A: Fundamentals of Life

- Definition of Life
- Logic of Molecular Biology
- History of Biology
- Becoming alive
- Soup of Life
- Selection: before and in life
- Three faces of Entropy
- Death and equilibrium
- Missing non-equilibrium
- Structure of Origin of Life
- Modes of non-equilibrium
- Examples of evolution

B: Physics for Chemistry

Polymerization
- Theory of polymerization
- P. by fast cooling
- P. by stacking with 3'-5'-Ph.
- Activation groups
- P. on clay
- P. by thermophoresis
- Phase transitions with DNA
- Sedimentation of DNA
- Drying and its problems
- Elegance of air interface

Replication
- Templated polymerization
- Ligation
- Strand separation problem
- PCR in convection
- Ribo-PCR in convection

C: Evolution Machines

Replication with accumulation
- Case of Ribo-PCR
- Spiegelman problem
- Case of trapped PCR
- Trapped PCR with flow
- Feeding problem
- Replication with heated tRNA
- Replication in driven Fog

Rebustness of evolution
- Error threshold
- Instability of four bases
- Hypercycles with ligation
- Spont. Symmetry breaking
- Spont. sequence selection
- Cooperation within cells

Replication only by RNA

to be submitted to PRL

A Thermal Habitat for RNA Amplification and Accumulation

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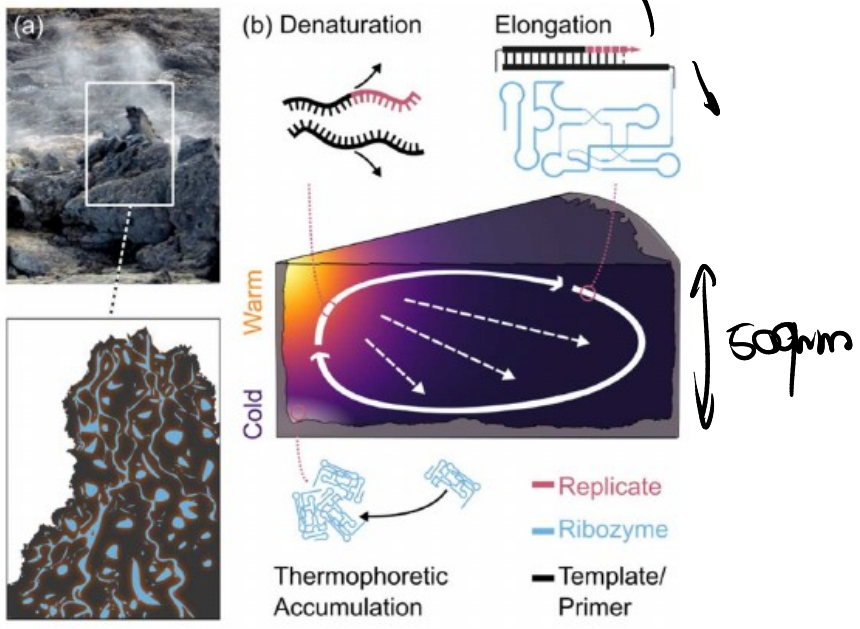
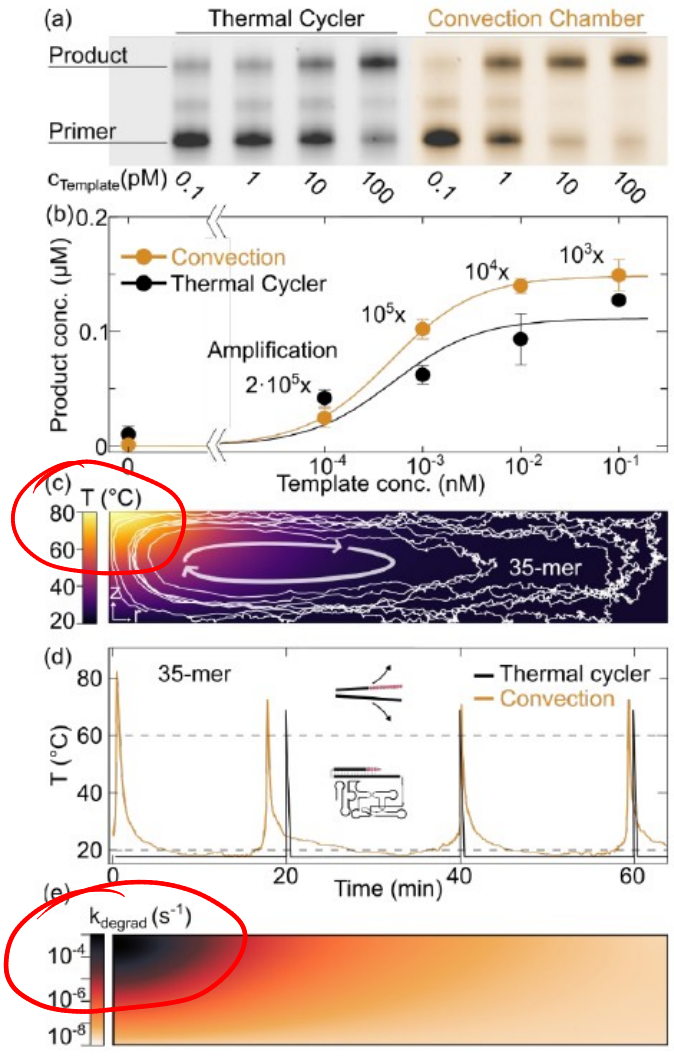
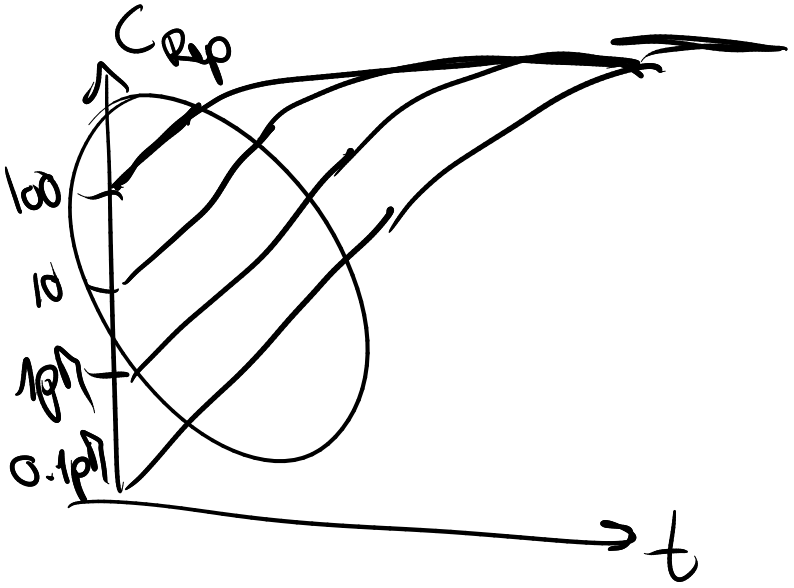
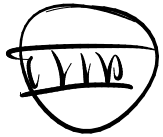
# Contributed equally

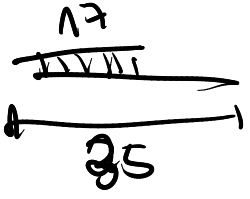


Replication only by RNA



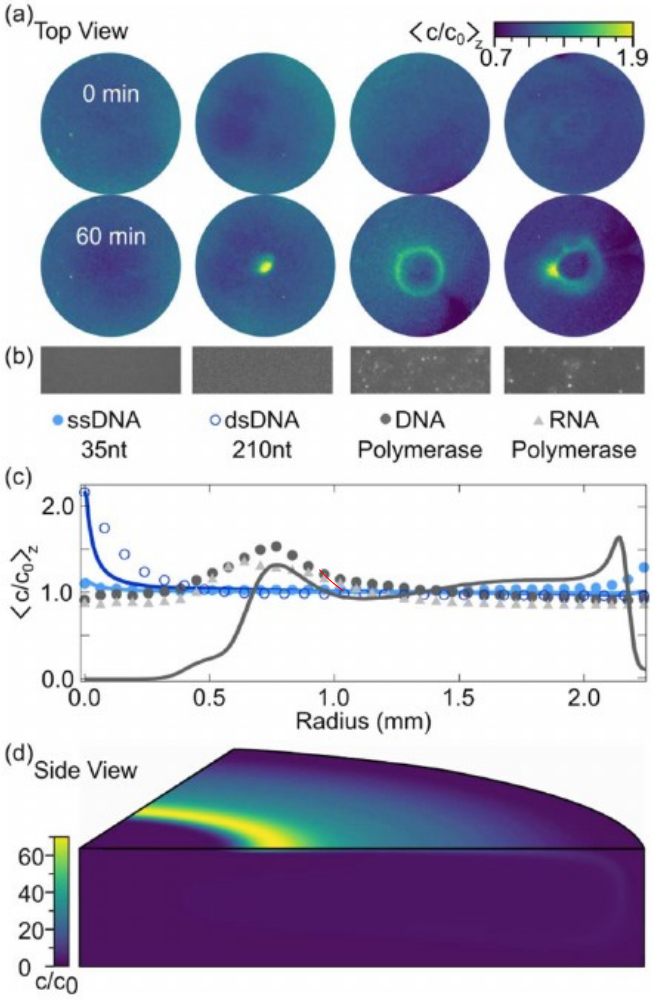


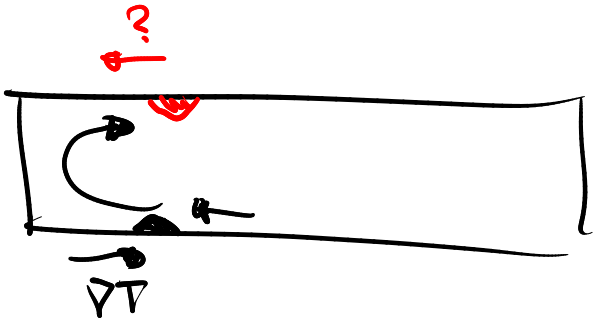


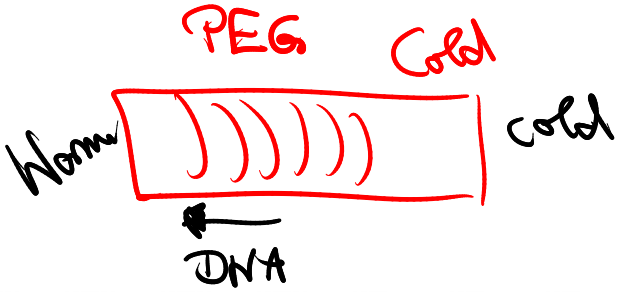


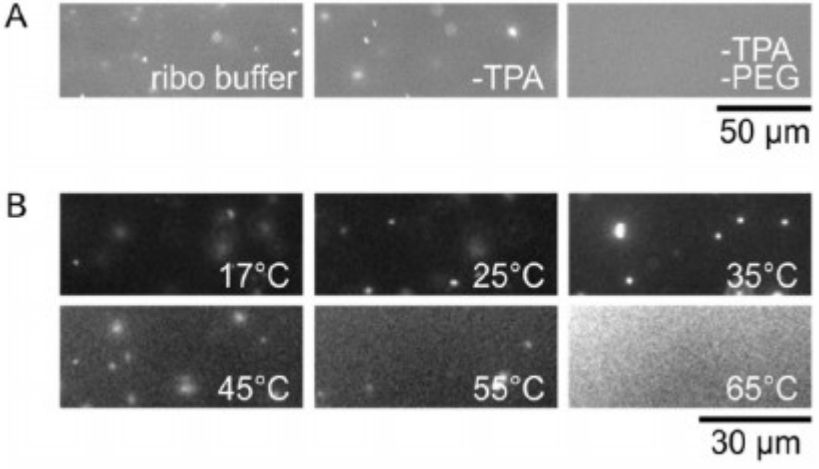
Ribozyme

Replication only by RNA

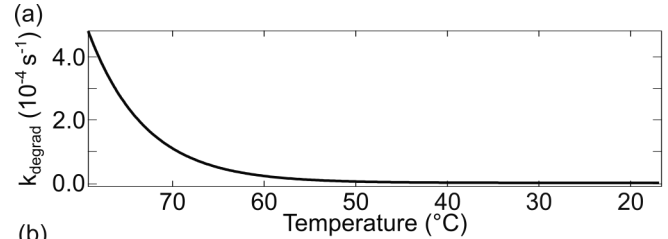


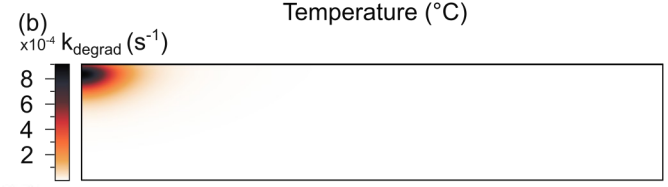


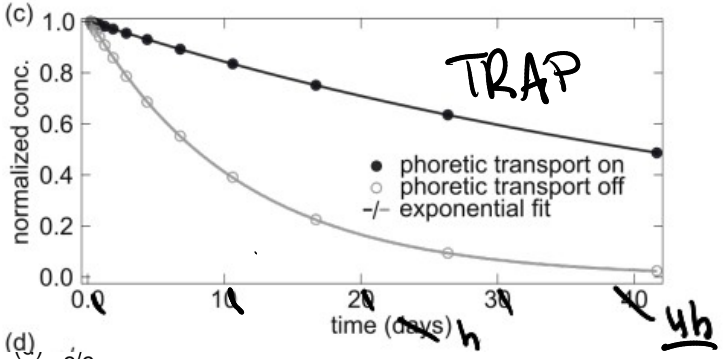


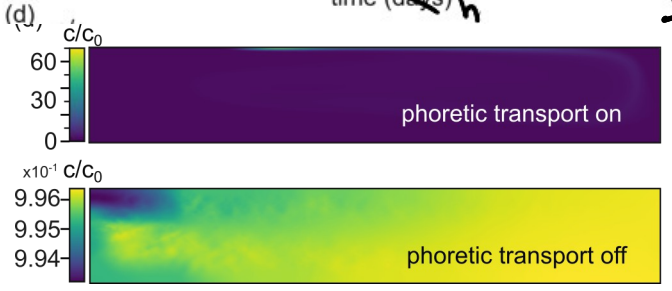


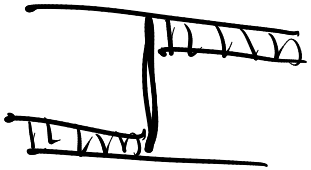
Protection by accumulation











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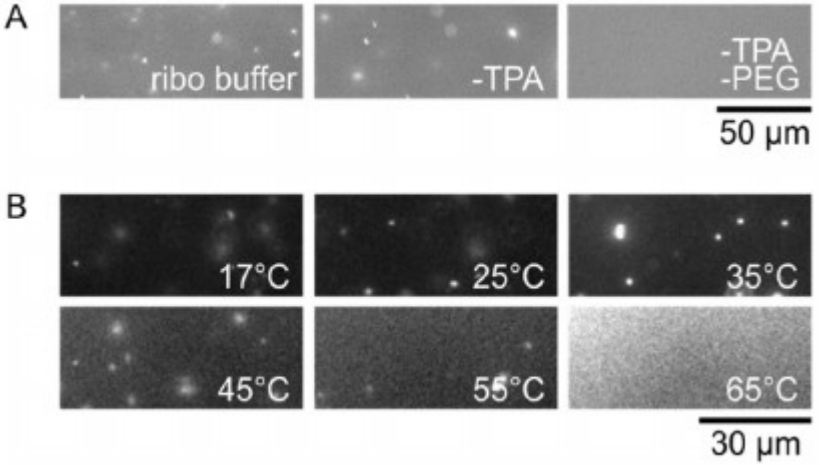
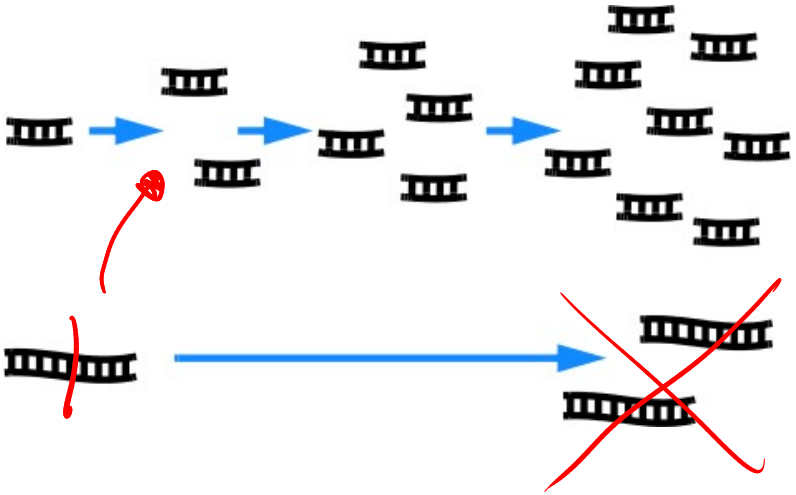
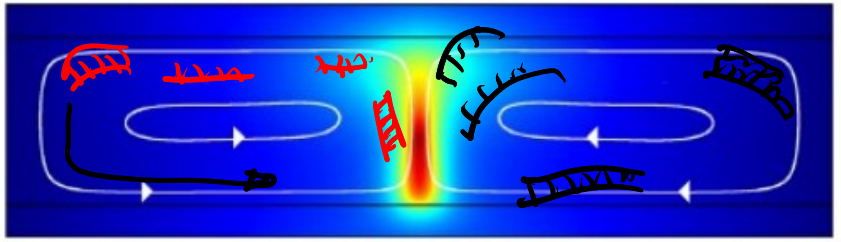


