Electronic Supplementary Material (ESI) for RSC Advances.

Supplementary Information

Enzyme immobilization on silicate glass through simple adsorption of dendronized polymer-enzyme conjugates for localized enzymatic cascade reactions

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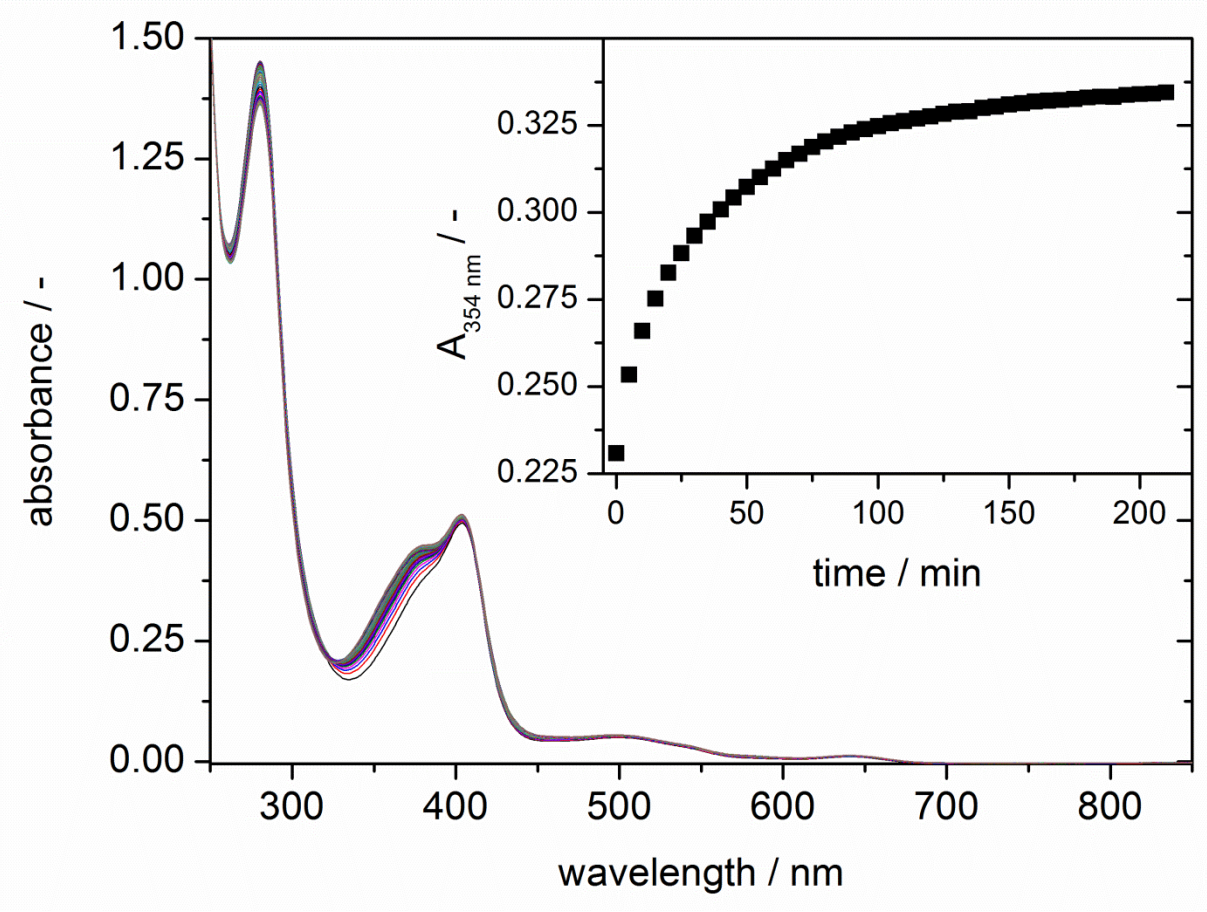

