# CHEMICAL & BIOMEDICAL IMAGING

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Review

# Single-Molecule Fluorescence Imaging of Energy-Related Catalytic Reactions

Published as part of Chemical & Biomedical Imaging special issue "Imaging in Energy Science". Qingdian Yan,<sup>#</sup> Xianghong Li,<sup>#</sup> Jianbin Luo,<sup>#</sup> and Ming Zhao\*

Cite This: Chem. Biomed. Imaging 2025, 3, 280–300		🔇 Read	Online	
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ABSTRACT: The pressing challenges of the energy crisis and environmental problems necessitate the pursuit of efficient and sustainable energy conversion technologies, wherein catalytic processes play a vital role in addressing these issues. Single-molecule fluorescence microscopy (SMFM) offers a transformative approach to understanding various catalytic reactions by enabling real-time visualization of molecular adsorption, diffusion, and transformation on catalytic surfaces. The unprecedented insights into the spatial distribution of active sites, catalytic heterogeneity, and the dynamics of key intermediates result in single- or subparticle level structure–property relations, thereby offering insightful perspectives for catalyst design and mechanistic understanding of energy-related catalytic processes. In this review, we provide an overview of the recent progress in using SMFM for investigating energy-related catalytic reactions. The advancement in SMFM imaging techniques for investigating nonfluorescent chemical processes is also highlighted. Finally, we conclude the review by commenting on the current challenges and prospects in advancing SMFM in energy research.



We hope that the capable SMFM imaging techniques and insights will promote the development and realistic application of various energy-related catalytic reactions, together with inspiring researchers to explore the power of SMFM in other applications.

**KEYWORDS:** single-molecule imaging, single nanoparticle, nanobubble, catalysis, energy, reaction dynamics, operando imaging, surface restructuring, molecular adsorption

# 1. INTRODUCTION

The intensive consumption of fossil fuels has led to severe climate change, environmental crisis, and energy shortage issues.<sup>1,2</sup> There is a pressing need to switch to clean and renewable sources to address the above challenges.<sup>3</sup> To this end, many researchers have devoted themselves to exploring various clean-energy applications and resources, such as green hydrogen production via water splitting, carbon dioxide utilization and conversion, and fuel cells, among others.<sup>4-</sup> Although promising, the practical applications of these energyrelated techniques are limited by unsatisfactory performance and cost-effectiveness.<sup>13,14</sup> One major bottleneck lies in the catalysts involved in these reactions, whose activity, selectivity, and/or durability as well as cost-effectiveness need significant improvements.<sup>15</sup> Despite the different mechanisms underlying these energy conversion processes, they all share fundamental steps such as the adsorption of reactants, the conversion of reactants to products on the surface, and the desorption of products from the catalyst surface.<sup>16-19</sup> To this end, similar strategies have been proposed for catalyst design, including phase engineering, defects engineering, doping, and morphol-ogy regulation.<sup>20-29</sup> Significant achievements have been made in the development of high-performance catalysts by  $\frac{30-33}{30-33}$ researchers under the guidance of these principles.<sup>3</sup>

However, the limited understanding of the mechanisms remains a major obstacle to transitioning these catalysts into industrial applications. Addressing these challenges is crucial to bridging the gap between research innovations and practical industrial implementation. Thus, far, many advanced characterization tools, such as infrared spectroscopy, Raman spectroscopy, scanning electrochemical microscopy, have been developed and/or applied to investigate various energy-related catalytic reactions.<sup>34–37</sup> The scientific discoveries and insights have greatly promoted an in-depth understanding of reaction mechanisms and catalyst design principles. However, these techniques predominantly provide information about the bulk properties of catalysts, where the intrinsic catalytic heterogeneities of individual nanoparticles with diverse sizes, shapes, and structures are smeared.

Received:December 30, 2024Revised:February 24, 2025Accepted:February 26, 2025Published:March 7, 2025





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**Figure 1.** (a) Schematic illustrating the total internal reflection fluorescence (TIRF) microscopy setup for visualizing H<sub>2</sub> nanobubbles formed during water electrolysis. The nanobubbles are labeled with individual R6G molecules. (b) TIRF images on an ITO electrode. Area:  $22.8 \times 22.8 \mu m^2$ . The images were captured at various potentials during a potential sweep. The R6G concentration is 10 nM, and the exposure time is 50 ms. Scale bar: 5  $\mu$ m. (c) Comparison of the H<sub>2</sub> nanobubble detection rate (blue trace) and the corresponding current–voltage relation (black trace). The red dashed line marks the Faradaic current baseline. Inset: schematic of nanobubbles. (d) Fluorescence intensity versus time of single fluorophore-labeled H<sub>2</sub> nanobubbles (five randomly selected nanobubbles, upper trace) at -1 V. The concentrations of R6G and Na<sub>2</sub>SO<sub>4</sub> are 1 nM and 1 M, respectively. Fluorescence images and corresponding intensity traces. (lower panel) The concentrations of R6G and Na<sub>2</sub>SO<sub>4</sub> are 1 nM and 1  $\mu$ M, respectively. The pixel size is 177.8 × 177.8 nm<sup>2</sup>. The inset shows the definition of nanobubble size. (e) Scatter plot displaying the fluorescence intensities of single H<sub>2</sub> nanobubbles, detected within the potential range of -0.5 to -2.0 V from the same data set. Figures a–e are reprinted with permission from ref 70. Copyright 2018, National Academy of Sciences. (f) SEM images of a Au/Pd-coated ITO surface, with the right half overlaid by EDS mapping results. Red dots indicate Au, while blue dots indicate Pd. (g) O<sub>2</sub> nanobubble detection rate (Detections per Frame) at varying potentials in a 1 M NaOH solution containing 15 nM R6G. (h) Representative CV response of a Au/Pd-coated ITO electrode, scanned from -1 to +0.5 V. The electrolyte is 1 M NaOH. (i) H<sub>2</sub> nanobubble detection rate (Detections per Frame) at varying potentials in a 1 M NaOH. (ii) H<sub>2</sub> nanobubble detection rate (Detections per Frame) at varying potentials in a 1 M NaOH. (ii) H<sub>2</sub> nanobubble detection rate (Detections per Frame) at v

Recently, optical microscopy-based imaging methods offer an alternative solution to solve these problems, enabling noninvasive, in situ observation of structures and dynamics under operando reaction conditions.<sup>38–43</sup> Among diverse microscopy methods for energy-related catalysis, singlemolecule fluorescence microscopy (SMFM) has received enormous attention for catalytic studies due to its capability to detect the fluorescence signal of single molecules. As the intensity distribution of single-molecule fluorescence can be well depicted by the Gaussian distribution, the centroid of each molecule can be super-resolved at nanometer resolution, much better than the  $\sim$ 300 nm resolution in conventional microscopic techniques. Moreover, single-molecule detection also enables the study of catalysis at the single turnover level, offering unprecedented insights into the reaction dynamics. Currently, SMFM imaging has drawn enormous attention due to its high spatial and temporal resolution, high sensitivity to individual molecules or particles, wide field-of-view (e.g., total internal reflection fluorescence (TIRF) and epifluorescence imaging), and ability to operate noninvasively in environmental conditions.<sup>44–48</sup> One compelling example can be found in the TIRF configuration for catalysis study, which relies on the generated evanescent wave during total internal reflection for exciting the fluorescence of probes. Because of the short penetration depth, i.e., typically <200 nm, the evanescent wave selectively excites fluorescent molecules very close to the substrate surface, resulting in minimal background fluorescence and high-quality single-molecule fluorescence signals. Widefield epifluorescence microscopy illuminates the entire sample with a specific wavelength of light, causing the fluorescent molecules in the sample to emit longer wavelengths of light. The emitted fluorescence is collected through an objective lens and detected by a camera, resulting in a two-dimensional image of the sample. By leveraging reactions involving fluorescent species and imaging the fluorescence signals of individual molecules, one can visualize single-particle or even subparticle catalysis at the single-molecule level, providing necessary insights into catalytic reaction mechanisms and catalyst design.  $\frac{42,49-53}{2}$ 

In this review, we provide a systematic overview of recent advances in applying SMFM to energy-related catalytic reactions, including the hydrogen evolution reaction (HER), oxygen evolution reaction (OER), carbon monoxide (CO) oxidation, water treatment, hydrogen oxidation reaction (HOR), methanol oxidation reaction (MOR), and oxygen reduction reaction (ORR). The working principles of SMFM in these reactions are explicitly discussed, while new scientific insights are also highlighted. Moreover, the advancement in new imaging technique development is also discussed, which enables the study of nonfluorescent chemical reactions/species using fluorescence imaging. Finally, we highlight the challenges and prospects in implementing SMFM for a broad range of energy-related applications.

# 2. ENERGY-RELATED CATALYTIC APPLICATIONS

#### 2.1. Water Splitting

Hydrogen  $(H_2)$  serves as the most promising energy carrier due to its high mass density and ability to significantly reduce the carbon footprint of energy systems.<sup>54–36</sup> Currently, H<sub>2</sub> production in the industry is majorly based on steam methane reforming, which, however, is energy-consuming and involves heavy carbon emissions.<sup>57–59</sup> To this end, there is a pressing need to replace fossil fuels with renewable energy, such as solar, wind, hydro, geothermal, ocean thermal energy conversion, and biomass sources, for producing H<sub>2</sub> via water electrolysis, where the produced H<sub>2</sub> is known as green H<sub>2</sub>.<sup>60–64</sup> Among various approaches, electrocatalytic and photocatalytic water splitting are two representative strategies to produce green H<sub>2</sub> and have received considerable attention.<sup>65–68</sup>

**2.1.1. Electrocatalytic Water Splitting.**  $H_2$  production via water splitting has emerged as a promising technology owing to its ability to produce high-purity hydrogen without carbon emissions and its potential for sustainable energy generation. During water splitting,  $H_2$  molecules are generated on the cathode via HER and subsequently nucleate and grow into  $H_2$  nanobubbles on the electrode surface, whose formation and desorption behaviors strongly affect the catalytic performance.<sup>69</sup> However, the intricate dynamics of these processes remain unexplored in view of the limitations of existing techniques in achieving high spatial and temporal resolution in order to examine the interfacial structure of nanobubbles.

