

# Role of fluctuations in Quinone Reductase hydride transfer: a combined quantum mechanics and molecular dynamics study.

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## Abstract

Quinone reductase (QR1; NQO1), a cytosolic phase 2 detoxification flavoenzyme, catalyzes the obligatory two-electron reduction of quinones to hydroquinones using either NADH or NADPH as the electron donor. The catalytic cycle of QR1 involves reduction of the FAD by NAD(P)H that takes place *via* a hydride transfer from the C4 of nicotinamide to the N5 of the flavin followed by the hydride transfer from N5 to the quinone. We carried out QM/MM calculations to shed light on the role of the enzyme in accelerating individual steps of the reaction. First, using *ab-initio* DFT calculations, we studied the energetics of the contribution of QR1 to the charge stabilization that follows the hydride transfer. Second, by additional quantum mechanical calculations we analyzed the energetic details of the reaction coordinate of the hydride transfer step. Third, we complemented the QM calculations with molecular dynamics simulations that utilized the *ab-initio* derived atomic charges. These simulations show the contribution to the enzymatic mechanism and energetics of the fluctuations of active-site side-chains and cofactors. This is best reflected by a result that shed light on one important aspect of the mechanism of the hydride transfer that remained unexplained. The long distance (4.2 Å) between the C4 of the nicotinamide (hydride donor) and the N5 of the flavin (hydride acceptor) in the x-ray structure appears to be too long for a direct hydride transfer. The calculations, however, show that due to fluctuations, C4 and N5 spend a large fraction of the time at distances shorter than 3.5 Å, ideally suited for hydride transfer.