Distinguishing Direct and Indirect Photoelectrocatalytic Oxidation Mechanisms Using Quantitative Single-Molecule Reaction Imaging and Photocurrent Measurements

Justin B. Sambur and Peng Chen*

Department of Chemistry and Chemical Biology, Cornell University, Ithaca, New York 14853, United States

ABSTRACT: Light-driven semiconductor-catalyzed oxidation reactions are of fundamental importance in photocatalysis and photoelectrocatalysis for removing organic contaminants in wastewater, solar energy conversion, and fine chemical synthesis. The underlying reaction mechanism is often unclear because it is difficult to measure directly and specifically the semiconductor-catalyzed reaction rates. For example, an organic molecule could be oxidized “directly” by photogenerated holes that are transported from the semiconductor interior to the semiconductor−electrolyte interface or “indirectly” by photogenerated intermediates (e.g., hydroxyl radical, superoxide anion, or hydrogen peroxide) that are produced at the semiconductor surface in aqueous solution. New experimental approaches that can distinguish these pathways are thus desirable. Here we introduce quantitative single-molecule, single-particle fluorescence imaging to measure the photoelectrocatalytic oxidation rate of a model organic substrate, amplex red, on the surface of individual rutile TiO2 nanorods. Our approach probes the oxidation product selectively before it becomes further degraded (which complicates bulk reaction kinetics measurements) while also avoiding interparticle charge transfer kinetics. By examining the reaction rate scaling relations versus light intensity at fixed potential and versus potential at fixed light intensity, together with the corresponding photocurrent scaling reactions, we demonstrate that amplex red oxidation on a TiO2-nanorod photoanode proceeds via an indirect mechanism.

1. INTRODUCTION

Semiconductor photo(electro)catalysis is a multistep process that involves photoexcitation of a semiconductor, followed by charge-carrier separation within the semiconductor and subsequent charge transfer reactions at the semiconductor−liquid interface.6,2 Solar-light-driven semiconductor-catalyzed chemical reactions are of broad interest for environmental remediation (e.g., decomposition of organic contaminants in wastewater),3 solar energy conversion,4 and fine chemical synthesis.2,5 Decades of research have been devoted to addressing the fundamental questions regarding the underlying photo(electro)catalytic oxidation mechanism. For example, a variety of oxidizing intermediate species may be produced by a photoexcited semiconductor in aqueous solution.6 Photogenerated electrons can react with dissolved O2 to generate superoxide anions (O2•−), which can then oxidize a substrate. O2•− can also further react with protons and additional photogenerated electrons to form hydrogen peroxide (H2O2) and hydroxyl radical species (OH•), which can also act as oxidants.8 On the other hand, photogenerated holes can directly oxidize a substrate or react with surface-adsorbed water molecules to generate OH•.8 Since both photogenerated electrons and holes can potentially produce oxidative intermediates, identifying the true oxidant in a photo(electro)catalytic oxidation reaction can be challenging. Even when anaerobic conditions are used to remove the O2-induced oxidation pathway, photogenerated holes could react “directly” or “indirectly” (e.g., via intermediate OH• with organic molecules, and therefore the dominant reaction pathway needs to be differentiated.8,9

Previous studies used ensemble-level photocatalysis and photoelectrochemical characterization tools to distinguish between the direct or indirect hole-induced oxidation mechanisms. Most experiments use TiO2-based photo(electro)-catalysts because the materials are commercially available, inexpensive, nontoxic, and photochemically stable.6 For photoexcited semiconductor particles in aqueous solution (i.e., photocatalysis), reaction mechanisms can be probed by analyzing product distributions and formation rates. By use of these analyses, the direct, indirect, and simultaneous direct−indirect oxidation mechanisms have been observed (see ref 10 and references therein); the mechanism depends on the...
photocatalyst, solution composition, and organic substrate. In some cases, such as formic acid oxidation by TiO₂ photocatalysts, some authors propose an indirect mechanism⁹,¹² while others propose a direct mechanism.²,¹³−¹⁵ This controversy is partly due to that in photocatalysis both photogenerated electrons and holes react on the same particle surface, making it difficult to differentiate which charge-carrier limits the oxidation rate of the organic substrate.¹⁶

To address this controversy, Salvador and co-workers combined electrochemical and photocatalytic methods (i.e., photoelectrocatalysis) to separate the oxidation and reduction processes to a TiO₂ photoanode and a metal cathode, respectively.¹⁰,¹¹ This approach effectively removed the electron-induced oxidation pathway by rapidly extracting photogenerated electrons via the external circuit. By analyzing the organic substrate-induced photocurrent enhancement as a function of light intensity at a fixed applied potential, they concluded that formic acid and methanol are oxidized by direct and indirect pathways, respectively. A critical requirement for this approach is that the organic substrate is oxidized by the so-called “current doubling” mechanism.¹⁶ Several other studies have been reported in using the scaling relation of photocurrent enhancement versus light intensity for other photoanode—substrate combinations.⁹,¹⁹,²⁰ On the other hand, the scaling relation of substrate-induced photocurrent enhancements versus the applied potential has not been explored quantitatively.

