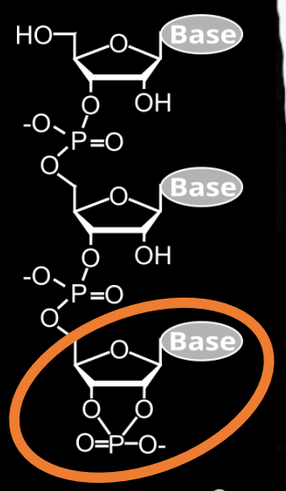
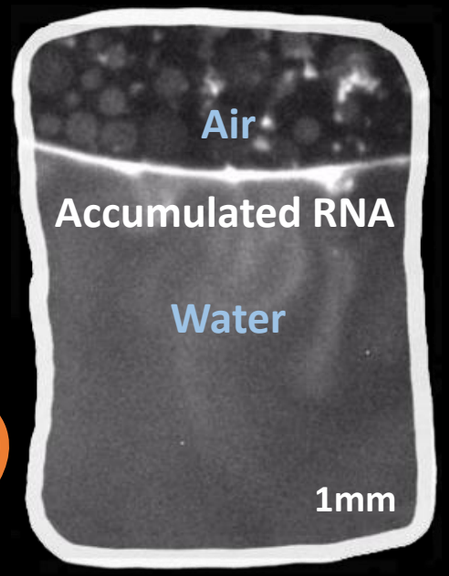
From a Few Molecules to Cells:
Exploring the Origins of Life and Advancing Biotechnology

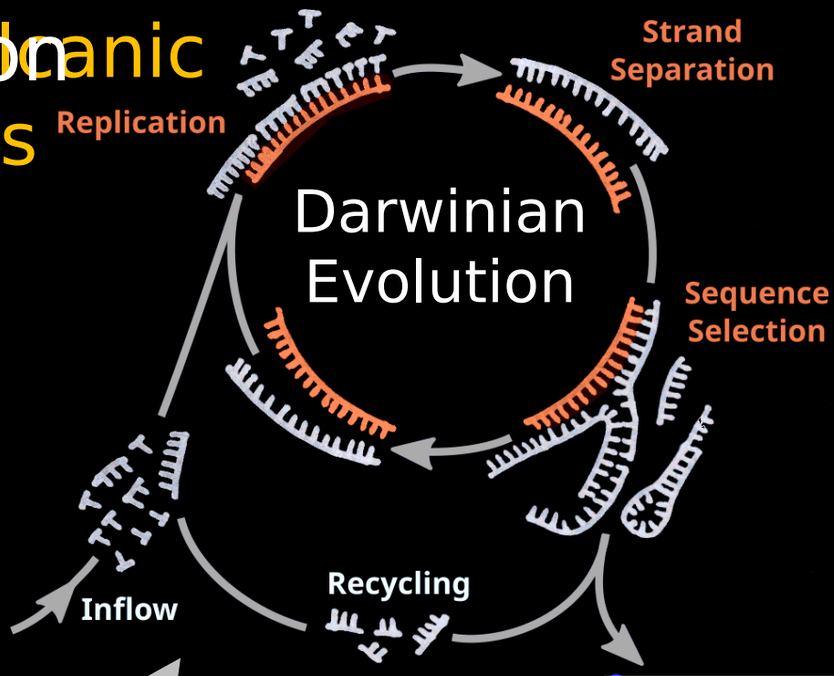
Dieter Braun
Biophysics, Center for NanoScience, LMU Munich

Biological Evolution

RNA evolution in volcanic wet-dry settings



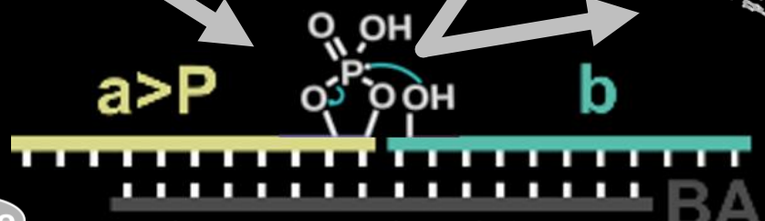




Bacteria

80% yield

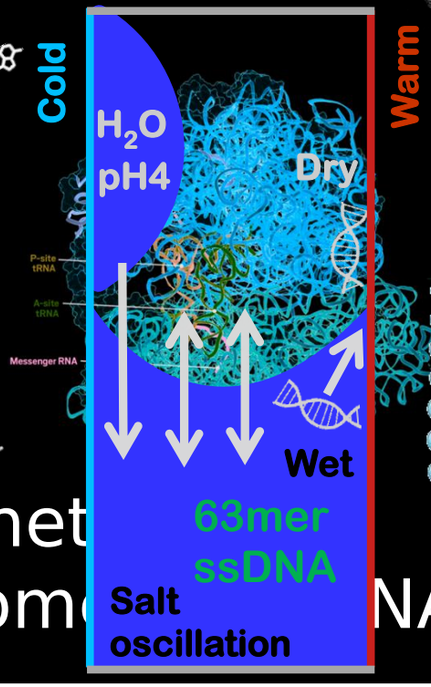
40% yield



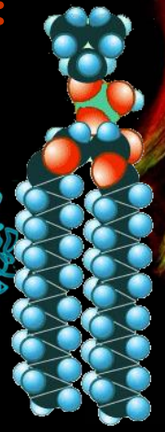
Replication by Ligation

Genet

Ribosom



NA

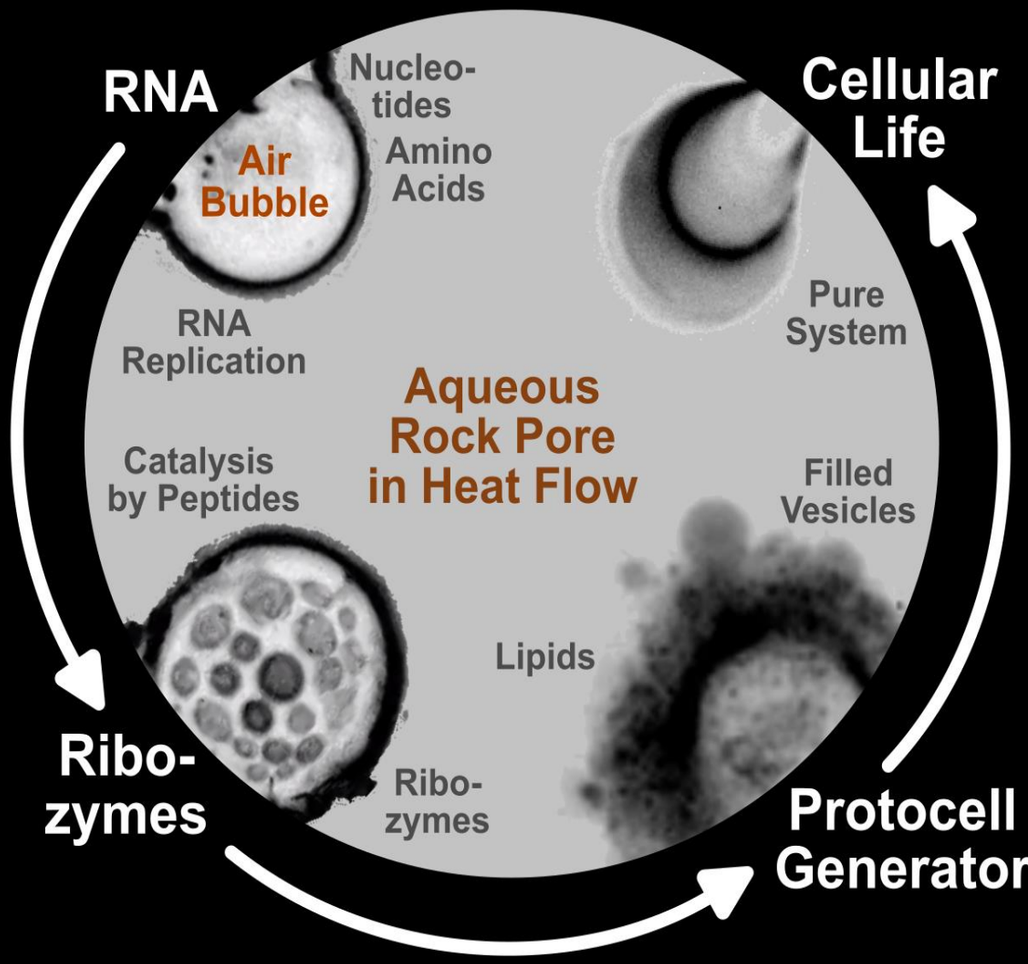


DOPC

E

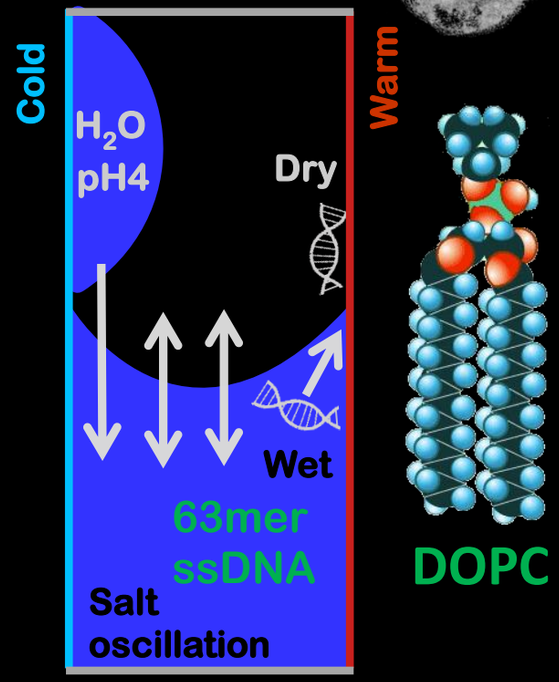
Time

From RNA to Cellular Life at heated Air Bubbles

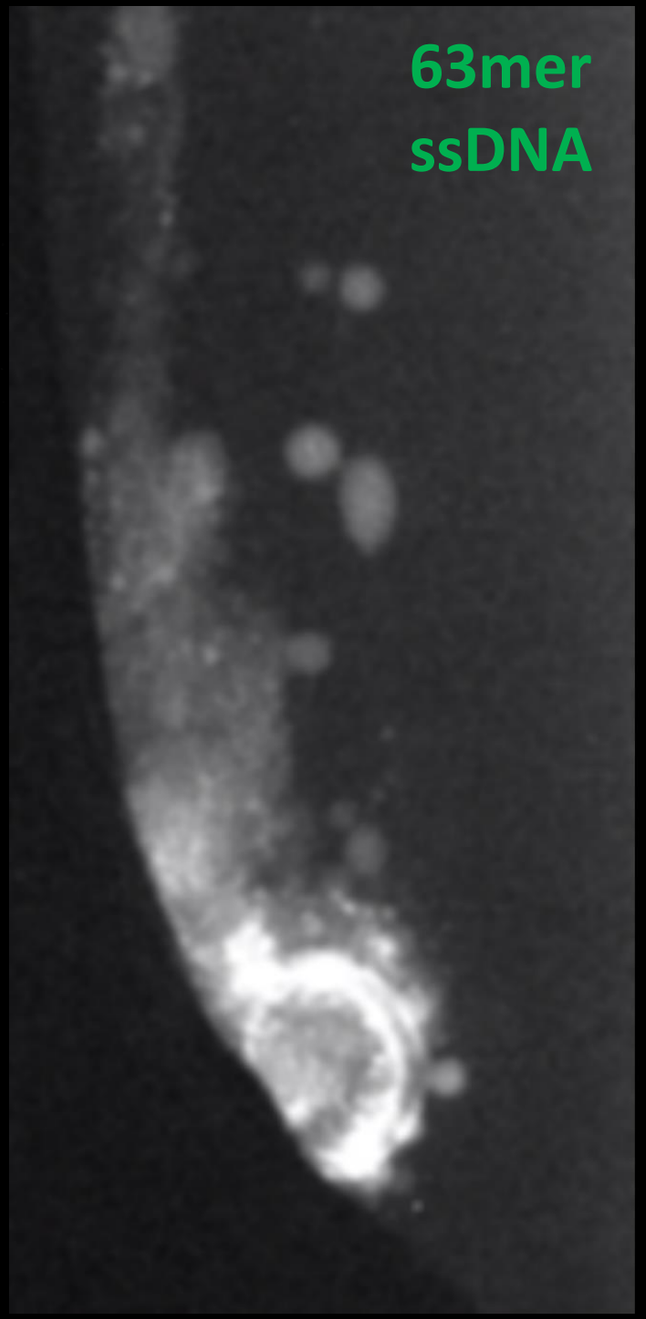


ERC Synergy Proposal

Hannes Mutschler



DOPC



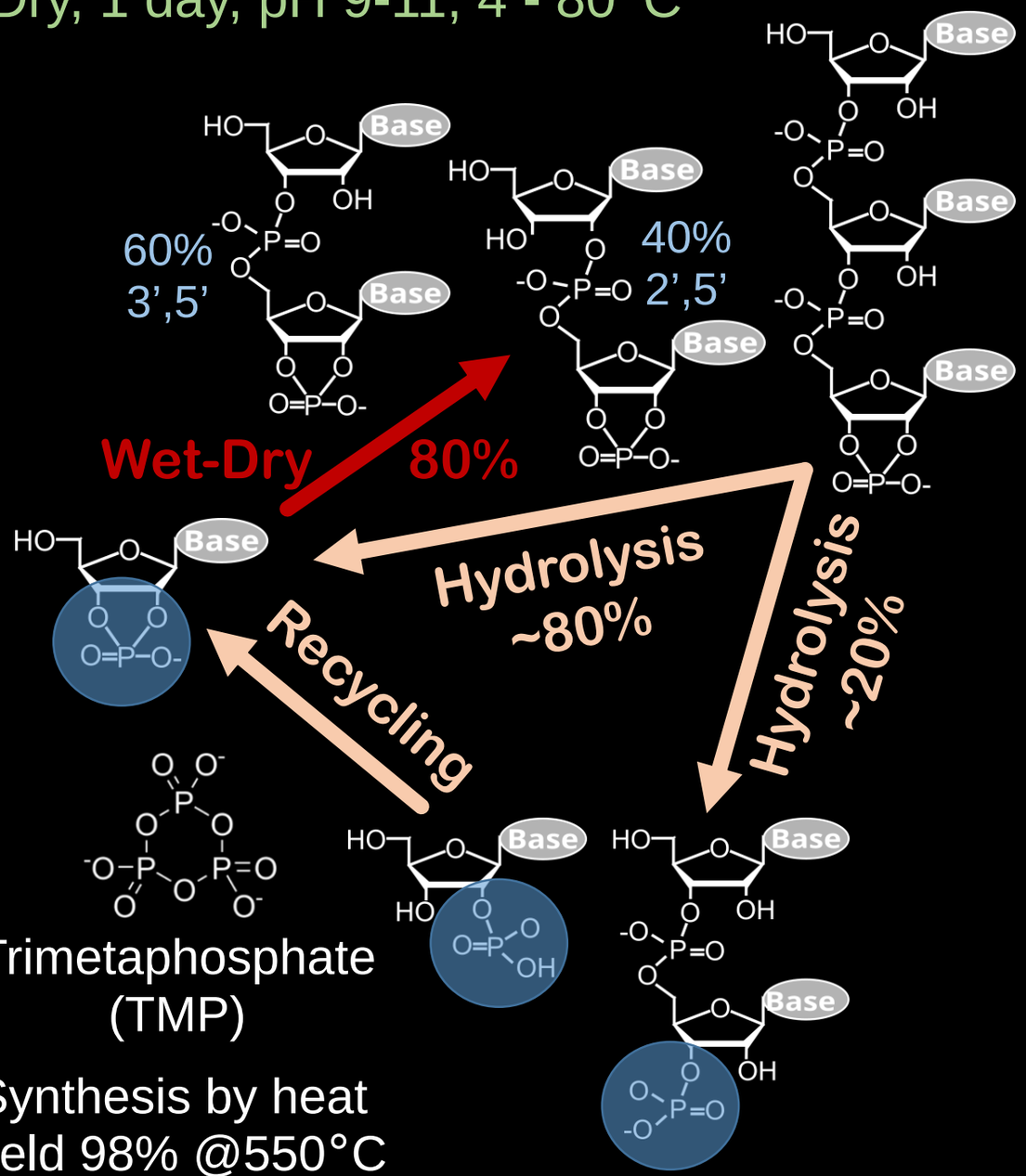
2019: **Scientific Reports, Nature Chemistry, Angewandte Chemie**
2020: **Physical Review Letters, PNAS**
2021: 2x **Nature Chemistry, PRX, Angewandte Chemie, PNAS**
2022: **ChemBioChem, ChemSystemsChem**
2023: **NAR, PNAS, Nature Communications, PCCP**
2024: **JACS, accepted; Nature accepted**

1. Emergence of RNA and Peptides
2. Replication by templated ligation
3. Darwinian evolution on an early Earth

Chemical Evolution

Dry, 1 day, pH 9-11, 4 - 80°C

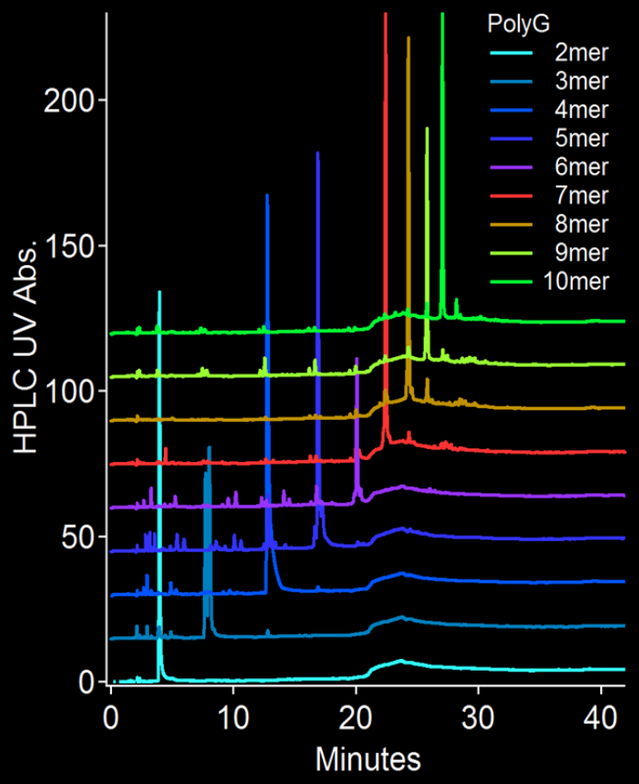
RNA oligomerization in “dry” state



Trimetaphosphate (TMP)

Synthesis by heat
Yield 98% @550°C

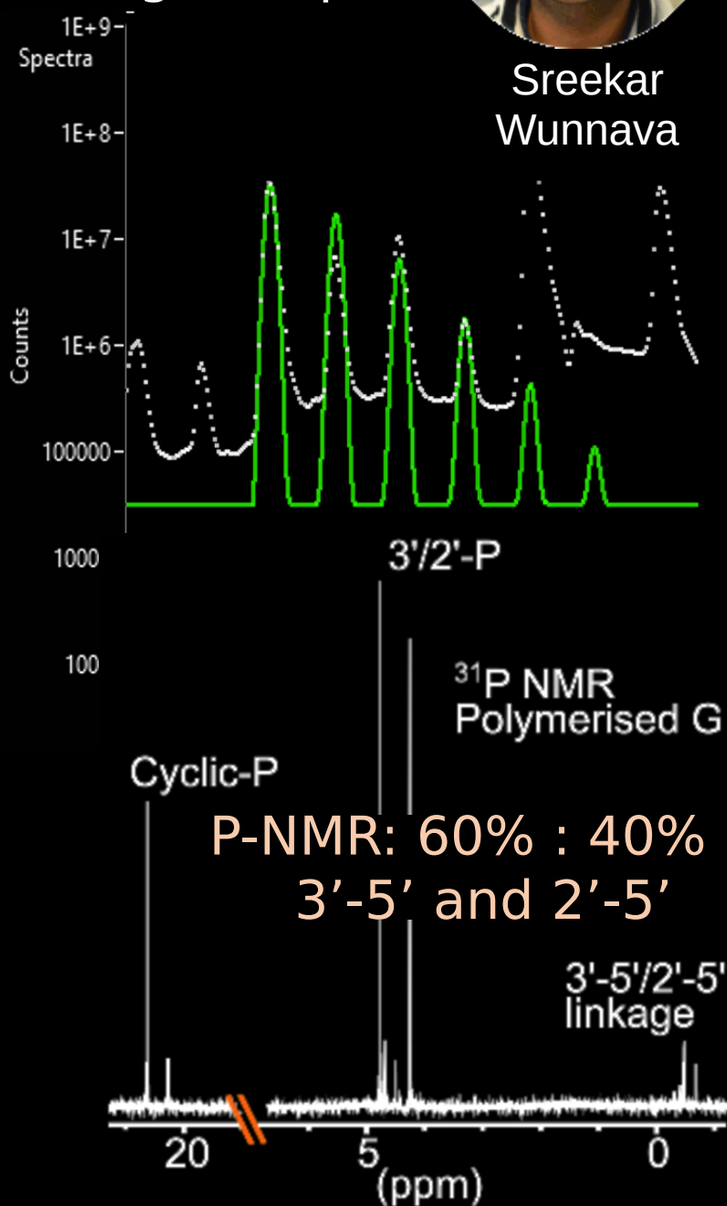
HPLC @ 60°C



ChemSystemsChem
doi.org/10.1002/syst.
202200026 (2022)

ESI-TOF-MS
Fitting isotopes

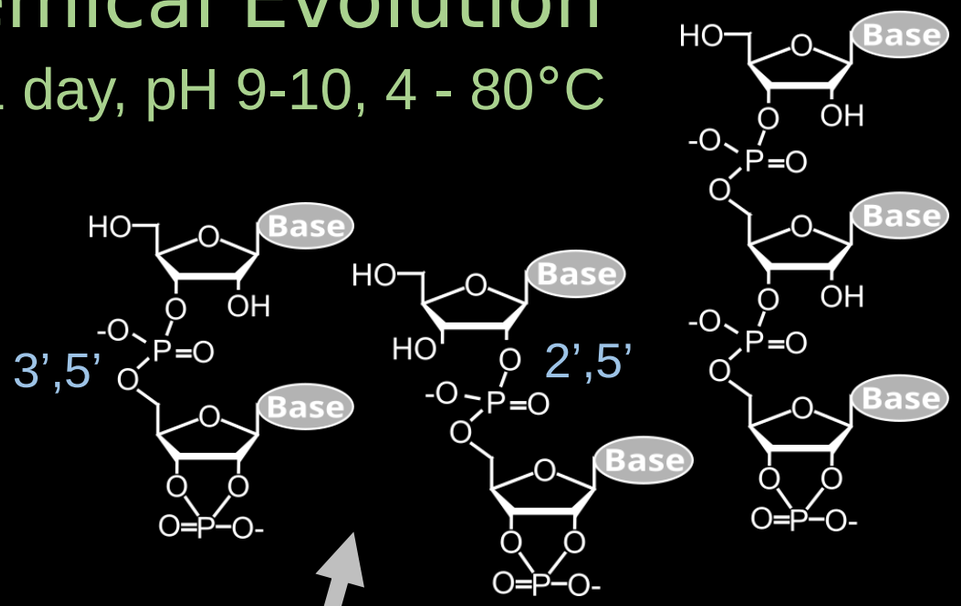
Sreekar Wunnava



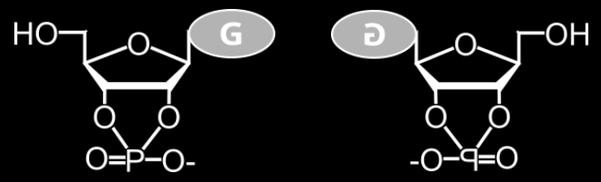
P-NMR: 60% : 40%
3’-5’ and 2’-5’

