

CYTOCHROME C OXIDASE - A KEY COMPONENT OF THE OXIDATIVE PHOSPHORYLATION: REGULATION AND MODULATION OF ITS CATALYTIC ACTIVITY



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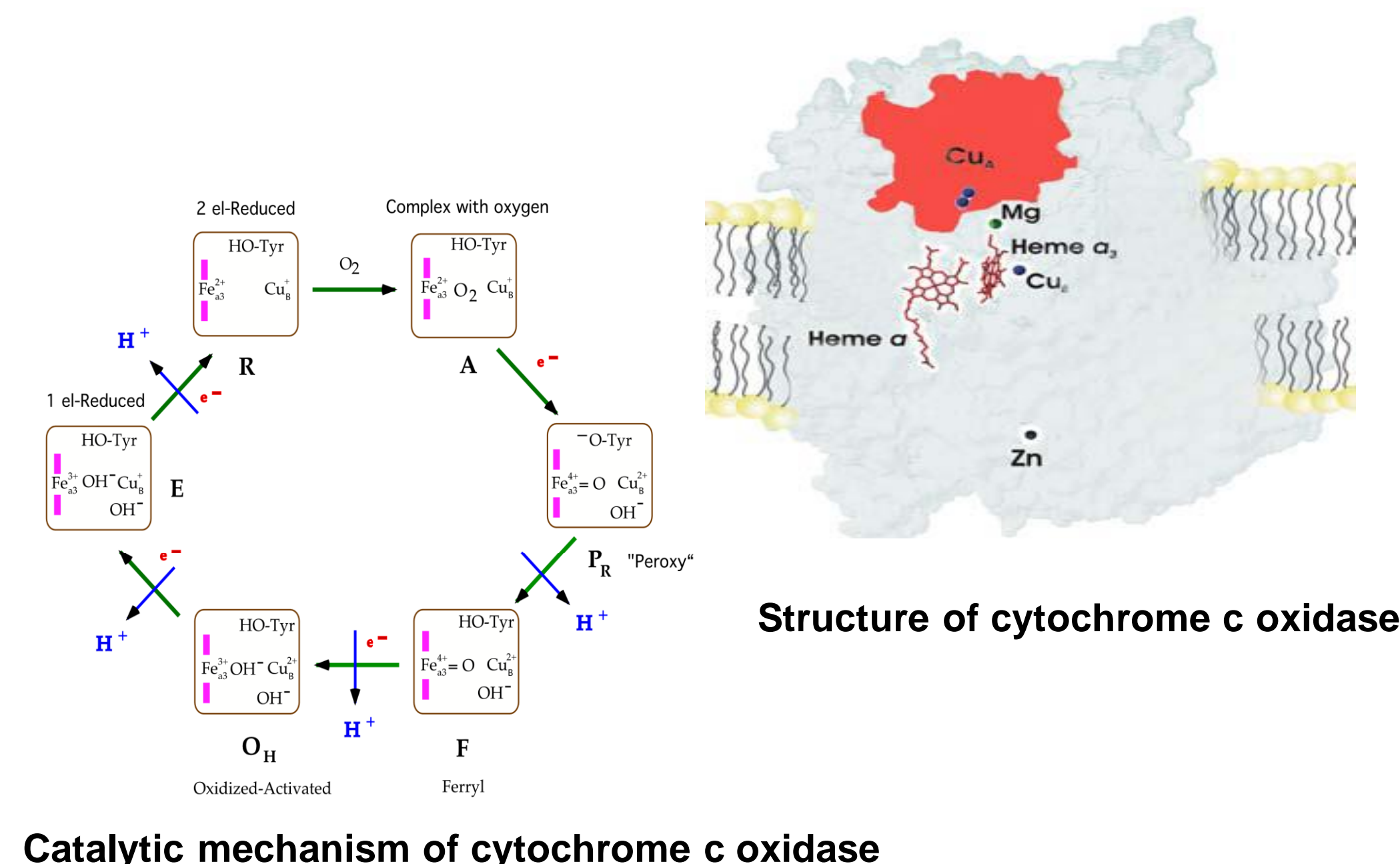
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Introduction

Mitochondrial cytochrome c oxidase (CcO) is the terminal enzyme of the respiratory electron-transfer chain in mitochondria of the eukaryotic cells. This molecular complex catalyzes reduction of dioxygen to water and converts the released free energy into an electrochemical proton gradient across the inner mitochondrial membrane. The full reduction of CcO requires four electrons and the transfer of two electrons to the oxidized catalytic site is accompanied with an uptake of two protons, ultimately from surrounding of the enzyme. The rate of heme a_3 reduction appears to be simultaneous with proton uptake and it has been concluded that the rate limiting process for this electron transfer is the stabilization of the electrons by protons. In this work we have studied the processes regulating the reduction of the catalytic site of the oxidized CcO. Moreover, we have investigated differences between the „high-energy“ metastable O_H and the “resting” O state of the oxidized CcO.

