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Electronic Supplementary Information

Boosting electrochemical oxygen reduction activity of hemoglobin onto fructose@graphene-oxide nanoplatforms

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Abstract

A metal-free oxygen reduction reaction (ORR) electrocatalyst with oustanding performance was obtained through an easy and one-pot synthesis of hemoglobin functionalized fructuose@graphene-oxide (GO) nanocomposites. The active pyridinic nitrogen sites of the highly unfolded proteins together with the excellent electronic properties of GO appears to be the main factors of the improved electrocatalytic activity.

Experimental Part

Synthesis of GO@Fruc-Hb nanocomposites

GO-Fruc@Hb nanocomposites were synthesized by a novel methodology at room temperature and 80 ^{o}C. For this, 5 wt% graphene oxide solution was prepared using acetonitrile as a solvent. Then, the reaction was initiated by adding 10 mmol of fructose to the mixture. After 45 minutes of mixing, a Hb aqueous solution ($5 \cdot 10^{-6}$ M) was added drop by drop to the reaction and it was left under continuous stirring at room temperature during 24 h. The resulting material was then centrifuged and the supernatant was discarded. The pellet containing the synthesized biomaterial was resuspended into a fresh acetonitrile solution by a moderate shaking. This purification protocol was repeated three times to assure the complete removal of the physically adsorbed Hb. Finally, the resulting nanobiomaterials were gently dried and stored until they were needed. It was named GO-Fruc@Hb-RT. To synthesize GO-Fruc@Hb-HT it was follow the same protocol described above. However after adding the Hb solution

it was transferred to a carrousel place station when remained under continuous stirring at 80°C during 24 h. The final material was subjected to the same purification protocol.

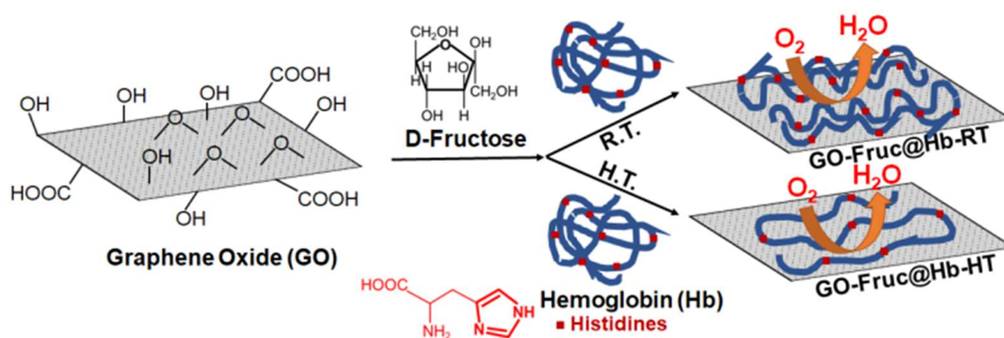
Material characterization

Samples for scanning electron microscopy (SEM) were prepared under ambient conditions. SEM images were obtained in a JEOL JSM 7800F SEM microscope. UV-visible spectra were recorded on a Cary 100 Bio UV-Vis spectrometer in disposable polystyrene cuvettes with 1.0 cm path length. XPS studies were performed on a Physical Electronics PHI 5700 spectrometer (non-monochromatic Mg-Kα radiation, 300 W, 15 kV and 1253.6 eV). Spectra were recorded in the constant pass energy mode at 29.35 eV, using a 720 µm diameter analysis area. Charge referencing was carried out using the adventitious carbon peak (C 1s at 284.8 eV). The energy scale was calibrated using Cu 2p3/2, Ag 3d5/2, and Au 4f7/2 lines at 932.7, 368.2 and 84.0 eV, respectively. A PHI ACCESS ESCAV6.0 F software package was used for acquisition and data analysis.Fourier-transform infrared (FTIR) spectroscopy was performed in an ALPHA-T Bruker spectrometer. Spectra were recorded at room temperature in a 4000-600 cm^{-1} wavenumber range, using the OPUS software.

