Sensors & Actuators: B. Chemical 274 (2018) 338–342

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

journal homepage: www.elsevier.com/locate/snb

Nanofluidic fluorescence microscopy with integrated concentration gradient generation for one-shot parallel kinetic assays

Pattamon Teerapanich[a], Martine Pugnière[b], Corinne Henriquet[b], Yii-Lih Lin[c], Antoine Naillon[d], Pierre Joseph[a], Chia-Fu Chou[c], Thierry Leïchlé[a,*]

[a] LAAS-CNRS, Université de Toulouse, CNRS, Toulouse, France
[b] IRCM, Institut de Recherche en Cancérologie de Montpellier, INSERM, U1194, Université de Montpellier, ICM, Institut Régional du Cancer, Montpellier, F-34090, France
[c] Institute of Physics, Academia Sinica, Taipei 11529, Taiwan
[d] CNRS, IMFT, F-31400 Toulouse, France

ARTICLE INFO

Keywords:
Affinity assay
Biosensor
Nanofluidics

ABSTRACT

We report a simple and cost-effective nanofluidic fluorescence microscopy platform with parallel kinetic assay capability for the determination of kinetic parameters in a single run. An on-chip microfluidic concentration diluter, or gradient generator, was integrated to a biofunctionalized nanofluidic chip, enabling simultaneous interrogation of multiple biomolecular interactions with a full titration series of analyte in a single experiment. We demonstrate that since the association and dissociation phases are induced by the on-chip gradient generator and a reverse buffer flow operation, complete kinetic sensorgrams for IgG/anti-IgG interactions can be achieved within 20 min on a single device, which is at least 10 times faster than traditional kinetic techniques. This method could contribute to low-cost, rapid and high-throughput drug-screening and clinical diagnostics.

1. Introduction

High-throughput measurement of biomolecular interactions with rapid determination of the affinity and kinetic parameters are of critical importance in drug discovery processes and proteomic analysis [1]. Surface plasmon resonance (SPR) is established as a powerful bioanalytical tool for quantitative characterization of molecular binding events owing to its real-time and label-free detection [2]. Traditional kinetic experiment requires the successive introduction of different analyte concentrations and the sensor surface is regenerated after each analyte injection, a protocol regarded as “multi-cycle kinetics”. These repetitive procedures are typically performed manually, and thus are laborious and time-consuming.

Over the past decade however, single cycle titration kinetic was implemented on SPR to decrease the number of regeneration steps: FastStep [3] and diSPR® [4] dynamic injections are now commercialized in the PIONEER FE SPR instrument, mainly used in drug screening [5]. Moreover, there has been a growing interest in high-throughput kinetic analysis using SPR imaging (SPRi) technology as demonstrated in the commercial cross-flow ProteOn XPR 36 assay system (BioRad) [6], or the flow cell based FlexChip (Biacore) [7]. Combined with protein arrays, SPRi uses a parallel detector such as a CCD camera to measure kinetics on multiple ligand surfaces in a single run without the need of surface regeneration. In the near future, SPR microscopy [8] and emerging SPR systems based on nanostructures [9] which propose higher throughput and sensitivity, could also stimulate the field.

Despite the capability to provide kinetic data in a parallel manner, the above mentioned systems necessitate a high-cost specialized optical instrument and specifically trained operators [10]. On the other hand, electrochemical and quartz crystal microbalance biosensors may provide low-cost solution for kinetic studies [11–13], but they encounter the similar issues, as SPR platform, of either sensor surface regeneration [14], difficulty in parallel kinetic assays [15], or slow assay time, particularly when target concentrations are close to the sensor detection limit [16,17], for kinetic analysis, though the later can be alleviated by implementing complex analyte enrichment strategies [18–22]. Therefore, there is still of great demand to develop simple, rapid and cost-effective methods that allow high-throughput access to kinetic information.

Miniaturized fluidic systems have lately emerged to address the challenges associated with present bioanalytical tools [23]. Microfluidics that offers a high degree of parallelization has the potential to increase the assay throughput while minimizing reagent consumption and analysis time. With Reynolds numbers being well below one, laminar flow has been an effective way to deliver concentration gradients of chemical species within microfluidic channels. Microfluidic diluters

* Corresponding author.
E-mail address: tleichle@laas.fr (T. Leïchlé).

https://doi.org/10.1016/j.snb.2018.07.167
Received 3 May 2018; Received in revised form 27 July 2018; Accepted 31 July 2018
Available online 02 August 2018
0925-4005/

offer means to automatically generate stable and reproducible concentration gradients on chip for cell-based experiments [24], pharmaceutical studies [25] and high-throughput fluorescent immunoassays [26]. Despite several successful applications, there have been a few efforts to implement gradient-generating microfluidic devices for kinetic assays. Most recently, we have reported the feasibility of using a nanofluidic fluorescence microscopy system (NFM) for low-noise real-time measurements of protein binding kinetics with optimized response time and target capture [27]. This system, relying on the combination of a functionalized nanofluidic device with a conventional fluorescence microscope, allows the generation of full kinetic sensorgrams (association and dissociation curves) with a single injection via a simple reversed flow operation. Thanks to the unique feature of nanometer scale channels, the reduced observation volume, leading to negligible levels of fluorescence background, allows the sensor surface to be directly probed without using sophisticated apparatuses such as SPR [28].

