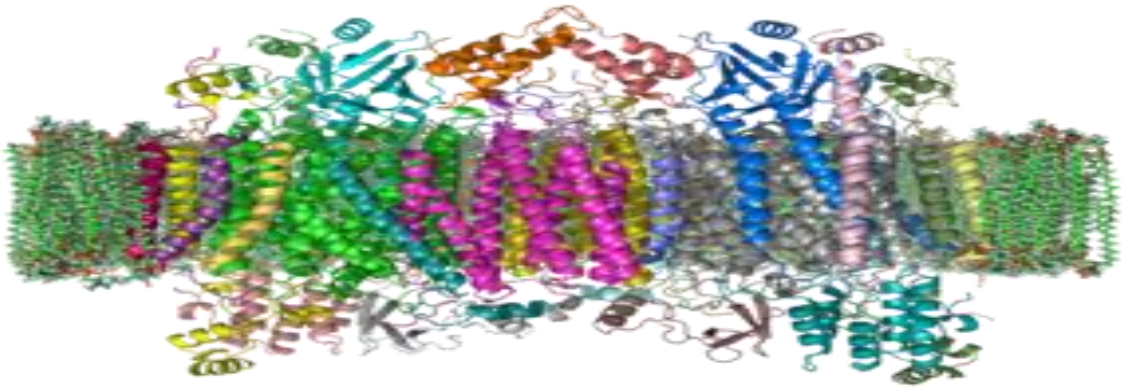
Cytochrome c oxidase a key component of oxidative phosphorylation - Regulation and modulation of its catalytic mechanism

Daniel Jancura

Department of Biophysics
Safarik University
Kosice,Slovakia

Structure and function of cytochrome c oxidase



- 13 polypeptide chains (3 encoded by mtDNA and 10 by nuclear DNA

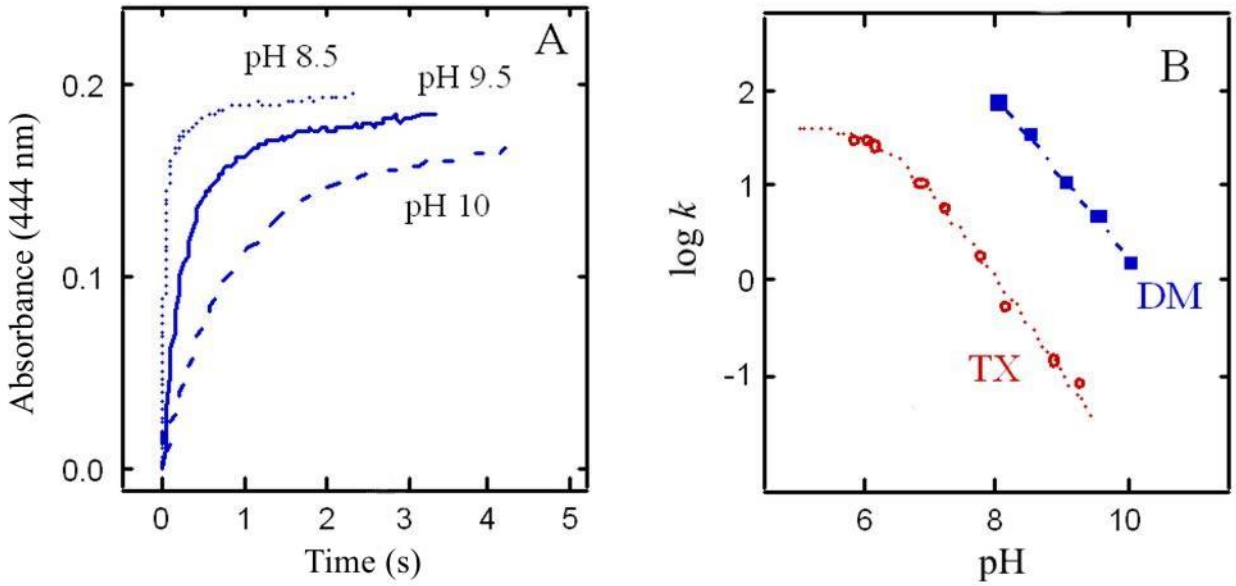
- 4 redox centers (heme *a*, heme a_3, Cu_A, Cu_B)

