DNA Hydrogels Hot Paper

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Heat-Flow-Driven Oligonucleotide Gelation Separates Single-Base Differences

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Abstract: *DNA phase transitions are often induced by the addition of condensation agents or by dry concentration. Herein, we show that the non-equilibrium setting of a moderate heat flow across a water-filled chamber separates and gelates DNA strands with single-base resolution. A dilute mix of DNA with two slightly different gel-forming sequences separates into sequence-pure hydrogels under constant physiological solvent conditions. A single base change in a 36 mer DNA inhibits gelation. Only sequences with the ability to form longer strands are concentrated, further elongated, and finally gelated by length-dependent thermal trapping. No condensation agents, such as multivalent ions, were added. Equilibrium aggregates from dry concentration did not show any sequence separation. RNA is expected to behave identically owing to its equal thermophoretic properties. The highly sequence-specific phase transition points towards new possibilities for non-equilibrium origins of life.*

Nucleic acids are one of the most abundant molecules in biology. They are able to store large amounts of information in a condensed state at an unprecedented density.[1] At the same time, they provide access for readout at any time by local unwinding and re-packing. Their ability to efficiently condense or self-organize while still being accessible for sequence-selective processes, such as replication or transcription, is an essential aspect for the development of life and has been intensively researched.[2,3]

Experimentally, such condensation processes are described as phase transitions between the dilute (sol) phase and a dense phase. The dense phase is usually induced by concentrating nucleic acids or adding multivalent ions or proteins. One example is the condensation of long DNA (>1 kbp), assisted by spermidine^{3+} or $Co(NH_3)_6^{3+}$ ions that bind to the negatively charged phosphate groups, allowing for DNA–DNA attraction.[2,4] Further condensation agents include cationic lipids, polymers, and metal complexes.[3] Monovalent ions, on the other hand, only partially shield the repulsive force between the DNA strands. This hinders strand condensation without the help of neutral polymers or the use of modified DNA hybrids.[5] In pure water, complementary DNA strands can self-organize into liquid crystals at very high concentrations (300–1000 mg mL^{-1}) achieved by drying.[6] Interestingly, this process was found to generate complementarity from a pool of random and short (<16 nts) sequences during their concentration,[7] forming initial conditions for abiotic ligation.[8] Longer random strands, however, formed liquid crystals without any sequence selective behavior.

The experiments discussed above illustrate the diversity of DNA self-assembly and aggregation. They focus only on the final equilibrium result of the ordering process, which is frozen and locked in a minimal state of the free-energy landscape. Herein, we demonstrate that a dynamic, non-equilibrium approach to a DNA phase transition allows for a highly sequence-selective accumulation and separation of initially dilute oligomers. Compared to the discussed methods, heat-flow-driven gelation of DNA occurs without the addition of condensation agents, such as multivalent ions, and under physiological buffer conditions (Supporting Information Movie M1).

The non-equilibrium condition is solely implemented by a heat flux across the probe vessel and causes simultaneous solvent convection and thermophoresis of biomolecules (Supporting Information Figure S1–S3).[9] Both effects superimpose and implement a length-dependent thermal trap for biomolecules. Thermal traps have been shown to push the concentration of fatty-acids above their critical micelle concentration,[10] they can also arrange micrometer-sized beads to 2D-crystals via slip-flow,[11] and drive the replication and selection of DNA in an open flow chamber.[12]

Herein, we use thermal traps to concentrate short DNA strands that can hybridize to their own kind at several binding sites. At higher concentrations, these building blocks assemble into larger DNA polymers that are subsequently accumulated more efficiently by thermal trapping (Figure 1).[13] We show that this escalated elongation of DNA by a thermal trap acts as a dynamic non-linear switch that amplifies even the smallest sequence differences: though initially mixed, different gel-forming sequences are separated into distinct, sequence-pure gels. In contrast to non-equilibrium thermal trapping, the equilibrated DNA assemblies obtained by dry-condensation did not show any sequence dependent separation.

