Correlative Laboratory Nano-CT and 360° Electron Tomography of Macropore Structures in Hierarchical Zeolites

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Hierarchical pore structures exhibit morphological features on several length scales, which govern important materials properties in catalysis, such as catalytic activity, diffusivity or selectivity. Correlative tomography offers unique opportunities for a comprehensive and scale-bridging 3D characterization of such complex pore morphologies, which is crucial to further optimize materials design and synthesis routines. This study explores the capabilities of correlative 360° electron tomography (ET) and lab-based nano X-ray computed tomography (Nano-CT) enabling 3D analyses of volumes of up to (60 µm)³ with down to nm resolution, as demonstrated for zeolite particles with embedded macropores. By first applying the two techniques to the same particle the higher resolution and fidelity of ET are used to improve the segmentation of pore space in the Nano-CT reconstruction. Extended statistical relevance and access to interparticle pore space are obtained from reconstructions of larger particle agglomerates, using the large-field-of-view mode of the Nano-CT. The presented correlative approach enables real space analyses of important pore characteristics for comparison with complementary pore characterization techniques. Moreover, by investigating samples from different stages of the synthesis, 360°-ET and Nano-CT provide unique insights into the formation mechanism of porous materials, as demonstrated for the steam-assisted crystallization of the macroporous zeolite particles.

1. Introduction

Functional materials often exhibit features on the nano- and microscale that extend into 3D like porous materials used in catalysis,[1] chromatography,[2] fuel cells[3] or nanoparticulate materials such as photonic pigments from colloidal self-assembled particles.[4] Features on different length scales in one sample require the development and application of complementary and scale-bridging tomography routines for 3D real space investigation.[5] On smaller length scales, atom probe tomography (APT)[6] and electron tomography (ET)[7] allow up to atomic resolution for samples typically below 100 nm in size. Whereas APT is a destructive technique, ET is nondestructive, so that the sample can be used for other experiments afterward. ET also enables the investigation of larger samples up to a few µm in size when the material’s density is low enough, either because the sample is composed of low atomic number elements or because of porosity.[8] Another versatile, but destructive 3D imaging
Recent examples of (noncorrelative) 3D studies of porous media include Micro-CT investigations of packed bed chromatography columns\cite{2b} and hierarchical zeolite structures.\cite{1,20} Furthermore, different studies applied conventional ET,\cite{21} synchrotron PXCT,\cite{5d,14a} and synchrotron micro X-ray computed tomography (Micro-CT)\cite{2b} and hierarchical zeolite structures.\cite{1,20} Weissenberger et al. used synchrotron PXCT and ET to investigate porous polymeric particles. Scale-bridging and correlative tomography experiments of porous structures using combinations of different aforementioned 3D techniques have been performed by, for instance, Becher et al. applying laboratory Micro-CT, synchrotron PXCT, and ET to investigate structured catalyst monoliths\cite{5a} or by Alvarez et al. using complementary FIB/SEM tomography, serial block-face scanning electron microscopy (SBFSEM) and ET to investigate porous polymeric particles.\cite{5b} Weissenberger et al. used synchrotron PXCT and conventional ET to reconstruct single macroporous zeolite crystals\cite{31} whereas Fam et al. combined synchrotron PXCT, FIB/SEM tomography, and ET to analyze nanoporous gold catalyst materials.\cite{32} One recent application, including lab Nano-CT for correlative studies in combination with FIB/SEM slice and view, was the 3D characterization of the internal structure of a
chromatography bead.[24] Further examples are given by Schurch et al. who applied SBFSEM, laboratory, and synchrotron Micro-CT and laboratory Nano-CT to electrical treeing channels[24] and Lenz et al. who used FIB–SEM–EDXS informed laboratory Nano-CT to investigate oxidized superalloys.[25]

Besides 3D imaging techniques, complementary and averaging analyses of porous materials are commonly obtained by gas adsorption techniques. They are typically applied for the analysis of materials with internal micro- and mesopores <50 nm.[26] Above that, mercury porosimetry can be employed to investigate solids with pore sizes ranging from about 4 nm to 400 µm.[27] Significant progress has been achieved in recent years concerning the textural characterization of hierarchical structured nanoporous materials with these techniques.[28] However, 3D imaging techniques can help to reconstruct even inaccessible pores and obtain deeper insights into details of their pore network.[29]

Here, we present a correlative and scale-bridging 360°-ET and Nano-CT approach, which is completely realized in a laboratory environment, and demonstrate it for macroporous silicalite-1 zeolite particles synthesized via a steam-assisted crystallization (SAC) process.[21,30] As it is sketched in Figure 1, the combination of 360°-ET and lab Nano-CT allows versatile, nondestructive 3D investigation over several length scales from nm-sized volumes with atomic resolution[31] up to objects with a size of several dozens of µm with resolutions down to 50 nm.[32] In this study, we explore the possibilities and benefits of lab-based Nano-CT in combination with 360°-ET by investigating identical samples featuring dimensions that exploit the limits of each technique (exemplary experiments are indicated in Figure 1 by encircled numbers 1 and 2). Whereas high-resolution (HR) Nano-CT overlaps on the low end with reachable sample sizes and spatial resolutions of the high end of 360°-ET, large-field-of-view (LFOV) Nano-CT opens up the possibility to extend 3D analyses to much larger and statistically more relevant volumes of hundreds of particles in the same lab instrument. To enhance the segmentation quality of 3D scans acquired at lower magnification, we employ high-resolution knowledge from 3D reconstructions of the same sample gained at higher magnification. This enables us to precisely reconstruct and quantitatively analyze the intra- and interparticle pore system of single particles and particle agglomerates. Above that, the 3D investigation of differently developed zeolite crystals extracted at different stages of the SAC process reveals a glimpse into the formation mechanism of the macroporous zeolite particles. This correlative approach has the potential to be applied to many more porous systems, where the hierarchical nature of the material spans over several length scales.

