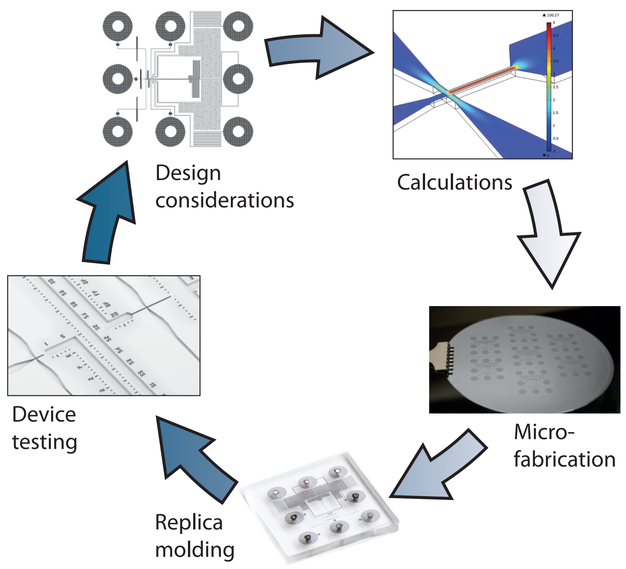
Microfluidics for single-molecule fluorescence spectroscopy

Niels Zijlstra, Fabian Dingfelder, Bengt Wunderlich, Franziska Zosel, Stephan Benke, Daniel Nettels, and Ben Schuler
Departments of Biochemistry and Physics, University of Zurich, Winterthurerstr. 190, 8057 Zurich, Switzerland

Introduction

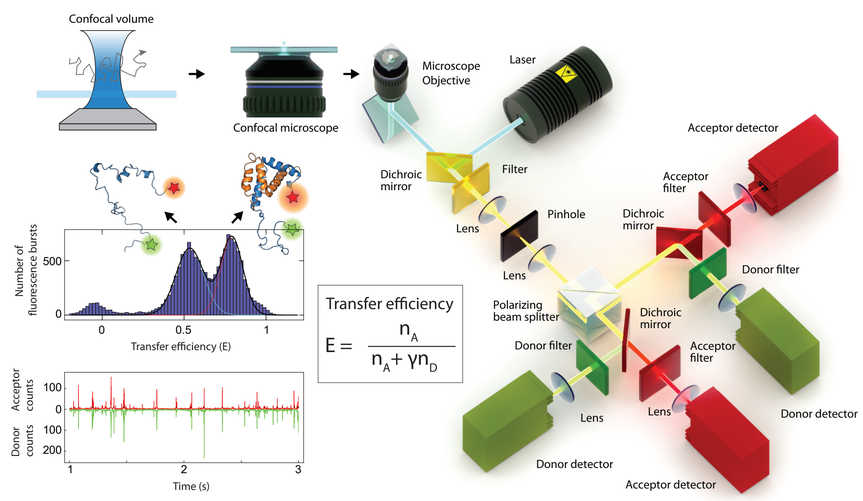
Single-molecule fluorescence spectroscopy has developed into a powerful strategy for investigating biomolecular structure, dynamics, and interactions, especially in combination with Förster resonance energy transfer (FRET). To be able to exploit the full potential of single-molecule detection for complex heterogeneous systems, it has to be combined with complementary methods that not only can be directly interfaced with the advanced instrumentation used for single-molecule detection, but also are sufficiently simple to use that they can be employed on a regular basis. Recently, microfluidic devices have been found to be particularly well suited as a flexible, easy to use, complementary method for single-molecule detection. During the past years, we have fully established the design and production of microfluidic devices and developed several devices that enable us to study protein folding dynamics and protein-protein interactions (1-3).