Electrochemical experiments

Voltammetric measurements were recorded on an AUTOLAB PGSTAT30 electrochemical analyser using a three-electrode system. A glassy carbon (GC) disc (5 mm in diameter; Pine Instruments Company) were used as working electrodes. A drop of 25 µL of sample was loaded onto the clean surface of GC electrode and then dried gently under room temperature. A platinum sheet and an Ag/AgCl electrode were used as counter and reference electrodes, respectively. Depending on the experiment, the electrolyte solution was purged prior to electrochemical measurements for 30 minutes using nitrogen (N_2) or oxygen (O_2) gas. An aqueous solution of 0.1 M PBS (pH 7.2) was used as supporting electrolyte. Mostly, the use of biological molecules requires very controllable experimental conditions because they are very sensitive to the environment. For this, extremely high or low pHs generally result in complete loss of activity for most redox proteins or enzymes, even producing irreversible agglomeration/aggregation. In addition, the optimal activity level of the protein, the glycosylated interaction between the Hb and the GO-fruc (i.e. sugars linked by Maillard reaction to proteins by a covalent bond between free amino groups of amino acids, mostly lysine and arginine) as well as the integrity of the histidine residues could be affected by extreme pH values.

Results and discussion



Scheme 1. One-pot synthesis of GO-Fruc@Hb nanocomposites.

Table S1. Surface chemical composition determined by XPS.			
Sample	**C 1s**	**O 1s**	**N 1s**
GO	96.51	2,90	n.d.
GO-Fruc@Hb-RT	87.94	9.14	0.56
GO-Fruc@Hb-HT	89.73	7.16	1.60

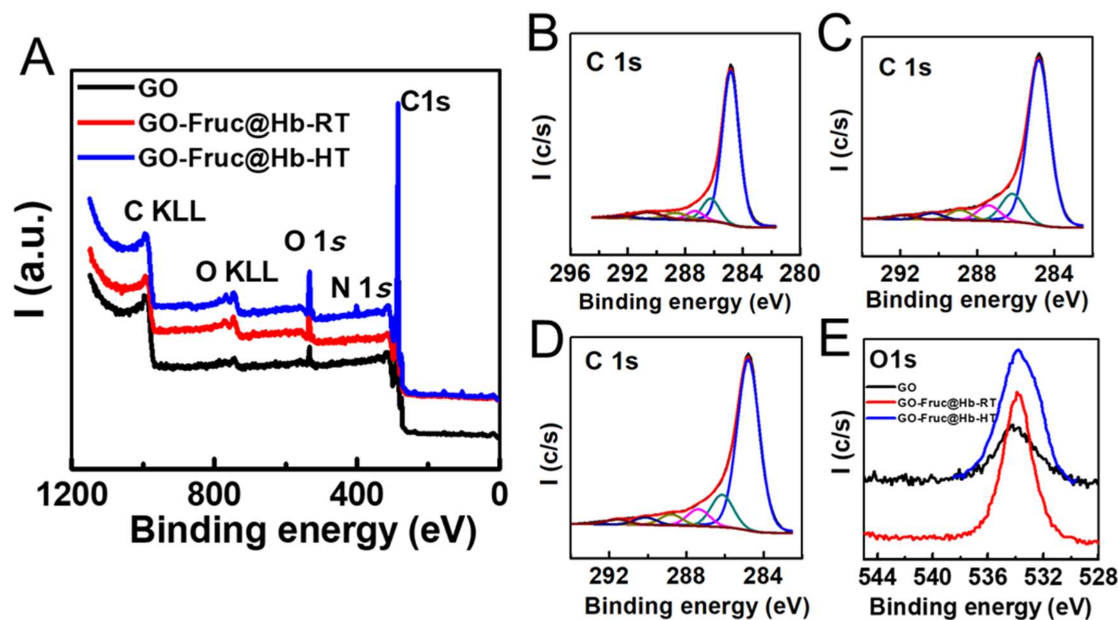


Fig. S1. (A) Survey and (E) Deconvoluted high-resolution O1s XPS spectra of GO, GO-Fruc@Hb-RT and GO-Fruc@Hb-HT, respectively. (B-D) Deconvoluted high-resolution C 1s XPS spectra of GO (B), GO-Fruc@Hb-RT (C) and GO-Fruc@Hb-HT (D), respectively.

