

# Molecular Recognition between Human Mitochondrial Cytochromes: The Cytochrome $c_1$ /Cytochrome $c$ Interaction

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The cytochrome  $bc_1$  ( $Cbc_1$ ) complex is a membrane-bound component of the mitochondrial electron transport chain. The complex, which catalyzes electron transport from ubiquinol to cytochrome  $c$  ( $Cc$ ), comprises three redox centers: cytochrome  $b$ , the Rieske protein and cytochrome  $c_1$  ( $Cc_1$ ).  $Cc_1$  is responsible for the reduction of  $Cc$ , which, in turn, results in the transfer of electrons to cytochrome  $c$  oxidase ( $CcO$ ). The reaction is essential for cellular bioenergetics, insofar as it is coupled with proton translocation leading to ATP formation. Recently, our group has reported that plant  $Cc$  shows two binding sites on  $Cc_1$ <sup>1</sup>. The first, or so-called *proximal* site, is suitable for electron transfer, whereas the second, or *distal* site, located near the Rieske protein, is involved in the channeling of  $Cc$  molecules towards  $CcO$ <sup>1-3</sup>.

Given this, this work aims to determine whether the  $Cc$ - $Cc_1$  two-binding site model is conserved through out evolution and, specifically, can be found in the interaction between the two corresponding human hemoproteins. First, in close collaboration with the *European Integrating Structure Platform (Instruct, PID1163)*, several constructions of the soluble N-terminal domain of  $Cc_1$  were designed. Among these, a triple  $Cc_1$  mutant lacking non-heme-coordinated cysteines and containing a bacterial periplasmic signal allowed for the expression of human  $Cc_1$  as a recombinant protein. Physicochemical properties, including redox potential, were then analyzed. Preliminary measurements by isothermal titration calorimetry suggest that the  $Cc:Cc_1$  stoichiometry of 2:1 is present in both plants and humans.

<sup>1</sup>Moreno-Beltrán, B *et al. Biochim. Biophys. Acta-Bioenerg.* (2014) 1837: 717-729.

<sup>2</sup>Moreno-Beltrán, B *et al. FEBS Lett.* (2015) 589: 476-483.

<sup>3</sup>Louro, R. O. & Díaz-Moreno I. (eds). *Redox Proteins in Supercomplexes and Signalosomes.* CRC Press (2015).