Supplementary Material (ESI) for New Journal of Chemistry

Hemoglobin Immobilized on Mesoporous Silica as Effective Material for the Removal of Polycyclic Aromatic Hydrocarbons Pollutants from Water

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Hemoglobin (Hb) is known to oxidize Polycyclic Aromatic Hydrocarbons (PAH) in water/acetonitrile (20%) medium in the presence of H_2O_2. PAH are carcinogenic and mutagenic pollutants found in various places of human industrial activities such as waste waters of oil refineries. Hb is pH sensitive and is effective at a pH of 5. In order to render the use of Hb compatible with wastewater pH conditions (6.5 <pH< 8.5), Hb was immobilized by adsorption in mesoporous silicas. A loading capacity of Hb as high as 300 mg Hb/g silica was obtained. Prior to use Hb-immobilized silica, an optimization of the oxidation conditions of 11 PAH (300 nM each) using H_2O_2 in water, with only 1% acetonitrile, was performed with free Hb at pH 5. The reaction of Hb with PAH is pseudo-catalytic because an excess of H_2O_2 ($[H_2O_2]/[PAH] > 2500$) and of Hb ($[Hb]/[PAH] > 2$) is necessary to reach an optimal PAH removal of 75%. The excess of Hb depends on the PAH physico-chemical characteristics. At pH = 7, the activity of free Hb decreased to 47% of PAH removal, whereas the Hb-immobilized silica allowed 82% of PAH removal. Immobilization of Hb in silicas leads to a protection of Hb towards pH, and also against solvent, temperature and inactivation by H_2O_2. All oxidized-PAH remained covalently linked to Hb inside the material. Only a small fraction of anthraquinone resulting from the oxidation of anthracene was observed in the solution. These results open the perspective for a new biotechnology process aiming at the cleaning of contaminated wastewaters by a reactive adsorption process followed by filtration.

Supplementary Informations

UFLC analyses. The UFLC system (Shimadzu), was equipped with a Supelco reversed phase column C18-PAH (50 mm length, 4.6 mm internal diameter and 3 µm particles size). The chromatographic apparatus is composed of two pumps LC-20AD, an automatic sampler SIL-20AHT, a diode array detector SPD-M20A, a column oven CTO-20A, and a communication bus module CBM-20A. Chromatograms were monitored with LabPower Shimadzu software. The separation method consists of a gradient between solvent A (deionized water) and solvent B (methanol) starting 65% B during 1 min, up to 90% B from 1 to 5 min, holding 90% to 8 min, back to 65% B from 8 to 9.5 min. Column oven is maintained at 45°C during the analysis. To enhance the detection limit, peaks integration has been done considering the maximum wavelength adsorption of each PAH. In these conditions, the retention times and integration wavelength of for each PAH were respectively: naphtalene (1.42 min; 220 nm), acenatphtene (2.37 min; 227 nm), phenanthrene (2.91 min; 250 nm), anthracene (3.26 min; 251 nm), fluoranthene (3.70 min; 235 nm), pyrene (3.92 min; 239 nm), benzo(b)fluoranthene (5.68 min; 255 nm), benzo(k)fluoranthene (5.93 min; 306 nm), benzo(a)pyrene (6.17 min; 295 nm), benzo(g,h,i)perylene (7.12 min; 298 nm), indeno(1,2,3,cd)pyrene (7.47 min; 249 nm). An example of PAH separation by UFLC is given in Figure 1 for the initial PAH mixture solution. Figure S1 shows an overlay of 11 chromatograms, each one corresponding to a PAH maximum adsorption wavelength. The analyzed sample presents the initial conditions of a typical experiment thus 300 nM of each PAH in citrate buffer pH = 5 (I = 50 mM) and 1% ACN (v/v). The same analysis has then been performed at several times of the reaction with H_2O_2 to follow PAH removal (not shown).

