Electronic supporting information

Correlated Super-Resolution Fluorescence and Electron Microscopy Reveals the Catalytically Active Nanorods within Individual H-ZSM-22 Zeolite Particles

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1. Experimental section

Correlative super-resolution fluorescence and scanning electron microscopy

ILEM (Figure S2) was used to perform the correlative super-resolution fluorescence and scanning electron microscopy experiments. The applied setup is based on the commercially available Secom platform for integrated wide-field fluorescence and electron microscopy, developed by DELMIC B.V., which is built into a FEI Quanta 250 FEG SEM, and has been in-house adjusted to enable super-resolution fluorescence microscopy. The latter was achieved by mounting a 100× 1.4 NA CFI plan APO VC oil immersion objective lens (Nikon) (Figure S2) into the sample stage that is used in combination with 1,3-EMIM acetate (BASF, Germany) as vacuum compatible immersion liquid. Secondly, an adjustable mount has been integrated into the optical excitation pathway that holds the wide-field lens. This allows for sufficient flexibility for the alignment of the wide-field system. The configuration of the microscope implies that both fluorescence excitation and detection are provided from the bottom side up and the SEM is achieved from the top of the sample. Samples are therefore mounted on top of optically transparent cover slides according to the procedure disclosed in the sample preparation section.

Excitation is provided at 532 nm by a diode pump solid state laser (Omicron laserage) and additionally passes through an excitation filter prior to being directed towards the objective lens and the sample by the 442/532 nm dichroic mirror (Chroma). The transmission of the excitation light into the sample chamber, where the objective lens is located, is enabled by an optically transparent window. The fluorescent signal is captured by the objective lens and passes through the optical chamber window, the dichroic mirror, a 542 nm long pass filter and a 2.5x camera lens, before detection using a highly sensitive electron multiplying charge-coupled device (EMCCD) camera (ImagEM Enhanced C9100-23B, Hamamatsu). As such, a 32.8 × 32.8 μm^2 FOV is obtained with a 64 × 64 nm^2 pixel size. Linear polarized excitation light experiments are enabled by introducing a Glan Thompson polarizer (Thorlabs) on a rotatable mount that has been introduced into the excitation pathway of the ILEM.

Zeolite synthesis

The H-ZSM-22 sample with intended bulk Si/Al ratio of 75 has been synthesized according to the procedure used by Hayasaka et al.[1] based on the original work by Ernst et al.[2] and Olson et al.[3] The different reactants and the necessary quantities for synthesis involved preparation of the following four solutions:

1. Solution A: 3.875 g of KOH dissolved in 13.5 ml of de-ionized water
2. Solution B: 1.059 g of $Al_2(SO_4)_3 \cdot 18H_2O$ dissolved in 10 ml of de-ionized water
3. Solution C: 8.35 g of 1, 6-diaminohexane diluted with 65 ml of de-ionized water
4. Solution D: 36 g of Ludox AS-40 (colloidal silica) diluted with 62 ml of de-ionized water

Solution A was added to B and mixed until complete aluminum dissolution. After adding solution C, the mixture of A, B and C was poured into solution D, while rigorous stirring at room temperature. The molar ratio in the synthesis mixture is: $150SiO_2$: $Al_2(SO_4)_3$: 44KOH: 45Diaminohexane: $6044H_2O$. An amount of 100 mg of previously synthesized ZSM-22 powder was added as seeds to assist crystallization. The resulting gel was divided over 2 stainless steel autoclaves with a volume of 120 ml. They were kept in an oven at 150°C for 60 h while tumbling at ca. 60 rpm. When synthesis was completed, the content was filtered and washed with de-ionized water. The resulting material was dried at 60°C overnight.

