PI: Luca De Gioia

Project title: Computational investigation of the molecular determinants responsible for enzymatic electron bifurcation: towards the design of molecular devices for biotechnological applications.

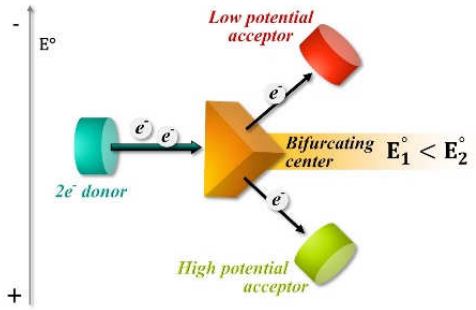
Abstract

It was recently discovered that some redox proteins can *thermodynamically* and *spatially* split two incoming electrons towards different pathways, resulting in the one-electron reduction of two different substrates, featuring reduction potential respectively higher and lower than the parent reductant.[1,2] This energy conversion mechanism, referred to as electron bifurcation, is relevant not only from an evolutionary and biochemical perspective, but also for the potentially ground-breaking applications that electron-bifurcating molecular devices could have in the field of energy conversion.[3,4,5]

We aim at combining different computational approaches (sequence and structure analysis, Molecular Dynamics, Thermodynamic integration (TI), QM/MM and QM approaches) to investigate the molecular determinants responsible for electron bifurcation, taking advantage of the now available diversity of enzymes that are known to bifurcate electrons. The ultimate goal of the project is the design of molecular devices for biotechnological applications in the field of energy conversion.

Background, aims and significance of the proposed work

Non-equilibrium states of matter can sustain themselves tapping on energy gradients. In particular, life on Earth depends on the free energy provided by the electrochemical gradient between reduced and oxidized substrates. Chemiosmotically driven redox reactions was considered for several years the only mechanism to increase the reducing power of environmentally provided reducing substrates to the levels demanded by metabolic needs. However, in 2008 it was reported that the some NADH-dependent flavoproteins can reduce acceptors having reduction potentials substantially more negative than NADH itself. Such a process, which is in apparent contradiction with the second law of thermodynamics, can take place because one-electron reduction of a low potential acceptor is concomitant with one-electron reduction of a second substrate with much higher reduction potential (Scheme 1).



Scheme 1. *Representation of the redox phenomenon of electron bifurcation.*

The functional *thermodynamic* features of electron bifurcation (EB) depends on the chemical properties of the redox cofactor (usually a flavin). However, electron bifurcation implies not only *thermodynamic* bifurcation but also *spatial* bifurcation. In fact, gating might be controlled by conformational rearrangement, but it is also possible that the differential rate of electron transfer between the redox centers acts as an intrinsic gating mechanism.[12]

Electron bifurcation is relevant not only from an evolutionary and biochemical perspective, but also for the potentially ground-breaking applications that electron-bifurcating molecular devices could have in the field of energy conversion.[3] In fact, such devices might allow to use low-cost mild reductants to convert chemically inert low-potential substrates, such as N_2 and CO_2, into high added-value products. Therefore, shedding light on the determinants responsible for electron bifurcation in natural systems is urgent and

fundamental both for providing hints about the functioning of natural EB cofactor and for driving the design of bifurcating biomimetic devices.

