Supporting Information

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Dendrimer-encapsulated Pt Nanoparticles with Peroxidase-mimetic Activity as Biocatalytic Labels for Sensitive Colorimetric Analyses

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Experimental Section

Chemicals and materials Amine-terminated fourth-generation polyamidoamine dendrimers (G4-NH_2 PAMAM dendrimers), potassium tetrachloroplatinate (K_2PtCl_4), sodium borohydride ($NaBH_4$), hydrogen peroxide (H_2O_2), 3,3',5,5'-tetramethylbenzidine (TMB), *o*-phenylenediamine (OPD), 3,3'-diaminobenzidine (DAB), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), catalase (from bovine liver), D-(+)-glucose, D-lactose monohydrate, D-(+)-maltose monohydrate, D-(-)-fructose, sucrose, sodium acetate (NaAc), dimethyl sulfoxide (DMSO), phosphate buffered saline (PBS), and cellulose dialysis sacks (MW cutoff of 12,000) were obtained from Sigma-Aldrich, Inc. (USA). *N*-hydroxysuccinimide ester of biotin (EZ-Link® NHS-PEG_4-Biotin), avidin and 4'-hydroxyazobenzene-2-carboxylic acid (HABA) were purchased from Thermo Fisher Scientific, Inc. (USA). Streptavidin-conjugated glucose oxidase (Streptavidin-GOx) was used as received from Fitzgerald Industries International (USA). 18 MΩ•cm deionized (DI) water was used in the preparation of aqueous solutions (Ultra370, Younglin Co., Korea).

Synthesis of dendrimer-encapsulated Pt nanoparticle (Pt DEN) Pt DENs were synthesized as previously reported with some modification.[1-5] Briefly, 55 mol equivalent of an aqueous 10 mM K_2PtCl_4 was added to an aqueous 10 μM G4-NH_2 PAMAM dendrimer solution (pH 5). The mixture was stirred for 76 h to complex Pt ions with the intradendrimer tertiary amines. Then, a 20-fold excess of an aqueous $NaBH_4$ solution was slowly added to the Pt ion-dendrimer complex solution with vigorous stirring. The mixture solution (pH 7) was kept in a closed vial for 24 h to ensure sufficient reduction of Pt. Finally, the Pt DENs solution was dialyzed using a cellulose dialysis sack for 2 days to remove impurities. Transmission electron

microscope (TEM) images of the as-synthesized Pt DENs were collected using a Tecnai G2 F30 instrument (FEI Co., USA) operating at 200 kV. TEM samples were prepared by evaporating a drop of aqueous Pt DEN solution on 200 mesh carbon-coated copper grids (Ted Pella Inc., USA).

Peroxidase-mimetic activity measurements The peroxidase-like activity of Pt DENs was measured at room temperature in reaction mixtures containing H_2O_2 and chromogenic substrates such as TMB, DAB, OPD, and ABTS in the presence of Pt DENs. Specifically, the steady-state kinetic assays were carried out in 3 mL of 200 mM NaAc reaction buffer (pH 4.0) containing various concentrations of H_2O_2 (10 to 300 mM) and TMB (25 to 800 μM) in the presence of 30 nM Pt DENs. The reaction buffer used for OPD and ABTS was 200 mM NaAc (pH 3.5) buffer, and for DAB was 200 mM NaAc (pH 6.0) buffer. All the reactions were monitored at 652 nm in kinetic mode using Agilent 8453 UV-vis spectrometer (Agilent Tech., USA). The Michaelis-Menten constants (K_m) were calculated based on Lineweaver-Burk plots, $1/v = (K_m/V_{MAX})\cdot(1/[S]+1/K_m)$ where v is the rate of conversion, V_{MAX} is the maximum rate of conversion, and [S] is the substrate concentration. To test stability of the peroxidase-like activity of Pt DENs, Pt DEN solutions were heat-treated at 95 ℃ for 30 min and cooled to room temperature. The catalytic reactions were carried out using 30 nM of heat-treated or non-treated Pt DENs in 200 mM NaAc buffer (pH 4.0) containing 100 mM H_2O_2 and 800 μM TMB.

