Single-Molecule Kinetics Reveals a Hidden Surface Reaction Intermediate in Single-Nanoparticle Catalysis

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Supporting Information

ABSTRACT: Detecting and characterizing reaction intermediates is not only important and powerful for elucidating reaction mechanisms but also challenging in general because of the low populations of intermediates in a reaction mixture. Studying surface reaction intermediates in heterogeneous catalysis presents additional challenges, especially the ubiquitous structural heterogeneity among the catalyst particles and the accompanying polydispersion in reaction kinetics. Here we use single-molecule fluorescence microscopy to study two complementary types of Au nanocatalysts—mesoporous-silica-coated Au nanorods (i.e., Au@mSiO2 nanorods) and bare 5.3 nm pseudospherical Au nanoparticles—at the single-particle, single-turnover resolution in catalyzing the oxidative deacetylation of amplex red by H2O2, a synthetically relevant and increasingly important probe reaction. For both nanocatalysts, the distributions of the microscopic reaction time from a single catalyst particle clearly reveal a kinetic intermediate, which is hidden when the data are averaged over many particles or only the time-averaged turnover rates are examined for a single particle. This intermediate is further resolvable by single-turnover kinetics at the subparticle level. Detailed single-molecule kinetic analysis leads to a quantitative reaction mechanism and supports that the intermediate is likely a surface-adsorbed one-electron-oxidized amplex red radical. The quantitation of kinetic parameters further allows for the evaluation of the large reactivity inhomogeneity among the individual nanorods and pseudospherical nanoparticles, and for Au@mSiO2 nanorods, it uncovers their size-dependent reactivity in catalyzing the first one-electron oxidation of amplex red to the radical. Such single-particle, single-molecule kinetic studies are expected to be broadly useful for dissecting reaction kinetics and mechanisms.

1. INTRODUCTION

Observing and characterizing reaction intermediates represents a powerful approach in elucidating the mechanism of chemical transformations, such as those in enzyme catalysis, homogeneous catalysis, and heterogeneous catalysis.1−8 But trapping and detecting reaction intermediates is difficult in general because of their very nature of being intermediates—their populations in a reaction mixture are low, and they exist only transiently. Synchronization of reaction progress (e.g., via the stopped-flow technique9,10) and trapping of reactive species (e.g., via rapid-freeze quenching11,12 or chemical trapping13,14) are often needed to obtain an appreciable amount of the desired intermediate, so as to characterize it by spectroscopic techniques.

In heterogeneous catalysis, studying reaction intermediates presents additional challenges.1,2,5−7,15,16 Besides the general low population of surface-adsorbed species, the ubiquitous heterogeneity among catalyst particles gives rise to polydispersion in reaction kinetics, making synchronization more difficult. Yet it is important to know whether and how many intermediates are involved in a surface catalytic reaction, so one can determine the number of rate-limiting steps in the reaction and how one could possibly modify the catalyst to accelerate the respective reaction steps for improving the catalyst performance.

Single-molecule kinetics provides advantages in dissecting reaction steps and uncovering reaction intermediates.17−23 By studying individual molecules/catalysts, this approach removes ensemble averaging, so dispersion in kinetics among different molecules can be circumvented. Synchronization of molecular actions is not needed either, as it monitors one molecule at a time. It also allows for following the actions of individual molecules in real time, and at any time point, only one molecular state is present even if the molecule can adopt multiple different states; this feature is particularly useful in capturing intermediates and elucidating reaction mechanisms.

The capability of single-molecule kinetics in uncovering intermediates and elucidating mechanisms has in particular been shown in single-molecule fluorescence studies of biological molecules. For example, Lu et al. studied the catalysis by single cholesterol oxidases and observed an enzyme−substrate complex as a kinetic intermediate.24 Others have observed kinetic intermediates in the catalysis by β-galactosidase,25 α-chymotrypsin,26 and nitrite reductase27 in the DNA unwinding by nonstructural protein 3 (NS3),28 and in virus fusion.29

Single-molecule kinetics via fluorescence microscopy has also been achieved in studying catalysis (or reactions) by layered
double hydroxides,\textsuperscript{31} zeolites,\textsuperscript{32,33} metal nanoparticles,\textsuperscript{34–41} semiconductor nanocrystals,\textsuperscript{42–48} carbon nanotubes,\textsuperscript{49} and small molecules,\textsuperscript{50–53} but no surface reaction intermediates were resolved in these studies. Other techniques, such as optical tweezers,\textsuperscript{54,55} magnetic tweezers,\textsuperscript{56} single-channel-recording,\textsuperscript{57} and atomic force microscopy,\textsuperscript{58} are also powerful in studying dynamic processes.

