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Ion-Protein Interactions

Submitted by Jan Heyda

"Oponentský posudek disertační práce"

Before going into details one has to state that the PhD thesis „Ion-Protein Interactions" written by Jan Heyda is based on 13 original papers. Twelve out of those 13 papers were published in *Journal of Physical Chemistry*, one in *Physical Chemistry Chemical Physics*. In 6 papers Jan Heyda acts as the first authors. When going through all those 13 papers, it becomes evident that Jan Heyda has considerably contributed to those papers. Considering the high publication productivity it would be very difficult to find reasons for not recommending his thesis. It is evident that this work goes beyond the usual standards found for PhD thesis at Czech Universities.

The thesis is aiming to a better understanding of the interaction of aminoacids, peptides, and proteins with ions. The main methods used by Jan Heyda are molecular dynamic simulations, thus the thesis is coming from the branch of computational chemistry. In order to verify the findings experimentally, techniques like capillary electrophoresis and second harmonic generation spectroscopy were applied apparently in collaborating laboratories. However, it becomes evident that Jan Heyda was focused on the computational part of those 13 publications.

The research strategy appears very systematic. First the binding of ions to simple amicoacids and selected short chain oligopeptides as well as n-methylacetamide as a model compound for a peptide bond was investigated. Next, J. Heyda focused on small peptides like the amyloid 1-16 or the 20 aminoacids containing Trp-cage miniprotein. Finally, "real" proteins like the HIV-1 protease or the dehalogenase enzyme LinB were investigated. Regarding the choice of the ions, Jan Heyda not only investigated representatives of the Hofmeister series, but also examined more complex cations like imidazolium, urea or guanidinium.

The thesis certainly fulfilled its aim and advanced the atomistic or molecular understanding of the nature of the interaction of proteins with ions. We are dealing here with cutting edge computational chemistry applied to relevant biological problems on –and I would like to emphasis that point- a realistic level.

In summary I strongly recommend the committee to accept this thesis. For the case, Jan Heyda will successfully defend his thesis, he should be awarded with the title PhD.

Prague, 2.09.2011

Prof. Dr. Martin Hof, DSc.

I would like to raise one issue for the candidate to answer: A certain part of the thesis is dealing with the molecular understanding of the denaturation pathways of the Trp-cage miniprotein. As also cited by J. Heyda [147] there is a single molecule study using the fluorescence quenching of an ATTO dye by the Trp chromophore. In my eyes this is an elegant and convincing study focusing on the determination of kinetic constants of protein conformational changes. I have two questions:

1) Could you relate this study to your findings?
2) Could you design an ATTO dye/ Trp experiment which could confirm your conclusions?