Reaction coordinate analysis for β-diketone cleavage by nonheme Fe^{2+}-dependent dioxygenase Dke1

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Contents:

Figure S 2. Determination of the apparent extinction coefficients (ε) for the β-dicarbonyl substrates used in this study.

Figure S 3. Representative fluorescence titration curves from the determination of substrates' dissociation constants (K_d) to Dke1.

Figure S 4. Estimation of the redox potential for the iron(III)-(2'-hydroxyacetophenone)$_3$ complex.

Figure S 5. Graphical frontier molecular orbital analysis for the substrate 2-acetylcyclopentanone and the non-convertible ligand 2'-hydroxyacetophenone.

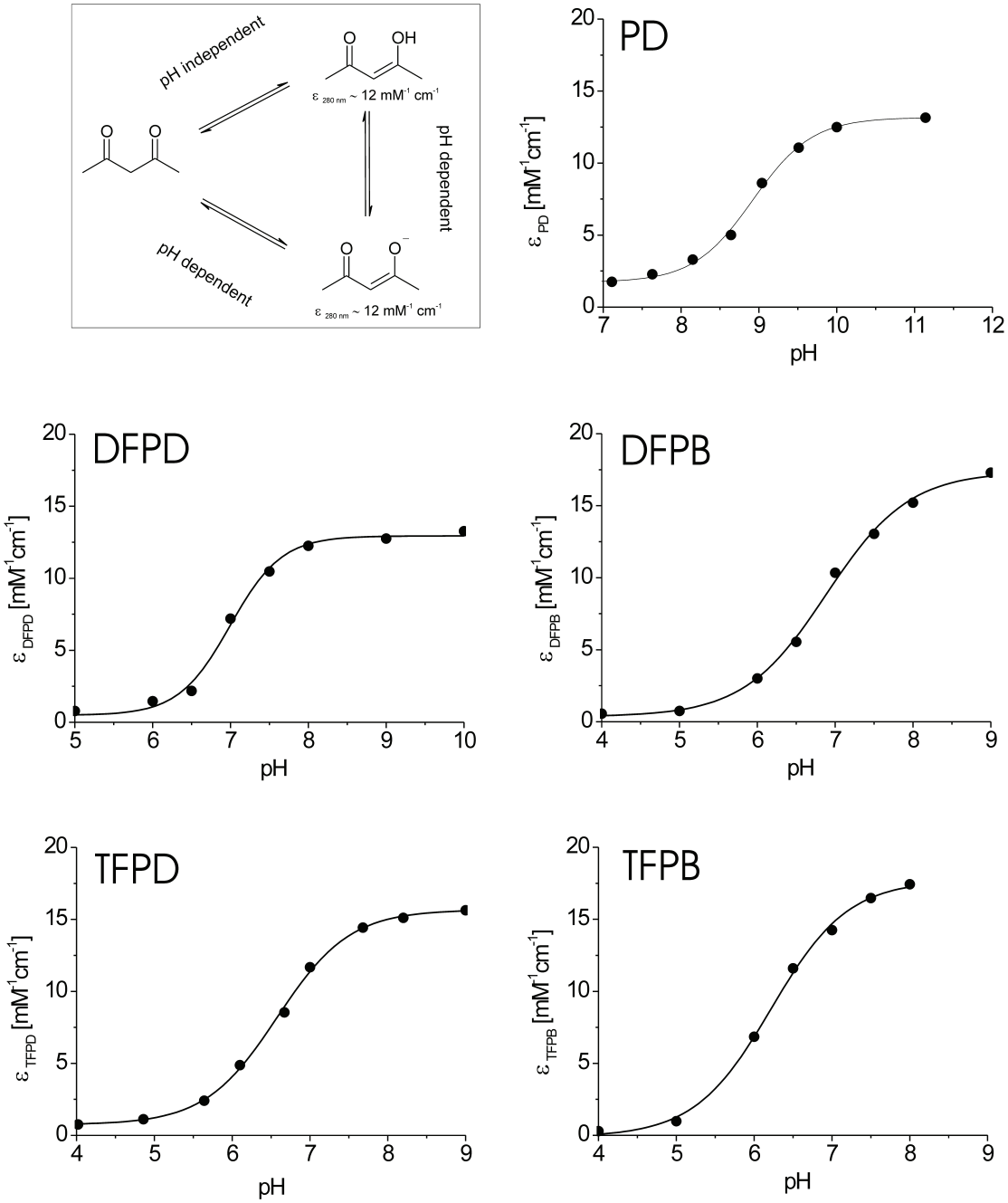

Figure S 2. Determination of the apparent extinction coefficients (ε) for the β-dicarbonyl substrates used in this study. Absorption bands at a wavelength $\geq$ 260 nm and an ε of about 12 mM^{-1} cm^{-1} have been reported previously for enol/enolate structures.[1,2] Apparent ε is governed by the fast keto-enol-enolate equilibrium, as outlined for PD (inset) and shows a sigmoid dependency on pH. ε of pentanedione (PD), 1,1-difluoro-2,4-pentanedione (DFPD), 1,1,1-trifluoro-2,4-pentanedione (TFPD), 4,4-difluoro-1-phenyl-1,3-butanedione (DFPB), 4,4,4-trifluoro-1-phenyl-1,3-butanedione (TFPB) were determined via UV/Vis spectra of a 50 μM buffered substrate solution by reading the absorbance of the enol/enolate transition at the wavelength of maximum intensity (λ_1). Mc Ilvaine type buffer systems were used. Note that apparent ε at pH 7.5 was applied for calculating the velocity of substrate depletion under steady state conditions, and they were identical with analogously determined dioxygen consumption rates, justifying the use of apparent ε values for kinetic analysis under the conditions used.