Spiegelman Problem

1967





Replication speed ∝ f(Length) : Not yet : RepSpeed(Long) > RepSpeed(Short)

Combining Replication with Accumulation using Polymerase

Molecule Trap

Selection

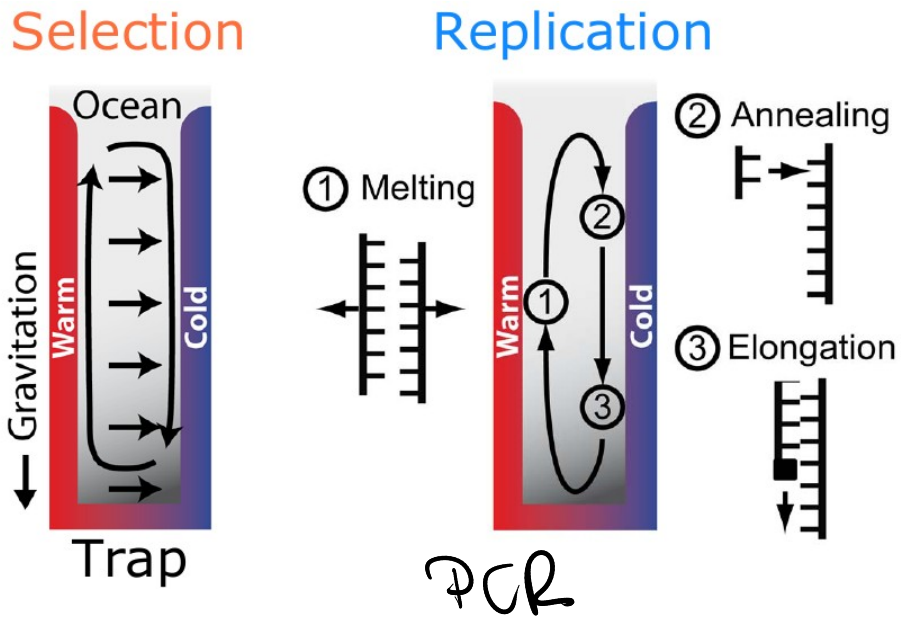
Convection

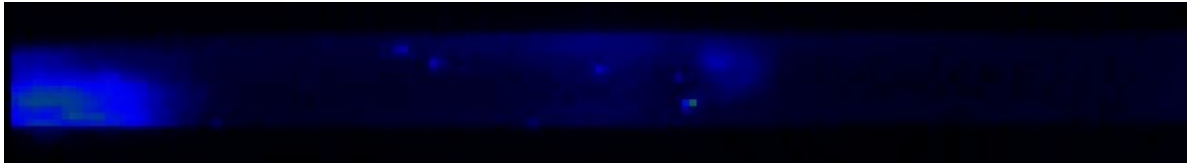
Replication

Minimal evolution machine driven by thermal gradient

Mast & Braun, PRL, 104, 188102 (2010)

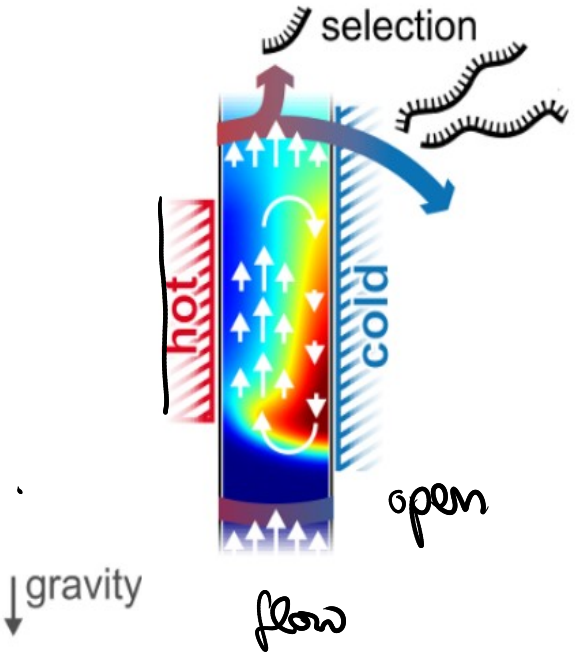
Combining Replication with Accumulation using Polymerase



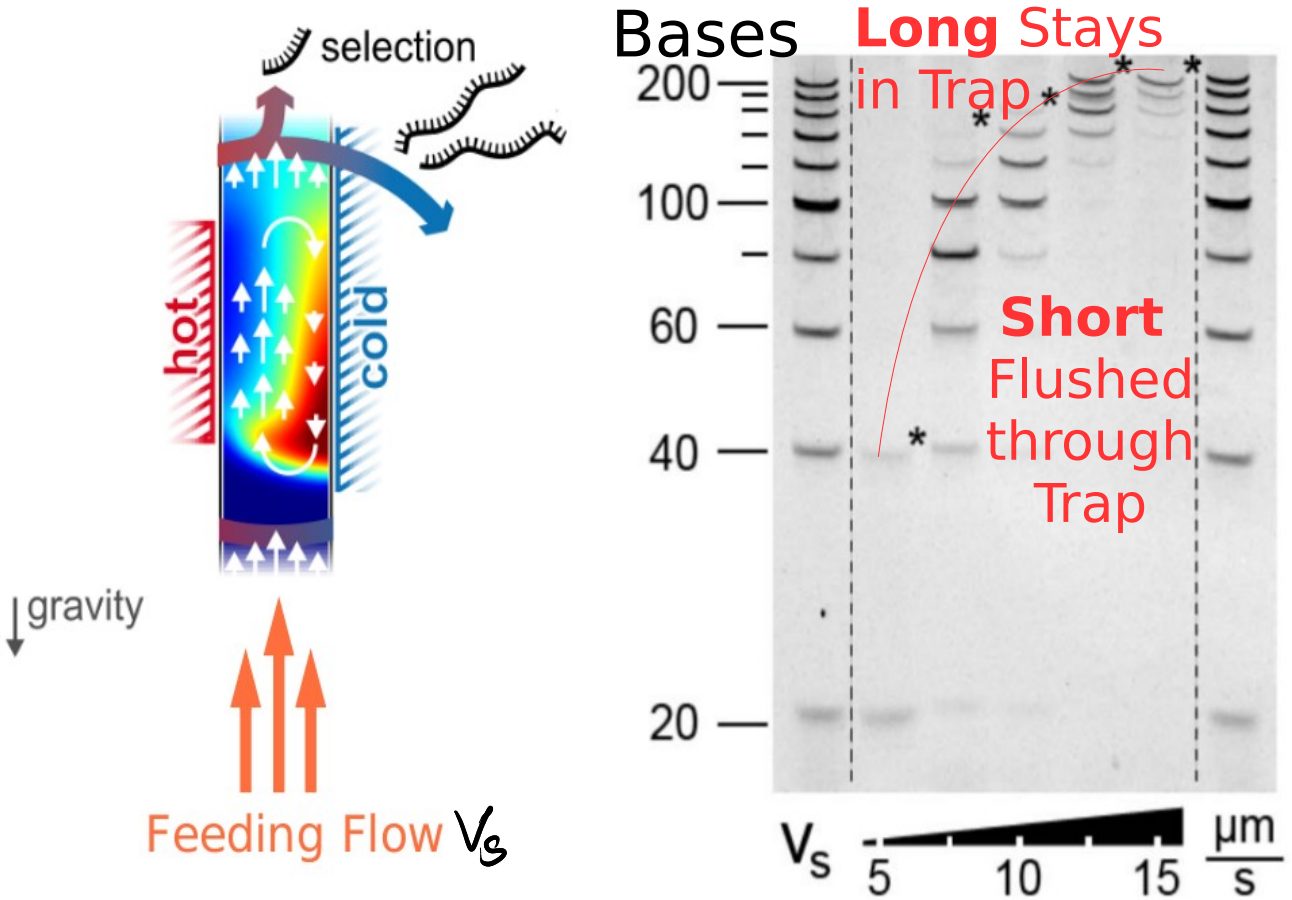


- Mast & Braun, PRL, 104, 188102 (2010)

DNA Length Selection

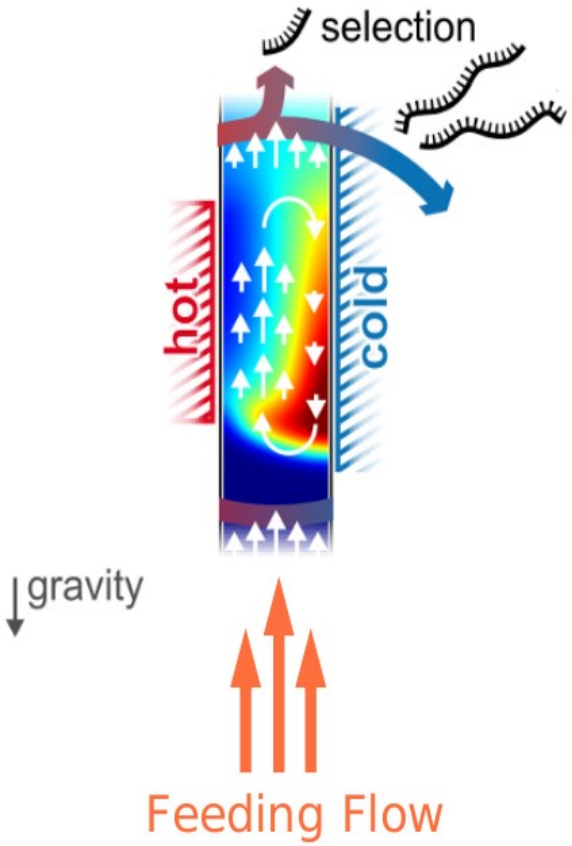


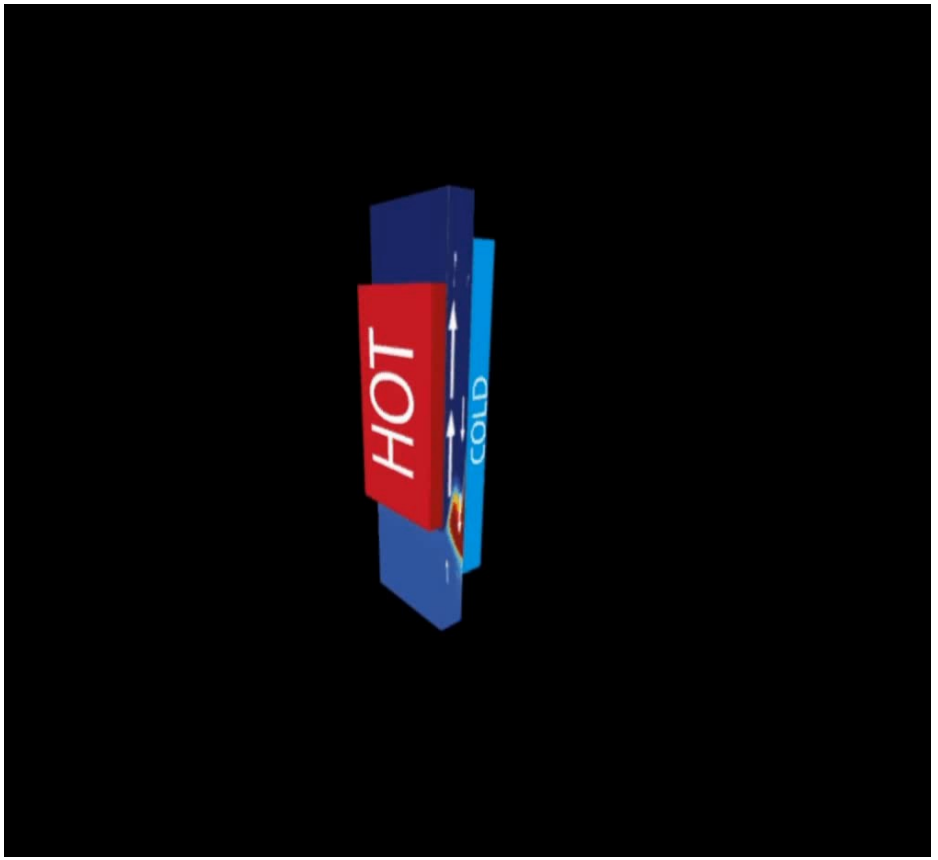
DNA Length Selection



Kreysing, Keil, Lanzmich & Braun, Nature Chemistry 2015

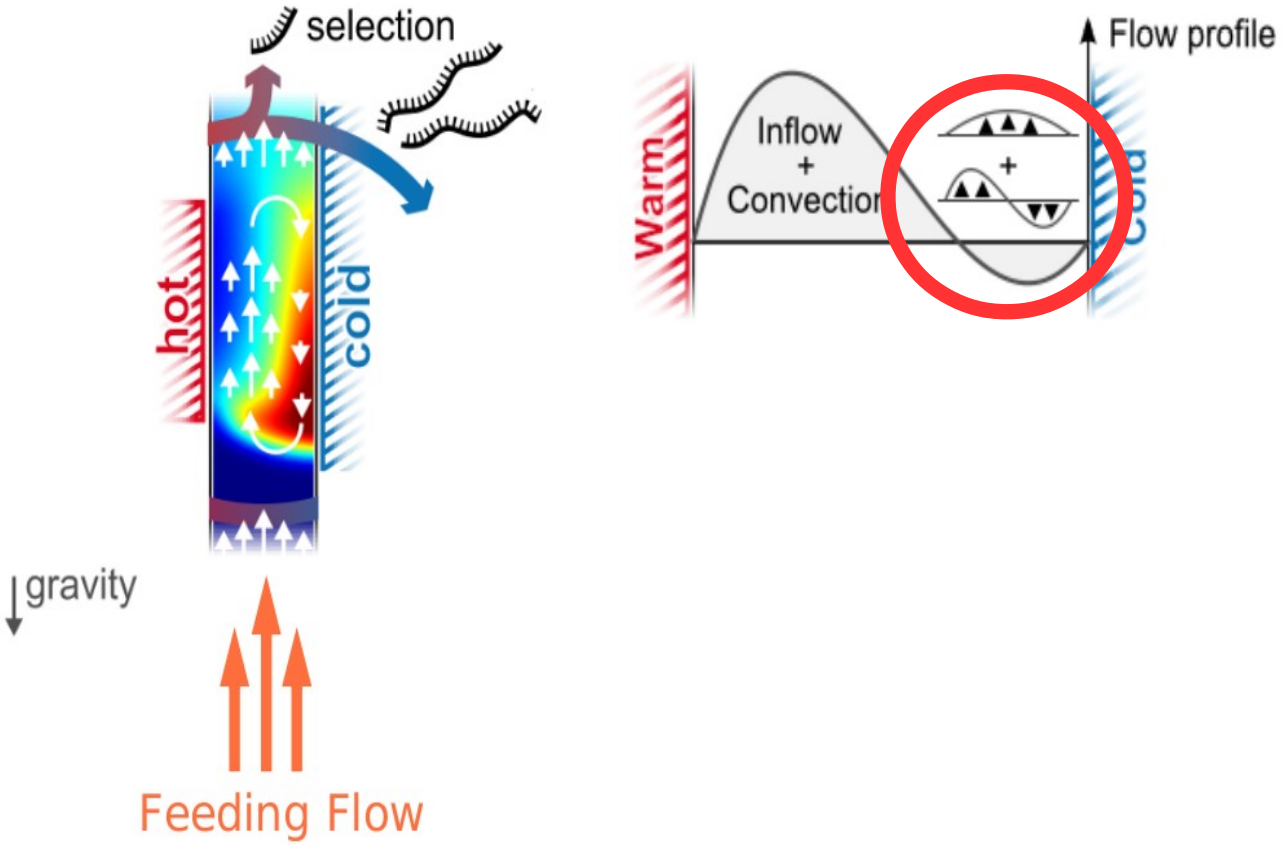
DNA Length Selection





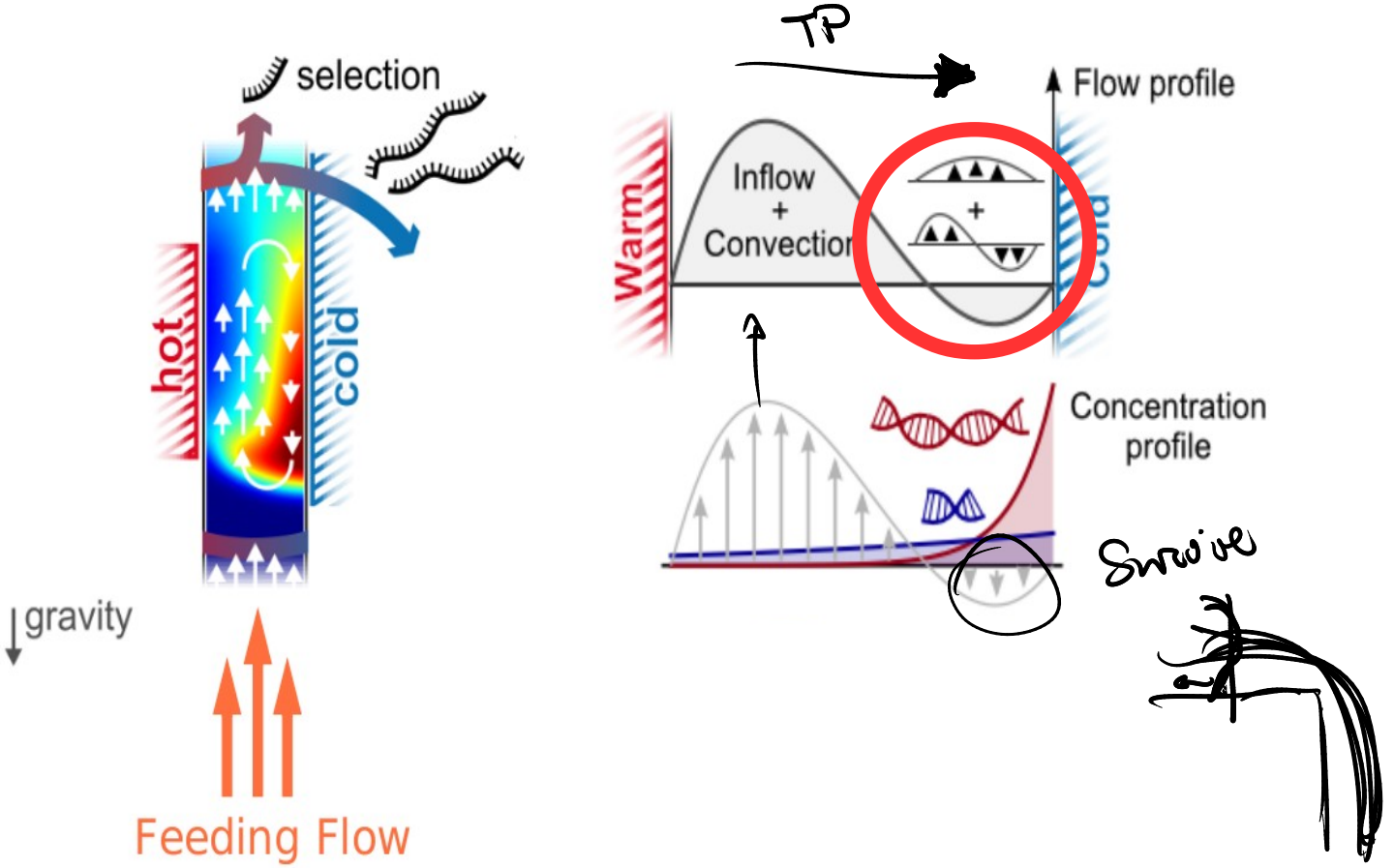
DNA 75bp 36bp

DNA Length Selection



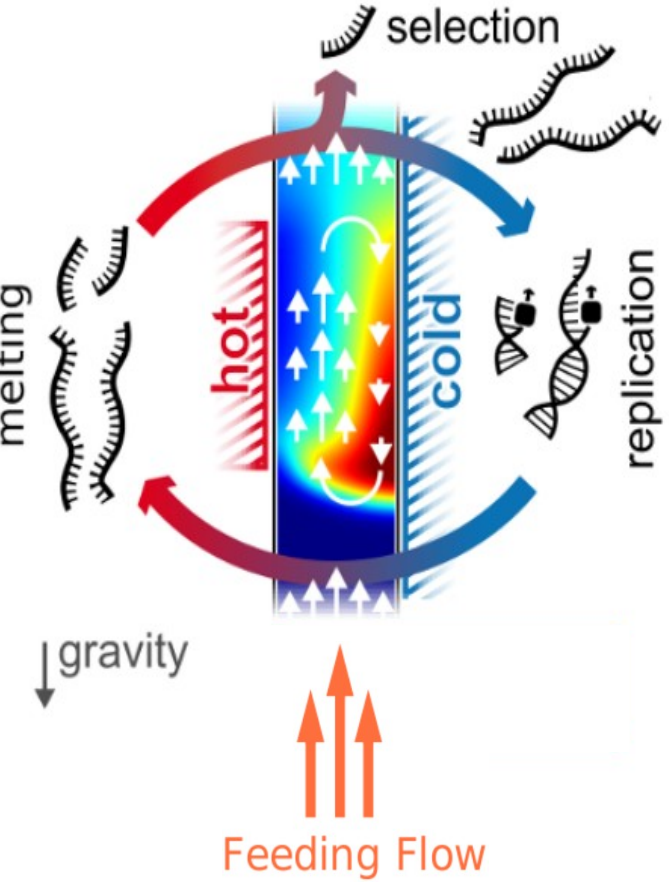
Kreysing, Keil, Lanzmich & Braun, Nature Chemistry 2015

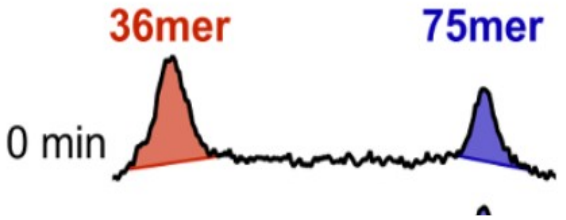
DNA Length Selection



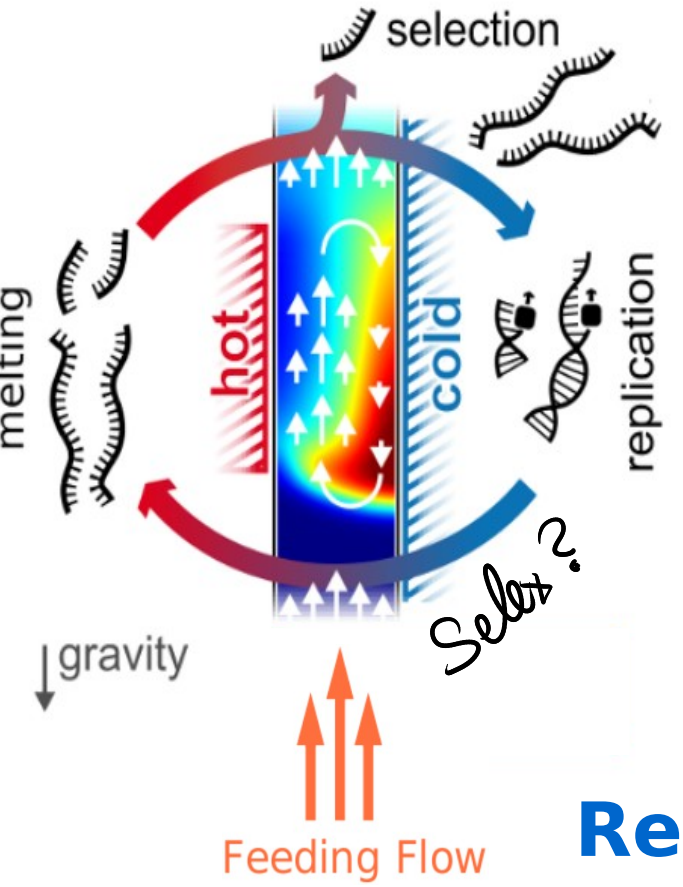
Kreysing, Keil, Lanzmich & Braun, Nature Chemistry 2015

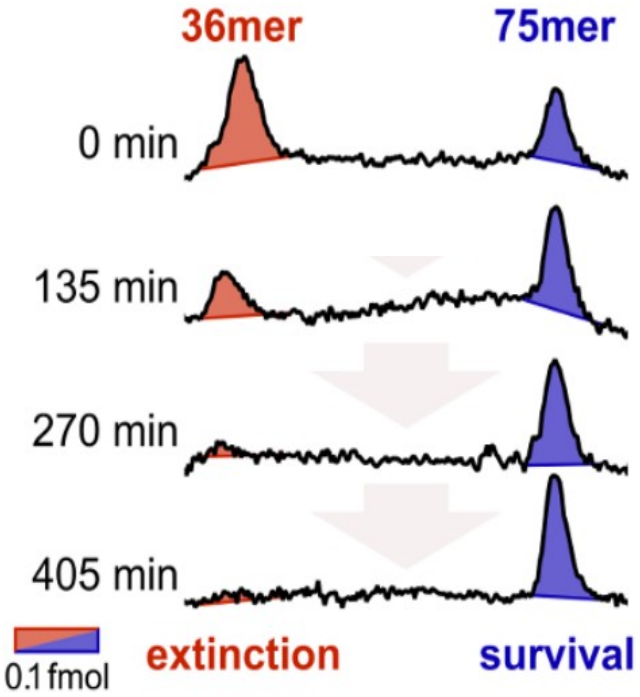
Replication and Length Selection





Replication and Length Selection

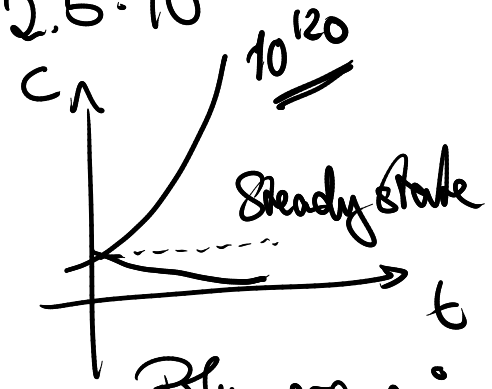




1 Min : 2x Replication
E-Coli : 20 Min 2x Repl.

2^{400} fold Replication

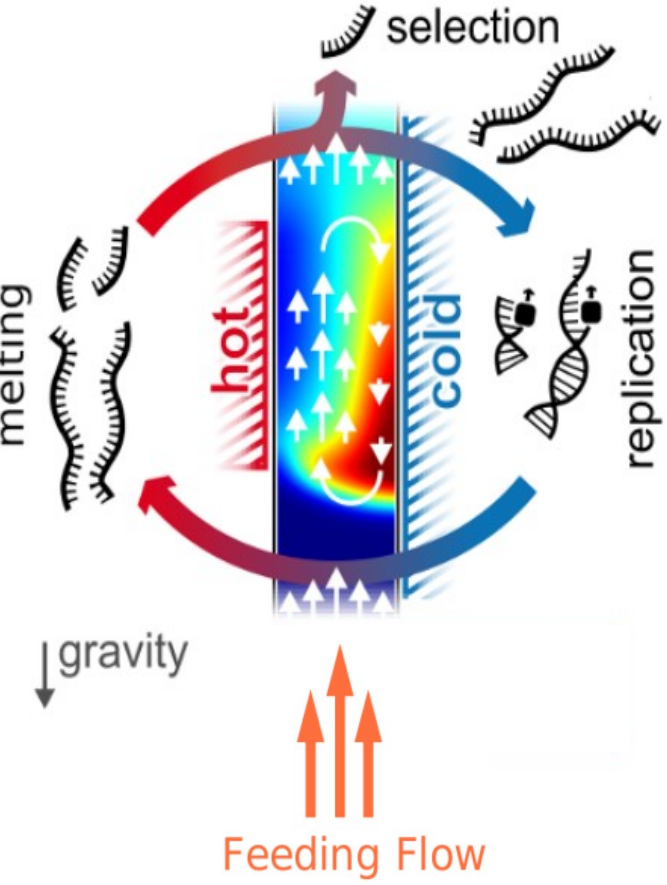
$= 2.5 \cdot 10^{120}$



Polymerase is too precise.

