Ribozymes at air-water interfaces

2.11.2021

Replication: Hen and Egg

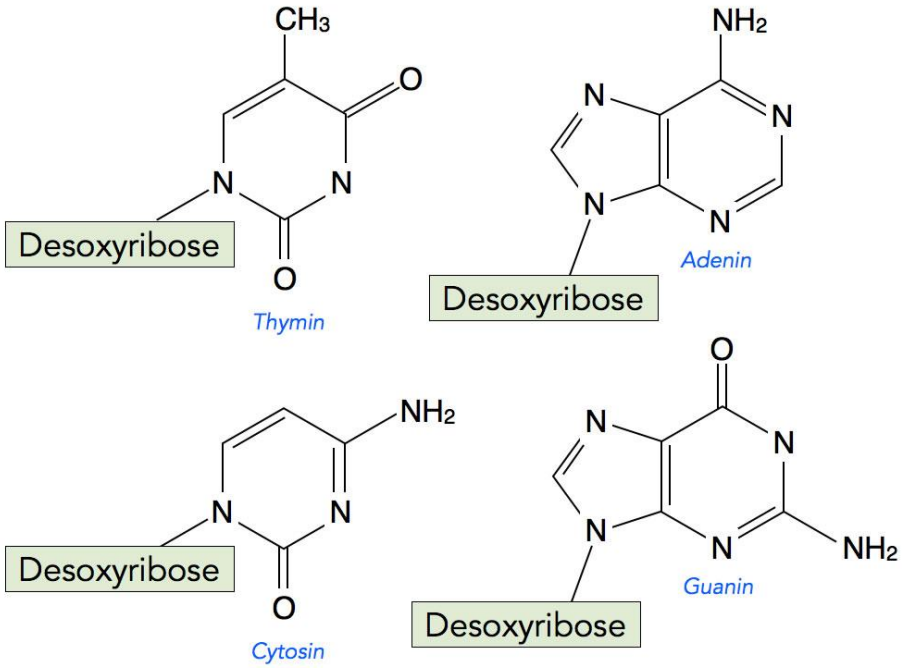
DNA

Information storage

Replication: Hen and Egg

DNA

4 basic building blocks = Bases



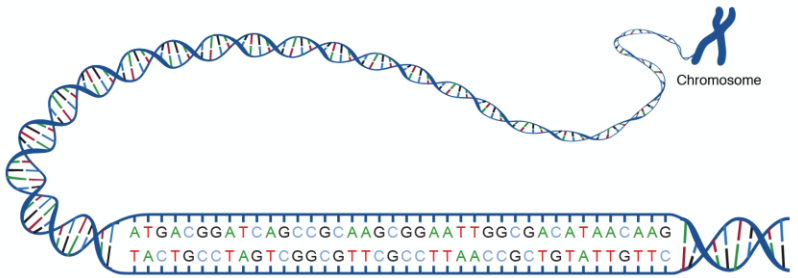
Information storage

Replication: Hen and Egg

DNA

4 basic building blocks = Bases

Multiple bases = Sequence



Information storage

Replication: Hen and Egg

DNA

4 basic building blocks = Bases

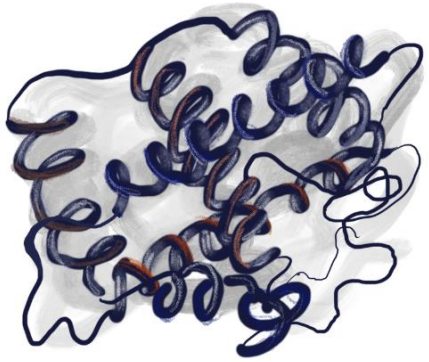
Multiple bases = Sequence

Sequence = Information

Information storage

Replication: Hen and Egg

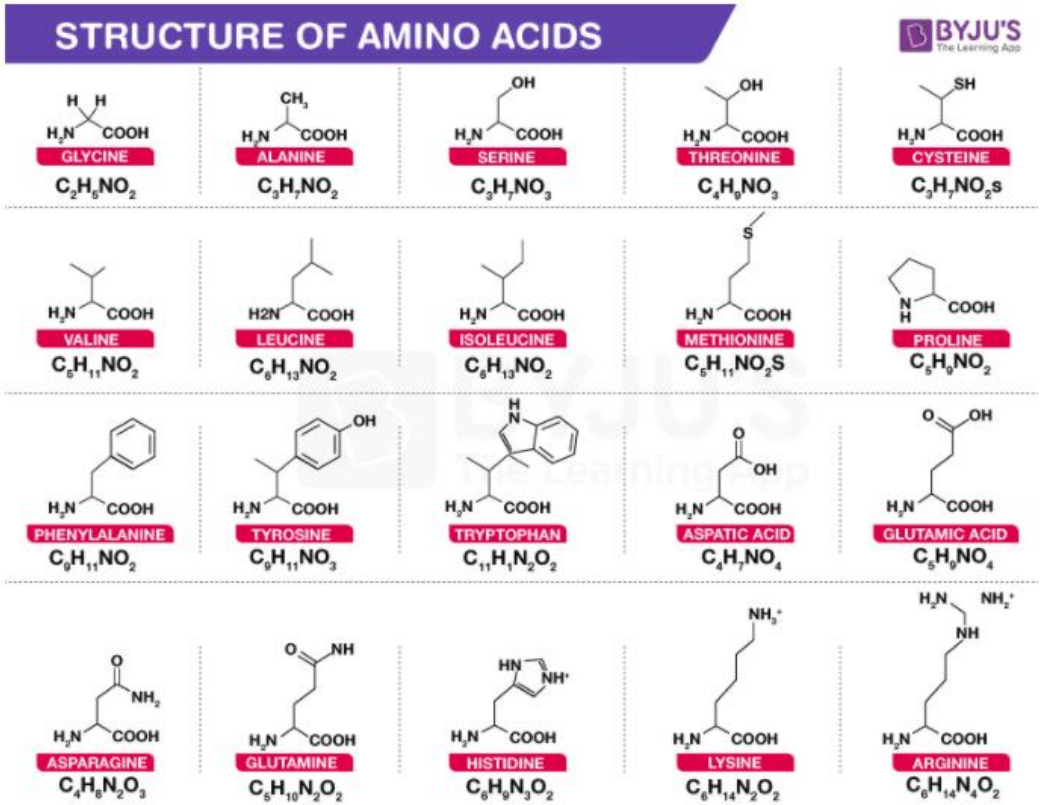
Proteins



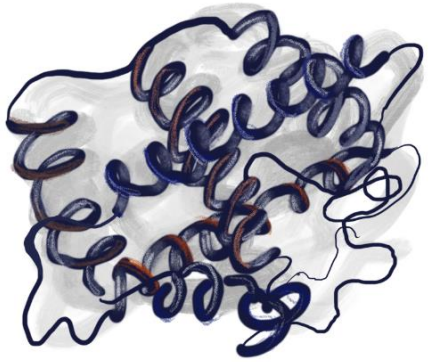
Functionality

Replication: Hen and Egg

20 building blocks = amino acids



Proteins



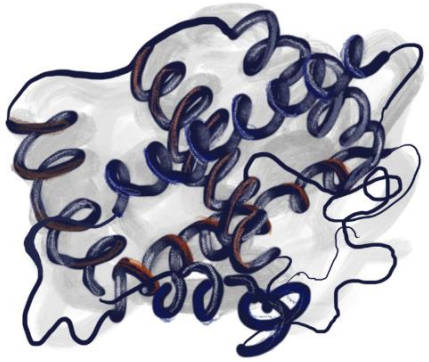
Functionality

Replication: Hen and Egg

20 building blocks = amino acids

Sequence of amino acids
= folding into 3D tructur

Proteins



Functionality

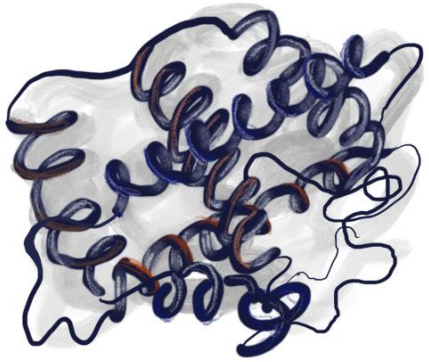
Replication: Hen and Egg

20 building blocks = amino acids

Sequence of amino acids
= folding into 3D tructur

3D Structur = Functionality

Proteins



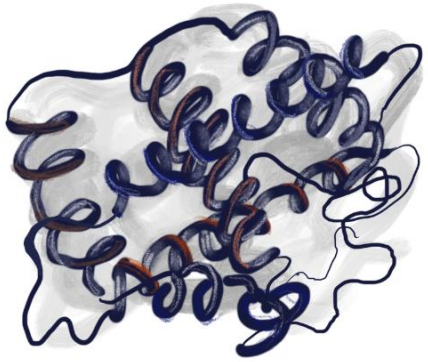
Functionality

Replication: Hen and Egg

DNA

Information storage

Proteins



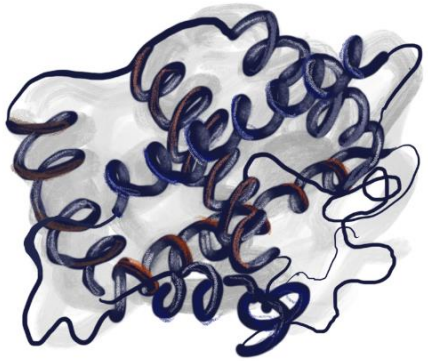
Functionality

Replication: Hen and Egg

DNA

RNA

Proteins



Information storage

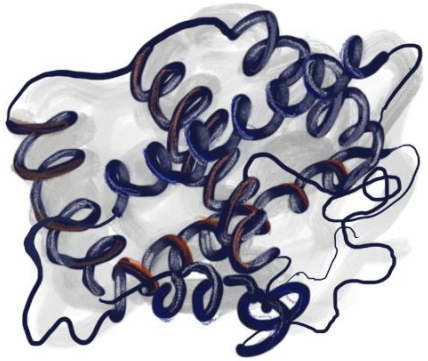
Functionality

Replication: Hen and Egg

DNA

RNA

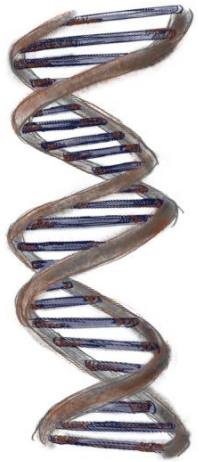
Proteins



Central Dogma of molecular biology
- Francis Crick (1958)

Replication: Hen and Egg

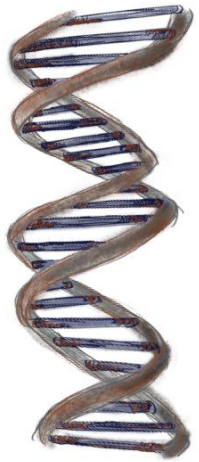
RNA



Ribozyme

Replication: Hen and Egg

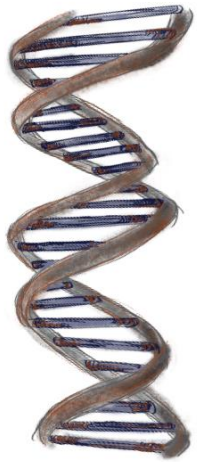
RNA

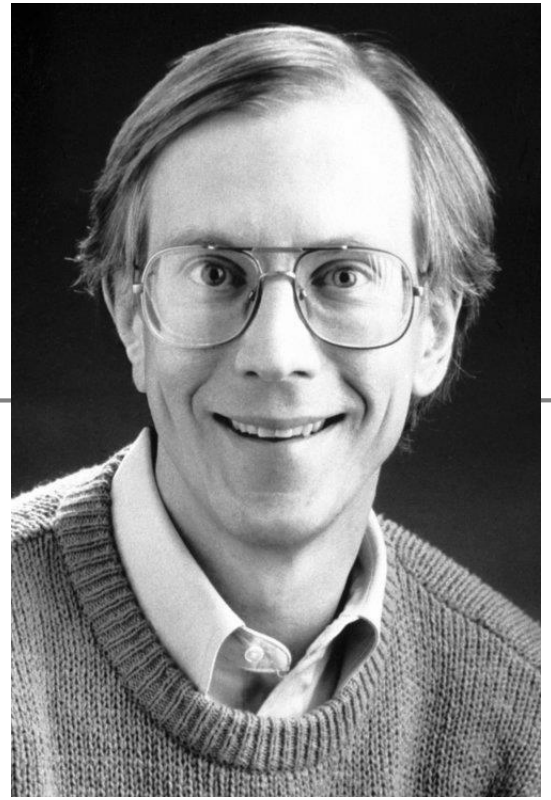


Ribozyme

Replication: Hen and Egg

RNA





Ribozyme

Nobelpreis für Chemie 1989

Thomas R. Cech Sidney Altman

Replication: Hen and Egg

RNA

Ribozyme

PERSPECTIVES: STRUCTURAL BIOLOGY

The Ribosome Is a Ribozyme

Thomas R. Cech

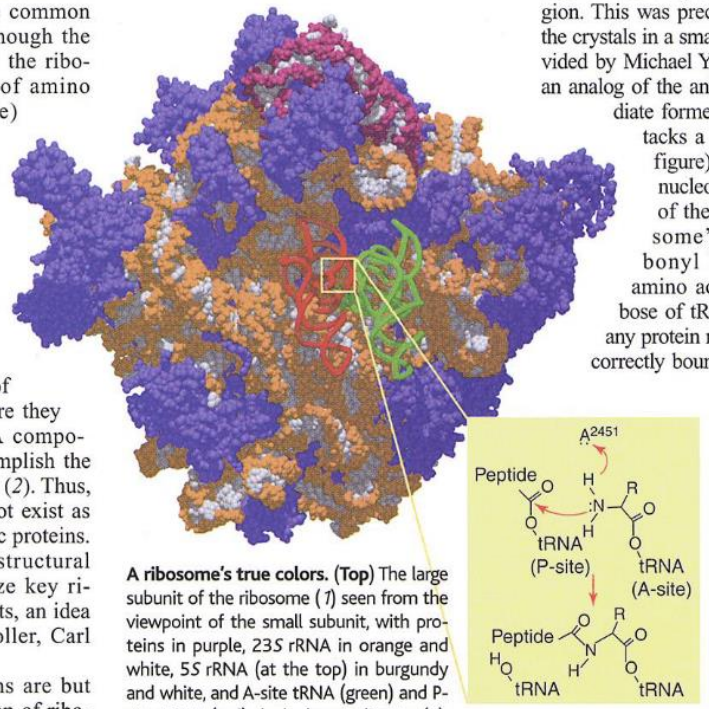
The amino acids we obtain by digestion of steak, salmon, or a lettuce salad are loaded onto transfer RNAs (tRNAs) and rebuilt into proteins in the ribosome, the cell's macromolecular protein-synthesis factory. The bacterial ribosome is composed of three RNA molecules and more than 50 proteins. Its key components are so highly conserved among all of Earth's species that a similar entity must have fueled protein synthesis in the common ancestor of all extant life. Although the chemical reaction catalyzed by the ribosome is simple—the joining of amino acids through amide (peptide) linkages—it performs the remarkable task of choosing the amino acids to be added to the growing polypeptide chain by reading successive messenger RNA (mRNA) codons. On page 905 of this issue, Steitz, Moore, and colleagues (*1*) now provide the first atomic-resolution view of the larger of the two subunits of the ribosome. From this structure they deduce on page 920 that RNA components of the large subunit accomplish the key peptidyl transferase reaction (*2*). Thus, ribosomal RNA (rRNA) does not exist as a framework to organize catalytic proteins. Instead, the proteins are the structural units and they help to organize key ribozyme (catalytic RNA) elements, an idea long championed by Harry Noller, Carl Woese, and others.

These landmark publications are but the latest chapter in a progression of ribo-

Enhanced online at www.sciencemag.org/cgi/content/full/289/5481/878

from the bacterium *Haloarcula marismortui* in the 1980s by Ada Yonath and H. G. Wittmann provided the first rays of hope, but it is only in the past few years that crystal structures have been determined for the large subunit (5 Å resolution) (*3*), the small subunit (5.5 Å resolution) (*4*), and the whole ribosome complexed with tRNAs (7.8 Å resolution) (*5*).

Now, at 2.4 Å, almost the entire chain of the 23*S* rRNA and its tiny 5*S* rRNA partner, totaling 3043 nucleotides, have been fitted



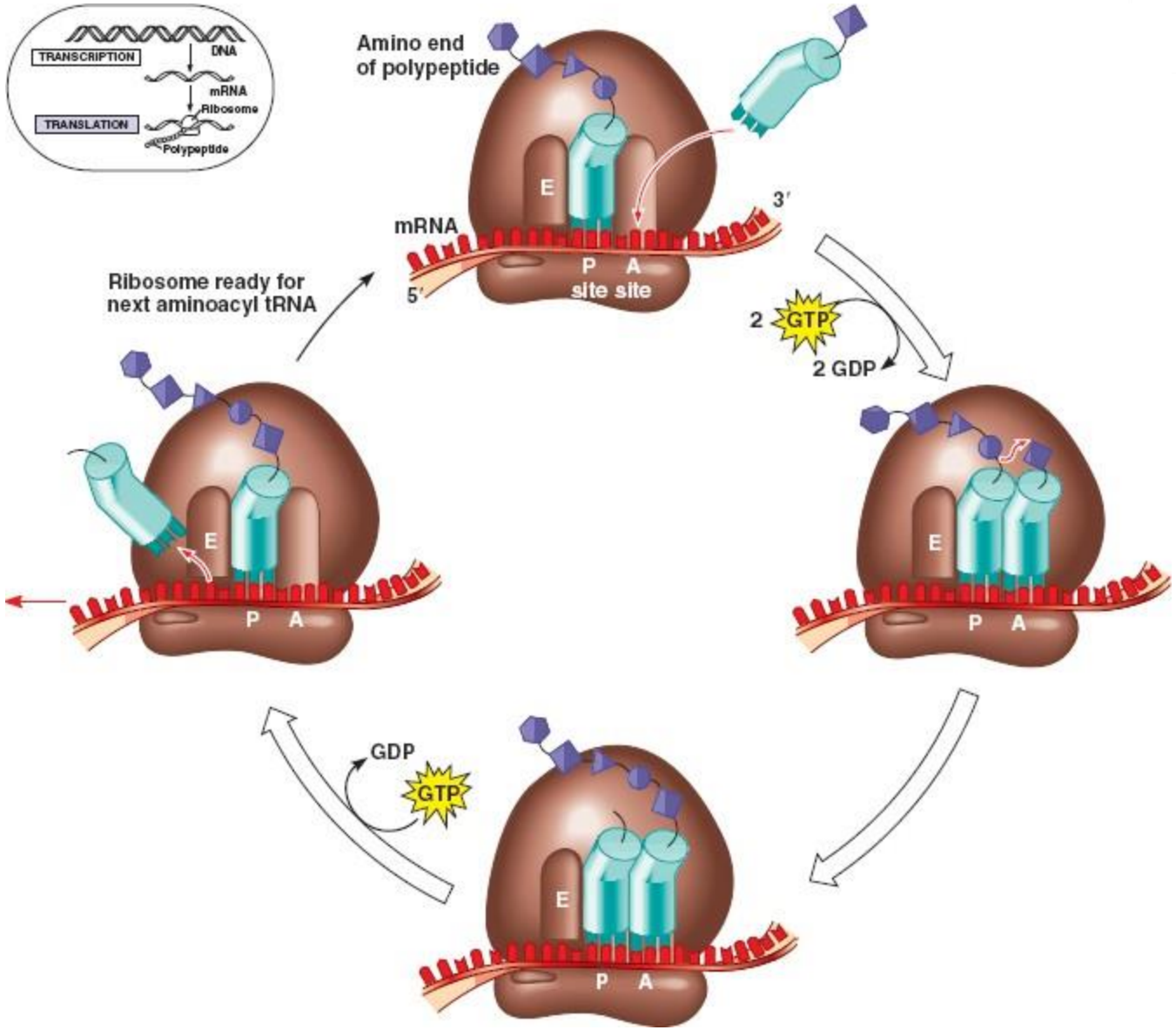
A ribosome's true colors. (Top) The large subunit of the ribosome (*1*) seen from the viewpoint of the small subunit, with proteins in purple, 23*S* rRNA in orange and white, 5*S* rRNA (at the top) in burgundy and white, and A-site tRNA (green) and P-site tRNA (red) docked according to (*5*).

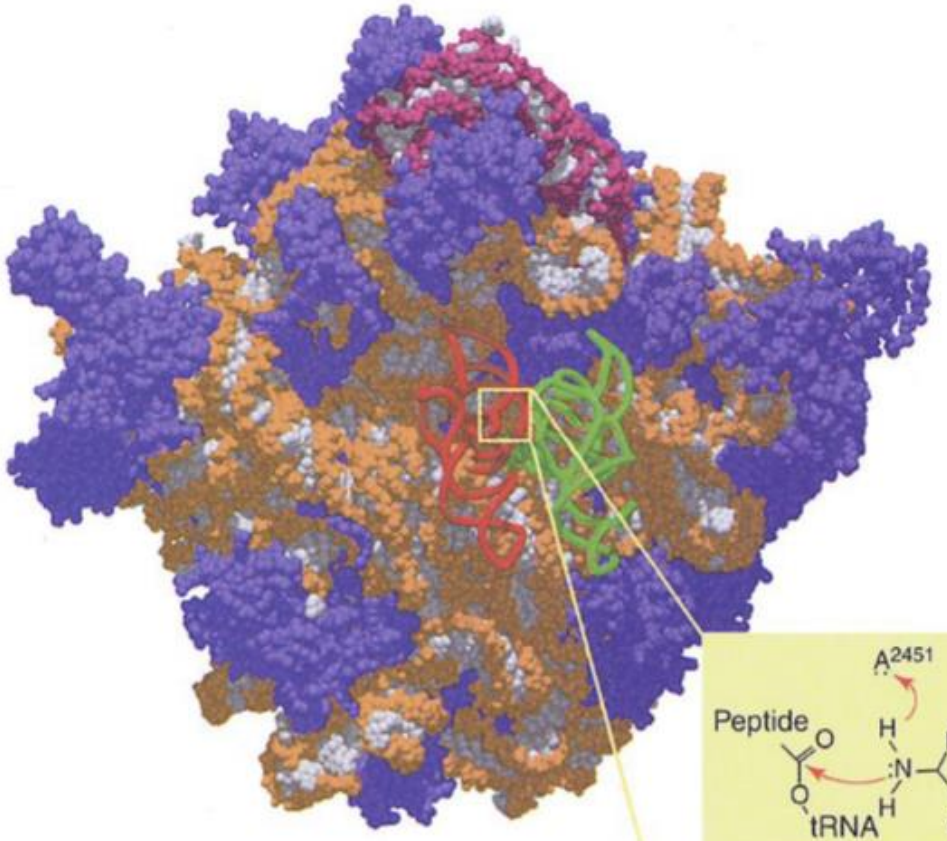
observer might predict from looking at the secondary structure diagram.

Where, then, are all of the proteins, and what is their function? The globular domains of 26 proteins are found largely on the exterior of the subunit (see the figure). Twelve of these proteins have unusual snake-like extensions, devoid of tertiary structure and in some cases even secondary structure, and an additional protein is entirely extended; their shapes are molded by their interactions with the RNA. From these pictures, and from what is known about protein cofactors that facilitate the action of some other ribozymes, it is likely that these ribosomal proteins buttress, stabilize, and orient the otherwise floppy RNA into a specific, active structure.