Considering the importance of photo(electro)catalytic oxidation reactions and the complications faced when determining the underlying mechanism, new or alternative experimental approaches are desirable to distinguish between potential mechanistic pathways. Here, building upon our recent work,²¹ we use a novel single-molecule, single-particle approach to distinguish between photoelectrocatalytic oxidation mechanisms. We chose solution-processable rutile TiO₂ nanorods with well-defined surface facet orientations (¹{100} sidewalls and {011} end tips) as model semiconductor photoanodes.²² We chose to study amplex red as a model organic substrate because it can be irreversibly oxidized to a highly fluorescent product resorufln (Scheme 1) and the reaction rate can be monitored via absorption and fluorescence spectroscopy at the ensemble level and via fluorescence at the single-molecule level.²³,²⁴ Amplex red is becoming increasingly popular as a probe reaction for (photo)catalytic oxidation reactions,²³−²⁵ but the reaction mechanism is often not defined. Recent ensemble-level photocatalysis studies showed that photoexcited TiO₂ nanoparticles could further oxidize the product resorufln,²⁴ which complicates the quantitation of amplex red to resorufln reaction kinetics because the fluorescent product is continuously generated as well as consumed during the bulk fluorescence assay experiment.

Here we use single-molecule fluorescence microscopy to quantitatively image individual product molecules while they are generated on individual TiO₂ nanorod photoanode surfaces (i.e., single reaction resolution). The single-molecule, single-particle approach to study photoelectrocatalysis has several unique advantages compared with ensemble-level measurements. First, fluorescence microscopy selectively probes product formation prior to its consumption. Second, the product fluorescence signal is distinct from the photoelectrochemical current signal, which is not selective to the substrate oxidation process and contains many other contributions such as water oxidation in an aqueous electrolyte. Third, as we only study isolated nanorods, complex charge-carrier transport processes between nanorods do not contribute to the observed reaction rates. Fourth, studying single particles also removes ensemble-averaging effects and allows for identification of champion or inactive catalysts.

We use the Gärtner model to describe the photogenerated hole transport rate to the semiconductor photoanode surface (step i in Scheme 2).³¹ The major assumptions of this model are the following. (1) Charge carrier generation follows a Beer–Lambert absorption profile into the semiconductor photoanode. (2) The applied potential E is entirely manifested as a potential drop within the depletion region of the semiconductor whose thickness is W (i.e., the depletion approximation; see Scheme 2 for schematic illustration). (3) All photogenerated holes within the depletion region are transported to the surface (i.e., no recombination with electrons within W). (4) Holes generated outside the depletion region (i.e., x > W in Scheme 2) are transported to the surface if they diffuse into the depletion region. (5) Due to the ~3.0 eV band gap of TiO₂, the thermally excited hole population at room temperature is orders of magnitude lower than the light-

### Scheme 1. Oxidative Decaylation of Amplex Red (AR) to Resorufln (P) by a Photoexcited TiO₂ Photoanode

**Amplex Red**

nonfluorescent

**Resorufln**

highly fluorescent

**Reactions:**

- **Scheme 1:** Oxidative Decaylation of Amplex Red (AR) to Resorufln (P) by a Photoexcited TiO₂ Photoanode

**Equation:**

\[
\text{[R]}_s = k_\text{R}[\text{ox}]]
\]

This equation describes the reaction rate of R (\(v_R\)) as a function of the concentration of the oxidizing species ([ox]).

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**Note:**

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Scheme 2. Illustration of Photoelectrooxidation of an Organic Substrate (R) to a Product (P) and of H₂O/OH⁻ to O₂ on a TiO₂ Photoanode?

See the main text for details on steps i–vii. Step i: hole transport to the TiO₂ surface. Steps ii–iv represent the major water oxidation reaction steps. Step v: direct oxidation pathway of R by surface holes. Steps vi and vii: indirect oxidation pathway of R by OH⁻ and H₂O₂, respectively.

Induced hole population, and therefore thermally excited holes are not considered. Under these conditions, the photogenerated hole transport rate \( \nu_h^i \) to the photoanode surface is given by

\[
\nu_h^i = \frac{d[(h^+_i)]}{dt} = I_0 \left(1 - \frac{1}{1 + aW_{fi}}\right)
\]  

(2)

Here \( q, I_0, \alpha, \) and \( L_p \) are the elementary charge (C), intensity of light impinging on the semiconductor photoanode (photons/s), absorption coefficient (cm⁻¹) of the semiconductor at the excitation light wavelength, and the hole diffusion length (cm).

\( W = \sqrt{2e\epsilon_0(E - E_{fb})/(qN_d)} \) (cm), where \( e \) is the relative dielectric constant of the semiconductor, \( \epsilon_0 \) is the vacuum permittivity, \( E \) is the applied potential relative to the Ag/AgCl reference electrode, \( E_{fb} \) is the flat band potential, and \( N_d \) is the doping density of the semiconductor.

In eq 2, \( aL_p \) and \( aW \) account for the fraction of absorbed photons within a diffusion length distance from \( W \) and the fraction of photons absorbed within \( W \), respectively. For the rutile TiO₂ nanorods we studied here, \( \alpha_{375nm} = 10^6 \text{ cm}^{-1} \) and \( L_p = 20 - 30 \text{ nm} \), yielding \( aL_p = 0.03 \), which is less than 5% and therefore can be neglected in the \( 1 + aL_p \) term in eq 2. In other words, the contribution of interior photogenerated carriers that diffuse to \( W \) can be neglected (i.e., for \( x > W \) in Scheme 2). To estimate the magnitude of the potential dependent \( aW \) term, we evaluate \( W \) at \( E - E_{fb} = 0.9 \text{ V} \) (corresponding to an \( E - E_{fb} \) value that is larger than any applied potential used in single-molecule imaging experiments, e.g., +0.2 V versus \( E_{fb} = -0.63 \) \text{ V}; see section 4.2 for \( E_{fb} \) determination) using an estimated \( N_d = 10^{16} \text{ cm}^{-2} \), where \( W = 100 \text{ nm} \) and \( \alpha W = 0.1 \). Thus, this small magnitude of \( aW \) allows for expanding the exponential term in eq 2 into a Taylor series and neglecting the higher order terms to yield a simplified Gärtnert equation (eq 3), as done by Butler:

\[
\nu_h^i = \frac{d[(h^+_i)]}{dt} = I_0 aW = \frac{2e\epsilon_0(E - E_{fb})}{qN_d}
\]

