Supplementary Information

Co-immobilization of enzymes with the help of dendronized polymers and mesoporous silica nanoparticles

Hanna Gustafsson[§a], Andreas Küchler[§b], Krister Holmberg[a], Peter Walde[b]*

§ Equal contributions

[a] Applied Surface Chemistry, Department of Chemical and Biological Engineering, Chalmers University of Technology, SE-412 96 Gothenburg, Sweden

[b] Polymer Chemistry, Department of Materials, ETH Zürich, Vladimir-Prelog-Weg 5, CH-8093 Zürich, Switzerland

* Corresponding author: email: *peter.walde@mat.ethz.ch*

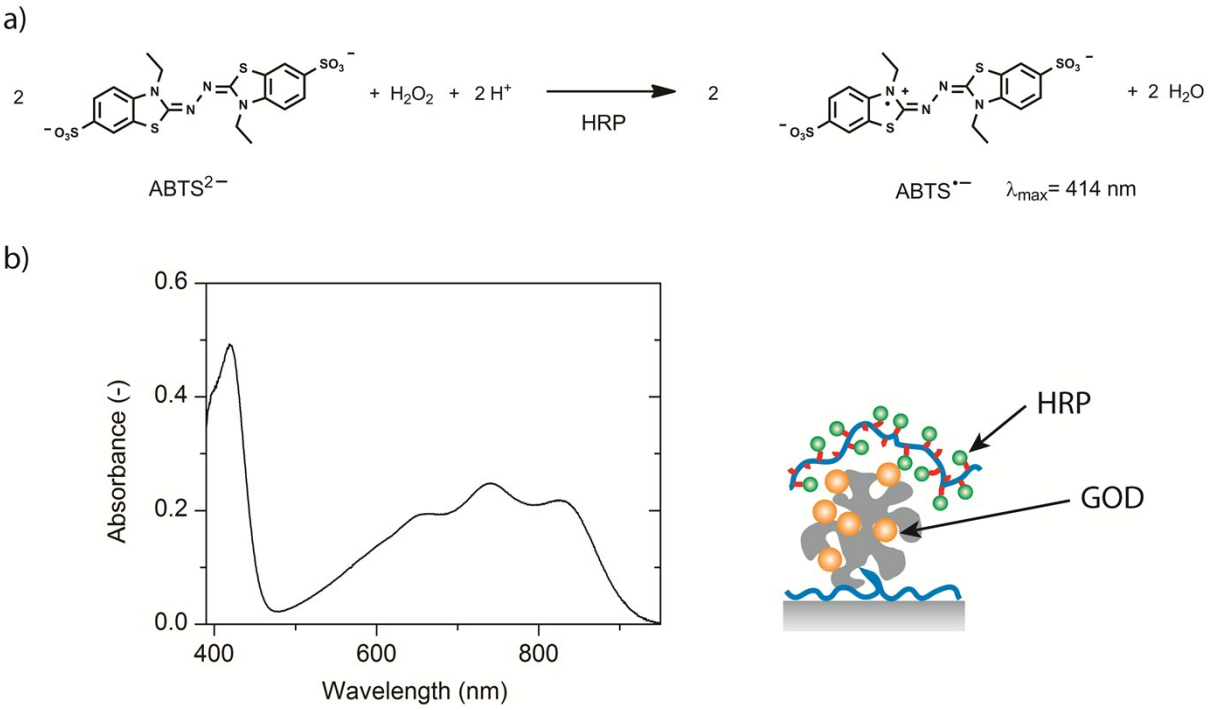

Figure S1

a) Activity assay for the quantification of HRP: Oxidation of $ABTS^{2-}$ with H_2O_2 is catalyzed by HRP, and the $ABTS^{\bullet-}$ formed is quantified by UV/vis spectrophotometry. Using a PBS solution of 1 mM $ABTS^{2-}$ and 0.2 mM H_2O_2 (10 mM phosphate, 150 mM NaCl, pH 7), a linear correlation of the HRP concentration in solution to the measured activity allows the quantification of HRP (see Ref. [S1]).

b) Example of a spectrum recorded for the quantification of the HRP activity on a microscopy glass coverslip with adsorbed *de*-PG2, HMM particles loaded with GOD and a covering layer of *de*-PG2-BAH-HRP. The glass coverslip was immersed in the substrate solution for 40 seconds and after removal the $ABTS^{\bullet-}$ concentration in the assay solution was determined by UV/vis spectrophotometry. By comparing the increase in absorbance at λ = 414 nm with a calibration curve prepared with known amounts of HRP in solution under the same conditions (Ref. [S1]) the HRP activity per surface area was determined.

