

Catalytic molecular motors: Fueling autonomous movement by a surface bound synthetic Manganese catalase

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Supporting information.

General:

Chemicals were purchased from Acros, Aldrich, Fluka or Merck. Solvents for extraction and chromatography were technical grade. All solvents used in reactions were freshly distilled from appropriate drying agents before use. All other reagents were recrystallized or distilled as necessary. Analytical TLC was performed with Merck silica gel 60 F₂₅₄ plates and visualisation was accomplished by UV light. Flash chromatography was carried out using Merck silica gel 60 (230-400 mesh ASTM). NMR spectra were obtained using a Varian Gemini-200 operating at 199.97 MHz for the ¹H nucleus or at 50.29 for the ¹³C nucleus. Chemical shifts are reported in δ units (ppm) relative to the residual deuterated solvent signals of CHCl₃ (¹H NMR: δ 7.26 ppm; ¹³C NMR: δ 77.0 ppm). The splitting patterns are designated as follows: s (singlet), d (doublet), t (triplet), m (multiplet). MS (EI) spectra were obtained with a Jeol JMS-600 spectrometer. Elemental analyses were performed in with a Foss-Heraeus CHN-O-Rapid or a EuroVector Euro EA Elemental Analyzer. Electrochemical measurements were carried out on a Model 630B Electrochemical Workstation (CHInstruments). Analyte concentrations were typically 0.5 to 1 mM in anhydrous acetonitrile containing 0.1 M tetrabutyl ammonium hexafluorophosphate (TBAP). Unless otherwise stated a Teflon shrouded glassy carbon working electrode or 10 μ m diameter platinum microelectrode (CHInstruments), a Pt wire auxiliary electrode and non-aqueous Ag/Ag⁺ ion reference electrode were employed (calibrated using 0.1 mM solutions of ferrocene (0.06 V vs Ag/Ag⁺ in 0.1 M TBAP/CH₃CN). Cyclic voltammograms were obtained at sweep rates of between 10 mV s⁻¹ to 100 mV s⁻¹. For reversible processes the half-wave potential values are reported. Redox potentials are +/- 10 mV. FTIR spectra were recorded (as intimate mixtures in KBr) in relectance mode using a Nicolet Nexus FTIR spectrometer. Images and movies of the moving particles were recorded using an Olympus BX 60 microscope, equipped with a Sony 3CCD DXC 950P digital camera, attached to a personal computer with Matrox Inspector 2.1 imaging software. Commercially available di-2-pyridyl ketone was converted into the oxime, which was reduced with zinc in ammonia to afford di-2-pyridyl-methylamine using literature procedures.¹

Experimental procedures:

2-({Di(2-pyridyl)methyl}imino)methylphenol. To a stirred solution of di-2-pyridyl-methylamine (1.85 g, 10 mmol) in 15 mL of methanol was added neat salicylaldehyde (1.22 g, 10 mmol). The solution immediately turned yellow. After stirring for about 10 min a yellow crystalline material separated. The reaction mixture was cooled to 0°C and stirred for another 30 min. The solids were isolated by vacuum filtration, washed with ice-cold MeOH, dried and a yellow micro crystalline powder was obtained. Yield 2.39 g (8.3 mmol), 83 %. ¹H-NMR (200 MHz, CDCl₃) δ 5.94 (s, 1H), 6.83-7.00 (m, 2H), 7.14-7.21 (m, 2H), 7.26-7.36 (m, 2H), 7.44 (dd, J = 7.8, J=0.5, 2H), 8.56-8.60 (m, 2H), 8.64 (s, 1H). ¹³C-NMR (50.3 MHz, CDCl₃) δ 80.1 (d), 116.9 (d), 118.5 (s), 118.8 (d), 122.0 (d), 122.5 (d), 131.9 (d), 132.7 (d), 136.9 (d), 149.5 (d), 160.2 (s), 160.9 (s), 166.5 (d). Anal. Calcd. for C₁₈H₁₅N₃O: C, 74.72 %; H, 5.23 %; N, 14.52 %; Found: C, 74.74 %; H, 5.21 %; N, 14.46 %.

