Electronic Supplementary Material (ESI) for Nanoscale.

Supplementary information

A long-term stable paper-based glucose sensor using a glucose oxidase-loaded, Mn_2BPMP-conjugated nanocarrier with a smartphone readout

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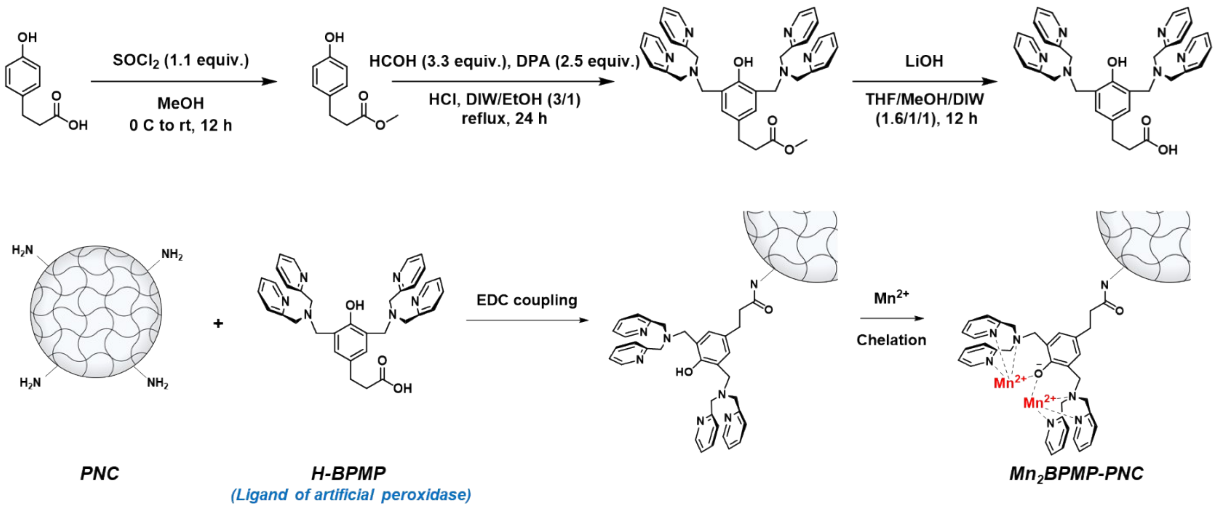
Synthesis of Mn_2BPMP-PNC



Scheme S1. Synthesis scheme for Mn_2BPMP-PNC.

Synthesis of methyl 3-(4-hydroxyphenyl) propanoate[1]

3-(4-hydroxyphenyl)propanoic acid (6.0 mmol, 1.0 g) was dissolved in 40 mL of methanol (MeOH) in an ice bath. Thionyl chloride (6.6 mmol, 0.5 mL) was slowly added dropwise to the solution and stirred at room temperature for 12 h. The solvent and residual thionyl chloride were subsequently evaporated under reduced pressure. The mixture was purified via column chromatography (ethyl acetate:hexane = 1:1) to yield a pale-yellow oil (1.1 g, yield 99.5%). ^{1}H-NMR (400 MHz, chloroform-D) δ 7.04 (d, J = 8.5 Hz, 2H), 6.75 (d, J = 8.5 Hz, 2H), 5.72 (s, 1H), 3.67 (s, 3H), 2.88 (t, J = 7.8Hz, 2H), 2.61 (t, J = 7.8 Hz, 2H).

Synthesis of methyl 3-(3,5-bis((bis(pyridin-2-ylmethyl)amino)methyl)-4-hydroxyphenyl) propanoate (H-BPMP-COOMe)[1]

Paraformaldehyde (3.3 mmol, 0.1 g) and di(2-picolyl)amine (2.5 mmol, 0.5 mL) were suspended in 12 mL of 75% aqueous ethanol. Methyl 3-(4-hydroxyphenyl)propanoate (1.0 mmol, 0.2 g) and 0.3 mL HCl (1.0 M) were added to the above solution and refluxed under stirring for 1 day. The solution was cooled to room temperature, neutralized with a saturated sodium carbonate solution, and extracted with excess chloroform. The organic layer was then collected, dried over sodium sulfate, and concentrated under reduced pressure. The mixture was purified via column chromatography ($CHCl_3$:MeOH =20:1) to yield a pale-yellow solid (0.5 g, yield 86.2%). ^{1}H-NMR (400 MHz, chloroform-D) δ 10.91 (s, 1H), 8.54-8.46 (m, 5H), 7.58 (td, J = 7.6, 1.5 Hz, 4H), 7.47 (d, J = 7.6 Hz, 4H), 7.14-7.07 (m, 4H), 7.01 (s, 2H), 3.84 (s, 8H), 3.80-3.73 (4H), 3.63-3.58 (3H), 2.82 (t, J = 7.9 Hz, 2H), 2.54 (t, J = 7.9 Hz, 2H).

