Optothermal biophysics:
Light driven molecule traps, Microscale Thermophoresis, Imaging of kinetics and Autonomous evolution

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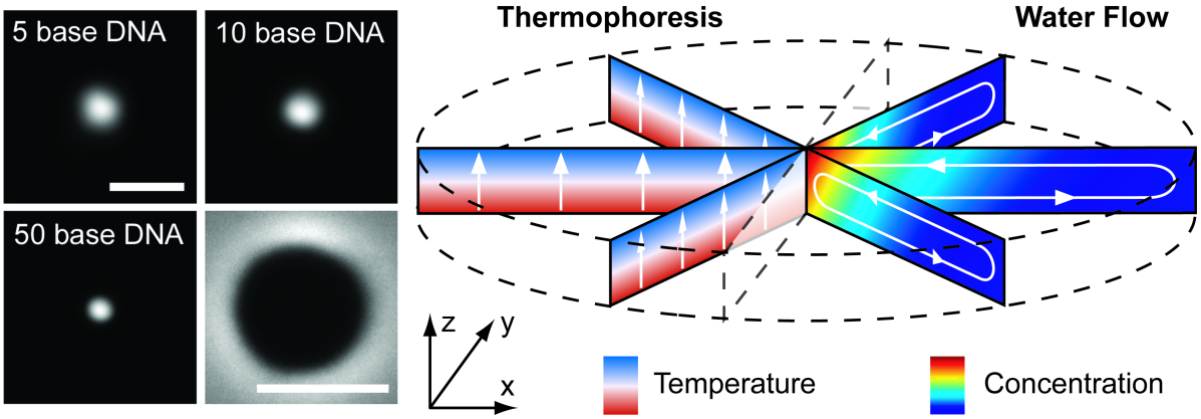
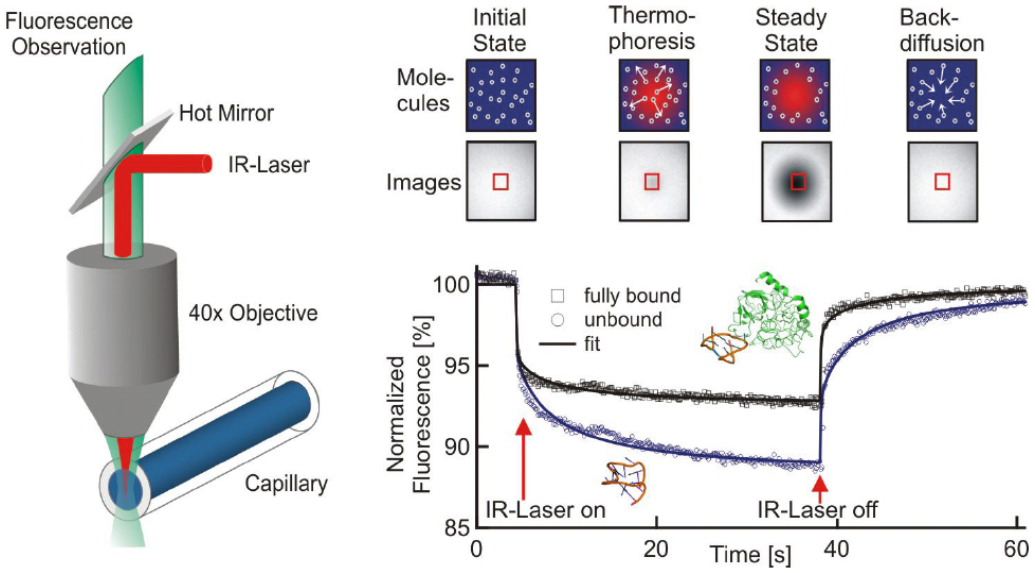
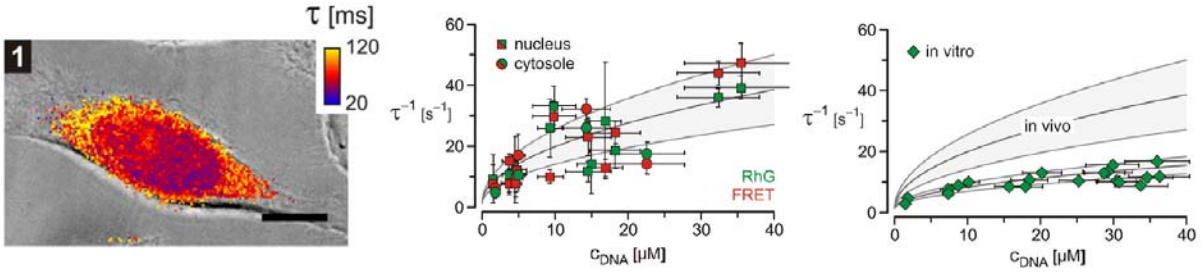
Light driven molecule traps. Trapping single ions under vacuum allows for precise spectroscopy in atomic physics. However the confinement of biological molecules in bulk water is hindered by the lack of comparably strong forces. Molecules have been immobilized to surfaces, often with detrimental effects on their function.
Here, we optically trap molecules by creating the microscale analog of a conveyor belt: a bidirectional flow is combined with a perpendicular thermophoretic molecule drift [1]. Arranged in a toroidal geometry, the conveyor accumulates a hundredfold excess of 5-base DNA within seconds. The concentrations of the trapped DNA scale exponentially with length, reaching trapping potential depths of 14kT for 50 bases.
The mechanism does not require microfluidics, electrodes or surface modifications. It uses the previously found light driven microfluidics of thermoviscous expansion [2][3]. As a result, the trap can be dynamically relocated. The optical conveyor can be used to enhance diffusion-limited surface reactions, redirect cellular signaling, observe individual biomolecules over a prolonged time or approach single-molecule chemistry in bulk water.