(3)

Assuming all holes transported to the surface will undergo interfacial charge transfer reactions on the photoanode (e.g., with \( \text{H}_2\text{O} \) or \( \text{R} \)), the corresponding photocurrent is given by

\[
i = qI_0 aW = qI_0 aW = \frac{2e\epsilon_0(E - E_{fb})}{qN_d}
\]

(4)

Equation 4 predicts that \( i \) is proportional to \((E)^{1/2}\) and \( I_0 \). The photocurrent increases with increasingly anodic potential because it is proportional to the number of photons absorbed within \( W \), whose thickness scales with \((E)^{1/2}\). Equation 4 also predicts that the flat band potential \((E_{fb})\) is also the photocurrent onset potential (i.e., when \( E > E_{fb} \)).

To describe the major interfacial charge transfer reactions by the photogenerated holes, we considered a mechanistic model that includes a limited number of major irreversible reaction steps for water oxidation toward \( \text{O}_2 \) on a TiO₂ photoanode surface, following Salvador et al.\(^{10,36,37}\). The relevance of these reactions is supported by experimental data obtained with bulk rutile TiO₂ photoanodes.\(^{35-39}\) These major reactions are schematically shown in Scheme 2, and their rate equations are listed in Table 1. In this model, water oxidation by photogenerated holes is approximated to start with the oxidation of surface adsorbed \( \text{H}_2\text{O} \) or \( \text{OH}^- \) to generate surface adsorbed \( \text{OH}^+ \) (step ii in Scheme 2 and eq 5 in Table 1). We note a more recent work by Salvador and co-workers using isotopically labeled TiO₂ photoelectrodes in anhydrous acetonitrile showed that terminal oxygen atoms in TiO₂ can initially capture photogenerated holes.\(^{39}\) The relevance of the aforementioned model in the context of this work is discussed in Supporting Information section 1, and the implications of this mechanism will be discussed following the derivation of the model. As a further approximation, we do not differentiate the oxidation rates of surface adsorbed \( \text{H}_2\text{O} \) and \( \text{OH}^- \). The surface adsorbed \( \text{OH}^+ \) can subsequently combine to form \( \text{H}_2\text{O}_2 \) (step iii in Scheme 2 and eq 6 in Table 1), which can be further oxidized by holes to generate \( \text{O}_2 \) (step iv in Scheme 2 and eq 7 in Table 1). The oxidation of \( \text{H}_2\text{O}_2 \) to \( \text{O}_2 \) consumes two \((h^+_i)_o\) but we assume that they are sequential reactions, as three-body reactions are microscopically unlikely, and assume that the first hole oxidation step is rate limiting so that the formation rate of

Table 1. Major Kinetic Reactions Included in the Model for Water Oxidation on Photoexcited TiO₂ under Anodic Conditions\(^a\)

<table>
<thead>
<tr>
<th>step</th>
<th>reaction</th>
<th>rate law</th>
<th>equation no.</th>
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| ii   | \((\text{H}_2\text{O} \text{ or } \text{OH}^-)_o + (h^+_i)_o \xrightarrow{k_{\text{cat}}^\text{OH}^+} (\text{OH}^+)_o\) | \[
v_{\text{OH}^+} = \frac{d[(\text{OH}^+)_o]}{dt} = k_{\text{cat}}^\text{OH}^+[(h^+_i)_o][(\text{OH}^-)_o \text{ or (H}_2\text{O}_2)]
\] | 5  |
| iii  | \((\text{OH}^+)_o + (\text{OH}^-)_o \xrightarrow{k_{\text{cat}}^\text{H}_2\text{O}_2} (\text{H}_2\text{O}_2)_o\) | \[
v_{\text{H}_2\text{O}_2} = \frac{d[(\text{H}_2\text{O}_2)_o]}{dt} = k_{\text{cat}}^\text{H}_2\text{O}_2[(\text{OH}^+)_o]^2
\] | 6  |
| iv   | \((\text{H}_2\text{O}_2)_o + 2(h^+_i)_o \xrightarrow{k_{\text{cat}}^\text{O}_2} \text{O}_2 + 2\text{H}^+\) | \[
v_{\text{O}_2} = \frac{d[\text{O}_2]}{dt} = k_{\text{cat}}^\text{O}_2[(h^+_i)_o][(\text{H}_2\text{O}_2)_o]
\] | 7  |

\(^a\)These reactions are schematically shown in Scheme 2.
O₂ is first order with respect to holes. It is also assumed that the applied potential is sufficiently positive so that all recombination reactions between electrons and holes, as well as electrons and photogenerated intermediates (e.g., OH⁻ and H₂O₂), are sufficiently slow to be negligible kinetically, similar to what was assumed by Salvador et al. in their dependences on I₀ and E, whereas [H₂O₂] is potential and light independent, and its magnitude depends on the ratio of rate constants for OH⁻ formation and O₂ evolution.

