Supporting information for

Non-heme iron(II/III) complexes that model the reactivity of lipoxygenase with redox switch

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Experimental Section

Materials and Physical Measurements. All chemicals were of analytical reagent grade, except that *ortho*-phenylene diamine (*o*-PDA) was chemical pure; linoleic acid (LA) and 2-thiobabituric acid (TBA) were biological reagents. These chemicals were used without further purification unless specially stated. All solvents were of analytical grade and were purified by literature methods.

Ultraviolet-visible spectra (UV-vis. 900–190 nm) were measured on a Shimadzu UV-265 spectrometer. Electrospray ionization mass spectral measurements in positive ion mode were carried out on an API 2000 mass spectrometer equipped with a Turbo Ion spray source. Cyclic voltammetry (CV) was carried out under Ar atmosphere with CHI 660C Electrochemical Analyzer on a conventional three–electrode: glassy–carbon electrode as working electrode, a platinum wire as auxiliary electrode and an Ag/AgCl electrode as reference. All the potentials were referenced to $Fc^{+/0}$ in methanol (+0.610 V *vs* SHE, ΔE = 67 mV, scan rate: 50 mV/s, 0.10 mol/L TBAP). The supported electrolyte of tetrabutylammonium perchlorate (TBAP) was recrystallized twice from methanol and dried overnight under vacuum.

Synthesis of N,N,N′,N′-tetrakis(2-benzimidazolyl-methyl)*ortho*-diamine-*trans*- cyclohexane (ctb). The synthetic route of ligand ctb was a modification to previously reported procedure according to literature.[1] CTA (*ortho*-bisamino-*trans*-cyclohexane-N,N,N′,N′-tetraacetic acid) and *o*-PDA were mixed in a molar ratio of 1:4 in glycol. After heating for 6-8 h at 140-180 °C, the product ctb was separated from the cooled reaction solution and recrystallized from ethanol to give a good yield (95%). NMR, UV-Vis and IR spectra of the product were identical with those reported earlier.[2]

[1] H. Hendris, P. Birker, J. Vanrijin, G. Verschoor, J. Reedijk, *J. Am. Chem. Soc.* 1982, **104**,

3607.

[2] Z. Liao, C. Liu, J. Wang, T. Zhang, J. Shi, B.T. Fan, L. Xiao, *J. Chim. Phys.* 1995, **92**, 668.

Synthesis of [Fe(ctb)]$(ClO_4)_2 \cdot C_2H_5OH$ (1).

Method 1: $Fe(ClO_4)_2 \cdot 6H_2O$ (0.363 g, 1.00 mmol) and ctb (0.635 g, 1.00 mmol) were dissolved in 200 ml ethanol to give a clear solution, which was filtered and stood under ambient atmosphere without disturbance for a week. Block, colorless crystals were collected, washed with ethanol and dried in air to give a yield of 0.86 g (92%).

Method 2: $Fe(ClO_4)_3 \cdot 9H_2O$ (0.516 g, 1.00 mmol) and ctb (0.635 g, 1.00 mmol) were dissolved in 80 ml ethanol with refluxing for 4 hours, then filtered and stood under ambient atmosphere without disturbance for a week. Two kinds of crystals were found: colorless and orange. The former was found to be complex **1** by X-ray analysis. Anal. Calcd. for $FeC_{40}H_{44}N_{10}O_9Cl_2$: C, 51.35; H, 4.74; N, 14.97%; Found: C, 51.32; H, 4.56; N, 14.92%; $\Lambda_m = 135$ S·cm^2·mol^{-1}; UV-vis (in MeOH): $\lambda_{max} = 278.2$ nm ($\varepsilon_m = 35750$ cm^{-1}·M^{-1}), 281.7 (33250 cm^{-1}·M^{-1}).

Synthesis of [Fe(C_2H_5O)(Hctb)]$(ClO_4)_3 \cdot C_2H_5OH$ (2).

Method 1: $Fe(ClO_4)_3 \cdot 9H_2O$ (0.516 g, 1.00 mmol) and ctb (0.635 g, 1.00 mmol) were dissolved in 200 ml ethanol with stirring, the resulting solution was filtered and stood under ambient atmosphere without disturbance for a week. Orange crystals were collected, washed with ethanol and dried in air to give a yield of 0.54 g (50%).