To quantify the sequence selectivity of heat-flow-driven gelation of oligonucleotides, we designed five slightly different 36 mer DNA strands. Strand **A** is a reference strand with three self-complementary binding sites (Table 1). Strands **B**–**E** have one, three, four, and six single nucleotide changes compared to **A**. The three hybridization sites of each strand can either form hairpins (Figure 1b, Box 1) or bind to corresponding sequences on other strands (Figure 1b, Box 3).

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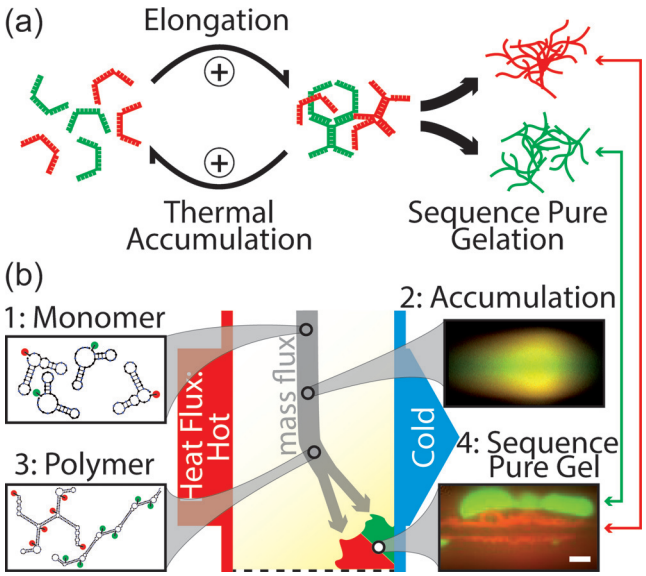
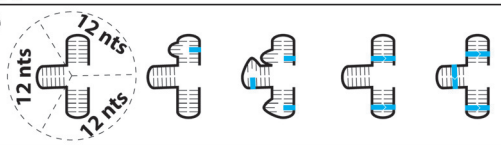


Figure 1. DNA gelation by thermal accumulation. a) DNA that contains self-complementary sequences can bind to its own kind (same color in scheme) after heat-flux-driven concentration. The longer complexes are more efficiently concentrated by length-dependent thermal trapping, leading to the formation of a DNA hydrogel. b) Even small differences in the sequence lead to a demixing or inhibition of DNA gelation. Sequences are distinguished using different fluorescent labels (FAM: green, Cy5: red). Scale bar: 20 μm.

Table 1: Properties of strands **A**–**E**.[a]

Strand	**A**	**B**	**C**	**D**	**E**
Dye	Cy5	FAM			
# Mutated Bases vs. A	0	1	3	4	6
Structure					
Builds Gel: (Fig. 2)	Yes	No	No	Yes	Yes
Separates From A (Fig. 3):					
Thermal Trap:	-	Yes	Yes	No	Yes
Drying (50°C):	-	No	No	No	No

[a] To show the sequence selectivity of the heat flow driven gelation of DNA, five different sequences were analyzed. Strand **A** is the reference strand with three self-complementary binding regions for hybridization, each made out of 12 nts and stained with Cy5. Strands **B** and **C** comprise one or three single base changes, respectively, which already inhibits their gelation (Figure 2). Both strands also separate well from the reference strand **A** in a competitive gelation (Figure 3). Strands **D** and **E** contain 4 or 6 single base changes, respectively, while retaining the full self-complementarity of the binding regions. In contrast to strand **E**, strand **D** is not separated from **A** during competitive gelation, probably due to its common center binding region (Figure 3)

All five sequences consist exclusively of G and C bases, which represents a worst-case scenario: The reduced alphabet size decreases sequence specificity while the high G-content facilitates G-quadruplex formation.[14,15] Our results therefore also apply to more moderate designs using four base letters. To show this, we additionally demonstrate the gelation of AT-only sequences or sequences that contain all canonical bases (Figure S4,S5).