Depending on the mechanism, R can react directly with (h⁺), (step v in Scheme 2) or with any of the intermediates of water oxidation reactions (e.g., OH⁻ and H₂O₂; steps vi and vii in Scheme 2). Superoxide, another potential oxidant that can form by reducing O₂ by the photogenerated electron and with which R can react, can be minimized by degassing the electrolyte and operating under dominantly anodic conditions. In either case, we further assume that the concentration of R is sufficiently small compared with that of H₂O or OH⁻ so that it contributes insignificantly to the overall kinetics and thus the photocurrent from all photoelectrocatalytic oxidation reactions on TiO₂ surfaces (we will justify this approximation in analyzing the experimental data in section 4.3). In other words, R can be treated as a probe molecule that samples the surface concentration of oxidizing species but does not significantly perturb the species' concentration.

Taking into account eq 2, as well as eqs 5–7 in Table 1, we can apply the steady-state approximation to the intermediates (h⁺), (OH⁺), and (H₂O₂):

\[
\frac{d[(h⁺)]}{dt} = v_h - v_{OH⁻} - 2v_O₂ = 0
\]

\[
\frac{d[(OH⁺)]}{dt} = v_{OH⁻} - 2v_{H₂O₂} = 0
\]

\[
\frac{d[(H₂O₂)]}{dt} = v_{H₂O₂} - v_O₂ = 0
\]

To help solve for [(h⁺)], [(OH⁺)], and [(H₂O₂)] from eqs 8–10, we approximate that the rate constant for hole capture by either surface adsorbed OH⁻ or H₂O is similar in magnitude to that by surface-adsorbed H₂O₂ (i.e., k₉H⁺ ≈ k₉O₂). In addition, due to the large excess of bulk OH⁻ or H₂O we approximate that the steady-state concentration of OH⁻ and H₂O₂ are both significantly less than surface adsorbed OH⁻ and H₂O₂. This approximation is supported by the maximum theoretical surface coverage of OH⁻ on bulk rutile TiO₂ of about (5 to 15) × 10¹⁴ cm⁻² whereas the calculated values of [(OH⁺)] and [(H₂O₂)] are 8 × 10¹² and 8 × 10¹³ cm⁻², respectively, from experimental photocurrent data. Using these approximations, we obtained

\[
[(h⁺)] = \frac{IDT}{k_{OH⁻}[(H₂O₂) or (OH⁻)]} \sqrt{\frac{2εE₀(E - E_b)}{qN_d}}
\]

\[
[(OH⁺)] = \frac{I DT}{2k_{H₂O₂}} \sqrt{\frac{2εE₀(E - E_b)}{qN_d}}
\]

\[
[(H₂O₂)] = \frac{k_{OH⁻}[(H₂O₂) or (OH⁻)]}{k_{O₂}}
\]

Equations 11, 12, and 13 indicate that [(h⁺)], [(OH⁺)], and [(H₂O₂)] differ in their dependences on I₀ and E, whereas [H₂O₂] is potential and light independent, and its magnitude depends on the ratio of rate constants for OH⁻ formation and O₂ evolution.

By use of eq 1, for R to react with (h⁺), (OH⁺), or (H₂O₂), the corresponding rate equations are

\[
v_R = k_R[(R)][(h⁺)] = \frac{k_R k_{H₂O₂}[(H₂O₂) or (OH⁻)]}{K_{OH⁻}[(H₂O₂) or (OH⁻)]} \sqrt{\frac{2εE₀(E - E_b)}{qN_d}}
\]

\[
v_R = k_R[(R)][(OH⁺)] = \frac{k_R}{2k_{H₂O₂}} \sqrt{\frac{2εE₀(E - E_b)}{qN_d}}
\]

\[
v_R = k_R[(R)][(H₂O₂)] = \frac{k_R k_{OH⁻}[(H₂O₂) or (OH⁻)]}{2k_{O₂}}
\]

The three equations predict distinct scaling relations for vᵣ versus the light intensity I₀ or the applied potential E. For the direct mechanism, vᵣ scales with I₀ and (E)¹/². For the indirect mechanism via (OH⁺), vᵣ scales with (I₀)¹/² and (E)¹/⁴. And for the indirect mechanism via (H₂O₂), vᵣ would be independent of I₀ and E.

We note here that the vᵣ dependencies on E and I₀ in eq 15 for the indirect mechanism are unaffected whether photogenerated holes are assumed to react with either adsorbed H₂O/OH⁻ to generate oxidizing OH⁺ species or terminal oxygen atoms in TiO₂ to generate oxidizing O²⁻ species (see Supporting Information section 1); additional characterization tools would be required to distinguish between the two oxidants. In section 4 below we will apply eqs 4 and 14–16 to analyze the results from the photoelectrooxidation of amplex red (AR), where we also validate many of the approximations in the above mechanistic model.

3. EXPERIMENTAL SECTION

3.1. Nanorod Synthesis. Rutile TiO₂ nanorods were synthesized via a molten-flux salt method, following Liu et al. The nanorods were synthesized by grinding a mixture of 250 mg of P25 TiO₂ nanoparticles (Aeros Organics), 1 g of NaCl (Aldrich), and 250 mg of Na₂HPO₄ (Aldrich) to a fine powder. The powder was transferred to a crucible and annealed in a box furnace (Thermo Scientific) at 850 °C for 12 h. After cooling to room temperature, the reaction product was washed 5 times with boiling 0.1 M hydrochloric acid to dissolve residual salts (presumably from NaCl and Na₂HPO₄ precursors). The nanorods were dispersed in a 4:1 (by volume) ethanol to H₂O (all water used was np-H₂O, unless specified otherwise).