Synthesis of 3-(3,5-bis((bis(pyridin-2-ylmethyl)amino)methyl)-4-hydroxyphenyl) propanoic acid (H-BPMP-COOH)[1]

H-BPMP-COOMe (1.0 mmol, 0.6 g) was dissolved in 130 mL of tetrahydrofuran/MeOH (1.6:1). Afterwards, 50 mL of LiOH solution (2.0 M) was added and the mixture was stirred for 12 h. Excess chloroform was then added to the solution and the pH was adjusted to 5–6 using a HCl solution (aq. 2 N). The organic layer was collected, washed with brine, dried over sodium

sulfate, and concentrated under reduced pressure to give H-BPMP-COOH (0.6 g, yield 97.0%). δ 8.52 (d, J = 4.3 Hz, 17H), 7.60 (td, J = 7.6, 1.2 Hz, 16H), 7.47 (d, J = 7.9 Hz, 16H), 7.14 (dd, J = 7.3, 5.2 Hz, 16H), 7.09 (s, 8H), 3.97 (s, 30H), 3.88 (s, 16H), 2.88 (t, J = 7.2 Hz, 8H), 2.65-2.55 (8H).

Synthesis of PNC[2]

Pluronic F127 (PF127) was diacrylated using acryloyl chloride and TEA in toluene, resulting in >95% degree of substitution. Methacrylated chitosan was obtained by reacting chitosan with glycidyl methacrylate. The degree of methacrylation was ~15% of the amine group of chitosan. Subsequently, an aqueous solution containing 0.77 wt% diacrylated PF127, 0.14 wt% of methacrylated chitosan, and Irgacure 2959 (0.05 wt%) as a photo-initiator was irradiated with 365 nm UV light at 1.3 mW/cm^2 using a UV lamp (VL-4.LC, 8 W, Viber Lourmat, France) for 15 min to induce photo-crosslinking. The crosslinked PNC was dialyzed against deionized water (DIW) using a 50 kDa dialysis membrane bag for 2 days for purification.

Synthesis of Mn_2BPMP-PNC

H-BPMP-COOH (44.6 μmol, 26.3 mg), N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDCI; 22.3 μmol, 42.8 g), and N-hydroxysuccinimide (NHS; 22.3 μmol, 25.7 g) were dissolved in 20 mL of acetonitrile (ACN), and the solution was stirred for 2 h. After PNC (0.1 g) in 50 mL of DIW was added dropwise to the above solution, the mixture was stirred for 24 h. The solution was subsequently dialyzed in a 20% ACN aqueous solution for two days and an aqueous solution for 1 day. The H-BPMP-PNC (0.1 g) was obtained by lyophilization. Afterwards, Mn_2BPMP-PNC was prepared by chelating 2 equivalents of $Mn(ClO_4)_2$ with respect to BPMP in H-BPMP-PNC for 15 min.

Glucose assay in solution

Optimization of the GOx loading ratio to Mn_2BPMP-PNC

GOx at various concentrations (from 0 to 10 wt%) was loaded into Mn_2BPMP-PNC (2.7 mg/mL). Glucose (1 mM) was added to the buffer solutions (phosphate, 20 mM, pH 7.0) containing GOx-loaded Mn_2BPMP-PNC (2 μM) and ABTS (2 mM). The absorbance of the solutions at 417 nm was recorded at 30 s intervals for 10 min.

Glucose titration using Mn_2BPMP-PNC in solution

Glucose at various concentrations (0 to 1.5 mM) was added to a buffer solution (phosphate, 20 mM, pH 7.0) containing GOx (10 wt%)-loaded Mn_2BPMP-PNC (2 μM) and ABTS (2 mM). The absorbance of the solutions at 417 nm was monitored at 30 s intervals for 10 min. Similarly, glucose at various concentrations (0 to 1.5 mM) was added to the buffer solution (phosphate, 20 mM, pH 7.0, ACN 1%) containing Mn_2BPMP (2 μM), GOx (10 wt%) and ABTS (2 mM). The absorbance of the solutions at 417 nm was monitored at 30 s intervals for 10 min.

Analysis of substrate selectivity for Mn_2BPMP-PNC in solution

Various saccharides (galactose, fructose, maltose, lactose, sucrose; 10 mM, and glucose; 1 mM) were added to a buffer solution (phosphate, 20 mM, pH 7.0) containing GOx (10 wt%)-loaded Mn_2BPMP-PNC (2 μM) and ABTS (2 mM). Absorbance of the solutions at 417 nm was monitored at 30 s intervals for 10 min.