We experimentally implemented a thermal trap by focusing an infrared laser inside the center of a water-filled, horizontally aligned borosilicate capillary (Figure S1).[13,16] The implementation with a laser allows for full optical inspection and ensures faster relaxation times of the system as compared to a purely gravitationally driven trap (Figure S6). The absorption of the moving IR laser simultaneously creates a thermal gradient as well as circular fluid convection via thermoviscous pumping (Figure S1–S3, Movie M3).[17] The peak and center temperature is 65 °C, while the bottom surface is actively cooled by a thermoelectric element to 49 °C.

While the setup is almost identical to the one shown in previous work, we are now extending the principle of length-dependent thermal trapping:[13] By including multiple DNA strands that cover a wide range of the smallest possible sequence differences, we demonstrate that thermal traps allow for a sequence selectivity on a single-base level by utilizing the non-equilibrium formation of hydrogels (Sequences: Table ST1).

We first checked strands **A**–**E** individually for their ability to form a hydrogel in physiological buffer at $c_0 = 10$ μM initial concentration (Figure 2a). As expected, the thermal trap gelated the strands **A**, **D**, and **E** after around 40 min because of their three full self-complementary hybridization sites. Strands **B** and **C**, however, did not form a gel. This shows that even a single base difference compared to **A** is enough to inhibit gelation.

This result can be explained by the calculated length distribution of the DNA assemblies at 55 °C shortly before gelation, at which strands were concentrated to 100 μM (Figure S7). Only solutions containing strands **A**, **D**, and **E** allow for larger DNA assemblies. The assemblies are then favorably accumulated by thermal trapping, while strands with sequence mismatches (**B**, **C**) stay unbound and cannot be further concentrated.

The ability to form branched assemblies is not a necessary requirement for heat-driven gelation of DNA: Strands with only two hybridization sites made out of 25 canonical bases each also form hydrogels, which we used to compare it to UV and calculated melting curves (Figures S5,S8).[13] We also excluded the possibility that the DNA could have formed highly ordered structures, such as crystals, using polarization microscopy (Figure S9). The DNA hydrogel showed a remarkable stability even outside the trap, where it remained stable for days under physiological conditions (see Movie M2).

We were also interested to see how two gel-forming strands compete against each other in the race for the limited space in the same solution. Thermal trapping of an initially mixed solution with strands **A** and **E** resulted in separated, sequence-pure hydrogels with a time difference of 15 min (Figure 3a, Movie M4). Both strands only differ by a change of one base pair per binding site, showing the strong sequence selecting abilities of the process.

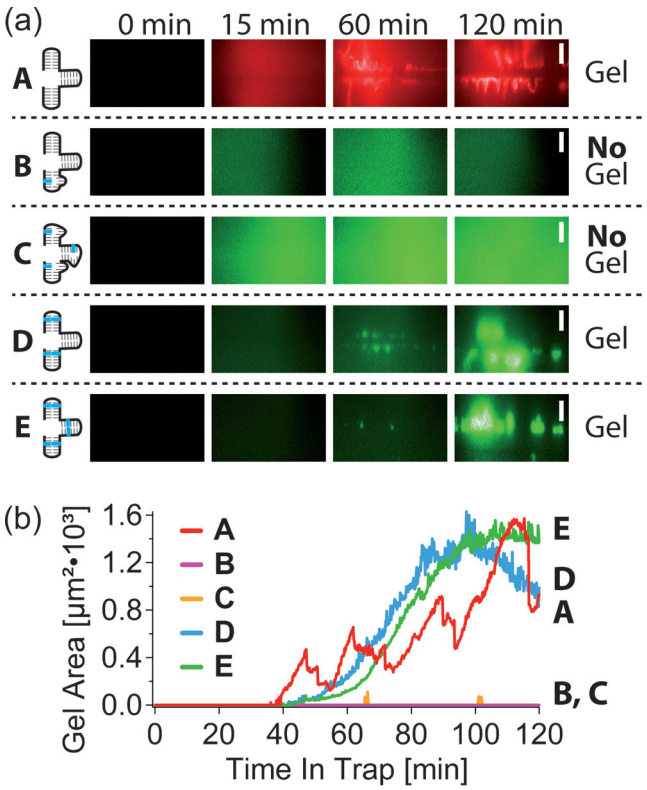


Figure 2. Sequence-selective gelation of DNA oligomers in a thermal trap. a) Strands **A**, **D**, and **E** are made of three fully self-complementary hybridization sections, and thus are elongated and further concentrated by length-selective thermal trapping. Strands **B** and **C** contain one or three changes in their sequence, respectively. This alone suffices to hinder heat-flow-driven gelation owing to their reduced ability to form longer strands that are favored by thermal trapping. The color of the pictures indicates the attached dye (red: Cy5, green: FAM). b) The measured hydrogel area reflects the snapshots shown in (a). The gelation dynamics and its fluctuating release from the glass surface lead to a damped oscillatory dynamics (e.g. strand **A**). Scale bars: 20 µm.

Trapping of a solution that contained both sequences **A** and **B** resulted in a sequence pure hydrogel from **A**, while strands **B** could not gelate, as expected (Figure 3b). This also corresponds to a separation of both strands, for example after flushing the capillary with buffer solution, and shows that sequence selection is possible with only a single base difference in the full 36 mer strand. No separation is achieved by thermally trapping and strands **A** and **D** gelate together. They differ only in one base pair in two full self-complementary hybridization sites, while the central binding site is unchanged. This allows for both strands to form large, mixed networks (Figure 3c). We compared the non-equilibrium gelation by a temperature gradient to the equilibrated DNA assemblies obtained by dry concentration and re-suspension to the initial volume of 10 µl at 55 °C. The formed aggregates did not show any signs of sequence separation (Figure 3d).

We showed experimentally that the heat-flux driven sol–gel phase transition of oligonucleotides is a highly sequence selective process. Already a single base difference between two DNA sequences was enough to separate both strands by gelation. One base pair per self-complementary binding site

