A non-invasive wearable stress patch for real-time cortisol monitoring using a pseudoknot-assisted aptamer

Supplemental Material

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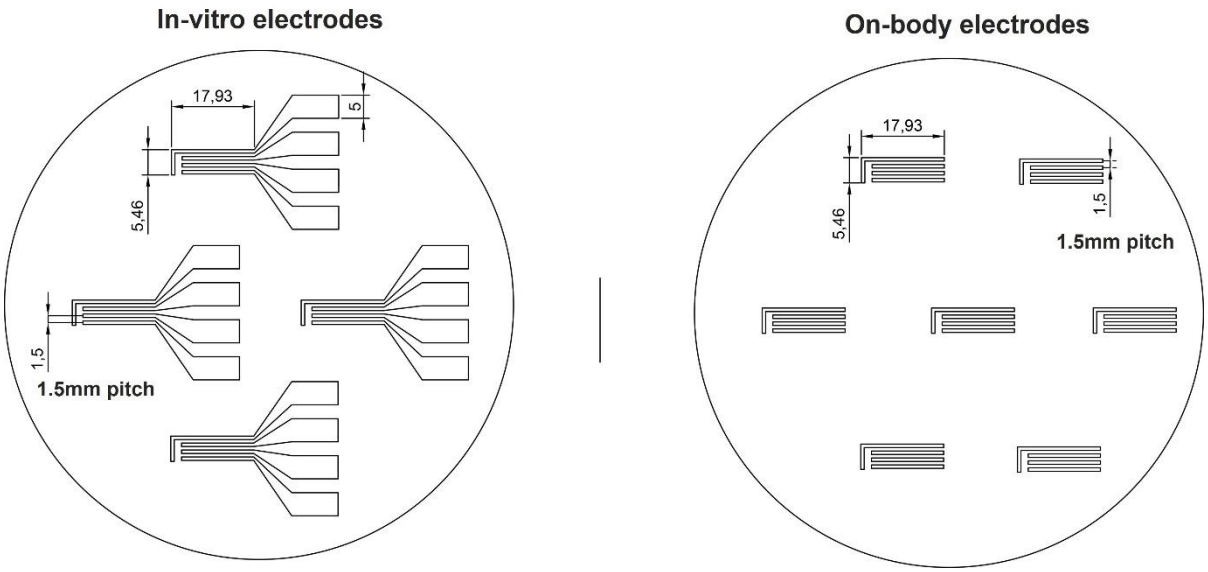

Figure S1. Electrode designs for the wearable electrochemical sensor.