Replication and Selection of the Largest

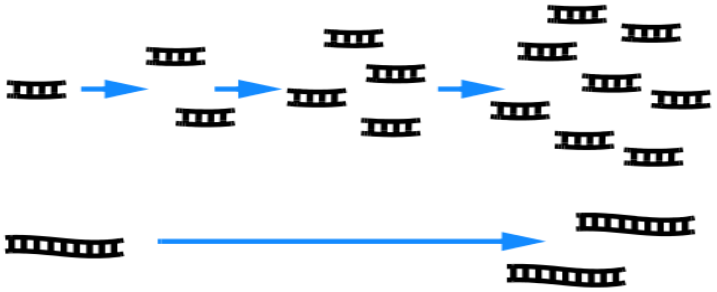
Replication and Length Selection



60° 2AI-Nt $MgCl_2$

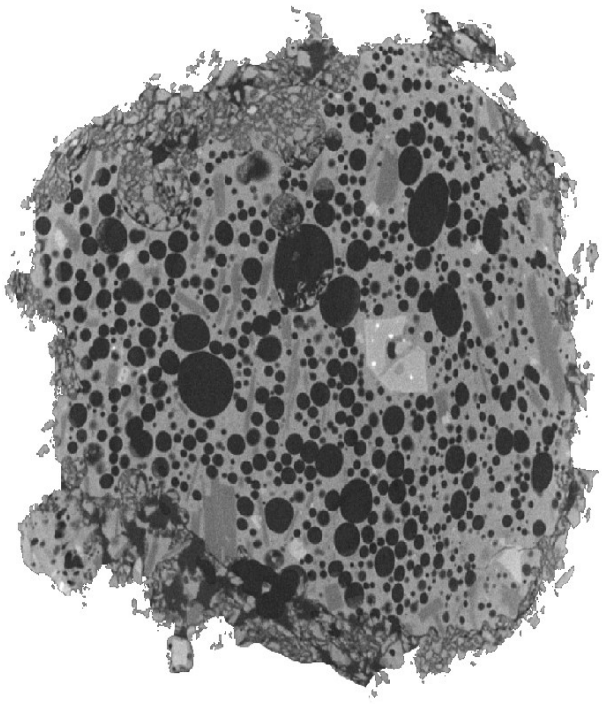
RNA → DNA

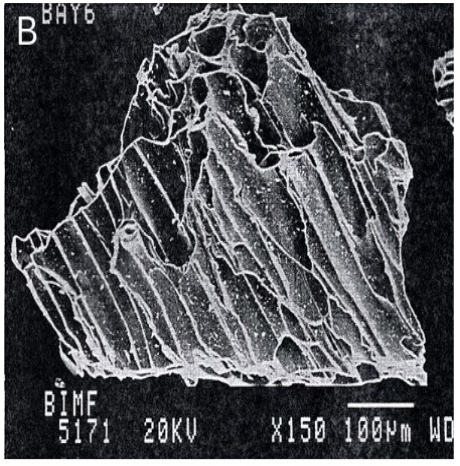
Overcomes
the tyranny of
the shortest

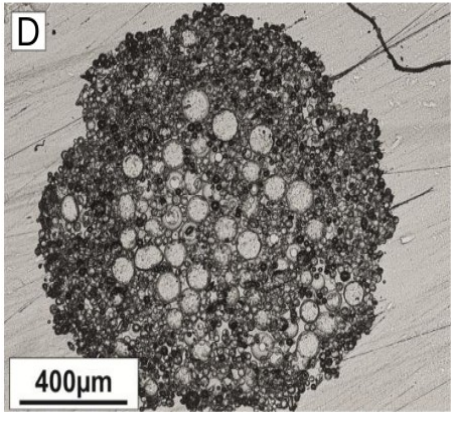


Spiegelman 1967

1. How can the **molecular feedstock** be chaperoned by early rocks?



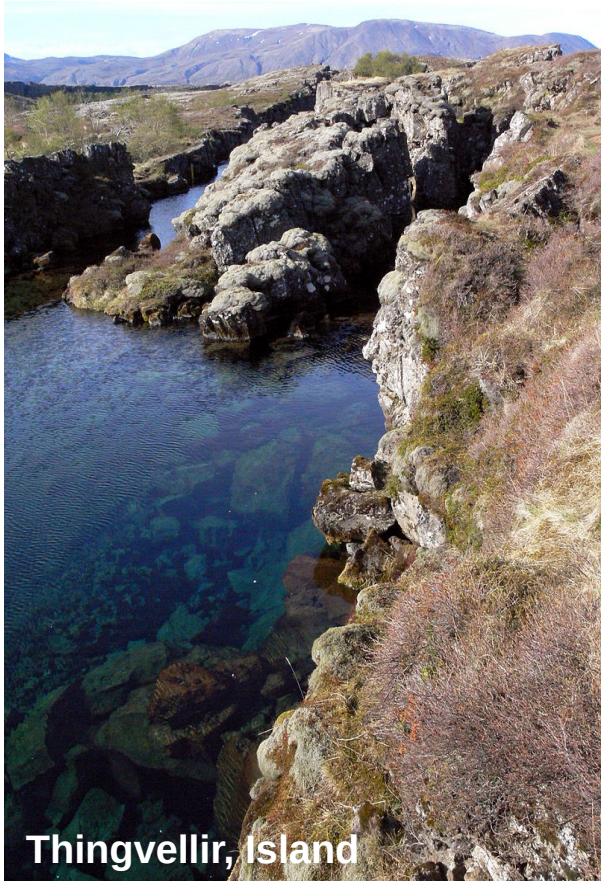


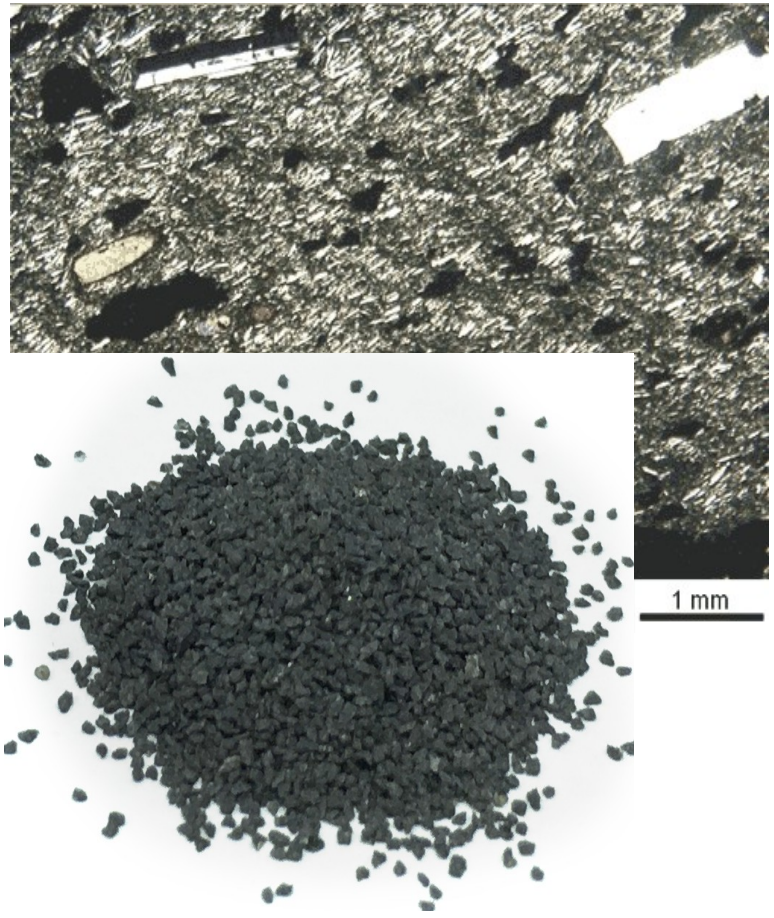


Natural vs. synthetic volcanic rocks

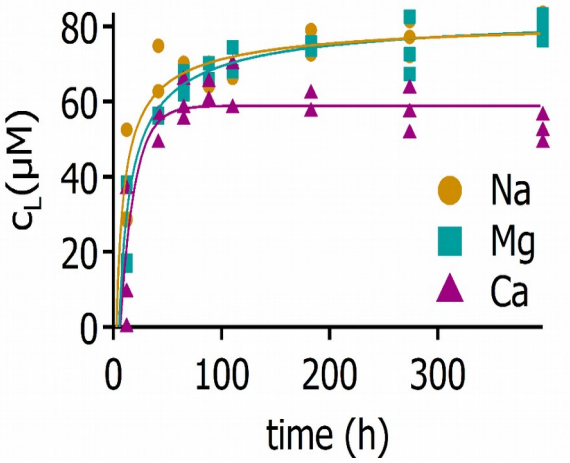
Tholeitic Basalt

most common igneous rocks on Earth,
produced by submarine volcanism


Thingvellir, Island



Salt leaching from tholeitic basalt



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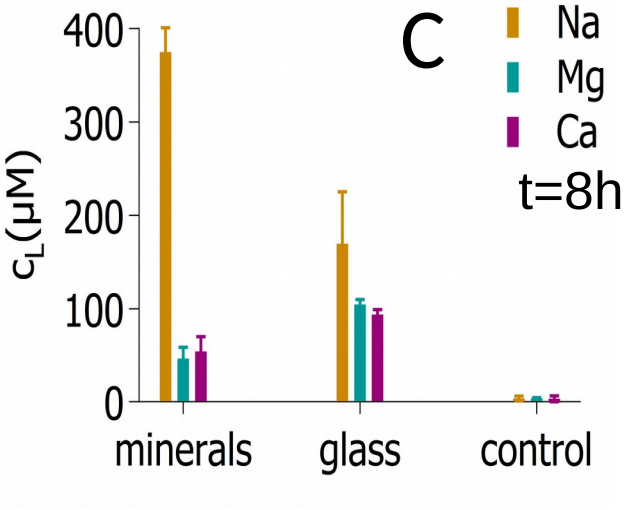
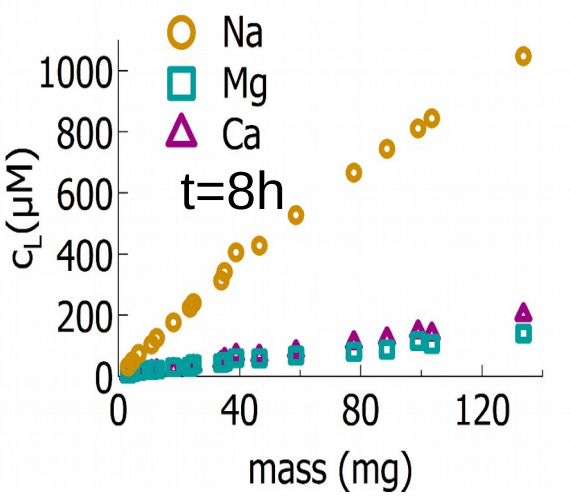


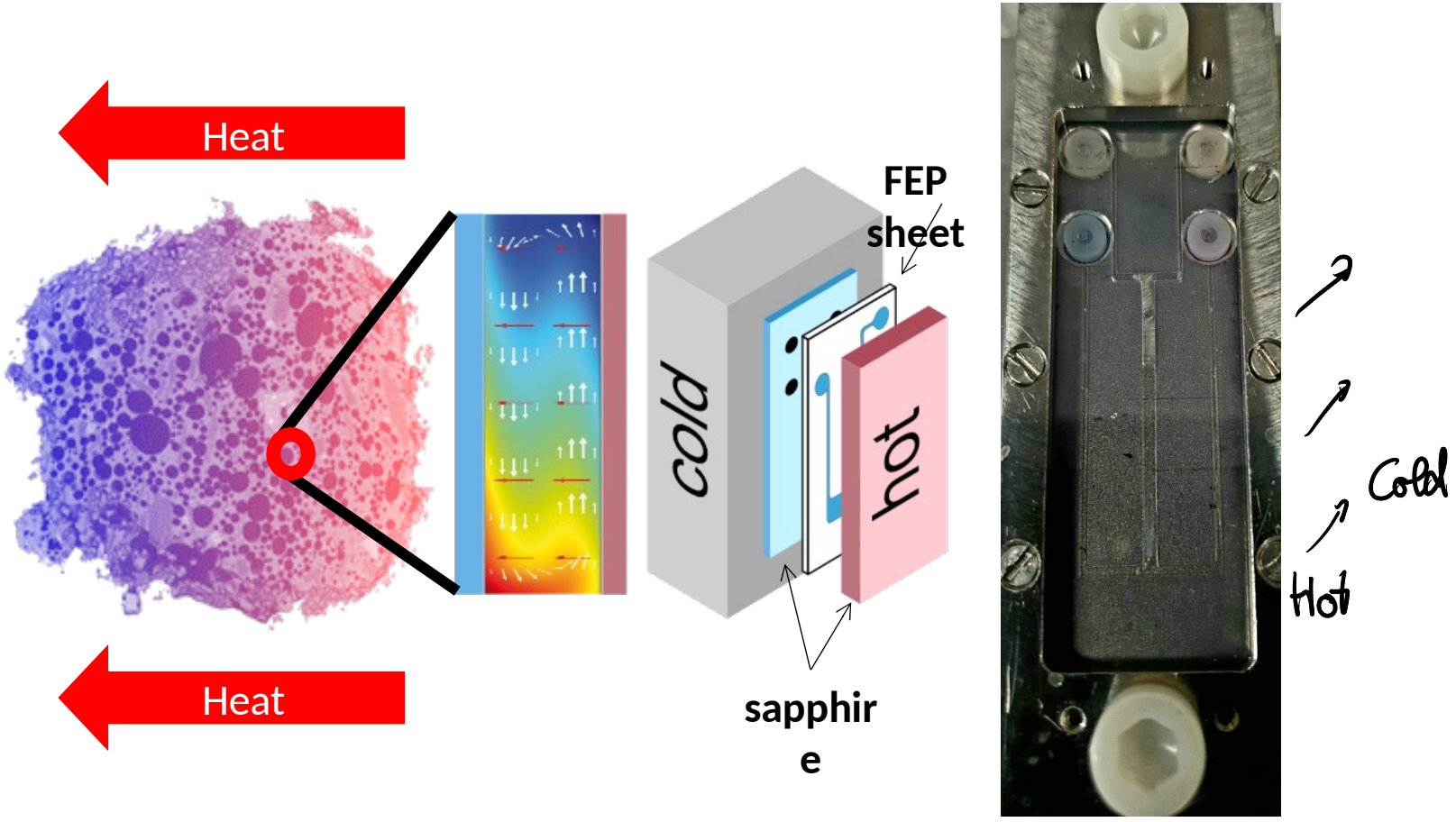


$MgCl_2$: leach 100 mM

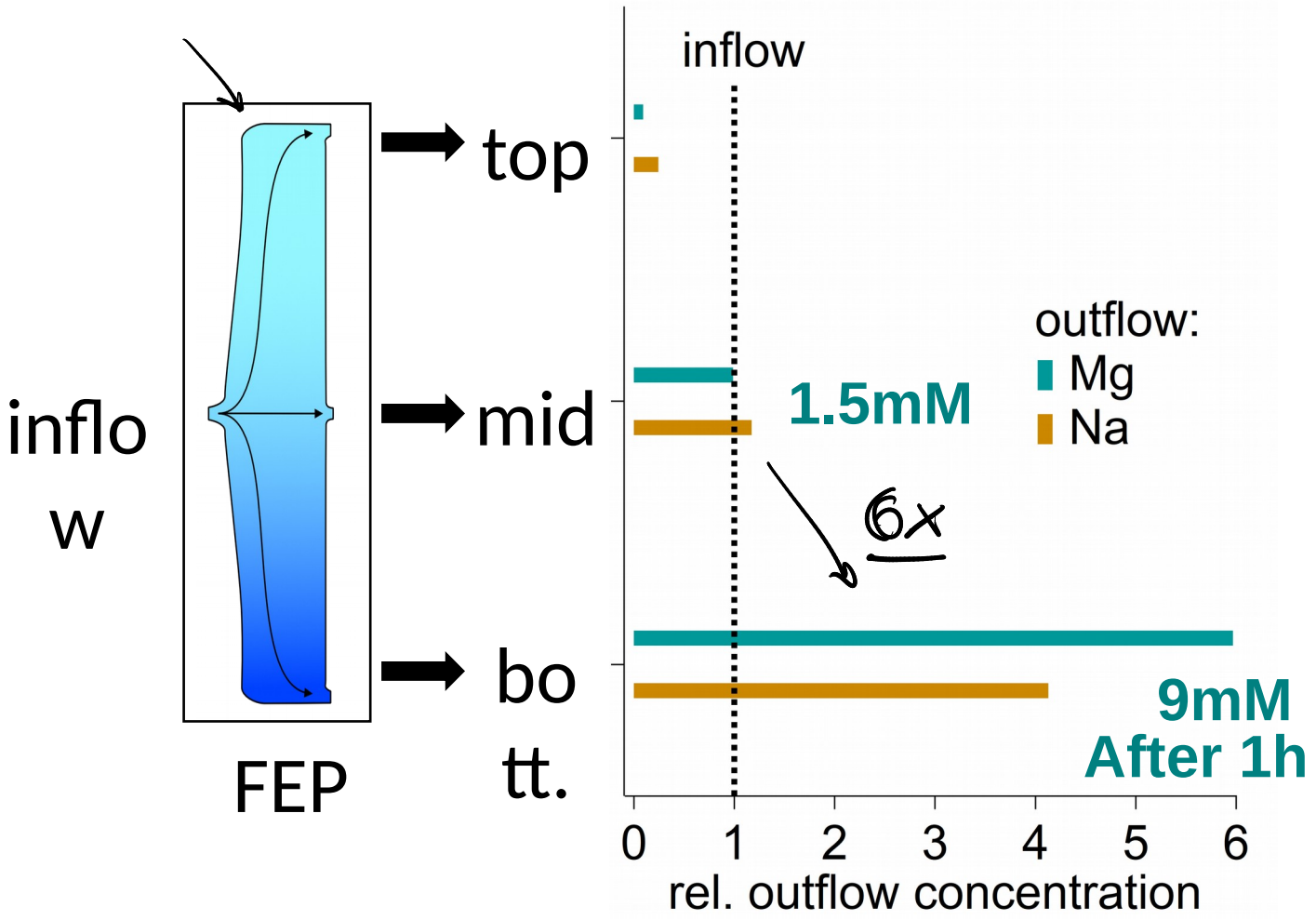
200 mM

100 mM

Thermal traps to accumulate salts

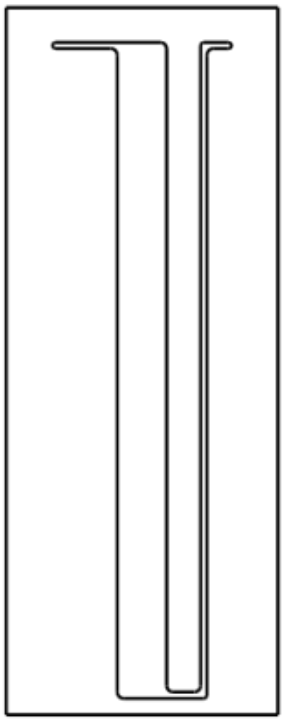


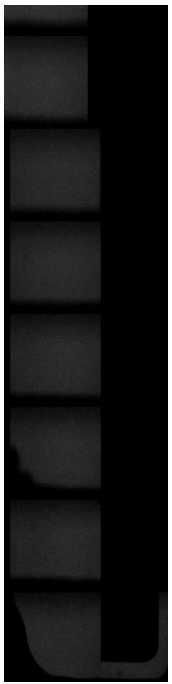
Heat flows separate salt species

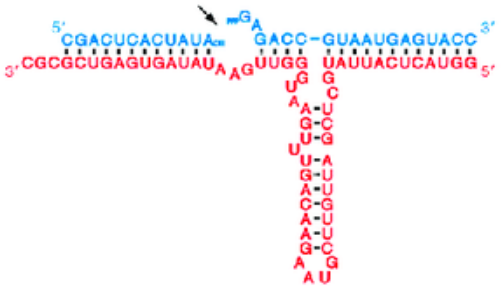


Salty habitat for ligase ribozyme

thermal trap: 20-60°C

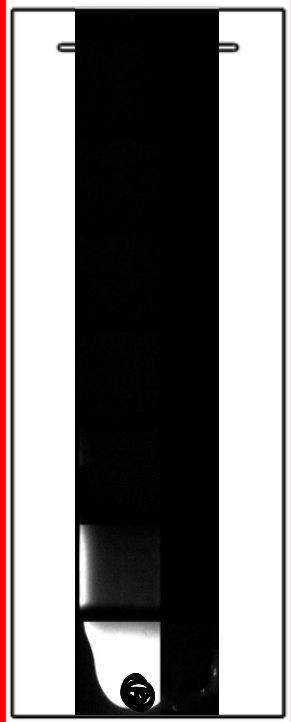


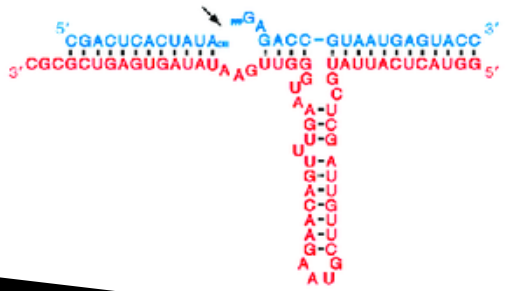




Salty habitat for ligase ribozyme

thermal trap: 20-60°C



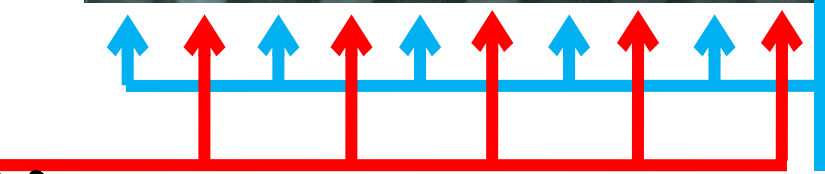


Mg concentration

4 2 1 0.5 0.2 5

10
4
2
S

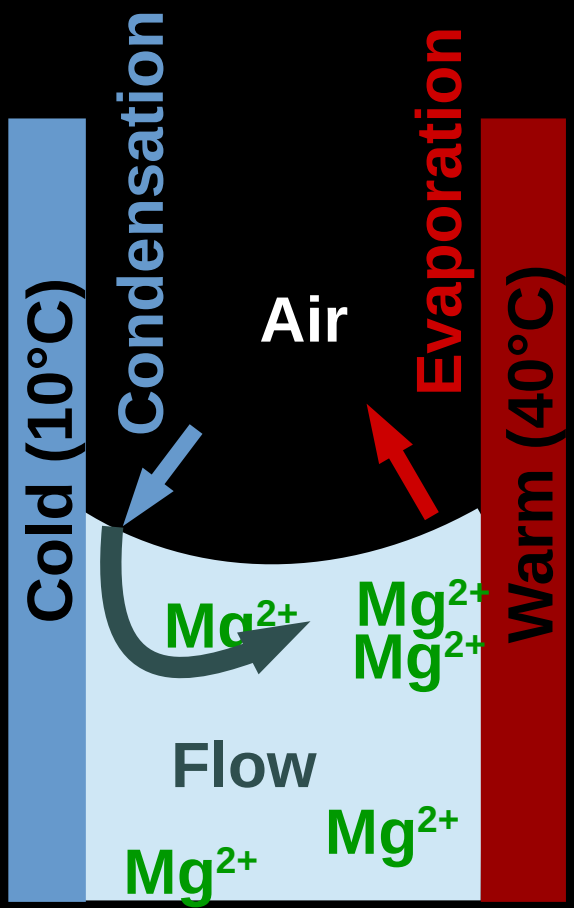
Cy5
Sybr



constant temperature
Topt = 35°C

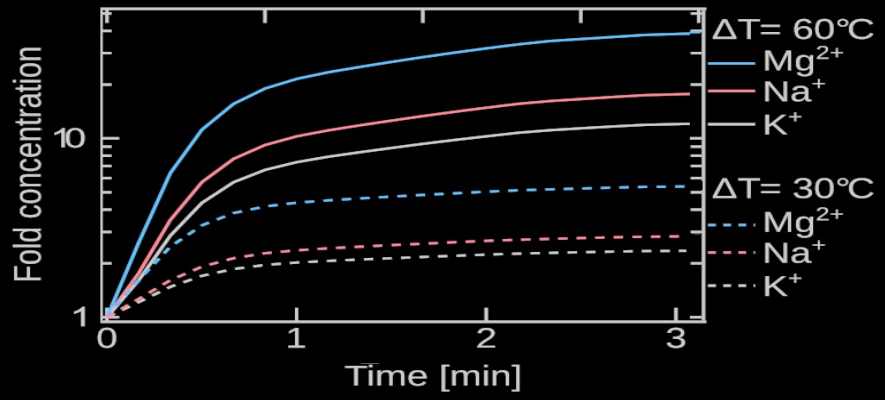
Air bubble

Accumulation at air-water interface



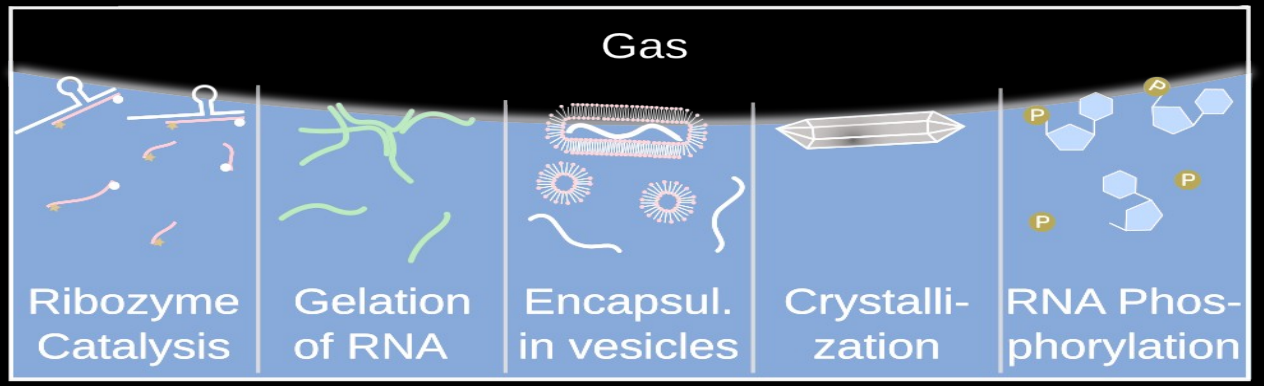