2-({Di(2-pyridyl)methyl}amino)methylphenol. To a stirred solution of 2-({di(2-pyridyl)methyl}imino)methylphenol (6.0 g, 20.7 mmol) in 150 mL methanol at 0°C was added NaBH₄ (0.78 g, 20.7 mmol) in 3 portions during 15 min. The reaction mixture was stirred for 4 h. The mixture was acidified with 2 M aq. HCl to pH 1 and stirred for 15 min. Subsequently the reaction was neutralized with 2 M aq. NH₃ and 30 mL water was added. MeOH was removed under vacuum and the mixture extracted twice with AcOEt. The combined organic layers were dried and solvents were removed under vacuum to yield a reddish oil which turned into a glass after several weeks. Yield: 5.2 g (17.8 mmol), 86 %. ¹H-NMR (200 MHz, CDCl₃) δ 3.91 (s, 3H), 5.10 (s, 1H),

6.70-6.92 (m, 3H), 7.11-7.19 (m, 3H), 7.26-7.32 (m, 2H), 7.57 (dt, 2H, J = 7.7, J = 2.0), 8.57-8.60 (m, 2H). ¹³C-NMR (50.3 MHz, CDCl₃) δ 50.1 (t), 67.0 (d), 116.3 (d), 118.9 (d), 122.4 (d), 122.5 (d), 122.6 (s), 128.6 (d), 128.7 (d), 136.7 (d), 149.3 (d), 158.0 (s), 159.7 (s). Anal. Calcd. for C₁₈H₁₇N₃O: C, 74.20 %; H, 5.88 %; N, 14.42 %; Found: C, 73.41 %; H, 5.80 %; N, 14.29 %.

2-[[[Di(2-pyridyl)methyl](methyl)amino]methyl]phenol. To a solution of 2-([di(2-pyridyl)methyl]amino)methylphenol (3.04 g, 10.45 mmol) in 50 mL of ClCH₂CH₂Cl was added aqueous 30 % formaldehyde (1.70 g, 20.9 mmol) and after 15 min of stirring NaBH(OAc)₃ (4.42 g, 20.9 mmol) was added in small portions during 20 min. Subsequently the reaction was vigorously stirred overnight. CH₂Cl₂ (50 mL) was added and the organic layer was washed consecutively with 2 M aq. NH₃, and twice with water. After drying and evaporation of the solvents under vacuum the product was obtained as a sticky oil which was purified by chromatography on silica using ether as the eluent to obtain white solid material. Yield: 2.6 g, (8.5 mmol), 81%. ¹H-NMR (200 MHz, CDCl₃) δ 2.17 (s, 3H), 3.62 (s, 2H), 4.88 (s, 1H), 7.73 (dt, J = 7.3, 1.2, 1H), 6.87 - 6.97 (m, 2H), 7.12 - 7.20 (m, 3H), 7.50 - 7.68 (m, 4H), 8.58 - 8.62 (m, 2H). ¹³C-NMR (50.3 MHz, CDCl₃) δ 39.6 (q), 57.6 (d), 77.4 (s), 116.2 (d), 118.6 (d), 122.5 (d), 122.7 (s), 123.6 (d), 128.6 (d), 129.5 (d), 136.8 (d), 149.1 (d), 157.4 (s), 159.5 (s). Anal. Calcd. for C₁₉H₁₉N₃O: C, 74.73 %; H, 6.27 %; N, 13.76 %; Found: C, 74.56 %; H, 6.18 %; N, 13.77 %.

[Mn₂L₂-(μ-O₂CC₆H₅)](ClO₄) (1a). To a mixture of Mn(ClO₄)₂·6H₂O (0.724 g, 2 mmol) and 2-[[[di(2-pyridyl)methyl](methyl)amino]methyl]phenol (0.610 g, 2 mmol) under N₂ atmosphere, 10 mL of MeOH were added while the mixture was stirred. Subsequently, benzoic acid (0.122 g, 1 mmol) was added. After all solids were dissolved, triethylamine was added (0.42 mL, 3 mmol) at once and the solution turned pale green. After stirring for about 5 min, a solid precipitated and stirring continued for another 5 min. The solution was heated until boiling and gradually 13 mL of CH₃CN were added, while keeping the mixture boiling. After the last few mL of CH₃CN were added, the solids suddenly dissolved and the solution was left overnight. The supernatant was removed and the pale green crystals were washed thrice with MeOH and thrice with Et₂O and dried under vacuum. Yield: 610 mg (0.65 mmol), 65 %. ES-MS: 839.4 [L₂Mn₂(O₂CC₆H₅)]⁺. IR (cm⁻¹): 623, 736, 763, 791, 888, 997, 1011, 1047, 1090, 1117, 1280, 1289, 1412. Anal. Calcd. for C₄₅H₄₁ClMn₂N₆O₈: C, 57.55 %; H, 4.40%; N, 8.95%; Found: C, 57.32%; H, 4.42%; N, 8.84 %.

Suitable light green colored 'cubic-shaped' crystals were obtained by crystallisation from a mixture of acetonitrile/methanol. The crystal, a block of approximate size 0.25 x 0.25 x 0.25 mm, used for characterisation and data collection was glued on top of a glass fiber by using inert-atmosphere handling techniques and was transferred into the cold nitrogen cold stream of the low temperature unit² mounted on an Enraf-Nonius CAD-4F³ diffractometer, interfaced to a INDY (Silicon Graphics) UNIX computer (Mo tube, 50 kV, 40 mA, monochromated MoK α radiation, $\Delta\omega = 0.80 + 0.34 \text{ tg } \theta$). Unit cell parameters⁴ and orientation matrix were determined from a least-squares treatment of the SET⁴ setting angles of 22 reflections in the range $16.65^\circ < \theta < 20.21^\circ$. The unit cell was identified as monoclinic; reduced cell calculations did indicate C-centered lattice symmetry.⁶ The |E| distribution statistics were indicative of a non-centrosymmetric space group: the structure was initially solved in P1. Molecular symmetry and coordinate equivalence,^{7,8} within their s.u.'s suggested a twofold axis symmetry be present in the molecule. The space group C2 was determined from considerations of the unit cell parameters, number of molecules in the unit cell required for space group C2, statistical analyses of intensity distributions. The intensities of three standard reflections, monitored every three hours of X-ray exposure time, showed no greater fluctuations during data collection than those expected from Poisson statistics. A 360° ψ -scan for a reflection close to axial (31-1) showed a variation in intensity of less than 3% about the mean value. Intensity data were corrected for Lorentz and polarization effects, scale variation, but not for absorption and reduced to F_o².⁹ The structure was solved by Patterson methods and extension of the model was accomplished by direct methods applied to difference structure factors using the program DIRDIF.¹⁰ The positional and anisotropic thermal displacement parameters for the non-hydrogen atoms were refined. Refinement was frustrated by a disorder problem: from the solution it was clear that one of the two oxygen positions of the perchlorate ion was disordered. The position of the disordered oxygen was split in two positions, which s.o.f.'s were refined. The s.o.f. of the major component of the disordered oxygen refined to a

value of 0.61(1). A subsequent difference Fourier synthesis resulted in the location of all the hydrogen atoms, which coordinates and isotropic thermal displacement parameters were refined. Flack's¹¹ \mathbf{x} -refinement give an ambiguous result, probably due to twinning in this respect. Enantiomorph twin refinement resulted in a lower R value ($\Delta R = 0.01$) and $\mathbf{x} = 0.428(15)$. Final refinement on F^2 carried out by full-matrix least-squares techniques converged at $wR(F^2) = 0.0778$ for 4691 reflections with $F_o^2 \geq 0$ and $R(F) = 0.0303$ for 4481 reflections with $F_o \geq 4.0 \sigma(F_o)$ and 375 parameters. The final difference Fourier map was essentially featureless: no significant peaks ($0.55(5) \text{ e}/\text{\AA}^3$) having chemical meaning above the general background were observed. The positional and anisotropic thermal displacement parameters for the non-hydrogen atoms and isotropic thermal displacement parameters for hydrogen atoms were refined on F^2 with full-matrix least-squares procedures minimizing the function $Q = \sum_h [w(F_o^2 - kF_c^2)]^2$, where $w = 1/[\sigma^2(F_o^2) + (aP)^2 + bP]$, $P = [\max(F_o^2, 0) + 2F_c^2] / 3$, F_o and F_c are the observed and calculated structure factor amplitudes, respectively; a and b were refined. Reflections were stated observed if satisfying $F^2 > 0$ criterion of observability. No missed symmetry (*MISSYM*) and solvent-accessible voids (total potential solvent accessible volume is 37.1 \AA^3) were detected by procedures implemented in *PLATON*.^{12,13} Crystal data and numerical details on data collection and refinement are given in Table S1. Neutral atom scattering factors and anomalous dispersion corrections were taken from *International Tables of Crystallography*.¹⁴ All calculations performed on the HP9000/735 computer at the University of Groningen with the program packages *SHELXL*¹⁵ (least-square refinements), *PLATON*¹⁶ (calculation of geometric data and the *ORTEP* illustrations).

[Mn₂L₂-(μ -O₂CC₆H₅-CHO)](ClO₄) (1b). To a mixture of Mn(ClO₄)₂.6H₂O (0.724 g, 2 mmol) and 2-[[[di(2-pyridyl)methyl](methyl)amino]methyl]phenol (0.610 g, 2 mmol) under N₂ atmosphere, 10 mL of MeOH were added while the mixture was stirred. Subsequently, 4-carboxybenzaldehyde (0.150 g, 1 mmol) was added. After all solids were dissolved, triethylamine was added (0.42 mL, 3 mmol) at once and the solution turned pale green. After stirring for about 5 min, a solid precipitated and stirring continued for another 5 min. The solution was heated until boiling and gradually 15 mL of CH₃CN were added, while keeping the mixture boiling. After the last few mL of CH₃CN were added, the solids suddenly dissolved and the solution was left overnight. The supernatant was removed and the pale green crystals were washed thrice with MeOH and thrice with Et₂O and dried under vacuum. Yield: 590 mg (0.61 mmol), 61 %. ES-MS: 867.5 [L₂Mn₂(O₂CC₆H₅CHO)]⁺. IR (cm⁻¹): 629, 760, 808, 883, 1014, 1097, 1159, 1203, 1276, 1403, 1454, 1483, 1558, 1602, 1660, 1760. Anal. Calcd. for C₄₆H₄₁ClMn₂N₆O₉: C, 57.12; H, 4.27; Cl, 3.67; Mn, 11.36; N, 8.69; O, 14.89; Found: C, 56.98%; H, 4.38%; N, 8.51 %.

Functionalisation of SiO₂ particles with [Mn₂L₂-(μ -O₂CC₆H₅-CHO)](ClO₄). Aminopropyl silicagel (40-63 μm) (200 mg) was added to a solution of [Mn₂L₂-(μ -O₂CC₆H₅-CHO)](ClO₄) (50 mg, 0.05 mmol) in EtOH / CH₃CN 2:1 (15 mL). The suspension was stirred at reflux for 2 h. and the functionalised particles were isolated by vacuum filtration and washed twice with EtOH, CH₃CN, CH₂Cl₂ and Et₂O, affording 220 mg of functionalised particles which showed strong catalytic activity (*vide infra* for IR and CV).

