Electronic Supplementary Material (ESI) for Sensors & Diagnostics.

Supporting Information

A CRISPR-amplified label-free electrochemical aptasensor for the sensitive detection of HbA1c

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Materials and reagents

Hemoglobin (Hb) and glycated hemoglobin (HbA1c) were purchased from Shanghai Xinfan Biotechnology (China). Healthy human serum was purchased from Shanghai Jiwei Biotechnology Co., Ltd (http://www.givei.cn/product/detail/18142.html). Cas12a (Cpf1, 100 μM), and 10× NEBuffer 2.1 (100 mM Tris-HCl, 500 mM NaCl, 100 mM $MgCl_2$, 1000 μg mL^{-1} BSA, pH 7.9) were provided by New England Biolabs (Beijing). Tris(2-carboxyethyl) phosphine (TCEP) and 6-mercapto-1-hexanol (MCH) were obtained from Sigma-Aldrich Co., Ltd (Shanghai). Disodium hydrogen phosphate dodecahydrate ($Na_2HPO_4 \cdot 12H_2O$, ≥99.0%), sodium dihydrogen phosphate dihydrate ($NaH_2PO_4 \cdot 2H_2O$, ≥99.0%), potassium ferricyanide ($K_3Fe(CN)_6$), potassium ferrocyanide ($K_4Fe(CN)_6$), uric acid (UA), ascorbic acid (AA), glucose, bovine serum albumin (BSA), magnesium chloride ($MgCl_2$), sodium chloride (NaCl) and potassium chloride (KCl) were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai). Phosphate buffer (PB) and phosphate buffered saline (PBS) were prepared by NaH_2PO_4 (0.2 M), Na_2HPO_4 (0.2 M) and different salts according to classical protocol. All reagents were analytical grade and used without further purification. Aqueous solutions were prepared by using ultrapure water (>18 MΩ·cm) obtained from the Millipore water purification system.

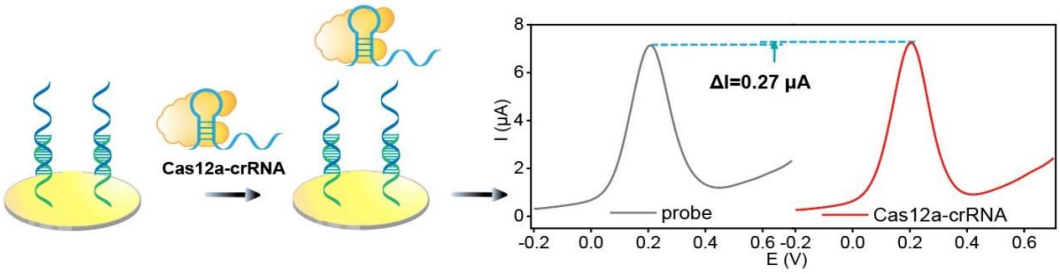

Figure S1. Schematic diagram and SWV curve of probe/Au before and after introducing CRISPR-Cas12a system without HbA1c in 0.5 mM $[Fe(CN)_6]^{3-/4-}$ solution containing 0.1 M KCl.