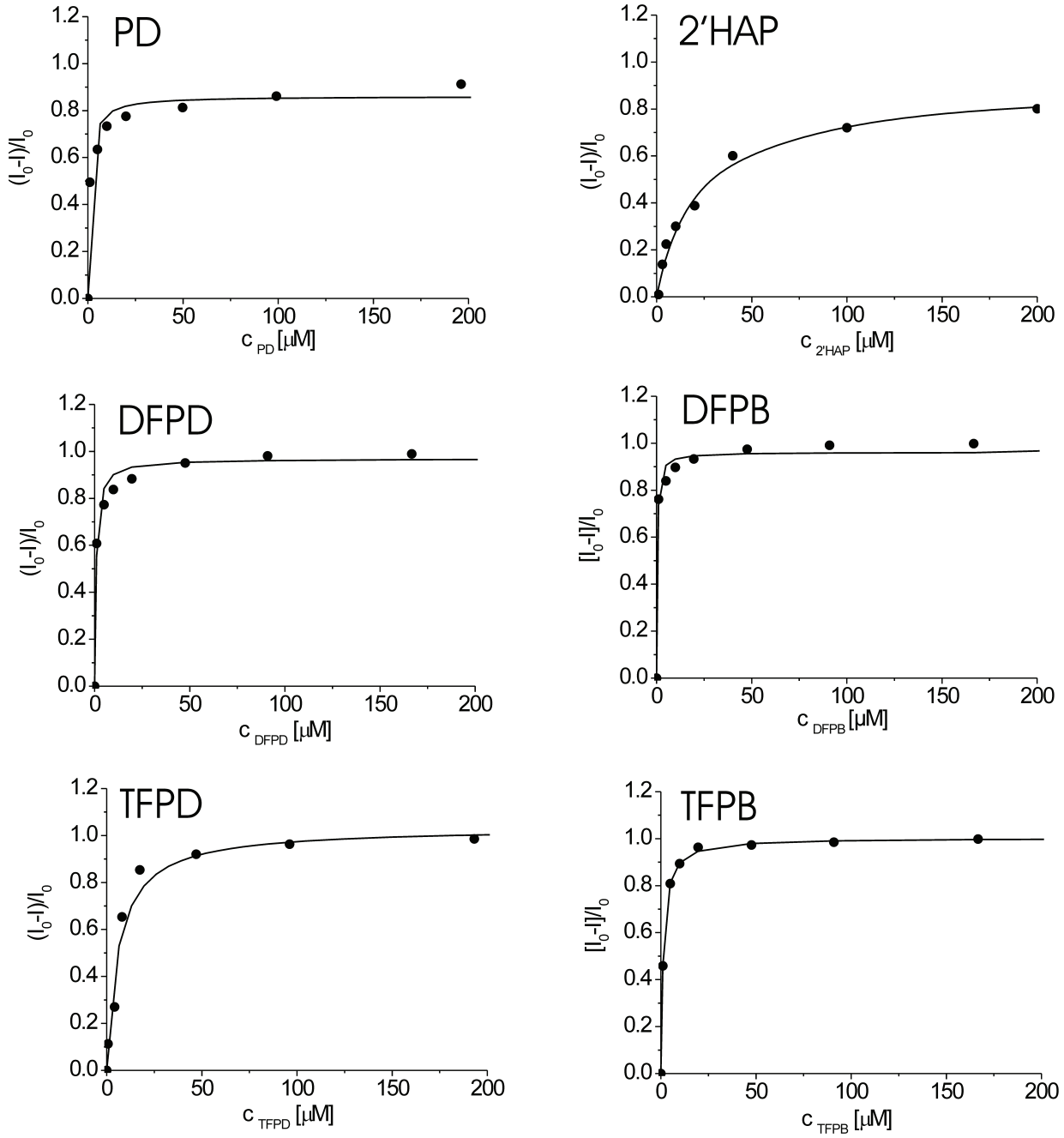

Figure S 3. Representative fluorescence titration curves from the determination of the substrates' dissociation constants (K_d) to Dke1 at 4°C.

Quenching of fluorescence of aromatic residues by addition of substrate was measured at 330 nm. The excitation wavelength was 290 nm. Representative titration curves are shown for the substrates investigated: PD, 2'-hydroxyacetophenone (2'HAP), DFPD, DFPB, TFPD and TFPB. K_d values were calculated by averaging the results from duplicate measurements.

