Abstract

The understanding of biological systems relies on the accurate description of the interaction among biomolecules. This knowledge can be obtained by in vitro assays involving interacting partners with well-defined compositional, spatial, and temporal constraints. The distinguishing features of living systems, namely, low copy number, crowded environment, and spatial compartmentalization, are usually absent in most in vitro experiments reported in the literature. This thesis discusses the implications of low copy number and spatial constraints using theoretical and computational methods in some model systems. Furthermore, two experimental platforms, based on the recent development of microfluidic techniques, are described in detail. In the first implementation, micron-sized chambers fabricated using soft lithography provide a high-throughput reactor array whose size and composition can be configured to mimic the in vivo environment. The second design reports the generation and manipulation of femtoliter-volume water-in-oil droplets. A model biochemical reaction catalyzed by β-galactosidase is observed in both reactors with precisely defined initiation time, opening the way to monitor transient kinetics in addition to steady-state behavior. Additionally, the enzymatic activity exhibits a negative correlation with the size of water-in-oil droplets when the nominal concentrations of reagents are kept the same. This surprising result is analyzed in detail by carefully designed control experiments, and attributed to the shear-induced redistribution of surfactant employed to stabilize the water–oil interface. Specifically, smaller droplets experience bigger shear stress, which change the surface concentration of surfactant and allow for the nonspecific binding of proteins to the interface. Surface-bound enzymes are denatured, leading to reduced catalytic activity. This highly dynamic process is hardly detectable by other methods such as tensiometry or direct fluorescence imaging of the interface.