

## How experiments and molecular simulations can help understand selective C25-hydroxylation of vitamin D by fungal peroxygenases

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25-Monohydroxylated vitamin D3 (cholecalciferol) and D2 (ergocalciferol), compounds of high interest in human health and animal feeding, can be obtained through reaction with fungal peroxygenases [1]. In this work we show the results from a combined experimental and computational study of the hydroxylation of vitamin D by *Agrocybe aegerita* and *Coprinopsis cinerea* peroxygenases [2]. To rationalize experimentally observed differences in conversion yields and regioselectivity, diffusion of D2 and D3 on the molecular structure of these two enzymes was performed with PELE software [3]. In good agreement with experimental conversion yields, simulations indicate more favorable energy profiles for the substrates' entrance in *C. cinerea* than for *A. aegerita* enzyme. On the other hand, GC-MS analyses show that while a full regioselective conversion into the active C25 form is catalyzed by *C. cinerea* peroxygenase for D2 and D3, *A. aegerita* yielded a mixture of the hydroxylated D3 products. From the molecular simulations, relative distance distributions between the haem compound I oxygen and H24/H25 atoms (hydrogens on C24 and C25 respectively) were plotted. Results show large populations for O-H25 distances below 3 Å for D2 and D3 in *C. cinerea* in accordance with the high reactivity observed for this enzyme. In *A. aegerita*, however, cholecalciferol has similar populations (below 3 Å) for O-H25 and O-H24 which can justify the hydroxylation observed in C24. In the case of ergocalciferol, due to the bulky methyl group in position C24, very few structures are found with O-H24 distances below 3 Å and thus, as expected, reaction was only observed at C25 position.

### References

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