Heterogeneous Catalysis

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High-Resolution Single-Turnover Mapping Reveals Intraparticle Diffusion Limitation in Ti-MCM-41-Catalyzed Epoxidation**

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Micro- and mesoporous materials offer unique opportunities for catalysis thanks to their large surface area. By introducing active elements inside the pore walls of such materials, a wide range of acid–base or redox catalysts has been developed.[1,2] For example, incorporation of Ti sites in silicalite resulted in the TS-1 catalyst, which is known for its high performance in the selective oxidation and epoxidation of hydrocarbons.[3] However, the small (0.55 nm) micropores of this catalyst hinder the uptake of larger olefins as substrates for the epoxidation.[4] To circumvent this limitation of TS-1, titanosilicates with larger pores, such as Ti-Beta[5] and Ti-MWW[6] zeolites, have been synthesized. Even mesoporous titanosilicates such as Ti-MCM-41 were developed with the aim of faster diffusion of more bulky substrates towards the inner active sites.[7,8] MCM-41 materials are characterized by a hexagonal array of pores with a uniform diameter that can be tuned between 1.5 and 10 nm.[9] Despite the relatively large pore size, maximal utilization of the Ti sites in diffusion unlimited conditions remains a major challenge. Typically, Ti-MCM-41 is prepared in the form of particles with sizes of a few micrometers. It was recently demonstrated that a decrease in particle size to about 100 nm was accompanied with a relevant increase in selectivity and reaction rate for the epoxidation of cyclohexene and cholesterol.[10,11] It was reasoned that intraparticle diffusion limitations in the mesopores of the large particles hindered an optimal use of the active titanium sites, similarly as previously described for the microporous TS-1 catalyst.[4]

The kinetics of a catalytic process are often governed by the interplay between diffusion and reaction. Such insights are classically gathered by macroscopic kinetic experiments, for example, by comparing reaction rates using crystals with different sizes, by varying space velocities of the feed, or by measuring apparent activation energies.[12,13] Pulsed-field gradient NMR spectroscopy has been used to determine intraparticle diffusion coefficients during catalysis, but this technique is restricted to extremely large particles ($>10\ \mu m$) and only yields ensemble-averaged results.[14,15] Recent technological evolutions in optical microscopy now offer the opportunity to confront these insights with in situ observations for single catalyst particles.

The high spatiotemporal resolution (submicrometer and milliseconds) of (single-molecule) fluorescence microscopy has proven to be extremely useful to study catalysis at the level of individual particles or even at the level of individual reaction events,[16–24] as well as to investigate diffusion processes in mesoporous materials.[25–29] However, so far these two phenomena, catalytic conversion and diffusion in porous materials, were treated separately in single-molecule studies; no direct information on the interplay between these two processes has been obtained.

Moreover optical microscopy is subject to the laws of diffraction, limiting the spatial resolution to a few hundred nanometers, whereas the interesting processes related to catalysis within porous particles typically occur on smaller length scales. The present contribution circumvents the resolution discrepancy by applying a single-turnover-based strategy in fluorescence microscopy to provide diffraction-unlimited resolution.[29] This approach allows mapping the catalytic activity with nanometer-scale spatial resolution, that is, in the order of 10 to 30 nm, which is competitive with the most recent, but more complex nanoscopy tools such as PALM, STORM, STED, and related techniques.[30–36] The high spatial resolution provides the direct visualization, and thus the immediate localization of active sites within individual particles, while recording the catalytic process under realistic conditions. By exploiting the milliseconds time resolution of the technique, the direct evaluation and quantification of the kinetics is within reach with a very limited number of experiments, as will be demonstrated below for epoxidation over Ti-MCM-41. Typical parameters such as the Thiele modulus and the related effectiveness

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factor[12,13] for catalysis in the condensed phase were calculated on the basis of the obtained experimental data.

Individual reactions were monitored by a versatile method based on a fluorogenic reporter. A phenylbutadienyl-substituted boron dipyrromethene difluoride probe (PBD-bodipy; see the Supporting Information for synthesis), the red fluorescence of which is not detected by the setup, was used as substrate for the catalytic reaction (see Figure 1).

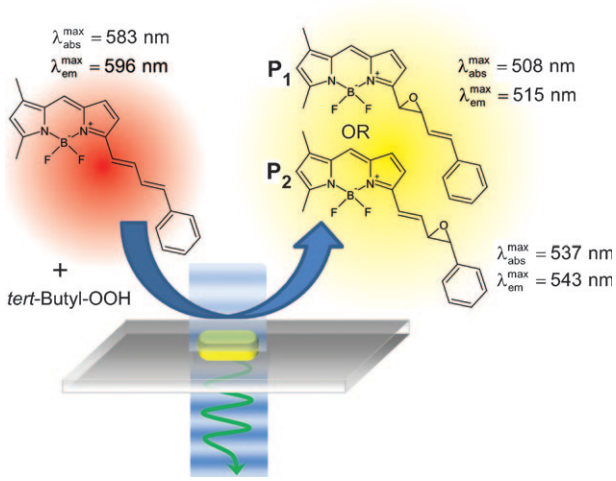

Figure 1. Strategy for monitoring individual epoxidation events. The catalyst is immobilized on a glass cover slide and mounted on a wide-field fluorescence microscope. The red fluorescent bodipy probe is epoxidized with *tert*-butylhydroperoxide (TBHP) on the Ti sites in the mesopores of a Ti-MCM-41 particle. Upon catalytic conversion, yellow-emitting bodipy products P_1 and P_2 are formed.

Upon epoxidation of the probe's butadienyl bridge, the emission undergoes a strong blue shift of 50 to 80 nm depending on the regioselectivity of the reaction (for spectral details, see Figure 1 and the Supporting Information).[37] By spectral selection one can thus specifically monitor the epoxidation in the fluorescence microscope. The high extinction coefficient (ca. 90000 $M^{-1}cm^{-1}$) and fluorescence quantum yield (ca. 1) of the yellow-emitting epoxides allow detection of their formation with single-molecule sensitivity. In addition, the choice of the PBD-bodipy probe is justified on the basis of its molecular size (ca. 1.8 nm and 0.5 nm along the long and short axes, respectively), which is comparable to that of large olefinic substrates such as cholesterol, which are frequently used for epoxidation with Ti-MCM-41.

The formation of individual emissive product molecules was monitored in a wide-field fluorescence microscope with 488 nm illumination. The micrometer-sized Ti-MCM-41 catalysts (see the Supporting Information for synthesis) were deposited on a glass cover slide by spin-coating from a suspension in *n*-butanol. Subsequently, a freshly prepared *n*-butanol solution containing the reactants (500 nM PBD-bodipy and 35 mM *tert*-butylhydroperoxide (TBHP)) was added. Images were recorded by moving the focal plane to the middle of a Ti-MCM-41 particle to avoid emission from catalytic events occurring at the bottom or top surfaces (see the Supporting Information for experimental details). Although the reaction mixture was not stirred during the in situ measurements, the relatively low volumetric reaction rate (0.1–1 $\mu mol\,m^{-3}_{catalyst}\,s^{-1}$) in the applied conditions assures a constant substrate concentration in the solution directly surrounding the particle without limitations of substrate supply from the bulk solution towards the particle (see the Supporting Information for calculations).

As expected, no fluorescent spots were observed on the particles in the absence of either of the two reactants (*tert*-butylhydroperoxide or PBD-bodipy). This result confirms that the observed activity does not result from photocatalytic generation of reactive oxygen species such as hydroxyl radicals from molecular oxygen, as observed in other Ti-containing systems.[38,39] When Ti-MCM-41 particles were contacted with both reactants, the formation of individual emissive product molecules, followed by fast photobleaching or diffusion out of the focal plane, was seen as short (in general < 100 ms), but very intense flashes of light with a spot size of about 250 nm, which is in agreement with the diffraction limits of the technique. A snapshot of one such epoxidation event is presented in Figure 2a. Mapping of the

