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ARTICLE

Single Molecule Fluorescence Imaging of Nanoconfinement in Porous Materials†

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This review covers recent progress on using single molecule fluorescence microscopy imaging to understand the nanoconfinement in porous materials. The single molecule approach unveils the static and dynamic heterogeneities from seemingly equal molecules by removing the ensemble averaging effect. Physicochemical processes including mass transport, surface adsorption/desorption, chemical conversions within the confining space inside porous materials have been studied at nanometer spatial resolution, at the single nanopore level, with millisecond temporal resolution, and under real chemical reaction conditions. By understanding these physicochemical processes, it provides the ability to quantitatively measure the inhomogeneities of nanoconfinement effects from the confining properties, including morphologies, spatial arrangement, trapping domains, etc. Prospects and limitations of current single molecule imaging studies on nanoconfinement are also discussed.

1. Introduction

Porous materials, in which guest molecules are encapsulated in nanoscale confining space within the porous network, have been used in various applications, such as catalysis,[1, 2] biological and environmental sensing,[3, 4] chromatography,[5, 6] energy generation and storage,[7-10] selective sequestration of contaminants,[11, 12] and drug delivery.[13, 14] Understanding the nanoconfinement in porous materials has been gaining a lot of interest because confined molecules can fundamentally change their chemical and physical properties comparing to those in bulk conditions.[15-17]

In chemical reactions, nanoconfinement can result in changes in specific reaction pathways by tuning geometrical constraints, selective adsorption, and potential energy surface, thus eventually influencing activity and selectivity.[18, 19] For instance, confinement of molecules has enabled the synthesis of select amino acids and sugars, the stabilization of polymers such as RNA and polyglycine, and restoration of misfolded proteins to their native structures.[15, 20] On the other hand, the mass transport of molecules under nanoconfinement is orders of magnitude slower than that in bulk liquids.[21] The mass transport and the accessibility of reactant molecules in porous materials can greatly influence their catalytic activities or the final products' selectivity. In many reactions, it is of great importance to understand the diffusion of reactants into the porous material because the efficiency in such applications mainly depends on the molecule's penetration and partitioning behaviors, which are strongly affected by the considerably enhanced steric and electrostatic interactions within the confining space.

Porous materials possess complicated structural features (e.g., spatial arrangement, types, morphologies, and inhomogeneity of confining space) and physicochemical properties (e.g., viscosity, hydrophobicity, and electronic charges) differing in their effects of nanoconfinement on confined chemical dynamics. In rational design and synthesis of porous materials with improved performance, one needs quantitative correlations between the properties of nanoconfinement and the corresponding chemical dynamics, ideally obtained under realistic conditions. High-resolution electron microscopy, such as scanning transmission electron microscopy (STEM) and scanning tunneling microscopy (STM), has an atomic level spatial resolution but requires low-pressure conditions and/or conductive surfaces. Ensemble methods such as optical microscopy and spectroscopy, neutron scattering, and pulsed-field gradient nuclear magnetic resonance (NMR) spectroscopy can provide the averaged information on pore morphology, pore integrity, pore environmental chemistry, etc. However, they lack the sensitivity down to the single molecule level and are unable to resolve the inhomogeneity of nanoconfinement in complex porous materials. Neither electron microscopy nor ensemble characterization methods can directly access molecular chemical dynamics under nanoconfinement in the porous materials.

In recent years, the development of single molecule fluorescence (SMF) imaging has brought new insights into biophysical studies and chemical measurements. For example, SMF imaging has been applied to investigating heterogeneous catalysis on solid catalysts for unveiling the hidden inhomogeneities in catalytic dynamics and activity fluctuations at different surface features in

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single catalysts and among individual catalysts.[22-25] SMF imaging can reveal static and dynamic heterogeneities from seemingly equal molecules by removing the ensemble averaging effect at nanometer spatial resolution, millisecond temporal resolution, and under true reaction conditions. The recovered static positions and dynamic trajectories of individual molecules in SMF imaging enable one to unveil the features of the local environment, including morphologies, spatial arrangement, trapping domains, etc., and determine dynamic behaviors of targeted molecules or objects such as diffusion rates and adsorption/desorption kinetics. By utilizing fluorogenic reactions, SMF imaging also enables the monitoring of single molecule reaction dynamics through counting individual turn-over events, deconvolution of individual kinetic steps, and uncovering hidden reaction dynamic inhomogeneities in both spatial and temporal domains.

Over the last decade, numerous SMF imaging studies, taking advantage of high spatial and temporal resolution, have been conducted to acquire direct experimental measurements and achieve a quantitative understanding of the nanoconfinement at the single molecule and single nanopore level. This review surveys the application of single molecule imaging in understanding the effects of nanoconfinement in porous materials, with a focus on the dynamic mass transport behaviours of individual molecules with various structural features and chemical environments. We will also discuss the nanoconfinement effects on heterogeneous catalysis, including catalyst effectiveness and reaction dynamics within well-defined structures. Further readings on the review articles on single molecule fluorescence imaging of chemical dynamics are strongly encouraged.[24-32]

2. Mass transport in confined space

Porous materials have gained a lot of interest due to their large surface area, controllable structure, and ease of preparation.[20] The porous structures act as pathways that can limit or enhance the mass transport of the incorporated molecules. Examples of the most commonly employed self-organized nanostructured materials with 1D pathways include mesoporous silica or metal oxides, lyotropic and thermotropic liquid crystals (LCs), microporous coordination polymers (MCPs), and block copolymers (BCPs). These nanostructured materials have the ability to selectively transport particular chemical species. The partitioning of guest molecules and the mass transport within those nanoscale domain affects the transport rate and the selectivity of such models. Those nanostructures may have different barriers that confine the molecular motion in one or more dimensions. Moreover, mass transport could be affected by molecular-level processes such as steric, chemical, and electrostatic interactions between guest molecules and nanostructured medium. Therefore, it is important to achieve a better understanding of the mechanism for mass transport in such confined space to design optimized materials for specific applications such as chromatographic separations.[30] So far, mass transport in confined space has been mainly studied using ensemble measurements. For instance, various studies have focused on the subject of molecular diffusion in porous materials by employing various methods such as fluorescence recovery after photobleaching (FRAP), infrared (IR) spectroscopy, pulsed-field gradient nuclear magnetic resonance (NMR), and quasi-elastic neutron scattering. In recent years, single molecule spectroscopy (SMS) has been developed to directly monitor the behaviors of individual molecules in confined space, thus enabling spatial, temporal visualization and better understanding of heterogeneous molecular dynamics under diverse nanoconfinement in complex porous materials.[33]

Two different SMS techniques can be used to study the diffusion of fluorescent dye molecules. The first method is fluorescence correlation spectroscopy (FCS), which uses a microscopy apparatus to observe the diffusion of individual molecules entering and exiting a well-defined small detection volume. The fluctuations of the detected fluorescence intensity in time $I(t)$ can be statistically analyzed to extract the diffusion coefficients.[33] The limitation of this method is that in order to obtain a diffusion coefficient, it requires the fitting of the intensity autocorrelation function with a theoretical expression derived from a model of the diffusion process. Moreover, the temporal and spatial information of individual molecules is not accessible in this method.[33] The second method is directly analyzing a series of fluorescence microscopy images, i.e., movies, of diluted fluorescent molecules. In those movies/images, the fluorescence of individual dye molecules is imaged on cameras in many frames as diffraction-limited patterns that can be tracked and analyzed; thus, this method is known as single molecule tracking (SMT). SMT does not require a model assumption and allows us to directly characterize the motion in space and time to observe various diffusion behaviors in the reconstructed trajectories. This method is not sensitive for fast processes in comparison to FCS and requires sufficient acquisition time (> ms) for obtaining single molecule images of enough signal to noise ratio (SNR). Both methods have their own pros and cons,[34-36] and here we will mainly cover studies on SMT in porous materials. SMT can be used to investigate single molecules diffusing in porous materials by revealing the presence of distinct diffusion behavior of individual molecules and the heterogeneous behavior in the trajectory when they are trapped in a nanometer-sized confined space. SMT also allows us to observe deviations in the distribution of step sizes which could be due to adsorption sites and molecular-size barriers present in the material or anisotropy in the material's structure.[33]

2.1. Overview of single molecule tracking in porous materials

To achieve single molecule detection in porous materials, the guest molecules (fluorescence probes) are loaded into the porous structures with very low concentrations at the nano- or pico-molar level so that it is possible to resolve individual molecules spatially. There are several ways to incorporate fluorescent molecules into porous materials, including entrapping the guest molecules inside the porous materials during synthesis or incubating guest molecules with the host material for a period. Typically, sequences of SMF

images (Figure 1A) are recorded with a wide-field imaging setup. A laser beam is used to illuminate a certain spot on the sample to excite the probe molecules causing them to fluoresce. The fluorescence photons are then collected by an objective and imaged onto a highly sensitive detector such as the electron-multiplying charge-coupled device (EMCCD).

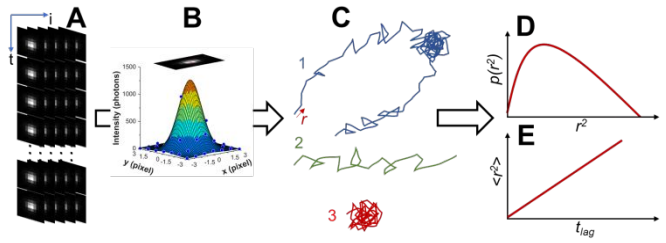

Figure 1. Single molecule tracking in porous materials. (A) Multiple time sequences of single molecule fluorescent images. (B) Localize the center positions of fluorescent molecules with nanometer precision using PSF (e.g., 2D elliptical Gaussian) fitting image intensity distribution. (C) Recovered single molecule trajectories. (D) Characterize MSD by fitting the distribution of step sizes or displacements (r) with PDF. (E) Determine the diffusion coefficient by fitting MSD versus lag time.

Localize and track molecular positions with nanometer precision. The actual sizes of fluorescent probe molecules are typically only a few nanometers; however, their fluorescence images are much larger, usually a few hundreds of nanometers, which is approximately one-half of the light wavelength. This effect can be described by the point-spread function (PSF) in light microscopes. Although the true shapes and sizes of the fluorescent probe molecules are not available in fluorescence microscopy imaging, their center positions (x_0, y_0) can be accurately determined by fitting the intensity distributions (I(x,y)) of these single molecule images with the PSF (Figure 1B). The Gaussian functions are often used as a suitable approximation of the PSF in fitting the intensity distributions.[37]

$$I(x,y) = A * exp\left(-\left(\frac{(x-x_0)^2}{2s_x^2} + \frac{(y-y_0)^2}{2s_y^2}\right)\right) \quad (1)$$

The accuracy in localizing the center positions depends on the collected photons of single molecule images and can be calculated using equation (2),[37] where S_j is the standard deviation of single molecule fluorescent image, a is the pixel size of the camera, N is the collected photon number from single fluorescence molecules, and b is the fluorescence background noise level.

$$\sigma_j = \sqrt{\left(\frac{S_j^2}{N} + \frac{a^2/12}{N} + \frac{8\pi S_j^4 b^2}{a^2 N^2}\right)} \quad (2)$$

With sufficient photons collected and low fluorescence background noise, the localization precision can be as high as 1 nm.[38] Trajectories of the movement of fluorescent molecules (Figure 1C) can be reconstructed from precisely localized positions as described above, thus enables one to directly visualize the mass transport, dynamic behaviors of confined guest molecules in porous materials.

Quantify diffusion behaviors from molecular trajectories. After obtaining the molecular positions in time series (i.e., trajectories), one can quantify the mass transport properties in porous materials using statistical methods, for instance, mean squared displacement (MSD) analysis. The MSD analysis is applied by taking the difference between two molecular positions along with the trajectory that is separated by a certain time, i.e., lag time t_{lag}. The step sizes or displacements (r^2) at a certain lag time are first calculated from the trajectories and their mean values ($\langle r^2 \rangle$) are then plotted versus the lag time. Alternatively, one can do the histogram distribution analysis of the obtained step sizes. The obtained histogram distribution could be fitted with a radial probability density function (PDF)[39] to extract the characteristic mean squared displacement (Figure 1D).

$$p(r^2,t) \cdot dr^2 = \frac{1}{\pi\langle r^2_{(t)}\rangle}\exp\left(\frac{-r^2_{(t)}}{\langle r^2_{(t)}\rangle + \sigma^2}\right)2\pi r \cdot dr^2 \quad (3)$$

One can fit $\langle r^2 \rangle$-t_{lag} relationship to extract the kinetic parameter in mass transport, i.e., diffusion coefficient, using the Einstein-Smoluchowski equation for random diffusion in n dimensions (Figure 1E).

$$\langle r^2 \rangle = 2nDt_{lag} \quad (4)$$

Furthermore, the measured diffusion coefficient can also be used to determine the apparent viscosity (η) of the medium inside the nanoconfinement using equation (5),[40] where k_B is the Boltzmann constant, T is the temperature, and r_0 is the size of fluorescent probe molecules.

$$D = \frac{k_B T}{2n\pi\eta r_0} \quad (5)$$

Uncover heterogeneous diffusion behaviors. Inhomogeneous diffusion dynamics can be seen either among or within individual trajectories. In the latter case, one may directly observe distinct regions of different diffusion behaviors as they spread out in space. Molecular trajectories within these regions can be quantified separately to examine their differences in diffusion coefficients, properties of the local nanoconfinement such as viscosity. However, this may not always be true where one can not readily tell the existence of such heterogeneous regimes within molecular trajectories. Such hidden heterogeneous diffusion behaviors can be recovered from the displacement analysis, where a single component PDF cannot fully describe the distribution of displacement. The multicomponent PDF with distinct diffusion coefficients can then be used to describe the heterogeneous diffusion behaviors of molecules.[14]

$$p(r^2,t) \cdot dr^2 = \sum_1^i c_i \frac{1}{\pi\langle r^2_{i(t)}\rangle}\exp\left(\frac{-r^2_{(t)}}{\langle r^2_{i(t)}\rangle + \sigma^2}\right)2\pi r \cdot dr^2 \quad (6)$$

Based on the measurement results, the mass transport of fluorescent probe molecules in the nanopores can be categorized into several sub-groups such as fast, moderate, and slow diffusion. Such results usually suggest that the mass transport of fluorescent probe molecules cannot be described simply as one Brownian motion, but rather as a combination of diffusion and adsorption behaviors associated with variable local nanoconfinement environments in porous materials.

