Buffer-free integrative nanofluidic device for real-time continuous flow bioassays by ion concentration polarization

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Supplementary Information S1

Numerical simulation for shear rate to calculate kinetic energy $E_k$

The modeling microchannel has a width of 100 μm, a height of 20 μm and a length of 5,000 μm. The dynamic viscosity of water is $\mu = 8.90 \times 10^{-4}$ Pa s (at 25 °C). The two-dimensional model is shown in Fig. S1. Because aptamer’s height was four orders of magnitude smaller than channel’s height ($h_{\text{channel}} = 20 \, \mu\text{m} \text{ vs. } h_{\text{aptamer}} \approx 2 \, \text{nm}$), we could simplify the model by neglecting the hydrodynamic resistance caused by immobilized aptamers on the channel’s bottom surface. The Laminar Flow module in COMSOL® Multiphysics version 5.0 was used to simulate the shear rate profiles under different input volumetric flow rates. Boundary conditions were used as follows: no-slip at the walls of the channel, the outlet was open reservoir so its pressure $P = 0$. The sensing part can be considered the fully developed region since it is located at the second half of the channel’s length ($x \approx 2,500–5,000 \, \mu\text{m}$). The model was meshed into the finer element size in COMSOL.

Fig. S1 Two-dimensional geometry and boundary conditions of the COMSOL model.
**Kinetic energy** $E_k$

The kinetic energy caused by a flow along a differential length $\delta x$ can be calculated as follows:

$$E_k = F \delta x = \gamma \mu w (\delta x)^2$$  \hspace{1cm} (1)

Note that $F$ is the lateral force exerting on the object to make a displacement $\delta x$.

**Table S1** Detailed calculations for the kinetic energy $E_k$ in Fig. 2b in the manuscript. Three values of kinetic energy $E_k$ highlighted in gray are discussed in the manuscript: $1.65 \times 10^{-21}$, $2.21 \times 10^{-21}$, and $2.76 \times 10^{-21}$ J, corresponding to the washing flow rates of 0.75, 1, and 1.25 $\mu$l min$^{-1}$, respectively. These values are calculated at the vicinity of the bottom surface of the microchannel, where the aptamer is immobilized ($z \approx 1$ nm). The differential displacement measured along the flow direction is $\delta x = 1$ nm, corresponding to the movement of an ATP molecule to a new position under flow. Note that an ATP characteristic dimension is $\approx 1$ nm and the characteristic length of one nucleotide is $\approx 0.3$ nm.

<table>
<thead>
<tr>
<th>Flow rate $Q$ ($\mu$l min$^{-1}$)</th>
<th>Shear rate $\gamma$ (s$^{-1}$)</th>
<th>Kinetic energy $E_k$ (J)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.25</td>
<td>6196.25</td>
<td>$5.51 \times 10^{-22}$</td>
</tr>
<tr>
<td>0.50</td>
<td>12392.50</td>
<td>$1.10 \times 10^{-21}$</td>
</tr>
<tr>
<td>0.75</td>
<td>18588.75</td>
<td>$1.65 \times 10^{-21}$</td>
</tr>
<tr>
<td>1.00</td>
<td>24785.00</td>
<td>$2.21 \times 10^{-21}$</td>
</tr>
<tr>
<td>1.25</td>
<td>30981.25</td>
<td>$2.76 \times 10^{-21}$</td>
</tr>
<tr>
<td>1.50</td>
<td>37177.50</td>
<td>$3.31 \times 10^{-21}$</td>
</tr>
<tr>
<td>1.75</td>
<td>43373.75</td>
<td>$3.86 \times 10^{-21}$</td>
</tr>
<tr>
<td>2.00</td>
<td>49570.00</td>
<td>$4.41 \times 10^{-21}$</td>
</tr>
</tbody>
</table>
Fluorescence spectra of the aptamer

The fluorescence quenching of FAM attached on the 5’ end of the aptamer induced by ATP at two different temperatures is shown in Fig. S2. The spectra were measured using a Fluorimeter Infinite M200 Pro, Tecan.