Conjugation of Pt DEN to glucose oxidase (GOx) For colorimetric assays of glucose, streptavidin-GOx enzymes were conjugated at the surface of Pt DENs via streptavidin-biotin chemistry. Specifically, a 20 μM Pt DEN solution was mixed with a 200 mM NHS-PEG_4-biotin solution (in DMSO) to yield a molar ratio of 1:1920. The mixture solution was stirred for 1 h and

then dialyzed using a cellulose dialysis sack for 4 days to remove any free NHS-PEG_4-biotin. After purification, the biotinylated Pt DENs were examined with HABA assay to confirm the presence of biotin moieties conjugated to the Pt DENs.[6, 7] The biotinylated Pt DENs were then mixed with streptavidin-GOx at a molar ratio of 1:1, and stirred for 2 h to conjugate the biotinylated Pt DEN to the streptavidin-GOx. The colorimetric analyses of glucose were performed as following. Glucose solutions were added into O_2-purged 200 mM NaAc buffer (pH 4.0) solutions containing 800 μM TMB and 30 nM Pt DEN-GOx conjugate, and the reaction mixture solutions were incubated at 37 ℃ for 60 min. Absorbance values of the reaction mixtures were then measured at 652 nm using Agilent 8453 UV-vis spectrometer (Agilent Tech., USA). The selectivity of the colorimetric assays of glucose was also tested using glucose analogues such as fructose, sucrose, maltose, and lactose even at 10 times higher concentration (*i.e.* 166.7 μM) than that of glucose (*i.e.* 16.67 μM).

Table S1 Comparison of apparent K_m value of Pt DENs with TMB to that of other previously reported Pt-based peroxidase mimics and natural HRP enzyme.

Nanomaterial	K_M value (mM)	Reference
Ferritin-platinum nanoparticle (Pt-Ft)	0.22	*Biomaterials*, **2011**, 32, 1611-1618
DNA Aptamer-Pt (cisplatin) complexes	1.49	*J. Biomater. Sci., Polym. Ed.*, **2010**, 21, 67-82
Pt nanoparticles	0.42	*Anal. Chim. Acta*, **2015**, 853, 360-367
BSA/Pt-NPs	0.217	*Microchim. Acta*, **2013**, 180, 1517-1522
BSA-stabilized Pt nanozyme	0.119	*Biosens. Bioelectron.*, **2015**, 66, 251-258
Pt-MoO_3 hybrid nanomaterials	0.106	*Nanoscale*, **2014**, 6, 12340-12344
DNA aptamer-Pt ($K_2[PtCl_4]$) complex	4.88	*Anal. Chem.*, **2008**, 80, 6580-6586
Pt-DNA complexes	N/A	*Anal. Methods*, **2012**, 4, 2183-2187
DNA-Pt complexes	N/A	*Biomacromolecules*, **2007**, 8, 2684-2688
Cubic Pt nanocrstals	N/A	*Colloids and Surface A ; Physicochem. Eng. Aspects.* **2011**, 373, 6-10.
DNA-based platinium nanozymes	0.056	*J. Phy Chem. C*, **2014**, 118, 18116-18125
Pt/Au nanoparticles	0.113	*Nanoscale*, **2012**, 4, 6823-6830
Au@Pt nanostructure	0.027	*Biomaterials*, **2011**, 32, 1139-1147
PdPt alloy nanodots on Au nanorods	0.0154	*Langmuir*, **2011**, 27, 2796-2803
Au@Pt core/shell nanorods	0.026	*Sens. Actuators B*, **2012**, 166-167, 708-714
HRP	0.434	*Nat. Nanotechnol.*, **2007**, 2, 577-583
Pt DENs	**0.091**	**This work**

Table S2 Comparison of selected nanomaterial-based colorimetric assays of glucose.