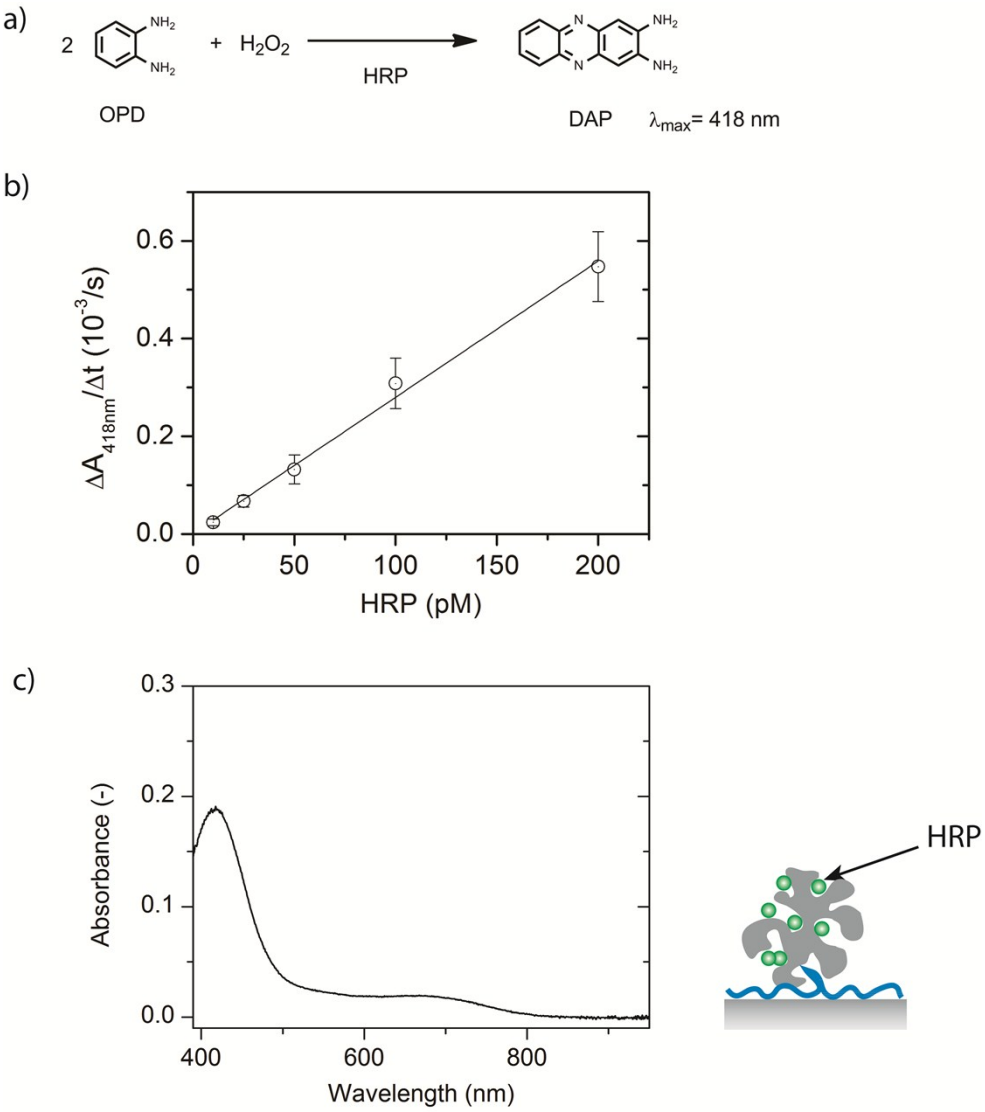

Figure S2

a) Activity assay for the quantification of HRP: Oxidation of OPD with H_2O_2 is catalyzed by HRP, and the DAP formed is quantified by UV/vis spectrophotometry.

b) Calibration curve prepared with known amounts of HRP and a substrate solution of 3.14 mM OPD and 80 µM H_2O_2 solution in PBS (10 mM phosphate, 150 mM NaCl, pH 7). A linear correlation of the HRP concentration in solution to the measured activity allows the quantification of unknown amounts of HRP.

c) Example of a spectrum recorded for the quantification of the HRP activity on a microscopy glass coverslip with adsorbed *de*-PG2 and HMM particles loaded with HRP. The glass coverslip was immersed in the substrate solution for 20 minutes and after removal the DAP concentration in the assay solution was determined by UV/vis spectrophotometry. By comparing the increase in absorbance at λ = 418 nm with the calibration curve prepared with known amounts of HRP in solution under the same conditions − see b) − the HRP activity per surface area was determined.