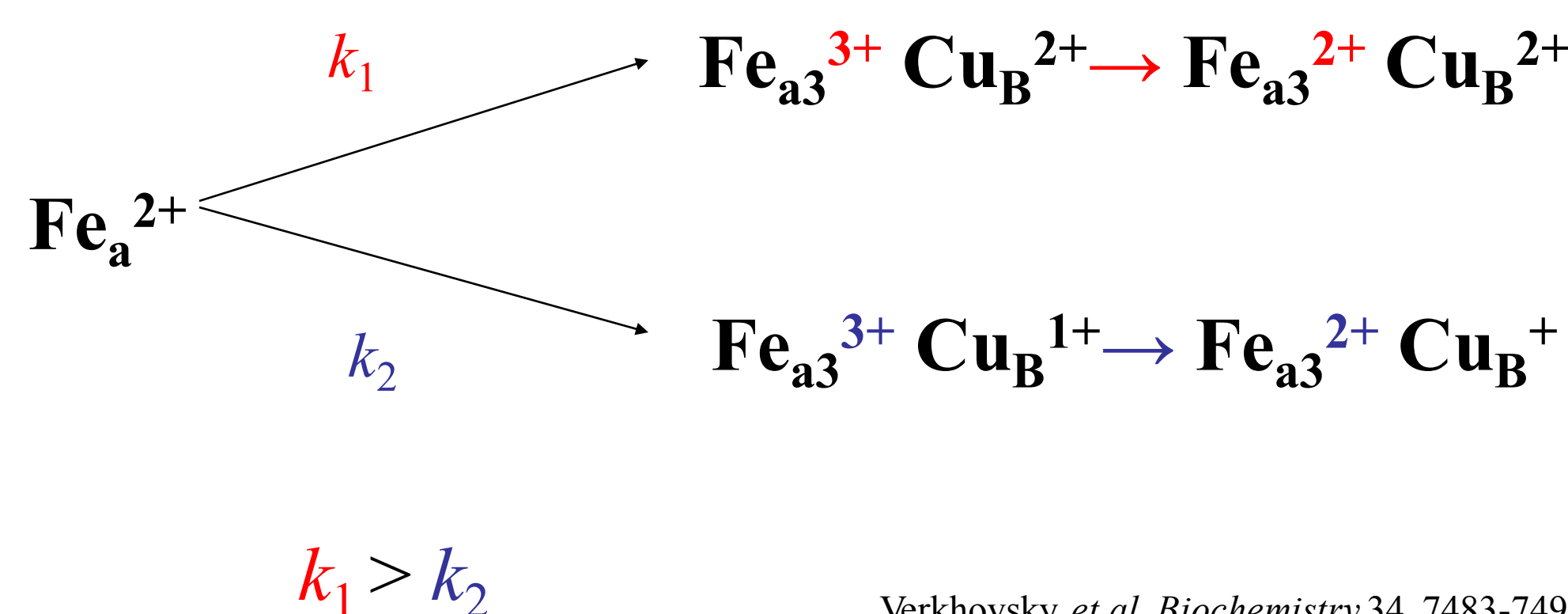


High - energy metastable state of oxidized CcO (O_H)

- ❖ formed immediately after oxidation of the reduced CcO
- ❖ the energy needed for the proton pumping during reductive phase stored in this form
- ❖ in the absence of external electron donors O_H form relaxes to the “resting” form of CcO (~several seconds) with dissipation of the stored energy and subsequent reduction of the resting CcO does not lead to proton pumping

Verkhovsky et al., *Nature* 400, 480, 1999
Bloch et al., *PNAS* 101, 529, 2004
Wikström et al., *BBA* 1757, 104, 2007

