Electronic Supplementary Material

A robust dual-readout ratiometric assay for dopamine based on $NiMn_2O_4$-catalyzed DHNP fluorophore conversion

Glowi Alasiri[a], Ali M. Alaseem[b], Razan Orfali[b], Ramadan Ali[c], Al-Montaser Bellah H. Ali[d], Mohamed M. El-Wekil[e*]

[a] Department of Biochemistry, College of Medicine, Imam Mohammad Ibn Saud Islamic University (IMSIU), Riyadh, 13317, Saudi Arabia.

[b] Department of pharmacology College of Medicine, Imam Mohammad Ibn Saud Islamic University (IMSIU), Riyadh 13317, Saudi Arabia

[c] Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Tabuk, Tabuk 71491, Saudi Arabia

[d] Department of Pharmaceutical Analytical Chemistry, Faculty of Pharmacy, Assiut University, Assiut, 71526, Egypt

[e] Pharmaceutical Chemistry Department, Faculty of Pharmacy, Badr University in Assiut (BUA), 2014101, Assiut, Egypt

Correspondence

*** mohamed.elwakeel@pharm.aun.edu.eg, mohamed.mohamoud@ymail.com**

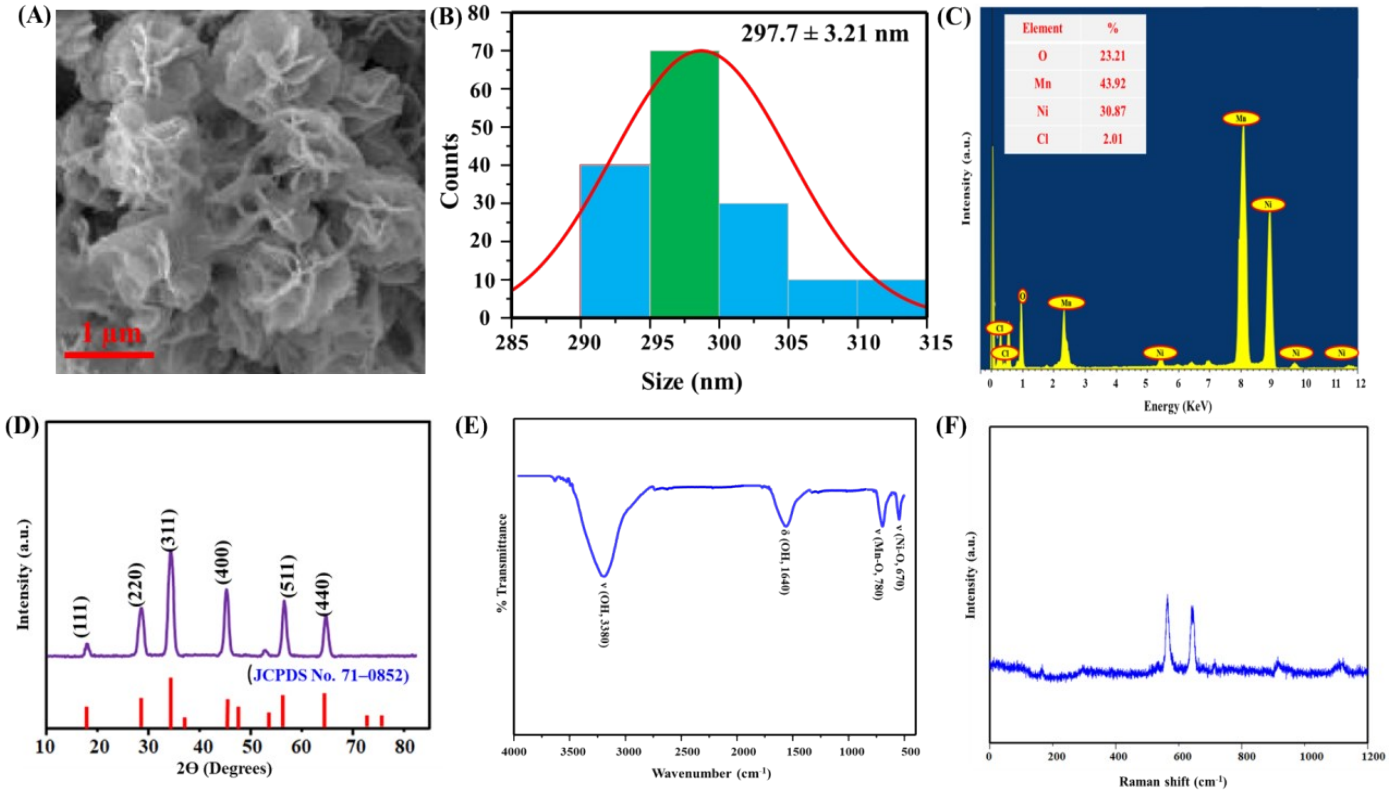

Fig. S1 SEM (A), size distribution (B), EDX (C), XRD (D), FTIR (E), and Raman spectrum of $NiMn_2O_4$ nanozyme.