Method 2: same as the method 2 for complex **1**, the above-mentioned orange crystals were found to be complex **2** by X-ray analysis. Anal. Calcld for $FeC_{42}H_{50}N_{10}O_{14}Cl_3$: C, 46.66; H, 4.66; N, 12.96%; Found: C, 46.90; H, 4.98; N, 12.45%. $\Lambda_m = 195$ S·cm^2·mol^{-1}; UV-vis (in MeOH): $\lambda_{max} =$ 278.2 nm ($\varepsilon_m = 35700$ cm^{-1}·M^{-1}), 280.2 (33215 cm^{-1}·M^{-1}), 368 (4743 cm^{-1}·M^{-1}).

Synthesis of [Fe(CH_3O)(Hctb)]$(ClO_4)_3 \cdot 3CH_3OH \cdot 4.5H_2O$ (3).

$Fe(ClO_4)_3{\cdot}9H_2O$ (0.516 g, 1.00 mmol) and ctb (0.635 g, 1.00 mmol) were dissolved in 40 ml methanol with stirring, the resulting solution was filtered and stood under ambient atmosphere without disturbance for a week. Orange crystals were collected, washed with methanol and dried in air to give a yield of 0.66 g (55%). Anal. Calcd. for $FeC_{42}H_{55}N_{10}O_{18}Cl_3$: C, 43.86; H, 4.82; N, 12.18%; Found: C, 44.02; H, 4.56; N, 11.92%; Λ_m = 196 $S{\cdot}cm^2{\cdot}mol^{-1}$; UV-vis (in MeOH): λ_{max} = 278.2 nm (ε_m = 35743 $cm^{-1}{\cdot}M^{-1}$), 280.6 (33266 $cm^{-1}{\cdot}M^{-1}$), 368 (4830 $cm^{-1}{\cdot}M^{-1}$).

Caution! Perchlorate salts may be explosive. It should be handled with a small amount.

Crystal data and structure refinement for the complexes

The crystal data of the complexes were collected on a Bruker AXS SMART 4000 CCD. The crystal structures were solved by Patterson methods option of SHELXS-97, and developed by conventionally alternating cycles of least-squares refinement on F^2 (SHELXL-97) and different Fourier synthesis. All the non-hydrogen atoms were refined anisotropically. The hydrogen atoms were geometrically generated and allowed to ride on their parent atoms. One perchlorate anion in **1** (Cl1, O1, O2, O3, O4; Cl1' O1', O2', O3', O4') and **3** (Cl3, O10, O11, O12, O13; Cl3', O10', O11', O12', O13'), and the carbons of ethoxide group (C39, C40; C39', C40') in complex **2** were refined disorderly in two positions. The crystal data and relevant experimental parameters used in the structure determination of the complexes are listed in Table S1. The crystal structures of complex **3** are shown in Fig. S1.

