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Electronic Supporting Information

for

Supramolecular Mimetic Peroxidase Based on Hemin and PAMAM Dendrimers

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Experimental section

Materials. Poly(amidoamine) PAMAM dendrimers (generation 2.0, G2, FW_{av} = 3256 and generation 3.0, G3, FW_{av} = 6910) as methanolic solutions (20 wt %) were purchased from Aldrich and used as received. Hemin chloride (bovine) and ABTS were obtained from Sigma. Solvents and other reagents were reagent grade, available from commercial suppliers (Lab-Scan Ltd.). The supramolecular adduct between hemin and PAMAM dendrimers (HPG) have been prepared by suspending 65 mg (0.1 mmol) of solid hemin in methanol solutions containing G2 (326 mg, 0.1 mmol) or G3 (691 mg, 0.1 mmol). The suspensions have been vigorously stirred at room temperature for about 5 h, till complete dissolution, filtered through Millipore filters (0.2 μm) and then dried under vacuum, affording a waxy dark red solid. Stock solutions were prepared in dust-free Millipore water or in methanol and stored at 4°C in the dark. The HPG supramolecular complexes are stable for months in these solvents. For EPR measurements solutions of HPG were prepared with a final concentration of 0.2 mM in

methanol or aqueous solution. Compound I was prepared by adding a known volume of a concentrated stock solution of hydrogen peroxide to a solution of HPG in 100 mM phosphate buffer at pH 7 with a 1:8 HPG:oxidant agent molar ratio with a final concentration of HPG 0.2mM and 1.3mM of H_2O_2. Milli-Q water was used throughout. The reaction was stopped after 10s by rapid immersion of the EPR tube in liquid nitrogen. ABTS in 100 mM phosphate buffer solution at pH 7 has been added with a 1:1.25 HPG:substrate molar ratio maintaining the HPG:H_2O_2 molar ratio equal to 1:8. Ascorbic acid has been added with a 1:1000 HPG:reducing agent molar ratio with a final concentration of HPG 0.2mM and 0.2M of ascorbic acid.

Methods. UV/Vis spectra were obtained on a Hewlett-Packard mod. 8453 diode array spectrophotometer using 1 cm path-length quartz cuvette. CW-X-band (9.4GHz) EPR measurements were carried out on a Bruker E500 Elexsys Series using an Oxford helium continuous flow cryostat (ESR900). ^{1}H NMR spectra were obtained on a Bruker AMX R-300 spectrometer operating at 300.13 MHz. The spectra were referenced to the residual solvent peak and chemical shifts (δ) have been reported in p.p.m. downfield from TMS.

Formation of thin films. One wall of a standard 1 cm fluorescence cuvette (Hellma) was used as substrate to adsorb the supramolecular systems affording a thin film. The cuvette was cleaned prior to use by a 1:1 mixture of concentrated NH_3 solution and 30% H_2O_2 (Caution! This mixture is highly corrosive and should be handle with care under a ventilated hood and avoiding skin contact), then rinsed with Millipore-Q water and dried under a N_2 stream. The quartz cuvette was placed on the long side and 500 μL of a methanol solution of HPG2 (9 mM) were placed in contact with the surface for at least 24 h. After removing the solution from the cuvette, the formed red-brownish film is

gently rinsed with methanol and dried with a stream of nitrogen. This specific cuvette is particularly suitable for spectroscopic investigations on the system (see Fig. SI 8), since it allows to follow a kinetic run for an oxidation catalytic cycle (through the clean windows) and to check the stability of the film.

Kinetic measurements. The catalytic oxidation of ABTS substrate was investigated for HPG2 and HPG3 in homogeneous phase and HPG2 in heterogeneous phase. Control reaction without catalyst shows no catalytic activity. All reactions were monitored through UV/Vis spectroscopy monitoring the increasing of the absorbance band at 415 nm. Absorbance at 415 nm was converted to ABTS concentration (ε 415 nm = 3.6 x 10^4 M^{-1} cm^{-1}) and the method of initial rate was applied for determining the rate constant.[1]

