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Referee's report on the PhD Thesis of Ewa Irena Gołaś entitled: "Molecular dynamics investigation of the structure-function relationships in proteins with examples from Hsp70 molecular chaperones, αA-crystallin, and sericin".

The submitted doctoral dissertation supervised by Professor Józef Adam Liwo, have been performed in the Laboratory of Molecular Modeling Faculty of Chemistry University of Gdańsk. Structure function relationships play a central role in chemistry and protein science. A look into the list of the literature devoted to the subject studied in the submitted PhD Thesis shows that mentioned relationships can be thought to span a very wide spectrum, where on one end, the function of a protein is to provide structure, and on the other end, the protein's structure lends to its function. Unraveling the structure function mystery therefore entails an investigation of the interplay between the notions of structure and function. The submitted work provides insight into the structure function relationships operational in three representative proteins: the Hsp70 (Heat shock protein 70kDa) molecular chaperone, whose function is to promote the proper structure of client proteins; αA-crystallin, which stands in the middle of the structure function spectrum, expressing both chaperone functionality and purpose of structual protein; and finally, a sericin based biopolymer, which puts structural relationships for engineering a novel biomaterial. Thus, the topic studied here by Ewa Irena Gołaś is undoubtedly actual, modern and the obtained results can have wide applicability.

The main goal of the present work was to contribute to the development of the qualitative theory of statistical thermodynamic of interactions between proteins and its environment.

The semi-quantitative theory of statistical thermodynamics of interactions between the Hsp70 molecular chaperone, which function as ATP-ase by use substrate binding domain (SBD) have been studied by all atom canonical molecular dynamics simulations using AMBER force field. The Principal Component Analysis enabled Ewa Gołaś a decomposition of the behavior of the domain into a series of eigenvectors describing important trend motions which occurred regardless of the binding state with accordance to experimental observations. More over, the protein dynamics reveals a complex nucleotide-dependent structure function relationship that is based on sub-domain rotation and internal allosteric network.

Recent decades have seen the emergence of a novel approach to scientific research, based on the exploitation of fast electronic digital computers. Computation provides a method of investigation which transcends the traditional division between theory and experiment. Computer-assisted simulation and design may afford a semi-quantitative solutions to complex problems which would otherwise be intractable to theoretical analysis, and may also provide a viable alternative to difficult or costly laboratory experiments. To select the simulation method, which would enable studies of whole chaperone (from *E. coli*, pdb 2KHO) Ewa

Gołaś have been employed the UNRES coarse-grained model and force field. The Langevin molecular dynamics were then performed, which enabled the observation and characterization of three types of binding between the SBD and NBD parts of chaperone. This part of work enabled a detailed description of the inter-domain communication between sub-domains, describing sub-domain behavior as the product of a structure-function relationship reliant on the state of the ATP-ase.

In part three of her thesis, Ewa Gołaś have been studied the αA-crystalin protein (from *B. Taurus*, pdb 3L1E) as model compound employing the AMBER all-atom model and force field. In this case, the effect of amino-acid racemization, that is, the substitution of an L-amino acid for its D-analogue, was investigated with respect to the mechanical and structural properties of the protein. Extracting accurate mechanical properties of materials from molecular dynamics (MD) simulations is notoriously difficult. There are special modern methods to be used to tackle this problem. Steered Molecular Dynamics (SMD) was the primary tool utilized by Ewa Gołaś, which introduces an external force in a molecular dynamics simulation of the system. In this case, the small heat-shock (sHsp) protein was stretched along its main long axis. An Essential Dynamics-like method was correctly employed as analysis methodology. A dominant feature found was a change in the stiffness of structural elements within the protein. Increased stiffness was associated with a re-organization of the structural links relative to native stiffness. Decreased stiffness was correlated to a decoupling of the force response from the typical structural-link pattern of αA-crystalin protein. For αA-crystalin protein, the structure-function relationship relating amino-acid chirality to the protein's mechanical properties is indeed highly sensitive and residue specific. Additionally Ewa Gołaś have been designed A sericin-based biopolymer and tested it mechanically by SMD simulation, using AMBER all-atom model and force field. It was her own scientific activity not suggested by Profesor Liwo. Since sericin is obtained as a byproduct of the silk processing industry, this part of work of Ewa Gołaś can have important practical consequences for this industry.

I conclude that all goals specified in the brief primers parts of the work were successfully reached and the problems outlined in the research findings were solved. The thesis is written very carefully and in perfect English, and the proofs are clear and easily understandable. I have found neither logical nor formal (typographical or linguistic) errors there. The work shows that Ewa Irena Gołaś is able to perform qualified independent scientific research and to present the results of his investigation in a cultivated way.

Conclusion:

I found the main results correct, new and interesting. The thesis is written clearly and without errors. I think that the results will be surely of considerable interest to specialists working in the field of chemical dynamic processes on time scales.

Summarizing the above facts, I conclude that the submitted work fulfills the requirements for the PhD Thesis and recommend to, issue the degree of PhD to Ewa Irena Gołaś.

Aleksander Herman

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