- Catalyzes oxidation of cytochrome c by molecular oxygen and pumps protons through the inner mitochondrial membrane

$$4\ \text{cyt}\ c^{2+} + O_2 + 8\ H^+_{mat} \Rightarrow 4\ \text{cyt}\ c^{3+} + 2\ H_2O + 4H^+_{int}$$

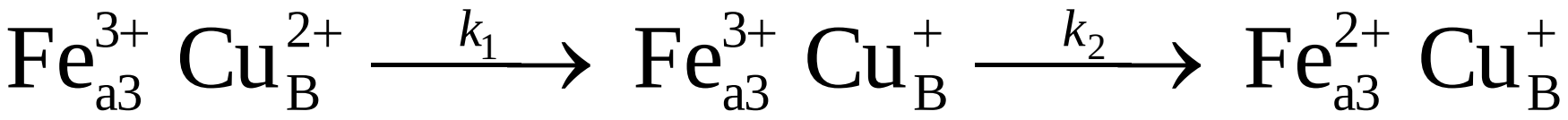
pH-dependence of the rate constant of the reduction of heme a_3

$$k = k_{max} / \left(1+10^{pH-pK_a}\right)$$



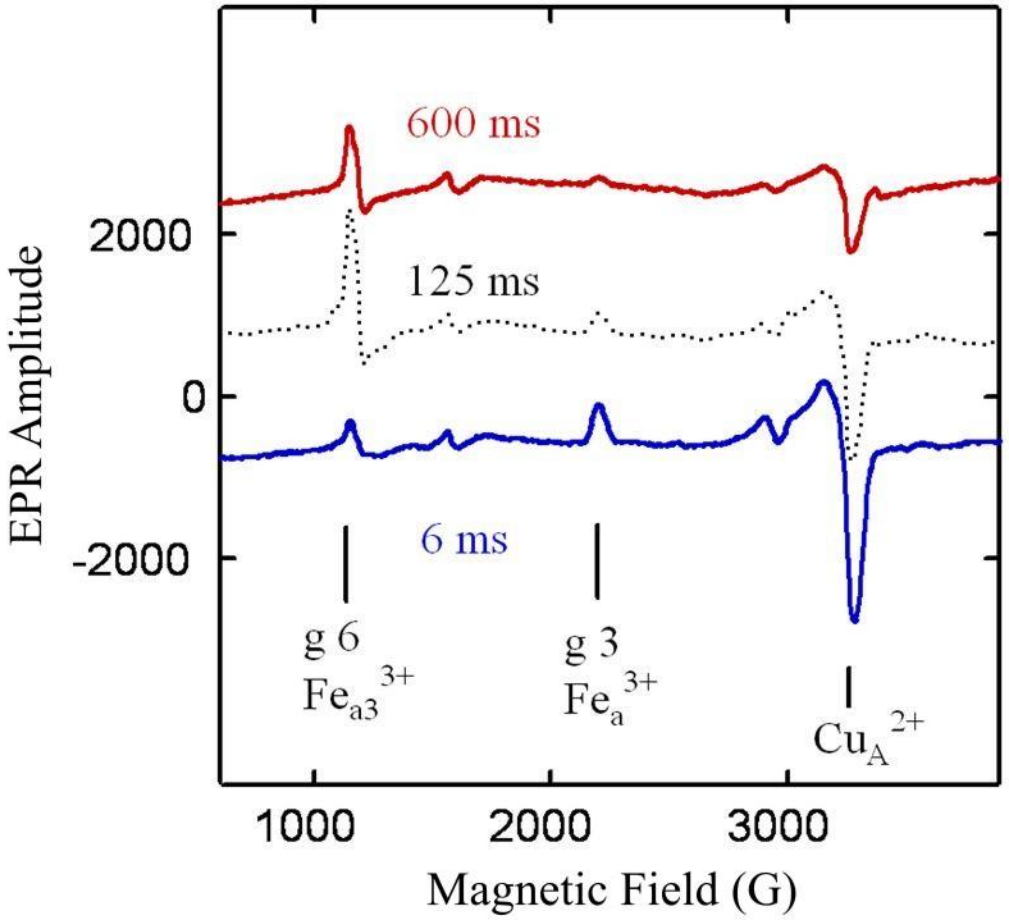
ET to the catalytic site is regulated by the protolytic group with pK_a=6.6