Figure S1. Continuous UV/vis-spectrophotometric monitoring of the formation of *de*-PG2-BAH-HRP at pH = 4.7 (reaction ④ in Fig. 1). In a quartz cuvette (0.1 cm path length, 0.35 mL) 205.9 µL buffer-2, 32.6 µL of *de*-PG2-HyNic (from a stock solution with a concentration of 690 µM HyNic), 53.6 µL HRP-4FB (from a stock solution with a concentration of 280 µM 4FB), and 7.9 µL aniline (from a 380 mM stock solution) were mixed. Immediately after mixing, recording of UV/vis spectra was started at an interval of 5 minutes. The inset shows the increasing absorbance at 354 nm which is characteristic for the BAH bond formed during the conjugation. The conjugate preparation was carried out several times with always the same conditions. As the concentrations of the stock solutions varied, the exact volumes were adjusted accordingly.

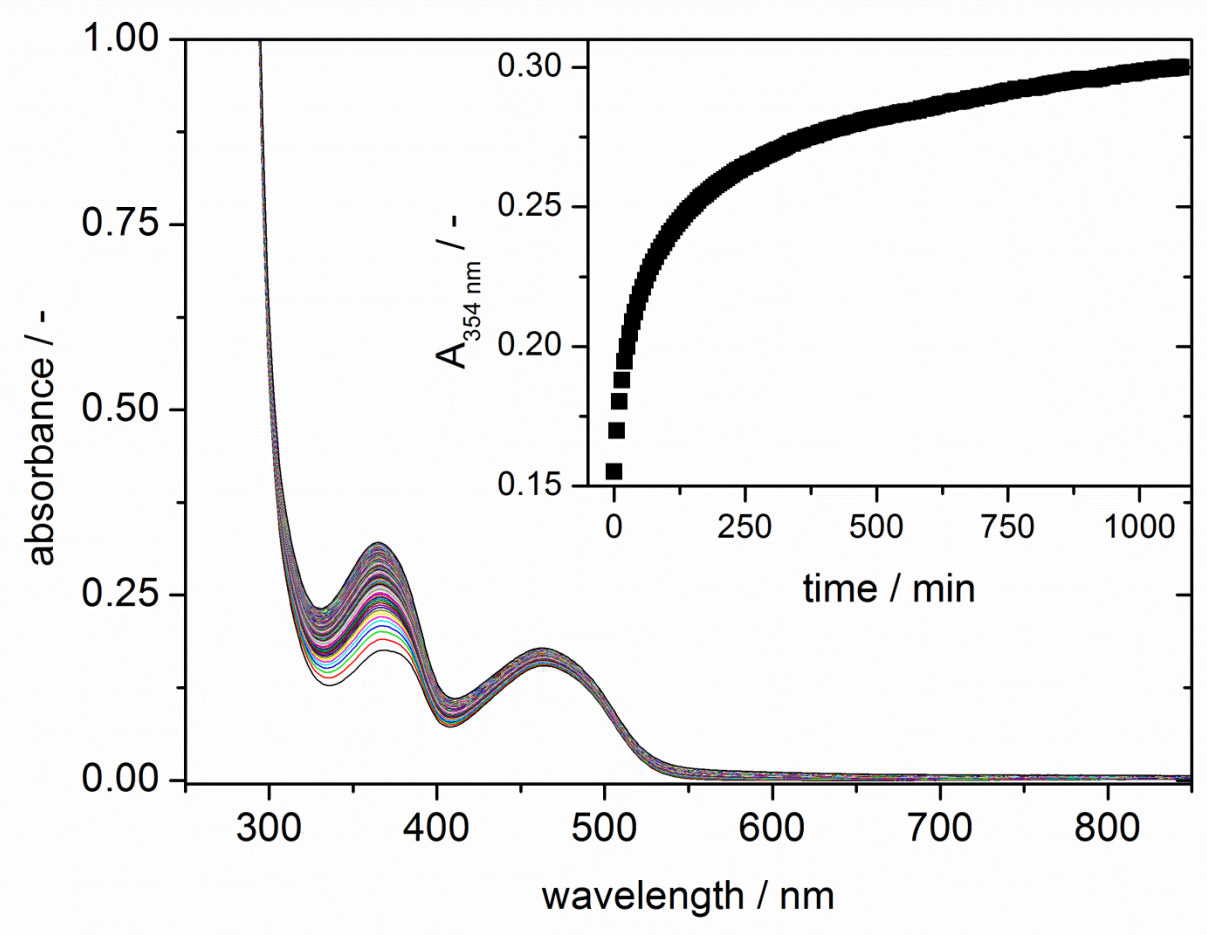

Figure S2. Continuous UV/vis-spectrophotometric monitoring of the formation of *de*-PG2-BAH-GOD at pH = 4.7 (reaction ⑤ in Fig. 1). In a quartz cuvette (0.1 cm path length, 0.35 mL) 215.6 µL buffer-5, 67.7 µL of *de*-PG2-HyNic (from a stock solution with a concentration of 388 µM HyNic), and 66.8 µL GOD-4FB (from a stock solution with a concentration of 262 µM 4FB) were mixed. Immediately after mixing, recording of UV/vis spectra was started at an interval of 5 minutes. The inset shows the increasing absorbance at 354 nm which is characteristic for the BAH bond formed during the conjugation. The conjugate preparation was carried out several times with always the same conditions. As the concentrations of the stock solutions varied, the exact volumes were adjusted accordingly.

