Spatiotemporal catalytic dynamics within single nanocatalysts revealed by single-molecule microscopy

Peng Chen,* Xiaochun Zhou,† Nesha May Andoy,‡ Kyu-Sung Han,§ Eric Choudhary, Ningmu Zou, Guanqun Chen and Hao Shen

This review discusses the latest advances in using single-molecule microscopy of fluorogenic reactions to examine and understand the spatiotemporal catalytic behaviors of single metal nanoparticles of various shapes including pseudospheres, nanorods, and nanoplates. Real-time single-turnover kinetics reveal size-, catalysis-, and metal-dependent temporal activity fluctuations of single pseudospherical nanoparticles (<20 nm in diameter). These temporal catalytic dynamics can be related to nanoparticles’ dynamic surface restructuring whose timescales and energetics can be quantified. Single-molecule super-resolution catalysis imaging further enables the direct quantification of catalytic activities at different surface sites (i.e., ends vs. sides, or corner, edge vs. facet regions) on single pseudo 1-D and 2-D nanocrystals, and uncovers linear and radial activity gradients within the same surface facets. These spatial activity patterns within single nanocrystals can be attributed to the inhomogeneous distributions of low-coordination surface sites, including corner, edge, and defect sites, among which the distribution of defect sites is correlated with the nanocrystals’ morphology and growth mechanisms. A brief discussion is given on the extension of the single-molecule imaging approach to catalysis that does not involve fluorescent molecules.

1. Introduction

Nanoscale particles are among the most important catalysts.1–3 They can be of diverse material compositions, such as metals, oxides, and sulfides, and they catalyze a wide range of transformations including oxidation, reduction, (de)hydrogenation, carbon–carbon or carbon–heteroatom bond coupling and cleavage reactions.4–8 Their catalytic versatility makes them widely applicable in petroleum processing, fine chemical synthesis, energy conversion, and pollutant removal.

It is thus important to characterize the catalytic activity of nanoparticles for any reaction of interest, but it is challenging. The first contributor to this challenge is the ubiquitous heterogeneity among nanoparticles,9 where individual nanoparticles differ in size, shape, and thus the exact number and types of surface sites. Second, even on a single nanoparticle, different types of surface sites are present, such as corner, edge, and facet sites, and their structural features depend significantly on the nanoparticle’s morphology. Third, when the nanoparticles are sufficiently small, their structural dynamics can occur at a timescale comparable to that of the catalytic turnovers, including overall morphology changes and nanoscale surface restructuring.10–21 These structural dynamics would give rise to temporal evolutions of catalytic properties that also differ from one nanoparticle to another. In order to address these challenges, a direct approach is to study catalytic reactions of nanoparticles at the single-particle level, in real time, and in a spatially resolved manner.

Single-molecule fluorescence microscopy has recently proved to be effective for this type of studies. By using fluorogenic catalytic reactions and imaging the fluorescence signal of a product, one can follow the reactions in real time on a single nanoparticle at single-turnover resolution under steady-state reaction kinetics. This approach was initially developed to study catalysis by single enzyme molecules,22–27 and was later implemented by Hofkens to study heterogeneous catalysis on layered hydroxide microcrystals,28 by Majima to study metal oxide semiconductors29 and by us to study metal nanoparticles.30 Several recent reviews31–41 cover these early and later studies on a number of heterogeneous catalytic systems. New studies continue to emerge.12–43 Relatedly, single-molecule fluorescence imaging has been used to track molecular motion in porous materials and on surfaces, which is relevant to heterogeneous catalysis.39–52
Wide-field imaging of single-molecule fluorescence also enables nanometer-precision localization of the individual product molecules.\textsuperscript{53–55} This localization allows resolving reactions on a single catalyst beyond the diffraction-limited optical resolution,\textsuperscript{41,42,46,47,56–58} i.e., super-resolution, in a way directly analogous to (f)PALM/STORM and other related super-resolution techniques based on single-molecule detection.\textsuperscript{59–64}

This review discusses the latest advances in single-molecule imaging of nanocatalysis from our group. The discussion focuses on the spatiotemporal catalytic dynamics within single nanocatalysts, which were obtained by combining real-time single-turnover kinetics, super-resolution catalysis imaging, and correlation with electron microscopy of catalyst structures. The catalysts included pseudospherical nanoparticles and shaped nanocrystals made of Au or Pt. Interested readers are also referred to the review papers cited above for other related studies using single-molecule microscopy. A forthcoming review article\textsuperscript{65} from us will discuss other techniques, including electrochemistry,\textsuperscript{66–72} surface plasmon resonance spectroscopy,\textsuperscript{73–78} scanning probe microscopy,\textsuperscript{79–81} scanning transmission X-ray microscopy,\textsuperscript{82,83} and tip-enhanced Raman spectroscopy,\textsuperscript{84} that have been applied to study catalysis by single nanoparticles.