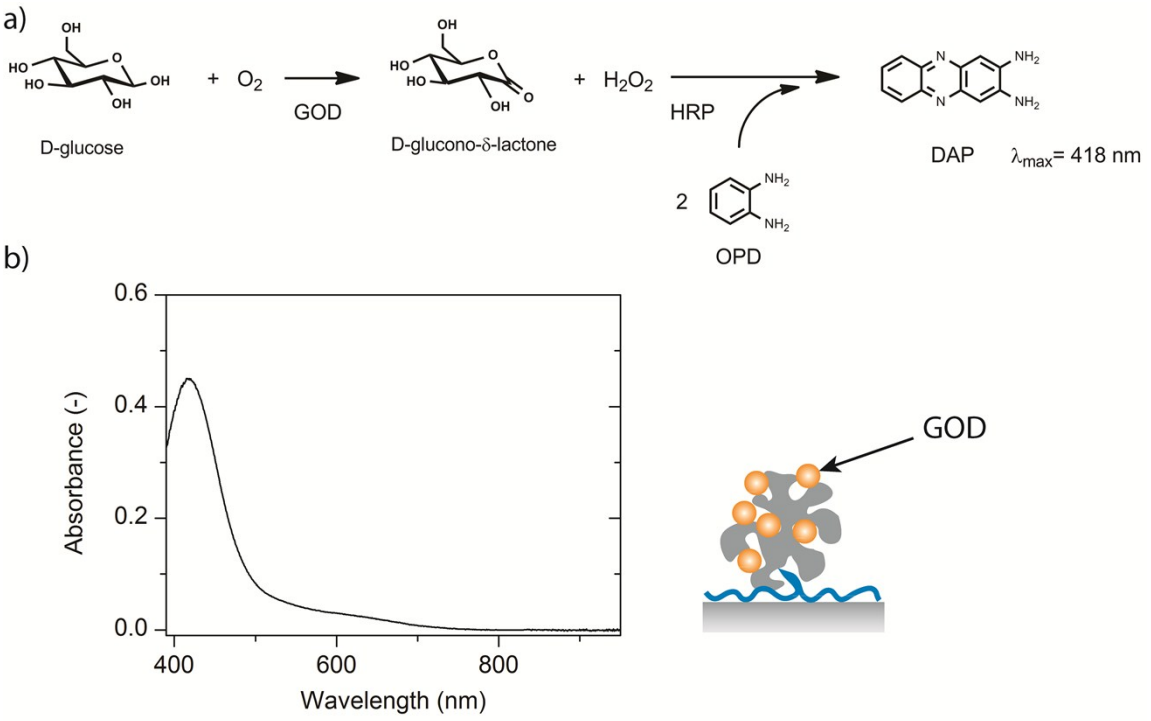

Figure S3

a) Activity assay for the quantification of GOD: Oxidation of D-glucose with O_2 is catalyzed by GOD. The H_2O_2 formed is used in a coupled assay for the HRP catalyzed oxidation of OPD to form the UV/vis detectable DAP. Using an assay solution containing 3.45 mM D-glucose, 3.14 mM OPD and 2 nM HRP in PBS (10 mM phosphate, 150 mM NaCl, pH 7), a linear correlation of the GOD concentration in solution to the measured activity allows the quantification of unknown amounts of GOD (see Ref. [S1]).

b) Example of a spectrum recorded for the quantification of the GOD activity on a microscopy glass coverslip with adsorbed *de*-PG2 and HMM particles loaded with GOD. The glass coverslip was immersed in the substrate solution for 20 minutes and after removal the DAP concentration in the assay solution was determined by UV/vis spectrophotometry. By comparing the increase in absorbance at $\lambda = 418$ nm with the calibration curve prepared with known amounts of GOD in solution under the same conditions (Ref. [S1]) the GOD activity per surface area was determined.