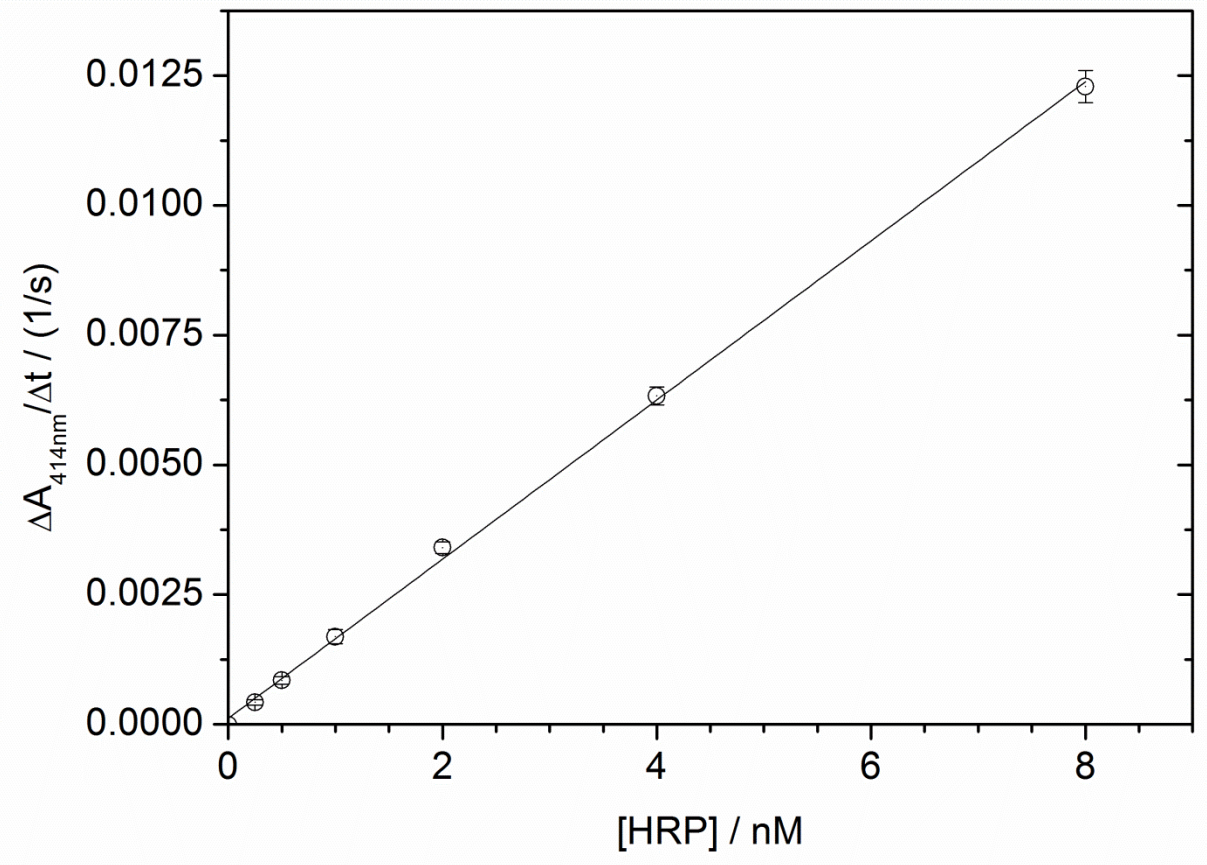

Figure S3. Calibration curve for the quantification of HRP activity at pH = 7.0. With known amounts of HRP in solution (0-8 nM), enzymatic oxidation of chromogenic $ABTS^{2-}$ (1 mM) with H_2O_2 (0.2 mM) yielding $ABTS^{\bullet-}$ was monitored by UV/vis spectrophotometry. The reaction rate, expressed as the increase in absorption at λ_{max} = 414 nm per time, is linearly dependent on the enzyme concentration. Determination of the apparent concentration of immobilized HRP on glass surfaces was performed by correlating the enzymatic activity measured to the corresponding amount of HRP read from the calibration curve.