Hao et al. utilized TIRF imaging to visualize the dynamic nucleation and growth processes of  $H_2$  nanobubbles at the electrode–solution interface, which were generated during water electrolysis.<sup>70</sup> Nanobubble imaging was realized in a twoelectrode system via single-molecule labeling (Figure 1a) by fluorescent dye rhodamine 6G (R6G), whose concentration is at the nM level to suppress the background fluorescence from dyes. Specifically, when  $H_2$  molecules were generated, the fluorescent dye R6G tended to bind to the gas-solution interface of the nanobubbles, where its fluorescence signal

could be temporarily captured and recorded. This labeling process enables individual fluorophores to mark H<sub>2</sub> nanobubbles for imaging. It is worth pointing out that during the imaging experiments, it is necessary to use fresh solutions for each measurement so as to avoid the potential interference from accumulated fluorescent products. Figure 1b illustrates the changes in the number of R6G-labeled H<sub>2</sub> nanobubbles on Indium Tin Oxide (ITO) at potentials ranging from 0 to -2.0V versus a Pt quasi-reference electrode (vs Pt QRE). It can be found that the nanobubbles start to appear from -0.75 V and the number gradually increases with increasing voltage. However, as the potential became more negative, the number of H<sub>2</sub> nanobubbles showed a gradual decline. This trend can be attributed to a shift in dominance from H<sub>2</sub> nanobubble nucleation at a lower negative potential to growth and coalescence at a higher negative potential. Specifically, at high negative potentials, the density of nanobubbles on the ITO surface is adequately high, and the newly produced H<sub>2</sub> molecules could find nearby nanobubbles easily, leading to the growth of nanobubbles. As the potential became more negative, HER was further enhanced, and more H<sub>2</sub> molecules were produced, thus resulting in nanobubble aggregation and a reduced number. The number of generated H<sub>2</sub> nanobubbles versus the applied potential was also quantified (Figure 1c), showing an increase-followed-by-decay trend that is harmonized with the microscopic observations in Figure 1b.

Furthermore, by comparing the electrochemical current signals and the potential-dependent nanobubble detection frequency (blue traces), the authors also found that the  $H_2$ nanobubbles could be easily formed on the macroscopic ITO electrode surface even if the Faradaic current was almost undetectable. This phenomenon may be due to some active sites on the ITO electrode surface, which facilitates the nanobubble formation. Although the Faradaic current is barely detectable at the electrode, the local reduction rate of water at these catalytic sites may be quite high, thus, promoting  $H_2$ nanobubble nucleation. Subsequently, the authors labeled H<sub>2</sub> nanobubbles with R6G fluorophores and recorded intensitytime trajectories (Figure 1d upper). The majority of nanobubbles (~97%) were found to exhibit an abrupt increase in intensity, attributed to single fluorophore adsorption, followed by subseconds of near-constant intensity and abrupt decay to baseline. As the fluorescence intensity of detected fluorophores at varied time periods has a similar amplitude, the authors claimed that the nanobubbles should be labeled by individual R6G fluorophores. However, nanobubbles labeled by multiple fluorophores were occasionally observed from their multilayer plots (Figure 1d lower). The high single-molecule coverage suggests that intermolecular repulsion may prevent additional fluorophores from being adsorbed on the nanobubble surface. The change in fluorescence intensity of individual nanobubbles as a function of the applied potential shows that the average fluorescence intensity per nanobubble decreased from  $\sim 270$ counts at -1.1 V to  $\sim 70$  counts at -2.0 V (Figure 1e). In addition, the average bubble radius increased from 42 nm at -1.1 V to 90 nm at a potential of -2 0 V, indicating that the nanobubbles are larger at more negative potentials. The authors assumed that all generated bubbles are hemispherical and that the dye molecules are located at the bubble vertices. The calculated intensity of a single R6G molecule depends on its distance z from the ITO surface, i.e., the diameter r of the nanobubble, as shown in the inset of Figure 1c. Based on the intensity versus z distance, the size of the nanobubbles can be



**Figure 2.** (a) Experimental setup used for SMFM imaging of photoelectrocatalysis based on a two-laser TIRF configuration in a three-electrode cell. The subnanorod photocurrent was measured by focusing the 375 nm laser on a nanorod segment. (b) Super-resolution hole and electron surface reaction mapping Left panel: scatter plot. Right panel: 2D histogram (Pixels:  $40 \times 40 \text{ nm}^2$ ). Both illustrate the distribution of Rf product molecules (orange dots) formed during  $h^+$ -induced AR oxidation at potentials of  $E \ge -0.3 \text{ V}$  (i) and  $e^-$ -induced Rz reduction at  $E \le -0.4 \text{ V}$  (ii) on a single TiO<sub>2</sub> nanorod. Two localized hotspots are visible. The solid white line is the nanorod contour, and dashed white circles indicate the focused 375 nm laser spots for photocurrent measurements and the OEC deposition. Blue dots represent Rf molecules formed from  $e^-$ -induced Rz reduction. (iii) SEM image showing the nanorods after OEC deposition. Scale bars: 400 nm. (c) Correlation between  $k_h$ ,  $k_e$ , and  $\eta$  for 78 spots of TiO<sub>2</sub> nanorods (open symbols). Cross-correlation coefficients  $\rho$  ( $k_h$ ,  $\eta$ ) = 0.45  $\pm$  0.06 and  $\rho(k_h, k_e) = 0.75 \pm 0.03$ . Binned and averaged data (solid symbols) show general trends, and linear fits are applied to highlight relationships. (d) Relationship between  $E_{onr/GB}$  and  $\eta$  before and after the OEC deposition for 78 nanorod spots. The top and side panels are histograms of  $\eta$  and  $E_{onr/GB}$ . Counts represent the number of nanorod spots. (e) Relationship between the relative change in absorbed-photon-to-current efficiency ( $\Delta\eta/\eta$ ) and initial  $\eta$  of TiO<sub>2</sub> nanorods. Open circles represent the 78 nanorod spots. (f) Relationship between  $\Delta\eta/\eta$  and initial  $\eta$  of OEC-modified TiO<sub>2</sub> nanorods. Open circles represent the 78 nanorod spots. (g) Relationship between relative change in onset potential ( $-\Delta E_{on,GB}/E_{on,GB}$ ) and initial  $E_{on,GB}$  (open circles) of OEC-modified TiO<sub>2</sub> nanorods. Negative values represent negative shifts in  $E_{on,GB}$ . (h) Schematic of block–deposit

determined. The size increase was attributed to the exponential growth in the electroreduction rate at higher negative potentials, which causes  $H_2$  to be supplied to the existing nanobubbles at an accelerated rate. As a result, more nanobubbles were generated, leading to their aggregation.

However, the authors found that they were unable to reproduce the images of  $O_2$  nanobubbles on ITO electrodes, which they attributed to the inherently low OER activity of ITO.<sup>70</sup> To address this challenge, Hao et al. utilized a similar setup to electrolytically generate  $H_2$  and  $O_2$  nanobubbles on the transparent ITO electrodes modified by the Au/Pd alloy

(Figure 1f).<sup>71</sup> The relationship between the number of  $O_2$  nanobubble detections per frame and the applied potential is shown in Figure 1g. The number of  $O_2$  nanobubbles increased around -0.4 V, peaked at approximately 0 V, and then decreased as the potential became more positive. The initial increase from -0.4 to 0 V was attributed to enhanced OER kinetics at elevated potentials, while the subsequent decline from 0 to +0.5 V was ascribed to the size growth of the  $O_2$  nanobubbles, which "lifts" the fluorescence probe (R6G) so that they could not be efficiently excited by the evanescent wave. Cyclic voltammetry (CV) measurements on the Au/Pd-

modified ITO electrodes (Figure 1h) indicated that the OER onset potential was approximately +0.1 V. The earlier onset of  $O_2$  nanobubble detection via fluorescence microscopy suggested that bubble formation began at the initial stages of OER, aligning with similar results for  $H_2$  nanobubbles on the ITO electrodes.

A comparison of detections per frame versus potential on ITO with (Figure 1c, blue traces) and without Au/Pd alloymodification (Figure 1i) indicates that the generation of  $H_2$ nanobubbles distinctly occurred at a more negative potential, below -1.4 V. This observation aligned with previous studies that the onset of H<sub>2</sub> nanobubble formation on ITO surfaces under similar conditions.<sup>70</sup> However, the generation of H<sub>2</sub> nanobubbles was somewhat "delayed", for which the authors attributed it to the capability of Pd or Au/Pd alloys in absorbing/storing H<sub>2</sub>. Therefore, electrochemically generated H<sub>2</sub> atoms were likely to dissolve in the metal electrode first before forming  $H_2$  molecules and nucleating into  $H_2$ nanobubbles. In addition, the high H<sub>2</sub> oxidation efficiency of Au/Pd allowed H<sub>2</sub> bubbles generated at the electrode surface to be rapidly consumed when a sufficiently positive potential was applied. This study further demonstrated the successful use of SMFM imaging to observe the transient nucleation and growth of individual nanobubbles under operando water splitting conditions, together with a new approach to fabricating conductive and transparent platforms for singlemolecule spectroelectrochemical studies.

Compared with traditional bulk experiments, single-molecule fluorescence imaging precisely monitors the formation, distribution, and dynamic behavior of individual nanobubbles and reveals the heterogeneity of individual nanobubbles, which deepens the understanding of the nanobubble generation and growth mechanisms, providing microscopic insights for optimizing the design of water decomposition catalysts.

**2.1.2. Photoelectrocatalytic Water Splitting.** Photoelectrochemical water splitting for green H<sub>2</sub> production offers an alternative approach to converting solar energy into renewable fuels. To materialize the technique for practical applications, one key obstacle is to ensure the efficient utilization of photogenerated holes for water oxidation reaction, which typically requires modifying the photoanode with an oxygen-extracting catalyst (OEC) to enhance the photocurrent by promoting holes ( $h^+$ )-electrons ( $e^-$ ) separation and lower the start-up potential. However, the deposition of OEC materials needs to be finely controlled, as excessive deposition would reduce light absorption and diminish the performance of the photoanode.<sup>72</sup> To this end, it is necessary to reveal the proper semiconductor surface sites for OEC deposition.

