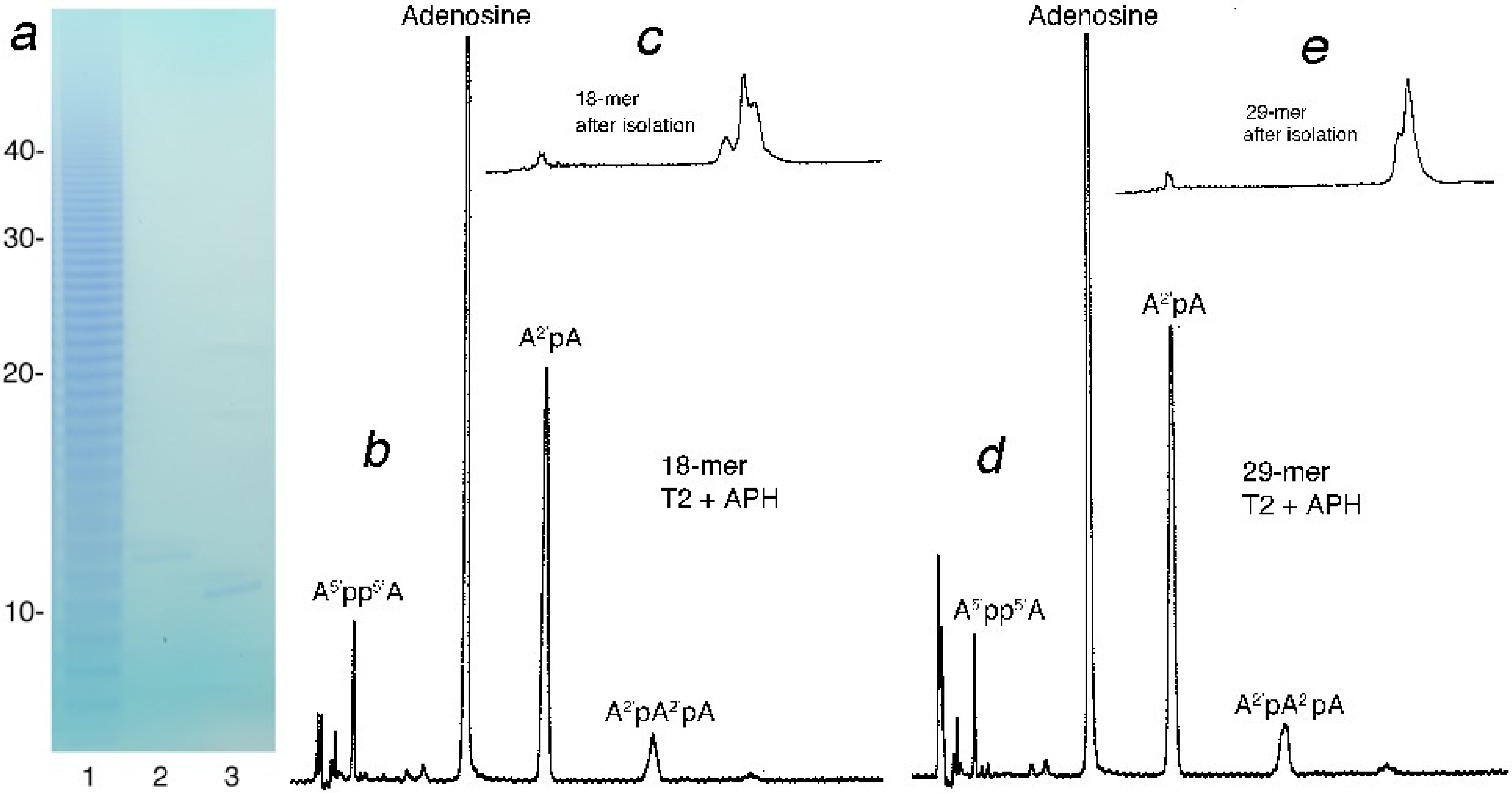
Gel Electrophoresis



Lane 1 = decamer

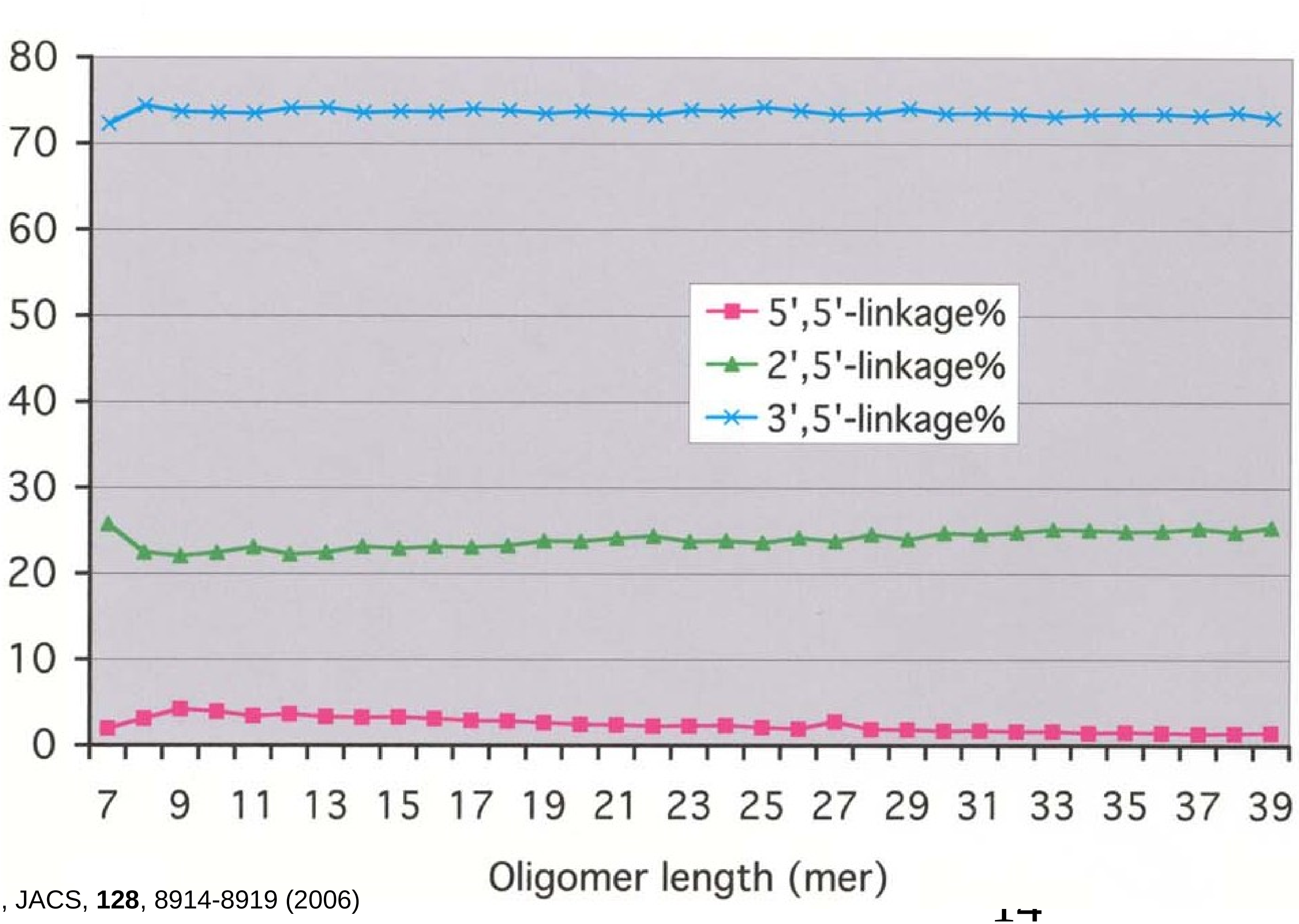
Huang Chem. Commun. 1458, 2003.