Experimental plan

- **WP1 (months 1-3):** *Collection, analysis and comparison of "classical" and bifurcating flavoproteins.* Aim of this task is the definition of stereoelectronic fingerprints of the redox cofactor molecular environment associated to electron bifurcation. In fact, the effect of the environment on redox cofactor properties is of central relevance in natural EB, where the same cofactor (such as flavin) can perform mono- or bielectronic redox transitions according to the amino acidic pattern surrounding it. Specific correlations between flavin environment and redox properties in different flavoproteins will be thus defined.
- **WP2 (months 3-24):** *Comparative MD simulations of homologous "classical" and electron bifurcating flavoproteins (in collaboration with Prof Carole Baffert, University of Marseille, FR).* Aim of this task is the investigation of conformational transitions responsible for spatial electron-transfer gating. Molecular dynamics will be thus used to study conformational changes of flavin environment and to detect structural rearrangements involved in electron gating, in order to propose mechanistic models associated with electron bifurcation.
- **WP3 (months 6-24):** *QM, QM/MM and TI investigation of electron transfer in electron bifurcating enzymes (in collaboration with Prof Frederic Biaso, University of Marseille, FR).* Aim of this task is the investigation of the electronic features responsible for thermodynamic electron-transfer gating. Different protocols for the calculation of flavin redox potentials in different flavoproteins will be developed.
- **WP4 (months 12-24):** *QM and QM/MM investigation of biomimetic systems potentially capable of electron bifurcation.* Aim of this task is the investigation of the effects of environment and chemical modifications on the redox properties of organic and inorganic compounds with electronic features suitable for thermodynamic electron-transfer gating. Pure quantum mechanical and hybrid methods will be used to disclose the relationships between the stereoelectronic features of biomimetic compounds and the energetics of their first and second reductions (or oxidations), in order to inspire the synthesis of molecular systems with tailored redox properties (see WP5).
- **WP5 (months 12-24):** *Computational design and characterization of electron bifurcating molecular devices (in collaboration with Prof. Philippe Schollhammer, University of Brest, FR)* Aim of this task is the design and computational characterization of organic and inorganic compounds with electronic features suitable for thermodynamic and spatial electron-transfer gating; i.e. electron bifurcation. This will be done by combining all the information gained in WP1-4, concerning both natural and biomimetic systems that are related to the electron bifurcation phenomenon.

Feasibility and financial support

- Yes

Literature references

1. G. Herrmann, E. Jayamani, G. Mai, W. Buckel, J. Bacteriol. **2008**, 190, 784–791.
2. F. Li, J. Hinderberger, H. Seedorf, J. Zhang, W. Buckel, R. K. Thauer, J. Bacteriol. **2008**, 190, 843–850.
3. J. W. Peters, D. N. Beratan, G. J. Schut, M. W. W. Adams, Chemical Communications **2018**, 54, 4091–4099.
4. J. Liang, H. Huang, S. Wang, Frontiers in Microbiology **2019**, 10, DOI 10.3389/fmicb.2019.00373.

5. F. Baymann, B. Schoepp-Cothenet, S. Duval, M. Guiral, M. Brugna, C. Baffert, M. J. Russell, W. Nitschke, Front. Microbiol. **2018**, 9, 1357.

PI recent papers related to the topic

1. Redox Potentials of Small Inorganic Radicals and Hexa-Aquo Complexes of First-Row Transition Metals in Water: A DFT Study Based on the Grand Canonical Ensemble
   F Arrigoni, R Breglia, LD Gioia, M Bruschi, P Fantucci
   The Journal of Physical Chemistry A 123 (32), 6948-6957 (2019)
2. H_2 Activation in [FeFe]-Hydrogenase Cofactor Versus Diiron Dithiolate Models: Factors Underlying the Catalytic Success of Nature and Implications …
   F Arrigoni, L Bertini, M Bruschi, C Greco, L De Gioia, G Zampella
   Chemistry–A European Journal 25 (5), 1114-1114 (2019)
3. Reactivation of the ready and unready oxidized states of [NiFe]-hydrogenases: mechanistic insights from DFT calculations
   R Breglia, C Greco, P Fantucci, L De Gioia, M Bruschi
   Inorganic chemistry 58 (1), 279-293 (2018)
4. FeMo heterobimetallic dithiolate complexes: Investigation of their electron transfer chemistry and reactivity toward acids, a density functional theory rationalization
   S Bouchard, M Bruschi, L De Gioia, C Le Roy, FY Pétillon, ...
   Inorganic chemistry 58 (1), 679-694 (2018)
5. Interaction of the H-cluster of FeFe hydrogenase with halides
   M del Barrio, M Sensi, L Fradale, M Bruschi, C Greco, L de Gioia, L Bertini, ...
   Journal of the American Chemical Society 140 (16), 5485-5492 (2018)