

Supporting Information

Plasmon-Activated Nanozymes with Enhanced Catalytic Activity by Near-Infrared Light Irradiation

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1. Experimental details:

1.1 Chemicals and equipment

Hexadecyl trimethyl ammonium Chloride (CTAC, 97%), Hexadecyl trimethyl ammonium Bromide (CTAB, $\geq 99\%$), sodium borohydride (NaBH_4 , $\geq 99\%$), L-ascorbic acid (AA, $\geq 99\%$), silver nitrate (AgNO_3 , $\geq 99\%$), hydrochloric acid (HCl, 37%), 3,3',5,5'-tetramethylbenzidine (TMB, $\geq 98\%$), hydrogen peroxide solution (30 wt. % in H_2O), acetic acid (HOAc, $\geq 99.5\%$), sodium acetate (NaOAc, $\geq 99\%$), dimethyl sulfoxide (DMSO, $\geq 99\%$). All aqueous solutions were prepared using deionized (DI) water with a resistivity of 18.0 M Ω cm.

UV-Vis absorbance spectra of the reaction solutions were performed using a UV-1750 UV-Vis spectrophotometer. The transmission electron microscope images of the morphologies of samples were characterized using FEI Tecnai G2 F20 S-TWIN microscope. The X-ray photoelectron spectroscopy spectra of samples were characterized using Thermo Scientific ESCALAB 250Xi. The temperature of the environment was controlled by using DK-S11 electric-heated thermostat water bath. The NIR light source of 808 nm was supplied by Diode Laser System for light irradiation to the catalytic reaction solutions. The temperature of the catalytic reaction solutions was recorded by the Infrared Thermal Imager (TVS-500, Avio). The cells were observed by a Nikon Eclipse Ti inverted fluorescence microscope (Nikon Japan).

1.2 Synthesis of GNRs

First, we synthesis of Au seed, which is performed in a water bath at around 27 °C. Adding 25 μL of 50 mM HAuCl_4 solution to 4.7 mL of 0.1 M CTAB, the mixture is slowly stirred for 5 min. Then, 300 μL of a freshly prepared 10 mM NaBH_4 solution is rapidly injected under vigorous stirring (1400 rpm) for 1 min, then the solution is mildly stirred (400 rpm) for 5 min, then left undisturbed at 27 °C for 2 hours. Prepared seed solution for further use. Then we synthesis of GNRs, which performed in a water bath at 30 °C. Adding 760 μL 1 M HCl and 400 μL 50 mM HAuCl_4 solution to 40 mL 0.1 M CTAB, the mixture is gently shaken and stirred for 5 min; Subsequently, 210 μL 10 mM AgNO_3 solution is added to the mixture, 400 μL 100 mM ascorbic acid solution is then added to the growth solution and thoroughly shaken, After few seconds, 96 μL of seed is added to the mixture and the solution is vigorously shaken and then left undisturbed at 30 °C for 2 hours. The resultant sample was centrifuged at 10000 rpm for 10 min. The precipitate was redispersed into the water for further use¹.

1.3 Synthesis of Pt-GNRs

Got the above GNRs 2 mL, make the longitudinal dipolar plasmon peak extinction value is ~ 1 when measured with a 0.5 cm cuvette. Then the Au NRs were centrifuged at 7000 rpm for 10 min. The precipitate was redispersed into a CTAC solution (2 mL, 0.08 M), After that, we added 10 μL 0.01 M AgNO_3 and 5 μL 0.1 M ascorbic acid into the mixture. The mixture solution was placed in an air-bath shaker (60 °C, 300 rpm) and kept for 4.5 h. The resultant sample was centrifuged twice at 6000 rpm for 10 min. The precipitate was redispersed into a CTAB solution (2 mL, 0.003 M). And then adding several volume of H_2PtCl_4 (0.001 M) and the same volume of ascorbic acid (0.01 M) under gentle shaking. The reaction was left undisturbed overnight at room temperature. The resultant sample was centrifuged at 2000 rpm for 10 min to remove spherical impurity. Then added some volume of 6 M HNO_3 to the supernatant to remove the residual silver. The resultant sample was centrifuged twice at 5000 rpm for 10 min. The precipitate was redispersed into water

for further use².

