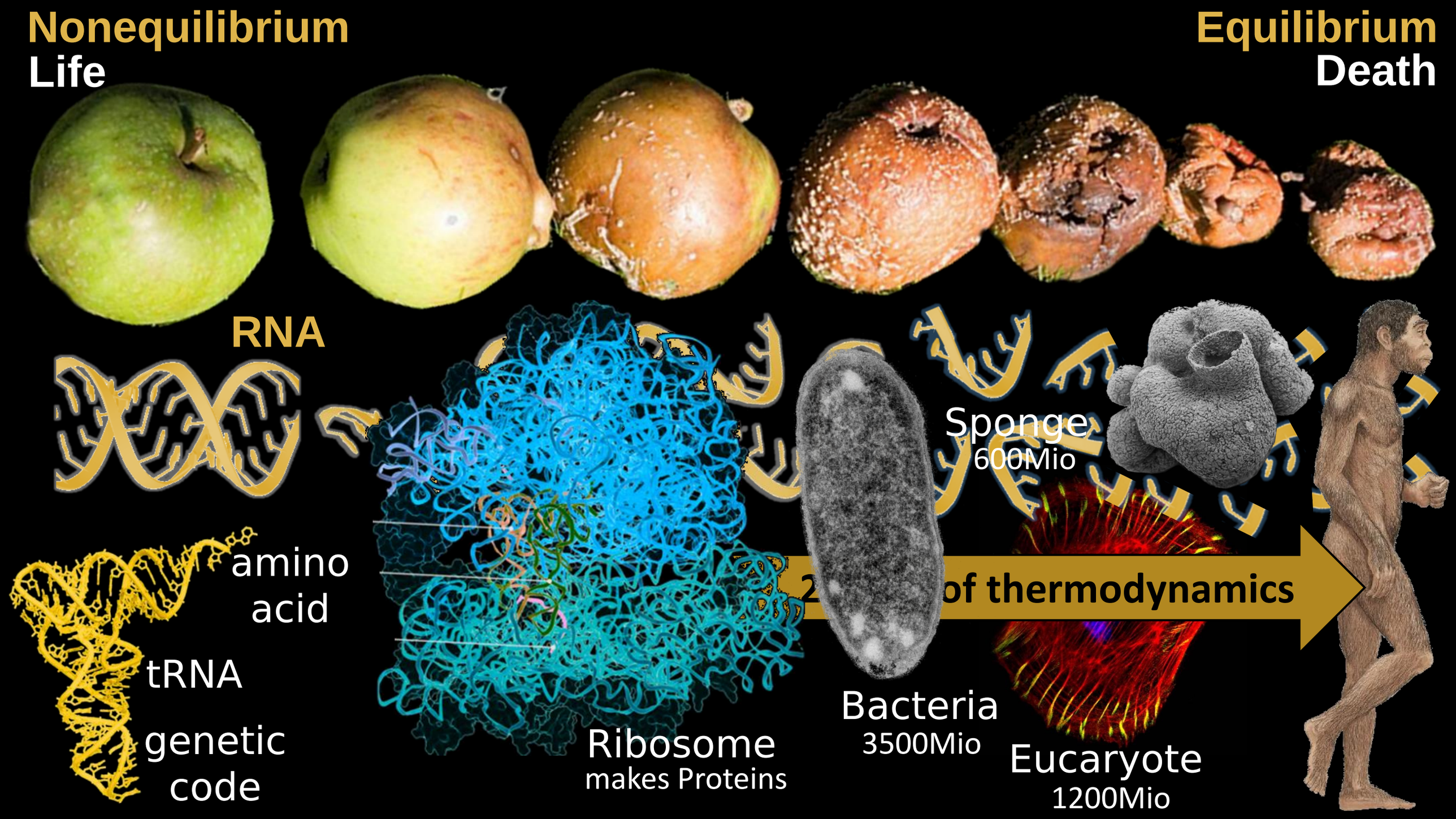
Lessons learned when trying to recreate the Origin of Life

Dieter Braun

Biophysics, Center for NanoScience, LMU Munich

0. Introduction
1. Molecules
2. Entropy
3. Early Earth


Nonequilibrium
Life
Equilibrium
Death
RNA
amino acid
tRNA
genetic code
Ribosome
makes Proteins
Bacteria
3500Mio
Sponge
600Mio
Eucaryote
1200Mio
of thermodynamics

Origin of Life in the lab

Equilibrium
Death

Nonequilibrium
Life

replication, mutation
and selection

Creating RNA-Life by triggering Darwinian Evolution

RNA

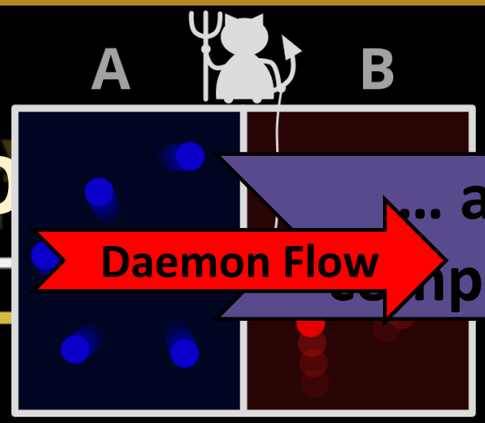
1.Molecules

2. Tackling Entropy

2nd law of thermodynamics

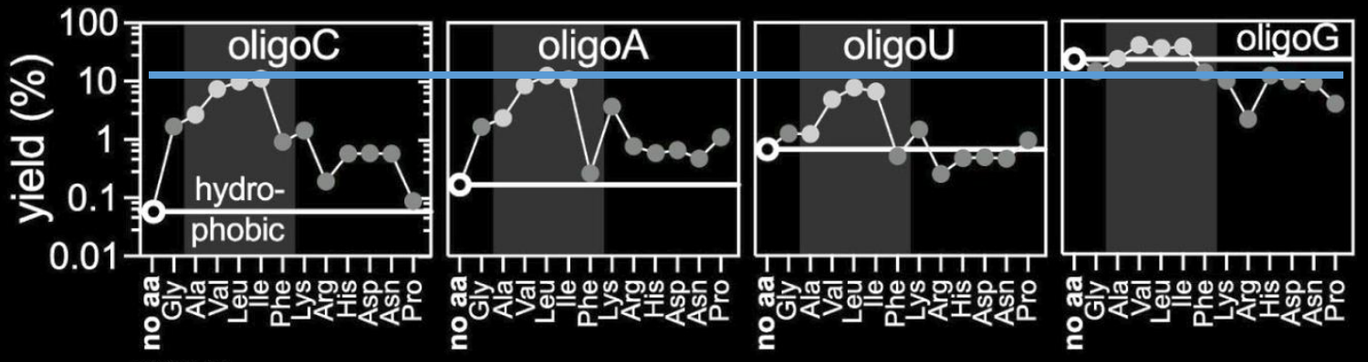
3. Early Earth driving evolutio

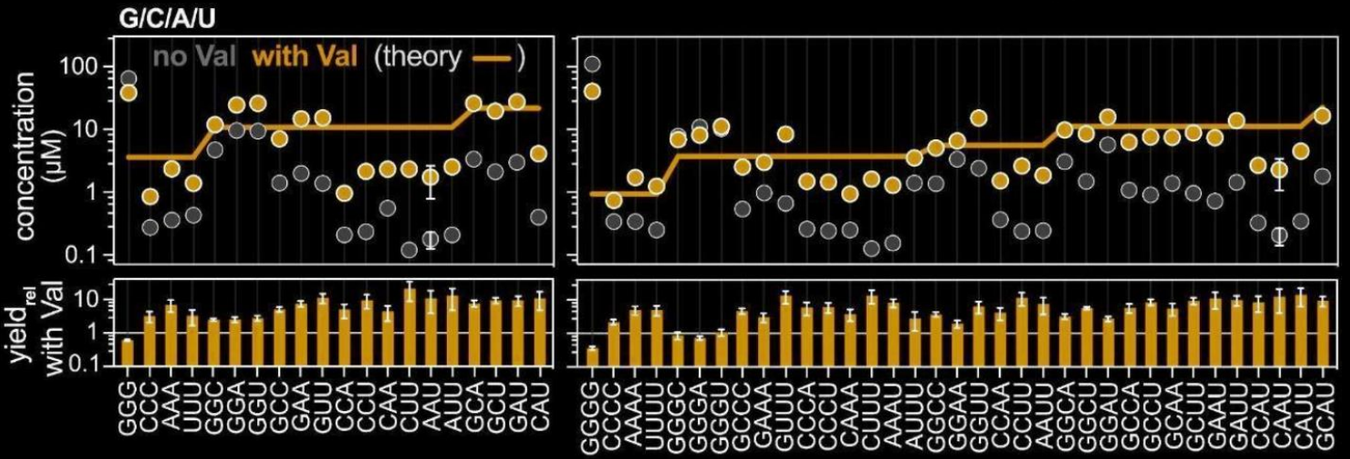
Maxwell's
Daemon

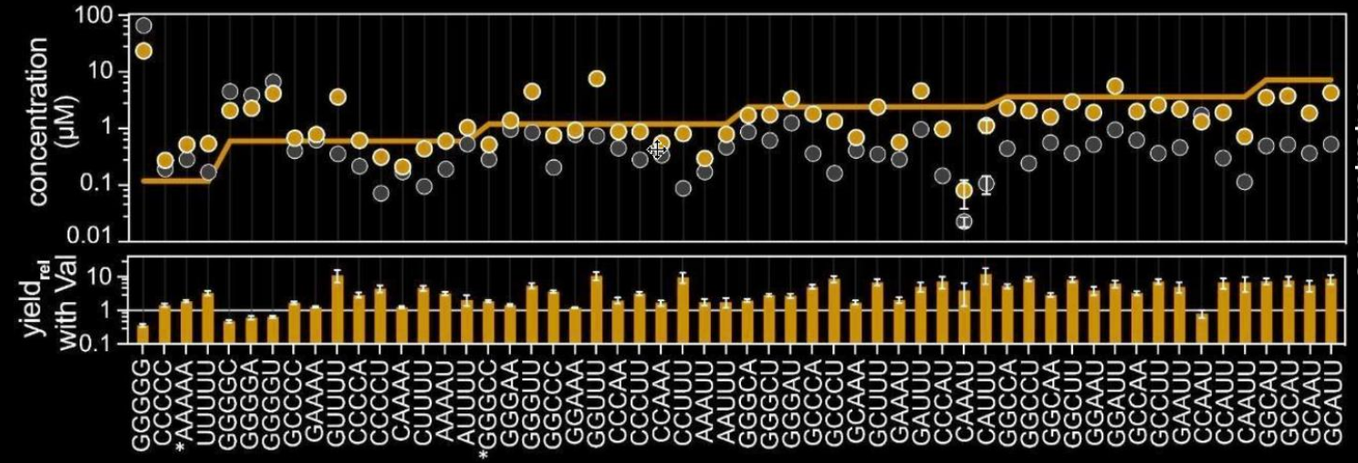


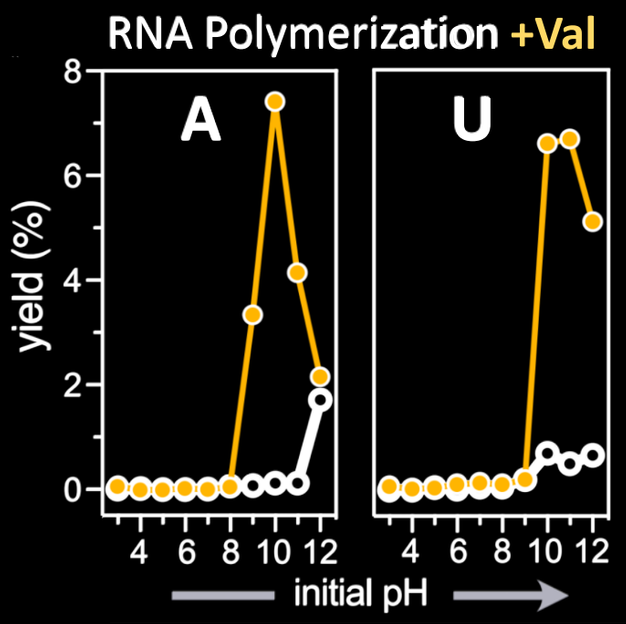
... and hence
not possible
in equilibrium

