Electronic supporting information for:

Biocatalytic oxidation by chloroperoxidase from *Caldariomyces fumago* in polymersome nanoreactors

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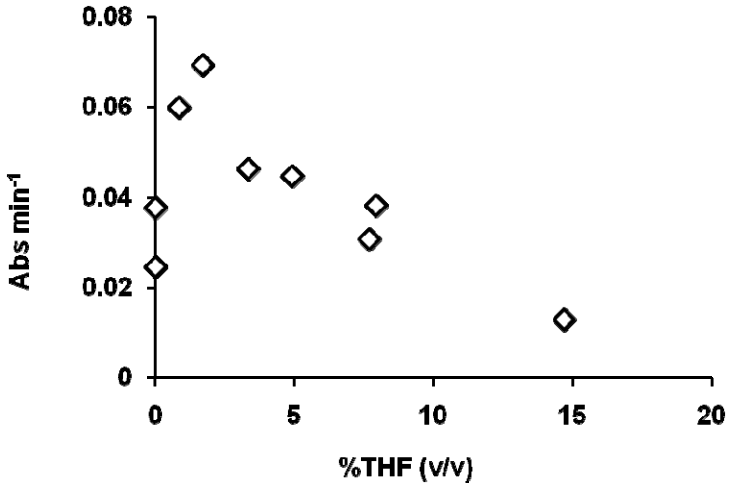


Figure S1; Variation of halogenation activity of CPO with increasing amounts of THF in 0.1 M phosphate / citric acid buffer, pH = 3.0.

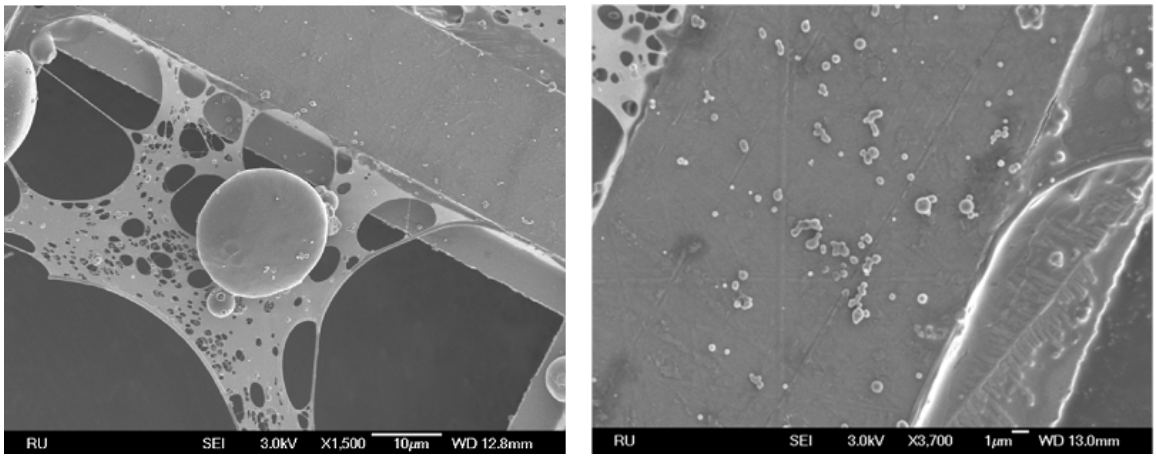


Figure S2; SEM imaging of spherical co-aggregates observed in PS-PIAT dispersions containing chloroperoxidase in 0.1 M phosphate / citric acid buffer buffer, pH = 2.8. The aggregates show abnormal diameters (up to 20 µM) and do not display the occasional holes observed for regular PS-PIAT polymersomes, which reveals that these polymersomes are hollow. The dispersions were deposited on holey-carbon grids.

