

HABILITATION THESIS REVIEWER'S REPORT

Masaryk University

Applicant

Mgr. Zdeněk Farka, Ph.D.

Habilitation thesis

Advanced Immunochemical Biosensors and Assays:
From Label-Free to Single-Molecule Detection

Reviewer

Doc. RNDr. Lucie Korecká, Ph.D.

**Reviewer's home unit,
institution**

Department of Biological and Biochemical Sciences, Faculty
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The habilitation thesis of Dr. Zdeněk Farka, titled “Advanced Immunochemical Biosensors and Assays: From Label-Free to Single-Molecule Detection”, represents commented summary of 22 selected author’s articles of the total of 43 listed in the list of publications. Two of them are review articles. Some of them were published in highly prestigious journals, namely Biosensors & Bioelectronics (IF 10.6), Angewandte Chemie Int. Ed. (IF 15.3), Chemical Reviews (IF 60.6). Together with the H-index (16 according to Web of Science) it confirms the significant scientific qualities of the author.

An introduction of more than 40 pages provides a theoretical basis for the issues studied in the research focus of the author. In his research, Dr. Farka focuses mainly on the development of biosensing platforms and devices with applicability in clinical diagnostics of protein biomarkers, small molecules like drugs or metabolites, food-safety monitoring via detection of bacteria, and honeybee disease detection etc. These devices are based on utilizing various nanoparticles as sensitive detection labels. Photon-Upconversion nanoparticles (UCNPs) appear to be very promising for various applications. Another possibility in biosensors is presenting a label-free approach based on direct signal recording caused by the interaction of a determined analyte and detection platform without the necessity of an additional reagent. Within the research, he uses a wide range of modern analytical methods.

The presented results are a significant scientific contribution and have great potential for applicability in routine analysis in the area of clinical laboratory practice and monitoring.

Reviewer's questions for the habilitation thesis defence (number of questions up to the reviewer)

Here I enclose some questions as points for discussion:

1. On p. 28 you state, that in NLISA and ELISA “...antibodies are becoming the main limiting element. Therefore, the overall stability of the detection label could be, in the future, improved by replacing antibodies with MIPs or aptamers.” From which point of view is limitation meant? Specificity? Stability? If you compare antibodies and aptamers from the point of routine applicability, could aptamers (in future) replace antibodies in these assays?

2. You present works dealing with the detection of *Salmonella typhimurium* in milk samples based on EIS, QCM biosensors and SPR biosensing. Did you try to develop similar sensors also for other types of bacteria, e.g. *Campylobacter*, *Listeria*, which are important in food-quality control? Antibodies are a really limiting element for these bacteria due to quality and cross-reactivity.
3. In paper XIV aimed at the development of an immunoassay for detection of *Melissococcus plutonius* based on UCNPs you used the carbodiimide method for the preparation of conjugates with streptavidin using relatively high amounts of cross-linking agents (EDC, sulfo-NHS). Wasn't the quality of the prepared conjugate influenced by undesirable aggregation or multipoint cross-linking of molecules due to the amount of these reagents?

Conclusion

To sum up, the habilitation thesis presented by Dr. Farka undoubtedly fulfils the requirements expected of a habilitation procedure in the field of Biochemistry.

Date: 23. 5. 2022

Signature: