# ASTROCHEMISTRY

## FROM THE BIG BANG TO LIFE

*Global Astrochemistry Lecture Series (GALS) 2024*

# EVOLUTION OF BIOMOLECULE PRECURSORS AND FORMATION OF BIOPOLYMERS AND FIRST CELLS

**Dieter Braun.** *Ludwig Maximilian University of Munich, Germany*

**11.04.2024 | 15:00 - 17:00 CET**

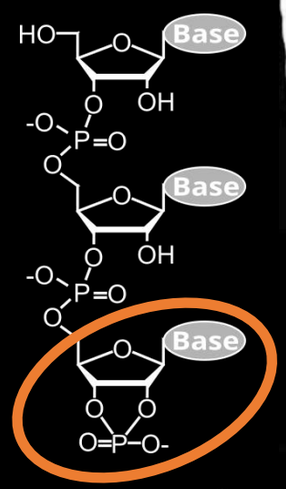
cost EUROPEAN COOPERATION IN SCIENCE & TECHNOLOGY
NanoSpace
Funded by the European Union

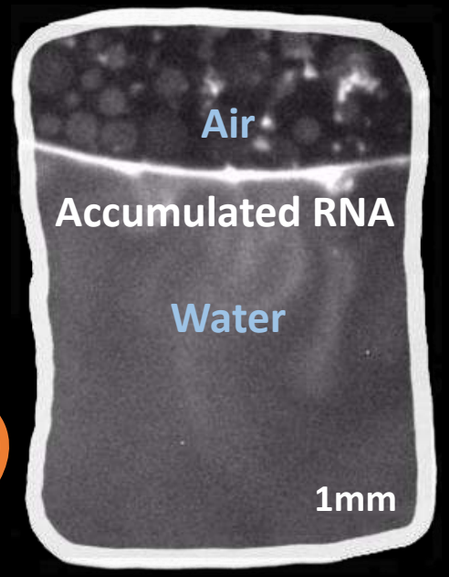
## Emergence of Life in the Lab

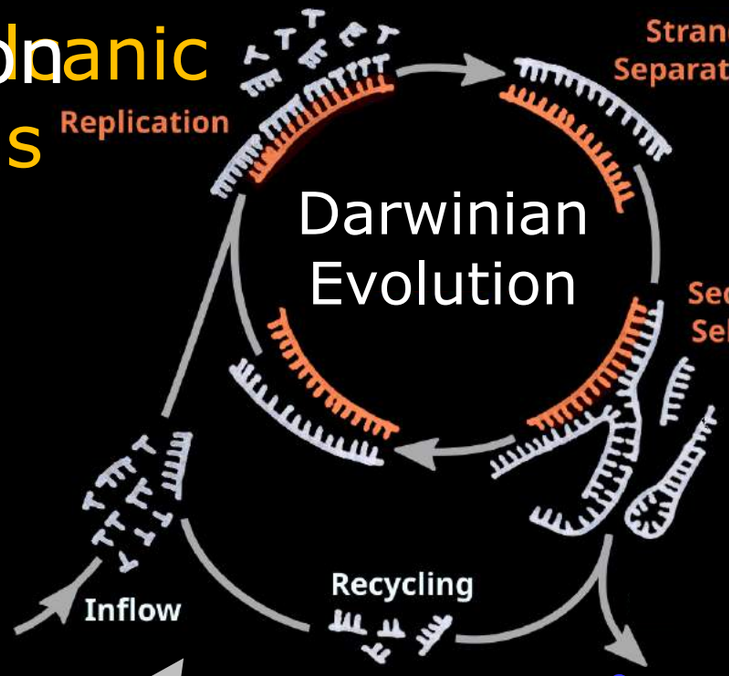
1. Biological History
2. Chemical Selection
3. Sequence Selection
4. Geophysical Space
5. Autonomous Evolution

# Biological Evolution

RNA evolution in volcanic wet-dry settings



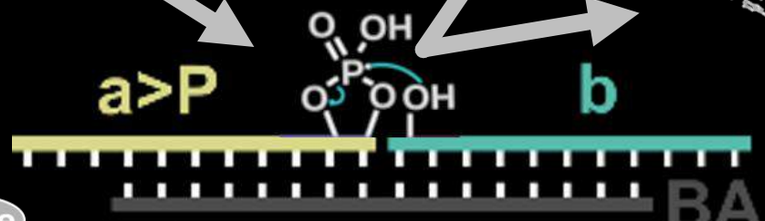

Bacteria





80% yield

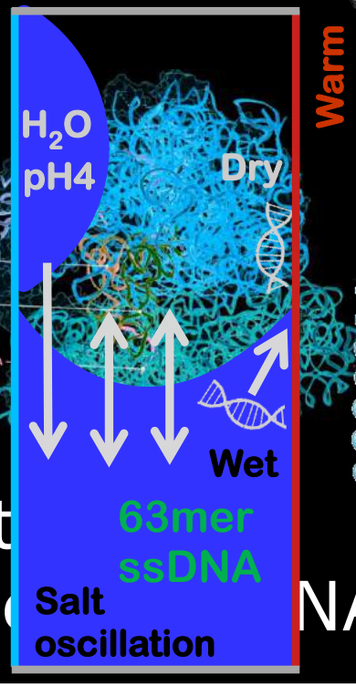
40% yield

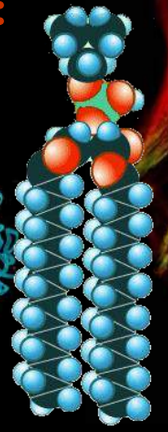



Replication by Ligation



Genet
Ribosom
NA



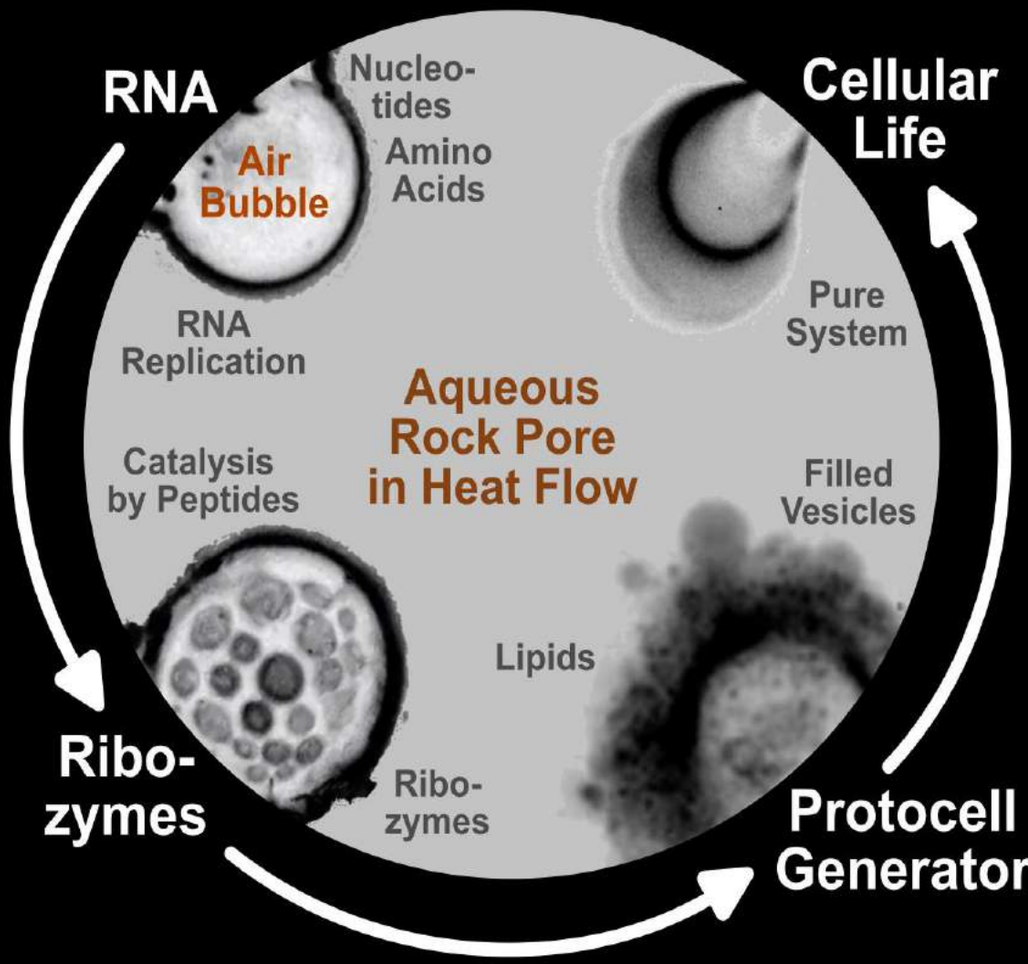



DOPC

E

Time

# From RNA to Cellular Life at heated Air Bubbles

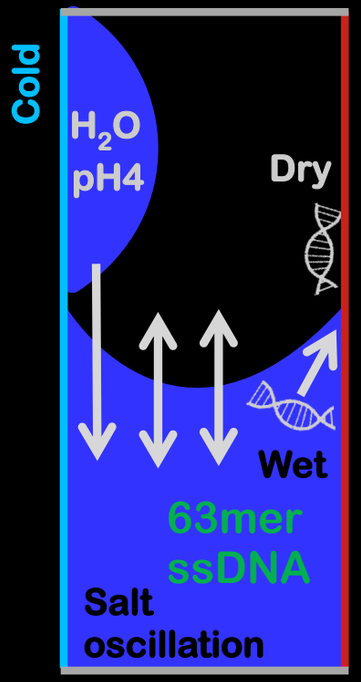


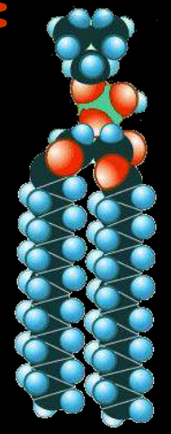

ERC Synergy Proposal



Hannes Mutschler





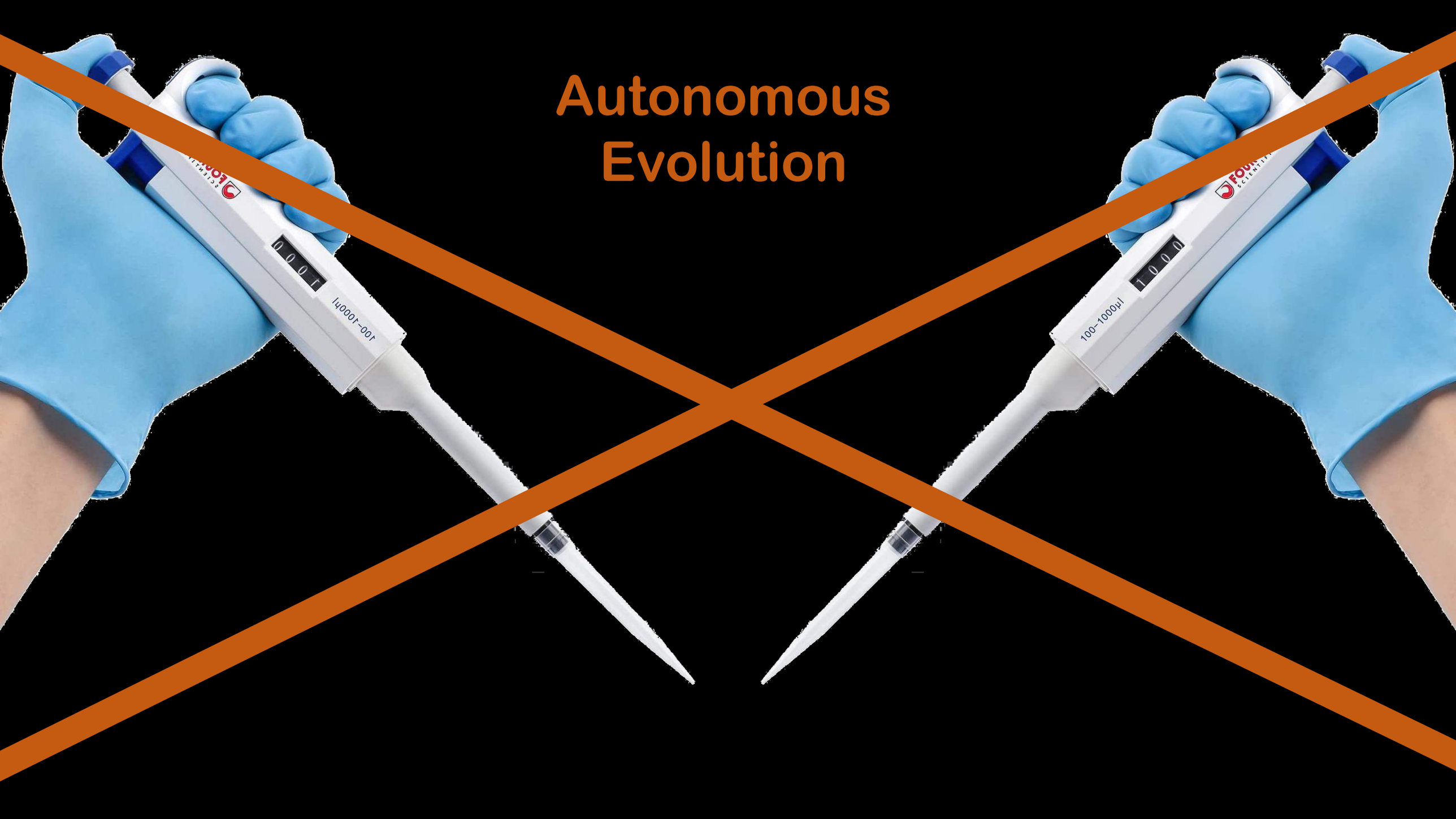



DOPC




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**

# Autonomous Evolution



## Emergence of Life in the Lab

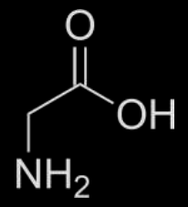
1. Biological History
2. Chemical Selection
3. Sequence Selection
4. Geophysical Space
5. Autonomous Evolution

# Chemical Evolution …

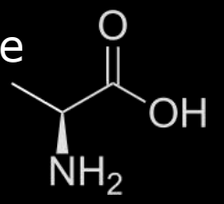
# …driven by physical Forces

**Amino Acids**

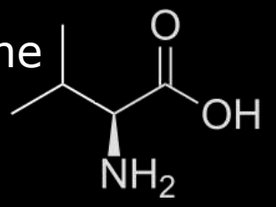
Glycine



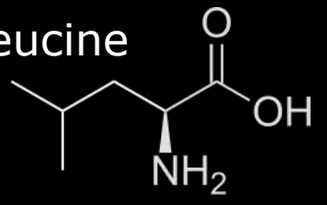
Alanine



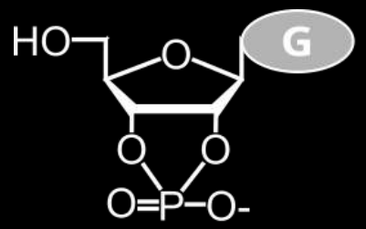
Valine

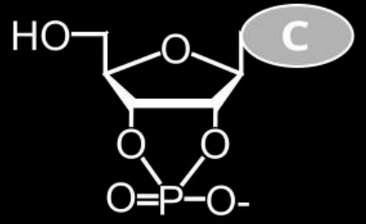


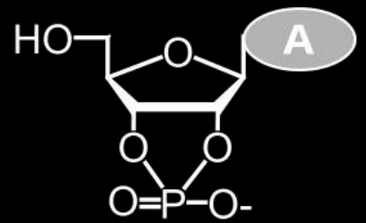
Leucine

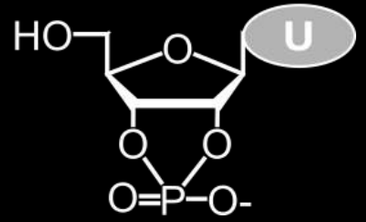


**Ribonucleotides**

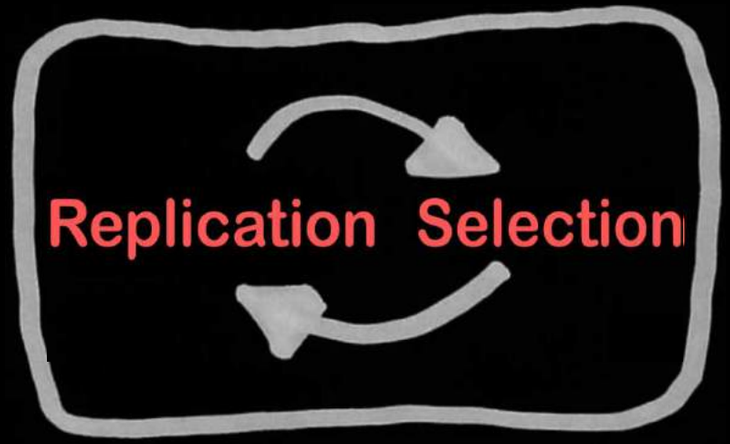


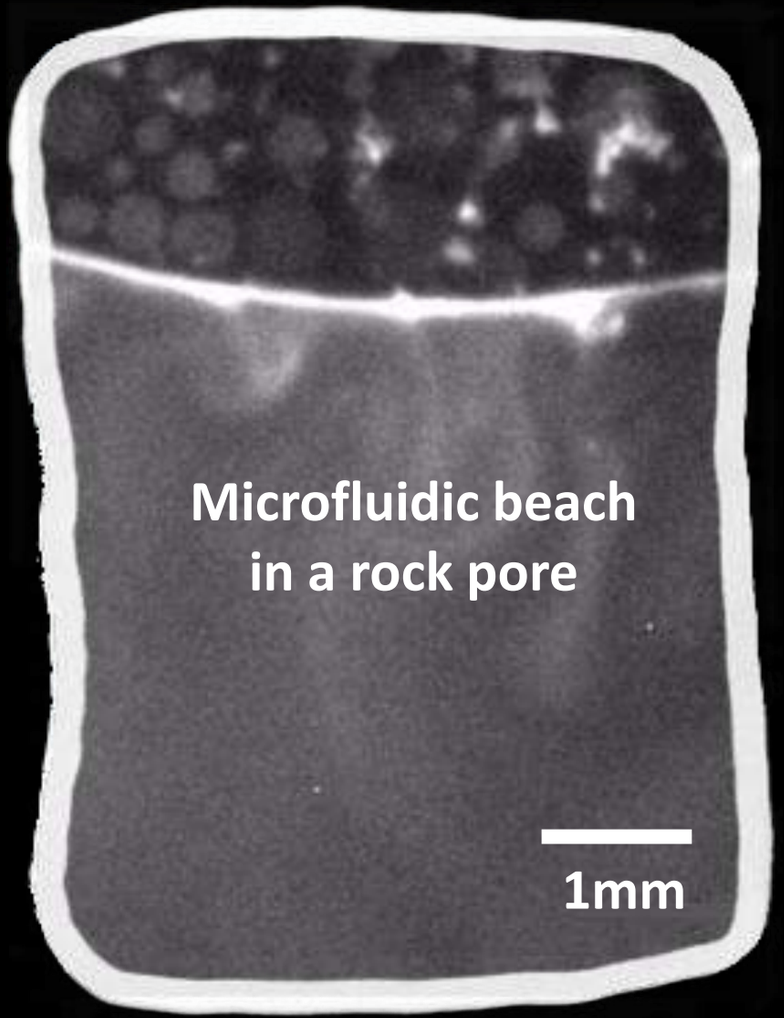




## Darwinian Evolution of Information




**Collaborators**: Matt Powner, Ram Krishnamurthy, Moran Frenkel-Pinter, Claudia Bonfio, Hannes Mutschler, Dora Tang, Kerstin Göpfrich, Tomoaki Matsuura, Gonen Ashkenasy

life

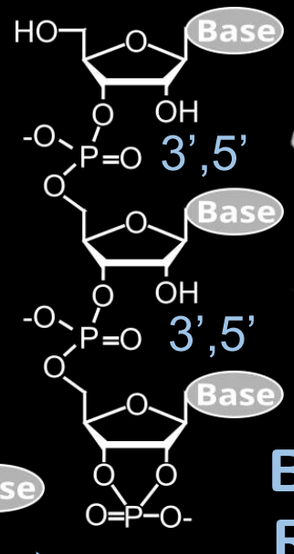
erc Starting 2010-15

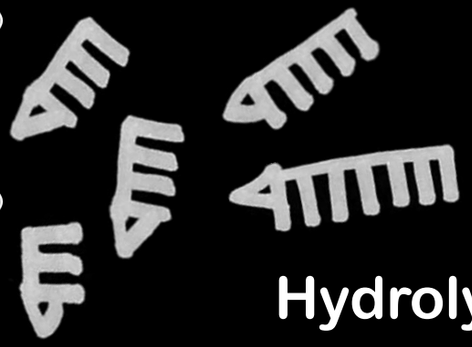
erc Advanced 2018-23

nano TEMPER

# Chemical Evolution

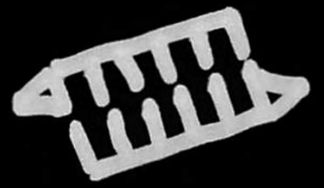
Dry, 1 day, pH 9-10, 4 - 80°C



Hydrolysis

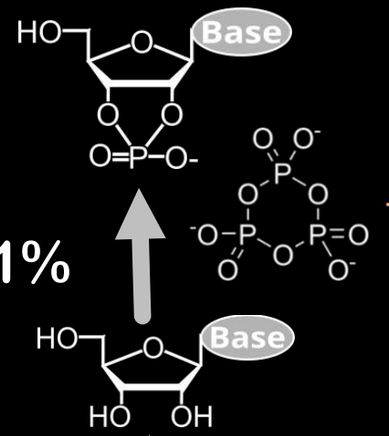
Selection of size



1000-fold Protection by Hybridization

Backbone Recycling

10-20%

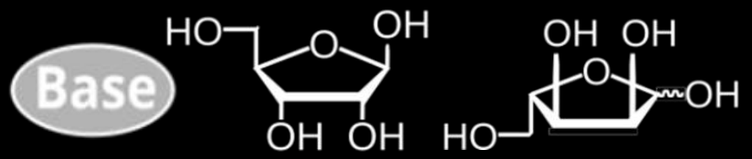



1%

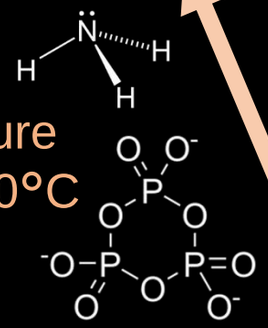
??

D-Ribose

L-Ribose

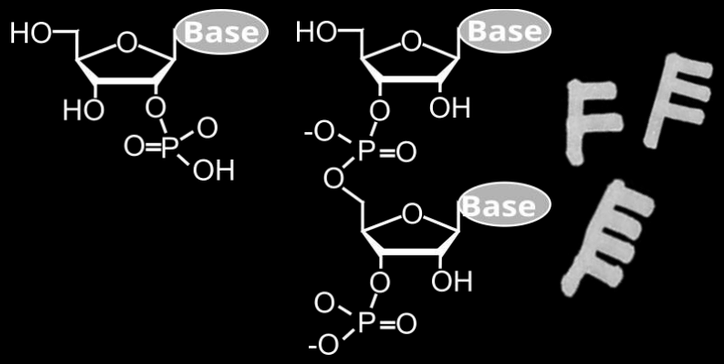



98% pure from 550°C



6%

2‘,3‘-cyclic Recycling



Chemical selection for:

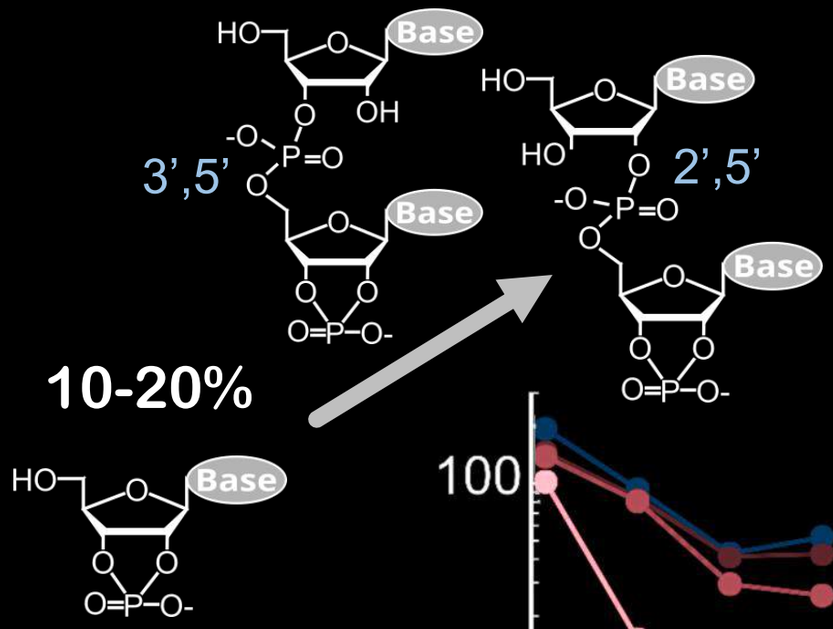
- Homo-Chirality
- 3‘,5‘-Linkage
- Nucleoside isomers
- Balancing [G]=[C], [A]=[U]
- Rudimentay Replication

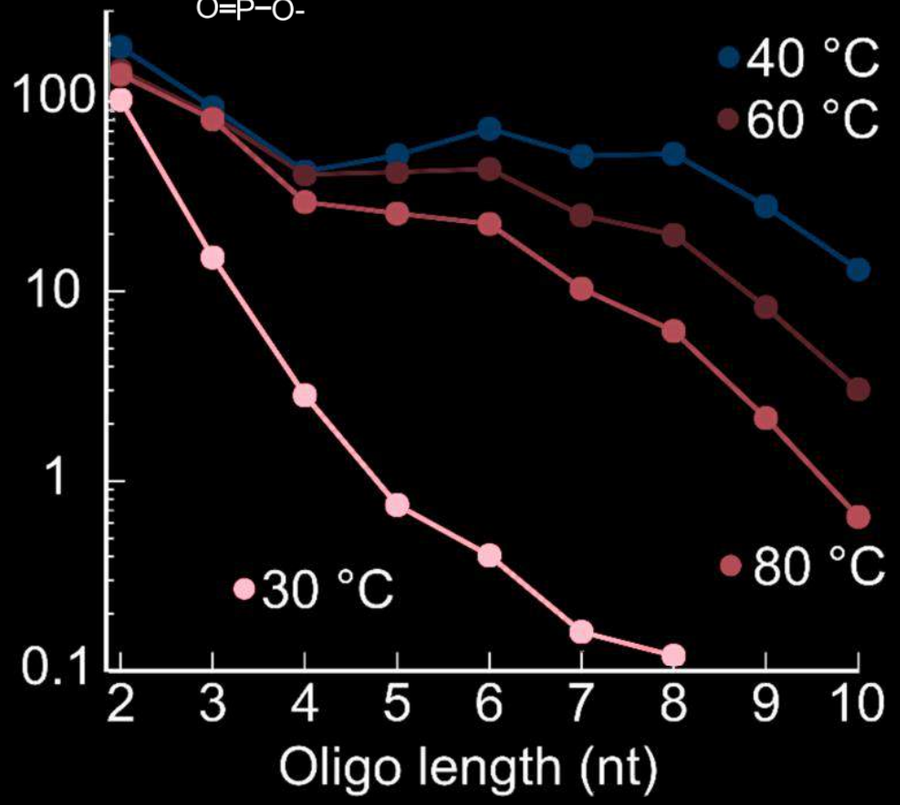


# Chemical Evolution

## RNA oligomerization in “dry” state

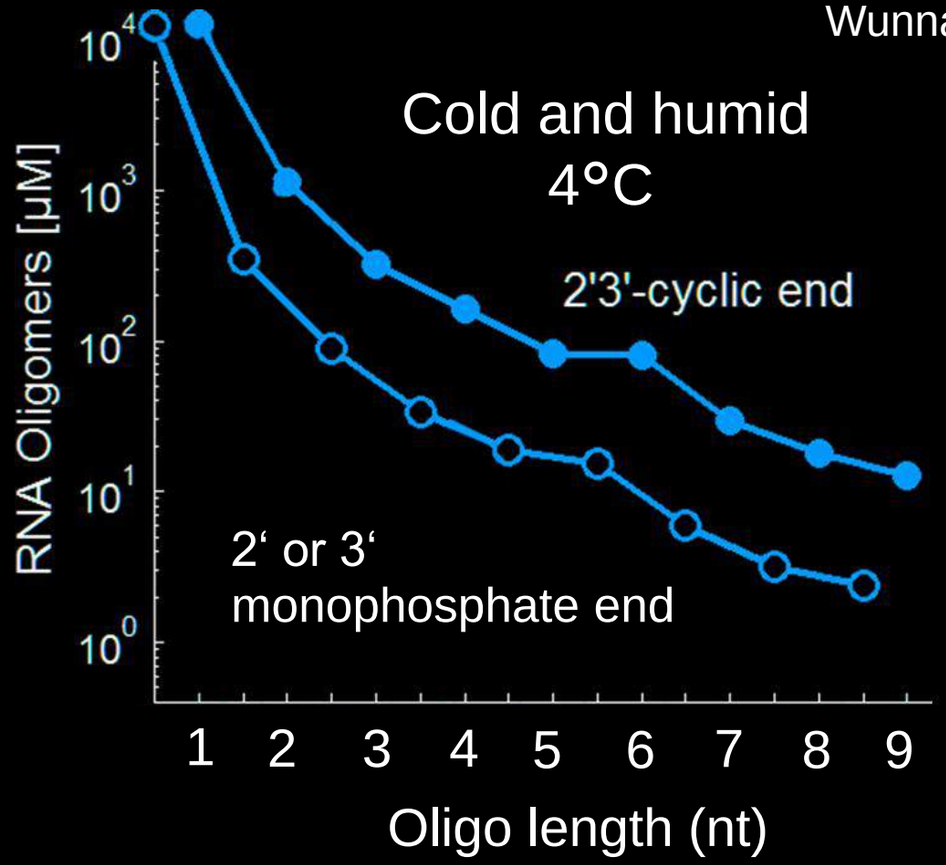
Dry, 1 day, pH 9-10, 4 - 80°C






Sreekar Wunnava




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

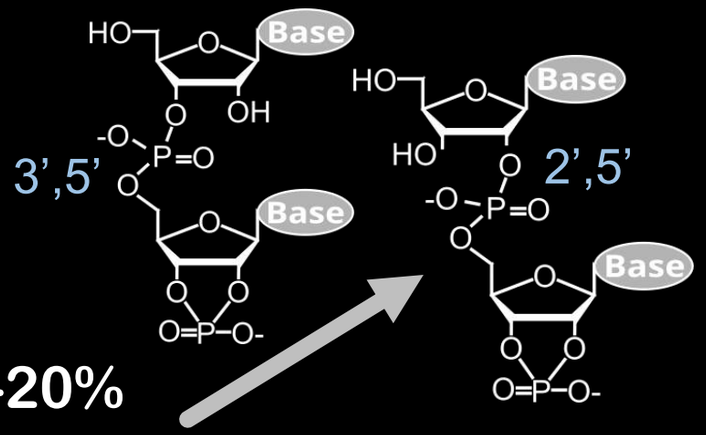
# Chemical Evolution

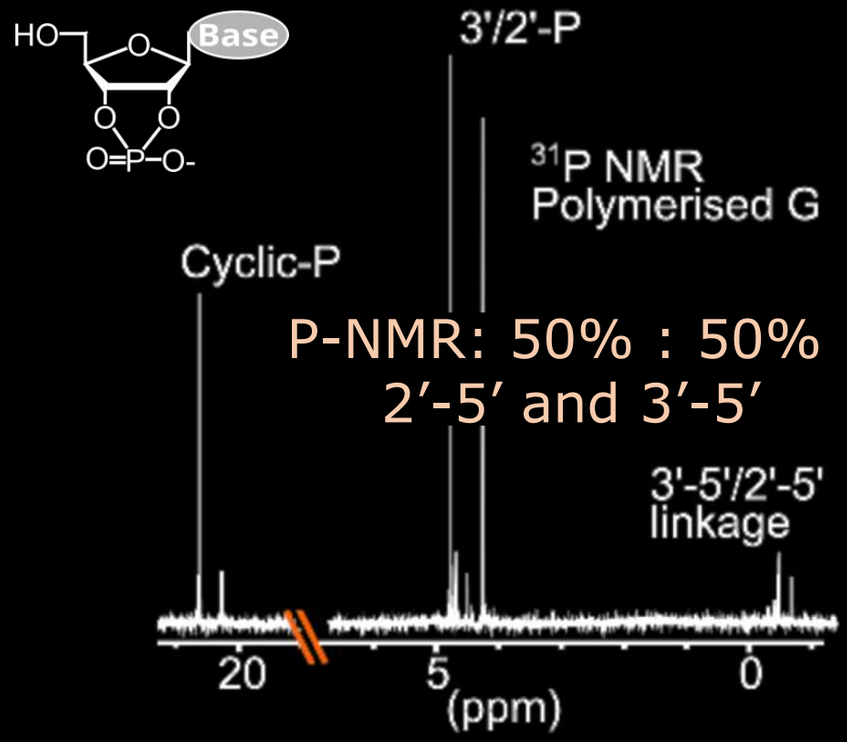
## Dry, 1 day, pH 9-10, 4 - 80°C

# RNA oligomerization in “dry” state

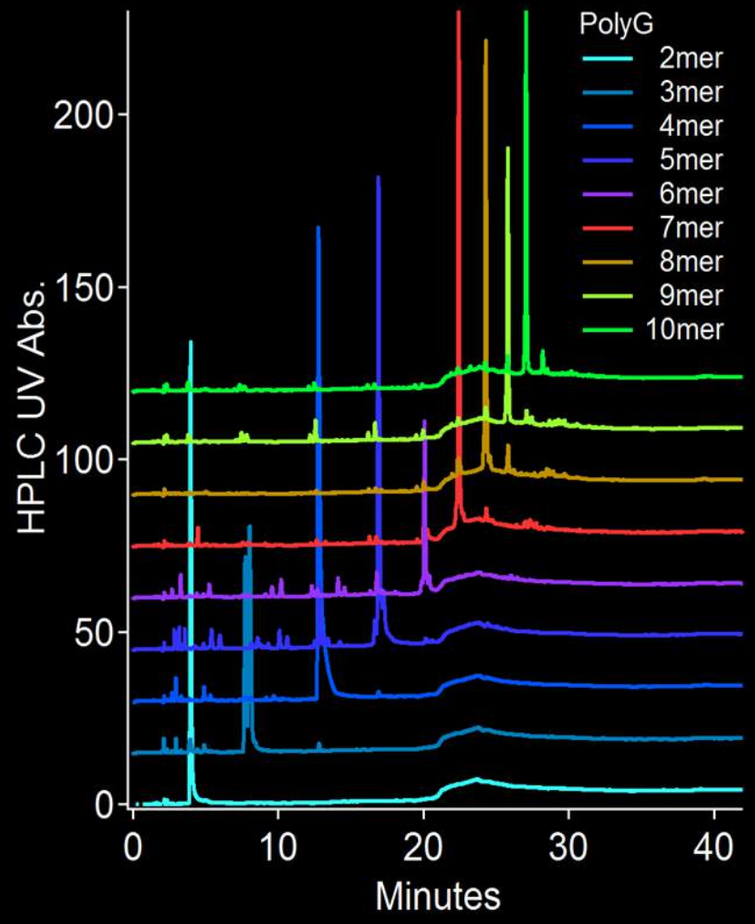


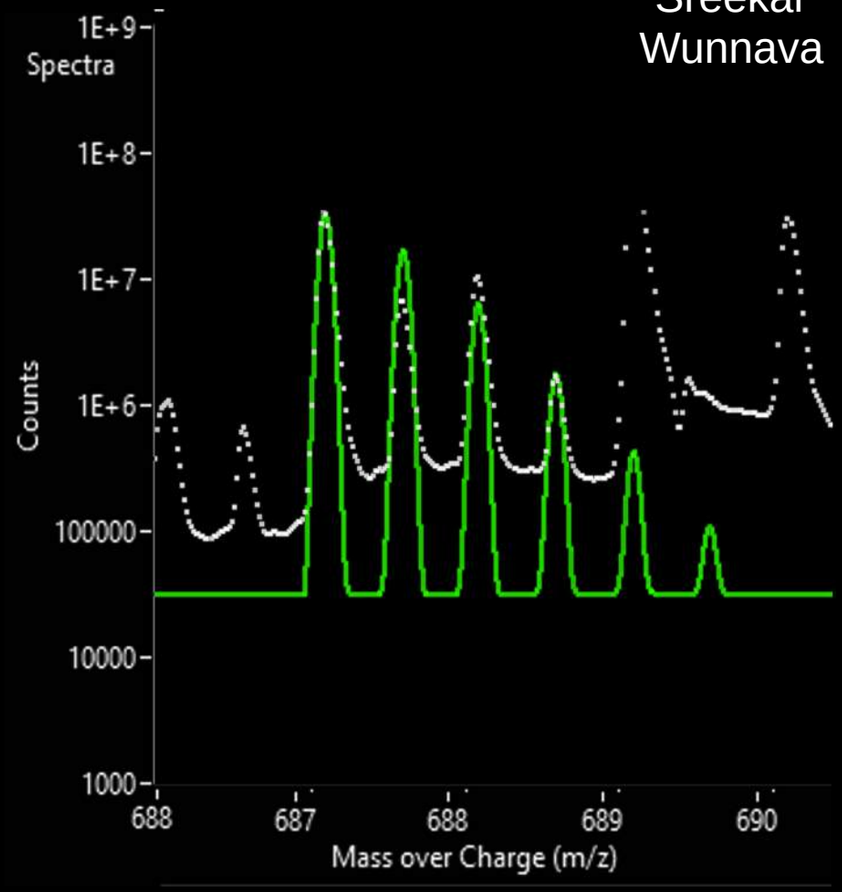
Sreekar Wunnava




HPLC ESI-TOF with fit of Isotope distribution

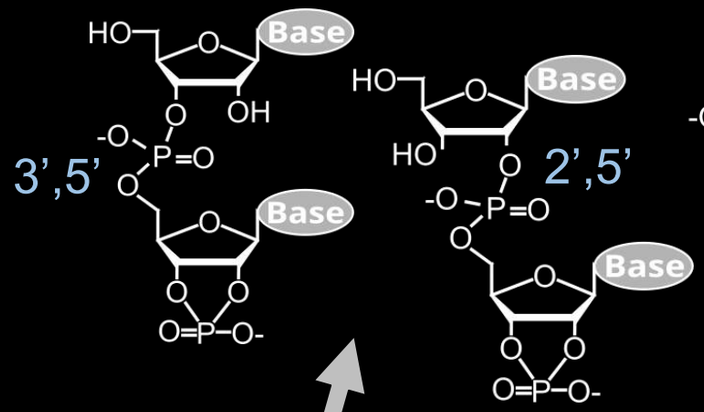


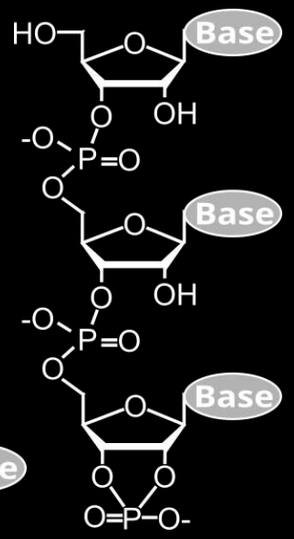



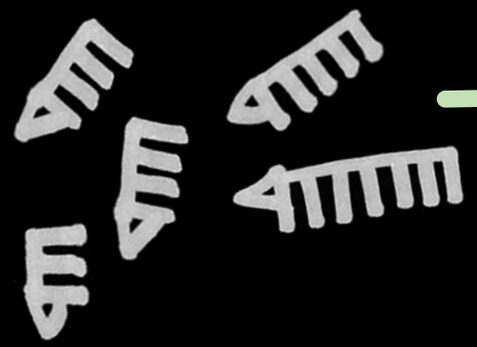

ChemSystemsChem
doi.org/10.1002/syst.202200026 (2022)

# Chemical Evolution

Dry, 1 day, pH 9-10, 4 - 80°C



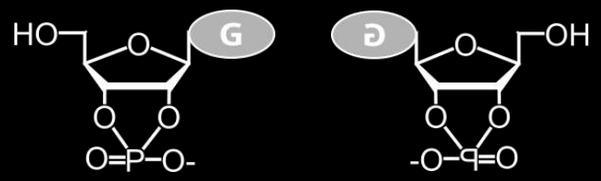








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D L




5x 15N
+5Da

Selection?

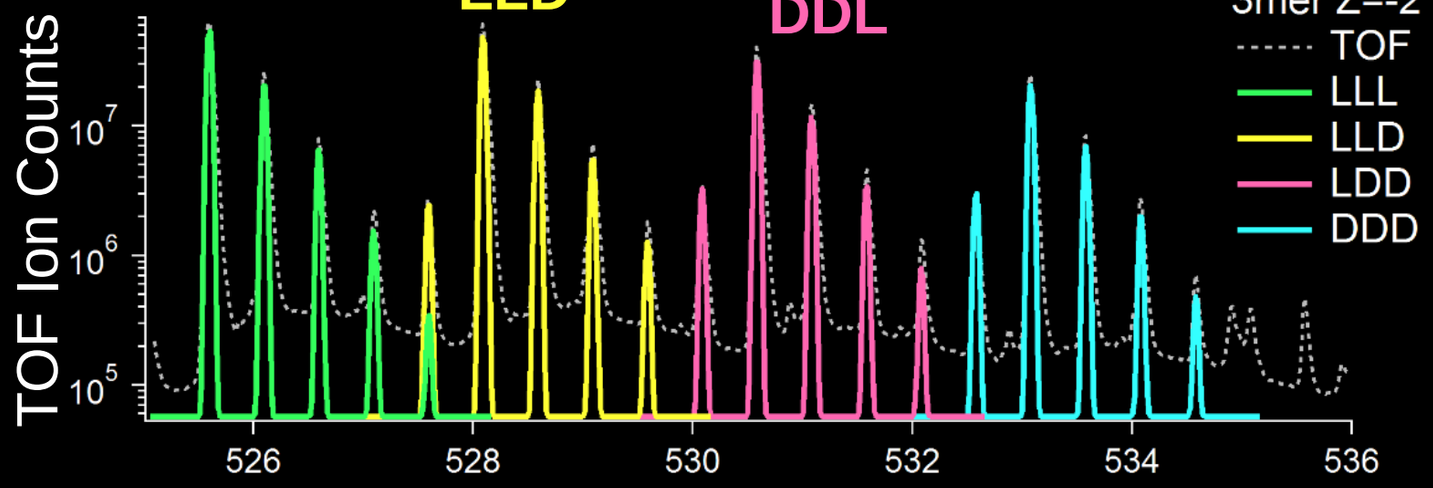
LLL

DLL,
LDL,
LLD

LDD,
DLD,
DDL

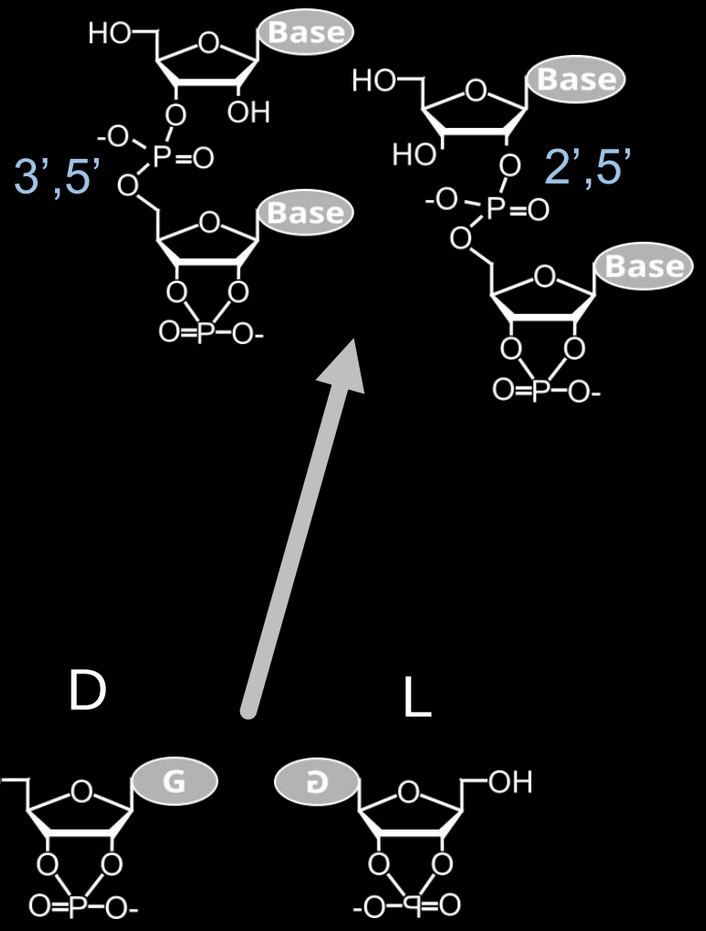
Selection?

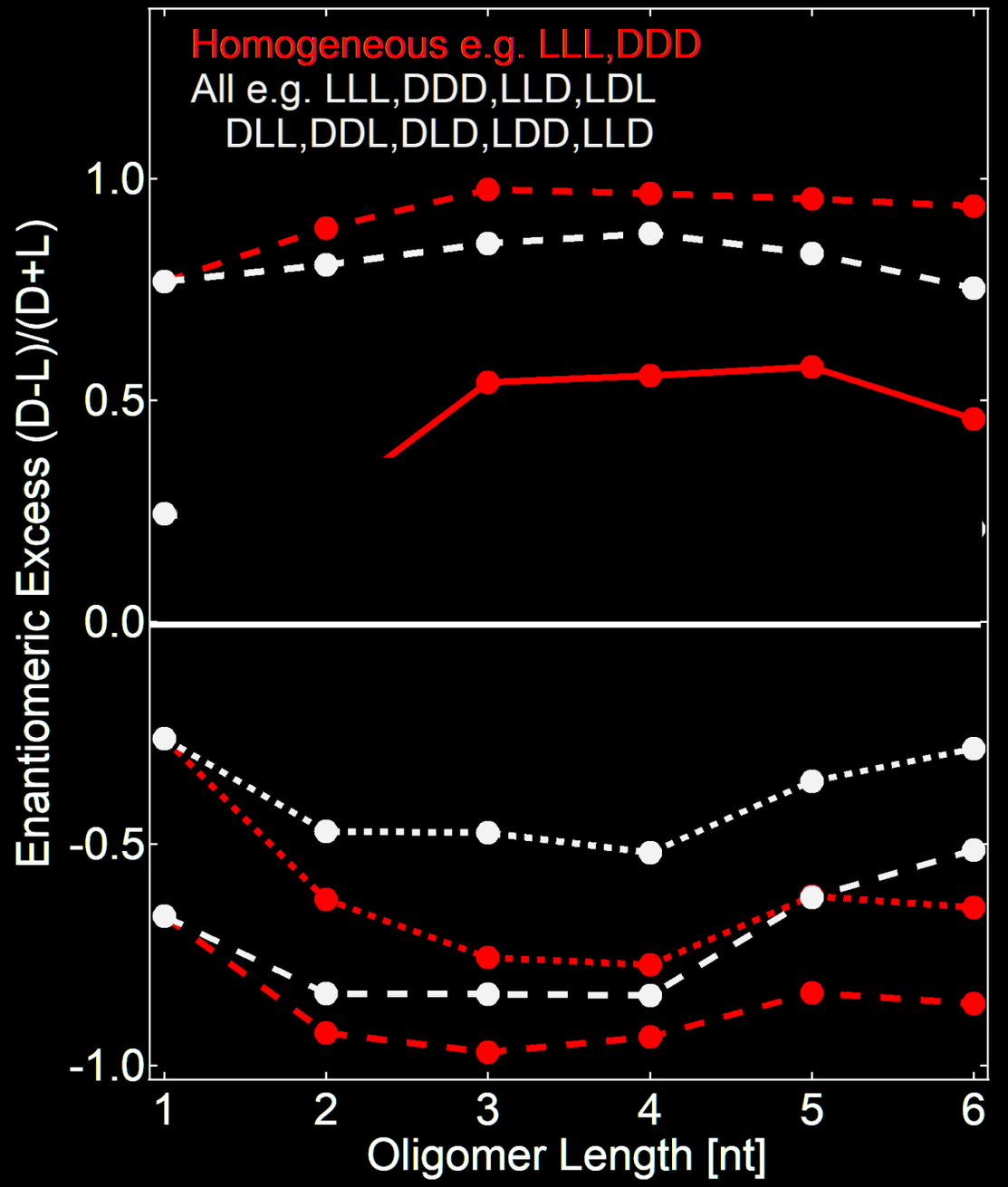
DDD



# Homochiral selection

Dry, 1 day, pH 10, 25°C






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Chirality is amplified for oligos that will hybridize

=> Chiral amplification by hydrolysis

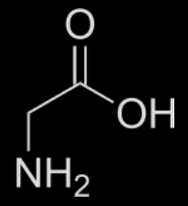
Upcoming Experiments with Dimitar Sasselov

# Catalytic Amino Acids

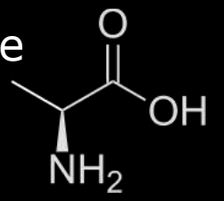
Dry, 1 day, pH 10, 20°C

**Amino Acids** 50mM

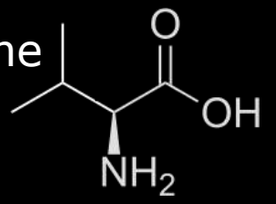
Glycine



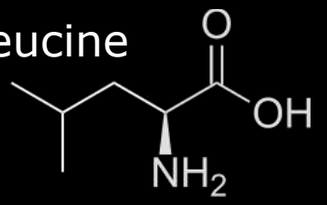
Alanine

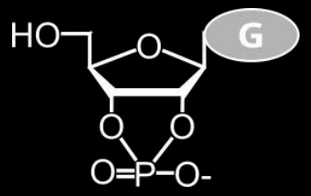


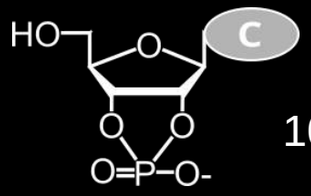
Valine



Leucine



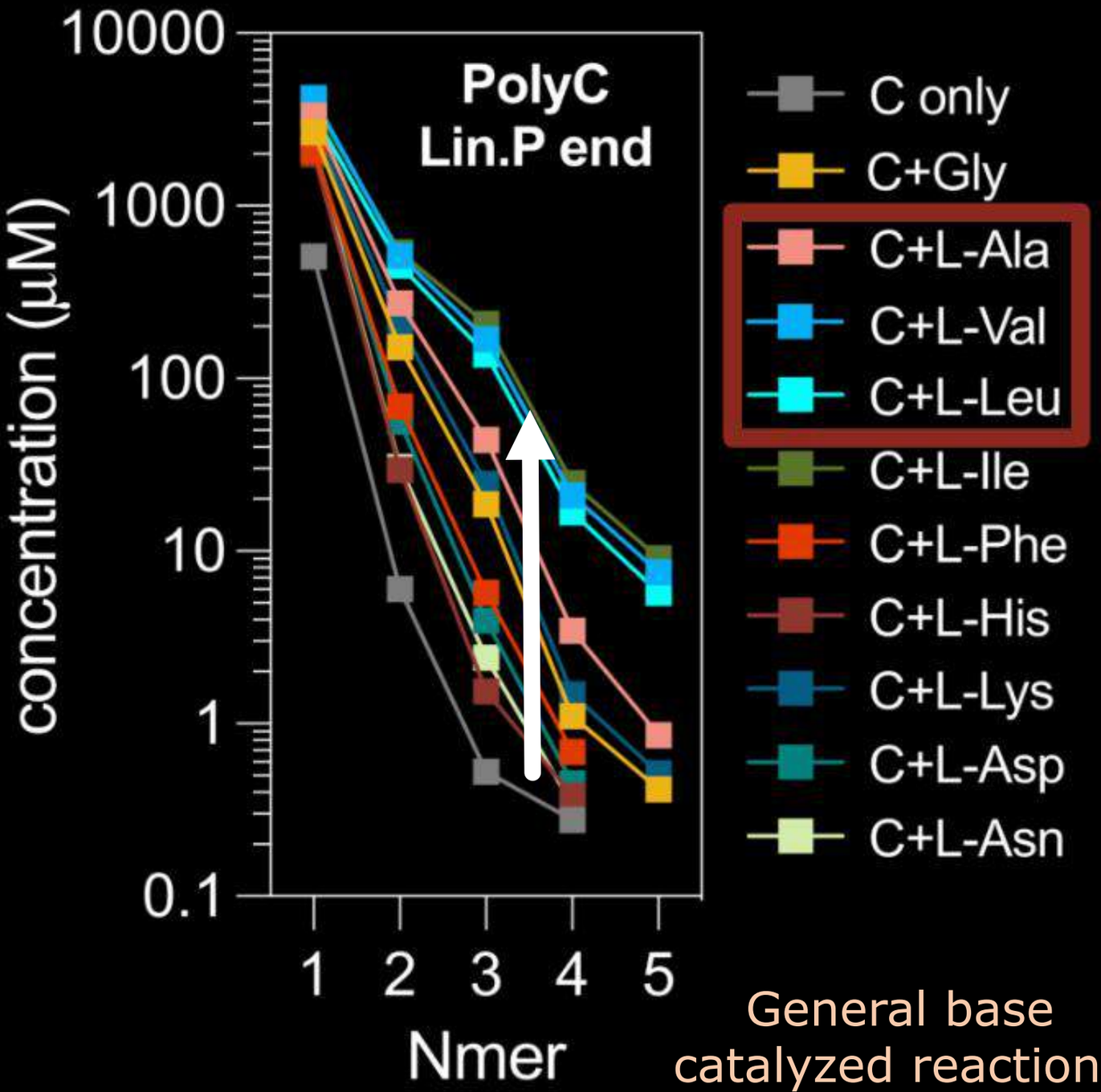



10mM



Saroj Rout



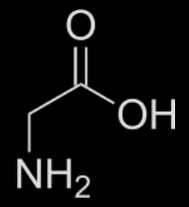

General base catalyzed reaction?

# Coevolution of RNA and Peptides?

Dry, 1 day, pH 10, 60°C

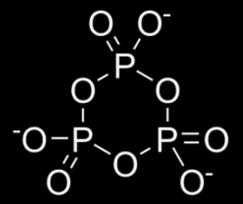
Glycine

10mM



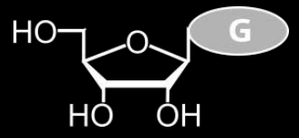

TMP

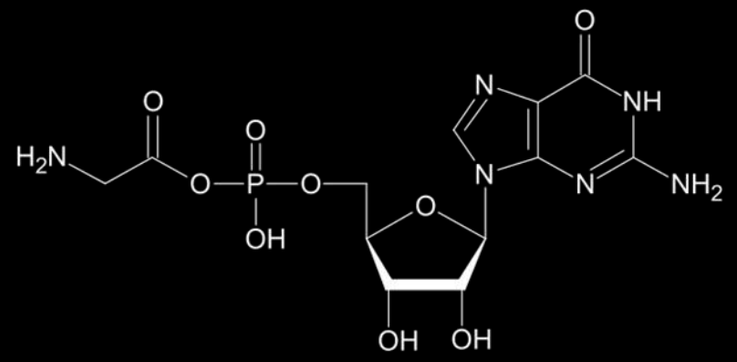
10mM



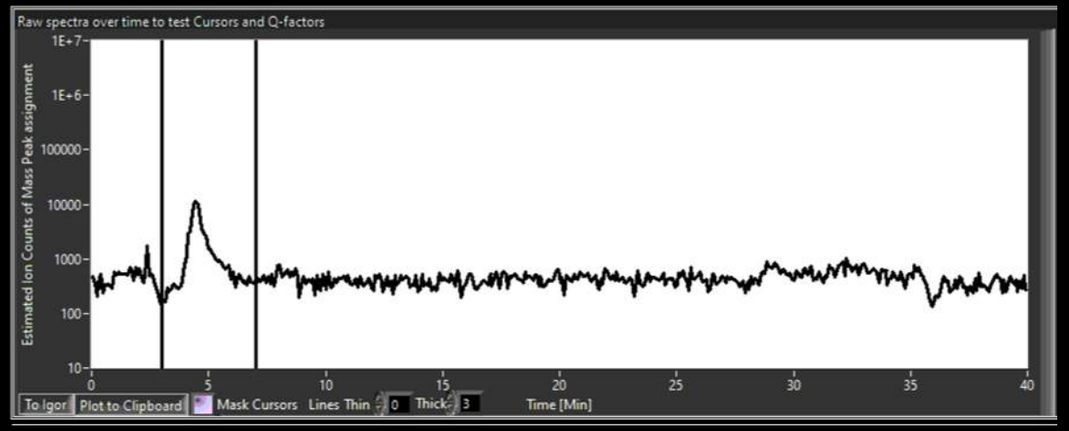

G Nucleoside

1mM




Molecule of Translation




Changed phosphorylation profile



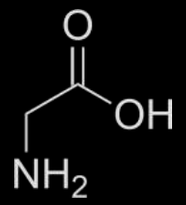
Juliette Langlais

Preliminary results

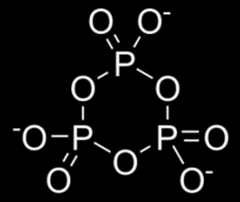
# Coevolution of RNA and Peptides?

## Dry, 1 day, pH 10, 60°C

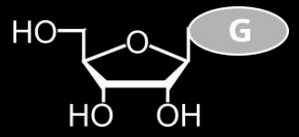
Glycine
10mM



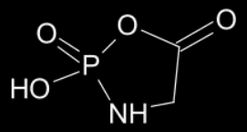
TMP
10mM

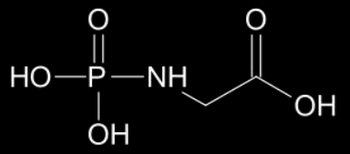


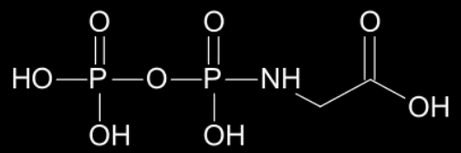
G Nucleoside
1mM



Phosphorylation of amino acids



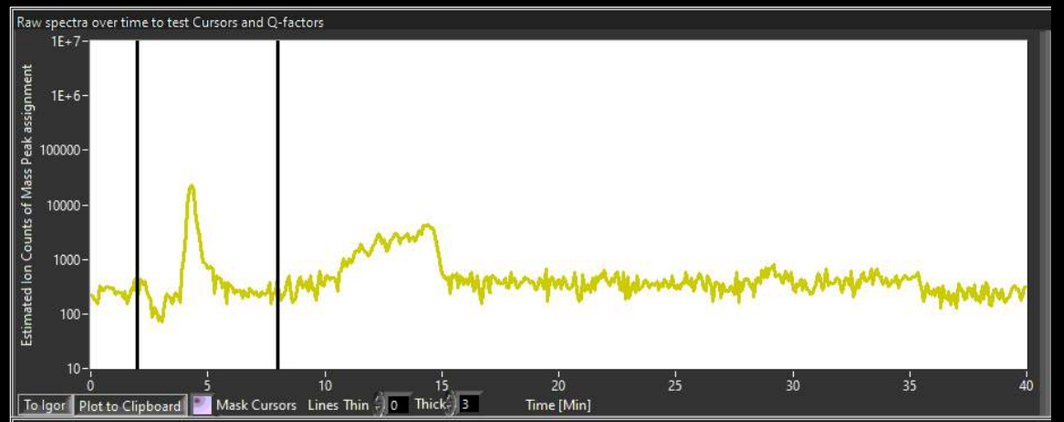


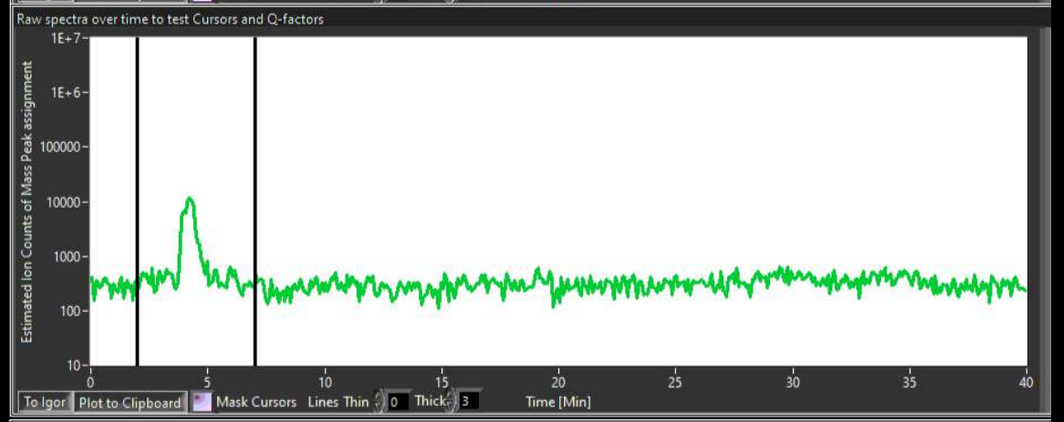


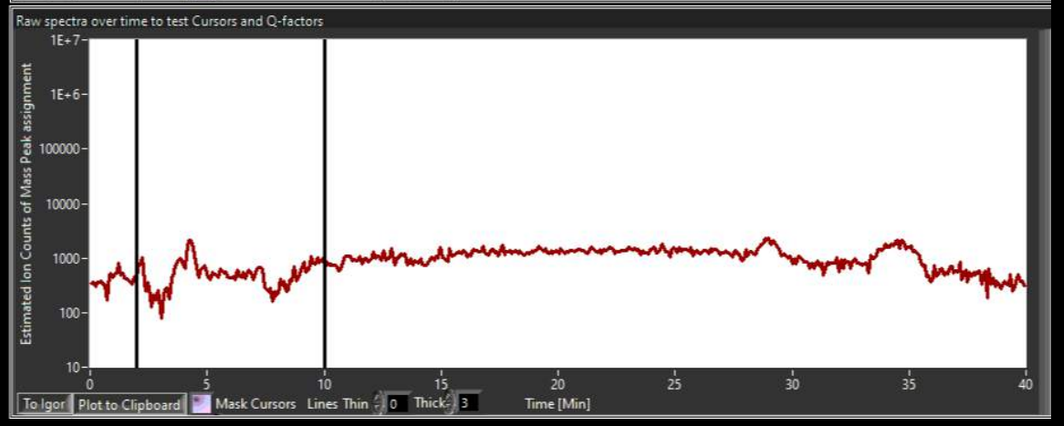


Juliette Langlais

Preliminary results



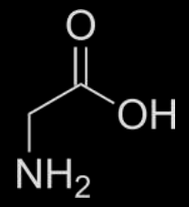



# Coevolution of RNA and Peptides?

## Dry, 1 day, pH 10, 60°C

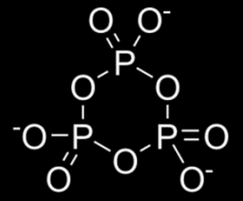
Glycine

10mM



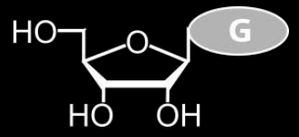
TMP

10mM



G Nucleoside

1mM

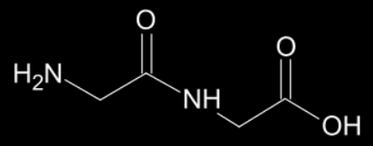


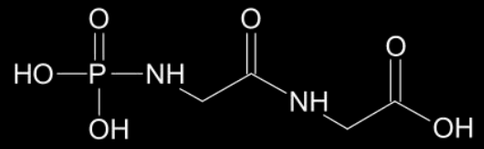


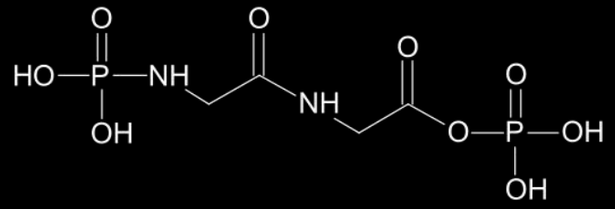
Juliette Langlais

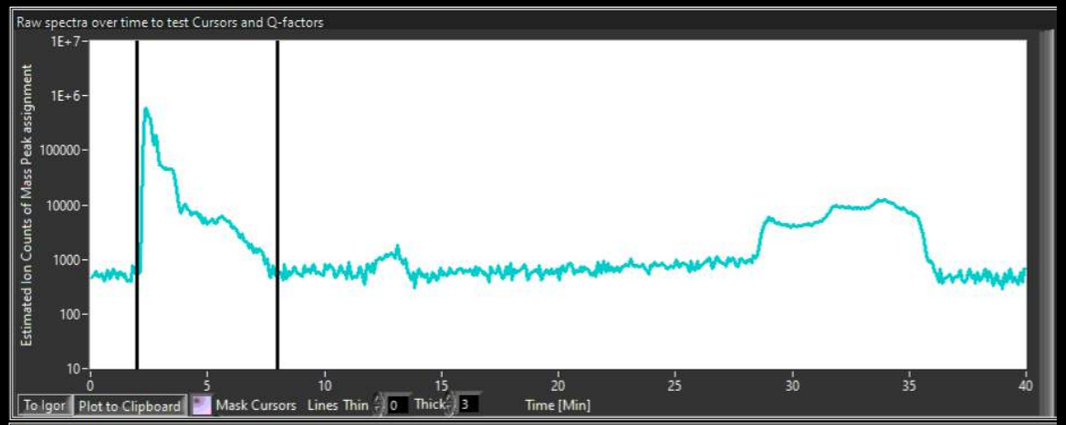
Preliminary results

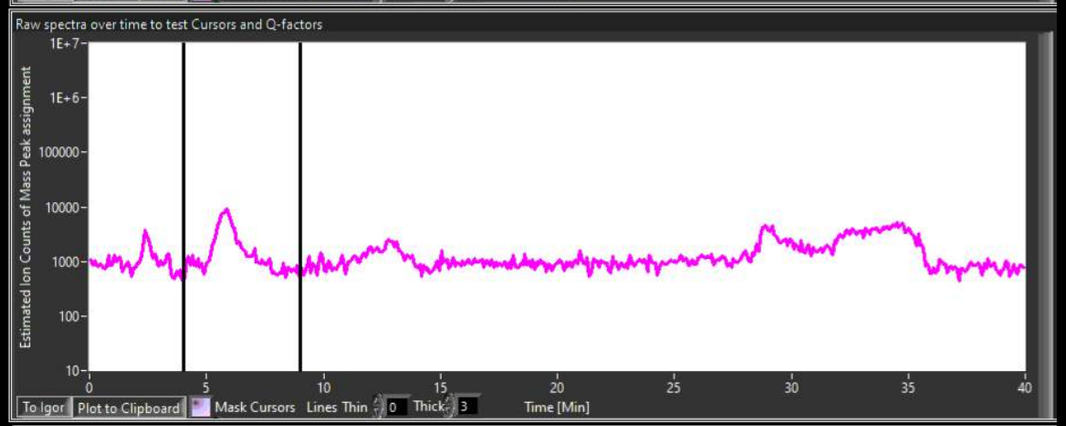
Polymerization of amino acids

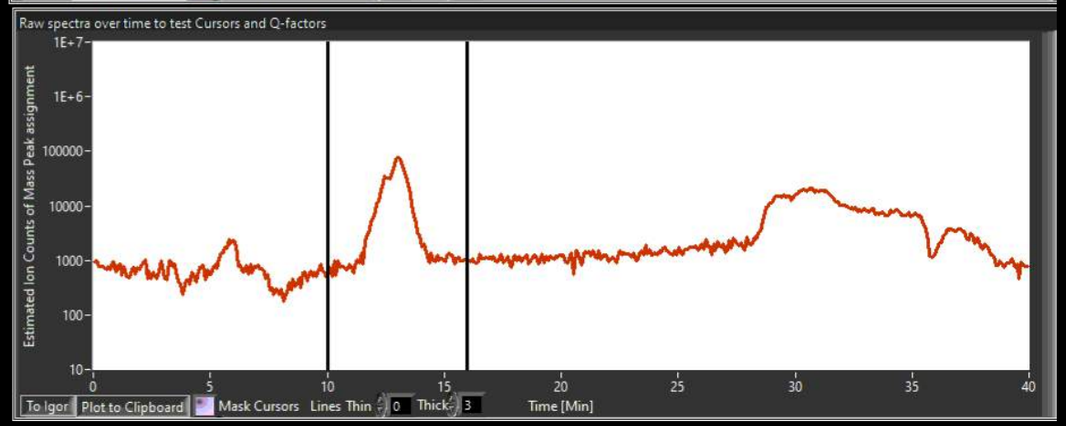








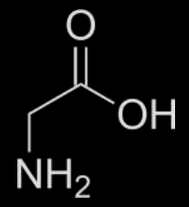



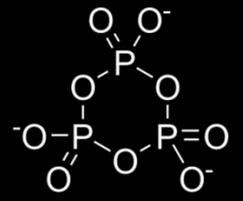
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## Dry, 1 day, pH 10, 60°C

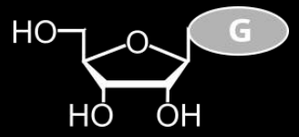
Glycine
10mM



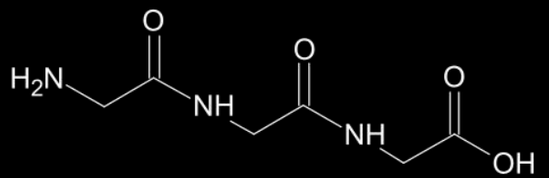
TMP
10mM

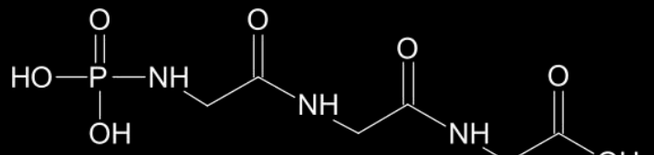


G Nucleoside
1mM




Polymerization of amino acids



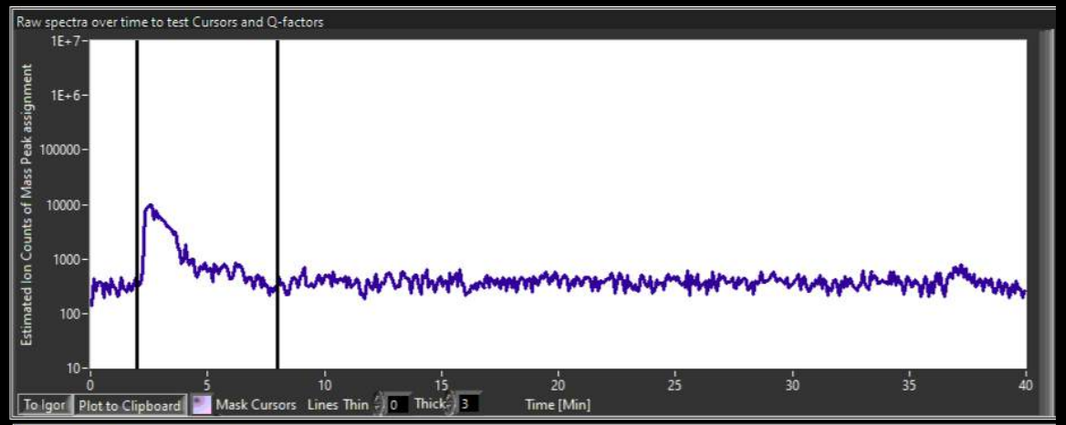


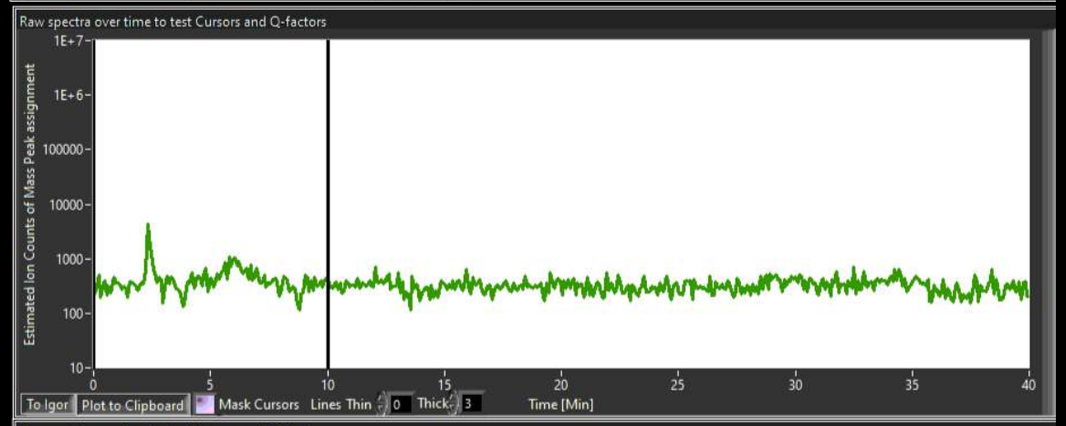


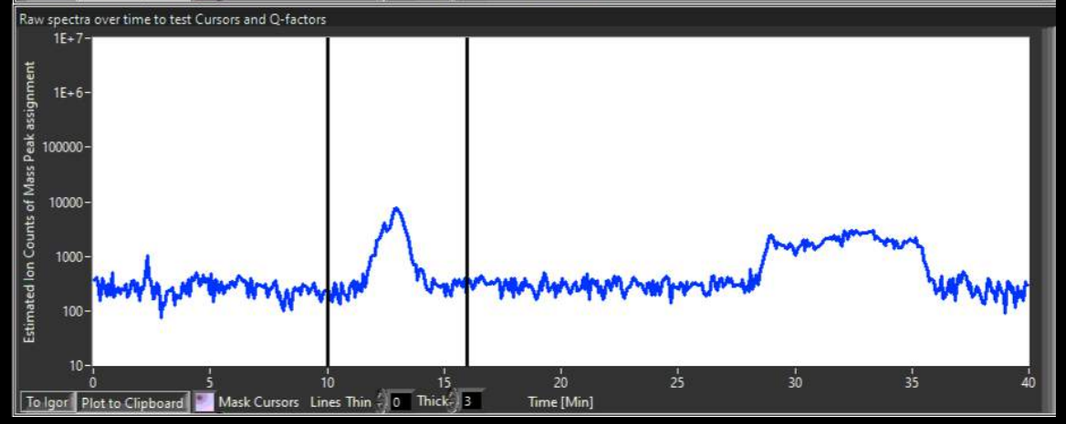


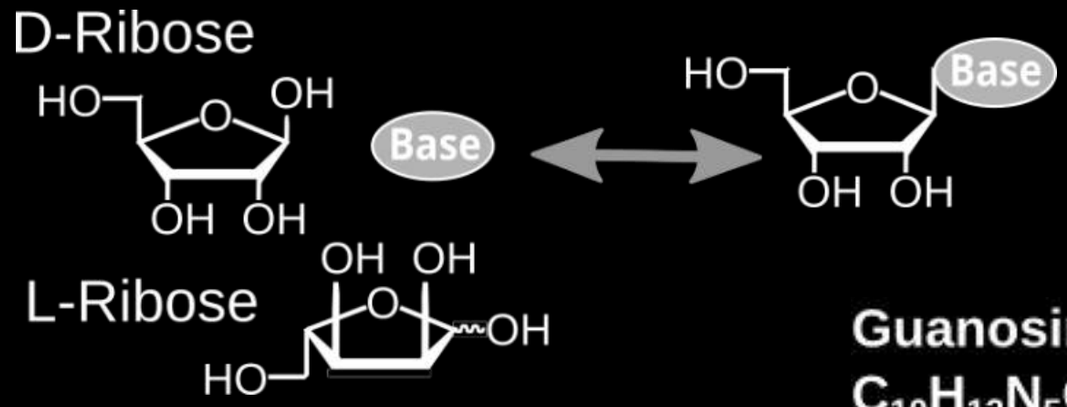
Juliette Langlais

Preliminary results



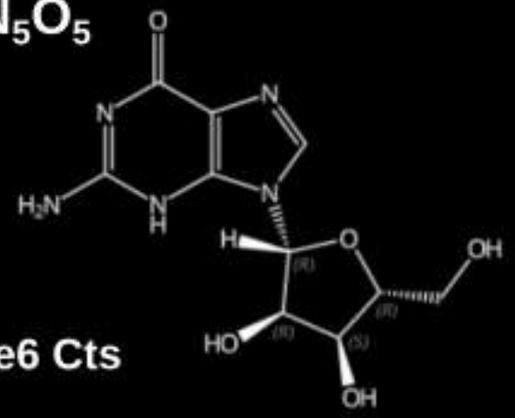






# Nucleoside synthesis, old school
# Detection by Orbitrap Fit

**Guanosine isoforms**

$C_{10}H_{13}N_5O_5$

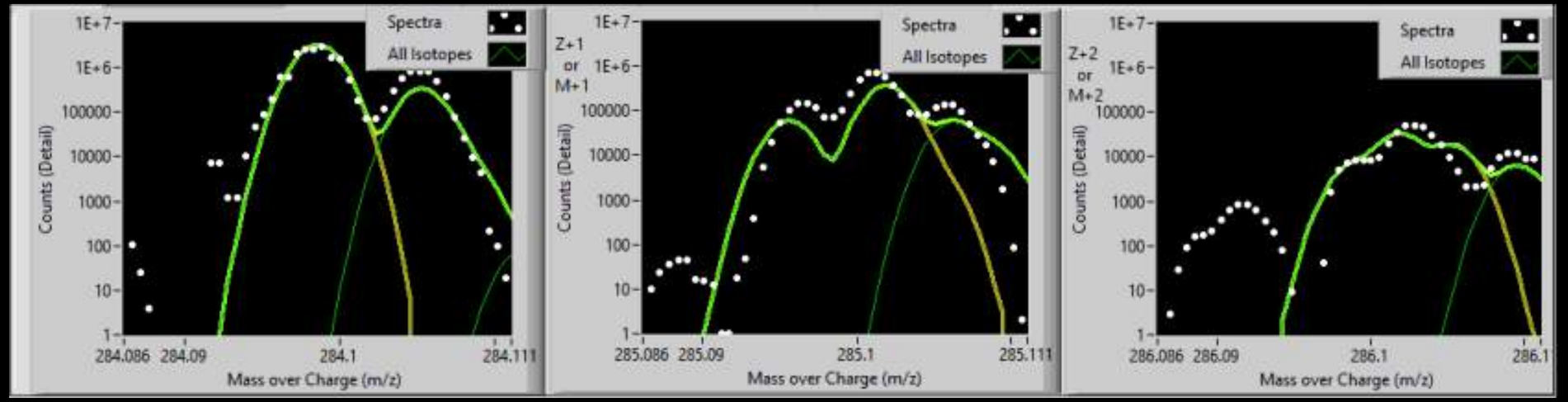



**4e6 Cts**

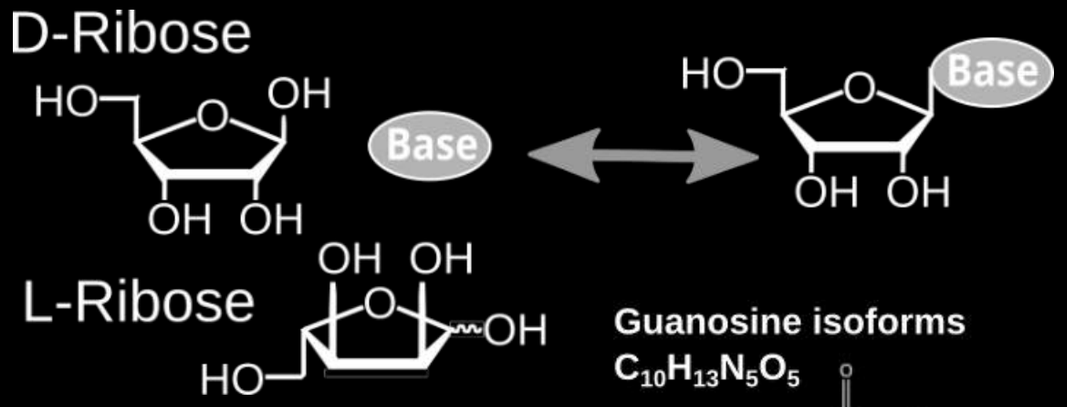
Carbonate buffer
dry reaction
70° at pH 10, 24h



Max
Weingart

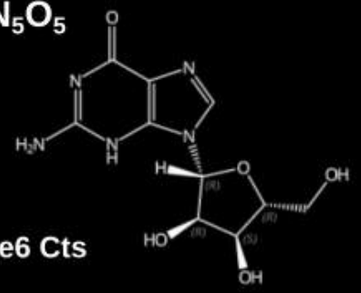


# Nucleoside synthesis, old school
# Detection by Orbitrap Fit



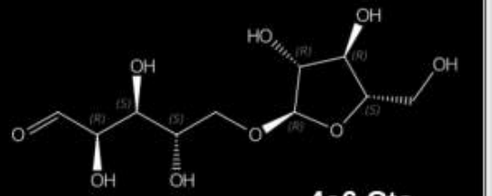

Carbonate buffer
dry reaction
70° at pH 10, 24h

**Guanosine isoforms**
$C_{10}H_{13}N_5O_5$



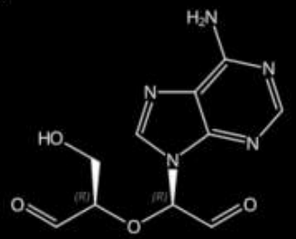
4e6 Cts

**Bi-Ribose isoforms**
$C_{10}H_{18}O_9$



4e6 Cts

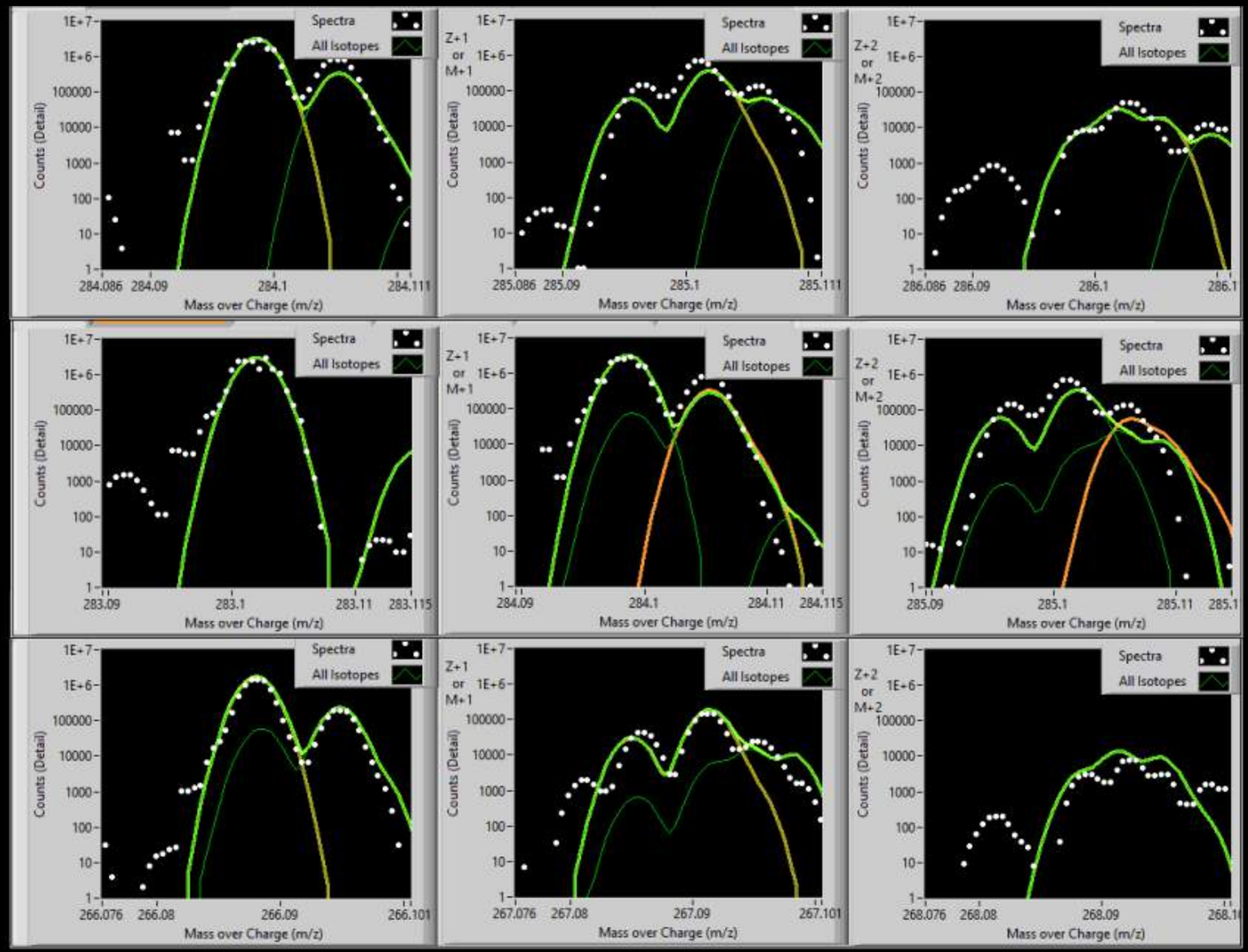
**Sugar-Guanine isoforms**
$C_{10}H_{11}N_5O_4$



2e6 Cts



Max
Weingart



## Emergence of Life in the Lab

1. Biological History
2. Chemical Selection
3. Sequence Selection
4. Geophysical Space
5. Autonomous Evolution

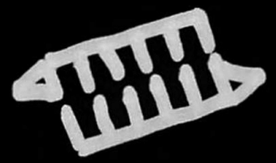
# Equal number of Bases

5x 25-40°C dry cycle, 3h+3h time

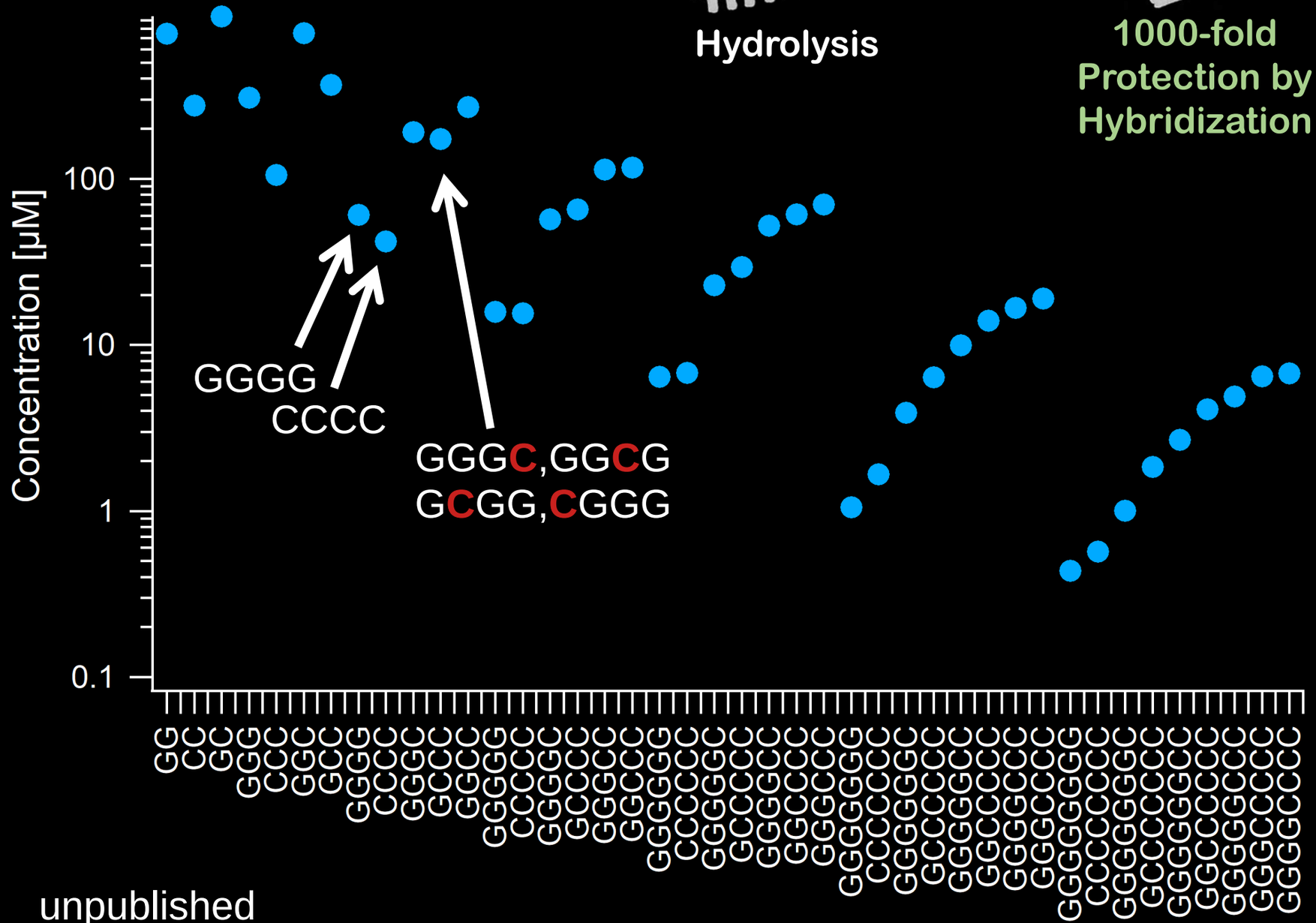


Hydrolysis

Selection of size



1000-fold Protection by Hybridization




Hypothesis: protection by hybridization against hydrolysis




GGGCCGGC
CCCGGCCG

McMaster Planet Simulator

~~GGGGGGGG~~

unpublished


Alix Dujardin
Avinash Dass
Ralph Pudritz
Maikel Rheinstadter

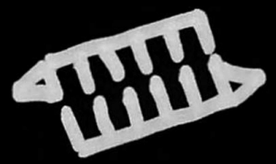
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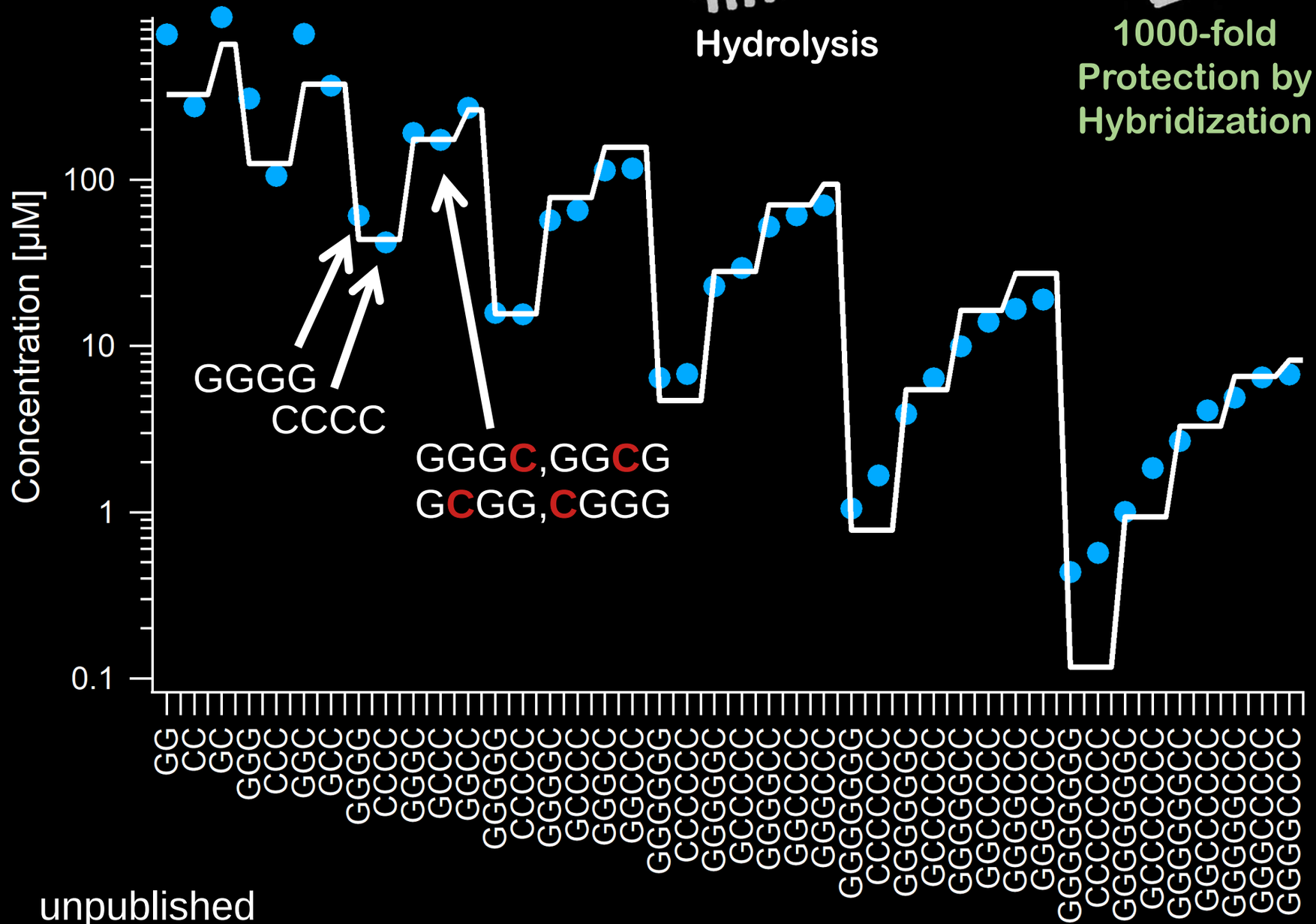


Hydrolysis

Selection of size



1000-fold Protection by Hybridization




Hypothesis: protection by hybridization against hydrolysis

GGGGGGGG
CCCCCCCC

~~GGGGGGGG~~

unpublished

# Equal number of Bases

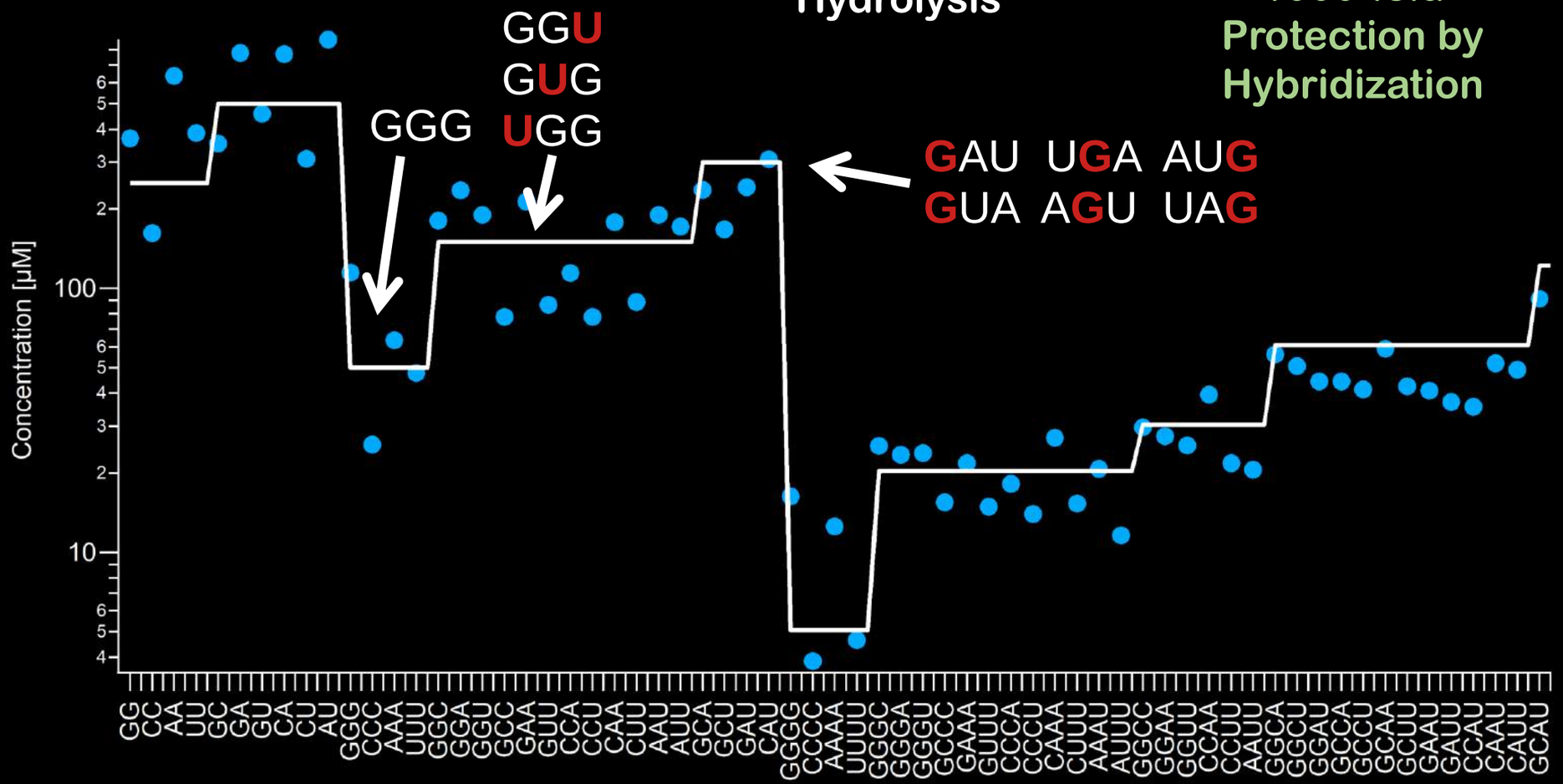
5x 25-40°C dry cycle, 3h+3h time




Selection of size



1000-fold Protection by Hybridization




Hypothesis: protection by hybridization against hydrolysis

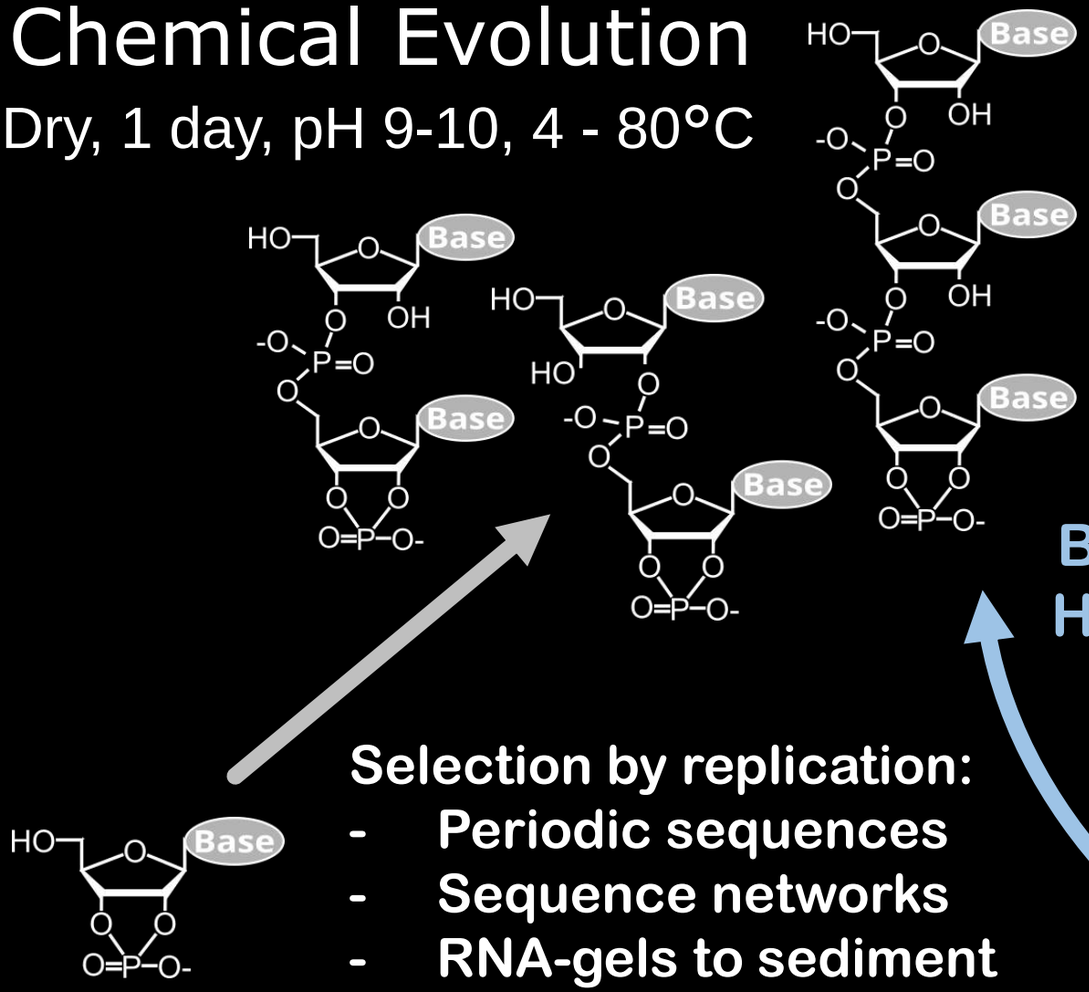
**AUGCAUGC**
**UACGUACG**

~~**UACGUACG**~~

unpublished

# Chemical Evolution

Dry, 1 day, pH 9-10, 4 - 80°C





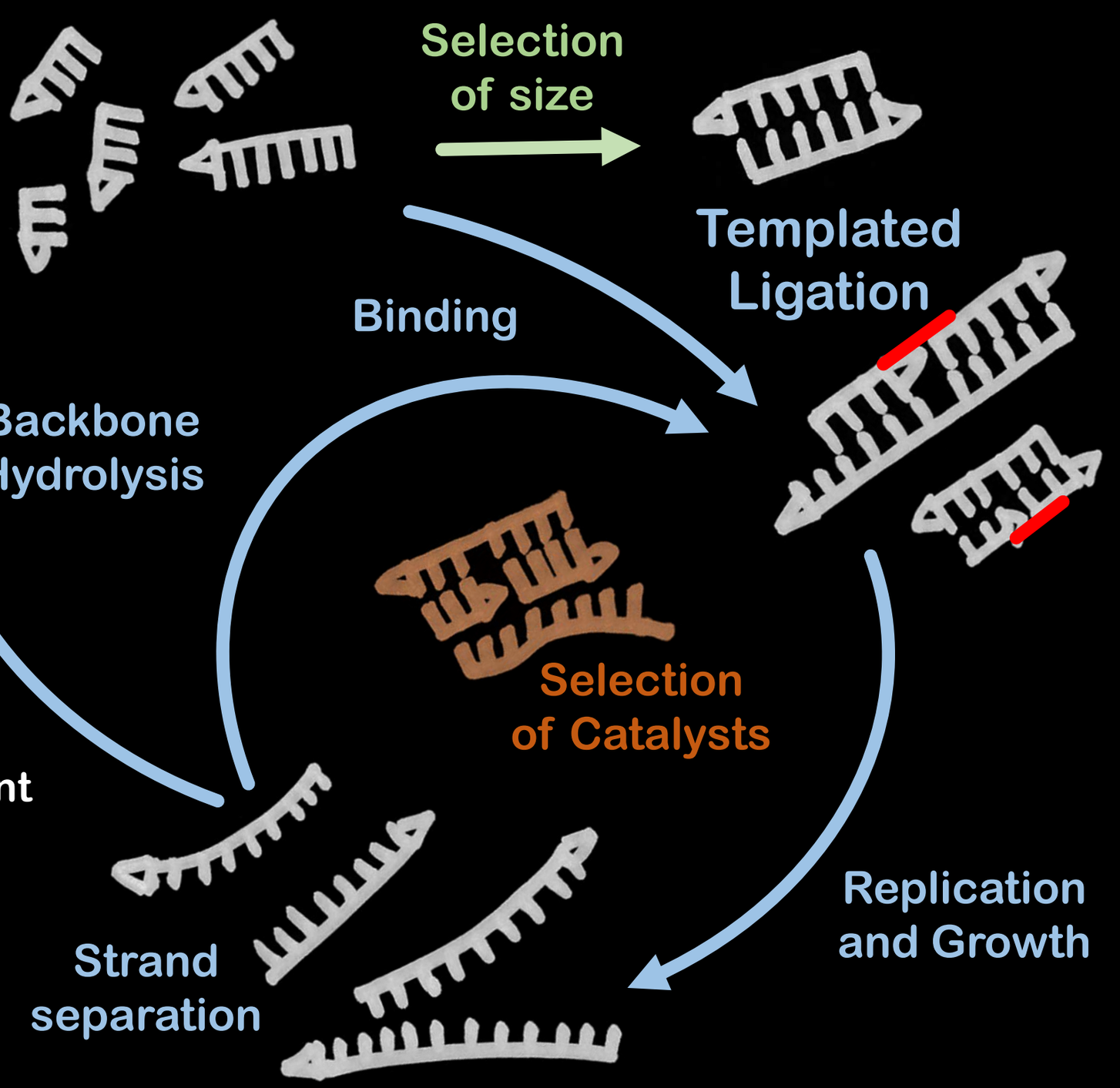


**Selection by replication:**

- Periodic sequences
- Sequence networks
- RNA-gels to sediment
- Adaptation to environment
- Replication networks
- Darwinian Evolution

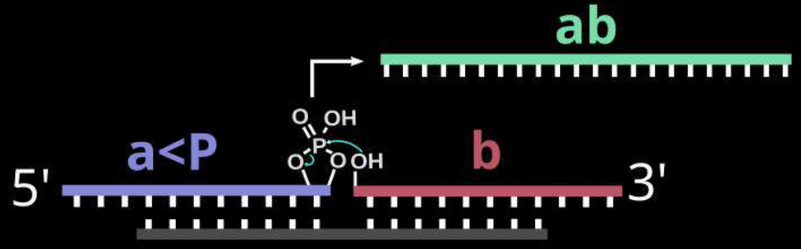
# Sequence Evolution

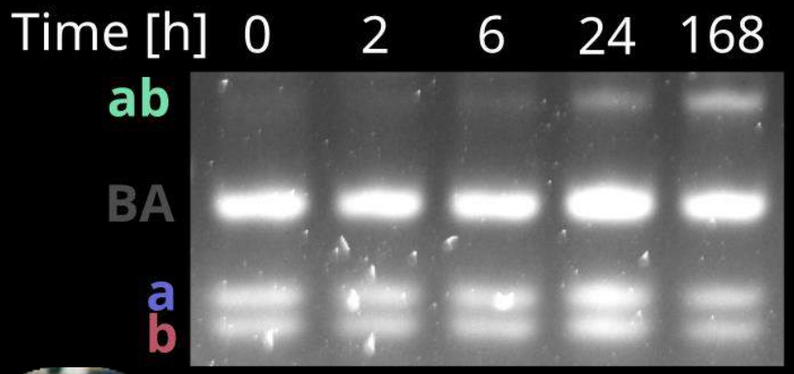
Wet, 1 day, pH 9-10, 4 - 80°C

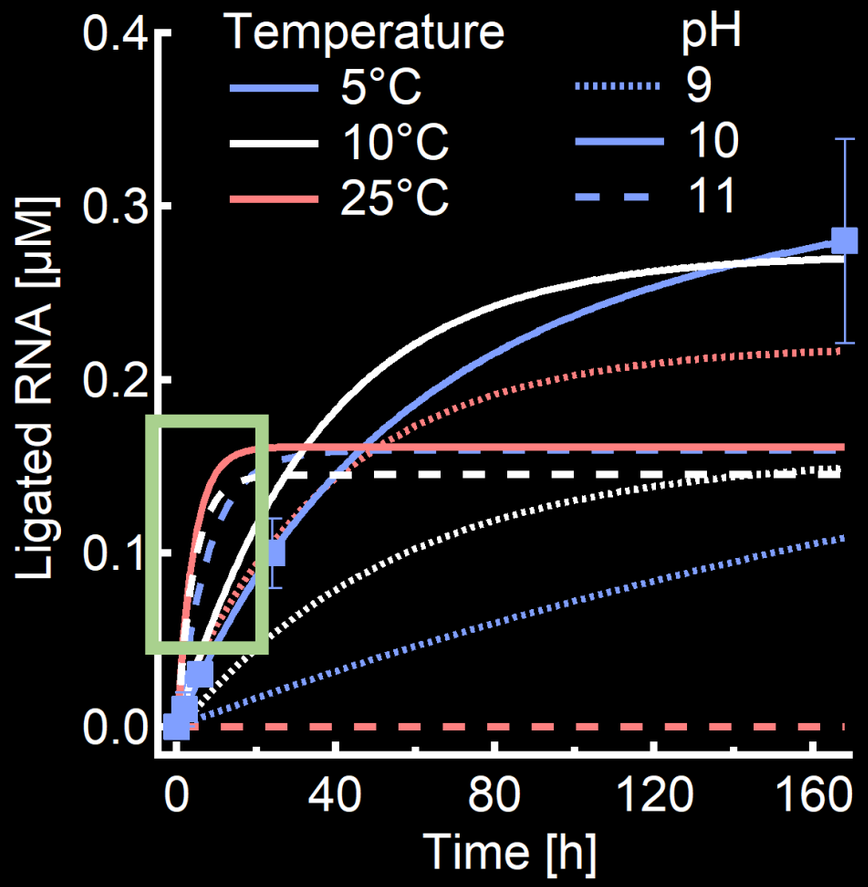


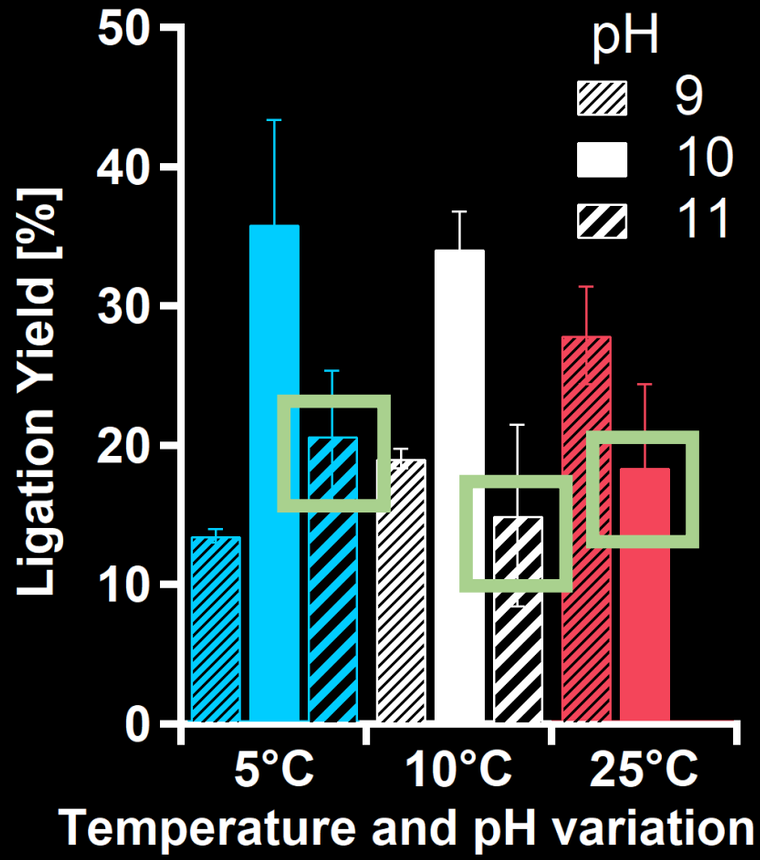
# Templated ligation of RNA at low $Mg^{2+}$ concentration

[a],[b],[ba] = 1µM; 50mM CHES
pH 10; 1mM $MgCl_2$; Incubation 7d



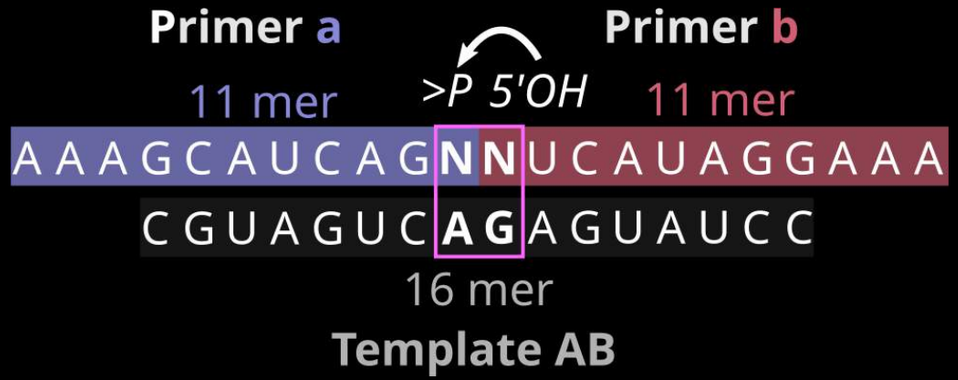





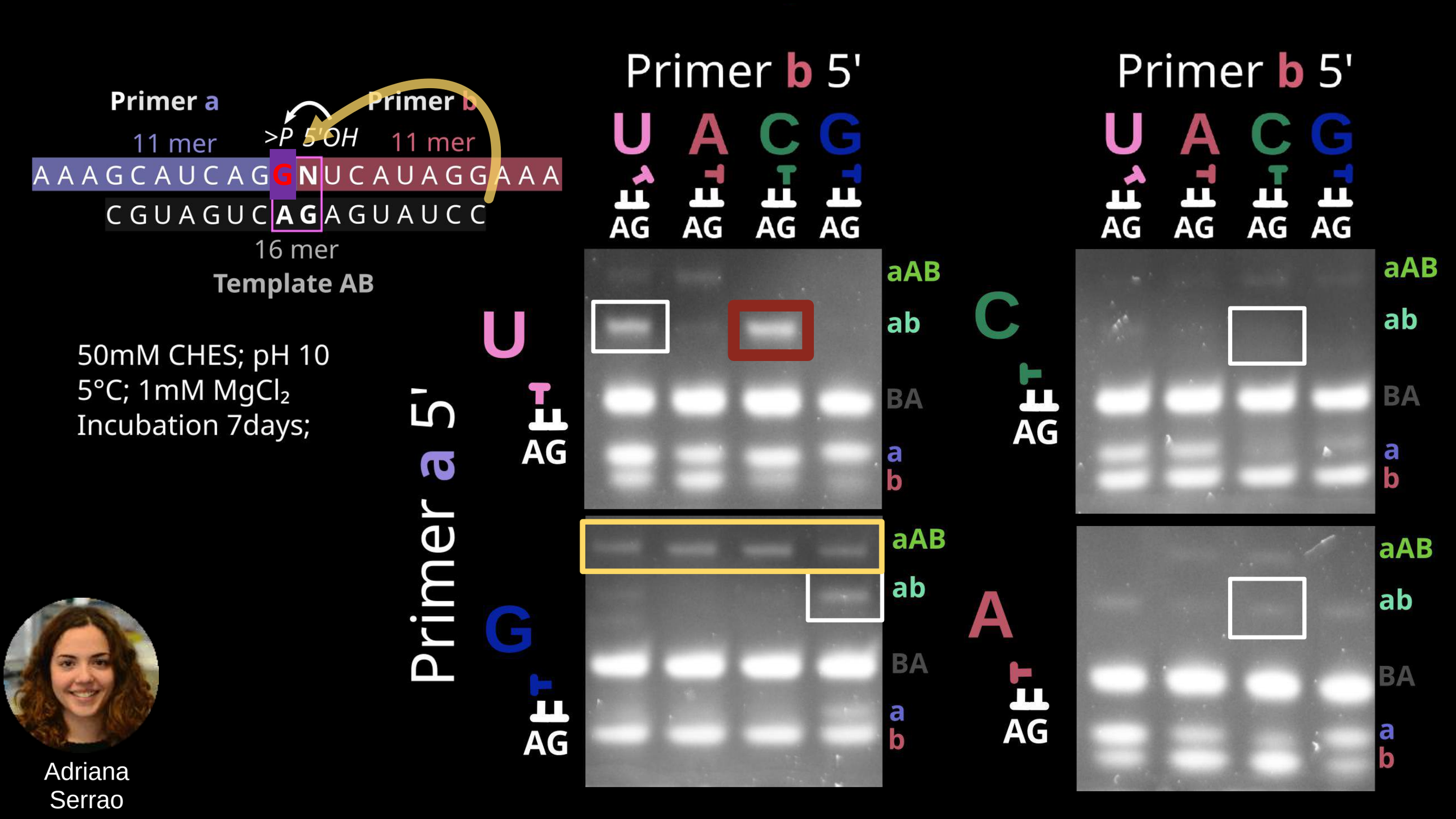

Adriana Serrao



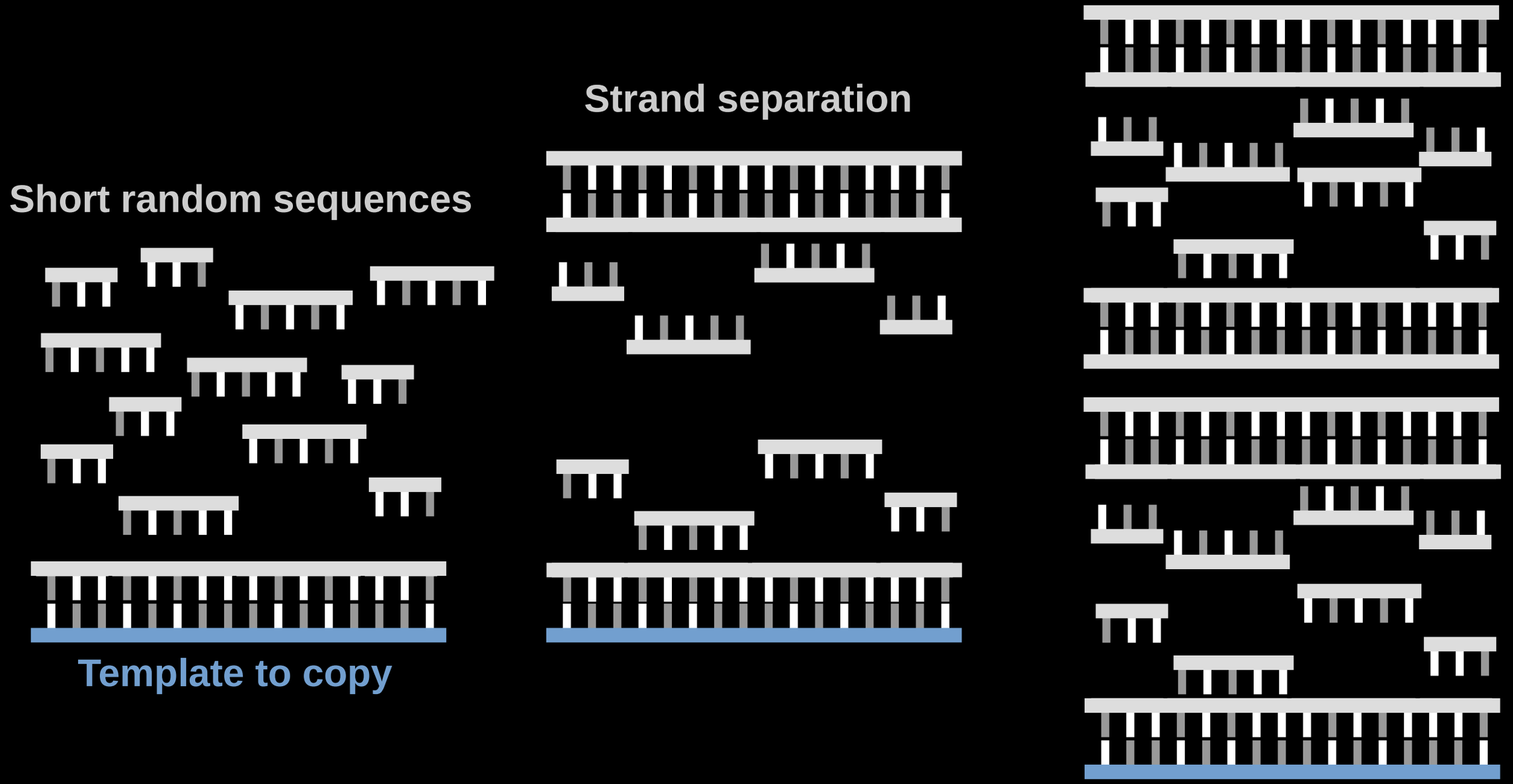

50mM CHES; pH 10
5°C; 1mM $MgCl_2$
Incubation 7days;



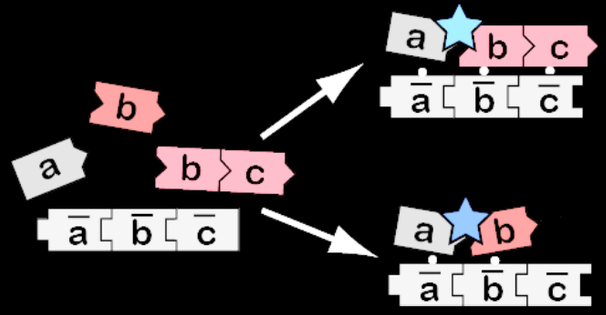
Adriana Serrao


Primer a
11 mer
>P 5'OH
Primer b
11 mer
AAAGCAUCAGGNUCAUAGGAAA
CGUAGUCAGAGUAUCC
16 mer
Template AB
50mM CHES; pH 10
5°C; 1mM MgCl₂
Incubation 7days;
Primer a 5'
U
G
Primer b 5'
U A C G
AG AG AG AG
aAB
ab
BA
a
b
Primer b 5'
C
A
U A C G
AG AG AG AG
aAB
ab
BA
a
b
Adriana Serrao

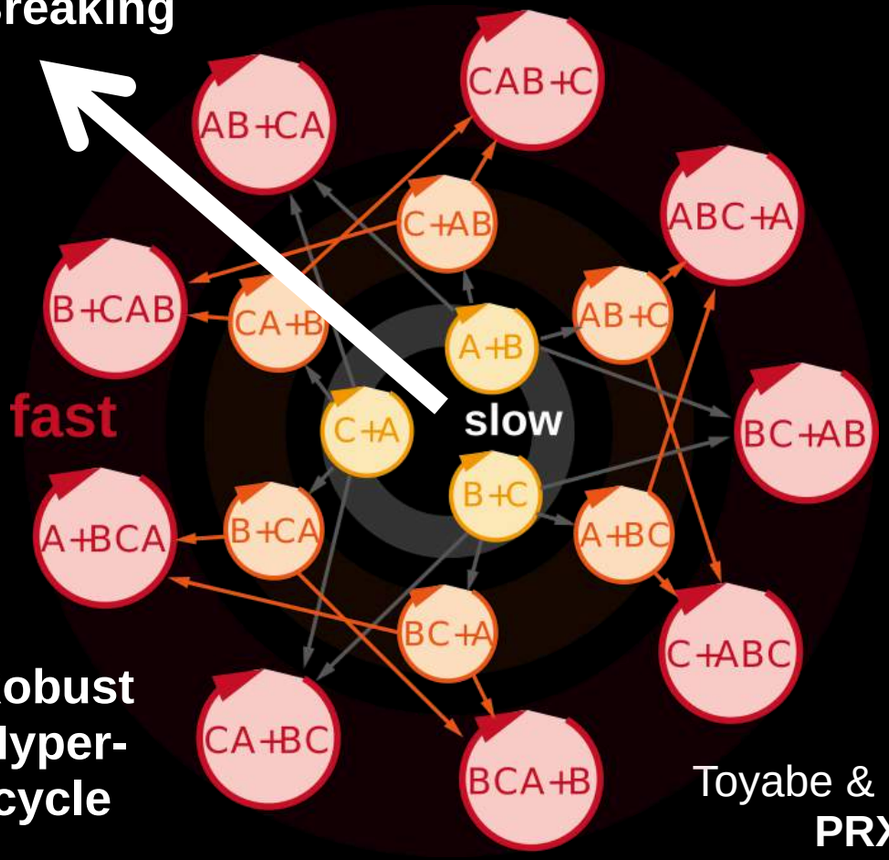
# Replication by templated Ligation



# Symmetry breaking in Replication by templated Ligation




Symmetry Breaking

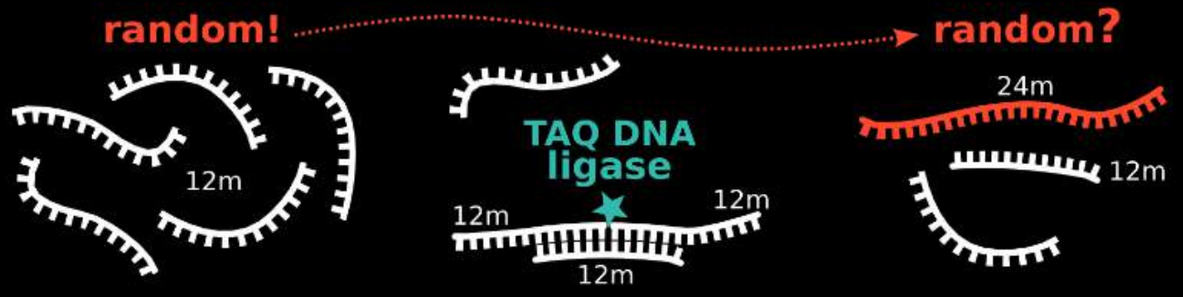



Robust Hyper-cycle

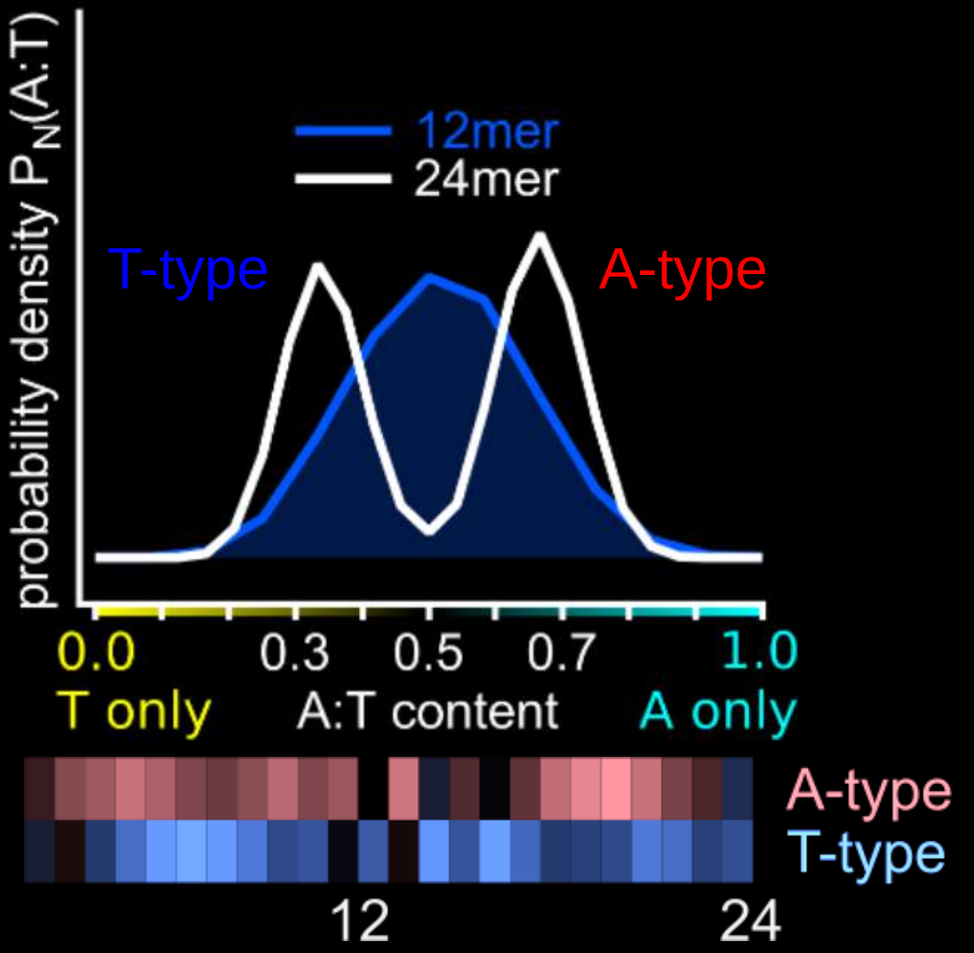
Toyabe & Braun **PRX** 2019

# Replication dynamics in sequence space

From Random to Non-random Sequences




12mer random



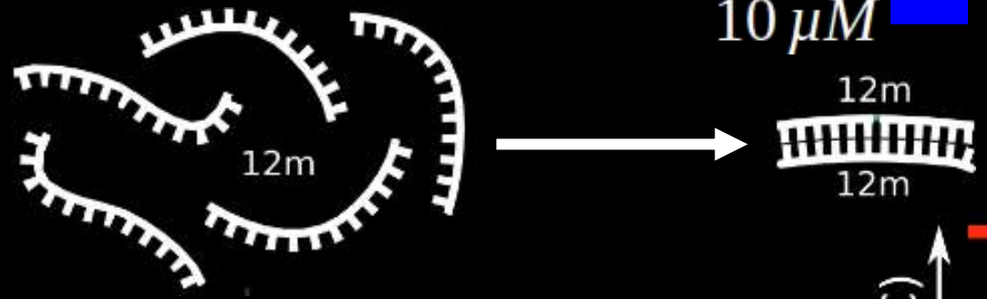

**Braun et.al. PNAS** 2021

# Non-equilibrium of Thermal cycling

## Dilution by Sequence Space

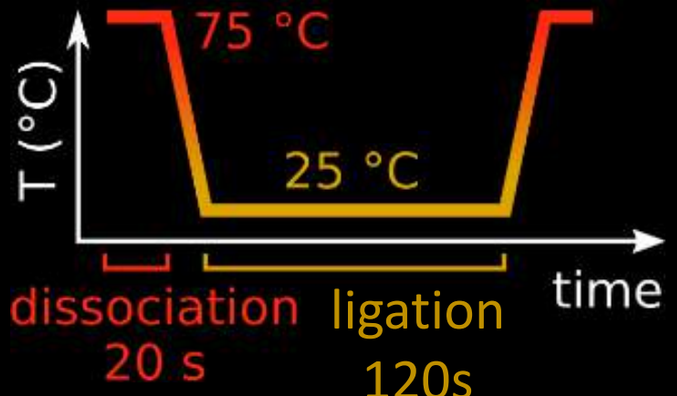
AT

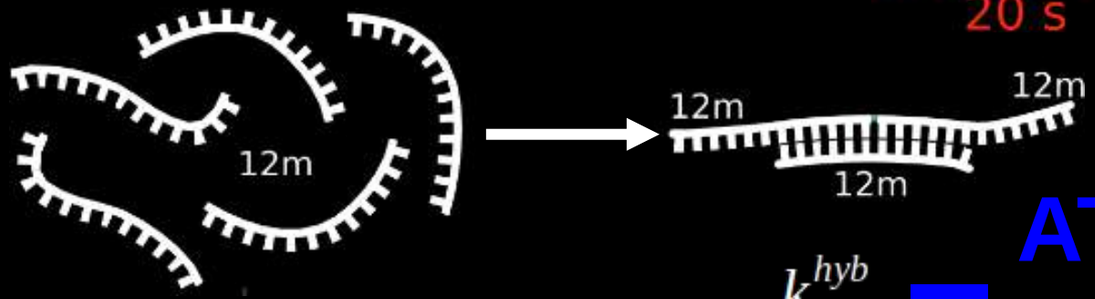
$$t = \frac{k_{on}^{hyb}}{10\,\mu M} 2^{12} = 410\,s$$




Hybridization
no competition

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



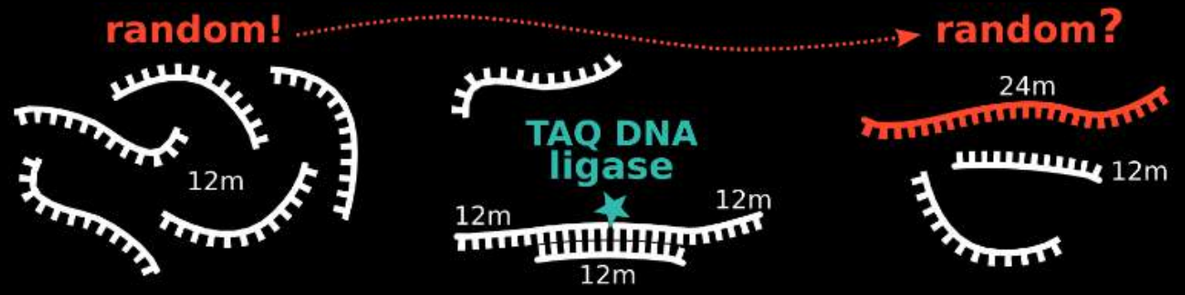




AT

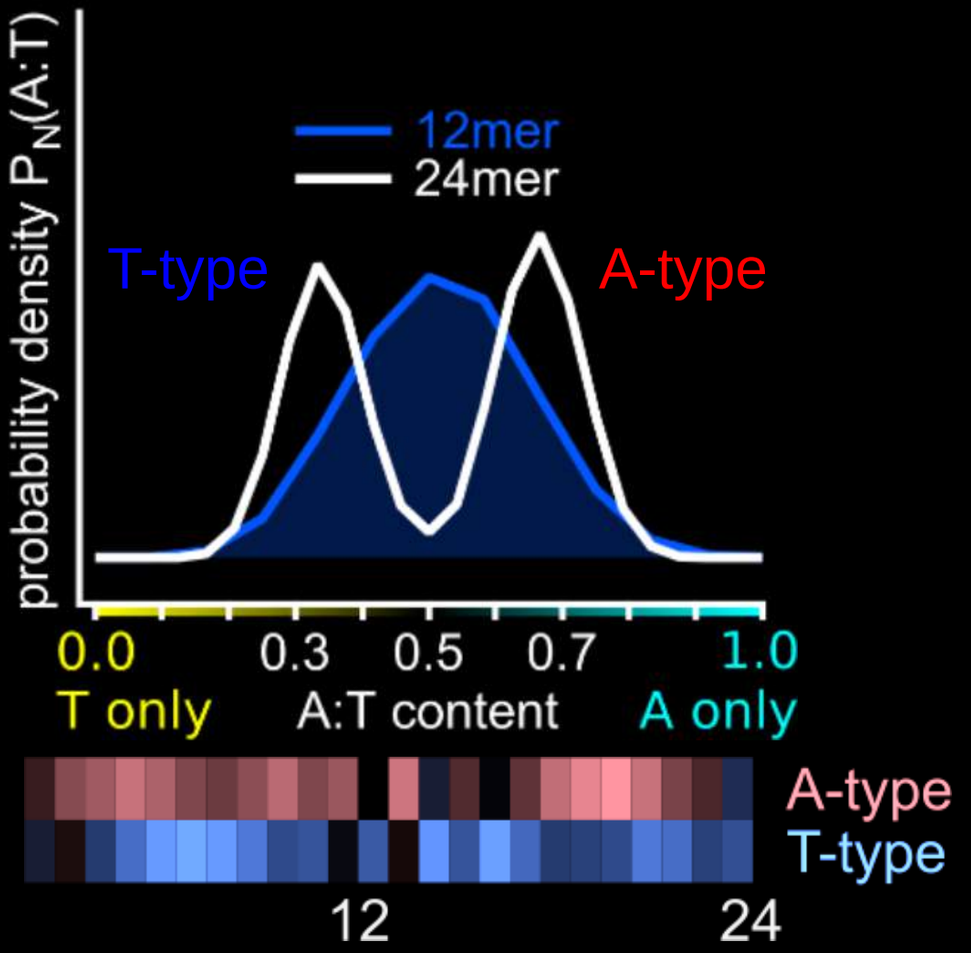
$$t = \frac{k_{on}^{hyb}}{10\,\mu M} 2^{6} = 6\,s$$

# Replication dynamics in sequence space

From Random to Non-random Sequences




12mer
random



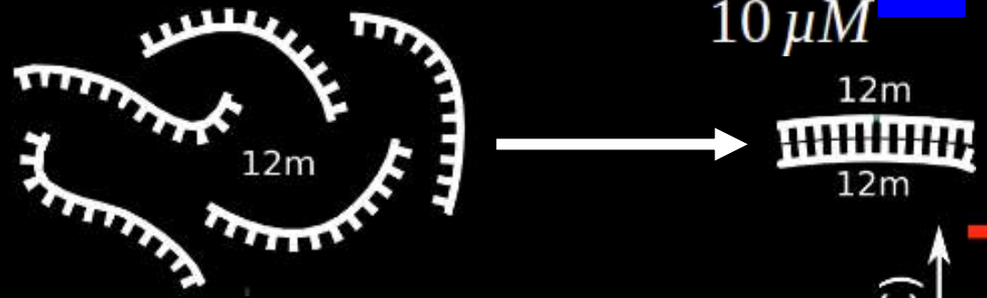

**Braun et.al.**
**PNAS** 2021

# Non-equilibrium of Thermal cycling

## Dilution by Sequence Space

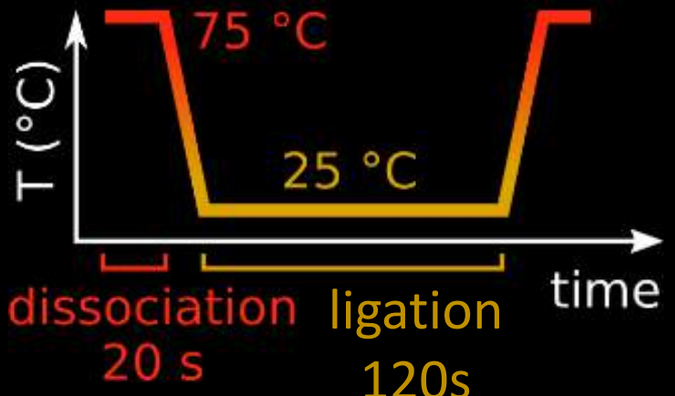
$$t = \frac{k_{on}^{hyb}}{10\,\mu M} 4^{12} = 19\,days$$

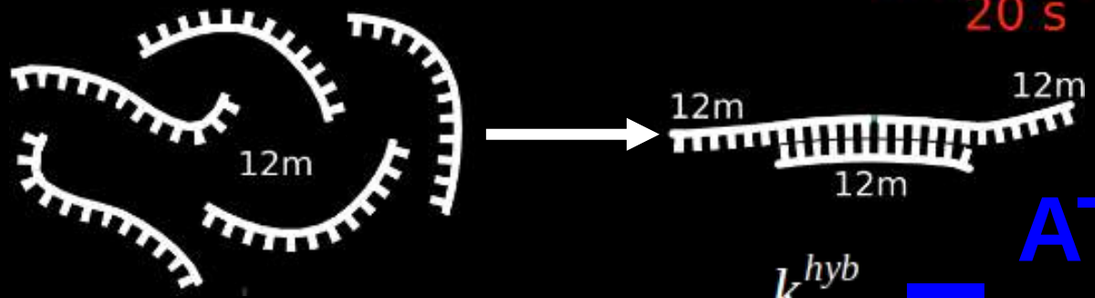
ATGC




Hybridization
no competition

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



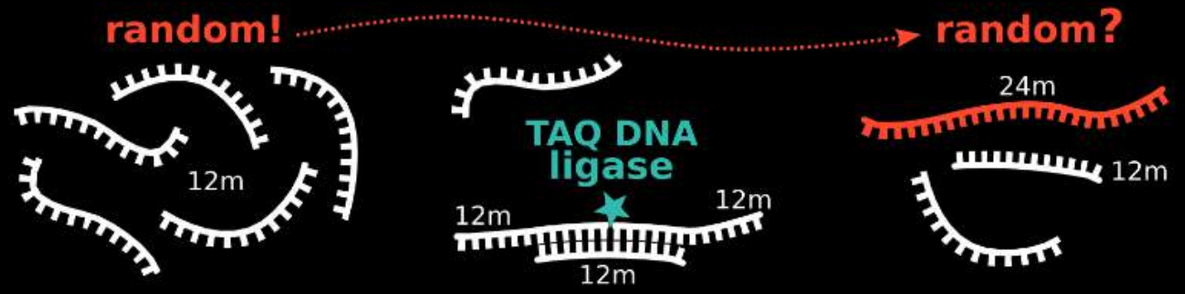




ATGC

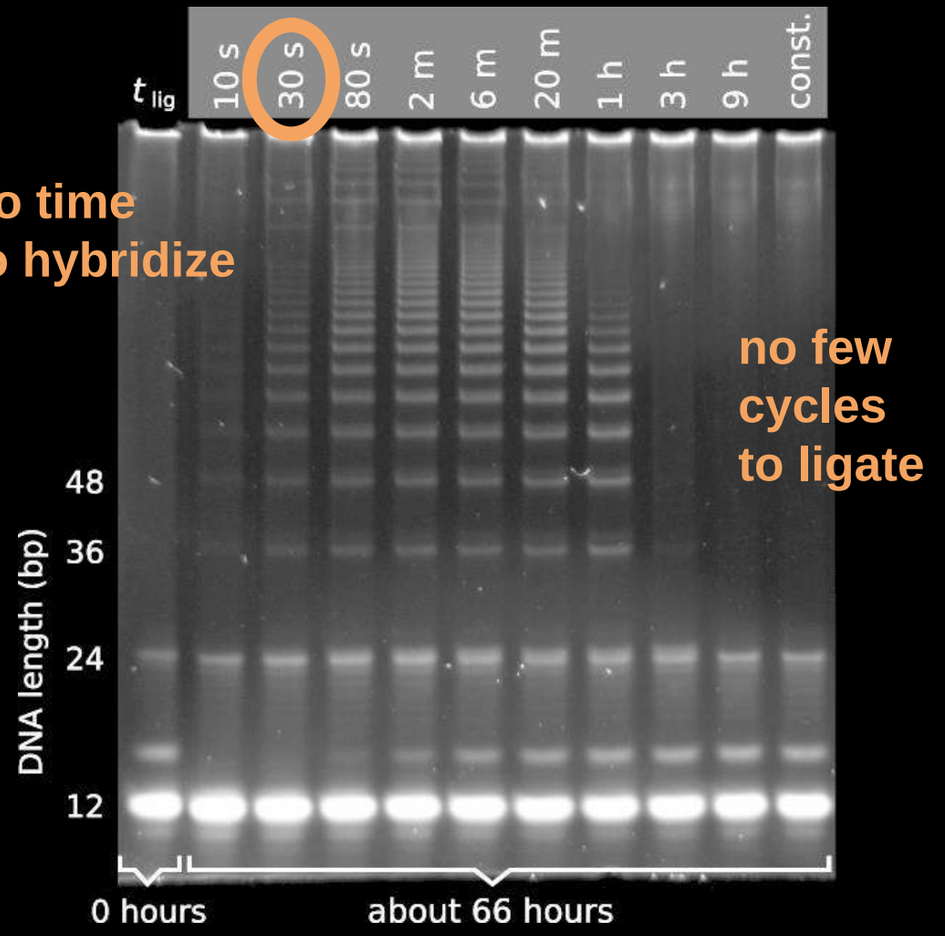
$$t = \frac{k_{on}^{hyb}}{10\,\mu M} 4^{6} = 410\,s$$

# Replication dynamics in sequence space

From Random to Non-random Sequences




12mer random




**no time to hybridize**

**no few cycles to ligate**

**Braun et.al.**
**PNAS** 2021

## Emergence of Life in the Lab

1. Biological History
2. Chemical Selection
3. Sequence Selection
4. Geophysical Space
5. Autonomous Evolution

# Early Earth and Exoplanets

4.5 Ga



Moon forming impact

3.8 Ga



Water world
with some volcanic islands

# Temperature difference across volcanic rock pores



# Temperature difference across volcanic rock pores






National Geographic

# Temperature difference across volcanic rock pores





Iceland, old eruption site

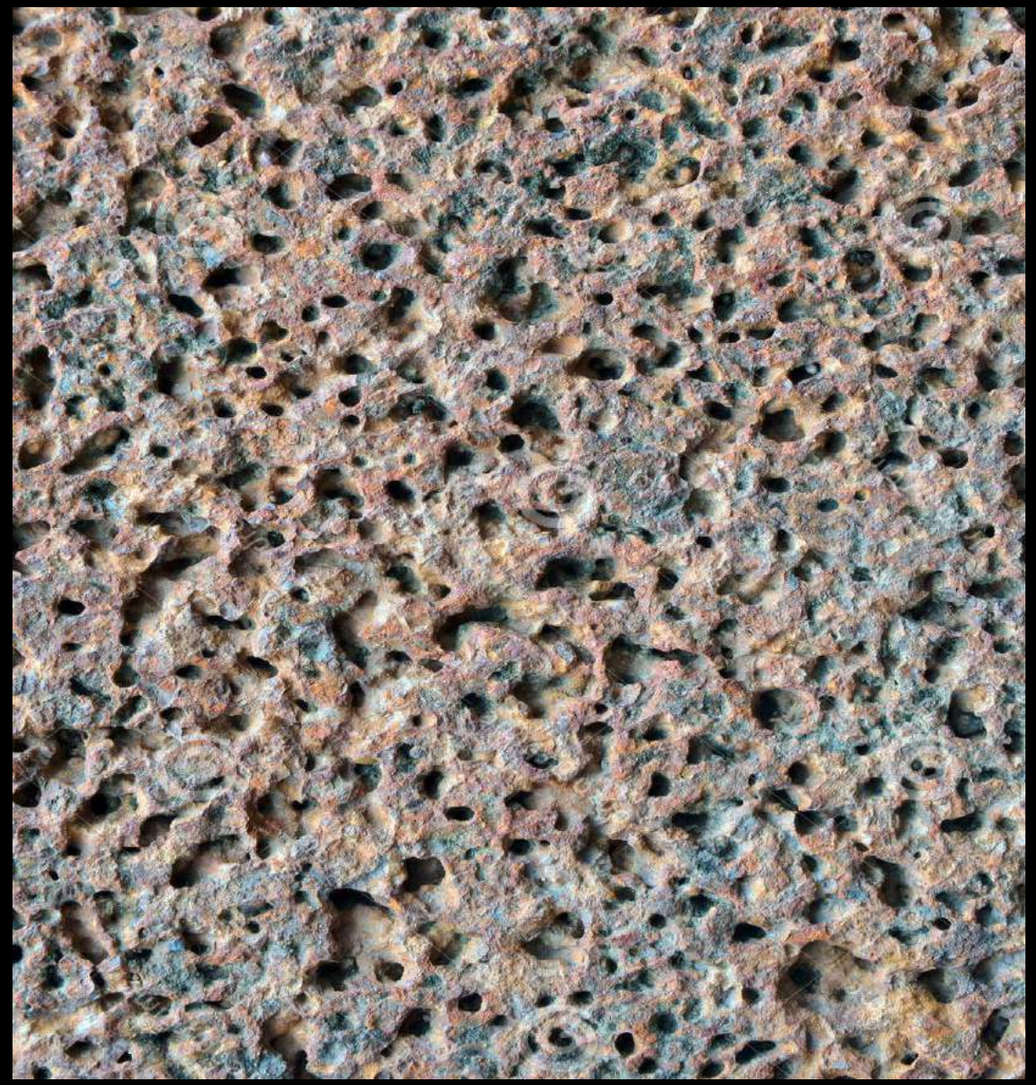
# Temperature difference across volcanic rock pores



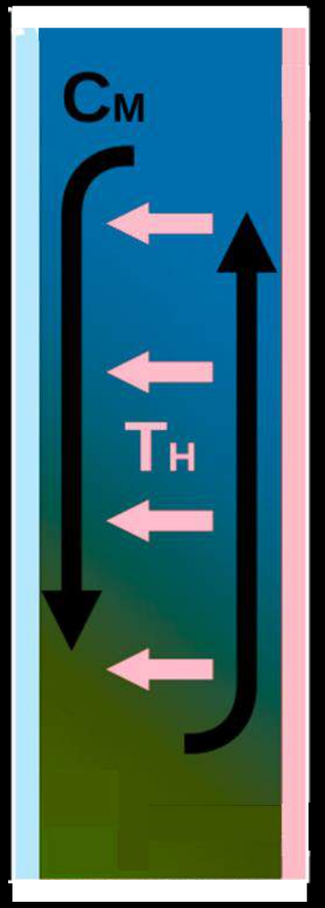


Iceland, old eruption site

# Temperature difference across volcanic rock pores



Cold ← Warm







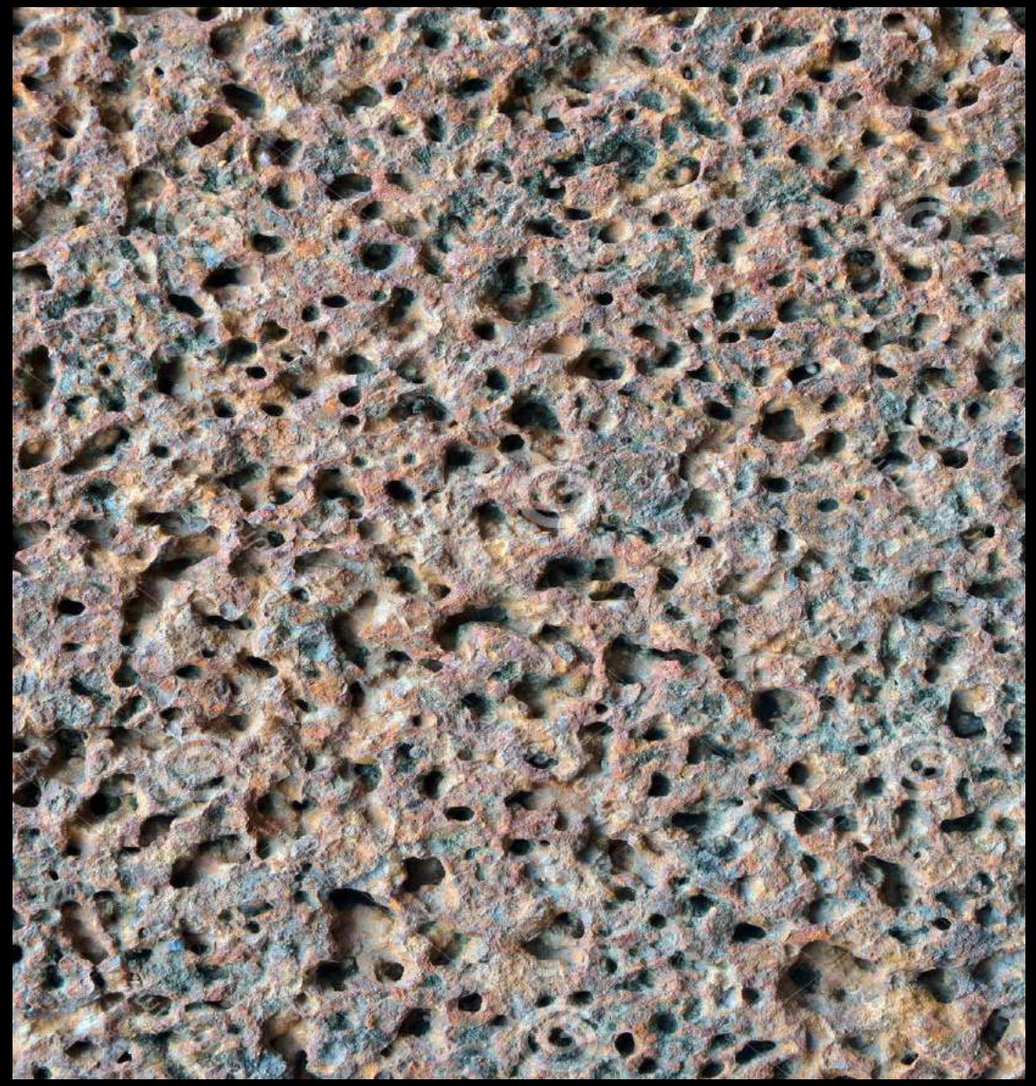

"Rock crack"

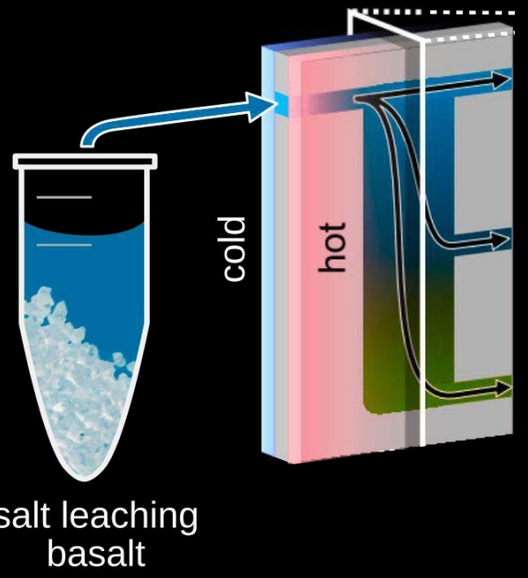
20mer DNA

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

nano TEMPER

# Temperature difference across volcanic rock pores





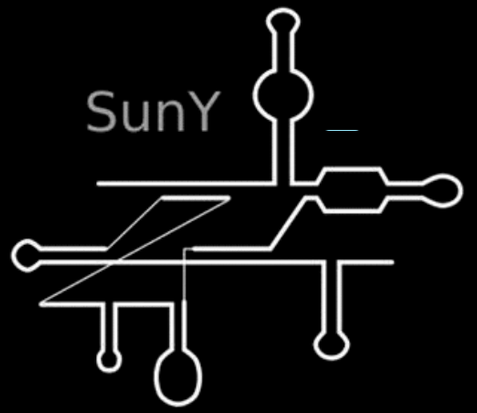

Prebiotic
HPLC



Christof
Mast



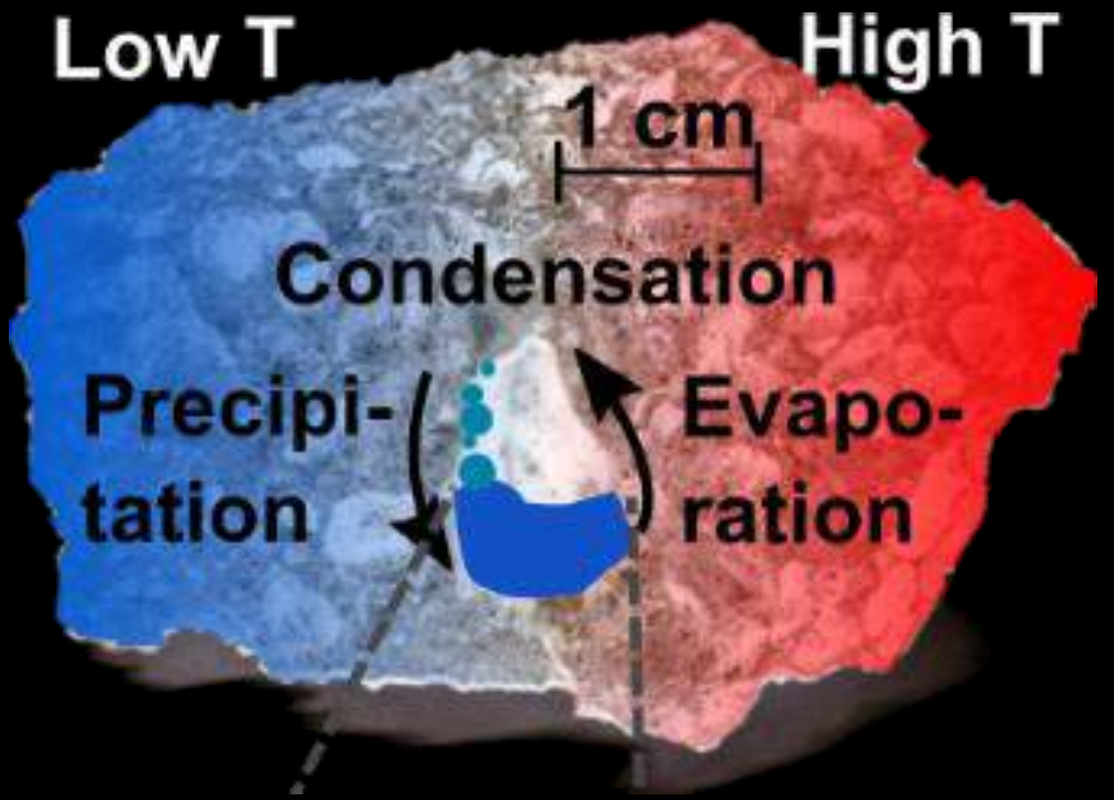
Thomas
Matreux




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

# Heated air-water interface

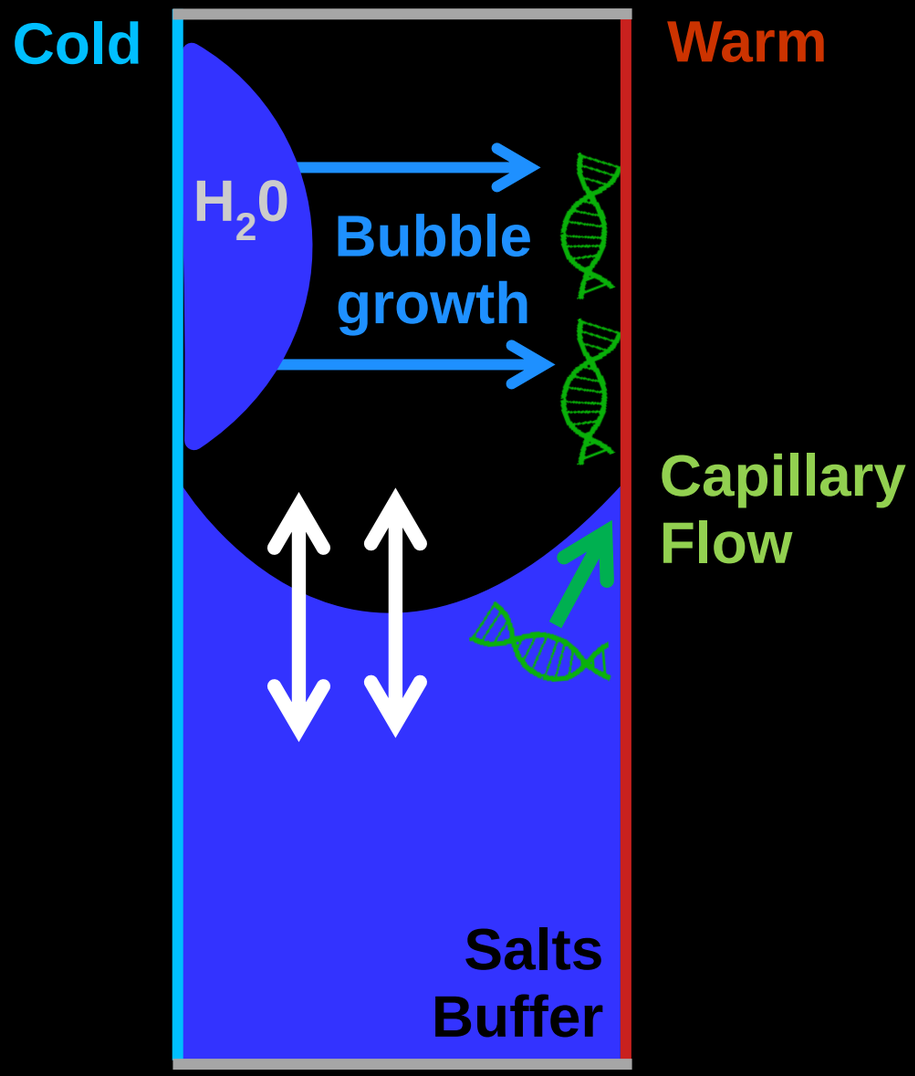


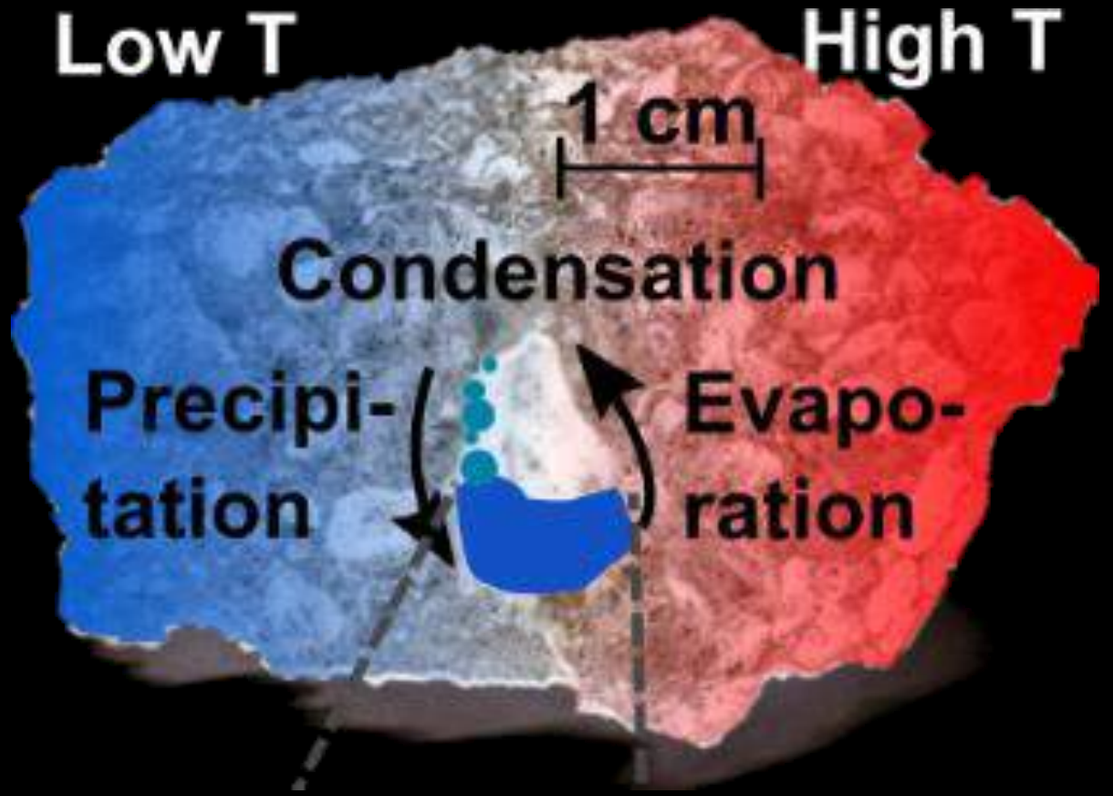



**Nature Chemistry** (2019)
doi.org/10.1038/s41557-019-0299-5

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

# Heated air-water interface

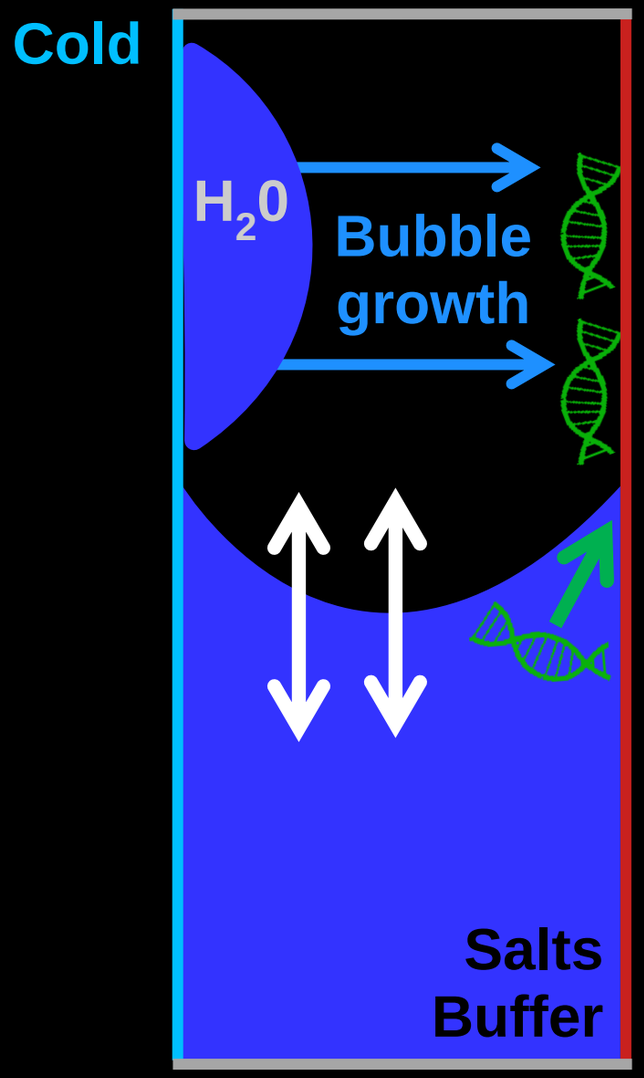


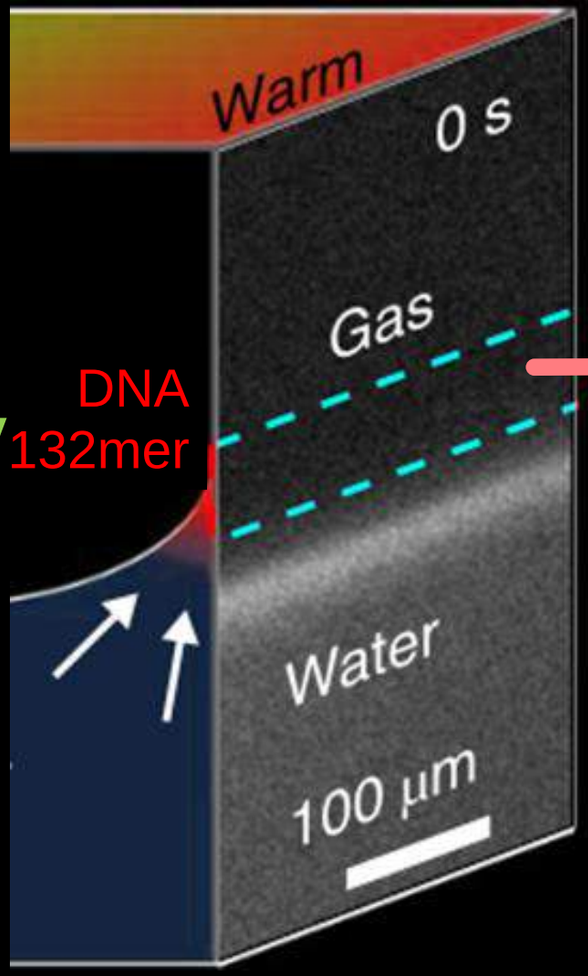




**Nature Chemistry** (2019)
doi.org/10.1038/s41557-019-0299-5

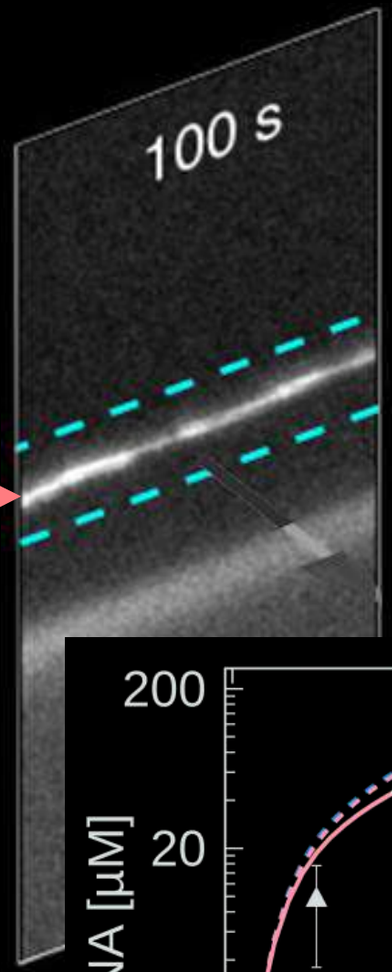
We also work on other scenarios: **fumaroles, humidity cycles, dry feeding**

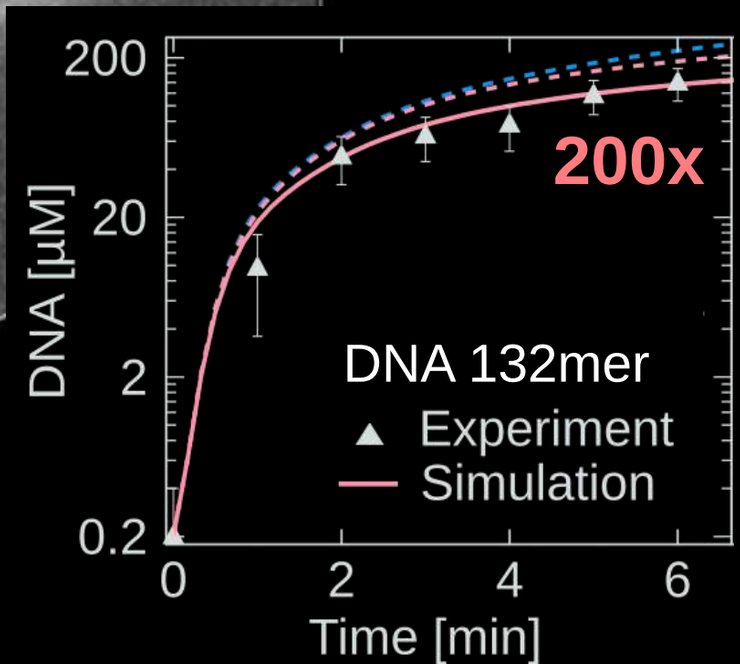
# Heated air-water interface … accumulate by size