2. Results and Discussion

The investigated single-crystalline zeolite crystals exhibit dimensions of ≈4 µm × 2.5 µm × 3 µm and are synthesized following a SAC process as described by Machoke et al.[21] and Weissenberger et al.[21] They exhibit a hierarchical pore system combining intrinsic zeolitic micropores of MFI-type structure to a mostly interconnected intracrystalline macropore network of spherical pores with diameters between 250 and 500 nm (Figure 2 and Figure 4). The macropores are induced via “inverse crystallization”[21,23] of the zeolite structure by the dissolution of mesoporous silica spheres (MSPs) during the

![Figure 2](https://example.com/figure2.png)

**Figure 2.** TEM characterization of macroporous zeolite particles. a) SEM overview image of final-state particles. b) High-angle annular dark-field (HAADF) scanning TEM (STEM) image of two particles on a Lacey carbon TEM sample grid. c) Bright field (BF) TEM image of cross-section sample prepared via FIB milling of a macroporous zeolite particle (dispersed on Si substrate and covered by and partly infiltrated with a carbon layer that protected the zeolite from ion damage during FIB preparation). d) HRTEM image of the marked area in (c) showing the microporous MFI-type zeolite framework directly connected to the macropore space—the remaining amorphous contrast at the interface is caused by carbon infiltration during FIB preparation. The indexed diffractogram (FFT) of the HRTEM image in the inset reveals that the MFI crystal is closely oriented along its [010] zone axis.
SAC process, which takes place preferably in the gaps between the MSPs creating the negative pattern of them. Figure S1 of the Supporting Information shows nitrogen adsorption and desorption isotherms of final-state zeolite particle powder which exhibit typical characteristics of a microporous material (IUPAC type 1) with a smaller mesoporous fraction hysteresis (micropore volume 0.173 cm$^{-3}$ and mesopore volume 0.072 cm$^{-3}$). The derived pore size distribution (PSD) shows mesoporosity with a peak at around 26 nm. The mesoporosity is likely due to remaining surface roughness of the macropores and smaller pore channels in between the macropores.

For transmission electron microscopy (TEM) and ET experiments, samples of several µm in size are often too thick. However, in this case, the particles can still be investigated since the mean density of the zeolite particle is relatively low (silicalite-1 mass density 1.76 g cm$^{-3}$[33] with an inelastic mean free path of ≈400 nm or higher for 300 keV electrons[34]) and the porosity caused by the macropores is quite high (27–45%[21,23]). Previous ET investigations showed that the zeolite particles also exhibit open pores toward the particle surrounding,[8a,21] so there is also a good interconnection to the external space around the particles. Since zeolite catalysts are applied as shaped catalyst bodies such as pellets, cylinders, or honeycombs, it is of high importance to investigate further how the intraparticle pores are connected to the interparticle pore space in larger volumes of particle clusters. This cannot be achieved by ET due to the limitation in sample volume. Therefore, Nano-CT is applied to both, single particles (for direct correlation with ET) and larger particle agglomerates.

The particle and pore size of the macroporous zeolite particles have just the right dimensions that both ET and Nano-CT are well applicable (Figure 1). We first applied 360°-ET and lab HR-PC Nano-CT to the identical single-particle samples. This allows for quantitative analysis of the pore space and, at the same time, a direct comparison regarding the 3D reconstruction quality of both techniques. Moreover, the higher resolution 3D data obtained by ET can be used to improve the segmentation of the Nano-CT reconstructions. Finally, the analysis of differently developed zeolite crystals additionally enables to directly investigate the formation process and morphological changes occurring during the SAC synthesis procedure.

2.1. Sample Preparation Using “Stamping Transfer Technique”

For 360°-ET and HR-PC Nano-CT investigations, we prepared fully crystallized, so-called final-state particles, after 96 h of SAC and partially crystallized, so-called transition-state particles, after 48 h of SAC on top of tomography tip plateaus according to the stamping transfer method developed earlier in our group.[8a] Figure 3 exemplarily shows the preparation procedure of one final-state particle. It starts with the selection of a suitable particle on a Lacey carbon TEM grid (Figure 3b), which is mounted onto a micromanipulator inside the SEM instrument. As shown in the sketch of Figure 3c, the identified particle and the tomography tip, which is fixed to the microscope stage below the particle, are approached toward each other. Here, in a previous step, the tomography tip had been tailored with an FIB instrument to obtain a flat plateau matching the particle size (Figure 3a), which facilitates the transfer and avoids shadowing artifacts while tilting the sample (Figure 3e). Then, the particle is transferred from the carbon grid to the tip plateau by bringing them in direct contact. Here, the adhesive forces of the particle toward the tip have been enhanced by putting some SEM-compatible glue onto the tip plateau, which is hardened by additional e$^-$-beam exposure after the particle transfer. The transferred particle on the tip plateau (Figure 3d,e) is mounted onto the 360°-ET sample holder or the stage of the Nano-CT instrument, so that corresponding tilt series in a tilt-angle range of 180° can be acquired without shadowing from the tip or any substrate.

![Figure 3](image-url). Stamping transfer sample preparation of final-state zeolite particle: a) SEM image in top-view of a tomography tip with a plateau tailored via FIB milling to fit the size of one particle. b) One selected representative final-state zeolite particle. c) Sketch of the set-up inside the SEM chamber to realize the stamping transfer. d) SEM image (perspective view) and e) HAADF STEM image of the zeolite particle from b) on top of the plateau of a tomography tip.
2.2. 360°-ET and HR-PC Nano-CT of Final-State Zeolite Particle

