

Supplementary information

For

**Design and Optimization of a Cu–MOF@Graphene Oxide Nanozyme for On-site
Colorimetric Determination of the Prostate Cancer Biomarker Sarcosine**

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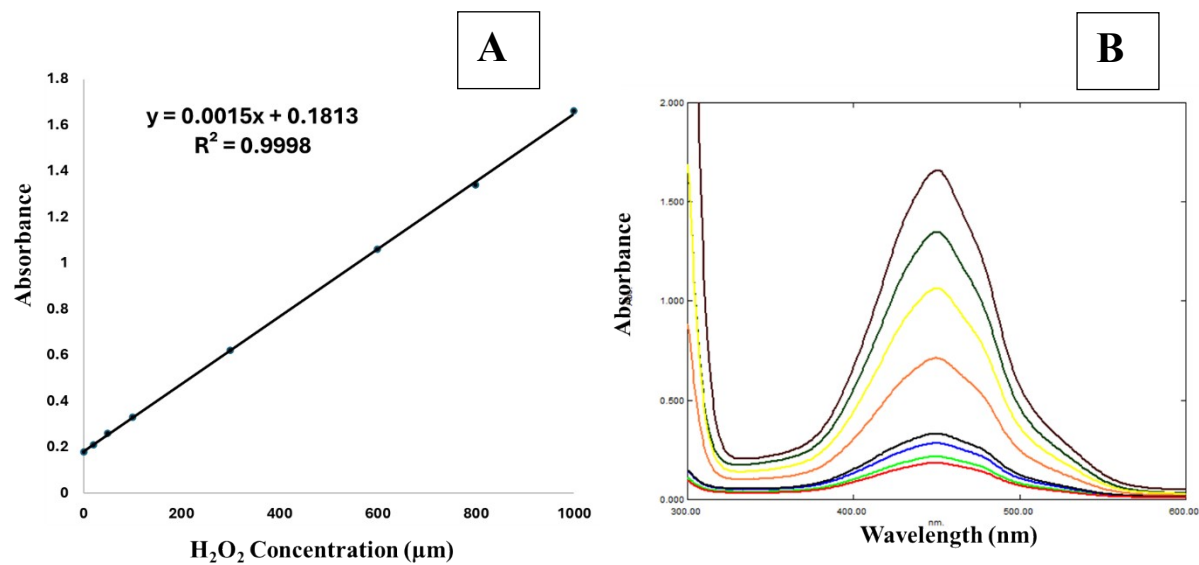


Figure S1. The linear calibration curve of different H₂O₂ concentrations using fixed concentration of OPD and catalyst (A), and the absorption spectra of H₂O₂ using the synthesized nanozymes as peroxidase mimetics (B).