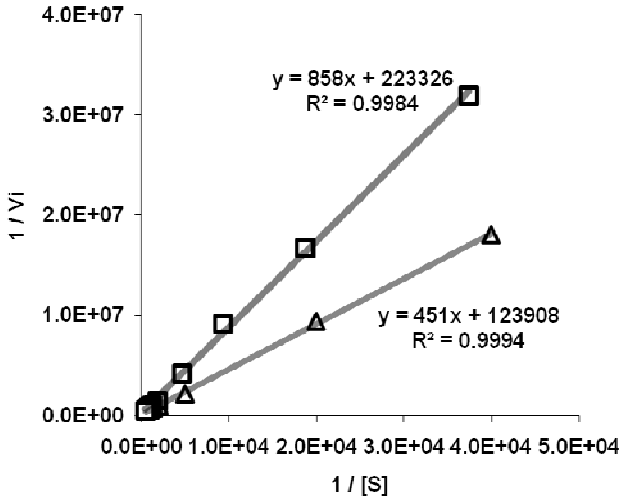


Figure S3; Double reciprocal plots of substrate concentration versus the initial velocity (V_i) of the sulfoxidation of thioanisole by free CPO in phosphate / citric acid buffer at pH = 5 and in Milli-Q (pH ~ 6).

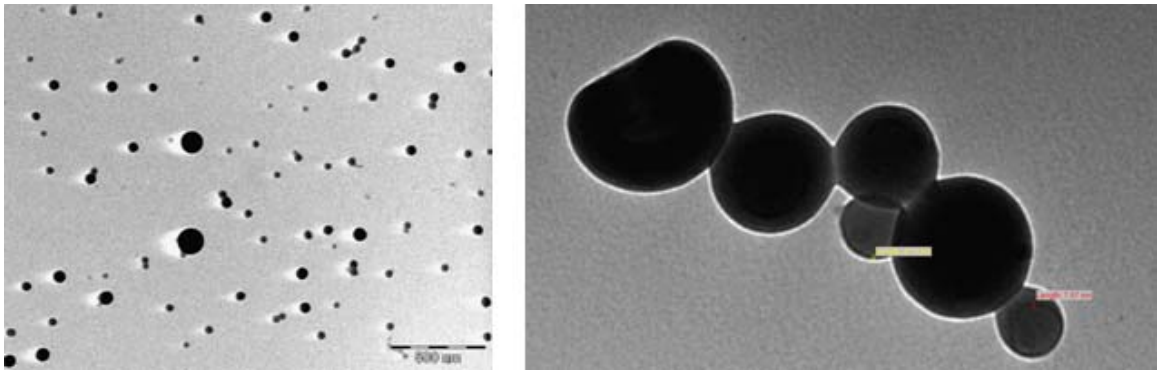


Figure S4; (Left) TEM-image of CPO-containing polymersomes in Milli-Q after removal of the non-encapsulated enzymes by filtration. (Right) The close-up shows the membrane structure of the polymersomes.