Nanomaterial	Linear range	LOD (μM)	Reference
PDDA-Fe_3O_4 nanoparticles	30 μM – 100 μM & 200 μM – 1000 μM	30	*Biosens. Bioelectrons*, **2010**, 26, 913-917
Au@Pt core/shell nanorods	45 μM – 400 μM	45	*Sens. Actuactors B*, **2012**, 166-167, 708-714
Fe_3O_4 magnetic nanoparticles	50 μM – 1000 μM	30	*Anal. Chem.*, **2008**, 80, 2250-2254
[Fe^{III}(biuret-amide)] on silica nanoparticles	20 μM – 300 μM	10	*Chem. Commun.*, **2012**, 48, 5289-5291
Positively-charged Au nanoparticles ((+)AuNPs)	18 μM – 1100 μM	4	*Chem. Commun.*, **2010**, 46, 8017-8019
Nanoporous nanocomposites entrapping Fe_3O_4 magnetic nanoparticles	30 μM – 1000 μM	3	*Chem. Eur. J.*, **2011**, 17, 10700-10707
Graphene oxides	1 μM – 20 μM	1	*Adv. Mater.*, **2010**, 22, 2206-2210
Fe(III)-based coordination polymer nanoparticles (FeCPNPs)	2 μM – 20 μM	1	*Catal. Sci. Technol.*, **2012**, 2, 432-436
Graphene oxide-Fe_3O_4 magnetic nanocomposite	2 μM – 200 μM	0.74	*Nanoscale*, **2012**, 4, 3969-3976
CoFe layered double hydroxide (CoFe-LDHs) nanoplates	1 μM – 10 μM	0.6	*Analyst*, **2012**, 137, 1325-1328
Carbon nitride dots (CNDs)	1 μM - 5 μM	0.5	*RSC Adv.*, **2012**, 2, 411-413
Carbon nanodots (C-Dots)	1 μM - 500 μM	0.4	*Chem. Commun.*, **2011**, 47, 6695-6697
$ZnFe_2O_4$ magnetic nanoparticles	1.25 μM – 1.875 μM	0.3	*Anal. Chem.*, **2012**, 84, 5753-5758
Chitosan stabilized Ag nanoparticles (Ch-Ag NPs)	5 μM - 200 μM	0.1	*Analyst*, **2012**, 137, 5560-5564
Hemin functionalized graphene nanosheets (H-GNs)	0.05 μM – 500 μM	0.03	*Sens. Actuators. B*, **2011**, 160, 295-300
FeTe nanorods	0.1 μM – 5 μM	0.38	*Chem. Commun.*, **2012**, 48, 4079-4081
Pt DENs	**1 μM – 50 μM**	**1**	**This work**

