Colorimetric and Fluorescence Dual Modes Biosensor Based on Peroxidase-Like Activity of the Co_3O_4 Nanosheets

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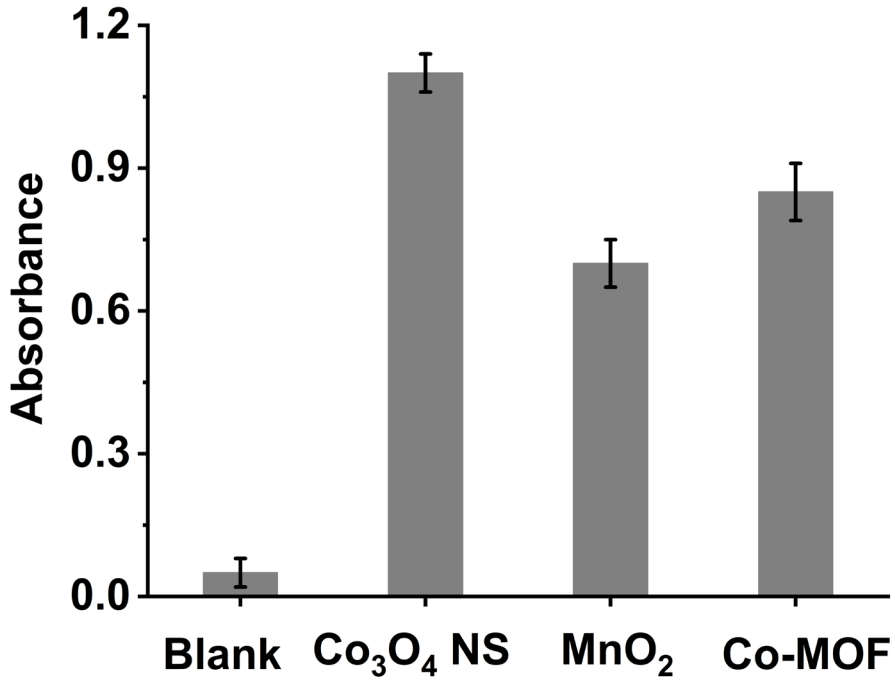

Fig. S1 Catalytic activity of Co_3O_4 nanosheets compared with the earlier published peroxidase-like nanomaterials MnO_2, 2D Co-MOF.

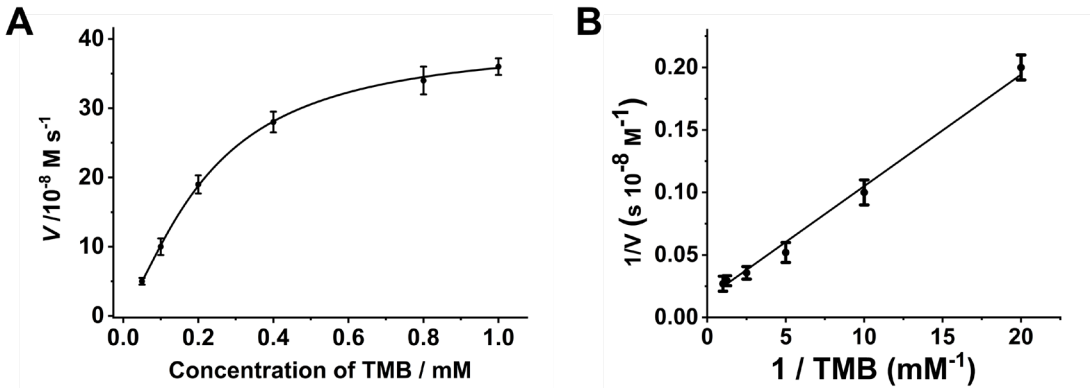

Fig. S2 The steady-state kinetic analysis of Co_3O_4 nanosheets. (A) 5 mM H_2O_2 with different concentration of TMB; (B) Double-reciprocal plots of (A). Experimental conditions: 20 μg·mL^{-1} Co_3O_4 nanosheets in acetate buffer (pH 4.5) at room temperature.