Inhomogeneities among individual molecular trajectories are also often observed. As shown in Figure 1C, three types of molecular trajectories can be identified, namely, mobile, immobile and hybrid modes. These distinct trajectories can be quantitatively analyzed separately, and the determined diffusion coefficients, in combination with nanometer-precision positions, can be used to reconstruct high-spatial-resolution mapping showing heterogeneous regimes of diffusivity in porous materials.

Three-dimensional single molecule tracking. By extending both methods to three-dimensional (3D) single molecule localization, additional vital information can be obtained, including the visualization of structures or motions extending in the axial direction and a better understanding of complex systems. 3D super-resolution images and 3D tracking can be achieved by interferometric detection, multiple focal plane imaging, and point-spread-function engineering.[41] For example, Zhao et al. employed 3D single-particle tracking method to monitor the microscopic motion of 100 nm nanoparticles in cylindrical alumina nanopores (~3 μm in diameter) with a z-span as large as several micrometers.[42] This study revealed that particles could be retained inside the pores over an extended period due to either increased solvent viscosity or increased pore wall affinity, demonstrating the ability to differentiate different mechanisms for slow diffusion in confined environments.

2.2 Mass transport and local structure of nanoconfinement

Mesoporous materials synthesized through surfactant self-assembly and framework building blocks can be tuned to adopt a variety of structures, which made them widely employed in various applications. The local structure of the confined environment can greatly influence molecular diffusion. Distinct regimes or modes of molecular diffusion could occur within the pore networks, which may arise from structural heterogeneities of the materials such as slight variations of pore diameter. The viscosity of the medium in the confinement also increases due to stronger molecular interactions. Therefore, it is important to understand the molecular movement inside the porous materials and how the local structures, such as domains and defects, influence the movement of confined molecules. Optical as well as transmission electron microscopies fail to directly image the mesoporous structure or provide dynamic information on molecular diffusion. The high spatial and temporal resolution of SMT enables one to obtain such information and provide insights into the heterogeneity and details of molecular diffusion as well as the structure of the porous host.

Spatially resolve heterogeneous nanoconfinement. SMF microscopy has been used to assess, quantify, and map heterogeneities in local diffusion properties within complex porous materials. For instance, Weckhuysen et al. used SMF microscopy to study single molecule diffusion inside a complex network of micropores (internal diameter < 2 nm), mesopores (2-50 nm), and macropores (> 50 nm) in real-life fluid catalytic cracking (FCC) particles.[43] FCC particles are used for producing half of the gasoline worldwide because they are the catalyst for one of the main conversion processes in the oil refinery. In this work, they studied the diffusion of feedstock-like probe molecule, i.e., N, N'-bis (2,6-dimethyl phenyl)-perylene-3,4,9,10-tetracarboxylic diimide (PDI), within the complex pores of micrometer-sized FCC particles (~ 20 μm in diameter). The recorded movies of single fluorescent molecules allowed the classification of their movement through the porous networks into three different states: immobile, mobile, and hybrid (Figure 2A). The average apparent diffusion coefficients of each type (Figure 2B) were measured to reveal such diffusive heterogeneity across the entire FCC particle (Figure 2C). This study shows the heterogeneity of the local pore structure and how it influences the molecular transport.[43]

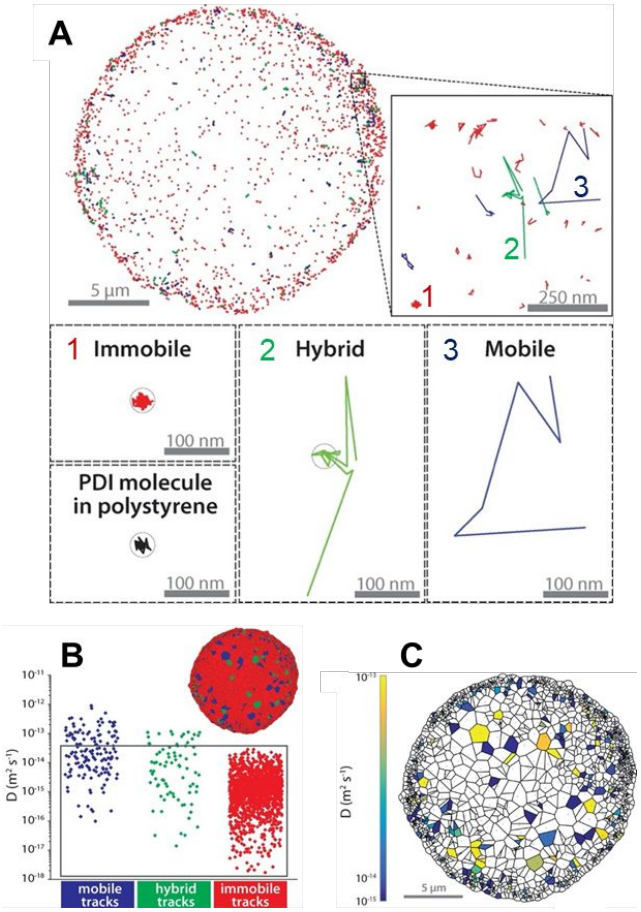

Figure 2. SMF imaging of heterogeneous diffusion in pore network of FCC particles. (A) Super-localization mapping of PDI molecules diffusion in an FCC particle. (B) Diffusion coefficients of each type of track. The gray rectangle includes diffusion coefficients falling within

the localization uncertainty of the single molecule analysis. The inset Voronoi diagram shows the spatial distribution of each track type. (C) Diagram showing localized diffusion coefficients in the middle cross section of the FCC particle. The color of each area indicates the diffusion coefficient, with areas around immobile tracks being white. Figures are adapted from ref. 43 with permission. Copyright 2017 American Chemical Society.

To determine the diffusion behaviors of analytes and obtain better separation efficiency in high performance separations, e.g., reversed phase liquid chromatography (RPLC) using porous particles, it is important to understand the time scale of processes responsible for analyte retention in the stationary phase. Harris et al. used SMF imaging to observe the transport of individual molecules within C_{18}-modified porous silica particles (Figure 3).[5] This study focused on

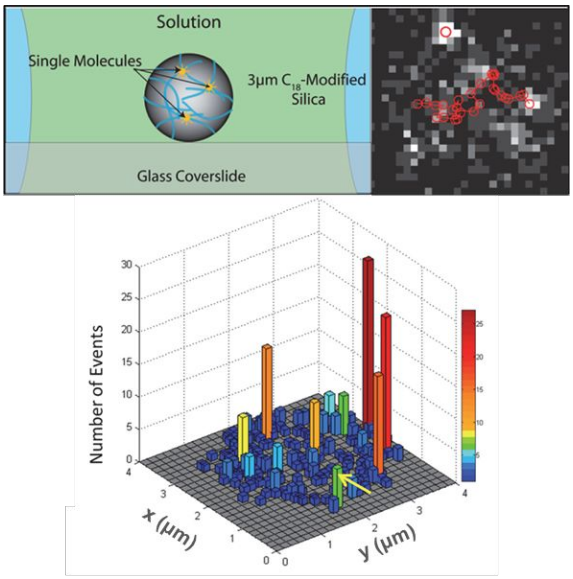

Figure 3. SMF imaging of R18 molecules diffusing in C_{18}-modified porous silica particles. Schematic view (top left), single molecule images of R18 dye molecules (top right), and a 2-D map of stuck events at resolved sites (bottom) where color bars describe the number of events. Figures are adapted from ref. 5 with permission. Copyright 2013 American Chemical Society.

characterizing intraparticle molecular transport when individual molecules visiting C_{18}-modified porous silica particles. The residence times and diffusion rates of octadecylrhodamine B (R18), as well as its spatial distribution within the porous silica particles, were extracted from SMT experiments. The recorded molecular trajectories were divided into the moving (N_m) and stationary (stuck, N_S) time segments. The stationary molecules represent the strong adsorption that would lead to peak tailing in chromatographic elution with higher residence time. These strong retention sites at the interfacial surface of solid materials such as defects of available silanols are often unavoidable during the materials synthesis and fabrication process, which can interact with analyte molecules thorough interfacial forces like hydrogen bonding and electrostatic interaction.[5, 44-46] SMF imaging enables the understanding of the separation mechanism at the molecular, nanoscale level and can inspire future work in modifying stationary phases to improve the separation performance both by synthetic methods and theoretical simulations, for example, designing strategies to reduce silanol prevalence.[47-49] Furthermore, the calculated capacity factor, k' = $N_S/N_m \sim 490$, suggested that R18 molecules spend only 0.2% of their time in the mobile phase. In other words, almost all of the intraparticle transport occurs as surface diffusion on the C_{18}-modified silica stationary phase.

Spatial arrangement, types, and morphologies of nanoconfinement. Heterogeneous nanoconfinement can be created by changing the spatial arrangement of a variety of pore structures. SMT has also been employed to show how the pore arrangements or types cause different confinement effects on molecular behaviors.

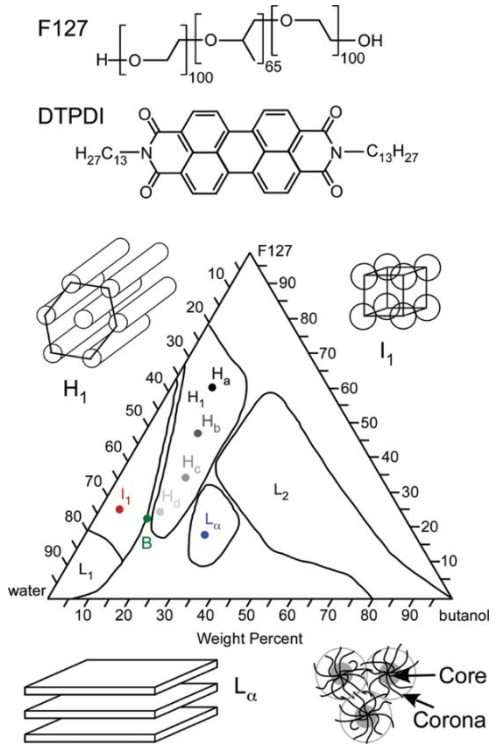

Figure 4. Phase diagram for F127/water/butanol mixtures. Shown are the normal hexagonal (H_1), isotropic (I_1), and lamellar (L_α) regions investigated, along with the normal (L_1) and reverse (L_2) micellar regions. The filled circles appended to H_1, I_1, and L_α regions depict the samples prepared and characterized. The diagrams at the upper left, upper right, and lower left show simple models for assembly of copolymer molecules in each phase. The diagram at the lower right depicts the core and corona regions of cylindrical and spherical micelles. Figures are adapted from ref. 50 with permission. Copyright 2011 American Chemical Society.

Higgins et al. investigated the diffusion of the hydrophobic perylene diimide (DTPDI) dye molecules within the mesophase structures, i.e., hexagonal, lamellar, and cubic regions, of Pluronic F127 gels that are a series of dye-doped F127/water/butanol mixtures (Figure 4).[50] In the hexagonally arranged cylindrical gels, DTPDI molecules exhibited distinct 1D diffusion within the viscous and hydrophobic micelle cores. In comparison, DTPDI molecules in

the lamellar and cubic mesophases exhibited isotropic 2D and 3D diffusion behaviors. The diffusion in the cubic regions were found to be much slower due to long-term confinement of the dye molecules in the micelle core. A bimodal diffusion coefficient distribution was observed: the slow diffusion reflected the 1D motion in the micelle core, while the faster component reflected the partitioning or 3D diffusion in the interconnected micelle coronas. These results provide vital information for understanding the individual roles played by the micelle core and corona in governing molecular diffusion.

Bräuchle et al. employed SMF microscopy to track strongly fluorescent terrylene diimide (TDI) dye molecules inside mesoporous silica channel systems.[51, 52] The mesoporous silica channel system was prepared as thin films via cooperative self-assembly of surfactant molecules with polymerizable silicate species, resulting in three different samples: a single pure hexagonal mesophase, a single pure lamellar mesophase, and the third sample in which the two mesophases coexisted. Molecules in the lamellar phase maintained a constant orientation perpendicular to the glass substrate and normal to the silica planes during diffusion because of the strong interactions of the dye molecule with the template molecules. Whereas molecules in the hexagonal phase diffused faster and reoriented constantly, indicating much weaker interactions with the template than in the lamellar phase. Their results demonstrated that different topologies strongly influenced the diffusion of the molecules inside the pores. This approach provides a detailed picture of the structure and connectivity of different nanopore systems and how they would influence molecular diffusion.[51] In another study, the same group investigated the influence of pore size on molecular diffusion in cast silica xerogels and on spatial heterogeneities.[53] Two types of monolithic silica gels were doped with different porosities with streptocyanine dye. The first type of gel had a pore size similar to the size of the dye molecule (3 nm mesopore), while the other had a bigger pore size (22 nm mesopore). The dye molecules were found largely (80%) trapped in regions of narrower pores (diameter 50 - 200 nm), while the wider pores had fewer physical traps. Statistical analysis of the single molecule trajectories revealed that the gel with a wider pore allowed the dye molecules to diffuse freely and had a higher diffusion coefficient compared to the gel with smaller pores. This shows the significance of the pronounced microscopic inhomogeneities and the distribution of diffusion coefficients due to the difference in pore size and the local structure.[53]

Structural domains and defects in nanoconfinement. Studies have also been conducted to obtain information on the nanoconfinement environment such as the domain size and how it affects the molecular transport.