The part of the subunit's surface that is most devoid of protein is the active-site region. This was precisely located by soaking the crystals in a small-molecule inhibitor provided by Michael Yarus (*7*). This inhibitor is an analog of the anionic tetrahedral intermediate formed when a nucleophile attacks a planar carbonyl (see the figure). (In protein synthesis, the nucleophile is the amino group of the amino acid in the ribosome's A-site, and the carbonyl belongs to the P-site amino acid esterified to the 3′-ribose of tRNA.) It is the absence of any protein moiety within 18 Å of the correctly bound inhibitor in their structure, coupled with earlier work that defined this conserved part of the large-subunit rRNA as the "peptide transferase center," that led the authors to conclude that RNA (and not protein) must be responsible for catalysis. The ribosome is a ribozyme, admittedly one dependent on structural support from protein com-

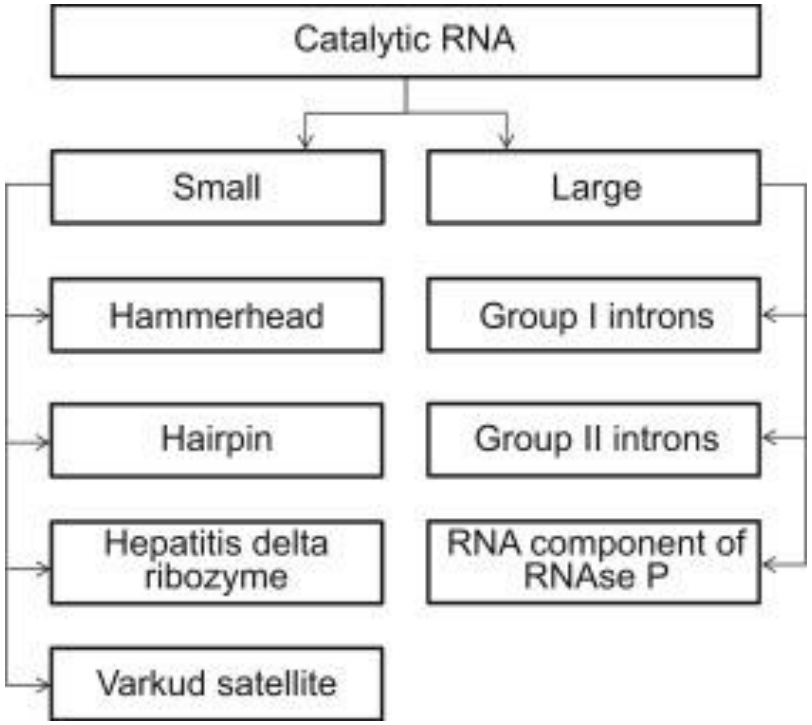
The ribosome is a ribozyme



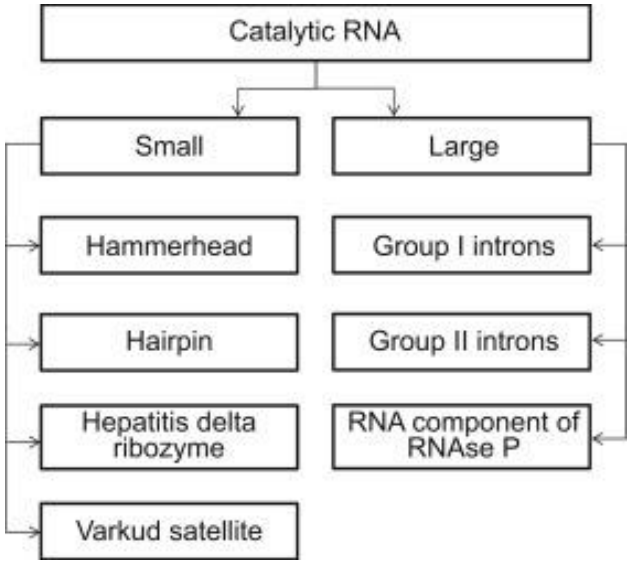


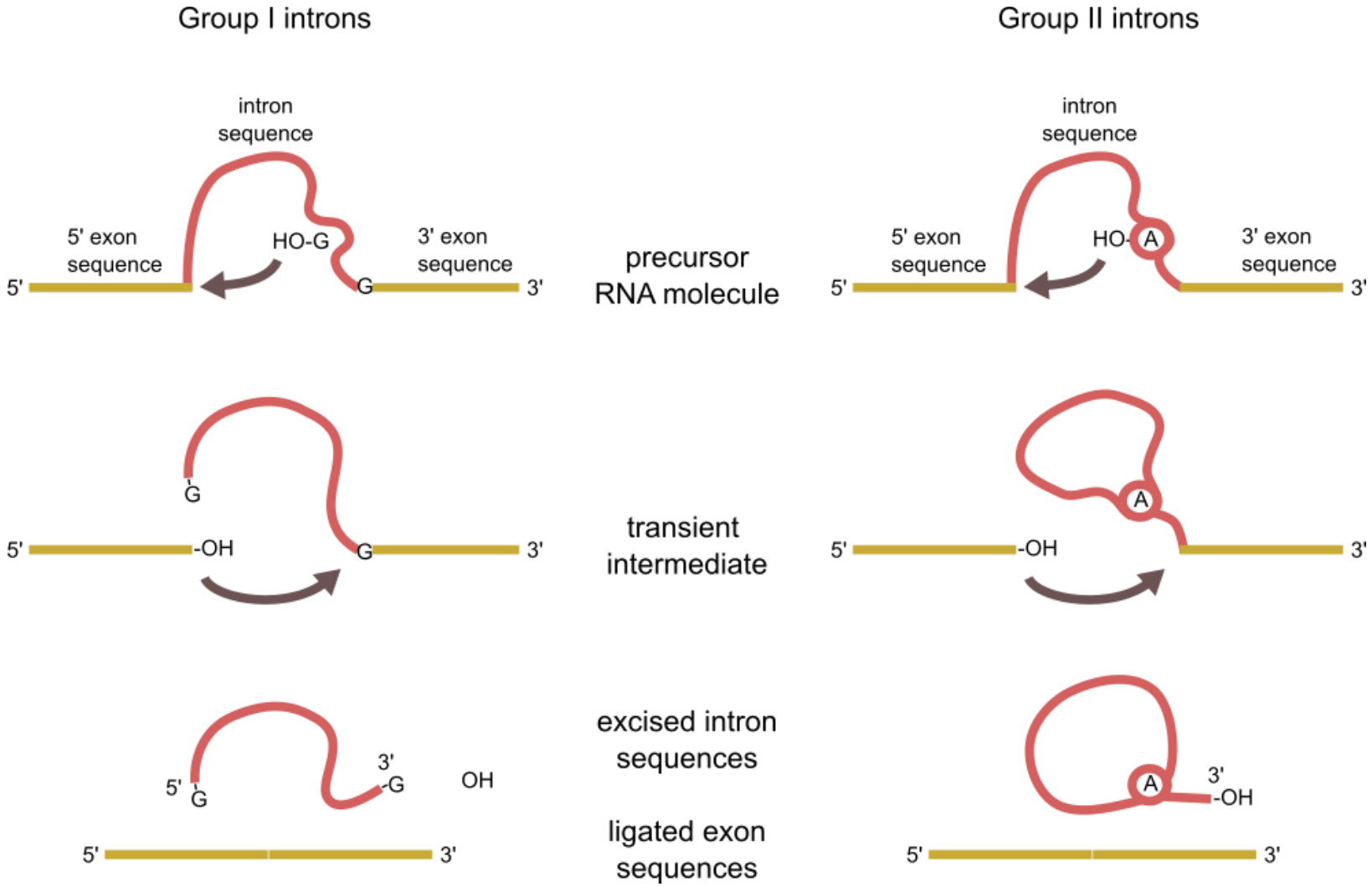
A ribosome's true colors. (Top) The large subunit of the ribosome (*1*) seen from the viewpoint of the small subunit, with proteins in purple, 23*S* rRNA in orange and white, 5*S* rRNA (at the top) in burgundy and white, and A-site tRNA (green) and P-site tRNA (red) docked according to (*5*). **(Bottom)** The peptidyl transfer mechanism catalyzed by RNA (*2*). The general base (adenine 2451 in *Escherichia coli* 23*S* rRNA) is rendered unusually basic by its environment within the folded structure; it could abstract the proton at any of several steps, one of which is shown here.

Classes of ribozymes

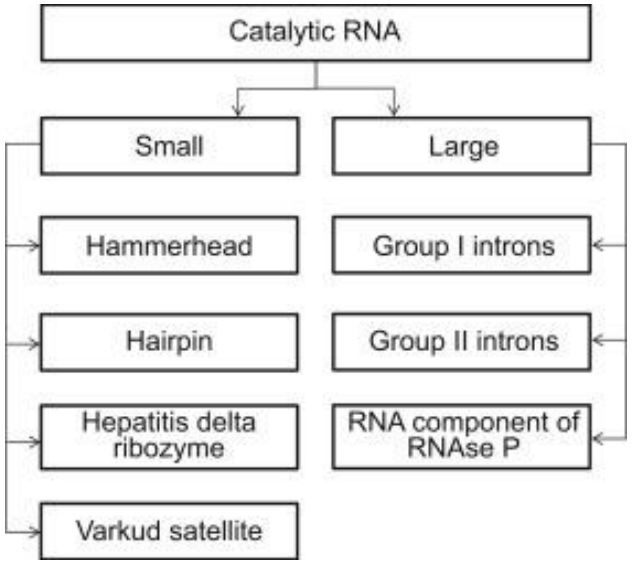


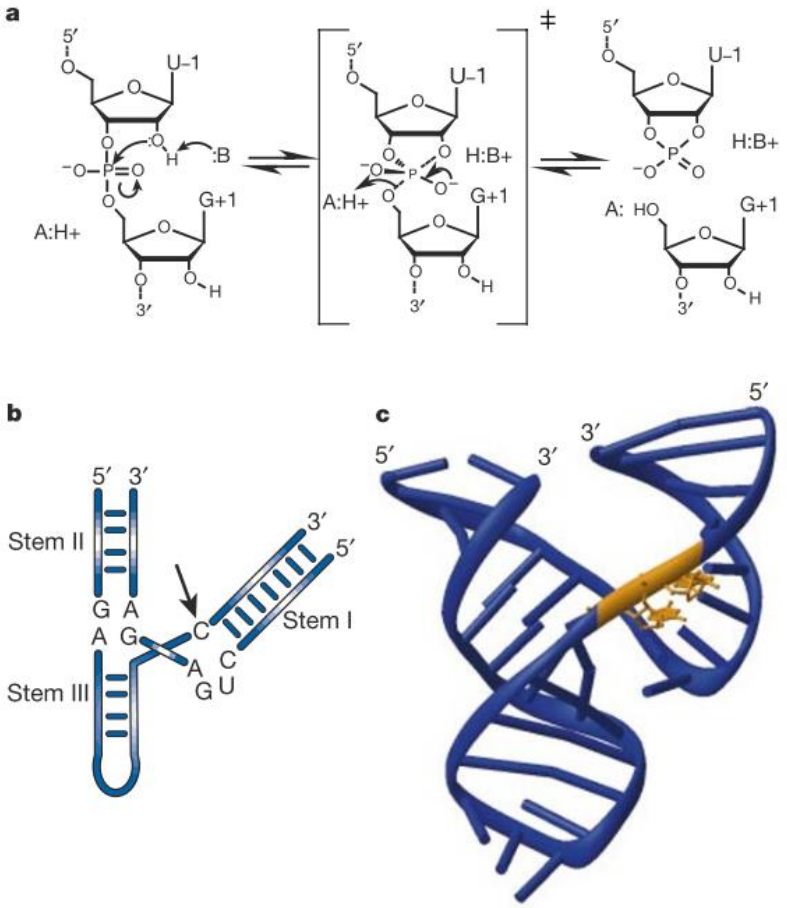
Classes of ribozymes – Group I + II introns



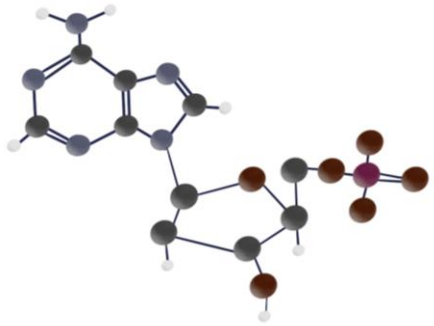


Classes of ribozymes – small ribozymes

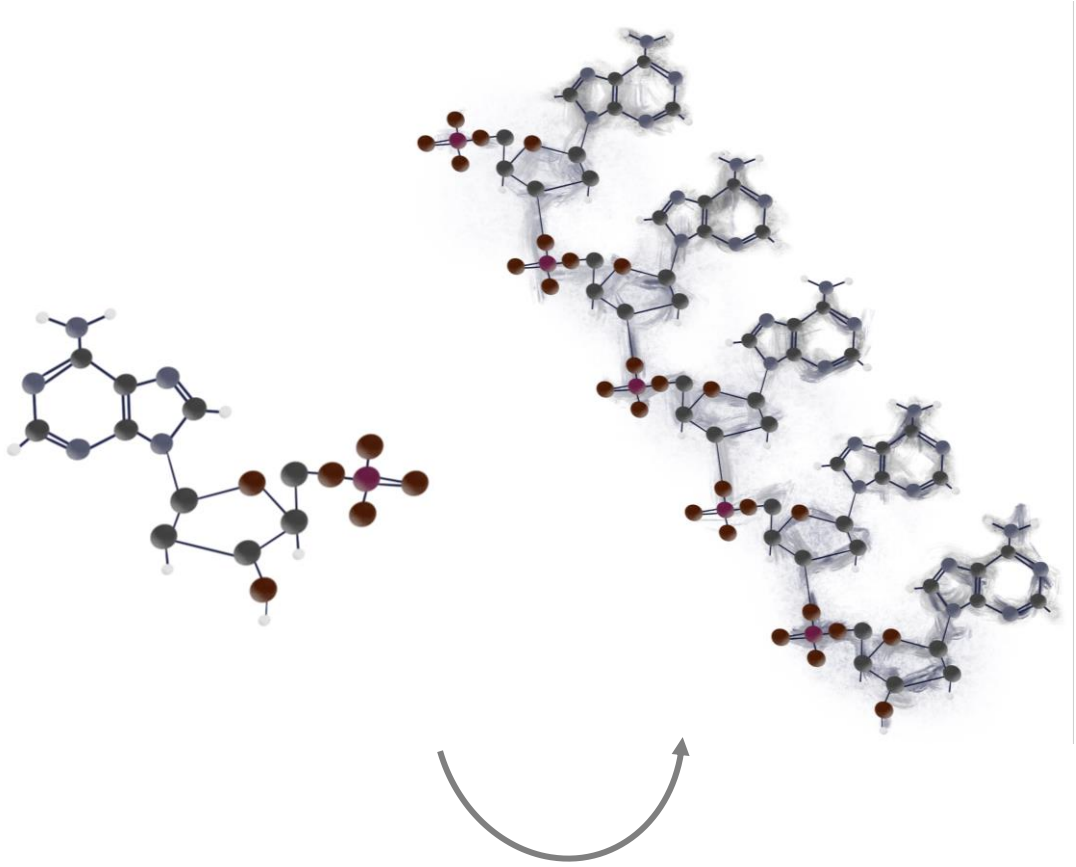




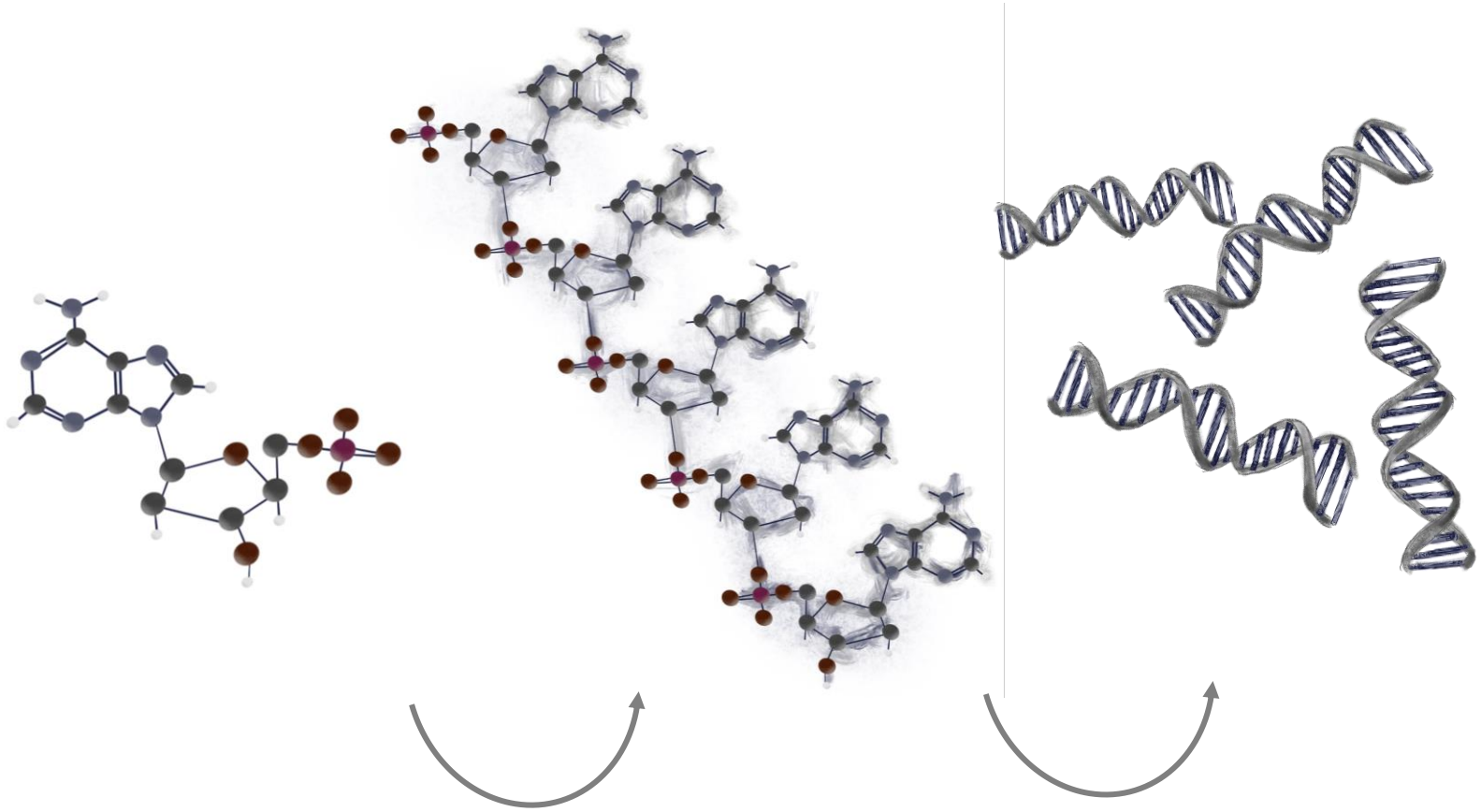
Origin of replication



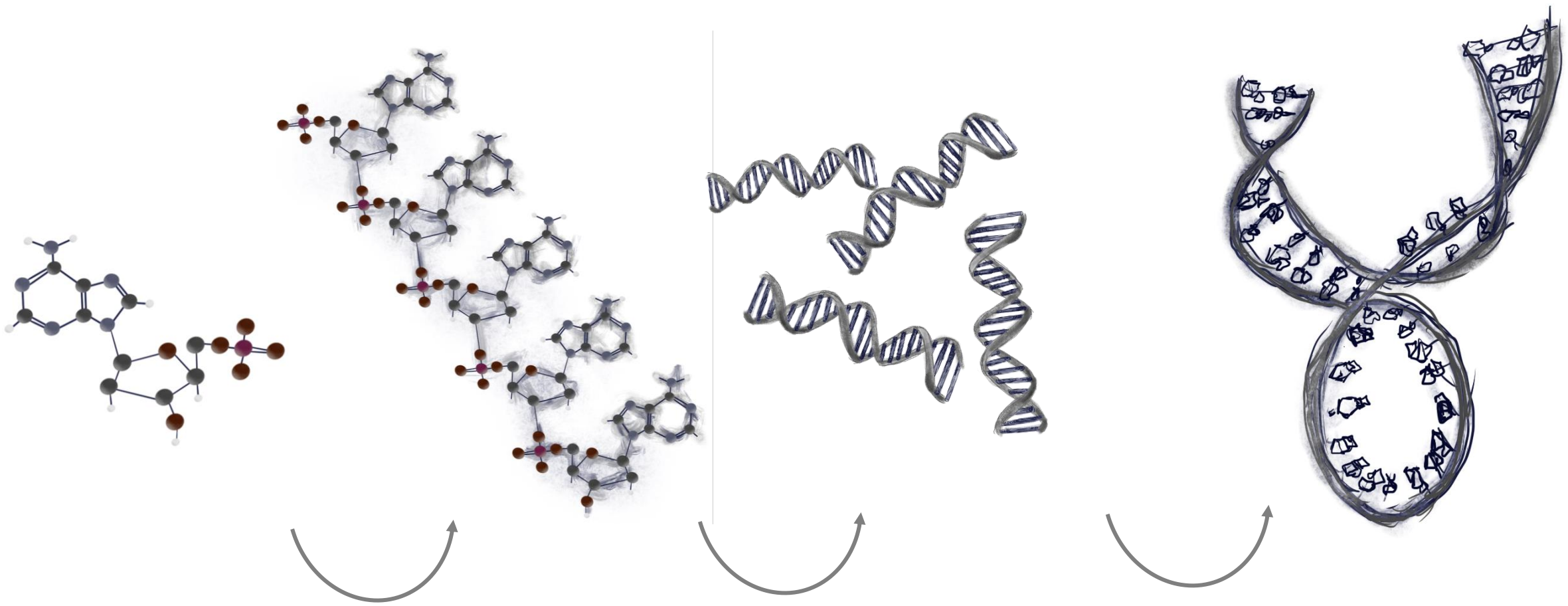
Origin of replication



Origin of replication

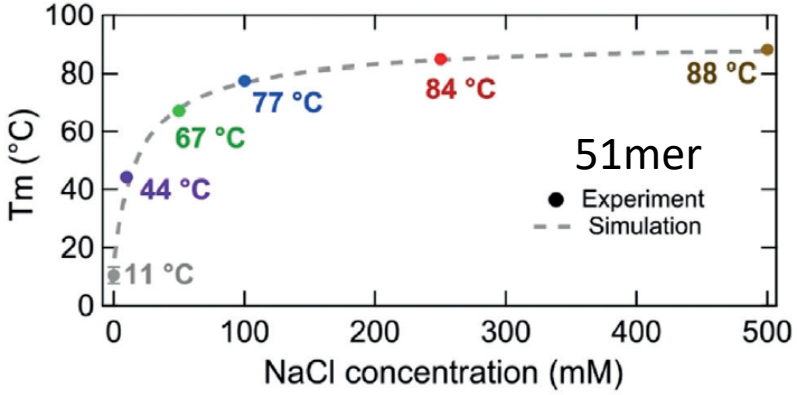


Origin of replication



Ribozymes – Problem salt

- Need high salt (Magnesium) for functionality such as replication
- Increased degradation → Loss of information and functionality
- Increased melting temperature for product-template complex → dead-end duplex

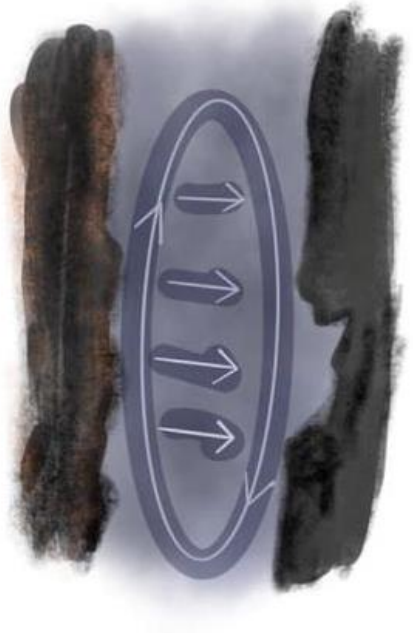


Ianeselli, A. *et al.* Periodic Melting of Oligonucleotides by Oscillating Salt Concentrations Triggered by Microscale Water Cycles Inside Heated Rock Pores. *Angew. Chemie Int. Ed.* **58**, 13155–13160 (2019).