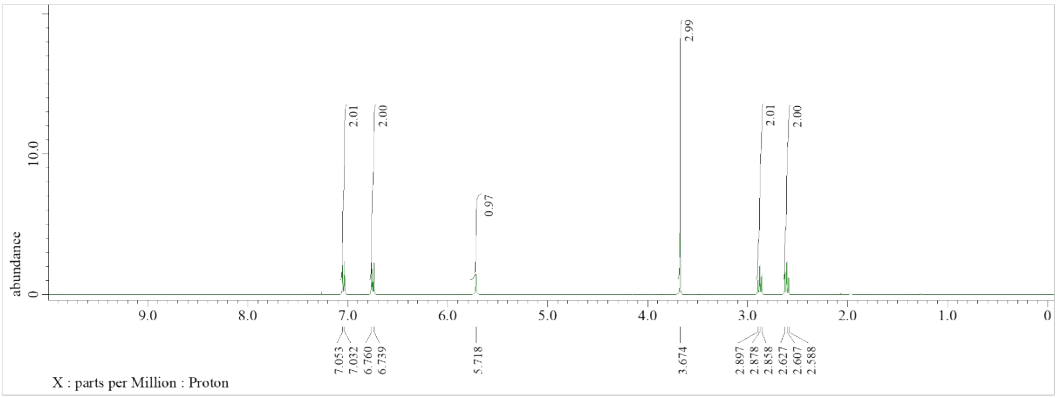


Fig. S1 ^{1}H NMR of methyl 3-(4-hydroxyphenyl) propanoate.

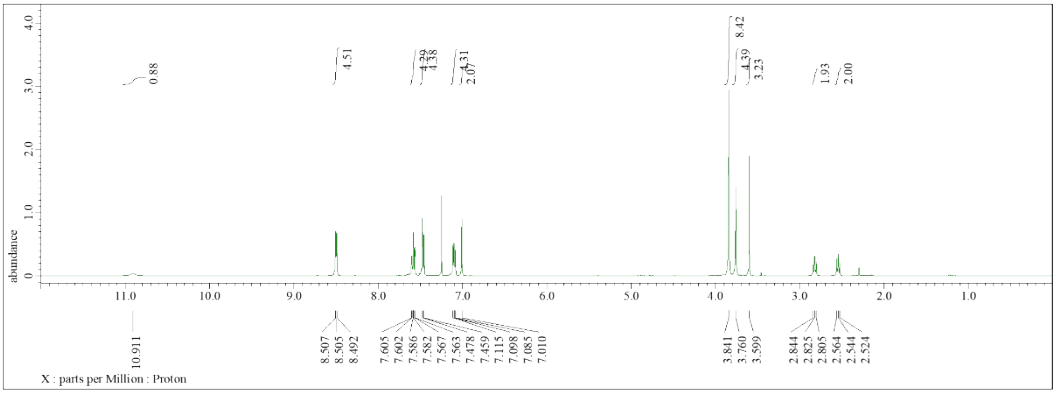


Fig. S2 ^{1}H NMR of H-BPMP-COOMe.

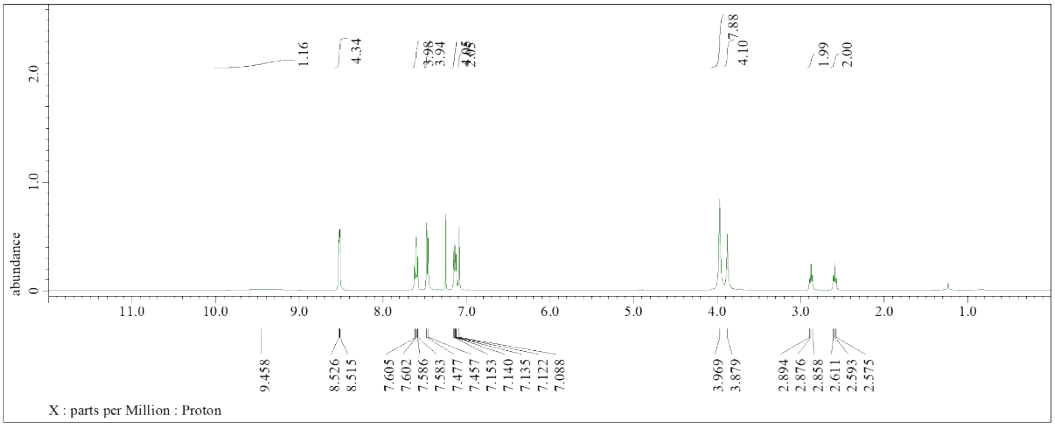


Fig. S3 ^{1}H NMR of H-BPMP-COOH.