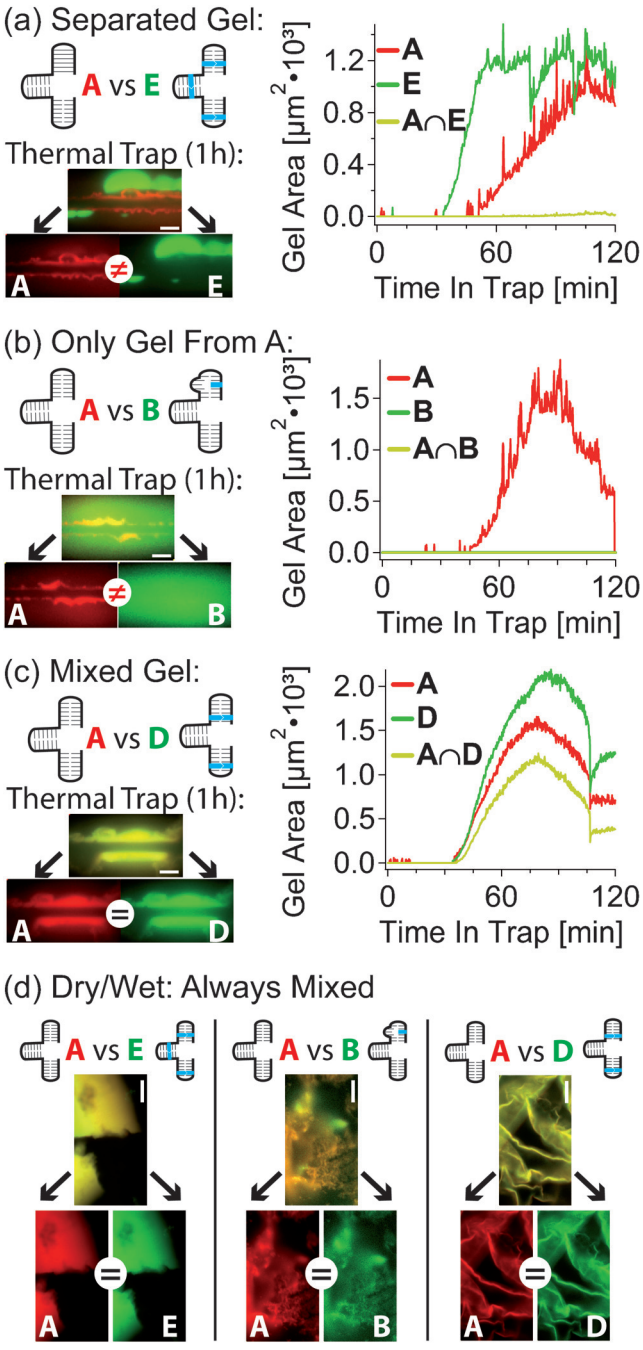


Figure 3. Competitive hydrogel formation: Strands **B**–**E** were each mixed with **A** in 1xPBS and concentrated by thermal trapping as well as in a dry-wet cycle. a) One base pair was modified in each of the three hybridization sites of strand **E**, which preserved self-complementarity. The sequence change alone sufficed to allow for the formation of spatially distinct and sequence pure hydrogels (no common gel area **A**∩**E**, plotted in yellow). b) Even a single base change in sequence suffices to inhibit the gelation of strand **B**. This allows for a sequence separation of strands **B** and **A** from a mixed solution. c) One base pair was modified in each of the two framing hybridization sites of strand **D**, preserving the self-complementarity of each 12 nts sequence block. The central sequence block is unchanged and therefore compatible to strand **A**, yielding a mixed gel made out of **D** and **A**. d) Dry-concentration and subsequent resuspension with MilliQ water of the same solutions used in (a–c) always produced mixed aggregates without strand separation or other indication of sequence selectivity. Scale bars: 20 µm.

sufficed to create two sequence-pure, distinct hydrogels. The gelation of sticky-ended DNA confirms the predicted hyper-exponential escalation in a thermal gradient.

The process could also be a promising mechanism to approach several questions concerning the origins of life. While recent advances demonstrate the prebiotically plausible synthesis of RNA oligonucleotides and their replication by RNA enzymes, it remains unclear how functional ribozymes came into existence in the first place.[18] If placed in porous rock and filled with a dilute mix of random oligonucleotides, thermal traps could have accumulated and selected for the most interacting RNA strands. Only those would be concentrated that were able to stick to each other and form hairpins, the most prevalent secondary structures in ribozymes. At the same time, the separation of these strands into sequence-pure gels could allow for high local ribozyme concentrations and therefore fast catalytic reaction times.

In contrast to crystalline phases, oligonucleotide hydrogels mostly consist of water and should still be accessible to small RNA substrates via diffusion. These could interact within the whole hydrogel volume and not only on its surface. Moreover, sequence-pure hydrogels act as sequence filters. From a mixed solution, they only incorporate sequences of their own kind. In the presence of a random oligomer pool, for example, built by dry polymerization, this corresponds to a replication reaction that enriches one family of sequences from a random input.[19] Even within the relatively unknown conditions during the origins of life, thermal traps should have been highly abundant. A heat flow is generated by almost all hydrothermal, chemical, and geological systems, making heat fluxes through porous systems a common scenario.

Experimental Section

The chamber geometry and laser heating are similar to the conditions in the previously described polymerization trap.[13] A borosilicate capillary with a rectangular cross section of 100 μm × 50 μm contains the analyte solution and is thermally coupled to a Peltier element with sapphire and silicon (see Figure S1 for experiments). The absorption of a moving 1940 nm infrared (IR) laser (20W, IPG Photonics) implements the thermal gradient and the thermoviscous convection flow. The thermal gradient of 7 K over 50 μm is measured using the temperature dependent fluorescence of the BCECF (2′,7′-bis-(2-carboxyethyl)-5-(and-6)-carboxyfluorescein) signal in 10 mM TRIS buffer. Flow speeds are measured using position tracking of silica beads (Figure S2). For DNA trapping, we use physiological salt concentrations (1xPBS) without any multivalent ions or proteins as standard buffer. All sequences were fluorescently labeled to measure gelation and subsequent sequence separation (Sequences: Table ST1). By gelating unstained DNA we excluded artifacts from a false-positive gelation mediated by interacting fluorophores (Figure S5). Multiple images at various LED currents are used to increase the dynamic range of the 12-bit CCD camera (PCO imaging). Determination of gel area from experimental data is done as described in the supplementary information.

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