Potential non-equilibrium settings on Early Earth

Potential non-equilibrium settings on Early Earth

Laminar convection
& thermophoretic accumulation



Potential non-equilibrium settings on Early Earth

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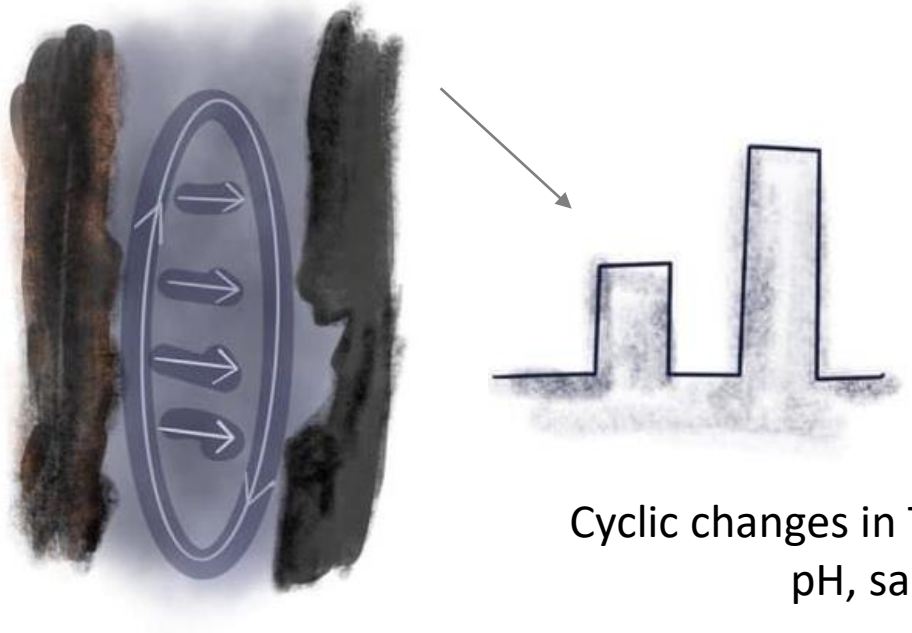
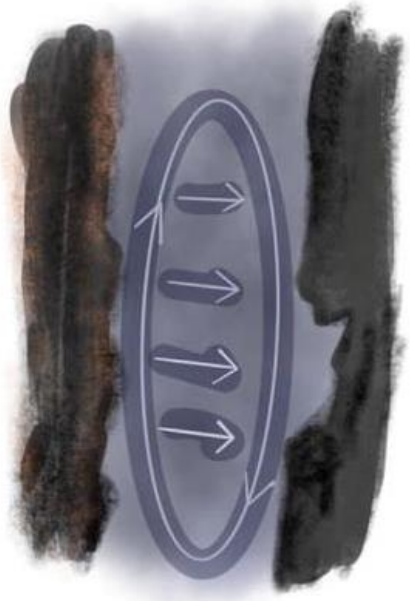
& thermophoretic accumulation

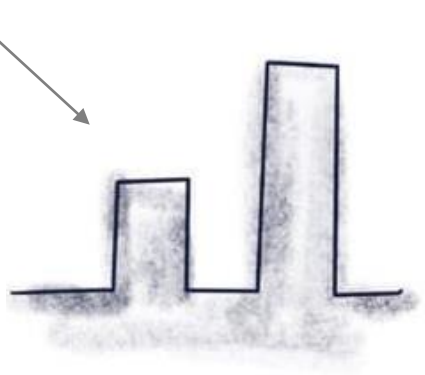


Cyclic changes in T,
pH, salt

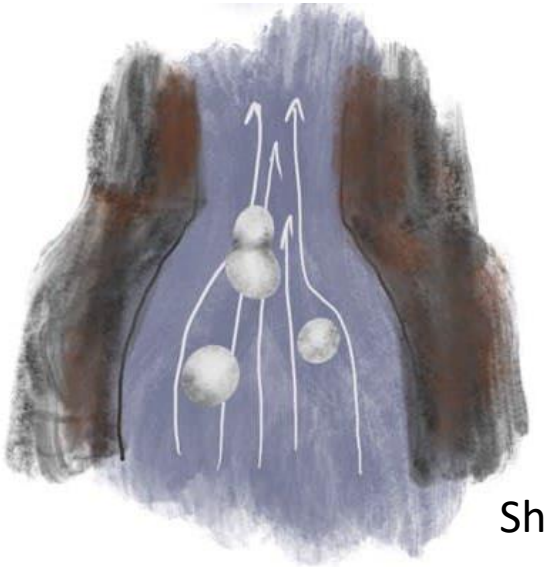
Potential non-equilibrium settings on Early Earth

Laminar convection
& thermophoretic accumulation





Cyclic changes in T,
pH, salt

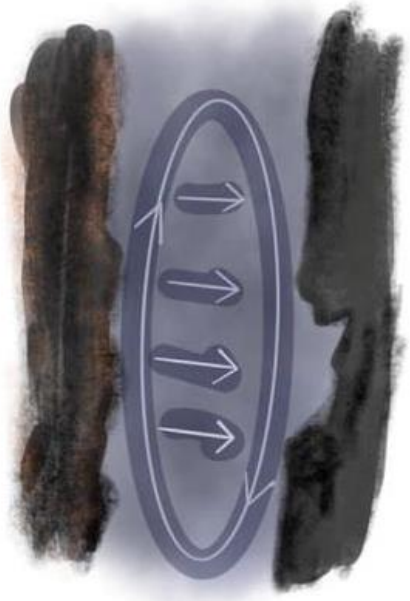


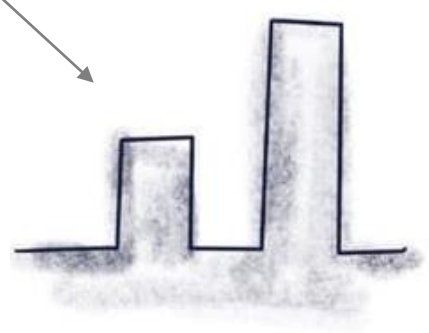
Shear flow leading to fission

Potential non-equilibrium settings on Early Earth

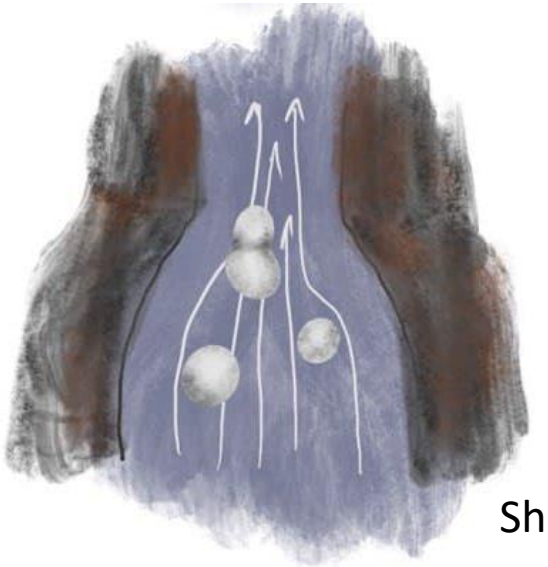
Accumulation by evaporation

Laminar convection
& thermophoretic accumulation





Cyclic changes in T,
pH, salt



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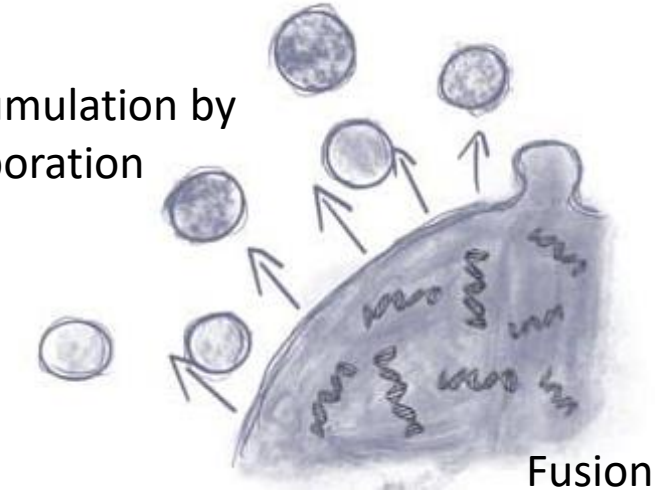
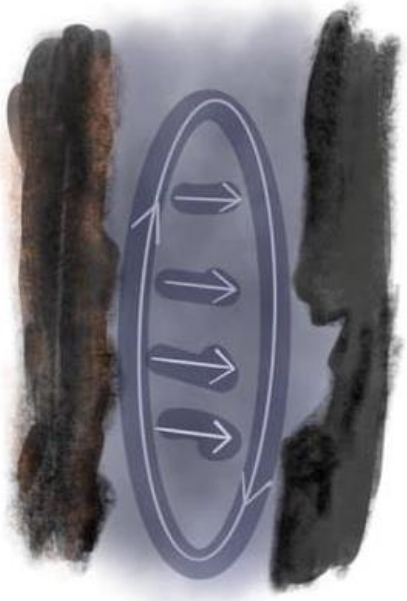
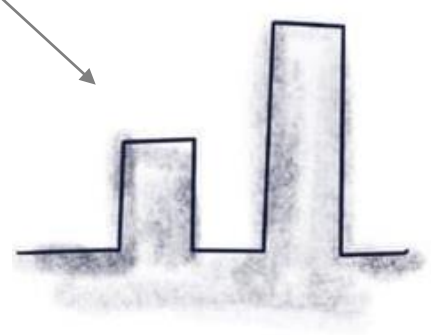


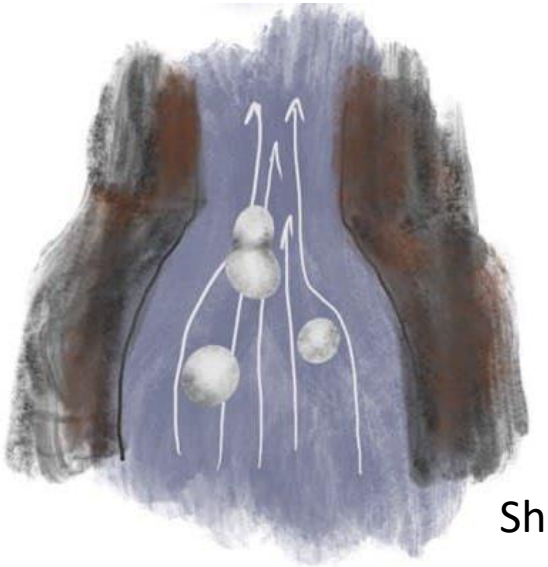
Laminar convection
& thermophoretic accumulation





Cyclic changes in T,
pH, salt

Fusion and condensation of droplets driven by surface tension

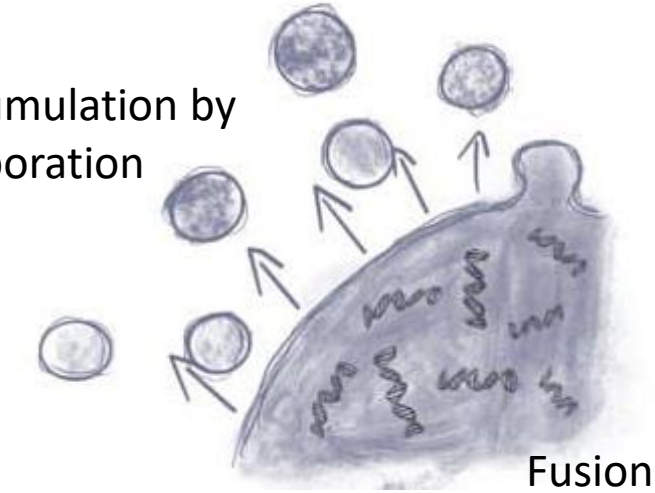


Shear flow leading to fission

Potential non-equilibrium settings on Early Earth

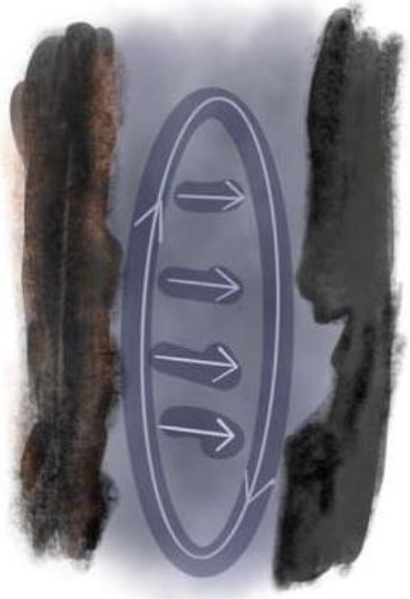
Selective adsorption and desorption

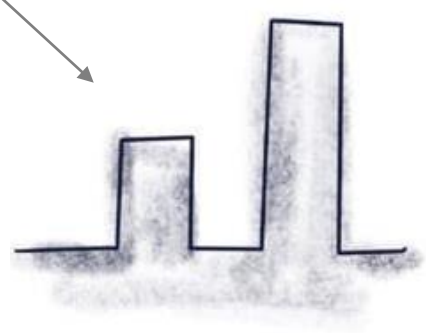
Accumulation by evaporation



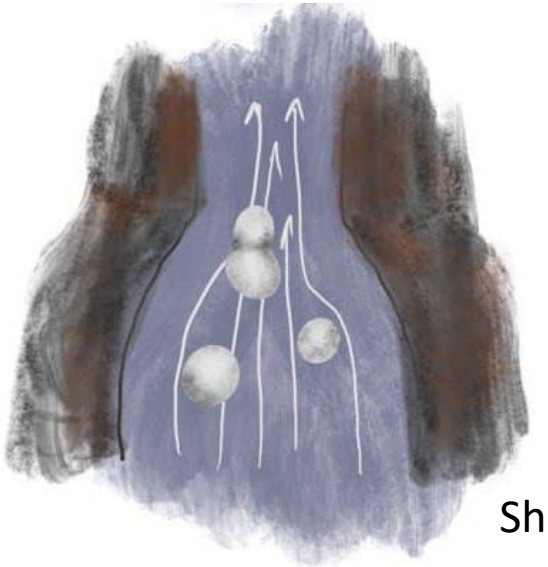
Fusion and condensation of droplets driven by surface tension

Laminar convection & thermophoretic accumulation





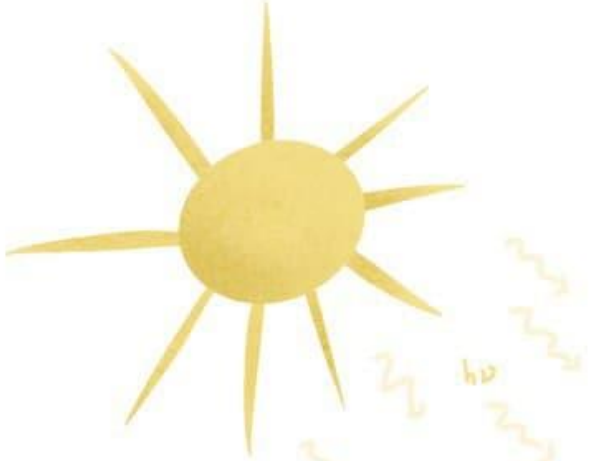
Cyclic changes in T, pH, salt



Shear flow leading to fission

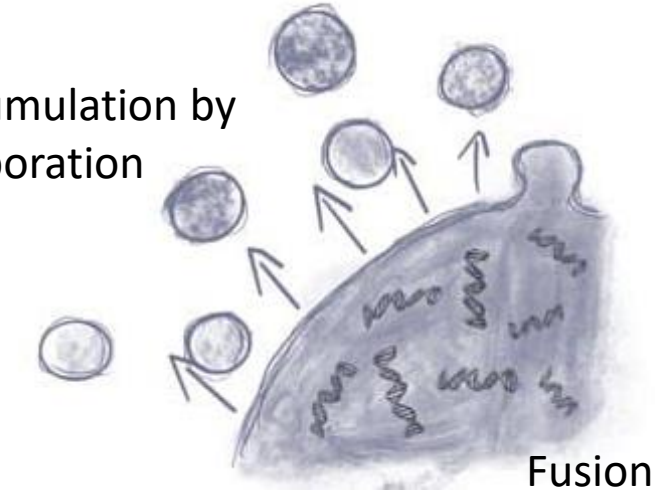
Potential non-equilibrium settings on Early Earth

Selective adsorption and desorption



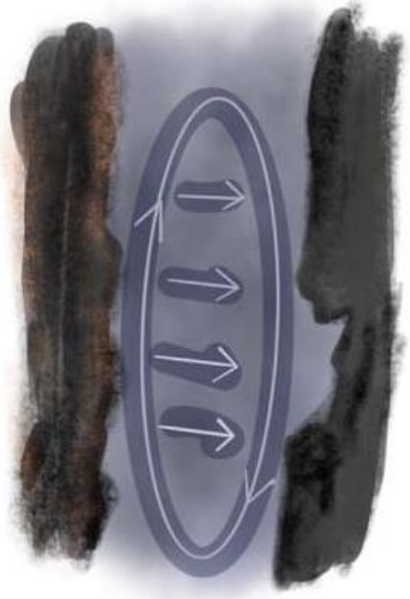
Sequence selection

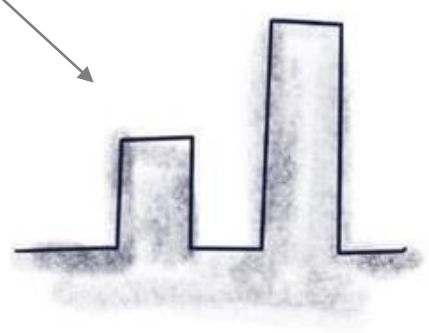
Accumulation by evaporation



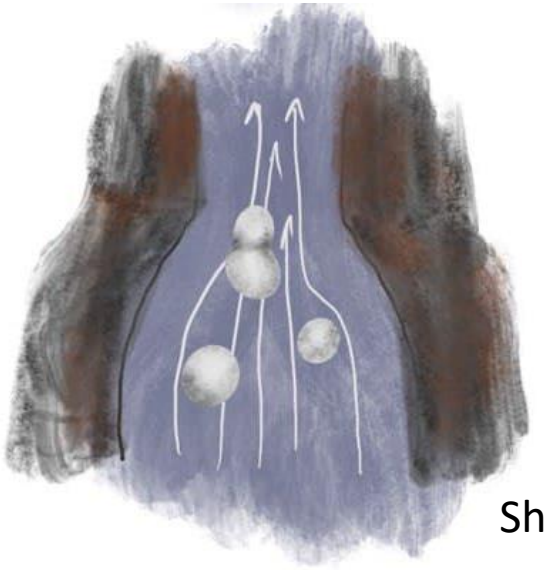
Fusion and condensation of droplets driven by surface tension

Laminar convection & thermophoretic accumulation



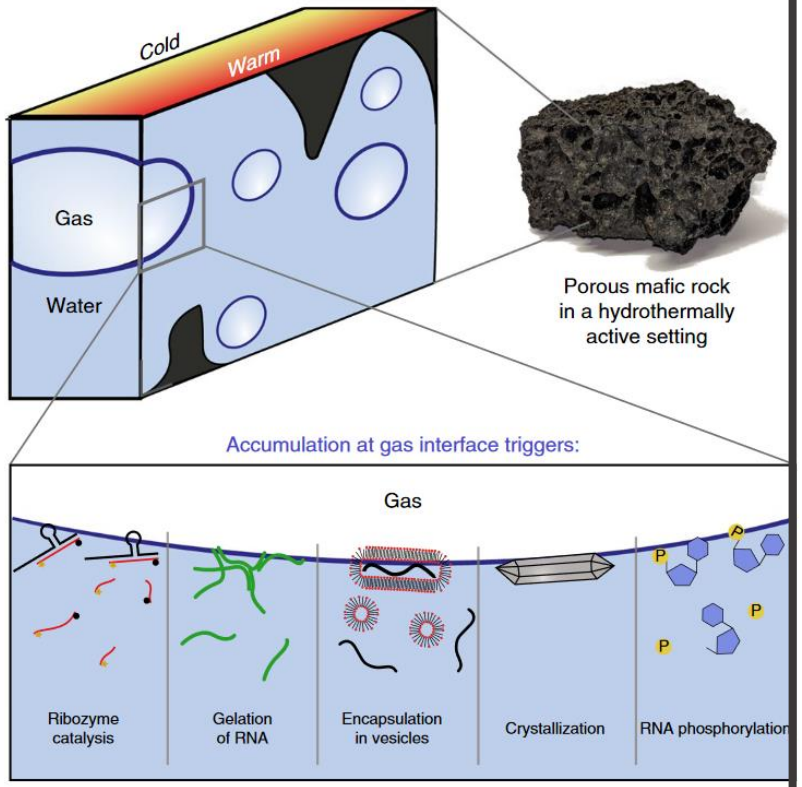


Cyclic changes in T, pH, salt



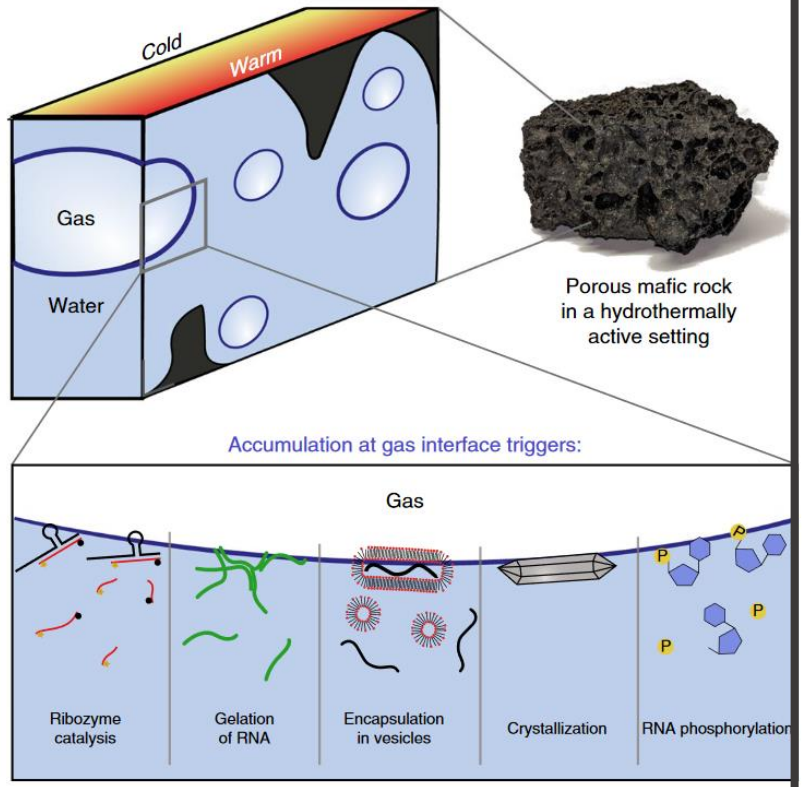
Shear flow leading to fission

Building plausible non-equilibria in the Lab

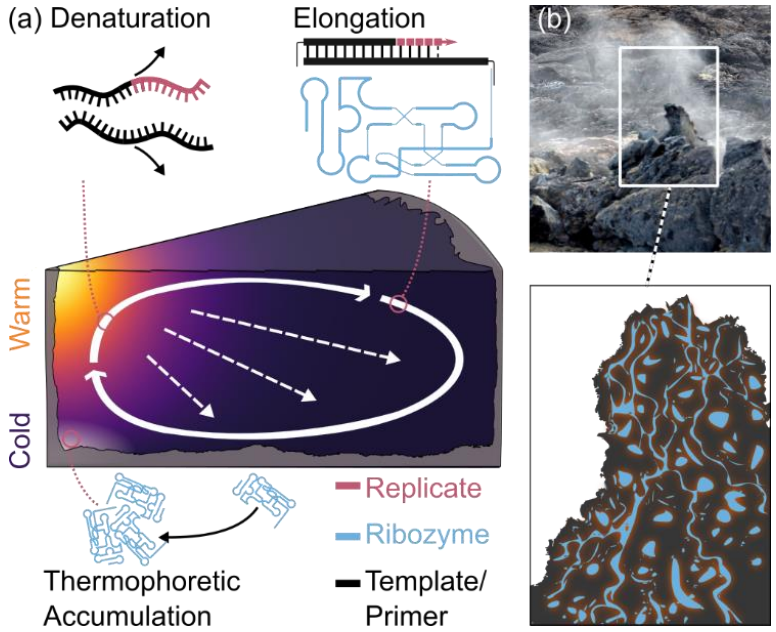


Morasch, M., *et al.* Heated gas bubbles enrich, crystallize, dry, phosphorylate and encapsulate prebiotic molecules. *Nat. Chem.* **11,** 779–788 (2019)

Building plausible non-equilibria in the Lab

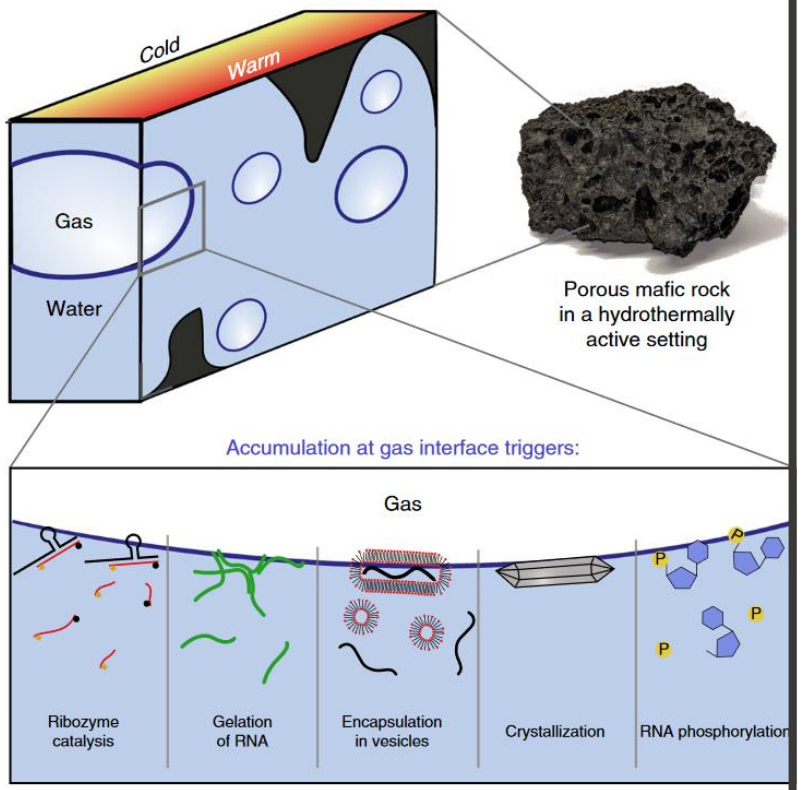


Morasch, M., *et al.* Heated gas bubbles enrich, crystallize, dry, phosphorylate and encapsulate prebiotic molecules. *Nat. Chem.* **11,** 779–788 (2019)

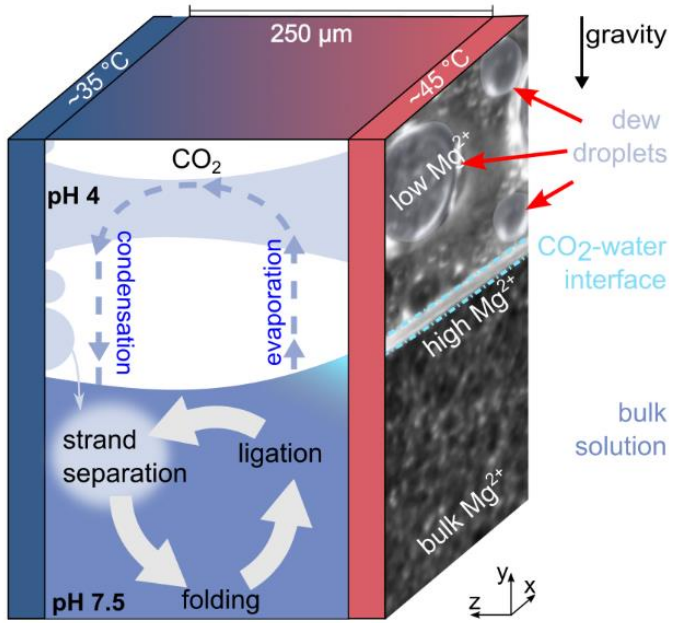


Salditt, A. *et al.* Thermal Habitat for RNA Amplification and Accumulation. *Phys. Rev. Lett.* (2020). doi:10.1103/PhysRevLett.125.048104

Building plausible non-equilibria in the Lab

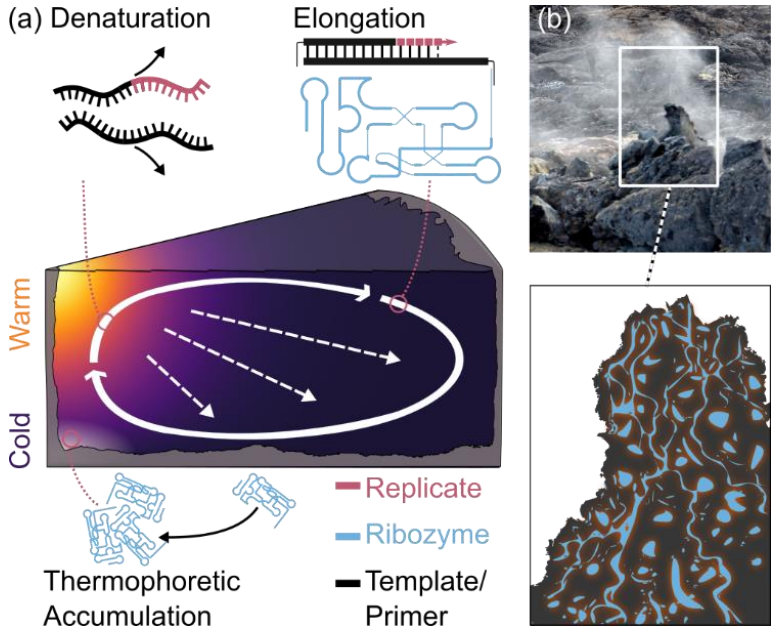


Morasch, M., *et al.* Heated gas bubbles enrich, crystallize, dry, phosphorylate and encapsulate prebiotic molecules. *Nat. Chem.* **11,** 779–788 (2019)



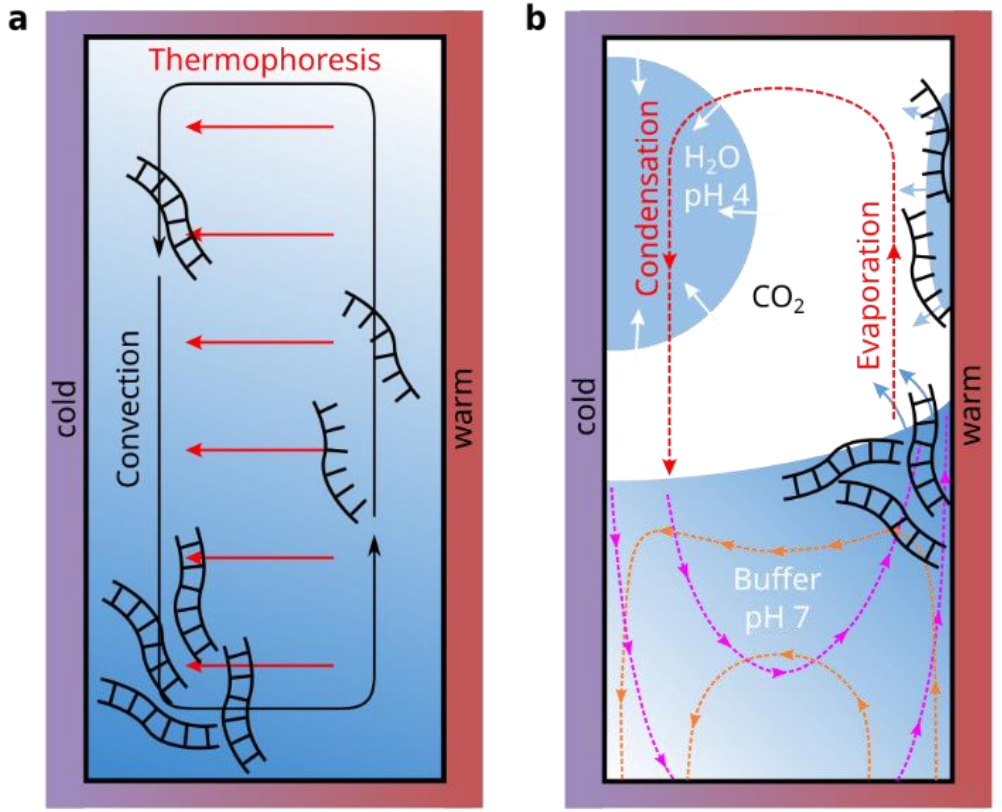
Salditt, A. *et al.* Ribozyme-mediated RNA synthesis and replication in a model Hadean microenvironment, Nature Communications (2023) doi.org/10.1038/s41467-023-37206-4

Ianeselli, A. *et al.* Periodic Melting of Oligonucleotides by Oscillating Salt Concentrations Triggered by Microscale Water Cycles Inside Heated Rock Pores. *Angew. Chemie Int. Ed.* **58**, 13155–13160 (2019).



Salditt, A. *et al.* Thermal Habitat for RNA Amplification and Accumulation. *Phys. Rev. Lett.* (2020). doi:10.1103/PhysRevLett.125.048104

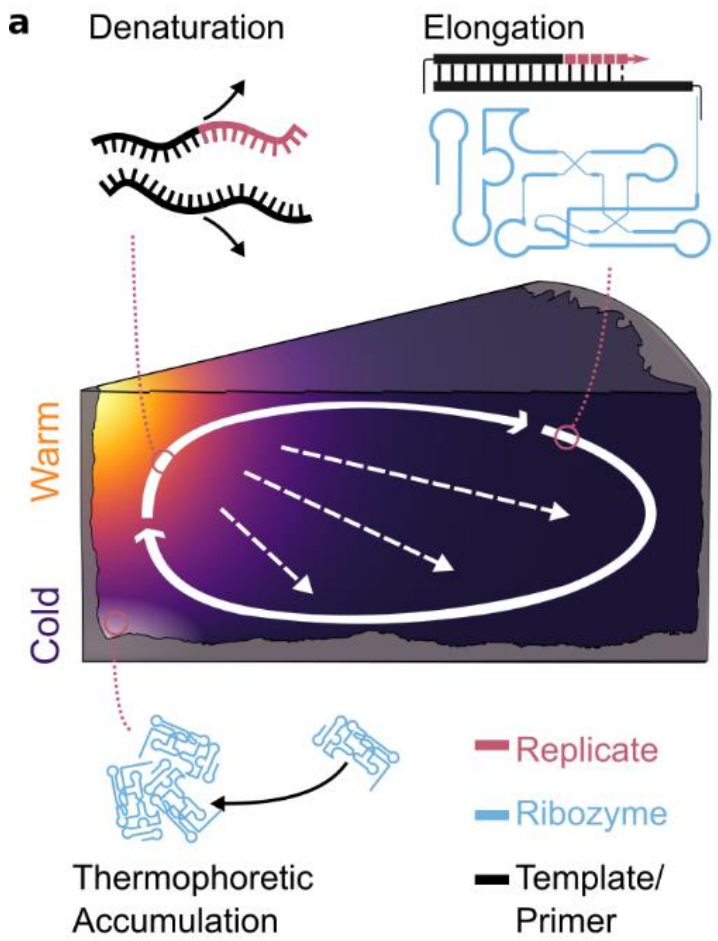
Thermal non-equilibria

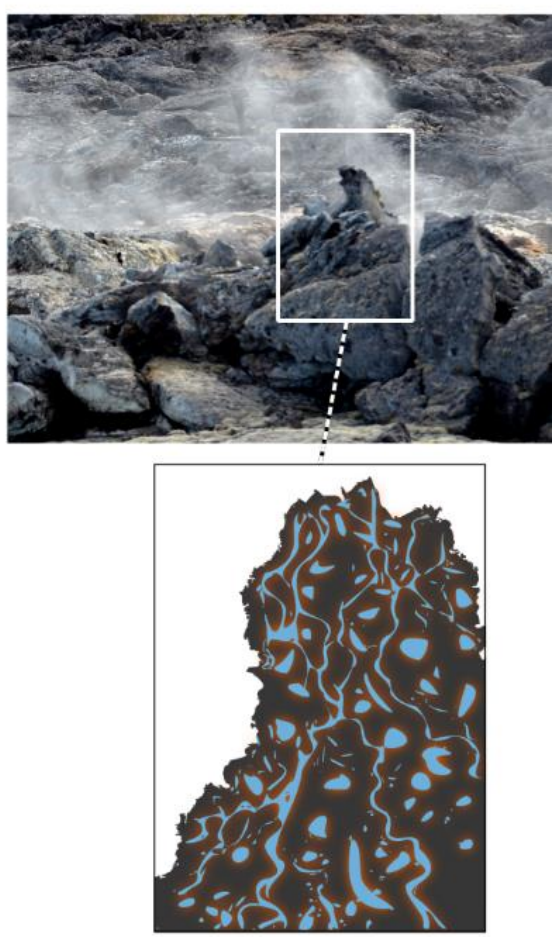


Thermogravitational traps

Air-water interfaces

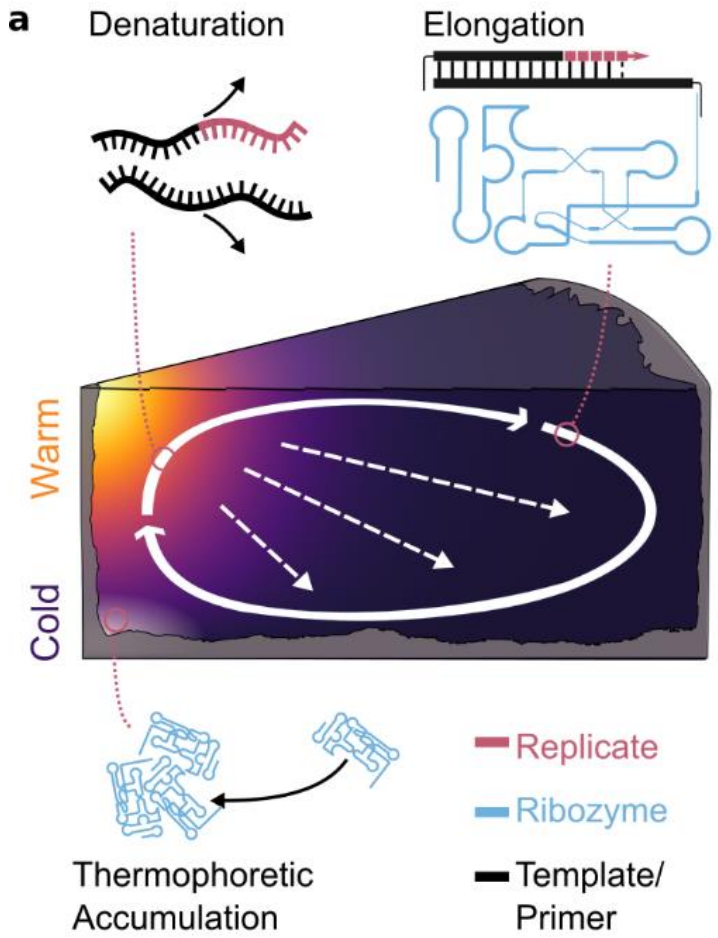
RNA amplifictaion in water-filled pores

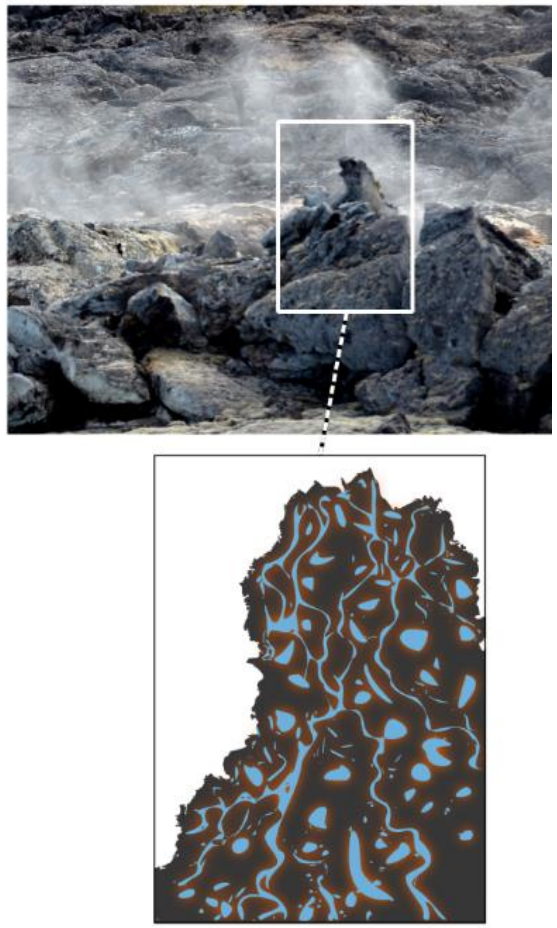




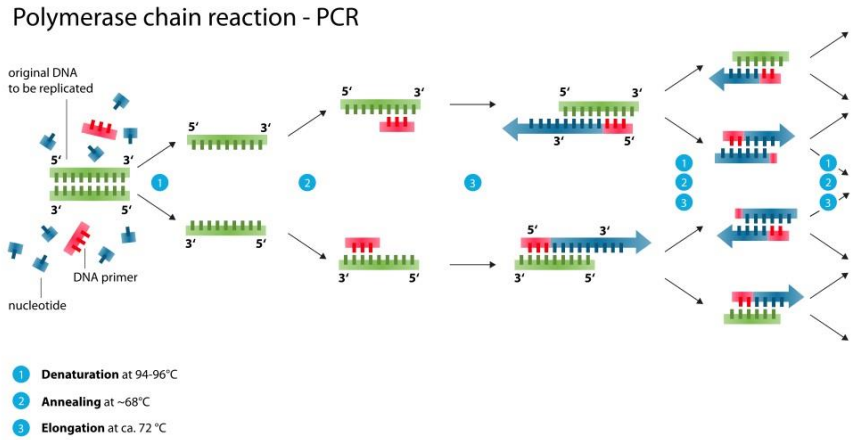
- 24-3 Polymerase
- Ribo PCR in realistic environment