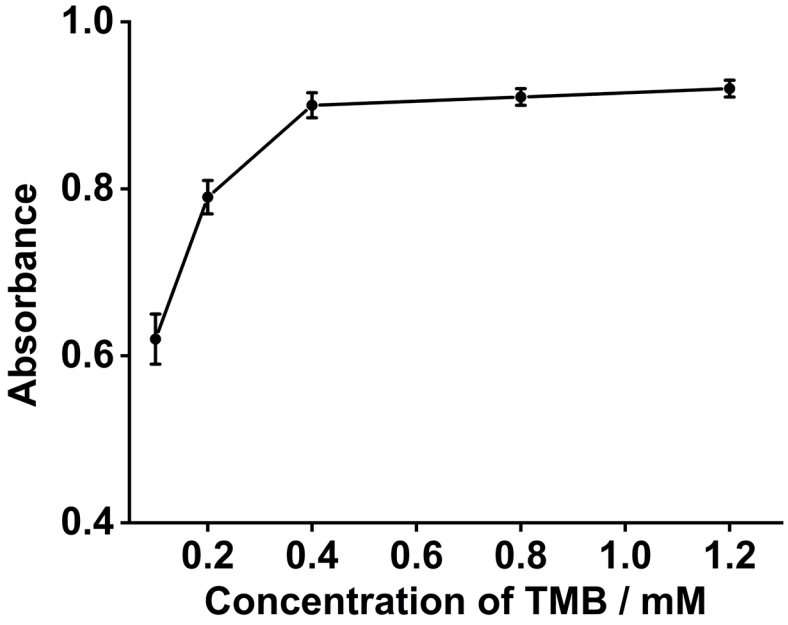

Figure S3. Optimization experiments of TMB concentration. All the colorimetric tests were conducted in HAc-NaAc (0.01 M, pH 4.5) buffer solutions. The concentrations of Co_3O_4 and H_2O_2 were 20 μg/mL and 0.05 mM, respectively.

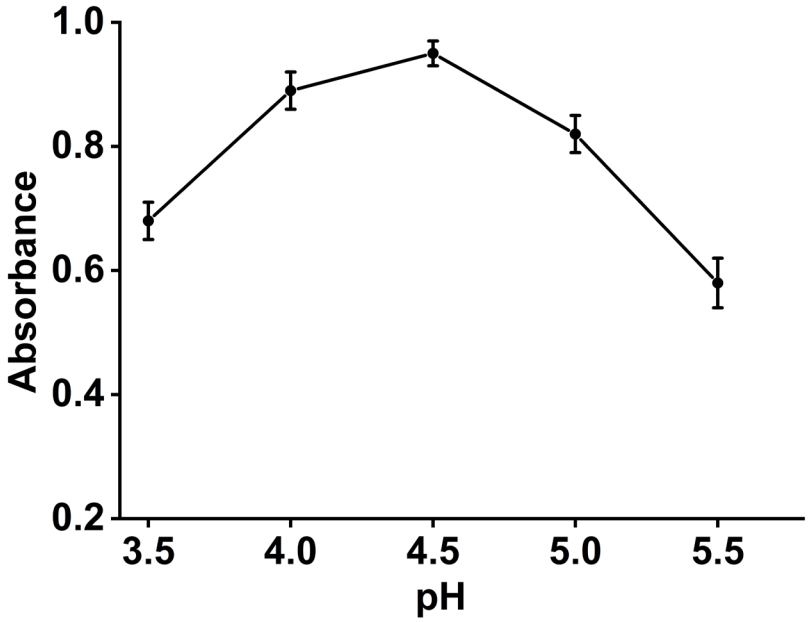

Figure S4. Optimization experiments of pH. All the colorimetric tests were conducted in HAc-NaAc (0.01 M) buffer solutions. The concentrations of Co_3O_4, TMB and H_2O_2 were 20 μg/mL, 0.5 mM and 0.05 mM, respectively.

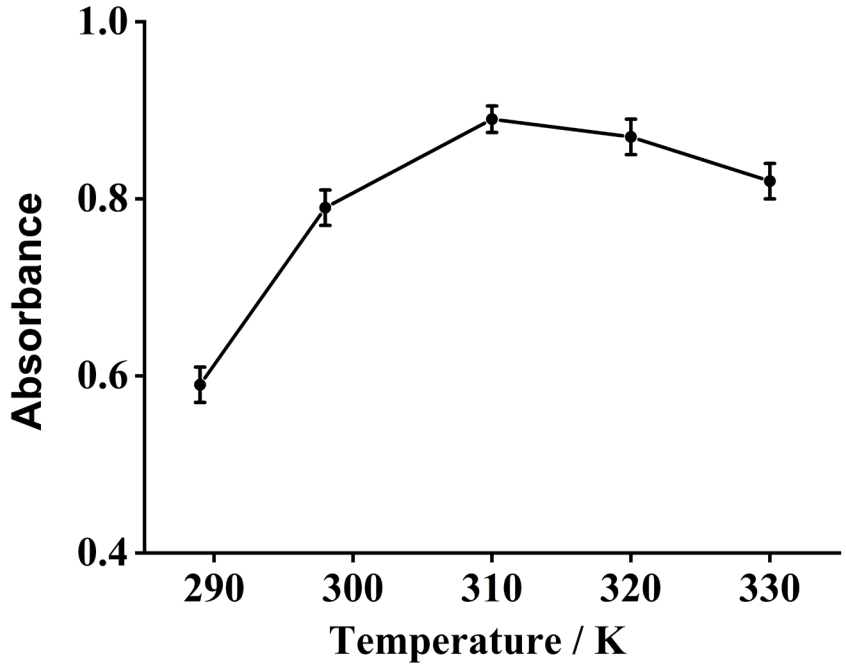

Figure S5. Optimization experiments of temperature. All the colorimetric tests were conducted in HAc-NaAc (0.01 M, pH 4.5) buffer solutions. The concentrations of Co_3O_4, TMB and H_2O_2 were 20 μg/mL, 0.5 mM and 0.05 mM, respectively.

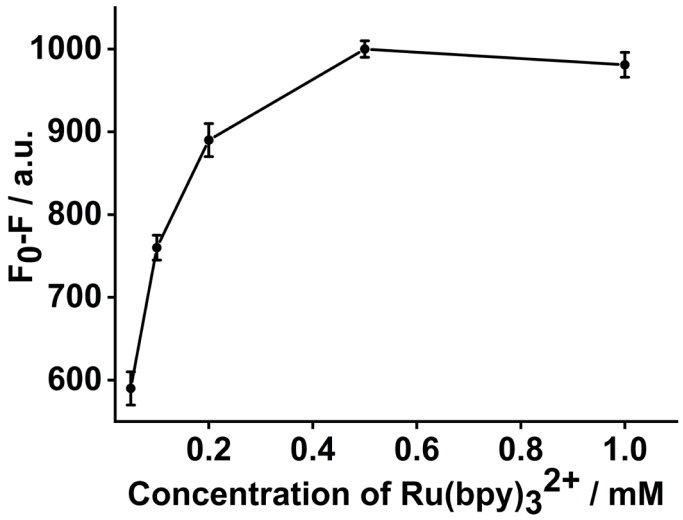

Figure S6. Optimization experiments of $Ru(bpy)_3^{2+}$ concentration. All the colorimetric tests were conducted in HAc-NaAc (0.01 M, pH 4.5) buffer solutions. The concentrations of Co_3O_4, TMB and H_2O_2 were 20 μg/mL, 0.5 mM and 0.05 mM, respectively.