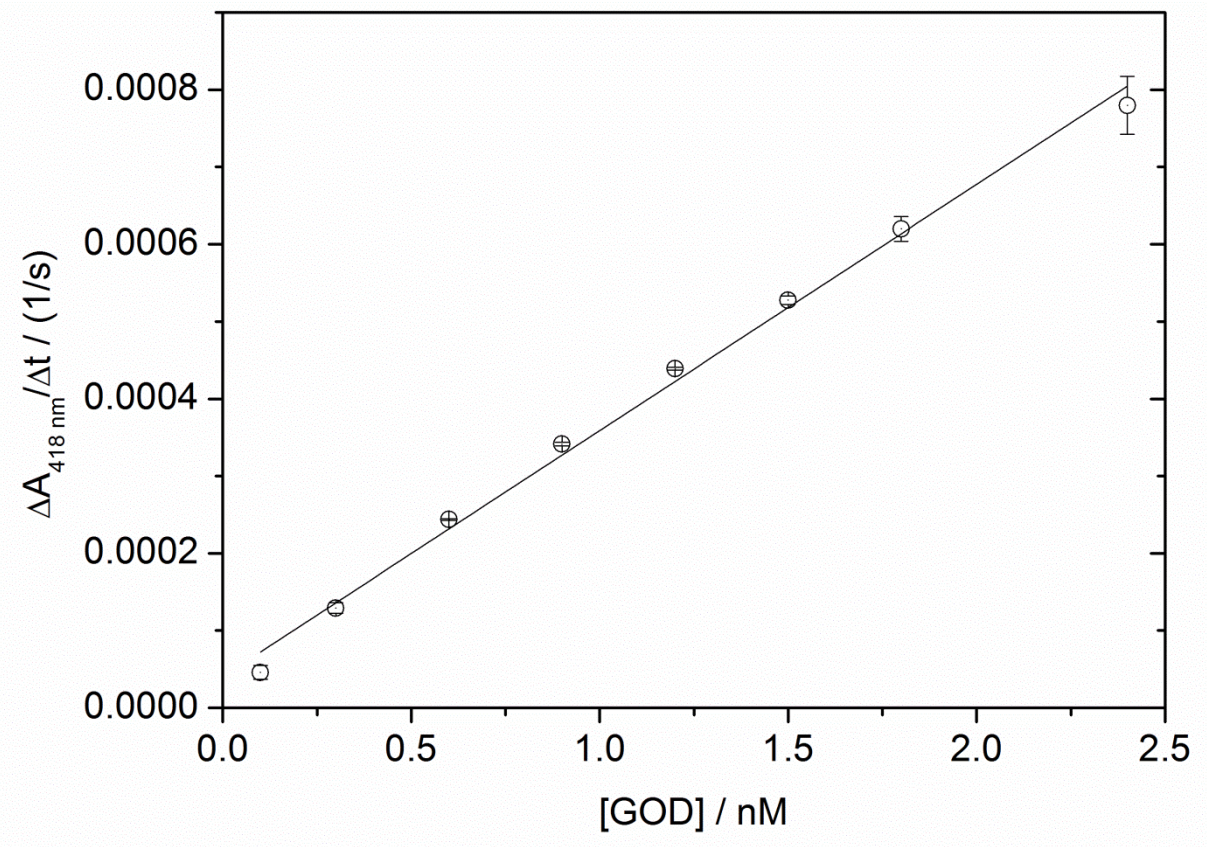

Figure S4. Calibration curve for the quantification of GOD activity at pH = 7.0. With known amounts of GOD in solution (0-2.4 nM), enzymatic oxidation of D-glucose (3.45 mM) by dissolved O_2 yielding glucono-δ-lactone and H_2O_2 was monitored exploiting a coupled HRP based assay for H_2O_2 detection (2 nM HRP, 3.14 mM OPD, as described in the EXPERIMENTAL SECTION). Product formation was monitored by UV/vis spectrophotometry. The reaction rate, expressed as the increase in absorption at λ_{max} = 418 nm per time, is linearly dependent on the enzyme concentration. Determination of the apparent concentration of immobilized GOD on glass surfaces was performed by correlating the enzymatic activity measured to the corresponding amount of GOD read from the calibration curve.