1. A. Fersht, *Enzyme structure and mechanism*, W.H. Freeman, San Francisco, 1977.

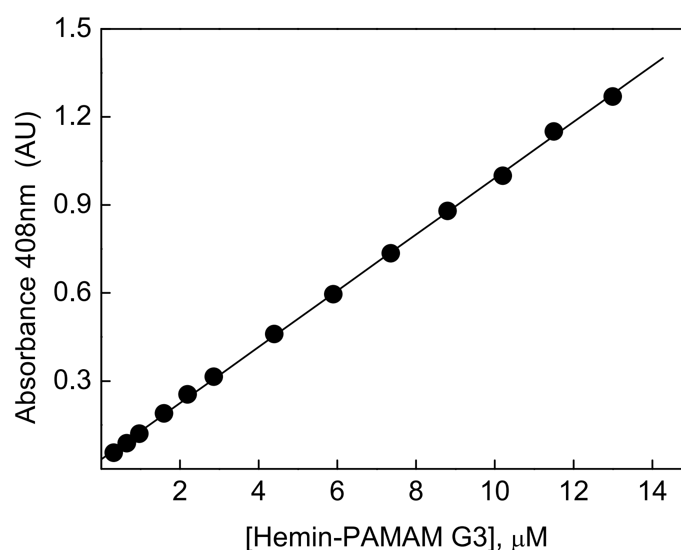

Figure SI1. Beer law on the HPG3 adduct in methanol solution.

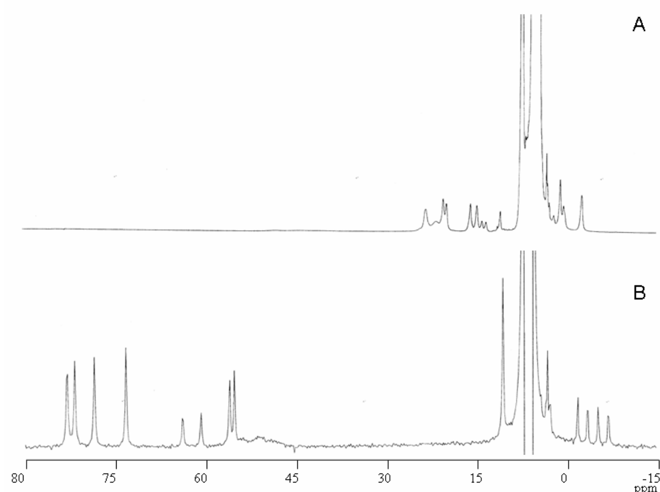

Figure SI2. ^{1}H NMR spectra of HPG2 (A) and Hemin (B) in MeOD at 298 K.

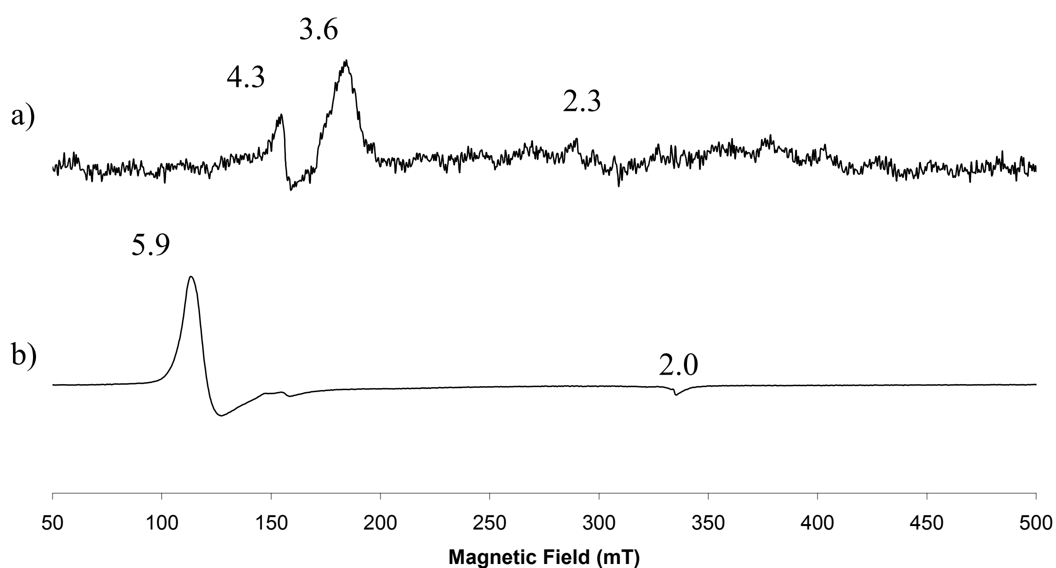

Figure SI3. 20K EPR spectra of HPG2 in methanol solution: a) in the native state and b) in the presence of ascorbic acid, 10s after the addition. Experimental conditions: ν= 9.39GHz, Mod. Amp. =1mT, 2mW microwave power, 100KHz modulation frequency.