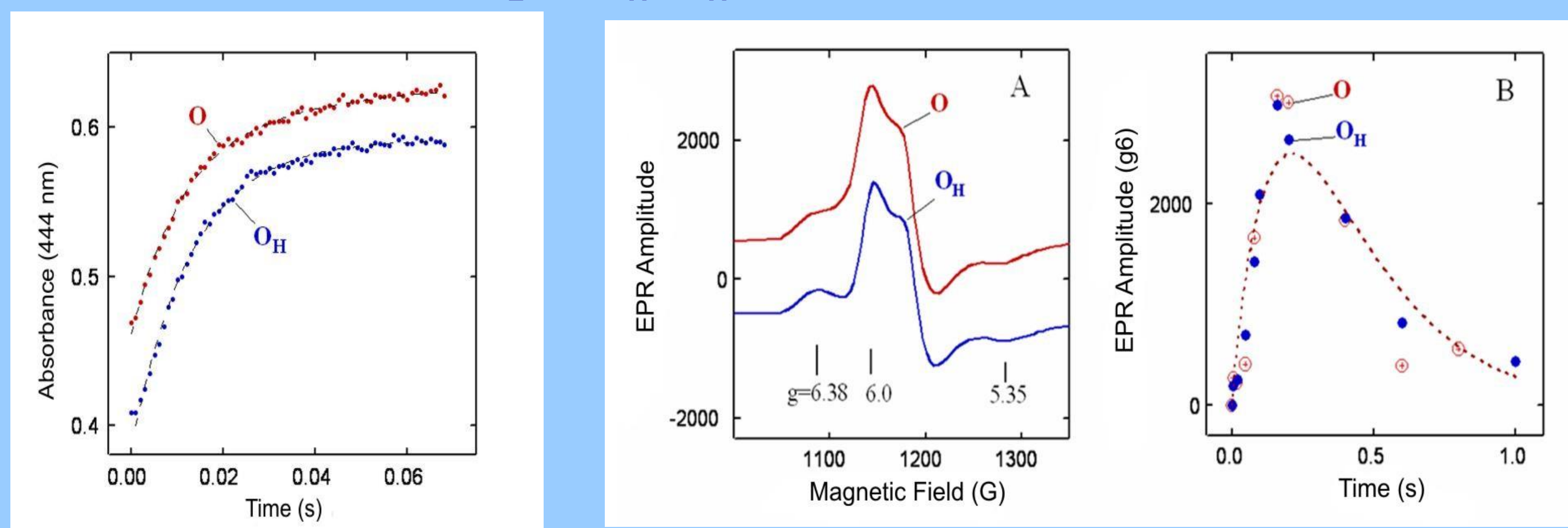
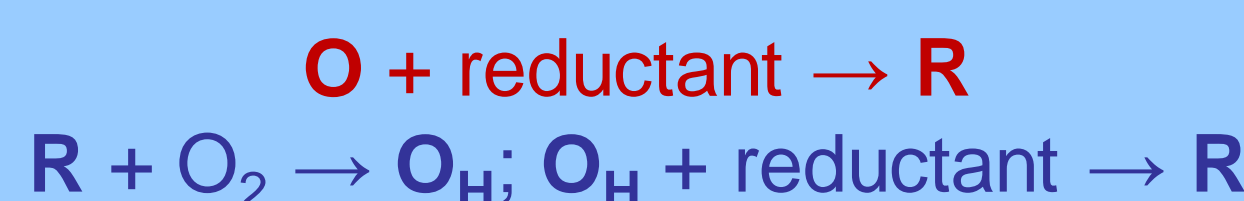
Two phases of the reduction of the heme a_3



Verkhovsky et al. *Biochemistry* 34, 7483-7491 (1995)
Malatesta et al. *PNAS* 87, 7410-7413 (1990)

