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Abstract: Provides information on a method discovered by Dieter Braun and Albert Libchaber that could inspire new microfluidic tools for handling the DNA and other large biomolecules. Use of thermal gradients in the method; Significance of the method; Thermophoresis phenomenon in the experiments of Braun.

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Thermal Gradients Can Boost the Local Concentration of DNA in Solution

Did thermal diffusion and convection initiate the creation of the first biomolecules 3.5 billion years ago?

Suppose you wanted to trap DNA near a surface. Dissolved DNA is charged, so an electrical method might come to mind. Or, applying osmosis, you might try a semipermeable membrane. Heat, though, would probably be at the bottom of your list of exploitable physics. Thermal processes, such as convection in a cup of hot tea, tend to mix things up, not concentrate them. Worse, DNA starts to fall apart at a modest 80 Celsius;.

But if you're not setting out to concentrate DNA, you're freer, perhaps, to make the serendipitous discovery that heat can indeed do the job. That's what happened to Dieter Braun of Rockefeller University in New York. Working as a postdoc in Albert Libchaber's lab, Braun found that an infrared laser can generate thermal flows in a small container. If the container is the right size, those flows can boost DNA concentrations by factors of 1000 or more.(n1)

Braun and Libchaber's method could inspire new microfluidic tools for handling DNA and other large biomolecules. More speculatively, the underlying process may have provided the initial impetus, more than 3.5 billion years ago, for the formation of DNA's molecular ancestors.

Out of curiosity

When he joined Libchaber's lab two years ago, Braun started work on developing a method for quickly heating and cooling reactants. His aim was to measure how the chemical equilibrium of certain biochemical reactions depends on temperature.

Biological samples tend to be small and precious, so Braun's setup had to be compact. For his heat source, he chose an infrared laser that could create a hot spot a few tens of microns across. A small container of transparent plastic held the reactants. To get information about the reactants' temperature and concentration, he laced the solution with heat-sensitive fluorescent dyes and watched intensity changes through an optical microscope. You can see some of the equipment in the photo below.

To identify what sorts of motions could occur in his setup, Braun first observed the motions of fluorescent beads rather than dissolved molecules. When he trained the laser on the beads to trigger convection, he found they grouped together as if held in a trap. Libchaber's lab examines the dynamical behavior of biological structures, especially DNA. Out of curiosity, Braun decided to see whether DNA molecules would behave the same way. To his surprise, they did.

Initially, the trapping utterly mystified Braun and Libchaber, but a chance remark from a fellow lab member put them on the scent of a diffusive phenomenon called thermophoresis.

In 1856, physiologist Carl Ludwig discovered that a temperature gradient could influence the diffusion of salt in water. Cooling one side of a heated vessel caused the salt to go one way and the water the other. Twenty years later, Charles Soret investigated the phenomenon further, but an explanation eluded him.

In what is now known as the Ludwig--Soret effect, the heavier molecules typically diffuse down the temperature gradient (that is, toward the cold), while the lighter molecules diffuse up the temperature gradient. Bizarrely, however, some mixtures behave in the opposite way. By itself, the effect is rather feeble, but it can become significant when it teams up with convection. In the oceans, for example, the Ludwig--Soret effect helps drive the vertical mixing of salts.

Thermophoresis refers to the carrying along of macromolecules or dispersed particles by the Ludwig--Soret effect. To see whether thermophoresis was at work in his experiments, Braun first had to suppress the stronger effect, convection. That's not hard to do. If you make your container thin enough, convection slows so much that thermophoresis predominates. Braun duly reduced the height of the container to 25 µm and illuminated the initially uniform DNA solution with a hot spot about 10 µm across. As the temperature increased, the DNA fled the region around hot spot. Raising the temperature in the spot by 2.3 K created a concentration deficit of 27%.

To trap DNA, rather than repel it, Braun needed to turn on convection. When he doubled the height of the container to 50 µm, thermophoresis still repelled the DNA. But, as the figure at the top of page 17 shows, convection grabbed the ousted DNA and piled it up in a distinctive ringlike pattern.

Determining the magnitude of the concentration increase was tricky because the images, which are taken from above, lack information about depth. But, with a few assumptions and by measuring the speed of fluorescent beads under the same conditions, Braun and Libchaber could estimate the ring pattern's vertical thickness and, with it, a concentration factor of 60.

More than a century after Ludwig's and Soret's investigations, a microscopic explanation of thermophoresis still hasn't been found. But it's straightforward to write down phenomenological equations that characterize the Ludwig--Soret effect in terms of two

diffusion coefficients: the familiar diffusion coefficient that appears in Fick's law and a thermal diffusion coefficient. By applying, the equations to their data, Braun and Libchaber measured DNA's thermal diffusion coefficient for the first time.

The phenomenological equations predict concentration factors of 1000 or more are possible. To realize those gains, Braun increased the height of the container yet again--this time, to 500 µm. And for simplicity, he brought the laser right up to the bottom of the container and created a divergent beam. With this configuration, the laser initially created a depletion, but, as the figure on the right shows, within a few minutes the concentration of the trapped DNA rose by more than 2000.

What's happening at the molecular level? In solution, electrolyte ions of opposite charge surround each DNA molecule and screen its charge. The more counterions there are in the solution, the more effective is the screening. Suspecting that electrostatic forces could have a role in thermophoresis, Braun added extra counterions--that is, extra salt. As he increased the salt content, the trapping effect weakened and eventually vanished, presumably when the screening was complete.

Another clue comes from the size of the DNA molecules. The vigor of thermophoresis depends strongly on the size of the particles. In his first set of experiments, Braun used plasmid DNA (rings of DNA used in gene substitution) that had 5600 base pairs per molecule. When he switched to bigger DNA molecules, the trapping strengthened.

When he attended a meeting on thermophoresis, Braun found that Roberto Piazza and Andrea Guarino of Milan Polytechnic in Italy were working on the thermophoresis of charged molecular assemblies known as micelles.(n2) To explain their data, Piazza and Guarino adapted an idea from the University of Buffalo's Eli Ruckenstein: If the interfacial energy of the solvent and solute depends on temperature--and that's the case for macroions--the solute will be pulled or pushed along a thermal gradient. Whether this picture is correct in the case of DNA will require further testing, but its quantitative predictions are consistent with Braun's results.

Apart from providing another instance of the enigmatic Ludwig--Soret effect, what are the implications of the DNA trapping experiment? Braun and Libchaber see two. First, their method, which requires just a laser and a small container, can both increase and decrease the local concentration of DNA or any other large molecule. It does so at relatively benign temperatures and works effectively on small quantities. Such a method could, they reason, form the basis of a new microfluidic manipulation technology, which, for DNA, would be especially valuable in the burgeoning field of applied genetics.

