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Opponent's report on a doctoral thesis:

STRUCTURAL STUDIES OF 14-3-3 PROTEIN COMPLEXES AND THEIR STABILIZATION BY SMALL MOLECULE COMPOUNDS

by

Domenico Lentini Santo.

The submitted doctoral thesis is focused on 14-3-3 proteins (the main interest of the supervisor lab), a family of eukaryotic adaptor and scaffolding proteins. The 14-3-3 proteins are involved in the regulation of many signalling pathways, and function as an interaction platform and critical regulators of many enzymes, receptors and structural proteins. The main aim was to structurally characterize selected 14-3-3 protein complexes and investigate their stabilization by small molecule compounds. MSc. Lentini Santo employed a combination of protein crystallography, differential scanning fluorimetry, fluorescence polarization and analytical ultracentrifugation. The protein-protein interactions (PPIs) between 14-3-3 and two physiologically important binding partners, the Ca^{2+}/calmodulin-dependent protein kinase kinase 2 (CaMKK2) and the nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha (IκBα), have been characterized. The stabilization of PPIs between 14-3-3 and CaMKK2 by fusicoccins have been investigated. Colleague Lentini Santo showed that the targeting of the fusicoccin-binding site by small-molecule compounds could be an alternative way how to suppress CaMKK2 activity by stabilizing its phosphorylation-dependent inhibited state. In addition, the screening of a fragment library designed to target the 14-3-3 protein surface enabled the identification of three molecules bound to two different surface sites of the 14-3-3 protein. This was aside the usual binding groove, which brings new possibilities for selective modulation of 14-3-3 complexes.

MSc. Lentini Santo is the author or co-author of three publications (he is the first author of one of them) published in international journals. The thesis is written in English, the formal level is

good and graphic design of the pictures is excellent. Of course, you can find some problematic expression and small errors, like mistypes, e.g. page 6: Diethylamonietil should be Diethylaminoethyl.

My favourite field is protein crystallography which brings me to my questions.

1) Data collection of the 14-3-3γΔC:CaMKK2 pS100 and 14-3-3γΔC:CaMKK2 pS511 complexes was performed on different diffractometers. Could you comment on what was the reason for this choice. And could you compare the two different data collections.
2) In your thesis you study stabilization of complexes upon binding using several methods. However, I didn't find the B factors analysis of the components-forming complex. This can provide many additional information about mutual stabilization of components in noncovalent interaction. Moreover, I miss the Wilson B in both data set, which is important for overall B factor analysis.
3) My last question is on the two structures of ternary complexes of the 14-3-3γ, the pepS100 peptide and the FC-A/16-Me-FC-H. Those two looks like the most problematic structures, concerning high B factors, low resolution and so on. Despite, it seems that you have good electron density for the both fusicoccanes molecules. This surprises me, because FC-A is almost tree order of magnitude worse binder than 16-Me-FC-H. To me, it would be much more convincing to see initial differential electron density or omit map, could you comment on that.

Submitted doctoral dissertation MSc. Lentini Santo represents a valuable contribution to the problematic of 14-3-3 proteins. The work is written clearly, carefully, the results have been published in impacted international journals. In his doctoral thesis author proved that he is capable of independent scientific work.

Since the presented work of MSc. Lentini Santo meets all the requirements for a doctoral defence, I fully recommend it for admission.

Prague, May 26.

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