Chemical Evolution

Dry, 1 day, pH 9-10, 4 - 80°C



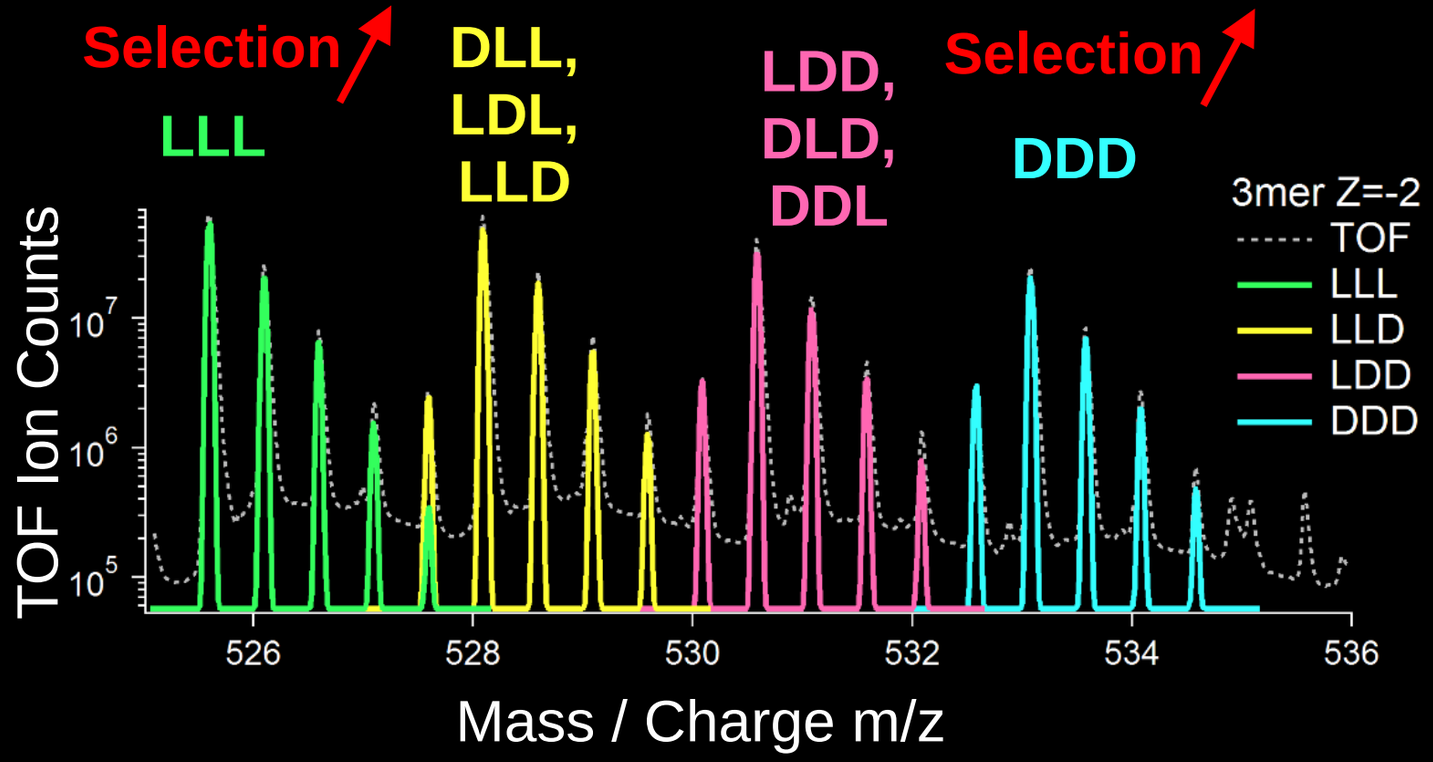
D L



5x 15N
+5Da

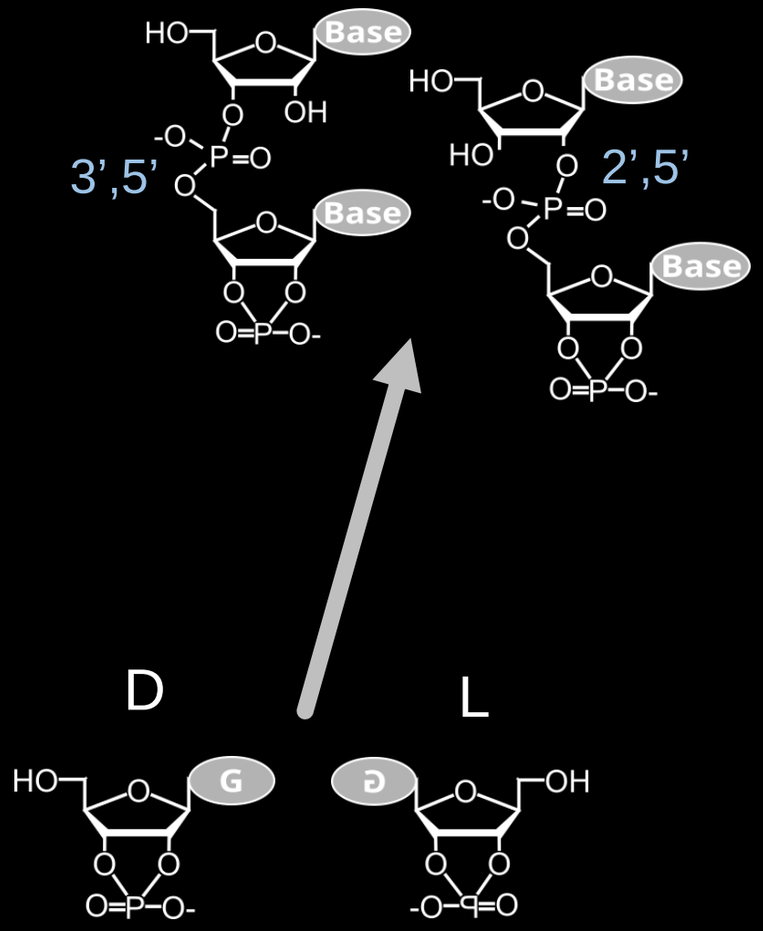
LLL DLL, LDL, LLD LDD, DLD, DDL DDD

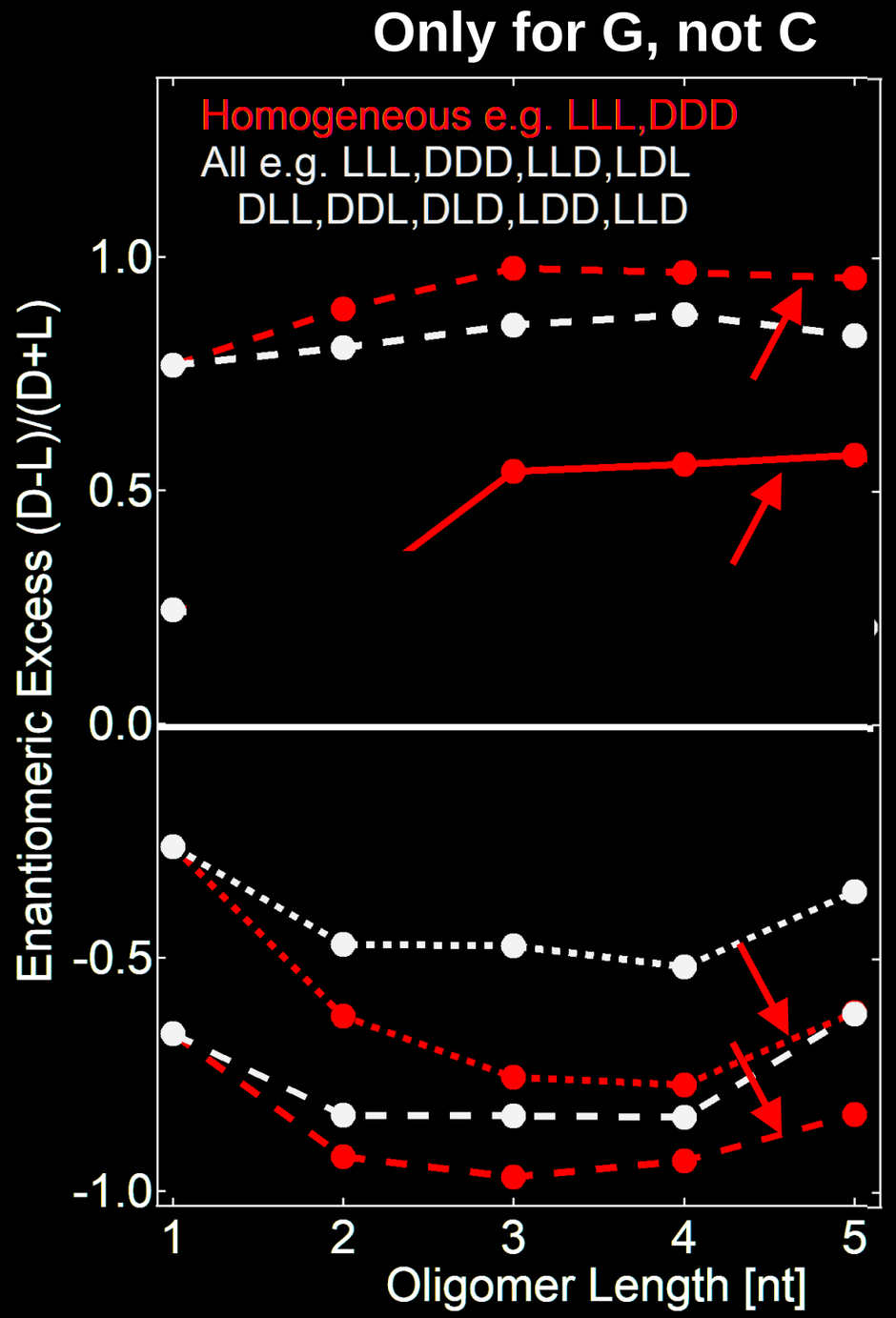
Sreekar Wunnava



Homochiral selection

Dry, 1 day, pH 10, 25°C





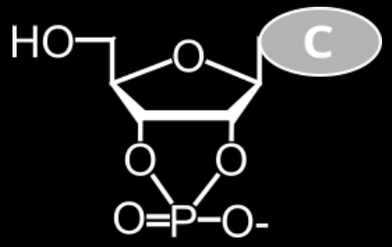
Sreekar Wunnava

Chirality is amplified for oligos capable to hybridize

Chiral amplification cycles by polymeri-zation and hydrolysis

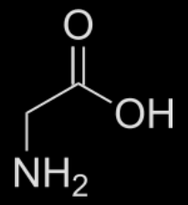
Catalytic Amino Acids

Dry, 1 day, 20µl, pH 10, **20°C**

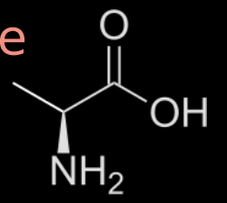


Amino Acids

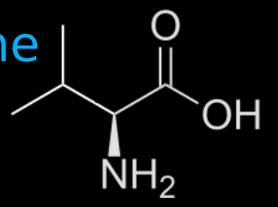
Glycine



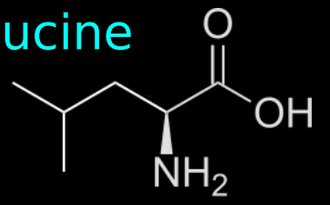
Alanine



Valine



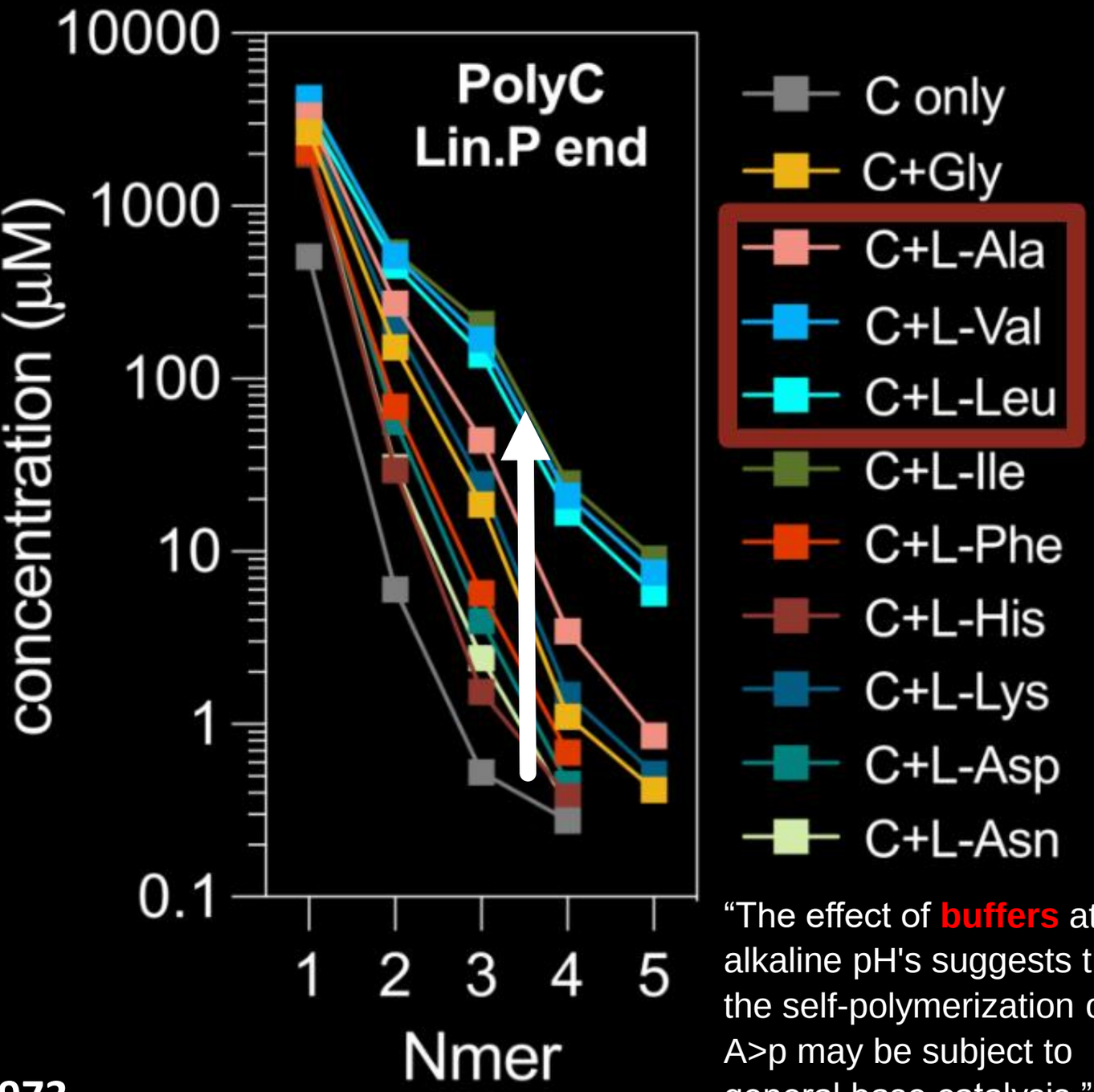
Leucine



Saroj Rout

With
Matt Powner

“Of the amino acids and peptides investigated as potential catalysts, only lysine and its oligomers proved effective, **and only on heating at 85°C**”

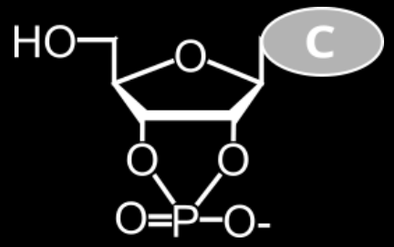


“The effect of **buffers** at alkaline pH's suggests that the self-polymerization of A>p may be subject to general base catalysis.”

Verlander, Lohrmann, Orgel, L. E. *J. Mol. Evol.* **1973**

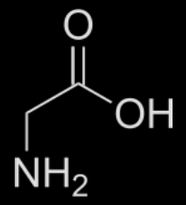
Catalytic Amino Acids

Dry, 1 day, 20µl, pH 10, 20°C

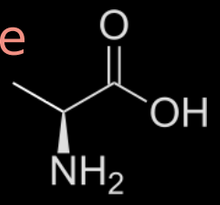


Amino Acids

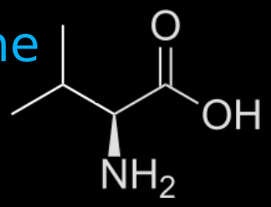
Glycine



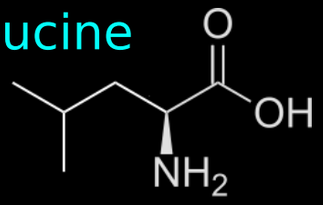
Alanine



Valine



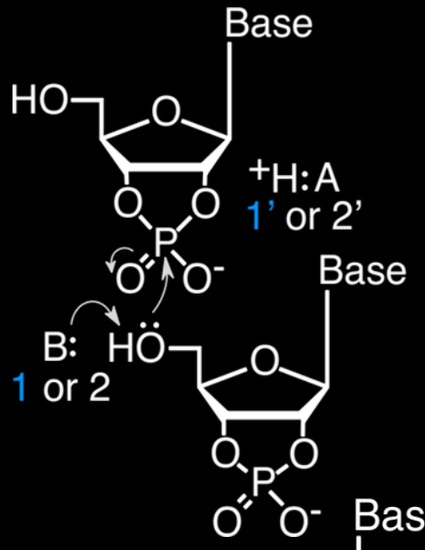
Leucine



Saroj Rout

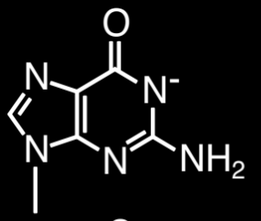
With
Matt Powner

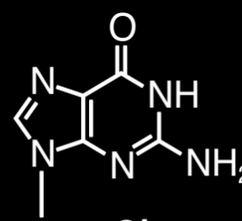
General Base Catalysis



1 1'

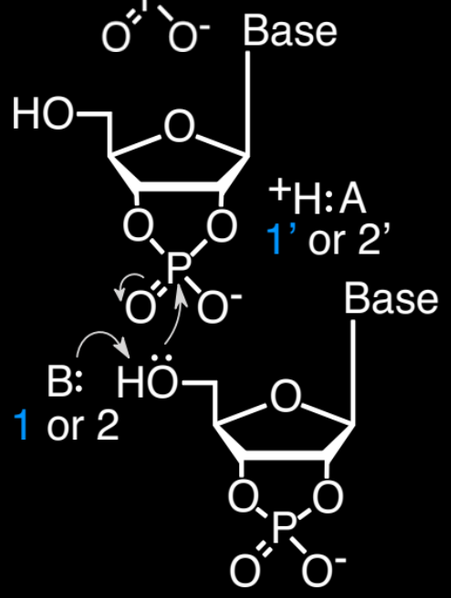
Amino acid : Valine

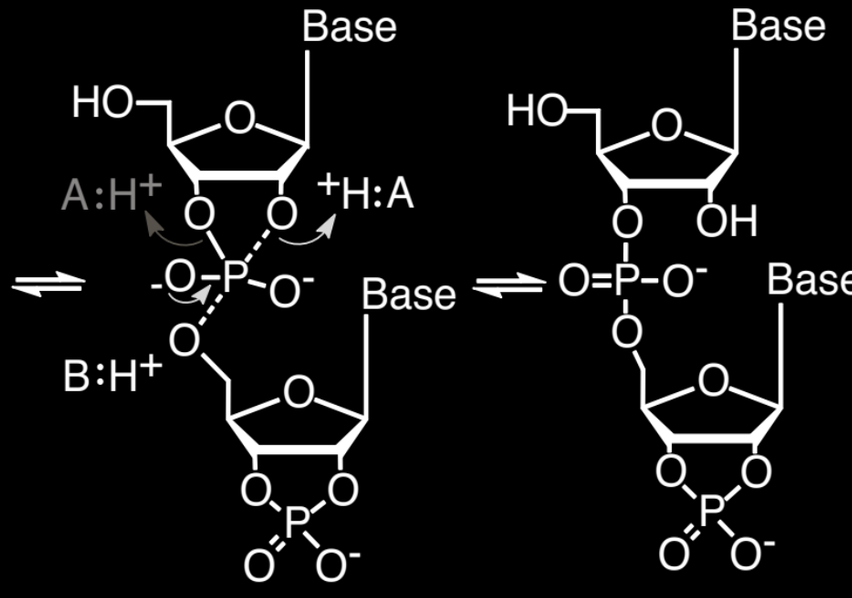




2 2'

Nucleobase : Guanine





Microscopic Reverse of Ribozyme hydrolysis

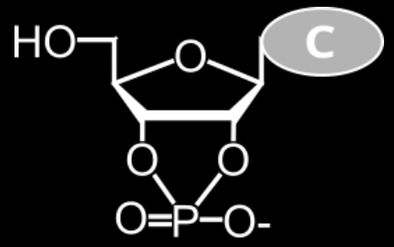
Direct pK_a Measurement of the Active-Site Cytosine in a Genomic Hepatitis Delta Virus Ribozyme

Andrej Lupták,[†] Adrian R. Ferré-D'Amaré,[§,¶] Kaihong Zhou,[‡] Kurt W. Zilm,[†] and Jennifer A. Doudna*[,§,‡]

In prep.

Catalytic Amino Acids

Dry, 1 day, 20µl, pH X, 20°C

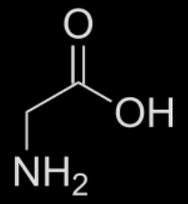


Saroj Rout

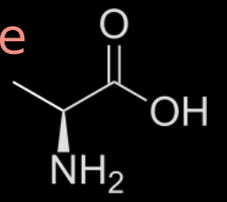
With
Matt Powner

Amino Acids

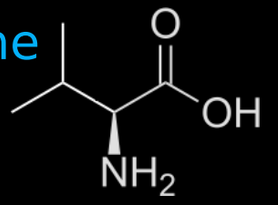
Glycine



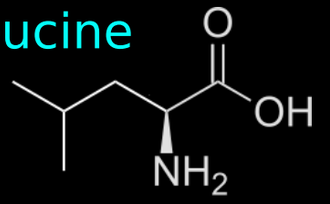
Alanine

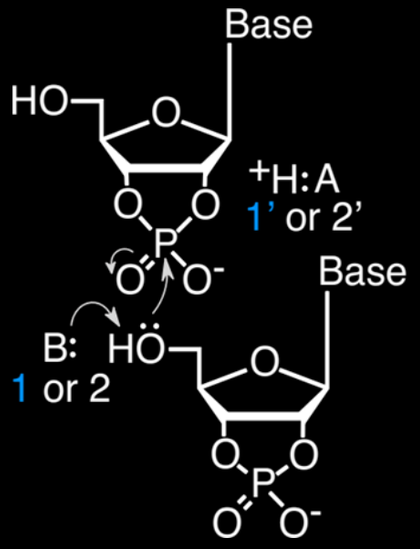


Valine

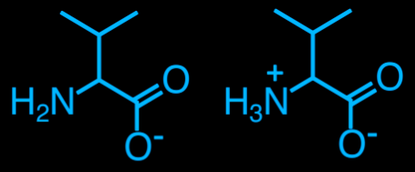


Leucine



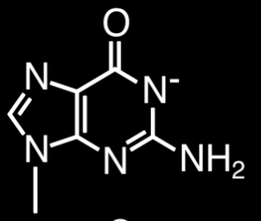


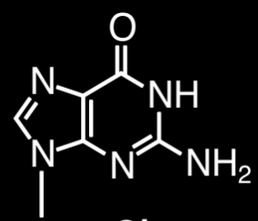
General Base Catalysis



1 1'

Amino acid : Valine

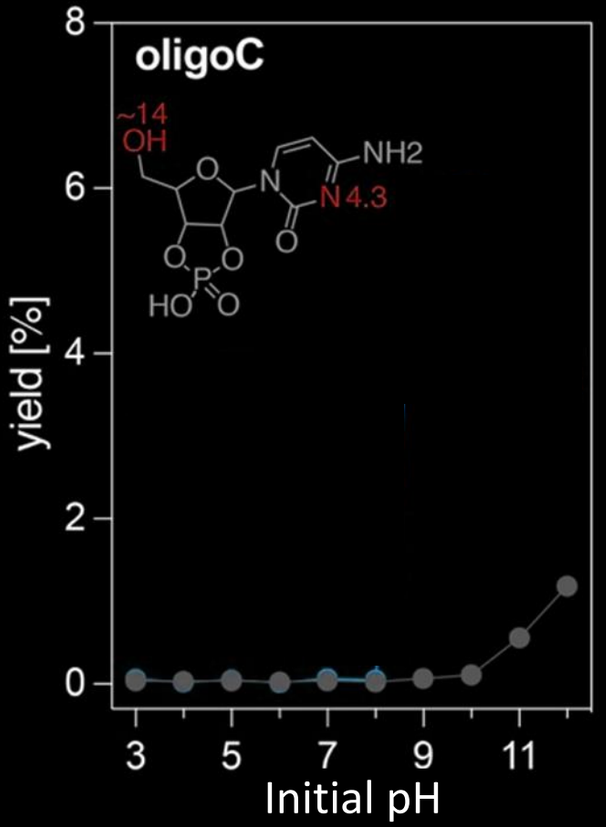




2 2'

Nucleobase : Guanine

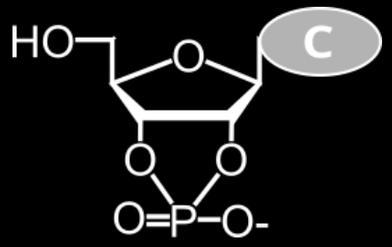
Nucleotide 10mM



In prep.

