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Supporting Information

Highly Active Enzymes Immobilized in Large Pore Colloidal Mesoporous Silica Nanoparticles

Dorothée Gößl, Helena Singer, Hsin-Yi Chiu, Alexandra Schmidt, Martina Lichtnecker, Hanna Engelke and Thomas Bein*

Department of Chemistry and Center for NanoScience (CeNS), University of Munich (LMU), Butenandtstr. 5 – 13(E), 81377 Munich, Germany

*To whom correspondence should be addressed: bein@lmu.de

Supporting Figures

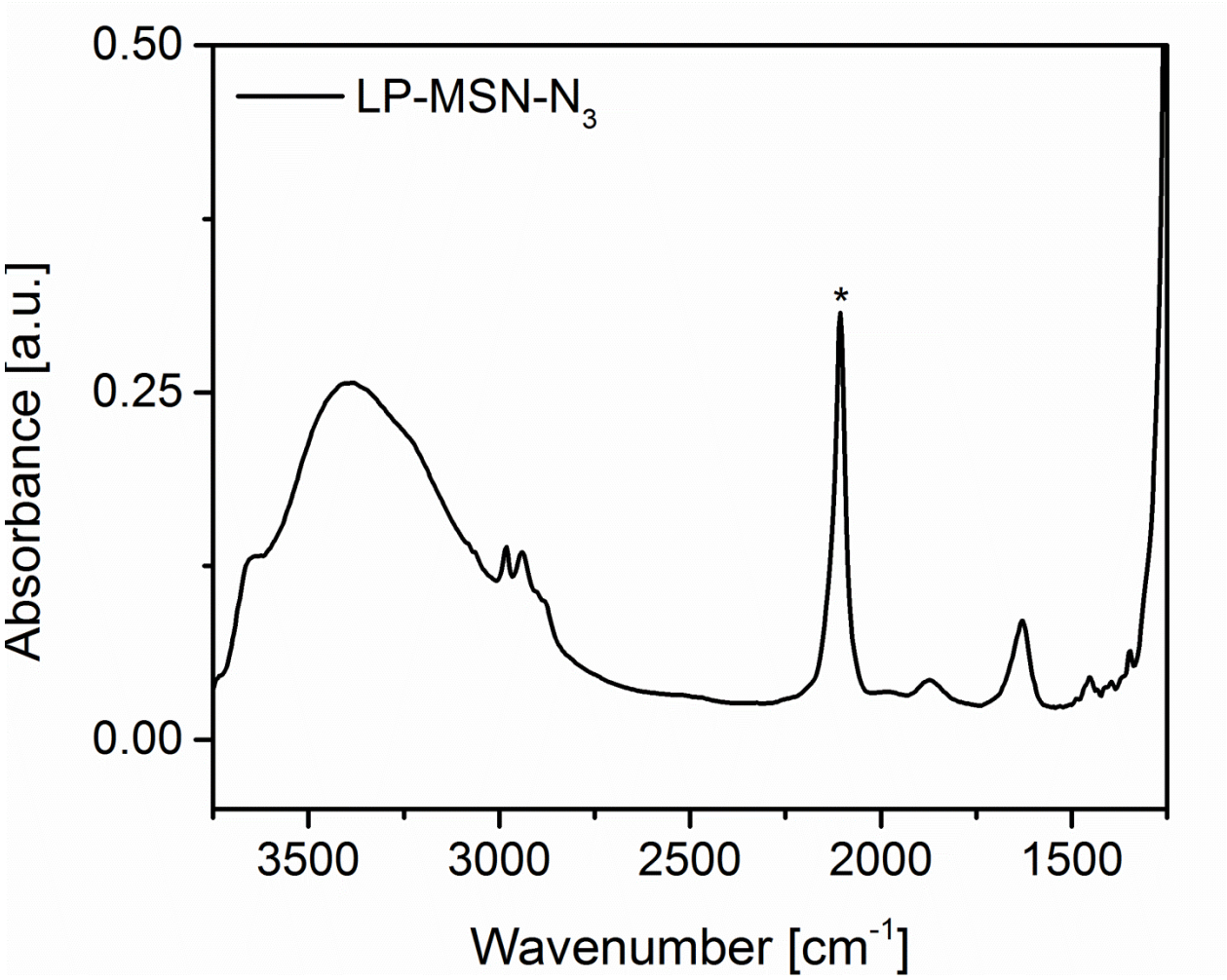

Figure S 1: Infrared spectrum of LP-MSN-N_3. The asterisked peak indicates the typical vibration arising from the azide-functionality.

IR spectroscopy was carried out to verify the presence of azide moieties in LP-MSN-N_3. The asymmetric CH_2 stretching vibrations of the azidopropyl-moieties are visible between 2990 cm^{-1} and 2940 cm^{-1}. Furthermore, the CH_2 bending vibration appears at 1451 cm^{-1}. The asterisked strong vibration at 2105 cm^{-1} is attributed to the stretching vibration of the N_3-groups. The vibration at 1633 cm^{-1} appears with lower intensity and can be attributed to the vibrations of residual amounts of water in the sample. Vibrations of the silica framework are visible below 1300 cm^{-1} with strong intensities.