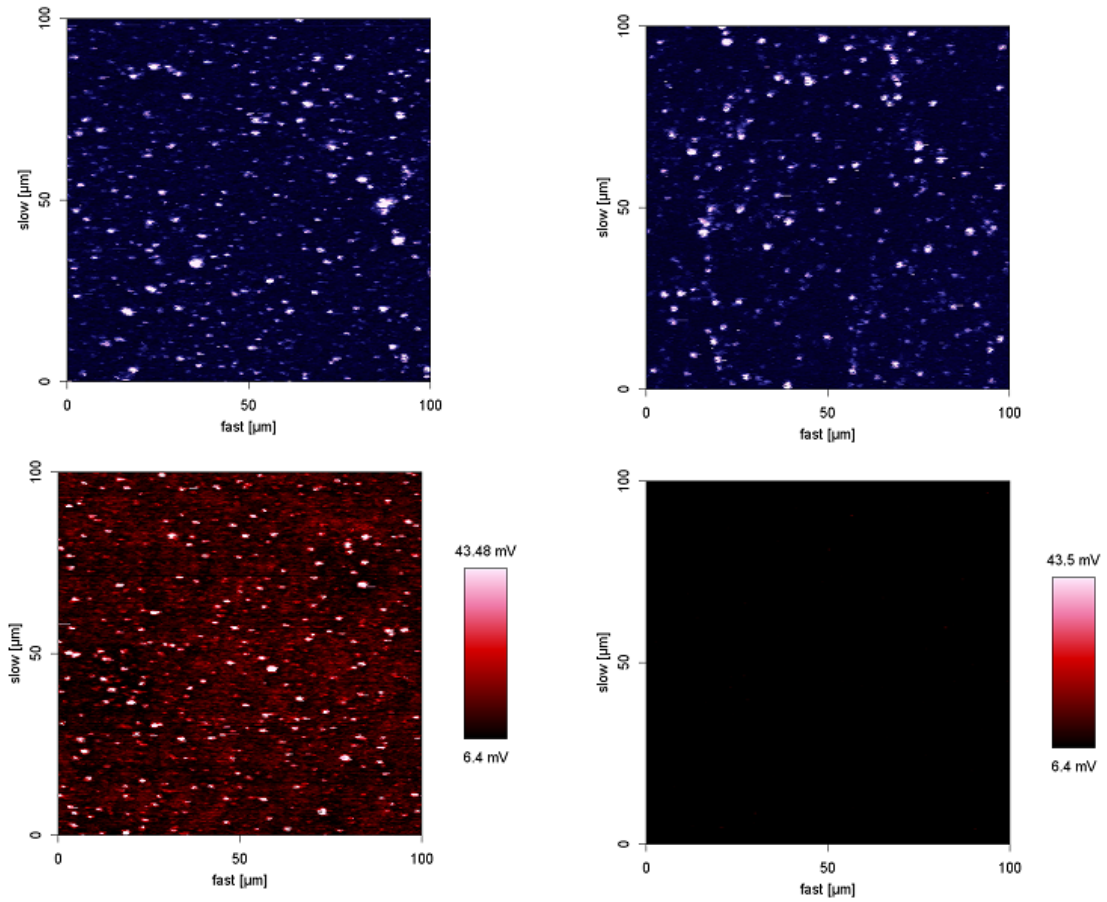


Figure S5; (Left) Confocal fluorescence images of alexa-labeled CPO encapsulated in PS-PIAT polymersomes at λ_{em} = 505 nm (above) and at λ_{em} = 650 nm (below) showing the fluorescence coming from the polymersomes themselves and the fluorescence of the dye-labeled enzyme respectively. (Right) Measurements at the same emission wavelengths on the non-labeled CPO encapsulated within the polymersomes.

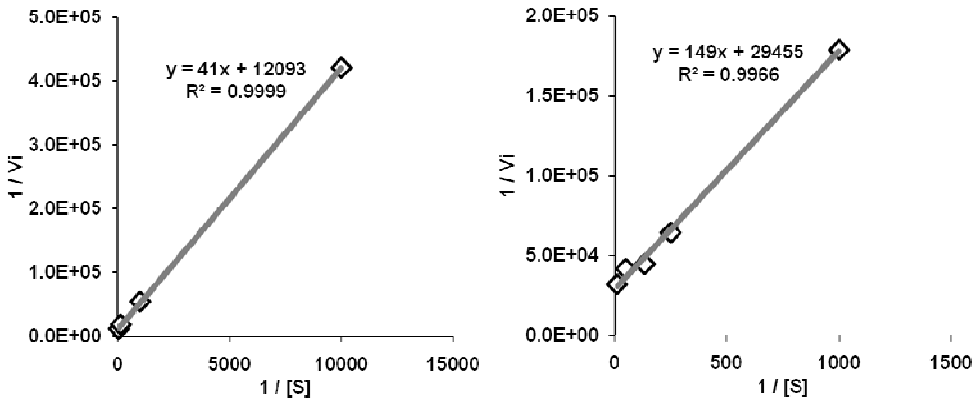


Figure S6; (Left) Double reciprocal plot of the substrate concentration versus V_i for the oxidation of thioanisole by CPO at a concentration of 300 nM. (Right) The same plot for CPs.

