Supporting Information for

Single Molecular Catalysis Identifying Activation Energy of Intermediate Product and Rate-limiting Step in Plasmonic Photocatalysis

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Experimental Procedures

1. Materials

Gold(III) chloride trihydrate (HAuCl$_4$·3H$_2$O ≥99.9%) was purchased from J&K Scientific Ltd. Ascorbic acid (AA ≥99.0%), silver nitrate (>99%), sodium borohydride (NaBH$_4$, 98%), cetyltrimethylammonium bromide (CTAB ≥99%) were obtained from Sigma-Aldrich. Amplex Red was obtained from Thermo Fisher Scientific. H$_2$O$_2$ was obtained from Sinopharm Chemical Reagent Co., Ltd. Au NRs were synthesized according to a seed-mediated growth approach.$^1$

2. Experimental Section

2.1 Fabrication of temperature-controllable flow cell

A temperature-controllable flow cell, 2 cm (length)×5 mm (width)×100 μm (height), formed by double-sided tapes sandwiched between quartz slide and borosilicate coverslip, was used as reactor. The borosilicate coverslip was silanized using (3-Mercaptopropyl) trimethoxysilane for immobilizing Au NRs. Briefly, 50 μL of 3.3 pM Au NRs solution was dropped on the silanized slide and incubated for 30 min, and then rinsed with excess water to remove unbound Au NRs. Substrate solution containing AR and H$_2$O$_2$ was continuously flowed into the inner pool at a rate of 30 μL/min through a syringe pump (LSP02-1B, Longer). Temperature of the reactor was controlled by circulating water from water bath, while the precise temperature inside the reactor was monitor by a thermocouple.$^2$ The picture of the flow cell setup was provided as Figure S1.
Figure S1. The picture of the flow cell: (A) The vertical view of the flow cell. (B) The lateral view of the flow cell. The red dash line represents the substrate cell. The area between red dash line and black dash line represents water bath part. The temperatures of the substrate cell and hot water were monitored by two thermocouples. (C) The readout of the two thermocouples.

To exclude the possible temperature gradient in the chamber, two thermocouples were used to monitor the temperature of water bath and reactor, respectively, in order to ensure the uniform temperature. After hot water was continuously flowed through the reactor for 5 min, we recorded temperature readout from the two thermocouples. Readout of the two thermocouples at different temperature was listed in Table S1.

Table S1 Temperature drop in the chamber

<table>
<thead>
<tr>
<th></th>
<th>Thermocouple 1 (T1)</th>
<th>Thermocouple 2 (T2)</th>
<th>ΔT (K)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Temperature (K)</td>
<td>Temperature (K)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(t = 5 min)</td>
<td>(t = 30 min)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>298</td>
<td>298</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>298</td>
<td>298</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>Temperatures (K)</td>
<td>ΔT (K)</td>
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</tr>
<tr>
<td></td>
<td>(t = 5 min)</td>
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<td>0</td>
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<tr>
<td></td>
<td>303</td>
<td>303</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Thermocouple 1 (T1)</td>
<td>Thermocouple 2 (T2)</td>
<td></td>
</tr>
<tr>
<td>---</td>
<td>---------------------</td>
<td>---------------------</td>
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<tr>
<td>4</td>
<td>313</td>
<td>313</td>
<td></td>
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</tbody>
</table>

2.2 Single molecule fluorescence experiments

Single molecule fluorescence experiments were conducted on a commercial total internal reflection fluorescence microscopy (N-storm, Nikon) with a high numerical aperture oil immersion 100× objective lens (NA 1.49) and electron multiplying charge coupled devices (EMCCD) camera (Andor, iXon 3). An external circularly polarized 785 nm laser beam (1-200 mW) was used to illuminate Au NRs. The laser beam was perpendicular to the interface between objective lens and the borosilicate coverslip, and focused on an area of ~40 × 40 μm² on the cover slide. The laser intensity in the reactor spot was measured by a laser power meter and suggested to be 54 mW. The fluorescence of Rf was excited by 561 nm laser and collected with a resolution of 30 ms/frame at different temperatures (298, 303, 308, 313 K). The fluorescence trajectories were analyzed through a home-written MATLAB software.
3. Control experiments confirming the fluorescence burst is a result of Au NRs-catalyzed fluorogenic reaction