Redox states of the catalytic site during its reduction



Oxidated	1- electron reduced	2-electron reduced
none EPR signal	g=6 signal	none EPR signal

Kinetics of the reduction of the catalytic site - EPR experiments



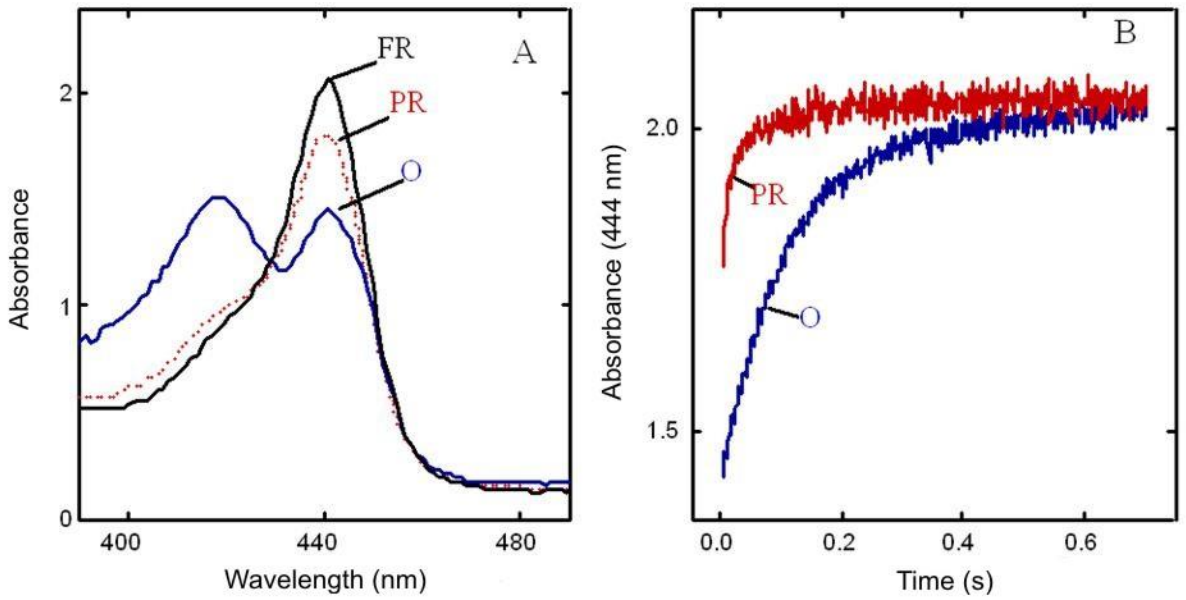
Rate constants of the reduction of the catalytic site by the first and the second electron

$$a_3^{3+}\ Cu_B^{2+} \xrightarrow{k_1} \left(a_3^{3+}\ Cu_B^{+} \xleftrightarrow{p} a_3^{2+}\ Cu_B^{2+}\right) \xrightarrow{k_2} a_3^{2+}\ Cu_B^{+}$$

$$g6(t) = \left(k_1 p/\left(k_2 - k_1\right)\right)\left(e^{-k_1 t} - e^{-k_2 t}\right)$$

