Electronic Supplementary Material (ESI) for Analyst.

Design of a label-free aptasensor for electrochemical determination of hemoglobin; Investigation of the peroxidase-like activity of hemoglobin for the sensing of different substrates

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Supplementary Material

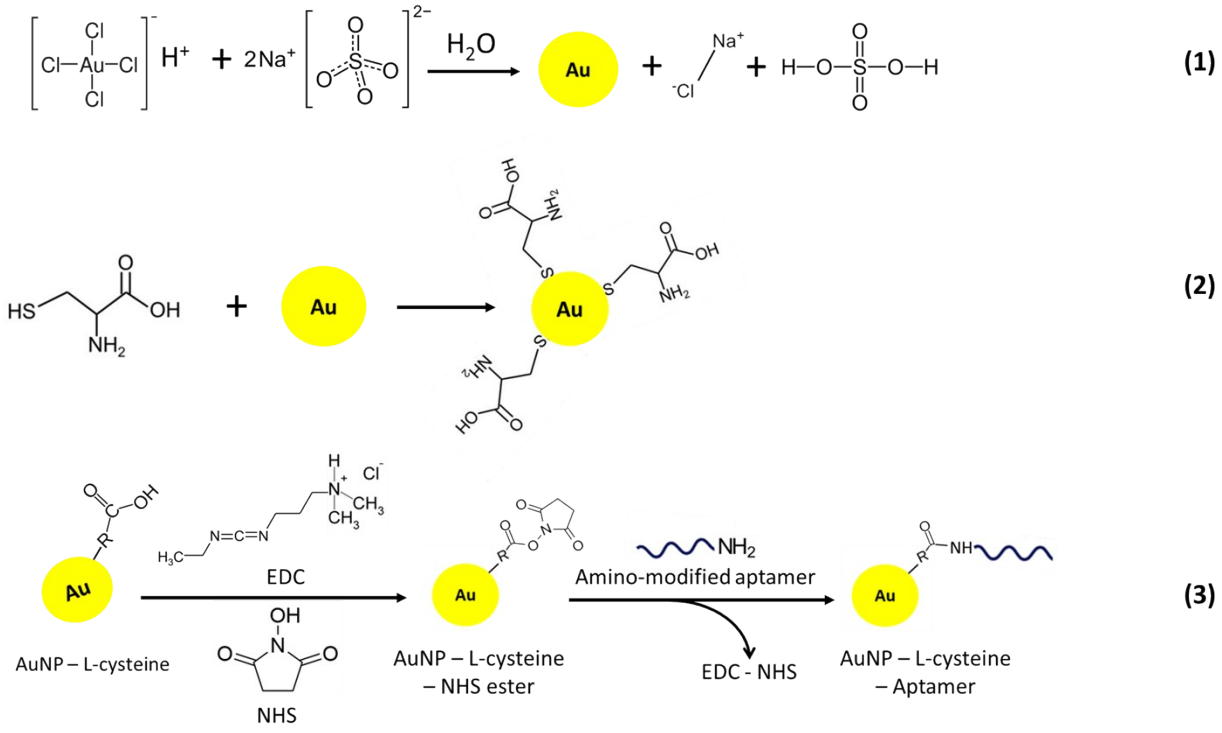

Fig. S1 Schematic representation of (1) the formation of gold nanoparticles by the interaction of Hydrogen tetrachloroaurate ($HAuCl_4$) and Sodium sulfate (Na_2SO_4); (2) the formation of cysteine modified gold nanoparticles via Au-thiol group (SH) interaction; (3) the immobilization of Aptamer on L-cysteine modified gold nanoparticles via EDC/NHS interaction.