At first, final-state particles were used to directly compare 360°-ET and Nano-CT regarding their 3D reconstruction performance with respect to a macroporous material of rather low density. We mention here that the 3D shape and pore structure of such particles have already been analyzed in some detail in previous ET and PXCT experiments. The final-state particle in Figure 3b,d,e, which had been prepared using the stamping transfer technique, was reconstructed from 360°-ET and Nano-CT tilt series and corresponding volume renderings are depicted in Figure 4a,b, respectively. The tilt series, animated slicings through the reconstructed volumes and volume renderings of the particles from the 360°-ET and HR-PC Nano-CT experiments are provided as Videos S1–S4 of the Supporting Information. Both applied 3D techniques consistently reveal the main morphological features of the zeolite particles and the two volume renderings of the reconstructed particle look astonishingly similar (Figure 4a,b). Corresponding macropores are clearly identifiable in both slices through the 3D datasets (Figure 4c,d) and most of the pores form an open-porous, interconnected pore network (Figure 4e). It is remarkable that it is possible to investigate such large structures of several µm in size using ET and that the same sample can be investigated using Nano-CT showing comparable results. A closer qualitative comparison of corresponding slices through the 360°-ET and HR-PC Nano-CT reconstructions shows that the ET dataset exhibits more detailed spatial information and more constant intensities of the pore space and zeolite regions. This is supported by the quantification of the spatial resolution of the 3D reconstruction. The Fourier shell correlation (FSC) analysis of the 360°-ET dataset determines a resolution of 16.4/22.3 nm at half-bit/one-bit criterion which is almost a factor of two better than the 30.4/38.6 nm calculated for the HR-PC Nano-CT dataset (Table 1; Figure S8, Supporting Information). Moreover, in the Nano-CT reconstruction the zeolite material shows a less uniform contrast. This can be attributed to the Zernike PC, which leads to edge enhancement and characteristic halo contrast in regions where the density and/or projected thickness of the material and thus the phase shift abruptly changes. One benefit of the correlative approach is that the ET reconstruction with higher resolution and more homogeneous contrast can be used to improve the segmentation of the Nano-CT dataset. In practice, prior knowledge from the ET reconstruction regarding pore sizes, pore wall borders, and porosity was employed to train a machine learning algorithm for improved segmentation of the Nano-CT reconstruction (see Figure S2 of the Supporting Information for more details of this procedure). Without prior knowledge from the ET reconstruction, the pore volume in the Nano-CT dataset is typically overestimated. This can be attributed to halo artifacts in PC images, which affect the image contrast of interfaces, including zeolite–pore interfaces and the particle surface, in an asymmetric and nonlocal way. The PC contrast at the pore–zeolite interfaces typically causes an overestimation of the segmented pore space (Figure S2d, Supporting Information). In the ET-informed Nano-CT segmentation the pore volume is reduced to fit the ET reconstruction. This adaption of the machine learning segmentation algorithm,
Table 1. Overview of the analyzed 3D reconstructions with important parameters of the used acquisition parameters and results.

| Sample            | Technique | Field of view of projection | # of pixels per projection | Pixel size of projection [nm] | # of projections | Acquisition time [s per projection] | FSC resolution of 3D reconstruction (½-/1-bit criterion) [nm] |
|-------------------|-----------|-----------------------------|---------------------------|-------------------------------|-----------------|-------------------------------------|-------------------------------------------------------------|
| Final state       | 360°-ET   | (5.6 µm)^2                  | 1024*1024                 | 5.45                          | 180             | 9.58                               | 16.4/22.3                                                   |
|                   | HR-PC Nano-CT | (16.5 µm)^2     | 1024*1024                 | 16.09                         | 721             | 250                                 | 30.4/38.6                                                   |
| Transition state  | 360°-ET   | (7.9 µm)^2                  | 1024*1024                 | 7.70                          | 180             | 9.58                               | 20.0/24.6                                                   |
|                   | HR-PC Nano-CT | (16.5 µm)^2     | 1024*1024                 | 16.09                         | 901             | 250                                 | 22.4/30.5                                                   |
| Agglomerate       | HR-PC Nano-CT | (16.5 µm)^2     | 1024*1024                 | 16.09                         | 901             | 250                                 | 53.1/62.8                                                   |
|                   | LFOV-PC Nano-CT | (65.4 µm)^2   | 1024*1024                 | 63.89                         | 901             | 100                                 | 89.6/115.0                                                  |

however, leads to an overestimation of the total zeolite volume (Figure S2c, Supporting Information), which causes a systematic underestimation of the total porosity. The porosity of the particle was determined to 13.0% ± 1.3% (360°-ET) and 11.1% ± 1.6% (Nano-CT; the error indicates the approximate underestimation). The result from the ET dataset is in line with previous investigations,[21,23] albeit the porosity of this specific particle is lower than the values stated in the literature. This is most probably a statistical variation among different particles or due to partial zeolite growth into the macropores.[23] A closer inspection of Figure 4c provides details, which point to another explanation for the lower porosity in Nano-CT. The particle exhibits enclosed macropores which are still partly filled with remains from the MSP dissociation. Obviously the inverse transformation process from the SAC into a spherical pore could not be completed. The arrows in the slice through the 360°-ET reconstruction (Figure 4c) indicate enclosed macropores with trapped residuals from the MSPs. These residuals exhibit a darker and less uniform contrast, which can be explained by remaining mesopores from the MSPs causing a lower average mass-density than the main crystalline zeolite structure. The corresponding regions in the HR-PC Nano-CT reconstruction (Figure 4d) also show a drop in intensity, but can only hardly be interpreted without the knowledge from the ET dataset. This underlines the importance of using ET data with higher fidelity to improve the interpretation and segmentation of the Nano-CT dataset.

