Supplementary Information (SI) for RSC Advances.

Enzymatic properties of iron oxide nanoclusters and their application as a colorimetric glucose detection probe

Dahyun Bae, Minhee Kim, Jin-sil Choi

Department of Chemical and Biological Engineering, Hanbat National University, Daejeon 34158, Korea

E-mail: jinsil.choi@hanbat.ac.kr

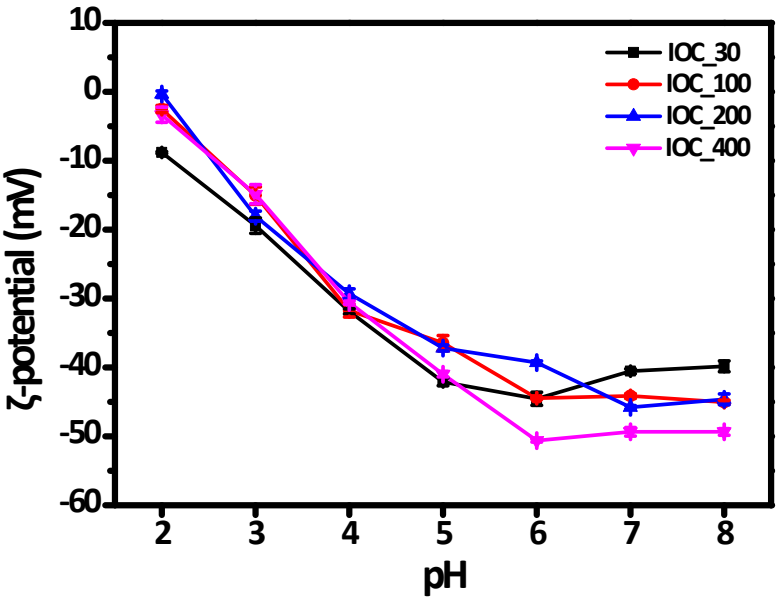

Figure S1. Zeta potential of IOCs dispersed in buffer solution (citrate buffer: pH2–6, phosphate buffer: pH7 and 8).

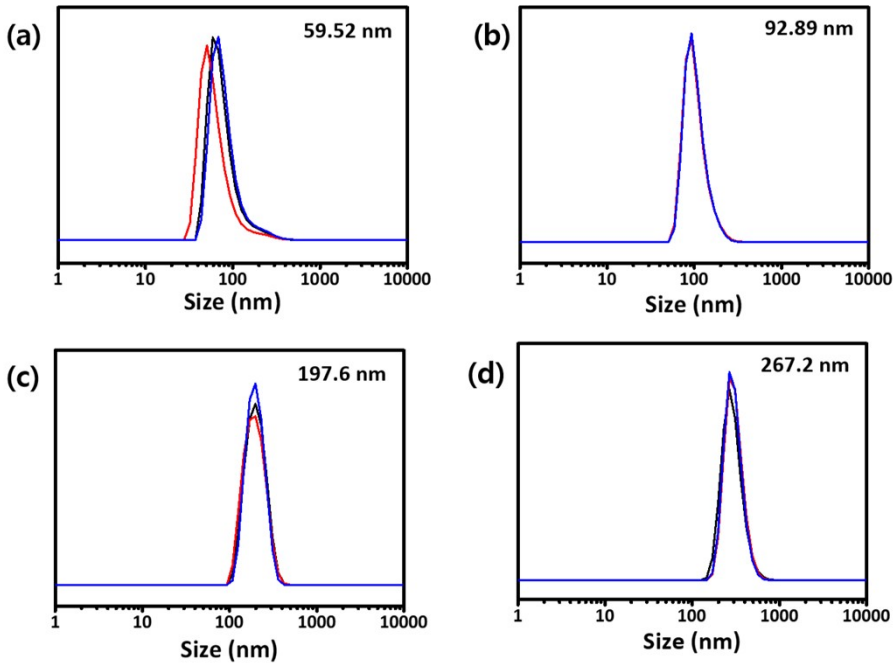

Figure S2. Hydrodynamic sizes of (a) IOC_30, (b) IOC_100, (c) IOC_200, and (d) IOC_400 (DIW).