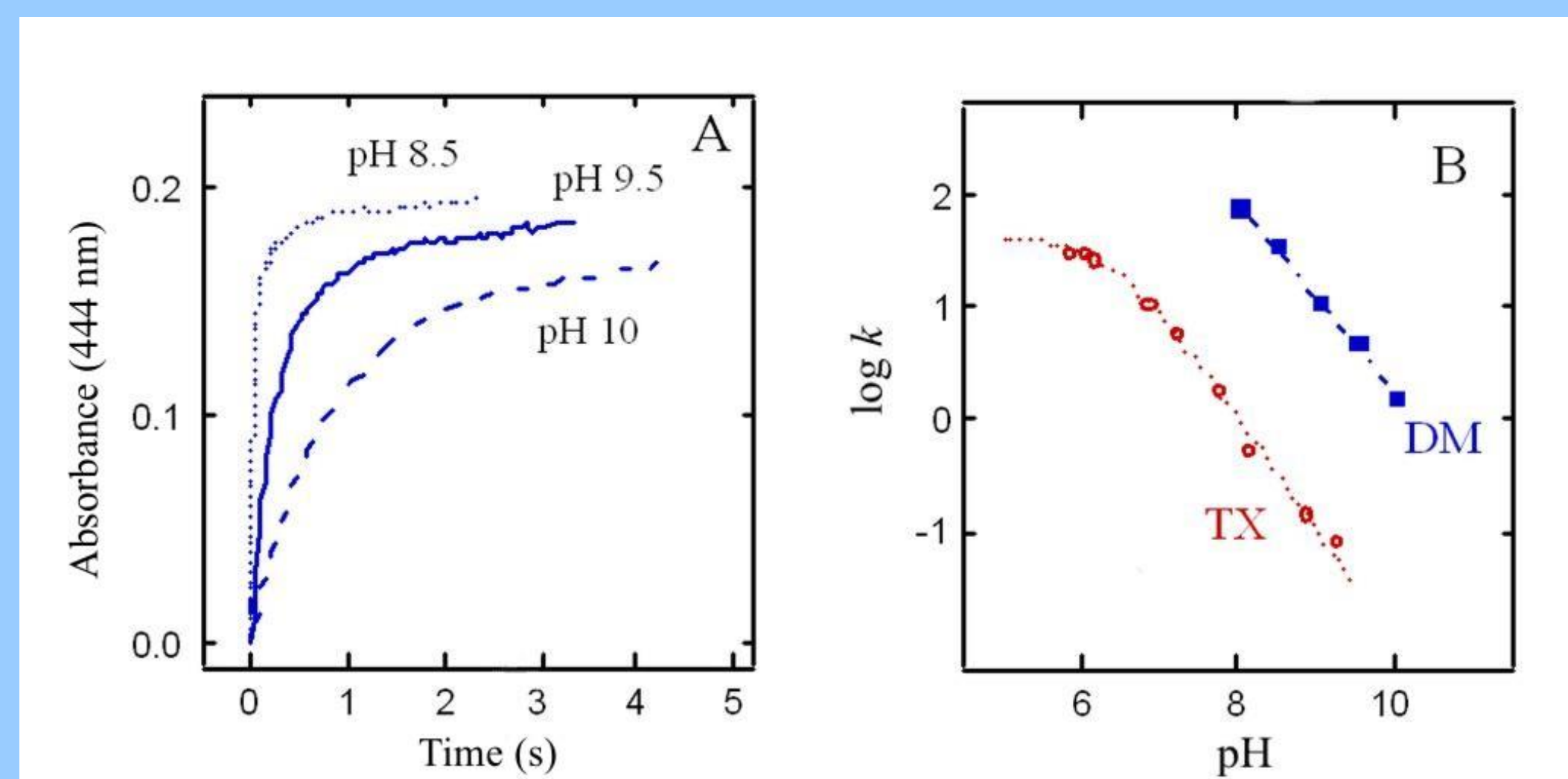
Results

Kinetics of the reduction of the catalytic site in O and O_H



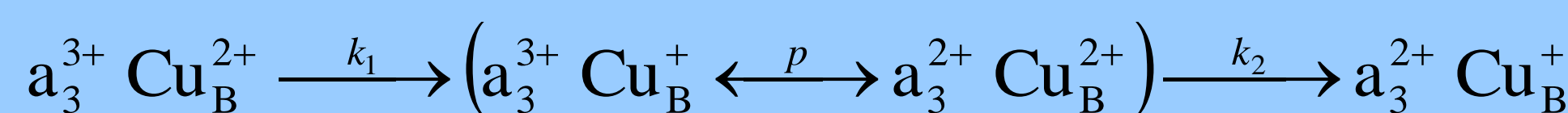