Fig. S2 Fluorescence spectra of FAM attached on to aptamer (1 μM) at an excitation wavelength $\lambda_{ex} = 475$ nm at two different temperatures in the presence of different concentrations of ATP: 0, 25, 200, 1000, and 2000 nM. The intensity of DI water is shown as a baseline for comparison.
Theoretical model for the binding energy

The emitted intensity value taken at 514 nm was used for the calculation of the Stern-Volmer plot.

\[ \frac{I_o}{I} = 1 + K_{SV}[ATP] = 1 + K_q \tau_o[ATP] \]  

(1)

where \( I_o \) and \( I \) represent the steady-state fluorescence intensity in the absence and presence of ATP, \( K_{SV} \) is the Stern–Volmer constant, \( K_q \) is the quenching rate constant, \( \tau_o \) is the average lifetime of FAM in the absence of ATP (4 ns), and \([ATP]\) is the molar concentration of ATP.

The absolute values of \( K_q \) for the ATP-aptamer complex (at 299.15 and 310.55 °K) were found to be greater than the maximum value for a diffusion-controlled quenching process \((10^{10} \text{ M}^{-1} \text{s}^{-1})\), which shows that the quenching mechanism of the aptamer is static. Moreover, it has been shown that the ground state complex formation is characterized by a decrease in \( K_{SV} \) with increasing temperature, which confirms that the fluorescence quenching mechanism is static quenching.

<table>
<thead>
<tr>
<th>Complex</th>
<th>Temperature (°K)</th>
<th>Stern–Volmer constant, ( K_{SV} ) (M⁻¹)</th>
<th>Absolute values of quenching rate constant, ( K_q ) (M⁻¹ s⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATP-aptamer</td>
<td>299.15</td>
<td>-76020</td>
<td>1.90050 × 10¹³</td>
</tr>
<tr>
<td></td>
<td>310.55</td>
<td>-77425</td>
<td>1.93563 × 10¹³</td>
</tr>
</tbody>
</table>

In the case of static quenching, the Stern-Volmer constant \( (K_{SV}) \) can be considered the binding constant \( (K) \).

Combining the Gibbs free energy

\[ \Delta G = -RT \ln(K) \]  

(2)

\[ \Delta G = \Delta H - T\Delta S \]  

(3)

We obtain

\[ \Delta G = -RT \ln(K) = \Delta H - T\Delta S \]  

(4)

The relationship between the binding constant and binding energy can be linked via the Van’t Hoff equation,

\[ \ln(K) = - \frac{\Delta H}{RT} + \frac{\Delta S}{R} \]  

(5)

where \( \Delta H \) is the enthalpy \((i.e., \text{the binding energy})\), \( \Delta S \) is the entropy of the system, \( R \) is the gas constant, and \( T \) is the absolute temperature.

At a given temperature \((T_1 \text{ or } T_2)\)
\[
\ln(K_1) = -\frac{\Delta H}{R T_1} + \frac{\Delta S}{R} \\
\ln(K_2) = -\frac{\Delta H}{R T_2} + \frac{\Delta S}{R}
\] (6)

\[
\ln(K_2) - \ln(K_1) = \left( -\frac{\Delta H}{R T_2} + \frac{\Delta S}{R} \right) - \left( -\frac{\Delta H}{R T_1} + \frac{\Delta S}{R} \right) = -\frac{\Delta H}{R T_2} + \frac{\Delta H}{R T_1}
\] (8)

The binding energy \(\Delta H\) for the reaction of one mole of ATP-bound aptamer is calculated as follows:

\[
\Delta H = -R \ln \left( \frac{K_2}{K_1} \right) \left( \frac{T_1 T_2}{T_2 - T_1} \right)
\] (10)

Thus, the binding energy between one complex of ATP and aptamer \((E_b)\) is determined as follows:

\[
E_b = \frac{\Delta H}{N_A}
\] (11)

where \(N_A\) is Avogadro's number, which is the number of molecules contained in one mole of ATP \((i.e., 6.022 \times 10^{23}\) molecules\).
**Supplementary Information S3**