RNA amplifictaion in water-filled pores

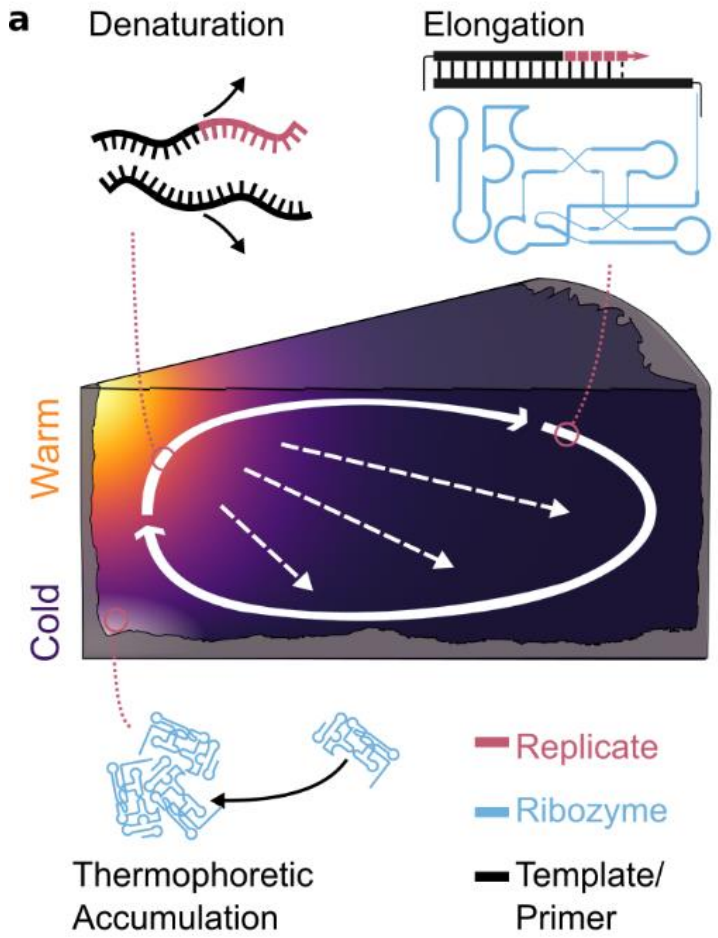


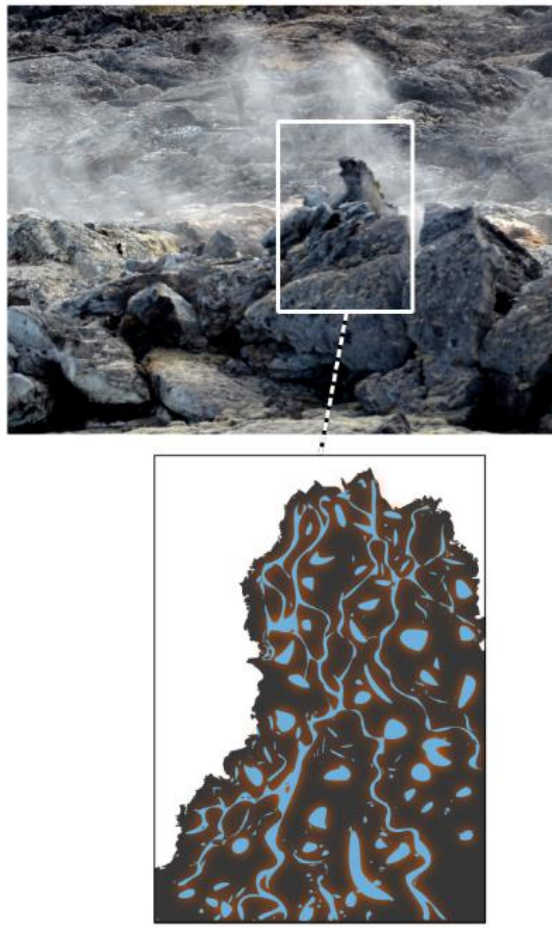


- 24-3 Polymerase
- Ribo PCR in realistic environment

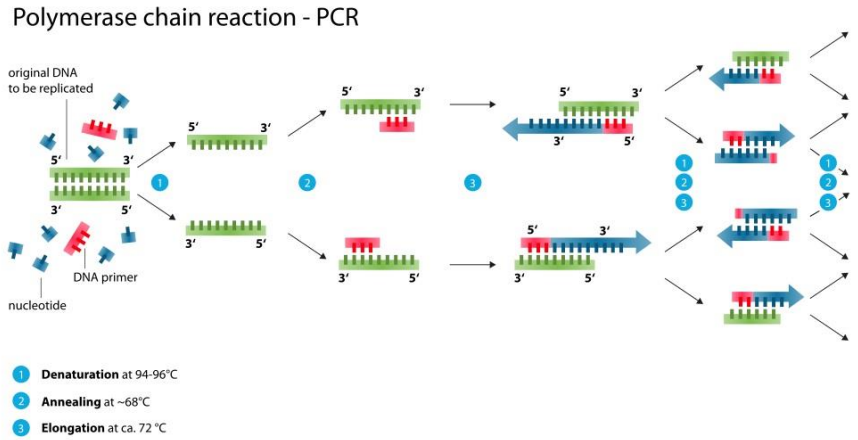


RNA amplifictaion in water-filled pores



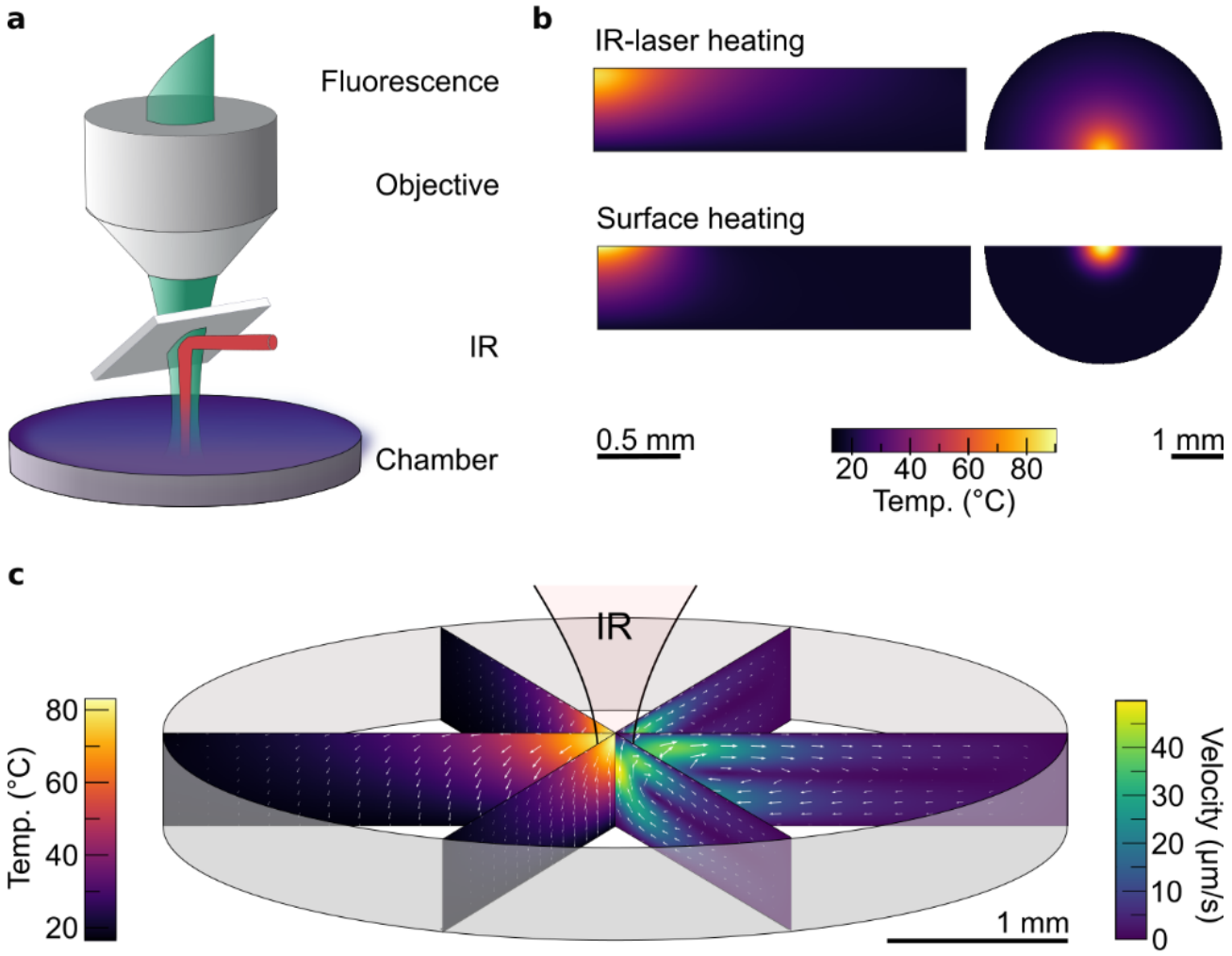


- 24-3 Polymerase
- Ribo PCR in realistic environment

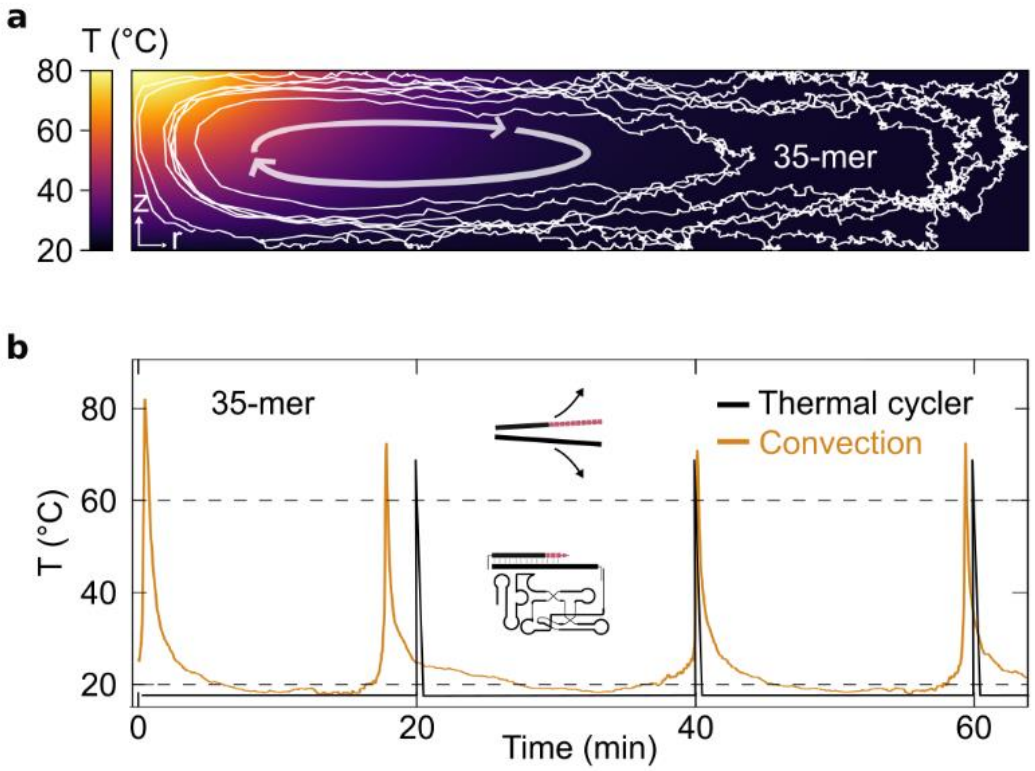


- Convection leads to temperature cycles
- Themophoresis leads to protection

Experimental impementation



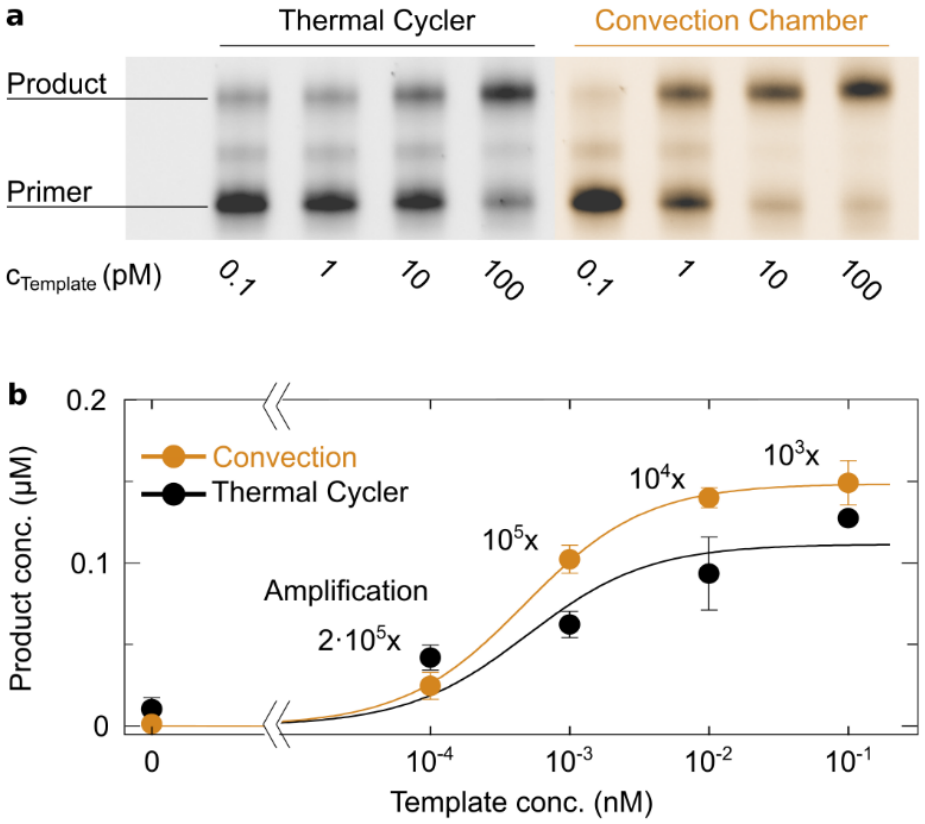
RNA amplifictaion in water-filled pores



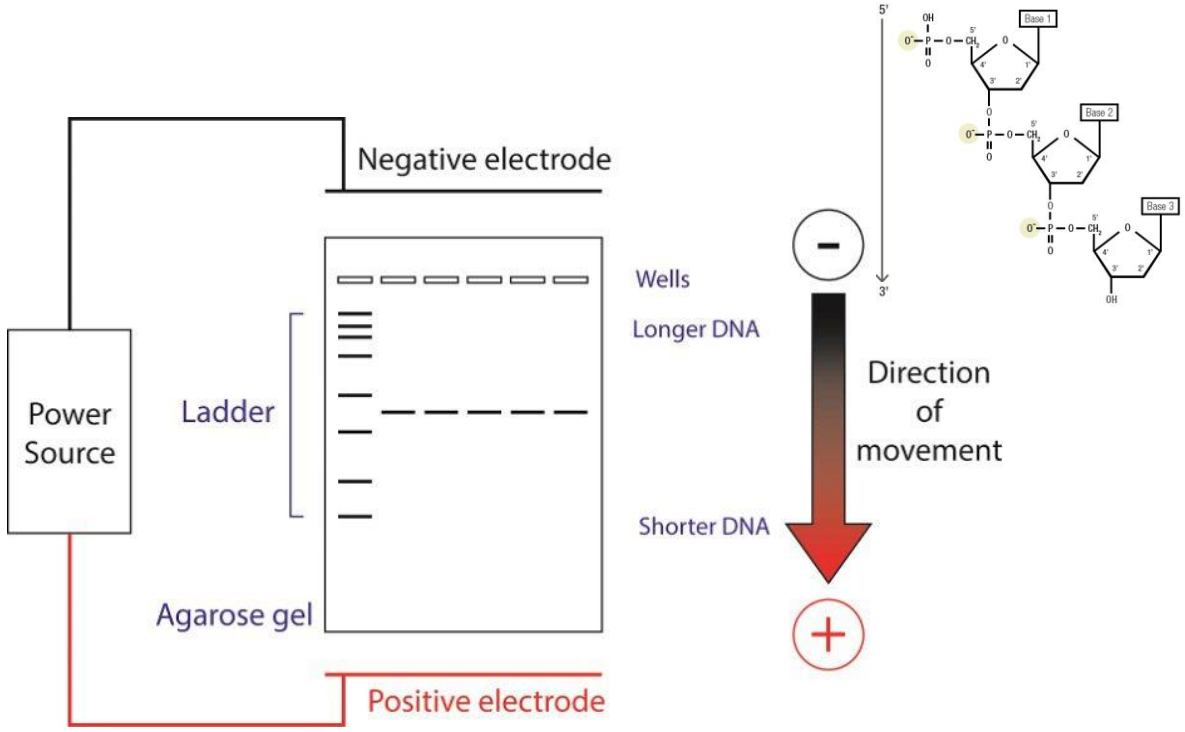
$$\vec{j_i} = \underbrace{-D_i \cdot \nabla c_i}_{} - \underbrace{S_{T_i} \cdot D_i \cdot \nabla T \cdot c_i}_{} + \underbrace{\vec{v} \cdot c_i}_{}$$

- Simulate trajectories by including diffusion, thermophoresis and convection

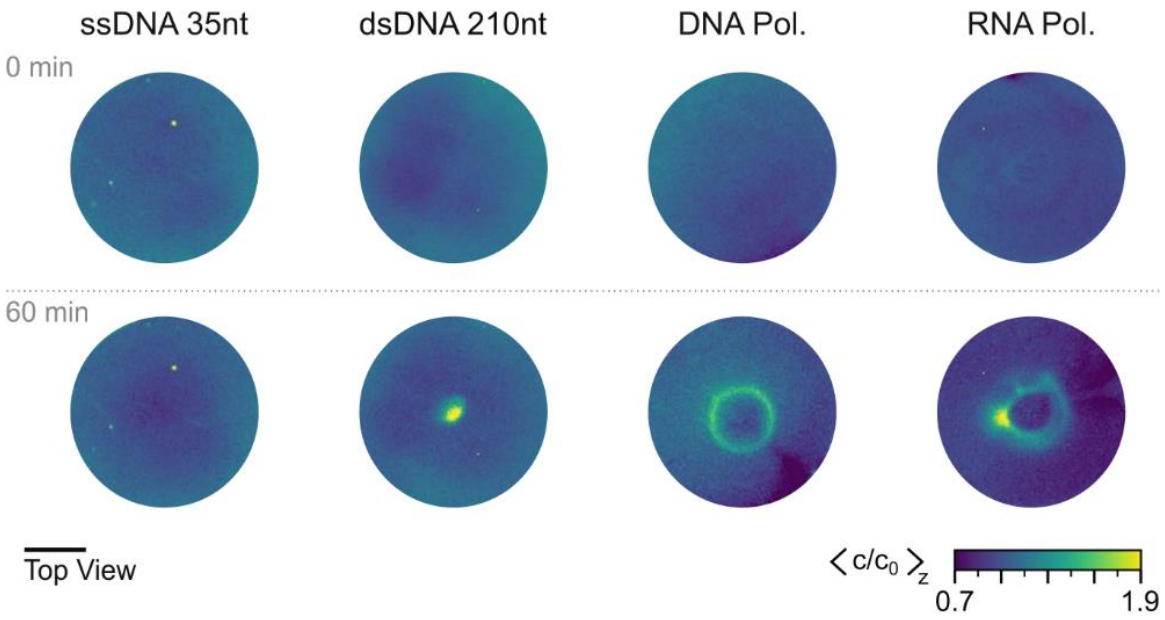
RNA amplifictaion in water-filled pores

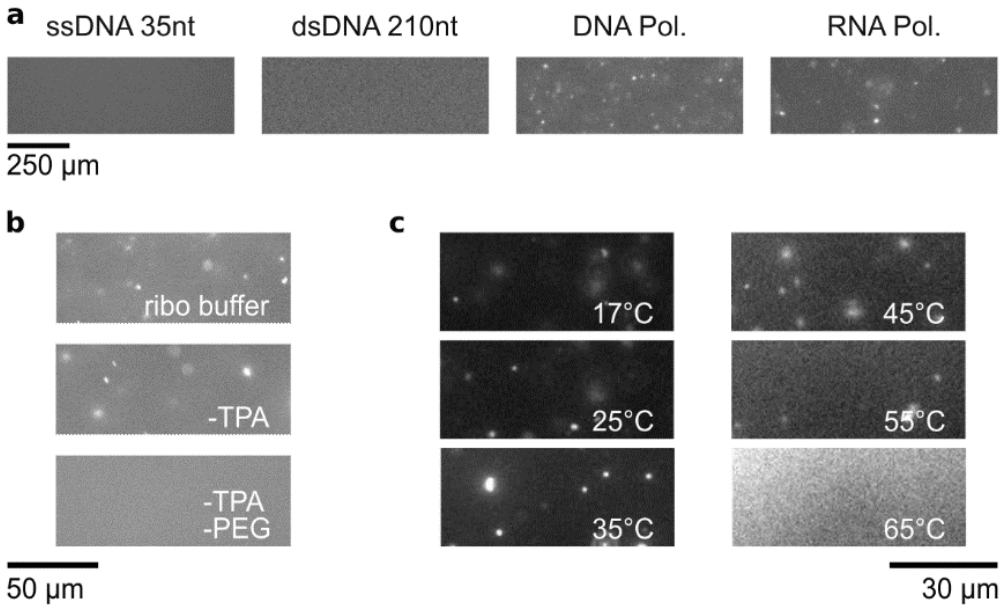


- Analyze by PAGE (polyacylamide gelelectrophoresis)
- Convection chamber performs equally well!



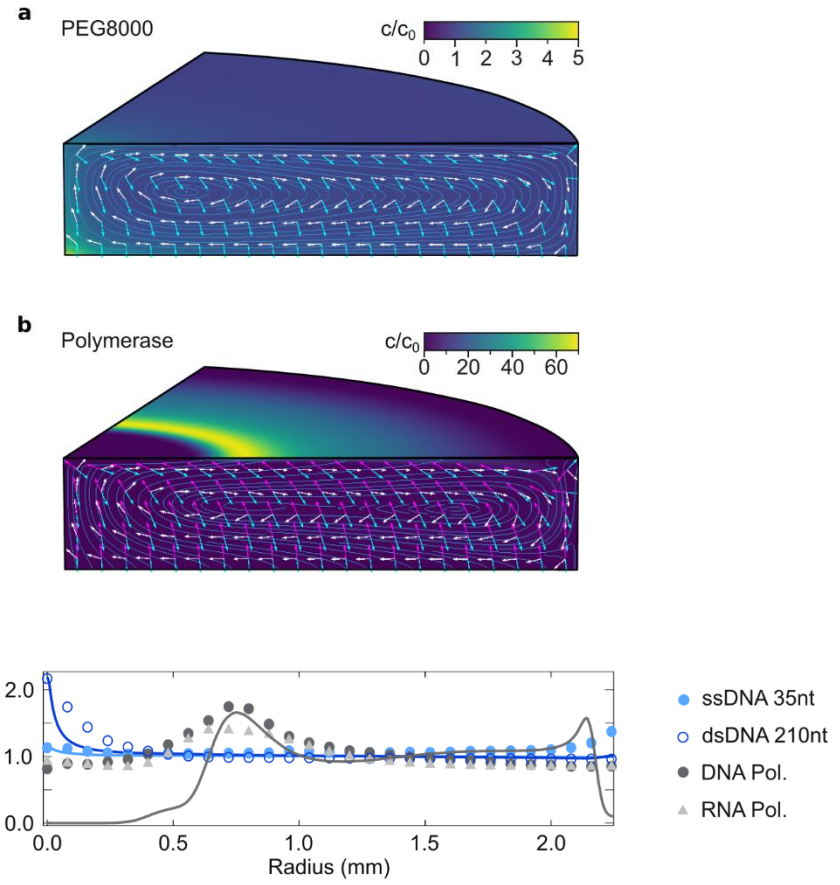
Accumulation pattern of RNA

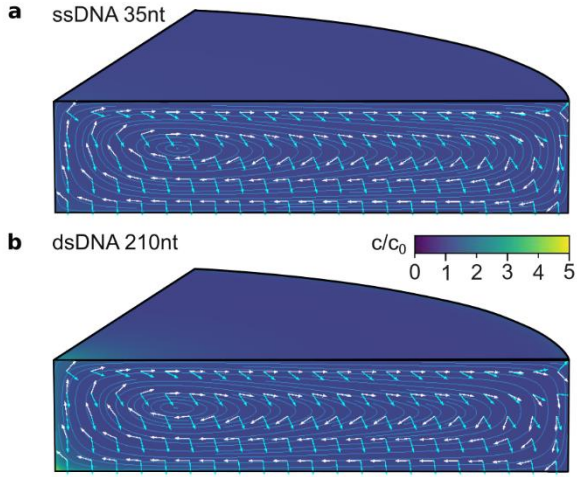




- Micrometer sized conglomerates
- Include diffusiophoresis -> movement along a concentration gradient