Design-Expert® Software

Desirability



X1 = A: buffer pH
X2 = B: OPD conc

Actual Factors
C: Nanozyme conc = 1.05
D: Temp = 39.74
E: Incubation time = 9.35

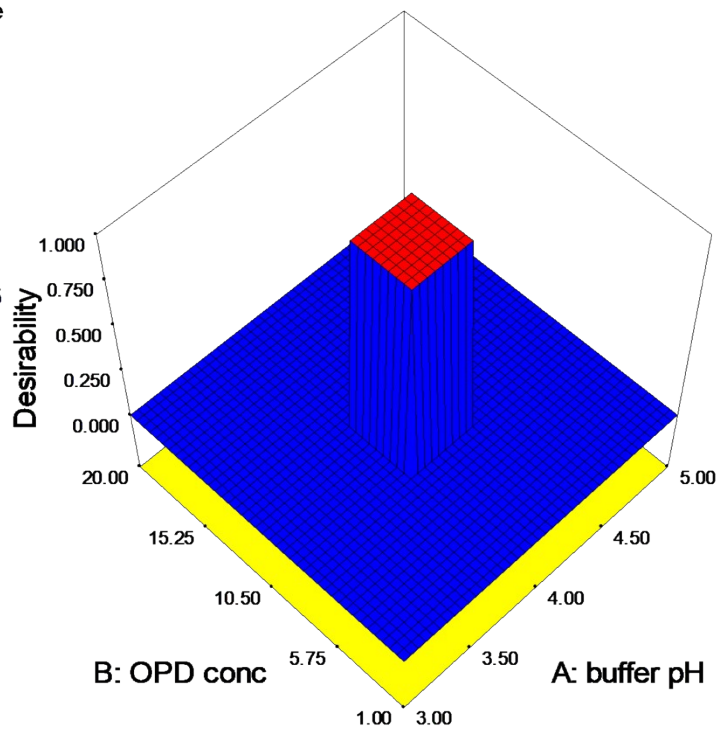


Figure S2. Experimental design desirability showing the optimum experimental conditions: buffer pH= 4, OPD concentration = 10 mM, nanozyme concentration = 1 mg/mL, temperature = 40 °C and incubation time = 10 minutes.

Design-Expert® Software

Absorbance

● Design Points

1.12

0.43

X1 = A: buffer pH

X2 = B: OPD conc

Actual Factors

C: Nanozyme conc = 1.00

D: Temp = 40.00

E: Incubation time = 10.00

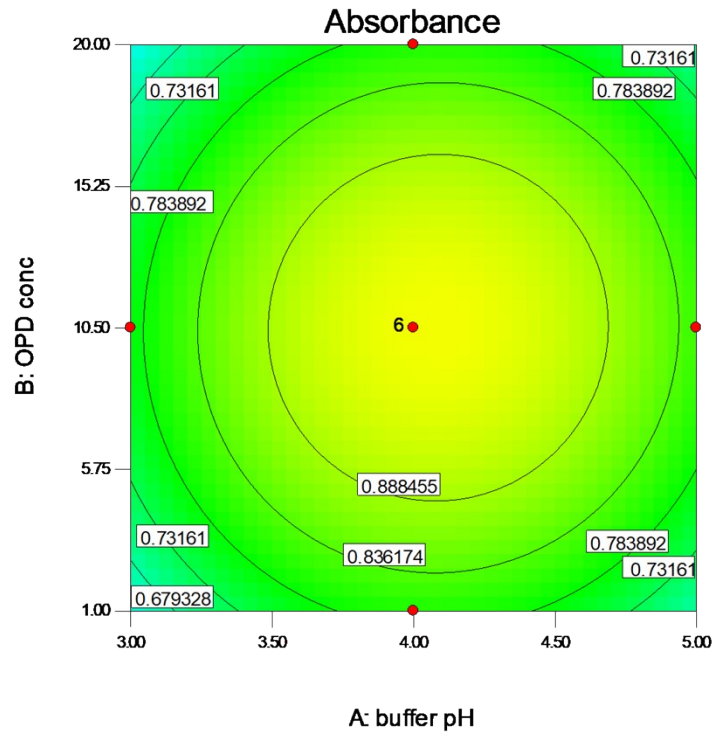


Figure S3. Experimental design results showing the optimum absorbance achieved at buffer pH= 4, OPD concentration = 10 mM, nanozyme concentration = 1 mg/mL, temperature = 40 °C and incubation time = 10 minutes.