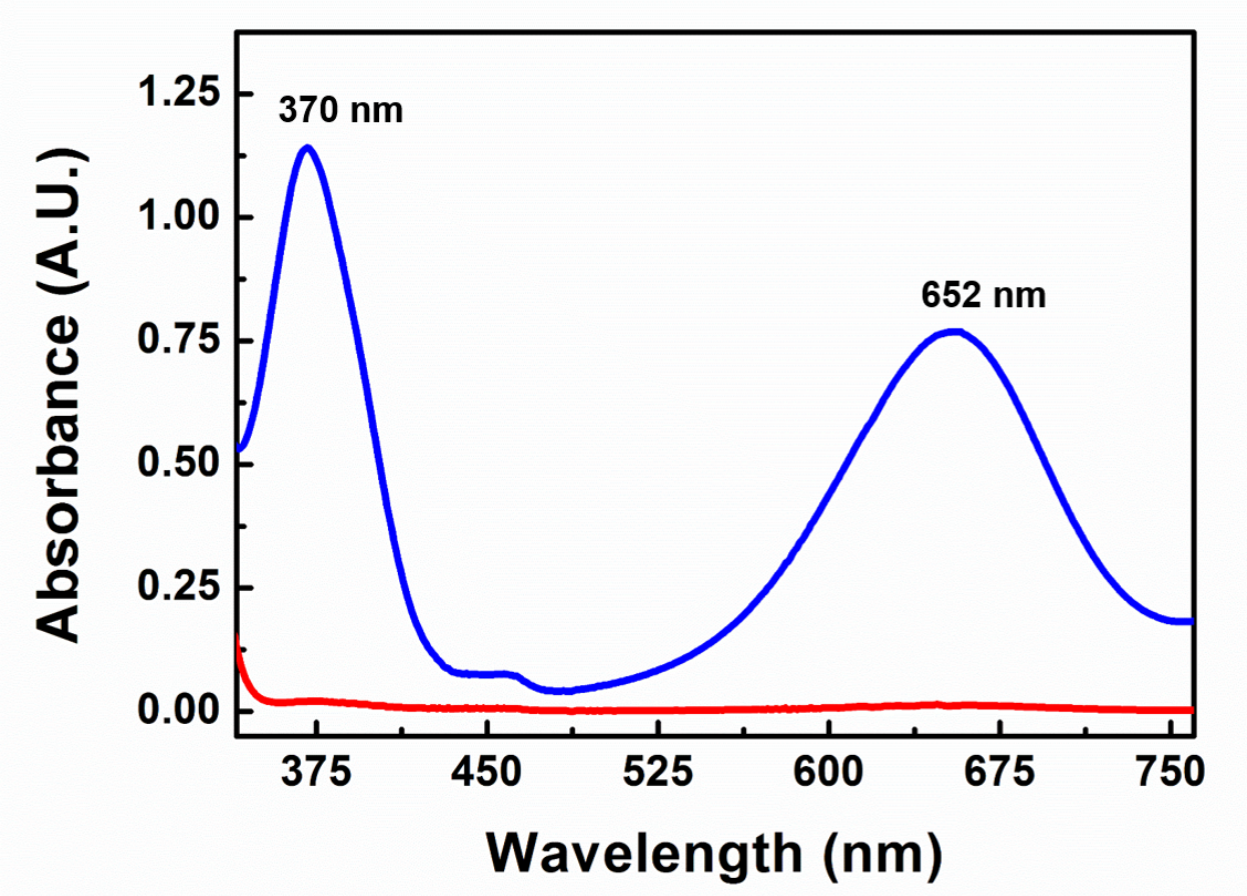

Fig. S1 Spectra of 800 μM TMB solutions (200 mM NaAc buffer, pH 4.0) containing 100 mM H_2O_2 in the presence of 30 nM G4-NH_2(Pt_{55}) DENs. Incubation times: (red) 0 s and (blue) 60 s.

Fig. S2 Photograph of an aqueous 30 nM G4-NH_2(Pt_{55}) DEN solution.

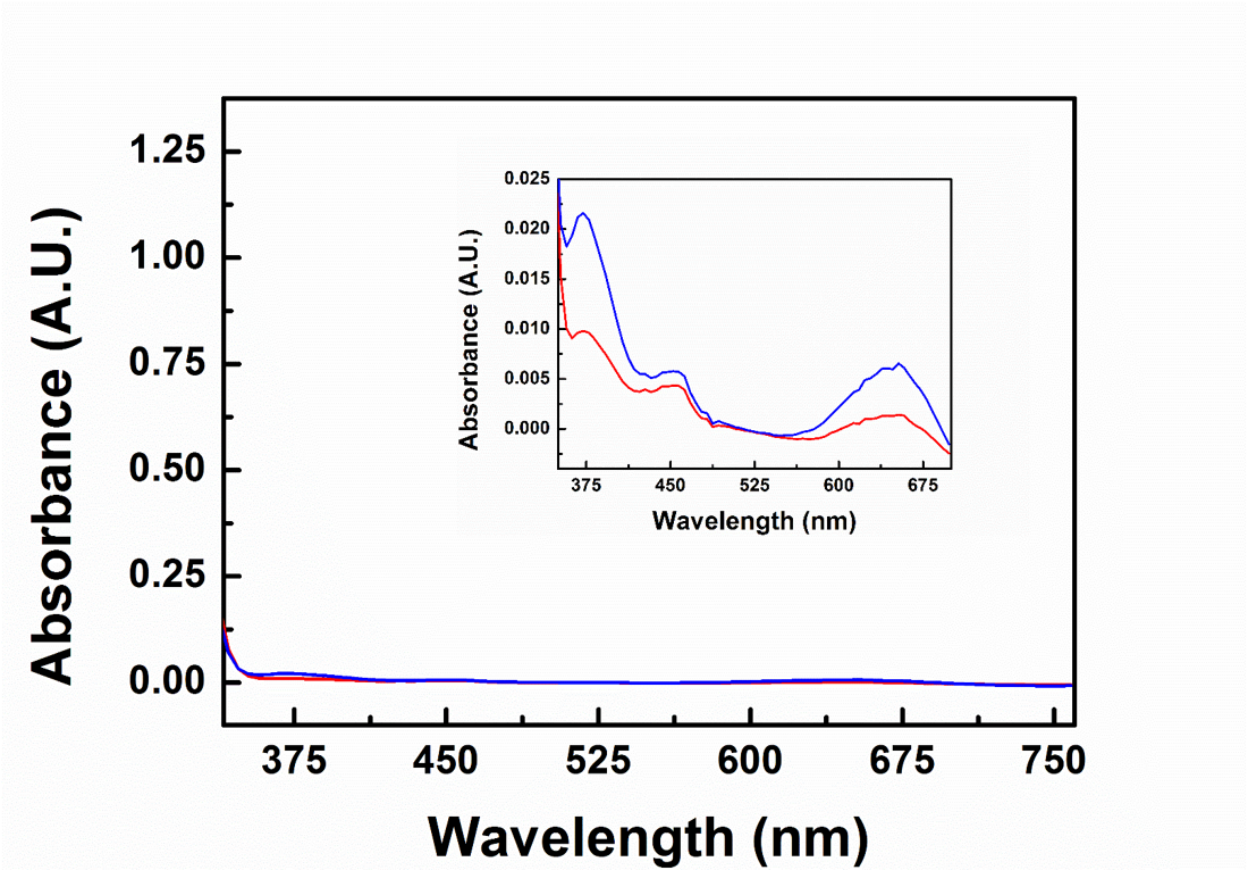

Fig. S3 Spectra of 800 μM TMB solutions (200 mM NaAc buffer, pH 4.0) (red) before or (blue) after purging oxygen gas for 10 min. Enlarged spectra are shown in inset for clear comparison. Flow rate of oxygen: ≥300 mL/min.