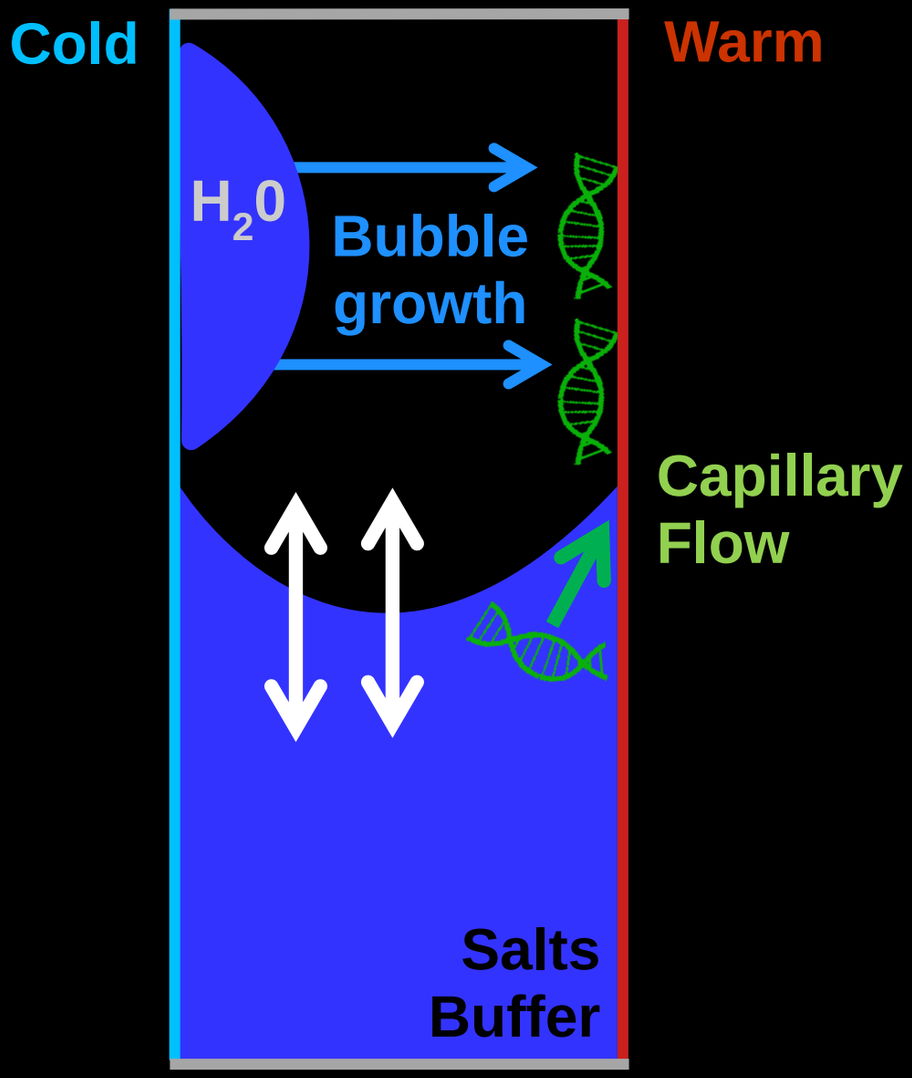



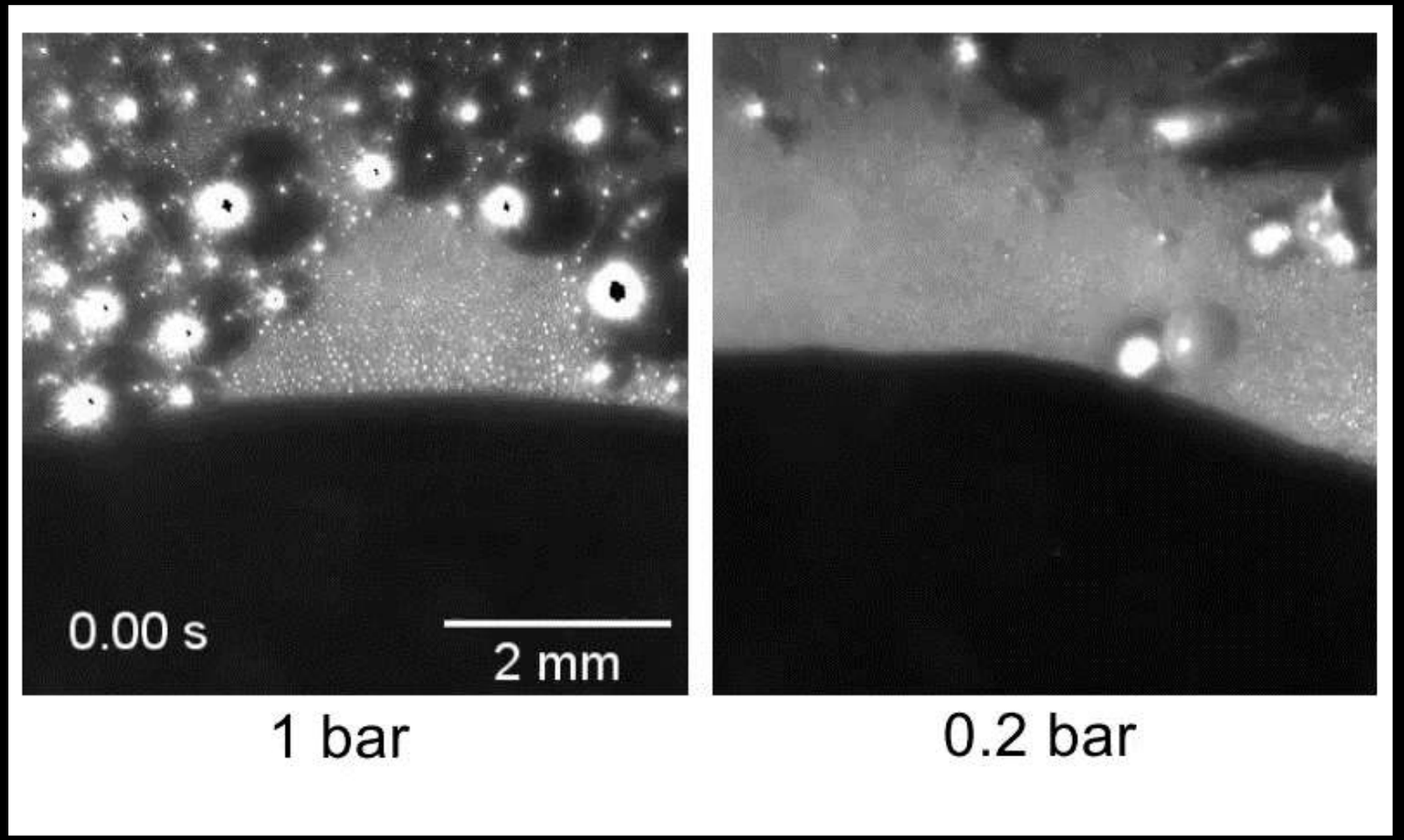

200x



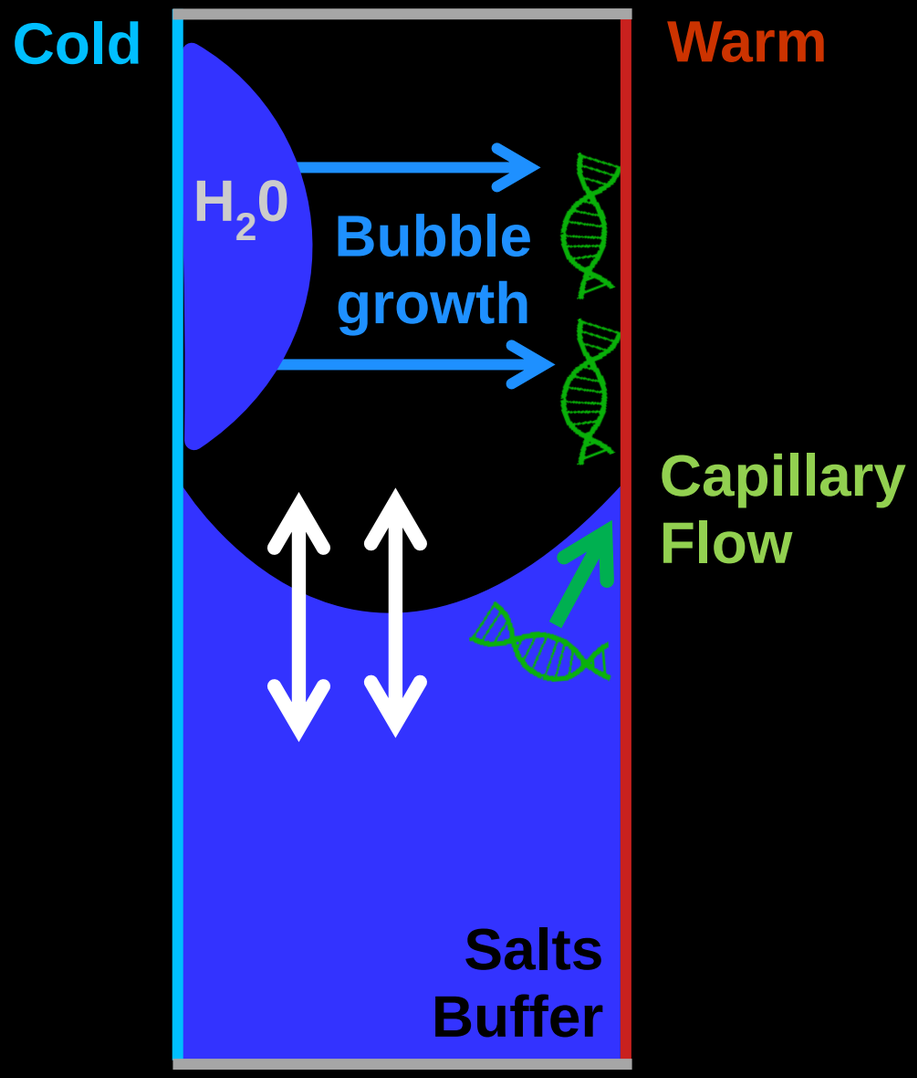



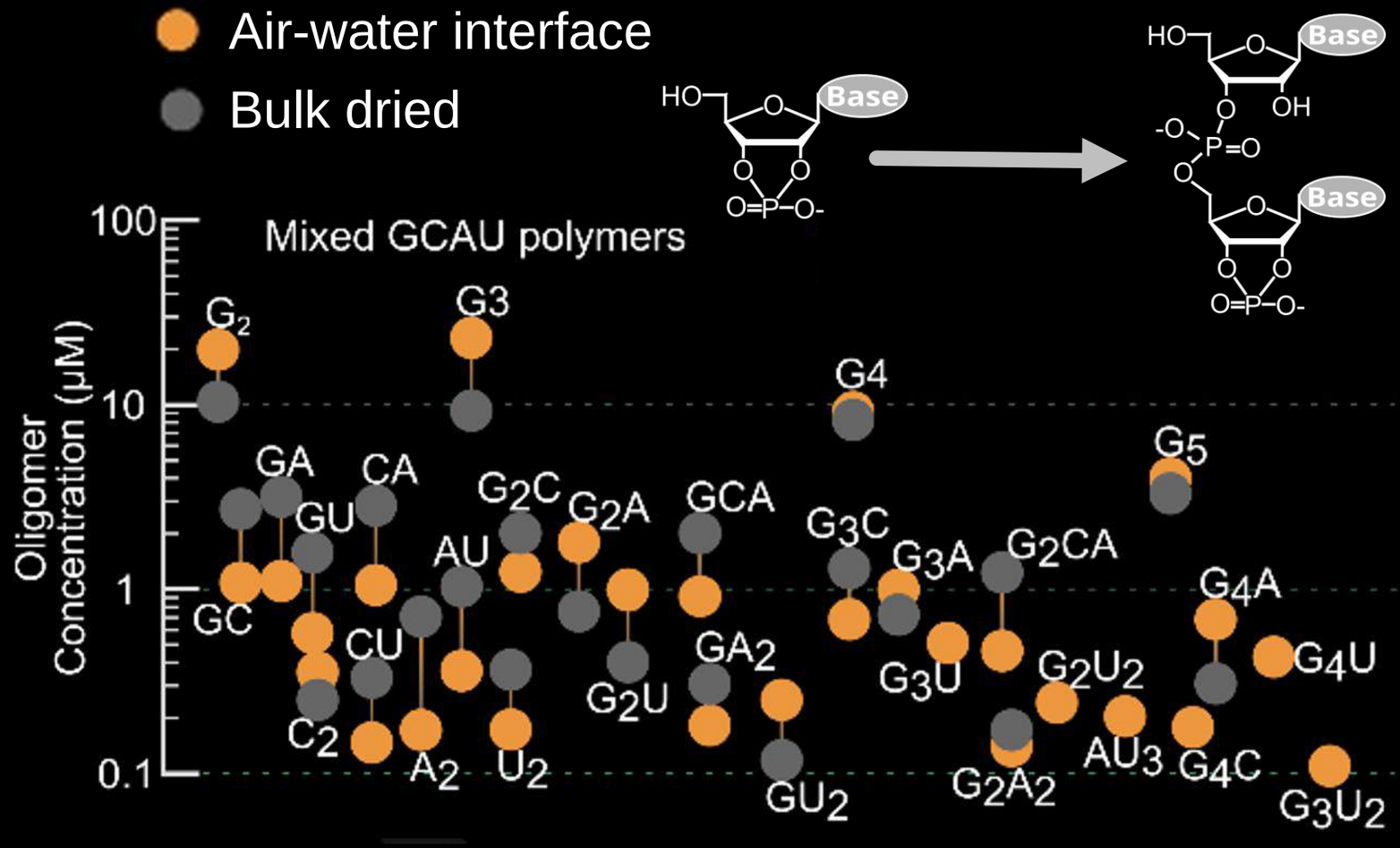
# Heated air-water interface … create pure water



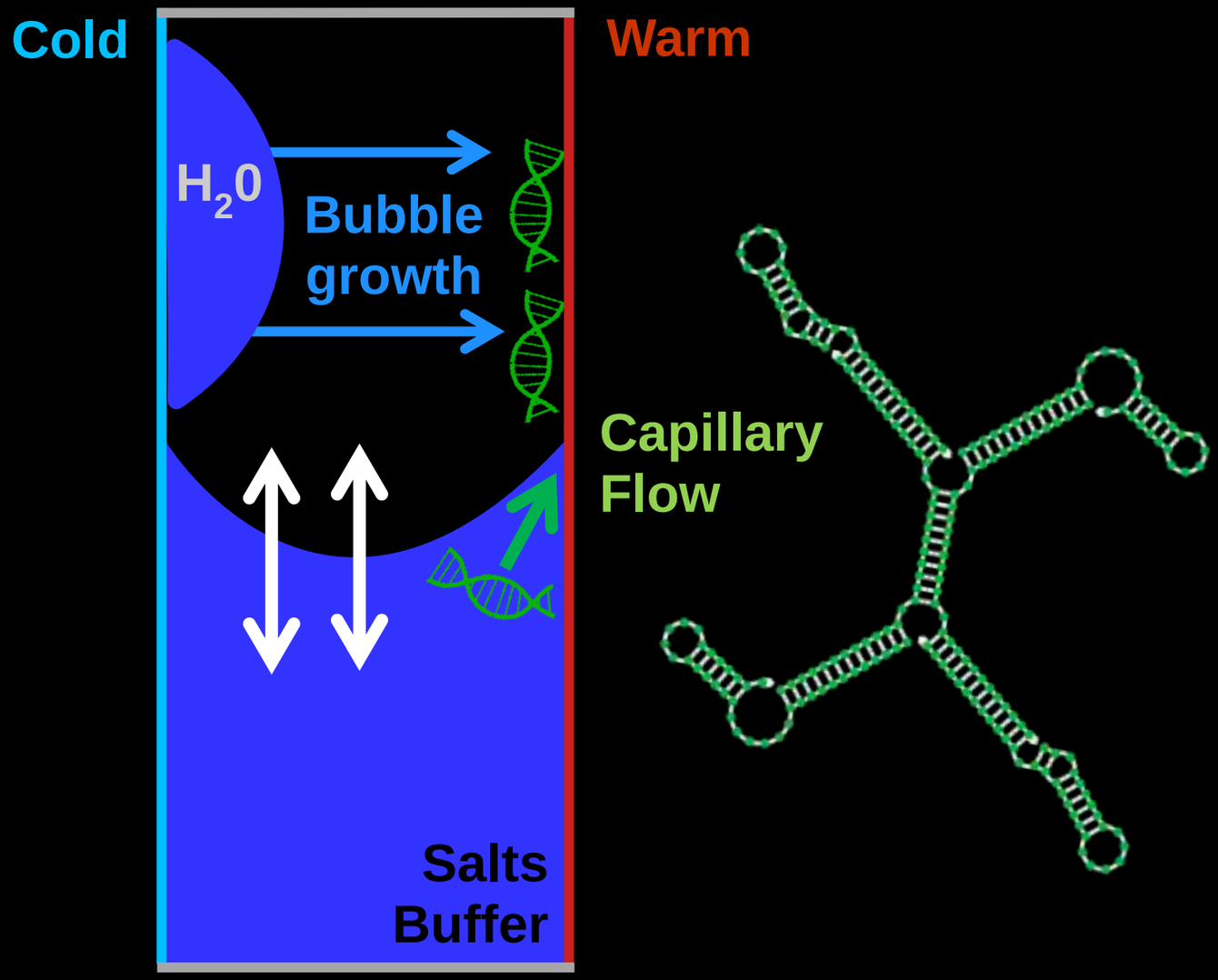
# Heated air-water interface … for polymerization



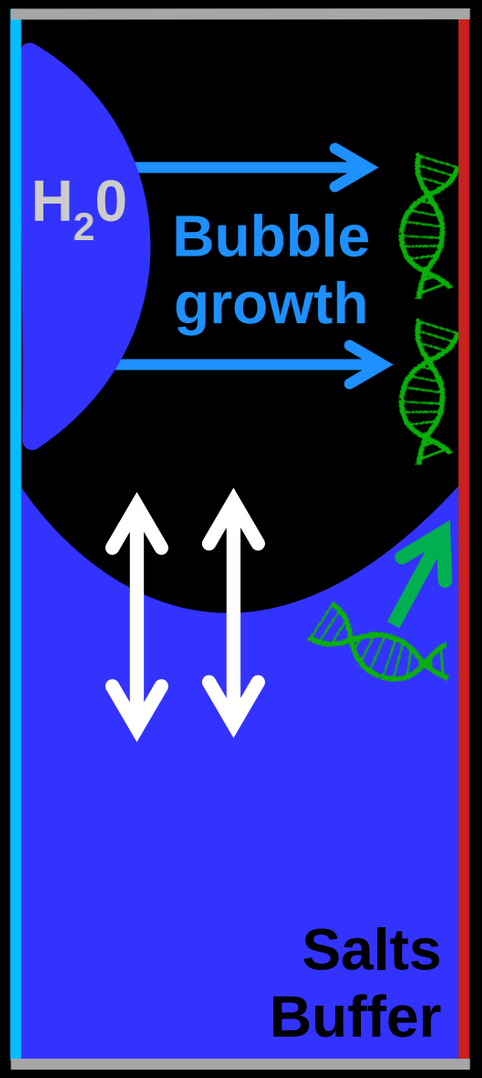

ChemSystemsChem
doi.org/10.1002/syst.202200026 (2022)

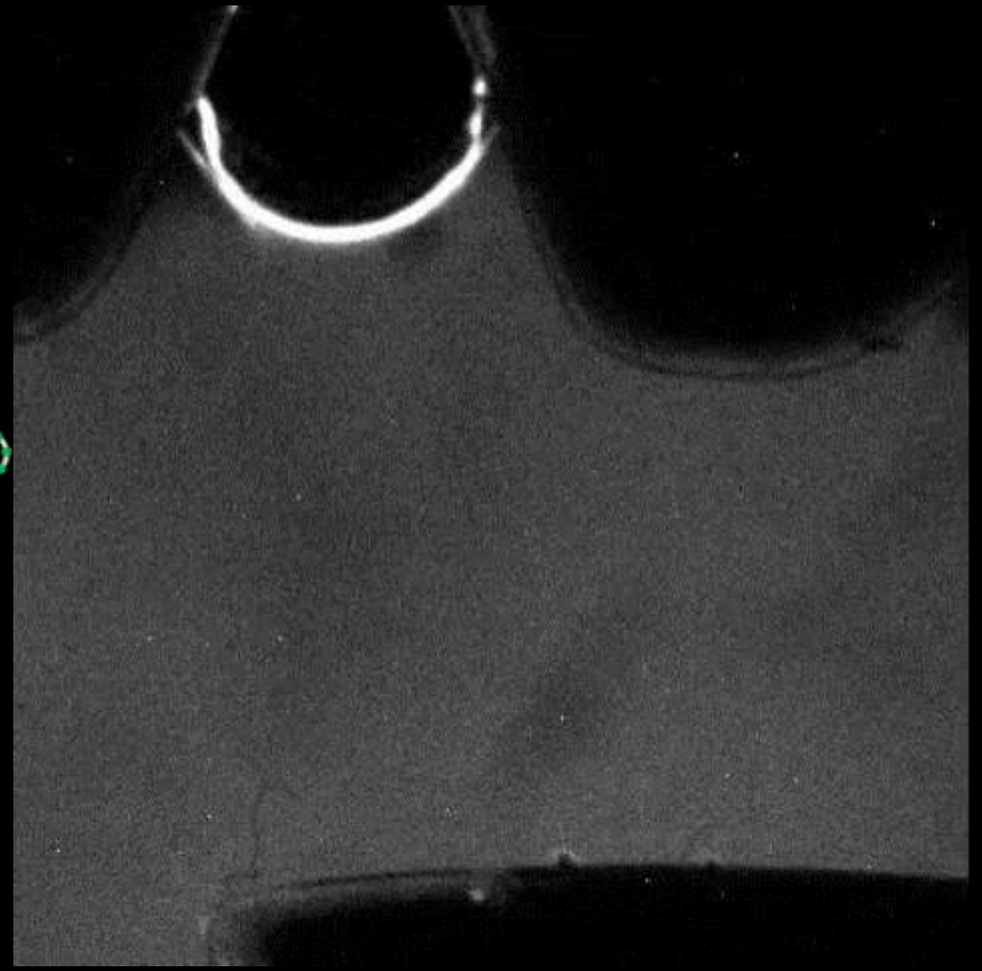
# Heated air-water interface … for RNA gelation




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

# Heated air-water interface … for RNA gelation



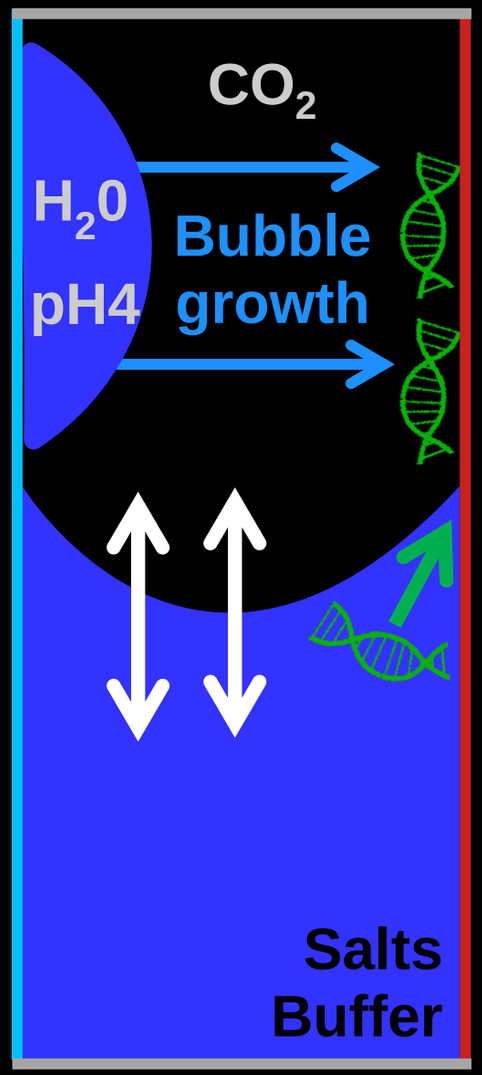



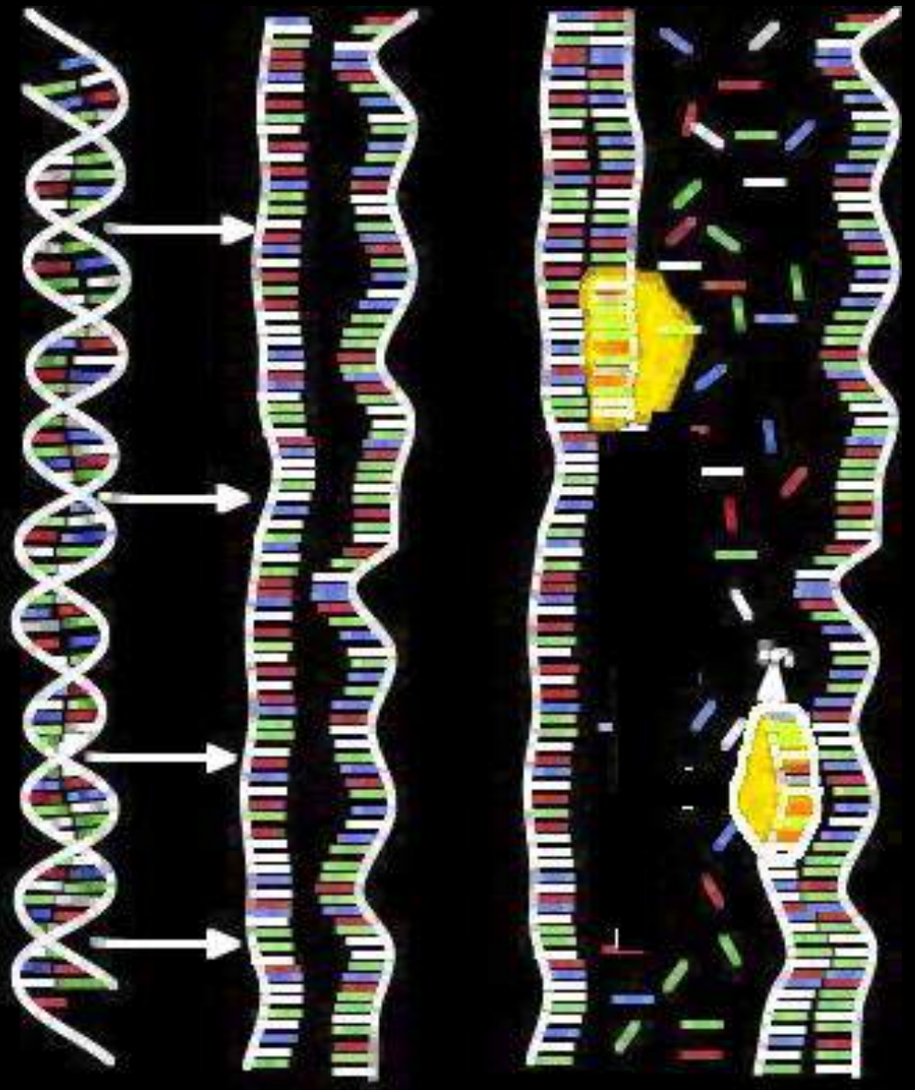

**Nature Chemistry** (2019)
doi.org/10.1038/s41557-019-0299-5

## Emergence of Life in the Lab

1. Biological History
2. Chemical Selection
3. Sequence Selection
4. Geophysical Space
5. Autonomous Evolution

# Replication at air-water interface





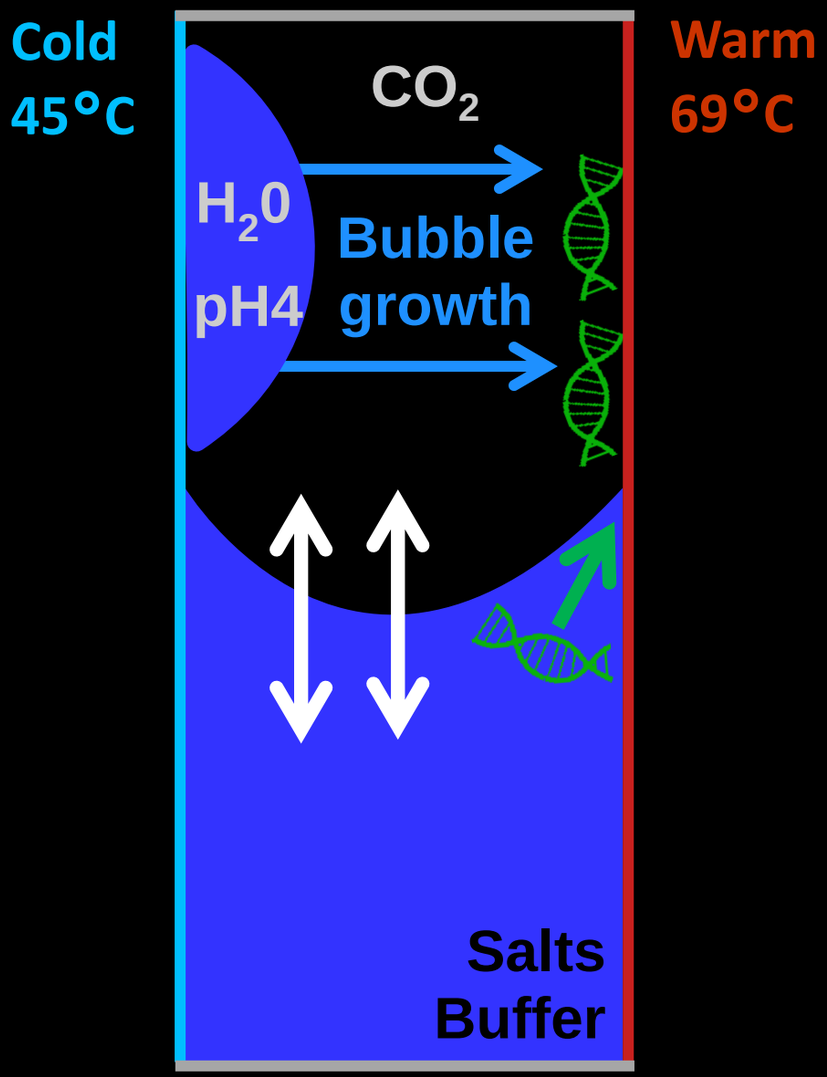
Alan
Ianeselli

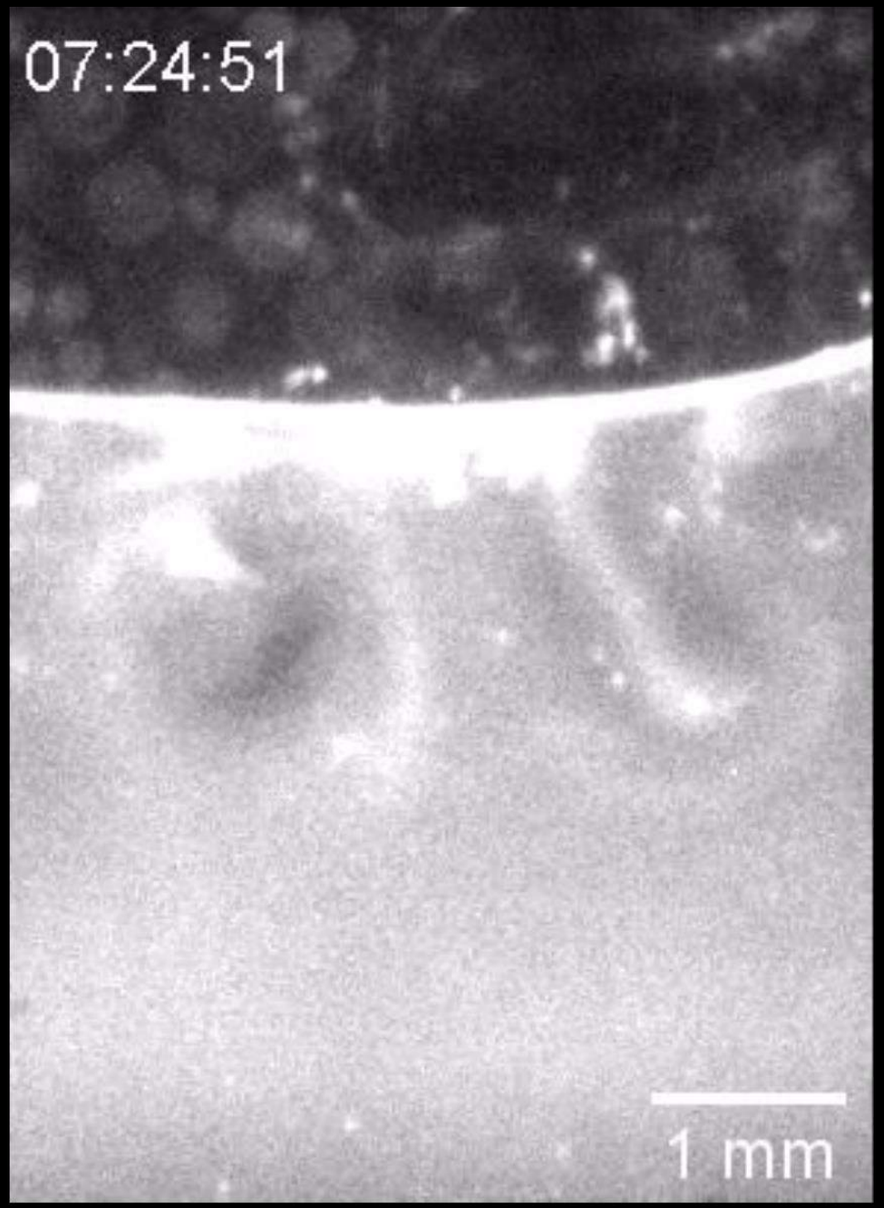
T gradient:
45 °C - 69 °C

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)

# Replication at air-water interface





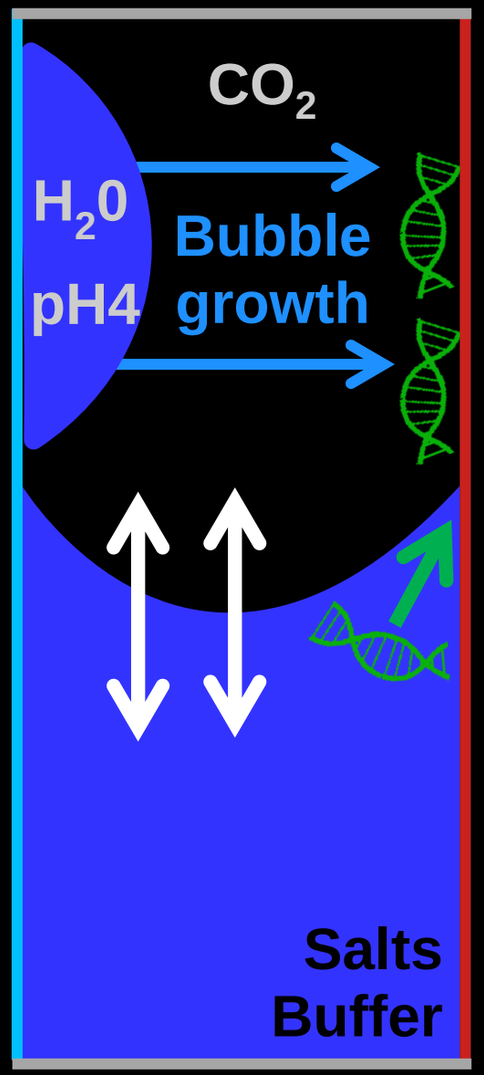

Alan
Ianeselli

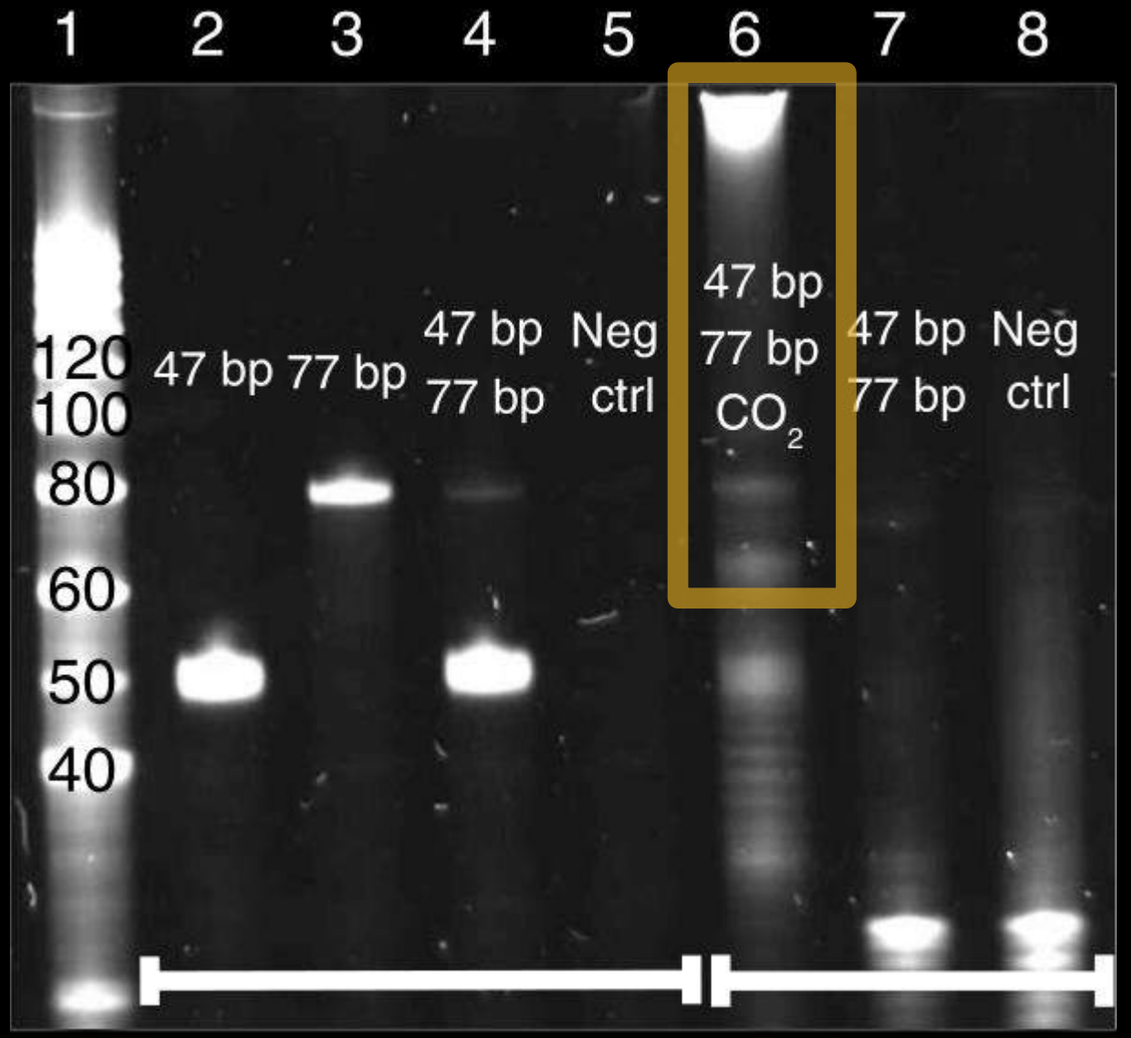
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Ianeselli, Nature Physics (2022)

# Replication at air-water interface





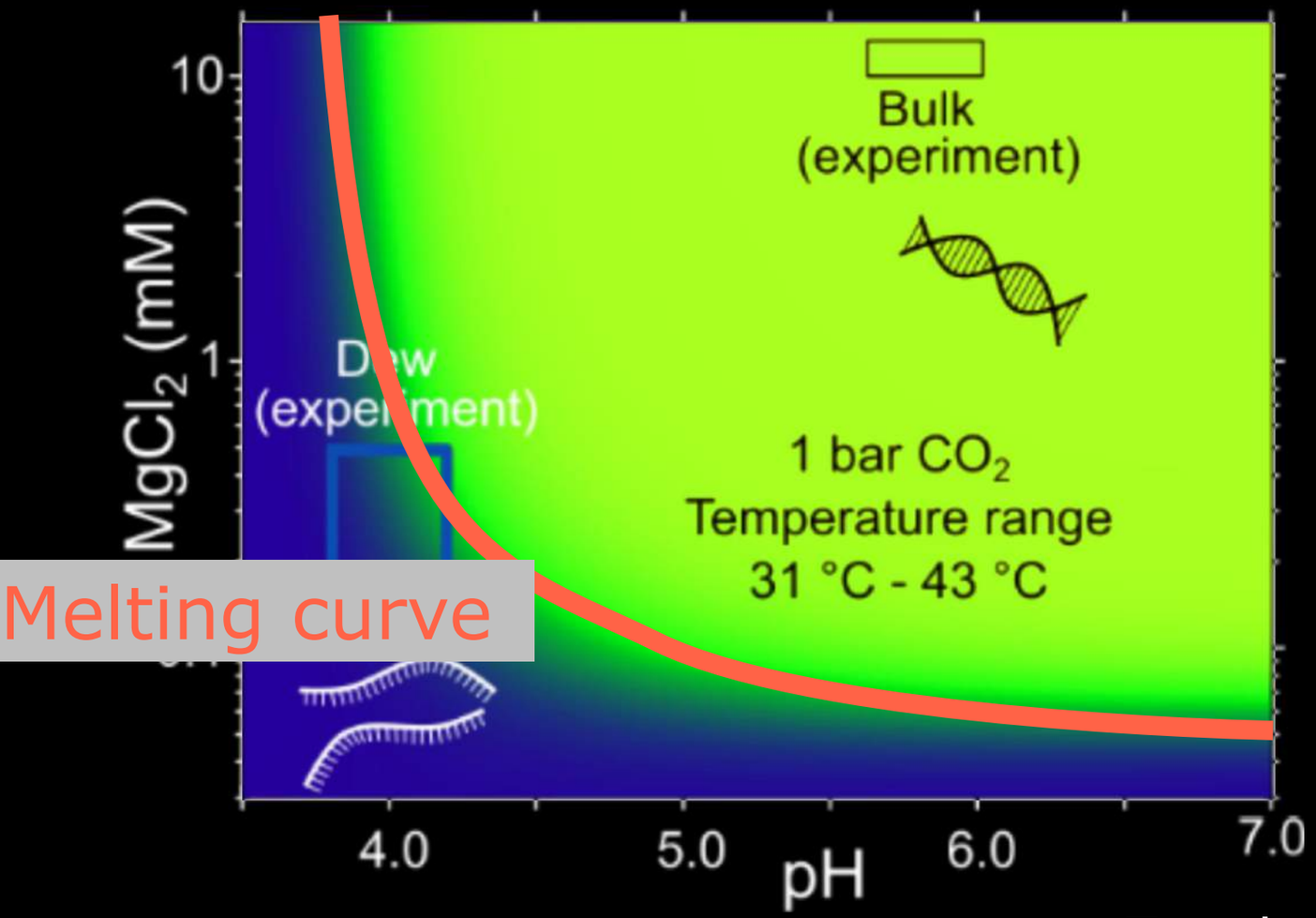

Alan
Ianeselli

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Taq polymerase
1.5 mM $MgCl_2$
0.1% BSA
2x SYBR Green

Ianeselli, Nature Physics (2022)

# Fast sequence evolution at interface




Melting curve



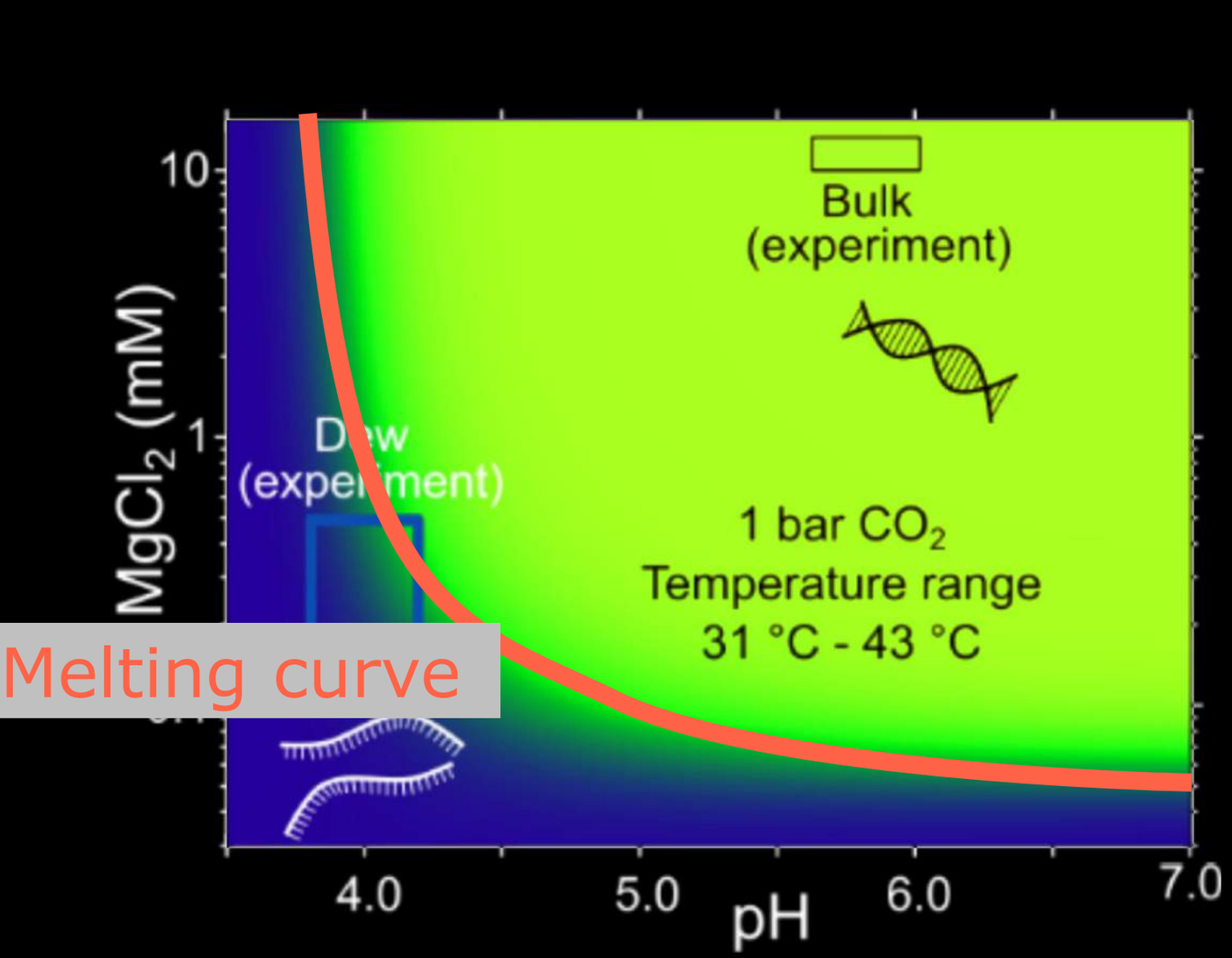

Selection for AT

Selection for longer sequences

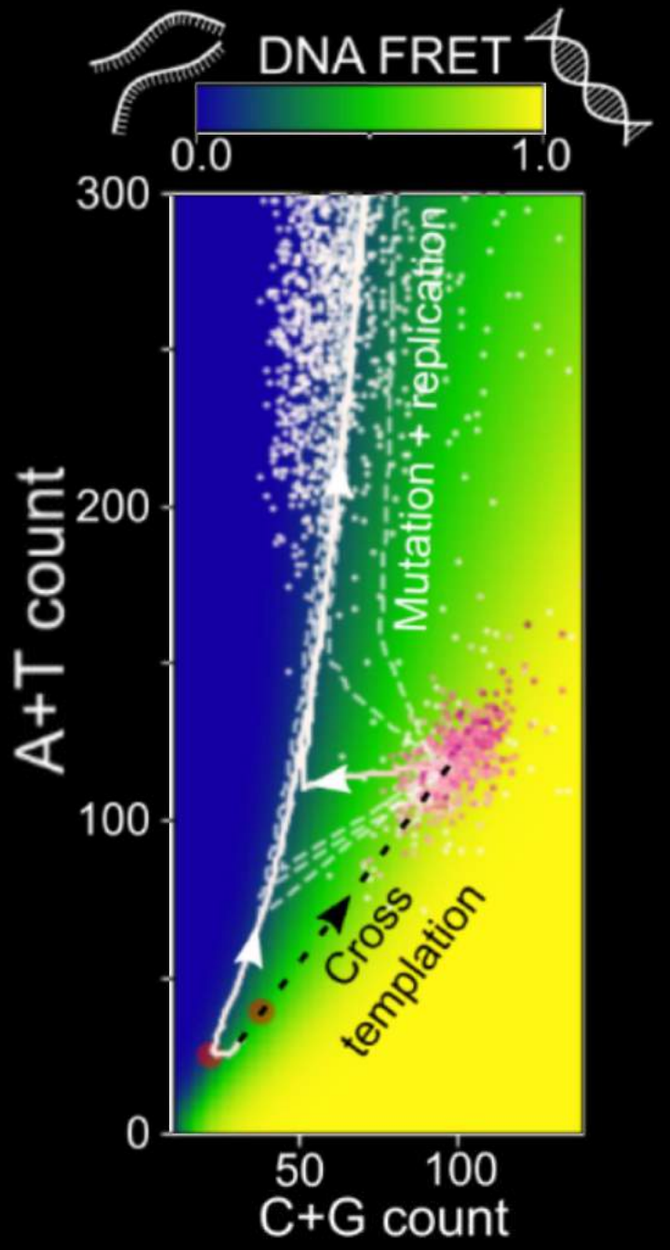
No tyranny of the shortest

Ianeselli, Nature Physics (2022)