IR Spectroscopy

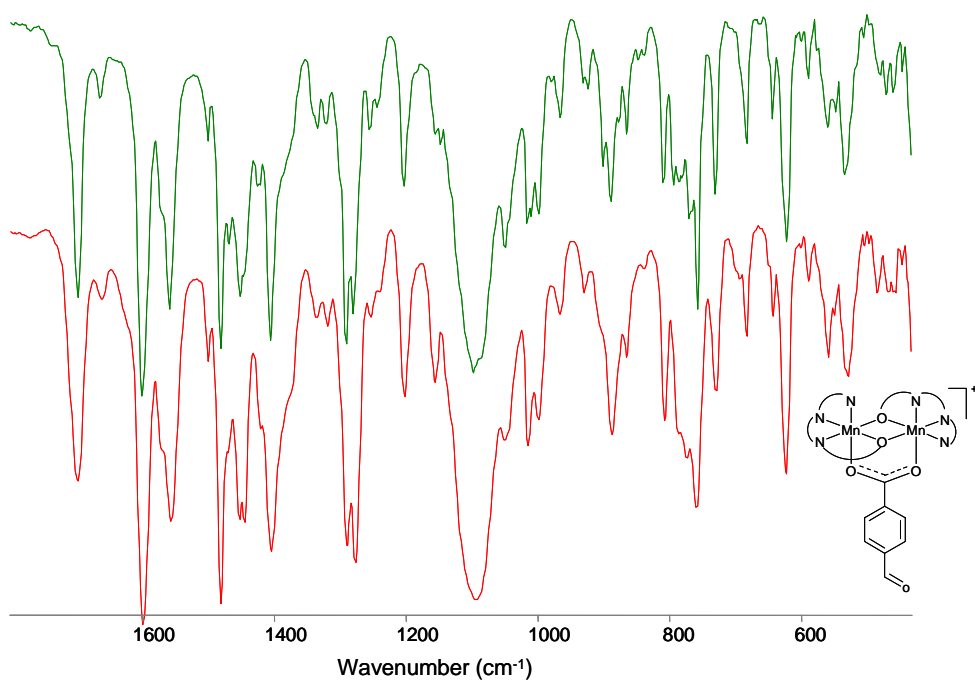


Figure S1. IR spectra of **1b** before (upper) and after (lower) treatment with H₂O₂.

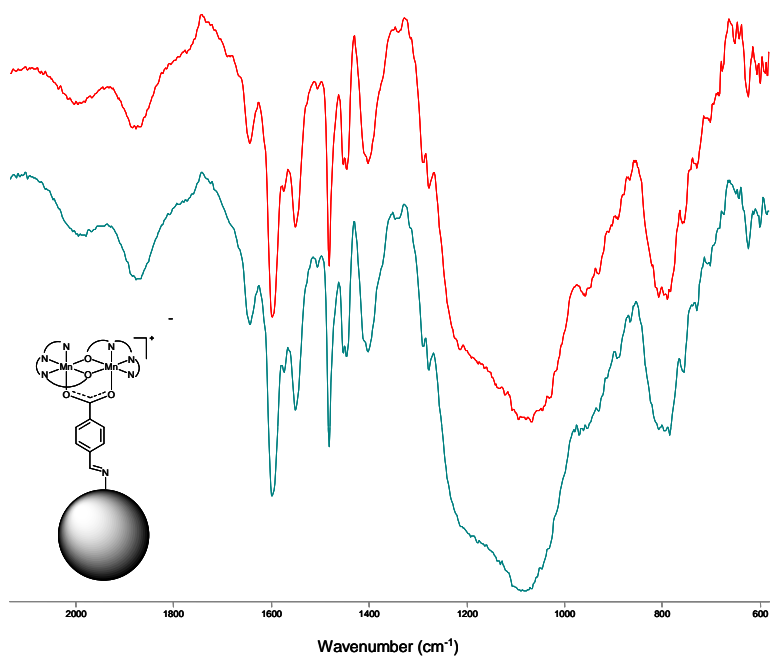


Figure S2 IR spectra of **1b**-modified microparticles before (upper) and after (lower) treatment with H₂O₂.

