Evaluation of the Dissertation Thesis of Mgr. Honzejkova

Mgr. Honzejkova's dissertation thesis focuses on 14-3-3 proteins, a unifying theme among its varied topics and also the primary interest of Prof. Obsil's and Dr. Obsilova's laboratories. The thesis is based on three publications, two of which have been accepted in renowned journals - Protein Science and the Journal of Biological Chemistry - while the third is currently under review at the prestigious journal eLife. Mgr. Honzejkova is the first author or co-author in two of these publications.

The thesis is exceptionally well-written, displaying excellent English and containing only a minimal number of typos (few examples below). It's organized traditionally into five chapters: Literature review, Aims, Materials and methods, Results and discussion, and Conclusions. Each section is crafted with excellence, especially the Literature review, which has more than plenty of current and relevant citations.

Additionally, it's worth noting Mgr. Honzejkova's adeptness in a variety of biophysical techniques related to biophysical chemistry. These skills include protein expression and purification, Analytical Ultracentrifugation (AUC), Small Angle X-ray Scattering (SAXS), cryo-Electron Microscopy (cryo-EM), and Hydrogen-Deuterium Exchange (HD exchange), making her thesis methodologically rich. The thesis is also visually appealing, with well-prepared figures enhancing its graphical quality.

In conclusion, I am pleased to recommend Mgr. Honzejkova's thesis for defense. However, I do have a few questions and comments:

1) " The 14-3-3 proteins are called "hub proteins" because they interact with a large number of protein partners (recently estimated to be more than 1,200), including signaling proteins, transcription factors, metabolic enzymes, cytoskeletal components, apoptotic proteins, or cell cycle proteins..."

In this statement, I miss a large group of proteins, where some were also shown to interact with 14-3-3 proteins. Could you comment on the role of 14-3-3 in the regulation of proteins from intracellular parasites?

2) What is the major physiological role of the PH domain of the ASK1 kinase? Usually, it is a lipid binding domain. Is anything known about ASK1:membrane interaction?

3) The models of 14-3-3γ:CAMKK are set to have $\chi 2 = 2.13$. How was that calculated? Could you show an overlay of the experimental data and theoretical data calculated from the 14-3-3γ:CAMKK models?

Few English typoes:

1) "The structure of ASK1-KD was crystallized in complex with the inhibitor staurosporine" should read:

The ASK1-KD was crystallized in complex with the inhibitor staurosporine. (You get the structure after crystallization).

2) "Another physiological inhibitor of ASK1 is the 14-3-3 proteins,"

Proteins are, one protein is. You can say: "The 14-3-3 proteins are another physiological inhibitor of ASK1."

Best wishes in your future (scientific) career,

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