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Annex No. 10 to the MU Directive on Habilitation Procedures and Professor Appointment Procedures

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	Ing. Ján Tkáč, DrSc.
Reviewer's home unit, institution	Institute of Chemistry, Slovak Academy of Sciences

The Habilitation thesis discusses selection of 22 papers authored by Dr. Zdeněk Farka published in top ranked journals, what underlines high scientific status of the scientific work. The thesis focuses on the development and subsequent application of immunoassays and is divided into two logical sections depending on the transduction protocol applied i.e. using label-free and label-based approaches. Each chapter is accompanied by a short discussion about the importance of the analyte detected by a particular bioanalytical device.

It is worth to mention that the author also published review papers describing the state of the art in the field in the journal like Chemical Reviews and Angewandte Chemie International Edition.

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

Here are my comments and questions driven by my curiosity to start discussion:

1. On p. 11 you mention beneficial properties of aptamers. Do you think that DNA aptamers have also some drawbacks compared to protein-based biorecognition molecules?
2. What would be your ideal bioanalytical device for detection of low molecular analytes (such as hormones, small active compounds, toxins), medium sized analytes (proteins) and large analytes (whole cells, pathogens, viruses, *etc.*)? Would you prefer label-free transducing protocols? What do you think is the main limitation of label-free bioanalytical approaches?
3. In paper III you used screen printed gold electrodes (SPGE) to design the biosensor device in combination with Faradaic impedance measurements using a ferri/ferro redox couple. Did you observe a significant variation in the electrochemical behaviour of SPGEs? What about the stability of EIS signal on such electrodes since it is known that a ferri/ferro redox couple can etch gold from the electrode?

4. Why did you modify SPGE by a short thiol? Did you try also longer thiols for SPGE modification? Did you test EIS biosensing for analysis of complex samples such blood serum, cell lysate, *etc.*? If yes, did you get reliable analysis and results?
5. How convenient was it to work with QCM?
6. When you discussed paper IX you made a conclusion that assay time by the bioanalytical device was 60 min, much shorter compared to ELISA. How many samples could you analyse within approx. 10 h (time needed for ELISA analysis) by such a device?
7. On p. 27 it would be better to be more specific that “oxidation of antibodies by sodium periodate” is rather oxidation of glycan/carbohydrate present within the antibody structure rather that the oxidation of the protein backbone.
8. What biosensing (bioanalytical) device would you recommend for analysis of disease biomarkers in serum samples and why?
9. Do you consider upconversion-linked immunosorbent assays as biosensing assays?

Conclusion

The habilitation thesis entitled “Advanced Immunochemical Biosensors and Assays: From Label-Free to Single-Molecule Detection” by Zdeněk Farka **fulfils** requirements expected of a habilitation thesis in the field of Biochemistry.

Date: 24.4.2022 Signature: