Imaging Catalytic Hotspots on Single Plasmonic Nanostructures via Correlated Super-Resolution and Electron Microscopy

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**Supporting Information**

**ABSTRACT:** Surface-plasmon (SP) enhanced catalysis on plasmonic nanostructures brings opportunities to increase catalytic efficiency and alter catalytic selectivity. Understanding the underlying mechanism requires quantitative measurements of catalytic enhancement on these nanostructures, whose intrinsic structural heterogeneity presents experimental challenges. Using correlated super-resolution fluorescence microscopy and electron microscopy, here we report a quantitative visualization of SP-enhanced catalytic activity at the nanoscale within single plasmonic nanostructures. We focus on two Au- and Ag-based linked nanostructures that present plasmonic hotspots at nanoscale gaps. Spatially localized higher reaction rates at these gaps vs nongap regions report the SP-induced catalytic enhancements, which show direct correlations with the nanostructure geometries and local electric field enhancements. Furthermore, the catalytic enhancement scales quadratically with the local actual light intensity, attributable to hot electron involvement in the catalytic enhancement mechanism. These discoveries highlight the effectiveness of correlated super-resolution and electron microscopy in interrogating nanoscale catalytic properties.

**KEYWORDS:** single-molecule catalysis imaging, linked nanostructures, plasmonic catalytic hotspots, hot electron mechanism

**M**etal nanoparticles, such as those made of Au and Ag, exhibit localized surface plasmon (SP) resonance upon excitation with visible light in which the collective oscillations of the metal valence electrons generate an intense oscillating electric field within a few nanometers to the nanoparticle surface.1–5 These metal nanoparticles can often also act as catalysts,6–12 and many studies have shown that their SP excitation can enhance their catalytic activity, for example, in Ag nanoparticle catalyzed CO oxidation,10 decomposition of organic molecules,11 and catalytic coupling reactions.12 This SP-enhanced catalysis opens opportunities to harvest light to drive catalytic reactions that are otherwise inefficient or to bias the reaction pathways toward more desirable products.6,7

The promise of SP-enhanced catalysis motivated many studies into understanding the underlying mechanisms of catalytic enhancement. Several mechanisms can operate on plasmonic metal nanoparticles. One obvious possible mechanism is the thermal effect in which the enhanced light absorption by plasmonic nanoparticles converts into heat to drive thermally activated reactions on the nanoparticle surface.13–15 A second mechanism is enhanced photoexcitation of the reactant molecules, if they are able to absorb light in the wavelength range where the SP resonance occurs; the intense local electric field on the plasmonic nanoparticle surfaces can thus enhance the photoexcitation of the reactant molecules, leading to more efficient photocatalytic reactions.16 A third mechanism involves hot electrons generated from SP excitation; these hot electrons could be injected into the surface adsorbed molecules, driving subsequent chemical transformations.8,10,17–19

In probing the mechanisms of SP-enhanced catalysis, ensemble-level measurements have been instrumental,10,20–25 in which the collective catalytic behaviors of a large number of plasmonic nanoparticles are measured, for example, as a function of light intensity,10 light wavelength,20 or temperature.21,22 Photoluminescence spectroscopy of the metal nanoparticles has also been used to probe indirectly SP-enhanced catalysis, even down to the single-particle level, but the catalytic activity there was still measured at the ensemble level.23 Although powerful, these ensemble measurements have limitations imposed by the intrinsic heterogeneity of plasmonic nanostructures. First, the electric field enhancement is spatially...
heterogeneous across a plasmonic particle. Depending on the particle shape, the enhancement could be much larger at particular locations of nanometers in dimension, so-called plasmonic “hotspots” (e.g., corners or crevices), than other locations; even for a pseudospherical particle, the electric field enhancement distribution is dependent on the polarization of the excitation light. Second, plasmonic hotspots often exist at nanoscale gaps between plasmonic particles; these gaps are few and occur irregularly within a cluster of particles, but they could be dominant contributors to the enhanced catalytic activity measured at the ensemble level.

The spatial heterogeneity of plasmonic enhancements also predicts different spatial patterns of catalytic enhancements on plasmonic nanostructures, depending on the underlying mechanism. For the thermal effect, as plasmonic metals have large thermal conductivity, the heat generated from SP excitation would quickly dissipate, resulting in a spatial uniformity of the corresponding catalytic enhancement over any single nanostructure. However, both the enhanced photoexcitation effect and the hot electron effect depend on the local electric field intensity; a spatially heterogeneous electric field enhancement would lead to spatial heterogeneity in the corresponding catalytic enhancement, forming nanoscale catalytic hotspots at plasmonic hotspots.

