Description for the general public

High throughput microfluidic system for fast determination of the equilibrium constant for biomolecular complexes: application to RNA-DNA interactions

Complex formation reactions play a vital role in nature and occur in all living systems. This type's key reaction is the hybridization of nucleic acids, which is the basis for replicating and transcribing genetic information. The stability of the DNA-RNA complex and the strength of the interactions between threads are determined by its equilibrium constant (*K*). Such a complex is formed during gen edition in CRISPR/Cas9 system.

CRISPR/Cas9 is a gene-editing method that utilizes hybridization between the first 20 nucleotides of an engineered guide RNA (gRNA) and a target genomic DNA sequence of interest. Previous studies have shown that genetic modification occurs when a DNA strand of the targeted locus is not complementary with gRNA. It may cause modification of wrong genomic loci during therapeutic applications. To better understand the processes behind the interactions of gRNA and targeted DNA, there is a need to determine the strength of those threads' interactions.

The most commonly used method to measure the equilibrium constant of such a reaction is Förster Resonance Energy Transfer (FRET). However, FRET requires labeling two substrates with different fluorescent dyes to ensure energy transfer. Additionally, high initial brightness and precisely tailor distance between fluorophores is necessitated. We have recently developed a novel method based on molecular brightness analysis that allows *K* quantification when only one substrate is inherently fluorescent or labeled. The DNA-RNA hybridization determination requires a series of dilutions with a different concentration ratio of the threads. To decrease experiment time and reagents consumption, only a few substrates ratios are tested to obtain quantitative results. Research on a bigger scale has become more laborious as the number of samples is increasing. To solve this problem, we intend to miniaturize and automatize the experimental setup by applying a microfluidics-based system. An automated method will allow shortening sample preparation time by over 10 000 times and quick mixing of components.

This project will develop a high throughput microfluidics-based device for the quantitative characterization of biochemical reactions using fluorescence-based methods. We intend that this improvement paves a way towards the quantitative study of biomolecule interactions in living systems and preliminary research after discovering new pharmaceuticals. Subsequently, we will use a newly developed device to quantify the stability of 20 base-pair DNA-RNA oligonucleotides with differently located multiple mismatches in various environmental conditions, such as ions concentration, pH, and temperature. As well, we will measure interactions between double-strand DNA and single-strand RNA.

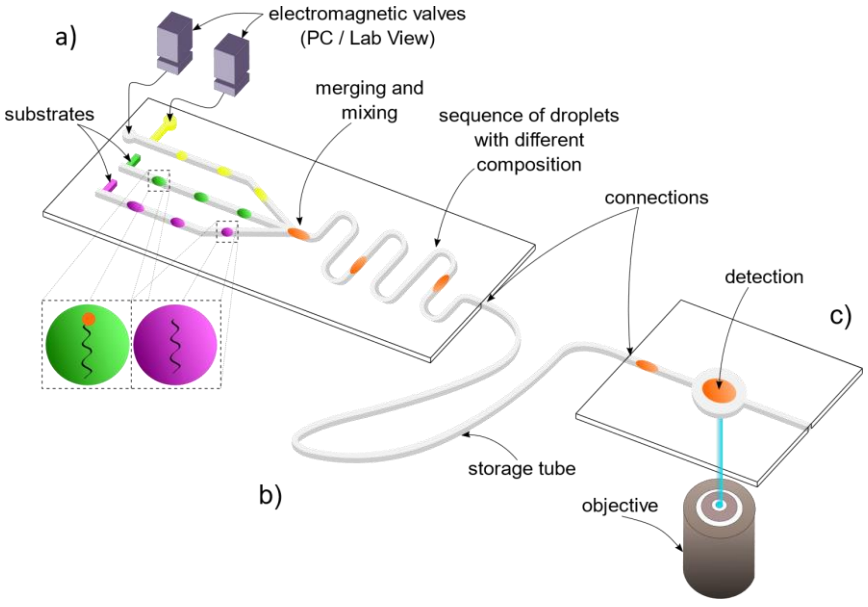

Figure 1: A schematic illustration of the integrated system with high throughput for determination of reaction equilibrium constant. The system consists of three interconnected subsystems: a) merging and mixing chip, b) storage tube and c) detection chip. Each element is easy to separate.