Electronic Supplementary Material (ESI) for Analyst.

Exceptional Peroxidase-Like activity of an Iron and Copper based organic framework for Consecutive Colorimetric Biosensing of Glucose and Kanamycin in Real Food Sample

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We did an experiment of peroxidase catalytic activity for FeCu-MOF with different peroxidase substrates such as OPDA and ABTS in the presence of H_2O_2 and form an oxidized OPDA and ABTS. Nevertheless, the FeCu-MOF + H_2O_2 system with TMB gives oxidization of TMB produced an Blue color, while in the presence of OPDA produced oxidized OPDA with orange color and ABTS gives oxidized ABTS and produced a green color. Moreover, OPDA and ABTS having some disadvantages when compared to TMB. The OPDA is less stability easily oxidized with an hour in room temperature and ABTS is slow oxidizing peroxidase substrate, expensive, poor stability and so on. To overcome these drawbacks we choose TMB as a peroxidase substrate for our sensor.

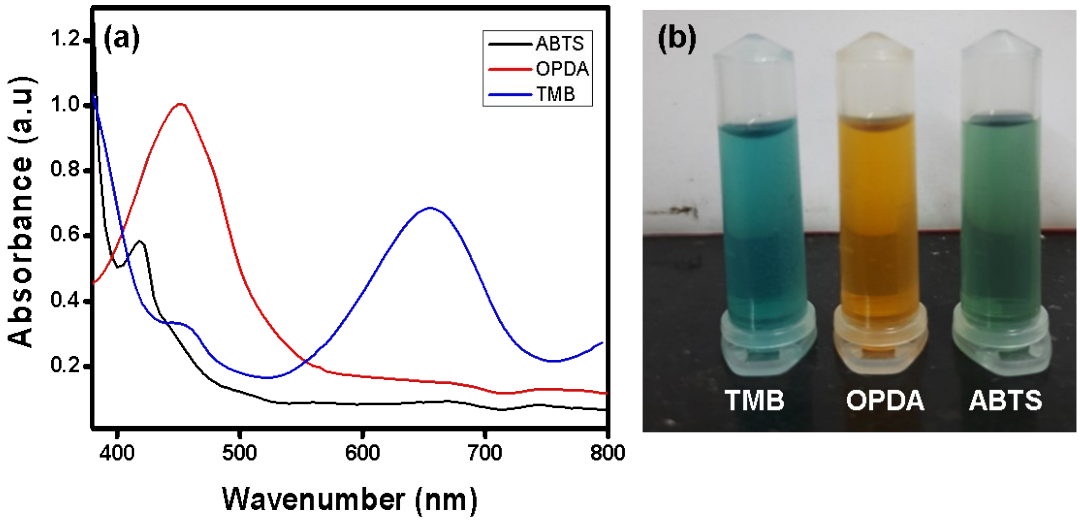

Figure S1. (a&b) Peroxidase-like activity of FeCu-MOF NS with TMB, OPDA and ABTS.

Kinetic analysis of FeCu-MOF nanozyme

Kinetics studies were accomplished by determining the A_{654} nm with respect to reaction time. The peroxidase-like catalytic performance of FeCu MOF with H_2O_2 and TMB as substrates was measured using steady-state kinetics. The standard kinetic parameters including Michaelis–Menten constant (K_m) as well as the maximum reaction velocity (V_{max}) for FeCu-MOF nanozyme were calculated via Lineweaver–Burk double reciprocal equation (1).

$$\frac{1}{V} = \frac{K_m}{V_{max}} \frac{1}{[S]} + \frac{1}{V_{max}} \qquad (1)$$

Where, V is the initial velocity, V_{max} represents the maximal reaction velocity, K_m is the Michaelis constant and [S] represents the concentration of substrate [4]. The K_m value and the V_{max} value of the FeCu-MOF nanozyme have been given in Table 1. Here, the K_m value indicates the affinity of the enzyme to the substrate and V_{max} indicates a catalytic activity of the enzyme. The Km value of FeCu-MOF using TMB as the substrate is close to HRP. Simultaneously, the K_m value of FeCu-MOF using H_2O_2 as the substrate is much lower than the individual catalyst and HRP, which demonstrates that FeCu-MOF have superior affinity to H_2O_2. The higher V_{max} value of the FeCu-MOF shows high catalytic activity. Furthermore, the efficiency of the FeCu-MOF has been demonstrated by evaluating the obtained K_m value and V_{max} value with different previously reported enzymes.