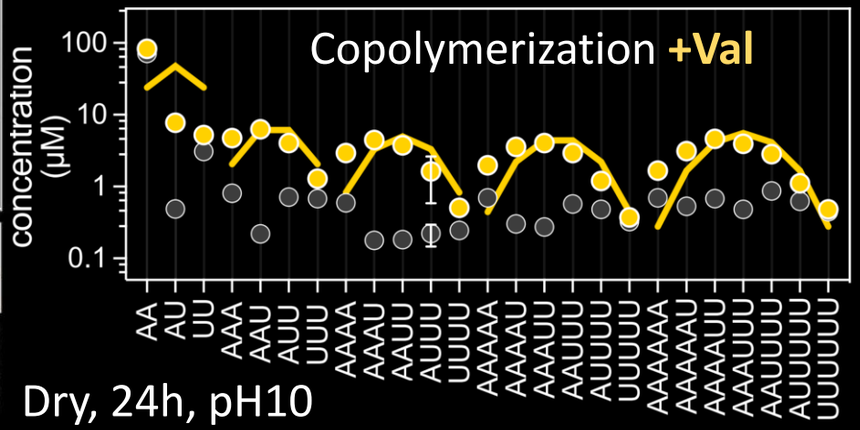
1. Molecules





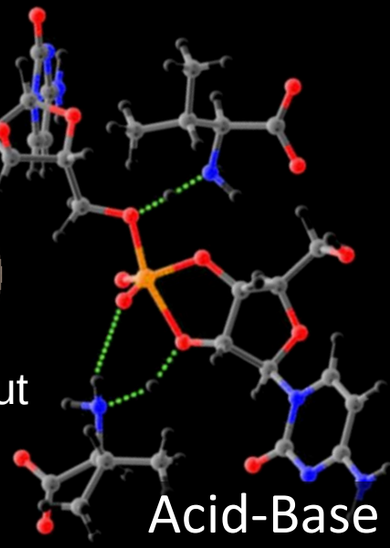






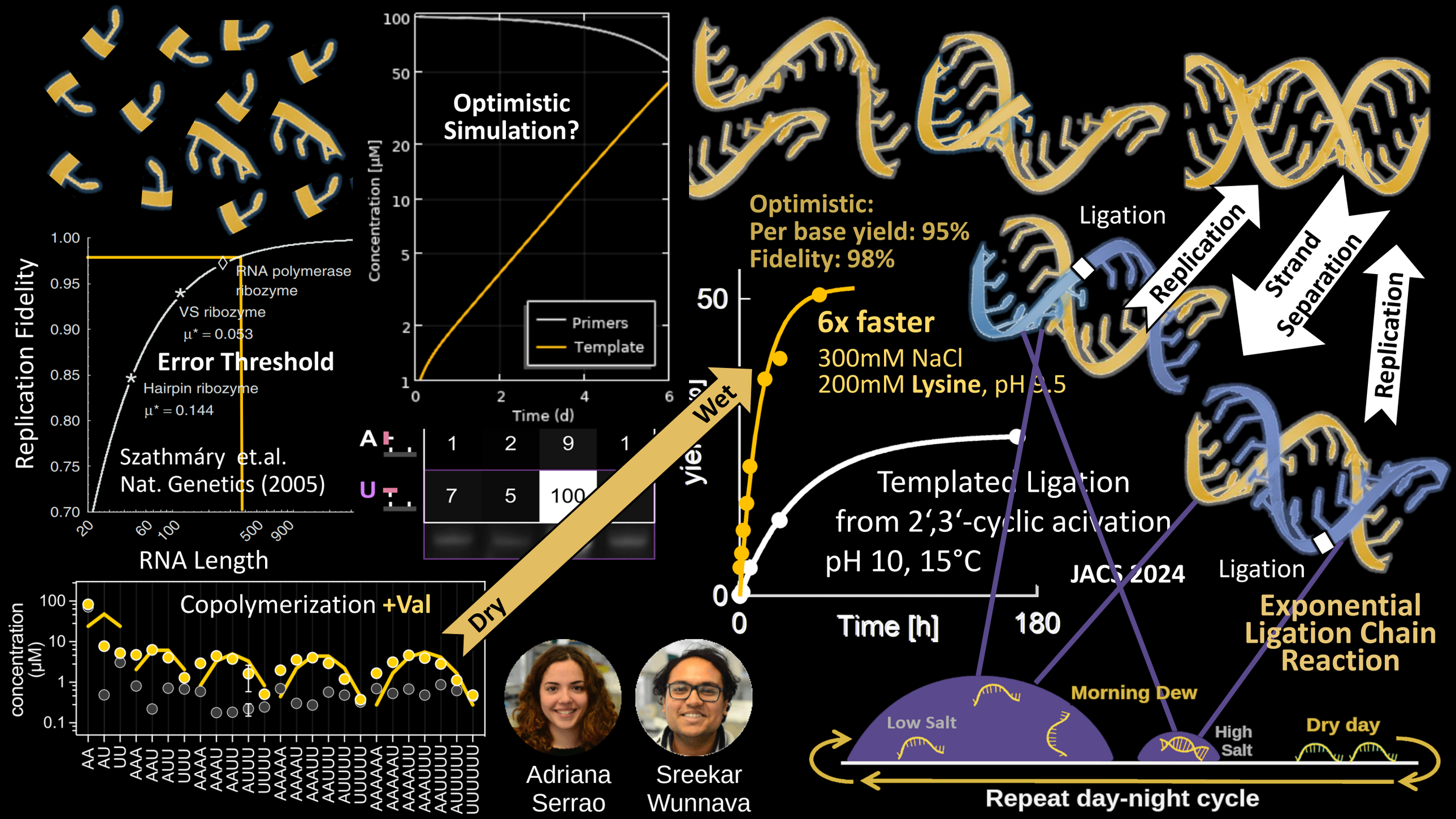
Saroj Rout

Sreekar Wunnava



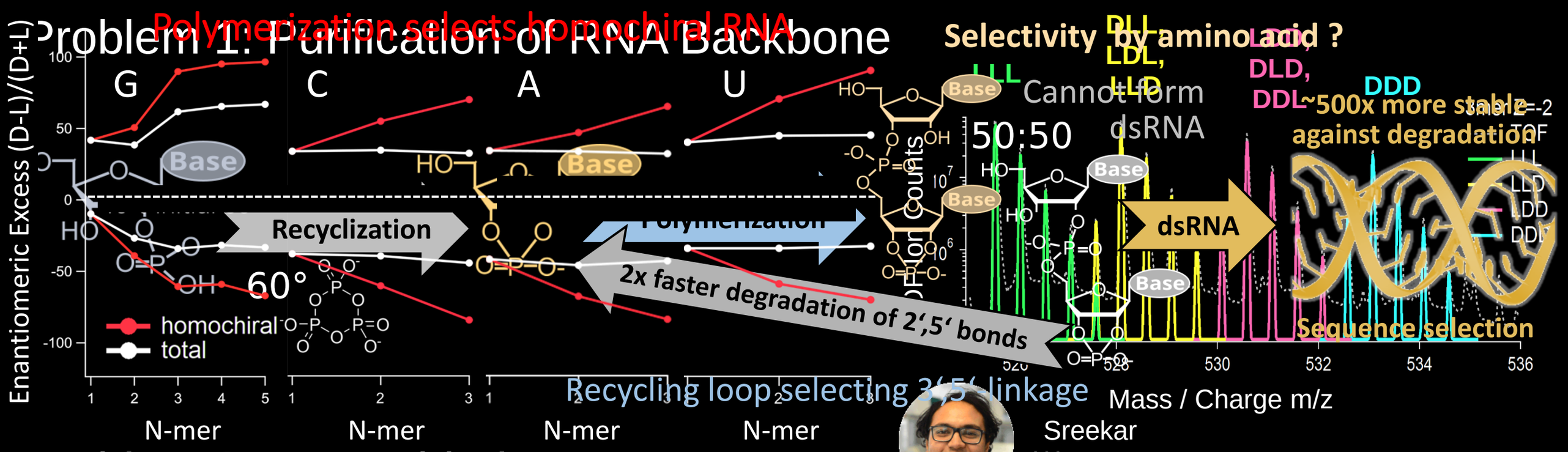
Acid-Base catalysis

Judit Sponer


Optimistic Simulation?
Concentration [µM]
Time (d)
Primers
Template
Optimistic:
Per base yield: 95%
Fidelity: 98%
6x faster
300mM NaCl
200mM Lysine, pH 9.5
Templated Ligation
from 2‘,3‘-cyclic activation
pH 10, 15°C
JACS 2024
Time [h]
Ligation
Replication
Strand Separation
Replication
Ligation
Exponential Ligation Chain Reaction
Morning Dew
Low Salt
High Salt
Dry day
Repeat day-night cycle
Wet
Dry
Replication Fidelity
RNA polymerase ribozyme
VS ribozyme
μ* = 0.053
Error Threshold
Hairpin ribozyme
μ* = 0.144
Szathmáry et.al.
Nat. Genetics (2005)
RNA Length
Copolymerization +Val
concentration (µM)
Adriana Serrao
Sreekar Wunnava

Problem 1: Purification of RNA Backbone

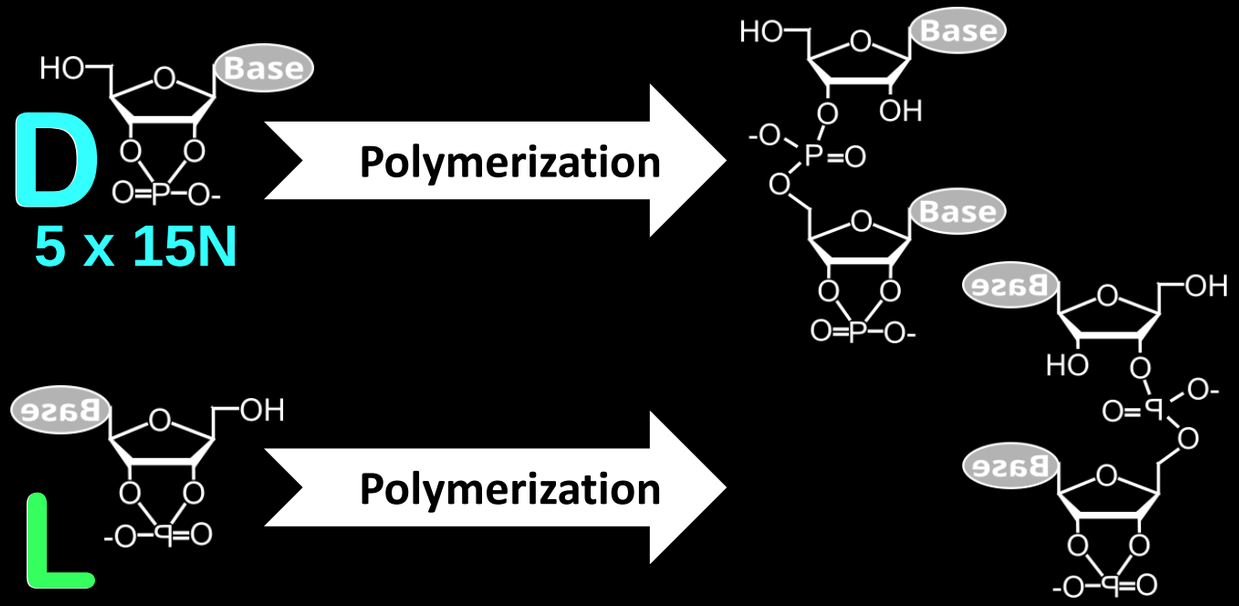
Polymerization selects homochiral RNA



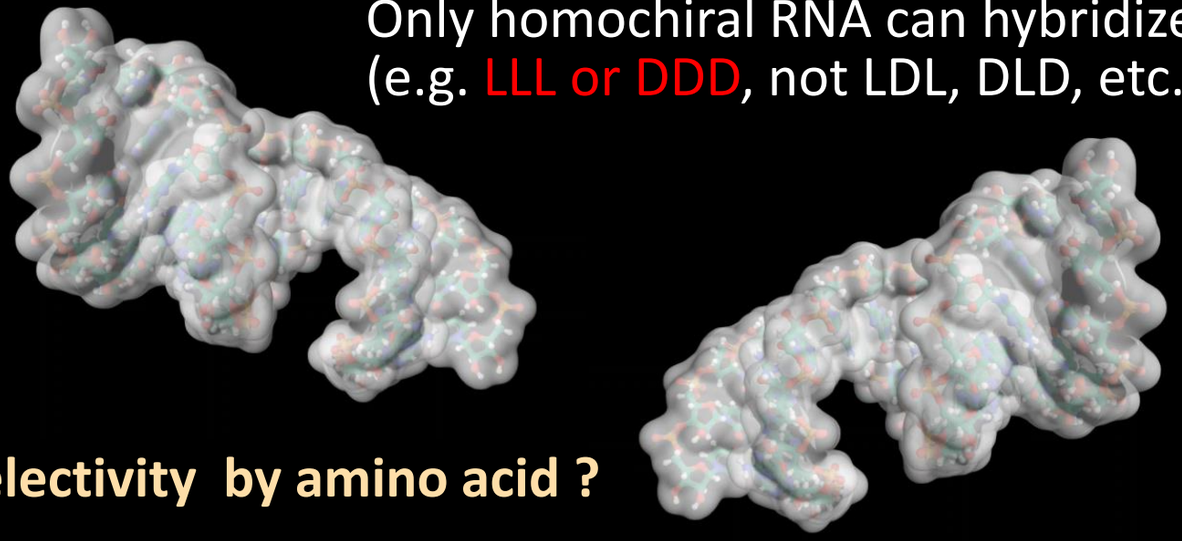
Recycling loop selecting 3‘,5‘ linkage

Sreekar Wunnava

Problem 2: Homochiral RNA



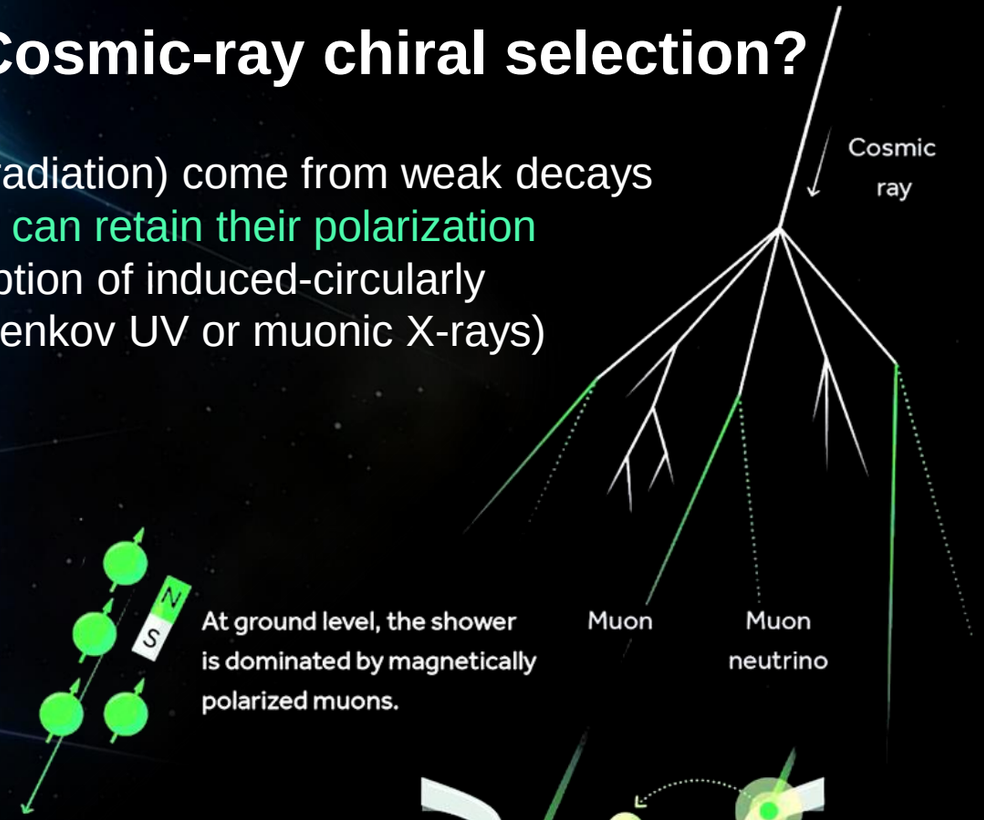
Only homochiral RNA can hybridize (e.g. LLL or DDD, not LDL, DLD, etc.)

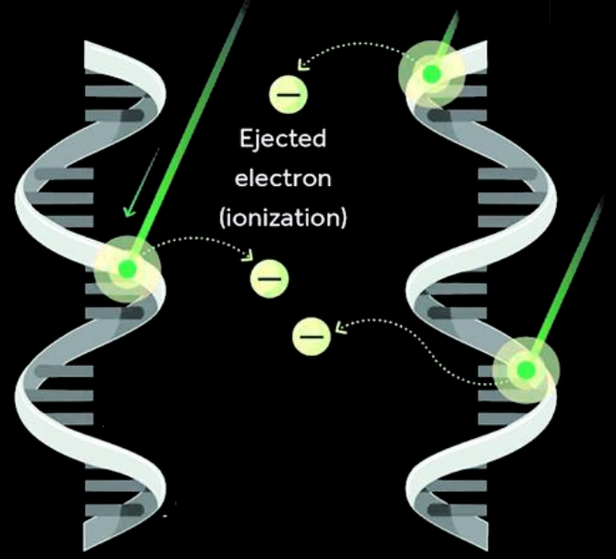


Selectivity by amino acid ?

Cosmic-ray chiral selection?

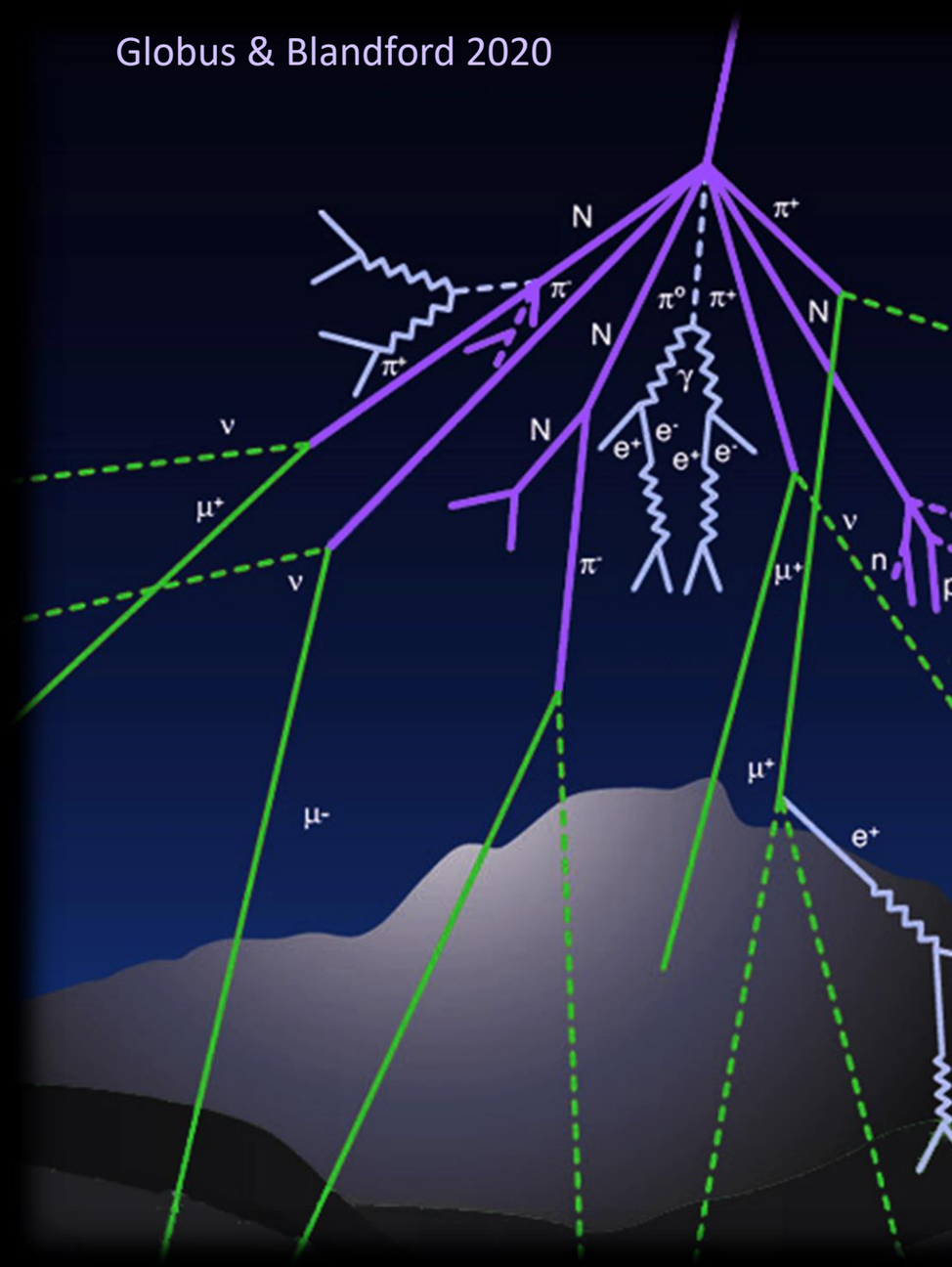
- Muons (85% of cosmic radiation) come from weak decays
- Unlike electrons, muons can retain their polarization
- Direct damage or absorption of induced-circularly polarized photons (Cherenkov UV or muonic X-rays)





Roger Blandford, Stanford
Stephen Blundell, Oxford
Dieter Braun, LMU/ORIGINS
Noémie Globus, UC Santa Cruz, Stanford
Stephan Paul, TUM/ORIGINS
Thomas Prokscha, **Paul Scherrer Institut**

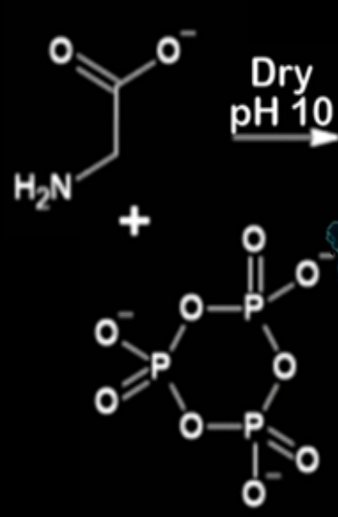
Globus & Blandford 2020



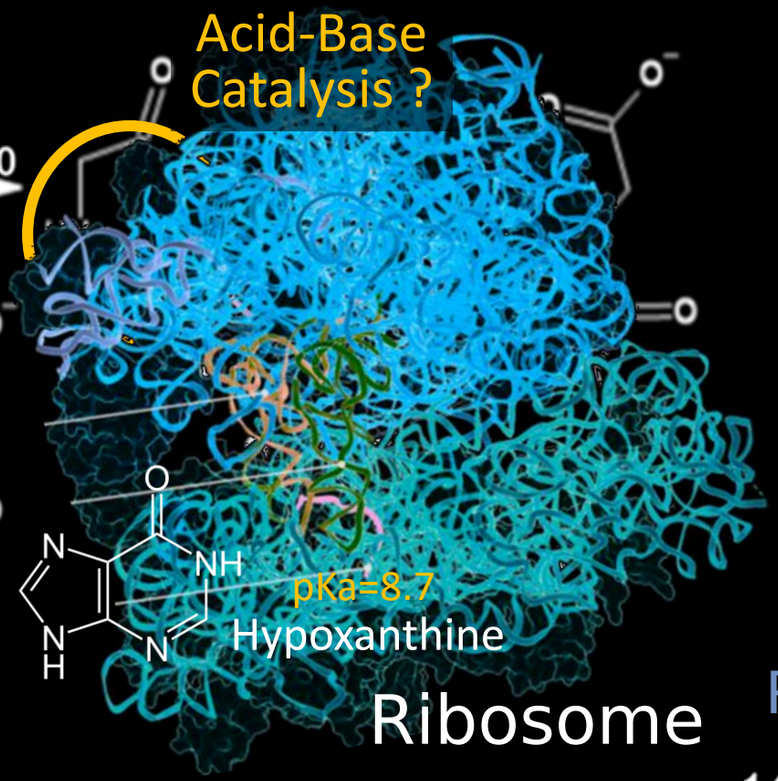
Interaction of primary cosmic ray (p^+) with atmospheric atoms (N, O)
→ Generation of $K^\pm$ and $\pi^\pm$ → Decay of $\pi^\pm$ → Generation of $\mu^\pm$ and ν_μ

Ring opening
Peptide synthesis

pH10
60°C

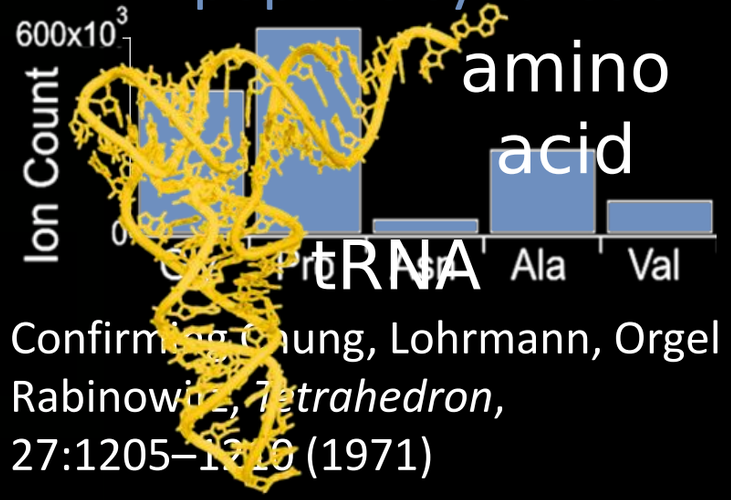


Acid-Base
Catalysis ?



Ribosome

Dipeptide synthesis



amino
acid

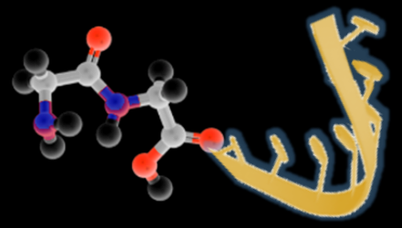
tRNA

Confirmation Chung, Lohrmann, Orgel & Rabinowitz, *Tetrahedron*, 27:1205–1210 (1971)

2‘,3‘-cyclic
Nucleotides

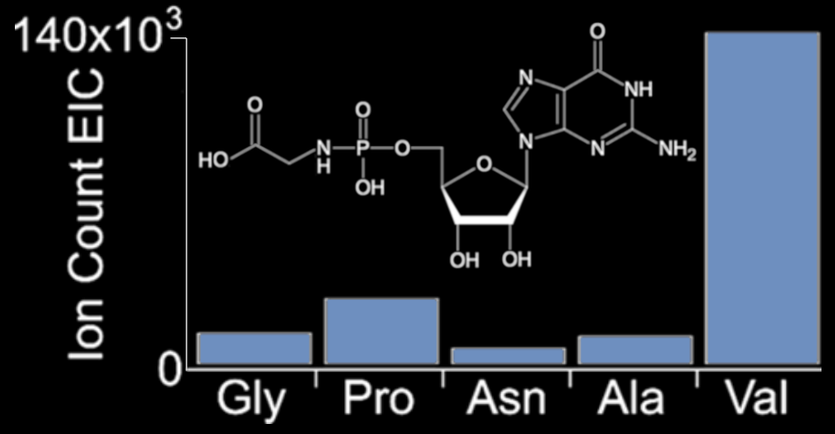
Amino
Acids

RNA



RNA-Peptide
Hybrids

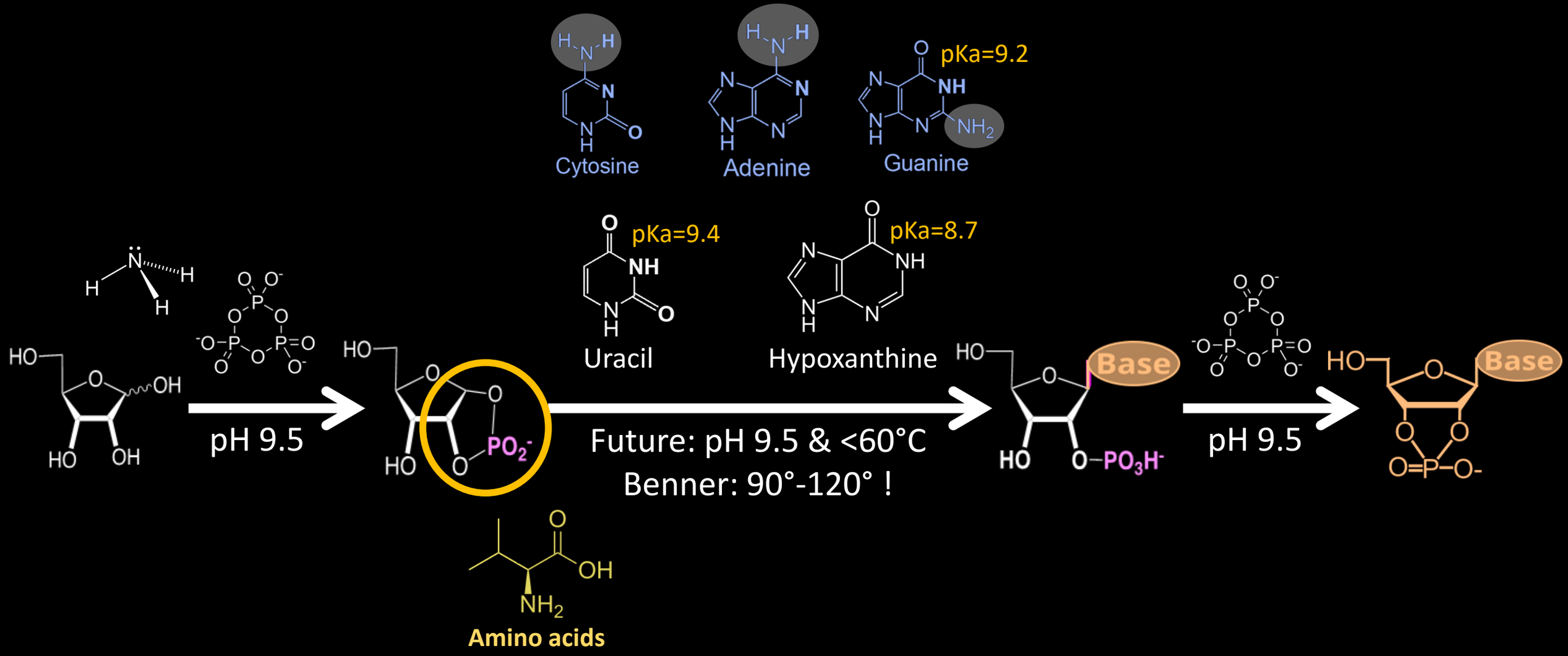
RNA-amino-acid conjugates



with Moran Frenkel-Pinter

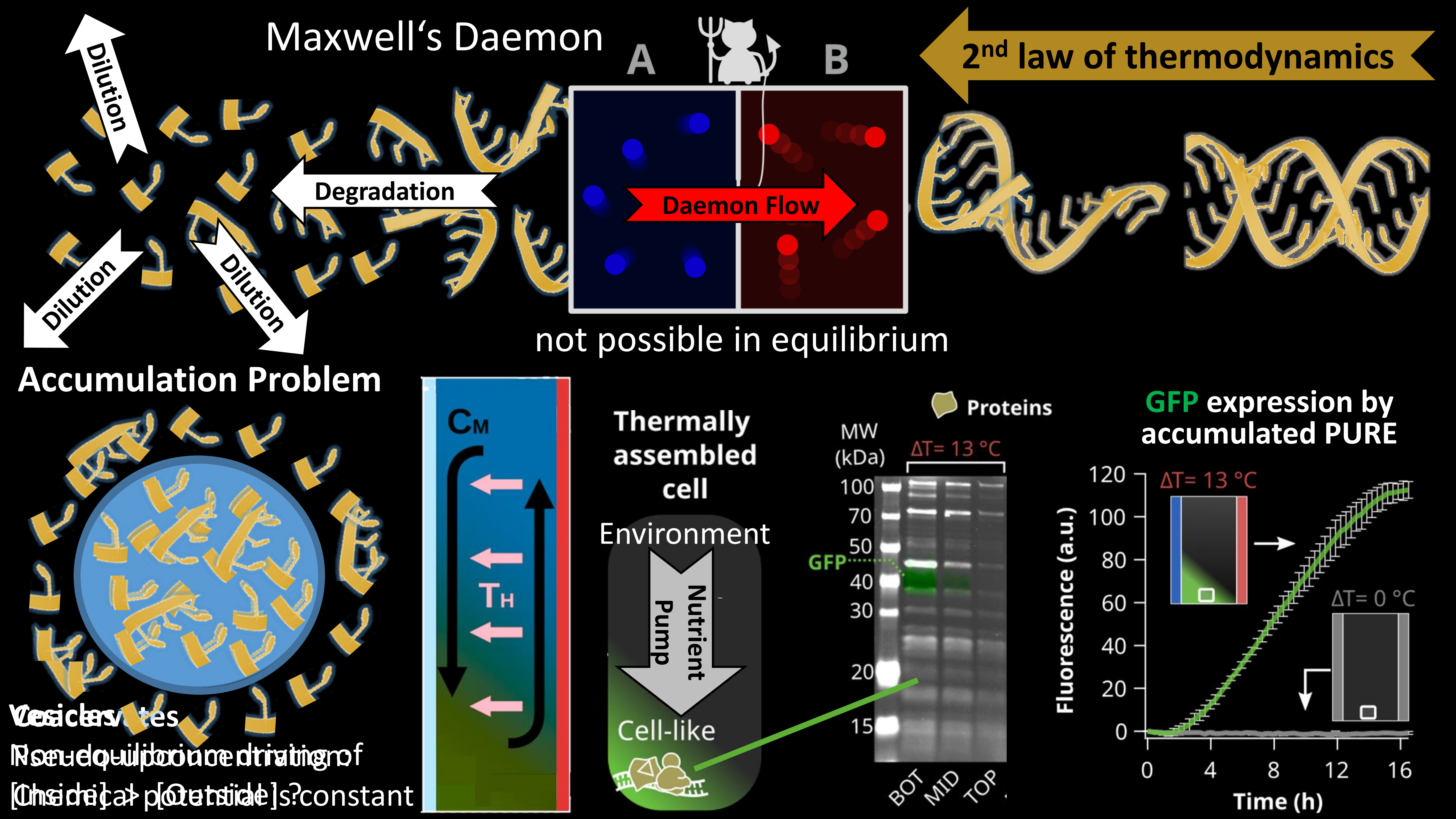
Prospect:
Coevolution of RNA and Peptides
towards early translation?

Synthesis of Nucleotides by Ring opening conjugation?



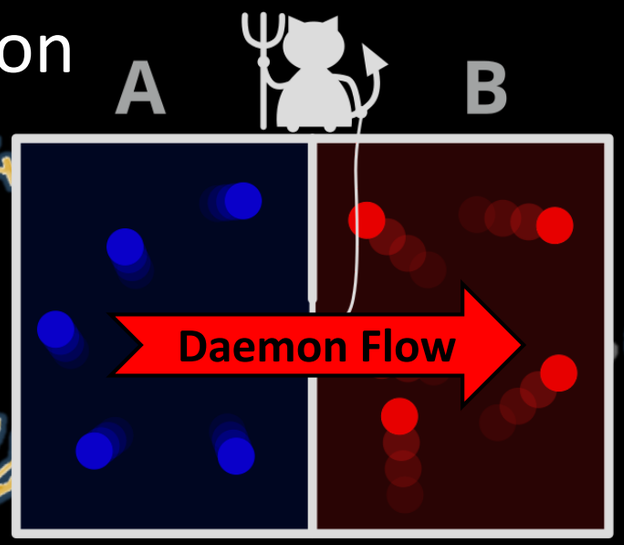
„Unboronified“ from Benner et.al.
Collaboration with Ram Krishnamurthy

2. Tackling Entropy



Maxwell's Daemon
A
B
2nd law of thermodynamics
Dilution
Degradation
Dilution
Dilution
Daemon Flow
not possible in equilibrium
Accumulation Problem
Vesicles
Coacervates
Non-equilibrium driving of
Pseudo-upconcentration:
[Inside] > [Outside]?
Chemical potentials constant
CM
TH
Thermally assembled cell
Environment
Nutrient Pump
Cell-like
Proteins
MW (kDa)
ΔT= 13 °C
100
70
50
GFP
40
30
20
15
BOT
MID
TOP
GFP expression by accumulated PURE
Fluorescence (a.u.)
120
100
80
60
40
20
0
ΔT= 13 °C
ΔT= 0 °C
0
4
8
12
16
Time (h)

