Experimental and theoretical studies into the structural importance of the imine versus the amine function of the histidine brace motif of LPMO's

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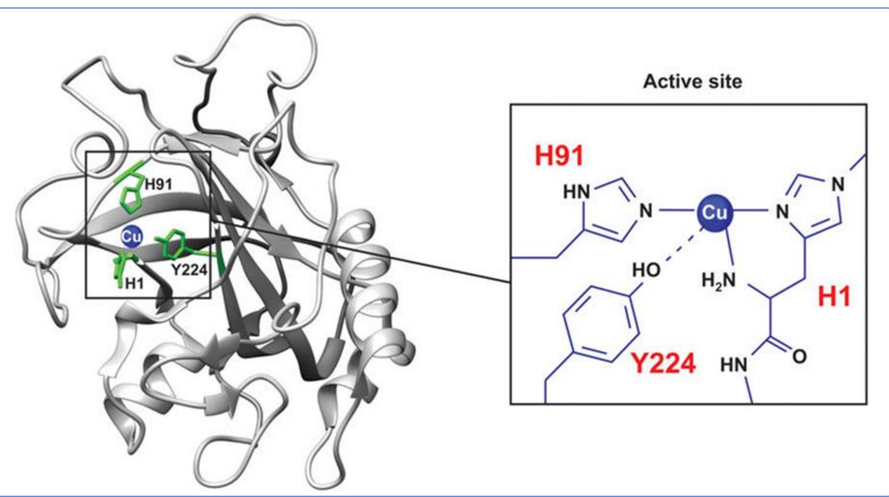
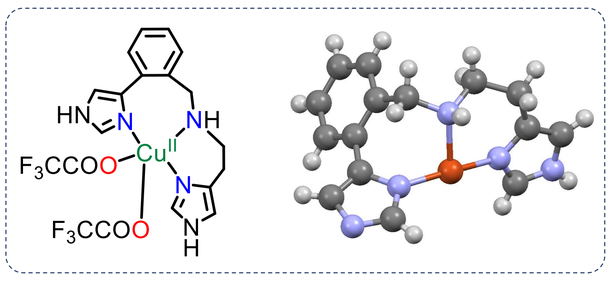
Introduction: Lytic polysaccharide monooxygenases (LPMO) are a family of copper containing metalloenzymes important for biofuel and biochemical production due to their ability to oxidatively cleave the strong glycosidic C-H bonds in cellulose. The active site of LPMO consists of a single copper atom bound in a T-shaped N3 coordination environment called the histidine brace. The same structure is also found in particulate methane monooxygenase suggesting that the histidine brace is responsible for the oxidative capabilities of the enzymes. However, the role of the histidine brace as well as many other structural features of the active site remain unclear. Synthesizing and studying synthetic models of the histidine brace can increase the understanding of the structural features important for reactivity and generate new powerful catalysts for the oxidation of organic substrates. Currently, the limited number of LPMO model complexes that have been described and the large structural variation between these makes comparison difficult, limiting the understanding of how different modifications affect reactivity.



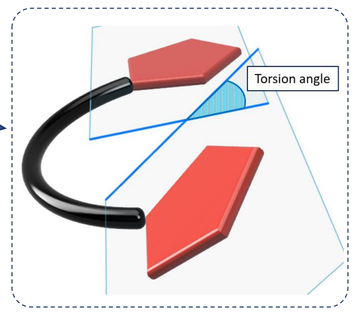
Background: The study of enzyme's active site can be done via the design and the synthesis of small molecular models, few model complexes aimed at mimicking the active site of LPMO's have been described in the literature. The model reported by Itoh exhibited the best structural and physicochemical properties so far as well as great oxidative power. Those properties were proposed to come from the large torsion angle between the imidazole rings.

Itoh's complex: great properties



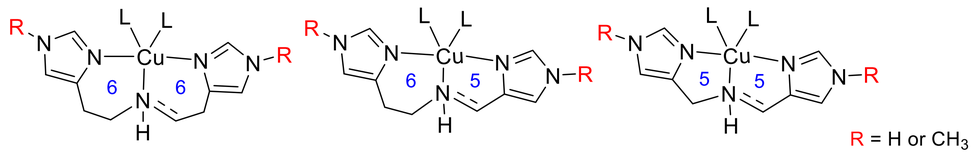
Origin?