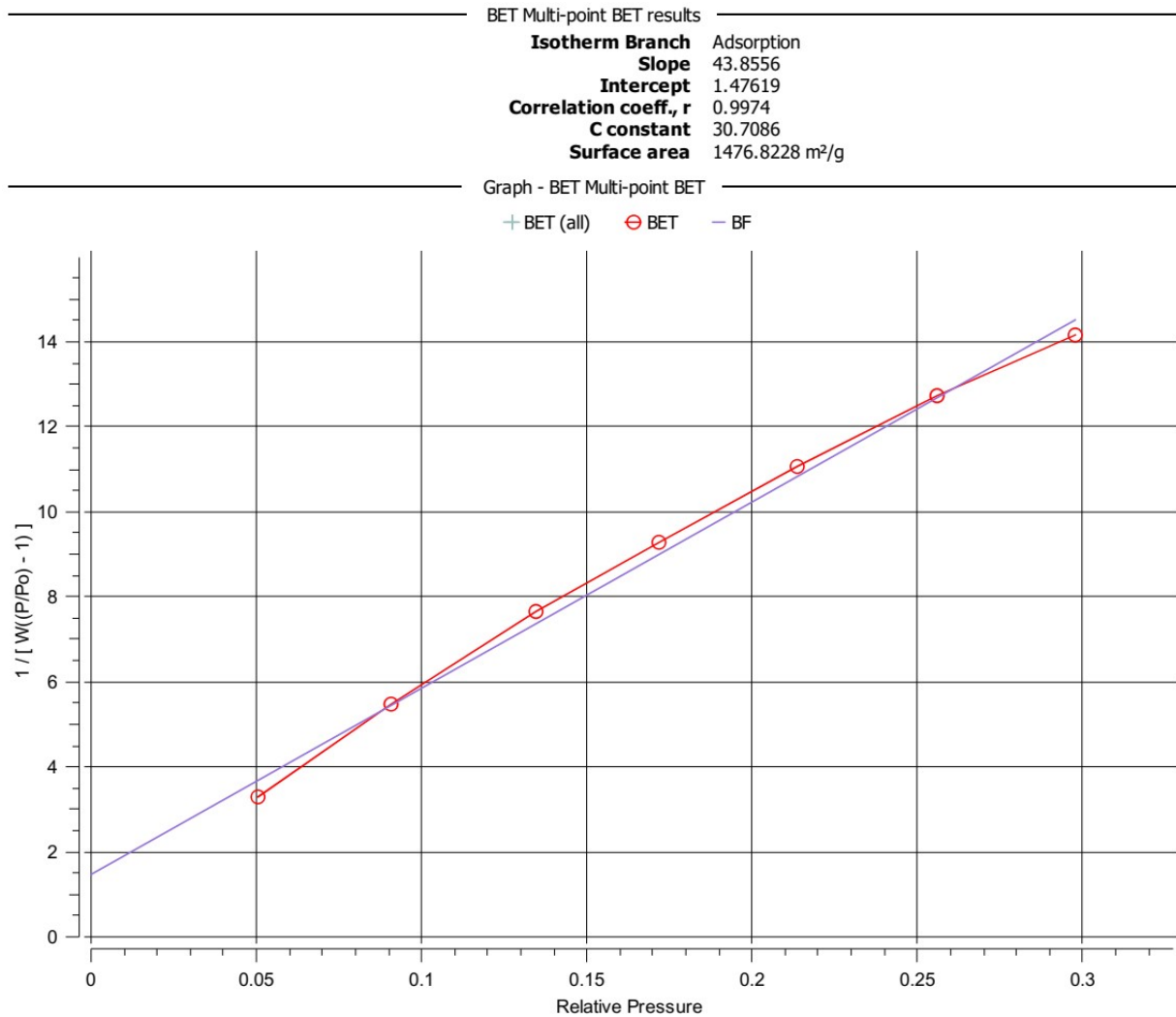


Figure S4. The N₂ adsorption, desorption isotherm plot for the prepared Cu-MOF@GO nanozymes.