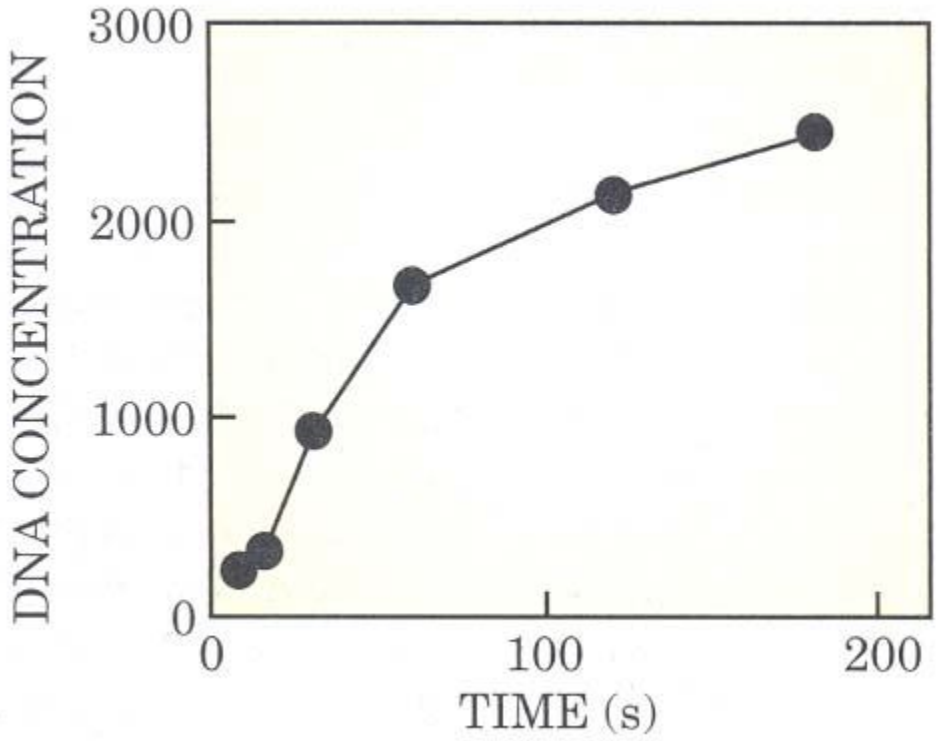
Precambrian events

The other implication is for prebiotic evolution. RNA, DNA, and other molecules of life are massive. Making such large molecules from scratch requires concentrating their smaller precursors somehow. But if the starting point is a dilute solution--the Precambrian ocean, say--that feat is all but impossible to pull off. Several lines of evidence suggest that the precursors congregated and reacted on surfaces, but concentrating the reactants is still necessary to give evolution time to pick the winners.

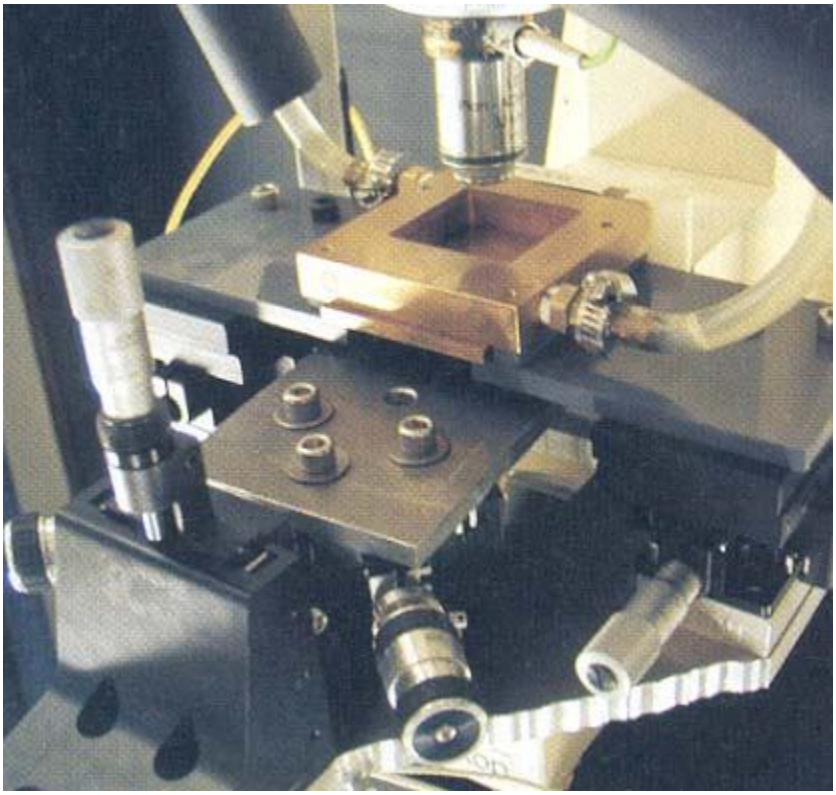
That's where Braun and Libchaber's mechanism could come in. Hot porous rocks of the sort spewed out of volcanoes have cavities where the combination of thermophoresis and convection could occur. But identifying a plausible site isn't enough. Salt quenches the trapping effect, so whatever solution the precursors occupied must have been less salty

than it is now (not, perhaps, a big problem, given that sea salt originates in the gradually accumulated runoff from land masses).