To address this issue, Sambur et al. used SMFM imaging to directly map the active sites for  $e^-$  and  $h^+$ -driven photocatalytic reactions on individual rutile titanium oxide (TiO<sub>2</sub>) nanorods, with approximately 30 nm spatial resolution and 15 ms temporal resolution.<sup>73</sup> They built a three-electrode microfluidic photoelectrochemical cell with TiO<sub>2</sub> nanorods sparsely dispersed on ITO (Figure 2a). A 375 nm laser excited the TiO<sub>2</sub> nanorods, generating charge carriers and inducing a steady-state anodic photocurrent from water oxidation. Mapping of  $e^-$  and  $h^+$  were realized using fluorogenic reactions:  $e^-$ -catalyzed resazurin (Rz) reduction and  $h^+$ -catalyzed amplex red (AR) oxidation, both producing fluorescent Rf molecules. The fluorescence of Rf, excited by a 532 nm laser, allowed nanometer-resolution mapping of the  $e^-$  and  $h^+$  distributions on individual TiO<sub>2</sub> nanorods, revealing electron-hole recombination sites. Additionally, subparticle photocurrent mapping was achieved by illuminating specific segments of a single TiO<sub>2</sub> nanorod with a focused 375 nm laser.

By applying different potentials ( $E_{t}$  referenced to the Ag/ AgCl electrode) ranging from -0.6 to 0.2 V, the positions of the oxidizing ( $E \ge -0.3$  V,  $h^+$  involvement) and reducing area  $(E \leq -0.4 \text{ V}, e^{-} \text{ involvement})$  of the catalysts were imaged at the nanoscale (Figure 2bi, bii). It was found that hotspots with high oxidizing activity also exhibited high reducing activity with the distance between the two hot spots on the same nanorod being about 40 nm. This suggests that photogenerated  $e^-$  and  $h^+$  tend to react not only on the same facet within a photocatalyst but also at the same sites on the same facet.<sup>73,74</sup> Notably, during the experiment, the particles underwent position variation owing to the stage drift of the microscope. To correct the stage drift, the authors employed 100 nm Au nanoparticles as position markers, whose constant emission of fluorescence could be monitored on a frame-byframe basis for drift correction.

To better understand the results of the experiment, the authors used the Gärtner-Butler and Reichman models to correlate the absorbed-photon-to-current efficiency within the  $TiO_2$  nanorod depletion layer  $\eta$ , the photocurrent onset potential  $E_{on/GB}$ , and the effective rate constants for the participation of electrons  $(k_e)$  and holes  $(k_h)$  in the reaction (Figure 2c). The results showed that the points with large values of  $\eta$  also had large  $k_{\rm h}$  while  $k_{\rm h}$  and  $k_{\rm e}$  were correlated with each other, suggesting that the hotspots of the oxidation and reduction reactions, where the electron-hole complexation occurred, were also the most efficient. Based on the above findings, the authors focused a 375 nm laser onto the nanorod sites and selectively deposited the cocatalysts (cobaltborate, Co-Bi) for the OER on the hot spots and low-activity zones (Figure 2biii). They found that OEC deposition enhanced  $\eta$  across most nanorod regions by an average of 60% (Figure 2d, top), with an average shift in  $E_{on,GB}$  of -70mV (Figure 2d, right), aligning with the reduced overpotential seen in the presence of the OEC-modified photoanodes. Interestingly, 16% of the sites exhibited positive shifts ( $\Delta E_{on,GB}$ > 0; Figure 2e). However, for sites with negative shifts  $(\Delta E_{\text{on GB}} < 0)$ , smaller shifts correlated with larger  $\Delta \eta$ , suggesting that photocurrent improvements were not always directly linked to negative  $E_{on,GB}$  shifts, and the magnitudes of these changes showed an inverse relationship. A notable negative correlation emerged for the relative change in  $\eta$ : sites with initially lower  $\eta$ , characterized by reduced  $h^+$  and  $e^$ activity, experienced greater relative enhancements, exceeding 200% (Figure 2f). This trend likely arose from an increased interfacial charge transfer efficiency in low- $\eta$  regions, as OECs typically did not influence charge transport. In addition, a strong negative correlation was observed between  $\Delta E_{on,GB}$  $E_{\text{on,GB}}$  and  $E_{\text{on,GB}}$  (Figure 2g), where sites with a more positive initial  $E_{on,GB}$  exhibited larger relative negative shifts. This was likely due to the ability of OEC to alleviate excess positive surface charge, enabling greater negative shifts in  $E_{on,GB}$  at highcharge sites. These findings suggested that the optimal sites for OEC deposition to enhance photocurrent were those with the lowest initial  $h^+$  and  $e^-$  activities, while the sites most effective at reducing the onset potential had the highest initial  $E_{on,GB}$ values.



**Figure 3.** (a) Diagram depicting an anisotropic BiVO<sub>4</sub> particle with {011} and {110} facets.  $L_{\{010\}}$  and  $L_{\{110\}}$ : size of the {010} and {110} facets, respectively. (b) Overview of a multimodal functional imaging setup under three laser illumination modes: focused 405 nm laser for subfacet photocurrent mapping (left), wide-field epifluorescence imaging with 532 nm laser excitation of the reaction products from charge-carrier reactions (middle), and iris-confined illumination for single-particle photocurrent measurements (right). (c) SEM image of a typical anisotropic BiVO<sub>4</sub> particle. (d) Super-resolution images of  $h^+$ -induced reactions.  $n_p$ : number of detected product molecules. Bin size: 37.1 nm<sup>2</sup>. The white lines indicates the SEM structural outlines. (e) Super-resolution images displaying  $e^-$ -induced reactions using the same bin size and structural contours as in d. (f) Dependence of PEC current  $i_{ph}$  on subfacet positions at specific potentials. (g)  $v_h$  and  $v_e$  with subfacet positions at different potentials. (h) Schematic of interfacet junction effects on the particle's overall PEC activity, with orange and green bars representing areas/contributions from the {010} and {110} facets, respectively. (i) Dependence of particle-wide photoelectrochemical current density  $j_{ph}$  on  $L_{\{010\}}$  at  $L_{\{110\}}$  at 1.13 V. Each circle corresponds to data from an individual particle. The shaded surface represents predictive models, while orange and green lines show  $j_{ph}$  trends for fixed  $L_{\{010\}}$  and  $L_{\{110\}}$ , respectively. (j) Relationships between  $L_{\{010\}}$ ,  $L_{\{110\}}$ , ABPCE<sub>max</sub> (bottom) and  $E_{opt}$  (top). Reprinted with permission from ref 75. Copyright 2022, Springer Nature.

From these results, they innovatively proposed a three-step design strategy, i.e., block-deposit-remove strategy, for the photoanode (Figure 2h): (1) blocking the sites with more negative  $E_{on,GB}$  and higher  $\eta$  using protective group deposition at increasingly positive potentials; (2) depositing the catalysts at sites with lower  $E_{on,GB}$  or  $\eta$ ; and (3) removing the blocking groups to obtain photoanodes with optimally positioned catalysts. This strategy provides a new strategy to engineer the photocatalysts at the subparticle precision for efficient photocatalysis.

By developing subfacet-level multimodal functional imaging methods, Mao et al. utilized subparticle photocurrent measurements to reveal the interfacial junction effects and associated intrafacet photoelectrochemical property changes of BiVO<sub>4</sub> particles with anisotropic shapes at roughly 40 nm resolution.<sup>75</sup> They first synthesized anisotropic BiVO<sub>4</sub> particles encased by  $\{010\}$  and  $\{110\}$  facets, hypothesizing that the facets adjoining edges form interfacial junctions. These interfacial junctions would lead to spatial heterogeneity in electronic and photoelectrochemical properties, even within the region of the same facet near the edge (Figure 3a, c). As the band gap of BiVO<sub>4</sub> is 2.4 eV, the authors utilized a 405 nm laser to effectively excite the charge carriers in BiVO<sub>4</sub> particles. The authors conducted three types of function imaging: (1) probing the photoelectrochemical current across the BiVO<sub>4</sub> particle interfacet edge via subparticle illumination by a focused 405 nm laser (Figure 3b, left); (2) wide-field epifluorescence microscopy imaging using redox-selective



**Figure 4.** (a) SMFM imaging experimental setup. (b) Representative TEM image of Pd-100 nanoparticles. Inset: size distribution of Pd-100. (c) Fluorescence image in the TIRF mode. (d) Time-dependent catalytic fluorescence trajectory for a single Pd-100 particle recorded at a temporal resolution of 100 ms. (e) TOF plots of individual nanoparticles versus different CO concentrations in phosphate buffer solution (pH = 7.4, Rz = 1  $\mu$ M). Each data point is averaged over >50 nanoparticles. The error bar is the standard error of the mean (s.e.m.). (f) Dependence of the TOF on Rz concentration during the CO oxidation reaction, with CO maintained at saturation. Each data point is averaged over >50 nanoparticles. Error bar is the s.e.m. (g) Distribution of  $\gamma_{app}$  from individual Pd nanoparticles. [Rz] = 1  $\mu$ M and [CO] = 0.5 mM. The solid line represents the Gaussian fit. (h) Activity fluctuation rates of Pd-100 and Pd-111 versus TOF, with solid lines representing horizontal fitting trends. (i) Adsorption energies for the reactants CO, Rz, and product Rf on {111} and {100} facets. Reprinted with permission from ref 82. Copyright 2024, American Chemical Society.

fluorogenic probes to map  $h^+$  and  $e^-$  distributions, with an additional 532 nm laser for fluorescence excitation. (Figure 3b, middle); (3) measuring the whole-particle photoelectrochemical currents using laser illumination confined by an iris (Figure 3b, right). At anodic potential (>0.5 V),  $h^+$ -induced reactions occurred less frequently on the  $\{010\}$  facet of the BiVO<sub>4</sub> particle, which was richer in  $e^-$  donors, compared to the {110} facet, which was richer in  $e^-$  acceptors (Figure 3d).<sup>73,75</sup> Using SMFM imaging, they further revealed the spatial heterogeneity in surface  $h^+$  activity, even within the same facet. The specific  $h^+$ -induced reaction rate  $(v_h)$  exhibited a pronounced s-like trend along the x-axis (Figure 3g, red), which paralleled the local anodic  $i_{ph}$  distribution (Figure 3f). At lower anodic potentials (<0.6 V), even if the overall photoelectrochemical current was anodic, the e-induced surface reactions were also detectable (Figure 3g). The specific rate  $v_e$  showed a reverse slike trend along the x-axis compared to  $v_h$  (Figure 3g, blue), indicating intrafacet heterogeneity. By correlating  $i_{\rm ph}$  and  $v_{\rm h}$  (or  $v_{\rm e}$ ) on the BiVO<sub>4</sub> particles surface edges, they further

discovered that all quantities  $(i_{ph}, v_{h}, and v_e)$  showed s-type leaps on the (010)I(110) edge (Figure 3g, f).