3.2. Photoelectrochemical Measurements. The design of the photoelectrochemical flow cell reactor and experimental setup has been described in detail. The working electrode was a TiO₂ nanorod-coated ITO electrode prepared by spin-casting 300 μL of a ~1 mg mL⁻¹ nanorod solution onto an ITO substrate at 750 rpm. The nanorod–ITO electrode was rinsed with H₂O and annealed in air at 450 °C for 30 min prior to use. The cell was mounted on the stage of an Olympus IX71 microscope and connected to a CH Instruments 1200A potentiostat. The nanorod-coated ITO working electrode was excited with a continuous wave, circularly polarized 375 nm laser (32.9–151.3 W/cm², CrystaLaser DL-375-020) using prism-type total internal reflection (TIR) illumination with an illumination area of ~80 × 95 μm². We measured photo-
current—time \((i-t)\) data in the absence of amplex red with 20 ms time resolution under chopped illumination (10 mHz, 50% duty cycle) at a constant applied potential. The background (dark) current measured from the macroscopic ITO electrode was subtracted from the photocurrent signal using a two-point linear background subtraction method to an average of five consecutive data points before and after illumination.

### 3.3. Single-Molecule Reaction Imaging

A continuous white light (18 mW), circularly polarized 532 nm laser beam (CrystaLaser CL532-025-L) was focused onto a \(50 \times 20 \mu m^2\) area (630 W cm\(^{-2}\)) of the ITO electrode to induce the fluorescence of the reaction product resorufin via TIR geometry. The TiO\(_2\) nanorods were also excited via TIR illumination using a 375 nm laser (60 W cm\(^{-2}\)). The fluorescence was collected by a 60X NA1.2 water-immersion objective (Olympus UPLSAPO60XW), filtered by a \(580 \pm 30\) nm band-pass filter (HQ580m60, Chroma), and detected by a back-illuminated ANDOR iXon EMCCD camera (DU897D-CS0-#BV) operated at a 15 ms frame rate. A 1.6X magnification changer was used to magnify the image 96X overall. The final image pixel size was \(155.8 \times 174.9\) nm. The slight asymmetry of the pixel size was due to a Princeton spectrograph (Acton SpectraPro) in the detection path that was used to acquire fluorescence spectra from the sample.

In a typical experiment, the electrode potential, laser powers, and reactant solution flow rate were fixed (i.e., steady-state conditions). A series of fluorescence images (i.e., catalytic movie) were acquired after a steady-state photocurrent response was reached (approximately 1 min after 375 nm laser excitation). The potential was held for 7.5 min at each potential and stepped anodically by 0.1 V from \(-0.6\) V to \(+0.2\) V. Following imaging experiments, the flow cell was disassembled and scanning electron microscopy (SEM, LEO 1550) imaging of the sample was performed at the Cornell Center for Materials Research (CCMR).

### 3.4. Image Processing and Data Analysis

Image processing and data analysis methods were described in detail in our earlier study.\(^{21}\) Fluorescence images were background subtracted according to ref 41, and all fluorescence bursts whose pixel intensity values were greater than the image mean pixel intensity plus 6 standard deviations were considered as potential candidate molecules. Each candidate was fitted with a two-dimensional (2D) Gaussian function. The width and integrated intensity of each fit were analyzed and used to quantitatively count single molecules. We developed an algorithm\(^{21}\) that considers (1) “hot” pixels or those with fit widths too narrow to represent a single molecule, (2) single molecules that appear in consecutive frames (i.e., product adsorption), (3) multiple molecules that appear within a diffraction-limited volume, and (4) single product molecules that diffuse on the surface during a single frame. Only those candidates whose fit widths and intensities were consistent with those of single product molecules were included in constructing the super-resolution reaction images. The sample drift was monitored in a frame-by-frame fashion by fitting the emission from individual 100 nm Au nanoparticle fluorescent markers adsorbed on the ITO electrode; the positions of products were subsequently corrected for in a frame-by-frame fashion.

### 4. RESULTS AND DISCUSSION

#### 4.1. Observation of Photocurrent Transients from Chopped Illumination and a Qualitative Explanation of the Potential-Dependent Transient Behavior

This section describes the photoelectrochemical current response of these single-crystalline rutile TiO\(_2\) nanorods initiated by a square-wave 375 nm laser pulse. The nanorods have an average length and diameter of 263 ± 113 nm and 62 ± 16 nm, respectively.\(^{21}\) To our knowledge, the photoelectrochemical properties of these materials have not been reported in the literature. The majority of nanorods are well-isolated from each other on the ITO electrode surface (surface coverage of \(~2\%)\), lying down with their long axes parallel to the electrode (Supporting Information Figure S2); charge transfer between adjacent or stacked nanorods does not contribute significantly to the photocurrent response.

