

# A comparative structure-based analysis of the pH-dependent reduction potentials of Rieske iron-sulfur proteins

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Rieske iron-sulfur proteins carry a [2Fe-2S] cluster coordinated by two histidine and two cysteine residues. Based on their electrochemical properties, Rieske proteins are grouped into two classes: Rieske proteins from hydroquinone-oxidising bc-type cytochrome complexes display high reduction potentials (300-400 mV) that depend on pH in the physiological range. Rieske proteins from bacterial aromatic ring hydroxylating dioxygenase complexes have low reduction potentials (-150 mV) that show no pH-dependence in the physiological range. The difference in pH-dependence between bc-type and dioxygenase Rieske proteins is due to the difference in protonation behavior of the histidine sidechains coordinating the iron-sulphur cluster. Ligand histidine pK-values lie within the physiological range in bc-type Rieske proteins, but above in dioxygenase Rieske proteins. In the presented study, structural differences between a bc-type and a dioxygenase Rieske protein were identified that account for the differences in pH-dependence of their reduction potentials. Based on available high-resolution structures, histidine pK-values and relative reduction potentials were calculated by a combined classical electrostatic/quantum chemical approach. Obtained histidine pK-values of the wildtype structures agree well with experiment. By introducing *in silico* mutational changes, differences between the two studied proteins were removed. From an analysis of the reduction potentials and pK-values of the mutated structures, differences in electrochemical behavior could be related to structural differences between the two proteins. Presence of hydrogen bonds towards the iron-sulfur cluster together with absence of acidic residues in bc-type Rieske proteins accounts for their higher reduction potentials and lower ligand histidine pK-values.

[1] Klingen, A.R. & G.M. Ullmann (2004): Negatively-charged Aminoacids and Hydrogen Bond Pattern Tune the  $pK_a$  Values of the Rieske-type Iron-Sulfur Proteins. *Biochemistry*, **43**, 12383-12389.

[2] Ullmann, G.M. ; Noodleman, L. & D.A. Case (2003): Density Functional Calculation of  $pK_a$  values and Redox Potentials in the Bovine Rieske Iron-Sulfur Protein. *J. Biol. Inorg. Chem.* **7**, 632-639.