250µm

Magnesium at interface

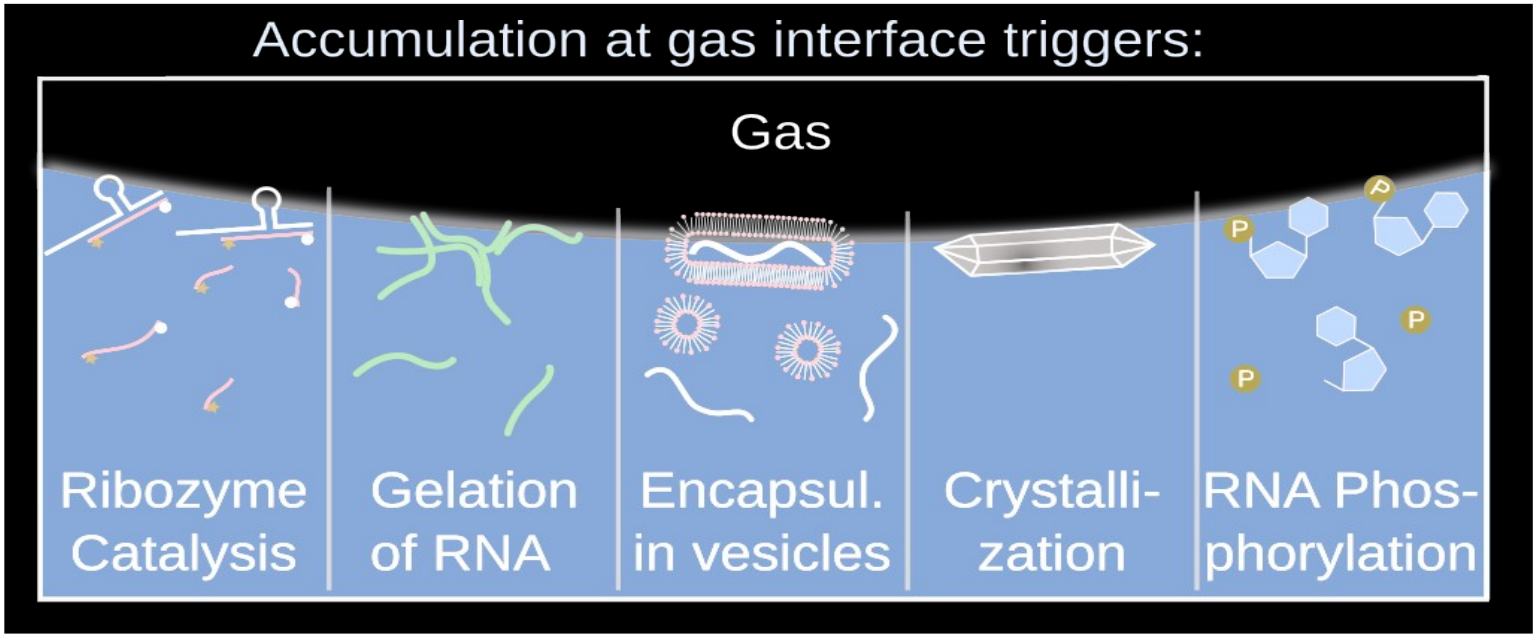


40x

5x



Nature Chemistry, 2019



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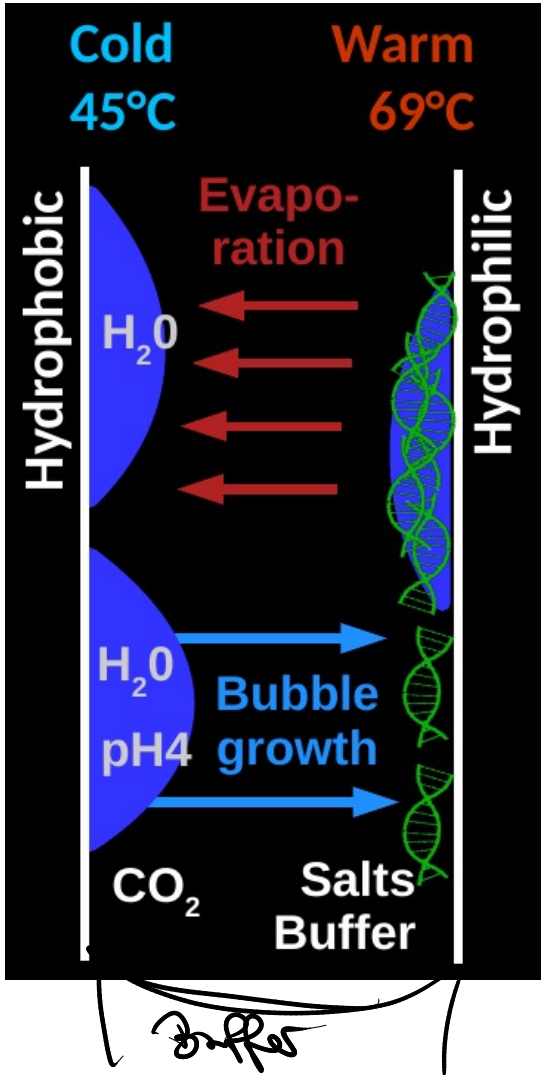
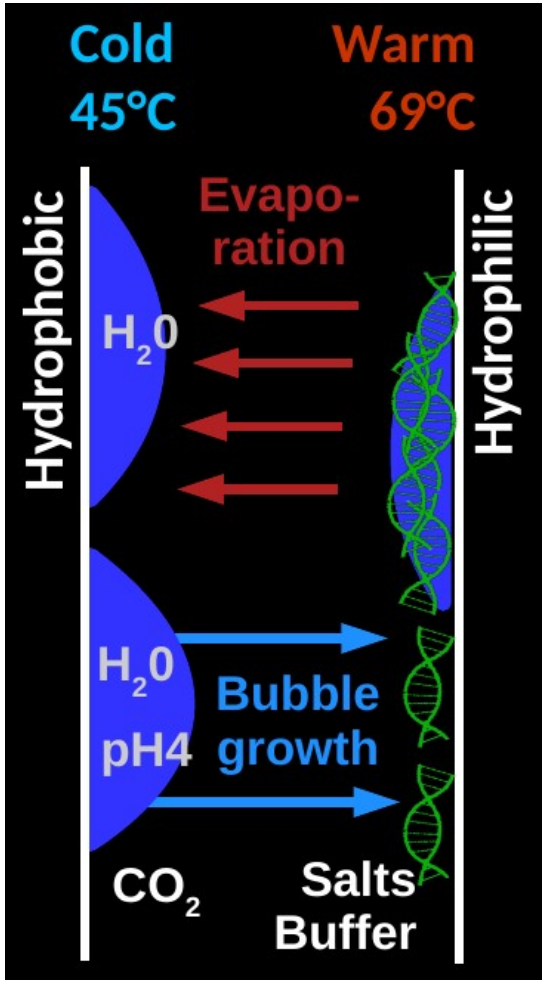
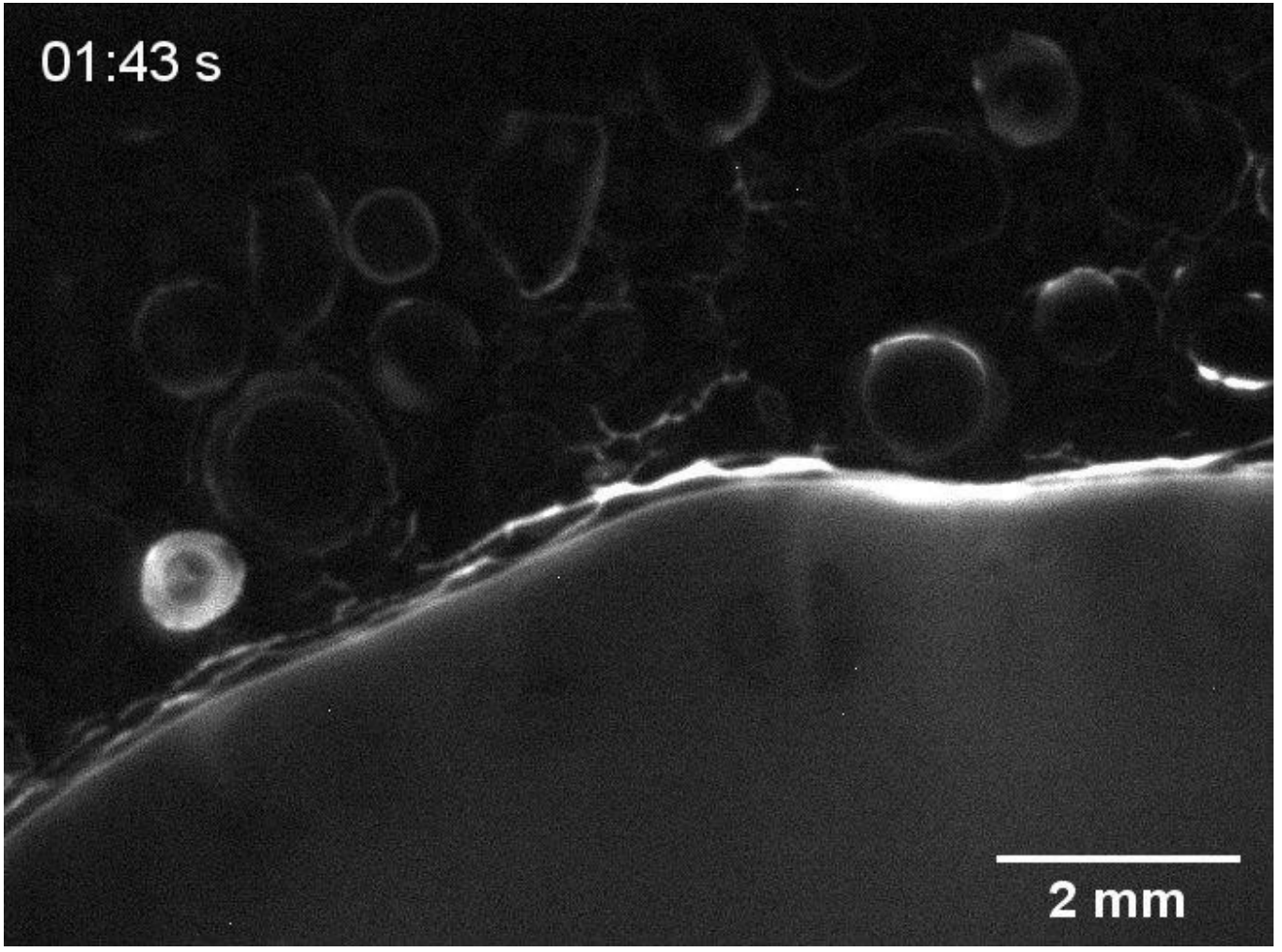
Nature Chemistry 2019

Fog PCR

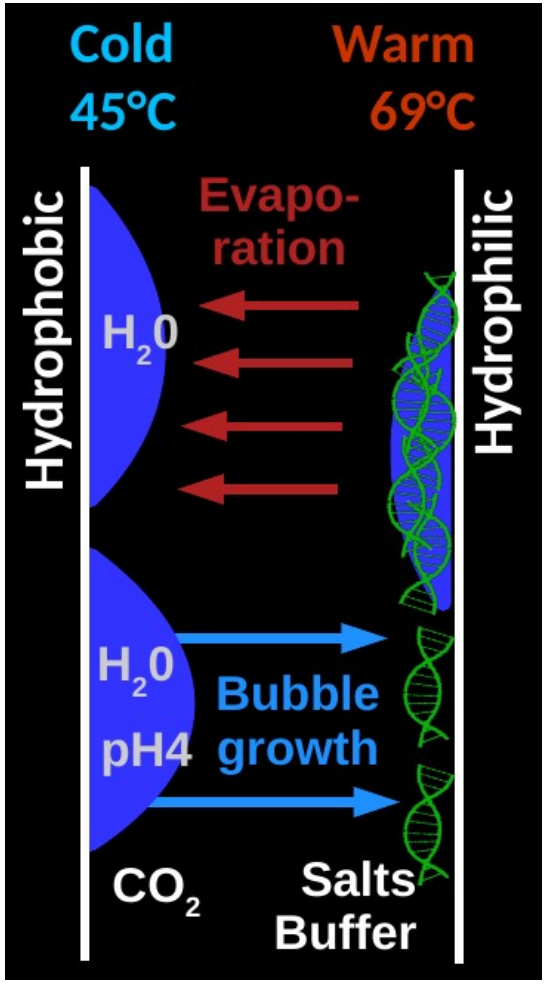

Cold
45°C
Warm
69°C
Hydrophobic
Hydrophilic
Evapo-
ration
H_20
H_20
pH4
Bubble
growth
CO_2
Salts
Buffer

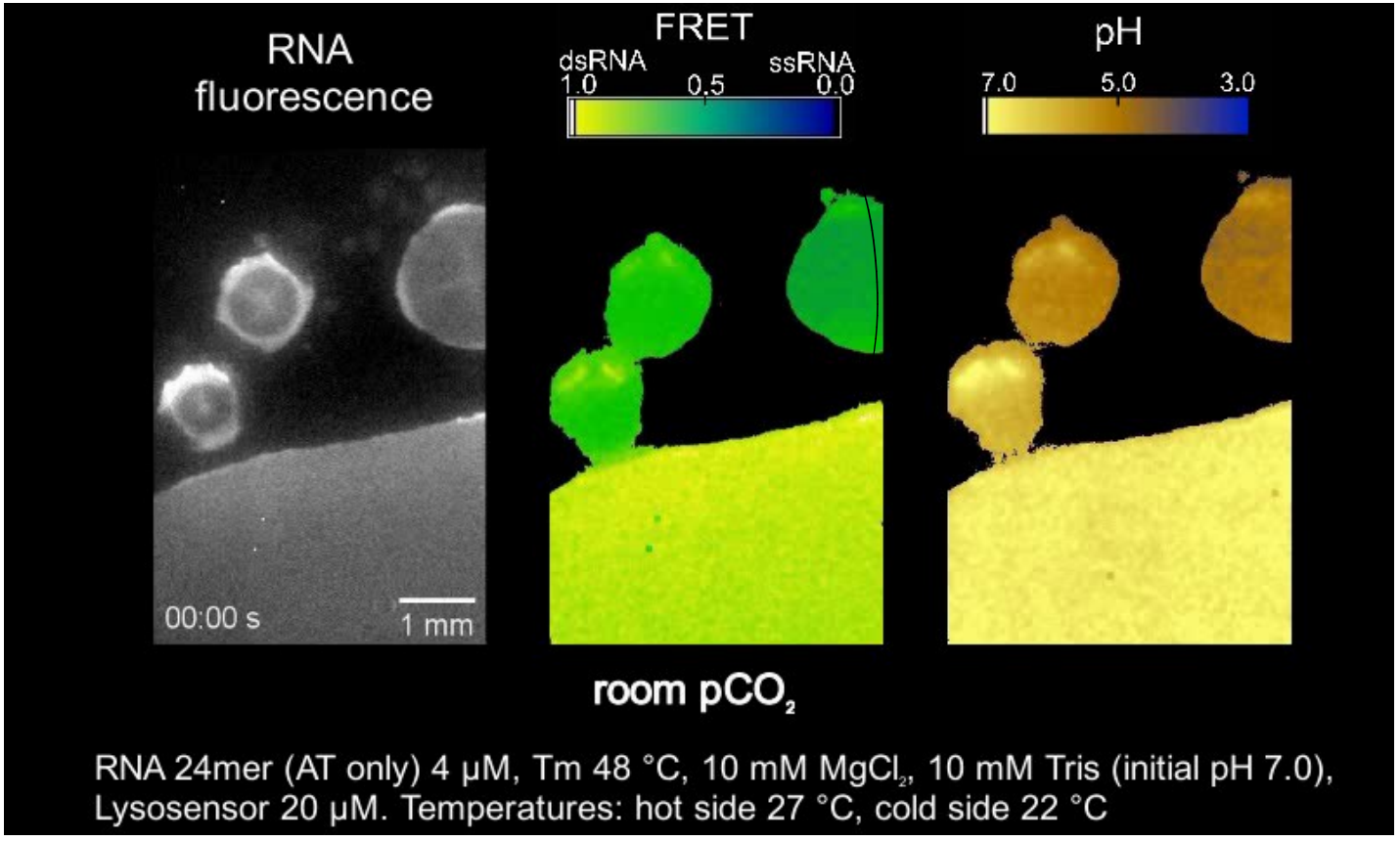
Fog PCR


Cold
45°C
Warm
69°C
Hydrophobic
Hydrophilic
Evapo-
ration
H_20
H_20
pH4
Bubble
growth
CO_2
Salts
Buffer

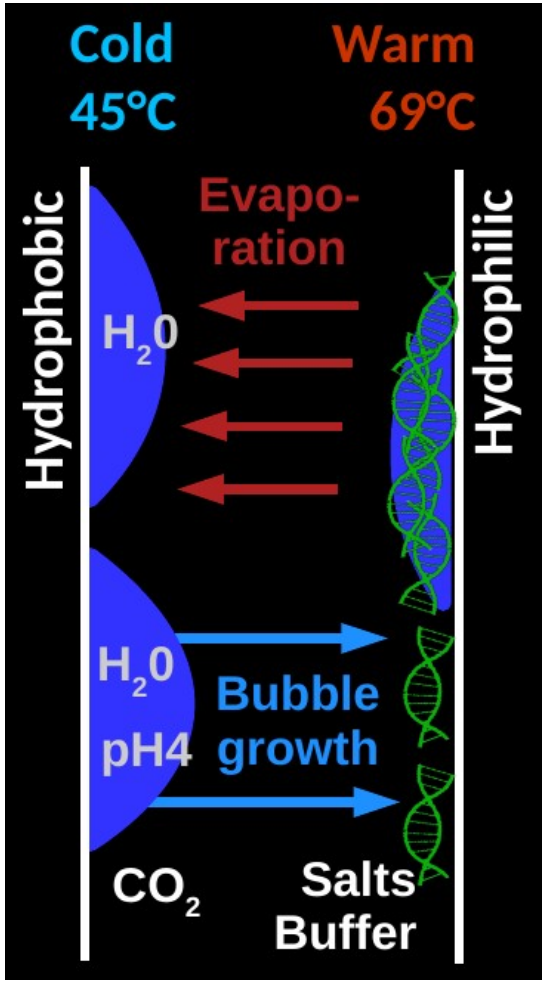

01:43 s
2 mm

Fog PCR

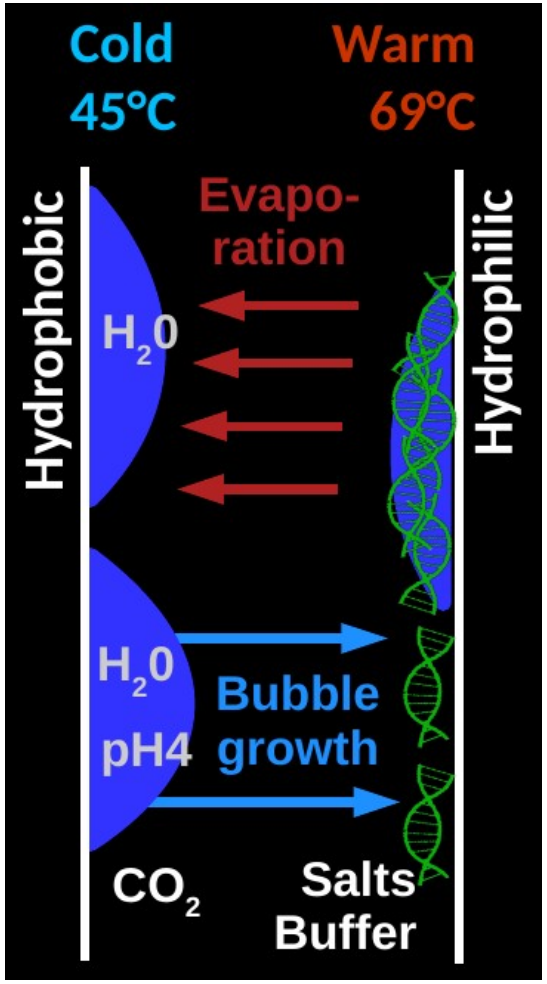

Cold
45°C
Warm
69°C
Hydrophobic
Hydrophilic
Evapo-
ration
H_2O
H_2O
pH4
Bubble
growth
CO_2
Salts
Buffer

