Supporting Information

Pd@Pt Nanodendrites as Peroxidase Nanomimics for Enhanced Colorimetric ELISA of Cytokines with Femtomolar Sensitivity

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Additional Materials and Methods

S1. Chemicals and Materials.

Sodium tetrachloropalladate(II) (Na_2PdCl_4, 98%), potassium bromide (KBr, ≥99%), L-ascorbic acid (AA, ≥99%), poly(vinylpyrrolidone) (PVP, M_W≈55,000), sodium hexachloroplatinate(IV) hexahydrate ($Na_2PtCl_6{\cdot}6H_2O$, 98%), 3,3',5,5'-tetramethylbenzidine (TMB, >99%), and horseradish peroxidase (HRP) were obtained from Millipore Sigma. Hydrogen peroxide solution (30 wt% in H_2O), sodium bicarbonate ($NaHCO_3$, ≥99.7%), sodium carbonate (Na_2CO_3, ≥99.5%), sodium phosphate dibasic (Na_2HPO_4, ≥99.0%), sodium chloride (NaCl, ≥99.0%), potassium phosphate monobasic (KH_2PO_4, ≥99.0%), potassium chloride (KCl, ≥99.0%), Tween 20, citric acid, bovine serum albumin (BSA, ≥98%), sucrose, and streptavidin (SA) were all obtained from VWR. Thiol-PEG-Carboxyl (M_w ≈ 3400, HS-PEG-COOH) was obtained from Laysan Bio, Inc. N-ethyl-N'-(3- (dimethylamino)propyl)carbodiimide hydrochloride (EDC, ≥98.0%) and N-hydroxysulfosuccinimide sodium salt (NHS, ≥98.0%) were obtained from Tokyo Chemical Industry Co., Itd. Human interleukin-6 (IL-6), mouse anti-cytokine capture antibody (CAb), biotin-conjugated detection antibodies (Biotin-DAb), and HRP-conjugated SA (HRP-SA) were obtained from Fisher Scientific, Inc. Deionized water (DI) with a resistivity of 18.2 MΩ·cm was used throughout the experiments. 10 mM Carbonate-bicarbonate buffer (pH 9.6) was prepared by dissolving 1.59 g of Na_2CO_3 and 2.93 g of $NaHCO_3$ in 1.0 L of DI water. 10 mM phosphate-buffered saline (PBS, pH 7.4) was prepared by dissolving 1.15 g of Na_2HPO_4, 0.24 g of KH_2PO_4, 0.2 g of KCl, and 8.0 g of NaCl in 1.0 L of DI water. Citrate-phosphate buffer (pH 4.0) was prepared by 11.825 g of Na_2HPO_4 and 2.3650 g citric acid in 1.0 L of DI water. Washing buffer was PBS (pH 7.4) containing 0.05% Tween 20 (PBST). Block-fix buffer was PBST containing 1% BSA and 15% sucrose. Dilution buffer was PBST containing 1% BSA.

S2. Characterizations.

The transmission electron microscope (TEM) images were taken using a Zeiss EM10 transmission electron microscope. The scanning electron microscopy (SEM) images and EDX spectra were obtained using a JEOL JSM-7000F microscope. The UV-vis spectra and kinetic curves of apparent steady-state kinetic assays were recorded using an Ultrospec 2100 pro UV-vis spectrophotometer (Amersham Biosciences). Dynamic light scattering (DLS) data were obtained using a Zetasizer Nano ZS90, Malvern. The absorbance of samples in wells of microplates was measured using a SpectraMax iD3 Multi-Mode Microplate Reader (Molecular Devices). Fourier-transform infrared (FT-IR) spectra were performed using a PerkinElmer Spectrum 400 FT-IR/FT-NIR Spectrometer.