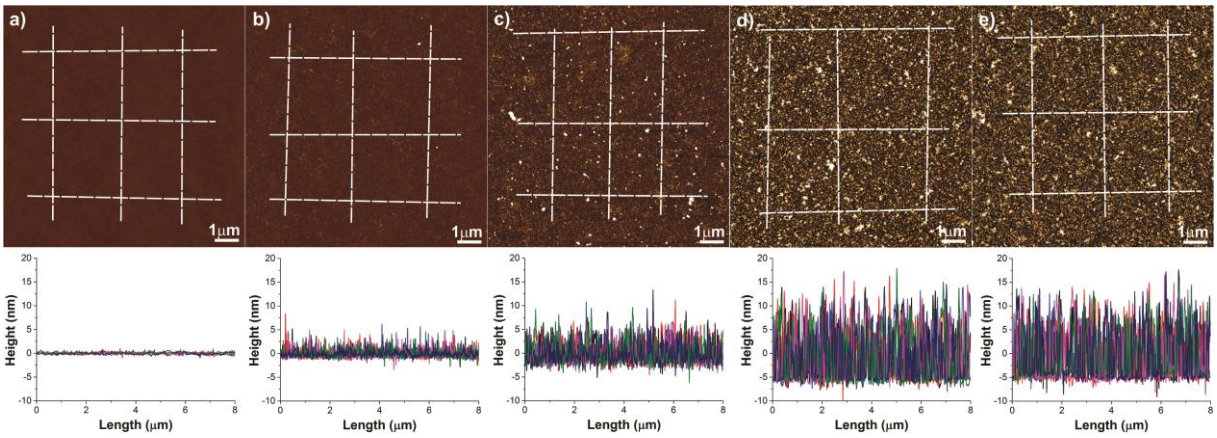

Figure S5. Tapping mode 3D AFM images of a microscopy glass coverslip (a) as used for the denpol-BAH-enzyme immobilization and images of immobilized *de*-PG2 (b), *de*-PG2-BAH-HRP (c), *de*-PG2-BAH-GOD (d), and *de*-PG2-BAH-(GOD,HRP) (e) on microscopy glass coverslips giving a large area for estimation of the homogeneity of the adsorbed layer. No large aggregates are visible on a several micrometer scale. The corresponding height profiles along the indicated white lines suggest a relatively homogeneous thickness of the adsorbed layers at this scale with a thickness corresponding to what is expected from the thickness of the individual conjugate chains observed on mica.