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Oponentský posudek doktorské disertační práce

Jiří KYSILKA: Intermolecular Interactions in Proteins

In his PhD thesis Jiří Kysilka tries to understand and explain intermolecular interactions in protein using on the one hand quantum chemistry and on the other hand methods of bioinformatics. One chapter is devoted to a DFT study of protein functional groups with a hydrophobic surface. The second chapter is devoted to the analysis of protein interfaces in a set of protein-protein complexes of high resolution solved by X-ray crystallography, and the third chapter is an analysis of crystal water in redundant lysozyme structures in the PDB database. In all three cases the underlying theme is the deeper understanding of non-covalent interactions in protein science. The thesis is backed by 3 papers already published in impacted journals and one manuscript submitted, including 3 as first author. The three published publications collected altogether 23 citations according to Web of Science, 10.4.13, which is for a PhD-student at the beginning of his career a very good result, clearly indicating that his research is internationally recognised. The thesis itself is written in a classical way and has 91 pages plus the attached publications. The comprehensive introduction of 27 pages introduces the problematics, methodology and the observed systems and finishes with aims of the thesis. This is followed by a methods part (10 pages), that gives all essential methodological details. Finally results are presented for all three main aims (or chapters) separately, followed by a conclusion that connects the three parts. In the thesis as well as in the publications the contribution of the candiate is evident and significant and certainly demonstrates the ability of Jiří Kysilka to conduct research independently (though under supervision, of course), and thus in my opinion he fullfills what is expected from a PhD student. Personally, I consider most interesting the results from the analysis of the protein-protein interfaces, as this might help the community in interface prediction or in developing better protein-protein docking algorithms.

However, I also have a few points to critize and a couple of remarks that might serve for discussion:

1. The thesis is written in english, with very little typing errors or other omissions. However, a large fraction of the articles "the" and "a" is used incorrectly, and careful proofreading would have improved the thesis significantly.

2. With respect to the results of the first aim Jiří Kysilka writes that "the data could serve as a benchmark for the intermolecular interactions in proteins". In the two DFT papers proteins are not mentioned at all, as to mimic the functional groups full molecules were used and described. When comparing the flat graphite surface with a

hydrophobic protein surface for sure the lateral translation is different, but also in the protein case you nearly never have "pure" hydrophobicity. I would have enjoyed to read a bit more about the idea of using this benchmarking, having this idea more developed. For what purpose would you enjoy to have this benchmark? Please comment and discuss.

3. page 20 "the various amino acid residues that are attached to alpha carbons are called side chains"
This is a wrong statement, a residue in biology refers to a specific monomer within the polymeric chain of a protein, and thus a residue is an individual amino acid in a peptide chain, not just the side chain! Please be aware of the definintion of an amino acid residue.

4. page 22 "This process is governed by the increase of entropy of the hydration shell, as the water molecules around the hydrophobic residues loose their degrees of freedom. It was shown that water near large hydrophobic surfaces is more mobile than bulk water (ref32)"

The second sentence on a first glance does oppose the first one and thus stimulates discussion. Indeed, hydrophobicity is the dominant force of protein folding, and entropy plays a major role in two ways: A gain in entropy of the solvent, a loss in entropy of the protein.
The gain in entropy arises from the water solvation around the unfolded, largly hydophobic protein chain. Hereby, water molecules surrounding the nonpolar amino acid side chains prefer to hydrogen bond with other waters instead of exposing their polar parts to the nonpolar species. This view is generally accepted and supported by early computer simulations (Geiger et al., 1979; Pangali et al., 1982; Ravishanker et al., 1982). In the cited reference I do not find the statement that "water near large hydrophobic surfaces is more mobile than bulk water". Nevertheless, the statement as given in the thesis seems to contradict with the first sentence, as folding would result in a loss of entropy, instead. (as it does for the protein itself, where the dominant opposing force to protein structure and stability is the loss of nonlocal conformational entropy due to steric contraints in the folded state). Rather the cited publication mentiones the "glassy" state of the hydration shell, and cites a couple of MD results that show shorter residence times for waters near nonpolar atoms than near polar atoms, but not versus bulk water. Please discuss this issue and explain your view on the entropic contribution to protein folding.

4. page 47-48, tables 3 and 4: Some of the obligate complexes have a positive total interaction energy, which would mean to my understanding that they do not interact, which is especially strange in the case of the obligate complexes in table 3. This might mean that an important energy contribution (in this case probably the most important) was neglected in these cases, please comment on that.

5. In the third sub-project the candidate uses crystal water from a large set of redundant X-ray structures to study the protein hydration shell. The position if the water molecules in oprotein crystals might be influenced by crystal packing, too. To which degree can we write the equation cystal water=hydration shell? Would a hydration shell generated by MD for lysozyme (taking a certain "mobility threshold") differ from yours?

6. The last paper was submitted in January. Is there already any feedback from the reviewers?

Finally, none of the above said should in any way diminish Jiří Kysilka's performance, and the fact that he conducted internationally recognised science. The 3 publications and the one submitted manuscript 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 Jiří Kysilka for being awarded the PhD degree.

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