Table S2. Percentages of each type of secondary structure determined by FT-IR deconvolution for both GO-Fruc@Hb nanocomposites

Secondary structure	Native Hemoglobin	GO-Fruc@Hb-HT	GO-Fruc@Hb-HT
β-sheet/random	39	65	75
α-helix	43	27	20
β-turn	18	8	5

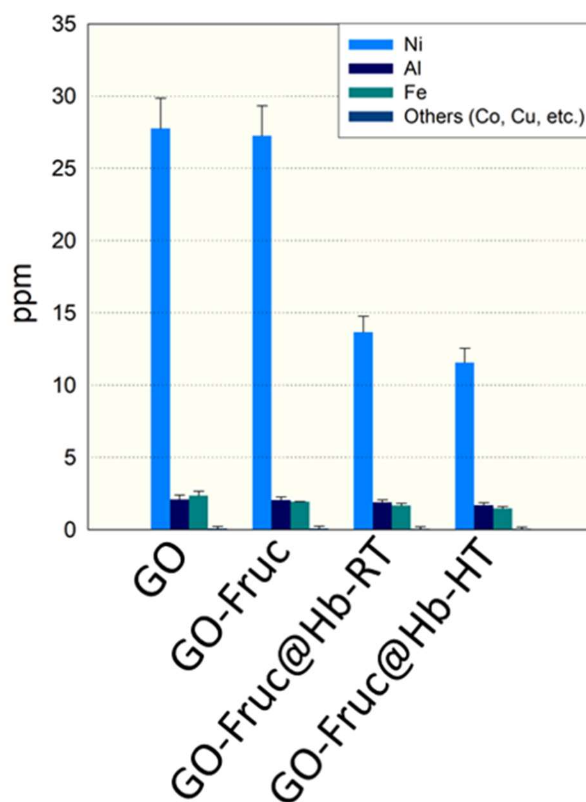


Fig. S2. Histograms obtained from ICP-MS analysis, after microwave-assisted acid digestion, for the different samples containing GO.