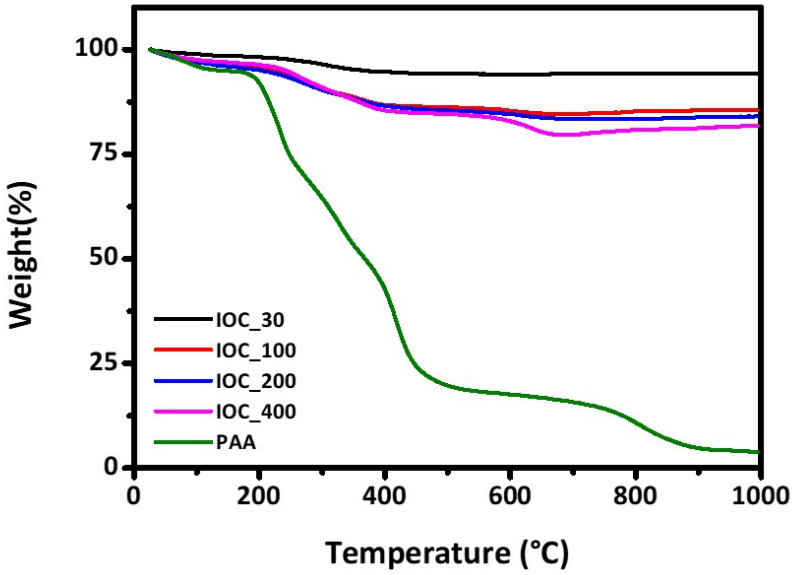

Figure S3. TGA analysis of the percentage of PAA present in IOCs.

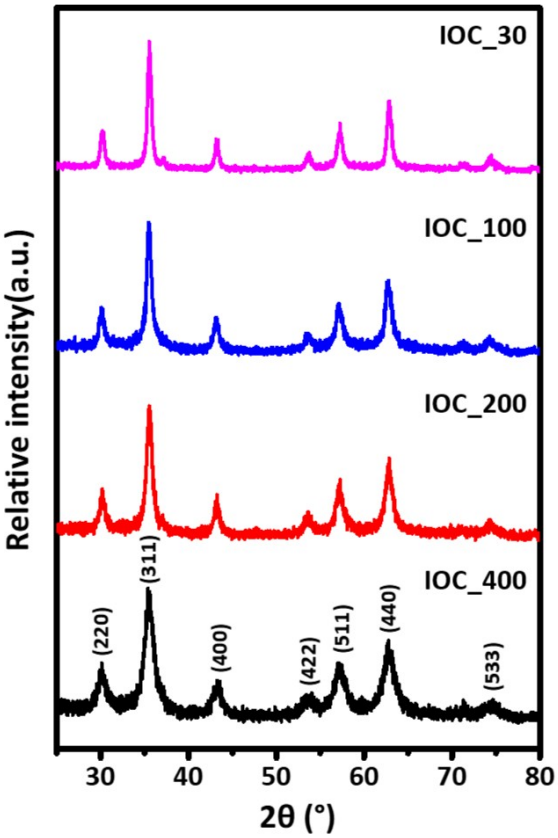

Figure S4. XRD diffraction pattern of IOCs.

