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The metalation process of coproporphyrin III by ferrochelatase from *Listeria monocytogenes.*

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Unlike humans, monoderm Gram-positive bacteria use the coproporphyrin-dependent heme biosynthesis pathway (CPD) to produce heme *b* [1], an iron porphyrin essential to pathogens for surviving and infecting the host. In the penultimate step of CPD, the coproporphyrin ferrochelatase (CpfC) catalyses the insertion and oxidation of Fe^{2+} into the coproporphyrin III (cpIII), producing ferric coproporphyrin III (coproheme). The latter is eventually decarboxylated by the coproheme decarboxylase to form heme *b* through a two-step decarboxylation of the propionate groups at positions 2 and 4 forming vinyl groups. In CpfC from *Listeria monocytogenes* (*Lm*), both the porphyrin substrate (cpIII) and product (coproheme) are stabilized by several H-bond interactions of different strength, between the four propionate groups and the polar amino acids of the protein active site [2].
By following, in solution and under anaerobic conditions, the *in vitro* insertion of Fe^{2+} into cpIII by wild-type *Lm*CpfC using UV-vis electronic absorption and resonance Raman spectroscopies, we proved that upon metalation of the native substrate, a stable and saddled-distorted catalytic intermediate is formed. The distortion is a consequence of the reorganization of the H-bonds interaction between the propionate groups and the protein matrix [3]. Therefore, the active site's environment controls the orientation and distortion of the porphyrin before and during metalation.
Moreover, preliminary data on the role of the His182 and Glu263 distal residues in the metalation process showed that, unlike what was suggested in the literature, these residues are not fundamental for iron insertion. Instead, Glu263 is involved in the iron oxidation of the product.

[1] H.A. Dailey et al., *Proc. Natl. Acad. Sci USA,* **2015**, *112*, 2210-2215.
[2] A. Dali et al., *Protein Sci.,* **2023**, *32*, e4534.
[3] T. Gabler et al., *Protein Sci.,* **2023**, *32*, e4788.