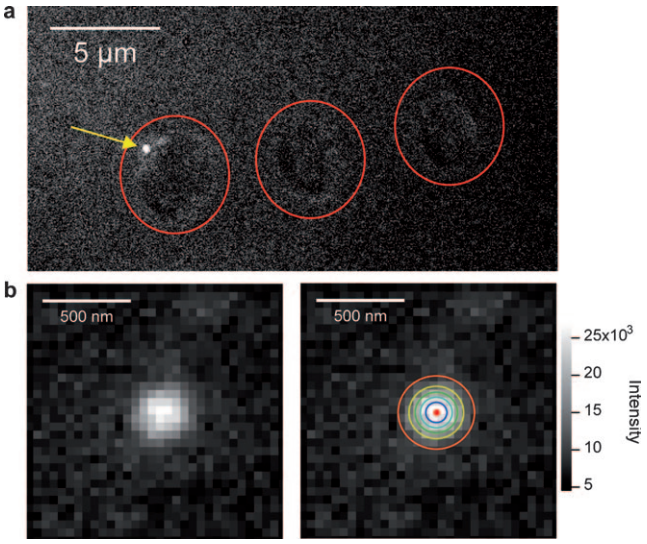

Figure 2. a) Wide-field fluorescence microscopy snapshot image upon 488 nm illumination of Ti-MCM-41 particles (indicated by the red circles) in the presence of the two reactants (*tert*-butylhydroperoxide and PBD-bodipy). The contours of the particles are vaguely visible because of scattering effects. The yellow arrow indicates a fluorescent spot representing one turnover event. b) Left: Enlargement of the emissive spot representing one epoxidized PBD-bodipy molecule. Right: Fitting the intensity profile with a 2D Gaussian function (colored contour lines; full width at half maximum = 223 nm) yields the central (*x*,*y*) position (red dot) of the single emitter in the focal plane.

epoxidation on a single particle reveals distinct spots within a short time frame. This stochastic behavior of the catalytic events allows accurate determination of the central (*x*,*y*) position in the focal plane, by simply fitting the intensity profile with a 2D Gaussian function, such as illustrated in Figure 2b.[29,40–41] It is important to note that, although in a

wide field microscope emission from out-of-focus planes is in principle also collected, only turnovers occurring in the focal plane will yield well-defined sharp spots with diffraction-limited size. These will be recognized and fitted by the analysis software (see the Supporting Information).

By superposition of many single catalytic turnover events and their respective fitted positions in one image, the spots where the product molecules are actually formed can be mapped with a resolution that is only limited by the signal-to-noise ratio of the product's emission. This mapping is illustrated in Figure 3a, in which a transmission microscopy

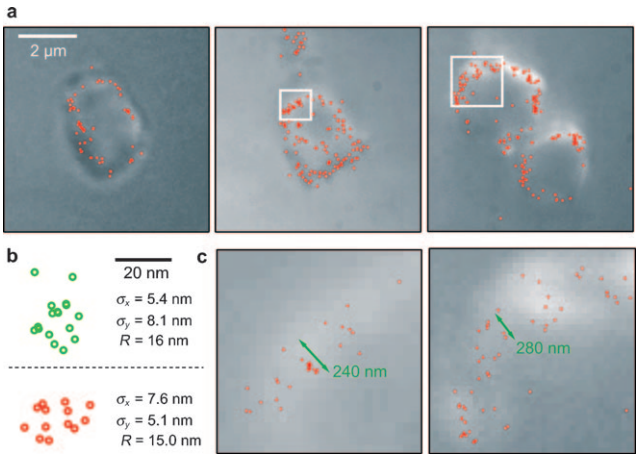

Figure 3. a) Three examples of individual turnover measurements on Ti-MCM-41 particles. The transmission images (grayscale) are overlaid with a scatter plot (red dots) of the fitted positions of fluorescent spots originating from individual product molecules. b) For two representative examples, the fitted positions of an individual adsorbed product molecule, emitting from the same location during several frames (up to 2 s), are displayed in a scatter plot. The standard deviations (σ) in the x and y dimensions are indicated, together with the obtained spatial accuracy (R). c) Enlargements of two regions of the images displayed in panel (a), indicated by the white rectangles. The positions of product formation are distributed over the outer 200 to 300 nm of the particles.

image is overlaid with the fitted positions of individual reaction events acquired over 150 s (1500 frames) for a few representative particles. As every spot corresponds to the formation of one product molecule, the reaction rate or catalytic activity per particle can easily be determined by counting the spots. For example, the first particle in Figure 3a produces 60 spots within 150 s seconds. The catalytic reaction clearly occurs predominantly at the outer rim of the Ti-MCM-41 particles.