Maxwell‘s Daemon



2nd law of thermodynamics

Dilution

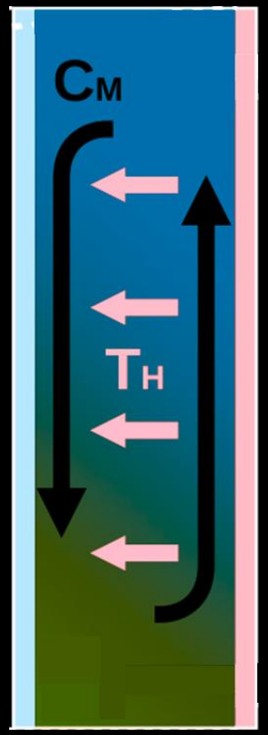
Degradation

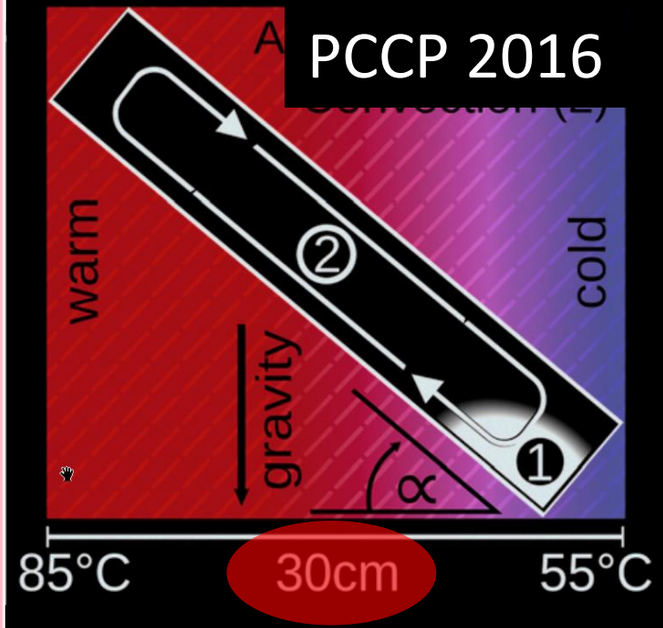
Dilution

Dilution

not possible in equilibrium

Accumulation Problem

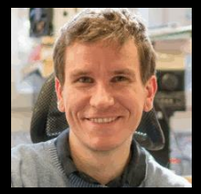




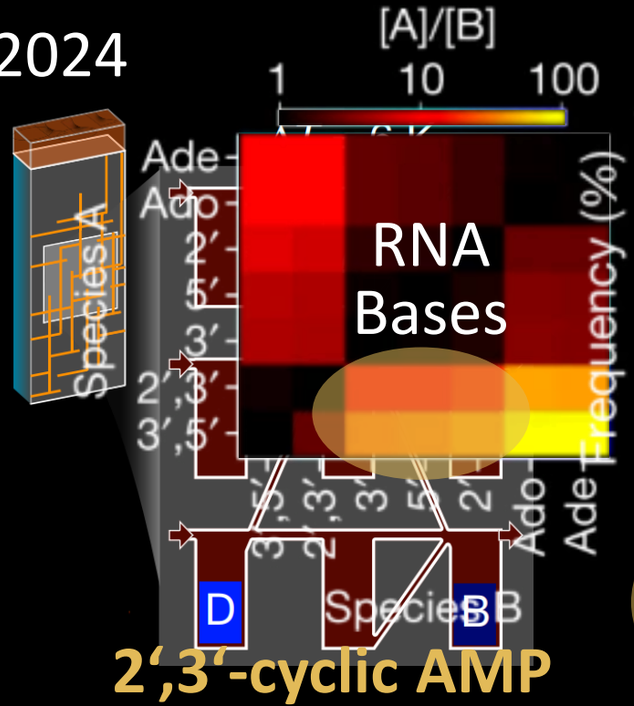
PCCP 2016

Nature 2024

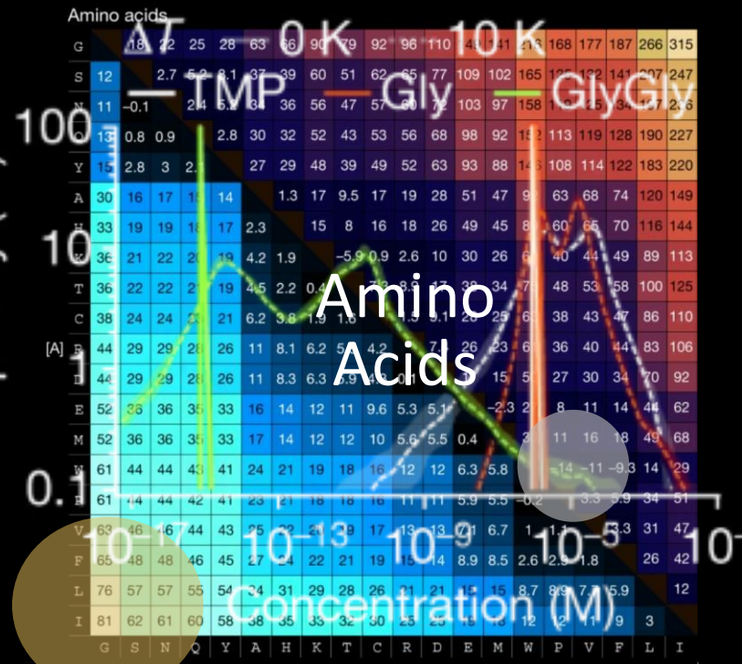
Thomas Matreux



Christof Mast



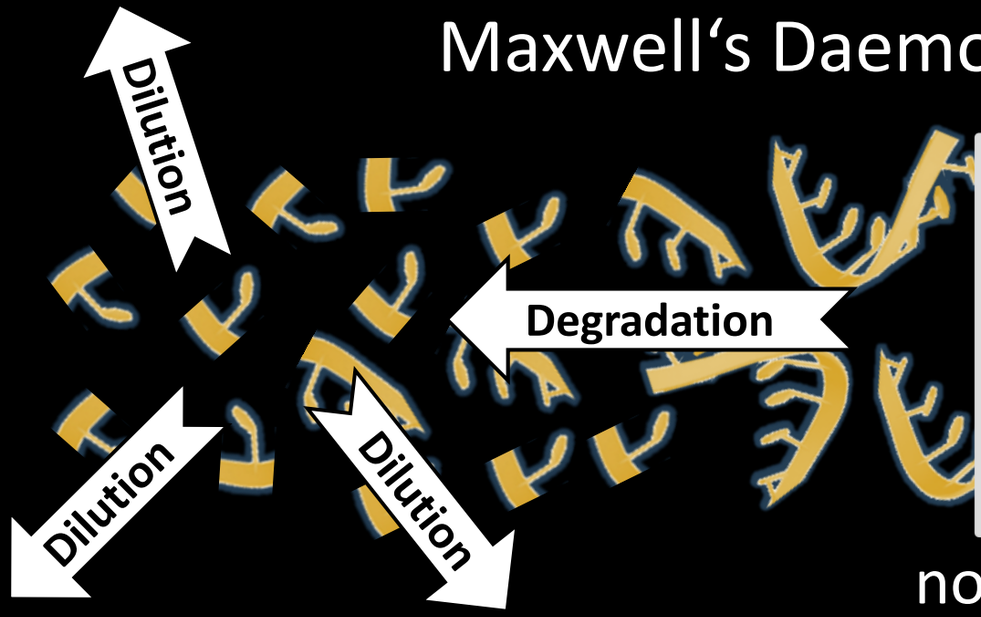
2‘,3‘-cyclic AMP

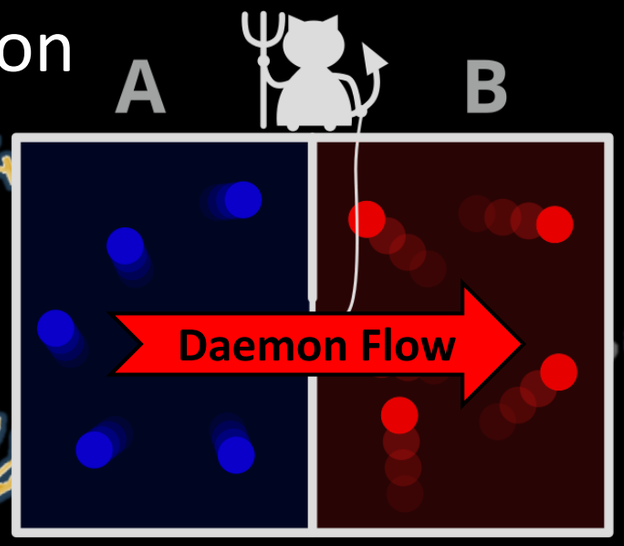


More Valine, Lysine and Gly !

V_2

Maxwell‘s Daemon





2nd law of thermodynamics

not possible in equilibrium

Lower sequence entropy
Information gain

Strand Inhibition

High sequence entropy
Random sequences

To ensure open-ended Evolution we need to…

… select for long sequences

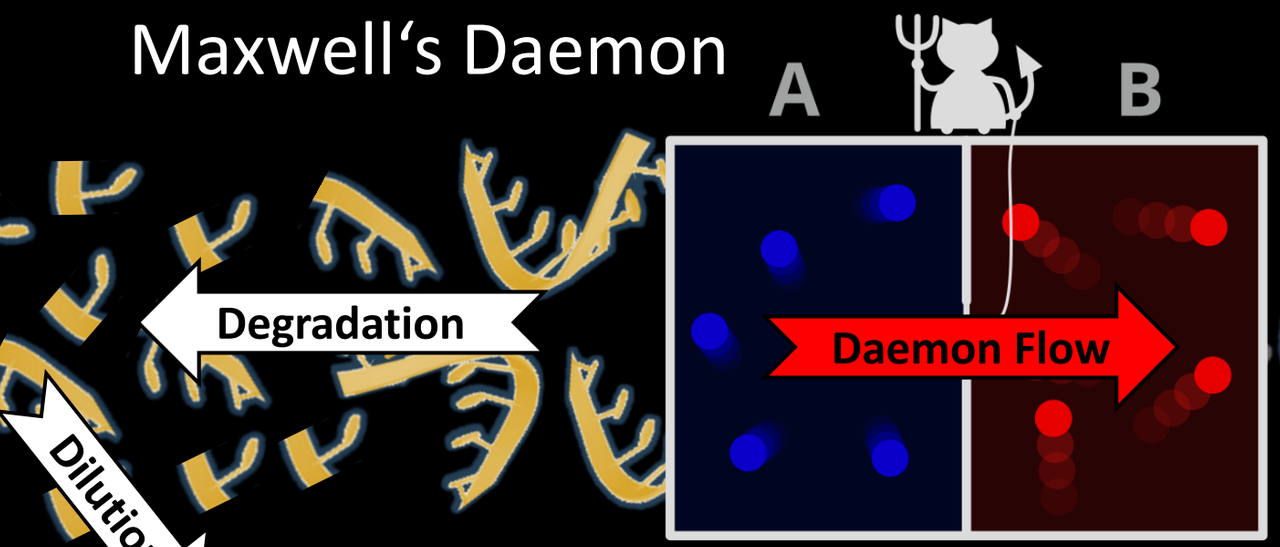
Replication
Mutation

Sequence information

$$\text{Sequence entropy} = -\sum_{c \in A,C,G,T} p(c) \log_2 p(c)$$

From random, AGCTTGAC: 2 bits / base
towards ATATATATAT: 1 bit / base
not leading to AAAAAAAA: 0 bit = no information

Maxwell‘s Daemon



2nd law of thermodynamics

Dilution

Degradation

Dilution

Dilution

High sequence entropy
Random sequences

not possible in equilibrium

Lower sequence entropy
Information gain

To ensure open-ended Evolution we need to…

… select for long sequences

Strand Inhibition

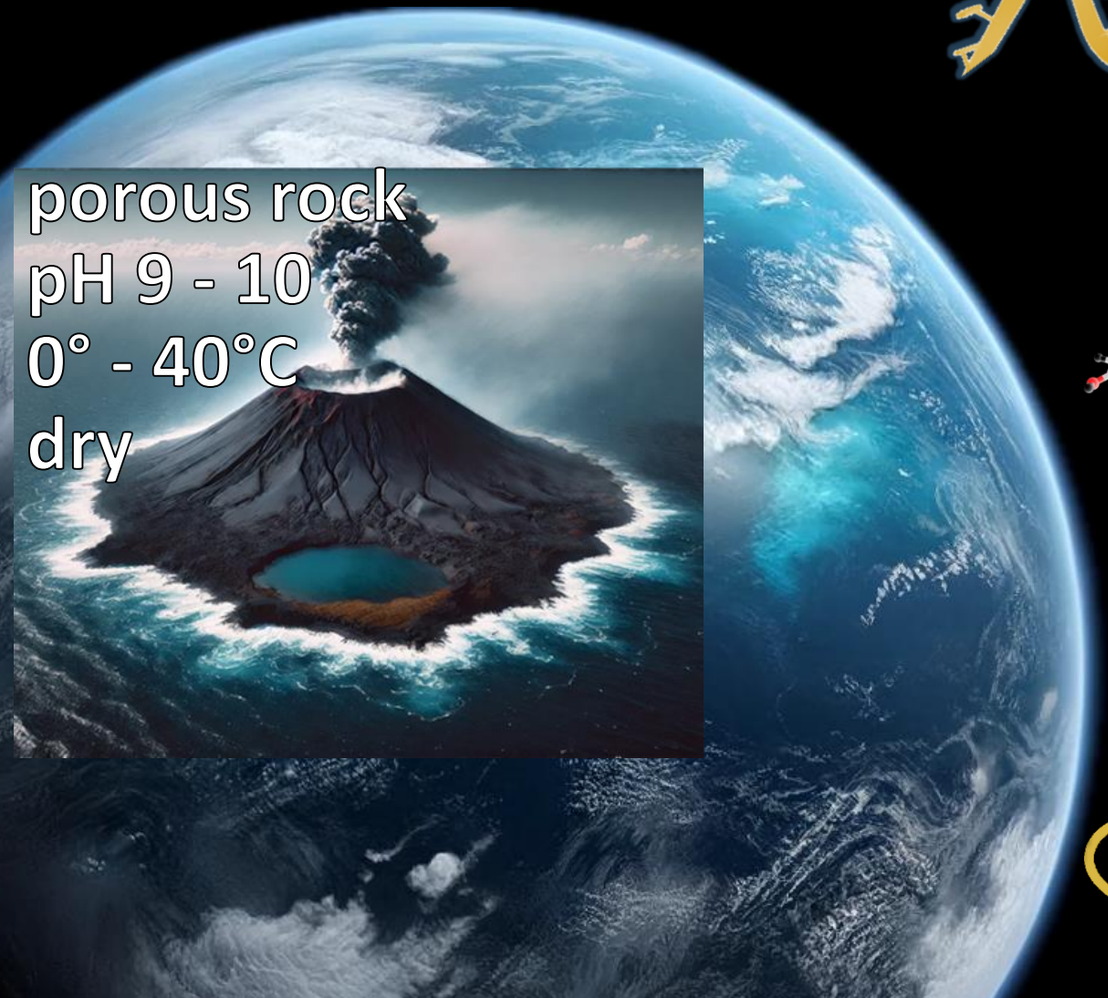
What is selected for?

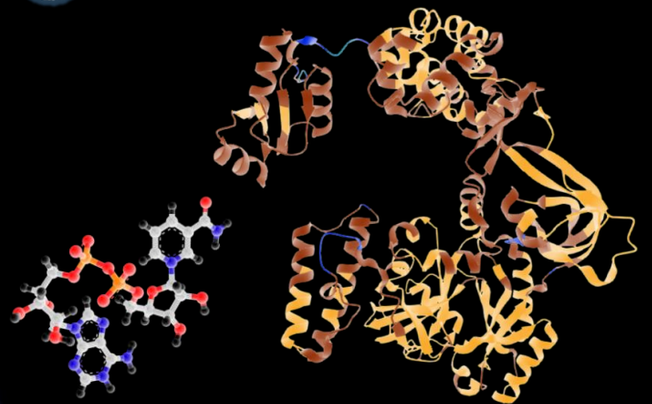
- No mysterious phenotype
- Speed is the selection pressure
- Increase length for open ended evolution
- Ribosome, not hard to reach
  Ribozymes are the goal?