Herein, we implement a microfluidic diluter in the NMF platform, allowing one-shot kinetic measurements to be carried out in a parallel manner on a single chip. This approach is capable of simultaneous monitoring multiple ligand surfaces through multiple analyte streams with up to ten different concentrations spanning almost 2 orders of magnitude using a single-analyte injection and without the need for lengthy surface regeneration procedure. The microfluidic diluter is employed to produce stable analyte concentration gradients within the device, thus eliminating tedious manual dilution process. In this work, generation of concentration gradients relies on diffusion-based partial mixing as described elsewhere [29], where desired concentration gradients can be achieved by regulating the input flow rates. The microfluidic dilution network is designed such that a suitable dynamic concentration range required for kinetic studies is achieved at the outputs and the target solutions are transported through each channel at identical flow speeds. In principle, different analyte concentrations generated from the concentration diluter can be introduced over the sensors located at independent nanochannels and the concentration-dependent binding data can be collected all at once using a bench-top fluorescence microscope and a CCD camera. We demonstrate the device performance for high-throughput measurements through kinetic studies of mouse immunoglobulin G (IgG)/anti-mouse IgG interaction.

2. Materials and methods

The integrated device is composed of three main components: the first component is a Y-shape microfluidic diluter, referred as the main microchannel, for the generation of partial-mixing concentration gradients; the second component is an array of 10 separated nanoslits with embedded probe-immobilized gold sensors for kinetic studies; the third component is a microchannel used for in-situ functionalization and reversed-buffer flow operation. The dimensions of each component are shown in Fig. 1. The Y-shape gradient network has two input fluid lines, one for the analyte solution with the maximum concentration for kinetic experiments, and the other for the diluent (buffer solution). Two confluent streams of liquid merge at the intersection and laterally mix via molecular diffusion in the main microchannel as they flow downstream side by side, thus generating traverse concentration gradients.

The geometry of microfluidic diluter was designed using COMSOL Multiphysics to target the desired concentration profile and flow speed (see details of the design in the ESI). After a fixed distance, the main microchannel is partitioned into a series of 10 parallel narrow microchannels to permanently isolate and homogenize distinct sample concentrations emanating from the main flow stream before entering the nanoslits. Furthermore, the Y-shape microfluidic gradient network is symmetrical in order to guarantee the same flow velocity at all outputs, which is indispensable for parallel kinetic measurements. The flow velocity has a major influence on the steepness of the output concentration gradient profile wherein a wide sigmoidal concentration profile is obtained at a low flow velocity. Nevertheless, too high of a flow velocity can lead to non-homogeneity of the sample solutions at the exit as they do not experience enough residence time to mix while flowing along the narrow microchannels. Hence, there is a tradeoff between the concentration range generated from the gradient device and the homogeneity of the sample solution. Still, the simulation results show (see ESI) that with the selected geometry and inlet pressure, the designed Y-shape microfluidic dilution network enables gradient generation with a range of concentrations spanning approximately two orders of magnitude, and all channel outputs possessed virtually identical flow velocities.

The fluidic chip (16×16 mm^2) was fabricated on a silicon substrate using standard photolithography and reactive ion etching techniques as described previously [27]. The microfluidic channels are 10 μm deep while the nanochannels are 450 nm deep. Selective immobilization of protein receptors within the nanochannel was achieved by patterning 100 nm thick gold patches onto the silicon substrate. The functionalization protocol used to immobilize the probe molecules onto the gold sensors embedded in the nanoslits was partly carried out prior to chip encapsulation (see details in the ESI). The fluidic chip was sealed with a hard-PDMS coated cover glass using a specifically developed room-temperature bonding process [30]. The fluidic chip was then filled with a blocking solution (a buffer containing 1% bovine serum albumin and 0.02% Tween-20) to prevent nonspecific adsorption of proteins on the channel surfaces. After chip bonding and channel passivation, the functionalization of the gold sensor with protein probes was finalized in flow as described in the ESI.

The device was mounted on a dedicated fluidic support and the reservoirs were connected to a pressure controller (MFC-8C Fluigent) to induce liquid flows. All experiments were monitored using a CCD camera (ANDOR iXonEM + 885) and an inverted microscope (Olympus IX70) equipped with a white light source (Lumencor SOLA light engine). Fluorescence images were acquired with a 10X microscope objective to cover an area of approximately 800 μm × 800 μm, allowing us to visualize the binding reactions in all channels.

3. Results and discussion

In order to investigate the effectiveness of the diluter for generating accurate concentration gradients of target solutions, we used fluorescent molecules to visualize the local concentration by means of fluorescence microscopy. A 20 nM Alexa Fluor-647 conjugated mouse anti-rabbit IgG (mIgG-AF 647) solution was injected at the analyte input port and a buffer solution at the diluent input port. To create a stable concentration gradient using the Y-type diluter, it was compulsory to maintain the pressure equilibrium between the two feeding streams at the input ports by using a single pressure source and a Y-barbed connector (Fig. 2).

An analyte-buffer interface was observed at the stagnation point (Fig. 3a) upon injection of the mIgG-AF 647 and buffer solutions. Both fluids were mixed by molecular diffusion as they progressed down the main channel resulting in a broader fluorescence region. The concentration gradient at the end of the main channel was constantly isolated and maintained in the downstream array of narrow microchannels. It was observed that the concentration gradient was stabilized within 2 min and controllable during the time span of the kinetic experiments (typically 10–15 min). This is particularly crucial to achieve robust and accurate kinetic information of the observed interactions. Besides, the time required to establish a steady-state equilibrium gradient was observed to be less than 1 min. This timescale is relatively short compared to the total kinetic analysis time (see next section), thus allowing the observation of the initial association phase of the binding reactions. As expected, the fluorescence intensity in the channel outputs increased from the left to the right (Fig. 3b), generating a characteristic diffusion-induced sigmoidal concentration profile [31] (error function [32]). The corresponding target concentrations were estimated by exploiting the observed linear relationship to correlate the fluorescence