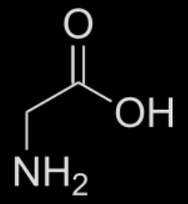
Catalytic Amino Acids

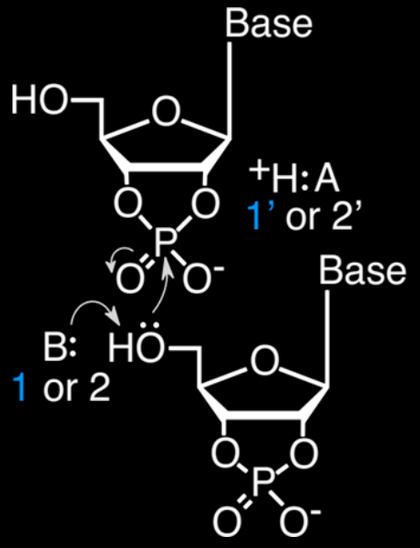
Dry, 1 day, 20µl, pH X, 20°C

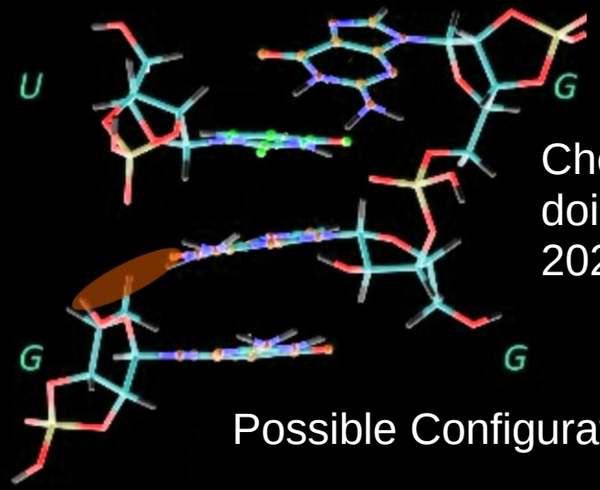


Amino Acids

Glycine







ChemSystemsChem
doi.org/10.1002/syst.
202200026 (2022)

Possible Configuration

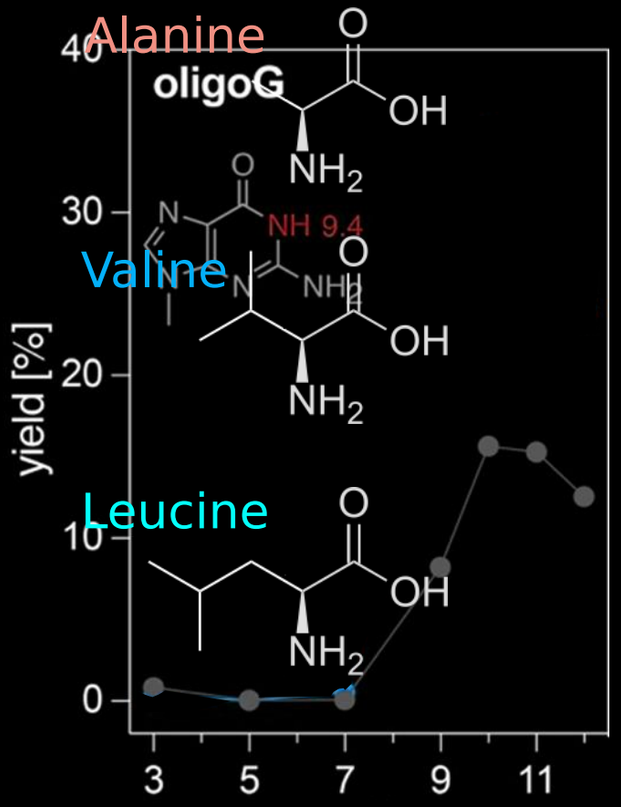
Saroj Rout

With
Matt Powner

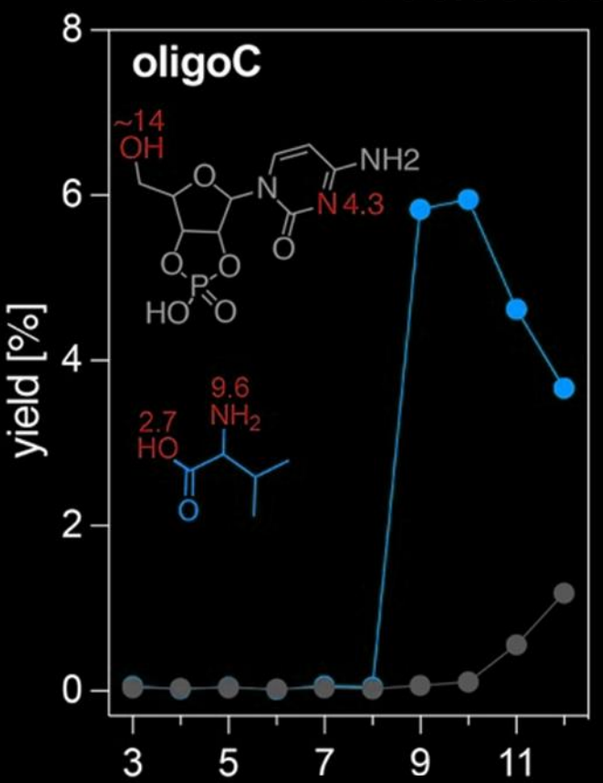
Alanine

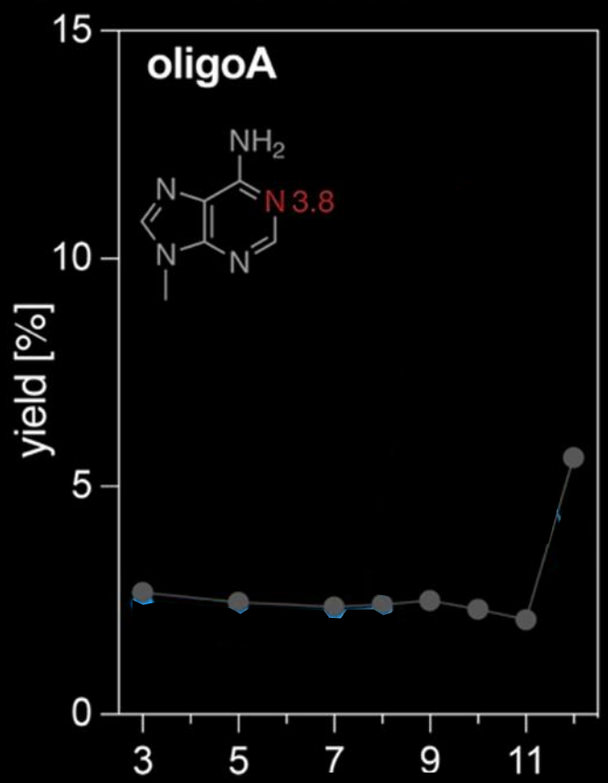
Valine

Leucine



Nucleotide 10mM **Valine 50mM**





In prep.

Crosscatalytic Network

Strand Separation

Recycling by Hydrolysis

RNA World

Replication by Ligation

Chiral Selection

RNA Polymers

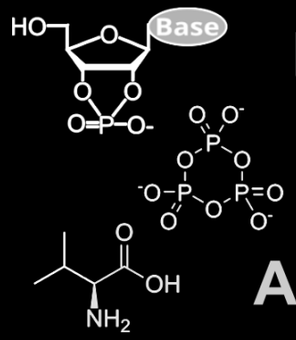
RNA Base

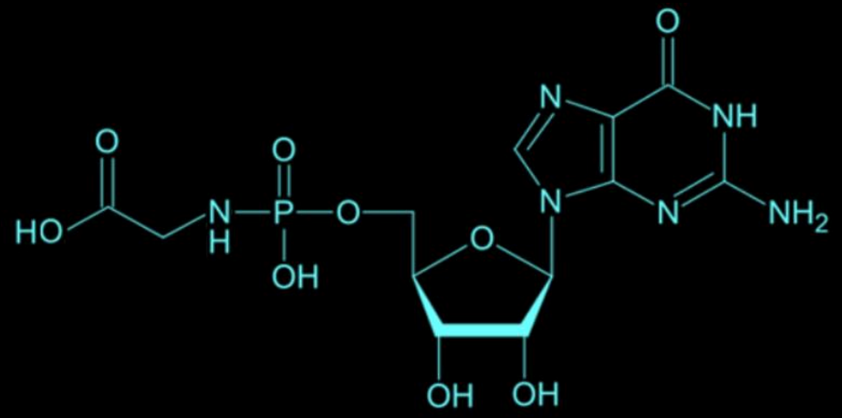
Activator

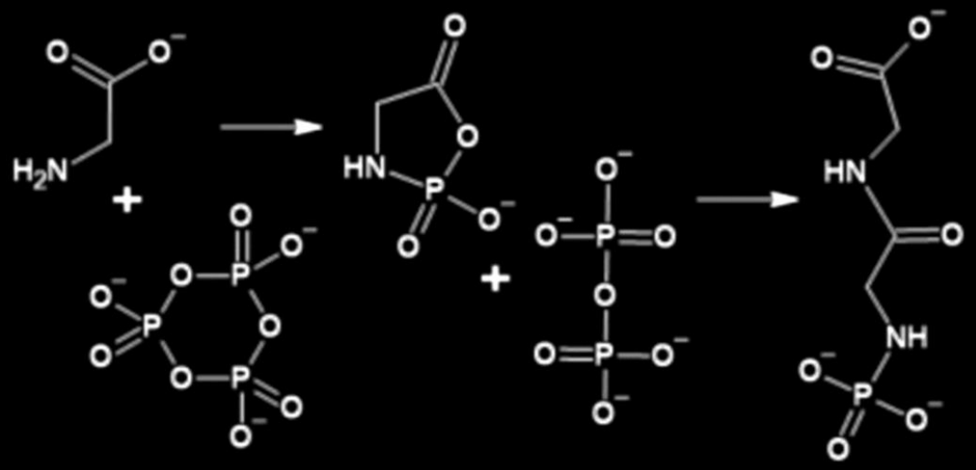
Amino acid

RNA-Peptide Hybrids

Peptide Polymers







10mM Glycine 10mM TMP **pH 10 60°C Dry**

The mechanism of the trimetaphosphate-induced peptide synthesis, Chung, Lohrmann, Orgel & Rabinowitz, *Tetrahedron*, 27:1205–1210 (1971)

Juliette Langlais

Crosscatalytic Network

Recycling by Hydrolysis

Strand Separation

RNA World

Replication by Ligation

Chiral Selection

RNA Polymers

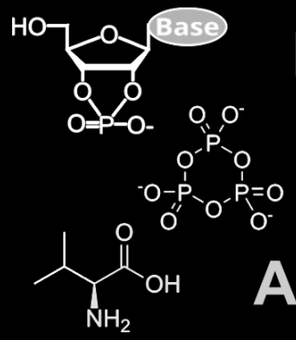
RNA Base

Activator

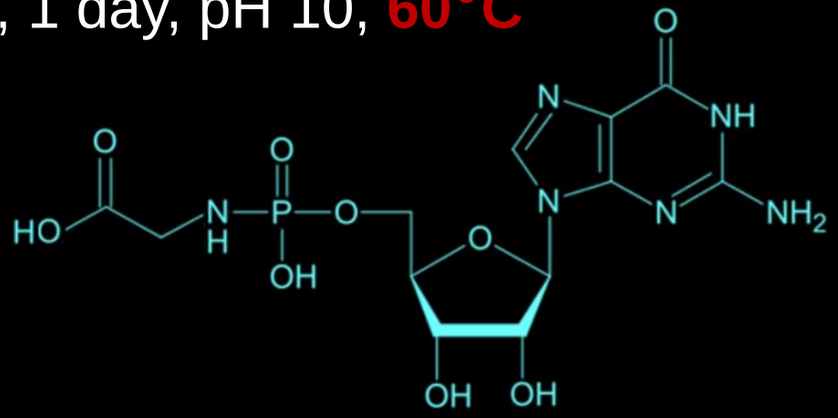
Amino acid

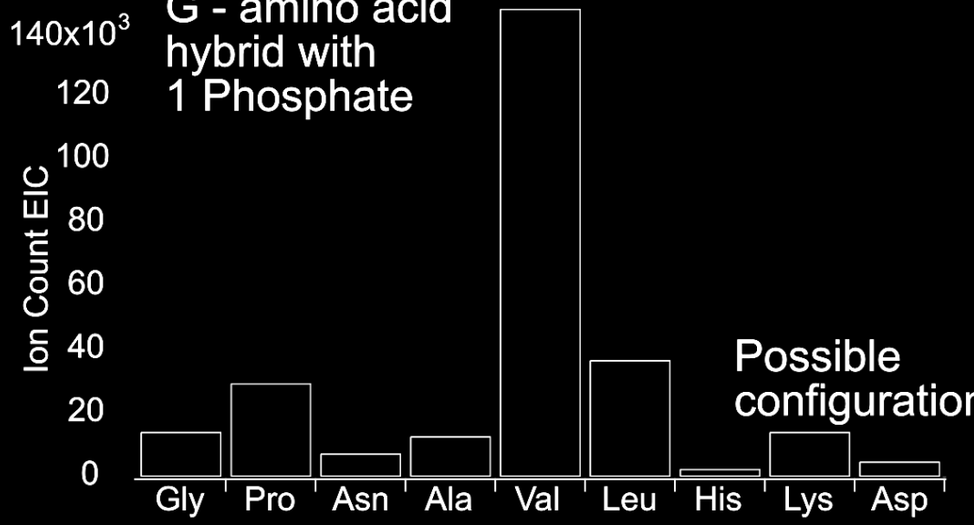
RNA-Peptide Hybrids

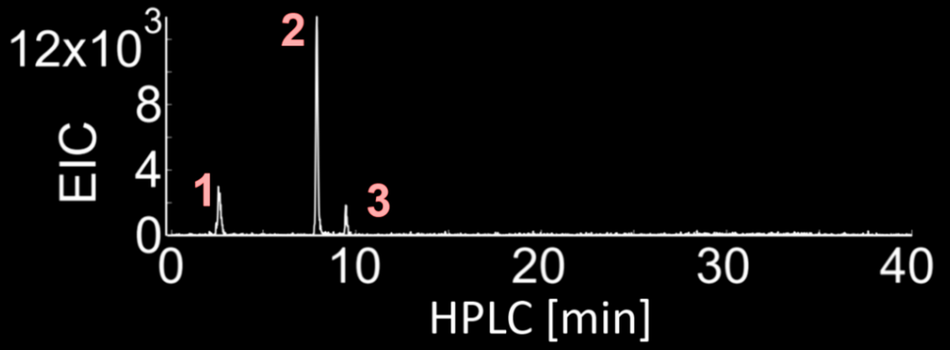
Peptide Polymers



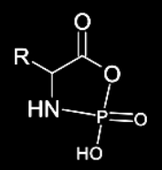
Dry, 1 day, pH 10, 60°C

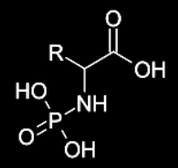


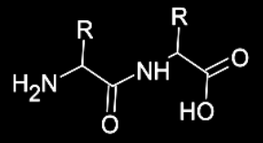


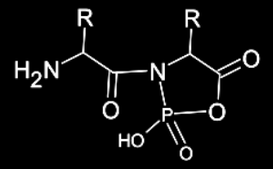


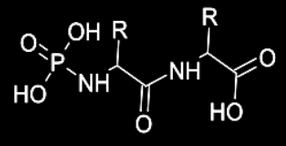
Juliette Langlais

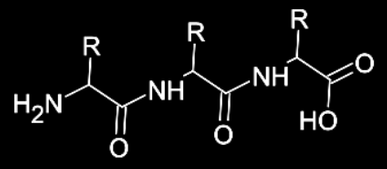


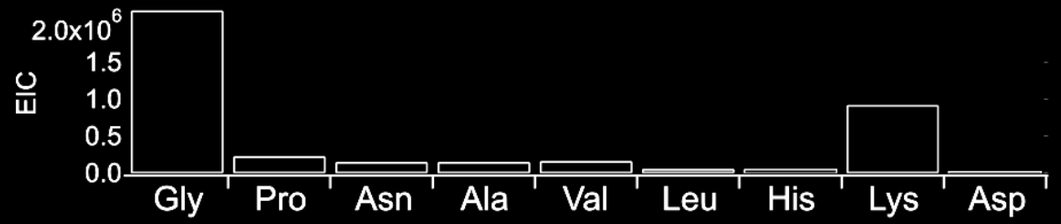


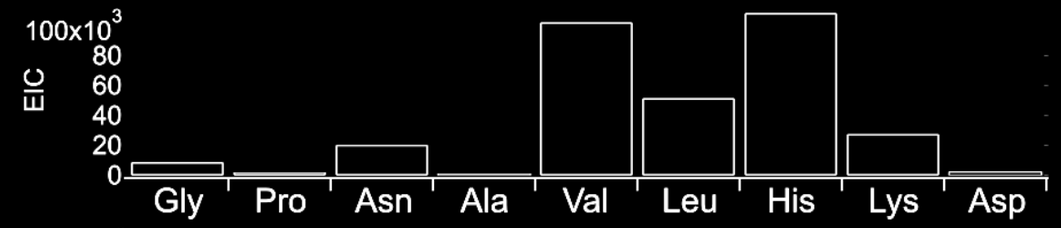


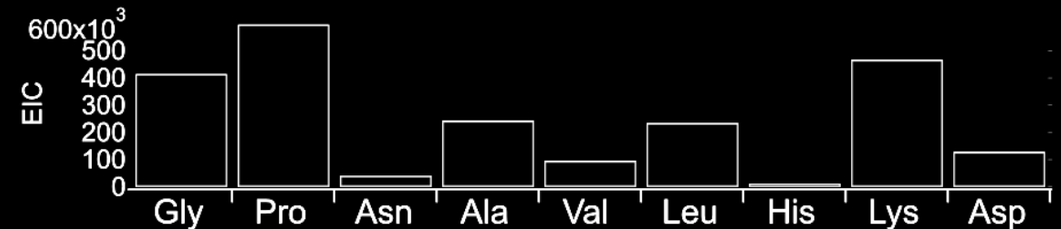


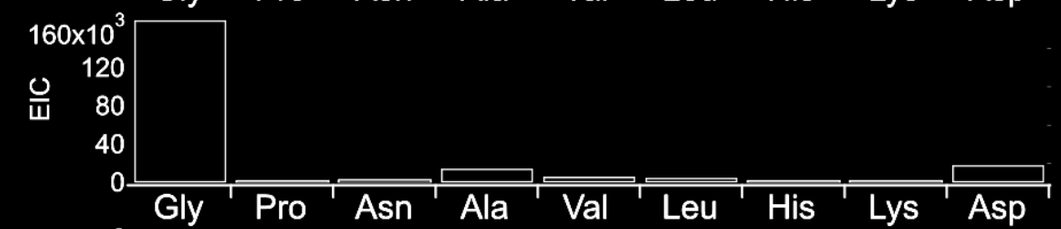


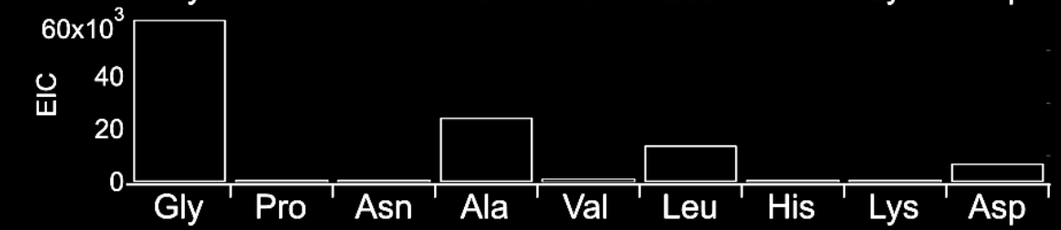


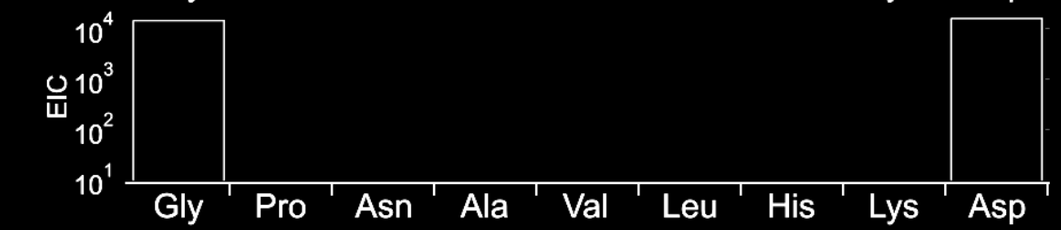




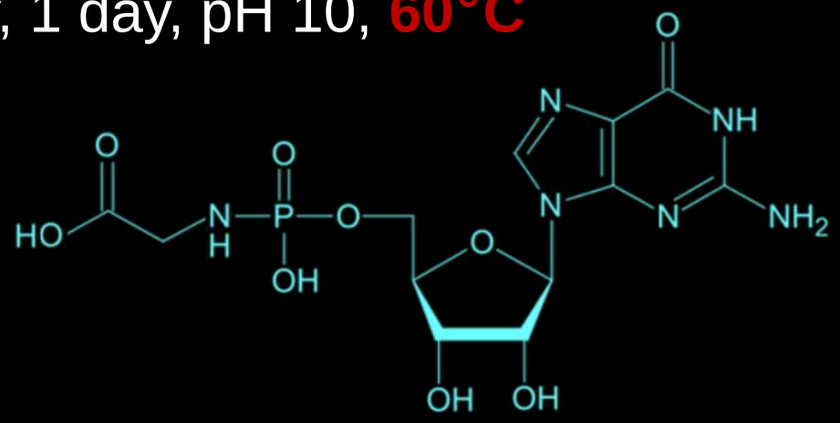




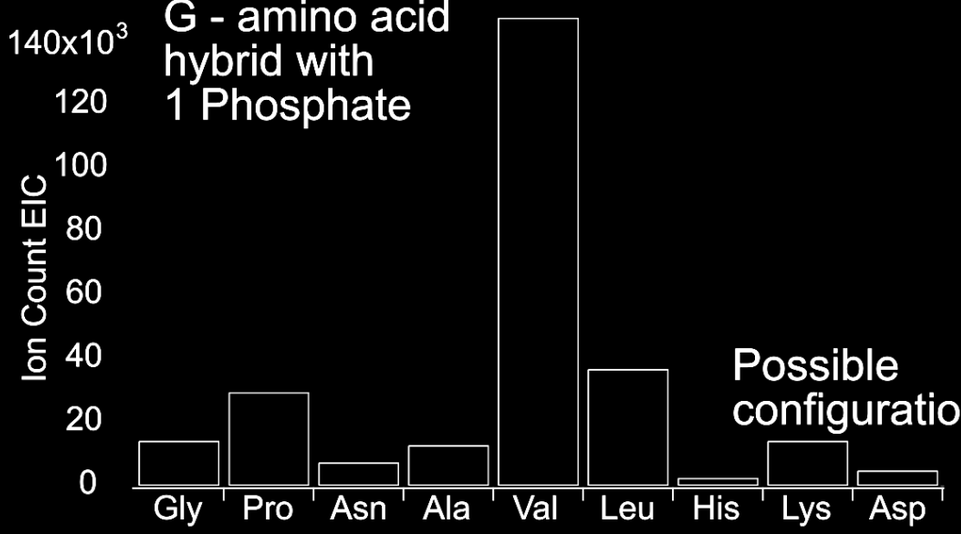




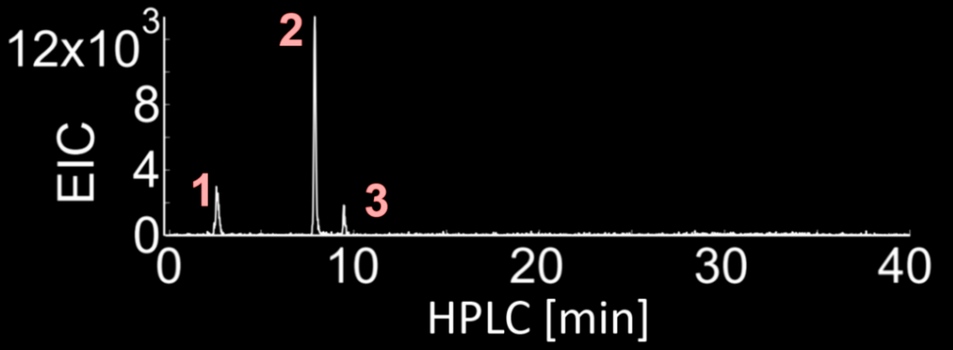
Dry, 1 day, pH 10, 60°C



G - amino acid hybrid with 1 Phosphate



Possible configuration



Juliette Langlais

Crosscatalytic Network

Recycling by Hydrolysis

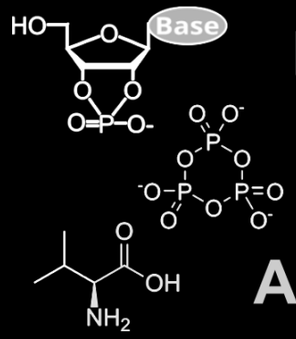
Strand Separation

RNA World

Replication by Ligation

Chiral Selection

RNA Polymers



RNA Base

Activator

Amino acid

RNA-Peptide Hybrids

Peptide Polymers

Reduction of 2',5'-links?