Peng Chen is the Peter J. W. Debye Professor of Chemistry at Cornell University. He received his BS from Nanjing University, China, in 1997 and obtained his PhD with Prof. Edward Solomon in bioinorganic/physical inorganic chemistry from Stanford University in 2004. He then did postdoctoral research in single-molecule biophysics with Prof. Sunney Xie at Harvard University, before starting at Cornell in 2005. His current research focuses on single-molecule imaging of nanocatalysis and bioinorganic chemistry. He has received a Dreyfus New Faculty award, a NSF Career award, a Sloan Fellowship, and a Paul Saltman Award.

Eric Choudhary received his BS in 2008 and MS in 2009 from Rensselaer Polytechnic Institute. He is currently a graduate student with Prof. Peng Chen working on single-molecule fluorescence imaging of nanoscale catalysis.

Ningmu Zou obtained his BS in Chemistry from Nanjing University, China, in 2011. He is currently a graduate student at Cornell University in the Department of Chemistry and Chemical Biology, working on single-nanoparticle catalysis in Prof. Peng Chen’s group.

Guanqun Chen received his BS in polymer materials and engineering from Zhejiang University, China, in 2011. He is currently a graduate student in Prof. Peng Chen’s group in the Department of Chemistry and Chemical Biology at Cornell University. His research is about single-molecule fluorescence imaging of metal nanoparticle catalysis.

Hao Shen obtained his BS in Chemistry from Nanjing University, China, in 2007. He is currently a graduate student in Prof. Peng Chen’s group in the Department of Chemistry and Chemical Biology at Cornell University. His research is on single-molecule study of electrocatalysis by carbon nanotubes and other carbon-based materials.

Xiaochun Zhou obtained his PhD in Physical Chemistry from the Chinese Academy of Sciences in 2007, and worked as a postdoctoral fellow in the Department of Chemistry and Chemical Biology at Cornell University on single-nanoparticle catalysis in Prof. Peng Chen’s lab from 2008 to 2013. He is currently a professor in the Suzhou Institute of Nano-Tech and Nano-Bionics, Chinese Academy of Sciences. His interests include catalysis from microns to the atomic scale, decomposition of formic acid to high quality hydrogen and optimization of thin layer electrodes.

Nesha May Andoy obtained her BS in Chemistry from the University of the Philippines in 2001 and her PhD in Chemistry from Cornell University in 2010 working on single molecule studies of metalloregulator–DNA interactions, bioinorganic enzymology, and nanoscale catalysis in Prof. Peng Chen’s group. Currently, she is a postdoctoral fellow at Harvard Medical School studying clathrin-coated pit assembly using single-molecule fluorescence techniques.
2. Real-time single-nanoparticle catalysis at single-turnover resolution

Single-nanoparticle catalysis at single turnover resolution

Using wide-field total-internal-reflection fluorescence microscopy and a microfluidic reactor (Fig. 1A and B), we have studied catalysis by metal nanoparticles in two fluorogenic reactions: a reductive N-deoxygenation reaction and an oxidative N-deacetylation reaction, both generating the fluorescent molecule resorufin (Fig. 1C). Fig. 2A is a wide-field fluorescence image of the catalytic products generated from single 6 nm pseudospherical Au nanoparticles in the N-deoxygenation reaction.\(^\text{30}\) Individual catalytic reactions on a single nanoparticle are reported by the probabilistic fluorescence bursts at a localized spot on the image, which is best presented in the corresponding fluorescence intensity vs. time trajectory (Fig. 2B). In this trajectory, each sudden intensity jump from the background marks a catalytic product formation event; each sudden intensity drop marks a product desorption event; and each off–on cycle represents a single catalytic turnover on a single nanoparticle. Owing to the microscopic nature of single-molecule processes, the reaction events are stochastic, i.e., the occurrence of each catalytic product formation and desorption event is probabilistic. As a result, \(t_{\text{off}}\) and \(t_{\text{on}}\), the two waiting times, are probabilistic, but their statistical properties, such as averages and distributions, are defined by the underlying reaction kinetics.