Lately, both fluorescence recovery after photobleaching (FRAP) and SMT measurements have been used to provide complementary information on molecular diffusion in porous materials and how the domain size of the nanoconfinement environment affects the molecular transport. Ito et al. employed both techniques (Figure 5) and obtained a direct comparison of the molecular diffusion information within identical regions of a cylinder-forming polystyrene-poly (ethylene oxide) diblock copolymer (PS-*b*-PEO) film (~4 μm thick) with aligned cylindrical PEO microdomains containing 10 μM sulforhodamine B (SRB).[54] FRAP was used to study SRB ensemble diffusion behavior in the domains at microlevel, while SMT was used to study the diffusion of individual SRB molecules at nanoscale. The obtained average diffusion direction and coefficient for the florescent molecule inside the cylindrical domain were similar for both methods. FRAP measurements assisted in studying longer range diffusion behavior in the ≥100 μm microdomain length, while SMT measurements were used to assess the distribution of mass transport properties of individual molecules. FRAP offered information on the microdomain morphology, such as its effective length, orientation, and dimensionality. SMT also provided information on the dimensionality and orientation of individual microdomains plus single molecule diffusion coefficients. By applying both methods on identical sample areas, they were able to minimize the influence of the compositional and morphological heterogeneity on accurate mass transport measurement. Therefore, permitting direct comparison of ensemble and single molecule diffusion behavior.

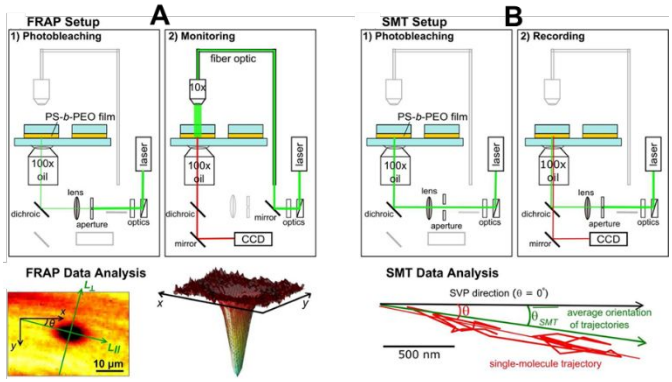

Figure 5. Experimental setups for FRAP and SMT measurements. (A) Top: For FRAP measurements, a circular region in a PS-*b*-PEO film was first photobleached using an intense laser pulse. Subsequently, the fluorescence was imaged by irradiating the sample with attenuated laser light from the top. Bottom: A representative fluorescence image of a photobleached region in the FRAP experiment. (B) Top: All SMT data were recorded (right) under broad laser illumination after the wider observation area was further photobleached (left) by more intense laser light. Bottom: A typical 1D single molecule trajectory (red) and its best-fit line using orthogonal regression methods. Here, θ represents the tilt angle (red) of the single trajectory with respect to the solvent-vapor penetration direction ($-90° \le \theta \le 90°$) while θ_{SMT} depicts the average trajectory orientation from all 1D trajectories found in each set of SMT data (green). Figures are adapted from ref. 54 with permission. Copyright 2015 American Chemical Society.

TDI dye molecules have also been incorporated into CTAB-templated hexagonal mesoporous silica films containing highly structured domains as a host (Figure 6).[55] The diffusional and orientational behaviors of the TDI molecules were monitored by

polarization modulated confocal microscopy. By examining different atmosphere conditions, it was shown that the TDI molecules were free to diffuse linearly along with the pores in chloroform, but they were immobile in the air (Figure 6A, B). Furthermore, the transition dipole moment did not change its orientation along the trajectories (Figure 6C), suggesting the host having highly linear channels with rare defects. Moreover, dead ends and connections between the adjacent pores were detected from the single molecule trajectories. The data also showed that the overall diffusion of the dye molecules could not be described by a 1D random walk, but it was more complicated because the diffusion was occasionally interrupted by temporary entrapment of molecules at the adsorption sites. Therefore, investigation of the translational and orientational dynamics via SMF techniques with very high positioning accuracy (down to 2-3 nm) gives structural as well as dynamics information, and it is quintessential for enhancing the performance of mesoporous solids for applications in separations, catalysis, chemical sensors, and host-guest chemistry.[55]

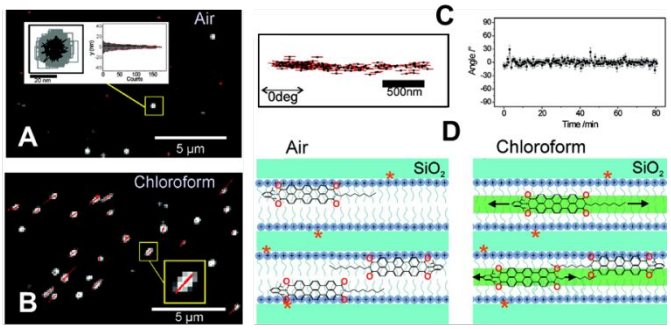

Figure 6. Parallel orientation and diffusion of single TDI molecules in highly structured domains. Single TDI molecules embedded in parallel pores in air atmosphere (A) and in chloroform (B). (C) A single molecule trajectory of TDI molecules embedded in parallel pores in chloroform atmosphere (left) and the calculated angular time trajectory of the same molecule (right). (D) Schematic of TDI molecules immobilized in the mesoporous material in air atmosphere (left) and diffusing in the mesoporous material in the presence of chloroform (right). The stars indicate the presence of active silanol groups. Figures are adapted from ref. 55 with permission. Copyright 2008 American Chemical Society.

2.3 Mass transport and chemical environment of nanoconfinement

It has been observed that the chemical environment in which the guest molecules are confined has a great influence on their molecular diffusion. The capability of controlling guest molecules in the host materials can greatly benefit a large variety of applications.

Viscosity. Wang et al. have developed a 3D single-particle localization technique to study how viscosity and the wall pore affinity influence molecular transport in confined environments.[42] The microscopic motion of carboxylated polystyrene nanoparticles in cylindrical alumina nanopores with a z-span as large as several micrometers were imaged for studying the effects of increased solvent viscosity and particle−surface interactions on diffusion in nanopores. This experiment showed that increasing the buffer viscosity slowed down particles' motions significantly throughout the entire pore structure (Figure 8A), while increasing the pore wall affinity only slowed down the particle's microscopic motion slightly (Figure 8B), likely because the increased pore wall affinity only slowed down the particle at the wall. This was a nice demonstration of 3D single particle tracking techniques for differentiating different factors in influencing molecular dynamics in confined environments.

Interfacial electrostatic force. The charges and electrostatic interactions between the host material and confined molecules have been shown to define the mass transport within porous materials.

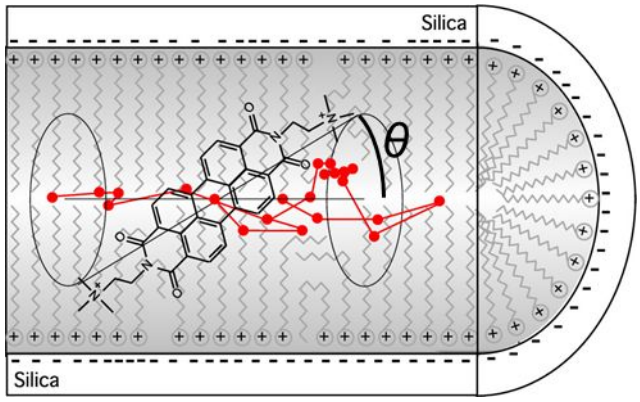

Figure 7. Schematic view mass transport of charged PDI molecules confined in mesoporous silica pore of nanometer sizes. Figures are adapted from ref. 20 with permission. Copyright 2020 American Chemical Society.

A recent study by Higgins et al. investigated the effects of charges on mass transport in one-dimensional (1D) nanoscale pores of surfactant-templated mesoporous silica films.[20] In this work, polarization-dependent single molecule tracking was employed to observe the translational and rotational diffusion of perylene diimide (PDI) dyes of different lengths and charges inside the silica mesopores (Figure 7). The acquired fluorescence videos revealed that the majority of the molecules exhibited 1D diffusion as well as a highly polarized fluorescence, consistent with their orientational confinement. The acquired step size distributions were fitted with a model of the 1D Fick diffusion in the presence of finite localization precision. The two neutral PDIs had 20−100% larger average diffusion coefficients compared to the charged PDIs, which could be explained by the electrostatic interactions of charged PDIs with the oppositely charged sites on the cationic surfactant headgroups and deprotonated silanol sites on the pore walls. The longest uncharged PDI was found to be more confined than the other three shorter dyes. Since the dyes diffuse in a highly oriented state, the orientation confinement of the 1D diffusing dye molecules was evident by the strongly polarized emission. A subtle depolarization of the fluorescence represents the confined orientational wobbling of the molecules and allows the measurement of the wobbling motions and the lateral dimensions of the pathway that each dye molecule

follows within the pore. It was found that cationic PDI explored more of the pore diameter as it produced a broader wobbling angle distribution compared to the others. The information on how the confined molecules interact with the surfactant filling the pores as well as on the pore surface is important to understand the mass transport within porous materials.[20]

Bräuchle et al. employed SMF techniques to study the orientational and spectral dynamics of single molecules in nanostructured host-guest materials.[56] Oxazine-1 dye molecules that were encapsulated tightly inside microporous $AlPO_4$-5 crystals (a zeotype structure (artificial zeolites)) did not show movement, while terrylenediimide (TDI) molecules inside the pores of a templated mesoporous silica (uncalcined M41S film) showed more orientational dynamics and interrupted movement supported by the molecule's fixed orientation for a certain period of time. Those movement interruptions could be due to the interactions with the silica wall and the electrostatic interactions with the cationic template or at defect sites.

Hydrophobicity. The hydrophobicity of the pore structure is another key factor in influencing molecular transport within the nanochannels. Recently Bein et al. functionalized the wall of mesoporous silica networks/films with alkyl molecules to study the diffusion behavior of single terrylene diimide (TDI) dye molecules using SMF microscopy.[57] The influence of functional-group polarity on the diffusion coefficients was revealed from comparing propyl-, cyanopropyl-, and phenyl-functionalized films. The strongly polar phenyl groups decreased the mean diffusion coefficient of the dye molecule while it was the opposite in the nonpolar propyl- and cyanopropyl- functionalized films. This study provided a direct experiment evidence that molecular diffusion can be controlled by varying the density and chemical nature of the functional groups within the confined space.

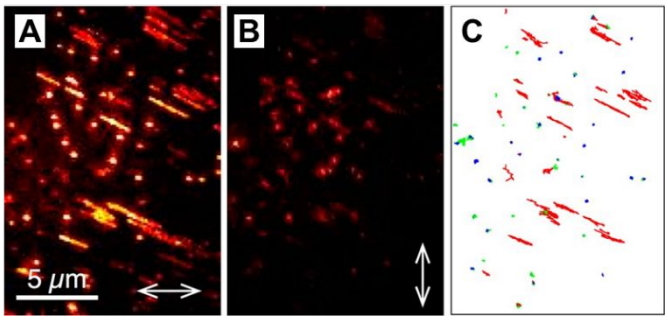

Figure 8. SMF imaging of one-dimensional diffusion of TDI dye molecules in surfactant templated mesoporous silica. (A, B) Z-projection images depicting Nile Red motions in a surfactant-templated mesoporous silica film under orthogonal emission polarization detection (white double-ended arrows). (C) Single molecule trajectories obtained by tracking the molecules where color describes immobile (blue), 1D diffusing (red), and 2D diffusing (green) molecules. Figures are adapted from ref. 21 with permission. Copyright 2016 American Chemical Society.

Higgins et al. employed spectroscopic and polarization-dependent SMT to determine the location of sensitive dye Nile Red (NR) confined in surfactant-filled mesoporous silica films incorporating hexagonally ordered cylindrical pores (Figure 8).[21] They observed that NR confined in the cylindrical pores exhibited 1D diffusion with ~ $\times 10^3$-fold smaller diffusion coefficients than in bulk solution. The hydrophobic NR molecules were found mostly located in the hydrophobic core regions of the micelles (having polarities similar to that of n-hexane), instead of being near the silica/surfactant interface. Furthermore, single molecule emission polarization (SMEP) measurements demonstrated that the NR molecules were orientationally confined to ~0.6 nm diameter pathways within the pores, which was much smaller than the physical diameter of the pores.