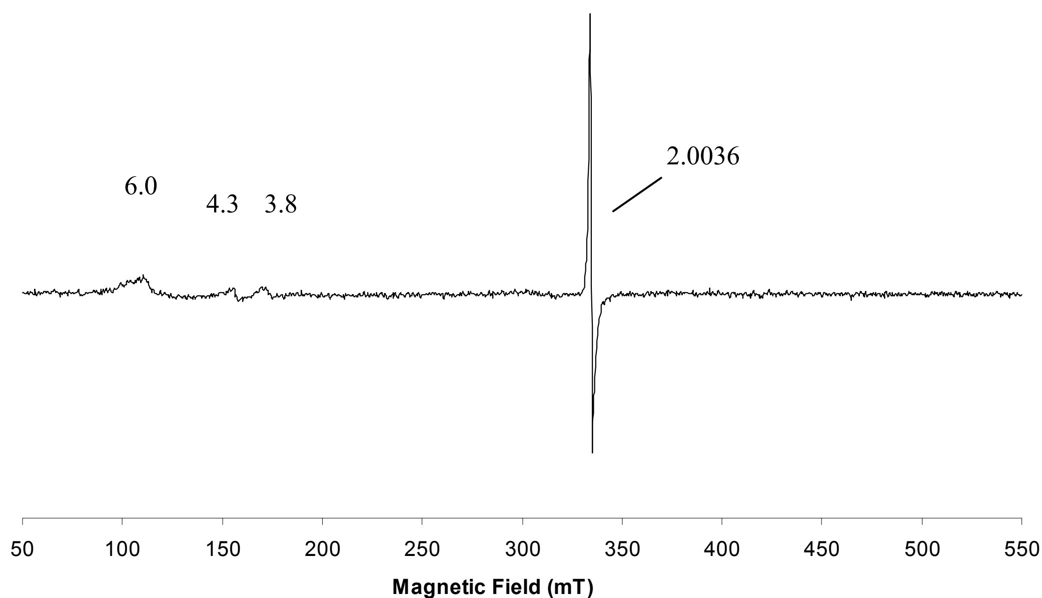

Figure SI4. EPR radical spectrum of ABTS obtained after the addition of H_2O_2 to HPG2/ABTS in water solution (T = 20K). Experimental conditions: [HPG2]= 0.14mM, [ABTS]= 1.7mM, $[H_2O_2]$= 1.3mM in phosphate buffer 100mM pH=7. ν= 9.39GHz, Mod. Amp. =1mT, 2mW microwave power, 100KHz modulation frequency.