OLIGO A 18 MER & 28 MER EXTRACTED FROM THE GEL AFTER ELECTROPHORESIS



Huang & Ferris, JACS, **128**, 8914-8919 (2006)

VARIATION OF REGIOSELECTIVITY OF PHOSPHODIESTER BONDS IN OLIGO(A)s WITH CHAIN LENGTH



Huang, JACS, **128**, 8914-8919 (2006)

MONTMORILLONITE IS LIKELY TO HAVE BEEN PRESENT ON THE PRIMITIVE EARTH

1. Formed by the weathering of volcanic ash.

2. Montmorillonite is a clay mineral.

3. Montmorillonite is be present on Mars

4. Found in ancient rock formations

5. Found in meteorites

J. D. Bernal, Guthrie Lecture 1947
Proc. Phys. Soc. A **62,** (357A), 537-558 (1949).

V. M. Goldschmidt, 1947, New Biology,12, 97-205 (1952).

NOT ALL MONTMORILLONITES ARE CATALYSTS

OUT OF 20 MONTMORILLONITES

- 5 are excellent catalysts
- 5 are good catalysts
- 10 are not catalysts

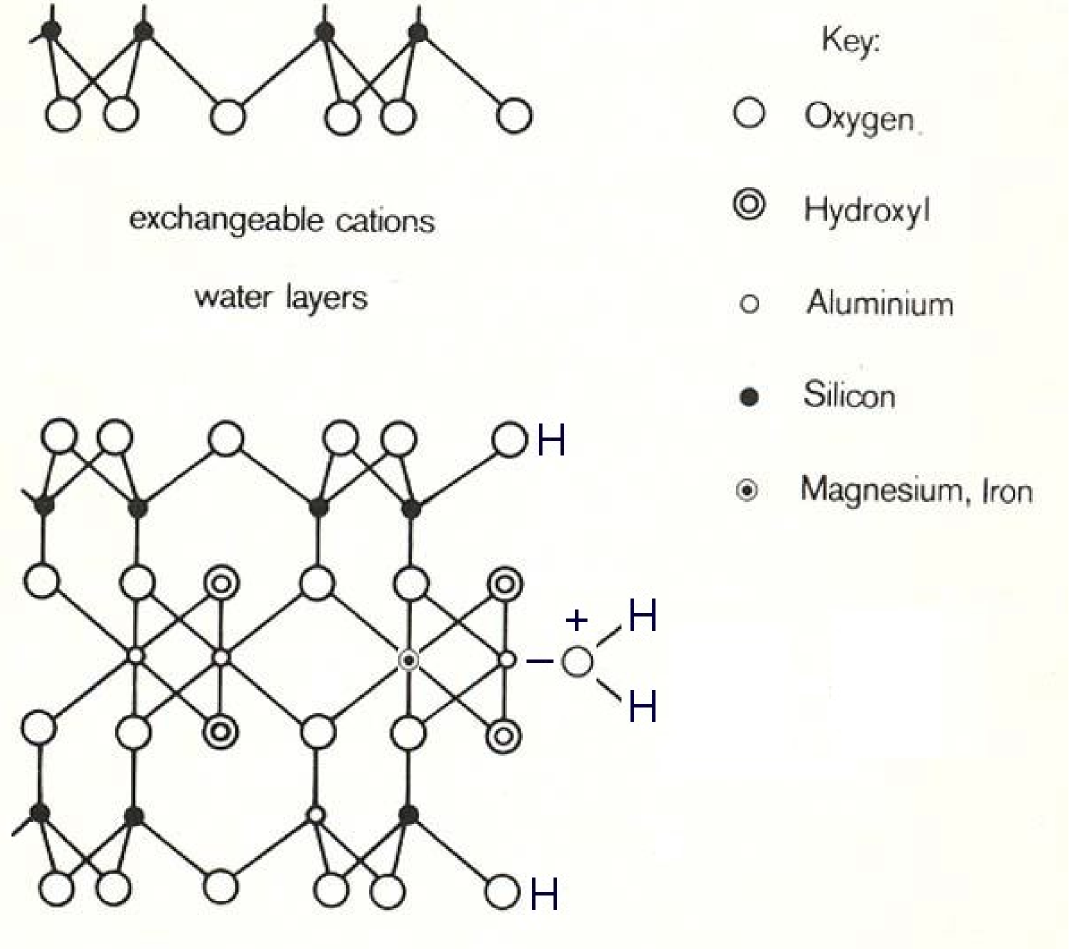
Catalysis takes place in the galleries between the clay platelets

Excellent catalysts have fewer negative charges so fewer cations in the galleries permits the entrance of activated monomers into the galleries

Non-catalysts have high amounts of negative charges so the high proportion of cations in the galleries blocks the entrance of activated monomers into the galleries

Breen and Moronta, J. Phys. Chem. B 103, 5675-5680 (1999)

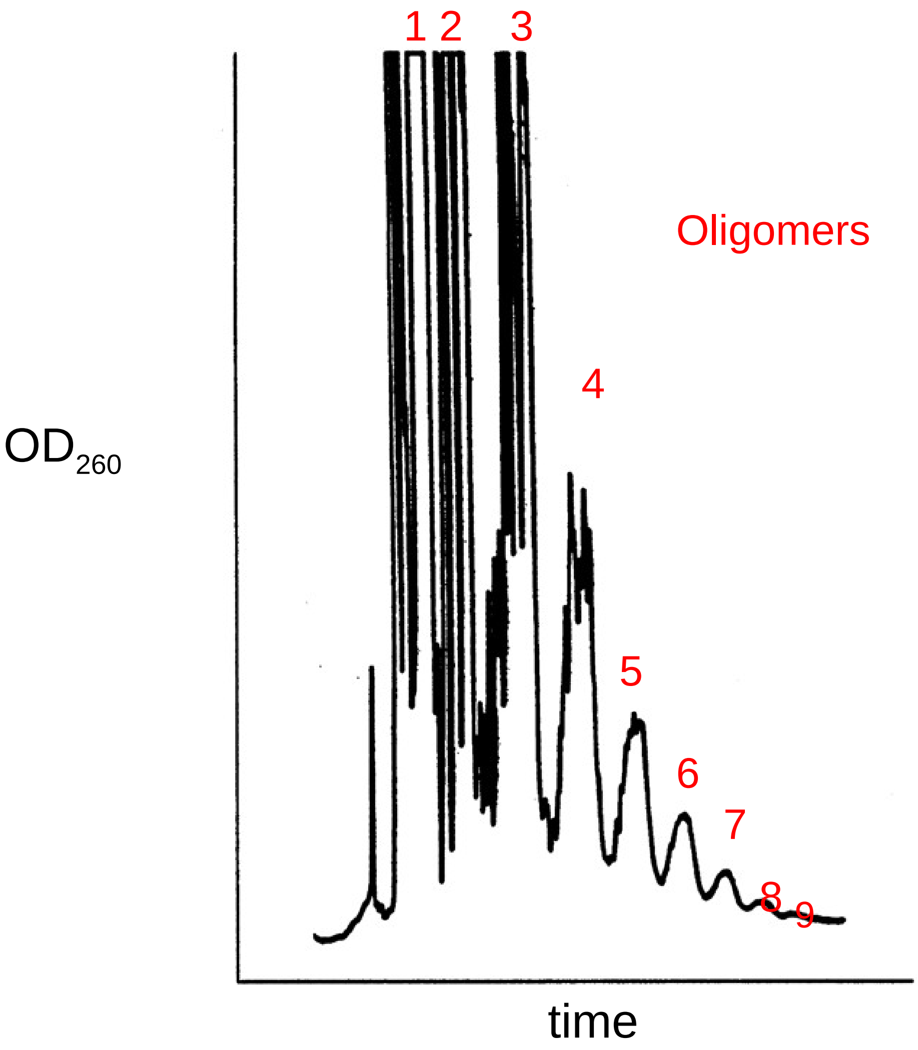
STRUCTURAL UNIT IN H^+- MONTMORILLONITE



Lattice has negative charge due to substitution of Mg^{+2} for Al^{+3} and Al^{+3} for Si^{+4}

Substitution is random and depends on the metal ions in the environment

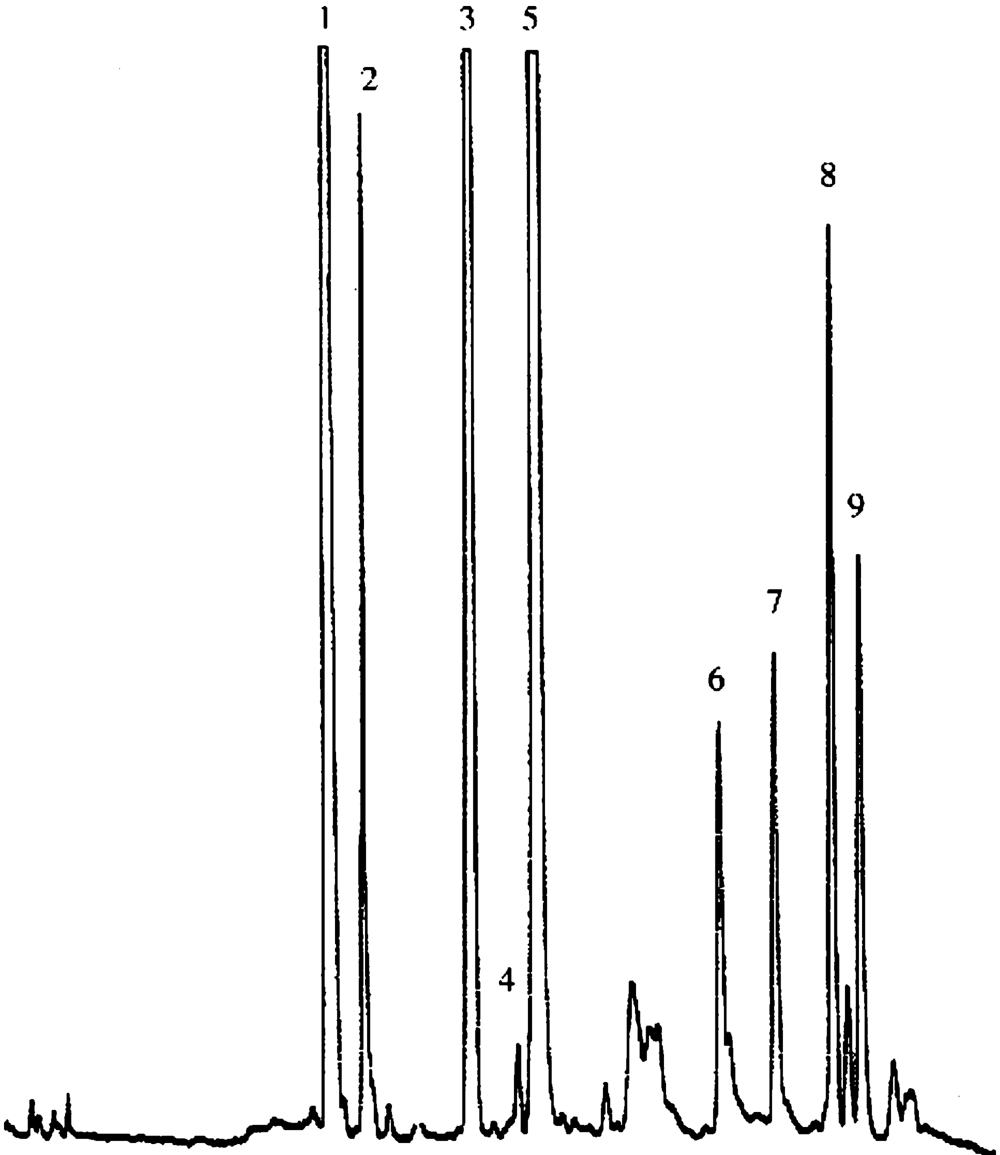
Oligomers from D-ImpA and D-ImpU



High Performance Liquid Chromatography (HPLC) Analysis of the Montmorillonite-Catalyzed Reaction of D-ImpA and D-ImpU

HPLC analysis of the dimers formed in the reaction of D, L-ImpA on montmorillonite followed by alkaline phosphatase hydrolysis

1) HEPES-pA adduct
2) D, L & L, D-c-$A^{3'}pA^{3'}p$
3) D, D & L, L-c-$A^{3'}pA^{3'}p$
4) 3', 5-c-AMP
5) Adenosine
6) D, D & L, L-$A^{2'}pA$
7) D, L & L, D-$A^{2'}pA$
8) D, D & L, L-$A^{3'}pA$
9) D, L & L, D-$A^{3'}pA$



Dimers formed by reaction of D, L-ImpA on montmorillonite followed by hydrolysis with alkaline phosphatase

Cyclic dimers		%
	D, L & L, D-c-$A^{3'}pA^{3'}p$	13.3
	D, D & L, L-c-$A^{3'}pA^{3'}p$	42.7
Linear dimers		
	D, D & L, L-$A^{2'}pA$	7.5
	D, D & L, L-$A^{3'}pA$	16.9
	D, L & L, D-$A^{2'}pA$	8.3
	D, L & L, D-$A^{3'}pA$	11.3
	Homochirality (%)	67.1

Reverse phase HPLC analysis of the products of the reaction of D, L-ImpU on montmorillonite followed by alkaline phosphatase hydrolysis.