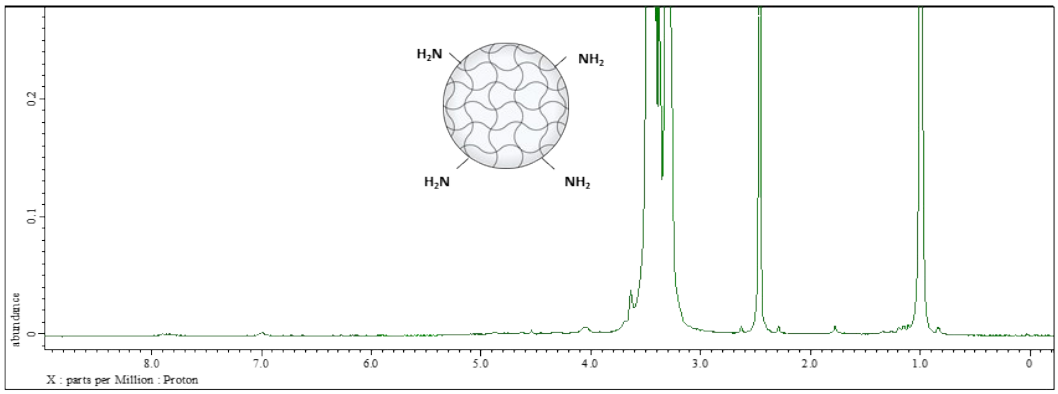


Fig. S4 ^{1}H NMR of PNC.

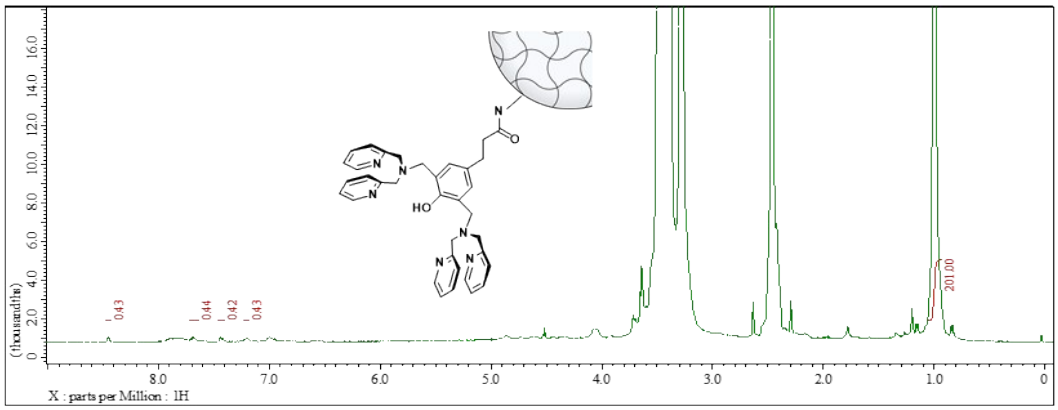


Fig. S5 ^{1}H NMR of H-BPMP-PNC.

