

applications, disulfide bonds are already used in the design of micelles for gene delivery and self-assembled monolayers because of the reversibility of crosslinking<sup>11</sup>.

The idea of force-activated caged structures also hints at a potential use as a flexible building block inside microfluidic channels. In elongational flow, molecules can be stretched and this can trigger secondary reactions that cause specific self-assembly mechanisms (Fig. 1b) — like the protein assembly that controls the formation of silk fibres in a spider's spinning duct. Potential applications of mutable disulfide bond arrangements also exist in the design of structural materials, where weak bonds can

enhance mechanical resilience by sacrificially dissipating large deformation forces, leading to great fracture toughness or ductility<sup>12</sup>. Moreover, controlling the arrangement of disulfide bonds through force-activated isomerization could lead to new materials with mutable fracture properties, where isomerization processes at high-stress sites such as crack tips could be controlled by external signals — for example, reducing agents or light — and thereby used to mitigate the risk of crack propagation when needed. □

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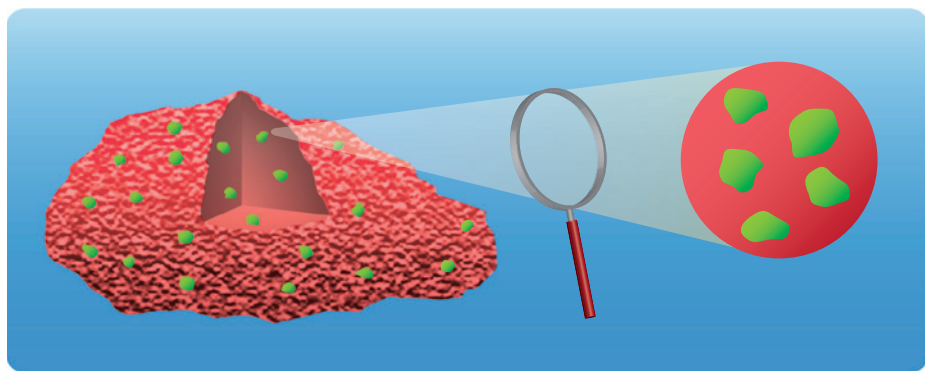
## SOLID ACID CATALYSTS

# Stain and shine

Catalyst particles for fluid catalytic cracking are vital for the oil-refinery industry, but their activity is hard to diagnose because of their inter- and intra-particle structural inhomogeneity. With fluorescence confocal microscopy and selective staining, one can now pinpoint the catalytic activity within single catalyst particles from an industrial reactor.

Peng Chen

One cannot stress enough the importance of oil in our lives. But for crude oil to be transformed into more useful products, it needs to be refined. One such refinery process is fluid catalytic cracking (FCC), in which the high-molecular-weight molecules of oil are broken down into smaller, lighter molecules, generating more valuable products such as gasoline. The efficiency of FCC depends largely on catalysts. To improve the performance of these catalysts, diagnosing their activity throughout their usage is crucial. Their diagnosis is challenging, however, at least from two aspects. First, like all solid catalysts, FCC catalyst particles are tremendously inhomogeneous. Each catalyst particle is a complex mixture of zeolites, which are the catalytic components of the particle, and matrix materials, which hold the zeolite particles together. As a result, each catalyst particle is different from the others; even within a single particle, the zeolite components distribute non-evenly, and the catalytic activity changes from one place to another. Second, the FCC catalysts do not live forever. They deactivate over their life cycle, and individual particles deactivate differently. These two aspects of FCC catalysts mean that methods of measuring the average activity of a collection of catalyst particles are inadequate for probing their catalytic performance. Now, writing in *Nature Chemistry*, Weckhuysen and co-workers report a fluorescence microscopy



**Figure 1** | Microscopic diagnosis of single catalyst particles used in oil refining. A fluorogenic catalytic reaction selectively occurs at the catalytic zeolite domains (green) within a single fluidic cracking catalyst particle, while a large non-reactive fluorescent probe stains the pores of the matrix material (red) of the catalyst.

approach for measuring and spatially resolving the catalytic activity of individual FCC catalyst particles, throughout their life stages, at sub-particle resolution<sup>1</sup>.

The researchers used a fluorescence confocal microscopy technique, in which a focused laser light is used to scan across a catalyst while the fluorescence emission from the focal point is detected. The catalyst particles are ~70 µm in diameter, and most of their zeolite catalytic domains are a few micrometres in size. Confocal microscopy further allows them to examine a section

within a particle (Fig. 1). To generate a fluorescence signal that reports the catalytic activity, they used the fluorogenic reaction of thiophene oligomerization, which is specifically catalysed by the acidic sites of the zeolite domains. The oligomerized thiophene emits green fluorescence, reporting the location of the zeolite domains, while the intensity of the fluorescence reports the acidity, that is, the activity, of the catalytic sites. They used another fluorescent probe, Nile Blue A, whose bigger size restricts its presence to the

matrix material and whose red fluorescence lights up the porous matrix. Optical detection of this signal makes the approach easily applied *in situ*, where catalysts can be interrogated under reaction conditions.

