# Unveiling the Effect of the Oxidation State in the Fully-folded Locally-unfolded Transition of Human Peroxiredoxin 5: A Molecular Dynamics Study

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Human peroxiredoxin 5 is an antioxidant enzyme that catalyzes the reduction of different peroxides using two cysteine residues. The first step of the catalytic cycle involves the nucleophilic attack of a peroxidatic cysteine "$C_P$" (C47) to the substrate to form a sulfenic acid derivative ($C_P$-SOH) and water. The second step is the resolution by reaction of $C_P$-SOH with a second "$C_R$" (C151) leading to a disulfide bond. Before the latter proceeds, a conformational change must occur as C47 and C151 are too far away each other (~13 Å) to react. This is known as the *fully-folded (FF) locally-unfolded (LU) transition* and involves partial unfolding of the N-terminal region of the α-helix that contains the active site and the movement of the loop carrying $C_R$. The aim of this work is to assess whether the oxidation of $C_P$ triggers the *FF-FU transition* or the protein may exist in both conformations in equilibrium disregarding the oxidation and ionization states of $C_P$. To cope with that accelerated molecular dynamics simulations were carried out for the reduced enzyme ($C_P$ as thiol and thiolate) and in the oxidized enzyme ($C_P$ as $C_P$-SOH and $C_P$-$SO^-$). Four replicas of 500 ns were run for each of the systems considered together with control conventional molecular dynamics simulations in the microsecond time scale. Our results show that whereas the oxidation of $C_P$ triggers the *FF-LU transition* disregarding the ionization state of reduced $C_P$, the ionization state of the oxidized $C_P$ does matter. When the sulfenate is present, it strongly interacts with a conserved Thr44 residue in the active site, preventing the α-helix to unfold. We conclude that for a proper catalysis C47 sulfenic acid must be formed at the active site, consistently with the findings of a previous QM/MM study conducted by us (*Portillo-Ledesma et al., Biochemistry, 2014*).

**keywords:** peroxiredoxin 5, fully-folded locally-unfolded transition, accelerated molecular dynamics

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