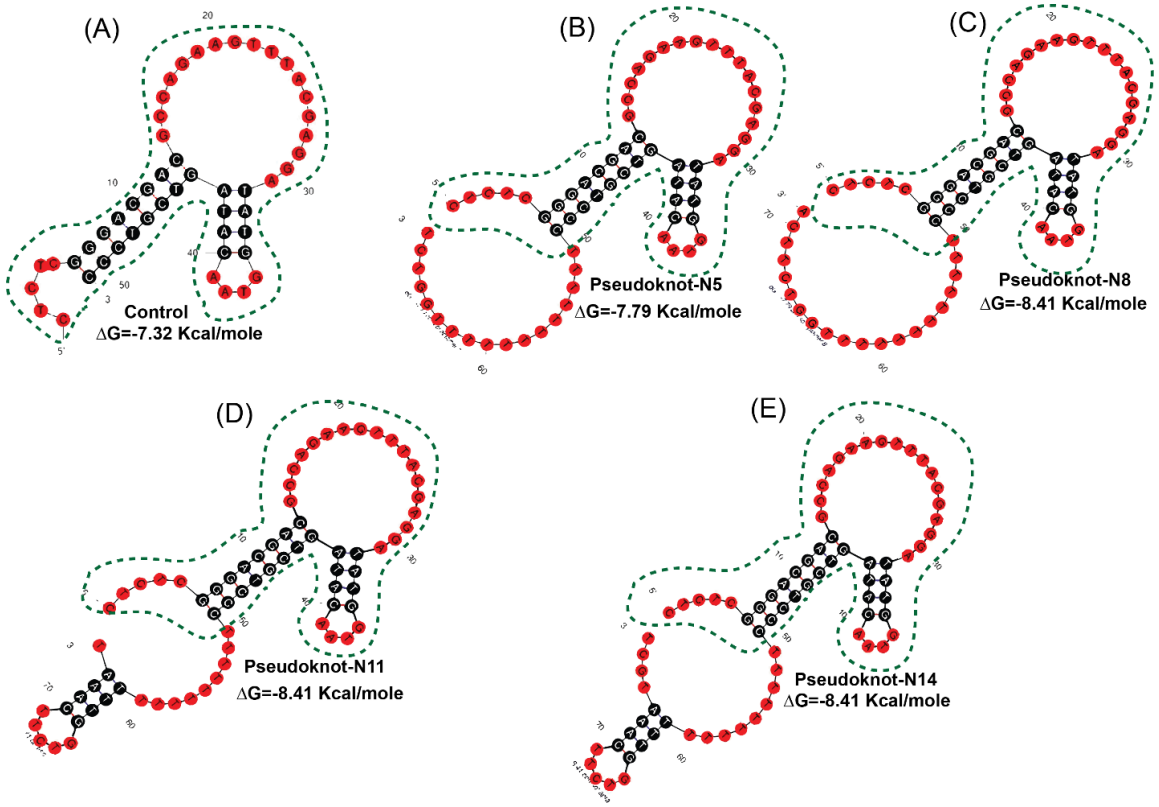

Figure S2. Aptamer structure. Secondary structure of **(A)** cortisol aptamer(Yang et al., 2017) and its modified pseudoknot enabled aptamer with different overlap regions, **(B)** N5, **(C)** N8, **(D)** N11, and **(E)** N14. Structurally conserved regions are represented by the green dashed line. Secondary structures were evaluated using Mfold (Zuker, 2003).

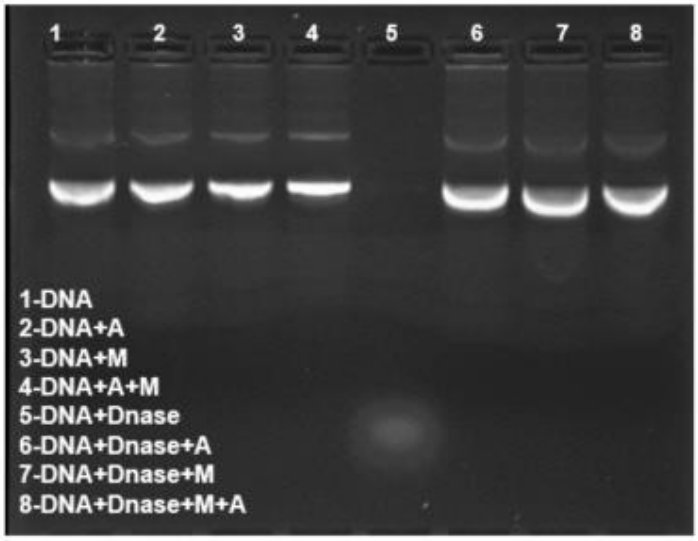

Figure S3. **DNA aptamer stability from DNase-I.** DNA was incubated for 30 min with the DNase-I enzyme (65 µU/mg) at RT. Actin (A) and 2 ME (M) were used at 100 µM concentration in 1× PBS.

