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Oponentský posudek doktorské disertační práce

Jan HEYDA: Ion-Protein Interaction

In his PhD thesis Jan Heyda tries to understand and explain the complex behaviour caused by ion-specific effects using computational modeling of ion-protein interactions with classical molecular dynamics as his main tool. The phenomena he is studying range from ordering in the Hofmeister series, enhancement of enzyme acivity, preferential interactions with functional groups up to osmolyte specific denaturation pathways. The thesis itself can be separated into two parts: 63 pages of a comprehensive introduction into the problemtatics and the methodology followed by a detailed introduction of the observed systems, including a summary and discussion of results for each studied system, and the attached publications to back up the thesis.

The thesis is backed by 13 papers in impacted journals, including 6 as first author, and Jan Heyda collected altogether 63 citations according to Web of Science, 7.9.11, which is for a PhD. student at the beginning of his career, who published his first paper just 2 years ago, an excellent record, clearly indicating that his research is internationally recognised.

The methodology part of the thesis focuses on advanced techniques in MD, describes the used simulation protocols in a general, easy understandable way, and explains the analystical tools used in the thesis. I especially like here the comparison of the radial distribution function, the spatial distribution function, and the correlated and uncorrelated proximal distrtribution functions, pointing clearly out the situations when each of the functions can be applied, when the spatial distribution function is superior in information content to the radial distribution function and so on. It must be noted that Jan Heyda himself programmed code for the calculation of the 3D spatial distribution function. The results part is nicely logically structured, starting with very simple systems, amino acid proxies, and then coming via single amino acids and short oligos finally to peptides and proteins. After the detailed results the thesis finishes with a three page conculsion section, linking the results together and putting them into a larger framework. I very much appreciate this structure, it makes the thesis easy to read and understand, and connects the different parts by telling a continuous story. A personal hightlight for me in the thesis is surely the dynamics of peptide denaturation demonstrated for two widely used denaturants (urea and guanidinium chloride) acting on the TrpCage minipeptide. Jan Heyda is able to describe in atomistic terms the full denaturation pathway from the native structure, then a proline shift followed by Asp-Arg saltbridge destabilization, followed by enhanced flexibility and increased solvent accessible surface area, finally leading to the denatured state. The ensemble of denatured states was characterized by three independent experimental techniques (CD, DSC, NMR). The way from the native to the extended conformation is described below, and may be viewed as the main outcome of the work, as well as the comparison of actions of urea and guanidinium chloride.

studied contexts. Due to the fact that protein folding still is not fully understood and hardly predictable, and that proteins are always exposed to solvents and salty solutions with denaturation being a major issue in molecular biology, this work has potential applications to many different fields, such as biophysics, biochemistry, biology or biotechnology. Another highlight, in my eyes, is the explanation for strong stacking of guanidinium moieties of arginine as arginine stacking motifs are regularly found in protein structures, either inside a single protein or stabilizing protein dimers. Information that leads to a better understanding of this very special amino acid, arginine, is highly appreciated in protein chemistry. The thesis is in good and clear english, with very little typing errors or other omissions (for example on page 22, second row, the citation of the protein database is missing).

Finally, I must state that Jan Heyda until now conducted internationally recognised excellent science. The 13 publications that back up this PhD. thesis and their citation record show, without leaving any doubts, that the applicant fullfills all criteria for being awarded a PhD degree, therefore there is nothing else I can do, and I do that with pleasure, than to recommend Jan Heyda for being awarded the PhD degree.

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

1. Urea and guanidinium are very different and have different affinities to backbone and side chains of varying polarity and charge. However, your calculations show, and experiments confirm this, that the unfolding mechanism of Trp-Cage in both denaturants is practically the same. Please discuss possible molecular explanations for this phenomenon.
2. High salt concentrations are said to enhance enzymatic activity in case of HIV-1 protease. In your simulations of HIV-1 protease in NaCl and KCl solutions you demonstrate the preference of the protein surface for sodium over potassium ions. However, in KCl we find a significantly higher catalytic efficiency in hand with overall higher enzymatic activity compared to NaCl. This would oppose one of the suggested mechanisms, that the increasing salt concentration increases the conformational stability of the enzyme and thus the activity, as according to this theory NaCl would probably better enhance the activity than KCl. Could you please comment on that, and discuss possible mechanisms how increasing salt concentrations might enhance enzyme activity and why sodium decreases the activity?

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