Supporting Information

Boronate-Modified Interdigitated Electrode Array for Selective Impedance-Based Sensing of Glycated Hemoglobin

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Figure S1. pH dependence of boronate-HbA1c interaction

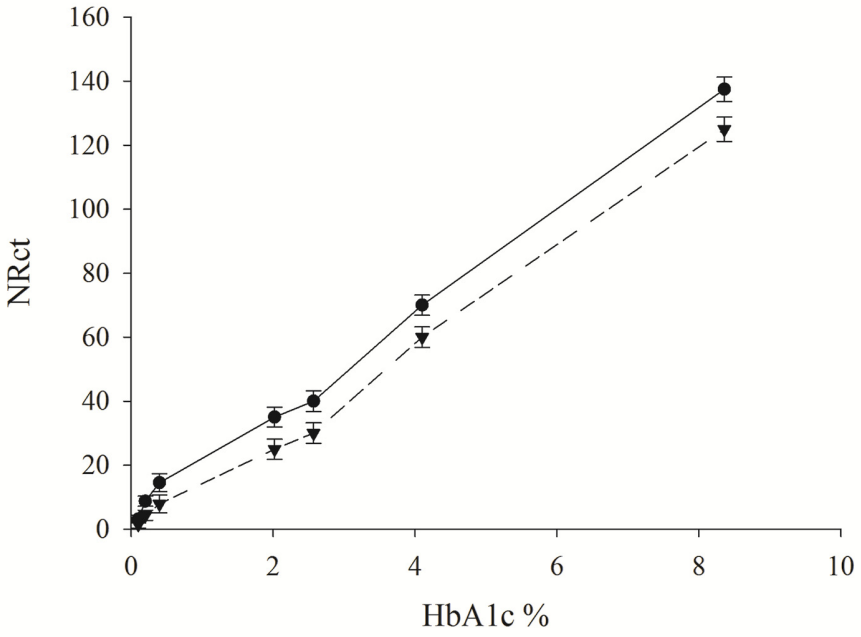

Figure S1. Comparison of HbA1c binding on APBA-modified electrodes under neutral (▼ - pH 7) and alkaline (● - pH 8.5) conditions after incubation with 0.10%, 0.20%, 0.40%, 2.02%, 2.57%, 4.10%, and 8.36% HbA1c. The response for each HbA1c concentration is expressed as normalized charge transfer resistance (average ± standard deviation, n = 24).

