



Fluorescence studies of bacterial membrane proteins and cellular signaling

Submitted by Radovan Fišer

“Oponentský posudek disertační práce”

Before going into details one has to state that the PhD thesis „ Fluorescence studies of bacterial membrane proteins and cellular signaling “written by Radovan Fišer is based on 5 original papers published in international Journals with an average IF of 6.7, studying mostly adenylate cyclase toxin (CyaA) from *Bordetella pertussis* and its interaction with biological membranes. According to the original publications shown in the PhD work, Radovan Fišer acts as the first author in 3 original papers. When going through those papers, it becomes evident that Radovan Fišer has considerably contributed to those papers, mainly by protein production and labeling, by incorporation of those proteins in model membranes, fluorescence microscopy experiments, and performing several fluorescence based assays. Considering both, the high publication productivity as well as the broad scientific interest of Radovan Fišer, it would be very difficult to find reasons for not recommending his thesis. It is evident that this work goes beyond the usual standards found for PhD thesis at Czech Universities.

The first study clarifies the membrane disruption mechanisms of CyaA and is mainly based on a fluorescence quenching method using liposomes. The resulting paper is published in BBA, having just two authors (i.e. corresponding author is the supervisor of the thesis).

Question:

- 1) It was found that the size of liposomes has influence on the validity of the fluorescence experiments. This should be explained during the examination in more details.

The second publication deals with the role of certain glutamates in the activity of CyaA, mostly on planar lipid membranes and erythrocytes (Published in JBC). As planar lipid membranes black lipid membranes formed by soy bean lecithin were used.

Questions:

- 2) Why did one use that particular lipid?
- 3) What are the advantages and drawback of this model lipid system for those particular experiments?
- 4) Could there also be other model membranes be used?

The third paper describes a new activity of CyaA that yields elevation of cytosolic calcium concentration (Published in *JBC*) in target cells. Here again Radovan Fišer serves as the first author. Here the authors used a known fluorescence assay based on Fura-2 for the Calcium concentration determination.

Question:

- 5) Would be there some alternative methods instead of Fura-2 fluorescence? What is the advantage of this particular assay?
- 6) How is the molecular mechanism of the used calcium channel blockers?

The forth paper is meant to address the recruitment of the integrin-CyaA complex into lipid rafts (Published in *PLOS PATHOGENS*) and is dealing with the co-localisation of fluorescently labeled CyaA and Cholera Toxin Subunit B. On top of that, also the fifth paper (Submitted to *PLOS PATHOGENS*) is dealing partially with that topic. I would like to focus the discussion here on paper 4, specifically on the co-localisation studies and their conclusions. To be honest, this part of the thesis is not fully convincing me. The co-localisation argumentation (see Figure 2) is based on purely intuitively co-localization findings in cellular imaging apparently without any quantitative arguments. Also, here detection of detergent resistant membranes and visualization of Cholera Toxin Subunit B binding to cells are mixed in the interpretation to yield information on rafts. Thus I would like to ask Radovan Fišer, to present a differentiated, defined explanation of those results and to present a careful interpretation of those findings during his defense.

Even the “raft part” at the end of the thesis might somewhat dilute the scientific significance of that PhD thesis, there is absolutely no doubt that this PhD thesis should be accepted by the PhD examination board. In particular, I very much appreciate the first 3 papers of the thesis, mainly, because all conclusions seemed to be “double checked” in the experiments. The forth paper is somewhat more speculative, possibly in the attempt to be more spectacular. The fifth paper is still under revision, and thus I took fewer efforts to judge that certainly interesting manuscript.

For the case, Radovan Fišer will successfully defend his thesis, he should be awarded with the title PhD.

Prague, 29.03.2011

Prof. Dr. Martin Hof, DSc.