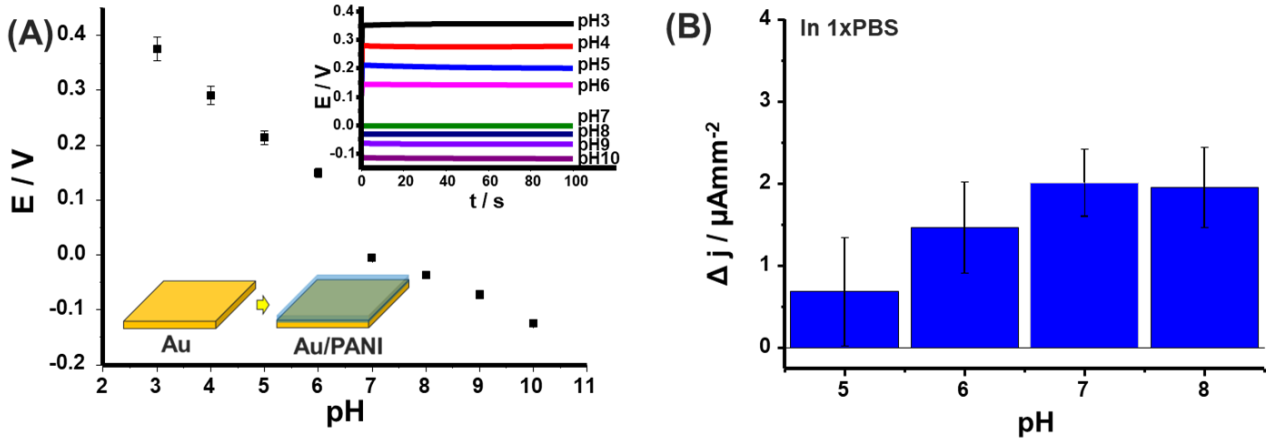

Figure S4. **pH sensor and sensor performance**. **(A)** Calibration plot of open circuit potential (OCP) response vs. increasing pH solution. **(B)** Sensor performance in various pH buffers with 100 nM cortisol.

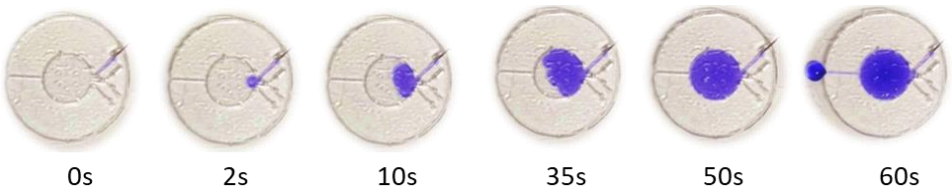

Figure S5. *in vitro* microfluidic reservoir filling test under 20 µL/min flow.