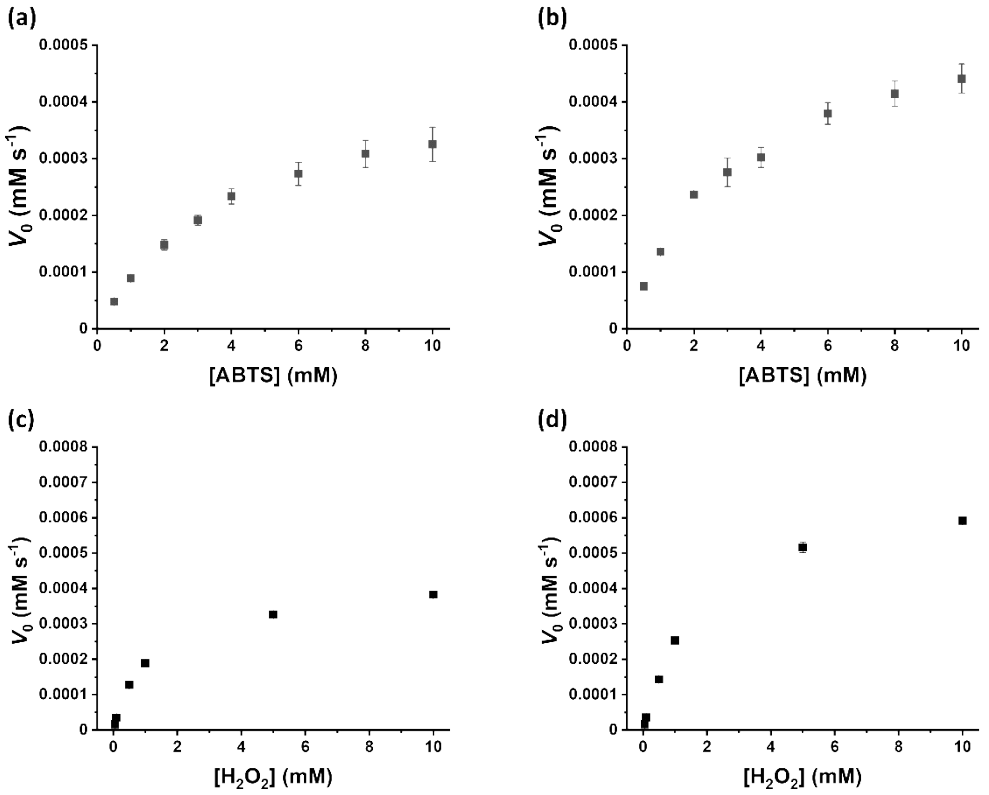


Fig. S6 Michaelis-Menten plots of the peroxidase-like activity of Mn_2BPMP (a, c) and Mn_2BPMP-PNC (b, d) *versus* the various concentrations of ABTS (a, b) or H_2O_2 (c, d) with a fixed concentration of the other substrate.

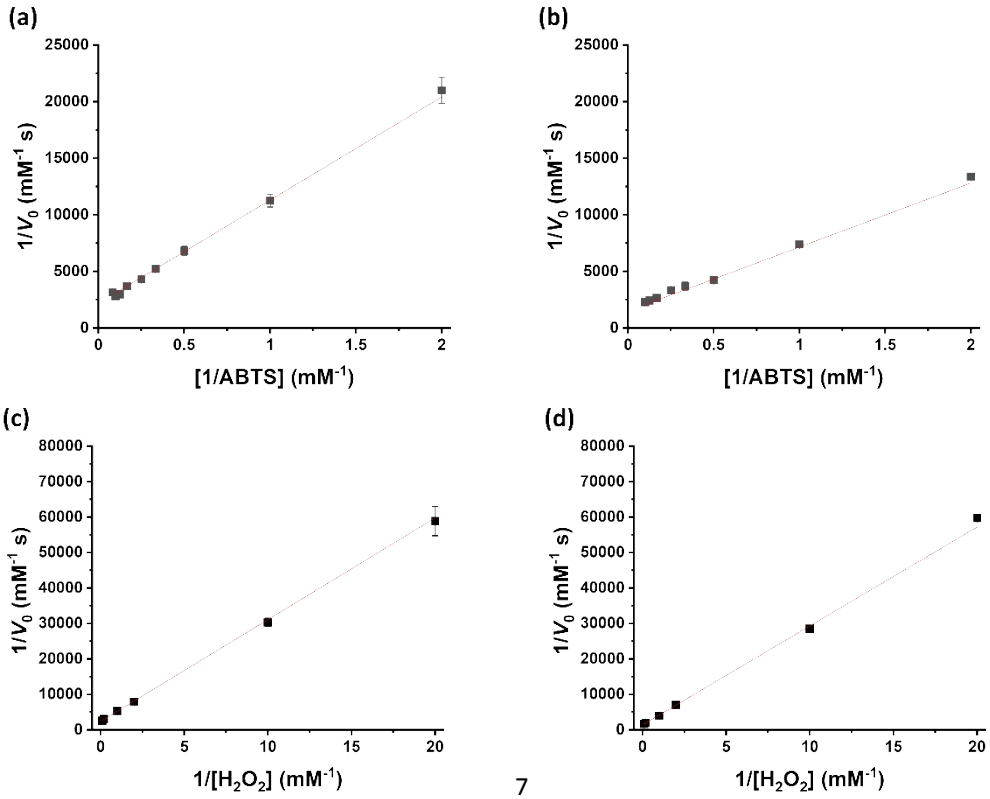


Fig. S7 Double reciprocal plots of the peroxidase-like activity of Mn_2BPMP (a, c) and Mn_2BPMP-PNC (b, d) *versus* concentration of ABTS (a, b) or H_2O_2 (c, d) with a fixed concentration of the other substrate.