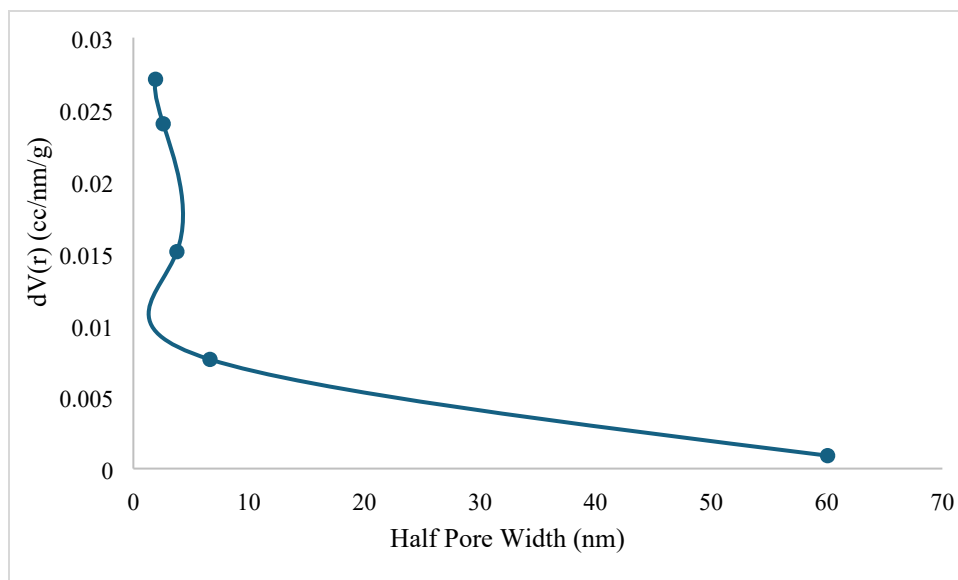


Figure S5. Pore size distribution of the prepared nanozymes

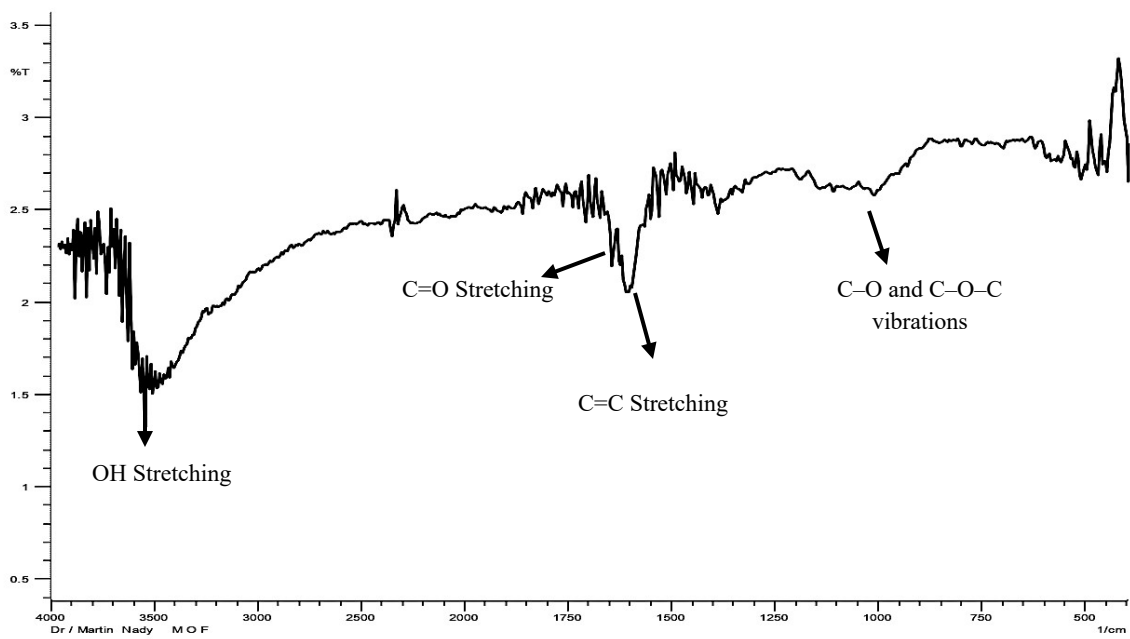


Figure S6. FTIR spectrum of the prepared Cu-MOF@GO nanozymes.

