

# Opponent's Review of the Ph.D. Thesis

## Maren Pflüger: Establishment of novel test systems for standardized screening of bioactive Substances

The aim of the work of Maren Pflüger was to establish several commercial cell-based *in vitro* techniques used for the screening of potentially bioactive substances. She also applied these methods for testing of various compounds isolated from natural sources, especially from cyanobacteria.

Ph.D. thesis of Maren Pflüger has 46 pages, and is divided into following parts: Introduction, Material, Methods, Results and Discussion. The work includes 33 references. Moreover, the work contains also 8 attachments with full texts of original papers. Maren Pflüger is listed as the first author in one case; (Journal of Biomolecular Screening). It was interesting for me to read this whole Ph.D. thesis, since I learned new things about several advanced methods for screening of bioactive substances. I think that this work can help, especially in its part "Introduction", in a similar way also to other people, especially to new students and collaborators in the lab.

I think the most important result of this work is the screening of more than 78 samples isolated from cyanobacteria. This effort resulted in the identification of 2 weak inhibitors of inflammation. Combination of several complementary assays, which have been used for this screening, also allowed to exclude compounds with potentially cytotoxic or barrier disintegrating effects. This strategy of application of complementary high-throughput assays is really notable.

### Comments:

The amount of work and results in this Ph.D. thesis is indisputable and admirable. However, I think that the main ideas, strategies and finally, the important findings of the whole work are hidden sometimes in the text, or are not mentioned at all and should be stressed in a more concise way. A brief conclusion in several points (put for example after the Discussion part) would help a lot.

The part "Discussion" could be more general and should stress not only what was done, but it should also fully evaluate the importance of these findings. The part Discussion written in the aforementioned article in the Journal of Biomolecular Screening could serve as a good example.

The way how the References are written is quite unsatisfying. Many styles are combined together; I found at least 5 different styles of writing author's names.

In scientific texts, the binomial names of species should be written in italics, by tradition; (e.g. *Nostoc muscorum*, pages 29 - 42).

**I have these questions:**

1. You have used compounds isolated from natural sources. Is it possible to guarantee the same or at least similar concentration of substances in aliquots tested?
2. You have used very interesting 3D artificial skin model. Could you describe it in more details? Which parameters does it share with normal skin and which parameters not?
3. Good positive and negative controls are usually important parts of similar screening experiments. What kinds of them were used for example during the screening of crude extracts from cyanobacteria; (Figures 6-9)?

I don't want to diminish the value of this work by previous comments. I think that this work fulfills well the requirements for Ph.D. thesis and I recommend it for the defense.

Prague, 30<sup>th</sup> November 2013

RNDr. Jiří Pavlíček, Ph.D.

A handwritten signature in cursive script, appearing to read "Jiri Pavlicek", with a horizontal line extending from the end of the signature.

**Ao.Univ.Prof. DI Dr. Johannes A. Schmid**  
**Center for Physiology and Pharmacology**  
**Dept. of Vascular Biology and Thrombosis Research**  
**Medical University Vienna**  
Schwarzspanierstr. 17, A-1090 Vienna, Austria,  
Tel: +43-1-40160-31155, FAX: +43-1-40160 931101;  
e-mail: [johannes.schmid@meduniwien.ac.at](mailto:johannes.schmid@meduniwien.ac.at)



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19 December, 2013

To  
University of South Bohemia, České Budějovice  
Faculty of Sciences

Review of the PhD thesis of Maren Pflueger

Dear ladies and gentlemen,

Thank you very much for considering me as appropriate reviewer for the PhD thesis of Maren Pflueger entitled: "Establishment of novel test systems for standardized screening of bioactive Substances".

In general, the thesis fulfills the requirements of a scientific work for obtaining a doctoral degree. The topic is clearly relevant given that professional cell-based assays for the purpose of screening anti-inflammatory drugs are important predominantly for translational aspects in life sciences. The fact that Maren Pflueger succeeded in publishing a peer-reviewed article in a scientific journal as first author furthermore proves that the necessary standards have been achieved. Moreover, as documented in the thesis, she is also co-author on a significant number of additional publications. While co-authorships per se cannot directly prove the proficiency of a scientist, they demonstrate at least that Maren Pflueger was embedded in a fruitful scientific environment thereby providing a professional educational basis for the development of scientific skills and know-how. Apart from the publications that are included in the thesis, the complementary text of the *Introduction* describes various aspects of inflammation and cancer in general, as well as the NF- $\kappa$ B signaling pathway as a central mediator of inflammatory processes and also various NF- $\kappa$ B inhibitors as therapeutic agents. Furthermore, different sophisticated assays and measurement techniques are described, which are suited for high-throughput screening of anti-inflammatory drugs. This part of the thesis is well written. The following *Methods* part appears quite elaborate and comprehensive and would allow repeating the experiments following the quite detailed protocols. Unfortunately, a section dedicated to describe the *Aims* of the study in a clear and structured manner is missing. It might be the case that this has not been defined as a requirement by the respective authorities.