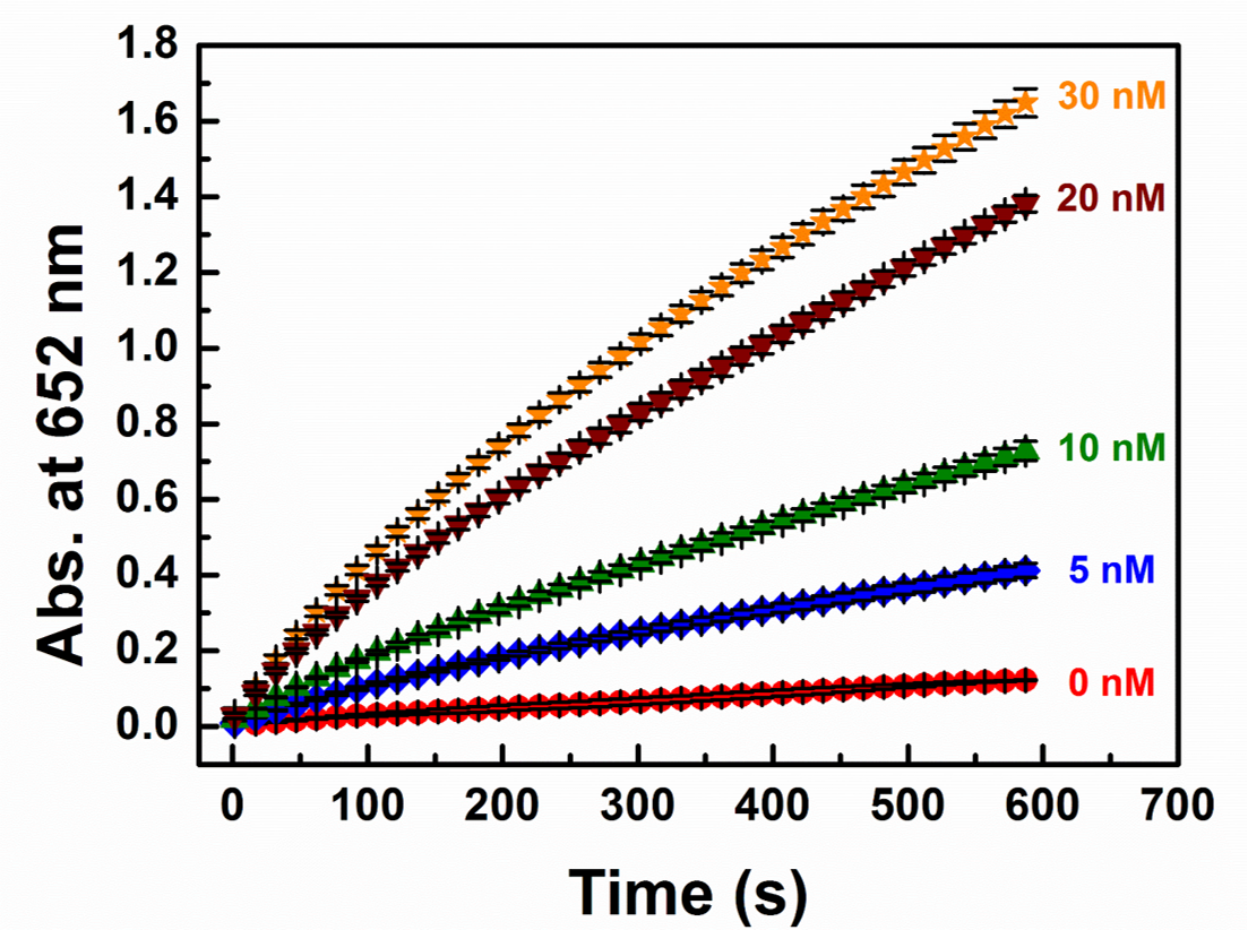

Fig. S4 Time-dependent absorbance changes at 652 nm obtained with 800 μM TMB solutions (200 mM NaAc buffer, pH 4.0) containing 100 mM H_2O_2 at different concentrations of G4-NH_2(Pt_{55}) DENs. The error bars represent the standard deviations derived from independent measurements (n=3).

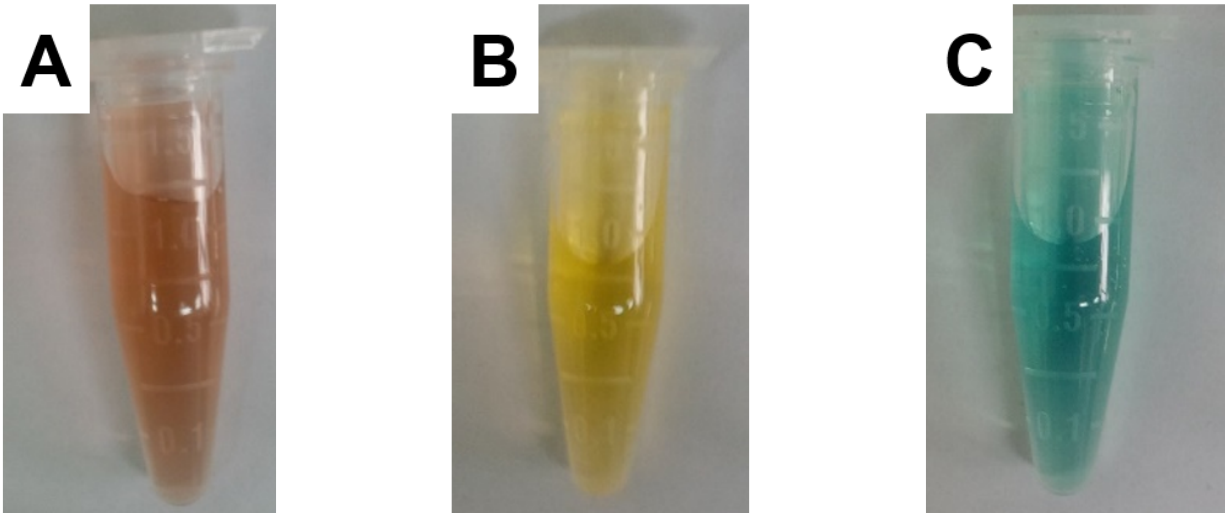

Fig. S5 Photographs of 800 μM chromogenic substrates, *i.e.* (A) DAB (200 mM NaAc buffer, pH 6.0), (B) OPD (200 mM NaAc buffer, pH 3.5), and (C) ABTS (200 mM NaAc buffer, pH 3.5), containing 100 mM H_2O_2 in the presence of 30 nM G4-NH_2(Pt_{55}) DENs. Incubation time: 30 min.