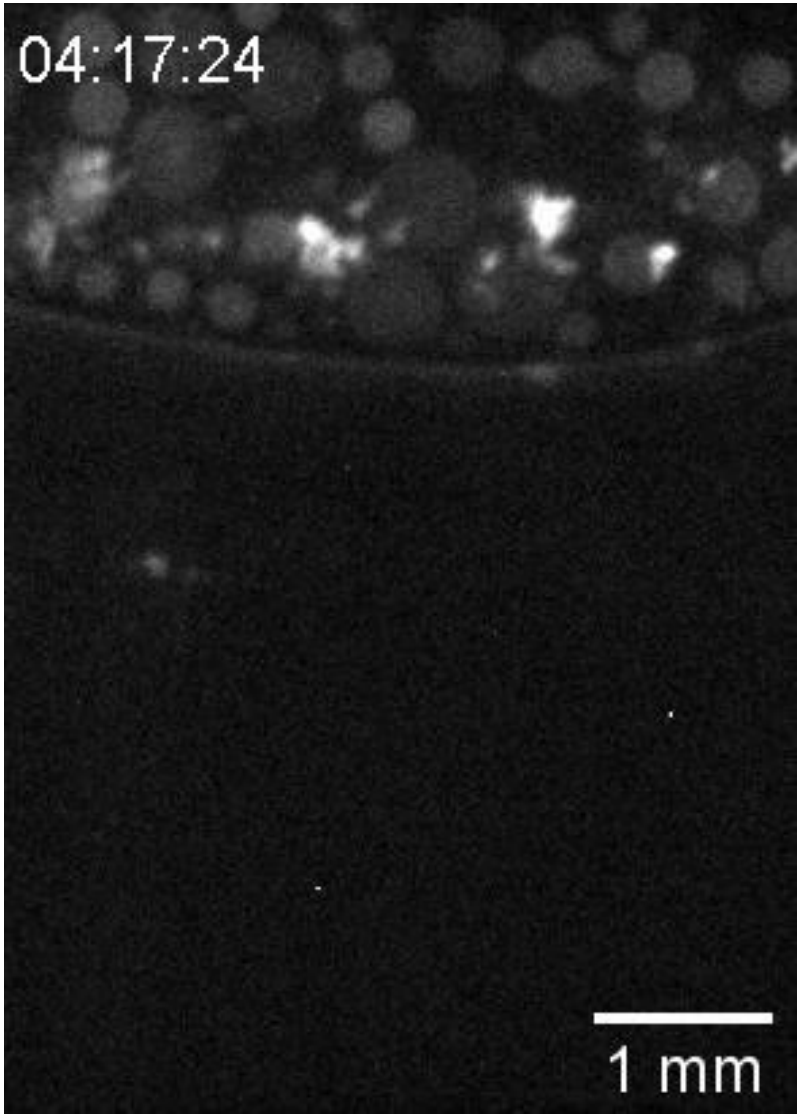

RNA
fluorescence
FRET
dsRNA
ssRNA
1.0
0.5
0.0
pH
7.0
5.0
3.0
00:00 s
1 mm
room pCO_2
RNA 24mer (AT only) 4 µM, Tm 48 °C, 10 mM $MgCl_2$, 10 mM Tris (initial pH 7.0),
Lysosensor 20 µM. Temperatures: hot side 27 °C, cold side 22 °C

Fog PCR


Cold
45°C
Warm
69°C
Hydrophobic
Hydrophilic
Evapo-
ration
H_20
H_20
pH4
Bubble
growth
CO_2
Salts
Buffer

Fog PCR

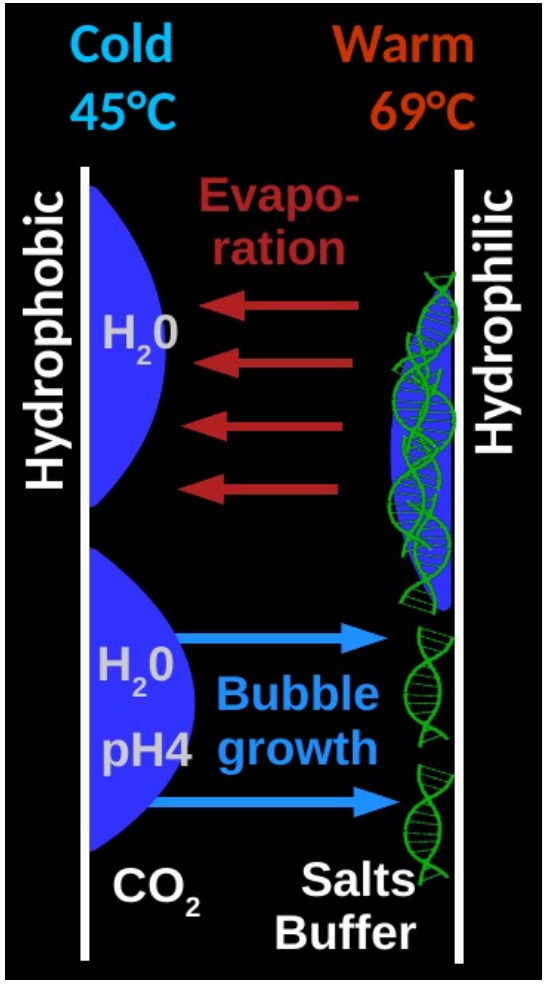


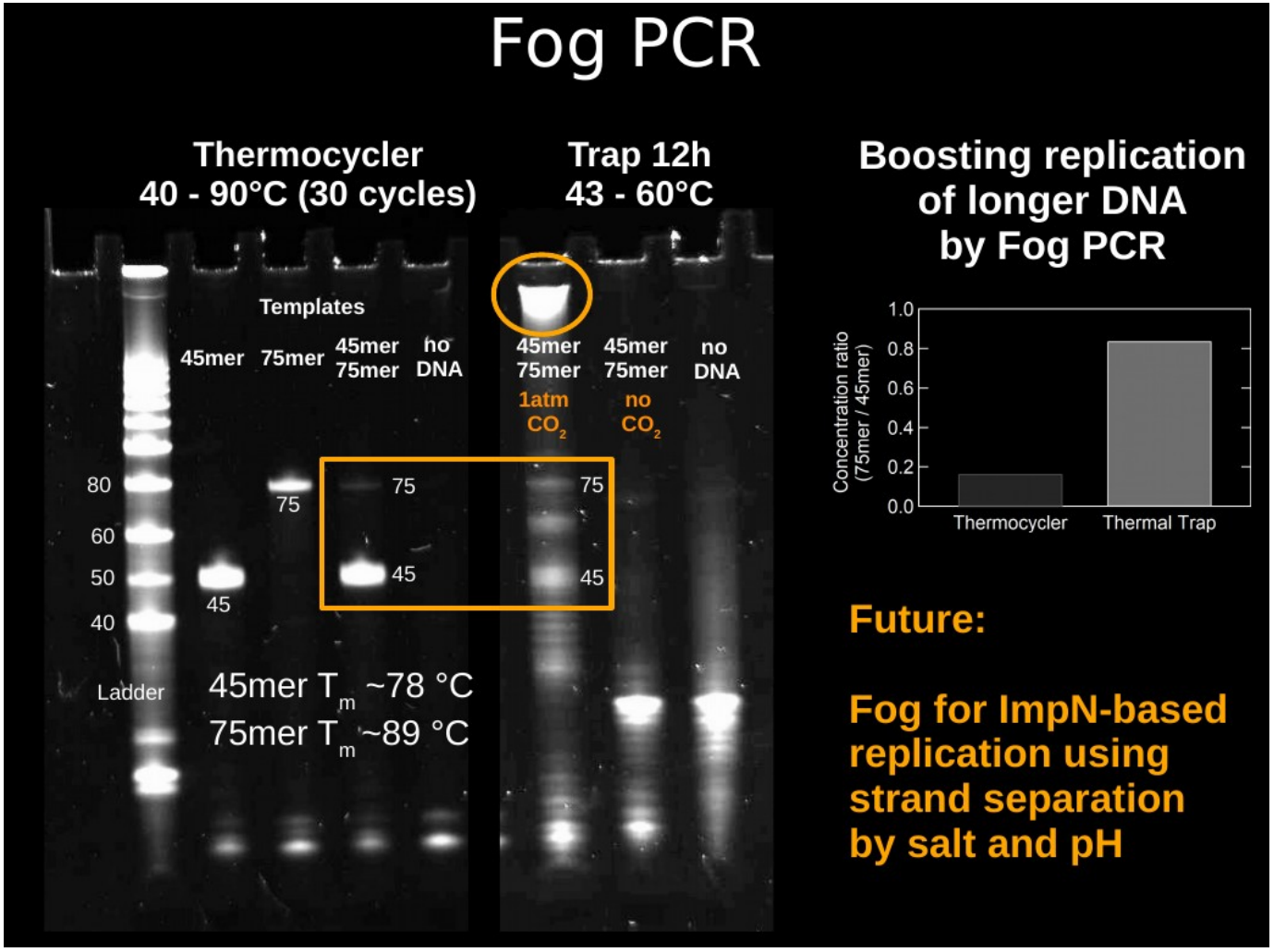


Alan
Ianeselli

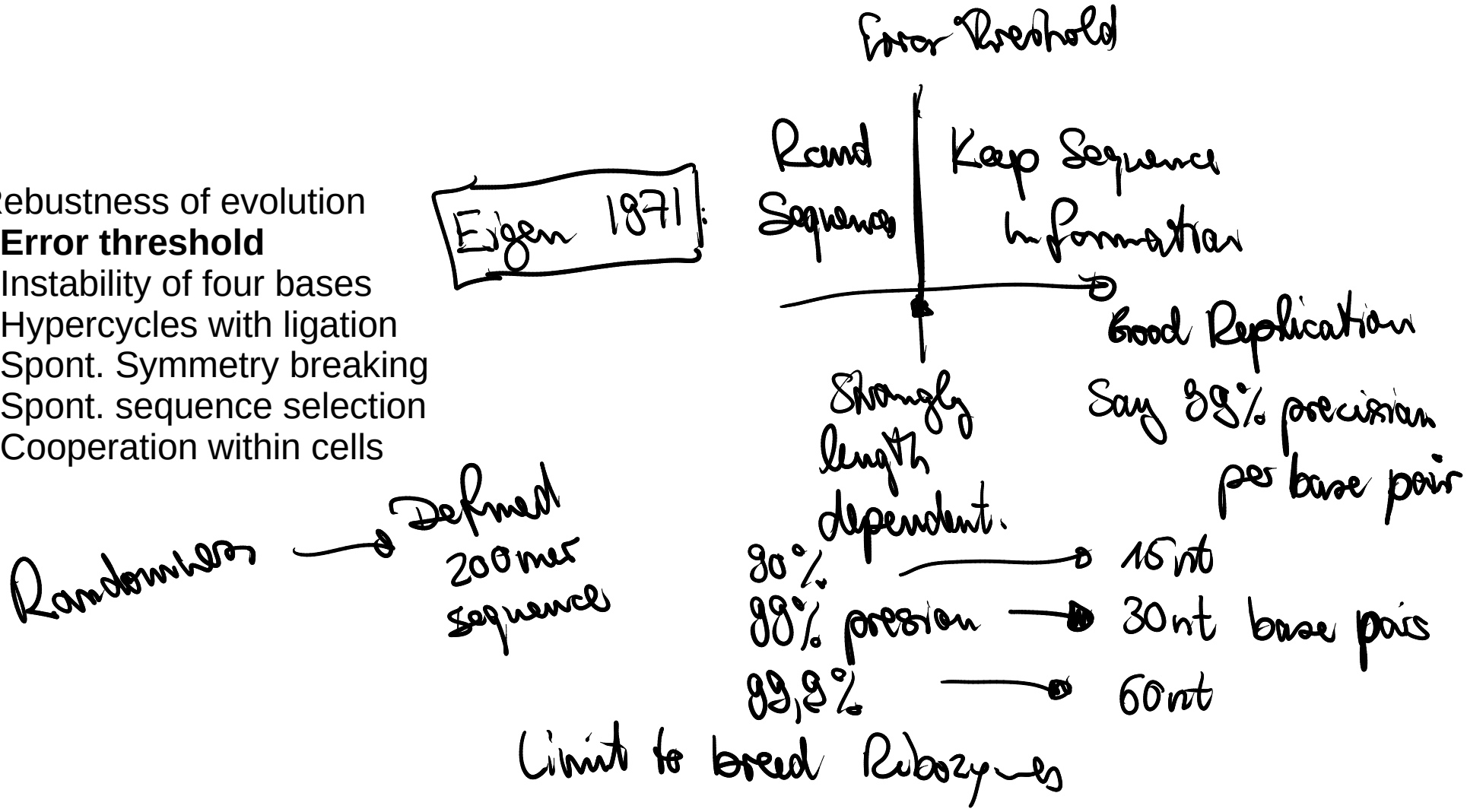
T gradient:
45 °C - 69 °C
51mer T_m = 83 °C
1 bar CO_2
1 nM template DNA
0.5 µM primers
Taq polymerase
1.5 mM $MgCl_2$
0.1% BSA
2x SYBR Green

Fog PCR


Cold
45°C
Warm
69°C
Hydrophobic
Hydrophilic
Evapo-
ration
H_2O
H_2O
pH4
Bubble
growth
CO_2
Salts
Buffer


Fog PCR
Thermocycler
40 - 90°C (30 cycles)
Trap 12h
43 - 60°C
Templates
45mer
75mer
45mer
75mer
no
DNA
45mer
75mer
1atm
CO_2
45mer
75mer
no
CO_2
no
DNA
80
60
50
40
Ladder
45
75
75
45
75
45
45mer T_m ~78 °C
75mer T_m ~89 °C
Boosting replication
of longer DNA
by Fog PCR
Concentration ratio
(75mer / 45mer)
1.0
0.8
0.6
0.4
0.2
0.0
Thermocycler
Thermal Trap
Future:
Fog for ImpN-based
replication using
strand separation
by salt and pH

Rebustness of evolution
- **Error threshold**
- Instability of four bases
- Hypercycles with ligation
- Spont. Symmetry breaking
- Spont. sequence selection
- Cooperation within cells



Rebustness of evolution
- Error threshold
- **Instability of four bases**
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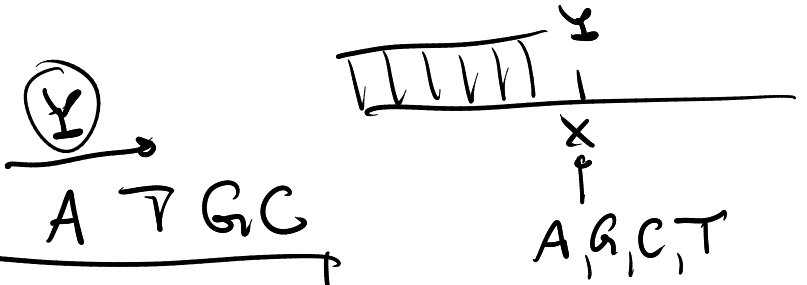
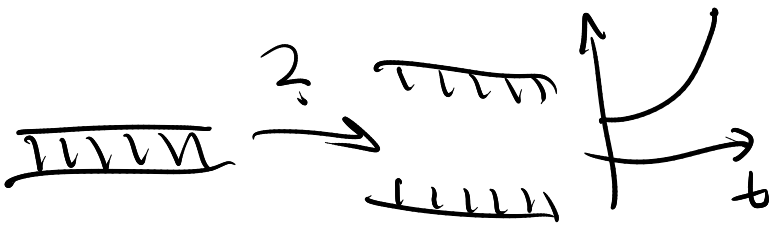


Ideal word



GC $\xrightarrow{a}$ AT

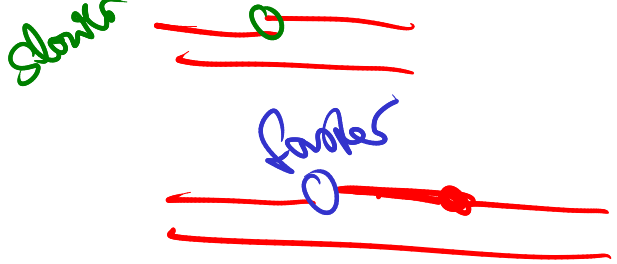
AT $\xrightarrow{g}$ GC

$a \neq g$

ore unlikely.