Accumulation pattern of RNA

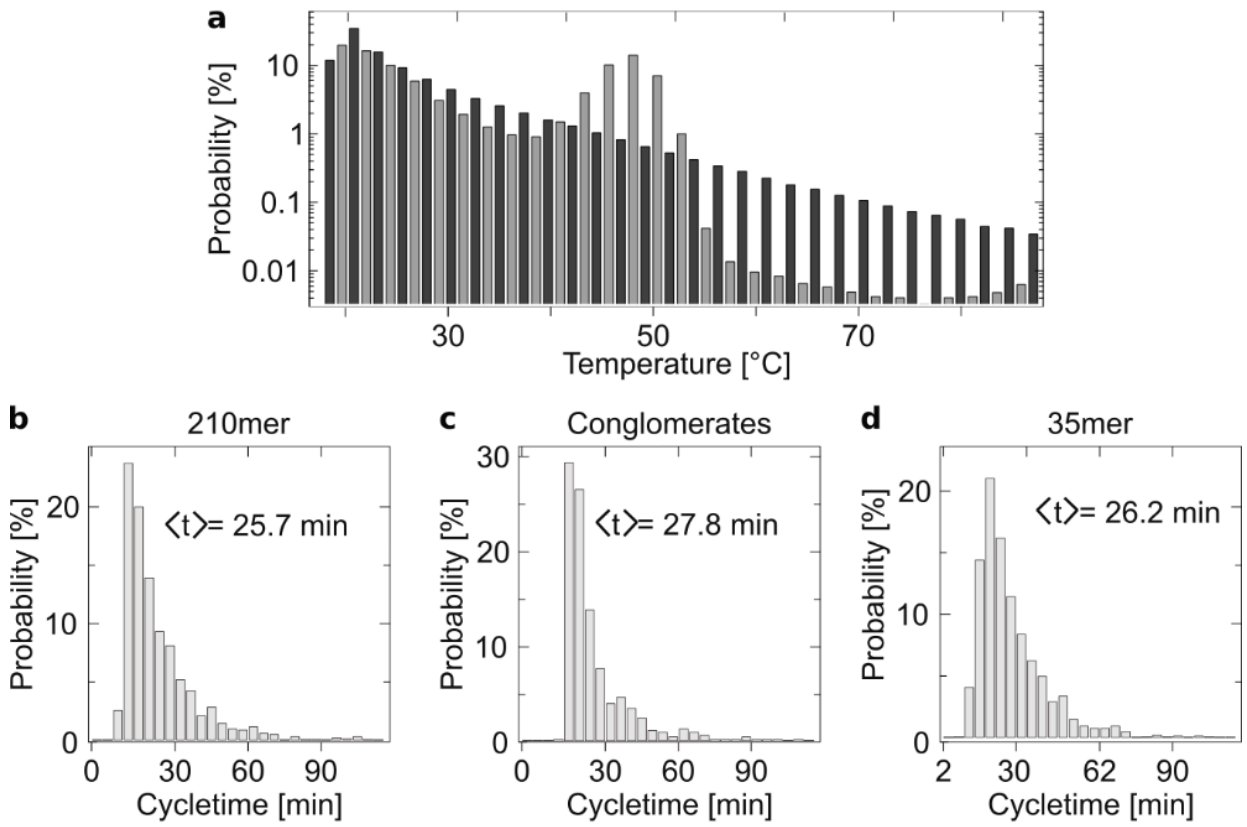


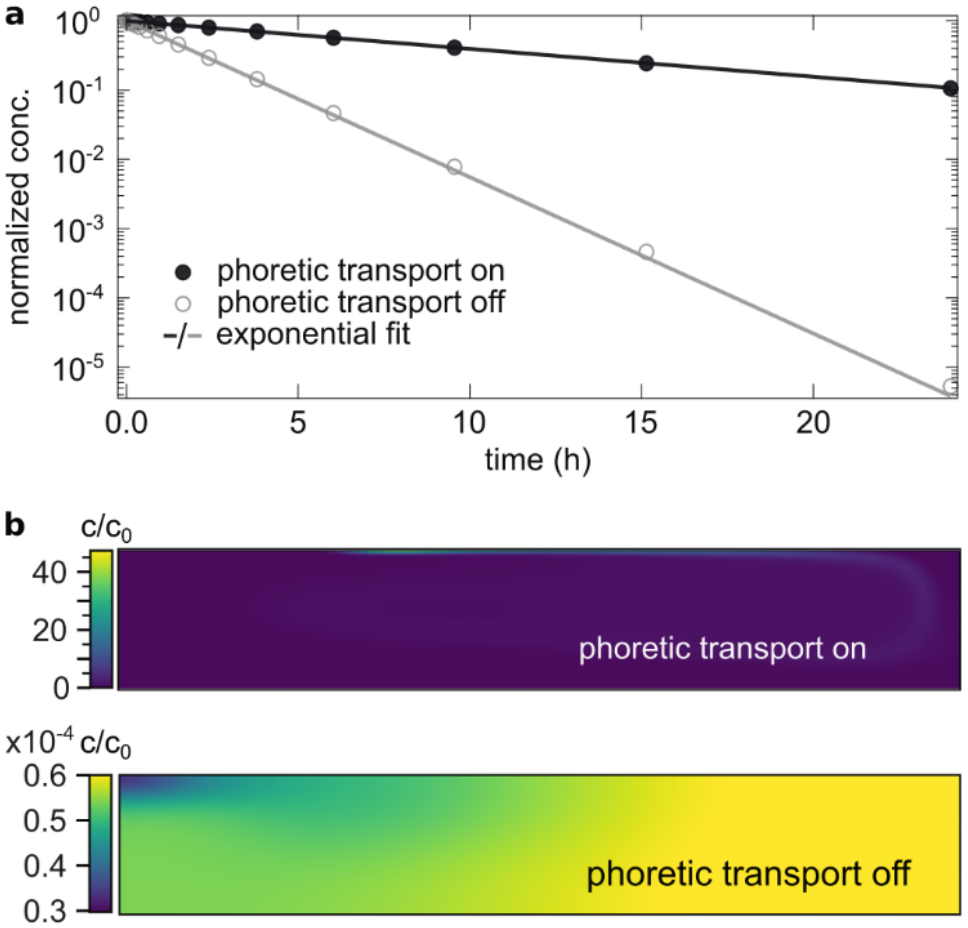


Diffusiophoretic velocity

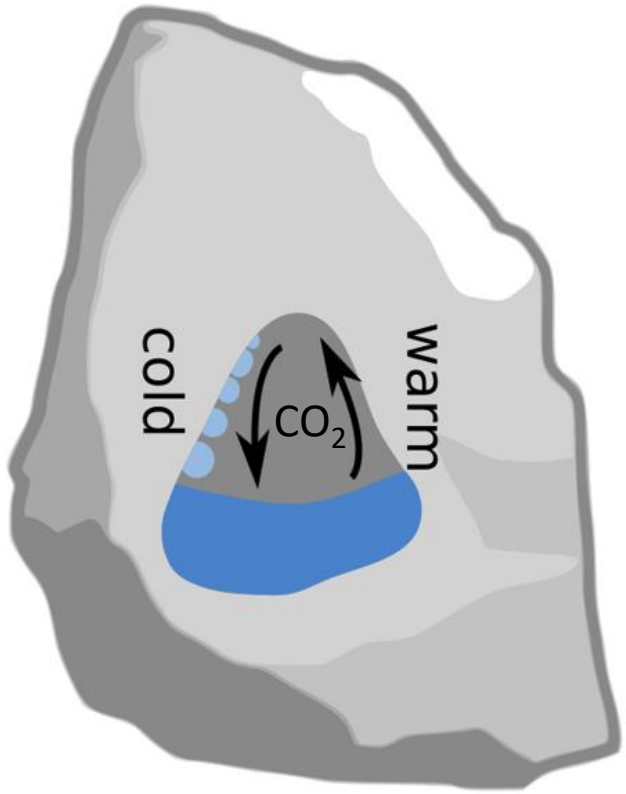
$$\vec{j}_i = -D_i \cdot \nabla c_i - S_{T_i} \cdot D_i \cdot \nabla T \cdot c_i + (\vec{v} + \vec{u}_D) \cdot c_i$$

RNA protection from heat





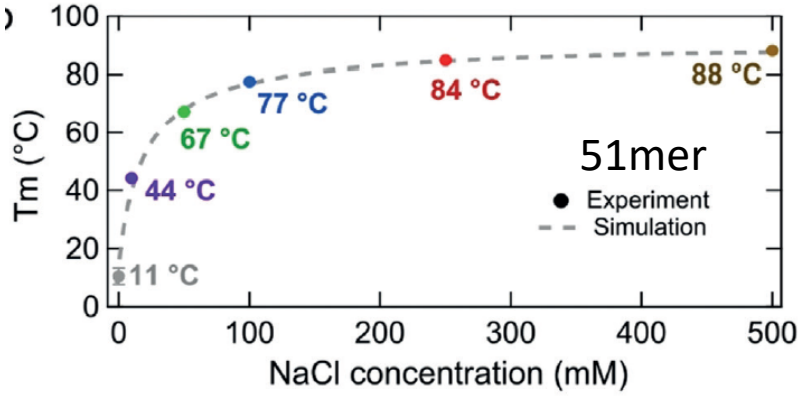
Limited denaturation by temperature



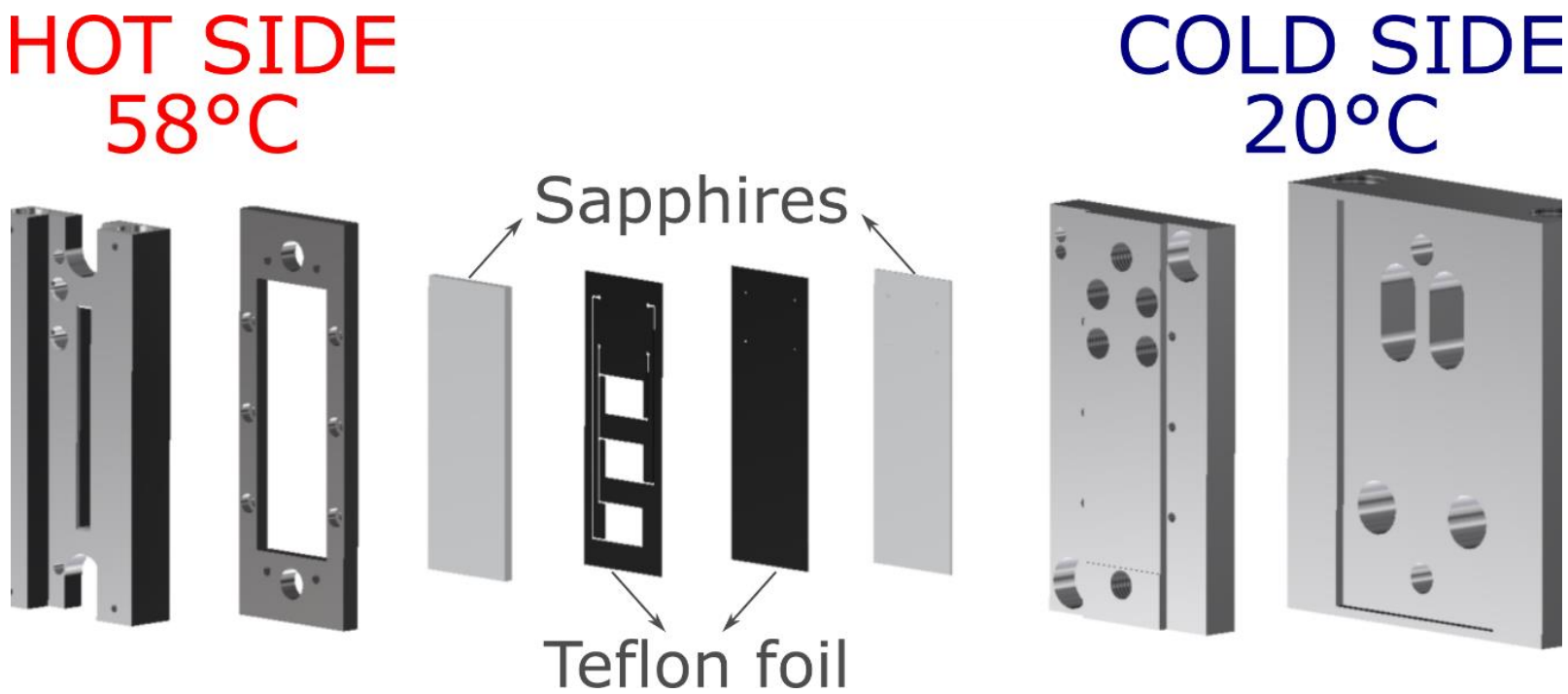
Water cycles in heated rock pores

→ Increased melting temperature for product-template complex

→ dead-end duplex

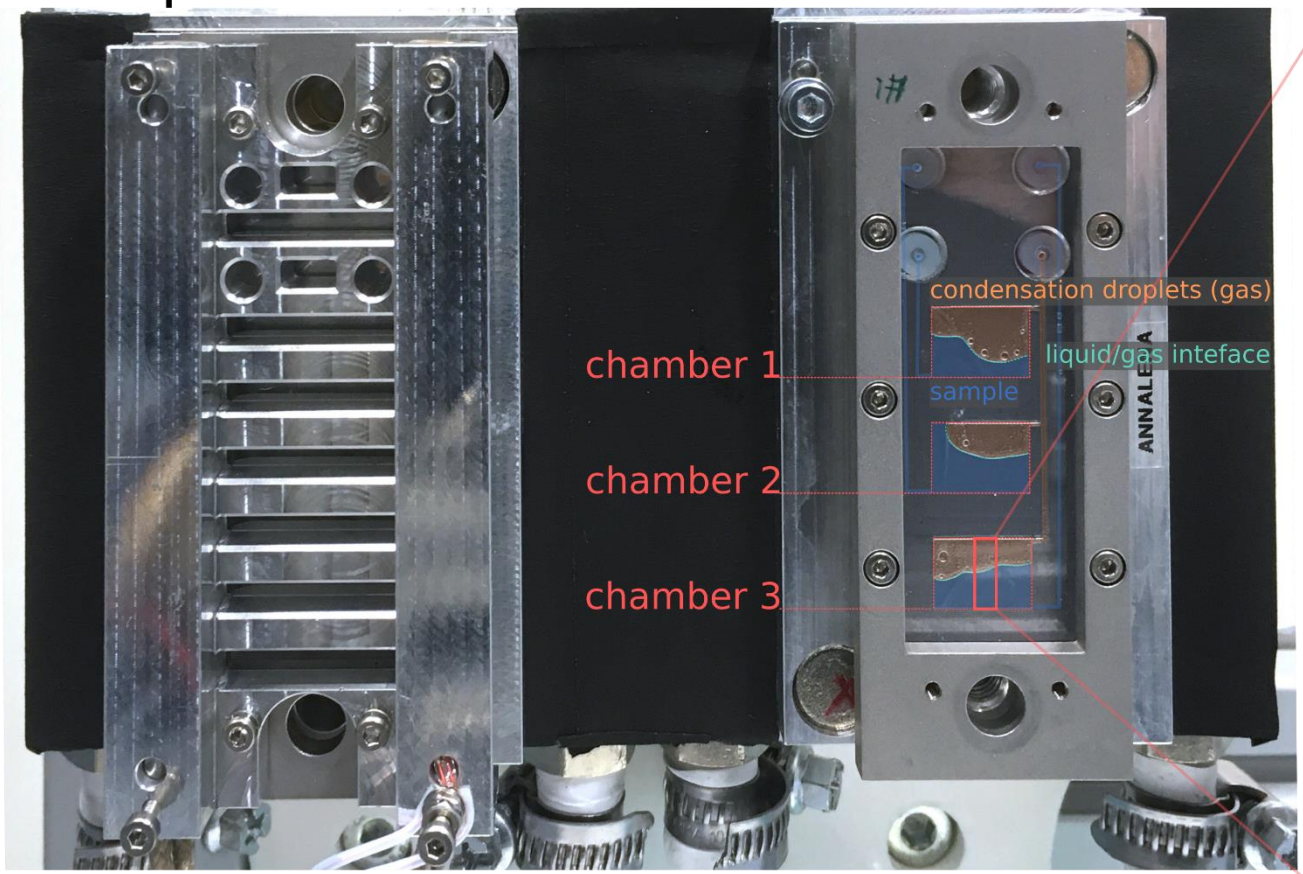


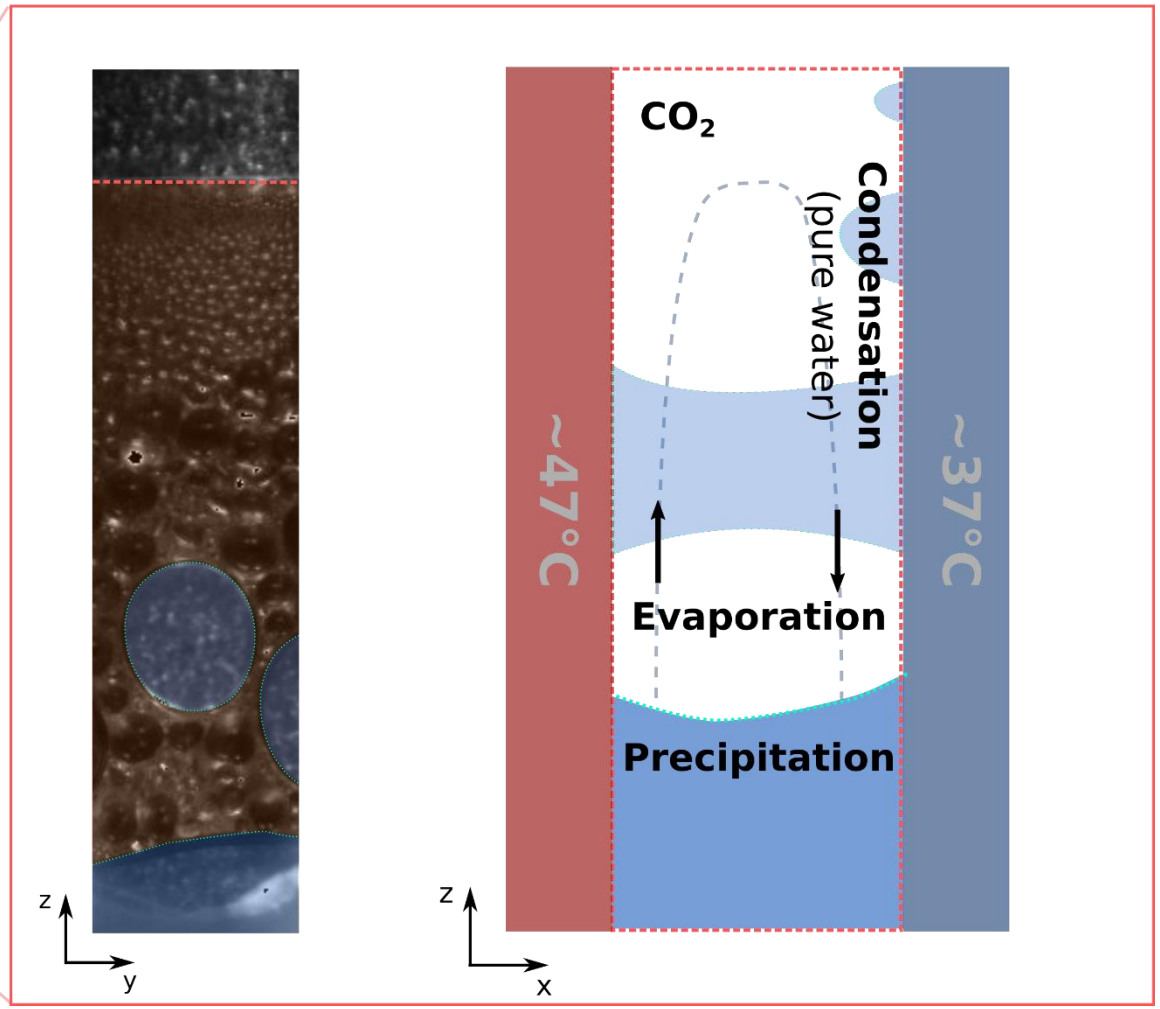
Ianeselli, A. *et al.* Periodic Melting of Oligonucleotides by Oscillating Salt Concentrations Triggered by Microscale Water Cycles Inside Heated Rock Pores. *Angew. Chemie Int. Ed.* **58**, 13155–13160 (2019).