Peptide World

Reduced Hydrolysis?

RNA encoded Peptides?

Ligation leaving group?

Darwinian Evolution

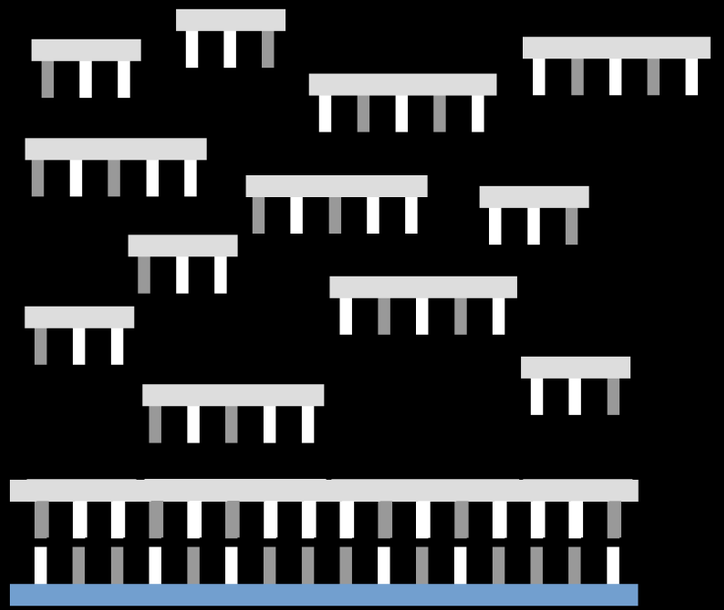
Life

1. Emergence of RNA and Peptides
2. Replication by templated ligation
3. Darwinian evolution on an early Earth

Replication by templated ligation

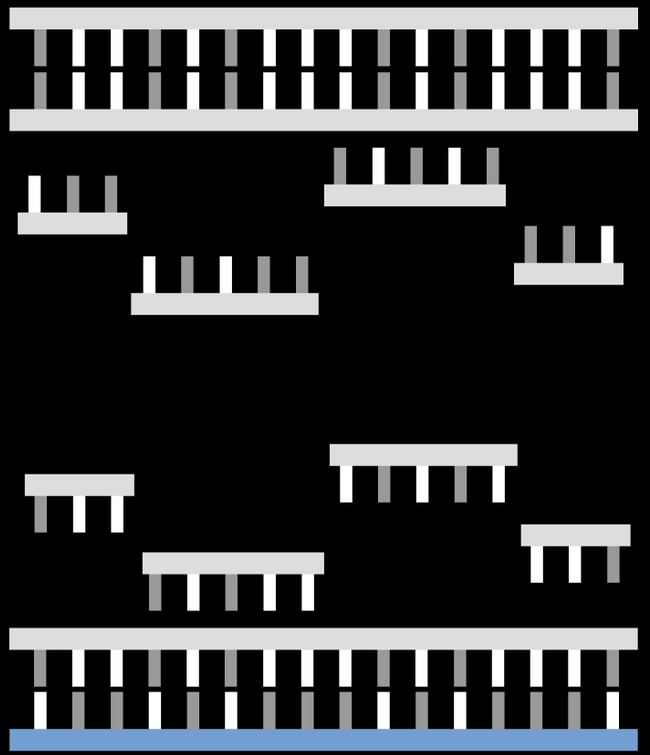
Length Independent Kinetics

Short random sequences



Template to copy

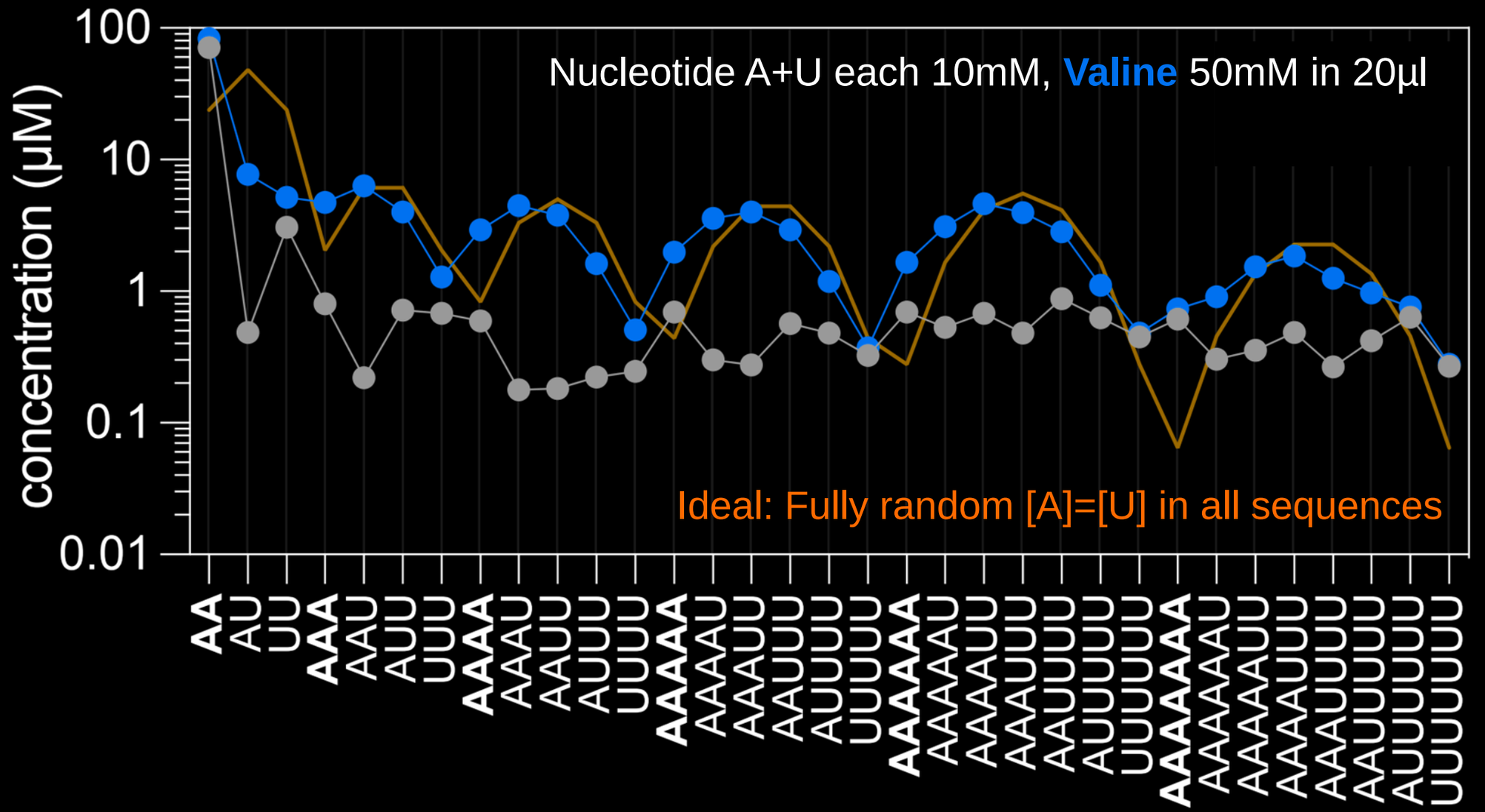
Strand separation



Template to copy

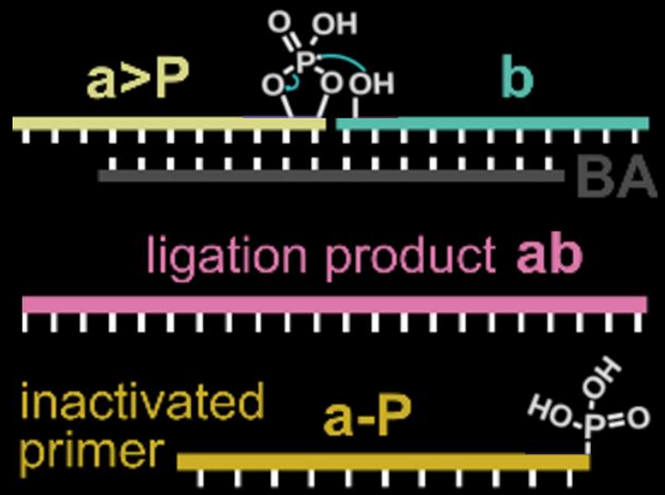
Copolymerization of A+U

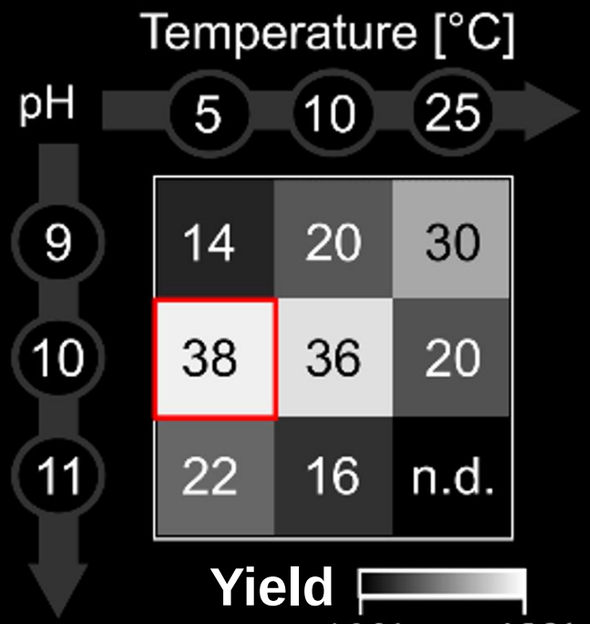
Dry, 1 day, 20µl, pH 10, 20°C

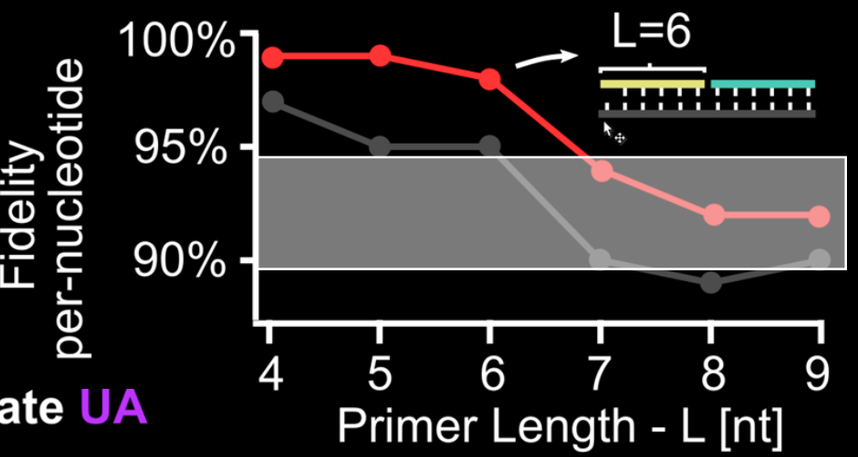


In prep.

Templated ligation of RNA at low Mg^{2+} concentration

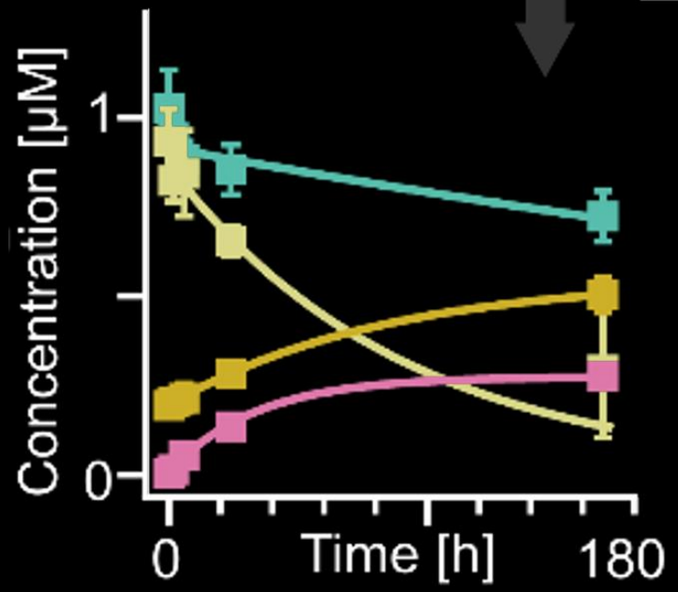




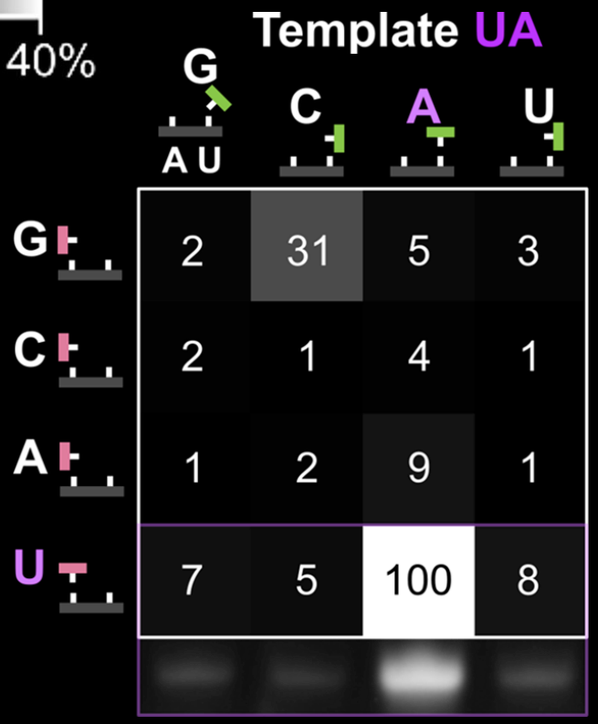


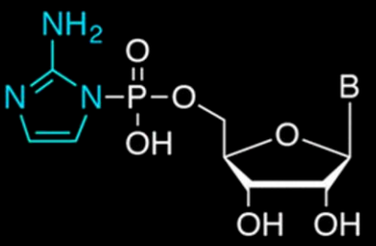
Adriana Serrao

Sreekar Wunnava



[a],[b],[ba] = 1µM 50mM CHES
1mM $MgCl_2$ pH 10, 10°C

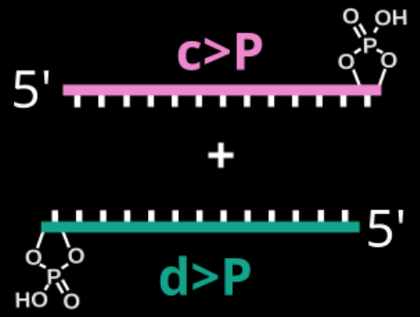


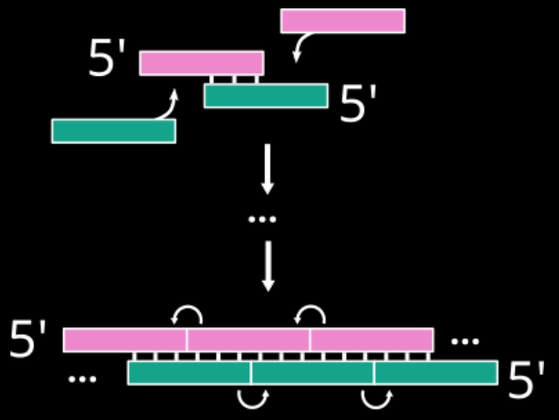


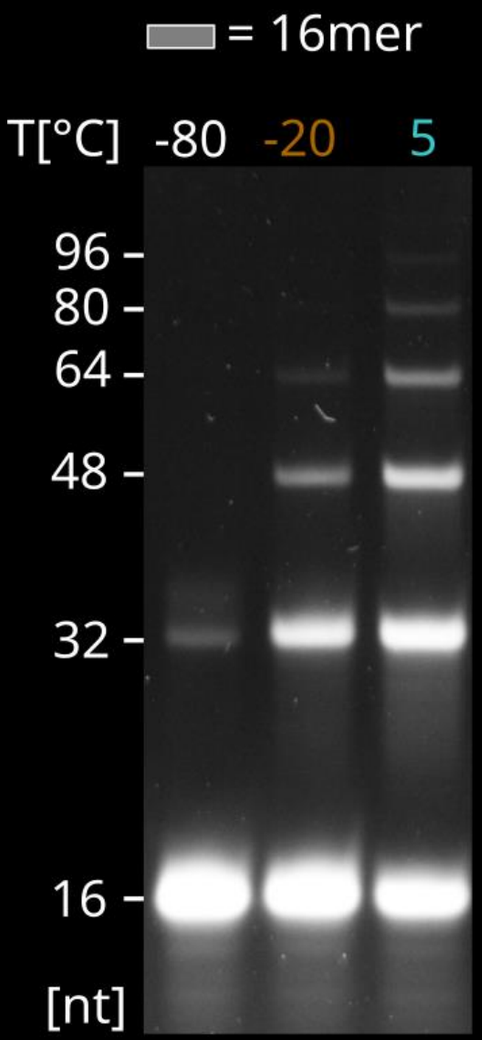
Jack Szostak et.al.

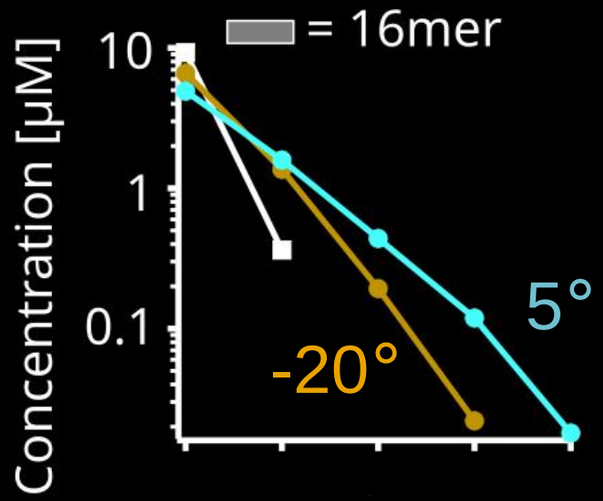
JACS
doi.org/10.1021/jacs.3c10813 (2024)

Splint ligation of RNA at low Mg^{2+} concentration



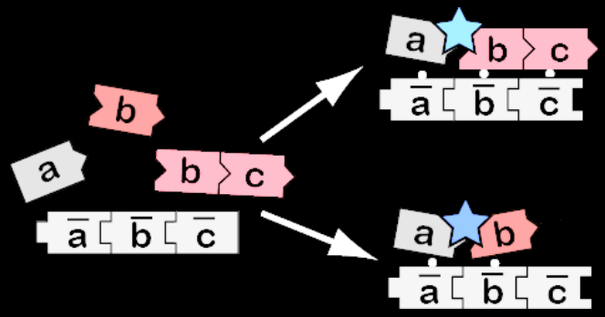




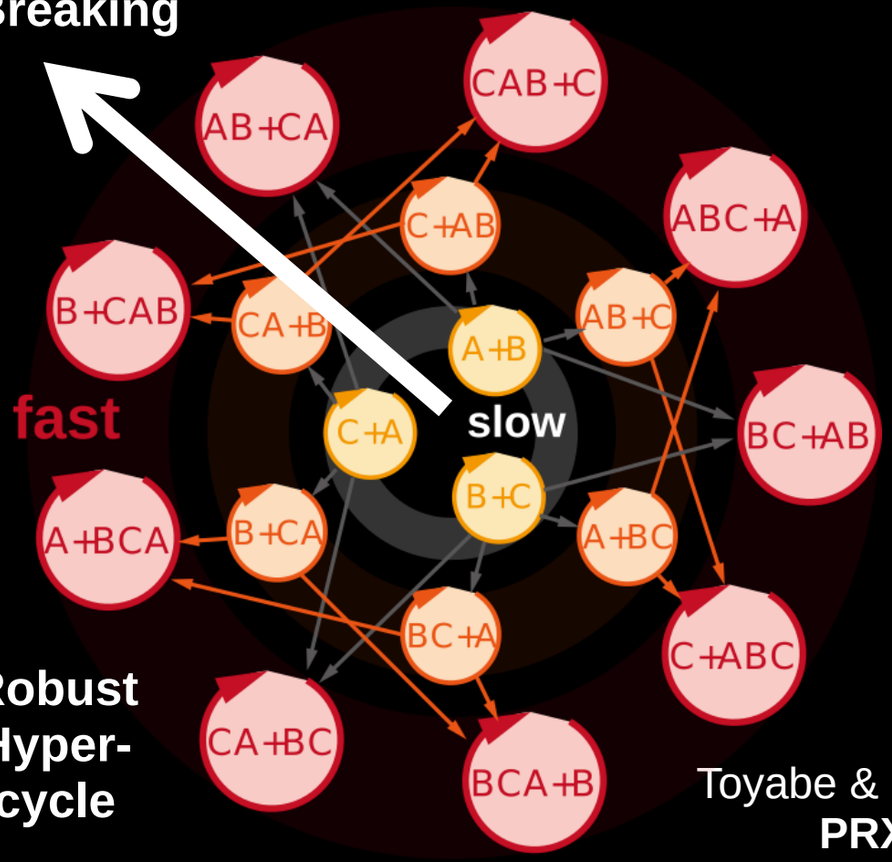


1mM $MgCl_2$ 50mM CHES pH 10 7 days

Symmetry breaking in Replication by templated Ligation (with ligase)