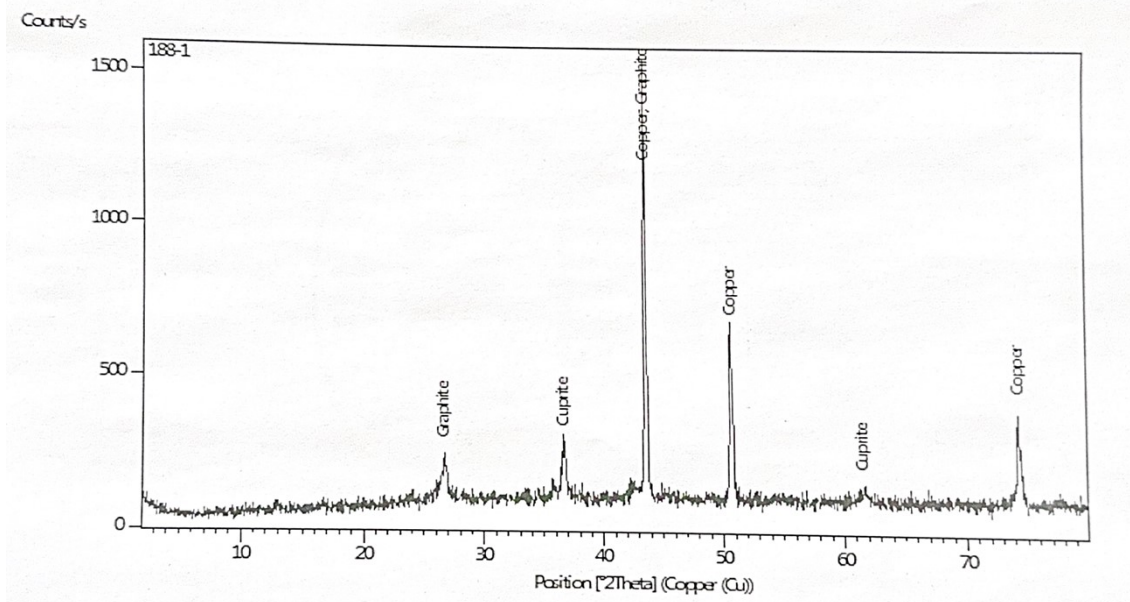


Figure S7. X-ray diffraction analysis (XRD) of the prepared Cu-MOF@GO nanozymes

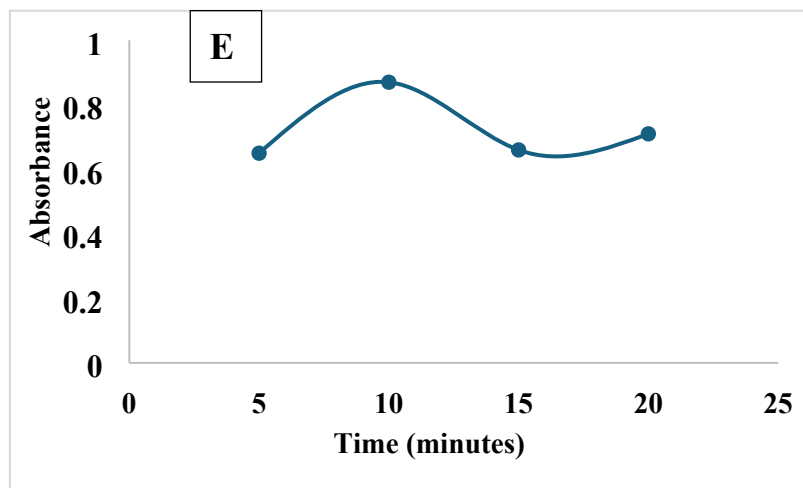