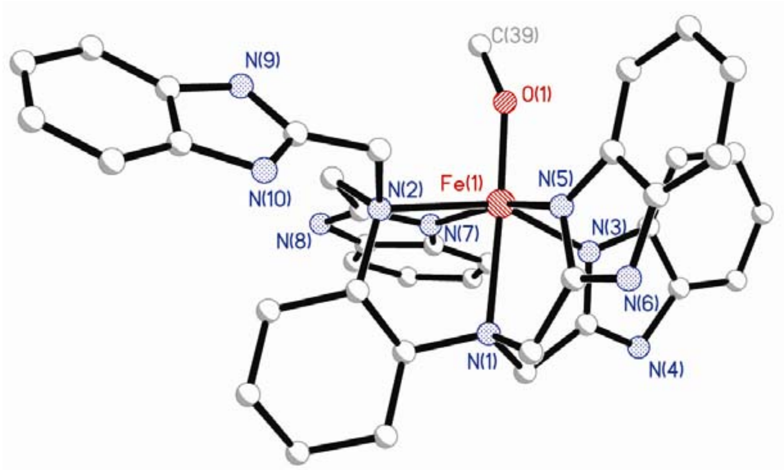

Fig. S1 Crystal structure of the cation of **3**, Fe^{III}(OMe)(Hctb)]$^{3+}$, showing the atom labeling scheme, omitting hydrogen atoms. Selected bond lengths [Å] or angles [°]: Fe1–O1 1.811(3), Fe–N1 2.334(4), Fe1–N2 2.300(4), Fe1–N3 2.051(4), Fe1–N5 2.062(4), Fe1–N7 2.127(4), O1–Fe1–N1 171.33(15), O1–Fe1–N2 97.26(16), O1–Fe1–N3 109.55(15), O1–Fe1–N5 95.95(15), O1–Fe1–N7 96.73(16), N1–Fe1–N2 76.77(14), N1–Fe1–N3 77.31(14), N1–Fe1–N5 78.33(14), N1–Fe1–N7 88.20(15).

Table S1 Crystal parameters of the complexes **1**, **2** and **3**.

Compounds	**[Fe(ctb)](ClO$_4$)$_2$·EtOH (1)**	**[Fe(OEt)(Hctb)](ClO$_4$)$_3$·EtOH (2)**	**[Fe(OMe)(Hctb)](ClO$_4$)$_3$·3MeOH·4.5H$_2$O(3)**
Empirical formula	$C_{40}H_{44}Cl_2FeN_{10}O_9$	$C_{42}H_{50}Cl_3FeN_{10}O_{14}$	$C_{42}H_{63}Cl_3FeN_{10}O_{20.5}$
Formula weight	935.60	1081.12	1198.22
Temperature, K	293(2)	293(2)	150(2)
Wavelength, Å	0.71073	0.71073	0.71073
Crystal system	Monoclinic	Monoclinic	Monoclinic
Space group	$P2_1$/n	$P2_1$/n	$C2$/c
Unit cell dimensions	a = 14.3899(9) Å b = 16.1238(10) Å c = 18.8845(12) Å β= 103.1700(10)°	a = 14.3060(10) Å b = 24.6698(17) Å c = 14.8559(10) Å β= 104.7840(10)°	a = 41.3876(14) Å b = 14.1082(5) Å c = 18.3504(4) Å β= 99.582(2)°
Volume, Å^3	4266.3(5)	5069.5(6)	10565.4(6)
Z	4	4	8
Number of reflections measured	23054	25366	15724
Number of independent reflections (R_{int})	8360 (0.0537)	8902 (0.0394)	9275 (0.0233)
D_c, Mg/m^3	1.457	1.417	1.507
Final R indices [I>2σ(I)]	R_1 = 0.0594 wR_2 = 0.1465	R_1 = 0.0662 wR_2 = 0.1924	R_1 = 0.0788 wR_2 = 0.2249

$R_1 = \Sigma||F_o|-|F_c||/ \Sigma|F_o|$, $wR_2 = \{\Sigma[w(|F_o|-|F_c|)^2]/ \Sigma[wF_o^2]\}^{1/2}$.