HOT SIDE
58°C
Sapphires
Teflon foil
COLD SIDE
20°C

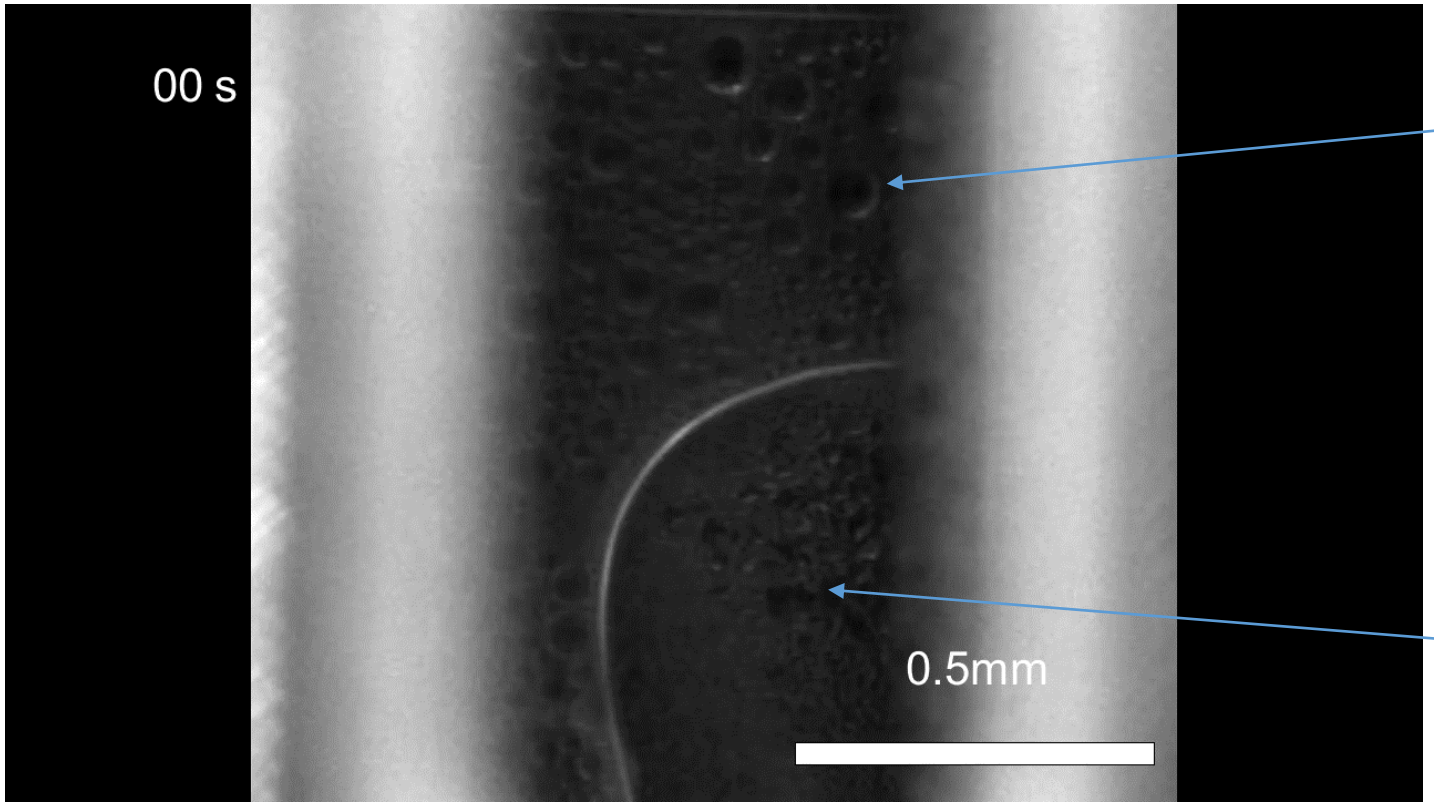
Fully assembled trap

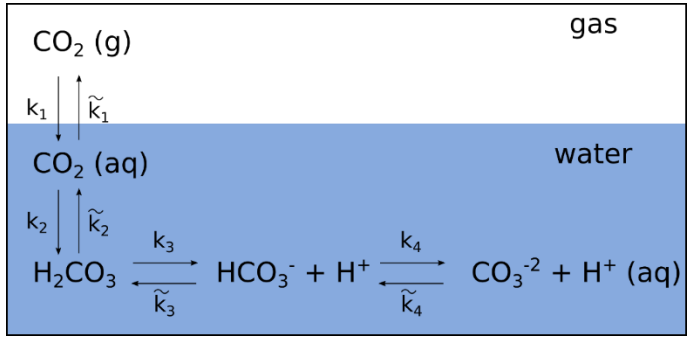
Trap without heater





Non-equilibrium setting to drive elongation

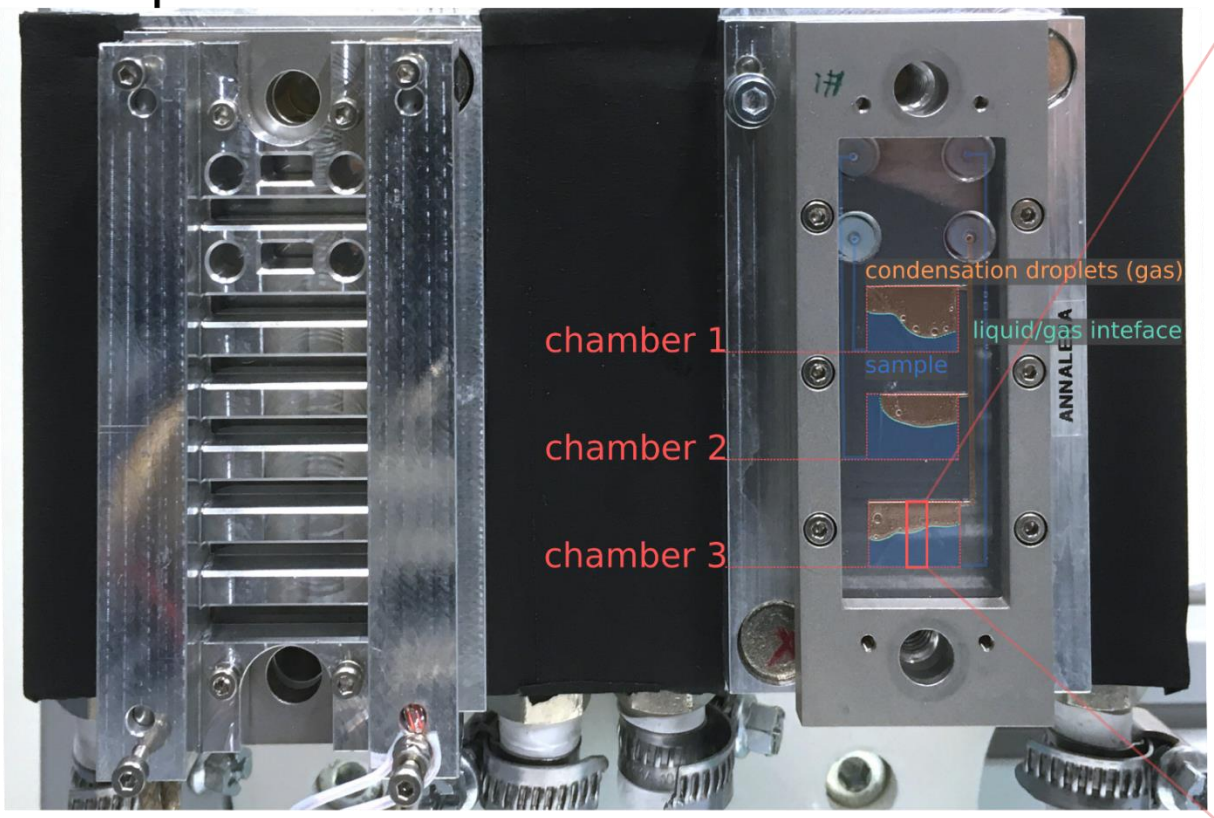


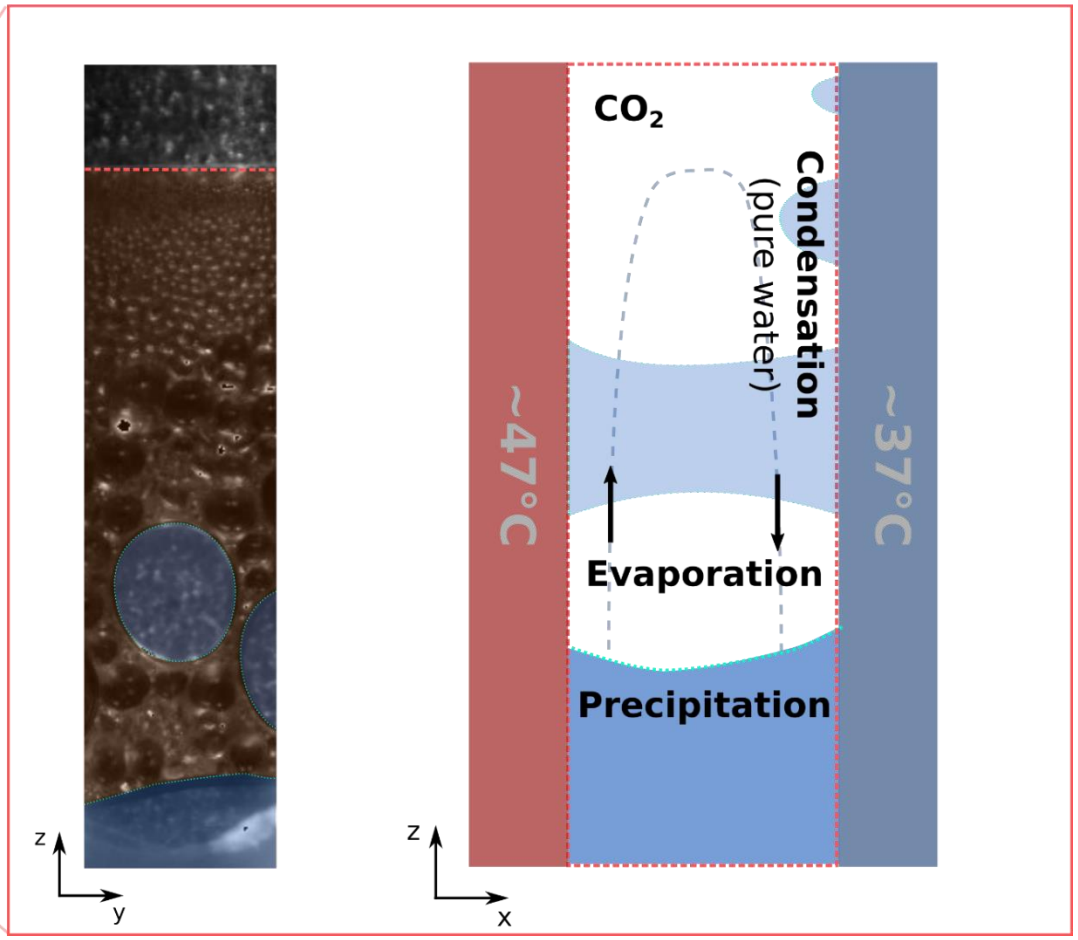


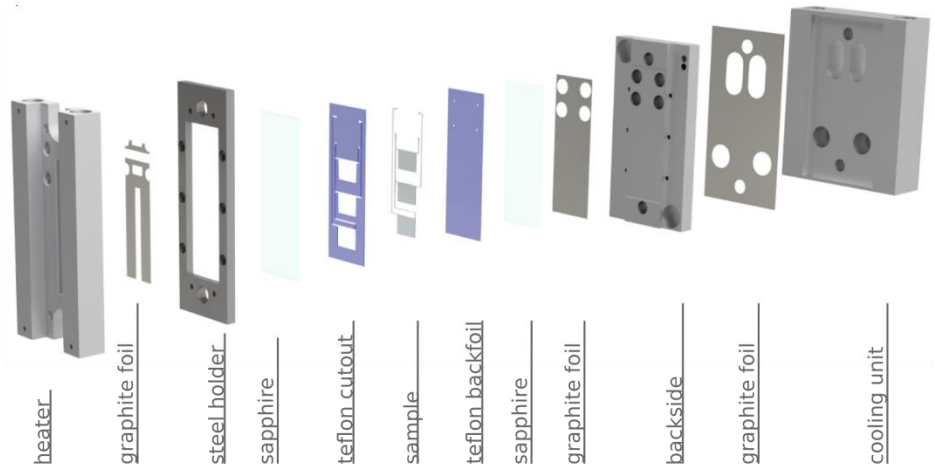
Buffered sample

Fully assembled trap

Trap without heater





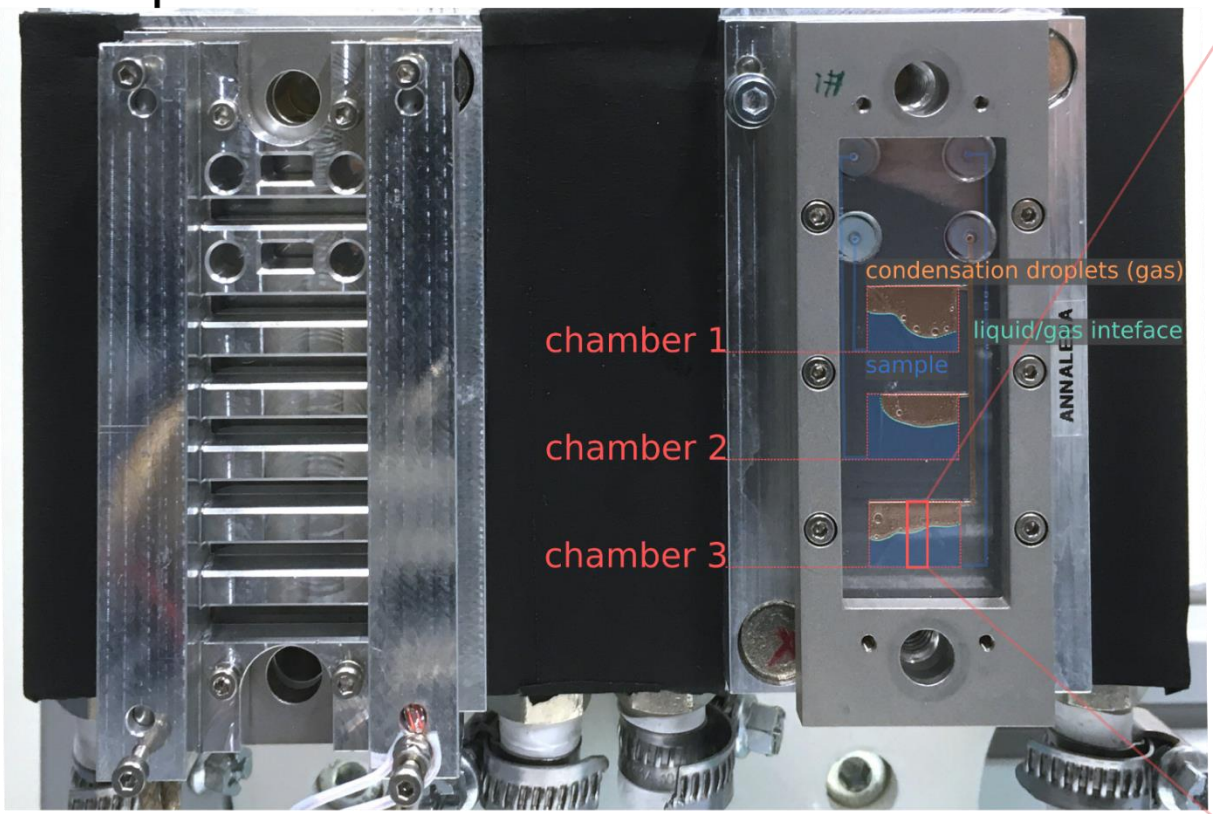


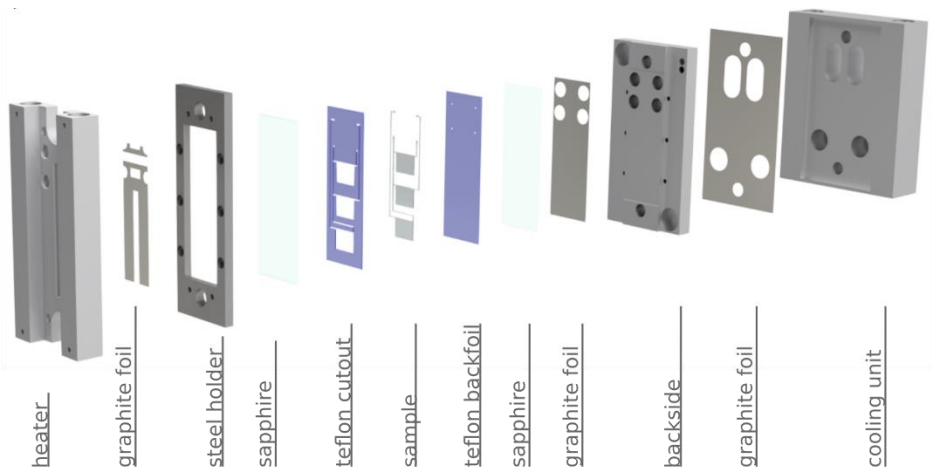
Ianeselli, A. *et al.* Periodic Melting of Oligonucleotides by Oscillating Salt Concentrations Triggered by Microscale Water Cycles Inside Heated Rock Pores. *Angew. Chemie Int. Ed.* **58**, 13155–13160 (2019).

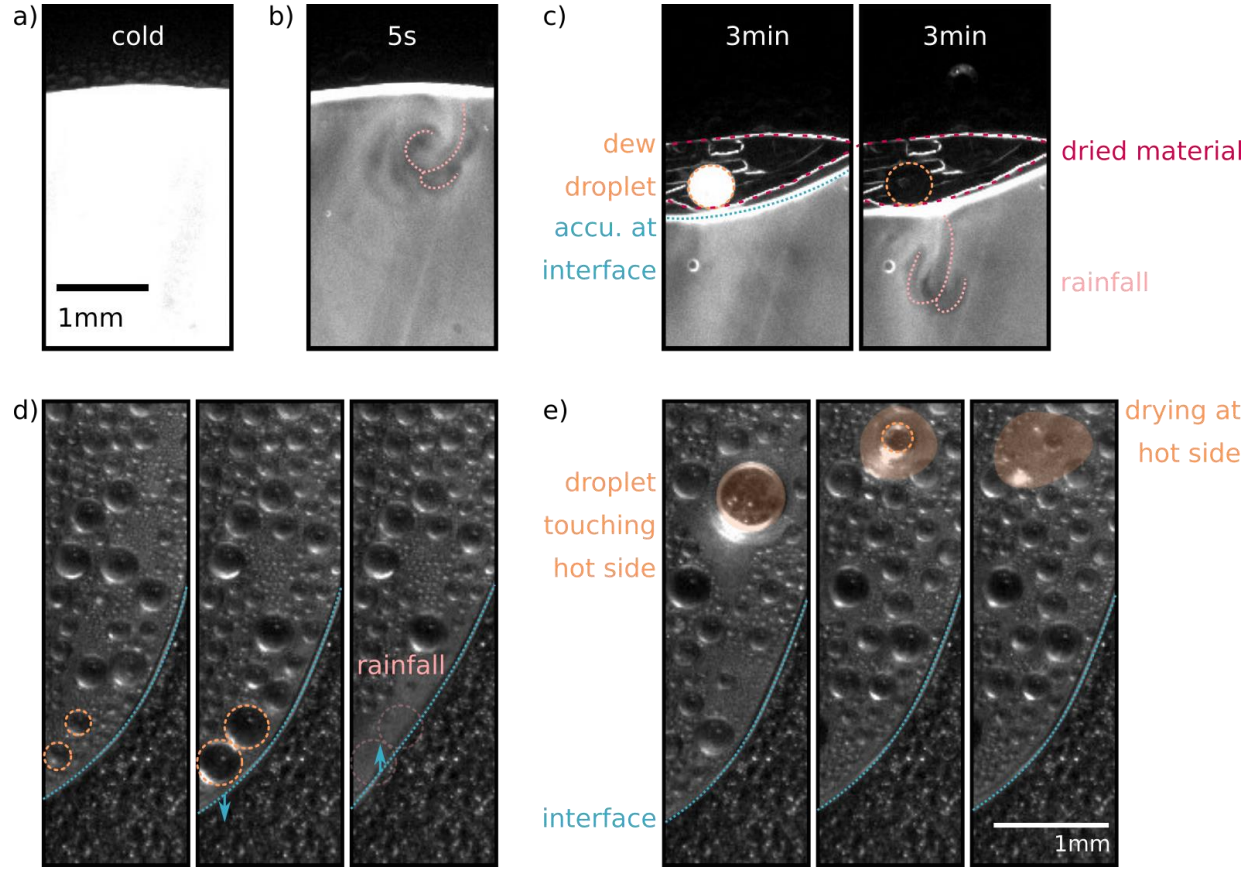
Ianeselli, A. *et al.* Water cycles in a Hadean CO2 atmosphere drive the evolution of long DNA. Nat. Phys. 18, 579–585 (2022).

Fully assembled trap

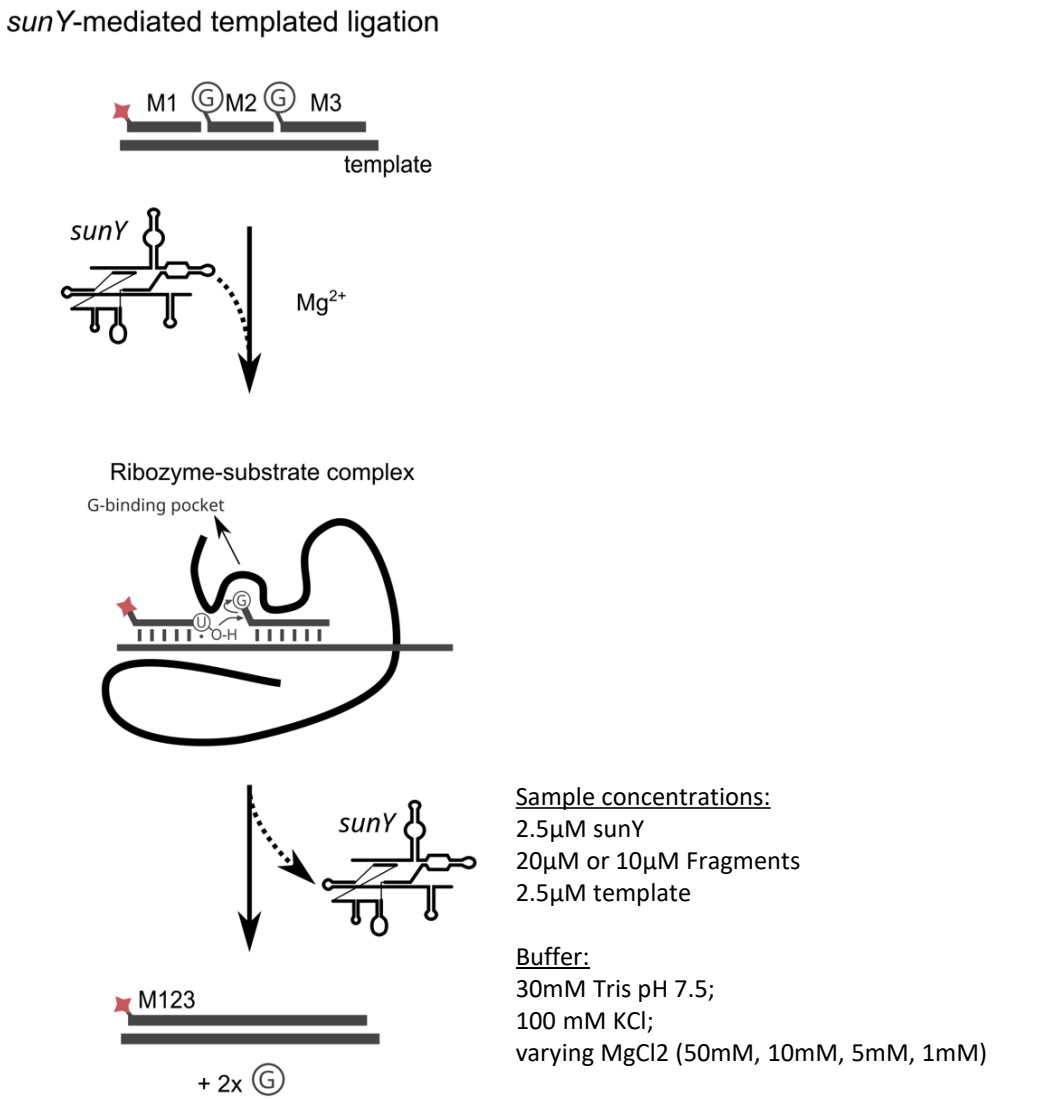
Trap without heater





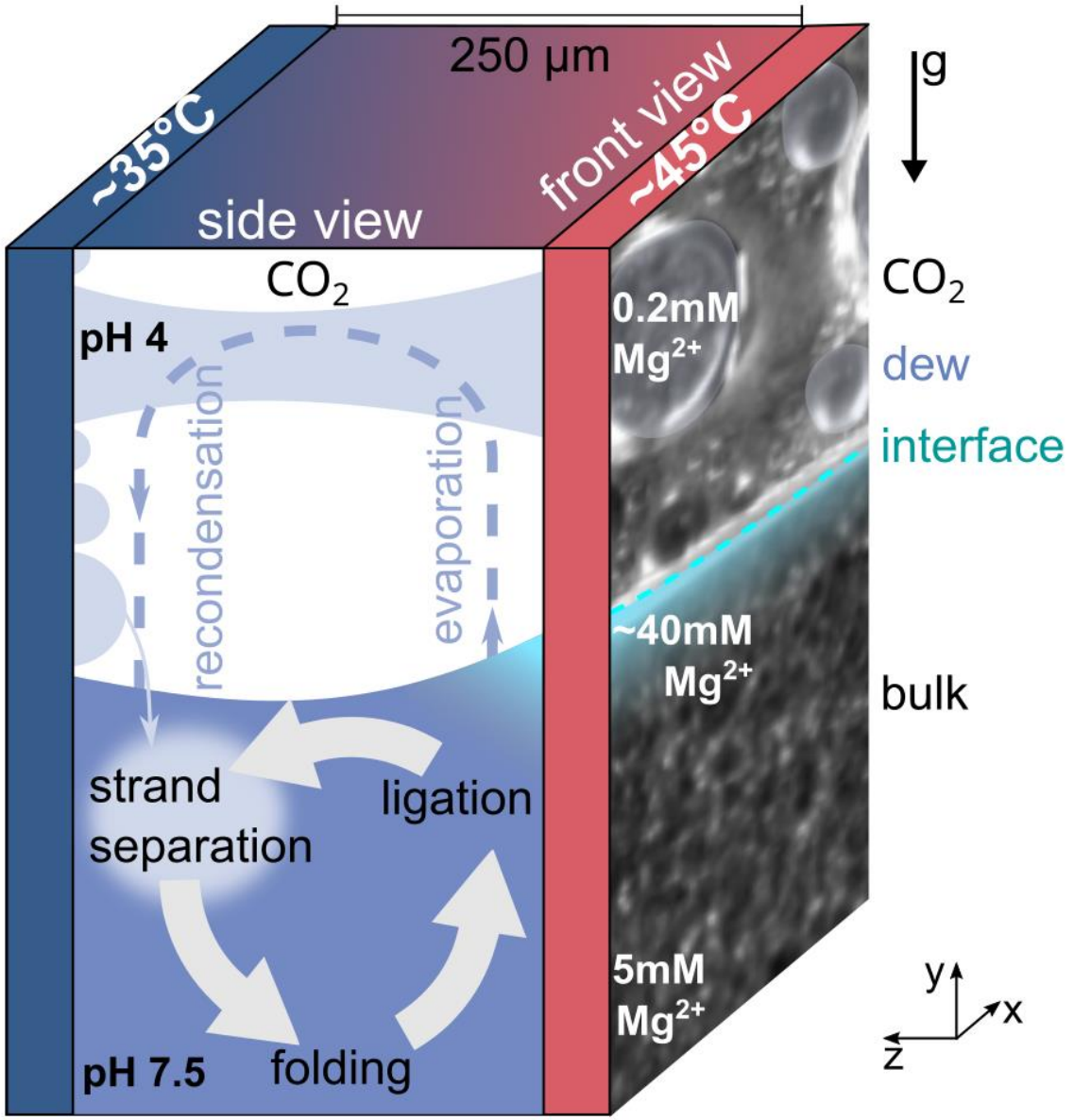


Reaction and template release



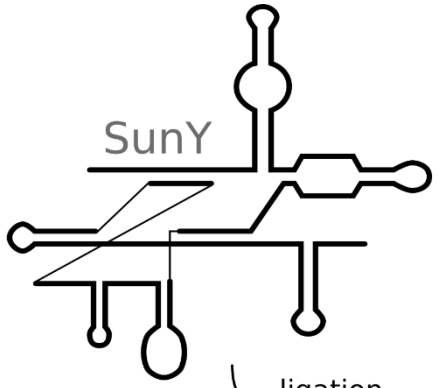
Sample concentrations:
2.5µM sunY
20µM or 10µM Fragments
2.5µM template

Buffer:
30mM Tris pH 7.5;
100 mM KCl;
varying MgCl2 (50mM, 10mM, 5mM, 1mM)



Salditt, A., Karr, L., Salibi, E., Le Vay, K., Braun, D., & Mutschler, H. (2022). Complete RNA replication cycles in a Hadean microcompartment.