# Fast sequence evolution at interface




Melting curve



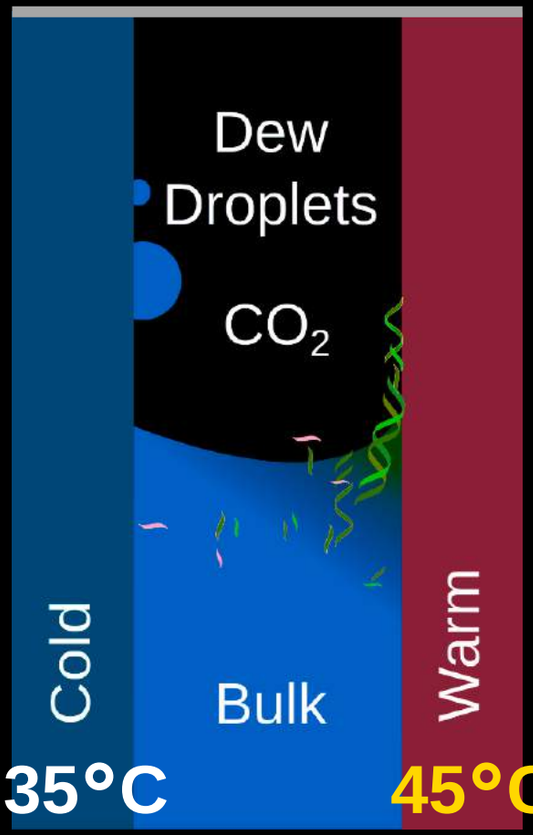

Selection for AT

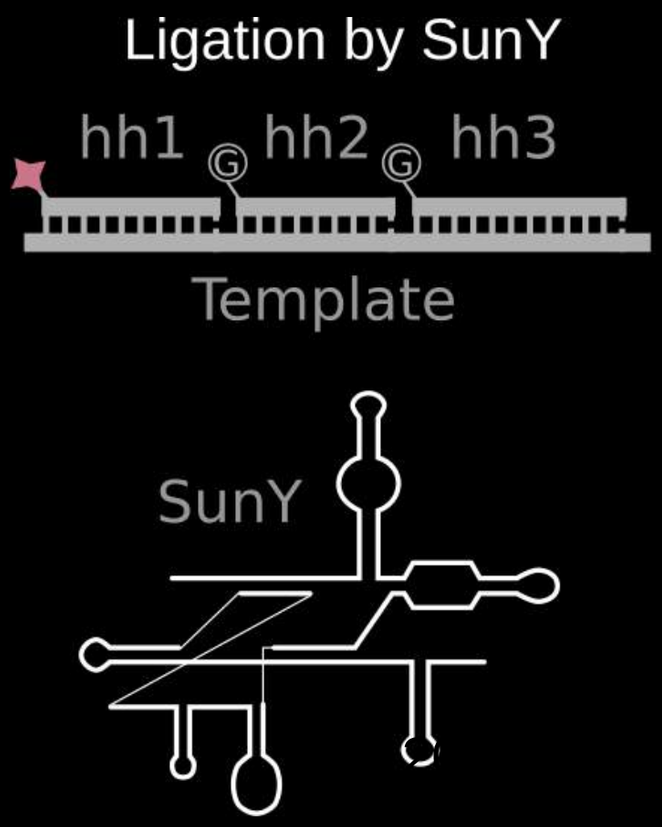
Selection for strand separation

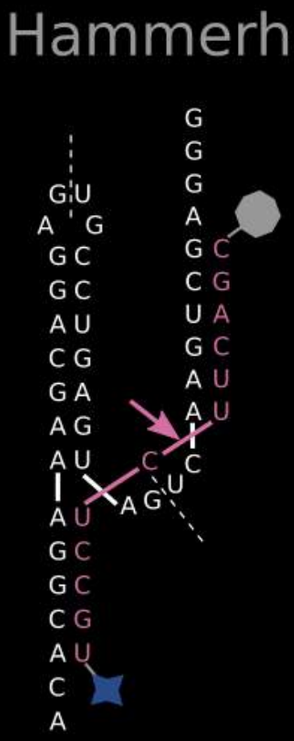
Selection for longer sequences

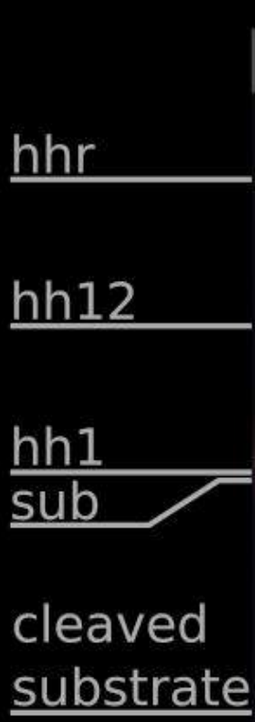
Ianeselli, Nature Physics (2022)

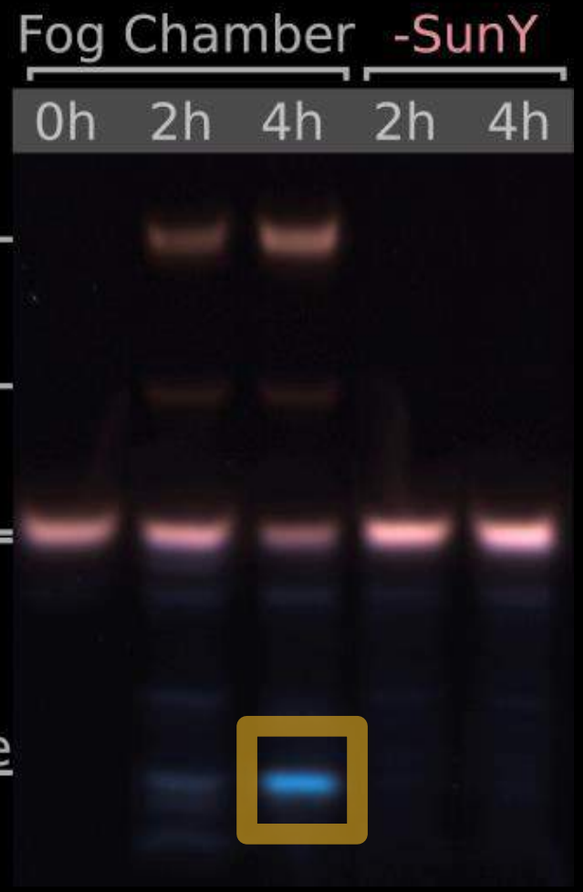
# Heated air bubbles … to host RNA ribozymes






With Hannes Mutschler

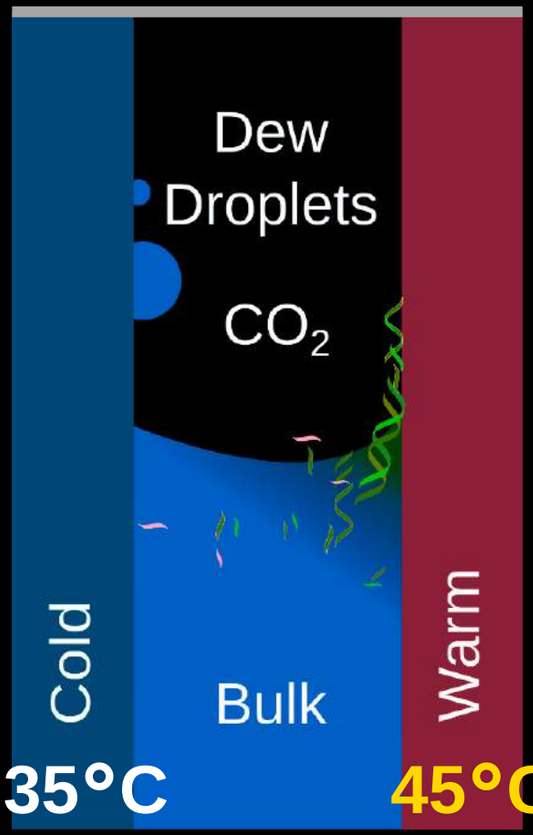


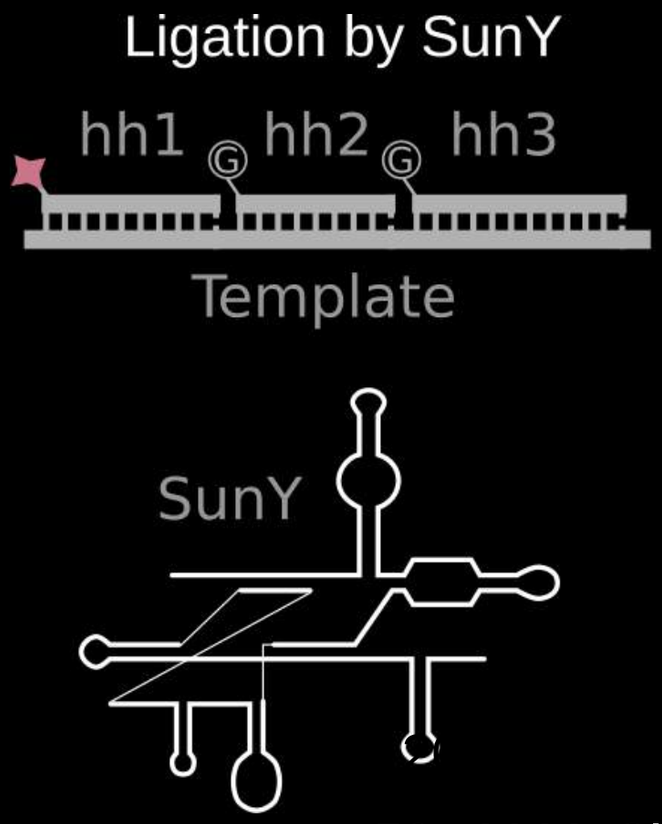
Annalena Salditt

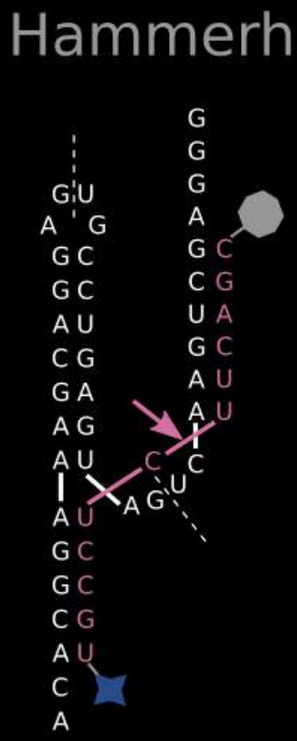
50mM $MgCl_2$
=> **1-5mM** $MgCl_2$

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

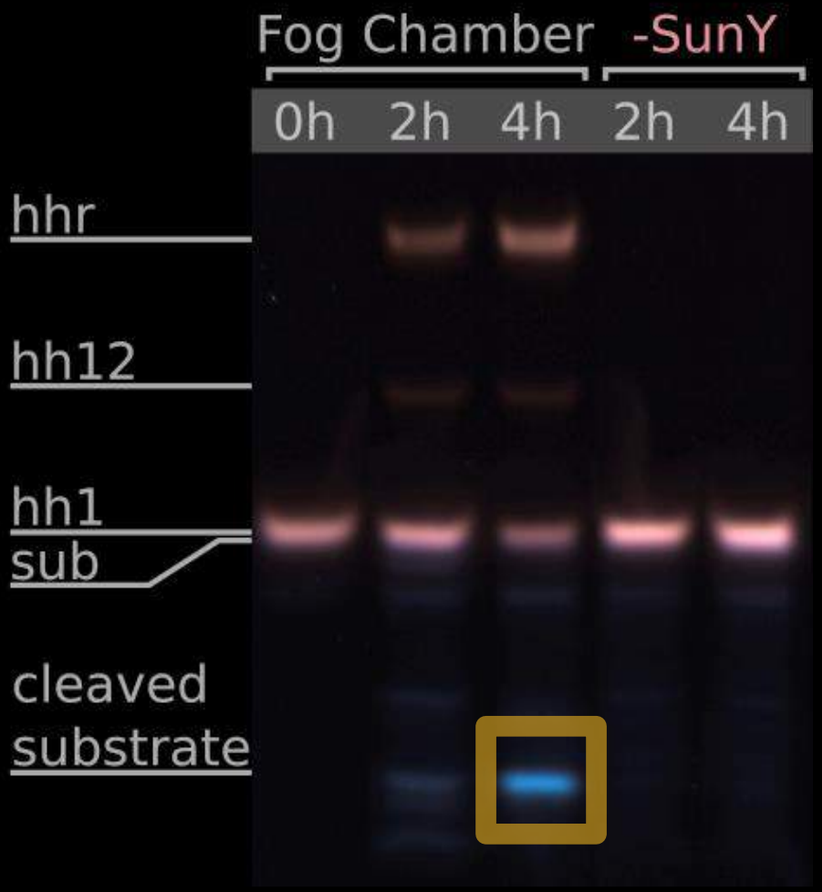
# Heated air bubbles … to host RNA ribozymes




50mM $MgCl_2$
=> **1-5mM** $MgCl_2$





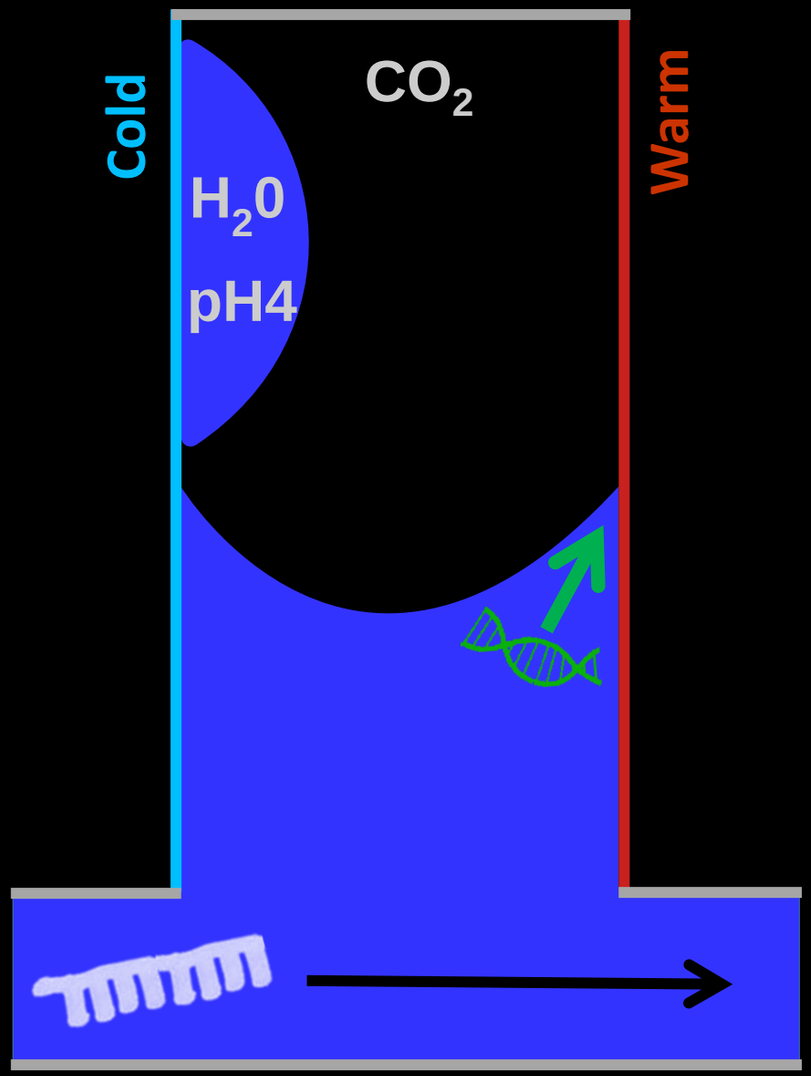

With Hannes Mutschler

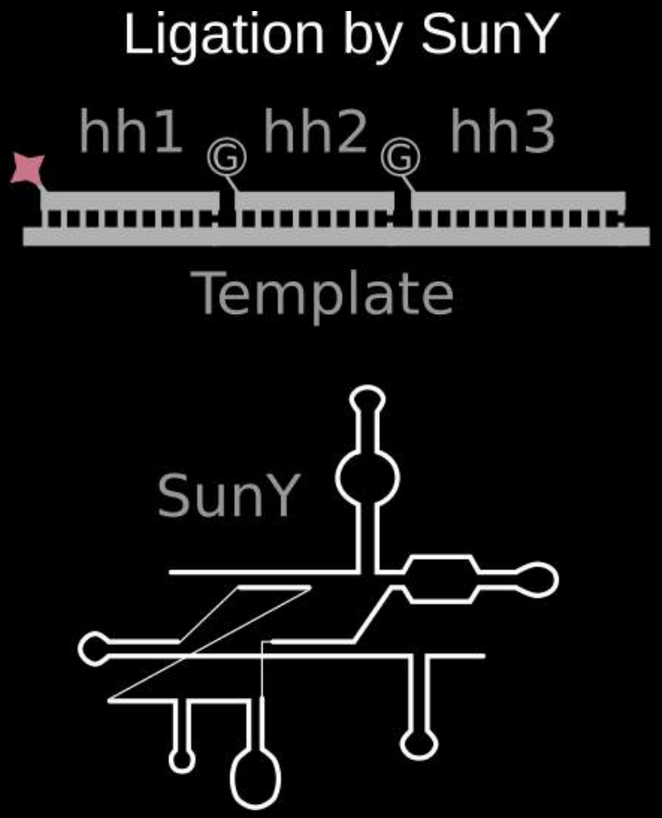


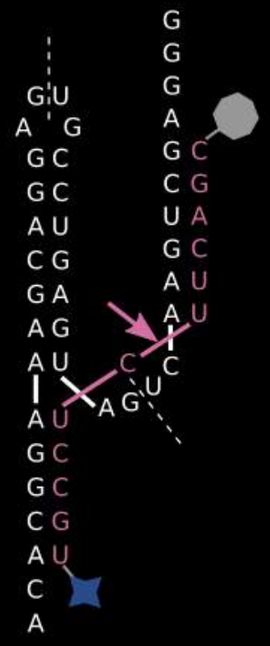
Annalena Salditt

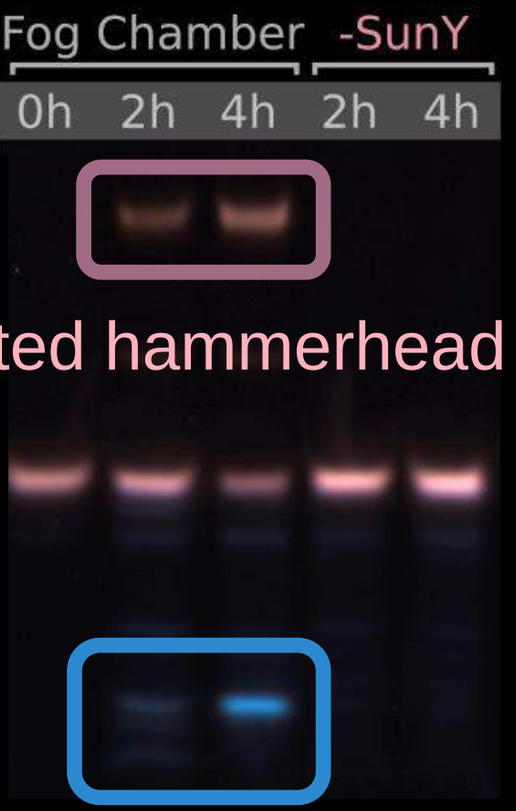
Nature Communications
doi.org/10.1038/s41467-023-37206-4 (2023)

# Heated air bubbles ... a how can Ribozyme





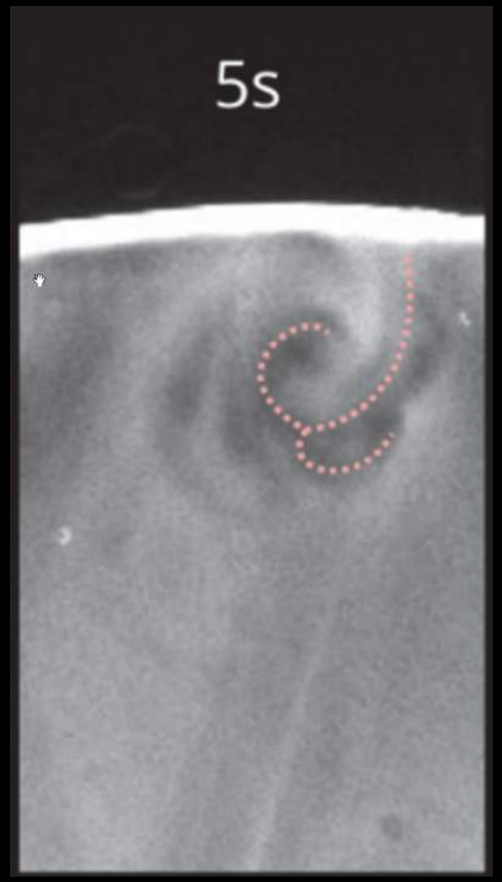

With Hannes Mutschler

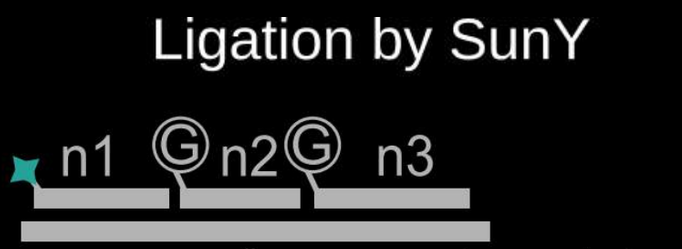


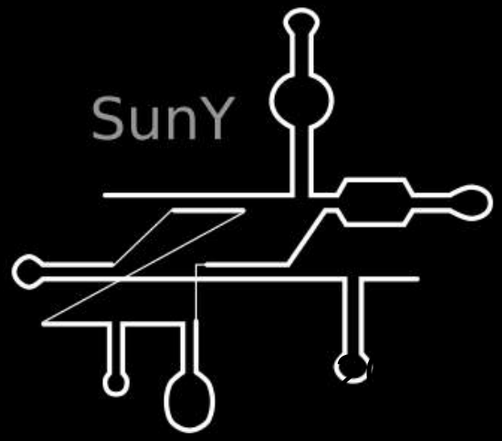
Annalena Salditt

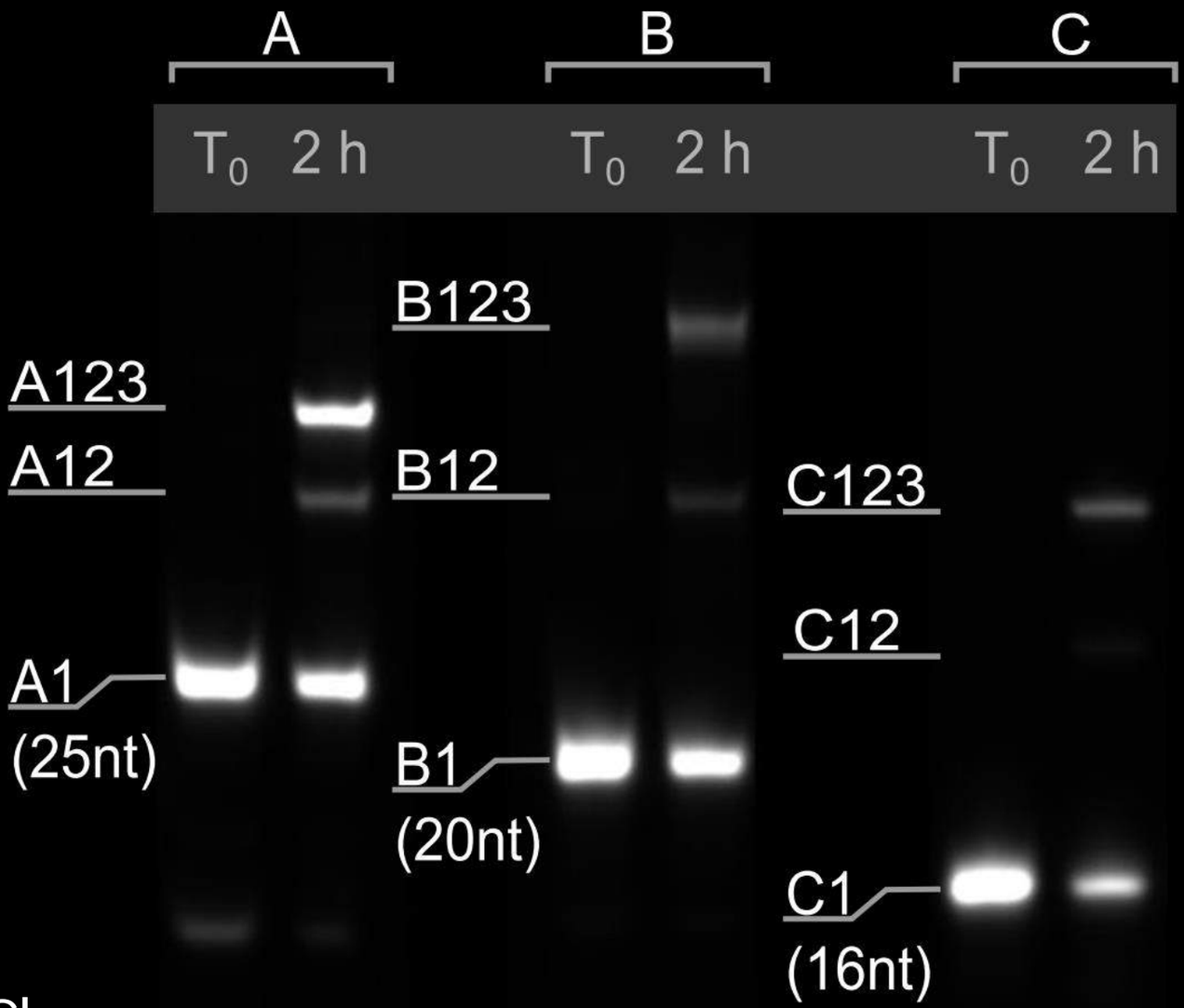
Nature Communications
doi.org/10.1038/s41467-023-37206-4 (2023)

# Heated air bubbles … towards **sunY-replication**






With Hannes Mutschler

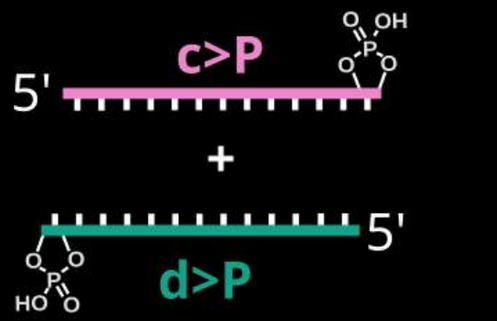


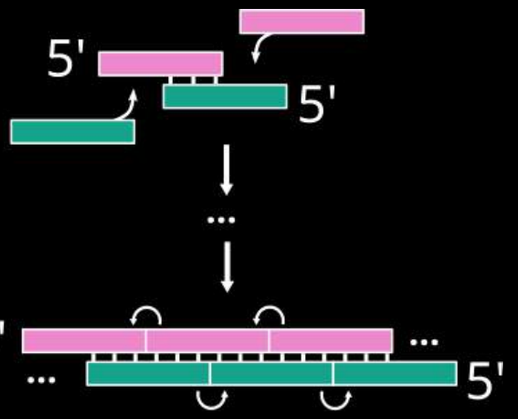
Annalena Salditt

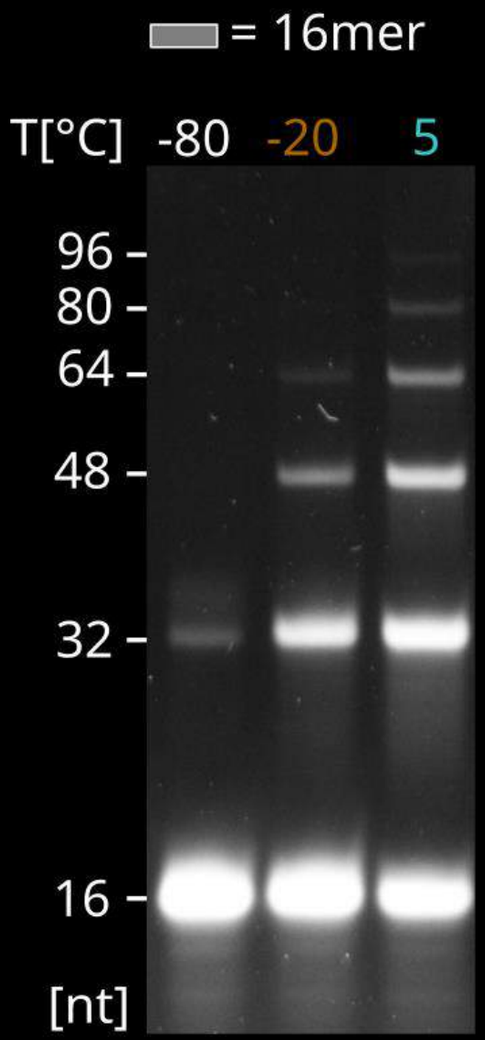
50mM $MgCl_2$
=> **1-5mM** $MgCl_2$

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

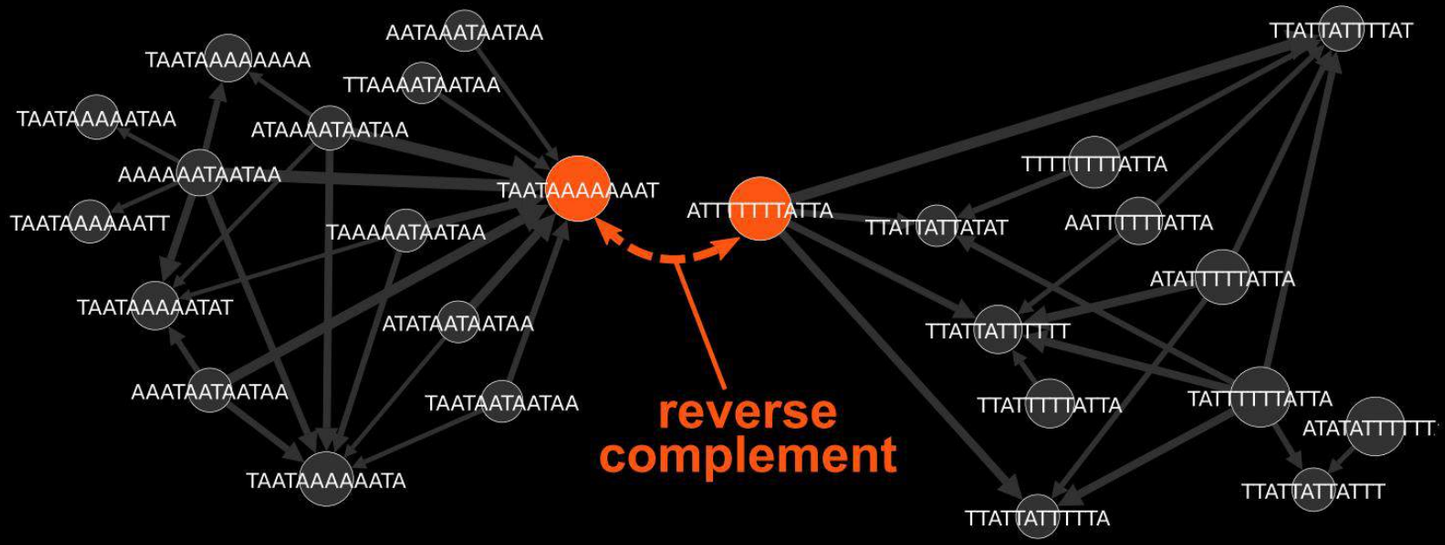
# Splint ligation of RNA at low $Mg^{2+}$ concentration

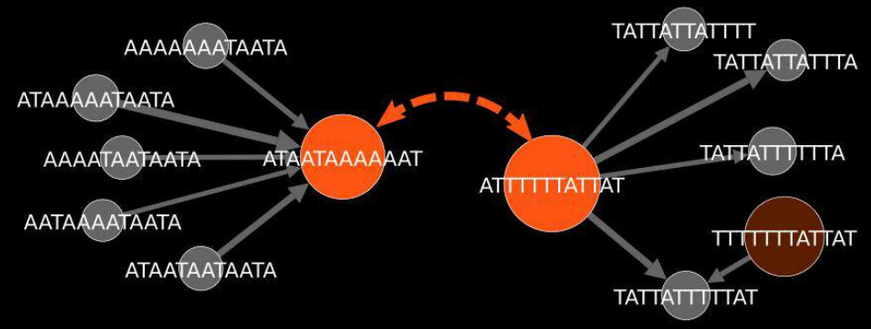



Expect a complementary sequence network

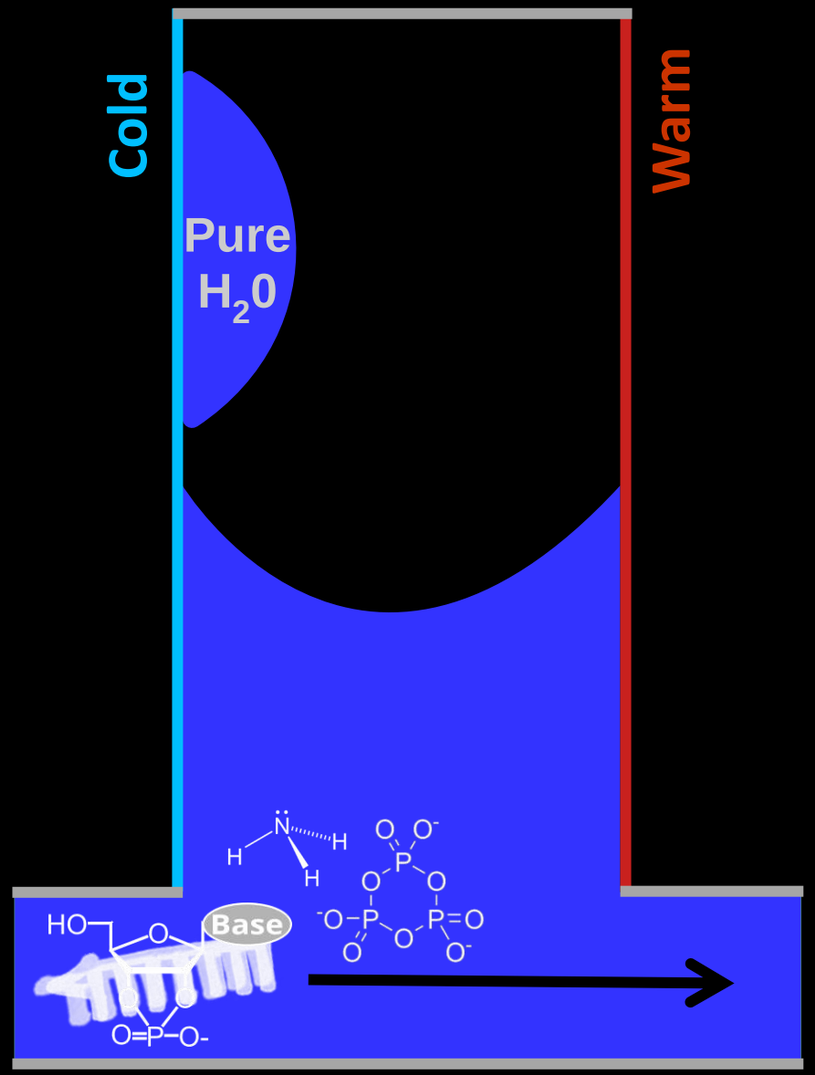


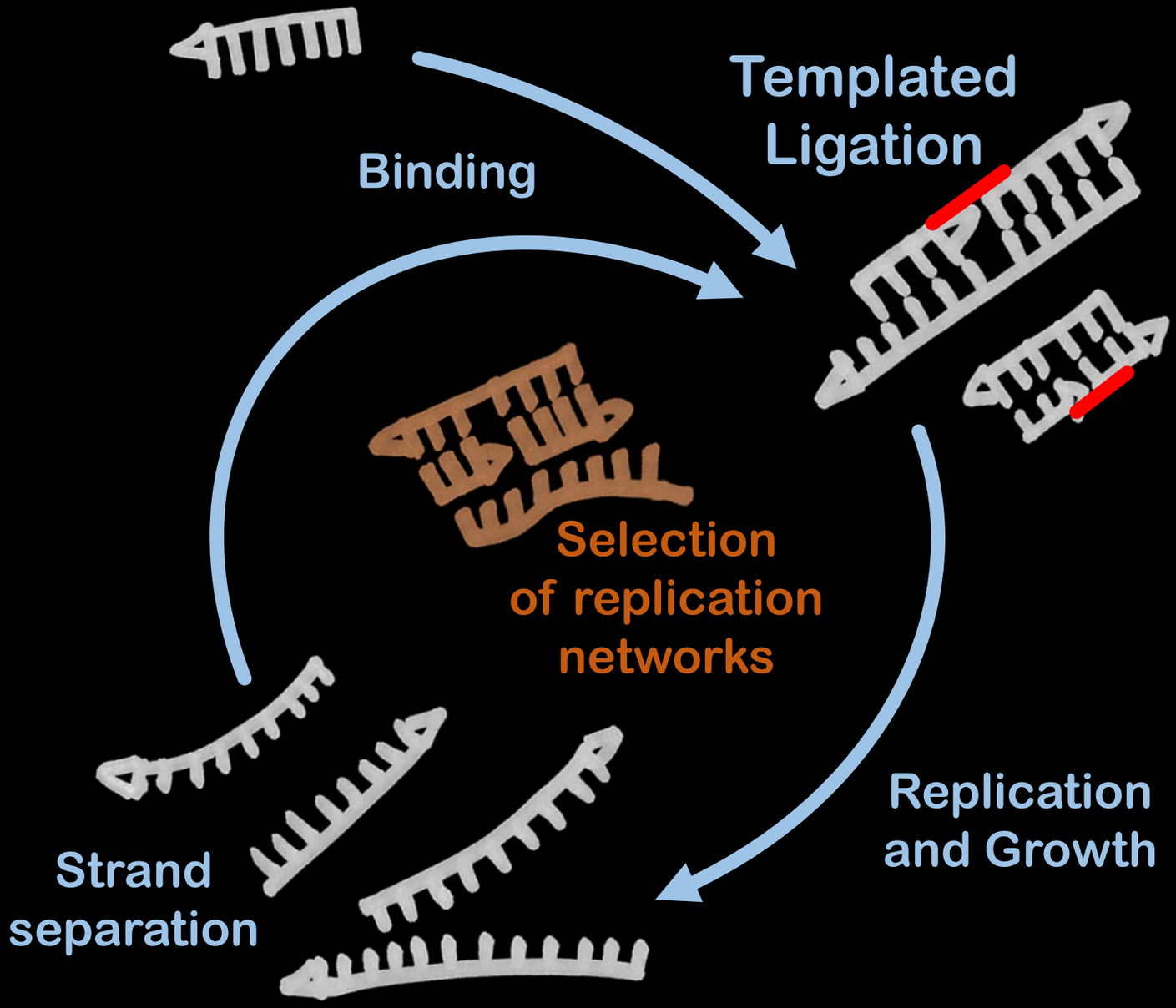




**Braun et.al.**
**PNAS** 2021

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

# Evolution from short Oligomers

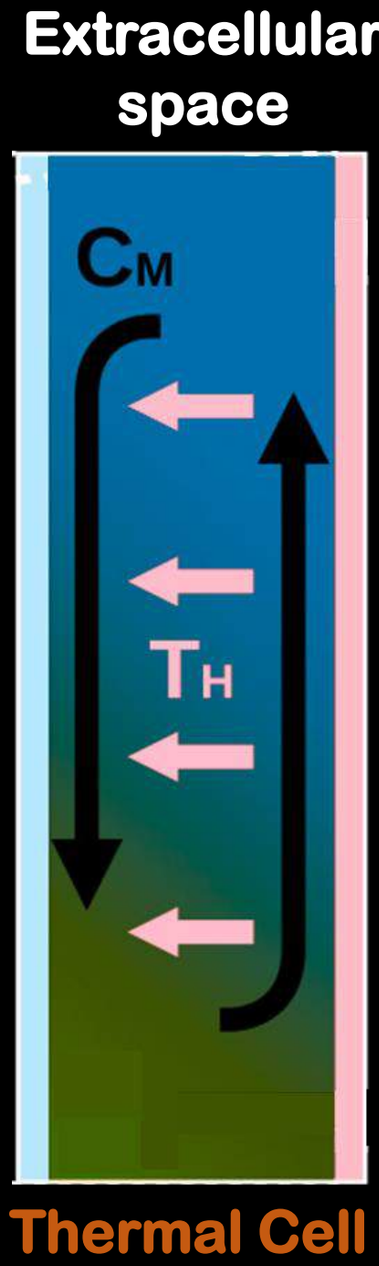


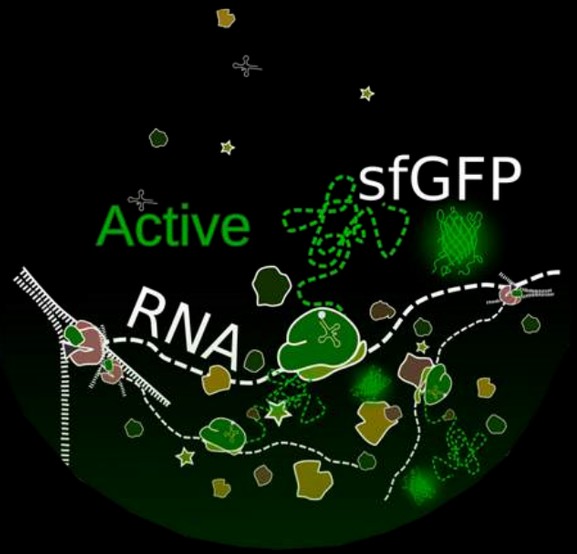
## Emergence of Life in the Lab

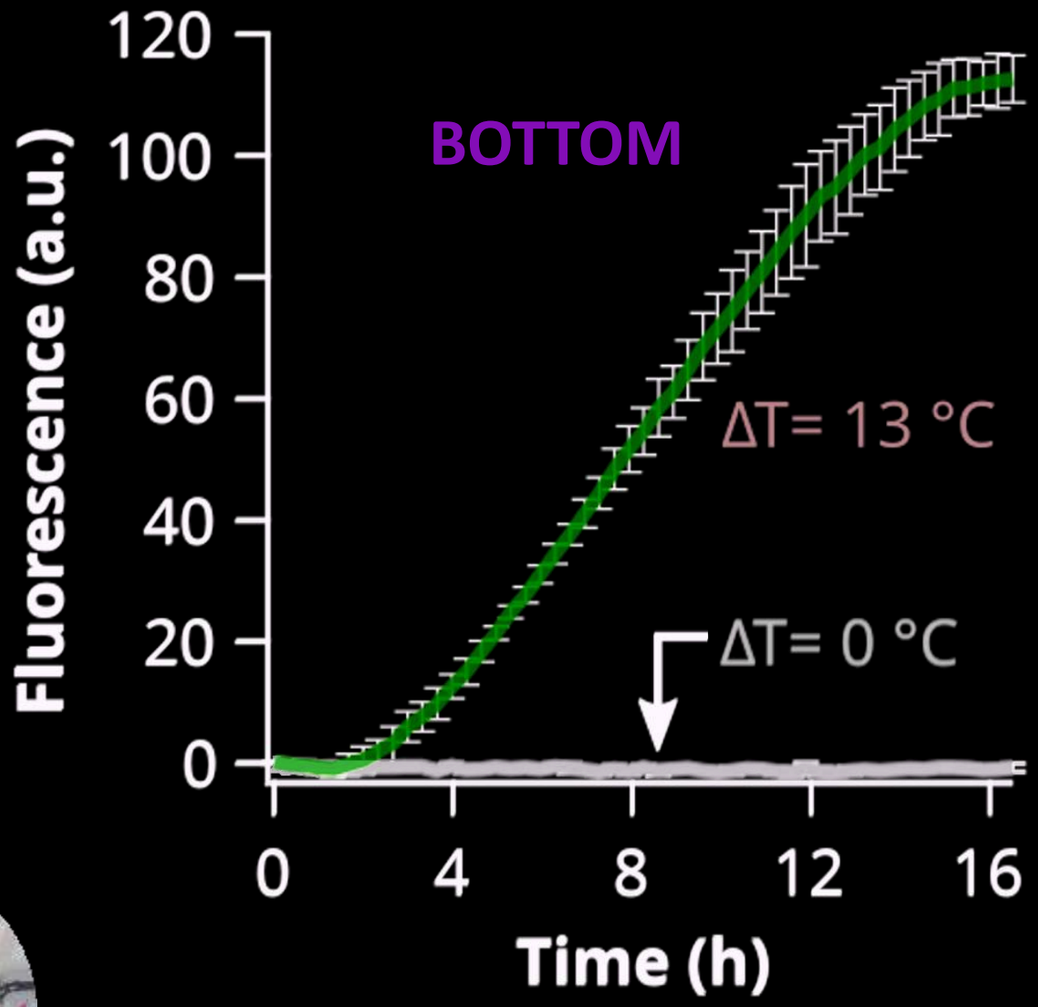
1. Biological History
2. Chemical Selection
3. Sequence Selection
4. Geophysical Space
5. Autonomous Evolution of the first cells