Biomolecule Binding quantified with Thermophoresis. Methods to measure biomolecule binding are essential for medicine, biology and the pharmaceutical industry. We use thermophoresis, the directed movement of molecules in a temperature gradient to quantify binding in titration experiments. By combining highly defined microfluidics with all-optical heating and detection, the thermophoretic depletion is measured with an error of <10^-3 down to nanomolar concentrations.
We fluorescently label one binder either specifically or unspecifically and track the changes of thermophoretic depletion under the titration of a binding target. The results are quantitative binding curves over a wide range of binding affinities. Advantages of the method are low volume consumption, fast response time and surface-free detection. However the measurement in various physiological buffers is the hallmark of the approach. Binding can be for example quantified in untreated serum.
Although thermophoresis is performed in bulk fluid, significant background signals from surface binding are commonly detected. Competing surface-based methods such as ELISA or surface plasmon resonance (SPR, e.g. Biacore) have to measure on unreliable surfaces. As result, they cannot measure binding quantitatively in untreated or undiluted physiological solutions.

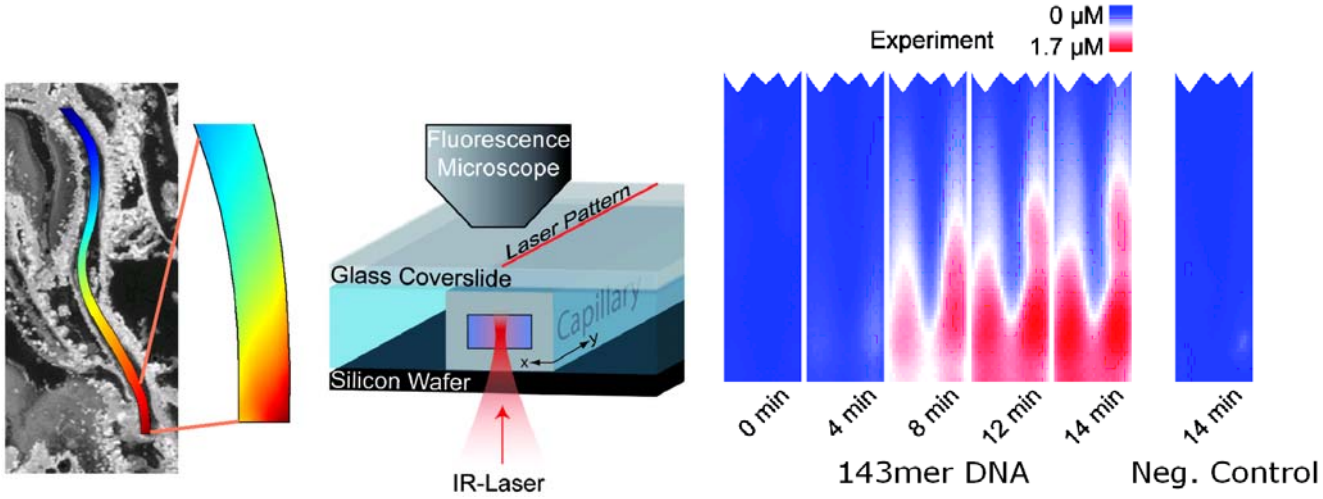


Imaging kinetics with TOOL. Molecules in a cell are subjected to significant crowding from their sister molecules. While measurements of anomalous diffusion inside cells point towards a marked effect of molecular crowding, its impact on the rate of reactions are hard to assess.
We have developed a novel technique to image kinetics in living cells using an optical lock-in approach[4]. The reaction time constant is resolved in frequency space with optical resolution under a moderate temperature oscillation and sinusoidal illumination.
DNA hybridization kinetics in living cells is strongly length selective: 16 base pair DNA has a seven-fold faster on-rate as compared to the in vitro situation, whereas 12bp DNA has a five-fold slower on-rate in vivo as compared to in vitro. Evidence points towards a catalytic acceleration for longer DNA and a slowing down by DNA binding proteins.
Above result is not expected from molecular crowding. We assessed molecular crowding with Dextran and Ficoll at high concentrations [20% (w/v)] and find no significant changes in the hybridization kinetics, indicating a minor role of molecular crowding for bi-molecular DNA hybridization.



Replication Trap. The hallmark of living matter is the replication of genetic molecules and their active storage against diffusion. We have argued in the past that thermal convection can host the million-fold accumulation even of single nucleotides and at the same time trigger exponential replication [5]. Accumulation is driven by thermophoresis and convection in elongated chambers, replication by the inherent temperature cycling in convection. Only now we could experimentally combine both and show replication and trapping of the replicated DNA in the same chamber [6]. As we are missing a solid chemistry of prebiotic replication, we used as a proxy reaction for to replication the polymerase chain reaction.
Convective flow both drives the DNA replicating polymerase chain reaction (PCR) while concurrent thermophoresis accumulates the replicated 143 base pair DNA in bulk solution. The time constant for accumulation is 92 s while DNA is doubled every 50 s. The length of the amplified DNA is checked with thermophoresis. Finite element simulations confirm the findings. The experiments explore conditions in pores of hydrothermal rock which can serve as a model environment for the origin of life and has

prospects towards the first autonomous evolution, hosting the Darwin process by molecular selection using the thermophoretic trap. On the other side, the implemented continuous evolution will be able to breed well specified DNA or RNA molecules in the future.



References

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