Application of QM/MM Methods in the Study of PNPOx

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INTRODUCTION

Pyridoxal 5'-phosphate (PLP), the active form of the vitamin B6, is an essential cofactor required by more than 160 families of enzymes. Its role as an electron sink makes it imperative for the catalysis of a myriad of chemical reactions. Contrarily to microorganisms and plants, humans and other mammals are not able to synthesize PLP *de novo*, resorting to a "salvage pathway" (**Fig. 1**) that helps to maintain PLP homeostasis in the cell [1].

Pyridoxine/pyridoxamine 5'-phosphate oxidase (PNPOx) is a FMN-dependent homodimeric enzyme essential in this pathway, being responsible for the recycling of pyridoxine 5'-phosphate (PNP) and pyridoxamine 5'-phosphate (PMP) into PLP [2]. Its malfunction has been directly correlated with severe neurological disorders [3].

Therefore, in order to better understand its role in these disorders, it is of the utmost importance to unveil the catalytic mechanism of PNPOx. To do so we used computational means, namely QM/MM hybrid methodologies [4], to evaluate the different mechanistic proposals related to PNPOx reactivity. Models were prepared having as starting point the crystalographic structure of the enzyme from E. coli (PDB 1JNW).

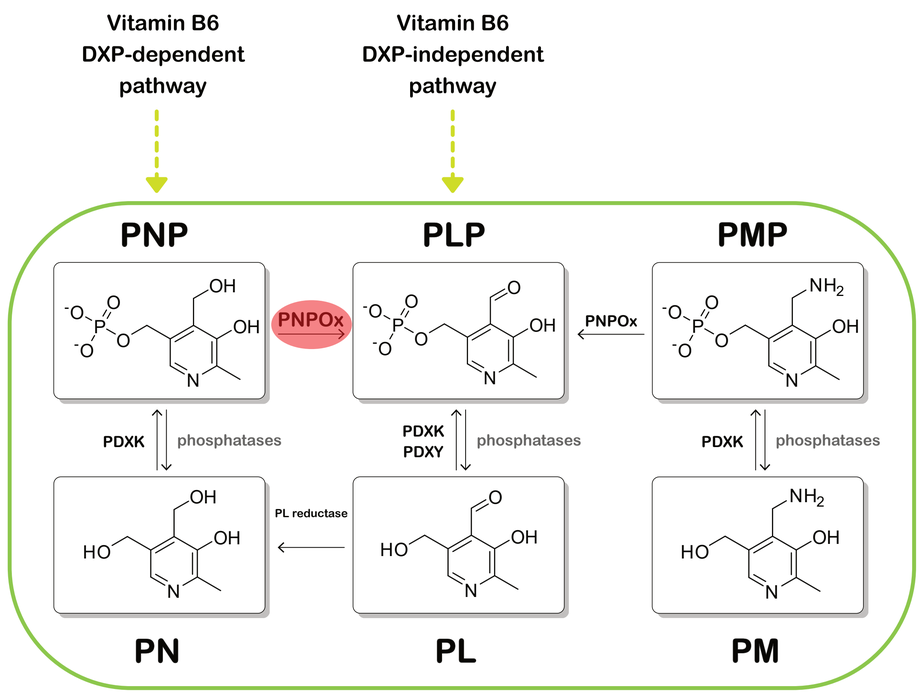
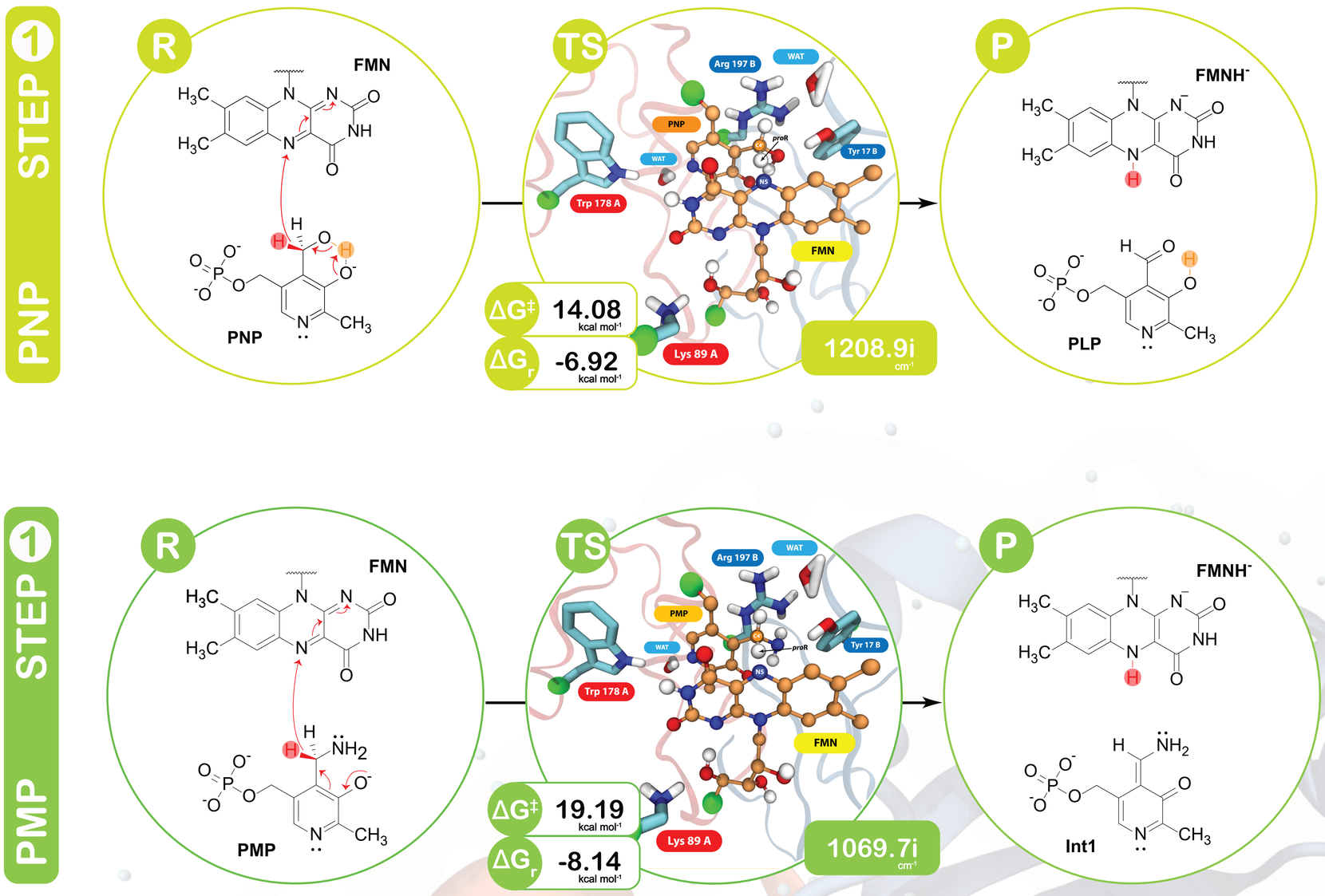


Fig. 1 - "Salvage pathway" of PLP

RESULTS



CONCLUSIONS AND FUTURE WORK

The results obtained in the present work provide important details about the catalytic mechanism of PNPOx, corroborating the proposed mechanism of a hydride transfer as the first step for the formation of PLP. Moreover, it was found that when PNP is the substrate, the catalysis proceeds in a single step. On the other hand, in the case of PMP, an intermediary structure is formed (Int1), that needs to be deaminated in order to originate PLP. Ongoing work intends to decipher this deamination step (**Fig. 2**), as well as, the role of neighbouring residues in the enzymatic turnover. In summary, these results provide important details about the catalytic mechanism of PNPOx, helping us to understand the importance of key residues in the active site that can have implications in some PLP-deficiency disorders.