3. Chemical reactions under nanoconfinement

Synthetically generated confinement systems could enhance the performance of encapsulated active centers, such as acid, base, and metal sites. The altered chemical and physical behaviors of substrate molecules within nanometer-size pore structures are often attributed to the nanoconfinement effects. For example, the confining materials can directly alter the chemical reaction mechanism by assisting the chemical bond formation/breaking in zeolites and carbon nanotubes.[58-61] Chemical reaction rate and product selectivity are often dependent on morphological properties of confining environment in both enzymes and synthesized porous materials.[62-64] Furthermore, interfacial interactions (i.e., electrostatic, van der Waals force, etc.) between substrate molecules and pore surface of confining materials can also dramatically change mass transport[14, 65, 66] and adsorption-desorption equilibrium,[5, 67] thus either enhancing or reducing catalytic reaction activities and selectivity significantly.

Porous catalysts are complex systems with different structural features and physicochemical properties, resulting in distinct local nanoconfinement environments. Such heterogeneities exist among individual catalyst particles as well as within a single catalyst at the sub-particle level. Nanoconfinement effects on chemical reactions in porous catalysts have been studied both theoretically with simplified model systems[68-71] and experimentally at the ensemble level.[10, 72-75] On one hand, theoretical modeling and studies, without any doubt, are important and useful for the understanding of nanoconfinement effects. However, it is limited due to the gap between the simplified model systems used in theoretical studies and the much more complex systems employed under realistic conditions. On the other hand, ensemble measurement of catalytic reactions in porous catalysts can investigate the nanoconfinement effects under operando conditions, but it lacks the sensitivity down to the single molecule level. To improve the efficiency of porous catalysts through rational design of nanoconfinement, it is imperative to know the quantitative correlation between the properties of nanoconfinement and the heterogeneous catalytic dynamics of confined active centers. Single molecule localization microscopy (SMLM) imaging of

heterogeneous catalysis in porous catalysts is one way to search for the answer. In this section, we will cover the principle of SMLM and its applications in the study of nanoconfinement effects on heterogeneous catalysis in various porous materials.

3.1 Single molecule localization microscopy imaging of catalytic reactions

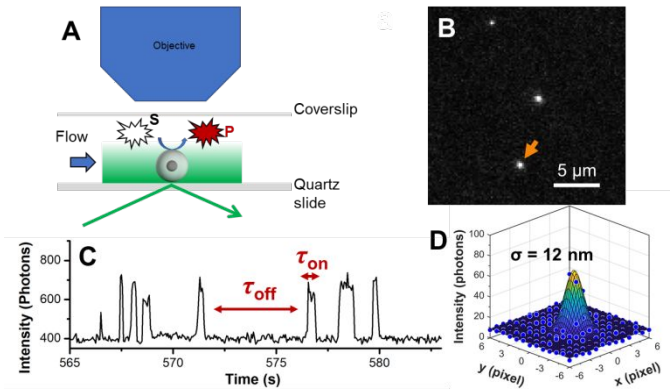

Figure 9. Single molecule localization microscopy imaging of catalytic reactions. (A) SMF imaging of the oxidation of amplex red catalyzed by platinum loaded core-shell mesoporous silica particles under TIRFM. (B) Typical fluorescent image of freshly generated fluorescent product molecules. (C) Segment of fluorescence intensity trajectory on a single nanocatalyst that is highlighted in (B). τ_{off} and τ_{on} are determined at turn-over resolution. (D) Gaussian fitting the intensity distribution of the highlighted single molecule image gives the molecular center position with 12 nm localization precision. Figures are adapted from ref. 118 with permission. Copyright 2020 American Chemical Society.

Single molecule localization microscopy (SMLM) relies on having sparsely distributed fluorescent molecules that can be isolated both temporally and spatially.[76] The positions of these individual molecules with nanometer precision can be used to reconstruct images with <10 nm lateral resolution and <20 nm axial resolution.[77] The switching between a fluorescent state (on state) and a nonfluorescent state (off state) for photoactivatable fluorophores is one of the working principles since it allows single molecule detection in condensed labeling conditions in samples. The on-off switching dynamics of dye molecules had also been achieved through a technique named points accumulation for imaging in nanoscale topography (PAINT),[78] where the reversible binding/unbinding of dye molecules to the substrate was utilized to achieve single molecule isolation. Similarly, fluorogenic chemical reaction, where a nonfluorescent reactant molecule is chemically converted to a highly fluorescent product molecule (Figure 9A), can also lead to on-off dynamics (Figure 9B, C).

The on-off dynamics of fluorogenic reactions on the catalysts can be monitored in real time with an optimized experimental setup. Technical considerations include minimizing the fluorescence background with total internal reflection fluorescence microscope (TIRFM), enhancing detection sensitivity with single-photon counting EMCCD cameras, using highly purified and clean reagents, and removing desorbed fluorescent product molecules using a flow system, etc. The positions of freshly generated fluorescent product molecules can be determined with nanometer precision (Figure 9D) to pinpoint the locations of catalytic active sites on a single catalyst. Transmission electron microscopy (TEM) and scanning electron microscopy (SEM) images are often taken to correlate the different structures on single catalyst particles with their catalytic activities from single molecule studies.[79] A recently published review article discussed the importance of correlating SFM imaging results with SEM images to establish the structure-activity relationship in heterogeneous catalysis, especially for catalysts containing complex structures.[80] Beyond the benefits of the high spatial resolution in SMLM for resolving locations of catalytic active sites, the single molecule on-off dynamics enables quantitative measurement of temporary properties, including reactant reaction kinetics, product dissociation kinetics, and catalyst stability at turn-over resolution.

Fluorogenic probes for single molecule fluorescent imaging. The success of single molecule fluorescence imaging of chemical reaction on catalyst surfaces relies on choosing an appropriate fluorogenic probe. First, the catalytic center should be active for the chosen fluorogenic reaction. Second, sufficient contrast of fluorescence emission brightness between reactant and product molecules. Ideally, the reactant should make no contribution to the fluorescence background. Third, the overlap between fluorescence signals of the product molecules and the fluorescence background from the catalyst, should be very small (or ideally avoided entirely). So far, many fluorogenic reactions that can be used for SMF imaging have been reported. For example, nonfluorescent fluorescein dye-based derivatives including APF, HPF,[81] H_2DCFDA, carboxyl-H_2DCFDA, chloromethyl-H_2DCFDA, deacetylated H_2DCFDA[82] can generate highly fluorescent product molecules upon oxidation; DN-BODIPY,[83] resazurin,[84] DCDHF-azide and its derivatives[85, 86] can generate highly fluorescent product molecules by reduction; CalFluors[87-89] can also be converted to highly fluorescent molecules upon copper-catalyzed click reaction with alkyne.[90] Furthermore, coupling reactions (such as Suzuki, Heck, or Stille reactions) catalyzed by noble metal NPs (such as Pd, Pt, Rh, etc.) to add aryl, vinyl, or alkyl groups can also turn on the fluorescence.[91-94] A more detailed summary is given in Figure 10. Some of these fluorogenic probes (e.g., amplex red, resazurin, APF, CalFluors, etc.) are readily available from commercial sources, while the synthesis protocols for other fluorogenic probes have been fully described in the previous publications.

Super-resolved mapping of catalytic active sites. SMLM has been used to recover heterogeneities in catalysis at different structures such as facets, edges, and defects in single solid catalysts, such as metal, semiconductor particles,[95-97] crystals,[98, 99] and nanowires.[100, 101] Quantitative correlations between structural properties of catalysts and the catalytic activity have been unveiled. Weckhuysen et al. used SMF imaging together with super-resolution optical fluctuation imaging (SOFI) analysis to investigate the inhomogeneity in zeolite aggregates distributions at the sub-micrometer level as

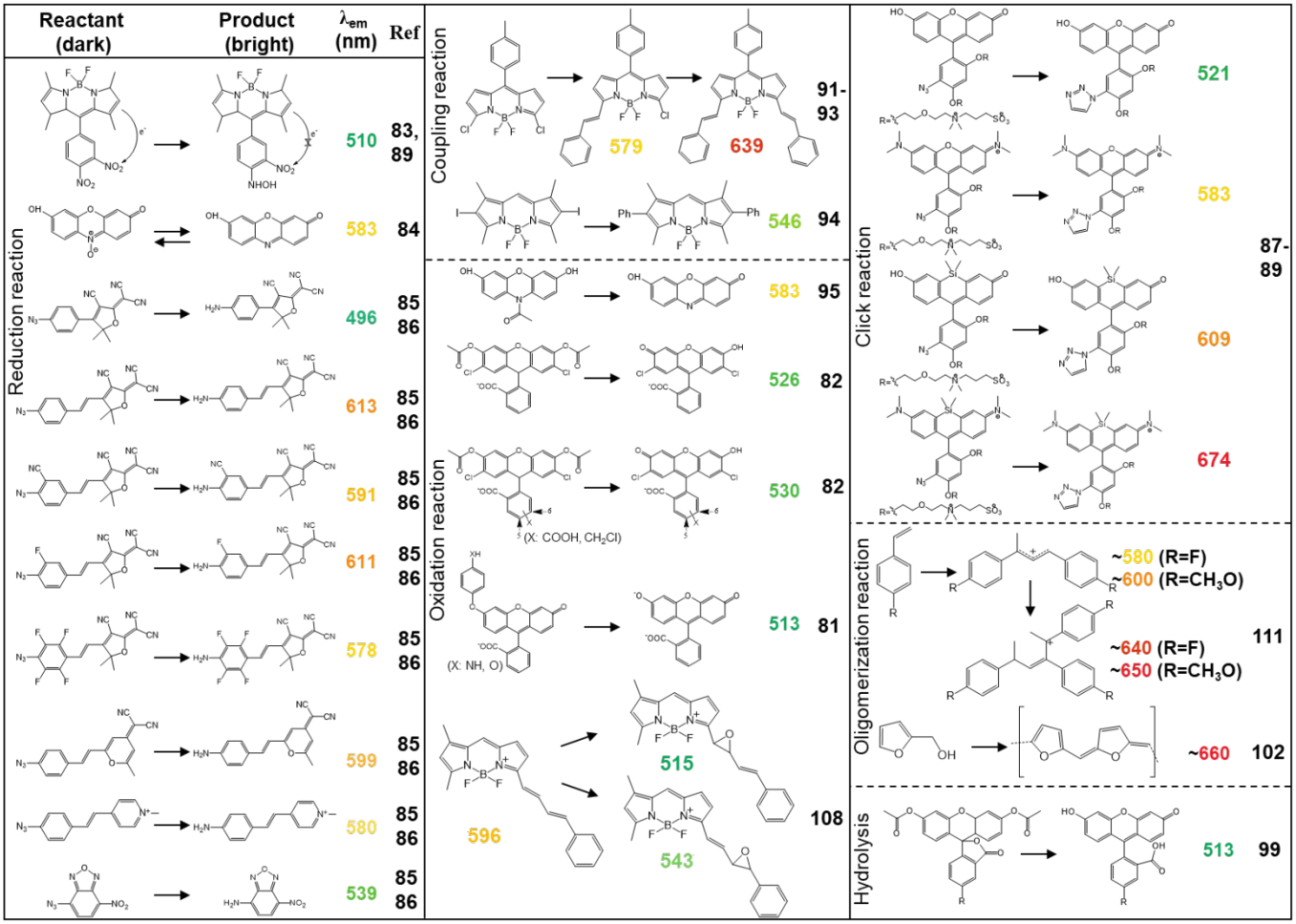

Figure 10. Fluorogenic probes for single molecule imaging of chemical reactions.

well as in acidic catalytic activities among different zeolite aggregates embedded inside real-life FCC particles with sizes of 50-150 μm.[102]

Typically, a quantitative correlation between structural features and their catalytic activity are explored to reveal the structure-property information, which can be used as the guidance for the rational design of high-performance catalysts. SMF imaging studies of these catalysts are carried out in real-time and under realistic conditions, unlike other methods such as TEM and STEM that allow the characterization at higher spatial resolution, but lack dynamic information and usually require vacuum or low-pressure conditions. Vibrational spectroscopy can measure adsorbed reactants and products on surface under realistic conditions, but lacks the single-molecule sensitivity without extraordinary enhancement. Often, a combination of results from all the aforementioned techniques is necessary to fully recover and understand the surface reactions on a single catalyst.

Deconvolute catalytic dynamics at nanoscale. Kinetic processes in a chemical reaction catalyzed on the catalyst surface involve the adsorption, diffusion, activation, chemical conversion of the reactant, and the desorption of the final product. The elementary processes that are related to the rate-limiting step(s) will determine the turnover frequency of a catalytic reaction. The physicochemical properties (e.g., structure, chemical composition, and electronic property) of active sites in single catalyst particle as well as between different catalyst particles are often heterogeneous, resulting in inhomogeneities at each kinetic step and overall catalytic reaction performance. The capability of deconvoluting these kinetic processes in a catalytic reaction and correlate them to specific structural, physicochemical properties of a catalyst is essential to establish the structure-property relationship and guide the rational design of highly efficient catalysts. SMF imaging offers such a crucial capability.

In SMF imaging of a fluorogenic reactions on a catalyst, one can obtain a typical fluorescence intensity curve over time as shown in Figure 9C with two distinguishable waiting periods: τ_{off} for the product formation, and τ_{on} for the product dissociation from the catalyst surface. Statistical analysis of these τ_{off} and τ_{on} values that collected over sufficient imaging time, by using either the arithmetic average or exponential decay fitting their distributions, gives the average waiting times $\langle\tau_{off}\rangle$ and $\langle\tau_{on}\rangle$. The inverse values, i.e., $\langle\tau_{off}\rangle^{-1}$

and $<\tau_{on}>^{-1}$, are used as the product formation rate and dissociation rate, respectively. Kinetic data of both product formation and dissociation can then be obtained by repeating such quantitative measurements under different reactant concentrations. Fitting the obtained kinetic data could resolve kinetic parameters, including adsorption/desorption equilibrium constant ($K_1 = k_1/k_{-1}$) and reaction rate constant (k_{eff}) in the individual dynamics processes in a catalytic reaction (Figure 11).