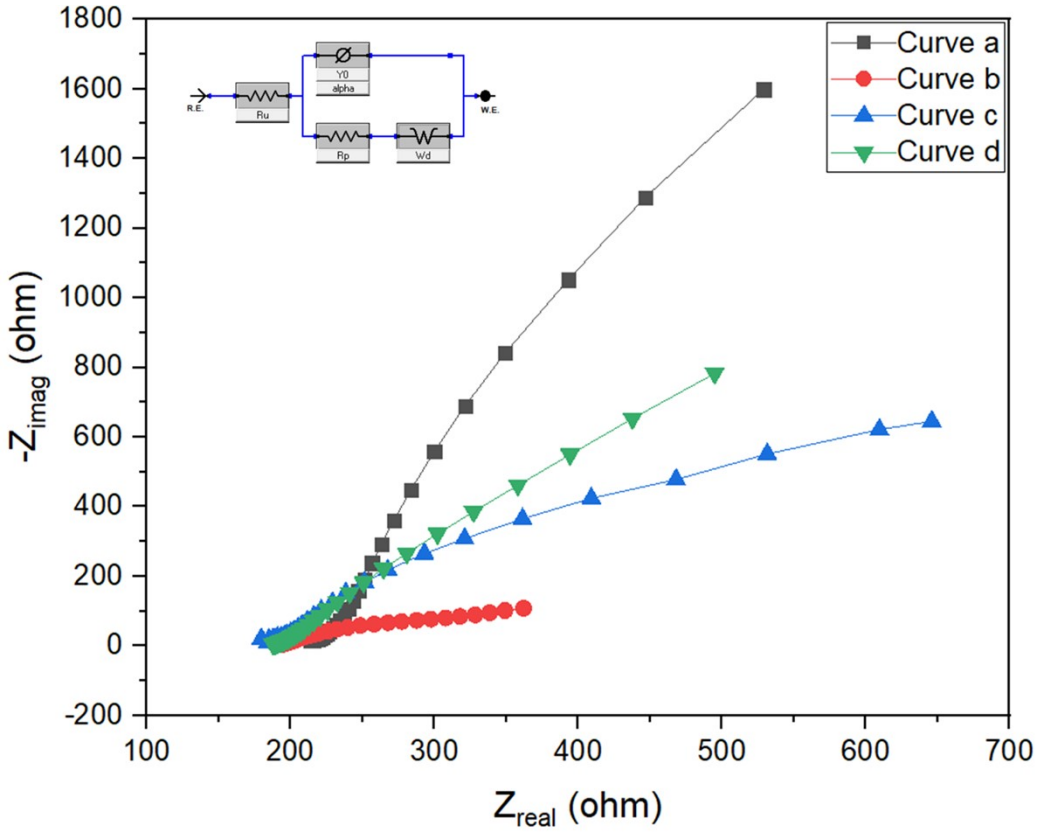

Fig. S2 Nyquist plots of 5 mM $[Fe(CN)_6]^{4-/3-}$ redox couple in PBS, pH 7.4, for bare SPCE (curve a), AuNPs /SPCE(curve b), L-cysteine/ AuNPs /SPCE (Curve c), and Aptamer/ L-cysteine/ AuNPs/ SPCE (curve d) using a frequency range of 100000 Hz - 0.1 Hz, 10 point/decade and an AC voltage of 10 mv ms. Inset: Equivalent circuit (CPE with diffusion) Ru(Ø[RpWd]) used to fit the frequency scans along with the impedance spectra.