The actual accuracy of the fitted position was estimated by calculating the spread on the center of the emission from the same product molecule that appears immobile for several successive frames. The fitted center positions for two such immobile molecules are depicted in Figure 3b. The spread in the x and y dimensions is typically in the order of a few nanometers, yielding an artificial resolution R of below 20 nm (see the Supporting Information for calculations). The high positioning accuracy in the microscopic measurements is the result of the excellent signal-to-noise ratio of the fluorescent spots originating from individual product molecules. Note that this spread is determined not only by the accuracy of the Gaussian fit, but also by possible diffusion of the product molecule inside the Ti-MCM-41 channels and by vibration in the experimental setup. The obtained resolution provides, to our knowledge for the first time, unique spatial information about the actual active regions for oxidation catalysis in individual porous particles (Figure 3a,c).

An enlargement on the active region of one particle reveals that product formation takes place only in the outer 300 nm (Figure 3c). A more accurate determination of the reactivity decay from the particle's outer surface towards the particle's center can be found in the Supporting Information. Since this value is considerably larger than the accuracy in determining the position of the individual turnovers, the measured width is not biased by the resolution. XPS measurements prove that Ti is homogeneously distributed throughout the whole particle (see the Supporting Information). It can therefore be excluded that Ti enrichment at the surface is the cause of the peripheral active zone. Moreover, sorption experiments in presence of excess of PBD-probe and in the absence of TBHP reveal complete accessibility of the inner part of the particle, thus excluding the hypothesis that pore discontinuities are the origin of the observed catalytic zoning effects (see the Supporting Information). Intraparticle diffusion must be the limiting factor in case of Ti-MCM-41 catalyzed epoxidation of substrates such as PBD-bodipy. In other words, the probability for the reactants to reach the inner parts of the micrometer-sized particle before being epoxidized is negligible and hence only the titanium sites in the outer 300 nm of the particle are responsible for the observed catalytic activity. In situ monitoring of the epoxidation activity with fluorescence microcopy thus provides direct evidence for the proposed size-dependent activity of Ti-MCM-41 catalysts.[10]

The data obtained from the microscopic experiment (Figure 3) also allow estimation of the effective intraparticle diffusion coefficient D_{eff} of the bodipy probe. The value is in the order of $10^{-16}\ m^2 s^{-1}$ (see the Supporting Information for calculations), which is a plausible value when compared with diffusion coefficients obtained by single-molecule tracking in other mesoporous materials.[27–28] For the first time, parameters such as the Thiele modulus and the effectiveness factor, which are commonly used in heterogeneous catalysis to identify diffusion limitations at the bulk scale, were successfully determined at the single-particle level. The dimensionless Thiele modulus (ϕ) is defined by Equation (1).

$$\phi = \frac{L_c}{\sqrt{D_{\mathrm{eff}}/k}} \qquad (1)$$

This modulus represents the characteristic size of the particle ($L_c = r/3$) relative to the typical length scale over which the concentration in the particle decays ($\sqrt{D_{\mathrm{eff}}/k}$, in which D_{eff} is the intraparticle diffusion coefficient and k is the rate constant). The value of ϕ is estimated at about 3, corresponding to an effectiveness factor (η) of only 30% (see the Supporting Information for calculations). This result seems to agree visually with the volume fraction of the

catalyst which actually contributes to the observed activity in the conditions applied, as observed in the fluorescence microscopic image. The Biot number (*Bi*) which compares the rate of film diffusion with intraparticle diffusion, was found to be in the order of 10^5 (see the Supporting Information for calculations), thus confirming that the concentration drop from the bulk solution towards the particle's surface, that is, film mass transfer limitation, is negligible.

In summary, we introduced a new versatile approach for studying diffusion limitations in realistic catalytic conditions for catalysis in the condensed phase using porous materials. A probe molecule was selected that upon epoxidation gives rise to bright fluorescent bursts, which represent the formation of individual epoxide molecules. Thanks to the distinct positions of the spots in time and space, their spatial coordinates could be determined to an accuracy of under 20 nm, that is, well below the diffraction limit of optical microscopy. The nanometer resolution was used to map the epoxidation activity of Ti-MCM-41 for large substrates. The results confirm that only a restricted portion of Ti of common micrometer-sized Ti-MCM-41 particles contributes to the observed activity because of the limitation of intraparticle diffusion, and suggest that only in submicrometer particles would the Ti sites be fully used for catalysis. Interestingly, the experimental data obtained from one microscopic measurement at the single-particle level contain all the required information to directly evaluate and calculate the macrokinetic parameters that are commonly used in heterogeneous catalysis.

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