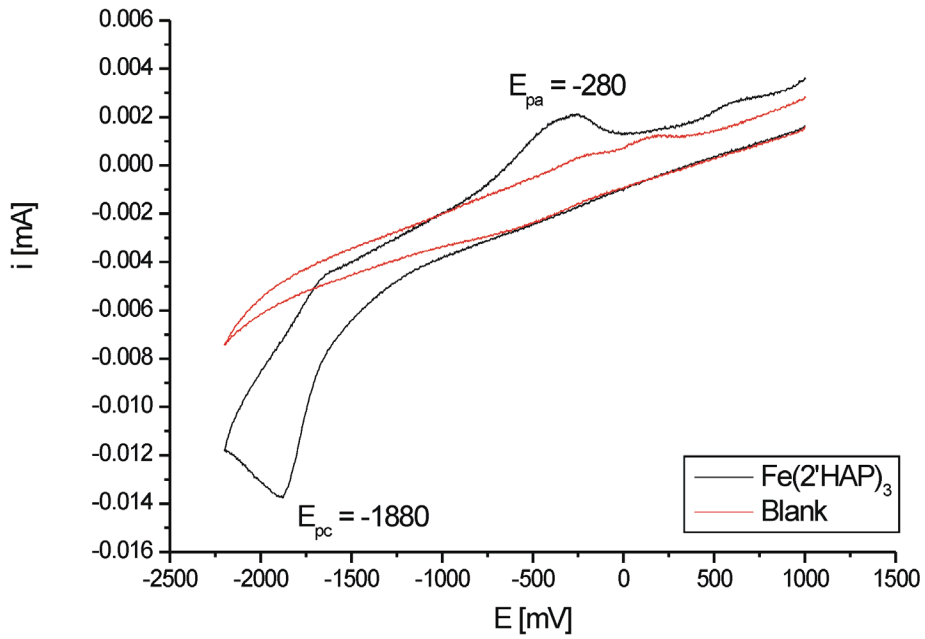

Figure S 4. The cyclic voltammogram of the iron(III)-(2'HAP)$_3$ complex measured with a scan rate of 2 V s^{-1} indicates E_{pa} (-0.28 V) and E_{pc} (-1.88 V). The redox potential was determined in analogy to the redox potential measurements described in literature.[3] As 2'HAP is only slightly soluble, a saturated solution of 2'HAP in dimethylformamide (DMF) was measured. A gap of 1.6 V between cathodic E_{pc} and anodic potentials E_{pa} indicates an irreversible system with an apparent midpoint potential (E_o) of ~ -1.1 V. The large difference between E_{pc} and E_{pa} may be explained by a subsequent chemical reaction, namely the reversible exchange of the 2'HAP-ligand with solvent molecules concomitant with reduction, resulting in two distinct redox-pairs. At comparably high scan-rates (≥ 0.5 V s^{-1}), a minor peak at -1.63 V becomes visible. This might account for the E_{pa} of a mixed iron (II)-(2'HAP)$_2$DMF complex, which can only be seen when the re-oxidation of the complex is faster than ligand exchange, while the true E_{pc} for iron(III)-(2'HAP)$_3$ is still invisible due to a fast ligand substitution. In any case E_o for iron(III)-(2'HAP)$_3$ is < 0, which is markedly more negative than E_o for the fluorinated complexes.

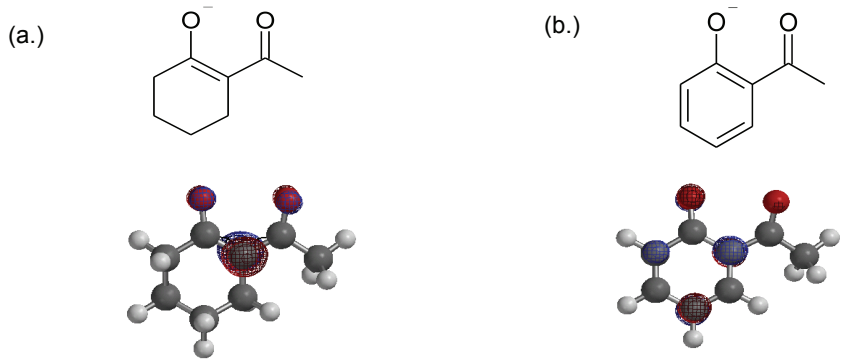

Figure S 5. Graphical frontier molecular orbital analysis for the substrate 2-acetylcyclopentanone (ACH) and the non convertible structural analog 2'-hydroxyacetophenone (2'HAP).

Enolate-structures (top) of (a) ACH and (b) the substrate analog 2'HAP and graphical representation of their calculated highest occupied molecular orbitals (HOMOs, indicated as a blue and red mesh, bottom of graph) are shown. HOMO energies (ε_{HOMO}) were rather similar with -3.13 eV and -3.31 eV respectively. Energy and localization of HOMOs were calculated by the program Spartan'02 (Wavefunction, Inc., CA, U. S. A.) via a semi-empirical metod using the AM1 basis set.

Literature:

(1) Rappoport, Z. E. *The chemistry of enols*. Wiley: Chichester, UK, 1990.

(2) Chang, J. A., Kresge, A. J., Nikolaev, V. A., Popik, V. V. *J. Am. Chem. Soc.* **2003**, *125*, 6478-6484.

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