Replication
Mutation

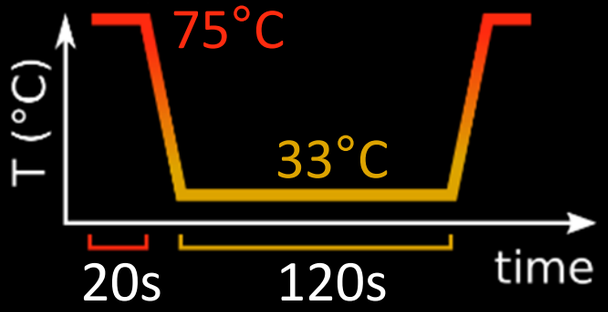
3. How Early Earth can drive evolution

Creating RNA-Life by triggering Darwinian Evolution





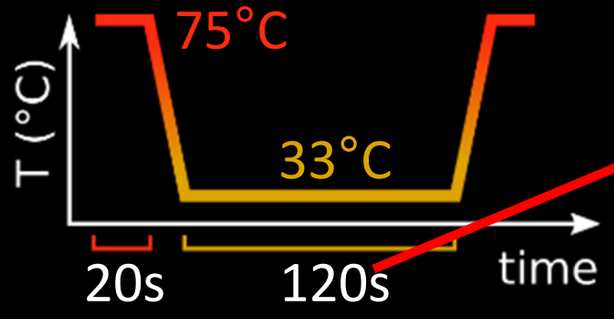
NAD Taq DNA Ligase



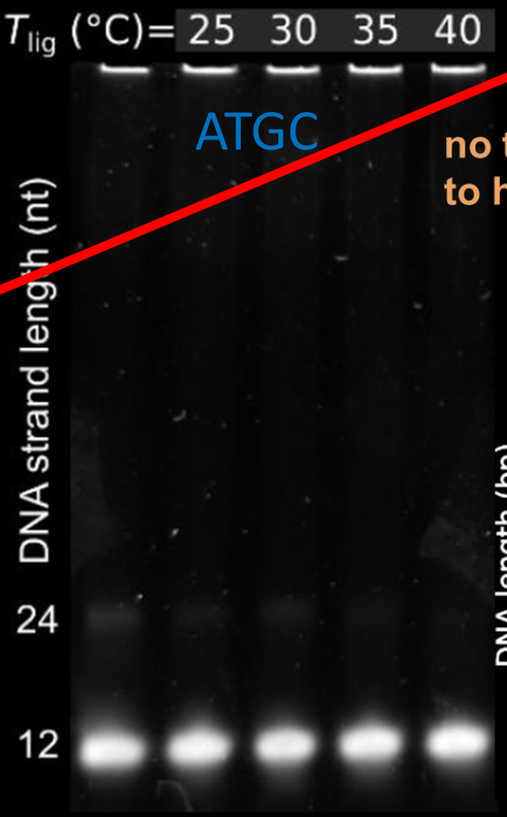
Nonequilibrium driving

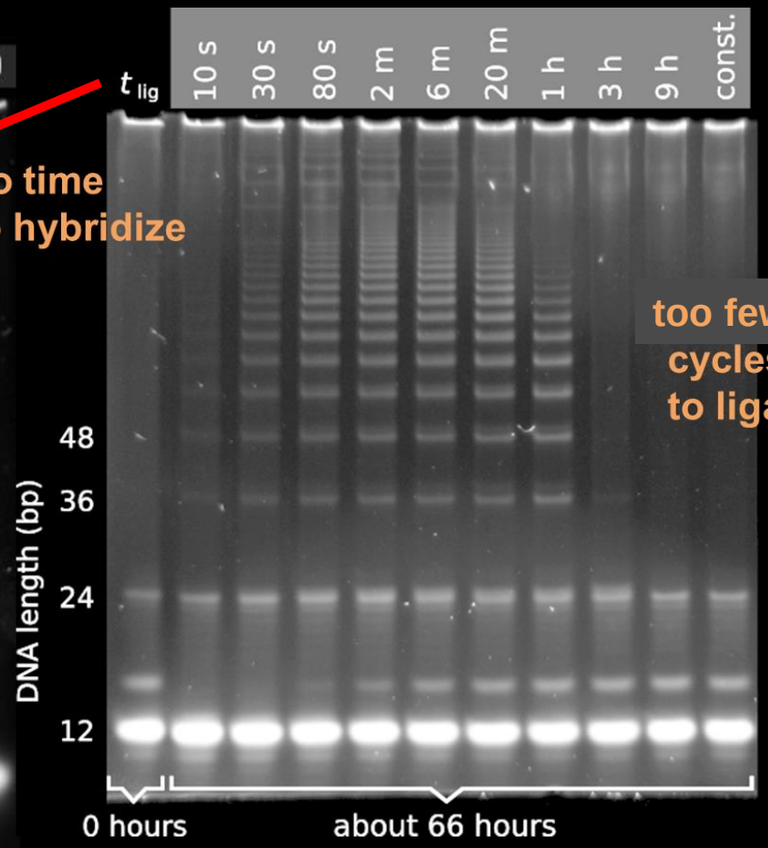
Creating RNA-Life by triggering Darwinian Evolution

NAD Taq DNA Ligase



Nonequilibrium driving





$k_{on}^{hyb} = 1\,\mu M^{-1} s^{-1}$

	AT	ATGC
	410s	19days
	6s	410s

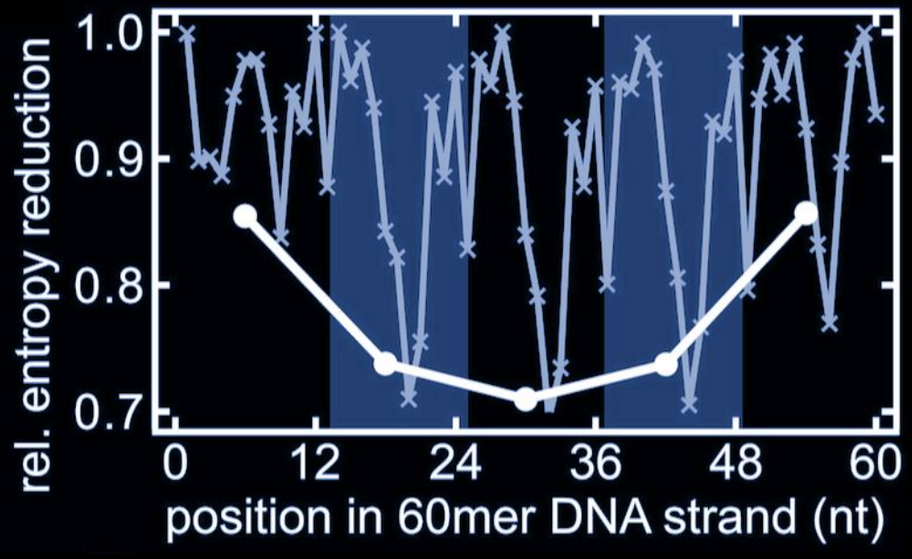
Elongation since the partner sequence is found much faster

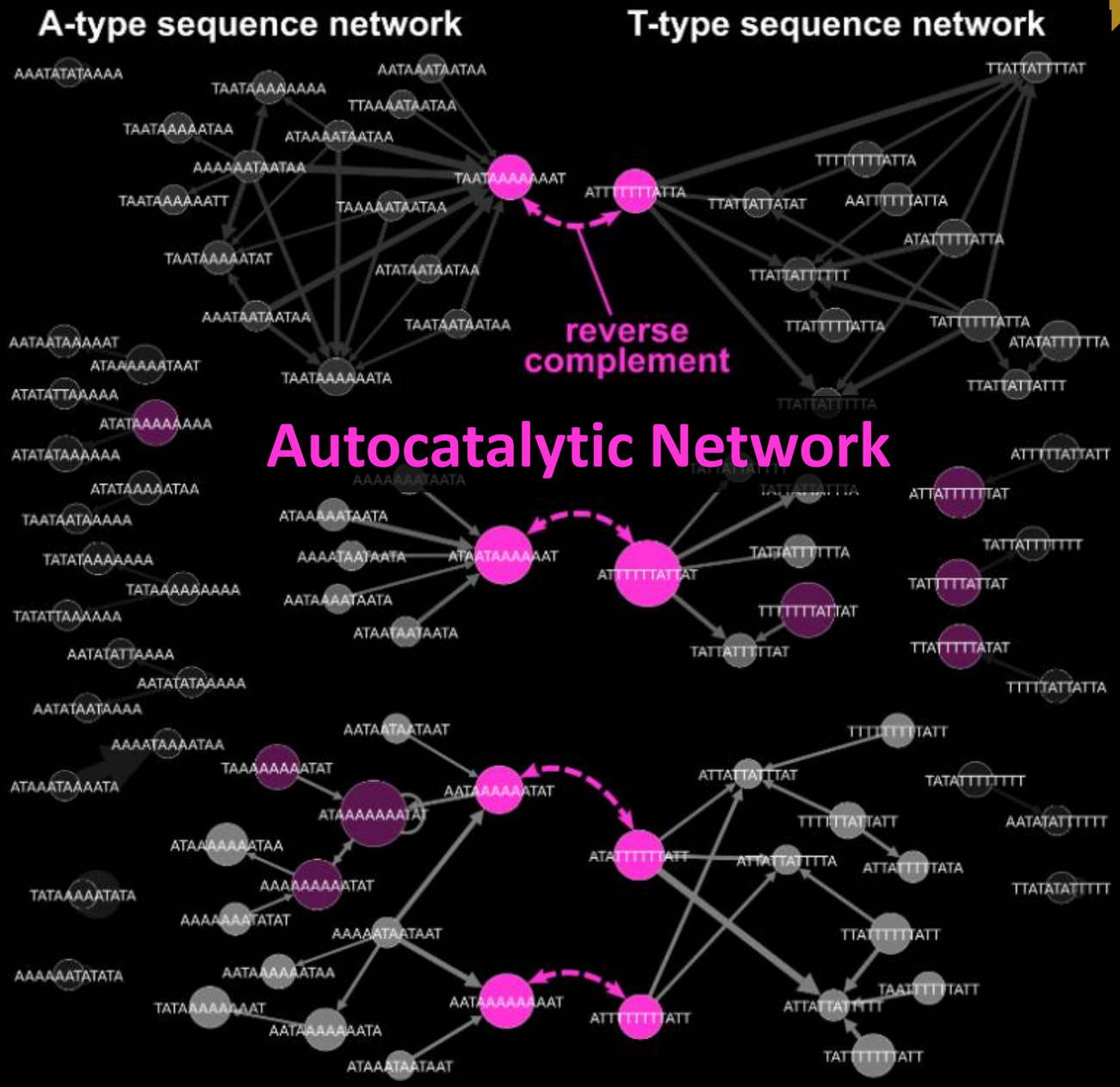
Patrick Kudella, Alexei Tkachenko, Annalena Salditt, **Sergei Maslov**
PNAS 2021

Evolution by Random RNA ligation is guided by kinetics

Creating RNA-Life by triggering Darwinian Evolution

NAD Taq DNA Ligase



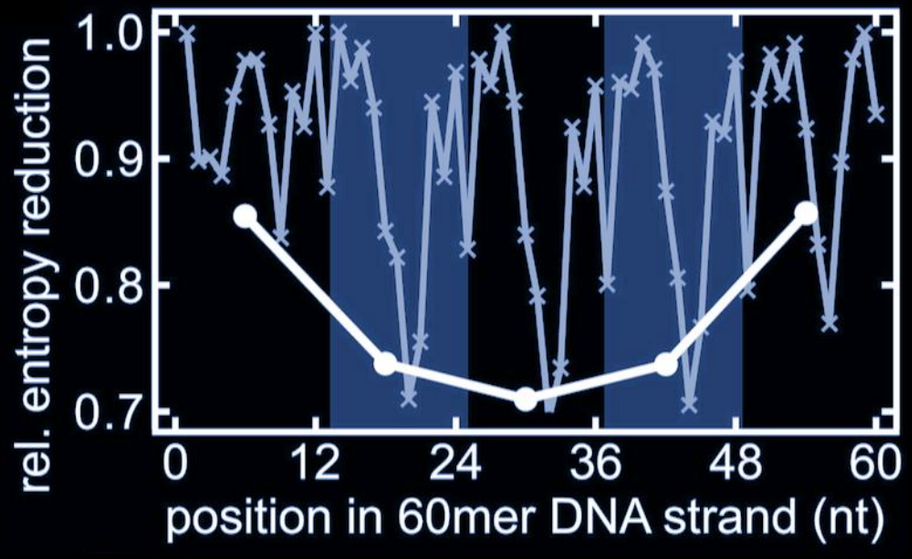


Patrick Kudella, Alexei Tkachenko,
Annalena Salditt, **Sergei Maslov**
PNAS 2021

Creating RNA-Life by triggering Darwinian Evolution

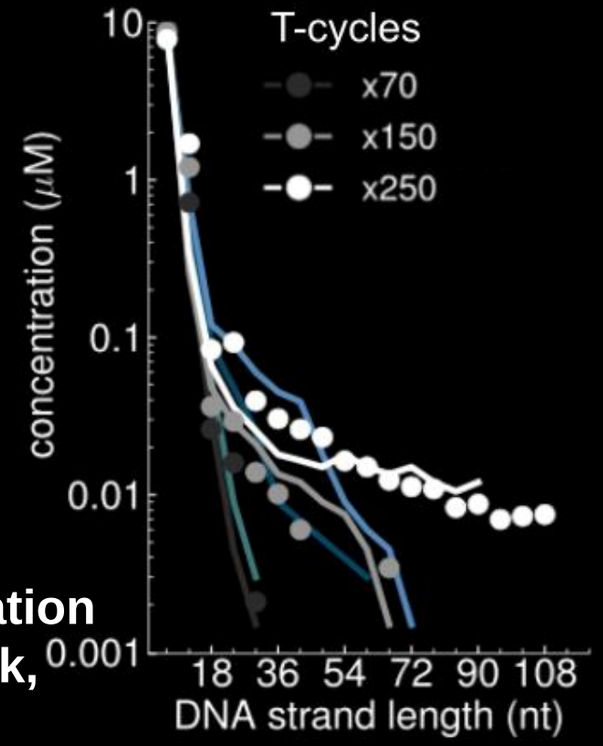
NAD Taq DNA Ligase

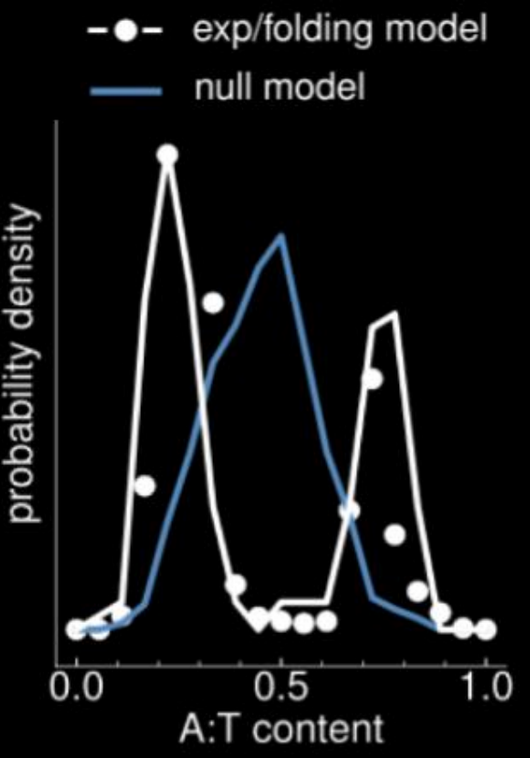
Random 12mers



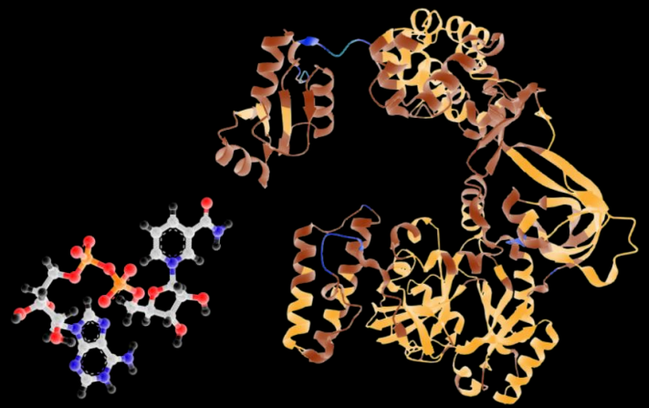
Patrick Kudella, Alexei Tkachenko, Annalena Salditt, **Sergei Maslov**
PNAS 2021

Stochastic simulation of ligation network, Collaboration with Ulrich Gerland, in prep.

