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SINGLE-MOLECULE SPECTROSCOPY

Communicating catalysts

The beauty and activity of enzymes inspire chemists to tailor new and better non-biological catalysts. Now, a study reveals that the active sites within heterogeneous catalysts actively cooperate in a fashion phenomenologically similar to, but mechanistically distinct, from enzymes.

Bert M. Weckhuysen

Enzymes are essential to life on earth. They have evolved into very powerful and effective catalysts with a complexity hardly encountered in their non-biological counterparts, including homogeneous and heterogeneous catalyst systems[1,2]. One of the distinguishing features, in part induced by the fascinating flexibility and structural complexity of enzymes, is the existence of allosterism. Allosterism is a change in the activity and conformation of an enzyme resulting from the binding of non-covalent effectors (molecules, ligands or ions) at a site (the allosteric site) on an enzyme other than the active binding site[3] and offers a way by which such effectors can influence biochemical processes.

Now, writing in *Nature Chemistry*, Chen and colleagues[4] propose that the surface sites within heterogeneous catalysts communicate with each other in a manner that is phenomenologically similar to allosteric enzymes. By using single-molecule fluorescence microscopy — a powerful method that is finding its way in heterogeneous catalysis research[5–8] — they were able to demonstrate communication between sites on heterogeneous catalysts. They observe communication between sites on the same catalyst particle over distances up to a few hundred nanometres as well as communication between different catalyst particles over distances up to tens of micrometres. These intriguing phenomena turn out to be rather generic as they have been uncovered for both palladium- and gold-based nanocatalysts, and for three distinct catalytic reactions; specifically, photo-induced disproportion, oxidative deacetylation and reductive deoxygenation.

The method employed by Chen and colleagues to explore these allosteric effects uses a total internal reflection fluorescence microscope (Fig. 1a) in combination with a fluorogenic catalytic reaction; for example, that of Amplex Red and H_2O_2, which produces acetate and the fluorescent resorufin over a mesoporous-SiO_2-coated gold nanorod (Fig. 1b). By positioning these catalyst particles in a microfluidic reactor cell and irradiating them with a laser, they were able to monitor the fluorescent bursts arising from the oxidative deacetylation reaction of Amplex Red at different locations within different gold nanorods as a function of time. This could be done with spatial and

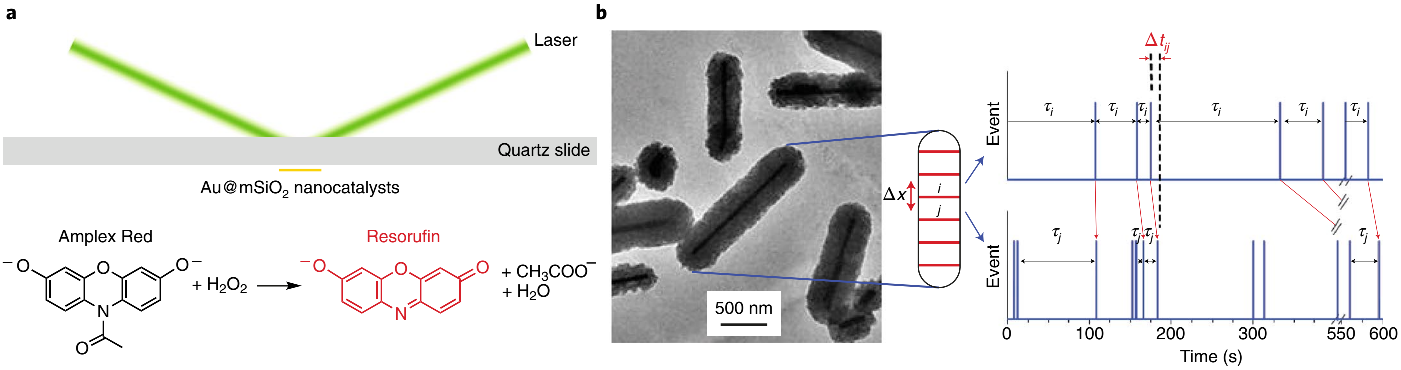

Fig. 1 | Monitoring communicating catalysts using single-molecule spectroscopy. a, Schematic of the single-molecule fluorescence approach to investigate inter- and intraparticle catalytic communication. The set-up is based on a total internal reflection fluorescence microscope with 532 nm (green) laser light and a liquid microfluidic reactor cell that hosts the fluorogenic catalytic reaction. **b**, Gold nanorods between 100 and 1,800 nm in length and approximately 20 nm in diameter were coated with a mesoporous silica. They were nominally segmented for analysis and two catalytic event-reaction time sequences are shown, each from a single segment *i* and *j*. In each catalytic event-reaction time sequence, the individual fluorescent product formation events (vertical blue lines) were plotted against the times when they were detected. Pairs of reactions that are temporally subsequent — but occur at different segments — are linked by the red arrows. The time separation (Δt_{ij}) is shown for one pair of catalytic events, each occurring at one of the two gold nanorod segments. Figure reproduced from ref. [4], Macmillan Publishers Ltd.

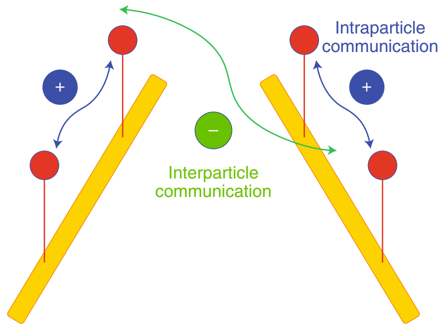

Fig. 2 | Intraparticle and interparticle communication. Electromagnetic field effects indicate that the messenger for intraparticle catalytic communication is a positively charged species and that the messenger for interparticle catalytic communication is a negatively charged species, probably acetate.

temporal resolution of approximately 40 nm and 25 ms respectively.