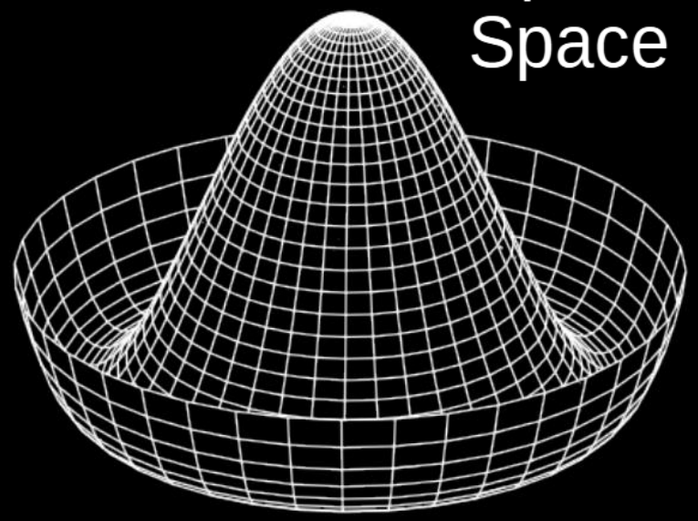


Symmetry Breaking

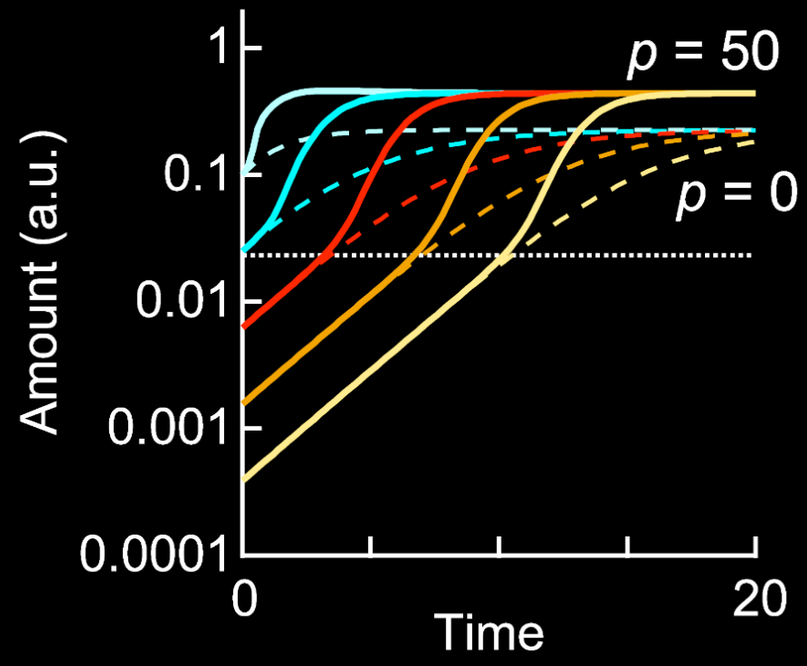


Robust Hyper-cycle

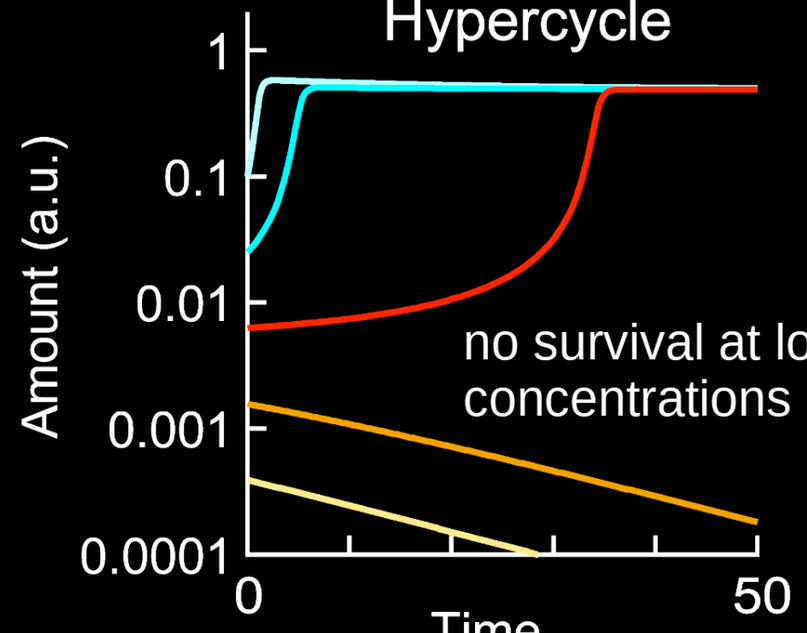
Toyabe & Braun **PRX** 2019



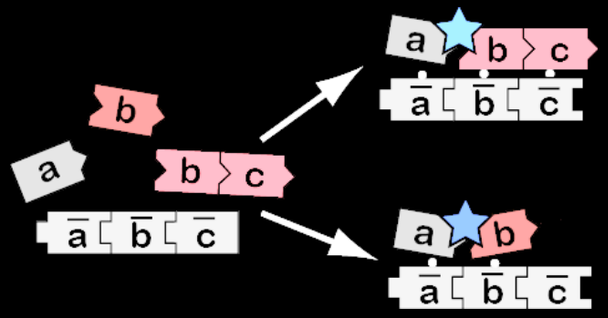
Cooperative cascade



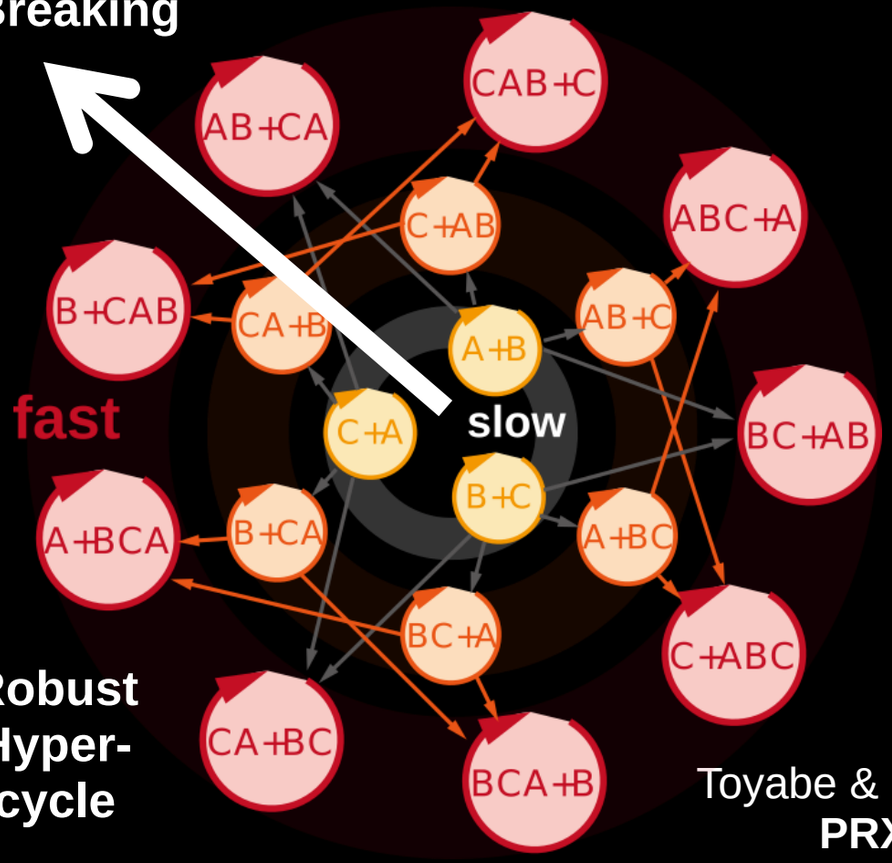
Hypercycle



Symmetry breaking in Replication by templated Ligation (with ligase)



Symmetry Breaking

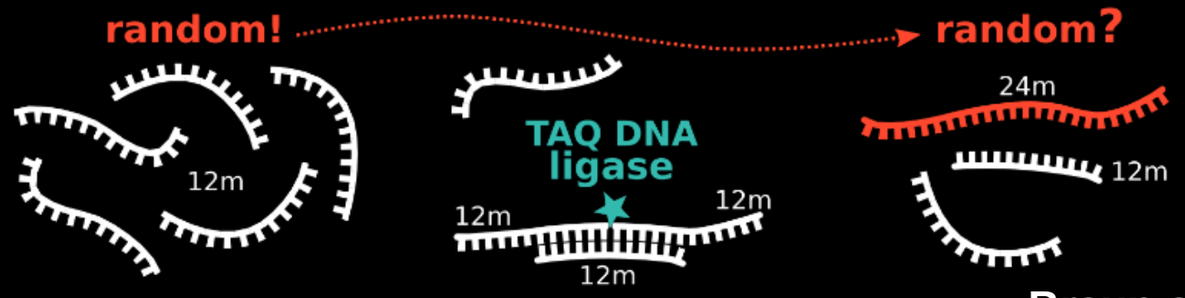


Robust Hyper-cycle

Toyabe & Braun **PRX** 2019

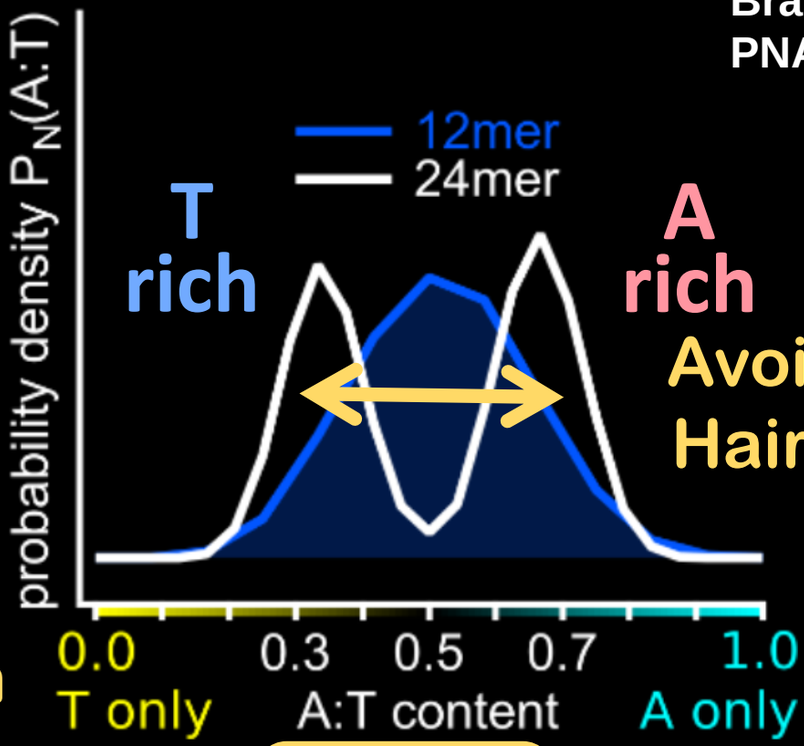
Replication dynamics in sequence space

From Random to Non-random Sequences



12mer random

Braun et.al. **PNAS** 2021



Replication Sequences

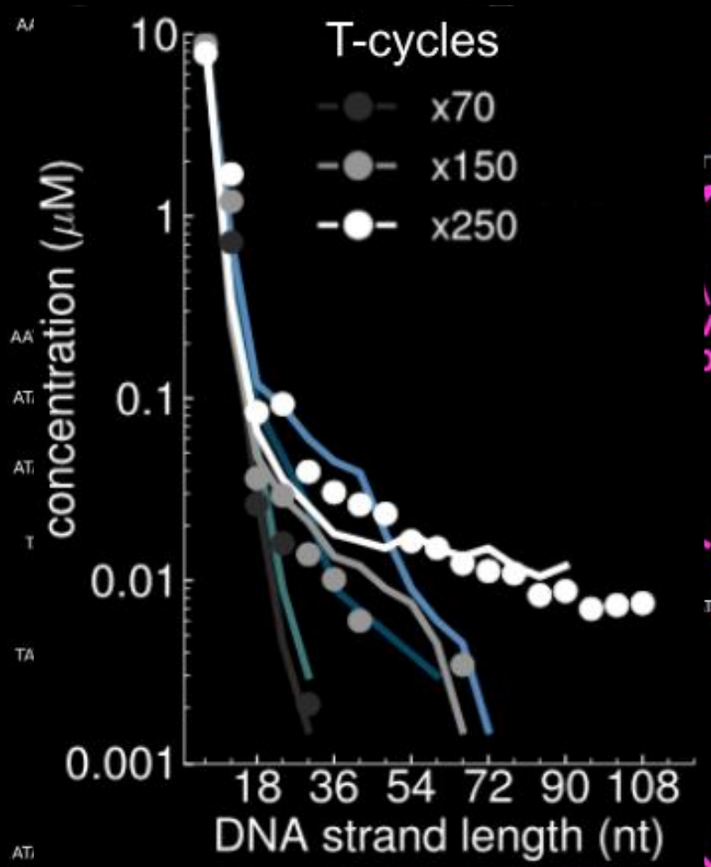
A-rich T-rich

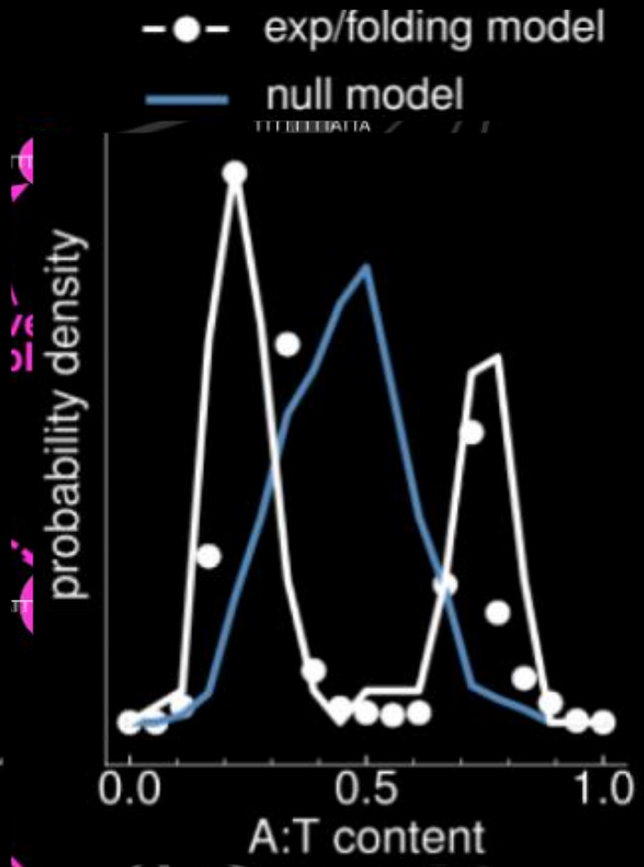
Replication dynamics in sequence space

From Random to Non-random Sequences

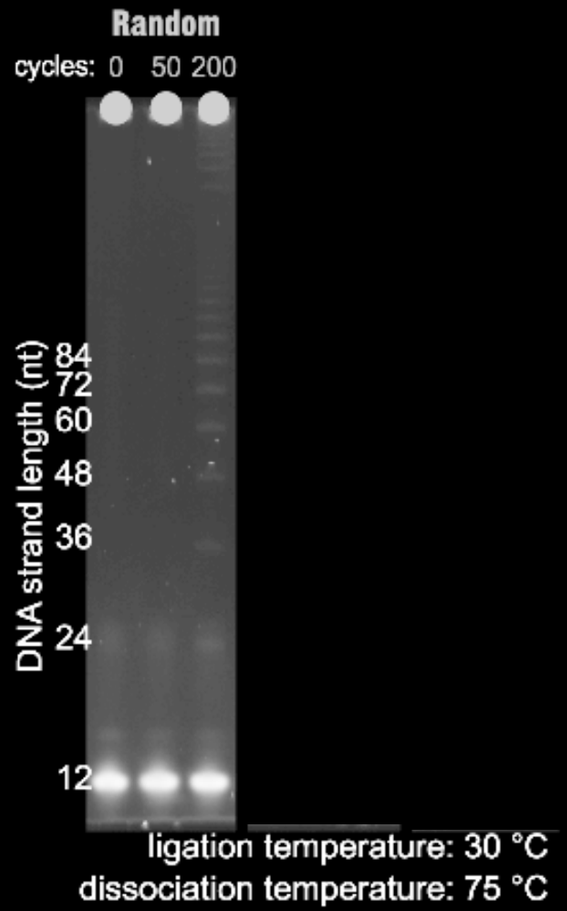
A-rich sequence network

T-rich sequence network





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



Random
AAAATAAAATAT
ATAATTAAATAA
TAAAAATTATTT
TTAAATTTTATA
TATTTAATTTTT
TAAAAATTAATA
AAAATAATTTAT
TTATATAAAATA

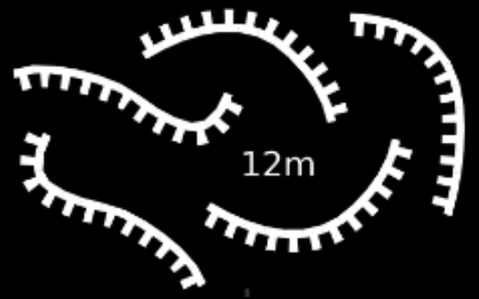
Braun et.al.
PNAS 2021

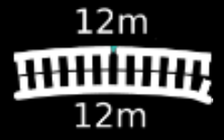
Kinetic selection of simpler, longer sequences

Dilution by Sequence Space

ATGC

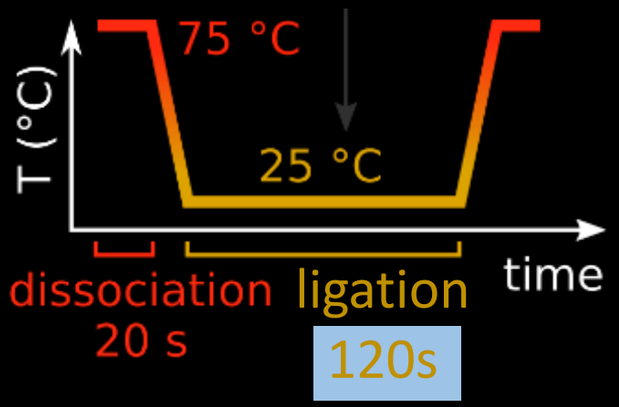
$$t = \frac{k_{on}^{hyb}}{10\,\mu M} 4^{12} = 19\,days$$

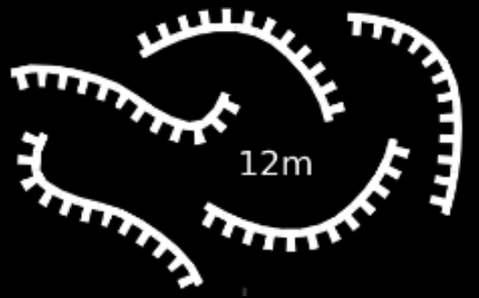


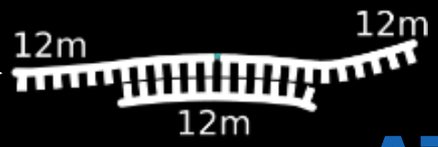


Hybridization

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

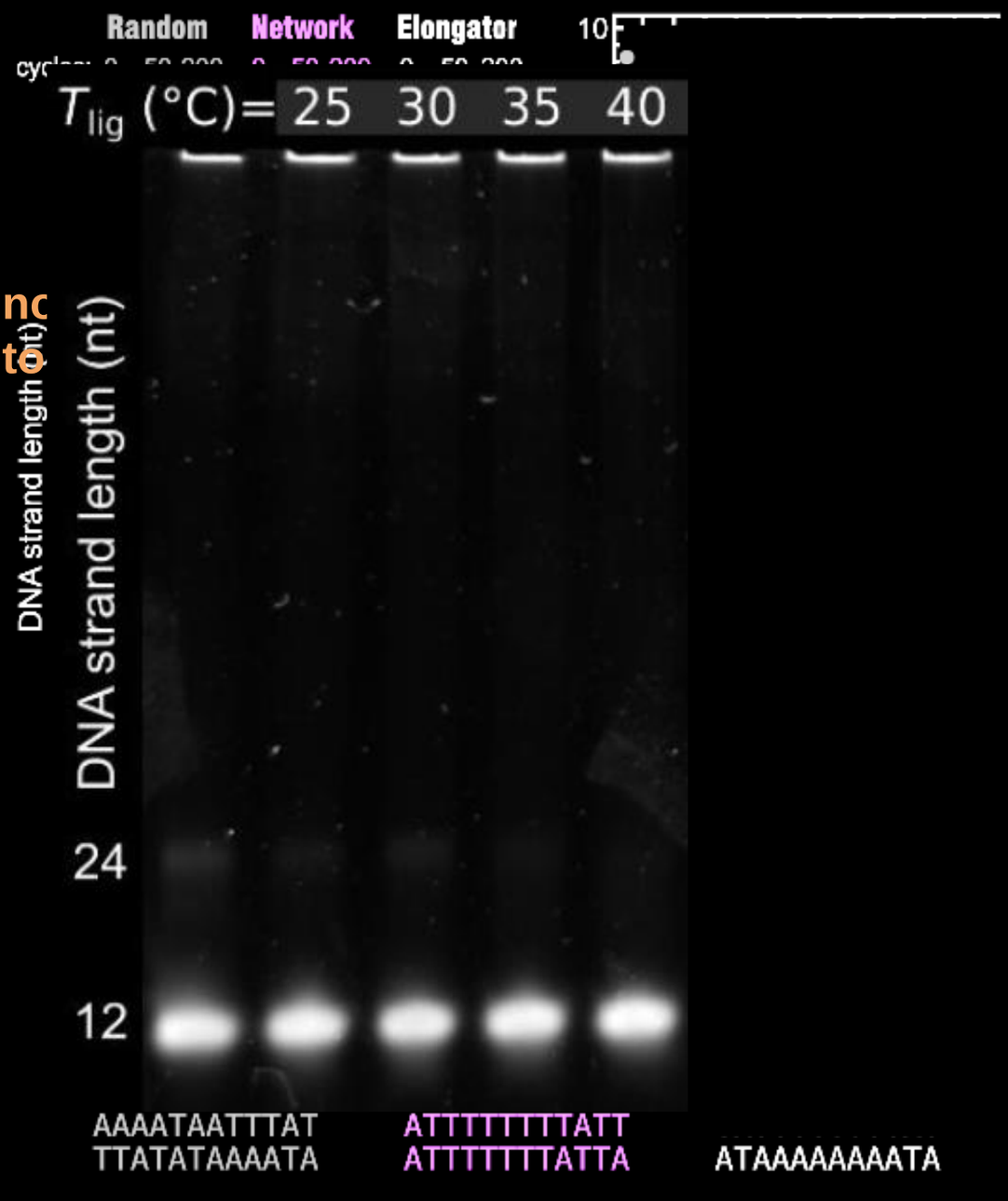




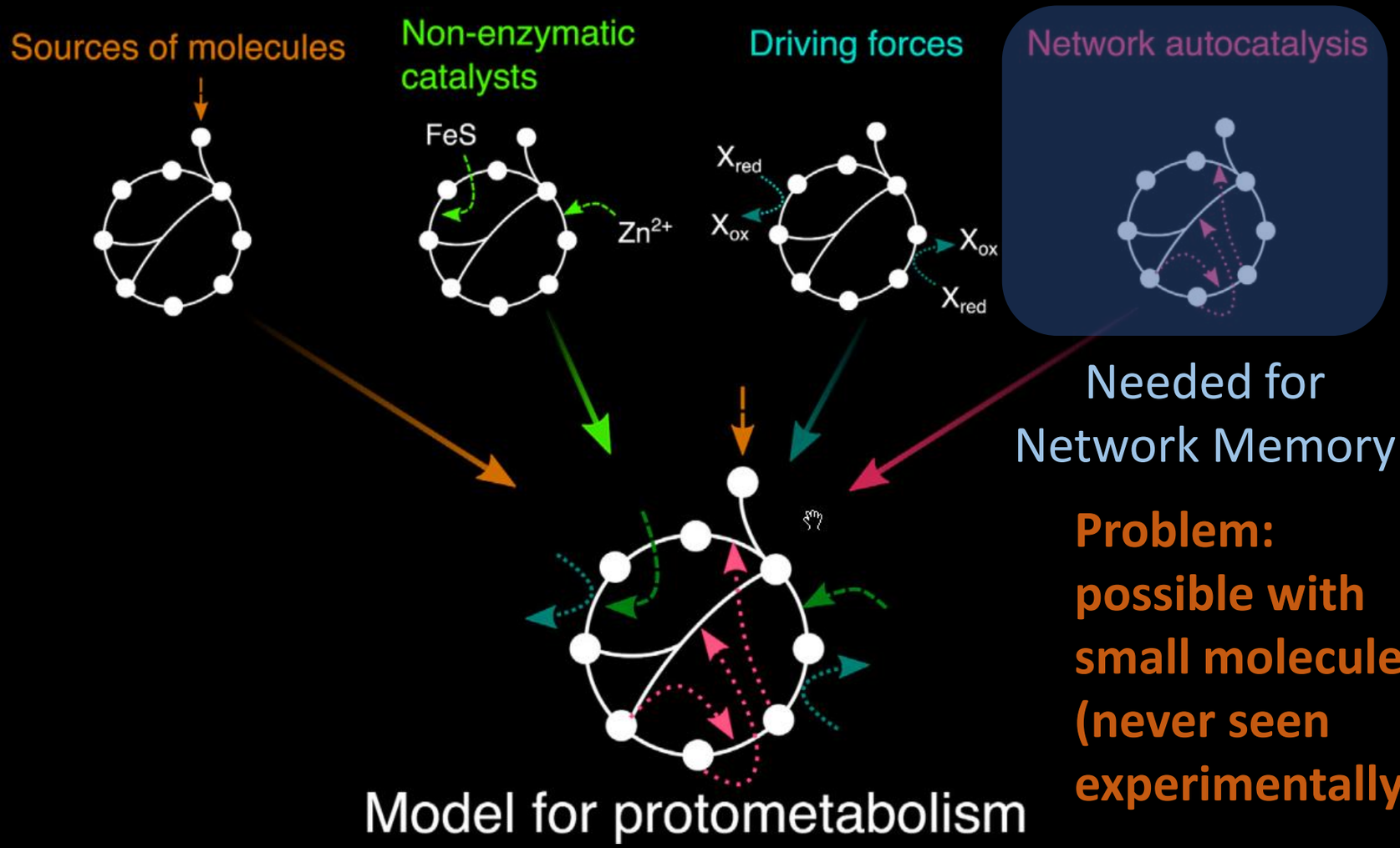


ATGC

$$t = \frac{k_{on}^{hyb}}{10\,\mu M} 4^{6} = 410\,s$$



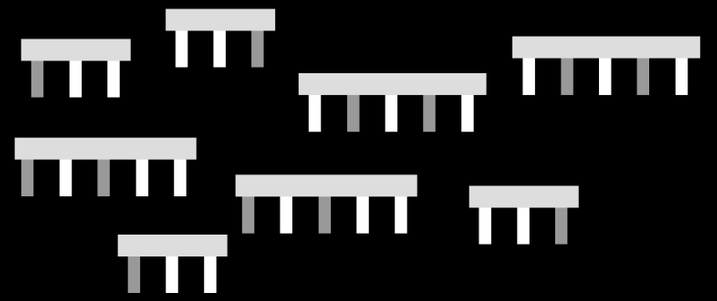
Cross-catalytic networks with 2’,3’-cyclic RNA

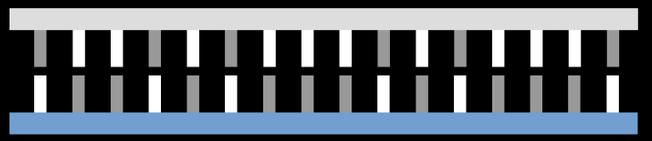


Needed for Network Memory

Problem: possible with small molecules? (never seen experimentally)

