Kinetic and theoretical comprehension of diverse rate laws and reactivity gaps in Coriolus hirsutus laccase-catalyzed oxidation of acido and cyclometalated Ru^II complexes

Sergey A. Kurzeev,[a,b] Alexander S. Vilesov,[a] Tatyana V. Fedorova,[a] Elena V. Stepanova,[a] Olga V. Koroleva,[a] Igor V. Kurnikov,[d] Christian Bukh,[c] Morten J. Bjerrum,[c] and Alexander D. Ryabov[d]*

[a] *A. N. Bach Institute of Biochemistry Russian Academy of Science, Leninskii pr. 33, Moscow, 117071 Russia;* [b] *Department of Chemistry, Moscow State University, Moscow, 119899 Russia;* [c] *Department of Basic Sciences and Environment, University of Copenhagen, Thorvaldsensvej 40, DK-1871 Frederiksberg C, Denmark;* [d] *Department of Chemistry, Carnegie Mellon University, 4400 Fifth Avenue, Pittsburgh, PA 15213, USA*
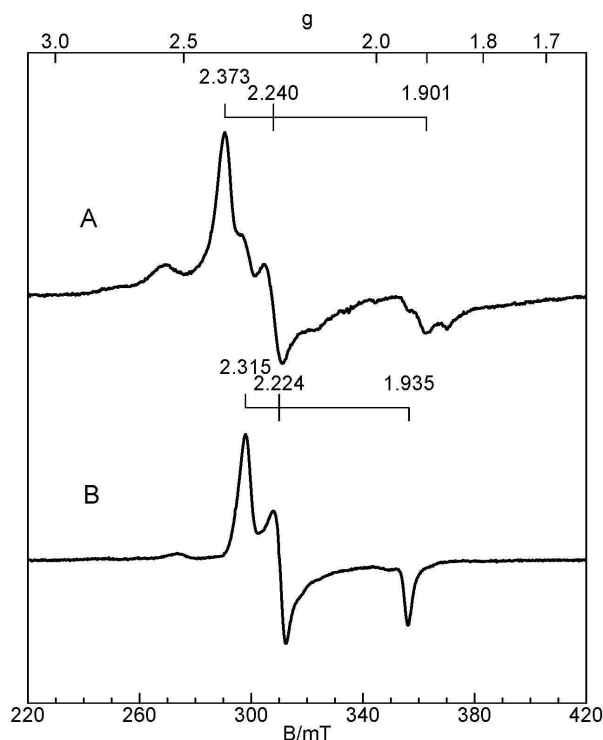
Figure 1S. (A) EPR spectrum of the reaction mixture obtained after reaction of complex **2a** (0.5 mM) with laccase (7.4×10^{-8} M) in 0.01 M phosphate buffer containing 10% methanol indicating generating of a major Ru^{III} species with g values 1.901, 2.240, and 2.373. There is a minor product with g values 1.935, 2.224, and 2.315. (B) EPR spectrum of the minor product obtained by irradiating of **2a** in MeOH for 10 min followed by dissolving the solution in in 0.01 M phosphate buffer. The irradiation leads to the dissociation of both MeCN ligands of **2a** suggesting the minor product in (A) to be $[Ru^{III}(o\text{-}C_6H_4\text{-}2\text{-py})(phen)(H_2O/OH)_2]^{n+}$, the major product being $[Ru^{III}(o\text{-}C_6H_4\text{-}2\text{-py})(phen)(MeCN)_2]^{2+}$. The presence of tiny amount of $[Ru^{III}(o\text{-}C_6H_4\text{-}2\text{-py})(phen)(MeCN)(H_2O/OH)]^{n+}$ cannot be excluded.