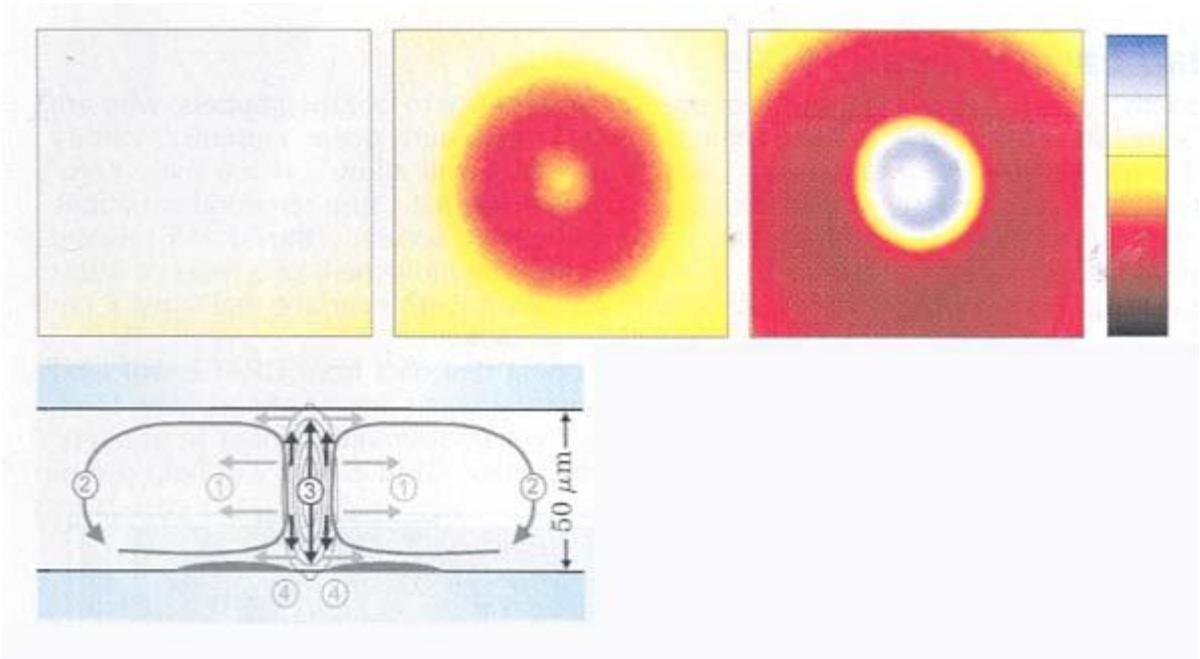
More problematic is the dependence of the trapping on molecular size. John Reader and Gerald Joyce of the Scripps Institute in La Jolla, California, have recently reported a possible RNA precursor, a ribozyme, that has about 100 base pairs.(n3) For such molecules, Braun and Libchaber's mechanism would raise concentrations by a factor of four, not a thousand. Braun believes that this limitation could be overcome if some of the cavities in the porous rocks were linked, like a series of antechambers in a palace. The concentration process could then proceed in steps. He and Libchaber are investigating this possibility.



Increasing the thickness of the container to 500 µm leads to huge concentrations of trapped DNA in a matter of minutes. (Adapted from ref. 1.)



The experimental setup. The gold-colored tray houses the plastic container for the DNA solution. Above it is the microscope objective for imaging the progress of the trapping. (Courtesy of Dieter Braun.)



DNA is trapped when the container is thick enough for convection to occur. The three top images, which are taken from above the container, show how the intensity of fluorescent dye changes after the onset of heating. In the linear color bar, black represents zero intensity; blue represents the maximum. The sequence runs from left to right and corresponds to intervals of 0 s, 10 s, and 60 s. In the last image, the blue ring-like

concentration is clearly visible. The lower diagram shows a side view of how the ring forms: Lateral thermophoresis (1 & 4) and axial thermophoresis (3) drive the DNA away from the heated spot at the center, but convection (2) creates a flow that halts depletion, holding the DNA in a pile at the bottom. (Adapted from ref. 1.)

References

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By Charles Day

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