Table S1 Comparison of the kinetic parameters obtained for HRP and other artificial peroxidases including Michaelis-Menten constants (K_m), maximum reaction rates (V_{max}), and catalytic constants (k_{cat})

	Substrate	K_m (mM)	V_{max} (10^{-3} mM s^{-1})	k_{cat} (s^{-1})	Optimal pH	Reference
Horseradish peroxidase (HRP)	ABTS	0.472	55.0×10^{-2}	4.837×10^{3}	4.5	3
	TMB	0.434	10.0×10^{-2}	4.00×10^{3}		4
	H_2O_2	3.70	8.71×10^{-2}	3.48×10^{3}		
Fe_3O_4 nanoparticles	TMB	0.098	3.44×10^{-2}	3.02×10^{4}	3.5 - 4.5	4
	H_2O_2	154	9.78×10^{-2}	8.58×10^{4}		
Gold nanoclusters	TMB	3.59	8.61×10^{-3}	2.87×10^{-3}	3.0 – 4.0	5
	H_2O_2	16.71	13.02×10^{-3}	4.34×10^{-3}		
Co_3O_4 nanoparticles	TMB	0.037	6.27×10^{-2}	1.83×10^{2}	5.0	6
	H_2O_2	140.07	1.21×10^{-1}	3.53×10^{2}		
Single-layer Rh nanosheets	TMB	0.264	12.56×10^{-2}	8.2×10^{4}	4.0	7
	H_2O_2	4.51	68.09×10^{-2}	44.5×10^{4}		
Cystein-MoS_2 nanoflakes	ABTS	0.15	1.61×10^{-1}	0.64×10^{4}	4.0	8
	H_2O_2	8.06	9.92×10^{-1}	3.97×10^{4}		
Cubic boron nitride	TMB	0.157	18.54×10^{-2}	5.98×10^{4}	4.0	9
	H_2O_2	10.88	10.69×10^{-2}	3.45×10^{4}		
Fluorescein	TMB	1.31	0.418×10^{-2}	4.18×10^{-5}	3.0	10
	H_2O_2	1.11	0.438×10^{-2}	4.38×10^{-5}		
Guanosine triphosphate	TMB	2.93	9.15×10^{-2}	9.15×10^{-3}	5.0	11
	H_2O_2	0.761	1.80×10^{-2}	1.80×10^{-3}		

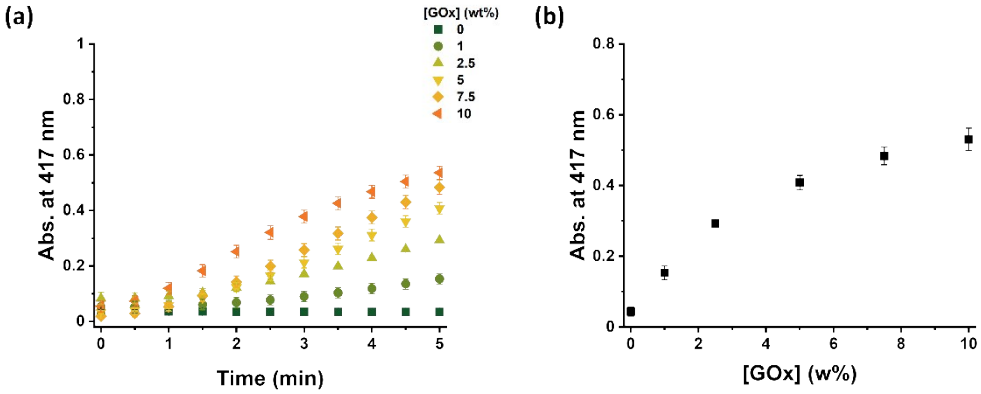


Fig. S8 (a) Absorbance changes of the ABTS/Mn_2BPMP-PNC/GOx system in the presence of glucose and various concentrations of GOx (from 0 to 10 wt% for Mn_2BPMP-PNC). (b) Plot of the absorbance at 417 nm *versus* GOx concentration at 5 min. [ABTS] = 2 mM, [Mn_2BPMP-PNC] = 2 µM, [glucose] = 1 mM in a buffer solution (phosphate, 20 mM, pH 7.0).