We have used single-molecule microscopy to study single Au nanoparticles and nanoplates in catalyzing a fluorogenic deoxygenation reaction at the single-turnover temporal resolution\textsuperscript{64–66} and later at nanometer spatial resolution as well.\textsuperscript{59} In this approach, the catalyzed reaction converts a nonfluorescent reactant to a highly fluorescent product on the surface of the nanoparticles, which are dispersed at a low density and spatially separated on a slide. Under continuous laser excitation, each catalytically produced reaction product emits a large number of fluorescence photons, enabling its easy detection and imaging at the single-molecule level (as well as its position localization down to nanometer precision). By imaging this fluorescent product one molecule at a time in real time, one can follow the catalytic reactions on a single nanoparticle at the single-turnover resolution in situ.

We have further applied this single-molecule microscopy approach to single Pt nanoparticles\textsuperscript{7} and single mesoporous-silica-coated Au nanorods (i.e., Au@mSiO\textsubscript{2} nanorods),\textsuperscript{38} using a different fluorogenic probe reaction: the oxidative deacetylation of amplex red (i.e., AR) by H\textsubscript{2}O\textsubscript{2} (Figure 1A). This deacetylation reaction has been an important probe reaction for assaying the activity of horseradish peroxidase and other enzymes that scavenge reactive oxygen species\textsuperscript{59–65} and is increasingly adopted by others as well in studying catalysis by semiconductor nanostructures.\textsuperscript{47,48} Despite these studies and the study\textsuperscript{66} on noncatalyzed photooxidation of AR where possible mechanistic steps and intermediates were proposed, no quantitative kinetic analysis is available, including the definition of the rate-determining steps and quantitation of their rate constants, on this important probe reaction.

In our previous study of single Au@mSiO\textsubscript{2} nanorods in catalyzing the oxidative deacetylation of AR\textsuperscript{38} we measured the time-averaged catalytic turnover rate as a function of the reactant concentration in a spatially resolved manner and discovered reactivity gradients along the side facets of individual nanorods, which were attributable to an underlying defect density gradient. Motivated by the increasing importance of this reaction as a probe in surface catalysis and the general relevance of deacetylation reaction to organic synthesis,\textsuperscript{67–69} we have been studying this reaction more mechanistically. Here we report that single-molecule kinetics further uncovers a hidden kinetic intermediate during the surface catalysis of AR deacetylation on single Au@mSiO\textsubscript{2} nanorods. We further discover that the same catalytic intermediate is resolved as well on single bare 5.3 nm pseudospherical Au nanoparticles. To our knowledge, these are the first examples of single-molecule kinetics in revealing surface reaction intermediates. By analyzing the distributions of the microscopic reaction time of individual catalyst particles, we were able to resolve two rate-determining steps, quantify the rate constant of each step, provide evidence for the nature of the captured intermediate, and formulate a quantitative working mechanism for the catalytic reaction.

2. EXPERIMENTAL SECTION

2.1. Synthesis and Characterization of Au Nanocatalysts. The 5.3 nm pseudospherical Au-nanoparticles, prepared from citrate reduction of H\textsubscript{2}AuCl\textsubscript{4} and capped with tannic acid, were purchased from Ted Pella (JME1052). Mesoporous silica-coated Au nanorods (i.e., Au@mSiO\textsubscript{2} nanorods) were prepared by making the Au nanorods first via stepwise seeded growth,\textsuperscript{70} coating them with silica, and subsequently etching the silica shell with base in the presence of cetyltrimethylammonium bromide to make it mesoporous,\textsuperscript{71} as reported previously (more details in Supporting Information).\textsuperscript{38} The Au@mSiO\textsubscript{2} nanorods were later calcinated at 500 °C to remove the organic ligands for activation for surface catalysis, during which the Au nanorod cores maintained their morphology as examined by transmission electron microscopy (TEM), as we reported previously.\textsuperscript{38} All samples were characterized by an FEI Tecnai 12 TEM at Cornell Center for Materials Research.

2.2. Catalytic Reaction Conditions. The oxidative deacetylation of AR, a nonfluorescent molecule, to resorufin, a highly fluorescent molecule, by hydrogen peroxide (H\textsubscript{2}O\textsubscript{2}) was studied in 50 mM pH 7.3 degassed sodium phosphate buffer. Ensemble reaction measurements were done via monitoring the fluorescence of the reaction product resorufin (excited at 532 nm and detected at 585 nm) using a Varian Cary Eclipse fluorometer.