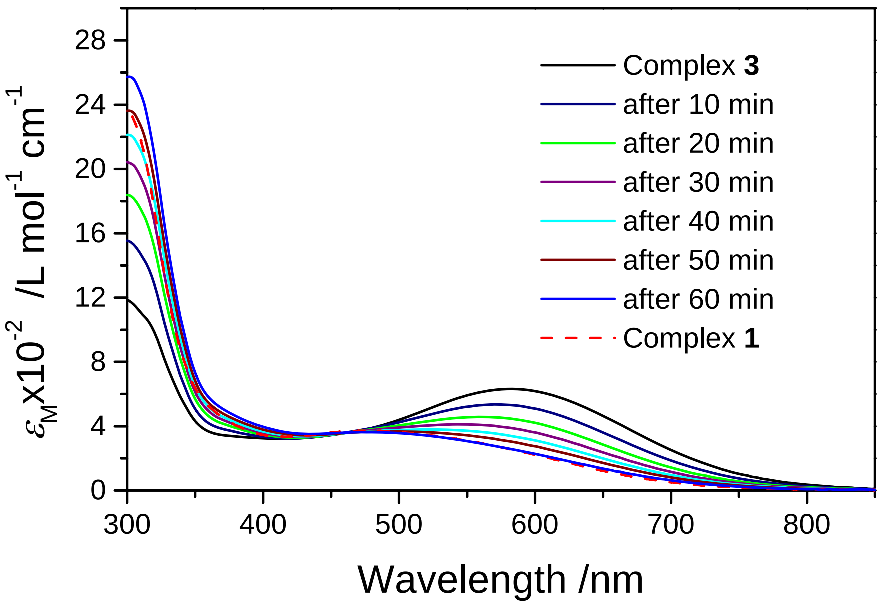

Fig. S2 UV-vis spectra for complex **3** in acetonitrile with time courses and complex **1** (dashed line) in acetonitrile for comparison.

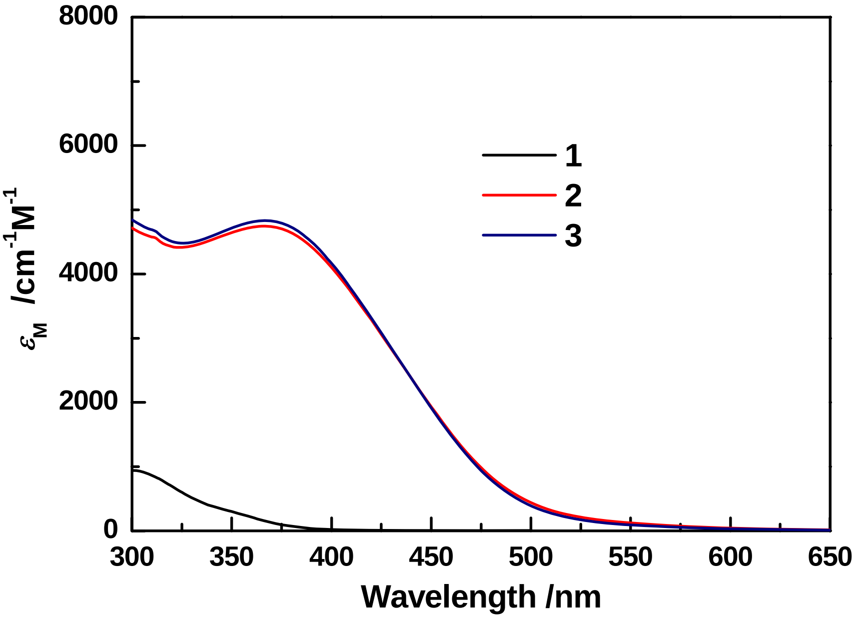

Fig. S3. UV-vis spectra of complexes **1**, **2** and **3** in MeOH.

Kinetic measurements

All kinetic runs were performed with an excess of substrate to achieve pseudo-first-order conditions for the reduction of the ferric complex. For each substrate, at least five measurements were made of k_{obs} and the absolute second-order rate constants k_2 were calculated by a least-squares fitting plot of k_{obs} versus substrate concentration.

1) **Peroxidation of linoleic acid:** The reactions were carried out at 333 K and pH 7.25 in ethanol exposed to air. The wavelength of 532 nm was chosen to monitor the processes. A constant concentration of the complexes (2.0×10^{-5} M) was used in all reactions, while linoleic acid (LA) concentration ranged from 0.0004 to 0.006 M. The model compounds and LA were dissolved in ethanol, respectively. The mixed solutions were first stirred for 10 min, and then TBA ethanol solution was added. Upon incubation at 333 K and at pH 7.25, the color of solutions was changed from colorless to yellow, orange, and pink after ca. 40 minutes. The electronic spectra for the solution were recorded with time courses between 450–600 nm. A control experiment was also checked for ferric perchlorate, but its spectra didn't show any change during the test for 10 hours. In order to study the oxidized products, the ultimate reaction solutions were introduced directly into electroionspray source at a flow-rate of 5 μL/min by a syringe.
2) **Oxidation of 1,4-cyclohexadiene (CHD), 9,10-dihydroanthracene (DHAn) and d_4-9,10-dihydroanthracene (d_4-DHAn)** [3] All the runs were performed under anaerobic environment. A constant concentration of the complexes (0.0016 M) was used in all reactions, while the substrate concentration ranged from 0.08 to 0.64 M. The rate constants were determined from absorption data collected in the wavelength range 300–600 nm.