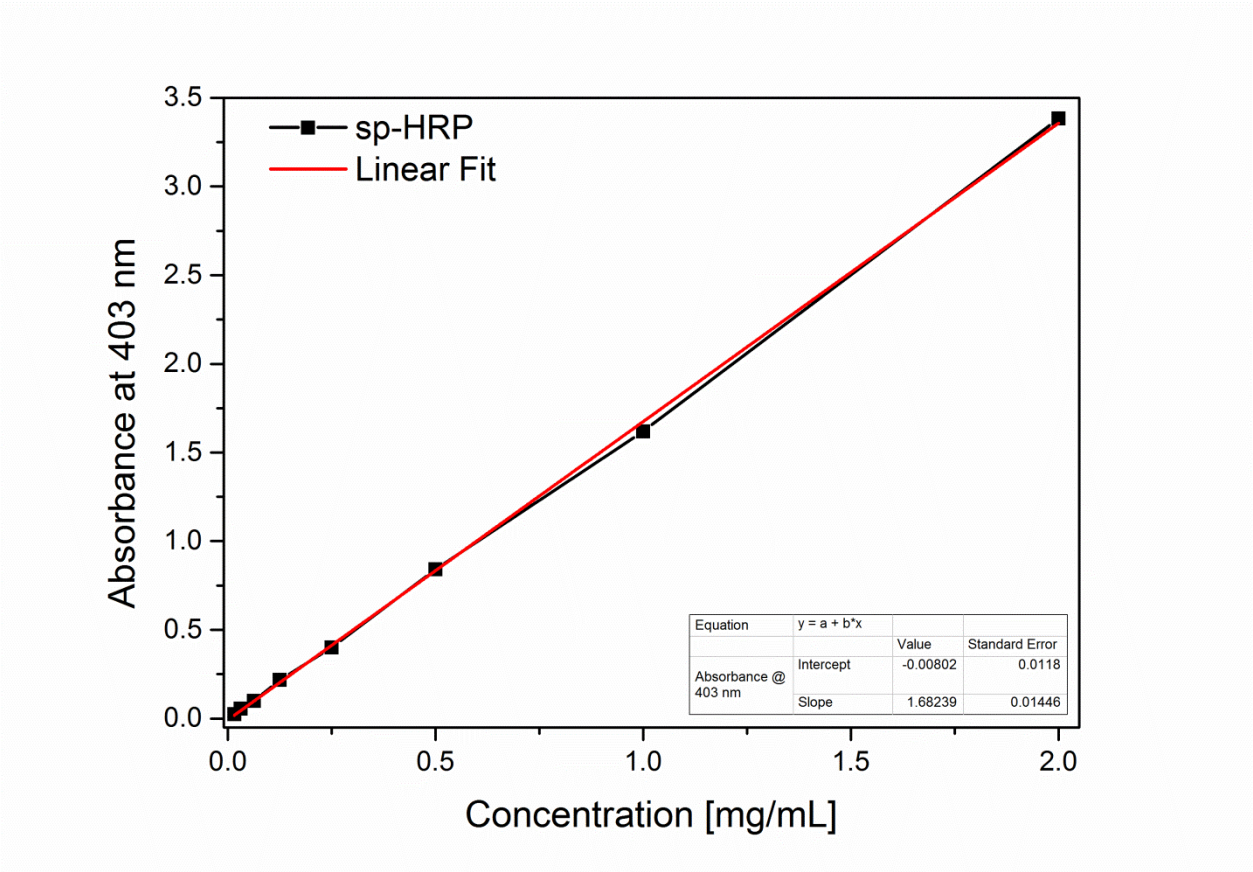

Figure S 2: Calibration curve of sp-HRP. Absorbance of different concentrated solutions of alkyne-functionalized horseradish peroxidase was measured at 403 nm *via* UV-Vis and fitted linearly with Origin 9.0 (inserted box).

To estimate the amount of sp-enzyme attached on the solid support, thermogravimetric analyses of the samples LP-MSN-N_3, LP-MSN-CA and LP-MSN-HRP were performed (Figure 2c). From the additional mass loss of 11.5% for the sample LP-MSN-CA the amount of attached enzyme can be estimated to be 4.7 µmol per g silica (for calculation see Calculation A1 below). This indicates that 0.5% of all azide moieties have reacted with sp-CA, as the amount of azide-moieties was estimated to be 1.02 mmol per g silica. From the additional mass loss of 9.4%, the amount of immobilized HRP can be estimated to be 2.6 µmol per g silica (see Calculation A1) and the amount of azide-moieties can be estimated at 1.02 mmol per g silica. Horseradish peroxidase is sterically somewhat more demanding than CA due to its larger dimensions (4.0 x 4.4 x 6.8 nm^3)[1] and was only able to react with 0.25% of all azide moieties of LP-MSN-N_3.