Washing under varying flow rates

<table>
<thead>
<tr>
<th>Flow Rate</th>
<th>1st Cycle</th>
<th>2nd Cycle</th>
<th>3rd Cycle</th>
<th>4th Cycle</th>
<th>5th Cycle</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.75 µl min⁻¹</td>
<td>![Image]</td>
<td>![Image]</td>
<td>![Image]</td>
<td>![Image]</td>
<td>![Image]</td>
</tr>
<tr>
<td>1.00 µl min⁻¹</td>
<td>![Image]</td>
<td>![Image]</td>
<td>![Image]</td>
<td>![Image]</td>
<td>![Image]</td>
</tr>
<tr>
<td>1.25 µl min⁻¹</td>
<td>![Image]</td>
<td>![Image]</td>
<td>![Image]</td>
<td>![Image]</td>
<td>![Image]</td>
</tr>
<tr>
<td>1.50 µl min⁻¹</td>
<td>![Image]</td>
<td>![Image]</td>
<td>![Image]</td>
<td>![Image]</td>
<td>![Image]</td>
</tr>
</tbody>
</table>

**Fig. S3** Fluorescence images showing the presence of biomarker bound aptamer. The images were collected at the peak intensity of each cycle (i.e., before washing). The blue box can be considered to represent the optimized washing flow rate, where the aptamer signal is regenerated. In contrast, the red box represents a high washing flow rate, where the aptamer signal is lost due to the complete removal of the aptamer from the surface. Scale bar = 200 µm.
Supplementary Information S4

ICP characterization

Fig. S4.1 (a) The experimental setup showing the syringe pump, source meter unit (SMU), and control PC. (b) The fabricated device with all electrical and fluidic connections is in operation. The scale bar is 1 cm. (c) Microelectrodes were deposited by gold sputtering onto a glass substrate for the in situ conductivity measurements inside the microchannel. The magnified view is an optical image showing the distance between the two electrodes $\Delta x = 100 \, \mu m$. The scale bar is 100 $\mu m$. (d) The effect of the flow rate and voltage on the shift of the ion depletion boundary tracked by fluorescence dye. The scale bar is 200 $\mu m$.

In situ conductivity measurement after the sensing part

The in situ conductivity was calculated using the following relation:

$$\sigma = \frac{1}{\rho} = \frac{J}{E} = \frac{I \Delta x}{AU}$$

where $\rho$ is the resistivity, $J$ is the magnitude of the current density estimated from the current $I$ passing through the microelectrodes divided by the cross-sectional area $A$ of the microchannel,
and $E$ is the magnitude of the electric field estimated from the applied voltage $U$ divided by the gap $\Delta x$ (see Fig. S4c) between two microelectrodes.

![Normalized conductivity vs. Time (min)](image)

**Fig. S4.2** Measured *in situ* conductivity of human serum mixed with different ATP concentrations during the washing process used to remove the ATP molecules from the aptamers. After 10 minutes of washing, the conductivity of the washing solution decreased to ~98% of its initial value, reaching as low as the conductivity level of clean solution with a negligible amount of remaining ATP molecules. The source meter unit Keithley 2410 used in experiment has a default measurement resolution of 10 pA for the measurement range under 1 μA. External electric field 5 V cm$^{-1}$ was applied through electrodes, so that 100 μm spaced electrodes experienced $U = 0.1$ V. The *in situ* conductivity values were calibrated by a fitting parameter against the conductivity values measured by a portable pH/conductivity meter to ensure the error bound within ~5%.

For calibration purposes, the conductivity of 0.01X human serum mixed with ATP at varying concentrations was also measured using a portable pH/conductivity meter (Thermo Scientific Orion 4-Star Plus, USA). These values (Table S4) were used to convert the measured *in situ* conductivity to concentrations of ATP in human serum.

**Table S4** Calibration conductivity measured using a conductivity meter of 0.01X human serum mixed with different ATP concentrations. The pH/conductivity meter used in measurements has a resolution of 4 significant digits down to 0.001 μS cm$^{-1}$.

