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# Supplementary Information

# Porous ceramic monoliths assembled from microbeads with high specific surface area for effective biocatalysis

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***Zeta-potential:***

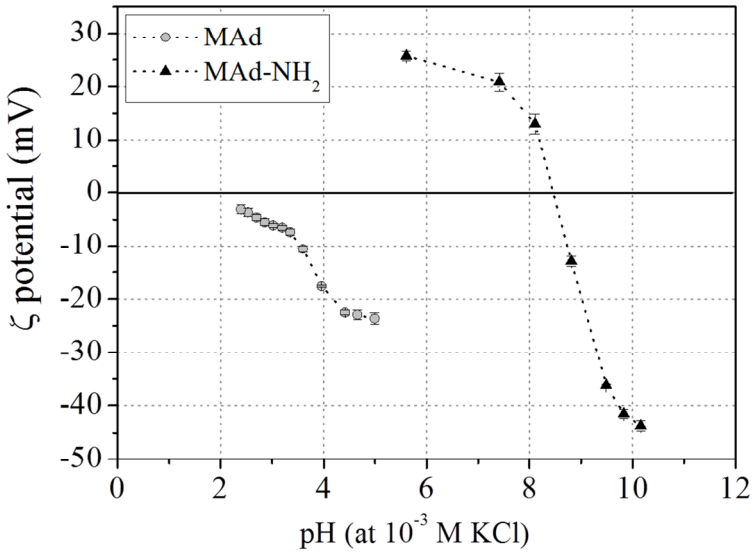


**Figure S.1:** Zeta-potential curves of non-functionalized and amino-functionalized samples, obtained via streaming potential measurements.

***MAds surface roughness***

**Table S.1** summarizes the results of the profilometer surface roughness measurements. The results were obtained from measuring 3 different MAds, for each MAd 10 independent microbeads were measured, using the topography mode of the profilometer including a correction for carved surfaces. The measuring area was 255*185 µm.

**Table S.1:** Surface roughness data for MAds sintered at $T_{sinter}$ = 1000 °C.

| | Roughness $R_a$ [µm] | Highest Hight [µm] | Deepest Deep [µm] |
|---|---|---|---|
| average* | 4.3 ± 2.8 | 27.1± 18.9 | 34.0 ± 29.5 |

*average of 30 single measurements.

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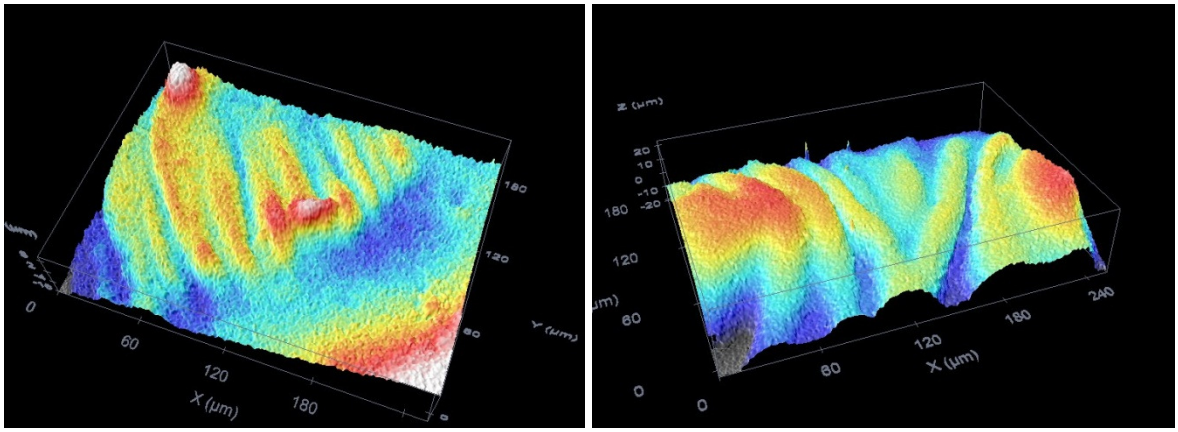