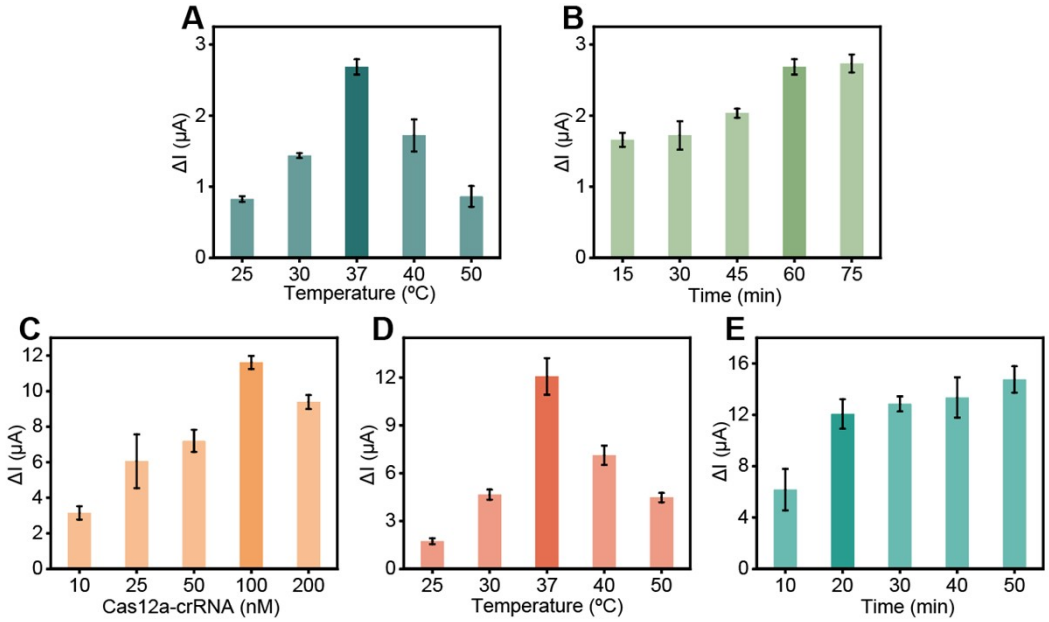

Figure S2. The effects of different (A) HbA1c incubation temperatures, (B) HbA1c incubation time, (C) Cas12a-crRNA concentration, (D) CRISPR-Cas12a system incubation temperatures, and (E) CRISPR-Cas12a system incubation time on the current variations ($\Delta I = I_{HbA1c} - I_{no\ HbA1c}$) of electrochemical aptasensor.

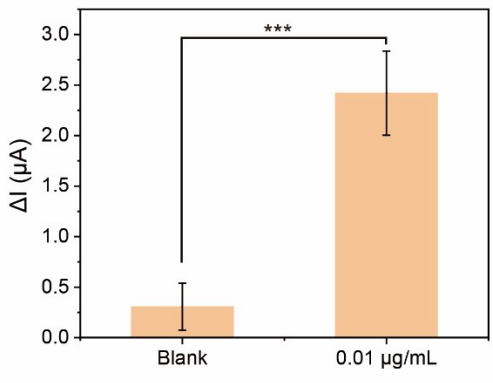

Figure S3. The statistical significance between 0.01 μg/mL HbA1c and blank in 50% human serum (***P<0.001).

Table S1. DNA and RNA sequences required in the experiment (5' to 3' ends).

Name	Sequence
Apt	ACACACCCACCCACCAGCCCCAGCATCATGCCCATCCGTCGTGTGT
cDNA	SH-TTTTTTTTTACACACGACGGATGGGCATGA
crRNA	UAAUUUCUACUAAGUGUAGAUUCAUGCCCAUCCGUCGUGUG

Table S2. Comparison of the analytical performance of the developed aptasensor with other reported biosensors for HbA1c detection.

Methods	**Linear range**	**Limit of detection**	**Ref.**
quartz crystal microbalance	13-108 nM	26.29 nM	1
field-effect transistor	3-600 nM	3 nM	2
Surface plasmon resonance	18–147 nM	1 nM	3
electrochemical	0.1-14 μg/mL	0.084 μg/mL	4
electrochemical	0.1-100 μg/mL	0.016 μg/mL	5
Luminol chemiluminescence	50–1000 μg/mL	350 ng/mL	6
electrochemical	1 ng/mL-100 μg/mL	0.84 ng/mL	This work

Table S3. Analysis of HbA1c in 10 % human serum by using the developed electrochemical aptasensor

Sample	Added lg (ng/mL)	Found lg (ng/mL)	RSD（%）	Recovery（%）
1	1	1.01	9.08	101.14
2	2	2.02	7.29	101.15
3	3	2.98	1.73	99.44
4	4	4.03	2.21	100.77

Table S4. Analysis of HbA1c in 20 % human serum by using the developed electrochemical aptasensor

Sample	Added lg (ng/mL)	Found lg (ng/mL)	RSD（%）	Recovery（%）
1	1	1.02	5.48	102.05

2	2	1.95	4.27	97.44
3	3	2.73	8.92	91.01
4	4	4.27	5.20	106.67

Table S5. Analysis of HbA1c in 50 % human serum by using the developed electrochemical aptasensor

Sample	Added lg (ng/mL)	Found lg (ng/mL)	RSD（%）	Recovery（%）
1	1	1.05	9.01	104.80
2	2	1.93	4.05	96.43
3	3	3.19	7.50	106.49
4	4	4.24	8.13	106.08

References:

1 Y. Sriondee, P. Vijitvarasan, A. Rattanachata, H. Nakajima, S. Oaew and S. Cheunkar, *Anal. Methods*, 2024, **16**, 599-607.

2 A. Anand, C.-Y. Chen, T.-H. Chen, Y.-C. Liu, S.-Y. Sheu and Y.-T. Chen, *Nano Today*, 2021, **41**, 101294.

3 C. G. Zhang, S. J. Chang, K. Settu, C. Jung Chen and J. T. Liu, *Sensor. Actuat. B-Chem.*, 2019, **279**, 267-273.

4 X.-Q. Feng, Y. Ju, W.-T. Dou, Q. Li, Z.-G. Jin, X.-P. He, T. D. James and B.-C. Ye, *Molecules*, 2021, **26**, 7707.

5 Y. Zheng, Y. Cen, T. Du, D. Zhu, S. Su and L. Wang, *Chem. Commun.*, 2024, **60**, 3942-3945.

6 K.-S. Ahn, J. H. Lee, J.-M. Park, H. N. Choi and W.-Y. Lee, *Biosens. Bioelectron.*, 2016, **75**, 82-87.