Figure S2. Optimization of glutaraldehyde and APBA concentration

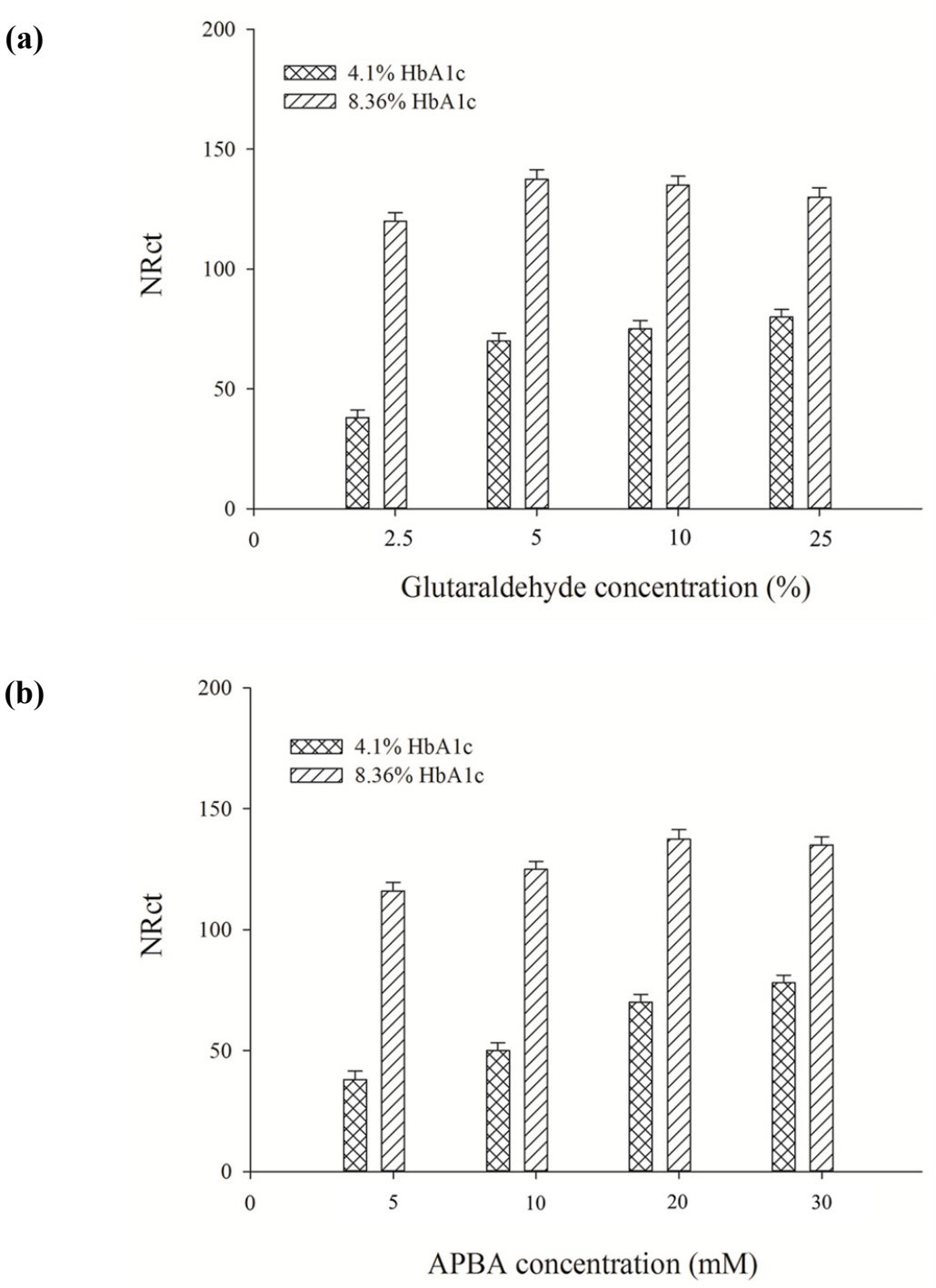

Figure S2. Optimization of (a) glutaraldehyde and (b) APBA concentration using normal (4.1%) and diabetic (8.36%) HbA1c level. The response for each modification and HbA1c concentration is expressed as normalized charge transfer resistance (average ± standard deviation, n = 24).