2.3. Single-Molecule Fluorescence Microscopy. Single-molecule fluorescence measurements were performed on a home-built prism-type total internal reflection fluorescence (TIRF) microscope based on an Olympus IX71 inverted microscope as previously described.\textsuperscript{34–37} A 5–6 mW continuous-wave circularly polarized 532 nm laser (CrystaLaser, GCL-025-L-0.5%) was focused onto an area of ~80 × 40 μm\textsuperscript{2} in a microfluidic reactor cell to directly excite the fluorescence of the product resorufin generated on immobilized nanocatalysts. The fluorescence of resorufin was collected by a 60× NA 1.2 water-immersion objective (UPLSAPO60XW, Olympus), filtered by two filters (HQ550LP, HQ580m60), and projected onto a camera (Andor iXon EMCCD) controlled by the Andor IQ software. The time resolution of image acquisition was 25 ms.

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The microfluidic reactor, ~100 μm (height) × 2 cm (length) × 5 mm (width), was assembled using double-sided tapes sandwiched between a quartz slide (Technical Glass) and a borosilicate coverslip (Gold Seal). The quartz slide was amine-functionalized by an aminosilane reagent (Vectorbond, Vector Laboratory) to have a positively charged surface to immobilize the negatively charged 5.3 nm Au nanoparticles. As for the Au@mSiO2 nanorods, they were drop casted on the quartz slide. The reactant solution was flowed in continuously at 25 μL/min, maintaining nonequilibrium steady-state reaction kinetics.

2.4. Scanning Electron Microscopy. The microfluidic reactor used for the fluorescence microscopy measurements was subsequently disassembled for characterizing the nanoparticles on the slide under a LEO 1550VP FESEM (operated at 2–5 keV). A carbon film of ~10 nm was coated on the quartz slide before the scanning electron microscopy (SEM) measurements.

3. RESULTS AND DISCUSSION

3.1. Catalytic Reaction and Nanocatalysts. We studied the catalyzed oxidative deacetylation of AR by H2O2 to generate resorufin and acetate (Figure 1A).37 The product resorufin is highly fluorescent, allowing for its single-molecule fluorescence imaging.

We studied two types of Au nanocatalysts: mesoporous silica-coated Au nanorods (i.e., Au@mSiO2 nanorods) and "bare" pseudospherical 5.3 ± 0.7 nm Au nanoparticles (Figure 1B,C and Supporting Information, Figure S1; here bare refers to the fact that particles have weak surface ligands, e.g., citrate or tannic acid, which can be readily washed off).

These two types of Au nanocatalysts are complementary model catalysts. The Au nanorods are pseudo-one-dimensional nanocatalysts with defined surface facets (e.g., their side facets are {110} and {100}).72,73 The Au nanorod cores have monodisperse diameters (21.4 ± 3.2 nm), but their lengths are variable (from ~100 nm to 600–700 nm);38 they are large enough to be imaged readily under SEM (Supporting Information, Figure S2) to be correlated with optical microscopy using the same sample preparation (i.e., dispersed on quartz slides). The mSiO2 shell (~80 nm in thickness) here was necessary for removing through calcination the capping ligands that were used in synthesizing these nanorods while maintaining their structural integrity and preventing aggregation. The mesopores here are large enough for reactants to access the gold surface, and the catalytic kinetics is not limited by mass transport of reactants, as we reported.38,39 The mSiO2 shell also helps temporarily trap the product resorufin, facilitating its single-molecule fluorescence detection before it desorbs, diffuses into surrounding solution, and gets carried away by the solution flow.

On the other hand, the 5.3 nm pseudospherical Au nanoparticles are pseudo-zero-dimensional nanocatalysts with many types of surface facets, which are hard to define. Their small sizes also make them harder to image by SEM. But, these pseudospherical particles can be prepared without using strong capping ligands, removing the need for the mSiO2 encapsulation.

3.2. Single-Turnover Kinetics of Single Au@mSiO2 Nanorods Reveal a Reaction Intermediate. Figure 2A shows an exemplary fluorescence intensity versus time trajectory from a single immobilized Au@mSiO2 nanorod in catalyzing the oxidative deacetylation of AR to resorufin by H2O2. The reactants were kept at constant concentrations and supplied continuously via a flow, giving steady-state reaction kinetics. This trajectory features stochastic fluorescence intensity bursts over a constant background emission of the Au nanorod; each burst reports the generation of a single product molecule resorufin. These bursts each typically last for ~50–100 ms (Supporting Information, Figure S3), before the product desorbs, diffuses out of the laser excitation volume, and gets carried away by the solution flow; the times (τ) between the bursts are the microscopic reaction times for catalytic product generation. These τ values are probabilistic in their individual values, but their statistical properties, such as averages and distributions, are defined by the underlying reaction kinetics.74,75 Although AR can also be oxidized by H2O2 spontaneously, the contribution of this spontaneous reaction to the detected catalytic events is many orders of magnitude lower than that from the Au-surface catalyzed reaction, as we previously showed (Supporting Materials in ref 38).