Pool of sequences





Template

Autocatalysis encoded by sequences from 2’,3’-cyclic Nucleotides

Modern views of ancient metabolic networks,
Joshua Goldford and Daniel Segrè, Curr. Opp. Sys. Biol. 8:117–124 (2018)

1. Emergence of RNA and Peptides
2. Replication by templated ligation
3. Darwinian evolution on an early Earth

Early Earth and Exoplanets

4.5 Ga

3.8 Ga

Moon forming impact

Water world
with some volcanic islands

Temperature difference across volcanic rock pores

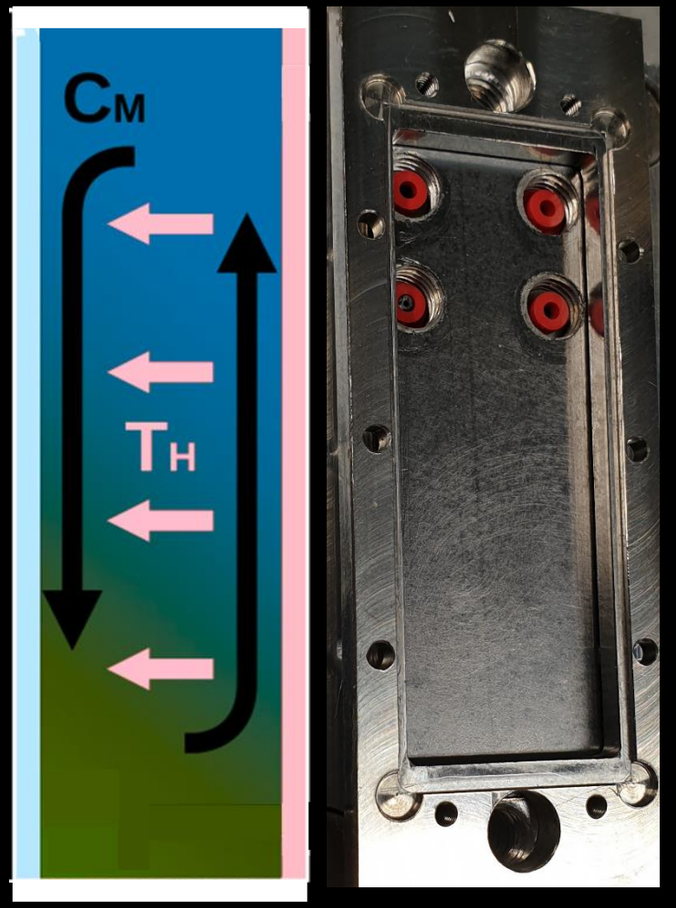
National Geographic

Temperature difference across volcanic rock pores

Iceland, old eruption site

Temperature difference: wasteless non-equilibrium

Cold ← Warm



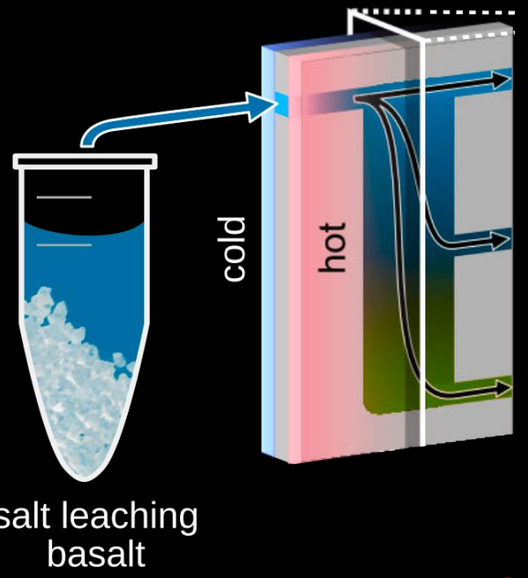
“Rock crack”

20mer DNA

PRL 112, 198101 (2014)
PNAS 103, 19678–19682 (2006)

nano TEMPER

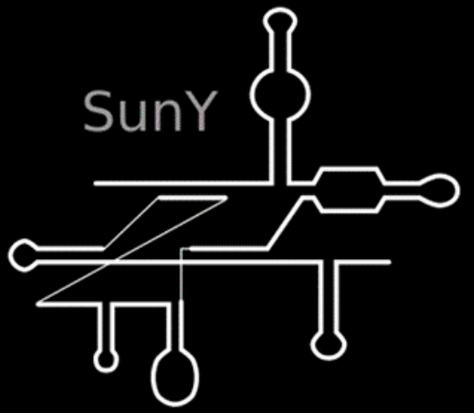
Temperature difference: wasteless non-equilibrium



Prebiotic
HPLC

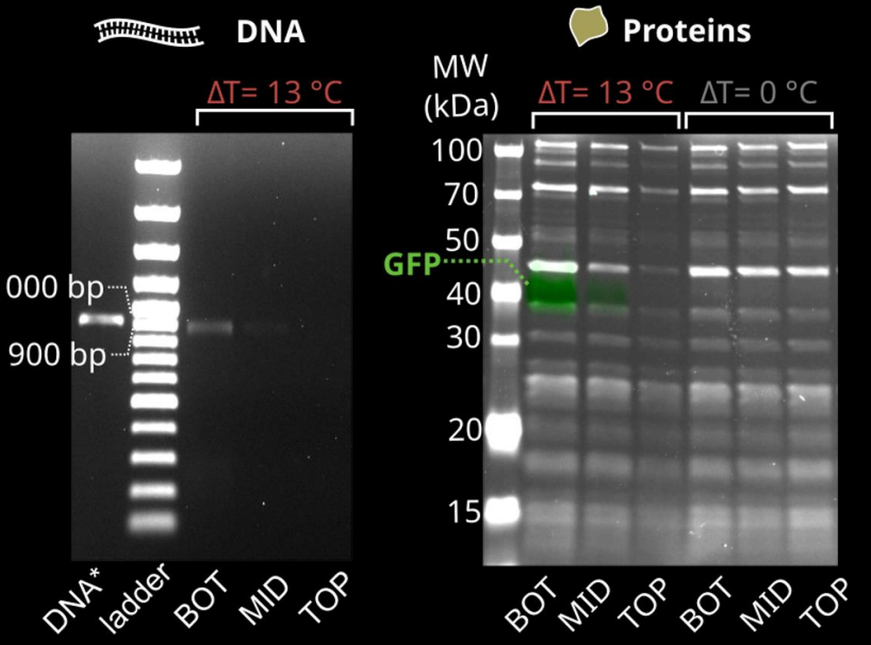
Christof
Mast

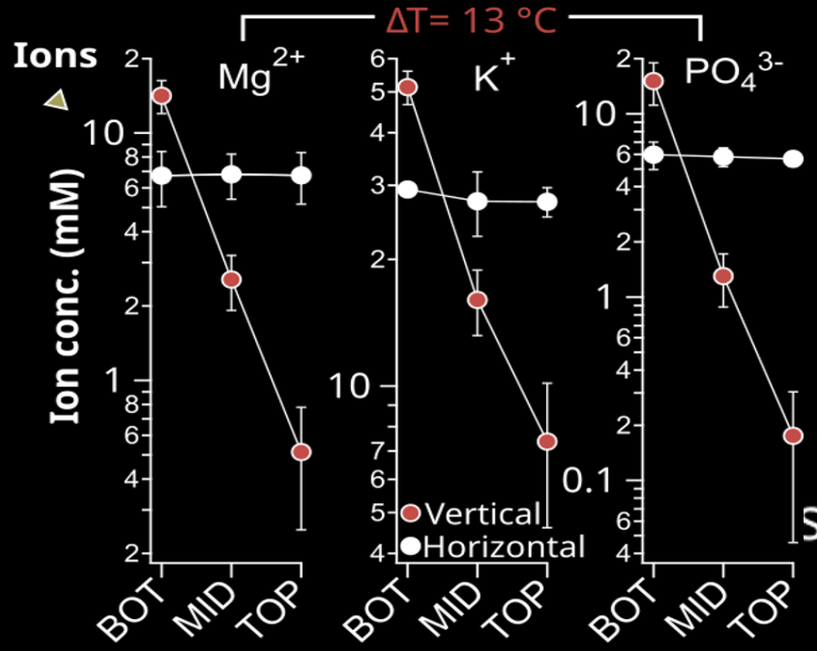
Thomas
Matreux



Nature Chemistry
doi.org/10.1038/s41557
-021-00772-5 (2021)

Assembling membrane-free cell by heat





Thermally assembled cell

environ-ment

Cell-like

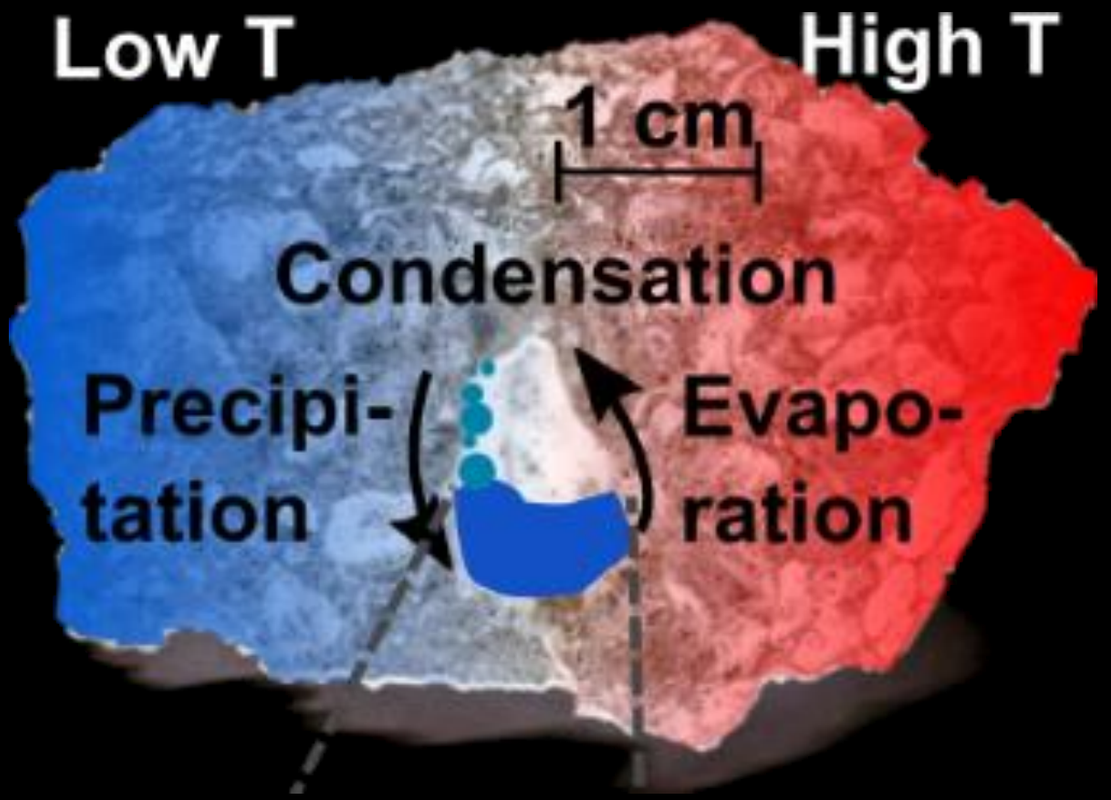
sion →

PURE

ON

PNAS, under resubmission

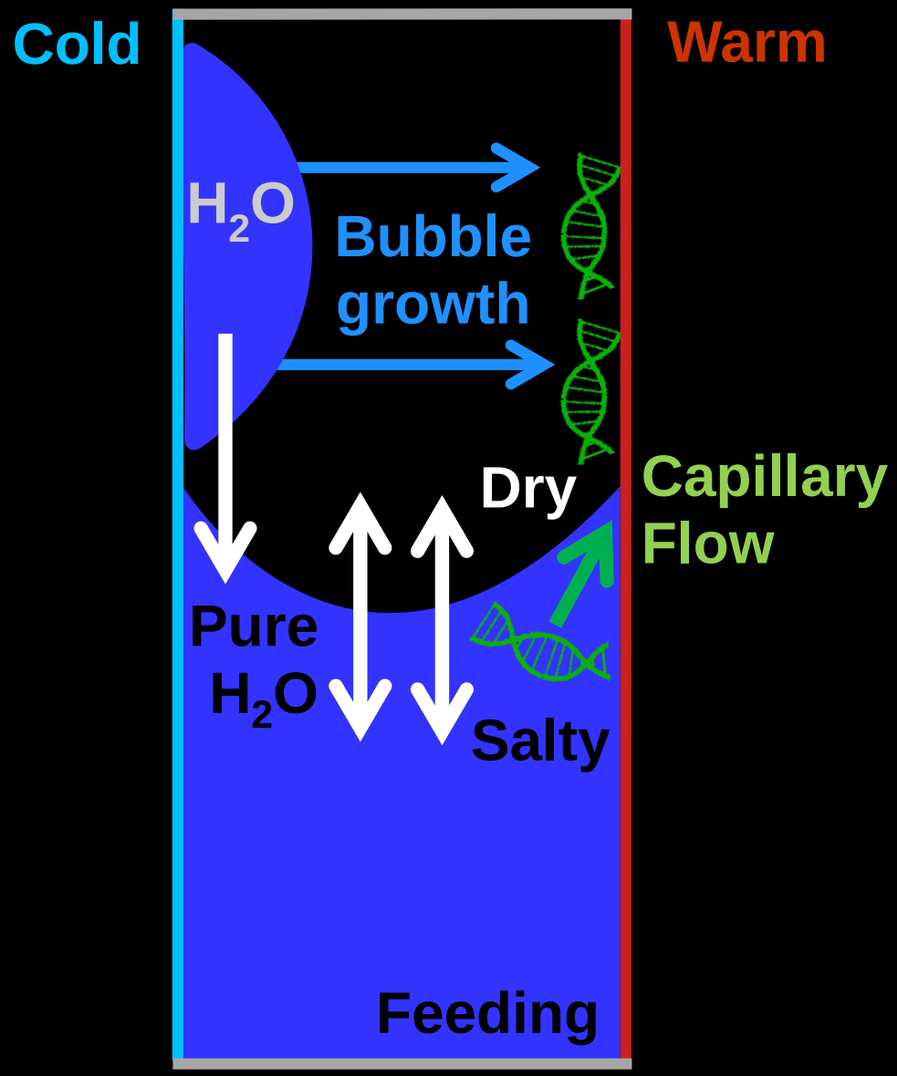
Heated air-water interface

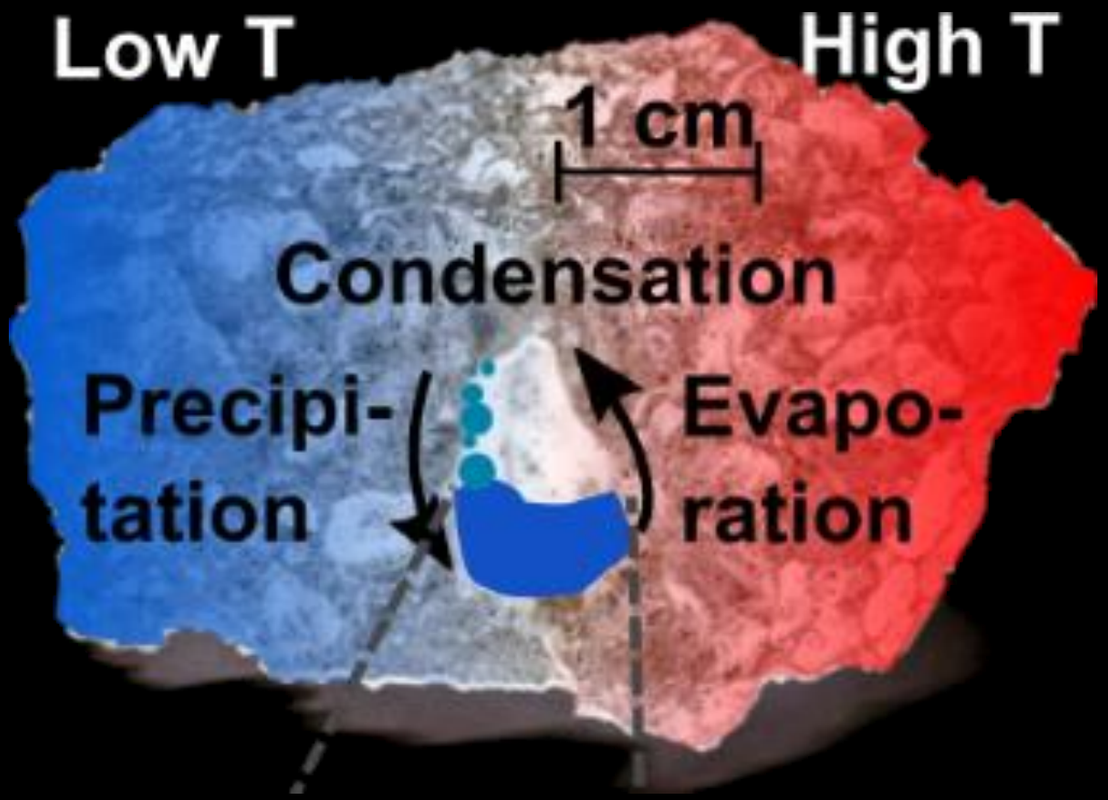


Nature Chemistry (2019)
doi.org/10.1038/s41557-019-0299-5

We also work on other scenarios: **fumaroles, humidity cycles, dry feeding**

Heated air-water interface

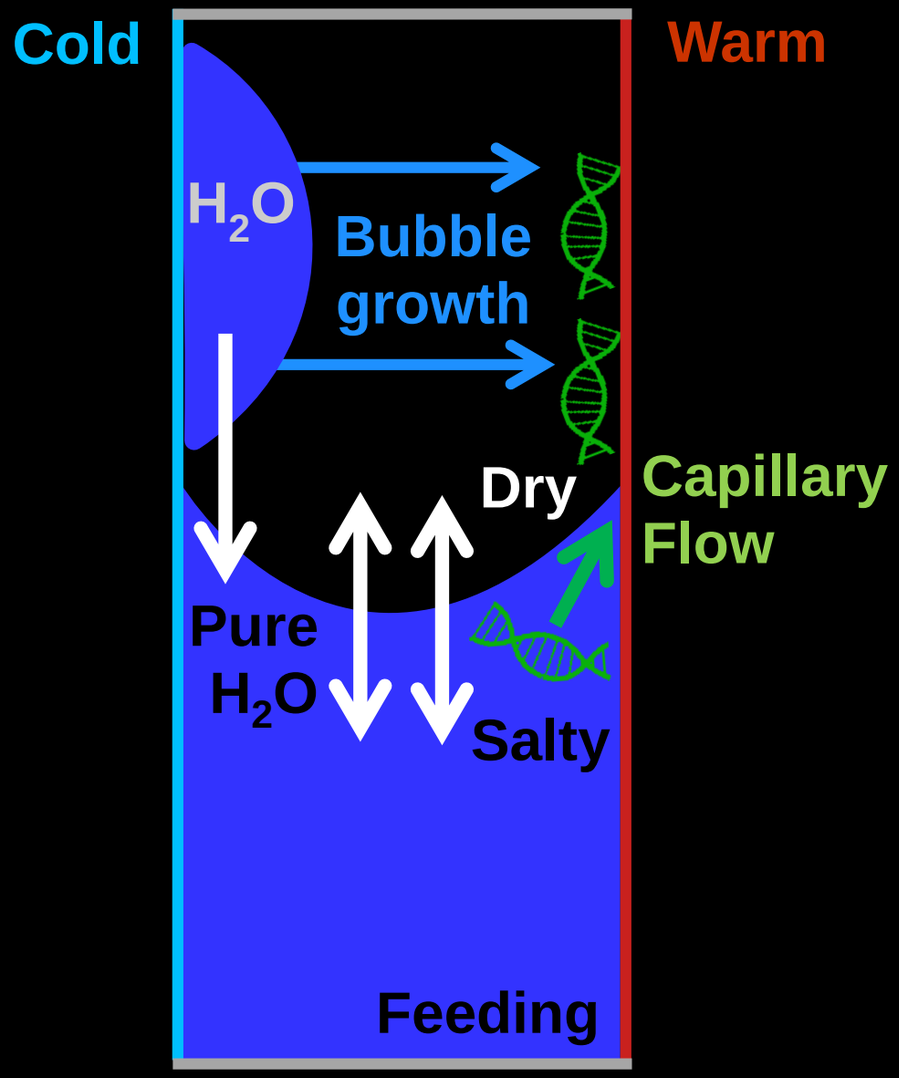


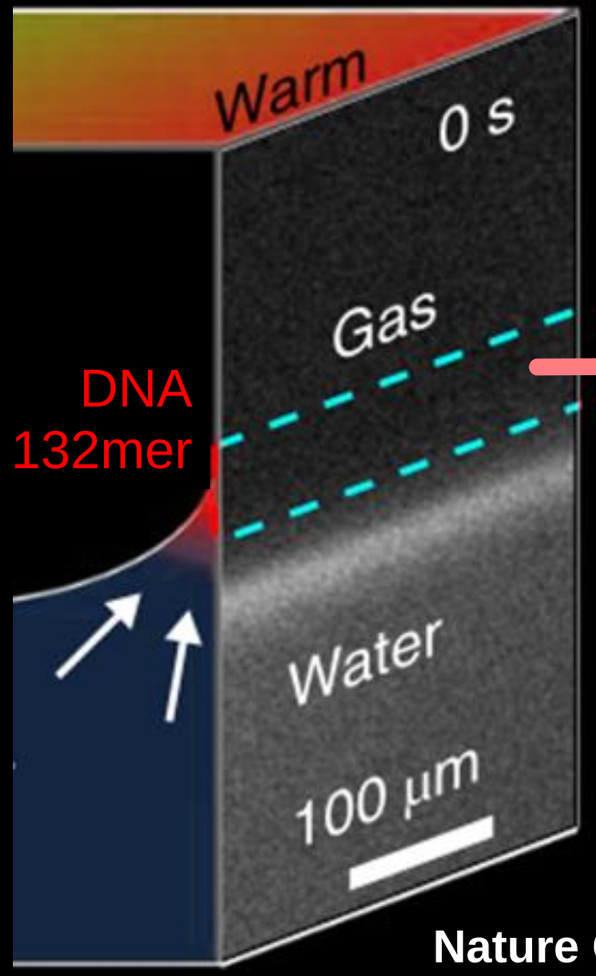


Nature Chemistry (2019)
doi.org/10.1038/s41557-019-0299-5

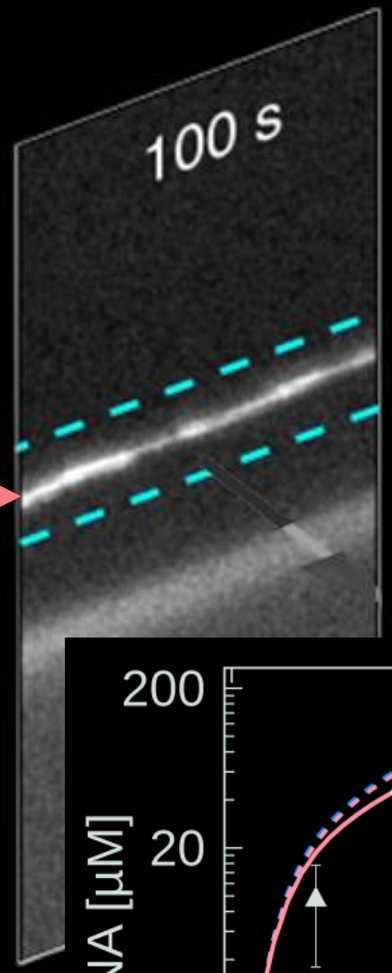
We also work on other scenarios: **fumaroles, humidity cycles, dry feeding**

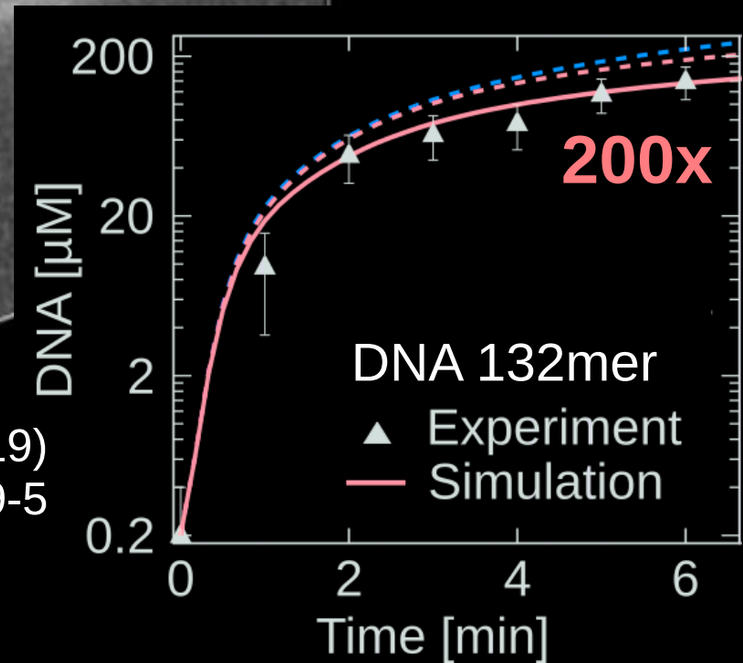
Heated air-water interface … accumulate by size