Calculation A1: Example for the calculation for thermogravimetric analysis (TGA) of sample LP_MSN-N_3 and LP_MSN-CA

1 g sample LP_MSN-N_3 contains 77 mg azide-residues (mass loss 9.2%, M_w ($C_3H_6N_3$) = 81,05 $gmol^{-1}$ 5:1 M_w (C_6H_5) = 77,1 $gmol^{-1}$→ mass loss for azide-moieties 7.7%)

$$\frac{77\ mg}{(100\% - 7.7\%)\ g} = 83\ mg\ azide - residues\ /\ g\ silica$$

$$\frac{0.083\ g}{81.05\ \frac{g}{mol}} = 1.02\ mmol\ azide - residues\ /\ g\ silica$$

1 g sample LP_MSN-CA contains 115 mg CA (additional mass loss 11.5%, M_w(CA) = 30 000 $gmol^{-1}$)

$$\frac{115\ mg}{(100\% - 7.7\% - 11.5\%)\ g} = 142\ mg\ CA\ /\ g\ silica$$

$$\frac{0.142\ g}{30\ 000\ \frac{g}{mol}} = 4.7\ \mu mol\ CA\ /\ g\ silica$$

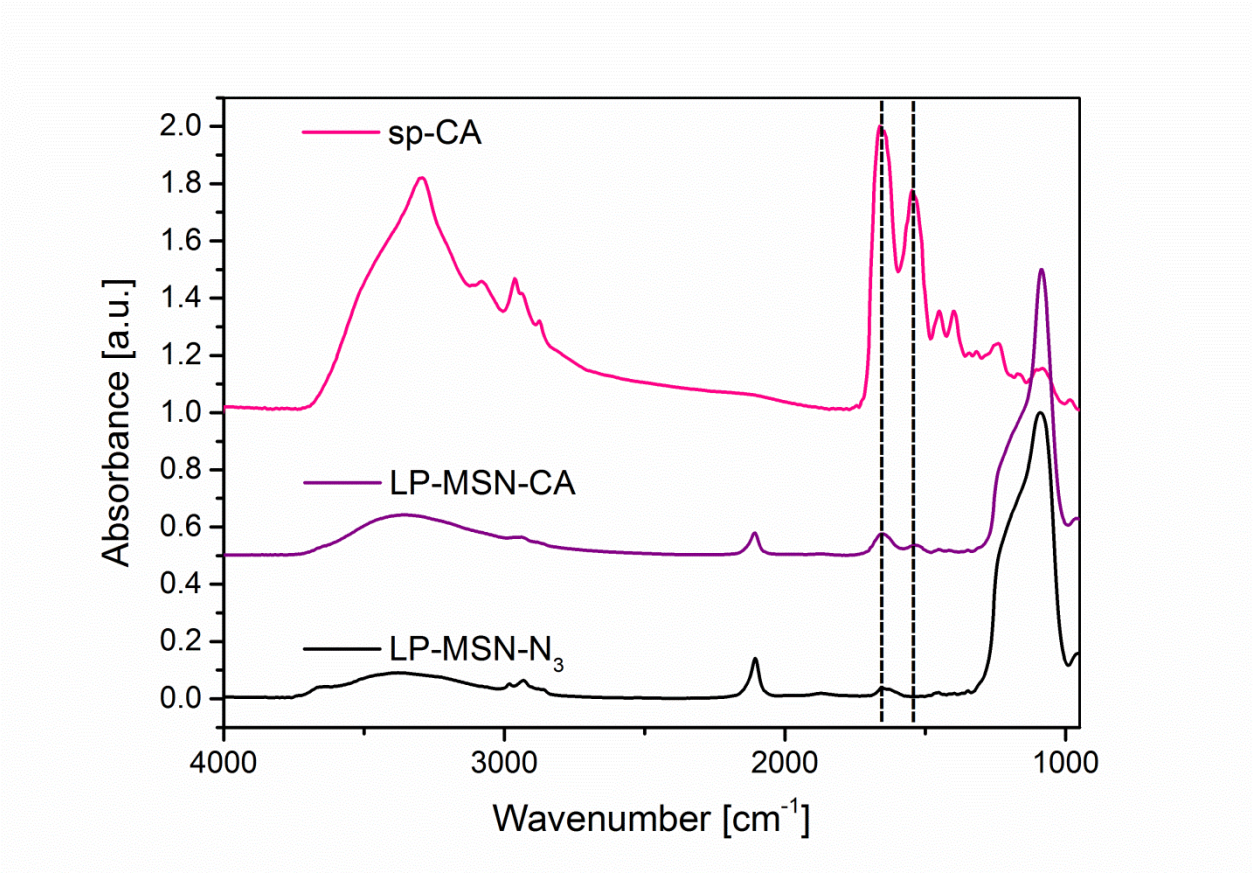

Figure S 3: IR spectra of sp-CA (pink), LP-MSN-CA (purple) and LP-MSN-N_3 (black). The spectrum of LP-MSN-CA was shifted along the y-axis by a value of 0.5 and the spectrum of sp-CA by a value of 1.5 for clarity reasons. The peak at 1658 cm^{-1} (dashed line) can be attributed to the C=O stretching vibration and that at 1530 cm^{-1} (dashed line) to the N-H bending vibration of the peptide bonds in sample LP-MSN-CA. Due to the large portion of unclicked azide-groups inside the particles, the N_3-stretching vibration at 2105 cm^{-1} can be seen in both samples – the particles without and with carbonic anhydrase.

