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The role of the propionate interactions in the *Listeria monocytogenes* coproporphyrin ferrochelatase bound to its physiological substrate

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The coproporphyrin-dependent heme biosynthesis (CPD) pathway has been discovered in 2015 and it is used by monoderm Gram-positive bacteria to produce heme *b* [1]. In the penultimate step, the coproporphyrin ferrochelatases (CpfCs) catalyzes the insertion of ferrous iron into the coproporphyrin III (cpIII) substrate, producing the iron coproporphyrin III (coproheme). In the final step, the coproheme decarboxylases (ChdCs) generate heme *b* by a two-step decarboxylation of the propionate groups of coproheme at positions 2 (p2) and 4 (p4), forming vinyl groups. After the cleavage of p2, the transiently formed monovinyl monopropionyl intermediate rotates by 90 degrees inside the protein pocket to bring p4 near the catalytic tyrosine, to allow the decarboxylation of p4 to form heme *b*.
Spectroscopic studies of wild-type (WT) CpfC and several variants, from *Listeria monocytogenes* (*Lm*CpfC) complexed with the product (coproheme), allowed us to conclude that the hydrogen bonding interactions to the propionate groups are fundamental for the stabilization and orientation of porphyrin ring inside the active site [2].
During my first year, the substrate (cpIII) free and complexed with *Lm*CpfC (WT protein and several variants) have been studied using UV-Vis electronic absorption and resonance Raman (RR) spectroscopies [3]. The results show that: i) the four propionate groups of cpIII form H-bonds with the protein matrix, and, as observed in the crystal, the H-bonds are characterized by different strengths; ii) unlike the coproheme complex, which is flat, cpIII shows a dome-like distortion when bound to the protein, as a consequence of the protein interaction with all four propionate groups. The differences between the RR spectra of the substrate and the product allowed us to highlight the differences in the H-bonds interactions established between the propionates and the conserved polar amino acids of the protein pocket. These differences will be used to follow *in vitro* the iron insertion process, upon addition of a solution of Fe (II), under anaerobic conditions, starting from the WT *Lm*CpfC - cpIII complex.

[1] H.A. Dailey et al., *Proc Natl Acad Sci USA.* **2015**, *112*, 2210-2215.
[2] T. Gabler et al., *Febs J.* **2022**, *289*, 1680-1699.
[3] A. Dali et al., *Protein Science*, **2022**, online (doi: 10.1002/pro.4534)