Several control experiments were implemented to confirm that the digital fluorescence bursts were originated from single Au NRs catalyzed reaction: (1) Substrate solution containing AR and H$_2$O$_2$ were flowed over the reaction cell in the absence of immobilized Au NRs, no digital fluorescence bursts were observed (Figure S2), suggesting the fluorescence bursts were results of catalytic reaction; (2) Rf was flowed over the reaction cell containing immobilized Au NRs, again no digital fluorescence bursts were observed (Figure S3), suggesting the fluorescence bursts are not results of binding/unbinding of free Rf to Au NRs; (3) laser intensities dependence $\tau_{off}^{-1}$ and $\tau_{on}^{-1}$ were measured, both $\tau_{off}^{-1}$ and $\tau_{on}^{-1}$ were independent on the 561 nm laser intensities, indicating that the fluorescence bursts were not photoinduced (Figure S4). Therefore, from these control experiments, we safely concluded that the variation of the fluorescence intensity was caused by the Au NRs-catalyzed fluorogenic reaction.

![Figure S2](image)

**Figure S2.** (A) TIRFM image and corresponding fluorescence trajectory on glass slide in the presence of AR and H$_2$O$_2$. (B) TIRFM image and corresponding fluorescence trajectory on Au NRs modified glass slide in the absence of AR or H$_2$O$_2$.

![Figure S3](image)

**Figure S3.** TIRFM image and corresponding fluorescence trajectory on Au NRs
modified glass slide in the presence of Rf.

Figure S4. (A, B) 561 nm laser intensity dependence of $\frac{1}{\langle \tau_{on} \rangle}$ and $\frac{1}{\langle \tau_{off} \rangle}$, respectively. (C) The effect of the 785 nm laser on the reaction. Product formation rates are positively related to 785 nm laser intensities.
4. Enhanced catalytic performance under laser illumination

The laser enhanced catalytic performance was confirmed by both single molecule and ensemble measurements. On single molecule level, we recorded the fluorescence trajectory collected from Au NRs modified glass slide in the presence of AR and H$_2$O$_2$ under dark, then 785 nm laser was turned on to illuminate the reactor. As shown in Figure S5, upon the illumination with 785 nm laser, the frequency of fluorescence burst was increased, indicating more products were generated.

![Figure S5](image)

**Figure S5.** A typical fluorescence trajectory collected from Au NRs modified glass slide in the presence of AR and H$_2$O$_2$, 785 nm laser was turned on at the point marked with arrow.

Resorufin is a highly fluorescent molecule with large extinction coefficients ($\varepsilon=570$ nm $\sim$57000 M$^{-1}$ cm$^{-1}$) and a high fluorescence quantum yield (up to 0.97 at neutral to basic conditions). On ensemble level, the catalytic performance of Au NRs under dark or laser illumination conditions were thereby evaluated by comparing the amounts of generated fluorescent product Rf in equal time scale (Figure S6), 785 nm laser illumination clearly enhanced the catalytic performance.

![Figure S6](image)

**Figure S6.** (A) The comparison of fluorescence intensity of Rf generated under dark reaction (blue) and laser illumination (red) in 60 min. (B) Reaction rate of the Au NRs catalyzed fluorogenic reaction under dark (black) or laser illumination (red).
5. Verification the radical involved reaction mechanism

In the reaction pathway shown in Figure 2A, OH− radical is one of the intermediates. We introduced dimethyl sulfoxide (DMSO) to scavenge OH− radicals to investigate the solidity of reaction mechanism. As shown in Figure S7, upon the injection of DMSO at 450 s, the fluorescence burst was gradually disappeared. This suggested that OH− is involved in the reaction and confirmed the reaction pathway in Figure 2A.

**Figure S7.** A typical fluorescence trajectory collected from Au NRs modified glass slide in the presence of AR and H₂O₂. DMSO was injected at the point marked with arrow.
6. Distribution of $\tau_{\text{off}}$ and $\tau_{\text{on}}$ for the oxidative N-deacetylation

**Figure S8.** Distribution of $\tau_{\text{off, dark}}$ (A) and $\tau_{\text{on, dark}}$ (B) from one Au NRs for oxidative N-deacetylation at [AR] = 100 nM, 300 nM, 500 nM, respectively. $\text{H}_2\text{O}_2$ concentration was kept at 88.3 mM. The solid lines are fit with $y = A(e^{-k_1\tau} - e^{-k_2\tau})$ or $y = Ae^{-k\tau}$. The average $\tau_{\text{on}}$ is less than 0.2 s.
**7. Derivation probability density of $\tau_{on}$, $f_{on}(\tau)$, product desorption rate $<\tau_{on}>^1$**

For the oxidative N-deacetylation, the average desorption rates are independent on [AR] and almost kept constant.

