Supporting Information

A Micro-cantilever Sensor Chip Based on Contact Angle Analysis for a Label-Free Troponin I Immunoassay

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I. Mechanics model of a multilayer cantilever for capillary forces detection

Figure S1 depicts a multilayer cantilever with the same layer composition of the cantilever structure fabricated by a standard CMOS process as shown in Fig. 2. The cantilever is of length $L$, width $W$, thickness $t$, and piezoresistor length $l$.

Assuming that the deflection of the cantilever is small and the materials of the cantilever are linearly elastic and follow Hook’s law. Under a uniformly distributed force loading of $2\Delta\gamma + \Delta PW$ along the cantilever length (induced by surface tension and Laplace pressure) and a tip force loading $\Delta\gamma W$ (induced by surface tension), the differential equation of the deflection curve of the cantilever is given by

$$
\sum_i E_i I_i v'' = M(x) \\
= -\left(2\Delta\gamma + \Delta PW\right)\frac{(L-x)^2}{2} - \Delta\gamma W (L-x)
$$

(S1)

where $v$ is the deflection of the cantilever along the x-axis, $M$ is the bending moment, and $E_i I_i$ is the flexural rigidity of each layer of the cantilever. According to
the strain-curvature relation of the cantilever, the mechanical strain in the
piezoresistor (considering the strain value on the mid plane) is given by
\[ \varepsilon_x = -\frac{z}{\rho} = \frac{z}{\sum_i E_i I_i} \left[ \left( \frac{\Delta P W}{2} + 2\Delta_\gamma \right)(L-x)^2 + \Delta_\gamma W (L-x) \right] \] (S2)

where \( \rho \) is the radius of curvature and \( z \) is the displacement from the neutral axis of the cantilever. The neutral axis (\( h_N \)) is defined as
\[ h_N = \frac{\sum_i E_i t_i h_i}{\sum_i E_i t_i} \] (S3)

where \( t_i \) is thickness of each layer and \( h_i \) is the position of the layer surface with respect to the neutral axis. The flexural rigidity of the composite cantilever can therefore be explicitly expressed as
\[ \sum_i E_i I_i = \sum_i E_i \left( \frac{W t_i^3}{12} + W t_i (h_i - h_N)^2 \right) \] (S4)

II. Force sensitivity of the piezoresistive cantilever

The piezoresistive layer in the cantilever is considered to be a u-shaped piezoresistor with leg width \( w \), leg length \( l \), and thickness \( t_p \). The resistance of the resistor is given by
\[ R = 2 \int_0^l \frac{\rho dx}{w t_p} = \frac{2\rho l}{w t_p} \] (S5)

According to the piezoresistive effect \( \Delta \rho \rho = G\varepsilon_x \), the resistance change induced by the mechanical strain is given by
\[ \Delta R = 2 \int_0^l \frac{\rho G\varepsilon_x}{w t_p} dx \] (S6)

Since the leg length of the piezoresistor is slightly smaller than the length of cantilever in the proposed CAMCS chips, the force sensitivity of the piezoresistive
cantilever, represented by the average values of the relative resistance change of the piezoresistor, can be approximately expressed as

\[
\begin{bmatrix}
\frac{\Delta R}{R} \\
0.5 \frac{\Delta R}{R}
\end{bmatrix}
\approx \frac{\Delta \Delta R}{\Delta \Delta R}
\tag{S7}
\]