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

Kinetics of the reduction of the catalytic site in the partially reduced CcO

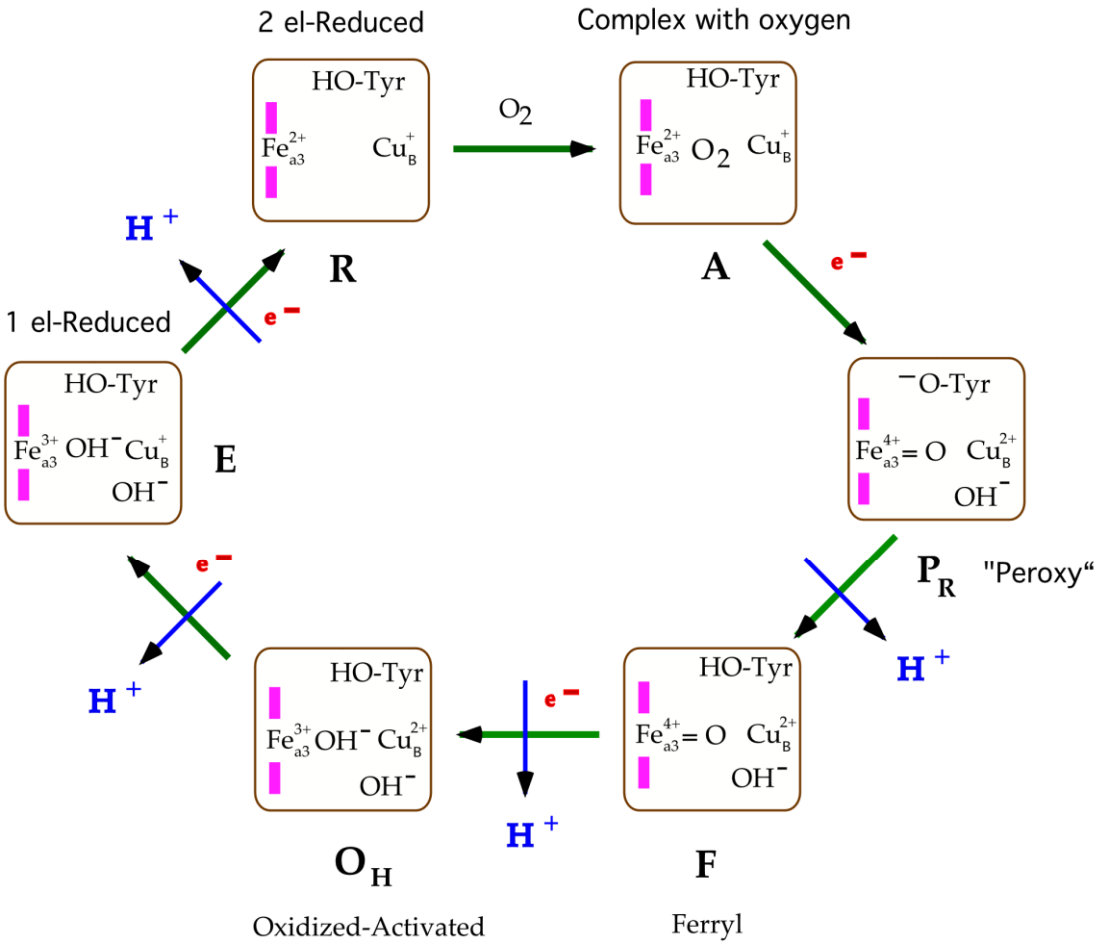


k_r = 10 s^{-1} (80%)
k_p= 3 s^{-1} (20 %)

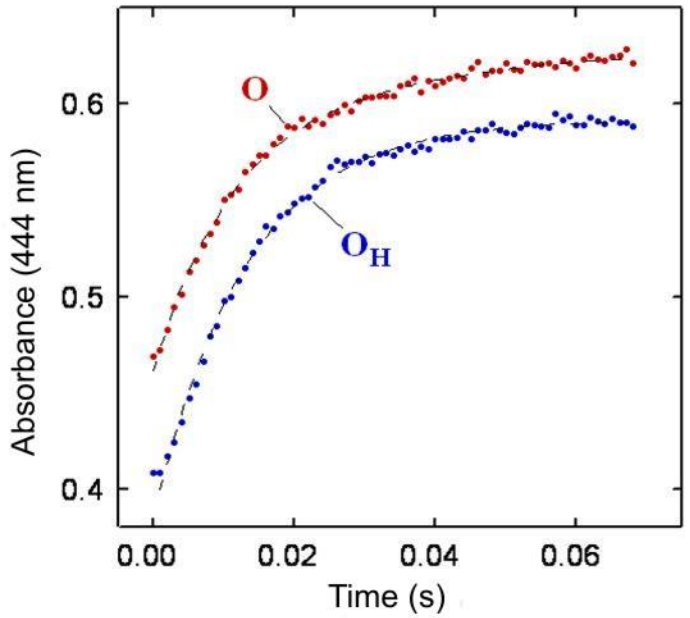
k_r =108 s^{-1} (65 %)
k_p=12 s^{-1} (35 %)

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

Spectral and kinetic equivalence of oxidized CcO as isolated and “activated” by reoxidation