For heterogeneous catalysis on surface, the reaction kinetic data can be fitted using the classic Langmuir-Hinshelwood surface reaction model and determined the kinetic parameters K_1 and k_{eff}. [103]

$$\upsilon_r = \langle \tau_{off} \rangle^{-1} = \frac{k_{eff} K_1 [S]}{1 + K_1 [S]} \quad (7)$$

On the other hand, two possible parallel product dissociation pathways: a direct dissociation pathway (step iv) and a reactant-assisted pathway involving the participation of reactant (step ii and iii) can exist in the waiting time τ_{on}. Fitting the dissociation kinetic data with the Langmuir-Hinshelwood model can determine kinetic parameters k_2, k_4, and K_2, where $K_2 = k_3/(k_{-3} + k_4)$. [103]

$$\upsilon_d = \langle \tau_{on} \rangle^{-1} = \frac{k_4 K_2 [S] + k_2}{1 + K_2 [S]} \quad (8)$$

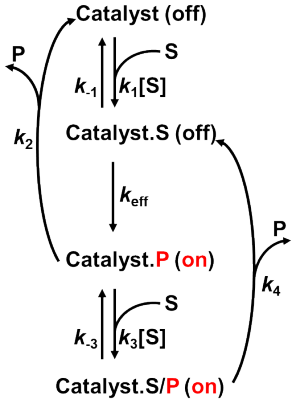

Figure 11. Reaction dynamics on catalysts. S: reactant/substrate, P: product.

Since this approach for quantitative analysis of kinetics in a catalytic reaction can be performed at the single-catalyst particle and single molecule level with turn-over temporal resolution, it enabled one to discover inhomogeneous behaviors in both catalytic activities and dissociation mechanisms among the same types of catalyst. Moreover, such quantitative analysis of dynamic processes can also be done at sub-regions on a single catalyst particle when the spatial resolution in SMLM is sufficiently higher than the physical sizes of the particle. In SMLM imaging, temporal information when the fluorescent molecule is detected is also stored accordingly with the molecular position. The super-resolved mapping enables one to differentiate subregions on a single catalyst particle and extract molecular spatial and temporal information at corresponding regions. Thus photocatalytic dynamics at isolated subregions can be quantitative measured. [98]

Unveil dynamics of catalytic reactions at nanoscale. Like enzyme,[104] the activities of solid catalysts vary temporarily. Many factors can contribute to temporal fluctuations of reaction activity on a catalyst, such as dynamic surface restructuring during chemical reactions. Inhomogeneity in fluctuation rates often exists at different surface structures, sites, as well as among individual catalysts. Such temporal dynamics and inhomogeneities are not accessible in ensemble-averaged measurements. In SMLM imaging, one can readily acquire temporal dynamics of chemical reactions by statistically analyzing the on-off times in the turn-over reaction (Figure 9C).

Weckhuysen et al. investigated the reversible proton transfer processes between two aromatic molecules (i.e., 4-methoxystyrene and 4-fluorostyrene) and Brønsted acid sites in porous materials, zeolite ZSM-5 (Figure 12).[105] The on-off fluorescence of probe molecules is caused by the conversion between its carbocationic (on) and neutral formats (off) through the proton-transfer reaction at Brønsted acid sites. The fluorescence intensity blinked differently between the two probe molecules and among individual molecules. The subsequent pairs of off-state (τ_{off}) and on-state (τ_{on}) over many individual molecules for the two probe molecules were statistically analyzed to reveal the distribution of long- and short-lived fluorescence events as well as their correlation in the time domain. The highly heterogeneous distributions suggests that the stability of fluorescent carbocationic species largely depends on their local environments. The on-off dynamics, i.e., the proton-transfer reaction rates, could be affected from changing the solvent environment, such as introducing n-heptane would cause faster protonation rates for 4-methoxystyrene; however, when using 1-butanol, an opposite effect would occur.

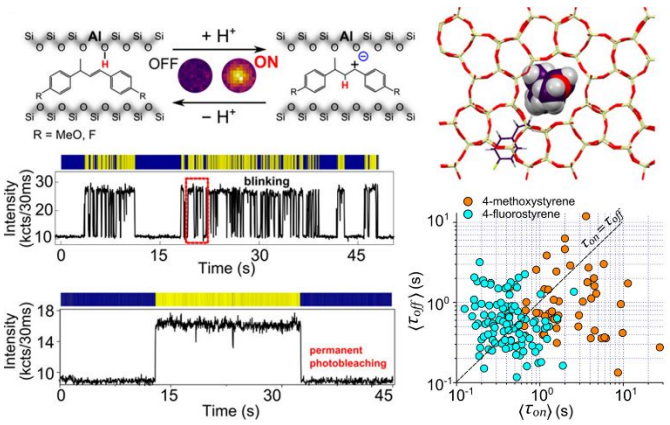

Figure 12. Single molecule fluorescence imaging of reversible proton-transfer reaction and the stability of fluorogenic probes formed from styrene oligomerization in zeolites. Figures are adapted from ref. 105 with permission. Copyright 2018 American Chemical Society.

Chen et al. studied the time dependence of the turnover rates on single gold nanoparticles by calculating the autocorrelation

function (C_t(m)).[103, 104] The decay times obtained from fitting with the exponential decay functions were used to determine the fluctuation rates of both product formation and dissociation processes. The unveiled fluctuation dynamics was attributed to small-scale surface restructuring at specific reactive sites. The results showed that the surface reconstructing rates were positively correlated to the catalytic turnover rates. A large difference in temporal fluctuation dynamics was detected between the two chemical processes (τ_{off} and τ_{on}), which indicated the docking sites where the product molecules dissociate could be different from the catalytic sites.

3.2 Effects of intraparticle mass transport

It is well known that heterogeneous catalysis is an interplay between mass transport and chemical reaction. Generally, the chemical conversion is considered as the rate-limiting step in catalysis on catalyst surfaces such as the aforementioned LDH, titanium dioxide crystal, and metal nanoparticles. The mass transport of reactant is usually much faster than the chemical reaction rate. However, mass transport of reactant cannot be ignored when catalytic reactions occur within the confined space in all types of porous materials (e.g., zeolites, metal-organic frameworks, carbon nanotubes, mesoporous particles, etc.). The accessibility of reactant molecules to the catalytic active sites confined in these porous materials are essential for optimal use of the materials. Undoubtedly, understanding the role of intraparticle mass transport in catalysts is even more important for industrial applications such as using ZSM-5 in the petroleum industry. The following are some examples of using SMF imaging to unveil the role of molecular mass transport during the catalysis in porous materials.

Mobil Composition of Matter (MCM). MCM is a type of mesoporous material composes of a hierarchical structure from a family of silicate and aluminosilicate solids, which are often used as catalysts or catalyst supports. The MCM-41 materials are composed of hexagonal array of pores with a uniform diameter that can be tuned between 1.5 and 10 nm. Ensemble measurements have shown that smaller Ti-MCM-41 particles have better catalytic selectivity and higher activity than bigger ones for the epoxidation of cyclohexene and cholesterol.[106, 107] It was believed that the intraparticle diffusion limits the full usage of Ti sites in the porous materials. Therefore, reducing the particle size will promote the optimal use of confined Ti sites in the MCM-41 particles.

Roeffaers et al. studied the effects of intraparticle diffusion on catalysis of titanium sites confined in porous MCM-41 at single-particle single molecule level (Figure 13).[108] The epoxidation of phenylbutadienyl-substituted boron dipyrromethene difluoride (PBD-BODIPY) probe molecules was used as a model reaction where a blue shift of fluorescence will be generated. Super-resolution mapping of the locations of active Ti sites in MCM-41 particles unveils that only ~300 nm region in the outer part of the particles is responsible for the catalytic reaction, thus contributing to the overall activity. Together with control experiments and material characterization of particles, the SMF imaging results uncover the fact that only in a sub-micrometer fraction of particles would the Ti sites be fully utilized for catalysis because of the limitation of intraparticle molecule transport.

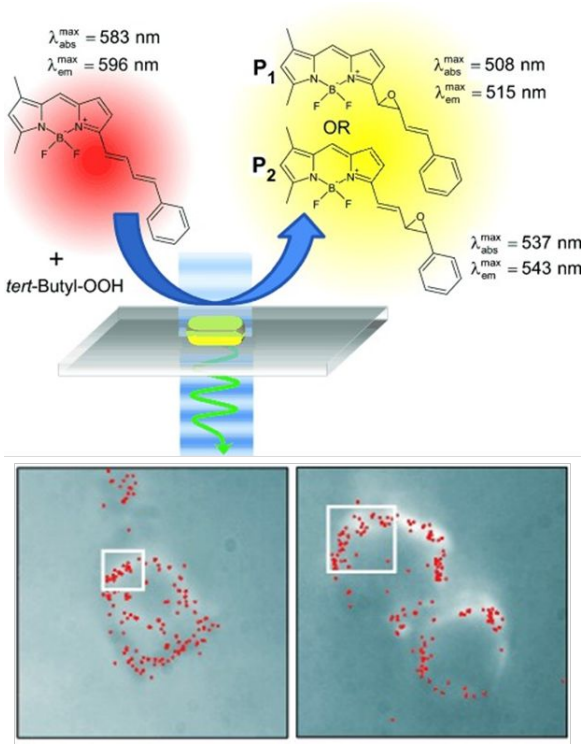

Figure 13. Single-particle single molecule imaging of the epoxidation of PBD-BODIPY in Ti-MCM-41 particles. Schematic view of the epoxidation of PBD-BODIPY in Ti-MCM-41 particles and two examples of individual turnover measurements on Ti-MCM-41 particles. Figures are adapted from ref. 108 with permission. Copyright 2010 Wiley-VCH.

Zeolites. Hofkens et al. investigated the catalytic reaction of furfuryl alcohol at acidic sites in zeolite socony mobil-5 (ZSM-5) to generate a highly fluorescent product.[109] ZSM-5 is widely used in the petroleum industry as a heterogeneous catalyst for hydrocarbon isomerization reactions. ZSM-5 has porous structures of medium pore sizes of ~0.5 nm. It contains two intersecting three-dimensional channels, including straight parallel pores and sinusoidal pores of 10-membered ring. Probed by the combination of focused-ion-beam (FIB), transmission electron microscopy, and electron diffraction pattern analysis, two zones with different crystallographic orientations of 90° intergrowth had been observed (Figure 14A). Using SMF imaging, they reconstructed a super-resolution mapping of activities in the ZSM-5 crystal. A highly active region of 20-60 nm wide at the boundary of the 90° intergrowth was uncovered (Figure 14B). The enhanced catalytic activity in this zone can be explained by the fact that locally there is reagent influx from two crystal faces via both the sinusoidal and the straight pores, rather than the difference in activity of local acidic sites. These heterogeneous catalytic activities at nanoscale are hidden in traditional ensemble measurement. They also used single molecule fluorescent imaging to investigate the effects of steaming on H-ZSM-5 crystal on the

catalysis efficiency (Figure 14C).[110] They found out that mild steaming of H-ZSM-5 crystals at 500 °C (H-ZSM-5-MT) notably increases the catalytic activity by enhancing the accessibility of acidic sites through altering the porosity via dealumination. The steaming also causes a highly heterogeneous distribution of accessible acid sites at the macroscopic level. However, a significant loss of acidic sites and much lower catalytic activity were observed if steaming at higher temperature (700 °C, H-ZSM-5-ST). Later, they also discovered that the catalytic activity of the oligomerization of styrene derivatives by H-ZSM-5 crystal largely depends on the local polarity and structure (pore types and sizes) of confined space, as well as the chemical property (moiety) of the reactant molecules using SMF microscopy imaging (Figure 14D).[111]

In another example, Roeffaers et al. studied the effects of hierarchical porous structure on the catalytic activity of dealuminated mordenite zeolites using SMF microscopy imaging.[112] The acid-catalyzed condensation reaction of furfuryl alcohol was used as the model reaction. It had been believed intracrystalline diffusion limitations could be overcome by introducing larger extraframework meso- and macro-porosity into the solid catalyst, which enhances the effectiveness of porous catalysts. However, the effects of the introduced extraframework pore structures on local nanoscale catalytic activities were not clear. The SMF imaging experiments show that catalytic turnovers mostly happen within aligned micropores while reaction activity in extraframework pores is much lower. Though the intraparticle diffusion can be overcome by the introduction of extraframework pores, it also causes the loss of original active sites at the location of the extraframework pores, thus reducing the optimal use of all catalytic sites inside the porous particles.