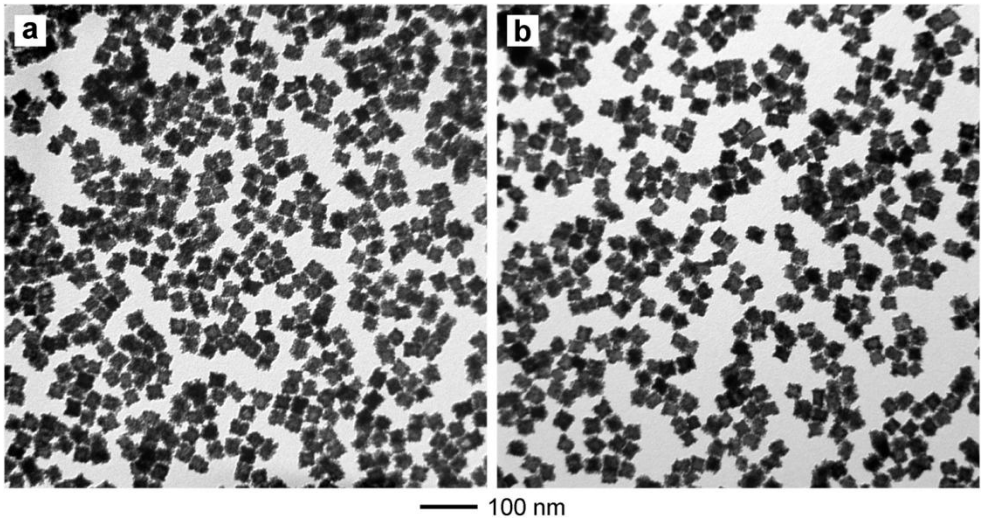

Figure S1. TEM images of Pd@Pt NDs synthesized from two other batches in addition to the batch shown in Figures 1d,e. It can be seen that these two-batch Pd@Pt NDs presented the same dendrite-like morphology as the Pd@Pt NDs shown in Figures 1d,e. The edge lengths of these two-batch Pd@Pt NDs and the Pd@Pt NDs shown in Figures 1d,e were measured to be 27.3 ± 1.4 nm, 27.2 ± 1.5 nm, and 26.8 ± 1.5 nm, respectively, with the coefficient of variation (CV) of 1.0% (n = 3). These results indicate that the Pd@Pt NDs can be readily reproduced with high uniformity.

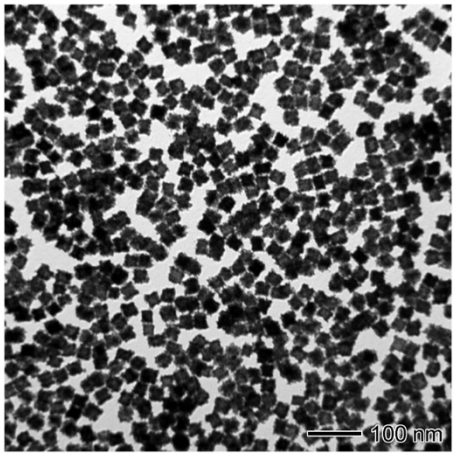

Figure S2. TEM image of Pd@Pt NDs prepared from 60-fold scale-up synthesis. Please see Materials and Methods for the detailed synthesis procedures.

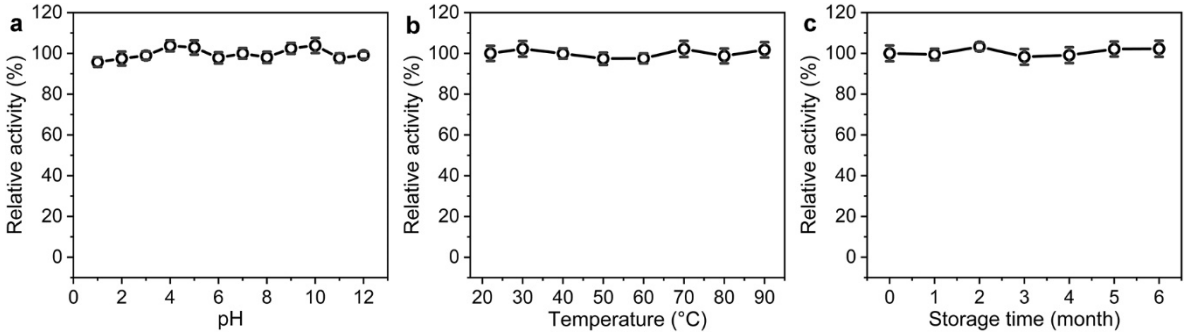

Figure S3. Stability evaluation for the peroxidase-like catalytic activity of Pd@Pt NDs. Relative catalytic activities of Pd@Pt NDs after incubation with acid or base (pH 1-12) for 2 h (**a**), treatment with heat (22-90 °C) for 2 h (**b**), and storage for different time (0-6 months, **c**), in which the activity at pH 4.0, 22 °C, and 0 month was set as 100%.