1) D, D & L,L-c-$U^{2'}pU^{2'}p$
2) D,L & L,D-c-$U^{3'}pU^{3'}p$
3) HEPES-pU adduct
4) D, D & L,L-c-$U^{3'}pU^{3'}p$
5) $U^{5'}pp^{5'}U$
6) uridine
7) 3, 5-c-UMP
8) D,D & L, L-$U^{2'}pU$
9) D, L & L,D-$U^{2'}pU$
10) D, D & L,L-$U^{3'}pU$
11) D, L & L,D-$U^{3'}pU$

Reverse phase HPLC analysis of the dimers of the reaction of D,L-ImpU on montmorillonite followed by alkaline phosphatase hydrolysis

Cyclic dimers		%
	D, D & L, L-c-$U^{2'}pU^{2'}p$	3.0
	D, L & L, D-c-$U^{3'}pU^{3'}p$	46.9
	D, D & L, L-c-$U^{3'}pU^{3'}p$	18.0
Linear dimers		
	D, D & L, L-$U^{2'}pU$	14.7
	D, D & L, L-$U^{3'}pU$	3.6
	D, L & L, D-$U^{2'}pU$	11.7
	D, L & L, D-$U^{3'}pU$	2.0
Homochirality (%)		39.4

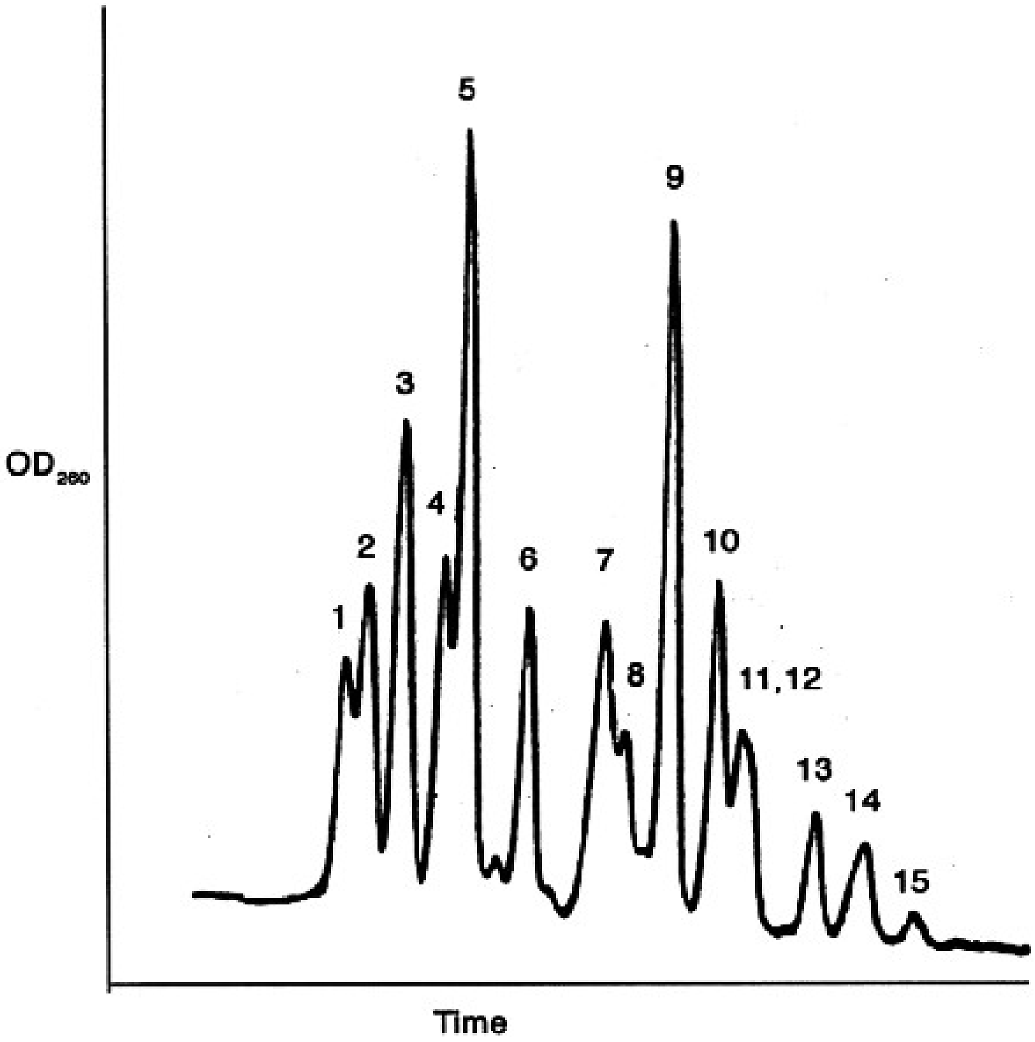
OD_{260}

5 10 15 20 25 30

Time, minutes

Reaction of D,L-ImpA with D,L-ImpU

HPLC analysis of the trimers formed in the reaction D, L-ImpA with D, L-ImpU on montmorillonite followed by alkaline phosphatase hydrolysis.



(1) D, D, D & L, L, L-$A^{2'}pA^{2'}pU$
(2) D, D, D & L, L, L-$A^{3'}pU^{2'}pU$
(3) D, D, D & L, L, L- $A^{3'}pU^{2'}pA$ &
D, D, D & L, L, L-$A^{3'}pU^{3'}pU$
(4) D, D, D & L, L, L-$A^{2'}pA^{3'}pU$
(5) D, D, D & L, L, L-$A^{3'}pA^{2'}pU$
(6, 7) non-standard nucleotides
(8) D, D, D & L, L, L-$A^{3'}pA^{3'}pU$
(9) D, D, D & L, L, L-$A^{3'}pA^{2'}pA$
(10) D, D, D & L, L, L-$A^{3'}pU^{3'}pA$
(11) D, L, D & L, D, L-$A^{3'}pA^{2'}pA$
(12) D, D, L & L, L, D-$A^{3'}pA^{2'}pA$
(13) D, D, D & L, L, L-$A^{3'}pA^{3'}pA$
(14) D, D, L & L, L, D- $A^{3'}pA^{3'}pA$
(15) D, L, D & L, D, L-$A^{3'}pA^{3'}pA$