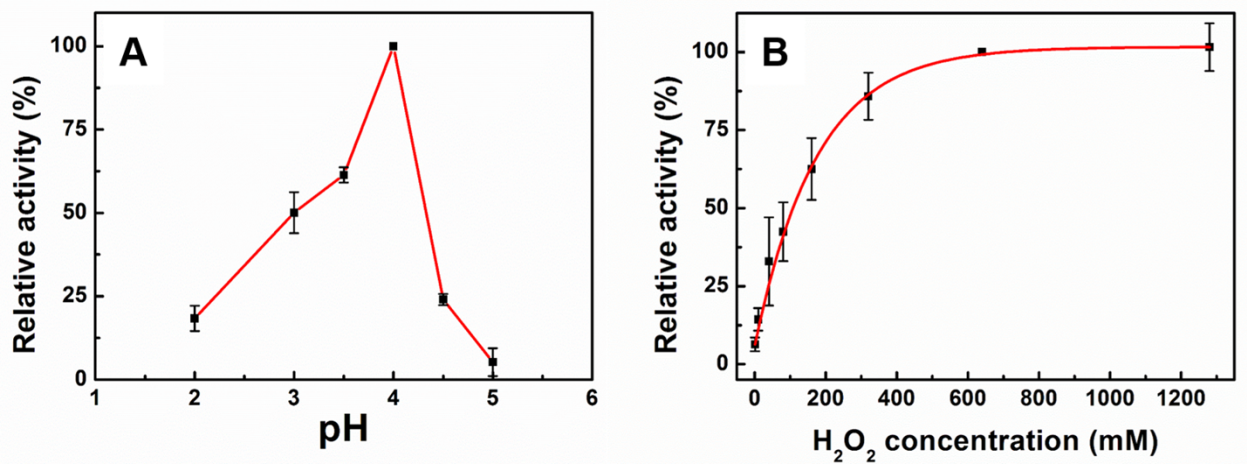

Fig. S6 Effects of (A) pH and (B) H_2O_2 concentration on the peroxidase-like activity of G4-$NH_2(Pt_{55})$ DENs. The peroxidase-like activity of G4-$NH_2(Pt_{55})$ DENs is dependent on pH and H_2O_2 concentration, which is in agreement with previously reported peroxidase-mimetic nanomaterials and natural peroxidases. The error bars represent the standard deviations derived from independent measurements (n=3).