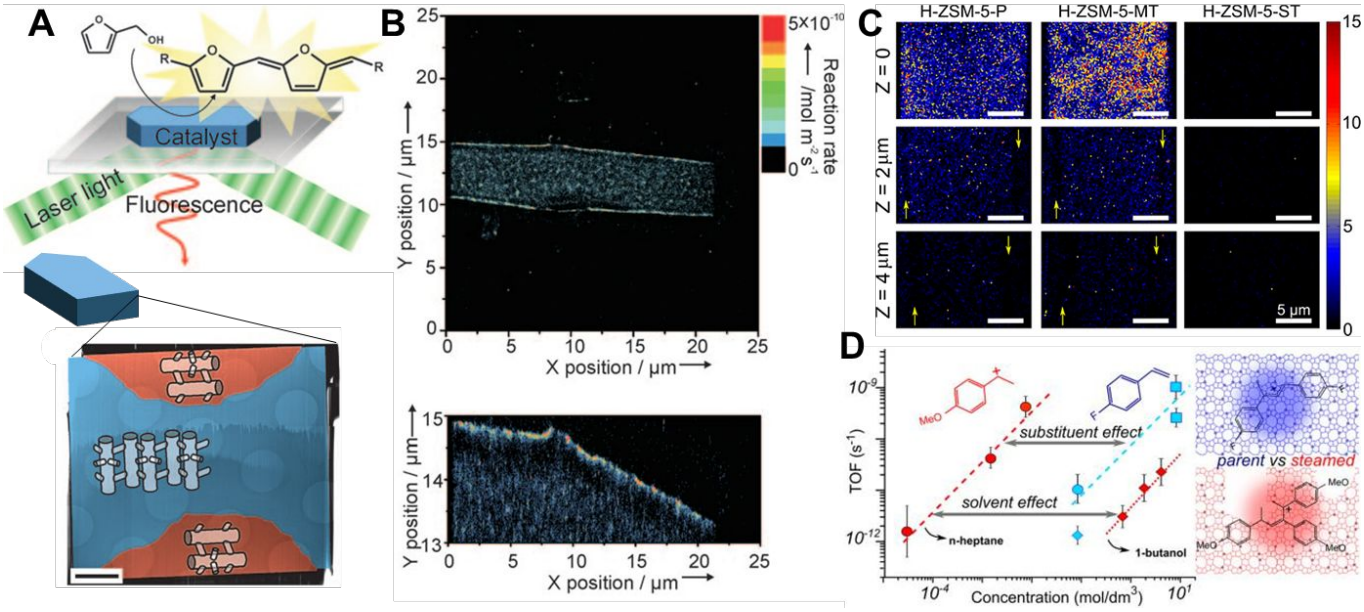

Figure 14. SMF imaging of heterogeneous catalysis in ZSM-5 zeolites. (A) Schematic view of acidic catalyzed reaction of furfural alcohol polymerization (top) and crystal orientation indicated by color and a schematic representation of the channel system from FIB–TEM characterization. (B) Super-resolution mapping of acidic sites and activities in ZSM-5. Figures (A, B) are adapted from ref. 109 with permission. Copyright 2009 Wiley-VCH. (C) 3D super-resolution mapping of acidic sites and activities in ZSM-5 after steaming treatment where H-ZSM-5-P, H-ZSM-5-MT, H-ZSM-5-ST represent parent, 500 °C, and 700 °C treated ZSM-5 respectively. Color bar is turnover rates per 200 × 200 nm². Figures are adapted from ref. 110 with permission. Copyright 2015 American Chemical Society. (D) Effects of pore physicochemical properties on acid-catalyzed reaction in ZSM-5. Figures are adapted from ref. 111 with permission. Copyright 2016 American Chemical Society.

Ruthenium catalyst complex. Blum et al. studied the polymerization reactions in porous ruthenium (Ru) catalyst complex that composes of numerous individual living polymer strands associated with ruthenium catalyst and untagged norbornene (Figure 15).[113] The catalytic active center of Ru catalysts were embedded inside the porous complex. Two types of Ru-catalyzed polymerization reactions were investigated, including chain elongation reaction and chain terminalization reaction. These two polymerization reactions were imaged at the single molecule level with turn-over resolution simultaneously using two different reagent molecules tagged with fluorescent probes of different fluorescent emission spectra. The super-resolved spatial and temporal results of the two catalyzed polymerization reactions showed different dynamic responses, reflecting the different effects of the local catalyst microenvironments on reaction dynamics. Considering the continuously catalyzed polymerization reactions surrounding the Ru active center would increase the local polymer density, induce conformational changes, and reduce the accessibility of reagents. Therefore, possible explanations for the different spatial-temporal variations of catalytic activities of investigated chain elongation and

termination reactions are attributed to diffusion differences between the two reagents since their chemical structures and molecular sizes are very different.

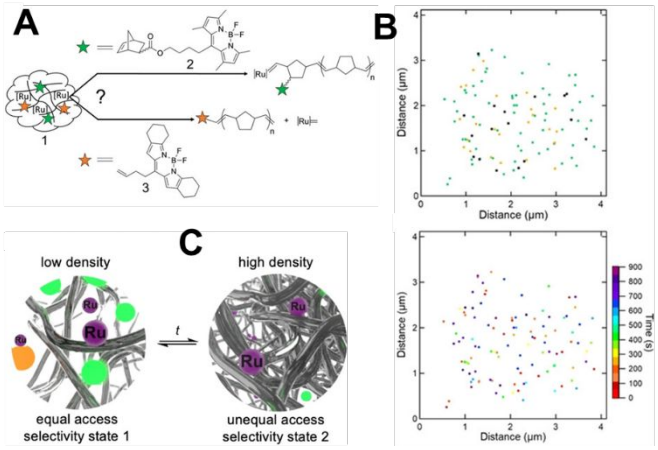

Figure 15. SMF imaging of polymerization reaction in Ru-complex. (A) Schematic for two different polymerization reaction pathways: single chain-elongation and -termination. (B) Super-resolution mapping image of single chain-elongation (green) and -termination (orange) events observed at a single polymer-particle aggregate, with events not identified with statistical confidence (top) and of all single-turnover events, where the color of data points indicates reaction time. (C) Hypothesized physical model for selectivity changes. Figures are adapted from ref. 113 with permission. Copyright 2020 Wiley-VCH.

3.3 Reaction dynamics of heterogeneous catalysis under nanoconfinement

To further understand the nanoconfinement effects on the dynamics of heterogeneous catalysis on active centers in porous materials, a well-defined catalyst system is needed. Ideally, a single reaction center in a well-defined and highly tunable nanoconfinement structure should be created for single molecule catalysis investigation. In such configuration, effects of the properties of nanoconfinement including structural features, physicochemical properties, and electrostatic forces on chemical reaction dynamics including mass transport, adsorption/desorption, chemical conversion of reactants and diffusion, desorption of products can be systematically investigated at single molecule level.

Platinum nanoparticles in mesoporous silica. Though, lots of single molecule imaging porous materials (e.g., thin films, porous particles) have been investigated to understand the physicochemical properties of the pore environment on mass transport. However, simultaneously measurements of the mass transport and reaction dynamics in nanopores inside the complicated porous materials have been have very challenging. To overcome this obstacle, we took the advantage of a well-defined core-shell catalyst platform where the catalytic centers (metal nanoparticles) are confined at the end of nanopores with controlled lengths in mesoporous silica dioxide ($mSiO_2$) shell (Figure 16). [114] We studied the dynamic behavior of catalytic processes under nanoconfinement including mass transport and catalytic reactions at the single molecule and single particle level in situ.

Confining catalytic active centers in mesoporous silica has been a very useful strategy for designing high-performance catalysts. Many benefits can be obtained through the confined structure. For example, encapsulating nanoparticles in mesoporous silica materials can stabilize the particle morphology, avoid the aggregation of particles during the removal of surfactant ligands. Moreover, mesoporous silica is essentially the same material like glass, making it transparent to create a clean background for fluorescence imaging. Furthermore, this catalyst structure provides a restricted pathway for reactant molecules in the bulk solution to diffuse a uniform distance, defined by the thickness of the $mSiO_2$ shell, to access the active sites on confined platinum NPs placed at the bottom of the nanopores. In this well-defined catalyst platform, it has two obvious benefits. First, one can confidently track the one-dimensional mass transport of fluorescent molecules in the linear nanopores. Local environments, including pore structures and viscosities, can be quantitatively evaluated. Second, the single molecular trajectory analysis provides accurate measurement of molecular diffusion in nanopores under reaction conditions, which then enables further analysis to decouple the influence of molecular transport and reaction kinetics. Quantitative measurement of molecular diffusion in nanopores using single molecule tracking experiments shows ~10^4 times slower mass transport in nanopores comparing to that in bulk condition.

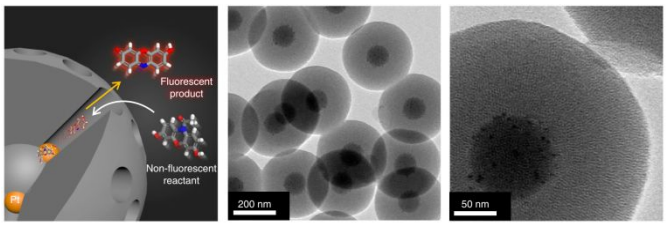

Figure 16. Multi-layer core-shell porous particle as a model platform for simultaneously studying mass transport and heterogeneous surface catalysis. Figures are adapted from ref. 114 with permission. Copyright 2018 Nature Publishing Group.

In this specific study, Platinum nanoparticles (Pt NPs) catalyzed oxidation reaction of amplex red was used as the model reaction to evaluate nanoconfinement effects. Under 0.02 μM of amplex red, the amount of amplex red molecules that approaches the Pt NPs in ~2 nm nanopore was estimated to be 4 × 10^{-26} mol s^{-1}, while the experimental measured single-particle catalytic activity is 3 × 10^{-26} mol s^{-1}. Therefore, the catalytic reaction rates are limited by the mass transport at the low concentrations of amplex red. However, the effect of mass transport on catalytic reaction rates is not significant at higher concentrations. A modified kinetic model taking into account the mass transport factor was established to accurately fit the kinetic data and determine kinetic parameters (e.g.,

adsorption/desorption equilibrium constant K_{AR} and reaction rate constant k_{eff}) under nanoconfinement. On the contrary, mass transport of reactant is negligible when no mesoporous shell presents around Pt NPs. The kinetic data can then be directly fitted by the Langmuir-Hinshelwood surface reaction model to determine K_{AR}, k_{eff}. From the single particle single molecule kinetic data, measured k_{eff} is around seven times higher and K_{AR} is around two times smaller for Pt NPs under nanoconfinement. Possible reasons, including effective concentrations and physical constrain of adsorbed substrate molecules, are used to explain the observed catalytic reaction dynamics under nanoconfinement.

Gold nanoparticles in carbon nanotubes. Carbon nanotubes (CNTs) have been widely used as the supporting materials for metal particle catalysts because of their well-defined hollow interiors, high surface area, unusual mechanical properties, and thermal stability. Metal particles as catalytic active centers have distinct activities when loaded inside the CNTs hollow structure comparing to those supported on the external CNTs surface.[72] When metal particles are loaded inside CNTs, nanoconfinement effect presents.

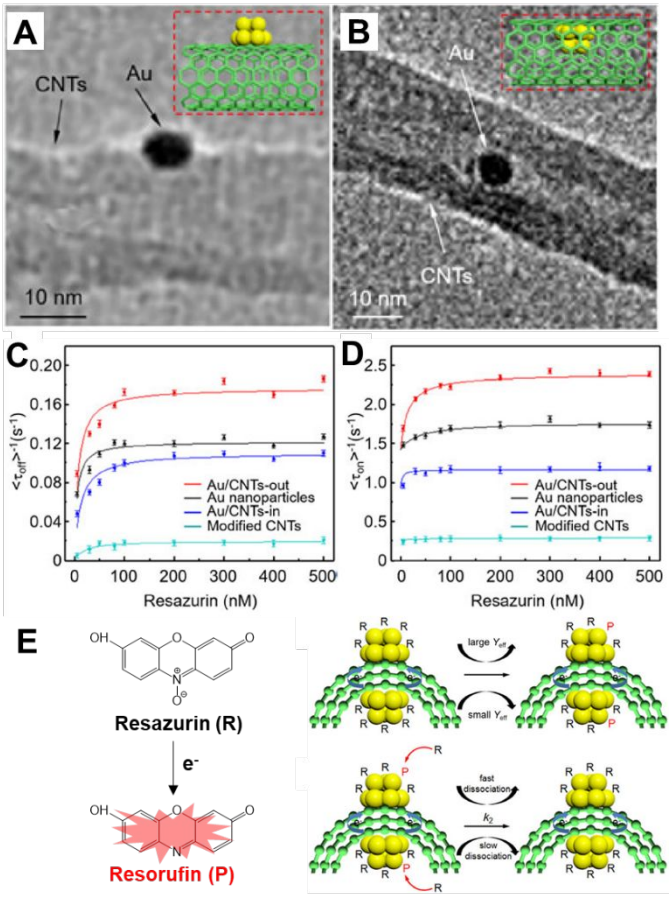

Figure 17. SMF imaging of support-effects of gold nanoparticles on carbon nanotubes. TEM images showing gold nanoparticle outside (A, Au/CNTs-out) and inside (B, Au/CNTs-in) carbon nanotubes. Catalytic reduction reaction kinetics of resazurin (C) and dissociation kinetics of resorufin (D) in four nanocatalysts. (E) Proposed kinetic mechanisms of product formation process and product dissociation process on Au/CNTs-out and Au/CNTs-in. Figures are adapted from ref. 115 with permission. Copyright 2018 Wiley-VCH.