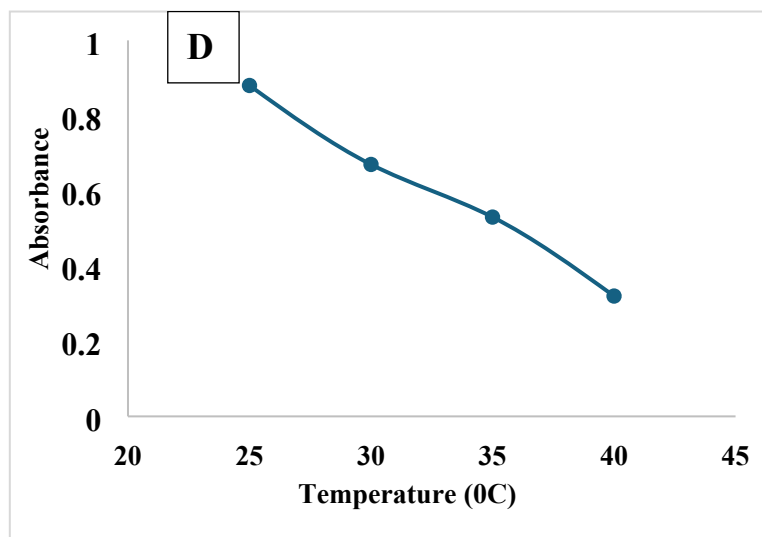
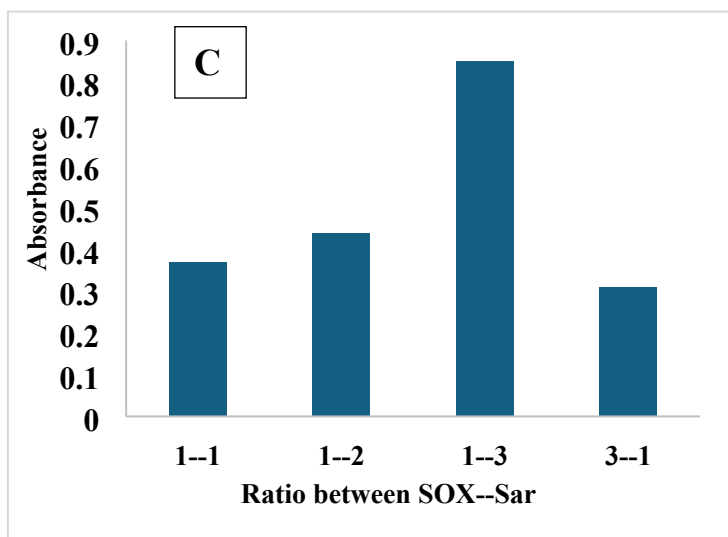
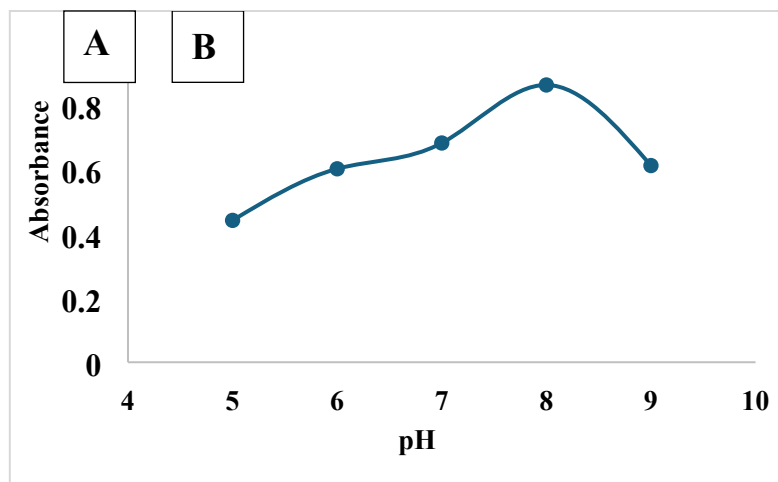
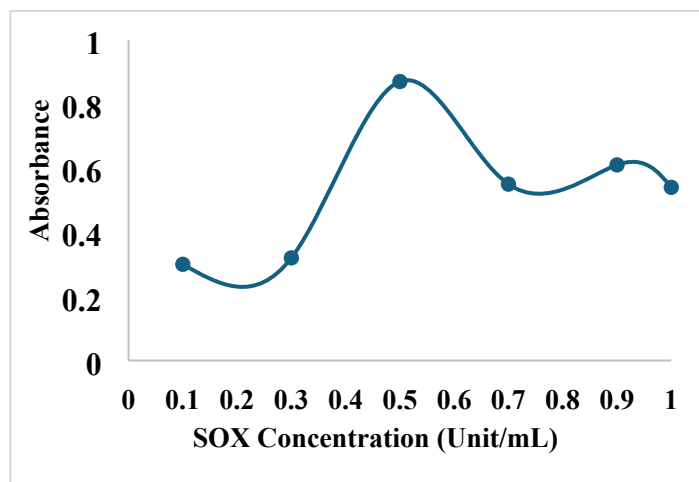