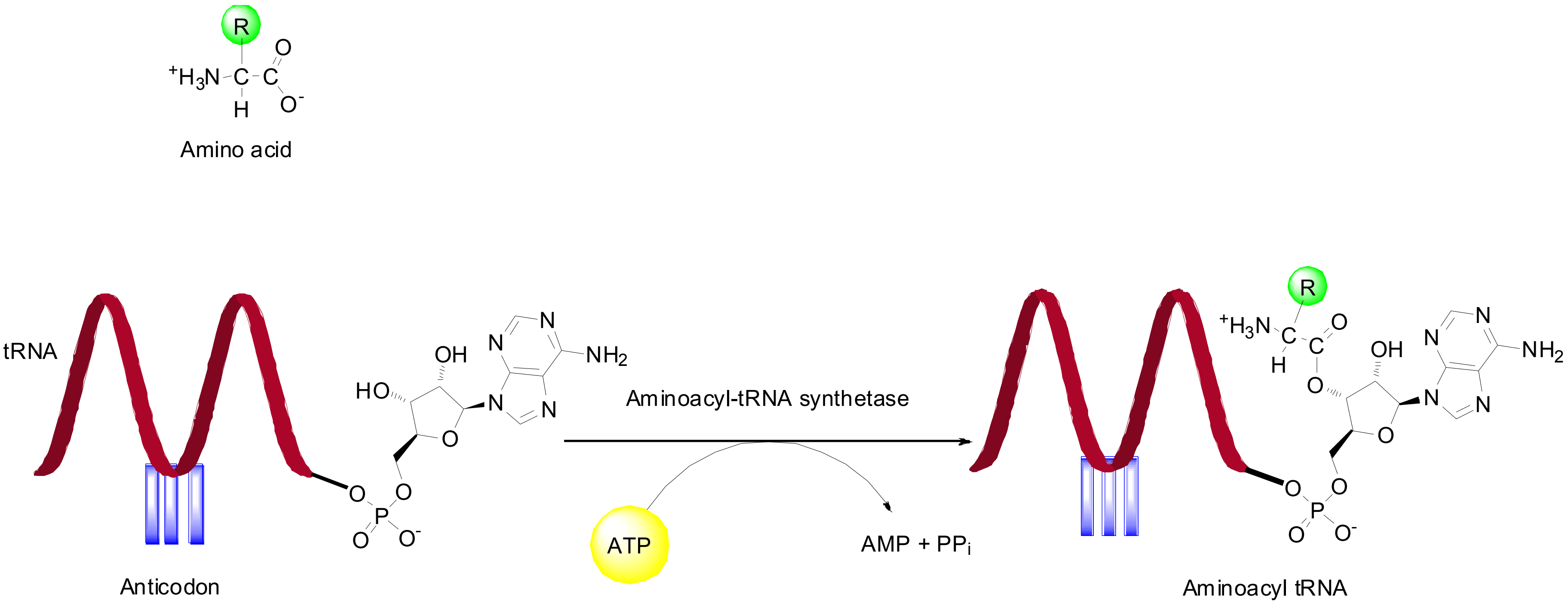
Trimers formed by reaction of D, L-ImpU with D, L-ImpA on montmorillonite followed by hydrolysis with alkaline phosphatase

Homochiral	D, D, D & L, L, L-$A^{2'}pA^{2'}pU$	4.74
	D, D, D & L, L, L-$A^{2'}pA^{3'}pU$	6.70
	D, D, D & L, L, L-$A^{3'}pU^{2'}pU$	9.95
	D, D, D & L, L, L-$A^{3'}pU^{2'}pU$ and	
	D, D, D & L, L, L-$A^{3'}pU^{2'}pA$	10.77
	D, D, D & L, L, L-$A^{3'}pU^{3'}pA$	2.23
	D, D, D & L, L, L-$A^{3'}pA^{2'}pU$	18.02
	D, D, D & L, L, L-$A^{3'}pA^{3'}pU$	7.60
	D, D, D & L, L, L-$A^{3'}pA^{2'}pA$	13.38
	D, D, D & L, L, L-$A^{3'}pA^{3'}pA$	2.64
Heterochiral		
	D, D, L & L, L, D-$A^{3'}pA^{2'}pA$ and	
	D, L, D & L, D, L-$A^{3'}pA^{2'}pA$	4.68
	D, D, L & L, L, D-$A^{3'}pA^{3'}pA$	3.21
	D, L, D & L, D, L-$A^{3'}pA^{3'}pA$	0.45
	Dihydro products	15.63
	Homochirality (%)	76.0

HOMOCHIRALITY OF THE REACTION PRODUCTS

	Dimers	Trimers
[a]D, L-ImpA	67%	33%
[a]D, L-ImpU	39%	11%
D, L-ImpA + D, L-ImpU	64%	76%

[a] Joshi, P C., Pitsch, S., Ferris, J. P. Orig Life Evol Biosph (2007)39: 3-26.


Amino acid
tRNA
Anticodon
Aminoacyl-tRNA synthetase
ATP
AMP + PP$_i$
Aminoacyl tRNA