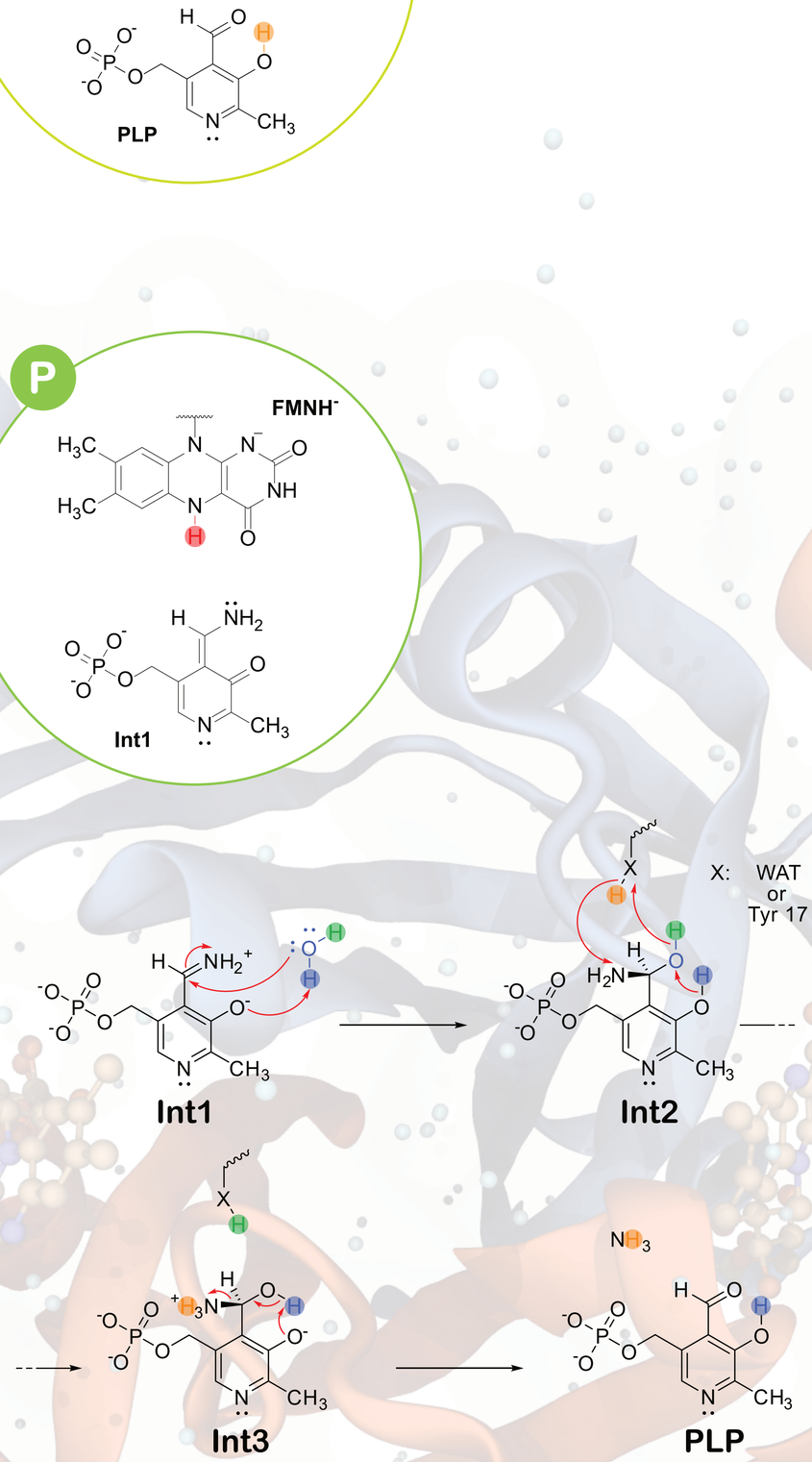


Fig. 2 - Mechanism proposal (PMP, step 2)

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