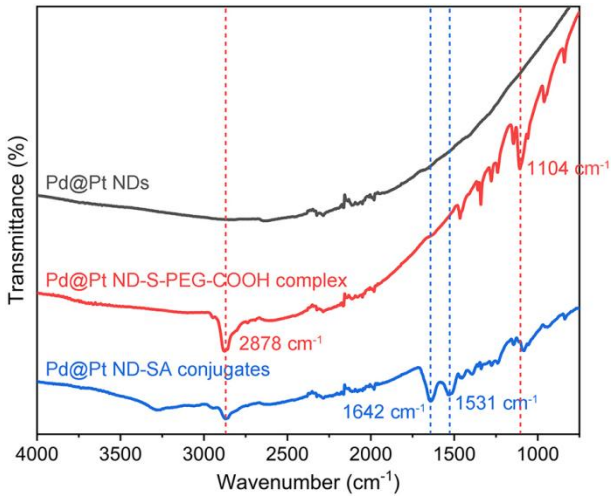

Figure S4. FT-IR spectra of Pd@Pt NDs (black curve), Pd@Pt ND-S-PEG-COOH complex (red curve), and Pd@Pt ND-SA conjugates (blue curve).

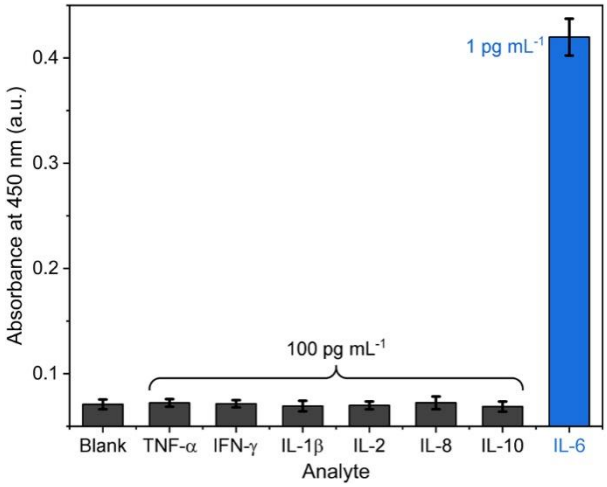

Figure S5. Specificity of Pd@Pt ND ELISA toward IL-6 detection. Bar graph showing the intensity of the detection signal for detection of IL-6 (1 pg mL^{-1}) and interfering cytokines including TNF-α, IFN-γ, IL-1β, IL-2, IL-8, and IL-10 (100 pg mL^{-1}) using Pd@Pt ND ELISA.

Table S1. Comparison of the kinetic parameters of various catalysts toward the TMB+H_2O_2 reaction.[a]