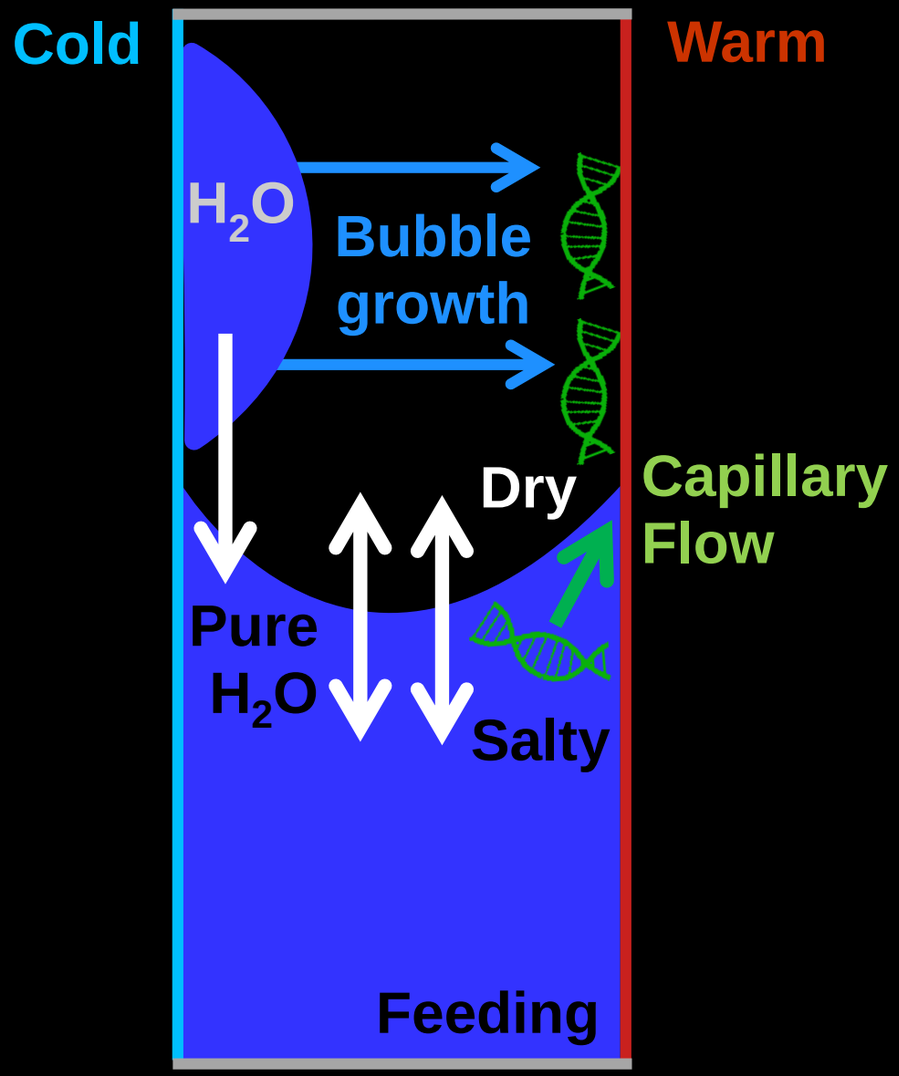
200x

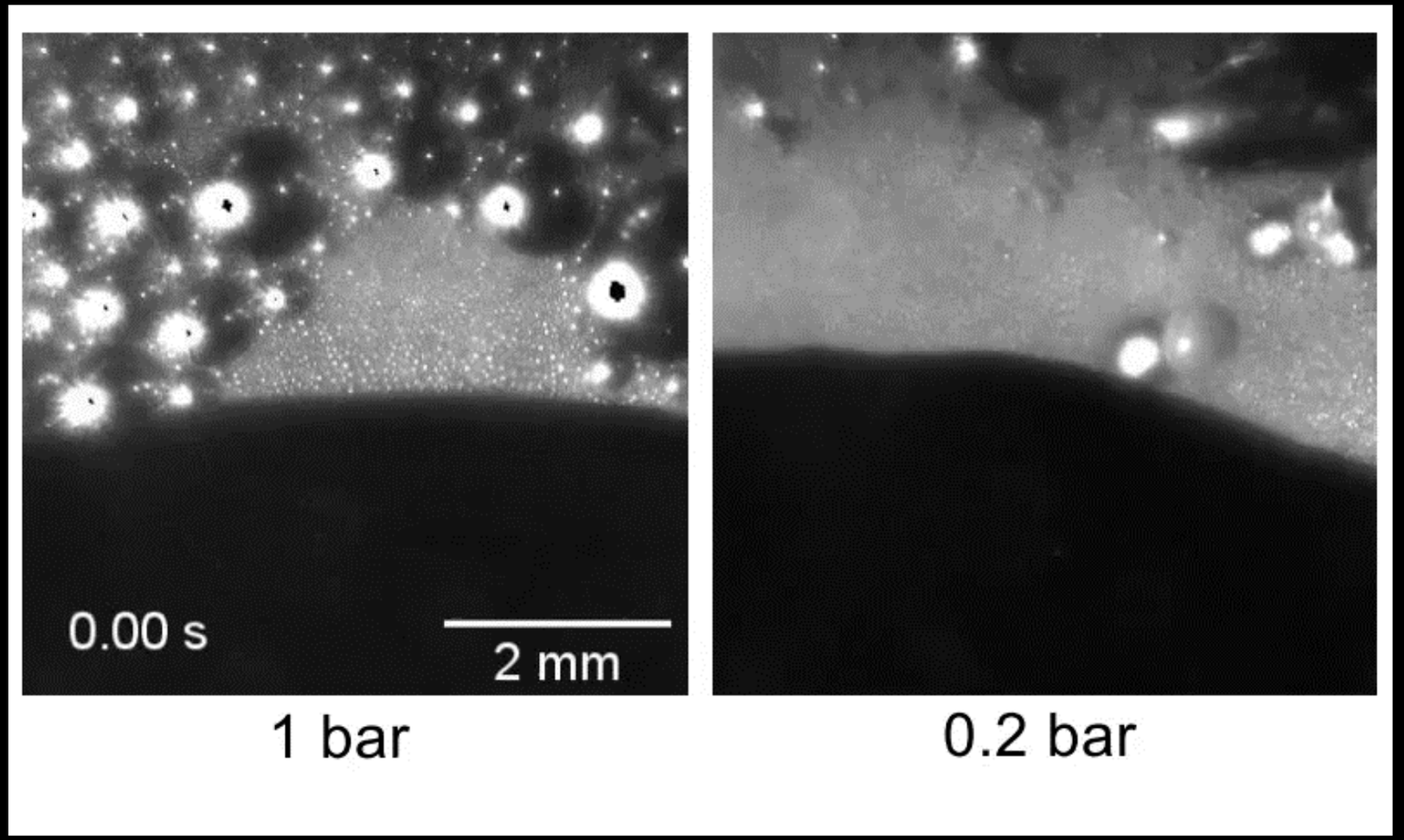




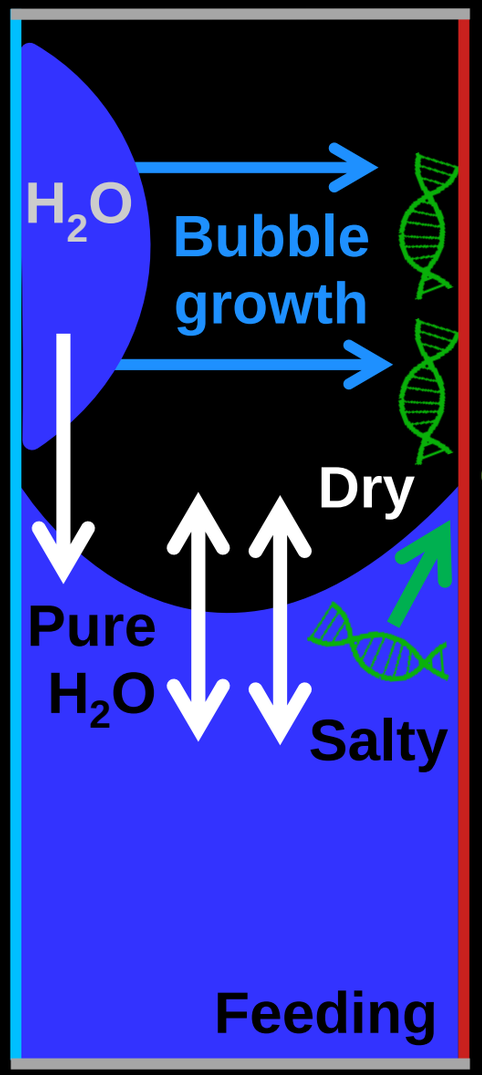
Nature Chemistry (2019)
doi.org/10.1038/s41557-019-0299-5

Heated air-water interface … with strand separation





PCR at air-water interface



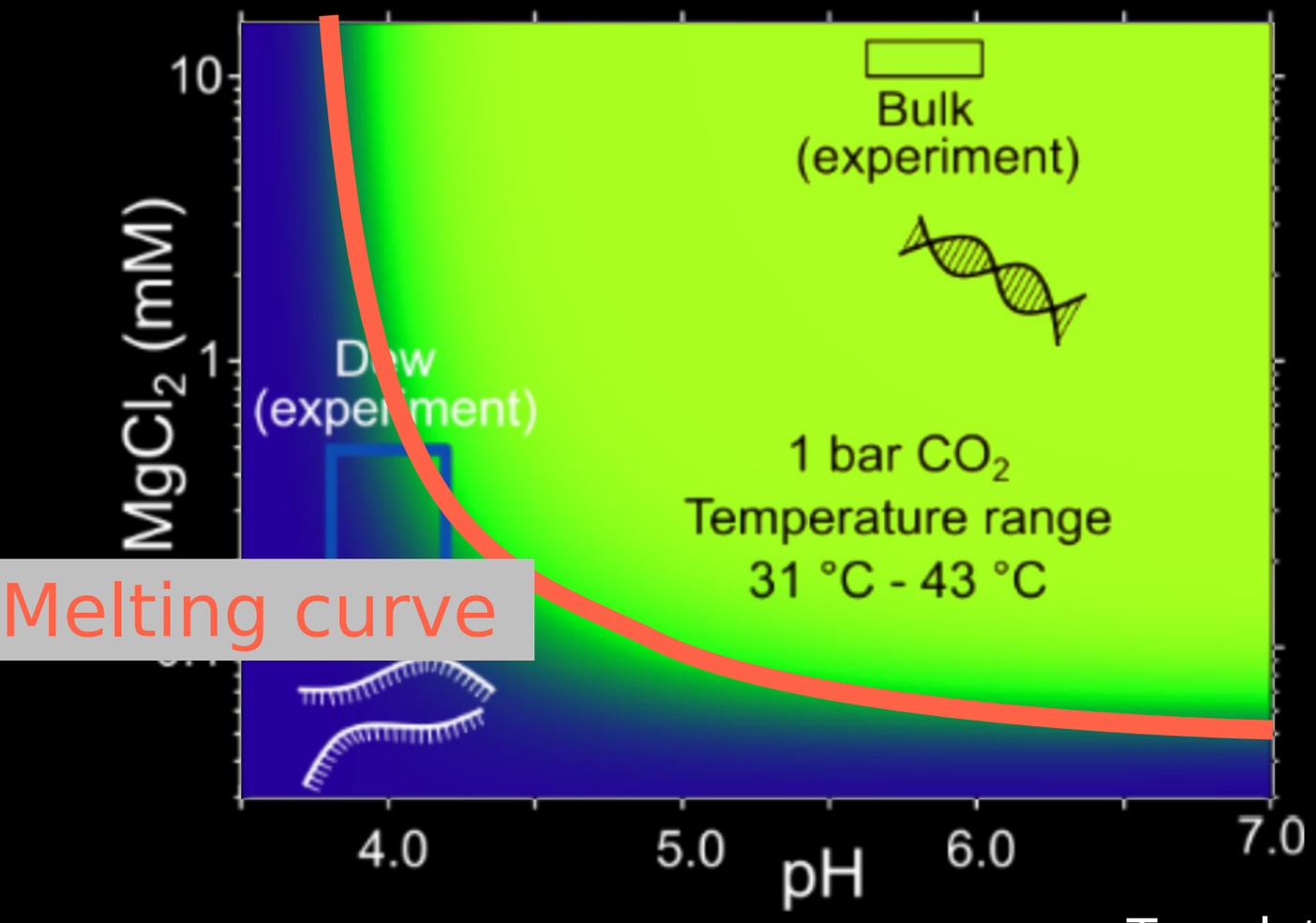
Alan Ianeselli

T gradient:
45 °C - 69 °C

51mer T_m = 83 °C
1 bar CO_2
1 nM template DNA
0.5 µM primers
Taq polymerase
1.5 mM $MgCl_2$
0.1% BSA
2x SYBR Green

Ianeselli, Nature Physics (2022)

PCR at air-water interface... shows fast evolution



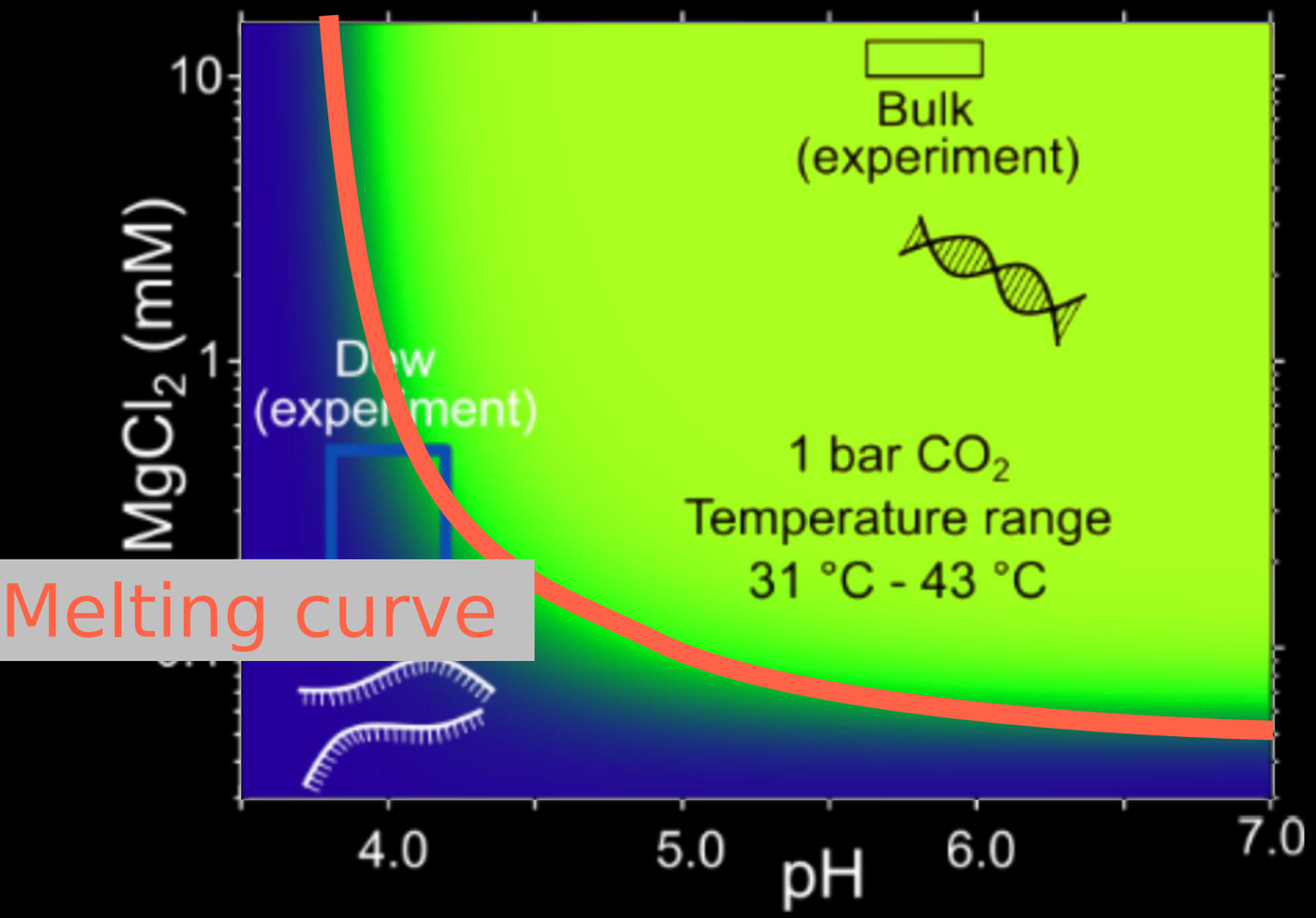
Selection for AT

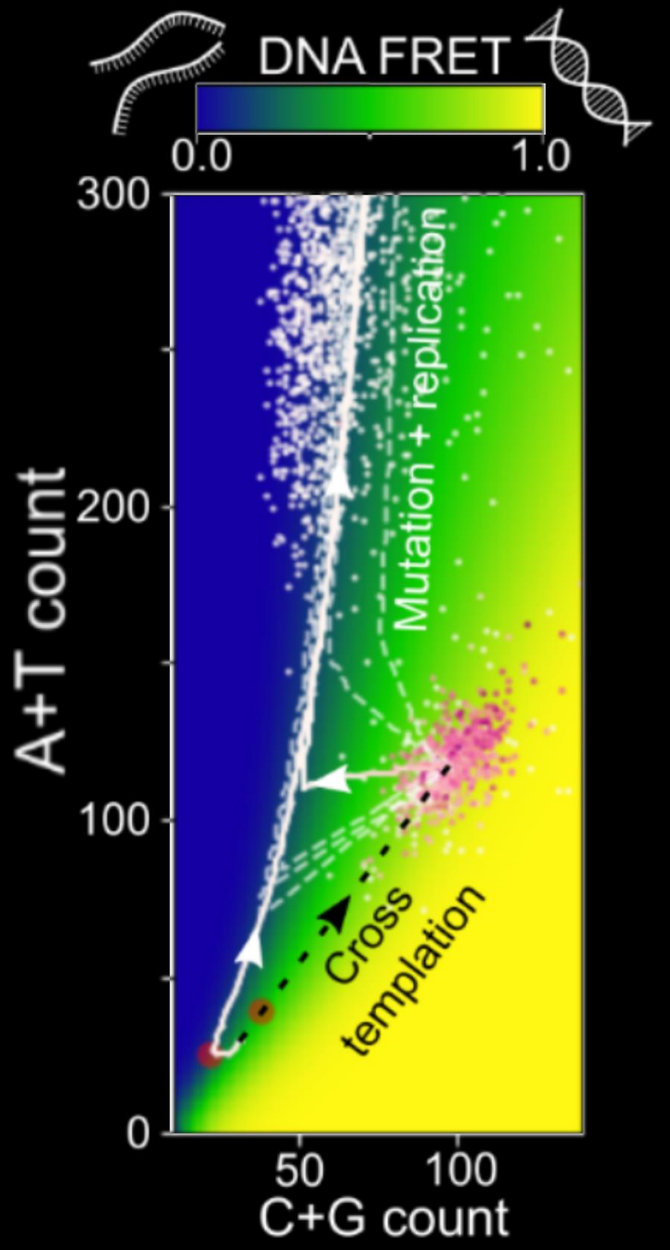
Selection for longer sequences

No tyranny of the shortest

Ianeselli, Nature Physics (2022)

Fast sequence evolution at interface





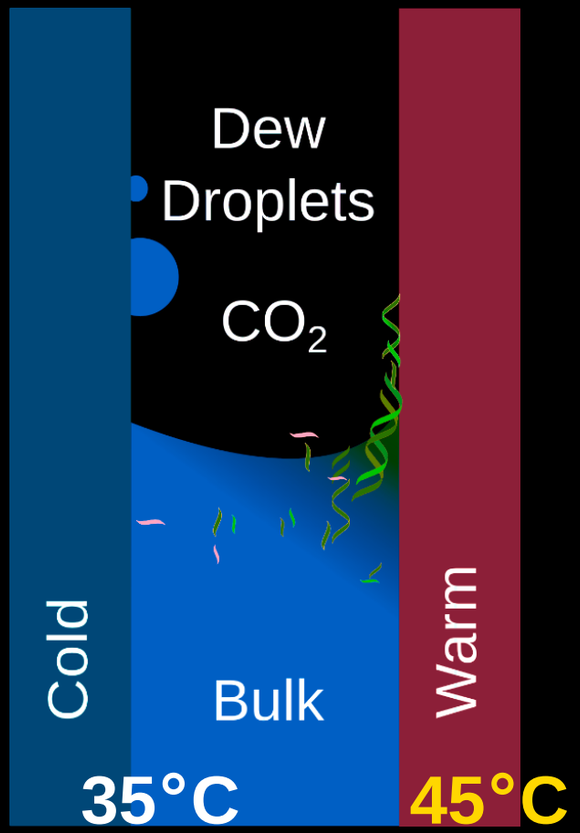
Selection for AT

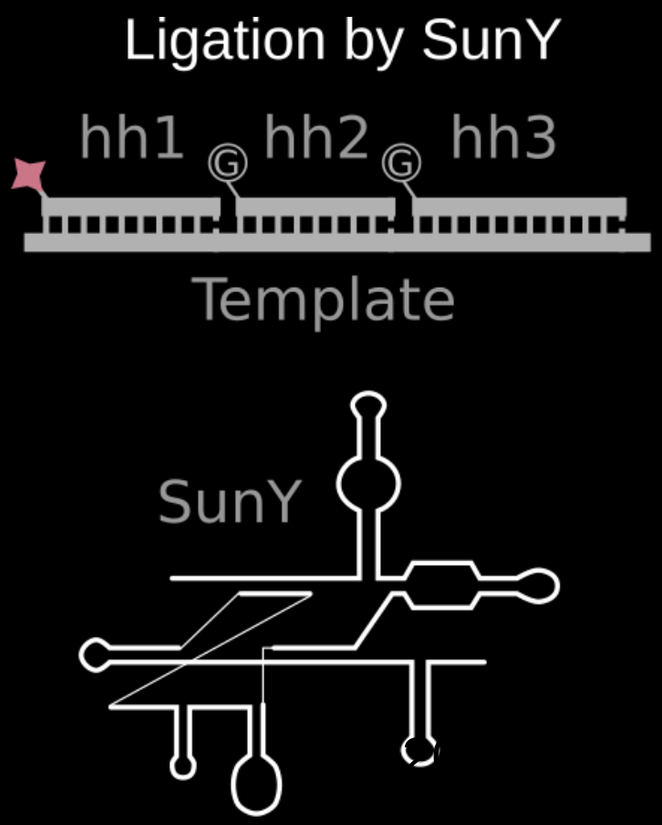
Selection for strand separation

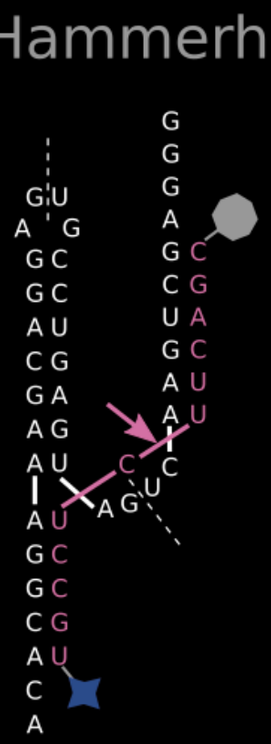
Selection for longer sequences

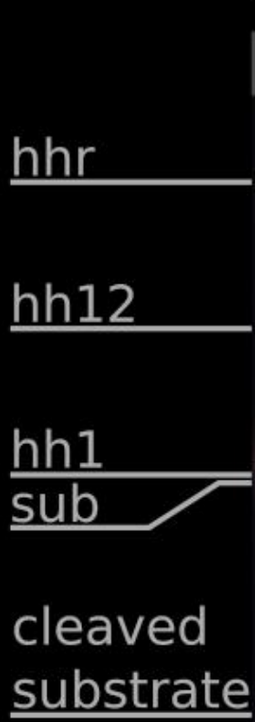
Ianeselli, Nature Physics (2022)

Heated air bubbles ... to host RNA ribozymes









Hannes Mutschler

Annalena Salditt

Nature Communications
doi.org/10.1038/s41467-023-37206-4 (2023)

1. Emergence of RNA and Peptides
2. Replication by templated ligation
3. Darwinian evolution on an early Earth
4. All of above in one experiment?