Figure 2B–D shows the distributions of τ from a single Au@mSiO2 nanorod at various AR concentrations (i.e., [AR]), while...
the H$_2$O$_2$ concentration was kept at large excess. (The catalytic activities of these Au@mSiO$_2$ nanorods were sufficiently stable so that each nanorod could be studied over a range of [AR] over a period of many hours.\textsuperscript{38} Strikingly, the distributions of $\tau$ show an initial rise and then decay behavior with a delayed maximum at $\tau > 0$ (especially clear at [AR] = 0.05 $\mu$M, Figure 2B), in contrast to the typical single-exponential decay behavior for single-molecule kinetics that contains just one rate-determining step.\textsuperscript{18,74,76–78} This initial rise and then decay behavior of $\tau$ distributions indicates that the catalytic kinetics in forming the fluorescent product contains at least two sequential rate-determining steps; that is, there is a hidden kinetic intermediate.\textsuperscript{21,26,74} Similar behaviors of $\tau$ distributions were also observed in the single-molecule kinetics of some enzyme reactions that contain kinetic intermediates.\textsuperscript{21,26–30} It is important to note that this initial rise and then decay behavior of $\tau$ distributions could not be reliably discerned when $\tau$ values from many nanorods are compiled because of the polydispersion in kinetics among the individual nanorods (Supporting Information, Figure S4); therefore, even at the single-turnover resolution, measurements at the single-particle level were also essential in unmasking the kinetic signature of this intermediate.

The distributions of $\tau$ from single Au@mSiO$_2$ nanorods can each be fitted by an empirical equation of a combination of two exponentials:

$$y = A(e^{-k_1 \tau} - e^{-k_2 \tau})$$

(1)

where $k_1$ and $k_2$ are the apparent rate constants for the two rate-determining steps straddling the kinetic intermediate (see later and Supporting Information, Section 4 for derivation of this functional form of the $\tau$ distribution), and $A$ is a scaling factor. Interestingly, in examining the results across a range of [AR], we find that $k_2$ is dependent on [AR]: it increases with increasing [AR] and eventually saturates (Figure 2E); this dependence indicates that the associated rate-determining step involves the reactant AR. In contrast, $k_3$ is virtually independent of [AR] (Figure 2F), indicating that the associated rate-determining step does not involve AR. Moreover, $k_3$ is in general an order of magnitude larger than $k_2$ across different [AR]. These trends of $k_1$ and $k_2$ versus [AR] persist, regardless of whether individual Au@mSiO$_2$ nanorods are examined or $k_1$ and $k_2$ are averaged over many nanorods (Figure 2E,F).

The single-turnover kinetics also readily gives the conventional rate of turnovers ($v$, in s$^{-1}$ particle$^{-1}$) for a single Au@mSiO$_2$ nanorod, which is equivalent to $\langle \tau \rangle^{-1}$, where $\langle \cdot \rangle$ denotes averaging. With increasing [AR], the single-particle rate of turnovers $\langle \tau \rangle^{-1}$ shows typical saturation kinetics, regardless of whether the data are from a single Au@mSiO$_2$ nanorod or are averaged over many nanorods (Figure 2G). These saturation kinetics of $\langle \tau \rangle^{-1}$ alone can be well-accounted for by the classic Langmuir–Hinshelwood mechanism for surface catalysis involving surface-adsorbed AR molecules, without the need of invoking an intermediate in the catalytic kinetics, as we previously showed.\textsuperscript{34,36–38} In other words, the conventional titration of turnover rate versus reactant concentration is insensitive here to the presence of this intermediate. This insensitivity again highlights the capability of single-turnover resolution kinetics to produce the distribution of the microscopic reaction time $\tau$ to unmask kinetic intermediates.