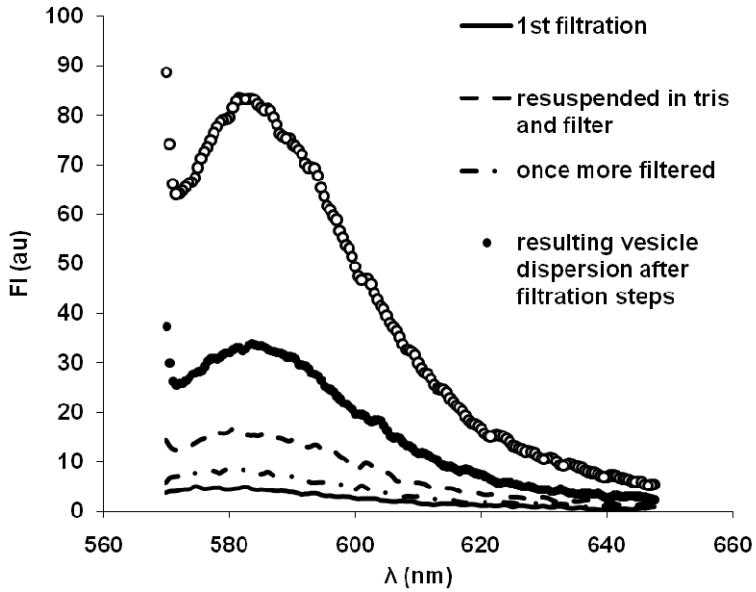


Figure S7; Change of fluorescence intensity of CPs upon changing from Milli-Q to buffer solution (phosphate buffer pH = 6.5, 50 mM) measured by the fluorescence of the rhodamine dye that was conjugated to the CPO. After preparation of the CPs, the solution was once again filtered and the fluorescence of the flow-through measured (─). The CPs were resuspended in phosphate buffer and the procedure was repeated twice (─ ─, and ─●)). The fluorescence of the initial solution (○) and the resulting solution (●) was also measured. It is clear that no fluorescence intensity can be discerned upon filtering the MQ solution, but that upon resuspension in buffer a considerable amount of enzyme ends up in the flow-throughs, showing that enzyme has been released from the vesicles.

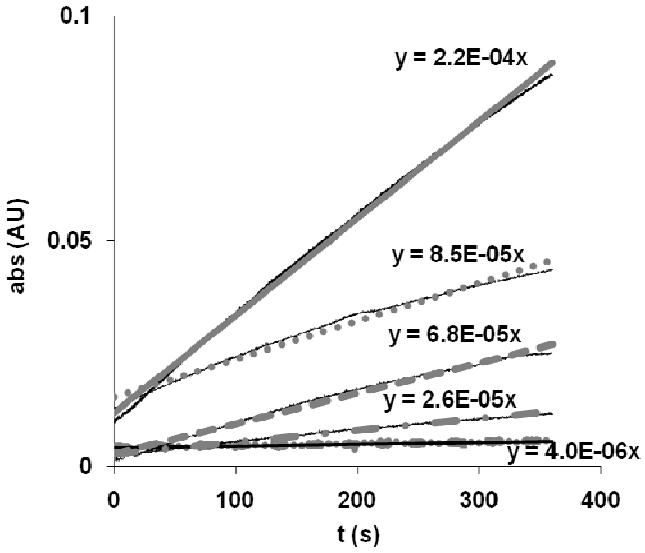


Figure S8; The same filtration procedure was followed as for **Figure S7**, but instead of measuring the fluorescence intensity the CPO-activity of the corresponding solutions was measured. The activity is represented by the derivative of the linear fit through the corresponding graph. Legend: initial solution: ─; flow-through 1st filtration ─ ─; flow-through 2nd filtration ─ •; flow-through 3rd filtration ─••; resulting solution •.