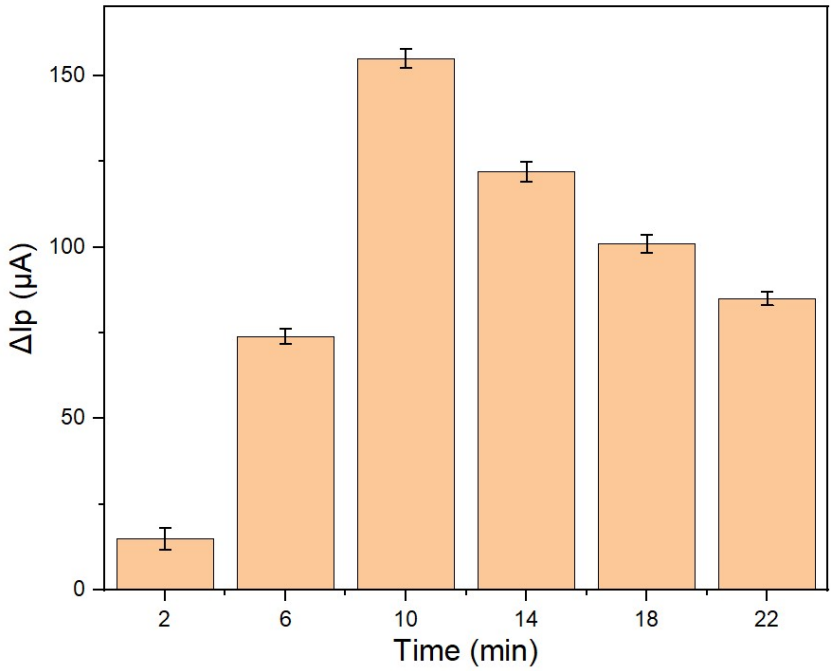

Fig. S3 Optimization of Hb-aptamer incubation time (2, 6, 10, 14, 18, 22 min). Plot of peak current difference vs. Hb incubation time obtaining from SWV in the potential range of -0.2 to +0.4 with a frequency of 7 Hz and an amplitude of 0.025V using 0.1 M PBS (pH 7.4) containing 5.0 mM $[Fe(CN)_6]^{3-/4-}$.

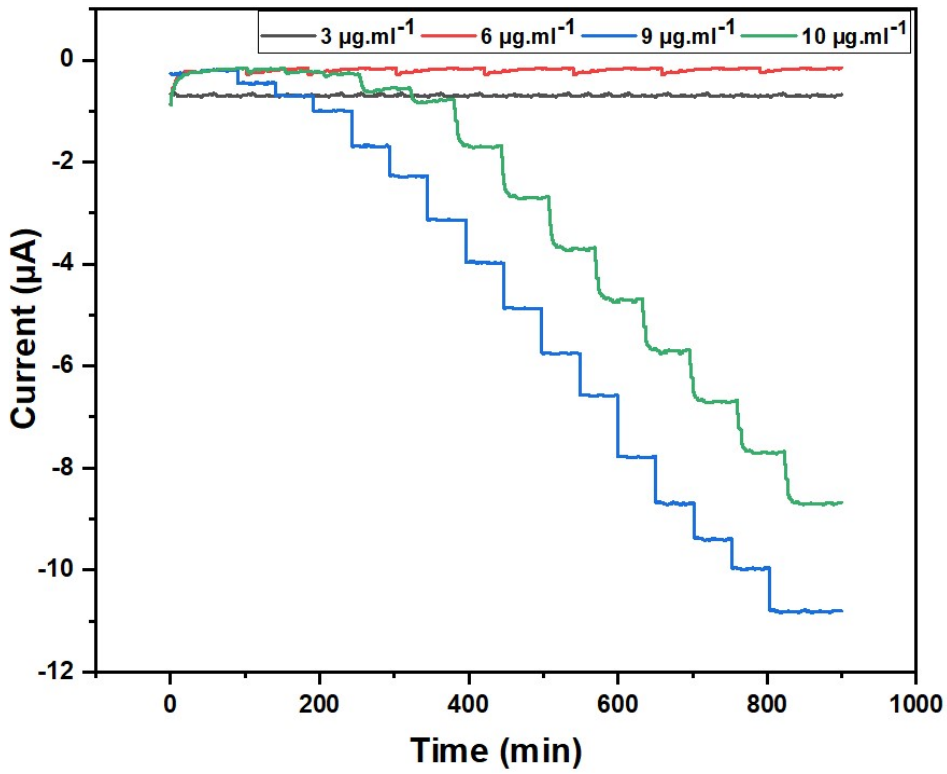

Fig. S4 Effect of Hb concentrations (3, 6, 9, 10 µg.ml^{-1}) on the H_2O_2 electrocatalytic activity using amperometry measurements toward successive additions of H_2O_2 in 0.1M PBS (pH 7) with an applied potential of -0.4V. Error bars correspond to the relative standard deviation of three independent experiments

Table S1. Comparison between this method and other reported electrochemical techniques for detection of H_2O_2.

Method	Target	Principle	Linear range (µM)	LOD (µM)	Ref
Amperometry	H_2O_2	Hb-PCL/GCE	2 – 30	6.07	[1]
Amperometry		Hb-AgNPs-PAMAM/GCE	6 – 91	4.9	[2]
CV		Hb/IL/CILE	100 – 500	40	[3]