**Fig. S1** Mechanics model of a multilayer cantilever for capillary force detection

### III. Calibration of CAMCS chips by using contact angle measurement of sessile drops

We performed contact angle measurement for sessile drops on Au substrates with the same modification as the cantilever surfaces (with DTSSP and BSA adsorbed on DTSSP) and compared the contact angle change of the sessile drops with the signal change of the cantilever sensors. The result from one of the experiments is shown in Fig. S2: A smaller contact angle change of PBS drops on a DTSSP modified Au substrate (\(\theta = 72 \pm 2\)) corresponded to a smaller signal change of the cantilever sensor (\(\Delta V_{out} \approx 2.5 \times 10^{-3}\) mV) with DTSSP modification (section c), while a larger contact angle change of PBS drop on BSA adsorbed substrate (\(\theta = 49 \pm 4\)) corresponded to a larger signal change of the cantilever sensor (\(\Delta V_{out} \approx 1.5 \times 10^{-2}\) mV) with adsorbed BSA on cantilever (section e). The initial contact angle of PBS drop on Au substrate is \(\theta = 77 \pm 0.5\). Based on the result of the contact angle measurement, the magnitude of signal changes in the cantilever sensors correlates well with the contact angle change of the sessile drops.
The sessile drop contact angle measurement was performed by using Kruess Drop Shape Analysis System, DSA 10 MK2 (Kruess GmbH, Hamburg, Germany). The experiments were conducted in room temperature without control of humidity.

**IV. Detection of cTnI binding by using CAMCS chips and ELISA assay**

A standard ELISA assay was performed to compare the data with those of the CAMCS chips. The standard ELISA assay and CAMCS chips demonstrated similar performance of cTnI binding in the critical concentration region 1pg/mL - 0.1 ng/mL.

**Performing the ELISA assay**

Flat bottom polystyrene microtiter plates (Nunc-Immuno™ LockWell™ Modules, Nunc GmbH & Co. KG, Germany) were coated with combination of
capture antibody 19C7 and 16A11 to final concentration of 10 \( \mu g \) ml\(^{-1} \) diluted in PBS. Incubation was carried out under cover for 2 h. After the incubation, the plate was washed. To saturate any binding sites not already occupied by the primary antibody, incubation with 200 \( \mu l \) of blocking solution was carried out 30 min at 25 °C on microplate shaker followed by overnight incubation at 4 °C without shaking. After consequent washing, the plate was ready to use.

Troponin IT complex was spiked into reagent diluents. For final optimization, undiluted cTnI free serum was used instead of reagent diluents. The calibrators were incubated for 2 hours followed by a washing step and 1 hour incubation with 5 \( \mu g \) ml\(^{-1} \) of biotinylated detection antibody MF4-b. After three wash cycles, 100 \( \mu l \) of a solution of glucose oxidase conjugated streptavidin diluted to 1 \( \mu g \) ml\(^{-1} \) in reagent diluent was added and the plates were incubated for 30 min at 25 °C. After subsequent washing, 50 \( \mu l \) of ABTS diluted in phosphate-citrate buffer to concentration 2,2 mg ml\(^{-1} \) were pipetted into each well. Solutions containing 160 mM of glucose and 0.8 mg ml\(^{-1} \) of HRP both in phosphate-citrate buffer were mixed in one to one ratio. Volume of 50 \( \mu l \) of this solution was pipetted into each well. The plate was incubated without shaking for 1 h at 25 °C and then immediately measured on absorbance microplate reader (SpectraMax 340PC384, Molecular Devices GmbH, Germany) preheated at 25 °C at 405 nm. Software SoftMax Pro (Molecular Devices GmbH, Germany) was used to collect the data.

Unless otherwise stated, every step of the immunoreactions was followed by three washing cycles on commercial ELISA microplate washer using wash buffer. Except for blocking solution, standard coating volume, 100 \( \mu l \) per well, was used for all immunoreactants. Plates were incubated sealed on a plate shaker (ThermoShaker PST-60 HL plus, BioSan, Riga, Latvia) preheated at 25 °C and at 350 rpm. Following
reagents were used:

- Phosphate buffer saline: 0.1 M PBS; 0.1 M NaCl, pH 7.4
- Wash buffer: 0.01 M phosphate buffer saline; 0.138 M NaCl; 0.0027 M KCl, 0.05% Tween 20; pH 7.4
- Blocking solution: 1% BSA, 0.5% casein; 0.01% NaN₃ in 0.1 M PBS; 0.1 M NaCl, pH 7.4
- Reagent diluent: 0.1% BSA in 0.01 M PBS; 0.01 M NaCl, pH 7.4