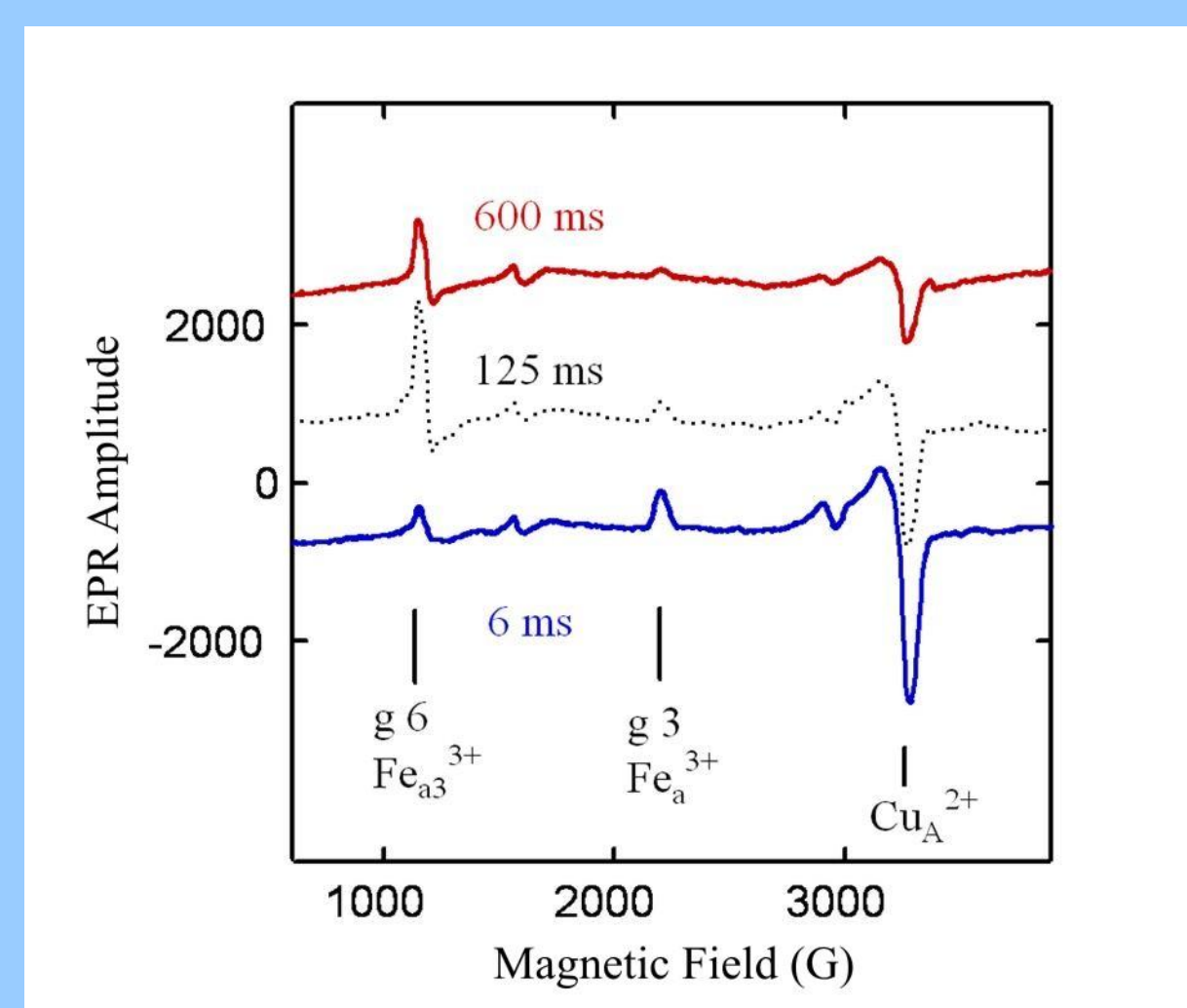
No difference between redox potentials of heme a_3 and Cu_B in O and O_H