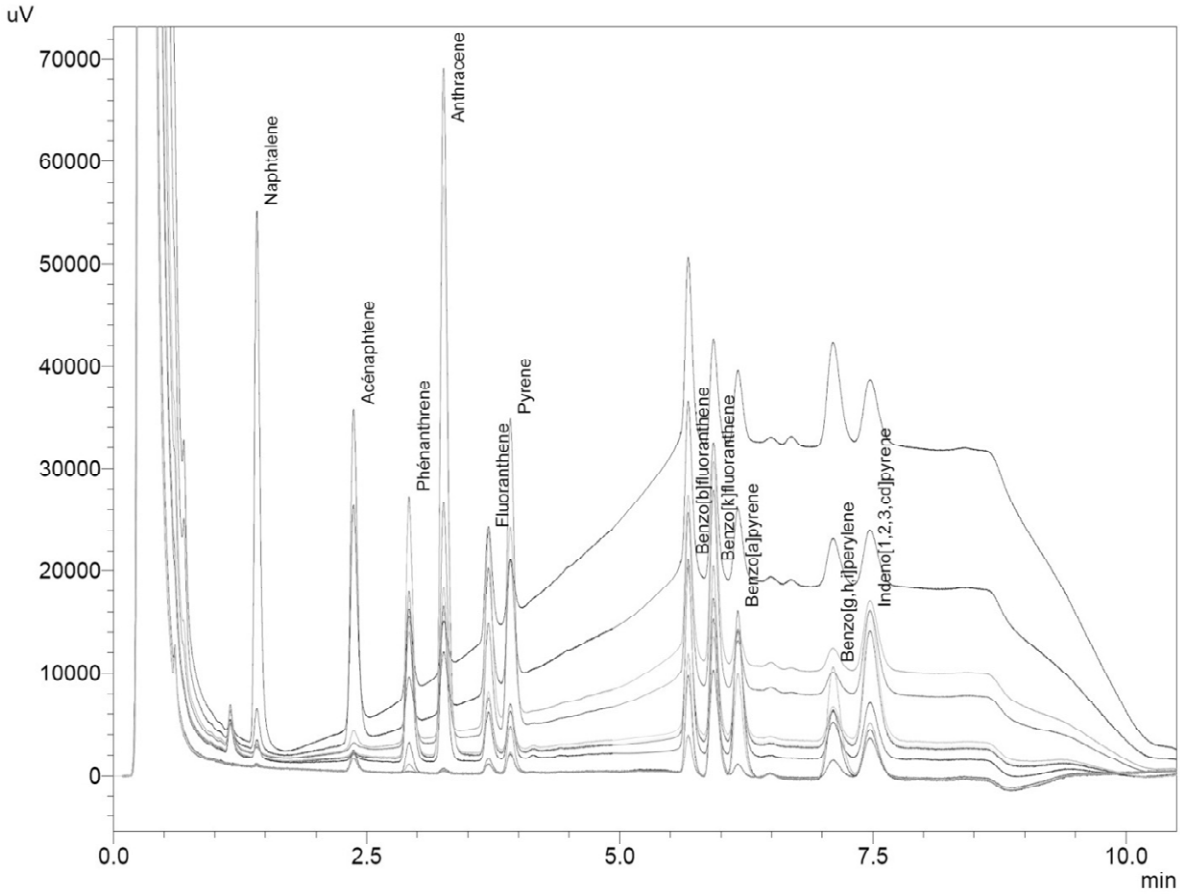

Figure S1: Example of PAH separation by UFLC. The figure shows an overlay of 11 chromatograms, each one obtained at the maximum adsorption wavelength of the PAH. (Initial concentration of PAH : 300 nM of each PAH in citrate buffer pH = 5 (I = 50 mM) and 1% acetonitrile (v/v)). The sample is diluted in 50% acetonitrile before analysis.