A number of spatially selective approaches have been used to exploit this spatial heterogeneity to observe catalytic reactions at plasmonic hotspots. Single-molecule surface-enhanced Raman spectroscopy (SERS) and tip-enhanced Raman spectroscopy (TERS) have been used to image redox reactions on single gold and silver colloidal particles, on bulk metal surfaces, and on particle aggregates down to subdiffraction-limited resolution. However, the colloidal aggregates are hard to control geometrically; the approaches only apply to SERS-active molecules; and the extent of local catalytic enhancements is not directly quantifiable because the reaction rates at plasmonic hotspots cannot be compared with those at nonplasmonic hotspots, where the SERS signals are undetectable. Atomic force microscopy (AFM) and scanning electron microscope (SEM) have been used to image polymerization reaction products on plasmonic nanostructures, but the measurements are ex situ, on dry samples, and do not provide quantitative reaction kinetics.

Single-molecule super-resolution fluorescence microscopy has been shown to be a complementary and powerful approach to study catalysis on nanoscale particles. With its single-molecule sensitivity and nanometer spatial resolution, catalytic (and (photo)(electro)catalytic reactions on individual metal nanoparticles, zeolite particles, semiconductor particles, and carbon nanomaterials can be imaged and quantified under operando conditions in real time with single-turnover resolution. Here, we use single-molecule super-resolution fluorescence imaging combined with electron microscopy to study SP-enhanced catalysis on two linked nanostructures that present plasmonic hotspots at nanoscale gaps. We directly observe and quantify the catalytic enhancements at these nanoscale plasmonic hotspots, define their correlations with the nanostructure geometry and local electric field enhancement, and show that the enhancement scales quadratically with the local actual light intensity, reflecting the involvement of plasmon-excitation induced hot electrons in the catalytic enhancement mechanism.

RESULTS

Au–Au and Au–Ag Nanostructures with Plasmonic Hotspots. We chose to make two Au- and Ag-based nanostructures with nanoscale gaps where high plasmonic enhancement is expected. One such plasmonic nanostructure is linked Au–Au nanorods with ~8 nm long spacers of biotin–streptavidin linkages, following the method of Murphy et al. (SI Section S1). We chose these Au nanorods for their high structural anisotropy (~21 nm in diameter and hundreds of nm in length; SI Figure S1) as well as for their visible wavelength localized SP resonance (the transverse mode peaks at ~515 nm; SI Figure S2J). We further coated these linked Au–Au nanorods with a ~70 nm thick mesoporous silica shell (i.e., mSiO2) to stabilize the linked geometry. This shell also allows for subsequent UV-ozone treatment to remove the organic components while preventing aggregation; the mesopores still allow the reactants to access the metal surface for catalysis without mass transport limitation, as we previously showed. SEM images of such linked Au–Au nanorods reveal variations of linkage geometries: V-shaped (e.g., Figure 1A,C) and T-shaped (e.g., Figure 1B) with variable angles in between.

Figure 1. Linked plasmonic nanostructures. (A–C) SEM images of exemplary linked Au–Au nanorods encapsulated in mSiO2. All scale bars are 200 nm. (D) Line profile analysis of the red box on the linked Au–Au nanorods in A. Gap size is defined as d = r1 − r2, where r is the radius of the nanorod/nanoparticle, determined from the fwhm of the Gaussian fit of the line profile, and d is the center-to-center distance between the nanorods/nanoparticles. Blue lines, Gaussian deconvolution; red line, overall fit. (E) Gap size distribution of linked Au–Au nanorods; average is 8.8 ± 2.2 nm. (F–J) Same as A–E, but for linked Au–Ag nanorod–nanoparticle encapsulated in mSiO2. Average gap size is 8.2 ± 2.1 nm.
readily determined via line profile analysis of the SEM images and are about 8.8 ± 2.2 nm (Figure 1D,E), consistent with the expected dimension of the original biotin–streptavidin linkages used in the synthesis (SI Section S1.4).