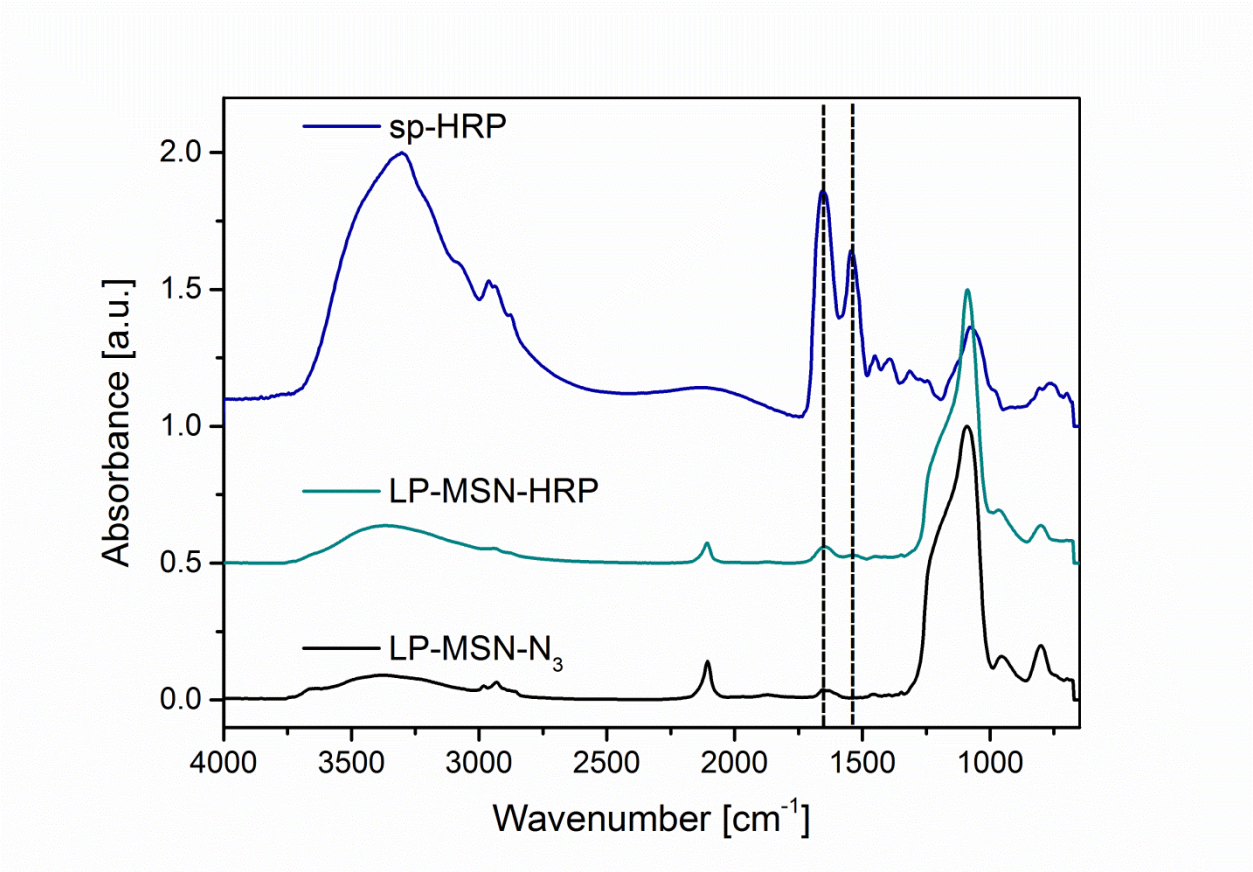

Figure S 4: IR spectra of sp-HRP (blue), LP-MSN-HRP (cyan) and LP-MSN-N_3 (black). The spectrum of LP-MSN-HRP was shifted along the y axis by a value of 0.5 and the spectrum of sp-HRP by a value of 1.25 for clarity reasons. The modes at 1658 cm^{-1} (dashed line) can be attributed to the C=O stretching vibration and those at 1534 cm^{-1} (dashed line) to the N-H bending vibration of the peptide bonds in sample LP-MSN-HRP. The stretching of the azide-moieties can also be observed at 2105 cm^{-1} (see explanation above).