The quantitative evaluation of the PSD of both segmented 360°-ET and HR-PC Nano-CT datasets using the maximum sphere inscription (MSI) method is depicted in Figure 4e,f (see also Videos S5 and S6 of the Supporting Information for animated slices through the MSI volumes). Both distributions peak at a pore diameter of around 290 nm representing most of the internal macropores. The ET MSI shows another sharp peak around 425 nm, which is due to few internal pores and concave open pores on the surface of the particle with this deviant diameter (dark green to red regions in Figure 4e). The PSD of the Nano-CT dataset shows the most prominent peak at a diameter of about 370 nm between the two peaks of the ET PSD. This can be explained by the more faint contrast and higher contrast fluctuations in the Nano-CT reconstruction leading to an average fading and broadening of peaks (cf. Figure S3, Supporting Information). Besides the main macropore peaks, smaller pore diameters below 200 nm corresponding to the interconnecting pores in the meso- to macroporous regime are revealed. Since the resolution of the HR-PC Nano-CT reconstruction is estimated to 30.4/38.6 nm (FSC half-bit/one-bit criterion), mesopores and smaller macropores cannot reliably be investigated using this method (see discussion in Section 2.5). In both ET and Nano-CT PSDs, peaks at larger diameters >450 nm (Figure S5a, Supporting Information) are mainly caused by the external empty space around the particles that was also included in the MSI analysis. The outside of the particles exhibits partly concave surfaces and inside-facing edges which for both techniques contribute to pores with smaller pore diameters in the PSD. Figure S4a,b of the Supporting Information shows a different type of PSD representation of both the 360°-ET and HR-PC Nano-CT datasets depicting the approximate number of pores assigned with a certain pore diameter (green curve). In this type of representation, it is more evident that the peak around 425 nm belongs to a small amount of larger pores. For both the 360°-ET and HR-PC Nano-CT datasets many smaller pores (∼200 nm) are present. These can be partly attributed to connecting pores. Moreover, the less spherical the pores are and the rougher their pore surface is, the broader the resulting PSD appears. As in the Nano-CT dataset the higher contrast fluctuations lead to a broader distribution of smaller pore diameters (Figure S4b, Supporting Information) than in the 360°-ET PSD (Figure S4a, Supporting Information).

2.3. 360°-ET and HR-PC Nano-CT of Transition-State Zeolite Particles

The applied SAC process is a stunning method to obtain zeolite crystals with intrinsic microporosity directly connected to spherical, intracrystalline macropores with adjustable size[35] (Figure 2c,d). Even though previous studies already proposed a formation mechanism based on observations by SEM, nitrogen physisorption measurements, and X-ray diffraction analyses,[21,23] a direct proof via a 3D investigation of zeolite crystals in a transition state of the SAC process has not been given yet. To realize the latter, both 360°-ET and HR-PC Nano-CT were applied to reconstruct the 3D morphology of zeolite particles in a transition state (Figure 5b–e). Two particles on a tip plateau were prepared using the particle stamping transfer method (Figure 5a). Videos S7–S10 of the Supporting Information show the tilt series, animated slicings through the reconstructed volumes and volume renderings of the particles from the 360°-ET and HR-PC Nano-CT experiments, respectively. The 3D reconstructions from both nondestructive techniques consistently reveal the main features of this even larger sample (compared...
to the final-state particle, cf. Figure 4), e.g., the characteristic morphology of the zeolite particles with the attached MSPs and the mostly spherical macropores. Most interestingly, remaining parts of MSPs can be observed that have not yet or only partly been dissolved and transformed into the inverse zeolite structure. The partly dissolved MSPs show remaining spherical, dense silica shells (Figure 5d,e) indicating that the SAC process consumed the inner parts of the MSPs first by transporting these parts to the MSP surface, where the silica crystallizes into the MFI crystalline bulk phase. Our observations in the 3D reconstructions support the model of macropore formation during the SAC process as described by Machoke et al. [21] and Weissenberger et al. [23]. The final-state crystals consequently resemble the final result of the SAC procedure, where the MSPs are entirely consumed and inversely transformed into the MFI zeolite crystal structure. Most notably, the MSPs act as a template for the resulting spherical macropores which adopt their shape with almost no shrinkage in size.

The insets in Figure 5d,e show enlarged sections through the 360°-ET and Nano-CT reconstructions, respectively, both consistently revealing the hollow sphere of a partly dissolved MSP and its surrounding region. Regarding Nano-CT this is quite amazing given the fact that the shell thickness already approaches the resolution limit of the instrument. The 360°-ET reconstruction profits from the higher resolution of electron microscopy and therefore shows finer details. For instance, the thin pore wall with a thickness of around 50 nm in the lower left corner of the inset of Figure 5d is reconstructed by 360°-ET, but is not visible in the corresponding Nano-CT slice in the inset of Figure 5e. On the other hand, the total size of the sample which consists of two zeolite particles is already close to the upper size limit of what can be studied with decent resolution by scanning TEM (STEM) at 300 kV acceleration voltage.

These qualitative observations are in accordance with the determined spatial resolution using the FSC method. The ET reconstruction supports the model of macropore formation during the SAC process as described by Machoke et al. [21] and Weissenberger et al. [23]. The final-state crystals consequently resemble the final result of the SAC procedure, where the MSPs are entirely consumed and inversely transformed into the MFI zeolite crystal structure. Most notably, the MSPs act as a template for the resulting spherical macropores which adopt their shape with almost no shrinkage in size.

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The Nano-CT PSD with a broader peak around 450 nm indicates that pores where MSP residuals are present are partly overestimated. This finding can also be observed in the slice through the reconstruction in Figure 5e. Since the pore regions with MSP residuals exhibit a lower mean density than the main zeolite crystal, the resulting darker contrast in the Nano-CT reconstruction is misleadingly interpreted as pores. Above that, the higher contrast fluctuations in the Nano-CT reconstruction lead to a generally broadened peak in between 200 and 500 nm. This is also apparent in the alternative representations of the PSDs in Figure S4c,d of the Supporting Information, which show the approximate number of pores assigned with a certain pore diameter. For both the 360°-ET and HR-PC Nano-CT datasets, many smaller pores (<200 nm) are present. This can be attributed to pore connections and pores in a transition state containing remains from the MSPs. The porosity was determined to 14.9% ± 1.5% (360°-ET) and 11.2% ± 1.6% (Nano-CT; refer to the Experimental Section and Figure S2 of the Supporting Information for an explanation and illustration of this systematic underestimation of the porosity). The generally higher value compared to the final-state particle results from the additionally analyzed interparticle pore space between the two particles (Figure 5d–f). Another reason might be the statistical variation between different particles.