<table>
<thead>
<tr>
<th>on state</th>
<th>off state</th>
</tr>
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<tbody>
<tr>
<td>$M_mA_{n-1}P$</td>
<td>$k_j$</td>
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</table>

The on-time, $\tau_{on}$, represents the waiting time for each Rf desorption from Au NRs after its formation, or the residence time of a Rf molecule on surface of Au NRs.

According to the conventional ensemble measurements, the kinetic equations for $\tau_{on}$ reaction are:

\[
\frac{d[M_mA_{n-1} - P]}{dt} = -k_3[M_mA_{n-1} - P] \quad (S1)
\]
\[
\frac{d[M_mA_{n-1}]}{dt} = k_3[M_mA_{n-1} - P] \quad (S2)
\]

$[M_mA_{n-1}P]$ represents the concentration of Au NRs on which one product molecule is generated. $[M_mA_{n-1}]$ represents the concentration of Au NRs on which one product molecule is dissociated from the surface of Au NRs.

However, under single-particle level, the concentration of Au NRs is meaningless. Therefore, the concentration of Au NRs should be replaced by probability of single nanoparticle, and Eq. S1 and S2 be deduced as:

\[
\frac{dP_{M_mA_{n-1} - P}(t)}{dt} = -k_3P_{M_mA_{n-1} - P}(t) \quad (S3)
\]
\[
\frac{dP_{M_mA_{n-1}}(t)}{dt} = k_3P_{M_mA_{n-1} - P}(t) \quad (S4)
\]

$P_i(t)$ (i is index, represent $[M_mA_{n-1}P]$ and $[M_mA_{n-1}]$), are the probabilities for finding the single particle in the states $[M_mA_{n-1}P]$ and $[M_mA_{n-1}]$. At the onset of each $\tau_{on}$ reaction (t=0), one product molecule has formed on the surface of Au NRs. So, the initial conditions for solving equations are:

\[
P_{M_mA_{n-1} - P}(0) = 1 , \quad P_{M_mA_{n-1}}(0) = 0
\]

And at any time within $\tau_{on}$, $P_{M_mA_{n-1} - P}(t) + P_{M_mA_{n-1}}(t) = 1$. The probability density of $\tau_{on}$ is $f_{on}(\tau)$:
\[ f_{on}(\tau) = \left. \frac{dP_{MnA_{n-1}(\tau)}}{dt} \right|_{t=\tau} = k_3 P_{MnA_{n-1}} - P(\tau) \quad \text{(S5)} \]

Solving equations S3 and S4 for \( P_{MnA_{n-1}} - P(\tau) \) with the initial conditions, we obtain:

\[ f_{on}(\tau) = k_3 \exp(-k_3 \tau) \quad \text{(S6)} \]

Then, \( <\tau_{on}>^{-1} \) represents the time-averaged product formation rate for a single nanoparticle:

\[ <\tau_{on}>^{-1} = \frac{1}{\int_0^\infty \tau f_{on}(\tau) d\tau} = k_3 \quad \text{(S7)} \]

For the reductive N-deoxygenation, \( <\tau_{on}>^{-1} = k_3 \).
8. Negligible plasmonic enhancement for reductive N-deoxygenation of Rz to Rf

We also investigated whether the plasmon could enhance the control N-deoxygenation reaction of Rz to Rf. We recorded the fluorescence trajectory collected from Au NRs modified glass slide in the presence of Rz and NH$_2$OH under dark, then 785 nm laser was turned on to illuminate the reactor. As shown in Figure S9, upon the illumination with 785 nm laser, the frequencies of fluorescence bursts remained almost unchanged, indicating plasmon did not generate more products for the reductive N-deoxygenation of Rz to Rf.

