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Advanced spectroscopic study aiming to the understanding of the heme-biosynthesis pathway of gram-positive bacteria

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The coproporphyrin-dependent heme biosynthesis (CPD) pathway, utilized by monoderm bacteria to produce heme *b*, has been discovered in 2015 [1]. The coproporphyrin III (cpIII) is the substrate of coproporphyrin ferrochelatases (CpfCs) which catalyze the insertion of ferrous iron into the porphyrin ring, producing the iron coproporphyrin III (coproheme). This is the penultimate step within the CPD pathway. In the next 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 [2,3], to allow the decarboxylation of p4 to form heme *b*.
During my master thesis, I studied the wild-type and several variants of CpfC from Firmicute *Listeria monocytogenes* (*Lm*) complexed with the product (coproheme) using UV-Vis electronic absorption and resonance Raman spectroscopies (carried out at different temperatures and by using polarized light). I selectively assigned the vibrations of the four propionates, and I found that some hydrogen bonds observed in the crystal are not present in solution [4].
As PhD student, I started the spectroscopic characterization of the wild-type *Lm*CpfC complexed with the substrate (cpIII). These studies are fundamental to follow *in vitro* the synthesis of coproheme, starting from the CpfC-cpIII complex, upon addition of a solution of Fe(II), under anaerobic conditions. I found that the propionate vibrations of the cpIII-complex are at different frequencies as compared to those of the coproheme complex, and therefore, can be used to follow the titration. Interestingly, the preliminary data suggest that at the beginning of the titration, upon addition of 0.1-0.3 equivalents of a Fe(II) solution, an intermediate species is formed.

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