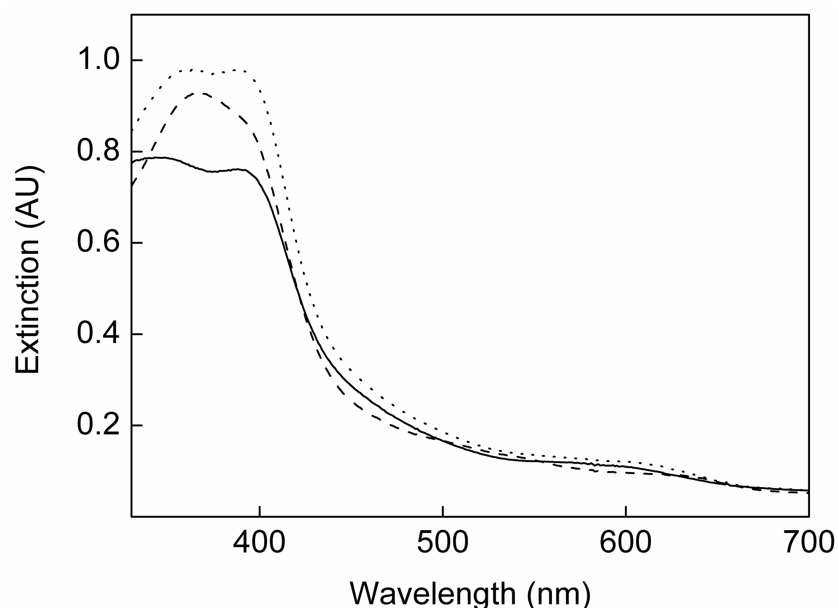

Figure SI5. UV-Vis spectra of HPG3 in aqueous solution (dotted line), in the presence of ascorbic acid (dashed line) and in presence of H_2O_2 (solid line). ([HPG3] = 1 M, [ascorbic acid] = 100 M, $[H_2O_2]$ = 8 μM, in 100mM phosphate buffer pH 7, T = 298 K).

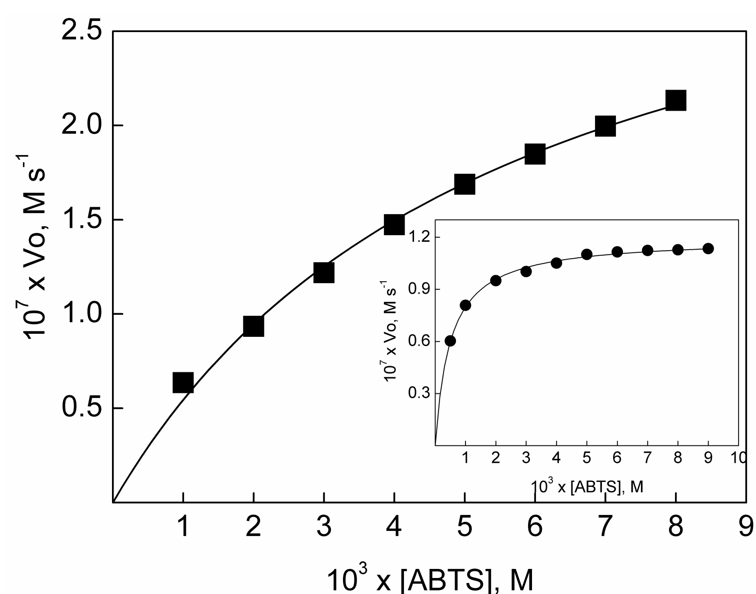

Figure SI6. Initial rates vs ABTS concentration in homogeneous solution. Absorbance at 415 nm was converted to ABTS concentration ($\varepsilon_{415\ nm}$ = 3.6 x 10^4 M^{-1} cm^{-1}). ([ABTS] = 1 – 8.5 mM, [H_2O_2] = 4 mM, [HPG2] = 1 μM in 100 mM phosphate buffer pH 7, T = 298 K). The inset reports the Initial rates vs ABTS concentration in heterogeneous catalytic process. ([ABTS] = 0.5 - 9 mM, [H_2O_2] = 2 mM, in 100 mM phosphate buffer pH 7, T = 298 K).

