Abstract

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Droplet microfluidic systems for formation and studies of lipid bilayers

In vitro formation of model lipid bilayers is an essential part of research on properties of lipid bilayers and membrane proteins. One of the methods to obtain stable lipid bilayers relies on submerging aqueous droplets in an oil phase containing dissolved lipid molecules and upon formation of a monolayer of phospholipids at the surface of droplets, bringing the droplets into contact. Thanks to the self-organization of phospholipid molecules at the interface of aqueous and continuous phase, a lipid bilayer is formed at the point of contact between the droplets (droplet interface bilayer, DIB). In comparison to other models of a lipid bilayer, DIB is characterized by an exceptional mechanical stability. In addition, droplets are separate microreactors, allowing for a control over the chemical composition on both sides of a bilayer.

In the recent 25 years a significant progress was made towards establishing miniaturized high-throughput systems dedicated for biological and chemical research. Microfluidic techniques allowed to develop experimental assays on droplets of volumes ranging from femto- to microliters, which is an attractive strategy to implement in developing experimental systems for formation of artificial lipid bilayers.

This dissertation presents results of development of pioneering microfluidic devices dedicated for formation of lipid bilayers. The main objective was to combine the advantages rising from the use of droplet microfluidic systems with the unique properties of the DIB technology. Research projects were focused both on development of microfluidic devices and on using them for experiments on membrane proteins.

The dissertation is composed of seven Chapters. Chapter 1 contains a literature review on the methods available for formation of artificial lipid bilayers, especially on the use of microfluidic techniques. Chapter 2 describes materials and methods used in the research projects. The results are presented from Chapter 3 through Chapter 6 as follows.

In Chapter 3 I present an automated system for repetitive formation of lipid bilayers and electrophysiological measurements on nanopores. The control over the formation of lipid bilayers in the presented system was achieved with programmable external valves. The electrodes inserted into the system allowed for electrophysiological measurements on activity of nanopores – alpha-

hemolysin protein molecules. The kinetics of inhibition of proteins pore with small molecule was determined.

Chapter 4 describes the signal transmission in a network of droplets. An automated droplet microfluidic system was used to generate networks of droplets interconnected with lipid bilayers. Electrodes inserted into the droplets at the edges of a network were used to measure the flow of electric current across several droplets. Various concentrations of alpha-hemolysin nanopores were added to droplets, allowing for measurements of an electric signal from the interior of a network and excluding the direct contact of electrode with a sample. A model of electric circuit describing the flow of electric current in a network of droplets was used to interpret the data.

Chapter 5 focuses on the automated dilution of a sample on-chip. A brief description of an attempt to construct an automated system for dilution of a sample is provided. The proposed system relied on metering of portions of samples on-chip and automated serial dilution.

Chapter 6 details the passive microfluidic device for a formation of lipid membranes in hydrodynamic traps. A system based on geometry of microfluidic channels, capable of splitting the sample into immobilized droplets, was adapted for formation of lipid bilayers. Permeability of lipid bilayers for small molecule dye and the transport of ions through nanopores inserted in the membranes were determined using the microfluidic device in which multiple bilayers were formed in parallel at the interface of 9-nanoliter droplets. The on-chip dilution of a sample in a series of hydrodynamic traps was also demonstrated.

Finally, Chapter 7 summarizes the results and provides an outlook for a future research.