The catalytic mechanism of the riboflavin kinase activity of a bifunctional FAD synthetase: An *In silico* study

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In organisms from all kingdoms, the FMN and FAD cofactors of flavoproteins are synthetized from riboflavin by its initial phosphorylation to produce FMN and the subsequent adenylylation of FMN yielding FAD. In eukaryotes, the FMN and FAD syntheses are catalyzed by two different enzymes, riboflavin kinases (RFK) and FMN adenylyltransferase (FMNAT) respectively, while in prokaryotes these two processes are catalyzed by a bifunctional enzyme, FAD synthetase (FADS) [1, 2]. The riboflavin kinase activity in the bifunctional FADS takes place at the C-terminal module, which shares structural and sequence homology with monofunctional eukaryotic RFKs but where, on the contrary to eukaryotic enzymes, binding of ligands triggers dramatic structural changes which affect large portions of the protein to putatively stabilize the catalytic complex [3]. The adenylyltransferase activity takes place at the N-terminal module of FADS, which does not present neither structural nor sequence homology with FMNATs from eukaryotes[4]. These differences with eukaryotic enzymes have identified prokaryoticbifunctional FADSs as potential drug targets for the design of inhibitors which could fight resistant pathogen microorganisms. In this study we have focused in the catalytic mechanism of the riboflavin kinase activity of bifunctional FAD synthetase from *Corynebacterium ammoniagenes*. Hybrid molecular dynamic simulations (QM/MM;AM1/CHARMM), coupled to Umbrella Sampling, finite-temperature string method and the WHAM method, have provided the potential of mean force (PMF) of the riboflavin phosphorylation processes. Our preliminary results confirm the relevant role of the residues E268 and T208 from catalytic site PTAN motif. In addition we were able to observe, at atomistic level, several interactions which can contribute to the riboflavin phosphorylation catalysis. These interactions facilitate the approach between the phosphate moiety of ATP and the ribityl end of riboflavin.

1 Herguedas, B., Martinez-Julvez, M., Frago, S., Medina, M. and Hermoso, J. A. (2010) Oligomeric state in the crystal structure of modular FAD synthetase provides insights into its sequential catalysis in prokaryotes. J Mol Biol. **400**, 218-230
2 Barile, M., Brizio, C., Valenti, D., De Virgilio, C. and Passarella, S. (2000) The riboflavin/FAD cycle in rat liver mitochondria. Eur J Biochem. **267**, 4888-4900
3 Herguedas, B., Lans, I., Sebastián, M., Hermoso, J. A., Martínez-Júlvez, M. and Medina, M. (2015) Structural insights into the synthesis of FMN in prokaryotic organisms. Acta Crystallogr D Biol Crystallogr. **71**, 2526-2542
4 Krupa, A., Sandhya, K., Srinivasan, N. and Jonnalagadda, S. (2003) A conserved domain in prokaryotic bifunctional FAD synthetases can potentially catalyze nucleotide transfer. Trends Biochem Sci. **28**, 9-12