Representative current—time data under 10 mHz chopped 375 nm illumination at different potentials are shown in Figure 1A. The photocurrent data in Figure 1 was obtained in the absence of AR; adding AR to the electrolyte does not affect much the photocurrent response presumably because of its low concentration. The 375 nm laser beam excites \(~1000\) TiO\(_2\) particles, containing mostly nanorods and pseudospherical particles (see also Supporting Information Figure S2). The chopping frequency is slow here so that each illumination cycle lasts 50 s to mimic the steady-state 375 nm illumination condition used in single-molecule reaction imaging conditions. Three distinct features were observed in the current—time data (denoted on the −0.4 V data by the black trace in Figure 1A): (1) an initial photocurrent spike \((I_{\text{initial}})\) when the light is turned on, (2) a decay of the photocurrent on a
millisecond to second time scale while the light is on toward a steady-state current ($i_{ss}$), and (3) an initial negative, cathodic photocurrent spike ($i_{cath}$) when the illumination is turned off, which decays to the background current level also on a millisecond to second time scale. For $E = -0.8 \, \text{V} \, \text{vs} \, \text{Ag/AgCl}$, we never observed (1) initial cathodic current spikes upon light illumination, (2) steady-state cathodic currents under continuous illumination (anodic currents have positive sign in Figure 1), or (3) photocurrent under sub-bandgap 532 nm illumination only.

The photocurrent transient behavior in Figure 1A can be qualitatively interpreted based on previous photoelectrochemical studies. Prior to illumination of the TiO$_2$ nanorods, the system is in a dark equilibrium condition, and for an N$_2$-purged aqueous electrolyte, the dominant h$^+$-accepting species on the nanorod surface is H$_2$O or OH$^-$ (and AR in our experiments, whose low nanomolar concentration makes its contribution negligible). Electron–hole pairs are generated in the TiO$_2$ nanorods when the square-wave light pulse impinges on the electrode. An anodic photocurrent is measured when the photogenerated electron–hole pairs are initially separated; electrons are transported to and collected by the ITO electrode, and holes are transported to the semiconductor–electrolyte interface and oxidize H$_2$O or OH$^-$. Depending on the potential, the photocurrent decays with illumination time on a millisecond to second time scale until $i_{ss}$ is reached. The magnitude of $i_{ss}$ depends on the applied potential and reflects the net rate of charge transfer across the interface at the steady state, where the surface concentration of h$^+$ acceptors (e.g., H$_2$O or OH$^-$) is lower than the initial, dark equilibrium condition. Upon turning off the light illumination, photogenerated electrons can recombine with photogenerated surface intermediates, such as surface trapped holes or chemisorbed intermediates that can accept electrons (e.g., OH$^*$, H$_2$O$_2$), leading to a cathodic photocurrent spike that decays while the photogenerated electrons and/or the surface intermediates are depleted.

4.2. Establishing a Potential Regime Where the Gärtnert Model Adequately Describes the Photocurrent Dependences on Potential and Light Intensity. As described in section 2, the Gärnter model assumes that all recombination reactions between electrons and holes, as well as electrons and photogenerated intermediates, are sufficiently slow to be negligible kinetically. Here we establish a potential regime where this approximation is valid.

First, we establish a potential regime where the photocurrent–time behavior is adequately described by the Gärnter model. Following Salvador and Sagara, we constructed $i$–$E$ curves by plotting the components of the current–time response versus potential (Figure 1B). For $E \geq -0.3 \, \text{V} \, \text{vs} \, \text{Ag/AgCl}$, $i_{ss}$ is $>70\%$ of $i_{initial}$ and $i_{cath}$ is almost negligible. The observation that $>70\%$ of the initial photocurrent is retained implies that the photocurrent–time behavior of these TiO$_2$ nanorods is adequately described by the Gärnter model because this model does not consider photocurrent decay behavior. The cathodic current spike is negligible over the same potential range presumably because the larger bending within the depletion region (curved black lines within W in Scheme 2) effectively separates and transports photogenerated electrons to the ITO electrode, and therefore a negligible amount of photogenerated electrons are transported to the surface for recombination.

Second, we establish a potential regime where the photocurrent dependences on potential and light intensity qualitatively follow the Gärnter model. For $E \geq -0.3 \, \text{V} \, \text{vs} \, \text{Ag/AgCl}$ and $i_{ss}$ are proportional to $(E)^{1/2}$ (see linear $i$ vs $E$ plot in Figure 1C). At fixed $+0.2 \, \text{V}$, where $i_{cath}$ is $97\%$ of $i_{initial}$, $i$ scales linearly with $I_0$ (Figure 1D). We note here that for our illumination geometry $I_0 = 0.02I_{incident}$ where $I_{incident}$ is the total incident light power on the ITO electrode and the 0.02 factor accounts for the fraction of ITO surface area covered by the TiO$_2$ nanorods and pseudospherical particles. We then calculated the apparent surface recombination probability (SRP) using SRP (%) = $\left[ \left( i_{initial} - i_{cath} \right) / i_{initial} \right] \times 100$ (Supporting Information Figure S3), following Kong et al. For $E \geq -0.3 \, \text{V} \, \text{vs} \, \text{Ag/AgCl}$, SRP < 30%, and in the same potential regime $i \propto I_0$ and $i \propto \sqrt{E}$ (Figure 1C,D). Therefore, for the potential regime of $E \geq -0.3 \, \text{V}$, the photocurrent dependences on potential, and light intensity are adequately described by the simplified form of the Gärnter model (eq 4).