Self-“buffering“ Oligomerization

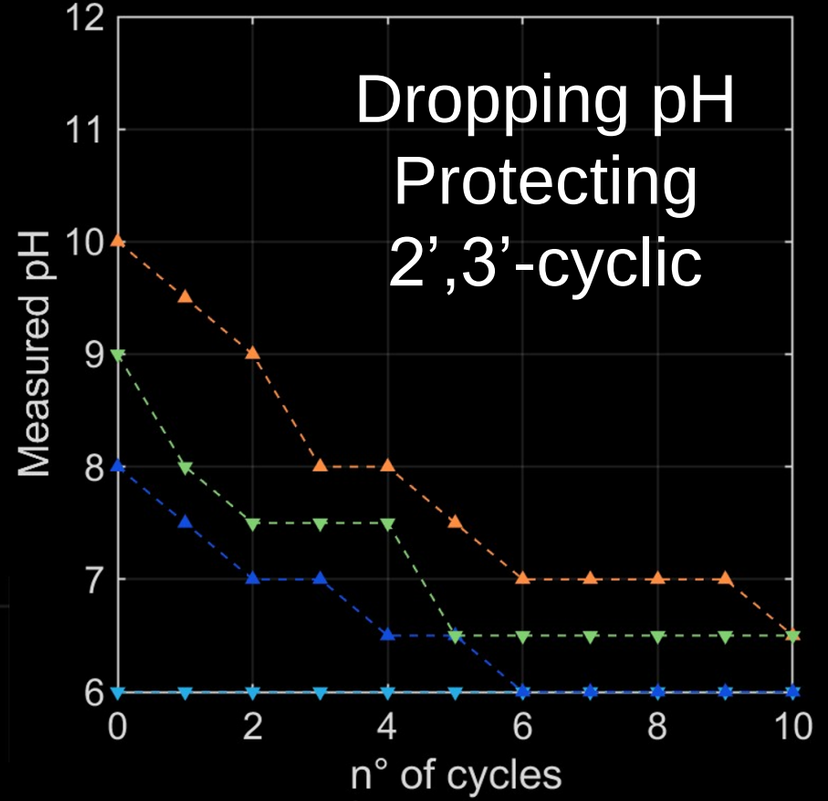
2‘,3‘-cyclic, no amino acid 25°C, dry, pH 10 initial

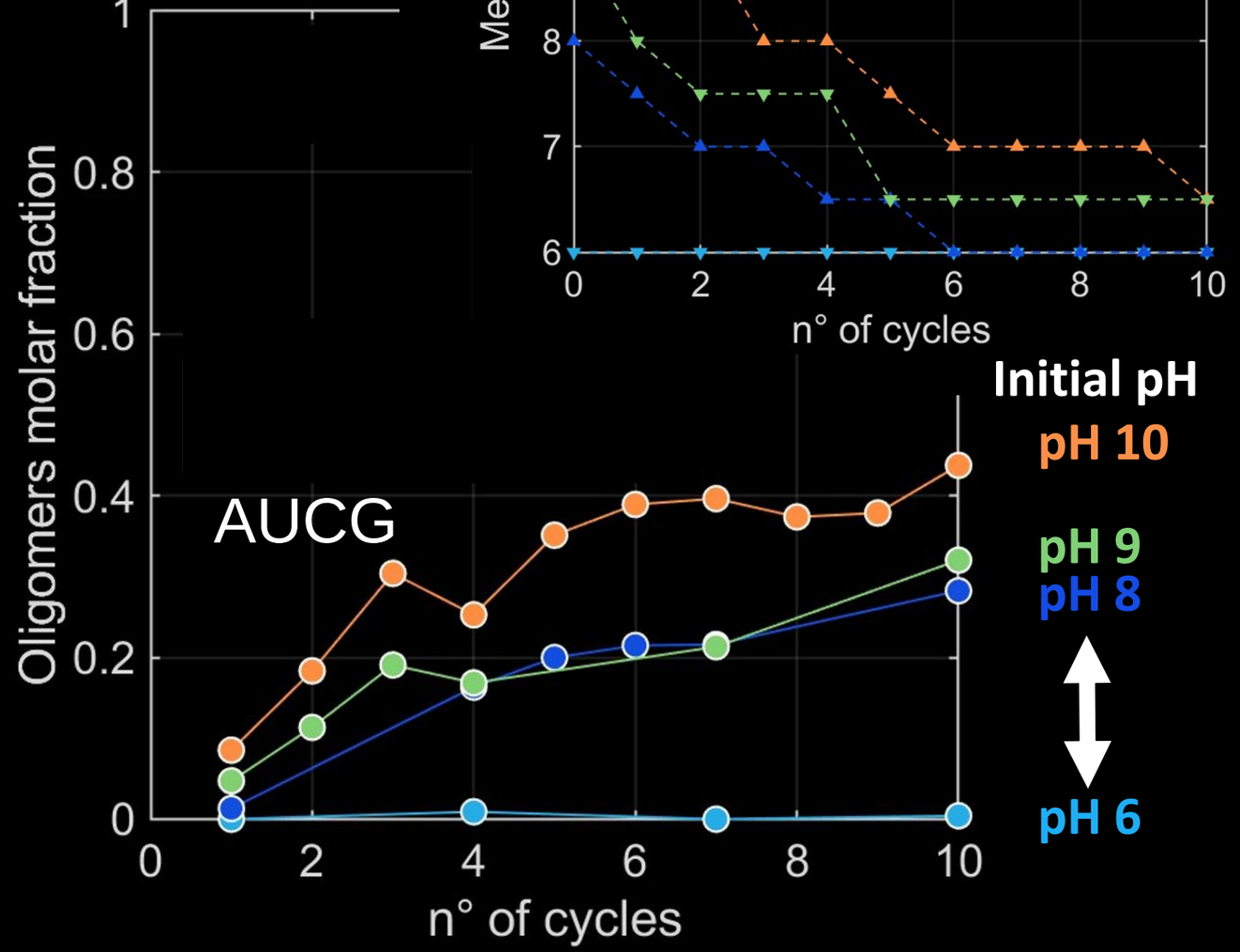
Tommaso Fraccia

Tommaso Bellini

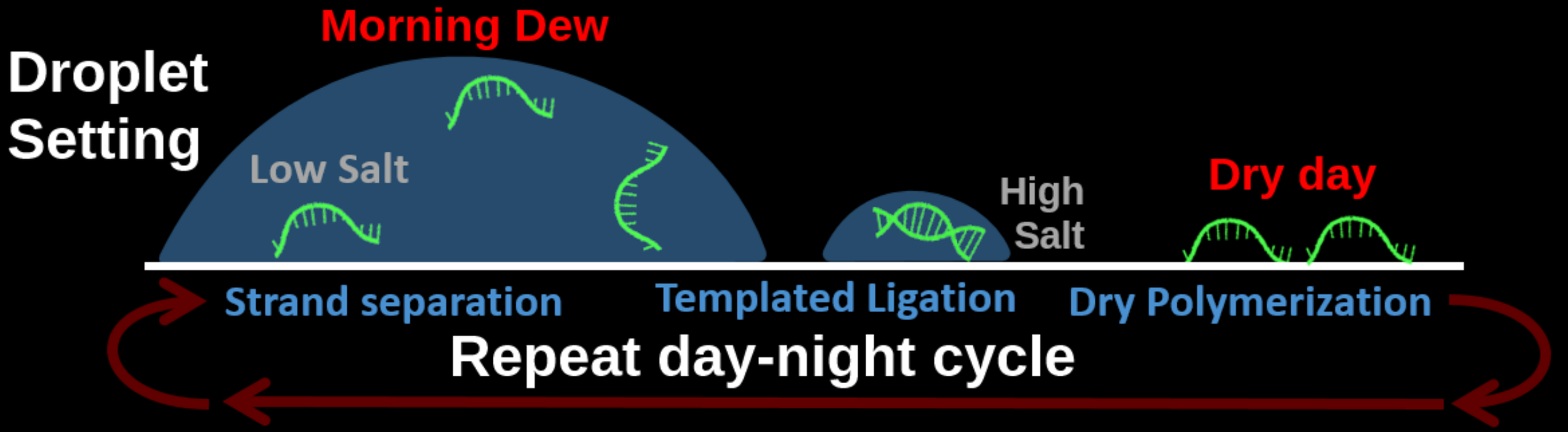
Milano University

In prep.

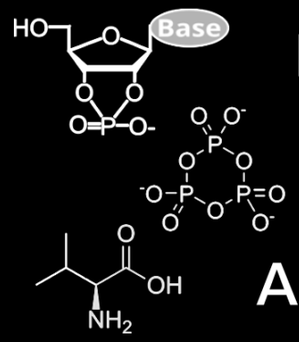




Polymerization and replication of RNA in day-night cycles



Salt-buffering by humidity and temperature using partial pressure of water



RNA Base

Activator

Amino acid

Crosscatalytic network

Recycling by hydrolysis

Strand separation

RNA World

Replication by ligation

Chiral selection

RNA Polymers

Vesicles

RNA-Peptide Hybrids

Peptide Polymers

Reduction of 2',5'-links

Coacervates

Reduced hydrolysis

Peptide World

RNA encoded Peptides

Ligation by leaving group

Selection by size

by replication speed

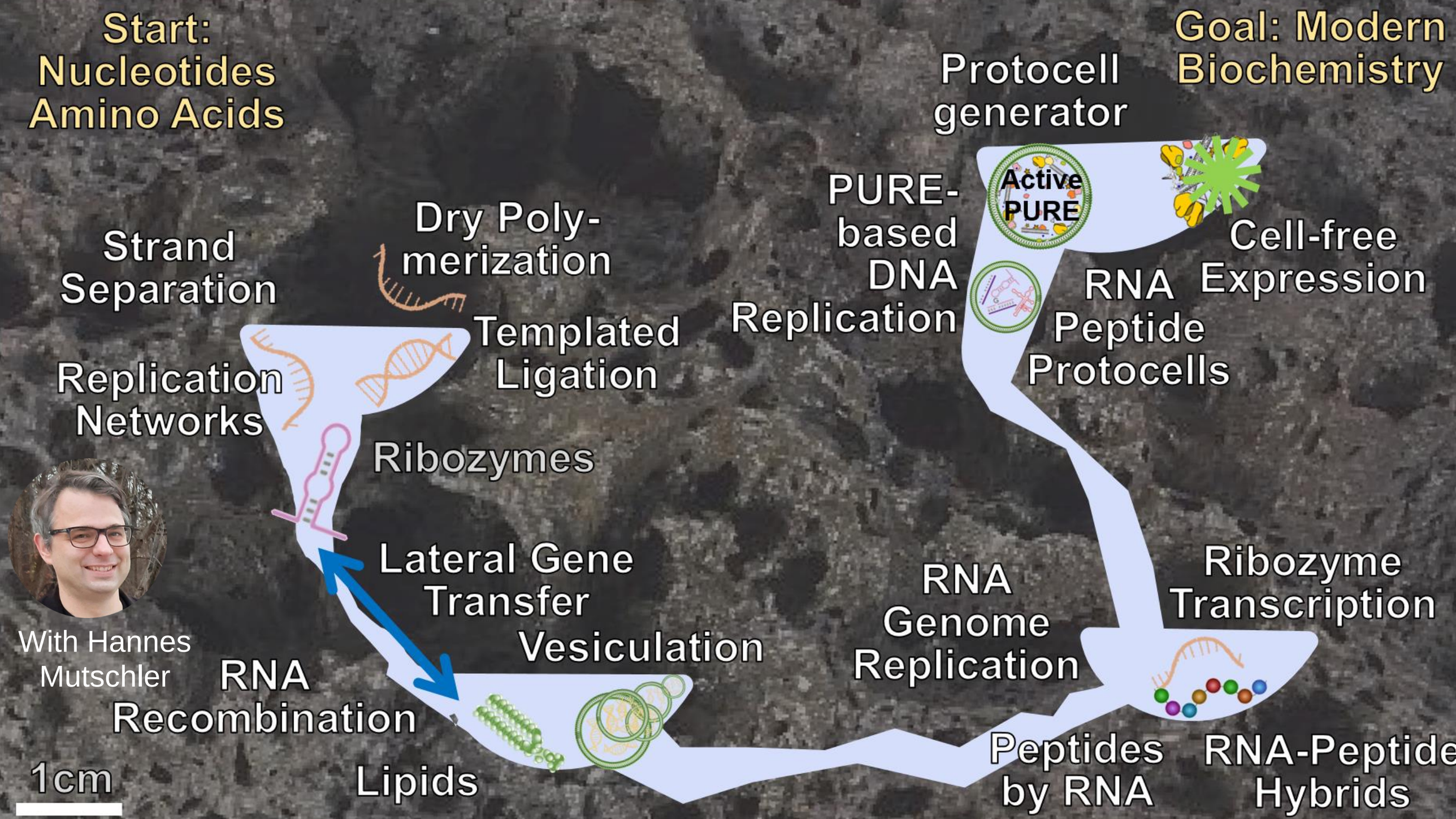
Darwinian Evolution

Life

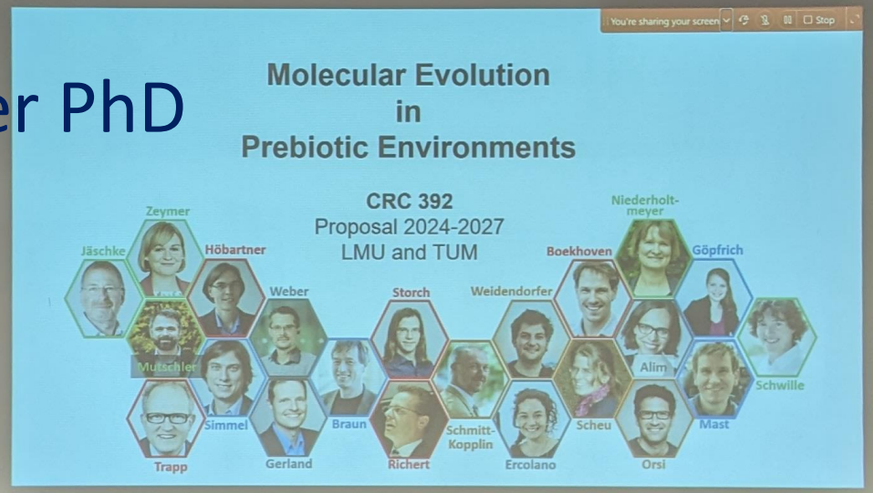
by translation

by sedimentation

by hydrolysis

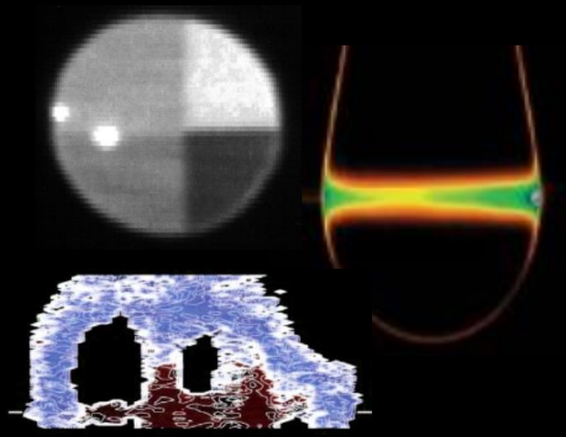

Start:
Nucleotides
Amino Acids
Goal: Modern
Biochemistry
Protocell
generator
Active
PURE
PURE-
based
DNA
Replication
Cell-free
Expression
RNA
Peptide
Protocells
Dry Poly-
merization
Strand
Separation
Templated
Ligation
Replication
Networks
Ribozymes
Lateral Gene
Transfer
Vesiculation
RNA
Recombination
RNA
Genome
Replication
Ribozyme
Transcription
Peptides
by RNA
RNA-Peptide
Hybrids
Lipids
With Hannes
Mutschler
1cm

Looking for
Executive Manager
100% E14, right after PhD



Advancing Biotechnology ?

Optical methods



Nanometer precision cell distance

Microcavity biomolecule detection

Voltage recording in silicon-neuron junction

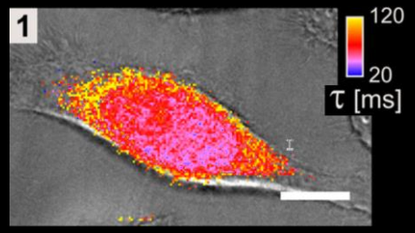
Thermophoresis of Biomolecules →

nanoTEMPER

>200 Employees

All-optical pumping in water and ice →

FLUCS inside cells
(Moritz Kreysing)

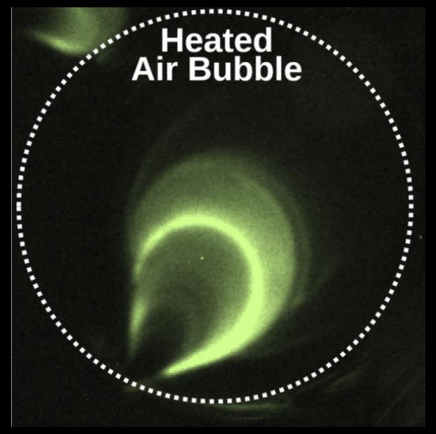


Imaging of kinetics in living cells →

Ultrafast freezing
and thawing?

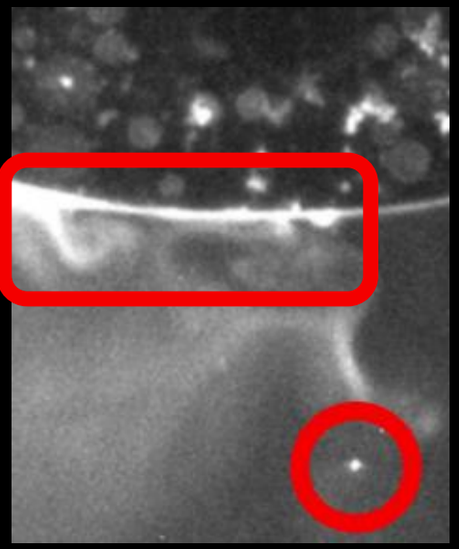
Advancing Biotechnology ?

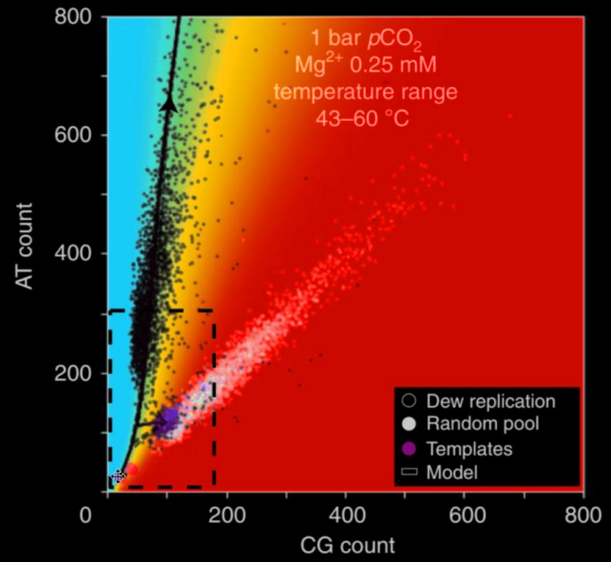
In situ evolution of artificicial cells and functional genetics



Accumulated PURE
Expressing GFP

Evolution of synthetic cells at air bubbles. Heated air bubbles accumulate and activate cell-free systems. With added lipids, it is packaged into vesicles. Both combined allow the in situ evolution of protocells.





Autonomous high-speed SELEX. Length-selective accumulation combined with replication (PCR or ligation) trigger local Darwinien evolution. By molecular selection, we expect very fast SELEX in the same reaction chamber.

Christof Mast

Annelena Salditt

Juliette Langlais

Christina Dirscherl

Alexander Floroni

Alan Ianeselli

Max Weingart

Avinash Dass

Thomas Matreux

Adriana Serrao

Sreekar Wunnava

Philipp Schwintek

Noel Martin

Simons Foundation
Klung-Wilhelmy Price
Volkswagen Life!

LMU W2, 1 Assistant, no TA, 10k€/a

life Under reconstruction

Resurrected!

ORIGINS Excellence Cluster

erc Starting 2010-15

erc Advanced 2018-23

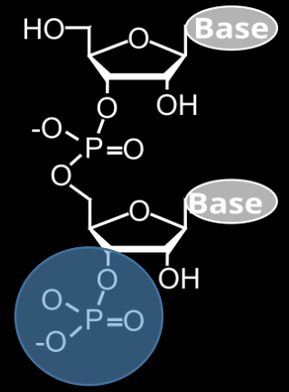
nano agents SFB 1032

QNM

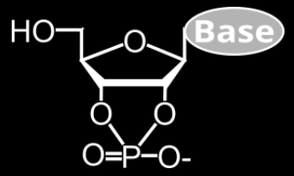
nano TEMPER

Polymerization and hydrolysis of RNA

Make RNA



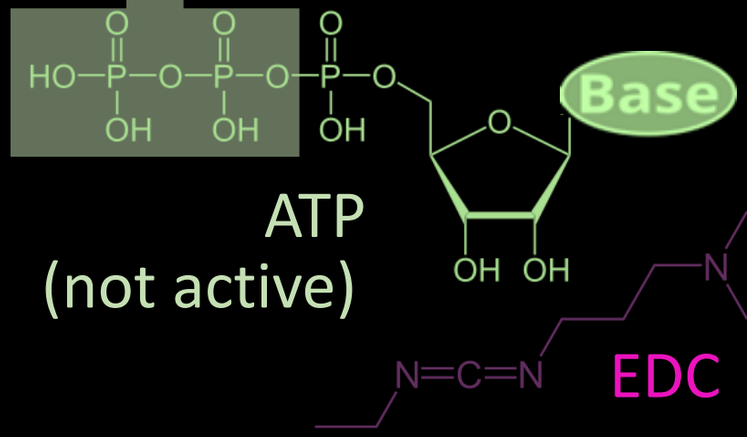
- H_2O



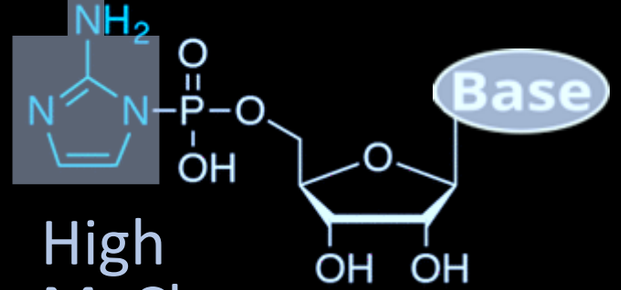
Leaving group

Waste

Biology

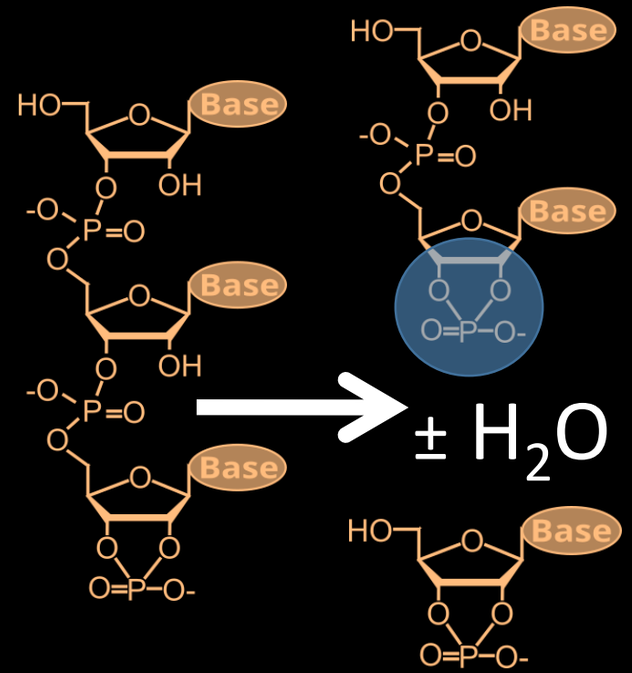


Waste

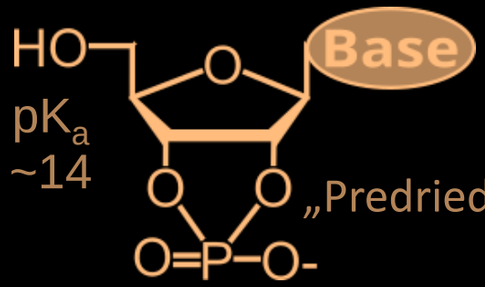


Strand separation?
Recycling? Activation?

Break RNA



Ring opening ligation



Findings:
- pH 9-11 sufficient
- No divalent ions
  (strands separable)

- Only shuffle around H_2O
- Minimal side products
- RNA bases G+U buffer at 9-10
- 70% polymerization yield
  (with Tommaso Fraccia, Milano)