The second plasmonic nanostructure is Au nanorods linked to ~50 nm Ag nanoparticles with nanoscale gaps, using the same biotin–streptavidin linkage method and also encapsulated in a mSiO2 shell (Figure 1F–H; SI Section S1.2). Ag nanoparticles here provide localized SP resonance at a different visible wavelength region (~415 nm;56 SI Figure S21). The Ag nanoparticle could be located on the side of a Au nanorod (e.g., Figure 1F,G) or near its end (e.g., Figure 1H). The gaps are about 8.2 ± 2.1 nm in size (Figure 1I,J).

**Super-Resolution Imaging of Catalytic Hotspots on Linked Au–Au Nanorods in Correlation with SEM.** Using single-molecule super-resolution fluorescence microscopy (SI Section S2.1), we imaged and localized individual fluorescent catalytic product molecules on single Au–Au nanorod structures with ~40 nm precision (Figure 2A). These nanostructures were immobilized on a quartz slide in a microfluidic reactor cell into which the reactants were supplied continuously to achieve steady state kinetics (SI Section S2.1). The catalytic reaction here is a fluorogenic reaction in buffered pH 7.1 aqueous solution: the reductive deoxygenation of resazurin by NH2OH to generate resorufin, a highly fluorescent molecule (SI Figure S7C). We have previously shown37 that (1) this Au particle catalyzed reaction follows the classic Langmuir–Hinshelwood kinetics; (2) at a large excess of NH2OH (e.g., 20 mM), the catalytic rate is initially first order to the resazurin concentration and then saturates to zeroth order when the resazurin concentration reaches ~0.2 μM (also Figure 7B); and (3) the fluorescence of the product resorufin is imaged, while it is temporarily adsorbed within the mesopores of the mSiO2 shell rather than on the metal surface (as its desorption off the Au nanorod surface is fast), before it desorbs and disappears into the surrounding solution. Moreover, during our experimental imaging time of ~6 h, the catalytic activities of these linked Au–Au nanorods stayed stable (SI Figure S9).

We further performed ex situ SEM on the same nanostructures, subsequent to our fluorescence imaging experiment (Figure 2B). Correlating the SEM image with the fluorescence images allowed us to map the positions of fluorescent catalytic products onto the structural contours of individual linked Au–Au nanorod structures with nanometer precision (Figure 2 and SI Section S4). The correlated images immediately reveal an enhanced catalytic activity at the gap region of linked Au–Au nanorod nanostructures, where the plasmonic enhancement is high. For the linked nanostructure in Figure 2A,B with a ~9 nm gap (Figure 2E), the detected number of catalytic products at the gap region (red circle) is ~2.5 times more than those detected at the nongap regions of the two linked nanorods (two black circles) (Figure 2A), representing a quantitative visualization of SP-enhanced catalysis at the nanoscale.

Control measurements indicated that (1) the higher detection rate of catalytic products at the gap region is not due to their enhanced fluorescence intensities there (which could occur depending on the plasmonic nanostructure, the fluorophore, and their relative locations58,59) because their fluorescence intensities do not show significant differences at the gap vs nongap regions (Figure 2C and SI Figure S11E,K). The absence of product fluorescence enhancement at the gap region is consistent with that the product is detected while temporarily adsorbed within the mesopores of the ~100 nm-thick mSiO2 shell (where the electric field enhancement is insignificant) rather than directly on the Au surface (where the electric field enhancement is localized) because product desorption off the Au surface is fast. This detection within the mSiO2 shell is also directly reflected by that, in the direction perpendicular to the nanorod long axis, many product molecules are located >40 nm away from the nanorod (note ~40 nm is our localization precision) (Figure 2A). It is worth noting that it is advantageous to detect the product molecules.
off the Au surface while trapped in the mSiO₂ shell, as it avoids plasmonic fluorescence enhancement that would bias the detection toward molecules at plasmonic hotspots. Such biases are often present for surface-enhanced detection techniques, such as surface-enhanced Raman scattering or surface-enhanced fluorescence. (2) It is not because the products stay adsorbed longer at the gap region, as the average residence time of individual products are the same at gap vs nongap regions (Figure 2D and SI Figure S11F,L). (3) It is not due to higher reactant access at the gap region because our reaction condition is at high enough reactant concentrations, where the catalytic kinetics is pseudo-zeroth order to reactant concentration in which variations of local reactant concentration would not cause significant changes in reaction rates. (4) To check if the fluorescent product resorufin might preferentially adsorb onto regions at the gap, which was observed for a tetramethylrhodamine dye,60 we performed the control experiment by flowing in a solution of resorufin (SI Section S5.2). Similar adsorption frequency at gap vs nongap regions were observed (SI Figure S13), ruling out this possibility. (5) Furthermore, FDTD simulations showed that possible coupling between the product fluorescence and the nanostructure plasmon only shifts the apparent product position by less than ~20 nm, smaller than our localization precision of ~40 nm (SI Section S6.3).

To quantify the catalytic enhancement, we computed the specific turnover rate (i.e., catalytic activity) within a circular region centered at the gap in comparison with that at nongap regions (red vs black circles, Figure 2A,B and SI Section S7). The typical circle radius is 70 nm, significantly larger than our spatial resolution of ~40 nm. We also varied the circle radius to 40 and 100 nm; the conclusions presented below stay the same (SI Figure S11D,J). Pooling results from 31 linked Au–Au nanorods illuminated by an average power density of 1.27 kW cm⁻² at 532 nm, we observed that the catalytic activity at the gap regions are always higher than the nongap regions, by ~1.9 times on average and up to ~3.1 times at the single nanostructure level (Figure 2F,H). These localized catalytic activity enhancements clearly demonstrate the catalytic hotspots on these linked plasmonic nanostructures.

To quantitatively connect the catalytic enhancement with the local plasmonic enhancement at the gap region, we performed two-dimensional FDTD simulations to calculate the local electric field enhancement patterns (SI Section S6.1). For each linked Au–Au nanorod structure, we used its experimentally determined geometry, nanorod dimension, and gap size, as well as the excitation light wavelength (532 nm), polarization, and k-vector direction. Figure 2G shows the simulated electric field intensity pattern for the nanostructure in Figure 2A. A strong electric field enhancement is clear at the gap region, as expected. We further determined the average electric field enhancement (i.e., |E₀/E|²) within the same circles at the gap vs nongap regions, as we did in calculating their catalytic activities. We only considered the electric field enhancement within 3 nm of the Au surface, as the catalytic reactions occur at the surface and the reactant molecules are ~1 nm in size (SI Section S6.1). For the gap vs nongap regions of the 31 linked Au–Au nanostructures, their electric field enhancement ratios are directly correlated with their catalytic activity ratios (Pearson’s correlation coefficient ρ = 0.76 ± 0.05, Figure 2H), directly supporting that the catalytic enhancement at the gap region arises from local electric field enhancement.

Taken altogether, the above results demonstrate the direct visualization of catalytic hotspots at plasmonic hotspots on these linked Au–Au nanorod structures. These localized catalytic hotspots within a single nanostructure also immediately rules out a thermal effect as an underlying cause for the catalytic enhancement, as the high thermal conductivity of Au would give a homogeneous temperature distribution within a single nanostructure without temperature hotspots (SI Section S5.3).

Super-Resolution Imaging of Catalytic Hotspots on Linked Au–Ag Nanostructures in Correlation with SEM. Using correlated single-molecule super-resolution fluorescence microscopy and SEM, we also studied linked Au–Ag nanorod–nanoparticle nanostructures in catalyzing the same reductive deoxygenation reaction of resazurin. Here, the Ag nanoparticles are not catalytically active, as shown by ensemble activity measurements (SI Figure S7E); they only act as SP enhancers. We also added a second laser excitation at 405 nm, which would preferentially excite the SP of the Ag nanoparticle (the SP of Au nanorod would also be excited to a certain extent by the 405 nm laser due to plasmon energy transfer).61

The distinct SP properties of Au and Ag allowed us to differentiate the isolated and the linked Au–Ag nanostructures readily using scattering and photoluminescence (PL) microscopy at 405 nm vs 532 nm excitations. For Au nanorods, their transverse mode of localized SP resonance peaks at ~515 nm; they thus scatter the 532 nm light more strongly than the 405 nm light (Figure 3B vs A). However, Ag nanoparticles have localized SP resonance peaked at ~415 nm and thus scatter the 405 nm light more strongly (Figure 3E vs F). Moreover, both Au nanorods and Ag nanoparticles exhibit some PL. The PL intensity of Au nanorods are much stronger under 532 nm excitation (Figure 3D vs C), whereas Ag nanoparticles show stronger PL under 405 nm excitation (Figure 3G vs H). For linked Au–Ag nanostructures, they have appreciable scattering and PL intensities under both 405 and 532 nm excitations (Figure 3I–L). (Note the fluorescence signal of the catalytic product resorufin is detected on top of the PL signal of the nanoparticle, which is stable under continuous wave laser excitation, as we showed previously.60)

Because of these optical properties, when we examined individual nanostructures in both scattering and PL images under 405 or 532 nm excitation, Au nanorods, Ag nanoparticles, and linked Au–Ag nanostructures cluster into distinct populations (Figure 3M) in which the linked Au–Ag nanostructures are readily identified. This optical identification can be further confirmed by subsequent SEM imaging (Figure 3M inset) as well as elemental analysis via energy-dispersive X-ray (EDX) spectroscopy (Figure 3N).