**Figure S.2**: Examples of surface roughness measurements.

### *Adsorption of model molecules*

**Figure S.3a-c** depicts examples of non-functionalized and APTES functionalized MAds before and after adsorption of model dye molecules. Methylene blue adsorbs to non-functionalized MAds while acid orange adsorbs to $NH_2$-functionalized MAds. Virtually no BL adsorption was observed for $NH_2$-functionalized MAds, and AO did not adsorb to non-functionalized MAds. **Figure S.3d** and **e** illustrate the distribution of the utilized model dye molecules within the whole cross-sectional area of single MBs while

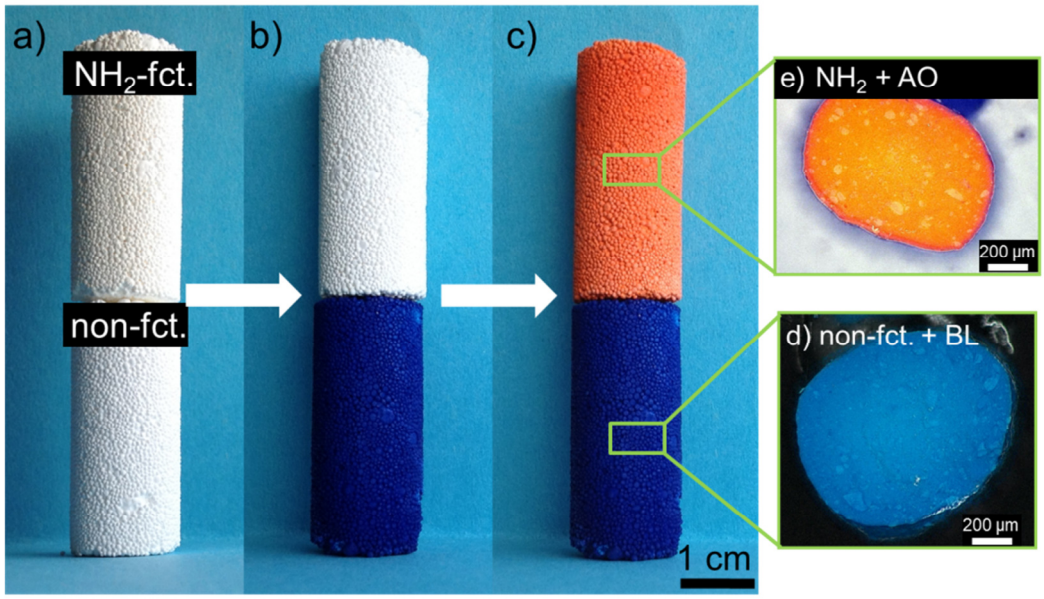


**Figure S.3:** Selective adsorption of model dye molecules on MAds: a) amino-functionalized and non-functionalized MAds before adsorption; b) after adsorption of methylene blue (BL); c) after adsorption of acid orange (AO); d and e) microscopic cross-sections of single beads after dye adsorption.

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### *Laccase immobilization:*

Laccase belongs to the family of multicopper oxidases. Multicopper oxidases contain one type 1 copper ion (T1), which is reported to be the first oxidation site during substrate transformation, and at least 3 other coppers (T2, T3). During catalytic reaction, the substrate binds to a cavity close to the Cu-T1 site.[1]