1.4 Peroxidase-like catalytic activity of Pt-GNRs.

To investigate the peroxidase-like catalytic activity of Pt-GNRs. TMB could be oxidized in the presence of H₂O₂ to produce a blue product, which has a strong absorption at 652 nm. A typical colorimetric experiment is as follows: in a reaction volume of 250 μ L NaOAc/HOAc buffer (0.2 M, pH 4.0) with 1.6 mM TMB, 2.3×10^{-11} M Pt-GNRs and 20 mM H₂O₂. After reaction for 20 min at room temperature, the UV-Vis absorption spectra of the sample can be got by the UV-Vis spectrometer.

1.5 Kinetic assays

The steady-state kinetic assays were performed in a typical colorimetric experiment in a reaction volume of 250 μ L of NaOAc/HOAc buffer (0.2 M, pH 4.0) containing 2.3×10^{-11} M Pt-GNRs, 1.6 mM TMB as substrate and different concentrations of H₂O₂ (from 10 to 50 mM) at room temperature. The Michaelis-Menten equation $1/V = K_m/V_m (1/[S] + 1/K_m)$, where V is the initial velocity, V_m represents the maximal reaction velocity, [S] corresponds to the concentration of substrate, and K_m is the Michaelis constant. Apparent kinetic parameters were calculated using Line weaver-Burk plots or the double reciprocal plots²⁶.

1.6 Cell culture and cytotoxicity assay

4T1 cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) medium containing 10% fetal bovine serum and 1% streptomycin-penicillin at 37 °C with a 5% CO₂ humidified atmosphere. Cells were seeded into 96-well plates at a density of 7×10^3 per well and incubated for 12 h. Then, the media of the 96-well plates were replaced by fresh media that containing various concentrations of GNRs or Pt-GNRs and incubated for 24 h. The cell viability was evaluated by 3-(4,5 dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. For live and dead cells observation, the cells of each group were incubated with calcein acetoxymethyl (Calcein AM) and propidium iodide (PI), and then observed by a Nikon Eclipse Ti inverted fluorescence microscope (Nikon Japan).

1.7 In vitro cytotoxicity assays of Pt-GNRs nanozyme

4T1 cells were seeded into 96-well plate at a density of 7×10^3 per well for 12 h, Then, the media of the 96-well plates were replaced by fresh media that containing various concentrations of GNRs or Pt-GNRs and incubated for 4 h. Subsequently, the cells were irradiated with an 808 nm laser at a power density of 1 W/cm² for 5 min. After incubated for another 20 h, the cell viability was evaluated by standard MTT assay. For live and dead cells observation, the cells of each group were stained by Calcein AM and PI, and then observed by a Nikon Eclipse Ti inverted fluorescence microscope (Nikon Japan).

1.8 Intracellular reactive oxygen species (ROS) assay.

The 2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA) was used as a probe to detect the ROS generation. 4T1 cells were seeded into 12-well plate at a density of 7×10^4 per well, after 12 h incubation, the media were replaced by fresh media that containing various concentrations of GNRs or Pt-GNRs. After 4 h of incubation, the cells were irradiated with an 808 nm laser at a

power density of 1 W/cm^2 for 5 min per well. After incubated for another 20 h, the cells were stained with DCFH-DA (Ex/Em = 495/529 nm) for 30 min, and observed by a fluorescence microscope (Nikon, Japan).

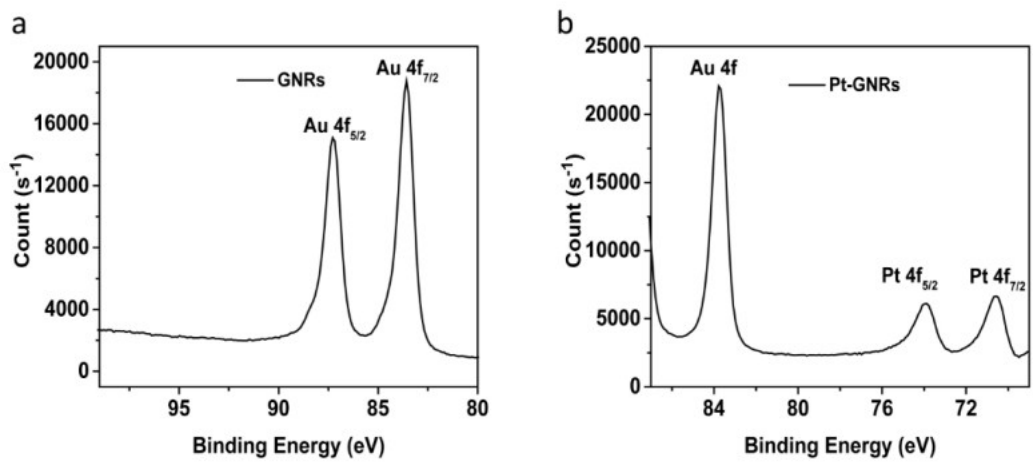


Figure S1 High-resolution XPS spectra of (a) GNRs, (b) Pt-GNRs.