# Thermophoretic traps … assemble and trigger TX-TL




PURE expression system at low concentration



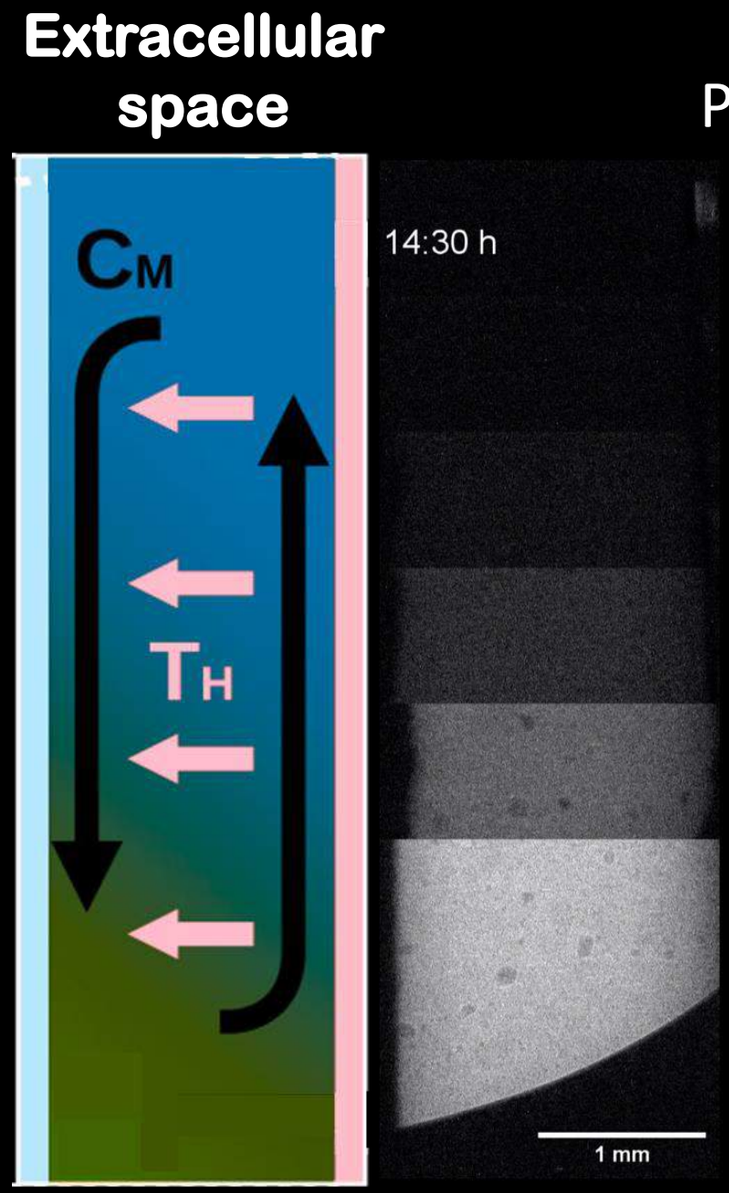



Alexander Floroni

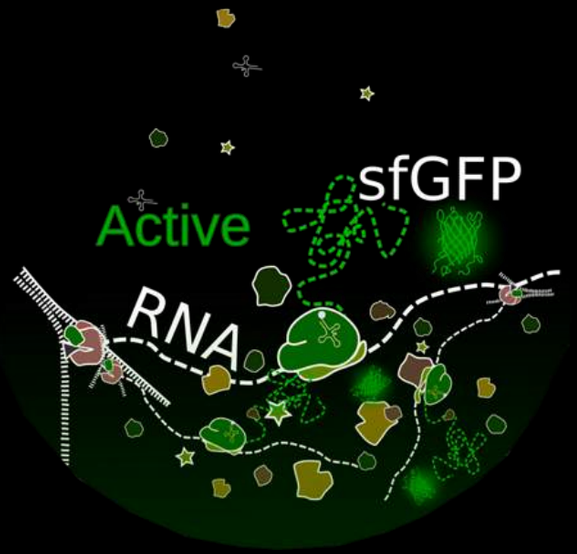


Noel Yeh Martin

# Thermophoretic traps … assemble and trigger TX-TL




PURE expression system at low concentration

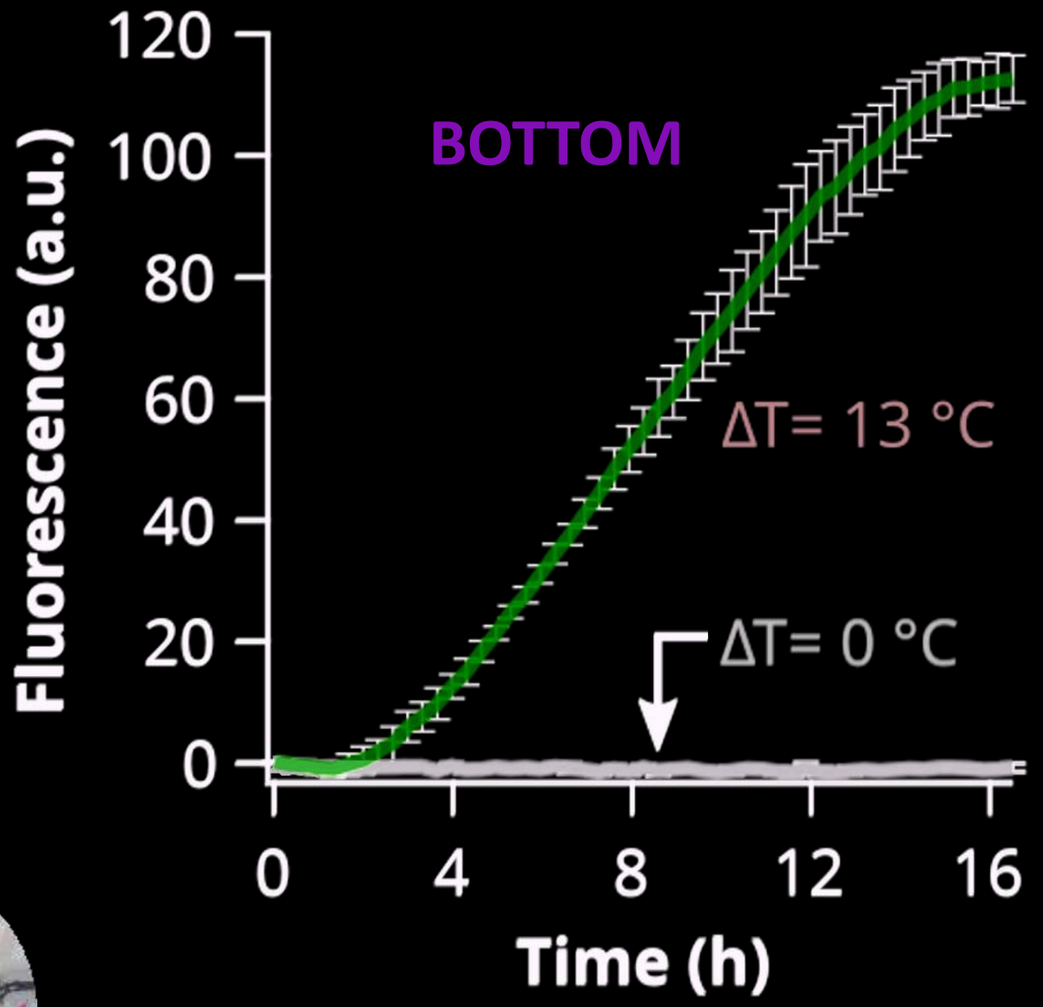




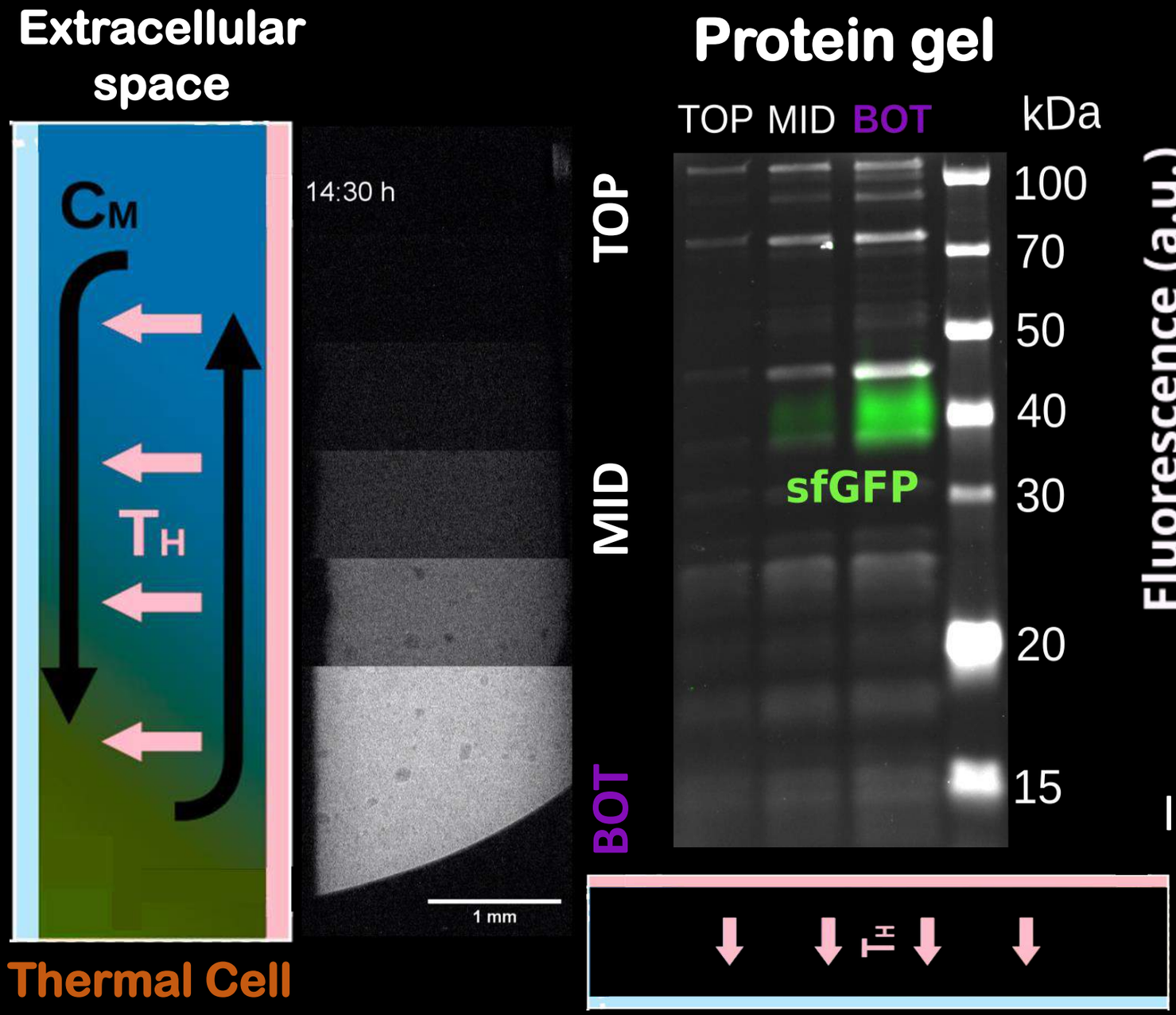

Alexander Floroni



Noel Yeh Martin



# Thermophoretic traps … assemble and trigger TX-TL



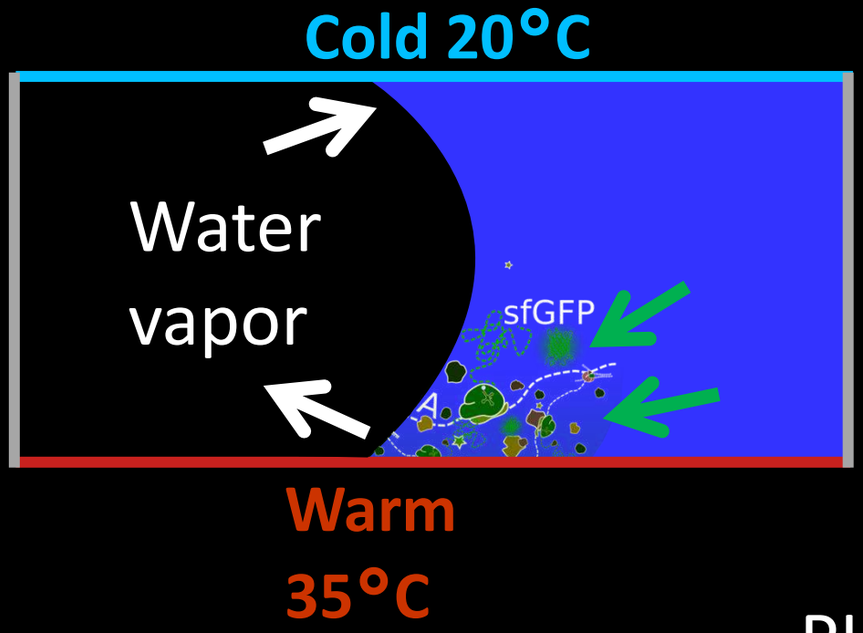

Ions and Proteins accumulate similarly

Tilted control shows no expression due to convection switched off

# Heated air-water interfaces … assemble and trigger TX-TL



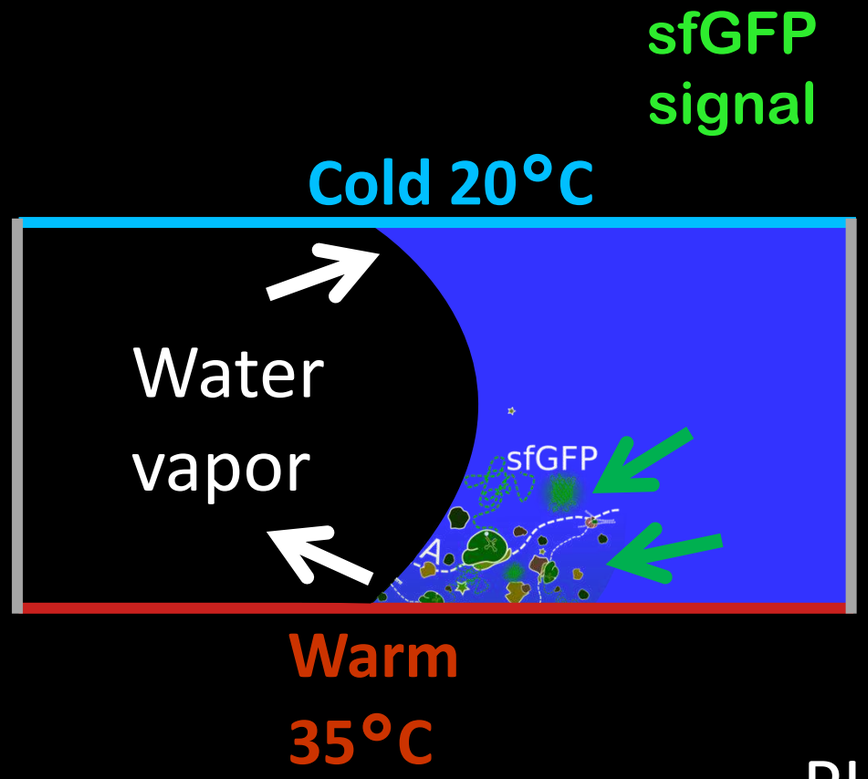
Alexander Floroni

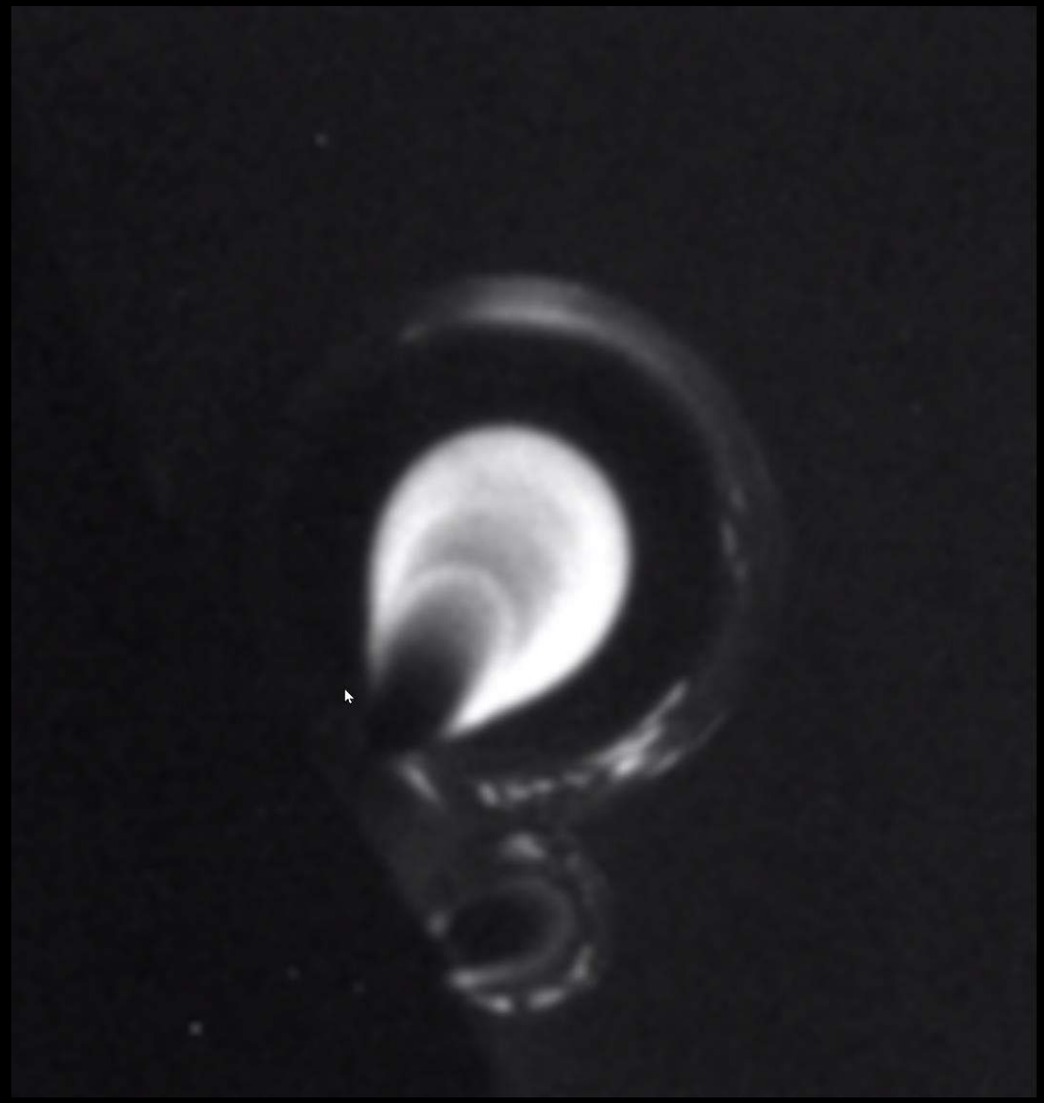



200µm

PURE system assembled at water-air-interface

# Heated air-water interfaces … assemble and trigger TX-TL



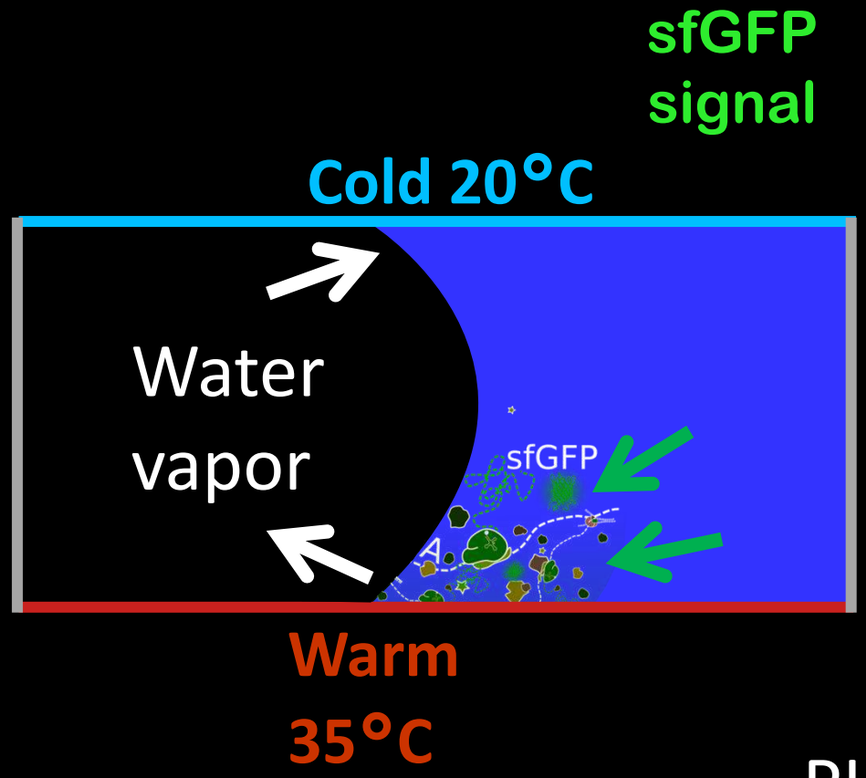


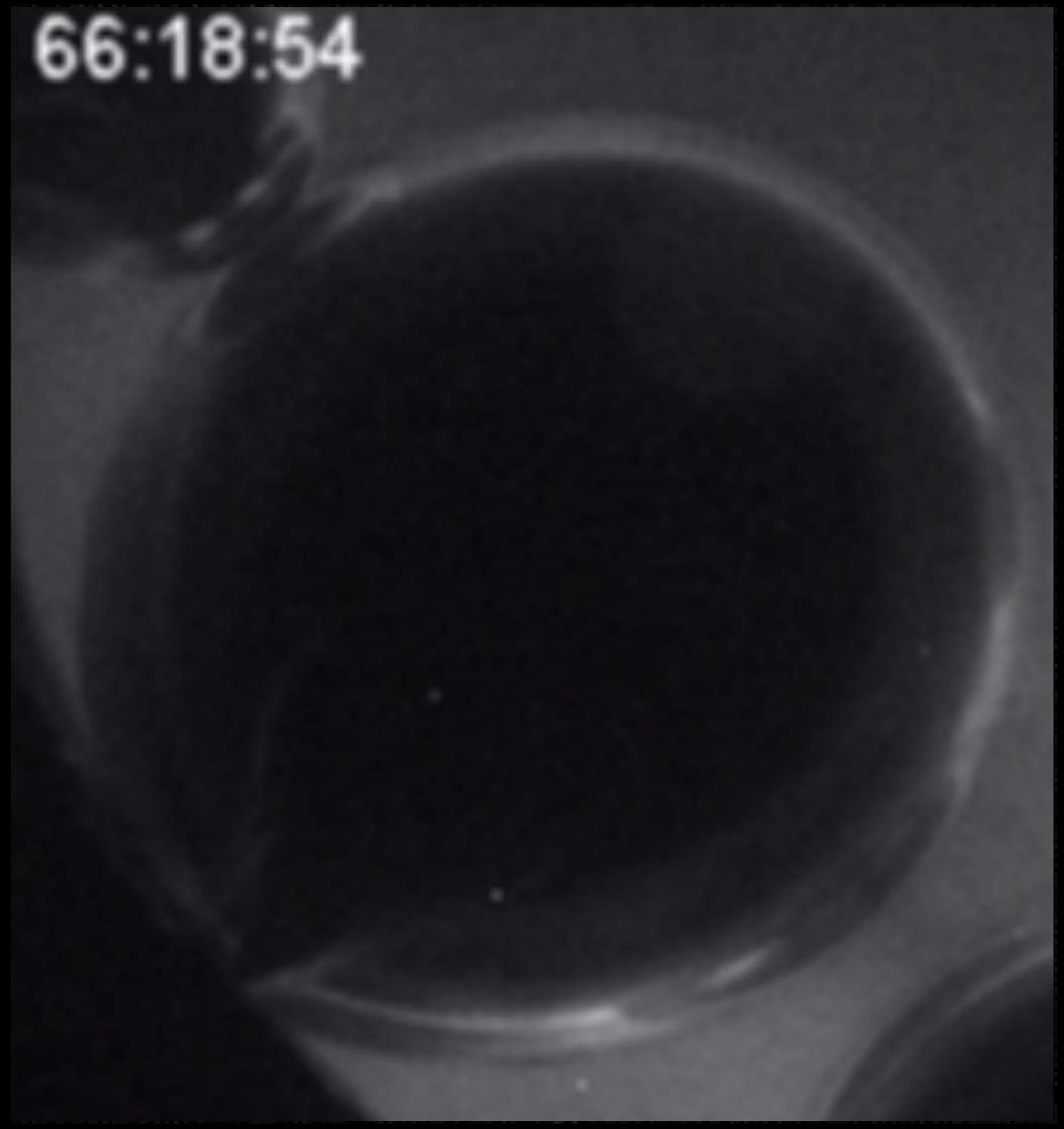
Alexander
Floroni

200µm

PURE system assembled at water-air-interface

# Heated air-water interfaces … assemble and trigger TX-TL



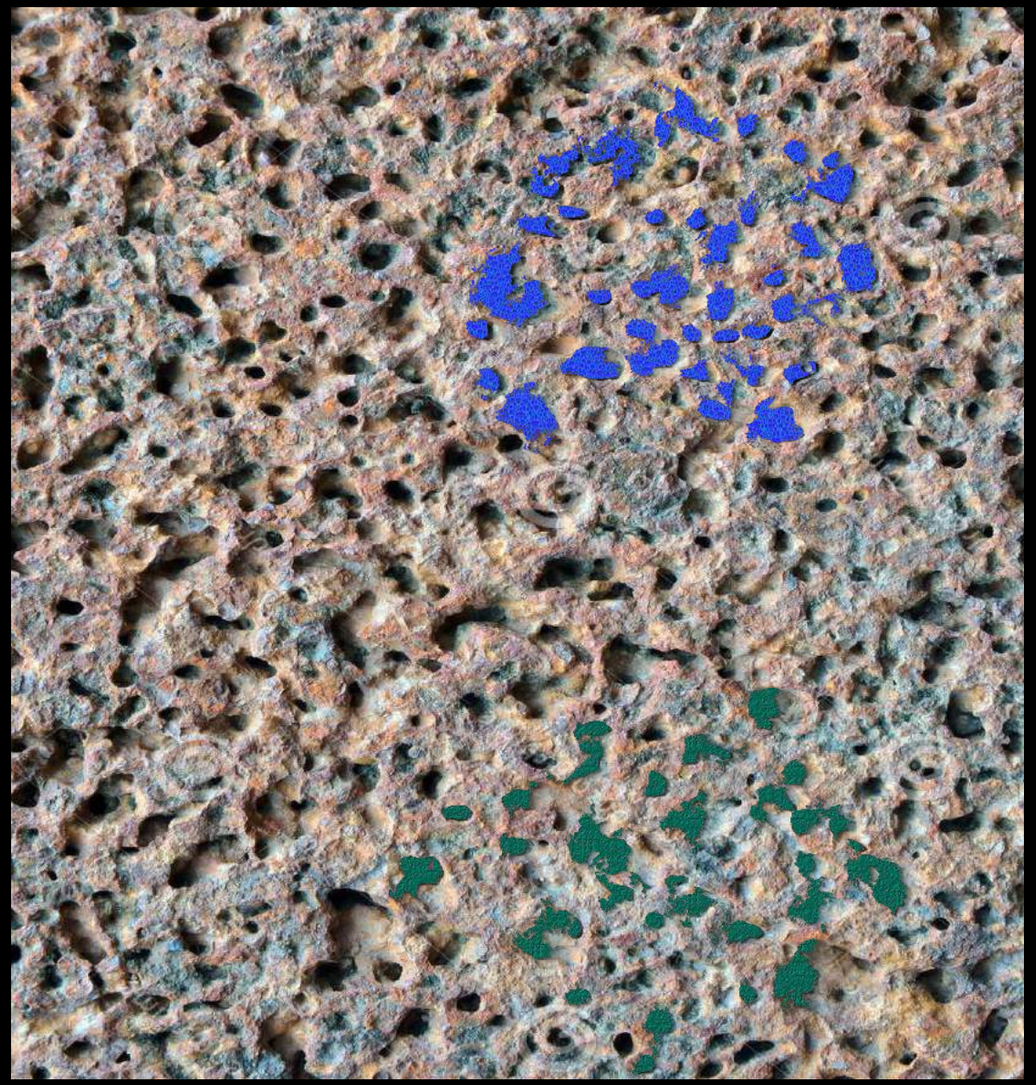






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PURE system assembled at water-air-interface

# Lateral gene transfer

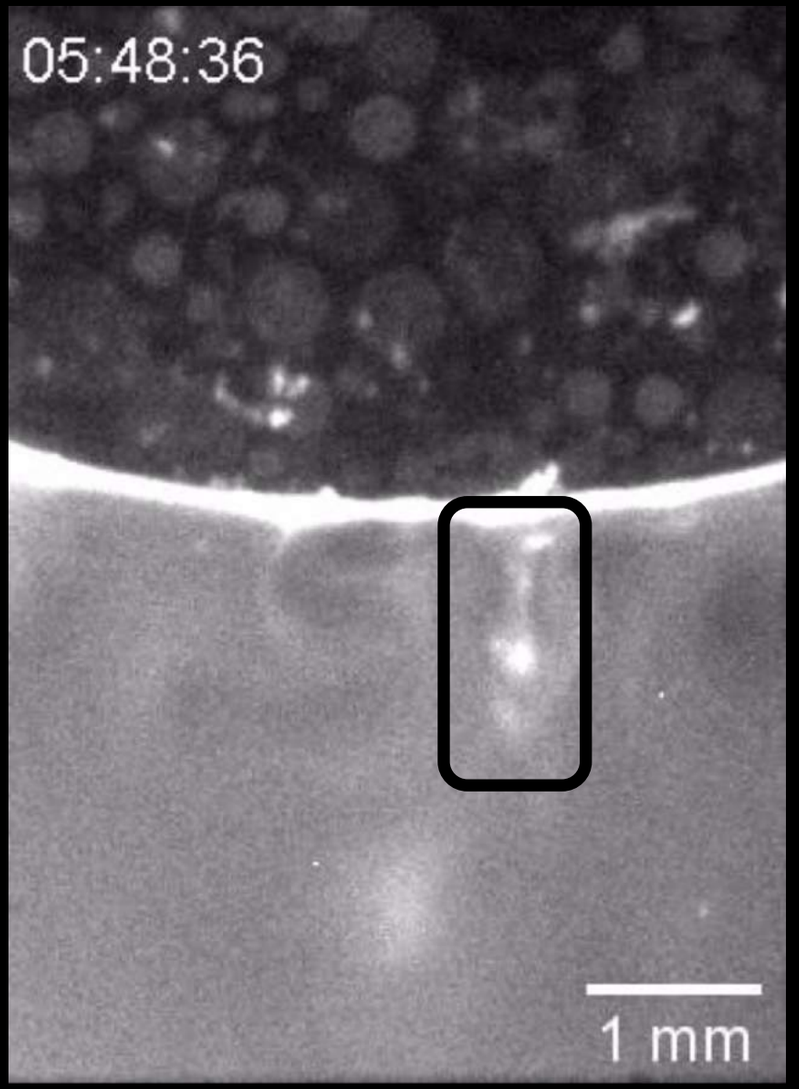


Growth +
Diffusion
+ Drift

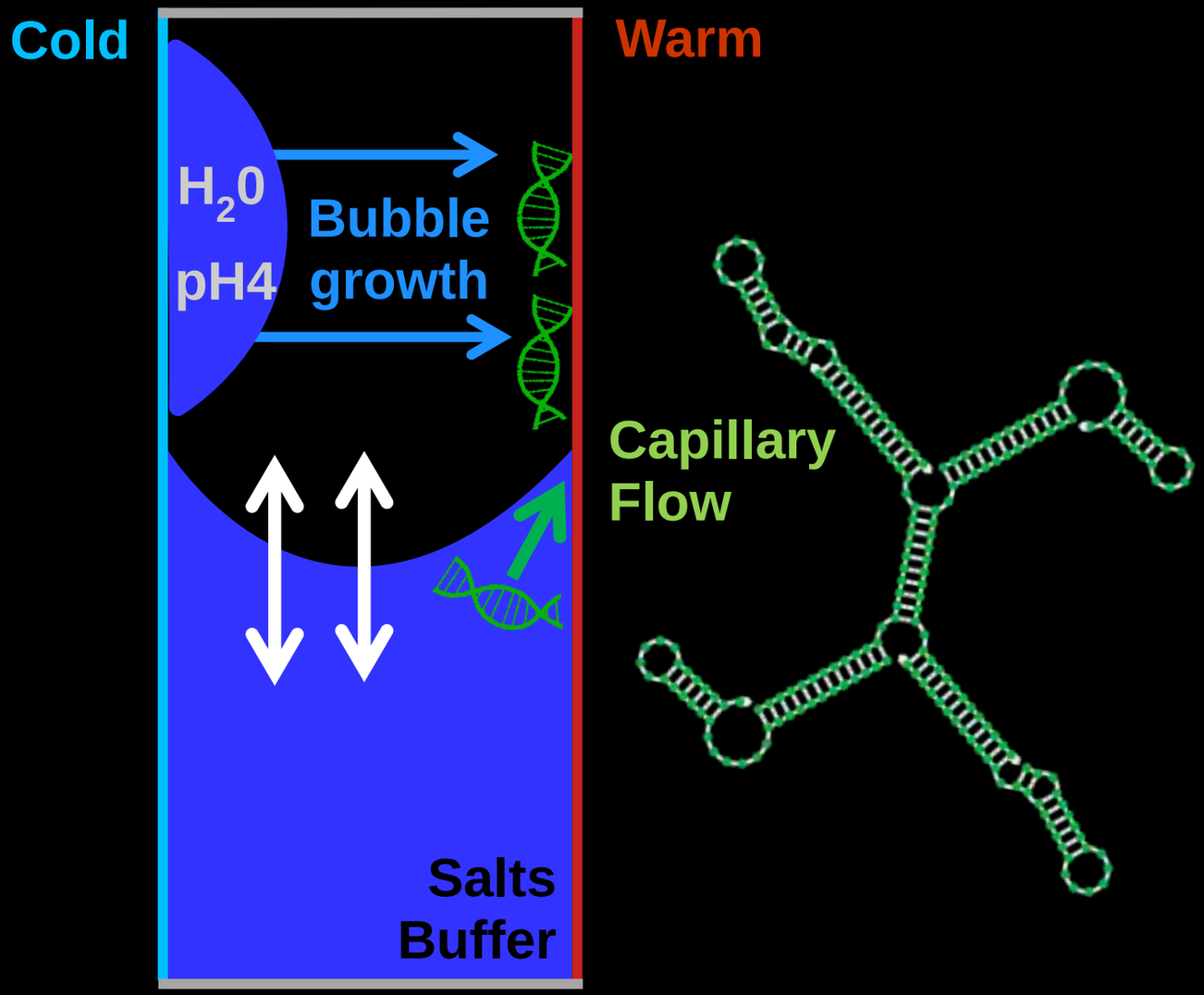
Cold
area

Exchange
of RNA
packages

Salty
area

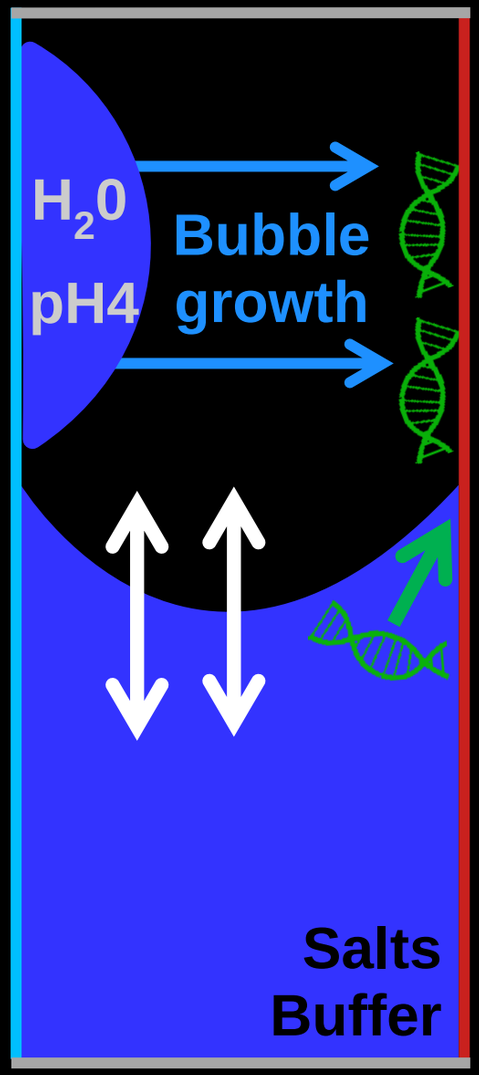


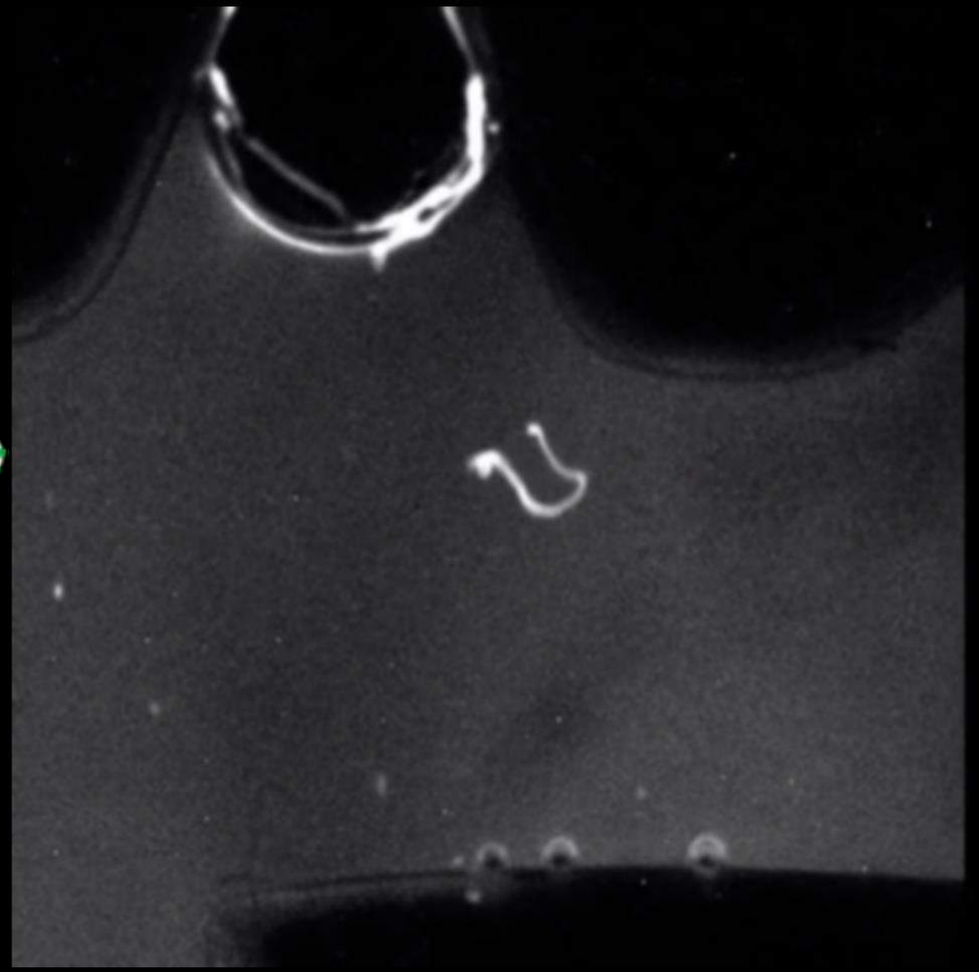
# Heated air-water interface ... for RNA gelation




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

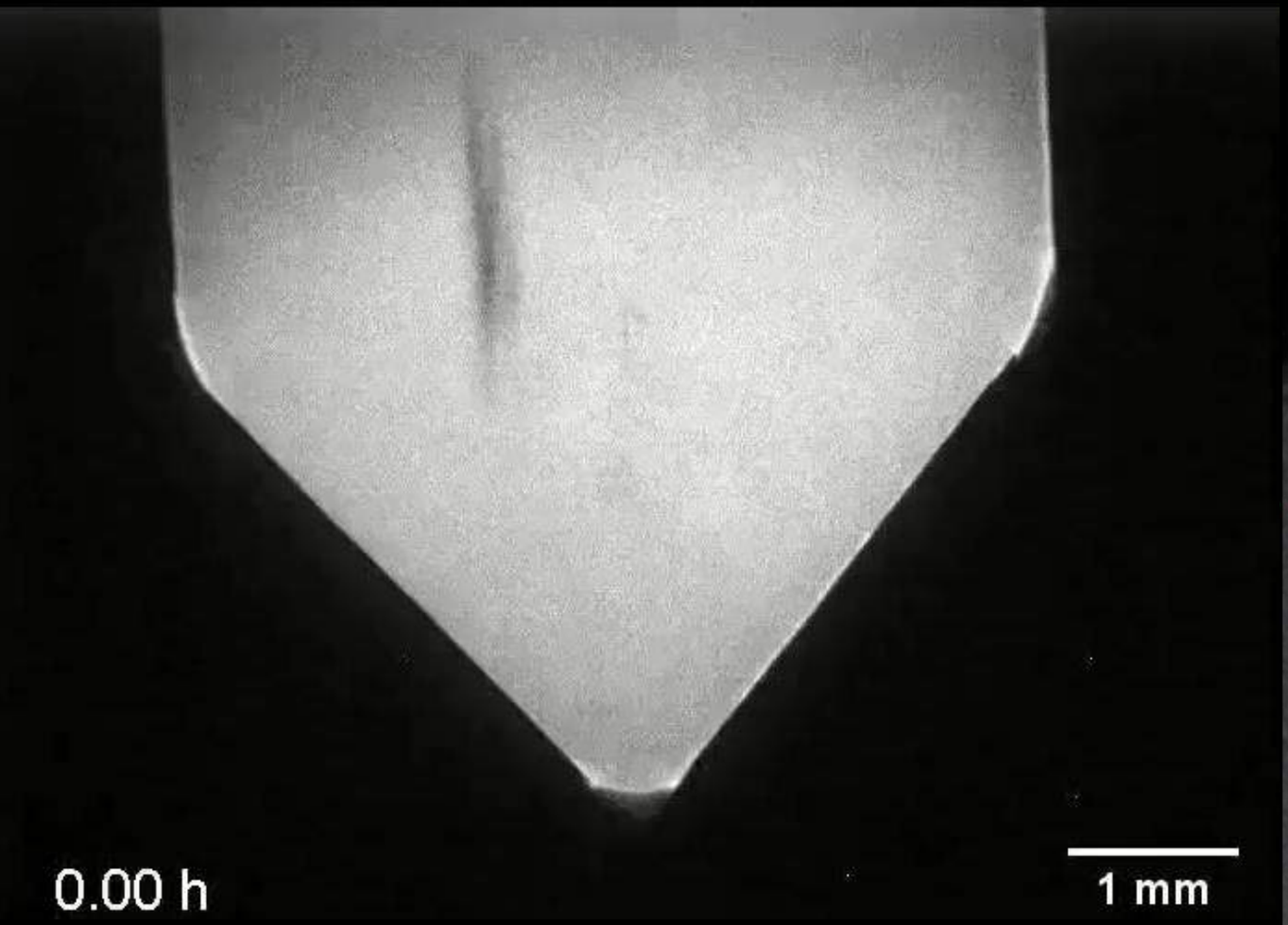
# Heated air-water interface … for RNA gelation