Through the modified Reichman model, the authors derived the intrinsic parameters for the photoelectrochemical OER at the solid-electrolyte interface (SEI), including the electronhole separation efficiency  $(\eta_{sep})$  and flat band potential  $(V_{FB})$ in the SEI depletion region, and the effective rate constants ( $k_{\rm h}$ for  $h^+$ ,  $k_e$  for  $e^-$ ). The s-type spatial variations of  $V_{\rm FB}$  and  $\eta_{\rm sep}$ along the interfacial junctions on the particle surface suggested that the properties of BiVO<sub>4</sub> particles vary with the size of the  $\{110\}$  and  $\{010\}$  surfaces. When the  $\{110\}$  surface area is unchanged, the specific activity gradually decreases as the {010} surface area increases (Figure 3h middle). In contrast, when the {010} surface area remains constant, the specific activity gradually increases as the area of the {110} surface increases (Figure 3h bottom). Therefore, optimizing BiVO<sub>4</sub> particle performance required reducing the {010} facet size to minimize regions outside the transition zone while increasing the {110} facet size to utilize its full transition zone and surrounding regions.

The authors then calculated the photocurrent density  $(j_{\rm ph})$  of whole particles at any potential and facet sizes. They found that  $j_{\rm ph}$  decreased with increasing {010} facet size  $(L_{\{010\}})$ , while it initially increased with  $L_{\{110\}}$  and tended to decrease as  $L_{\{110\}}$  reached tens of micrometers. It was found that the calculations (Figure 3i, green lines) aligned closely with the experimental results (Figure 3i, circles), further proving the rationality of the model. Moreover, they determined the maximum achievable applied-bias photon-to-current efficiency (ABPCE<sub>max</sub>), and the corresponding optimal potential ( $E_{\rm opt}$ ) for particles with any face size (Figure 3j). These performance metrics provide quantitative guidance for optimizing the performance of shaped-particle photoelectrodes by adjusting the facet size of the interfacet junctions.

# 2.2. CO Oxidation

CO oxidation reaction holds significant environmental and industrial value due to its widespread application in air pollution control, energy conversion, and various industrial processes.<sup>76,77</sup> Over the past decades, considerable efforts have been devoted to developing noble-metal catalysts with desirable catalytic activity and durability, with a focus on palladium (Pd) and platinum (Pt) catalysts.<sup>78–81</sup> To improve the performance of catalysts, it is necessary to understand explicitly the structure–property relationship, which, however, remains a challenge for conventional techniques based on ensemble measurements.

Recently, Yan et al. utilized the SMFM imaging technique to investigate the facet-dependent catalytic properties of Pd nanoparticles at the single-nanoparticle level in response to CO fluorescent probes (Figure 4a).<sup>82</sup> The surfaces of Pd nanoparticles were covered by different proportions of {100} and {111} facets, respectively, with 45.2% {100} facets in Pd-100 (Figure 4b) and 68.8% {111} facets in Pd-111. Then, a reaction solution containing a certain amount of CO and Rz continuously flowed through the channel. A circularly polarized laser of 532 nm was employed to excite the fluorescence product molecules Rf generated by reducing Rz with CO on the surface of Pd nanoparticles (Figure 4c). Each fluorescence burst indicated the production of a reduced fluorescent molecule of resorcinol on the surface of Pd nanoparticles, accompanied by a complete CO oxidation process, known as catalytic turnover. The catalytic fluorescence time trajectory of a single Pd-100 nanoparticle revealed multiple bursts of fluorescence intensity, each corresponding to a complete catalytic turnover. The time between two consecutive fluorescence bursts was the time interval  $(\tau)$  for a single molecule generation. The short burst times of fluorescence on the trajectory reflect the rapid desorption of new product molecules after their generation. Thus, time interval  $\tau$  served as an approximate measure of the time required for one catalytic turnover.

To clarify how the turnover frequency (TOF) depended on reactant concentration, they constructed a TOF versus substrate concentration plot to further explore the kinetic mechanism of the CO oxidation reaction and quantify the TOF of individual Pd nanoparticles (Figure 4e). It was found that when the CO concentration was increased while keeping the Rz concentration constant, the TOF initially rose, reaching a peak, and subsequently declined at higher CO concentrations. A similar pattern occurred when the Rz concentration was increased with the constant CO concentration (Figure 4f), suggesting that the adsorption of Rz and CO follows a competitive model, with both adsorbing on the same surface site of the Pd nanoparticles. Equation 1 quantitatively describes this type of Langmuir–Hinshelwood kinetics.

$$\text{TOF} = \langle \tau \rangle^{-1} = \frac{1}{\int_0^\infty \tau f(\tau) d\tau} = \gamma_{\text{eff}} \frac{\alpha_{\text{A}}[\text{A}]\alpha_{\text{B}}[\text{B}]}{\left(1 + \alpha_{\text{A}}[\text{A}]\alpha_{\text{B}}[\text{B}]\right)^2}$$
(1)

Here,  $\gamma_{\rm eff}$  is the effective catalytic rate constant, signifying the reactivity of a single Pd nanoparticles.  $\tau$  is the interval time required to complete a reaction turnover. A and B represent CO and resazurin, respectively. [A] and [B] and  $\alpha_{\rm A}$  and  $\alpha_{\rm B}$ represent the concentrations and the adsorption equilibrium constants for these two reactants, respectively. Therefore, when one reactant was at a very high concentration and occupied a majority of surface sites, the TOF decayed because of the insufficient adsorption of the other reactant on the catalyst surface for reacting. A similar conclusion was confirmed when the CO concentration. Specifically, the TOFs of both nanoparticles tended to increase initially along with the increase in the CO concentration and then decreased inversely as the CO concentration reached a certain amount (0.3 mM for Pd-100 and 0.2 mM for Pd-111). Obviously, for the CO oxidation process, individual Pd-100 nanoparticles exhibited significantly higher catalytic reactivity compared to that of Pd-111, suggesting facet-dependent catalytic activity.

To understand the difference in catalytic activity among individual Pd nanoparticles, the authors introduced the factor  $\gamma_{app\prime}$  the apparent rate constant of Rf generated on a single nanoparticle surface,  $\gamma_{app} = \gamma_{eff} \frac{a_A[A]a_B[B]}{(1 + a_A[A]a_B[B])^2}$ , with the larger  $\gamma_{app}$  representing a faster rate of product generation and is essentially the same as TOF. Figure 4g shows the distribution of  $\gamma_{app}$  for multiple Pd nanoparticles, where the wide distribution indicated significant activity heterogeneity among individual Pd nanoparticles. Specifically, the  $\gamma_{app}$  of Pd-100 was  $0.041 \pm 0.004 \text{ s}^{-1}$ , larger than that of Pd-111 of  $0.035 \pm 0.002$  $s^{-1}$ , indicating that the {100} facets of Pd nanoparticles were more reactive than the {111} facets. In addition, the timedependent frequency of single catalytic conversion trajectories also reflected nanoparticle activity fluctuations caused by surface atom restructuring. Interestingly, the average fluctuation rate of Pd-100  $(0.015 \pm 0.003 \text{ s}^{-1})$  was found to be three times as high as that of Pd-111 (0.005  $\pm$  0.001 s<sup>-1</sup>), both of which were independent of the TOF (Figure 4h). The different surface restructuring rates indicated that Pd-111 is more stable than Pd-100, which was due to the lower surface energy of  $\{111\}$  relative to  $\{100\}$  facets. Further density functional theory (DFT) calculations showed that the  $E_{ad}$  value of CO was stronger than those of Rz and Rf on both the {100} and {111} facets (Figure 4i), consistent with the experimental results. Moreover, the adsorption energy of the product Rf on the Pd  $\{111\}$  facets were slightly larger than that on the  $\{100\}$ facets, suggesting that the product was more readily desorbed from the  $\{100\}$  relative to  $\{111\}$  facets. These findings offer critical insights into the structure-activity relations of Pd nanocatalysts, providing guidance for efficient catalyst design for CO oxidation reactions.

Compared with traditional bulk studies, single-molecule fluorescence imaging features nanometer resolution, allowing us to unveil the intrinsic catalytic heterogeneity and dynamics among individual nanoparticles. The as-obtained single-particle information is key to establishing a precise structure-property



**Figure 5.** (a) Schematic illustrating the single-molecule imaging of a photocatalyst particle. (b) Single-frame image of dye signals, marked by yellow circles. Dashed line: the contour of InSe flake. (c) Typical fluorescence intensity versus time trajectories of InSe flake by the green rectangle in (b). (d) Long-time photocatalytic activity image of an InSe flake. Inset: optical image of the InSe flake. (e) Distributions of turnover rates ( $v_T$ ) on different local characteristic regions. (f) Statistic plot of  $\langle v_T \rangle$  at different defects ( $v_{T-D}$ ) and basal plane ( $v_{T-BP}$ ). (g–i) Reaction rate constant  $k_{eff}$  (g), adsorption equilibrium constant  $K_{APF}$  (h), dissociation rate constant  $k_3$  (i) of various surface sites and as a function of the flake layer number. Reproduced from ref 84. Copyright 2021 American Association for the Advancement of Science.

relation for heterogeneous catalysis, offering unprecedented insights into the reaction mechanism and catalyst design principles.

#### 2.3. Water Treatment

Degrading emerging micropollutants in wastewater imposes a key challenge in advance of water security. Photocatalysis, a promising technique that can effectively degrade emerging micropollutants, such as plasticizers and pesticide, is limited by the low efficiency of solar to chemical energy conversion.<sup>70,87</sup> During photocatalysis, the adsorption behaviors of micropollutants on the catalyst surfaces significantly affect the electron transfer and structural transformation of the micropollutant molecules. However, little is known about the quantitative adsorption behaviors of micropollutant molecules, especially at the nanoscale. To address this challenge, single-molecule fluorescence imaging techniques have emerged as a powerful tool owing to its capability to quantify molecular adsorption and the high spatiotemporal resolution.

