Review of the doctoral thesis

Domenico Lentini Santo: Structural studies of 14-3-3 protein complexes and their stabilization by small molecule compounds

Domenico Lentini Santo presents a doctoral thesis based on 4 scientific publications. All of them have multiple authors. The candidate is the first author of one and significantly contributed to all of them. There is one more paper of the candidate, which was not involved into this thesis. The overall goal of the dissertation was to study various complexes of 14-3-3 proteins with their partners and to investigate the effect of stabilization of such complexes, which can be achieved by binding of some small molecules (like fusicoccins) into specific binding sites. For this research, many methods have been used, for example protein crystallography, differential scanning fluorimetry, fluorescence polarization, analytical centrifugation, and many others.

The Introduction of the thesis is setting the background of the research, discussing various aspects of 14-3-3 proteins, mainly focusing on the ability of these proteins to form protein-protein complexes. Specifically, role of 14-3-3 interaction with Ca^{2+}/calmodulin-dependent protein kinase kinase 2 (CaMKK2) and with the inhibitor of nuclear factor NFκB (IκBα) is described. The thesis defines in further chapters several aims of the work and describes the methods used. The chapter 4 represents the core of the thesis, going through individual publications, specifying the contribution of the candidate, and showing, and discussing all results obtained. The thesis continues with the general Conclusions, the list of references and it is finished with the Supplements, consisting of the copies of 4 scientific publications of the candidate. The thesis has a good graphical quality (except for the Supplements) and is written in an English of acceptable level.

I my opinion, this thesis represents a good example of research in the scientific area, where biophysical chemistry borders the structural biology. The flexibility and other problematic aspects of studied proteins makes every new published 14-3-3 protein complex with its binding partner an important step toward the understanding of regulation of physiological processes. I consider the experiments with the fragment screening as the most valuable part of this thesis and the results showing the potential binding sites outside of the usual binding groove, as the most challenging ones. The publication with these experiments is the one, where is Domenico Lentini Santo mentioned as the first author.

I have several questions about the thesis:

1) If I am right, all studied small molecules used for the stabilization of 14-3-3 complexes were from fungi, or of an artificial origin. Is there anything known about similar molecules in animals or humans?

2) Is it possible to observe some structural changes on bound partners of 14-3-3 if we would compare the state with and without fusicoccin? Did you observe some similar differences in your own studies?

3) Is there any experience that crystallization of 14-3-3 with their protein partners can be facilitated by the presence of molecules like fusicoccin?

4) Why was one of your papers (Alblova et al. 2019) not included into the thesis? Wasn't it close enough to the main topic of the work?

In conclusion, I can say that I recommend accepting the thesis of Domenico Lentini Santo and I award this work the score of "passed".

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