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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	Dr. hab. Tomasz Grzyb, Associate Prossesor at Adam Mickiewicz University
Reviewer's home unit, institution	Faculty of Chemistry, Adam Mickiewicz University, Poznań, Poland

The presented for evaluation habilitation thesis, entitled "Advanced Immunochemical Biosensors and Assays: From Label-Free to Single-Molecule Detection," is a collection of 22 scientific articles in the field of biochemistry and sensors. Dr. Farka attached copies of the publications to the thesis with an over 40-page introduction and comments on the articles' material. The number of papers composing the habilitation is impressive and a substantial scientific achievement. Dr. Farka published these works in a relatively short time, between 2014 and 2022. In 7 articles, Dr. Farka is the first author, 10 as the second author, and 7 as the corresponding author. The presented in the thesis contribution statements prove the researcher's significant and sometimes crucial participation in preparing these works. All articles have were published in excellent high-impact journals, e.g., Chem. Rev. (IF = 60,622), Angewandte Chemie (IF = 15,336), Analytical Chemistry (IF = 6,986), Biosensors and Bioelectronics (IF = 10,618) or Nanoscale (IF = 7,790). These are very prestigious and well-known journals.

Dr. Farka has an outstanding academic record. His Hirsch index is currently 18, and the number of citations is 1328, according to Google Scholar on the day of writing this review. However, the most important information deduced from bibliometric data is the dynamic increase in citations of Dr. Farka's works. Such a dynamic proves the interest in the research results and is promising for Dr. Farka's future scientific career. It is worth adding that some works published only a year or two ago have a dozen or more citations.

The subject of the habilitation thesis is immunochemical biosensors and assays of various types of analytes such as small molecules (diclofenac, zearalenone), bacteria (*Salmonella*, *M. plutonius*, and *P. larvae*), proteins (human serum albumin, troponin, prostate-specific antigen). From the presented thesis, it is clear that Dr. Farka has a great experience in the investigated subject. The author does not focus on a single technique but extensively checks various analytical methods, from label-free biosensors, through catalytic labels to detection based on luminescence. Dr. Farka achieved significant results by using upconverting nanoparticles. The researcher is also an expert in biofunctionalization and surface modification, including the surface of nanoparticles, which is an important element of his scientific achievements. While reading the thesis, it is also clear that Dr. Farka is also a proficient chemist, which is required

to design efficient functional groups to trap molecules. In addition, the presented research concerns aspects important for the global community, such as the detection of *Salmonella* or monitoring of diseases of the honey bees. The author often presents the application in the real systems in the published works, which significantly increases the rank of the presented research.

In his habilitation thesis, Dr. Farka presents alternative methods of detecting analytes that can compete with such well-known techniques as ELISA or PCR and, in some cases, even significantly exceed them in terms of sensitivity, detection limits, or analysis time. This is a significant achievement, especially when it comes to detecting analytes important for human health, such as cancer markers, which, even at low concentrations, may indicate tumor development. Often the time of detection is also essential to stop the development of the disease or for rapid screening purposes. It is worth emphasizing that Dr. Farka's field of research has been developing very quickly recently because many key analytes still cannot be effectively determined using known techniques. Therefore, studies in the field of biosensors are important and definitely worth continuation.

The most significant achievements of Dr. Farka include:

- Employment of the electrochemical immunosensor for the detection of *Salmonella*. The effectiveness of the sensor was also confirmed using milk samples.
- Obtainment of a biosensor based on the quartz crystal microbalance phenomenon, which was used to detect bacteria in aerosols.
- Development of human serum albumin (HAS) biosensor based on surface plasmon resonance biosensing (SPR) with LOD of 50 ng/mL. Here, a significant achievement was also the optimization of the surface of the SPR sensors, which increased the response to HAS.
- Development of a chronoamperometric biosensor for the detection of *M. plutonius* with a short analysis time and high selectivity.
- Development of a highly sensitive, robust, and straightforward assay for detection of *Salmonella* using SPR immunosensor enhanced by biocatalyzed precipitation. The assay time was significantly shorter than commercial, and additionally, the method was successfully tested in food samples.
- Introduction of a method for the conjugation of Prussian blue nanoparticles with antibodies and application in nanozyme-linked immunosorbent assay (NLISA) for detecting HSA in urine and *Salmonella* in powdered milk. The developed NLISA offers comparable LODs to ELISA in case of HSA or better for detection of *Salmonella*.
- Development of assays based on upconverting nanoparticles (ULISA). Dr. Farka utilized the exceptional properties of upconverting nanoparticles, which exhibit anti-Stokes emission under irradiation with near-infrared light, to detect such molecules as diclofenac (DCF), zearalenone (ZEA), HSA - markers of albmuminuria, PSA – markers of prostate cancer, antibodies to *P. larvae* or bacteria *M. plutonius*. Upconversion-based tests are pioneering in the field and provide excellent results, thanks to a large Stokes shift between excitation and emission, high photostability, and low background signals. The obtained LODs of the analytes mentioned above were comparable with ELISA assays in the case of DCF or 400 times better for detecting *M. plutonius.* ULISA

allowed for even 200 times better detection of ZEA than previously reported bioluminescence immunoassays. A really impressive LOD of 24 fg/mL PSA was obtained when digital ULISA was employed.

- Upconverting nanoparticles were also used for labeling human epidermal growth factor receptors biomarker on the surface of breast cancer cells in immunocytochemistry imaging.
- Development of detection method of Ag and Au nanoparticles by laser-induced breakdown spectroscopy, which allows for detecting labels without luminescence properties.

Reviewer's questions for the habilitation thesis defence:

1. What was the emission quantum yield of studied upconverting nanoparticles? If the emission quantum yield was not studied, can the author estimate how important this property can be for a limit of detection?

2. Some of the results were obtained with core@shell nanoparticles and another with just bare $NaYF_4$:Yb,Er nanoparticles. Has the author analyzed how important is the thickness of the shell on the nanoparticles in terms of their performance in ULISA?

3. Has the author considered a different method of detection in digital ULISA? sCMOS cameras have some limitations, such as sensitivity range, whereas CCD, for example, allows for detection up to 1100 nm, making it possible to use different than 980 nm excitation laser wavelength and collect signal in the 830-1100 nm range omitted on the currently used setup.

Conclusion

In summary, the habilitation thesis entitled "Advanced Immunochemical Biosensors and Assays: From Label-Free to Single-Molecule Detection" by Zdeněk Farka fulfills the requirements expected of a habilitation thesis in the field of Biochemistry. The presented research results are important and breakthrough for biochemical and biological research—also other areas such as clinical diagnosis, food safety, and monitoring threats to the environment will benefit from the research results obtained by Dr. Farka.

Date: 05.05.2022 Signature