Figure S8. Optimization of the reaction between Sarcosine oxidase and sarcosine to produce H_2O_2 .

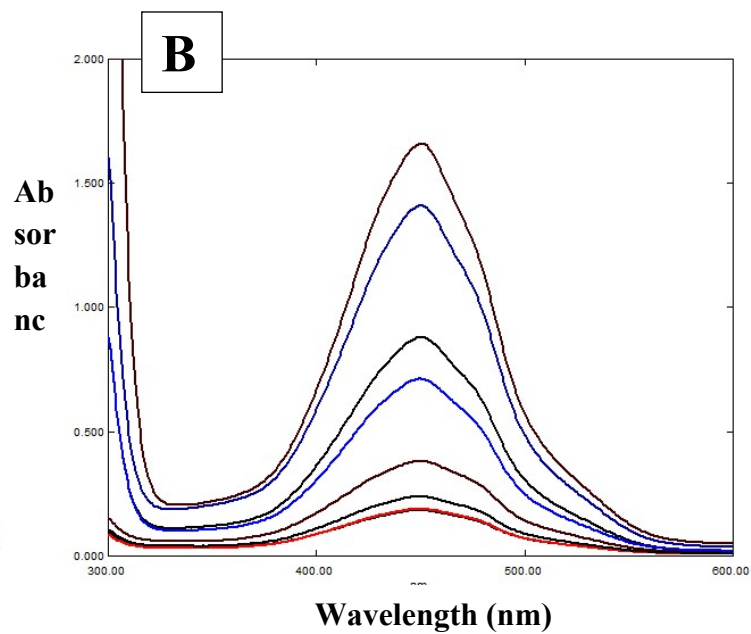
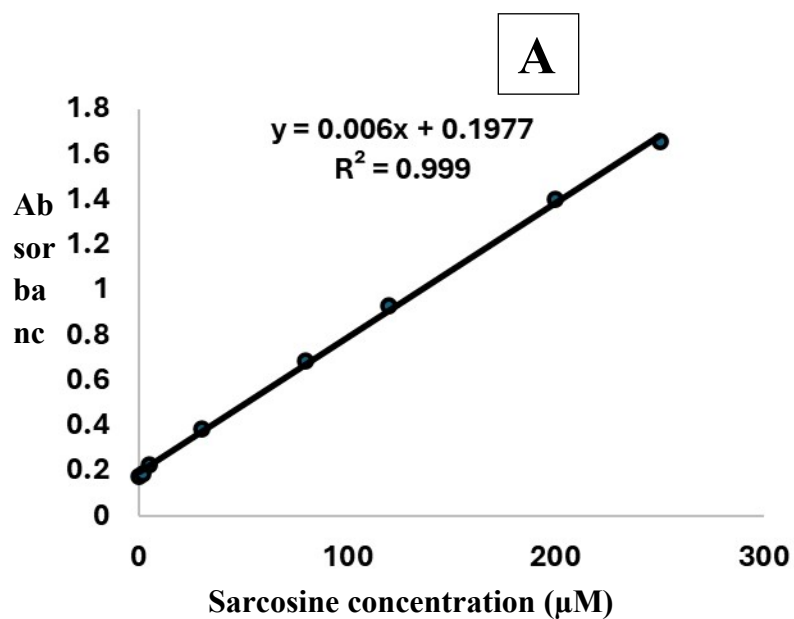


Figure S9. The linear calibration curve of different sarcosine concentrations (A) and the absorption spectra of using the synthesized nanozymes as peroxidase mimetics (B).