Torsion angle

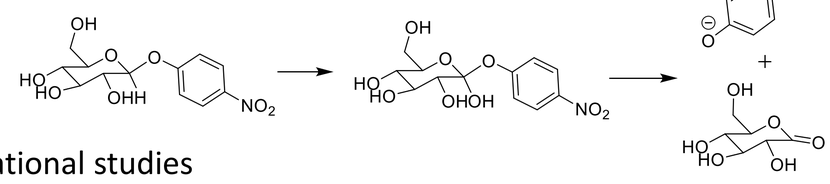


Objectives: Due to the lack of comparison in between the reported LPMO models, the impact of the structural parameters of the histidine brace motif such as the torsion angle and the imidazole methylation is still unknown. To get insight in the important structural features, the objectives were the following:

- Preparation of LPMO active site models containing different chelate ring sizes 5 or 6), methylation state and with an amine or an imine function

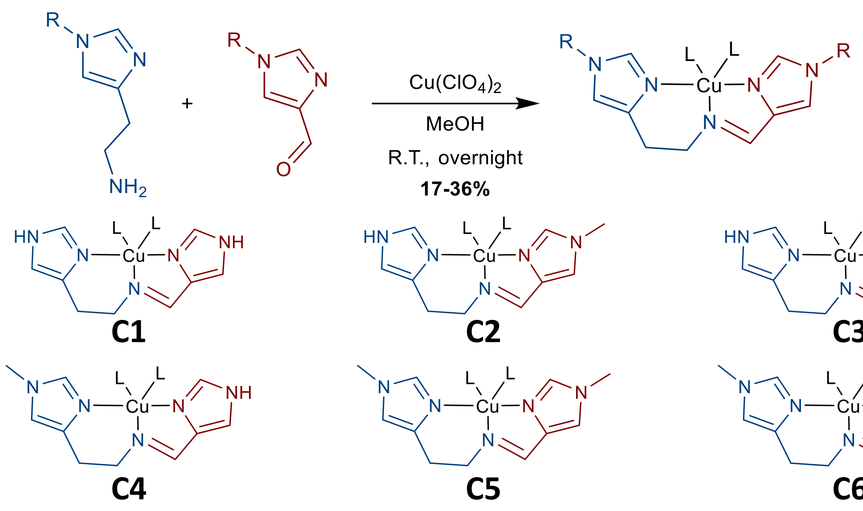


- Characterization of the models (UV, CV, XRD, …)
- Reactivity assays on a model substrate

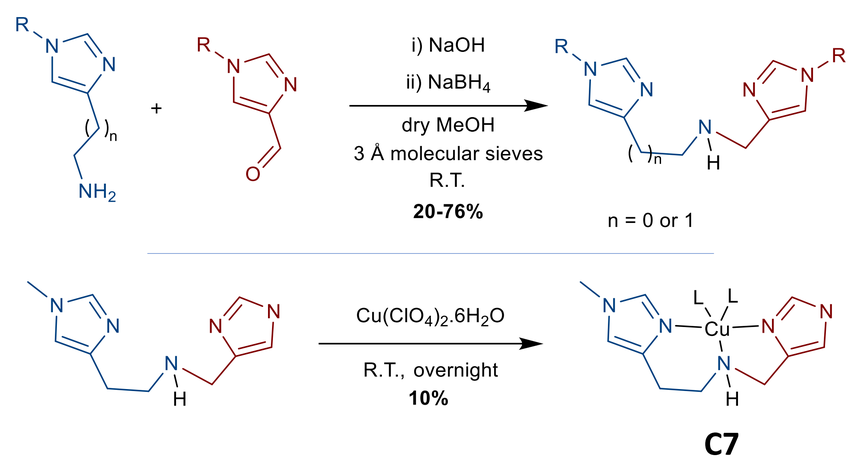


- Computational studies

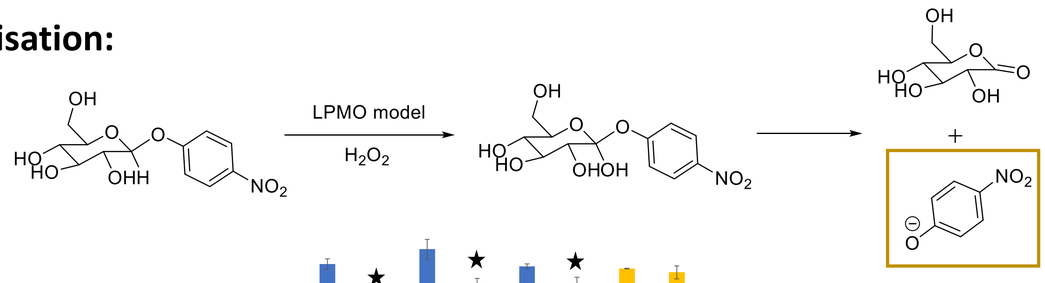
Imine complexes synthesis:

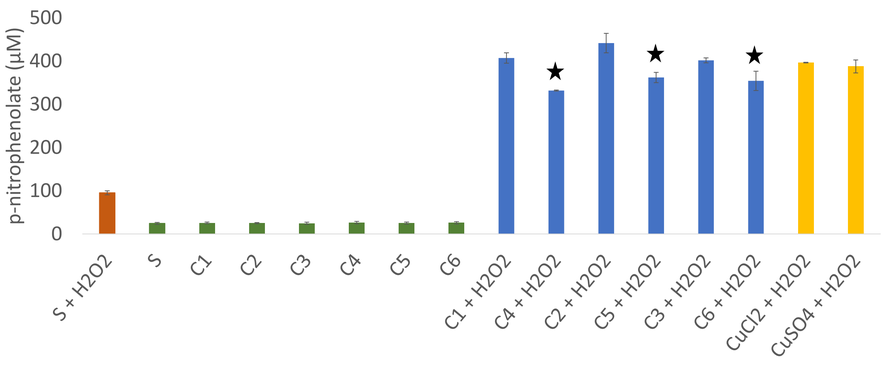


Amine complex synthesis:

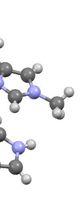


Characterisation:





- Imine models are active
- Copper salts equally good
- Negative impact of the methylation on the 6-membered ring ★

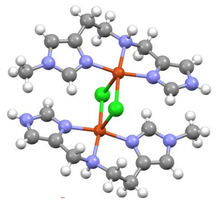


C4

5,6°

Imine linker

Low torsion angle

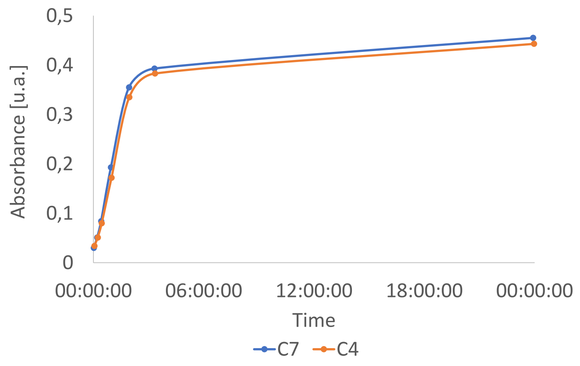


C7

24,6°

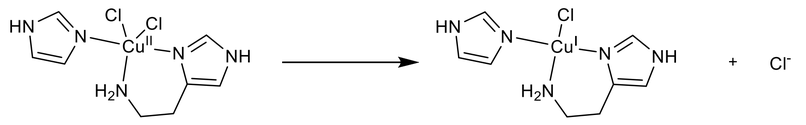
Amine linker

larger torsion angle

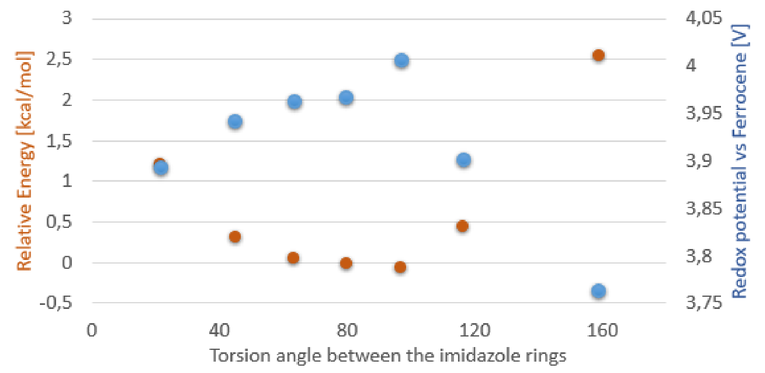


No influence of the C-N bond oxidation state of the linker on the reactivity

Computational:



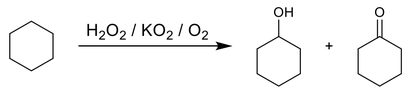
Scan at different torsion angles



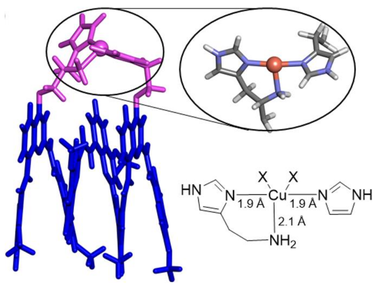
- Most stable conformation around 80° like in the enzyme
- Correlation between torsion angle and redox potential

Perspectives:

- More small molecular LPMO models
- Reactivity assays with other substrates and oxidants



- New generation of AOF scaffold based LPMO models



References: Castillo I., *et al., Inorg. Chim. Acta*, **2014**, 422, 152-257, Concia A. L., *et al., Inorg. Chem.*, **2017**, 56, 1023-1026, Mayilmurugan R., *et al., J. Catal.*, **2019**, 372, 352-361, Itoh S., *et al., Chem. Commun.*, **2020**, 56, 5123-5126