Catalyst	Size (nm)	[*E*] (M)	Substance	K_m (M)	V_{max} ($M\ s^{-1}$)	K_{cat} (s^{-1})	Refs.
HRP	N/A	2.5×10^{-11}	TMB	4.3×10^{-4}	1.0×10^{-7}	4.0×10^{3}	[30]
		2.5×10^{-11}	H_2O_2	3.7×10^{-3}	8.7×10^{-8}	3.5×10^{3}	
Fe_3O_4 particles	300 (diameter)	1.1×10^{-12}	TMB	9.8×10^{-5}	3.4×10^{-8}	3.0×10^{4}	[30]
		1.1×10^{-12}	H_2O_2	1.5×10^{-1}	9.8×10^{-8}	8.6×10^{4}	
Co_3O_4 cubes	20 (edge length)	3.4×10^{-10}	TMB	3.7×10^{-5}	6.3×10^{-8}	1.8×10^{2}	[31]
		3.4×10^{-10}	H_2O_2	1.4×10^{-1}	1.2×10^{-7}	3.5×10^{2}	
MnO_2 particles	4.5 (diameter)	3.0×10^{-8}	OPD	3.1×10^{-4}	8.2×10^{-8}	2.7×10^{0}	[66]
		3.0×10^{-8}	H_2O_2	1.2×10^{-4}	5.7×10^{-8}	1.9×10^{0}	
V_2O_5 wires	100×500 (width×length)	1.1×10^{-4}	ABTS	4.0×10^{-7}	2.8×10^{-1}	2.5×10^{3}	[33]
		N/A	H_2O_2	2.9×10^{-6}	N/A	N/A	
Au particles	40 (diameter)	6.7×10^{-12}	TMB	N/A	4.8×10^{-8}	7.2×10^{3}	[52]
		N/A	H_2O_2	N/A	N/A	N/A	
Ru frames	10 (diameter)	1.1×10^{-12}	TMB	6.0×10^{-5}	1.3×10^{-7}	1.3×10^{4}	[42]
		1.1×10^{-12}	H_2O_2	3.8×10^{-1}	7.4×10^{-8}	7.0×10^{3}	
Au@Pt rods	30×70 (width×length)	1.3×10^{-11}	TMB	2.7×10^{-5}	1.8×10^{-7}	1.4×10^{4}	[63]
		N/A	H_2O_2	N/A	N/A	N/A	
Pt particles	5-7 (diameter)	8.1×10^{-11}	TMB	1.2×10^{-4}	1.3×10^{-6}	2.3×10^{4}	[62]
		8.1×10^{-11}	H_2O_2	7.7×10^{-1}	1.9×10^{-6}	1.6×10^{4}	
Pd cubes	18 (edge length)	1.4×10^{-12}	TMB	5.4×10^{-5}	9.7×10^{-8}	6.9×10^{4}	[65]
		1.4×10^{-12}	H_2O_2	7.0×10^{-1}	6.5×10^{-8}	4.6×10^{4}	
Pd-Ru cubes	20 (edge length)	N/A	TMB	N/A	N/A	4.8×10^{5}	[67]
		N/A	H_2O_2	N/A	N/A	N/A	
Pt cubes	7.4 (edge length)	4.1×10^{-13}	TMB	7.3×10^{-4}	3.3×10^{-7}	8.2×10^{5}	[68]
		N/A	H_2O_2	N/A	N/A	N/A	
Au@Pt particles	42 (diameter)	6.3×10^{-14}	TMB	N/A	9.7×10^{-8}	1.5×10^{6}	[52]
		N/A	H_2O_2	N/A	N/A	N/A	
Pd-Ir cubes	19.2 (edge length)	3.4×10^{-14}	TMB	1.3×10^{-4}	6.5×10^{-8}	1.9×10^{6}	[65]
		3.4×10^{-14}	H_2O_2	3.4×10^{-1}	5.1×10^{-8}	1.5×10^{6}	
Pd@Pt cubes	20 (edge length)	2.6×10^{-13}	TMB	3.4×10^{-4}	6.0×10^{-7}	2.3×10^{6}	[69]
		2.6×10^{-13}	H_2O_2	7.7×10^{-1}	5.8×10^{-7}	2.2×10^{6}	
Concave Pt cubes	44 (diameter)	2.5×10^{-14}	TMB	N/A	1.5×10^{-7}	6.0×10^{6}	[51]
		2.5×10^{-14}	H_2O_2	N/A	1.3×10^{-7}	5.1×10^{6}	
Pd@Pt NDs	27 (edge length)	2.44×10^{-14}	TMB	6.63×10^{-4}	2.21×10^{-7}	9.06×10^{6}	This work
		2.44×10^{-14}	H_2O_2	3.82×10^{0}	1.86×10^{-7}	7.62×10^{6}	

[a] [*E*] is the catalyst concentration, K_m is the Michaelis-Menten constant, V_{max} is the maximal reaction rate, K_{cat} is the catalytic constant, where $K_{cat}=V_{max}/[E]$, and N/A is "not applicable". The mechanism behind the catalysis of Pd@Pt NDs toward the TMB+H_2O_2 reaction was proposed as shown in the following schematic, in which the catalytic effect of Pd@Pt NDs on H_2O_2 decomposition could be better understood [62,65].