Using this differential fluorescence staining approach, Weckhuysen and co-workers analysed the acidity of individual FCC catalyst particles that were freshly made or were treated in ways that mimicked the deactivation processes they undergo in an industrial FCC unit, such as coke formation, metal deposition and hydrothermal ageing. They directly visualized and mapped the acidity of individual zeolite domains in each catalyst particle, and uncovered large heterogeneity in the acidity within each one and among different particles, as well as at every stage of the deactivation process; none of this information is available in ensemble-averaged measurements. The deactivation processes were found to lead to loss of strong acid sites in individual zeolite domains, whereas access to the zeolite domains through the matrix material stayed unchanged.

More excitingly, the loss of strong acid sites at the single-particle level is directly correlated with the loss of the catalysts' activity in catalytic cracking. This correlation makes

acidity mapping by fluorescence confocal microscopy a viable approach for diagnosing the cracking activity of individual catalyst particles, including for those catalyst particles taken from an industrial FCC reactor. Such a reactor contains a huge mixture of unequally deactivated catalysts as well as fresh ones that are continually added to compensate for the catalyst deactivation. With a small amount of sample, one can now screen the effectiveness of the catalyst particles from various parts of a reactor bed and at various stages during their usage in an industrial process.

The fluorogenic reaction approach further opens a way to push the spatial resolution down to the nanometre scale. Traditional fluorescence confocal microscopy has a diffraction-limited resolution of about half-a-micrometre; it cannot see catalytic domains that are nanometres in dimension, but which could be highly active. The fluorogenic reaction offers a natural bridge towards single-molecule fluorescence imaging of catalysis, which has a spatial resolution down to ~20 nm owing to the super-resolution imaging analysis<sup>2-7</sup>.

The current results have uncovered large heterogeneity in the deactivation of individual particles and catalytic domains.

But how the individual particles and domains deactivate in real time is unclear. Do domains and particles that are more active deactivate faster or slower? Real-time measurements, while the catalyst particles are operating, are needed to investigate the mechanism of deactivation. With clever designs of reactor cells, real-time optical detection should be possible. Although still early to say how we can use this knowledge to improve FCC catalysts, a petroleum engineer would definitely like to have a tool that can pinpoint the activity in a single catalyst particle with nanometre resolution in real time. □

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## BIOENERGETICS

# Proton fronts on membranes

Proton migration on membranes is a crucial step in the bioenergetics of the cell. It has typically been regarded as slow successive proton transfers between ionizable moieties within the membrane, but recent measurements suggest fast lateral diffusion in the membrane's hydration layer.

Noam Agmon and Menachem Gutman

Adenosine triphosphate (ATP) is the most common free-energy carrier in the biosphere. Its synthesis, first suggested by Mitchell<sup>1</sup>, involves two sets of membrane-bound enzymes — one (proton pumps) creating a transmembrane proton gradient and the other (ATP synthase) consuming this potential to drive ATP synthesis.

The general acceptance of this scenario sparked interest in the behaviour of protons at the membrane/solution interface<sup>2,3</sup> — a very narrow layer, only a few water molecules across, where protons are released and consumed. Monitoring such proton dynamics is extremely difficult<sup>3</sup> because of the proton's high diffusivity: unlike other ions, protons (and hydroxyl ions) do not propagate in water by mass transport. Rather, they diffuse by the Grotthuss mechanism,

where the rearrangement of covalent and hydrogen bonds around the solvated proton propagates the protonic charge<sup>4</sup>. Even faster proton translocation from acidic to basic moieties at close proximity can occur along ordered chains of water molecules ('water wires') connecting them, by a concerted multi-proton tunnelling mechanism, leading to an inverse temperature effect for the rate function for traversing the wire<sup>5</sup>.

In respiratory membranes, the high density of proton-pumping proteins can generate a local state of quasi-equilibrium, and the resulting 'localized pH gradient' may be in disequilibrium with the surrounding solution. This depends on the rate of proton pumping, on the presence of mobile buffers in solution and, to a large extent, on the buffer capacity of the surface itself. Junge and McLaughlin provided<sup>6</sup> a general theory

for diffusion in the presence of both mobile and fixed buffers — fixed ones including the anionic (phosphate) head-groups of the lipids, and other molecules that can be protonated and are embedded in the lipid bilayer. If protons were transferred predominantly by fixed buffers, the protons' lateral diffusion rate would diminish by several orders of magnitude as compared with water, commensurate with their  $pK_a$  value.

Protons pumped by bacteriorhodopsin, however, have been observed to diffuse laterally on the surrounding membrane surface about as fast as in water<sup>7</sup>. Moreover, they were detected in solution considerably after their arrival at target membrane sites, suggesting that protons are somehow retained on the surface for a long time.

Now, building on their earlier experimentation<sup>8</sup>, Peter Pohl and co-workers<sup>9</sup>