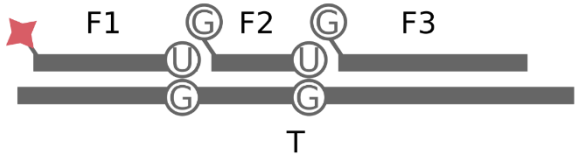
sunY activity in the AWI-system at low Mg2+



Mango aptamer

ligation

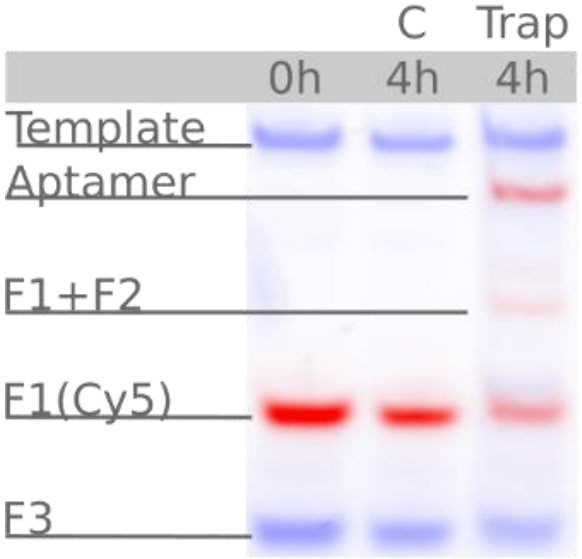
PAGE



melting and binding

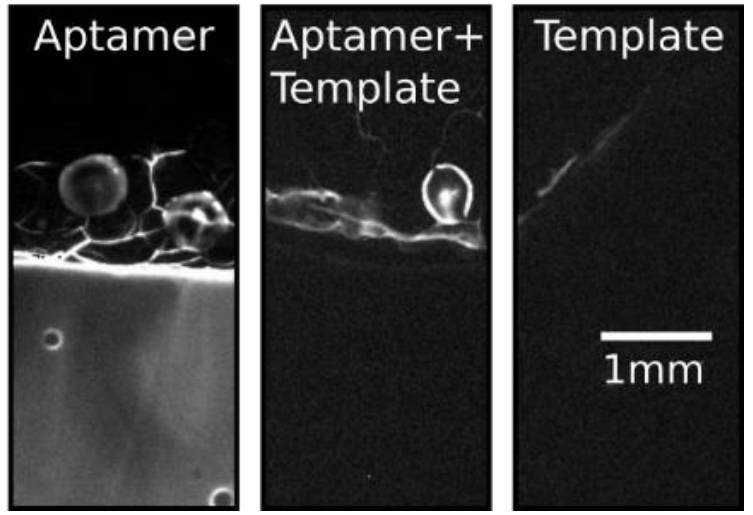
Fluorescent readout

reaction setup

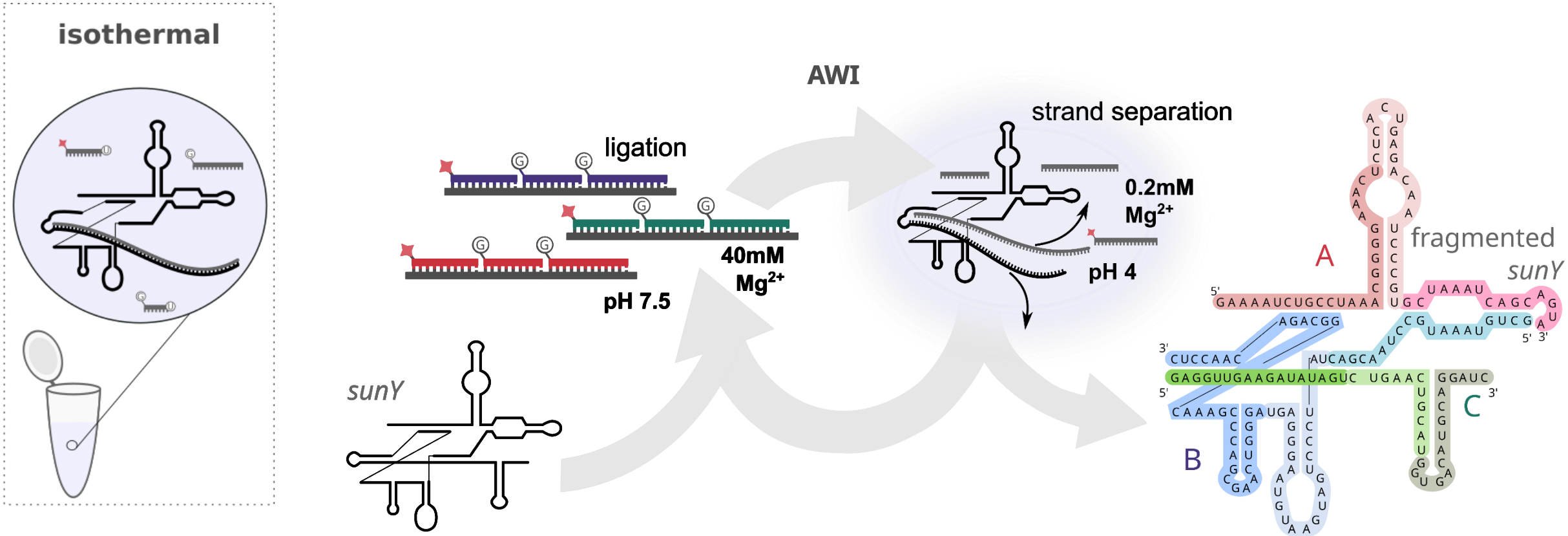


Sample concentrations:
2.5µM sunY
20µM Fragments
2.5µM template

Buffer:
30mM Tris pH 7.5;
100 mM KCl;
5mM MgCl2



Synthesis of similar sequences



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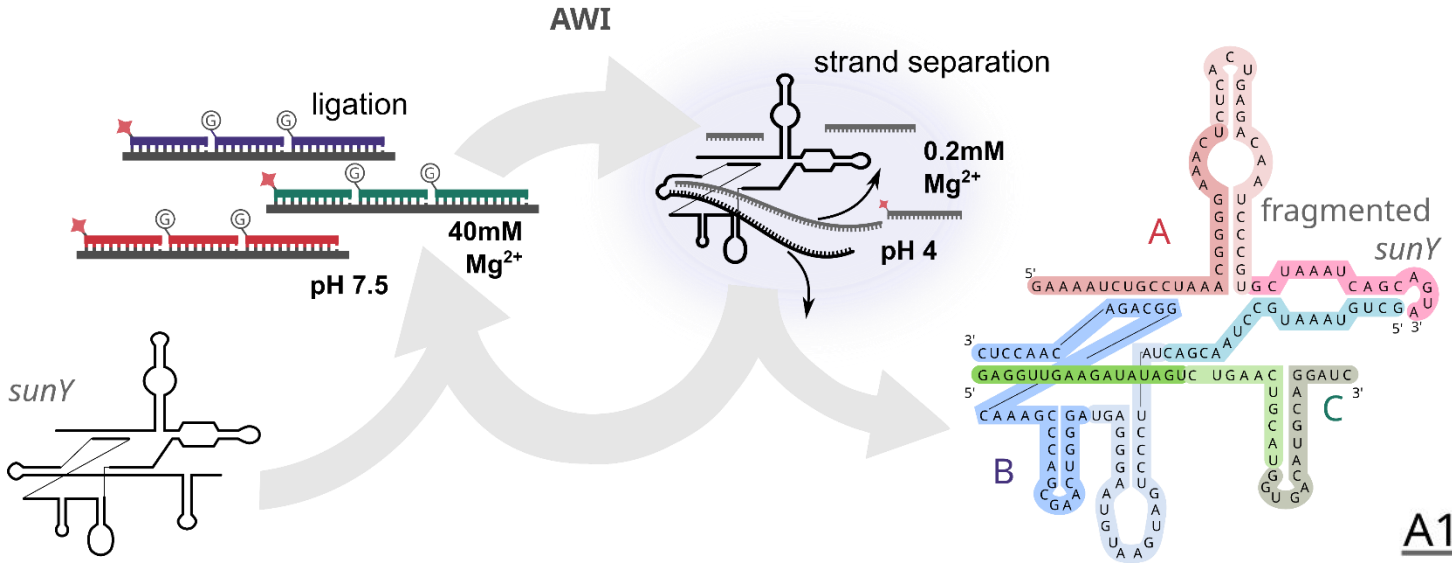
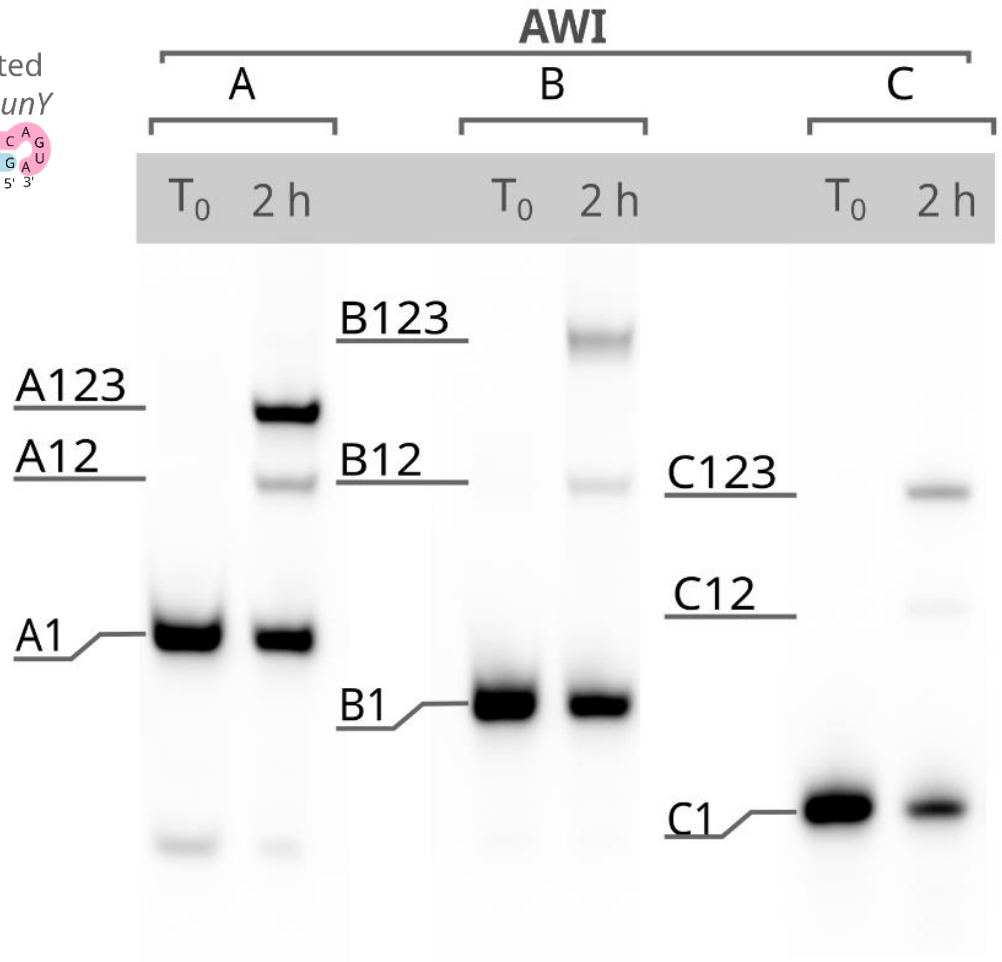
Synthesis of similar sequences





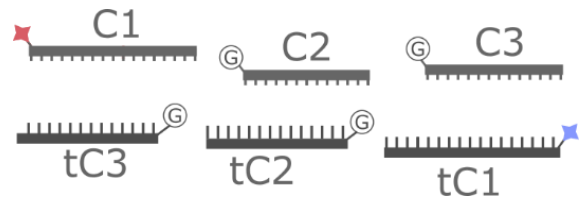
Sample concentrations:
2.5µM sunY
10µM Fragments
2.5µM template

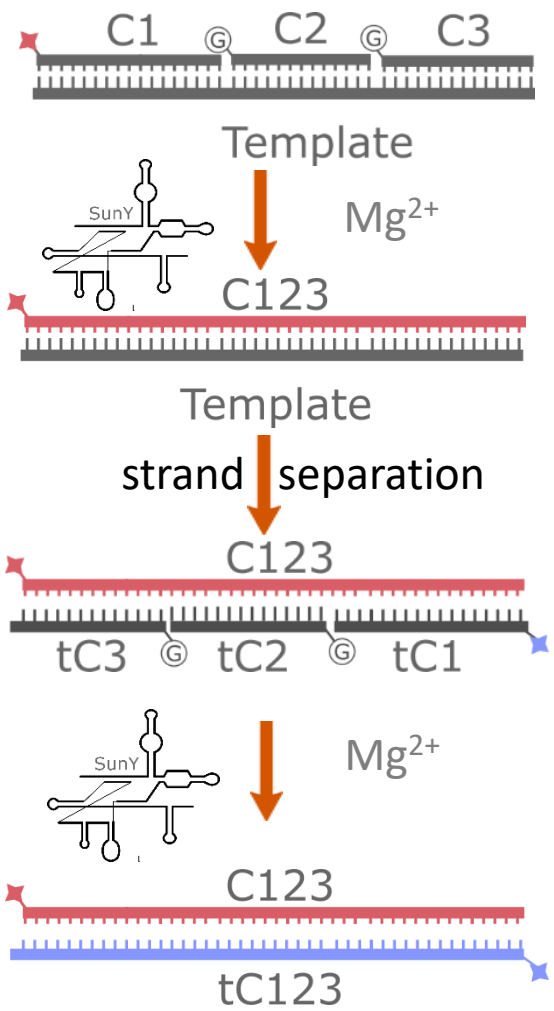
Buffer:
30mM Tris pH 7.5;
100 mM KCl;
10mM MgCl2

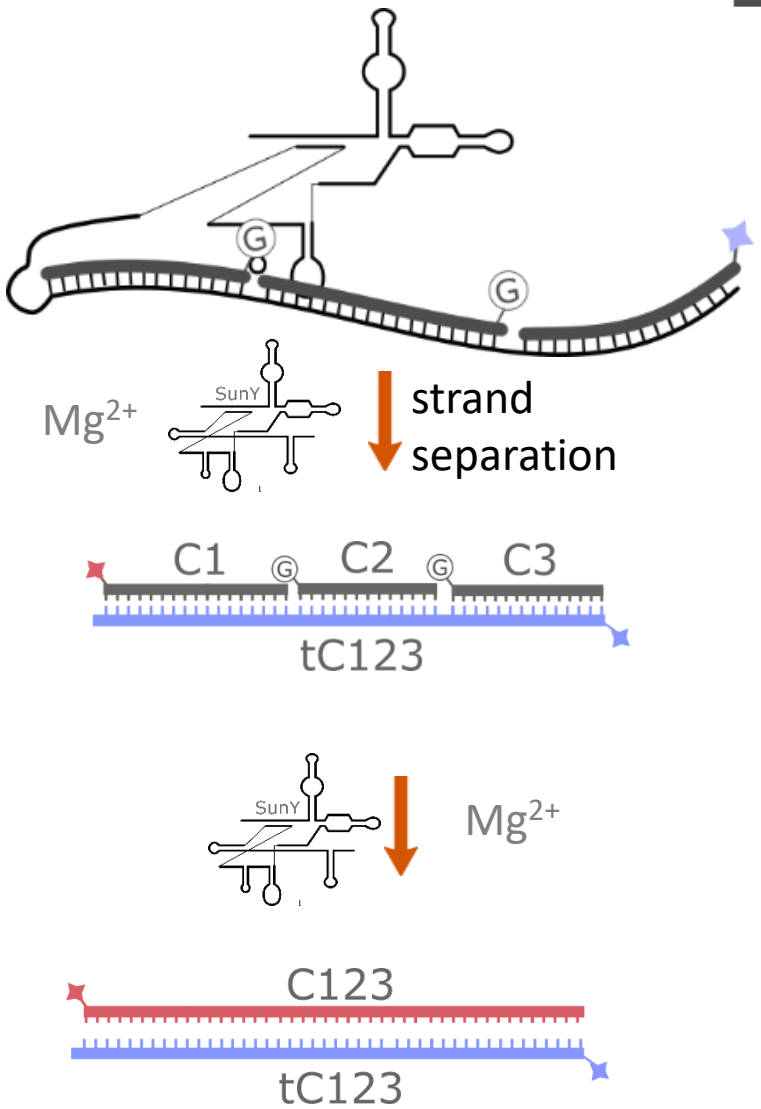
Salditt, A., Karr, L., Salibi, E., Le Vay, K., Braun, D., & Mutschler, H. (2022). Complete RNA replication cycles in a Hadean microcompartment.

Towards a full replication cycle

Fragments of both (+ and -) side ⟶ C1-C3 and tC1-tC3

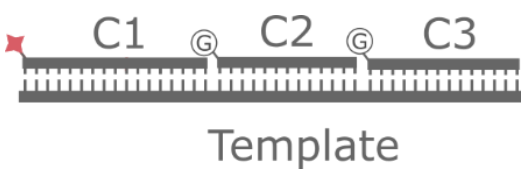




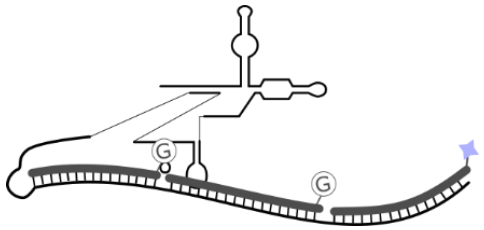


Salditt, A., Karr, L., Salibi, E., Le Vay, K., Braun, D., & Mutschler, H. (2022). Complete RNA replication cycles in a Hadean microcompartment.

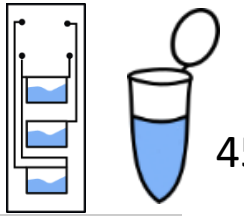
Towards a full replication cycle



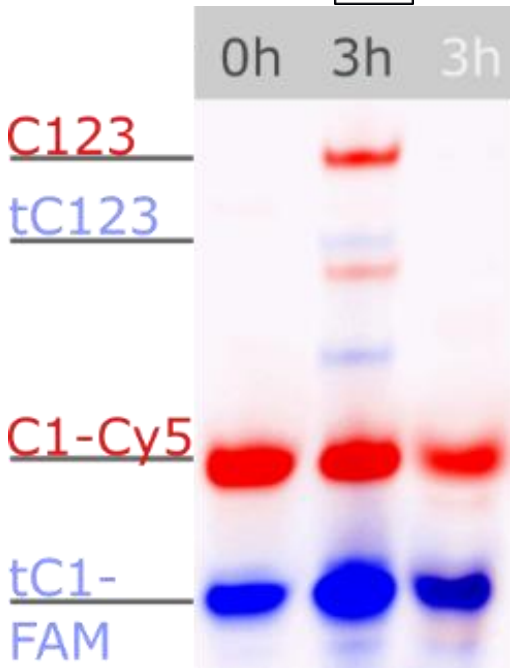
with template



without template

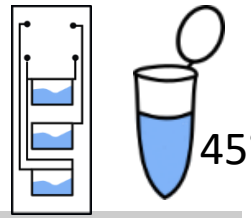


45°C

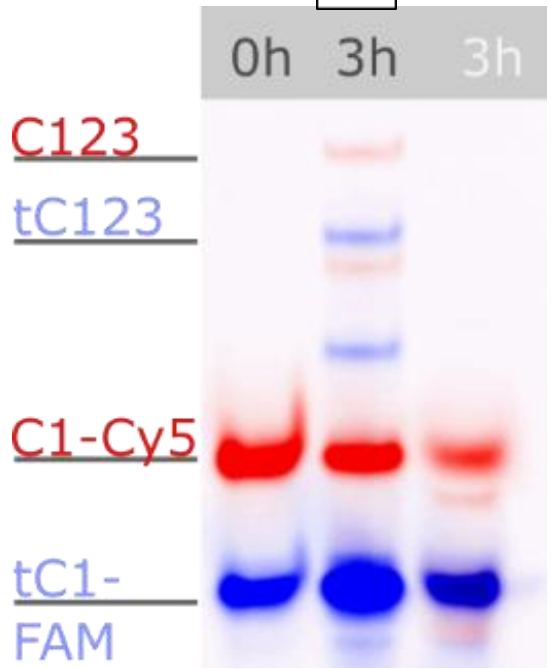


<u>Sample concentrations:</u>
2.5µM sunY
10µM Fragments
2.5µM template

<u>Buffer:</u>
30mM Tris pH 7.5;
100 mM KCl;
10mM MgCl2



45°C



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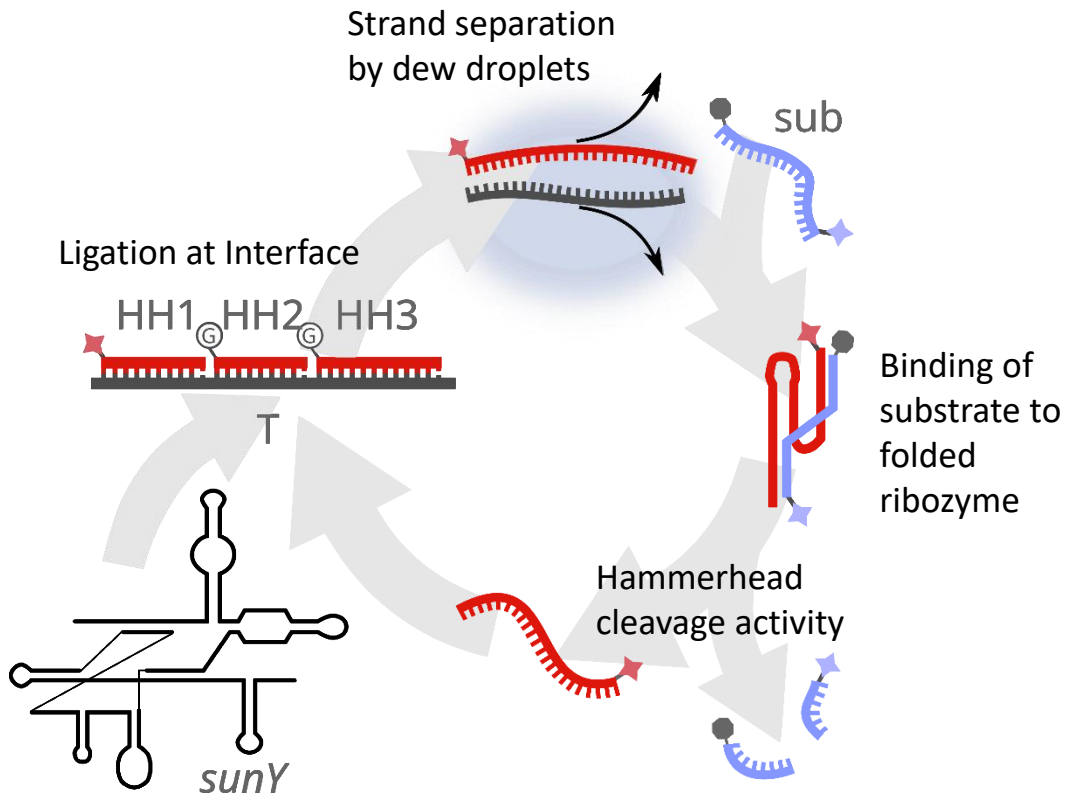
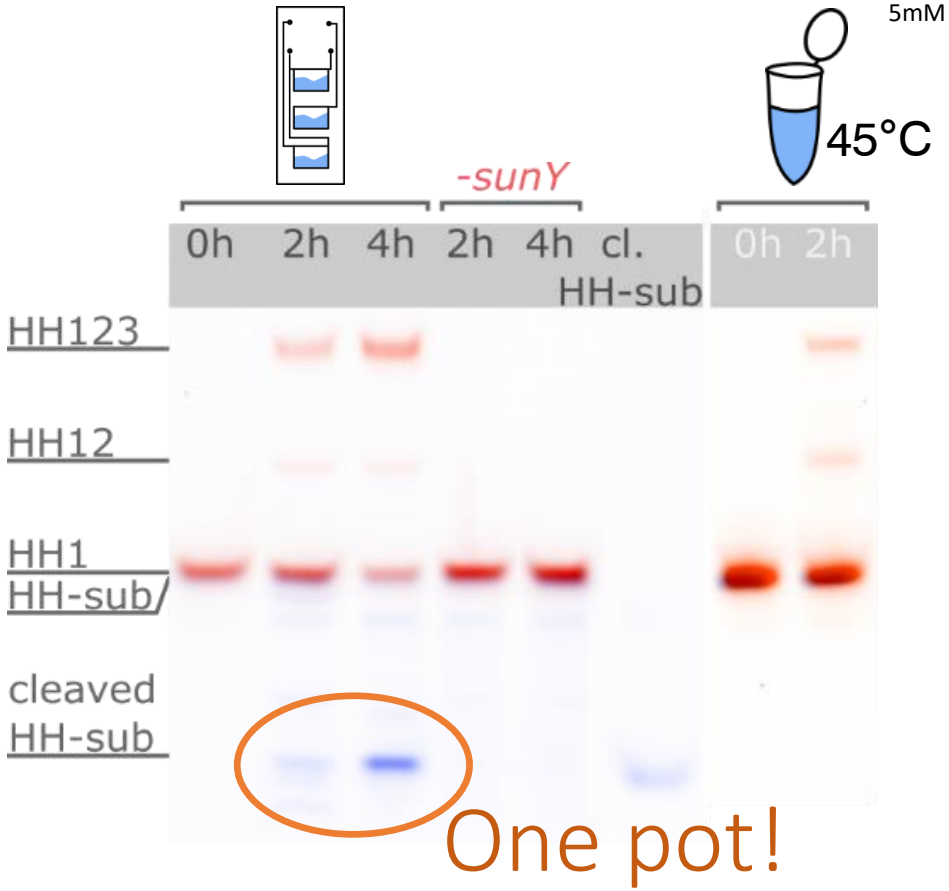
Synthesis of active ribozyme

Sample concentrations:
2.5µM sunY
10µM Fragments
2.5µM template

Buffer:
30mM Tris pH 7.5;
100 mM KCl;
5mM MgCl2





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