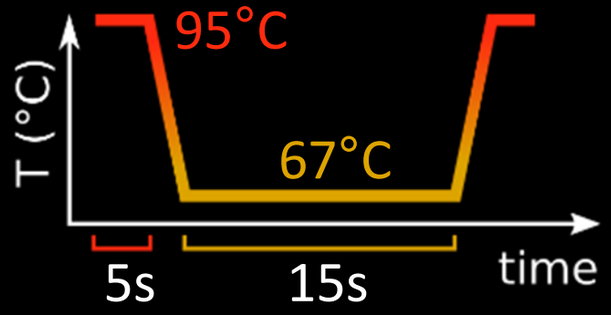




Creating RNA-Life by triggering Darwinian Evolution



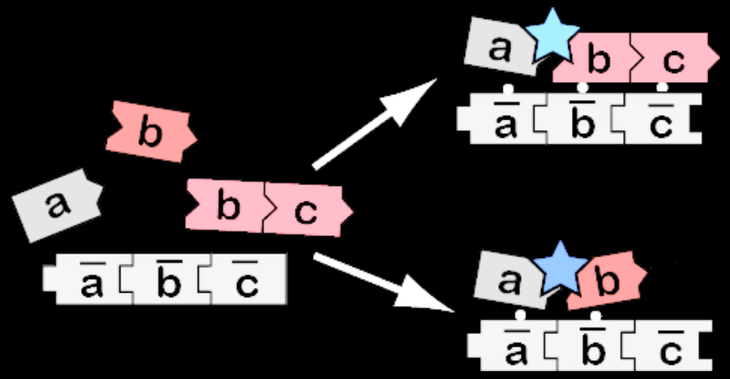
NAD Taq DNA Ligase



Nonequilibrium driving

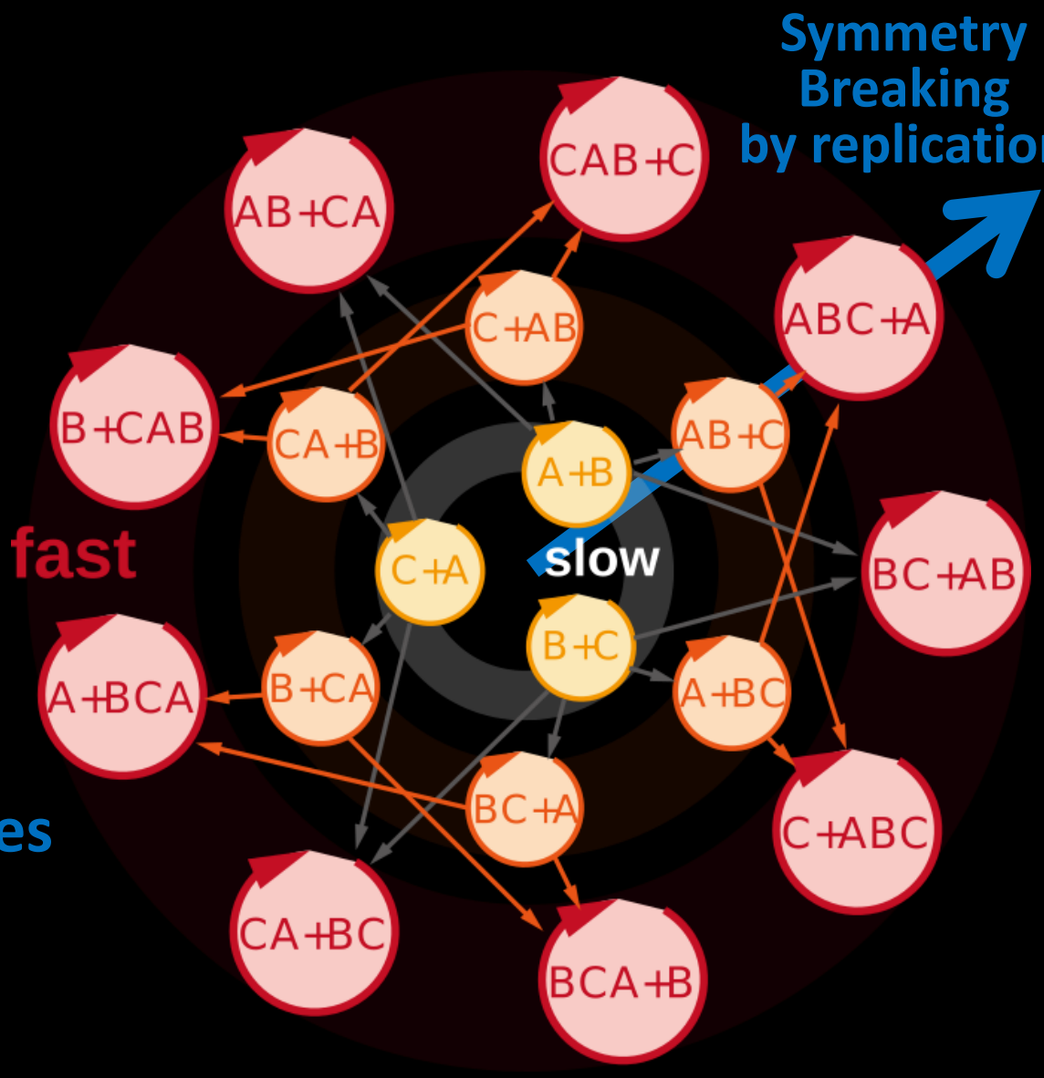
Shoichi Toyabe
Professor, Tohoku University

PRX 2019



Starting with 6 Sequences, a,b,c & complement; 20mers under ligation & serial dilution

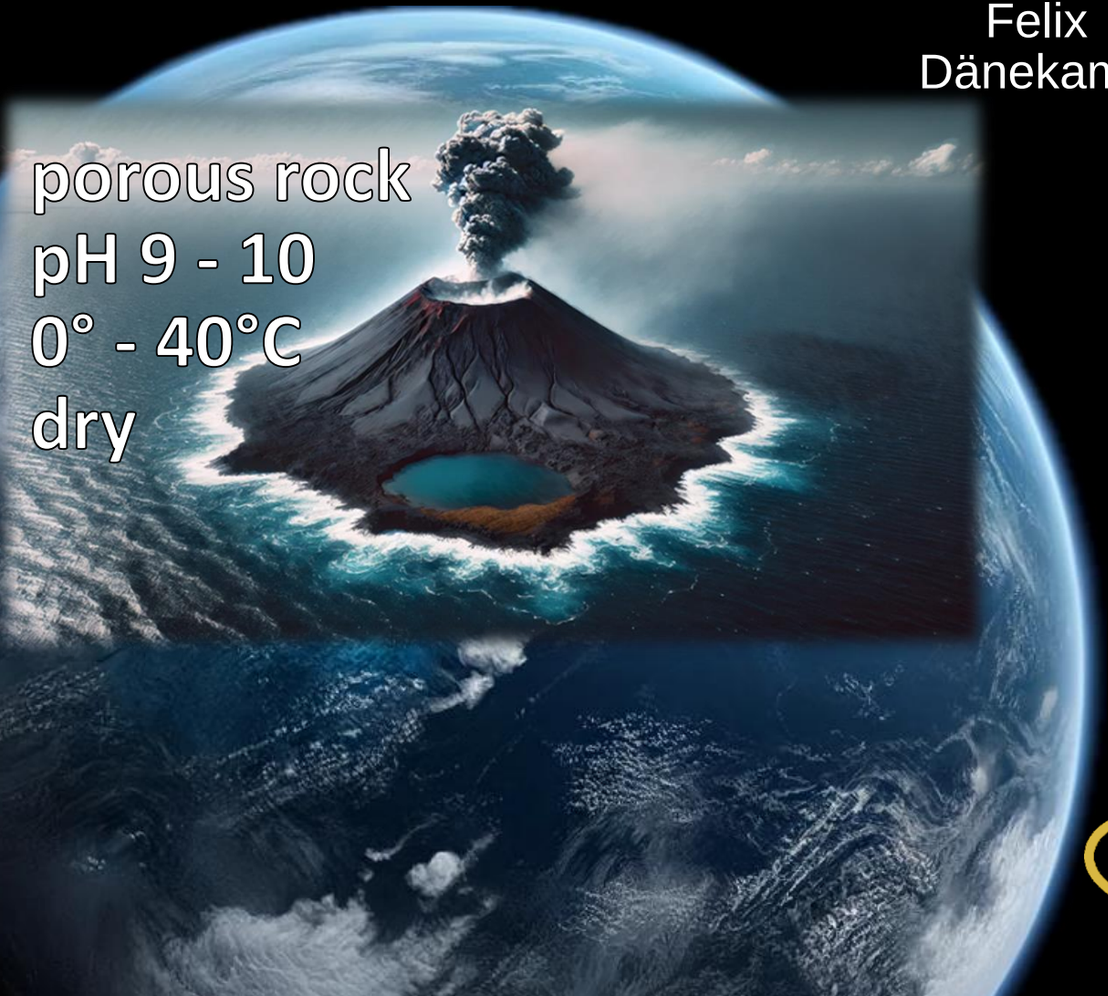
Binding competition creates long term memory in a replicating network



Prebiotic Volcano Surface Simulator for RNA

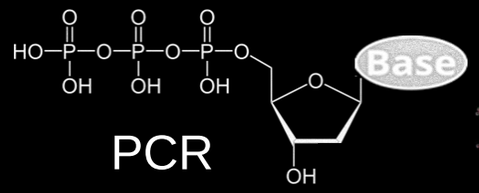
Felix Dänekamp

Almuth Schmid

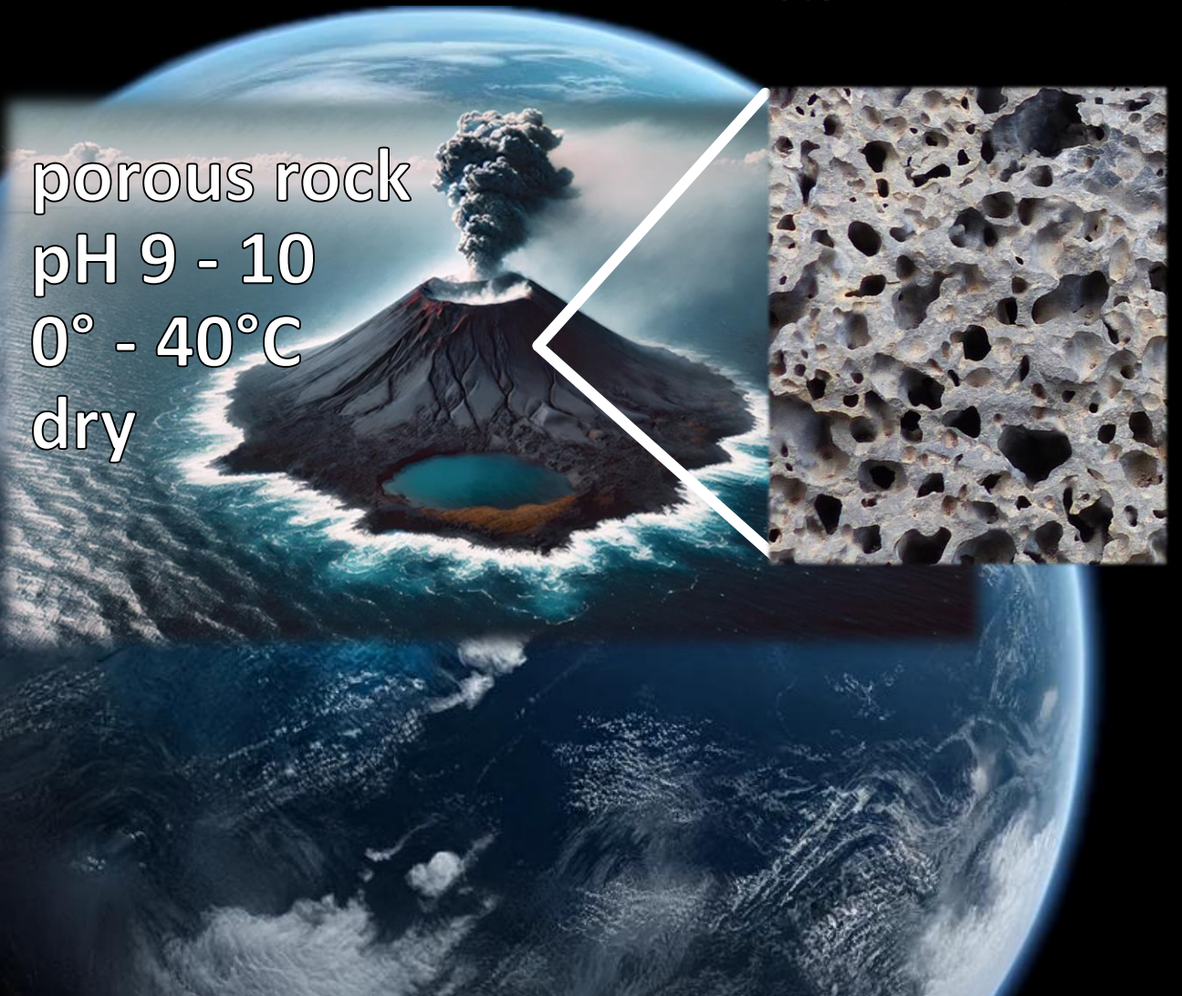


Humidity control to set salt concentration

Isothermal replication by evaporative air flow



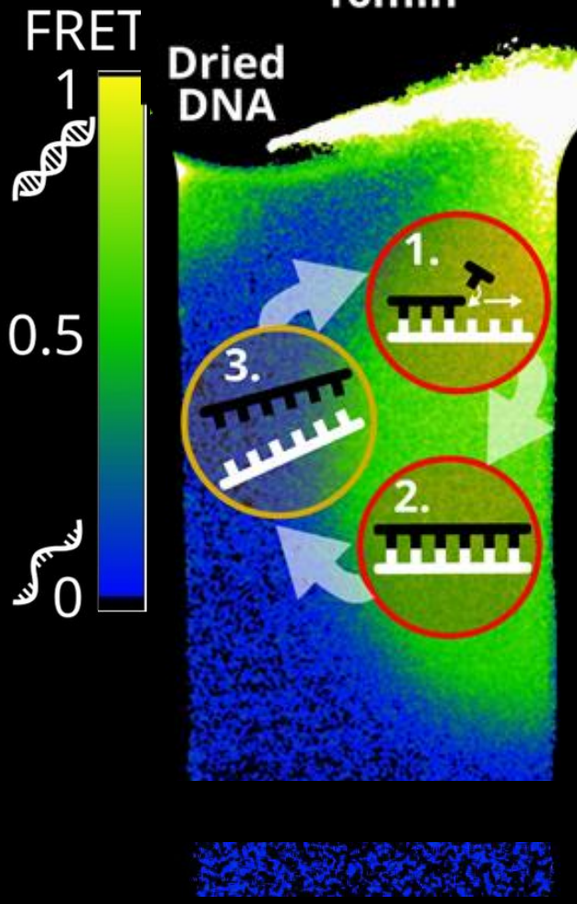
Taq Polymerase
Protein



68°C, 0.25 µM primers, 5 nM template,
200 µM dNTPs, 0.5x PCR buffer,
2.5 U Taq polymerase, **2x SYBR Green I**