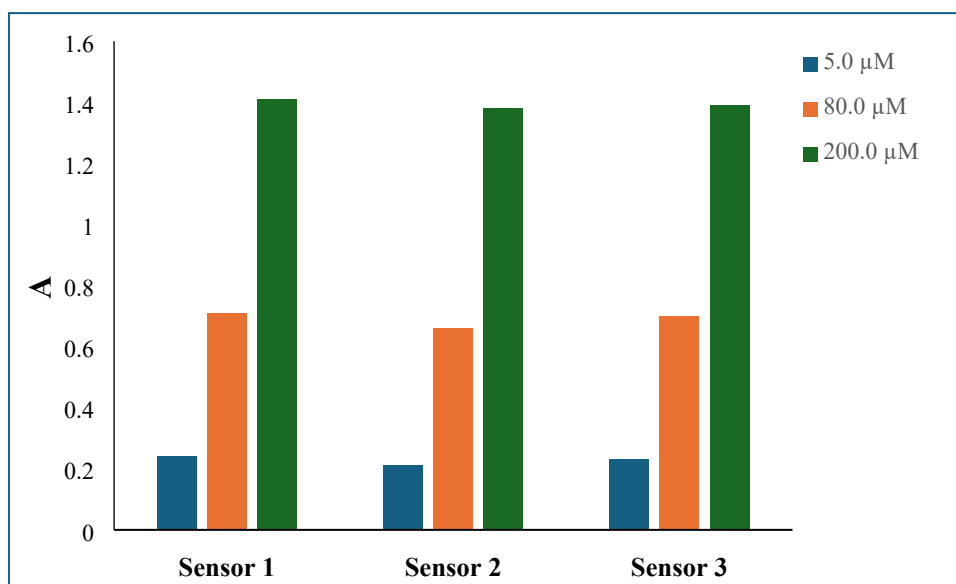
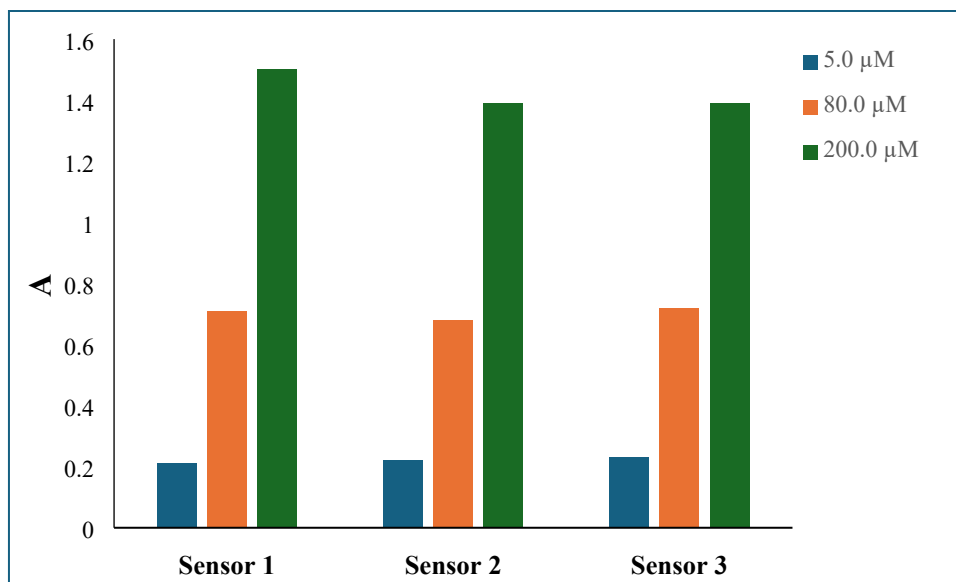


Figure S10. Diagrammatic illustration for (a): intra-batch, (b): inter-batch reproducibility of sensor fabrication process.

Table S1. Design of experiment showing the 5 factors: buffer pH, OPD concentration, nanozyme concentration, temperature and incubation time, also showing measured responses (Absorbances).

Run	Factor 1 buffer pH	Factor 2 OPD conc. (mM)	Factor 3 Nanozyme conc. (mg/mL)	Factor 4 Temp (°C)	Factor 5 Incubation time (Minutes)	Response 1 Absorbance
1	3	1	0.5	45	5	0.56
2	4	10.5	1	40	10	1.1
3	5	20	1.5	35	5	0.52
4	3	10.5	1	40	10	0.52
5	4	10.5	1.5	40	10	1.12
6	5	1	1.5	35	15	0.54
7	5	10.5	1	40	10	0.83
8	4	1	1	40	10	0.62
9	4	10.5	1	40	10	1.1
10	4	10.5	1	45	10	0.7
11	3	1	0.5	35	15	0.51
12	3	20	0.5	45	15	0.43
13	3	20	1.5	35	15	0.66
14	4	10.5	1	40	5	0.66
15	3	20	1.5	45	5	0.64
16	5	20	0.5	35	15	0.62
17	4	20	1	40	10	0.74
18	5	1	0.5	45	15	0.71
19	4	10.5	1	40	10	1.1
20	5	20	0.5	45	5	0.78
21	5	1	0.5	35	5	0.69
22	5	1	1.5	45	5	0.72
23	3	1	1.5	35	5	0.74
24	4	10.5	0.5	40	10	0.77
25	5	20	1.5	45	15	0.71
26	4	10.5	1	35	10	0.82
27	4	10.5	1	40	10	1.1
28	4	10.5	1	40	10	1.1
29	4	10.5	1	40	15	0.81
30	3	20	0.5	35	5	0.8
31	4	10.5	1	40	10	1.1
32	3	1	1.5	45	15	0.81

Table S2. Controls for the proposed colorimetric sensor using Cu MOF@GO using optimum experimental conditions.

	SOX	Sar	Cu MOF@GO	OPD	Absorbance
Full reaction	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	1.01
Control 1	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	0.13
Control 2	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	0.10
Control 3	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	0.12
Control 4	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	0.04