Michael Yarus and Coworkers Discovered
A Similar Non-Specific System

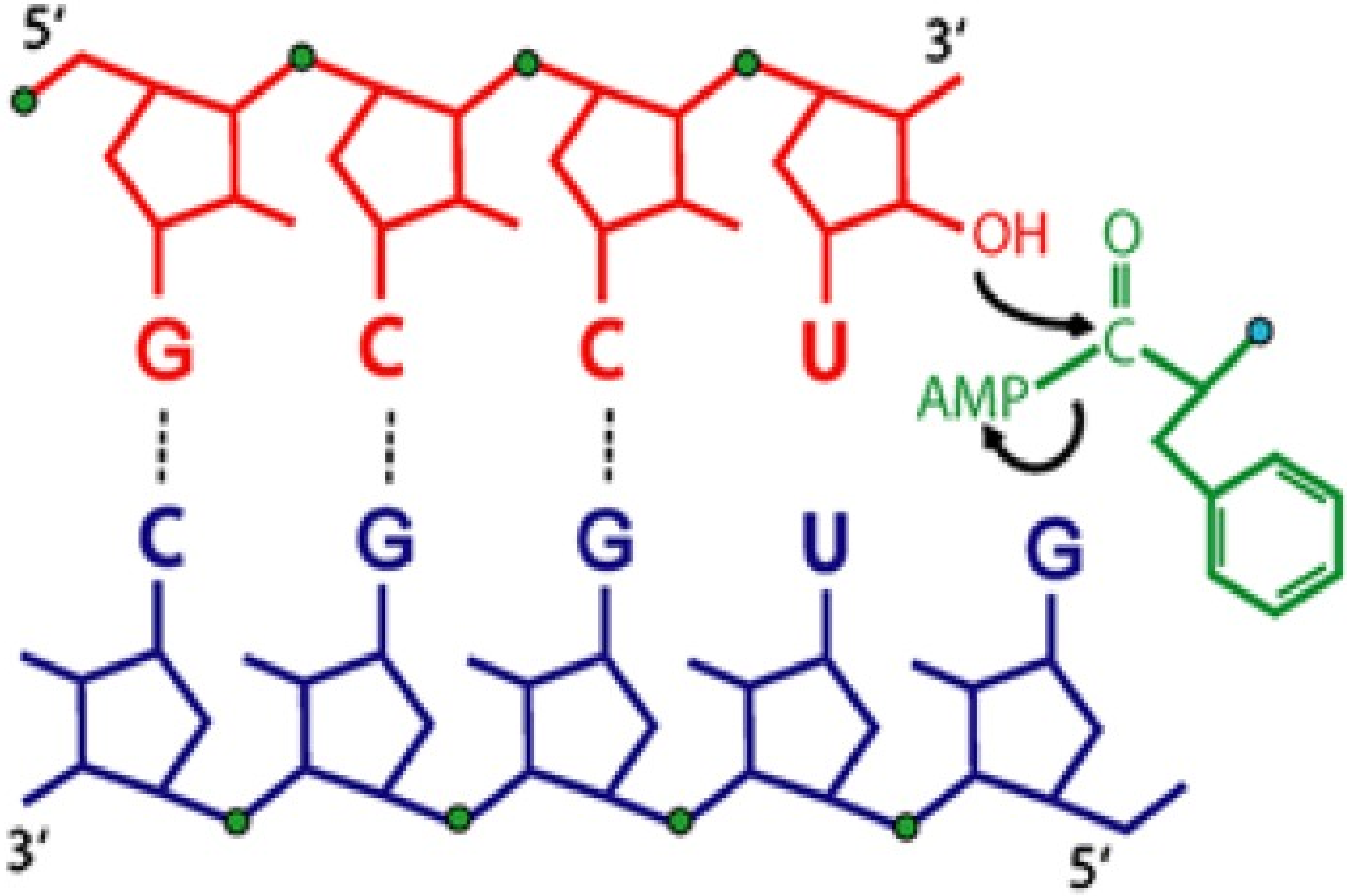
1. An important step in contemporary biosynthesis is the enzymatic formation of an aminoacyl-RNA.

2. A similar catalyst exists in a tiny 5’-nucleotide-long ribozyme. The small ribozyme initially phenylalanylates selectively at its terminally 2’-hydroxyl using PheAMP.

3. The small size and minimal requirements for regio-specific translational activity supports the hypothesis that the miniscule ribozyme participated in early forms of translation.

R. M. Turk, N. V. Chumachenko and M. Yarus,
PNAS, 107, 4585-4589, 2010

SMALLEST RIBOZYME



RANDOM SYNTHESIS DEALT WITH CATALYSIS

RNA

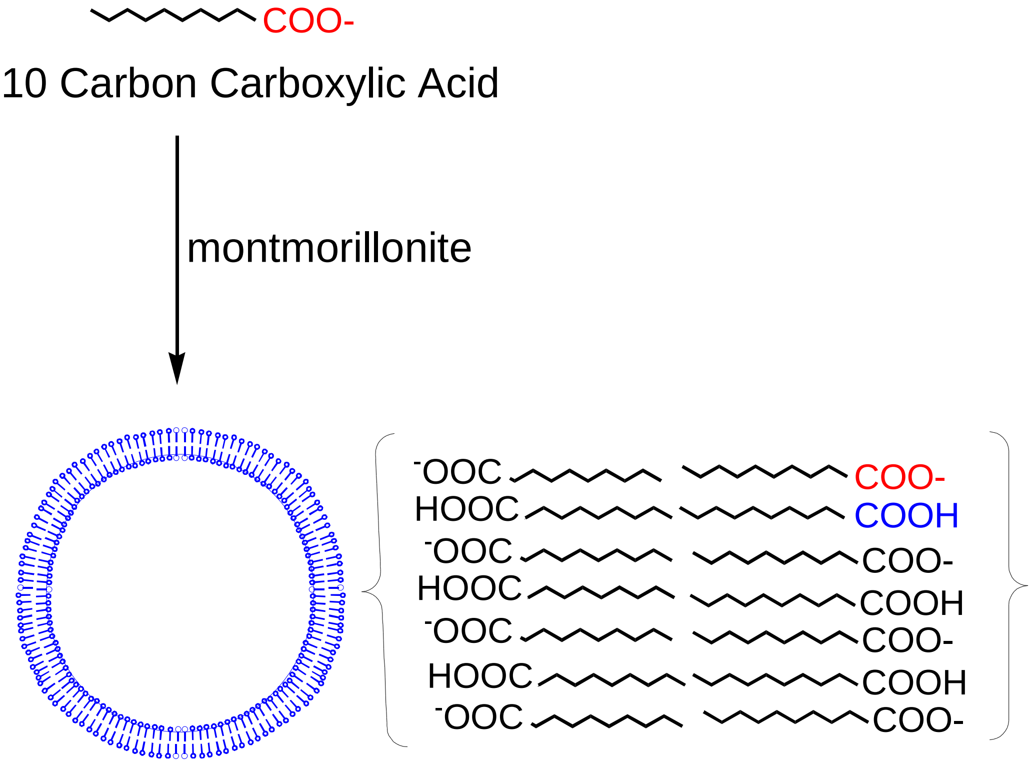
One copy of every 50 mer would result in 10^{30} RNAs weighing 10^{10} grams.

Joyce and Orgel The RNA World, 1993

Montmorillonite Catalysis

Montmorillonite is a selective (not specific) catalyst that generates a limited number of oligomer sequences.

MONTMORILLONITE -CATALYZED VESICLE FORMATION



COO-

10 Carbon Carboxylic Acid

montmorillonite

$^{-}$OOC COO-
HOOC COOH
$^{-}$OOC COO-
HOOC COOH
$^{-}$OOC COO-
HOOC COOH
$^{-}$OOC COO-

Spherical Vesicle
Cross-Section

Section of Vesicle Wall

Hanczyc et al., Science 2003, **302**, 618-622

STATUS OF OUR UNDERSTANDING OF THE ORIGIN OF THE RNA WORLD

“Prebiotic Chemists Nightmare: how to make any kind of self-replicating system from the intractable mixtures that are formed in experiments designed to simulate the chemistry of the primitive earth.”

G. F. Joyce and L. E. Orgel, *The RNA World*, 2nd Edition, 1999.

Formation of the monomers (RNA or pre-RNA) is still a big problem.

“ …it is difficult to imagine plausible routes by which such a ribozyme could emerge in a single step from prebiotic pools of random mono- and polynucleotides.”

D. B. McKay and J. E. Wedekind *The RNA World, 2nd Edition, 1999*.

Catalysis provides the way from monomers to non-random polymers.