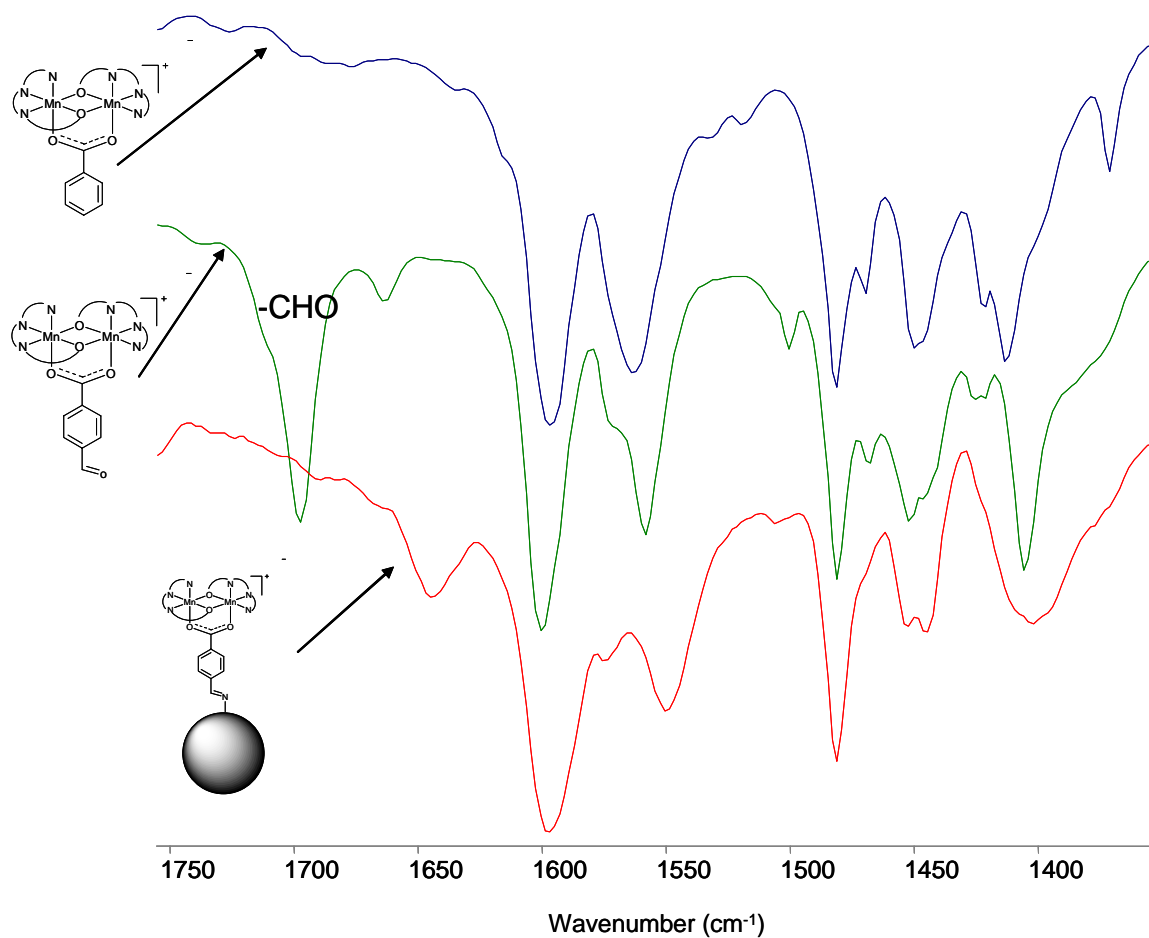


Figure S3 IR spectra of **1a** (upper), **1b** (middle) and **1b**-modified microparticles (lower).

Electrochemistry

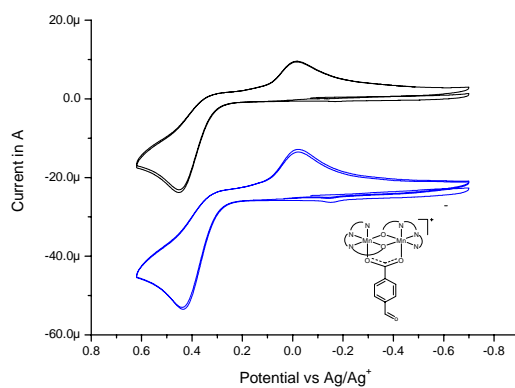


Figure S4. Cyclic voltammetry of **1b** before (upper) and after (lower) treatment with H_2O_2 . In CH_3CN (0.1 M TBAP)