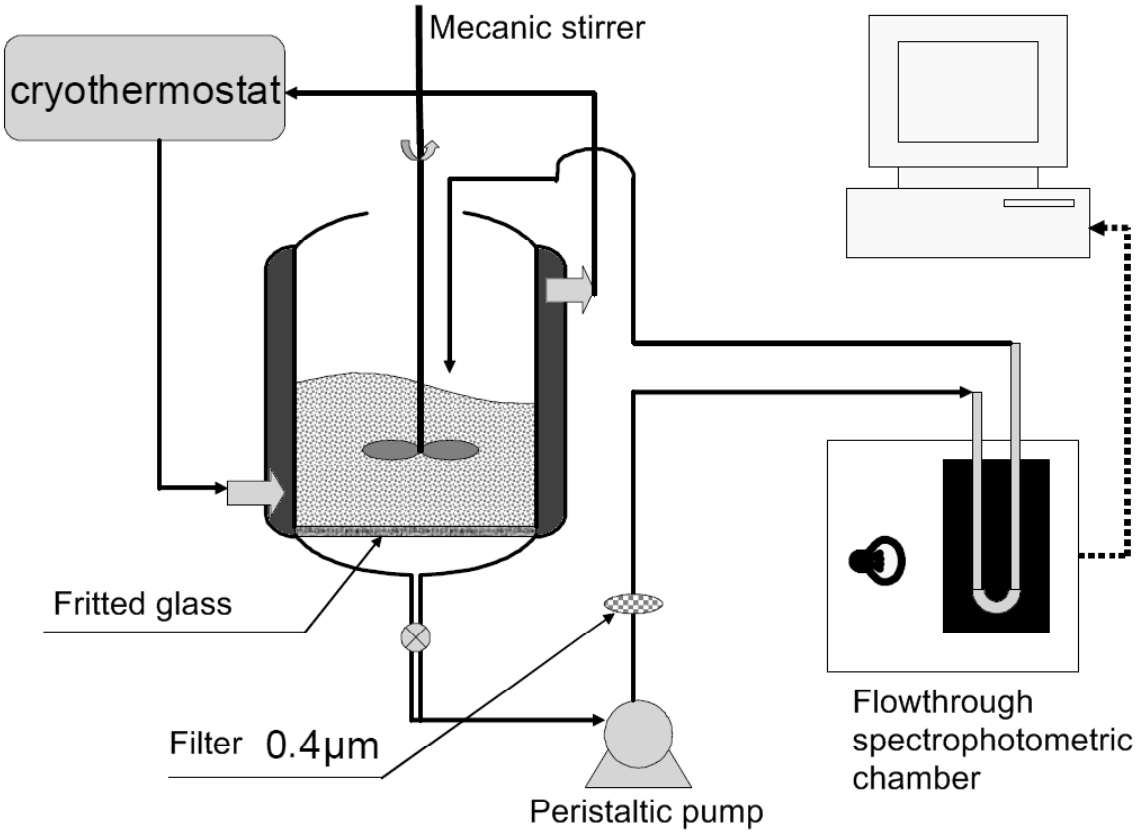

Figure S2: Schematic representation of the continuous flow reactor for the study of ABTS oxidation by Hb-immobilized silica.