**2.3.1. Dye Micropollutant Degredation.** Photocatalysis can effectively degrade emerging pollutants and produce energy, wherein the low solar energy conversion efficiency remains a challenge. Understanding in situ the catalytic dynamics at isolated structural defects of two-dimensional (2D) layered materials is crucial for developing excellent catalysts via defect engineering.<sup>83</sup> To this end, Huang and co-workers employed single-molecule total internal reflection fluorescence (TIRF) imaging to resolve the photocatalytic

properties of 2D layered indium selenide (InSe) flake and quantitatively revealed heterogeneous dynamic features and surface diffusion behaviors of photocatalytic reaction.<sup>84</sup>

The imaging of InSe flakes was based on a fluorogenic oxidation reaction enabled by the generated holes in InSe under 365 nm light (Figure 5a), where the weakly fluorescent reactant molecule 3-aminophenyl fluorescein (APF) was oxidized by holes and formed a highly fluorescent product fluorescein (excitation wavelength = 490 nm and emission wavelength = 512 nm). A typical TIRF image of fluorescein molecules (excited by a 488 nm laser) produced on InSe is shown in Figure 5b, proving the feasibility to map InSe flakes using SMFM imaging. Figure 5c presents a segment of a typical fluorescence intensity trajectory on the basal plane (BP), demonstrating the stochastic on-off fluorescence signals indicative of single photocatalytic events. Through localization mapping of individual product molecules, the distribution of photocatalytic activities on an InSe flake was mapped (Figure 5d), showing heterogeneous catalytic activity on the same InSe flake. Specifically, higher photocatalytic activities at the edge, wrinkle, and vacancy were observed as compared to the BP. Based on the average specific catalytic turnover rates  $\langle v_{\rm T} \rangle$ , the activity of various structures was found to follow the order of BP  $(1.1 \pm 0.6 \text{ s}^{-1} \ \mu\text{m}^{-2}) < \text{edge} (2.6 \pm 0.6 \text{ s}^{-1} \ \mu\text{m}^{-2}) < \text{wrinkle} (3.6 \pm 0.3 \text{ s}^{-1} \ \mu\text{m}^{-2}) < \text{vacancy} (6.5 \pm 2 \text{ s}^{-1} \ \mu\text{m}^{-2}),$ with the vacancy showing the highest photocatalytic activity (Figure 5e). Pooling from 20 InSe flakes comparing  $\langle v_T \rangle$  at defects  $(v_{T-D})$  versus those on the BP  $(v_{T-BP})$  (Figure 5f), the



**Figure 6.** (a) Schematic COMPEITS setup. (b) SEM image of the BiVO<sub>4</sub> particle with {010} and {110} facets. (c) Definition of type-I and type-II edge regions in a BiVO<sub>4</sub> particle. (d–g) 2D histogram plot of  $n_p$  without HQ (d) and with 250  $\mu$ M HQ (e), and the images derived from  $\Delta n_p$  (f) and  $\Delta n_p^{-1}$  (g) between (d) and (e). (h)  $K_{\text{HQ}}$  values of {010} and {110} (top) and  $K_{\text{HQ}}^{\{010\}}/K_{\text{HQ}}^{\{110\}}$  (bottom) over the accessible ranges of L and  $\xi$ . The yellow dashed line is mean  $K_{\text{HQ}}^{\{010\}} = K_{\text{HQ}}^{\{110\}}$ . Reproduced from ref 86. Copyright 2019 Springer Nature.

authors confirmed that all defects, including edge, wrinkle, and vacancy, exhibited higher photocatalytic activity than the BP.

In addition, Huang et al. further explored the impact of layer thickness (~0.7 nm for a single-layered InSe nanoflake) on photocatalytic kinetics at various structural features in InSe. Regarding the reaction rate constant  $k_{\text{eff}}$  at the BP, edge, and vacancy, they found that  $k_{\rm eff}$  was increased initially and then became stable as the layer number was decreased (Figure 5g). For the adsorption equilibrium constant of APF ( $K_{APF}$ ), it was found that APF adsorption was strongest on the BP and weakest in the vacancy of the InSe flake. Moreover,  $K_{\rm APF}$ showed a clear decrease when the InSe flakes had 2-7 layers but remained constant at all structural features for InSe flakes >8 layers (Figure 5h). In contrast, the fluorescein dissociation rate  $k_3$  was found to be higher on the BP compared to defects for InSe flakes in all layers (Figure 5i). Overall,  $k_3$  did not show a clear InSe layer dependence, but suggested an opposite trend when compared with the layer-dependent  $K_{APF}$ . These results revealed that the thickness of 2D materials significantly impacts their catalytic properties, offering a new perspective on optimizing the performance of 2D material catalysts through defect engineering. In summary, this study not only clarifies the photocatalytic dynamics at defects in 2D materials but also provides a scientific basis for designing high-performance catalysts by rationally controlling defects and layer thickness.

**2.3.2. Hydroquinone Degradation.** SMFM imaging has revolutionized the understanding of heterogeneous catalysis by providing novel insights into the reaction dynamics, catalysis mechanisms, and catalyst restructuring at the single-molecule level and single-turnover resolution.<sup>85</sup> However, a majority of the studies are limited to fluorescent entities, excluding a broad range of chemical and biological processes that do not involve fluorescence. To address this challenge, Mao and co-workers developed a novel imaging technique, competition-enabled imaging with super-resolution (COMPEITS), which broke this limitation by allowing the imaging of nonfluorescent processes at the nanometer scale.<sup>86</sup> This technique was particularly significant as it provided more precise information on unlabeled, nonfluorescent substances under operando conditions, which was crucial for understanding and optimizing processes in heterogeneous catalysis and energy engineering.

Figure 6a shows the concept of COMPEITS with a surfacecatalyzed reaction, where a solid particle catalyzed an auxiliary fluorogenic reaction, with the product molecules imaged at nanometer resolution. The reaction of interest, which is nonfluorescent, competed for the same surface sites on the catalyst particle, resulting in the suppression of the fluorogenic reaction rate. This suppression can be imaged at nanometer resolution, providing super-resolution spatial information on the nonfluorescent reaction. As a proof of concept, the authors



**Figure 7.** (a) Schematic illustration of the concept of adCOMPEITS imaging. (b) SEM image of the Au/TiO<sub>2</sub> nanostructure. (c,d) 2D histogram images of Cy3.5 adsorption events on a single Au/TiO<sub>2</sub> without (c) and with 100  $\mu$ M PM (d). (e) adCOMPEITS image derived from  $\Delta n/n$  between (c) and (d). (f,g) 1D project of the  $\Delta n/n$  (f) and the fitted K of TiO<sub>2</sub> segments as dissected in (e). (h,j) Comparison of K<sup>d</sup> versus K<sup>p</sup> of Au (h) and TiO<sub>2</sub> interface segment (i). (j) SEM image of a Au/TiO<sub>2</sub> nanostructure. (k) Subparticle photoanodic current  $i_{photo}$  and subparticle V<sub>FB</sub> of the Au/TiO<sub>2</sub> nanostructure. Reproduced from ref 88. Copyright 2024 Springer Nature.

applied the COMPEITS imaging technique to study the photoelectrocatalytic oxidation of hydroquinone (HQ), a popular micropollutant in wastewater, on BiVO<sub>4</sub> truncated bipyramid morphology, wherein the fluorogenic AR oxidation to fluorescent Rf was employed as the auxiliary reaction. Specifically, the BiVO<sub>4</sub> nanoparticles were covered by well-defined {010} basal facets and {110} lateral facets (Figure 6b), providing a great platform for investigating the facet-dependent adsorption behavior of a nonfluorescent micropollutant. Moreover, there existed two types of edges on the shaped BiVO<sub>4</sub> nanoparticles, one between the basal and lateral facets, and the other between the lateral facets (Figure 6c).

The authors first conducted single-molecule imaging of the photocatalytic AR oxidation, where the 375 nm laser was introduced to generate charge carriers in BiVO<sub>4</sub> while the 532 nm laser was introduced to excite the fluorescence of the fluorescent product Rf. Owing to the ~40 nm resolution of SMFM imaging, the reaction rate of AR oxidation could be derived based on the following eq 2:

$$\nu_{\rm AR} = \frac{k_{\rm AR} K_{\rm AR} [\rm AR]}{1 + K_{\rm AR} [\rm AR] + K_{\rm HQ} [\rm HQ]} \tag{2}$$

where  $v_{AR}$  and  $k_{AR}$  represent the rate and rate constant of AR oxidation, respectively;  $K_{AR}$  and  $K_{HQ}$  stand for the adsorption equilibrium constants for AR and HQ, respectively. Figure 6d shows the 2D histogram of detected single-molecule events on a single nanoparticle relative to its SEM image, suggesting heterogeneous activity. It is important to note that HQ adsorption was less strong at the corners where two lateral facets met at an edge. As the HQ concentration gradually increased, the number of detected fluorescent events  $n_p$  decreased dramatically (Figure 6e), meaning that competitive

adsorption was indeed happening. Hence, Figure 6f shows the difference image  $(\Delta n_p)$  derived by calculating the difference of  $n_p$  with and without introducing 250  $\mu$ M HQ, clearly indicative of competitive adsorption.

Most excitingly, by calculating the difference in  $n_p^{-1}$  with and without competitive adsorption  $(\Delta n_p^{-1})$ , the authors found that  $\Delta n_p^{-1}$  was proportional to  $K_{\text{HQ}}$  (Figure 6g). Based on this relation, they derived the informative COMPEITS image that immediately showed the  $K_{\rm HO}$  distribution on single BiVO<sub>4</sub> nanoparticles (Figure 6h). Notably, in Figure 6h, it can be observed that the HQ adsorption was stronger on the  $\{010\}$ facets than on the {110} facets, while the type-I edge showed stronger HQ adsorption relative to the type-II edge. Furthermore, based on facet-dependent HQ adsorption behaviors, the authors also distilled the optimal size and shape for BiVO<sub>4</sub> nanoparticles under various catalytic conditions. Specifically, when the particle size was very small, a plate-like shape with more  $\{010\}$  facets would adsorb more HQ molecules and display outstanding performance. In contrast, when the particle was very large, a bipyramid-like shape with more {110} facets would benefit the HQ adsorption for enhanced catalytic performance. The COM-PEITS technique demonstrated in this study not only enables the super-resolution imaging of nonfluorescent processes but also provides valuable insights into the role of particle size and shape on adsorption properties.