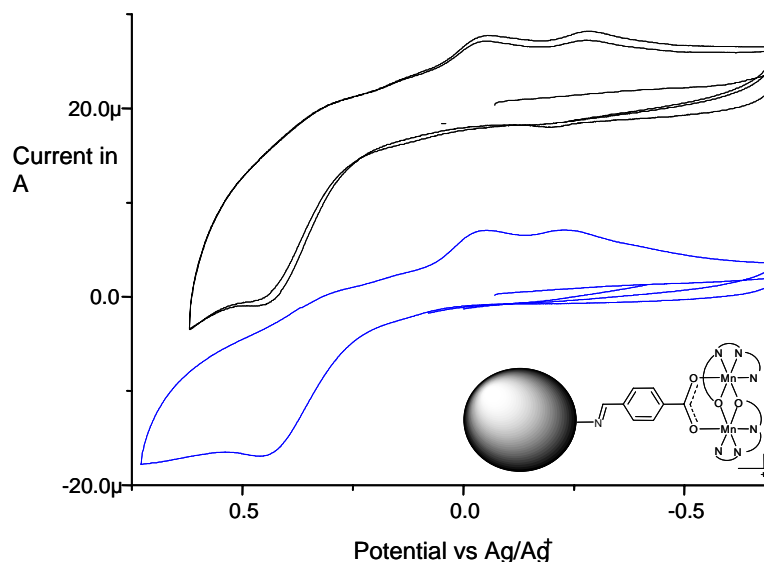


Figure S5. Cyclic voltammety of **1b**-modified microparticles before (upper) and after (lower) treatment with H_2O_2 . In CH_3CN (0.1 M TBAP)

References

- 1 E. Niemers, R. Hiltmann, *Synthesis*, 1976, 593.
- 2 F. van Bolhuis, *J. Appl. Cryst.* 1971, 4, 263.
- 3 Enraf-Nonius *CAD4-UNIX* Version 5.1, Utrecht modified version October 1994. Enraf-Nonius Delft, Scientific Instruments Division, Delft, The Netherlands.
- 4 A. J. M. Duisenberg, *J. Appl. Cryst.* 1992, 25, 92.
- 5 J.L. de Boer, A.J.M. Duisenberg, *Acta. Cryst.* 1984, A40, C-410.
- 6 A.L. Spek, *J. Appl. Cryst.* 1988, 21, 578.
- 7 Y. Le Page, *J. Appl. Cryst.* 1987, 20, 264.
- 8 Y. Le Page, *J. Appl. Cryst.* 1988, 21, 983.
- 9 A.L. Spek, 1993. *HELENA. Program for Reduction of CAD4 Data*. Utrecht Univ. The Netherlands.
- 10 P.T. Beurskens, G. Beurskens, W.P. Bosman, R. de Gelder, S. Garcia-Granda, R.O. Gould, R. Israël, J.M.M. Smits, 1997. The *DIRDIF-97* program system, Crystallography Laboratory, University of Nijmegen, The Netherlands.
- 11 H.D. Flack, *Acta. Cryst.* 1983, A39, 876.
- 12 A.L. Spek, *Acta Cryst.* 1990, A46, C-34.
- 13 A.L. Spek, *Am. Crystallogr. Assoc. Abstr.* 1994, 22, 66.
- 14 *International Tables for Crystallography*, 1992. Vol. C. Edited by A.J.C. Wilson, Kluwer Academic Publishers, Dordrecht. The Netherlands.
- 15 G.M. Sheldrick, 1997. *SHELX-97. Program for the Solution and Refinement of Crystal Structures*. Univ. of Göttingen, Germany.
- 16 A.L. Spek, 1998. *PLATON. Program for the Automated Analysis of Molecular Geometry*. Version of March 1998. Univ. of Utrecht, The Netherlands.