Sample preparation for microscopy experiments

Single molecule clean H-ZSM-22 samples (Si/Al ratio: 75 based on precursor ratio) were prepared according to an optimized sample preparation method. Initially, regular glass cover slides

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(# 1) were calcined in a static air oven at 450°C for at least 24 h and subsequently ozone treated for 30 min in a UV-reactor (Ultra violet products, PR-100). The H-ZSM-22 sample was spin-coated onto the cleaned cover slides from a 1 mg/10 ml H-ZSM-22 in Milli-Q water suspension at 2000 rpm for 60 s. Two droplets were added to the cover slide for spin coating. Additionally, the sample containing cover slides were calcined in an oven to remove any organic contaminants from the microporous structure of the zeolite that could lead to fluorescent background. For this purpose, a static air over was used in combination with a specialized heating program in order to prevent any structural changes to the zeolite framework. The sample was heated from room temperature to 80°C at a rate of 1°C/min, after which the sample was kept at 80°C for 1 h. In a second step, the temperature was further increased to 120°C at 1°C/min and this temperature was held for 1 h again. In a final heating stage, the temperature was further increased from 120°C to 450°C, again with a heating rate of 1°C/min, and this temperature was maintained for at least 24 h. Afterwards, the sample was cooled down to room temperature and immediately mounted into the ILEM for investigation.

Correlated experiments

All correlative super-resolution fluorescence and scanning electron microscopy experiments were performed at room temperature. After spin coating and subsequent calcination, the sample was mounted on the stage of the ILEM. Unless stated otherwise, SEM imaging was performed prior to the NASCA experiments throughout the presented research. Therefore, the sample chamber was pumped down to allow SEM imaging (2 kV, SE, high vacuum mode), which was applied to locate a suitable region of interest and to perform detailed structural imaging of the contained samples. Afterwards, the SEM chamber was brought back to atmospheric pressure. This allowed us to perform the cathodoluminescent grid scan that was part of the quantitative overlay procedure (further described in the overlay section) and to perform the NASCA imaging. The latter was performed after attaching a perfusion chamber (PC8R-1.0-CoverWell, Grace Bio labs) onto the sample and after a waiting time of approximately 15 min needed to eliminate mechanical drift that could be present after bringing the sample back to atmospheric pressure. 50 µL of a 10 vol% FFA (Sigma Aldrich, 98%, purified through vacuum distillation prior to use) in milli-Q water was added to the perfusion chamber, which acted as a reactor cell. This reagent concentration has been established as being optimal through several experiments that were conducted using various reagent concentrations. By adding the FFA reagent, fluorescent oligomers are formed on the catalytically active sites which are excited with 3.5 kW/cm^2 laser power at 532 nm, and detected using 30 ms exposure time and 297 electron multiplying gain. The reaction scheme for the FFA oligomerization reaction that leads to the fluorescent reaction products is described elsewhere. [4]

Data Analysis

During all experiments performed in this research, 10.000 frame movies were acquired. One exception was made for the experiment performed using linear polarized excitation light perpendicular to the one dimensional porous structure. In case of such light polarization orientation, no catalytic turnovers were observed, so the movie was recorded for only 1000 frames. All resulting movies were analyzed using the Localizer software for Igor Pro (Wavemetrics).[5] The localization of individual catalytic turnovers is achieved by fitting a 2D Gaussian function to their point spread function. This approach yields the positions of the fluorescent catalytic reaction products with up to 20 nm resolution.[6] By counting the turnovers within 20 × 20 nm^2 areas, a quantitative catalytic activity map is obtained. The quantitative catalytic activity maps that are described in the current research have undergone an additional consolidation procedure in order to remove reappearing fluorescent events (120 ms of blinking time is maximally allowed and an intermolecular distance of up to 50 nm). A series of different parameters was carefully assessed for properly analyzing the sample. On the quantitative activity maps that are displayed in the main article, the color bar indicates the chemical reactivity. This is obtained by considering the number of turnovers within the 20 × 20 nm^2 bins in combination with the average thickness of the needle-shaped particles. The latter is assumed to be 160 nm, based on their geometry, which implies that the height of the particles is similar to their width. These dimensions result in the voxel volume. By using the Avogadro number, the mol of product that is formed within every voxel and per time unit can be determined. It is important to also consider the detection limits of the used NASCA technique. These range from individual turnovers at the lower end, up to a maximum set by the diffraction limited resolution which is about one turnover per 250 × 250 × 160 nm^3 volume, every 120 ms. This results in an upper detection limit of 1.4×10^{-6} M/s.