<table>
<thead>
<tr>
<th>[ATP] (nM)</th>
<th>Conductivity $\sigma$ (μS cm$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>115.234</td>
</tr>
<tr>
<td>100</td>
<td>118.713</td>
</tr>
<tr>
<td>500</td>
<td>132.631</td>
</tr>
<tr>
<td>750</td>
<td>141.330</td>
</tr>
<tr>
<td>1000</td>
<td>150.029</td>
</tr>
<tr>
<td>1250</td>
<td>158.727</td>
</tr>
</tbody>
</table>
Supplementary Information S5

Current tracking during real-time ATP monitoring

Fig. S5 (a) Current flowing through the nanojunction during five cycles of washing away ATP via ICP. The inset is an AFM image showing the Nafion® morphology with the average deposition thickness of 600 nm. Since the Nafion® resin is adhered onto the glass substrate simply by physical absorption after solvent evaporation, its functionality is susceptible to degradation. Consequently, the current passing through the nanoporous network will decrease progressively with repeated washing. (b) Optical images of the channel at the sensing part showing the fluorescence intensity in the presence and absence of applied voltage. Steps 2 and 3 were repeated in turn. Scale bar = 100 μm.
Supplementary Information S6

Image processing by MATLAB®

The MATLAB® script for calculating intensity values of fluorescence images:

```matlab
close all; clear all; clc;
inputfld = uigetdir;
inputfldAllFile = [inputfld '/*.png'];
inputfiles = dir(inputfldAllFile);
filesnumber = length(inputfiles);
fid = fopen('intensity_01.txt','w');
fprintf(fid, '%20s %20s n', 'Filename', 'Pixels intensity (A.U.)');
cycles = 6;
interval = 2; % time interval in minutes
datapoints = 5*cycles*interval;

for i=1:datapoints
    filename = inputfiles(i).name;
    disp(filename)
    infilename = [inputfld ' ' filename];
    croppedImage = imread(infilename);
    figure('Name',filename)
    imshow(croppedImage);
    gray = rgb2gray(croppedImage);
    intensity = mean2(gray);
    disp(intensity)
    time = i*interval;
    figure;
    plot(time,intensity,'ro');
    xlabel('Time (mins)');
    ylabel('Intensity (A.U.)');
    hold on;
    fprintf(fid, '%20s %20s n', filename, intensity);
end
fclose(fid);
```
Supplementary Information S7

Operation of ICP under different human serum solutions with and without dilution

![Graph showing current-time response through the nanojunction during ICP operation under three different human serum solutions: no diluted (1X), diluted 10-fold (0.1X), and diluted 100-fold (0.01X). The vertical dashed lines are considered the time when the ion depletion boundary stabilized.](image)

**Fig. S6** Current-time response through the nanojunction during ICP operation under three different human serum solutions: no diluted (1X), diluted 10-fold (0.1X), and diluted 100-fold (0.01X). The vertical dashed lines are considered the time when the ion depletion boundary stabilized.

Forming ICP in non-diluted serum with high ionic strength required a certain current and voltage (~3 μA with an applied voltage ~50 V).\(^5,6\) In several previous reports, ICP was formed to extract clean water directly from seawater with an ultra-high ionic strength (conductivity up to ~45 mS cm\(^{-1}\)) by applying an electric field \(|E| \approx 75\) V cm\(^{-1}\).\(^7\) In the case of non-diluted serum, the ionic strength is ~100 mM (conductivity ~10 mS cm\(^{-1}\)), which is much lower than the ionic strength of seawater. 50 V cm\(^{-1}\) of electric field was applied to generate ICP in non-diluted serum to extract the clean buffer solutions. In our experiment, the time period to form and stabilize ICP in non-diluted serum was ~2 minutes, which was longer than the time period to form and stabilize ICP in diluted serum (*i.e.*, less than one minute). This observation is in a good agreement with results reported before.\(^8\) With optimization of ion exchange membrane and device design, low voltages (5 – 10 V) could be applied to form ICP in a solution with high ionic strength, showing promising energy efficiency for future portable/wearable device design.\(^9,10\)
Supplementary Information S8

Continuous flow human serum extraction

**Fig. S7** Scheme of an integrated system for human serum extraction from whole blood. The filtered serum will be directed to the ICP-based aptamer sensor.
References