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Oponentský posudek doktorské disertační práce

Petr JEŘÁBEK: Teoretická studie enzymů spojených s procesem karcinogeneze: DNA polymerázy β a cytochromů P450

In his PhD thesis Petr Jeřábek tries to understand and explain the mechanistic principles underpinning the complex behaviour of DNA polymerase β and cytochromes P450, two enzymes participating in the process of carcinogenesis, using computational modeling as his main tool. In case of DNA polymerase β he studied mainly the ability of pol β to discriminate between "right" and "wrong" nucleotides, a phenomenon called fidelity. This includes calculations of relative free energies by LRA, demonstrating the stabilization of the transition state in case of the right nucleotide, as well as point mutations studied by FEP and LIE. This approach did not only lead to results that could be correlated with experimental data for six single-point mutants and wild-type, but also to a improved methodology, as the hybrid FEP/LIE and the modified FEP methods developed within the thesis provided quantitative estimation of fidelity within acceptable margin of errors.

With respect to cytochromes P450, Petr Jeřábek studied mainly the interaction of cytochrome b5 with cytochrome P450 and the influence of this interaction on the catalytic mechanism using flexible protein-protein docking and steered MD. Additionally he studied the activation of the xenobiotic aristolochic acid I catalyzed by two different cytochromes P450, CYP1B1 and CYP1A1/2, that leads to metabolites with genotoxic properties. He did so by using flexible protein-substrate docking. The thesis itself can be separated into two parts: 70 pages of comprehensive introduction into the problematics, methodology and the observed systems, including a summary and discussion of results for each studied system, and the second part which are actually the publications that back up the thesis. The thesis is written in czech, with very little typing errors or other omissions. The introduction mainly describes the studied systems in detail and is clearly focused on a biochemistry readership. The computational part introduces only in a very short manner the underlying principles of classical MD, steered MD and computational docking, omitting any equations or mathmatical descriptions, which is probably okay for a thesis in a biochemistry department. The methods part then summerizes the used methods citing the published papers for experimental detail.

The thesis is backed by 3 papers already published in impacted journals and one manuscript draft, including 2 as first author and one with shared first authorship. During his thesis Petr Jeřábek obviously had several overseas stays in the lab of Jan Florian where he also had the great opportunity to collaborate with Arieh Warshel, one of the leading scientists in computational chemistry with an H-index close to one hundred. The publication with Arieh Warshel as a co-author is certainly a great honour for any PhD-student and confirms the high

quality of the work. Additionally, from his former undergraduate work Petr Jeřábek has three publications and collected altogether 34 citations according to Web of Science, 8.12.12, which is for a PhD-student at the beginning of his career an excellent record, clearly indicating that his research is internationally recognised.

In the thesis I especially like that Peter did not stay just on the level of a user, applying highly sophisticated computational methods on two essential biosystems, but also got involved in the further development of the methodology. This deeper understanding for the different energy terms contributing to the free energy, the recognition of the strengthes and weaknesses of each method and the expertise gained in participating in the development of a smart way how to get around the shortcomings of the individual methods will greatly profit him in his future career. The results part is nicely logically structured into two blocks, one for each biosystem. The thesis finishes with a "take home message" like list of conclusions, summing up the main results and putting them into a larger framework. The work of Petr Jeřábek is on the forefront of computational biology and demonstrates the immense gain of calculations of these types for experimental biochemistry due to the predictive potential of the calculations. Information that leads to a better understanding of molecular mechanism underlying enzyme function is highly needed and appreciated in protein chemistry.

Finally, I must state that Petr Jeřábek until now conducted internationally recognised science. The 3 publications and the one manuscript draft that back up this PhD-thesis support the fact, that the applicant fullfills all criteria for being awarded a PhD degree, therefore I can certainly recommend Petr Jeřábek for being awarded the PhD degree.

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

1. In your thesis you worked on the one hand with very sophisticated methods to calculate the free energy as accurate as possible, on the other hand you used molecular doking to gain binding free energies predicted on the basis of a scoring function that ranks the docked poses. Is it possible to say how good these predicted binding free energies compare with experimental values and with more sophisticated methods?
2. Cyt b5 induces changes in the charge distribution on the CYP1A2 surface when forming the complex. Would you expect polarization effects to play a major role in the interaction, and would the inclusion of charge polarization in the MD improve the calculations?
3. Wouldn't it make sense to use the steered MD results of the dissociation of the identified stable complex A to set up umbrella-sampling to calculate the Potential Mean Force?

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