Ph.D. Thesis Review Report

Thesis by: Ing. Césare Rodríguez Emmenegger

Study programme: F-4, Biophysics, Chemical and Macromolecular Physics

Title: Sensitive Layers for Optical Biosensors and Protein Chips

This thesis represents a research on development of non-fouling surfaces for biosensors, the subject of extraordinary scientific significance. Both fundamental and applied issues have been addressed.

The thesis has rather unconventional structure and is divided into fourteen sections, almost each having its own brief introduction, results and discussion, summary and references parts. Furthermore, eight manuscripts authored by Ing. Rodríguez Emmenegger and published in peer-reviewed journals are included at the end. I find such arrangement reasonable and helpful in view of the vast amount of experimental work done. It allows the author's focusing on the analysis of the most important aspects within the main body of the thesis while leaving important but overwhelming details to the appendices.

The thesis first offers introduction where quite exhaustive state-of-the-art in the field of affinity biosensors is presented and general issues to be solved are classified. The second chapter sets up the goals of the thesis which are very clearly formulated. Each of the three goals specified are highly challenging in science and might have been a content of a separate PhD thesis. Chapter 3 gives all the required details of chemical syntheses and characterization techniques used. The chapters 4 to 14 analyze the results obtained by the author. The strong point of the narrative is its organization in a highly logical way showing the evolution of the author's ideas and the progress in his research. Three types of surfaces with different architectures have been progressively studied including self-assembled monolayers, end-tethered polymers and polymeric brushes. The SAMs and end-tethered polymers were effective in preventing protein adsorption from the single-protein solutions but mostly failed at interaction with blood plasma. The most outstanding results were obtained with polymeric brushes for preparation of which the author developed unique controlled polymerization pathways.

Among the numerous benefits of the work, I would like to emphasize several most important.

- A generally accepted paradigm of biofouling has been critically argued. New insight into this agenda has been developed. It was proven that at least hydrophilicity and the lack of hydrogen bond donors should not be taken as criteria for a surface to prevent biofouling.
- It was proven that model plasma protein solutions can not be used for unambiguous prediction of fouling from complex biological media.

- Novel biosensors for detection of *Cronobacter* in milk and a herpes virus in blood serum were developed and tested clinically on real samples.

From the formal view, the thesis has only a few minor drawbacks which by no means deteriorate the high quality of the work.

1) A few mistype errors are present in the text: page 6-1 "Figure C*6*-1…" instead of "Figure C4-1…"; page 6-5 "…in reactors to which new polymerization solution…*was added*"; page 7-6 "…hydrogen bond *donor* NH_3^+ groups…"; page 9-3 the legend to Figure C9-3 does not mention the green HAS line present in the figure; page 11-10 "Figure *C11-9*…" instead of "Figure C9-11…"; page 12-3 "…that not only were *they* stable but also that only…"; page 13-6 "Figure C13-*3B*…" instead of "Figure C13-B…"; page 13-6 "binding" instead of "biding".
2) Several figures are not cited in the text at all: Fig. C1-2, C1-6, C3-2, C5-2, C11-7, C11-8.
3) The chapter 12 deals with modification of nanocarriers to improve their stability in biological media. Although giving highly actual and important results, this chapter, in my opinion, is not completely relevant in this thesis dealing with sensitive layers and it could have been omitted.

In addition, I suggest the following issues to be discussed by Ing. Rodríguez Emmenegger at his defence.

1) The packing density of SAMs and polymeric brushes is extremely important parameter which strongly influences their performance. In this work, it was calculated. Furthermore, at least a couple of times (page 8-5 and 11-5) the thesis claims that AFM proved full and homogeneous coverage of surface by polymeric brushes. Methodologically, this is not very much convincing because AFM has limited lateral resolution, given mainly by the tip radius of curvature. The standard tips have approximately 10 nm radii and in real measurements the lateral resolution can be even worse. Therefore, AFM can not be sufficient for such analysis. Can the packing density of SAMs and polymeric brushes be assessed experimentally for these surfaces, if at all?
2) The non-fouling surfaces obtained in this work were tested against biological fluids, mainly blood plasma, at 25 °C. Can we directly correlate the results obtained for blood plasma at room temperature to those obtained at human body temperature? Can the non-fouling properties be affected by raising the temperature of the biological analyte? Certainly, this is a subject that goes beyond the scope of this thesis. I suggest it only for academic discussion.

To conclude, this thesis goes beyond the mainstream of the research, suggests innovative solutions and by far exceeds the requirements for a doctoral degree. Undoubtedly, the results obtained will be acknowledged by the world's leading research laboratories working in this field.

Doc. Ing. Andrey Shukurov, PhD
Associate professor
Charles University in Prague
Faculty of Mathematics and Physics
Department of Macromolecular Physics
V Holesovickach 2
18000 Prague
Czech Republic