Review of Doctoral Thesis

MSc. Alma Carolina Sanchez Rocha

Faculty of Science, Charles University, Prague

Evolution of protein-RNA and protein-cofactor interactions

In her dissertation thesis, MSc. Alma Carolina Sanchez Rocha presents and analyses three aims focused on the evolution of amino acid selections via – (Aim I) study of ancient ribosomes, (Aim II) characterization of structural properties of random peptide sequences using evolutionary sets of amino acids, and finally, (Aim III) study of protein-coenzyme interactions through evolutionary sets of amino acids.

The thesis is written in good English and is 97 pages long. After the introduction (20 pages) to the topic of early evolution of proteins follows a section about aims of the thesis. Materials and methods section (7 pages) is short but concise. Result section is organized according to individual aims listed above with the largest emphasis to Aim III about protein-coenzyme interactions. Results are followed by short discussion. Author have published 4 manuscripts (1 as a first author).

The thesis typesetting would benefit from more careful setting e.g. headings on new pages and not in the last paragraph. Slightly more problematic are References, which were not proofread - exemplified by refs. 3 + 59 + 102 + 161 (books?), 9 (version?), 36 + 41 + 96 + 97 + 100 + 104 +110 + 127 + 185 (name of journal), 153 (missing journal). But these are only a minor negligible problem to the scientific content gathered out of 188 references.

Hence, this thesis shows that the candidate is ready for its defense. I recommend the acceptance of the submitted thesis as a part of the procedure of awarding the Ph.D. degree.

I have attached my questions for the author on the following page.

Conclusion: **I suggest the acceptance of the thesis**

Olomouc 31st May 2024

doc. RNDr. Karel Berka, Ph.D.
Department of Physical Chemistry
Faculty of Science, Palacký University in Olomouc

Questions to the author:

1. What was author's contribution to individual publications listed in thesis?
   Aim I:
2. Have you considered to test possible conformational change of peptide pL22 (p. 42) in the presence of rRNA experimentally?
   Aim II:
3. Why there was no library 20 (p.33)? Why there was no Cys even in 19F library, considered that Cys is not the latest amino acid according to temporality order listed in Table II?
4. Can you estimate the error of assignment of secondary structures to random peptide libraries, e.g. from bootstrap analysis (e.g. Figure 14)?
5. Do you have any explanation, why the PRIPRED predictions (Figure 15) show much higher percentage of beta-sheets than PEPstrMOD (Figure 14)?
   Aim III:
6. Given that ancient coenzymes bind to backbone atoms, could it be the reason why alpha-aminoacids were selected, or do distance measurements allow for different backbones as well?