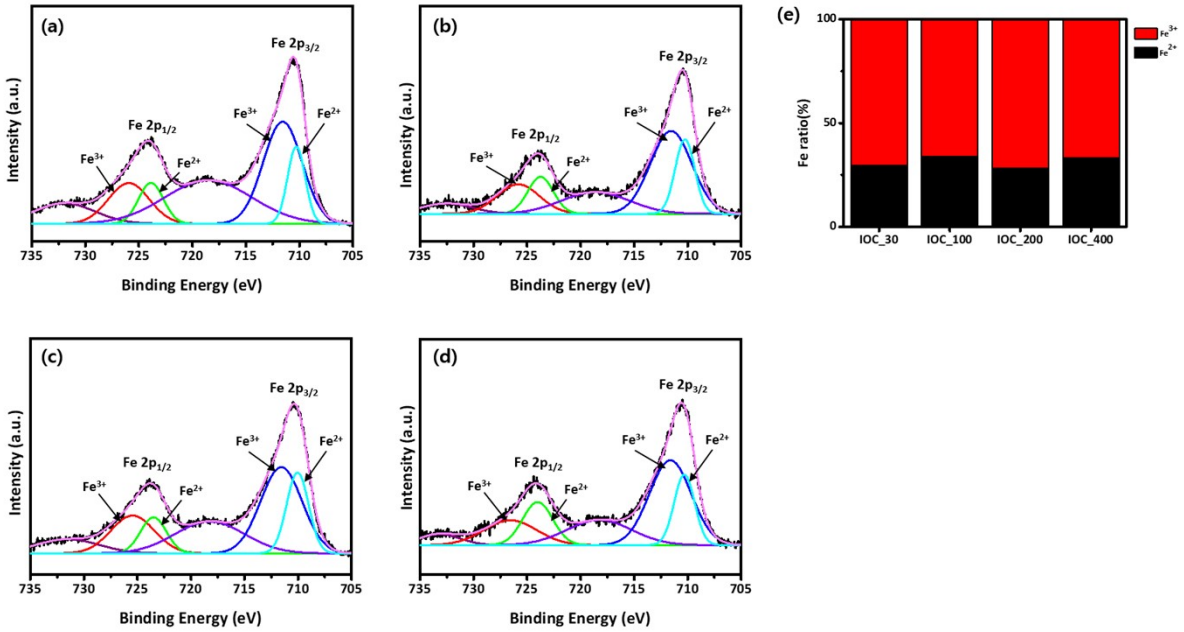

Figure S5. Fe 2p XPS spectra of (a) IOC_30, (b) IOC_100, (c) IOC_200, and (d) IOC_400. (e) Fe^{2+} to Fe^{3+} ratio of IOCs based on correlated peak area in XPS spectra.

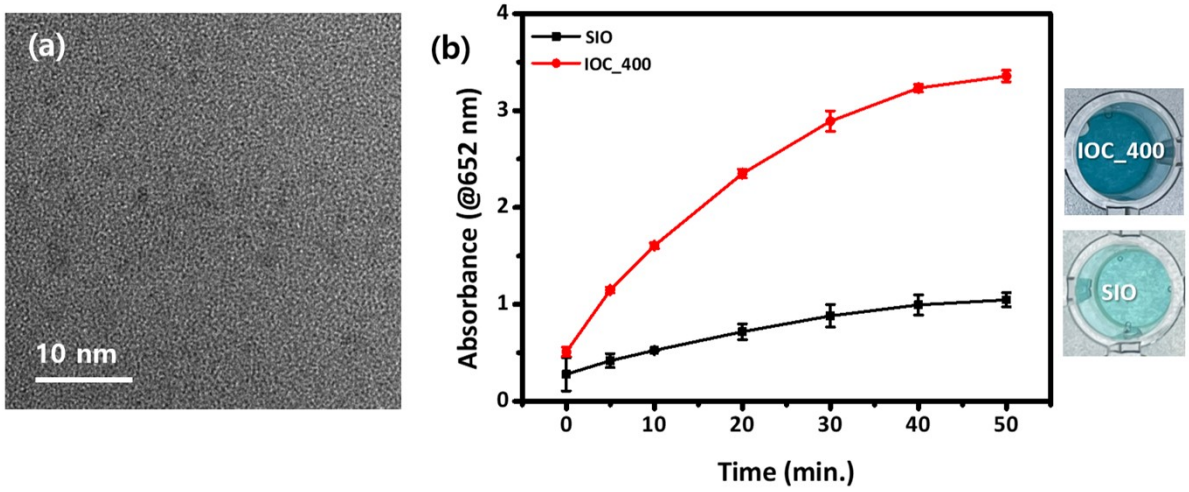

Figure S6. (a) TEM image of SIO. (b) Nanozyme activity of IOC and SIO color change and absorption intensity at 652 nm for indication solution containing TMB (3.12 mM) and H_2O_2 (100 mM) at 5 min at pH 4 at room temperature.

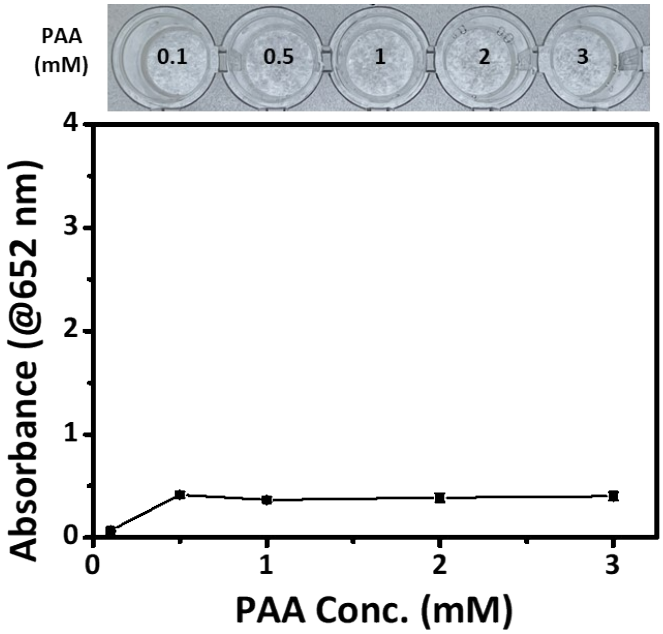

Figure S7. PAA concentration-dependent color change and absorption intensity at 652 nm for indication solution containing TMB (3.12 mM) and H_2O_2 (5 mM) at 10 min at room temperature.