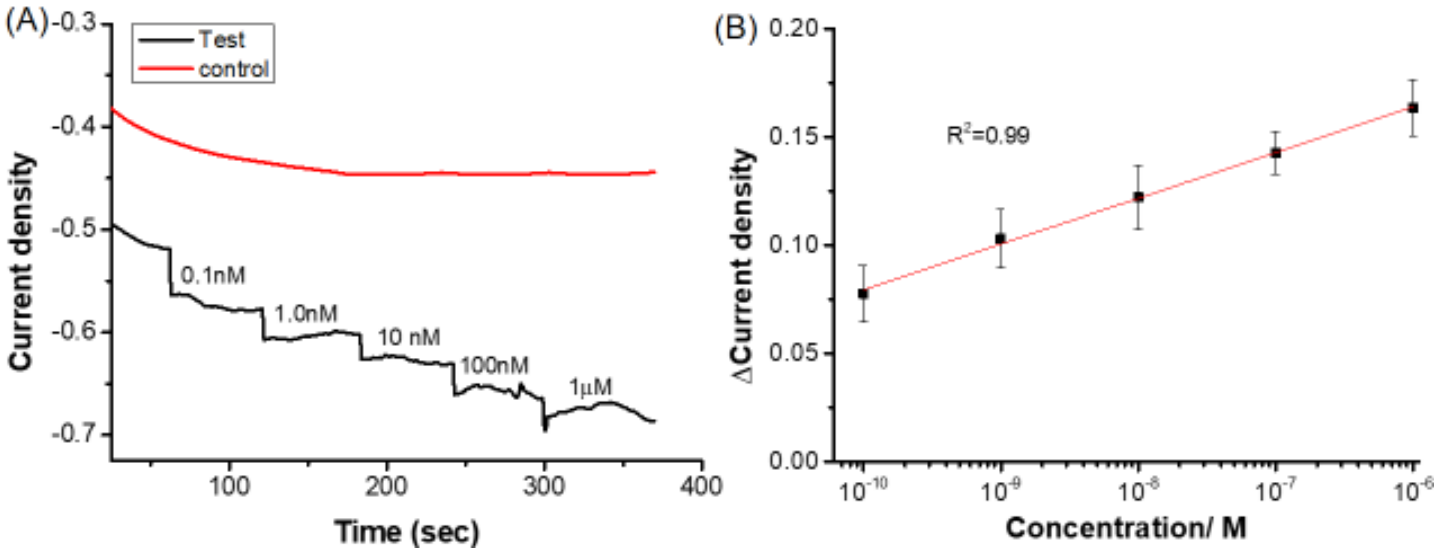

Figure S6. Real-time measurement. (A) Chronoamperometry response of the aptasensor and **(B)** calibration plot as a function of cortisol concentration (black) and without cortisol (red) in binding buffer at −0.25 V versus Ag/AgCl.

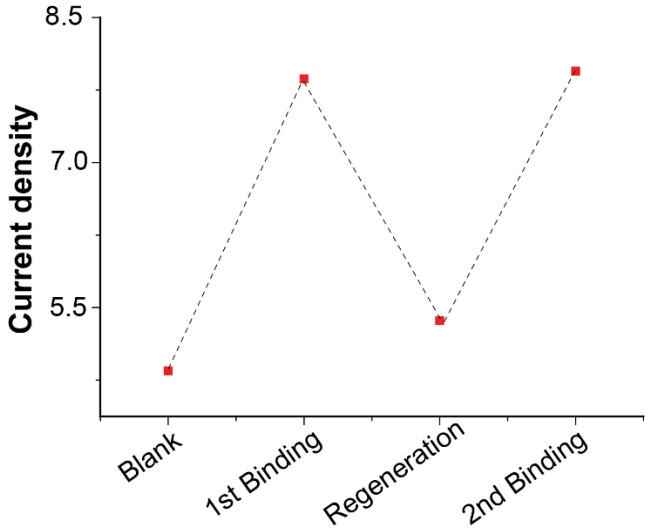

Figure S7. Sensor reproducibility and sweat patch on body testing. Sensor reproducibility and signal regeneration was performed with 30 mM NaOH.

Table S1. Composition of buffer solutions.

No	Name	Concentration
1	Washing buffer	1x Phosphate buffer saline, pH 7.2
2.	Binding buffer	1x Phosphate buffer saline, 0.90 mM $CaCl_2$, 0.5 mM $MgCl_2$, 2.5 mM KCl
3.	Sensor characterization buffer	1x PBS, 5mM K_3/K_4[Fe(CN)]6

Table S2. Comparison with state-of-the-art aptamer-based sensor for cortisol sensing.

Sensor	Detection Principle	Readout Method	Linear Range (nM)	LOD (nM)	Sample	Wearable platform?	Microfluidics and *in-situ* sweat replacement?
MWNT-Cu-PP Electrode (Fernandez et al., 2017)	Aptamer-assisted electrochemical reduction of cortisol	DPV	0.1 − 50	0.01	Saliva	No	No
In_2O_3-Au/Polyimide (Wang et al., 2022)	Measuring gate voltage of cortisol binding with aptamer	FET	0.001 − 1000*	0.001	Saliva Sweat	Yes	Yes
PAN-PEDOT/Silk Film (An et al., 2022)	Measurement of the shift in drain current on cortisol binding with aptamer	Liquid ion FET	0.001 − 10,000	0.01	Saliva Sweat	Yes	Yes
ZnO nanopores/ Polyimide (Pali et al., 2021)	Aptamer-based impedance measurement	CA	2 − 700	2.76	Sweat	No	No
AuNW-BSA/Au (Singh et al., 2021)	Conformation-switching aptamer-based amperometry measurement	DPV	1 – 1000	0.68	Serum	No	NA
CNT-Carbon cellulose/PDMS (Mugo et al., 2021)	Change in capacitance of a conductive nanoporous carbon nanotube-cellulose nanocrystals (CNC/CNT) film impregnated with aptamers	Capacitance	6.9 – 96	4.97	Sweat	No	NA
PDMS/Gold/Actin/2 ME (**This work**)	Pseudoknot-assisted aptamer with pH-calibrated real-time response	DPV, CA	0.001 − 1000	0.002	Sweat	Yes	Yes

***Dynamic range**

DPV: Differential pulse voltammetry, **CA:** Chronoamperometry

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