We then mapped super-resolution catalysis images of individual linked Au–Ag nanostructures onto their SEM images, as we did on linked Au–Au nanorods (Figure 4A,B). Catalytic hotspots are clearly observed at the Au–Ag gap, which is a few nanometers in size (Figure 4E) (no significant reaction products are detectable on isolated Ag nanoparticles, which are catalytically inactive). Again, this enhanced catalytic activity at the gap regions are not due to enhanced fluorescence intensity of the product molecules (Figure 4C), longer product residence time (Figure 4D), or more reactant access there (SI Section S5.2).

Similarly, we quantified the specific turnover rate at the gap vs nongap regions on these linked Au–Ag nanostructures; here, only the surface area of Au nanorods was considered because Ag nanoparticles are catalytically inactive (SI Section S7 and Figure S7E). Pooling results from 29 linked Au–Ag
nanostructures, the catalytic activities of gap regions are ∼2.1 times higher on average than nongap regions and can be ∼3.9 times higher for individual nanostructures (Figure 4F,H,J), clearly demonstrating the catalytic enhancements at plasmonic hotspots on these nanostructures.

We performed FDTD simulations on each linked Au−Ag nanostructure that we experimentally studied. Both the 405 and 532 nm laser sources were included in the simulations, and their intensity ratios were taken from the experimental local power densities at each nanostructure. At both the 532 and 405 nm wavelength, the electric field intensity patterns always show localized enhancements at the gap region (Figure 4G), as expected. More important, the observed catalytic enhancement at the gap vs nongap regions shows a direct correlation with the relative electric field intensity enhancement (Figure 4H), clearly demonstrating that the catalytic enhancements came from local plasmonic enhancements and ruling out thermal effect as the underlying cause.

Figure 3. Differentiation of isolated Au nanorod and Ag nanoparticle, and linked Au−Ag nanostructures via optical microscopy. (A,B) Scattering images of a single Au nanorod under 405 nm (A) or 532 nm (B) light excitation. (C,D) Photoluminescence image of a single Au nanorod in the >425 nm wavelength range under 405 nm light excitation (C) or in the 550 to 610 nm wavelength range under 532 nm light excitation (D). (E−H) Same as A−D, but for a single Ag nanoparticle. (I−L) Same as A−D, but for a linked Au−Ag nanostructure. All scale bars in A−L represent 500 nm. (M) Scattering intensity ratio at 405 or 532 nm excitation vs the photoluminescence intensity ratio at 405 or 532 nm excitation for individual particles of Au nanorods (open orange squares), Ag nanoparticles (open black circles), or the sample of linked Au−Ag nanostructures (open triangles), which is a mixture of linked and unlinked nanoparticles. Au nanorods, Ag nanoparticles, and linked Au−Ag nanostructures appear as distinct populations (areas circled by red-, black-, and blue-dashed lines, respectively). Inset: SEM image of a linked Au−Ag nanostructure encapsulated in mSiO2; scale bar = 200 nm. (N) Elemental analysis on the Au−Ag nanostructure in the inset in M via EDX spectroscopy (red box). The peaks are annotated by the elemental origin. Both Au and Ag are seen. Na and Si came from the residual cations in the experimental buffer solution and the mesoporous silica shell.