To understand the difference in catalytic properties of gold nanoparticles at the two types of loading sites, Kang et al. synthesized Au nanoparticles deposited on the outer surface (Au/CNTs-out) and inner surface (Au/CNTs-in) of CNTs and monitored the reduction reaction of resazurin using single molecule fluorescent imaging (Figure 17).[115] Both reaction kinetics of reactant resazurin ($<\tau_{off}>^{-1}$) and dissociation kinetics of product resorufin ($<\tau_{on}>^{-1}$) for four types of catalyst, i.e., Au/CNTs-out, Au/CNTs-in, Au nanoparticles, and CNTs, were quantitatively determined at single molecule level with turn-over resolution. Fitting the reaction kinetics gives the highest catalytic activity (k_{eff}) for Au/CNTs-out. Au/CNTs-out also has the largest adsorption strength (K_1) of resazurin. For product dissociation, the same dissociation mechanisms are discovered for all four catalysts. The reactant assisted dissociation pathway (k_2) is faster than the direction dissociation pathway (k_3). Both k_2 and k_3 are larger for Au/CNTs-out, suggesting weaker binding strength of product resorufin than that for Au/CNTs-in. By comparing to unsupported gold nanoparticles, the experimental results show that gold nanoparticles confined inside CNTs have lower catalytic activity, weaker adsorption strength of reactant molecule, and stronger binding strength of product molecule. On the other hand, the opposite trend was observed for gold nanoparticles supported on the external surface of CNTs. Kang et al. attributed this opposite trend to a higher density of electrons on the external surface of CNTs as electrons would shift from the inner to the outer surface of CNTs due to their curved walls. The more electron-rich environment for gold nanoparticles on the external surface of CNTs would facilitate the reduction reaction of resazurin.

3.4 Effects of pore morphology

Using the well-defined core-shell nanocatalyst platform (Figure 16), we studied nanoconfinement effects on the catalytic reaction dynamics under variable nanopore morphologies, including pore length and diameters at the single-molecule and single-particle level.[116] The nanoconfinement effects on catalytic reaction kinetics (i.e., molecular adsorption strength and reaction rate) were discovered to be nanopore morphology dependent. Intuitively, one would expect a decreasing activity when catalytic center (Pt) is blocked from reactant molecules by obstacle objects such as mesoporous silica shell in this work. Moreover, one would also expect the catalytic activity reduction due to the blocking effects from $mSiO_2$ shell would be stronger when the shell gets thicker and the nanopore gets narrower. However, we observed that catalytic reaction activity (k_{eff}) increases, rather than decrease, when shell become thicker (0-120 nm) and nanopores changes from ~3 to 2 nm (Figure 18). The ensemble measurements of catalytic activities of the same core-shell particles also show the same results. On the other hand, the nanoconfinement on molecular adsorption strength (K_{AR}) is only sensitive to nanopore diameter where weaker adsorption strength presents inside nanopore of smaller diameter due to the less motion freedom in the narrower pore. Furthermore,

experimental results also show that the activation energy (E_a) for the oxidation of amplex red catalyzed by Pt NPs in 2.2 and 3.3 nm nanopore is reduced but independent of pore length. The reduced activation energy agrees with the enhanced catalytic activity under nanoconfinement.

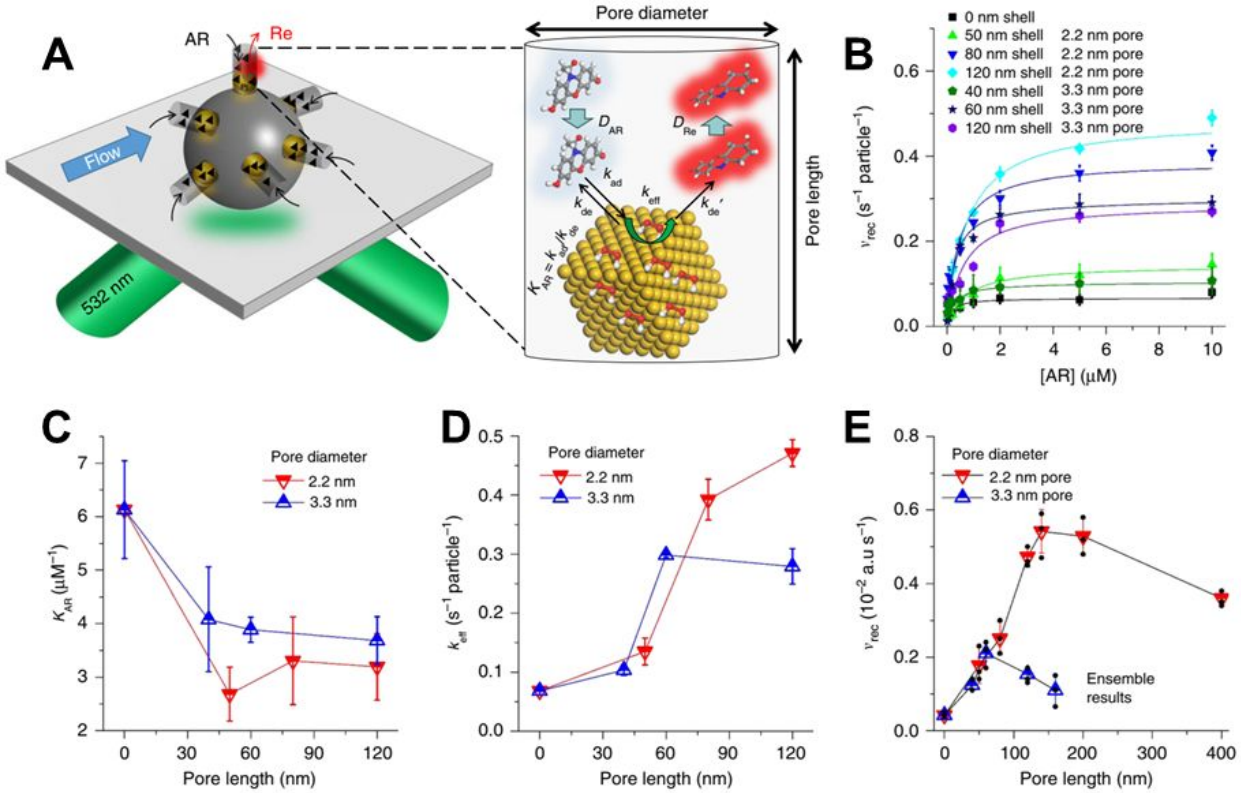

Figure 18. Effects of nanopore morphologies on reaction kinetics in nanopores. (A) Schematic of single-particle single molecule imaging setup (left) and chemical conversion processes inside nanopore (right). (B) Reaction kinetics for different nanopore lengths and diameters at single-particle single molecule level. Adsorption/desorption equilibrium constant K_{AR} (C) and rate constant k_{eff} (D) are obtained from fitting the single-particle single molecule kinetics data. (E) Ensemble results of reaction rates of nanocatalysts with variable porous shell thickness and pore diameter. Figures are adapted from ref. 116 with permission. Copyright 2019 Nature Publishing Group.

3.5 Effects of chemical environment on catalytic dynamics

The surface properties of porous materials play pivotal roles for their applications in separation, drug delivery, and catalysis. In catalysis science, modifying porous silica materials by grafting the silica surface with acid, base, or other organic functional groups could induce new or enhanced reactivity in chemical reactions. For example, organosulfonic acid-functionalized mesoporous silica materials have been used in the esterification of fatty acid for biodiesel production.[117] For chemical reactions that involve hydrophobic reactants and/or hydrophilic products, modifying the pore surface with hydrophobic functional groups could enrich reactants within the pores and repel products from the pores, and thus enhance the reaction rate.

Using SMF imaging, we revealed the effects of environmental hydrophobicity on molecular dynamics during the Pt-catalyzed oxidation of hydrophobic amplex red in ~3 nm silica nanopores (Figure 19).[118] The silica nanopore surface was functionalized with -SO_3H or -CF_3, creating hydrophilic or hydrophobic environments. Higher catalytic activity, stronger adsorption strength, and higher activation energy were unveiled in hydrophobic nanopores as compared to that in hydrophilic nanopores. The seemingly counterintuitive results between higher catalytic activity and higher activation energy in nanopores are due to the confinement effects on trapping intermediate species. The local enrichment of the intermediate amplex red (AR) radicals in the hydrophobic nanopores is more significant than that in the hydrophilic nanopores, which is supported by evidence from both single molecule tracking and incubating resorufin (Re) with core-shell mesoporous particles where lower mass transport rates and stronger trapping capabilities of Re in hydrophobic nanopores than that in the hydrophilic nanopores were observed. Moreover, the product molecules resorufin also have very different dissociation kinetic behaviors in the two types of nanopores (Figure 19c). At low concentration of AR, the direct dissociation pathway dominates. Smaller dissociation and mass transport rates are smaller in the hydrophobic nanopores, which suggests its strong confinement effects for trapping the molecules.

At high concentration of AR, the reactant assisted dissociation mechanism becomes the dominant dissociation pathway and the interplay and competition between reactant molecule AR and product molecule Re is important for their dynamic behaviors. Larger dissociation rate in the hydrophobic nanopores was measured, which can be explained by the stronger confinement effects of hydrophobic nanopores and much higher concentrations of reactant molecule AR. Furthermore, the faster dissociation rate of product molecule Re can also speed up the catalytic reactions.

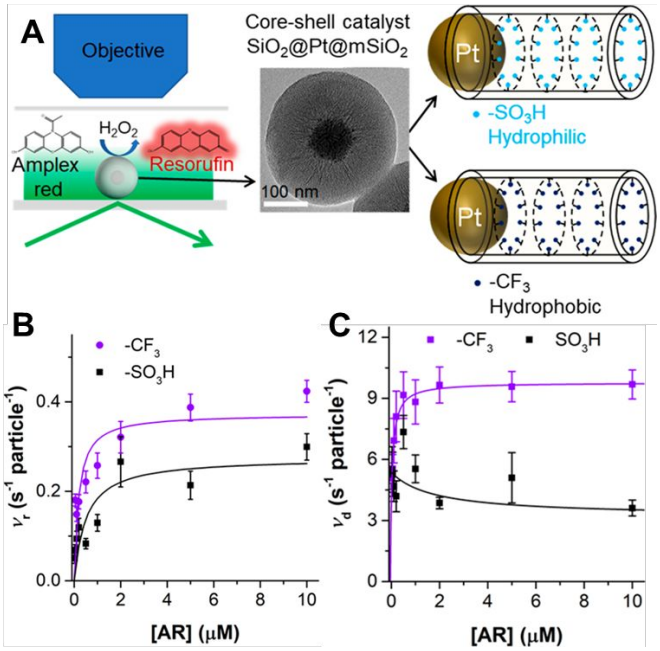

Figure 19. Single molecule investigation of nanoconfinement hydrophobicity in heterogeneous catalysis. (A) Schematic view of the imaging experiments and high-resolution TEM images of core-shell nanocatalysts. The pore surface in the mesoporous shell was modified to be hydrophilic using -SO_3H functional groups or hydrophobic using -CF_3 functional groups. Oxidation reaction kinetics of amplex red (B) and dissociation kinetics of resorufin (C) on Pt NPs in single core-shell nanocatalysts. Figures are adapted from ref. 118 with permission. Copyright 2020 American Chemical Society.

3.6 Decipher nanoconfinement effects by single molecule imaging

Restricted molecular orientation reduces adsorption strength in nanopore. The orientation of molecules will be restricted in nanopore when molecule size and pore diameter are comparable. In combination with the well-defined nanocatalyst platform and single molecule polarization fluorescence microscopy imaging, we showed that the confined molecular orientation was the reason for the reduced adsorption strength in nanopore (Figure 18).[116] For asymmetric aromatic molecules like amplex red and resorufin, a preferred molecular orientation where the long axis of molecule aligns perpendicular to Pt NPs surface and parallel with the nanopore would be expected. Also, the resorufin molecule has absorption and emission dipole moments (μ) along the long axis of its planar structure. Using linear polarized excitation light source, only resorufin molecules with their absorption dipole moments parallel to the illumination light polarization direction would show strong fluorescence. Suppose the long axis of resorufin molecules are aligned parallel with the nanopore. In that case, more resorufin molecules will be excited when the nanopore is aligned parallel with the excitation light's polarization direction. On the contrary, if the long axis of resorufin molecules is randomly oriented in the pore, polarized light will excite resorufin regardless of the orientation of the nanopore with the polarization direction of the excitation light. An elliptical distribution of resorufin molecules was observed with linearly polarized excitation light (Figure 20D), while such asymmetrical distribution pattern disappeared when switching to a circularly polarized excitation light (Figure 20E). Moreover, more product molecules were detected under circularly polarized excitation light. The super-resolution imaging results here suggest the parallel alignment of resorufin molecules with nanopore. The preferential orientations of amplex red and resorufin molecules in nanopores should be same since their molecular structures are similar. Without the confinement of the nanopore, aromatic molecules typically adsorb strongly on a precious metal surface in laying down configuration due to the preferred interaction of aromatic pi-orbital with metal's d-orbital. Therefore, the restricted molecular orientation of amplex red in nanopore would prevent the laying down adsorption configuration, resulting in reduced adsorption strength.