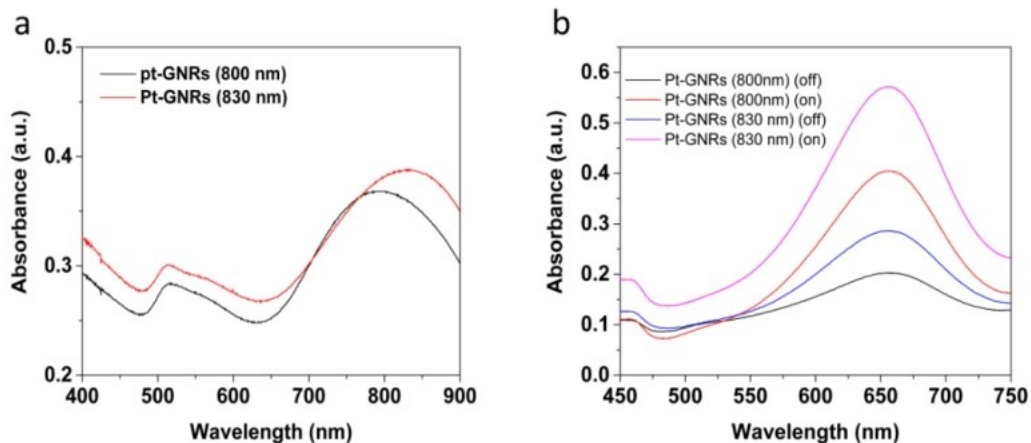


Figure S2 (a) UV-Vis-NIR spectra of the Pt-GNRs (800 nm) and Pt-GNRs (830 nm). (b) UV-Vis spectra of the enzyme-like system containing 1.6 mM TMB and 20 mM H₂O₂ at a reaction time of 20 min with and without illumination (808 nm, 400 mW/cm²). on (light on), off (light off).