2.4. HR- and LFOV-PC Nano-CT of Final-State Zeolite Particle Agglomerate

To investigate a statistically more relevant number of final-state zeolite particles and their respective intra- and interparticle pore space, a particle agglomerate was prepared on top of a suitable Nano-CT needle. The agglomerate exhibits a height of ≈60 µm and a diameter of ≈40 µm (Figure 6a) suitable for LFOV Nano-CT. Since the agglomerate was directly transferred as a larger piece of the dry particle powder, the packing density is expected to be lower than, for instance, in a compressed pellet. Due to this and the high particle porosity, the sample with an X-ray attenuation length at 5.4 keV for nonporous silicalite-1 of about 52 µm does not generate sufficient contrast for AC Nano-CT imaging, thus necessitating PC Nano-CT.

Figure 6a,b shows a volume rendering of the LFOV-PC Nano-CT 3D reconstruction and a virtual slice through the reconstruction (see Videos S13 and S14 of the Supporting Information for the tilt series and an animation of the reconstruction). The agglomerated zeolite crystals with their internal, mostly spherical macropores are revealed in the reconstruction. Beyond that, the tomogram provides unique insight into the packing behavior of the particles.

Figure 6c compares two corresponding slices through the HR and LFOV Nano-CT reconstructions of the particle agglomerate.
(see Videos S16 and S17 of the Supporting Information for the HR tilt series and an animation of virtual slices through the HR reconstruction). The sample region imaged with the HR scan is indicated with a red rectangle at the top of the LFOV reconstruction in Figure 6a. Both slices exhibit comparable features. Since the HR Nano-CT volume has a higher fidelity, it was used to enhance the segmentation precision of the LFOV reconstruction in a similar way as for the ET-supported HR Nano-CT segmentation. The segmented LFOV Nano-CT reconstruction was then used to extract the PSD of the intra- and interparticle pore space of the particle agglomerate utilizing the MSI method (refer to Video S15 of the Supporting Information for animated slices through the MSI volume). The resulting PSD in Figure 6d exhibits a broadened peak in between 150–300 nm, which represents the internal macropores in accordance with the results from the HR Nano-CT and 360°-ET measurements of a final-state particle (Figure 4). This proves that the macropores are well quantifiable using LFOV-PC Nano-CT. The corresponding PSD is in good agreement with the results from measurements at higher resolution, but now averaged over dozens of zeolite particles. The extended PSD of the LFOV Nano-CT reconstruction in Figure S7a of the Supporting Information shows pore diameters up to 13 µm. Here, values of up to ~300 nm belong to the intraparticle pores, between about 300–2500 nm to the interparticle pore space and above 2500 nm to the external space around the particle agglomerate as demonstrated in Figure S6 of the Supporting Information. These ranges are only approximate values and also overlap with each other. The FSC analysis of the LFOV Nano-CT dataset determines a resolution of 89.6/115.0 nm at half-bit/one-bit criterion (cf. Table 1; Figure S8, Supporting Information). These results impressively demonstrate that an LFOV Nano-CT reconstruction enables the full quantitative examination of both the main internal macropores and the pore space between the agglomerated particles.

2.5. Comparison of 360°-ET and Nano-CT Results

Table 1 gives an overview of all tomography datasets analyzed in this work with important parameters of both the raw data and the 3D reconstructions. The 360°-ET experiments necessitate much shorter acquisition times than the lab-based Nano-CT experiments due to a higher signal-to-noise ratio. To obtain a comparable measure for the spatial resolution, we applied the same FSC method to all 360°-ET, HR-PC Nano-CT, and LFOV-PC Nano-CT datasets. In Table 1, the resolutions determined from the corresponding FSCs (Figure S8, Supporting Information) using the half-bit and one-bit criterion are listed. Whereas the half-bit criterion is recommended for cryo-ET datasets, the one-bit criterion is rather suitable to analyze X-ray crystallography data. The resulting trend is as expected, calculating the best spatial resolution for the 360°-ET experiments, followed by HR and LFOV Nano-CT. In the case of 360°-ET, the sampling rate of the pixel size in the tilt-series projections with respect to the FSC resolution complies with the Nyquist–Shannon sampling theorem, so that the spatial information is sampled with sufficient accuracy. For both Nano-CT acquisition modes, this is not the case, even when using the one-bit criterion for the FSC resolution. Since, moreover, the obtained values of the FSC resolution are smaller than the nominal optical resolution limit for the used Nano-CT instrument of about (50 nm)³ for the HR mode and (150 nm)³ for the LFOV method, the FSC resolution has to be treated with care. It does not necessarily correspond to the actual spatial 3D resolution, but rather gives a measure to compare 3D reconstruction quality. Potential reasons for the overestimation of the FSC resolution is its calculation using sub-tomograms reconstructed from sub-tilt series partitioned from the aligned full tilt series and PC artifacts which enhance edge features and pretend an increased resolution. The determined FSC resolution of about (16 nm)³ to (20 nm)³ for the ET tomograms might surprise since STEM tomography generally allows down to nm or even atomic resolution for small samples like nanoparticles. However, the µm-sized zeolite particles lead to multiple scattering of the penetrating electrons and therefore a broadening of the STEM probe allowing for only a moderate 3D resolution. This circumstance renders Nano-CT competitive to ET for samples of this scale.

A comparison of the resulting PSDs determined from the MSI analyses of all datasets is given in Figure S7b of the Supporting Information. The PSDs ascertain that all applied techniques can reconstruct the internal macropores of the zeolite particles, all exhibiting a main peak around 200–450 nm. LFOV Nano-CT further allows to bridge scales by investigating larger particle agglomerates and the corresponding interparticle pore space with pore diameters in between 300 and 2500 nm.

3. Conclusion

Correlative Nano-CT and 360°-ET is a powerful tool to study the 3D structure of particulate and porous materials across different length scales, as exemplarily demonstrated in this work for the model system of macroporous zeolite structures. The scale-bridging nanotomography analyses enable to obtain reliable measures for the PSD and porosity, and, in particular, confirm the formation mechanism of macropores during the SAC process.