ZIRCONS PROVIDE INFORMATION ABOUT THE EARTH IN THE 4.0-4.5 Gyr TIME PERIOD

Zircons ($ZrSi0_4$) don’t melt when subducted during plate tectonics
so information frozen in them when formed is not lost.

Continental crust on Earth at 4.4-4.5 Gyr. Earth cooled rapidly ($10x10^6$ years)

Harrison et al. Science **310**, 1947-1950 (2005)

Temperature of formation of zircons at 4.3.-4.4 Gyr the same as it is today.
Liquid water was present when the zircons formed.

Watson and Harrison Science **308**, 841 (2005)

It is likely that water was present on the prebiotic Earth

PREPARATION OF MONTMORILLONITE FOR STUDY AS A CATALYST

Montmorillonite has exchangeable cations like an ion exchange resin. Cations with montmorillonite are mainly Na^+ and Ca^{2+} and smaller amounts of other metal ions

- Convert to Homoionic Form Two Ways
  a. Add concentrated NaCl 3 times to montmorillonite.

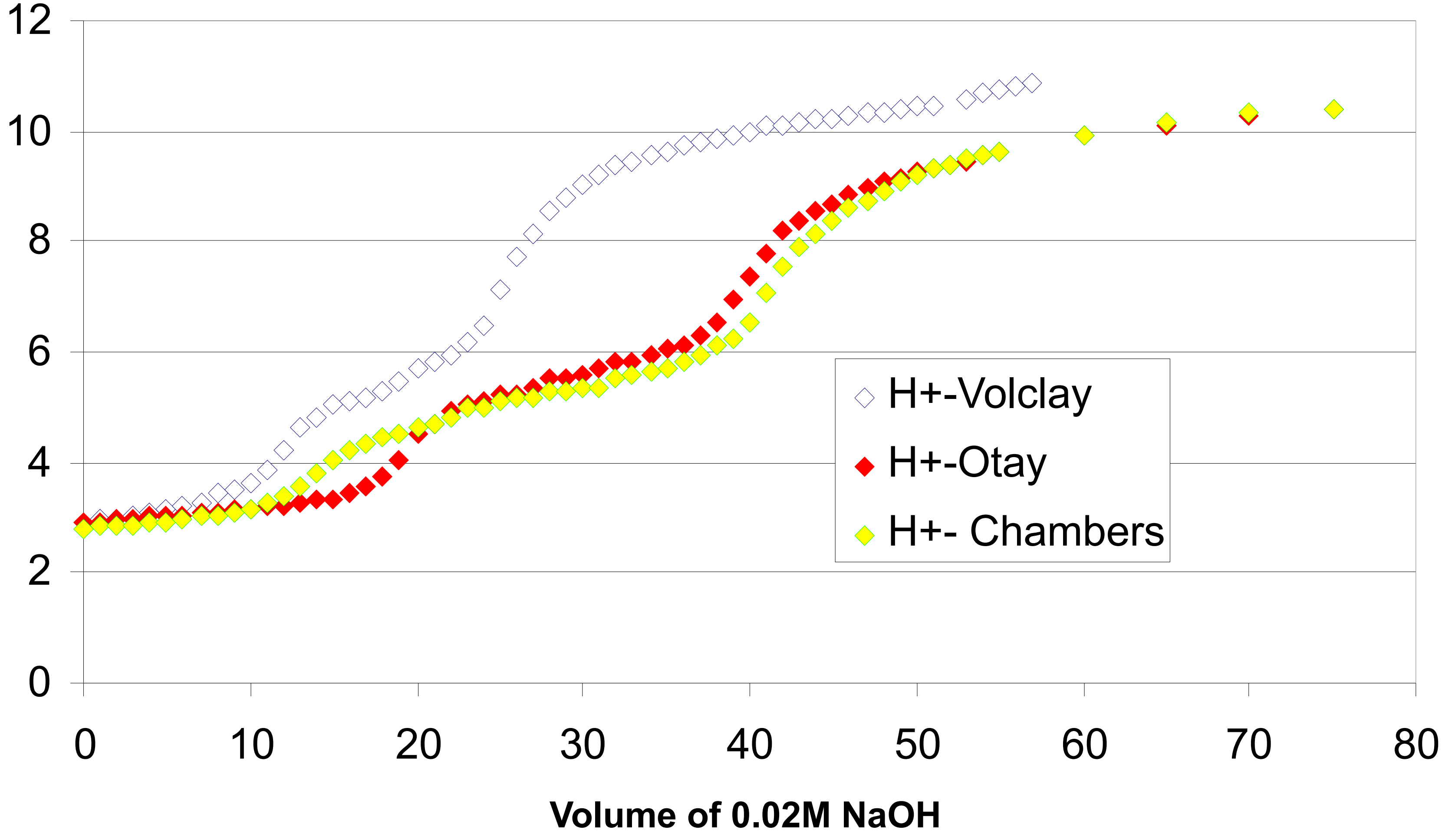
  b. Add cold concentrated HCl to montmorillonite 3 times to replace cations with H^+. Add NaOH to bring pH to 6-7 (Banin et al. Origins Life Evol. Biosph. 1985) (Bill Hagan).

