Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information

A three-in-one point-of-care electrochemical sensing platform for accurate monitoring of diabetes

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Experimental section

Reagents and materials. SPCE was purchased from Ningbo Yuangan Biotechnology Co., Ltd. (Ningbo, China). Healthy human serum was purchased from Shanghai Jiwei Biotechnology Co., Ltd. (Shanghai, China). Chloroauricacid trihydrate ($HAuCl_4 \cdot 3H_2O$, ≥99%), polyacrylic acid (PAA), polyethyleneimine (PEI), tris(hydroxymethyl) aminomethane hydrochloride (Tris-HCl, ≥99.0%)，tris(2-carboxyethyl) phosphine (TCEP) and 6-mercapto-1-hexanol (MCH) were obtained from Sigma-Aldrich Co., Ltd. (Shanghai, China). Disodium hydrogen phosphate dodecahy-drate ($Na_2HPO_4 \cdot 12H_2O$, ≥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, China). Total hemoglobin (tHb) and glycated hemoglobin (HbA1c) were purchased from Shanghai Xinfan Biotechnology (China). Phosphate buffer (PB) and phosphate-buffered saline (PBS) were prepared using NaH_2PO_4 (0.2 M), Na_2HPO_4 (0.2 M) and different salts according to the standard protocol. All reagents were of 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.

Total Hb aptamer (Apt1) and HbA1c aptamer (Apt2) were provided by Sangon Biotech (China). Their sequences were listed as follows:

Apt1: 5'-SH-TTTTACGCACACCAGAGACAAGTAGCCCCCCAAACGCG-3'

Apt2: 5'-ACACACCCACCCACCAGCCCCAGCATCATGCCCATCCGTCGTGTGT-3'

Preparation of SPCE-HFGNs. SPCE-HFGNs were prepared according to our previous works.[1, 2] Briefly, SPCE was alternately immersed in PAA and PEI solution to form a PAA/PEI film on the surface of SPCE. After washing and drying, 20 μL 50 mM chloroauric acid solution was added on SPCE to prepared SPCE-HFGNs by electrodeposition method.[3] After preparation, scanning electron microscope (SEM, S-4800, HITACHI, Tokyo, Japan) used to character the morphology.

Construction of an electrochemical sensing platform. At first, 1 μM Apt1 was assembled on the surface of SPCE-HFGNs for 12 h via Au-S bond,[4, 5] naming Apt1/SPCE-HFGNs. Then, 6-mercapto-1-hexanol (MCH) and 1% bovine serum albumin (BSA) were employed to block the remaining active sites of SPCE-HFGNs and reduce the nonspecific adsorption. After simultaneously

capturing target HbA0 and HbA1c, 1 μM Apt2 was further employed to specifically recognize HbA1c for accurate detection. All electrochemical experiments were performed by using CHI electrochemical workstation (Shanghai Chenhua, China). It should be noted that $[Fe(CN)_6]^{3-/4-}$ was used as an electrochemical indicator for label-free detection. To demonstrate the successful modification of HFGNs and Apt1, contact angle/surface tension measurement instrument (SDC 350KS, Kunshan Shengding) and X-ray photoelectron spectroscopy (XPS, Thermo Scientific K-Alpha, America) were used to characterized SPCE, SPCE-HFGNs and Apt1/SPCE-HFGNs.

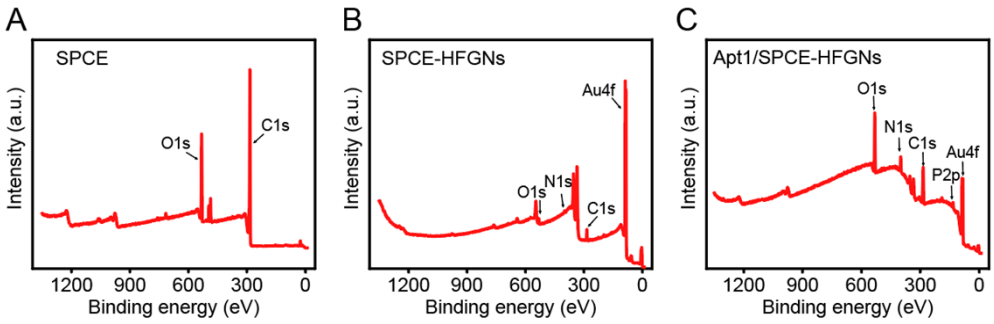

Figure S1. XPS spectra of (A) SPCE, (B) SPCE-HFGNs and (C) Apt1/SPCE-HFGNs.