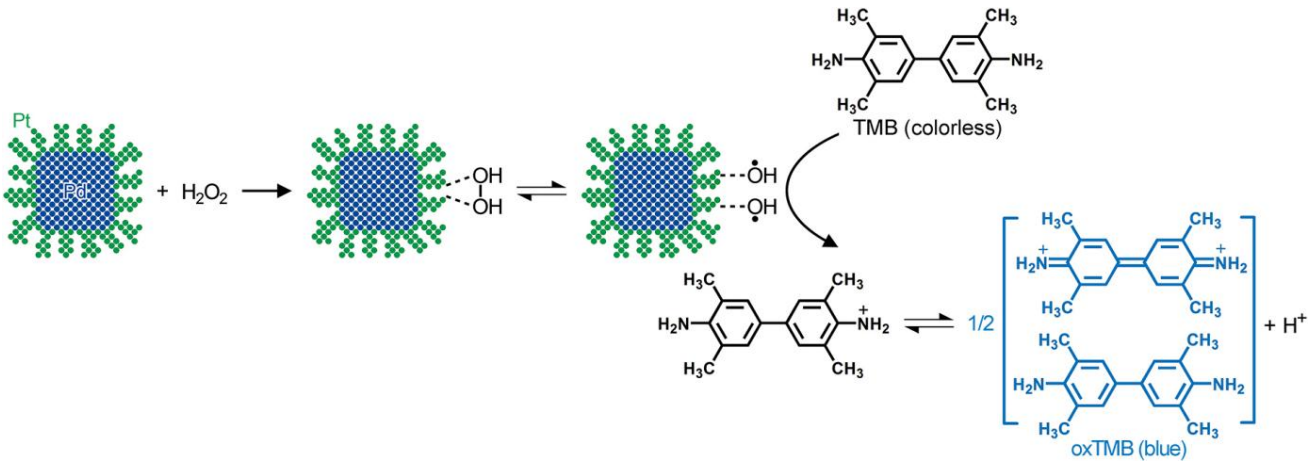

Table S2. Comparison of the limits of detection (LODs) of colorimetric Pd@Pt ND ELISA with some different commercial colorimetric ELISA kits in IL-6 detection.

Detection method	Manufacturer	Catalog number	LOD (pg mL^{-1})	LOD (fM)
Commercial ELISA kits	Fisher Scientific	KAC1261	2	76.9
		KHC0061	2	76.9
		EH2IL6	1	38.5
		BMS213-2	0.92	35.4
	Milllipore Sigma	RAB0306-1KT	3	115.4
		RAB0307-1KT	3	115.4
	Abcam	ab100573	30	1,153.8
		ab100572	3	115.4
		ab46027	2	76.9
		ab178013	1.6	61.5
		ab46042	0.8	30.8
	R&D Systems	QK206	2.95	113.5
		D6050	0.7	26.9
Pd@Pt ND ELISA	This work	This work	0.044	1.7

Table S3. Intra- and inter-batch coefficients of variation (CVs, n = 6) of Pd@Pt ND ELISA in detecting 0.1, 1, and 10 pg mL^{-1} IL-6 standards.

	IL-6 conc. (pg mL^{-1})	Absorbance at 450 nm (a.u.)						Mean (a.u.)	Standard deviation (SD, a.u.)	CV (%, n = 6)
		1	**2**	3	4	5	6			
Intra-batch assay	**0.1**	**0.0992**	**0.0981**	0.0911	0.1020	0.0992	0.0894	0.0965	0.0050	5.22
	1	0.4276	0.4005	0.4325	0.4105	0.4390	0.4044	0.4191	0.0160	3.82
	10	2.1794	1.9950	2.2002	2.2437	2.1337	1.9646	2.1194	0.1142	5.39
Inter-batch assay	0.1	0.1052	0.1024	0.0990	0.0891	0.0833	0.0943	0.0956	0.0083	8.70
	1	0.4447	0.4303	0.4253	0.4052	0.3862	0.4328	0.4208	0.0213	5.06
	10	2.2067	2.3171	2.0749	2.0527	1.9167	2.2948	2.1438	0.1558	7.27