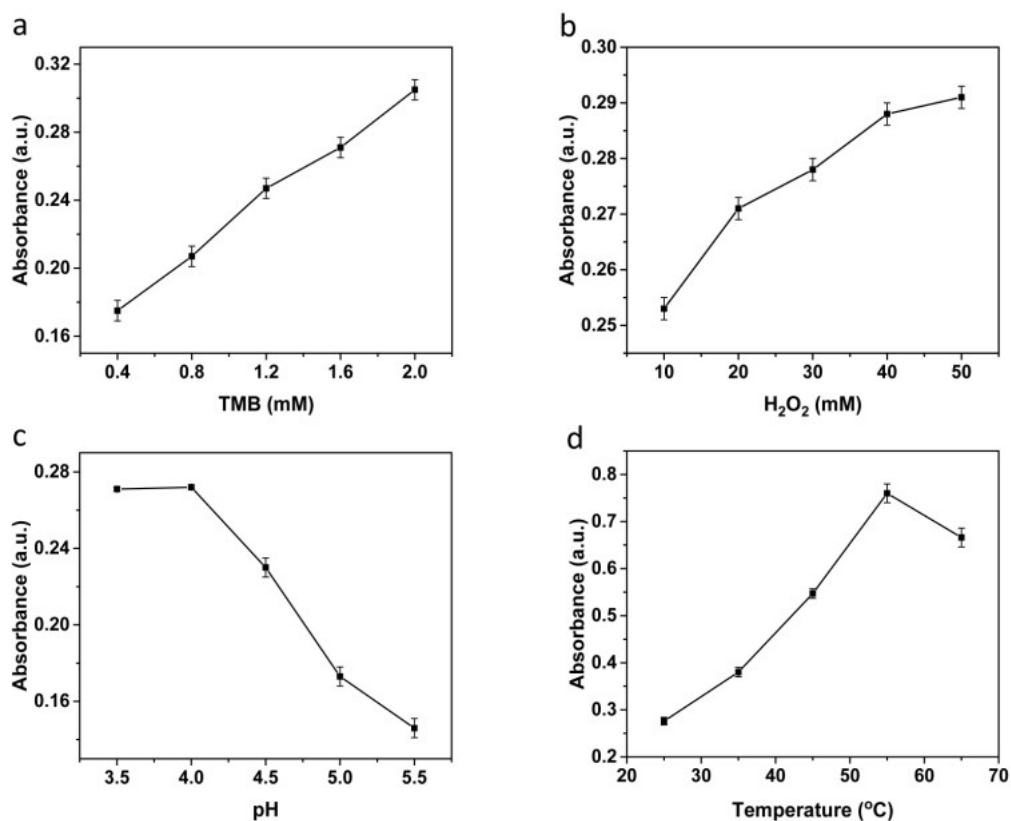


Figure S3 Response of the enzyme-like activity of Pt-GNRs: (a) TMB concentration, (b) H₂O₂ concentration, (c) pH, (d) temperature.

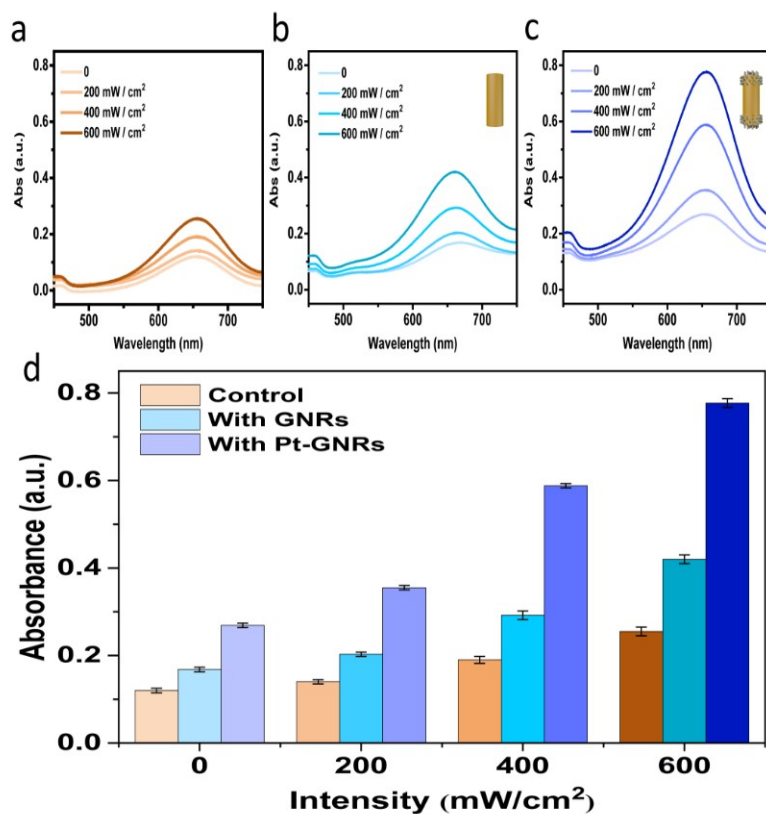


Figure S4 The total volume of the system is 250 μL , containing 1.6 mM TMB, 20 mM H_2O_2 , (a) the UV/Vis spectra absorbance were recorded after 20 min under different light intensity (0, 200, 400, 600mW/cm²) without adding enzyme, (b) with adding GNRs, (c) with adding Pt-GNRs. (d) the absorbance at 652 nm was recorded after 20 min under different light intensity(0, 200, 400, 600mW/cm²) without adding an enzyme, with adding GNRs and adding Pt-GNRs

