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Oponentský posudek doktorské disertační práce

Michal Grulich: Studium enantioselektivity a syntézy beta laktamových antibiotic katalyzované penicilin-G-acylasou: biokatalýza a *in silico* experimenty.

In his PhD thesis Michal Grulich makes an attempt to characterize and understand the enantio-slectivity of penicilin-G-acylase PGA from *E.coli* and *Achromobacter*, and later to use that knowledge to design an enzyme with increased enzymatic function. The bullet points of his work are: He compares PGA^{Ec} and PgA^{A} enantio-selectivity demonstrating the higher selectivity of PgA^{A} for the given set of substrates. A model of PgA^{A} was generated and used for reproducing the experimental data for the same group of substrates. This work out nicely and thus confirmed the validity of the model. The confirmed model then was used to predict the enantio-preferences for 7 new substrates. In analogy to the just mentioned approach the model was used also for the study of the synthesis reaction for semi-synthetic β-lactamic antibiotics, where PGA is a catalyst for the synthesis of anoxicilin and ampicilin. The model was finally used to predict modifications in the entrance to the catalytic site to influence/increase the activity. One of these point mutations was then prepared experimentally and the mutant enzyme indeed exhibited a better conversion (81% to 77%) in anoxicilin synthesis under industrial conditions and was therefore further characterized. The thesis consists of 115 pages and is written in czech in a classical way as a full thesis and not as a collection of papers. Nevertheless 4 papers are attached to thesis. 22 pages of literature review form the introduction, 35 pages of detailed materials and methods give an overview about the broad range of methods used by the candidate. Then 25 pages of results are followed by 10 pages of discussion and a 2 page conclusion. It is visible that the author invested a lot of time and care in writing up his research and did care even about small details that are often overseen. Michal Grulich has six publications so far, 5 publications in Web of Sciences, and one more in print in the Journal of Molecular Catalysis B (Enzymes). Four of those are included in the thesis. On three of this papers Michal Grulich is the first author. Of the papers directly related and attached to the thesis, papers 1 and 2 are reviews, and especially the review in Biotechnology advances with an IF of 9.7 demonstrates his deep knowledge about PGA selectivity and promiscuity. Paper 3 deals with biochemical characterization and paper 4 represents the core work of this thesis including most of the theoretical as well as experimental work described in the thesis. The research has been conducted with great care and the paper is technically sound without any flaws in the experiments and the conclusions are drawn appropriately.
The remarkable broadness of the whole thesis demonstrates the broad theoretical and practical knowledge of the candidate and the reader is left with the feeling that the candidate knows what he is writing about.

Questions and remarks for the defense that should be addressed by the candidate:

1. The declaration on page 2 is for all publications and does not differentiate on which paper the candidate contributed just 20% and on which 80%. In my opinion such a declaration should be for each paper separately. I actually in the whole thesis did not find a list publications of the candidate but had to look myself in WOS., even the “obsah” is mentioning just “prilohy” without naming or numbering the papers.

2. In paper 4 Jan Brezovsky from Masaryk University is one of the co-authors and a well known modeler. As the paper in addition to the experimental work contains an extensive modeling and docking section, the question comes to my mind which parts of the theoretical work were performed by the candidate and which part by the co-author?

3. Question 2 actually raised also another follow-up question: The thesis describes in detail a huge variety of experimental and theoretical methods ranging from molecular biology, enzymology via crystallization to targeted computational modeling and protein engineering. I can hardly imagine that all of the mentioned methods are mastered by the candidate with the same perfection. I would be glad if the candidate could describe a bit to which degree he manages the various techniques with his "own hands" and where he sees his main strength.

3. The homology model was built on the x-ray structure 1GM7 of PGA^{Ec}. What is the resolution of the structure? As not only the sequence identity determines the consequent model resolution, but a model can't hardly be better then the input structure.

3. You deposited the model in Protein Model Database. While I generally appreciate that you uploaded the model to give the community the possibility to use it further or the verify your results, I don't think this is a good database. It was last updated (when you look in the news-section) in 2006 and I am not sure how well it is recognized. I would recommend to you to submit your model to modelarchive.org, where your model gets it's own DOI, and where additional information (methods etc) is presented similarly as in the PDB database, as this archive collaborates with PDB.

3. When using AutoDock VINA you describe that you increased the parameter 'exhaustiveness' to 200 (from how many?). Why did you do so, and what does the parameter mean (as this is not a standard physical parameter)?

4. Additionally to the excellent and convincing work done, did you consider refining your docked-poses with MD, or using a QM/MM approach to estimate deltaG with better confidence?

6. For getting the initial crystals you used the sitting-drop method. Why were the crystals harvested after 9 month only? This is an unusual long time in which anything can happen in the solution (even growth of microorganisms). Can the long time play a role in the fact that you were not able to repeat the crystal growth with the same success and these initial crystals were the largest/best? Did you/ will you try alternative methods like crystallization in a capillary for example to gain better crystals?

7. You mentioned the measurement of the crystals in Germany. Have you been in Bessy, Berlin or Desy, Hamburg? Did you mount the crystal and collect the data yourself?

Finally, it is my pleasure to state that Michal Grulich until now conducted internationally recognized high quality science, clearly manifested in the 32 citations that his publications gained so far (Web of Science, 20.11.2015). The well written thesis tells a compact story, the combination of experiment and computation is convincing, and the publications that back up the thesis show, without leaving any doubts, that the applicant fulfills all criteria for being awarded a PhD degree. Therefore I can fully recommend Michal Grulich for being awarded the PhD degree.

(Český doplneľk: Michal Grulich jásně prokázal tvůrčí schopností, prácé bez sebemenších pochybů splňuje požadavky kládené na disertační přáce v oboru biochemie)

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