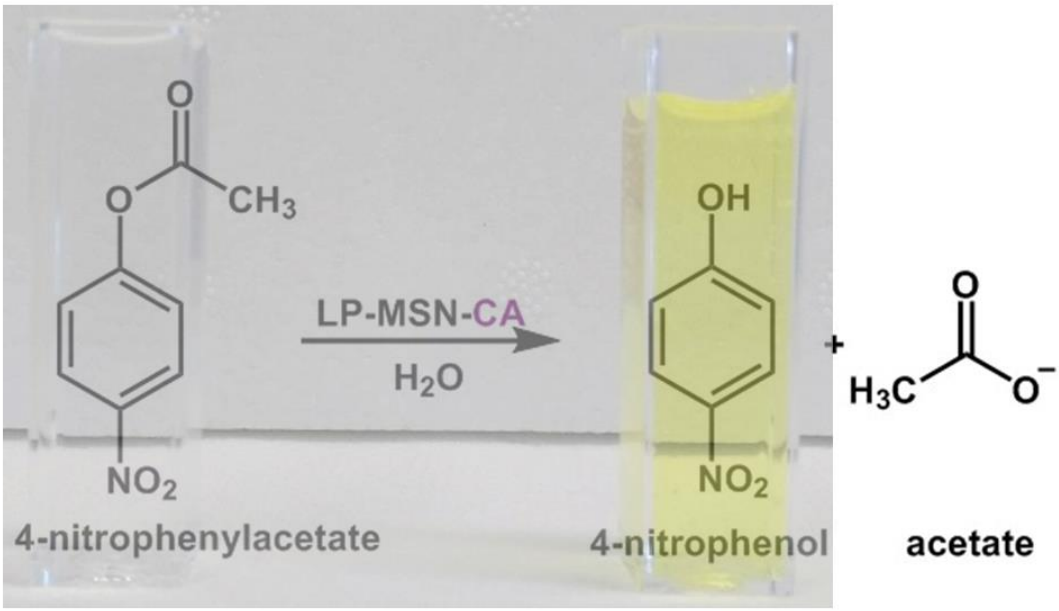

Figure S 5: Image and reaction scheme of catalytic activity assay for immobilized carbonic anhydrase. On the left: Sample LP_MSN-CA at the time points t = 0 sec (colourless) and t = 120 sec after the start of the hydrolysis reaction of 4-nitrophenyl acetate (4-NPA) in water and TRIS buffer (pH 8).

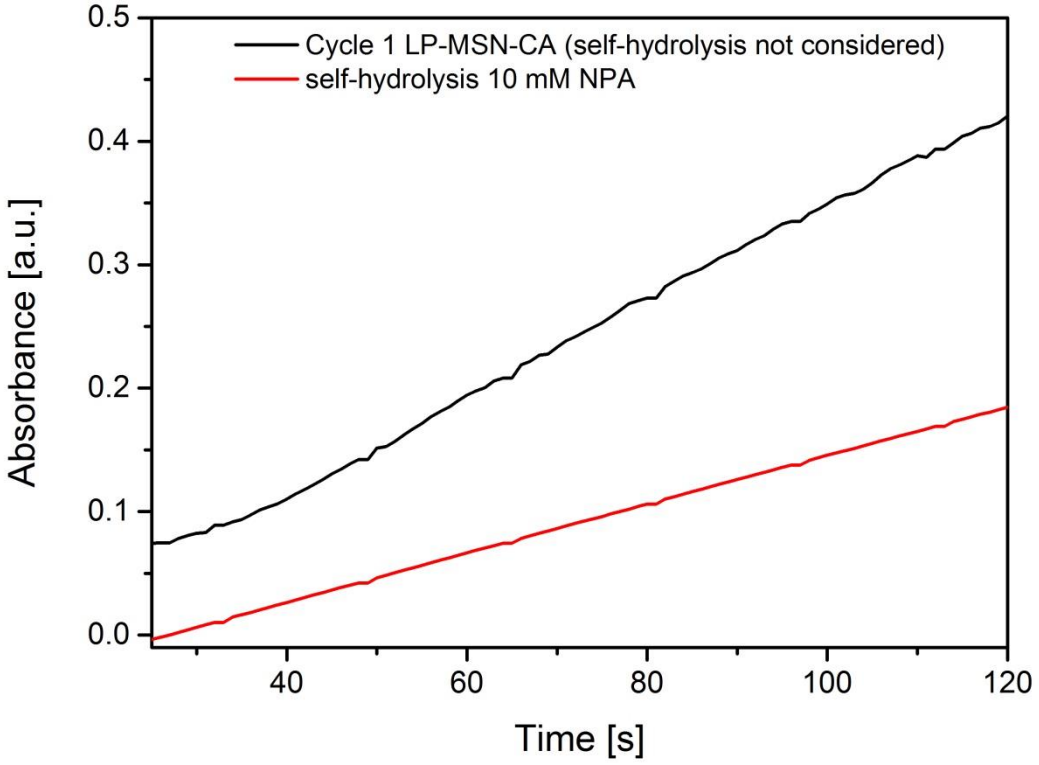

Figure S 6: Comparison of 4-NPA's self-hydrolysis and catalytic performance of LP-MSN-CA. UV-Vis absorption measurement of LP-MSN-CA (Cycle 1; black curve) within a period of 120 s at 400 nm with 10 mM NPA and TRIS buffer versus 10 mM NPA only and TRIS buffer (red curve).