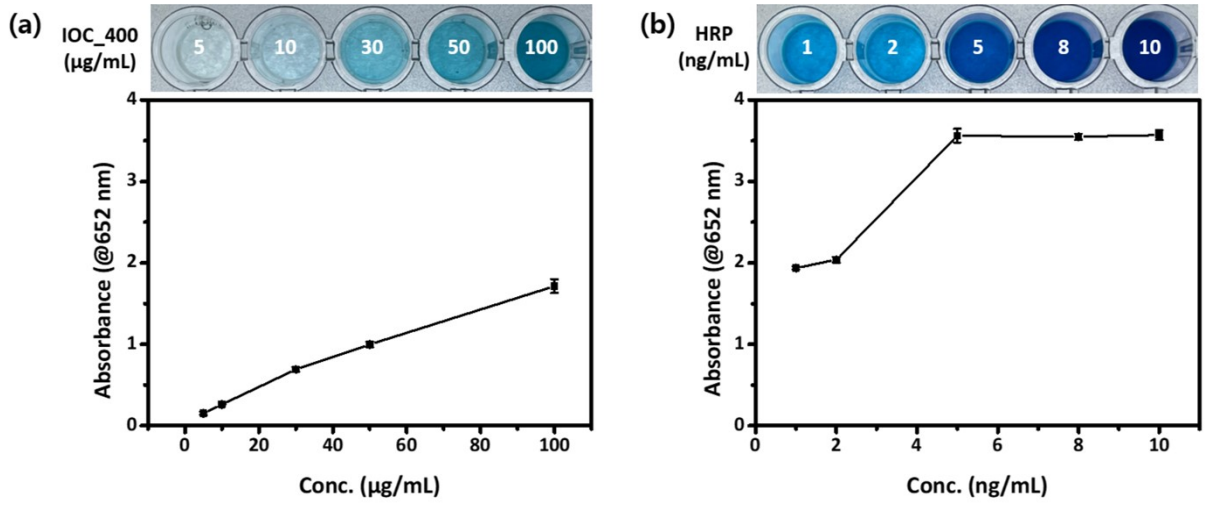

Figure S8. (a) IOC_400 and (b) HRP concentration-dependent color change and absorption intensity at 652 nm for indicator solution containing TMB (3.12 mM) and H_2O_2 (5 mM) at 10 min at room temperature.

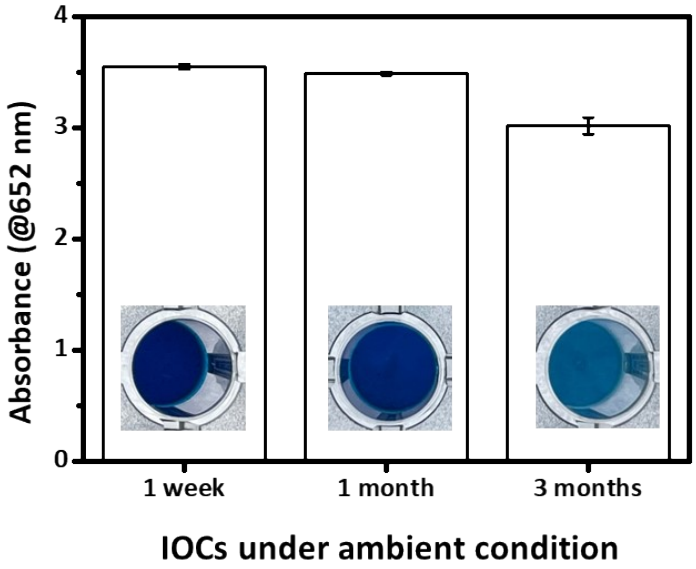

Figure S9. Nanozyme activity of IOC dispersed in water and stored at room temperature after 1 week, 1 month, and 3 months. IOC size-dependent color changes at 30 min and absorption intensities at 652 nm for indication solution containing IOCs (100 µg Fe/mL), TMB (3.12 mM) and H_2O_2 (50 mM) at pH 4.