**2.3.3.** Pesticide and Plasticizer Degradation. An essential aspect of this process is the efficient adsorption of micropollutants onto the surfaces of the photocatalysts. Quantifying this adsorption is challenging, particularly on heterogeneous photocatalysts under diverse conditions. To address this challenge, Zhao and co-workers developed a novel



**Figure 8.** (a) Principle of leveraging a fluorogenic reaction to image CO species. (b) SEM image of a Pt nanotube. Inset: the corresponding optical image. Scale bar in the inset is 2  $\mu$ m. (c,d) Scatter and 2D histogram plots of individual Rf molecules on a Pt nanotube. (e) Potential dependence of  $\nu_{Rz}$  at three segments on the nanotube in d. (f) Distributions of  $k_2/k_1$  and  $E_p$  for the center and end segments of different Pt nanotubes. (g) 2D histograms of Rf molecules detected on the Pt nanotube during 0–2 h (T1), 2–4 h (T2), and 4–6 h (T3). (h) Highest transition state free energy in the free energy profiles of CH<sub>3</sub>OH dehydrogenation (G<sub>1</sub>) and CO oxidation (G<sub>2</sub>). (i) Schematic illustrating the volcano plot of MOR performance vs defect density of Pt nanotubes. Reproduced from ref 90. Copyright 2024 National Academy of Sciences.

imaging technique by replacing the auxiliary reaction with a simple fluorescent reporter probe, namely, adsorption-based COMPEITS (adCOMPEITS), which great broadened the scope of nonfluorescent species/reactions and materials for study at nanometer resolution.<sup>88</sup>

Figure 7a illustrates the principle of adCOMPEITS, which was based on the competitive adsorption between fluorescent reporters and micropollutant molecules. Specifically, an adCOMPEITS imaging experiment started with the single-molecule imaging of the fluorescence reporter dye molecules, by which the adsorption strength of the dye could be quantified at nanometer resolution. Subsequently, the micro-pollutant at different concentrations was introduced into the same dye solution, where the competitive adsorption of micropollutant molecules on the same surface sites would suppress the adsorption of dye molecules. By quantifying the change in the dye adsorption, the adsorption strength of the micropollutant was quantified based on the following competitive Langmuir–Hinshelwood adsorption model:

$$\nu_{\mathrm{R}} = \frac{k_{\mathrm{R}}[\mathrm{M}]_{\mathrm{T}}[\mathrm{R}]}{1 + K_{\mathrm{L}}[\mathrm{L}] + K_{\mathrm{R}}[\mathrm{R}]} \xrightarrow{[\mathrm{R}] \to 0} \frac{k_{\mathrm{R}}[\mathrm{M}]_{\mathrm{T}}[\mathrm{R}]}{1 + K_{\mathrm{L}}[\mathrm{L}]}$$
(3)

where  $k_{\rm R}$  and  $[M]_{\rm T}$  represent the specific adsorption rate constant of the fluorophore reporter and the total surface concentration of adsorption sites, respectively;  $K_{\rm R}$  and  $K_{\rm L}$  are the adsorption equilibrium constants for the reporter fluorophore and the nonfluorescent molecule, respectively. Compared with COMPEITS, adCOMPEITS did not require the substrate materials to catalyze an auxiliary reaction, thus enabling the study of all materials in principle under any condition, such as nonreaction conditions or dark conditions in photocatalysis. Moreover, as shown in the reduced equation in eq 2, the imaging experiments could be simplified by conducting competitive adsorption experiments only at fixed dye concentration, by which  $K_{\rm L}$  could be effectively derived.

Employing sulfo-cyanine 3.5 alkyne (Cy3.5) as the fluorescence reporter for adCOMPEITS imaging, the authors measured the adsorption of pirimiphos methyl (PM, a pesticide) and diethyl phthalate (DP, a plasticizer), two representative micropollutants in wastewater, on individual heterostructured Au/TiO<sub>2</sub> photocatalysts under dark, light or photocatalytic, and photoelectrocatalytic conditions. To ensure the accuracy of the data, the microscope stage drift was monitored in a frame-by-frame fashion by calculating the point spread function-fitted centroid position of the stable intrinsic photoluminescence of Au nanoparticles. Clearly, singlemolecule imaging of Cy3.5 solutions with and without micropollutant molecules suggested that the introduction of PM led to a substantial decrease in the number of reporter fluorophores detected, indicating competition for adsorption sites (Figure 7c, d). By quantifying the adsorption difference and following eq 2, the authors derived the adCOMPEITS image directly showing the adsorption affinity pattern on a single Au/TiO<sub>2</sub> nanostructure (Figure 7e), from which one could immediately differentiate the stronger adsorption of

micropollutants on Au nanoparticle than on the  $TiO_2$  nanorod (Figure 7e-g).

Based on the adCOMPEITS image, the authors first investigated the micropollutant adsorption at the Au-TiO<sub>2</sub> interface by dissecting the interface TiO<sub>2</sub> segment and Au into proximal and distal halves. Interestingly, they observed a consistent short-range adsorption enhancement at the Au-TiO<sub>2</sub> interface for both PM and DP, with a stronger adsorption affinity on the Au or TiO<sub>2</sub> segments closer to the interface compared to those farther away (Figure 7h, i). The short-range adsorption enhancement was ascribed to the localized electric field at the Au-TiO<sub>2</sub> interface, which was induced by the plasmonic effects of Au under 561 nm illumination. More importantly, by dissecting the Au-attached TiO<sub>2</sub> nanorod into 300 nm segments, the authors discovered that the adsorption affinity of micropollutants showed a peak value and then exponentially decayed to the distal TiO<sub>2</sub> segments from the Au-TiO<sub>2</sub> interface, with the decay distance approaching one micrometer (Figure 7f, g). Given that the micrometer adsorption enhancement disappeared on TiO<sub>2</sub> without attached Au, the authors attributed the long-range adsorption enhancement to the energy band bending in TiO2 caused by Au. To experimentally prove the assumption, they measured the subparticle photoelectrochemical current on a single Au/  $TiO_2$  nanostructure (Figure 7j).

Consistently, the subparticle photocurrent also suggested a peak value at the Au-TiO2 interface and then decayed exponentially to the distal TiO<sub>2</sub> segments, with a similar decay constant of 1  $\mu$ m (Figure 7k). By measuring the subparticle photocurrents at varied potentials, the authors derived the flatband potential  $E_{\rm FR}$  that directly reflected the energy band bending (Figure 71). Significantly, the positiondependent  $E_{\rm FB}$  showed a similar trend and an identical distance constant of roughly 1  $\mu$ m, validating that the long-range adsorption enhancement was indeed caused by the band bending effect in metal-semiconductor heterojunctions. Building on this knowledge, the author further manipulated the band bending degree in the metal-semiconductor heterojunction by replacing Au with Pt which had a lower Fermi energy to deepen the band bending and doping TiO<sub>2</sub> with nitrogen to shallow the band bending. Excitingly, the imaging results indicated that the band bending degree was positively correlated with the long-range effect magnitude and the distance constant, further confirming the key role of band bending in inducing the long-range effect. The ability to manipulate this long-range effect through band-bending engineering offers new strategies for optimizing photocatalyst performance, particularly for water decontamination where regulating micropollutant adsorption can significantly tune the catalyst-adsorbate interaction and improve the conversion efficiency of solar energy to chemical activity.

#### 2.4. MOR

Direct methanol fuel cells (DMFCs) have received significant interest owing to their high volumetric energy density, fast start-up, and easy accessibility. However, the commercialization of DMFCs is still impeded by the unsatisfactory performance of the anodic MOR and the high cost of the state-of-the-art Pt catalysts.<sup>89</sup> To develop highly efficient catalysts for MOR, it is necessary to understand explicitly the reaction mechanisms on Pt nanocatalysts. Recently, Xiao and co-workers employed SMFM imaging to study MOR on sub-Pt nanotube catalysts and unveiled the structure–property

relationship at nanometer resolution.<sup>90</sup> Specifically, the subparticle imaging was accomplished by leveraging the reductive fluorogenic reduction of nonfluorescent Rz molecules with CO (Figure 8a), an intermediate species generated during Pt-catalyzed MOR. Consequently, the fluorescent product Rf molecules were produced and mapped at the single-molecule level.

Leveraging the 40 nm resolution of SMFM, the authors dissected the nanocubes into one center (S2) and two end segments (S1 and S3), which allowed the study of sitedependent reactivity within a Pt nanotube (Figure 8b-d). As the applied potential (saturated calomel electrode (SCE)) was increased, the three segments displayed different reaction rates but had the same trend, wherein the reaction rate  $v_{\rm Rz}$  exhibited a rise-followed-by-decay behavior (Figure 8e). This potentialdependent phenomenon was rationalized by the quantitative comparison of the formation and consumption rate constants  $(k_1 \text{ and } k_2, \text{ respectively})$  of adsorbed CO (CO<sub>ads</sub>) under different potentials (Figure 8f). At the low potential range, the rise of  $v_{Rz}$  was due to the faster formation of  $CO_{ads}$  than its consumption. As the potentials were increased, the consumption of CO<sub>ads</sub> was enhanced significantly and surpassed that of  $CO_{ads}$  formation, resulting in the decay of  $v_{Rz}$ . Despite the same potential-dependence, the end and center segments within the same Pt nanotube were found to show notable differences. Specifically, both CO<sub>ads</sub> formation and consumption on the two ends were faster than those of the center segment, which was attributed to the larger number of lowcoordinated atoms, including defects, corners, and edges. Interestingly, the authors also found that the catalytic activity and anti-CO poisoning ability of catalysts toward MOR were strongly dependent on the defect density and show a volcano trend, suggesting that there existed an appropriate defect density on Pt nanocubes for achieving an optimal MOR activity.

In addition, the authors discovered that Pt catalysts also showed distinctive catalytic dynamics within a single nanotube. As shown in Figure 8g, the upper end dominated the reactivity in the initial stage while the center segment and bottom end showed much lower activity. As the reaction proceeded, the activity of the upper end reached its peak and then started to decay, whereas the activity of the center segment and bottom end increased significantly. Further analyses revealed that the superior activity of Pt ends over the Pt center was due to the existence of a larger number of surface defects. However, those highly active sites on Pt ends also deactivated faster than those on the Pt center with lower activity, suggesting the inferior anti-CO poisoning ability of Pt ends. The defect-dependence behavior was further confirmed by DFT calculations (Figure 8h). Specifically, both the highest transition state free energies of CH<sub>3</sub>OH dehydrogenation and CO oxidation showed a volcano shape as the function of defect density (Figure 8i), consistent with the experiment results. The findings from this study enhanced the comprehension of defect-driven catalysis and facilitated the rational design of highly efficient defectbased catalysts.