Figure S3. Optimization of immobilization (APBA) and incubation (HbA1c) time

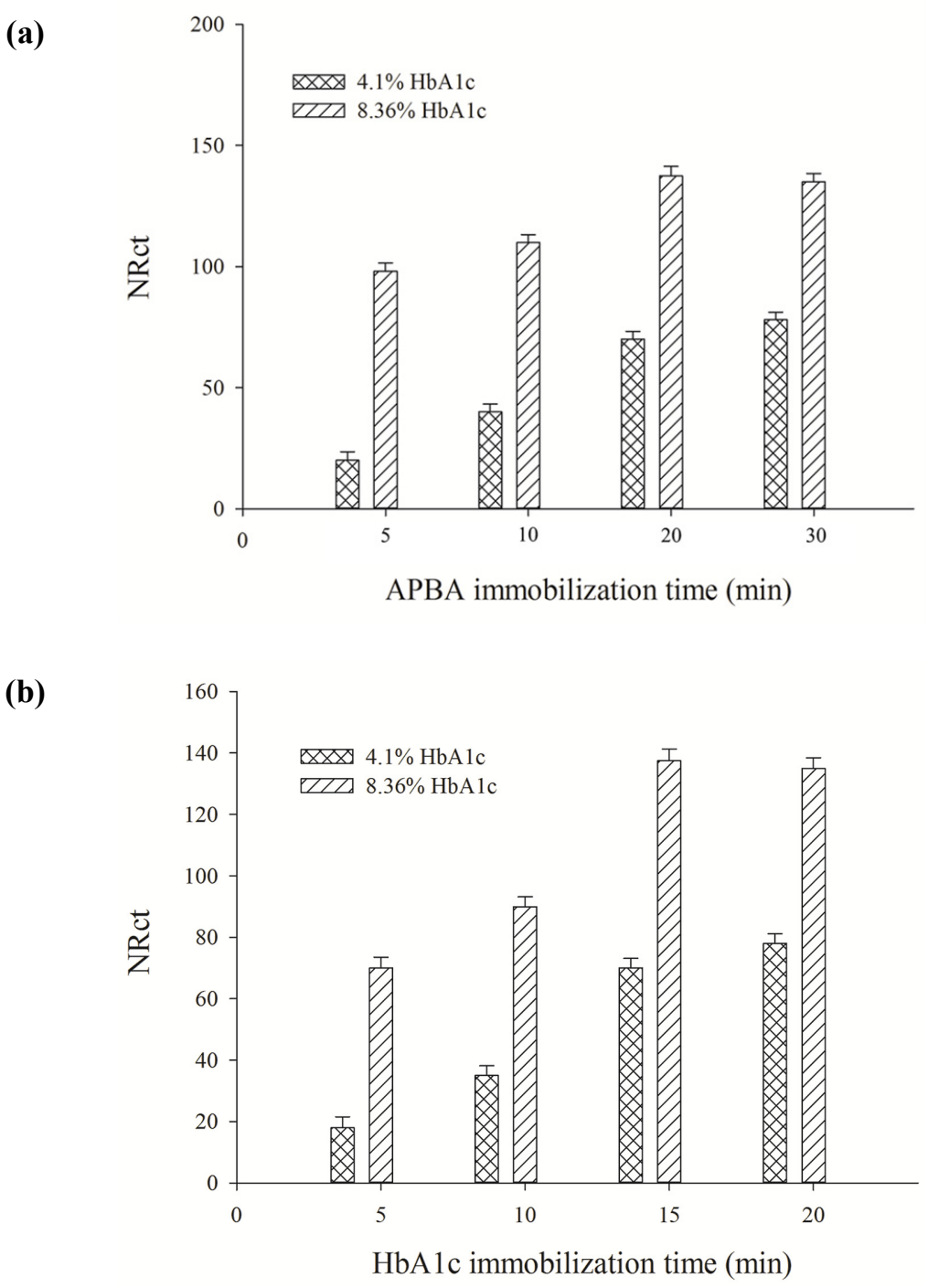

Figure S3. Optimization of time for (a) APBA immobilization and (b) HbA1c incubation using normal (4.1%) and diabetic (8.36%) HbA1c level. The response for each time and HbA1c concentration is expressed as normalized charge transfer resistance (average ± standard deviation, n = 24).