Herein, ET allows the analysis of a small number of particles in one measurement. Benefits are a high spatial resolution of, in this case, about (16 nm)³ or better and an easy-interpretable contrast achieved by high-angle annular dark-field (HAADF) STEM imaging. Furthermore, ET analyses can be complemented by a plethora of available TEM techniques, including electron diffraction for crystallographic information, HRTEM to image the micropore structure (Figure 2) or analytical TEM for chemical analyses. Nano-CT unveils its strength when particle agglomerates or, in general, larger structures are investigated, thus allowing for the analysis of a statistically more-relevant number of particles at a spatial resolution down to about (50 nm)³. We showed that 360°-ET and HR Nano-CT enable the investigation of the same sample of several µm thick, and we explored the limits of both techniques in terms of sample size and resolution, since the few-particle samples are big for STEM tomography and rather small for Nano-CT. One significant advantage of this correlative approach is to take high-resolution information to enable a segmentation with improved fidelity of a larger volume acquired at a lower...
resolution. Future experiments could focus on the acquisition of several neighboring HR Nano-CT tilt series to cover the complete agglomerate sample, thus significantly extending the field of view up to about (100 µm)$^3$ at nearly constant spatial resolution (cf. Figure 1). This means that the field of view of one LFOV Nano-CT measurement, where the spatial resolution is limited to about (150 nm)$^3$, could be covered by several stitched HR-Nano-CT datasets, similar to the mosaic tomography approach reported for synchrotron Micro-CT by Vescovi et al.$^{[39]}$ In the same way, this approach could be applied to LFOV Nano-CT even further extending the sample volume to larger sizes, still providing a sufficiently high resolution to reliably reconstruct macropores. Thus, LFOV Nano-CT bridges to Micro-CT in terms of volume size and resolution enhancing the opportunities for scale-bridging 3D analyses of larger samples.

Since the applied methods are nondestructive, the samples may be employed for additional investigations using complementary analysis techniques or set-ups for in situ or ex situ experiments. Lab Nano-CT reaches almost the same spatial resolution as synchrotron Nano-CT. However, the latter technique enables shorter acquisition times, which are beneficial for in situ and operando experiments like in situ X-ray pychography.$^{[5d,40]}$ operando Micro-CT of Li-ion battery materials,$^{[36,41]}$ interrupted in situ Nano-CT of mechanical testing$^{[42]}$ in situ and operando Nano-CT of fuel cell electrodes$^{[43]}$ or environmental in situ Nano-CT of a fuel cell.$^{[44]}$ Similar experiments can also be realized on lab Nano-CT instruments, albeit at lower temporal resolution or by interrupted in situ experiments, where before and after an experiment, e.g., mechanical testing$^{[45]}$ or in situ heating$^{[46]}$ Nano-CT datasets are collected. In addition, future work should focus on a finer analysis of Nano-CT imaging artifacts by addressing the above-mentioned Zernike PC artifacts for the used lab Nano-CT instrument with X-ray energies of 5.4 keV, as examined for other lab and synchrotron Nano-CT and Micro-CT set-ups.$^{[9,47]}$ Since pores with sizes >50 nm are typically not accessible using nitrogen physisorption measurements (see Figure S1, Supporting Information), future experiments should include mercury porosimetry to obtain relative measures for the intracrystalline macropores as well as the larger interparticle pore space of for instance particle agglomerates or larger amounts of particles pressed to pellets for catalytic applications.

In summary, we demonstrated the potential of combining 360°-ET and lab-based Nano-CT for correlative 3D studies of hierarchical porous materials with dimensions spanning over several length scales covering pores sizes from the meso- to the larger macropore range. Our approach does not only enhance the range of accessible sample sizes from about (10 nm)$^3$ to (60 µm)$^3$, but also exploits 3D reconstructions with higher fidelity to improve the reconstruction quality of larger field-of-view scans enabling quantitative analyses of statistically relevant volumes.

4. Experimental Section

Particle Synthesis: The studied macroporous MFI-type zeolite particles were provided by Weissenberger and co-workers from the Institute of Chemical Reaction Engineering (FAU Erlangen-Nürnberg, Germany). Details about the synthesis are listed as follows and can further be found in ref. [21]. Here, 0.25 g calcined MSPs were impregnated with a mixture of 0.28 g aqueous 40 wt% tetrapropylammonium hydroxide and 0.07 g of an 40 wt% aqueous tetrapropylammonium bromide solution and dried at 313 K for 2 h. SAC of the impregnated powder was carried out at 383 K for 96 h to obtain a fully crystallized final-state sample and 48 h to obtain the partially crystallized transition-state sample. The solid product was recovered by centrifugation, washed three times with a water/ethanol mixture and dried overnight at 348 K. To remove the structure directing agent, the samples were calcined at 823 K for 6 h under airflow (heating ramp 1.2 K min$^{-1}$).

Nitrogen Physisorption: $N_2$ sorption isotherms of final-state zeolite particle powder were measured at 77 K by using a Quantachrome Autosorb AS-1. Before the measurement, the sample was degassed at 523 K for 12 h under vacuum. The pore size distribution was calculated using a density functional theory model for cylinder pores in silica and is displayed as differential pore volume distribution representation dV(t) in cm$^3$ Å$^2$ g$^{-1}$ with pore radius r.

Particle Preparation via Stamping Transfer for 360°-ET and HR Nano-CT: Samples for the stamping transfer were prepared by dispersing powders containing silicate-1 zeolite particles (final-state or transition-state) onto standard Lacey carbon TEM grids (200 mesh). The preparation of the tomography samples with one or two particles on top of the tip plateau was conducted using the direct particle stamping transfer routine$^{[46]}$ using an FEI Helios Nanolab 660 DualBeam FIB instrument equipped with a micromanipulator (MM3A, Kleindiek Nanotechnik GmbH). Standard 360° electron tomography tips (E.A. Fischione Instruments, Inc.) were milled with the Ga+ ion beam (30 kV acceleration voltage) in the FIB instrument down to sizes of a few µm to match the distinct particle sizes, respectively. FIB milling was performed in top and side view to ensure a flat plateaus surface. The micromanipulator was further equipped with a 360° rotational axis (tilt-angle step size 0.1°) controllable plug-in tool (ROTIP-EM, Kleindiek Nanotechnik GmbH), which allows precise adjustments and flexible sample positioning inside the SEM instrument chamber, and a gripper at its end, which enables to clamp the TEM grid with the dispersed particles. SEM-compatible glue (SEM GLU, Kleindiek Nanotechnik GmbH) was placed on top of the tip plateaus using a standard tungsten needle micromanipulator (FEI Easy Lift) before the particle transfer to enhance the adhesion forces toward the tip to enable the direct transfer of the particles from the Lacey carbon film of the TEM grid.