Figure 4. Catalytic hotspots on linked Au−Ag nanorod−nanoparticle nanostructures. (A) Quantitative super-resolution mapping of catalytic products on the Au−Ag nanostructure in B. White line: structure contour of the nanostructure from B. (B) SEM image of the linked Au−Ag nanostructure encapsulated in mSiO2 in A. The red and black circles (70 nm radius) define the gap and nongap regions. (C) Spatial distribution of the average single-molecule fluorescence intensity of the catalytic product resorufin per image frame (30 ms) on the nanostructure in A. (D) Spatial distribution of the average residence time of the catalytic product on the nanostructure in A. (E) Gap size determination via line profiling of the SEM image in B (red box). The gap here is 8.2 ± 1.1 nm. (F) Box plot of specific turnover rate ρ of gap vs nongap regions on 29 linked Au−Ag nanostructures. (G) FDTD simulation of electric field enhancement pattern on the nanostructure in A at 532 nm with circularly polarized incident light. (H) Correlation between electric field enhancement ratios of gap vs nongap regions and their catalytic activity ratios. Each open circle is one nanostructure. Solid squares are binned and averaged results. All error bars represent SD. All scale bars represent 200 nm. (I,J) Same as G,H, but using electric field enhancement pattern at 405 nm.
Catalytic Enhancement Decreases with Larger Gap. For plasmonic nanostructures with nanoscale gaps, the local electric field enhancement at the gap region is known to depend sensitively on the gap size; the larger the gap, the smaller the enhancement. The ability to determine the gap sizes of individual linked Au–Au and Au–Ag nanostructures from SEM allowed us to evaluate how the catalytic enhancement at the gap regions depends on the gap size at the single nanostructure level.

For the linked Au–Au nanorods, there is no clear dependence on the gap size for the gap vs nongap activity ratio (Figure 5A, open black squares). This is not surprising, as for these linked nanorods, the local electric field enhancement depends not only on the gap size, but also on the relative orientation of two nanorods; the latter differs greatly from one nanostructure to another (e.g., Figure 1A–C). (It also depends on the incident light propagation direction, but this dependence is much smaller, contributing to ~15% difference and thus less significant here, as shown by FDTD simulations; SI Section S6.2.) We thus performed FDTD simulations to evaluate the effect of relative nanorod orientation on the local electric field enhancement (Figure 5C). With a fixed 5 nm gap and keeping all other conditions constant, the electric field enhancement could differ by as much as ~55%, when the angle between the two nanorods is varied from 0 to 90° (Figure 5D). Using this angle dependence, we normalized all experimental data of the individual Au–Au nanorod structure to the case of the 70° angle to factor out the orientation effect. Expectantly, after this normalization, the gap vs nongap activity ratios of these linked Au–Au nanorods show a clear exponential decay with increasing gap size, with a decay constant of 7.8 ± 1.6 nm (Figure 5A, open red circles). This decay behavior is also reproduced in FDTD simulations, where the gap size is increased while the orientations of the two nanorods are kept constant; and the decay constant there is 7.6 ± 0.3 nm (Figure 5B).

For the linked Au–Ag nanorod–nanoparticle structures, the gap vs nongap activity ratio shows a clear exponential dependence on increasing gap sizes (Figure 5E); here, because of the pseudo-spherical shape of the Ag nanoparticle, the relative orientation of the Au nanorod is less important. This exponential dependence has a decay constant of 7.1 ± 0.8 nm (Figure 5E), in agreement with the FDTD simulations of electric field enhancement at either 405 or 532 nm (Figure 5F). Altogether, the gap size dependences of the catalytic activity enhancement at gap regions further support that the local plasmonic enhancement is the cause of the catalytic hotspots in which the catalytic enhancement depends not only on the gap size but also on the geometries of the linked nanostructures.

Activity of Catalytic Hotspots Shows a Quadratic Dependence on Light Intensity. We further examined the excitation light intensity dependence of the specific turnover rate at the gap regions of the linked Au–Au and Au–Ag nanostructures, where catalytic enhancement is observed. We determined the local incident light power density by mapping out the laser beam profile on the sample and also taking into account the evanescent field illumination geometry (SI Section S2.4). Strikingly, for both types of nanostructures, the specific turnover rates of the gap regions increase quadratically with increasing local incident power density of 532 or 405 nm laser (Figure 6A–C, open circles; linear fits to the data clearly failed; SI Figure S20A–C and Table S4). This quadratic dependence indicates that the underlying mechanism for the catalytic enhancements at these catalytic hotspots must involve two photoexcited species, such as SP-induced hot electrons or photoexcited resazurin molecules, which absorb near the excitation wavelengths (λabs ≈ 570 nm). Consistently, at any local incident power density, the specific turnover rates at the gap regions, where SP enhancement is large, are always higher than those at nongap regions, where SP enhancement is negligible and for which the dependence on the local incident power density cannot be reliably differentiated between first and second orders (Figure 6A–C, open squares).
Figure 6. Quadratic dependence of specific activity on light intensity at catalytic hotspots. (A) Specific turnover rates \( v \) of individual linked Au–Au nanorods at gap and nongap regions vs their local incident light power density \( I_{\text{incident}} \) at 532 nm. Each open symbol represents a single nanostructure. Solid symbols are binned and averaged data to obtain general trends. Lines are quadratic fits. (B) Same as A, but for linked Au–Ag nanorod–nanoparticle structures and for 405 nm light. (C) Same as B, but for 532 nm light. (D–F) Corresponding to A–C, respectively, in which the incident local light power density has been converted to the actual local power density using the electric field enhancement factor obtained from FDTD simulations. \( x \) error bars are SD; \( y \) error bars are SEM.