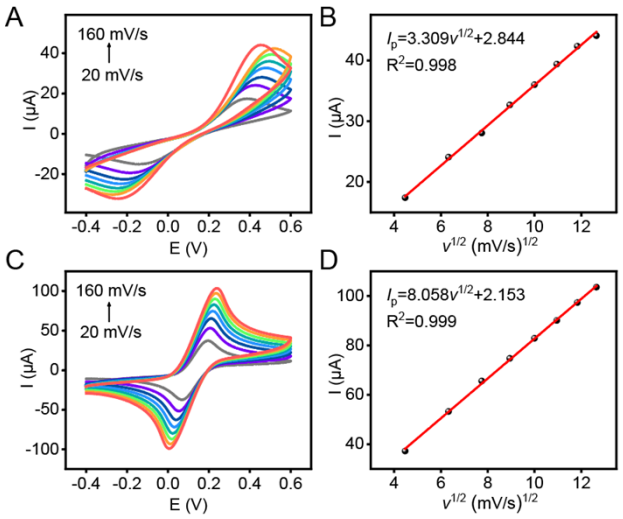

Figure S2. (A) CVs of SPCE in 5 mM $[Fe(CN)_6]^{3-/4-}$ containing 0.1 M KCl at different scan rate ranging from 20 to 160 mV/s. (B) The linear relationship between peak currents of SPCE with the square root of scan rates. (C) CVs of SPCE-HFGNs in 5 mM $[Fe(CN)_6]^{3-/4-}$ at different scan rates ranging from 20 to 160 mV/s. (D) The linear relationship between peak currents of SPCE-HFGNs with the square root of scan rates.

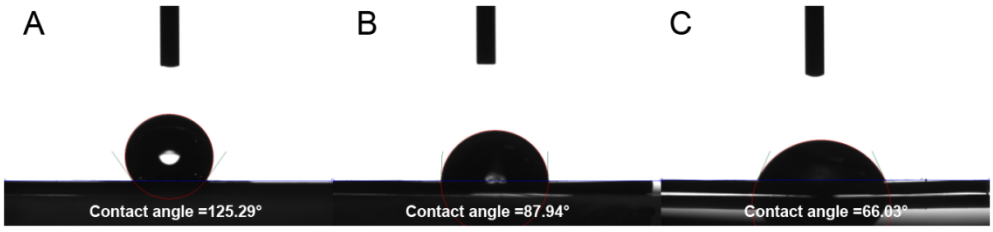

Figure S3. Contact angles of (A) SPCE, (B) SPCE-HFGNs and (C) Apt1/SPCE-HFGNs.

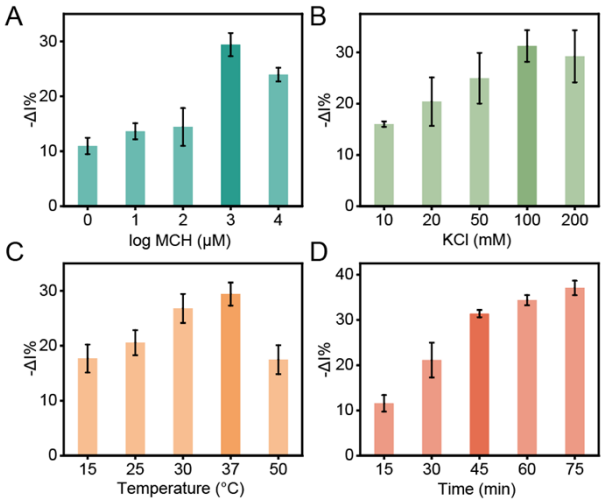

Figure S4. The impact of different (A) MCH concentrations, (B) KCl concentrations, (C) tHb incubation temperatures, and (D) tHb incubation times on the developed aptamer-based biosensor's electrochemical signal.

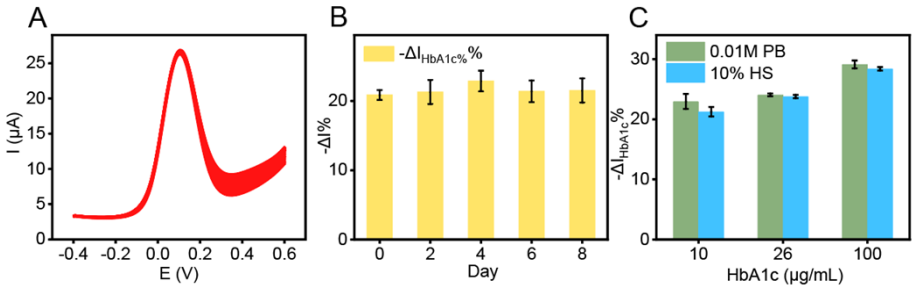

Figure S5. (A) SWV curves of tHb/Apt1/SPCE-HFGNs tested continuously 20 times in 5 mM $[Fe(CN)_6]^{3-/4-}$. (B) The storage stability of this sensing platform. (C) Electrochemical signal changes of electrochemical sensing platform for HbA1c detection in 0.01 M PB and 10% human serum (HS).