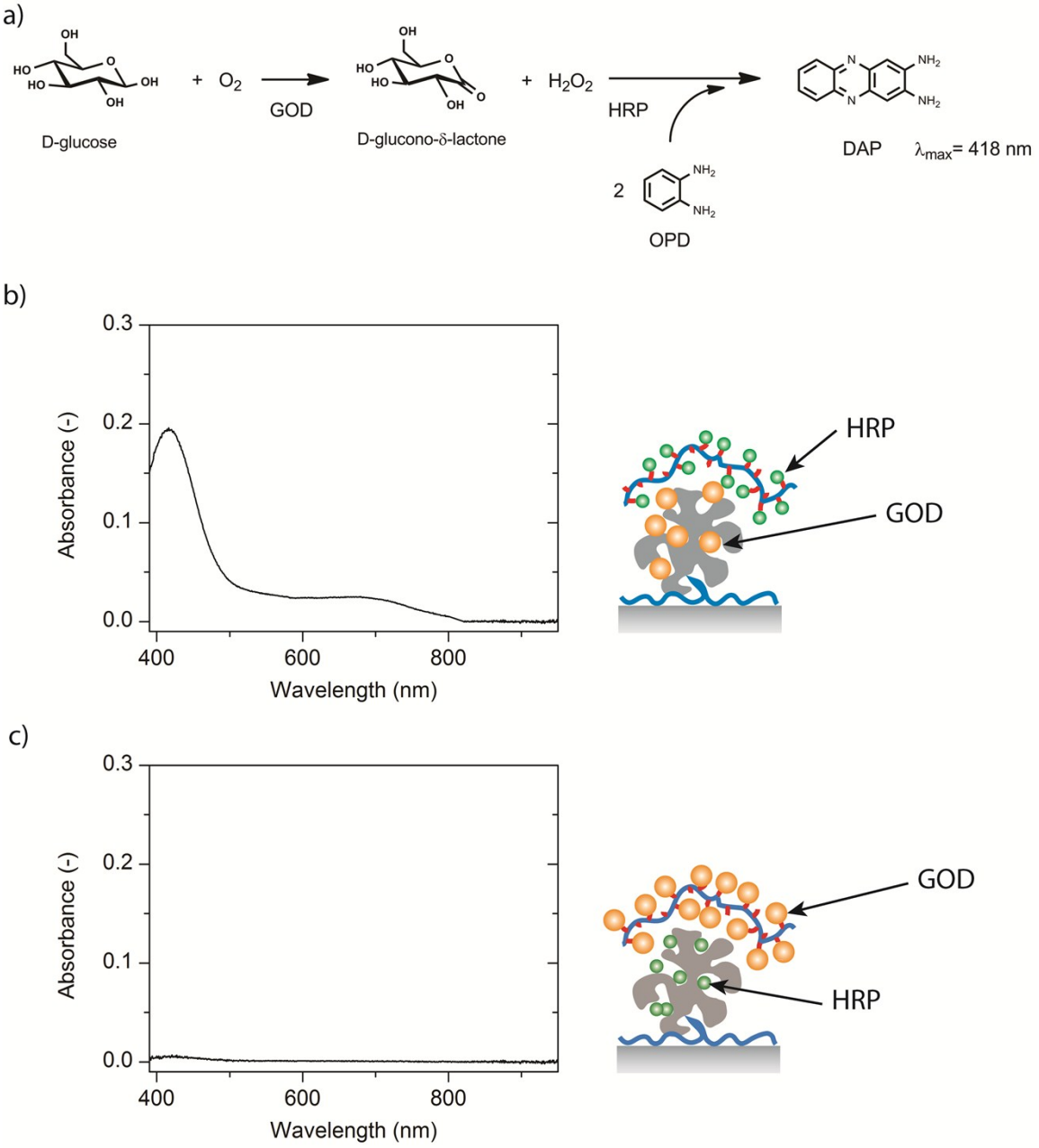

Figure S4

a) Activity assay for the characterization of the catalytic cascade reaction catalyzed by GOD and HRP: Oxidation of D-glucose with O_2 is catalyzed by GOD. The H_2O_2 formed is used in a subsequent step for the HRP catalyzed oxidation of OPD to form the UV/vis detectable DAP. An assay solution containing 3.45 mM D-glucose and 3.14 mM OPD in PBS buffer (10 mM phosphate, 150 mM NaCl, pH 7) was used (see Ref. [S1]).

b) Example of a spectrum recorded for the characterization of the enzymatic cascade reaction catalyzed by GOD and HRP immobilized on a microscopy glass coverslip with adsorbed *de*-PG2, HMM particles loaded with GOD and covered with *de*-PG2-BAH-HRP. The glass coverslip was

immersed in the substrate solution for 20 minutes and after removal the DAP concentration in the assay solution was determined by UV/vis spectrophotometry.

c) Example of a spectrum recorded for the characterization of the enzymatic cascade reaction catalyzed by GOD and HRP immobilized on a microscopy glass coverslip with adsorbed *de*-PG2, HMM particles loaded with HRP and covered with *de*-PG2-BAH-GOD. The glass coverslip was immersed in the substrate solution for 20 minutes and a spectrum recorded after removal of the coverslip from the assay solution.