### 3.3. Subparticle-Level Single-Turnover Kinetics Can Still Reveal the Reaction Intermediate on a Au@mSiO$_2$ Nanorod.

In imaging the fluorescence signal of the individual reaction products on a Au@mSiO$_2$ nanorod, the position of each product can be localized to a few to tens of nanometers precision, as we showed previously.\textsuperscript{38} Figure 3A shows the image plot of the two-dimensional position histogram of all the reaction products detected on the particular Au@mSiO$_2$ nanorod, whose single-turnover kinetics is presented in Figure 2B–D. This image clearly resolves the nanorod shape at tens of nanometers resolution, that is, superoptical resolution. This spatial resolution allows for analyzing the single-molecule kinetics of a nanorod at the subparticle level, for example, by dissecting the nanorod into two halves (Figure 3A). By extracting the real-time sequence of the catalytic product formation events for each half, we obtained the distributions of the respective microscopic reaction time $\tau$ for the two halves separately: Figure 3B–D for the left half and Figure 3E,F for the right half at various [AR]. (Note we could also dissect the nanorod into smaller segments, but with decreasing segment size, the number of catalytic events per segment also decreases, limiting the statistical significance of the distributions of $\tau$.)

The average $\tau$ for each half segment is about twice longer than that for the whole nanorod (e.g., Figure 3B–D vs Figure 2B–D). This is expected, as a half segment contains about half of the number of catalytic events over the entire observation time. Strikingly though, the distributions of $\tau$ for each half
segment still show clear initial rise and then decay behaviors, indicating the presence of the reaction intermediate. Therefore, even at the subparticle level, single-turnover kinetics can still unmask reaction intermediates in surface catalysis.

### 3.4. Single-Turnover Kinetics of Single 5.3 nm Pseudospherical Bare Au Nanoparticles Again Reveals a Reaction Intermediate.

To probe if the observed kinetic intermediate is unique to Au@mSiO2 nanorods or is somehow related to the presence of its mSiO2 shell, we further studied bare pseudospherical Au nanoparticles of 5.3 nm diameter. Figure 4A shows an exemplary fluorescence intensity versus time trajectory from a single 5.3 nm Au nanoparticle in catalyzing the deacetylation of AR by H2O2. This trajectory exhibits the characteristic intensity bursts reporting the generation of the fluorescent product molecule resorufin, similarly as in Figure 2A. The distributions of the microscopic reaction time τ from a single particle also show clear initial rise and then decay behaviors (Figure 4B–C), indicating the presence of a hidden reaction intermediate straddled by two rate-determining steps in converting AR to the product resorufin. Again, this initial rise and then decay behavior is masked when the τ values from many particles are compiled together (Supporting Information, Figure S5), further stressing the importance of having both single-particle and single-turnover level resolution in measuring kinetics here.

Compared with the Au@mSiO2 nanorods, these 5.3 nm Au nanoparticles do not have the mSiO2 shell, and the product resorufin is detected while it is temporarily adsorbed on the particle surface. Therefore, the persistent observation of a hidden kinetic intermediate indicates that this intermediate stems from the catalytic process on the Au surface and is unrelated to the presence or not of an mSiO2 shell.

The distribution of τ for a single 5.3 nm Au nanoparticle here again can be satisfactorily fitted by eq 1, where k1 and k2 are the apparent rate constants of the two rate-determining steps (Figure 4B,C). We further studied many 5.3 nm Au nanoparticles across a range of [AR], while [H2O2] was kept at large excess. (Note that because of particle inactivation that becomes significant after 2 h, we could not study the same set of 5.3 nm Au nanoparticles over the entire range of [AR]; instead, a different set of particles were studied at each [AR] within a <2 h time window.) Once averaged over the many Au nanoparticles, the extracted k1 shows a clear dependence on [AR]; it increases with increasing [AR] until saturation (Figure 4D), whereas k2 is essentially independent of [AR] (Figure 4E); both trends are similar to those for Au@mSiO2 nanorods (Figure 2E,F).

(τ)−1, the single-particle rate of turnovers, for the 5.3 nm Au nanoparticles shows the typical saturation kinetics with increasing [AR] for surface-mediated catalysis (Figure 4F), similar to that of Au@mSiO2 nanorods (Figure 2G). Again, this saturation kinetics of (τ)−1 is insensitive to the presence of the kinetic intermediate that is unmasked by the distribution of τ in Figure 4B,C.

### 3.5. Mechanism of Catalysis.

The single-turnover kinetics of single Au@mSiO2 nanorods and single 5.3 nm Au nanoparticles above show that the surface-catalyzed oxidative deacetylation of AR by H2O2 involves a kinetic intermediate straddled by two rate-determining steps (Figure 2B–D and Figure 4B,C). The kinetics of one of the two steps is dependent on [AR], whereas the other is independent (Figure 2E,F and Figure 4D–E). The catalysis also directly involves surface-adsorbed AR molecules, as evidenced by the saturation behavior of the single-particle turnover rate (τ)−1 versus [AR] (Figure 2G and Figure 4F).