~ GCAT → GC only sequence.

~ Start with a 2 base code. ~ Ribozyme

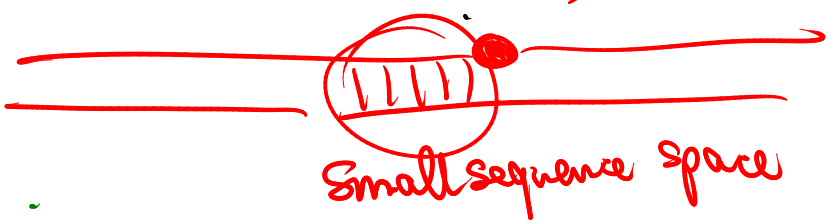


Random Sequences

Rebustness of evolution
- Error threshold
- Instability of four bases
- **Hypercycles with ligation**
- Spont. Symmetry breaking
- Spont. sequence selection
- Cooperation within cells

Reduction of sequence entropy

Ligation

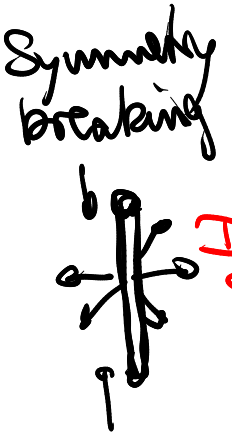


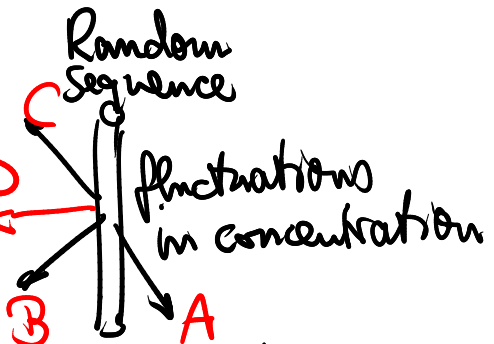
Thermo-dynamics dominance

Dead end for evolution

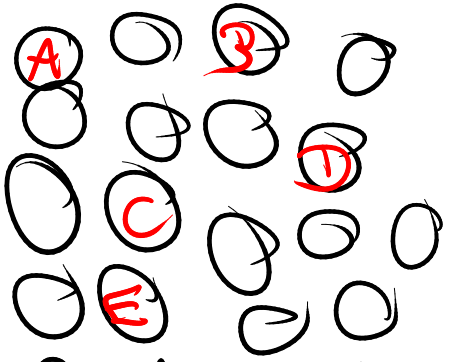
Specific sequence

GCGCGCGC
GGGGGGGG





Locations with

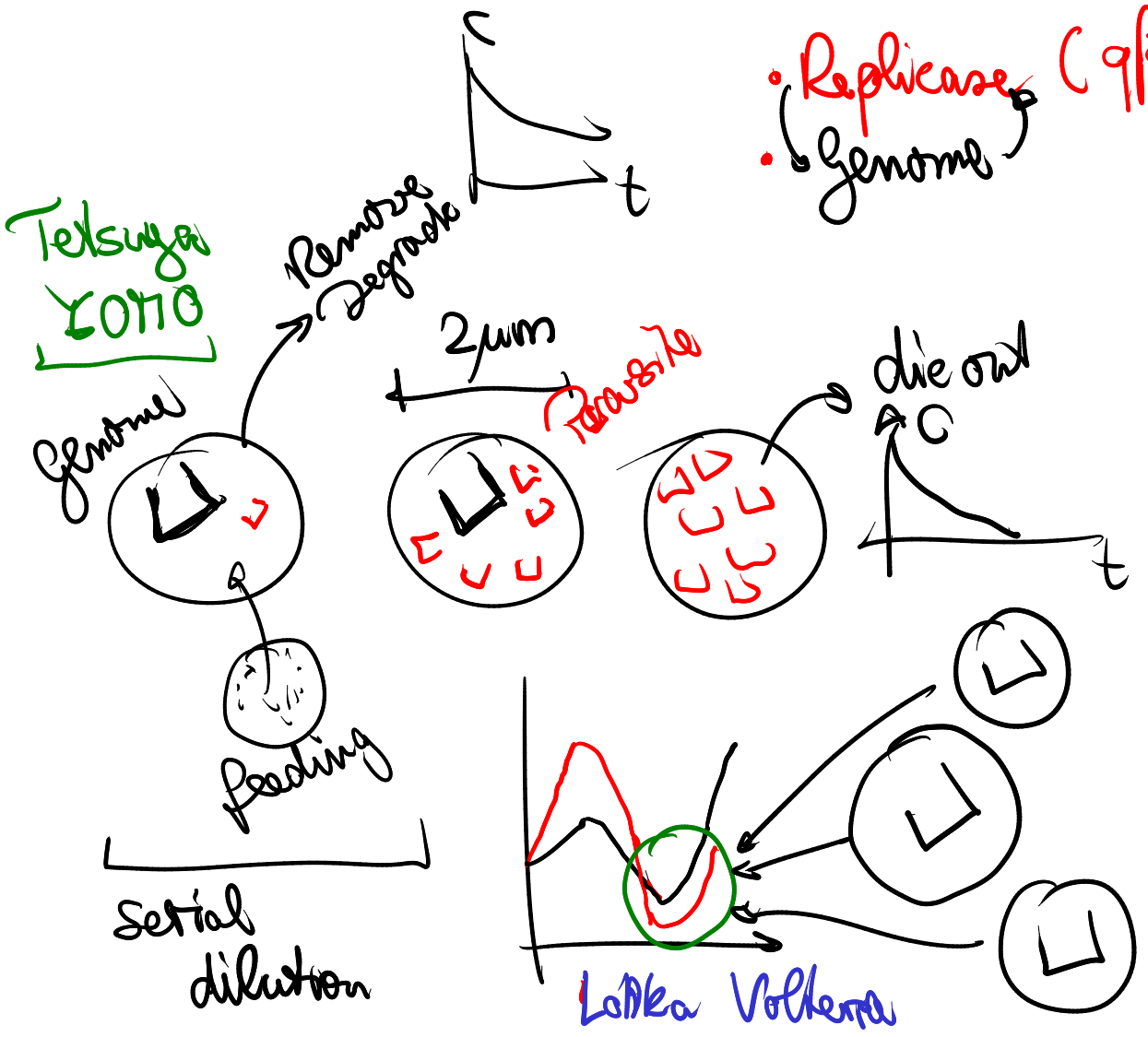


Random initial conditions

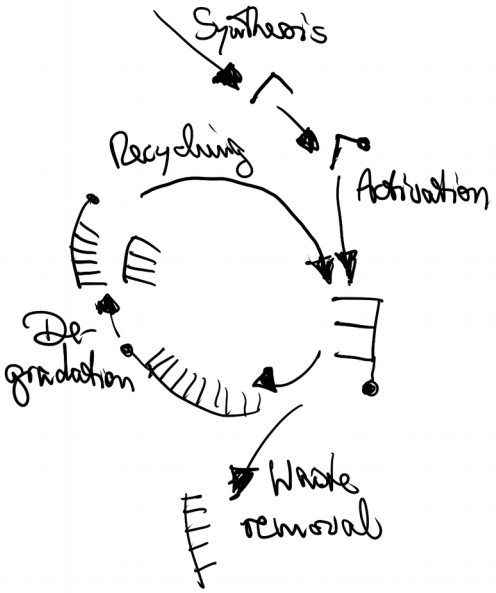
Rebustness of evolution
- Error threshold
- Instability of four bases
- Hypercycles with ligation
- **Spont. Symmetry breaking**
- Spont. sequence selection
- Cooperation within cells

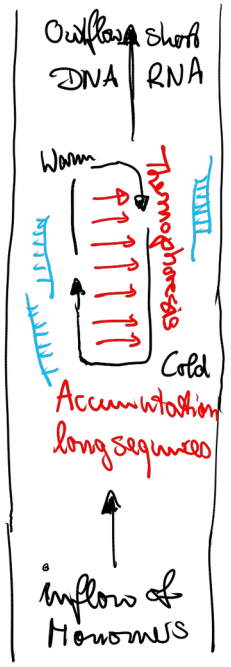
Rebustness of evolution
- Error threshold
- Instability of four bases
- Hypercycles with ligation
- Spont. Symmetry breaking
- **Spont. sequence selection**
- Cooperation within cells

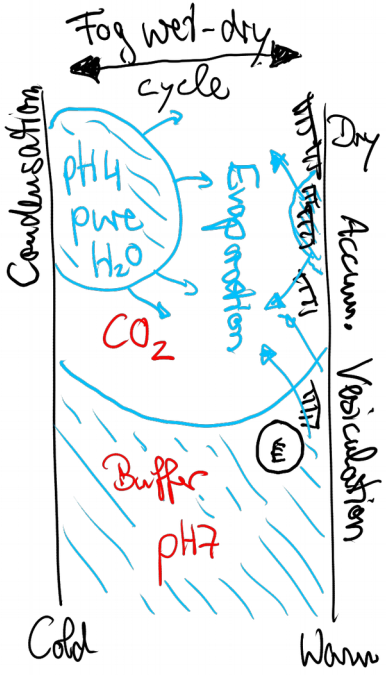
Rebustness of evolution
- Error threshold
- Instability of four bases
- Hypercycles with ligation
- Spont. Symmetry breaking
- Spont. sequence selection
- **Cooperation within cells**



Chemical nonequilibrium in physical nonequilibrium

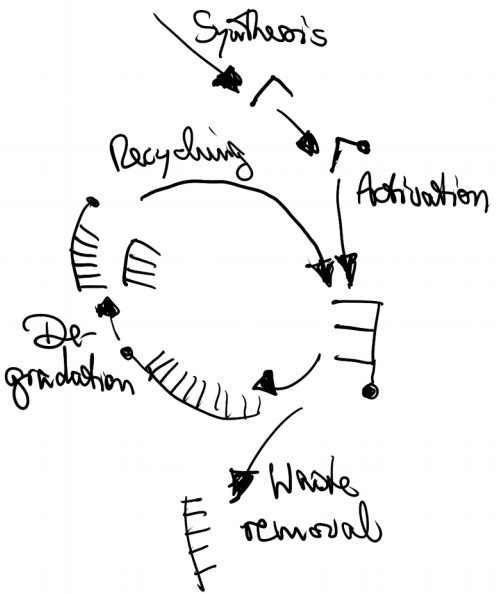


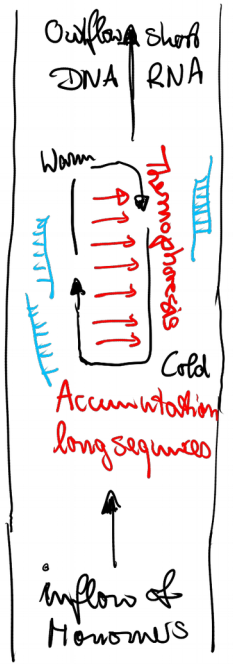


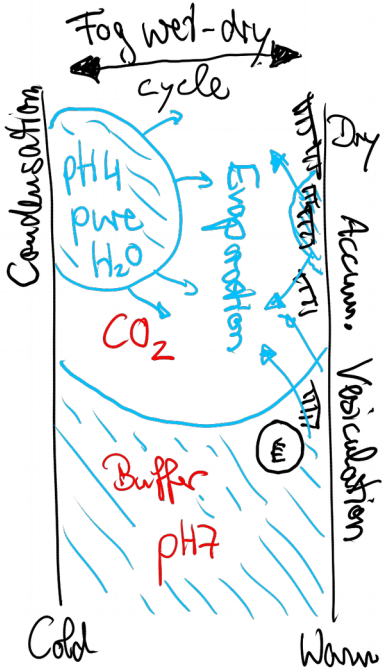


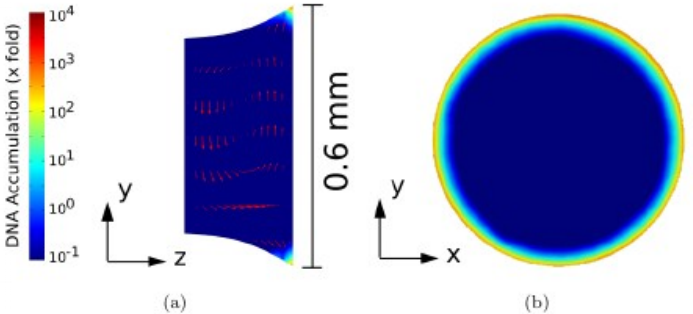
=> Simulation in Comsol

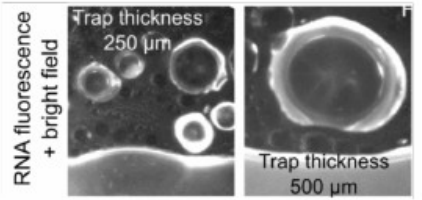
Chemical nonequilibrium in physical nonequilibrium

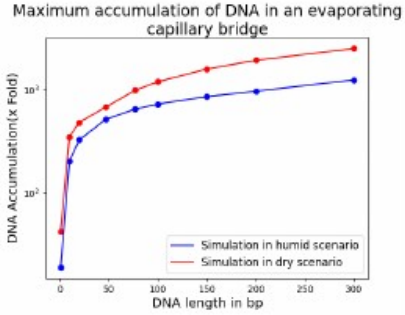






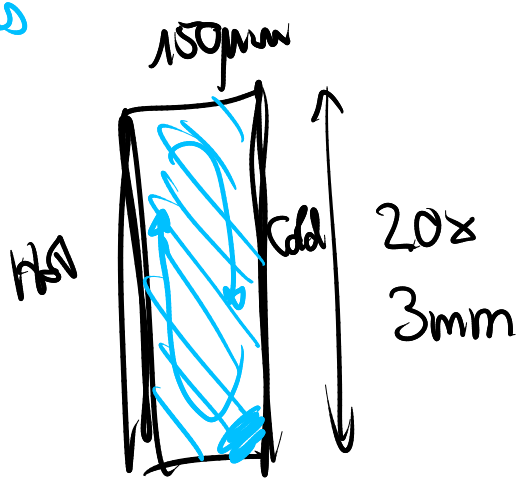






=> Simulation in Comsol

2D thermal trap:
- heat flow ✓
- water flow : Navier Stokes
- molecule diffusion
  - + Thermoph.
  - Reaction



=> Simulation in Comsol