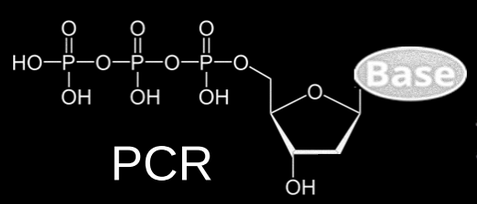
Philipp
Schwintek

16min



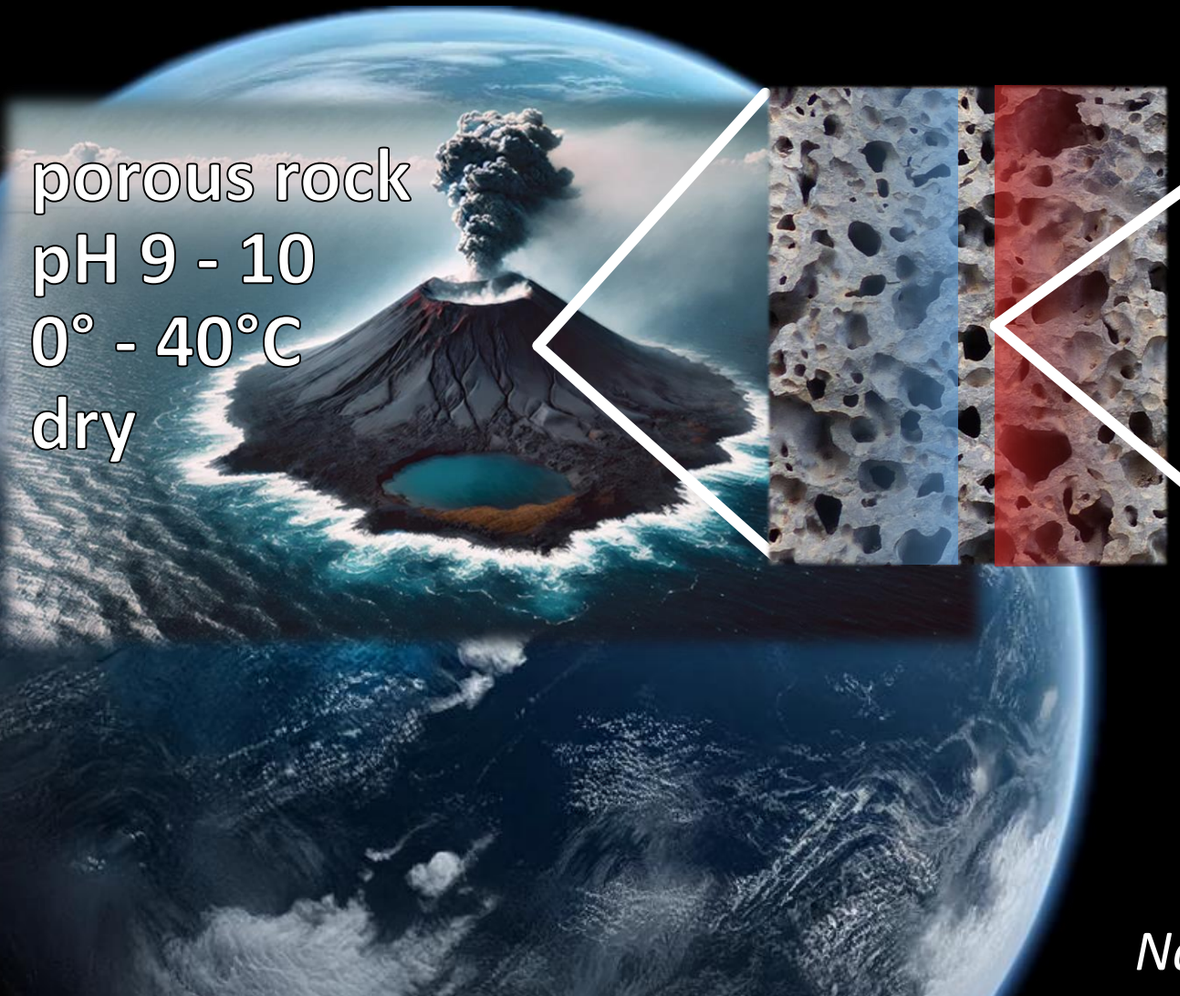
eLife 2024

Alan
Ianeselli

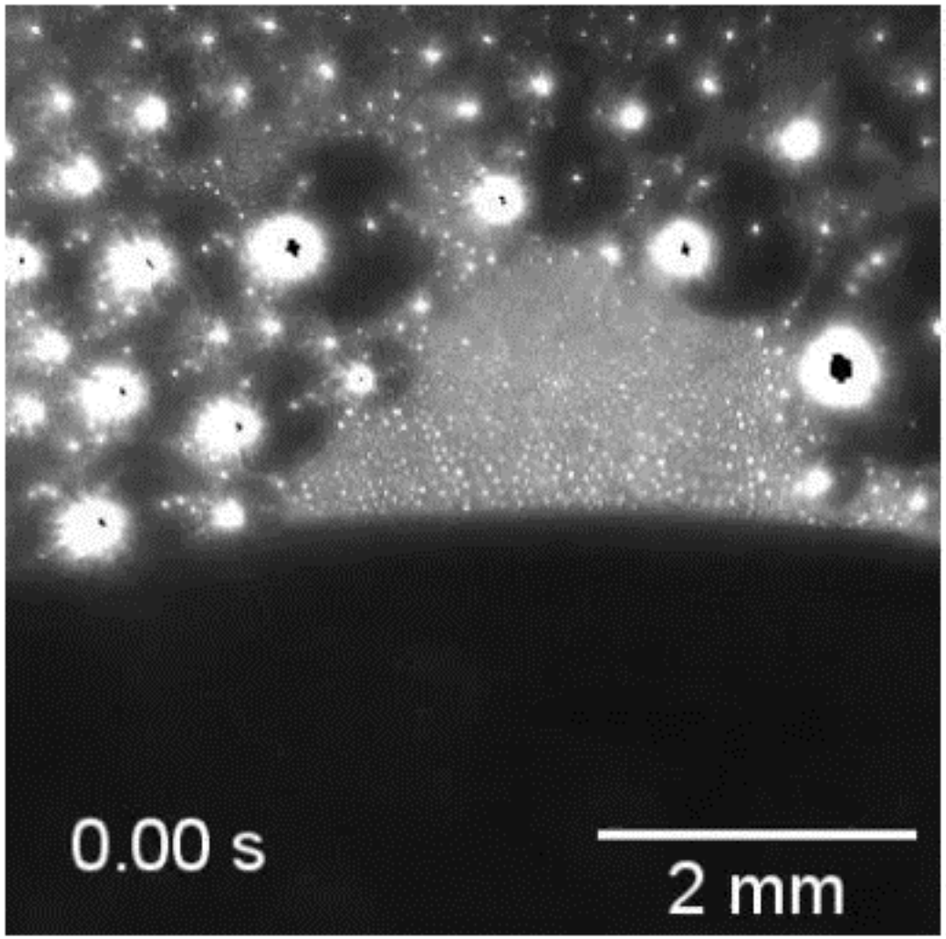


Taq Polymerase
Protein

porous rock
pH 9 - 10
0° - 40°C
dry



cold



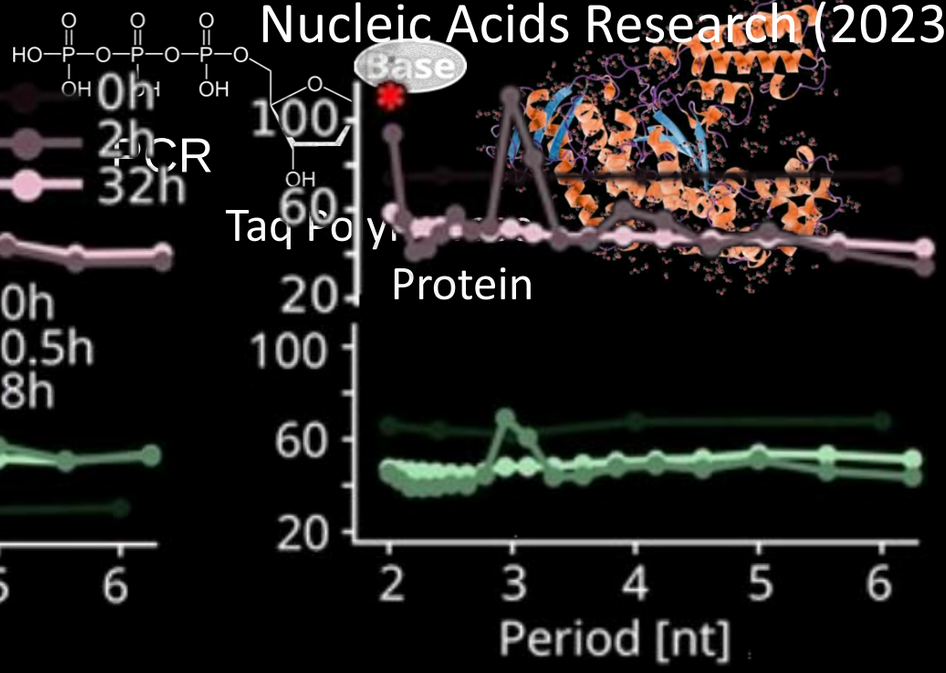
1 bar

1 mm

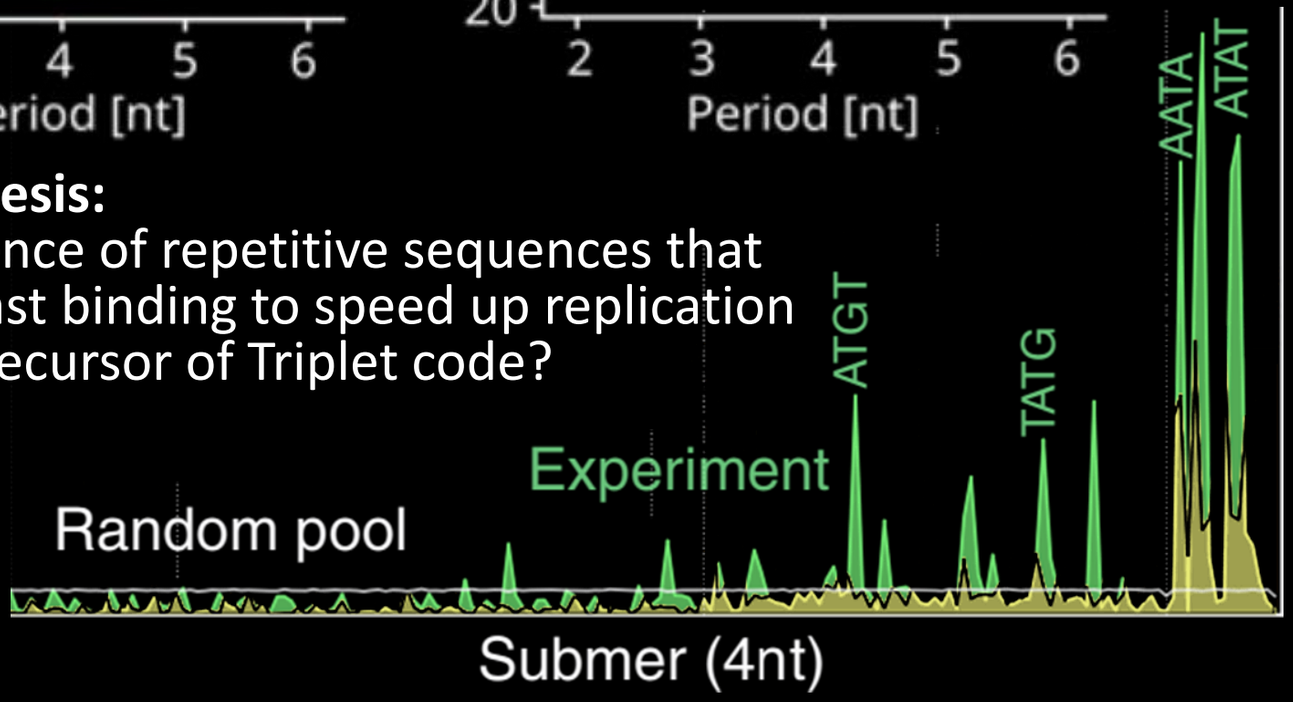
Nat. Phys. **2022** 18, 579–585

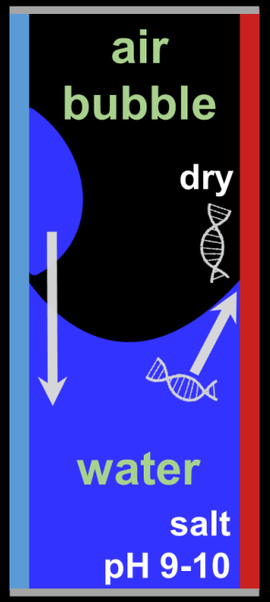
Ilan Jayaseeli

Nucleic Acids Research (2023)

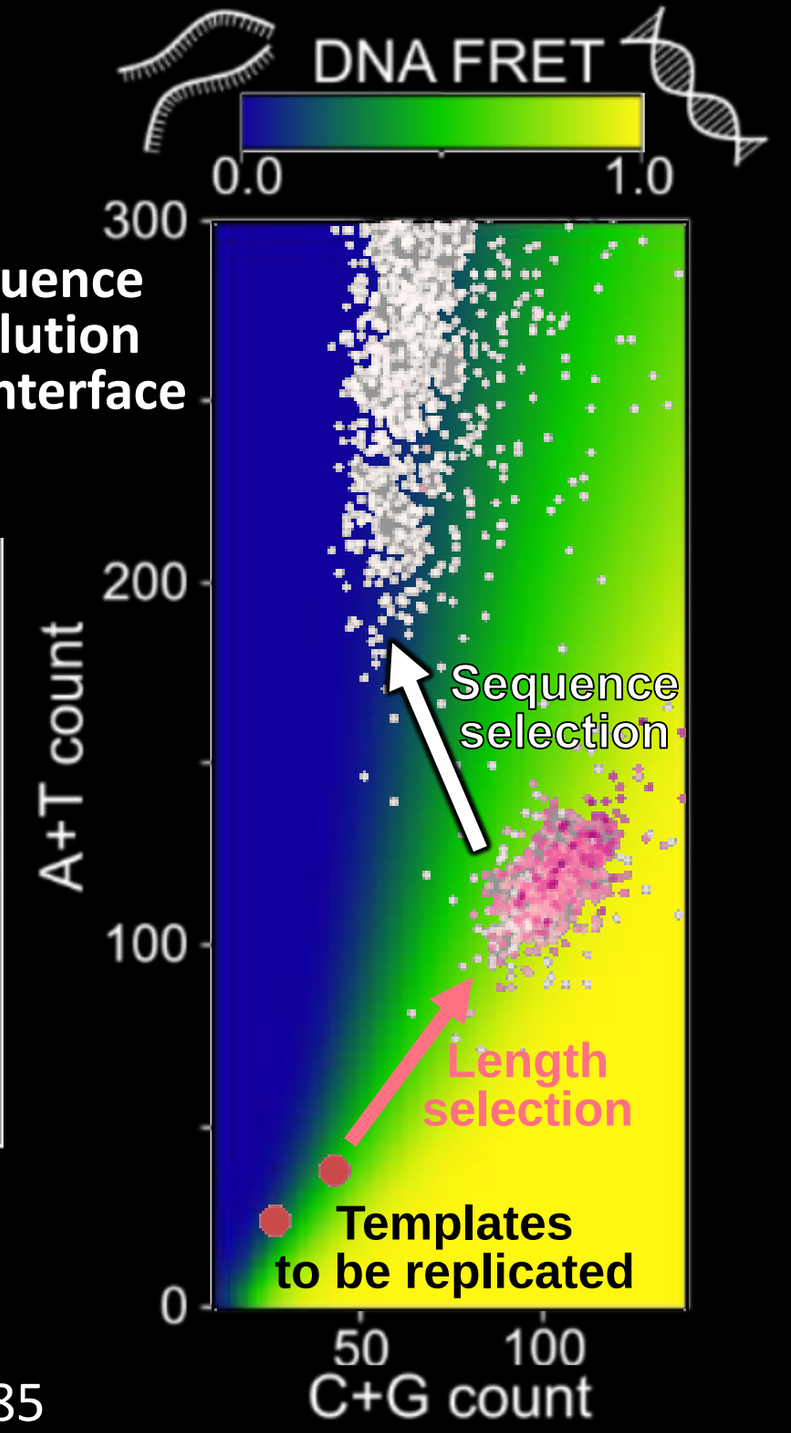


Hypothesis:
Emergence of repetitive sequences that allow fast binding to speed up replication
=> Precursor of Triplet code?



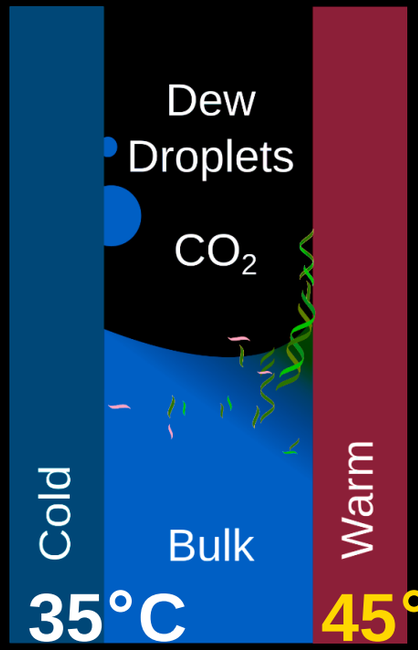


Sequence evolution at interface



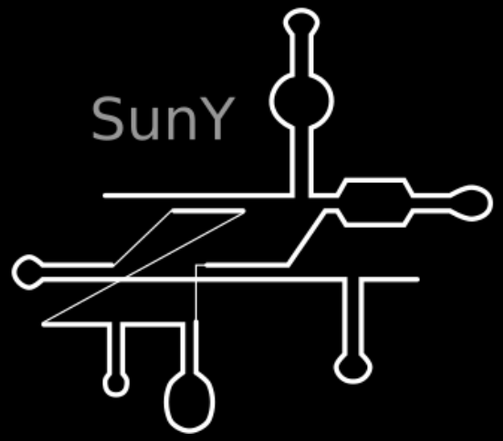
Nat. Phys. **2022** 18, 579–585

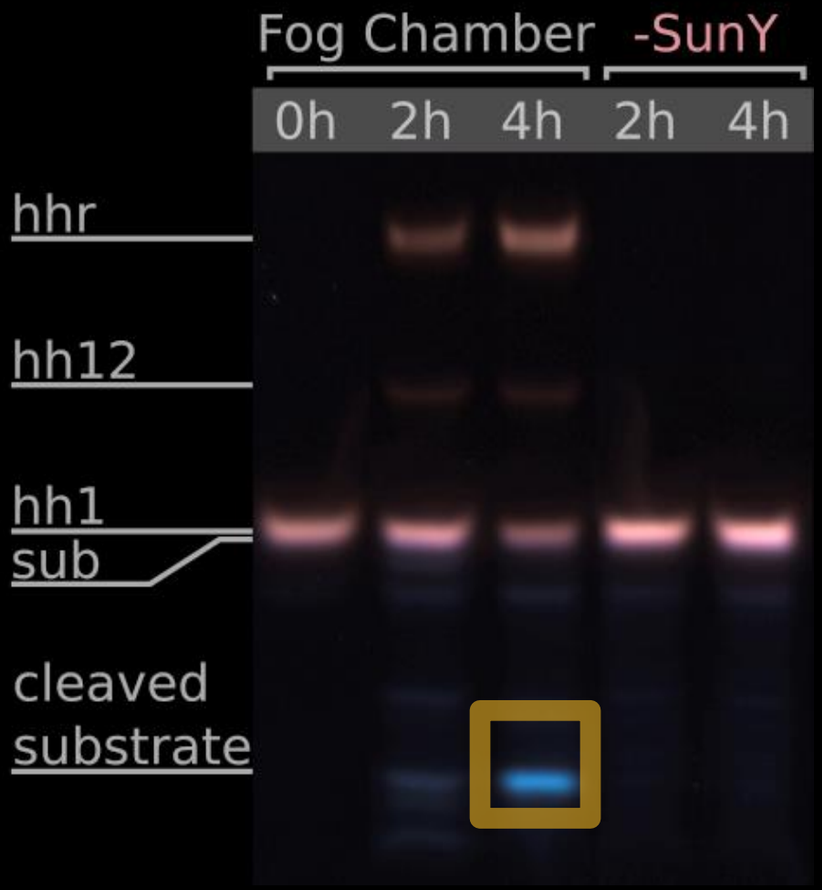
ribozymes



Hannes Mutschler

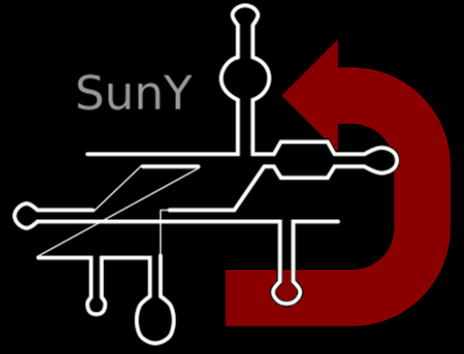
Annalena Salditt

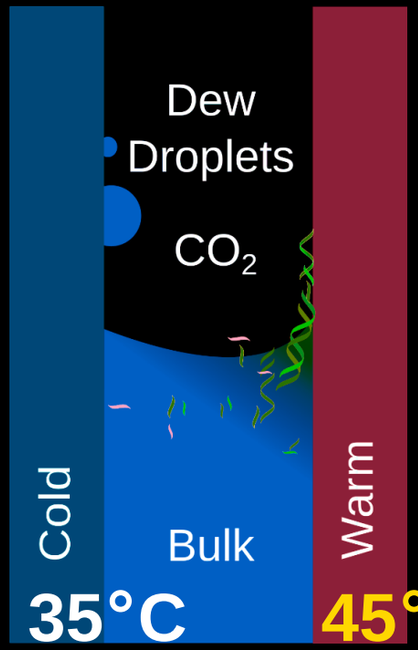




Nat Commun **2023 14**, 1495

Heated air bubbles host ribozyme self-replication?

