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Evaluation of thesis of Mgr. Petr Těšina
Structural Studies of LEDGF/p75 Interactions

The thesis of Petr Těšina presents important results on the protein LEDGF/p75 and its binding parters. This work and corresponding articles provide an interesting information about these attractive anticancer and anti-HIV targets. The thesis is classically written with separate Literature overview, Materials and methods, Results and Discussions. Experimental methods used in this work comprise recombinant expression of five proteins, studies of their interaction by a FRET-based assay, NMR and calorimetry titration, structure determination, gel permeation chromatography, site-directed mutagenesis, bioinformatics and other techniques.

The author and his coworkers successfully identified the interface of the interaction between LEDGF/p75 and its binding partners MLL1, JPO2 and PogZ. These interactions were characterized at the level of identification of interacting residues; for LEDGF/p75-MLL1 and LEDGF/p75-PogZ also in the form of a three-dimensional structure of the complex of interacting domains. On the basis of these results they obtained a complete picture of the interplay between proteins MLL1, menin and LEDGF/p75.

Another protein JPO2 was identified as an example of intrinsically disordered proteins. The author characterized its interaction with LEDGF/p75 and demonstrated that their association leads only to partial structuring of JPO2.

Finally, the author identified a consensus motif responsible for interaction with LEDGF/p75. He scanned a sequence database and predicted and experimentally verified a new LEDGF/p75-binding protein IWS1.

The results presented in the thesis were published in high quality publications (*Cancer Research*, *Nature Communications*). I appreciate that authors did not fragment their results into multiple lower-quality publications. I can hardly find anything to criticize in the thesis, for example I would appreciate a figure showing structures of inhibitors presented in Literature overview. I would also appreciate some description of the cyclic peptide CP65. As far as I understood, it is cyclic due to presence of a disulfide bridge, thus word “cyclic” seems to me confusing. The thesis is written in very good English and, despite number of abbreviations, it is fluent and easy to read.

I would like to ask author following questions:

1. It can be found in many signaling pathways that signal transduction is relatively non-specific at the beginning of the pathway, for example activated receptors promote formation of relatively general second messengers and different signaling pathways may cross-communicate. Signaling becomes more specific in the middle of the pathway, e.g. certain protein kinase activates only the downstream protein kinase and at this point there is no cross-communications between different signaling pathways. Finally, signaling becomes again nonspecific at the end of the pathway, where one signaling protein may activate many different transcription factors, and signaling pathways intensively cross-communicate. Do the author think that the variety of roles played by LEDGF/p75 fits into this scheme?

2. The author have demonstrated that JPO2 does not fully structure upon binding to LEDGF/p75 and remains mostly unstructured. This is in the agreement with the fact that loss of flexibility leads to loss of entropy with a negative effect on binding. Is there any study showing how many of intrinsically disordered proteins fully structure upon binding to a partner and how many of them remain mostly unstructured?

3. The author used differential scanning fluorimetry and compared profiles of intrinsically

disordered protein and their complexes with binding proteins. How often is use of this method in studies of intrinsically disordered proteins and formation of their complexes?

The thesis of Petr Těšina elegantly combines molecular and structural biology to study potential targets of antiretroviral and anticancer therapies. Its results were published in high quality journals and make an important impact in the field. The thesis shows that Petr Těšina is an independent researcher, who is able to perform and report scientific work. Therefore I strongly support granting the Ph.D. degree to the candidate.

In Prague, 2 January 2016

Vojtěch Spiwok