Overlay procedure

The quantitative activity maps and SEM micrographs are spatially correlated by an in-house developed approach based on cathodoluminescence (CL, for schematic see Figure S3 and S4). After performing the SEM imaging, the electron beam is used to generate optical reference signals by means of CL. A grid is projected onto the glass cover slide by the electron beam in spot mode and the corresponding positions are precisely localized based on these diffraction limited CL spots in the optical image using localization software. The determined optical positions are subsequently correlated to their corresponding position in SEM, which are accurately known as they have been used as input for the grid projection. This approach enables an accurate determination of the translation, rotation and magnification parameters between both imaging modalities. This approach enables sub-5 nm precise image registration.[7] The software needed to perform the grid scan is provided as a Micromanager script and the application that has been developed to perform the actual overlay is provided as a Matlab procedure, both are publicly available at “https://github.com/KrisJanssen/QuickCLEM”.

Electronic supporting information

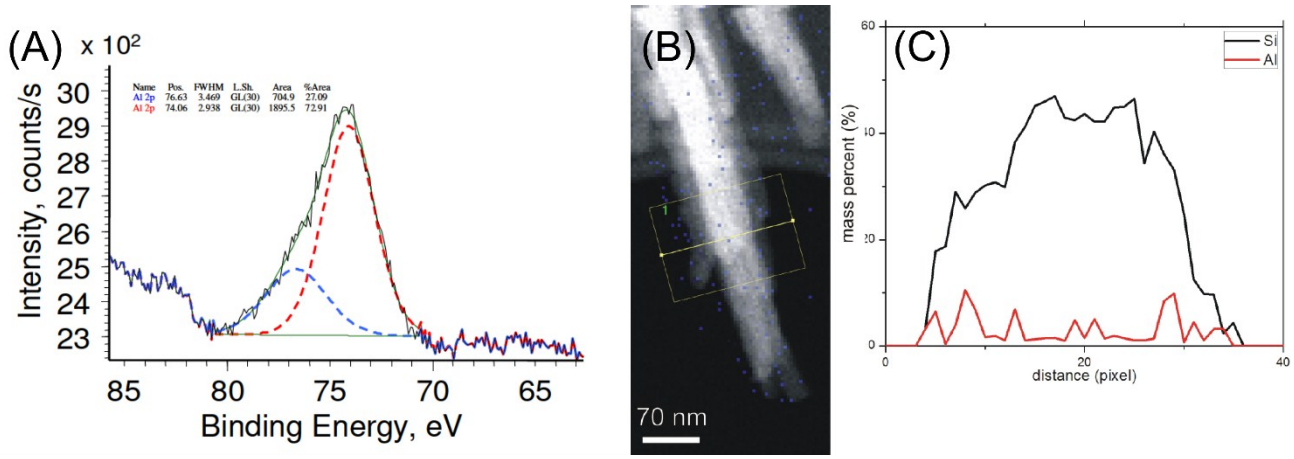

Figure S1. (A) XPS spectrum of H-ZSM-22 (Si/Al=75), with Al (IV) (red line) and Al (V+VI) (blue line) components. (B) HAADF-STEM image of H-ZSM-22 (Si/Al=75) crystal overlaid with the EDX signal of Al (the blue dots). (C) The line profiles of mass percentage comparison of Al and Si. The line profile was obtained from the highlighted region in panel (B).