Unfortunately, titrating [H2O2] in single-molecule imaging of single-nanoparticle catalysis is unreliable: the concentration of dilute H2O2 solutions is unstable, but the single-nanoparticle catalysis measurements need a long observation time (>1 h) to observe a sufficient number of catalytic turnovers per particle for accumulating statistics on τ. Nevertheless, we performed titration of the catalytic reaction rate versus [H2O2] at the ensemble level, using 5.3 nm pseudospherical Au nanoparticles as a representative because they are more homogeneous in size (Figure 1C) (the Au@mSiO2 nanorod sample contains a mixture of pseudospherical particles, triangular particles, and variable-length nanorods, which are more problematic for ensemble-averaged measurements; Figure 1B and Supporting Information, Figure S1,C). The reaction rate shows saturation kinetics with increasing [H2O2] (Figure 5A), supporting that the catalysis involves adsorption of H2O2 onto the particle surfaces.

To account mechanistically for the observed single-turnover catalytic kinetics of Au@mSiO2 nanorods and 5.3 nm Au nanoparticles, we first considered the following reaction sequence for the surface-catalyzed oxidative deacetylation of AR to resorufin by H2O2 (Scheme1, and Supporting Information, Section 4). This catalyzed reaction has overall a 1:1 reaction stoichiometry between AR and H2O2, as we
determined previously (Figure 1A), and for AR, it is overall a two-electron oxidation reaction.

In this overall reaction sequence (Scheme 1), the two reactants first adsorb reversibly to the Au surface (step (i)). The adsorbed H$_2$O$_2$ can undergo a reversible hemolytic O–O bond cleavage to generate surface-bound OH$^\bullet$ radicals (step (ii)). Then the reaction proceeds via a one-electron oxidation of AR by a surface-bound OH$^\bullet$ radical, generating a radical species AR$^\bullet$ (step (iii)). A subsequent second electron oxidation generates the product resorufin (step (iv)). The deactivation of AR could be accompanying the first or the second oxidation step, or occur as a separate step that is not explicitly included here, and both or either step can be coupled with proton transfer as well.

It is known that Au surface can catalyze H$_2$O$_2$ decomposition to form OH$^\bullet$ radicals, and this reaction is reversible. To test the involvement of OH$^\bullet$ radical in the reaction pathway, we examined the effect of dimethyl sulfoxide (DMSO), a known effective scavenger of OH$^\bullet$ radicals, on the catalytic kinetics at the ensemble level, again using 5.3 nm Au nanoparticles as the representative. The catalytic reaction rate is strongly quenched by DMSO in the solution (Figure 5B), indicating the involvement of OH$^\bullet$ radical in the reaction pathway. The involvement of the AR$^\bullet$ radical in the oxidation of AR to form resorufin has been described in the catalysis by the enzyme horseradish peroxidase. However, we could directly detect the fluorescence of the product resorufin on their surfaces. For Au@mSiO$_2$ nanorods, the generated resorufin on the Au nanorod surface could be fluorescently quenched due to the proximity to the Au surface, before desorbing from Au nanorod surface and getting temporarily trapped in the mSiO$_2$ shell. Regardless, this fluorescence-quenched state of the product cannot be the observed kinetic intermediate, as the intermediate is still present for the bare 5.3 nm Au nanoparticles.

In Scheme 1, step (i) can be assumed to follow a fast adsorption–desorption equilibrium, in which AR and H$_2$O$_2$ each follow a Langmuir adsorption behavior onto different types of surface sites, which would be consistent with the saturation kinetics observed with increasing reactant concentrations. The two rate-determining steps observed experimentally should come from the remaining three reaction steps (i.e., steps (ii), (iii), and (iv)).

Scheme 1. Possible Reaction Steps of Amplex Red Oxidation by H$_2$O$_2$ Catalyzed on Au Nanoparticle Surfaces