Linear square wave	————	NF-Hb-Cys- AuNPs/SPCE.	10 – 450	4.4	[4]
Amperometry		Hb/Ag NPs-BDDE	0.5 – 20	2.5	[5]
Amperometry		Hb-SWCNTs-CTAB/GCEs.	23.6 – 134	7.87	[6]
CV + Amperometry		Hb/CMC–TiO2-NTs/GC	4 – 64	4.6	[7]
Amperometry		GE/Fe_3O_4/Hb/GCE	0.25 – 1700	06	[8]
Amperometry		Hb/Aptamer/L-cysteine/AuNPs/SPCE	0.2 – 2100	0.044	This work
Amperometry	Nitrite	Hb/AuNPsPTH/PtNPs/GCE	70×10^{-3} – 1200	0.02	[9]
		Hb/HS-CdS/GCE	0.3 – 182	0.08	[10]
		Hb/Au/GACS/GCE	0.05 -1000	0.01	[11]
		Nafion/Hb/MX-Ti_3C_2/GCE	0.5 - 11800	0.12μM	[12]
CV		AuNPs@MoS_2/GCE	10 – 1100	5	[13]
Amperometry		Hb/Apt/L-cysteine/AuNPs/SPCE	3.6×10^{-3} – 1300	0.00055	This work

Abbreviations: **SPCE**: screen printed carbon electrode; **GCE**: glassy carbon electrode; **PCL**: poly(ε-caprolactone); **AgNP**s: silver nanoparticles; **PAMAM**: poly(amidoamine) dendrimer; IL: ionic liquid; **CILE**: carbon ionic liquid electrode; **NF**: nafion; **Cys**: cysteine; **BDDE**: Boron doped diamond electrodes; **SWCNTs**: single walled carbon nanotubes; **CTAB**: cetyltrimethylammonium bromide; **CMC**: Carboxymethyl cellulose; **TiO_2-NTs**: Titanium oxide nanotubes; **GE**: Graphene. **GCE**: Glassy carbon electrode; **PTH**: Polythionine; **PtNPs**: Platinium nanoparticles, **AuNPs**: Gold nanoparticles, **HS-CdS**: CdS hollow nanospheres; **GACS:** Graphene with biocompatible chitosan; **MX-Ti_3C_2**: MXene- Ti_3C_2; **MoS_2**: Molybdenum disulfide; Hb: Hemoglobin; **APT**: Aptamer; **CV**: Cyclic voltammetry

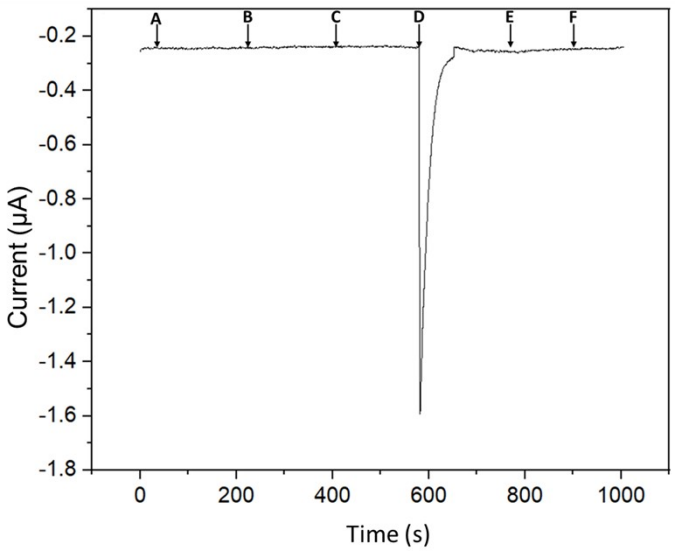

Fig. S5 Amperometric response at constant potential of -0.4 V for Hb/Aptamer/L-cysteine/AuNPs/SPCE in phosphate buffer with successive addition of 35 μM H_2O_2 (D), 100μM ascorbic acid (A), 500μM dopamine (B), 100μM Glucose (C), 100μM Fructose (E), and 100μM Arginine (F)

Table S2. Determination and recovery results corresponding to H_2O_2 and nitrite determination in rain water and river water samples, respectively (n = 3).

Sample	Added (μM)	Founded (μM)	RSD %	Recovery %
	4.5	4.3	0.9	95.55
Rain water	100	103	2.01	103
	800	793	1.56	99.12
	1500	1496	1.3	99.73

River water	5	5.18	1.5	103.6
	80	82.63	3.01	103.28
	500	497.23	1.26	99.44
	1000	1000.96	0.98	100.09

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