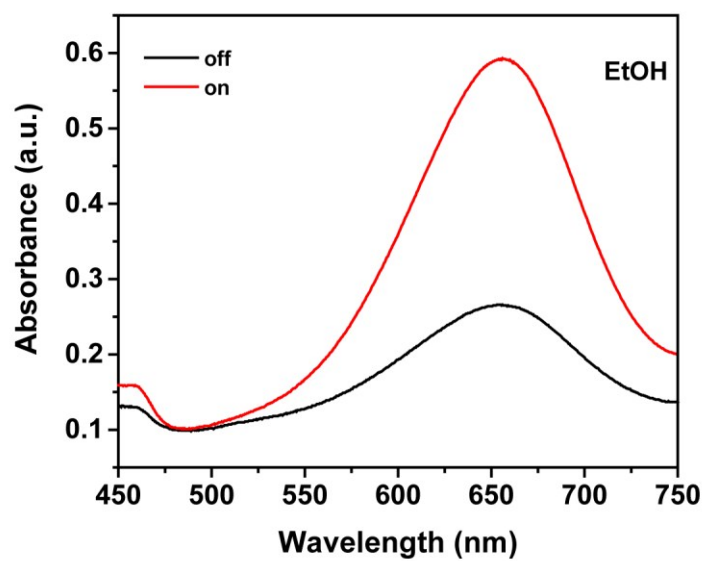


Figure S5 The total volume of the system is 250 μL , containing 1.6 mM TMB, 20 mM H_2O_2 , UV/Vis spectra of the EtOH system with the light on at 400 mW/cm^2 and off. The concentration of EtOH solution was 1 vol%

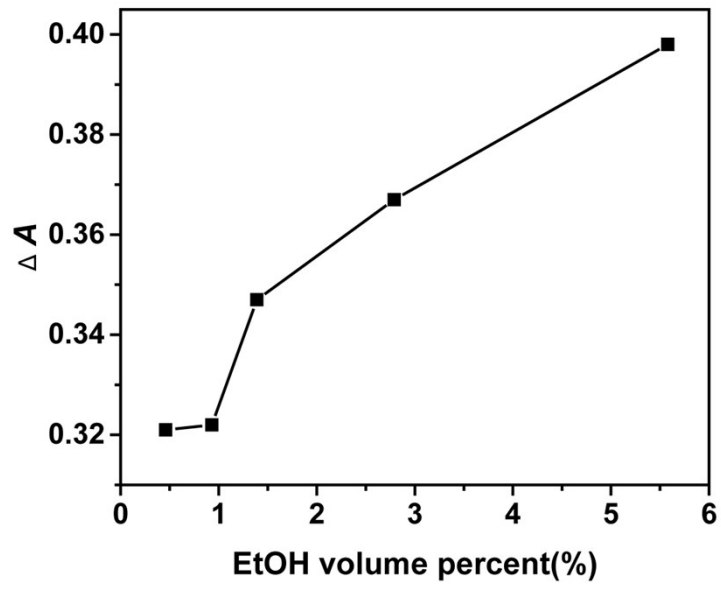


Figure S6 The change of the absorbance at 652 nm of the EtOH system with light on at 400 mW/cm² for 20 min with different EtOH volume percent.

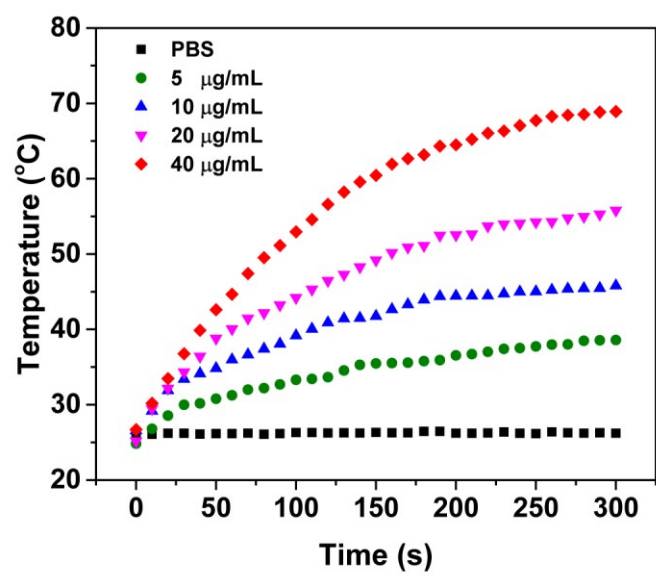


Figure S7 Concentration-dependent temperature increase after Pt-GNRs exposed to NIR laser.