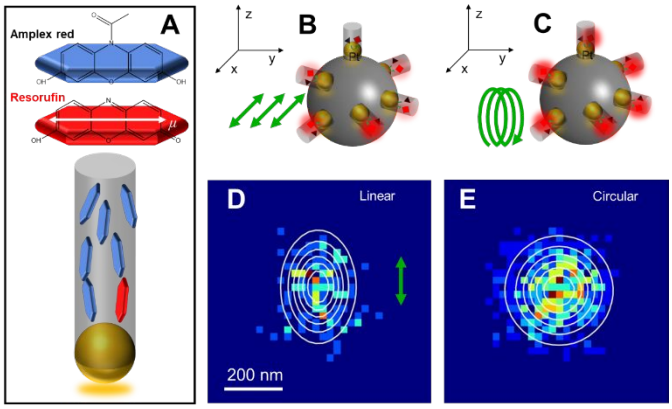

Figure 20. Unveil molecular orientations in nanopore using SMF polarization microscopy. (A) Dipole moments of AR, Re molecules and schematic view of molecular arrangement inside nanopore. Single-particle single molecule imaging experiments under linearly polarized excitation (s-pol, B, D) and circularly polarized excitation (c-pol, C, E). Figures are adapted from ref. 116 with permission. Copyright 2019 Nature Publishing Group.

Enrichment of reaction intermediate enhances the overall catalytic activity. The restricted molecular orientation of amplex red where the phenol group directly faces toward the surface of Pt NPs in nanopore can facilitate the catalytic reaction by the probability of the phenol group reacting with reactive oxygen species (ROS), e.g., chemisorbed oxygen on Pt surface.[119] This is in agreement with and

also evident by the measured lower activation energy under nanoconfinement. Nonetheless, the reduced E_a under the nanoconfinement effects still does not explain the dependence of the activity enhancement on nanopore length, i.e., catalytic activity increases when nanopore lengths increases (0-120 nm, Figure 18D). We attribute this phenomenon to the enrichment of reaction intermediate species in nanopores. For the oxidation of amplex red on Pt NPs, it involves three sequential chemical conversion steps, including forming amplex red radicals ($AR^{\bullet}$), the following up disproportionation reaction of two $AR^{\bullet}$ to produce one AR molecule and AR^{+} cation, and finally the hydrolysis of AR^{+} to produce resorufin (Figure 21A). With no mesoporous shell, the intermediate $AR^{\bullet}$ could dissociate from Pt NPs and diffuse into the bulk solution before encountering another $AR^{\bullet}$ to go through the disproportionation reaction. On the contrary, the dissociated $AR^{\bullet}$ would be temporarily trapped in nanopores inside the mesoporous shell, thus resulting high local concentrations of $AR^{\bullet}$ and fast reaction rate to form fluorescent product Re.

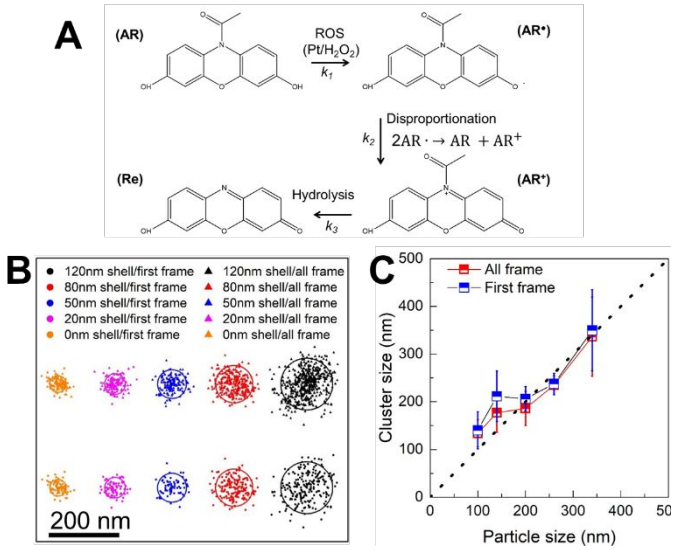

Figure 21. Cluster analysis of Re positions with and without mesoporous shell. (A) Reaction mechanism of the oxidation of amplex red to resorufin. (B) Typical cluster distributions of molecular positions of Re when first detected and during their whole lifetime (all frame) inside nanopore at different pore lengths. The solid line represents the overall average diameter of core-shell nanoparticles. (C) Average cluster sizes from multiple core-shell nanoparticles versus the overall diameter of core-shell nanoparticles. Figures are adapted from ref. 116 with permission. Copyright 2019 Nature Publishing Group.

Super-resolution mapping of the locations, where Re molecules were generated in mesoporous materials of variable pore lengths, was used to experimentally verify the proposed nanoconfinement effect of increasing local concentration of intermediate species $AR^{\bullet}$ in nanopores. Two super-resolution images were reconstructed (Figure 21B): mapping of molecular positions where resorufin first formed (down panel, symbol: ●) and through the whole resident time (up panel, symbol: ▲). As shown in the mapping results, both super-resolution images show similar cluster sizes at the same nanopore length. The cluster size matches very well with the physical size of the core-shell particle at all pore lengths (Figure 21C). Based on these results, we conclude that Re molecules are formed anywhere within the nanopores, rather than just formed on/near the Pt NPs. This can only be explained by the fact that $AR^{\bullet}$ were indeed trapped inside nanopore after dissociation from Pt NPs surface. Considering the size of core-shell particles are at nanoscale within the diffraction-limit, the nanometer spatial resolution and turn-over temporal resolution in SMLM are essential to resolve the nanoscale processes involving the intermediate species. Nonetheless, we also discover that the confinement-induced enhancement of catalytic activity will eventually be canceled out, and the catalytic activity will be dominated by the mass transport of reactant molecules.

4. Prospects and limitations

Significant progress has been made, and exciting results have been obtained in understanding the nanoconfinement effects in porous materials using SMF imaging. Yet, limitations still exist, and challenges are to be overcome.

Fluorescent probe molecules are usually several nanometers in size. Nanoconfinement with smaller physical sizes such as micropores (< 2 nm) in metal-organic frameworks (MOFs) and interlayer spacing in two-dimensional materials (< 1 nm)[120] are not easily accessible. Therefore, chemical dynamics in these types of small nanoconfinement cannot yet be investigated by SMF imaging. Understanding of these types of nanoconfinement is essential for modulating chemistry in small space and rationally design advanced materials for varieties of applications such as separation, heterogeneous catalysis, energy conversion, and energy storage. On the other hand, SMF imaging of heterogeneous catalysis under nanoconfinement relies on detecting fluorescent product molecules generated in fluorogenic reactions. Many fluorogenic probes have been reported, but the types of chemical reactions or reaction mechanisms that can be studied are still limited. Designing and synthesizing fluorogenic probes that can be correlated to important chemical transformations such as water splitting, oxygen reduction, carbon dioxide reduction, and small alcohol formation are worthy of effort. Furthermore, intermediate species in currently available fluorogenic reactions are commonly nonfluorescent, which makes them invisible in fluorescence microscopy. However, these intermediate species are essential for understanding the heterogeneous catalysis under nanoconfinement. For example, tandem catalysis where cascade chemical transformations occur in sequence to generate the desired products in one reactor without the need for separation, purification, and transfer of intermediates produced in each step can be realized with a multifunctional catalyst. Being able to directly monitor each step of chemical transformations, localize where it happens, and determine corresponding kinetics enables one to understand the cause of the better performance of certain catalyst configurations, and in turn will largely help in

designing highly efficient multifunctional catalysts. One possible strategy would be the design and synthesis of fluorogenic probes that give different fluorescence emission spectra at each step of chemical transformations in tandem catalysis.

The information acquired from SMF imaging on molecular dynamics at the catalyst surface can still be quite limited. Vibrational spectroscopy, such as Raman spectroscopy[121] and attenuated total reflectance Fourier-transform infrared (ATR-FTIR) spectroscopy[122], is a highly valuable companion to probe the vibrational states of molecules at the interface. Rich molecular fingerprint information from vibrational spectroscopy can reveal the interactions between the catalyst and molecules and the bond breaking/formation in the chemical transformations. Besides molecular dynamics, the structural and electronic properties of the catalyst also significantly affect the catalytic dynamics. For instance, the adsorbate-surface interactions during chemical reactions can induce dynamic surface reconstruction that can vary the surface electronic properties, and in turn, cause oscillatory chemical dynamics. Spectroscopy, such as Raman and photoluminescence (PL) spectroscopies, can also be used to monitor the evolution of lattice vibration, electronic band structures and electron density during the catalytic reaction. However, spectroscopic methods still have compatibility issues with the SMF imaging. On the one hand, spectroscopic methods often lack the sensitivity and temporal resolution to monitoring single molecules during a chemical reaction. The ensemble spectroscopic measurement can only provide limited understanding of molecular behaviors. On the other hand, most spectroscopic methods have insufficient spatial resolution to correlate the molecular behaviors or catalyst properties with heterogeneous chemical dynamics obtained by SMF imaging. Tip-enhanced spectroscopies, such as tip-enhanced Raman spectroscopy (TERS),[123-125] tip-enhanced photoluminescence spectroscopy (TEPL),[126, 127] and nanoscale infrared spectroscopy (Nano IR),[128, 129] have high spatial resolution down to 10 nm[130] in air and sub-nanometer[131] under UHV. They are mostly useful in revealing the molecular behaviors at the surface structures, such as island[132] and edge.[133, 134] However, the use of tips (tens of nanometer) in tip-enhanced spectroscopies limits the study of molecule behaviors inside the porous materials. Hence these techniques can only characterize the molecule confined at the exposure catalyst surface (e.g., defect) but lack the ability to reveal the molecular behaviors in a cavity. Moreover, the much longer acquisition time makes it impractical to monitor catalytic reactions in real time at the nanoscale. Although it is still challenging to combine spectroscopy with SMF imaging where merits from both methods can be obtained, continued efforts, such as the improvement of temporal spatial resolution and sensitivity and the development of new spectroscopic methodology, are worthy of devotion.

Statistical analysis of single molecule chemical dynamics under nanoconfinement enables one to quantify the heterogeneous mass transport, chemical reaction kinetic, and product dissociation kinetics. Both high localization precision and fast temporary resolution are critical. Despite efforts and progress made in this field, it is still challenging to acquire both merits in SMF imaging due to the following inherent limitations. (1) The total number of collectable photons is finite, resulting in a shot noise-limited localization precision. (2) Low signal makes the image quality susceptible to interfering background, which is frequently encountered in real-world systems, such as impurities in polymer substrates, Rayleigh and Raman scattering from porous nanoparticles/microparticles, etc. (3) In order to obtain additional information such as 3D spatial position (e.g., using PSF engineering),[135-138] increased time resolution, spectroscopic intensity, etc., the limited number of photon signals will be distributed to different channels, which worsens these two problems (low S/N and high background). So far, typical 2D or 3D localization methods (such as astigmatism[139], double-helix[140], defocused[141], parallax[142, 143], etc.) are ranging from non-linear least squares (NLLS) fitting, simple correlation coefficient (CC) method,[144] to supervised machine learning algorithms (such as maximum likelihood estimation and Bayesian parameter estimation).[145-148] Several strategies can be used to improve the spatial and temporal resolution in SMF imaging. Deep neural networks (DNNs) have been well recognized for pattern recognition, which abstracts the features from the images at multiple levels by applying various filter matrices for convolution.[149, 150] DNNs can be trained to estimate the background patterns,[151] then enables one to remove interference background like that from porous materials thus obtain higher localization precision in determining the molecule positions. Another recently developed optical imaging method developed by Hell et al.,[152] namely minimal photon fluxes (MINFLUX), are very promising to bring new insights for understanding nanoconfinement at higher spatiotemporal resolution (~ 1 nm localization precision and > 100 times faster).

Porous materials such as metal-organic frameworks (MOFs) and covalent organic frameworks (COFs) have well-defined uniform pore structures but have rarely been studied using SMF imaging. The MOFs can be used to down reach the microporous scale (< 2 nm) in comparison to the industry standard of zeolite catalysts. COFs are recently developed materials with either two-dimensionally aligned or three-dimensional mesopores. The presence of organic linkers in MOFs and COFs is also beneficial to control the chemical environment of the pores, similar to the functionalization of silica pores but in a more ordered fashion due to the crystalline nature of MOFs and COFs. The advantages of MOFs and COFs are apparent: (i) versatile and exquisite structural design can be achieved by the judicious selection of preferred topologies, guided by the principles of reticular chemistry,[153, 154] (ii) exceptional porosity and highly tunable pore sizes have given rise to an extensive library of crystalline structures,[155, 156] (iii) multivariate functionalization allows for the incorporation of multiple metals and/or organic ligands into the MOF/COF backbone while preserving their structural integrity,[1, 157, 158] and (iv) post-synthetic modifications of pre-formed MOFs/COFs have proven to be a potent tool for engineering the environment of the interior pores, as well as modulating the stereoelectronic character of the active sites.[159-161] These distinct features mentioned above have undoubtedly contributed to the extensive study of

MOFs/COFs in a plethora of applications in areas ranging from gas storage and separation,[162-167] chemical sensors,[168, 169] drug delivery,[13, 170] catalysis,[171-173] energy transfer and migration,[174] to energy storage devices.[175, 176] Knowledge of the nanoconfinement effects and mass transfer of confined molecules to these well-defined MOFs and COFs are of great interest.

Theoretical studies and modeling of nanoconfinement effects, combined with chemical dynamic data from SMF imaging, are also important directions. Peruchena et al. employed density functional theory (DFT) methods and the quantum theory to study the nanoconfinement effects on methylation of benzene in H-ZSM-5 and H-Beta zeolites cavities.[59] They found out that zeolites with the larger cavity exhibited higher interaction strength related to adsorption and co-adsorption processes, while smaller zeolites exhibited higher nanoconfinement effects where the stabilization energy is higher in H-ZSM-5 than in H-Beta from an electronic viewpoint. Such theoretical studies and modeling could be readily realized in well-defined porous materials, thus providing complementary insights into chemical dynamics under nanoconfinement.

Acknowledgements

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