Particle Agglomerate Preparation for LFOV Nano-CT Measurements: The particle agglomerate used for the Nano-CT experiments shown in Figure 6 was transferred onto the top of an iron needle$^{[46]}$ using the following approach. First, the particle powder containing particle agglomerates of different size was distributed onto a glass plate. Then, a round-shaped sticky carbon tab (EM-Tec high purity conductive double sided adhesive carbon tab), which is typically used for SEM/ FIB applications, was cut in half and the straight edge was dipped into the particle powder on the glass plate using tweezers to pick up several agglomerates. After a first scan in the Nano-CT instrument (mosaic of single projections in LFOV-PC imaging mode) of the agglomerates attached to the carbon-pad edge, one agglomerate with suitable shape and size was selected. Finally, it was carefully transferred onto an iron needle covered with prehardened UV light sensitive glue under observation using the Zeiss prealignment microscope, which comprises two light microscopes with a micromanipulation stage each. During the transfer process the glue is completely cured under light irradiation of the light microscope.

TEM Characterization: A cross-section sample of a macroporous zeolite particle (taken from a synthesized batch as described by Machoke et al.$^{[21]}$) dispersed onto a Si wafer was prepared by FIB milling (FEI Helios Nanolab 660 FIB instrument). The samples were investigated using bright field TEM and HRTEM imaging using a both probe- and image-side aberration-corrected FEI Titan$^3$ Themis 60–300 transmission electron microscope operated at 300 kV. ET: The tomography tips with the attached particles were mounted onto a Fischione Model 2050 On-Axis Rotation Tomography holder (E.A. Fischione Instruments, Inc.), which allows the acquisition of tilt
series within a full tilt-angle range of at least 180°. 360°-ET of the zeolite particle was carried out on a both probe- and image-side aberration-corrected FEI Titan® Themis 60–300 transmission electron microscope at an acceleration voltage of 300 kV in HAADF STEM imaging mode. The semiconvergence angle of the STEM probe (microprobe STEM imaging mode) was reduced to about 0.5 mrad to increase the depth of field to be able to image all parts of the sample entirely in focus throughout the entire tilt series acquisition procedure.[57] The diffraction-limited resolution regarding a semiconvergence angle of 0.5 mrad is 2.4 nm. The zeolite particles in a transition state were recorded with 13 kx magnification, 1024×1024 pixels, and a pixel size of 7.70 nm; and the top part of the particle at 18 kx magnification, 1024×1024 pixels and a pixel size of 5.45 nm. There is a further decrease of resolution due to a broadening of the STEM probe with increasing sample thickness (mainly due to multiple elastic scattering).[52] which results in estimated STEM probe diameters of about 15–25 nm (one particle) and 20–50 nm (two particles) when leaving the sample. The camera length was reduced to 91 mm (collection-angle range 61–200 mrad) to obtain mainly mass–thickness contrast conditions (Rutherford scattering) and to keep nonlinear contrast contributions (diffraction contrast) very low. Since the unit cell of the MFI-type zeolite crystals is rather large, most diffraction spots exhibit little diffraction angles, which are not covered by the HAADF detector. The tilt series were acquired using the FEI Tomography 4.0 software in a tilt-angle range of 180° with 1° tilt increment, continuous and linear tilting scheme, and autofocus and tracking before the acquisition. For each 180° tilt series, two subsequent tilt series were acquired in a tilt-angle range of ±55° each. After the acquisition of the first tilt series, the sample was rotated by 90° along the tilt-axis using the mechanical rotation mechanism of the sample holder. In the next step, the tilt series were merged using Imag[57] and FEI Inspect 3D software. Overlapping and duplicate projections were deleted from the merged tilt series. Tilt-series alignment of the final-state particle tilt series was performed using FEI Inspect 3D software (cross-correlation technique). The Imagej plugin “linear stack alignment with scale-invariant feature transform (SIFT)”[52] was used to align the tilt series of the transition-state particle sample. The nonlinear sample thickness–intensity relationship in HAADF STEM imaging, especially for samples with increased mass–thickness,[53] can lead to so-called cupping artifacts in electron tomographic reconstructions.[54] In the case of the investigated MFI-type zeolite crystals, up to a thickness of about 300 nm, a linear relationship between thickness and the measured STEM signal using synthesized MFI-type zeolite crystals with only intrinsic micropores and without additional macropores (see Figure S9, Supporting Information) was able to be determined. A reliable thickness–intensity relationship for thicknesses larger than 300 nm was not able to be measured. However, the projected thickness of the macroporous zeolite particles often exceeds 300 nm and a nonlinear decrease in the HAADF STEM signal is expected since, without correction, slight cupping artifacts were present in preliminary 3D reconstructions of thicker sample parts. According to Van den Broek et al.[54] the thickness-corrected values for the projected intensity I measured in every pixel of each projection and with incident electron beam intensity I₀ measured on the HAADF STEM detector. The thickness-corrected values for the projected intensities I were used as input to reconstruct the 3D volumes. 3D reconstruction was performed employing the simultaneous iterative reconstruction technique (SIRT)[55] algorithm (100 iterations) implemented via an in-house Python script based on the Astra-Toolbox.[56] Avis Vision4D, combined with Imagej,[57] was used for visualization, segmentation, and quantitative 3D analysis (see below in section Data Processing and Analysis).

