Heme-copper oxidases: could they be stochastic machines?

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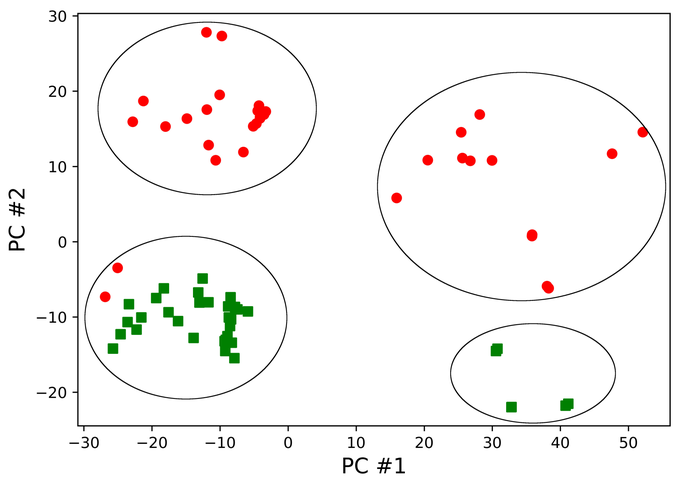
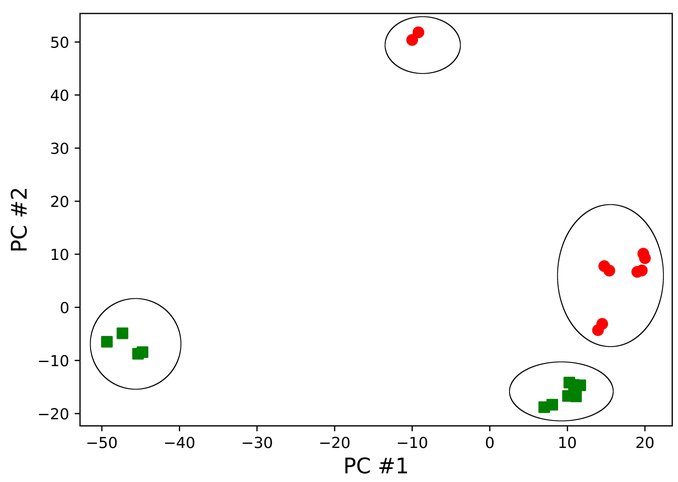
1. Introduction

Heme-copper oxidases (HCOs) are the terminal enzymes of many aerobic respiratory chains, including the mitochondrial one. HCOs reduce molecular oxygen in a process coupled with proton pumping.[1,2] Despite decades of intense work, some features of this proton pump mechanism still remain controversial.[1–3] Currently accepted models require, more or less explicitly, an ordered sequence of events, and can be considered deterministic. Taking into account the cytochrome *c* oxidase clusters of structures and experimental data on which there is a general consensus, we suggested a stochastic pump mechanism for this enzyme class.[4] From a biochemical point of view, the model is essentially based on the decoupling of the redox linked events at the proton loading site from the fluctuations of the access barriers to the intramolecular proton conduction pathways. This model predicts some pump features that can be hardly explained by deterministic models, such as the convex dependence of the stoichiometry of the pump on the electron transfer rate.[1,3,5] Furthermore, this stochastic model provides a rational explanation for contrasting evidences from single-molecule experiments performed on HCOs incorporated in proteoliposomes and predicts when it is more likely to observe leak states during HCO turnover.[6,7]

2. Principal component analysis

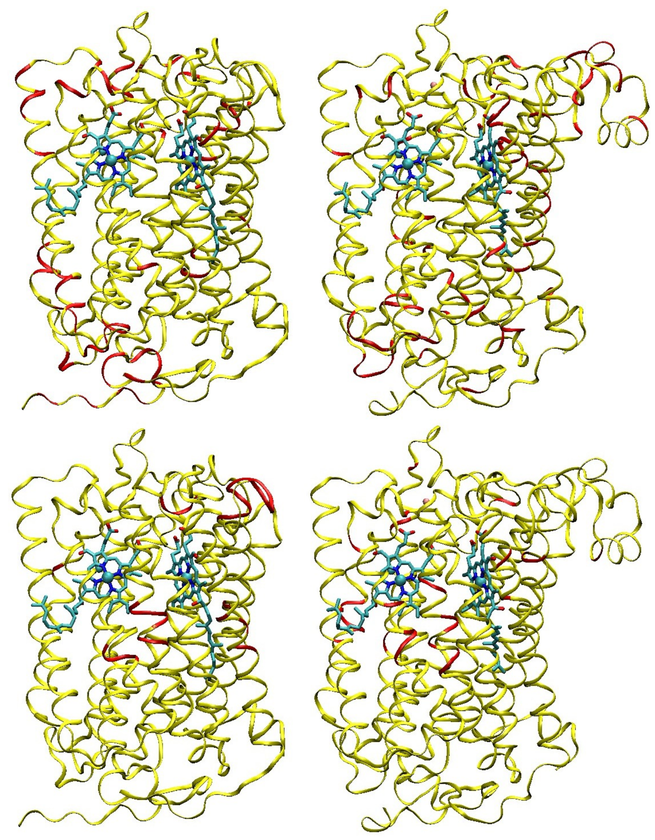
Principal component analysis (PCA) of the bovine (left panel) and *Rhodobacter sphaeroides* (right panel) subunit I data sets; oxidized and reduced forms are reported as green squares and red circles, respectively. HCO structures can be grouped into four clusters. The separation along PC #2 is related to the redox state of the enzyme. The separation along PC #1 is more difficult to explain: all trivial causes can be excluded. A tentative (but plausible) interpretation is that it can be attributed to thermal motion of the protein. Note that, if so, **both motions are stochastic** at single-molecule level.