Table S1. Comparison of K_m and V_{max} with other previously reported literature

Catalyst	Substrate	K_m (mM)	V_{max}	Reference
HRP	TMB	0.275	1.24 (10^{-8} Ms^{-1})	[1]
	H_2O_2	0.214	2.46 (10^{-8} Ms^{-1})	
Cu-MOF	TMB	0.456	2.478 (10^{-8} Ms^{-1})	[2]
	H_2O_2	28.58	5.45 (10^{-8} Ms^{-1})	
2D Fe-BTC	TMB	0.2610	7.95 (10^{-8} Ms^{-1})	[3]
	H_2O_2	0.0334	2.65(10^{-8} Ms-1)	
$CuFe_2O_4$	TMB	2.26	2.07(10^{-8} Ms^{-1})	[4]
	H_2O_2	0.50	2.61(10^{-8} Ms^{-1})	
FeCu-MOF NS	TMB	0.286	8.17(10^{-8} Ms^{-1})	This work
	H_2O_2	0.30	2.68(10^{-8} Ms^{-1})	

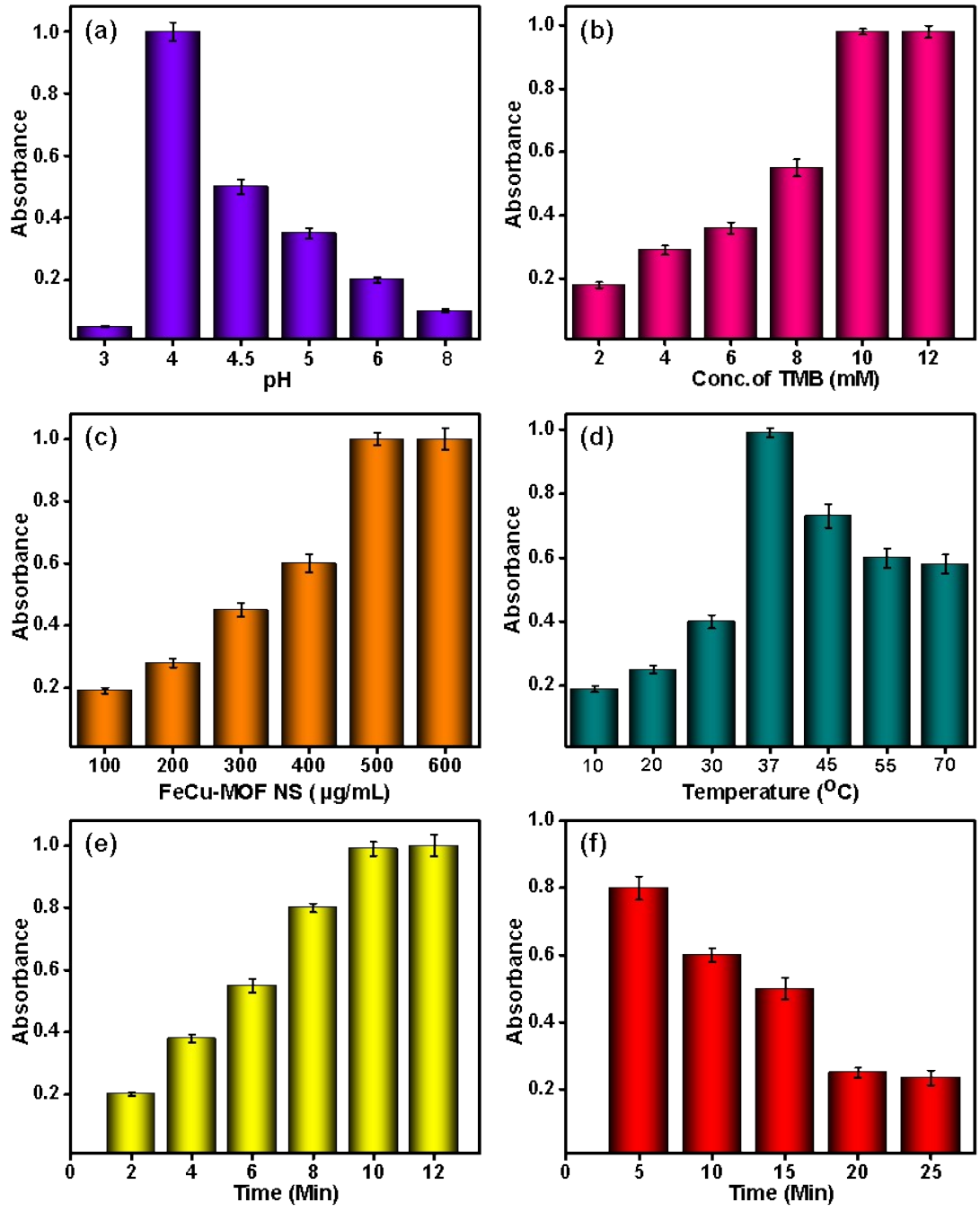

Figure S2. Optimization of (a) incubation of pH (b) concentration of TMB (c) amount of FeCu-MOF NS (d) incubation temperature (e-f) sensing time of glucose and KAN.

Table S2. Comparison of the proposed glucose sensor with other reported methods

Sensing Method	Sensing Probe	Linear Range	LOD	Reference
CM	Au-PtNCs-GMP	0.05–0.4 mM	11 μM	[5]
CM	Ba-hemin@GOX	9.25 μM to 0.74 mM	3.083 μM	[6]
CM	Fe-COF	5 to 350 μM	1.0 μM	[7]