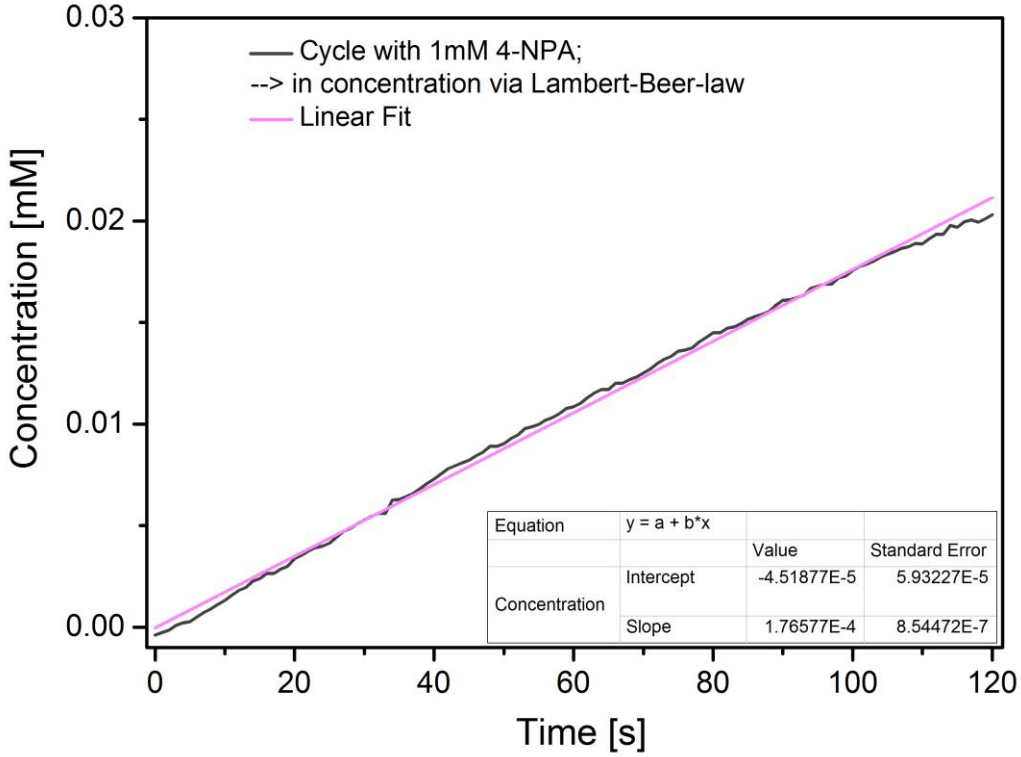

Figure S 7: Activity determination for LP-MSN-CA. UV-Vis absorption measurement of LP-MSN-CA within a period of 120 s at 400 nm with 1 mM NPA converted into concentration vs. time via Lambert-Beer law ($\varepsilon = 16\,300\ M^{-1}\ cm^{-1}$).[2] The rate of 4-NPA's self-hydrolysis is considered and already subtracted from the measured curve (not shown here).

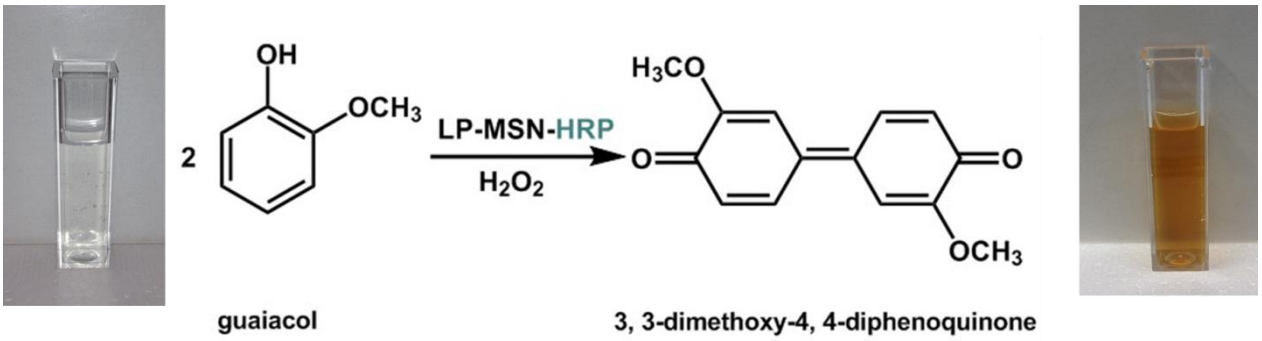

Figure S 8: Image and reaction scheme of catalytic activity assay for immobilized horseradish peroxidase. On the left: Colourless mixture of particles (LP-MSN-HRP) and guaiacol solution which is oxidized under addition of hydrogen peroxide to a light brownish coloured diphenoquinone.