![Fluorescence trajectory](image)

**Figure S9.** A typical fluorescence trajectory collected from Au NRs modified glass slide in the presence of Rz and NH$_2$OH, 785 nm laser was turned on at the point marked with arrow.
9. Laser illumination induced temperature change

We employed a laser with weak density (54 mW) to illuminate Au NRs (Figure S10A), the laser intensity was not as high as in usual plasmonic photothermal conversion. The laser illumination induced temperature change of reactor solution containing immobilized Au NRs were explored both theoretically and experimentally. The temperature change induced by photothermal effect of Au NRs could be calculated according to Pustovalov theory.\(^3\)

\[
\Delta T = \frac{\sigma_{abs} I}{4\pi R_{eq} \beta k_{water}} \quad (S8)
\]

where \(\sigma_{abs}\) = absorption cross section (m\(^2\)), \(I\) = intensity of the incident light (W/m\(^2\)), \(R_{eq}\) = radius of a sphere with the same volume as the particle \((R_{eq} = (3V_{NP}/4 \pi )^{1/3} \text{m})\), \(\beta\) = thermal capacitance coefficient dependent on nanoparticle aspect ratio (AR) \((\beta = 1 + 0.96587(\ln^2(\text{AR}))\)), and \(k_{water}\) is thermal conductivity of water. In our experiments, the aspect ratio for Au NRs is AR = 3.6, \(\sigma_{abs}= 2.75 \times 10^{-15} \text{m}^2\), \(I = 33.6 \text{ W/cm}^2\), \(R_{eq} = 13.6 \text{ nm}\), \(\beta = 2.58479\) and \(k_{water} = 0.6/\text{mK}\). On the basis of these parameters, the theoretical temperature increased by laser at the surface of Au NRs is \(\Delta T = 0.0038 \text{ K}\).

The temperature change of reactor containing Au NRs was also monitored by a thermocouple. Compared to dark condition, the temperature change induced by 785 nm laser illumination was only increase 0.7 K (Figure S10B).

At the single molecule experiments, the temperature of aqueous solution upon illumination by the 785 nm laser revealed negligible rise (Figure S10C).
Figure S10. (A) The power of 785 nm laser measured by a laser power meter. (B) The temperature of the reaction under dark or laser illumination as measured by a thermocouple. (C) The temperature change of aqueous solution upon illumination by the 785 nm laser. (Left) The experimental setup. The temperature of the substrate cell was monitored by thermocouple. (Right) Thermocouple readout before and after 785 nm laser illumination.
10. Morphology changes of Au NRs after plasmonic photocatalysis

The morphology of Au NRs might be altered after laser illumination through the photothermal effect. The morphology of Au NRs before and after the plasmonic photocatalysis in ensemble experiments and in single-molecule experiments were characterized by TEM and SEM, respectively (Figure S11). We found negligible morphology change.

Figure S11. (A) TEM images of Au NRs and aspect ratio distributions of Au NRs before and after plasmonic photocatalysis in ensemble experiments. (B) SEM images of Au NRs and aspect ratio distributions of Au NRs before and after plasmonic photocatalysis in single-molecule experiments.
11. Dynamic Surface Restructuring

The catalytic activity of Au NRs is closely related to its surface atoms, ordered crystal structure leads to low surface energy. However, TEM characterization is less effective for exploration surface restructuring.

Dynamic surface restructuring of nanocatalysts could be related to temporal fluctuations of catalytic activity. These time-varying fluctuations in catalytic activity are likely attributable to spontaneous and catalysis-induced dynamic surface restructuring. The time scale of the activity fluctuations, which is also the time scale of the underlying surface restructuring, can be obtained from the autocorrelation function $C_\tau(m) = \langle \Delta \tau(0) - \Delta \tau(m) \rangle / \langle \Delta \tau^2 \rangle$, $\tau$ represents $\tau_{off}$, m is the turnover index number in the sequence, and $\Delta \tau(m) = \tau(m) - \langle \tau \rangle$ ($\langle \rangle$ denotes to averaging).

As shown in Figure S12A and B, $C_{\tau_{off}}(m)$ shows an exponential decay, indicating the fluctuations of catalytic activity for product formation exist in both dark reaction and under laser illumination. The decay constants are $m_{off} = 15.2 \pm 1.2$ and $m_{off} = 10.0 \pm 1.2$ turnovers. For the dark reaction, both spontaneous and catalysis-induced dynamic surface restructuring can induce the fluctuations of catalytic activity. However, under laser illumination, it is still not clear whether the distribution of active site on the surface of gold nanorods can be altered by plasmon.

We then investigate the relationship between surface restructuring rates, $r_{off}$ and laser intensity or temperature. As shown in Figure S12C, $r_{off}$ increased with the rising temperature, indicating that the temperature could induce the surface restructuring or alter the distribution of the active sites. However, $r_{off}$ was independent of laser intensities, indicating that the laser power in our experiments was not sufficiently high to surface structure. Therefore, the plasmon enhanced catalytic could be not attributed to laser induced rearrangement of active sites.
Figure S12 Autocorrelation functions of $\tau_{\text{off}}$ for dark reaction (A) and laser illumination (B) derived from the turnover trajectory of a single gold nanorod. Solid lines are exponential fits with decay constants of $m_{\text{off}} = 15.2 \pm 1.2$ (A) and $m_{\text{off}} = 10.0 \pm 1.2$ (B) turnovers. Insets: Histograms of fluctuation correlation times. (C) Temperature and laser intensity dependence of the activity fluctuation rate of Au NRs.

