Influence of protein environment on the EPR properties of flavoprotein radicals: a QM/MM study

E. Pauwels[1], R. Declerck[1], T. Verstraelen[1], B. De Sterck[1],
C. W. M. Kay[2], V. Van Speybroeck[1], M. Waroquier[1]
[1] *Center for Molecular Modeling, Ghent University, Technologiepark 903, B-9052 Zwijnaarde, Belgium*
[2] *Institute of Structural and Molecular Biology and London Centre for Nanotechnology, University College London, Gower Street, London WC1E 6BT, United Kingdom*

Flavins are frequently encountered cofactors in enzymes that catalyze a broad variety of biochemical reactions. These cofactors can exist in three different oxidation states: fully oxidized, one-electron reduced (radical) and two-electron reduced. The radical forms of flavin are involved in various interesting redox processes, such as DNA photorepair [1] or even circadian timing [2]. Insight into the (electronic) structure of these various cofactor forms in interaction with the protein is vital for a better understanding of their role in catalysis.

Electron paramagnetic resonance (EPR) is an important spectroscopic tool in the identification and characterization of these species. These experiments are increasingly complemented by ab initio quantum mechanical calculations of the EPR quantities within Density Functional Theory (DFT) [3]. By comparing experimental EPR properties with those calculated from first principles, it is possible to get a better understanding of the microscopic structure of the radicals. Efficient application of computational methods on large biomolecular systems requires the use of periodic boundary conditions and a mixed quantum mechanics/molecular mechanics (QM/MM) description of the system. The prediction of EPR quantities within the same approach is challenging from a theoretical perspective. Previously, a scheme was introduced to calculate the EPR hyperfine tensor in extended systems under periodic boundary conditions, using the Gaussian and augmented-plane-wave DFT method [4]. This scheme was implemented in the freely available CP2K code (http://cp2k.berlios.de). A more recent implementation features the calculation of the g tensor [5].

In this work, QM/MM molecular modeling methods are applied to examine the neutral and anionic semiquinone radicals of the flavin adenine dinucleotide (FAD) cofactor non-covalently-bound in glucose oxidase from *A. niger*. Specifically, the EPR characteristics – the anisotropic g tensor and all the significant hyperfine couplings – of these flavoprotein radicals are calculated on the fly during MD simulations and compared with the results of a recent high-resolution experimental study [6]. The general agreement between calculated and experimental EPR properties is found to be very good. In the experiment, the most significant difference between the neutral and anionic radical forms was found to be a shift in the g_y principal value. This shift is well reproduced by the QM/MM simulations, but required incorporation of at least the nearest protein environment of the cofactor radicals, indicating that this shift is a *global feature* of the protein rather than a *local* one. In addition, a prediction is made for relative angles between important spectroscopic principal directions, which are not readily determined by conventional EPR experiments. Remarkably, the directions of the g_x and the g_y components of the g-tensor that lie in the plane of the isoalloxazine moiety are rotated by approximately 60° between the neutral and the anionic radicals.

[1] Weber, S. *Biochim. Biophys. Acta* **2005**, *1707*, 1. [2] Berndt, A.; Kottke, T.; Breitkreuz, H.; Dvosrky, R.; Hennig, S.; Alexander, M.; Wolf, E. *J. Biol. Chem.* **2007**, *282*, 13011. [3] Kaupp, M.; Bühl, M.; Malkin, V. G. Calculations of NMR and EPR parameters: Theory and Applications (Wiley-VCH, Weinheim, 2004). [4] Declerck, R.; Pauwels, E.; Van Speybroeck, V.; Waroquier, M. *Phys. Rev. B* **2006**, *74*, 245103. [5] Weber, V.; Iannuzzi, M.; Giani, S.; Hutter, J.; Declerck, R.; Waroquier, M. *J. Chem. Phys*. **2009**, *131*, 014106. [6] Okafuji, A.; Schegg, A.; Schleicher, E.; Möbius, K.; Weber, S. *J. Phys. Chem. B* **2008**, *112*, 3568.