Figure S4. Sensing surfaces without boronate modification

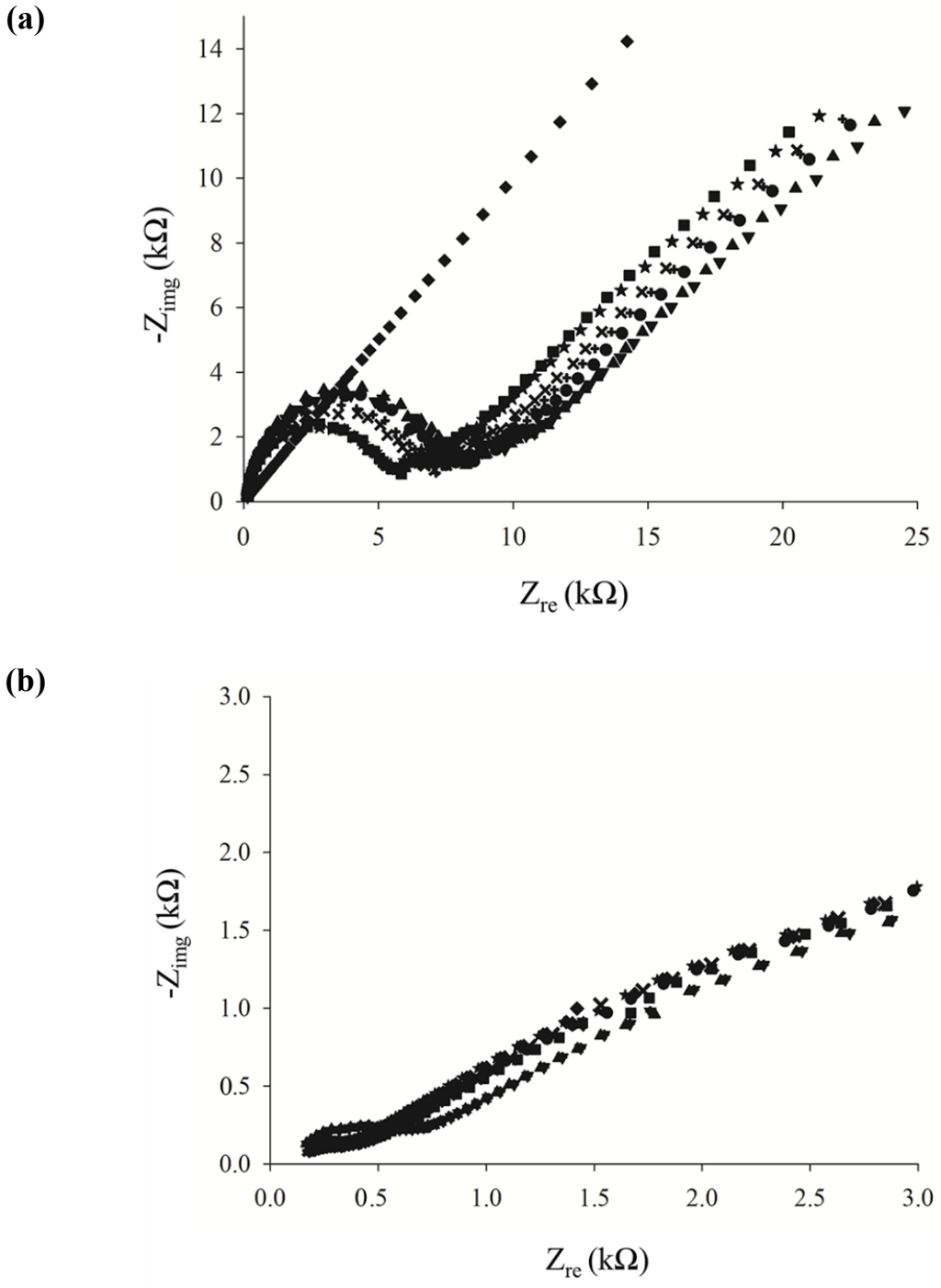

Figure S4. Characteristic Nyquist plots for a (a) glutaraldehyde activated and (b) cysteamine-modified electrode before and after incubation with (◆) 0%, (★) 0.10%, (✕) 0.20%, (✚) 0.40%, (●) 2.02%, (■) 2.57%, (▲) 4.10%, and (▼) 8.36% HbA1c standard solution. The increase in Rct due to covalent binding of HbA1c to glutaraldehyde is practically independent of HbA1c concentration, whereas no significant increase in Rct is observed on a cysteamine-modified electrode due to lack of any interaction between cysteamine and HbA1c.

Figure S5. Precision profile (RSE% vs. HbA1c concentration)

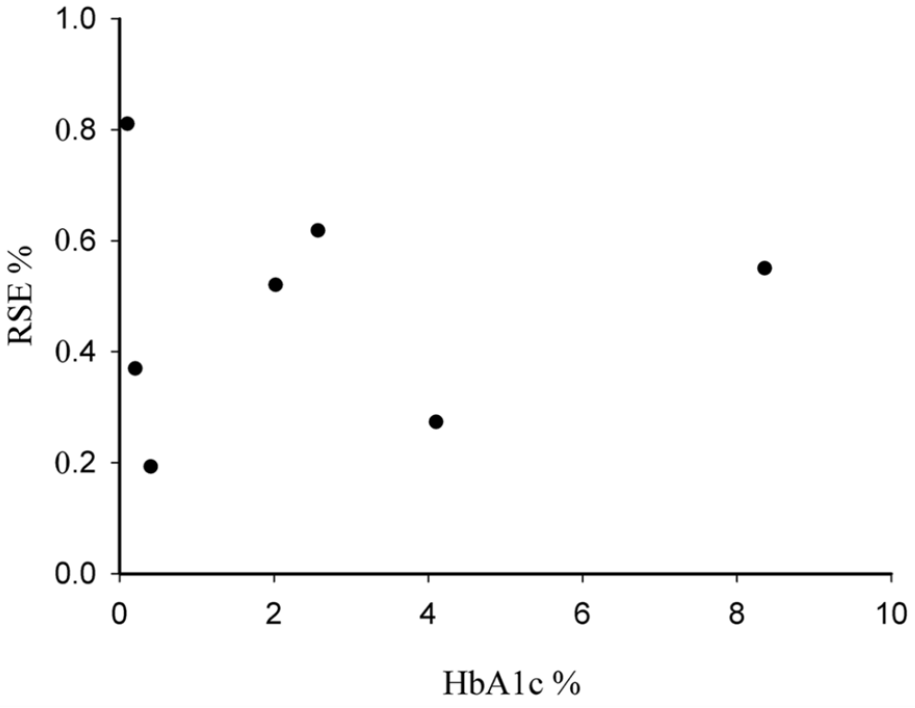

Figure S5. Variation of RSE% as a function of HbA1c concentration. The values have been determined during calibration shown in Figure 3(b) of the article. Each RSE% value is based on duplicate measurements using the 12 IDEs of a sensor chip (n = 24).

Figure S6. HbAo interference

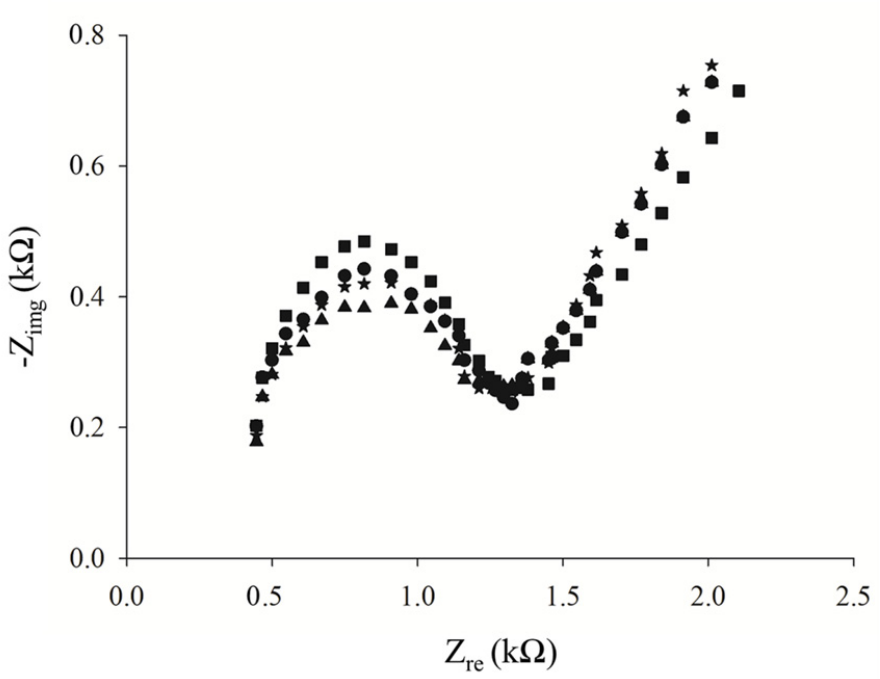

Figure S6. Characteristic Nyquist plots for an APBA-modified electrode before (▲) and after incubation with (■) 10, (★) 15, and (●) 20 g dL^{-1} HbAo.

Figure S7. Regression plot for method bias (ΔHbA1c % vs. HbA1c concentration)