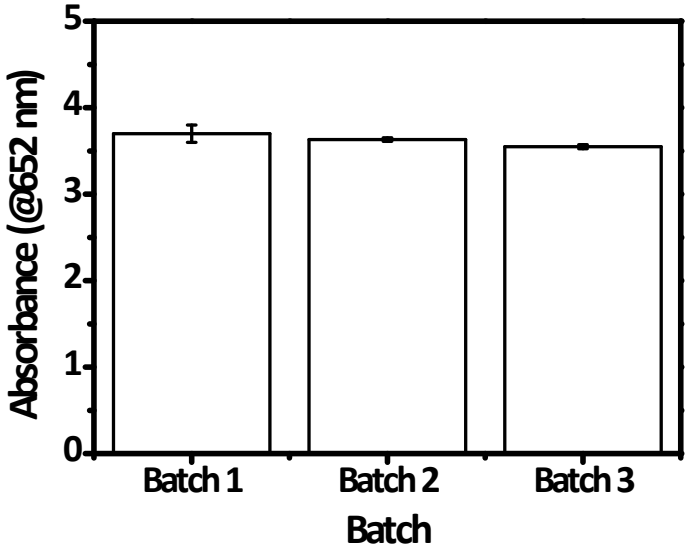

Figure S10. Batch-to-batch reproducibility of IOC nanozyme activity. Color changes at 30 minutes and absorption intensities at 652 nm for indication solution containing IOCs (100 µg

Fe/mL), TMB (3.12 mM), and H_2O_2 (50 mM) at pH 4 demonstrate consistent activity across three independent batches.

Table S1. K_m, V_{max} and K_{cat} values obtained using H_2O_2 and TMB as a substrate, respectively.

		Substrate						
		H_2O_2			TMB			
catalyst	diameter	K_m (mM)	V_{max} (mM/min.)	K_{cat} (/min.)	K_m (mM)	V_{max} (mM/min.)	K_{cat} (/min.)	ref
IOC_30	30 nm	59.588	0.229	2.43×10^5	1.060	0.138	1.46×10^5	This work
IOC_100	100 nm	30.189	0.330	1.30×10^7	0.490	0.442	1.74×10^7	This work
IOC_200	200 nm	20.780	0.371	1.17×10^8	0.429	0.522	1.64×10^8	This work
IOC_400	400 nm	19.711	0.737	1.85×10^9	0.297	0.682	1.72×10^9	This work
Fe_3O_4	300 nm	154	5.87×10^{-6}	5.15×10^6	0.098	1.87×10^{-6}	1.81×10^6	*Nat. Nanotechnol.* **2007**, *2*, 577–583
Fe_3O_4	500 nm	458.9	1.84×10^{-5}	1.87×10^7	101.4	2.60×10^{-6}	5.30×10^7	*Chem. Commun.* **2017**, 53, 424–427
HRP		0.964	0.969	4.27×10^7	1.647	0.457	2.01×10^7	This work

Calculation of K_{cat}

We used IOCs containing 3 μg Fe (~5.67×10^6 nanoparticles) or 0.1 ng of HRP in 100 μL.

The K_{cat} was calculated as below:

$$K_{cat} = \frac{V_{max}}{[E]}$$

[E] = Enzyme concentration

Table S2. Comparison of the glucose detection capability between iron oxide based colorimetry sensor system.

Material	TMB (mM)	NP concentration (μg/mL)	Volume (mL)	LOD (μM)	Reference
Fe_3O_4	55.56	3.89	4.5	0.37	*J. Colloid interface Sci.* **2017**, 506, 46–57
Fe_3O_4+ATP	1	30	0.2	50	*Colloids Surf. B Bi ointerfaces* **2017**, 153, 52–60
Casein-MNP/GOx	0.08	25	3	1	*Biosens. Bioelectron.* **2014**, 52, 391–396
graphene-Fe_3O_4	0.49	29.13	1.03	0.8	*ACS Appl. Mater. Interfaces* **2017**, 9, 7465–7471
Fe_3O_4@SiO_2/ GOx	0.5	2764.02	0.24	3	*Chem. Eur. J.* **2011**, 17, 10700–10707
Fe_3O_4/m-GC	0.044	11.11	0.9	0.24	*Microchim. Acta* **2021**, 188
Fe single iron site nanozyme	0.24	40	0.25	2.1	*Small* **2020**, 16
IOC_400	3.12	138.20	**0.1**	**252**	This work

Calculation of the limit of detection (LOD) and quantitation (LOQ)

The limit of detection (LOD) and quantitation (LOQ) were calculated as below:

$$LOD = \frac{3.3\,\sigma}{S}$$

$$LOQ = \frac{10\,\sigma}{S}$$

σ = standard deviation of the response

S = slope of the calibration curve