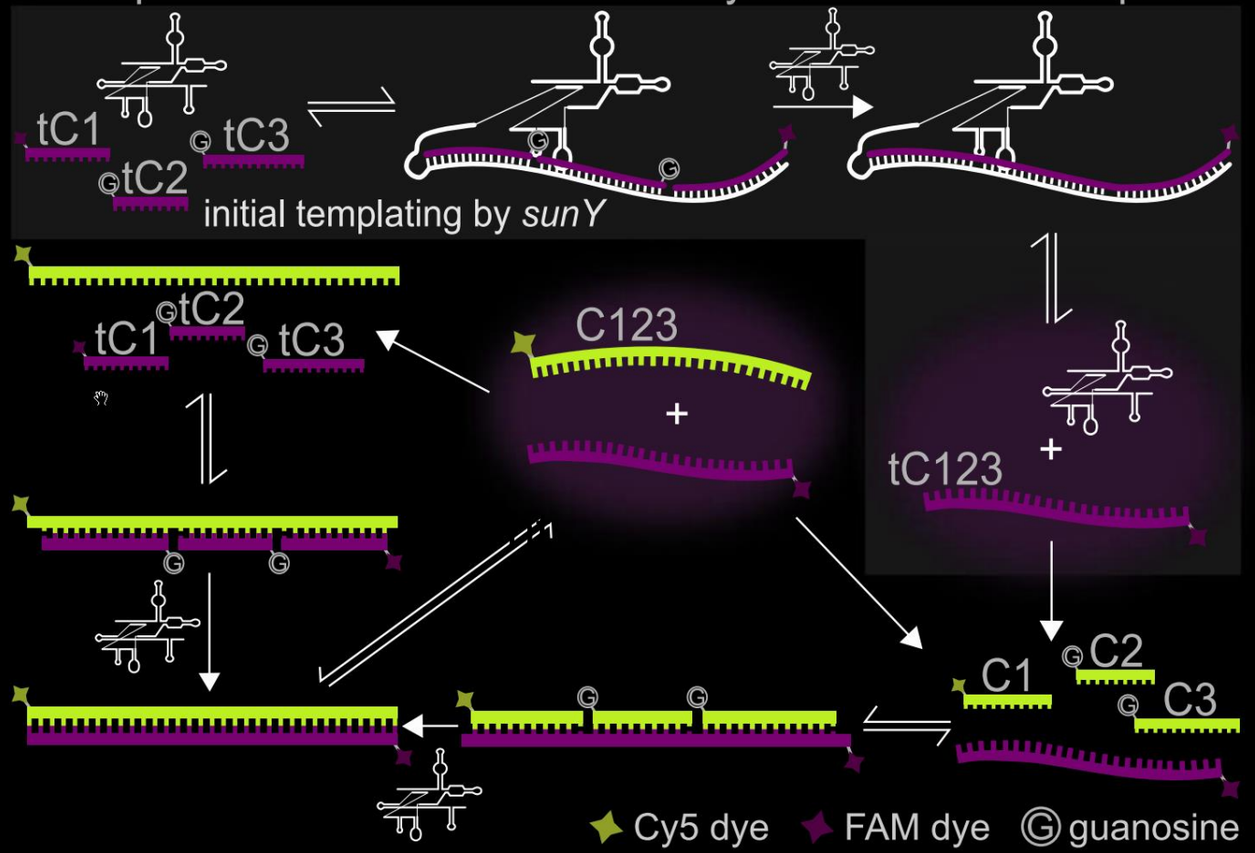




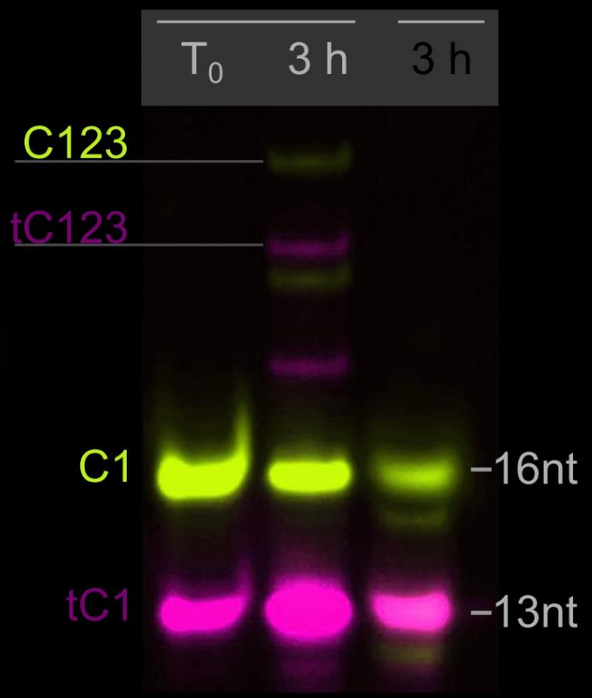
Hannes Mutschler

Annalena Salditt

One-pot sense + antisense strand synthesis without template



without template



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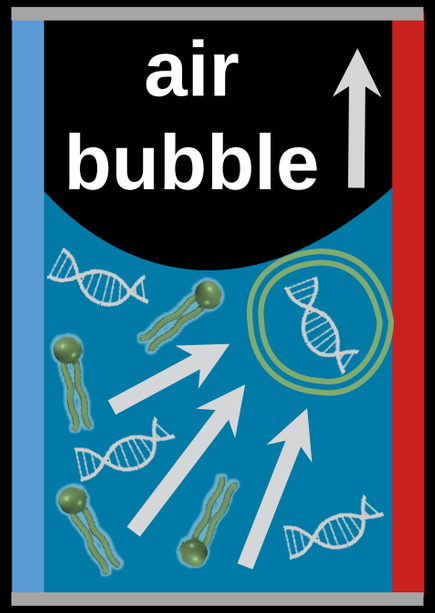
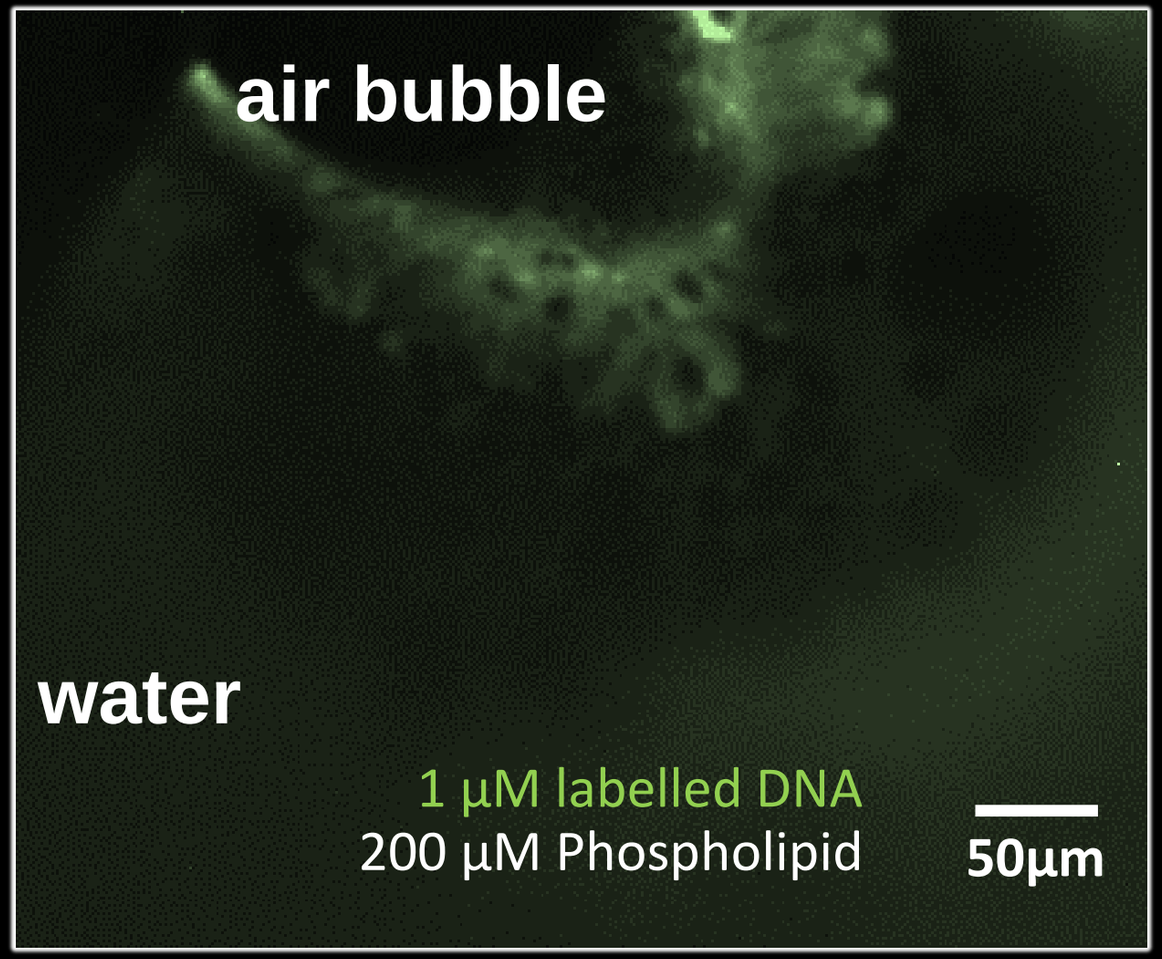
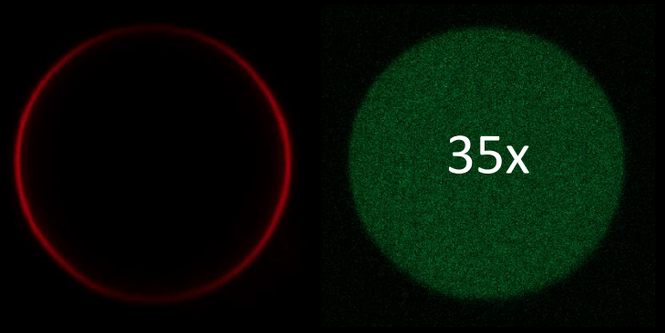
Heated air bubbles host vesicle formation and encapsulation





Alexander Floroni

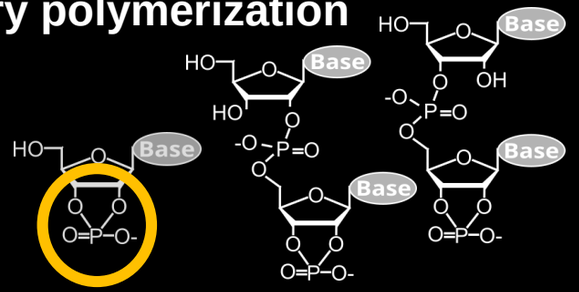


Nonequilibrium and Chemistry driving early Darwinian Evolution

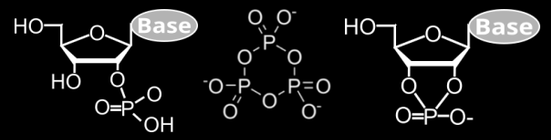
cold

warm

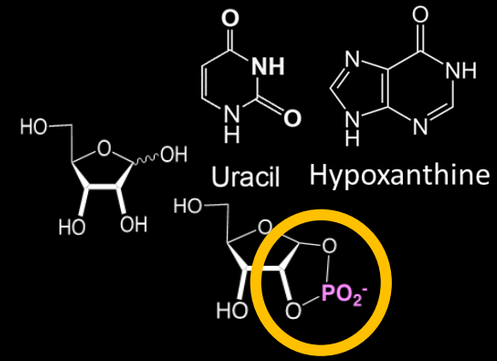
Dry polymerization



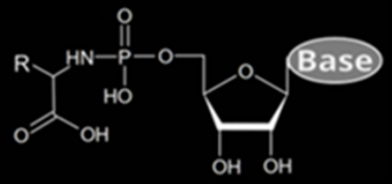
Dry Phosphorylation and 2‘-3‘ Reactivation



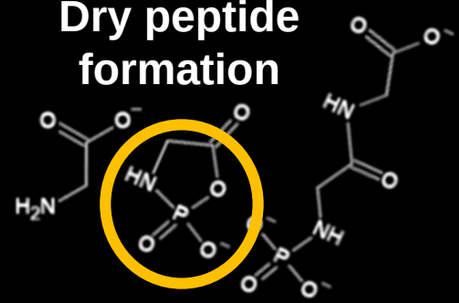
Dry Nucleosynthesis



Peptide-RNA hybrids



Dry peptide formation



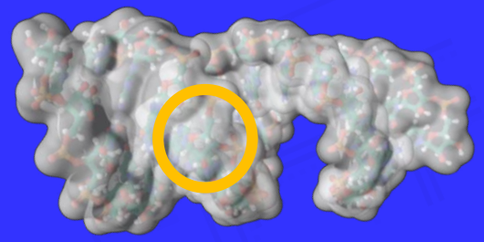
CO_2

Interfacial movement

pH 5
low salt

Strand separation

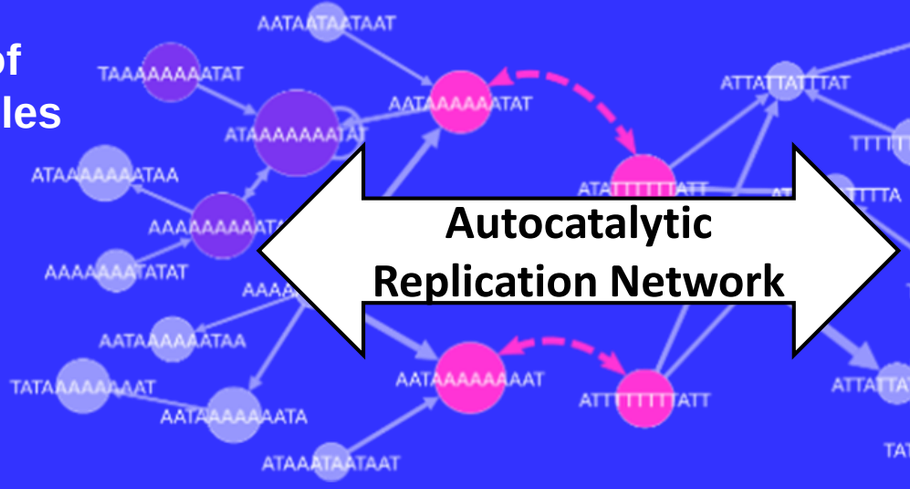
pH 9-10
high salt



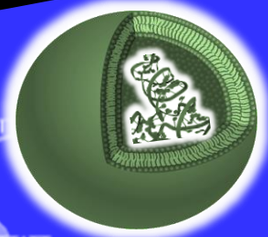
dsRNA to amplify chirality and 3‘-5‘ links

Selection of large molecules

Replication by templated ligation



Autocatalytic Replication Network



Vesicles

Ben-Gurion University of the Negev

Minerva Center for Studying the Planetary Emergence of Life

Minerva Centers

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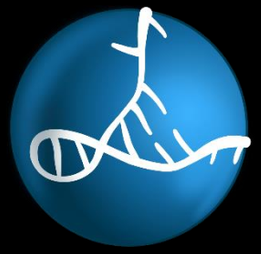

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