# The conserved amino acid motif –GSSYN- is essential for the *E. coli* flavorubredoxin NO reductase its activity

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Flavodiiron proteins (FDPs) are a family of modular and soluble enzymes endowed with nitric oxide and/or oxygen reductase activities, producing N2O or H2O, respectively [1]. The FDP from Escherichia coli, which apart from the two core domains, possesses a rubredoxin-like domain at the C-terminus and therefore named flavorubredoxin, FlRd, is a bona fide NO reductase, exhibiting an O2 reducing activity that is approximately ten times lower than the one for NO [2, 3,4]. Among the flavorubredoxins, there is a strictly conserved amino acids motif, -G[S,T]SYN-, which is located close to the catalytic diiron center. To assess its role in FlRd's activity, we designed several site-directed mutants, replacing the conserved residues by hydrophobic or anionic ones [5].

While maintaining the general characteristics of the wild-type enzyme, including cofactor content (iron and FMN) and the integrity of the diiron center, the mutants revealed a significant decrease in both of their oxygen (up to 60% reduction) and NO reductase activity (up to 99%). The later, its physiological function, was almost completely abolished in some of the mutants. Molecular modelling of the mutant proteins pointed to subtle changes in the predicted structures, that result in the reduction of the hydration of the regions around the conserved residues as well as in the elimination of hydrogen bonds, which may affect proton transfer and/or product release.

References

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