#### 2.5. HOR

As a crucial reaction in utilizing hydrogen energy, HOR has attracted extensive attention.<sup>91</sup> This anodic reaction is widely considered as one of the major contributors of the catalyst deactivation in hydrogen-based fuel cells.<sup>92</sup> However, the quantitative understanding of individual nanoparticles' deacti-



**Figure 9.** (a) Process of tandem Pt-catalyzed R1 and R2 reactions for HOR imaging. (b) Schematic showing the dispersion of Pt/G on carbon fiber microelectrode. (c) Activity profiles of two individual particles: one with high initial activity and rapid decay, and the other with low initial activity and gradual decay. (d) Dependence of the remaining observable active particles ( $N_{tot}$ ) on R1 reaction time. (e) R1 reaction time dependence of  $P_{tot}$  and ECSA for all observable active particles, including the exponential fitting curve (black line). (f,g) TEM images of 1 wt % Pt/G before (f) and after (g) 12 h electrocatalytic HOR. (h, (i) XPS spectrum of Pt 4f before (h) and after (i) 12-h CV scan at 50 mV s<sup>-1</sup> (-0.6 V ~ 0.6 V). (j) H<sub>upd</sub> region from ensemble static linear sweep voltammetry after different reaction times. (k) Spontaneous regeneration ("•") and the incubation period (" $\blacksquare$ ") of a single Pt nanoparticle. Reprinted with permission from ref 94. Copyright 2016, WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim.

vation process remains ambiguous. Compared with conventional electrochemical characterization tools featuring bulk measurements, SMFM provides a novel method to investigate, at the single-particle level, the catalytic reactivity and dynamics, thus offering novel insights into the reaction mechanisms and catalyst design.<sup>93</sup>

In a representative study, Zhang et al. utilized SMFM to investigate the deactivation process of graphene-supported Pt (Pt/G) electrocatalysts during HOR at the single-particle level.<sup>94</sup> The imaging of Pt-catalyzed HOR was realized in an alternative two-step process (Figure 9a): (1) HOR catalysis on Pt nanoparticles, conducting CV scanning from -0.6 to 0.6 V vs SCE at a rate of 50 mV s<sup>-1</sup> for 4 h (R1); (2) fluorogenic reduction of Rz by H<sub>2</sub>, generating Rf on the same Pt nanoparticles for 30 min (R2), whose fluorescence was excited by a 532 nm laser. Notably, the Pt/G nanoparticles were sparsely dispersed to a microelectrode with flagella-like nanofibers, which were connected to the work electrode as well as dipped in phosphate buffer (Figure 9b). By calculating the average of TOF per particle (TOF<sub>Ave</sub>) based on the fluorescence bursts over time, the deactivation behavior of Pt nanoparticles was found to decay in an exponential way (Figure 9c). Further, by identifying the deactivation rates of individual Pt nanoparticles, the authors classified the Pt nanoparticles into two categories: fast- and slow-decaying particles. Specifically, the percentages of fast- and slow-deactivating particles were approximately 77% and 23%, respectively. In addition, the distinction in decay rates was attributed to the different rates of catalyst reconstruction, where faster reconstruction led to faster electro-etching on the



**Figure 10.** (a) Bifunctional catalysis of  $Fe_3O_4$  nanoparticles used to investigate the  $2e^-$  ORR process at the single-particle level, including its elementary reactions. (b, c) Plots showing size-dependent  $\langle \tau_{off} \rangle^{-1}$  (b) and  $\langle \tau_{on} \rangle^{-1}$  (c) for  $Fe_3O_4$  nanoparticles. (d) Potential-dependent product formation for individual  $Fe_3O_4$  nanoparticles (dotted lines) and the average across multiple nanoparticles (solid line). (e, f) Distributions of rate constant k (e) and electron transfer coefficient  $\beta$  (f) for individual nanoparticles during  $2e^-$  ORR process. (g) Constable plot showing the relationship between  $A_{off}$  and  $\beta$ , derived from  $Fe_3O_4$  nanoparticles. (h) Dependence of kinetic rate constants ( $\gamma_{app}$ ) on individual nanoparticles at two distinct potentials. (i) Isokinetic analysis of three groups of  $Fe_3O_4$  nanoparticles with varying  $\beta$ , showing the relationship between potential E and  $\ln(\langle \tau_{off} \rangle^{-1})$  with linear fittings. (j) Summary of single-particle and mean TOF for over 70 nanoparticles, observed at different reaction times during a single-molecule  $2e^-$  ORR experiment. (k) Reaction time dependence of mean TOFs for three nanoparticle groups, based on the data in (j). Reprinted with permission from ref 99. Copyright 2020, American Chemical Society.

surface of catalyst nanoparticles with higher initial performance and resulted in a faster decay rate, and vice versa.

By tracing the step during which the electro-etching occurred, the authors revealed that HOR (R1)-induced reaction acted as the major contributor of deactivation, while R2-induced reaction had a negligible impact. To quantify HOR (R1)-induced decay process of total performance ( $P_{tot}$ ) for R2, the decay of catalysts was described by the following equation:

$$P_{\text{tot}} = P_{\text{f}} \cdot \exp(-b \cdot t) \cdot \exp(-r_{\text{f}} \cdot t) + P_{\text{s}} \cdot \exp(-d \cdot t) \cdot \exp(-r_{\text{s}} \cdot t)$$
(4)

where  $P_{\rm f}$  and  $P_{\rm s}$  describe the performance contributions of fastand slowly decaying nanoparticles, respectively;  $r_{\rm s}$  and  $r_{\rm f}$ represent the rate constants of slowly- and fast-decay nanoparticles, respectively; *b* and *d* stand for the decay rate constants of the particle numbers, respectively. Based on eq 4 (Figure 9d, e),  $r_{\rm f}$  was calculated to be four times greater than  $r_{\rm sr}$  and  $P_{\rm f}$  was significantly higher than  $P_{\rm s}$ . This quantitatively indicated that the initial performance of the catalyst primarily arose from the fast-decaying particles (Figure 9c, e). Additionally, eq 4 highlighted another factor driving the fast-decay process: b was nearly ten times higher than d, consistent with the fact that 90% of Pt/G particles disappeared due to electroetching and dissolution behavior after the long-term HOR process (Figure 9f, g). This result illustrated that highly reactive Pt nanoparticles decayed at least 1 order of magnitude faster than those with lower reactivity. By comparing XPS spectra before and after CV scanning (Figure 9, h-j), it was further confirmed that the rapid decrease in active Pt nanoparticles  $(N_{tot})$  observed from R2 after HOR was primarily due to physical loss or electro-etching of Pt nanoparticles, rather than the chemical deactivation caused by strong chemical binding or poisoning of intermediates on the Pt nanoparticle surface. Interestingly, although accounting for only 3% of the total number of Pt nanoparticles, these fastdecaying particles exhibited an incubation period, which was attributed to the binding and unbinding of the byproducts from carbon oxidation (Figure 9k).95 These observations suggested that stable and slow-decaying nanoparticles with low initial performance serve longer durations, which provides new insights into designing rational, high-efficiency functional catalysts.

#### 2.6. ORR

ORR is widely considered as the rate-determining step in the redox reaction of hydrogen and oxygen.<sup>96,97</sup> Different from the four-electron ORR (4e<sup>-</sup> ORR) that generates  $H_2O$  as the product, the two-electron ORR (2e<sup>-</sup> ORR) is an efficient and energy-saving pathway to produce  $H_2O_2$ , which requires high selectivity on the cathode. However, in practice, it is challenging to distinguish 2e<sup>-</sup> and 4e<sup>-</sup> ORR independently through the ensemble electrochemical method.<sup>96,98</sup> Thanks to the different oxidizing activities of products from 2e<sup>-</sup> and 4e<sup>-</sup> ORR, SMFM emerges as a powerful tool for gaining novel insights into ORR by deploying fluorogenic oxidation reactions as a probe.

Combining SMFM with electrochemistry, Xiao et al. successfully investigated the electrocatalytic kinetics of Fe<sub>3</sub>O<sub>4</sub>-catalyzed 2e<sup>-</sup> ORR at the single-particle level.<sup>99</sup> Under negative potentials in a phosphate buffer, the 2e<sup>-</sup> ORR proceeded in the presence of  $O_2$  and  $Fe_3O_4$ , generating reactive oxygen species (ROSs), which were detected using AR as the probe, whose oxidation produced the fluorescent Rf as products (Figure 10a). Specifically, the rate of 2e<sup>-</sup> ORR was strongly associated with the formation rate of Rf ( $\langle \tau_{\text{off}} \rangle^{-1}$ ) and was increased with the particle sizes (3-8 nm) due to the more active sites of larger Fe<sub>3</sub>O<sub>4</sub> nanoparticles, while the dissociation rate of Rf  $(\langle \tau_{on} \rangle^{-1})$  was size-independent (Figure 10b, c). Furthermore, in the potential range of -0.2 V to -0.8V,  $\langle \tau_{\rm off} \rangle^{-1}$  exhibited linear positive potential dependence, which was attributed to the regeneration of Fe (II), the active valence for absorbing O<sub>2</sub>, from Fe(III) proceeded faster in the more negative potential (Figure 10a,d). To quantitatively describe the relation between potential and  $\langle \tau_{\rm off} \rangle^{-1}$ , the authors derived the following equation for 2e<sup>-</sup> ORR based on the classic Butler-Volmer model:

$$\langle \tau_{\rm off} \rangle^{-1} = A_{\rm off} \exp(-\beta fE)$$
 (5)

where  $A_{\text{off}}$  represents the frequency factor of the reduction process, *f* is a constant derived from temperature,  $\beta$  and *E* are

the electron-transfer coefficients and applied potential, respectively. By fitting eq 5, both k obtained from  $A_{\text{off}}$  and  $\beta$ of individual particles were found to basically follow standard distribution in the wide board, reflecting the large static heterogeneity of the electrocatalytic activity of these nanoparticles (Figure 10e, f). Interestingly, the author found that  $\beta$ and  $\ln A_{\rm off}$  showed a good linear relationship, exhibiting a compensation effect between the electron transferring process and the reduction process (Figure 10g).<sup>7</sup> Fitting results from eq 4 indicated that the isokinetic potential  $(E_{iso})$  for 2e<sup>-</sup> ORR was  $-0.6 \pm 0.03$  V, where all the elementary reactions involved in the entire  $2e^-$  ORR process proceeded at the same rate. Also, as shown in Figure 10h,  $\ln \gamma_{app,1}$  and  $\ln \gamma_{app,2}$  represented different apparent rates for generating a single H<sub>2</sub>O<sub>2</sub> molecule on the surface of a single Fe<sub>3</sub>O<sub>4</sub> nanoparticle under different arbitrary applied potentials  $E_1$  and  $E_2$  ( $E_1 < E_2$ ). The  $\ln \gamma_{app,1}$ and  $\ln \gamma_{app,2}$  exhibited a strong linear relationship, with a correlation coefficient of R = 0.92, and a positive slope at the two different applied potentials. Furthermore, based on the observation that the slope between  $ln\gamma_{app,1}$  and  $ln\gamma_{app,2}$  was less than 1, the authors found that the selectivity of 2e<sup>-</sup>ORR faded away with the increasing overpotential, especially when  $E < E_{iso}$ (-0.6 V).