Development cycle



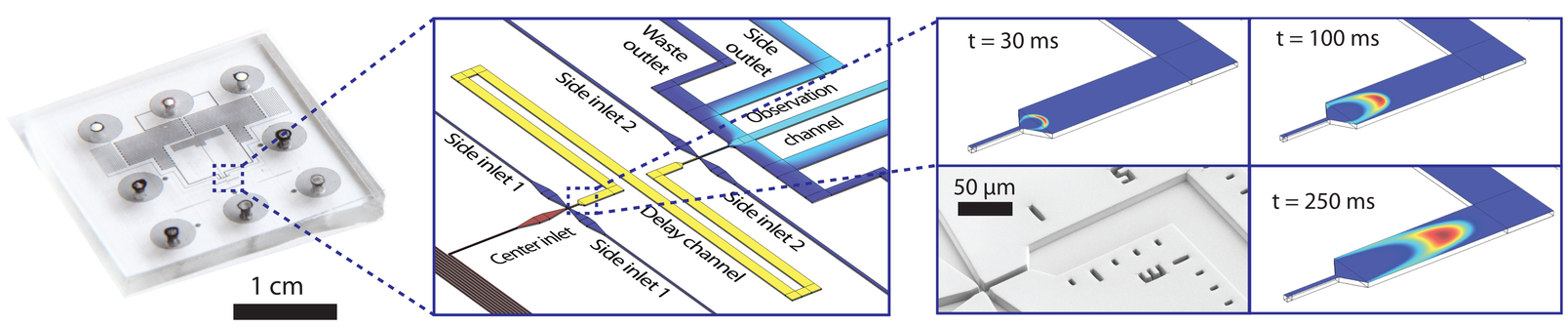
A typical development cycle of a new microfluidic device starts with an initial design idea that is modelled and optimized using finite-element calculations. Subsequently, the silicon master is fabricated in the cleanroom using photolithography and reactive ion etching (1). The microfluidic devices are produced from the master via replica molding with PDMS. The quality of the structures is monitored with electron microscopy. Finally, the devices are tested, characterized and compared to the finite-element calculations. Refinement of the initial design might be necessary, which will lead to a repetition of the cycle.

Single-molecule FRET Spectroscopy



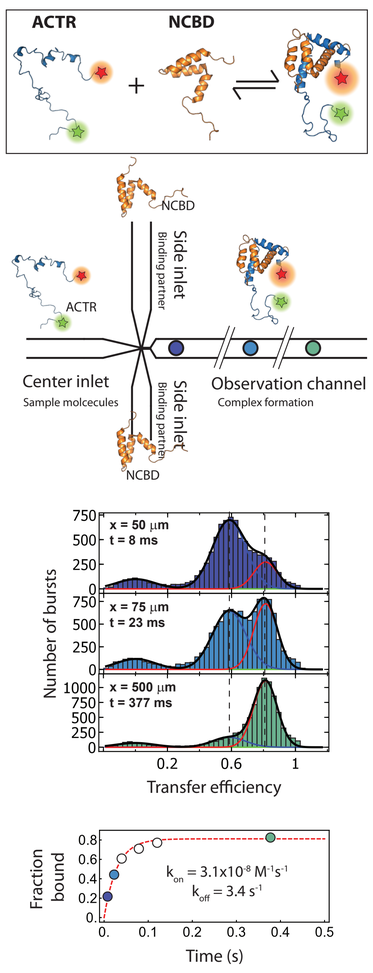
Confocal single-molecule FRET on freely diffusing molecules: The fluorescence signal is first separated by polarization and then by wavelength into the detection channels corresponding to emission from donor and acceptor chromophores. By analyzing each of the fluorescence bursts, a histogram of transfer efficiencies can be calculated and different subpopulations exhibiting different transfer efficiencies can be distinguished.

Finite-element calculations and characterization



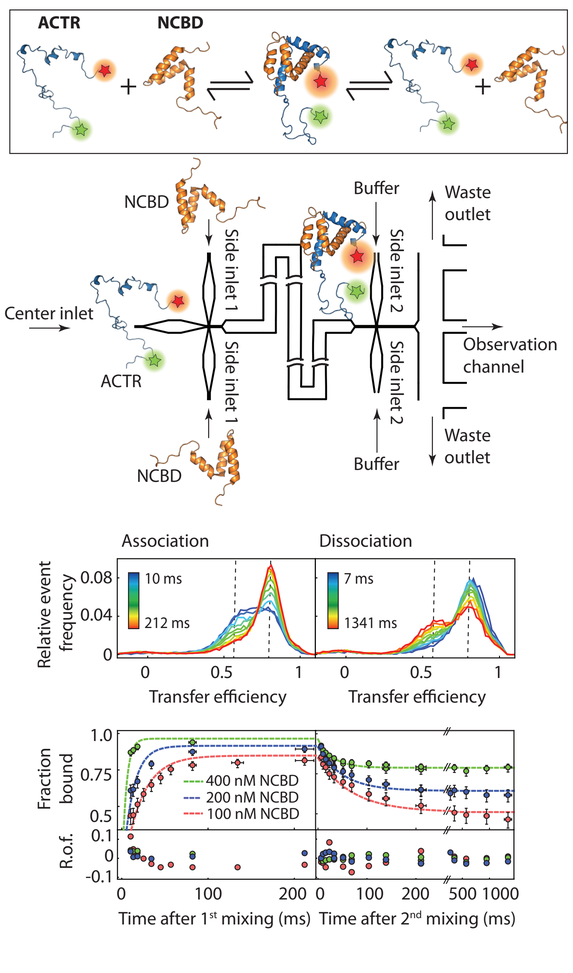
An added advantage of microfluidic devices is that, due to the absence of turbulence, the full mechanics underlying these laminar flow systems can be calculated even for complex geometries with the help of finite-element methods. Experimentally determined flow velocities can therefore be directly compared to calculations to determine the performance of the device.
Additionally, time-dependent calculations enable us to accurately convert positions within the device to times after mixing and quantify the uncertainty in the observation times due to Taylor dispersion (4).