pH dependence of the rate of heme a_3 reduction



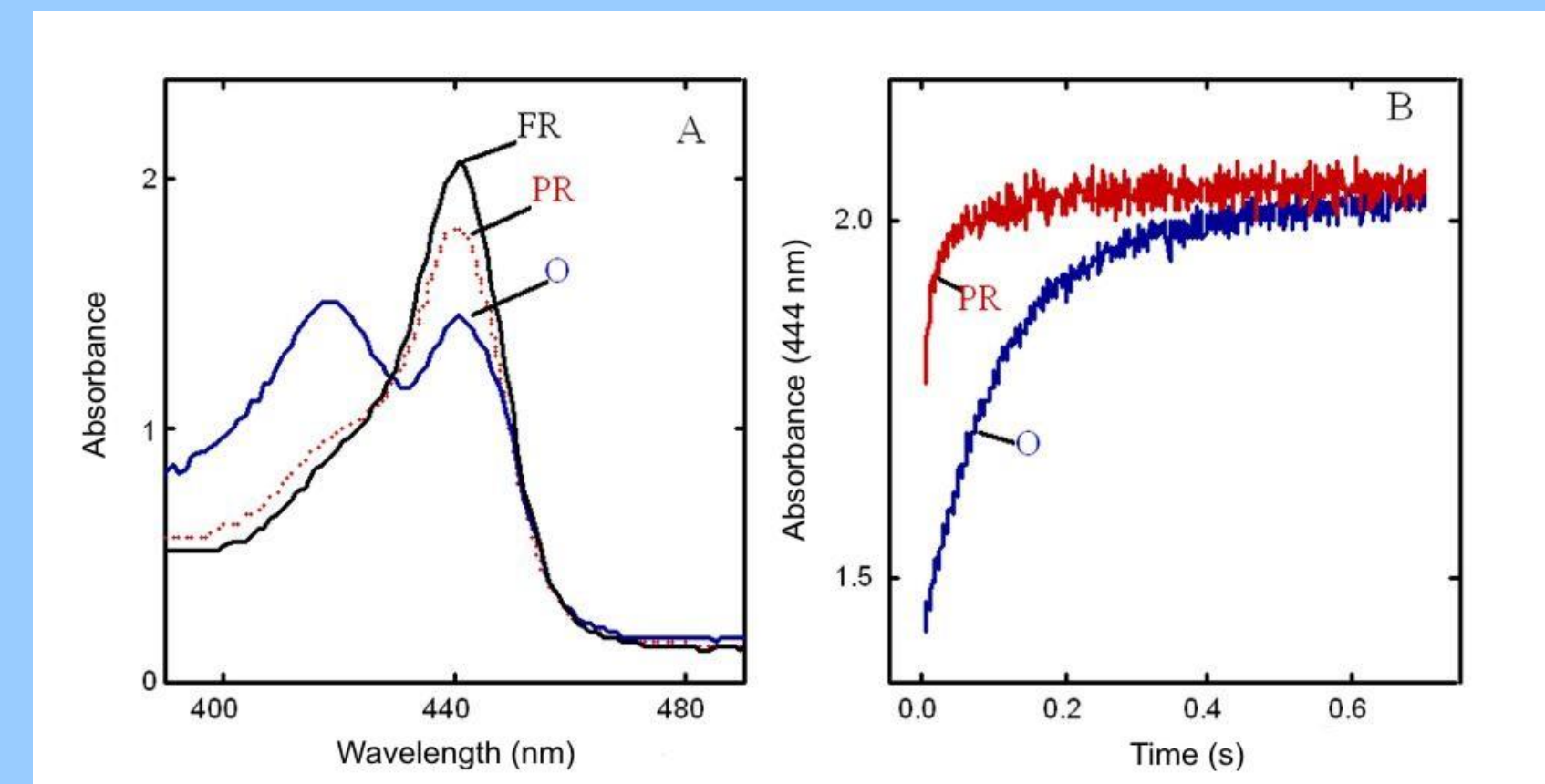
Electron transfer to the catalytic site CcO is regulated by the protolytic group with $\text{pK}_a = 6.6$

Kinetics of the reduction of the CcO catalytic site – EPR experiments



$$g_6(t) = (k_1 p / (k_2 - k_1)) (e^{-k_1 t} - e^{-k_2 t})$$

Ru (mM)	k_1 (s ⁻¹)	k_2 (s ⁻¹)
0.025	5 ± 1	5 ± 1
0.1	7 ± 4	30 ± 18
3.0	120 ± 30	ND



$k_r = 10 \text{ s}^{-1}$ (80%)
 $k_p = 3 \text{ s}^{-1}$ (20%)

$k_r = 108 \text{ s}^{-1}$ (65%)
 $k_p = 12 \text{ s}^{-1}$ (35%)

Rate limiting step of the reduction of the catalytic site in CcO is the transfer of the first electron to this site

Conclusions

- ❖ Electron transfer to the catalytic site of CcO is regulated by the protolytic group with $\text{pK}_a = 6.6$
- ❖ Rate limiting step of the reduction of the catalytic site in CcO is the transfer of the first electron to this site
- ❖ Functional equivalence of as „isolated“ and „high-energy“ metastable forms of the oxidized CcO

Acknowledgment

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