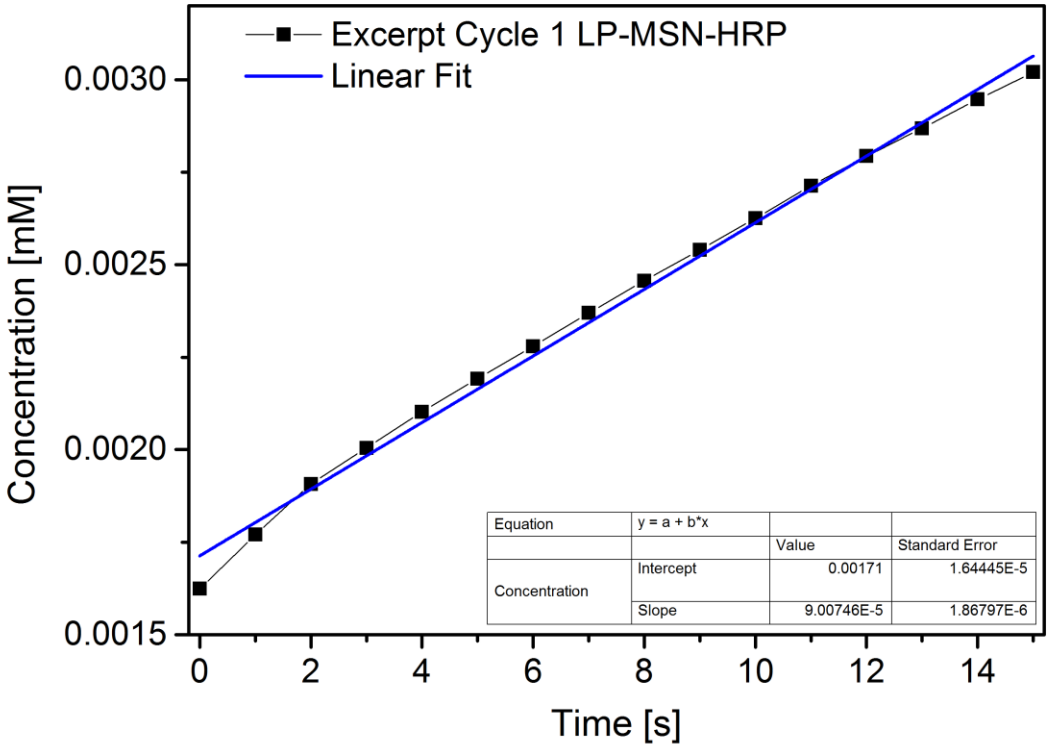

Figure S 9: Activity determination for LP-MSN-HRP. Activity determination of LP-MSN-HRP by measuring the absorbance at 470 nm and converting the linear region of the first cycle into concentration via Lambert-Beer law (ε = 26.6 $mM^{-1}cm^{-1}$).[3]

