

Thinking a little more about Lcp (Latex clearing protein), our team applied some bioinformatic tools to try and get more information about this protein's properties and structure. Unlike RoxA (Rubber oxygenase A), Lcp crystallographic structure has not been solved yet (RoxA PDB: 4B2N). It has been indeed shown that Lcp is able to link itself to a prosthetic heme group in a non-covalent way (b-type), unlike what happens in RoxA (c-type)¹.

We tried but we couldn't obtain a good structure model using structure predictors like I-TASSER² (figure 1). On the other hand, it is possible to observe in secondary structure prediction that Lcp has a lot of loops and unstructured regions that are also observed in RoxA (32% helical and 7% beta sheet)³, fact that might suggest some similarity between these two proteins. For RoxA, a complex structure composed by three enormous loops forming a tower (each loop formed by helices and non-structured regions) was proposed to determine the exo-cleavage rubber polymer³ (figure 3). The difficulty of finding a model might be due to the protein's high complexity, distinctly of RoxA because it performs endo-cleavage of the rubber polymer. In fact, I-TASSER was not able to predict even a heme ligand.

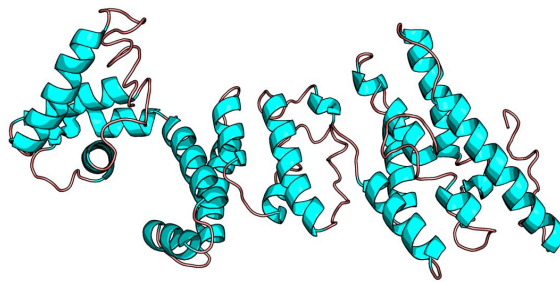


Figure 1: The best model predicted by I-TASSER. It is composed of helices present in sequence and approximately in the same plan, not looking like a center that can receive a heme group. The C-score and TM scores are under acceptable values (-3.22 and 0.35 ± 0.12 respectively).

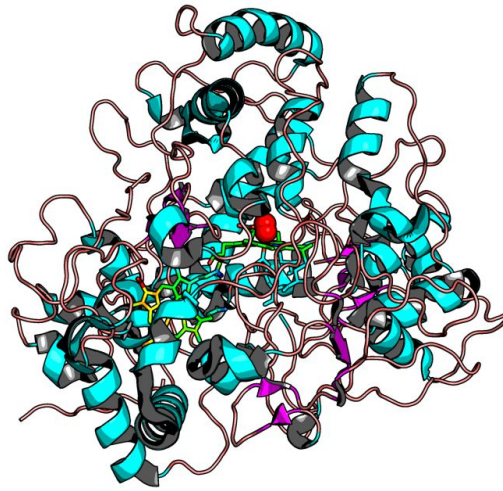


Figure 2: RoxA structure is a complex globular protein that contains two heme groups in its core. The catalysis happens in group heme 1³. The heme groups are surrounded by alpha helices as it is characteristic of this kind of protein. The oxygen responsible for attacking the polyisoprene chain is shown in red, the helices are shown in cyan and in purple, is the beta sheet.

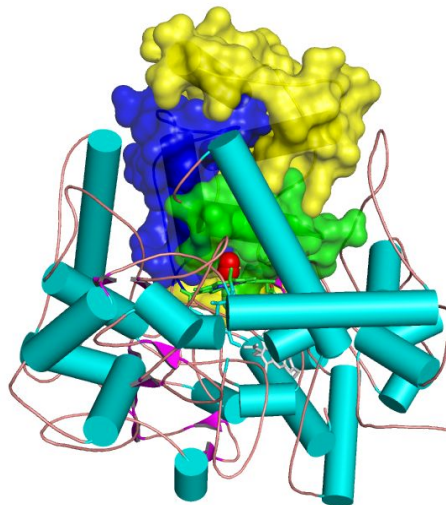
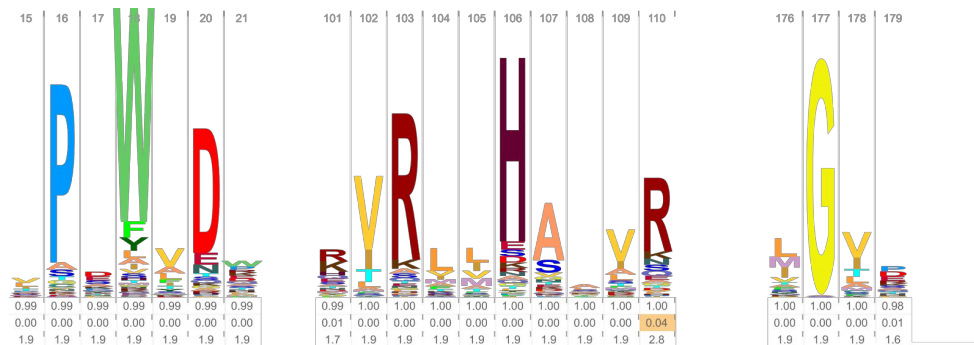


Figure 3: The RoxA structure simplifying helices and loops. In this figure, loop one is highlighted in dark blue, loop two in green and loop 3 in yellow.

We have performed a HMM model using the Jackhammer tool in HMMER package on-line server⁴. In this analysis we used the lcp sequence without TAT signal as a start sequence. Eight iterations in the program were sufficient for the model to converge. The HMM model was

designed using Skylign online server (<http://skylign.org/>⁵). Three main regions in the HMM model with highly conserved amino acid were recognized (figure 4). The second one has a highly conserved histidine amino acid, that suggests an oxygen-amino acid coordination, known to heme groups.

(a)



(b)

	Lcp	--RMVHAAVRHLLPQSP----
H6R6V5	NOCCG/24-397	--L-VHAAVRHLLPQ----
D9T3F1	MICAI/34-405	--L-AHAGVRHLLPQ----
D2BAA8	STRRD/13-72	-----
N1PCE9	DOTSN/137-552	--S-SRAGKERDANG-----
M7WYJ4	RHOT1/63-464	--M-LHAQVRRRIANGK----
G0SUZ7	RHOG2/63-463	--M-LHAQVRRRIANGK----
V6KXG1	STRRC/9-367	--L-LHAAIRYHLTR-----
V6JJB9	STRNV/2-361	--L-LHASIRHHLRR-----
G0G5M6	AMYMS/13-344	--L-IHAAVRHFITR-----
W5W8M2	9PSEU/4-322	--L-IHAAVRHLITE-----

Figure 4: The upper image (figure 4a) showing the final HMM logo was analyzed and three regions were recognized showing considerable conservation. The middle one called our attention because a histidine (H) is necessary to coordinate the oxygen of the heme group to chappen catalysis. The lower image (figure 4b) show the alignment used in the HMM model revealing that Lcp has that same histidine⁶.

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4. Finn, Robert D., Jody Clements, and Sean R. Eddy. "HMMER web server: interactive sequence similarity searching." *Nucleic acids research* (2011): gkr367.

5. Wheeler, Travis J., Jody Clements, and Robert D. Finn. "Skyline: a tool for creating informative, interactive logos representing sequence alignments and profile hidden Markov models." *BMC bioinformatics* 15, no. 1 (2014): 7.
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