Temporal catalytic dynamics of small nanoparticles

The single-turnover catalysis trajectory immediately allowed the examination of temporal catalytic behaviors of a single nanoparticle under steady-state reaction conditions. Strikingly, the time-binned turnover rate of a single nanoparticle fluctuates significantly (e.g., Fig. 3A inset for a 6 nm Au nanoparticle\(^\text{30}\)), suggesting temporal fluctuations of single-nanoparticle catalytic activity, a phenomenon termed “dynamic disorder” in chemical kinetics. Although more intuitive, this time-binned turnover rate as in Fig. 3A inset is not a reliable representation of temporal activity fluctuations because of the probabilistic nature of single turnover events and the limited turnover events binned in each data point. A reliable and quantitative representation is the autocorrelation function of the waiting times\(^\text{85,86}\) (e.g., \(C_{\text{off}}(m)\)), the autocorrelation function of \(t_{\text{off}}\) where \(m\) is the event index in a single-turnover catalysis trajectory; (Fig. 3A).\(^\text{30}\) The exponential decay of this autocorrelation function unambiguously reflects the temporal fluctuations of the single-nanoparticle activity, and the exponential time constant gives the timescale of the activity fluctuations.

This temporal activity fluctuation of a single Au nanoparticle is attributable to the nanoparticle’s dynamic surface restructuring that is coupled to catalytic kinetics. The timescale of the activity fluctuation here reflects the timescale of the underlying surface restructuring dynamics. Fundamentally, the dynamic surface structure of a nanoparticle results from its nanometer dimension, which renders it higher surface energy and lower restructuring activation energy compared with its bulk counterpart, and it can be observed directly by electron microscopy and other techniques.\(^\text{19–21,87–89}\) During catalysis, the continually changing adsorbate–surface interactions can further induce surface reconstruction. Consistently, the activity fluctuation becomes faster when the catalytic turnover rate increases (e.g., through increasing reactant concentrations), and the fluctuation slows down when the size of the nanoparticle increases (Fig. 3B).\(^\text{90}\) By analyzing the size- and catalysis-dependent activity fluctuations using a thermodynamic model,\(^\text{90}\) we obtained the activation energy and the rate of spontaneous surface restructuring, both of which show a clear size dependence as expected (Fig. 3C). With increasing size to about 40 nm, the activation...
corresponding to a timescale of $10^{-2}$ to $10^{-3}$ s. \cite{90} Further theoretical analysis of this single-nanoparticle temporal catalytic dynamics suggests that the underlying fluctuations occur in a more concerted manner across all surface sites on a nanoparticle, rather than independently at localized individual sites. \cite{91}

Moreover, the timescale of this restructuring is expected to be dependent on the catalyst material. Compared with Au nanoparticles of the same size, single 4.6 nm Pt nanoparticles show significantly slower temporal fluctuations of their activity within the same range of catalytic turnover rates (Fig. 3D), \cite{45} consistent with Pt being a more thermodynamically stable metal than Au. \cite{92} Regardless of the catalytic reaction being the N-deoxygenation or the N-deacetylation reaction (Fig. 3D), the activity fluctuation rate of single Pt nanoparticles is the same, which is consistent with the observation that the underlying dynamic surface restructuring is inherent to the nanoparticle, rather than determined by the catalytic reaction. The activity fluctuation rate of Pt nanoparticles is also independent of the catalytic turnover rate (Fig. 3D), indicating that the catalysis-induced contribution to their dynamic surface restructuring is insignificant.

3. Spatially resolved activity patterns within single shaped nanocrystals

Super-resolution imaging of single-nanoparticle catalysis

Wide-field imaging of single-molecule fluorescence enables the localization of a molecule’s position down to nanometer accuracy. \cite{53, 54} This is typically done by fitting the fluorescence point spread function with a 2D Gaussian function (Fig. 2C). By applying this localization analysis, one can immediately map the locations of individual fluorescent catalytic products on a single nanoparticle at super-optical resolution. For a 6 nm pseudospherical Au nanoparticle, its apparent size from the locations of catalytic product molecules cannot resolve its physical dimension because of the limited spatial resolution (Fig. 4A). However, if the particle is large enough, for example a ~200 nm pseudospherical Au@mSiO$_2$ particle, the apparent size from the product locations matches closely the particle’s physical dimension from SEM (Fig. 4B). Using this super-resolution catalysis imaging approach, we extracted the apparent sizes for a series of pseudospherical Au and Au@mSiO$_2$ particles (Fig. 4C). For larger particles, their apparent sizes are essentially the same as their true physical sizes from TEM. With decreasing particle size to <40 nm, the apparent sizes from super-resolution catalysis imaging overestimate the true sizes and reach a limit value of about 15–40 nm, which reflects the resolution limit of this approach.

Site-specific catalytic activity on single shaped nanocrystals

We further applied the nanometer localization method to map catalytic reactions on two types of shaped nanocrystals: Au nanorods...
and nanoplates, both encapsulated in mesoporous silica of tens of nanometer thickness (i.e., Au@mSiO₂ nanorods and nanoplates, Figs. 5A and B). The mSiO₂ coating was necessary to stabilize the nanocrystals' morphology and prevent their aggregation after removing their capping ligands for catalysis; these capping ligands were essential for achieving shape control in synthesizing these nanocrystals,93–96 but were detrimental for catalysis. The mesopores were large enough to allow reactants to access the Au surface readily so that mass transport did not limit the catalytic kinetics. 46,47

Fig. 5C and D show the maps of reaction products on a single Au@mSiO₂ nanorod and a single Au@mSiO₂ nanoplate in catalyzing the N-deacetylation and N-deoxygenation reaction, respectively, overlaid on their respective SEM structural contour or SEM image. The apparent sizes were extracted from the super-resolution catalysis imaging across a series of pseudospherical Au and Au@mSiO₂ particles. These site-specific activity patterns can be understood by assuming that the catalytic active sites are low-coordination surface sites, which include corner and edge atoms and defects sites, and which are often more active due to their coordination unsaturation. 1,11 For nanorods, their ends have in general higher percentages of low-coordination sites than their side facets. For nanoplates, the percentages of low-coordination sites are the highest in the corner regions, lower in edge regions, and the lowest on the top flat facets. A recent study by Katz et al.97 further supports that for the N-deoxygenation reaction, the low-coordination sites on Au nanoparticles are the catalytic active sites.

Catalytic activity gradient within the same facets on a single nanocrystal

Spatially resolved catalysis imaging at the nanometer scale also enabled the examination of catalytic activity at different locations within the same surface facets on a single nanocatalyst. Surprisingly, we observed that the specific catalytic activity is...
not only non-constant but also shows consistent gradients within the same surface facets on a single nanocatalyst. For a Au@mSiO$_2$ nanorod, a pseudo-1-D nanocatalyst, the specific activity along the length of its side facets shows a linear gradient in catalyzing the N-deacetylation reaction: highest in the center and decaying gradually toward its two ends (Fig. 6A and B; and Fig. 5E as well). For a Au@mSiO$_2$ nanoplate, a pseudo-2-D nanocatalyst, the specific activity within its flat facets shows a radial gradient in catalyzing the N-deoxygenation reaction: highest in the center and decaying gradually toward its periphery (Fig. 6D and E). For individual Au@mSiO$_2$ nanorods, the specific activity at the center of the nanorod can be a factor of ten larger than the extrapolated specific activity for perfect side facets.

These catalytic activity gradients within the same surface facets of a single nanocatalyst are attributable to the underlying density gradients of surface defect sites, whose low-coordination renders them the catalytic active sites as described earlier. These density gradients of surface defects likely arise from the decaying growth rates during the solution-phase syntheses of the Au nanorods and nanoplates via seeded growth. The Au nanorods grow linearly from a seed$^{93–95,98}$ and the Au nanoplates are believed to grow radially$^{96,99–102}$ to form 1-D and 2-D nanostructures, respectively. However, their growth rate is not constant with their increasing length or size, and instead, the rate decays gradually. This decaying growth rate has been determined experimentally for Au nanorods by Hafner et al.$^{103}$ —with increasing length, the growth rate of a nanorod decays linearly until the rate becomes zero when the growth stops. Therefore, there is a gradient of growth rate from the middle toward the two ends for a nanorod, and a similar one is expected from the center toward the periphery for a nanoplate. These growth rate gradients would lead to surface defect density gradients, as faster crystal growth rates tend to result in more crystal defects$^{104}$ giving rise to the specific activity gradients we observed experimentally.

Interestingly, the magnitudes of the specific activity gradients show size dependence. The longer or larger the nanorod/ nanoplate is, the smaller the specific activity gradient it exhibits (Fig. 6C and F). This trend indicates that for longer or larger nanorods/ nanoplates, their surface defect densities have shallower gradients from the center toward their ends/ periphery. These shallower gradients further suggest slower decays of their seeded growth rates during the syntheses of the longer or larger nanorods/ nanoplates, which contribute to their eventual sizes.

The discovery of specific catalytic activity gradients within the same crystal facets on pseudo-1-D and pseudo-2-D nanocrystals has broad implications for studying and understanding the catalytic activity of nanocrystal catalysts. This discovery reinforces the importance of surface defects in determining the catalytic properties of metal surfaces, as well known in the surface science of heterogeneous catalysts.$^{1,105,106}$ For shape-controlled colloidal nanocrystals, for which facet information is often used to explain activity, it is challenging to determine their surface defects, and the spatial distribution of defects is strongly affected by the nanocatalysts' growth pattern and synthesis procedure. But it is imperative to consider them, so one can better use the knowledge from surface science to understand their activities.

### 4. Scalable, parallel screening of nanoparticle catalytic activity

Owing to the wide-field imaging format, the super-resolution imaging approach to nanoparticle catalysis can be scaled up readily to screen the activity of a large number of catalyst particles in parallel. We recently demonstrated this scalability, where quantitative activity can be obtained at the single-particle level that enables identification of high activity particles.$^{46}$ Fig. 7A shows the super-resolution catalysis image of ~1000 particles from a mixture of pseudospherical 21@42 and 102@32 nm Au@mSiO$_2$
particles in catalyzing the reductive N-deoxygenation reaction, in direct correlation with their SEM image (Fig. 7B). Individual particles are clearly resolved, even within aggregates, and with quantitative activity information (i.e., their rates of turnovers). For example, particle 1 is clearly more active than particle 2 (Fig. 7A inset), even though they are similar in size (Fig. 7B inset). Statistical distribution can be readily obtained among the large number of particles, for example for particle activity and size (Fig. 7C), where the two subpopulations are clearly resolvable in the corresponding 2-D histogram (Fig. 7D). This statistical analysis not only immediately shows the general trend (i.e., large particles are more active on a per-particle basis), but also identifies outliers: some smaller particles show significantly higher activities (e.g., particle 3, Fig. 7C), whereas some larger ones show lower activities (e.g., particle 7, Fig. 7C). This direct identification of catalyst activity at the single-particle level is exciting, because one can now pinpoint the particle of desired activity for subsequent structural characterizations, although our structural characterization is currently just at the SEM level, which is insufficient to identify the structural basis of activity differences.

We have also demonstrated this parallel screening of a large number of pseudospherical particles in catalyzing the oxidative N-deacetylation reactions, as well as of particles that have a mixture of different shapes. With motorized fluorescence microscopes and larger camera formats, this screening approach can be scaled up significantly to identify highly active particles among many thousands, which can then be selected for subsequent high-resolution structural and compositional analysis, for example using high-resolution electron microscopy. Coupled with combinatorial or parallel synthesis of catalysts, one can envision that this approach can be powerful for assessing catalyst preparation processes and the performance of resulting catalysts. The information can then be fed back to the next round of catalyst synthesis and optimization, which would accelerate the discovery and development of new or better catalysts.

5. Concluding remarks

The single-molecule fluorescence microscopy approach is generally applicable for studying chemical catalysis, electrocatalysis, and photocatalysis. The approach is amenable to any catalyst material, such as metals, metal oxides, and metal sulfides, and to a wide range of catalyst sizes, such as nano, micro, or bulk dimensions, or even small molecule catalysts. This approach certainly has limitations, such as the requirement of fluorescent molecules, as discussed in detail in our previous review. However, the chemical transformations to be studied are not limited; for example the two reactions in Fig. 1C represent two distinct chemical transformations. Other fluorogenic reactions are available too (e.g., trans-esterification and nitro reduction reactions). With clever synthetic chemistry, one can design reagent molecules that undergo the desired chemical transformations to generate a fluorescent product molecule.

Moreover, it may not even be necessary to design fluorogenic reactions in order to evaluate the activity of catalysts in a particular chemical transformation at the single-particle level.
In combination with other detection methods, as well as other manipulation methods such as electrical or optical manipulations, one can access a plethora of information towards understanding the structure-activity correlations of catalysts.

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