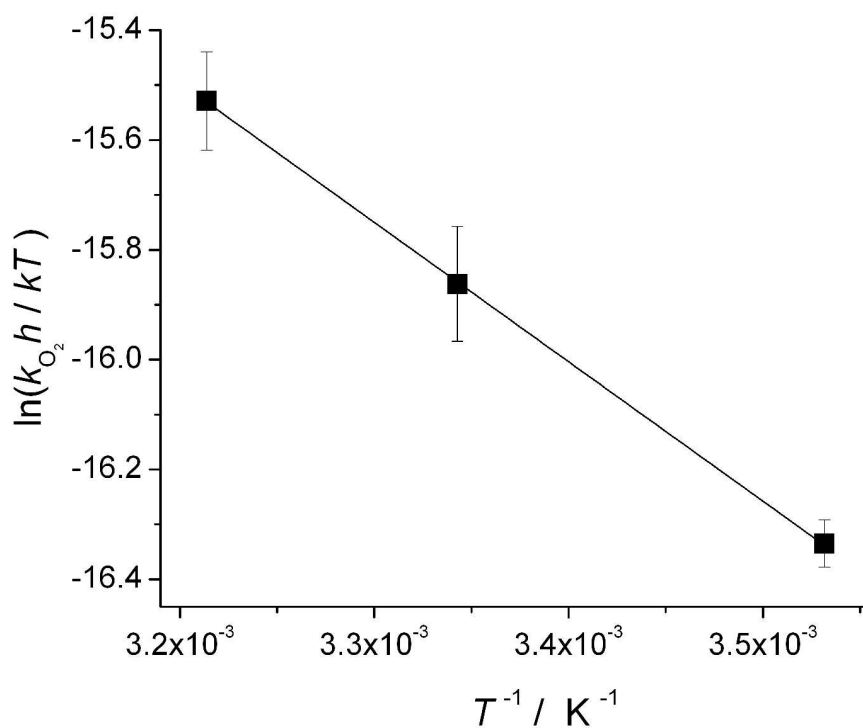
Figure 2S. Determination of the activation entropy and enthalpy for the reaction between reduced laccase and dioxygen by a secondary plot of ln ($k_{O_2}h/k{\cdot}T$) versus T^{-1}. The second order rate constants at three temperatures for the reaction between reduced laccase and dioxygen, k_{O_2}, were derived from the slopes in Figure 5; k is Boltzmann constant and h is Planck's constant.
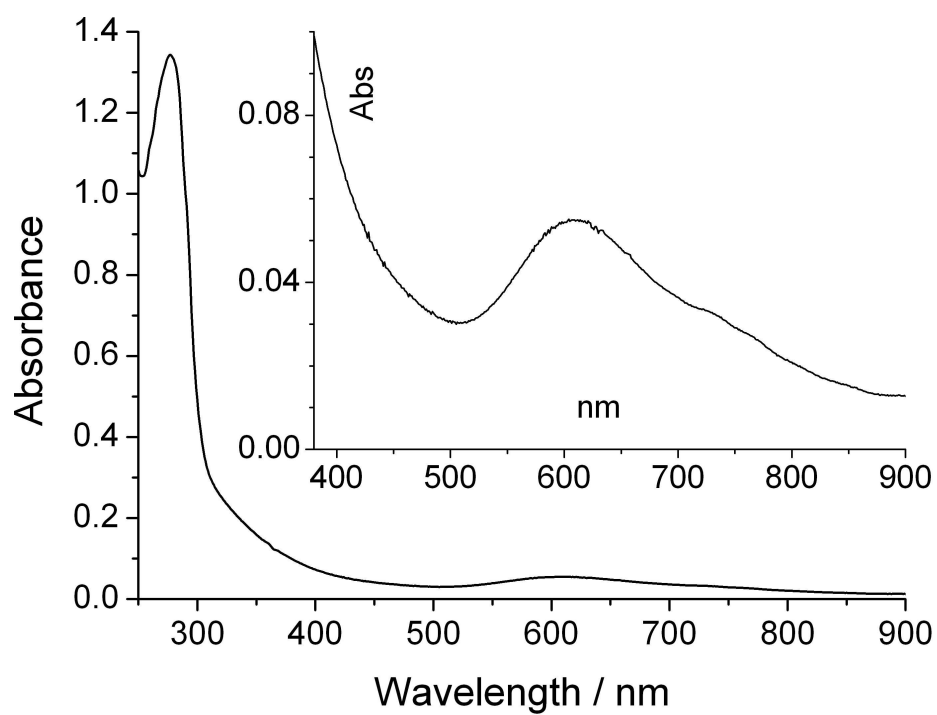
Figure 3S. UV-Vis spectrum of *C. hirsutus* laccase at pH 6.2. The stock solution of laccase was diluted 40 times in a buffer containing 50 mM citrate, 50 mM sodium phosphate and 50 mM boric acid adjusted to pH 6.2 with NaOH.