[3] C. R. Goldsmith, R. T. Jonas, T. D. P. Stack, *J. Am. Chem. Soc.* 2002, **124**, 83

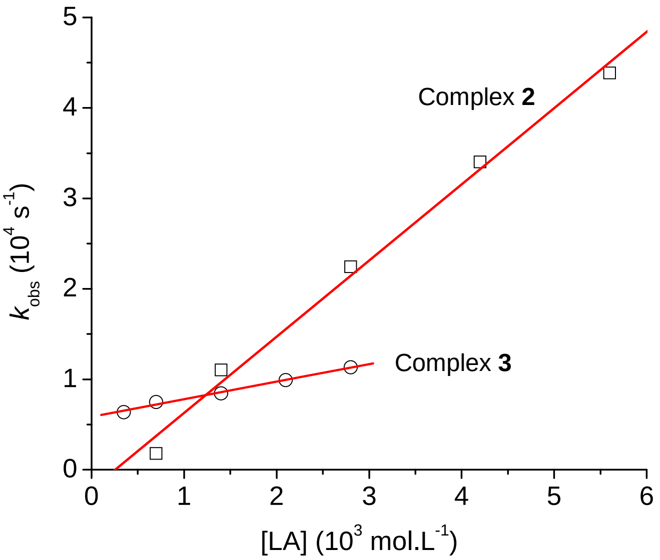

Fig. S4. First-order rate constants for the peroxidationof linoleic acid (LA) with complexes **2** and **3** in the presence of thiobabituric acid (TBA) in EtOH at 333 K.

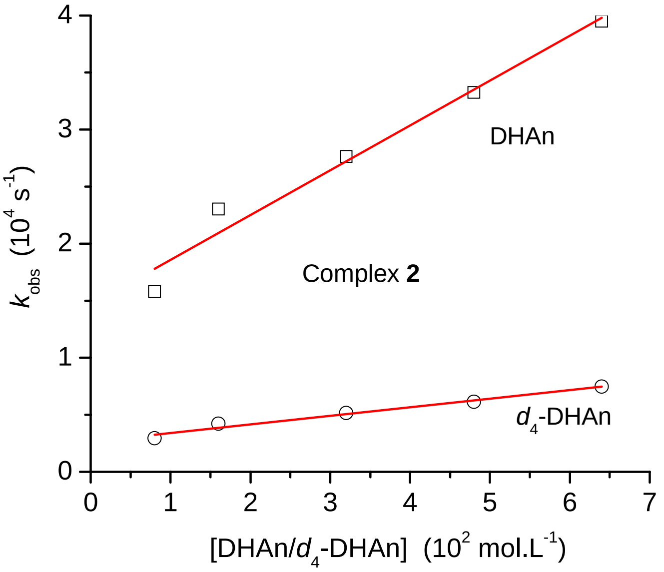

Fig. S5. First-order rate constants for the reduction of complex **2** in the presence of 9,10-dihydroanthracene (DHAn) or d_4-9,10-dihydroanthracene (d_4-DHAn) in MeOH at 323 K.

Table S2. Second-Order Rate Constants for the Reactions of Substrates with Complexes **1**, **2** and **3** in MeOH.

Complex	Substrate	Temp (K)	k_{2corr} ($M^{-1}s^{-1}$)[a]	KIE[b]
1	linoleic acid	333	0	-
2	linoleic acid	333	$4.2 \pm 0.2 \times 10^{-2}$	-
3	linoleic acid	333	$1.0 \pm 0.1 \times 10^{-2}$	-
2	1,4-cyclohexadiene	313	$1.3 \pm 0.1 \times 10^{-3}$	-
3	1,4-cyclohexadiene	313	$7.7 \pm 0.2 \times 10^{-4}$	-
2	9,10-dihydroanthracene	323	$9.8 \pm 0.2 \times 10^{-4}$	5.1
2	d_4-9,10-dihydroanthracene	323	$1.9 \pm 0.1 \times 10^{-4}$	

[a] Second-order rate constants per available C-H bond were adjusted for reaction stoichiometry to yield k_{2corr}. [b] Kinetic isotope effect determined in MeOH at 323 K.