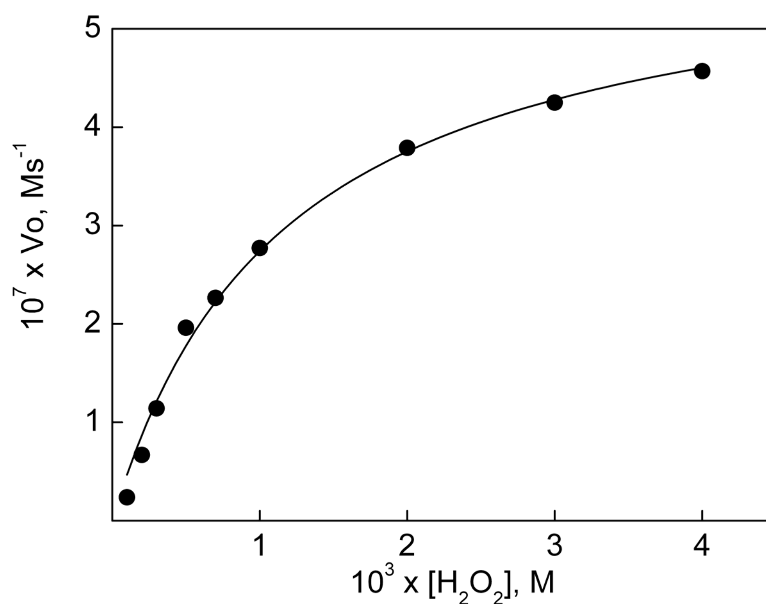

Figure SI7. Initial rates vs oxidant concentration in homogeneous solution. Absorbance at 415 nm was converted to ABTS concentration (ε_{415} = 3.6 x 10^4 M^{-1} cm^{-1}). ([ABTS] = 9 mM, [H_2O_2] = 0.1 - 4 mM, [HPG2] = 1μM in 100 mM phosphate buffer pH 7, T = 298 K).

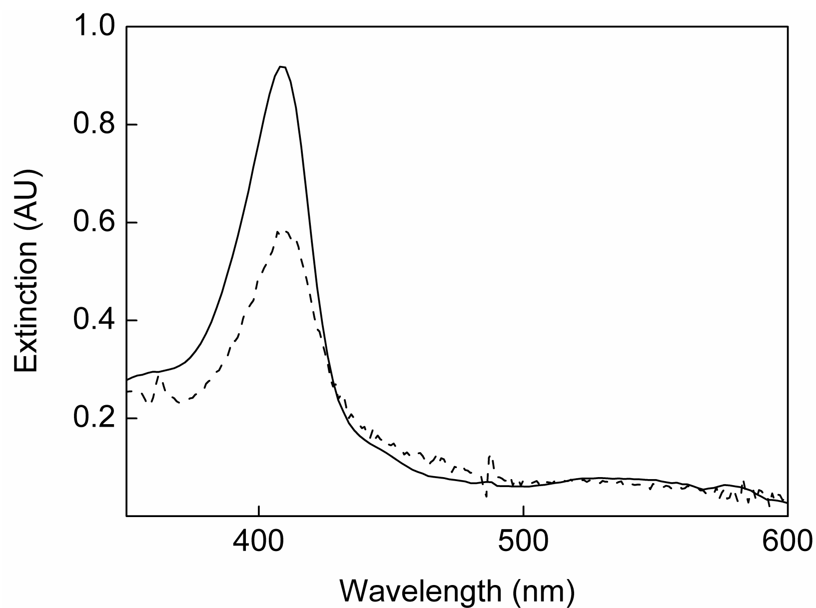

Figure SI8. UV-Vis spectra of 1 μM HPG2 in methanol solution (solid line) and as thin film (dashed line).

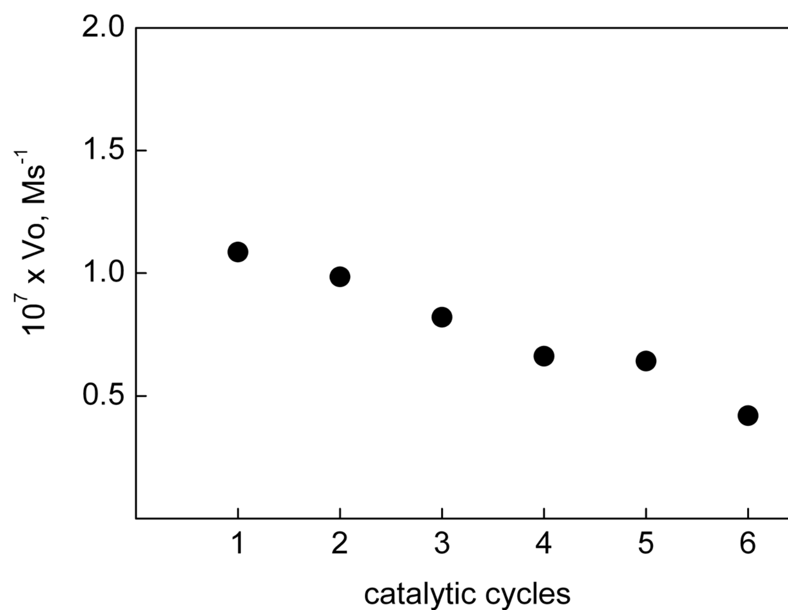

Figure SI9. Degradation of catalyst at high oxidant concentration for the heterogeneous process. Initial rates plot as function of catalytic cycles. ([ABTS] = 9 mM, [H_2O_2] = 4 mM in 100 mM phosphate buffer pH 7; HPG2 thin film, T = 298 K).