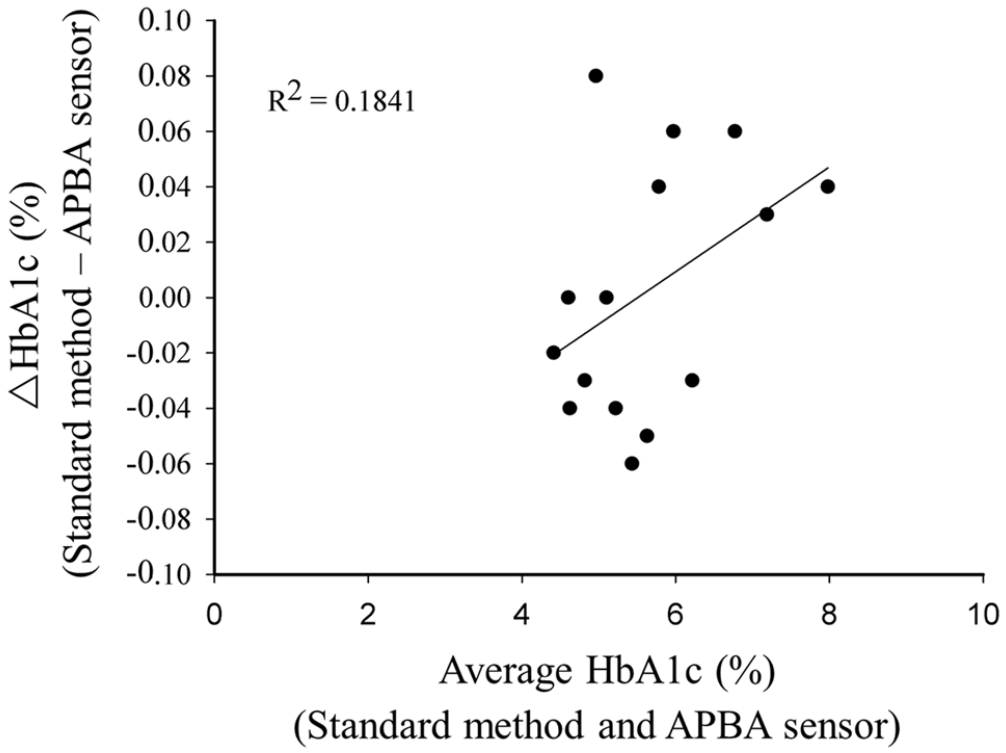

Figure S7. Regression line showing the trend of the calculated differences between the standard method and boronate sensor in determining HbA1c % of analyzed blood samples. The plotted values are the same as shown in the Bland-Altman bias plot of Figure 4(a) in the article.

Table S1. Analytical characteristics of electrochemical boronate-based HbA1c sensors

Approach (recognition element/ electrode/ redox indicator/ label)	Linear range[a]	LOD[a]	Reproducibility[b]	Stability[c] (%, Period)	Real samples	Ref.
Electrochemical Impedance Spectroscopy (EIS)						
T3BA[d]/ Au disk/ HCF[d]/ None	0.1-1 µg/mL	ND[e]	ND[e]	ND[e]	ND[e]	1
T3BA[d]/ Au thin film/ None/ None	10-100 µg/mL	ND[e]	ND[e]	ND[e]	ND[e]	2
T3BA[d]/ Au thin film/ None/ None	10-100 µg/mL	1 µg/mL	ND[e]	ND[e]	ND[e]	3
APBA[d]-modified ESM[d]/ Screen printed Pt/ HCF[d]/ None	2.3-14%	0.19%	RSD[b] 2.0% (n = 30)	70%, 4 days	Hemolysate	4
APBA[d]-modified ESM[d]/ Screen printed carbon/ HCF[d]/ None	2.3-14%	0.21%	RSD[d] 2.15% (n = 30)	92%, 1 week	Hemolysate	5
APBA[d] / Au thin film / HCF[d] / None	0.10-8.36%	0.024%	RSE[b] 1.1% (n = 30 assays, 12 measurements each)	96%, 28 days	Hemolysate	This work
Electrochemistry others (technique)						
FcBA[d]/ PG[d]-ZrO_2NP/ Fc[d]/ None (SWV[d])	6.8-14%	ND[e]	RSD[b] 12.7% (n = 3)	ND[e]	Hemolysate	6
FcBA[d]/ Au thin film/ FcM[d]/ GOx[d] (CV[d])	2.5-15%	ND[e]	RSD[b] 5% (n = 3)	ND[e]	ND[e]	7
FPBA[d]/ Au thin film/ FcM[d]/ GOx[d] (CV[d])	4.5-15%	ND[e]	ND[e]	ND[e]	Hemolysate	8
APBA[d]/ Screen printed carbon-AuNP/ H_2O_2/ None (CAm[d])	0.1-1.5%	0.052%	RSD[b] 5.1% (n = 5)	92%, 1 month	Hemolysate	9
PBA[d]/ GC[d]/ ARS[d]/ None (Pm[d])	ND[e]	ND[e]	ND[e]	ND[e]	Hemolysate	10
APBA[d]/ GC[d] disk-graphene oxide/ Immobilized PQQ[d]/ None (DPV[d])	9.4-65.8 µg/mL	1.25 µg/mL	RSD[b] 8.5% (n = 6)	95%, 1 month	Hemolysate	11