The apparent rate constants of the two rate-determining steps would both be predicted to be dependent on [AR] (Supporting Information, Section 4.2) and [H$_2$O$_2$] (Supporting Information, Equation S16 (Supporting Information, Section 5)) at saturating [H$_2$O$_2$]. [H] is the H$_2$O$_2$ concentration. The O–O bond of H$_2$O$_2$ to generate OH$^\bullet$ on Au surfaces is estimated to be ~0.1 μmol cm$^{-2}$ s$^{-1}$. For Au@mSiO$_2$ nanorods and 5.3 nm Au nanoparticles with average surface areas of 10$^{-10}$ cm$^2$ and 10$^{-12}$ cm$^2$, respectively, this O–O bond cleavage rate would correspond to 10$^2$ and 10$^3$ s$^{-1}$ particle$^{-1}$, at least 6 orders of magnitude larger than their observed highest turnover rates at ~2 and ~0.04 s$^{-1}$ particle$^{-1}$ (Figure 2G and Figure 4F). The apparent rate constants of the two rate-determining steps would both be predicted to be dependent on [AR] (Supporting Information, Section 4.2) and [H$_2$O$_2$] (Supporting Information, Equation S16 (Supporting Information, Section 5)) at saturating [H$_2$O$_2$]. [H] is the H$_2$O$_2$ concentration. The O–O bond of H$_2$O$_2$ to generate OH$^\bullet$ on Au surfaces is estimated to be ~0.1 μmol cm$^{-2}$ s$^{-1}$. For Au@mSiO$_2$ nanorods and 5.3 nm Au nanoparticles with average surface areas of 10$^{-10}$ cm$^2$ and 10$^{-12}$ cm$^2$, respectively, this O–O bond cleavage rate would correspond to 10$^2$ and 10$^3$ s$^{-1}$ particle$^{-1}$, at least 6 orders of magnitude larger than their observed highest turnover rates at ~2 and ~0.04 s$^{-1}$ particle$^{-1}$ (Figure 2G and Figure 4F). The apparent rate constants of the two rate-determining steps would both be predicted to be dependent on [AR] (Supporting Information, Section 4.2) and [H$_2$O$_2$] (Supporting Information, Equation S16 (Supporting Information, Section 5)).

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following forms (see derivations in Supporting Information, Section 4).

\[
k_{app1} = \frac{G_A[A]}{1 + G_A[A]} \frac{G_B^{1/2}[H]^{1/2}}{1 + G_B^{1/2}[H]^{1/2}} \gamma_{eff1} \frac{G_A[A]}{1 + G_A[A]}
\]

(k app2 = \frac{G_A[A]}{1 + G_A[A]} \frac{G_B^{1/2}[H]^{1/2}}{1 + G_B^{1/2}[H]^{1/2}} \gamma_{eff2}
\]

Here G_A is the adsorption equilibrium constant of AR. G_B is the equilibrium constant for H_2O_2 adsorption to directly become two adsorbed OH^·. γ_{eff1} = γ_1 n_A n_B, γ_{eff2} = γ_2 n_B, where γ_1 and γ_2 are two rate constants and n_A and n_B are, respectively, the total numbers of adsorption sites for AR and OH^· on a particle. [A] (i.e., [AR]) and [H] are the solution concentrations of AR and H_2O_2, respectively. Moreover, when H_2O_2 in the solution goes to saturation concentrations (e.g., at 60 mM in our experiment), ((G_B^{1/2}[H]^{1/2})/(1 + G_B^{1/2}[H]^{1/2})) → 1. Clearly, k app1 is dependent on [A] (the AR concentration)—it increases with increasing [A] and eventually saturates—whereas k app2 is independent of [A], as observed experimentally for the two exponents in the distributions of τ (Figure 2E,F and Figure 4D,E).

\[(\tau)^{-1} = \frac{\gamma_{eff1} G_A[A]}{1 + \left(1 + \frac{\gamma_{eff2}}{\gamma_{eff1}} G_A[A]\right)} \frac{\gamma_{eff2} G_A[A]}{1 + G_A[A]}\]

(5)

With increasing AR concentration, (τ)^{-1} is predicted to show the typical saturation kinetics, consistent with experimental results (Figure 2G and Figure 4F), and be non-informative about the presence of the kinetic intermediate. The limiting form of eq S at γ_{eff} ≫ γ_{eff1} is identical to the classic Langmuir–Hinshelwood rate equation for the case there is merely one rate-determining step and no kinetic intermediate is present, which we used previously.38

3.6. Inhomogeneity and Size Dependence of Nano-catalyst Reactivity. The formulation of the effective kinetic mechanism in Scheme 2 and the corresponding expressions for f(τ), k app1, and k app2 in eqs 2–4 allowed us to fit the experimental results to determine the kinetic parameters for each catalyst particle. For each Au@mSiO2 nanorod, the data collected at all [AR] were globally fitted (e.g., fitting the distributions of τ in Figure 2B–D, or the k_1 and k_2 in Figure 2E,F) to obtain its γ_{eff} (= γ_1 n_A n_B, γ_{eff2} = γ_2 n_B, and G_A. These nanorods are sufficiently large to be easily identifiable in their SEM image (Supporting Information, Figure S2), from which the core length of each nanorod can be obtained using known mSiO2 shell thickness (~80 nm, Supporting Information, Section 1.3).38 For each 5.3 nm Au nanoparticle, its distribution of τ at an [AR] was fitted to obtain its γ_{eff}, γ_{eff2}, and G_A (e.g., Figure 4B,C).