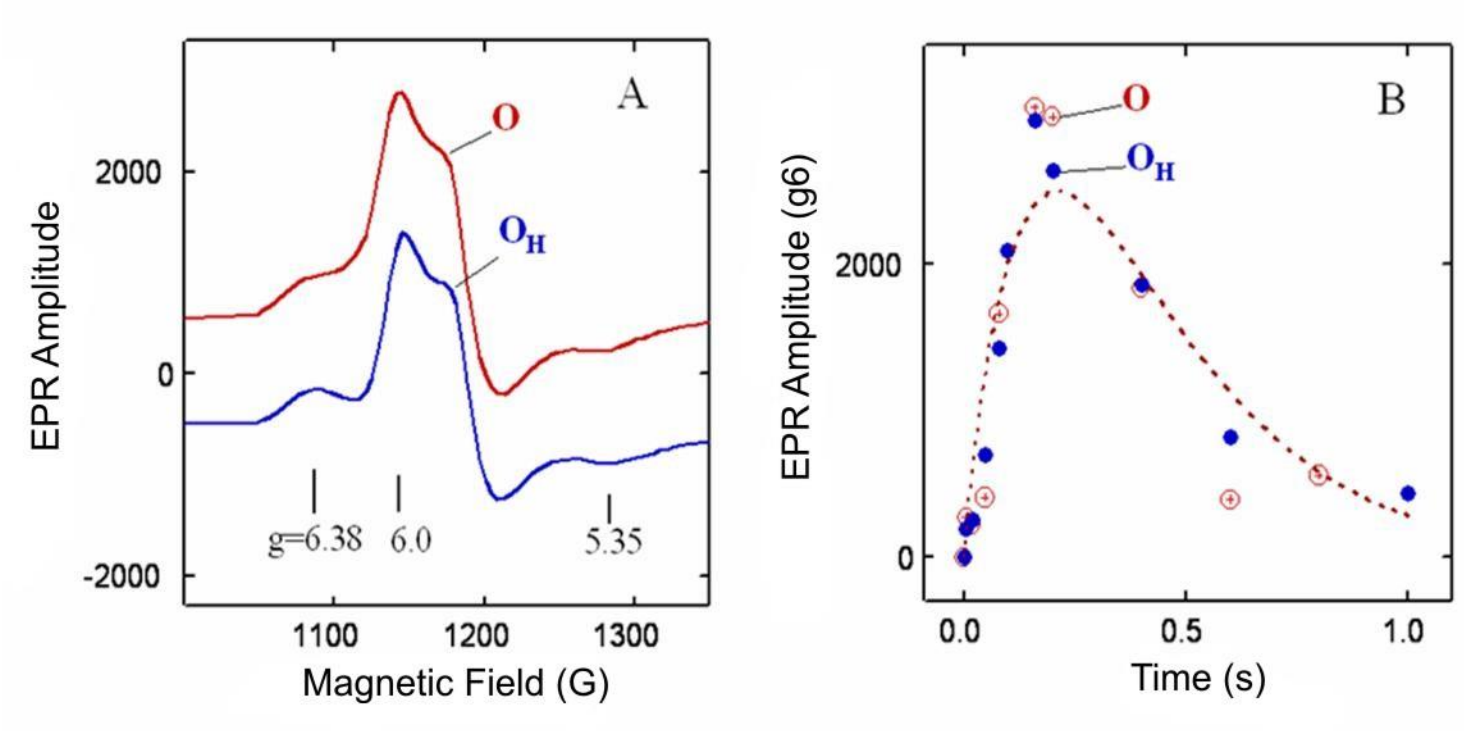


Kinetics of the reduction of heme a_3 in "as isolated" (O) and "activated" (O_H) CcO



	k_{a3} (s^{-1})	
	O	O_H
CcO	87	78
ETČ	121	123

EPR spectra and kinetics of g=6 signal in “as isolated” and “activated” oxidized CcO



Spectral (EPR+ UV-VIS absorption) and kinetic equivalence of O and O_H forms of oxidized CcO

The most important findings

Revealing that the electron transfer to the catalytic center of CcO during reductive phase is regulated by a protolytic group with pK_a=6.6. The confirmation of the importance of the type of detergent, which solubilize CcO, for the catalytic activity of CcO

Determination of the rate limiting step in the catalytic cycle of CcO - the transfer of the first electron to the catalytic site

Determination of the kinetic equivalence of oxidized CcO as isolated and “activated” by reoxidation

Thank you for your attention