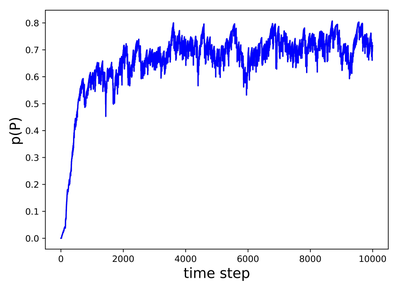


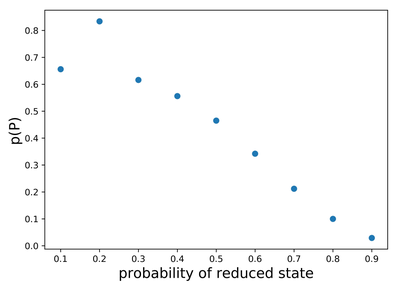
3. Protein involvement in PCs

PC #1 involves mainly residues at the N- and P-sides of the coupling membrane (in red, in the upper part of the figure), which suggests for this PC a role on proton entry barriers. PC #2 related displacements are located at the internal helices (in red, in the lower part of the figure) and can modulate the proton loading site. Left structures refer to the bovine enzyme, right ones to the *R. sphaeroides*.

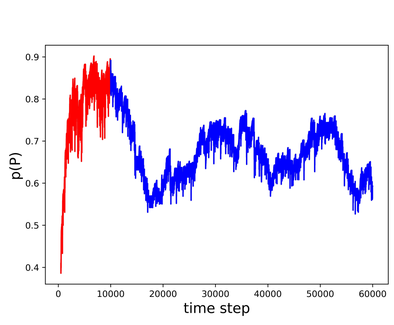


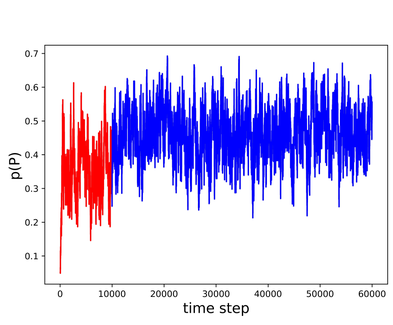
5. Stochastic model *vs* experiments





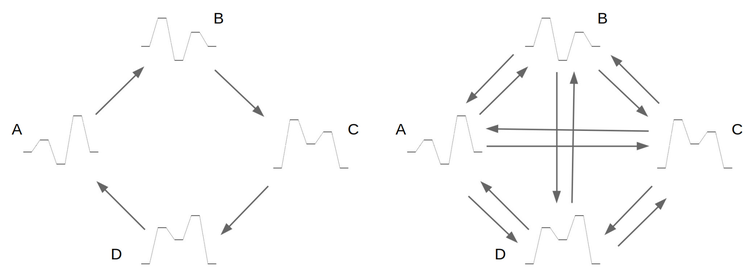
The stochastic four-state model for the HCO proton pump has been implemented numerically as a random discrete time Markov chain.[4] If the state **C** is more probable than that indicated as **D** (see box 4) the system pumps protons from the N to the P side of the membrane (above, left panel). The proton pump efficiency depends on the probability of finding the redox centers in the reduced state (above, right panel).[3,5] If the system is in high pumping efficiency conditions, small fluctuations of reducing substrates lead to a **reversal of the pump** (below, left panel; red and blue traces are before and after the fluctuation).[6] This effect is hardly observable at higher electron transfer rates (below, right panel; colors as above).[7]





4. Possible models

Structural data can be adapted to a deterministic pump mechanism (left scheme), but the simplest possible hypothesis is the one in which the switch from one state to another is purely probabilistic (right scheme). Since it is a good principle to explain phenomena with the simplest possible hypothesis (Occam's razor), we have explored the consequences of this stochastic mechanism.



6. References

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