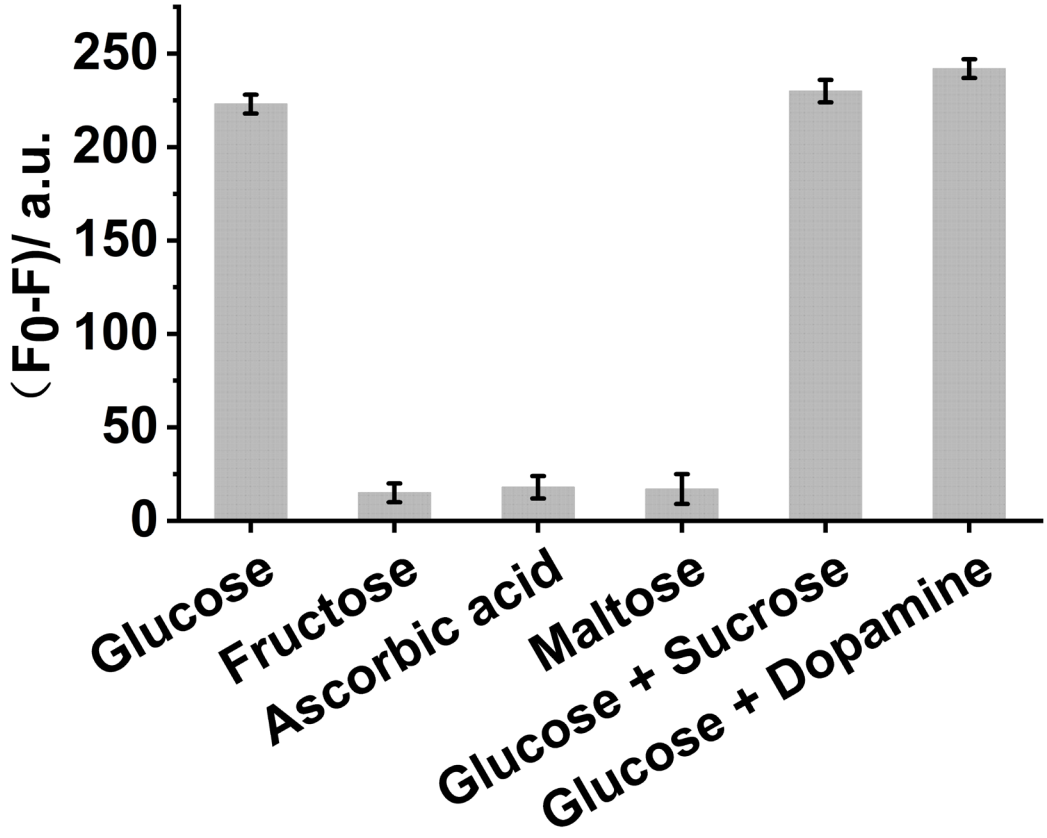

Figure S7. Fluorescence intensity (at the emission wavelength of 610 nm) of the $Ru(bpy)_3^{2+}$ in the presence of glucose, fructose, ascorbic acid, maltose, mixture of glucose and sucrose, mixture of glucose and dopamine, respectively. The concentrations of the above analogue were all 10 μM except that glucose concentration was 0.5 μM. The error bars represent the standard deviation of three measurements.

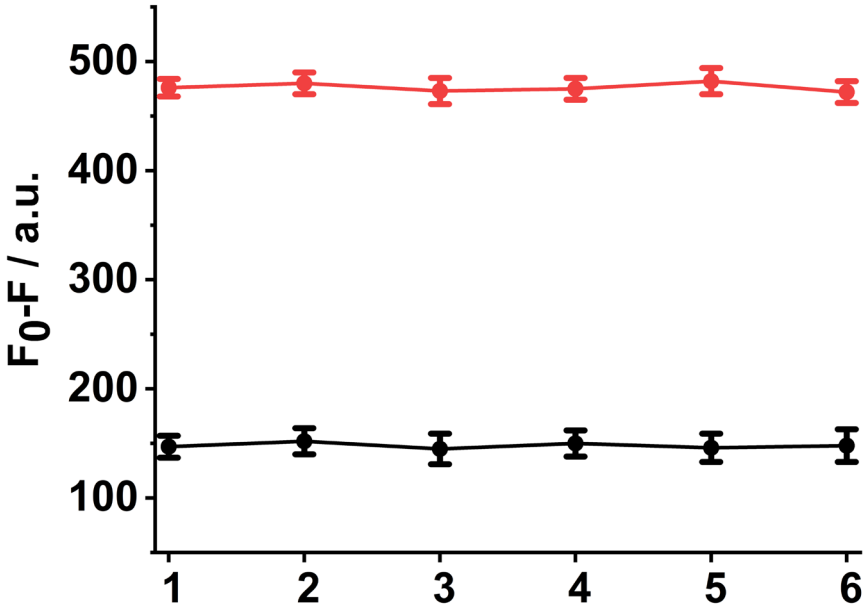

Figure S8. The reproducibility of the fluorescence intensity (at the emission wavelength of 610 nm) of the $Ru(bpy)_3^{2+}$ in the presence of 0.2 and 1.5 μM glucose based on 6 times detections of the proposed biosensing system. The error bars represent the standard deviation of three measurements.

Table S1 Comparison of various glucose assay methods

Detection method	Linear range	LOD	References
Colormetry	5 – 300 μM	2.9 μM	(Lin et al., 2014)
Colorimetry	0.5 – 150 μM	0.2 μM	(Zhang et al., 2021)
Colorimetry	1 – 50 μM	65 nM	(Peng and Weng, 2017)
Electrochemical	0.1– 5.0 mM	0.1μM	(Balouch et al., 2015)
Fluorescence	0.5 – 24 μM	7.54 nM	(Lin et al., 2018)
Fluorescence	0.02 – 2 μM	5.0 nM	This work

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Table S2 Colorimetric detection for H_2O_2 in artificial lake water samples

Samples	Amount measured (μM)	Amount added (μM)	Amount measured (μM)	RSD (%) (n=6)	Recovery (%)
Artificial lake water	5.40	5.00	10.73	4.05	106.6
		20.00	25.83	3.71	102.1
		100.00	104.62	6.66	99.2

Table S3 Fluorescence detection for glucose in fruit juice and blood samples

Samples	Amount measured (mM)	Amount added (mM)	Amount measured (mM)	RSD (%) (n=6)	Recovery (%)
Fruit juice 1	335	50.0	389	5.05	108
Fruit juice 2	750	100.0	855	4.12	105
Fruit juice 3	250	50.0	297	4.91	94
Blood sample 1	5.20	5.00	10.55	6.30	107
Blood sample 2	4.90	10.0	14.66	6.68	97.6