Nano-CT: Nano-CT was performed using a lab-scale ZEISS Xradia 810 Ultra X-ray microscope equipped with a 5.4 kV rotating anode Cr-source and a Zernike phase ring for (PC imaging. Two magnification modes can be selected, either LFOV or HR mode, allowing imaging with a field of view (FOV) of 65.4 µm x 65.4 µm or 16.5 µm x 16.5 µm with optical resolutions down to 150 nm (pixel size of 63.89 nm) or 50 nm (pixel size of 16.09 nm), respectively. The tomography samples with one or two zeolite particles completely fit into the FOV of the HR mode. The tomography sample with the particle agglomerate was prepared for fit into the FOV of the LFOV mode. The Nano-CT tilt series contain a total number of 721 (HR-PC Nano-CT of final-state particle) and 901 (HR-PC Nano-CT of transition-state particles, LFOV-PC Nano-CT) projections over the full tilt-angle range of 180° with an acquisition time of 250 s per projection (HR) and 100 s per projection (LFOV), respectively. The tilt series were acquired in the native ZEISS microscope software (XMCcontroller and Scout&Scan). The Imagej plugin “linear stack alignment with SIFT”[52] was used to align the tilt series, except for the transition-state sample and the HR-PC Nano-CT tilt series of the top part of the sample. In those cases, the preliminary tilt series alignment of the ZEISS microscope software based on cross-correlation was already suitable for reconstruction. 3D reconstruction was performed employing the SIRT algorithm[55] (100 iterations) implemented via an in-house Python script based on the Astra-Toolbox.[56] ET reconstructions utilized the non-negative constraint provided by the Astra-Toolbox to improve the reconstruction quality.[54] Avis Vision 4D and Imagej[57] were used for visualization, segmentation, and quantitative 3D analysis.

Data Processing and Analysis: Data processing and analysis were realized by a combination of in-house Java and Python coding together with Imagej[54] and the commercial software package Avis Vision4D. The machine learning algorithm based on[57] the software Arivis Vision4D was employed to segment the pore space of all Nano-CT reconstructions. Since the Nano-CT data are recorded in PC mode, halo-like edge-enhancement artifacts complicate the thresholding process. To suppress the influence of these artifacts, the reconstructed Nano-CT data were preprocessed in two steps in Arivis Vision 4D by iterative machine learning-based segmentation.[57] First, the zeolite phase was selected and segmented as bulk material. Then, the internal and open surface pores were segmented as pore space, separated from the external pore space. The open, concavely shaped surface pores were segmented by manually, but machine-learning supported, separating the external space around the zeolite from the internal pore space by drawing virtual lines (or surfaces) between the salient, confining zeolite features. Like this, it was possible to separate the halo artifacts around the zeolite from the actual pore space, since both features exhibit similar image intensities and a simple segmentation induced huge errors. Where a 3D reconstruction of the same sample at higher magnification was presented, the resulting segmentation was refined by using this prior knowledge within the machine learning routine, as depicted in Figure S2 of the Supporting Information. In the case of the HR-PC Nano-CT datasets, by adjusting the pore space with respect to the prior knowledge from the 360°-ET reconstructions, the previously overestimated pore space is corrected, but the zeolite volume at the same time is slightly overestimated. This leads to a systematic underestimation of the determined porosity for the HR-PC Nano-CT datasets of both the final-state and transition-state sample. The error due to this underestimation is estimated by finally adjusting the overestimated zeolite volume on the basis of the corresponding 360°-ET reconstructions. Segmentation of the 360°-ET datasets was performed using the Imagej segmentation function “convert stack to binary” using the IsoData Iterative procedure based on the isodata algorithm.[58] The internal pores were separated from the external pore space for porosity determination by an additional machine-learning segmentation in Arivis Vision 4D, as described above for the Nano-CT datasets. The segmented volumes were used to calculate porosity values and pore size distributions (PSDs). The PSDs were calculated using the maximum sphere inscription (MSI) technique[59] utilized by utilizing the (Imagej) plugin “local thickness.”[60] For the MSI analyses, the external pore space and the internal pores were included. The MSI determines in a binarized dataset for each voxel the largest inscribable sphere (pore diameter) into the pore space before touching the pore walls. Relative frequencies of assigned pore diameters in the resulting PSDs (cf. Figures 4f, 5g, and 6d; Figure S6, Supporting Information) are calculated by dividing the number of voxels assigned with a certain MSI pore diameter by the total voxel number in a certain pore diameter range. The relative frequency representation is most comparable to
the differential pore volume distribution representation known from gas sorption methods. The PSD representation showing the number of pores (cf. Figure S4, Supporting Information) is calculated by multiplying the number of voxels, which are assigned with a certain MSI pore diameter $d$, with the volume of one voxel divided by the volume occupied by one sphere with the respective assigned MSI pore diameter $d$, which is calculated by $4/3 \pi (d/2)^3$. One spherical pore contributes to a sharp peak in the PSD with a value close to 1 at its corresponding pore diameter. The less spherical the pores are, e.g., elongated pore channels, and the rougher their pore surface is, the broader is the resulting PSD. Since in this calculation cubic voxels are divided by spheres, the smaller the MSI diameter, the larger the deviation of the resulting number of pores from an integer value.

Porosity values have been determined using the segmented datasets in which the internal pores had been separated from the external pore space by dividing the number of internal pore voxels by the number of total voxels (zeolite and pore volume). The resolution of the reconstructed volumes was estimated by using the FSC method,[16,38] using the half-bit and one-bit criterion (Figure S8, Supporting Information). Sub-tomograms were reconstructed using the SIRT algorithm from sub-tilt series of even and odd members partitioned from the aligned full tilt series, respectively. In the case of the 360°-ET transition-state dataset and the HR-PC Nano-CT tilt series from the top and the MSI diameter, the larger the deviation of the resulting number of pores from an integer value.

Supporting Information
Supporting Information is available from the Wiley Online Library or from the author.

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Conflict of Interest
The authors declare no conflict of interest.

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