Equation 4 also predicts that a linear fit to $i^2$–$E$ data yields $E_{fb}$ at $i = 0 \, \text{A}$. Following refs 36 and 43, we extrapolated the linear fit of the $i_{initial}$–$i^2$–$E$ data in Figure 1C to $i = 0 \, \text{nA}$, yielding an ensemble-averaged $E_{fb} = -0.63 \pm 0.01 \, \text{V}$, which is in agreement with literature values for bulk rutile TiO$_2$ at pH 8.5. This linear $i^2$–$E$ extrapolation method was previously shown to yield an $E_{fb}$ value equivalent to that determined in the dark from Mott–Schottky analysis of capacitance–potential data. The steady-state photocurrent falls to zero at about $-0.6 \, \text{V}$ because the steady-state photocurrent is proportional to the depletion region thickness $W$, which is nearly 0 cm at $-0.6 \, \text{V}$. According to eq 4, $i = 0 \, \text{nA}$ when $E = E_{fb}$. Therefore, $-0.6 \, \text{V}$ is very close to the determined flat band potential ($E_{fb} = -0.63 \, \text{V}$), and according to eq 4, the flat band potential represents the photocurrent onset potential (i.e., for $E > -0.63 \, \text{V}$, $i > 0 \, \text{nA}$).

We note it is generally observed and specifically for bulk TiO$_2$ electrodes, that $i$–$E$ data qualitatively follows the Gärnter model for sufficiently positive potentials (e.g., $+0.5 \, \text{V}$ versus $E_{fb}$). For the dilute TiO$_2$ nanorod-coated ITO electrode studied herein, the Gärnter model satisfactorily describes the photocurrent dependences on $E$, as well as $I_0$ for $E > 0.25 \, \text{V}$ versus $E_{fb}$ (i.e., $\geq -0.3 \, \text{V}$ vs Ag/AgCl electrode here).

Furthermore, it is worth discussing the photocurrent dependences on potential and potential over the potential regime where the data are not adequately described by the Gärnter model. Beginning at $-0.4 \, \text{V}$ and toward more negative potentials, the three components, $i_{initial}$, $i_{o}$, and $i_{cath}$, show different potential dependences, and the scaling relation of $i = (E)^{1/2}$ no longer holds for $i_{initial}$ and $i_{cath}$. Specifically, $i_{o}$ decreases rapidly to zero at a potential slightly more negative than $-0.45 \, \text{V}$. $i_{initial}$ increases to a magnitude greater than the photocurrent observed at positive potentials, reaching a maximum at $-0.6 \, \text{V}$ before decreasing again with more negative potentials; and $i_{cath}$ increases rapidly to an absolute magnitude larger than that of the anodic photocurrent at positive potentials, reaching a maximum at $-0.6 \, \text{V}$ before decreasing at more negative potentials. Similar behaviors of $i_{o}$, $i_{initial}$, and $i_{cath}$ were also reported for bulk TiO$_2$ electrodes previously. $i_{o}$ closely follows $i_{initial}$ while $i_{cath}$ is negligible over a range of more positive potentials, and the three current components differ in their potential dependences at some critical potential when polarizing the electrode at negative potentials. It is worth noting that when $i_{initial}$ and $i_{cath}$ reach their maximal values at $-0.6 \, \text{V}$, $i_{o} = 0$, in agreement with previous studies that used chopped light to measure the photocurrent response. In some specific cases with TiO$_2$ thin films and TiO$_2$.
nanotube arrays, the peak feature was larger in magnitude than the photocurrent at positive potentials, as we observed here. In those studies, the peak in \(i_{\text{initial}}\) and \(i_{\text{cath}}\) has been attributed to photoexcited electrons that originally populate energy levels within the band gap (gap states). That both photocurrent components reach a maximum value at approximately \(-0.6\) V can be explained as follows. Upon illumination of TiO\(_2\), the electrons that occupy the gap states are excited, besides those from the valence band, leading to an additional instantaneous photocurrent at negative potentials. A peak is observed in the plot of \(i_{\text{initial}}\) versus \(E\) presumably for two reasons: (i) if the energy distribution of the gap state−approximately energy levels within the band gap (gap states). That both attributed to photoexcited electrons that originally populate excitation from the gap states will reach a maximum as the potential is made increasingly more negative. Correspondingly, when the light is interrupted at negative potentials, photogenerated electrons react to recombine with holes or intermediates that have accumulated at the surface during illumination, as well as repopulate the band gap states, accounting for the peak in \(i_{\text{cath}}\) at the same potentials as \(i_{\text{initial}}\).

### 4.3. Super-Resolution Imaging of AR Oxidation Reactions and Reaction Rate Dependences on Potential and Light Power.

Here we use quantitative single-molecule reaction imaging to establish AR oxidation rate scaling relations versus \(E\) and \(I_0\) at fixed 50 nM AR concentration.

Parts A, C, and E (left panels) of Figure 2 show positions of product molecules (each orange dot represents a molecule) generated from single catalytic turnovers (i.e., single-molecule reaction imaging) on the surfaces of individual nanorods over the potential range of −0.6 to +0.2 V. Product positions were mapped onto the nanorod structural contour determined via SEM imaging (parts B, D, and F of Figure 2; see ref 21 for details of this mapping). Quantitative super-resolution activity maps were then constructed by binning the product positions into \(40 \times 40\) nm\(^2\) pixels in a two-dimensional histogram (parts A, C, and E (right panels) of Figure 2).

These activity maps indicate significant nanorod-to-nanorod activity heterogeneity as well as significant intranannorod activity heterogeneity along the nanorod length. These data motivate single-particle-level studies because they reveal nanoscale activity heterogeneities that are averaged in ensemble-level experiments.