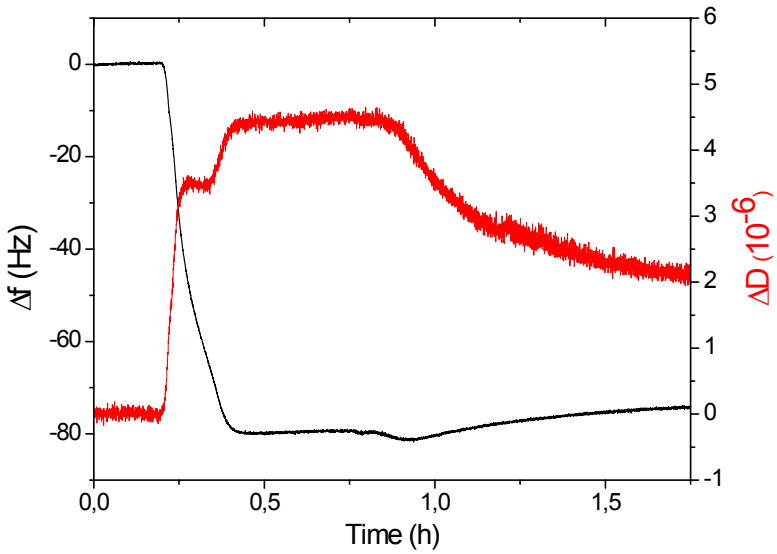

Figure S5

QCM-D results, presented as shift in frequency (Δf, black) and dissipation (ΔD, red) as a function of time, showing the adsorption of the denpol *de*-PG2 onto a silica-coated sensor.

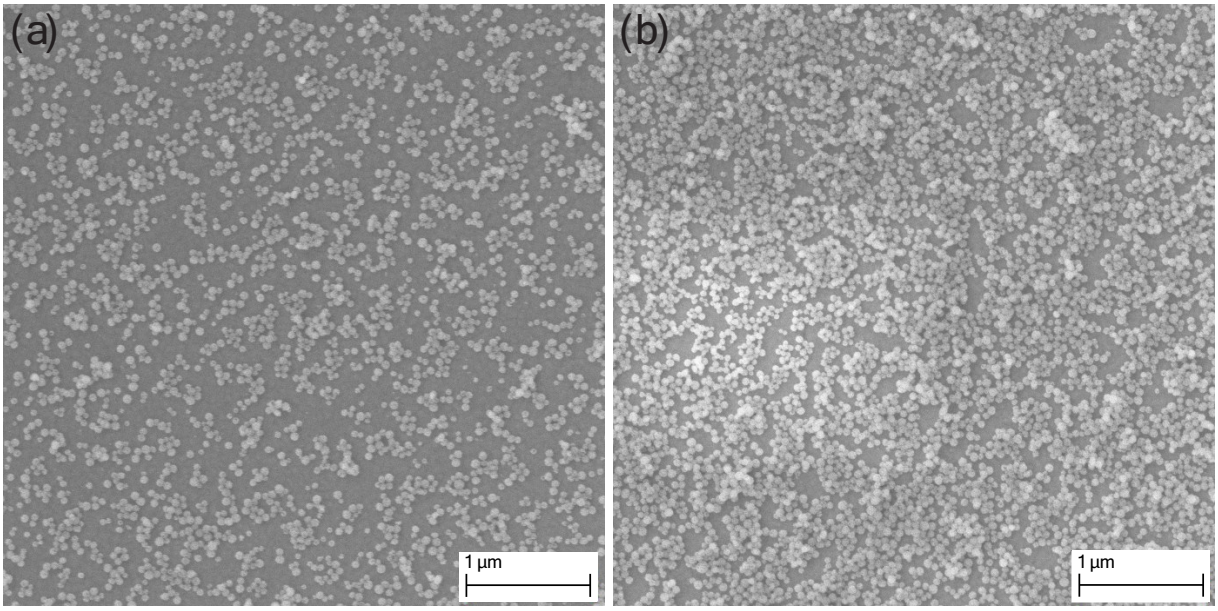

Figure S6

SEM images of mesoporous silica particles (HMM) attached to the surface of a silica-coated QCM-D sensor with (a) aminopropyltrimethoxysilane (APTMS), and (b) the second generation polycationic denpol *de*-PG2 as linker or binding layer, respectively.

Reference

[S1] A. Küchler, J. Adamcik, R. Mezzenga, A. D. Schlüter and P. Walde, *RSC Adv.*, 2015, **5**, 44530-44544