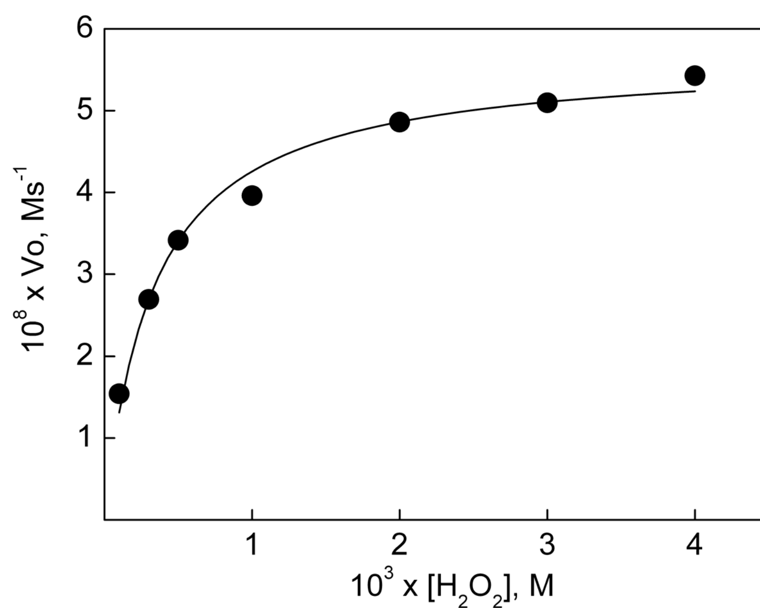

Figure SI10. Initial rates vs oxidant concentration for the heterogeneous process. Absorbance at 415 nm was converted to ABTS concentration (ε_{415} = 3.6 x 10^4 M^{-1} cm^{-1}). ([ABTS] = 9 mM, [H_2O_2] = 0.1 - 4 mM, HPG2 thin film in 100 mM phosphate buffer pH 7, T = 298 K)

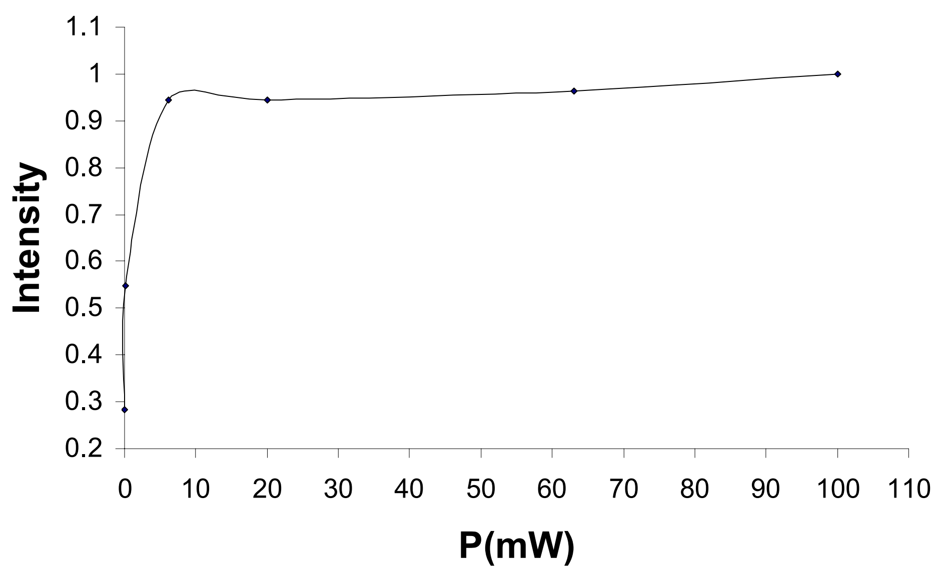

Figure SI11. Intensity (I) of the normalized radical signal at g=2.00 versus microwave power (P) (in milliwatts). EPR spectra have been recorded at 10K.