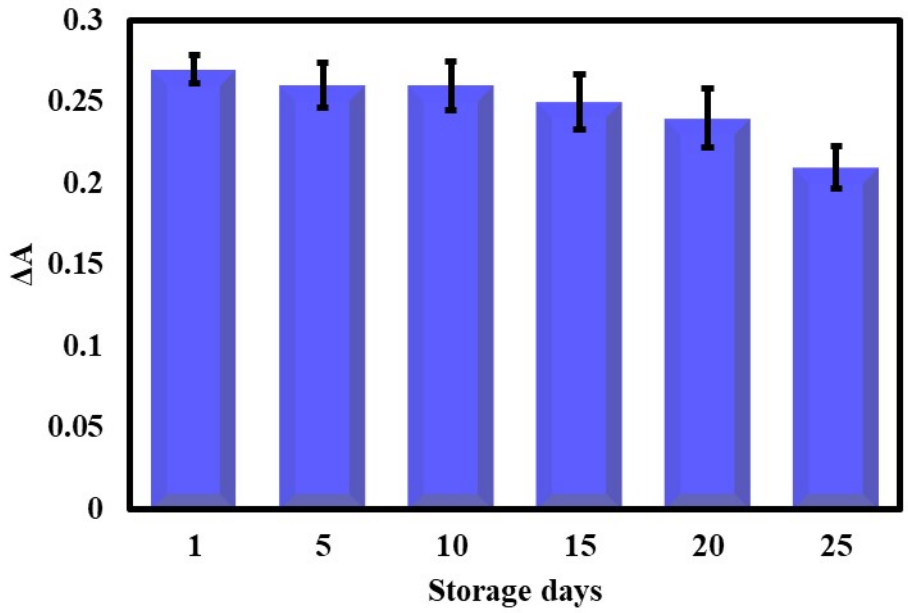

Fig. S2. Long-term stability of the nanozyme, evaluated by its colorimetric response toward 40 μM DA over 25 days. All measurements were performed in triplicate.

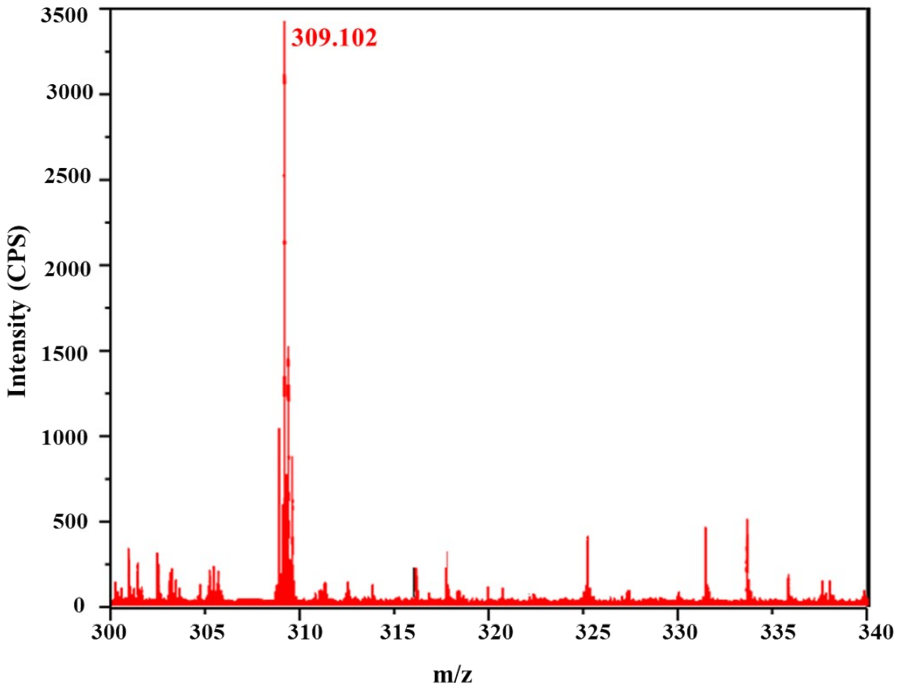

Fig. S3 LC-MS/MS analysis of the cyclization reaction between DA and DHNP.