Rapid mixing



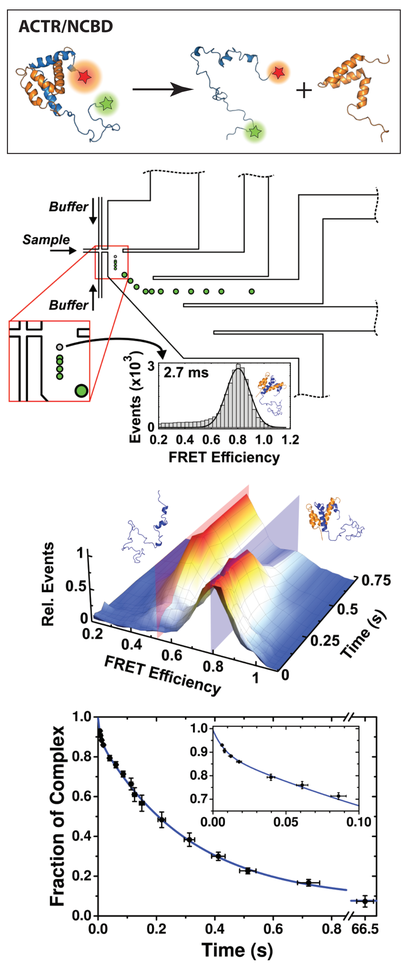
The microfluidic mixing device (1) can be used to obtain kinetics on timescales from milliseconds to minutes.
In the example shown, the device is used to probe the interaction of two intrinsically disordered proteins, ACTR and NCBD, on a millisecond timescale. Knowing the affinity of the protein complex, the association and dissociation rate coefficients can be calculated.

Double-jump mixing



Recently, we extended microfluidic mixing to also enable double-jump experiments (2). After a first mixing step, the sample molecules spend a defined time in a delay channel and can then be probed also after a second mixing step. This technique is particularly suited to investigate kinetic properties of transient intermediate species, but can also be used to study the association and dissociation of e.g. intrinsically disordered proteins, as shown here.

Rapid dilution



A major challenge in studying biomolecular complexes by single-molecule spectroscopy is that their affinity is often low, resulting in rapid dissociation of the complexes at the exceedingly low concentrations required for single-molecule detection.
To circumvent this limitation, we have developed a microfluidic device that allows a concentrated sample to be diluted by up to five orders of magnitude within milliseconds (3).
We demonstrate the capabilities of the device by studying the dissociation kinetics and structural properties of low-affinity protein complexes between ACTR and NCBD using single-molecule FRET. The versatility of the device makes it suitable for studying complexes with dissociation constants from low nanomolar up to 10 µM, thus covering a wide range of biomolecular interactions.

Conclusion and Outlook

Microfluidics has developed into an easy-to-use and versatile methodology. Especially in combination with advanced single-molecule fluorescence approaches it substantially extends the range of molecular mechanisms that can be studied. Here, we show the advantageous usage of several microfluidic devices we developed over the years to study a pair of intrinsically disordered proteins, ACTR and NCBD. Of course, these novel microfluidic devices are not limited to studying ACTR and NCBD, but can be used to study a wide range of biomolecular recognition, such as chaperone binding or protein-nucleic acid interactions.

References

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