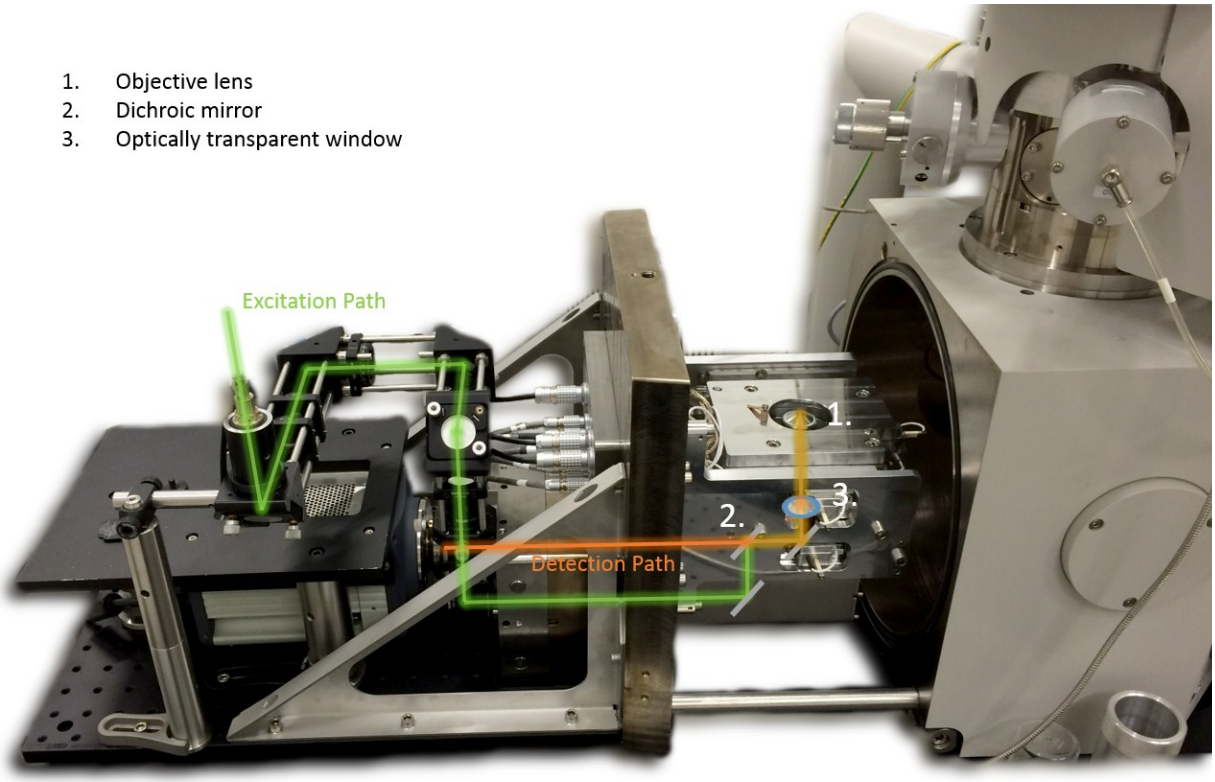

Figure S2. Picture of the ILEM in the configuration applied for super-resolution fluorescence microscopy (SRFM), *i.e.* SEM door opened and sample at atmospheric pressure. Both the excitation (green) and detection (orange) paths are depicted, as well as the position of the dichroic mirror (1), the position of the optically transparent window (2) that enables excitation and detection without the need to transfer the sample between dedicated setups, and the objective lens (3). SEM images are obtained under high vacuum conditions prior to SRFM imaging.

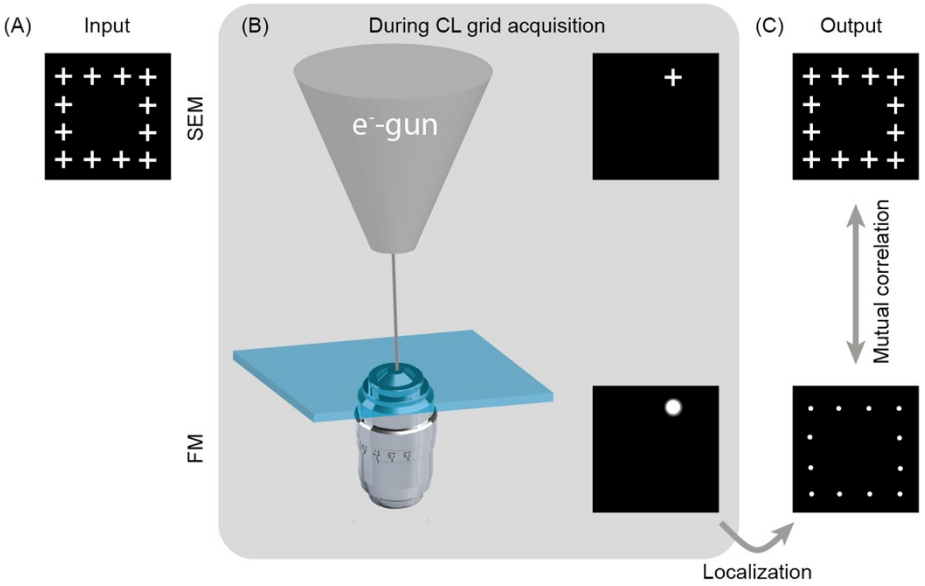

Figure S3. A schematic representation of the cathodoluminescence (CL) based overlay procedure used to quantitatively produce the correlative micrographs from an SEM image and corresponding catalytic activity map. (A) The SEM beam is used in spot mode to project a grid pattern on the cover slide. (B) These SEM beam projections generate a CL signal that can be detected using the integrated optical microscope and this is done in consecutive images for the individual grid positions. (C) The detected CL signals are localized using the localizer software that is also at the base of the NASCA technique. By subsequently correlating the localized positions with the initially set SEM grid input parameters, the magnification, translation and rotational parameters that are needed for an accurate image registration are obtained.

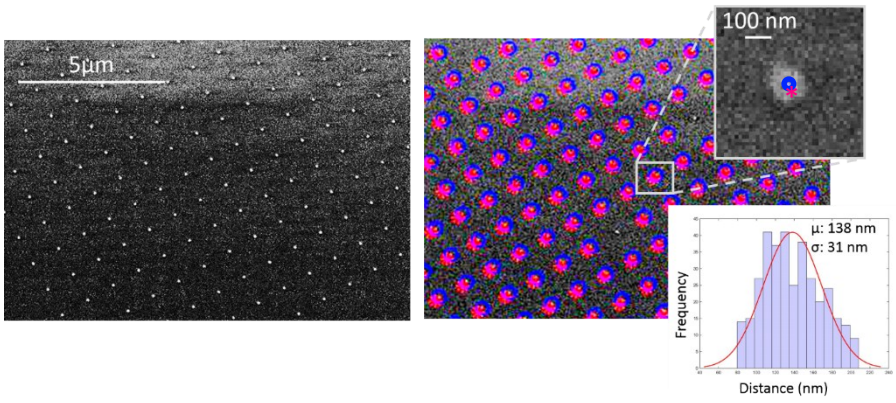

Figure S4. The estimation of the precision of the CL-based correlation. Left: Gold nanoparticle grid; Right: the centroid positions (blue circles, SEM) and localized positions (red stars, NASCA) superimposed onto the SEM image by means of the autocorrelation procedure. The top inset is an enlarged FOV of one such gold nanoparticle, showing, in detail, the discrepancy between the localized position based on the photoluminescence data and centroid position determined from the SEM image. The bottom inset shows the distribution function of the mutual distance between every localized and centroid position in the sample, by fitting this to a Gaussian function and determining the standard deviation and average, a measure for the performance of a certain procedure can be obtained.

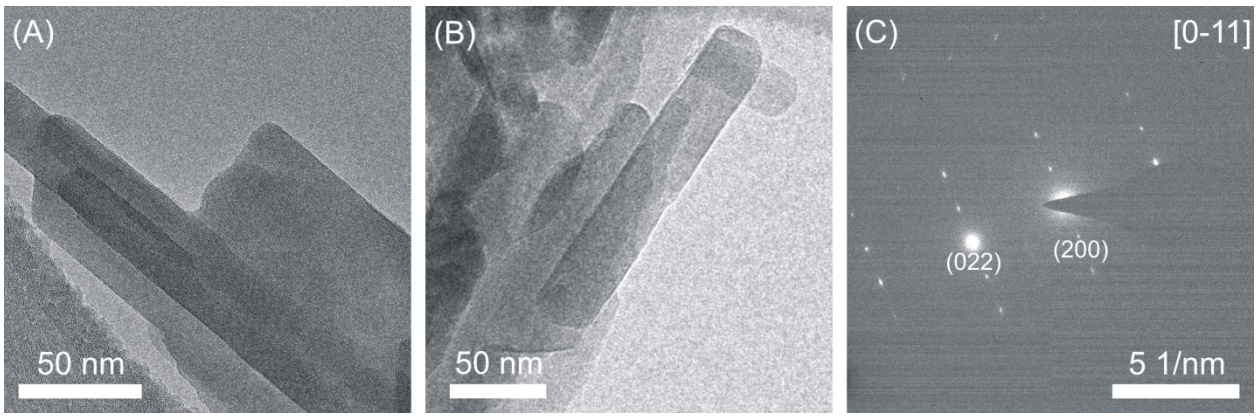

Figure S5. (A) HR-TEM image of needle shaped H-ZSM-22 particles (B) a H-ZSM-22 nanorod with a length of 170 nm and a width of 30 nm. (C) An electron diffraction pattern from a ZSM nanoparticle in [0-11] zone axis orientation.

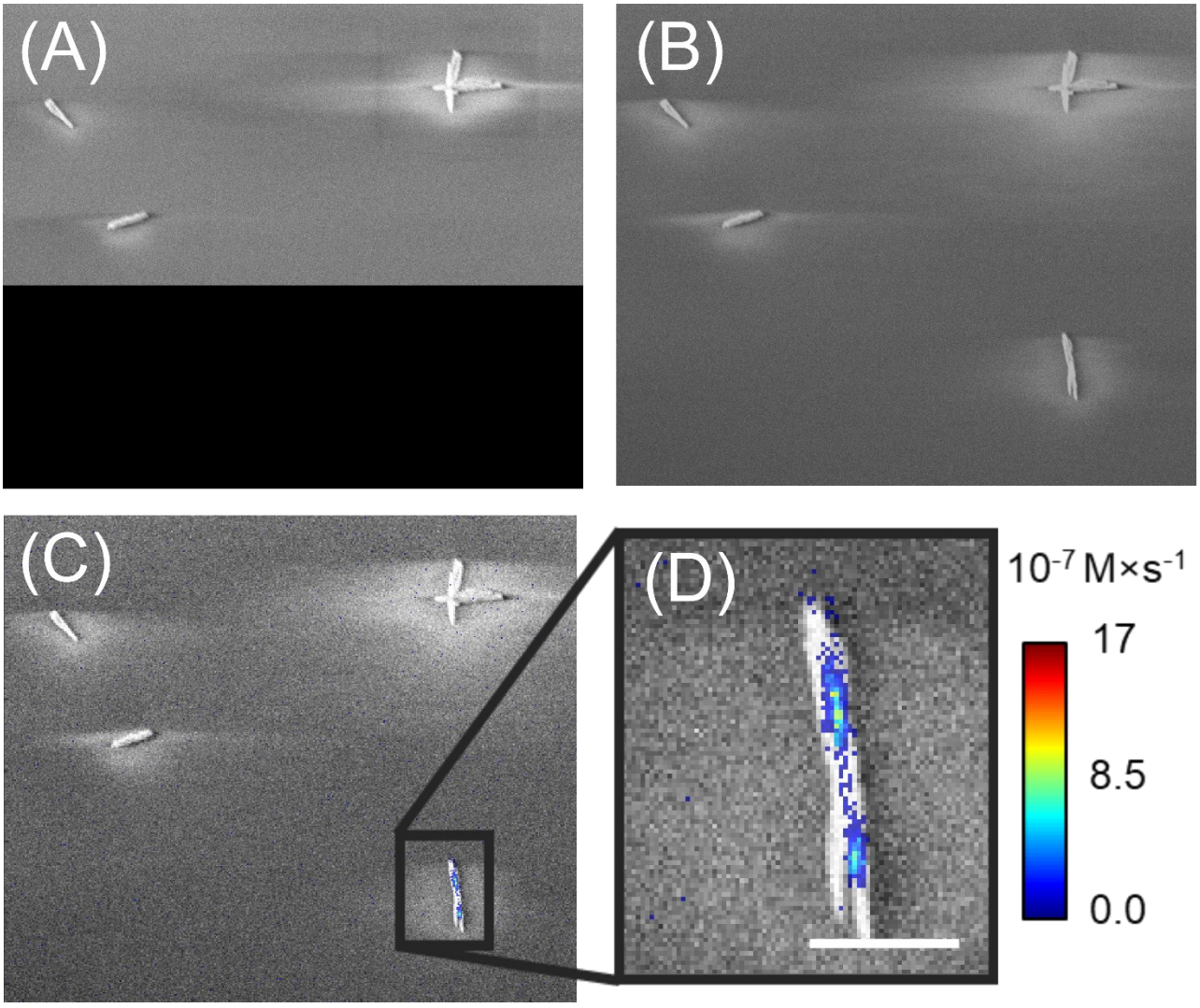

Figure S6. (A) SEM image of the intensely irradiated area containing three H-ZSM-22 particles (SEM imaging conditions: FOV scanned halfway in 2 min, 5 keV acceleration voltage, 0.19 nA). (B) SEM image of the full field of view (FOV), acquired with the imaging conditions used throughout the experiments (FOV fully scanned in 1.4 min, 2 keV acceleration voltage, 0.10 nA), revealing an additional H-ZSM-22

particle that was not intensely irradiated. (C) the correlative micrograph with quantitative activity map obtained for the complete FOV; only the particle at the bottom right in the FOV shows catalytic activity. (D) enlargement of the correlative micrograph. Scale bar = 500 nm.