Table S1. Michaelis-Menten kinetic parameters of $NiMn_2O_4$ in comparison with various reported nanozyme systems.

Enzyme	K_m (mM)		V_{max} (10^{-8} M s^{-1})		Reference
	TMB	H_2O_2	TMB	H_2O_2	
HRP	0.434	3.702	10.000	8.710	[1]
NH_2-MIL-88(Fe)	0.219	0.029	4.104	2.519	[2]
Ag/Fe_3O_4@h-BN	0.162	0.494	7.21	10.25	[3]
FeOOH	2.81	3.85	0.54	0.33	[4]
Fe-CDs	0.0086	0.00672	2.035	2.817	[5]
BSA@Pt NPs	0.17	104	23.0	24.1	[6]
Co_3O_4	0.511	140.56	12.41	3.43	[7]
CeO_2/Co_3O_4 HNs	0.495	1.225	27.06	8.87	[8]
Au-NCs	3.59	16.71	0.86	1.3	[9]
AuPtCo	0.137	2.593	1.79	10.16	[10]
PDA@Cu-CeO_2	0.197	0.196	77,0000	128,0000	[11]
CeO_2 NSs	8.17	4.33	3400	1450	[12]
$NiMn_2O_4$	0.119	1.671	19.621	21.750	**This work**

Table S 2 Precision DA detection in buffer solution by fluorescence method.

Added (μM)	Intra-batch (n = 6)			Inter-batch (n = 6 * 3)		
	Measured (μM)	Recovery %	RSD %	Measured (μM)	Recovery %	RSD %
5.0	4.92± 0.17	98.4	1.76	5.13± 0.43	102.6	2.76
50.0	48.87± 0.43	97.7	1.88	51.31± 0.65	102.6	3.19
100.0	101.32± 0.65	101.3	2.55	102.67± 0.87	102.7	2.56

Table S3 Precision DA detection in buffer solution by colorimetric method.

Added (μM)	Intra-batch (n = 6)			Inter-batch (n = 6 * 3)		
	Measured (μM)	Recovery %	RSD %	Measured (μM)	Recovery %	RSD %
5.0	4.98± 0.14	99.6	2.67	5.24± 0.22	104.8	3.62
50.0	52.43± 0.24	104.9	2.15	47.63± 0.32	95.3	2.89
100.0	97.67± 0.37	97.7	3.10	104.21± 0.57	104.2	3.87

Table S4 Precision DA detection in human serum by fluorometric method.

Added (μM)	Intra-batch (n = 6)			Inter-batch (n = 6 * 3)		
	Measured (μM)	Recovery %	RSD %	Measured (μM)	Recovery %	RSD %
5.0	5.35± 0.36	107.0	3.76	4.75± 0.54	95.0	3.10
50.0	47.76± 0.57	95.8	4.18	53.47± 0.47	106.9	4.26
100.0	96.34± 0.87	96.3	4.46	95.78± 0.82	95.8	3.01

Table S5 Precision DA detection in human serum by colorimetric method.

Added (μM)	Intra-batch (n = 6)			Inter-batch (n = 6 * 3)		
	Measured (μM)	Recovery %	RSD %	Measured (μM)	Recovery %	RSD %
5.0	4.87± 0.54	97.4	3.87	5.24± 0.76	104.8	2.87
50.0	53.21± 0.76	106.4	3.26	53.89± 0.44	106.9	3.66
100.0	103.76± 0.65	103.8	4.00	106.32± 0.76	106.3	4.34

Table S6 Precision DA detection in human urine by fluorometric method.

Added (μM)	Intra-batch (n = 6)			Inter-batch (n = 6 * 3)		
	Measured (μM)	Recovery %	RSD %	Measured (μM)	Recovery %	RSD %
5.0	5.26± 0.44	105.2	3.00	4.78± 0.43	95.6	2.98
50.0	52.25± 0.54	104.5	3.23	52.43± 0.65	104.8	3.10
100.0	102.14± 0.55	102.1	3.87	104.21± 0.57	104.2	2.76

Table S7 Precision DA detection in human urine by colorimetric method.

Added (μM)	Intra-batch (n = 6)			Inter-batch (n = 6 * 3)		
	Measured (μM)	Recovery %	RSD %	Measured (μM)	Recovery %	RSD %
5.0	4.89± 0.43	97.8	2.78	4.88± 0.43	97.6	3.10
50.0	47.43± 0.35	94.9	1.87	52.03± 0.65	104.1	1.89
100.0	97.43± 0.67	97.4	2.10	103.28± 0.57	103.3	2.21