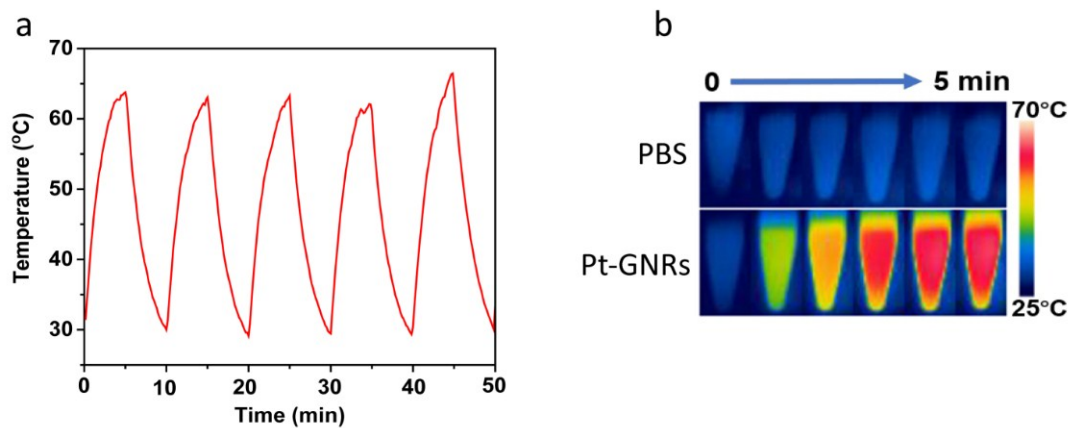


Figure S8 (a) Photothermal stability of Pt-GNRs nanozyme in five cycles of laser irradiation. (b) the infrared thermal images of PBS solution and Pt-GNRs nanozyme with a concentration of 40 $\mu\text{g}/\text{mL}$ under 808 nm laser irradiation ($1 \text{ W}/\text{cm}^2$).

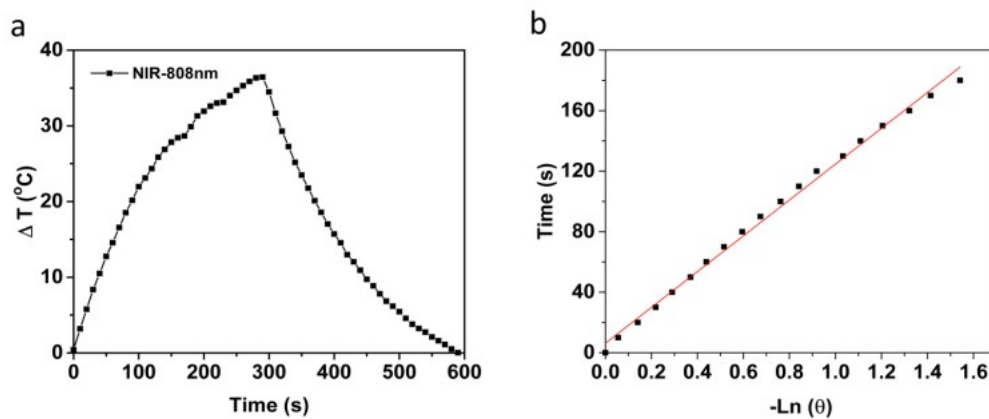


Figure S9 (a) The change of the temperature of Pt-GNRs (40 $\mu\text{g}/\text{mL}$) nanozyme under laser irradiation of 808 nm laser with the power density of $1 \text{ W}/\text{cm}^2$ in one cycle. (b) The time constant for heat transfer from our system is determined to be $\tau_s = 118.2 \text{ s}$ applying the linear time data from the cooling period versus negative logarithm of driving force temperature, which is obtained from the cooling stage of curve of (a)

Table S1 The result of element statistics of Pt-GNRs measured by EDX.

Element	Type	<i>k-factor</i>	Wt %
Pt	L	2.373	15.62
Au	L	2.399	84.38
Total:			100

Table S2 Comparison of the kinetic parameters of Pt-GNRs and GNRs between light on and off, K_m is apparent Michaelis–Menten constant, V_m is maximum reaction rate, and k_{cat} is catalytic constant.

		K_m (mM)	V_m (M·S ⁻¹)	K_{cat} (S ⁻¹)
Pt-GNRs	off	6.73	7.73×10^{-9}	3.34×10^2
Pt-GNRs	on	14.43	1.89×10^{-8}	8.21×10^2
GNRs	off	3.98	4.96×10^{-9}	2.15×10^2
GNRs	on	9.28	8.59×10^{-9}	3.73×10^2

References

- 1 L. Scarabelli, A. Sánchez-Iglesias and J. Pérez-Juste, Chem. Lett, 2015, 6, 4270-4279.
- 2 X. Zhu, H. K. Yip, X. Zhuo, R. Jiang and L. Chen, J. Am. Chem. Soc, 2017, 139, 13837-13846.