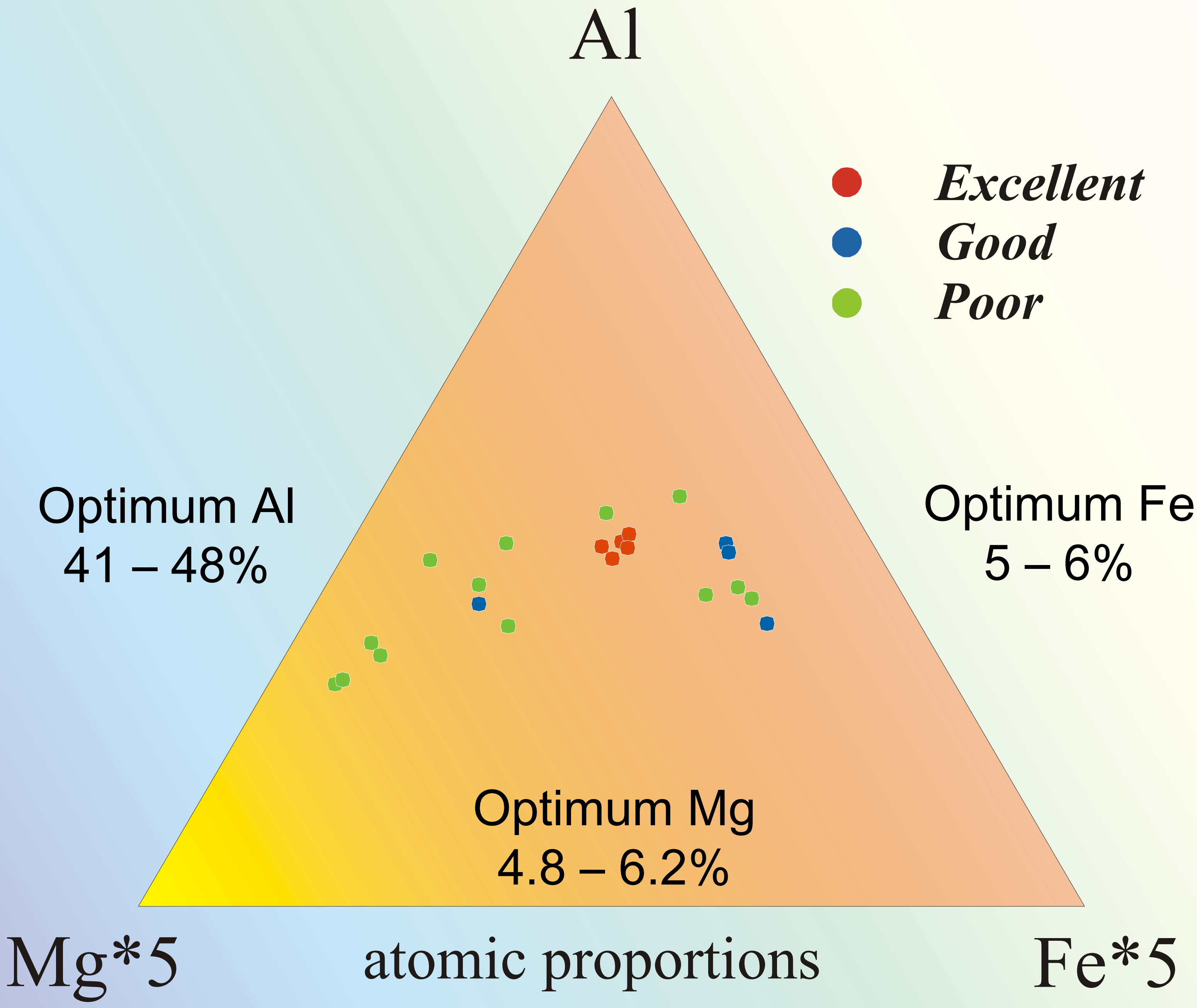
- No Montmorillonites Are Catalysts Using The Concentrated NaCl Procedure.

- Not All Montmorillonites Are Catalysts Even After Using The Banin Procedure

[redacted]

Titration of H$^+$-Volclay, H$^+$-Otay and H$^+$-Chambers clays with sodium hydroxide





Optimum Al
41 – 48%

Optimum Fe
5 – 6%

Optimum Mg
4.8 – 6.2%

Binding of ImpN To Catalytic And Non-Catalytic Montmorillonites (mol)

	ImpA	ImpU	ImpC
Volclay	20	1.5	2
Chambers	2	0.2	4
Otay	1.5	0.2	3

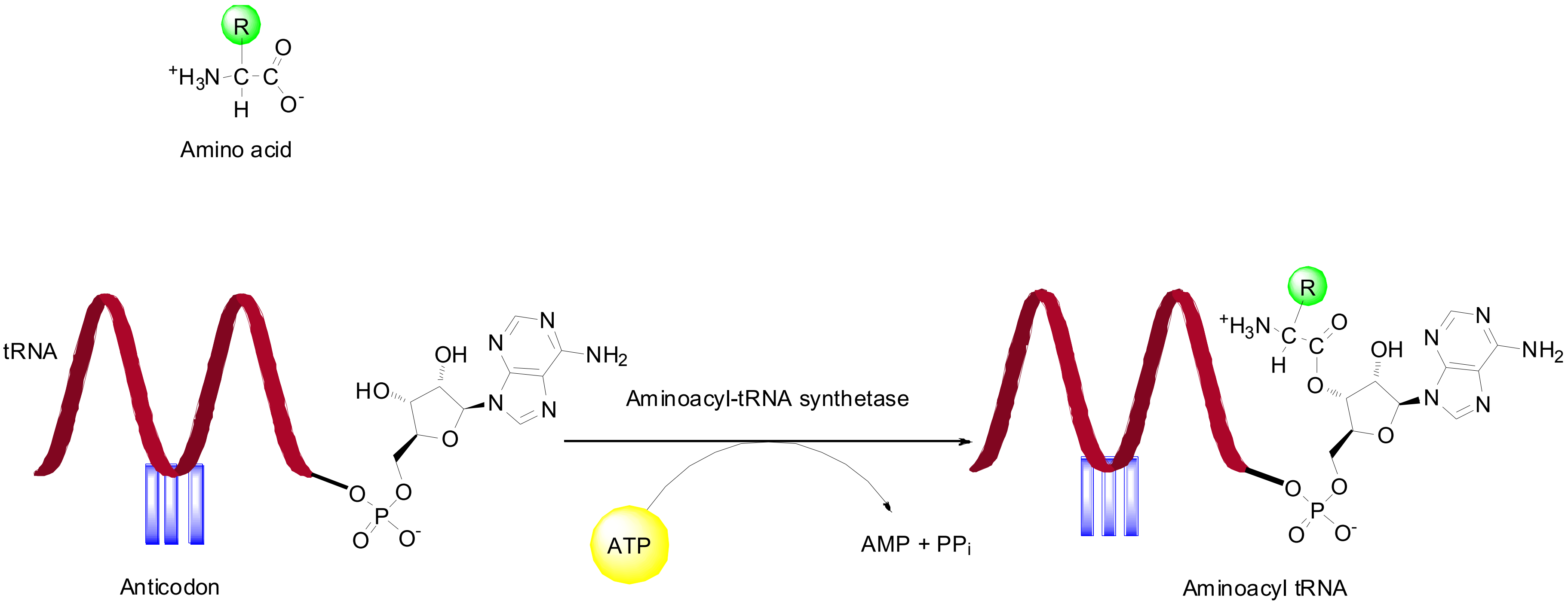
PREPARATION OF MONTMORILLONITE FOR STUDY AS A CATALYST

Montmorillonite has exchangeable cations like an ion exchange resin. Cations with montmorillonite on Earth are mainly Na^+ and Ca^{2+} with smaller amounts of other metal ions

- Add cold concentrated HCl to montmorillonite 3 times to replace cations with H^+. Add NaOH to bring pH to 6-7 (Banin et al. Origins Life Evol. Biosph. 1985)

- About 20% of the montmorillonites have catalytic activity after conversion to the Na^+ form by this procedure. No catalytic activity without acid treatment.

P. Joshi and G, Gluzak


R
^+H_3N
C
C
O
O^-
H
Amino acid
tRNA
HO
OH
N
N
NH_2
N
N
O
O
O
P
O
O^-
Anticodon
Aminoacyl-tRNA synthetase
ATP
AMP + PP_i
R
^+H_3N
C
C
O
H
O
OH
N
N
NH_2
N
N
O
O
O
P
O
O^-
Aminoacyl tRNA