Table S1. Comparison of the analytical performance of the developed sensing platform with other reported biosensors for HbA1c detection.

Electrodes	Linear range (μg/mL)	Detection limit (μg/mL)	References
IgG-FITC/Au	10–100	1	6
PQQ/GCE/PQQ-ERGO	9.4–65.8	1.25	7
Apt/SPCE	0.1-14	0.084	8
C-Ab/GNF/SPCE	20-1000	2	9
Ab/AuNPs/SPCE	20-200	15.5	10
Apt/SPCE-HFGNs	0.1-100	0.016	this work

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

Electrodes	Linear range (%)	Detection limit (%)	References
AuNPs- pTTBA-APBA/SPCE	0.1-1.5	0.052	11
ZrMOF/Fe_3O_4(TMC)/AuNCs /SPCE	2-18	0.072	12
4-MPBA-Au NFs /SPCE	2-20	0.65	13
GA/MWCNT/BSA/SPCE	2-15	0.4	14
3-APBA/IDA	0.1-8.36	0.024	15
Apt/SPCE-HFGNs	4.12-15.63	0.016	this work

Table S3. The recoveries of serum samples were detected by this biosensor.

Sample	Added (%)	Found (%)	Recovery (%)	RSD (%)
1	4.12	3.94	95.71	7.99
2	7.00	6.86	98.00	2.92
3	12.75	12.65	99.24	5.12

References:

1 S. Su, Y. Wu, D. Zhu, J. Chao, X. Liu, Y. Wan, Y. Su, X. Zuo, C. Fan and L. Wang, *Small*, 2016, **12**, 3794-3801.

2 L. Wan, J. Ma, J. Yi, Y. Dong, R. Niu, Y. Su, Q. Li, D. Zhu, J. Chao, S. Su, C. Fan, L. Wang and Y. Wan, *Chem. Commun.*, 2022, **58**, 8826-8829.

3 J. Ma, L. Feng, J. Li, D. Zhu, L. Wang and S. Su, *Biosensors*, 2023, **13**, 746-755.

4 F. Gao, C. Gao, S. He, Q. Wang and A. Wu, *Biosens. Bioelectron.*, 2016, **81**, 15-22.

5 C. Erkmen, G. A. Tig and B. Uslu, *Sensor. Actuat. B-Chem.*, 2022, **358**, 131420-131430.

6 K.-M. Hsieh, K.-C. Lan, W.-L. Hu, M.-K. Chen, L.-S. Jang and M.-H. Wang, *Biosens. Bioelectron.*, 2013, **49**, 450-456.

7 Y. Zhou, H. Dong, L. Liu, Y. Hao, Z. Chang and M. Xu, *Biosens. Bioelectron.*, 2015, **64**, 442-448.

8 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**, 7077.
9 M. Thapa and Y. S. Heo, *Biosens. Bioelectron.*, 2023, **221**, 902.
10 Y. Zhao, H. Zhang, Y. Li, X. Wang, L. Zhao, J. Xu, Z. Zhan, G. Zhang and W. J. Li, *Biosensors*, 2022, **12**, 902.
11 D.-M. Kim and Y.-B. Shim, *Anal. Chem.*, 2013, **85**, 6536-6543.
12 A. Ahmadi, S. M. Khoshfetrat, S. Kabiri, P. S. Dorraji, B. Larijani and K. Omidfar, *Microchim. Acta*, 2021, **188**, 296.
13 X. Wang, J. Su, D. Zeng, G. Liu, L. Liu, Y. Xu, C. Wang, X. Liu, L. Wang and X. Mi, *Talanta*, 2019, **201**, 119-125.
14 Z. Li, J. Li, Y. Dou, L. Wang and S. Song, *Biosensors*, 2021, **11**, 118.
15 Y. Boonyasit, W. Laiwattanapaisal, O. Chailapakul, J. Emneus and A. R. Heiskanen, *Anal. Chem.*, 2016, **88**, 9582-9589.