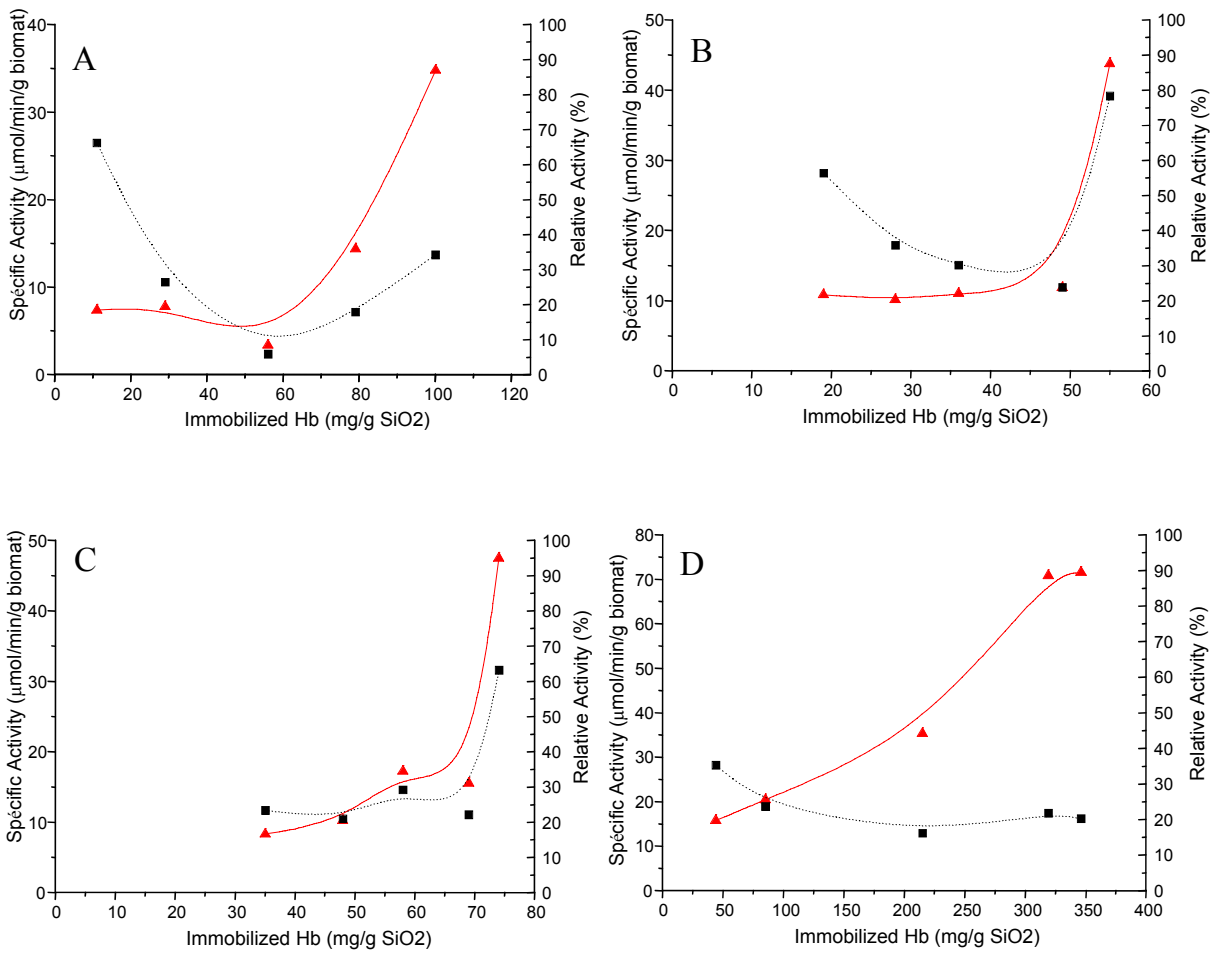

Figure S3: Specific (triangle) and relative (square) activities for ABTS oxidation in aqueous medium of the Hb-immobilized silica in function of the amount of Hb immobilized in the different silica support: (A) LiC60, (B) Dav6, (C) Dav10, (D) Dav20.

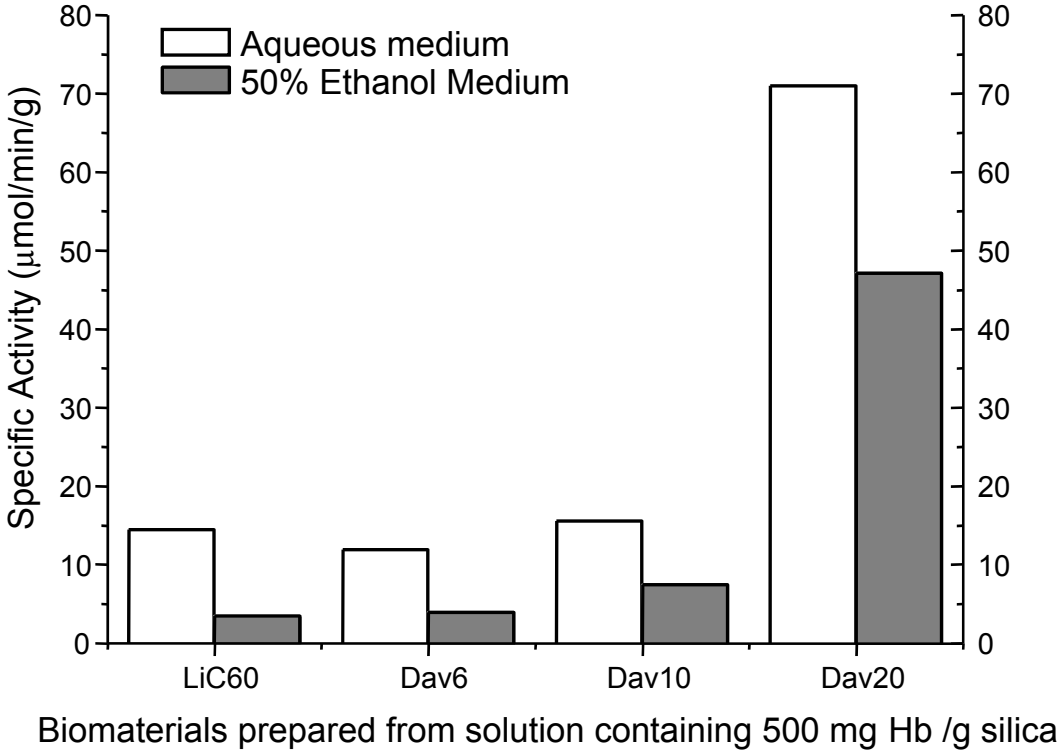

Figure S4: Comparison of specific activities for ABTS oxidation in aqueous medium (white bars) and in 50 (v/v)% ethanol medium (grey bars) for biomaterials prepared with impregnation solution of 500 mg Hb/g silica.