In general, Au@mSiO2 nanorods are more active on a per particle basis than the 5.3 Au nanoparticles, reflected by their ~20 times and ~2 times larger average γ_{eff1} and γ_{eff2} values, respectively (Figure 6A,B vs E,F). It is not surprising, as the nanorods are much larger and thus have more surface sites per particle. The distributions of γ_{eff} γ_{eff2}, and G_A for the Au@mSiO2 nanorods are all broad (Figure 6A–C), reflecting the large reactivity inhomogeneity among the individual nanorods. This large reactivity inhomogeneity is also observed among the individual 5.3 nm Au nanoparticles (Figure 6E–G). For these inhomogeneities, single-nanoparticle catalysis measurements are uniquely capable of quantifying them, for example, here by the distributions of the respective kinetic parameters.

For the individual Au@mSiO2 nanorods, the availability of their lengths from SEM also allowed for examining their size-dependent catalytic properties. Their γ_{eff} (= γ_1 n_A n_B) value represents the combined reactivity of all the sites on a single nanorod in the first one-electron oxidation of AR to AR^· by OH^· (the first rate-determining step in Scheme 2). γ_{eff} shows a clearly positive correlation with the nanorod length (i.e., L; Figure 6A, left), attributable to a larger number (i.e., n_A and n_B) of reactive sites on longer nanorods. However, once normalized by the nanorod length, γ_{eff}/L decreases with increasing length (Figure 6D), suggesting that longer Au@mSiO2 nanorods have smaller specific reactivity (i.e., γ_1) for the reaction of AR oxidation to AR^·. In contrast, γ_{eff2} (= γ_2 n_B), which represents the combined reactivity of all sites on a single nanorod for the second one-electron oxidation of AR^· to P (the second rate-determining step in Scheme 2), shows no clear dependence on the nanorod length (Figure 6B, left), even though longer nanorods presumably have more reactive sites of the n_B type.
This independence might result from opposite dependences of \( \gamma \) and \( n_p \) on nanorod length, thus canceling each other. \( G_{\text{AR}} \) being the adsorption equilibrium constant of AR, does not show any clear dependence on the nanorod length, either (Figure 6C, left).

4. CONCLUSION

Using single-molecule fluorescence microscopy, we have studied the single-molecule kinetics of individual Au@mSiO\(_2\) nanorods and bare 5.3 nm pseudospherical Au nanoparticles in catalyzing the oxidative deacetylation of AR to resorufin by H\(_2\)O\(_2\), a synthetically relevant and increasingly important probe reaction. For both nanocatalysts, the distributions of the microscopic reaction time from a single catalyst particle, as well as from part of a single particle, clearly reveal a kinetic intermediate straddled by two rate-determining steps. This surface reaction intermediate is hidden when the data are averaged over many particles or only the time-averaged turnover rates are examined for a single particle, demonstrating the necessity of both single-turnover and single-particle resolution in unmasking this reaction intermediate. The kinetic (in)dependence of the two rate-determining steps on the reactant concentration allows for the formulation of a reaction mechanism that can describe quantitatively the catalytic kinetics, which, in combination with literature results, supports that the intermediate is likely a surface-adsorbed one-electron-oxidized AR radical. The quantitative kinetics also gives kinetic parameters of the reaction steps for each catalyst particle, allowing the evaluation of the large reactivity inhomogeneity among the individual nanorods and pseudospherical nanoparticles. Parallel SEM imaging further enables the correlation of each Au@mSiO\(_2\) nanorod’s reactivity with its length, uncovering its size-dependent reactivity in catalyzing the first one-electron oxidation of AR to the radical. To our knowledge, this study represents the first examples of single-molecule kinetics in revealing surface reaction intermediates. We envision that such single-molecule kinetic studies should be broadly useful for dissecting reaction kinetics and mechanism in heterogeneous catalysis, where the catalyst heterogeneity is a ubiquitous challenge for which ensemble-averaging could mask the kinetic signatures of reaction intermediates.

ASSOCIATED CONTENT

Supporting Information

Detailed procedures of catalyst synthesis and structural characterization. Procedures for extracting the microscopic reaction time \( \tau \) from single-molecule fluorescence movies of nanocatalyst catalysis. Additional results on the single-molecule kinetics of Au@mSiO\(_2\) nanorods and 5.3 nm pseudospherical Au nanoparticles. Detailed formulation of possible reaction mechanisms, and the corresponding derivations of the probability density functions of \( \tau \). This material is available free of charge via the Internet at http://pubs.acs.org.

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