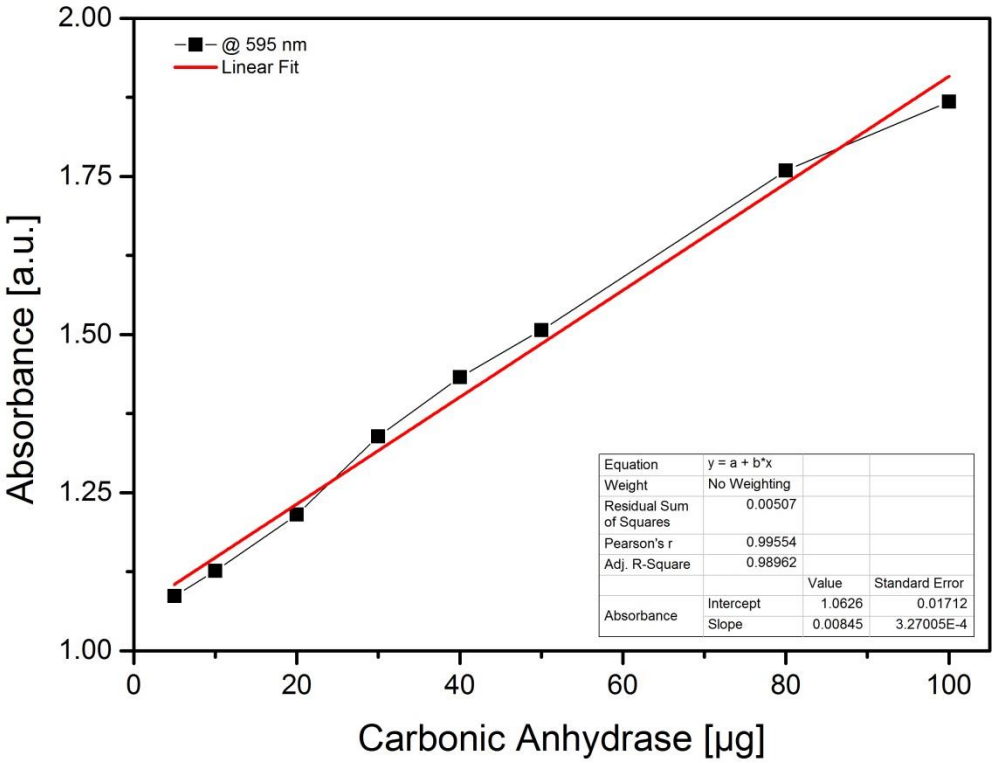

Figure S 10: Standard curve of carbonic anhydrase for Bradford assay.[4-5] The amount of immobilized carbonic anhydrase determined via the standard curve (shown above) is 3.4 µmol CA per g silica. In comparison to the via TGA determined amount of 4.7 µmol CA per g silica a deviation of almost 28% is asserted. This deviation is attributed to additional components in the channels of the mesoporous silica that contribute to weight changes during TGA measurements.

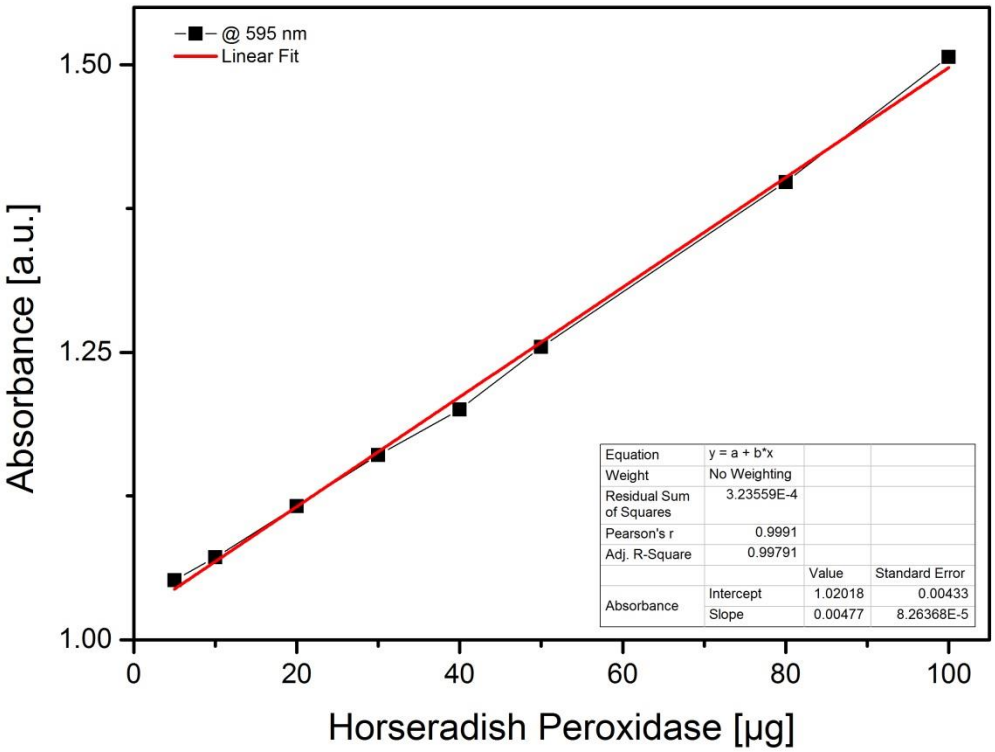

Figure S 11: Standard curve of horseradish peroxidase for Bradford assay. In the case of immobilized horseradish peroxidase the amount determined with the Bradford assay differs very little in comparison to carbonic anhydrase. Evaluation of the protein assay results in 2.4 µmol HRP per g silica whereas the amount determined with TGA is 2.6 µmol HRP per g silica which corresponds to a deviation of only ~ 8%.

The expressed activity of the biocatalysts was determined according to the following definitions:

1. Carbonic anhydrase: One international unit of activity (IU) was defined as the amount of enzyme that hydrolyzes 1 µmol of 4-NPA per liter per minute under the conditions described in the manuscript.

The resulting activity of immobilized carbonic anhydrase is 8,4 units (per 5 mg MSNs) i.e. 1680 units / g support. The respective amount of free enzyme shows an activity of 85,6 units.

2. Horseradish peroxidase: One international unit of peroxidase activity is defined as the

amount of enzyme catalyzing the oxidation of 1 µmol of guaiacol per liter per minute under the conditions described in the manuscript.

The resulting activity of immobilized horseradish peroxidase is 0,86 units (per 0,16 mg MSN) i.e. 5343 units / g support. The respective amount of free enzyme shows an activity of 3,7 units.

References

1. Hudson, S.; Cooney, J.; Magner, E., Proteins in mesoporous silicates. *Angew. Chem., Int. Ed. Engl.* **2008,** *47,* 8582-8594.
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3. Maehly, A. C.; Chance, B., The assay of catalases and peroxidases. *Methods Biochem. Anal.* **1954,** *1,* 357-424.
4. Bradford, M. M., A Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein-Dye Binding. *Anal. Biochem.* **1976,** *72,* 248-254.
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