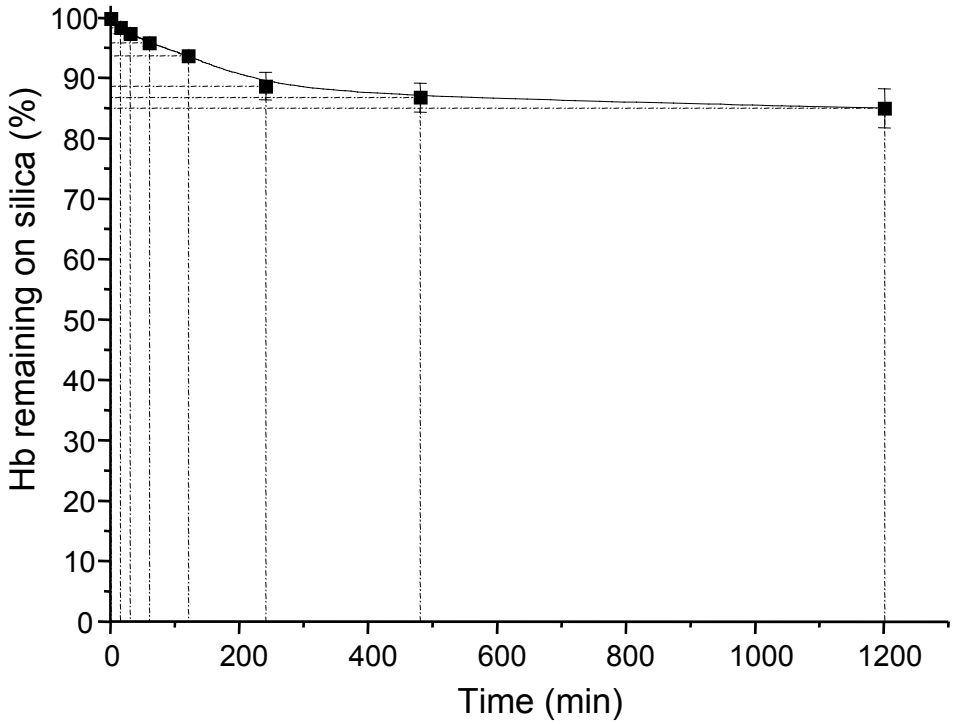

Figure S5: Hb leaching experiments represented in terms of percentage of remaining Hb on the support (Hb-Dav20 – 320 mg Hb/g silica) after vigorous stirring at various times and pH 5.