Chen and colleagues observed correlated fluorescent bursts across the length of the gold nanorods. By segmenting the nanorods (Fig. 1b), it was possible to evaluate the temporal sequence of fluorescent bursts for each segment. The time separation, τ, between fluorescent bursts is the microscopic reaction time for generating resorufin at the corresponding nanorod segment. They then developed a quantitative approach to evaluate the statistical nature of these time–space correlation events. They computed correlation coefficients between the microscopic reaction times of catalytic events at one segment and the immediate subsequent event at another segment on the same gold nanorod. This coefficient is a quantitative measure of how the microscopic reaction kinetics of any catalytic event at one location in the nanorod is correlated with that of a subsequent event at a different location in the nanorod.

By performing this detailed analysis on a large number of gold nanorods (that is, more than 1,100 segments from more than 220 nanorods), trends could be obtained for the correlation coefficients. They found that the correlation coefficient is positive and exponentially decays with increasing intraparticle-distance separation and with increasing average-time separation, which implies that the catalytic events within a single gold nanorod do communicate: a reaction with a short τ (representative of a fast reaction) at one segment tends to be followed by another fast reaction nearby, giving rise to a positive cooperativity. It is important to note here that reported correlation coefficients are very small; however, the trends of these correlation coefficients, which have been obtained through many control experiments, are much more sensitive to changes and corroborate their findings. Another intriguing finding is that the exponential distance-decay constant is approximately 500 nm, showing that this intraparticle communication distance is around 200 times longer than that in allosteric enzymes, the sites of which are only separated by a few nanometres or less.

The cross-correlation analysis between temporarily subsequent reactions was repeated to assess whether different gold nanorods could communicate with each other. Chen and colleagues again observed positive correlation coefficients that decayed exponentially with increasing interparticle distance and average-time separations between the catalytic events. Interestingly, although intraparticle catalytic communication could be observed for each reaction under study, as well as for palladium- and gold-based nanocatalysts, interparticle communication was surprisingly (and inexplicably) only present in the gold-based catalysts.

So what is the origin of these intra- and interparticle communication pathways? Chen and co-workers have tried to answer this question — at least partially — by designing a set of advanced experiments. For this they have made use of two electrodes to change the electrical field vector relative to the orientation of the gold nanorods under study. They found that, for both intra- and interparticle communication, the correlation coefficient was affected if the field was upfield or downfield and observed co-sinusoidal modulations with respect to the orientation angle φ of the nanorod relative to the field direction. Intriguingly, these modulations, which increase with increasing voltage, are opposite in size for intra- and interparticle communication, indicating that the catalytic messenger for intraparticle communication is a positively charged species, whereas it is a negatively charged species for interparticle communication (Fig. 2).

To further pinpoint the nature of these messengers Chen and colleagues looked at how reaction rate and communication distances were affected by the concentration of K^+ (present in the buffer solution) and CH_3COO^- (being one of the reaction products) in the deacetylation of Amplex Red. They saw that intra- and interparticle communication were not affected by the concentration of K^+, whereas CH_3COO^- did influence the communication between different gold nanorods. This again suggests that catalytic communication between different catalyst particles operates through negatively charged reaction products, namely acetate, and occurs over distances extending to tens of microns. By contrast, catalytic communication between sites within the same catalyst particle seems to be related to an as yet unknown positively charged species on or near the catalyst surface and extending for hundreds of nanometres.

Clearly, more research is required to grasp the true impact of this study for the field of heterogeneous catalysis. Firstly, it should be noted that the reported correlation coefficients are very small and indeed close to zero, which raises the question how much the communication pathways affect the overall catalytic performance. Other practitioners in the field have now to put the concepts discussed in this work to the test and explore how generic these findings are. Can they find catalyst–reaction combinations for which the correlation coefficients are (much) more significant? A field to be explored is certainly zeolite-based catalysis, which can also be studied in detail with single molecule fluorescence microscopy[9,10]. Secondly, the observed allosteric coupling for heterogeneous catalysis seems also to be much lower than for allosteric enzymes. Hence, the effectors at the allosteric site in a heterogeneous catalyst have still to be uncovered because the same interaction principles do not seem to be operative. This may be of no real surprise when considering the limited structural flexibility of most heterogeneous catalysts. Finally, it is intriguing to note that a characterization method that originated from the life sciences has enabled the investigation of such phenomena; phenomena that have so far gone unnoticed using more standard bulk characterization methods. The observations further underpin the importance of single-molecule fluorescence microscopy to evaluate catalyst heterogeneities[4]. Here, the integration of a single-molecule fluorescence microscope within a transmission electron microscope may help as it allows nanoscale defects to be related to differences in spatiotemporal fluorescence bursts within and between catalyst particles[10].

The recent developments in the field of single-molecule fluorescence microscopy[9,10], together with the study by Chen and colleagues[4], show that catalyst scientists are on their way to appreciate the similarities as well as the differences in the mechanism of enzymatic and heterogeneous catalysis. Although very different in terms of chemical composition, it is clear that both types of catalysts share several common features; hence, both research fields should further inspire each other to develop the most active and selective catalyst for a particular chemical reaction. ❐

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