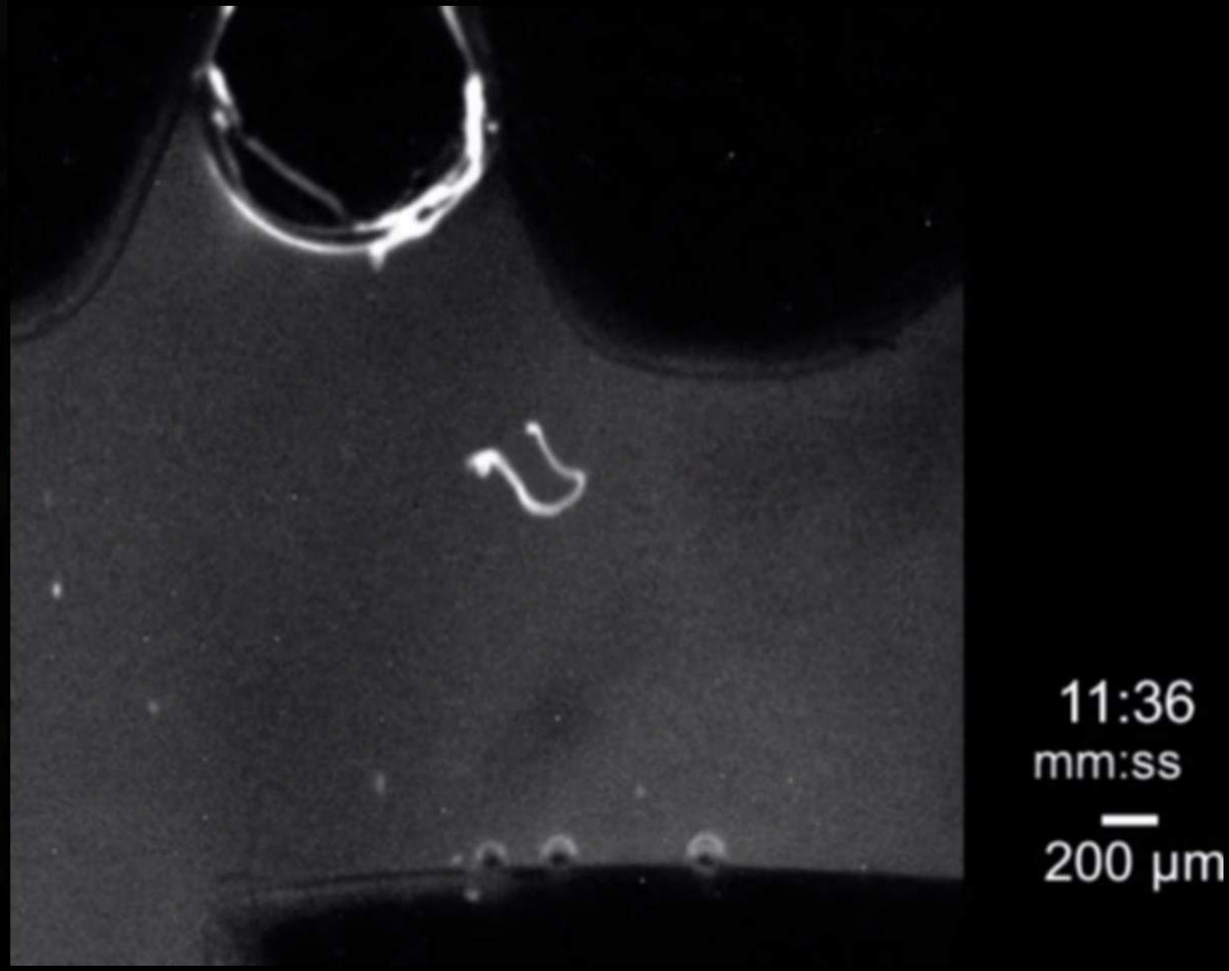


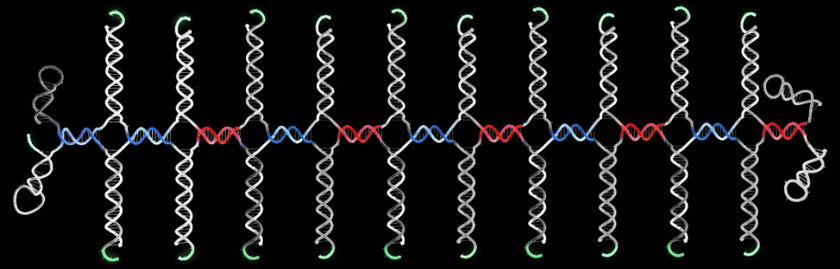




**Nature Chemistry** (2019)
doi.org/10.1038/s41557-019-0299-5

# Heated air-water interface … for RNA gelation



**eLife** (2021)
doi: 10.7554/
eLife.63431

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

# Heated air-water interfaces … fill and make vesicles

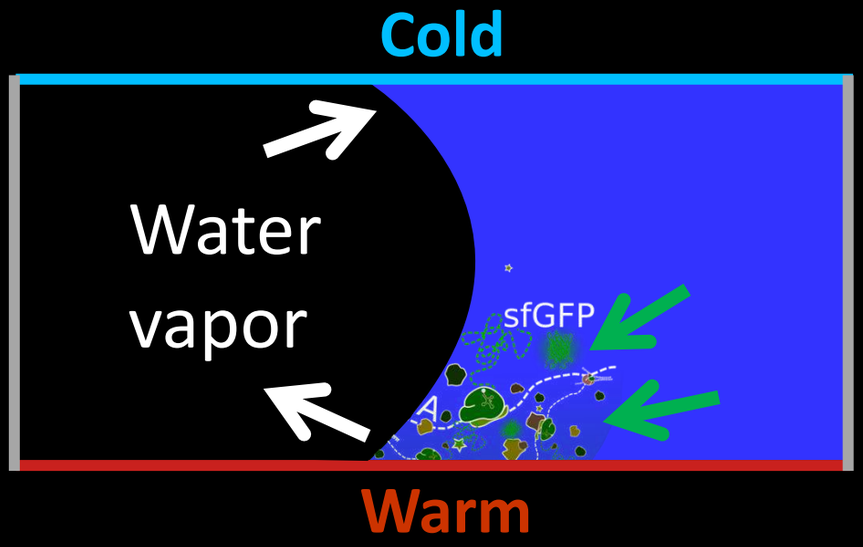
**63mer ssDNA**

**DOPC**

**<30mM NaCl**



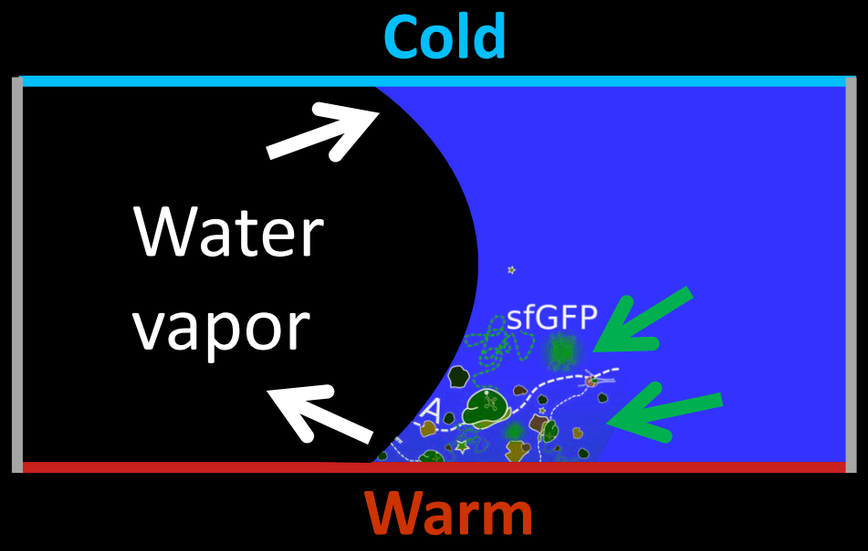
Alexander Floroni




200µm

# Heated air-water interfaces … fill and make vesicles

**63mer ssDNA**
**DOPC**
**<30mM NaCl**




**63mer ssDNA**





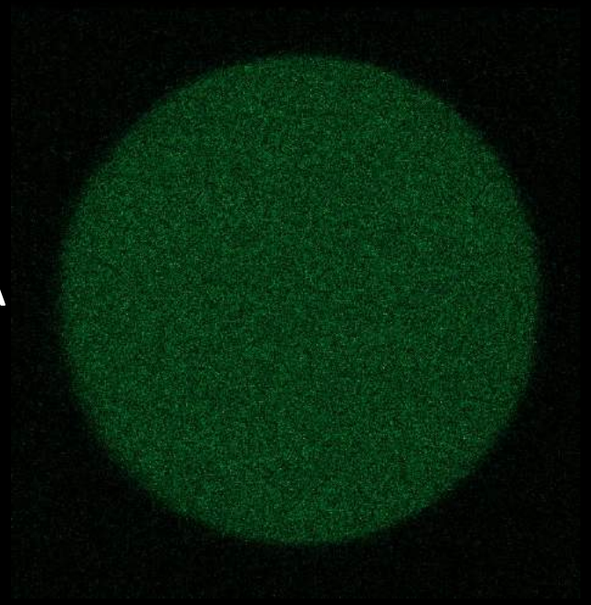

Alexander Floroni

# Heated air-water interfaces … fill and make vesicles

**63mer ssDNA**
**DOPC**
**<30mM NaCl**



Lipid label DiD



63mer DNA

20µm

**63mer ssDNA**



200µm



Alexander Floroni

**Encapsulate PURE ?**

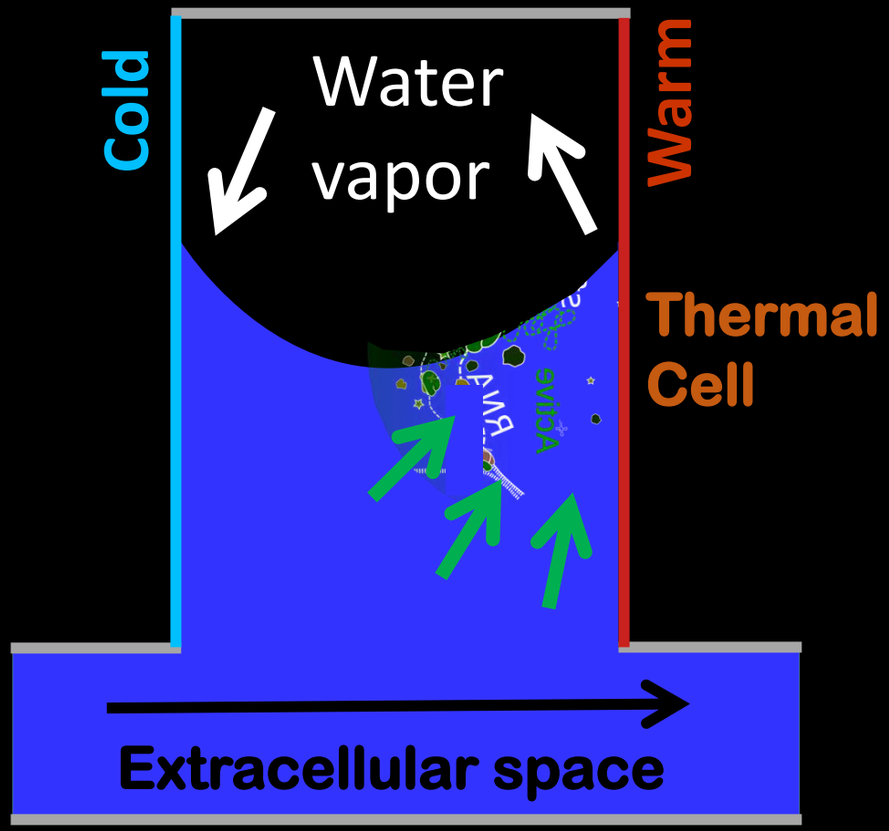
**Evolutionary Advantage ?**



with Kerstin Göpfrich



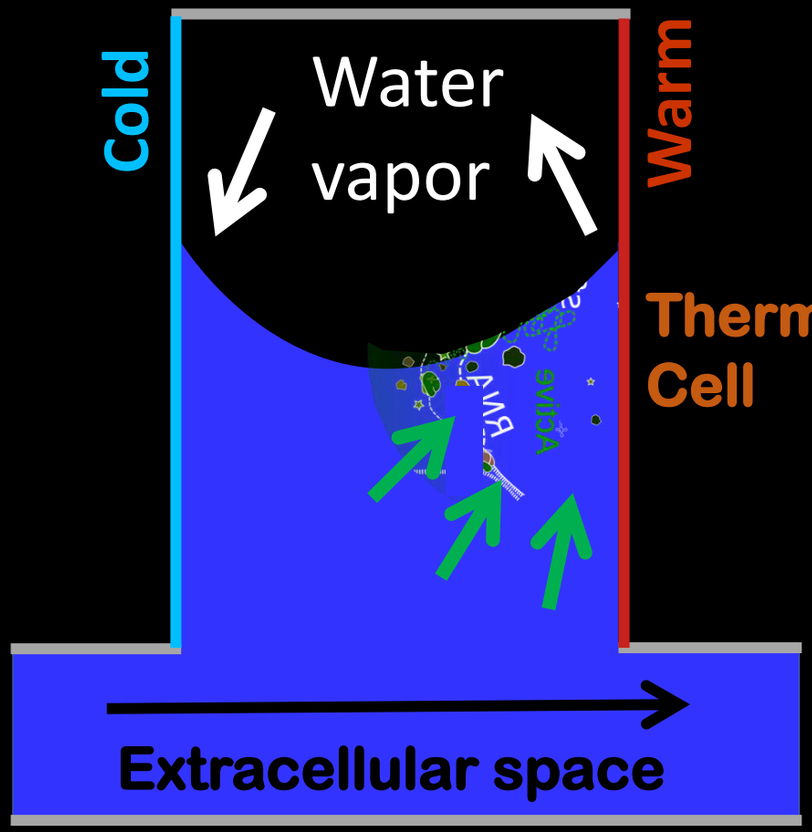
# Early thermal Cell



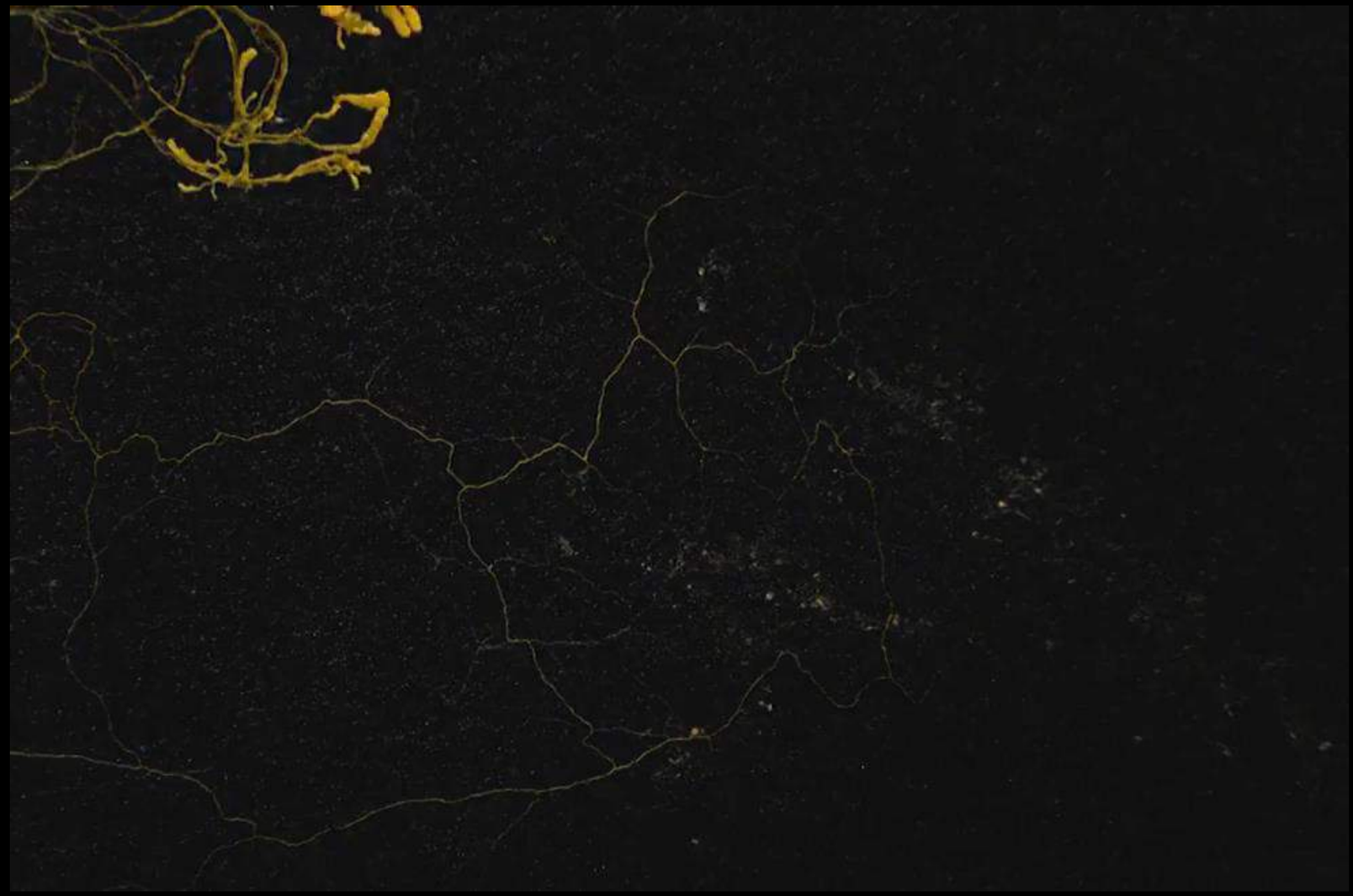

**Easy synthetic cell for studying evolution**



# Early thermal Cell



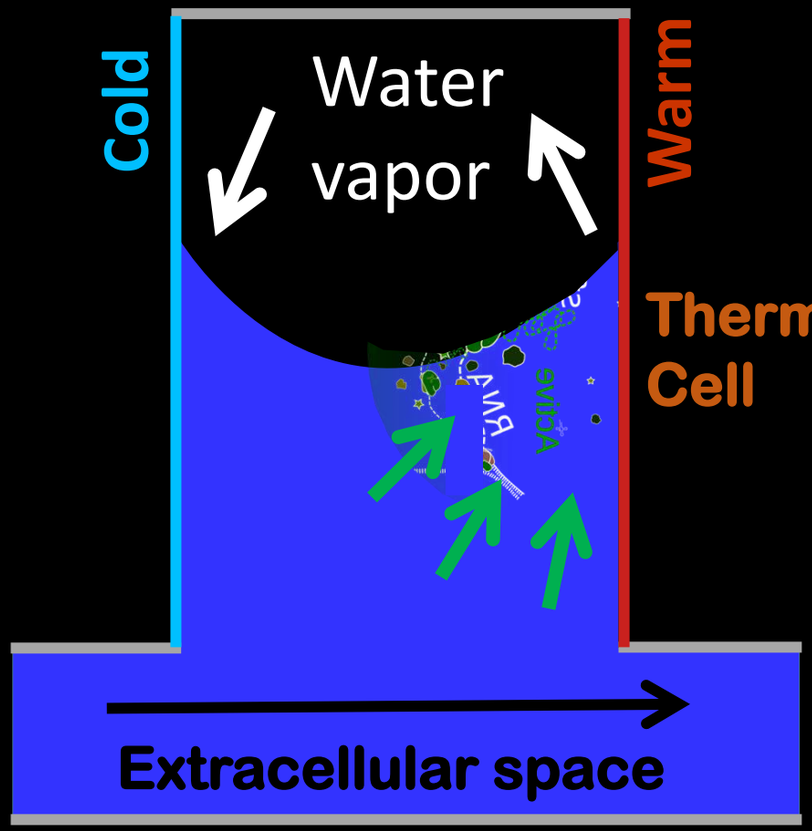

**Easy synthetic cell for studying evolution**



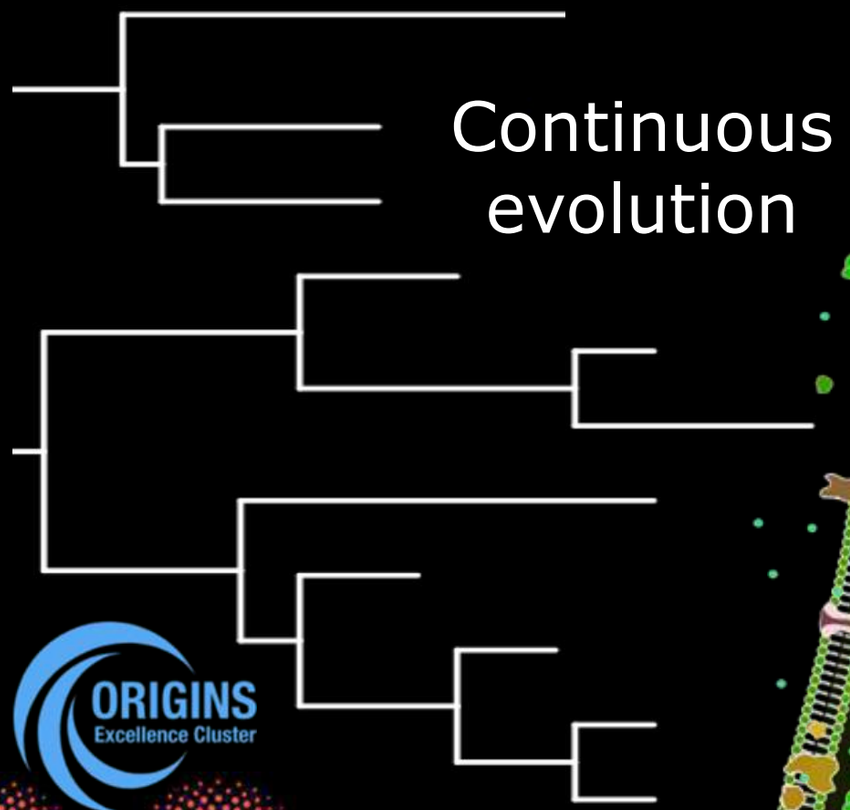
Physarum polycephalum, by Heather Barnett



## Early thermal Cell




**Easy synthetic cell for studying evolution**




ORIGINS Excellence Cluster

erc Starting 2010-15

erc Advanced 2018-23

HFSP

## Late membrane Cell




Membrane Transport

Confinement

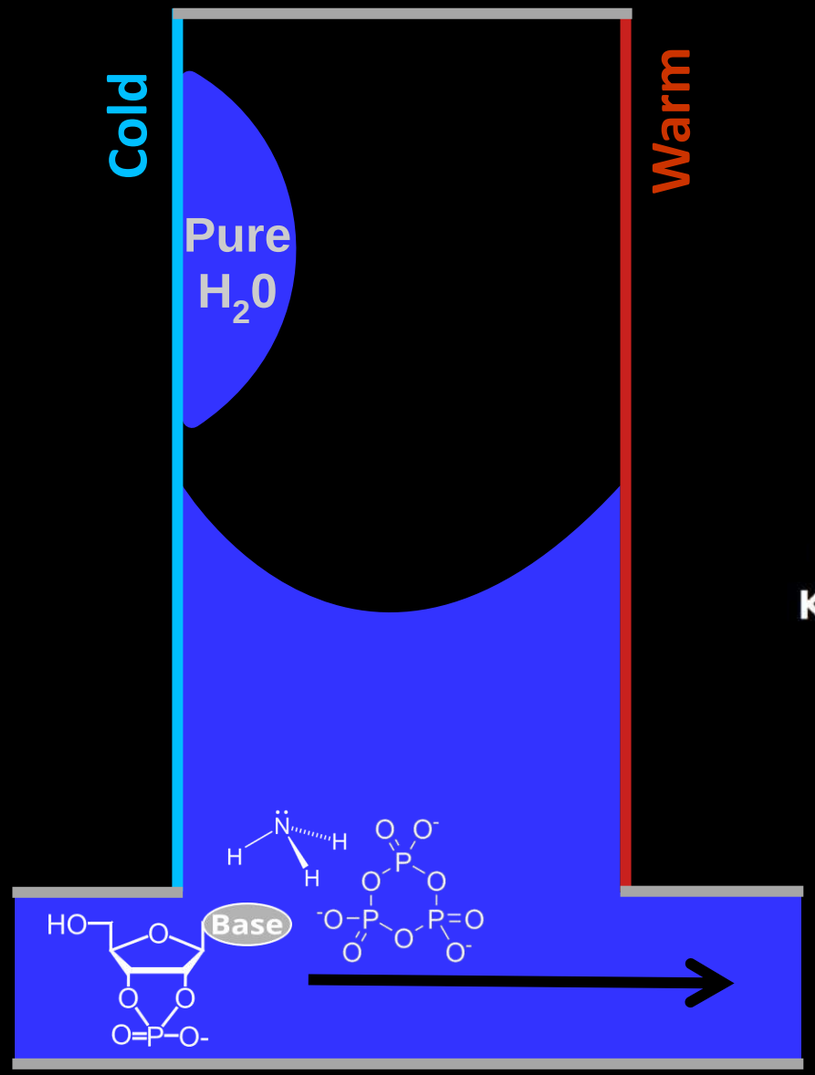
Concentration Gradient

Active Metabolism

**with Kerstin Göpfrich and Tomoaki Matsuura**

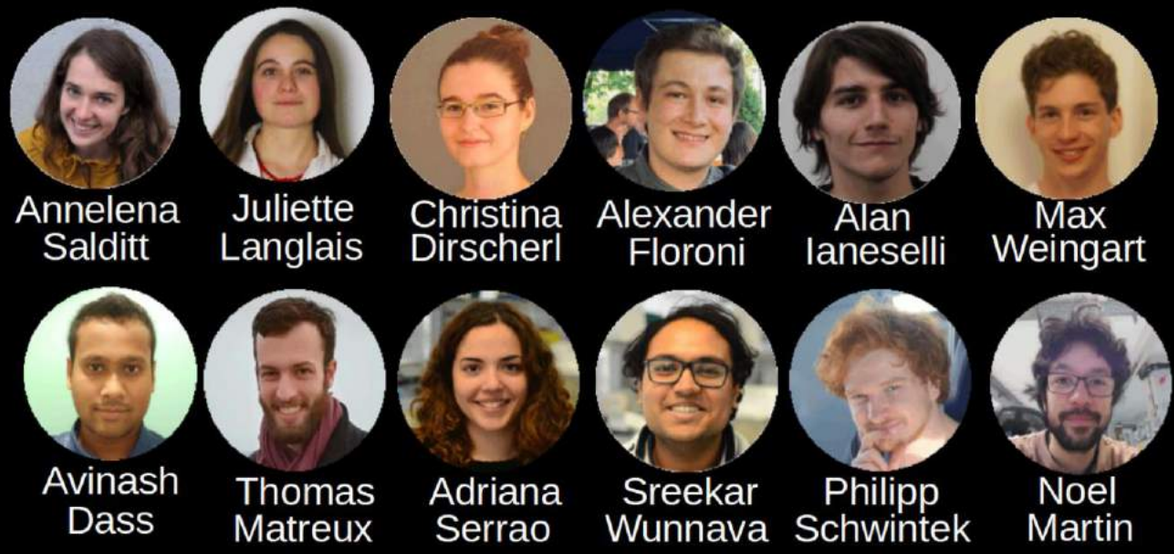
**Protein-based Biology**

# Physical Chemistry of early Evolution






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

CRC 392
Molecular evolution
in prebiotic environments

ORIGINS Excellence Cluster

erc Starting 2010-15

erc Advanced 2018-23

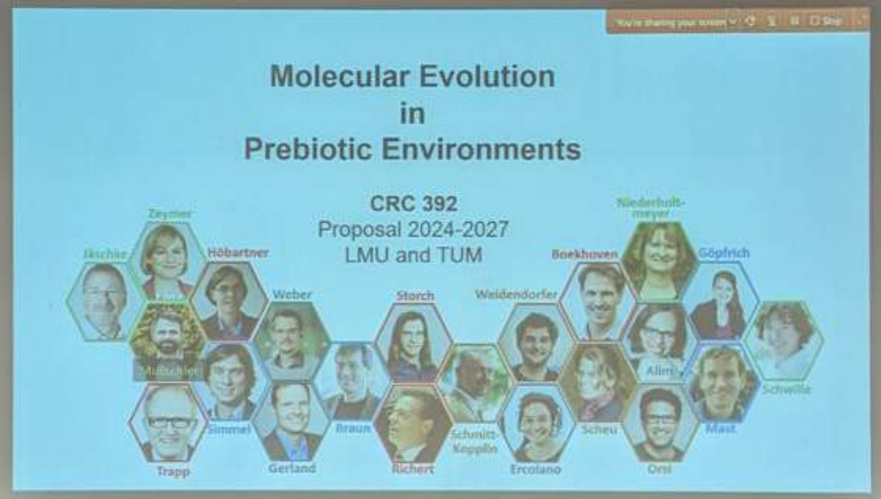
nano agents SFB 1032

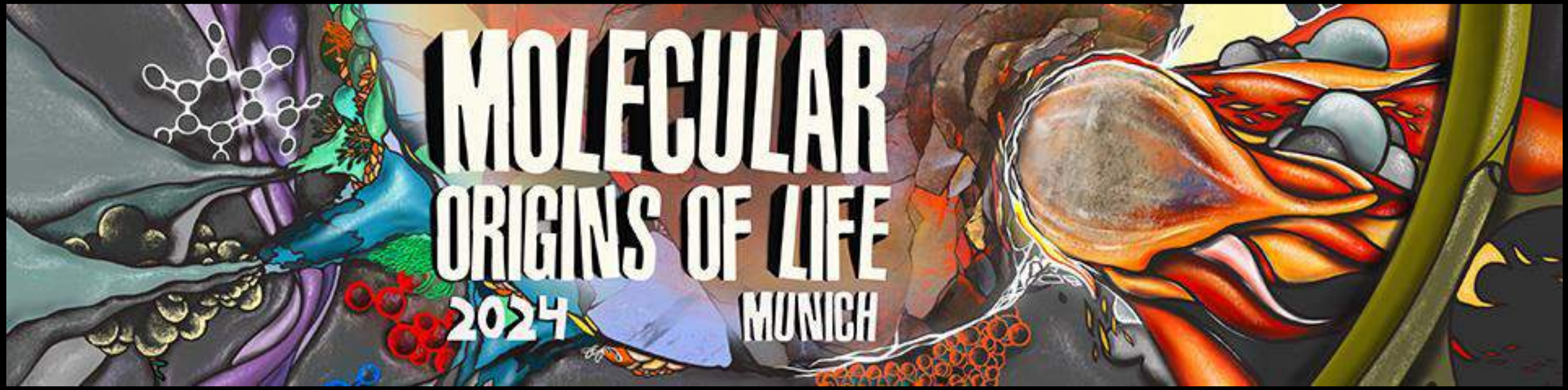
QB

nano TEMPER

# www.molecular-evolution.de/positions

## Please apply for PhD positions!






At **July 19th-20th**, the Molecular Origins of Life Munich conference addresses one of the most fundamental questions of science: How could life originate? With 24 talks by renowned scientists accompanied by panel discussions and poster sessions, this international conference brings together scientists from a wide range of disciplines, namely: astronomy, bio-/chemistry, bio-/physics, geosciences and theoretical chemistry and physics. Website: **www.lmu.de/mom2024**

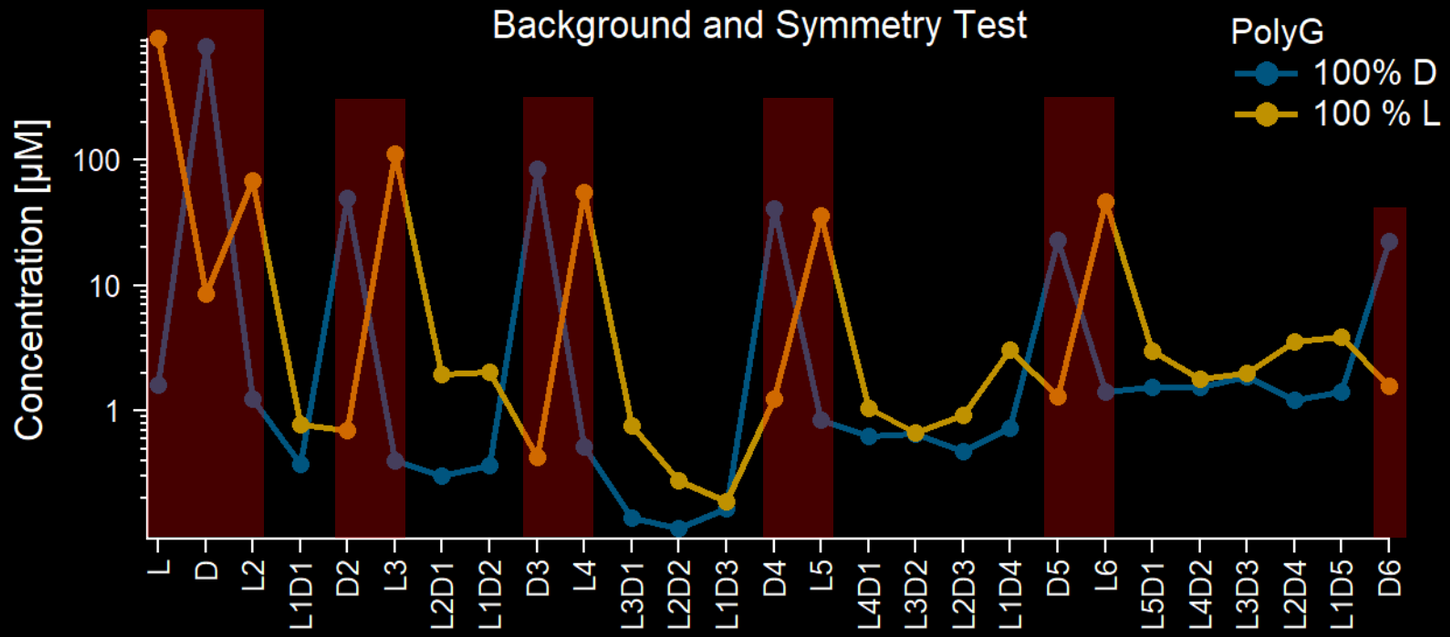
# Homochiral selection

Dry, 1 day, pH 10, 25°C



Sreekar Wunnava




Selection?

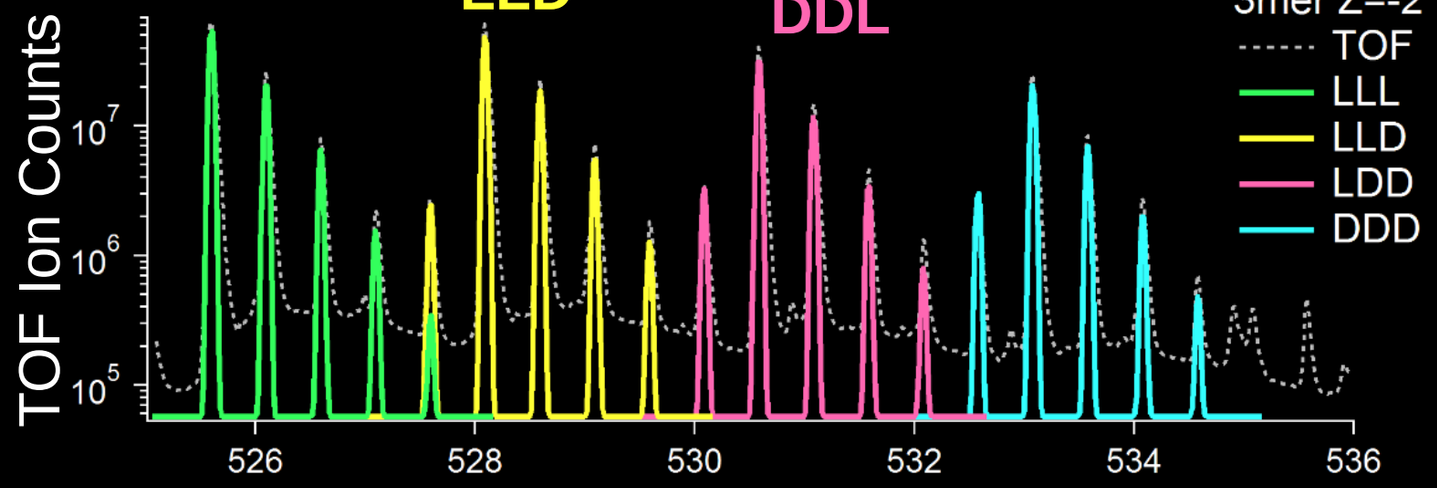
**LLL**

**DLL, LDL, LLD**

**LDD, DLD, DDL**

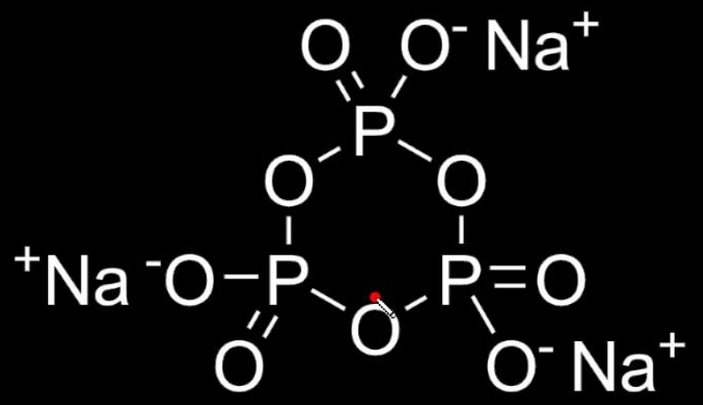
Selection?

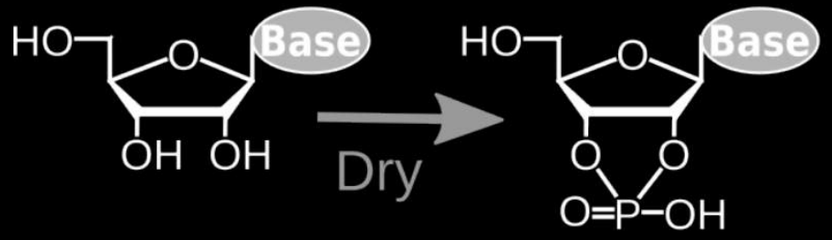
**DDD**

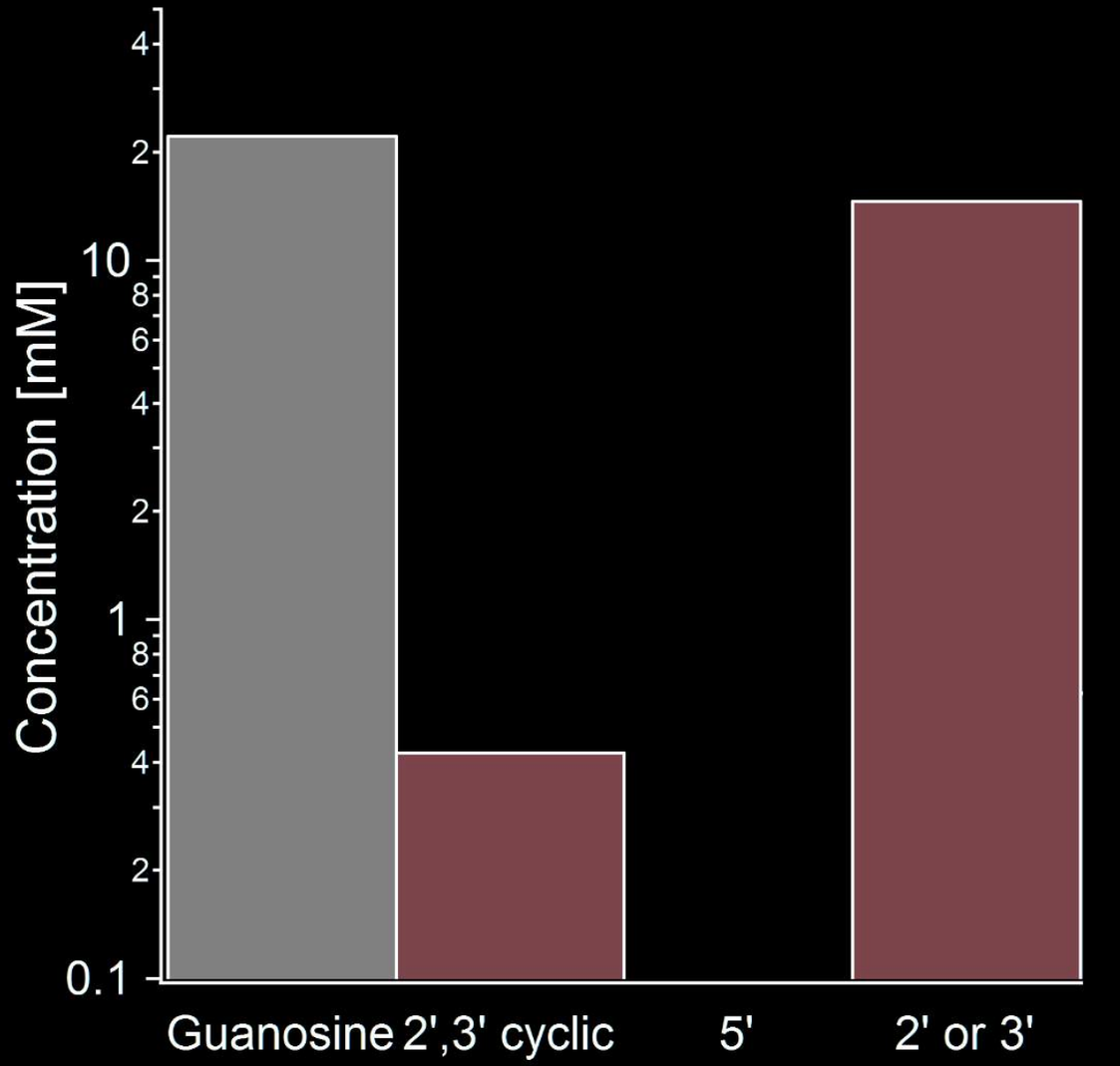


# Phosphorylation

1:1 Mixture of Guanosine
and Trimetaphosphate
Dry / 80°C / 3days / pH10




Guanosine Monophosphate

# Phosphorylation + Polymerization

1:1 Mixture of Guanosine and Trimetaphosphate
Dry / 80°C / 3days / pH10