In addition, the authors observed notable heterogeneity in the catalytic activity between individual Fe<sub>3</sub>O<sub>4</sub> nanoparticles. Based on the magnitude of  $\beta$ , they divided the Fe<sub>3</sub>O<sub>4</sub> nanoparticles into three groups:  $\beta \in [0, 0.04], [0.04, 0.055],$ and [0.055, 0.08] (Figure 10f). Based on eq 5 and Figure 10i, it can be concluded that nanoparticles with a higher  $\beta$  value exhibited a steeper slope in the linear relationship between  $\ln(\langle \tau_{off} \rangle^{-1})$  and E, indicating a stronger dependence of the reaction rate on the applied potential and suggesting that their kinetic response was more sensitive to changes in E. This conclusion revealed that for 2e<sup>-</sup> ORR, the activity of Fe<sub>3</sub>O<sub>4</sub> nanoparticles with a higher electron transfer coefficient was more strongly influenced by the changes in potential. Surprisingly, three independent fitting lines with different  $\beta$ values intersected in one point whose x-axis was located at -0.61 V, showing the consistency of the calculated isokinetic potential of -0.6 V. Moreover, the Fe<sub>3</sub>O<sub>4</sub> nanoparticles also showed heterogeneous decaying rates and could also be classified into three types: gradually activated (10%), constantly stable (29%), and slowly deactivated nanoparticles (61%) (Figure 10i,k). The authors claimed that the heterogeneity of individual particles was attributed to the different dominant processes during 2e<sup>-</sup> ORR, which led to different damaging and intermediate-blocking rates on active sites on Fe<sub>3</sub>O<sub>4</sub> nanoparticles. In summary, this work leveraged SMFM to investigate Fe<sub>3</sub>O<sub>4</sub>-catalyzed 2e<sup>-</sup> ORR at the singleparticle level and discovered the linear relationship between electron-transfer coefficients and the reaction rate constant, which provided necessary insights into the mechanistic understanding of 2e<sup>-</sup> ORR.

## 3. CONCLUSIONS AND PERSPECTIVE

In summary, this review systematically highlights recent progress in utilizing SMFM imaging for studying energyrelated processes at the single-molecule or single-particle level. SMFM enables noninvasive in situ monitoring of reaction kinetics, maps the active site distributions, and provides a powerful approach for analyzing the behavior of individual catalyst particle surfaces. These insights deepen our understanding of catalytic reaction mechanisms and offer valuable guidance for catalyst design and optimization. For instance, SMFM imaging enables the precise identification of active sites on catalyst surfaces with nanoscale spatial resolution. By providing real-time monitoring of the adsorption behaviors of individual molecules, it reveals the heterogeneity in reactivity among different sites, thereby offering critical fundamental insights into the design and optimization of catalysts. Furthermore, this technique dynamically captures real-time fluctuations in catalytic activity during reaction processes, providing novel research perspectives and methodological support for in-depth investigations into catalyst stability and deactivation mechanisms.

Despite notable achievements in the energy field, several major challenges remain in further extending this technique to a broad range of reactions. First, the utilization of SMFM for investigating energy-related catalytic applications needs further broadening. Specifically, a majority of the fluorescent probes require neutral buffer solutions (Table 1), whereas most energy-related catalytic processes, such as electrocatalysis, operate under acidic or basic conditions. Therefore, the availability of fluorescent probes suitable for energy-related processes is quite limited, and special probes are often required to couple with specific reactions or processes. In addition, current SMFM devices and systems are mainly focused on

Table 1. Excitation Wavelength and pH Values ofRepresentative Fluorophores

Molecule structure	Excitation wavelength	pH value	Refs
	(nm)		
.0, COO- COCO- COCO-	488	7.4	84
	561	5.6	88
	532	6.5, 7.3, 7.4, 8.3, 8.5	73, 86, 90, 94, 99
	420	1.8-11.9	101
XHOCK X	532	14	71
	550	7.0-8.0	102
quito	420	3.3-4.6	103
	400	5.0-7.3	104
$\mathcal{O}_{N+N}^{(P)} = \mathcal{O}_{N}^{(P)} \mathcal{O}_{N}^{(P)} \mathcal{O}_{N}^{(P)}$	470	2.0-7.0	105
N CL Colored Olicoha Olicoha	405	6.3-8.0	106

reactions under ambient conditions and at room temperature. However, many heterogeneous catalytic reactions involve elevated temperatures and high pressures, which required the development of new imaging devices capable of achieving these harsh reaction conditions for imaging.

Second, the signal-to-noise ratio of fluorescence imaging for chemical reactions needs further improvement, which is key to improving the spatial resolution of imaging and obtaining highquality data for the following analyses. Meanwhile, this limits the study of the precise distribution and dynamic changes of the active sites on the surfaces of certain catalysts. This issue is mainly due to the involvement of physical adsorption or weak chemical adsorption of fluorescent dye molecules on the catalyst surface as well as the short residence time. Moreover, the intrinsic photoluminescence of nanoparticles under light illumination, particularly the photocatalysts, also contributes severely to the background noise and notably deteriorates the signal-to-noise ratio for the following scientific discoveries. In addition, the temporal resolution should also be improved to capture the transient reaction intermediates in chemical reactions. Technically, shot noise and dark current noise can be addressed by the implementation of advanced cameras with higher spatial resolution and faster response times. Experimentally, the imaging quality could be improved by optimizing the flow cell design and/or selecting dyes with outstanding brightness and proper emission wavelength to minimize the photoluminescence from particles and reduce the number of floating fluorescent molecules. However, considering the limited number of fluorescent probes so far, more efforts are expected to be devoted to synthesizing fluorescent probes with various optimal properties. In addition, employing advanced data analysis methods can also effectively improve the data quality by minimizing contamination by various noises. For example, background deduction and image enhancement can be performed by mathematical correction algorithms, while single-molecule event picking can be precisely realized via machine learning techniques.

Third, frame recording and analysis are time-intensive, making analyzing the results a laborious process. To improve efficiency, leveraging advanced techniques such as machine learning and efficient computational models could be a promising strategy. Specifically, leveraging machine learning can not only reduce the time required for manual model construction and data analysis but also improve the quality of data analysis by avoiding artifacts, significantly boosting research efficiency, and enabling more comprehensive insights. For instance, White et al. developed a new top-down machine learning approach called divisive segmentation and clustering (DISC) which enhances the analysis of single-molecule trajectories.<sup>100</sup> It segments time-series data into distinct molecular states with minimal user input, achieving results significantly faster and more accurately than traditional methods, which greatly improves the speed of data analysis.

Lastly, most studies so far are centered on entities or processes that fluoresce, whereas most chemical or biological processes do not involve fluorescent species. Although new imaging techniques such as (ad)COMPEITS have been developed and demonstrated for studying nonfluorescent entities, imaging nonfluorescent chemical processes is still limited. In the future, more efforts should be devoted to exploring nonfluorescent reactions and developing new imaging techniques to explore processes beyond energy-related catalytic reactions.

## **Chemical & Biomedical Imaging**

In conclusion, although single-molecule fluorescence imaging technology faces challenges in probe selectivity, signal-tonoise ratio, and data processing, its unique advantages in revealing the microscopic mechanism of catalytic reactions are irreplaceable. In the future, through technological innovation and multidisciplinary crossover, this technology is expected to play a greater role in the field of catalyst design and provide more efficient and precise solutions for catalytic reactions related to the energy sector.

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#### **Author Contributions**

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The authors declare no competing financial interest.

#### ACKNOWLEDGMENTS

M.Z. acknowledges the financial support by the National University of Singapore start-up grant, Centre for Hydrogen Innovations (grant no. CHI-P2023-04), National Research Foundation of Singapore (grant No. U2411D4005), and Ministry of Education (grant no. 23-0646-A0001 and T2EP50124-0006). This research is also supported by the National Research Foundation (grant no. 24-1637-A0004), Prime Minister's Office, Singapore under its Campus for Research Excellence and Technological Enterprise (CREATE) programme (Development of advanced catalysts for electrochemical carbon abatement).

# VOCABULARY

Single-molecule fluorescence microscopy: technique with high temporal and spatial resolution that utilizes fluorescent probes to study reaction kinetics, conformational relationships, etc.

Fluorescence probe: molecule or material that can emit fluorescence. For example, rhodamine 6G, resorufin, amplex red.

Interfacial junction effects: physical and chemical phenomena that occur at the interface of two materials, such as a heterojunction or semiconductor interface. Fluctuation rate: change or variability of a value over a specific time period, and in the review, it refers to the dynamic change of palladium nanoparticle catalytic activity over time.

Two-dimensional layered materials: materials composed of single atomic layers with unique properties, used in electronics, photonics, and energy applications.

Competition-enabled imaging with super-resolution: nonfluorescent reaction competes for the same surface sites on the catalyst particle where the fluorogenic reaction occurs, which can be imaged at the same nanometre resolution as the fluorogenic reaction.

Adsorption-based competition-enabled imaging: technique using competitive adsorption to achieve super-resolution imaging of molecular interactions on surfaces.

Methanol oxidation reaction: chemical process converting methanol to formaldehyde or formic acid, using catalysts and oxidizing agents, important in industrial applications.

Cyclic voltammetry: widely used electrochemical characterization whose electrochemical active surface area describes the performance of catalyst.

Fluorogenic reaction: the redox reaction which turns the nonfluorescent material to the fluorescent material which generates fluorescent signals.

Elementary reactions: minimized steps during the reaction which involve several intermediates and simple processes, such as adsorption/desorption and electron transfer.

Microflow cell: device that serves as a chamber where the catalyst is loaded and the fluorescent reaction occurs and, in specific cases, as a work electrode on which a designed potential is applied.

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