We then examined the magnitudes of the single-nanorod and nanorod-averaged AR oxidation rates. Figure 2G shows representative AR oxidation rate (\(v_{\text{AR}}\)) versus potential (\(v_{\text{AR}} = v_{\text{AR}}\)) data for 37 individual nanorods (red circles) and the nanorod-averaged rate from 37 nanorods (black squares). There is a large heterogeneity in the single nanorod rates. The onset of AR oxidation occurs at about −0.5 V and increases with positive \(E\), in qualitative agreement with \(i_{\text{ox}}\). The average product formation rate per nanorod at +0.2 V is about 32 molecules s\(^{-1}\) or 64 holes s\(^{-1}\) nanorod\(^{-1}\) (note AR oxidation is overall a two-electron oxidation reaction). Comparing the maximum single-nanorod hole-induced oxidation rate of AR (180 molecules s\(^{-1}\) nanorod\(^{-1}\) or 360 holes s\(^{-1}\) nanorod\(^{-1}\)) with \(i_{\text{ox}}\) at +0.2 V (30 nA/1000 nanorods or 10\(^4\) holes s\(^{-1}\) nanorod\(^{-1}\)) indicates that AR contributes insignificantly to \(i_{\text{ox}}\) and also validates our earlier assumption that AR oxidation reactions do not affect steady-state surface concentrations of the oxidizing species (i.e., ox) that react with AR.

We then examined the scaling relations of \(v_{\text{AR}}\) with \(E\) and \(I_0\). Figure 2H shows the nanorod-averaged \(v_{\text{AR}}^4\) data scales linearly with \(E\) (i.e., \(v_{\text{AR}}\) scales with \((E)^{1/4}\)) for \(E \geq -0.3\) V. These data also rule out the possibility that the observed photoelectrocatalytic product formation is due to impurity P in the electrolyte that diffuses to the surface of the TiO\(_2\) nanorod because the diffusion of P to the surface is not expected to exhibit \((E)^{1/4}\) dependence. At fixed positive potentials (e.g., +0.2 V), \(v_{\text{AR}}\) scales linearly with the square root of light power impinging on the nanorods \(I_0\) in the presence of 50 nM bulk AR and +0.2 V. These data were obtained from 23 nanorods different from those in (G). The red dots indicate data from individual nanorods, and the black squares represent nanorod-averaged data (error bars represent SD).

4.4. AR Is Oxidized via an Indirect Pathway (OH\(^*\)).

Equations 14−16 predict distinct scaling relations for \(v_{\text{AR}}\) vs. the light intensity \(I_0\) or the applied potential \(E\) for various reaction mechanisms. For the direct mechanism, \(v_{\text{AR}}\) should scale with \(I_0\) and \((E)^{1/2}\) (eq 14), which is inconsistent with the data in parts H and I of Figure 2. For the indirect mechanism via reaction with surface adsorbed H\(_2\)O\(_2\), \(v_{\text{AR}}\) should be independent of the applied potential and light power (eq 16), again inconsistent with the data. Furthermore, \(v_{\text{AR}}\) should increase with decreasing \((H_2O_2)\) however we did not observe an increase in \(v_{\text{AR}}\) in ensemble level photocatalysis measurements that contained 161 mM H\(_2\)O\(_2\) (Supporting Information Figure S2). On the other hand, for the indirect mechanism via surface adsorbed OH\(^*\), \(v_{\text{AR}}\) is predicted to scale with \((I_0)^{1/2}\) and \((E)^{1/4}\) (eq 15). We experimentally observed that \(v_{\text{AR}}\) scales with \((I_0)^{1/2}\) and \((E)^{1/4}\) at fixed 50 nM bulk AR (parts H and I of Figure 2). Therefore, quantitative single-molecule reaction imaging reveals that photoelectrocatalytic AR oxidation by these TiO\(_2\) nanorods...
occurs via an indirect pathway and specifically via reacting with surface adsorbed OH\(^{\cdot}\). We note here that our conclusion of the indirect pathway applies to the low concentration range of AR we studied; it remains to be determined if the indirect pathway also applies at very high AR concentrations.

5. CONCLUSION

We have used single-molecule fluorescence microscopy to image quantitatively organic substrate oxidation reactions on the surfaces of individual single-crystalline rutile TiO\(_2\) nanorod photoanodes under photoelectrocatalytic water oxidation conditions in operando. The single-molecule approach allowed for quantification of amplex red oxidation reaction kinetics that is otherwise difficult to achieve in a conventional ensemble-level experiment. The scaling relations for the substrate oxidation rate versus applied potential at a fixed light intensity and versus light intensity at a fixed applied potential, together with the photocurrent scaling relations, were consistent with a kinetic model whereby the substrate molecule was oxidized indirectly by photogenerated surface adsorbed hydroxyl radical species (or terminal O\(^{2\cdot}\) species on the surface) rather than directly by photogenerated holes.

■ ASSOCIATED CONTENT

Supporting Information
The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jpcc.6b01848.

Explanation of an alternative indirect oxidation mechanism, determination of surface coverage of TiO\(_2\) nanorods, surface recombination probability, and amplex red oxidation in the presence of H\(_2\)O\(_2\) (PDF)

■ AUTHOR INFORMATION

Corresponding Author
*Phone: (607) 254-8533. E-mail: pc252@cornell.edu.

Author Contributions
J.B.S. and P.C. designed the research. J.B.S. performed research. J.B.S. and P.C. analyzed the data and wrote the manuscript.

Notes
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■ ABBREVIATIONS

AR, amplex red; \(i\), photocurrent; \(E\), applied potential vs Ag/AgCl reference electrode

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