CM	COF_{HD}–GOx	0.005 to 2 mM	0.54 μM	[8]
CM	FeCu-MOF NS	0.25-1 mM	0.1 μM	This work

Table S3. Comparison of the proposed KAN sensor with other reported method

Sensing Method	Sensing Probe	Linear Range	LOD	Reference
CM	OFL-Ti-MN	15.28 nM to 46.14 μM	15.28 nM	[9]
FM	UCNPs-BHQ3-cDNA	0.05–50 μM	18.90 nM	[10]
FM	DNA Cu/Ag NCs	80 nM-10 μM	13.3 nM	[11]
FM	FDNA-QDNA	100-600 nM	13.52 nM	[12]
CM	FeCu-MOF NS	0.020-0.1 μM	8 nM	This work

We looked a selectivity analysis for kanamycin with sugar, ions and amino acids (**Figure S3a**). However, sugars and ions does not interfere with the detection of kanamycin. Meanwhile, amino acids have a minor interference but it takes double the time compared to detection time of kanamycin so it's negligible. However, the minor interference also prohibited by using NEM masking agent as shown in **Figure S3b**.

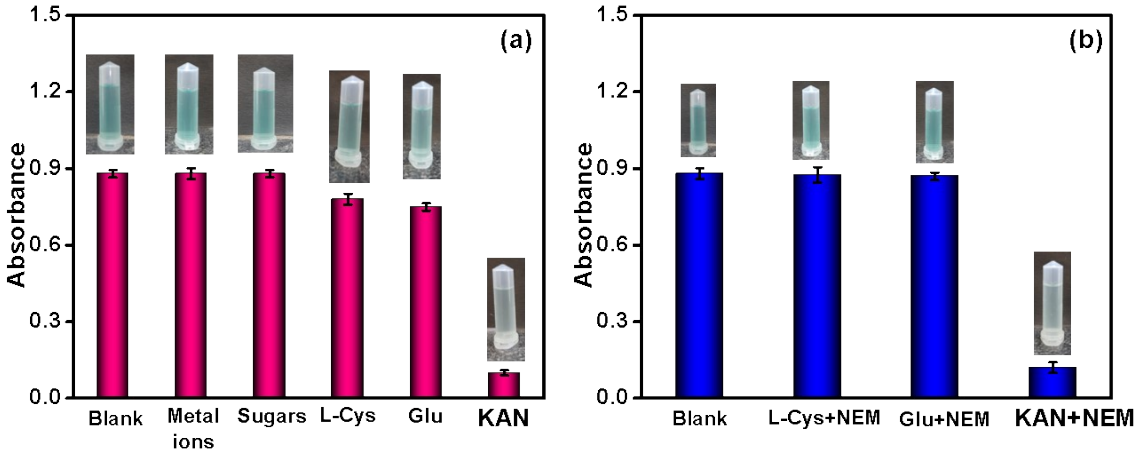

Figure S3. Selectivity analysis for kanamycin with sugar, ions and amino acids.

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