[a] Linear range and limit of detection (LOD) are usually given in either µg/mL or % HbA1c of the total Hb contents. [b] The published evaluations of sensor reproducibility (expressed as relative standard deviation, RSD, or relative standard error of mean, RSE) are based on repeated determination of the same concentration of HbA1c using one or multiple electrodes. [c] The stability % refers to the initial response obtained with the tested sensor. [d] Abbreviations: APBA (aminophenylboronic acid); ARS (alizarin red); CAm (chronoamperometry); CV (cyclic voltammetry); DPV (differential pulse voltammetry); ESM (eggshell membrane), Fc (ferrocene); FcBA (ferroceneboronic acid); FcM (ferrocenemethanol); FPBA (formylphenylboronic acid); GC (glassy carbon); GOx (glucose oxidase); HCF (hexacyanoferrate); PBA (phenylboronic acid); Pm (potentiometry); PG (pyrolytic graphite); PQQ (pyrroloquinoline quinone); SWV (square wave voltammetry); T3BA (thiophene-3-boronic acid). [e] ND – not determined.

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Table S2. Characteristic values of equivalent circuit parameters: charge transfer resistance (Rct) and constant phase element impedance (Z_{CPE} expressed as Q and α) after different electrode modifications and incubation with various concentrations of HbA1c as well as the calculated effective capacitance (C_{eff}) based on Z_{CPE}.

Treatment	Rct (kΩ)[c]	Q (nS s^{α})[c]	α[c]	C_{eff} (nF)[d]
Chemical cleaning[a]	26.12	43.17	0.903	20.65
Electrochemical cleaning[a]	16.01	18.65	0.899	7.70
Cysteamine modification[a]	0.61	18.21	0.705	5.34
Glutaraldehyde activation[a]	0.81	14.57	0.690	3.33
APBA-modification[a]	1.51	38.35	0.817	4.49
0.10% HbA1c[b]	2.68	13.21	0.934	6.48
0.20% HbA1c[b]	7.19	19.77	0.935	10.40
0.40% HbA1c[b]	13.49	13.70	0.937	7.70
2.02% HbA1c[b]	26.89	12.05	0.942	7.30
2.57% HbA1c[b]	30.36	12.33	0.940	7.40
4.10% HbA1c[b]	48.46	19.52	0.945	13.07
8.36% HbA1c[b]	103.20	19.77	0.944	13.62

[a] The values of the parameters correspond to the Nyquist plots shown in Figure 2a of the article.
[b] The values of the parameters correspond to the Nyquist plots shown in Figure 3a of the article.
[c] During the nonlinear regression analysis of the impedance spectra, the value of solution resistance (Rs) has been 210 Ω.
[d] The calculation of C_{eff} was done according to the approach presented by Hsu and Mansfeld (Corrosion 2001, 57, 747-748).

Table S3. Evaluation of regeneration efficiency (RE) on an APBA-modified electrode

Treatment	Rct[a] (kΩ)	RE[b] (%)
APBA-modification	1.51 ± 0.12	
Incubation with 0.10% HbA1c[c]	2.67 ± 0.26	
Regeneration[d]	1.53 ± 0.15	99.25
Incubation with 0.20% HbA1c[c]	7.18 ± 0.53	
Regeneration[d]	1.58 ± 0.13	99.03
Incubation with 0.10% HbA1c[c]	2.60 ± 0.30	

[a] The Rct values represent mean ± standard deviation of duplicate measurements.
[b] The regeneration efficiency was determined as described in section "Data handling and analysis".
[c] The chip was incubated with HbA1c for 15 min.
[d] The chip was regenerated for 30 min using 10 mM sodium acetate buffer (pH 5).