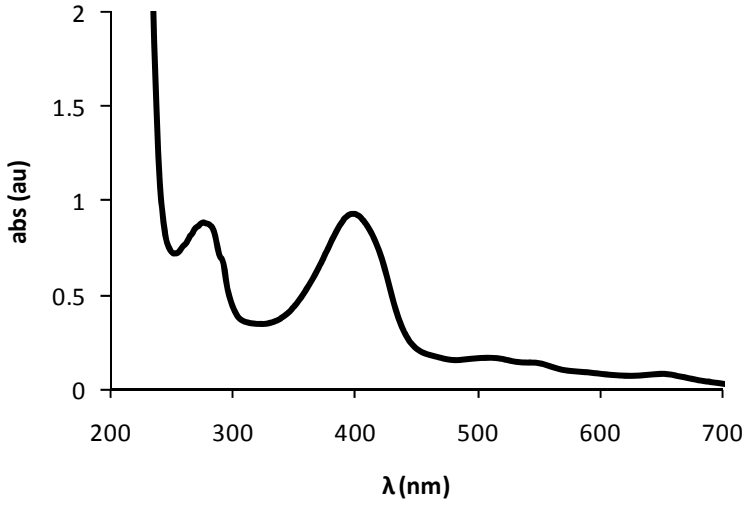
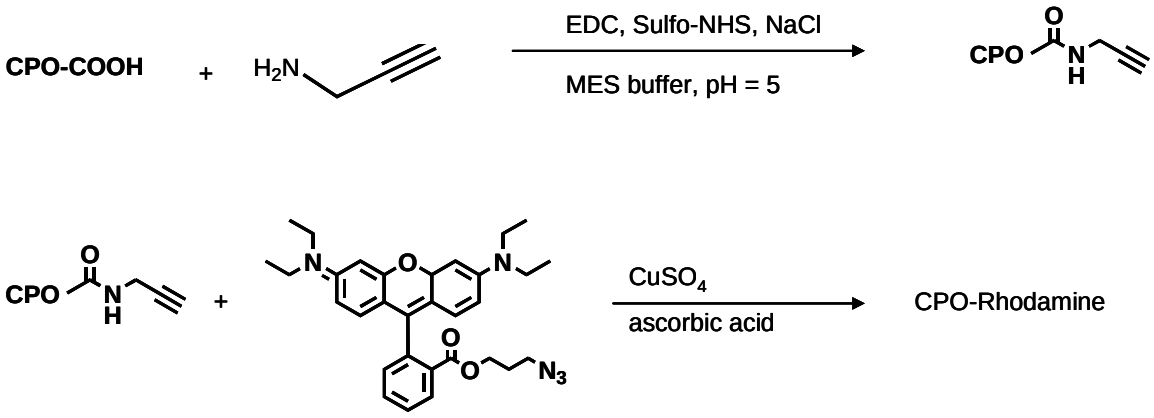


Figure S9; UV-spectrum of a 50 times diluted stock solution of CPO at pH = 5.5 in 100 mM MES buffer, showing the absorption of the heme group at λ = 400 nm. By dividing this absorbance value (abs = 0.93) by that of the protein at 280 (abs = 0.89), the Rz can be calculated, yielding 1.04. This number indicates a good purity.[1]

Procedure for labeling CPO with the azide functionalized rhodamine:

For labeling the CPO with the azide-functionalized rhodamine dye, the CPO was first functionalized with acetylene-groups by coupling of propargyl-amine to the accessible COOH-groups functions on the enzymes. To 1.0 ml CPO-solution (10 mg, 0.24 μmole) in MES-buffer (pH = 5.5. 0.1 M, 0.1 M NaCl) was added: 200 μl of 3.3 mg ml^{-1} propargylamine in water (12 μmole), 200 μl of 11.4 mg ml^{-1} EDC (12 μmole) and 200 μl of 14.1 mg ml^{-1} sulfo-NHS (13 μmole). After incubation overnight, the solution was filtered three times over a 10 K MWCO centrifugal filter, to remove the reactants and the CPO was resuspended in Milli-Q to 1.0 ml. This solution was mixed with 150 μl 1.0 mg ml^{-1} azide rhodamine (0.58 μmole) and 100 μl of a premixed solution of $CuSO_4$ (5.1 μmole) and ascorbic acid (22.7 μmole), and once again incubated overnight. The excess dye and reactants were removed by first filtering twice over a 10K MWCO filter and separation over a size-exclusion column (Sephadex G50, Milli-Q)). The average degree of labelling was 1.7 moles of rhodamine dye per mole of enzyme. The labeled enzyme retained 80% of its initial activity. The azido-rhodamine derivative was kindly provided by Dr. M. Koepf.



[1] Seelbach, K. *et al*., *Biotechnol. Bioeng.*, **1997**, 55 (2), 283