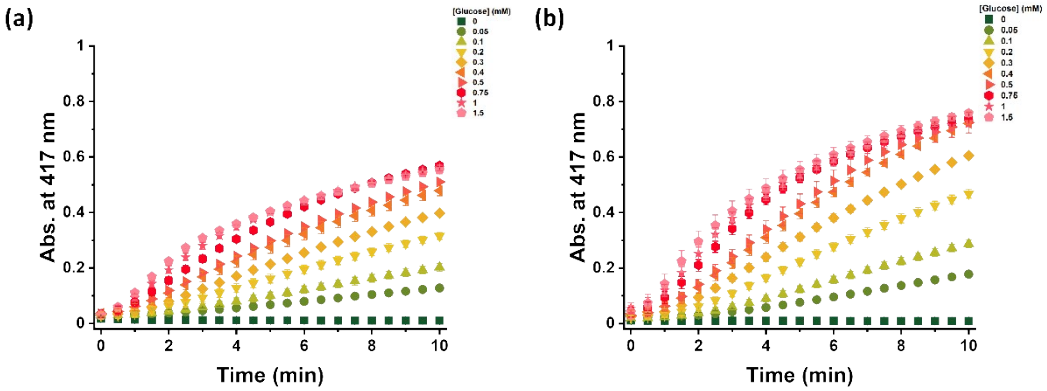


Fig. S9 Absorbance changes of (a) ABTS/Mn_2BPMP/GOx system and (b) ABTS/Mn_2BPMP-PNC/GOx system in the presence of various concentrations of glucose (from 0 to 1.5 mM). [ABTS] = 2 mM, [Mn_2BPMP-PNC] = [Mn_2BPMP] = 2 μM, [GOx] = 10 wt% in a buffer solution (phosphate, 20 mM, pH 7.0).

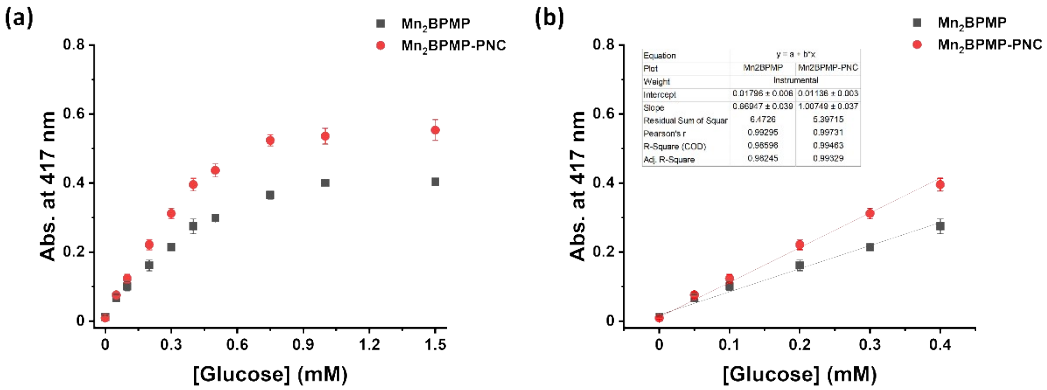


Fig. S10 (a) Absorbance at 417 nm for the ABTS/Mn_2BPMP/GOx (black square) and ABTS/Mn_2BPMP-PNC/GOx (red circle) systems at various glucose concentrations after 5 min. (b) Linear range of the plot of absorbance against the glucose concentrations. [ABTS] = 2 mM, [Mn_2BPMP-PNC] = [Mn_2BPMP] = 2 μM, [GOx] = 10 wt% in a buffer solution (phosphate, 20 mM, pH 7.0).

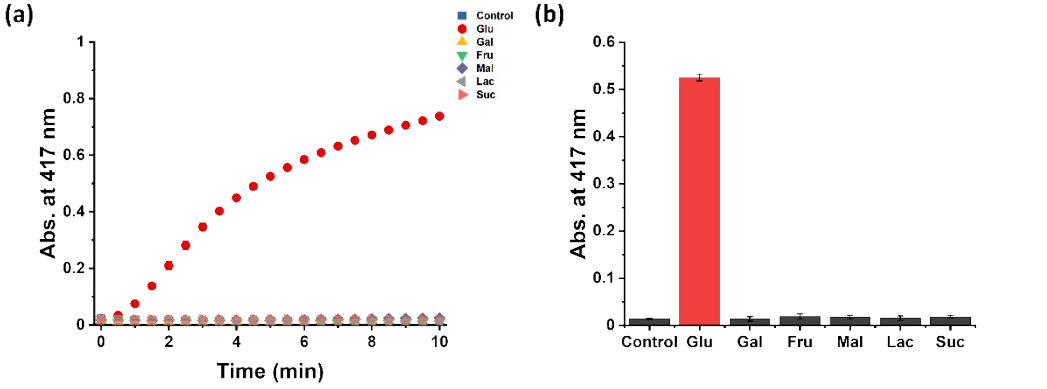


Fig. S11 (a) Absorbance change against time and (b) absorbance at 5 min of the ABTS/Mn_2BPMP-PNC/GOx system in the presence of various saccharides. [ABTS] = 2 mM, [Mn_2BPMP-PNC] = 2 μM, [GOx] = 10 wt%, [glucose] = 1 mM, [other saccharides] = 10 mM in a buffer solution (phosphate, 20 mM, pH 7.0).