With respect to the *Results* section the following criticism has to be raised:

- Alamar Blue assay (Figures 6 – 9): Proper dose response curves would have been fine including the calculation of IC50 (or EC50) values (such as in Fig. 25).
- Fig. 10, 11, 12: It is not sufficiently explained in the legend what “rel. induction level” (Y-axis) exactly means.  
Furthermore, it is not clear why two ratios are shown. Wouldn't be one sufficient – as it would also specify the second one (Live/Dead versus Dead/Live)?
- The NF- $\kappa$ B nuclear translocation assay (Fig. 29) was evaluated only in a qualitative manner. A quantitative assessment would have been possible by calculating ratios of cytosolic to nuclear fluorescence intensity – e.g. using the scientific freeware *CellProfiler*

Concerning the *Discussion* section it can be stated that it is rather short. This is probably due to the rather focused translational nature of the work, which does not lead to significant possibilities of scientific interpretation. In addition to these minor weaknesses, there are also some negligible typographic errors. Overall, the minor flaws that are criticized here are not hampering a principally positive assessment of the thesis.

Despite this principal positive assessment, I have a couple of questions for the purpose of discussion:

1. The AlphaLISA principle involves the formation of reactive singlet oxygen, which is diffusing towards nearby acceptor beads leading to a detectable signal only in case of close proximity: Does the medium influence the performance of the assay given that the diffusion distance of the reactive oxygen might depend on the composition of the medium?
2. The electrical impedance measurement to assess the barrier function requires special plates. Can a combined assay be established by using the ECIS plates and measuring the supernatant of these plates (e.g. for IL-8) with the AlphaLISA system – so that the same cell culture is measured with both principles?
3. What would be alternative methods to determine the barrier function of cells so as to exclude potential artifacts from the ECIS measurement?

In conclusion, I recommend to accept the thesis handed in by Maren Pflueger for defense, and to award her the Ph.D. degree.

With best regards,



Johannes A. Schmid

Gernot Schabbauer, PhD, Assistant Prof.

Center for Physiology and Pharmacology

Inst. of Physiology

Medical University Vienna

Schwarzspanierstr. 17, A-1090 Vienna, Austria, Tel: +43-1-40160-31427, FAX: +43-1-40160-93101

E-mail: [gernot.schabbauer@meduniwien.ac.at](mailto:gernot.schabbauer@meduniwien.ac.at)



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Review for the PhD thesis "Establishment of novel Test Systems for standardized Screening of Bioactive Substances" by Maren Pflüger Msc. Dec. 9<sup>th</sup>, 2013

Dear Members of the PhD committee

This is an evaluation of the PhD thesis handed in to the University of South Bohemia, Faculty of Science, by the candidate Maren Pflüger, Msc. in 2013.

In the introduction the candidate thoroughly describes the scientific background of bioactive substances and the development of those over the past decades. Then the interaction of NFκB as an example for a prototypic signalling pathway important in cancerogenesis and the development of cancer in general are discussed.

The results section is a summary of all the experiments and test systems, exclusively in vitro, she performed over time during her PhD. Most of those assays are focused on the activation of the NFκB signalling pathway in most cases by human TNFα and the pharmacologic intervention to inhibit effective outside-in signal transduction. Those assays are the cornerstones for the successful completion for some of the publications that are listed in the annex of this PhD thesis.

In the discussion the candidate elaborated on the different assay systems as well as the substances tested in the results section.

However I miss the detailed information (apart from the single sentence explanation in the preface) on the direct contribution of her work to some of the publications listed. Therefore I would be delighted to hear more about that during the *defensio* on the 19<sup>th</sup> of December.

Furthermore I would like to hear a detailed description of the "LanthaScreen" IκB assay, since, after reading the thesis, it is not clear to me on what basic biologic principles this assay is based on.

In general, this is a comprehensive study and through the establishment of valuable tools for the evaluation of biological test systems for bioactive substances the candidate has proven to contribute to scientifically relevant, hypothesis driven research (total IP: approx. 27). Those findings could have implications for the development of therapeutics.

With kind regards,

Gernot Schabbauer