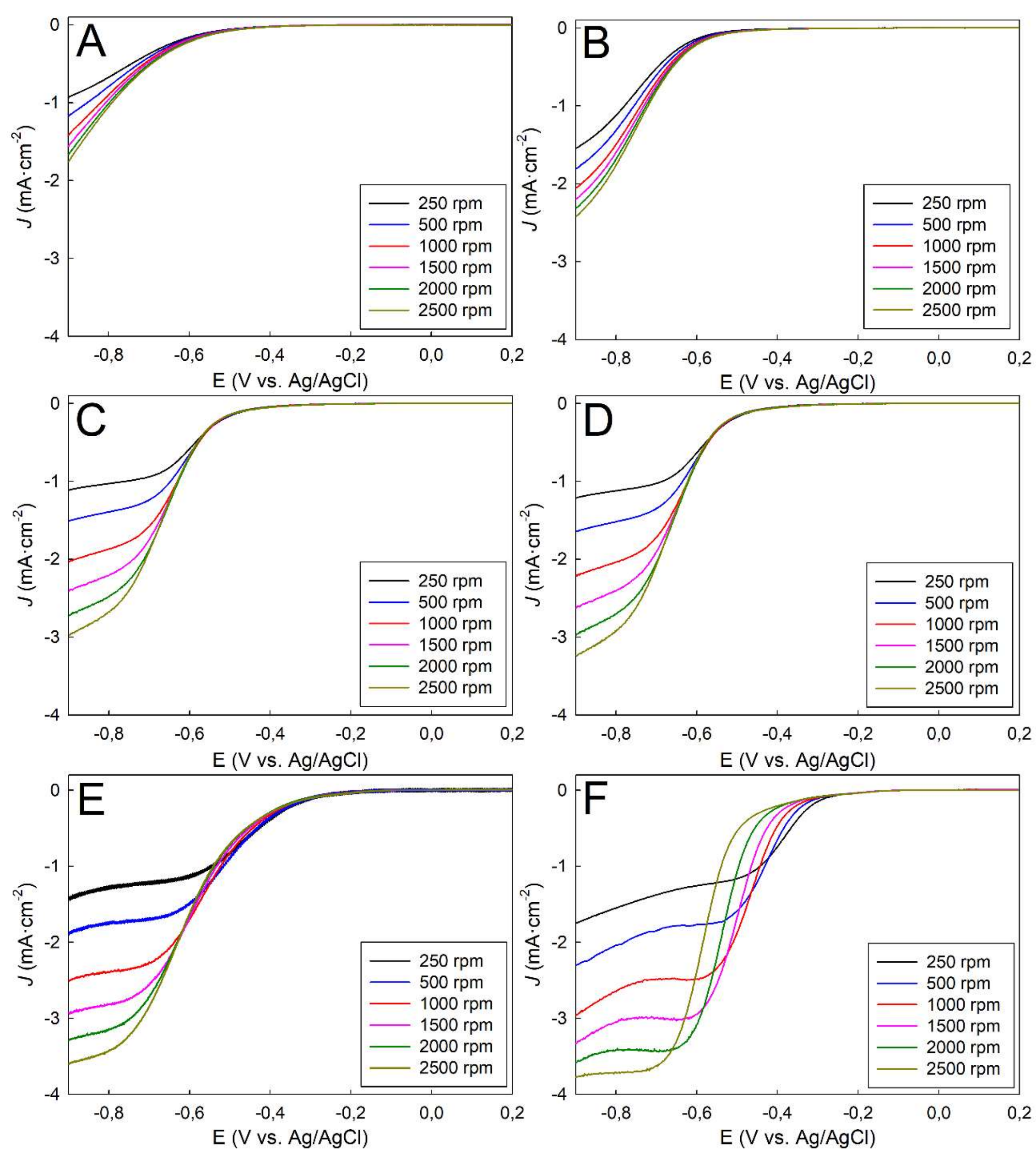


Fig. S3. Rotating-disk voltammograms of GC electrodes modified with all samples: Bare GC (A), Hb (B), GO (C), GO-Fruc (D), GO-Fruc@Hb-RT (E) and GO-Fruc@Hb-HT (F) in O_2-saturated 0.1 M PBS (pH 7.2) at different rotation rates. Scan rate: 10 mV·s^{-1}.

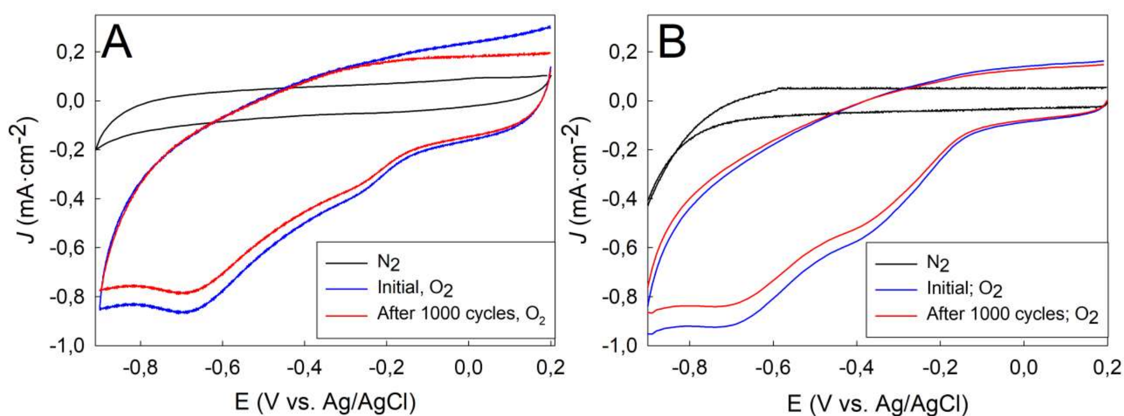


Fig. S4. CVs of GO-Fruc@Hb-RT (A) and GO-Fruc@Hb-RT (B) catalysts in N_2- and O_2-saturated 0.1 M PBS buffer (pH 7.2) before and after 1000 cycling stability tests. Scan rate of 0.1 V·s^{-1}.

Table S3. Onset potential values (E_{on}) and average number of electrons transferred (n_e) for O_2 molecules obtained at -0.75 V from Fig. 2C and S3, respectively.

Catalysts	E_{on} (V)	n_e at -0.75V
GC	-0.324	2.1
Hemoglobin	-0.321	2.9
GO	-0.237	2.2
GO-Fructose	-0.237	2.4
GO-Fruc@Hb-RT	-0.139	2.6
GO-Fruc@Hb-HT	-0.139	3.2

Table S4. Comparison of the ORR performances of GO-Fruc@Hb-HT nanobiomaterials with other related electrocatalysts reported in the literature.

Catalysts	E_{on} (V)	n_e	j (mA cm^{-2}) at -0.6V*	Ref.
PyPOP-Hb@G	-0.160	4	-0.90	1
BP350C1000	-0.008	3.5	-1.40	2
MW@N6	-0.169	3.5	-2.90	3
N-GQDs/G-12	-0.090	3.6-4.1	-3.25	4
G–Co/CoO	-0.176	4	-5.20	5
GO-Fruc@Hb-HT	-0.139	3.2	-2.50	This work

* Values have been taken at 2500 rpm and 0.01 V s^{-1}.

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