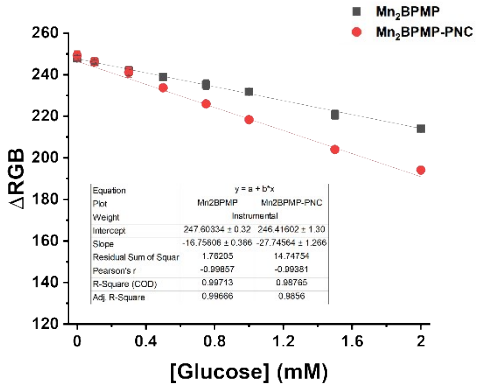


Fig. S12 Plot of the ΔRGB against the glucose concentrations in the PADs prepared using the ABTS/Mn_2BPMP/GOx (black square) and ABTS/Mn_2BPMP-PNC/GOx (red circle) systems.

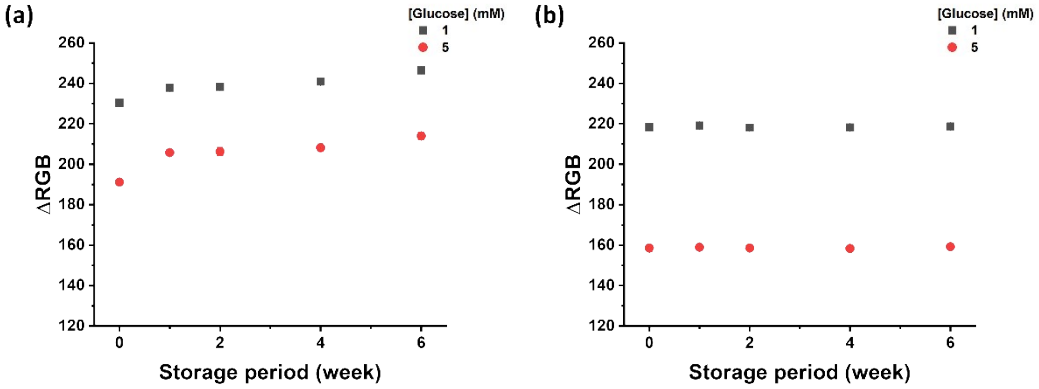


Fig. S13 Plot of the ΔRGB against the storage period in the PADs prepared with the (a) ABTS/Mn_2BPMP/GOx and (b) ABTS/Mn_2BPMP-PNC/GOx systems.

Table S2 Previous reports of paper-based glucose sensors

Sensing system	Signal type	Readout	Detection range for glucose	Total sensing time and step	Real sample	Storage period and Temp.	Reference
Chitosan supported GOx/HRP/TMB	Colorimetric	Scanner and Corel Photo-Paint™ software	0.1 - 1 mM	15 min, 1 step	Tear		12
GOx/HRP-$Cu_3(PO_4)_2$ hybrid nanoflowers/TMB	Colorimetric	Digital camera and ImageJ software	0.1 - 10 mM	10 min, 1 step	Serum	3 days, R.T.	13
Chitosan supported GOx/HRP/TMB	Colorimetric	Smartphone and ImageJ software	0.02 - 4 mM	10 min, 1 step	Serum and tear		14
GOx/Au nanocluster/fluorescent graphene oxide	Fluorescent	Digital camera	0.5 - 10 mM	1h 10 min, 2 step	Serum		15
Chitosan supported GOx/HRP/TBHBA	Colorimetric	Scanner and GIMP 2.8.22 software	0 - 90 $mgdL^{-1}$		Saliva	10 days, - 4 ^oC	16
GOx/Antimony-doped tin oxide nanoparticles/TMB	Colorimetric	Smartphone and Image J software	0.5 - 80 mM	1h 6 min, 2 step	Glucose drink		17
Chitosan supported GOx/HRP/TMB	Colorimetric	Smartphone and Color Name application	50 - 600 µM	6 min, 1 step	Sweat		18
GOx/Co_3O_4-CeO_2 nanosheets/TMB	Colorimetric	Smartphone and Color Picker application	0.005 - 1.5 mM	5 min, 1 step	Serum	15 days, 4 ^oC	19
GOx/starch-iodine-gelatin	Colorimetric	Scanner and Adobe Photoshop CS 5 software	0.5 - 5 mM	15 min, 1 step	Serum	6 week, − 20 ^oC	20
GOx/HRP/quantum dot	Fluorescent	Digital camera and ImageJ software	1- 10 µM	30 min, 1 step	Serum and urine		21
Gox loaded Mn_2BPMP-PNC	Colorimetric	Smartphone and Color Grab application	0.1-10 mM	10 min, 1 step	Serum	6 week, R.T.	This work

HRP: horseradish peroxidase
TMB: 3,3,5,5-tetramethybenzidine
TBHBA: 2,4,6-tribromo-3-hydroxyl benzoic acid
R.T.: room temperature

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