The underlying mechanism of LSPR-enhanced catalytic activity was investigated through DFT calculation by using a nanometer rod-like Au149 cluster as a model system.

In aqueous solution, H$_2$O$_2$ and AR molecules were absorbed on the surface of Au NRs. The adsorbed H$_2$O$_2$ was homolytically cleaved as two OH$^{ads}$ radicals, which is consistent with previous reports. Under laser illumination, hot carries (electron–hole pairs) are excited on the surface of Au NRs, which can alter the energetic states of adsorbed H$_2$O$_2$ (HO-OH$^{ads}$) and AR$^{ads}$, thus accelerating the reaction rate. As shown in Figure S14 A, the HOMO of AR$^{abs}$ is at least 7.60 eV higher than the hot holes of Au NRs, and the hot electrons are at least 2.07 eV higher than the LUMO of OH$^{ads}$. This means the hot electrons can simultaneously inject into the LUMO of OH$^{ads}$ and the electrons in HOMO of AR$^{abs}$ can transfer into the hot holes, thus facilitating the AR radical formation and the redox reaction. Due to the significant increase of reaction sites on the surface of Au NRs, the reaction rate accelerates remarkably relative to the dark reaction which involves only direct molecular collision of reactants.

More importantly, the hot carriers can reduce the activation energy of intermediate product formation by activating the Au-OH$^{ads}$ bond and Au-AR$^{ads}$ bond along two reaction pathways. One is that the hot carriers excited the the Au-OH$^{ads}$ and Au-AR$^{ads}$ in vibronic levels (red dashed arrow and box in Figure S14C): hot electrons transfer into OH$^{ads}$ and hot holes transfer into AR$^{ads}$, activating the the Au-OH$^{ads}$ and Au-AR$^{ads}$. The activated the Au-OH$^{ads}$ bond and Au-AR$^{ads}$ bond poesss higher energy and are easier to transfer into intermediate product with a lower activation barrier (red and green dotted lines in Figure S14C).

Another possibility for activation of the Au-OH$^{ads}$ bond and Au-AR$^{ads}$ bond is inelastic electron-vibrational dipole scattering process. According to this process, the plasmon excited electrons, which process energies greater than the vibrational quantum, can induce multiple vibrational transitions of the Au-OH$^{ads}$ bond and Au-AR$^{ads}$ bond. As the vibrational energy stored in Au-OH$^{ads}$ bond and Au-AR$^{ads}$ bond increases, results in the reduced $T_{iso}$ and lowered the activation energy (blue solid arrows in the Franck-
Figure S13 (A) Scheme of the efficient charge transfer pathway under laer illumination. Red and green dashed arrows denote possible hot carrier transfer into or electronic excitation of the Au-OH$^{\text{ads}}$ bond and Au-AR$^{\text{ads}}$. Blue solid arrows denote multiple vibrational excitations of the electronic ground state. (C) Schematic energetics of elementary reaction steps for AR$^-$ formation. The ‡ denotes transition state. OH$^*$ and AR$^*$ refer to excited Au-OH bond and Au-AR: either vibronic levels in an excited electronic state (red dashed arrow and box) or vibrational excitations in the electronic ground state (red solid arrow and box). The activation barriers after excitation (red, green, and blue dotted lines) are all lower than that of the ground state (black dotted line). e$^-$: electron; h$^+$: hole.
**Table S2** Rate constants of each step under dark or laser illumination at different temperature

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<thead>
<tr>
<th></th>
<th>298 K</th>
<th>303 K</th>
<th>308 K</th>
<th>313 K</th>
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<td><strong>Dark</strong></td>
<td></td>
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<td></td>
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<tr>
<td>$k_1$ (s$^{-1}$)</td>
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<tr>
<td><strong>illumination</strong></td>
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<td>$k_1$ (s$^{-1}$)</td>
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<td>$k_2$ (s$^{-1}$)</td>
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<td>25.51 ± 3.5</td>
<td>26.33 ± 3.4</td>
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Reference