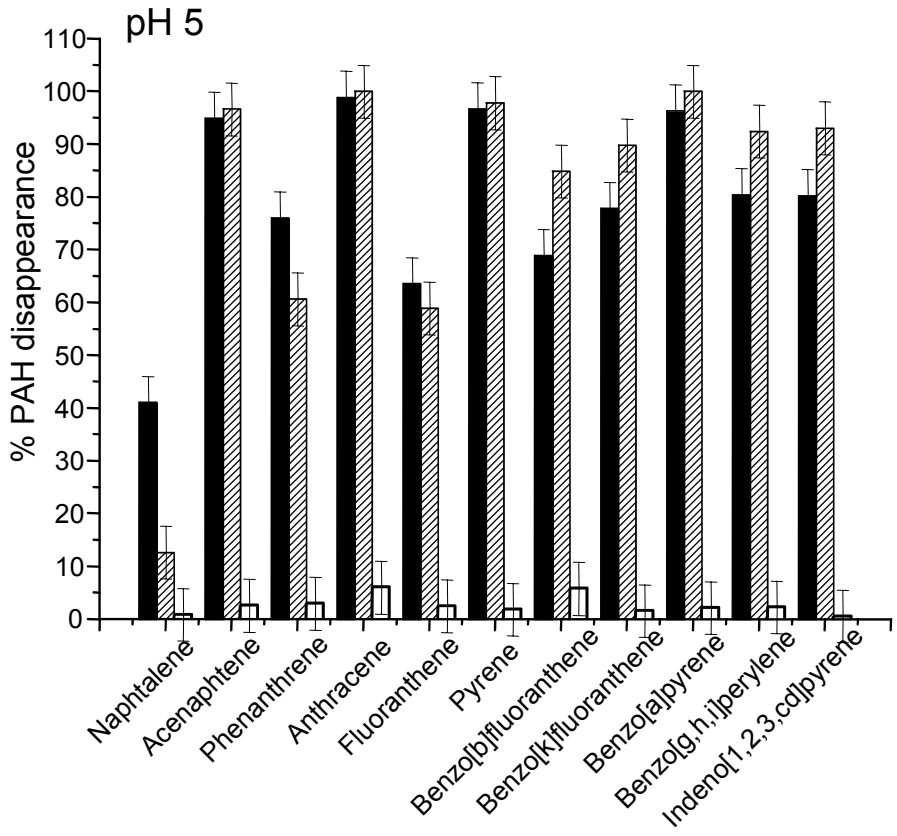

Figure S6: Percentage of removal at pH 5 of 11 PAH using (black) free Hb, (striped) Hb-Dav20 containing 320 mg Hb/g silica and (white) blank reaction with H_2O_2 only, without Hb. Conditions are: [PAH] = 300 nM each, [H_2O_2] = 8.2 mM, 25°C. For free Hb, the reaction was performed with [Hb] = 16.5 µM and for Hb-Dav20, with 17 g material/l. Analyses were done by UFLC.

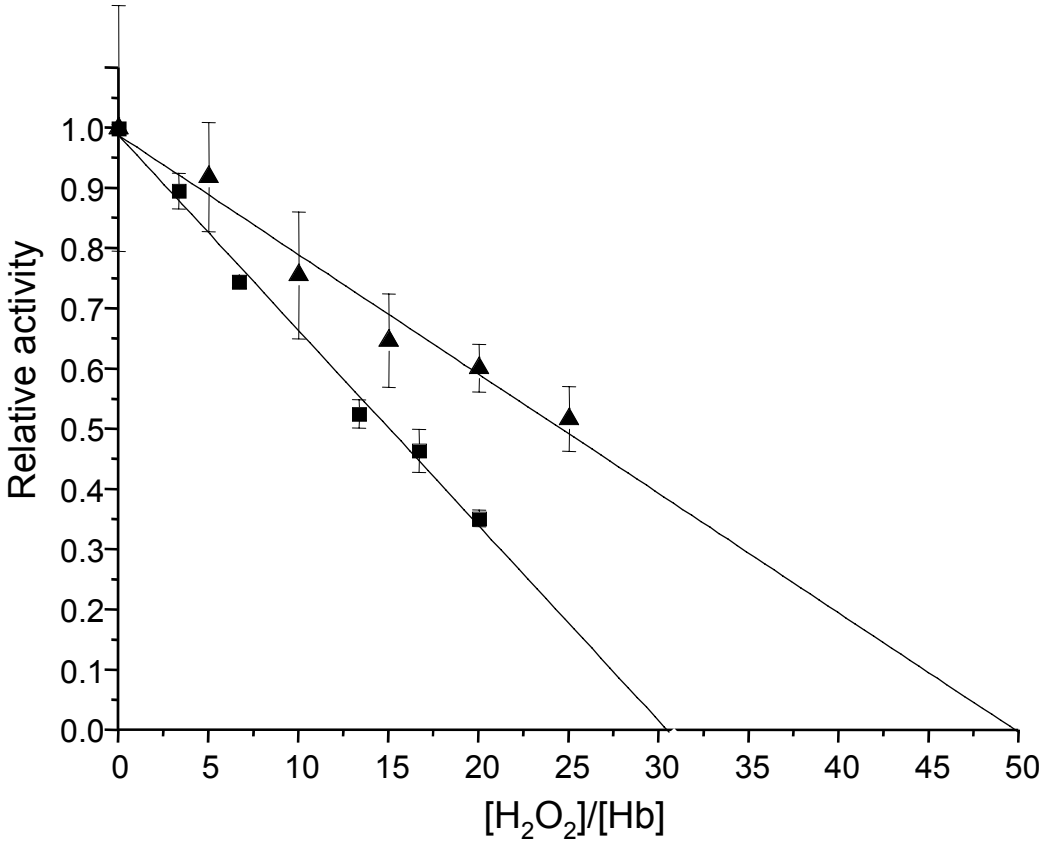

Figure S7: Relative activities for ABTS oxidation remaining after an incubation of 3 hours with various H_2O_2 concentrations for (square) free Hb and (triangle) Hb-Dav20 (320 mg Hb/g silica). The intersection of the extrapolated linear fit with the x axis gives the ratio $[H_2O_2]/[Hb]$ for which Hb is totally inactivated.