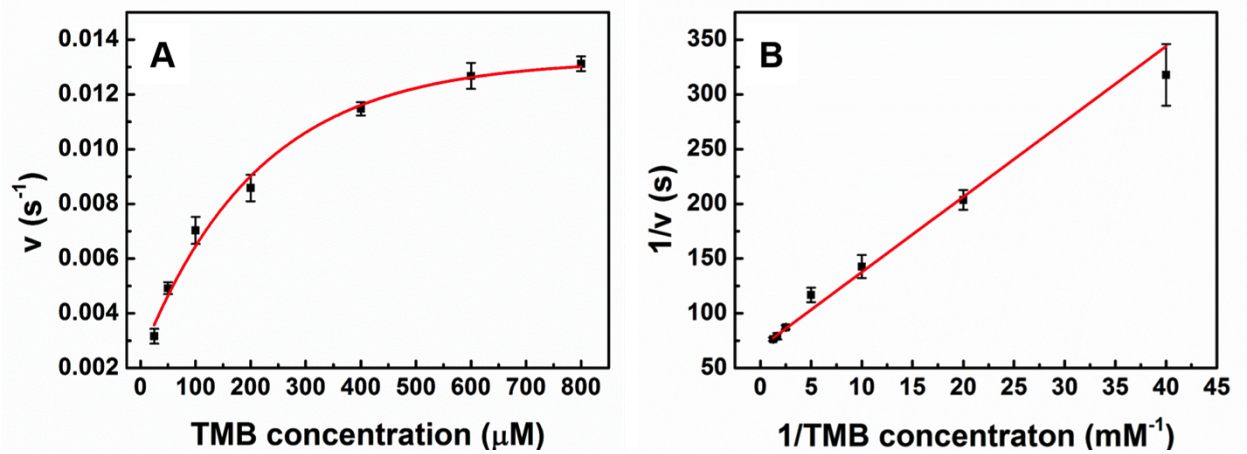

Fig. S7 Steady-state kinetic assays of heat-treated G4-NH_2(Pt_{55}) DENs. (A) Michaelis-Menten and (B) Lineweaver-Burk plots for TMB substrate. Experimental details are provided in Experimental Section. The error bars represent the standard deviations derived from independent measurements (n=3).

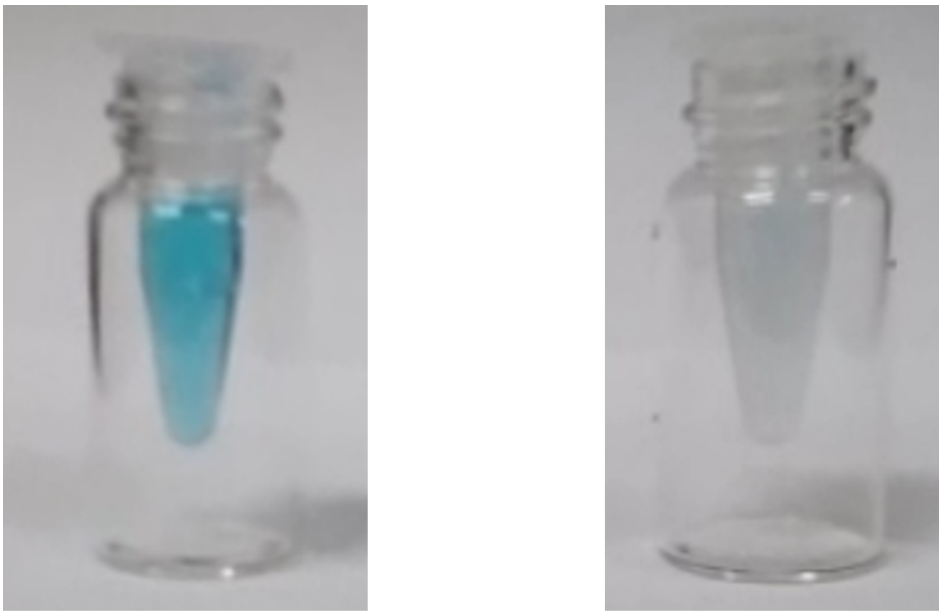

Fig. S8 Photographs of 800 μM TMB solutions (200 mM NaAc buffer, pH 4.0) containing 100 mM H_2O_2 in the presence of 300 nM G4-NH_2(Pt_{55}) DENs (left photograph) or 300 nM HRP (right photograph) after heat treatment of G4-NH_2(Pt_{55}) DENs and HRP in boiling water for ca. 3 min. Incubation time: ca. 10 s. The movie file is also provided separately, which shows the colorimetric assays carried out as described above.

References

1. R. M. Crooks, M. Zhao, L. Sun, V. Chechik and L. K. Yeung, *Acc. Chem. Res.*, 2001, **34**, 181-190.
2. H. Ju, C. M. Koo and J. Kim, *Chem. Commun.*, 2011, **47**, 12322-12324.
3. J. M. Kim, J. Kim and J. Kim, *Chem. Commun.*, 2012, **48**, 9233-9235.
4. S. B. Lee, Y. Ju, Y. Kim, C. M. Koo and J. Kim, *Chem. Commun.*, 2013, **49**, 8913-8915.
5. Y. Kim and J. Kim, *Anal. Chem.*, 2014, **86**, 1654-1660.
6. N. M. Green, *Biochem. J.*, 1965, **94**, 23c-24c.
7. V. G. Janolino, J. Fontecha and H. E. Swaisgood, *Appl. Biochem. Biotechnol.*, 1996, **56**, 1-7.