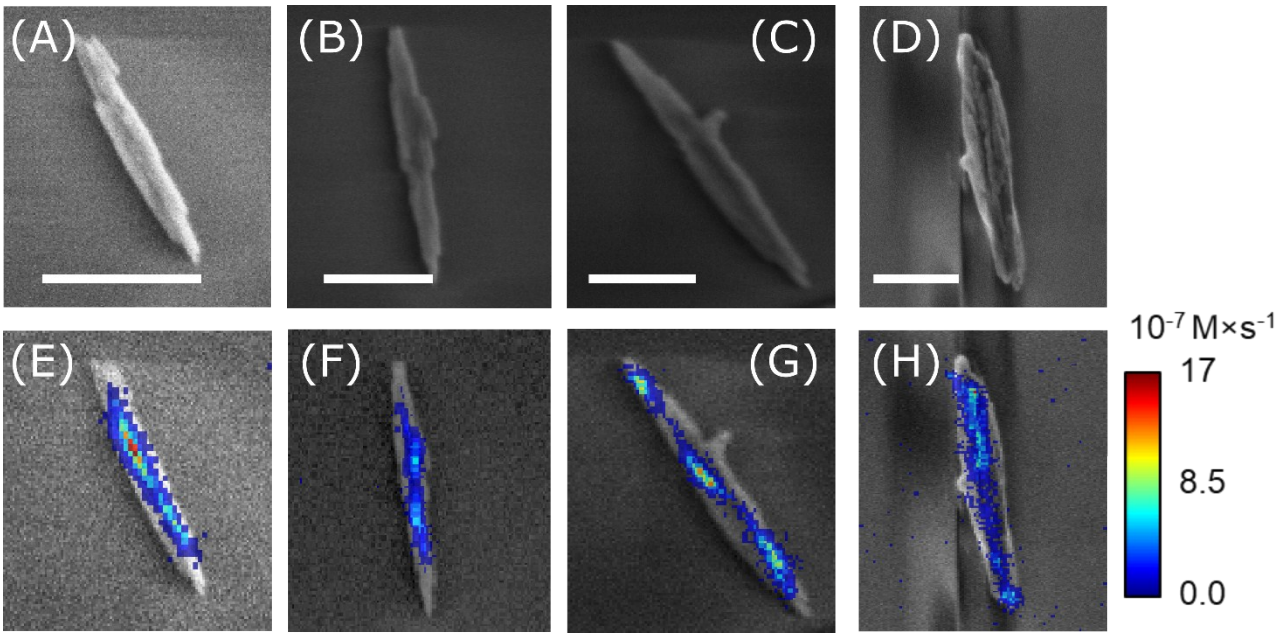

Figure S7. The resulting images of correlative experiments performed in a reversed order compared to the results shown in the main article, *i.e.* catalytic activity mapping is performed prior to SEM imaging. (A-D) SEM images and (I-L) resulting correlative micrographs with catalytic activity maps. The SEM images represented in B and C reveal a possible drawback of performing the experiments in the reversed order. A film has deposited on top of the samples due to the removal of the reagent solution after activity mapping. This reduces the obtained resolution, leaving more structural features undetected. Scale bars = 500 nm.

Notes and references

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