Because of the large electric field enhancement at the gap regions, the actual light power density there is much larger than the incident light power density. Using our earlier FDTD simulations, which gave the local electric field enhancement pattern around each nanostructure, we corrected for these enhancements to obtain the actual light power density at both gap and nongap regions. Consequently, the specific turnover rates of the gap regions now fall on the same curve as that for the nongap regions as a function of increasing actual local power density for both the linked Au–Au and Au–Ag nanostructures at 532 or 405 nm (Figure 6D–F). In the range of smaller than \( \sim 1.5 \) kW cm\(^{-2} \), the specific turnover rates show little dependence on the light power density, consistent with our earlier studies\(^{49,57} \) on individual Au nanoparticles at an illumination power density of 0.5 to 0.75 kW cm\(^{-2} \). In the higher range of \( >1.5 \) kW cm\(^{-2} \), the specific turnover rates increase rapidly. Overall, the specific turnover rate \( v \) follows a second order dependence on the actual local power density \( I \) in all cases, sufficiently described by the following relationship (SI Section S8):

\[
v = A + CI^2
\]

Here, \( A \) (about \( 2 \times 10^{-5} \) s\(^{-1} \) nm\(^{-2} \)) is a light independent term, consistent with what we determined earlier\(^{49,50} \) that, under low light conditions, Au-particle catalyzed reduction of resazurin by \( \text{NH}_2\text{OH} \) follows the classic Langmuir–Hinshelwood mechanism, in which the excitation light plays no significant roles. \( C \) is the coefficient for the second-order dependence, and it gives the catalytic enhancement at plasmonic hotspots (i.e., gaps) where the local actual light power density is large. This second-order term again indicates that the underlying mechanism of enhancement involves two photoexcited species.

**DISCUSSION**

Using correlated single-molecule super-resolution fluorescence microscopy and scanning electron microscopy, we have visualized and quantified the catalytic hotspots at nanoscale gaps on two types of linked plasmonic nanostructures. These spatially localized enhancements directly rule out the thermal effect as the enhancement mechanism, and they are also correlated with the local electric field enhancement, tunable by the gap size.

Moreover, the specific catalytic activities at these gap regions, along with those at the nongap regions, follow an overall second order dependence on the local actual light power density. This second-order dependence suggests that the rate-limiting step in the enhancement mechanism involves two photoexcited species. One possibility is that both of these photoexcited species are the hot electrons (\( e_{\text{hot}} \)) from the SP excitation, which are injected into a surface adsorbed resazurin to reduce it to the product resorufin (Figure 7A, mechanism 1).\(^{10} \) In this mechanism, the reaction rate \( v \) would scale as \( v \propto \left[ e_{\text{hot}} \right]^2 \left[ \text{S}_{\text{ad}} \right] \propto F^2 \left[ \text{S}_{\text{ad}} \right] \), where \( \left[ \text{S}_{\text{ad}} \right] \) is the concentration of surface adsorbed resazurin. A second possibility is that one hot electron is involved and the other photoexcited species is a photoexcited resazurin (\( \text{S}^*_{\text{ad}} \)) adsorbed on the catalyst surface, as resazurin’s absorption band overlaps significantly with the SP resonance of Au (Figure 7A, mechanism 2). This mechanism would also predict a second order light dependence of the reactant rate: \( v \propto \left[ e_{\text{hot}} \right]^2 \left[ \text{S}^*_{\text{ad}} \right] \propto F^2 \left[ \text{S}^*_{\text{ad}} \right] \). A third possibility is that both the photoexcited species are photoexcited resazurin molecules on the surface, and no hot electrons are involved; in this case, \( v \propto \left[ \text{S}^*_{\text{ad}} \right]^2 \propto F^2 \left[ \text{S}^*_{\text{ad}} \right]^2 \) (Figure 7A, mechanism 3).
All three mechanisms in Figure 7A would result in a second order dependence on the light power density, but they predict different dependences on the concentration of the reactant resazurin. The reaction rates of the first two mechanisms scale linearly with \([S_{eq}]\), while that of the third mechanism scales with \([S_{eq}]^2\). Previously we have shown that resazurin adsorption on these Au nanocatalysts follows effectively the Langmuir adsorption behavior, \(^9 \), \(^{49,57} [S_{eq}] \propto K_s[S]/(1 + K_s[S]), \) where \([S]\) is the concentration of resazurin in the surrounding solution, and \(K_s\) is the equilibrium constant of resazurin adsorption on the catalyst surface. Consequently, mechanisms 1 and 2 would give:

\[
\nu = k_{eq} K_s[S]/(1 + K_s[S])
\]

in which \(k_{eq}\) is an effective rate constant that can contain the light power density dependence. In contrast, mechanism 3 would give:

\[
\nu = k_{eq}^2 [S]^2/(1 + K_s[S])^2
\]

We thus examined the resazurin concentration dependence of the specific turnover rate to differentiate these mechanisms, using the linked Au–Au nanorods as an example. For nongap regions where there is little catalytic enhancement and for which our previous studies \(^9 \), \(^{49,57} \) showed that the kinetics follows a Langmuir saturation kinetics, the specific turnover rate follows eq 2 satisfactorily, giving \(K_s = (1.01 \pm 0.09) \times 10^{-3} \) M\(^{-1}\) (Figure 7B). For the gap regions where the catalytic enhancement is prominent, eq 2 also fits the data satisfactorily, giving \(K_s = (0.94 \pm 0.12) \times 10^{-3} \) M\(^{-1}\) (Figure 7C, red line), effectively the same as that for the nongap regions and supporting that mechanisms 1 and 2 are likely. Alternatively, fitting the results from the gap regions with eq 3 gives \(K_s = (2.93 \pm 0.49) \times 10^{-2} \) M\(^{-1}\) (Figure 7C, blue line), almost three times larger than that of the nongap regions. As SP enhancement is not expected to affect significantly the reactant adsorption affinity to the surface, this three times larger magnitude of \(K_s\) argues against mechanism 3 that involves two photoexcited resazurin to account for the second order light power dependence. Therefore, mechanisms 1 and 2 are more likely, and both of them involve hot electrons from the SP excitation in which the plasmonic hotspots are the catalytic hotspots.

CONCLUSIONS

In conclusion, we have used single-molecule super-resolution fluorescence microscopy, in combination with electron microscopy, to visualize directly catalytic hotspots on plasmonic nanostructures at nanometer resolution. The spatially resolved, quantitative activity information also allowed for gaining insights into the underlying enhancement mechanism, demonstrating the power of this correlative approach in interrogating nanoscale catalytic properties.

METHODS

Details of materials and methods are described in the Supporting Information. Au nanorod synthesis was based on previous work with some modification. \(^9 \) Ag nanoparticles were purchased from Ted Pella. The linked Au–Au and Au–Ag nanorod–nanoparticle nanostructures were prepared using a biotin–streptavidin linkage strategy \(^3 \) and then coated with mesoporous silica shell as previously reported, \(^9 \), \(^{49,57} \) after which the organic ligands were removed via UV-ozone treatment. \(^9 \) SEM was done on a LEO 1550VP FESEM operated at 10–15 keV. EDX was done using a Bruker Quantax X-ray detector attached to the SEM. Catalysis on plasmonic nanostructures was imaged using single-molecule total internal reflection fluorescence (TIRF) microscopy on an Olympus IX71 microscope as previously described. \(^9 \), \(^{49,57} \) All single-molecule image processing was done via home-written MATLAB codes. \(^9 \) FDTD simulations were done using FDTD Solutions from Lumerical Solutions, Inc.

ASSOCIATED CONTENT

3 Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsnano.8b01338.

Detailed procedures of catalyst synthesis and characterization; single-molecule fluorescence imaging experiments and data analyses; super-resolution catalysis image and SEM image correlation; FDTD simulations; catalyst surface area calculation; control experiments; and additional results (PDF).

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Notes

The authors declare no competing financial interest.

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