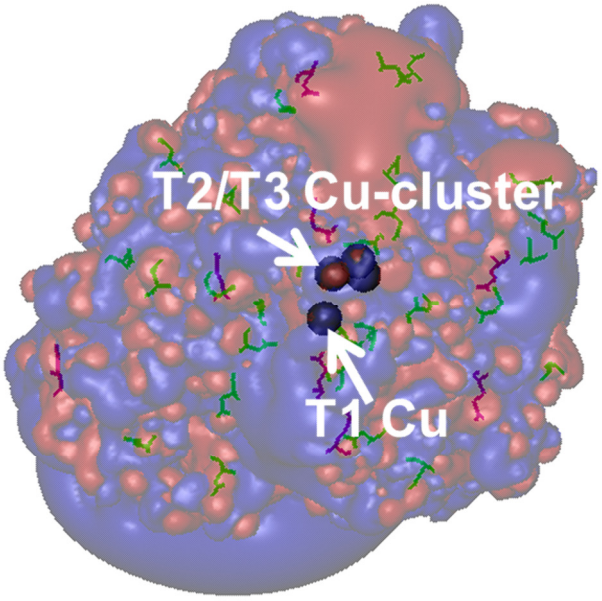


**Figure S.4:** Spatial surface potential distribution of laccase from *Trametes versicolor* calculated by PDB2PQR, APBS and VMD from the RCSB PDB data file 1GYC; negative areas (red), positive areas (blue) with Cu sites (grey spheres), and glutamic acid residue (purple) and aspartic acid residue (green) locations.[2-3; 5-6]

For covalent immobilization amino functionalized MBs/MAds were used. The linker molecules used for covalent immobilization (EDC/sulfo-NHS) activate enzymes carboxyl groups, the latter can then form amide bonds with primary amines [4] which covalently bind the enzyme to an aminated surface. Carboxyl groups vacant for EDC/NHS coupling are provided by aspartic acid (ASP) and glutamic acid (GLU) residues.[1] The schematic representation of laccase within **figure S.4** gives the locations of ASP and GLU. No carboxyl groups are present in the positively charged region of the enzyme, suggesting that the orientation of EDC/sulfo-NHS linked laccase on MBs/MAds will differ from that of non-specific adsorbed laccase.

### *Performance of immobilized laccase:*

To test the long term applicability of immobilized LAC under flux conditions one LAC-MAd was inserted to the measuring cell that was used for the permeability measurements. First, 50 mL of fresh buffer were pumped through the measuring circuit to remove excessive or loosely bound LAC. Then a 20 µM solution of ABTS was pumped continuously through the monolith and the absorbance at 420 nm of the eluate was measured. **Figure S.5** depicts the change in absorbance due to enzyme catalyzed ABTS decompositions over a time period of 8 h.

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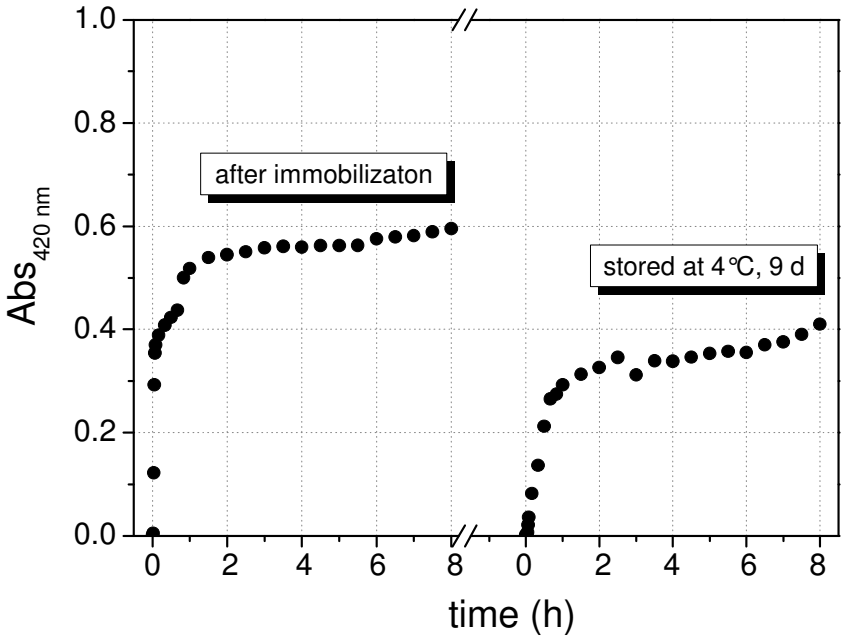


**Figure S.5:** Absorbance vs. time for laccase catalyzed degradation of ABTS over time.

After the first 8 h the sample was thoroughly rinsed with fresh buffer and stored in a fridge at 4°C for 9 d. After 9 d the test was repeated with the same sample. Laccase immobilized on MAds remains active under continuous flux conditions for at least 16 h (2 * 8 h + storage 9 d). The activity for the second run is approx. 30-40% less, compared to freshly immobilized LAC.

***References:***

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